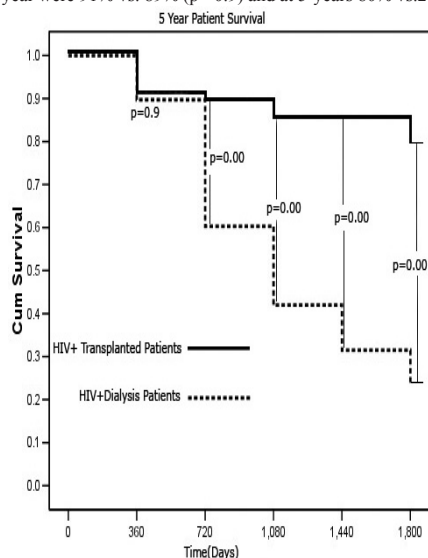


**AMERICAN TRANSPLANT
CONGRESS 2008
ABSTRACTS**

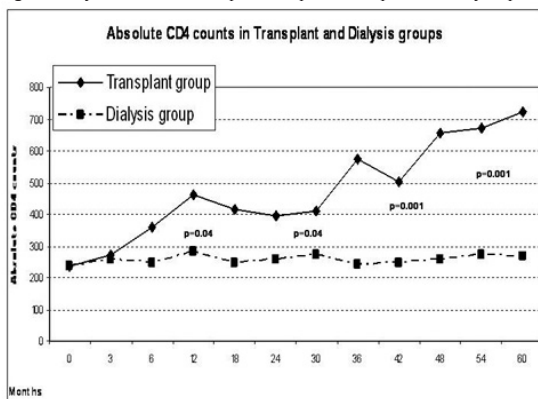
Abstract# 1

In HIV+ Patients with End Stage Renal Disease (ESRD) Kidney Transplantation Significantly Prolongs Long-Term Patient Survival Compared to Chronic Dialysis Treatment. Mysore S. Anil Kumar,¹ Shahid M. Khan,¹ Karthik Ranganna,¹ Gregory E. Malat,¹ Arjun M. S. Kumar,¹ Jeffrey M. Jacobson.² ¹*Surgery/Division of Transplantation, Drexel University/Hahnemann University Hospital, Philadelphia, PA;* ²*Infectious Disease and HIV Medicine, Drexel University/Hahnemann University Hospital, Philadelphia, PA.*

The proportion of HIV+ patients on dialysis is increasing due to 2 reasons: HIV infection is the third most common cause of ESRD in the African American population and highly active anti-retroviral therapy (HAART) has significantly prolonged survival in HIV+ patients. This prospective study analyzed long-term patient survival in 132 consecutive HIV+ patients with ESRD who were placed on the wait list with the intent for kidney transplantation during February 2001 and December 2006; 65 received kidney transplantation and 67 patients on the wait list continued dialysis treatment due to non availability of kidneys. The selection criteria for waitlist placement included maintenance on HAART, plasma HIV RNA < 50 copies and absolute CD4 count of ≥ 200 cells per mm³ of blood. Primary end point was patient death. Patient demography was comparable in transplant and dialysis groups. Patient survivals in transplant vs. dialysis groups at 1 year were 91% vs. 89% ($p=0.9$) and at 5-years 80% vs. 26% ($p=0.000$).



Graft survival in transplant group was 85% at 1 year and 54% at 5 years. Plasma HIV RNA remained < 50 copies in all patients. The mean absolute CD4 counts significantly increased in transplant recipients compared to dialysis patients.



Despite equal therapeutic suppression of HIV infection with HAART and similar comorbid factors kidney transplantation significantly prolonged 5-year patient survival in HIV+ patients compared to dialysis treatment.

Abstract# 2

Spare-the-Nephron (STN) Trial in Liver Transplant Recipients: Interim Efficacy and Safety of Mycophenolate Mofetil (MMF)/Sirolimus (SRL) Maintenance Therapy after CNI Withdrawal. L. Sher,¹ P. Marotta,² L. Teperman,³ A. Sebastian,⁴ D. Moonka,⁵ B. Koneru,⁶ D. Patel,⁷ G. Klintmalm.⁸ ¹*USC, Los Angeles, CA;* ²*London Health Sciences Hospital, London, ON;* ³*NYU, NY, NY;* ⁴*Integrus Baptist Med Ctr, Oklahoma City, OK;* ⁵*Henry Ford Hosp, Detroit, MI;* ⁶*UMDNJ, Newark, NJ;* ⁷*Roche, NJ;* ⁸*Baylor Med Ctr, Dallas, TX.*

Purpose: To compare the efficacy and safety of maintenance immunosuppression with MMF and SRL to that of MMF and a CNI in liver allograft recipients.

Methods: In a 2-year open-label, prospective, randomized, controlled multicenter study, 340 planned patients maintained on MMF and a CNI were randomized 30-90 days after transplantation to receive MMF (1-1.5 g BID) plus SRL (2-4 mg/day; trough 5-10 ng/mL) and discontinue the CNI (MMF/SRL group) or continue their current regimen (MMF/CNI group). Antibody induction and/or corticosteroids were administered according to individual center practice. Primary efficacy variables included the percent of patients with biopsy-proven acute rejection (BPARG), graft loss, and death, and mean percent change in calculated glomerular filtration rate (GFR; MDRD-6 equation) at month 12. BPARG was assessed by Banff grade and Rejection Activity Index (RAI). Safety outcomes included incidence of adverse events, malignancies, and opportunistic infections at months 12 and 24.

Results: Interim 12-month data from 276 patients (MMF/SRL, n=139; MMF/CNI, n=137) were analyzed; baseline characteristics were similar between groups. Mean time from transplant to randomization was 54 days in the MMF/SRL and 52 days in the MMF/CNI groups.

	MMF/SRL	MMF/CNI
Mean GFR increase, baseline to month 12 (n)	22.1% (52)	5.2% (59)
Patient survival to date	137 (99)	132 (96)
Graft survival to date	137 (99)	132 (96)
Withdrew from study	51 (35)	27 (18)
BPARG $\geq$ II and RAI $\geq$ 4	15 (11)*	4 (3)
BPARG Grade I or RAI < 4	13 (9)	11 (8)

*Includes 1 severe BPARG

Grade $\geq$ II and RAI $\geq$ 4 BPARG occurred in 6 (10%) HCV+ patients receiving MMF/SRL and 1 (2%) receiving MMF/CNI; Grade I or RAI < 4 BPARG occurred in 11 (19%) HCV+ patients receiving MMF/SRL and 6 (11%) receiving MMF/CNI. The impact of these regimens on HCV recurrence and severity will be presented. 31% of patients in the MMF/SRL vs. 15% in the MMF/CNI group withdrew for safety reasons.

Conclusions: MMF/SRL appears to improve renal function at 1 year compared to MMF/CNI-containing regimens in patients but with more frequent rejections. At least 69% of patients are able to tolerate this maintenance regimen and may benefit long term.

Abstract# 3

Antibody Mediated, Complement Independent Transplant Arteriopathy in Mice. Tsutomu Hirohashi,¹ Shuichi Uehara,³ Catharine M. Chase,¹ Joren C. Madsen,¹ Paul S. Russell,¹ Robert B. Colvin.² ¹*Department of Surgery, Massachusetts General Hospital, Boston, MA;* ²*Department of Pathology, Massachusetts General Hospital, Boston, MA;* ³*Department of Pediatric Surgery, Osaka University Graduate School of Medicine, Osaka, Japan.*

Purpose: Based on experimental and clinical studies, alloantibodies are believed to be one of the pathogenetic mechanisms responsible for chronic transplant arteriopathy, a major cause of late graft loss. Whether complement fixation is necessary, as it is for acute antibody mediated rejection, is unknown, but is suggested by the observation that antibody alone can activate endothelial cells in culture. Therefore, we have tested whether complement is required for antibody-mediated chronic transplant arteriopathy in mice.

Methods: Hearts from B10.BR (H-2k) donors were transplanted heterotopically to C57BL/6 recipients (H-2b) lacking the RAG1 gene (B6RAG^{-/-}) or both the RAG1 and C3 genes (B6RAG^{-/-}C3^{-/-}). Recipients were given mouse monoclonal antibody to H-2Kk, either IgG2a (complement fixing, 36-7-5) or IgG1 (non-complement fixing, AF3-12.1.3). Controls received either monoclonal anti-H-2Dd (IgG2a) or no treatment. 30 μ g of purified antibody was given i.p. on day +1 and 2x/week thereafter. On day 28, the transplanted hearts were removed and evaluated for coronary allograft vasculopathy (CAV) by histologic and morphometry (neointimal index) and C4d deposition by immunohistochemistry.

Results: Transplanted hearts in recipients treated with IgG2a or IgG1 anti-H-2Kk had prominent vascular lesions (8/9 and 6/9, respectively; $p<0.005$ and $p<0.05$, vs. untreated group). C4d was deposited in the IgG2a- but not the IgG1- treated hearts. A minority treated with anti-H-2Dd had vascular lesions (2/7; $p>0.05$ vs. untreated). The neointimal index was higher in IgG2a and IgG1 anti-H-2Kk treated recipients compared with untreated recipients (IgG2a group $p<0.01$). To further rule out subtle participation of late complement components, we bred C3 deficient B6RAG^{-/-} mice. IgG2a anti-H-2Kk administered to B6RAG^{-/-}C3^{-/-} mice induced CAV in all recipients (5/5). The neointimal index of the IgG2a treated recipients was $62.6 \pm 23.8\%$ vs. $11.4 \pm 18.4\%$ in the untreated B6RAG^{-/-}C3^{-/-} controls ($p<0.01$).

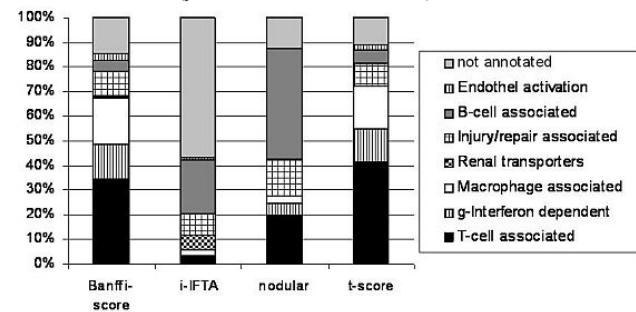
Conclusions: These data indicate that antibody can trigger chronic vascular lesions in vivo without complement participation. Thus, even non-complement fixing antibodies, which do not cause C4d deposition, may participate in late graft injury.

Abstract# 4

Heterogeneity in Molecular Associations on Infiltration: Inflammation in Areas of Interstitial Fibrosis and Tubular Atrophy Correlates with B Cell/Plasma Cell Associated Transcripts and Inferior Prognosis of Renal Allografts. Michael Mengel,¹ Jeff Reeve,¹ Gunilla Einecke,¹ Banu Sis,^{1,2} Julie Riopel,² Philip F. Halloran.¹ ¹Alberta Transplant Applied Genomics Centre, University of Alberta, Edmonton, AB, Canada; ²Laboratory Medicine and Pathology, University of Alberta, Edmonton, AB, Canada.

According to the current Banff classification, infiltrates in cortical areas of interstitial fibrosis and tubular atrophy (IFTA) should not be considered for the diagnosis of rejection.

In 129 renal allograft biopsies we assessed semi-quantitatively the % cortex with inflammation in areas with IFTA (i-IFTA). The extent of i-IFTA was correlated with established Banff criteria, gene expression by microarrays, and allograft function. Extent of i-IFTA correlated significantly with the extent of IFTA. Of the 54675 microarray probe sets 202 correlated with the extent of i-IFTA ($r>0.4$, $p<0.0001$). 57% of these transcripts were not annotated by previously described pathogenesis based transcript sets of renal allograft rejection. 22% were annotated as B/plasma cell associated transcripts (predominating also with nodular infiltrates), 9% as injury-and-repair, 6% as kidney transcripts decreased in epithelial cells, but only 4% as T cell associated transcripts. In contrast, of the 483 transcripts found to be significantly correlated with infiltrates in non-scarred cortex (i.e. Banff i-score), none were B/plasma cell associated while 34% were T cell associated (predominating also with tubulitis).



B cell richness in i-IFTA was confirmed by immunohistochemistry. The extent of i-IFTA ($r=0.29$, $p=0.002$) and IFTA ($r=0.31$, $p=0.0009$) showed an inverse correlation with future renal allograft function.

Infiltrates in IFTA show an association with B/ plasma cell transcripts, while infiltrates in non-scarred cortex are predominantly related to T cell associated transcripts. This data suggest heterogeneity of the molecular events in these two infiltrate patterns, and that i-IFTA has information that should not be ignored. Since i-IFTA is related to an inferior prognosis, the evaluation of i-IFTA should be added to routine biopsy assessment.

Abstract# 5

Combining Life Years from Transplant, Dialysis Time, Sensitization Points and Donor Profile Index. M. D. Stegall,¹ K. P. McCullough,² P. G. Stock,³ A. M. Rodgers,² A. B. Leichtman,² R. A. Wolfe.² ¹Department of Surgery, Mayo Clinic, Rochester, MN; ²SRTR, Ann Arbor, MI; ³Department of Surgery, UCSF, San Francisco, CA; ⁴Dept of Medicine, Division of Nephrology, SRTR, U of Michigan, Ann Arbor, MI.

Introduction. A new kidney allocation system is being considered by the OPTN/UNOS Kidney Committee that seeks to increase the overall utility of the system and while still offering an opportunity for transplant to a wide range of candidates.

Methods. More than 20 schemas were simulated using the Kidney-Pancreas Simulated Allocation Model (KPSAM) including combinations of: 1) a continuous ranking schema for donors (termed the donor profile index, DPI); 2) Points assigned to candidates based on: (a) Life years from transplant (LYFT); defined as the expected median years of life with kidney transplant (KTx) compared to remaining on the waiting list (WL) with WL years weighted at 0.8 vs years with a functioning KTx; (b) time elapsed from the start of dialysis allowing candidates to move up the list over time and (c) points for sensitization. Pediatric priority was unchanged.

Results. Several models increased overall LYFT compared to the current schema. Age matching of donors/recipients reduced transplants in recipients 65+ years the greatest. Ranking candidates using LYFT points alone for standard criteria kidneys increased overall LYFT the most. Several models employed DPI—one allocated the best DPI kidneys by LYFT points alone and a second allocated these kidneys by a combination of LYFT (80%) and Time (20%). Both included up to 4 additional points for sensitization. This last simulation decreased overall LYFT, but increased the number of kidney

transplants in African Americans and was supported by the Committee as their initial proposal for further public discussion.

Conclusions. Simulations demonstrate how changes in kidney allocation can increase both patient and graft survival, increase transplantation rates among African Americans while still enabling a wide spectrum of candidates to receive a transplant.

	Current Schema	Age Matching	Quintiles LYFT	LYFT Alone	LYFT Cont DPI	Max DPI = 80%LYFT/20% Time
ALYFT	48,424 (ref)	+7871	+198	+11346	+7079	+3402
Rec AfAm	32.5	34.9	35.3	33.0	36.7	37.7
Rec. Caucasian	47.4	45.3	44.0	44.7	41.9	41.1
PRA 80+	13.1	4.7	5.6	7.2	13.4	14.0
Rec 18-34	12.9	21.2	10.5	33.7	23.8	21.6
Rec 35-49	30.5	35.3	27.6	31	33.4	33
Rec 50-64	37.8	30.8	41.2	21.9	27.8	29.5
Rec 65+	12.2	5.3	13.6	6.2	7.9	8.5

Abstract# 6

Low Allo-Specific CD3-Cell Reactivity Pre-Desensitization (PRE-DES) as Detected by Intracellular Cytokine Flow Cytometry (CFC) Predicts Low Risk for Antibody (AB) Mediated Rejection (AMR) in Highly-Sensitized Patients (HS). Shili Ge,¹ Andy Pao,¹ Nancy Reinsmoen,¹ Ashley Vo,¹ Robert W. Chang,¹ Alice Peng,¹ Rafael Villicana,¹ Stanley C. Jordan,¹ Mieko Toyoda.¹ ¹Transplant Immunology Laboratory, HLA Laboratory, Comprehensive Transplant Center, Cedars-Sinai Medical Center, Los Angeles, CA.

Background: We have previously reported that allo- and donor (DO)-specific CD3- cell numbers as analyzed by CFC are elevated in many HS, but not normal controls (NC). HS w/ high(+) CFC are at higher risk for AMR. Measuring CFC against DO peripheral blood mononuclear cells (PBMCs) is ideal in living DO (LD) transplants (Tx). However, using a mixture of PBMCs from multiple NC (NC-PBMCs) might also be informative, especially in deceased DO Tx. Here, we report on pre-DES allo-reactivity by CFC in 25 HS who received DES with IVIG and Rituximab followed by Tx. **Methods:** Pre-DES samples in 25 HS (14 LD) were submitted for CFC. Whole blood was incubated overnight w/ NC-PBMCs prepared from 5 NC. IFN γ /CD3- cells were enumerated and results expressed as a ratio against IFN γ + cells w/o stimulation. A ratio >5 represents high(+) response. This ratio was compared with contemporaneous clinical data except where stated. **Results:** 14 HS showed a ratio >5 (11.5 $\pm$ 7.5, range 5.1-30.5), 11 showed <5 (1.6 $\pm$ 1.0, 0.6-3.9). Higher CFC were found more often in females (12/18, 8.5 $\pm$ 8.2) than males (2/7, 3.4 $\pm$ 3.3, $p=0.08$). No significant correlation between CFC and age, previous Tx number, or anti-HLA class I/II Abs by ELISA was found. However, there was an association between CFC responses and current or peak flow PRA (f-PRA). HS w/ f-PRA <30% showed low CFC, while all with high CFC had f-PRA >30%. However, some HS w/ f-PRA >30% also showed low CFC. In LD HS, no significant correlation between CFC responses and flow crossmatch against LDs was noted. 16/25 HS received Tx (11 LD). 0/7 HS w/ CFC <5 (1.2 $\pm$ 0.7) developed AMR, while 4/9 HS w/ >5 (10.9 $\pm$ 8.0) did ($p=0.04$). **Conclusions:** 1) CFC using NC-PBMCs is a novel assay to measure global allo-specific CD3- cell responses, 2) Low CFC (ratio <5) pre-DES predicts successful Tx w/o AMR, 3) Allo-specific response by CFC differs from concurrent anti-HLA Ab by ELISA or crossmatch levels, but partially mirrors f-PRA, maybe due to its high sensitivity, 4) The trend of high CFC in female HS may explain their higher rates of AMR, 5) Measurement of allo-specific response by CFC using NC-PBMCs in HS may identify those at higher risk for AMR and in need of additional immunosuppression.

Abstract# 7

Cost Analysis of Renal Transplantation in Highly Sensitized Recipients Compared to Maintenance Hemodialysis. Ahmed H. Aljedai,¹ Mohammed S. Alsultan,² Khalid A. Al Meshari,¹ Khalid Al Shaibani,¹ Mai Altalhi,¹ Abdulaziz Alotaibi,¹ Mennah Sharaf,¹ Khaled F. Hamawi.¹ ¹Renal and Pancreas Transplant Program, King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia; ²College of Pharmacy, King Saud University, Riyadh, Saudi Arabia.

Background:

Renal transplantation in positive cross match recipients applying various desensitization protocols has increased over the past few years. Cost analysis data of renal transplantation utilizing such expensive regimens is scarce.

Methods:

We performed an actual cost analysis study comparing highly sensitized renal transplant recipients and hemodialysis patients. Group A (n=25) consists of all highly sensitized renal transplant recipients who underwent successful desensitization at our institution using immunoadsorption or plasmapheresis, high dose immunoglobulin, anti-CD20 and polyclonal antibodies induction and had at least two years of follow up. Group B (n=49) consists of matched group of patients who remained on hemodialysis during the study period. Actual cost was calculated for all surgical procedures, out-patient visits, in-hospital admissions, medications, hemodialysis, immunoadsorption, plasmapheresis, laboratory and radiology investigations for both groups including the pre-transplant evaluation period. Donor costs were added to the recipient's first year total cost in

group A. All health care related services for both groups during the study period were provided solely by our institution.

Results:

With the exception of female gender predominance in group A, no difference in patients' demographics was noted. Mortality rate was similar between both groups. Average follow up period was 3 years. The average 3-year actual total cost was \$157,751 and \$118,440 in group A and B; respectively ($p=0.017$). Average annual cost for subjects in group B was \$79,759 while the annual cost in group A was \$133,291, \$14,233, \$5,536, \$4,402 in the first, second, third and fourth year post transplantation; respectively. In group A, the first year accounted for 84.5% of the total cost acquired over 4 years post transplantation. Using a simplified cost modeling, the total cost in both groups became equal in month 25. The predicted annual cost savings in group A after 25 months is equal to \$33,943.

Conclusion:

Despite using costly desensitization protocols in the first year, renal transplantation in highly sensitized subjects provides long-term cost savings when compared to maintenance hemodialysis.

Abstract# 8

Use of IVIG + Single Dose Rituximab for Desensitization of Highly-HLA Sensitized Patients Awaiting Kidney Transplantation. Ashley A. Vo,¹ Robert Chang,¹ Mieko Toyoda,² Nancy Reinsmoen,³ Alice Peng,¹ Rafael Villicana,¹ Stanley C. Jordan.¹ ¹Comprehensive Transplant Center; ²Transplant Immunology Laboratory; ³HLA Laboratory, Cedars-Sinai Medical Center, Los Angeles, CA.

Introduction: The demand for kidney transplants exceed the supply, thus increasing the wait times on dialysis. This is especially true for patients who are broadly sensitized to HLA (HS). We showed that high-dose IVIG (2gm/kg x 2) + rituximab (1gm x2) was effective in lowering anti-HLA antibody titers and improving rates of transplantation. Here we report on a new approach to desensitization using IVIG (2gm/kg X2) + single dose rituximab. **Patients & Methods:** From 7/06-11/07 64 HS patients were desensitized using IVIG 2gm/kg (day#1 & day#30) + rituximab (1gm on day#15). Parameters evaluated included: Rates of transplantation, previous transplants, PRA values, Crossmatch (CMX) at transplant, patient and graft survival, acute rejection (C4d+ AMR/C4d- CMR), serum creatinine values (1M, 3M, 6M, 12M) and infections. **Results:** 55/64 (86%) treated patients (27LD/28DD) were transplanted. All transplanted patients received induction therapy with Campath 1H (#33), Zenapax® (#19) or Thymoglobulin® (#3). Patients were maintained on Prograf®, Celcept® and steroids. Time on wait list for DD recipients prior to treatment was (89±43M, range: 60-180M). After treatment, time to transplant was (1.73±1.1M, range: 1-5M). 61% of patients had PRAs >50% and 19% had PRAs 20-50% at time of desensitization. 52% of patients had +FCMX at time of transplantation. Six patients (LD) needed additional plasmapheresis to achieve transplantation. After transplant, 10 patients (18%) experienced AR (11% C4d+ 7% C4d-). Patient & graft survival at 12M was 96%/94%. Three graft losses occurred (1 non-compliance at 12M, 2 AMR at 1M and 12M). One patient death at 6M was related to sepsis. The mean serum creatinine values were: 1M 1.67±1.1, 3M 1.28±0.6, 6M 1.28±0.6 and 12M 1.4±0.4mg/dl. Viral infections were seen in 4 patients (2 polyoma BK and 2 CMV). **Conclusions:** 1). The use of IVIG 2gm/kg X2 and single dose 1gm rituximab appears to offer significant benefits in reduction of anti-HLA antibodies and improving rates of transplantation for HS patients, especially those on the DD list. 2). Significant reductions in AMR rates (11%) were seen in this patient population and this was associated with excellent 1 year graft/patient survivals. 3). Thus, IVIG + single dose rituximab reduces the time and costs needed for desensitization.

Abstract# 9

Low-Dose Rituximab Injection as an Effective Preconditioning Therapy for Prevention of Antibody-Mediated Rejection (AMR) in Donor-Specific Anti-HLA Antibody (DSA) Positive Renal Transplant Recipients. Kazunari Tanabe,¹ Hideki Ishida,¹ Kazuya Omoto,¹ Tomokazu Shimizu,¹ Hiroki Shirakawa,¹ Daisuke Toki,¹ Hayato Nishida,¹ Tadahiko Tokumoto.¹ ¹Department of Urology, Tokyo Women's Medical University, Shinjuku, Tokyo, Japan.

INTRODUCTION:

Recently, it was reported that AMR occurred in patients although they had only low levels of DSA. Anti-CD20 antibody (rituximab, RIT) has recently been used in immunologically high-risk kidney transplant recipients. The objective of this study was to determine whether low-dose RIT could reduce the incidence of AMR in recipients with DSA and improve graft survival.

MATERIALS AND METHODS:

Between January 2005 and May 2007, 43 patients who had DSA underwent living-related renal transplantation under a preconditioning regimen with a single low-dose of RIT (200-500mg/body) and plasmapheresis (PP). Tacrolimus, mycophenolate mofetil and methylprednisolone were started 7 days before transplantation. Thirty-nine DSA-positive recipients who underwent kidney transplantation without RIT treatment were employed as a matched control group. We performed protocol biopsies within and over 6 months after transplantation.

RESULTS:

None of the RIT-treated patients died or lost their graft during the observation period (patient and graft survival: 100%), whereas 4 of the 39 RIT-non-treated patients lost their grafts (graft survival: 88%). The incidence of AMR or acute vascular rejection (AVR) was significantly reduced in the RIT-treated patients compared to the RIT-non-treated patients (RIT-treated patients, 7% vs. RIT non-treated patients, 54%) ($p<0.001$). Also, the RIT-treated patients showed a significantly lower incidence of chronic AMR compared to the RIT-non-treated patients (RIT-treated group vs. RIT-non-treated group; 11% vs. 43%). The post-transplant incidence of DSA-positive patients was significantly higher in the RIT-non-treated patients (70%) than in the RIT-treated patients (30%) and in these patient cohorts, the incidence of chronic AMR was also significantly higher in the RIT-non-treated patients (42%) compared to the RIT-treated patients (8%) ($p<0.01$).

CONCLUSION:

The incidence of AMR/AVR in pre-transplant DSA-positive patients was significantly reduced in RIT-treated patients compared to non-treated patients. The incidence of posttransplant DSA production was significantly higher in RIT-non-treated patients compared to RIT-treated patients. Low-dose RIT induction therapy seems to be a very effective treatment option for DSA- positive kidney transplant recipients.

Immunosuppression: Protocols for Sensitized and ABO Incompatible Renal Transplantation: A Period of Consolidation

Abstract# 10

Correlation between Post-Transplant Donor Specific Alloantibody (DSA) Levels and Acute Humoral Rejection (AHR). J. M. Burns,¹ L. D. Cornell,¹ D. K. Perry,¹ H. S. Pollinger,¹ J. M. Gloor,¹ W. K. Kremers,¹ M. D. Stegall.¹ ¹Mayo Clinic, Rochester, MN.

Introduction: The aim of this study was to assess the association between post-transplant DSA levels and histologic changes of AHR in positive crossmatch kidney transplants (+XMatch KTx).

Methods: We retrospectively examined 53 +XMatch KTx recipients (defined by T or B cell flow cytometric crossmatches (FXM) and Single Antigen Flow Beads (SAFB)) at our institution from 1/05 to 7/07. Anti-donor HLA antibody level was defined as the channel shift on the B cell FXM or the total of all normalized values (TotNlval) against donor HLA types by SAFB. All patients underwent protocol biopsy at least once in the 28 day post-transplant period. DSA levels were performed on serum collected on the same day. Additional biopsies were performed for cause. Biopsies were scored as: no rejection (0), C4d deposition alone (1), moderate AHR (2; mild tubular damage/neutrophil infiltration) and severe AHR (3; glomerular thrombosis, necrosis, mesangiolysis). Linear regression was used to model DSA levels as a function of rejection severity. A jackknife estimate of variance was used for all tests to account for dependencies of multiple observations deriving from individual recipients.

Results: Over the 28 day period, 25 patients had persistently normal biopsies, 3 had C4d deposition alone, 12 had moderate AHR, and 13 severe AHR. DSA level was found to have a direct relationship to histologic injury of the allograft ($p<0.0001$; Figure 1). No patient with a B cell FXM channel shift <350 developed AHR. 93% (28/30) of biopsies with AHR had a TotNlval > 9,000 while 89% (24/27) of biopsies without AHR were found to have a TotNlval < 9,000.

Discussion: These data suggest a strong association between DSA level and the occurrence of AHR after +XMatch KTx. Persistently low levels of DSA after transplant did not result in AHR suggesting that clinical protocols should be designed to maintain these low levels. C4d deposition alone appeared to be associated with intermediate levels of DSA. Post-transplant SAFB assays using TotNlval appears to be a useful method for monitoring for AHR and can decrease the need for frequent crossmatches and protocol allograft biopsies

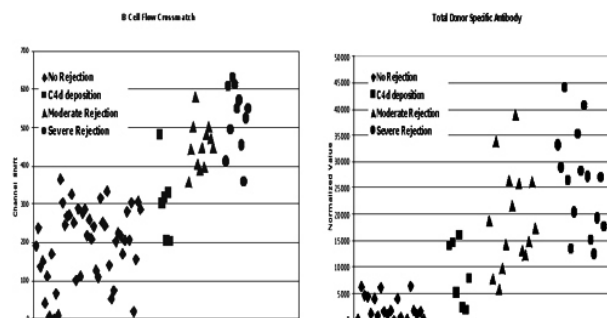


Figure 1: Correlation of antibody level to histologic evidence of rejection in B cell FXM and SAFB (total DSA level).

Abstract# 11

Long Term Outcome Following Anti-Human Globulin-Enhanced Complement Dependent Cytotoxicity (AHG-CDC) Positive Crossmatch (+XM) Kidney Transplantation. James M. Gloor,¹ Fernando G. Cosio,¹ Steven R. DeGoeij,² Jeffrey L. Winters,² S. Breannan Moore,² Donna J. Lager,² Mark D. Stegall.¹ ¹Transplant Center, Mayo Clinic, Rochester, MN; ²Department of Pathology and Laboratory Medicine, Mayo Clinic, Rochester, MN.

Introduction: Protocols have been developed which allow kidney transplantation despite the presence of donor specific anti-HLA antibodies. In this investigation we report long term outcome in a series of +XM kidney transplants.

Methods: Patients with +XM (AHG-CDC) confirmed with HLA antigen coated flow bead analysis underwent pretransplant desensitization using plasmapheresis/low dose intravenous immunoglobulin (PP/IVIg). Immunosuppression consisted of rATG induction, tacrolimus, mycophenolate mofetil and prednisone. 47 patients received anti-CD20 antibody induction, and 19 underwent splenectomy. Antibody mediated rejection was treated with plasmapheresis/IVIg and corticosteroids.

Results: Between 4/2000 and 8/2005 53 patients underwent transplantation. Baseline XM titers ranged from 1 to 512 (median 1:4). Patients underwent 5.9±3.2 pretransplant PP/IVIg treatments (range 2-15), and 4.3±5.6 (range 0-30) posttransplant sessions. A negative crossmatch at transplantation was achieved in 44/53 (83%) patients. In patients with a negative XM at transplantation antibody mediated rejection (AMR) occurred in 18/44 (41%), and was successfully reversed in 13/18 (72%). Transplant glomerulopathy (TG) occurred in 19/44 (43%) patients. Allograft loss was associated with both AMR (10/18, 56%; 14±21 months posttransplant, range 3-54) and TG (8/19, 42%; 26±21 months, range 3-54). Patient survival was 95, 95, 85 and 63% while allograft survival was 81, 77, 59, and 49%, at one, three, five and seven years, respectively. Nine patients were transplanted despite persistent +XM after unsuccessful preconditioning. Six of nine developed AMR, including 2 hyperacute rejections. Five of nine have lost function by 36 months posttransplant.

Conclusion: PP/IVIg preconditioning permits +XM transplantation with good early allograft survival in patients who achieve a negative crossmatch. Transplantation of patients with persistent +XM is associated with hyperacute and early AMR, with poor allograft survival. Although +XM kidney transplantation represents an important option for highly sensitized patients unlikely to be transplanted otherwise, the long-term outcome appears less favorable than conventional transplants. +XM transplantation should be pursued only when other options have been investigated.

Abstract# 12

Increased Alloantibody Production Associated with Acute Humoral Rejection (AHR): Evidence of Immunologic Memory. M. D. Stegall,¹ J. M. Burns,¹ J. M. Gloor,¹ S. B. Moore,¹ S. R. DeGoeij,¹ H. S. Pollinger,¹ D. K. Perry,¹ P. I. Terasaki,² N. El-Awar.² ¹Mayo Clinic, Rochester, MN; ²One Lambda, Canoga Park, CA.

Introduction. Pre-existing donor-specific antibody (DSA) has been shown to cause AHR. This study examined the role of new post-transplant antibody production in AHR in positive crossmatch kidney transplants (+XMatch KTx).

Methods. We retrospectively reviewed 25 +XMatch KTx between 1/05 to 7/07 who had "low levels" of DSA at baseline defined as a negative T cell AHG Xmatch and positive T and/or B cell flow Xmatch (FXM) with channel shifts <300 at baseline. These patients did not receive pre-transplant plasmapheresis. AHR was diagnosed as a rise in serum creatinine confirmed by biopsy. Serum antibody levels were assessed as channel shift of T/B FXM and/or normalized values of single antigen flow beads (SAFBs). In addition, we assessed the specificities and levels of anti-HLA antibody in lymphoid tissue in 9 high level DSA patients during AHR.

Results. AHR occurred in 36% (9/25) of low level DSA recipients. AHR only developed when DSA levels increased from baseline and no patient developed AHR when their DSA levels remained low (B FXM <350 (0/17)). AHR occurred in 7/8 cases when B cell FXM increased to >350 and in 6/7 cases when the SAFB normalized value increased to >5900. AHR occurred despite pre-transplant treatment with anti-CD20, high dose IVIG, and rATG induction. During AHR: 1) DSA always increased against donor specificities for which there was antibody present at baseline; 2) New anti-donor specificities were detectable in 50% of patients; 3) Unexpectedly, pre-existing third-party alloantibody levels also increased in all patients; and 4) Computer models previously used to identify "public epitopes" suggested some, but not all, of these apparent increases in third party alloantibody could be explained by public epitopes shared with donor HLA. Finally, antibody secreting cells assayed in the spleen, lymph nodes and marrow during AHR (from both high and low level DSA patients) showed increased alloantibody production in all three compartments.

Conclusions. AHR in recipients with low level DSA at baseline is associated with an increase in DSA levels from baseline suggesting that immunologic memory is an important component of AHR in this setting. Increased serum alloantibody production against both donor and third party HLA occurs in all lymphoid compartments and the apparent increase against third-party HLA is only partially explained by cross-reactivity of alloantibody generated against the donor.

Abstract# 13

Breaking ABO Barrier in Kidney Transplantation: Excellent Outcome of ABO-Incompatible Kidney Transplantation in Japan. Kota Takahashi,¹ Kazuhide Saito,¹ Kazunari Tanabe,¹ Shiro Takahara,¹ Kazuharu Uchida,¹ Atsushi Aikawa,¹ Tsuneo Kinukawa,¹ Takaaki Kobayashi,¹ Norio Yoshimura,¹ Yoriaki Kamiryo,¹ Kazuhiko Yamada.¹ ¹Japanese ABO-Incompatible Kidney Transplantation Committee, Niigata, Japan.

(INTRODUCTION) We have been making continuing efforts in ABO-incompatible kidney transplantation since 1989 to expand the opportunities for kidney transplantation from living donors.

From the Japanese registry, we have reviewed the patient and graft survival of ABO-incompatible kidney transplantation.

(PATIENTS AND METHODS) This survey focused on 1,012 patients who received ABO-incompatible kidney grafts from January 1989 to December 2006 in whom monitoring follow-up could be achieved in 92 institutions all over Japan. The mean age of patients at surgery was 38 years (4 to 71) comprised of 638(63%) males and 374(37%) females. The mean observation period was 3 years and 3 months. Donor/ recipient relationships were 611 parents/children (60%), 213 spouses(21%), 109 siblings(11%) and others(8%). Blood type O recipients were 53%, type A were 25% and type B were 22%. A- and B- incompatible cases were 534 and 467, respectively.

(RESULTS) The overall patient survival rate at 1, 3, 5 and 10 years after transplantation were 95, 93, 91 and 87%, with overall graft survival rate of 90, 86, 80 and 63%, respectively.

The graft survival rate has been significantly improved especially after 2001, at 1, 3 and 5 years with 96, 94 and 90% (n=567), compared with those in the period of 1995-2000 at 84, 79 and 76 % (n=277) and in the period of 1989-1994 at 81, 78 and 68%(n=168), respectively. The graft survival rate was significantly higher in patients aged 29 and younger compared with those aged 30 and older (p<0.01). There were no significant differences in A- and B-incompatible recipients, number of HLA mismatches, induction and maintenance calcineurin inhibitors and donor/recipient relationships with respect to clinical outcomes.

Recent trend in Japan is the induction of rituximab desensitization and avoidance of splenectomy. Among 168 cases in 2006, rituximab was used in 69 cases and splenectomy was avoided in 63 cases. Overall splenectomy avoidance were up to 148 (15%) among 1,012 cases.

(CONCLUSION) This study confirms that the short-term and long-term outcome of ABO-incompatible living kidney transplantation is excellent, and are similar to that in ABO- compatible cases. ABO-incompatible kidney transplantation has become one of a standard, safety and effective treatment choice for end-stage renal disease.

Abstract# 14

Effect of Induction Agent on Cellular and Humoral Responses to Renal Transplants in Sensitized Patients. Mary S. Leffell,¹ Dessilava Kopchliiska,¹ Donna P. Lucas,¹ Annette M. Jackson,¹ Robert A. Montgomery,¹ Jayme E. Locke,¹ Daniel S. Warren,¹ Andrea A. Zachary.¹ ¹Medicine and Surgery, Johns Hopkins University School of Medicine, Baltimore, MD.

More than 90% of patients with antibody to donor HLA (DSA), who undergo treatment with plasmapheresis (PP) and hyperimmune anti-CMV immunoglobulin, either eliminate DSA or reduce it to very low levels and are transplanted successfully. Despite this high degree of success, these patients are at increased risk for both cell-mediated and antibody-mediated rejection which, in turn, may be greatly affected by the choice of induction agent. In a randomized, clinical trial, patients undergoing desensitization were treated with either Zenapax (Z) or Thymoglobulin (T), for induction. We evaluated 3 parameters in the two groups of patients: cellular immune function (measured by release of cATP, Cylex ImmunKnow); T cell subpopulations (%CD4+ of CD3+ cells, % CD25+ of CD4+ cells, and % CD45RA-/62L- of CD3+CD4+ cells) measured by three and four color fluorescence in a FACSCalibur cytometer using Cell Quest software; and elimination of DSA as determined by multianalyte bead assays performed on the Luminex platform. Time points examined were pre-PP, immediately pre-transplant (Tx), and 6 months post-Tx. Statistical significance was evaluated with chi squared and two-tail t tests. There were no significant differences between the Z and T groups for any parameter at any pre-Tx time point. Differences post-Tx, either significant or approaching significance, are shown below. Lymphocyte activation tended to be more suppressed in T treated patients and correlated with significantly lower numbers of CD4+ T cells post-TX. The proportion of T cells with the memory phenotype, CD45-/62L-, was significantly lower in Z treated patients which was associated with a higher % of DSA elimination among these patients. These results suggest that induction therapy and the associated impact on lymphocyte populations and function may substantially impact desensitization outcomes and warrants further study.

Effect of Induction Therapy at 6 mos Post-Tx						
	T Lymphocyte Activation ATP ng/ml	# Patients	T Lymphocyte Subset Analysis		# Patients	DSA Elimination
			% CD4+	% CD45-/62L-		% Eliminating
Thymo- globulin	190	6	53.4	24.8	9	53.8
Zenapax	286	6	68.0	13.6	6	86.7
	p=0.09		p=0.05	p=0.05		p=0.06

Infections: A Broad Perspective

Abstract# 15

Management of Post Transplant CMV Disease: Lessons Learned from the VICTOR Trial. Mark D. Pescovitz,¹ Anders Hartmann,² Atul Humar,³ Halvor Rollag,² Alan G. Jardine,⁴ Angelo A. Bignamini,⁵ Anders Åsberg.⁶
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We previously showed that oral valganciclovir (VGCV, 900 mg BID) was non-inferior to intravenous ganciclovir (GCV, 5 mg/kg BID), in the treatment of CMV disease. To provide guidance for CMV treatment efficacy, we analyzed for predictive parameters from pooled 1-year follow-up data on the 321 patients that received randomized treatment (VGCV: n=164 or GCV: n=157) for 21 days, followed by VGCV maintenance till day 49 in all. Blood samples were collected at days 0, 21 and 49 for central analyses of anti-CMV IgG titers (Abbott Architect), plasma viral loads (Amplicor, cut-off 600 copies/mL) and UL97/UL54 resistance (PCR).

The study population consisted of kidney (74.1%), heart (5.6%), liver (7.2%), lung (5.9%) and other/combined (7.2%) recipients. 12.5% of patients were CMV IgG negative at day 0. At 21 and 49 days, 31.8% and 4.6% of patients remained viremic, respectively. Recurrence during follow-up was 13.9%.

Patients with persistent CMV viremia at day 21 were significantly more likely to recur (abs. yearly risk: 23.2% [95% CI: 15.7-34.1%] vs. 9.6% [6.3-14.7%]). Lung transplant patients were more likely to recur (37.5%) than kidney (14.4%), heart (6.3%) and liver (0%) recipients. Patients viremic at day 21 had a rate of resistance mutation of 7/94 (absolute risk: 7.4% [3.7-14.9%]) while those still positive at day 49 had a rate of 4/13 (30.8% [14.3-65.0%]). Patients CMV IgG negative at day 0 had a slower initial viral clearance (lag-phase of median 7 days) and higher rates of recurrence: 8/29 (27.6% [15.6-48.6%]) vs. CMV IgG positive patients: 26/200 (13.0% [9.1-18.5%; P=0.039, chi square]).

This supports the use of VGCV for treatment of CMV disease and indicates that the CMV IgG status at start determines the response. Induction therapy should be given for at least 21 days and viral load measured prior to reduction to maintenance dose. Patients with persistent viremia at 21 days should continue on treatment doses, but are not likely to have resistant mutations, whereas patients with viremia at 49 days should be evaluated for resistance. These data support a patient-specific treatment regimen based on CMV IgG status, viral load at days 21 and 49, and identification of CMV resistance at day 49.

Abstract# 16

A Prospective Molecular Surveillance Study Evaluating the Clinical Impact of Community Acquired Respiratory Viruses in Lung Transplant Recipients. Deepali Kumar,¹ Shahid Husain,² George Moussa,³ Karen Doucette,¹ Oriol Manuel,¹ Justin Weinkauff,¹ Joseph Pilewski,² David Himsworth,⁴ Richard Janeczko,⁴ Kenneth R. McCurry,² Sean M. Studer,² Atul Humar.¹ ¹Transplantation and Infectious Diseases, University of Alberta, Edmonton, AB, Canada; ²University of Pittsburgh, PA; ³Mount Sinai Hospital, Toronto, Canada; ⁴Luminex Corporation.

Background: In lung transplant patients, community acquired respiratory viral infections of the allograft may upregulate allo-reactive immune response and lead to the development of acute rejection and bronchiolitis obliterans syndrome (BOS). The use of sensitive molecular methods that are simultaneously able to detect a large panel of respiratory viruses may help better define their effects.

Methods: Lung transplant recipients undergoing serial surveillance and diagnostic bronchoalveolar lavages (BAL) over a period of several years were enrolled. BAL samples underwent molecular multiplex testing for a panel of 21 respiratory viruses using the Luminex assay. Confirmation of positive results was done by sequencing. Demographics, symptoms, and FEV-1 results were all prospectively collected.

Results: We enrolled 93 lung transplant recipients whose mean age was 51.6±12.1 years and mean follow-up was 870±312 days. 49/93 (52.7%) had a respiratory virus isolated on at least one BAL sample. The majority of samples had rhinovirus (n=41) followed by parainfluenza 1-4 (n=14), coronavirus (n=10), influenza (n=4), metapneumovirus (n=4) and RSV (n=2). Dual viral infections were seen in 3/49 (6.1%) patients. 17/49 (34.7%) patients had more than one positive BAL sample over time. The majority of infections (72.2%) were asymptomatic. Biopsy-proven acute rejection (≥ grade 2) or a decline in FEV-1 ≥20% occurred in 17/49 (34.7%) patients within three months of the respiratory virus infection. No significant difference was seen in the incidence of acute rejection between symptomatic or asymptotically infected patients (p=0.11). Either biopsy proven obliterative bronchiolitis (OB) or a sustained decline in FEV-1 (consistent with BOS) was diagnosed in 9/49 (18.4%) patients within one year of infection. Prolonged shedding was not observed except with rhinovirus infection.

Conclusion: Symptomatic and asymptomatic respiratory viral infections may be a trigger for acute rejection and OB/BOS. Routine molecular surveillance using multiplex assays may be useful for clinical monitoring of lung transplant patients.

Abstract# 17

Bacterial Infections in HIV Positive Transplant Recipients. Emily Blumberg,¹ Burc Barin,² Kim Olthoff,¹ Peter Stock,³ Douglas Hanto,⁴ Barbara Murphy,⁵ David Simon,⁶ Jimmy Light,⁷ Margaret Ragni,⁸ Michelle Roland,⁹ Michelle Roland.⁹ ¹University of Pennsylvania; ²Emmes Corporation; ³Univ of California, San Francisco; ⁴Beth Israel Deaconess Med Center; ⁵Mt. Sinai Med Center; ⁶Rush Presbyterian Med Center; ⁷Washington Hosp Center; ⁸Univ Pittsburgh; ⁹California Dept of Public Health.

The incidence of and factors associated with bacterial infections (BI) in HIV positive transplant patients (pts) are unknown. We report the incidence of BI in a cohort of HIV+ pts and describe infection characteristics, risk factors and outcomes in these pts. **Patients/Methods:** All pts were enrolled in an NIH sponsored multicenter trial of liver and kidney transplantation in HIV infected individuals. Data collected included pt demographics, type of BI (organism and site), time of onset, comorbid conditions, immunosuppressive therapies, CD4 count, HIV viral load, CMV status, and outcome. Univariate and multivariate Cox proportional hazard models were performed to assess the association of these factors with the development of BI. **Results:** 29/84 (35%) of kidney (K) and 36/83 (43%) of liver (L) pts had at least one BI; 15K and 23L had >1 BI. BI in K and L recipients occurred at a median of 158 days (range 1-1175) in K pts and 80 days (range 0-709) in L pts. Early BI (≤30 days) accounted for 30% of BI episodes (24% in K pts, 34% in L pts). Enteric Gram negatives were most common, accounting for 62 (36%) of the isolates; 35 (20%) of isolates were Staphylococci (18 coagulase negative Staphylococci and 17 S. aureus) and 13% C.difficile. Bloodstream infections were most common (21%) followed by genitourinary sites (18%), intestinal (15%), wound/skin (10%), and respiratory (9%). Both K and L pts were hospitalized for or during 87% of BI. 2/29 (7%) of K and 7/36 (19%) of L pts with infection died. Although diabetes, HCV, rejection, and lower baseline CD4 counts occurred more frequently in L pts with BI, only thymoglobulin use (HR 22.1; CI 2.1, 234.4) and HCV (HR 3.8; CI 1.3, 10.8) were significantly associated with first BI in multivariate analysis. There were no significant associations with K pts. Sirolimus use was not associated with BI. **Conclusions:** BI are common in HIV+ transplant pts, occurring throughout the post transplant period and associated with death, especially in L pts. Risk factors for BI include thymoglobulin and HCV in L pts. Close observation for BI should be performed in all HIV+ transplant pts.

Abstract# 18

CNS Cryptococcal Lesions in Solid Organ Transplant Recipients with Cryptococcosis. Nina Singh, O. Lortholary, B. D. Alexander, S. Husain, Crypto Collaborative Transplant Study Gp. Univ Pitt; Institut Pasteur; Duke Univ.

Background: Precise frequency, clinical characteristics and outcomes associated with CNS cryptococcal lesions in SOT recipients has not been defined.

Methods: Of 116 consecutive SOT recipients with cryptococcosis in a prospective, multicenter study between 1999-06, 61 had CNS cryptococcosis (+ CSF culture or CSF cryptococcal antigen). Patients with cryptococcal disease who had CNS lesions (defined as abnormal neuroimaging studies or lesions at autopsy) comprised the study population for this study. Mortality was assessed at 90 days.

Results: CNS lesions were documented in 16 (29%) of 55 patients with CNS cryptococcal disease who underwent neuroimaging studies. These included leptomeningeal lesions in 50% (8/16), parenchymal lesions or cryptococcomas in 37.5% (6/16) and hydrocephalus in 12.5% (2/16). Five of 6 parenchymal and all meningeal lesions were present at diagnosis. One parenchymal lesion and both cases of hydrocephalus however, were new onset and developed a median of 18 days (range 14-21 days) after antifungal therapy; all 3 cases fulfilled the definition of immune reconstitution syndrome (IRS). 50% of the patients with meningeal, 17% with parenchymal lesions, and none of those with hydrocephalus had fungemia. CSF antigen titers were significantly higher with meningeal vs. parenchymal lesions, and hydrocephalus (p=0.015). CSF antigen titers (p=0.04) and CSF protein (p=0.04) were significantly higher for patients with meningeal lesions on imaging studies than patients with meningitis but without meningeal lesions. Mortality at was 50% (3/6) for patients with parenchymal, 12.5% (1/8) for meningeal, and 0% (0/2) for patients with hydrocephalus. Mortality rate was 31% (4/13) in patients with cryptococcal CNS lesions at baseline and 0% (0/3) in those with new onset lesions consistent with IRS.

Conclusions: CNS lesions were documented in 29% of the SOT recipients with CNS cryptococcal disease. Despite a greater fungal burden in patients with meningeal lesions as indicated by the presence of fungemia and higher CSF antigen titers, outcomes were insignificantly worse for patients with parenchymal as compared to meningeal lesions or hydrocephalus. New onset CNS lesions may represent IRS in these patients and appeared to be associated with better outcome.

Abstract# 19

Histoplasmosis in Solid Organ Transplant Recipients 1997-2007. Jennifer M. Cuellar,¹ Michelle Lard,¹ Steven Mawhorter,¹ Robin Avery,¹ Nabin Shrestha,¹ Marie Budev.¹ ¹Cleveland Clinic, Cleveland, OH.

Post-transplant histoplasmosis has been reported mainly in case series of renal and liver recipients. This study sought to define the clinical feature, diagnosis, therapy, and outcome at one center in an endemic area, including those with donor or recipient

histoplasmosis at time of lung transplantation. **Methods:** Cases of histoplasmosis from 1997-9/07 in SOT recipients were identified by billing codes. Clinical data was obtained retrospectively. Descriptive statistics were used. **Results:** During the study period 3226 transplants were performed and 32 (1%) cases of histoplasmosis were identified. Six were excluded as diagnosis was solely clinical. Of the remaining 26, 13 had active histoplasmosis; 9 had histologic evidence of histoplasmosis in the explanted organ, and in 4 histoplasmosis was identified in donor tissue at the time of transplant. Of 13 (0.4%) patients with active histoplasmosis, 12 were Caucasian and resided in Ohio. Distribution by transplanted organ was: 4 heart, 3 lung, 3 kidney, 1 liver, 1 pancreas and 1 kidney-pancreas. Median time from transplant to diagnosis was 540 (55-2494) days, and from onset of symptoms to diagnosis was 18 (4-96) days. Most common symptoms were fever 12/13, dyspnea 7/13, diarrhea 6/13 and cough 6/13. All had lung involvement on radiology, culture or histopathology. *Histoplasma* urine antigen (UAg) and *Histoplasma* antibodies (Ab) were positive in 66.7% and 36.4%, respectively. Six were UAg+, Ab-, while 2 were Ab+, UAg-. All patients were treated with amphotericin B and/or itraconazole. Three received voriconazole because of itraconazole intolerance or inefficacy. One patient had recurrence after 90 days of treatment, but was eventually cured. All other patients were cured or are still on treatment, but symptom-free. Median duration of completed therapy was 203 (135-1661) days. Of those with histoplasmosis from explanted organ or donor tissue (12 lung and 1 liver), none developed active histoplasmosis, but all received itraconazole prophylaxis for 6-18 mos. **Conclusions:** Active histoplasmosis is a rare event post-transplant (0.4%) even in endemic areas. The prognosis of SOT recipients with histoplasmosis is good with active case finding and early but protracted therapy; delays in diagnosis are common. Empiric therapy often must be started before lab confirmation. In this series, patients with latent infection in the explanted or donor organ who received prophylactic itraconazole did not develop active histoplasmosis.

Abstract# 20

A Prospective Study of Viral Genetic Polymorphisms in CMV Glycoprotein B and Their Association with Clinical and Virologic Outcomes in Patients with CMV Disease. Oriol Manuel,¹ Xiaoli Pang,¹ Jutta K. Preiksaitis,¹ Vincent C. Emery,² Deepali Kumar,¹ Anders Asberg,³ Anders Hartmann,³ Mark D. Pescovitz,⁴ Angelo A. Bignamini,⁵ Halvor Rollag,² Alan G. Jardine,⁶ Atul Humar.¹ ¹University of Alberta, Canada; ²University College London, United Kingdom; ³University of Oslo, Norway; ⁴Indiana University; ⁵University of Milan, Italy; ⁶University of Glasgow, United Kingdom.

Background: It is unknown if specific viral polymorphisms affect *in vivo* virulence in patients with CMV disease. Polymorphisms in the CMV UL55 gene encoding the glycoprotein B (gB), allow discrimination of four distinct genotypes (gB1-4). We assessed the influence of gB genotypes on CMV disease in a large prospective cohort of patients.

Methods: Solid organ transplant recipients enrolled in a trial of CMV disease treatment were included in this study. Quantitative and sensitive CMV gB genotyping was performed using a real-time PCR at Day 0 (start of antiviral therapy) and at days 3, 7, 14, and 21 after starting antiviral therapy. Mixed infection was defined as CMV infection with more than one gB genotype in a single sample.

Results: 229 patients with CMV disease were analyzed. The prevalence of gB strain types at day 0 was gB1 (28%), gB2 (14%), gB3 (14%), gB4 (6%) and mixed-infection in 38%. Seropositive patients were more likely to have mixed gB infection compared to seronegative patients (41% vs. 12%, $p=0.003$). Mean baseline viral loads ($\log_{10}$) were similar for different gB types but were higher for mixed vs. non-mixed infections (4.36 vs. 4.16; $P=0.056$). Viral clearance kinetics including viral half-life and slope of viral decay were similar for the four gB infections types. Time to viral eradication was longer in mixed vs. non-mixed infections (median of 22.0 ± 2.0 vs. 19.0 ± 1.0 days, respectively, $p=0.06$). In a multivariate model accounting for baseline viral load, CMV IgG at baseline, ganciclovir resistance, and antiviral treatment, mixed gB infection was a significant predictor of failure of therapy at Day 21 (mixed vs. non-mixed OR 2.87; 95% CI: 1.38 to 5.95; $P=0.005$). No effect of gB genotype was seen on virological or clinical CMV recurrence.

Conclusion: No specific strain of CMV based on gB genotyping appears to confer a specific viral virulence advantage. However, mixed infection with different gB genotypes is associated with higher viral loads and delayed virological clearance suggesting a role for viral polymorphisms in influencing *in vivo* viral fitness.

Abstract# 21

Incidence and Mortality of Invasive Candidiasis in Orthotopic Liver Transplant Recipients in 2006: The Emergence of Non-*Albicans* Species. A. Restrepo,¹ S. Safadjou,² A. Bozorgzadeh,² R. Kashyap,² D. J. Hardy,³ R. F. Betts,¹ C. E. Koval.¹ ¹Dept. of Medicine/Division of Infectious Diseases; ²Dept. of Surgery/Division of Transplantation; ³Dept. of Microbiology, University of Rochester Medical Center, Rochester, NY.

Background/Purpose: In 1999, Winston et al reported the incidence of Invasive Candidiasis (IC) following Cadaveric Orthotopic Liver Transplant (cOLT) with fluconazole prophylaxis to be 6%, with *C. albicans* the predominant species. We have since given fluconazole prophylaxis in high risk individuals for 3 months post-OLT. Our purpose is to update the incidence and mortality of IC following cOLT in 2006 and

to characterize the *Candida* species involved.

Methods: Charts of patients with IC after cOLT from 1/06 to 12/06 were reviewed. IC was defined by *Candida* in cultures from sterile sites. *Candida* susceptibilities were determined by E-test (AB Biodisk, Sweden). We compared outcomes of cOLT patients with and without IC.

Results: 209 patients received cOLT. 24/209 (11.5%) developed IC a median of 29d (range 0-409d) post-cOLT. 13/24 (54%) were cultured from blood, 10/24 from peritoneal fluid and 1/24 from a biliary cast. Non-*albicans Candida* accounted for 18/27 (67%) of the isolates, with 9/27 (33%) *C. parapsilosis*, 5/27 (18.5%) *C. glabrata*, 3/27 (11%) *C. dublinensis* and 1/27 (3.7%) *C. krusei*. Of 24 isolates with susceptibility data, 0/7 *C. albicans* were fluconazole-resistant(R), 6/8 *C. parapsilosis* were fluconazole-R (MIC ≥ 64), 0/8 were voriconazole-R, and 0/8 were caspofungin-R. 2/5 *C. glabrata* were fluconazole-R (MIC ≥ 64), 1/5 voriconazole-R and 1/5 likely caspofungin-R (no interpretive criteria). For IC and non-IC cOLT, overall mortality was 15/24 (62.5%) and 44/185 (23.7%) ($p \leq 0.001$), and mean survival after cOLT was 210d (range 11-587d) and 396d (range 0-700d), respectively. For *C. albicans*, non-*albicans* and *parapsilosis* IC, the overall mortality was 28.5%, 80%, and 100%, respectively ($p=0.149$), despite routine use of caspofungin as empiric therapy for candidemia.

Conclusion: In 2006, our incidence of IC after cOLT was 11.4%. This is high compared to previously reported incidence of 6% in the late 1990s. Our findings are coincident with the emergence of non-*albicans Candida* with fluconazole-resistance as predominant pathogens in IC. For non-*albicans* IC, mortality was 80-100% despite echinocandin therapy. These findings warrant investigations to detail the risk for non-*albicans* IC and to assess the impact of antifungal therapy and prophylaxis strategies on outcomes.

Abstract# 22

Extended-Spectrum β -Lactamase Bacterial Infections in Solid Organ Transplant Recipients. Holli A. Winters,¹ Stanley I. Martin,¹ Ellen A. Keating,² Negin Soufi-Siavash,¹ Debra A. Goff.¹ ¹Pharmacy, The Ohio State University Medical Center, Columbus, OH; ²Infectious Diseases, The Ohio State University Medical Center, Columbus, OH.

Purpose: The incidence of infections due to extended-spectrum β -lactamase (ESBL)-producing bacteria has increased significantly. Patients with ESBL infections have increased mortality, hospital stay, and cost. This is the first study to evaluate the risk factors and antibiotic prescribing patterns of solid organ transplant recipients with ESBL infections.

Methods: A retrospective review was performed of abdominal transplant patients who had an ESBL infection from January 2003-August 2006. Data collected included transplant date and type, ESBL organism, source of infection, risk factors, and empiric and treatment antibiotics.

Results: Twenty transplant recipients (17 kidney, 1 kidney/pancreas, 2 liver) with 44 total hospital admissions were evaluated. Sixty five ESBL isolates (54 *Klebsiella pneumoniae*, 11 *Escherichia coli*) were analyzed. Urine (51%) and blood (26%) were the most common sources. Sixty percent of patients were started on ineffective empiric antibiotics (27% ciprofloxacin, 42% piperacillin/tazobactam). Time to switch to an effective treatment antibiotic was 3.9 ± 1.2 days. Average length of inpatient and post-discharge outpatient therapy was 8.6 and 17.1 days, respectively. Sixty five percent of patients had been hospitalized within 3 months prior to the ESBL infection, 25% had an intensive care unit stay within 6 months prior to the ESBL infection, and 30% had resided in a nursing home within the prior year. In addition to PCP prophylaxis, 70% of the patients had been treated with antibiotics within 3 months prior to the ESBL infection. Sixty percent of patients were readmitted with a second ESBL infection. Mean time to readmission was 55 days (range 5-455 days). Upon readmission, empiric antibiotics were ineffective in 18% of patients. Patients were 2 times more likely to be readmitted to the hospital if they received ineffective empiric therapy for the first infection compared to those who received effective empiric therapy. Risk factors included a recent hospitalization, ICU and/or nursing home stay, and recent antibiotic therapy.

Conclusion: Solid organ transplant patients with ESBL infections are at risk for multiple hospitalizations and recurrence if not treated empirically with appropriate antibiotics. If patients are readmitted within 90 days of the first ESBL infection a carbapenem should be considered empirically.

Abstract# 23

Low Rate of CMV Infection across Everolimus-Based Regimens in *De Novo* Heart Transplant Patients. Cristoph Bara,¹ Hannah A. Valentine-von Kaeppler,² Thomas Dengler,² Angelo Branzi,² Shoen-Sheng Wang,² Heather Ross,² Juan Delgado,² Randall C. Starling,² Howard J. Eisen.² ¹Hannover Medical School, Hannover, Germany; ²On Behalf of the RAD B253, A2403 and A2411 Study Groups.

Introduction. CMV infection contributes to cardiac allograft vasculopathy via endothelialitis and vascular smooth muscle proliferation. In the B253 study, the proliferation signal inhibitor everolimus (evl) was shown to be associated with a low CMV infection rate vs azathioprine (AZA) in heart tx recipients. Evl administration has since evolved from fixed-dose (FD) regimens with standard-exposure calcineurin inhibitor (CNI) to concentration-controlled (CC) dosing with reduced-exposure CNI.

Methods. The incidence of CMV infections and CMV-related adverse events was captured in 3 multicenter, randomized, open-label studies (B253 [N=634], A2403 [N=199], A2411 [N=176]) in which *de novo* heart tx patients received FD-evl or CC-

evl (target 3-8ng/mL), AZA or MMF with standard (SD) or reduced (RD) CsA. Data at 6 months post-tx is presented here. **Results.** The proportion of patients with CMV events, including serious adverse events and laboratory evidence of CMV infection, has remained low and broadly similar following introduction of CC administration of evl and use of concomitant RD-CsA.

	Evl fixed-dose (1.5mg) + SD CsA (B253)	AZA + standard CsA (B253)	Evl concentration-controlled + standard CsA (A2403)	Evl concentration-controlled + reduced CsA (A2403)	Evl concentration-controlled + reduced CsA (A2411)	MMF 3g + standard CsA (A2411)
CMV infection	7.2%	21.5%	7.1%	3.0%	3.3%	10.8%
CMV serious adverse event	1.4%	8.4%	1.0%	1.0%	2.2%	4.8%
Laboratory evidence of CMV	6.2%	16.4%	12.1%	12.0%	8.8%	32.5%
CMV syndrome	2.4%	8.9%	0.0%	1.0%	1.1%	8.4%
CMV organ involvement	1.4%	4.7%	1.0%	2.0%	1.1%	1.2%

CMV syndrome and CMV organ involvement occurred in <3% of recipients receiving evl in each of the studies. The highest rates of all CMV parameters occurred with MMF + SD-CsA or AZA + SD-CsA. **Conclusion.** The low incidence of CMV infection and CMV-related events observed in heart tx patients receiving FD-evl and SD-CsA as *de novo* immunosuppression has been maintained in newer regimens of CC-evl with RD-CsA. CMV syndrome and CMV organ involvement are rare with evl-based immunosuppression after cardiac tx. The low rate of CMV infections in evl-treated patients may contribute to improved long-term outcome and a lower incidence of CAV; confirmatory data are awaited.

Ischemia and Reperfusion Injury: New Mechanistic Insights

Abstract# 24

Fibronectin- α_5 Interactions Enhance p38 MAPK Phosphorylation and Metalloproteinase-9 Expression in Cold Liver Ischemia/Reperfusion Injury. Sergio Duarte,¹ Xiu-Da Shen,¹ Takashi Hamada,¹ Constantino Fondevila,¹ Ronald Busuttil,¹ Ana J. Coito.¹ ¹The Dumont UCLA Transplant Center.

Expression of endothelial fibronectin (FN) is an early event in liver ischemia/reperfusion (I/R) injury. We have recently shown that CS1 peptide facilitated blockade of FN- $\alpha_5\beta_1$ leukocyte interactions regulates metalloproteinase-9 (MMP-9) expression in steatotic orthotopic liver transplants (OLT). This study tests the function of the CS1 peptide therapy upon MMP-9, and further dissects putative mechanisms, in an alternate model of cold Ischemia/Reperfusion (I/R) Injury.

Methods and Results: CS1 peptides were administered through the portal vein of Sprague-Dawley (SD) rat livers before and after 24 h cold storage (500 μ g/rat). SD recipients of OLTs received an additional dose of CS1 peptides 1h post-OLT. CS1 therapy significantly increased the 14d OLT survival rate (100% vs. 50%, n=8/gr, p<0.005). CS1 peptides reduced sGOT levels (U/L) at 6h (1413 $\pm$ 420 vs. 2866 $\pm$ 864 p<0.008) and 24h (1350 $\pm$ 142 vs. 4000 $\pm$ 1358, p<0.006) post-OLT. CS1 treated OLTs showed good preservation of lobular architecture, contrasting with severe necrosis and sinusoidal congestion in controls. Moreover, CS1 treated livers were characterized by a profound decrease in T (31 $\pm$ 3 vs. 64 $\pm$ 8, p<0.0002), NK (19 $\pm$ 2 vs. 41 $\pm$ 3, p<0.0003) and ED1 (21 $\pm$ 7 vs. 57 $\pm$ 16, p<0.008) cells as early as 6h after I/R. Neutrophils, as indicated by MPO activity (0.48 $\pm$ 0.02 vs. 3.18 $\pm$ 0.94, p<0.02) were depressed by CS1 therapy. This correlated with decreased mRNA expression of TNF- α (0.032 $\pm$ 0.04 vs. 0.83 $\pm$ 0.26, p<0.005) and cyclooxygenase-2 (0.8 $\pm$ 0.1 vs. 2.2 $\pm$ 0.6, p<0.05). Leukocyte transmigration is dependent upon adhesive and focal matrix degradation mechanisms. MMP-9, which is inducible and expressed by infiltrating leukocytes, was profoundly depressed in 6h CS-1 peptide treated OLTs, at both mRNA (0.1 $\pm$ 0.1 vs. 0.5 $\pm$ 0.2, p<0.03) and protein (0.15 $\pm$ 0.07 vs. 0.7 $\pm$ 0.14, p<0.03) levels. Moreover, MMP-9 activity evaluated by zymography was reduced by ~3-fold in the CS-1 group. Interestingly, phosphorylation of mitogen-activated protein (MAP) kinase p38 (0.05 $\pm$ 0.01 vs. 0.14 $\pm$ 0.06, p<0.03) was selectively downregulated in the 6h CS-1 treated OLTs. **In conclusion,** this work supports a broad regulatory role for FN- $\alpha_5\beta_1$ interactions on MMP-9 expression by leukocytes, likely mediated through activation of p38 MAPKinase, and matrix pathological breakdown associated with leukocyte infiltration. This data provides the rationale for the development of novel therapeutic approaches in cold liver I/R injury.

Abstract# 25

Cyclooxygenase-2 Deficiency Disrupts the Traffic of Metalloproteinase-9+ Neutrophils in Hepatic Ischemia/Reperfusion Injury. Takashi Hamada,¹ Seiichiro Tsuchihashi,¹ Sergio Duarte,¹ Ronald W. Busuttil,¹ Ana J. Coito.¹ ¹The Dumont UCLA Transplant Center, Los Angeles, CA.

Neutrophils are critical mediators in warm liver ischemia/reperfusion (I/R) injury. This study tests the hypothesis that cyclooxygenase-2 (COX-2), a potent mediator of inflammation, regulates neutrophil recruitment in liver I/R injury. **Methods and Results:** COX-2 $-/-$ mice (COX-2 KO) and matched COX-2 $+/+$ controls (WT) were submitted to 90 minutes warm ischemia followed by 6h and 24h of reperfusion. Liver function, as evidenced by the sGOT levels (IU/L) (6h: 1,505 $\pm$ 1,125 vs. 15,502 $\pm$ 11,344; p<0.03; 24h: 348 $\pm$ 3 vs. 974 $\pm$ 193; p<0.01, n=5-6/gr), was significantly improved in COX-2 KO mice as compared to WT controls after I/R. COX-2 KO livers showed overall improved histological preservation after I/R insult, contrasting with extensive sinusoidal congestion and necrosis observed in the controls. COX-2 deficiency reduced

neutrophil recruitment in livers after I/R as indicated by the decreased levels of MPO activity (U/g) (6h: 2.0 $\pm$ 1.0 vs. 13.7 $\pm$ 4.3; p<0.01; 24h: 1.2 $\pm$ 0.4 vs. 3.4 $\pm$ 1.5; p<0.01), and by the reduced Ly-6G neutrophil infiltration at both 6h (35%, p<0.01) and 24h (50%, p<0.01). CXCL-2 mRNA expression, a major neutrophil chemoattractant (6h: 9.1 $\pm$ 0.7 vs. 11.1 $\pm$ 0.7; p<0.05; 24h: 3.1 $\pm$ 3.0 vs. 10.0 $\pm$ 3.5; p<0.05) was decreased in COX-2 KO livers after I/R. Overall, leukocyte transmigration is dependent upon cell activating chemokines, adhesive interactions, as well as matrix degradation mechanisms. MMP-9 activity, which is induced and mediates liver I/R injury, was significantly reduced in COX-2 KO livers at both 6h (~2-fold) and 24h (~4-fold) of reperfusion. Indeed, Ly-6G neutrophils co-expressing MMP-9 were highly detected in damaged WT livers and profoundly depressed in COX-2 KO livers after I/R insult. Interestingly, the addition of NS-398, a selective COX-2 inhibitor, to fMLP-stimulated neutrophils *in vitro*, markedly depressed MMP-9 activation (~5 to 10 fold) by these cells in a dose-dependent manner. Furthermore, the treatment of isolated neutrophils with MMP-9 inhibitor-I, a specific MMP-9 inhibitor, significantly disrupted their migration across fibronectin in transwell filters (35% vs. 63%, p<0.004), compared to controls. **Conclusion:** Our novel findings support the view of an important function for COX-2 in the pathogenesis of hepatic I/R injury. Moreover, they evidence a regulatory role for COX-2 on MMP-9 activation by neutrophils, which is likely a key factor in the recruitment of these cells in warm liver I/R injury.

Abstract# 26

The Role of Matrix Metalloproteinase 9 and Lipocalin-2 Complex in Ischemia and Reperfusion of the Transplanted Heart. Herbert T. Maier,¹ Albert Muigg,² Evelyn A. Wallnoefer,² Hubert Schwelberger,² Stephan Sickinger,² Stefan Schneeberger,¹ Jakob Troppmair,² Raimund Margreiter,¹ Felix Aigner.¹ ¹Department of General and Transplant Surgery, Innsbruck Medical University, Innsbruck, Tyrol, Austria; ²Daniel Swarovski Research Laboratory, Innsbruck Medical University, Innsbruck, Tyrol, Austria.

PURPOSE: Matrix Metalloproteinase 9 (MMP-9) is known to be involved in cell migration, tissue repair and remodelling. Lipocalin-2 (NGAL/Lcn-2) forms a complex with MMP-9 preventing it from degradation. Retrieved from previous data about the role of NGAL/Lcn-2 during ischemia and reperfusion (IR) following murine heart transplantation, this study focuses on the interaction between MMP-9 and NGAL/Lcn-2 in analogous settings.

MATERIAL AND METHODS: Male inbred C57BL/6 and the Lcn-2 $-/-$ mouse were used for heterotopic heart transplantations. RT-PCR, Western blot, gelatin zymography and immunohistochemistry were performed to visualize MMP-9 and NGAL/Lcn-2 expression in the graft at different time points (0, 2, 12, 24 and 48h). Polymorphonuclear cells (PMN) isolated from wildtype and Lcn-2 $-/-$ mice were used for *in vitro* experiments.

RESULTS: MMP-9 expression was upregulated 8 fold and peaked at 48h compared to NGAL/Lcn-2 expression which peaked at 24h. Similar expression patterns were observed in the different experimental settings (Lcn-2 $-/-$ donor Lcn-2 $-/-$ recipient, wildtype donor and recipient and mixed wildtype/Lcn-2 $-/-$ transplantations). However, MMP-9 protein levels were significantly lower in Lcn-2 $-/-$ grafts corresponding to a reduced infiltration of PMN in Lcn-2 $-/-$ recipients. *In vitro* PMN retrieved from Lcn-2 $-/-$ mice showed lower levels of MMP-9 than from wildtype mice. Stimulation of PMN with recombinant NGAL/Lcn-2 did not increase MMP-9 expression.

CONCLUSION: Our data demonstrate an interaction between MMP-9 and NGAL/Lcn-2 expression during IR in heart transplantation. Reduced MMP-9 expression in Lcn-2 $-/-$ grafts is directly related to the simultaneously decreased infiltration of PMN described previously, thus suggesting a chemotactic function of the MMP-9/Lcn-2 complex during IR of the transplanted heart.

Abstract# 27

FGL2/Fibroleukin Is a Novel Mediator of Hepatic Reperfusion Injury in Mice. Markus Selzner,¹ Nazia Selzner,¹ Oyedele Adeyi,² Itay Shalev,¹ Yi Zhu,¹ Agata Bartczak,¹ Gary A. Levy,¹ Ian D. McGilvray.¹ ¹Multiorgan Transplant Program, Toronto General Hospital, Toronto, ON, Canada; ²Department of Pathology, Toronto General Hospital, Toronto, ON, Canada.

Ischemia and reperfusion (I/Rp) injury is associated with increased morbidity and mortality following liver surgery and transplantation. T lymphocytes regulate the I/Rp induced inflammatory process through secretion of various cytokines. FGL2, a member of the fibrinogen superfamily, is secreted by CD4 $^{+}$ and CD8 $^{+}$ T cells and has potent immunomodulatory effects on the adaptive immune system. **Aim:** To determine the role of CD4 $^{+}$ cells/FGL2 on the mechanism of I/Rp injury. **Methods:** I/Rp injury was induced in C57BL/6 mice by clamping of the portal vein and hepatic artery supplying the left and median lobes of the liver (70%) for 60 minutes. Plasma levels of FGL2 were quantified in wild type and FGL2 KO mice after sham operation, 60 minutes ischemia only, and 60 minutes ischemia and 3hr of reperfusion. Hepatic CD4 $^{+}$ and CD8 $^{+}$ cells were investigated in wild type and FGL2 KO mice by immunohistochemistry. Serum AST and percentage of necrosis (H&E) were determined as markers of liver injury after 3hr and 24hr of reperfusion. **Results:** In wild type mice, hepatic I/Rp resulted in a 70-fold up-regulation of FGL2 levels (0.49 vs 0.007 ng/ml, p= 0.002), while ischemia alone did not affect FGL2 levels when compared to the sham mouse (0.009 vs 0.007ng/ml, p=0.5). 60 minutes of ischemia and 3hr of reperfusion resulted in a 4-fold increase of CD4 $^{+}$ cells in liver tissue when compared to the sham operated mice (42 vs 11 cell/10X,

field $p=0.001$). In contrast, no increase was observed in the FGL2 KO mice (8 vs 11 cells/10x field, $p=0.1$). CD8+ cells were significantly increased in liver tissue following I/Rp in comparison to sham mice (50 vs 12 CD8+ cells/10x field, $p<0.0001$), but not in FGL2 KO mice (10 vs 12 CD8+ cells/10x field, $p=0.6$). AST was significantly decreased after 60 min ischemia and 3hr of reperfusion in FGL2 KO compared to wild type mice (2509 vs 4250 U/L, $p=0.03$). Accordingly, following 60 min of ischemia and 24hr of reperfusion, FGL2 KO had significantly less necrosis when compared to the wild type (10% vs 35%, $p=0.02$). **Conclusion:** FGL2 up-regulation following I/Rp injury is associated with increased hepatic lymphocyte infiltration. Inhibition of FGL2 reduces reperfusion injury possibly by prevention of the T cell induced inflammatory response. Thus anti-FGL2 strategies might open a novel avenue for the prevention of reperfusion injury.

Abstract# 28

The Role of B Cells during the Healing Phase of Kidney Ischemia Reperfusion Injury. Hye Ryoung Jang,¹ Maria-Teresa Gandolfo,² Shailesh Satpute,¹ Hamid Rabb.¹ ¹Medicine, Johns Hopkins University School of Medicine, Baltimore, MD; ²Pathology, Johns Hopkins University School of Medicine, Baltimore, MD.

Though B cells have been implicated in the early pathogenesis of kidney ischemia reperfusion injury (IRI) (*J Immunol* 2003) and in the healing phase of CCl₄-induced liver injury (*J Clin Invest* 2005), the role of B cells in healing from IRI is not known. We hypothesized that B cells could participate in the healing phase of kidney IRI. We therefore examined B cell trafficking during the healing phase of kidney IRI in wild type mice. We then compared renal histologic changes during healing from IRI in μ MT (mature B cell-deficient) mice and their wild type littermate (C57BL/6 mice).

Unilateral renal pedicle clamping was performed for 45 min in C57BL/6 mice ($n=6$ per time point) and μ MT mice ($n=3$ per time point). On day 3 or 10 after ischemia-reperfusion injury (IRI), renal histologic examination and FACs with isolated kidney mononuclear cells (KMNC) were performed.

KMNC were significantly increased in the postischemic kidneys compared to the contralateral kidneys on the 3rd day after IRI. Total infiltrating B cells (expressing CD19) increased in the postischemic kidneys on the 3rd day (postischemic vs. contralateral, $33.4\pm1.5\%$ vs. $14.4\pm1.6\%$), and activated mature B-2 cells (expressing MHC class II, CD21/35, and CD69) remained increased on both 3rd and 10th day after IRI. B-1 B cells (expressing IgM & CD5) increased in the postischemic kidneys on the 10th day after IRI. Plasma cells (expressing CD126 & CD138) appeared at the early healing phase and matured as time passed. Increased numbers of T cells, though less activated, trafficked into the postischemic kidneys of μ MT mice. There was little difference in kidney histology between μ MT mice and wild type mice on the 3rd day after IRI. However on the 10th day after IRI, postischemic kidneys of μ MT mice showed extensive regeneration of both cortex and outer medulla, more normal tubules (30–50%), and less tubular atrophy (5–10%). Postischemic kidneys of wild type littermates showed focal regeneration with more tubular atrophy (30–60%) and less normal tubules (0–20%).

Infiltrating B cells expressing both maturation and activation markers increased in the healing phase of AKI in C57BL/6 mice. Postischemic kidneys of B cell-deficient mice showed more extensive regeneration and less tubular atrophy, suggesting that B cells can influence regenerative process during recovery from kidney IRI.

Abstract# 29

T-Cells and Increased Renal Vascular Permeability in Kidney Ischemia-Reperfusion Injury in Mice. Manchang Liu,¹ Chu-Chun Chien,¹ Dmitry N. Grigoryev,¹ Robert B. Colvin,² Hamid Rabb.¹ ¹Medicine, Johns Hopkins University, Baltimore, MD; ²Pathology, Massachusetts General Hospital, Boston, MA.

Ischemia reperfusion injury is the most common cause of acute kidney injury in both native and allograft kidneys. The pathogenic mechanisms of renal ischemia reperfusion injury include changes at the level of the microvasculature as well as the tubules. Within the microvasculature, leukocyte-endothelial interactions likely play a role and contribute to the well-established microvasculature dysfunction (e.g., endothelial permeability) and alterations in endothelial-leukocyte interaction occur during ischemic acute kidney injury. Our previous studies have demonstrated that T cells modulate renal ischemia reperfusion injury in a murine model, we hypothesized that T cells could mediate changes in renal vascular permeability during ischemia reperfusion injury. We performed a 30 min bilateral renal ischemia followed by reperfusion in C57BL/6 wild type mice and in T cell deficient (*nu/nu*) mice with or without T-cell adoptive transfer from their wild type littermates and evaluated RVP by Evans blue dye extravasations (EBDE). The time course studies of RVP showed marked increases in renal EBDE within the early 3–6 hrs after ischemia. CD3 positive pan T-cells but neither CD4 nor CD8 T cells were found to infiltrate into post ischemic kidney within 6 hrs, and comparison was made with other leukocytes using immunohistochemistry technique. Gene microarray analysis demonstrated that the gene for TNF- α (Tnfaip1), a potent mediator of microvascular permeability as well as a T cell product, was increased in the kidney early after ischemia. TNF- α , IFN- γ and IL-4 protein by an intracellular cytokine staining technique was found increased early in peripheral circulating T cells and later in renal T cells after renal ischemia. The rise in RVP was significantly attenuated in T cell deficient mice *nu/nu* at 6 hrs compared to the wild type littermates and this attenuated RVP in T cell deficient mice was restored after adoptive transfer of splenic T cells from wild type

littermates into these nude mice. These data demonstrate that T cells traffic early into post ischemic kidney, produce TNF- α and other cytokines that can increase RVP, and directly participate in the increased RVP during acute ischemia reperfusion injury. T cell-endothelial interactions are a likely mechanisms underlying the pathophysiologic role of T cells in renal ischemia reperfusion injury.

Abstract# 30

CD4+ T Cells Mediate Mouse Intestinal Injury Induced by Ischemia and Reperfusion. Melissa J. Watson,¹ Bibo Ke,¹ Xiu-Da Shen,¹ Feng Gao,¹ Ronald W. Busuttil,¹ Jerzy W. Kupiec-Weglinski,¹ Douglas G. Farmer.¹ ¹The Dumont-UCLA Transplant Center, David Geffen School of Medicine at UCLA, Los Angeles, CA.

Background: Ischemia/reperfusion injury (IRI) is a major cause of organ dysfunction after intestinal injury and transplantation. The interaction between the innate and adaptive immune systems has become a major area of focus in this field. Our preliminary work showed that mice undergoing intestinal IRI experienced worse survival and tissue injury in conjunction with increased infiltration of PMN and CD3+ cells as compared to sham mice. The purpose of this study was to investigate the role of the T cell in intestinal IRI through the use of genetically deficient mice.

Methods: Under anesthesia, male C57BL/6 wild-type mice (WT) and CD4 knockout mice (CD4KO) underwent 100 min of warm intestinal IRI by clamping of the SMA. Separate survival and analysis groups were performed. Intestinal tissue was harvested at 4h and 24h. Tissue was analyzed by histology, CD3 immunostaining, myeloperoxidase activity (MPO), and semi-quantitative PCR for several cytokines/chemokines.

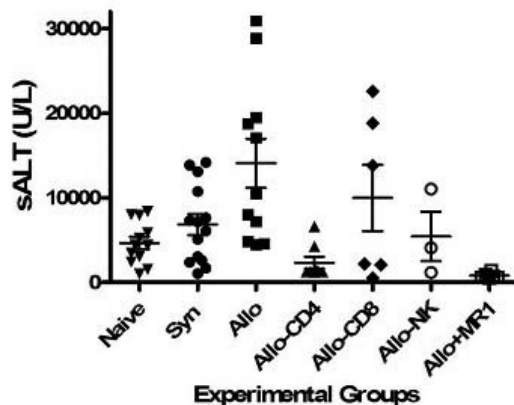
Results: WT had significantly worse survival compared to CD4KO (30% vs. 100%, $p=0.002$), and worse histopathological injury mostly involving mucosal sloughing. WT had higher MPO activity than CD4KO (1.9 ± 0.88 vs. 0.70 ± 0.73 at 4h, $p=0.059$, and 1.5 ± 0.05 vs. 0.22 ± 0.065 at 24h, $p=0.004$) and increased CD3+ cell infiltration. There was also increased mRNA production of cytokines/chemokines in WT vs. CD4KO at both timepoints; specifically, the data with respect to B-actin at 24h was: IL-2 (0.67 ± 0.05 vs. 0.30 ± 0.06 , $p=0.02$), MIP-2 (0.37 ± 0.03 vs. 0.11 ± 0.006 , $p=0.007$), MCP-1 (0.78 ± 0.03 vs. 0.42 ± 0.13 , $p=0.06$), RANTES (0.43 ± 0.09 vs. 0.14 ± 0.03 , $p=0.05$).

Conclusion: This study demonstrates for the first time in the intestine that CD4+ T cells are important mediators of IRI. In the absence of CD4+ T cells, better outcomes were associated with less PMN infiltration implying a link between the innate and adaptive immune systems. An alteration in chemotactic signaling potentially effected by CD4+ T cells may play a role in this process. These results confirm the important function of CD4+ T cells in intestinal IRI and justify further investigation into their complex role in this process.

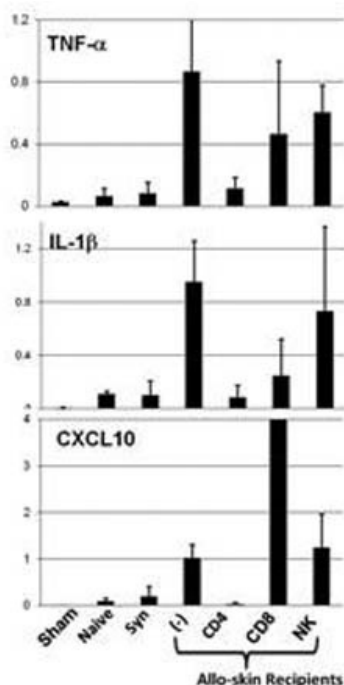
Abstract# 31

Alloimmunity Enhances Tissue Inflammation and Injury. Xiuda Shen,¹ Yue Wang,¹ Feng Gao,¹ Ronald W. Busuttil,¹ Jerzy W. Kupiec-Weglinski,¹ Yuan Zhai.¹ ¹Surgery, Dumont-UCLA Transplant Center, Los Angeles, CA.

Background: It has been recognized that innate immune activation is the prerequisite to trigger adaptive immunity. However, it is unknown whether Ag-specific immune response may impart the innate immune reaction, particularly local inflammation and tissue injury. **Methods:** We analyzed the effect of skin Tx-induced alloimmunity on the development of liver ischemia and reperfusion injury (IRI). Groups of B6 mice were challenged with syngeneic vs. allogeneic (B/c) skin Tx. Ten days later, these skin Tx recipients were subjected to 90 min cephalad liver lobe “warm” ischemia, followed by 6 h of reperfusion. To determine the role of T cell subsets in alloimmune-mediated tissue inflammation/injury, we selectively depleted CD4, CD8 or NK cells (Ab: GK1.5, 53.6.72, or PK136, resp.; 1mg/mouse, i.v., at day -1) in skin Tx recipients prior to IR onset. To determine molecular mechanisms in the alloimmune - liver innate immune cross talk, anti-CD154 mAb (MR1, at 0.5 mg/mouse, p.v.) was administered prior to reperfusion. **Results:** Allogeneic skin Tx recipients suffered a more severe liver IRI, compared with naive or syngeneic skin Tx recipients, as shown by higher sALT levels and liver histology.



Correlated with hepatocellular damage, IR-induced liver inflammation, as measured by local TNF- α , IL-1 β and CXCL10 gene expression, was also significantly upregulated selectively in allo-skin Tx recipients.



The depletion of CD4, but not CD8 or NK cells, abrogated alloimmune-mediated enhanced tissue inflammation/injury, as determined by both sALT and liver pro-inflammatory gene profiles. Furthermore, anti-CD154 treatment, which failed to suppress effector T cells to reject allografts, effectively diminished liver IRI in allo-skin Tx recipients. **Conclusion:** Alloreactive CD4 T cells: i/ promote liver inflammation and IRI, and ii/ function via CD154-dependent pathway.

Abstract# 32

The CXCR3 Expression on Non-Parenchymal Cells Plays an Integral Role in the Development of Liver Ischemia and Reperfusion Injury (IRI). Elizabeth R. Benjamin, Danyun Zhao, Chrissy Wang, Xiu-Da Shen, Feng Gao, Bo Qiao, Yuan Zhai, John A. Belperio, Ronald W. Busuttill, Jerzy W. Kupiec-Weglinski. *Surgery, Dumont-UCLA Transplant Center, Los Angeles, CA.*

Background: The IRI is a debilitating process that affects transplantation outcomes. Although CXCR3 signaling is known to play a role in the development of liver IRI, the underlying mechanisms remain to be elucidated. **Methods:** We used CXCR3 KO mice and WT counterparts in a model of partial liver warm ischemia (90min), followed by 0min, 30min, 2h (n=2-3/gr), 6h (n=10, and 24h (n=9) reperfusion. Serum was collected to measure sALT levels, tissue was analyzed with H&E/immunohistochemistry. Protein/mRNA were assessed by Western/qPCR analyses, resp. Bone marrow (BM) chimeras between CXCR3 KO and WT were generated in four groups: WT/KO, KO/WT, WT/WT, and KO/KO (n=5/gr). **Results:** CXCR3 KO mice were protected from liver IRI. Early post-reperfusion, sALT were elevated in all groups reflecting the ischemic insult. By 6 and 24 h, however, sALT levels in the CXCR3KO mice were markedly lower than in WT (p<0.01). Similarly, histology at early time points showed hepatocyte changes in both groups with scattered areas of hypereosinophilic cytoplasm. By 6 and 24h, CXCR3 KO had preserved architecture while the WT exhibited patchy necrosis, suggesting that early ischemia fails to convert to injury in the absence of CXCR3 signaling. Ab staining for CD4+ and CD8+ cells at 24 h showed similar infiltration in CXCR3 KO and WT groups. The qPCR analysis shows decreased levels of CXCL10 mRNA expression in the CXCR3 KO animals at 6h, consistent with the protection noted on histology/serum enzymes. In addition, CXCR3 KO animals had decreased levels of DR5 expression, while maintaining similar levels of FasL and TRAIL. The WT/WT chimeras showed large areas of necrosis and elevated sALT while the KO/KO animals were largely protected. Interestingly, the WT/KO animals were protected, whereas the KO/WT animals suffered significant liver injury. **Conclusion:** The disruption of CXCR3 signaling blunted local inflammatory response, and protected livers from converting ischemic insult into full-blown IRI. The cytoprotection in CXCR3 KO animals involves an interaction between resident non-parenchymal cells and hepatocytes that results in a reversal of ischemic damage, inhibiting conversion to apoptotic and/or necrotic injury. These data identify key players in the mechanisms of CXCR3 mediated liver damage and identify possible targets for clinical application.

Kidney - Acute Rejection: Markers and Outcomes

Abstract# 33

Increased mRNA Expression of Transforming Growth Factor-beta and FoxP3 in Urine from Renal Allograft Recipients Is Associated with Occurrence of Graft Rejection. Michael Eikmans,¹ Dave L. Roelen,¹ Cees van Kooten,² Hans W. de Fijter,² Frans H. J. Claas.¹ *¹Immunohematology and Bloodtransfusion, Leiden University Medical Center, Leiden, Netherlands; ²Nephrology, Leiden University Medical Center, Leiden, Netherlands.*

INTRODUCTION

Messenger RNA assessment in urine sediments from renal transplant patients is rapidly evolving as a non-invasive diagnostic tool. T cells, macrophages, and often B cells are present in rejecting kidney grafts. We questioned whether urinary mRNA levels of markers representing these cell types ((regulatory) T cells: CD3 ϵ , FoxP3, CD25, TGF- β ; macrophages: CD68, S100A9; B cells: CD20) are associated with rejection.

MATERIALS

In our institute 520 urine samples a year from over 100 renal transplant patients are being collected for mRNA quantitation purposes. Urine sediments are pelleted and stored in RNA-preserving solution. We initially investigated the effect of incubation time of urine on mRNA integrity. Next, in a case-control study 17 urine samples taken at time of biopsy-proven rejection were compared to 17 samples taken during stable graft function. Median time of sampling (55 days *versus* 43 days posttransplant) did not significantly differ between groups. RNA (0.90 $\pm$ 1.18 μ g) was extracted using RNeasy spin columns. Message of the markers mentioned above was quantified with Q-PCR and normalized.

RESULTS

Incubation of urine for 7h or longer at room temperature after acquisition resulted in a 75% decrease in 18S rRNA signals (P<0.05). However, storage of urine for up to 24h at 4°C did not result in decreased mRNA expression. In the case-control study, all markers tested showed the highest expression levels in urine rejection samples. When corrected for multiple comparisons, only TGF- β (26-fold, P=0.007) and FoxP3 (21-fold, P=0.002) expression was significantly increased in rejection samples compared to non-rejection samples. TGF- β levels highly correlated with FoxP3 levels (r = 0.90, P<0.00001), suggesting a mechanistic relationship *in vivo* between the two molecules.

CONCLUSION

RNA integrity in urine samples stored at 4°C is maintained for at least 24h. Detection of increased TGF- β and FoxP3 message in urine from patients with a kidney transplant represents a means for indicating occurrence of graft rejection. This implies that mRNA urinalysis renders a suitable molecular tool for non-invasive patient monitoring in clinical practice. The data furthermore suggest that rejection is associated with TGF- β -mediated immune mechanisms in which regulatory T cells play a role.

Abstract# 34

Expression of B Cell Associated Transcripts and Immunoglobulin Transcripts Is Time Dependent and Is Related to the Inflammatory Burden. G. Einecke, J. Reeve, M. Mengel, P. F. Halloran. *Department of Medicine, University of Alberta, Edmonton, Canada.*

The significance of B cell and plasma cell infiltration in renal allografts remains controversial. We previously established that transcript sets associated with Ifng effects or T cells reflect the inflammatory burden in renal allografts. In the present study, we identified B cell associated transcripts (BATs) and immunoglobulin transcripts (IGTs), reflecting B cell and plasma cell infiltration, respectively. Using microarrays, we analyzed BAT and IGT expression in relationship to histologic lesions, diagnosis, and renal function in 177 renal allograft biopsies.

Immunostaining confirmed that BAT and IGT expression was associated with B cells and plasma cells in the graft. Expression of BATs and IGTs was increased in biopsies with rejection (1.2 $\pm$ 0.2, 1.6 $\pm$ 0.9) compared to non-rejection (1.1 $\pm$ 0.2, 1.1 $\pm$ 0.5), but was not different between T cell (1.3 $\pm$ 0.3, 1.5 $\pm$ 1.1) and antibody mediated rejection (1.2 $\pm$ 0.2, 1.5 $\pm$ 0.9) and also occurred in some non-rejecting biopsies (recurrent GN). BAT and IGT scores correlated strongly with time post transplant (Fig1), which was best modeled as a dichotomous relationship: biopsies $\leq$ 5 months did not express BATs or IGTs above the level of control kidneys. Other inflammatory markers were not time dependent. In biopsies $\geq$ 5 months, BAT and IGT expression correlated with interstitial inflammation, tubular atrophy, and interstitial fibrosis. In a multiple regression analysis, only time post transplant and interstitial inflammation were independently related to BAT and IGT scores. When correcting for time post transplant, BAT and IGT scores did not correlate with renal function at the time of biopsy or future function 6 months post biopsy.

BATs and particularly IGTs are a time dependent feature of injured and inflamed renal allografts. Corrected for the effect of time, BATs and IGTs do not correlate with outcomes, indicating that B cell and plasma cell infiltrates have no specific role for the

mechanism of injury independent of the inflammatory burden. Their accumulation in late allografts may indicate emergence of specialized lymphoid compartments in tissues with long standing low level inflammation.

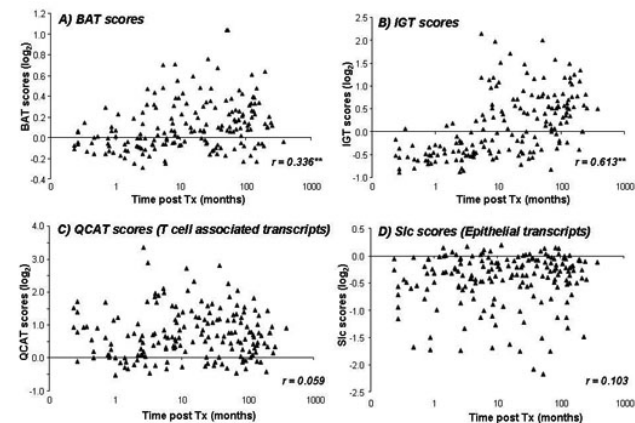


Figure 1. Relationship between BAT and IGT scores and time post transplant

Abstract# 35

Association of G-137C IL-18 Promoter Polymorphism with Acute Allograft Rejection in Renal Transplant Recipients. Chan-Duck Kim,¹ Hye-Myung Ryu,¹ Ji-Young Choi,¹ Hee-Jeong Choi,¹ Hyuk-Joon Choi,¹ Ji-Hyung Cho,¹ Sun-Hee Park,¹ Yong-Lim Kim.¹ ¹Division of Nephrology, Department of Internal Medicine, Kyungpook National University School of Medicine, Daegu, Korea.

Background. Interleukin 18 (IL-18) is a potent proinflammatory cytokine, which is postulated to play a role in mechanism of renal allograft rejection and strongly induces IFN- γ production. The literature on IL-18 polymorphisms and their importance in acute allograft rejection is not conclusive. The aim of this study was to investigate whether the G-137C IL-18 promoter polymorphism is associated with acute allograft rejection in renal transplant recipients (RTR).

Methods. For association analysis, 226 RTR and 148 controls were recruited. Genotyping was performed by using a real-time polymerase chain reaction which was carried on the LightCycler 480. Acute rejection episodes were defined by typical biopsy proven appearances or an improvement in renal dysfunction with pulsed methylprednisolone. Patients were separated into acute rejection (AR) group (n=37) and no AR group (n=189) according to the experience of acute rejection episode. IL-18 and IFN- γ serum levels of 73 RTR who were randomly selected were measured by ELISA.

Results. Acute rejection episode occurred in 16.4% of the patients. No significant differences in genotype and allele frequencies were observed between the RTR and the controls. There were significant differences in genotype frequency (GG:GC:CC, 89.2%:8.1%:2.7% vs 69.3%:29.1%:1.6%, $\chi^2=8.377$, $P=0.013$) and allele frequency (G:C, 93.2%:6.8% vs 83.9%:16.1%, $\chi^2=4.367$, $P=0.037$) between the AR group and no AR group. The frequency of the -137GG genotype significantly increased in patients with AR ($P=0.015$, odds ratio=3.653, 95% CI=1.237 to 10.785). Serum levels of IL-18 (348.9 $\pm$ 108.8 pg/mL vs 279.3 $\pm$ 109.2 pg/mL, respectively, $P=0.016$) and IFN- γ (11.9 $\pm$ 7.6 pg/mL vs 9.2 $\pm$ 3.3 pg/mL, respectively, $P=0.039$) were significantly elevated in the AR group as compared to the no AR group. In the AR group, the -137GG genotype had significantly higher IL-18 serum levels compared to other genotypes.

Conclusion. These data demonstrate that the -137GG genotype of the IL-18 gene, encoding higher IL-18 production, seems to be associated with acute rejection and may be useful markers of AR risk in RTR.

Abstract# 36

Changes in Gene Expression Reflect Renal Function at Time of Biopsy and Predict Future Function in Human Renal Allografts. Sakarn Bunnag,¹ Thomas F. Mueller,¹ Gian S. Jhangri,² Gunilla Einecke,¹ Konrad Famulski,¹ Luis G. Hidalgo,¹ Banu Sis,³ Bruce Kaplan,⁴ Philip F. Halloran.¹ ¹Medicine, University of Alberta, Canada; ²Public Health Sciences, University of Alberta, Canada; ³Laboratory Medicine and Pathology, University of Alberta, Canada; ⁴Medicine, Surgery and Pharmacology and UIC Transplant Center, University of Illinois.

The relationship between gene expression in biopsies and renal allograft function remains unclear. Using Affymetrix microarrays, we analyzed 194 human renal transplant biopsies for cause from 152 patients. We correlated estimated GFR (Cockcroft-Gault) at time of biopsy and 6 months after biopsy with the mean expression of pathogenesis-based transcript sets (PBTs) defined in mouse kidney transplants. These reflect the major processes during renal allograft rejection: cytotoxic T cell associated (QCATs); IFN γ effects (GRITs); injury and repair induced transcripts that peak at day 1 (IRIT_D1), day3 (IRIT_D3) or day 5 (IRIT_D5); and kidney transcripts lost in injury (KT2). Higher

QCATs, GRITs, IRIT_D3, IRIT_D5 and lower KT2 were associated with lower GFR at time of biopsy and loss in GFR from baseline to time of biopsy, indicating that PBTs reflect dysfunction. All the PBTs except IRIT_D1 and KT2 were associated with lower GFR at 6 months, but IRIT_D3 showed the strongest correlation with GFR, including improvement in GFR within 6 months after biopsy.

PBTs	Pearson correlation coefficient between PBTs expression and renal function			
	eGFR at Biopsy	6 months after biopsy	Baseline to biopsy	Biopsy to 6 months after
QCATs	-0.17*	-0.16*	-0.19*	-0.05
GRITs	-0.23**	-0.25**	-0.20**	-0.10
IRIT_D1	-0.02	-0.01	-0.09	0.13
IRIT_D3	-0.51**	-0.29**	-0.36**	0.16*
IRIT_D5	-0.28**	-0.22**	-0.19*	-0.02
KT2	0.27**	0.13	0.22**	-0.02

* $p<0.05$, ** $p<0.01$

Thus the PBTs have prognostic value. Surprisingly, the transcripts in the biopsy with greatest prognostic value for future GFR or recovery of GFR were not related to rejection (cytotoxic T cell or IFN γ associated) but to the degree of injury response. We propose that the common pathway linking various injuries (immune and non immune) to GFR is through the degree of injury response these events induce in the epithelium, particularly those in the IRIT_D3 set.

Abstract# 37

Early Rejection from Anamnestic Response in Offspring-to-Mother or Husband-to-Wife Kidney Transplant. Kwan Tae Park,¹ Song Chul Kim,¹ Duck Jong Han.¹ ¹Surgery, Asan Medical Center, Seoul, Korea.

Accelerated rejection can be developed in the immediate post-transplant period resulting from anamnestic response due to the exposure to fetal HLA antigen during the previous pregnancy in case of offspring-to-mother or husband-to-wife. However, accelerated rejections in these groups have been rarely reported.

81 cases of offspring-to-mother (Offspring group) and 53 cases of husband-to-wife (Spouse group), who has been sensitized to spouse via their children, underwent kidney transplants from January of 1997 to August of 2007 at our institution and retrospectively reviewed. Control group was female kidney recipients transplanted at the same period from living related donors other than offspring and from living unrelated donors other than husband.

Acute rejection (AR) rate within 3 months after transplant were 19.7% (16/81) in offspring group and 18.8% (10/53) in spouse group. The AR rates were not different between the two groups, however they were significantly higher than control group in both groups, namely 10.2% (24/235) for offspring control and 13.5% (10/74) for spouse control. The mean onset of AR was 7.5 day (0-29) and it was significantly later than that of control groups (16.5 day, $p<0.05$) and 54% of AR were accelerated rejections within 7 days after transplant. Proportion of acute humoral rejection was significantly higher in both groups than control group (37.5% vs 8.3% in offspring group, 60% vs 10% in spouse group). Most of the AR was successfully reversed by steroid pulse and/or plasmapheresis, IVIG, rituximab. Any risk factors for the AR such as number of pregnancy, preoperative CDC cross matching, PRA, immunosuppressant and antibody induction couldn't be identified. Serum creatinine level in AR patients were higher than AR free patients by postoperative 1 month, but last follow up creatinine level didn't show any statistical difference (1.43 mg/dl in AR vs 1.14mg/dl in AR free). 5 year graft/patient survival rate were not different between AR and AR free groups and study and control groups.

Risk of higher rejection rate accompanied with higher accelerated and humoral rejection was identified in female kidney recipients from offspring or spouse donor in comparison with control group. Considering the anamnestic response of this cohort of female recipients, more prudent preoperative screening and careful immediate postoperative immune monitoring are required for the avoidance of rejection and impaired graft survival.

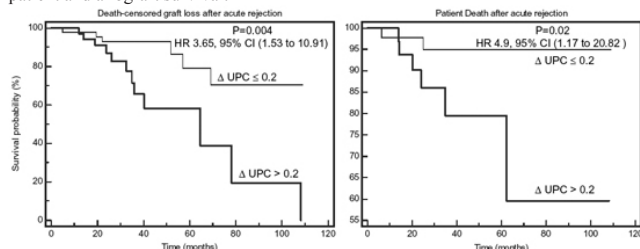
Abstract# 38

Increase in Proteinuria Greater Than 200 mg after Rejection Is Associated with Poor Patient/Allograft Outcomes. Brenda Muth,¹ Millie Samaniego,¹ Neeraj Singh,¹ Jose Torrealba,¹ John Pirsch,¹ Bryan Becker,¹ Arjang Djarnali.¹ ¹Nephrology, UW Madison, Madison, WI.

The impact of proteinuria after acute rejection on kidney allograft and patient outcomes is unknown. We retrospectively analyzed the effects of a change from baseline to 1 month after rejection in urine protein:creatinine ratios (Δ UPC) on patient and allograft outcomes in 82 patients between 1998 and 2007. Only first acute rejection episodes occurring after the 3d month post-transplant were studied. The average time interval prior to rejection was 19M and patients were followed for an average of 40M after transplant. There were 63, 51 and 31 cases of acute cellular, acute antibody-mediated and mixed rejection, respectively. Baseline eGFR, Scr and UPC levels were 48 ml/min, 1.7 mg/dL and 0.63 g/g respectively. One month after rejection, these values were 42 ml/min, 1.9 mg/dL and 1.06 g/g respectively. The average Δ UPC was 0.44 $\pm$ 0.16 g/g. There were 18 death-censored graft losses and 8 deaths. Univariate analyses evaluating more than twenty clinical and biochemical characteristics including the Banff classification of acute rejection, causes of ESRD and rejection therapy showed that a Δ UPC > 0.2 g/g was a significant predictor of death-censored graft loss. These results were confirmed by stepwise multivariable Cox regression analyses.

Death-censored allograft loss						
		Univariate analyses		Multivariate analyses		
	p	HR	95% CI	P	HR	95% CI
AUPC > 0.2	0.008	3.8	1.4-10.3	0.002	7.3	2.05-25.8
African-American	0.04	3.4	1.05-11.1	0.008	7.8	1.7-35.5
Deceased donor kidney	0.03	3.2	1.1-9.3	-	-	-
Living donor kidney	0.04	0.3	0.08-0.9	0.008	0.1	0.03-0.6
Scr at rejection	0.01	2.1	1.03-4.3	-	-	-
Number of previous transplants	0.004	2.5	1.3-4.8	0.0004	5.2	2.1-13

Kaplan-Meier survival curves showed that $\Delta\text{UPC} > 0.2$ were associated with decreased patient and allograft survival.



These data show for the first time that regardless of the histopathology, $\Delta\text{UPC} > 0.2$ one month after rejection is associated with poor patient/allograft outcomes.

Abstract# 39

From Histology to Microarrays and Back: Refining Histopathological Diagnosis of Renal Allograft Rejection Using Microarrays. Michael Mengel,¹ Jeff Reeve,¹ Banu Sis,^{1,2} Gunilla Einecke,¹ Sakarn Bunnag,¹ Julie Riopel,² Thomas Mueller,¹ Philip F. Halloran.¹ ¹*Alberta Transplant Applied Genomics Centre, University of Alberta, Edmonton, AB, Canada;* ²*Laboratory Medicine and Pathology, University of Alberta, Edmonton, AB, Canada.*

The histopathological hallmark of T cell mediated rejection (TCMR), is interstitial infiltration. Assessing infiltration by histology is arbitrary, limited in reproducibility, and has never been assessed against independent standards. We recently reported that a set of cytotoxic T cell-associated transcripts (QCATs) could quantitatively assess the T cell burden in tissue. Objective of the present study was to re-examine the current diagnostic criteria in relationship to the QCATs.

In 129 renal allograft biopsies, we assessed how histology predicts the QCAT burden. An independent diagnostic threshold for QCATs was established in control samples. Applying this QCAT threshold revealed current diagnostic criteria for histology to be flawed; the threshold for interstitial infiltration is too high (100% specificity, 37% sensitivity), the types of infiltration to be considered (i.e. i-Banff) are wrongly defined, and tubulitis is not increasing diagnostic accuracy. Changing the criteria by lowering the histological threshold for cortical infiltration from 25% to 10%, taking into account all interstitial cellular infiltration (=total) and ignoring tubulitis increased sensitivity to 91% with a decreased specificity of 50%. But, this includes biopsies having interstitial infiltration without QCATs, indicating that histology might not discriminate between 'active' and 'inactive' infiltration. Both the refined histological and the QCAT threshold had prognostic value in terms of future renal allograft function.

Table 1: Association with renal function

	eGFR at biopsy ml/min	p-value	eGFR 6 months after biopsy ml/min	p-value	delta eGFR ml/min	p-value
i-Banff (<10% vs. ≥10%)	48.3 vs. 42.5	0.05	55.9 vs. 50.6	0.18	6.4 vs. 5.2	0.92
i-total (<10% vs. ≥10%)	44.9 vs. 45.3	1.0	66.3 vs. 51.3	0.03	22.5 vs. 3.9	0.00002
OCATS (<1xSD vs. ≥1xSD)	50.5 vs. 42.8	0.01	61.8 vs. 48.5	0.0007	10.8 vs. 3.1	0.009

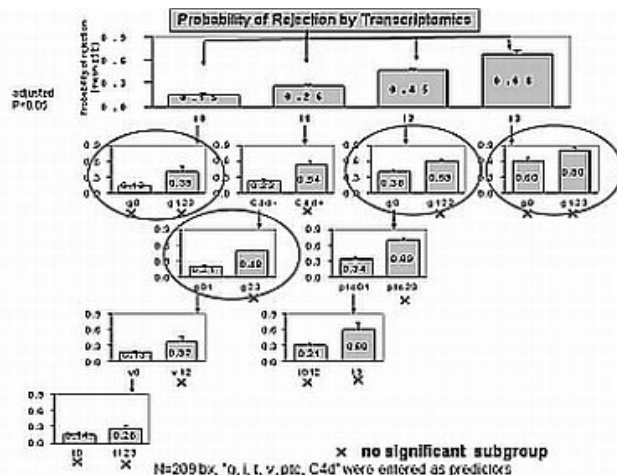
We propose a refined histological scoring system that better predicts the active T cell burden and outcome than the current Banff criteria for renal allograft rejection.

Abstract# 40

The Independent Correlates of Rejection Are Interstitial Inflammation and Glomerulitis but Not Tubulitis. Banu Sis,¹ Julie Riopel,¹ Gian Jhangri,¹ Jeff Reeve,¹ Philip F. Halloran.¹ ¹Medicine, University of Alberta, Edmonton, AB, Canada.

The significance of glomerulitis (“g”) in renal allografts is still unknown. We previously defined pathogenesis based transcript sets (PBTs) that capture major biologic events during allograft rejection. We aimed to 1) study gene expression by Affymetrix microarrays in 223 renal allograft biopsies taken for deteriorating function and relate g-score to changes in gene expression, C4d deposition, and Banff lesions, 2) use transcriptomics as an external standard to test the relations of g-score to the probability of rejection defined by a classifier built using PBTs.

We used a classification tree analysis (SPSS 15.0) using acute Banff lesions (g, i, t, v, ptc) and C4d-staining to investigate the best combinations of histologic lesions that predict probability of rejection defined by transcriptomics in 209 biopsies for cause. This analysis showed the i-score (interstitial inflammation) as the strongest predictor of rejection, which is followed by the g-score (adjusted $p < 0.05$). At any given degree of i score, the probability of rejection was significantly higher if there is glomerulitis.



Glomerulitis was more frequent in antibody-mediated rejection (ABMR) (80%) than in T-cell mediated rejection (TCMR) (33%) ($p<0.05$), and correlated with i-score and ABMR features i.e. C4d deposition, peritubular capillaritis (ptc), multilayering of peritubular capillary basement membranes, and glomerular double contours ($p<0.05$). The g-score correlated with PBTs reflecting CTL-associated ($r=0.29$) and Ifng dependent genes ($r=0.40$). Compared to TCMR-glomerulitis, ABMR-glomerulitis biopsies showed higher expression for selective endothelial genes ($p<0.001$) plus additional 6 genes some of which are involved in complement regulation i.e. THBD, CD59 ($p<0.05$). Biopsies with glomerulitis show an intense inflammatory burden as measured by PBTs. Surprisingly, the power of g-score, as a predictor of rejection, was much greater than t-score or v-score, lesions considered pathognomonic for rejection. We propose that in the Banff meeting the “i” and particularly the “g” scores should be emphasized, and the t score should be re-evaluated.

Abstract# 41

Screening for De Novo Anti-HLA Antibodies Was Not Predictive of a First AR Episode, but May Be Useful in Identifying Patients at Risk for a Second AR. John S. Gill,¹ Olwyn Johnston,¹ Vivian Wu,¹ Gary Nussbaumer,¹ Paul A. Keown,¹ Ruth J. Shapiro,¹ Alex Magil,² David Landsberg.¹ ¹*Division of Nephrology, University of British Columbia, Vancouver, BC, Canada;* ²*Department of Pathology, University of British Columbia, Vancouver, BC, Canada.*

It is not known if screening for anti-HLA antibodies (Abs) after kidney transplantation (KTX) is predictive of AR. We prospectively tested for anti-HLA Abs (flow-PRA analysis and anti-HLA Ab specificity determination using purified single antigen beads) in 84 non-HLA identical KTX recipients at 7, 14, 30, 60, 90, 180, 365 days post-KTX, and at the time of any clinical acute rejection (AR). Protocol biopsies (including C4d staining) were performed at the time of engraftment, 3 and 12 months post KTX, when patients developed new anti-HLA Abs, or when clinically indicated.

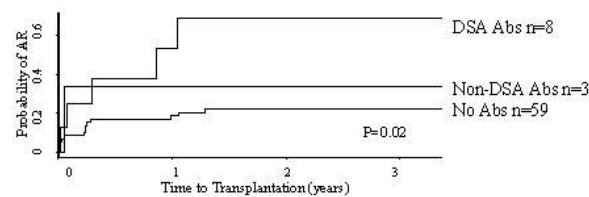
N = 14 patients with anti-HLA Abs prior to KTX were excluded from study. 11/ 70 included patients developed de novo anti-HLA Abs (N= 8 donor specific antibodies (DSA)) at a median of 30 days (q1-q3 = 10-180 days) after KTX. Patients who developed de novo anti-HLA Abs had a shorter time to AR than patients who did not develop de novo Abs (figure).

However, among the 11 patients who developed de novo anti-HLA Abs, 6 developed Abs in the setting of AR. The other 5 patients who developed de novo anti-HLA never had AR. Thus de novo Abs were not predictive of AR that would not have been identified clinically. Among the 6/11 patients who developed anti-HLA Abs in the setting of AR, 2/6 had a subsequent AR (table). There was no difference in glomerular filtration rate (GFR) at last follow up (median follow up=3.7 years (Q1, Q3=1.0, 4.4) between patients who did and did not develop de novo anti-HLA Abs (median GFR 54.8 and 56.0 ml/min/1.73m² respectively, $p=0.79$).

We conclude that de novo anti-HLA Abs after KTX are not predictive of AR or kidney function. Whether patients who develop de novo anti-HLA Abs during an AR (with or without C4d) are at risk for a second AR, warrants further study.

Pathology at time of de novo anti-HLA antibody formation	Subsequent AR during f/u
AR C4d negative n = 3	33 % (n=1)
AR C4d positive n = 3	33 % (n=1)
No abnormality n = 5	0%

(Figure on Page 190)



Kidney: Living Donor Issues I

Abstract# 42

Effects of Living Kidney Donation on 24-Hour Ambulatory Blood Pressure and Cardiovascular Risk: A Prospective Study. G. V. Ramesh Prasad,^{1,2} Deborah Lipszyc,² Michelle M. Nash,² Jennie Huckle,² Michael Huang,² Lindita Rapi.² ¹Medicine, University of Toronto; ²Renal Transplant Program, St. Michael's Hospital, Toronto, ON, Canada.

Background: Recent meta-analyses show that long-term small elevations in blood pressure (BP) occur after living kidney donation. The purpose of this prospective study was to determine 24-h ambulatory BP (ABP) changes and the occurrence of nocturnal non-dipping and other cardiovascular (CV) risk factors in donors over 6 mos. **Methods:** 24-h ABP monitoring was performed in 46 consecutive healthy normotensive live kidney donors prior to donation, and at 6 mos post-donation using a calibrated DynaPulse 5000A ABP monitor. Donors provided 24-h urine for protein, 75g oral glucose tolerance testing (ADA 2003), and fasting lipids at each time point. For 24-h ABP monitoring, awake and sleep times were recorded and 60-min BP measurements performed. Nocturnal non-dipping was defined as night-to-day systolic BP (SBP) ratio ≥ 0.9 . Pre-to-post donation comparisons were made by paired student t or chi² test. All donors provided informed consent. **Results:** Out of 46 donors, 36 were women, 29 white, and age was 45.5 ± 13 y (range 21-69). At baseline, 31 were dippers and 15 non-dippers. 24-h SBP was 115 ± 8 and 119 ± 9 mmHg ($p=0.19$); eGFR 95.5 ± 15 and 86.7 ± 19 ml/min/1.73m² ($p=0.11$); and 24-h urine protein 0.095 ± 0.04 and 0.113 ± 0.04 g ($p=0.25$) in dippers and non-dippers respectively. There was no difference in DBP, BMI, fasting or 2-h BG, or fasting lipids. At 6 mos, there were 8 non-dippers. 11/15 baseline non-dippers became dippers while 4/31 dippers became non-dippers. 4 remained non-dippers throughout. For the whole group, eGFR declined from 92.8 ± 16 to 61.3 ± 12 ml/min/1.73m² ($p<0.0001$), SBP from 117 ± 8 to 113 ± 9 mmHg ($p=0.04$), and 24-h urine protein increased from 0.109 ± 0.04 to 0.135 ± 0.06 g/d ($p=0.08$). At 6 months, non-dippers had a higher SBP (121 ± 11 v 111 ± 8 mmHg, $p=0.004$) than dippers but there were no differences in BMI (25.2 ± 1.1 v 23.5 ± 3.1 kg/m², $p=0.28$), eGFR (62.0 ± 13 v 57.4 ± 11 ml/min/1.73m², $p=0.38$) or proteinuria (0.130 ± 0.05 v 0.160 ± 0.11 g/d, $p=0.65$). **Conclusions:** Normotensive live kidney donors show significant BP reduction over the first 6 mos post-donation. Many pre-donation non-dippers subsequently demonstrate normal dipping. Post-donation non-dippers had a higher 24-h SBP unlike prior to donation, but increased proteinuria or diminished eGFR could not be demonstrated in this study. Other CV risk factors also remain unchanged in this timeframe.

Abstract# 43

Predictors of Reduced Measured Glomerular Filtration Rate (GFR) and Albuminuria in a Large Cohort of Previous Kidney Donors. Hassan N. Ibrahim,¹ Robert F. Bailey,² Hongfei Guo,³ Aleksandra Kukla,¹ Abhinav Humar,² Arthur J. Matas.² ¹Medicine, University of Minnesota, Minneapolis, MN; ²Surgery, University of Minnesota, Minneapolis, MN; ³Biostatistics, University of Minnesota, Minneapolis, MN.

There has been an increasing and well justified interest regarding the long term renal consequences of kidney donation. The collective evidence suggests, however, that kidney donors enjoy a normal life span and their lifetime risk of ESRD may not be different from non-kidney donors. Assessing kidney function utilizing serum creatinine is not without limitations. Therefore, to better assess kidney function, 5 years ago, we began a large effort to measure GFR using the plasma disappearance of iohexol and first void urinary albumin/creatinine ratio (ACR) in randomly selected kidney donors who donated at our institution. **Results:** We have performed over 3600 donor uninephrectomies since the inception of our program in 1963. Of these, 242 donors underwent iohexol GFR at our General Clinical Research Center. Microalbuminuria is defined as ACR between 30-300mg/g and macroalbuminuria as ACR>300mg/g. The mean age was 53.0 ± 9.6 years, 11.9 ± 8.8 years have elapsed since donation, hemoglobin was 13.7 ± 1.23 g/L, systolic blood pressure (SBP) was 121.7 ± 14.7 mmHg and diastolic blood pressure (DBP) was 73.2 ± 9.1 mmHg. 82.6% of donors had a GFR greater than 60ml/min/1.73m² and 17.4% had a GFR between 30-60ml/min/1.73m². The detailed renal profile of these donors is shown in the table below.

Measured GFR and ACR in Previous Kidney Donors	
n	242
Serum Creatinine	1.11 ± 0.21 mg/dL
ACR	27.8 ± 180 mg/g
Iohexol GFR	71.5 ± 11.7 ml/min/1.73m ²
% Normoalbuminuric	91.7%
% Microalbuminuric	7.4%
% Macroalbuminuric	0.9%

Multivariate analysis that adjusted for age, gender, ethnicity, time from donation, SBP, DBP and body mass index (BMI) identified age at donation, female gender and BMI as independent predictors for GFR<60ml/min/1.73m² and time from donation and systolic blood pressure as independent predictors for micro and macroalbuminuria. **Conclusion:** This is the largest effort describing measured GFR in previous kidney donors. It is reassuring to find out that the majorities have a preserved GFR and only a minority has albuminuria. The risk factors for reduced GFR and albuminuria are analogous to what has been described in the general population.

Abstract# 44

Cardiovascular Disease and Hypertension Risk in Living Kidney Donors: An Analysis of Health Administrative Data in Ontario, Canada. A. X. Garg,^{1,2,3} G. V. Ramesh Prasad,⁴ H. R. Thiessen-Philbrook,¹ Li Ping,³ M. Melo,³ E. M. Gibney,⁵ G. Knoll,⁶ M. Karpinski,⁷ C. R. Parikh,⁸ J. Gill,⁹ L. Storsley,⁷ M. E. O. Vlasschaert,² M. Mamdani.^{3,10} ¹Division of Nephrology, University of Western Ontario, London, ON, Canada; ²Department of Epidemiology and Biostatistics, University of Western Ontario, London, ON, Canada; ³Institute for Clinical Evaluative Sciences, Toronto, ON, Canada; ⁴Division of Nephrology, University of Toronto, Toronto, ON, Canada; ⁵Division of Nephrology, Virginia Commonwealth University, Richmond, VA; ⁶Division of Nephrology, University of Ottawa, Ottawa, ON, Canada; ⁷Division of Nephrology, University of Manitoba, Winnipeg, MB, Canada; ⁸Section of Nephrology, Yale University School of Medicine, New Haven, CT; ⁹Division of Nephrology, University of British Columbia, St. Paul's Hospital, Vancouver, BC, Canada; ¹⁰Keenan Research Centre, Li Ka Shing Knowledge Institute of St. Michael's Hospital, Toronto, ON, Canada.

To determine whether kidney donation increases the risk of death and major cardiovascular events, and the risk of hypertension, we conducted a natural history retrospective cohort study using administrative data for donations which occurred in Ontario, Canada between the years 1993 and 2005. Non-donor controls were randomly selected from healthy residents of Ontario and were matched on age, sex, neighbourhood income, and number of non physician visits. We evaluated 1278 living donors and 6359 healthy adults who acted as a control group. Individuals were followed for a mean of 6.2 years (range 1 to 13 years) after donation. The primary outcome was a composite of time to death or first cardiovascular event (myocardial infarction, stroke, angioplasty, bypass surgery). The secondary outcome was time to a diagnosis of hypertension. There was no significant difference in death or cardiovascular events between donors and controls [1.3% vs. 1.7%; hazard ratio 0.7, 95% confidence interval (CI) 0.4 to 1.2]. Donors were more frequently diagnosed with hypertension than controls [16.3% vs. 11.9%, hazard ratio 1.4, 95% CI 1.2 to 1.7] but were also seen more often by their primary care physicians [median (inter quartile range) 3.6 (1.9-6.1) vs. 2.6 (1.4-4.3) visits per person year, $p < 0.001$]. Based on administrative data, the risk of cardiovascular disease was unchanged in the first decade after kidney donation. The observed increase in diagnosed hypertension may be due to nephrectomy or more blood pressure measurements amongst donors in follow-up, and requires further study.

Abstract# 45

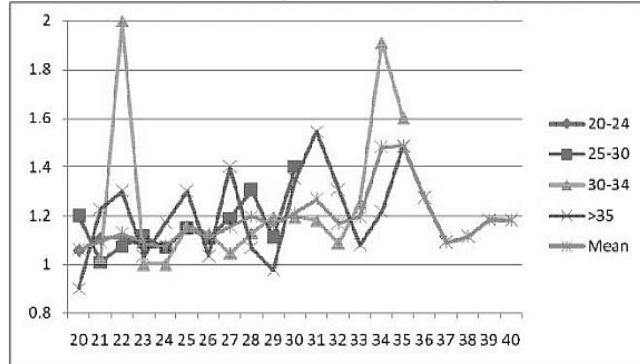
I Would like To Donate a Kidney. Can You Tell Me What My Kidney Function Will Be in 20-40 Years after I Donate? Hassan N. Ibrahim,¹ Sanjeev K. Akkina,¹ Robert F. Bailey,² Arthur J. Matas.² ¹Medicine, University of Minnesota, Minneapolis, MN; ²Surgery, University of Minnesota, Minneapolis, MN.

Kidney donation is generally accepted as safe but little data exists regarding donors outcome beyond 20 years. We have previously reported our experience with 1359 donors who donated over 20 years ago and found that roughly a third of the survivors to be hypertensive but with preserved estimated glomerular filtration rate (GFR); ≥ 60 ml/min in the majority.

Results: Herein, we expand our previous observation and report on 1431 kidney donors who donated > 20 years ago in two domains: serum creatinine and requirement for anti-hypertensive medications. 231 have died. Unknown cause of death and cardiovascular disease accounted for 75% of deaths. 54% were women, 95% were white and the mean age at donation was 34.8 ± 11.2 years. Main results are shown in the table below.

Years from Donation	Serum Creatinine at Last Year of Follow-Up	Requiring Anti-Hypertensive Medications
20-24 (n=428)	1.07 ± 0.30 mg/dL	16.3%
25-30 (n=430)	1.10 ± 0.33 mg/dL	22.3%
30-34 (n=336)	1.9 ± 2.4 mg/dL	30.4%
>35 (n=236)	1.1 ± 0.35 mg/dL	31.4%

The graph below depicts the serial serum creatinine in donors with multiple measurements starting at 20 years after donation and the overall mean. The graph was constructed by dividing the cohort into 20-24 years post donation, 25-30, 30-34 and > 35 years from donation to minimize possible confounding of the period effect.



Conclusions: Just as in the general population, there was a stepwise increase in the need for anti-hypertensive medications after donation. Serum creatinine remained stable during follow-up and showed minimal change over time. It is necessary to develop efforts to obtain 100% long-term follow-up for donors > 20 years post-donation in order to provide accurate information to future donor candidates.

Abstract# 46

Cystatin C is a Strong Predictor of Renal Dysfunction and Cardiovascular Disease Risk in Long-Term Kidney Donors. Michael Frederick,¹ Mehdi Tavakol,¹ Hamid Assadi,¹ John Roberts,¹ Andrew Posselt.¹ *Transplant Surgery, University of California San Francisco, San Francisco, CA.*

Background: Cystatin C (CysC) is a serum marker of renal function that is independent of age, sex, and lean muscle mass and has been found to be an accurate predictor of all-cause mortality and cardiovascular disease risk. The goal of the present study was to determine whether CysC levels could identify long-term kidney donors at greater risk for development of renal insufficiency, proteinuria, dyslipidemias and cardiovascular disease.

Methods: 107 patients who donated a kidney at our institution between 1975-2004 were evaluated. Evaluation included review of medical records, physical exam, blood draw and 24h urine collection to determine proteinuria and albuminuria. Glomerular filtration rate (GFR) was determined using the MDRD formula. Serum CysC was measured using immunonephelometry and study participants were grouped according to levels of the protein (<1.0 and ≥1.1). Serum CysC values in individuals with normal renal function typically range from 0.6-1.0 mg/l. Cardiovascular disease risk and all-cause mortality are known to be increased in individuals with CysC levels >1.1.

Results: Mean follow-up for the entire cohort was 10±7 years. CysC was higher in older and overweight (BMI>25) donors even though serum Cr levels in these groups were not significantly different from controls. As expected, both CysC ≥1.1 mg/l and elevated serum Cr were associated with markers of renal dysfunction (proteinuria, renal insufficiency); however, only CysC was strongly associated with several known cardiovascular risk factors (low HDL levels, hypertension).

Cystatin C Levels and Long-Term Donor Health Markers

	Cystatin-C <1 (n=65)	Cystatin-C ≥ 1.1 (n=42)	Relative Risk	p-value
Age at Donation (y)	41 ± 11	50 ± 9	-	<0.001
Mean F/U (y)	9 ± 7	10 ± 9	-	0.43
Hypertension	29.2%	76.2%	3	<0.001
MDRD GFR <60	26.1%	76.2%	3.05	<0.001
24h-Uprotein>150mg/dl	13.8%	38.1%	2.75	0.008
24h-Ualbumin >30md/dl	6.6%	11.9%	1.8	0.49
HDL < 50mg/dl	20.0%	52.3%	2.62	<0.001
Cholesterol (mg/dl)	203 ± 33	198 ± 29	-	0.41

Conclusions: CysC is a strong predictor of both renal insufficiency and cardiovascular disease risk in long-term kidney donors. We recommend that CysC measurements be included in the postoperative evaluation and follow-up of kidney donors.

Abstract# 47

Similar Short-Term Renal Outcomes among African Americans and Caucasians Following Live Kidney Donation. Amber Reeves-Daniel,¹ Kurt Daniel,¹ Dean Assimos,² Carl Westcott,² Jeff Rogers,² Alan Farney,² Robert Stratta,² Erica Hartmann,¹ Patricia Adams,¹ Anthony Bleyer,¹ Barry Freedman.¹ *Internal Medicine, Wake Forest University, Winston-Salem, NC;*

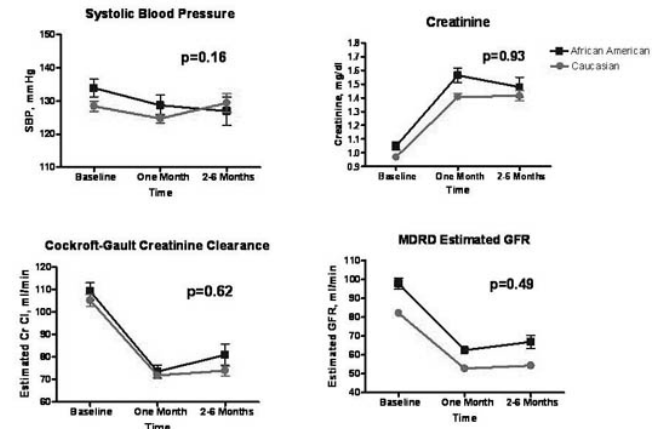
Surgery, Wake Forest University, Winston-Salem, NC.

Introduction: African Americans (AA) are considered higher risk kidney donors than Caucasians because of the higher prevalence of end-stage renal disease among select AA families. However, little data are available regarding the post-donation outcomes of AA donors. The purpose of this study was to evaluate ethnic differences in renal function and blood pressure after kidney donation.

Methods: We reviewed the records of kidney donors at our center who donated from

1998 to 2007 and collected data from the time of donation (baseline), one month post donation, and at 2-6 months post-donation. We evaluated ethnic differences in the rates of change in serum creatinine concentration, creatinine clearance (by Cockcroft-Gault method), estimated glomerular filtration rate (eGFR, by MDRD method), and systolic blood pressure using linear mixed models.

Results: We evaluated 148 living kidney donors (49 AA and 99 Caucasian) during the study period. African Americans had higher baseline serum creatinine concentrations (mean 1.05 mg/dL AA vs. 0.97 mg/dL Caucasian, p=0.02), higher eGFR (mean 97.8 mL/min AA vs. 82.1 mL/min Caucasian, p<0.001), and higher systolic blood pressures (mean 134 mm Hg AA vs. 128 mm Hg Caucasian, p=0.07). There was no significant baseline difference in creatinine clearance. Both Caucasians and AA experienced reductions in kidney function following donation; however, no differences were observed in the rates of change in systolic blood pressure or any index of renal function between the two ethnic groups within the first six months after donation



Conclusion: Although AA in our study had higher pre-donation serum creatinine, eGFR, and systolic blood pressure values compared to Caucasians, the rates of change in renal function and blood pressure following kidney donation did not differ between ethnic groups. Although long term studies are needed, our study suggests that AA and Caucasians may experience similar short-term risks of donation.

Abstract# 48

Health Insurance Status and Living Kidney Donor Follow-Up: Is There a Safety Net? Eric M. Gibney,¹ Anne L. King,¹ Mona D. Doshi,² Chirag R.

Parikh,³ Amit X. Garg.⁴ *¹Nephrology, Hume-Lee Transplant Center, Virginia Commonwealth University, Richmond, VA; ²Division of Nephrology, Wayne State University, Detroit, MI; ³Nephrology and Clinical Epidemiology Research, Yale University and VAMC, West Haven, CT; ⁴London Kidney Clinical Research Unit, London Health Sciences Center, London, ON, Canada.*

Introduction: Living kidney donor follow-up is essential for long-term preventive health care. We sought information on health insurance status of U.S. living kidney donors, with particular attention to age, gender, and ethnicity, as previous studies noted that African Americans, young donors, and males were more likely to be placed on the kidney transplant waiting list following kidney donation. **Methods:** The UNOS/OPTN database was queried for associations among age at donation, race, gender, and health insurance status, and whether health insurance status was associated with likelihood of completing 6-month follow up. **Results:** Between 7/2004 and 9/2006, 10,021 kidney donors were studied. 1765 (17.6%) lacked health insurance at the time of kidney donation. Younger donors, (age 18-34: 26.2%, age 35-49: 15.2%, age 50-64: 11.2%, age >65: 3.8%; p<0.0001), males (19.5% vs. 16.3% for females; p<0.0001), and members of ethnic minorities (Caucasian: 13.4%, AA: 21%, Hispanic: 35.6%, Asian: 26.7%; p<0.0001) were less likely to have health insurance at donation. Rates of uninsured patients were particularly high in 18-34 year old Hispanic males (40%), all Hispanics (36%), 18-34 year old AA males (31%), and Asian males (34%). Uninsured patients were less likely to have a 6-month serum creatinine reported (p=0.03). **Conclusion:** Younger patients, ethnic minorities, and males are less likely to have health insurance when donating a kidney. Similar subgroups may be at higher risk of kidney failure following donation. Lack of health insurance is likely to affect follow-up care, even in the brief window when transplant programs cover follow-up. Fair health and insurance policies limiting disincentives to obtaining preventive care may avoid sequelae in donors at risk.

Abstract# 49

Psychosocial Follow-Up Practices for Living Kidney Donors (LKD) at US Transplant Centers. Nina Schroder,¹ Abdolreza Haririan,² Christopher Welsh,³ Matthew Cooper.⁴ ¹Department of Social Work; ²Medicine; ³Psychiatry; ⁴Surgery, University of Maryland Medical Center, Baltimore, MD.

Psychosocial and physical health of LKDs following donation is of utmost importance. Unfortunately, this issue has been neglected to a large extent. We sought to examine the current practices regarding psychosocial follow-up of LKDs after surgery across transplant (Tx) centers.

We conducted a 68-question online survey regarding this practice and issues related to LKD. The survey was e-mailed via listservs of 2 professional Tx societies. Several questions allowed for more than one response.

Characteristics of the 69 Tx centers that participated in the survey are found in Table 1.

Tx Center Characteristics			
Number per year	Number of Tx per year (% of centers)	Number of LKD Evaluations (% of centers)	Number of LKD Tx (% of centers)
≤ 50	21.9	43.9	63.4
51-100	37.5	22.8	22.2
101-200	23.4	19.3	11
200+	17.7	14	3.2

Only 38.3% actively follow donors regarding mental health, substance abuse, or quality of life issues after donation. The professionals most often involved are social workers (72%) and nurse coordinators (69%). The majority of follow-up is conducted via phone (83.3%); though appointments (69.4%) and questionnaires (11.1%) are also utilized. 65% initiate follow-up with donors 1-4 weeks post-operatively, whereas only 27% of programs maintain contact at both 3-6 months and 12 months.

83.3% offer post-operative psychosocial support; 62% only under certain circumstances. This support is provided by social workers (90%), psychologists (30%), and/or psychiatrists (28%). Support is available indefinitely in 68% of programs.

30.5% assume the costs of post-operative medical and psychosocial care indefinitely; 50.8% for a specified period of time. 40.7% bill the recipient's insurance and 15.2% bill the donor's insurance. 83.1% of centers accept donors without health insurance and only 5.1% purchase insurance on behalf of donors to cover post-operative health care needs.

The results of this survey clearly demonstrate that post-operative psychosocial follow-up of LKDs is uncommon and that current practices are widely variable. Without routine follow-up of donors, Tx centers are less likely to capture post-operative psychosocial issues that may result from organ donation. Standardized post-operative follow-up of LKDs should become a mandatory part of their care, which will require increased support from health care policy makers.

Abstract# 50

The Role of Hepatitis C and Race in Patient and Graft Survival in Combined Kidney and Liver Transplantation. Dilip Moonka,¹ Ravi K. Parasuraman,² Kim A. Brown,¹ Alissa Kapke,³ Dean Y. Kim.⁴ ¹Gastroenterology, Henry Ford Health Systems, Detroit, MI; ²Nephrology, Henry Ford Health Systems, Detroit, MI; ³Biostatistics, Henry Ford Health Systems, Detroit, MI; ⁴Transplant Institute, Henry Ford Health Systems, Detroit, MI.

The influence of hepatitis C (HCV) and race are not well understood in combined kidney-liver transplant (KLT). HCV has a negative impact on patient and graft survival in liver recipients whereas African-American (AA) race is a negative prognostic factor in kidney recipients.

AIM: To determine the influence of HCV and AA race on patient and graft survival in KLT.

METHODS: The UNOS Public Use Database was used to identify 2296 patients undergoing KLT who had known HCV Ab status. 13% were AA and 68% were non-Hispanic white (NHW). 39.5% were HCV Ab positive. Groups were assessed for patient and graft survival.

RESULTS: There is a significant gradient in patient survival and both kidney and liver survival from NHW patients without HCV to AA patients with HCV (Table). The patient survival at five years drops from 72% to 54% along this gradient. There is also a 14% drop in liver and an 18% drop in kidney graft survival.

Survival Estimates Stratified by Race and HCV Status				
Patient Survival	One Year	Three Year	Five Year	
AA/HCV pos	79%	66%	54%	p = 0.003
AA/HCV neg	81%	71%	65%	
NHW/HCV Pos	78%	66%	61%	
NHW/HCV Neg	82%	77%	72%	
Liver Graft Survival				
AA/HCV pos	76%	64%	54%	p = 0.003
AA/HCV neg	79%	70%	67%	
NHW/HCV Pos	74%	62%	56%	
NHW/HCV Neg	79%	73%	68%	
Kidney Graft Survival				
AA/HCV pos	76%	62%	49%	p = 0.014
AA/HCV neg	75%	67%	57%	
NHW/HCV Pos	76%	63%	55%	
NHW/HCV Neg	79%	72%	67%	

*Calculated from Log-rank test

On pairwise testing, the difference in patient survival at 5 yr between all patients without HCV and those HCV Ab positive drops from 72% to 60% (p=0.0003). The difference in 5 yr survival between all NHW and AA patients (regardless of HCV status) drops from 68% to 57% (p=0.221). On multivariate analysis, HCV and the combination of HCV and AA race remains associated with diminished survival but race alone does not.

CONCLUSIONS: Patients with HCV who undergo a KLT transplant are at increased risk for poor overall survival and poor survival of kidney and liver grafts. This effect appears even more pronounced in AA patients. This knowledge is critical to individual centers in assessing risk and benefit from KLT in these groups and these groups represent an opportunity for improved interventions.

Kidney: Pediatrics

Abstract# 51

A Phase II Exploratory Study To Evaluate the Safety of Induction Therapy with Campath-1H®, Combined with Chronic Immunosuppression with MMF and Sirolimus: A Study of the Cooperative Clinical Trials in Pediatric Transplantation. Ruth McDonald,¹ Jodi Smith,¹ Kevin Meyers,² Robert Mathias,³ Anthony Portale,⁴ David Ikle,⁵ Yvonne Morrison,⁶ Nancy Bridges,⁶ William Harmon.⁷ ¹University of Washington; ²CHOP; ³Nemours Children's Services; ⁴UCSF; ⁵PPD; ⁶NIH/NIAID; ⁷Children's Hospital Boston.

Background: Pediatric renal transplant recipients have excellent short-term outcomes but long-term success is compromised by complications of chronic immunosuppressive medications and chronic allograft nephropathy. Studies show that calcineurin inhibitors and steroids can be individually avoided in pediatric renal transplantation. Building on that experience we designed this study to optimize short and long-term renal allograft function with minimal chronic immunosuppression using a steroid-free, calcineurin-inhibitor withdrawal protocol in low risk pediatric renal transplant recipients.

Methods: Unsensitized pediatric recipients of a first living donor kidney transplant received 2 doses of Campath-1H® (0.3 mg/kg), 1 day pre- and post-transplant. Subjects received tacrolimus and MMF immediately post-transplant until week 8-12 when they underwent protocol renal biopsy and were changed to sirolimus and MMF if rejection free. The planned 35 subjects have been enrolled; this report describes the clinical outcomes of the 23 with 1 year of follow up.

Results: The mean subject age is 12.9 yrs; 59.3% are female and 70.4% Caucasian. At transplant, 16/23 were CMV seronegative and 16/23 were EBV seronegative. Protocol therapy was discontinued in eight subjects due to: rejection (3), mouth ulcers (2), leukopenia (1), unrelated (2). Clinical acute rejection (AR) occurred in 4 subjects (17%) and 2 had subclinical AR; AR was cellular rejection in 5 subjects, and humoral in 1 at 4 days post-transplant who had an undetected positive crossmatch to Class II HLA. There were two graft losses, one due to recurrent FSGS and one due to medication non-adherence. There were no cases of PTLN and no deaths. Leukopenia occurred in 11 subjects (47.8%). There were 9 infections of which 7 were urinary tract infections (34.7%) and 2 were pneumonia (8.7%).

Conclusions: Minimization of immunosuppression using a steroid-free, calcineurin-withdrawal protocol in low risk pediatric renal transplant recipients appears to be well tolerated with acceptable rates of clinical AR and no serious infections 12 months after transplantation.

Abstract# 52

One Year Results of a Prospective, Randomized, Multicenter Trial of Steroid Avoidance in Pediatric Renal Transplantation. Minnie Sarwal,¹ M. Benfield, R. Ettenger, V. Dharnidharka, R. Mathias, R. McDonald, W. Harmon, D. Kershaw, V. Vehaskari, E. Kamil, H. Baluarte, B. Warady, D. Ikle, N. Bridges, O. Salvatierra. ¹CCTPT (NIAID), NIH.

Background: Single center non-randomized results with steroid avoidance have shown patient and graft benefits.

Methods: 130 unsensitized, primary kidney recipients, 0-21 yrs of age, were enrolled from 12 US transplant programs (2004-2006), in a prospective 1:1 randomized multicenter study of steroid-free (SF) vs. steroid-based (SB) immunosuppression with matched demographics. 24.1 % of SF and 27.5% of SB were African Americans and 15.5% of SF vs. 8.7% of SB had ESRD from FSGS. SF patients received extended (6 mo) vs. standard (2 mo) Daclizumab induction in the SB group. Patients in both arms received tacrolimus and MMF maintenance. Protocol biopsies were performed at 0, 6, 12 and 24 mo, and for renal dysfunction. Primary end-points were differences for standardized height scores and biopsy proven acute rejection (BPAR) at 1 year.

Results at 1 year: 60 SF and 70 SB patients were enrolled; 10 SF and 16 SB were 0-5 yrs of age. Patient survival was 100% in both arms. Graft survival was similar (96.7% in SF vs. 98.6% in SB). Intent to treat median delta height SDS scores from baseline for different age groups were: 0.58 for SF and 0.48 for SB in the 0-5 yr old (p=0.80); 0.34 for SF and 0.34 for SB in the 6-12 yr old (p=0.86). BPAR was 23.3% for SF vs. 21.4% for SB (p=0.83) with 28.6% and 29.4% steroid-resistance respectively. 25% of SB rejections occurred within 3 months vs. none in SF. Mean calculated Schwartz GFR (ml/min/1.73m2) was 90.0 vs. 90.6 respectively for SF vs. SB for intent to treat; and 104.6 for SF and 109.3 for SB when stratified for absence of BPAR. Percent first hospitalizations for SF vs SB were: 48% vs. 60% at 6 mo, and 55% vs. 70% at 12 mo,

with no differences in bacterial, fungal or viral infections between groups. Neoplasms were noted in 0% SF vs. 6.3% SB (n=1 PTL; 2.3%) and PTDM in 0% SF vs. 4.5% SB (n=2). There were no significant differences for hypertension, hyperlipidemia, leukopenia, anemia, AEs or SAEs between groups.

Conclusions: Preliminary analysis does not reveal a growth advantage at 1 year in children receiving SF vs SB immunosuppression. There was no difference at 1 year in BPAR and graft survival, and the SF group did not demonstrate excess morbidity. Protocol biopsy analysis and continued monitoring is planned for both groups until 3 years post-transplantation.

Abstract# 53

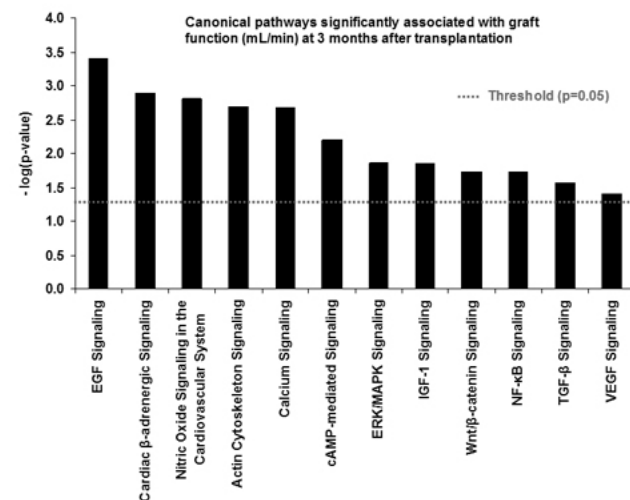
Physiological and Molecular Mechanisms Underlying the Functional Adaptation of Adult-Sized Kidneys Transplanted into Pediatric Recipients. Maarten Naesens,¹ Neeraja Kambham,¹ Oscar Salvatierra,¹ Li Li,¹ Minnie Sarwal.¹ ¹Stanford University, CA.

Background: The discrepancy between the vasculature size of pediatric renal allograft recipients and the adult-sized kidney grafts leads to renal hypoperfusion and decrease in absolute GFR, referred to as functional adaptation of these kidneys to the recipients' size. It was reported recently that this functional adaptation is not completely reversible in the smallest recipients and is associated with irreversible histological damage, but the underlying mechanisms remain unknown.

Methods: We selected 21 pediatric recipients (age 9.1±6.8 years; range 1.0-21) of an adult-sized kidney who did not have delayed graft function or rejection episodes. Protocol biopsies were obtained at implantation and at 3 months after transplantation and scored according to the Banff classification. Whole-genome expression profiles of the biopsies obtained at 3 months were assessed using Affymetrix cDNA microarrays.

Results: Despite the pristine condition of the kidneys at implantation and the absence of DGF or rejection episodes, renal allograft function at 3 months after transplantation was highly variable, with an absolute creatinine clearance (cCr_{cl}) of 63±23 mL/min (range 31.2-106). cCr_{cl} was lowest in the smallest recipients (p<0.0001). Using quantitative SAM (FDR <5%), 724 unique genes correlated with cCr_{cl}. Ingenuity canonical pathway analysis identified significant overrepresentation of relevant and overlapping pathways (fig.1; all p<0.05) involved in regulation of intracellular Ca²⁺ and in cAMP signaling, important for the regulation of renal vascular tone and blood flow, as well as multiple growth factor signaling pathways. Expression of these overlapping genes correlated significantly with cCr_{cl} at 3 months (all q-values <0.05).

Conclusion: The adaptation of adult-sized kidney grafts to the pediatric recipients' size is not only an automatic, passive physiological consequence of low renal blood flow, but is associated with gene expression alterations likely involved in the regulation of renal vascular resistance and GFR autoregulation, which could add to the irreversible tubulointerstitial injury observed in the smallest recipients.



Abstract# 54

Evaluation of Modification to OPTN/UNOS Policy on Pediatric Priority for Kidneys from Deceased Donors under Age 35. J. Smith,¹ W. Cherikh,² J. Magee,³ W. Harmon,⁴ S. Sweet,⁵ P. Healey,¹ S. Bartosh,⁶ R. McDonald.¹ ¹U. of Washington; ²UNOS; ³U. of Michigan; ⁴Children's Hospital Boston; ⁵Washington University; ⁶University of Wisconsin, Madison.

Background: On 9/28/05 a modification to OPTN/UNOS policy regarding deceased donor (DD) kidney allocation was implemented which provided pediatric candidates relative priority for kidneys from standard criteria donors < 35 yrs; after zero ABDR mismatched (MM), PRA ≥ 80%, combined kidney/non-renal organ candidates, but before kidney paybacks. The intent was to allocate donor kidneys better suited to children to address established goals of rapid transplantation.

Methods: Ongoing analysis has been performed using the OPTN database specifically evaluating age at TX, time to TX, donor source, sensitization status, ethnicity, HLA and DR matching, and blood group both prior to and following implementation of Share 35 (9/04-9/07).

Results: Comparing numbers of DD and LD TXs performed in the 12 months pre and post policy, there are 59% more DD TXs and 23% fewer LD TXs. Following a "bolus effect" in the first two 6 month periods post policy, there appears to be stabilization of the number of DD TXs with 252 DD TXs in the most recent period (3/07 to 9/07), vs. 190 in the 6 months pre policy. The total number of TXs (LD + DD) done 6 months pre policy compared to the most recent 6 month period is unchanged (429 vs. 424). There was no significant difference in the number and % of adults receiving DD TXs pre vs. post policy. Wait time decreased post policy with 60% of patients being transplanted within 6 months of listing (vs. 40%). The probability of being transplanted within 12 months of listing increased for all categories of sensitized patients. Post policy, only 51% (vs. 44%) of nonsensitized children awaiting DD TX are transplanted within 12 months of listing. There was no significant difference in patient and graft survival in the first year after policy implementation despite increasing TXs with higher HLA mismatch.

Conclusion: The number and % of DD TXs in children has increased post-policy with a decrease in LD TXs and no overall increase in TX numbers. Although wait times have decreased, only 51% of unsensitized children are being transplanted within 12 months of listing. Preliminary analysis shows no significant difference in patient and graft survival in the first year after the policy implementation, however, the impact on longer term graft survival and degree of sensitization has yet to be determined.

Abstract# 55

Pediatric Living Donor Kidney Transplantation Using Alemtuzumab Pre-Conditioning and Tacrolimus Monotherapy. Henkie P. Tan,¹ Demetrius Ellis,¹ Michael L. Moritz,¹ Amit Basu,¹ Abhay N. Vats,¹ Elif Erkan,¹ Joseph Donaldson,¹ Amadeo Marcos,¹ Gerri James,¹ Diane Connors,¹ Corde McFeaters,¹ Mary Grosso,¹ Thomas E. Starzl,¹ Ron Shapiro.¹ ¹UPMC.

Introduction: We have previously reported that Alemtuzumab (Campath-1H) pre-conditioning with tacrolimus monotherapy provides effective immunosuppression for solid organ transplantation. We extend our study to a 4-year experience of recipient pre-transplant lymphoid depletion and minimal post-transplant steroid-free immunosuppression in pediatric living donor renal transplantation.

Methods: Alemtuzumab pre-conditioning was used in 35 consecutive unselected living donor renal transplant recipients between January 14th 2004 and November 7th 2007. Donor kidneys were removed laparoscopically (LLDN). Post-operatively, patients were treated with steroid-free tacrolimus monotherapy, and immunosuppression was weaned when possible. The mean follow up was 21.5±14.5 mos.

Results: The mean donor and recipient ages were 34.5 ± 7.7 and 8.3 ± 6.0 years, respectively. The mean HLA mismatch was 2.6 ± 1.1. LLDN was associated with 0% delayed graft function (DGF). The mean creatinine (mg/dL) at 1, 2, 3-yr was 0.89 ± 0.44 (n=24), 0.87±0.37 (n=15), and 0.86±0.48 (n=7), respectively. The mean GFR (mL/min/1.73m²) at 1, 2, 3-yr was 69.2±38.1, 76.1±36.1, and 85.4±64.5, respectively. Patient survival at 1, 2, and 3-yr was 100%, 95%, and 95%, respectively. Graft survival at 1, 2, and 3-yr was 100%, 89.7%, and 89.7%, respectively. The loss of 2 renal allografts was related to vascular torsion of an intraperitoneal allograft 645 days after transplantation and a patient death unrelated to the transplant. The cumulative incidence of acute cellular rejection (ACR) at 1, 2, and 3 yrs was 2.9% (day 22, preweaning), 2.9%, and 5.7% (day 834, postweaning), respectively. Weaning was attempted in 46% of recipients. At follow-up, 12 (35%) of children were tapered to spaced tacrolimus monotherapy, and 88.2% (n=30) were steroid free and on tacrolimus monotherapy. There was no tissue invasive cytomegalovirus (CMV) infection, no post-transplant lymphoproliferative disease, and no BK viral nephropathy. There was no donor mortality or morbidity.

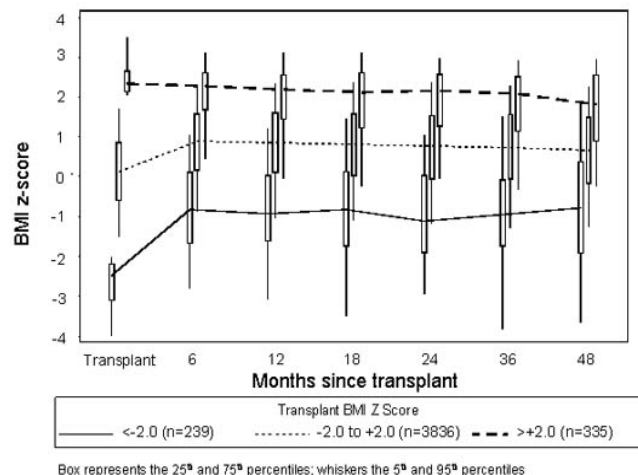
Conclusion: This study represents the largest series to date of unselected live donor pediatric kidney transplant recipients receiving alemtuzumab preconditioning, and confirms the mid-term safety and efficacy of this approach. LLDN is not a risk factor for DGF or ACR in pediatric kidney transplant recipients.

Abstract# 56

Natural History of Weight Changes and Risk Factors for Unhealthy Weight Gain in Pediatric Renal Transplantation: Analysis of the North American Pediatric Renal Trials & Collaborative Studies Registry (NAPRTCS). Bethany J. Foster,¹ Karen Martz,² Manjula Gowrishankar,³ Donald Stablein,² Amira Al-Uzri.⁴ ¹Dept. of Pediatrics, McGill University, Montreal, QC, Canada; ²EMMES Corporation, Rockville, MD; ³Dept. of Pediatrics, University of Alberta, Edmonton, AB, Canada; ⁴Dept. of Pediatrics, Oregon Health & Sciences University, Portland, OR.

Weight gain resulting in overweight (body mass index for age z-score (BMIz) >1.04) is common after pediatric renal transplantation (Tx). We aimed to describe the natural history of changes in BMIz after renal Tx, and to identify risk factors for unhealthy weight gain (defined as BMIz >1.04 and >1 unit above baseline BMIz) at 12 mo., and for persistence of unhealthy gain at 48 mo. Using the NAPRTCS registry, we performed a retrospective cohort study of children (2 to 18 y.) who received a Tx between 1995 and 2006. BMI data were collected every 6 months. Stepwise logistic regression was used to identify risk factors (including sex, age, race, baseline BMIz, donor source, transplant year, and primary diagnosis). BMIz was recorded at baseline in 4410 children (59%

male; 57% white). Substantial increases in BMIz were observed within the first 6 mo.; no changes were seen from 6 to 48 mo. Baseline BMIz category (<-2.0, -2.0 to +2.0, >+2.0) influenced the pattern of change. Subjects with low BMIz (<-2.0) at baseline experienced the greatest increases in BMIz, but overweight was rare; increases tended to result in a BMIz in the healthy range. Those with high BMIz (>+2.0) at baseline demonstrated no significant change in BMIz post-Tx.



Younger age at Tx (highest risk for those 2 to 5 y. at Tx) more remote date of Tx, and baseline BMI between the 25th and 75th percentiles were significant independent risk factors for unhealthy weight gain both at 12 mo. and persisting at 48 mo. post-Tx. Weight gains occur early after Tx and tend to persist. Counseling focused on prevention of weight gain should be a routine part of post-Tx care, with the most intense efforts concentrated on the highest risk patients: young, healthy weight children. Avoidance of early weight gains may have an important impact on BMI over the long term.

Abstract# 57

High Rate of Graft Loss in Pediatric Kidney Recipients Who Transition to an Adult Transplant Program; Roles of Non-Adherence and Late Referral. Lindsey A. Pote,¹ Jennifer Trofe,¹ Erin H. Wade,¹ Jorge Baluarte,³ Alden Doyle,² Simin Goral,² Karen Warburton,² Robert Grossman,² Jo Ann Palmer,³ Roy D. Bloom.² ¹Pharmacy, Hosp of the Univ of Penn; ²Nephrology, Univ of Penn; ³Transplant Surgery, Children's Hospital of Philadelphia.

Intro: Few data exist on outcomes in pediatric kidney recipients who transition to an adult transplant program. **Purpose:** To examine outcomes in pediatric kidney recipients who transition to an adult transplant program and to identify characteristics associated with non-adherence related graft loss. **Methods:** Retrospective, single center analysis of 41 pediatric kidney recipients who transitioned to an adult program. **Results:** For the cohort overall, transition to the adult program occurred a mean of 82 ± 8.7 months following transplantation. Mean serum creatinine at transition was 2.6 ± 3.2 mg/dL and mean patient age 21.2 ± 0.3 years. 61% of patients received living donor kidneys. Within 31 ± 3.5 months of transition, 13 (31.5%) of patients experienced graft loss. Causes of graft loss included admitted non-adherence (n=6), recurrent disease (n=3), chronic progressive graft dysfunction (n=3) and BK nephropathy (n=1). Graft loss occurred a mean of 16 ± 7 months post transition for non-adherent patients and 20.5 ± 3 months for adherent patients (P=ns). The characteristics of the cohort is shown in the table according to whether or not patients had documented non-adherence related graft loss. **Conclusions:** 1) Graft loss commonly occurs within 3 years following transition and is attributable to both patient non-adherence and late referral by the pediatric transplant program, 2) Non-adherence related graft loss was more common in males, 3) Factors unassociated with non-adherence include ethnicity, prior transplantation, age at transplant, & duration of dialysis, 4) The development of collaborative pediatric-to-adult transition clinics may enhance adherence and lead to improved graft outcomes in this population.

Characteristics of transitioning patients according to documentation of non-adherence

	Non-adherent (n=6)	Adherent (n=35)	P value (NS=not significant)
African-American	67%	29%	NS
Male	100%	63%	0.025
Living donor	50%	62%	NS
Taking tacrolimus at time of transition	50%	57%	NS
Prior transplant	33%	14%	NS
Mean age at transplant (yrs)	13 ± 2.2	15 ± 0.7	NS
Mean age at transition (yrs)	21 ± 0.7	21 ± 0.3	NS
Time from transplant to transition (months)	96 ± 22	79 ± 9	NS
Duration of dialysis pre-transplant (months)	23 ± 15	30 ± 8	NS

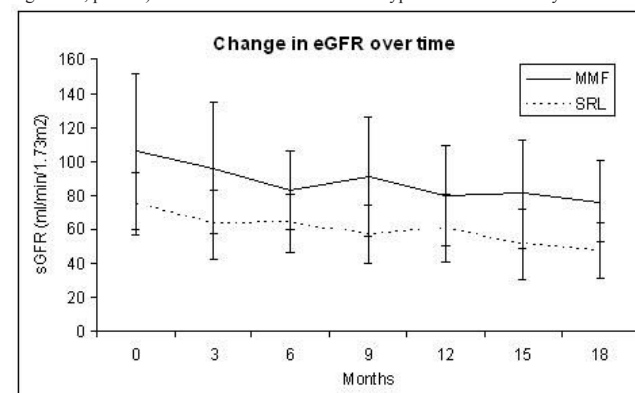
Abstract# 58

Treatment of CAN with Sirolimus vs. MMF and Low-Dose Tacrolimus in Pediatric Renal Transplants. 2-Year Safety Analysis. Tom Blydt-Hansen,¹ Patricia Birk.¹ ¹University of Manitoba, Winnipeg, MB, Canada.

This 2-year, prospective randomized pilot study compares the effect of conversion to sirolimus (SRL) vs. continued mycophenolate mofetil (MMF) in patients with chronic allograft nephropathy (CAN), on histological progression. We present 2-year data on safety and renal function.

Participants >1 year post-transplant with CAN (Banff ≥ci1, ct1) on tacrolimus, MMF and prednisone were randomized to continue MMF or convert to SRL (target 8-12 ng/ml). TAC dose was minimized (target 3-5 g/L), and renal biopsy performed at baseline, 1, 2 years. 3-month interval monitoring included adverse event (AE) reporting, eGFR (Schwartz) and immunosuppressant levels.

16/20 (MMF=10, SRL=10) have completed 2-year follow-up. Baseline gender, ethnicity, previous acute rejection episodes, CAN grade, proteinuria (uPCR) were similar. SRL had lower baseline eGFR (79 ± 17 vs. 109 ± 49 ml/min/1.73m², p=0.22). 285 AEs were reported (MMF=117, SRL=168), 43 serious AE (SAE: MMF=25, SRL=28). Common SAEs were similar: dehydration & elevated creatinine (30), gastroenteritis (10) and rejection (AR). 2 episodes (1 patient) of AR occurred in MMF group and 5 (2 patients) in SRL group, all attributable to non-adherence. There were no sustained change in electrolytes, Hg and total WBC counts in the 1st 2 year, except neutrophil counts were lower in the SRL group (24 mo: mean 4.7 vs. 2.8, p<0.05). Platelets were significantly lower at 3 months (SRL) but not thereafter (24 month: mean 271 ± 71 vs. 196 ± 61 x10⁹). Cholesterol and TG increased early (p<0.05), but only cholesterol persisted after 2 years (mean 5.2 vs. 3.7 mmol/L, p<0.05). In SRL group only, uPCR increased over 2 years (ΔuPCR 94 ± 87 mg/mmol, p<0.05). This was not associated with hypoalbuminemia at 2 years.



eGFR was lower in SRL vs. MMF after 18 months (p<0.01), but the rate of change in eGFR from baseline was similar between groups (-28 ± 23 vs. -30 ± 39 ml/min/1.73m², p=NS).

Serious adverse events did not differ significantly between the SRL and MMF groups. Sustained SRL treatment was associated with mild neutropenia, hypercholesterolemia and proteinuria after 2 years. eGFR was reduced at baseline compared with MMF, and both groups had similar rates of decline in GFR over time.

Abstract# 59

Maturation of Tacrolimus Pharmacokinetics in Pediatric Kidney Allograft Recipients. Maarten Naesens,¹ Oscar Salvatierra,¹ Li Li,¹ Minnie Sarwal.¹ ¹Department of Pediatrics, Stanford University School of Medicine, Stanford, CA.

Background: In contrast to adult kidney recipients, little is known about the long-term evolution of tacrolimus pharmacokinetics in pediatric kidney transplant recipients.

Methods: One-hundred five pediatric recipients of a kidney allograft, all treated with a corticosteroid-free immunosuppressive protocol, were included. The evolution of tacrolimus doses and exposure was recorded at 3, 6, 9, 12, 18 and 24 months after transplantation, as well as all pre-dose trough levels (C₀; N=9376) obtained in the first 2 years after transplantation.

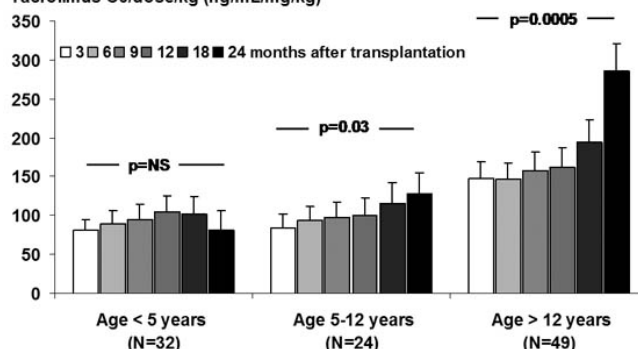
Results: Dose-corrected tacrolimus exposure (C₀/dose/kg) increased in the first 2 years after kidney transplantation in pediatric recipients (table 1, figure 1). This decrease in dose requirement by time was only significant in children older than 5 years at the time of transplantation (figure 1). In addition, the younger patients had significantly higher dose requirements compared to older recipients, which translated in marked underexposure in 72-79% of patients <12 years of age in the first days after transplantation.

Conclusion: Pediatric kidney transplant recipients exhibit maturation of tacrolimus pharmacokinetics with time after transplantation. This can not be explained by differences in corticosteroid use, as all patients were treated with a corticosteroid-free protocol. The higher dose requirements for younger recipients and the absence of tacrolimus maturation in the youngest recipients suggest that age-dependent changes in tacrolimus intestinal first-pass effect, metabolism or distribution play a role. Whether age-specific tacrolimus dosing algorithms will improve outcome needs further study.

Determinants of dose-corrected pre-dose trough levels (C0/dose/kg)

Parameters	Univariate	Multivariate full model	Multivariate after backward elimination
Time after transplantation	0.0001	0.0236	0.0167
Creatinine clearance	0.1128	0.9401	
Age	<0.0001	0.0001	<0.0001
Gender	NS		
Donor gender	NS		
Corticosteroid use	0.0321	0.1379	
Sirolimus use	NS		
Hematocrit	NS		
Race	0.1389	0.1490	

Tacrolimus C0/dose/kg (ng/mL/mg/kg)



Liver: Hepatitis

Abstract# 60

Autoimmune Hepatitis (AIH) Presenting as Acute Liver Failure (ALF): Distinguishing Clinical and Histologic Features. R. Todd Stravitz,¹ Jay H. Lefkowitz,² Richard K. Sterling,¹ William M. Lee,³ The ALF Study Group. ¹Virginia Commonwealth University; ²Columbia University; ³University of Texas-Southwestern.

AIH may present as acute hepatitis in 25% of patients (pts) and result in ALF. Early administration of corticosteroids may obviate the need for liver transplantation (LT), but features which identify AIH in pts with ALF have not been determined. AIM: To identify clinical and histologic features which distinguish ALF due to AIH. METHODS: 197/1033 pts in the ALF Study Group Registry had no evidence of viral, metabolic, vascular, and drug/toxic liver injury. All had ALF defined by acute disease, encephalopathy, and coagulopathy. Based upon admission clinical features, 52/197 had probable AIH, and 145 were considered "indeterminate." Liver biopsies (LBx) available from 56 pts were reviewed by a blinded expert hepatopathologist. Clinicopathologic correlations were analyzed retrospectively. RESULTS: All LBx available from pts with suspected AIH had classical histologic features of AIH. Moreover, 23/46 (50%) LBx from pts with indeterminate ALF also had AIH features: extensive necrosis (100%), fibrosis (84%), cirrhosis (22%), interface hepatitis (75%), and plasma cell-rich inflammation (69%). Of all 33 LBx with AIH features, centrilobular lesions associated with acute, severe AIH (Am J Surg Pathol 2004;28:471) were frequent: plasma cell-rich central venulitis (97%), exclusive centrilobular necroinflammation (19%), and pericentral dilatation/congestion (46%). Clinical characteristics and outcomes of the 75 pts with AIH based upon laboratory and histologic findings differed significantly from pts whose ALF remained indeterminate: AIH pts were older (44 v 38 y), predominantly female (75 v 56%), and had longer jaundice-encephalopathy interval (20 v 12 d), lower ALT (660 v 1949 U/L), higher globulins (3.7 v 2.7 g/dL), lower creatinine (1.7 v 2.3 mg/dL), and a higher prevalence of ANA (68 v 24%) and ASMA (51 v 29%) (all P<.01). Although spontaneous survival did not differ, more AIH pts underwent LT (63 v 34%), and more survived 1 month after enrollment (75 v 59%; P<.0001). CONCLUSIONS: Using histologic criteria to classify pts with indeterminate ALF, AIH accounted for at least 7% of the ALF Study Group registry, and represented 50% of indeterminate ALF. LBx should be performed in all patients with ALF of obscure etiology, in particular to identify centrilobular necroinflammatory lesions, which appear to be specific indicators of acute and severe AIH. (U-01 58369 from NIDDK).

Abstract# 61

Results of the Spanish Experience in HIV-Infected Patients Treated with Orthotopic Liver Transplantation: A Prospective Cohort of 89 Cases (2002-2006). Mikel Gastaca,¹ Miguel Montejo,¹ Lluís Castells,² Antonio Rafecas,³ Antonio Rimola,⁴ Ramon Barcena,⁵ Federico Pulido,⁶ Magdalena Salcedo,⁷ Martin Prieto,⁸ Manuel de la Mata,⁹ Jose R. Fernandez,¹ Jose M. Miro,⁴ The Spanish LT in HIV-Infected Patients Working Group. ¹Hospital de Cruces, Bilbao, Spain; ²Hospital Vall d'Hebron, Barcelona, Spain; ³Hospital Vellbige-IDIBELL, Barcelona, Spain; ⁴Hospital Clinic-IDIBAPS, Univ. of Barcelona, Barcelona, Spain; ⁵Hospital Ramon y Cajal, Madrid, Spain; ⁶Hospital 12 de Octubre, Madrid, Spain; ⁷Hospital Gregorio Marañón, Madrid, Spain; ⁸Hospital La Fe, Valencia, Spain; ⁹Hospital Univ. Reina Sofía, Cordoba, Spain.

Background and Aim: We report on the preliminary results of the prospective multicenter Spanish study in HIV-1 infected patients who underwent OLT.

Methods: The prospective multicenter Spanish study FIPSE-OLT-HIV 05-GESIDA 45-05 was initiated in January 2002. Inclusion criteria follows the rules previously described in the Spanish Consensus Document. HIV-infected patients transplanted between January 2002 and December 2006 were included in this study.

Results: 89 OLT were consecutively performed in 85 HIV-1 infected patients in the period of the study. Median (IQR) follow-up is 16 (8-29) months. Median (IQR) age was 42 years (39-46), 74% of the recipients were male and former drug abuse was the most common HIV-1 risk factor (78%). 83% of the patients were transplanted due to HCV-related cirrhosis and 6% due to HBV cirrhosis. Median (IQR) MELD score was 14 (10-19). Pre-OLT median (IQR) CD4 cell count was 288 (165-486) cells/mm³ and 64% patients had undetectable plasma HIV viral load. Immunosuppression was based on tacrolimus in 70% of the patients. HAART was re-started in a median (IQR) time of 10 (5-21) days after OLT and was based on efavirenz in 48% of the cases. Acute rejection occurred in 38 patients (43%). Twenty patients died (22.5%) mainly due to HCV recurrence (8%). Antiviral therapy with peg-interferon plus ribavirin was initiated in 18 patients obtaining a sustained viral response in 6 of them (33%). Patient survival (95% confidence intervals) at 1, 2, 3 and 4 years was 90% (81-95%), 74% (60-84%), 67% (52-79%) and 67% (52-79%), respectively.

Conclusion: For selected HIV-1 infected patients under HAART therapy, OLT is a safe and effective procedure at mid-term. As in the HIV-negative population, HCV recurrence is the mayor cause of concern and response to antiviral therapy is still disappointing.

Abstract# 62

Hepatitis C Virus and Liver Transplant: 12-Year Experience Treating Chronic Hepatitis after Liver Transplantation. Raquel S. B. Stucchi,¹ Ilka F. S. F. Boin,¹ Isabel W. Pereira,¹ Marília I. Leonardi.¹ ¹Unit of Liver Transplantation, State University of Campinas, Campinas, São Paulo, Brazil.

Objectives: Describe the 12-year experience of a University Hospital treating chronic hepatitis C relapse, histologically diagnosed after liver transplant. **Material & Methods:** From September 1991 through July 2006, all the patients subject to liver transplant with histological diagnostics of chronic hepatitis relapse were submitted to antiviral treatment. Treatment was suspended if there was a severe adverse reaction to the drugs used, non-adherence, severe rejection or no response after at least 12 months. HCV positive serology patients, ALT increase (1.5x nl) and hepatic biopsy with Metavir rating showing structural 1 and/or periseptal or parenchymatous portal inflammatory activity /= 2 were included. From 1995 to 2003, all patients were treated with conventional interferon and ribavirin, regardless of the genotype. From October 2003 on, patients with genotype 1 were treated with ribavirin and pegylated interferon and genotype 3 were treated with conventional interferon and ribavirin. **Results:** 47 patients were treated during this time period, thirty-seven male (78.7%). Their average age was 48. 51.1% were genotype 1, 25.5% were genotype 3. Average time between transplant and beginning of treatment was 30 months. Average treatment was 16 (4-36) months. 74.5% received conventional interferon, 6.4%, pegylated alpha 2a and 19.1%, pegylated alpha 2b. 48.9% had one or more cellular rejection episodes before VHC relapse diagnostics and were treated with corticosteroids. Sustained virological response (SVR) occurred in 48.8%. Three patients had a virological response at the end of treatment (EVR) and have not completed six months post treatment. Out of 21 patients with SVR, 20.9% were genotype 3, 9.3% genotype 1 and 18.6% were not genotyped. Considering genotypes, SVR was detected in 16.7% with genotype 1, in 75% with genotype 3. SRV was detected when we examined hepatic biopsy, in 85.7% of the F1/F2 patients (18 out of 21), 9.5% of the F3 patients (2), 4.8% of the F4 patients (1). Survival period of 5 years for SVR patients was 94% (20 out of 21) and 68.2% for patient without SVR (p = 0.03 - Kaplan-Meier). Five-year survival was 79.2% for patients with genotype 1 and 100% for patients with genotype 3. **Conclusions:** Our results show it is possible to get good five-year survival rates for treated patients. However, handling adverse reactions and long term treatment still pose difficulties in pursuing better SVR rates.

Abstract# 63

The Impact of Alcoholic Liver Disease (ALD) and Hepatitis C Virus (HCV) Infection on Liver Waitlist and Post-Transplant Mortality, and Transplant Survival Benefit. M. R. Lucey,¹ D. E. Schaubel,² M. K. Guidinger,³ S. Tome,⁴ R. M. Merion.⁵ ¹*Dept of Medicine, University of Wisconsin, Madison, WI;* ²*Dept of Biostatistics, University of Michigan, Ann Arbor, MI;* ³*Scientific Registry of Transplant Recipients (SRTR), Arbor Research Collaborative for Health, Ann Arbor, MI;* ⁴*Dept of Internal Medicine, Universitario de Santiago, Santiago, Spain;* ⁵*Dept of Surgery, University of Michigan, Ann Arbor, MI.*

Background: Although liver transplant survival benefit has been shown to be associated with MELD score, disease-specific analyses have not been reported. We evaluated, using SRTR data, the effect of ALD and/or HCV infection on transplant waitlist and post-transplant mortality, and survival benefit of deceased donor liver transplants. **Methods:** 38,889 patients age ≥ 18 , who were listed between Sept 2001 and Dec 2006, and followed until Dec 31, 2006, were classified into 4 cells according to HCV or ALD status: HCV+, ALD-: 12,322; HCV+, ALD+: 4,220; HCV-, ALD+: 6,581; HCV-, ALD-: 15,776. Cox regression was used to estimate waiting list mortality and post-transplant mortality separately. Survival benefit, which encompasses both pre- and post-transplant events, was assessed using sequential stratification; an extension of Cox regression which matches transplant recipients by MELD and organ procurement organization to patients on active on the waitlist at the time of transplant. **Results:** HCV significantly ($p=0.0001$) increased waitlist mortality, with a covariate-adjusted hazard ratio (HR) for HCV+ vs. HCV- of HR=1.19 ($p=0.0001$). The impact of HCV+ was greater among ALD+ candidates (HR=1.36; $p<0.0001$), but was also significant among ALD- candidates (HR=1.11; $p=0.02$). The contrast between ALD+ and ALD- waitlist mortality was only significant among HCV+ candidates (HR=1.14; $p=0.006$). Post-transplant mortality was significantly higher among HCV+ vs. HCV- recipients (HR=1.26; $p=0.009$); there was no difference between ALD+ vs. ALD- recipients. Survival benefit of liver transplantation was significantly lower among HCV+ compared to HCV- recipients with MELD 9-29, but significantly higher for HCV+ recipients with MELD scores of ≥ 30 . A diagnosis of ALD did not influence the survival benefit of transplantation at any MELD score. **Conclusion:** Despite higher waitlist mortality, HCV+ recipients had significantly lower liver transplant survival benefit than HCV- recipients, within categories defined by MELD score. In contrast, transplant survival benefit was not influenced by ALD.

Abstract# 64

One-Year Extended Anti-HBV Vaccination with an MPL-Adjuvanted Vaccine Combined with Anti-HBs Immunoglobulins in Patients Liver Transplanted for HBV-Related Cirrhosis. Giuseppe Tisone,¹ Daniele Di Paolo,¹ Ilaria Lenci,¹ Laura Taricciotti,¹ Andrea Monaco,¹ Manuele Berlanda,¹ Linda De Luca,¹ Giuseppe Iaria,¹ Alessandro Anselmo,¹ Irene Bellini,¹ Mario Angelico.¹ ¹*Hepatic Surgery and Transplantation, University of Rome tor Vergata, Rome, Italy.*

Background and Aim: Post-transplant active immunization with HBsAg vaccine is a potential prophylaxis strategy against HBV-recurrence after liver transplantation due to HBV-related disease. Previous studies showed conflicting results using standard vaccines, whereas the use of the new adjuvant 3-deacylated monophosphoryl-lipid-A (MPL) significantly increased patient's immunization rate. We investigated the efficacy of a long-term (12 months) accelerated (monthly doses) vaccination schedule using the MPL-adjuvanted vaccine administered with and without concomitant HBIG. **Methods:** 18 patients (M/F: 13/5) transplanted for HBV-related cirrhosis 73 $\pm$ 38 months earlier were recruited. All were HBsAg and HBV DNA negative in serum and cccDNA negative in liver tissue; 5 (27.7%) were co-infected with HCV and 5 (27.7%) with HDV. Study protocol consisted of 12 consecutive monthly intramuscular vaccine doses (HBsAg 20mg plus MPL 50mg) given together with lamivudine (100 mg/daily). Each of the initial 6 doses (first cycle) was administered within 7 days after 2000 IU HBIG i.v. infusion, while the last 6 doses were given after complete HBIG withdrawal (second cycle). HBsAb titre was determined before each vaccine dose and during the follow-up. All patients were maintained on low-level immunosuppression. **Preliminary results:** all patients completed first vaccination cycle; 14 (77.7%) patients received 12 adjuvanted vaccine doses (first and second cycles) and were monitored during 3 months follow-up after vaccination end. No side effects occurred, nor evidence of HBV recurrence. At the end of first cycle all patients achieved an anti-HBs titre >100 IU/L (mean 343 $\pm$ 209 IU/L) and 3 (16.6%) a titre greater than 500 IU/L. At the end of follow-up 8/14 (57.1%) and 4/14 (28.5%) had an anti-HBs titre greater than 100 (mean 411 $\pm$ 599 IU/L) and 500 IU/L, respectively. **Conclusions:** nine months after HBIG withdrawal more than half of the patients reached and maintained a protective anti-HBs titre (>100 IU/L). This intensive schedule using the MPL-adjuvanted vaccine, given in combination with HBIG and lamivudine, seems to be more effective than previous HBV vaccination protocols, although a longer follow-up is needed to assess its final effectiveness.

Abstract# 65

Heterologous Immunity Via Alloimmune Responses to Hepatitis C Virus Replication after Liver Transplantation. Hideki Ohdan,¹ Masahiro Ohira,¹ Yuka Tanaka,¹ Kohei Ishiyama,¹ Nobuhiko Hiraga,² Michio Imamura,² Kazuaki Chayama,² Toshimasa Asahara.¹ ¹*Surgery, Hiroshima University, Hiroshima, Japan;* ²*Internal Medicine, Hiroshima University, Hiroshima, Japan.*

The immunosuppressive environment in liver transplantation (LT) recipients infected with HCV is believed to be associated with the progression of HCV reinfection. In the present study, we simultaneously monitored HCV levels and alloimmune status in HCV-infected LT recipients. For evaluating the immune status of 33 HCV-infected LT recipients, we employed a mixed lymphocyte reaction (MLR) assay using a CFSE-labeling technique. The kinetics of the stimulation index of anti-donor reactive CD4⁺ T cells clearly mirrored that of the HCV RNA titer, and a significant reverse correlation was observed between the 2 parameters ($R^2 = 0.67$). We did not observe a similar relationship between the stimulation index of an anti-third party CD4⁺ T cells and the HCV RNA titer ($R^2 = 0.03$). One possible explanation for this phenomenon might be that cytokines outputted by T cells responding to allostimulation display the anti-HCV activity either directly or indirectly through the activation of bystander immunocytes. To investigate such possibilities, we performed a transwell culture assay comprising a MLR culture in the upper chamber and genomic HCV replicon cell culture in the lower chamber to mimic the anatomical features of the interaction between HCV-infected hepatocytes and alloreactive T cells that infiltrate the portal area in the liver. When one-way MLR was performed using one-half identical combination in the upper chamber, HCV replication was significantly suppressed in the lower chamber containing HCV replicon cells. HCV replication was further suppressed when MLR was carried out with a complete allogeneic combination. This inhibiting effect was dependent on their IFN- γ secreting activity. In addition, a similar result was obtained when IFN- γ -secreting NK/NKT cells stimulated with IL-2 were cultured in the upper chamber. In conclusion, there was a close relationship between the anti-donor and the anti-HCV immune status in the LT recipients infected with HCV. Cytokines such as IL-2 and IFN- γ may be produced in response to allostimulation, and even these cytokines do not cause graft rejection, display anti-HCV activity. The elucidation of such a possible heterologous immunity via alloimmune responses to HCV replication might lead to the establishment of a novel method to prevent the progression of HCV reinfection in HCV-infected LT recipients.

Abstract# 66

Hepatitis C E2 Region Diversity after Liver Transplantation: A Report from the Hepatitis C III Trial. Juan F. Gallegos-Orozco,¹ Hugo E. Vargas,¹ Georges Netto,² Gary L. Davis,³ Devin E. Eckhoff,⁴ Goran B. Klintmalm.³ ¹*Mayo Clinic Arizona, Scottsdale, AZ;* ²*Johns Hopkins Medical Institutions, Baltimore, MD;* ³*Baylor University Medical Center, Dallas, TX;* ⁴*University of Alabama at Birmingham, Birmingham, AL.*

Purpose: Hepatitis C III Trial is a multicenter, randomized study to assess safety and efficacy of a steroid-free immunosuppressive (IS) protocol (daclizumab, tacrolimus and MMF) in patients transplanted for HCV-related disease. We hypothesize that HCV quasispecies behavior after liver transplantation (LT) is influenced by IS regimen and can predict histological outcome.

Methods: Patients with minimum 90-day follow-up and available pre-LT, 90-day and 1-year serum samples were studied. Hypervariable region 1 of the HCV E2 gene was amplified by RT-PCR. Quasispecies diversity was analyzed by heteroduplex mobility assay (HMA). Viral diversity was defined using Shannon entropy and median mobility shift (MMS). Genetic diversity before, 90 days and 1 year after LT were correlated to liver biopsy. Histologic recurrence was defined as inflammation grade ≥ 3 and/or fibrosis stage ≥ 2 (Batts-Ludwig score).

Results: 312 patients were enrolled. HMA analysis was performed in 96 patients (42 in the steroid-free group). Day 90 and 1-year protocol liver biopsies were available in 95 and 91 patients, respectively. Recurrence was present in 14 patients (15%) at 90-day biopsy and in 57 patients (63%) at 1-year biopsy. Rates of recurrence were not significantly different in the steroid-free and steroid-treated groups: 19% vs. 11% at 90 days ($p=0.4$) and 66% vs. 60% at 1 year ($p=0.7$). No significant change in viral diversity was seen regardless of IS regimen. There were no significant changes in viral diversity when comparisons were made between patients with and without histologic recurrence at 1 year. A subanalysis of 21 patients with complete quasispecies and histological data at 2 years revealed that viral populations were less diverse among patients with recurrence at 2 years compared to recurrence-free recipients ($p<0.05$).

Conclusions: The current findings do not support the proposed hypothesis that steroid-free IS has an impact on viral quasispecies behavior. The role of viral diversity on histologic recurrence is unclear and may be reflective of other factors, such as donor age, degree of steatosis, and other factors that also contribute to development of recurrent HCV.

Abstract# 67**Inoculation with IL-2/OKT3-Treated Lymphocytes Extracted from Liver Allograft Perfusate Elicits an Anti-HCV Response after Liver Transplantation.** Masahiro Ohira,¹ Hideki Ohdan,¹ Kohei Ishiyama,¹ Kazuaki Chayama,¹ Toshimasa Asahara.¹ ¹*Hiroshima University, Japan.*

Following liver transplantation (LT) in HCV-infected patients, the viral load exceeds the pretransplant levels. The immunosuppressive environment is thought to play a major role in the rapid progression of recurrent HCV infection following LT. We recently demonstrated that administration of activated donor liver-derived lymphocytes has the potential to promote the anti-HCC activity after LT. In this study, we demonstrate that this treatment also elicits an anti-HCV response in LT recipients infected with HCV even in an immunosuppressive environment. The therapy comprised intravenous injection of IL-2-stimulated lymphocytes extracted from the perfusate of donor liver grafts into recipients at 3 days after LT ($2-5 \times 10^6$ /subject). We added OKT3 in the culture medium for 1 day prior to inoculation to prevent GVHD. Seven HCV-infected LT recipients were treated with this immune therapy, while another 7 recipients with similar backgrounds served as untreated controls. During the first month after LT before the initiation of IFN- α therapy, the HCV RNA titers in the sera of the recipients who underwent the immune therapy were markedly lower than those of the untreated recipients ($p < 0.05$). In order to determine the cell type responsible for the anti-HCV activity, the IL-2/OKT3-treated lymphocytes extracted from liver allograft were separated into fractions of NK cells, NKT cells, and T cells, and all the cells were cocultured with genomic HCV replicon cells. Among these fractions, the NKT cell fraction displayed the strongest inhibitory effects on viral replication. Omission of exposure to either IL-2 or OKT3 resulted in a reduction in this anti-HCV activity. The anti-HCV effect depended on the IFN- γ -secreting activity of the cells. We also verified that OKT3 binding to the NKT cells significantly promoted their IFN- γ -secreting activity. The findings indicate that IFN- γ -secreting NKT cells play a pivotal role in the anti-HCV response induced by IL-2/OKT3-treated liver allograft lymphocytes. Consistently, the number of IFN- γ -secreting cells in the peripheral blood was remarkably increased in the recipients treated with the immune therapy compared to the untreated recipients (The percentage of IFN- γ -secreting cells was 25.8 ± 7.1 and 4.0 ± 0.3 on day 14, respectively; $p < 0.05$). Further elucidation of the mechanisms underlying this phenomenon would result in a novel concept to prevent the progression of HCV reinfection after LT.

Abstract# 68**Gene Expression Patterns in Acute Cellular Rejection and Hepatitis C Recurrence Post-Liver Transplantation.** Valeria R. Mas, Daniel G. Maluf, Kellie J. Archer, Kenneth Yanek, Shannon Levesque, Xiangron Kong, Todd R. Stravitz, Robert A. Fisher. *Transplant Surgery, VCUHS, Richmond, VA.*

Background. Recurrent hepatitis C (HCVrec) and acute cellular rejection (ACR) share common histological features. The differential diagnosis is important for targeting treatment. We aimed to characterize molecular insight into the pathophysiology of ACR and HCVrec.

Samples and methods. Liver tissue from patients with histological diagnosis of ACR by Banff criteria (N=11) and HCVrec (N=12) were evaluated using microarrays. Immunosuppression was similar in both groups. For each probe set, the one-sided Wilcoxon rank test was used to detect up or down regulated in a determined sample set condition. To identify differentially expressed genes simultaneously while controlling FDR, the qvalue method was used to adjust the p-values from the one-sided Wilcoxon rank tests. Microarray results were confirmed using qRT-real time PCR. Differential expression profiles were analyzed using Interaction Networks and Functional Analysis.

Results. We examined differences in gene expression occurring in ACR when compared to HCVrec. When examining the raw p-values from the Wilcoxon rank tests, 221 probe sets were statistically differentially expressed between groups ($p < 0.01$). From the analysis of the over expressed genes in ACR liver tissues we identified genes related to immune response (i.e., IL6R, CX3CL1), inflammatory response (i.e., TNFAIP1, KNG1), apoptosis (i.e., FAM8, TIAF1, MAP3K5), and complement (i.e., C8G, CFHR2). The expression of TCRalpha was also up regulated in ACR liver biopsies as well as genes related to growth factor receptor signaling (i.e., TGFBR3, IGF2R, EGFR) and genes involved in protein modification process and ubiquitin cycle (i.e., UBE2B, USP47). The down-regulated differentially expressed in ACR when compared with HCVrec were classified as related to cell cycle and cell division (i.e., CCND2, JUMB), cell proliferation (i.e., S100A6, SPON2, SEMA4D), and blood coagulation (i.e., TFP11, EFEMP2, FBN1). Using Interaction networks and Functional Analysis the top molecular and biological functions for the down-regulated genes were classified as cell growth and proliferation (p -value= $3.4E-06$ - $1.6E-2$), cellular development (p -value= $5.8E-06$ - $1.4E-02$), and cell cycle (p -value= $3.7E-06$ - $1.6E-02$).

Conclusions. We identified a characteristic gene expression signatures associated with post-Ltx ACR and HCVrec. These gene signatures may conduct to biomarkers identification to increase diagnostic accuracy.

Non-Organ Specific: Disparities in Outcome and Access to Healthcare**Abstract# 69****Obesity, Access to Transplantation, and Survival Benefit.** Dorry L. Segev,¹ Lauren M. Kucirka,¹ Robert A. Montgomery.¹ ¹*Johns Hopkins Medical Institutions.*

Background: 18.1% of new adult dialysis patients are obese (BMI 30-35), 8.9% are severely obese (BMI 35-40), and 7.3% are morbidly obese (BMI 40-60). The goal of this study was to quantify the effect of BMI on access to transplantation (ATT), likelihood of receiving a transplantation (LRT), turnaround rates (TR), and survival benefit (SB).

Methods: ATT was defined as either registering for the deceased donor waiting list or receiving a live donor transplant, and was analyzed based on the USRDS registry using logistic regression. LRT was based on time spent in active status on the waiting list before receiving a transplant, and was analyzed based on the UNOS waiting list dataset using Cox proportional hazards time-to-event analysis. TR was defined as the relative likelihood of being turned down for an organ offer by a provider other than the patient, and was analyzed based on the UNOS organ turnaround dataset using negative binomial regression. SB was defined as survival after kidney transplantation versus survival on the deceased donor waiting list, and was analyzed based on the UNOS waiting list dataset using a time-dependent Cox survival benefit model. All models were adjusted for known factors influencing those outcomes.

Effect of BMI on Access to and Benefit of Transplantation

	BMI 30-35	BMI 35-40	BMI 40-60
Access to Transplantation (OR)	1.19 (1.16-1.23)	0.90 (0.87-0.93)	0.48 (0.46-0.50)
Likelihood of Receiving a Transplant (HR)	0.93 (0.90-0.97)	0.72 (0.68-0.77)	0.56 (0.50-0.62)
Turndown Rate (IRR)	1.05 (1.02-1.08)	1.11 (1.07-1.14)	1.22 (1.13-1.32)
Survival Benefit (HR)	1.67 (1.49-1.87)	1.42 (1.21-1.66)	1.36 (1.15-1.62)

* $p < 0.001$ for all cells

Results: Of 132,353 patients listed between 2000 and 2005, obese patients had significantly lower ATT. Even once listed, obese patients experienced much longer waiting times (lower LRT) and higher TR, despite a statistically significant survival benefit from transplantation. These effects were most pronounced in morbidly obese patients, who had a 36% benefit from transplantation but were 52% less likely to be listed, 44% less likely to be transplanted once listed, and 22% more likely to be turned down for an organ offer.

Conclusions: Although obese patients benefit significantly from transplantation, their ability to receive a kidney transplant, both in terms of access to the waiting list and waiting time once listed, is significantly compromised.

Abstract# 70**Laparoscopic Bariatric Surgery Is Well-Tolerated and Improves Candidacy in Morbidly Obese Patients Awaiting Transplantation.**

Michael Frederick,¹ Mehdi Tavakoli,¹ Mark Takata,¹ Charlotte Rabl,¹ Guilherme Campos,¹ John Cello,² Stanley Rogers,¹ Nancy Ascher,¹ Andrew Posselt.¹ ¹*Transplant Surgery, University of California San Francisco, San Francisco, CA;* ²*Medicine, University of California San Francisco, San Francisco, CA.*

Background: Patients with morbid obesity are often considered unsuitable for transplantation due to a higher risk for perioperative morbidity and mortality. We evaluated the safety and efficacy of laparoscopic Roux-en-Y gastric bypass (LGBP) in patients with end stage renal disease (ESRD) and laparoscopic sleeve gastrectomy (LSG) in patients with cirrhosis or end stage lung disease (ESLD), and examined whether these procedures could help patients become better candidates for transplantation.

Methods: Retrospective review of selected patients with end stage organ failure not eligible for transplantation due to morbid obesity who underwent LGBP or LSG. Prospectively collected data included demographics, operative details, complications, percentage excess weight loss (EWL), post-operative laboratory data, and status of transplant candidacy.

Results: Seven patients with ESRD underwent LGBP, 6 patients with cirrhosis and 2 with ESLD underwent LSG.

Follow-up, Weight Loss and Transplant Candidacy

	ESRD (n=7)	Cirrhosis (n=6)	ESLD (n=2)
Procedure	LGBP	LSG	LSG
Mean Follow-up (months)	15.4 (range 3-24)	9 (range 3-18)	12.5 (12, 13)
Patients > 9 months since operation (n)	6/7	4/6	2/2
Mean % EWL at > 9 months	61 (range 41-75)	33 (range 24-40)	62 (50, 73)
Transplant candidate at > 3 months	7/7	5/6	2/2
Underwent transplant	0/7	1/6	1/2

Complications developed in two patients (both with cirrhosis) and there was no mortality. Mean follow-up was 12.3 months, and mean EWL at 9 months or later was 61% (ESRD), 33% (cirrhosis) and 61.5% (ESLD). Obesity associated comorbidities were improved or resolved in all patients. Serum albumin and other nutritional parameters 9 months or later after surgery were similar to preoperative levels in all 3 groups. At most recent follow-up, 14 out of 15 patients (82%) have reached our institution's body mass index (BMI) limit for transplantation and are awaiting transplant; one patient with ESLD has undergone a successful lung transplant and one patient with cirrhosis has undergone a successful liver transplant.

Conclusions: This pilot study provides evidence that LGBP in patients with ESRD and LSG in patients with cirrhosis or ESRD is safe, well-tolerated, and improves candidacy for transplantation.

Abstract# 71

Barriers to Transplantation of HIV-Positive Kidney Transplant Candidates. Deirdre Sawinski,¹ Christina Wyatt,¹ Lisette Casagrande,¹ Paul Myoung,¹ Inci Bijan,¹ Enver Akalin,¹ Bernd Schroppel,¹ Marla Keller,¹ Shirish Huprikar,¹ Graciella de Boccardo,¹ Vinita Sehgal,¹ Rajani Dinavahi,¹ Susan Lerner,¹ Scott Ames,¹ Jonathan Bromberg,¹ Barbara Murphy.¹ ¹Mount Sinai School of Medicine, NY, NY.

With improved survival in the era of antiretroviral therapy (ART), HIV patients can be safely transplanted. The disproportionate burden of HIV-related ESRD in minority populations may impose additional obstacles to successful completion of the transplant evaluation. We retrospectively reviewed 344 HIV patients evaluated for kidney transplant at our institution since 2001. Results. Thirty-five patients were excluded for medical reasons, including active or recent malignancy (n=9), anticipated failure to achieve viral suppression (n=6), no antiretroviral therapy (n=5), recent opportunistic infection or tuberculosis (n=4), severe comorbidity (n=4), active substance abuse (n=3), and history of noncompliance (n=2). Of the 309 patients remaining, the mean age was 46 +/- 9 years, 75% were black, and 72% were male. At the time of initial evaluation, the median CD4 was 379, and the HIV viral load was undetectable in nearly two-thirds. 90% of patients were on ART. Patients who did not meet HIV disease-specific criteria at the initial evaluation were encouraged to re-evaluate their ART regimen with the referring HIV provider. Only 63/309 patients (18%) have been listed, compared to 2130/3463 (62%) HIV-negative patients evaluated at our institution (p<0.00001). Characteristics associated with failure to complete the transplant evaluation included CD4<200, detectable viral load, and family history of kidney disease. History of illicit drug use and black race demonstrated non-significant trends. Hepatitis virus co-infection (43% of patients), marital status, education level, and time on dialysis did not significantly impact completion. In multivariate analysis, factors independently associated with failure to complete the transplant evaluation included CD4 < 200 at initial evaluation (OR 18.12, 95% CI 2.29-143.43), black race (OR 2.51, 95% CI 1.12-5.62), history of illicit drug use (OR 2.58, 95% CI 1.20-5.53), and family history of kidney disease (OR 3.88, 95% CI 1.35-11.18). **Conclusions.** Control of HIV infection at the time of initial evaluation predicts successful completion, and along with a history of illicit drug use may be a surrogate for compliance. As previously observed in the general transplant population, racial disparities to transplantation may be particularly significant in HIV patients.

Abstract# 72

Access to Liver Transplantation in the MELD Era: Role of Ethnicity and Insurance. Nyingi Kemmer,^{1,2} Tiffany E. Kaiser,¹ Victoria Zacharias,¹ Guy W. Neff.¹ ¹Digestive Disease, University of Cincinnati, Cincinnati, OH; ²Cincinnati VA Medical Center.

Factors that contribute to inequitable access to the transplantation network (UNOS/OPTN) include socioeconomic status, geographic location and delayed referral. The goal of the MELD allocation system was to assign priority to the sickest candidate and assure equitable access to liver transplantation (LT). The MELD has been validated as a reliable marker for liver disease severity. At the time of referral to the transplant center or listing a high MELD (HM) is an indirect measure of delayed referral. Therefore, the aim of this study is to identify factors associated with a HM at the time of listing for LT. **Method:** Using the UNOS database, we identified all adult candidates listed for LT from 2002 to 2006. Patients who received MELD upgrade (i.e. HCC, FHF) and those listed for multi-organ transplant were excluded. The data collected included demographics, insurance payor, diagnosis, and MELD score. MELD score at time of listing was categorized as low (≤ 20) and high (>20) and insurance type as Private, Medicaid and Government (excluding Medicaid). **Results:** During the study period 15,323 candidates were added to the LT waiting list. Of these, there were 10,302 (67.2%) males with a median age of 53 (range 18 - 83). Caucasians were 11,511 (75.1%), Hispanic (11.6%), African Americans (8.9%) and the rest (4.4%). The underlying liver disease was Hepatitis C (31.9%), Alcohol (15.1%), Alcohol/Hepatitis C (7.2%), PBC/PSC (9.9%), Cryptogenic (7.5%) and others (28.4%). Age, gender and liver disease etiology were not associated with a HM at listing. A HM was associated with Ethnicity: AA (49.5%), Hispanic (44.2%), Caucasian (36.6%)[p<0.001] and Insurance type: Medicaid (46.3%), government (38.8%) and private (36.5%)[p<0.001]. There was no strong interaction between ethnicity/race and insurance in combination as predictors of HM i.e. both were independent. **Conclusion:** (1) AA and LT candidates with Medicaid insurance are more likely to have a HM score at initial listing (2) Hispanics had the lowest rate of private insurance compared to Caucasians with the highest rate. The above results suggest that type of insurance and ethnicity are independently associated with a HM (i.e. sicker patients) at listing. Since a HM score is associated with increased mortality, implementation of strategies that result in timely and equitable access to the transplantation network regardless of the insurance type or ethnicity of the candidate(s) should be a priority.

Abstract# 73

Marked Increase in the Use of Inactive Status on the Kidney Transplant Waiting List. Kim Nguyen,¹ Valerie Ashby,^{1,2} John Kalbfleisch,^{1,2} Sangeetha Krishnan,^{1,2} Panduranga Rao,^{1,2} Alan Leichtman.^{1,2} ¹University of Michigan; ²SRTR, Ann Arbor, MI.

Under prior OPTN policy, US waitlisted (WL) kidney (KI) transplant (Tx) candidates accrued waiting time only during their first 30 days in Status 7 (Inactive Status, S7); further wait time accrued only after active status was restored. On 11/5/03, the OPTN implemented a change in policy that provides for the accrual of waiting time during the entire interval that WL candidates are designated as S7.

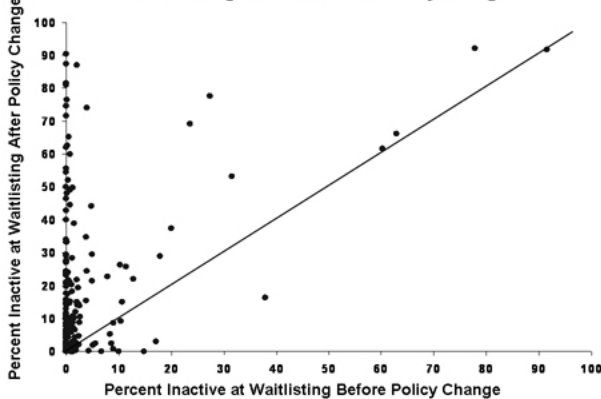
Methods: We investigated the impact of this policy change on the patterns of S7 designation, using the SRTR/OPTN database. Initial and subsequent status on the WL were determined for 130,986 candidates placed on the KI Tx WL from 11/5/00-11/4/06. The probability of becoming active (including receiving a living donor Tx) on the WL was calculated for those who were S7 at listing before and after the policy change. **Results:** Table 1 shows trends in the use of S7 before and after the policy change. Of the 1,396 candidates listed as S7 before the policy change, 76% became active within 6 months and 83% within 1 year of WL. For the 11,248 candidates listed as S7 after the policy change, the corresponding figures are 48% and 60% respectively. Figure 1 demonstrates that, since the implementation of the new policy, the % of patients initially WL in S7 at most US Tx centers has increased.

Table 1: Inactive Status by Listing Date

LISTING DATE	Candidates	INACTIVE STATUS					
		Never Inactive*		Inactive at Listing		Inactive after Listing*	
		N	%	N	%	N	%
11/5/00-11/4/01	18669	13364	71.6	448	2.4	4857	26.0
11/5/01-11/4/02	19312	13592	70.4	492	2.5	5228	27.1
11/5/02-11/4/03	20475	13908	67.9	456	2.2	6111	29.8
11/5/03-11/4/04	21877	13758	62.9	2153	9.8	5966	27.3
11/5/04-11/4/05	24668	15452	62.6	3616	14.7	5600	22.7
11/5/05-11/4/06	25985	15980	61.5	5479	21.1	4526	17.4

*As of 11/1/07

Figure 1: Percent of Candidates in Transplant Center Inactive at Waitlisting Before and After Policy Change



Conclusion: Since the implementation of this policy, the number and % of pts WL as S7 at most KI Tx centers, and the duration of S7 for those initially WL as S7 has increased dramatically. The implications of this practice on access to KI Tx and survival warrants further investigation.

Abstract# 74

Can Donor Race Dictate Graft Outcomes in African American Cadaveric Kidney Transplant Recipients? Benjamin T. Duhart, Jr.,¹ Samir J. Patel,² Amy G. Krauss,¹ Maung Mya,¹ William Bastnagel,¹ James Eason.¹ ¹University of Tennessee/Methodist University Hospital Transplant Institute, Memphis, TN.

Previous literature indicates that African American and Caucasian cadaveric kidney transplant recipients have similar short term graft outcomes, but differences in long term graft outcomes continue to exist. The purpose of the study was to determine transplant characteristics and evaluate graft outcomes of African American recipients with either African American or Caucasian donors. **Methods:** This was a retrospective analysis of 231 kidney recipients that were transplanted between January 2000 and April 2005. All patients included in this study received Thymoglobulin induction during this time period. Maintenance immunosuppression consisted of calcineurin- or sirolimus-containing regimens. Ninety-nine per cent of patients continued steroid maintenance immunosuppression after transplant. Univariate analyses were performed to determine significant transplant characteristics and differentiate graft outcomes between African American recipients with African American or Caucasian cadaveric donors. **Results:** The mean length of follow-up was 3.1 years. Of the recipients (n=150) included in the study, 109 (73%) were recipients of Caucasian donor transplants, 41 (27%) were recipients of African American donor transplants, 92 (61%) patients were male, 16

(11%) patients were retransplanted, and 13 (9%) patients had panel reactive antibody >30. The mean cumulative Thymoglobulin dose administered was 5.7 mg/kg, and the primary maintenance immunosuppression started prior to discharge was a sirolimus-containing regimen (59%). At the end of the follow-up period, 98 (65%) patients had functioning grafts and 52 (35%) patients experienced graft loss. Of the 52 patients with graft loss, 15 (10%) patients experienced graft loss secondary to death. No pertinent differences were identified between groups. The graft survivals for African American recipients with African American or Caucasian donors were 95% vs. 91% at 1 year and 83% vs. 72% at year 2. Conclusions: Our study results suggest that donor race does not adversely affect graft outcomes in African American cadaveric kidney recipients with modern immunosuppression. Donor racial differences may not play a primary role in the inferior graft outcomes of African American cadaveric kidney recipients.

Abstract# 75

Cardiac Evaluation before Kidney Transplantation: Are We Screening Too Often or Not Enough? Krista L. Lentine,¹ M. A. Schnitzler,¹ J. J. Snyder,² D. C. Brennan,³ P. Hauptman,¹ P. R. Salvalaggio,¹ B. Kasiske.⁴ ¹Saint Louis University Center for Outcomes Research; ²MN Medical Research Fdn; ³Washington University; ⁴U Minnesota.

Evaluation for ischemic heart disease (IHD) is a common but non-standardized practice before kidney transplant. We retrospectively studied pre-transplant cardiac evaluation (CE) practices among a national sample of renal allograft recipients.

Methods: We examined USRDS data for Medicare beneficiaries transplanted in 1991-2004 with Part A and B benefits from dialysis initiation through transplant. Clinical traits defining "high" expected IHD risk were defined as diabetes, prior IHD, or ≥2 other coronary disease risk factors. Pre-transplant CE were identified by billing claims for non-invasive stress tests and angiography. We quantified individuals with claims for coronary revascularization procedures between CE and transplant, and abstracted post-transplant acute myocardial infarction (AMI) events from claims and death records.

Results: Among 27,786 eligible patients, 46.3% (65.4% of high-risk and 20.4% of lower-risk) underwent CE before transplant. Overall, 9.5% of patients who received CE also received pre-transplant revascularization, including only 0.3% of lower-risk patients studied by CE (Table A). The adjusted odds of transplant without CE (higher OR for no CE, Table B) increased sharply with younger age and shorter dialysis duration. Increased likelihood of transplant without CE also correlated with black race, female sex, and certain geographic regions.

A. Pre-transplant Revascularization Practices Following CE

Most recent CE by Test Type & Risk Group	Angioplasty and/or Stent Number (%)	Coronary Artery Bypass Grafting Number (%)	Any Revascularization Number (%)	Number of CE per Revascularization
CE of any form				
Full cohort (N=12,876)	705 (5.5)	557 (4.3)	1,228 (9.5)	10.5
High risk (N=10,482)	701 (6.7)	554 (5.3)	1,221 (11.6)	8.6
Lower risk (N=2,394)	4 (0.2)	3 (0.1)	7 (0.3)	342.0
Noninvasive Stress Test				
Full cohort (N=11,341)	254 (2.3)	174 (1.5)	411 (3.6)	27.6
High risk (N=9,120)	251 (2.7)	173 (1.9)	407 (4.5)	22.4
Lower risk (N=2,221)	3 (0.1)	1 (0.1)	4 (0.2)	555.3
Coronary angiography				
Full cohort (N=5,355)	697 (12.4)	531 (9.9)	1172 (21.8)	4.6
High risk (N=4,567)	654 (13.4)	529 (10.7)	1187 (23.3)	4.2
Lower risk (N=828)	3 (0.7)	2 (0.5)	5 (1.2)	65.6

B. Adjusted Correlates of Transplantation Without CE by Multivariable Logistic Regression

Characteristic	Full Cohort (N=27,786) Adjusted Odds Ratio (95% CI)	High Risk (N=16,028) Adjusted Odds Ratio (95% CI)	Lower Risk (N=11,757) Adjusted Odds Ratio (95% CI)
Age			
18-30 yrs	0.84 (0.11-7.65)	3.24 (2.80-3.78)	19.89 (16.43-24.07)
31-44 yrs	2.89 (2.64-3.16)	1.81 (1.63-2.02)	7.18 (6.11-8.43)
45-60 yrs	1.77 (1.62-1.93)	1.50 (1.35-1.66)	2.64 (2.25-3.11)
≥60 yrs	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
Female gender	1.12 (1.00-1.19)	1.08 (1.00-1.17)	1.34 (1.20-1.49)
Race			
Black	1.39 (1.20-1.48)	1.41 (1.20-1.64)	1.20 (1.00-1.36)
White	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
Other	1.33 (1.16-1.52)	1.31 (1.10-1.57)	1.00 (Reference)
BMI at transplant			
Underweight	1.36 (1.15-1.61)	1.00 (Reference)	1.66 (1.24-2.22)
Normal weight, overweight or obese	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
Cause of ESRD			
Diabetes	0.48 (0.43-0.54)	0.52 (0.46-0.58)	-
Hypertension	0.74 (0.59-0.81)	0.79 (0.71-0.88)	0.75 (0.67-0.85)
Other causes	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
Pre-transplant dialysis duration			
1-12 months	8.15 (7.18-9.24)	8.00 (7.74-9.48)	8.14 (6.64-9.99)
13-24 months	3.48 (3.10-3.92)	3.43 (2.92-4.04)	3.54 (2.94-4.26)
25-60 months	1.92 (1.72-2.16)	1.82 (1.55-2.13)	2.02 (1.71-2.41)
>60 months	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
Diabetes mellitus	0.61 (0.55-0.67)	0.82 (0.73-0.92)	-
Ischemic heart disease	0.21 (0.19-0.23)	0.23 (0.21-0.25)	-
Coronary heart failure	0.55 (0.51-0.59)	0.64 (0.59-0.69)	0.35 (0.30-0.41)
Cerebrovascular disease	0.82 (0.72-0.94)	0.87 (0.76-1.00)	0.96 (0.80-0.87)
Peripheral vascular disease	0.76 (0.71-0.81)	0.77 (0.72-0.84)	0.74 (0.62-0.87)
Dyslipidemia	0.77 (0.67-0.88)	-	-

OR, odds ratio. OR >1.0 (<1.0) indicates increased (decreased) likelihood of transplant without CE, compared to the reference. Regional data not shown due to space constraints.

Post-transplant AMI rates in patients transplanted without CE allow assessment of whether CE was appropriately deferred in those who indeed face low IHD-risk after transplant. Among patients transplanted without CE, the 3-yr incidence of post-transplant AMI was 3% and 10% in lower and high-clinical risk groups, respectively, but varied by clinical traits within these groups. In lower-risk patients transplanted without CE, blacks patients faced increased AMI-risk compared to whites (adjusted HR 1.52, 95% CI 1.16-2.00).

Conclusions: Observed CE practices demonstrate a low yield of pre-transplant revascularization but also raise concern for socio-demographic barriers to evaluation access.

Abstract# 76

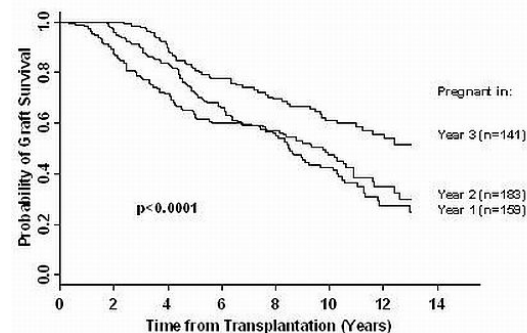
Women Who Become Pregnant in the First Two Years after Kidney Transplantation Have a Higher Risk of Graft Loss. Nadia Zaluendo,¹ Olwyn Johnston,¹ Caren L. Rose,¹ John S. Gill.¹ ¹Division of Nephrology, University of British Columbia, Vancouver, BC, Canada.

Existing information regarding the risks of pregnancy is derived from voluntary data sources. Using Medicare Claims files from the USRDS (1990-2003), we examined pregnancies (defined by presence of an inpatient ICD-9 billing code for pregnancy or pregnancy-related complication) in the first 3 years after kidney transplantation (KTX) among women aged 15-45 years whose primary insurance payor was Medicare (N=16,195). Patients were followed until graft failure, death or December 31, 2003.

There were 530 pregnancies identified in 483 women during the first 3 post-transplant years. Women who became pregnant during the 1st or 2nd post-transplant year had a shorter time to graft loss than women who became pregnant during the 3rd year (figure).

To minimize the survivor bias among women who became pregnant during the 3rd post-transplant year, we determined the association between the timing of pregnancy and graft survival among the subset of women (n=455) who had graft survival of at least two years using a Cox multivariate regression adjusted for age, race, cause of ESRD, donor source, calendar year of transplant, dialysis vintage, maintenance immunosuppression, and GFR at 6 months after KTX. Pregnancy in the 1st year (HR 2.01, 95% CI 1.35-2.99), and second year (HR 1.79, 95% CI 1.23 to 2.62) was associated with an increased risk of graft loss compared to pregnancy during the third post-transplant year.

We concluded that women who are able to wait should be counseled to become pregnant after the second post transplant year.



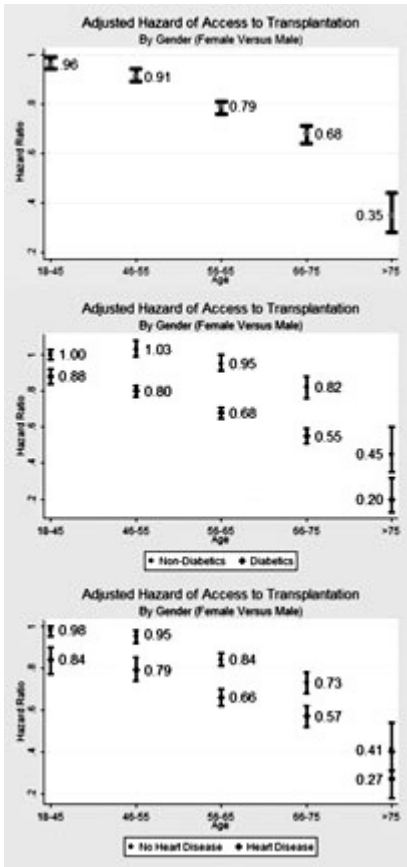
Abstract# 77

Gender Disparities in Access to Transplantation Are Worsened by Age and Comorbidities. Lauren M. Kucirka,¹ Robert A. Montgomery,¹ Dorry L. Segev.¹ ¹Johns Hopkins Medical Institutions.

Background: Multiple studies have associated female gender with decreased access to transplantation (ATT). However, little is known about the underlying causes of this disparity. Our goal in this study was to better characterize the relationship between gender, ATT, and post-transplant survival benefit. We hypothesized that older age and comorbidities might further decrease access for women.

Methods: We analyzed 563,197 adult patients with ESRD as captured in USRDS between 2000 and 2005. ATT was defined as either registering for the deceased donor waiting list or receiving a live donor transplant. Survival benefit was defined as relative survival after transplantation versus dialysis. Cox proportional hazards models were adjusted for age, gender, ethnicity, BMI, comorbidities, type of insurance, and primary cause of ESRD, and smoking. All models were stratified by age category with or without comorbidity.

(Continued on Page 200)



Results: Women aged 18-45 had equivalent ATT compared to men of the same age (HR = 0.96, $p < 0.001$). With each decade of age, ATT for women was further decreased, with women over 65 having 32% less ATT compared to men of the same age (Figure 1, top panel). We further subdivided the groups by comorbidity. In every age group, the presence of diabetes (Figure 1, middle panel) or heart disease (Figure 1, lower panel) significantly worsened ATT for women of that age group with the specified comorbidity, when compared to their male counterparts. However, post-transplant survival benefit did not significantly differ between men and women in any of the analyses.

Conclusions: Older age and the presence of comorbidities both play significant roles in gender disparities for ATT. We theorize that at some level (patient or provider) elderly women and women with comorbidities are perceived as more frail and less able to tolerate a surgical procedure than their male counterparts, and thus suffer from decreased access. These perceptions may not be accurate, as post-transplant survival benefit is similar for men and women regardless of age or comorbidities that we studied. A better understanding of the underlying causes of this disparity is needed so that interventions can be developed to increase ATT for women.

Pancreas Transplantation

Abstract# 78

Experience with Extended Donors and Recipients in Simultaneous Kidney-Pancreas Transplantation. Robert J. Stratta,¹ Alan C. Farney,¹ Jeffrey Rogers,¹ Rajinder P. Singh,¹ Erica L. Hartmann,² Amber Reeves-Daniel,² Michael D. Gautreaux,¹ Samy S. Iskandar,³ Patricia L. Adams.²
¹General Surgery; ²Internal Medicine; ³Pathology, Wake Forest Univ., Winston-Salem, NC.

The aging donor and recipient populations have led to new challenges in simultaneous kidney-pancreas transplantation (SKPT). The purpose of this study was to retrospectively review our single center experience in SKPT with respect to extended (EX) donor (D) and recipient (R) criteria. **Methods:** Over a 65 month period, we performed 83 SKPTs with enteric drainage (79 portal venous drainage). EX Ds were defined as age < 10 ($n=4$), > 45 ($n=12$, mean age 50.2 yrs), or donation after cardiac death (DCD, $n=4$). All DCD donors were managed with extracorporeal support. EX Rs were defined as age > 50 ($n=20$, mean age 55.8 yrs) or those with a pretransplant serum C-peptide level > 2.0 ng/ml ($n=7$, mean 5.7 ng/ml). All Rs received depleting antibody induction (57 rATG, 26 alemtuzumab) with tacrolimus, MMF, and tapered steroids (39 steroid-free). **Results:** A total of 20 Ds (24%) and 23 Rs (28%) met the above EX criteria. Median waiting time was 10 months, mean pancreas preservation was 17 hours, and median length of stay was 10 days. With a mean follow-up (f/u) of 28 months, patient (pt) (95% EX D vs 94% non-EX D), kidney (90% EX D vs 89% non-EX D) and pancreas

graft survival (85% EX D vs 81% non-EX D) rates were similar between D groups (all $p=NS$). The incidences of delayed kidney graft function (5% in each group) and early pancreas graft loss due to thrombosis (5% EX D vs 8% non-EX D) were also comparable between D groups. With regard to R groups, pt (87% vs 97%, $p=0.13$) and kidney (87% vs 92%, $p=NS$) graft survival (GS) rates were slightly lower in the EX R group compared to the non-EX R group, respectively. However, death-censored kidney GS rates (100% EX R vs 95% non-EX R) were comparable between groups. Uncensored pancreas GS rates (83% EX R vs 82% non-EX R) were similar. The incidences of acute rejection, surgical complications, infection, and other morbidity were comparable regardless of D or R group. At 1 year (or latest) follow-up, renal and pancreas functional parameters were similar between D groups. However, the EX R group demonstrated slightly compromised renal and pancreas allograft function and a greater need for oral hypoglycemic agents.

Conclusion: Intermediate-term outcomes in SKPT from selected EX Ds or Rs have comparable outcomes, although EX R criteria may represent a risk factor for pt survival and functional outcomes.

Abstract# 79

12 Month Pancreas Graft Function Significantly Influences Kidney Graft and Patient Survival Following Simultaneous Pancreas-Kidney (SPK) Transplantation. Andrew S. Weiss,¹ Gerard Smits,² Alexander C. Wiseman.¹
¹Division of Renal Diseases and Hypertension, University of Colorado Health Sciences Center, Denver, CO; ²CSC, Inc, Santa Barbara, CA.

Background: Simultaneous pancreas-kidney transplantation (SPK) is regarded as the treatment of choice for type 1 diabetes (DM1) and kidney dysfunction. However, the morbidity associated with pancreas transplant leads many patients to select kidney alone (KA) rather than SPK. The aim of this study was to quantify the impact of early pancreas graft failure (within 12 months of transplant) on kidney graft and patient survival following SPK. **Methods:** From the SRTD database, we evaluated all patients transplanted from the SPK waitlist from 1997-2005. Patients were placed into 4 groups: Group 1: SPK recipients with pancreas graft function at 12 months post-transplant, Group 2: SPK recipients with loss of pancreas graft function within 12 months post-transplant, Group 3: Recipients of deceased donor (DD) KA, Group 4: Recipients of living donor (LD) KA. Exclusion criteria included death or kidney graft loss prior to 12 months post-transplant. We then compared patient and kidney graft survival at 72 months.

Results: There were 9630 transplants from the SPK waitlist from 1997-2005. Of those, 7952 received SPK, 616 received DD KA, and 1062 received LD KA. 12 month patient and kidney graft survival was 95.9% and 92.0% vs 95.6 and 90.3% vs 97.2 and 94.8%, respectively (SPK vs LD KA, $p=0.036$ and 0.001 , LD KA vs DD KA, $p=0.099$ and < 0.001). For 12 month survivors with functioning kidney grafts, we then compared kidney and patient graft survival at 72 months.

12m Survivors	72m Kidney Graft Survival %	p value *vs 1, **vs 4	72m Patient Survival %	p value *vs 1, **vs 4
1) SPK, functioning pancreas (n=6486)	72.0	—	88.6	—
2) SPK, failed pancreas (n=371)	59.8	* < 0.001 , **0.204	73.9	* < 0.001 , **0.127
3) DD KA (n=520)	49.7	* < 0.001 , **0.063	64.8	* < 0.001 , **0.085
4) LD KA (n=904)	63.6	*0.015	80.0	* < 0.001

Conclusion: SPK recipients with functioning pancreas grafts have significantly improved kidney and patient survival compared to LD KA and DD KA. However, early pancreas graft failure results in kidney and patient survival rates similar to KA recipients. SPK provides optimal outcomes for patients with DM1, but long-term risk associated with early pancreas loss may be a consideration when selecting SPK vs KA transplantation.

Abstract# 80

The Impact of Long-Term Metabolic Control on Renal Allograft and Patient Survival in Type 1 Diabetes. Christian Morath,¹ Martin Zeier,¹ Bernd Dohler,² Jan Schmidt,³ Peter P. Nawroth,⁴ Gerhard Opelz.²
¹Nephrology, University of Heidelberg; ²Transplantation Immunology, University of Heidelberg; ³Transplantation Surgery, University of Heidelberg; ⁴Endocrinology, University of Heidelberg.

It is a matter of debate whether pancreas allografts independently contribute to renal transplant and patient survival in type 1 diabetics who received a simultaneous pancreas kidney transplant (SPK). Using the data of the Collaborative Transplant Study (CTS), we studied type 1 diabetic recipients of deceased donor kidneys (DDK), living donor kidneys (LDK), or SPK performed during two time periods: 1984 to 1990 and 1991 to 2000. We analyzed graft and patient survival rates for a maximum of 18 years. DDK recipients showed inferior graft and patient survival compared to LDK and SPK recipients in both time periods. LDK recipients had a superior graft survival rate initially, but the survival rate of kidneys in SPK recipients reached the level of LDK toward the end of the follow up period. The results of patient survival paralleled those of kidney graft survival: an early advantage of LDK as compared to SPK faded away during follow up. Multivariate analysis, in which pretransplant cardiovascular risk assessment was appropriately considered, showed that patient survival of SPK recipients was superior to that of LDK recipients beyond the tenth year after transplantation (hazard ratio HR = 0.55, $p = 0.005$).

Results of multivariate Cox regression analysis (patient survival)

Follow up years	Year of transplant	N	SPK compared to DDK			SPK compared to LDK		
			hazard ratio	p value	N	hazard ratio	p value	
2 to 5	1984 to 1990	2783	0.70	0.008	1435	1.34	0.12	
	1991 to 2000	4245	0.82	0.064	3038	1.31	0.085	
6 to 10	1984 to 1990	1671	0.56	<0.001	930	0.91	0.62	
	1991 to 2000	2395	0.64	<0.001	1662	0.89	0.53	
11 to 18	1984 to 1990	741	0.52	<0.001	449	0.55	0.005	

SPK - simultaneous pancreas kidney transplant; DDK - deceased donor kidney transplant; LDK - living donor kidney transplant

This was reflected by a lower cumulative cardiovascular death rate in recipients of SPK (37.0 %) compared to recipients of DDK (45.8 %) or LDK (49.3 %). The early survival benefit of LDK compared to SPK is lost during long-term follow up, probably related to improved glycemic control in SPK recipients.

Abstract# 81

Impact of Obesity in the United States: Outcomes of Simultaneous Pancreas-Kidney Transplants in Modern Immunosuppression Era. An Analysis of UNOS Data from 1996 to 2006. Kanae Ishihara, James Rice, Luca Cicalese, Phillip Thomas, Kristene Gugliuzza, J. Pesi, Yong-Fang Kuo. *The University of Texas Medical Branch, Galveston, TX.*

SKP has become a preferred treatment modality in diabetic ESRD patients with lack of insulin production, providing euglycemic benefit. Whereas the negative impact of obesity to renal allografts and native kidneys is recognized, the impact of obesity on SPK outcomes is conflicting.

Methods: Retrospective analysis of data from the UNOS Star file on primary adult SKP (1996-2006)(N=8461) was performed to examine the impact of obesity on SKP. Pancreas graft survival (PGS), kidney graft survival (KGS), and patient survival (PS) were compared among various BMI ranges. Cox proportional hazard model was used to obtain adjusted graft survival and hazard ratios were calculated.

Results: 60% of the SKP patients were male, and the mean age was 39.7 yr $\pm$ 8.0. 79.5% were white, 12.2% were African-American, and 6.9% were Hispanic. The mean duration of diabetes was 25.5 yrs $\pm$ 7.7, and 11.6% had history of CAD. The mean donor age was 26.7 yrs $\pm$ 11.1. 52.7% (N=4247) of the recipients had BMI of 20-25, whereas 8.5% had BMI > 30 (mean:24.5 $\pm$ 4.2). The preservation time was $\leq$ 12 hrs in 49.1% and 12-24 hrs in 47.9%.

DGF rate was 7.6%. Kidney graft failure (KGF) rates were 3.5, 4.5, 15.4, and 26.3%; pancreas graft failure (PGF) rates were 10.9, 12.6, 27.8, 76.6%; the patient survival rates were 96, 94.7, 85.8, 78.9% at 6, 12, 60, and 96 months respectively. The thrombosis and acute rejection causing KGF occurred in 4.1% and 6.2% respectively. The causes of PGF were thrombosis (35.5%), chronic rejection (21.2%), acute rejection (10.3%), graft infection (6.6%), and pancreatitis (3.2%). Donor age $\geq$ 50 was associated with poor KGS (HR:1.64, CI:1.20-2.24) and poor PGS (HR: 1.85, CI: 1.43-2.38) but not with PS. BMI $\leq$ 20 was associated with poor PS (HR:1.25, CI:1.0-1.55), however, obesity did not affect over all PS. BMI also did not affect K-GS. However, obesity was associated with poor PGS (BMI 30-35;HR:1.49, CI:1.12-1.98, BMI >35;HR:1.60, CI:1.02-2.51). The effect of BMI was similar among all ethnic groups.

Conclusion: There was significant impact of obesity on PGS in SKP, however, it did not affect KGS or PS. Low BMI patients had the worst PS but had the best PGS. Obese SPK patients had the worst PGS but similar KGS and PS compared to non-obese patients. As obesity becomes epidemic in the United States, recipient BMI may be an underrecognized and important factor for outcome of SKP.

Abstract# 82

10-Year Outcomes of Simultaneous Pancreas-Kidney Transplantation from Donation after Cardiac Death. Janet Bellingham,¹ Jeremy Goodman,¹ Glen Levenson,¹ Yolanda Becker,¹ Jon Odorico,¹ Hans Sollinger,¹ Luis Fernandez.¹ ¹*Division of Organ Transplantation, Department of Surgery, University of Wisconsin, Madison, WI.*

Objective: Although the number of organ donors and transplantations in the United States from donation after cardiac death (DCD) has increased exponentially over the last decade, the lack of long-term safety data has resulted in a stagnant utilization of pancreata from DCD donors.

Methods: A total of 970 simultaneous pancreas-kidney (SPK) recipients from January 1982 to July 2007 were retrospectively reviewed. The outcomes of SPK transplantation with organs procured from DCD donors (n=56) were compared with transplants performed with donor organs recovered from donation after brain death (DBD) (n=914). Patient and graft survival, episodes of acute cellular rejection, and short- and long-term allograft function were evaluated.

Results: Ten-year patient survival between SPK recipients from DBD and DCD donors was similar (80% vs. 84%, respectively; p=0.97). Pancreas graft survival at 5 years was 76% in the DBD group compared to 75% in the DCD group (p=0.57), and 64% and 60%, respectively, at 10 years. Renal allograft survival in SPK transplantation approaches 80% for DBD recipients versus 76% for DCD recipients at 5 years, whereas the 10-year comparison is 64% for DBD and 53% for DCD organs. In the DBD group, acute cellular rejection was seen in 52% of kidney transplants and 27% of pancreas transplants. In contrast, the incidence of acute cellular rejection in the the DCD group was 39% for kidneys and 20% of pancreata (p=0.55 and 0.83, respectively). Although

delayed graft function and a statistically lower glomerular filtration rate (GFR) at 7 days post-transplant occurs more frequently in DCD kidneys (p=0.001 for both), GFR was equivalent at month 1 and remained similar for the remainder of the follow-up period. Pancreas function as evaluated by fasting blood sugar and hemoglobin A1C was identical between the two groups (p=0.88).

Conclusions: Donation after cardiac death continues to be a viable method to expand the organ procurement pool. Long-term outcomes are similar in SPK transplantation between organs recovered from DBD and DCD donors. Based on the current results, we recommend a wider utilization of DCD donors for simultaneous pancreas-kidney transplantation.

Abstract# 83

BANFF Schema for Grading Pancreas Allograft Rejection: Working Proposal by a Multidisciplinary International Consensus Panel. C. B. Drachenberg,¹ J. Odorico,¹ A. J. Demetris,¹ L. Arend,¹ I. Bajema,¹ J. Bruijn,¹ D. Cantarovich,¹ H. Cathro,¹ J. Chapman,¹ K. Demosthenous,¹ B. Fyfe-Kirschner,¹ L. Gaber,¹ O. Gaber,¹ J. Goldberg,¹ E. Honsova,¹ S. Iskandar,¹ D. Klassen,¹ B. Nankivell,¹ J. C. Papadimitriou,¹ L. Racusen,¹ P. Randhawa,¹ F. Reinholt,¹ K. Renaudin,¹ P. Revelo,¹ P. Ruiz,¹ J. Torrealba,¹ E. Vazquez-Martul,¹ L. Voska,¹ R. Stratta,¹ S. Bartlett,¹ D. E. R. Sutherland.¹ ¹*Consensus from the 2005-2007 Banff Conferences on Allograft Pathology.*

Proposal for a grading rejection schema in pancreas allograft biopsies from a multidisciplinary panel of pathologists, surgeons and nephrologists.

1.Normal

2.Indeterminate Septal inflammation that appears active but the overall features do not fulfill the criteria for mild cell mediated acute rejection.

3.Acute cell mediated rejection

- Grade I / Mild acute cell mediated rejection

Active septal inflammation involving septal structures **and/or** Focal acinar inflammation.

- Grade II / Moderate acute cell mediated rejection

Multifocal (but not confluent or diffuse) acinar inflammation with spotty acinar cell injury and drop-out **and/or** Minimal intimal arteritis

- Grade III / Severe acute cell mediated rejection

Diffuse, (widespread, extensive) acinar inflammation with focal or diffuse multicellular /confluent acinar cell necrosis **and/or** Moderate or severe intimal arteritis **and/or** Transmural inflammation - Necrotizing arteritis

Chronic active cell-mediated rejection. Chronic allograft arteriopathy (arterial intimal fibrosis with mononuclear cell infiltration in fibrosis, formation of neo-intima)

4.Antibody mediated rejection =C4d positivity + confirmed donor specific antibodies + graft dysfunction

- Hyperacute rejection

- **Accelerated antibody mediated rejection** Severe, fulminant form of antibody mediated rejection with morphological similarities to hyperacute rejection but occurring later (within hours or days of transplantation).

- **Acute antibody mediated rejection** Specify percentage of biopsy surface with intercapillary capillaries positive for C4d.

5.Chronic allograft rejection/graft sclerosis

Stage I (mild graft sclerosis) <30% of the core surface

Stage II (moderate graft sclerosis) 30-60% of the core surface.

Stage III (severe graft sclerosis) >60% of the core surface

6. Other histological diagnosis e.g. CMV pancreatitis, PTLD, etc.

A simple, reproducible, clinically relevant and internationally accepted schema for grading rejection should improve the level of diagnostic accuracy and positively affect patient care.

Abstract# 84

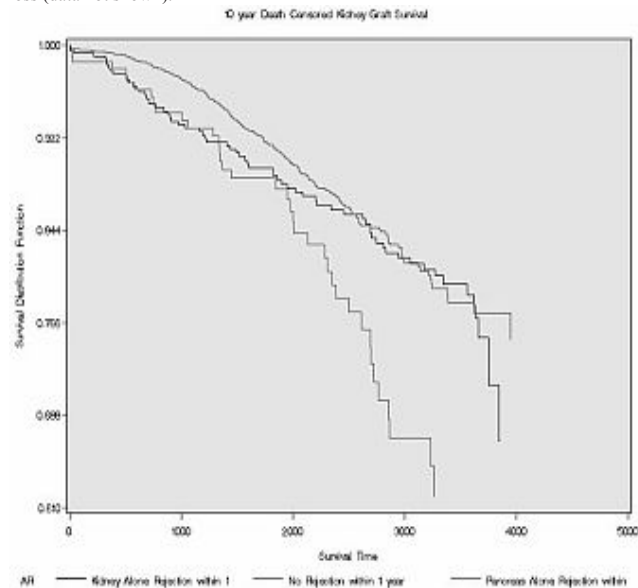
Pancreas Alone Acute Rejection Is Associated with Worse Renal Allograft Survival Than Kidney Acute Rejection: Strong Support for Concept of High Degree of Concordant and Often Missed Acute Rejection. Heather L. Herren,¹ John Gill,² Patricia M. West-Thielke,¹ Greg Knoll,³ Jose Oberholzer,¹ Howard Sankary,¹ Enrico Benedetti,¹ Bruce Kaplan.¹ ¹*Transplant Surgery, University of Illinois Medical Center at Chicago, Chicago, IL;* ²*Nephrology, University of British Columbia, Vancouver, BC, Canada;* ³*Nephrology, University of Ottawa, Ottawa, ON, Canada.*

Purpose: To examine the effect of pancreas alone rejection (PAR) within the first year on long term kidney graft survival compared to kidney alone rejection (KAR) in patients who received simultaneous pancreas kidney (SPK) transplant.

Methods: We examined 4475 adult only SPK transplants performed between 1/1/1995 through 12/31/2005 using OPTN data. We analyzed the type of rejection within the first year and its effect on long term kidney graft survival. Kaplan-Meier curves were compared using SAS v.9.1.

Results: Among the 4475 SPK recipients PAR and KAR occurred in 4.5% and 9.4% of patients during the first post-transplant year. Figure 1 shows that patients with PAR had decreased death censored kidney allograft survival than patients with either KAR (p = 0.024, log rank test) or patients without rejection in either organ (p = 0.01, log

rank test). Similar results were obtained when death was included as a cause of graft loss (data not shown).



Conclusion: PAR is associated with worse long term kidney graft survival than KAR. It is likely that patients that experience PAR within the first year have also experienced undiagnosed sub-clinical KAR. This adds associative data to the argument that the likelihood of concordance between PAR and KAR is high and therefore, the need for increased monitoring of kidney function after PAR is warranted.

Abstract# 85

Alemtuzumab Is Not Superior to Basiliximab in Preventing Antibody-Mediated Rejection (AMR) of the Kidney after Simultaneous Pancreas-Kidney Transplantation (SPKT). J. Pascual, J. Pirsch, J. Torrealla, J. Odorico, A. Djamali, Y. Becker, G. Levenson, S. Knechtle, H. Sollinger, M. Samaniego. *University of Wisconsin, Madison, WI.*

The most efficacious immunosuppressive (immuno) regimen for SPKT is debated, and information on the best regimen to prevent AMR is particularly scarce. We performed a retrospective comparative cohort study that included 136 SPKT patients (Pts) transplanted in 2002-2005, who received maintenance immuno with TAC, MMF and steroids. Two groups were compared: Pts who received induction with alemtuzumab (Alem) (n=97) and Pts who were induced with basiliximab (Basilmab) (n=39). Donor and recipient characteristics were similar. KT acute cellular rejection (ACR) was more frequent with Basilmab (2-yr 12.8% vs 3.1%, p=0.04), but the incidence of biopsy-proven KT AMR was similar (2-yr 18% with Basilmab vs 13.8% with Alem, p=NS). No differences in prevalence were detected considering early AMR (<90th day) or late AMR (>90th day) separately. Multivariate analyses showed that the only significant risk factor for AMR was female gender (adjusted HR 2.97, p=0.016). The biopsy-proven KT rejection was associated with clinical PT rejection in 13 out of the 21 AMR cases (62%), without differences between the groups. Post-rejection KT graft survival was similar in both groups (2-yr Basilmab/Alem 94.7/91.2%), but death-censored KT survival was lower with Alem (100/91.2%, p=0.05). The predominant cause of KT loss in Pts induced with Basilmab was death-with-function (n=3), while in Pts induced with Alem acute or chronic AMR was the most common cause of KT attrition (n=5). Pancreas survival was similar between both groups. More PT losses due to AR occurred in Alem-treated group than in the Basilmab group (4 vs 1). Nearly all early episodes resolved with treatment and were not associated with worse graft survival. Conversely, late AMR episodes carried a worse prognosis, with decreased 2-yr KT (61.5% vs 96.2%, p<0.0001) and PT graft survival (47.7 vs 92.3%, p<0.0001). Late AMR was associated with graft loss in multivariate Cox models (kidney loss HR 3.34, p=0.05 and pancreas HR=3.22, p=0.049).

AMR is common in SPKT recipients. ACR is better prevented with Alem than with Basilmab, but no relevant difference is found between Alem and Basilmab in AMR. Late (as opposed to early) AMR episodes are associated with significant reduction in SPKT survival rates despite treatment. Preventive and/or different treatment strategies are required to address late AMR in SPKT.

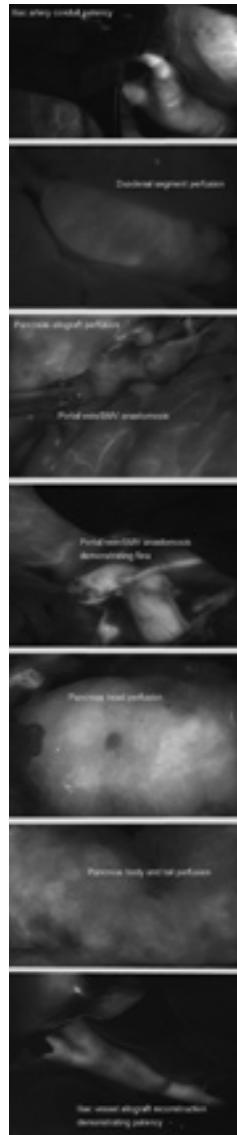
Abstract# 86

Intraoperative Fluorescence Imaging (IFI) in Pancreas Transplantation (PT) To Determine Vascular Patency and Allograft Perfusion. Edmund Q. Sanchez,¹ Srinath Chinnakotla,¹ Marlon F. Levy,¹ Robert M. Goldstein,¹ Goran B. Klintmalm.¹ *¹Baylor Regional Transplant Institute, Dallas/Ft. Worth, TX.*

Thrombotic complications of PT are well known. We report IFI using the SPY device to assess immediate vascular patency and allograft perfusion. Our controlled experience is presented here.

Methods: PTs were imaged intraoperatively using the SPY device under our IRB approved study. Indocyanine green solution (2.5 mg/ml) was injected into central venous catheters after completion of the vascular anastomoses of whole pancreaticoduodenal allografts. The pancreas transplants were performed in the retroperitoneal portal and enteric drained technique described by Boggi, et al. Imaging of the allograft vasculature, perfusion of the pancreas, and perfusion of the duodenal segments was performed and recorded intraoperatively. All video sequences were archived for later review.

Results: IFI on 7 pancreas transplants (6 Simultaneous Pancreas-Kidney and 1 Pancreas after Kidney) was performed and video sequences were recorded. All pancreas allografts demonstrated intraoperative vascular patency and complete pancreatic and duodenal perfusion. There were no side effects seen. All pancreas transplants had immediate graft function. One patient was re-explored on postop day 0 due to persistent acidosis and hypotension. Repeat IFI demonstrated vascular patency and perfusion, despite an ischemic external physical appearance. There were no vascular complications that required reoperation, nor were there any graft thromboses. Characteristic slow venous outflow was seen in each case.



Conclusion: IFI with the SPY device is a simple and effective method to determine immediate patency and perfusion of the whole pancreaticoduodenal allograft. Its use is beneficial in re-exploration to rule out infarcted pancreas allografts. Further development in quantification of vascular flow rates and allograft perfusion indices by this device is

in progress. Once these are established, this information will be studied in a controlled fashion, and will be compared with clinical outcomes. We have demonstrated safety and developed a protocol for using the SPY device in IFI of pancreas transplants.

Tolerance/Immune Deviation I

Abstract# 87

Tolerance without Immunosuppression: Exploiting Epigenetic Regulation as a New Approach To Achieving Donor-Specific Allograft Tolerance. Liqing Wang,¹ Ran Tao,¹ Joel A. Friedlander,¹ Wayne W. Hancock,¹ ¹Pathology & Immunology, Children's Hospital of Philadelphia & UPenn, Philadelphia, PA.

Given toxicities of all current immunosuppressive agents, plus complications of malignancy, infection and chronic rejection, maintaining the search for new approaches to tolerance induction is essential. While weaning of immunosuppression is fraught with risks and costimulation blockade has not yielded the expected boon, use of a broad histone deacetylase inhibitor (HDACi), such as trichostatin A (TSA) or suberoylanilide hydroxamic acid (SAHA), promotes Foxp3 acetylation of Foxp3 and binding to target genes, leading to enhanced Treg suppressive function, and 2 wks of therapy with HDACi and low-dose rapamycin can induce allograft tolerance. We now show that in addition to acetylation, modulation of a second major epigenetic mechanism, DNA methylation, has salutary effects, such that combined use of an HDACi and a DNA methyltransferase inhibitor (DNMTi, e.g. 5-aza-2 deoxycytidine) has potent effects. Microarray analysis of the effects of HDACi on Tregs showed down-regulation of multiple genes associated with DNA methylation, including DNMT, methyl-CpG-binding domain and associated proteins, leading us to test the effects of DNMTi use on Treg function. DNMTi administration decreased Foxp3 gene methylation, enhanced Treg gene expression, and increased Treg suppression *in vitro*, and led to a dose-dependent prolongation of cardiac allograft survival (BALB/c->C57BL/6) *in vivo* (p<0.01). The combination of HDACi and lower doses of DNMTi, administered for just 2 wks, led to permanent engraftment (>100 d, p<0.001), and the permanent acceptance of second donor allografts but acute rejection of third-party (CBA) cardiac allografts indicated induction of donor-specific tolerance post-epigenetic therapy. Analysis of long-surviving cardiac allografts showed a minor infiltrate consisting primarily of Foxp3+ Tregs and an absence of chronic rejection (transplant arteriosclerosis, myocardial fibrosis), whereas combined epigenetic therapy led to only minor prolongation of allograft survival in Treg-depleted recipients. In summary, brief use of 2 clinically approved agents (an HDACi plus a DNMTi), can synergistically enhance Treg function and induce Treg-dependent donor-specific allograft tolerance without use of any immunosuppression. We conclude that insights gained through epigenetic targeting may provide completely new approaches to the taming and regulation of otherwise powerful immune responses post-transplantation.

Abstract# 88

A Novel Epigenetic Approach To Generate Mouse and Human CD4⁺CD25⁺Foxp3⁺ Regulatory T Cells. Girdhari Lal,¹ Nan Zhang,¹ William van der Touw,¹ Yaozhong Ding,¹ Jonathan S. Bromberg,¹ ¹Gene and Cell Medicine, Mount Sinai School of Medicine, New York City, NY.

Background: Constitutive Foxp3 expression is required for stable and suppressive CD4⁺CD25⁺regulatory T cells (Treg). We previously showed that demethylation of an upstream CpG island of the *Foxp3* promoter is characteristic of stable and suppressive Treg. Using DNA methyltransferase inhibitors, we present a novel method to generate antigen-specific Treg from mouse and human CD4⁺CD25⁺ T cells.

Methods: Naïve CD4⁺CD25⁺ T cells and natural Treg (nTreg) were purified from wild type or Foxp3-GFP transgenic mice. Human naïve CD4⁺CD25⁺ T cells were purified from PBMC. T cells were cultured with irradiated antigen presenting cells in the presence of IL-2, anti-CD3ε mAb, TGFβ or the DNA methyltransferase inhibitor 5-aza-2'-deoxycytidine (ZdCyd), which demethylates the upstream promoter. Foxp3 expression was determined by flow cytometry and qRT-PCR.

Results: Naïve T cells cultured under stimulatory conditions with ZdCyd express increased *Foxp3* mRNA (30 fold) and intracellular Foxp3 protein (35% of cells vs 2% without ZdCyd). Foxp3 expression is synergistically enhanced with TGFβ (76 ± 4.6% vs 38.8 ± 5.3% ZdCyd alone or 44.6 ± 1.4% TGFβ alone), similar to nTreg (92.4 ± 2.5%). TGFβ in combination with other DNA methyltransferase inhibitors (procainamide, hydralazine, RG108) also induces Foxp3. ZdCyd plus TGFβ induced Treg stably express Foxp3 and similar surface markers and cytokine mRNA as nTreg. ZdCyd plus TGFβ induced Treg suppress proliferation of CD4⁺CD25⁺ T cells, prevent CD4⁺CD25⁺CD45RB^{hi} induced colitis in SCID mice, and enhance islet allograft survival. ZdCyd plus TGFβ induce Foxp3 expression in antigen-specific wild type or T cell receptor transgenic CD4⁺ T cells stimulated with alloantigen or peptides, and in naïve CD8⁺CD25⁺ T cells. *In vivo* administration of ZdCyd preferentially preserves thymic CD4⁺CD25⁺Foxp3⁺ Treg generation. ZdCyd alone or ZdCyd plus TGFβ induce strong Foxp3 expression in human CD4⁺CD25⁺ T cells compared to TGFβ alone. ZdCyd plus TGFβ induced human Treg suppress the proliferation of naïve CD4⁺CD25⁺ T cells, whereas TGFβ-induced Treg do not.

Conclusion: DNA methyltransferase inhibitors induce stable and suppressive Foxp3⁺ Treg from peripheral CD4⁺CD25⁺ T cells. These finding have important implications

for understanding T cell development and differentiation, and provide a clinically applicable technique for manipulating epigenetic regulation for the generation of Treg for tolerance.

Abstract# 89

Macrophages Driven to a Novel State of Activation Can Promote Tolerance. Katharina Kronenberg,¹ Seiichiro Inoue,¹ James A. Hutchinson,² Beate G. Brem-Exner,² Gudrun E. Koehl,¹ Hans J. Schlitt,¹ Fred Fandrich,² Edward K. Geissler,¹ ¹Surgery, University of Regensburg, Regensburg, Germany; ²Surgery, University Hospital Schleswig Holstein, Kiel, Germany.

Background: The use of immunomodulatory cells in organ transplantation (Tx) is a promising tolerance-induction strategy. Here we describe a novel macrophage population capable of enriching T regulatory cells (Treg) and promoting tolerance. **Methods:** BALB/c bone marrow, spleen and blood mononuclear cells were cultured with M-CSF for 5 days, with a 24 hr pulse of IFN-γ on day 4; the resultant adherent cells are referred to as IFNγ-induced monocyte-derived cells (IFNγ-MdC). Residual lymphocytes from these cultures were recultured with the adherent IFNγ-MdC for an additional 3 days. IFNγ-MdC were phenotyped by FACS and immunomodulation of lymphocytes was determined by cell counting and FACS analysis for Tregs. Mouse heterotopic heart Tx was used for *in vivo* testing. **Results:** IFNγ-MdC highly express CD11b/c, F4/80 and PD-L1. Compared to classically-activated (M1) macrophages, IFNγ-MdC express less CD40/CD86, but higher CD11c. IFNγ-MdC profoundly delete activated, but not resting, lymphocytes (>60%), with cell contact (via transwell system) and caspases (via inhibitors) being essential. Most interestingly, IFNγ-MdC highly enrich lymphocytes for CD4⁺CD25^{hi}Foxp3⁺ Treg (41±2%; n=27). The same, or higher, proportions of Treg developed when IFNγ-MdC were produced from MACS-purified CD11b⁺ and CD4⁺ cells (1:1). IFNγ-MdC culture supernatant showed increased IL-10 (ELISA) vs. monocyte control cultures (183±42 pg/ml vs. <30 pg/ml, respectively; n=6), suggesting IL-10 as one potential mediator. IFNγ receptor signaling and CD40 interactions are required for Treg enrichment, as confirmed using IFNγ receptor and CD40 knock-out mice (C57BL/6 mice). IFNγ-MdC derived from CD11b⁺ cells and lymphocytes of OVA-transgenic OTII mice showed enrichment of Treg by 10-fold with high-affinity OVA peptide (323-339) vs. Treg levels using a low-affinity OVA peptide (OVA 323-334), suggesting Ag-specific Treg cells can be enriched with IFNγ-MdC. Finally, a single post-heart Tx (d+1) i.v. injection of 5x10⁶ donor (C3H)-derived IFNγ-MdC prolonged allograft survival in BALB/c recipients from 8.5±0.8d in controls (n=5) to 21.4±8.3d (n=11; p=0.001). **Conclusions:** IFNγ-MdC are a novel macrophage population capable of deleting activated T cells and highly enriching remaining lymphocytes for Tregs. Therefore, IFNγ-MdC could be useful in a clinical setting for promoting transplant tolerance.

Abstract# 90

Plasmacytoid DC Therapeutic Potential Is Independent of IDO Induction Due to Elevated DAP12 Expression. Tina L. Sumpter,¹ Bridget L. Colvin,¹ Zhiliang Wang,¹ Andrew L. Mellor,³ Angus W. Thomson.^{1,2} ¹Starzl Transplantation Institute, Dept. of Surgery, University of Pittsburgh, Pittsburgh, PA; ²Immunology, University of Pittsburgh, Pittsburgh, PA; ³Immunotherapy Center, Medical College of Georgia, Augusta, GA.

Optimizing the mechanisms of pDC tolerogenicity may facilitate their use in the development of cell-based tolerance induction strategies. pDC may undermine T cell function via inducible expression of IDO, a tryptophan catabolizer that enhances T cell apoptosis. IDO is negatively regulated by DAP12. In some systems, loss of DAP12 correlates with immunostimulatory activation of pDC, while in others, its absence correlates with enhancement of pDC tolerogenicity through induction of IDO. In these studies, we characterized the ability of WT and IDO-deficient pDC to attenuate murine heart allograft rejection in order to define the contribution of IDO and its regulation by DAP12 relative to the immunoregulatory potential of pDC.

Methods: pDC and control myeloid (m) DC were generated from BM cultures of WT or IDO KO C57BL/6 (B6; H2^b) mice. WT mDC, pDC or IDO KO pDC were pulsed with alloAg (BALB/c; H2^d), stimulated with CpG, and then injected i.v. into syngeneic (B6) recipients 7d before heart transplant. Donor Ag-specific activation of T cells was analyzed by MLR and ELISA. DAP12 expression was evaluated by RT-PCR and abrogated with siRNA.

Results: Administration of IDO KO pDC 7d before transplant prolonged graft survival beyond that seen with control mDC, but not to the extent seen with WT pDC, suggesting involvement of IDO. pDC cultures from IDO KO mice exhibited a more mature phenotype than their WT counterparts with reduced expression of co-regulatory molecules (e.g., ICOSL). Although IDO KO pDC induced enhanced T cell alloactivation compared to WT pDC, use of the IDO inhibitor 1-MT indicated that WT pDC do not produce functional IDO. Further analyses showed WT pDC exhibited high levels of DAP12 mRNA expression. Silencing DAP12 had no effect on the ability of WT pDC to activate allogeneic T cell proliferation, but significantly enhanced IFNγ secretion. Addition of 1-MT to MLR with DAP12-silenced pDC increased T cell proliferative responses to alloAg, verifying IDO induction in DAP12-silenced pDC.

Conclusions: These data underscore the prophylactic potential of pDC for cell-based therapy that may be independent of IDO, due, in part, to elevated DAP12 expression. Additionally, our data support a role for DAP12 in both immunostimulation and immunoregulation by pDC.

Abstract# 91

Rapamycin Preferentially Blocks the Expansion of Potentially Tolerogenic Plasmacytoid Dendritic Cells In Vivo. Heth R. Turnquist,¹ Angus W. Thomson.^{1,2} ¹*Starzl Transpl Inst and Dept of Surgery;* ²*Dept of Immunol, Univ of Pittsburgh Sch of Med, Pittsburgh, PA.*

Rapamycin (RAPA), a 'tolerance-sparing' immunosuppressant with anti-proliferative properties, inhibits myeloid (m) dendritic cell (DC) differentiation/maturation in vitro. RAPA decreases CD11c⁺ DCs in the mouse spleen and suppresses the expansion of total CD11c⁺ DC following administration of the DC growth factor, flms-like tyrosine kinase 3 ligand (Flt3L). However, the influence of RAPA on plasmacytoid (p) DC, -the principal type-1 IFN producers in the body, known to regulate innate and adaptive immune responses, and reported to promote experimental transplant tolerance, has not been evaluated.

Methods: DC were propagated from bone marrow for 10 days (d) in Flt3L, in the absence or presence of a clinically-relevant dose of RAPA (10 ng/ml). In addition, DCs were mobilized in C57BL/10 mice by i.p. Flt3L (10 mg/d for 10 d; d1-10). Untreated and Flt3L-treated groups also received RAPA (0.5 mg/kg/d i.p.; d3-10). On d11, DC were isolated by density gradient centrifugation and/or CD11c⁺ positive selection. mDC (CD11c⁺CD11b⁺) and pDC (CD11c⁺CD11b⁺B220⁺) were identified by flow cytometry.

Results: RAPA suppressed pDC and mDC generation in vitro; RAPA-DC-treated cultures had only 22% of the pDC and 40% of the mDC found in control conditions. In normal mice, both mDC (54% of control) and pDC (24.5%) numbers were reduced significantly by RAPA administration. RAPA, when given concurrently with Flt3L, blunted the typical profound expansion of mDC. Specifically, Flt3L and RAPA-treated mice displayed a 46±8X increase over control (steady state) numbers compared to a 145±39X increase in mice treated with Flt3L alone. Flt3L-induced expansion of pDC was to a much greater extent impacted by RAPA, as absolute numbers of pDC increased only 6.5±2.2X over control numbers compared to 51±3X increase in absolute splenic pDC in Flt3L-treated mice.

Conclusion: These data identify RAPA as a selective suppressor of pDC generation, as described for corticosteroids. This has significant implications, given the use of RAPA following organ transplantation, and the suggested importance of secondary lymphoid tissue pDC for promotion of transplant tolerance mediated by Treg. Due attention to these disparate effects of RAPA on regulating cell populations will be necessary to optimize therapeutic regimens for safe promotion of tolerance.

Abstract# 92

CD103^{hi} Liver Dendritic Cells Are Resistant to the Effects of Retinoic Acid and Transforming Growth Factor-β on Induction of Foxp3⁺ Regulatory T Cells. Tina L. Sumpter,¹ Daisuke Tokita,¹ C. Shekhar Gandhi,¹ Angus W. Thomson.^{1,2} ¹*Starzl Transplantation Institute, Dept of Surgery, University of Pittsburgh, Pittsburgh, PA;* ²*Immunology, University of Pittsburgh, Pittsburgh, PA.*

The liver is tolerated better than other transplanted organs. This may reflect the inherent tolerogenicity of liver dendritic cells (DC) and interactions with Foxp3⁺ regulatory T cells (Treg). Recent studies have shown that CD103 expression on DC correlates with induction of Foxp3⁺ Treg, and can be enhanced in the presence of transforming growth factor-β (TGF-β) and retinoic acid (RA), both of which are produced in the liver. This study evaluates CD103 expression on liver plasmacytoid (p) and myeloid (m) DC and the potential of liver DC subsets to induce Tregs.

Methods: pDC and mDC were magnetically isolated from livers and spleens of C57BL/10 mice and co-cultured with CFSE-labeled allogeneic (BALB/c) CD4⁺CD25⁺ T cells. After 4 or 5d of co-culture, T cells were assessed for CFSE dilution and intracellular Foxp3 expression. rhTGF-β and either all trans or cis-RA were added to co-cultures. CD103 expression was evaluated by flow cytometry.

Results: CD103 expression was elevated on liver pDC and mDC compared to spleen pDC or mDC, with higher expression on liver mDC. pDC from the liver and the spleen were poor inducers of Foxp3 in CD4⁺CD25⁺ T cells compared to mDC. Foxp3 induction was enhanced with TGF-β when splenic pDC were used as stimulators. However, when liver pDC were used as stimulators, TGF-β had no effect on Foxp3 induction. RA (either all trans or cis) enhanced induction of Foxp3⁺ cells in CD4⁺CD25⁺ T cells in the presence of TGF-β when splenic pDC but not liver pDC were used as stimulators. TGF-β and TGF-β with RA also enhanced Foxp3 induction in CD4⁺CD25⁺ T cells when either spleen or liver mDC were used as stimulators, though splenic mDC were superior inducers of Foxp3.

Conclusions: Many studies have focused on naturally-occurring Foxp3⁺ Tregs in systemic tolerance. Our data show that liver mDC and pDC are poor inducers of Foxp3 in T cells, even in the presence of TGF-β and RA. These data suggest that the inherent tolerogenicity of liver DC subsets may be independent of Treg induction or that liver DC interact with Treg through alternative mechanisms.

Abstract# 93

Prevent Heart Graft Rejection by Silencing Costimulatory Molecules Using siRNA. Xusheng Zhang,¹ Dameng Lian,¹ Xiufen Zheng,¹ Hongtao Sun,¹ Weihua Liu,¹ Motohiko Suzuki,¹ Marianne Beduhn,¹ Norihiko Kubo,¹ Aminah Shunnar,¹ Bertha Garcia,¹ Anthony Jevnikar,^{1,2} Wei-Ping Min.^{1,2} ¹*Surgery, University of Western Ontario, London, ON, Canada;* ²*Multi-Organ Transplant Program, London Health Sciences Centre, London, ON, Canada.*

Background: T cell-mediated immune rejection occurs in organ transplantation. In addition to MHC-TCR signaling, T cell activation requires costimulation from antigen presenting cells. B7 molecules (CD80/CD86) and CD40 are critical costimulatory molecules in T cell activation. Insufficient or lack of costimulation results in inactivation or tolerance. We hypothesized that blocking the costimulation pathway using small interfering RNA (siRNA) expression vector can prolong allogeneic heart graft survival.

Method: Vectors that express hairpin siRNAs specifically targeting CD40 and CD80 were prepared. Recipients (BALB/c mice) were treated with CD40 and/or CD80 siRNA vectors, 3 and 7 days prior to heart transplantation. Control groups were injected with a blank vector and sham treatment (PBS). After siRNA treatment, a fully MHC-mismatched (BALB/c to C57/BL6) heart transplantation was performed.

Result: Allogeneic heart graft survival (>100days) was approximately 70% in the mice treated simultaneously with CD40 and CD80 siRNA vectors. In contrast, allogeneic hearts transplanted into recipients treated with blank vector and PBS stopped beating within 10 days. Hearts transplanted into CD40 or CD80 siRNA vector-treated recipients had an increased graft survival time compared to negative control groups, but did not survive longer than 40 days. Real time PCR and flow cytometric analysis showed an upregulation of FoxP3 expression in spleen lymphocytes and a concurrent downregulation of CD40 and CD80 expression in splenic dendritic cells of siRNA-treated mice. An MLR, using splenic dendritic cells (DCs) isolated from tolerant recipients, showed a significantly lower T cell proliferation capacity in CD40- and CD80-siRNA vector-treated mice, compared to control groups. Tolerant DCs from CD40- and CD80-treated recipients promoted CD4⁺CD25⁺FoxP3⁺ regulatory T cell differentiation. Finally, tissue histopathology demonstrated an overall reduction in lymphocyte interstitium infiltration, vascular obstruction, and edema in mice treated with CD40 and CD80 siRNA vectors.

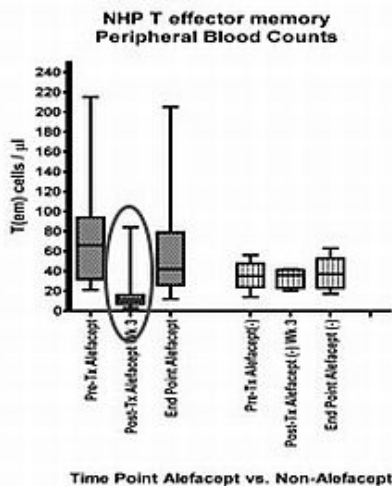
Conclusion: This study demonstrates that the simultaneous silencing of CD40 and CD80 genes has synergistic effects in preventing allograft rejection, and may therefore have therapeutic potential in clinical transplantation.

Abstract# 94

Targeting of Costimulation Blockade Resistant T Effector Memory (TEM) Cells in Non-Human Primate Renal Transplantation with LFA-3-Ig (Alefacept) Prolongs Allograft Survival. T. A. Weaver,¹ A. H. Charafeddine,¹ A. Agarwal,¹ F. Leopardi,¹ R. Kampen,¹ M. F. Starost,² C. P. Larsen,¹ A. D. Kirk.¹ ¹*Emory Transplant Center, Atlanta, GA;* ²*Veterinary Pathology, NIH, Bethesda, MD.*

Costimulation blockade (CoB) of the CD28/B7 pathway has long been suggested as a means of attaining indefinite, well-tolerated, antigen-specific prophylaxis from allograft rejection. Although effective in rodent models, CD28/B7 blockade is not alone sufficient to prevent rejection in more robust primate models. The relative presence of allo-crossreactive memory T cells is one mechanism of CoB resistance rejection. LFA3-Ig is an approved agent for the treatment of psoriasis, shown clinically to deplete TEM cells in psoriatic lesions. We hypothesize that LFA3-Ig specifically targets CoB resistance cells, including heterologous alloreactive T cell memory, while avoiding interference of peripheral mechanisms that foster CoB-mediated allograft acceptance. Rhesus monkeys underwent MHC mismatched renal allotransplantation. CTLA4-Ig was given 20 mg/kg IV on days -1, 0,3,7,14,21,28 and 10 mg/kg IV on days 35,42,49,56. LFA3-Ig, (0.3 mg/kg IV) was given on day -1,0,3,7 then weekly (8 wks). Sirolimus was given orally (1mg/kg) days 0-90 and donor specific transfusion (7 ml/kg IV) on day -1. Animals were followed by polychromatic flow-cytometry to quantify T cell subsets. LFA3-Ig synergized with CTLA4-Ig successfully preventing renal allograft rejection in this model and leading to indefinite survival in 2/5 animals (Table). This enhanced survival was associated with a significant reduction in TEM cells in animals treated with LFA3-Ig compared to animals without LFA3-Ig treatment (figure). LFA3-Ig withdrawal led to TEM re-population and rejection. This therapy regimen, all clinically available agents, promotes CoB-mediated graft acceptance without the use of calcineurin inhibitors, steroids, or gross T cell depletion.

Treatment Group	Rejection Free Survival	p-value compared to Group 7
1. No Treatment	5, 6, 7, 7, 8	0.015
2. Sirolimus Alone	8, 10, 21, 23, 28	0.021
3. Sirolimus, DST	10, 6, 5	0.016
4. CTLA4-Ig, Sirolimus, DST	13, 33, 44	0.028
5. LFA3-Ig, Sirolimus, DST	18, 41, 49	0.033
6. CTLA4-Ig, LFA3-Ig, Sirolimus	89, 83, 131	0.336
7. CTLA4-Ig, LFA3-Ig, Sirolimus, DST	56, 105, 108, >168, >243	*****

**Abstract# 95**

BAFF Alters T-Cell-Dependant Immune Responses Allowing Allograft Acceptance. Stacey N. Walters,¹ Andrew Sutherland,¹ Jessica Thaxton,¹ Joanna Groom,¹ David Liuwantara,¹ Eliana Marino,¹ Fabienne Mackay,¹ Jonathon Sprent,¹ Shane T. Grey.¹ *Immunology, Garvan Institute of Medical Research, Sydney, New South Wales, Australia.*

The aim of this study was to investigate the immunological pathways that regulate T cell dependant allograft responses in the hope of finding novel immunomodulatory compounds. Islet (and skin) allografts were transplanted into full MHC-mismatched Wild Type (WT) and BAFF-Transgenic (BAFF-Tg) recipient mice. Allograft function/rejection was monitored by blood glucose levels and/or confirmed by nephrectomy. Histology, splenocyte populations & T cell functions were analyzed. The B cell activation factor from the TNF family (BAFF) is critical for B cell survival. T cells express BAFF receptors suggesting that BAFF may also play a role in T cell responses. Contrary to expectations, we found that BAFF-Tg mice accepted a full MHC-mismatched islet allograft for >100 days. In addition, BAFF-Tg mice also showed delayed skin graft rejection. This was due to a T cell intrinsic change in allo-responsiveness as shown by a failure of purified BAFF-Tg T cells to reject an allograft in adoptive transfer experiments. However, BAFF-Tg T cells were not anergic *per se* as they proliferated normally to mitogens *in vitro* and to antigenic challenge *in vivo*. Intriguingly, BAFF-Tg mice harbored an increased frequency of T regulatory cells in the periphery as compared to WT mice. Elimination of CD25⁺ T cells restored normal allograft rejection to BAFF-Tg mice, demonstrating that the increased number of Treg cells were responsible for their altered allo-immunity. In a second approach, BAFF-Tg T cells depleted of CD4⁺ CD25⁺ T cells were adoptively transferred to transplanted RAG recipients, and in this case the BAFF-Tg CD4⁺ CD25⁺ T cells rejected their allograft. Proliferation assays showed that excessive BAFF was not promoting Treg expansion by driving proliferation in the periphery. Adoptive transfer of GFP expressing syngeneic splenocytes did not exhibit enhanced survival in the presence of excessive BAFF. However, analysis of Treg cells in the thymus of BAFF-Tg mice showed an increased intrathymic frequency. Together these results demonstrate that BAFF-Tg mice harbor an expanded number of Treg cells that prevent Th1-type immune responses including allograft rejection. Furthermore, we demonstrate that manipulating BAFF levels may provide a means to generate immunosuppression free dominant allograft tolerance.

Xenotransplantation**Abstract# 96**

Average Survival Greater Than 50 Days in Baboon Recipients of Life-Supporting GalT-KO Pig Thymokidneys. Adam Griesemer, Hideyuki Iwaki, Atsushi Hirakata, Yoshinori Ishikawa, Shannon Moran, Patrick Schule, Scott Arn, Akira Shimizu, David Sachs, Kazuhiko Yamada. *Transplantation Biology Research Center, Massachusetts General Hospital, Boston, MA.*

We have previously reported the outcome of pig-to-baboon thymokidney transplants from GalT-KO miniature swine using an immunosuppressive regimen designed to facilitate the induction of tolerance. Although the results were superior to results using hDAF thymokidneys, there was a high rate of early post-operative complications. We investigated whether the elimination of steroids and whole body irradiation (WBI) from the treatment protocol would decrease the complication rate in the perioperative period. **Methods:** 7 baboons received thymokidney transplants from GalT-KO miniature swine. The immunosuppressive regimen was based on the previously published protocol, but eliminated steroids and substituted Rituximab for WBI. It consisted of thymectomy day -21, splenectomy day 0, ATG, Rituximab, MMF and anti-CD154 mAb. Renal function

was assessed by serum creatinine levels. Evidence for baboon thymopoiesis in the pig thymus was assessed by FACS and immunohistochemistry. **Results:** One animal died due to a drug reaction at POD 18 with normal renal function and no evidence of infection. The remaining 6 baboons showed no signs of rejection although IgM deposition was observed, likely due to preformed non-Gal nAb. There were no deaths due to rejection. Although early infectious complications were not seen, late complications leading to mortality were still observed, including systemic CMV infection (POD 28), pleural effusions (POD 40, 57), ARDS with pulmonary hemorrhage (POD 49, 81) and acute myocardial infarction (POD 83). Two animals showed evidence for early thymopoiesis in the pig thymus by FACS and immunohistochemistry. CD4⁺/CD8⁺ thymocytes were seen in the thymic grafts, indicating T cell development was supported by the pig thymus. The average survival of the steroid-free group was 51 days including the animal with the drug reaction and 56 days excluding it, compared to 34.6 days in the regimen that included steroids/WBI. **Conclusions:** A steroid-free and WBI free regimen designed for the induction of tolerance across the pig-to-baboon xenogeneic barrier had fewer early post-operative complications than previous regimens. Greater than 50-day average survival of recipients of life-supporting xenogeneic thymokidneys was observed without rejection. This regimen also appears to permit baboon thymopoiesis in the pig thymus.

Abstract# 97

Addition of HDAF to the GalT-KO Background Improves Survival and Function of Pig Kidneys in Baboons. Shahrooz S. Kelishadi,¹ Tianshu Zhang,¹ Tiffany Stoddard,¹ Agnes M. Azimzadeh,¹ Emily Wely,¹ Christopher J. Avon,¹ Amal Laaris,¹ Xiang-Fei Cheng,¹ Rolf Barth,¹ Richard N. Pierson III.¹ *Surgery, University of Maryland School of Medicine and Baltimore VAMC, Baltimore, MD.*

Purpose: The effects of human decay accelerating factor (hDAF) addition to the galactosyl transferase knock-out (GalT-KO) background in transgenic pig kidney xenotransplantation in baboons has not been previously studied.

Methods: Baboon recipients of GalT-KO (n=4) or GalT-KO/hDAF (n=2) pig kidneys received Flolan, heparin and/or aspirin. Steroids (S), cobra venom factor (V), ATG (A), MMF (M), and/or a low-dose CD154 blockade (C) regimen were given.

Results: Pre-transplant (tx) anti-non-Gal antibody (Ab) titers were inconsistently associated with early GalT-KO kidney xenograft failure. High D-Dimer levels 4 hours post-tx were closely associated with early GalT-KO graft loss but not when assessing levels of C3a, BTG, or increased CD62P expression on circulating platelets. Regimens lacking MMF and/or CD154 blockade elicited by day 7-14 large anti-donor non-Gal Ab responses (3-5-fold IgM increase; 20-40-fold IgG increase). Post-operative creatinine levels for the GalT-KO/hDAF recipients were lower along with superior graft survival compared to GalT-KO; D-Dimer, anti-donor non-Gal Ab, C3a, BTG, and F1+2 measurements are in progress for the GalT-KO/hDAF recipients.

Animal ID	Genetics	Regimen	Survival Days	4 hr D-Dimer (ng/mL)	Pre-Tx odonor non Gal IgM	Creatinine Day (1); (5)
B18925	GalT-KO	V,A,M,S	0	7565	31	NA; NA
B15779	GalT-KO	V,S	2	5231	43	6.4; 21.5
B19955	GalT-KO	V,M,C,S	5	2575	57	1.5; 7.5
B19967	GalT-KO	V,A,S	7	1292	71	2.8; >5
BGE07	GalT-KO/hDAF	V,A,M,S,C	>10*	TBP	105	0.6; 0.6
BGB36	GalT-KO/hDAF	V,A,M,S,C	>10*	TBP	239	1.9; 1.5

* Graft survival at time of abstract submission as graft still functioning at time of abstract submission; TBP -- To be processed

! -- anuric; non-life supporting, but appeared viable, pink, and well-perfused until day 7

Conclusion: Early failure of vascularized GalT-KO organs is closely associated with activation of coagulation pathways; whether this is triggered primarily by anti-non-Gal Ab or by other mechanisms is not addressed by our study. Preliminary results using GalT-KO/hDAF pig kidneys show promise in attenuating or possibly preventing these and/or other such mechanisms. The established efficacy of CD154-based costimulation blockade with MMF to prevent induced anti-non-Gal antibody elaboration is confirmed by our preliminary findings.

Abstract# 98

Recovery of Cardiac Function after Pig-to-Primate Orthotopic Heart Transplant. Christopher G. A. McGregor,¹ William R. Davies,¹ Keiji Oi,¹ Henry D. Tazelaar,¹ Randall C. Walker,¹ Krishnaswamy Chandrasekaran,¹ Guerard W. Byrne.¹ *Mayo Clinic, Rochester, MN.*

Xenotransplantation has been proposed as a method to alleviate the shortage of donor organs. We report survival of up to 8 weeks of three orthotopic transplants after pig-to-baboon cardiac xenotransplantation.

Pig-to-baboon orthotopic transplantation was performed using an adult baboon recipient and CD46 transgenic pig. The recipients were treated with an α -Gal polymer to block the effects of anti-Gal antibody and with ATG induction and a tacrolimus and sirolimus based maintenance immunosuppression. Heart function was continuously monitored by intramyocardial electrocardiography and every 3-4 days by serial echocardiography. Standard hematological, serum chemistry and cardiac troponin levels were monitored every 2-3 days.

The animals survived 34, 40, and 57 days in a healthy condition. Mortality was a result of pneumonitis, respiratory failure and bowel infarction respectively. One recipient (survival 40 days) showed impaired perioperative left ventricular function with an

ejection fraction of 25% on echo. Over the next two weeks, the ejection fraction in this animal returned to normal (60%) and troponin levels normalized. This study shows the longest survival of orthotopic cardiac xenografts to date with recovery of impaired heart function after early ischemia reperfusion injury. This significant improvement in ventricular function suggests that the normal reparative processes appear to function across the xenotransplantation barrier, supporting the potential clinical potential of cardiac xenotransplantation.

Abstract# 99

GalT-KO and GalT-KO/hDAF Pig Lungs Are Protected from Hyperacute Lung Rejection by Human Blood. Shahrooz S. Kelishadi,¹ Tiffany Stoddard,¹ Tianshu Zhang,¹ Nitin Sangrampurkar,¹ Mitch Higuchi,¹ Bao N. Nguyen,¹ Emily Welty,¹ Christopher J. Avon,¹ Amal Laaris,¹ Xiang-Fei Cheng,¹ David Ayares,² Agnes M. Azimzadeh,¹ Richard N. Pierson III.¹ ¹*Surgery, University of Maryland School of Medicine and Baltimore VAMC, Baltimore, MD;* ²*Revivicor, Blacksburg, VA.*

Purpose: To determine whether the galactosyl transferase gene knock-out (GalT-KO) protects pig lungs from hyperacute lung rejection (HALR) by human blood. The effects of adding human decay accelerating factor (hDAF) to the GalT-KO background will also be examined for the first time.

Methods: Heparinized fresh human blood was used to perfuse GalT-KO pig lungs (n=10). Lung function was assessed by flow, pulmonary vascular resistance (PVR), oxygen transfer, and tracheal edema using pre-defined survival endpoints. Historical wild-type (WT) pig lungs (n=15) provide context for analysis. Additionally, two pig lungs expressing GalT-KO/hDAF were recently examined, one of which received treatment with Xigris.

Results: Median lung survival in GalT-KO lungs was 114 minutes (range 15min to >240min) vs. 10 minutes in WT lungs (range 4min to 36min, p=0.01); 2/2 GalT-KO/hDAF pig lungs survived >240 min. PVR at 5 minutes for GalT-KO lungs was 62±35mmHg-min/L vs. WT lungs (308±178 mmHg-min/L at 5 minutes) vs. 23mmHg-min/L for each GalT-KO/hDAF lung. Complement activation (ΔC3a) at 15 minutes was significantly lower with GalT-KO (ΔC3a 133±243 ng/mL) than WT lungs (vs. 2080±1332 ng/mL, p=0.039). GalT-KO was also associated with reduced platelet activation: only 4±4% of circulating platelets express CD62P at 15 min. vs. 16±13% in the WT group (p=0.05); ΔBTG at 15 min [223±151 IU/mL (GalT-KO) vs. 1196±1186 IU/mL (WT)], and less thrombin formation (ΔF1+2) at 15 minutes (GalT-KO: 5±7.9 nM vs. WT: 22±39 nM). ΔC3a, % of circulating platelets expressing CD62P, ΔBTG, and ΔF1+2 measurements are in progress for GalT-KO/hDAF. Platelet sequestration was delayed as mean % of the initial platelets remaining in the GalT-KO lung perfusate was 52±19% at 15min vs. 27±9% in the WT lung group (p=0.02). Neutrophil sequestration was also diminished in GalT-KO (58±15% residual) vs. WT lung (5±3% residual, p=0.004). **Conclusion:** GalT-KO pig lungs are significantly protected from several facets of HALR: complement and coagulation cascade activation, neutrophil sequestration, and platelet activation are all partially attenuated by this modification alone. Preliminary results suggest that GalT-KO/hDAF lungs offer even further protection.

Abstract# 100

Disseminated Intravascular Coagulation Is Associated with Tissue Factor Expression on Recipient Platelets and Monocytes. Chih Che Lin,^{1,3,4} Mohamed Ezzelarab,¹ Corin Torres,¹ David Ayares,² Anthony Dorling,³ David K. C. Cooper.¹ ¹*Thomas E. Starzl Transplantation Institute, University of Pittsburgh, Pittsburgh, PA;* ²*Revivicor Inc., Blacksburg, VA;* ³*Department of Immunology, Imperial College London, Hammersmith Hospital, London, United Kingdom;* ⁴*Department of Surgery, Chang Gung Memorial Hospital, Kaohsiung, Taiwan.*

Purpose

Acute humoral xenograft rejection (AHXR), frequently associated with disseminated intravascular coagulation (DIC), remains a challenge in pig-to-primate xenotransplantation (Tx). A previous *in vitro* study showed that recipient platelets and monocytes were induced to express tissue factor (TF) after incubation with porcine endothelial cells, and we speculated this may contribute to thrombosis. The present study investigated whether circulating (extragraft) monocytes and platelets express TF and whether this relates to the development of DIC after pig Tx.

Methods

Baboons (n=7) received an aortic patch or kidney from either a wild-type (WT) or α1,3-galactosyltransferase gene-knockout (GTKO) pig, with or without immunosuppressive therapy. Baboon monocytes and platelets were isolated from blood before and after Tx. Surface TF phenotype was determined by flow cytometry. Functional TF activity was determined by clotting time assays after mixing monocytes and platelets with recalcified Factor VII (FVII)-deficient plasma with or without added FVII.

Results

Before Tx, monocytes and platelets did not express TF or display TF-dependent procoagulant activity. After GTKO aortic patch Tx, the baboons were euthanized by day 35 without developing DIC. However, by day 28, circulating platelets (but not monocytes) expressed TF and promoted TF-dependent clotting in recalcified plasma. In the absence of immunosuppression, WT kidneys survived <1 day before the onset

of DIC, at which time TF activity was detected on both platelets and monocytes. After GTKO kidney Tx in immunosuppressed baboons, platelets expressed TF as early as day 3. In contrast, monocytes began to express TF only at the onset of DIC.

Conclusions

This study links expression of TF on recipient monocytes and platelets with the development of DIC. Expression on platelets appeared to predict the subsequent development of DIC, whereas that on monocytes was associated with the onset of DIC. Whilst our observations do not establish a causal relationship, they provide the basis for further study.

Abstract# 101

International Human Xenotransplantation Inventory. Antonino Sgroi,¹ Leo Buhler,¹ Megan Sykes,² Luc Noel.³ ¹*Surgical Research Unit, Department of Surgery, Geneva University Hospital, Geneva, Switzerland;* ²*Transplantation Biology Research Center, Massachusetts General Hospital, Boston, MA;* ³*World Health Organization, Geneva, Switzerland.*

BACKGROUND: Xenotransplantation carries inherent risks of infectious disease transmission to the recipient and even to society at large, and should only be carried out with tight regulation and oversight. A collaboration between the *International Xenotransplantation Association*, the *University Hospital Geneva* and the *World Health Organization* has established an international inventory (www.humanxenotransplant.org) aiming to collect basic data on all types of xenotransplantation practices on humans that are currently ongoing or have been recently performed.

METHODS: We collected information using publications in scientific journals, presentations at international congresses, internet-based information, and declarations of IXA members. An electronic questionnaire is available on the website www.humanxenotransplant.org, which can be filled out and sent to the office in Geneva.

RESULTS: We identified a total of 16 recent or current human applications of xenotransplantation, eight were currently ongoing and one will start soon. The source animal was: pig (n=7), sheep (n=3), calf (n=1), rabbit (n=2), blue shark (n=1), hamster (n=1), and unknown (n=1). All trials transplanted xenogeneic cells, i.e. islets of Langerhans (n=6), hepatocytes (n=1), kidney cells (n=1), chromaffin cells (n=1), embryonic stem cells (n=2), fetal (n=4) and adult cells (n=1) of various organs. The treatments were performed in 10 different countries, 7 in Europe, 2 in Russia, 2 in Asia, 2 Mexico, 1 in USA and 1 in Africa. Six countries had no national regulation on xenotransplantation.

CONCLUSION: Several clinical applications of cell xenotransplantation are ongoing around the world, often without any clear governmental regulation. This information should be used to inform national health authorities, health care staff and the public, with the objective of encouraging good practices, with internationally harmonized guidelines and regulation of xenotransplantation.

Abstract# 102

Cells from α1,3-Galactosyltransferase Gene-Knockout Pigs Additionally Transgenic for Human Membrane Cofactor Protein Demonstrate Resistance to Human Complement-Mediated Cytotoxicity. Hidetaka Hara,¹ Cassandra Long,¹ Mohamed Ezzelarab,¹ Peter Yeh,¹ Carol Phelps,² David Ayares,² David K. C. Cooper.¹ ¹*Surgery, Thomas E. Starzl Transplantation Institute, University of Pittsburgh, Pittsburgh, PA;* ²*Revivicor, Inc., Blacksburg, VA.*

Purpose: (1) To compare the antibody binding and complement-mediated cytotoxicity (CDC) of human sera to pig peripheral blood mononuclear cells (PBMC) and porcine aortic endothelial cells (PAEC) from (i) wild-type (WT), (ii) α1,3-galactosyltransferase gene-knockout (GTKO), (iii) human membrane cofactor protein transgenic (MCP) and (iv) GTKO/MCP pigs. (2) To investigate the effect on binding and CDC of human sera following activation of PAEC.

Methods: Pooled human serum was tested by flow cytometry for binding of IgM and IgG (5% serum concentration) and CDC (25% serum concentration) to PBMC and PAEC from WT, GTKO, MCP, and GTKO/MCP pigs. PAEC from all 4 pig types were activated by IFN-γ, and again tested for antibody binding and CDC.

Results: There was higher binding of IgM and IgG to WT and MCP than to GTKO and GTKO/MCP PBMC and PAEC, but there were no differences in binding (i) between WT and MCP cells or (ii) between GTKO and GTKO/MCP cells. CDC of WT PBMC and PAEC was significantly greater than of GTKO, MCP, and GTKO/MCP PBMC (WT 73%; GTKO 37%; MCP 30%; GTKO/MCP 4%) and PAEC (WT 51%; GTKO 18%; MCP 1%; GTKO/MCP 0%) (both p<0.05). Importantly, there was no lysis of GTKO/MCP PAEC. After activation of PAEC, although CDC was increased against WT and GTKO PAEC, MCP and GTKO/MCP PAEC demonstrated significant resistance to lysis (WT 73%; GTKO 41%; MCP 5%; GTKO/MCP 0%).

Conclusions: (1) CDC of all 3 types of genetically-engineered PBMC and PAEC was significantly lower than of WT PBMC and PAEC, with lysis of GTKO/MCP PAEC being significantly lower than to GTKO or MCP cells. (2) PAEC from both MCP and GTKO/MCP showed resistance to lysis even after activation of the cells. (3) Organs from GTKO/MCP pigs should provide considerable protection against CDC.

Abstract# 103

Role for CD47-SIRP α Signaling in Human T Cell Proliferation in Response to Stimulation with Porcine Antigen Presenting Cells. Hiroyuki Tahara,¹ Hideki Ohdan,¹ Kentaro Ide,¹ Toshimasa Asahara.¹ ¹*Department of Surgery, Hiroshima University, Hiroshima, Japan.*

We have previously proven that genetic induction of human CD47 on porcine cells provides inhibitory signaling to signal regulatory protein(SIRP) α on human macrophages; this provides a novel approach to preventing macrophage-mediated xenograft rejection. A recent report indicated that the similar CD47-SIRP system negatively regulates the functions of both T cells and antigen presenting cells(APCs) in humans. We hypothesize that the interspecies incompatibility of CD47 may also act as an additional barrier of T cell-mediated xenograft rejection. We have analyzed the frequency and proliferative activity of human T cells responding to either porcine or allogenic APCs by using *in vitro* mixed lymphocyte reaction (MLR) assay with a CFSE-labeling technique. Irradiated stimulator porcine or human peripheral blood mononuclear cells(PBMCs) were cultured with CFSE-labeled responder human PBMCs. By FCM analysis, the number of division precursors was extrapolated from the number of daughter cells of each division and from the mitotic events, and precursor frequencies in CD4⁺ and CD8⁺ T cell subsets. Using these values, stimulation index(SI) was calculated. The frequencies of alloreactive and xenoreactive CD4⁺ T cell precursors were almost identical, 4.5 $\pm$ 0.8% and 4.4 $\pm$ 0.9%, respectively (n=10-12, for each type of precursor). However, the SI of xenoreactive CD4⁺ T cells was significantly higher than that of alloreactive CD4⁺ T cells, indicating a stronger reaction by a single xenoreactive CD4⁺ T cell. In the presence of human CD47-Fc(containing the extracellular domain of human CD47 fused to the Fc portion of human Ig, 10 μ g/ml), the SI of xenoreactive CD4⁺ T cells was significantly reduced to a level similar to that of alloreactive CD4⁺ T cells (n=12, *P*<0.05), although the frequencies of their precursors were absolutely uninfluenced. The frequencies of alloreactive and xenoreactive CD8⁺ T cell precursors were also identical, i.e. 3.5 $\pm$ 0.3%, 3.9 $\pm$ 0.9%, respectively (n=10-12). The SI of both alloreactive and xenoreactive CD8⁺ T cells did not differ; however, that of xenoreactive CD8⁺ T cells was significantly suppressed in the presence of human CD47-Fc (n=12, *P*<0.05). These results suggest that the T cell responses to porcine cells are stronger than those to allogeneic cells because of the interspecies incompatibility of CD47. Moreover, genetic manipulation of porcine APCs to induce human CD47 expression might attenuate human T cell-mediated xenograft rejection.

Abstract# 104

Efficient Lytic Infection of Porcine Endothelial Cells by Human Cytomegalovirus Strains. Anne-Laure Millard,¹ Lea Häberli,¹ Maddalena Ghielmetti,¹ Mårten K. J. Schneider,¹ Jörg D. Seebach,³ Walter Bossart,² Nicolas J. Mueller.¹ ¹*Division of Infectious Diseases and Hospital Epidemiology, University Hospital, Zurich, Switzerland;* ²*Institute for Medical Virology, University Hospital, Zurich, Switzerland;* ³*Laboratory for Immunology and Allergology, University Hospital, Geneva, Switzerland.*

Reactivation of human cytomegalovirus (hCMV) is a potential risk following the clinical application of pig-to-human xenotransplantation. Since little is known about undesirable side-effects arising from hCMV infection of porcine organs, we investigated the capabilities of various hCMV strains to infect porcine endothelial cells (pEC) and putative immunological consequences thereof.

pEC from different anatomical origins were incubated with the hCMV laboratory strains AD169 and TB40/E or a clinical isolate at a multiplicity of infection (MOI) ranging from 0.1 to 100. Viral replication kinetics, evolution of cytopathology, and lytic end points were analyzed. Consequences of pEC infection on human NK cell activation were evaluated using xenostimulation assay assessing NK cell IFN γ secretion and cytotoxicity.

Infection was evident and a maximum percentage of infected cells was reached at a MOI of 10. hCMV replicated in all tested pEC types, with a fraction of infected cells ranging from 1% to 50%. AD169 infection of 2A2 (microvascular EC) resulted in cytopathic effect (CPE) development by 3 dpi and in lysis of 70% of the cells at 7 dpi. Contrary, no CPE was observed in PED (aortic EC) up to 15 dpi. Infection of 2A2 with TB40/E was non-lytic and resulted in accumulation of both extra- and intracellular virus. Virus titers reached a maximum at 5 dpi, peaking at levels 20-fold higher than residual input virus. We then determined whether infected pEC supported a complete replication cycle and produced viral progeny. After 5 dpi pEC supernatants and lysates revealed the production of significant amounts of virus. Preliminary results showed that coculture of human NK cells and infected pEC resulted in increased NK killing and IFN γ production.

Altogether, these findings provide evidence that pEC are permissive and support the complete productive replication cycle of hCMV. However, CPE are cell-type and strain dependent. Moreover, pEC infection leads to modification of the xenogeneic cytotoxicity mediated by human NK cells. Our findings allow a better estimation of the potential role of hCMV cross-species infection following xenotransplantation and may be crucial to guide future clinical trials.

Chronic Rejection/Injury: Innate and Adaptive Immunity I

Abstract# 105

Testing for Donor-Specificity of Antibodies Post-Transplantation Increases the Predictability of Chronic Rejection. Mikki Ozawa,¹ Arundhati Panigrahi,² Paul I. Terasaki,³ Narindar Mehra.² ¹*One Lambda, Inc., Canoga Park, CA;* ²*All-India Institute of Medical Sciences, New Delhi, India;* ³*Terasaki Foundation Laboratory, Los Angeles, CA.*

Purpose: Post-transplant HLA antibodies have been shown to be linked to chronic graft failure, yet some recipients do well in the presence of antibodies. We investigated whether testing for donor-specificity of antibodies improves the predictive value of antibodies in regards to chronic rejection.

Methods: In a prospective study of 400 renal transplant recipients, post-transplant sera were tested for HLA and MICA antibodies, and positive sera were tested by single antigen beads to determine whether the antibodies were donor-specific. One year after testing, biopsy and clinical data on the graft status were collected. Patients who did not have follow-up or died with function were excluded from analysis.

Results: Of the 350 patients with follow-up and ABO typing info, 102 (29%) were found to have antibodies. Sixteen of those had donor-specific A, B, or DR antibodies (DSA), while 26 had non-donor specific ab's (NDSA). Sixty patients had only DP, DQ, Cw, or MICA antibodies, and were grouped into "Donor-specificity unknown", as these typing were not available at the time of this report. Table 1 summarizes the antibody groups and transplant outcome one year later. Interestingly, the mean serum creatinine values at the time of antibody testing were very similar among the groups, regardless of the presence of antibody or the type (ranged 1.34 to 1.73mg/dL). One year later, however, a striking 63% of DSA patients had either returned to hemodialysis, were regrafted, had died, or had biopsy-proven chronic rejection, compared to only 23% in NDSA patients and 13% in those who were negative (DSA vs. Neg, *p*=0.00009). MICA antibodies also showed significant association with graft failure or CAN (*p*=0.03), and donor MICA typing is underway.

Conclusion: In this prospective study of 350 renal patients, DSA detected post-transplantation were highly correlated with chronic rejection. Inclusion of specificity analysis in post-transplant antibody monitoring would significantly improve the predictability of chronic rejection.

Table 1		# Patients n=350	Mean SCR at time of Ab testing (mg/dL)	Returned to HD, regrafted, CAN, or died	% fail or CAN	p-value
DSA	A, B, DR	16	1.56	10	63%	0.00009
NDSA	A, B, DR	26	1.34	6	23%	0.21
Donor- specificity unknown	MICA only	21	1.56	7	33%	0.033
	DQ or DP only	28	1.64	6	21%	0.27
	Cw only	11	1.73	2	18%	0.63
Negative		248	1.41	31	13%	reference

Abstract# 106

Donor CD4 T Cells within Solid Organ Transplants Contribute to Graft Rejection. Thet Su Win,¹ Sylvia Rehakova,¹ Margaret Negus,¹ Kourosh Saeb-Parsy,¹ Martin Goddard,² Eleanor Bolton,¹ Andrew Bradley,¹ Gavin Pettigrew.¹ ¹*University of Cambridge;* ²*Papworth Hospital, United Kingdom.*

This study examines the contribution of donor CD4 T cells within heart allografts to the development of graft vasculopathy, by providing help to recipient B cells for generating effector autoantibody responses.

B6 mice were transplanted with MHC class II-disparate bm12 hearts. The *allo*- and *auto*-antibody responses were quantified, and their contribution to allograft vasculopathy assessed by incorporating B cell deficient mice as recipients. The role of donor CD4 T cells in providing help for autoantibody was examined by removing CD4 T cells from donor hearts before transplantation by three approaches: treating with anti-CD4 mAb, administering 1300Gy lethal irradiation, and using bm12 donors that are genetically deficient in T cells. The contribution of donor CD4 T cells to autoantibody development was further assessed by challenge with bm12 CD4 T cells two weeks prior to transplantation.

Bm12 heart grafts developed progressive vasculopathy and were rejected slowly (MST=95 days, n=17). The contribution of antibody-mediated effector mechanisms was confirmed by histopathological evidence (fibrinoid necrosis and vascular proliferation), by complement C4d staining, and by a reduction in the severity of vasculopathy and long-term graft survival in B cell deficient recipients. Surprisingly, no autoantibody was detected, but recipients instead developed autoantibody. The autoantibody response was completely dependent upon the provision of help from donor CD4 T cells; it was abrogated by transplanting CD4 T cell deficient hearts.

To further confirm an effector role for autoantibody in vasculopathy development, B6 mice were primed for humoral autoimmunity, but not for alloimmunity, by injecting with highly purified bm12 CD4 T cells prior to transplantation. Heart grafts were rejected more rapidly (MST=29 days, n=4, *p*<0.0001), and demonstrated severe vasculopathy. Finally, BM-chimeric recipients that lacked MHC class II expression only on B cells did not develop autoantibody, suggesting that cognate interaction between the donor CD4 T cells and MHC class II of recipient B cells is crucial for post-transplant humoral autoimmunity.

Our results demonstrate the novel finding that help for autoantibody production is provided by graft-versus-host cognate recognition of recipient B cell MHC class II by

donor CD4 T cells. This autoantibody contributes to the development of vasculopathy independently of alloantibody.

Abstract# 107

TLR4-MyD88 Signaling Is Required for the Development of Mouse Chronic Cardiac Allograft Damage in a Model of Chronic Cardiac Allograft Rejection. Gang Chen,¹ Huiling Wu,¹ Jainlin Yin,¹ Josette M. Eris,¹ Steven J. Chadban,¹ ¹*Collaborative Transplantation Research Group/Renal Medicine, University of Sydney/RPAH, Sydney, NSW, Australia.*

Toll like receptors (TLRs) are innate immune receptors that play an important role in innate immunity but also provide a link between innate and adaptive immunity. MyD88 is a key TLR signal adaptor. A recent clinical study reported that activation of innate immunity in heart-transplantation recipients through TLR4 contributes to the development of chronic rejection after cardiac transplantation. In this study, we aimed to determine whether TLR4-MyD88 signaling is required for the development of chronic allograft damage using TLR4^{-/-} and MyD88^{-/-} mice in a murine model of chronic cardiac allograft rejection. **Methods:** Cardiac transplants were performed: B6.C-H-2^{bm12} (B6.H-2^{bm12}) hearts to WT, MyD88^{-/-} and TLR4^{-/-} mice (all C57BL/6-H-2^b – single MHC class II mismatch) as allografts (n = 6-8/group) and C57BL/6 to C57BL/6 isografts (n = 5). Blood and tissue were harvested at day 49 after transplantation. Cell infiltration, fibrosis and vasculopathy were assessed histologically (grade 0–5). CD4⁺Foxp3⁺ cells in the blood and spleen were measured by flow cytometry analysis. **Results:** All hearts remained pulsatile until sacrifice. Histology of TLR4^{-/-} and MyD88^{-/-} recipients showed protection from leukocyte infiltration (1.2±1.1 & 1.0±1.1 vs 3.2±1.4, p < 0.01), fibrosis (0.75±1.0 & 0.6±1.2 vs 2.6±2.0, p < 0.05) and vasculopathy (0.28±0.6 & 0.04±0.1 vs 1.9±2.2) at day 49 post transplantation compared to WT recipients. By FACS, the ratio of CD4⁺Foxp3⁺ regulatory T (Treg) cells to total CD4⁺ cells at day 49 post transplantation in spleen was significantly increased in TLR4^{-/-} and MyD88^{-/-} recipients versus WT allograft and isograft mice (18.1±0.2 & 19.0±0.9% vs 12.9±1.1 & 11.8±0.2%, P<0.01). **Conclusion:** Absence of TLR4 and MyD88 signaling reduces chronic allograft damage in a murine model of chronic cardiac rejection. One mechanism of protection may be enhancement of regulatory T cell function. Promotion of Treg generation via the TLR4/MyD88 pathway may be one important consequence of cross-talk between innate and adaptive immunity.

Abstract# 108

Activation of the Notch Pathway in Vascular Endothelial Cells Prevents Apoptosis by Promoting Protective Gene Expression and Survival Pathway: Implication in Transplant Arteriosclerosis. Thibaut Quillard,¹ Stéphanie Coupel,¹ Flora Coulon,¹ Juliette Fitau,¹ Maria-Cristina Cuturi,¹ Elise Chiffolleau,¹ Béatrice Charreau,¹ ¹*INSERM U643, ITER, Nantes, France.*

Endothelial cell (EC) activation, injury and apoptosis are the key events associated with transplant arteriosclerosis (TA) and chronic allograft rejection (CR). Notch is a major signalling pathway controlling vascular function and injury. Nonetheless, the involvement of Notch, at endothelial level, in both TA initiation and progression remains unknown. Using a fully MHC mismatched rat cardiac allograft model of CR, we found that TA at Day100 correlates with a strong decrease in both expression and activity of the Notch pathway in transplant as compared to tolerant and syngeneic controls. The present study investigates the contribution of Notch activity to EC survival. In this purpose, a recombinant adenovirus, encoding the Notch2 intracellular Domain (NICD) and the reporter GFP cDNAs was constructed and used to maintain Notch activity in cultured primary human arterial EC. Our results demonstrate that NICD protects EC from cell death induced by TNFα in the presence of CHX or by anoikis as measured by AnnexinV and DNA content staining. NICD mediates cytoprotection through the regulation of key-apoptosis molecules. Using an apoptosis-dedicated qPCR array, we found that 14 out of a panel of 88 apoptosis-related genes were significantly regulated. NICD upregulated Bcl2, Bcl2a1 and Bcl-xl (2.5-, 8.2- and 2.8-fold compared to non-infected cells, respectively). In addition, pro-apoptotic genes Bim, Drp1, Bok and CD40 were markedly downregulated in transduced cells (6.7-, 11.4-, 3.9- and 14.5-fold decrease, respectively). Western blotting analysis confirmed the induction of protective molecules (Bcl2 and Bcl-xl) and the inhibition of pro-apoptotic proteins (bad, bak and bax). Consistent with these data, NICD conducted phosphorylation of Akt as well as a reduction of PTEN expression, suggesting that the cytoprotective activity of NICD may be mediated by recruitment of the PI3K survival pathway. To conclude, our findings indicate that TA correlates with a decreased Notch signaling in transplant and that activation of the Notch pathway in vascular EC prevents apoptosis by promoting protective gene expression and survival pathway. These data suggest that controlling Notch activity may prevent EC dysfunction associated with TA and CR.

Abstract# 109

Donor Age Intensifies the Early Immune Response. Christian Denecke,¹ Xupeng Ge,¹ Irene Kim,¹ Daman Bedi,¹ Anne Weiland,¹ Anke Jurisch,¹ Steven McGuire,¹ Johann Pratschke,² Stefan G. Tullius.¹ ¹*Div. of Transplant Surg, BWH, Transplant Surg Res Lab, Boston;* ²*Dept. of Surgery, Charite Berlin, Germany.*

Increasing numbers of organs from elderly donors are currently utilized for transplantation. Advanced donor age may not only be associated with physiological impairments but also with a modified immune response of the recipient. We hypothesized a more potent early immune response following the transplantation of elderly donor organs and we analyzed the immune response in a mouse heart transplant model. Young B6 mice received heart allografts from 3, 12 and 18mths old bm12 donors. The recipients immune response and intragraft changes were analyzed.

Elderly, non-manipulated hearts contained overall significantly elevated frequencies of CD4⁺ and CD8⁺ T-cells and DCs (CD11c⁺) (18mths vs. 3mths: CD4⁺: 2.77% vs. 1.35%, CD8⁺: 3.90% vs 1.71%, CD11c⁺: 46.1% vs. 11.8%, p<0.05).

Following engraftment of 18mths old heart grafts numbers of activated DC's (CD11c⁺I-A^b and CD11c⁺CD40⁺) had significantly increased in recipient spleens (day 14:p<0.05). In parallel, frequencies of effector/memory phenotype T-cells (CD4⁺CD44^{high}CD62L^{low} and CD8⁺CD44^{high}CD62L^{low}) were significantly elevated with increasing age (3 vs. 12 vs. 18mths: CD8⁺CD44^{high}CD62L^{low}: 6.7% vs. 8.4% vs. 12.2%, respectively, p<0.01). In addition, Tregs (CD4⁺CD25⁺Foxp3⁺) were also elevated (3 vs. 12 vs. 18mths: 2.9%vs.9.6%vs.11%,p<0.05) T-cell alloreactivity, as measured by IFNγ-production, increased with donor age (3mths vs. 12mths vs.18mths: 24.4±1.5 vs 74.4±21.9 vs. 73.2±7.8 IFNγ-producing spots/5x10⁶ cells, p<0.05). Mixed Lymphocyte Reaction (MLR) at day 14 revealed a gradual increase in splenocyte proliferation with advancing donor age (p=0.0002) indicating an enhanced immunogenicity of older organs.

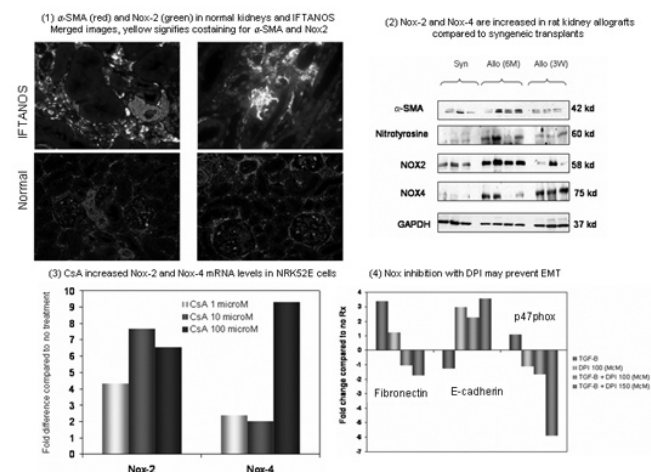
Immunohistochemical staining confirmed augmented CD4⁺ and CD8⁺ T-cell infiltrates and an intense Ki67 positivity of GICs in 18mths old heart grafts, emphasizing an intensified immune response towards organs from old donors.

In summary, old native heart transplants contain an overall higher number of passenger leukocytes contributing to an increased immunogenicity of these organs. After transplantation, a more potent DC and T-cell activation and intragraft T-cell infiltration was observed in elderly organs.

Abstract# 110

Nox and Chronic Kidney Allograft Interstitial Fibrosis. Shannon Reese,¹ Madhu Adulla,¹ Surmeet Bedi,¹ Jose Torrealba,¹ Deb Hullett,¹ Arjang Djamali.¹ ¹*Nephrology, UW Madison, Madison, WI.*

To determine the role of oxidative stress (OS) in kidney allograft fibrosis, we are developing strategies that would decrease the **generation** of reactive oxygen species (ROS) instead of using ROS scavengers, the standard approach to antioxidant therapy so far. We therefore started to examine the expression of NADPH oxidase (Nox) subunits (Nox2, Nox4, p22 and p47phox) and their distribution in human and rat kidney allografts with chronic interstitial fibrosis (IFTANOS). Using double-staining immunofluorescent studies in human allografts (n=16) we showed that Nox2 was present in injured tubules costained with αSMA (Figure 1.1)



Similarly, interstitial macrophages (CD68⁺-Nox²⁺) and myofibroblasts (αSMA⁺-Nox²⁺) but not CD3⁺ T cells or S100A4⁺ fibroblasts expressed high Nox2 levels, suggesting that Nox is involved in the pathogenesis of allograft fibrosis via epithelial-to-mesenchymal transition (EMT), macrophage and myofibroblasts activation. We then examined the coexpression of Nox2, Nox4 and p22phox in normal and transplant kidneys with IFTANOS and showed that these molecules were upregulated in the latter group and that Nox2 and Nox4 were associated with p22phox expression. These results were confirmed in the Fisher to Lewis rat kidney transplant model (Figure 1.2). Immunoblot analyses at 3 weeks and 6 months showed that Nox4 and Nox2 levels were increased in the allogeneic (n=7) compared to syngeneic transplants (n=3). Greater Nox levels were

associated with increased OS (nitrotyrosine) and fibrosis (α SMA). We then performed *in vitro* studies in NRK52E cells to evaluate the effects of potential inducers/inhibitors of Nox. Cyclosporin A increased Nox2 and Nox4 mRNA levels in a dose-dependent manner (Figure 1.3), whereas treatment with DPI, a Nox inhibitor downregulated p47phox and prevented EMT (Figure 1.4). In aggregate, these studies demonstrate that Nox enzymes are involved in the pathogenesis of IFTANOS and suggest that the prevention of ROS generation through Nox inhibition may have beneficial effects on kidney allograft fibrosis.

Composite Tissue Allotransplantation

Abstract# 111

The Banff Composite Tissue Allotransplantation 2007 Working Classification of Skin Allograft Pathology. Linda Cendales,¹ Jean Kanitakis,² Stefan Schneeberger,³ Carolyn Burns,⁴ Phillip Ruiz,⁵ Luis Landin,⁶ Myriam Rummelink,⁷ Charles Hewitt,⁸ Anthony Landgren,⁹ Bruce Lyons,¹⁰ Cynthia Drachenberg,¹¹ Kim Soles,¹² Allan Kirk,¹³ David Kleiner,¹⁴ Lorraine Racusen.¹⁵ ¹Emory University and the Atlanta Veterans Affairs Medical Center, Atlanta; ²Ed.Herriot Hospital, Lyon, France; ³UPMC and Medical University Innsbruck, Pittsburgh and Innsbruck; ⁴Jewish Hospital and Sr Mary's Healthcare, Louisville; ⁵University of Miami, Miami; ⁶Pedro Cavadas Foundation, Valencia, Spain; ⁷CUB-ULB Hopital Erasme, Brussels, Belgium; ⁸Robert Wood Johnson Medical School/UMDNJ, Camden; ⁹Royal Melbourne Hospital, Victoria, Australia; ¹⁰Derriford Hospital, Plymouth, United Kingdom; ¹¹University of Maryland, Baltimore; ¹²University of Alberta, Edmonton, Canada; ¹³Emory University, Atlanta; ¹⁴National Cancer Institute, Bethesda; ¹⁵Johns Hopkins, Baltimore.

Composite Tissue Allotransplantation (CTA) is a recently introduced option for limb replacement and reconstruction of tissue defects. As other allografts, a CTA grafts can undergo immune-mediated rejection, and standardized criteria are required for characterizing and reporting severity and types of rejection. This manuscript documents the conclusions of a symposium on CTA rejection held at the Ninth Banff Conference on Allograft Pathology in La Coruna, Spain on June 26, 2007, and proposes a working classification scheme, the Banff CTA-07, for the categorization of CTA rejection. This classification was derived from public international consensus discussions regarding all published scoring systems for CTA rejection. Given the current limited clinical experience in CTA, a formal histological classification was established for acute skin rejection with the understanding that other types of rejection involving other tissues will be developed with periodic review of this emerging field. It was agreed that the defining features to diagnose acute skin rejection would include inflammatory cell infiltration with epidermal and/or adnexal structure involvement, epithelial apoptosis, dyskeratosis, and necrosis, and that severity of rejection will be graded under five categories. This classification refines proposed schemas, represents international consensus on this topic, and establishes a working collective classification system for CTA reporting.

Abstract# 112

The Role of Skin Biopsies in Diagnosing Clinical Rejection in Hand Composite Tissue Allotransplantation (CTA). Christina L. Kaufman,^{1,4} Ruben N. Gonzales,¹ Kadiyala V. Ravindra,^{3,4} Brenda W. Blair,¹ Joeshp F. Buell,^{3,4} Warren C. Breidenbach,^{1,2,4} ¹Christine M. Kleinert Institute, Louisville, KY; ²Kleinert Kutz and Associates, Louisville, KY; ³Jewish Hospital Transplant Center, Louisville, KY; ⁴Surgery, University of Louisville, Louisville, KY.

Aim: Traditionally allograft biopsy has dictated treatment of allograft rejection. We hypothesize that histological grade of the biopsy may over diagnose rejection in CTA. Hand CTA is unique in that the organ is external and early signs of rejection can be viewed directly. Skin is the first tissue in the CTA graft to show rejection.

Methods: For this study, rejection by defined by requirement of treatment. These episodes were compared with histological grade, and hand appearance. We reviewed 127 skin biopsies taken during 9, 7 and 1 years of follow up.

Results: Three observations surfaced. **First,** a rejection grading scale for CTA is still evolving. A review of the literature showed at least four different criteria are in use. The criteria used to grade biopsies at our center changed over the 9 year follow up. Bias towards calling higher grades of rejection, and more aggressive treatment occurred in the first two patients. A re-read of random biopsies taken from the first two patients showed a down-grade of rejection to grade II or I in five cases. **Secondly,** concomitant CMV infection in the last patient prevented the aggressive treatment of rejection. Despite high grade histology, the swelling, rash and redness responded to topical immunosuppression. Systemic treatment was not necessary. Analysis indicated that swelling and/or rash, and the percentage of skin involvement seemed to be more informative markers of existing (or resolving) alloreactivity than was histology. The biopsies taken from the graft shown below showed a grade III histology.



Thirdly, changes in hand function were not associated with changes in biopsy grade.

Conclusion: The histologic grade of skin biopsies from hand allografts appears to over estimate rejection. Alterations in immunosuppression should be based on appearance of the allograft, and clinical course as much as on the histologic grade of the biopsy.

Abstract# 113

Expression of Molecular Mechanisms of Lymphocyte Trafficking Correlates Closely with Skin Rejection in Human Hand Transplantation.

Theresa Hautz,¹ Bettina Zelger,² Gerald Brandacher,¹ Hans G. Mueller,³ Andrew W. P. Lee,⁴ Raimund Margreiter,¹ Stefan Schneeberger.¹ ¹Dept. of General and Transplant Surgery, Innsbruck Medical University, Austria; ²Dept. of Pathology, Innsbruck Medical University, Austria; ³Dept. of Dermatology, Innsbruck Medical University, Austria; ⁴Div. of Plastic Surgery, University of Pittsburgh.

Introduction: To understand in greater depth the molecular mechanisms involved in skin rejection in hand transplantation, we investigated 8 key molecular markers of lymphocyte trafficking, cellular rejection and antibody mediated rejection in human hand transplantation.

Methods: A total of 130 skin biopsies taken from three bilateral hand transplants were assessed by H&E histology (grades as per previously published classification 0-4b) as well as immunohistochemistry using antibodies for following markers: Lymphocyte Function-Associated Antigen (LFA)-1 = CD11a, Intercellular Adhesion Molecule (ICAM)-1 = CD54, Selectin E = CD62E, Selectin P = CD62P, VE-Cadherin = CD144, Human Leukocyte Antigen (HLA) II (DP, DQ, DR), Psoriasis = S100A7 and C4d. Levels of expression were assessed (0, +, ++, +++) and read in the light correlated with the rejection as well as time after transplantation.

Results: Rejection ranged between grade 0 and 4a with an average score of 0.9. In healthy skin, none of the markers investigated was consistently up-regulated. Upon rejection, CD54, CD62E and CD62P staining in endothelial cells was significantly increased. Expression of CD62E and CD62P correlated well with severity of rejection. The majority of infiltrating lymphocytes stained positive for CD11a. Interestingly, also keratinocytes were highly positive for CD11a at the onset of rejection. CD144 was detected on endothelial cells, but its occurrence did not correlate with rejection. Psoriasis expression was observed in keratinocytes in a basal and focal pattern and correlated well with rejection. For C4d, no consistent staining pattern was observed indicating that antibody mediated rejection did not play a role in these patients.

Conclusion: Molecular markers involved in lymphocyte trafficking are up-regulated upon skin rejection after hand transplantation and represent promising target for prophylaxis and treatment of rejection in Composite Tissue Allotransplantation.

Abstract# 114

Investigation of the Immunomodulatory Phenotype of Infiltrating Lymphocytes in Skin Rejection of Human Hand Allografts.

Theresa Hautz,¹ Gerald Brandacher,¹ Bettina Zelger,² Hans G. Mueller,³ Andrew W. P. Lee,⁴ Raimund Margreiter,¹ Stefan Schneeberger.¹ ¹Dept. of General and Transplant Surgery, Innsbruck Medical University, Innsbruck, Austria; ²Dept. of Pathology, Innsbruck Medical University, Austria; ³Dept. of Dermatology, Innsbruck Medical University, Austria; ⁴Div. of Plastic Surgery, University of Pittsburgh.

Introduction: Skin rejection has complicated the postoperative course in human hand transplantation. To better define the characteristics of the lymphocytic infiltrate human hand transplant biopsies have been investigated for expression of FoxP3 and Indoleamine 2,3-dioxygenase (IDO), a key regulatory enzyme to induce T-lymphocyte unresponsiveness.

Methods: A total of 104 skin biopsies taken from three bilateral hand transplant recipients during the first 6 years after transplantation were assessed by H&E histology (graded as per previously published classification 0-4b) as well as immunohistochemistry for IDO and FoxP3. Levels of expression were assessed (0, +, ++, +++) and interpreted in the light of clinical courses, time after transplantation, severity of rejection as well as markers for lymphocyte migration.

Results: Overall, rejection ranged between grade 0 and 4a with an average score of 0.94. IDO was found constitutively expressed in the endothelium independent of rejection. IDO expression in the cellular infiltrate was significantly increased upon and correlated well with severity of rejection (rejection grade 1, 0.91+/-0.85; rejection

grade 3, 2.30 ± 0.82 $p < 0.001$). FoxP3 positive T-cells were mainly found in severe rejection (rejection grade 1, 0.14 ± 0.35 ; rejection grade 3, 0.75 ± 0.75 $p = 0.019$). IDO expression correlated well with FoxP3 expression, although the overall staining intensity for FoxP3 was lower. A strong tendency towards higher expression of IDO as well as FoxP3 towards later time-points after transplantation was observed (year 1 – FoxP3 0.07 ± 0.26 , IDO 0.75 ± 0.92 [$n = 68$], year 3 – FoxP3 0.45 ± 0.74 , IDO 1.29 ± 0.99 [$n = 24$], year 5 – Fox 0.25 ± 0.45 , IDO 1.4 ± 0.97 [$n = 12$]). Expression of IDO correlated closely with expression of E-Selectin, P-Selectin, ICAM1 and LFA-1.

Conclusion: Characteristics of the cellular infiltrate indicate a strong tendency towards self limitation of the alloimmune response towards the skin with both time after transplantation as well as severity of rejection in human hand transplantation. Further studies are warranted to clarify the clinical relevance of these findings.

Abstract# 115

Prospective Analysis of the Immunologic Profile of a Hand Transplant Recipient in the First Year. Kadiyala V. Ravindra,¹ Warren C. Breidenbach,² Joseph Buell,¹ Suzanne T. Ildstad.³ ¹Department of Surgery, University of Louisville, Louisville, KY; ²Kleinert, Kutz, and Associates, Louisville, KY; ³Institute for Cellular Therapeutics, University of Louisville, Louisville, KY.

Introduction: A major obstacle to the wider application of hand transplantation is the long term complications associated with immunosuppression. Minimization of immunosuppression is an important goal in all transplant recipients. Currently there are no accurate tools to evaluate the immunological responsiveness which might help tailor the level of immunosuppression for an individual patient. The response of recipient lymphocytes to PHA, Candida, and alloantigen may represent laboratory tool towards this end. It has been reported that these 3 responses are hierarchical with response to alloantigen being the first to be lost, followed by *candida* and finally PHA.

Patient & Method: A 54 year old male received a proximal forearm transplant in November 2006. Immunosuppression included induction with a single 30 mg dose of alemtuzumab and maintenance with tacrolimus and mycophenolate mofetil. The patient developed an episode of cytomegalovirus infection followed by acute rejection after reduction of his immunosuppression during the 3rd post-operative month. These were successfully treated with Ganciclovir and topical tacrolimus & steroids respectively. Blood samples were drawn at selected time points, and subjected to phenotyping of lymphocyte subsets and immune monitoring for circulating peripheral blood regulatory T cells (T_{reg}) and proliferative responses to phytohemagglutinin (PHA), *candida*, and alloantigen.

Results: Alemtuzumab induction resulted in profound lymphopenia. At week 2 and 1 month, the response to PHA was intact (stimulation index 110 and 24 respectively), but response to alloantigen and *candida* suppressed ($SI < 3$). A similar immunologic profile persisted up through 6 months. At 1 year, the PHA and *candida* responses are robust (SI 69 and 38 respectively), but alloresponses have not returned. Current immunosuppression consists of tacrolimus (6-9 ng/ml) and mycophenolate mofetil (500 mg b.i.d.). There is no gross evidence of acute or chronic rejection.

Conclusions: Induction with alemtuzumab alters the recovery of immune response: recovery to *candida* was delayed beyond 6 months and to alloantigens beyond a year. In light of this, further reduction of immunosuppression may be contemplated in future hand transplants without the risk of rejection.

Abstract# 116

Prolonged Survival of Composite Facial Allografts in Non-Human Primates and Development of Donor-Derived Post-Transplant Lymphoproliferative Disorders. Author J. Nam,¹ Matthew G. Stanwich,¹ Eduardo D. Rodriguez,¹ Debra Kukuruja,¹ Cynthia Drachenberg,¹ Rachel Bluebond-Langner,¹ Aruna Panda,¹ Steven T. Shipley,¹ Stephen T. Bartlett,¹ Rolf N. Barth.¹ ¹University of Maryland School of Medicine, Baltimore, MD.

Composite tissue allotransplantation has achieved significant clinical advances despite an adequate preclinical model to study technical and immunosuppressive strategies. We have developed a non-human primate model of facial composite tissue allografts (CTA). Unilateral lower hemi-facial CTA (bone, muscle, skin) transplants were performed between mismatched cynomolgus monkeys. Immunosuppression consisted of 28 days of continuous IV tacrolimus monotherapy followed by tapered daily IM doses. Six animals received prophylactic ganciclovir. All animals had serial transplant biopsies. Ten transplants have been performed, with one loss secondary to line infection on day 27 without evidence of rejection. Two CTAs had evidence of chronic rejection (day 56, 99); with development of alloantibody after 30 days. Five CTAs had prolonged survival (day 60, 86, 108, 150, 177), but developed PTLDs resulting in experimental endpoints. All animals had clinically normal grafts, but 3 animals showed histological evidence of mild rejection not treated with any additional immunosuppressive therapy. PTLD tumors were analyzed using short tandem repeats (STR) to define donor or recipient origin. STR analysis demonstrated donor origin of 4 PTLD tumors and recipient origin in 1 animal. None of the PTLD animals had clinical evidence of rejection of skin, bone, or muscle. EBV was not detected in the serum of 2 tested animals, and ganciclovir therapy had no effect on the development of tumors. Tacrolimus levels of PTLD animals were higher than animals with rejected grafts (45 vs. 34 ng/mL; $p = 0.02$). Two additional animals have healthy grafts (day 24+, 52+) without evidence of rejection

or PTLTD, and have been converted to rapamycin after day 28. We have developed a preclinical model for facial CTA transplantation that achieves prolonged graft survivals with tacrolimus monotherapy. The high incidence of PTLTD tumors of donor origin represents an outcome similar to bone marrow transplantation in contrast to its rarity in solid organ transplantation. Our findings are a cautionary note regarding CTAs that include vascularized bone elements. Immunosuppressive protocol modifications have been made in an effort to decrease the incidence of these donor-derived PTLTDs.

Heart Transplantation

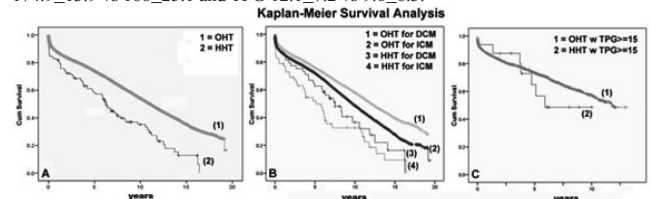
Abstract# 117

Heterotopic Heart Transplantation: The United States Experience. Jama Jahanyar,¹ Tarek A. Sibai,¹ Matthias Loebe,¹ Michael M. Koerner,¹ Guillermo Torre-Amione,¹ George P. Noon.¹ ¹Dept. of Surgery, Baylor College of Medicine, Houston, TX.

Heterotopic heart transplantation (HHT) is utilized in patients (pts) who do not qualify for standard orthotopic heart transplantation (OHT). Specific indications include refractory pulmonary hypertension and a donor-recipient size mismatch. The objective of this study was to analyze the UNOS database and compare outcomes of HHT to OHT.

The UNOS database with more than 58000 pts undergoing thoracic organ transplantation in the U.S. between 1987 and 2007 was reviewed (based on OPTN data as of May 1, 2007). Primary endpoint of this study was overall survival and subgroup survival [pts with transpulmonary gradient (TPG) ≥ 15 , ischemic (ICM) and dilated cardiomyopathy (DCM)]. Secondary endpoint was assessment of pretransplant criteria. Exclusion criteria were retransplantation and missing transplant dates.

Of 41379 who underwent OHT and 178 who underwent HHT, 32361 and 111 respectively, were enrolled in this study. Overall 1-, 5- and 10 year survival was significantly ($p < 0.001$) better in OHT (87.7%, 74.4%, 54.4%) than HHT (83.8%, 59%, 35.1%) (A). Survival in patients with DCM who underwent OHT (89.5%, 75.7%, 57.9%) was significantly ($P \leq 0.001$) better than in pts with ICM (86.1%, 72%, 48.5%). Although survival in pts with DCM and HHT (87.7%, 62.7%, 36.5%) was better than in pts. with ICM (78.8%, 52.1%, 32.7%), this difference did not reach statistical significance ($p = 0.19$) (B). Survival in pts with TPG ≥ 15 was 86.6%, 73.3%, 57.4% in OHT and 93.8%, 64.8%, 48.6% in HHT (C). This difference however, was not significant ($p = 0.5$). Pretransplant criteria (HHT vs OHT) with statistically significant differences ($P \leq 0.05$) were as follows (mean $\pm$ SD): recipient weight 78.9 ± 19.9 vs 74.1 ± 23.4 , recipient height 174.9 ± 13.9 vs 168 ± 25.1 and TPG 12.1 ± 7.2 vs 9.6 ± 6.3 .



Survival after OHT is superior to HHT. This survival benefit however, disappears in pts with a TPG ≥ 15 . Overall the survival after HHT is superior to the reported survival in pts who undergo LVAD implantation as destination treatment (REMATCH-trial/1-year survival of 52%). Thus in selected pts, especially those with elevated TPGs, HHT should be considered a viable option with overall good results.

Abstract# 118

Ventricular Assist Device as a Bridge to Heart Transplantation in Children: Can We Afford It? William T. Mahle, Glen Ianucci, Robert N. Vincent, Kirk R. Kanter. Sibley Heart Center Cardiology, Emory University School of Medicine, Atlanta, GA.

Ventricular assist devices (VADs) allow children with severe heart failure to be bridged to successful heart transplantation (HT). VADs are being used with increasing frequency in the pediatric population and newer devices allow even young infants to be supported. VAD implantation and maintenance, however, is quite expensive and the cost-effectiveness of VAD use in adults has been questioned. To date, an economic analysis of VAD support in children has not been undertaken.

Methods: We used Pediatric Health Information System (PHIS), an administrative database of the Child Health Corporation of America (a consortium of Children's Hospitals in North America), to determine the costs related to VAD use in children. Data on subjects < 18 yrs of age from 2002-2007 were reviewed. Hospital charges were converted to costs based on hospital-specific cost-to-charge ratios. Projected survival for subjects who were successfully bridged to HT was derived from published data. Cost-utility was expressed as cost per quality-adjusted life years (QALYs) saved, expressed in 2006 US dollars. All future costs and benefits were discounted at 3%.

Results: The median age at implantation from the PHIS database was 11.2 years, range 2 days to 17 yrs. The mean hospital cost per patient was \$522,489. Estimated survival to heart transplantation was 77% and estimated successful explantation without transplantation was 4%. The calculated cost-utility for VAD as a bridge to transplantation was \$123,232 /QALY saved. If one assumes that the children who survived to VAD explantation would otherwise have a high risk of hospital death (65%) without VAD support, then the calculated cost-utility would be \$117,235/ QALY saved. Even if

survival to transplantation exceeds 90%, VAD implantation does not achieve a favorable cost-utility ratio. VAD support in pediatric heart only becomes cost-effective if recovery and explantation can be achieved in over 22% of subjects. Conclusions: VAD supports serves as an effective bridge to heart transplantation in children. However, the cost-utility of this strategy is above the generally accepted threshold for cost-effectiveness (\$100,000/QALY). In a setting of limited healthcare resources VAD as a bridge to transplantation may not be justified.

Abstract# 119

Virtual Crossmatch Has High Accuracy and Improves Organ Allocation in Sensitized Patients. Josef Stehlik,¹ N. Islam,¹ D. Hurst,¹ A. Fuller,¹ J. Delgado, M. Movsesian, E. Gilbert, F. Bader, A. Kfoury, D. Renlund, P. Fisher, M. E. Hammond, D. Eckels. ¹UTAH Cardiac Transplant, Salt Lake City, UT.

Purpose: Heart transplantation (tx) in patients with HLA sensitization presents challenges in organ allocation. Virtual crossmatch (vXM), in which recipient HLA antibodies, identified by LabScreen PRA beads, are compared to the prospective donor HLA-type, could increase the use of allografts from distant donors (DD). Accuracy of vXM and outcomes of this approach in heart tx are not known.

Methods and Materials: To increase time-efficiency at the time of organ allocation, crossmatch testing is frequently initiated on pre-set trays which contain sera of multiple prospective sensitized recipients. We used results from these studies to determine expected accuracy of vXM.

We assessed outcomes of allocation algorithm implemented in 2001 in sensitized patients. Conventional prospective crossmatch was done when allografts were procured from local donors (LD), while vXM was utilized when allografts were offered from DD.

Results:

There were 257 direct T-Cell AHG crossmatch tests done with sera of 12 potential allograft recipients who had preformed HLA-antibodies of known Class I HLA specificities. As shown in table 1, the positive and negative predictive values (PPV, NPV) of vXM were 92% and 79%.

	T-cell AHG Crossmatch Incompatible (+)	T-cell AHG Crossmatch Compatible (-)	
Virtual Crossmatch Incompatible (+)	89	23	PPV = 79%
Virtual Crossmatch Compatible (-)	12	133	NPV = 92%

Table 1. Crossmatch results in recipients with preformed Class I HLA antibodies.

Table 2 shows outcomes in 30 sensitized patients who were eligible for vXM approach. 14 received allografts from LD with negative prospective crossmatch while 16 (53%) received allografts from DD with negative vXM. Three DD patients had a positive retrospective crossmatch - NPV of vXM was 81% in this cohort.

	Virtual Crossmatch (n=16)	Prospective crossmatch (n=14)	p-value
Age (yr)	44	48	NS
PRA (%)	39	27	NS
Graft Ischemic Time (min)	239	138	NS
Postoperative use of inotropic agents (%)	68	33	NS
Antibody Mediated Rejection, 1st Year (%)	31	13	NS
Cellular Rejection >1R, 1st Year (%)	19	7	NS
Mortality* (% / mean follow-up)	0% / 17 months	21% / 29 months	NS (log rank)

Table 2. Clinical outcomes in sensitized patients

Conclusions:

vXM has high negative and positive predictive values, accurately predicting results of standard direct crossmatch in most patients.

vXM allows use of allografts from DD and is likely to improve organ allocation in the disadvantaged group of sensitized patients.

Abstract# 120

Single Center Experience with New Heart Allocation System Implemented by United Network of Organ Sharing. Biljana Pavlovic-Surjanec,¹ Nilamkumar Patel,¹ Linda Dusek,¹ James Sinacore,¹ Jennifer Johnson,¹ Cassie Bessert,¹ Alain Heroux.¹ ¹Heart Failure/Heart Transplant Program, Loyola University Medical Center, Maywood, IL.

Purpose: In July 2006, United Network of Organ Sharing (UNOS) implemented a new allocation system for adult heart transplant (tx) candidates with the following sequence: Local Status 1A→Local Status 1B→Zone A Status 1A→Zone A Status 1B→Local Status 2. The purpose of this study is to evaluate the impact of new system on heart tx patients at a single center in region 7.

Methods: Patients transplanted during 1 year (y) prior to new system (7-11-05 to 7-11-06, Group 1, N=19) and 1 y following new system (7-12-06 to 7-12-07, Group 2, N=26) were compared for UNOS status at the time of transplant, waiting time, ischemia time, length of the hospital stay (LOS) before and after transplant, donor age, procurement-team travel distance and cost.

Results: New system significantly decreased median waiting time, but increased median ischemia time without affecting short-term survival: 1 patient died in each group. Number of transplants increased 37% in Group 2 mostly due to increased number of Status 1A patients supported by IABP without change in number of Status 1B and 2 patients.

	Status 1A	Status 1B	Status 2	VAD	IABP	Inotrope	Waiting time-days m (M)
Group 1 (N=19)	9	9	1	5	1	12	182 (114)*
Group 2 (N=26)	18	7	1	5	8	15	107 (35)*

m=mean (M=Median), *p<0.05 VAD=Ventricular Assist Device, IABP=Intra-Aortic Balloon Pump

Median pre-transplant LOS increased 16-fold in Group 2 (p=0.072), whereas mean pre-transplant LOS increased by 5 days. In Group 2, thirteen patients had donor heart procured in Zone A and thirteen patients had donor heart procured locally, whereas in Group 1, all donor hearts were procured locally. Median procurement-team travel distance increased 15-fold and median travel cost 10-fold in Group 2 (p<0.05).

	Donor age-y m	Ischemia time-min m(M)	Distance-miles m(M)	Procurement travel cost-\$ m(M)	Total procurement cost-\$ (M)	Pre tx LOS-d m(M)	Post tx LOS-d m(M)
Group 1 (N=19)	26.7	172 (159)*	45 (12)*	1,431 (620)*	(27,109)*	15 (1)	15 (12)
Group 2 (N=26)	27.1	203 (210)*	238 (178)*	4,982 (6,902)*	(34,215)*	21 (16)	18 (13)

m=mean (M=Median) *p<0.05

Conclusions: New allocation system had following consequences:

1. Waiting time decreased and number of transplants increased, particularly status 1A candidates,
2. Pre-transplant length of hospital stay and ischemia time increased with no effect on short-term survival.

Abstract# 121

Everolimus with Reduced-Exposure Cyclosporine Versus MMF and Standard-Exposure Cyclosporine in De Novo Cardiac Transplant Recipients: 12-Month Results from a Multicenter, Randomized Trial.

Jose Arizon,¹ Hans Lehmkuhl,² Ugolino Livi,² Mario Viganò,² Angelo Branzi,² Luis Almenar,² Gino Gerosa,² Francesco Musumeci,² Shaida Varnous,² Massimo Maccherini,² Mark Hexham,² Kevin C. Mange.² ¹Hospital Universitario Reina Sofia, Córdoba, Spain; ²On Behalf of the RAD B2411 Study Group.

The risk of acute rejection and the severity/incidence of cardiac allograft vasculopathy are significantly lower in heart transplant patients receiving everolimus compared to azathioprine. Use of concentration-controlled everolimus also permits reduction of cyclosporine (CsA) exposure without loss of efficacy. **Methods:** In a multicenter study, renal function and efficacy (biopsy-proven acute rejection; BPAR) were compared in *de novo* heart transplant recipients randomized to (i) concentration-controlled everolimus (target trough level 3-8ng/mL) + reduced-exposure CsA or (ii) MMF 1500mg b.i.d + standard-exposure CsA. All patients received corticosteroids. **Results:** 176 patients were randomized (92 everolimus, 84 MMF). Mean CsA C₀ at 6 and 12 months was 157ng/mL and 109ng/mL with everolimus, versus 219ng/mL and 180ng/mL with MMF. At baseline, creatinine clearance (CrCl, Cockcroft-Gault) was not balanced between treatment groups: everolimus 73±28mL/min, MMF 77±32mL/min. At 12 months, CrCl (everolimus 69±28mL/min, MMF 72±30 mL/min) and change in CrCl since baseline, were clinically comparable and not statistically different between groups. After month 1, CrCl remained stable in the everolimus group, but declined in the MMF group. Everolimus was statistically non-inferior to MMF for both BPAR grade ≥3A and efficacy failure (BPAR grade ≥3A, acute rejection with hemodynamic compromise, death, graft loss or lost to follow-up). BPAR grade ≥3A occurred in 22.8% everolimus patients and 29.8% MMF patients; efficacy failure occurred in 32.6% and 41.7% of patients, respectively. The rate of discontinuations due to adverse events was similar in both arms (everolimus 15.2%, MMF 15.5%). **Conclusions:** Concentration-controlled everolimus with reduced-exposure CsA results in comparable renal function and preserves efficacy compared to MMF with standard-exposure CsA to month 12 after cardiac transplantation.

Abstract# 122

Clinical Outcomes Associated with Simultaneous Heart-Kidney Transplantation. Tariq Shah,^{1,3,4,5} Suphamai Bunnapradist,² Jagbir Gill,² Steven K. Takemoto.¹ ¹National Institute of Transplantation, Los Angeles; ²Medicine, UCLA, CA; ³MOTC, St. Vincent Medical Center, LA, CA; ⁴Medicine & Urology, USC, LA, CA; ⁵Dept. of Pharmacy, Western University, LA, CA.

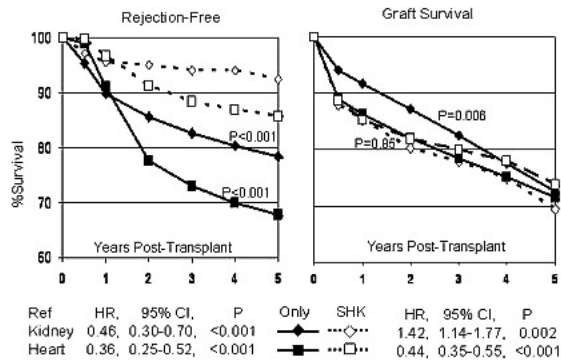
The 20-60 simultaneous heart-kidney (SHK) transplants performed in the United States every year provide a unique setting for comparing transplant care provided by nephrologists and cardiologists. This study examines rates of heart and kidney rejection and graft loss in the thoracic and kidney analytic files provided by the Organ Procurement Transplant Network.

Methods: A total of 346 SHK, 19,832 heart-only and 150,197 kidney-only deceased donor recipients transplanted during 1997-2006 were included in this study. Rates of steroid or antibody-treated rejection and graft loss as a function of time after transplantation were calculated by Kaplan-Meier product limit and Cox proportional hazards with adjustment for recipient and donor confounders.

Results: The figure below indicates recipients of SHK transplants (open symbols) had lower rates of rejection when compared to heart (square) or kidney (diamond) recipients. Survival rates were initially higher for kidney recipients with rates for SHK similar to heart recipients. The hazard ratio (HR) for graft loss was higher for SHK recipients compared to kidney, but lower when compared to heart recipients. The lower hazard for SHK in heart allografts might be attributed to risk associated with pretransplant dialysis (HR=4.09, 3.81-4.39, $P<0.001$). Approximately 10% (2/122) of cardiac transplant recipients initiated dialysis prior to transplantation.

The differing rates of SHK rejection observed in the heart and kidney analytic files might be attributed to susceptibility or treatment of heart and kidney allografts, rejection monitoring or reporting.

Conclusion: Retrospective examination of data provided to the OPTN indicates simultaneous heart-kidney transplantation seems to be effective for cardiac transplant candidates who require dialysis.



Islet and Stem Cell Transplantation

Abstract# 123

Local Delivery of T Regulatory (Treg) Cells in Combination with KRP203 Induces Tolerance to Pancreatic Islets. Stanislaw Stepkowski, Tammy Phan, Kloc Malgorzata, Barry D. Kahan, Wenhao Chen. *Surgery, The University of Texas Medical School, Houston, TX.*

Objective: KRP203, a novel structural analog of FTY720, has been documented to display 5-fold greater selectivity of agonism for sphingosine-1-phosphate (S1P) type 1 (S1P₁) receptor compared with S1P₃ receptor. Clinical trial has shown that FTY720 produced dose-limiting toxicity—bradycardia, due to its effect on S1P₃ receptor. We have tested the effect of KRP203 on the survival of islet allografts either alone or combined with local delivery of CD4⁺CD25⁺Foxp3⁺ T regulatory (Treg) cells. **Methods/Results:** Fresh B10 (H-2^b) islets transplanted under the kidney capsule to Balb/c (H-2^d) mice were acutely rejected at a mean survival time (MST) of 13.8±2.7 days (n=5). Although a 7-day therapy (p.o.) with 0.3 or 1 mg/kg KRP203 extended islet allograft survivals to 14.3 ± 1.2 days (n=3; 0.05) and 21 ± 1.2 days (n=6; $P<0.05$), respectively, 3 mg/kg prolonged survivals to 75.2 ± 30.5 days (n=6; $P<0.006$). Balb/c CD4⁺ T cells were cultured for 5-7 days with irradiated donor B10 splenocytes in medium supplemented with interleukin (IL)-2 and S-Nitroso-N-acetyl-DL-penicillamine (SNAP). Thereafter, CD4⁺CD25^{high} cells were purified by anti-CD4-FITC Ab and anti-CD25-PE Ab using FACSAria cell sorter and were confirmed to be Foxp3⁺ by intracellular staining (eBioscience kit). The number of 7x10⁵ CD4⁺CD25^{high}Foxp3⁺ Treg cells were mixed with 500 B10 islets and transplanted under the kidney capsule of Balb/c recipients. Such Treg/islet allograft transplants prolonged survivals to 54.3 ± 13.3 days (n=4; $P<0.001$) without any additional immunosuppression. When recipients of Treg/islet allografts were additionally treated with 1 mg/kg KRP203 for 7 days, they survived 61.5 ± 42.2 with 3 out of 6 surviving more than 100 days. Furthermore, addition of 3 mg/kg KRP203 for 7 days induced permanent acceptance of Reg/islet allografts (>100 days; n=4; $p=0.001$). **Conclusions:** These results showed that local delivery of donor-specific Treg cells extended islet allograft survival, whereas additional short therapy of KRP203 induced transplantation tolerance.

Abstract# 124

Depletion of CD103 Expressing Cells Promotes Long-Term Islet Allograft Survival. Lei Zhang, JiaoJing Wang, Alice Gaughan, Kelly Nye, Bryan Anthony, Gregg A. Hadley. *Division of Transplantation, The Ohio State University Medical Center, Columbus, OH.*

Previous studies using knockout mice have documented a key role for the integrin CD103 in promoting allograft rejection. These data are consistent with a critical role for CD103 expressing cells in this process. However, a direct test of this hypothesis has proven problematic due to the lack of mAbs that efficiently deplete CD103⁺ cells in wild type hosts. To circumvent this problem, we conjugated the non-depleting anti-CD103 mAb, M290, to the toxin, saporin (SAP), to produce an immunotoxin (M290-SAP) that selectively depletes CD103-expressing cells in vivo. Treatment of naive mice with M290-SAP selectively depleted CD103⁺CD8⁺ cells and dramatically

reduced the overall frequency of CD8⁺ lymphocytes in diverse compartment including intestinal intraepithelial lymphocyte, spleen and mesenteric lymph node. M290-SAP also depleted CD103⁺ dendritic cells (CD11c⁺) and T regulatory cells (Tregs, CD4⁺CD25⁺) in the above compartments. In the thymus, M290-SAP depleted CD103-expressing cells in both CD4⁺CD8⁺ and CD4⁺CD8⁺ subpopulations, both of which express CD103, leading to a dramatic reduction in the number of thymocytes (77.08 ± 5.74 × 10⁶ vs. 2.71 ± 0.82 × 10⁶, $P<0.0001$, in control vs. treated respectively). We next assessed the effect of M290-SAP in a fully allogeneic islet transplantation model (Balb/c→C57BL/6). M290-SAP produced long-term (LT) graft survival (>100d, n=9, vs. untreated median survival time 13d, n=8). Unconjugated M290 or isotype control (IgG-SAP) did not significantly prolong islet allograft survival. Graft histology showed little, if any, lymphocyte infiltration surrounding islet transplants in LT mice. In contrast, intense lymphocyte infiltration with disruption of islet morphology was observed in untreated mice. Pretreatment of donor islets with M290-SAP did not significantly prolong allograft survival indicating that the immunosuppressive effect of M290-SAP resides at the level of host. Interestingly, we found a 3-4 fold increase in both percentage and number of FoxP3⁺CD4⁺CD25⁺ cells in the spleen and draining lymph node from LT mice, though it remains to be determined whether such cells account for graft acceptance in M290-SAP treated recipients. In summary, these data document that depletion of CD103 expressing cells promotes long-term islet allograft survival. These findings point to a novel strategy for therapeutic intervention in islet allograft rejection.

Abstract# 125

Pancreatic Islets Engineered to Display on Their Surface an Exogenous FasL Protein with Potent Apoptotic Activity Achieve Tolerance in Allogeneic Recipients. Hong Zhao,¹ Esma S. Yolcu,¹ Laura Bandura-Morgan,¹ Chantale Lacelle,¹ Nadir Askenasy,² Haval Shirwan.¹ ¹Institute for Cellular Therapeutics and Department of Microbiology and Immunology, University of Louisville, Louisville, KY; ²Department of Pediatric Hematology Oncology, Schneider Children's Medical Center of Israel, Petach Tikva, Israel.

Objective: To engineer pancreatic islets in a rapid and efficient manner with a novel form of FasL protein chimeric with core streptavidin and test the efficacy of engineered islets for long-term survival in allogeneic hosts.

Methods: BALB/c pancreatic islets were engineered first by cell surface modification with biotin followed by the display of a chimeric form of FasL protein that consists of extracellular domain of FasL fused C-terminus with core streptavidin (SA-FasL). SA-FasL-engineered islets were transplanted into streptozotocin diabetic C57BL/6 mice under transient cover of rapamycin. Unmodified islets or those engineered with streptavidin protein (SA) served as controls.

Results: All the islets showed effective engineering with SA-FasL, which persisted on the surface of islets for weeks in vitro as assessed by confocal microscopy. All the islets (n=23) engineered with SA-FasL survived over the observation period of 100-400 days without detectable signs of rejection. In marked contrast, all the unmodified (n=9) and SA-engineered (n=14) islets underwent acute rejection within 40 days. The observed tolerance was localized to the engineered islets as unmodified second set of islets transplanted under contralateral kidney of long-term (>90 days) graft recipients were rejected in a normal tempo (MST=17 ± 9 days) without any effect on the survival of primary islets.

Conclusions: Engineering pancreatic islets with exogenous immunomodulatory molecules, such as SA-FasL, in a rapid (~2 hrs) and efficient (100% of targeted islets) manner represents a novel means of immunomodulation with considerable therapeutic potential for the treatment of type 1 diabetes.

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Abstract# 126

HOXB4 Regulates the Generation of Hematopoietic Cells from Embryonic Stem Cells. Nicholas Zavazava,¹ Kun-Ming Chan,^{1,2} Sabrina Bonde.¹ ¹Internal Medicine, University of Iowa & VAMC, Iowa City, IA; ²Department of General Surgery, Chang Gung Memorial Hospital, Chang Gung University College of Medicine, Taoyuan, Taiwan.

The ability of embryonic stem (ES) cells to form cells and tissues from all three germ layers can be exploited for the generation of cells that can be used to treat diseases. In particular, successful generation of hematopoietic cells from ES cells could provide safer and less immunogenic cells than bone marrow cells, that require severe host preconditioning, when transplanted across MHC barriers. In the past, it has been difficult to derive hematopoietic cells from ES cells. It has now become clear that this was due to the lack of self-renewal properties by these newly developed progenitor cells. Here, we exploited the self-renewal properties of ectopically expressed HOXB4, a homeobox transcription factor, to generate hematopoietic progenitor cells (HPCs) that successfully induce high level mixed chimerism and long-term engraftment in recipient mice. HOXB4-transduced 129SvJ ES cells (H2^b) were allowed to form embryoid bodies. These were dismantled after 5 days and the cells treated with a cocktail of hematopoietic cytokines. By day 26, ES cells had formed HPCs. These newly generated HPCs were CD45⁺, CD34⁺, CD117⁺ but poorly expressed MHC class I molecules and no class II. The HPCs fully restored splenic architecture in Rag2^{-/-} immunodeficient mice,

comparable to bone marrow. Additionally, HPC-derived newly generated T cells were able to mount a peptide-specific response to lymphocytic choriomeningitis virus (LCMV) and specifically secreted IL-2 and IFN- γ upon CD3 stimulation. Further, HPC-derived antigen presenting cells (APCs) in chimeric mice efficiently presented viral antigen to wild type (WT) T cells. In syngeneic recipient mice, HPCs engrafted and formed more robust T and B cell populations. The majority of the HPC-derived cells were however, Gr-1⁺, suggesting a bias towards myeloid cells by the HOXB4. Interestingly, these cells successfully engrafted in allogeneic MRL (H2^k) and Balb/c (H2^d) recipients without the need for immunosuppression. This ability to form mixed chimerism across MHC barriers is a consequence of their lack of MHC, CD80 and CD86 expression. Our results demonstrate for the first time that leukocytes derived from ES cells ectopically expressing HOXB4 are immunologically functional and escape immunological rejection when transplanted across MHC barriers allowing the induction of mixed chimerism.

Abstract# 127

Normalization of Factor VIII Activity in Mice with Hemophilia A by Transplantation of Embryonic Stem Cell-Derived Definitive Endodermal Cells. Kun-Ming Chan,^{1,2} Litao Xie,³ Marie E. Sarvida,³ Paul B. McCray, Jr.,³ Nicholas Zavazava.¹ ¹Department of Internal Medicine, University of Iowa and Veterans Affairs Medical Center, Iowa City, IA; ²Department of General Surgery, Linkou Chang Gung Memorial Hospital, Taoyuan, Taiwan; ³Pediatrics, University of Iowa, Iowa City, IA.

Embryonic stem (ES) cells differentiate into most cell types, under appropriate culture conditions, offering great opportunities for new treatments. We herein capitalized on these unique ES properties and developed a protocol for directing ES differentiation towards the definitive endoderm (DE) *in vitro*. The DE is derived from the anterior segment of the primitive streak which corresponds to the early and mid-gastrula organizer during early embryo development, from which many of the major visceral organs, including the liver, pancreas, lung, thyroid and intestines are derived. ES cells were cultured in a serum-free medium containing Activin A and bFGF for 6-8 days, the differentiated cells developed into an epithelial monolayer yielding more than 70% of CXCR4 expressing cells. CXCR4 has been reported as a cell surface marker of the definitive endoderm. Molecularly, the differentiated ES cells express typical definitive endodermal genes in particular, FoxA2, Sox-17, GSC, and HNF4 α . The CXCR4⁺ definitive endodermal cells were further purified using immunomagnetic bead separation to more than 99% purity in order to eliminate teratoma-forming cells. To study their engraftment and regenerative capacity, these newly differentiated cells were intravenously infused into a mouse with carbon tetrachloride-induced liver injury. Harvested livers from these animals showed large DE-derived cells positive for albumin suggesting that they were *de novo* generated hepatocytes. A second cell type was CK-19 expressing, suggesting that the engrafted cells also differentiated into cholangiocytes. Therefore, we further transplanted these DE cells into a factor VIII^{null} mouse and asked whether these cells could correct factor VIII activity. Plasma factor VIII activity (Coamatic assay) fully normalized to that of wild type mice and has remained stable over 60 days. These data suggest that ES cell-derived DE progenitor cells can restore factor VIII activity in hemophilia A mouse model, presumably through protein production in *de novo* generated hepatocytes. More importantly, none of these animals developed teratomas. Thus, ES-cell derived cells can potentially be coaxed to form cellular transplants with curative capabilities.

Abstract# 128

Establishment of Allogeneic Mixed Chimerism Using Bone Marrow Cells Engineered To Display on Their Surface an Exogenous FasL Protein with Potent Apoptotic Activity. Zhengda Sun,¹ Haval Shirwan,¹ Nadir Askenasy,² Esma S. Yolcu.¹ ¹Institute for Cellular Therapeutics and Department of Microbiology and Immunology, University of Louisville, Louisville, KY; ²Department of Pediatric Hematology Oncology, Schneider Children's Medical Center of Israel, Petach Tikva, Israel.

Objective: We have established a novel approach, ProtEx, to rapidly and efficiently engineer primary cells, tissues, or organs to display on their surface exogenous proteins of interest for immunomodulation. This approach involves generation of chimeric proteins with core streptavidin, biotinylation of cells, and the transient display of chimeric proteins on the cell surface. In this study, we displayed a chimeric form of FasL (SA-FasL) on the surface of bone marrow cells and tested the efficacy of these cells to establish mixed chimerism in allogeneic hosts under nonmyeloablative conditions.

Methods: BALB/c bone marrow cells were engineered with SA-FasL and 30 million of these cells were transplanted into C57BL/6 mice subjected to various doses of total body irradiation 2 days earlier. A short course of rapamycin was used to enhance the tolerogenic effect of SA-FasL. Bone marrow cell recipients were typed for multilineage chimerism at various times post-transplantation and tested for donor-specific tolerance using skin grafts.

Results: All the animals (n=8) treated with 300 cGy total body irradiation and transplanted with SA-FasL-engineered donor cells showed significant levels of chimerism (10-60%) on day 14 post-transplantation that showed a steady increase overtime and reached to 40-90% on day 60 post-transplantation. In marked contrast, none of the control animals (n=11) receiving bone marrow cells and a short course of rapamycin showed detectable chimerism. Chimerism was multilineage and associated with donor specific tolerance

since chimeric animals accepted donor, but rejected third party skin grafts.

Conclusions: Engineering bone marrow cells in a rapid (~2 hrs) and efficient (100% targeted cells) manner with exogenous proteins having immunoregulatory functions provides a new and effective means of immunomodulation to establish mixed allogeneic chimerism under nonmyeloablative conditions with significant potential in clinical bone marrow transplantation.

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Kidney Immunosuppression: CNI Minimization – Avoidance Protocols

Abstract# 129

Efficacy and Safety of Mycophenolate Mofetil (MMF)/Sirolimus (SRL) Maintenance Therapy after Calcineurin Inhibitor (CNI) Withdrawal in Renal Transplant Recipients: Final Results of the Spare-the-Nephron (STN) Trial. T. C. Pearson,¹ S. Mulgaonkar,² A. Patel,³ J. Scandling,⁴ H. Shidban,⁵ M. Weir,⁶ D. Patel.⁷ ¹Emory Univ Hospital, Atlanta, GA; ²St. Barnabas Med Ctr, Livingston, NJ; ³Henry Ford Hospital, Detroit, MI; ⁴Stanford Univ, Palo Alto, CA; ⁵NIT, Los Angeles, CA; ⁶Univ Maryland, Baltimore, MD; ⁷Roche, NJ.

Purpose: To evaluate the efficacy and safety of a maintenance immunosuppressive regimen of MMF and SRL compared with that of MMF and a CNI in renal allograft recipients over a 2-year period.

Methods: This open-label, prospective, multicenter study, randomized 305 patients (maintained on MMF and a CNI) 30-180 days post-transplant to receive either MMF (1-1.5 g BID) plus SRL (2-10 mg followed by at least 2 mg/day; trough 5-10 ng/mL) and discontinue the CNI (MMF/SRL) or to continue their current regimen (MMF/CNI). Antibody induction and/or corticosteroids were administered according to individual center practice. Efficacy endpoints included the proportions of patients with biopsy-proven acute rejection (BPAR), graft loss, and death at 12 months, and the percent change in measured glomerular filtration rate (GFR; cold iohalamate) from randomization.

Results: 12-month data from the first 249 patients receiving either MMF/SRL (n=123) or MMF/CNI (n=126) are described here; final outcomes of all 305 patients will be presented at the congress. Mean time from transplant to randomization in both groups was 117 days. Baseline characteristics and measured GFR values were similar in the treatment groups; 98 patients in the MMF/CNI group were receiving tacrolimus. Within the first 12 months, BPAR was reported in 8/123 (6.5%) MMF/SRL-treated patients and 9/126 (7.1%) receiving MMF/CNI. Graft loss occurred in 2/123 (1.6%) in the MMF/SRL group and 3/126 (2.4%) of those in the MMF/CNI group. No patients receiving MMF/SRL and 3/126 (2.4%) in the MMF/CNI group died. The number and proportion of patients withdrawn for adverse events at 12 months were 23/123 (18.7%) in the MMF/SRL group and 9/126 (7.1%) in the MMF/CNI group. The percent change from baseline in GFR at 12 months was 25.8 $\pm$ 75.0 and 11.3 $\pm$ 61.5 for the MMF/SRL and MMF/CNI groups, respectively.

Conclusions: MMF/SRL maintenance therapy appears to be associated with improved renal function when compared with MMF/CNI-containing regimens without increasing the risk of acute rejection and is tolerated in almost 80% of patients. Final results presented at the congress will provide definitive conclusions about the effects of this regimen.

Abstract# 130

Analysis of Allospecific T Cells in Kidney Transplant Patients on Various Immunosuppressive Protocols. Martin Gasser,¹ Igor Tsaur,¹ Beatriz Avilez,² Jens Lutz,² Martin Grimm,¹ Kai Lopau,¹ Uwe Heemann,² Anil Chandraker,³ Ana Maria Waaga-Gasser.¹ ¹Dept. of Surgery I, University of Wuerzburg, Wuerzburg, Germany; ²Dept. of Nephrology, University of Munich, Munich, Germany; ³Transplantation Center, Brigham and Women's Hospital, Boston.

Chronic allograft dysfunction is still a major clinical problem in organ transplantation. Morphologically it is characterized by changes suggestive of an alloantibody mediated mechanism such as glomerulopathy and vasculopathy or by non-specific changes such as interstitial fibrosis and tubular atrophy. Alloantigen dependent as well as -independent factors contribute to the pathogenesis of these changes. In this study we analysed allospecific T cells from the peripheral blood of kidney transplant patients under different immunosuppressive protocols with or without chronic allograft dysfunction. 107 renal allograft recipients of our renal transplant clinic were screened at least six months after transplantation. All patients were on a calcineurin-based immunosuppressive protocol consisting of cyclosporine (CsA)/mycophenolate mofetil (MMF)/Steroid, Tacrolimus (Tac)/MMF/Steroid, or CsA/Steroid. Patients had to be mismatched for one or more of the five candidate HLA-DR antigens for which synthetic peptides were available (DR1, DR2, DR3, DR4, and DR7). Patients with biopsy proven chronic allograft nephropathy (CAN) with an elevated serum creatinine level of ≥ 1.6 mg/dl were compared with patients with stable allograft function (serum creatinine <1.6 mg/dl). T cell lines were generated from peripheral blood lymphocytes of renal transplant recipients against donor-derived HLA-DR peptides presented by self APC. T cell lines generated from

patients with CAN produced significantly more IFN- γ , while those generated from stable patients produced IL-10 associated with a low proliferation index in response to the donor-derived mismatched HLA-DR allopeptide *in vitro*. Moreover, significantly more CD4+CD25+Foxp3+ T cells were found in stable patients on Tac and MMF as compared to patients on CsA and MMF. Interestingly, a higher gene expression of CD4, CD25, CTLA-4, Foxp3, and IL-10 was observed in those patients. Taken together a Th1 (IFN- γ) alloimmune response is deleterious and promotes chronic graft damage, while a Th2 (IL-10) response seems to be associated with a lower incidence of chronic allograft nephropathy. An immunosuppression based on Tac and MMF seems to favour CD4+CD25+Foxp3+ T cells and to allow long-term engraftment with stable renal function.

Abstract# 131

Cyclosporin Induces Epithelial to Mesenchymal Transition in Renal Grafts. Marc Hazzan,¹ Alexandre Hertig,² David Buob,³ Christian Noel,¹ Marie-Christine Copin,³ Eric Rondeau,² Yi-Chun Xu-Dubois.² ¹*Nephrologie, Hopital de Lille, Lille, France;* ²*Urgences Nephrologiques et Transplantation Renale, APHP, Hopital Tenon, Paris, France;* ³*Pathologie, Hopital de Lille, Lille, France.*

Introduction:

The expression of epithelial to mesenchymal transition (EMT) markers is a reliable predictor of the progression towards interstitial fibrosis and tubular atrophy of the renal grafts. *In vitro* experiments suggest that calcineurin inhibitors (CNI) can induce EMT of tubular epithelial cells. Although no evidence was ever provided *in vivo*, this suggests that EMT could be involved in the pathogenesis of renal fibrosis induced by CNI.

Patients and Methods:

We have previously reported the results of a prospective randomized trial comparing the elimination at month 3 of either cyclosporine (CsA, n=54) or mycophenolate (MMF, n=54) from a triple drug regimen in 108 *de novo* renal transplant patients. All of them had 2 systematic graft biopsies at months 3 and 12 post engraftment. In the leftover material, we retrospectively detected in tubular cells and by immuno-histochemistry the expression of two validated markers of EMT: the *de novo* expression of vimentin (Vim) and the cytoplasmic translocation of b-catenin (Cat).

Results:

We were able to measure the EMT score at both months 3 and 12 in a total of 68 patients (34 in each group). In the CsA group, the Vim and Cat scores had progressed between 3 and 12 months from 1.53 ± 0.86 to 1.99 ± 0.97 ($p=0.004$) and 1.55 ± 0.79 to 1.88 ± 1.04 ($p=0.041$), respectively, while they remained stable in the MMF group. Remarkably, the 3-month EMT scores were correlated with the 1-year eGFR and the Chronic Allograft Damage Index.

Since acute cellular rejection (ACR) may trigger EMT, we performed a subgroup analysis and excluded the 10 patients who had presented an ACR (CsA group, n=2; MMF group, n=8). Vim and Cat scores respectively progressed from 1.55 ± 0.86 to 2.04 ± 0.98 ($p=0.007$) and from 1.57 ± 0.79 to 1.89 ± 1.06 ($p=0.065$) under CsA, but, strikingly, these scores decreased in patients under MMF and without CsA: Vim from 1.67 ± 0.92 to 1.38 ± 0.91 ($p=0.13$) and Cat from 1.60 ± 0.85 to 1.26 ± 0.84 ($p=0.047$).

Conclusion:

First, we confirm that the early expression of EMT markers is of bad prognostic value regarding graft function and histology. Moreover, we found that the EMT score is durably influenced by CsA: maintenance of CsA increases the EMT score, while disruption of CsA attenuates it, as long as the patients don't experience rejection. Thus, in renal grafts, CsA induced fibrosis might involve EMT.

Abstract# 132

Vascular Calcineurin Inhibitor Toxicity in Early Protocol Biopsies from Human Renal Allografts. Verena Broecker,¹ Victoria Schubert,¹ Michael Mengel,¹ Irina Franz,² Anke Schwarz,² Hermann Haller,² Hans Kreipe,¹ Wilfried Gwinner.² ¹*Institute of Pathology, Medizinische Hochschule Hannover, Hannover, Germany;* ²*Department of Nephrology, Medizinische Hochschule Hannover, Hannover, Germany.*

Calcineurin inhibitors (CNI) are efficacious but nephrotoxic immunosuppressives. Arteriol hyaline sclerosis is one of the characteristic histological correlates of this toxicity. Yet, time course of this lesion, its reversibility, dose-dependency and the discrimination between drug-related effects, diabetic and hypertensive vasculopathy remain unclear. Aim of this study was to evaluate the prevalence and time course of CNI-related vascular changes after renal transplantation (tx) in protocol (pBx) as well as in indication biopsies (iBx) and to correlate this to the CNI blood levels, blood pressure and diabetes. From 491 patients, a total number of 1239 pBx, taken at 6 weeks (n=380), 3 (n=420) and 6 months (n=439) after tx and 360 iBx were classified according to Banff-criteria. Assessment of CNI toxicity included: isometric vacuolisation of tubules (ISOVAC), intimal arteriolar hyaline sclerosis (IAH) and nodular arteriolar hyaline sclerosis (NAH) and vacuolisation of small vessel smooth muscle cells (VSM). 96% of patients received either cyclosporine or tacrolimus.

In pBx, ISOVAC was present in 15%, 17% and 15% of patients at 6 weeks, 3 and 6 months post-tx, respectively; IAH in 11%, 9% and 10%; NAH in 5%, 5% and 7%; VSM in 19%, 19% and 15%. In late iBx (>2 years post-tx) the prevalence of the analyzed parameters apart from ISOVAC (9%) was markedly higher: 42% for IAH, 22% for NAH and 38% for VSM. In pBx, VSM, IAH and NAH were associated with each other but

not significantly dependent on blood pressure, rejection episodes or diabetes. Through levels of CNIs were not different between patients with and without vascular hyaline sclerosis. In patients with VSM and IAH in late iBx one third had these lesions already present in earlier biopsies.

Conclusion: The prevalence of presumed morphological signs for vascular CNI-toxicity in pBx is low and constant and apparently, not associated with CNI blood levels, hypertension or diabetes. In contrast, in iBx later than two years after transplantation, prevalence of vascular CNI-toxicity signs is much higher. This emphasises that CNI reduction protocols should be regarded within the first six months after transplantation, when vascular changes are still marginal. Further elucidation of precursor lesions in pBx would help to find out patients at risk for CNI-induced vascular changes.

Abstract# 133

Donor Marrow Infusion in Kidney Transplantation: Results of Preliminary Study. Kadiyala Ravindra, Roger Herzig, Kristie Paris, Joseph Buell, Suzanne T. Ildstad. *Departments of Surgery, Blood & Marrow Transplant Program, Radiation Oncology, Institute for Cellular Therapeutics, University of Louisville, Louisville, KY.*

Introduction: Achieving donor specific tolerance has been the goal of the transplant community. Success has been reported with donor stem cell transfusion in animal studies and prevention of chronic rejection in cardiac allograft recipients in the clinic. This report details the results of the initial phase of a study in humans.

Methods: A prospective phase I/II FDA approved pilot protocol was initiated to evaluate the effects of donor graft facilitating cell (FC)/stem cell infusion in kidney transplant recipients. Conditioning was performed with 200 cGy of total body irradiation. Bone marrow processed to remove GVHD-producing cells but retain CD8⁺/TCR- FC and stem cells was infused 24 hours post-operatively. The dosage of stem cells was limited by the T cell dosage. The starting dose was 1×10^6 T cells. This was increased in steps of 2×10^6 per patient. As the study spans a 7-year period, the immunosuppression changed: 3 patients received cyclosporine (CyA), MMF and Prednisone; 3 were induced with Basiliximab and maintained on CyA(2)/FK(1), Celcept and Prednisone; and 3 received Alemtuzumab induction and maintenance with FK and MMF. All patients underwent tolerance testing and immunoprofiling studies.

Results: Of the 9 patients, 6 received live donor kidney transplants. One graft was lost from arterial thrombosis on day 2. Delayed graft function was seen in 3 patients. Good long-term graft function was seen in the other 8 patients. Acute rejection was noted only in 1 and infectious complications (CMV-1, Histoplasma-1) in 2 patients. Two patients died with functioning grafts – one at 4 years from lung cancer and another from complications from diabetes at 6 years. Six patients are alive with functioning grafts at mean follow-up of 4.3 years with a mean creatinine of 1.3 mg/dl. No GVHD was detected in any of the patients. Macrochimerism was not detected in any of the patients at any point. Notably, in spite of the fact that durable engraftment was not yet achieved, none of the patients were sensitized as a result nor did they experience immunologic sequelae.

Conclusions: In our patients there were no untoward sequelae related to either the conditioning regimen or marrow infusion. The incidence of acute rejection even on long-term follow up has been low. We currently propose to reduce the immunosuppression further in these patients.

Abstract# 134

Sirolimus Monotherapy in Kidney Transplantation: Results of an ITN-Sponsored Trial. Stuart Knechtle,¹ Debra Bloom,¹ Julio Pascual,¹ Jose Torrealba,¹ Ewa Jankowska-Gan,¹ Katarzyna Bourcier,² Lorraine Harper,² Vicki Seyfert-Margolis,² Robert Colvin,³ Hans Sollinger,¹ William Burlingham.¹ ¹*University of Wisconsin, Madison, WI;* ²*Immune Tolerance Network, San Francisco, CA;* ³*Massachusetts General Hospital, Boston, MA.*

A pilot study was performed to evaluate whether immune cell depletion with alemtuzumab would permit post-transplant weaning of maintenance immunosuppression in well-matched renal transplant recipients. Patients received alemtuzumab 30 mg intravenously on the day of the transplant and the subsequent 2 days while sirolimus and tacrolimus were started on day 1. Tacrolimus was discontinued at day 60 in all patients. Extensive immune monitoring was performed at 1 year. At current follow-up (22 to 34 months), all patients are alive with a functioning graft (median MDRD GFR=46 ml/min). One patient experienced clinical and biopsy-proven rejection at 9 months. All other patients remain on sirolimus monotherapy. Four patients have been weaned to 1 mg of sirolimus daily as their sole immunosuppressive agent, with resulting blood levels of 3-4 ng/ml. These 4 patients have no evidence of donor-specific alloantibody, are unresponsive or hyporesponsive to donor cells by the cytokine kinetics test, and have a regulator phenotype to soluble donor antigens by trans-vivo DTH. Flow cytometry of peripheral blood demonstrated increased Foxp3 expression in the CD3+CD4+ population ($p=0.002$). Naive B cells (CD19/CD27neg) cells increased in 9 of 10 patients ($p=0.02$) and memory B cells increased in all 10 recipients ($p=0.0005$) when comparing pretransplant to 1 year timepoints.

Other than the patient with rejection, 12-month protocol biopsies did not show any evidence of rejection, although 2 of 9 showed focal C4d positivity and 1 diffuse positivity. These 3 patients also had evidence of alloantibody by Luminex xMAP testing. In conclusion, the cytokine kinetics test, alloantibody testing, and trans-vivo DTH assay

results correlated with clinical evolution of patients who successfully weaned both tacrolimus and sirolimus without rejection or alloantibody. The flow cytometry findings described occurred regardless of clinical evolution and may represent alterations of the immune system inherent in the treatment protocol independent of individual patient responses to the graft. However, the functional assays of cytokine kinetics assay and trans-vivo DTH may be of potential use to correlate with the clinical immune status of the kidney transplant recipient.

Kidney Immunosuppression: Induction Therapy I

Abstract# 135

12 Month Results of a Multicenter, Randomized Trial Comparing Three Induction Agents (Alemtuzumab, Thymoglobulin and Basiliximab) with Tacrolimus, Mycophenolate Mofetil and a Rapid Steroid Withdrawal in Renal Transplantation. Michael Hanaway, E. Steve Woodle, Shamkant Mulgaonkar, Ram Peddi, Gilda Harrison, Karen Vandeputte, William Fitzsimmons, Roy First, John Holman. *Surgery, Univ. of Alabama, Birmingham, AL; Surgery, Univ. of Cincinnati, Cincinnati, OH; Transplantation, St. Barnabas Med. Ctr., Livingston, NJ; Transplantation, CPMC, San Francisco, CA; R & D Dept., APUS, Deerfield, IL.*

To compare the safety and efficacy of three induction agents (Alemtuzumab (C1H), Thymoglobulin (Thymo) and Basiliximab (Bas) in renal transplantation, in combination with tacrolimus (TAC), mycophenolate mofetil (MMF) with a rapid steroid withdrawal.

Method: 474 adult patients were randomized and transplanted based on risk profile: High Risk (HR=African American (AA), PRA $\geq$ 20% or re-transplants) were assigned to C1H or Thymo. Low Risk (LR=non-AA, PRA<20% or primary transplant) were assigned to C1H or Bas. Both C1H groups received 30mg of C1H on day (D) 0; the Thymo group received 4 doses of Thymo (1.5mg/kg/d) on D 0-4; the Bas group received 2 doses of 20mg of Bas on D 0-4. One gram of methylprednisolone was given prior to discharge. Tacrolimus and MMF were used as maintenance immunosuppression. Primary endpoint was the incidence of biopsy-confirmed acute rejection (BCAR).

Results: Mean results at 12 mo are shown below. Adverse events are shown as % of patients with ≥ 1 event through 12 mo.

	HR		LR	
Parameter	C1H N=70	Thymo N=69	C1H N=164	Bas N=171
BCAR %	9.5	13.0	2.5	19.6*
Treated Rejections %	17.2	17.4	5.6	23.8*
Antibody for AR %	4.8	8.0	1.9	9.2*
TAC levels (12 mo mean, ng/mL)	7.30	7.26	7.41	7.50
MMF Daily Dosing (12 mo mean, mg/d)	1647	1582	1580	1650
Pt Survival %	98.6	98.6	98.7	98.7
Graft Survival %	97.1	95.3	98.1	95.1
All Infection %	41.4	68.1*	56.7	57.9
CMV (infection/viraemia) %	10.0	10.1	9.1	7.6
BK %	2.9	8.7	4.2	2.3
Malignancy %	2.9	0.0	2.4	1.8
Total Lymphocyte (cells/mm ³)	928	974	923	1460
Serum Creatinine mg/dL	1.6	1.5	1.4	1.3
Creatinine Clearance mL/min	60	55	62	61
Study Discontinuation %	12.9	4.3	4.9	7.0

*P<0.05 for C1H vs Bas or Thymo

Conclusions: In HR patients C1H provides similar efficacy and fewer patients with infection than Thymo. In LR patients C1H provides superior prophylaxis of acute rejection (AR), less use of antibody for treatment of rejection and no increase in infections.

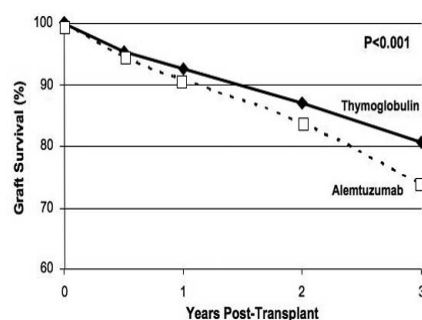
Abstract# 136

Kidney Transplant Outcomes with Alemtuzumab and Thymoglobulin Induction. Suphamai Bunnapradist,¹ Jagbir Gill,¹ Steven Takemoto,² Tariq Shah.² ¹Medicine-Nephrology, UCLA, LA, CA; ²Medicine-Nephrology, National Institute of Transplantation, LA, CA.

Introduction: The use of alemtuzumab for induction therapy in kidney transplantation has been increasing, although long-term follow-up data is lacking. The objective of this study was to compare the outcomes of deceased donor kidney transplant recipients treated with either alemtuzumab or Thymoglobulin.

Method: UNOS data was analyzed from 30,524 deceased donor, solitary kidney transplant recipients treated with antibody induction therapy with either Thymoglobulin (84%, n=25,640) or alemtuzumab (16%, n=4,884) between 2002 and 2007. Logistic regression was used to compare the odds of rejection at discharge, 6 months and 12 months. Kaplan-Meier was used to estimate time to graft loss and log-rank test was used to compare outcomes between groups. Cox proportional hazards model was used to identify risk factors for graft loss.

Results: Compared to Thymoglobulin, the odds of rejection at hospital discharge was 0.48 (0.34-0.68, p<0.001) for alemtuzumab, but at 6 months it increased to 1.46 (1.22-1.74, P<0.001) and at 12 months it was 1.64 (1.20-2.25, P<0.001). Three-year graft survival rates were 80.7% for patients given Thymoglobulin compared to 73.9% for alemtuzumab (P<0.001) (Figure). After adjustment for other risk factors, the risk of graft loss for patients receiving alemtuzumab compared to Thymoglobulin was 1.36 (1.19 - 1.55, p<0.001).



Discussion: Our findings suggest that although alemtuzumab is very effective in preventing early rejection, it is associated with a higher risk of late acute rejection and worse overall graft survival at 3-years post-transplant compared to Thymoglobulin.

Disclosure - Grant/Research Support: Genzyme.

Abstract# 137

411 Living Donor Kidney Transplants Using Alemtuzumab Pre-Conditioning and Tacrolimus Monotherapy: 5 Year Experience. Henkie P. Tan,¹ Joseph Donaldson,¹ Amit Basu,¹ Claire Morgan,¹ Mark Unruh,¹ Jerry McCauley,¹ Christine Wu,¹ Nirav Shah,¹ Amadeo Marcos,¹ Parmjeet Randhawa,¹ Thomas E. Starzl,¹ Fadi Lakkis,¹ Adriana Zeevi,¹ Ron Shapiro.¹ ¹UPMC.

We have previously reported the short-term results of Alemtuzumab (Campath-1H) pre-conditioning with tacrolimus monotherapy and subsequent spaced weaning in living donor kidney transplantation (LDKT). We report here our 5 year experience.

Methods: We performed 411 consecutive unselected LDKT (donor kidneys were removed laparoscopically) from 12/11/2002 to 11/26/2007 using 30 mg (0.5mg/kg) alemtuzumab and tacrolimus monotherapy. At 6 months post-transplant and every 2 to 6 months interval, we used clinical data (including ELISA antibody titers, Cylex T-cell activation assay, and identification of donor specific antibodies) to wean tacrolimus when possible (bid-->qd-->qod-->tiw-->biw-->qwk). The recipients included 5 HIV+, 35 pediatric recipients, and 60 re-transplants. The mean follow up was 843.1 $\pm$ 478.7 days.

Results: Actuarial recipient survivals at 1-, 2-, 3-years were 98.4%, 95.6%, and 92.7%, respectively. Graft survivals at 1-, 2-, 3-years were 97.6%, 90.4%, and 85.4%, respectively. The mean creatinine (mg/dL) at 1-, 2-, 3-years were 1.46 $\pm$ 0.62, 1.56 $\pm$ 1.08, and 1.54 $\pm$ 0.89, respectively. The mean GFR (mL/min/1.73m²) at 1-, 2-, 3-years were 73.0 $\pm$ 28.5, 71.8 $\pm$ 28.8, and 68.9 $\pm$ 28.6, respectively. The cumulative incidence of acute cellular rejection (ACR) at 6-, 12-, 18-, 24-, 30-, 36-, 42-, and >42 months were 4.4%, 10.0%, 15.3%, 17.0%, 19.2%, 19.7%, 21.4%, and 22.1%, respectively. 23.4% of recipients had been weaned to spaced-dose (qod or less) tacrolimus monotherapy. The incidence of preweaning ACR was 10.5%, and postweaning was 24.9%. A total of 91 recipients had ACR. Most (>80%) episodes of ACR were Banff I and were sensitive to steroid pulses. The degree of chronic allograft nephropathy score (cg, ci, cv, and ct, with a maximal score of 12) in pts with ACR appeared, as expected, to increase with time. The total CAN score for the 91 patients with ACR at 6-, 12-, 18-, 24-, 30-, 36-, 42-, and >42 months were 2.2 $\pm$ 2.0, 4.3 $\pm$ 2.3, 3.4 $\pm$ 2.0, 2.8 $\pm$ 1.6, 4.1 $\pm$ 1.2, 6.0 $\pm$ 0.0, 5.1 $\pm$ 1.8, and 8.3 $\pm$ 2.1, respectively. At least 82.7% of recipients were still steroid-free on tacrolimus monotherapy from the time of transplantation.

Conclusion: This report represents the largest series to date of LDKT under alemtuzumab preconditioning with tacrolimus monotherapy and subsequent weaning and confirms the 5-year safety and efficacy of this approach.

Abstract# 138

Are There Any Benefits To Using Depleting Antibodies in Low Risk Kidney Transplant Recipients? John S. Gill,¹ Olwyn Johnston,¹ Caren L. Rose,¹ David Landsberg.¹ ¹Division of Nephrology, University of British Columbia, St. Paul's Hospital, Vancouver, BC, Canada.

The role of depleting antibodies for induction (DPI) in low risk kidney transplant recipients (KTRs) is unclear. Using data from the USRDS, we determined the incidence of acute rejection (AR), and allograft survival in adult (≥ 18 years), non-Black, non-sensitized (0% PRA), first KTRs of a standard criteria deceased donor (SCD), between 1997-2004. We restricted the analysis to include KTRs who received maintenance immunosuppression with a CNI (tacrolimus or cyclosporine), MMF, and steroids. Induction agents were classified as Thymoglobulin (rabbit), other DPI (including OKT3, ALG and ATGAM (equine), anti-CD25 (basiliximab, daclizimab), or no induction. The independent association of antibody induction with AR at one year (AR), and short and long-term graft survival was determined in multivariate logistic and Cox regression models that adjusted for differences in donor age, cause of death, pre-transplant creatinine; HLA matching; cold ischemic time; delayed graft function; recipient age, gender, cause of ESRD; comorbid conditions; duration of pre-transplant dialysis; and transplant year (table).

Conclusions: In the current era low risk patients infrequently have AR, and have excellent short and long term graft survival without the use of depleting antibodies. Given the increased costs of these drugs, the indications for using depleting antibodies in low risk KTRs of SCD kidneys should be further clarified.

Patient group	AR at 1 year (%)	Adjusted odds of AR at 1 year **	1 year graft survival (%)	Adjusted risk of graft loss from any cause at 1 year ***	5 year graft survival (%)	Adjusted long-term risk of graft loss from any cause ***
No induction (n=5,657)	9.3	1.00	94.3	1.00	79.5	1.00
Anti-CD25 (n=3,446)	8.1*	0.82 (0.70, 0.96)*	93.5	1.05 (0.88, 1.26)	79.3	0.98 (0.88, 1.10)
Thymoglobulin (n=1,057)	6.3*	0.72 (0.54, 0.94)*	93.5	0.94 (0.71, 1.24)	76.5	1.01 (0.83, 1.22)
Other DPI (n=737)	15.3*	1.20 (0.96, 1.49)	92.1*	1.09 (0.82, 1.45)	75.2*	1.12 (0.96, 1.30)

* p<0.05 compared to patients with no induction; ** Odds ratio; 95%CI; *** HR; 95%CI

Abstract# 139

Randomised Controlled Trial of Campath and Tacrolimus-Monotherapy with Daclizumab, Tacrolimus and Mycophenolate Mofetil in Renal Transplantation; Interim Results. Kakit Chan,¹ David Taube,¹ Jack Galliford,¹ Rawya Charif,¹ Antony Dorling,¹ Dawn Goodall,¹ Nadey Hakim,¹ Vassilios Papalois,¹ Anthony Warrens,¹ Adam McLean.¹ ¹West London Renal and Transplant Centre, Imperial College Kidney and Transplant Institute, Hammersmith Hospital, London, United Kingdom.

Although Campath induction is widely used in transplantation, there have been no formal controlled trials using this agent with Tacrolimus [Tac] monotherapy. In this study, we report the interim results of our CamTac trial which compares Campath induction and Tac monotherapy with Daclizumab, Tac and Mycophenolate Mofetil [MMF]. Neither arm received steroids except during the first week post transplantation.

This study is stratified for live vs deceased donors, and randomization is 2:1 to the Campath and Daclizumab arms. The target enrollment of 120 patients is powered to detect a 10% difference in rejection rates in the first year. Over a 2 year period 109 patients have been enrolled with mean follow-up 12.3 months. (Campath arm 74 patients LD:DD 48:26, Daclizumab arm 35 patients LD:DD 23:12):

Immunosuppression:

	Daclizumab	Campath
Induction dose	2x2mg/kg IV	1x30mg IV
Steroids	Days 0-6	Days 0-6
Tacrolimus	Trough target 8-12ng/ml	Trough target 5-8ng/ml
MMF	Trough target 1.5-3.0mg/L	None

Results:

	Daclizumab	Campath
Patient survival	96.6%	100%
Graft survival	91%	98.6%
Graft function (eGFR ml/min)	45.1 [38.4-51.9]	52.8 [48.1-57.4]
Tacrolimus level (LC-MSMS) ng/ml	9.4 [8.2-10.7]	6.8 [6.1-7.6]

Patient and graft survival is similar in both arms. Allograft function is better in the Campath arm but does not reach statistical significance at 12 months [p=0.07] Crude rejection rate in the Campath arm is 5.4% with a median time to rejection of 3.2 months. The rejection rate in the Daclizumab arm is 17% with a median time to rejection of 4.4 months.

Conclusions: In this ongoing randomized controlled trial, Campath induction with Tacrolimus monotherapy provides a steroid sparing regime with excellent patient and graft survival with low rejection rates and improved graft function compared to a similar regime based on Daclizumab, Tacrolimus, and MMF.

Abstract# 140

T-Cell Depletion Does Not Potentiate the Progression of Liver Cirrhosis in HCV+ Renal Transplant Patients. Alp Sener,¹ Rolf N. Barth,² Matthew Cooper,¹ Luis Campos,¹ Eugene J. Schweitzer,¹ Bartlett T. Stephen,¹ Benjamin Philosophie.¹ ¹Surgery, Division of Transplantation, University of Maryland School of Medicine, Baltimore, MD.

Kidney transplantation prolongs survival in hepatitis C virus-positive (HCV+) patients with end-stage renal disease (ESRD). However, the effects of induction therapy and chronic immunosuppression are unknown on the course of HCV infection and potential for cirrhosis in renal transplantation (RTx) recipients. We have retrospectively assessed parameters of liver function, Child-Pugh (CP) and MELD scores in HCV+ ESRD patients who received induction therapy with T-cell depletion (Group 1: Thymoglobulin, n=30) or an IL-2 inhibitor (Group 2: Basiliximab, n=46). Pre-RTx liver biopsies were similar in Group 1 and 2. Patients were followed for a mean of 826 days (range 47 to 2679 days) following RTx and received tacrolimus, mycophenolate mofetil, and sometimes steroids post-transplant. Overall graft survival was 84% in Group 1 and 85% in Group 2 (p > 0.05). Data were analyzed pre-RTx, at 30 and 365 days and at time of last follow-up. Serum AST, ALT, platelets, INR, albumin and bilirubin did not change following RTx in either group. CP scores in Group 1 were not significantly changed after RTx (5.5 ± 0.9 to 5.7 ± 0.9 at last follow-up, p=0.53). Group 1 patients on steroid-free protocols (n=8) demonstrated declining CP scores from 6.0 ± 1.0 to 5.3 ± 0.5 at last follow-up that were not statistically significant (p=0.31). Group 1 patients on steroids showed

opposite trends of CP: 5.3 ± 0.6 pre-RTx vs 5.8 ± 1.1 at last follow-up that similarly did not reach statistical significance (p=0.18). Group 2 CP scores declined from 5.8 ± 0.8 to 5.4 ± 0.9 at last follow-up (p=0.04). There was no difference between CP or MELD scores at any point between the groups. As expected, MELD scores improved significantly following RTx and remained low up until the final visit (p=0.001); this was attributed to the drop in serum creatinine post-RTx. Neither the use of Thymoglobulin or Basiliximab resulted in acute hepatitis resurgence or the development of cirrhosis post-transplant. We have not identified any association between choice of induction agent or maintenance immunosuppression regimens, including steroid withdrawal, with impaired hepatic function or progression to liver cirrhosis in HCV+ RTx patients. T cell depletion was well-tolerated by HCV+ RTx patients and resulted in good graft outcomes. Interestingly, CP scores declined after renal transplantation in the Basiliximab induction group.

Kidney: Complications I

Abstract# 141

Prevalence of Post-Transplant Lymphoproliferative Disorder with Monoclonal Gammopathy of Unknown Significance in Patients Undergoing Kidney Transplantation. Harris V. K. Naina,¹ Robert Kyle,¹ Thomas M. Habermann,¹ Samar Harris,¹ Fernando G. Cosio,¹ Mark D. Stegall,¹ Leung Nelson.¹ ¹MayoClinic College of Medicine, Rochester, MN.

Background: The development of monoclonal (M) protein following liver transplantation is associated with the development of post-transplant lymphoproliferative disorder (PTLD). In this study, we investigate this relationship in the kidney transplantation (KT) population.

Methods: A total of 3518 patients underwent KT between 1963 to March 2006. These patients were cross referenced with the Monoclonal Gammopathy of Undetermined Significance (MGUS) database.

Results: We identified 97 patients who had a M protein either before or after KT. Patients with amyloidosis, multiple myeloma, heavy and light chain deposition disease and multi-organ transplantation were excluded from the analysis. A total of 69 patients met the inclusion criteria. Ten of the 69 (14.5%) patients developed PTLD. Median follow up was 14.8 years. Twenty three patients had pretransplant MGUS, 20 patients developed MGUS following the KT, and the other 26 did not have a M protein study prior to the KT. Of the 23 patients who had a positive MGUS prior to the KT, 4 (17.3%) patients developed PTLD, 1 developed EBV positive diffuse large cell lymphoma (DLCL), 1 developed EBV negative DLCL, 1 developed Hodgkins lymphoma and 1 developed increased plasma cells in bone marrow (20%) with stable M protein with no evidence of progression to multiple myeloma. None of these patients had a quantifiable M-protein prior to KT. The mean duration from diagnosis of MGUS to PTLD was 8.2 years (range 3 to 14 years). Of the 20 patients with a negative pre-transplant study for M protein, 2 (10%) developed PTLD (T cell lymphoproliferative disorder). Two patients developed MGUS after the transplant at 1 and 12 years post KT. It took an average of 15 years to develop PTLD after the diagnosis of MGUS. Four of the 26 patients who did not have a pretransplant study for MGUS developed PTLD. These included an EBV positive gamma delta type T cell lymphoproliferative disorder, an EBV positive plasmablastic lymphoma, one multiple myeloma and a plasmacytoma. The latter two patients had M-protein > 3g/dL. It took an average of 14 years after their transplant for these patients to develop PTLD.

Conclusion: Our study showed that the development of a M protein in patients undergoing KT is a strong risk factor for PTLD. Monoclonal protein study should be performed pretransplant and monitored post transplant as a surveillance of PTLD.

Abstract# 142

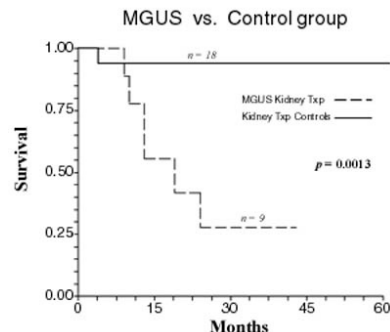
Decreased Survival in Kidney Transplant Patients with Monoclonal Gammopathy of Undetermined Significance. Hector Carbajal,¹ Liliana Soltero,² A. Osama Gaber,¹ Wadi N. Suki,³ Horacio E. Adrogué.⁴ ¹The Methodist Hospital Transplant Center, The Methodist Hospital, Houston, TX; ²Department of Medicine, The Methodist Hospital, Houston, TX; ³The Kidney Institute, Houston, TX; ⁴Department of Medicine, Baylor College of Medicine, Houston, TX.

BACKGROUND. Monoclonal gammopathy of undetermined significance (MGUS) has an estimated prevalence of 3% in the general population, and a 1% annual risk of transformation to lymphoproliferative disease. Protein electrophoresis is part of the evaluation of kidney transplant (KT) candidates in our center. The incidence of MGUS and its impact on patient and graft survival is unknown.

PATIENTS AND METHODS. Retrospective, case-control study. Starting in the year 2000, patients undergoing evaluation for a KT found to have MGUS were included. Clinical and hematological data were collected, as well as current status of the grafts and patients. MGUS patients who received a KT (9 patients) were paired with controls at a 1:2 ratio by age, gender, race, diabetes, type of donor, and date of transplant. Kaplan Meier survival analysis was done.

RESULTS. Of the 501 kidney transplant candidates evaluated since 2000, 36 patients were found to have MGUS (7.2%). Patients were 58% male, age 63.2 SD10.3 y; 52.8% Caucasian, 38.9% African-American, 8.4% other. The type of paraproteinemia was IgG

in 83% of the patients. All the patients had a Hematology evaluation. The presence of MGUS was not considered in itself a contraindication for KT. Nine patients were transplanted. This subgroup of patients was paired accordingly. Of this group, six patients (67%) died during a median follow-up of 17.8 months. Causes of death were cerebral abscess, lung cancer with myocardial infarction, sepsis, melanoma, and two unknown. Patients with MGUS that received a KT had a decreased survival compared to KT patients without MGUS.



CONCLUSION. MGUS is a common finding in patients undergoing evaluation for a kidney transplant. In this group, MGUS was associated with a decreased patient survival.

Abstract# 143

Kidney Transplantation in Patients with Systemic Amyloidosis. Tale N. Wien,¹ Torbjørn Leivestad,² Anna V. Reisæter,¹ Bartłomiej J. Witczak,¹ Aksel Foss,³ Anders Hartmann.¹ ¹Department of Medicine; ²Department of Transplant Surgery; ³Institute of Immunology, Rikshospitalet, University of Oslo, Norway.

We report clinical outcome of 98 patients with systemic amyloidosis (AMY) in Norway receiving a first renal graft from 1989 throughout 2006. All transplant recipients with diabetic nephropathy (DIA, n=360) and chronic glomerulonephritis (CGN, n=1061) served as control groups representing inferior and superior outcome, respectively. The 98 transplanted AMY patients constituted 39% of all AMY patients established in renal replacement therapy (RRT) in the same period. At start of RRT 71% of all AMY patients were considered eligible for transplantation work-up, a significantly lower percentage than in controls (CGN (85%) and DIA (81%)). 55% of AMY patients considered as transplantation candidates received a kidney transplant.

Mean age (SD) at first transplantation was 51 (14) years for AMY, 48 (12) years for DIA and 49 (16) years for CGN (n.s.). The AMY group comprised more females (52%) compared with controls (CGN 25% and DIA 30%). The proportion of live donors was lower in DIA (27%) than AMY (47%) and CGN (48%). With respect to donor age and PRA positivity the groups were not significantly different.

Patient survival post transplantation, overall graft survival and censored graft survival were:

	Patient survival		Graft survival		Censored graft survival	
	1 yr	5 yrs	1 yr	5 yrs	1 yr	5 yrs
Amyloid (n=98)	91%	67%	87%	61%	94%	83%
CGN (n=1061)	95%	85%	91%	78%	95%	89%
DIA (n=360)	93%	77%	88%	70%	92%	85%

Patient and censored graft survival curves for AMY patients were not significantly different from DIA, but differed from CGN patients, $p<0.003$. The major causes of death were cardiovascular events and infections in all groups. Septicaemia was the cause of death in 22% of AMY patients, significantly higher than in CGN (8%) and DIA patients (11%).

In a multivariable Cox model for mortality independent risk factors were: AMY vs CGN and DIA (HR 2.01, $P<0.001$), recipient age (HR 1.05/year, $P<0.001$), months in dialysis (HR 1.015/month, $P<0.003$), deceased versus live donor (HR 1.35, $P<0.02$). Recipient sex and donor age were not independent risk factors.

Conclusion: AMY patients are at increased risk of death compared with CGN and almost comparable to DIA patients. However, renal transplantation appears an acceptable treatment option, given that two thirds are alive after 5 years, which is superior to dialysis treatment. Septicaemia contributes to excessive mortality in AMY.

Abstract# 144

Cytomegalovirus-Induced Vd2^{neg} gd T Cells Have a Potential Protective Role Against Malignancies in Renal Transplant Recipients. Lionel Couzi,^{1,2} Yann Le Vaillant,¹ Abdellah Jamaï,¹ Régis Lassalle,³ Karin Martin,³ Jean-François Moreau,^{2,4} Julie Dechanet-Merville,² Pierre Merville.^{1,2} ¹Unité de Transplantation Renale, Département de Néphrologie, CHU Bordeaux, Bordeaux, France; ²CNRS UMR 5164, Université Bordeaux 2, Bordeaux, France; ³Unité de Pharmacologie-Epidémiologie, Département de Pharmacologie, Université Bordeaux 2, Bordeaux, France; ⁴Département de Virologie et d'Immunologie, CHU Bordeaux, Bordeaux, France.

Background: A few years ago we observed an expansion of blood gd T cells following cytomegalovirus (CMV) infection in kidney transplant recipient (KTR). We recently demonstrated that these cells share a strong reactivity against CMV infected cells and tumor epithelial cells *in vitro*. An implication of gd T cells in the immune surveillance against cancer has been demonstrated in mouse and strongly suggested in human.

Methods: We tested here the hypothesis of a protective role of CMV-induced gd T cells against neoplasia in KTR through: 1/ a longitudinal case / control (KTR with cancer / KTR without cancer) study where gd T cell percentages were determined before and after cancer diagnostic (n=63), 2/ a retrospective follow-up of 131 KTR for 8.23 years looking for risk factors for malignancy.

Results: The median of gd T cell percentage in patients with malignancies was significantly lower when compared to control patients 18, 12 and 6 months before the diagnostic of the cancer ($p<0.005$). Using a conditional logistic model, we determined that patients with a gd T cell percentage above 4 % were protected from cancer ($p<0.008$). A significant association between increase of the Vd2^{neg} gd T cell subset and lower cancer occurrence was only retrieved in the KTR who experienced pre- or post-graft CMV infection. Finally, using univariate and multivariable analysis, absence of pre- or post-graft CMV infection in KTR was associated with a risk of cancer 5.69 times more elevated ($p=0.006$).

Conclusion: This study reveals an unexpected protective role of CMV against cancer in KTR most probably via the expansion of gd T cells cross-reactive against CMV-infected and tumor cells.

Abstract# 145

CMV-Specific CD8+ T Cells (CMV-Tc) Remain Active in Campath-1H® (CAM) Depleted Lymphocytes in Sero-Positive Transplant Patients, but Not Zenapax® (ZEN) Treated Patients. Andy Pao,¹ Shili Ge,¹ David Thomas,¹ Ashley Vo,¹ Raju Radha,¹ Stanley C. Jordan,¹ Mieko Toyoda.¹ ¹Transplant Immunology Laboratory and Comprehensive Transplant Center, Cedars-Sinai Medical Center, Los Angeles, CA.

Background: Viral infection (VI) is a morbidity factor in transplant recipients (Tx pts). Induction therapy (Ind-Rx) is a known risk factor for VI. Although CAM is thought to be a more potent Ind-Rx than ZEN, we have previously shown similar CMV infection rates in each. We have also shown that CMV-Tc analyzed by cytokine flow cytometry (CFC) are consistently detectable in CMV sero(+), but not sero(-) individuals. CMV-Tc(-) was associated with persistence of CMV infection in Tx pts. Here, we report on the effect of Ind-Rx on CMV-Tc in kidney Tx pts. **Methods:** 58 pre-Tx samples from 39 CMV-sero(+) pts and 62 post-Tx samples from 44 pts were submitted for CMV Tc-CFC. Whole blood was incubated with a pooled overlapping peptide mixture consisting of 138 peptides from CMV pp65 and Bfrefeldin A at 37 degrees for 6 hours and room temperature overnight. IFN γ +CD8+ cells were enumerated by CFC and results were expressed as IFN γ +CD8+ cell%. Results $>0.2\%$ were considered as (+). **Results:** 54/58 (93%) pre-Tx samples showed CMV-Tc(+) (1.4 $\pm$ 1.6%, median 0.95), while only 35/62 (57%, $p<0.001$ vs. pre-Tx) post-Tx samples did so (2.2 $\pm$ 4.2%, median 0.22). Of 62 post-Tx samples, 18 were CAM-Rx and 26 ZEN-Rx. In the CAM-Rx group, 14/18 (78%, 4.8 $\pm$ 6.4%) showed CMV-Tc(+), while only 10/26 ZEN pts (39%, 0.5 $\pm$ 0.9%, $p=0.001$ vs. CAM) did so. Of 9 samples obtained within 6 months post-Tx in the CAM group, 7 showed low lymphocyte count, but 6/9 (69%) samples showed CMV-Tc(+). In contrast, only 4/23 ZEN-Rx samples (17%, $p=0.001$ vs. CAM) showed low lymphocyte count and only 8/23 (35%, $p=0.10$ vs. CAM) showed CMV-Tc(+). Two patients in each group developed CMV infection. **Conclusions:** 1) CMV-Tc activity is significantly reduced post-Tx in many CMV sero(+) Tx pts, 2) CMV-Tc remain active in many CAM-Rx pts despite lymphocyte depletion, while CMV-Tc is inactive in many ZEN-Rx pts with normal lymphocyte numbers, 3) This may explain the equivalent frequency of viral infections seen in ZEN- and CAM-Rx pts, 4) Lymphocytes remaining post-CAM-Rx may retain more function than lymphocytes post-ZEN-Rx despite greater numbers, 5) Pts treated with either CAM or ZEN require equivalent attention to anti-viral prophylaxis to manage post-Tx viral infection.

Abstract# 146

Significant Increase in Infection-Associated Renal Allograft Failure over the past Decade: UNOS Data Analysis. Ravi Parasuraman,¹ Attallah Nizaar,¹ Vanji Karthikeyan,¹ Dean Kim,² Jason Denny,² Lauren Malinzak,² Marwan Abouljoud,² Gordan Jacobsen,³ Mariella Goggins,¹ K. K. Venkat.¹ ¹*Division of Nephrology and Hypertension; ²Transplant Institute; ³Department of Research and Biostatistics, Henry Ford Hospital, Detroit, MI.*

Long-term renal allograft survival has not improved despite significant decline in acute rejection rate. This may be due to increase in infection-associated allograft failure offsetting the beneficial effect of potent immunosuppression on allograft rejection.

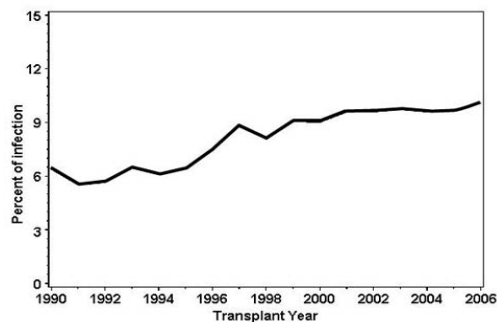
AIM: To study the role of infections in (death censored) renal allograft failure.

Method and Results: The UNOS data from 1990-2006 was analyzed and 38,286 first and only renal transplants that resulted in graft failure were identified with 31,420 having known contributory infection status. Results compared between two periods (period I [1990-1996] and period II [1997-2006]) showed a significant increase in infection contributed graft loss from 6.3% (1037/16419) to 9.1% (1360/14907) $p < 0.001$ and a significant decrease in treated for rejection rate from 20.3% (3316/16302) to 12.2% (1635/13350) $p < 0.001$ respectively. Other significant findings are shown in table1.

Summary of transplants that resulted in graft failure

Transplant year	Number of grafts failed	Infection contributing to graft failure(%)	Recipient death(%)	Any induction medication use(%)	Treated for rejection(%)
1990	1735	6.4	20	27.4	27.7
1992	2176	5.7	21.1	25.7	23.5
1994	2847	6.1	19.1	83.1	18.5
1996	2542	7.5	20.4	87.9	14.2
1998	2215	8.1	23.5	84.1	11.3
2000	1871	9.0	22.3	83.8	9.8
2002	1509	9.6	22.2	86.3	10.4
2004	949	9.6	20.3	87.2	10.4
2006	407	10.1	17	89.3	7.1

Infection associated graft loss during the entire study period is shown in figure1.



Conclusion: Infections contributing to renal allograft loss increased significantly from 1990 to 2006. This may be due to increase use of both induction agents and potent maintenance regimens. This is an important cause for poor long-term graft outcome despite decreasing rejection rates and a balance has to be maintained between prevention of rejection and avoidance of infection.

Kidney: Expanding Criteria Donor Issues

Abstract# 147

A Simple Composite Clinical and Histological Score Is Highly Predictive of Graft Outcome in Marginal Donors (MD). Dany Anglicheau,¹ Alexandre Loupy,¹ Pessione Fabienne,² Noel Laure-Hélène,³ Patey Natacha,³ Droz Dominique,⁴ Martinez Frank,¹ Thervet Eric,¹ Legendre Christophe.¹ ¹*Transplantation Rénale, Hôpital Necker, Paris, France; ²Biostatistique, Agence de la Biomédecine, Paris, France; ³Laboratoire d'Anatomie Pathologique, Hôpital Necker, Paris, France; ⁴Laboratoire d'Anatomie Pathologique, Hôpital Saint-Louis, Paris, France.*

Background: The usefulness and value of preimplantation (D0) biopsies compared to clinical scores have not been well studied in the population of MD in kidney transplantation. Our aim was to assess in MD the predictive performance of clinical vs histological scores and to define a simple composite algorithm to predict graft outcome.

Methods: All donor clinical and histological risk factors, as well as known clinical (Pessione, Transplantation 2003; Nyberg, AJT 2002) and histological scores (Remuzzi, NEJM 2006; CADI score, Isoniemi, KI 1992) were analyzed in 191 kidneys from deceased MD. The primary end point was a 1-year post-transplant eGFR < 25 ml/min/1.73m² (low eGFR).

Results: donor age was 60.1 ± 7.4 yrs. Serum creatinine (Scr) at procurement was 101 ± 51 μ mol/L. The incidence of donor hypertension, diabetes, and death from cerebrovascular origin was 31%, 15%, and 55% respectively. Multivariate analysis showed that the only clinical parameters associated with a low eGFR were donor Scr and donor hypertension. Nyberg or Pessione scores were not significantly associated with a low eGFR. Regarding D0 biopsies, univariate analysis showed that % of sclerotic glomeruli (SG, $p=0.02$),

arteriolar hyalinosis ($p=0.03$), mean Remuzzi score ($p=0.03$) and mean CADI score ($p=0.04$) were all significantly associated with a low eGFR. A logistic regression showed that an integrated score including: i) donor Scr (± 150 μ mol/L), ii) hypertension, and iii) SG ($\pm 10\%$) had the highest performance in predicting a low eGFR at 1 yr compared to clinical or histological parameters alone. Using this composite score, the adjusted OR for the prediction of a low 1-yr eGFR ranged from 1 if none of the 3 factors were present, to 7.1 (if SG $> 10\%$ was associated with one of the 2 clinical factors), and to 27.5 (if the 3 factors were present, $p=0.0003$).

Conclusion: this study highlights that D0 biopsies are useful to predict graft outcome particularly in MD population, and may perform better than clinical scores alone. In this population, a simple and routinely applicable integrated scoring strongly predicts a poor graft outcome, which may allow an optimized allocation of marginal donors.

Abstract# 148

Prospective Evaluation of Donor Pre-Treatment with Dopamine for the Prevention of Delayed Graft Function after Kidney Transplantation: A Randomized Controlled Trial. Peter Schnuelle,¹ Uwe Goettmann,¹ Simone Hoeger,¹ Detlef Boesebeck,² Werner Lauchart,³ Mike Smith,⁴ Axel Rahmel,⁴ Fokko J. van der Woude,¹ Benito A. Yard.¹ ¹*5th Medical Clinic, University Hospital Mannheim, Mannheim, Germany; ²DSO-Region of Bavaria, Muenchen, Germany; ³DSO-Region of Baden-Wuerttemberg, Stuttgart, Germany; ⁴Eurotransplant International Foundation, Leiden, Netherlands.*

Background: Retrospective clinical data indicate that donor dopamine (DA) may ameliorate cold preservation injury and improve initial graft function after kidney transplantation. This RCT investigates a standardized regimen of donor preconditioning with dopamine in human renal transplant recipients from a brain dead donor. **Methods:** Heart beating donors were randomized to receive or not to receive DA (4μ g/kg*min) after confirmation of brain death and given consent for donation. Eligibility criteria included hemodynamic stability with low dose noradrenaline ($< 0.4 \mu$ g/kg*min), a serum-creatinine < 1.3 mg/dl on admission and an increase < 2.0 mg during intensive care. Allocation of organs was centrally directed according to current Eurotransplant standards. DGF defined by the requirement of > 1 dialysis session post-transplant was the primary endpoint. Study analysis was done on intent-to-treat. **Results:** Donor DA infusion was given with an average duration of 368 ± 268 min before harvesting of the kidneys (range 0-1929 min). Treated and untreated donors were very similar in demographics, s-creatinine, and 24hr urine production. 505 subsequent kidney transplants from 62 collaborating centers were available for study analysis (DA treated $n=234$, Ctrl $n=271$). DA resulted in less DGF after transplantation (26.1% vs. 34.7%, $p=0.034$). DA mediated effects on hemodynamics and urine production had no measurable effect on the primary endpoint in the multivariate analysis, despite a marginal increase in systolic blood pressure (132 vs. 128 mmHg, $p=0.02$) and last hour urine production (207 vs. 176 ml, $p=0.005$). **Conclusion:** Donor DA ameliorates DGF after kidney transplantation. It remains to be seen if the beneficial effect translates in improved long-term graft survival.

Abstract# 149

Correlation of Histologic Findings on Pre-Implant Biopsy with Kidney Graft Survival. Liise K. Kayler,¹ Ravi Mohanka,² Claire Morgan,² Amit Basu,² Ron Shapiro,² Parmjeet S. Randhawa.³ ¹*Surgery, University of Florida Medical Center, Gainesville, FL; ²Surgery, University of Pittsburgh Medical Center, Pittsburgh, PA; ³Pathology, University of Pittsburgh Medical Center, Pittsburgh, PA.*

Kidney biopsies are being used to evaluate donors, but literature on the utility of this practice remains somewhat conflicting. We critically re-examined this issue by performing a multivariate logistic regression analysis of 596 kidney transplant recipients: this represents the largest single center study to date where biopsy findings were systematically recorded over a period of 20 years and carefully correlated with clinical parameters. A p -value < 0.10 was considered significant. Moderate arteriosclerosis defined as $> 25\%$ luminal compromise was confirmed to be a significant predictor of graft outcome ($p=0.0579$), independent of donor history of hypertension or diabetes mellitus. One-year allograft failure stratified by moderate arteriosclerosis was 29% vs. 15% ($p=0.0188$). Moderate arteriosclerosis correlated with degree of glomerulosclerosis ($p=0.0014$), and increasing percentage of sclerotic glomeruli showed a trend to earlier graft failure ($p=0.0339$). Glomerulosclerosis $> 20\%$ and interstitial fibrosis $> 25\%$ had a low frequency in the material reviewed, likely reflecting our organ utilization practices, and did not have a demonstrable effect on graft outcome. Moderate arteriolar hyaline change was significantly associated with donor history of hypertension and diabetes mellitus, but did not correlate with donor age, degree of glomerulosclerosis or graft outcome. Clinical parameters associated with worse graft function were ECD status ($p=0.0488$), retransplantation ($p=0.0048$), recipient age ($p=0.0286$), delayed graft function ($p<0.0001$), and long waiting time ($p=0.0870$).

Abstract# 150

Single Versus En-Bloc Kidney Transplantation from Pediatric Donors ≤ 15 kg. Ravi Mohanka,¹ Amit Basu,¹ Ron Shapiro,¹ Liise Kayler.² ¹Thomas E Starzl Transplant Institute, University of Pittsburgh Medical Center, Pittsburgh, PA; ²Department of Surgery, University of Florida Medical Center, Gainesville, FL.

Kidney transplantation from small pediatric donors is increasingly being utilized as a means to optimize the organ supply, however the single most common specified reason for the discard of pediatric kidneys is vascular damage, such as shortening of the suprarenal aorta or injury to the renal artery orifices, which often precludes en bloc transplantation (EBK). At our center, damaged kidneys were salvaged by transplantation as singles (SK).

Methods: Between 2002-2006, kidneys from pediatric donors ≤ 15 kg were transplanted en-bloc (n=19) or as singles (n=14) in recipients over 15 years of age. Mean donor age was 15 ± 10 months (median 13 months, range 21 days-3 years). Donor weight ranged between 4-15 kg.

Results: Graft survival at one year was 86% for SK and 79% for EBK recipients (p=0.6184). There were no differences between the two groups in terms of donor age, donor and recipient weight, cold ischemia time, incidence of acute rejection, and graft thrombosis, however delayed graft function was more frequent amongst SK recipients (EBK=0%; SK=25%, p=0.0253).

Etiology of graft loss

Number of Kidneys	Recipient Age (years)	Cause of Failure	Post-transplant days
Single	X	Primary Non-function	0
Single	X	Primary Non-function	0
Double	26	Non-compliance	358
Double	58	Death with graft function	130
Double	49	Primary Non-function	0
Double	18	Non-compliance	229

Conclusion: Short-term outcomes of kidneys from small pediatric donors are excellent when transplanted as singles or as en bloc.

Abstract# 151

Transplantation of Single Pediatric Kidneys under 5 Years of Age into Adult Recipients: Risky or Routine? Michael J. Goldstein,¹ Govind Nandakumar,¹ Peter Henderson,¹ Maria Goris,¹ Sandip Kapur.¹ ¹Surgery, Weill-Cornell Medical College, New York, NY.

BACKGROUND: In an effort to maximize the number of recipients transplanted per donor, transplant centers in our donor service area (DSA) voted to preferentially allocate local and imported en-bloc pediatric donor renal allografts to centers willing to transplant two individuals with single allografts. After 1 year of implementing this policy into action, we report on our initial experience.

METHODS: From July 2006 to June 2007 we reviewed our experience with 12 adult single allograft recipients of pediatric donors less than 60 months of age. There were no exclusions based on age or size with exclusion criteria consisting of donor age < 2 months and single allograft size < 5 cm. All but 1 recipient received rabbit anti-thymocyte globulin induction, tacrolimus, mycophenolate mofetil, and rapid steroid withdrawal. Results from this cohort were compared to 86 consecutive recipients of adult single allografts from standard criteria donors with the same immunosuppression protocol used as historical controls.

RESULTS: 12 pediatric single allografts with median donor age of 24 months (range 8-58) were transplanted into 12 adults with median age 46 years (range 24-67). The incidence of delayed graft function (DGF) was 8/12 (66.7%) in the pediatric recipients and 18/86 (20.9%) in the controls, p=0.001. All pediatric allografts experienced significant hypertrophy by 3 months with a median increase in size by 34.9% (range 21.3-67.2%). Measured 24-hour creatinine clearance (CrCl) increased with allograft hypertrophy. Median pediatric allograft CrCl at 1 month, 3 months, and 6 months was 15.0, 48.5, and 53.5 cc/min. Comparatively, historical controls experienced CrCl at 1 month, 3 months, and 6 months of 49, 57, 61 cc/min (p<0.001, 0.08, 0.54, respectively). 1 year graft survival was 100% for the pediatric recipients and 96.2% for controls, p=0.479.

CONCLUSION: Adoption of a policy to split all pediatric en-bloc renal allografts for adult recipients is safe and successful with outcomes comparable to those receiving SCD adult allografts at one year. Pediatric renal hypertrophy provides allograft function equal to adult SCD allografts by 6 months post-transplantation.

Abstract# 152

Similar Results of Single and Dual Transplantations with Expanded Criteria Donor Kidneys Allocated According to Donor's Estimated Glomerular Filtration Rate. Renaud Snanoudj,¹ Marc-Olivier Timsit,² Leila Tricot,³ Alexandre Loupy,¹ Julien Zuber,¹ Henri Kreis,¹ Frank Martinez,² Eric Thervet,¹ Arnaud Mejean,² Thierry Lebre,⁴ Christophe Legendre,¹ Michel Delahousse.² ¹Renal Transplantation, Necker Hospital, Paris, France; ²Urology, Necker Hospital, Paris, France; ³Nephrology, Foch Hospital, Suresnes, France; ⁴Urology, Foch Hospital, Suresnes.

Background: few studies have directly compared results of single and dual-kidney transplantations from expanded criteria donors (ECD) older than 65 years and appropriate criteria of allocation remain to be determined. **Methods:** in a prospective cohort study, we

assessed outcome of 70 dual and 65 single-kidney transplantations performed between august 2003 and august 2007 at two institutions, with a minimal follow-up of three months. In ECD deceased donors older than 65 years, two kidneys were allocated to a recipient older than 65 years if highest Glomerular Filtration Rate (eGFR) estimated with Cockcroft and Gault formula was less than 60 ml/min (dual group), while a single kidney was allocated to a recipient of whatever age if eGFR was greater than 60 ml/min (single group). Results: donors were significantly older in the dual group (74.0 ± 7.8 versus 71.3 ± 4.0 years, p=0.02). Donor mean eGFR was 47.7 ± 9.6 ml/min in the dual group and 86.9 ± 26.0 in the single group, p<0.0001. Recipients were older in the dual group (69.4 versus 60.2 years, p<0.0001). Waiting time on dialysis was shorter for recipients of the dual group: 33.6 ± 32.7 versus 57.7 ± 40.1 months, p<0.0005. At three months, death and graft loss occurred in 4.3 % and 2.9% of patients in the dual group, and in 3.1% and 9.2% of patients in the single group (ns). One-year actuarial patient and graft survival was similar (98.6 and 95.7 % in the dual versus 98.3 and 89.0% in the single group, ns). eGFR was not statistically different at month 3 and 12 post-transplantation: 46.3 ± 15.0 ml/min and 46.6 ± 17.6 ml/min in the dual group versus 45.6 ± 15.8 and 47.7 ± 16.9 ml/min in the single group. Length of first hospitalization was similar and hospitalizations within the first year occurred more frequently in the single group: 2.3 ± 1.8 versus 1.3 ± 1.2 , p<0.005.

Conclusion: Allocation of ECD kidneys for single or dual kidney transplantation according to eGFR (more or less than 60 ml/min) results in good and similar short-term results, despite a significantly older age of donors and recipients of dual kidney transplantation, suggesting that this approach is safe and appropriate.

Liver Transplantation: Allocation and MELD

Abstract# 153

Race and Liver Transplant Waitlist Survival. Young-Suk Lim,¹ Rachel A. Pedersen,² Terry M. Therneau,² W. Ray Kim.¹ ¹Division of Gastroenterology and Hepatology, Mayo Clinic College of Medicine, Rochester, MN; ²Division of Biostatistics, Mayo Clinic College of Medicine, Rochester, MN.

Background: African Americans (AA) have been shown to be at a higher risk of death while waiting for liver transplantation (LTx) than Caucasian Americans (CA). The reason for this difference is not clear. In this work, we evaluated whether the racial disparity in waitlist outcome may be explained by confounding factors other than the race per se. **Methods:** Patient level data on all waitlist registrants between 2003 and 2005 were obtained from the Organ Procurement and Transplantation Network. The relationship between patients' race, pre-LTx clinical status and waitlist survival was analyzed using multivariable Cox regression analysis.

Results: There were 20,672 adult primary LTx candidates with end stage liver disease (i.e., excluding patients with diagnosis of acute liver failure or malignancy), which included 15,351 (74.3%) CA, 1,579 (7.6%) AA, 657 (3.2%) Asian/Pacific Islanders (API), and 3,085 (14.9%) with other race. The overall waitlist survival at 3, 6, and 12 months was 85.0%, 76.5%, and 62.1%, respectively. Mean MELD ($\pm$ SD) at listing was higher in AA and API than CA registrants (16.3 ± 7.43 for CA, 18.4 ± 8.43 for AA, 17.3 ± 9.20 for API, and 16.8 ± 7.76 for other races, P<0.05). In the table, the univariate analysis [column A] showed that non-white race was associated with increased risk of death (p<0.01). This effect of race was attenuated when LTx center was taken into account [B]. Finally, with the addition of MELD in the model, the effect of race on waitlist mortality all but disappeared [C].

Conclusions: On the surface, minority patients may appear to have higher mortality on LTx waitlist compared to Caucasian counterparts. However, this association is predominantly a result of minority patients having a higher MELD score, although LTx center-specific mortality may contribute. These data suggest that waitlist outcome may be improved by optimizing referral of minority patients.

Table. Proportional hazards model predicting 6 month survival on waitlist

	HR(95% CI)* adjusted for		
RACE	None [A]	LTx Center [B]	Center and MELD [C]
AA**	1.24 (1.05-1.46)	1.18 (0.999-1.40)	0.87 (0.73-1.03)
API**	1.40 (1.08-1.82)	1.23 (0.94-1.60)	0.96 (0.73-1.26)
Other**	1.28 (1.13-1.44)	1.13 (0.98-1.29)	1.00 (0.87-1.15)
MELD	-	-	1.17 (1.16-1.17)

* HR, hazard ratio; CI, Confidence Interval. ** Reference group: Caucasian

Abstract# 154

MELD-Na90: An Improved Index for Predicting Pre-Liver Transplant Mortality in Cirrhotic Patients. Douglas M. Heuman,^{1,2} Nevin Yilmaz,^{1,2} Richard T. Stravitz,² HoChong S. Gilles,^{1,2} Robert A. Fisher.³ ¹Medicine, HH McGuire Dept. of Veterans Affairs Medical Center, Richmond, VA; ²Medicine, Virginia Commonwealth University, Richmond, VA; ³Surgery, Virginia Commonwealth University, Richmond, VA.

Background: In cirrhotic patients awaiting liver transplantation (LT), low serum sodium (Na) predicts short term pre-LT mortality, independently of MELD. Incorporation of Na into MELD has been recommended to improve prognostic accuracy (MELD-Na; *Gastroenterology* 130:1652, 2006). However, short term interventions such as water restriction that improve Na may have little effect on prognosis.

Hypothesis: the lowest level of serum sodium in the preceding 30 (Na30), 90 (Na90)

or 180 days (Na180) may be better than the current serum sodium (NaC) for predicting pre-LT cirrhotic mortality.

Methods: We reviewed electronic records of 764 cirrhotic veterans referred for consideration of LT, 2/28/02-6/30/07. Date of most recent Na at referral was chosen as time zero for determining NaC, Na30, Na90, and Na180 and for assessing subsequent survival.

Findings: Within 90 days, 94 patients died pre-LT (12%) and 27 underwent LT (3%). Na at all time points was associated strongly ($p<.001$) with preLT death (censored at LT). Areas under receiver operating characteristic curves (AUROCs) for Na30, Na90 and Na180 as predictors of 90d preLT mortality (mean $\pm$ SE) were .808 $\pm$.026, .807 $\pm$.026 and .783 $\pm$.025, respectively, compared to .714 $\pm$.032 for NaC (all $p<.05$ vs. NaC). On multivariable logistic regression analysis, MELD and Na90 were independent predictors of 90d preLT mortality, with best discrimination given by the following model:

MELD-Na90 = MELD + (135-Na90)*1.04

with value of Na90 capped at 135. AUROCs for MELD-Na90, MELD-Na, and MELD were .875 $\pm$.027, .865 $\pm$.025 and .838 $\pm$.027, respectively. Findings were similar when patients with HCC at referral (n=155) were excluded (AUROCs .884 $\pm$.025, .876 $\pm$.026 and .840 $\pm$.031, respectively), and when 90 day survival endpoint was changed from "death censored at LT" to "death or LT" (AUROC's .890 $\pm$.021, .882 $\pm$.022, and .855 $\pm$.026, respectively).

Conclusion: Short term improvement in Na may mask true preLT mortality risk. The lowest Na in the preceding 90 days is a better prognostic indicator than current sodium. Substitution of MELD-Na90 for MELD would permit more accurate "sickest first" organ allocation, while at the same time allowing preLT correction of Na without loss of priority. Prospective validation of MELD-Na90, in comparison to MELD-Na and MELD, is warranted.

Abstract# 155

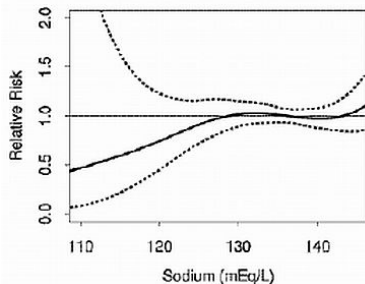
Hyponatremia Does Not Affect Survival Following Liver Transplantation.

Byung Cheol Yun,¹ W. Ray Kim,¹ Y. S. Lim,¹ Joanne T. Benson,¹ Walter K. Kremers,¹ Terry M. Therneau.¹ ¹*Gastroenterology and Hepatology, Mayo Clinic College of Medicine, Rochester, MN.*

Background: Hyponatremia is a common yet important complication of cirrhosis. Serum sodium (Na) has been found to be an important predictor of survival in patients with cirrhosis. Models incorporating Na have been proposed for liver allocation. Concerns have been raised, however, that liver transplantation (LTx) in hyponatremic patients will adversely affect the outcome. In this work, we assessed the effect of pre-LTx Na on the short term survival following LTx.

Methods: Patient-level data on all waitlist registrants in the US for 2005 and 2006 were obtained from the Organ Procurement and Transplantation Network. Demographic, clinical and laboratory data at the time of LTx and outcomes following LTx were extracted. The relationship between Na pre-LTx and survival post-LTx was analyzed using multivariable regression analyses.

Results: There were 7411 primary transplants that met the inclusion criteria between 2005 and 2006. The median Na in mEq/L at the time of LTx was 136 (interquartile range, IQR: 132-139). There were 1255 patients who had a Na $\leq$ 130 mEq/L. The mean MELD score was 22.5 (SD 9.2). Median follow up was 279 (IQR: 155-371) days. The overall 30- and 90-day survival post-OLT was 96% and 93%, respectively. In a multivariable logistic regression model, MELD was associated with 1.04-fold increase in 90-day mortality (95 confidence interval: 1.03-1.05), while Na did not have impact on survival (HR=0.99, 95% CI: 0.98-1.02). The figure represents the risk of 90-day mortality according to Na after adjustment for MELD, which clearly shows absence of mortality increase over a wide range of Na.



Conclusions: Hyponatremia at the time of LTx has no detrimental impact on short term patient survival following LTx. Although these data do not address morbidity (e.g., central pontine myelinolysis), there is no evidence that incorporation of Na in organ allocation will lead to diminished survival.

Abstract# 156

MELD and Prediction of Quality of Life One-Year after Liver

Transplantation. James R. Rodrigue,¹ Gail L. Lisson,² David R. Nelson,³ Alan I. Reed,⁴ Michael Curry,¹ Douglas W. Hanto.¹ ¹*Beth Israel Deaconess Medical Center, Boston, MA;* ²*Independent Practice, Rocky Mount, NC;* ³*University of Florida, Gainesville, FL;* ⁴*University of Iowa, Des Moines, IA.*

Objective. This study examined the relationship between MELD at liver transplantation (LT) and post-LT quality of life (QOL).

Methods. Adult LT recipients (n = 247) at two centers completed the SF-36 and Transplant Symptom Frequency Questionnaire (TSFQ) 1-year post-LT. High SF-36 scores indicate better QOL; high TSFQ scores indicate more symptomatology. Clinical (lab) MELD at LT, demographic characteristics, presence of ascites, encephalopathy, and variceal bleeding pre-LT, current employment status, presence of co-morbid medical conditions, and BMI were collected from medical records.

Results. Primary LT indication was viral hepatitis (57%), cholestatic liver disease (17%), or hepatocellular disease (27%), and 63% had ascites, 51% encephalopathy, and 29% gastroesophageal bleeding. Mean MELD at LT was 20 $\pm$ 9. There was almost no correlation between MELD and SF-36 Physical ($r = 0.11$) and Mental ($r = 0.001$) functioning. Statistically significant yet weak correlations were found between MELD and Physical Functioning ($r = -0.15$) and Role Functioning - Physical ($r = -0.15$). MELD was not significantly correlated with any other SF-36 scales (r 's -0.11 to -0.01). MELD was not significantly correlated with any TSFQ domains: affective distress ($r = 0.08$), neurocognitive symptoms ($r = 0.05$), gastrointestinal distress ($r = -0.02$), physical appearance changes ($r = 0.09$), appetite and weight changes ($r = 0.09$), and miscellaneous symptoms ($r = 0.08$). Older age ($\beta = -0.28$), female sex ($\beta = -0.26$), viral hepatitis ($\beta = 0.67$) or cholestatic disease ($\beta = 0.45$), higher BMI ($\beta = -0.62$), and >1 medical co-morbidity ($\beta = 0.57$) were significant predictors of lower QOL as measured by the SF-36 (adj $R^2 = 0.12$, $F = 4.4$, $p < 0.001$). Older age ($\beta = 0.27$), female sex ($\beta = 0.29$), higher BMI ($\beta = 0.71$), history of variceal bleeding ($\beta = 0.19$), and >1 medical co-morbidity ($\beta = 0.17$) were predictive of more symptoms on TSFQ (adj $R^2 = 0.10$, $F = 4.1$, $p < 0.001$). MELD was not predictive of QOL.

Conclusions. Higher disease severity, as measured by MELD, at LT does not portend a worse QOL outcome for patients 1-yr after transplantation. Other pre-LT indicators of decompensation also do not predict post-LT QOL. Post-LT QOL is affected more by other variables, including age, sex, BMI, and medical co-morbidities.

Abstract# 157

Waiting for Liver Transplantation in the United States: Does Race

Matter? C. A. O'Mahony,¹ N. S. Becker,¹ O. A. Taylor,¹ S. Kreml,¹ R. J. Stribling,¹ N. L. Sussman,¹ J. M. Vierling,¹ J. A. Goss.¹ ¹*Michael E DeBakey Department of Surgery, Baylor College of Medicine, Houston, TX.*

Introduction: Racial disparities in access to cadaveric renal allografts have been well described for renal transplantation. However, little is known about differences in orthotopic liver transplantation (OLT) rates for patients of minority racial groups following listing. The purpose of the current study was to determine if there is difference in rate of transplantation among racial groups and to examine the potential reasons for the disparity.

Methods: The United Network for Organ Sharing (UNOS) database was obtained. Data was extracted for adult OLTs greater than 18 years of age performed from 2/2002-12/2005. Transplants for which recipient race or Model for End-Stage Liver Disease (MELD) score at listing were unknown and patients active on the list were excluded. Rates of transplantation as well as differences in reasons for de-listing (transplantation, death/deterioration, and improvement) were examined. In an effort to examine only patients with chronic liver disease, further analysis was performed excluding patients with acute fulminant liver failure and retransplants.

Results: The database contained complete MELD and race information on 17,916 OLTs. Seventy-four percent of patients were Caucasian, 12% Hispanic, 9% African-American, and 4% were Asian. As seen in Table 1, laboratory MELD score at removal differed between racial groups. Examining pair-wise comparisons of the three minority groups to Caucasians, only Hispanics differed in reason for delisting (Table 1). Subgroup analysis excluding acute hepatic failure patients and retransplants showed similar results with Hispanic patients being more likely to die/deteriorate as compared to other racial groups (31% deaths vs. 24% deaths for Caucasians), and being less likely to receive a transplant (69% of Hispanics vs. 75% of Caucasians, $p<0.001$).

Conclusion: Hispanic patients, although listed with higher MELD scores, are transplanted less often than Caucasian patients and are more likely to die/deteriorate while awaiting OLT. Reasons for this discrepancy are unclear and merit further attention.

Table 1

	N	MELD	Rate of Transplantation	Reason for Delisting		p Value
				Death/Deterioration	Improvement	
Caucasian	13260	22	74%	23%	3%	Reference
Hispanic	2222	25	69%	29%	2%	<0.001
African-American	1645	26	73%	24%	3%	0.104
Asian	789	21	75%	22%	3%	0.561

Abstract# 158

Effect of Serum Creatinine on Survival Benefit of Liver Transplantation. Pratima Sharma,¹ Douglas E. Schaubel,^{2,3} Mary K. Guidinger,^{3,4} Robert M. Merion,^{5,3} ¹Internal Medicine, U. Michigan, Ann Arbor, MI; ²Biostatistics, U. Michigan, Ann Arbor, MI; ³SRTR, Ann Arbor, MI; ⁴Arbor Research, Ann Arbor, MI; ⁵Surgery, U. Michigan, Ann Arbor, MI.

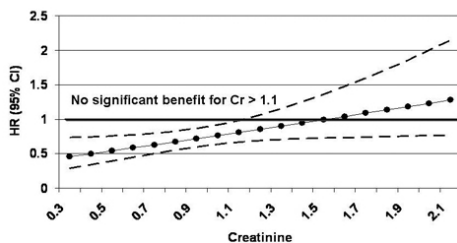
Background: Since the implementation of the Model for End-Stage Liver Disease (MELD) for liver allocation, an increasing number of candidates with renal insufficiency have undergone orthotopic liver transplantation (OLT). Since candidates with renal insufficiency have higher post-transplant morbidity and mortality, MELD-based allocation may be shifting some waiting list mortality to the post-transplant period in these candidates. The objective of this study was to evaluate the survival benefit among candidates with renal insufficiency who underwent OLT.

Methods: Scientific Registry of Transplant Recipients data for adult candidates age ≥ 18 initially listed for OLT between 9/1/01 and 12/31/2006 (n=38,899) were analyzed. The effect of serum creatinine on the survival benefit (contrast between waiting list and post-transplant mortality) was assessed by sequential stratification, an extension of Cox regression. Each recipient was matched with candidates active on the waiting list in the same organ procurement organization with the same MELD score.

Results: For MELD scores 12-40, the survival benefit of OLT significantly decreased as serum creatinine increased. Among candidates transplanted at MELD 12-14, the 23% with serum creatinine >1.1 mg/dl (23%) experienced no significant survival benefit (Figure). Candidates transplanted at MELD ≥ 15 experienced significant OLT benefit irrespective of serum creatinine level.

Conclusions: Comparing two patients with MELD ≥ 12 , the patient with higher creatinine experiences significantly less survival benefit from liver transplantation. Almost one-quarter of patients transplanted at MELD 12-14 experienced no survival benefit from OLT based on 5 years of follow-up. Therefore, more careful assessment of candidates is required in order to maximize the survival benefit gained by the wait-listed end-stage liver disease population as a whole.

Hazard Ratio of post-transplant to waitlist mortality at varying values of creatinine at MELD 12-14



Liver: Living Donors and Parial Grafts I

Abstract# 159

Assessment of Risk Tolerance in Live-Donor Liver Transplantation – A Comparison of the View of Donors, Non-Donors, and Transplant Professionals. Annie Fecteau, Sylvia Lee, Ivan R. Diamond, Linda Wright, Vicky Ng, David R. Grant. *Departments of Surgery and Pediatrics, and the Joint Center for Bioethics, The University of Toronto, Toronto, ON, Canada.*

Introduction: Consideration of the risks and benefits of a procedure are critical in medical decision making. However, relatively little is known about risk tolerance amongst donors and transplant professionals in live-donor liver transplantation (LDLT).

Method: We conducted confidential semi-structured interviews in a convenience sample of donors, non-donors (individuals who had been assessed for donation but did not donate) and transplant team members. In addition to examining issues surrounding decision making for LDLT donation, we sought to assess the tolerance of participants, above which they would no longer contemplate donation, for a number of potential outcomes following LDLT. The outcomes that participants were asked to consider included their tolerance for risk of donor death, risk of serious donor complication, as well as risk of recipient death following transplantation. The interviews were conducted sequentially, data was coded quantitatively, and the study terminated once saturation was reached.

Results: 24 donors, 5 non-donors and 22 transplant professionals participated in this IRB approved study. While 19/24 donors would consider donation despite a risk of donor death exceeding 5%, only 7/21 of the transplant team members viewed this risk to be acceptable ($p = 0.002$). Similarly, while 17/24 donors considered a donor risk of serious morbidity of 20% to be acceptable, only 9/22 of the transplant team considered this to be acceptable ($p = 0.07$). Interestingly, 11 of the donors would have accepted morbidity exceeding 50%. Donors and non-donors did not differ statistically in terms of tolerance for donor morbidity or mortality. In terms of recipient survival, 16/23 donors stated that they would donate even if the probability of recipient survival were less than 20%,

whereas only 1/21 transplant team member considered donation in this situation to be acceptable ($p < 0.0001$). All 5 non-donors surveyed stated that recipient survival should exceed 20% to make donation acceptable ($p = 0.008$ compared to donors).

Conclusion: The results of this study suggest that individuals who proceed with live donor liver donation have significantly higher tolerance of risk than transplant professionals. These data have important implications when considering informed consent for such donors.

Abstract# 160

Ambivalence in Adult Living Liver Donors. Mary Ann Simpson,¹ Julia Kendrick,¹ Jennifer E. Verbesey,¹ James J. Pomposelli,¹ Elizabeth A. Pomfret,¹

¹Liver Transplantation, Lahey Clinic, Burlington, MA.

Introduction: All candidates for right lobe liver donation undergo extensive evaluation prior to actual donation. A subset of donors was identified as being ambivalent about donation, either by staff during evaluation, or through self-identification on quality-of-life (QOL) surveys. In this study, we have identified factors associated with candidates who complete RHL donation despite ambivalence.

Methods: The records of 93 RHL donors were reviewed, including 6 QOL surveys (pre, weeks 1, 4, 12, 26 and 52 post-donation). Ambivalence detected by staff or described by donor was recorded. Donor and recipient characteristics were examined and compared between ambivalent and non-ambivalent groups.

Results: Staff identified and self identified ambivalent donors were not equivalent. Staff assessments indicated 20 ambivalent donors (16 male, 4 female). 18 donors self-identified as ambivalent (11 male, 7 female); 7 donors were on both lists (5 male, 2 female). The combinations of brother to brothers and sons to fathers were the most common pairs among ambivalent donors and more common than in total donor cohort. Recipient diagnosis of alcohol or hepatitis C related liver disease was more common in ambivalent donors. Ambivalent donors were more likely to be college educated and to express significant religious affiliations than the total RHL donor group. All but 1 ambivalent donor indicated that they would donate again on the 1 year QOL survey.

Conclusions: Ambivalence about RHL donation is present in approximately 20% of candidates who complete donation. Staff-identified and self-identified groups showed only 20% overlap; however, both groups showed similar characteristics. Brother-to-brother and son-to-father pairings and recipients with perceived self-induced liver failure were more common in both groups compared to total donor cohort. Ambivalent donors had more education and stronger religious or spiritual identification than the entire cohort. Only 1 donor indicated persistent doubt about donation. These results suggest that expressed or perceived donor candidate ambivalence may represent a process of careful consideration and should not be used sole basis for donor disqualification.

Group	%M/F	%Recipient EtOH	%Recipient HCV	%1st/Last Born	%College	%Religious	%Br-Br/ Son-father
Staff ID	80/20	20	55*	25/20	66*	73*	40*/25
Self ID	61/39	11	44	29/26	52*	59	22/27
All Donors	63/37	13	38	21/40	35	52	18/25

* $p < 0.05$ compared to All Donors

Abstract# 161

The Use of Older Donors for Right Lobe Living Donor Liver Transplantation Adversely Affects Biliary Complications, but Not Patient or Graft Survival. Markus Selzner,¹ Mark S. Cattral,¹ Ian D. McGilvray,¹ Paul D. Greig,¹ Lesley E. Adcock,¹ Arash Kashfi,¹ Nazia Selzner,¹ Eberhard L. Renner,¹ Les Lilly,¹ George Therapondos,¹ Gary A. Levy,¹ David R. Grant,¹ ¹Multiorgan Transplant Program, Toronto General Hospital, Toronto, ON, Canada.

The impact of donor age on recipient outcome for adult right-lobe living donor liver transplantation (RLDLT) is unclear. **Aim:** To analyze the effect of donor age on recipient outcome following RDLDT. **Methods:** Since 2000 we have performed 226 RDLDT (mean donor age 37 years, range 18-60 years), including 20 donors age 55 years or older. We analyzed the effects of donor age, as a continuous or categorical (< 54 vs ≥ 55 years) variable, on recipient outcome. Recipient outcome measures included biochemical markers of hepatocytes injury (AST, ALT) and graft function (INR, bilirubin), postoperative infections, bleeding, biliary complications, acute cellular rejection, as well as patient and graft survival. Analyses were carried out stratified for higher recipient MELD scores (< 25 vs ≥ 25), recipient age (< 60 vs ≥ 60 years), and hepatitis C virus (HCV) infection (presence vs. absence). **Results:** 5-year patient and graft survival after RDLDT was 80% and 78%, respectively. Donor age as a continuous variable was associated with increased AST ($p = 0.04$) and ALT ($p = 0.022$) release after transplantation, while no effect was observed on INR or bilirubin. RDLDT using donors above 55 years of age resulted in an increased incidence of biliary strictures (20% vs. 6%, $p = 0.028$), postoperative cholangitis (25% vs. 9%, $p = 0.023$). No effect of donor age was found for the following recipient outcome measures: the number of bile ducts supplying the graft, type of biliary reconstruction required; rejection, hemorrhage, pulmonary or urinary tract infections, renal failure, or length of hospital stay. 5-year patient survival was identical for patients receiving grafts from donors below or above 55 years of age (81% vs 77%, $p = 0.86$). Similarly, 5-year graft survival was comparable for young and old grafts (78% vs 77%, $p = 0.71$). Recipient age (< 60 vs ≥ 60 years), recipient MELD score (< 25 vs ≥ 25), or Hepatitis C status of the recipient did not impact on the effect of age on patient or graft survival. **Conclusion:** In this single center series of 226 RDLDT, the

use of selected older donors did not impair graft and patient survival, but was associated with an increased rate of biliary strictures.

Abstract# 162

Risk Factors for the Development of Biliary Strictures Following Right Lobe Living Donor Liver Transplant in Adults. Jose Almeda,¹ Trina Steljes,¹ Linda Sher,¹ Maria Stapfer,¹ Rod Mateo,¹ Rick Selby,¹ Yuri Genyk,¹ ¹University of Southern California, Los Angeles, CA.

Background: Biliary stricture rate after living donor liver transplant (LDLT) in adults remains relatively high in comparison to the stricture rate after adult cadaveric liver transplant or LDLT in pediatric patients. The etiology or risk factors for biliary stricture development at present time are uncertain.

Purpose: To determine the risk factors for biliary stricture after right lobe (RL) LDLT.

Methods: From 5/99 to 12/07, 109 LDLT procedures were performed in 109 adult recipients. Eleven patients were excluded from analysis due to <90 days follow up or need for retransplant. The following data was prospectively collected: 1. demographics, 2. acuity of illness, 3. number of bile ducts, 4. type of biliary reconstruction, 5. graft to recipient weight ratio, 6. hemodynamic parameters, 7. outcomes. These parameters were compared in patients with and without strictures.

Results: Mean follow-up for 98 patients is 1642 days (range: 120-3052). 8 of 109 patients died during the follow-up range and 3 required whole liver re-transplants. 33 patients (34%) developed a biliary strictures during the follow-up period. Comparison of risk factors in patients with and without strictures revealed the following results:

	Mean MELD	>1 Bile Duct	GRWR* < 1.1	Day 10 TB** >2 mg/dl	Duct vs Roux en Y
Stricture (n=33)	13	11 (33%)	17 (52%)	28 (85%)	15 vs 15
No stricture (n=65)	13	22 (33%)	15 (23%)	29 (43%)	38 vs 23
P Value	NS	0.8	0.006	0.0002	0.366

*GRWR: Graft to recipient weight ratio; TB: total bilirubin;

Conclusions: Neither MELD score, number of bile ducts or type of biliary reconstruction appear to be contributing factors to the development of bile duct stricture following RL LDLT. The biliary stricture rate was related to the volume of transplanted liver and post transplant graft recovery. Therefore, the development of biliary strictures in some patients may represent yet another feature of small-for-size syndrome.

Abstract# 163

Comparisons of Biliary Complications for Living and Deceased Donor Liver Transplants at a Single Large Transplant Center. Sandy Feng,¹ Nick Meo,¹ ¹Department of Surgery, University of California San Francisco, San Francisco, CA.

BACKGROUND: It is widely known that biliary complications occur more frequently after living donor (LD) compared to deceased donor (DD) liver transplantation (tx). Direct comparison of biliary complication frequency, type, timing, and resolution characteristics between contemporaneous LD and DD tx cohorts from a single transplant center with a high volume of both LD and DD tx has not been reported.

METHODS: Biliary complications of adult LD (N = 90) and DD (N = 548) txs performed between 2000 and 2005 were identified and analyzed. Complications were classified as either stricture or leak. Fisher's exact, Mann-Whitney, and Kruskal-Wallis tests were used to compare incidence, type, timing, and resolution characteristics of LD vs DD biliary complications.

RESULTS: LD txs had lower MELD score (16.4 ± 6.2 vs 24.7 ± 12.3; p < .0001), higher bile duct number (1.68 ± .63 vs 1.004 ± .06; p < .0001), and less frequent use of duct-duct anastomoses (47.8% vs 93.2%; p < .0001).

Figure 1: Living and Deceased Donor Liver Transplant Characteristics

CHARACTERISTIC	90 LDLTs	548 DDLTs	P Value
D Age	55.8 ± 9.4y	40.8 ± 36.8y	0.053
D Gender: N (%) male	322 (58.8)	47 (52.2)	0.274
R Age	53.0 ± 10.3y	52.4 ± 10.2y	0.89
R Gender: N (%) male	52 (57.8)	357 (65.1)	0.18
R Diagnosis: N (%) HCV	40 (44.4)	275 (50.2)	0.36
R MELD	16.4 ± 6.2	24.7 ± 12.3	<0.0001
Cold ischemia time		9.35 ± 2.6h	
Warm ischemia time	46.6 ± 15.6m	44.1 ± 8.0m	0.21
Bile Duct Number	1.68 ± 0.63	1.004 ± .06	<0.0001
N (%) of Grafts with 1 or >1 Duct	36 (40.0)	546	<0.0001
Anastomosis: N (%) Duct to Duct	43 (47.8)	511 (93.2)	<0.0001
Anastomosis: N (%)	62 (68.9)	548 (100)	<0.0001
	28 (31.1)	0	

Biliary complications occurred in 32.2% of LD vs 19.3% of DD txs (p = .0079). Seven (7.8%) LD vs 12 (2.2%) DD txs experienced >1 biliary complication (p = .011). LD and DD complications were similarly divided between strictures (2/3) and leaks (1/3). Strictures occurred much later for LD than DD txs [182 (19-2058)d vs 33 (4-1392)d; p = .0004]; 70% of LD vs 30% of DD strictures occurred >90d after tx. Complications

were successfully managed in 86.2% of LD and 85.8% of DD txs (p = 1.00). Time to resolution was comparable [54 (0-673)d vs 49 (0-1060)d; p = .17].

Figure 2: Biliary Complication Characteristics after LD and DD LTxs

CHARACTERISTIC	90 LDLTs	548 DDLTs	P Value
Transplants with Complications	29 (32.2)	106 (19.3)	0.0079
Number per Transplant	0.41 ± .67	0.22 ± .46	0.0033
Transplants with 0, 1, 2, or 3 Complications:			
0	61 (67.8)	442 (80.7)	0.0033
1	22 (24.4)	94 (17.2)	
2	6 (6.7)	12 (2.2)	
3	1 (1.1)	0	
Initial Complication Type: N (%)			0.66
Stricture	18 (62.1)	72 (67.9)	
Leak	11 (37.9)	34 (32.1)	
Timing of Initial Complication			
Stricture or Leak	67 (6 - 2058) d	24 (1 - 1392) d	0.0029
Stricture	182 (19 - 2058) d	33 (4 - 1392) d	0.0004
Leak	17 (6 - 57) d	11.5 (1 - 80) d	0.23
Frequency of Resolution for Initial Complication			
Stricture or Leak	25 / 29 (86.2)	91 / 106 (85.8)	1.000
Stricture	17 / 18 (94.4)	60 / 72 (83.3)	0.45
Leak	8 / 11 (72.7)	31 / 34 (91.2)	0.15
Time to Resolution of Initial Complication			
Stricture or Leak	54 (0 - 673) d	49 (0 - 1060) d	0.17
Stricture	72 (34 - 673) d	67.5 (0 - 1060) d	0.13
Leak	17.5 (0 - 129) d	8 (0 - 365) d	0.51

CONCLUSIONS: Biliary complications are more frequent after LD than DD tx. The distribution of complications between strictures and leaks is comparable. Strictures occur much later for LD compared to DD txs. The vast majority of biliary complications can be resolved and over a similar timeframe for both LD and DD txs.

Abstract# 164

A Decade Experience of 252 Adult-to-Adult Right Liver Living Donor Liver Transplantation in Hong Kong. Sheung Tat Fan,¹ Chung Mau Lo,¹ Chi Leung Liu,¹ See Ching Chan,¹ Barbara Chik,¹ Vanessa de Villa,¹ ¹Department of Surgery, The University of Hong Kong, Pokfulam, Hong Kong, China.

In 1996, we performed the world's first adult-to-adult right liver living donor liver transplantation (A-A RLDT). Up till December 2006, we have accumulated experience of 252 operations. In the initial period, A-A RLDT was offered only to patients with high urgency status, i.e. cirrhosis with acute deterioration, chronic active hepatitis with acute flare, and fulminant hepatic failure. Since case No. 17, the operation has been offered to all elective patients as well. All right liver grafts contained the middle hepatic vein except recipient No. 87. The patients were categorized into those with cirrhosis (n=138), cirrhosis with acute deterioration (n=51), chronic active hepatitis with acute flare (n=39), and fulminant hepatic failure (n=23). The median pre-transplant Model for End-stage Liver Disease scores of the four categories were 16, 35, 37, and 36, respectively. Their median blood transfusions during transplants were 4, 7, 7, and 7 units, respectively. The overall hospital mortality rate was 4.7%. During 2000-2004, there were 110 consecutive recipients without hospital death. The hospital mortality rates of the 4 groups were 3.6%, 5.9%, 7.7%, and 4.3%, respectively. There were 6 retransplants for graft failure using living donor (n=1) and deceased donor grafts (n=5). The one-year and 3-year survival rates of these 4 groups of patients were 93.5%, 92.1%, 92.3%, 95.7%, and 82.2%, 89.5%, 89.7%, 94.4%, respectively. In conclusion, A-A RLDT has achieved satisfactory early and mid-term results and is applicable to all categories of terminal liver failure patients. It has now established an important role in regions with scarce deceased organ donation.

New Developments in the Regulation of Tregs

Abstract# 165

OX40 Controls Transplant Tolerance in CD154 Deficient Mice by Regulating Foxp3+ Tregs. Gulcin Demirci,¹ Ming Chen,¹ Xiang Xiao,¹ Alexandar Kroemer,¹ Xian C. Li,¹ ¹Transplant Research Center, Beth Israel Deaconess Med Ctr, Boston, MA.

Background: OX40 and CD154 can be expressed by both Foxp3+ Tregs and activated T effector cells. However, the question as to how OX40 and CD154 function, individually or collectively, in regulating such functionally different T cell subsets in transplant models remains poorly understood. In some models, blocking CD154 costimulation is remarkably effective in prolonging graft survival, but targeting CD154 alone rarely creates tolerance. But the role of OX40 in regulating the CD154 blockade induced tolerance is completely unknown.

Methods: In the present study we critically examined the role of OX40 in the activation of CD154 deficient T effector cells as well as in the regulatory function of Foxp3+ Tregs. We also examined the effect of OX40 on the induction of new Foxp3+ Tregs/Th17 cells from activated CD154 deficient T effector cells. The impact of OX40 in the induction of allograft tolerance was examined using an islet transplant model.

Results: We found that CD154 deficient Foxp3+ Tregs constitutively expressed OX40 on the cell surface, but the CD154 deficient T effector cells did not. However, when the T effector cells were sorted and stimulated in vitro, OX40 expression could be

readily induced on the T effector cells. To further examine how OX40 regulates such functionally different T cell subsets, we found that OX40 delivers potent costimulatory signals to T effector cells, which prevent the induction of new Foxp3⁺ Tregs from activated T effector cells but promote their differentiation to Th1 cells but not Th17 cells. Surprisingly, OX40 costimulation to CD154 deficient Foxp3⁺ Tregs completely inhibited their regulatory functions. In an islet transplant model, we showed that CD154 deficient mice can reject the DBA/2 islet allografts, but blocking OX40 costimulation readily induced donor specific tolerance (MST>150 days), and this tolerant status was critically dependent on the induction of Foxp3⁺ Tregs. In contrast, treatment of CD154 deficient recipients with a agonist anti-OX40 mAb precipitate rapid islet allograft rejection, suggesting that OX40 costimulation is critically important in the induction of transplant tolerance.

Conclusions: Our data suggest that OX40 is a costimulatory molecule to T effector cells but a powerful negative regulator for Foxp3⁺ Tregs. Thus, a key role for OX40 in the induction of transplant tolerance is the control of T cell mediated regulation.

Abstract# 166

DNA Methylation Has a Major Role in Regulating Foxp3 Transcription.

Girdhari Lal,¹ Yaozhong Ding,¹ Jonathan S. Bromberg.¹ ¹*Gene and Cell Medicine, Mount Sinai School of Medicine, New York City, NY.*

Background: Foxp3 is a winged-helix family transcription factor that is the master regulator for the development and function of regulatory T cells (Treg). We investigated the molecular mechanisms important for regulation of Foxp3 expression, and defined the structure of the active Foxp3 promoter in CD4⁺ T cell lineages.

Methods: Purified CD4⁺CD25⁺Foxp3⁺GFP⁺ T cells (naïve) and CD4⁺CD25⁺Foxp3⁺GFP⁺ Treg were cultured with antigen presenting cells in the presence of IL-2, anti-CD3ε mAb, TGFβ or the DNA methyltransferase inhibitor 5-aza-2'-deoxycytidine (ZdCyd). Foxp3 promoter structure and activity were monitored with methylation-specific PCR, disulfite-sequencing, chromatin immunoprecipitation (ChIP) assays, electrophoretic mobility shift assay (EMSA) and luciferase promoter assay.

Results: The Foxp3 promoter has an upstream CpG island ~5kb from the transcriptional start site. Disulfite-sequencing and methylation-specific PCR analysis showed that this region is heavily methylated in naïve CD4⁺ T cells and TGFβ induced peripheral Treg, but demethylated in thymic derived natural Treg (nTreg). ChIP analysis showed that the methylated CpG island is bound specifically by the DNA methyltransferases 1 and 3b. ZdCyd causes demethylation of the CpG island, and in combination with TGFβ, synergistically induces Foxp3 expression. ChIP assays for acetylated histone 3 and Sp1, both markers of gene activation, showed that the CpG island is acetylated and bound by Sp1 in nTreg and ZdCyd plus TGFβ induced Treg, but not in activated CD4⁺ T cells or TGFβ induced Treg. EMSA likewise shows the CpG island binds Sp1. In contrast to the upstream promoter, the structure of the first intronic promoter differs markedly between nTreg and TGFβ induced Treg, but is not affected by ZdCyd. The upstream CpG island also possesses enhancer activity that is repressed by DNA methyltransferases. ZdCyd plus TGFβ induced Treg have stable Foxp3 expression and enhanced suppressive functions *in vitro* and *in vivo*.

Conclusion: These results demonstrate that nTreg and TGFβ induced Treg are genetically distinguished from each other by the epigenetic structure of a unique upstream CpG island of the Foxp3 promoter. The function of this region is regulated by DNA methylation and histone acetylation. ZdCyd demethylates the promoter, leading to enhanced and stable expression of Foxp3 and suppressor activity, similar to nTreg. This has important implications for biology, and generating Treg for tolerance.

Abstract# 167

Chemokine Receptors Important for Treg Lymphatic Migration and Suppressive Function.

Nan Zhang,¹ Bernd Schroppel,¹ Girdhari Lal,¹ Jonathan S. Bromberg.¹ ¹*Gene and Cell Medicine, Reconati/Miller Transplantation Institute, Mount Sinai School of Medicine, New York, NY.*

Background: Trafficking of lymphocytes through lymphatics to secondary lymphoid organs is crucial for immune responses. We previously showed that regulatory T cell (Treg) function required trafficking from the inflammatory graft site to the local draining lymph node (dLN). Since the mechanisms that regulate migration through afferent lymphatics are poorly understood, we explored the role of chemokine receptors on Treg for afferent lymphatic migration in an islet transplantation model.

Methods: Islets were transplanted from BALB/c mice into Foxp3^{GFP} C57BL/6 mice. Treg from wild type, CCR2^{-/-}, CCR4^{-/-}, CCR5^{-/-}, or CCR7^{-/-} C57BL/6 mice were isolated, labeled with red dye PKH26, and transferred intravenously, or locally into the islet allograft. Treg migration to islet grafts and dLN was determined by flow cytometry and immunohistochemistry. Endogenous Foxp3^{GFP} Treg and transferred PKH26 labeled Treg were sorted from the islet grafts and the dLN, and chemokine receptor and sphingosine 1-phosphate receptor (S1P1) expression were determined by RT-PCR. Islet allograft survival was determined by measurement of blood glucose.

Results: Freshly isolated Treg expressed S1P1 and the chemokine receptors CCR2, CCR4, CCR5, and CCR7. Endogenous Treg, and both intravenously and locally transferred Treg, that were recovered from islet allografts and dLN expressed similar levels of CCR2 and CCR5. CCR4 was expressed preferentially on islet migrating Treg, while S1P1 and CCR7 were expressed preferentially in dLN migrating Treg. Locally transferred Treg migrated to the dLN, but CCR7^{-/-} Treg were not able to migrate to the dLN. CCR2^{-/-} and CCR5^{-/-} Treg were impaired in their ability to migrate to the dLN.

This suggested that these three chemokine receptors all regulated Treg entry into afferent lymphatics and migration from the graft to the dLN. In contrast, CCR4^{-/-} Treg migrated normally from the islet to the dLN. Importantly, CCR2^{-/-}, CCR5^{-/-} and CCR7^{-/-}, but not CCR4^{-/-} Treg, were impaired in their ability to prolong islet allograft survival when transferred locally in the islet allograft.

Conclusion: Treg migrate from the inflammatory site of the allograft to draining secondary lymphoid tissue through afferent lymphatics. This process depends on CCR2, CCR5, and CCR7; and is crucial for full Treg function *in vivo*. These results demonstrate a novel role for sequential migration from the graft to the dLN in Treg function and suppression.

Abstract# 168

HDAC6 Is a Key New Epigenetic Target for the Enhancement of Treg

Production and Function *In Vitro* and *In Vivo*. Wayne W. Hancock,¹ Liqing Wang,¹ Edwin F. de Zoeten,¹ James E. Bradner,² Ralph Mazitschek.³ ¹*Pathology and Laboratory Medicine, Children's Hospital of Philadelphia and University of Pennsylvania, Philadelphia, PA;* ²*Dana-Farber Cancer Institute, Boston, MA;* ³*Broad Institute of Harvard University and MIT, Boston, MA.*

Epigenetic regulation of gene expression provides a major, and especially beyond oncology, largely unexplored means to regulate host immune cell functions. Our ongoing analysis of histone deacetylase (HDAC) expression by Foxp3⁺ naturally occurring murine regulatory T (Treg) cells showed TCR-activated Tregs had 5-6 fold more HDAC6 mRNA than corresponding resting Treg or non-Treg cells. In various cell types, HDAC6 deacetylates alpha-tubulin, cortactin, and HSP90, abrogates formation of the aggresome, and blocks the unfolded protein response, though nothing is known regarding these pathways in Tregs. We found that an HDAC6-specific inhibitor, tubacin (but not the control compound, niltubacin), increased Treg suppressive function *in vitro* (p<0.01), in association with increased expression of CTLA, IL-10, GITR, PD-1 and other Treg-associated genes (p<0.05), and increased Treg Foxp3 protein (though not mRNA) expression. Tubacin enhanced the conversion of CD4⁺CD25⁺ cells into CD4⁺ Foxp3⁺ Treg *in vitro*, and globally decreased cytokine production, with the exception of IL-10 and IL-17 mRNA. Comparable and dose-dependent effects were seen using the HSP90 inhibitor, geldanamycin, suggesting that the effects of HDAC6 inhibition were mediated, at least in part, by blocking the chaperone effect of HSP90. Use of tubacin *in vivo* significantly decreased the severity of colitis in two murine inflammatory bowel disease models (p<0.05), dextran sodium sulfate-induced colitis and the CD4⁺CD62L^{high} adoptive transfer model of colitis, as assessed by standard clinical and histologic criteria. In addition, 14 days combined use of tubacin and a subtherapeutic dosage of rapamycin led to significantly prolonged cardiac allograft survival (BALB/c->C57BL/6) compared to use of either agent alone (p<0.05). Our data show that use of the first known small molecule inhibitor of one specific HDAC has important therapeutic effects, including enhancing the production and suppressive function of Tregs. While ongoing studies are directed towards unraveling the interactions of HDAC6-dependent pathways and Treg functions, the current data indicate the importance of understanding the functions of HDACs to the development of entirely new ways to regulate host immune responses.

Abstract# 169

Dendritic Cells Supply Paracrine IL-2 for Treg Cell Functional Activity.

Mohamed Nasr,¹ Katarina Kulhankova,² Todd Rouse,¹ Lauren Jensen,³ Elizabeth H. Field.^{1,2} ¹*Research Program, Department of Veteran Affairs Medical Center, Iowa City, IA;* ²*Internal Medicine, Carver College of Medicine, University of Iowa, Iowa City, IA;* ³*Harvard College, Cambridge, MA.*

Regulatory CD4⁺CD25⁺ T cells (Tregs) are important for the maintenance of immune tolerance, and immunotherapy with Tregs is being explored for organ and cell transplantation. Treg development, expansion and function depend on IL-2. Because Tregs do not make IL-2, they must obtain IL-2 from another cell. Although CD4⁺ T effectors are a logical candidate, the identity of the paracrine source of IL-2 for Tregs is not substantiated. We explored whether dendritic cells (DCs) could serve as the paracrine source of IL-2 for Treg and RD6, a CD4⁺CD25⁺ regulatory hybridoma. Using four dimensional live cell imaging we demonstrate that Treg and RD6 cells establish tight contact with DCs, and CD25 is localized at these contacts. Using the IL-2 ELISPOT and real-time RT-PCR we found that splenic DCs and the JAWSII DC cell line constitutively make IL-2. LPS and CpG increases DC production of IL-2. Co-Culture with JAWSII DC cell line significantly upregulates CD25 expression on alloreactive DO11.10 Tregs and RD6 cells, but not on DO11.10 CD4⁺ T effector cells. Tregs and RD6 cells are functionally suppressive after activation by wild type but not IL-2 knock-out allogeneic DCs, and anti-CD25 inhibits the function of Treg and RD6 cells in a dose response fashion. In contrast, wild type and IL-2 knock-out DCs are equally able to activate alloreactive CD4⁺CD25⁺ cells. Supplemental IL-2 at high (1000 U/ml) but not low doses (100 U/ml) restores the function of alloreactive Tregs and RD6 that were activated by IL-2 KO DCs. These data indicate that Treg cells acquire IL-2 from dendritic cells for their gain of function and validate dendritic cells as a paracrine source of IL-2 for Treg.

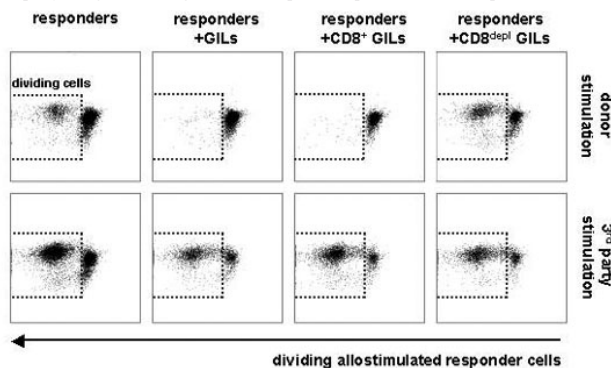
Abstract# 170

Donor-Specific Regulation by CD8⁺FoxP3⁺ Lymphocytes Expanded from Rejecting Human Cardiac Allografts. Esmé Dijke,¹ Aggie Balk,² Mariska Klepper,¹ Ronella de Kuiper,¹ Kadir Caliskan,² Lex Maat,² Willem Weimar,¹ Carla Baan.¹ ¹Internal Medicine; ²Thorax Center, Erasmus MC, Rotterdam, Netherlands.

Introduction: Previously, it has been demonstrated that FOXP3, a gene required for the development and function of regulatory T cells, was highly expressed in the graft during cardiac rejection, suggesting infiltration of regulatory T cells in the transplanted organ during an allogeneic response. In this study, we investigated whether graft-infiltrating T cells expanded from rejecting human cardiac allografts exhibit immune regulatory activities.

Methods: Graft-infiltrating lymphocytes (GILs) cultured from endomyocardial biopsies (EMB; n=13) with histological signs of acute cellular rejection were expanded in the presence of donor-antigens in IL-2/IL-15-enriched medium for 2-3 weeks. Flow cytometry was used to analyze the expression of CD3, CD4, CD8 and FoxP3. To analyze the immune regulatory function, we performed MLRs with peripheral blood mononuclear cells (PBMC) of the patients and irradiated donor or third party spleen cells in the absence and presence of GILs (ratio 5:1).

Results: Of the CD3⁺ GILs, 9% (median; range: 1–21%) stained positive for FoxP3. This FoxP3 expression was detected in both CD4⁺ and CD8⁺ T-cell population (median: 8% and 9%, respectively). Functional analysis demonstrated that GILs suppressed the anti-donor proliferation of responder T cells (range % inhibition: 55–81%). Interestingly, this suppression was predominantly achieved by CD8⁺ GILs: depletion of CD8⁺ cells from the GILs population diminished the inhibitory effect, whereas addition of solely CD8⁺ GILs to the MLR abundantly suppressed the anti-donor response (range % inhibition: 62–77%). In contrast, GILs did not inhibit the proliferation of T cells stimulated with third-party antigens. The figure below depicts a representative example.



Conclusion: Graft-infiltrating lymphocytes expanded from rejecting cardiac allograft exhibit donor-specific immune suppressive activities. These results suggest that during acute cellular rejection, graft-infiltrating lymphocytes not only consist of graft-destructing effector T cells, but may also comprise immune regulatory cells of the CD8⁺ phenotype.

Joint Plenary Session II

Abstract# 171

The Combined-Organ Effect: Protection Against Rejection? Abbas Rana,¹ Susanne Robles,¹ Mark J. Russo,¹ Karim J. Halazun,¹ David C. Woodland,¹ Lloyd E. Ratner,¹ Mark A. Hardy.¹ ¹Department of Surgery, Columbia University, New York, NY.

Context: It has been previously suggested that a liver allograft is immunoprotective and able to decrease the rate of rejection of a donor-specific allograft of another organ. It has been recently proposed that allografts other than the liver may also be immunoprotective.

Objective: The aim of this analysis was to examine one year rejection rate and the incidence of rejection free survival of all combined transplants in the collective US experience to gain insight to any possible protective effect of one organ for another.

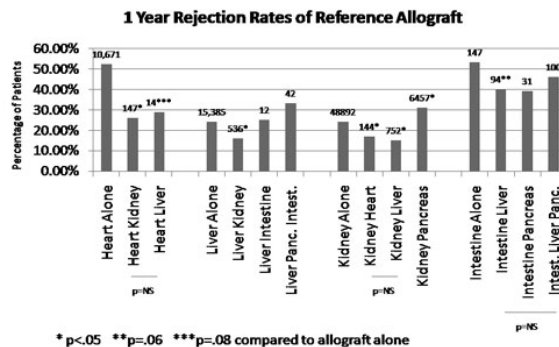
Methods: The United Network of Organ Sharing (UNOS) provided de-identified patient-level data. Analysis included all recipients transplanted between January 1, 1994 and October 6, 2005 who were 18 years or older (except intestinal transplants). Rejection at one year was defined as treatment for one or more episodes of rejection.

Results: Analysis included a total of 83,424 patients who received either one, or combined, simultaneous or sequential, organ transplants in all possible combinations. Results are summarized in figure 1 (one-year organ allograft rejection rate). The collected data demonstrate that the rejection rate of donor-specific organ allografts which accompanied primary liver, kidney, and heart transplants was significantly lower in combined transplants as compared to that of the primary allograft transplanted alone. This was not true, however, for intestinal and pancreatic allografts where protection for the accompanying organ was not observed. We further demonstrate that transplantation of two organs of the same type (double kidneys or double lungs), i.e. increase in antigen

load, also leads to decreased rates of rejection of the allografted organs.

Conclusions: In combined simultaneous transplants, the heart, liver, and kidney allografts appear themselves to be protected, and to protect the other organ from rejection. Increased antigenic load of identical antigens in case of double lung and double kidney transplants appears to also offer immunologic protection against rejection, perhaps by different mechanisms.

Figure 1

Combined Organ Transplants**Abstract# 172**

Comparison of A2ALL and National Experience with Adult-to-Adult Living Donor Liver Transplantation (AALDLT) Outcomes: Analysis of Center Experience and Mortality Risk Factors. K. M. Olthoff,¹ M. M. Abecassis,¹ J. C. Emond,¹ R. M. Merion,¹ B. W. Gillespie,¹ L. Tong.¹ ¹A2ALL Study Group, NIH-NIDDK.

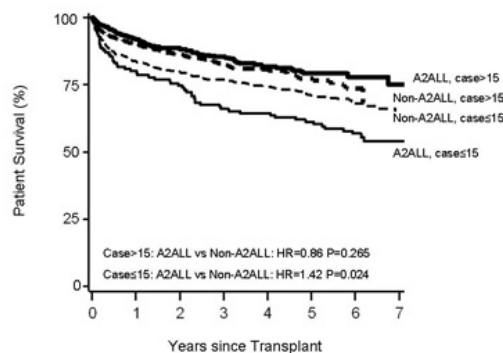
Background: A2ALL (9-center Adult-to-Adult Living Donor Liver Transplantation Cohort Study) has identified risk factors for mortality after AALDLT, including center experience. The aim of this study was to determine if A2ALL findings are reflected in the national experience.

Methods: AALDLT at A2ALL (n=681) and non-A2ALL centers (n=1598) from 1/1/98 to 8/31/07 in the Scientific Registry of Transplant Recipients database were analyzed. Cox regression models adjusted for recipient and donor characteristics were fitted to test associations with mortality risk after AALDLT, including center type (A2ALL vs. non-A2ALL) and case number (for each AALDLT at each center).

Results: AALDLT were performed at 9 A2ALL and 63 non-A2ALL centers. There was no significant difference in overall mortality risk between A2ALL and non-A2ALL centers. Significant predictors of death (both groups combined) included donor age (hazard ratio (HR)=1.14 per 10 years, P=0.003), recipient age (HR=1.25 per 10 years, P<0.001), diagnosis of HCV (HR=1.22, P=0.04) or HCC (HR=1.99, P<0.001), and earlier center experience (AALDLT case number ≤15, HR=1.58, P<0.001). There was no significant effect of transplant year after adjusting for experience. Cold ischemia time >4.5 hours was associated with higher mortality (HR=1.83, P=0.004); this effect was similar in A2ALL and non-A2ALL centers. There were no significant interactions between center type and any predictor except center experience (Figure). Compared to later experience, earlier center experience was associated with significantly higher mortality risk in both A2ALL (HR=2.24, P<0.0001) and non-A2ALL centers (HR=1.38, P<0.004). Survival during early experience was significantly worse at A2ALL vs. non-A2ALL centers (HR=1.42, P=0.024), but survival in later experience was similar.

Conclusions: After the first 15 cases, AALDLT survival was similar at A2ALL and non-A2ALL centers, and similar significant mortality risk factors were identified, including center experience. These analyses support the generalization of findings from A2ALL centers to others performing AALDLT.

Cox-based Adjusted Patient Survival after LDLT Transplant by Center Type (A2ALL, Non-A2ALL) and Case Number (≤15 Transplants, >15 Transplants)



Abstract# 173

Genetic Polymorphisms Impact the Risk of Rejection with Hemodynamic Compromise and Chronic Allograft Vasculopathy – A Multi-Institutional Study. D. M. Girmata,¹ M. Brooks,¹ S. Webber,¹ G. Burckart,² R. Ferrell,¹ G. Zdanowicz,¹ R. Chinnock,³ C. Canter,⁴ L. Addonizio,⁵ D. Bernstein,⁶ J. Kirklin,⁷ D. Naftel,⁷ A. Girmata,¹ A. Zeevi.¹ ¹Univ of Pittsburgh; ²Univ Southern California; ³Loma Linda Univ; ⁴Washington Univ; ⁵Columbia Univ; ⁶Stanford Univ; ⁷Univ Alabama Birmingham.

Rejection with hemodynamic compromise (HC) and chronic allograft vasculopathy (CAV) impact survival in pediatric heart transplantation (PHTx). We showed that high pro-inflammatory / lower regulatory cytokine gene polymorphism (GP) profile increased the risk for acute rejection. In this analysis, we assessed the effect of genetic factors on HC and CAV.

Methods: 406 PHTx with clinical and GP data for cytokines (TNF- α A-308G; INF- γ T-874A; IL-10 G-1082A, C-819T, C-592A; IL-4 C-590T; IL-5 T-746C; IL-6 G-174C), growth factors (TGF β -1 T+869C, C+915G; VEGF A-2578C, C-460T, G+405C), effector molecules (Fas A-670G; FasL C-843T) and pharmacogenomics (*ABCB1* C3435T, G2677T/A) were analyzed regarding HC and CAV.

Results: Adjusting for recipient black race and age with Cox regression models, we identified the following risk factors: IL-10 High was associated with lower rates of HC. Low Th1 (INF- γ , TNF- α) with high Th2 (IL-4, IL-5) cytokine GP profiles were protective for HC in combination with IL-10 High. Carriers of Fas High experienced higher rates for HC and CAV and High Fas-FasL combination doubled the relative risk for CAV. *ABCB1* 3435CC/2677GG genotypes were also associated with lower rates of HC (Table 1).

Conclusion: In this large multi-center study GPs with higher regulatory profiles and increased drug transport were associated with a lower incidence of HC. A genetic pro-apoptotic profile might contribute to the pathogenesis of CAV.

Sponsorship: This work was supported by 5P50 HL 074 732-03 from the National Heart Lung and Blood Institute, National Institutes of Health.

Table 1. Estimated adjusted Hazard Ratios of HC and CAV post-transplant.

Variable	Hazard Ratio		95% Hazard Ratio Confidence Limits		p value	
	HC	CAV	HC	CAV	HC	CAV
IL-10 HIGH	0.44	0.59	0.23 - 0.85	0.23 - 1.52	0.015	0.28
IL-10 HIGH and INF- γ LOW or IL-10 HIGH and IL-4 HIGH or IL-10 HIGH and IL-5 HIGH	0.47	0.47	0.25 - 0.91	0.17 - 1.32	0.024	0.15
IL-10 HIGH and TNF- α LOW or IL-10 HIGH and IL-4 HIGH or IL-10 HIGH and IL-5 HIGH	0.48	0.49	0.25 - 0.92	0.17 - 1.37	0.028	0.17
FAS HIGH	1.66	1.87	1.10 - 2.51	0.99 - 3.52	0.017	0.053
FAS HIGH+FASL HIGH	1.34	2.19	0.84 - 2.13	1.10 - 4.37	0.22	0.026
<i>ABCB1</i> (2677GG and 3435CC)	0.63	1.18	0.41 - 0.96	0.62 - 2.22	0.031	0.62

Abstract# 174

Differential Role of Invariant NKT Cells in the Production of Antibodies Against Blood Group Carbohydrates and Histocompatibility Complex Allopeptides. Toshimitsu Irei,¹ Hideki Ohdan,¹ Toshimasa Asahara.¹ ¹Hiroshima University, Hiroshima, Japan.

It has recently been reported that CD1d-restricted NKT cells that express invariant TCR (iNKT cells) play an important role in the production of autoantibodies through the interaction with B-1 cells. This observation prompted us to investigate the possible role of iNKT cells in the production of antibodies (Abs) against transplant-related antigens, such as ABO blood group carbohydrates and histocompatibility complex allopeptides, in a mouse model. We have previously demonstrated that B cells with receptors for blood group A carbohydrates were found exclusively in a CD11b⁺ CD5⁺ B-1 subpopulation of mice, resembling humans with blood group O or B. Immunization with human blood group A red blood cells (A-RBCs) elicited the extensive production of anti-A IgM and IgG. Furthermore, the number of B-1 cells with receptors for A carbohydrates increased in the peritoneal cavity. In CD1d^{-/-} and V α 14^{-/-} Balb/c mice, which lack iNKT cells, such elicited production of anti-A IgM was not observed, even after immunization with human A-RBCs. However, class II^{-/-} Balb/c mice, which lack CD4⁺ T cells but maintain normal levels of iNKT cells, exhibited levels of anti-A IgM production comparable to those in wild-type (WT) Balb/c mice. Moreover, anti-A IgG production was absent in CD1d^{-/-} Balb/c mice even after the immunization, indicating that although iNKT cells crucially contribute to anti-A IgM production and IgG class switching, helper T cells do not. Notably, the proportion of B-1 cells in the livers of CD1d^{-/-} Balb/c mice was significantly reduced ($2.66 \pm 0.43\%$, $n = 4$) when compared to that in WT mice ($4.76 \pm 1.93\%$, $n = 4$). We next immunized CD1d^{-/-} and WT Balb/c mice twice with 2×10^6 allogeneic B6 mouse thymocytes, and thereafter detected the anti-B6 (allopeptides) Abs by flow cytometry. In the CD1d^{-/-} mice, anti-B6 IgM production was comparable to that of WT mice, and IgG class switching also occurred normally. These findings indicated that iNKT cells play a pivotal role in the production

of Abs specific for blood group carbohydrate determinants that are believed to be T cell independent, but are not required in the production of Abs for allopeptides that are believed to be T cell dependent. The depletion of iNKT cells or the suppression of their function might constitute a novel approach for preventing antibody-mediated rejection in ABO-incompatible transplantation, or in xenotransplantation, which involves similar carbohydrate antigens.

Abstract# 175

Transplantation after Hypothermic Machine Perfusion Versus Static Cold Storage of Deceased Donor Kidneys: A Prospective Randomized Controlled Trial. Cyril Moers,¹ Jacqueline M. Smits,⁸ Mark-Hugo J. Maathuis,¹ Jürgen Treckmann,² Frank Van Gelder,³ Bogdan P. Napieralski,² Margitta van Kasterop-Kutz,⁸ Jaap J. Homan van der Heide,⁶ Jean-Paul Squifflet,⁴ Ernest van Heurn,⁵ Günter R. Kirste,⁷ Axel Rahmel,⁸ Henri G. D. Leuvenink,¹ Andreas Paul,² Jacques Pirenne,³ Rutger J. Ploeg.¹ ¹Surgery, University Medical Center Groningen, University of Groningen, Groningen, Netherlands; ²General, Visceral, and Transplantation Surgery, University Hospital Essen, Essen, Germany; ³Abdominal Transplant Surgery - Transplant Coordination, University Hospital Leuven, Leuven, Belgium; ⁴Abdominal Surgery and Transplantation, CHU Sart Tilman, University of Liège, Liège, Belgium; ⁵Surgery, University Hospital Maastricht, Maastricht, Netherlands; ⁶Nephrology, University Medical Center Groningen, University of Groningen, Groningen, Netherlands; ⁷Deutsche Stiftung Organtransplantation, Frankfurt am Main, Germany; ⁸Eurotransplant International Foundation, Leiden, Netherlands.

Background

Static cold storage (CS) is the most widely used organ preservation method for deceased donor kidney grafts. Retrospective analyses have indicated that preservation by hypothermic machine perfusion (MP) may lead to improved outcome after renal transplantation. However, there is a lack of sufficiently powered prospective studies to test the presumed superiority of MP.

Methods

In an international prospective randomized controlled trial we enrolled kidney pairs of 336 consecutive deceased donors and randomly assigned one organ to MP and the contralateral kidney to CS preservation. Follow-up was directed at all 672 recipients of these grafts. The primary endpoint was delayed graft function (DGF).

Results

MP significantly reduced the risk of DGF (OR 0.63; $P=0.02$) and more than halved the incidence of primary non-function after transplantation, when compared to CS (2.1 vs. 4.8%; $P=0.04$). Furthermore, MP significantly reduced the risk of graft failure in the first 6 months post-transplant (HR 0.46; $P=0.05$). In recipients who developed DGF, 6-month graft survival was better if their transplanted kidney was machine perfused (87 vs. 76%; $P=0.05$).

Conclusions

Hypothermic machine perfusion reduces the risk of delayed graft function, primary non-function, and graft failure in deceased donor kidney transplantation when compared to static cold storage. Furthermore, MP alleviates the deleterious effect of DGF on graft survival.

Antigen Presentation/Allorecognition

Abstract# 176

Cellular Trafficking in Transplantation – Dendritic Cells in and out of the Graft: An *In Vivo* Microscopy Approach. Georges Tocco,¹ Pilhan Kim,² Cavit D. Kant,¹ Charles Lin,² Seok-Hyun Yun,² Gilles Benichou.¹

¹Surgery/ Transplantation Unit, Massachusetts General Hospital, Boston, MA; ²Wellman Center for Photomedicine, Massachusetts General Hospital, Boston, MA.

We investigated the trafficking of cells after skin and heart transplantation in a dynamic fashion through the use of *in vivo* microscopy. Antigen presenting cells were followed using MHC-Cl-II-GFP and CD11c-GFP transgenic mice. Vascularized and non vascularized skin grafts as well as heart transplants were used in syngeneic as well as allogeneic settings.

After syngeneic non-vascularized skin transplantation, we observed an early and massive cellular infiltration of host cells into the graft as early as 3 hours post-transplant with a gradual accumulation in the dermis. The accumulation of host-derived cells was accelerated after graft vascularization at day 4/5 post transplantation. This graft infiltration by recipient cells was more pronounced with vascularized skin grafts, and to a higher degree in heart transplants. Recipient cells similarly infiltrated allogeneic grafts early on and in larger numbers than for syngeneic grafts by day 4/5 post-transplantation. When visualizing MHC-Cl-II-GFP recipient cells in a syngeneic skin transplant, recipient DCs invaded the graft early on and, by 3 weeks post transplant gradually replaced graft DCs in the dermis (dermal DCs) and the epidermis (Langerhans cells). Donor DCs could still be seen in the graft up to 8 days post transplant. However, virtually all donor Langerhans cells were eventually replaced by recipient ones in a concentric fashion

suggesting that the new Langerhans cells originate from the recipient skin adjacent to the graft and not from centrally-derived precursor cells. The vascular endothelium of a syngeneic transplant was partially replaced by recipient vascular endothelial cells in a centripetal fashion with more recipient-derived vascular endothelium present at the periphery of the graft and more donor-derived endothelium remaining in the center of the graft. Therefore, the graft can be seen as a "chimera" of cells from donor and recipient origin. The presence of recipient endothelial vascular cells and DCs within the graft may be important for maintaining the indirect response thought to be responsible for chronic rejection.

Abstract# 177

IL-1 β -Driven ST2L Expression Promotes Maturation Resistance in Rapamycin-Conditioned Dendritic Cells. Heth R. Turnquist,¹ Tina L. Sumpter,¹ Allan Tsung,¹ Alan F. Zahorchak,¹ Atsunori Nakao,¹ Gerard J. Nau,² David A. Geller,¹ Angus W. Thomson.^{1,3} ¹*Starzl Transplantation Institute and Dept. of Surgery;* ²*Dept. of Immunology;* ³*Dept. of Molecular Genetics and Biochemistry, University of Pittsburgh School of Medicine, Pittsburgh, PA.*
Objective: Maturation resistance and tolerogenicity can be conferred on dendritic cells (DC), -crucial regulators of T cells, by exposure to rapamycin (RAPA), a tolerance-sparing immunosuppressant. The mechanisms underlying this acquired unresponsiveness, typified by diminished responses to Toll-like receptor (TLR) or CD40 ligation, have not been identified. Thus, our objective was to elucidate a molecular basis for RAPA-induced DC maturation resistance.
Methods: RAPA administration was used to condition splenic DC in vivo and bone-marrow derived DC in vitro. DC maturation was monitored by assessment of co-stimulatory molecule expression, cytokine production, and T cell allostimulatory capacity. To identify negative regulators of maturation, microarray analysis and quantitative RT-PCR was completed, and findings confirmed via Western and flow cytometric analyses.
Results: In vitro or in vivo exposure of myeloid DC to RAPA elicited de novo production of IL-1 β by otherwise immature DC (CD86⁺). Interestingly, DC IL-1 β production, acting in an autocrine/paracrine fashion, promoted DC overexpression of the IL-1 receptor (R) family member, ST2L, and enhanced its surface expression. ST2L is the receptor for IL-33, an IL-1 family member, and has also been implicated as a negative regulator of TLR signaling. Consistent with this regulatory function, IL-1 β -induced ST2L expression suppressed the responsiveness of RAPA-conditioned DC to TLR or CD40 ligation.
Conclusion: RAPA causes de novo production of IL-1 β by immature DC, upregulating ST2L, and establishing a barrier to DC maturation following exposure to TLR or CD40 ligation. As such this work identifies a novel mechanism by which a clinically-important immunosuppressant impedes the capacity of DC to mature and consequently stimulate effector/adaptive T cell responses. These findings are particularly relevant to the potential use of RAPA-conditioned DC as "negative" cellular vaccines to block alloAg-specific responses, as exposure to endogenous and exogenous inflammatory stimuli can induce DC maturation and negate the tolerogenic properties of immature DC.

Abstract# 178

Transference of Exosomes as a Local Mechanism To Spread Allo-Antigen between Dendritic Cells in Secondary Lymphoid Organs. Angela Montecalvo,^{1,2} William J. Shufesky,^{1,2} Sherrie Divito,^{1,2,3} Donna Beer Stoltz,⁴ Zhiliang Wang,^{1,2} Paul D. Robbins,⁵ Adriana T. Larregina,^{3,6} Adrian E. Morelli.^{1,2,3} ¹*T.E. Starzl Transplantation Institute;* ²*Dept. of Surgery;* ³*Immunology;* ⁴*Cell Biology;* ⁵*Molecular Genetics and Biochemistry;* ⁶*Dermatology, University of Pittsburgh, Pittsburgh, PA.*
Exosomes are nanovesicles (50-100nm) released to the extracellular milieu by different cell types. Exosomes secreted by dendritic cells (DCs) and other APCs express MHC Ag, adhesion molecules and costimulatory molecules oriented on the membrane surface with their binding domains facing outwards. Thus, exosomes released by graft-infiltrating leukocytes (GILs) could function as "Ag-presenting vesicles" or as vehicles to transfer alloAg between recipient's APCs during elicitation of T-cell allo-immunity. **Aims:** to test if (i) GILs activate anti-donor T-cells in secondary lymphoid organs by releasing exosomes with alloAg into systemic circulation; or (ii) GILs that traffic to the spleen as passenger leukocytes use exosomes as a local mechanism to transfer alloAg to recipient's DCs. **Methods:** Exosomes were isolated from supernatants of BM-derived [C57BL/6(B6), IA^b] DCs pulsed with the BALB/c IEA₅₂₋₆₈ allopeptide and purified by ultra-filtration and ultra-centrifugation on a 30% sucrose/D₂O gradient. We used PKH67⁺ exosomes and CD45.1 congenic B6 mice for traffic studies, heart (heterotopic) and skin transplantation models (BALB/c $\rightarrow$ B6, *Thy1.2*⁺), and CFSE-labeled 1H3.1 TCRtg CD4 T-cells (*Thy1.1*⁺) specific for IA^b (B6) loaded with IEA₅₂₋₆₈ (BALB/c). DCs were genetically engineered to release exosomes expressing green fluorescent protein (GFP). **Results:** We have previously shown that blood-borne exosomes carrying BALB/c alloAg are reprocessed by different subsets of splenic DCs for presentation to indirect pathway 1H3.1 CD4 T-cells. Here, we demonstrated that although GILs of cardiac and skin allografts release exosomes ex vivo, they did not secrete enough concentrations of exosomes with alloAg into circulation to stimulate donor-reactive T-cells in lymphoid organs. Instead, our findings indicate that migrating DCs (generated in vitro or isolated from GILs), once homed in the spleen, they transfer exosomes expressing GFP and carrying allopeptides to spleen-resident DCs of the recipient, identified by the congenic

marker CD45.1. **Conclusion:** Exchange of exosomes between DCs in lymphoid organs might be a mechanism by which passenger leukocytes transfer alloAg to recipient's APCs in secondary lymphoid organs.

Abstract# 179

PKC θ Mediates the Alloimmune Response: (i) PKC θ Defective T Cells Fail To Elicit Allograft Rejection. Lei Wang,¹ Santhakumar Manicassamy,² Lianli Ma,¹ Zhidan Xiang,¹ Brian T. Caldwell,¹ Phillip Williams,¹ Ravi Chari,¹ Zuoming Sun,² Dengping Yin.¹ ¹*Surgery, Vanderbilt University Medical Center, Nashville, TN;* ²*Immunology, Beckman Research Institute of the City of Hope, Duarte, CA.*
T cell activation is critical in initiating adaptive immunity, and PKC θ , a novel member of the PKC family, mediates non-redundant functions in the T cell receptor; however, its role in the mediation of allograft rejection remains unclear. This study is aimed at investigating whether alloimmune response can be alleviated by a deficiency of the PKC θ molecule, and whether transgenic expression of anti-apoptotic Bcl-x_L in PKC θ ^{-/-} (PKC θ ^{-/-}/Bcl-x_L) T cells can reverse unresponsiveness of PKC θ ^{-/-} T cells. **Methods:** Wild-type (WT) cardiac allografts were transplanted into PKC θ ^{-/-} mice, with or without sub-therapeutic anti-CD154 mAb. Purified PKC θ ^{-/-} or PKC θ ^{-/-}/Bcl-x_L T cells were adoptively transferred into Rag2^{-/-} mice engrafted with cardiac allografts. Lymphocyte proliferation assays were performed (CFSE). NF-kB activation was assessed by bioluminescence imaging (BLI) using luciferase transgenic mice under the control of a NF-kB promoter. **Results:** The cardiac allografts were rejected in a delayed fashion in PKC θ ^{-/-} mice with increased NF-kB activation; however, sub-therapeutic anti-CD154 mAb (that normally delays rejection of cardiac allograft) induced long-term survival of cardiac allografts. The cardiac allografts were permanently accepted in Rag2^{-/-} mice with adoptive transfer of PKC θ ^{-/-} T cells, and the rejection can be elicited by transfer of PKC θ ^{-/-}/Bcl-x_L T cells. In a lymphocyte proliferation assay, PKC θ ^{-/-} T cells displayed greatly reduced proliferation. In response to CD3 and CD28 stimulation, PKC θ ^{-/-} T cells underwent accelerated apoptosis and reduced Th1, Th17, and Treg subsets compared to the WT T cells. Bcl-x_L restored the survival of the PKC θ ^{-/-} T cells. **Conclusions:** The results suggest that PKC θ mediates the alloimmune response. PKC θ ^{-/-} T cells have a great increase of apoptosis and inhibition of proliferation and NF-kB activation. Bcl-x_L transgene prevents PKC θ ^{-/-} T cell apoptosis and re-elicits allograft rejection.

Abstract# 180

Donor-Derived Tolerogenic Dendritic Cells Are Reprocessed into and Presented as AlloAg by Recipient DC to Down-Regulate Anti-Donor T Cells and Prolong Allograft Survival. Sherrie J. Divito, William J. Shufesky, Zhiliang Wang, Angelina Montecalvo, Adrian E. Morelli. *T.E. Starzl Transplantation Institute, Department of Surgery, University of Pittsburgh, Pittsburgh, PA.*
Tolerogenic dendritic cells (DC) are immature, maturation-resistant(MR) or alternatively-activated DC that express MHC molecules and low levels or absent co-stimulatory signals. Although MRDC administration has successfully prolonged allograft survival in murine models, the mechanism of action in vivo remains unknown. **Aim:** to test in vivo if the down-regulation of the anti-donor response induced by donor-derived tolerogenic DC is due to: (i) direct interaction of the tolerogenic DC with donor-reactive T cells or (ii) by reprocessing of the tolerogenic DC into alloantigen (alloAg) by recipient APC for interaction with indirect pathway T cells. **Methods:** DC were generated in vitro by culturing BALB/c bone marrow cells for 6-8 days in medium with GM-CSF + IL-4 supplemented with 10nM 1 α ,25-(OH)₂ vitamin D₃ (VD₃). We used a model of heterotopic vascularized allogeneic heart transplantation [BALB/c into C57BL/6 (B6)] and CD4 T cells from 1H3.1 TCRtg mice that recognize B6 IA^b loaded with the BALB/c allopeptide IEA₅₂₋₆₈ (indirect pathway). **Results:** we demonstrated that VD₃ renders DC maturation resistant (VD₃-MRDC) as VD₃-MRDC fail to up-regulate co-stimulatory molecule expression, release IL-12p70, or stimulate allo-responsive T cells after challenge with potent DC-maturation stimuli. Adoptive transfer (i.v.) of BALB/c VD₃-MRDC (day -7) significantly prolonged survival of BALB/c heart grafts in B6 mice in the absence of immunosuppressive therapy. Interestingly, we found that in vivo, BALB/c VD₃-MRDC induced proliferation of indirect pathway 1H3.1 CD4 T cells in the spleens of B6 recipient mice, indicating that reprocessing of the BALB/c DC by host (B6) APC does occur. Proliferation of 1H3.1 CD4 T cells in response to BALB/c VD₃-MRDC resulted in defective activation (CD62L^{high}, CD69^{low}) of 1H3.1 T cells, leading to their peripheral deletion and outgrowth of CD4⁺Foxp3⁺ Treg cells. Reprocessing of BALB/c VD₃-MRDC was performed by recipient splenic CD11c^{high}CD8 α ^{neg} DC, and donor alloAg continued to be presented through the indirect pathway for 3 days after donor DC administration. **Conclusion:** These results suggest that DC-based therapies down-regulate T cell allo-immunity and prolong allograft survival, at least in part, through reprocessing of the tolerogenic DC into alloAg by recipient APC.

Abstract# 181

Early Allorecognition by CD8 Memory T Cells Optimizes Subsequent Recruitment of Primed Effector T Cells. Austin D. Schenk,^{1,2} Taiji Nozaki,¹ Anna Valujskikh,¹ Robert L. Fairchild.¹ ¹*Dept. Immunology, Cleveland Clinic Fdn., Cleveland, OH;* ²*Dept. Pathology, Case Western Reserve University, Cleveland, OH.*

Introduction: Alloreactive memory T cells are present in all transplant recipients due to prior direct sensitization or heterologous immunity. These cells are known to circumvent tolerance induction and/or prevent indefinite graft survival in several models, but mechanistic details of their function are unknown. The goal of this study was to test the hypothesis that CD8 memory T cells initiate allorecognition and express effector functions within hours of reperfusion.

Methods: Syngeneic or A/J (H-2^b) hearts were transplanted into WT C57BL/6 (H-2^b), CD4^{-/-}, CD8^{-/-}, or Rag1^{-/-} recipients. RNA and protein were prepared from total graft homogenates and analyzed by qRT-PCR and ELISA. Rag1^{-/-} mice received 1x10⁶ WT or IFN γ -2C cells and were used as recipients 10 weeks after reconstitution. Donor-specific CD8 memory cells were purified from WT spleens 8 weeks after A/J skin grafting, and donor-specific effector CD4 cells were purified from spleens of CD90.1 mice 8 days after A/J heart transplantation. Flow cytometry was used to quantify graft infiltrates.

Results: Allografts contained elevated levels of IFN γ and CXCL9 mRNA at 24, 48 and 72 hrs post-transplant vs. isografts. Detectable CXCL9 protein was produced in allografts from WT and CD4^{-/-} recipients but not in isografts or allografts from CD8^{-/-} or Rag1^{-/-} recipients. Treatment with CTLA4-Ig and MR1 failed to reduce CXCL9 production. Reconstitution of Rag1^{-/-} mice with IFN γ sufficient or deficient 2C TCR transgenic CD8 cells indicated that early allospecific CXCL9 production absolutely requires IFN γ made by recipient CD8 cells. Although donor-specific IFN γ production was undetectable in splenocytes until day 4-5 post transplant, graft-infiltrating CD44^{hi}CD62L^{lo} CD8 T cells were present as early as 24 hrs post-transplant. In adoptive transfer studies, effector-memory CD8 T cells reconstituted early allospecific CXCL9 production in CD8^{-/-} mice. Lastly, primed CD4 T cells adoptively transferred at day 2 post-transplant readily infiltrated allografts in control but not CD8 depleted recipients.

Conclusions: CD8 memory T cells infiltrate allografts rapidly post-transplant, produce IFN γ , and propagate an inflammatory environment which optimizes recruitment of primed effector T cells. Successful neutralization of this early allorecognition pathway should provide valuable adjunctive therapy to improve graft function and survival.

Abstract# 182

Intracellular Retention of CD80/86 by Dendritic Cells, Unlike Tryptophan Catabolism, as a Mechanism To Induce Regulatory T Cell-Mediated Donor-Specific Tolerance. Adnan H. Khan,¹ Jennifer E. Harper,¹ Sven C. Beutelspacher,² Giovanna Lombardi,³ Myra O. McClure,¹ Andrew J. T. George.¹ ¹*Department of Immunology and Jefferris Research Trust Laboratories, Imperial College London;* ²*University Eye Hospital, University of Heidelberg;* ³*Department of Nephrology and Transplantation, King's College London.*

Background: Allogeneic T cell stimulation requires not only antigen-specific signals but also costimulatory signals, most importantly between CD80/86 on the antigen presenting cell (APC) and CD28 and CTLA4 on the T cell. Engagement of the T cell receptor without costimulation can lead to anergy and the induction of regulatory T cells (Tregs). T cell activation is also controlled by expression of the tryptophan-catabolising enzyme indoleamine 2,3-dioxygenase (IDO). Depletion of this essential amino acid, and/or the production of tryptophan metabolites inhibits T cell proliferation.

Methods: A genetic approach to confer tolerogenic properties on murine dendritic cells (DCs) has been explored using lentiviral vectors, based on the Equine Infectious Anaemia Virus. Firstly, an intracellular method that prevents costimulation has been developed: A fusion protein consisting of CTLA4 and KDEL [an endoplasmic reticulum (ER) retention signal] is expressed in DCs. The CTLA4-KDEL binds to CD80/86 in the ER and prevents expression of these proteins on the DC surface. A second approach uses an elevated expression of the IDO enzyme by transduced DCs.

Results: CTLA4-KDEL- or IDO-transduced DCs were unable to induce allogeneic T cell proliferation. However, using two-stage DC:T cell co-culture assays, it was shown that CTLA4-KDEL-, but not IDO-transduced DCs, can induce donor-specific T cell anergy *in vitro* and *in vivo*. Tolerance to both the direct and indirect pathways was shown using CTLA4-KDEL-transduced DCs. Linked suppression was mediated by the generation of donor-specific Tregs. IDO-transduced DCs did not generate Tregs. Furthermore, it was shown separately that DCs expressing IDO whilst lacking CD80/86 expression for potential ligation by CTLA4 (although CTLA4-CD80/86 ligation upregulates IDO, it downregulates T cell activation) failed to generate or even sustain FoxP3⁺ Treg populations. The ability of the transduced DCs to induce tolerance to allografts was assessed in a complete mismatch and CBK→CBA (indirect pathway) corneal graft model.

Conclusions: These results support a clinical strategy to induce Treg-mediated, donor-specific transplantation tolerance using CTLA4-KDEL-, rather than IDO-expressing DCs.

Abstract# 183

Indirect Pathway CD4 T Cells Provide Help for Generating Cytotoxic Effector Responses through Recognition of MHC II on CD8 T Cells. Gavin J. Pettigrew,¹ Thomas M. Conlon,¹ K. Saeb-Parsy,¹ Susanne Negus,¹ Margaret Negus,¹ John A. Bradley,¹ Eleanor M. Bolton,¹ Taylor L. Anna.¹ ¹*University of Cambridge.*

CD4 T cells that recognise processed alloantigen on recipient APC can provide help to alloreactive cytotoxic CD8 T cells that recognise intact MHC I alloantigen on donor APC, but exactly how such 'un-linked' help is provided is not clear.

The respective abilities of direct and indirect pathway CD4 T cells to provide help for cytotoxic CD8 alloimmunity were examined in a mouse model of heart graft rejection in which the recipients contain only monoclonal helper CD4 T cells, specific for self-restricted H-Y antigen (female B6 Mar/RAG1^{-/-} mice). Mice were additionally reconstituted with 10⁶ B6 CD8 T cells, and then challenged with female BALB/c (no CD4 T cell help), or male BALB/c (indirect pathway help), or male B6xBALB/c F1 hearts (direct pathway help).

Un-reconstituted Mar/RAG1^{-/-} mice lack effector B and CD8 T lymphocytes, and consequently all heart grafts survived indefinitely. In contrast, reconstituted Mar/RAG1^{-/-} mice rejected male F1 grafts rapidly (MST 8d), whereas female BALB/c grafts survived indefinitely, confirming a CD4-dependent effector role for the transferred CD8 T cells. CD4 T cell help through the indirect pathway, although sufficient to elicit graft rejection, was less efficient than direct pathway help, because male BALB/c grafts were rejected more slowly than the F1 grafts (MST 12d, p<0.001).

We next considered whether indirect pathway CD4 T cells provide help through recognition of MHC II complexes on the surface of alloreactive CD8 T cells, in analogous fashion to the cognate interaction between B and T lymphocytes. In support, reconstitution of Mar/RAG1^{-/-} recipients with instead, MHC II-deficient CD8 T cells, resulted in slower rejection of male BALB/c hearts (MST 21d, p<0.01), whereas male F1 grafts, that still permit provision of linked help, were rejected at the same tempo. Most tellingly, Mar/RAG1^{-/-} mice that received simultaneously a female BALB/c heart and male B6 APC (to activate Mar CD4 T cells) rejected their grafts rapidly when reconstituted with male CD8 T cells (MST 9d). In contrast, grafts survived indefinitely when female CD8 T cells were transferred. Flow cytometric analysis of mitogen-stimulated CD8 T cells revealed surface MHC II expression.

Indirect allorecognition can provide help for generating cytotoxic alloimmunity, but not as effectively as through the direct pathway. Indirect pathway help is potentiated by linkage through recognition of CD8 MHC II.

Abstract# 184

Rapamycin-Conditioned, Alloantigen-Pulsed Dendritic Cells Induce the Apoptosis of Directly Reactive, Alloantigen-Specific CD8⁺ T Cells by Presentation of Acquired Intact Donor MHC. Ryan Fischer,¹ Heth R. Turnquist,¹ Simon C. Watkins,³ Angus W. Thomson.^{1,2} ¹*Starzl Transplantation Institute and Dept. of Surgery;* ²*Dept. of Immunology;* ³*Center for Biological Imaging, University of Pittsburgh School of Medicine, Pittsburgh, PA.*

PURPOSE: Tolerogenic properties of dendritic cells (DC) are supported and preserved by conditioning with the immunosuppressant rapamycin (RAPA). The ability of RAPA-conditioned, recipient-derived DC pulsed with alloantigen (alloAg) to suppress both direct and indirect alloAg-specific T cells in the absence of immunosuppression has been demonstrated in a murine allograft model. DC can acquire intact MHC from cells or cell lysates. However, the ability of alloAg-pulsed RAPA-DC to immunomodulate directly-reactive alloAg-specific T cells has not been formally demonstrated.

METHODS: DC were generated from C57BL/6 (B6; H2^b) bone marrow cells in GM-CSF and IL-4. RAPA was added to indicated cultures (RAPA-DC) beginning on day (d) 2. On d7, CD11c⁺ bead-purified RAPA-DC or non-treated control DC (CTR-DC) were incubated with BALB/c (H2^d) splenocyte lysates ("alloAg pulsing"). Following incubation, the DC were harvested and the level of donor and recipient MHC molecules on CD11c⁺ cells determined by flow cytometry and immunofluorescent imaging. Surface levels of CD86, B7-H1 (Programmed Death Ligand-1; PD-L1), and Fas-L were compared. Pulsed-DC were also incubated with CD8⁺ T cells from Rag^{-/-} 2C mice for 3d. 2C CD8⁺ cells express T cell receptors specific for H2-L^d, a MHC class I molecule of BALB/c. Following incubation, 2C cell proliferation and apoptosis were both assessed.

RESULTS: CTR- and RAPA-DC presented detectable levels of directly-transferred MHC class I and II on their surface after incubation with allogeneic BALB/c cell lysate. Donor MHC presented by "pulsed" recipient DC stimulated directly-reactive, alloAg-specific 2C T cell proliferation. However, only RAPA-DC induced apoptosis in the overwhelming majority of these cells responding via the direct pathway. Induction of apoptosis correlated with an increased level of surface Fas-L on RAPA-DC and their comparatively low level of CD86 relative to PD-L1.

CONCLUSIONS: RAPA-conditioned DC can present intact MHC molecules acquired from lysates of allogeneic splenocytes and concurrently induce apoptosis of directly-reactive alloAg-specific CD8⁺ T cells. As such, we provide mechanistic insight into a mechanisms by which alloAg-pulsed, recipient-derived RAPA-DC may facilitate allograft tolerance.

Immunosuppression: Preclinical Studies

Abstract# 185**A Novel Clinically Relevant Therapeutic Strategy To Promote Regulation in Transplantation: T Cell Depletion Plus B7 Blockade and Sirolimus.**

Francesca D'Addio,¹ Xueli Yuan,¹ John M. Williams,² Melanie Ruzek,² John Iacomini,¹ Mohamed H. Sayegh,¹ M. Javed Ansari.¹ ¹Transplantation Research Center, Renal Division, Brigham & Women's Hospital and Children's Hospital, Harvard Medical School, Boston; ²Genzyme, Cambridge.

Background: T_{regs} actively regulate alloimmune responses and promote transplant tolerance. ATG, a widely used induction therapy in organ transplantation, depletes peripheral T cells but may preferentially spare T_{regs}. Sirolimus is thought to expand natural T_{regs}. B7 T cell costimulatory blockade inhibits effector T cell (T_{eff}) expansion and may promote regulation. We investigated the effect of combining mouse ATG (mATG), CTLA4Ig and Sirolimus on stringent skin allograft survival, and studied the mechanisms by determining T_{reg}/T_{eff} balance in vivo using a unique model (ABM-TCR-tg-Foxp3/gfp reporter mouse).

Methods: C57BL/6 (B6) recipients of fully mismatched BALB/c skin were treated with mATG (0.5mg; d 0,4); CTLA4Ig (0.5mg; d 0, 0.25mg; d 2,4,6,8,10); Sirolimus (0.3 mg/Kg/d; d 0,2,4,6,8,10) and followed for survival. 0.3x10⁶ Thy1.2⁺CD4⁺ABM/Foxp3/gfp⁺ or gfp⁻ cells were adoptively transferred into Thy1.1⁺B6 mice transplanted with bm12 skin and treated with above therapies to test T_{eff} generation and T_{reg} expansion and/or conversion by flow cytometry.

Results: Both mATG alone (MST 15.5 d, p<0.0004; control MST 9 d) and mATG+CTLA4Ig (MST 36 d, p<0.003) prolonged fully allogeneic skin allograft survival (CTLA4Ig MST 12 d). Addition of Sirolimus to mATG+CTLA4Ig provided significant synergy (MST 55 d, p<0.003) (Sirolimus alone MST 14.5 d). Although mATG profoundly depletes peripheral T cells, T_{regs} tend to be preserved (0.5 vs. 0.3 x10⁶ in mATG vs. controls). Further, more Foxp3/gfp cells converted to Foxp3/gfp⁺ phenotype and fewer Foxp3/gfp⁺ cells acquired the T_{eff} phenotype resulting in increased ratio of T_{reg}/T_{eff} cells in mATG compared to controls (5±0.3 vs 3.6±0.2, p<0.03); and more remarkably in mATG+CTLA4Ig and mATG+CTLA4Ig+Sirolimus groups (6.8±2.3 and 7±0.7 respectively, p<0.01).

Conclusion: This is the first report to establish that T cell depletion with mATG combined with CTLA4Ig and Sirolimus synergize to prolong stringent fully allogeneic skin allograft survival by promoting regulation and tipping the T_{reg}/T_{eff} balance by both preserving T_{regs} and facilitating generation of new T_{regs} by a conversion mechanism. These results provide the rationale for translating such a novel therapeutic combination to promote regulation and tolerance in primates and human organ transplantation.

Abstract# 186**Expansion of Cynomolgus CD4⁺CD25⁺FoxP3⁺ Regulatory T Cells Using Low Dose Anti-Thymocyte Globulin.**

Olaf Boenisch,¹ Marta Lopez,¹ Timothy M. Millington,² Svetlana Boskovic,² Joren C. Madsen,² Nidyanandh Vadivel,¹ Nader Najafian,¹ Mohamed H. Sayegh.¹ ¹Transplantation Research Center, Brigham and Women's Hospital and Children's Hospital, Boston, MA; ²Massachusetts General Hospital, Boston, MA.

Low dose ATG (10% of depleting dose) can rapidly (within 24 hours) expand human CD4⁺CD25⁺FoxP3⁺ regulatory T cells (Tregs) ex vivo (JASN, 2006). As both ex-vivo expansion of Tregs for cellular therapy or approaches to promote Tregs in vivo are promising novel strategies for tolerance induction, both principles were tested in a primate model.

To generate Tregs ex vivo, blood lymphocytes (PBL) from cynomolgus monkeys were cultured with different concentrations of ATG or control Rabbit-Ig (Rbt-Ig) for various incubation periods (1-6 days) in the presence of alloantigen (irradiated PBL). ATG triggered expansion of Tregs as compared with Rbt-Ig after allostimulation. Treg expansion peaked at 120 hours (baseline: 2±0.7%, 27±6% with 10ug/ml, 22±3.7% with 100ug/ml, and 23±3% with 200ug/ml of ATG vs. <3±1% with Rbt-Ig, p=0.03, n=3). ATG-generated Tregs suppressed the alloresponse of the cells from which the Tregs were generated (69±24% inhibition vs. 0% for Rbt-Ig, n=2). When CFSE-labeled PBL were allostimulated with ATG vs. Rbt Ig, CD4⁺CD25⁺ but not CD4⁺CD25⁻ T cells demonstrated proliferation (62±11% vs. 7±2%), while neither cells showed proliferation with Rbt-Ig (3±1%). CD25-depleted PBLs showed only slight conversion into CD4⁺CD25⁺FoxP3⁺ Tregs after incubation with ATG (3.1±1.3% vs. 0.4±0.2% with Rbt-Ig, n=4), suggesting proliferation of natural Tregs as the main mechanism of primate Treg expansion.

To test low dose ATG in vivo, 2 mg/kg (10% of depleting dose) was administered thrice (day 0, 1 and 2) to a naïve monkey and to a monkey that was treated concurrently with sirolimus (tough 15-20 ng/ml) after heart transplantation. In the naïve monkey, low-dose ATG led to expansion of CD4⁺CD25⁺FoxP3⁺ Tregs in peripheral blood (baseline 0.57%, day 7=4.09%, day 12=2.7%) and in lymph nodes (baseline 2.47%, day 7=9.35%, day 12=7.08%) without causing T cell depletion. Similarly, in the transplanted monkey peripheral blood Tregs expanded from 1.4% at baseline to 4.64% on day 6.

Low dose ATG is not only able to expand Tregs ex vivo by proliferation of natural CD4⁺CD25⁺ cells, but can equally induce Tregs in vivo without lymphodepletion. These findings provide the rationale for development of tolerance inducing strategies based on enhancing regulatory mechanisms in human transplant recipients.

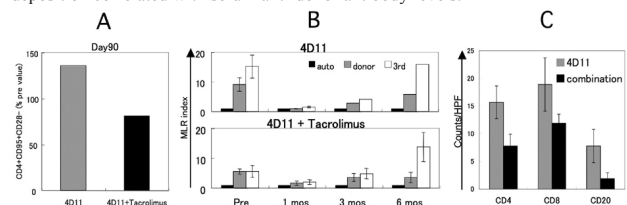
Abstract# 187**Immunological Aspects of Non-Human Primates Renal Transplant Recipients Treated with a Novel Human Anti-CD40 MAB, 4D11 and Tacrolimus.**

Motohiro Uno,¹ Kenichiro Yamashita,¹ Tomomi Suzuki,¹ Takeshi Aoyagi,¹ Ryoichi Goto,¹ Tetu Oura,¹ Masaaki Watanabe,¹ Toru Miura,² Nobuaki Takahashi,² Kazumitsu Okimura,² Tomoo Itoh,³ Akira Shimizu,⁴ Masahiko Taniguchi,¹ Tsuyoshi Shimamura,¹ Hiroyuki Furukawa,¹ Satoru Todo.¹ ¹1st Dept. of Surgery, Hokkaido Univ., Sapporo, Japan; ²Kirin Pharma Company, Ltd, Takasahi, Japan; ³Dept. of Surgical Pathology, Hokkaido Univ., Sapporo, Japan; ⁴Dept. of Pathology, Nippon Medical School, Tokyo, Japan.

Background/Aim: Previously, we have shown that combination of human anti-CD40 mAb, 4D11 and tacrolimus exerts additive immunosuppressive effect and markedly prolongs renal allograft survival in cynomolgus monkeys. In this study, we further evaluated the immunological aspects among these transplant recipients.

Method: Kidney transplantations were performed across MHC mismatched cynomolgus monkeys. Transplant recipient was given either no-treatment, tacrolimus (1 mg/kg/day, po), 4D11 (5 mg/kg, iv) or tacrolimus+4D11 (n=3/group). Peripheral lymphocyte population, MLR and serum anti-donor antibody levels and graft histology were assessed.

Results: Mean graft survival for no-treatment, tacrolimus, 4D11 and tacrolimus+4D11 treatment groups was 6.0±1.0, 27.7±3.2, 120.3±90.6 and 213.7±28.0 days, respectively. Peripheral CD20⁺ cells partially declined in both 4D11 alone and 4D11+ tacrolimus given animals at the early post-operation period, although the numbers recovered thereafter. CD4⁺ and CD8⁺ cells were unaffected. CD4⁺ effector memory population was reduced by addition of tacrolimus to 4D11 (Fig. 1A). MLR against donor and 3rd party antigens were suppressed in both 4D11 and tacrolimus+4D11 groups (Fig. 1B). Addition of tacrolimus further reduced graft CD4⁺, CD8⁺ and CD20⁺ cellular infiltration (Fig. 1C). Anti-donor antibodies were detected in sera during the treatment course of 4D11; however, they did not develop under the tacrolimus+4D11 treatment. Graft C4d deposition correlated with serum anti-donor antibody levels.



Conclusion: The 4D11 inhibits both cellular and humoral responses against donor antigens. Addition of tacrolimus strengthens these immunosuppressive effects of 4D11, leading to further prolongation of graft survival.

Abstract# 188**A Novel Costimulation Blockade Regimen for Engraftment and Function of Allogeneic Islets in Diabetic Rhesus Macaques.**

Maria C. Russell,¹ Kenneth Cardona,¹ Virginia L. Oliva,¹ Alexandra P. Turner,¹ Jose Cano,¹ Jose Avila,¹ Wanhong Jiang,¹ Frank Leopardi,¹ Brandi Johnson,¹ Erin Scott,¹ Elizabeth Strobert,² Thomas C. Pearson,¹ Allan D. Kirk,¹ Christian P. Larsen.¹ ¹Emory Transplant Center, Department of Surgery, Emory University, Atlanta, GA; ²Yerkes National Primate Center, Emory University, Atlanta, GA.

Objectives: Allogeneic islet transplantation offers the potential for cure from diabetes. Application of this therapy, however, is limited by immunologic mechanisms requiring medical therapy to prevent rejection of the islets. Costimulatory blockade of the CD28/CD80/CD86 and the CD40/CD154 pathways has shown promise in ameliorating the immune response to allow engraftment and function of islets. We have evaluated a new drug regimen consisting of induction therapy with 3A8, a murine anti-CD40 antibody, and basiliximab and maintenance treatment with CTLA4Ig and sirolimus in diabetic rhesus macaques which received allogeneic islets.

Methods: Allogeneic rhesus macaque islets (14,100 IE/kg ± 1,802) were transplanted intraportally into diabetic rhesus macaques (n=4) under the following immunosuppressive regimen: short term administration of anti-IL-2 receptor (basiliximab) and anti-CD40 (3A8), with maintenance immunosuppression using sirolimus for 134 days and abatacept (CTLA4Ig) for long term therapy. Weekly peripheral blood flow cytometric and CMV viral load monitoring was performed.

Results: Recipients treated with this immunosuppressive regimen had immediate return to normoglycemia following islet transplant. The graft survival in the first three animals was 141, 283 and 297 days. The fourth animal continues to exhibit good glycemic control at his current post-operative day 30. Each of these animals had monthly intravenous glucose tolerance tests with monitoring of blood glucoses and c-peptides with further evidence of glycemic response and c-peptide generation. Flow cytometry confirms CD40 blockade during the administration of 3A8 and return of CD40 after cessation of therapy. The treatment was well tolerated with minimal evidence of CMV reactivation and no evidence of thrombocytopenia or thromboembolism.

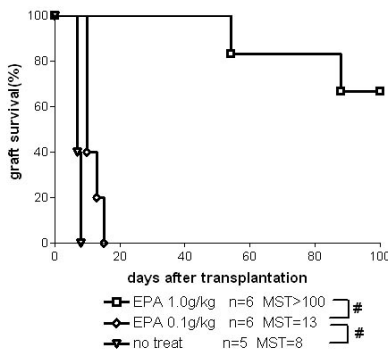
Conclusions: These preliminary results indicate that CD28/CD40 costimulatory-based immunosuppressive regimens can protect allogeneic islets from rejection. Furthermore,

3A8 appears to adequately block CD40 to facilitate this engraftment and function as demonstrated by flow cytometry.

Abstract# 189

Purified Eicosapentaenoic Acid Induces Prolonged Survival of Fully Allogeneic Cardiac Grafts and Generates Regulatory Cells. Daiki Iwami,^{1,2} Qi Zhang,¹ Osamu Aramaki,¹ Nozomu Shirasugi,¹ Katsuya Nonomura,² Masanori Niimi.¹ ¹*Surgery, Teikyo University, Tokyo, Japan;* ²*Renal and Genitourinary Surgery, Hokkaido University, Sapporo, Japan.*

Many studies have shown immunosuppressive effects of dietary intake of fish oil containing eicosapentaenoic acid (EPA) in various models such as autoimmune diseases and transplantation. However, its mechanisms remain uncertain. Furthermore, there have been no studies examining the effect of purified EPA. Here we determined the ability of purified EPA to inhibit alloimmune response in mouse cardiac transplantation model. **Methods:** CBA recipients (H-2^b) were given single injection of purified EPA intraperitoneally on the same day as transplantation of a heart from C57BL/10 donors (H-2^b). Mixed leukocyte reaction (MLR) assay and enzyme linked immunosorbent assay (ELISA) were also performed to evaluate the effect of purified EPA on cell proliferation and cytokine production. To determine the presence of regulatory cells, adoptive transfer study was conducted. **Results:** Untreated CBA recipients rejected C57BL/10 cardiac allografts with median survival time (MST), 8 days. In contrast, CBA recipients treated with purified EPA (1.0g/kg) had significant prolongation of allograft survival (MST, >100 days). CBA recipients treated with 0.1g/kg purified EPA eventually rejected allografts (MST, 13 days).



In MLR assay, treatment with 1.0 g/kg purified EPA suppressed alloproliferation of splenocytes in the recipients. The treatment also inhibited production of IL-2, IL-12 and IFN γ by the splenocytes in the recipients. When splenocytes were harvested from the recipients treated with 1.0g/kg purified EPA 50 days after cardiac allografting and were adoptively transferred into naïve secondary recipients, the adoptive transfer induced significant prolongation of cardiac allograft in naïve secondary recipients (MST >30 days, compared to that in the recipients with adoptive transfer of naïve splenocytes, MST, 10 days). **Conclusions:** Purified EPA induced significantly prolonged survival of fully mismatched cardiac allografts, and generated regulatory cells.

Abstract# 190

Reduction of Chronic Allograft Nephropathy by Inhibition of Extracellular Signal-Regulated Kinases 1/2 Signaling. Caigan Du,¹ Shuang Wang,² Jifu Jiang,³ Qionong Guan,¹ Hao Wang,³ Christopher Y. Ngan,¹ Anthony M. Jevnikar.² ¹*Department of Urologic Sciences, The University of British Columbia, Vancouver, BC, Canada;* ²*Department of Medicine, The University of Western Ontario, London, ON, Canada;* ³*Department of Surgery, The University of Western Ontario, London, ON, Canada.*

Background: Chronic allograft nephropathy (CAN), the most common cause of late kidney allograft failure, is not effectively prevented by the current regimens. Activation of extracellular signal-regulated kinases 1/2 (ERK1/2) mediating intracellular signal transduction from various growth factor stimuli is required for TGF- β production, which plays a key role in the development of CAN. Hence, the therapeutic potential of disruption of ERK1/2 signaling to prevent CAN was examined in an experimental model.

Methods: Kidney donors from C57BL/6J mice (H-2^b) were transplanted to bilaterally nephrectomized Balb/c recipient mice (H-2^d). The recipients were treated with CI1040 (MEK-ERK1/2 inhibitor) or vehicle after 14 days post-transplantation for 28 days. CAN was evaluated with the Banff 97 working classification.

Results: All six allografts receiving CI1040 treatment were survived, while two out of seven grafts were lost in vehicle-treated group. At the end of experiment, the function of grafts in CI1040 treated recipients had been maintained, indicated by lower levels of serum creatinine and BUN (30 $\pm$ 6 μ M and 22 $\pm$ 8 mM, n=6) as compared to those (94 $\pm$ 39 μ M and 56 $\pm$ 25 mM, n=5) in vehicle group (creatinine, p=0.0015; BUN, p=0.0054). Pathological evaluation indicated that CI1040 reduced CAN, reflected by a lower CAN score in CI1040-treated group (3.93, n=4) as compared to that (9.96, n=5) in vehicle controls (p=0.0234). Further examinations showed that CI1040 treatment resulted in

inhibition of phosphorylation of ERK1/2 and reduction of TGF- β levels in grafts. *In vitro* CI1040 potentially suppressed not only growth factors-stimulated ERK1/2 activation and TGF- β biosynthesis in renal tubular epithelial cells, but also attenuated alloantigen-stimulated T cell proliferation.

Conclusion: Our data suggest that interference of ERK1/2 signaling with pharmacological agent (i.e. CI1040) has therapeutic potential to prevent CAN in kidney transplantation.

Abstract# 191

R348, a Novel JAK3 and Syk-Inhibitor, Attenuates Obliterative Airway Disease (OAD). Jeffrey B. Velotta,¹ Tobias Deuse,¹ Munif Haddad,² Vanessa Taylor,³ Esteban Masuda,³ Gary Park,³ David Carroll,³ Robert C. Robbins,¹ Sonja Schrepfer.¹ ¹*Cardiothoracic Surgery, Stanford University School of Medicine, Stanford, CA;* ²*Clinical Chemistry, University Hospital, Hamburg, Germany;* ³*Rigel Pharmaceuticals, South San Francisco, CA.*

Objective: This is the first study to investigate the role of a novel JAK3 and Syk-inhibitor, R348, in the prevention of obliterative airway disease (OAD), the major obstacle after lung transplantation.

Methods: Trachea from Brown-Norway (BN) donors were heterotopically transplanted in the greater omentum of Lewis (Lew) rats. Recipients were treated for 28 days with R348 (10, 20, 40, or 80 mg/kg), rapamycin (0.75 or 3 mg/kg), or left untreated. Allografts were recovered and processed for histological evaluation determining degree of luminal obliteration, percentage of respiratory epithelial coverage, and mononuclear cell infiltration. Donor reactive (IgG) antibodies from the recipient's serum were determined using flow cytometry.

Results: R348 at 20, 40, and 80 mg/kg significantly inhibited luminal obliteration in a dose dependent manner (69 $\pm$ 20%, 20 $\pm$ 13%, 15 $\pm$ 7%; p=0.003 vs. no medication). Rapamycin in both concentrations significantly inhibited luminal obliteration (37 $\pm$ 15%, 11 $\pm$ 6%; p<0.001 vs. no medication) similarly to R348 at 40 and 80 mg/kg. R348 at 40 and 80 mg/kg significantly preserved respiratory epithelium compared to R348 at 10 and 20 mg/kg (49 $\pm$ 35%, 76 $\pm$ 27% vs. 0 $\pm$ 0, 3 $\pm$ 7%; p=0.004) and was superior to rapamycin in epithelial preservation (49 $\pm$ 35%, 76 $\pm$ 27% vs. 27 $\pm$ 17%, 36 $\pm$ 15%; p=0.01). All R348 and rapamycin-treated recipients expressed decreased numbers of peritracheal mononuclear cells in a dose dependent manner (p<0.0001). R348 20, 40, and 80 mg/kg treated recipients had significantly reduced IgG levels versus untreated recipients (381 $\pm$ 136, 371 $\pm$ 61, 230 $\pm$ 56 vs. 853 $\pm$ 207; p<0.03). All R348 treated recipient thymus and spleen weights were significantly lower compared to the untreated group (p=0.001). BUN, Cr, and cholesterol levels were unaffected in R348 treated recipients.

Conclusion: R348 potentially exhibits its inhibitory effect by preserving the respiratory epithelium, rather than by rapamycin's mechanism of reduced smooth muscle cell (SMC) proliferation. R348 occupies a beneficial pharmacokinetic profile, lacks nephrotoxic and atherogenic properties, and provides a favorable alternative to rapamycin in the treatment of chronic rejection in lung transplant recipients.

Abstract# 192

Pre-Clinical Assessment of Genz-29155, a Novel Small Molecule Inhibitor of Apoptosis with Anti-Rejection Effects in Non-Human Primates. Timothy A. Weaver,¹ Ali H. Charafeddine,¹ Frank V. Leopardi,¹ Avinash Agarwal,¹ Scott D. Batty,² John M. Williams,² Sharon R. Nahil,² Allan D. Kirk.¹ ¹*Emory Transplant Center, Atlanta, GA;* ²*Genzyme Corporation, Framingham, MA.*

Genz-29155 is a novel, oral immune-modulatory agent identified in a high-throughput screen designed to find inhibitors of TNF α -induced apoptosis. The molecular target of the compound remains under investigation but is likely downstream of the TNF α cell surface receptor. *In vitro* studies have shown Genz-29155 to be an effective inhibitor of the TNF α -triggered caspase cascade but not anti-CD3 or Fas-mediated apoptosis, and thus may act by inducing allograft resistance to immune attack rather than suppressing the alloimmune response per se. It has been shown to synergize with sirolimus in murine heterotopic cardiac allotransplant models. In order to test this promising new agent in a more clinically relevant model of solid organ transplantation, we studied Genz-29155 in a mismatched NHP (rhesus macaque) renal transplant model. Genz-29155 (n=3) was administered (3mg/kg, IV, Days 0-14) with sirolimus (1mg/kg, PO, Days 0-30). Five control animals received only sirolimus and vehicle (1mg/kg, PO, Days 0-30). All animals were followed serially by polychromatic flow cytometry to determine the relative and absolute number of CD4⁺ and CD8⁺ T cell subsets. Time to allograft rejection, the primary end point, was determined by a significant rise in serum creatinine and BUN, as identified with biweekly monitoring. After diagnosis of rejection, allografts were removed for histological and transcriptional studies, along with splenocytes for immune function assays. In this pilot study, prolongation of rejection-free survival was significantly improved with Genz-29155 and sirolimus combined vs. sirolimus alone (42.67 days vs. 17.20 days, respectively, p=0.05). Given these initial results, we have initiated a larger study (n=12) to optimize the dose and duration of Genz-29155. Five animals, transplanted within the past month remain alive and well in this study. Further investigation of this agent will allow us to better understand the benefit of inhibiting TNF α -mediated apoptotic effects in both cellular alloimmune response and allograft injury in solid organ transplantation.

Abstract# 193

Targeting LFA-1 Inhibits CD8-Mediated In Vivo Cytotoxic Effector Function in Sensitized Recipients. Jason M. Zimmerer,¹ Phillip H. Horne,¹ Kartika Jayashankar,¹ Mason Fisher,¹ Ginny L. Bumgardner.¹ ¹*Department of Surgery, Division of Transplantation, The Ohio State University, Columbus, OH.*

Purpose: Allospecific T memory cell responses are present in transplant recipients from exposure to cross-reacting antigens. We have previously reported that LFA-1 inhibition suppresses primary CD8-dependent rejection responses which are not controlled by any conventional immunosuppressive strategy. These studies were conducted to analyze the efficacy of this anti-LFA-1 Ab for control of CD8-dependent responses in sensitized hosts. **Methods:** FVB/N (H-2^d) donor hepatocytes were transplanted into C57BL/6 (H-2^b) or CD4 KO (H-2^b) recipients. Memory responses were analyzed by re-transplantation with a second FVB/N allogeneic hepatocyte transplant. Cohorts of mice were treated with anti-LFA-1 mAb and observed for hepatocyte survival or magnitude of CD8⁺ T cell mediated allospecific cytolytic activity. **Results:** The untreated secondary CD4 KO and C57BL/6 recipients rejected hepatocyte allografts with enhanced kinetics in comparison to the primary graft (MST= day 10 vs day 14, and MST= day 7 vs. day 10, respectively; $p < 0.05$). Anti-LFA-1 mAb treated CD4 KO recipients demonstrated delayed rejection (MST= day 35 vs day 10; $p=0.001$) compared to secondary rejection in untreated CD4 KO hosts. Anti-LFA-1 mAb treatment did not delay rejection in sensitized C57BL/6 recipients (MST= day 7) but did significantly reduce the in vivo allospecific cytotoxic effector function in C57BL/6 secondary recipients (20.6±4.1%; $p=0.001$) as compared to untreated controls (97±1.3%). The residual cytotoxicity observed in anti-LFA-1 mAb treated C57BL/6 recipients is comparable to the in vivo cytotoxicity of CD8-depleted C57BL/6 secondary recipients (12.1±8.9%) and is likely mediated by alloantibody. In fact, the level of allospecific cytotoxicity in anti-LFA-1 mAb treated sensitized C57BL/6 recipients correlated with the amount of alloantibody present in recipient serum. **Conclusion:** In conclusion, treatment with anti-LFA-1 mAb delayed (CD4-independent) CD8-dependent rejection in sensitized recipients but did not delay rejection in sensitized CD4-sufficient C57BL/6 recipients. Despite the efficacy of treatment with anti-LFA-1 mAb to significantly reduce the in vivo allospecific cytotoxic effector function in sensitized C57BL/6 mice this strategy did not delay rejection. This is likely due to alloantibody mediated rejection in sensitized C57BL/6 (but not CD4 KO recipients) which is not suppressed by treatment with anti-LFA-1 mAb.

Infections and Host Response

Abstract# 194

PD-1 Expression and IL-2 Loss of Cytomegalovirus-Specific T-Cells Correlate with Viremia and Reversible Functional Anergy. Martina Sester,¹ Daniela Preßer,¹ Jan Dirks,¹ Barbara C. Gärtner,² Hans Köhler,¹ Urban Sester.¹ ¹*Department of Internal Medicine IV, University of the Saarland, Homburg, Germany;* ²*Department of Virology, University of the Saarland, Homburg, Germany.*

Cytomegalovirus (CMV) represents a major cause of infectious complications after transplantation. Recently, chronic infections with LCMV, HIV or HCV were shown to be associated with functionally anergic T-cells characterized by high expression of the programmed death (PD)-1 molecule. This study was carried out to characterize functional exhaustion of CMV-specific CD4 T-cells as determinant of impaired CMV-control and to elucidate whether the PD-1 pathway may be operative in active CMV-infection after renal transplantation. CMV specific CD4 T cells from 13 controls, 31 hemodialysis patients, and 51 renal transplant patients were quantified using flow cytometry and analysed for their expression of PD-1 and cytokines IFN γ and IL2. CMV specific proliferation was analysed by CFDA-SE dilution. In viremic transplant-recipients, a significantly higher proportion of CMV-specific CD4 T-cells were PD-1 positive (median 40.9%) as compared to non-viremic transplant patients (8.8%), dialysis-patients (8.8%) or controls (3.1%, $p<0.0001$). In line with functional impairment, PD-1 positive T-cells produced significantly less IFN γ per single cell as compared to PD-1 negative T-cells (mean fluorescence intensity 129.4±52.6 versus 256.6±96.1, $p<0.0001$). Moreover, unlike controls or non-viremic patients, the majority of CMV-specific T-cells from viremic patients showed a long-term loss of IL-2 production. Interestingly, functional anergy of PD-1 positive CMV-specific CD4 T-cells was reversible in that antibody-mediated blockade of PD-1 signaling with its ligands PD-L1/L2 led to a 10fold increase in CMV-specific proliferation. In conclusion, expression of PD-1 defines a reversible defect of CMV-specific CD4 T-cells, and blocking PD-1 signaling may provide a potential target for enhancing the function of exhausted T-cells in chronic CMV-infection.

Abstract# 195

Differential Decay Kinetics of CMV DNAemia after Antiviral Therapy in Patients with Simultaneous Co-Infections with Different Viral Strains. Vincent Emery,¹ Oriol Manuel,² Anders Asberg,³ Anders Hartmann,³ Xiaoli Pang,² Mark Pescovitz,⁴ Angelo Bignamini,⁵ Halvor Rollag,³ Alan Jardine,⁶ Atul Humar.² ¹*University College London;* ²*University of Alberta;* ³*University of Oslo;* ⁴*Indiana University;* ⁵*University of Milan;* ⁶*University of Glasgow.*

Background: Some patients with CMV disease may be simultaneously infected with multiple viral strains. It is unknown if different strains clear differently with the commencement of antiviral therapy. We assessed response to antiviral therapy in patients with simultaneous co-infection with multiple strains of CMV. **Methods:** PCR-based strain typing of CMV was performed using the glycoprotein B gene of CMV (gB1-4) in a cohort of organ transplant recipients with CMV disease. From this, 89 patients were identified that had simultaneous infection with ≥ 2 CMV strains. Quantitative assessment of each of the strain types was performed at regular intervals after starting antiviral therapy. **Results:** The different types of multi-strain infections were gB1+gB2 (11/89, 12%), gB1+gB3 (22/89, 25%), gB1+ gB4 (7/89, 8%), gB2+gB3 (10/89, 11%), gB2+gB4 (6/89, 7%) and gB3+gB4 (8/89, 9%). 25/89 (28%) were simultaneously infected with 3 or 4 different genotypes. Within individual patients, there was trend for gB1 CMV load (4.27log genomes) to be lower than the other genotypes ($p=0.05-0.07$) at the onset of disease. Decay kinetics for all genotypes showed a biphasic response with a 1st phase decline of ~0.7 days and a 2nd phase of ~3days. 1st phase declines were fastest for gB1 ($p=0.0001$ vs gB2 and 4) while gB4 decline was slower than gB3 during the 1st phase. 2nd phase declines were similar between gB1 and 3 (3days and 2.9 days) but were slower for gB2 and 4 (3.45 days; $p=0.01$). There was a significant correlation between 1st phase decline and log decline from baseline by day 21 ($r=0.37$; $p=0.002$). Relative fitness calculations revealed complex fitness dynamics between genotypes although gB3 was always less fit than gB1, 2 and 4, and gB4 and 2 were always less fit than gB1. **Conclusion:** In patients with CMV disease who have simultaneous co-infection with multiple strains, the 1st and 2nd phase declines in gB1 are significantly slower than either gB2 or 4 and have a lower log decline from baseline by day 21. These data indicate that either a significant fitness difference exists between CMV strains or that antiviral control of replication may be linked to CMV gB genotype and should aid our understanding of treatment success and failure.

Abstract# 196

One-Year Experience with Testing for Antibodies and Evaluating the Risk Factors for Exposure to *Trypanosoma cruzi* among Organ Donors in Southern California. Marek J. Nowicki,¹ Claudia Chinchilla,¹ Thomas Mone,² Robert Mendez,¹ Dem Brucal,¹ Maria Stadler.² ¹*National Institute of Transplantation, Los Angeles, CA;* ²*OneLegacy, Los Angeles, CA.*

Trypanosoma cruzi, a parasite that causes Chagas' disease, is confined to South and Central America. Chagas disease primarily affects people living in poorly constructed homes (mud, adobe, or thatch) in rural areas and is usually spread by the reduviid bugs (vector). The acute disease generally occurs in children, whereas chronic symptoms usually appear later in life. Transmission of *T. cruzi* infection by solid organ transplantation has been reported in Latin America and the US (Transplantation, 2006; 81:477). Our laboratory implemented testing anti-*T. cruzi* for donors at risk in September 2006 and began to test all organ donors for anti-*T. cruzi* using FDA approved EIA on April 15, 2007.

Aim: To evaluate the prevalence of donors with risk factors for exposure to the Chagas disease vector and to compare the results with the data obtained using the serological screening test (EIA+RIPA).

Material and Methods: We used a modified questionnaire originally developed by Galel et al. (Transfusion, vol. 36, No. 3, 1996). Our questionnaire included 5 questions assessing the risk of the prospective organ donor's exposure to vectors potentially carrying parasite and/or diagnosed with Chagas disease. We applied this tool to 376 cases (133 females and 243 males, age 0.5-80 y) approached for donation between 09/2006 and 11/2007 by our OPO located in Southern California. To test for anti-*T. cruzi* we used FDA approved EIA (Ortho Diagnostics) confirmed by RIPA (Quest Diagnostics).

Results: 156 donors (41.5%) were of Latino descent. Three donors (0.8%) were reported to be diagnosed with Chagas disease in the past. Sixty donors (15.9%) were reported to have traveled to areas where *T. cruzi* is endemic and/or stayed for an extended time in housing potentially infested by the vectors of Chagas disease. One donor with risk factors tested positive (EIA+RIPA) for antibodies to *T. cruzi* (0.27%).

Conclusions: Our survey indicates that a large proportion of donors in S. California are at risk of being exposed to the vectors carrying *T. cruzi*. However, few have evidence of the actual infections as determined by self report and/or EIA+RIPA testing. Our finding underscores the need to monitor this new and emerging infection risk among organ donors using serological tests rather than self reported risk factors.

Abstract# 197

Incidence and Outcomes of Parovirus B19 Infection in Kidney Transplant Recipients: A Single Center Experience. Rafael Villicana,¹ Alice Peng,¹ Ashley Vo,¹ Robert Chang,¹ Donald Dafeo,¹ Stanley Jordan,¹ Mieko Toyoda.¹
¹Comprehensive Transplant Center, Cedars Sinai Medical Center, Los Angeles, CA.

Introduction: Parvovirus B19 (PVB19) is a single-stranded DNA virus that was first reported to affect transplant (tx) recipients around 20 years ago. In the kidney tx setting PVB19 has been reported to cause anemia and proteinuria. Reported incidence in a general kidney transplant cohort has been reported to be between 0%-12% and as high as 38% in an anemic kidney tx population. Here we report our incidence of PVB19 infection over a 12 year span.

Patients and Methods: All records of kidney tx recipients from 1996 until 2007 were reviewed for the presence of PVB19 infection. There were 834 kidney tx performed during this period. Diagnosis of PVB19 infection was made either by detection of PVB19 via PCR in a blood/tissue sample or by detection of virus on renal tissue by immunostain. In patients found to have PVB19 infection; presence of anemia, proteinuria, concurrent infection and acute rejection rates were examined. Response to treatment with IVIG was also evaluated.

Results: Incidence of infection was 2.4% as 20 patients were found to have evidence of infection. Average time from tx to diagnosis of infection was 21.6 months (range 3 days- 86 months). Average creatinine at diagnosis was 2.32 mg/dl. Anemia was present in 85% of patients with an average hematocrit of 30.8%. Proteinuria was present in 45% of patients with evidence of PVB19 infection. Co-infection was noted in 5 patients (4 CMV, 1EBV) and acute rejection was noted in 30% of individuals within 3 months of diagnosis. Collapsing glomerulopathy (CG) was present in 5 patients and they all had subsequent graft loss at an average of 6 months after diagnosis. 4 of the 5 patients with CG had proteinuria along with anemia and were Caucasian. 90% (18/20) of all patients with evidence of PVB19 infection received IVIG and cleared their infection. One of the remaining 2 pts without IVIG spontaneously cleared their virus.

Conclusion: Although the incidence of PVB19 infection in our kidney tx cohort was very low, its presence portends an unfavorable outcome. The presence of CG associated with PVB19 is an especially devastating lesion with very poor outcomes. Response to treatment with IVIG and reduction of immunosuppression is variable. Based on our data it seems reasonable to screen all tx patients with unexplained anemia and concurrent proteinuria as early detection of PVB19 may be crucial.

Abstract# 198

Comparison of the Quantiferon-TB Gold to Tuberculin Skin Test for the Diagnosis of Latent Tuberculosis Infection in Renal Transplant Candidates. Karen E. Doucette,¹ Deepali Kumar,¹ Anthony Chow,² Gregory Tyrrell,² Isabel Cobos,¹ Atul Humar,¹ Dennis Kunimoto.¹
¹Division of Infectious Diseases, University of Alberta, Edmonton, AB, Canada;
²Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, AB, Canada.

Background: Prior to transplant, screening for latent tuberculosis (LTBI) by tuberculin skin test (TST) is recommended. The accuracy of TST in end stage renal disease however may be limited. The Quantiferon®-TB Gold assay (QFT) detects interferon- γ produced by peripheral blood T-cells in response to TB specific antigens and may be more accurate for diagnosis of LTBI.

Methods: This prospective single center study compared the TST to QFT for the diagnosis of LTBI in a cohort of adult patients listed or undergoing workup for renal transplantation. All patients had both TST and QFT performed. Additional data collected included demographics, TB risk history and chest x-ray results. Based on demographic and radiographic findings, patients were classified as high or low risk for LTBI. A positive TST was defined as ≥ 5 mm and positive QFT as ≥ 0.35 IU/ml.

Results: A total of 45 patients were enrolled. Complete data was available for 38 subjects (5 did not return to have TST read). The mean age was 50.4 $\pm$ 12.2 years with 21 (55.3%) males and 17 (44.7%) females. The most common etiologies of renal diseases were diabetes (52.6%) and glomerulonephritis (26.3%). Most subjects (35 of 38) were on renal replacement therapy (hemodialysis in 73.7% and peritoneal dialysis in 18.4%). Twenty (52.6%) subjects had received BCG and 10 (26.3%) were born in or lived in a country in with TB prevalence rate $> 20/100,000$ population. Fifteen (39.5%) subjects were considered to be at high-risk for LTBI. Overall 5 (13.2%) had a positive TST and 3 (7.9%) had a positive QFT. The QFT was indeterminate in 1 subject due to a low mitogen response. Agreement between the tests was 92% ($K=0.72$, $p<0.0001$). In low-risk subjects ($n=23$) the TST was negative in all and the QFT was negative in 22 and indeterminate in 1. In clinically high-risk subjects, 5 (33%) had a positive TST and 3 (20%) had a positive QFT. The 2 subjects with discordant results, both from TB endemic countries, had both completed treatment for LTBI 4 years prior and remained TST positive, but were QFT negative.

Conclusion: In renal transplant candidates, the TST and QFT are comparable for the diagnosis of LTBI. The QFT has the advantage of being completed in a single visit and in our cohort indeterminate results were uncommon.

Abstract# 199

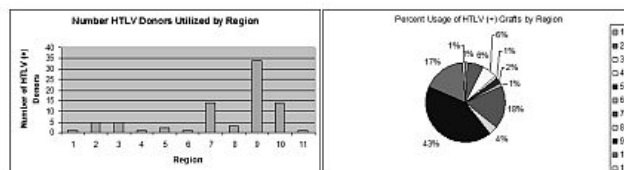
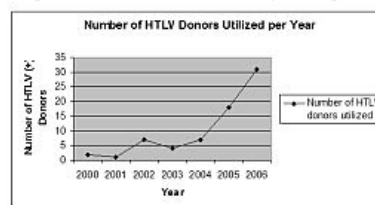
Optimal Utilization of HTLV I/II Positive Organs – A Nationwide Survey. Ravi Nagubandi,¹ Guy N. Brock,² Kadiyala V. Ravindra,¹ Mary Eng,¹ Joseph F. Buell,¹ Michael R. Marvin.¹
¹Division of Transplantation, Dept. of Surgery, University of Louisville, Louisville, KY; ²Bioinformatics and Biostatistics, University of Louisville, Louisville, KY.

Objective: We recently presented data from the UNOS database that demonstrated no significant difference in graft or patient survival between HTLV I/II (+) and (-) liver recipients. Several Organ Procurement Organizations (OPO) including our own, do not offer HTLV I/II positive organs while many others find it difficult to place them. Despite this, the number of HTLV (+) organs is increasing with 31 utilized in 2006 alone. This prompted us to evaluate the practical difficulties in placing these organs so as to improve utilization of these “high risk” life saving organs.

Method: A telephone/email survey of all the 65 OPOs in USA was done over a 2 month period from October to November 2007.

Results: Of the 65 OPOs, 42 responded. All screen patients for HTLV I/II with ELISA. 38 centers confirm with repeat ELISA, 27 confirm with Western Blot and 6 centers do not pursue further. 36 of the 42 centers offer the HTLV I/II positive organs. There were a total of 231 positive donors in the past 5 years of which organs from 109 donors (47.2%) were placed.

27 centers offer all the organs while 15 offer one or more organs selectively based on accepting centers. None have been able to place the pancreas. Only liver and kidney were commonly accepted. Several centers noted a high false positive rate.



Based on the UNOS regional analysis data, 43% of the organs are utilized in NY state alone. Many OPOs did not know which particular centers accept these organs and consequently spend a lot of time and effort in order to place them. A majority wanted to have a list of transplant centers that accept these organs.

Conclusions: HTLV I/II organs are being underutilized. Moreover, our prior analysis of UNOS data shows that these life saving organs are shared more nationally than loco-regionally which is associated with a poorer outcome. Increased knowledge of successful HTLV (+) donation and the centers that are willing to utilize these organs in the appropriate setting will help expand the donor pool and decrease mortality on the waiting list.

Abstract# 200

Increasing the Donor Pool – Transplantation of Kidneys of Anti HCV Positive Donors. Jürgen Treckmann,¹ Knut Nowak,¹ Silvo Nadalin,¹ Anja Gallinat,¹ Oliver Witzke,² Andreas Paul.¹
¹Clinic for General-, Visceral- and Transplant Surgery, University Essen, Essen, Germany; ²Clinic for Nephrology, University Essen, Essen, Germany.

Strategies for increasing the pool of kidney donors include acceptance of donors with chronic infections like hepatitis c virus (HCV) infection. 5% of all potential donors in Europe are positive for antibodies to HCV. Only few centres in Europe include acceptance of kidneys of HCV positive donors in their centre profile leading to the fact that nearly all organs from HCV positive donors in the Eurotransplant region are transplanted at the University of Essen (Germany). The outcome of the recipients of kidneys of anti HCV positive donors is analysed in this study.

Patients and Methods:

Between September 2002 and May 2007 48 patients (23 women, 25 men) were transplanted with kidneys of anti HCV positive donors. 24 recipients had a chronic HCV infection before transplantation. The remaining 24 recipients were HCV negative. 23 of them were transplanted after HCV PCR was performed with negative result. One patient (age 70) was transplanted with a PCR positive kidney and infected. All patients signed an informed consent concerning the risk of new infection and possible deterioration of the underlying HCV infection. Peripoperative data as well as data concerning graft function, liver function and serological examinations (anti HCV antibodies, HCV PCR) were collected and analyzed prospectively.

Results

Graft survival after one year was 87 % and patient survival 95 %. Mean age of donors was 32 years (18- 48 years) One patient was newly infected with HCV without actual

consequences concerning liver function but received a well functioning graft of a 25 year old donor (s. patient and methods). Two patients with underlying HCV infection showed deterioration of liver function one without correlation to transplantation one with genotype crossing. Of the 23 recipients without HCV infection transplanted with kidneys of anti HCV positive donors with negative HCV PCR none got infected.

Conclusion

Acceptance of kidneys of anti HCV positive donors increases the donor pool and is safe for recipients with chronic HCV infections. Also recipients without HCV infection can be transplanted safely if PCR is negative. In rare cases especially in old recipients HCV infection can be accepted as consequence of transplantation.

Abstract# 201

Alemtuzumab Preconditioning Followed by Tacrolimus Monotherapy in Kidney Transplant Recipients Renders Circulating EBV-Specific CD8⁺ T Cells Anergic. Camila Macedo,¹ Iulia Popescu,¹ Yun Hua,¹ Sheila Fedorek,¹ Ron Shapiro,¹ Diana Metes.¹ ¹*Surgery, Thomas E Starzl Transplantation Institute, Pittsburgh, PA.*

Traditional two-drug chronic immunosuppression (IS) used in organ transplantation (Tx) is associated with development of EBV-driven complications because of impairment of anti-viral CD8⁺ T cell surveillance. Since the long-term impact of alemtuzumab preconditioning combined with tacrolimus monotherapy on EBV immunity after Tx has not been studied, here we aim to analyze the frequency and function of peripheral blood EBV-specific CD8⁺ T cells. Thirteen EBV⁺ stable kidney transplant (KTx) recipients and 12 EBV⁺ healthy controls were recruited to this cross-sectional study. All patients received alemtuzumab preconditioning, followed by tacrolimus monotherapy. Blood samples were collected at least 1 year post-Tx to allow immune reconstitution. The EBV-specific CD8⁺ T cell phenotype and function were screened by flow cytometry and IFN γ ELISPOT assay. HLA-A201 restricted EBV-lytic (BMLF1) and latent (LMP2a) peptides were used to generate Tetramer (TMR) probes, and for functional screening in ELISPOT. Circulating CD8⁺ T cells from KTx patients had recovered by 1 year, and were comparable to those of healthy controls (28.6% $\pm$ 12.9 vs 23% $\pm$ 6.6, $p=0.2$). Moreover, the memory distribution and the frequency of EBV-specific CD8⁺ T cells detected in patients and controls (BMLF1-specific: 0.56% $\pm$ 0.96 vs 0.88% $\pm$ 1.08, $p=0.46$, and LMP2-specific: 0.05% $\pm$ 0.06 vs 0.24% $\pm$ 0.40 $p=0.09$) were similar. In contrast, the frequency of functional Type-1 (IFN- γ producing) EBV-specific CD8⁺ T cells was significantly lower in KTx patients than in healthy controls (BMLF1: 47 $\pm$ 24 spots/10⁵ CD8 T cells vs 164 $\pm$ 134 $p=0.06$, and LMP2: 9 $\pm$ 8 vs 64 $\pm$ 54 $p=0.03$). Accordingly, on average, only 8-12% of circulating EBV-specific CD8⁺ T cells from KTx patients produced IFN- γ , while 20-30% of effector cells were functional in healthy controls. Addition of IL-2 (20IU/ml) during the ELISPOT assay reversed the hypo-responsiveness of Type-1 (IFN- γ) EBV-specific CD8⁺ T in patients (range 3-8 fold increase), suggesting that these effector cells were anergic. These results support the notion that alemtuzumab-induced lymphocyte depletion followed by tacrolimus monotherapy renders EBV-specific CD8⁺ T cells anergic *in vivo*, a state that can be readily reversed by cytokines such as IL-2, which are commonly released during immune activation.

Abstract# 202

An Assessment of Donor to Recipient Transmission Patterns of Human Cytomegalovirus by Analyzing Viral Genomic Variants. Oriol Manuel,^{1,2} Xiaoli Pang,¹ Atul Humar,² Deepali Kumar,² Karen Doucette,² Jutta K. Preiksaitis.¹ ¹*Provincial Laboratory for Public Health; ²Transplant Infectious Diseases, University of Alberta, Edmonton, Canada.*

Background: The epidemiology of the transmission of cytomegalovirus (CMV) from organ donors to recipients is not completely understood. We studied donor to recipient transmission patterns by analyzing viral genomic variants through the use of CMV glycoprotein B (gB) genotyping by real-time PCR. Polymorphisms in gB UL55 allow discrimination of 4 distinct genomic variants (gB 1-4).

Methods: Organ transplant recipient pairs or triplets were included in the study if: a) they had CMV infection, b) they received an organ from a CMV seropositive donor, and c) there was at least one other recipient from the same donor that also developed CMV infection. Genotyping (gB 1-4) was performed by quantitative real-time PCR on stored blood samples. Clinical charts were reviewed to evaluate the clinical characteristics and outcome of CMV infection.

Results: Of the 78 CMV seropositive donors screened, 21 were multiple organ donors for which 2 or more of their recipients developed CMV infection. The total number of recipients from these 21 donors was 86 (median of 4 recipients per donor). Of these recipients, 47 (55%) had CMV infection (16 recipient pairs and 5 recipient triplets). The prevalence of genotypes was gB1 (n=22; 47%), gB2 (n=11; 23%), gB3 (n=4; 9%), gB4 (n=0). Mixed infection with two concurrent genotypes was present in 10 patients (21%). Overall concordance between CMV gB genotype in recipient pairs was 62.5% (10/16). If both recipients were CMV seronegative (D+/R-) the gB concordance in recipients was 67% (2/3 pairs). gB concordance was 70% (7/10 pairs) if one of the recipients was seronegative and the other seropositive. Concordance was 33% (1/3 pair) if both recipients were seropositive. Concordance between genotypes was seen in 2/5 (40%) recipients triplets. In seropositive recipients with CMV viremia, the origin of the CMV strain was thought to be donor derived in 11/16 (69%) and of reactivation of the recipients own virus in 5/16 (31%) of the cases. No difference in clinical outcome or organ tropism was seen between genotypes.

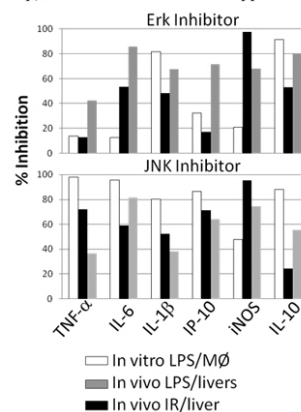
Conclusion: Based on an analysis of strain concordance within recipients from common donors, transmission patterns of CMV can be assessed. In D+/R+ transplant patients, donor strain superinfection accounts for the approximately two-thirds of CMV infection.

Ischemia/Reperfusion Injury and Sentinel TLR System

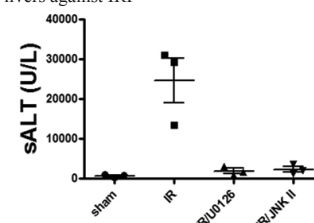
Abstract# 203

Both ERK and JNK MAP Kinase Activation Contribute to Liver Inflammation in Ischemia/Reperfusion Injury (IRI). Duanyun Zhao, Xiuda Shen, Feng Gao, Ronald W. Busuttil, Yuan Zhai, Jerzy W. Kupiec-Weglinski. *Surgery, David Geffen School of Medicine at UCLA, Los Angeles, CA.*

Background: Although MAP kinases have been implicated in the pathophysiology of liver IRI, their functional significance in the mechanism of TLR4 mediated pro-inflammatory immune regulation, remains to be elucidated. **Methods:** MAP kinase activation in a murine model of liver warm IRI (90 min. ischemia, 6 h reperfusion) was determined by Western blots. Chemical inhibitors of Erk (U0126, 10 μ M *in vitro* or 160 mg/kg *in vivo*), JNK (SP600125, 50 μ M or 30 mg/kg), and p38 (SB203580, 10 μ M, 100 mg/kg) MAP kinases were utilized *in vitro* in primary BM-derived macrophage cultures stimulated with LPS (10 ng/ml); or *in vivo* in liver pro-inflammatory immune responses induced by LPS (1 μ g/mouse, i.p.) or IRI. **Results:** Erk and JNK, but not p38, MAP kinase activation were readily detected in liver IRI. In primary macrophage cultures, LPS induced pro- and anti-inflammatory genes, including TNF- α , IL-1 β , IL-6, IL-10, iNOS and CXCL10. Erk inhibitor mainly suppressed IL-1 β and IL-10 (80% and 90% resp), whereas JNK inhibitor suppressed the majority of genes.



In LPS-induced liver inflammation, Erk inhibitor suppressed IL-6, IL-1 β , iNOS and IL-10 by >50%, but failed to affect TNF- α /CXCL10. JNK inhibitor, on the other hand, preferentially inhibited pro-inflammatory genes, but marginally affected IL-10 (<30%). When tested *in vivo*, both Erk and JNK MAP kinase inhibitors effectively protected livers against IRI.



and produced comparable suppression of pro-/anti-inflammatory genes (figure1). Interestingly, TNF- α was the least responsive gene subjected to MAP kinase regulation.

Conclusion: Erk and JNK MAP kinase activation: 1/ are required for TLR4 activation-induced pro-inflammatory gene induction; 2/ play critical role in the development of IR-mediated liver immune response/tissue injury.

Abstract# 204

JAK/STAT Signaling Pathways in the Inflammation Response during Liver Ischemia/Reperfusion Injury. Maria Cecilia S. Freitas,¹ Xiu-Da Shen,¹ Yoichiro Uchida,¹ Takashi Hamada,¹ Ronald W. Busuttil,¹ Jerzy W. Kupiec-Weglinski.¹ ¹*The Dumont-UCLA Transplant Center, David Geffen School of Medicine at UCLA, Los Angeles, CA.*

Background: The JAK/STAT signaling is one of the major pathways for cytokine signal transduction. The signal transducer and activator of transcription 1 (STAT1) is mainly activated by IFN- α /IFN- γ . The activation of STAT1 by IFN- γ has been implicated in hepatic inflammation. We have shown that activation of Toll-like receptor (TLR) 4 complex initiates pro-inflammatory response leading to liver ischemia/reperfusion

injury (IRI). Indeed, TLR4 signaling *in vitro* activates STAT1, which in turn triggers production of type-1 IFN-dependent CXCL10 (IP-10). This study was designed to analyze the cross-talk between STAT1 and the MAP kinase (ERK) downstream of JAK/STAT signaling pathways. **Methods & Results:** We used a mouse liver model of partial warm ischemia (90 min), followed by reperfusion (6 h). First, we employed STAT1 KO (n=17) and control WT (n=16) mice. The hepatocellular damage, as measured by sALT levels (IU/L), was significantly decreased in 10/17 STAT1 KO mice ($p < 0.005$); the remaining 7/17 of STAT1 KO showed sALT levels comparable with WT. Hence, we distinguished two groups of STAT1 “protected” vs. “nonprotected” KO recipients. Histology revealed minimal sinusoidal congestion without edema/vacuolization or necrosis in STAT1 KO “protected” group. The induction of mRNA coding for TNF α /IL-6 was higher in STAT1 KO “nonprotected” livers. The expression of CXCL10, the product of STAT1 activation downstream of TLR4 in Type I IFN pathway, was profoundly and selectively depressed in livers from STAT1 KO “protected” mice, as compared to IRI susceptible livers. Similarly, Western blot-assisted phospho-ERK expression was up regulated selectively in the STAT1 KO “protected” group. In the second series, C57BL/6 mice were treated 1 h prior to liver ischemic insult with JAK-2 inhibitor (Tyrostatin AG490; 40 mg/kg, i.p., n=5), or vehicle (n=5). The hepatocellular damage, as measured by sALT levels (IU/L), and histology was significantly decreased in AG490 group, as compared with controls (mean = 12770 vs. 28770; $p < 0.05$). **Conclusion:** The disruption of JAK/STAT signaling by inhibiting JAK2 uniformly ameliorates the inflammatory immune response in liver IRI. However, the blockage of STAT1 alone is insufficient to reproducibly exert cytoprotection. As JAK2 is upstream of STAT1 as well as upstream of MAP kinase (ERK), this study highlights the role of both signaling pathways in hepatic IRI.

Abstract# 205

Toll-Like Receptor 4 (TLR4) on Endothelia Contributes to Murine Ischemic Renal Injury. JianLin Chen,¹ Yanxia Wang,¹ Christopher Lu.¹ ¹Internal Medicine, UT Southwestern Medical Center at Dallas, Dallas, TX.

Purpose: TLR4 is required for maximal ischemic injury of the heart, liver, lung, and kidney. To better understand the mechanisms of TLR4 action, we investigated a murine model of ischemic kidney disease and examined endothelial TLR4 expression.

Methods: 1. Animal ischemia reperfusion injury (IRI): the right kidney of wildtype (WT) C57BL/10, or TLR4-deficient (KO) C57BL/10ScN mice was removed. The left pedicle was clamped for 23 min, followed by 4 hr reperfusion. Sham animals were controls. 2. Bone-marrow chimera: four groups of BM chimeras were created: WT→WT; KO→WT; WT→KO; KO→KO (8 recipients/grp). 8 wks later, chimerism was confirmed by tail and blood genotyping, and mice subjected to renal IRI. 3. TLR4 mRNA detected by DIG-labeled antisense; TLR4 on endothelium by anti-TLR4 and anti-CD31. 4. Total genome mRNA expression on ischemic vs. sham WT mice, ischemic vs. sham KO mice (4 mice/grp) determined using Affymetrix Mouse Genome 430.2 GeneChips followed by Genesifter analysis. Quantitative real-time RT-PCR confirmed candidate genes. 5. MS1 endothelial cells were treated with H₂O₂ (500uM) for 30 min, cultured for 4 hr and then expression of TLR4 and endothelial genes determined.

Results: TLR4-deficient mice had less renal injury as assessed by pathology and function. Radiation chimeras showed that radioresistant parenchymal cells and radiosensitive leukocytes were both required for maximal injury. Immunohistology and *in situ* hybridization identified endothelia in the outer medulla as a major TLR4 expressing cell type at 4 hr post-reperfusion. Genechip analysis revealed a panel of cytokine, chemokines, proinflammatory and cell-cycle related genes that were differentially expressed on IRI versus sham kidneys. Real-time RT-PCR confirmed that the following pro-inflammatory endothelial genes increased after ischemia in wildtype but not TLR4 KO mice: TLR4, Pentraxin related gene (PTX3), and Endothelial cell-specific molecule 1 (ESM1). Real-time RT-PCR showed that *in vitro* H₂O₂ treatment of endothelial cells induced expression of TLR4 (6.6-fold), PTX3 (4.6-fold), and ESM1 (69-fold).

Conclusions: We found that endothelia in the outer medulla increase their expression of TLR4 after ischemia, and that the endothelial genes PTX3 and ESM1 increase only in WT ischemic kidneys. We also found that H₂O₂, which mimics reactive oxygen species generated during IRI, directly increases these same endothelial genes *in vitro*.

Abstract# 206

Kidney Ischemia Reperfusion Injury Upregulates Expression of NKG2D Ligands (RAE-1 and MULT-1) by Intrinsic Kidney Cells through TLR4-MyD88 Signaling. Gang Chen,¹ Steven J. Chadban,¹ Kate R. Wyburn,¹ Stephen I. Alexander,¹ Alexandra F. Sharland,¹ Huiling Wu.¹ ¹Collaborative Transplantation Research Group/Renal Medicine, University of Sydney/RPAH, Sydney, NSW, Australia.

NKG2D is an activating receptor expressed on NK cells and CD8⁺ T cells. Ligands for NKG2D including RAE-1, MULT-1 and H60 in mouse, may be upregulated by tissues in response to stress. A study in mouse macrophages described upregulation of RAE-1 in response to stimulation through TLR4 by LPS. We have shown that TLR4 mediates kidney ischemia reperfusion injury (IRI) and also observed RAE-1 upregulation in IRI kidneys. We now determined whether: 1) kidney IRI could induce the expression of NKG2D ligands; 2) expression of NKG2D ligands is TLR4 dependent; 3) bone marrow (BM) derived cells or parenchymal kidney cells express NKG2D ligands. **Methods.** Kidney-ischemia was induced in TLR4^{-/-}, MyD88^{-/-} and WT mice for 22 min.

Blood and tissue were harvested at days 1, 3 and 5. Primary cultures of mouse tubular cells (TECs) were also subjected to ischemia (mineral oil overlay for 1 hr) or TLR4 activation (LPS stimulation). BM chimeric mice were generated by transplanting BM into irradiated recipient mice before IRI was induced. **Results.** RAE-1 mRNA level in IRI kidney was increased from day 1 to day 5, peaking at day 3 compared to sham operated kidney (14-48 fold increase, $P < 0.001$) measured by real time PCR. RAE-1 protein was detected in renal tubular cells from ischemic kidney but not from sham-operated control by flow cytometry. MULT-1 mRNA expression was also increased from day 1 to day 5 (15- 51 fold increase, $P < 0.005$). Both RAE-1 and MULT-1 mRNA levels were reduced in TLR4^{-/-} and MyD88^{-/-} IRI kidneys (2-8 fold reduction) versus WT controls ($P < 0.01$). TLR4^{-/-} and MyD88^{-/-} primary cultured TECs submitted to IRI *in vitro* also showed less RAE1 and MULT-1 mRNA expression than WT controls ($p < 0.01$). LPS stimulated RAE-1 and MULT-1 expression in WT but not in TLR4^{-/-} TECs. TLR4^{-/-} mice bearing WT hemopoietic cells had significantly lower kidney RAE-1 and MULT-1 mRNA expression after IRI versus WT mice with TLR4^{-/-} BM ($p < 0.05$). **Conclusion.** Kidney IRI causes RAE-1 and MULT-1 expression and kidney parenchymal cells are the dominant source. TECs can be stimulated to express RAE-1 and MULT-1 by ischemia or LPS via the TLR4 pathway *in vitro* and deficiencies in this pathway provide protection against IRI and RAE-1 and MULT-1 expression *in vitro* and *in vivo*. Thus, kidney IRI causes upregulation of NKG2D ligands by parenchymal kidney cells via TLR4.

Abstract# 207

Absence of Toll-Like Receptor 4 Ameliorates Intestinal Ischemia/Reperfusion Injury by a Reduction in Both Neutrophil and CD3+ Lymphocyte Activation and Infiltration. Melissa J. Watson,¹ Bibo Ke,¹ Xiu-Da Shen,¹ Feng Gao,¹ Ronald W. Busuttill,¹ Jerzy W. Kupiec-Weglinski,¹ Douglas G. Farmer.¹ ¹The Dumont-UCLA Transplant Center, David Geffen School of Medicine at UCLA, Los Angeles, CA.

Background: Intestinal ischemia/reperfusion injury (IRI) is a major clinical problem. Although Toll-like receptor 4 (TLR4) has been implicated as a potential link between the innate and adaptive immunity, little is known on its role in intestinal IRI. Our preliminary research in intestinal IRI has shown that, compared to sham controls, WT mice had decreased survival, worse tissue injury/apoptosis, increased PMN infiltration, increased CD3+ cell infiltration, and increased production of TLR4, chemokines, and adhesion molecules. Here we used TLR4KO mice to further investigate the role of TLR4 in intestinal IRI and its effects on cytokine/chemokine programs and apoptotic signaling.

Methods: C57BL10 WT and TLR4KO mice underwent 100 min of total jejunoileal warm IRI by clamping of the SMA. Separate survival and analysis groups were performed. Intestinal tissue was harvested at 4h and 24h. Tissue analysis included histopathology, CD3 immunostaining, myeloperoxidase (MPO) activity, RT-PCR for chemokines/cytokines, and Western blots for apoptotic and HO-1 protein expression.

Results: TLR4KO had superior survival compared to WT (100% vs. 33%, $p < 0.02$). On histopathology TLR4KO had near normal-appearing villous architecture, while in contrast, WT showed mucosal erosions and villous congestion/hemorrhage. TLR4KO had reduced CD3+ cell infiltration as compared to WT (3.9±0.7 vs. 6.2±1.4 per hpf at 4h, $p < 0.001$; and 4.8±1.1 vs. 7.0±2.3 per hpf at 24h, $p < 0.02$). Early MPO activity was also reduced in TLR4KO (0.11±0.06 vs. 0.88±0.5 U/g at 4h, $p < 0.005$). RT-PCR analysis demonstrated decreased production of mRNA for IP-10, MCP-1, RANTES, and IFN- γ and increased production of IL-13 in TLR4KO. There was decreased protein expression of caspase-3 and increased expression of bcl-2 and HO-1 in TLR4KO mice.

Conclusion: The genetic absence of TLR4 exerts protection against intestinal IRI, demonstrating for the first time that TLR4 is required for intestinal IRI. The absence of TLR4 signaling reduces IRI through reduced neutrophil and T cell chemotaxis, and up-regulation of protective molecules. These results support data that TLR4 is a mechanistic link between the innate and adaptive immunity, implicating TLR4 as a potential therapeutic target for the prevention of intestinal IRI.

Abstract# 208

TLR4 Is Responsible for Steatotic Liver Damage after Ischemia/Reperfusion. Justin D. Ellett,¹ Zachary P. Evans,¹ Kenneth D. Chavin.¹ ¹Surgery, Division of Transplant, Medical University of South Carolina, Charleston, SC.

It is well known that liver steatosis increases hepatic vulnerability to ischemia/reperfusion (I/R) injury as part of the transplantation process. Endotoxin (LPS) is thought to be a major contributing factor to the pathogenesis of I/R. During portal occlusion, LPS is translocated across the mesenteric tissue barrier into the portal circulation, and is delivered as a large bolus to the liver at the point of reperfusion. At this time, LPS is mainly recognized by toll-like receptor 4 (TLR4). This leads to downstream signaling and the production of proinflammatory products that ultimately lead to cellular inflammation, necrosis, and apoptosis. It is well known that steatotic livers are highly sensitive to endotoxin as compared to their lean counterparts post-I/R, and we have previously seen that monoclonal antibody blockade of endotoxin dramatically improves animal survival after I/R. Therefore, we propose the novel hypothesis that TLR4 signaling is a major contributor to cellular damage after steatotic hepatic I/R. To test this hypothesis, we subjected male 4-week-old C57BL/10J (control) or C57BL/10ScN (TLR4 deficient, TLR4KO) mice to a high-fat diet (HFD) for four weeks. Then, we subjected the animals

to 35 minutes of total hepatic ischemia and 1 or 24 hours of reperfusion. There was a dramatic improvement in animal survival in the HFD TLR4KO animals versus control HFD animals at 24 hours (74% vs. 31% in control HFD animals, $p < 0.05$). There was significantly more liver necrosis (as measured by a grading scale from 0-3) in the control HFD animals as compared to the TLR4KO HFD animals (1.4 ± 0.3 in control vs. 0.4 ± 0.2 in TLR4KO, $p < 0.05$). In addition, we see significant increases in the message level of the proinflammatory cytokines IL-6, IL-12, and IFN- γ at one hour in the control HFD animals that is abrogated dramatically in the TLR4KO HFD animals. We do not see these dramatic changes in the control animals fed a normal diet. Despite the significant increases in inflammation in the control HFD animals versus the TLR4KO HFD and normal diet control animals, we do not see changes in the TLR4 message level or endotoxin boluses, implying an increased sensitivity in the absence of an increased number of receptors. TLR4 is a critical molecule in the pathogenesis of steatotic liver ischemia/reperfusion, and represents a potential therapeutic target for expansion of the donor pool.

Abstract# 209

The Novel Role of Neutrophil Elastase in Liver Ischemia and Reperfusion Injury. Yoichiro Uchida,¹ Cecilia S. Freitas,¹ Ronald W. Busuttil,¹ Jerzy W. Kupiec-Weglinski.¹ ¹*The Dumont-UCLA Transplant Center, Department of Surgery, David Geffen School of Medicine at UCLA, Los Angeles, CA.*

Background: Neutrophils are considered crucial effector cells in the pathophysiology of organ ischemia and reperfusion injury (IRI). Particularly, neutrophil elastase (NE) accounts for a substantial portion of the neutrophil function. This study was designed to explore the role of, and mechanism by which NE exerts its function in a mouse model of liver warm IRI. **Methods:** Partial warm ischemia was produced in the left and middle hepatic lobes of C57BL/6 mice for 90 min, followed by 6-24h of reperfusion. Mice were treated with NE inhibitor (NEI; 2mg/kg p.o.; GW311616A; $n=6$) or control ($n=6$) at 60 min prior to the ischemia insult. After 6h or 24h of reperfusion, sAST/sALT levels and intrahepatic neutrophil accumulation (Myeloperoxidase [MPO] activity) were assessed. The pro-inflammatory cytokine (TNF- α , IL-6), chemokine (CXCL-1, CXCL-2, IP-10) and Toll-like receptor (TLR) 4 gene expression profiles were screened by RT-PCR. Liver samples were collected for histological grading, and detection of neutrophil infiltration by the naphtol AS-D chloroacetate esterase stains. **Results:** NEI treatment significantly reduced sAST/sALT levels, as compared with controls (17695 ± 3413 vs 10990 ± 2411 ; $p < 0.05$ / 33650 ± 3793 vs 13510 ± 6809 ; $p < 0.01$ at 6h, and 11778 ± 3113 / 14483 ± 3972 vs 3565 ± 957 / 4810 ± 1063 ; $p < 0.01$ at 24h). The expression of pro-inflammatory cytokines, and chemokines was significantly reduced in the NEI treatment group (TNF- α in 6h; $p < 0.05$, IL-6 in 6h and 24h; $p < 0.01$ and $p < 0.05$, CXCL-1 in 24h; $p < 0.01$, CXCL-2 in 6h and 24h; $p < 0.01$ and $p < 0.05$, IP-10 in 24h; $p < 0.01$). The MPO activity (U/g) was also significantly reduced following NEI treatment (14.1 ± 7.7 vs 4.0 ± 1.2 ; $p < 0.05$). TLR4 expression was selectively diminished in NEI pretreated livers (6h; $p < 0.01$). Histological examination of liver sections has revealed that unlike in controls, NEI treatment markedly reduced edema, diminished centrilobular ballooning/sinusoidal congestion, ameliorated hepatocellular necrosis, and decreased local neutrophil infiltration. **Conclusion:** The inhibition of NE ameliorated hepatocellular damage, reduced local inflammatory responses, and neutrophil activity/infiltration in a stringent mouse liver model of warm IRI. Interestingly, it also downregulated the innate TLR4 signaling. This study documents the previously unrecognized NE - TLR4 cross talk, and implies neutrophil elastase in the signal transduction pathway instrumental for liver IRI.

Abstract# 210

Lymphocyte Trafficking during Repair from Kidney Ischemia-Reperfusion: Effects of Corticosteroids and Mycophenolate Mofetil. Maria Teresa Gandolfo,¹ Patricia Agreda,² Michael T. Crow,² Mark J. Soloski,² Serena Bagnasco,¹ Hamid Rabb.² ¹*Pathology; ²Medicine, Johns Hopkins University School of Medicine, Baltimore, MD.*

Lymphocytes are involved in the early pathogenesis of ischemia-reperfusion injury (IRI) in kidney; however, their role during healing is unknown. This has direct clinical consequence since lymphocyte-targeting agents are currently administered to prevent rejection during recovery from IRI in renal transplants.

C57BL/6 mice underwent unilateral clamping of renal pedicle for 45 min, followed by reperfusion, and were sacrificed at day 10. Mice were treated with saline (C), methylprednisolone (Pred) or mycophenolate mofetil (MMF) i.p. daily from day 2 until sacrifice ($n=12$ /group). Lymphocytes were isolated from the kidneys, counted and stained with monoclonal antibodies. Kidney damage (% damaged tubules) and proliferation (Ki67 assay) were assessed. Flow cytometry analysis demonstrated increased numbers of TCR β ⁺CD4⁺ and TCR β ⁺CD8⁺ T and TCR β ⁺NK1.1⁺ NK, but not CD19⁺ B cells at day 10 in the ischemic (IR) kidneys compared to contralateral. Regulatory T cells, TCR β ⁺CD4⁺CD25⁺Foxp3⁺, and T cell subsets TCR β ⁺CD8⁺CD122⁺ and TCR β ⁺NK1.1⁺ also increased. Moderate tubular damage in cortex, severe injury in outer medulla and increased proliferation in both compartments characterized the repair phase. Pred improved histological damage in IR kidneys, while MMF worsened it. Proliferative index correlated with histology in outer medulla. Pred reduced the total counts and activation of TCR β ⁺CD4⁺ and TCR β ⁺CD8⁺ T cells in IR kidneys, and increased the percentage of TCR β ⁺CD8⁺CD122⁺ among total TCR β ⁺CD8⁺ T cells. MMF reduced all lymphocyte subsets, decreased the percentage of TCR β ⁺CD4⁺CD25⁺Foxp3⁺ among total TCR β ⁺CD4⁺ T cells, and lowered IL-10 tissue levels. IL-6 and Platelet Derived Growth Factor-BB

protein levels were also decreased in IR kidneys from MMF-treated mice.

In conclusion, specific trafficking and phenotypic changes of kidney-infiltrating lymphocytes occur during recovery from renal IRI, and lymphocyte-targeting agents, Pred and MMF, alter tubular cell structure, proliferation, and inflammatory response in the repair phase.

Abstract# 211

Small Interfering RNA (siRNA) Targeting Heme Oxygenase-1 (HO-1) Enhances Ischemia/Reperfusion-Induced Liver Apoptosis Via Activation of Caspase-3 Pathway. Bibo Ke,¹ Xiu-Da Shen,¹ Feng Gao,¹ Bo Qiao,¹ Ronald W. Busuttil,¹ Hans-Dieter Volk,² Jerzy W. Kupiec-Weglinski.¹ ¹*The Dumont-UCLA Transplant Center, David Geffen School of Medicine at UCLA, Los Angeles, CA;* ²*Institute of Medical Immunology, Humboldt University, Berlin, Germany.*

Background: HO-1 plays an important cytoprotective role in a variety of organ injury models. We have shown that HO-1 exhibits potent cytoprotective effects against liver I/R injury. This study explores the function and mechanism of HO-1 in liver I/R injury by using siRNA that suppress HO-1 expression both *in vitro* and *in vivo*. **Methods:** Using a partial liver warm ischemia model, C57BL/6 wide-type (WT) mice ($n=6$ /gr) were injected with HO-1 siRNA/nonspecific control siRNA (2 mg/kg, i.v. at day -1) or Ad-HO-1/Ad- β -gal (2.5×10^8 pfu, i.v. at day -2). Sham control WT underwent the same procedures, but without vascular occlusion. Mice were sacrificed at 6 h of reperfusion; liver tissue and blood samples were collected for future analysis. *In vitro* studies, YPEN-1 endothelium cells were transfected with HO-1 siRNA (100 nM) or Ad-HO-1/Ad- β -gal. **Results:** HO-1 siRNA treated mice showed significantly increased sGOT levels (IU/L), as compared with nonspecific control siRNA or Ad-HO-1 (7593 ± 2859 vs. 3104 ± 1777 and 211.5 ± 40 , respectively; $p < 0.05$). These correlated with histologic Suzuki's grading of liver I/R injury, with HO-1 siRNA showing significant edema, sinusoidal congestion/cytoplasmic vacuolization, and severe hepatocellular necrosis; nonspecific control siRNA showed moderate edema, sinusoidal congestion/cytoplasmic vacuolization. In contrast, Ad-HO-1 revealed only minimal sinusoidal congestion without edema or necrosis. HO-1 siRNA significantly increased local neutrophil accumulation and caspase-3 activity, and increased the frequency of apoptotic cells (31.8 ± 5.5 vs. 18.5 ± 5.4 and 3.5 ± 2.2 , respectively; $p < 0.005$), as compared with nonspecific control siRNA or Ad-HO-1. Both YPEN-1 endothelium cells and WT mice treated with HO-1 siRNA revealed markedly increased Caspase-3 activity and reduced HO-1 expression. In contrast, Ad-HO-1 significantly decreased caspase-3 activity and increased HO-1 and anti-apoptotic Bcl-2/Bcl-xL expression. **Conclusion:** This study provides evidence that HO-1 exerts cytoprotection against I/R injury by regulating liver apoptosis and inhibiting caspase-3 activation pathway. Organ specific siRNA is not only a powerful tool to study local gene function, but it may also provide novel therapeutic application in transplant recipients.

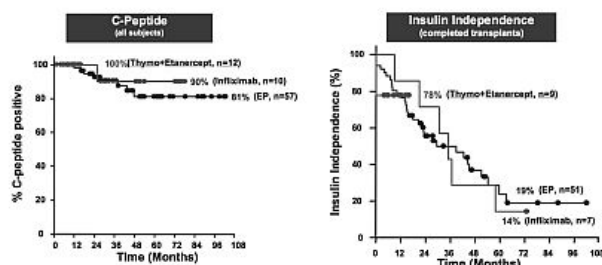
Islet Transplantation

Abstract# 212

Impact of Different Induction Therapies on Long Term Durability of Insulin Independence after Clinical Islet Transplantation. A. M. James Shapiro,¹ Angela Koh,¹ Abdul Salam,¹ Tatsuya Kin,¹ Parastoo Dinyari,¹ Andrew Malcolm,¹ Christian Toso,¹ Juliet Emamaullee,¹ Mohammed Al Qhatani,¹ Sharleen Imes,¹ Sonal Asthana,¹ Norman Kneteman,¹ David Bigam,¹ Peter Senior.¹ ¹*Clinical Islet Transplant Program, University of Alberta, Edmonton, AB, Canada.*

Background: The Edmonton Group and others have previously demonstrated evanescence of durable insulin independence at 3-5 years, with only 17% of subjects remaining free at 5 years. Hering et al reported on high rates of single donor success and 50% insulin independence at 5 years with a series of 7 protocol modifications and T-cell depletion with either thymoglobulin or hOKT3- α - α . 101 subjects have received islet infusions at a single institution in the past 8.5 years. We specifically compare long term outcomes with 3 alternative induction therapies - daclizumab alone (Edmonton Protocol, $n=57$, up to 8.5 years follow-up) vs daclizumab + infliximab ($n=10$, up to 6 years follow-up) vs thymoglobulin + etanercept ($n=12$, up to 2 years follow-up).

Results: Overall, 76% of subjects remain C-peptide positive ($n=101$), and currently 23% remain insulin-independent with good glycemic control at 8.5 years of follow-up (Kaplan-Meier, KM). C-peptide rates were 76% for daclizumab alone, 90% for daclizumab + infliximab, and 100% with thymo + etanercept ($p=NS$). Insulin independence rates for completed transplants were 19% for daclizumab alone, 14% for daclizumab + infliximab, and 78% with thymo + etanercept ($p=NS$).



Conclusions: The addition of anti-TNF alpha (infliximab) offered no additional benefit to long term outcome. Longer follow up is required to determine the true benefit of thymo + etanercept. However, early graft function is excellent in that group.

Abstract# 213

Outcomes Following Alemtuzumab Induction in Clinical Islet Transplantation. A. M. James Shapiro,¹ Angela Koh,¹ Tatsuya Kin,¹ Parastoo Dinyari,¹ Andrew Malcolm,¹ Christian Toso,¹ Juliet Emamullee,¹ Abdul Salam,¹ Sharleen Imes,¹ Sonal Asthana,¹ Mohammed Al Qhatani,¹ Norman Kneteman,¹ David Bigam,¹ Peter Senior.¹ ¹Clinical Islet Transplant Program, University of Alberta, Edmonton, AB, Canada.

Background: While the Edmonton Protocol led to improvement in outcomes of clinical islet transplantation, the need for multiple donors, high rates of side-effects from high sirolimus exposure, and inexorable loss of insulin independence over time highlight the need for innovative strategies.

Methods: Alemtuzumab (anti-CD52) was used as induction therapy in a total of 16 subjects with unstable Type 1 diabetes. Maintenance immunosuppression consisted of either sirolimus (12-15 ng/ml, n=9) or tacrolimus (8-10ng/ml) + MMF (2g/day, n=7). Alemtuzumab (30mg) was given IV 1-2 days prior to islet infusion. Outcomes and metabolic function (IVGTT, arginine and engraftment index) were compared with subjects transplanted under the original Edmonton Protocol (n=58).

Results: Profound lymphocyte depletion was achieved in all cases, with preservation of neutrophil counts. CD3 and absolute lymphocyte counts remained <20% of baseline with up to 19 months of follow-up. When sirolimus monotherapy was used as maintenance, high rates of acute rejection were seen, with only 33% of subjects remaining C-peptide positive at 5 years, compared to 78% in the Edmonton Protocol. Outcomes with alemtuzumab, tacrolimus + MMF were far superior. 100% of subjects remain C-peptide positive at 2 years of follow-up. Of the 4 subjects with completed islet infusions, all remain insulin free with up to 2 years of follow-up. In terms of metabolic function, the Engraftment Index (acute C-peptide/islet mass per kg) was 1.79±0.2 for alemtuzumab, Tac + MMF, 0.54±0.1 for alemtuzumab + sirolimus, and 0.85±0.1 for the Edmonton Protocol (p=0.01). A surprisingly high proportion of subjects (57%) achieved an entirely normal insulin response to arginine or IVGTT stimulation in the alemtuzumab, tacrolimus + MMF group, in contrast to the Edmonton Protocol (6%, p=0.002). The side effect profile for the tacrolimus-based regimen was far superior to that containing sirolimus.

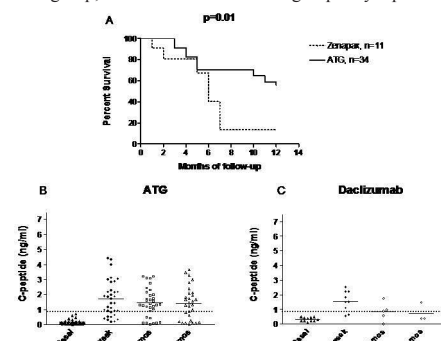
Conclusions: The short term outcomes with alemtuzumab, tacrolimus + MMF are excellent, with high rates of insulin independence and normal metabolic reserve. Graft function remains stable with up to 2 years of follow-up. It remains to be seen whether these results can be maintained in longer term follow-up.

Abstract# 214

Anti-Thymoglobulin vs. Daclizumab Induction Therapy in Kidney-Islet Transplanted Patients. Andrea Vergani, Paolo Fiorina, Alessandra Petrelli, Elena Frati, Francesca De Taddeo, Chiara Gremizzi, Paola Maffi, Antonio Secchi. ¹Transplantation Research Center, Children's Hospital/Brigham and Women's Hospital/Harvard Medical School, Boston, MA; ²Transplantation Medicine, San Raffaele Scientific Institute, Milan, Italy.

Islet cell transplantation has recently emerged as one of the most promising therapeutic approaches for diabetic patients to improve glycometabolic control. One major problem of the procedure is the requirement of an immunosuppression protocol capable of counteract both auto and allo-immune response. Recent data suggest that Anti-Thymoglobulin (ATG) can halt efficiently the mounting of an alloresponse and the recurrence of autoimmunity in NOD mice by expanding antigen specific T-regulatory cells. We retrospectively reviewed our casuistry type 1 diabetic kidney-transplanted patients who underwent islet transplantation using an immunosuppressive protocol based on ATG or Daclizumab (as induction treatment) plus cyclosporine and MMF as maintenance therapy. 45 patients underwent islet after kidney transplantation in our center. Thirty-four patients received a time course of ATG as induction (125 mg per day for 7-10 days), (number of islet infused=499,596±28,780), and 9 patients received Daclizumab at induction (1 mg/kg every 2 weeks for 10 weeks); (number of islets=486,108±70,644). No major adverse events were recorded in our center; no malignancies or infections outbreaks were evident. Patients in the ATG induction group showed a better islet survival rate compared to Daclizumab (p=0.01), according to

C-peptide>Ing/ml. A sustained and prolonged C-peptide secretion was evident in the ATG group; while in the Daclizumab group only 1 patient was functioning at 1 year.



Interestingly, in the ATG, which reached a longer follow-up, we cannot observe a loss of beta cell mass according to our metabolic test, suggesting the preservation of islet mass. In conclusion, ATG can provide a good protection towards both allo and auto immune response. The next step will be to use ATG in a calcineurin free protocol, allowing a better expansion of T-reg.

Abstract# 215

Differences in the Outcome of Islet-Alone (IA) vs Islet-after-Kidney (IAK) Transplants. James F. Markmann,¹ Shaoping Deng,¹ Chengyang Liu,² Jing Wang,² Moh-Moh Lian,¹ Yi Gu,¹ Heidi Yeh,¹ Maral Palanjian,² Eileen Markmann,² Michael Rickels,² Clyde F. Barker,² Ali Naji.² ¹Surgery, Massachusetts General Hospital, Boston, MA; ²Surgery, University of Pennsylvania, Phila., PA.

Introduction: Despite consistent achievement of insulin-independence, recent data indicate that the long term success of islets transplants using the Edmonton protocol is <10% at 5 years. The cause of the nearly universal late islet allograft failure remains unknown but hypotheses include: allo or autoimmune injury, marginal mass exhaustion, hepatic site related dysfunction, and immunosuppression toxicity. We examined the series of islet transplants performed at our institution and noted marked differences in the outcome and complications in IA and IAK groups. Our results may provide insight as to the cause of chronic islet loss.

Methods: Thirty-one islet infusions were administered to IA (n=9) and IAK (n=8) Type-1 diabetics between 9/2001-11/2007. IA and IAK had similar demographics and transplanted islet mass (13,838 vs 13,411 IEq/kg). IA received Edmonton like immunosuppression with Zenapax induction and CNI/SRL, whereas IAK patients received Zenapax and: CNI/MMF/pred(6), CNI/MMF(1), CNI/SRL(1).

Results: Insulin-independence was achieved in all but 2 patients who completed therapy (2 others withdrew). Compared with IA, IAK exhibited better glycemic control (6-month mean HbA1c 6.5 vs 6.1, stimulated C-peptide 2.3 vs 6.0), and improved islet survival; all IA eventually failed and only 1/8 were insulin free for >2-years, whereas only 3/7 IAK grafts have failed with 4 exhibiting continued robust function at >38, >38, >46, and >58 months with 3/4 fully insulin independent. In addition, all IA patients demonstrated immune sensitization post graft failure versus 0/8 IAK. All 9 IA developed mouth ulcers versus only 1/8 IAK.

Conclusions: IA and IAK exhibit striking differences in outcome and complications. The absence of mouth ulcers and lack of sensitization in IAK may relate to steroid use and continued immunosuppression for the kidney graft, respectively. The superior outcome of the IAK cohort may be a result of differences between the two groups including the use of maintenance steroids, prior exposure to Thymo or the chronically immunosuppressed state of the IAK recipient. Perhaps the most interesting correlation with outcome is the absence of the anti-proliferative agent sirolimus in the IAK group. Our results provide clues to the cause of chronic islet transplant failure and may lead to novel approaches to avoid it.

Abstract# 216

Islet Allograft Survival in Type 1 Diabetics Using a Calcineurin-Free Protocol Based on Efalizumab and Sirolimus. Andrew Posselt,¹ Greg Szot,² Lynda Frassetto,² Umesh Masharani,² Lawrence Fong,² Joan McElroy,¹ Deborah Ramos,¹ Tara Rojas,¹ Bernhard Hering,³ Flavio Vincenti,² Jeffrey Bluestone,² Peter Stock.¹ ¹Transplant Surgery, University of California, San Francisco, San Francisco, CA; ²Medicine, University of California, San Francisco, San Francisco, CA; ³Surgery, University of Minnesota, Minneapolis, MN.

Background: Although islet transplantation has become an option for treatment of type 1 diabetes, all currently used immunosuppressive protocols have significant renal and islet toxicity. We describe a novel immunosuppressive protocol using sirolimus and the anti-LFA 1 antibody efalizumab that permits prolonged islet allograft survival without the need for steroids or calcineurin inhibitors (CI).

Methods: Between February and August 2007, 4 consecutive type 1 diabetic patients with hypoglycemic unawareness and normal renal function received allogeneic

pancreatic islet transplants. Induction immunosuppression consisted of 2 doses of thymoglobulin given on pre-transplant days -2 and -1, efalizumab (5mg/kg sq/week starting on d -1), and sirolimus. Maintenance immunosuppression consisted of sirolimus and efalizumab.

Results: All patients achieved insulin independence after single islet infusions (mean IEQ/kg=8,905). Three of 4 remain insulin independent 4 or more months after transplant (table 1). Patient 1 resumed low dose insulin (approximately 15% of original dose) 6 weeks after transplant and is awaiting a second islet infusion. Her blood glucose control is markedly improved and she has not experienced any hypoglycemic episodes. All patients show persistent c-peptide secretion and have stable renal function. Side effects due to efalizumab were limited to transient irritation at the injection site.

table 1				
Patient	1	2	3	4
# of Islet Infusions	1	1	1	1
IEQ	574,950	482,500	630,165	459,290
IEQ/kg	8,711	8,041	11,458	7,408
C-peptide at 4 mos	Yes	Yes	Yes	Yes
Duration of Insulin Independence	1.5mos	>6mos	>5mos	>4mos
Currently on Insulin	Yes (3-5U/d)	No	No	No
HbA1c decrease>2.5%	No	Yes	Yes	Yes

Conclusions: Thymoglobulin induction followed by sirolimus and efalizumab maintenance is well tolerated and allows prolonged islet allograft survival. This protocol is the first CI/steroid free islet regimen resulting in insulin independence with a single donor islet infusion. By eliminating CI, this protocol minimizes renal and islet toxicity and may thus improve long-term islet survival and function.

Abstract# 217

Long Term Insulin Independence and Restoration of First Phase Insulin Release after Supplemental Islet Infusion under Exenatide Treatment in Subjects with Islet Graft Dysfunction. Raquel N. Faradji,¹ Thipaporn Tharavanij,¹ Tatiana Froud,¹ Shari Messinger,¹ Antonello Pileggi,¹ David A. Baidal,¹ Davide Mineo,¹ Cristiane Leitao,¹ Pablo Cure,¹ Aleida Saenz,¹ Andrea Curry,¹ Lourdes Dinkins,¹ Gennaro Selvaggi,¹ Camillo Ricordi,¹ Rodolfo Alejandro.¹ ¹Diabetes Research Institute, University of Miami, Miami, FL. **Objective:** To assess the effects of exenatide on supplemental islet infusions (SI).

Methods: Five subjects with islet graft dysfunction underwent SI under Edmonton-like protocol in 2003 without exenatide (SI-C group) and 4 with exenatide in 2006 (SI-EXN group). Clinical and metabolic profiles were assessed every 3 months for 18 months.

Results: SI-EXN group was on this drug for median time of 171 days pre-SI. Both groups were similar except for duration from post-completion to graft dysfunction (648±34 vs 221±58 in SI-EXN and SI-C group respectively, p<0.05) and duration of graft dysfunction before SI (664±83 vs 326±93, p=0.03). SI-C and SI-EXN groups received mean of 8713±2123 and 5613±485 IEQ/kg, respectively (NS). Only 3/5 of SI-C patients achieved insulin independence for 303, 403 and >1670 days after SI. All subjects in SI-EXN group achieved insulin independence for more than 443, 621, 641, 654 days. At 18 months insulin independence was 20% in SI-C and 100% in SI-EXN group. Comparing pre and post-SI, SI-EXN group had significantly lower A1C at 3-18 months and lower AUC glucagon at 6 and 15 months (p<0.05). AUC C-peptide in SI-EXN group was significantly higher than SI-C at 3 and 9 months. Intravenous glucose tolerance test showed significantly increased acute insulin responses to glucose at 3-18 months in SI-EXN and at 3 and 6 months in SI-C group. SI-EXN group had more acute C-peptide response to glucose than SI-C at 3 and 6 month (p<0.05). Acute exenatide administration during intravenous glucose tolerance test at 15 month in SI-EXN revealed significantly increased acute insulin and c-peptide response to glucose which indicates improved first phase insulin release.

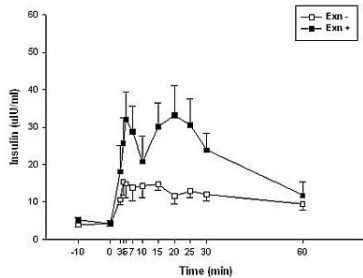


Figure 1: Insulin levels in SI-EXN group after intravenous glucose tolerance test with (Exn+) and without exenatide (Exn-) administration before the test at 15th month post-SI.

Conclusion: Supplemental islet infusions under exenatide lead to insulin independence, restore first phase insulin secretion and result in long term insulin independence.

Abstract# 218

Exenatide and Etanercept in De Novo Islet Transplantation for Type 1 Diabetes. Payam Salehi,¹ Antonio Gangemi,¹ Betul Hatipoglu,² Joan Martellotto,¹ Barbara Barbaro,¹ Joseph Kuechle,¹ Meirigeng Qi,¹ Yong Wang,¹ Pablo Pallan,¹ Charles Owens,³ James Bui,³ Derek West,³ Bruce Kaplan,¹ Enrico Benedetti,¹ Jose Oberholzer.¹ ¹Surgery, University of Illinois at Chicago, Chicago, IL; ²Medicine, University of Illinois at Chicago; ³Radiology, University of Illinois at Chicago.

This prospective phase 1/2 trial aimed to demonstrate safety and reproducibility of allogeneic islet transplantation (Tx) in type 1 diabetic (T1DM) patients and implement a strategy to achieve and maintain insulin-independence with minimal islets.

Ten C-peptide negative T1DM subjects with hypoglycemic unawareness received 1-3 intraportal allogeneic islet Tx. Four subjects (Group 1) received the Edmonton immunosuppression regimen (daclizumab, sirolimus, tacrolimus). The next 6 subjects (Group 2) received etanercept, exenatide and the Edmonton regimen. We followed all subjects for 15 months after the first Tx. The primary efficacy end point was insulin independence. Secondary endpoints were HbA1c, fructosamine, OGTT, mixed meal test, glucagon stimulation test, IVGTT and hypoglycemia. To study the effect of exenatide, we compared frequently sampled IVGTT, C-peptide, proinsulin, amylin and glucagon with and without exenatide.

Two self-limiting bleeds occurred in 18 infusions. All subjects became insulin independent. Group 1 received a mean total number of islets (EIN) of 1,460,080±418,330 in 2 (n=2) or 3 (n=2) Tx, whereas Group 2 became insulin independent after 1 Tx (537,495±190,968 EIN, p=0.028). All Group 1 subjects remained insulin free through the 15-month follow-up. Two Group 2 subjects resumed insulin: one after immunosuppression reduction during an infectious complication, the other with severe gastroparesis and exenatide intolerance. HbA1c reached normal range in both groups (6.5±0.6 at baseline to 5.6±0.5 after 2-3 Tx in Group 1 vs. 7.8±1.1 to 5.8±0.3 after 1 Tx in Group 2). Baseline HbA1c was significantly higher in Group 2 than Group 1 (p=0.046). Pre- and post-Tx HYPO scores were 841.8±1217.9 and 0 in group 1 vs 812.8±941.7 and 33.5±50.0 in Group 2. Glucagon levels decreased significantly in all subjects. In Group 2, the decrease in glucagon levels after challenge tests with and without exenatide was 18.7 fold more significant after exenatide in all subjects (Δ change 8.9±18.4 vs. 165.7±46.4 w/o and with exenatide, respectively, P=0.04).

Combined treatment of etanercept and exenatide improves islet graft function and facilitates achievement of insulin-independence with minimal islets.

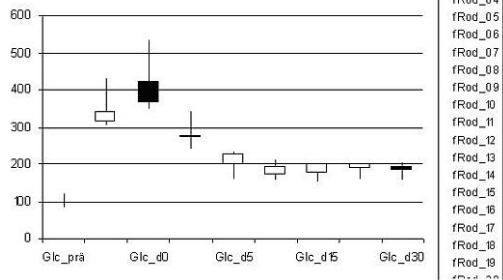
Abstract# 219

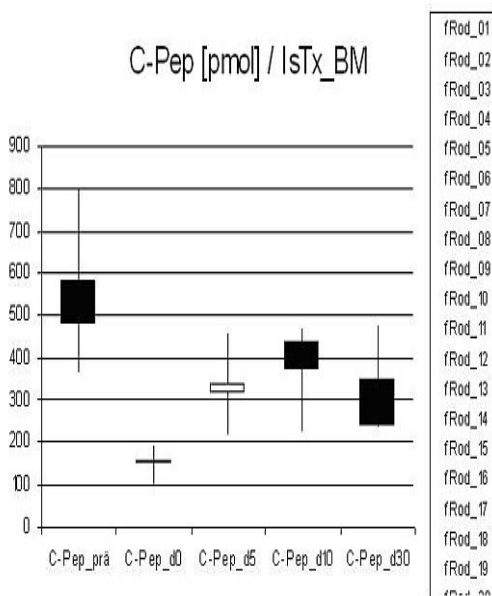
Efficacy of Islet Cell Transplantation into Bone Marrow (Os Femoris) Compared to Portal Liver Engraftment and Full Organ Transplantation. Guido Junge,¹ R. Duschka,¹ S. Kohler,¹ J. Klupp,¹ J. Pratschke,¹ P. Neuhaus,¹ L. Schewior.² ¹Dept of Transplant Surgery, Charite; ²Dept of Nephrology, Charite.

Background IsTx has been investigated as treatment for type 1 DM. However, the hope this approach would result in long-term freedom from exogenous insulin has failed in practice. Techniques for isolating islets have advanced and with availability of new IS agents, strategies can now be developed specifically for IsTx that will provide greater immunologic protection w/o diabetogenic side effects. Rejection/vascularization still remain major limitations for success of IsTx. We hypothesize that BM is an accessible, immunologic privileged space with natural well-developed vasculature and may be a suitable site for IsTx. **Method** Wistar rats were used as donors/recipients. DM was induced by iv-streptozocin. Rats who had morning Glc levels > 300 mg/dl on two separate occasions were used as recipients. PTx was performed as normal rodent standard. Islets were isolated from pancreas by distending pancreatic duct with Liberase. Islets were separated on a discontinuous histopaque density gradient, further purified, counted, divided into aliquots transplanted into different sites (liver, BM). BW, Glc and C-Pep were measured in all groups before/after Tx. **Results** PTx; IsTx_liver; IsTx_BM

Evolution of Glc [mg/dl] after PTx, IsTx_liver and IsTx_BM							
group	Glc_preTx	Glc_d2	Glc_d5	Glc_d10	Glc_d15	Glc_d20	Glc_d30
PTx	413	195	144	128	161	191	204
IsTx_liver	429	273	146	135	115	112	109
IsTx_BM	424	280	205	181	175	180	177

Blood Glc [mg/dl] / IsTx_BM





Discussion IsTx into BM opens new avenues in treatment of DM. This experimental model showed functionality of transplanted β -cells in a diabetic animal-model. Despite the fact of an insufficient number of islet cells it could be shown that engraftment/functionality are at least comparable to portal engraftment and full organ Tx.

Abstract# 220

Successful Clinical Islet Isolation and Purification Using a Bovine Product-Free GMP Collagenase and Neutral Protease. Gregory Szot,¹ Michael Lee,¹ Jiena Lang,¹ Pavel Koudria,¹ Jeffrey Bluestone,¹ Peter Stock,² Andrew Posselt.² ¹Diabetes Center; ²Department of Surgery, University of California San Francisco, San Francisco, CA.

Background: In February 2007, the islet community was notified of a possible bovine product contamination in the collagenase enzyme (Liberase HI) used for human islet isolations. To eliminate the potential hazard of bovine spongiform encephalopathy, we successfully adapted our human islet processing procedure to utilize a different GMP Collagenase and Neutral Protease (Nordmark/Serva). Here we describe what we consider the most important factors for achieving reproducible and clinically useable islet isolations.

Methods/Results: A standard isolation protocol involving controlled enzymatic digestion followed by density gradient purification was used. Seventeen donor pancreata were processed and ten were ultimately used for clinical transplantation. Eight of these successful isolations were performed using the Nordmark/Serva Enzymes (table 1). The following factors were identified as being important for ensuring successful islet yields: 1) **Donor Age and Size:** Male donors 17-45 years old who were tall (>180cm) and heavy (>100kg), rather than all patients with high BMIs. 2) **Cold Ischemia Time and Preparation:** Cold ischemia time < 9hrs; transport in cold UW solution; immediate trimming of organ upon receipt by an experienced surgeon. 3) **Enzyme perfusion:** Perfusion with cold enzyme solution (1600 Wunsch Units Collagenase and 200 Units Neutral Protease) containing 11mM CaCl₂ for 10-12 minutes while increasing pressure (80 psi for 4 min., then 180 psi for 6-8 min). 4) **Digestion/Dilution:** Addition of DNase (Pulmozyme) to digestate and digestion at 37°C in a 600cc Ricordi chamber; dilution of digestate into RPMI containing 5.0% human serum albumin (HSA) at 4°C while keeping chamber temperature at 30°C for the entire dilution; immediate centrifugation and pooling into a dilution solution with 0.625% HSA at 4°C.

Conclusions: Incorporation of several important modifications into our existing islet isolation protocol has allowed us to routinely obtain high quality islet isolations using an alternative, bovine product-free GMP enzyme.

Table 1. Islet Isolation Summary

	Transplanted IEQ (mean $\pm$ SD)	%Viability (mean $\pm$ SD)	Glucose Stimulation Index (mean $\pm$ SD)
Nordmark/Serva (n=8)	514,510 $\pm$ 140,606	98.05 $\pm$ 2%	3.20 $\pm$ 2.54
Roche Liberase HI (n=9)	508,883 $\pm$ 115,815	99.63 $\pm$ 0%	4.04 $\pm$ 5.2

Kidney - Acute Rejection: Immunosuppressive Agents and Registry Data

Abstract# 221

Rejection Characteristics of 200 Living Donor Kidney Transplants Using Alemtuzumab Induction and Tacrolimus Monotherapy: 3 Year Follow-Up. Henkie P. Tan,¹ Joseph Donaldson,¹ Amit Basu,¹ Claire Morgan,¹ Mark Unruh,¹ Jerry McCauley,¹ Christine Wu,¹ Nirav Shah,¹ Amadeo Marcos,¹ Parmjeet Randhawa,¹ Thomas E. Starzl,¹ Fadi Lakkis,¹ Adriana Zeevi,¹ Ron Shapiro.¹ ¹UPMC.

Living donor kidney transplantation was performed under a regimen of recipient pretreatment with Alemtuzumab and tacrolimus monotherapy with subsequent weaning.

Methods: We performed 200 consecutive unselected living donor kidney transplants (donor kidneys were removed laparoscopically) from 03/14/2003 to 12/12/2005 using 30 mg (or 0.5mg/kg) alemtuzumab and tacrolimus monotherapy. At 6 months post-transplant and every 2 to 6 months interval, we used clinical data (including ELISA antibody titers, Cylex T-cell activation assay, and identification of donor specific antibodies) to wean tacrolimus when possible (bid $\rightarrow$ qd $\rightarrow$ qod $\rightarrow$ tiw $\rightarrow$ biw $\rightarrow$ qwk). The recipients included 4 HIV+, 17 pediatric patients, and 25 re-transplants. The mean follow up was 35.7 $\pm$ 9.9 months.

Results: Actuarial recipient survivals at 1-, 2-, 3-years were 99.0%, 96.4%, and 93.3%, respectively. Graft survivals at 1-, 2-, 3-years were 98.0%, 90.8%, and 86.3%, respectively. The mean creatinine (mg/dL) at 1-, 2-, 3-years were 1.4 $\pm$ 0.5, 1.6 $\pm$ 1.2, 1.5 $\pm$ 0.7, respectively. The mean GFR (mL/min/1.73m²) at 1-, 2-, 3-years were 74.1 $\pm$ 29.3, 73.2 $\pm$ 28.6, and 71.6 $\pm$ 31.7, respectively. The cumulative incidence of acute cellular rejection (ACR) at 6, 12, 18, 24, 30, 36, 42, and >42 months were 2.0%, 9.0%, 16.5%, 19.5%, 23.5%, 24.0%, 25.0%, and 25.0%, respectively. 60.1% of recipients had been weaned to spaced-dose (qod or less) tacrolimus monotherapy. The preweaning incidence of ACR was 7.0%, and the postweaning incidence was 23.5%. A total of 50 recipients had ACR, with a total of 89 ACR episodes. Most (88.7%, 79/89) episodes of ACR were Banff 1 and were sensitive to steroid pulses. At least eight had antibody-mediated rejection (with donor specific antibody and C4d) and received IVIg and/or plasmapheresis. 13 patients were treated with alemtuzumab with resolution/improvement of ACR. 94.4% of recipients are still completely steroid-free on tacrolimus monotherapy from the time of transplantation. There was no DGF or CMV disease, and the incidence of post-transplant insulin dependent diabetes mellitus was 0.5%.

Conclusion: This report represents the largest series to date with the longest mean follow-up (3 years) of living donor kidney transplantations under alemtuzumab preconditioning with tacrolimus monotherapy, and confirms the 3-year safety and efficacy of this approach.

Abstract# 222

Antibody-Mediated Rejection (AMR) in Renal Allograft Recipients Treated with Alemtuzumab Induction. J. Pascual, J. Pirsch, J. Torrealba, A. Djamali, G. Levenson, N. Singh, J. Odorico, S. Knechtle, H. Sollinger, M. Samaniego. *University of Wisconsin, Madison, WI.*

Alemtuzumab (Alem) is an effective depletion agent used in induction of kidney transplants (KT). Prevalence, risk factors (RF) and impact of AMR in Alem-treated patients (Pts) are not well known. From 2002-2005, 829 adult KT alone were performed at our institution. Alem induction was used in 613 of these Pts (74%) (602 were CNI-based at the time of discharge). In addition to MMF and steroids, 481 Pts received CNI-free immunosuppression (immuno) [CsA (n=294) or TAC (n=187)], and in 121 Pts immuno was CNI-free. TAC-treated Pts were younger, more sensitized and more frequently re-KT. DGF was more frequent in Pts receiving CNI-free immuno. As a group, these Pts were older, more frequently received a KT from a donor after cardiac death and less frequently from a living donor.

Acute cellular rejection (ACR) was diagnosed in 41 Pts (6.8%), 13 occurred before day 90th (E-ACR), and 28 after this date (L-ACR). C4d-positive AMR was diagnosed in 128 Pts (21.2%), of which 58 were early (E-AMR) and 70 were late episodes (L-AMR). Donor age (HR 1.02, p=0.001), recipient age (HR 0.97, p=0.001), peak PRA (HR 1.008 per each 1% increase of PRA, p=0.06), and full mismatch at the B-locus (HR 1.57, p=0.02) were significant RF for AMR in the multivariate analysis. Peak PRA (HR 1.017, p=0.001), pregnancies (HR 1.13, p=0.01) and CNI-free immuno were RF for E-AMR, while donor age (HR 1.032, p=0.001), recipient age (0.96, p=0.001), peak PRA (HR 1.017, p=0.001), CMV D+R- status (HR 1.77, p=0.03) and time on dialysis (in months, HR 1.009, p=0.02) were significant RF for L-AMR.

Post-rejection graft survival was similarly affected by all types of AR (Kaplan-Meier, all p<0.001 vs. non-rejectors (NR)). RF for graft loss in the multivariate analysis were pre-KT diabetes (HR 1.61, p=0.01), ACR (HR 3.90, p<0.0001) and AMR (HR 3.83, p<0.0001). Post-rejection Pt survival was more affected by AMR (Kaplan Meier, E-AMR p=0.001, L-AMR p=0.01, E-ACR p=0.03, L-ACR p=0.90, all vs. NR). RF for post-rejection death in the multivariate analysis were recipient age (HR 1.06, p<0.001), pre-KT diabetes (HR 2.39, p<0.001), time on dialysis (in months, HR 1.009, p=0.006) and AMR (HR 1.74, p=0.006).

AMR is very frequent (more than ACR) in Alem treated KT recipients, and the reasons are not well-known. In Alem induced Pts, CNI-free immuno is associated to E-AMR episodes, whereas young recipients receiving old kidneys are predisposed to late forms.

Abstract# 223

Rates of Acute Rejection (AR) and Treatment Outcomes in Highly-HLA Sensitized Patients (HS) Transplanted after Desensitization with IVIG + Rituximab. Richard Lee,¹ Alice Peng,¹ Rafael Villicana,¹ Ashley Vo,¹ Donald Dafoe,¹ Robert Chang,¹ Miekko Toyoda,¹ Nancy Reinsmoen,¹ Stanley Jordan.¹ ¹*Comprehensive Transplant Center, Cedars Sinai Medical Center, Los Angeles, CA.*

Background: An increasing number of HS patients are being transplanted using desensitization protocols. These patients are considered high risk for AR, particularly antibody mediated rejection (AMR). Here we examined our AR rates and treatment outcomes for our HS kidney transplant (KT) recipients using our most current desensitization strategies. **Methods:** Between June 2004 (when rituximab was introduced to our desensitization and treatment protocols) to July 2007, 130 HS KT patients were transplanted using combinations of high-dose IVIG, rituximab, and plasmapheresis (PP) for desensitization. We examined the overall AR, C4d- cell-mediated (CMR), and C4d+AMR rates. AMR episodes were treated with steroids, high-dose IVIG, and rituximab. Refractory and rapidly progressive AMR was treated with PP. Treatment outcomes and differences in AR rates between deceased (DD) and living donors (LD) was examined. **Results:** Of 130 HS pts, there was an overall 23.8% AR rate, with 14.6% (19/130) AMR and 14.6% (19/130) CMR. There were 4 graft losses in those with AMR (graft survival 79% in AMR pts) and 9 total graft losses (graft survival 93% overall). The majority of pts were treated with HD IVIG + rituximab. PP was additionally performed in 6 AMR pts, 3 of whom lost their grafts. In the 66 HS pts who underwent DD KT, the overall AR rate was 34.8%, 12 had AMR (18.1%) and 14 had CMR (21.2%). The remaining 64 pts who underwent LD KT had an overall 18.8% AR rate with (12.5%) AMR and (7.8%) CMR. HS DD KT pts had significantly higher overall AR (p=0.04) and CMR (p=0.04) rates. There was no significant difference in AMR rates. The graft survival at 12M for DD & LD recipients was 91/95%, respectively. Overall, AMR pts with functioning grafts at 6M and 12M had a SCr of 1.5 ± 0.6 mg/dl and 1.5 ± 0.4 mg/dl respectively. In those with CMR, the average SCr at 6M and 12M was 1.8 ± 0.8 mg/dl and 1.6 ± 0.7 mg/dl respectively. **Conclusions:** HS KT pts have a higher overall AR rate, particularly AMR. 79% of pts who developed AMR responded to treatment with IVIG + rituximab compared to 40% prior to our use of rituximab (p<0.03). HS DD KT recipients had a higher incidence of CMR but no significant difference in AMR rates compared to HS LD pts. Despite higher AR rates in HS pts., the 6M and 12M graft survival is comparable to UNOS outcomes.

Abstract# 224

Low-Dose Rituximab To Salvage Renal Transplants from Refractory Antibody-Mediated Rejection. William R. Mulley,¹ Brian D. Tait,² Fiona Hudson,² Alison M. Skene,³ John P. Dowling,³ Peter G. Kerr,¹ John Kanellis.¹ ¹*Department of Nephrology, Monash Medical Centre, Clayton, Victoria, Australia;* ²*Victorian Transplantation and Immunogenetics Service, Parkville, Victoria, Australia;* ³*Department of Pathology, Monash Medical Centre, Clayton, Victoria, Australia.*

Recent reports suggest that treatment with the monoclonal anti-CD20 antibody, Rituximab (Rtx) may improve renal graft survival in antibody mediated rejection (AMR); however optimal dosing for this indication is unknown. We examined the efficacy of a single low dose (500mg) of Rtx for treatment of refractory AMR in order to limit significant infective complications associated with conventional dosing regimens (375mg/m²). Rtx was used in seven consecutive patients who had refractory AMR as judged by ongoing biopsy evidence of AMR after 4 weeks of standard therapy consisting of pulse methylprednisolone and plasma exchange with low dose IVIg (100mg/kg) (PE/IVIg). All patients received Tacrolimus and Mycophenolate Mofetil. AMR was defined as 1) characteristic histology on biopsy (Banff criteria), 2) graft dysfunction and 3) presence of a donor specific anti-HLA antibody (DSA). B-cell counts (CD19) and serum creatinine (SCr) were monitored. PE/IVIg was ceased in all cases after Rtx dosing. The average follow-up since Rtx dosing is 16.8 months (range 5.3-28.9 mths). All patients still have functioning grafts (100% 12 month graft and patient survival), with current mean SCr levels (156 ± 17 μ mol/L) significantly lower than mean peak rejection levels (514 ± 355 μ mol/L) p=0.05. CD19 counts fell to zero and remained <5x10⁶/L for >6 months in all patients. DSAs remain detectable by Luminex Flow Beads in all patients despite stabilization of SCr. Two of 7 patients developed an infective complication post Rtx dosing. One patient developed bacterial pneumonia requiring hospital admission whilst a second patient developed CMV viraemia and later BK nephropathy which has not led to significant graft dysfunction. Hence, infectious complications are far less than those reported for multiple standard dose regimens in similar patient groups. Large clinical trials are required to confirm the efficacy of Rtx in treating AMR as well as optimal dosing. Standard dosing is based on oncology treatment regimens which are likely to be excessive for AMR in patients already markedly immunosuppressed. Our data suggests single low-dose Rtx for refractory AMR results in excellent patient and graft survival and leads to low rates of serious infective complications in the short-term.

Abstract# 225

Renal Allograft Outcome among Recipients of Positive vs Negative Crossmatch Combined Liver-Kidney Transplantation: UNOS Data Analysis. Ravi Parasuraman,¹ K. K. Venkat,¹ Anita Patel,¹ Dilip Moonka,² Jason Denny,² Atsushi Yoshida,² Lauren Malinzak,² Kimberly Brown,² Marwan Abouljoud,² Dean Kim.² ¹*Division of Nephrology, Henry Ford Hospital;* ²*Transplant Institute, Henry Ford Hospital, Detroit, MI.*

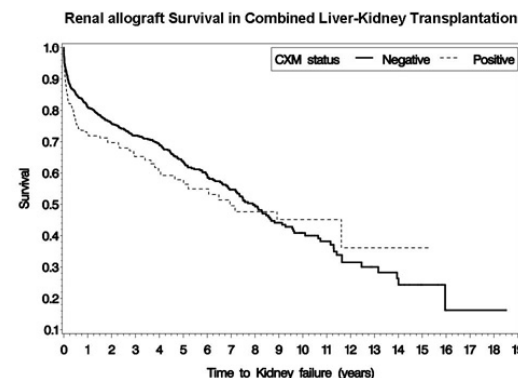
Successful combined liver kidney transplantation (CLKT) despite pre-transplant positive cross match (+ CXM) has been reported implying immunoprotective effect of liver allograft (LA) on renal allograft (RA). However, severe acute humoral rejection with RA loss has been documented suggesting LA may not always protect RA.

AIM: To examine the effects of pre-transplant + CXM on RA outcome in CLKT.

Method: A total of 1401 CLKT recipients for whom CXM data available were identified from UNOS data base from 1986–2006. Of these, 242 (17.3%) had +CXM and data was analyzed for acute rejection rate and RA outcome. The significant results are summarized below.

Variable	Negative CXM N = 1159	Positive CXM N = 242	p-value
Recipient: Female	374(32%)	136 (56%)	0.001
% Class I PRA	25.9±26.5	66.3±40.5	0.001
% Class II PRA	31.0±29.3	56.4±36.3	0.013
treated for rejection	69/606 (11%)	24/150 (16%)	0.026
Length of stay: Admission to discharge	Days 28.8±33.2(N=838)	Days 37.3±46.0(N=155)	0.028

Results: The demographic and clinical data were similar between +CXM and Negative CXM groups. The rejection rate was significantly higher and the length of stay was significantly longer in +CXM group as shown in the table. The RA survival rates were 8%, 7% and 6% lower at 1, 3 and 5 years post transplant respectively among +CXM recipients. However, this did not reach significance mostly due to small sample size (p=0.11). The inferior RA outcome is seen during the initial few years after transplantation that disappears after 9 years.



Conclusion: In CLKT, pre-transplant +CXM can result in higher RA rejection rate and longer length of stay in hospital. LA may not always confer immunological protection to RA in +CXM CLKT and its true burden may be under recognized since CXM is not routinely performed in all CLKT. Study of antibody specificities or LA volume to determine the effect of +CXM on RA outcome is essential.

Abstract# 226

Simultaneous Kidney Pancreas Transplantation in Patients with Positive CDC B-Cell and/or Flow Cytometry Crossmatch. R. Heilman,¹ A. Moss,¹ M. Mazur,¹ H. Chakkerla,¹ K. Mekeel,¹ D. Post,¹ D. Mulligan,¹ K. S. Reddy.¹ ¹*Divisions of Transplant Surgery and Medicine, Mayo Clinic Arizona, Phoenix, AZ.*

We reviewed our experience with simultaneous kidney pancreas transplantation (SKPT) in patients with positive CDC B-cell and/or flow cytometry crossmatch and compared to SKPT recipients with negative crossmatch performed during a concurrent period.

We performed 64 SKPTs from Jan 2005 to Nov 2007, 12 in patients with positive CDC B-cell and/or flow cytometry crossmatch and 52 in patients with negative crossmatch. Patients with positive crossmatch received induction with low dose IVIG (100 mg/kg x 3 doses), r-ATG (1.5 mg/kg x 4 doses) or Alemtuzumab (30 mg single dose) and maintenance with tacrolimus, MMF and steroids while negative crossmatch recipients received r-ATG or Alemtuzumab and maintenance with tacrolimus, MMF and rapid steroid taper (discontinued on day 4). One patient in the positive crossmatch group also received high dose IVIG as part of desensitization protocol. Protocol biopsies were performed at 1, 4 and 12 months and every year thereafter. Acute cellular rejection was treated with solumedrol or r-ATG depending upon the severity of rejection and antibody mediated rejection (AMR) episodes were treated with solumedrol, plasmapheresis, IVIG and rituximab.

Transplant characteristics, incidence of acute rejection, infection rates, graft and patient survival rates are shown in the table.

	positive crossmatch SKPT (n=12)	Negative crossmatch SKPT (n=52)	p value
Previous transplant	42%	2%	0.0006
PRA 0%	8%	94%	0.0001
PRA >80%	42%	0%	0.0001
Biopsy confirmed clinical acute rejection	42%	10%	0.015
Subclinical rejection (protocol biopsy)	8%	6%	NS
Antibody mediated rejection	42%	0%	0.0001
CMV infection	8%	17%	NS
BK nephropathy	0%	4%	NS
Kidney graft survival	83%	96%	NS
Pancreas graft survival	67%	92%	0.03
Patient survival	92%	100%	NS
Mean followup in months (median)	20 ± 11 (18)	17 ± 10 (16)	NS

SKPT in patients with positive CDC B-cell and/or flow cytometry crossmatch is associated with high AMR rate despite low dose IVIG and r-ATG/Alemtuzumab induction. Pancreas graft survival is inferior in patients with positive crossmatch while kidney graft and patient survival are similar to that of negative crossmatch recipients. Majority of AMR can be reversed with treatment but further long-term follow-up is needed to determine the impact on graft survival.

Abstract# 227

Ten-Year Follow-Up of the First Multicenter, Randomized, Kidney Transplant Study Shows Better Graft Function in Tacrolimus-Treated Patients. José M. Morales,¹ Y. Vanrenterghem,² J. P. Squifflet,² B. Suwelack,² F. Pietruck,² L. Wennberg,² S. Ball,² F. Mühlbacher,² J. P. Van Hoof,² M. Fischereder,² C. Short.² ¹H. 12 de Octubre, Madrid, Spain; ²For the European Tacrolimus Study Group.

The first European Phase III trial with tacrolimus in the early 90's clearly showed advantages for tacrolimus in terms of acute rejection (AR) vs the original cyclosporin formulation. However, no advantages were seen in survival rates. The present investigator-initiated, observational follow-up study collected data on patients included in the original cohort of 448 patients who participated in this large study and who were randomised to a triple immunosuppressive regimen consisting of tacrolimus, azathioprine and steroids (TAC/AZA/STER, n=303) or cyclosporine, AZA and steroids (CSA/AZA/STER, n=145). Efficacy and safety parameters assessed at follow-up included: acute rejection; patient and graft survival; renal function, vital signs, basic lab results and immunosuppressive regimen for the patients 10 years after completion of the original study.

Results: Currently, data are available from 75% of the patients. All assessments were conducted following the intent-to-treat principles. More patients in the TAC than in the CSA group remained on the randomised treatment. While Kaplan-Meier (K-M) estimates for patient survival at year 11 show comparable (72% TAC vs 72%CSA) results, K-M estimates for graft survival demonstrate an advantage for the tacrolimus cohort (51% TAC vs 46% CSA). Graft half-life estimates (Gjertson and Terasaki method) yielded overall results of 14.2 years (TAC) and 11.4 years (CS). In both treatment groups, AR was associated with inferior long-term results. For patients with AR, half-life estimates were 11.1 and 7.8 years for TAC and CSA, respectively, while in patients without AR half-lives were 19.2 (TAC) and 15.8 (CS) years. Mean serum creatinine after 10 years was significantly lower in the TAC cohort vs the CSA group (1.65mg/dL TAC vs 2.01mg/dL CSA, p<0.05). Mean glomerular filtration rates (MDRD4 estimate) were 48.7 mL/min in TAC and 41.9 mL/min in CS. After ten years of follow-up, the mean (C-0) levels were 7.6 mg/mL (TAC) and 120 mg/mL (CS).

Conclusion: Analysis of this long term follow-up of a large study confirms the benefits of a tacrolimus-based therapy. It also confirms the concept that freedom from early acute rejection is associated with superior long term results in renal function. Therefore, long term renal allograft function is remarkably good in TAC patients.

Abstract# 228

Cyclophosphamide a Novel Therapy for IVIG/PLEX-Resistant Antibody-Mediated Rejection. Wael Habhab,¹ Yeys Caumartin,¹ Andrew House,¹ Norman Muirhead,¹ Faisal Rehman,¹ Patrick Luke,¹ Vivian McAlister,¹ Madelaine Moussa,¹ Edward Ball,¹ Anthony M. Jevnikar.¹ ¹Nephrology, University of Western Ontario, London, ON, Canada.

Introduction: Despite advances in immunotherapy, acute antibody-mediated rejection (AMR) poses a significant risk to graft survival. Standard treatment (ST) of AMR currently consists of plasma exchange (PLEX) and intravenous immunoglobulin (IVIG) infusion. Since neutrophils and antibody-producing plasma cells contribute to AMR, cyclophosphamide (CYP) has been used in our centre to treat ST-failures. To our knowledge, we present the first description of salvage CYP for AMR.

Methods: From January 2001 to July 2007, 23 of 450 transplants performed at our center developed biopsy-proven C4d+ AMR treated with ST. Seven patients were refractory to ST as defined by persistent biopsy-proven rejection (persistence of glomerular and interstitial neutrophils infiltrate, C4d+) and no clinical improvement within 10 days of ST. These patients received 1 to 3 doses of CYP iv (500 mg). We compared functional outcomes achieved in CYP-treated patients with ST-responders. Mean follow-up was 20±15 and 30±25 mo in CYP and ST groups, respectively.

Results: All patients were transplanted through a negative preoperative AHG-CDC T cell cross-match. Patients salvaged with CYP had higher pre-transplant immunological risk as demonstrated by a mean PRA of 85±25% (vs. 15±25% in ST group) in association

with retransplantation (100 vs 50%). All the CYP-treated patients received prophylactic peritransplant PLEX/IVIG compared to 38% for the ST group. Patients salvaged with CYP received more IVIG (4.8±1.5 vs 2.8±2 g/kg, p=0.02) and PLEX (19±5 vs 10±7, p=0.003) in comparison to ST-treated patients. In the CYP-treated group, sCr following ST did not initially improve (346±256 µmol/L at diagnosis vs. 337±171 µmol/L one day before CYP). However, CYP administration was effective in treating refractory AMR as demonstrated by 6 month sCr (153±41 µmol/L, p=0.02 vs. pre-treatment level) and not significantly different than sCr of ST responders (149±46 µmol/L, p=NS). One-year sCr and graft survival, following the AMR episodes, were equivalent between the two groups as well. Importantly, CYP was well tolerated with no serious adverse effects.

Conclusions: For the first time, we have shown that CYP is capable of salvaging ST-resistant AMR with excellent functional outcomes and minimal morbidity. Its eventual role in relation with anti-CD20 therapy and splenectomy for ST-resistant AMR remains to be determined.

Abstract# 229

Influence of ABO-Compatible Transplantation on Long-Term Outcome in Cardiac Transplant Recipients. Arezu Z. Aliabadi,¹ Daniela Dunkler,¹ Stephane L. Mahr,¹ Matthias Paireder,¹ Martina Groemmer,¹ Gernot Seebacher,¹ Daniel Zimpfer,¹ Ernst Wolner,¹ Michael Grimm,¹ Alfred Kocher,¹ Andreas O. Zuckermann.¹ ¹Dept. of Cardiothoracic Surgery, Medical University of Vienna, Vienna, Austria.

Background: Cardiac transplantation is best therapeutic option for end-stage heart disease. Previous reports on the influence of the ABO system on cardiac transplant outcome indicate that blood group identical transplants have a better outcome than blood group compatible transplants. As the demand for donor organs largely exceeds the supply, it is our policy to use blood group compatible local donors for transplantation if no suitable ABO identical recipient is available. The aim of this study was to determine outcome after blood group compatible transplants.

Patients & Methods: Between 1984-2003 a total of 915 cardiac transplants have been performed at our center. Median follow-up was 127 months. Patients were analyzed according blood group matching (AB0 identical (I) vs. AB0 compatible(C)). Moreover subgroup analyses within the different groups were made to identify potential differences. Groups were analyzed for survival, graft rejection and graft vasculopathy (CAD). Kaplan-Meier analysis was used and log-rank test was performed to detect differences.

Results: A total of 81 transplants (8.9%) were blood group compatible. The majority (n=32) were 0A transplants (40%) followed by 0B (n=19; 23%), AAB (n=17; 21%), BAB (n=7; 9%) and 0AB (n=6; 7%). Overall survival comparison showed no significant difference in long-term survival (10-year) between the two groups (I: 52.2% vs. C: 42.1%; p: n.s.). Yet there was a clear trend towards lower survival within the C group. (10a survival: 0B: 73.3%, AAB:58.8%, BAB:32.1%, 0A:27.6%; p=0.0568). In contrast, Freedom from acute rejection was significantly different between I and C groups (71.4% vs. 49.9%; p<0.0001). There was no difference in incidence of CAD (62.5% vs. 60.3%) as well as severe CAD (76.8% vs. 77.9%).

Conclusion: The results of our analysis show that AB0 blood group compatible transplants have similar outcomes in behalf of survival and CAD as AB0 identical transplant. Yet rejection rates are significantly higher in AB0 compatible transplants. This finding needs further investigation.

Kidney: Complications II

Abstract# 230

Outcomes of Solid Organ Transplantation in HIV Infected Individuals. A Systematic Review. Ravikiran Barigala,¹ James Sosman,¹ Nasia Safdar.¹ ¹Medicine, University of Wisconsin, Madison, WI.

Purpose: Increasingly, patients with HIV meet indications for receiving an organ transplant. However, data on the outcomes of solid organ transplantation (SOT) in HIV infected individuals is limited. We undertook a systematic review to determine mortality, graft survival and HIV-related outcomes in patients with HIV and SOT.

Methods: Multiple computerized databases for studies that reported outcomes for HIV positive patients who underwent SOT from 1981-2007. The main outcomes were graft and patient survival, HIV status (viral load or CD4 count), rejection, drug interactions and opportunistic infection. **Results:** 479 patients met inclusion criteria. 220 (46%) underwent kidney transplantation, 256 (53%) patients received a liver transplant, 2 heart transplants and 1 kidney-pancreas transplant. For patients that received kidney transplantation, most were on highly active antiretroviral treatment (HAART) before transplant and the 94 patients for whom this data was provided had no detectable viral load. The graft survival was 75.5% (166/220) and the overall patient survival was 78% (172/220) at a mean followup time of 27 months. The mean absolute CD4 count decreased by 235.56 cells/mm3 after transplant. The proportion of pts with absolute CD4 cell count >200 also decreased from 100% to 54%. No major increase in HIV viral load was seen following SOT. An increase in incidence of new/recurrence of opportunistic infection before and after SOT was not seen. Rejection was noted in 35% of patients at 27 mos mean f/u. For patients that received liver transplantation, 77% were on HAART pre-transplant. For the studies reporting this data, the graft survival was 78.4% (51/66) and the overall survival was 70% (179/256) at at 22.9 m mean f/u. The HIV viral load was undetectable by any method in 87.1% (115/132) post-transplant. Increase in incidence

of new/recurrence of OI after transplantation was seen 4.1% (4/97) pre-transplant vs. 14.5 % (16/110) (CMV-13, candida-2, KS-1). Rejection occurred in 33% (37/111) of patients at a mean followup of 22.9 months. For both kidney and liver transplants, significant drug interactions were noted in 60 % of studies but data on the impact of these interactions was limited. **Conclusions:** HIV positive patients undergoing SOT have favorable graft and survival outcomes similar to HIV negative patients reported in the literature. Further research is necessary to determine the impact of drug interactions and the epidemiology of opportunistic infections following SOT.

Abstract# 231

Impact of Delayed Graft Function on Graft and Patient Outcomes: A Meta Analysis. Sri G. Yarlagadda,¹ Steven G. Coca,¹ Chirag R. Parikh.¹ ¹Medicine/Nephrology, Yale School of Medicine, New Haven, CT.

Introduction: Delayed Graft Function (DGF) is a frequent complication of renal transplantation. The long term impact of DGF on graft survival in the published literature is controversial with varying outcomes for patient and graft survival. We conducted a systematic review and meta-analysis to precisely estimate the impact of DGF on graft and patient outcomes.

Methods: We searched the MEDLINE and EMBASE databases for original studies published through March 2007 pertaining to long-term (> 6 months) outcomes of DGF. The primary outcome was graft survival. Secondary outcomes were patient survival and long-term kidney function. Weighted mean differences (WMD) for outcomes with continuous variables and relative risks (RR) for dichotomous outcomes were pooled from individual studies.

Results: Our search yielded 51 studies on prognosis (graft survival=35, patient survival=9, kidney function=13). Compared to patients without DGF, patients with DGF had an increased risk of graft loss [RR 1.63 (95% CI 1.4-1.8)] on long-term follow-up of > 6 months. The RR of graft loss persisted [1.50 (95 % CI 1.23-1.84)] in studies with longer follow-up (>4 years). When studies that clearly excluded acute rejection after transplant in the DGF group were pooled, the RR of graft loss increased to 2.11 (95 % CI 1.75-2.54) There was no significant effect of DGF on patient survival at 1 year [RR=1.06 (95% CI 0.94-1.2)]. Patients with DGF experienced an increase in serum creatinine at 1 year of 0.65 mg/dl (95% CI 0.5-0.7) compared to patients without DGF.

Conclusions: DGF results in inferior graft function at 1 year and increases graft loss by 63%. Patient survival appears to be unchanged at one year but longer term studies are needed. Efforts on preventing and treating DGF should be aggressively investigated in order to improve graft survival in an era with a deficit in the number of kidney donors.

Abstract# 232

Utility of Urinary Biomarkers in Predicting Long-Term Allograft Function Following Renal Transplantation. Kalathil K. Sureshkumar,¹ Tina Y. Ko,¹ Barbara J. Carpenter,¹ Sabiha M. Hussain,¹ Dai D. Nghiem,¹ Stephen E. Sandroni,¹ Richard J. Marcus.¹ ¹Division of Nephrology and Hypertension, Allegheny General Hospital, Pittsburgh, PA.

Recent data has shown the utility of urinary biomarkers to predict delayed graft function early following renal transplantation, but their ability to predict longer-term allograft function is less clear. We evaluated whether urinary biomarkers measured in the early post-transplant period would predict allograft function at 6 and 12 months after renal transplantation. Urinary biomarkers including N-acetyl-β-D-glucosaminidase (NAG), kidney injury molecule-1 (KIM-1) and matrix metalloproteinase-9 (MMP-9) were measured in 20 patients at hours 0, 6, 12, 24 and days 2 and 3 following renal transplantation. Glomerular filtration rate (GFR) at 6 and 12 months were calculated using the MDRD equation. All 20 patients had functional allografts at 6 months and 2 patients lost allografts after 6 months. Levels of individual biomarkers at different post-transplant time points were correlated with 6 and 12 month GFR using Spearman's correlation (rho) and are shown in the table.

Correlation of urinary biomarkers with 6 and 12 month GFR						
	NAG		KIM-1		MMP-9	
Post-transplant time	6-month GFR	12-month GFR	6-month GFR	12-month GFR	6-month GFR	12-month GFR
Hour 0	-.487*	-.417	-.174	-.157	-.129	-.068
Hour 6	-.481*	-.311	-.104	-.031	-.223	.038
Hour 12	-.438	-.250	-.236	.006	-.109	.072
Hour 24	-.709***	-.506*	-.454*	-.466*	-.114	-.078
Day 2	-.626**	-.457*	-.348	-.521*	-.417	-.215
Day 3	-.640**	-.534*	-.271	-.357	-.105	-.037

* = p < 0.05, ** = p < 0.01, *** = p < 0.001

Urinary NAG levels at post-transplant hours 0, 6, 24 and days 2 and 3 showed significant negative correlation with 6-month GFR. NAG levels at hour 24 and days 2 and 3 maintained the significant negative correlation with 12-month GFR. Urinary KIM-1 levels at the 24 hour point showed significant negative correlation with 6-month GFR and KIM-1 levels at hour 24 and day 2 displayed significant negative correlation with 12-month GFR. Urinary MMP-9 levels at no time points had significant correlation with either 6-month or 12-month GFR.

Urinary biomarkers particularly NAG shows promise as a tool in the early prediction of longer-term allograft function following renal transplantation.

Abstract# 233

In Renal Transplants with Delayed Graft Function (DGF) Chemokines and Chemokine Receptor Expression Predict Longterm Allograft Function. Ralf Dikow,¹ Luis Becker,² Matthias Schaefer,¹ Lars P. Kihm,¹ Martin Zeier,¹ Marie L. Gross.² ¹Nephrology, University Hospital, Heidelberg, Germany; ²Pathology, University Hospital, Heidelberg, Germany.

Introduction

In kidney disease chemokines are involved in the recruitment of leukocytes causing kidney damage and progression to endstage renal failure. Similar mechanisms are proposed for chronic allograft failure in renal transplant recipients. The chemokine attracted leukocytes produce proinflammatory and profibrotic cytokines contributing to fibroblast proliferation, matrix production and tubular atrophy. The role of chemokines in the acute post-ischemic tubular necrosis early after renal transplantation and their impact on long term allograft outcome has not been investigated yet.

Methods

30 patients (23m, 7f, mean age 49.9yrs) with DGF (with a median number of 6.4 dialysis sessions) developed biopsy proven acute tubular necrosis during the first three weeks after transplantation. The biopsies were studied by immunostaining for KI67, CCR1, CCR2, RANTES, MCP-1, CD20 and CD68, respectively. The patients were followed for a mean time of 4.2yrs. None of them developed a rejection episode in the follow up time.

Results

After follow up the mean serum creatinine was 2.4mg/dl (0.8-6.2, SA 1.5). Serum creatinine values after 4.2yrs were positively correlated with expression of KI67 per mm² (.582, p=0.001), expression of CCR1 per mm² (.495, p=0.01), expression of CCR2 per mm² (.504, p=0.007) and expression of RANTES in glomerular cells (.543, p=0.004) in early biopsy after renal transplantation. KI67 was positively correlated with CCR1 (.64, p<0.001), CCR2 (.67, p<0.001) and CD20 (.62, p<0.001).

Conclusion

High expression of CCR1 and CCR2 and of the chemokine RANTES in kidneys with DGF predict an impaired transplant function in the long follow-up even in the absence of rejection episodes. This may correlate with enhanced inflammation due to ischemic damage.

Abstract# 234

2202 Kidney Transplant (Tx) Recipients with 10 Yrs of Graft Survival: What Happens Next? Arthur Matas, K. Gillingham, A. Humar, T. Dunn, R. Kandaswamy, W. Payne, D. Sutherland, J. Najarian. *Surgery, U of MN, Mpls, MN.*

The goal of a tx is for the recip to achieve long-term survival (surv.), with continued graft function, equivalent to the gen. population. We studied subsequent outcome in 2202, 10-yr kidney tx survivors (tx 1963-1997); 62% living donor (LD); 87% 1st tx; 41% female; 28% type 1 diabetes; mean age at tx (±SE), 33±15 yrs. Actuarial 25-yr surv. is shown in Table 1; LD patient, graft, and death-censored graft surv. is signif. ↑ vs. deceased donor (p<.0001).

25-Yr Actuarial Survival (for 10-Yr Survivors)				
	LD- 1st (n=1,234)	DD - 1st (n=690)	LD - retx (n=96)	DD - retx (n=182)
Patient	56%	39%	69%	34%
Graft	43%	27%	49%	16%
DC - graft	69%	56%	63%	35%

DC = death censored

Mean creatinine (± SE) at 10 yrs was 1.7±.8; 15 yrs, 1.7±.9; 20 yrs, 1.5±1; 25 yrs, 1.6±1. The 2 major causes of late graft loss (GL) were death with function (DwF) and chronic rejection (CAN). By multivariate analysis, risk factors for GL after 10 yrs were age ≥50 yrs at tx; type 1 diabetes, retx, HLA mm ≥3; ≥1 acute rejection episode, and pretx cardiac, peripheral vascular, or liver disease (p<.05). The most common cause of DwF was cardiovascular disease (CVD); however, >20% deaths were due to malignancy. Mean age at death (±SE) (range) for those dying of CVD (nondiab/diab) was 54±13 (16-82 yrs) / 53± 7 (36-63 yrs); of CVA was 60±13 (38-80 yrs) / 53±9 (41-76 yrs) (p<.05); of malig. was 56±13 (30-91 yrs) / 53±9 (38-73 yrs); of infection was 57±16 (12-82 yrs) / 54±10 (39-71 yrs). By 20 yrs posttx, morbidity was common.

% Morbidity at 20 Years After a 1st Transplant				
	nondiabetic		diabetic	
	LD	DD	LD	DD
Skin cancer (ca.)	36%	49%	40%	56%
Nonskin ca.	10%	23%	14%	22%
PTLD	2%	5%	3%	7%
CVD	26%	33%	56%	50%
CVA	5%	14%	21%	12%
PVD	12%	13%	35%	32%
Respiratory	26%	37%	37%	38%

PVD = peripheral vascular disease

The mean age (±SE) (range) of 1st diagnosis for skin ca. (nondiab/diab) was 53±13 (13-81 yrs) / 49±8 (28-69 yrs) (p=.003); for non-skin, non-PTLD ca. was 53±16 (11-78 yrs) / 46±9 (28-65 yrs) (p<.001); for PTLD was 47±20 (5-79 yrs) / 49±8 (38-68 yrs); for CVD was 53±15 (3-79 yrs) / 47±9 (22-76 yrs) (p<.0001); for CVA was 58±14 (9-79 yrs) / 48±10 (20-75 yrs) (p<.003); for PVD was 50±15 (6-77 yrs) / 47±9 (25-76 yrs) (p<.004); for respiratory 36±22 (1-75 yrs) / 45±10 (23-70 yrs) (p=.001). Larger numbers are needed to compare to the age-matched gen.population, but morbidity appears to occur at a young age. Our data show need for improvement of long-term pt. and graft surv. and minimization of morbidity.

Abstract# 235

New Tool To Analyse Confounding Factors in Kidney Graft Outcome: A DIVAT* Data Base Study. Magali Giral,¹ Yohann Foucher,¹ Jean Pierre Daures,² Jean Paul Souillou.¹ ¹Kidney Transplantation, ITRT CHU, Nantes, France; ²Statistics, IURC, Montpellier, France.

Introduction. Cox regression is currently the most commonly used analysis to assess the impact of covariates on graft survival. It allows to study the effect of each independent covariate (age, sex etc.) on the speed to jump directly from a initial state (the transplantation) to graft failure and death which are generally pooled as a unique final event. Here, we compared the results of a Cox analysis with a new approach using a semi-markov multistate analysis that can differentiate the role of each covariate on several competitive states of outcome.

Patients and methods : We prospectively studied 839 first kidney adult transplant patients between 1996 and 2006. We defined 3 competitive states of outcome from the initial transplantation event: 1) a graft deterioration state defined by the loss of at least 30% of MDRD clearance compared with the entry level, 2) graft failure state and 3) death. For an initial validation of the semi markov model in kidney transplantation, we only chose 7 well defined covariates (donor and recipient age, sex, cold ischemia time, DGF, anti T PRA, HLA incompatibilities and induction therapy (ATG vs anti IL2R)).

Results : The Cox model showed that only 2 covariates are risk factors of graft failure : 1) DGF (RR 1.72) and 2) recipient age (RR 2.89) in the restricted case of donors older than 55 years. However interestingly, the semi markov analysis showed that all 7 chosen covariates influence graft outcome, some on the probability and others on the speed to jump from one state to another. For instance, female recipients receiving a kidney >= 55 years, treated with an anti RIL2 receptor induction therapy and with a DGF >= 6 days, represent the worst association to jump frequently and rapidly from the initial state to the state of graft deterioration (loss of >= 30% of their graft function). Moreover, when patients reach this first state of gravity, a high level of PRA >=25% or of HLA inc. >= 4 accelerates graft failure. Finally, the model shows that male recipients with a DGF >= 6 days die the quickest, all characteristics ignored by the Cox model.

Conclusion : We show, for the first time, that it is possible to better describe the role of the covariates on several stages of graft outcome. This could help to make physicians aware of "at risk population" in whom graft function deterioration could be predicted before irreversible graft loss or death.

* DIVAT: Computerized and Validated Data in Transplantation.

Abstract# 236

Regional Changes in Kidney Transplantation Outcomes across the United States over Time. Harini Chakkerla,¹ Ann O'Hare,² Glenn Chertow,³ William Amend,⁴ Thomas Gonwa.⁵ ¹Mayo Clinic, AZ; ²University of Washington; ³Stanford; ⁴UCSF; ⁵Mayo Clinic, Jacksonville.

Background: Clinical outcomes after kidney transplantation in the US have improved considerably over time. However, the degree to which such improvements have occurred uniformly across the country is unknown.

Methods: Using the USRDS database, we examined 1 and 3 year allograft survival across UNOS regions during three different time periods.

Era 1: CsA use:- 1/1/1988 to 1/1/1990 (n = 13,669). **Era 2:** Introduction of tacrolimus and MMF:- 1/1/1994 to 1/1/1996 (n = 17,456). **Era 3:** Widespread use of tacrolimus and MMF:-1/1/1998 to 1/1/2000 (n = 20,375).

Results: Overall graft survival improved from 80.5% to 90.6% (1 year) and 69.4% to 81.8% (3 year) between Eras 1 and 3 respectively. Multivariate models of regional effects for both 1 and 3 year graft survival demonstrated modest regional differences in transplant outcomes that diminished over time.

UNOS Region	Era 1	Era 2	Era 3
	Odds ratio (95% CI)*	Odds ratio (95% CI)*	Odds ratio (95% CI)*
Overall average	Referent 1.00	Referent 1.00	Referent 1.00
Region 1	0.90 (0.76-1.07)	0.95 (0.78-1.16)	0.93 (0.76-1.13)
Region 2	0.93 (0.82-1.05)	1.09(0.95-1.23)	1.13 (1.00-1.30)
Region 3	0.90 (0.80-1.00)	0.91 (0.81-1.04)	0.87 (0.73-1.02)
Region 4	1.26 (1.04-1.54)	1.20 (1.08-1.34)	1.17 (1.02-1.34)
Region 5	1.26 (1.10-1.44)	0.96 (0.87-1.07)	0.90 (0.77-1.04)
Region 6	1.03 (0.85-1.25)	0.87 (0.72-1.05)	0.77 (0.54- 1.08)
Region 7	0.94 (0.76-1.17)	0.85 (0.74-0.98)	0.95 (0.80- 1.13)
Region 8	0.78 (0.64-0.96)	1.11 (0.90-1.26)	1.10 (0.91- 1.32)
Region 9	1.09 (0.92-1.30)	1.12 (1.00-1.24)	1.20 (1.05- 1.39)
Region 10	0.99 (0.87-1.14)	1.06 (0.92-1.21)	1.03 (0.85-1.25)
Region 11	0.84 (0.71-1.00)	0.97 (0.85-1.10)	1.02 (0.90-1.15)

* adjusted for 30 donor and recipient variables

Conclusions: From 1988 to 2000, the 1 and 3 year graft survival improved substantially in all regions, and regional variations, which were modest to begin with, diminished over time.

Abstract# 237

Transplantation Improves Survival for ESRD Patients across the Body Mass Index Spectrum. Amy L. Friedman,¹ Eli A. Friedman,² Jeanne Macrae,³ Paul Eggers.³ ¹Surgery, SUNY Upstate Medical University, Syracuse, NY; ²Medicine, SUNY Downstate Medical Center, Brooklyn, NY; ³NIDDK, Bethesda, MD.

It is well known that morbid obesity increases the risk of morbidity and mortality following kidney transplantation. Yet, it has been unclear whether the individual morbidly obese patient is more likely to enjoy prolonged survival with transplantation vs dialysis. To answer this question, we searched the United States Renal Data System files for all persons waitlisted from 1996 through 2004, by initial BMI category, to determine the effect of sex, age, race, diabetes and transplant on survival.

BMI	Male	Diabetes		Transplant		
	Hazard Ratio	p value	Hazard Ratio	p value	Hazard Ratio	p value
<15	1.010	0.93	1.775	0.000	0.533	0.000
15-19.9	1.132	0.007	1.974	0.000	0.489	0.000
20-24.9	1.072	0.007	2.055	0.000	0.549	0.000
25-29.9	0.985	0.568	1.932	0.000	0.546	0.000
30-34.9	1.070	0.048	1.844	0.000	0.520	0.000
35-39.9	1.029	0.581	1.928	0.000	0.546	0.000
40-44.9	1.183	0.060	1.937	0.000	0.670	0.000
45-49.9	1.205	0.199	1.693	0.000	0.667	0.023
50+	0.827	0.259	1.546	0.011	0.588	0.014

During the study period 109,474 persons were waitlisted. One year and seven year survival was 95.7 % and 55.2 % for dialysis versus 95.6 % and 77.9 % for transplant. Obesity (BMI > 29) was present in 14% of waitlisted persons and in 25 % of those transplanted. BMI >39 was present in 4.1 % of waitlisted and in 7.3 % of transplanted persons and BMI was 50 + in 644 (.59 %) waitlisted and 265 (.43 %) transplanted patients.

Obesity is common among ESRD patients placed on the waitlist. In all BMI categories, transplantation affords a statistically significant survival advantage compared to dialysis. Although few transplants are performed in the US for patients with BMI of 50+, outcomes are equivalent to those for leaner patient cohorts. Increased BMI should not be considered an absolute contraindication to kidney transplantation.

Abstract# 238

Most Cases of Kidney Graft Failure Are Due to an Identifiable Cause and Not Chronic Allograft Nephropathy (CAN). Ziad M. El-Zoghby, Donna J. Lager, Hatem Amer, James M. Gloor, Mark D. Stegall, Fernando G. Cosio. *William von Liebig Transplant Center, Mayo Clinic, Rochester, MN.*

Background: Death censored kidney graft loss is commonly attributed to CAN now called fibrosis/atrophy (IF/TA) and not assigned to a specific etiology. The goal of this study was to assess the specific causes of graft loss in the last decade.

Methods: Included are 1317 conventional kidney grafts recipients transplanted between 1/1/96 and 7/1/06. Clinical and histologic data were reviewed to determine the cause of graft loss. When the information was not conclusive, biopsies were reviewed and additional histologic studies (C4d and/or BK staining) were done.

Results: Mean patient age was 48.7±17 years; 60% were male and 73% were living donor recipients. During a median follow up of 50.3 months (range: 0-138), 136 patients died (10%) and 182 allografts were lost (14%) not due to patient death. Allograft histology was available in 180 of the 182 allografts that failed. The median time from last biopsy to graft loss was 3.6 months (range: 0-104). 70 allografts were lost during the first year, 82 between years 1-5 and 30 beyond 5 years. Causes of graft loss are summarized in the Table. In 95% of cases a specific cause of graft loss was identifiable. Death with function was the single most common cause of graft loss. The most frequent histologic lesions in the failed graft were glomerular, particularly recurrent disease and transplant glomerulopathy (TG). In patients with IF/TA a cause was identified in 79%, most commonly secondary to BK nephropathy and recurrent acute rejections. Primary non-function was the 4th most common cause of graft loss and included vascular thrombosis, medical complications and poor allograft quality. Twelve of the 18 cases of acute rejection occurred after the first year. Medical causes included graft loss secondary to severe medical conditions, e.g. sepsis and recurrent non-glomerular diseases.

Cause of graft loss	Number	Percent of losses (N=318)
Death	136	43%
Glomerular disease	54	17%
Recurrent	22	7%
TG	22	7%
De novo	10	3%
Fibrosis/atrophy	43	14%
Secondary	34	11%
Idiopathic	9	3%
Primary non-function	40	13%
Acute rejection	18	6%
Medical	22	7%
Unknown	5	2%

Conclusions: In most cases kidney graft failure can be attributed to a specific cause defined by clinical and histologic criteria. A high proportion of death-censored graft failures (30%) are due to glomerular pathology. Among these, TG and recurrent disease are the most common causes of kidney graft failure.

Kidney: Deceased Donor Issues I

Abstract# 239

Seventeen Years of Experience Using Kidneys from HCV Positive Donors into HCV Positive Recipients: A Safe Policy To Expand the Donor Pool.

Beatriz Domínguez-Gil,¹ Nuria Esforzado,² Maria I. Delgado,³ Federico Oppenheimer,² Amado Andrés,³ Gregorio Castellanos,⁴ Jose M. Campistol,² Jose M. Morales.³ ¹Medical, Organización Nacional de Trasplantes, Madrid, Spain; ²Nephrology, Clinic i Provincial Hospital, Barcelona, Spain; ³Nephrology, 12 de Octubre Hospital, Madrid, Spain; ⁴Gastroenterology, 12 de Octubre Hospital, Madrid, Spain.

The use of kidneys from donors with a positive serology for HCV into HCV(+) recipients still remains controversial. In 1990, our units adopted this policy, subsequently modified in 1993, so these kidneys were limited to recipients with a positive RNA HCV before transplantation.

The aim of the present analysis was to review the long-term safety of our policy. Since January 1990 to February 2007, 474 HCV(+) patients with a negative HbsAg and not treated with interferon received a kidney transplant in our units. 313 patients were transplanted from an HCV(-) donor (Group 1) and 161 from an HCV(+) donor (Group 2). Median follow-up time was 65 (IR 24-117) months.

Remarkably, Group 2 showed a significantly higher donor age (46.6±13.6 versus 41.1±18.8 years; p<0.0001) and recipient age (50.4±13.1 versus 44.9±14.6 years; p<0.0001), as well as a worse HLA compatibility than Group 1. Immunosuppressive therapy did not significantly differ between the two groups.

MAIN OUTCOME RESULTS IN HCV (+) KIDNEY TRANSPLANT PATIENTS

	Group 1 HCV (-) donors (N=313)	Group 2 HCV (+) donors (N=161)	p
5 year death-censored graft survival*	69.5%	62.6%	0.1433
10 year death-censored graft survival*	58.4%	45.1%	0.1433
5 year patient survival*	84.3%	81.6%	0.5631
10 year patient survival*	73%	70.4%	0.5631
Chronic liver disease**	10.5%	15.5%	0.138
Severe liver disease***	5.5%	7.9%	0.285

*Life table analysis **ALT > 2.5 times the upper normal limit > 6 months ***Signs of hepatocellular insufficiency and/or portal hypertension

Notably, only 5 patients died because of a liver disease (3 in Group 1 versus 2 in Group 2) and only 2 patients developed a hepatocarcinoma (both in Group 1).

In summary, no significant differences were observed in HCV(+) recipients according to HCV serology of the donor, in terms of death censored graft survival, patient survival and evolution of liver disease. Therefore, our experience clearly demonstrates that the use of kidneys from HCV(+) donors into HCV(+) recipients is a safe strategy in the long-term and a wise way of using these kidneys, that otherwise would be lost at a moment of shortage.

Abstract# 240

Consequences of Deceased-Donor Kidney Allocation Systems Incorporating Life-Years from Transplant (LYFT).

Robert A. Wolfe,¹ K. P. McCullough,¹ A. M. Rodgers,¹ M. D. Stegall,² P. G. Stock,³ J. Kalbfleisch,¹ A. B. Leichtman.⁴ ¹SRTR, Ann Arbor, MI; ²Mayo Clinic College of Medicine, Rochester, MN; ³Department of Surgery, UCSF, San Francisco, CA; ⁴SRTR, Dept of Medicine, Div. of Nephrology, U. of Michigan, Ann Arbor, MI.

Introduction: Deceased donor kidneys are allocated to adult candidates in the U.S. primarily by waiting time and HLA similarity. We investigated two alternative allocation systems, LYFT-SCD and LYFT-DY, that prioritize offers of kidneys to adults based in part on LYFT.

Methods: Based on characteristics of each candidate and donor, LYFT was calculated as the difference between expected median years of life for the candidate with that kidney donor transplant (tx) versus without a kidney. Years on dialysis were weighted at 0.8 of years with a functioning graft. The relative risk of graft failure for each donor was calculated from donor factors using a Cox model and the Donor Profile Index (DPI), the percentile score of that risk among kidneys used in 2003 (100%=greatest risk of graft failure). Using actual candidates and deceased donors and acceptance patterns from 2003, we modeled allocation with the Kidney-Pancreas Simulated Allocation Model (KPSAM). KPSAM sequentially offers kidneys to candidates according to user-specified allocation rules, and models the probability for kidney placement and post-tx survival. The LYFT-SCD model allocated SCD kidneys by LYFT and ECD kidneys by Dialysis Years (DY). The LYFT-DY model allocated organs according to the formula [.8LYFT(1-DPI)]+[DY(.8DPI+.2)]+[4PRA/100] to adult kidney tx candidates.

Results: The table compares a year's worth of kidney-alone recipient characteristics, lifespan after tx and additional lifespan (v. dialysis) due to tx among all kidney (including SPK) recipients, and the donor/recipient age correlation (R) under current national kidney allocation, allocation using LYFT for SCD, and allocation using LYFT-DY within each donation service area.

Conclusions: Allocation systems incorporating LYFT have the potential to increase the number of years of life attainable from tx and are being considered for the allocation of kidneys within the U.S.

	%Age <50	%DM, age <50/>50	African Amer.	Life after Tx	Add. Life due to Tx	Don/Rec. age R
Current	48.8	6.6/19.8	32.5	107,798	48,044	.10
LYFT-DY	62.0	5.7/12.4	37.7	118,133	51,589	.31
LYFT-SCD	71.1	4.4/9.4	33.0	143,504	59,691	.29

Abstract# 241

Over Half of Kidney Candidates over Age 60 Now Listed Will Die Prior to Receiving a Deceased Donor Transplant. Jesse D. Schold,¹ Titte R. Srinivas,¹ Herwig-Ulf Meier-Kriesche.¹ ¹Medicine, University of Florida, Gainesville, FL.**Introduction**

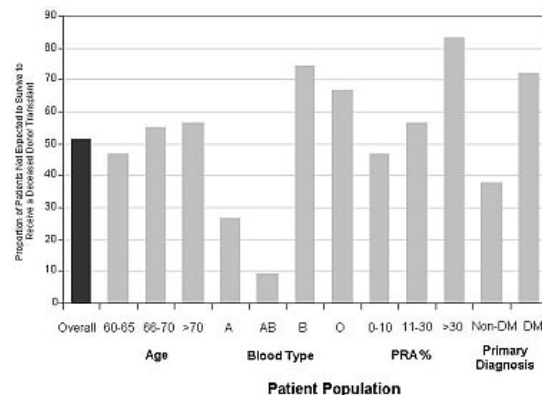
The expanding waiting time to kidney transplantation has significantly increased mortality for transplant candidates. In particular, frailer candidates are at growing risk for not surviving the interval at which they would receive a transplant. While this trend is recognized, data concerning the actual likelihood of receiving a transplant for the overall population and by certain patient characteristics are not widely available yet may provide critical information to transplant candidates and their caregivers.

Methods

The study examined adult solitary renal transplant candidates from 1995-2006 from the national SRTR database. Parametric survival models were utilized to estimate time to receiving a deceased donor transplant and time to death prior to transplantation based on known factors for individual candidates. The proportion of patients with a shorter expected time to death as compared to time to transplant was calculated and compared among subgroups.

Results

Among all adult candidates, the proportion of patients with shorter life expectancy than expected time to transplant increased from 4% to 30% from 1995-2006. As expected, these proportions were significantly higher in older candidates (age at listing > 60) and varied substantially by blood type, PRA and primary diagnosis. Overall in 2006, over 52% of older candidates were expected to die prior to receiving a deceased donor transplant (Figure 1).

**Conclusions**

This tangible data should be disseminated to patients, especially higher-risk transplant candidates, to help illustrate the incentives for seeking living donors and allow for informed decision-making.

Abstract# 242

Dual-Kidney Transplants as an Alternative for Very Marginal Donors: Long-Term Follow-Up in 63 Patients.

Sacha De Serres,¹ Yves Caumartin,² Alain Naud,³ Yves Fradet,³ Thierry Dujardin,³ Real Noel,¹ Jean-Guy Lachance,¹ Isabelle Cote,¹ Isabelle Houde.¹ ¹Division of Nephrology, Hotel-Dieu de Quebec Hospital, Laval University, Quebec City, QC, Canada; ²Division of Urology, University of Western Ontario, London, ON, Canada; ³Division of Urology, Hotel-Dieu de Quebec Hospital, Laval University, Quebec City, QC, Canada.

Introduction :

Organ shortage for transplantation has led to the use of marginal kidneys, which has been associated with poorer but still acceptable results. In our center, the transplantation of two very marginal kidneys into a single recipient was used as a strategy to increase the number of organs available. The present study reports renal function and survival of dual-kidney transplants (DKT) performed at our institution.

Methods :

From October 1999 to June 2007, 392 transplants were performed at our center, from which 63 were DKT. Kidney selection for a DKT was based on refusal of the kidneys for single kidney transplantation (SKT), donor's age ≥ 60 years and ≥15% of glomerulosclerosis on pre-implantation biopsy. The calculated creatinine clearance (crcl, ml/min), graft and recipient survival from DKT were compared to those of SKT from ECD (based on UNOS criteria, n=66) and ideal donors (ID, aged 10 to 39 y, n=63) over a seven-year period.

Results :

DKT donors were significantly older than the two other groups (DKT: 69 year old, ECD: 62, ID: 24). Dual transplants were offered to older recipients by design (60, 50, 44). Delayed graft function was more prevalent for DKT and ECD than with ID (27%, 29%, 11%). Twelve, 36 and 84-month crcl were similar for DKT and ECD but lower than ID (twelve months: 58, 59, 81; 36 months: 54, 60, 82; 84 months: 62, 51, 86, respectively). Patient survival, actuarial graft survival and actuarial graft survival censored for death were similar between the 3 groups.

Conclusion :

To our knowledge, this study reports short and long-term outcome of the largest cohort of DKT performed at a single institution. It shows that dual-kidney transplantation is a more complex intervention with more risks of surgical and post-op complications (data not shown). However, with adequate surgical expertise, DKT patients can expect long-term results comparable or even better than ECD. In our center, the use of DKT has increased the number of transplantations by 19% for the period of study. It allowed older patients to receive a graft within shorter delays. Moreover, it permitted a more judicious allocation of a resource that is still limited.

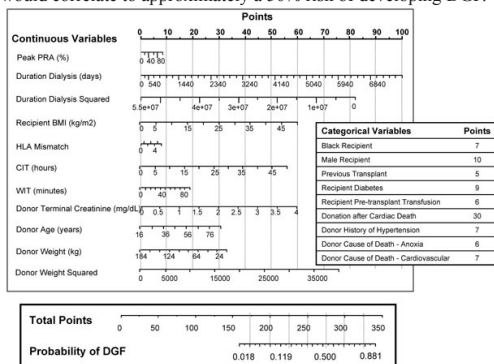
Abstract# 243**An Updated Nomogram for Predicting the Likelihood of Delayed Graft Function in the Current Era of Deceased Donor Renal Transplantation.**

W. D. Irish,¹ M. A. Schnitzler,² S. Feng,³ D. C. Brennan.⁴ ¹Research Triangle Institute, Research Triangle Park, NC; ²St. Louis University, St. Louis, MO; ³University of California San Francisco, San Francisco, CA; ⁴Washington University, St. Louis, MO.

Introduction: Previously, a predictive nomogram for DGF risk was developed using USRDS registry data (1995-1998) (Irish et al., JASN 2003). Given the changing landscape since the institution of a new kidney allocation policy in 2001, we aimed to refine the previously published model using more recent data. We addressed criticisms of the original model by excluding multiple-organ transplants, preemptive transplants, and organs with pulsatile preservation, while including additional risk factors of warm ischemia time (WIT), dialysis duration, donor weight, and recipient body mass index (BMI) in the updated analysis.

Methods: UNOS data on adult, solitary, non-preemptive, non-machine perfused, deceased donor renal transplants (2003-2005) were obtained. Grafts lost within the first 24 hours (n=245) or missing a DGF code (n=1) were excluded from further analysis. A multivariable logistic regression model was fit to the data for the remaining 18,276 patients. The model was validated using a dataset of patients transplanted in 2006 (n=3601). An updated nomogram quantifying the relative contribution of each risk factor was created.

Results: DGF incidence (25.8%) in the current era did not significantly differ from the previous report (23.7%). Although the relative impact of most risk factors remained consistent, the weight of peak PRA and previous transplant was significantly attenuated, while the impact of donor terminal serum creatinine was enhanced by approximately 2-fold. Using the nomogram below, DGF risk can be calculated by adding points associated with each continuous and categorical risk factor. A score of 270 for example, would correlate to approximately a 50% risk of developing DGF.



Overall accuracy of the updated model was 70.4%.

Conclusions: The updated nomogram provides a tool for developing a pre-transplant index of DGF likelihood in the current era of kidney transplantation. With the availability of this index, more informed treatment decisions can be made.

Abstract# 244**Transplantation of Older Patients Does Not Improve Survival Compared with Those Wait Listed. Analysis of 9,700 Wait Listed Patients in the UK.**

David Ansell,¹ John O'Neil,² Rachel Johnson,² David Collett,² Chris Dudley.¹ ¹UK Renal Registry, Bristol, United Kingdom; ²UK Transplant, Bristol, United Kingdom.

The survival benefit of receiving a kidney transplant over remaining on the waiting list is often quoted from the American study by Wolfe. Recent USA papers have also supported a survival benefit in the over 65s. In Europe survival on dialysis is better

than the USA across all age bands and survival benefit of transplantation may not be as great. Using the same statistical methodology as Wolfe, we have calculated the time to survival benefit by age group in the UK, for patients receiving a deceased donor (DD) kidney transplantation over those remaining on the waiting list.

Methods: 9739 adult patients listed for a DD first kidney only transplant in the UK, 1995-2000, were analyzed. Patients were followed from date of listing to Sept 2005 or death. Of the patients analyzed, 6819 received a DD transplant. Cox regression was used to analyze the combined effect of age, ethnicity, diagnosis, waiting time and time to be put on the waiting list on patient survival and also to evaluate the time to equal risk, equal survival, life years gained by transplantation for different age groups. We also examined the effect of periods of suspension from the waiting list (as a surrogate marker for comorbidity) on the survival benefit.

Results: Transplantation was of most benefit to younger patients with risk of death at the time of transplant increasing with age, resulting in increasing time to equal risk and equal survival with increasing age. In contrast to the USA, UK patients aged over 65 years receiving a renal transplant did not show a significant survival benefit.

Age band	N	RR of death tx vs list	Equal risk (days)	Equal survival (days)	Relative Risk immediate post tx
18 - 34	1956	0.3** (0.2-0.5)	34	82	3.2
35 - 44	2060	0.4** (0.3-0.5)	126	279	4.1
45 - 54	2464	0.5** (0.4-0.7)	158	362	4.5
55 - 64	2269	0.7** (0.6-0.8)	186	480	5.2
65+	990	0.9 (0.7-1.1)	190	581	8.0

** p< 0.0001

The survival benefit after receiving a DD transplant was similar for whites, south Asians and Blacks with no reduced relative benefit seen in ethnic minority groups who have better dialysis survival.

Summary: In the UK patients aged over 65 years receiving a renal transplant did not show a significant survival benefit. As well as age, the survival benefit of transplantation varied by primary diagnosis and time spent suspended.

Abstract# 245**The Effect of DonorNet on Allocation Efficiency of Exported Organs.**

Dorry L. Segev,¹ Robert A. Montgomery.¹ ¹Johns Hopkins Medical Institutions.

Background: Prior to national implementation of DonorNet, we showed that use of regionally or nationally exported kidneys was clustered at certain high-utilization centers (HUCs). The goal of this study was to evaluate the effect of DonorNet on allocation efficiency of these organs.

Methods: We analyzed all deceased donor kidney transplants reported to UNOS between two time periods: (1) the time since national launch of DonorNet (May 1, 2007 through Nov 1, 2007), and (2) a reference time period (Jan 1, 2005 through Dec 31, 2006). Because of expedited allocation, zero-mismatch kidneys were dropped from analysis.

Effect of DonorNet on Cold Ischemia Time (CIT)		
	Before DonorNet	After DonorNet
All Centers:		
Regional Export, Mean CIT	22.9h	23.3h
Regional Export, % with CIT>36h	6.4%	9.0%
National Export, Mean CIT	27.3h	26.8h
National Export, % with CIT>36	17.9%	16.1%
High Utilization Centers:		
Regional Export, Mean CIT	23.1h	23.4h
Regional Export, % with CIT>36h	7.0%	10.4%
National Export, Mean CIT	27.2h	27.4h
National Export, % with CIT>36	17.5%	16.8%

Results: Very few differences resulting from DonorNet were seen. For regionally exported kidneys, cold ischemia time (CIT) was 22.9h before DonorNet (BD) and 23.3h after DonorNet (AD); 6.4% of regionally exported kidneys had CIT>36h BD, as compared with 9.0% AD. For nationally exported kidneys, CIT was 27.3h BD and 26.8h AD; 17.9% of nationally exported kidneys had CIT>36h BD, as compared with 16.1% AD.

The same HUCs of exported kidneys BD were also HUCs AD. Of the top 10 centers utilizing exported kidneys BD, only one was not within the top 10 centers utilizing exported kidneys AD. When we looked at CIT for kidneys exported to HUCs, again there was little difference before or after DonorNet, with a trend to possibly longer CIT for HUCs. For kidneys regionally exported to HUCs, cold ischemia time (CIT) was 23.1h before DonorNet (BD) and 23.4h after DonorNet (AD); 7% of regionally exported kidneys had CIT>36h BD, as compared with 10.4% AD. For kidneys nationally exported to HUCs, CIT was 27.2h BD and 27.4h AD; 17.5% of nationally exported kidneys had CIT>36h BD, as compared with 16.8% AD.

Conclusion: The first 6 months of national implementation of DonorNet were not successful in decreasing CIT for regionally or nationally exported kidneys. Furthermore, the centers to which a high proportion of these kidneys were exported did not change as a result of DonorNet, and for these centers allocation efficiency is no better and might even be worse than before.

Abstract# 246

Machine Preservation of Kidneys Donated after Cardiac Death Does Not Reduce Delayed Graft Function. Christopher Watson,¹ Antonia Wells,¹ Rebecca Roberts,¹ Joanne Blackwell,⁶ Jacob Akoh,² Peter Friend,³ Murat Akkol,⁴ Francis Calder,⁵ Andrew Bradley,¹ David Collett.⁶ ¹University Dept. of Surgery, Addenbrooke's Hospital, Cambridge, United Kingdom; ²Renal Transplant Unit, Derriford Hospital, Plymouth, United Kingdom; ³Oxford Transplant Centre, Churchill Hospital, Oxford, United Kingdom; ⁴Transplant Unit, Royal Infirmary, Edinburgh, United Kingdom; ⁵Renal & Transplant Unit, Guys & St Thomas's Hospital, London, United Kingdom; ⁶Statistics & Audit Directorate, UK Transplant, Bristol, United Kingdom.

Background

Cold machine preservation has been shown to improve outcome following transplantation of deceased heart-beating donor kidneys. To determine whether cold machine preservation also improves outcome of kidneys donated after cardiac death (DCD) we undertook a multicentre randomized controlled trial in the UK comparing machine perfusion with simple cold storage.

Methods

One kidney from each DCD donor was randomized to machine perfusion (Organ Recovery Systems LifePort device with KPS-1 solution), the other to perfusion with UW (ViaSpan) solution followed by simple cold storage. The primary outcome measure was the need for dialysis in the first 7 days (delayed graft function, DGF); secondary outcome measures included GFR at 1 week (MDRD technique); 3 and 12 month graft and patient survival. At the time of writing, one week outcome data are available for all patients.

A sequential design was used with the data inspected after 60 transplants and then every 20. All recipients received basiliximab, mycophenolate sodium, tacrolimus and prednisolone.

The significance of the initial results has been tested using paired T tests and McNemar's exact tests.

Results

Recruitment was stopped after 46 donors when the unadjusted sequential analysis concluded that there was no difference in the incidence of DGF. 91 of the 92 kidneys were transplanted; one was not used for anatomical reasons. One kidney suffered primary non function; it had been machine preserved. The results shown below represent intention to treat.

	Machine perfusion (n=45)	Simple cold storage (n=45)	Significance (p-value)
Recipient Age: Mean (SD)	50 (14)	49 (14)	0.55
Cold ischaemic time: Mean (SD)	14h44m (4h36m)	14h53m (4h41m)	0.98
DGF incidence	25 (56%)	24 (53%)	0.99
GFR on day 7: Mean (SD)	17.0 (14.7)	13.4 (11.1)	0.09

Conclusion

Cold machine perfusion of kidneys from DCD donors does not reduce the incidence of delayed graft function in the context of the short ischaemic times seen in the study.

Liver: Living Donors and Parial Grafts II

Abstract# 247

Single-Center Experience of First 1000 Cases of Adult-to-Adult Living Donor Liver Transplantation. Shin Hwang, Sung-Gyu Lee, Chul-Soo Ahn, Ki-Hun Kim, Deok-Bog Moon, Tae-Yong Ha, Gi-Won Song, Kwang-Min Park. Department of Surgery, Asan Medical Center, University of Ulsan, Seoul, Korea.

The outcomes of first 1000 adult recipients undergoing living donor liver transplantation (LDLT) in a single center from February 1997 to January 2006 were retrospectively analyzed. Recipient mean age was 47.9±8.6 years. Major primary diagnoses included hepatitis B virus-associated cirrhosis (n=845), acute liver failure (n=56), hepatitis C virus-associated cirrhosis (n=33) and alcoholic liver disease (n=30). Primary liver malignancy was detected in 300 patients. Graft types were right liver grafts (n=651), left liver grafts (n=176) and dual liver grafts (n=173). Most right lobe grafts had middle hepatic vein outflow pathway through reconstructed or native vessel trunk. Median graft-to-recipient weight ratio was 0.97. Retransplantation was performed in 9. Overall 1-year, 3-year and 5-year patient survival rates were 89.3%, 84.7% and 82.9%, respectively. Five-year patient survival rate was 73.8% and 87.1% in patients with and without hepatocellular carcinoma, respectively. The most common causes of patient mortality and morbidity were hepatocellular carcinoma recurrence and biliary complication, respectively. Five-year hepatitis B virus reinfection rate was 7.3% after high-dose hepatitis B immunoglobulin monotherapy combined with rescue antiviral therapy. Overall perioperative recipient mortality rate within first 3 months was 5.8%, whereas perioperative mortality rates in the 1st, 2nd, 3rd, 4th and 5th 200 cases were 12%, 6%, 5.5%, 3% and 2.5%, respectively. A total number of 1175 living donors recovered without sequelae except 1 case of renal failure requiring kidney transplantation. The most important point of donor selection criteria for right liver harvest was preservation of at least 30% of the whole liver mass with full preservation of hepatic venous drainage. In conclusion, the perioperative mortality rate decreased much according to the accumulation of surgical experience. Prudent selection of patients with hepatocellular carcinoma appears to be critical to achieve favorable long-term survival.

Biliary complication was not overcome by technical refinement and remains as the most serious drawback of adult LDLT leading to deterioration of quality of life.

Abstract# 248

Preliminary Experience on Living Donor Exchange Program for Adult Living Donor Liver Transplantation at the Asan Medical Center, Korea. Deok-Bog Moon,¹ Sung-Gyu Lee,¹ Shin Hwang,¹ Ki-Hun Kim,¹ Tae-Yong Ha,¹ Gi-Won Song,¹ Dong-Hwan Jung.¹ ¹Division of Hepatobiliary Surgery, Department of Surgery, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea.

Purpose: When performing donor screening for living donor liver transplantation (LDLT) for an adult patient with end-stage liver disease, ABO blood group incompatibility is the most common cause of donor exclusion. To cope with this problem without performing ABO-incompatible LDLT, living donor exchange program has been maintained at the Asan Medical Center, Seoul, Korea since September 2003. Here we introduce the 8 cases of adult LDLT allocated by living donor exchange program.

Methods: From February, 1997 to December 2006. 1221 cases of adult LDLT were performed in our institution. Among them, there were 8 cases allocating through donor exchange program to avoid ABO mismatching. Four sets of 2 donor-recipient combination pairs were made after direct one-to-one donor-recipient matching.

Results: Three sets of donor exchange LDLT were performed on elective basis, but one in emergency situation. Three living donors and 3 recipients underwent LDLT operations at the same time at the same institution. All 8 living donors recovered well. All of the 8 recipients are doing well to date although 1 recipient had undergone prolonged intensive care for surgical complications. There was no conflict related to donor exchange program.

Conclusions: This is the world-first report on donor exchange adult LDLT. Although this is a preliminary report with only 4 sets, donor exchange program for adult LDLT appears to be a feasible modality to promote LDLT. We believe it can be applicable to a wider population of LDLT after widespread consensus formation.

Abstract# 249

Split Liver Transplantation: 10 Years Experience at a High Volume Center. Vittorio Corno,¹ Domenico Pinelli,¹ MariaClara Dezza,¹ Alessandro Lucianetti,¹ Michela Guizzetti,¹ Marco Zambelli,¹ Mara Giovanelli,¹ Fabio Tagliabue,¹ Michela Bravi,² Paola Stroppa,² MariaGrazia Luca,³ Stefano Fagioli,³ Daniela Codazzi,⁴ Giuliano Torre,² Michele Colledan.¹ ¹Liver and Lung Transplantation Center, Ospedali Riuniti, Bergamo, Italy.

Introduction: Split liver transplantation has been adopted as an alternative to expand the organ donor pool. While the Adult/Child Split Liver (A/CSL) in which an extended right graft (ERG) is transplanted into an adult and a left lateral segment (LLS), transplanted into a child, has gained a wide acceptance within the transplantation community, Adult/Adult Split Liver (A/ASL) in which the liver is split into a full right (FR) and full left (FL) is still performed very rarely. We analyzed the experience at our Center with split liver grafts over a period of 10 years. **Material and Methods:** Between October 1997 and October 2007 we performed a total of 676 liver transplants in 625 recipients (370/328 children and 306/297 adults). Among the 593 (310 children and 283 adults) recipients of a primary isolated liver transplant, 281 (247 children and 34 adult) were transplanted with a A/C or A/A SL. The recipients of a whole size graft (249 adults and 63 children) were used as a control. **Results: Adults:** 34 patients received a split liver graft (26 ERG graft and 8 FL/FR grafts) and 249 a whole liver graft. Overall the incidence of biliary complications using a split liver graft was 29% and with a whole liver graft 15% (p=0,0506). For the recipients of a split liver graft 1 and 5 years patient and graft survival was 91%/88% and 88%/85%. Recipients of a whole liver graft had a 1 and 5 years patient / graft survival of 85%/84% and 80%/78% respectively. **Children:** 247 children received a split graft (230 LLS, 12 ERG and 5 FL) and 63 a whole size graft. Biliary complications occurred in 42% of the recipients of a split liver graft and 5% of the recipients of a whole size graft (p < 0,0001). Among the recipients of a split graft 1 and 5 years patient / graft survival was 91%/84% and 87%/80% respectively. The recipient of a whole size graft had a 1 and 5 years patient / graft survival of 84%/78% and 83%/74%. **Conclusion:** Incidence of biliary complications is significantly higher using a split graft. At a high volume Center, use of split liver grafts reached comparable and even better results of a whole liver graft in terms of patient and graft survival. Thus, the use of split grafts should be strongly encouraged to expand the donor pool.

Abstract# 250

A Matched-Paired Analysis of Adult Living vs. Deceased Donor Liver Transplant. Tun Jie,¹ Elizabeth Gross,¹ Michael Hughes,¹ Mark Hill,¹ Raja Kandaswamy,¹ John Lake,¹ William Payne,¹ Donna Anderson,¹ David Radosevich,¹ Abhinav Humar.¹ ¹Department of Surgery, University of Minnesota, Minneapolis, MN.

Introduction:

Clinical outcomes of adult living donor liver transplant (LDLT) have improved with refined surgical techniques and careful patient selection. The healthcare expenditure of LDLT remains uncertain, in comparison to deceased donor liver transplant (DDLT).

Method:

Groups of primary LDLT and DDLT recipients were identified by matching diagnosis, MELD score, and recipient age. Cost data, acquired from the hospital financial database and inflation adjusted and clinical outcomes were compared between the 2 groups.

Result:

A total of 78 recipients (39 pairs of DDLT and LDLT) were identified from 348 primary adult transplants performed between 2000 and 2006. Mean age, calculated MELD score, and other major diagnostic features did not differ between two groups. The total hospital cost of the transplant was significant higher in DDLT group (\$131,434 vs \$105,244). Between 2000 and 2006, the deceased donor organ acquisition cost has increased 87%, while the living donor cost increased only 30%. Even after excluding the donor expenditure, recipient hospitalization cost of DDLT remain significantly higher secondary to increased incidence of perioperative cardiac, pulmonary, renal complications and re-explorations for bleeding. Incidence of biliary stricture was higher in the LDLT recipients post-transplant (12 in LDLT vs 6 in DDLT). Patient survival rate at 1 month, 1 year and 3 years were 92.3%, 89.7%, and 87.0% for DDLT recipient vs 97.4%, 94.9%, and 86.1% for LDLT (p=0.311, 0.402, and 0.970).

Adult Deceased vs Living Donor Liver Transplant

Groups	DDLT	LDLT	p-value
Age (years)	52.5±6.1	52.9±6.3	0.78
MELD	16.4±5.2	16.5±5.2	0.93
Men (%)	62%	67%	0.64
LOS	17±16	14±10	0.271
Complications			
Cardiac complications	10	4	<0.05
Post-tx dialysis	4	0	<0.02
Bleeding complications	15	9	>0.05
Financial cost (\$1000)			
Total cost	\$131±\$66	\$105±\$39	0.035
Recipient cost	\$84±\$63	\$58±\$37	0.026
Donor cost (2000 vs 2006)	\$31 vs \$58	\$39 vs \$51	

Financial cost and clinical outcomes

Conclusion:

Compared to DDLT, LDLT is cost-effective with comparable clinical outcomes. Increased biliary complications in LDLT did not affect graft or patient survival.

Abstract# 251

Predictors of Short and Long-Term Survival after Adult Living Donor Liver Transplantation. Walid Mohamed Elmoghazy,¹ Yasuhiro Ogura,¹ Kouji Harada,² Hiroto Egawa,¹ Akio Koizumi,² Shinji Uemoto.¹ ¹Department of Surgery, Kyoto University Graduate School of Medicine, Kyoto, Japan; ²Department of Health and Environmental Sciences, Kyoto University Graduate School of Medicine, Kyoto, Japan.

The aim of study is to determine the pre-operative and operative risk factors for short and long-term survival after adult living-donor liver transplantation (ALDLT). We analyzed a series of 408 patients (188 females, 220 males, mean age 47.3 ± 12.4 years) received primary ALDLT for end-stage liver disease at Kyoto University Hospital between 1998 and 2006. The follow-up periods ranged from 4 days to 112 months, with a median of 42 months.

The outcome was defined as 90-day, 1-year and 5-year survival of the patients or survival of the grafts. 44 pre-operative and operative variables were included for statistical analyses using Cox's proportional hazard regression model. In statistical analysis, to prevent masking of the actual risk factors affecting long term survival, we excluded 90-day survival during 1-year survival analysis and excluded 90-day and 1-year survival during 5-year analysis using Cox's proportional hazard regression model with Landmark method. We thus defined cases that died between 0 days and 90days, or 91 days and 1 year, or 1 years and 5 years and controls who sustained at each time point.

Graft or patient survivals were 86%, 79% and 74% at 90-day, 1-year and 5-year respectively, more than 50% of graft or patient loss happened during the first 3 months post LDLT.

For 90-day survival multivariate Cox regression analysis showed that 2 HLA-DR mismatches (Hazard Ratio (HR) = 4.78, CI = 1.473-3.799), operative blood loss (HR = 3.75, CI = 1.263-4.346), MELD score>25 (HR = 3.13, CI = 1.012-1.094), history of prior upper abdominal surgery excluding laparoscopy (PUAS) (HR = 2.78, CI = 1.355-4.647), creatinine clearance (HR = 2.43, CI = 1.323-3.088) and diabetes (HR = 2.25, CI = 1.018-3.872) were the risk factors.

For 1-year survival, MELD score>25 (HR = 2.30, CI = 1.029-1.097) and ABO-incompatibility (HR = 1.88, CI = 1.523-4.121) were independent risk factors. For 5-year survival, hepatocellular carcinoma (HR = 2.72, CI = 0.997-3.437) and ABO-incompatibility (HR = 2.11, CI = 1.056-1.886) were independent risk factors.

For short-term survival, general condition of the patient and operative blood loss are determinants, while pathogenesis of the liver disease is determinant for long-term outcome after ALDLT. In contrast, immunological incompatibilities are constantly risk factors for ALDLT.

Abstract# 252

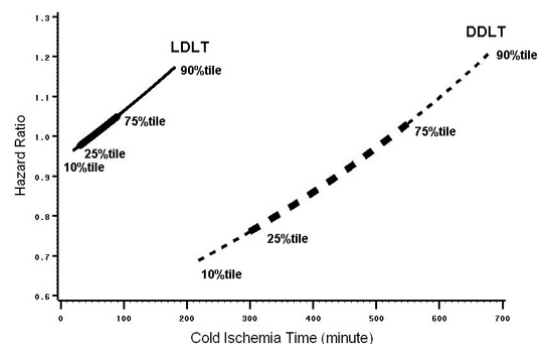
Association of Cold Ischemia Time and Acute Rejection after Living Donor and Deceased Donor Liver Transplantation. A. Shaked,¹ R. M. Ghobrial,¹ R. M. Merion,¹ T. H. Shearon,¹ J. C. Emond,¹ J. H. Fair,¹ R. A. Fisher,¹ L. M. Kulik,¹ T. L. Pruett,¹ N. A. Terrault.¹ ¹A2ALL Study Group, NIH-NIDDK.

Previous analysis from the Adult-to-Adult Living Donor Liver Transplantation Cohort Study (A2ALL) revealed that the overall rate of biopsy-proven acute cellular rejection (ACR) in recipients of living donor (LDLT) or deceased donor liver transplants (DDLT) was similar. However, because variables may be differentially associated with ACR depending on donor type, this study focused specifically on the relationships between cold ischemia time (CIT), donor type (DDLT vs. LDLT), and ACR.

Methods: The A2ALL Retrospective Study included 819 subjects who had a potential living donor evaluated between 1/1/98 and 2/28/03 at 9 U.S. transplant centers. Of these, 593 patients received a transplant (380 LDLT; 213 DDLT). Median post-transplant follow-up was 778 and 694 days for LDLT and DDLT recipients, respectively. Biopsy-proven rejection was defined as the presence of rejection on confirmatory liver biopsy and treatment for rejection. Cox regression models were fitted to investigate predictors of time from transplant to first biopsy-proven rejection. Covariate effects are presented as adjusted hazard ratios (HR) with 95% confidence intervals (CI).

Results: CIT was much longer for DDLT (median 440 min, range 10 to 1570) vs. LDLT (median 50 min, range 4 to 574; p<0.001). The risk of rejection for all liver transplant recipients was higher with longer CIT (HR=1.08 per hour, p=0.02). The risk of rejection for an LDLT at the median CIT of 50 minutes was similar to that of a DDLT at the median CIT of 440 minutes, although for a given CIT, LDLT was associated with significantly higher risk of rejection than DDLT (Figure)(HR=1.78, p=0.03).

Conclusions: We found a significant relationship between CIT and ACR for both LDLT and DDLT. Reducing CIT in LDLT may not be practicable. However, efforts to shorten CIT for DDLT may result in a lower risk of ACR. The significantly higher rejection rate in LDLT vs. DDLT, given similar CIT, suggests a testable hypothesis that liver regeneration after LDLT may predispose to a higher risk of ACR.

Hazard Ratio for Rejection vs. CIT by Transplant Type**Abstract# 253**

Graft Weight to Recipient Weight Ratio (GW/RW): How Well Does It Predict Outcome after Partial Graft Liver Transplant? Mark Hill,¹ Michael Hughes,¹ Elizabeth Gross,¹ Tun Jie,¹ John Lake,² Raja Kandaswamy,¹ William Payne,¹ Melissa Cohen,¹ Abhinav Humar.¹ ¹University of Minnesota, Minneapolis, MN.

Background: Liver transplant recipients with (GW/RW) < 0.8% are thought to have a higher incidence of post-operative complications, including small for size syndrome (SFSS), and overall inferior outcomes. We analyzed a cohort of partial liver graft recipients and compared those with GW/RW < 0.8% to those with GW/RW > 0.8%.

Results: Between 1999 and 2007, 107 adult patients underwent partial graft liver transplant. Seventy-six grafts were from live donors (LDLT) and the remaining 31 were from deceased donor split-liver transplants (SLT). Of these, 22 patients had GW/RW < 0.8% (12 LDLT, 10 SLT), and 85 had GW/RW > 0.8% (64 LDLT, 21 SLT). Median follow-up was 46.1 months and did not differ between the two groups. Baseline demographics including donor and recipient age, and MELD at the time of transplant also did not differ between groups (Table). Three-month and 1-year graft survival for the two groups was comparable. Three recipients with GW/RW < 0.8% developed SFSS (13.6%); all three had inflow modification with splenic artery ligation (SAL), and 1 of the 3 grafts was lost at one-year. Eight recipients with GW/RW > 0.8% developed SFSS (9.4%) (p = ns); six underwent SAL and none had graft loss at one-year. There was a trend toward increased hepatic artery thrombosis with the smaller grafts that did not reach statistical significance (p = 0.10). The incidence of other surgical complications was similar between the two groups (Table).

Baseline demographics and outcomes for the two groups			
	GW/RW < 0.8%	GW/RW > 0.8%	p value
Donor Age (±SD)	29.8 ± 11.1	34.8 ± 10.8	0.052
Recipient Age (±SD)	52.7 ± 7.35	49.6 ± 10.5	0.196
Mean MELD	15.7	17.7	0.195
Mean GW/RW	0.71	1.21	< 0.001
Median F/U (months)	55.3	44.4	0.116
Graft Survival 3/12 mos.	95.5% / 90.9%	96.5% / 91.8%	1.0/1.0
SFSS	3 (13.6%)	8 (9.4%)	0.693
Infectious complication	6 (27.3%)	29 (45.9%)	0.148
Hemorrhage	8 (36.4%)	23 (27.1%)	0.434
Biliary complication	3 (13.6%)	26 (30.6%)	0.177
Hepatic art. thrombosis	3 (13.6%)	3 (3.5%)	0.099
Portal vein thrombosis	1 (4.5%)	5 (5.9%)	1.00

Conclusion: GW/RW alone does not appear to be the primary determinant of outcome after partial liver transplant. With proper manipulation, selected recipients of smaller grafts may achieve acceptable outcomes.

Abstract# 254

Outcome Comparison of Whole Versus Split-Liver Grafts in Adults in the MELD Era: Analysis of UNOS/OPTN Database. Trina Steljes,¹ Yong Cho,^{1,2} Jose Almeda,¹ James Ciccirelli,² Maria Stapfer,¹ Rodrigo Mateo,¹ Linda Sher,¹ Rick Selby,¹ Yuri Genyk.¹ ¹*Surgery, University of Southern California Medical School, Los Angeles, CA;* ²*National Institute of Transplantation, Los Angeles, CA.*

Background: A chronic shortage in donor liver grafts spurred the evolution of split liver transplantation (SLT). Some earlier studies comparing the whole liver transplants (WLT) to SLT for adult recipients demonstrated worse outcomes with regards to primary nonfunction, biliary complications, and graft survival. Recent studies demonstrate more comparable outcomes between WLT and SLT, however their results often utilize recipients from both pre and post model-for-end-stage- liver-disease (MELD) implementation.

Aims: This study retrospectively compares SLT and WLT in adults in the MELD era by examining the UNOS/OPTN database with the intent to identify recipient factors that may impact outcomes.

Materials and Methods: From January 1, 2002 to December 31, 2006, 11,286 deceased donor (DD) liver transplants in recipients age>18 years from donors between 12 and 45 years old were reported to the OPTN/UNOS. We excluded suboptimal liver grafts such as donation from cardiac death (DCD) and body mass index (BMI)>32 kg/m2. Of these, 10,982 WLT and 304 SLT were included for the univariate and multivariate analyses using the Cox regression model. The log-rank test was used for comparison of the two survival curves.

Results: Patient survival rates of SLT (90% at 1-yr, 84% 3-yr, and 72% 5-yr) were not significantly different to those of WLT (89% at 1-yr, 81% 3-yr, and 70% 5-yr, P=0.36). Overall (unadjusted) graft survival rates of SLT (85% at 1-yr, 78% 3-yr, and 66% 5-yr) were similar to those of WLT (86% at 1-yr, 77% 3-yr, and 63% 5-yr, P=0.74). Incidence of primary graft failure was 2.6% for SLT compared with 1.9% for WLT (P=0.38). Incidence of graft loss due to vascular thrombosis in SLT group (1.6%) also was not significantly different than that of WLT group (1.1%), (P=0.33). Incidence of biliary tract complications was significantly higher in SLT versus WLT recipients (2.0% vs 0.3%, P<0.001). However, graft survival rates of SLT were not significantly different than the WLT group (P=0.656) after adjusting for confounding factors such as MELD, recipient age and cold ischemia time.

Conclusion: In the MELD era, SLT in adults yielded comparable graft and patient survival to WLT from ideal donors.

Proteomics and Genomics

Abstract# 255

A Microarray-Based Classifier for Diagnosing Rejection in Kidney Transplants: The Top Diagnostic Genes for Rejection Are Interferon-γ Induced. Jeff Reeve,¹ Gunilla Einecke,¹ Bruce Kaplan,¹ Phil F. Halloran.² ¹*Medicine, University of Alberta, Edmonton, AB, Canada;* ²*Medicine and UIC Transplant Center, University of Illinois, Chicago, IL.*

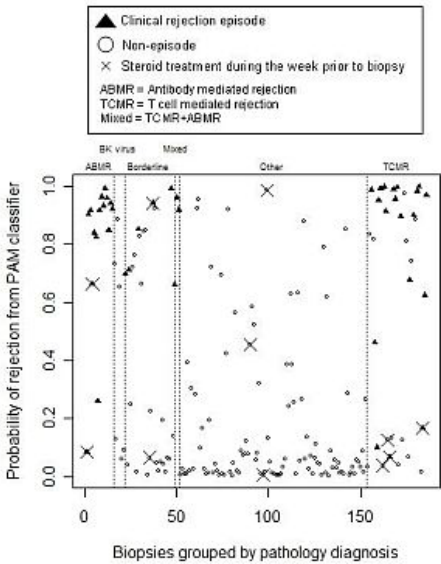
The diagnosis of rejection in post-transplant kidney disease depends largely on the assessment of biopsy pathology, which suffers from arbitrary scoring, subjectivity, and sampling variance. With microarray gene expression data from 186 biopsies for cause, we used Predictive Analysis of Microarrays (PAM) to build a rejection classifier. Because of the imperfect gold standard, the classifier cannot and should not have extremely high predictive accuracy. The goal was to see if microarrays could detect a robust rejection signature despite this uncertainty, and to identify the top genes distinguishing rejecting from non-rejecting biopsies. By examining discrepancies between pathology diagnoses and PAM predictions, and using clinical follow-up information, we combine the strengths of each method to produce a more accurate diagnostic system.

Biopsies with rejection plus clinical episodes (Fig. 1) have higher positive predictive value than those lacking episodes. The majority of episodes occur in the top part of Fig.1, indicating that a valid rejection signature is being detected. Of the exceptions, most had been treated with steroids before the biopsy, suppressing their gene expression patterns. Samples called borderline rejection separated into two distinct high/low probability

groups using PAM, with all the cases classified as clinical rejection episodes occurring in the high probability group.

Of the 39 genes chosen by the classifier, 36 had previously been annotated in our studies of mouse kidney graft rejection. GZMA, GZMB, and PRF1 ranked 26th, 32nd, and 38th respectively. The top five genes were: CXCL11, GBP1, CXCL9, INDO, and CXCL10, all previously annotated in rejecting mouse kidneys as interferon-γ induced genes.

In summary, gene expression-based classifiers, combined with histopathology, promise more accurate diagnostic assessment of the state of kidney transplants at the time of biopsy. Moreover our data driven classifier independently identifies the genes previously annotated in experimental studies.



Abstract# 256

Influence of Donor Toll-Like Receptor 4 Genotype on Delayed Graft Function after Kidney Transplantation. Bernd Krüger,^{1,2} Shay Harris,³ Moheeb al Awawami,³ John Vella,³ Barbara T. Murphy,¹ Bernd Schröppel.¹ ¹*Division of Nephrology, Mount Sinai School of Medicine, New York, NY;* ²*Klinik und Poliklinik für Innere Medizin II, Universität Regensburg, Regensburg, Germany;* ³*Division of Nephrology, Maine Medical Center, Portland, ME.*

Background: Renal-cell associated TLR4 activation was found to be important in mediating ischemia reperfusion injury to murine kidneys. We hypothesized that genetic variations within the TLR4 gene of the kidney donor affects the rate of delayed graft function (DGF).

Methods: We genotyped the functional TLR4 polymorphisms (SNPs) D299G (rs4986790) and T399I (rs4986791) in 268 kidney donors from 2 centers and correlated them with the occurrence of DGF. DGF was defined as the need of dialysis within 7 days post-transplant or less than 25% drop in creatinine within the first 24 hours after transplant. In a sample of 67 patients from one center, we analysed intragraft HMGB-1 (High Mobility Group Box Protein-1) gene expression in pre- and post-implantation biopsies. Statistical analyses were performed using the SPSS statistical package.

Results: Both TLR4 SNPs were in Hardy-Weinberg Equilibrium, and were combined for further analysis. Recipients of a TLR4 mutated kidney showed a significant lower rate of DGF (p=0.005), which was persistent after correction for known donor-derived risk factors (donor age, ECD vs. DCD-kidney, cold ischemia time, p=0.007, HR 4.42, CL 1.51-12.94). Additionally, we confirmed the presence of a possible TLR4 ligand, HMGB-1, in pre- and post-implantation biopsies. We also confirmed the presence of a functional TLR4 receptor in human proximal tubular cells which expressed MCP-1, IL-1-β, IL-6 and TNF-α after specific TLR4 agonist treatment.

Conclusion: Human renal tubular epithelial cells express TLR4 and a functional TLR4 SNP in donor kidney is associated with lower rate of delayed graft function.

Abstract# 257

High-Throughput Assessment of CpG Site Methylation in HCV-Infected Hepatocellular Carcinoma Patients. Kellie Archer,¹ Valeria Mas,¹ Daniel Maluf,¹ Robert Fisher.¹ ¹*Hume-Lee Transplant Division, VCUHS, Richmond, VA.*

Background: Methylation of promoter CpG islands has been associated with gene silencing and demonstrated to lead to chromosomal instability. We postulate that differences in methylation patterns observed in tissues ranging from normal to cirrhosis to HCC may lead to the discovery of direct precipitating events that contribute to tumorigenesis in HCV-HCC patients.

Methods: DNA from 20 HCV-HCC tumors and corresponding non-tumor HCV cirrhotic tissues as well as 20 independent HCV cirrhotic tissues and 20 normal liver tissues were bisulfite treated and hybridized to the Illumina GoldenGate Methylation BeadArray Cancer Panel I for interrogating CpG sites in the promoter regions of 808 distinct genes. For each CpG site, a summary statistic representing “percent methylated” was estimated. For each CpG site, a Jonckheere-Terpstra test was applied to identify whether there was a significant monotonic trend in percent methylated across the independent normal, cirrhotic, and HCC tissues. In addition, the paired HCC and non-tumor cirrhotic tissues were analyzed using a paired t-test. The q-value method for estimating gene-wise false discovery rates (FDR) was used to adjust for multiple comparisons; CpG sites with an FDR<0.05 were considered significant.

Results: Scatterplots and Pearson's correlation for the 12 samples hybridized in duplicate revealed high reproducibility of the assay (median rho=0.975, range 0.935, 0.996). When comparing the paired HCV-HCC and HCV-cirrhotic non-tumor liver tissue samples, 43 CpG sites (corresponding to 36 unique genes) were identified as differentially expressed using a paired t-test (FDR<0.05). When examining CpG islands with a significant monotonic trend across the independent normal, HCV cirrhosis, and HCV-HCC samples, 319 CpG sites had a significant increasing trend (hypermethylated) while 198 CpG sites had a significant decreasing trend (hypomethylated)(FDR<0.05). Restricting attention to those CpG sites with an FDR<0.05 and with a difference in mean percent methylation between normal and HCV-HCC of at least 0.1, 161 CpG sites (128 unique genes) had an increasing trend while 108 (90 distinct genes) had a decreasing trend. CpG sites with significant hypermethylation were located in the promoter regions of genes known to be important in carcinogenesis, including the tumor suppressor gene *CDKN2B*, and genes associated with other cancers *FLT3*, *HOXA9*, *RUNX1T*, *HOXB2*, and *MET*.

Conclusions: Methylation events are important in hepatocellular carcinogenesis.

Abstract# 258

Integrative Genomics To Identify Non-HLA Allogenic Kidney-Specific Targets after Kidney Transplantation. Li Li,¹ Persis Wadia,¹ Rong Chen,¹ Maarten Naesens,¹ Shiquan Wu,¹ David Miklos,¹ Atul Butte,¹ Minnie Sarwal.¹ ¹Pediatrics and Medicine, Stanford University, Stanford, CA.

AIMS: To identify if serological responses to allogenic non-HLA renal compartment-specific antigens can be detected after renal transplantation (txp).

METHODS: 36 paired pre- and post-transplant (mean time 25 months) serum samples from 18 pediatric kidney allograft recipients were used for identification of *de novo non-HLA* antibody formation assessed using Invitrogen ProtoArray (v3), measuring signal post-tx. Probes from the Protoarray and cDNA microarray platforms were re-annotated to current NCBI Entrez gene identifiers using AILUN. 34 cDNA arrays (GSE:3931) from 7 normal kidney regions (inner and outer cortex, inner and outer medulla, papillary tips, renal pelvis and glomeruli; see Table) were analyzed for compartment-specific genes by SAM, and the highest-ranked genes in each compartment (KS two-sample test $p < 0.001$) and were ranked against each individual patient's numerical antibody response across the 5057 proteins on the Protoarray.

RESULTS: In 83% of patients (15/18), kidney-specific serological responses against the renal pelvis were the first to be detected, followed by responses against renal cortex. Control gene expression data sets from other solid organs (GSE: 1133; lung, heart, pancreas) did not demonstrate immune-response enrichment. The presence of these antibodies was not associated with donor specific HLA class I or II antibody levels.

CONCLUSION: We demonstrated *de novo* formation of circulating non-HLA non-ABO antibodies after txp, irrespective of HLA antibodies, are detected against kidney-specific protein targets. As these antibodies are not seen against other solid organs, the response is likely allogeneic. The renal pelvis and renal cortex have the highest immunogenic potential.

Table 1: Allo-antigenic targets by anatomic regions mapped between cDNA microarray and ProtoArray Human Protein Microarrays.

Anatomic Location	# of Probes (FDR < 5%)	# of Up Probes (log Fold > 1)	# of Up and Map to Single Gene ID (AILUN)	Mis-Annotation Rate	Detected on ProtoArray	Overlapped Percentage (cDNA and ProtoArray)
Glomeruli	4957	1522	842	45%	161	19%
Inner Cortex	1354	1354	900	34%	201	22%
Outer Cortex	2404	2095	1405	33%	336	24%
Inner Medulla	57	57	37	35%	9	24%
Outer Medulla	270	245	150	39%	29	19%
Pelvis	7932	3438	2299	33%	465	20%
Papillary Tip	2237	1076	675	37%	167	25%

Abstract# 259

Urinary Cell mRNA Profiles Are Diagnostic of Chronic Allograft Nephropathy. T. Muthukumar, R. Wanchoo, C. Chang, C. Snopkowski, R. Ding, B. Li, V. Sharma, S. Seshan, D. Dadhania, M. Suthanthiran. *Nephrology, Cornell University Medical College, New York, NY.*

Chronic allograft nephropathy (CAN) is the commonest cause of allograft failure 3 months after renal transplantation. Interstitial fibrosis and tubular atrophy are the histological characteristics of CAN and their presence can only be ascertained by allograft biopsy.

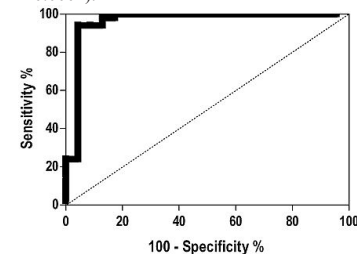
We reasoned that mRNA phenotyping of urinary cells that includes measurement of mRNA for tubule specific proteins & proteins implicated in epithelial to mesenchymal transition (EMT) would be informative of renal allograft status with respect to presence or absence of CAN.

We tested our hypothesis by measurement of urinary cell levels of mRNA for TGFβ1, βIGH3, αSMA, USAG1 & NKCC2 in urine samples collected from 73 human kidney transplant recipients. Samples were collected at the time of allograft biopsy from 23 subjects with biopsy confirmed CAN (Banff 97) & 50 subjects with stable graft function & normal allograft protocol biopsy. We isolated total RNA from urine cell pellets, reverse transcribed to cDNA & measured mRNA levels using gene specific primers and probes in the pre-amplification enhanced RT-PCR assays. Transcript levels were expressed as copies / ug total RNA.

Urinary cell mRNA levels of genes associated with interstitial fibrosis and tubular atrophy were significantly higher in the 23 recipients with CAN compared to the 50 recipients with stable graft function and normal allograft protocol biopsy.

Type of mRNA	CAN (Banff 97) N=23	Normal Biopsy N=50	P
	median copies/ ug total RNA		
TGFβ1	742000	313000	0.04
βIGH3	118000	39850	0.03
αSMA	15600	5475	<0.01
USAG1	30	793	<0.0001
NKCC2	450	1785	<0.01

A linear combination of mRNA levels of βIGH3, αSMA and USAG1 predicted the presence of CAN with high accuracy (Sensitivity 94%, Specificity 96%, AUC 0.96, $P < 0.0001$).



We conclude that urinary cell mRNA profiles that include levels of mRNA encoding tubular proteins and mRNA encoding proteins implicated in EMT offers a noninvasive means ascertaining renal allograft status with respect to presence or absence of CAN.

Abstract# 260

Functional Pathways Involved in Operational Allograft Tolerance. Marc Martinez-Llordella,¹ JuanJose Lozano,¹ Isabel Puig-Pey,¹ Carlos Benitez,¹ Antoni Rimola,¹ Alberto Sanchez-Fueyo.¹ ¹Liver Transplant Unit, Hospital Clinic Barcelona, IDIBAPS, Ciberehd, University of Barcelona, Barcelona, Spain.

Spontaneous allograft acceptance (operational tolerance) is sporadically reported in humans and constitutes a proof-of-principle that immunological tolerance can be achieved in clinical transplantation. The search for common features among tolerant recipients of different organs is a fundamental challenge to advance our understanding of the mechanisms of clinical allograft tolerance. In the current study we have conducted molecular profiling of peripheral blood from a cohort of 22 operationally tolerant and 31 non-tolerant liver recipients employing Affymetrix microarrays. Gene expression patterns have been compared with Affymetrix data obtained from non-transplanted healthy individuals and with a previously published cDNA array dataset (Brouard S. PNAS 2007; 104:15448) comprising 22 kidney recipients (11 tolerant, 11 chronic allograft nephropathy). Differential gene expression between tolerant and non-tolerant samples has been computed employing Significant Analysis of Microarray (SAM). Functional profiling of expression data has been conducted utilizing both gene-set enrichment analysis (GSEA) and Ingenuity Pathway Analysis (IPA). Comparison of tolerant and non-tolerant liver samples employing SAM yielded a total of 2341 probes with FDR<5%. Based on this dataset, GSEA identified hypertrophy pathway (e.g. ILR1, IFNg, VEGF), CTLA4 pathway (e.g. CTLA4, ICOS, CD28), hypoxia-inducible factor pathway (e.g. HIF1A, JUN), NTHI pathway (e.g. TNF, IL1B), LAIR pathway (e.g. C5, ICAM1) and IL1R pathway (e.g. ILR1, IL1B, TRAF) as the most significantly enriched in non-tolerant recipients, while natural killer related genes (e.g. NKG7, KLRD1, KIR2SD2) were significantly enriched in tolerant samples. IPA confirmed natural killer cell signaling as the most significant pathway associated with tolerance status. Comparison of the liver and kidney datasets yielded 61 identical genes. Functional annotation of these genes identified cell cycle pathway and carboxylic acid metabolism pathway as potentially relevant for both datasets. Altogether, these data suggest that as compared with recipients requiring on-going immunosuppression, operationally tolerant liver recipients exhibit traits of immunological quiescence and activation of natural killer related pathways. Liver and kidney tolerant states appear to be fundamentally different biological processes, although some common pathways could be relevant to both settings.

Abstract# 261

Genetic Risk Factors of Posttransplant Diabetes Mellitus in Hispanic Kidney Transplant Patients: A Single Center Study. Jae W. Yang,^{1,4} Ian V. Hutchinson,^{2,4} Vera Pravica,^{2,4} Gilbert Burckart,² Tariq Shah,^{1,2,3,4} David I. Min.^{1,3,4} ¹Health Sciences, Western University, Pomona, CA; ²University of Southern California, Los Angeles, CA; ³Saint Vincent Medical Center, Los Angeles, CA; ⁴National Institute of Transplantation, Los Angeles, CA.

Incidence of type 2 diabetes mellitus (DM) are significantly different among different ethnic groups in a general population. A new onset post transplant diabetes mellitus (PTDM) is an important complication after kidney transplantation and is associated with reduced overall patient and graft survival.

Objectives: to determine the genetic risk factors for PTDM in Hispanic kidney transplant patients.

Methods: A total of 572 Hispanic patients who have received kidney allograft at St. Vincent Medical Center, between January 1, 2001 and September 30 2005 were reviewed and a total of 230 patients were included in this study. The patients who have DM as a primary diagnosis prior to transplantation, with transient hyperglycemia or extra renal transplantation were excluded. Recipients' DNAs were genotyped for a total of 14 diabetes associated genes reported in the literatures and incorporated for binary logistic regression analysis to determine the association by using SPSS. $P < 0.05$ was regarded as of statistical significance.

Results: A total of 59 patients were fit to our definition of PTDM (fasting glucose ≥ 126 mg/dL in two occasions). In the case of genotypes, significant associations were found between the development of PTDM and the distributions of mutant alleles in two hepatocyte nuclear factor 4-a (HNF4A) of rs2144908 ($p = 0.008$) and rs1800961 ($p = 0.004$), hepatocyte nuclear Factor 1-a (HNF1A, rs=1800574) ($p < 0.002$), and Insulin Receptor Substrate 1 (IRS1, rs1801278) ($p = 0.006$) genes. But all other genes were not significantly associated with PTDM in kidney transplant patients with Hispanic ethnicity.

Conclusion: This study indicates that HNF-4a, HNF-1a, and IRS1 gene mutant alleles are significantly associated with PTDM in kidney transplant patients with Hispanic ethnicity.

Abstract# 262

Transcriptome Analyses Identify Common Pro-Inflammatory Changes in Renal Allografts during the First Year Post Transplant. Walter D. Park,¹ Matthew D. Griffin,¹ Bruce Morlan,² Fernando G. Cosio,¹ Mark D. Stegall.¹ ¹Transplant Center, Mayo Clinic, Rochester, MN; ²Biostatistics, Mayo Clinic, Rochester, MN.

Introduction: Both normal and fibrotic renal allografts develop a large number of persistent changes in pro-inflammatory gene transcripts early after transplantation (1). The aim of the current study was to determine if this "transplant effect" is attenuated by immunosuppression, fibrosis or HLA-match.

Methods: We studied 41 histologically normal implantation biopsies (T0) and 45 12m protocol biopsies (T12) from living donor kidney transplant recipients who had no identifiable post-transplant complication (no acute rejection, polyoma virus, etc). Patients were stratified into 4 distinct clinicopathologic groups. The first three groups had normal histology at both T0 and T12 and included: 1) HLA identical-Tac treated ($n = 10$); 2) non-HLA identical-Tac ($n = 19$); 3) non-HLA identical Srl (Tac-free, $n = 8$). A fourth group was histologically normal at T0 but developed mild fibrosis by T12 ($n = 7$). mRNA expression from these biopsies was measured using the U133Plus2.0 microarray and custom Taqman Low Density Arrays (TLDA). Data for each group was tested using a generalized linear model and changes common to each dataset identified ($p < 0.05$).

Results: In addition to transcript changes specific for each clinicopathologic condition, a large number of transcripts ($n = 1152$) were altered in all groups. 68% ($n = 778$) had higher expression in all comparisons at T12 versus T0, including fibronectin and collagen IV. 32% ($n = 374$) of transcripts were considered down-regulated, including interleukin 6 and 1 β . Gene Ontology and Signaling pathway analyses showed many of the transcripts to be involved in pro-inflammatory and immune response signaling pathways (ex. TLR, IL-10/-2, etc). Custom TLDA were used to study several genes considered not detected by microarray. This included TGF- β 1, CD-3E/-28/-74 and VEGF, all of which were significantly up-regulated in multiple comparisons.

Conclusions: Persistent changes in pro-inflammatory transcripts occur in all renal transplants. This "transplant effect" cannot be explained by calcineurin-inhibitors (occurs in Tac-free immunosuppression), increased alloreactivity (similar in HLA identical/non-identical) or fibrosis. A likely cause is persistent changes from IR injury. The role of the "transplant effect" in long-term allograft survival is unclear, but it likely interacts with other forms of graft injury.

1. Park et al. *Transplantation*. 2007;1466-76.

Abstract# 263

A Minimal, Highly Specific and Sensitive Gene-Set for Peripheral Blood-Based Diagnosis and Prediction of Acute Renal Transplant Rejection. Lihua Ying, Li Li, Jonathan Martin, Sue Hsieh, Minnie Sarwal. *Pediatrics, Stanford University School of Medicine, Stanford, CA.*

BACKGROUND: A critical 26 gene-set for acute renal rejection (AR) diagnosis in peripheral blood has been previously identified by our group using cross-platform

hybridization of 91 unique peripheral blood samples with matched biopsy diagnosis, with AR ($n = 39$) and without AR (STA; $n = 52$), with cross-hybridization across 3 human microarray platforms: 30k cDNA Lymphochip, 44k Agilent and 54k Affymetrix HU133plus. In this study we proposed to verify and validate this gene-set for AR diagnosis and prediction by peripheral blood PCR-based analysis.

METHOD: 57/91 peripheral blood samples from the microarray gene-set were selected for 384-well format multiplex ABI-Taqman qPCR validation of AR diagnosis across the 26 gene-set. An independent set of 66 new, time and immunosuppression matched, peripheral blood samples (30STA, 36AR) were used as a test-set for blinded prediction of AR diagnosis using qPCR across the most informative 8 genes. 42 sequential samples from 14 AR patients (at AR, 1-3 months prior to, and after AR) were also examined by qPCR for these 8 genes for blinded AR prediction, prior to biopsy proven AR. Prediction models were generated using logistic regression analysis and $p < 0.05$ considered significant.

RESULT: In the validation set of 57 samples, 8/26 genes were highly significant for confirming peripheral blood-AR diagnosis ($p < 0.03$). In the test group of 66 samples, AR was predicted with a very high level of sensitivity and specificity (PPV and NPV > 90%). In the group of 14 AR patients with sequential samples pre- and post-AR, expression for these 8 genes were significantly elevated in the pre-AR samples (vs. STA, $p < 0.01$) but were not different between pre-AR and AR values.

CONCLUSION: A highly specific and sensitive biomarker panel of 8 genes has been derived and tested by cross-platform microarray analysis. This gene-set has now been validated and further verified by multiplex PCR, for AR diagnosis and prediction, even 3 months prior to the rejection event. Utility of this gene-set should be further validated in real-time by serial longitudinal peripheral blood testing, for more sensitive and specific minimally invasive monitoring for graft rejection.

Tolerance/Immune Deviation II

Abstract# 264

Treg Trafficking from the Islet Allograft to the Draining Lymph Node Is Required To Inhibit Antigen-Specific Immunity. Nan Zhang,¹ Jordi C. Ochando,¹ Jonathan S. Bromberg,¹ *Gene and Cell Medicine, Recanati/Miller Transplantation Institute, Mount Sinai School of Medicine, New York, NY.*

Background: Our previous studies showed that regulatory T cells (Treg) execute suppressive function in both the inflammatory site of the allograft and the draining lymph node (dLN) to suppress allograft rejection. We explored how Treg influenced the migration and function of antigen specific effector T cells and dendritic cells (DC) at these two sites in an islet transplant model.

Methods: Treg were sorted from wild type or CCR7^{-/-} C57BL/6 mice, labeled with PKH26, and transferred directly to islet allografts (BALB/c into C57BL/6). Effector CD4 T cells (Te) from alloantigen specific T cell receptor transgenic mice were labeled with CFSE and transferred intravenously. Treg and Te migration to islet grafts and dLN were analyzed with flow cytometry and immunohistochemistry. Chemokine expression was determined by RT-PCR. CX3CR1^{GFP} mice, in which DC are internally labeled with GFP, served as islet donors, and donor DC migration was tracked with fluorescence microscopy.

Results: During priming and rejection, islet allografts expressed a panel of inflammatory chemokines shortly after transplantation that recruited Te to the islets. Te accumulated and proliferated in both the islet allograft and the dLN. Concomitantly, donor derived DC migrated from the islet to the dLN as early as 1 day after transplantation. Local transfer of Treg into the islet allograft inhibited islet chemokine expression, inhibited donor DC migration in an IL-10 and TGF β dependent fashion, and reduced Te migration to and proliferation in the graft. The transferred Treg also migrated from the islet allograft to the dLN, and directly inhibited Te accumulation and proliferation in the dLN, and migration of Te to the islet. CCR7^{-/-} Treg, which could not migrate to the dLN but were retained within the graft, and were far less effective than wild type Treg in inhibiting Te migration and proliferation into both the islets and dLN.

Conclusions: Treg migrate to the islet and suppress parenchymal cell chemokine expression, DC migration, and Te responses in the islet allograft. Treg also migrate to the dLN to suppress Te proliferation and migration. Treg trafficking from the allograft to the dLN is crucial for suppressive function in order to target the separate effector mechanisms. These results demonstrate novel and important functions for migration in Treg induced suppression and graft survival.

Abstract# 265

Tregs Undergo a High Basal Rate of Homeostatic Proliferation That Is Augmented by Anti-CD45RB and Regulated by TCR and Calcineurin, but Not TGF β , Signaling. Geoffrey Camirand,¹ Ying Wang,² Richard Flavell,² Fadi Lakkis,¹ David Rothstein.² ¹University of Pittsburgh; ²Yale Medical School.

CD4⁺Foxp3⁺ regulatory T cells (Tregs) play an important role in transplant tolerance, yet basic aspects of Treg biology including the mechanisms involved in their induction remain unclear. α -CD45RB is a potent tolerogenic agent that induces a 2X increase in number of Tregs in wt mice, even in the absence of allo-antigen. Here we use Foxp3-red fluorescent protein reporter knock-in mice to study Treg homeostasis and identify the mechanisms by which α -CD45RB induces Tregs. CFSE-stained highly sort-

purified Foxp3⁺ or Foxp3⁻ cells were adoptively transferred into fully replete naive wt congenic mice. Whereas only 5-10% of transferred Foxp3⁻ cells undergo homeostatic proliferation (HP) over 10d, 50-70% of Foxp3⁺ cells proliferate - a surprisingly high rate of HP. Moreover, treatment with α -CD45RB markedly enhanced HP by transferred Foxp3⁺ cells, resulting in a 10X increase in cell number. α -CD45RB induced de novo Foxp3 expression in 13-25% of transferred Foxp3⁻ cells (many of which subsequently underwent HP). Thus, α -CD45RB induces both conversion of Foxp3⁻ to Foxp3⁺ cells and promotes HP in Foxp3⁺ cells in the absence of exogenous antigen. We then addressed the signals controlling basal HP of Tregs and both HP and conversion mediated by α -CD45RB. CFSE-stained congenic CD4⁺ cells adoptively transferred into naive wt mice were untreated, or received CsA, α -CD45RB, or both and Foxp3⁺ and Foxp3⁻ CD4 cells were assessed on d10. Calcineurin inhibition greatly reduced basal HP of transferred Foxp3⁺ cells compared to untreated mice. Moreover, CsA completely abrogated increased Treg HP induced by α -CD45RB, although conversion still occurred. Next we assessed the role of TGF β . We found that blocking TGF β signaling with α -TGF β shortened allograft survival, but had no effect on basal Treg HP, or on Treg HP or conversion by α -CD45RB. Finally, although exogenous antigen is not required, basal and α -CD45RB-induced HP may still require recognition of self-Ag by Tregs. Indeed, preliminary results reveal that when CFSE-labeled CD4⁺ cells are transferred into MHCII KO mice, basal HP by Tregs is reduced and not restored by α -CD45RB. Thus, α -CD45RB alters normal controls regulating HP by Treg. Moreover, both basal and α -CD45RB-mediated HP requires intact calcineurin activity and TCR signaling, but not TGF β . Understanding the signals controlling HP in Treg may reveal new therapeutic targets for tolerance induction.

Abstract# 266

Induction of Heart Transplant Tolerance by Post-Transplant Administration of Allo-Ag Specific Treg and Rapamycin. Giorgio Raimondi,¹ Tina L. Sumpter,¹ Benjamin Matta,¹ Daisuke Tokita,¹ Zhiliang Wang,¹ Angus W. Thomson.¹ ¹*Surgery, Starzl Transplantation Institute, University of Pittsburgh, Pittsburgh, PA.*

We report the first demonstration of a tolerance-inducing strategy based on post-transplant administration of allo-Ag-specific Treg (AAsTreg) and rapamycin (Rapa), in a fully allogeneic, unmanipulated mouse heart transplant model.

Enrichment of naturally-occurring AAsTreg represented the first step to counterbalance the high frequency of alloreactive T cells. Selection was achieved by co-incubation of freshly-isolated CD4⁺CD25⁺ T cells with donor bone marrow-derived dendritic cells (DC). The source of stimulatory factors necessary to sustain Treg proliferation was the supernatant of CD4⁺CD25⁺ T cells co-cultured with allogeneic DC (MLRsup). Use of mature (CD86^{high}) DC favored extensive expansion of Foxp3⁺ cells capable of IL-17 production (Th17). Co-culture of Treg with immature DC in the presence of MLRsup rendered a T cell population with regulatory phenotype: Foxp3⁺, GITR⁺, CTLA-4⁺, CCR7⁺, CD62L⁺. These cells inhibited in vitro effector T cell proliferation at 1:20 and 1:40 Treg:T cell ratios. The suppressive activity was Ag-specific; no significant inhibition was evident when effector T cells were stimulated by third party DC.

AAsTreg were then tested for their ability to induce transplant tolerance in a mouse heterotopic heart allograft model (BALB/c to C57BL/10; unmanipulated). Rapa was used: i) to inhibit effector T cell proliferation, while sparing Treg activity and thus enhancing the in vivo Treg:effector T cell ratio; ii) to promote resolution of the inflammatory state and preserve the susceptibility of conventional T cells to Treg suppression. Graft recipients received Rapa (1mg/kg/d; d0-9) and 2x10⁶ AAsTreg i.v. on d7. In comparison to the untreated group (median survival time, MST=11d; n=14), Rapa alone extended MST to 30 d (n=7), with no long-term graft survival. Under cover of Rapa, AAsTreg exerted a profound tolerogenic effect: >80% recipients exhibited long-term graft survival (MST>150d; n=6). This effect was stronger than polyclonal Treg administration: 40% long-term survivors (MST>50d; n=5). Moreover, the tolerogenic effect was Ag-specific, as AAsTreg selected against third party DC (C3H/HeJ) did not prolong graft survival in comparison to the Rapa-only control group.

These results indicate the feasibility and therapeutic potential of Ag-specific tolerogenic cell therapy based on post-transplant administration of selected AAsTreg.

Abstract# 267

Direct Functional Activation of CD4⁺CD25⁺Foxp3⁺ T Cells by Intravenous Immunoglobulins Promotes Skin Allograft Acceptance. Thanayalak Tha-In,^{1,2} Jaap Kwekkeboom,¹ Andrew R. Bushell,² Herold J. Metselaar,¹ Kathryn J. Wood.² ¹*Dept. of Gastroenterology and Hepatology, Erasmus MC, Netherlands;* ²*Nuffield Dept. of Surgery, University of Oxford, United Kingdom.*

Intravenous Immunoglobulins (IVIg) is an effective treatment for T-cell mediated graft rejection and autoimmune diseases. IVIg treatment is associated with rapid clinical improvements without the side effects of global immunosuppression. This prompted us to ask whether IVIg might enhance directly the suppressive function CD4⁺CD25⁺Foxp3⁺ regulatory T cells *in vitro* and *in vivo*.

In vitro, mouse CBA/Ca (H2^b) total CD4⁺ or CD4⁺CD25⁺ responder cells were stimulated with C57BL/10 (H2^b) splenocytes, and human CD4⁺ or CD4⁺CD25⁺ cells were stimulated with allogeneic antigen presenting cells. *In vivo*, 1*10⁵ total CD4⁺ or CD4⁺CD25⁺ T cells of CBA/Ca mice were adoptively transferred into CBA/RAG1^{-/-} mice, and one day later transplanted with a tail skin graft of C57BL/10 mice. IVIg was administered i.v.

on day 1,3,7,10 and 14. Human Serum Albumin (HSA) was used as a control. Binding of IVIg and activation status of Foxp3⁺ CD4⁺ T cells were determined.

In vivo, only when total CD4⁺ T cells, but not CD25⁺ T cells, were adoptively transferred, IVIg protected against T-cell mediated rejection of the fully mismatched skin graft (MST: IVIg >100 vs HSA 16 days, p<0.01). IVIg binds to 16±2% of Foxp3⁺ T cells, while binding to Foxp3⁻ T cells was minimal. This binding was partially Fc γ receptor mediated. Furthermore, IVIg treatment resulted in activation of CD25⁺ T cells as detected by increased expression of phosphorylated ZAP70/Syk, which could be abrogated by specific tyrosine kinase inhibition. *In vitro*, IVIg inhibited the alloproliferative response of murine CD4⁺ T cells by 75±25%, but after depletion of CD25⁺ T cells, this inhibition decreased to 17±12% (N=6, p<0.01). Similar results were found in human MLR, where depletion of CD25⁺ T cells resulted in twofold reduction in IVIg mediated suppression. Significantly, incubation of human CD4⁺CD25⁺ with IVIg enhanced their ability to suppress allogeneic T-cell proliferation (IVIg 63±8% vs HSA 37±10% of inhibition, N=5, p<0.05).

Our results identify a novel pathway through which IVIg treatment induces direct functional activation of both mouse and human regulatory T cells. Immediate binding and activation of regulatory T cells is one of the mechanisms in the immunomodulatory repertoire of IVIg, which allows rapid inhibition of allogeneic responses, and therefore can be a valuable tool after organ transplantation.

Abstract# 268

Generation of Active Foxp3 Requires Proteolytic Cleavage of an Inactive Pro-Form: New Insights into the Mechanisms and Function of Foxp3. Engin Özkaynak,¹ Edwin de Zoeten,¹ Iris Lee,¹ Guanghui Ge,¹ Chunxia Chen,¹ Andrew D. Wells,¹ Wayne W. Hancock.¹ ¹*Pathology and Laboratory Medicine, Children's Hospital of Philadelphia and University of Pennsylvania, Philadelphia, PA.*

Foxp3 is a DNA-binding protein that is necessary for regulatory T cell (Treg) function, though recent data indicate that detection of Foxp3 mRNA or protein alone does not necessarily indicate whether the associated Foxp3⁺ cell is functional or not. To that end, our analysis of Foxp3 sequence showed the presence of an RXXR motif, conserved between mice and humans, that is located 12 amino acids (aa) from the carboxy terminus of Foxp3 and is a potential recognition sequence for cleavage by proprotein convertase enzymes. We found several proprotein convertases are expressed by naturally occurring Tregs, with further upregulation upon TCR activation. We generated an antibody against the 12 aa carboxyl peptide of Foxp3, and by Western blotting detected a peptide of about 1.3-kDa, consistent with a 12-aa long carboxy-terminal Foxp3 cleavage product. Both cleaved and uncleaved forms of Foxp3 were resolved by SDS-PAGE, and the cleaved form was found only in the DNA-bound fraction. The requirement of proteolytic cleavage, at the intact tetrabasic RXXR motif (414RKRR417), for full Treg function was shown by abolishing the motif through mutagenesis, followed by retroviral expression of mutants, and analysis of proteolytic cleavage by Western blotting. Assays of Treg function by cells expressing either long- or short-Foxp3 mutants showed short-Foxp3 (missing the carboxy-terminal 12-aa) suppressed Teff cell proliferation significantly more effectively than WT Foxp3 or an engineered and cleavage-resistant mutant, termed long-Foxp3 (p<0.01). Moreover, adoptive transfer of cells expressing short-Foxp3 prevented experimental colitis more effectively than WT-Foxp3 (p<0.01), and both were more effective than cells expressing long-Foxp3. Animals that received cells expressing short-Foxp3 gained weight and were protected from disease. Thus, function of Foxp3 is regulated at a post-translational level by proteolytic cleavage and the short form of Foxp3 represents the active form. Our data shows proteolytic cleavage of Foxp3 is key to the generation of functional Tregs, with enzymes(s) of the proprotein convertase family likely playing an important role in this process and providing an extra and hitherto unrecognized important level of regulation for Foxp3.

Abstract# 269

Blocking Both Th1 and Th2 Pathways Favors TGF β Driven Treg Generation. Yu Yang,^{1,2} Jiangnan Xu,^{1,2} Yanyan Niu,^{1,2} Jonathan S. Bromberg,^{1,2} Yaozhong Ding.^{1,2} ¹*Recanati/Miller Transplantation Institute, Mount Sinai School of Medicine;* ²*Department of Gene and Cell Medicine, Mount Sinai School of Medicine, New York, NY.*

Background: CD4⁺CD25⁺Foxp3⁺ suppressive regulatory T cells (Treg) play a critical role in mediating transplantation tolerance. While the involvement of Th1, Th2, and Th17 signaling pathways in negatively regulating *Foxp3* gene expression has been recognized, the specific mechanisms regulating *Foxp3* remain unclear. Using CD4⁺ T cells deficient in the Th2 and Th1 transcription factors Stat6 and T-bet, we discovered that blocking both Th1 and Th2 development profoundly enhanced TGF β driven Treg generation.

Methods: Naive CD4⁺CD25⁺ T cells were purified from wild type BALB/c and Stat6/T-bet (BALB/c background) double knock out (DKO) mice. Cells were cultured with irradiated T cell depleted splenocyte antigen presenting cells (APC) or bone marrow derived dendritic cells (DC) in IL-2, anti-CD3 ϵ mAb and TGF β . Foxp3 expression was measured by flow cytometry, and Treg functional activities were monitored by in vitro proliferation and in vivo inhibition of colitis.

Results: TGF β induced higher levels of Foxp3 expression and Treg cells in APC-stimulated Stat6/T-bet DKO than in wild type CD4⁺CD25⁺ T cells (DKO, 81.9% Foxp3⁺ cells; BALB/c, 15.1%). Similar results were obtained using DC-stimulated cultures: TGF β induced 91.5% Foxp3⁺ cells in DKO compared to 52.6% in wild type

CD4 T cells. Dose-response studies showed that the requirement for TGFβ was much lower in DKO than wild type CD4 T cells, so that 0.05 ng/ml TGFβ induced 25.4% Foxp3+ cells in DKO, while 5 ng/ml TGFβ induced 16.3% Foxp3+ cells in wild type. IL-6, which can divert T cell differentiation from the Treg to the Th17 lineage, reduced Foxp3 expression in both DKO and wild type CD4+CD25- cells, however higher levels of Foxp3 persisted in DKO (23.7%) compared to BALB/c cells (6.9%). Both thymic derived natural Treg, and peripherally derived TGFβ driven Treg, from the Stat6/T-bet DKO strain suppressed proliferation of CD4+CD25- T cells, and prevented CD4+CD25- CD45RBhi induced colitis in SCID mice.

Conclusion: These data demonstrate that double deficiency in both the Th1 transcription factor T-bet and the Th2 transcription factor Stat6 block Th1 and Th2 development, and strongly favor Treg development. This has important implications for understanding the pathways necessary for generating Treg, and for inducing transplant tolerance by targeting both the Th1 and Th2 developmental cascades.

Abstract# 270

The Synergistic Effect of TGF-beta/Fc and Rapamycin on Generating Regulatory T Cells and Inducing Islet Allograft Tolerance. Wensheng Zhang,¹ Wenda Gao,² Dong Zhang,¹ Yun Liu,¹ Yan Tian,¹ XinXiao Zheng,¹ ¹*Surgery, Starzl Transplant Institute, School of Medicine, University of Pittsburgh, Pittsburgh, PA;* ²*Transplantation Research Center, Beth Israel Deaconess Medical Center, Boston, MA.*

CD4+CD25+ regulatory T cells (Tregs) play a pivotal role in suppressing autoimmunity and inducing transplantation tolerance. Recent studies, including our own, demonstrate that naïve peripheral CD4+ CD25- T cells can be converted into CD4+ CD25+ regulatory cells through TGF-β induction of Foxp3 in context of TCR signaling and costimulation. This prove TGF-β to be important in generating regulatory T cells. In this study we test the hypothesis that the combination of hmTGF-β/Fc with Rapamycin will tip the balance of transplant immunity toward tolerance by strengthening the cadre of immunoregulatory T cells.

Since TGF- β is secreted in a latent form and active TGF- β is rapidly cleared from circulation. In order to make long-lasting active form of TGF- β, the mutant TGF- β was fused to a human IgG Fc component. The secreted human mutant TGF-β/Fc (hmTGF β /Fc) fusion protein stained with both anti-human TGF-β and anti-human IgG antibodies, confirming the cytokine and isotype specificity of TGF-β moiety and Fc domain. Moreover, in vitro bioassay, hmTGF- β/Fc inhibited IL-4 dependent HT-2 cell proliferation in a dose dependent manner and had a circulating half-life of 36 hours in mice.

In vitro, anti-CD3 and anti-CD28 triggered CD4+ or CD8+ T cell proliferation, measured by 3H-thymidine uptake, could be synergistically inhibited by TGF- β and Rapamycin. At the same time, the two reagents when added together could synergistically promote the induction of CD4+Foxp3+ T cells from peripheral naïve CD4+Foxp3- T cells.

In a pancreas islet transplantation model in which the fully MHC mismatched DBA/2 islets were transplanted into C57 BL/6 mice rendered diabetic by streptozotocin, mean survival time of islet was 19 days in control recipients (n=6). Administration of mutTGF-β/Fc resulted in a delayed islet allograft rejection (MST; 38 days, n=5). Treatment with Rapamycin prolonged islet grafts survival in 63% of recipients. In contrast, combined treatment with TGF-β/Fc and Rapamycin by four doses produced indefinite islet allograft survival in 83% cases.

We have produced the long-lasting active hmTGF-β/Fc fusion protein. The TGF-β/Fc is synergistic with Rapamycin to convert naïve CD4+Foxp3- T cells into CD4+Foxp3+ Tregs and promote long-term islet allograft engraftment.

Abstract# 271

The Reversal of Established Cardiac Allograft Tolerance Is Dependent on IL-6 and IFNα/b Receptor Signaling. Tongmin Wang,¹ Luqiu Chen,¹ Jing Xu,¹ Jing Tao,¹ Emily B. Ahmed,¹ Chyung-Ru Wang,¹ Maria-Luisa Alegre,¹ Anita S. Chong.¹ ¹*Surgery, Medicine, and Pathology, The University of Chicago, Chicago, IL.*

The recognition of microbial motifs by the innate immune system leading to the stimulation of adaptive immune responses may explain the relationship between infections and susceptibility to graft rejection. A number of infectious agents have been reported to prevent the induction of allograft tolerance, however, none of these can reverse established tolerance, a situation that is highly relevant to clinical transplantation. We here report that established allograft tolerance (induced with anti-CD154 + donor-specific transfusion) can be reversed in a cardiac transplantation mouse model following infection with *Listeria monocytogenes*. This reversal of tolerance was dependent on the presence of both CD4+ and CD8+ cells, as well as of the TLR/IL-1/IL-8 adaptor molecule, MyD88.

We hypothesized that the reversal of tolerance requires that alloreactive conventional T cells (Tconv) escape dominant regulation by pre-existing Tregs. These alloreactive Tconv can then proliferate resulting in an increased ratio of Tconv:Tregs favoring the reversal of established tolerance. Indeed, we observed that tolerant grafts harbored low numbers of infiltrating CD4+ and CD8+ T cells (1-1.4 x 10⁴/heart), with 10-43% of the infiltrating CD4+ cells co-expressing Foxp3 (N=10). Following the reversal of tolerance and allograft rejection, a 7-10 fold increase in CD4+Foxp3+ and CD8+Foxp3+ Tconv was observed in the graft, but no increase in the Foxp3+ subset. In contrast, we observed no significant changes in the splenic T cell subsets, raising the possibility that the escape

from Tregs occurs locally within the graft. Infection of tolerant IL-6- or IFNαR1-deficient recipients with *Listeria monocytogenes* failed to reverse tolerance, consistent with a hypothesis that the initial escape of Tconv from regulation may depend on IL-6, while their proliferation may be Type I IFN-dependent. In summary, the conditions for the reversal of tolerance is more stringent than the prevention of tolerance, and requires MyD88-signalling and the presence of both CD4+ cells and CD8+ cells, as well as IL-6 and type I IFN signaling. These studies point to the potential impact of bacterial infections on established tolerance, and to novel strategies to monitor and facilitate the maintenance of tolerance in the clinic.

Abstract# 272

Th17 Response in Tolerance Resistant T-bet Deficient Recipients of MHC Fully Mismatched Cardiac Allografts. Jesus R. Paez-Cortez,¹ Joyce Popoola,¹ Francesca D'Addio,¹ M. Javeed Ansari,¹ Laurie Glimcher,² John Iacomini,¹ Mohamed H. Sayegh,¹ Xueli Yuan.¹ ¹*Transplantation Research Center, Brigham and Women's Hospital & Children's Hospital – Harvard Medical School., Boston, MA;* ²*Department of Immunology and Infectious Diseases, Harvard Medical School of Public Health, Boston, MA.*

Introduction: We have previously shown that C57/BL6 (B6) T-bet deficient mice are resistant to tolerance induction. Tolerance resistance is mediated by CD8+ T cells and occurs despite a profound IFN-γ deficiency. In this work we examined the role of novel Th17 T cells subset in resistance to tolerance induction.

Methods and Results: Combined costimulatory blockade with MR1 and CTLA4-Ig consistently induces long-term allograft survival of fully mismatched BALB/c heart transplants in C57BL/6 wild-type (WT) mice (>100 days). However, T-bet deficient and IFN-γ deficient B6 mice reject their grafts in 12.3±1.2 and 13.6±1.1 days respectively. Examination of cytokine production by Luminex revealed that splenocytes from untreated T-bet deficient recipients produced significantly lower levels of Th1 cytokines (IFN-γ) and greater levels of Th2 cytokines (IL-5, IL-10 and IL-13) than WT recipient splenocytes (217.3±26 vs 45098±3101 pg/ml for IFN-γ and 178±20, 803.3±37.5, 701.3±107.5 vs 0±0, 219±28, 67.2±37.3 pg/ml for IL-5, IL-10 and IL-13 respectively) (p<0.01 for all four cytokines), when stimulated by irradiated donor Balb/c splenocytes. Most interestingly the production of the Th17 cytokine, IL-17, was dramatically elevated in T-bet deficient mice when compared to WT recipients (44966.7±15805.2 vs 8.4±26.2 pg/ml) (p<0.01). Confocal analysis of transplanted hearts revealed a massive CD4+ and CD8+ IL-17 producing T cell infiltration in untreated T-bet deficient recipients, while no IL-17 producing cells were observed in untreated WT and IFN-γ deficient groups. Interestingly, graft infiltrating CD8+ T cells of MR1/CTLA4-Ig treated T-bet deficient mice did not display significant levels of IL-17 expression. However, after allogeneic stimulation, recipient splenocytes produced higher levels of IL-17 when compared to treated WT mice (530.8±147 pg/ml vs 6.6±8.7 pg/ml, p<0.01). Other cytokines, such as IL-2, IL-5, IL-13 and TNF-α showed no dramatic increase between groups.

Conclusion: These data indicate that resistance to tolerance induction by costimulatory blockade in T-bet deficient mice may be related to increased IL-17 production.

Chronic Rejection/Injury: Innate and Adaptive Immunity II

Abstract# 273

Alloimmunity May Lead to Autoimmunity Against Epithelial Auto-Antigens Following Lung Transplantation (LTx): Role in Chronic Rejection. D. Saini,¹ S. Ramachandran,¹ A. Golochekine,¹ A. Aloush,⁴ N. Steward,¹ E. Trulock,³ G. A. Patterson,⁴ T. Mohanakumar.² ¹*Surg, WUSM;* ²*Path & Immunol, WUSM;* ³*Int Med-Pul, WUSM;* ⁴*Cardio, Washington Univ Sch Med, St. Louis, MO.*

Our laboratory has identified Kα1 tubulin, as an epithelial autoantigen to which immune response occurs following human LTx. Further, we have shown a strong correlation between the presence of antibodies (Abs) to Kα1 tubulin and development of chronic rejection ie Bronchiolitis Obliterans Syndrome (BOS). Goal of our study is to test the hypothesis that epithelial damage due to allo immunity results in remodeling exposes otherwise cryptic self antigens including Kα1 tubulin and collagen type V (coll V) leading to cellular immune reactivity and Ab production against these auto-antigens which may play a role in the pathogenesis of BOS. 10 patients who developed anti-HLA Ab and 10 patients who had no detectable anti-donor HLA Abs post-LTx by Luminex assay were analyzed for development of Abs to Kα1 tubulin and coll V using ELISA method developed in our laboratory. The ELISA for Kα1 tubulin used recombinant protein (1μg/ml) purified on affinity resin. The ELISA for collagen V uses commercially available human Collagen V (1μg/ml). Elispot assay for IFNγ and IL-10 were performed for 5 BOS- and 5 BOS+ patient PBLs (at the time of BOS diagnosis), after 3 stimulations with Kα1 tubulin protein. The levels of anti-tubulin Abs and anti-coll V Abs were increased significantly in BOS+ LTx recipients with anti-HLA compared to those with no anti-HLA, Table 1 (p<0.05).

Table 1: Development of Abs to Kα1 tubulin and collagen V in LTx recipients developing anti-HLA allo-Abs

Patients	Anti Kα1 tubulin Abs (μg/ml)	Anti-Collagen Abs (μg/ml)
Normal	132±43	92.6±34.2
HLA-	147.5±94.5	190.3±113.9
HLA+	458.9±342.7	365.7±219.8

8 out of 10 patients who were anti-HLA+ showed high autoAbs. In contrast, only 3 out of 10 anti-HLA- patients showed auto Abs. Further, cells from BOS+ patients at the time of BOS diagnosis demonstrated 5 fold more (256 vs 54 spots/million cells) IFN γ secreting cells and 2 fold less IL-10 secreting cells (120 vs 240 spots/million cells) by stimulation with recombinant α 1 tubulin compared to BOS- patients or normal PBLs ($p=0.025$). Autoimmunity triggered by alloimmune response plays a pivotal role in the immunopathogenesis of chronic rejection following human LTx and therefore prevention of autoimmune development by early intervention of alloimmunity may prevent or delay the development of BOS.

Abstract# 274

Regulatory T Cells Induced by Allochimeric MHC Class I Therapy Inhibit Cardiac Chronic Rejection. Natalya V. Semiletova,¹ Xiu-Da Shen,¹ D. Edbreg,¹ R. W. Busuttil,¹ J. W. Kupiec-Weglinski,¹ M. R. Ghobrial.¹ ¹UCLA Medical School.

MHC class I allochimeric protein therapy induces tolerance and inhibits chronic rejection. Allochimeric proteins showed its effectivity given peri- or post-operative through portal vein. Tolerant hosts demonstrated a restricted alloreactive T cell repertoire, attenuated alloantibody, and upregulated IL-10 response. This study investigated the role of allochimeric therapy in the development of T regulatory cells (T-regs) and T-reg's role in abrogation of transplant vascular sclerosis (TVS). **Methods:** Irradiated (130R) ACI hosts of WF hearts were injected with syngeneic splenic CD4⁺; CD8⁻; CD4⁺CD25⁺ cells (10^7 cells >95% purity; iv) from long-term allochimeric (AlloP) or high-dose CsA treated cardiac hosts. CDR3 size restriction was tested in splenic T cells of tolerant AlloP hosts. **Results:** AlloP therapy induces CDR3 size restriction in host's spleen cells. V β genes 10, 12, & 18 were limited when expression of V β 8.1 and 5 were upregulated. Unlike T cells from spleen, LN and graft infiltrated T cells demonstrated expression of most of V β genes. *In vitro*, T-regs from AlloP and CsA rats inhibited proliferation of ACI cells against WF (750 ± 100 1600 $\pm$ 200 ³H-TdR cpm/min), but not against third-party BN (3200 ± 500 ; $P < 0.001$). *In vivo*, T-reg's activity from AlloP and CsA rats was confirmed by their ability to prolong WF graft survival (>120 d) in ACI hosts ($n=17$ and 8, respectively). However, only AlloP induced T-regs abrogated TVS, as evidenced by intact architecture and absence of neointimal proliferation, as assessed by neointimal index (NI= 15 ± 5) with only 26 $\pm$ 6 % of vessels affected. In contrast, allografts of secondary ACI hosts treated with CsA-induced T-regs, exhibited severe TVS, strong neointimal proliferation and increased NI (NI= 50 ± 16 in 79 $\pm$ 1 % vessels affected). Surprisingly, both CD4 and CD8 subsets induced long-term survival of secondary test grafts (>100 days). However, only the CD4⁺ T-reg prevented TVS (NI= 19 ± 5 , 21 $\pm$ 6 % of vessels), as compared to CD8⁺ T-reg (NI= 40 ± 9 in 50 $\pm$ 15 % of vessels). The cytokine profile indicated a dominant IL-10 response. Allo-antibody analysis showed up-regulation IL-4/IL-12 dependent IgG1 and IgG2c. **Conclusion:** Allochimeric protein-therapy and CsA treatment generate T-reg that prolong graft survival, but only CD4⁺ T-reg generated from allochimeric protein-therapy prevent TVS. This CD4⁺ T-reg may be responsible for long-term graft maintenance through its unique ability to control anti-inflammatory and alloantibody responses.

Abstract# 275

Immunodominant H60-Specific CD8 T Cells Play a Key Role in Acute Rejection but Not in Chronic Allograft Vasculopathy. Jean Kwun,¹ Huaizhong Hu,¹ Jose Torrealba,² William J. Burlingham,¹ Stuart J. Knechtle.¹ ¹Surgery, University of Wisconsin-Madison, Madison, WI; ²Pathology, University of Wisconsin-Madison, Madison, WI.

Background: Minor histocompatibility Ags (miHAs) are self peptides, derived from proteolytic processing of normal cellular proteins. miHA incompatibility can induce T cell response to dominant miHAs and facilitates expansion of CTLs and graft rejection. However, the contribution of CTLs recognizing these miHAs in solid organ transplantation has not been fully evaluated.

Methods: BALB.B (H-2^b) donor hearts were transplanted heterotopically to C57BL/6 (H-2^b) recipients. *In vivo* alloreactive CD8 T cells were monitored with peptide/MHC multimers. α -LFA-1 mAb was given to recipients to prevent rejection. Various numbers of H60-specific CD8 T cells or CD8 T cells lacking H60 specificity were adoptively transferred into BALB.B graft bearing B6.SCID recipients.

Results: 50% of transplantation recipients developed acute rejection and showed markedly increased numbers of H60-specific CD8 T cells at day 8-12 in spleen. Abundant CD8 infiltration was found and selective infiltration of H60-specific CD8 T cells in the graft was confirmed with flow cytometry and *in situ* tetramer staining. α -LFA-1 mAb treatment prevented acute rejection (MST>100) and allogeneic T cell expansion. It also profoundly attenuated neointimal hyperplasia (61.6 ± 6.6 vs. 23.8 ± 7.6 ; $p < 0.01$), diseased vessel (55.3 ± 7.4 vs. 15.9 ± 5.0 ; $p < 0.01$), and fibrosis (3.29 ± 0.4 vs. 1.8 ± 0.6 ; $p < 0.05$). To evaluate the role of H60 specific CD8 T cells on vascularized cardiac allografts, sorted H60 specific CD8 T cells were adoptively transferred to BALB.B graft-bearing B6.SCID mice. We found that the degree of acute rejection positively correlated with the number of H60-specific CD8 T cells transferred. However, transferred H60-specific CD8 T cells did not cause chronic rejection. Interestingly, greater numbers of CD8 T cells with H4 immunodominance were found in spleen, blood and graft at 50 days after adoptive transfer of CD8 lacking H60 specificity compared to H60-specific CD8 T cell transfer.

Conclusion: H60 responses dominate other miHA immune responses during acute rejection after BALB.B to B6 cardiac allograft. We confirmed a role of immunodominant H60 specific CD8 T cells as pathological effector T cells in acute rejection but not in chronic rejection. Maintaining a H60 response may be beneficial in allotolerance to suppress miHA responses that could induce chronic rejection in long-term grafts.

Abstract# 276

Synergistic Effects of Anti-HLA Antibodies (Abs) and Defensins in the Pathogenesis of BOS Following Human Lung Transplantation (LTx). D. Saini,¹ S. Ramachandran,¹ N. Steward,¹ A. Aloush,³ E. Trulock,⁴ G. A. Patterson,³ T. Mohanakumar.² ¹Surg, WUSM; ²Path & Immunol, WUSM; ³Cardio Surg, WUSM; ⁴Int Med-Pul, Washington Univ Sch Med, St. Louis, MO.

Chronic lung allograft rejection, bronchiolitis obliterans syndrome (BOS), affects up to 60% of transplant survivors 5 years post-LTx. Human Neutrophil peptides (HNP 1-3/ α defensins), have been identified in the lavage fluids from patients with chronic rejection by proteomics. Goals of our study were to determine the role of anti-HLA Abs and defensins in BOS and to define the interactions between α -1- antitrypsin (AAT), and defensins in regulating inflammation leading to epithelial cell proliferation and BOS pathogenesis. BAL and serum samples (post-LTx and pre BOS) from 21 BOS+, 15 BOS- patients and 12 normals were analyzed by ELISA for α defensins (HNPI-3), human β defensin2 (HBD2) and AAT. Small Airway Epithelial Cells (SAEC) were treated with HNPI or 2, with or without equimolar AAT or with anti-HLA Abs and analyzed for levels of HBD2 production (ELISA), cytokine and chemokine (Luminex), and cell surface adhesion molecules (ICAM, VCAM) by FACS. BAL and serum from BOS+ patients had high levels of HNPI-3 and HBD2 compared to BOS- recipients or normal sera ($p < 0.05$) (Table 1). There was also a significant decrease in AAT levels in BOS+ compared to BOS- or normal serum ($p=0.01$) (Table 1).

Table 1

	Normal (n=12)	BOS- (n=15)	BOS+ (n=21)
α Defensins (HNP 1-3) (pg/ml)	242 $\pm$ 56	442.6 $\pm$ 226.2	564.4 $\pm$ 152.1
β Defensin (HBD2) (pg/ml)	14.9 $\pm$ 9.6	81.1 $\pm$ 41	127.9 $\pm$ 78.8
AAT (mg/dl)	308.6 $\pm$ 54.4	296.1 $\pm$ 56.8	256.0 $\pm$ 17.6

SAEC produced human β defensins following anti-HLA Ab stimulation or by HNP treatment (Table 2).

Table 2

	Untreated	SAEC + anti-HLA	SAEC + α
	SAEC	Ab (W6/32)	defensins (HNPI/2)
β defensin (HBD2) (pg/ml)	420 $\pm$ 12	520 $\pm$ 23	590 $\pm$ 10

There was increase in adhesion molecules (ICAM, VCAM, 2 folds) cytokines [IL-6, IL-1R α (2 folds), IL-1 β , IL-13 (20 folds)], IL-10 (2 folds decrease), chemokines (IL-8, MCP1, 2-4 folds increase) and growth factors (EGF and VEGF, 2.5 folds increase) in response to treatment with HNPI or HNP2 compared to untreated SAEC, that was inhibited by AAT. Increased defensins and decreased AAT levels were seen in lavage and serum of LTx recipients with BOS. Anti-HLA Abs further stimulate defensin production by SAEC. We conclude that chronic stimulation of epithelial cells both by defensins and Anti-HLA Abs can lead to increased growth factor production contributing to the pathogenesis of BOS.

Abstract# 277

Under a Mac Attack: Macrophages Are an Important Agent of Fibrosis in Human Kidney Allografts. Raphael Thuillier,¹ Orlena Cheng,¹ Roslyn B. Mannon.¹ ¹Kidney Disease Branch, NIH/NIDDK, Bethesda, MD.

Tubular atrophy/interstitial fibrosis (TA/IF) remains a major cause of late kidney allograft loss and epithelial mesenchymal transformation (EMT) is now appreciated as a key feature in this process. We hypothesize that macrophages play a direct role in the development of TA/IF by creating a profibrotic microenvironment and stimulating EMT within the allograft. In human kidney allografts with TA/IF ($n=128$), macrophage infiltration detected by immunostaining was significantly upregulated (mean score 2.8 ± 0.5) compared to biopsies without corresponding pathological changes from recipients with stable function ($n=51$; 2.0 ± 0.1 ; $p < 0.0001$) and the extent of this staining also correlated to the magnitude of graft dysfunction ($p < 0.0001$). RT-PCR in TA/IF grafts also showed marked upregulation for EMT markers α SMA (3.0 ± 1.1 -fold; $p < 0.05$), S100A4 (8.7 ± 2.7 -fold; $p < 0.05$), and vimentin (4.2 ± 2.3 -fold; $p < 0.01$), compared to stable function allografts. To explore the macrophage-EMT relationship, we co-cultured freshly isolated human monocytes over a primary culture of human proximal tubular epithelial cells (PTECs) using culture inserts allowing media exchange but forbidding contact between the two cell populations. By RT-PCR, co-cultured epithelial cells showed significant downregulation of BMP7 (0.45 ± 0.09 -fold; $p < 0.001$), a negative regulator of EMT, and epithelial marker E-cadherin (0.001 ± 0.001 -fold; $p < 0.01$), with marked upregulation of EMT genes S100A4 (2.43 ± 0.40 -fold; $p < 0.001$) and α SMA (5.31 ± 2.23 -fold; $p < 0.05$) compared to PTECs cultured in media alone. Investigating the mechanism of monocyte driven EMT, we evaluated the effects of Am80, a synthetic retinoid that also inhibits IL-6 and VEGF signaling. Am80 markedly reversed the transcriptional profile with reduction of EMT markers S100A4 (0.34 ± 0.10 -fold; $p < 0.01$), α SMA (0.29 ± 0.06 -fold; $p < 0.05$) and vimentin (0.45 ± 0.13 -fold; $p < 0.01$), and a simultaneous increase in expression of BMP7 (151.7 ± 53.9 -fold; $p < 0.01$), thus reversing the pro-EMT milieu. These results indicate that human monocytes induce transcription of EMT related genes in PTECs, even in the absence of physical contact.

Moreover, this phenomenon appears to be mediated in part by IL-6, VEGF, and other pathways mediated by the retinoic acid receptor. Thus, in addition to their typical immune functions, macrophages support a milieu within allografts that promotes TA/IF in humans. Further investigation into this novel pro-TA/IF pathway could ameliorate chronic injury and improve graft survival.

Abstract# 278

Greater Acute and Chronic Kidney Damage in Late-Generation Telomerase (TERC) KO Mice after Ischemia-Reperfusion Injury (IRI).

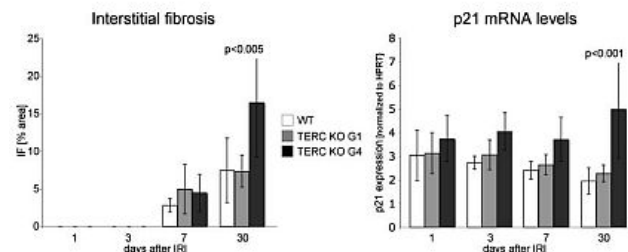
Jens H. Westhoff,¹ Carolin Schildhorn,¹ Heidi Braun,¹ Bettina Kraenzlin,² Norbert Gretz,² Anette Melk.¹ ¹Children's Hospital, University of Heidelberg, Heidelberg, Germany; ²Medical Research Center, University of Heidelberg, Mannheim, Germany.

Old donor kidneys are more likely to develop long-term graft failure, especially if they are exposed to stresses (e.g. rejection). This limited ability of old tissue to withstand stress may be due to its reduced capacity of replication and regeneration. Telomere shortening determines lifespan and regenerative capacity. We showed that telomere shortening occurs in old human kidneys. Late-generation TERC KO mice with critically short telomeres have a reduced lifespan, show an accelerated aging phenotype and are an ideal model to resemble the situation in old human donors.

This study addressed the question whether IRI causes greater damage in kidneys from late-generation TERC KO mice. We studied TERC wildtype (WT), early- (G1) and late-generation (G4) TERC KO mice 1 day (WT:n=7; G1:n=8; G4:n=8), 3 (WT:n=8; G1:n=8; G4:n=5), 7 (WT:n=6; G1:n=8; G4:n=8) and 30 days (WT:n=8; G1:n=9; G4:n=6) after IRI.

Acute tubular necrosis was found mainly on days 1 and 3 and was significantly higher in TERC KO G4 kidneys. Tubular atrophy (TA) and interstitial fibrosis (IF), reflecting chronic damage, were first detected at day 7 and increased in all mice with a significantly higher extent of TA/IF in TERC KO G4 kidneys at day 30 (fig.). The cell cycle inhibitor p21, a downstream mediator of senescence induced by telomere shortening, was significantly upregulated in all groups after IRI. P21 levels were highest in TERC KO G4 kidneys at day 30 (fig.). Proliferative capacity, as measured by Ki-67 immunostainings at day 30, was significantly lower in tubular, glomerular and interstitial cells of kidneys from TERC KO G4 mice.

We show that late-generation TERC KO mice with critically short telomeres have a greater susceptibility towards acute injury and develop more chronic renal damage. This is likely due to the reduced capacity of these kidneys to proliferate and thereby to regenerate. Our data strongly suggest a pathogenetic role of telomere shortening, an important senescence mechanism, for the development of long-term graft failure.



Kidney Immunosuppression

Abstract# 279

Sirolimus-Induced Infertility in Male Renal Transplant Recipients.

Julien Zuber,¹ Dany Anglicheau,¹ Lynda Bererhi,¹ Caroline Elie,² Magali Cioldi,¹ Marie-France Mamzer-Bruneel,¹ Frank Martinez,¹ Eric Thervet,¹ Christophe Legendre.¹ ¹Kidney Transplantation, Necker Hospital, Paris, France; ²Biostatistics, Necker Hospital, Paris, France.

Background: Hormonal studies in organ transplant recipients suggest that sirolimus may impair testicular function. The aim of this study was to evaluate the frequency and severity of sirolimus-induced alterations in sperm parameters and their impact on fathered pregnancy rate.

Methods: An observational study was carried out in male patients aged 20-40 years who received a kidney transplant during 1995-2005. Patients were sent a questionnaire by post, and sperm analysis was proposed. The fathered pregnancy rates according to the immunosuppressive regimen were estimated and compared using the Poisson model. Findings: Questionnaires were sent to 116 patients, and complete information was obtained from 95 recipients. Patients treated with sirolimus throughout the post-transplant period had a significantly reduced total sperm count compared to patients who did not receive sirolimus (28.6±31.2 x 10⁶ and 270.7±278.5 x 10⁶, respectively; p=0.01), and a decreased proportion of motile spermatozoa (22.2±12.3% and 40.6±15.5%, p=0.02). Of six patients in whom sirolimus treatment was interrupted, only three showed a significant improvement in sperm parameters.

Moreover, the fathered pregnancy rate (pregnancies/1000 patient years) was 5.9 [95% CI, 0.8-42.1] and 92.9 [95% CI, 66.4-130.0] in patients receiving sirolimus-based and sirolimus-free regimens, respectively (p=0.007).

Pregnancy rate according to sirolimus exposure

Parameters	Sirolimus	No Sirolimus	p
Spontaneous pregnancies (n)	1	34	
Cumulative treatment duration (patient-years)	168.6	366.1	
Pregnancy rate (per 1000 patient-years) [95CI]	5.9 [0.8-42.1]	92.9 [66.4-130.0]	0.007

Interpretation: Sirolimus strongly impairs spermatogenesis and, as a corollary, significantly reduces male fertility. Male candidates for sirolimus therapy, or male patients who are already receiving sirolimus, should be informed about this sensitive side effect. We recommend sperm banking before introduction of sirolimus in view of the uncertainty about complete and sustained recovery of testicular function after sirolimus cessation.

Abstract# 280

IMPDH Activity on Day 6 after Kidney Transplantation Is Significantly Related to the Risk of Acute Rejection in MMF Treated Patients.

Ferdi Sombogaard,¹ Ron Mathot,¹ Han-Yo Ie,¹ Petra Glander,² Willem Weimar,¹ Teun van Gelder.¹ ¹Erasmus University Medical Center, Rotterdam, Netherlands; ²Charité University, Berlin, Germany.

Introduction: Mycophenolic acid (MPA) –the active metabolite of MMF– inhibits the enzyme inosine monophosphate dehydrogenase (IMPDH). Monitoring IMPDH activity, as a measure of the immunosuppressive effect of MMF, could be a target to optimize MMF therapy. A previous study reported that high pre-transplant IMPDH activity was associated with biopsy proven acute rejection (BPAR) of the graft. So far, data on post-transplant IMPDH activity and BPAR are not available. In this study we investigated the relationship between IMPDH activity and risk of BPAR in renal transplant patients.

Methods: A cohort of 100 patients, transplanted between 4/06 and 9/07, was prospectively monitored for pharmacokinetics (MPA plasma levels) and pharmacodynamics (IMPDH activity) of MMF treatment. Patients received triple immunosuppressive therapy, consisting of MMF, tacrolimus and steroids. Blood samples were taken pre transplantation and on day 6 post transplantation pre-morning dose and 0.5, 1, 2, 6 and 12h after MMF intake. The enzyme activity of IMPDH in PBMCs was measured using a validated HPLC method. IMPDH activity and MPA exposure was calculated as the area under the activity or concentration-time curve (AUEC_{act} and AUC_{MPA}) using a linear trapezoidal rule. All acute rejection episodes were biopsy proven (Banff classification grade 1A or higher). This interim analysis includes patients with a follow-up after transplantation of at least two months.

Results: Samples for IMPDH activity measurement were available from 78 patients (age 19-76 years) on day 6 (range 3-11) post transplantation. In 16 (21%) of the patients a BPAR was documented within one year after transplantation. The mean AUEC_{act} of patients with BPAR was significantly higher compared to non-BPAR patients (401±294 and 255±155 h*μmol/s/mol AMP, p=0.017). No significant correlation was found between IMPDH activity pre (51±26 vs. 53±41 h*μmol/s/mol AMP; p=0.68) and at single time points post transplantation compared to BPAR. The mean AUC_{MPA} of patients with BPAR was not significantly different to non-BPAR patients (48.5±23.9 and 52.1±38.9 h*mg/L; p=0.99).

Conclusion: In the largest patient series to date, IMPDH activity, measured as AUEC_{act} on day 6, is significantly correlated with BPAR after renal transplantation. Monitoring IMPDH activity is a promising and novel biomarker to optimize MMF treatment in renal transplant patients.

Abstract# 281

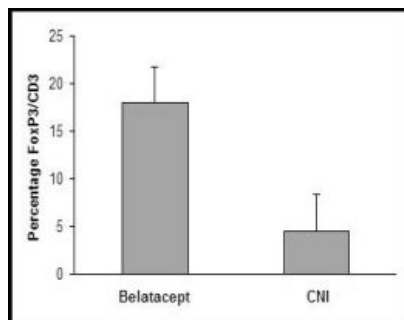
Effect of Belatacept on Circulating and Intragraft Regulatory T Cells in Kidney Transplant Patients.

Julie Yabu,¹ Weihong Liu,² Amy Putnam,² Mirco Belingeri,³ Dawn Gross,⁴ Zoltan Laszik,⁵ Jeffrey Bluestone,² Flavio Vincenti.¹ ¹Medicine, Univ California San Francisco, San Francisco, CA; ²Diabetes Center, Univ California San Francisco, San Francisco, CA; ³Clinica Pediatrica de Marchi, UO di Nefrologia, Milano, Italy; ⁴Medicine, Stanford Univ, Palo Alto, CA; ⁵Pathology, Univ San Francisco, San Francisco, CA.

Background: FoxP3+CD25+ regulatory T cells (Treg) are important in regulating immune homeostasis. Increased expression of Foxp3 mRNA in the urine of patients with rejection is associated with better resolution of rejection. Because Treg survival is dependent on the CD28-CD80/CD86 signaling pathway, belatacept could potentially alter the Treg population. We report the effect of belatacept on circulating Treg cells in renal transplant patients on maintenance belatacept therapy and intragraft Treg cells in patients with acute rejection from the phase II trial (Vincenti NEJM 2005).

Methods: Peripheral blood mononuclear cells (PBMC) were analyzed for CD4, CD25 and FoxP3 in 12 pts receiving belatacept for 3-5 years compared to pts treated with calcineurin inhibitors (CNI). Isolated Treg cells were studied for functional effects in T cell suppression assays. Kidney biopsy specimens from 32 pts receiving belatacept or CNI with acute rejection were stained for Treg cells by CD3-FoxP3 immunohistochemistry.

Results: Belatacept treatment had no effect on the number of peripheral blood Treg cells and T cell suppression assays. The percentage of FoxP3 cells was significantly elevated in rejecting kidney allografts in belatacept-treated patients compared to CNI-treated patients.



Conclusions: Following chronic belatacept therapy, the number and function of peripheral blood Treg cells are maintained in kidney transplant patients. Belatacept enhances the Treg population in the allograft in patients with acute rejection. Therefore, our data suggest that co-stimulation blockade with belatacept does not affect Treg homeostasis. The increased number of Treg cells in rejecting allografts in belatacept-treated patients may provide a novel mechanism whereby belatacept can mitigate the severity of acute rejection and improve graft survival.

Abstract# 282

mTOR Inhibition Reduces the Number of Colony-Forming Units of Erythroid Progenitor Cells. E. Marcelo Arellano,^{1,2} Maribel Díaz,³ Jordi Rovira,¹ Josep M. Jou,³ Josep M. Campistol,¹ Joan Ll. Vives-Corrons,³ Ginés Escolar,³ Fritz Diekmann.^{1,4} ¹Laboratori Experimental de Nefrologia i Trasplantament (LENIT), Hospital Clinic, Barcelona, Spain; ²Nephrology, Hospital Universitario "José E. González", Monterrey, Mexico; ³Hemotherapy-Hemostasis, Hospital Clinic, Barcelona, Spain; ⁴Nephrology, Charité Campus Mitte, Berlin, Germany.

Introduction:

mTOR inhibition has been associated with microcytosis and/or anemia in kidney transplant patients. The aim was to evaluate the influence of mTOR inhibition on erythropoiesis in kidney transplant patients and healthy controls.

Methods:

Erythroid progenitor cells were isolated from peripheral blood of healthy control persons (HC; n=8), or kidney transplant patients with chronic sirolimus treatment with (SRL+MC; n=8) or without microcytosis (SRL-MC; n=8). The isolated progenitor cells were then cultured in a semi-solid medium, containing 3 U/ml erythropoietin, in the absence or presence of SRL (5ng/mL) for 14 days. Burst forming unit erythroid (BFU-E) derived colonies were then counted through an inverted microscope considering that each colony consists of more than 40 cells. Cultures were performed in duplicate and colonies were counted in the entire culture dish.

Results:

Hemoglobin was 13.1 (SRL+MC) and 13.5 (SRL-MC) g/dL (p=ns). RBC count was 5.1 and 4.7x10⁶/μL in SRL+MC and SRL-MC respectively (p=0.034). MCV was 76 in SRL+MC and 87 fL in SRL-MC (p<0.0001). Presence of SRL in the culture medium led to a decreased number of colonies in healthy controls and kidney transplant patients (without SRL: 34.2±11.4 vs. with SRL: 27.5±9.9 BFU-E derived colonies p=0.03). The same difference was seen if the three groups were analyzed separately (HC, SRL-MC, SRL+MC). Interestingly, culture dishes of SRL+MC patients tended to contain an increased number of colonies when cultured in the absence of SRL when compared to SRL-MC and HC (39.9 vs 29.9 p=0.17), which might indicate that microcytic patients present an increased number of circulating progenitor cells.

Conclusion: mTOR inhibition leads to a reduced number of erythroid colonies in culture; microcytosis in SRL treated patients (if it occurs) might be compensated by a higher number of erythrocytes and circulating progenitor cells.

Abstract# 283

Steady-State Pharmacokinetics of the Protein Kinase C Inhibitor AEB071 in De Novo Kidney Transplant Patients. J. M. Kovarik,¹ K. Budde,² F. Pietruck,² M. Zeier,² J. Klempnauer,² J. Steiger,² J. Grinyo,² M. Weber,² T. Jung,¹ M. Soergel.¹ ¹Novartis Pharmaceuticals, Basel, Switzerland; ²AEB071 Renal Transplant Study Group.

The steady-state pharmacokinetics of the protein kinase C inhibitor AEB071 were characterized in the context of a randomized, multicenter trial in de novo kidney transplant recipients. A total of 63 evaluable patients received 200 mg bid AEB071 in a multidrug immunosuppressive regimen. An AUC-profile was obtained on day 8 posttransplant and blood samples were measured at a central laboratory for concentrations of AEB071 and its pharmacologically active N-desmethyl metabolite. Data were compared to those previously characterized in psoriasis patients receiving 200 mg bid AEB071 as monotherapy. **Drug exposure:** As tabulated below, steady-state drug exposure in renal transplant patients was similar to that in psoriasis patients (p=NS for all parameters). This exposure was associated with a significant clinical response (35% reduction in PASI score vs placebo) in the psoriasis trial (see ATC 2007 abstract 1725).

Parameter	Renal transplant patients (n=63)	Psoriasis patients (n=6)
C ₀ (ng/ml)	381 ± 298	300 ± 66
t _{max} (h)	2 (1 - 6)	2 (2 - 4)
C _{max} (ng/ml)	1560 ± 649	1163 ± 536
AUC (ng.h/ml)	9780 ± 4820	7837 ± 3877

The steady-state AUC of N-desmethyl-AEB071 was minor in comparison to AEB071 and similar between the patient groups: 200 ± 90 ng.h/ml in transplantation vs 393 ± 220 ng.h/ml in psoriasis (p=0.08). **Demographic covariates:** In the first week posttransplant with patients receiving fixed-dose AEB071, intersubject variability for C₀ was 78% and for AUC was 49%. AUCs were similar in men vs women (8350 ± 4163 vs 10784 ± 5369, p=0.26). Age, which ranged from 18-64 years, did not influence AUC based on regression analysis (r² = 0.005, p=0.65). There was a borderline-significant negative correlation between weight (range, 51-110 kg) and AUC (p = 0.07); however, its clinical relevance was low in that it could explain <9% of the variability in AUC (r² = 0.086). There was a significant positive correlation between AEB071 C₀ and AUC (r² = 0.724, p<0.001).

Conclusions: (1) In the first week posttransplant, patients achieved AEB071 blood levels anticipated for this regimen. (2) There was notable intersubject pharmacokinetic variability at this time but it was not attributable to standard demographic factors such as sex, age, or weight. (3) A good correlation was noted between C₀ and AUC suggesting that C₀ might serve as a marker for total drug exposure.

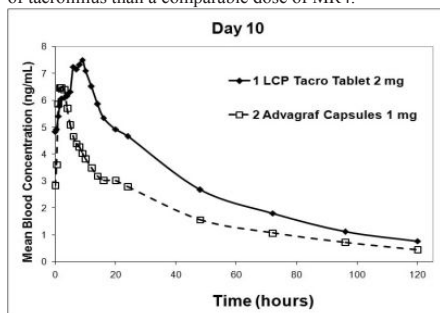
Abstract# 284

A Two-Way Crossover, Open-Label, Multiple-Dose, Fasting, Bioequivalence Study of LCP-Tacro 2 mg Tablets (q.d.) Versus Advagraf® 2 x 1 mg Capsules (q.d.) in Normal, Healthy, Caucasian Male Subjects. Michael Beckert,¹ Robert D. Gordon,¹ Juan He,² Zia R. Tayab.² ¹LifeCycle Pharma A/S, Hørsholm, Denmark; ²Biovail Contract Research, Toronto, Canada.

Extended release of tacrolimus along different intestinal regions could lower the inter-subject variability of drug absorption, increase bioavailability, and potentially improve the therapeutic use of the product. LCP-Tacro™ Tablets (LifeCycle Pharma) are a modified/extended release (MR) formulation of tacrolimus produced using MeltDose®, a novel proprietary technology that increases the bioavailability of low water soluble drugs via the solid formulation of drug substance at the molecular state.

In phase 1 studies in healthy volunteers, LCP-Tacro Tablets™ demonstrated approximately a 50% greater bioavailability than Prograf® Capsules (Astellas Pharma, Japan) and a PK profile supporting once-a-day administration. Advagraf® (MR4, Astellas Pharma) is also a modified, extended release formulation of tacrolimus designed for once-a-day administration. This study is a phase I, two-way crossover, open label, multidose, bioequivalence study to compare the pharmacokinetics (C_{max}, C₂₄, and AUC_{tau}), and safety of LCP-Tacro Tablets versus MR4 Capsules in steady state, fasting conditions. Twenty healthy male volunteers were randomized to receive either one LCP-Tacro 2 mg tablet or two Advagraf 1 mg Capsules daily for 10 days. After a two week washout period, each subject then received the alternative treatment.

Nineteen patients completed the study. There were no serious adverse events. The PK profile after 10 days of each treatment is illustrated in the figure below. The results demonstrate that LCP-Tacro Tablets provide approximately 50% greater bioavailability of tacrolimus than a comparable dose of MR4.



Kidney Immunosuppression: Minimization – Avoidance Protocols

Abstract# 285

Extended Enrollment and Analysis of a Prospective Steroid-Free Immunosuppression Trial Supports Study Safety and Efficacy. L. Li,¹ O. Salvatierra,¹ W. Concepcion,¹ C. Wong,¹ S. Alexander,¹ P. Grimm,¹ J. Martin,¹ Minnie Sarwal.¹ ¹Pediatric Department, Stanford University, Stanford, CA; ²Surgery Department, Stanford University, Stanford, CA.

AIM: To evaluate the long-term safety and efficacy of a prospective, single-center, pilot, steroid-free (SF) pediatric renal transplantation.

METHODS: 112 consecutive pediatric renal transplant recipients of living (n=88) and deceased donors were enrolled since 1999 in a steroid avoidance protocol with extended Daclizumab induction (6mo), TAC and MMF. 105 matched recipients on a steroid-based

(SB) protocol of induction (Daclizumab/Thymoglobulin) +steroids+TAC+MMF were controls (33% prospective). All SF and prospective SB patients had protocol biopsies at 3, 6, 12, and 24 mo and for graft dysfunction. Statistical analysis including T, survival analysis, chi-square, repeated measures were done by SAS.

RESULTS: Mean follow-up for SF patients was 53.6 mo. SF patient survival and death-censored graft survival at 1 yr was 99% and 100% and at 4 yrs was 92% and 100%. SF patients had a low incidence of acute rejection (AR) (7% vs. 28%, $p=0.0001$), with mean time to 1st AR at 21 mo. Significant benefits in SF were seen for linear growth with maximal benefit in the <6 yr old and excellent graft function (see figure). SF patients had significantly less hypertension and hyperlipidemia ($p<0.01$) upto 4 yrs by mixed model. At 1 yr SF required more erythropoietin (25% vs 10%, $p=0.004$) and GCSF (18% vs 0%, $p<0.0001$) skewed towards early study enrollment, but there were no differences in hematocrit, EPO use, leucopenia or GCSF usage at 4 yrs. In the SF group, there was 1 nodal PTLD, 2 CMV disease and 2 BK nephritis. 14% (n=16) broke SF protocol at mean 10 mo post-tpx, with recurrence of primary disease in 6 patients. 3% SF vs 13% SB patients had PTDM at 4 yrs ($p=0.0035$).

CONCLUSIONS: SF immunosuppression protocol offers excellent continued graft and patient outcomes for pediatric renal txp recipients with significant benefits for growth, rejection, graft function, hypertension, hyperlipidemia, PTDM, and graft survival.

	N (SF,SB)	SF	SB	P (Mixed Model)
Growth				
Age < 6 (Z Score)				
txp	34; 32	-1.26±1.36	-1.51±1.23	0.5
12mo	29; 32	-0.42±1.36	-1.24±1.23	0.0073
48mo	10; 22	-0.13±1.62	-1.61±0.94	
6 < Age < 12 (Z Score)				
txp	22; 17	-1.68±1.30	-1.50±1.44	0.7
12mo	21; 17	-1.24±1.39	-1.39±1.24	<.0001
48mo	12; 10	-0.96±2.06	-2.37±0.70	
Graft Function				
Calculated Creatinine Clearance (ml/min)				
txp	112; 97	16.08±13.27	22.08±23.96	0.03
12mo	100; 91	108.42±27.63	91.90±26.81	<.0001
48mo	39; 50	105.39±26.57	83.54±32.16	

Abstract# 286

Prospective Randomized Trial of Maintenance Immunosuppression (IS) in Prednisone (P)-Free Recipients: 5-Year Results. Raja Kandaswamy,¹ A. Humar,¹ T. Dunn,¹ E. Gross,¹ M. Hughes,¹ M. Hill,¹ T. Jie,¹ K. Gillingham,¹ A. Matas.¹ ¹Surgery, U of MN, Minneapolis, MN.

We studied which IS protocol would be best after rapid discontinuation of P. Between 9/01 and 4/06, 440 1st and 2nd kidney tx recipients were randomized: CSA-MMF (n=151) vs. high TAC-low SRL (n=149) vs. low TAC-hi SRL (n=140) (for TAC and SRL levels, high = 8-12, low = 3-7). All received thymoglobulin (TMG) 5 doses, and P for 5 days; TMG was continued in DGF. Min f/u = 1 yr; mean = 42±19 mos. There was no diff between groups in recip age, gender, ethnicity, prim dis, donor source, % retx, PRA; or in donor age, gender, ethnicity.

There was no signif diff. between groups (intention to treat) in actuarial patient, graft, death-censored (DC) graft, acute rejection (AR), or biopsy-proven chronic rejection rates (CR), serum Cr level or calculated (MDRD) GFR or in studied side effects.

Survival and renal function (1/2/3/4/5 yrs)			
	CSA/MMF(n=151)	High TAC(n=149)	Low TAC(n=140)
%patient	97/97/92/90/90	98/97/94/93/93	96/93/91/89/87
%graft	91/90/85/82/79	96/93/86/82/80	94/89/87/83/81
%DC-graft	97/93/92/91/87	98/96/91/87/85	96/95/91/91/91
%AR-free	80/77/76/72/70	86/81/79/76/73	86/81/80/77/75
%CR-free	90/87/82/78/73	98/91/84/78/71	96/88/87/82/76
Cr(SD)	1.6(.7);1.7(.9);1.8(1.3);1.9(2);1.6(.7)	1.5(.4);1.7(1.1);2(2.5);1.7(1.1);1.6(.6)	1.5(.5);1.5(.7);1.6(.6);1.6(.6);1.7(.7)
MDRD	63(24);58(21);60(26);	64(20);65(24);61(27);	65(20);62(22);
GFR(SD)	61(26);62(20)	56(23);63(28)	67(22);63(26);62(24)

No sig diff between groups		
CMV	polyoma virus	skin cancer
life threatening event	drug toxicity	hospitalizations
anemia	neutropenia	MI
tropoin leak	CVA	lymphocele
fascial/wound complications	PE	DVT
kidney thrombosis/infarction	pnumonia	recurrent disease
need for antihypertensives		

The most common cause of graft loss was death with function (CSA 40%, high TAC 35%, low TAC 57%).

The majority of recipis in each group remained P-free (CSA 72%, high TAC 86%, low TAC 74%) but a large % were not on the medications which they were randomized to (CSA 16%, high TAC 51%, low TAC 42%).

We found a signif ↑ of new onset diabetes (NODM) ($p=.03$) in the TAC-SRL groups: CSA 2%, high TAC 11%, low TAC 5%. There was a trend towards more use of lipid lowering medications in the TAC/SRL groups.

In summary, all 3 IS protocols were effective; with min f/u 1 yr for each group, patient and graft survival and AR rates are similar. We found an increased NODM and possibly hyperlipidemia in the TAC/SRL groups.

Abstract# 287

A Randomized, Open-Label Study To Compare the Efficacy and Safety of Two Different Sirolimus (SRL) Regimens with Tacrolimus (TAC) + Mycophenolate Mofetil (MMF) in De Novo Renal Allograft Recipients: Preliminary 2-Year Efficacy Results from the ORION Trial. Stuart M. Flechner,¹ Sandra Cockfield,² Josep Grinyo,³ Graeme Russ,⁴ K. Martin Wissing,⁵ Christophe Legendre,⁶ J. Brian Copley,⁷ The ORION Trial Investigators. ¹Cleveland Clinic Foundation, Cleveland, OH; ²University of Alberta, Edmonton, AB, Canada; ³Hospital Bellvitge, Barcelona, Spain; ⁴Queen Elizabeth Hospital, Woodville South, Australia; ⁵Hopital Erasme, Brussels, Belgium; ⁶Hopital Necker, Paris, France; ⁷Wyeth Pharmaceuticals, Collegeville, PA.

Purpose: We present preliminary 2-year results from a worldwide trial comparing 2 SRL regimens with TAC+MMF.

Methods: Renal transplant recipients (N=451) were randomly assigned to the following treatment regimens: Group 1: SRL (8-15 ng/mL, then 12-20 ng/mL after week 13) + TAC (6-15 ng/mL) with elimination at 13 weeks (n=155); Group 2: SRL (10-15 ng/mL through week 26, 8-15 ng/mL thereafter) + MMF (up to 2 gm/day) (n=155); or Group 3: TAC (8-15 ng/mL through week 26, 5-15 ng/mL thereafter) + MMF (up to 2 gm/day) (n=141). All patients received corticosteroids and daclizumab. In June 2006, Group 2 was terminated (after all patients were accrued) because of increased acute rejection (AR) rates.

Results: Demographic characteristics were similar between groups except for more females in Group 3. Patient and graft survival were also similar among groups (see Table). Biopsy-confirmed AR (BCAR) was significantly greater in Group 2 compared with Groups 1 and 3, $p<0.001$, and most occurred in the first 6 months posttransplant with preponderance during the first 3 months. Subtherapeutic SRL trough concentrations were reported in a large number of rejectors in Group 2. Most of the rejections in Group 1 occurred within the first 3 months before TAC was eliminated. All ARs were mild to moderate; grade II ARs were proportionally greater in Group 3. Mean Nankivell GFR was numerically higher in Group 2.

	Group 1 (SRL+TAC-Elim)	Group 2 (SRL+MMF)	Group 3 (TAC+MMF)
Patient survival, n (%)	147/155 (94.8)	147/155 (94.8)	136/140 (97.1)
Graft survival, n (%)	145/155 (93.5)	147/155 (94.8)	138/140 (98.6)
BCAR, n (%)	20/148 (13.5)	43/152 (28.3)*	13/139 (9.4)
Grade II (Mild)	15/20 (75.0)	33/43 (76.7)	7/13 (53.8)
Grade II (Moderate)	5/20 (25.0)	10/43 (23.3)	6/13 (46.2)
Grade III (Severe)	0 (0)	0 (0)	0 (0)
Mean Nankivell GFR, modified ITT, mL/min (n)	64.9 (118)	72.4 (10)	66.7 (110)
Mean serum creatinine, modified ITT, mg/dL (n)	1.68 (123)	1.35 (10)	1.61 (112)

*Not death censored. *No Therapy. *p<0.001

Conclusion: Preliminary results at 2 years show excellent patient and graft survival and similar renal function among treatment groups, despite higher AR rates in Group 2. Early adequate exposure to sirolimus is mandatory to achieve desired (low BCAR rates) results.

Abstract# 288

Long-Term Graft Survival (t½) after Rapid Discontinuation of Prednisone. Arthur J. Matas, Kristen J. Gillingham, Raja Kandaswamy, Ty Dunn, William Payne, David Sutherland, Abhinav Humar. *Surgery, U of MN, Mpls, MN.*

The goal of rapid discontinuation of prednisone (RDP) after kidney transplantation is to minimize prednisone-related side effects without increasing acute rejection (AR) rates or decreasing long-term graft survival. To date, studies have shown that RDP is associated with decreased prednisone (P)-related side effects, and randomized trials of RDP (vs. long-term P) have shown little or no ↑ in AR rates. However, concern remains that long-term graft survival will be worse with RDP protocols. We studied t½ (the time it takes for ½ of the grafts surviving at 1 year to subsequently fail) for 1st tx recipients treated with RPD (1999-2007) (n = 652) (antibody, CNI, antimetabolite [MMF or SRL], and RDP) vs. 1st tx historical controls (1995-2000) (n = 388) treated with a protocol of antibody, CNI, antimetabolite [MMF], and long-term P.

Table 1 shows characteristics of the 2 groups.

Table 1 - Characteristics of Control and RDP Groups			
	Control	RDP	p
% female	39%	40%	NS
% white	82%	86%	NS
Mean age (± SE)	51±13	48±16	NS
% type 1 diabetes	13%	14%	NS
% LURD	14%	30%	<.01
% PRA @ tx = 0%	78%	88%	<.001
% peak PRA = 0%	68%	76%	<.001
mean donor age (± SE)	40±16	41±13	NS

There was no difference gender, race or age between groups. Historical controls were more likely to have received a deceased donor transplant and less likely to have 0%

PRA; RDP were more likely to get a living unrelated donor transplant. T ½ is shown separately for living (LD) and deceased (DD) donor transplants in Table 2. There was no significant difference in t½ between groups.

Table 2 - 1st Transplant t½ (yrs) (± SE)

	Control	RDP
Deceased donor		
t½	8.9 ± 0.8 yrs	15.0 ± 3.3 yrs
D-C t½	17.2 ± 2.2 yrs	22.5 ± 6 yrs
Living donor		
t½	14.7 ± 2.5 yrs	16.1 ± 2.2 yrs
D-C t½	25.0 ± 5.6yrs	33.4 ± 6.7 yrs

D-C = Death-censored

We conclude that, compared to historical controls, rapid discontinuation of prednisone can be done without any detrimental impact on long-term outcome. A prospective, randomized study to confirm this observation is necessary.

Abstract# 289

Successful Calcineurin-Inhibitor (CI) Discontinuation Following Early Steroid Withdrawal (ESW) in Renal Allograft Recipients; Reduced Chronic Allograft Nephropathy (CAN) and Improved Renal Function without Increased Rejection or Graft Loss. R. Brian Stevens,¹ Lucile Wrenshall,¹ David Mercer,¹ Theodore Rigley,¹ Kathleen Nielsen,¹ Megan Henning,¹ Jill Skorupa,¹ Anna Skorupa,¹ John Sandoz,¹ Anna Kellogg,¹ Clifford Miles,¹ Vinaya Rao,¹ Gerald Groggel.¹ *Divisions of Transplantation and Nephrology, University of Nebraska Medical Center, Omaha, NE.*

We hypothesized that rATG induction would enable safe discontinuation of CI maintenance in renal transplant patients on steroid-free immunosuppression. We compared two rATG induction protocols (6 mg/kg x 1 dose vs. 1.5 mg/kg x 4 doses) in a prospective randomized trial of CI discontinuation (tacrolimus/sirolimus vs. mycophenolate mofetil/sirolimus) combined with ESW in kidney recipients (Fig. 1A).

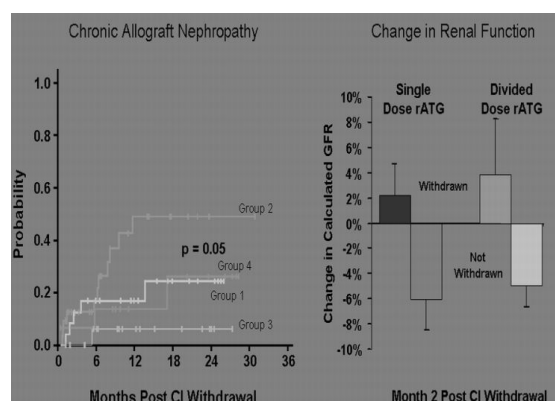
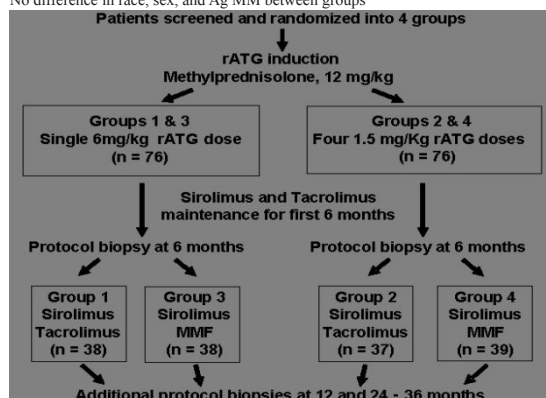
We enrolled 152 patients (Table 1) with an average follow-up of 19.7 ± 11.7 months. Subjects 19 – 65 years old included primary and re-transplants, with primary endpoints of renal function and CAN. Follow-up averaged 16.6 ± 9.5 months in 47 patients withdrawn from CI (26 from Group 3, 21 from Group 4). We found no increased rejection after CI discontinuation and that both rATG induction dosing regimen and CI discontinuation significantly impacted renal function and the development of CAN.

Conclusions: We successfully discontinued both calcineurin inhibitors and steroids with either single or divided-dose rATG induction, and single-dose rATG induction independently associated with improved renal function and reduced CAN.

Study Demographics

	Study Demographics			
	Single-dose rATG (6 mg/kg x 1)		Divided-dose rATG (1.5 mg/kg x 4)	
	Group 1 (n = 38)	Group 3 (n = 38)	Group 2 (n = 37)	Group 4 (n = 39)
Age	45.7 ± 11.9	45.3 ± 12.6	47.4 ± 10.9	51.8 ± 9.6
Deceased Donor (DD)	42%	42%	43%	41%
DD CIT	14.9 ± 6.8	14.5 ± 5.8	13.6 ± 4.1	15.4 ± 11.3
High Risk	11%	18%	22%	15%

No difference in race, sex, and Ag MM between groups



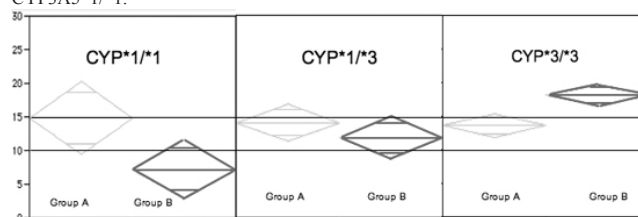
Abstract# 290

A Prospective Randomized Study of Pharmacogenetic Adaptation of Tacrolimus (TAC) Treatment after Renal Transplantation. Eric Thervet,¹ Marie Anne Lorient,² Stephane Barbier,³ Matthias Buchler,⁴ Maxence Fichoux,⁵ Gabriel Choukroun,⁶ Corinne Alberti,⁷ Christophe Legendre,⁸ Philippe Beaune.⁹ *¹Service de Transplantation, Necker, Paris, France; ²Service de Biochimie, HEGP, Paris, France; ³Service de Nephrologie, CHU, Rouen, France; ⁴Service de Nephrologie, CHU, Tours, France; ⁵Service de Nephrologie, CHU, Caen, France; ⁶Service de Nephrologie, CHU, Amiens, France; ⁷Laboratoire de Biostatistique, Robert Debre, Paris, France.*

Introduction: Retrospective studies have demonstrated that cytochrome P4503A5 (CYP3A5) polymorphisms are associated with TAC dose requirements. CYP3A5 expressors (CYP3A5*1 carriers) need a higher daily dose to achieve therapeutic trough (C0) levels. The aim of the study was to demonstrate that pharmacogenetic allows a more appropriate initial adaptation of TAC treatment.

Patients and methods: This multicenter, prospective, open-label trial included 280 renal recipients randomized at day0 to receive TAC at day7 according to CYP3A5 genotype (group A, 0.075 mg/kg bid in CYP3A5*1 carriers or 0.15 mg/kg bid in CYP3A5*3/*3) vs. usual TAC dose (group B, 0.10 mg/kg bid). All patients received a biological induction, MMF and steroids. Primary endpoint was the proportion of patients within therapeutic C0 range (10-15 ng/ml) at day10. 33 patients were excluded because of the use of drug interacting with CYP.

Results: Baseline characteristics were not different between the 2 groups. Genotype frequency was 79.8 % CYP3A5*3/*3, 15.8% CYP3A5*1/*3 and 4.4% CYP3A5*1/*1. The mean TAC daily dose was not different among the 2 groups (group A : 12.9±3.4 vs. 15.3±8 mg, p=NS). A significantly higher proportion of patients reached targeted C0 at day 10 (43 % vs. 26 %, p=0.009). In CYP3A5*1/*1, the mean C0 was 14,87±2,35 in group A vs. 7,23±2,35 in group B (p=0.035). These values were respectively 14.2±1.4 vs. 11.8±1.6 (p=0.26) in CYP3A5*1/*3, and 13.7±0.9 vs. 18.2±0.8 (p=0.0002) in CYP3A5*1/*1.



Conclusion: We report the first demonstration that the prospective adaptation of TAC daily dose according to CYP3A5 polymorphisms is associated with a higher proportion of patients reaching the targeted C0. The clinical results are being analyzed to determine the clinical consequences.

Kidney: Complications III

Abstract# 291

Review of 90,886 Renal Transplants: Survival Impact of Sirolimus. Jon VonVisger, Kyle Porter, Lianbo Yu. *Medicine, The Ohio State University, Columbus, OH; Center for Biostatistics.*

Univariable analysis of UNOS data previously suggested a potential negative survival effect of Sirolimus (Srl) on renal transplant recipients. We present a follow-up multivariable analysis of survival from UNOS data obtained for 90,866 renal recipients 18 years and older, transplanted 1/1/2000 to 1/1/2007. Comparison was restricted to patients initiating Srl at transplant before discharge. Using a piece-wise Cox proportional hazards model to account for recipient age, diabetes, race, and gender as well as donor

type (alive or deceased), and age, we observe a negative survival impact of Srl in patients with or without delayed graft function (DGF).

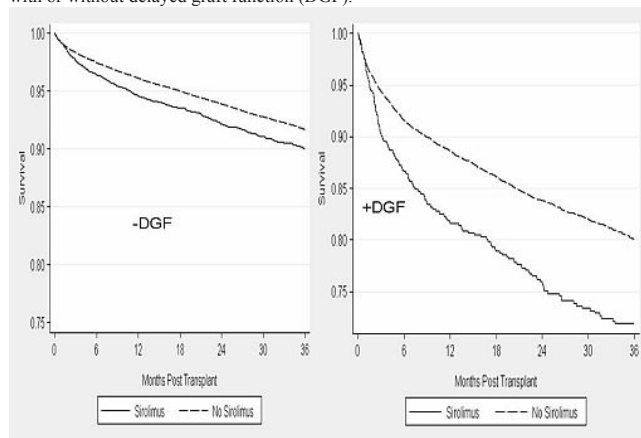


Figure 1. Kaplan-Meier Survival Curves For Patients +/- Srl, +/- DGF

Table 1. Covariate Adjusted Hazard Ratios (HR) +/- Srl

HR	0-6 Mos	7-12 Mos
+DGF: Srl vs -Srl	1.63(1.31-1.99)*	1.74(1.22-2.51)*
-DGF: Srl vs -Srl	1.57(1.35-1.84)*	1.48(1.17-1.86)*

(95%CI). *p<.005

In subset analysis, we also find an enhanced negative impact of Srl when combined with steroids (Str) in patients without DGF.

Table 2. Srl vs Str Covariate Adjusted Hazard Ratios For Each Pair No DGF

Treatment Group	0-6 Months	13-36 Months
+Srl/-Str vs +Str/-Srl	1.42(1.05-1.92)*	0.63(0.42-0.94)*
+Srl/-Str vs Both	0.78(0.55-1.09)	0.42(0.27-0.65)*
+Str/-Srl vs Both	0.55(0.45-0.66)*	0.67(0.56-0.80)*

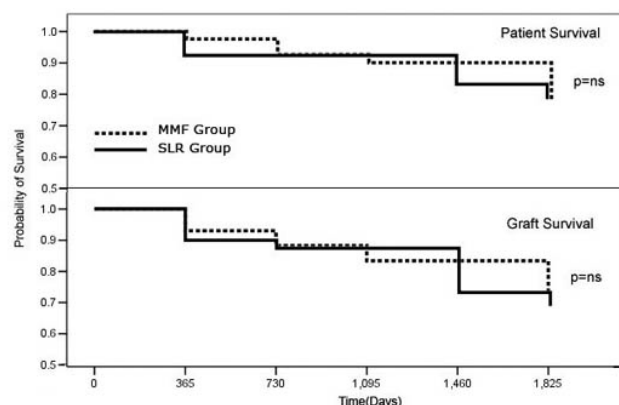
(95%CI). *p<.05

Conclusion: This data supports previous findings that Srl may be associated with a sustained survival disadvantage apparent early post transplant, and that this effect appears exacerbated when combined with DGF or Str. Several potential explanations for this effect may include Srl-associated prolonged early graft dysfunction, hyperlipidemia or proteinuria. However, patients initiating Srl after discharge may not evidence this survival disadvantage, and this question was not addressed here. These findings may be of particular importance to centers employing combined Srl and Str therapy, as well as to define the best mode of support during recovery from DGF. Prospective randomized studies would be necessary to evaluate these.

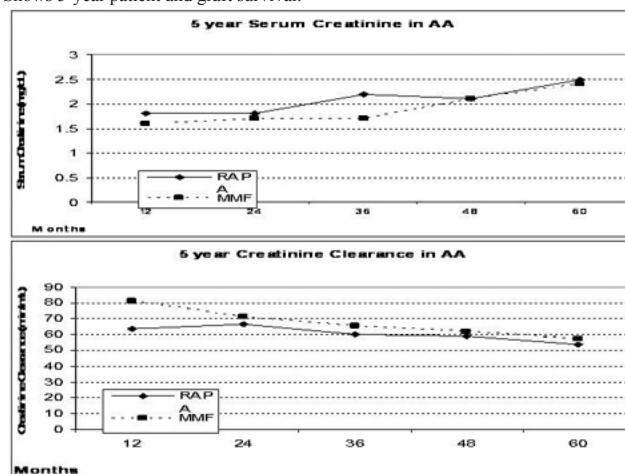
Abstract# 292

Sirolimus (SLR) Versus Mycophenolic Acid Therapy (MMF) in Non-African American (Non-AA) Recipients of Deceased Donor Kidneys without Chronic Steroid Therapy: Five Year Outcomes. Muhammad I. Saeed,¹ Arjun M. S. Kumar,¹ Gregory E. Malat,¹ Karthik Ranganna,¹ Mysore S. Anil Kumar.¹ ¹*Surgery/Transplantation, Drexel University/Hahnemann University Hospital, Philadelphia, PA.*

This study prospectively analyzed the effect of SLR vs. MMF on 5-year outcomes in non-AA recipients of deceased donor kidneys treated with calcineurin based immunosuppression(CIN) without chronic steroid therapy. 81 recipients transplanted between July 2000 and October 2004 were analyzed; 39 in SLR group and 42 in MMF group. The demography was comparable. Surveillance biopsy was performed to evaluate sub clinical acute rejection (SCAR) and chronic allograft injury (CAI). Biopsy proven clinical acute rejections (BPAR) were treated.



Shows 5-year patient and graft survival.



Shows renal function in MMF and SLR groups. BPAP was seen in 12%vs.10% in MMF vs. SLR groups. SCAR was 21% vs. 10%, 12% vs. 10%, 7% vs. 7%, 8% vs. 6% and 6% vs. 6% at 1, 2, 3, 4 and 5 years in MMF vs. SLR groups respectively.

Cumulative chronic allograft injury CAI at 5 years	MMF group	SLR group	P value
CAI-hypertension	50%	46%	0.9
CAI calcineurin toxicity	4%	8%	0.4
CAI-viral disease	6%	4%	0.8
CAI-pyelonophritis	0%	0%	1.0
CAI-obstruction	0%	0%	1.0
CAI chronic active antibody mediated rejection	6%	4%	0.8
CAI-chronic active T cell mediated rejection	12%	4%	0.3
CAI-Interstitial fibrosis/tubular atrophy	38%	16%	0.02

Table 1 shows CAI in both groups from 1 to 5 years. In SLR group the doses of CIN were significantly lower from one through 5 years (p=0.05). CAI due to interstitial fibrosis/tubular atrophy was significantly lower in SLR group at 5 years. Our data shows that 5 year patient and graft survival, graft function, BPAP and SCAR were comparable between MMF and SLR groups despite lower prevalence of interstitial fibrosis/tubular atrophy in SLR group.

Abstract# 293

Tacrolimus/Sirolimus (TAC/SRL) vs Tacrolimus/Mycophenolate (TAC/MPA) in Kidney Transplantation: Comparable 3-Year Graft and Patient Survival in Recent Era. Jane Gralla,¹ Alexander C. Wiseman,² ¹*Department of Pediatrics;* ²*Division of Nephrology, University of Colorado Health Sciences Center, Denver, CO.*

Registry analyses suggest that TAC/MPA immunosuppression is associated with superior kidney graft survival vs. TAC/SRL. Large single-center experience may assist in clarifying these findings, by examining outcomes related to specific utilization practice. We retrospectively examined the outcomes of 529 consecutive first renal transplants (55% deceased donor, 45% living donor) at a single center, treated with TAC/SRL or TAC/MPA. Graft and patient survival, acute rejection rates, and 1yr eGFR were analyzed by era of transplant (2000-2002 vs. 2003-2006). Changes in TAC/SRL utilization between eras included elimination of the SRL loading dose and a reduction in TAC target trough concentrations.

Group: Era (N)	% 3-Yr Patient Survival	% 3-Yr Graft survival	% 3-Yr Graft survival (Death Censored)	Mean 1Yr eGFR	%AR at 1 Yr
TAC/SRL: 00-02 (121)	84.8*	80.6*	93.3	54.6	14.1
TAC/SRL: 03-06 (191)	95.9	88.1	90.8	58.3†	7.9
TAC/MPA: 00-02 (54)	96.1	90.3	92.1	58.3	13.0
TAC/MPA: 03-06 (163)	92.9	87.5	93.0	63.1	12.9

*p<0.001, compared to remaining groups, †p=0.02 compared to TAC/MPA 03-06

Graft survival with TAC/SRL was significantly lower when first utilized (00-02) due to increased death. Causes of death were variable, with cardiovascular etiology (33%) as the leading cause of death. TAC/SRL use in 03-06 demonstrates lower rejection rates and comparable graft and patient survival, but with statistically lower 1yr eGFR compared to TAC/MPA. Multivariate analysis of graft and patient survival controlling for donor and recipient risk factors confirmed no significant difference in outcomes between TAC/SRL and TAC/MPA groups during the period 03-06, with improved patient survival for TAC/SRL over time. In patients alive with graft function, 81.6% remained on TAC, 83.2% on SRL, and 90.1% on MPA at last follow-up. These data, as the largest single center report representing ~7% of published registry data with TAC/SRL with additional follow-up, suggest that the poorer outcomes initially with TAC/SRL may be due to patient death. With greater experience, TAC/SRL can produce similar graft and patient survival to TAC/MPA without compromising rejection rates and graft function over time.

Abstract# 294

The Effect of Sirolimus on De Novo Cancer after Kidney Transplantation: An OPTN Database Analysis. Wida S. Cherikh,¹ Douglas W. Hanto,² Allan D. Kirk,³ ¹Research, UNOS, Richmond, VA; ²Division of Transplantation, Beth Israel Deaconess Medical Ctr & Harvard Med School, Boston, MA; ³Emory University, Atlanta, GA.

Background. A recent OPTN database analysis demonstrated that sirolimus (SRL) is associated with an increased risk of PTLD in kidney transplant (tx). Using the same database, we sought to determine the association of SRL with non-PTLD de novo cancer (CA). Since the physiologies of solid and skin cancers are different, we have examined the effect of SRL on these cancers separately.

Data and Methods. We included 66,192 adult recipients of primary living and deceased donor kidney txs from the OPTN database during 2000-2005. Patients were categorized into one of the following discharge regimens: SRL alone (N=2,257), SRL with cyclosporine (SRL+CYA) (N=2,664), SRL with tacrolimus (SRL+TAC) (N=4,659), CYA no SRL (CYA) (N=18,783) and TAC no SRL (TAC) (N=37,829). Incidence of de novo CA was computed using the Kaplan-Meier method. Multivariate Cox regression models were used to determine the association of discharge regimen and de novo solid and skin CA within 3 years of tx in the presence of various other characteristics.

Results. Incidence of de novo solid CA within 3 years was 19 (1.14%) with SRL, 19 (0.86%) with SRL+CYA, 46 (1.21%) with SRL+TAC, 211 (1.32%) with CYA and 412 (1.42%) with TAC (p=0.01). Incidence of skin CA within 3 years was 19 (1.13%) with SRL, 10 (0.45%) with SRL+CYA, 31 (0.85%) with SRL+TAC, 198 (1.25%) with CYA and 331 (1.15%) with TAC (p=0.04). Table below shows the adjusted hazard ratio (HR) of solid and skin CA, 95% confidence limit (CL) of the HR and p-value by drug regimen.

Drug	Solid CA			Skin CA		
	HR	[95% CL]	P	HR	[95% CL]	P
TAC (baseline)	1.00		-	1.00		-
SRL	0.739	[0.463, 1.180]	0.206	0.951	[0.593, 1.527]	0.836
SRL+CYA	0.682	[0.417, 1.117]	0.128	0.446	[0.231, 0.864]	0.017
SRL+ TAC	0.940	[0.668, 1.323]	0.722	0.817	[0.543, 1.228]	0.331
CYA	1.045	[0.872, 1.253]	0.634	1.139	[0.936, 1.385]	0.193

Summary. Compared to TAC, SRL+CYA was associated with a 55% decreased risk of skin CA that was statistically significant. Although SRL+CYA was associated with a 32% decreased risk of de novo solid CA, it did not reach statistical significance. This reduced risk may not reflect the unique aspects of SRL itself, but may be a reflection of practice pattern, patient selection or center effect.

Abstract# 295

The Changing Patterns of Post-Transplant Malignancies by Immunosuppressive Era-Analysis of 694 Renal Transplant Recipients for 37 Yrs in Japanese Single Center. Masahiko Okamoto,¹ Kazuki Sakai,² Tomohiko Suzuki,² Shuji Nobori,² Atsushi Yoshizawa,¹ Hidetaka Ushigome,² Seisuke Sakamoto,² Kiyokazu Akioka,³ Norio Yoshimura.^{1,2} ¹Department of Organ Interaction Research Medicine, Kyoto Prefectural University of Medicine, Kyoto, Japan; ²Department of Transplantation and Regenerative Surgery, Graduate School of Medicine, Kyoto Prefectural University of Medicine, Kyoto, Japan; ³Department of Surgery, Omihachiman Community Medical Center, Shiga, Japan.

Backgrounds: A number of studies have observed increase of malignancies following renal transplantation. However, the incidence and the site of malignancies were quite different by the follow-up times, the era and the region.

Methods: We reviewed the records of 694 renal transplant recipients in our institute between 1970 and 2007 and recorded the incidence and types of de novo malignancies. They were divided into two groups by immunosuppressive era; azathioprine (AZA) era (1970.4-1982.3: n=172) and calcineurin inhibitor (CNI) era (1982.4-: n=522).

Results: A total of 57 (27 in AZA era and 30 in CNI era) kidney recipients out of 694 developed 60 malignancies. The tumors included 10 GI-tract cancers, 9 liver cancers, 12 skin cancers, 4 tongue cancers, 6 breast cancers, 6 renal cell carcinomas, 2 thyroid cancers, 5 leukemia, 3 lymphoma, one lung cancer, one uterus cancer and one Kaposi's sarcoma (KS). The average interval between transplantation and development of malignancy was 134±86 (8-340) months. Mortality was high in liver cancer (89%) and leukemia (100%). Cumulative incidence of malignancies of all 694 recipients in 5, 10, 20, 30 years were 2.3%, 4.5%, 10.7% and 14.3%, respectively. Graft-loss censored cumulative incidence, which was calculated to see the incidence among graft survivors under continuing immunosuppression, of all recipients in 5, 10, 20, 30 years were 2.8%, 6.3%, 16.3% and 26.2%. That of 5, 10 and 20 years in CNI era was 3.0%, 6.8% and 13.9%, while that in AZA era was 1.8%, 4.9% and 19.5%, showing early higher incidence in CNI era outstripped by AZA era by 12 years. Site of malignancy in CNI era occurring within 3 years, which was never observed in AZA era, was focused on liver, leukemia (including ATL), KS and PTLD.

Discussions: Our results demonstrated that recent potent immunosuppressive regimen shortened the interval between transplantation and viral-related malignancies. However, long-term incidence of whole malignancies has been decreasing by minimizing chronic immunosuppression in our institute.

Kidney: Deceased Donor Issues II

Abstract# 296

A Virtual Crossmatch Protocol Significantly Increases Access of Highly Sensitized Patients to Deceased Donor Kidney Transplantation. Adam W. Bingaman,¹ Cathi Murphey,² Juan Palma-Vargas,¹ Jorge Ortiz,¹ Preston Foster,¹ Francis Wright.¹ ¹Texas Transplant Institute, San Antonio, TX; ²Southwest Immunodiagnostics Laboratory, San Antonio, TX.

Background: Patients with pre-existing anti-HLA antibodies (sensitized patients) are more likely to have a positive crossmatch with possible donors, and have a lower likelihood of receiving a kidney transplant with significantly longer wait times. A virtual crossmatch protocol (VCP) utilizing solid phase technology to determine the specificity of anti-HLA antibodies should improve the probability of identifying a cross-match negative compatible donor and increase access of highly sensitized patients to kidney transplantation.

Methods: A VCP was initiated 10-1-2006 with solid phase HLA antibody characterization for all sensitized patients on the wait list. Transplant rates for the VCP period from 10-2006 through 9-2007 were compared with rates from 10-2005 through 9-2006 when only preliminary crossmatching was used for screening sensitized patients. SRTR data from 2005 were used to determine national transplant rates for sensitized patients.

Results: During 2005-06, prior to initiation of the VCP, 52 deceased donor transplants were done, with 3 highly sensitized (PRA >80%) patients (5.8%) receiving kidneys, with 1 (1.9%) patient having a PRA>90%. Similarly, SRTR data for 2005 showed that nationally 479 of 9914 transplants (4.8%) were in patients with PRA >80%. During 2006-07 after initiation of the VCP, 62 deceased donor transplants were done, with 9 highly sensitized (PRA>80%) patients (14.5%) receiving kidneys, with 6 (9.7%) patients having a PRA>90%. (p = 0.003 compared to SRTR national data). The VCP was highly predictive of a negative final crossmatch. The VCP eliminated the use of preliminary crossmatching and reduced the number of final crossmatches performed with attendant cost savings of over \$100,000.

Conclusion: Initiation of a virtual crossmatch protocol utilizing solid phase histocompatibility techniques significantly increased access of highly sensitized patients to kidney transplantation and was more cost effective. Utilization of a virtual crossmatch may facilitate greater sharing of kidneys to improve access to transplantation for highly sensitized recipients.

Abstract# 297

The Impact of Transplant Center Practice on the Association between Pulsatile Perfusion and Delayed Graft Function. Jagbir Gill,¹ David Gjertson,¹ Suphamai Bunnapradist,¹ Michael Cecka.¹ ¹UCLA, LA, CA.

The use of pulsatile perfusion (PP) is increasing in the US, but practice varies widely among transplant (Tx) centers. We describe the variability of PP use and its impact on post transplant outcomes.

Methods: We identified all cadaveric kidney Tx from 2000-2005 using OPTN/UNOS data. The cohort was stratified by Tx center PP use as follows: Low PP centers (0-10% PP use), med PP centers (10-30% PP use), and high PP centers (>30% PP use). Donor characteristics and the incidence of DGF were compared between and within each strata.

Results: PP was used by 97 % of centers, however most centers used PP <10% of the time (70.7%). Compared to low and med PP use centers, kidneys pumped in high PP centers were from donors that were younger, had a lower mean terminal serum creatinine, and had a lower incidence of CVA and hypertension. The overall incidence of DGF was lowest in the high PP centers (13.3%), compared to the med (24.7%) and low PP (24.8%) centers.

The rates of DGF within each strata (high, med, and low PP centers) for Tx performed using PP versus cold storage (CS) are outlined in the Table below. Within each strata, the rate of DGF did not differ between Tx performed with and without PP. In ECD transplants, PP was associated with lower rates of DGF across all center groups. However, the impact of PP on SCD and DCD transplants was less significant and varied across centers by PP use.

Rates of DGF by TX Center in Pulsatile Perfusion (PP) VS. Cold Storage (CS) Cases			
	Low Pump Center (0-10% PP use)	Medium Pump Center (10-30% PP use)	High Pump Center (>30% PP use)
N	42,293 (70.7%)	9280 (15.5%)	6164 (10.3%)
Overall			
PP	30.7%	25.2%	13.3%
CS	25.1%	25.7%	14.7%
P value	<0.001	0.260	0.157
SCD			
PP	26.8%	22.3%	9.8%
CS	22.6%	23.2%	12.5%
P value	0.003	0.430	0.003
ECD			
PP	27.8%	25.4%	17.5%
CS	34.7%	35.6%	24.0%
P value	<0.001	<0.001	0.048
DCD			
PP	38.4%	39.8%	38.1%
CS	42.8%	47.5%	47.5%
P value	0.047	0.113	0.173

Conclusion: While the majority of centers use PP, most use it less than 10% of the time. The impact of PP on DGF is not significant when restricted to centers with similar PP use. When stratified by donor type and Tx center, only ECDs demonstrate a consistent reduction in DGF with PP vs CS.

Abstract# 298

Donor Service Area Transplantation Rate for HLA Sensitized Patients Is Increased by HLA Class I Unacceptable Antigen Entry into Unet.

Christopher F. Bryan,¹ Alan M. Luger,¹ John L. Smith,¹ Brad A. Warady,¹ Erik Schadde,¹ Daniel Murillo,¹ Paul W. Nelson.¹ ¹Midwest Transplant Network, Westwood, KS.

Introduction. Unacceptable antigen (UA) entry into Unet has been shown to improve the likelihood of transplanting HLA sensitized patients at single centers (AJT 2006; 6:2307-15) but the effect on the transplantation rate in a donor service area (DSA) is unknown. **Methods.** We compared the rate of deceased donor (DD) renal transplants in our five transplant center-DSA since beginning (7/1/07) UA entry of class I (A, B, C), but not class II, into Unet with the rate for the preceding period (1/1/06 to 3/1/07) when no UA entry occurred (a positive AHG T cell crossmatch, with current serum, was used to "strike" candidates from the match run). From 7/1/07 DD were typed (PCR-SSP) for HLA-A,B,C locus antigens and all those donor antigens were entered into Unet so a "virtual A, B & C crossmatch" occurred. The transplant rate for each group was stratified by the patient's peak HLA class I IgG flow PRA level of either low (0-19%), intermediate (20-79%) or high (80-100%). The crossmatch criteria for transplantation was either a negative AHG or flow T cell IgG crossmatch. **Results.** The data in the following table show the distribution of transplant rates before and since class I-UA entry into Unet began for renal candidates in our DSA.

Peak Class I PRA Level	DSA Transplant Rate from when Unacceptable HLA Class I Antigens were Entered into Unet		p Value (χ^2)
	Before	Since	
Low	77% (146/189)	60% (43/72)	0.02
Intermediate	13% (25/189)	21% (15/72)	0.03
High	10% (18/189)	19% (14/72)	0.02

HLA sensitized patients (20-100%) in our DSA are now transplanted at a rate of 40%, which is significantly higher ($p = 0.02$) than the 23% rate when UA weren't entered into Unet. Furthermore, of 9 kidneys imported into our DSA and allocated to the DSA-wide renal candidate list (since UA entry started), 63% (5/9) were transplanted into sensitized candidates (85% to 27%), each of whom had a negative flow or AHG T cell IgG crossmatch. **Conclusion.** The higher transplantation rate for HLA sensitized patients in our DSA shows that virtual A, B, & C crossmatching yields a DSA-wide ranked list of sensitized candidates likely to have a negative final class I (T cell) crossmatch and be transplanted. The data also lend support to the notion that sharing kidneys across DSA boundaries for HLA sensitized candidates, based on a negative virtual HLA class I crossmatch, has merit.

Abstract# 299

Predicting Graft Outcome with a Pre-Transplant Kidney Biopsy Using a Cumulative Pathology Score.

Raghava B. Munivenkatappa,¹ John C. Papadimitriou,² Cinthia B. Drachenberg,² Flavia Rasetto,³ Aldo Leizaola Haririan,⁴ Eugene Schweitzer,¹ Matthew M. Cooper,¹ Rolf Barth,¹ Luis Campos,¹ Stephen T. Bartlett,¹ Benjamin Philosophie.¹ ¹Surgery; ²Pathology; ³Nephrology; ⁴Pharmacy, University of MD, Baltimore, MD.

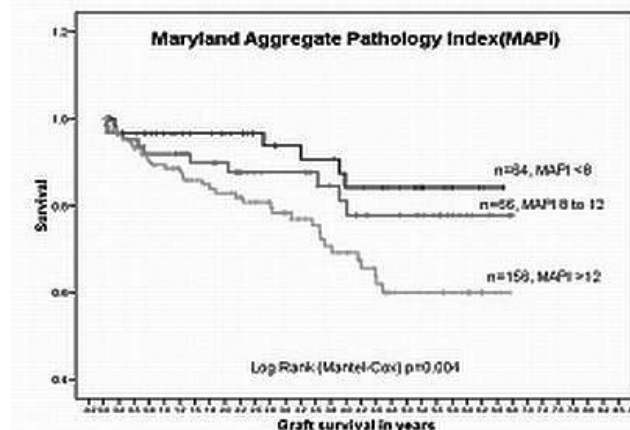
Introduction: Predicting graft outcome after renal transplantation based on donor histological features has remained elusive and is subject to institutional variability. We propose a pre-transplant donor path scoring system that reliably predicts graft outcome regardless of recipient characteristics.

Methods: We retrospectively analyzed 286 imported cadaveric renal transplants which were initially rejected by other centers due to donor parameters between 1/00-6/05. All kidneys were re-biopsied at our center prior to implantation. Morphometric analysis performed consisted of measuring glomerular-size arterioles, interlobular, and arcuate/interlobar arteries and wall to lumen ratio (WLR) was calculated; calculating % glomerulosclerosis (GS); the presence of arteriolar hyalinosis (AH), scar, periglomerular fibrosis (PGF), and acute tubular necrosis in the biopsies. The patients were followed for a mean of 33 months. Multivariate Cox analysis was done to evaluate the predictive value of these path variables to graft outcome. **Results:** AH, GS>15%, WLR>0.5, PGF, and scar were found to independently predict graft outcome.

Variable	Multivariate (RR)	p	Index Score	
			Present	Absent
AH	2.9	0.01	+3	0
GS>15%	2.2	0.02	+2	0
WLR>0.5	2	0.03	+2	0
PGF	5	0.03	+5	0
Scar	2.5	0.057	+3	0

The relative risk for each variable was rounded to an index score and the sum created the Maryland Aggregate Pathology Index (MAPI) which ranged from 0 to 15. MAPI predicted graft outcome regardless of R characteristics, including age, immunosuppression, induction, and the presence of DGF. Kaplan Meier curves for

transplants with low (0-8), intermediate (8-12), and high (>12) MAPI scores were significantly different.



Seven year graft survival was 84 %, 77% and 60% for low, intermediate and high MAPI, $p = 0.004$. The relative risk of graft loss increased 16% for every MAPI point increase.

Abstract# 300

The Use of Biochemical Perfusion Parameters in Predicting Delayed Graft Function of Machine Preserved Kidneys.

Ivan R. Zendejas-Ruiz,¹ Maximilian Polyak,¹ Joseph F. Magliocca,¹ Shiro Fujita,¹ Robin D. Kim,¹ Alan Reed,¹ Alan Hemming,¹ Richard J. Howard,¹ Liise Kayler.¹ ¹Department of Surgery, Division of Transplantation and Hepatobiliary Surgery, University of Florida, Gainesville, FL.

Background: Machine perfusion (MP) is increasingly utilized for pre-transplant evaluation of deceased-donor (DD) kidneys. The influence of perfusion solution biochemical characteristics, however, has rarely been evaluated. **Methods:** Sixty-four consecutive DD kidney transplant recipients were assessed for factors associated with delayed graft function (DGF) utilizing a multivariate logistic model. Covariates included perfusion solution [Na⁺], [K⁺], [Cl⁻], [iCa⁺⁺], pH, and lactate concentrations, perfusion flow and resistance at 2 hours and 4 hours MP, recipient age, gender, race, retransplantation, etiology of end-stage renal disease, waiting time, donor age and gender, expanded criteria (ECD), cardiac death (DCD), cold ischemia time, and induction medication type. Significance was considered at a p -value <0.05. **Results:** The incidence of DGF was 25%. DCD and ECD kidneys comprised 17% and 23% of the sample, respectively. Univariate analysis showed that final lactate concentration, resistance of ≥ 0.35 at 4 hours of MP, waiting list time, and DCD status were associated with DGF. On multivariate analysis only final lactate ($p=0.0026$) and DCD status ($p=0.0319$) remained significantly associated with DGF. Final lactate concentrations > 2, 3, 4, and 5 mmol/L result in DGF frequencies of 40%, 63%, 70% and 100% of recipients, respectively. **Conclusion:** Final lactate concentration in the perfusate solution is a major independent indicator of DGF. These results may aid in the assessment of machine perfused kidneys for transplant suitability.

Abstract# 301

Consequences of Allocating Deceased Donor Organs Based on Recipient Antibody Specificities (CPRA).

Ronald H. Kerman,¹ Charles T. Van Buren,¹ Stephen M. Katz,¹ Barry D. Kahan,¹ Jacqueline Lappin,¹ Carolyn Olivarez,² Teresa Shafer.² ¹Surgery-Organ Transplantation, University of Texas Medical School, Houston, TX; ²LifeGift OPO, Houston, TX.

The UNOS Board of Directors has approved the change from using Panel Reactive Antibodies (PRA) to calculated PRA (CPRA). The CPRA is a formulated PRA based upon the frequency of the specificities of HLA antigens found in the donor pool and is expected to standardize the degree of patient sensitization. Donor organs expressing unacceptable antigens will not be offered to a recipient with donor (HLA) antigen specific antibodies (DSA). Highly sensitive, single antigen bead and solid phase assays (Flow PRA and Luminex) are used to identify these HLA antibodies (Abs). Each transplant center can determine the criteria used for identifying an unacceptable antigen, for example, based upon DSA titer or the fluorescence intensity (FI) coming from the donor-specific single antigen bead. It is unclear whether Abs identified by these techniques are clinically relevant for organ allocation.

We retrospectively evaluated Flow-PRA, flow cytometry crossmatching (FCXM), HLA Ab specificities and titers of 300 pre-transplant (Tx) sera from transplant recipients of deceased renal allograft donors transplanted following a negative cytotoxic-anti-human globulin crossmatch.

The two year graft survival of 91% for the recipients (44/54, 81%) with low-titer ($\leq 1:16$) donor specific HLA Ab and a negative (-) FCXM was significantly better when compared to the 60% two year graft survival of the 19% (10/54) of recipients presenting with (+) DSA but a (+) FCXM ($p < 0.001$). Recipients (55/110, 50%) with non-donor

specific HLA Abs (high or low titer) and a (-) FCXM also experienced a better two year graft survival of 89% compared to the 74% for the other 50% of recipients with (+) FCXMs ($p < 0.001$).

These data suggest that in the presence of donor-specific or non-donor-specific HLA Abs you can not predict the crossmatch outcome without actually performing the crossmatch which will then influence donor organ allocation and graft survival outcome. In the face of low-titer DSA and a (-) FCXM recipients experienced excellent graft outcome when compared to recipients with (+) FCXMs. Therefore, donor organ allocation based on CPRA (Ab specificity and unacceptable Ags) utilizing highly sensitive single antigen bead and solid phase Luminex assays may disadvantage recipients (no donor crossmatch) who could otherwise be successfully transplanted.

Liver: Hepatocellular Carcinoma and Cholangiocarcinoma Malignancies

Abstract# 302

Vascular Endothelial Growth Factor Receptor 2 (VEGFR2) Expression in Non-Tumorous Cirrhotic Liver Is Higher When Hepatocellular Carcinoma (HCC) Is beyond Milan Criteria (MC). Federico Aucejo,¹ Richard Kim,¹ Nizar Zein,¹ Teresa Diago Urso,¹ Cristiano Quintini,¹ Koji Hashimoto,¹ Kenzo Hirose,¹ Ibrahim Hanouneh,¹ Rocio Lopez,¹ Dymna Kelly,¹ Bijan Egtesad,¹ John Fung,¹ Charles Miller,¹ Lisa Yerian.¹ ¹Liver Transplant Unit, Cleveland Clinic, Cleveland, OH.

Aim: To assess VEGFR2 expression in HCC and the adjacent benign cirrhotic parenchyma and its correlation with tumor differentiation, vascular invasion, and tumor morphologic parameters.

Background: HCC is a highly vascular tumor in which angiogenesis is mediated in part by VEGF. VEGF is highly expressed in HCC and mediates its effects through multiple receptors including VEGFR2. The tyrosine kinase inhibitor Sorafenib inactivates the VEGFR2 receptor and exhibits anti-tumor effects in HCC. Clinical significance of VEGFR2 expression with respect to tumor parameters has not been evaluated.

Patients and Methods: Immunohistochemical staining for VEGFR2 was performed in HCC and corresponding adjacent cirrhotic liver from 79 patients undergoing liver transplant. 57 patients had HCC within MC. Stains were scored by estimating the % of positive surface area in veins, arteries, and sinusoidal lining cells. Data are presented as Median [P25, P75]; Wilcoxon signed rank and Wilcoxon rank sum tests were used.

Results: VEGFR2 levels in HCC were significantly correlated to levels in adjacent non-tumorous liver. Higher levels of VEGFR2 in non-tumorous liver were associated with higher levels in HCC from the same patient. VEGFR2 levels were significantly higher in HCC compared to adjacent areas ($P < 0.05$). VEGFR2 levels in HCC were not significantly different between patients who fell within MC and those beyond MC. However, VEGFR2 levels were significantly higher in the adjacent arteries of non-tumorous liver (15.8 [0, 60] vs. 0 [0, 35]; $P = 0.03$) in those patients with HCC beyond MC. Subjects with moderate or poor differentiation had significantly higher levels of VEGFR2 in sinusoids and veins of HCC and in the sinusoids of adjacent non-tumorous liver. There was no correlation with vascular invasion.

Conclusions: Elevated VEGFR2 in HCC correlates with elevated VEGFR2 in adjacent cirrhosis, suggesting that high expressing HCC arise in a high VEGFR2 expression, pro-angiogenic environment. Moreover, higher VEGFR2 expression in background cirrhosis correlates with advanced HCC (beyond MC), suggesting that anti-angiogenic agents may prevent tumor formation or progression in cirrhotic patients. This novel concept warrants further study.

Abstract# 303

Which Patients with Hepatocellular Carcinoma Need a Biopsy before Transplantation? Nicholas Onaca, Gary L. Davis, Linda W. Jennings, Robert M. Goldstein, Goran B. Klintmalm. *International Registry of Hepatic Tumors in Liver Transplantation, Baylor University Medical Center, Dallas, TX.*

Purpose: Results of liver transplantation for hepatocellular carcinoma (HCC) are adversely impacted by poor histological differentiation (PDIFF) of the liver tumor(s). Our aim was to try to identify clinical parameters associated with poor tumor differentiation, which may warrant a pretransplant liver biopsy to potentially exclude patients at high risk of tumor recurrence after transplantation.

Results: Data from 1035 patients transplanted for HCC entered in a large registry were analyzed. 180 (17%) had poorly differentiated tumors in the liver at transplantation. PDIFF tumors were associated with alpha fetoprotein (AFP) > 400 U [positive predictive value (PPV) 22.8%, negative predictive value (NPV) 89.4%, $p < 0.0001$], tumors > 6 cm maximum diameter (PPV 15.3%, NPV 92.5%, $p = 0.0027$), > 4 tumor nodules (PPV 25.2%, NPV 81.1%, $p = 0.0001$) (Fishers Exact test) and younger median age (53 vs. 56 years, $p = 0.004$, Wilcoxon test). A Cox multivariate analysis showed higher risk of PDIFF with > 3 tumor nodules [odds ratio (OR) 2.285, $p = 0.0001$], tumors > 6 cm (OR 2.694, $p = 0.005$), and AFP > 400 U (OR 2.179, $p = 0.0014$). We further attempted to find a subgroup of patients combining tumor size, number of nodules and various levels of AFP which together would correlate strongly with PDIFF tumors. However, the distribution was erratic and even in extreme outliers (> 4 nodules, > 8 cm in maximum size, with or without high AFP), where transplantation is currently not indicated according to any current expanded tumor inclusion criteria, the incidence of PDIFF did not exceed 42%.

Conclusions: PDIFF is most highly associated with tumors that exceed the Milan criteria and the expanded criteria currently used for liver transplantation. Thus, tumor biopsy would not appear to be of benefit except perhaps in those patients with a high AFP level. However, if tumor criteria are further extended in the future, tumor biopsy may be justified in those with higher tumor burdens.

Abstract# 304

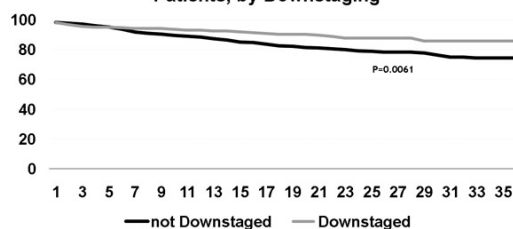
Longer Term Results for Hepatocellular Carcinoma (HCC) Liver Transplant (LT) Recipients: Down-Staging Has Benefit. Ann M. Harper,¹ Erick B. Edwards,² Richard B. Freeman.³ ¹Research Department, United Network for Organ Sharing, Richmond, VA; ²Dept. of Surgery, Tufts-New England Medical Center, Boston, MA.

Background: The OPTN database provides a very large, comprehensive cohort of LT recipients transplanted with HCC. In this study, we analyzed 3,909 patients transplanted with an HCC exception between 2/27/2002 and 3/31/2006.

Methods: We used the Kaplan-Meier survival method to determine factors affecting post transplant survival within 36 months. Median follow-up was 2.4 years. Post-transplant pathology data were available for 2,929 (75%) cases. 307 cases (10.5%) were transplanted at listing stage (LS) = T1 (1 tumor < 2 cm), 2,511 (85.7%) at LS=T2 (1 tumor < 5cm or 3 tumors ≤ 2 cm), and 111 (3.8%) with LS>2.

Results: Overall survival at 36 months was 76.5%, with significant differences seen for listing stage (LS 1= 82.2%, LS 2=75.9% LS>2=73.5%, $p = 0.0395$) and by histologic stage as determined by pathology ($p < 0.0001$). Survival for patients with histologic stage 4b HCC was only 29% at 36 months, versus 83% for stage 1. Patients with tumors greater than 3cm both by listing stage and by pathology fared poorly compared to those with smaller tumors ($p = 0.0002$). Patients listed who received ablation treatment (AT) preoperatively and who had their tumors "down-staged" had better results ($p = 0.0061$).

Post-Transplant Patient Survival for HCC Patients, by Downstaging



We observed no difference in AT types (TACE vs RFA). Those with micro or macrovascular invasion had lower survival rates, at 66.8% and 30.5%, respectively ($p < 0.0001$). AFP > 500 continues to be a significant predictor of lower post-transplant survival, with a survival rate of 57.9% at 36-months ($p < 0.0001$).

Conclusions: We conclude that listing and histologic tumor size, presence of micro/macrovascular invasion, and high AFP are associated with poorer LT results. AT is emerging as potentially effective treatment for improving LT outcomes for HCC recipients.

Abstract# 305

Recurrence of Hepatocellular Carcinoma after Liver Transplantation

- Comparison of Patients with and without Pretreatment by TACE. Gerd Otto,¹ Michael Heise,¹ Axel Herber,² Maria Hoppe-Lotichius,¹ Anja Victor,³ Marcus Schuchmann,⁴ Christoph Dueber.² ¹Transplantation Surgery, Johannes Gutenberg University of Mainz, Mainz, Germany; ²Radiology, Johannes Gutenberg University of Mainz, Mainz, Germany; ³Institute for Medical Biometry and Informatics, Johannes Gutenberg University of Mainz, Mainz, Germany; ⁴Internal Medicine I, Johannes Gutenberg University of Mainz, Mainz, Germany.

Introduction: So far, Milan criteria are used to select patients with hepatocellular carcinoma (HCC) for liver transplantation (LT). Herein we compare prognostic markers in patients with pretreatment by transarterial chemoembolization (TACE) to a second cohort transplanted without TACE pretreatment.

Patients and methods: Between September 1997 and October 2007, 134 patients with HCC underwent LT at our institution. Eighty-two patients were pretreated by repeatedly performed TACE whereas in 52 patients none or other forms of pretreatment had been used (non-TACE group). TACE was performed using lipiodol and mitomycin. Every 6 weeks TACE was repeated until transplantation. Tumor response was assessed by CT scans (6-week intervals).

Results: Sixty-seven percent of the patients transplanted after TACE pretreatment exceeded the Milan criteria compared to 37 % in non-TACE patients. The proportion of recurrence-free patients was comparable in the TACE and non-TACE group (77.32 % and 78.45 %, respectively). In the univariate analysis grading, angioinvasion and progress-free TACE were significant predictors for recurrence after LT in patients with TACE pretreatment. Progress-free TACE was the only significant predictor of recurrence in the multivariate analysis. In the non-TACE group T classification, number of nodules, grading, angioinvasion, Milan criteria and underlying disease (HCV versus

all other diseases) were significant. After TACE pretreatment freedom from recurrence was 92.3 % in patients with stable disease or regress but only 38.4 % in patients with progress during TACE.

Conclusions: TACE pretreatment is capable of selecting a biological entity of tumors which differs significantly from untreated tumors. This statement is deduced from the remarkable differences of predictors for tumor recurrence in patients with and without TACE pretreatment. Moreover, TACE patients can be separated into two groups: those who experienced tumor progress during repeatedly performed TACE and those who did not. Stable disease during the continued TACE before transplantation resulted in remarkably low tumor recurrence.

Abstract# 306

Liver Transplantation for Patients with Hepatocellular Carcinoma That Exceed the Milan Criteria. Derek DuBay,¹ Sean Cleary,¹ Paul Greig,¹ Mark Cattral,¹ Ian McGilvray,¹ George Therapondos,¹ Eberhard Renner,¹ Lesley Lilly,¹ Gary Levy,¹ David Grant.¹ ¹*Liver Transplantation, University of Toronto, Toronto, ON, Canada.*

Introduction: The optimal criteria for offering liver transplantation for hepatocellular carcinoma (HCC) are disputed. We offer transplantation for HCC that meets these conditions: HCC confined to the liver, no vascular invasion, no constitutional symptoms, and not poorly differentiated. The purpose of this study is to measure survival in patients with M+ tumors and analyse risk factors for HCC recurrence following transplantation.

Methods: Patients were stratified into Milan (M) or beyond Milan (M+) groups based upon clinical pre-transplant imaging. Patients with incidental HCC were excluded. Kaplan-Meier survival plots and univariate/ multivariate analyses were performed.

Results: Between 1996 and 2006, 215 patients were transplanted for HCC. There were 131 patients in the M and 84 patients in the M+ groups (38 uT3 and 46 uT4a UNOS staging). Basic demographics were equivalent. The M+ tumors received less preoperative ablation (p=0.04), more TACE (p=0.02) and by definition had higher staged tumors (p<0.001). Explant pathology revealed discordant staging in 22% of the M and 20% of the M+ groups.

With a median follow-up of 30 months, there was no statistical difference in the 5 year overall survival in the M (76%) and M+ (69%) groups (p=0.40). The 5-year disease free survival was significantly higher in the M (80%) vs. M+ (61%) groups (p=0.02). When stratifying for UCSF criteria tumors, the 5-year disease free survival was Milan (80%) vs. UCSF (72%) vs. beyond UCSF (54%) groups (p=0.01).

Univariate analysis demonstrated the following factors to be associated with disease recurrence: intermediate waiting time 2-4 months (p=0.01), preoperative TACE (p=0.002) or resection (p=0.04), more than 3 tumors (p=0.03), and max tumor size over 5cm (p=0.03). Multivariate analysis controlling for age, gender, and waiting time demonstrated that preoperative resection HR2.78 (95%CI 1.1-7.2), more than 3 tumors HR2.3 (95%CI 1.1-4.7) and max tumor size greater than 5cm HR2.68 (95%CI 1.2-5.9) were independently associated with disease recurrence.

Conclusions: The overall survival after liver transplantation is excellent in the M and M+ groups, far exceeding survival rates that can be obtained via any other modality. The current UNOS HCC algorithm should be reconsidered, to allow extra listing points for selected patients with HCC that exceed both the Milan and UCSF criteria.

Liver: Medical Complications

Abstract# 307

Postoperative Use of Intense Insulin Therapy in Liver Transplant Recipients. Lama M. Hsaiky,¹ Iman E. Bajjoka,¹ Dhaval Patel,¹ Marwan S. Abouljoud.¹ ¹*Transplant Institute, Henry Ford Hospital, Detroit, MI.*

Hyperglycemia and insulin resistance are common post liver transplant, even in patients with no history of diabetes. In the general surgical intensive care unit (SICU) population, the use of intensive control of blood glucose has recently been shown to reduce both morbidity and mortality. Thus far, limited data exist in the liver transplant population.

Purpose: Assess the impact of intensive insulin therapy to maintain blood glucose at or below 110 mg/dL immediately post liver transplantation in the surgical intensive care unit (SICU).

Methods: A retrospective evaluation of liver transplant recipients who received two different insulin protocols in the SICU was performed. Prior to January 2003, patients were assigned to receive sliding scale insulin therapy (SSIT) to maintain blood glucose (BG) less than 180 mg/dL. The intensive insulin therapy (IIT) was implemented in August 2005 with a goal BG level between 80-110mg/dL. The following data was analyzed: BG ranges, need for mechanical ventilation, blood transfusions, infection rate in the SICU, rejection episodes and patient survival.

Results: A total of 97 liver transplant patients were evaluated; of which 47 patients were in the SSIT group and the other 50 in the IIT group. Demographic characteristics were comparable between the two groups. In the IIT, 24% of the BG readings were maintained at $\leq$ 110 mg/dL versus 5% in the SSIT. The incidence of hypoglycemia (BG <60mg/dL) was less than 1% in both groups. The need for mechanical ventilation was 3.0 days vs. 2.3 days and the overall number of blood transfusion was on an average of 5.0 vs. 2 units (P<0.05) in the SSIT vs. IIT group, respectively. IIT also reduced overall SICU infection rate by 15% (P=0.01). The rate of acute cellular rejection at 3 months post transplant was less in the IIT, 14% vs. 25% in the SSIT group (P=0.02).

Moreover, mortality during hospital stay was reduced from 5% in the SSIT to 2% in the IIT group.

Conclusions: The use of intense insulin therapy immediately post liver transplantation has resulted in reducing infection rate and rejection episodes and a trend for reduced morbidity and mortality among post surgical liver transplant recipients without the adverse effects of hypoglycemia.

Abstract# 308

Medical Epidemiology of Patients Surviving Ten Years after Liver Transplantation. Kerri A. Simo,¹ Stephanie E. Sereika,¹ David A. Gerber.¹ ¹*Abdominal Transplantation Division, Department of General Surgery, University of North Carolina, Chapel Hill, NC.*

Background: As the population of long term survivors of liver transplantation (OLT) grows, their medical epidemiology has become increasingly important. The goals of this study were to define a collective profile of liver transplant recipients $\geq$ 10 years post OLT and to compare their co-morbidities with those of the general population. In 2005, the National Health Survey reported that 22% of the US population had hypertension, 14.4% had diabetes/impaired fasting glucose, and 16.8% had chronic kidney disease.

Methods: A retrospective review of a prospectively collected database of 125 adult patients who underwent OLT at a single transplant center from September 30, 1991 to October 1, 1997 was performed. Inclusion criteria consisted of survival $\geq$ 10 years post OLT with >1 year follow up.

Results: Seventy-one patients met inclusion criteria. Ninety percent of patients had $\geq$ 10 years follow up. The mean age at transplant was 43 (range 18-67). The mean calculated MELD score was 22 (range 9-62, median=22). Indications for OLT were HCV(32%), alcohol(28%), cryptogenic(18%), autoimmune(10%), HBV(7%), PSC(7%), PBC(7%), and other(10%). Seven patients required retransplant during the first 10 years. An additional 38 patients underwent other operations: 10 arterial or biliary revisions, 13 hernia repairs and 19 non-transplant related (7 abdominal).

During analysis, the following medical co-morbidities were found: 51 patients(72%) had hypertension (41 new onset), 20(28%) diabetes (10 new onset), 33(46%) renal insufficiency and 8 renal failure (28 new onset), 10(14%) cardiovascular disease (all new onset). Nine patients (13%) were diagnosed with de novo cancer.

Medications for chronic health problems included 18 patients on diabetic medications, 46 on antihypertensives and 18 on lipid lowering agents. Initial immunosuppression consisted of 100% on steroids, 61% on cyclosporine, 40% on tacrolimus, 24% on mycophenolate mofetil (MMF) and 23% on azathioprine. Immunosuppression at 10 years consisted of 42% on cyclosporine, 41% on tacrolimus, 18% on steroids, 13% on MMF, 8% on sirolimus, 1% on mycophenolate sodium, 1% on azathioprine. No patients were on triple therapy, 29 were on dual therapy, and 42 were on monotherapy.

Summary: Patients alive 10 years post OLT have a significantly higher incidence of hypertension, diabetes, and renal disease than the general population. This study supports conscientious medical follow up to ensure continued meaningful survival.

Abstract# 309

Liver Transplantation in the Morbidly Obese (BMI>40). C. Quintini,¹ L. Kauzman,¹ K. Hashimoto,¹ P. Ding,¹ T. Doago Uso,¹ N. Sopko,¹ J. Rosenblum,¹ F. Aucejo,¹ C. Winans,¹ D. Kelly,¹ B. Eghtesad,¹ D. Vogt,¹ J. J. Fung,¹ C. Miller.¹ ¹*General Surgery - Liver Transplant Service, Cleveland Clinic OH.*

INTRODUCTION. Morbid obesity (MO) is a problem seen with increasing frequency among candidates for OLT. MO is considered a contraindication for OLT in some centers without clear evidence to support such a practice. Our aim is to describe outcomes for OLT in patients with a BMI>40kg/m² in a single center.

METHODS. Between 1/2004 and 4/2007, 364 OLTs were performed in 347 patients. 20/347 patients with a BMI>40 were compared to all other OLT patients with a BMI<40. We analyzed patient and graft survival, operative time, blood transfusion requirements, and post operative events (ICU and overall length of stay LOS, surgical complications and infections). We also analyzed the post transplant weight records of our study group at 1-3 and 6 months.

RESULTS. Results are summarized in table 1.

Table 1			
	BMI<40	BMI>40	P value
OVERALL SURVIVAL	302/347 (77.1%)	18/20 (90.0%)	ns
GRAFT SURVIVAL	228/302 (74.9%)	16/20 (80.0%)	ns
MELD SCORE	21.94 +/- 6.29	20.4 +/- 4.83	ns
SURGERY TIME (minutes)	506 +/- 70 min	556 +/- 76 min	ns
PRBCs (Units)	7.7 +/- 7.5	8.0 +/- 7.15	ns
ICU LOS (Days)	5.4 +/- 8.8	5.8 +/- 5.7	ns
HOSPITAL LOS (Days)	16.5 +/- 14	21 +/- 14.6	ns
WOUND INFECTIONS (pts/%)	42/347 (12.1%)	5/20 (25.0%)	ns

Median follow up was 23.1 months (6-45 mos). Log rank test showed comparable patient and graft survival in the two groups (p=0.8). There was no statistical difference between the groups in terms of surgery time, blood products transfused, ICU and hospital Lengths of stay, surgical complications and episodes of infections. 2/20 patients were retransplanted in the study group vs 16/347 in the BMI<40 group (p=ns). In the BMI>40 group, 16/20 patients (73%) reported a dramatic weight loss at 3 mos after the transplant with a statistically significant decrease in overall mean BMI, 42.33.5 vs 33.5 5.9 (p=0.001). After 3 mos the weight curve plateaued.

CONCLUSIONS. Outcomes of OLT in the morbidly obese are no worse than those of other patients undergoing OLT. BMI is often artificially elevated in end-stage cirrhotic due to severe fluid retention. These patients exhibit rapid weight loss following transplantation that is likely due to extra-cellular fluid loss from the improved homeostatic milieu provided by the new liver and possible improvement in renal function.

Abstract# 310

Impact of Weight and Body Mass Index on Biliary Complications, Graft Loss, Rejection, and Survival Post Orthotopic Liver Transplantation.

Scott A. Fink,¹ Elizabeth C. Verna,¹ Eleonora De Martin,² Patrizia Burra,² Paul Gaglio,¹ Jean C. Emond,¹ Brown S. Robert.¹ ¹Center for Liver Disease and Transplantation, NewYork-Presbyterian Hospital/Columbia University Medical Center, New York, NY; ²Department of Surgical and Gastroenterological Sciences, University of Padova, Padova, Italy.

Background: Concern has arisen regarding the suitability of overweight and obese patients for liver transplantation. Data is conflicting regarding the impact of body mass index (BMI) on complication rates, mortality, and morbidity following orthotopic liver transplant (OLT). Our group has previously shown increased extrahepatic biliary complications (BC) in patients receiving OLT for hepatitis C virus (HCV) induced cirrhosis. **Aim:** To assess the impact of BMI on BC rate and one year graft and patient survival following OLT in patients with and without HCV.

Methods: We performed a retrospective cohort study of all patients at NewYork-Presbyterian Hospital who underwent OLT between 2000 and 2005. Recipient height and weight at the time of listing for OLT were used to calculate BMI.

Results: Data on 256 patients were available. Mean age of patients at time of OLT was 53 years. Mean height and weight of the cohort were 66.6 inches and 175.1 pounds respectively and mean BMI was 27.6. 27% of patients were obese (BMI $\geq$ 30). 33% of patients had normal weights (BMI 18.5 - 24.9). 9% of patients were morbidly obese (BMI $\geq$ 35). There was no association noted between BMI in patients with and without BC (p=0.25). No significant differences in rates of BC were noted between normal weight (p=0.75) and obese (p=0.7) patients. Patients with BC had higher weights (185 vs. 173 lbs, p=0.05). No associations were seen between BMI and one year patient and graft survival. Subgroup analysis showed similar results in HCV and non-HCV groups.

Conclusions: Neither BMI nor obesity predicts BC, rejection, mortality, and graft survival. Weight alone without height was associated with more BC.

Rates of graft loss, rejection, biliary complications, and mortality in obese (BMI $\geq$ 30) and non-obese (BMI $\leq$ 29.9) patients

All Patients	One year graft loss	One year mortality	BC	Rejection
Obese	10%	10%	20%	28%
Non-obese	16%	13%	18%	27%
p	0.23	0.49	0.7	0.97
Patients with HCV etiology	One year graft loss	One year mortality	BC	Rejection
Obese	12%	12%	17%	29%
Non-obese	25%	19%	26%	31%
p	0.09	0.31	0.27	0.82

Abstract# 311

The Impact of Obstructive Coronary Artery Disease on Outcomes after Liver Transplantation.

Anton I. Skaro,¹ Paul B. Alvord,¹ Alex Toledo,¹ Josh Levitsky,² Mihai Gheorghiade,³ Michael I. Abecassis.¹ ¹Surgery, Division of Organ Transplantation, Northwestern University Feinberg School of Medicine, Chicago, IL; ²Medicine, Division of Hepatology, Northwestern University Feinberg School of Medicine, Chicago, IL; ³Medicine, Division of Cardiology, Northwestern University Feinberg School of Medicine, Chicago, IL.

Background: The increasing prevalence of coronary artery disease (CAD) among patients with cirrhosis awaiting transplantation poses a considerable dilemma. Obstructive CAD is a contraindication for liver transplantation at most centers. However, the impact of obstructive CAD on outcomes following transplantation has not been studied.

Methods: We performed a retrospective review of 422 adult patients who underwent liver transplantation between January 2001 and December 2005. 157 patients had coronary angiography for pre-transplantation evaluation and were included in the analysis.

Results: The patients were categorized into the following groups: no CAD (n=62), mild CAD (<50% stenosis; n=55), moderate CAD (50-69% stenosis; n=12), obstructive ($\geq$ 70% stenosis; n=28). Patients with moderate and obstructive CAD were older (63 versus 53, p<0.05) males (86 versus 50, p<0.0001), and had a higher prevalence of hypercholesterolemia (80 versus 56, p<0.02) and diabetes (61 versus 35, p<0.03) than patients without CAD. There were no differences in early (30 day) or late (1 year) patient and graft survival. Moreover, there were no differences in cardiac events including: myocardial infarction, acute coronary syndrome, congestive heart failure, cardiac mortality or cardiac related hospital re-admission. Most strikingly, 11 (40%) patients with obstructive CAD were not revascularized.

Conclusions: The presence of obstructive CAD was not associated with increased peri-operative morbidity or mortality. These patients experienced similar patient and graft survival irrespective of the degree of CAD. Significant unmodified CAD should not represent an absolute contraindication to liver transplantation.

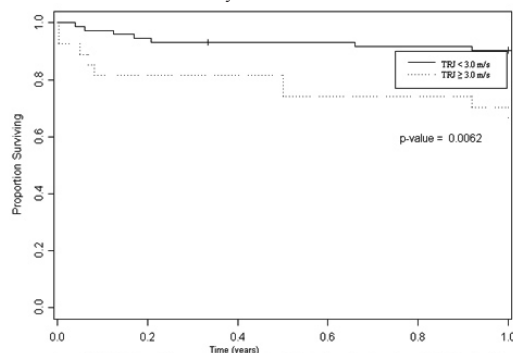
Abstract# 312

Relationship between Preoperative Transthoracic Echocardiographic (TTE) Estimate of Tricuspid Regurgitant Jet (TRJ) and Survival after Orthotopic Liver Transplantation (OLT).

H. James Ford,¹ Robert Aris,¹ Kenneth Andreoni.² ¹Medicine, Pulmonary and Critical Care, University of North Carolina, Chapel Hill, NC; ²Surgery, University of North Carolina, Chapel Hill, NC.

Portopulmonary hypertension (PPH) is widely recognized as being associated with worse outcomes after OLT. The initial screening tool usually employed is TTE with doppler measure of TRJ as an estimate of right ventricular systolic pressure (RVSP). Pulmonary hypertension is confirmed by right heart catheterization. Literature suggests that an RVSP of > 50 mmHg should prompt clinicians to perform right heart catheterization. For a right atrial pressure (RAP) of 5 mmHg, this correlates to a TRJ of 3.2-3.3 m/s, using the Bernoulli equation.

We examined our OLT population retrospectively to see how the preoperative screening TTE TRJ correlated with immediate and long term survival through Kaplan-Meier analysis, examining all-cause mortality. The total number of patients screened was 596. Patients were included if they had a documented numeric value for the TRJ at any time pre-OLT up to and including the day of transplant. The number of patients with a TRJ of <3.0 m/s was 70, with 27 having a TRJ $\geq$ 3.0 m/s. The vast majority of patients had not undergone right heart catheterization. The balance of 499 patients either had no TTE available, had no quantification of the TRJ, or were <18 years old. Indication for OLT was also examined. We found that using a TRJ of 3.0 m/s as a cut-off created two age-matched groups with significantly different survival curves at one year (p = 0.006) with a relative risk of mortality of 3.98 for TRJ $\geq$ 3.0 m/s.



This study underscores the importance of screening TTE for the presence of PPH in the evaluation of patients for OLT, suggesting that clinically significant PPH may be present at lower TRJ than typically prompt right heart catheterization. Some of the limitations of the study are the variability of the time from pre-OLT echo to transplant, and the assumption of RAP = 5 without assessment of IVC size. We are using these data as a framework for further prospective trials to better assess the clinical applications of the findings.

Lung Transplantation: Outcomes, Renal Function and Mechanisms of Graft Dysfunction

Abstract# 313

Hyperlipidemia Predicts Faster Loss of Renal Function within the First Month after Lung Transplantation.

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Hyperlipidemia has been shown to predict faster chronic kidney disease (CKD) progression over the long term in lung allograft recipients. It is unknown whether disordered lipid metabolism may also aggravate the early loss of renal function often seen in this patient population in the immediate post-operative period. We studied 230 lung allograft recipients transplanted between January 1997 and December 2003. Pertinent demographic and clinical variables were recorded at baseline and one month post-transplant, including creatinine levels and fasting lipid panels. Logistic regression models were created to investigate an independent association between lipid levels and change in renal function by one month post-transplant. Mean $\pm$ SD baseline creatinine was 0.8 $\pm$ 0.2 mg/dl and low density lipoprotein (LDL) was 110 $\pm$ 35 mg/dl, the latter remaining unchanged at one month. In contrast, by one month post-transplant the mean creatinine level of survivors increased significantly to 1.2 $\pm$ 0.6 mg/dl (p < 0.001), with a overall mean increase of 52% above baseline. The highest quartile of patients that fared the worst experienced a rise in creatinine $\geq$ 72% above baseline. On univariate analysis, there was a strong trend toward those with one month LDL values in the highest quartile (i.e., $\geq$ 140 mg/dl) having an increased odds of experiencing a rise

in creatinine by one month $\geq 72\%$ above baseline (OR 1.8, $p=0.09$). After controlling for age, gender, pre-transplant creatinine, BMI, and the presence of diabetes prior to transplant an LDL value in the highest quartile by one month post-transplant was the only variable independently predictive of a rise in creatinine $\geq 72\%$ above baseline at one month (OR 2.5, $p=0.05$). In summary, hyperlipidemia occurring early post-lung transplant predicts faster loss of renal function soon after surgery. Though speculative, given the known beneficial effects of lipid lowering agents such as statins on the rate of CKD progression, perhaps timely initiation of these medications after surgery may also attenuate the early decline in renal function often observed in this patient population.

Abstract# 314

Phenotypes of Acute Cellular Rejection of Lung Transplant Recipients Following Induction Therapy with T-Cell Depleting Agents. Adriana Zeevi,¹ Kathy Spichty,¹ Alin Girmata,¹ Diana Zaldonis,¹ Joseph Pilewski,¹ Yoshiya Toyoda,¹ Kenneth McCurry.¹ ¹University of Pittsburgh, Pittsburgh, PA.

The risk for acute cellular rejection (ACR) following lung transplantation (LTx) is still a problem despite heavy multidrug immunosuppression. Induction therapy with potent T-cell depleting agents have facilitated the implementation of minimal post-transplant immunosuppression. The impact of this protocol on the activation of proinflammatory cytokines and effector molecules that affect the cellular rejection process is not well determined. In this study, we evaluated the relationship between upregulation of T-cell and macrophage-dependent inflammatory cytokines detected by molecular methods and the clinical status of LTx patients.

We studied 28 LTx patients who received anti-lymphocytic induction therapy (Thymoglobulin n=8 or Campath-1H n=20) followed by maintenance immunosuppression with Tacrolimus and low-dose steroids. We analyzed 137 bronchoalveolar lavage (BAL) mRNA samples (3-8 per LTx patient) by Real-Time PCR for GZMB, IFN- γ , IL-15, MCP-1, RANTES, TNF- α , and GAPDH (control). The ABI Prism 7700 SDS and 7500 Fast Real-Time PCR Systems were used and data were analyzed by the dCT and 2-ddCT methods. We determined the relationship of categorical outcomes (rejection-no rejection) with continuous variables (number of cycles) by ANOVA.

Early ACR that occurred within the first 60 days post-LTx was associated with an increase (>3 -6 fold) of macrophage-specific mRNA (MCP-1, RANTES, TNF- α). 7/8 Thymo-treated LTx patients experienced early ACR while only 5/20 Campath-1H-treated patients had early ACR ($p<0.005$). In contrast, late ACR that occurred greater than 60 days post-LTx was associated with a significant upregulation (4-64 fold) of T-cell dependent mRNA (GZMB and IFN- γ) in addition to increases of RANTES and MCP-1 mRNA. Overall, LTx patients who experienced early or late ACR (n=20) exhibited higher mRNA levels for the above mediators compared to stable patients (n=8).

Our data indicated that in early post- T-cell depletion, the ACR phenotype was characterized mainly by macrophage activation. In contrast, greater than 3 months post-LTx with the recovery of CD8+ T-cells, the ACR phenotype was associated with cytotoxic T-cell activation. Furthermore, sensitive molecular methods may detect the activation of pro-inflammatory mediators within the allograft prior to the diagnosis of rejection by transbronchial biopsies and may impact optimal patient management.

Abstract# 315

Antibody-Mediated Rejection in Lung Transplant Patients. A. L. Girmata, A. Zeevi, S. A. Yousem, J. Pilewski, Y. Toyoda, S. Studer, L. Jelinek, J. Lomago, K. Spichty, G. Zdanowicz, J. Howe, D. Zaldonis, J. Martell, K. R. McCurry. University of Pittsburgh Medical Center.

Antibody-mediated rejection (AMR) is defined by the presence of donor-specific alloantibodies, markers of complement activation and the clinical phenotype of organ dysfunction. Compared to renal and cardiac allografts, very few AMR cases are documented in lung transplantation (LTx).

Methods. We report here on seventeen LTx patients exhibiting: 1) donor-specific HLA antibodies (DSA); 2) linear, continuous subendothelial C4d deposition in lung allograft; 3) lung allograft dysfunction. The presence and specificity of DSA were determined by ELISA and/or Luminex. C4d deposition was assessed by immunohistochemistry in transbronchial biopsy paraffin blocks. Allograft dysfunction was considered when either biopsy-proven acute cellular rejection (ACR, $\geq$ ISHLT A2) or bronchiolitis obliterans (BOS) were diagnosed.

Results

Pts	POD	Sex	DSA Pattern	PRA	Class I DSA	Class II DSA	Vascular C4d	Acute Rejection	BOS
1	487	F	De novo	9	No	DR11	Yes	Yes	No
2	480	F	De novo	59	No	DR17, DQ2	Yes	Yes	No
3	370	M	De novo	15	A24	No	Yes	Yes	No
4	380	M	De novo	11	No	DR1	Yes	Yes	Yes
5	456	F	De novo	21	B65	DQ8	Yes	Yes	Yes
6	734	F	De novo	7	A11	No	Yes	Yes	Yes
7	29	F	Memory	80	No	DR4, DQ3	Yes	Yes	Yes
8	129	M	De novo	25	A1	DR11	Yes	Yes	Yes
9	68	F	Memory	59	B18,35	No	Yes	Yes	Yes
10	14	F	Memory	80	A3, B44	DR13,52, DQ8	Yes	Yes	No
11	362	F	De novo	16	B55	No	Yes	No	Yes
12	220	F	De novo	59	A24	DQ1,2	Yes	No	Yes
13	60	M	De novo	30	B8	No	Yes	No	Yes
14	26	F	Memory/De novo	64	B8	DQ7	No	No	Yes
15	203	F	De novo	8	A1	No	Yes	No	No
16	218	F	De novo	18	A24	No	Yes	No	No
17	561	F	De novo	37	No	DQ8	Yes	No	No

The average detection of DSA occurred in the first year post-LTx, on 283 ± 218 post-operative day (POD), range 14 to 734 days. Thirteen (76%) of AMR patients were females. An anamnestic humoral response was encountered in 4 cases (one pregnancy-related and three re-transplant patients), while *de novo* DSA were detected in 14 cases (82%). The percent-reactive antibody (PRA) was lower in *de novo* cases (24%) when compared to memory response (71%, $p<0.05$). Anti-class I DSA were found in 7 cases, anti-class II in 5 cases, while 5 patients exhibited both class I and class II DSA. Specific vascular C4d deposition was detected in 16 (94%) patients. Lung allograft dysfunction was considered in 14 (82%) cases, while three patients with DSA and specific C4d deposition fulfilled the criteria of sub-clinical AMR. In patients where plasma-exchange/IVIg were applied, antibody titers dropped from 1:32 to 1:4 or 1:2 in two cases; in a third case, antibody titer remained high post-pheresis (1:512) and the graft failed.

Conclusions: Both anti-class I and anti-class II, low PRA or high PRA, pre-formed or *de novo* DSA can be detrimental for lung allograft. The presence of donor-specific alloantibodies, vascular C4d deposition and allograft dysfunction shows that AMR criteria can also be met in LTx.

Abstract# 316

Cyclosporine Alters Airway Epithelial Cell Cytokine Secretion: A Potential Mechanism To Explain the Mechanism of Action of Inhaled Cyclosporine. Robert M. Aris,¹ Patrick McNeillie,¹ Catherine Hammett-Stabler,¹ Olusegun Olusesi,¹ Sarah Thomas,¹ Lisa Rothlein,¹ Isabel P. Neuringer.¹ ¹Division of Pulmonary and Critical Care Medicine, University of North Carolina, Chapel Hill, NC.

Rationale: Recently, inhaled cyclosporine has been shown to reduce mortality and BOS. Previously, we demonstrated that apically-dosed cyclosporine poorly transmigrated differentiated human airway epithelial cells in vitro (using a transwell system). Only ~5% of the apically deposited cyclosporine passed through the epithelial layer whereas most of the drug accumulated within the epithelium. Thus inhaled cyclosporine may not be capable of inactivating airway allogeneic T cells since it may not reach the airway wall in high concentration. In this study, we hypothesized that inhaled cyclosporine alters airway epithelial signaling cascades that may ultimately result in reduced inflammatory cell recruitment to the lung.

Methods: Human tracheobronchial epithelial (hTBE) cells from healthy donors were grown in ALI media to confluence at an air-liquid interface in micellids. Differentiated hTBES were treated on their apical surface with cyclosporine concentrations of 1000 and 10,000 ng/ml or vehicle for 24hr to mimic the effects of inhaled cyclosporine. The basilar and apical compartments (n=8-12 for each) were then assayed for cytokine secretion (IL8, IL6, IL1, IL-12p40, TNF, eotaxin, MCP-1, GM-CSF, RANTES and EGF) by luminex assays in pg/ml.

Results: 10,000ng /ml of cyclosporine markedly blunted the basilar secretion of IL-6 (250 ± 142 vs 71 ± 24 , $p=0.002$), RANTES (22 ± 8 vs 9 ± 3 , $p=0.003$), GM-CSF (21 ± 16 vs 3 ± 3 , $p=0.008$) and MCP-1 (181 ± 112 vs 28 ± 19 , $p=0.002$). IL8 and eotaxin were unaffected; TNF α , IL1 β and IL12p40 were undetectable. At 1000ng/ml cyclosporine, cytokine secretion was decreased to a lesser extent. A similar pattern of diminished cytokine secretion was seen in the apical compartment of this system. Last, apical, but not basal, EGF secretion was augmented by cyclosporine (2231 ± 817 vs 1114 ± 614 pg/ml, $p=0.02$).

Conclusion: Cyclosporine decreased the secretion of critical cytokines and chemokines from human airway epithelial cells. These mediators are known to enhance mononuclear and T cell recruitment in a large variety of animal models of disease as well as in clinical studies. Inhaled cyclosporine may work by reducing cell recruitment. This hypothesis will require in vivo testing.

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Abstract# 317

Mannose Binding Lectin within the Lung Allograft and Peripheral Blood after Lung Transplantation. Isabel P. Neuringer,¹ Ayorinde A. Medaiyese,¹ William K. Funkhouser,² Robert M. Aris.¹ ¹*Pulmonary Medicine, UNC, Chapel Hill, NC;* ²*Division of Pathology and Lab Medicine, UNC, Chapel Hill, NC.*

Mannose binding lectin (MBL) is a soluble pattern recognition molecule which activates complement, binds to apoptotic cells, modulates inflammation, and participates in host defense and innate immunity. We hypothesize that MBL is deposited within injured lung allograft tissue, and elevated in the blood and bronchoalveolar fluid (BAL) of patients with bronchiolitis obliterans syndrome (BOS) and acute rejection, respectively, compared to patients who are clinically stable. Methods: Lung allograft tissue from patients at the time of transplant (cold ischemia, n=6), retransplant for BOS (BOS, n=8), and diffuse alveolar damage (DAD, n=6), were immunostained for MBL. Slides were graded semi-quantitatively for percent vasculature, airways, and alveolar/capillary membrane staining positive per slide. MBL levels in the peripheral blood of lung transplant patients (past 4 years) were compared between patients with BOS 0 (n=12) and BOS Op-BOS 3 (n=10). MBL levels in 27 BAL samples from 8 different patients were correlated with the presence or absence of cellular rejection on transbronchial biopsy. Results: MBL deposition was detected in all cold ischemia samples, localizing to vasculature (80.1±12%), bronchial epithelial basement membranes (72.6±11%), and along alveolar/capillary membrane (33.4±6%). BOS specimens demonstrated MBL along vasculature (88.5±8%), remnant bronchiolar basement membranes (52.3±15%), and alveolar/capillary membranes (18.4±6%). DAD tissue exhibited fainter deposition along vascular structures (27.7±11%) and bronchiolar basement membranes (54.3±8%). Patients with BOS Op-BOS 3 (n=10, 3198 ng/ml) had significantly higher levels of MBL in the peripheral blood compared to patients with BOS 0 (n=12, 1808 ng/ml), p = 0.033. In 27 BAL fluid samples, 8/8 patients with MBL > 2ng/ml/100ug total protein had A2 rejection or DAD, while 18/19 patients with MBL < 2ng/100ug total protein had A0 or A1 rejection. Conclusions: Localization of MBL to the lung allograft may stimulate innate immune responses and contribute to graft injury. Elevated MBL in the 1) peripheral blood of patients with early to late stages of BOS, and 2) BAL fluid of patients with DAD or A2 rejection, may prove to be a biomarker for an increased risk of chronic graft injury. Reduction of MBL levels and the elucidation of MBL ligands expressed by the lung allograft may provide targets for therapeutic inhibition.

Abstract# 318

β-Herpesvirus Infection and the Risk of Bronchiolitis Obliterans Syndrome (BOS) in Lung Transplant Recipients. Oriol Manuel,¹ Shahid Husain,² Deepali Kumar,¹ George Moussa,³ Maggie H. Chen,³ Joseph Pilewski,² Kenneth R. McCurry,² Atul Humar.¹ ¹*Transplant Infectious Diseases, University of Alberta, Edmonton, Canada;* ²*University of Pittsburgh;* ³*University of Toronto, Canada.*

Background: Cytomegalovirus (CMV), human herpes virus -6 and -7 (HHV-6 and -7) are β-herpesviruses that commonly reactivate and have been proposed to trigger acute rejection and chronic allograft injury. The role of these viruses in the development of BOS after lung transplantation remains unclear. We assessed the contribution of β-herpesvirus infection in the allograft by a prospective molecular assessment of serial broncho-alveolar lavage (BAL) samples in lung transplant recipients.

Methods: Quantitative real-time PCR of BAL samples were performed for CMV, HHV-6 and HHV-7 in a prospective cohort of lung transplant recipients. A time-dependent Cox regression analysis was used to correlate the risk of BOS and acute rejection in patients with and without β-herpesviruses infection.

Results: 93 patients were included in the study over a period of 3 years. A total of 581 samples from BAL were obtained (median 6 per patient). 61/93 patients (66%) had at least one positive result for one of the β-herpesviruses: 48 patients (52%) for CMV, 19 patients (20%) for HHV-6, and 19 patients (20%) for HHV-7. Median time to detection was 152 days (range 8-839) for CMV, 309 days (range 28-928) for HHV-6, and 286 days (range 17-928) for HHV-7. Median peak viral load was 3,419 copies/ml (range 102-41,600,000) for CMV, 258 copies/ml (range 106-131,075) for HHV-6, and 665 copies/ml (range 103-66,100) for HHV-7. Acute rejection (≥ grade 2) occurred in 46% and BOS (≥ stage 2) in 19%. In the time dependent Cox regression model, the relative risk of BOS or acute rejection was not increased in patients with CMV, HHV-6, or HHV-7 reactivation. For example, the hazard ratio of CMV and BOS was 1.04 (95% CI 0.62-1.73, p=0.9) and for CMV and acute rejection was 0.81 (95% CI 0.55-1.20, p=0.3). In many of the patients, β-herpesvirus reactivation occurred after the acute rejection episode likely reflecting augmented immunosuppression.

Conclusion: In this large cohort of lung transplant recipients, local reactivation of CMV, HHV-6 and HHV-7 in the allograft was very common. However, despite high viral loads in many patients, infection was not significantly associated with the development of acute rejection or BOS.

New Insights into T Cell Regulation

Abstract# 319

Dysregulation of the CD4 T Cell Response to Single Class II MHC-Disparate Heart Allografts in CCR5 Deficient Mice. Taiji Nozaki,^{1,2} Austin Schenk,¹ Kazunari Tanabe,² Robert L. Fairchild.¹ ¹*Dept. of Immunology and Glickman Urological and Kidney Inst., Cleveland Clinic, Cleveland, OH;* ²*Department of Urology, Tokyo Women's Medical University, Tokyo, Japan.*

Introduction: The role of chemokine receptors in regulating donor-specific responses to allografts is poorly understood. CD4⁺CD25⁺ T cells regulate alloreactive CD4 T cell responses and acute rejection of single class II MHC-disparate cardiac allografts in C57BL/6 mice. CCR5 is expressed by a small proportion of CD4⁺CD25⁺ T cells but the requirement for these cells in regulating alloreactive T cell responses remains poorly understood. The goal of this study was to investigate the role of CCR5⁺ T regulatory cells in acute rejection of single class II MHC-disparate cardiac allografts.

Methods: Wild-type C57BL/6 (H-2^b) and B6.CCR5^{-/-} received heterotopically transplanted B6.H-2^{bmi12} mice heart grafts. The presence of CD4⁺Foxp3⁺ T cells in the recipient spleen and in heart allografts was determined by flow cytometry. Foxp3 mRNA expression in the heart grafts was analyzed by qRT-PCR. Donor-specific CD4⁺ T cells producing IFN-γ or IL-4 in allograft recipient spleens were enumerated by ELISPOT assay. Cell sorted naïve wild-type CD4⁺CD25⁺ T cells and CD4⁺CD25⁻ T cells were adoptively transferred to wild-type and CCR5^{-/-} mice before the cardiac transplant.

Results: In wild-type recipients >80% B6.H-2^{bmi12} cardiac grafts survived more than 100 days whereas CCR5^{-/-} recipients rejected the allografts within 24 days (18 days mean survival) with intense CD4⁺ T cell infiltration in the graft. Donor-reactive IFN-γ and IL-4 producing CD4⁺ T cell numbers were increased 2-fold in the spleens of CCR5^{-/-} vs. wild-type recipients at day 7 post-transplant and in contrast to wild-type recipients these numbers were sustained for at least 7 more days. Allograft infiltrating CD4⁺CD25⁺Foxp3⁺ cells and intra-graft Foxp3 mRNA expression were clearly present in allografts from wild-type recipients and were virtually absent in allografts from CCR5^{-/-} recipients. Transfer of purified wild-type CD4⁺CD25⁺ T cells to CCR5-deficient mice resulted in the long-term survival of 60% of B6.H-2^{bmi12} cardiac allografts.

Conclusion: CCR5⁺ regulatory T cells control the magnitude and function of the alloreactive T cell immune response to single class II MHC-disparate cardiac allografts.

Abstract# 320

A New Pathway of Differentiating Double-Negative Regulatory T-Cells: Novel Cell-Based Therapeutic Approach. Dong Zhang,¹ Wensheng Zhang,¹ Yun Liu,¹ Yan Tian,¹ XinXiao Zheng.¹ ¹*Surgery, Starzl Transplantation Institute, University of Pittsburgh School of Medicine, Pittsburgh, PA.*

Recent studies have demonstrated that in the peripheral lymphoid tissues of normal mice and human 1-5% of αβTCR⁺ T-cells are CD4⁺CD8⁻ (double-negative, DN) T-cells, capable of down-regulating the immune response. Moreover, we identified a new differentiation pathway for the conversion of peripheral CD4⁺ T-cells into DN regulatory T-cells. Our study showed that a subset of proliferated CD4⁺ T-cells converted to DN T-cells after 4-5 rounds of antigen triggered or homeostatic proliferation in vitro and in vivo. The converted DN T-cells express a unique set of cell-surface markers and gene profile that were distinct from those of CD4⁺CD25⁺ Tregs, naïve CD4⁺CD25⁻ T-cells, and activated CD4⁺ T-cells. Furthermore, the CD4⁺ converted DN T-cells were highly potent in suppressing antigen specific alloimmune responses in vitro.

In this study, we further characterized and test the functional potential of the converted DN T-cells in vivo. We showed that the converted DN T-cells retained a stable phenotype after re-stimulation in vitro and in vivo. IL-2 was capable of breaking the anergic status and reserving the suppressive function of DN T-cells.

In an immunocompetent MHC completely mismatched islet transplant model, the transfer of 13 x 10⁶ DN T-cells (converted from CD4⁺CD25⁻ T-cells of naïve C57BL/6 mice by co-culture with mature DBA/2 DC plus rIL-15 in MLR for 6 days) resulted in a statistically significant prolongation of alloantigen specific DBA/2 strain, but not third party C3H strain, islet allograft survival in C57BL/6 recipients in comparison with that of untreated control group.

As IL-2 was capable of breaking the anergic status and reserving the suppressive function of DN T-cells, we added IL-2/Fc, a long-lasting form of IL-2, and low dose rapamycin with DN T-cells in a MHC mismatched skin allograft model. The single transfer of 5 x 10⁶ DN T-cells plus 28 days IL-2/Fc and low dose rapamycin treatment significantly prolong DBA/2 skin allograft survival in C57BL/6 recipients in comparison with untreated group (MST 71 days vs. 11 days, p=0.0049) and IL-2/Fc plus rapamycin treated group MST (71 days vs. 34 days, p=0.0091).

The results of using ex vivo CD4⁺ T-cells converted DN T-cells in skin and islet transplantation models support the concept and the feasibility of potentially utilizing this novel cell-based therapeutic approach clinically for the prevention of allograft rejection.

Abstract# 321**NK Cells Prevent the Development and Function of Regulatory T Cells.** Jessamyn Bagley,¹ Jonathan G. Godwin,¹ Joren Madsen,² John Iacomini,¹¹*Transplantation Research Center, Brigham and Women's Hospital and Children's Hospital Boston, Harvard Medical School, Boston, MA;* ²*MGH Transplant Center, Massachusetts General Hospital, Boston, MA.*

Introduction: It has been suggested that natural killer (NK) cells are critical mediators that connect the innate and adaptive immune response. Cytokine production by NK cells contributes to the polarization of immune responses to T helper 1, and NK cells express co-stimulatory molecules that may affect T cell proliferation. Recent work has shown that NK cells are involved in the chronic rejection of parental cardiac grafts by F1 recipients. We hypothesized that given the role of NK cells in T cell activation and proliferation, NK cells may play a role in the development and function of T regulatory cells (Treg) which control alloreactive responses.

Methods: NK, CD4+ T cells and CD4+CD25+ Treg were purified from the spleens of C57BL/6J mice using MACS bead separation followed by fluorescence activated cell sorting. Naïve CD4+CD25- T cells from C57BL/6J mice (H-2b) were placed in culture with TGF- β in the presence or absence of NK cells, and the development of Treg was monitored by assessing FoxP3 expression by intracellular cytokine staining and flow cytometry. In addition, the effect of NK cells on the function of Treg was measured by ELISPOT assay.

Results: Following 4 days of culture with TGF- β and anti-CD3 antibody, CD4+CD25- T cells acquire FoxP3 expression. The addition of activated NK cells to cultures with CD4+CD25- T cells and TGF- β prevented the acquisition of FoxP3 expression by CD4 cells. Tregs induced by stimulation of CD4+CD25- T cells with anti-CD3 in the presence of TGF- β are capable of suppressing the production of IL-2, IFN- γ and IL-4 by CD4 T cells in response to fully allogeneic BALB/c stimulators. However, in the presence of activated NK cells, induced Tregs fail to function, and production of IL-2, IFN- γ and IL-4 by CD4 T cells is restored. These experiments were repeated with natural CD4+CD25+ Tregs isolated directly from mice without induction, and found that the ability of natural Treg to suppress a CD4 T cell response to alloantigen was impaired in the presence of activated NK cells.

Conclusions: The presence of activated NK cells in culture can prevent the development of induced Treg. Furthermore, the presence of activated NK cells interferes with the function of mature Treg in culture and allows a productive cytokine response by CD4 effector cells in response to alloantigen.

Abstract# 322**Mechanisms of Graft Rejection Mediated by NK Cells in the Complete Absence of Adaptive Immune Cells.** Alexander Kroemer,¹ Xiang Xiao,¹ Nicolas Degauque,¹ Karoline Edtinger,¹ Gulcin Demirci,¹ Xian C. Li,¹¹*Transplant Research Center, Beth Israel Deaconess Med Ctr, Boston, MA.*

Background: Transplant rejection is mediated primarily by adaptive immune cells such as T cells and B cells. However, dismantling the allografts involves many other cell types including cells in the innate immune system. The daunting difficulty in creating transplant tolerance under stringent conditions stimulates renewed interests in the role of innate immune cells, especially NK cells, in the induction of transplant rejection.

Methods: We used Rag-/- and Rag-/-IL-2Rg-/- double mutant mice as transplant recipients to critically examine the alloreactivity of NK cells in a stringent skin transplant model. Both Rag-/- and Rag-/-IL-2Rg-/- mice are deficient for T and B cells. In contrast to the Rag-/-IL-2Rg-/- mice that are also deficient for NK cells, the Rag-/- mice have a large population of functional NK cells. Thus, the role of NK cells in mediating skin allograft rejection can be precisely studied in the absence of any adaptive immune cells.

Results: Both the syngeneic B6 cells and allogeneic DBA/2 cells survived nicely in the Rag-/-IL-2Rg-/- mice. However, the allogeneic DBA/2 cells, but not the syngeneic B6 cells, were readily killed by the NK cells in the Rag-/- mice. However, both Rag-/- and Rag-/-IL-2Rg-/- mice accepted the DBA/2 skin allograft long term without any sign of rejection (>100 days). Thus, NK cells by themselves, though cytolytic to DBA/2 cells, fail to reject the DBA/2 skin allografts. To test the hypothesis that the activation status of NK cells may dictate their alloreactive potential, we treated the Rag-/- mice with IL-5/IL-15Ra complex to maximally stimulate the NK cells in vivo. We found that IL-15 is remarkably potent in stimulating NK cells in vivo; and NK cells stimulated by IL-15 express an activated phenotype and are surprisingly potent in mediating acute skin allograft rejection in the absence of any adaptive immune cells, as IL-15 treated Rag-/-, but not the Rag-/-IL-2Rg-/- mice, readily rejected the DBA/2 skin allografts (MST=18 days). NK cell-mediated graft rejection doesn't show features of memory responses.

Conclusions: Our finding provides new insights on the role of NK cells in rejection and suggests that the fate of the allografts may depend on the activation status of NK cells and the availability of NK stimulating cytokines.

Abstract# 323**Cytokine Regulation of Th17 in T-Bet Deficient Allograft Recipients.**Bryna E. Burrell,¹ Keri Csencsits,¹ Svieta Grabauskienė,¹ Guanyi Lu,¹ D. Keith Bishop,¹ ¹*Department of Surgery, University of Michigan, Ann Arbor, MI.*

Background: T-bet is a transcription factor that promotes Th1 development. Both T-bet and the cytokines IFN γ and IL-4 have been implicated as negative regulators of

Th17. In contrast, IL-6 promotes Th17 development. IL-17 production is associated with granulocytic pathologies in several disease states. Hence, this study assessed the relationship between T-bet, IFN γ , IL-4 and IL-6 in Th17 induction and granulocytic infiltration of cardiac allografts.

Methods: C57BL/6 wild type (WT), T-bet deficient (T-bet-/-) and IFN γ deficient (IFN γ -/-) mice were transplanted with BALB/c cardiac allografts. Recipients were left untreated, depleted of CD4+ or CD8+ cells, treated with anti-CD40L mAb, and/or treated with neutralizing anti-IL-4 or anti-IL-6 mAb. Graft histology was assessed and primed donor-reactive Th responses were quantified by ELISPOT. Intra-graft expression of IL-17 and the Th17 transcription factor ROR γ T were quantified by real-time RT-PCR.

Results: WT and T-bet-/- mice rejected their allografts at a similar tempo but with distinct pathologies: allografts in T-bet-/- recipients were heavily infiltrated with granulocytes while graft infiltrating cells in WT recipients were primarily mononuclear. While Th1 and Th17 responses were readily detectable in T-bet-/- recipients, Th1 dominated the response in WT mice and Th17 were not detectable. Depletion of CD4+ cells prolonged graft survival in WT, but not in T-bet-/- recipients suggesting that CD8+ cells mediated rejection independent of CD4+ help in T-bet-/- mice. CD8+ cells were the source of IL-17 in T-bet-/- recipients. Anti-CD40L therapy promoted long-term allograft survival in WT recipients, but not T-bet-/- mice unless CD8+ cells were depleted. Additionally, anti-CD40L therapy inhibited Th1 responses in both WT and T-bet-/- recipients, but not the CD8+ Th17 response in T-bet-/- mice. Eliminating IFN γ and IL-4 failed to induce IL-17 production, while neutralizing IL-6 reduced the Th17 response in T-bet-/- mice.

Conclusions: While CD4+ Th17 have been described in detail, CD8+ Th17 have received less attention. In T-bet-/- allograft recipients, CD8+ Th17 emerge independent of CD4+ help. CD8+ Th17 are resistant to anti-CD40L therapy and are associated with granulocyte infiltration of the graft. These data implicate T-bet, as opposed to IFN γ and IL-4, as a negative regulator of the Th17 response while IL-6 is required for CD8+ Th17 induction.

Abstract# 324**Regulatory T Cells Sequentially Migrate from the Site of Tissue Inflammation to the Draining LN To Suppress Allograft Rejection.**Nan Zhang,¹ Bernd Schroppel,¹ Girdari Lal,¹ Jordi C. Ochando,¹ Jonathan S. Bromberg,¹ ¹*Gene and Cell Medicine, Recanati/Miller Transplantation Institute, Mount Sinai School of Medicine, New York, NY.*

Background: CD4+CD25+Foxp3+ regulatory T cells (Treg) are important in suppressing immunity to prolong allograft survival. Treg migration and its effects on suppressive activity are poorly understood. We determined Treg migration patterns and effector function in an islet allograft model.

Methods: Receptor expression of Treg was analyzed by flow cytometry and RT-PCR. CCR2^{-/-}, CCR4^{-/-}, CCR5^{-/-}, CCR7^{-/-}, L-selectin (CD62L)^{-/-}, and fucosyltransferase (FucT) IV-VII^{-/-} C57BL/6 mice were used to generate Treg. Treg were transferred intravenously, or locally to islet allografts, following islet transplantation (BALB/c into C57BL/6). Transferred Treg were labeled with red dye PKH26 and migration to islet grafts, draining LNs (dLN), peripheral LNs and spleen were tracked with fluorescence microscopy and flow cytometry. Islet allograft survival was determined by measurement of blood glucose.

Results: Treg expressed P-selectin ligand, CD62L, and a panel of chemokine receptors similar to other T cell subsets. Intravenously transferred wild type Treg migrated to both islet grafts and dLN, and prolonged allograft survival. CD62L^{-/-} or CCR7^{-/-} Treg, which migrated to islets but not dLNs, prolonged allograft survival as potentially as wild type Treg. FucT IV-VII^{-/-} Treg, which lack E-selectin and P-selectin ligands, migrated to dLN, but not islets, and did not prolong graft survival. Similarly, CCR2^{-/-}, CCR4^{-/-}, and CCR5^{-/-} Treg migrated to dLN, but not islets, and did not prolong graft survival. When locally transferred to the islet graft, Treg also migrated from the allograft to the dLN, and prolonged graft survival even longer than after intravenous transfer. Locally transferred CCR2^{-/-}, CCR5^{-/-}, or CCR7^{-/-} Treg were not able to migrate from the islet to the dLN, and were impaired in their ability to prolong islet survival.

Conclusion: Treg migration to allografts is essential for their suppressive function; migration to lymphoid tissues alone is not sufficient to prolong graft survival. Treg migration from the islet allografts to the dLNs, via afferent lymphatics, is also required for optimal suppressive function and graft survival. The sequential migration from the site of inflammation and then to dLNs is necessary for Treg to execute fully their suppressive program. These results demonstrate a novel and important aspect of migration in Treg suppression and tolerance.

Non-Organ Specific: Economics,
Public Policy, Allocation, Ethics I

Abstract# 325**Kidney Biopsy Is Safe and Useful in Guiding Kidney Allocation to Liver Transplant Candidates with GFR<40ml/Min.**Hani M. Wadei,¹ Martin L. Mai,¹ Nasim Ahsan,¹ Winston Hewitt,¹ Christopher Hughes,¹ Hani Grewal,¹ Barry Rosser,¹ Thomas A. Gonwa,¹ ¹*Transplantation, Mayo Clinic Jacksonville, Jacksonville, FL.*

The utility and risk of kidney biopsy to guide simultaneous-liver-kidney transplantation (SLK) versus liver transplant alone (LTA) in liver transplant candidates with renal

failure is unknown. **Methods:** 40 patients with iohalamate GFR <40ml/min (n=34) or on dialysis (n=6) at the time of liver transplant evaluation had undergone a percutaneous CT guided renal biopsy. Prior to the biopsy an INR ≤1.5 and platelet count ≥50,000/ml were achieved in the majority of cases. All patients were monitored overnight for complications. Candidates were listed for SLK if pathology showed ≥ 40% glomerulosclerosis (GS) or ≥30% interstitial fibrosis (IF). **Results:** 13 patients were eligible for SLK and 27 for LTA. Creatinine was higher in SLK candidates but not clinically different.

Results of the evaluation of 40 liver transplant candidates at time of kidney biopsy

	LTA (n=27)	SLK (n=13)	P
S. Cr (mg/dl)	1.6	1.9	0.04
Number (%) on HD	5 (19)	1 (8)	0.4
GFR (ml/min)	30	26	0.2
24 hr urine protein (mg)	420	1554	0.2
24 hr urine Na (mEq)	68	75	0.8
Mean % GS	18	30	0.02
Mean % IF	12	33	0.001

After a median of 70 days from the biopsy, 8 patients received SLK and 16 LTA. All recipients including the 5 LTA on dialysis at time of tx recovered kidney function. Two LTA and 2 SLK recipients became HD dependant following infectious complications. Renal function at 4 month, 1 year and last follow-up was similar between the 13 LTA and the 6 SLK recipients not on dialysis.

Post-transplant renal function

	LTA (n=16)	SLK (n=8)	P
Number (%) on HD at Tx	5 (31)	2 (25)	0.8
Days on HD	11	56	0.1
Number (%) on HD at 1 month	2 (13)	1 (13)	1
4-month GFR (ml/min)	38	44	0.5
1-year GFR (ml/min)	40	53	0.2
Follow-up days	322	365	0.7
Last crt (mg/dl)	1.4	1.4	0.9
Number (%) on HD last FU	3 (20)	2 (25)	0.7

Serious bleeding from the biopsy developed in 5 (12.5%), 4 of whom required selective embolization. **Conclusions:** 1) Liver transplant candidates with GFR <40ml/min have variable degree of GS and IF that can not be predicted from the results of their evaluation. 2) Lack of significant GS or IF on kidney biopsy identifies reversible renal failure and guides kidney allocation. 3) Post-tx ESRD can still develop mainly due to post-tx infectious complications. 4) Kidney biopsy can be done in liver tx candidates with an acceptable risk.

Abstract# 326

Cost of Delayed Graft Function in Medicare Covered Cadaveric Renal Recipients. Paula M. Buchanan,¹ Paolo Salvalaggio,² Krista Lentine,¹ Thomas Burroughs,¹ Mark A. Schnitzler.¹ ¹Center for Outcomes Research, Saint Louis University, Saint Louis, MO; ²Surgery, Saint Louis University, Saint Louis, MO.

Background - It is well known that delayed graft function (DGF) is costly for those who received a renal transplant. However, the true cost of DGF is unknown. **Methods -** We estimated the cost of DGF for adult cadaveric renal recipients in the USRDS 1995-2004 who had Medicare as their primary payer. Those included were restricted to single organ recipients as well as those who had no previous transplants. Cost was defined as the accumulated average cost per day for everyone with a functioning graft on that day. We examined the total cost of DGF, cost associated with dialysis, and non-dialysis cost for all patients combined and separately by donor type; standard criteria donors (SCD), expanded criteria donors (ECD), and non-heart beating donors (DCD) as well as time to graft failure or no graft failure.

Results -

One year post transplant average total cost for Medicare patients who received a cadaveric renal transplant according to the USRDS 1995-2004.

Donor Type	Time to Graft Failure	DGF			NO DGF		
		All Claims	Dialysis Claims	Non-Dialysis Claims	All Claims	Dialysis Claims	Non-Dialysis Claims
All	Note	75,767	28,610	47,157	65,111	8,005	57,106
	<60 days	146,823	96,336	50,487	148,679	80,281	68,398
	60-365 days	180,191	95,776	84,415	168,703	64,471	104,232
SCD	Note	73,905	27,947	45,957	64,257	7,753	56,504
	<60 days	144,329	95,284	49,045	148,357	79,261	69,095
	60-365 days	182,986	95,625	87,362	165,251	61,932	103,319
ECD	Note	83,957	31,045	52,912	71,812	9,563	62,248
	<60 days	154,271	98,820	55,451	151,382	85,551	65,831
	60-365 days	172,738	93,249	79,488	178,093	68,526	109,567
DCD	Note	75,175	30,269	44,905	63,483	9,874	53,610
	<60 days	139,860	99,141	40,719	139,779	75,516	64,262
	60-365 days	183,497	111,453	72,044	210,092	140,192	69,900

We identified 41,049 subjects. Of these 83.2% received SCD kidneys, 14.2% received ECD kidneys, and 2.6% received DCD kidneys. 23.6% of the SCD recipients experienced DGF while 35.9% of the ECD and 44.2% of the DCD recipients experienced DGF (p <0.0001). Almost immediately after transplant there is \$5,000 in additional cost for those

who experience DGF, while the one year post transplant difference is \$20,037 regardless of graft failure status. This difference is similar for all three donor types (SCD \$18,604, ECD \$21,179, and DCD \$18,615). For recipients who experience no graft failure DGF is most costly for the ECD recipients, followed by DCD recipients and SCD recipients (\$12,145, \$11,692, and \$9,648 in additional costs respectively). Dialysis costs makes up a higher percentage of total cost for patients who experience DGF.

Conclusions - Our results indicate that DGF leads to an average of \$20,000 in additional cost the first year after transplant. Donor type does not play a role in these additional costs, unlike graft failure which overrides the cost of DGF. However, due to the various rates of DGF in the different donor types, costs difference between the donor types could be confounded by DGF.

Abstract# 327

Developing Better Measures of Transplant Center Performance. Michael J. Englesbe,¹ Zhaohui Fan,¹ Onur Baser,¹ John D. Birkmeyer,¹ Justin B. Dimick.¹ ¹Michigan Surgical Collaborative for Outcomes Research and Evaluation, University of Michigan, Ann Arbor, MI.

Introduction: The individual quality measures used for transplant center-specific reporting are potentially unreliable due to statistical noise. In other settings, the unreliability of quality measures has been addressed with composite measures. An empirically-based composite measure of center performance in kidney transplantation may more accurately reflect "true" center-specific performance and forecast future performance.

Methods: Using the national Medicare claims database, we identified all kidney transplant recipients in 2000-2003 (N=40,784). "True" transplant center-specific mortality rates (2000-01) were determined by developing a composite measure using Bayesian-derived modeling techniques. Input variables for this composite measure included adjusted center-specific 30 day mortality and center-specific procedure volume. The composite measure (2000-01) was then validated by assessing its ability to forecast future performance (30 day mortality rates in 2002-03) and to forecast future costs (2003 Medicare outlier payments).

Results: In contrast to historical mortality, the composite measure for 2000-01 performance accurately forecasts future performance (risk adjusted mortality rates in 2002-2003). (Figure 1) Specifically, the composite measure was better able to discriminate between the future low and high performing centers (0.9 vs. 2.1) compared to historical mortality (1.8 vs. 2.1). In addition, the composite measure for performance (2000-01) also accurately predicts the future center specific Medicare outlier payments (2003). (Figure 2)

Figure 1: The relationship between mortality, a composite measure of performance, and future center-specific mortality rates

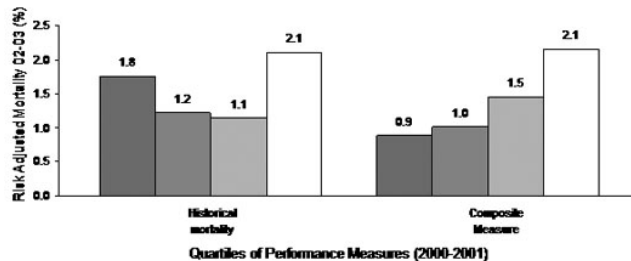
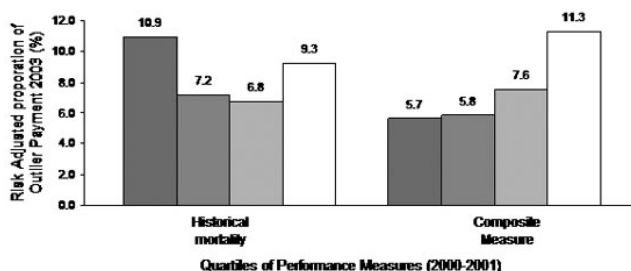


Figure 2: The relationship between performance measure of quality (2000 - 2001) and future Medicare outlier payments (2003)



Conclusion: Compared to historical mortality, an empirically-derived composite measure is superior in forecasting future performance. With further refinement, composite measures may be invaluable to improving the reliability of center-specific reporting.

Abstract# 328

Can Medicare Save Money by Providing Lifetime Immunosuppression Coverage to Kidney Transplant Recipients? Robert S. Woodward,¹ Timothy F. Page.¹ ¹Economics, UNH, Durham, NH.

While Medicare in the United States only added prescription medications to its benefit package in 2006, it has provided three-year coverage of immunosuppression

medications for transplant recipients since 1995. On 1/1/2000, Medicare provided lifetime immunosuppression medication coverage to transplant recipients over 65 or disabled, but the immunosuppression coverage for non-disabled transplant “ineligible” recipients under 65 remained at only 3 years following transplantation.

Purpose: This research estimates the changes in Medicare’s net cash flow that would have occurred in the years following 2000 if Medicare had extended its lifetime coverage of maintenance immunosuppression medications to all kidney transplant recipients. Providing lifetime coverage may create a cash outflow increase determined by the cost of the medications and the numbers of transplant recipients whose coverage would not have been cancelled. Lifetime coverage, however, may reduce cash outflows because fewer patients would have lost their transplanted kidney and therefore would not have incurred the expenses of returning to the dialysis. The net impact of these competing effects is unclear.

Methods: We used data from the United States Renal Data Systems (USRDS) for the years 1995 to 2003. Our sample included all 38,861 first, cadaveric, single organ kidney transplant recipients transplanted between 1995 and 2002 with Medicare as a primary payer for whom we could attach zip code median incomes. We divided our sample into quartiles by zip code median income and defined “high income” and “low income” as being from a zip code with median incomes in the highest and lowest quartiles, respectively.

Results: We found that if Medicare had extended lifetime coverage of immunosuppression medications to all transplant recipients as of 1/1/2000, Medicare’s cash outflows would have increased. Although there would have been savings associated with avoiding graft failure through increased drug coverage, these incremental benefits would have been outweighed by the incremental costs of covering all transplant recipients, most of whom would have experienced no improvement in graft survival outcomes. On the other hand, extending lifetime coverage only to “low income” individuals would have created a net Medicare savings.

Abstract# 329

A Proposed New Methodology for Flagging Transplant Programs for Outcome Review. Robert A. Wolfe,¹ Charlotte J. Arrington,¹ Jack D. Kalbfleisch,² Greg N. Levine,¹ Gregory V. Fant,³ ¹SRTR, Arbor Research, Ann Arbor, MI; ²SRTR, University of Michigan, Dept. of Biostatistics, Ann Arbor, MI; ³Division of Transplantation, HRSA, US Dept. of Health and Human Services, Bethesda, MD.

Background: Based on adverse outcomes during the first year post-transplant, the OPTN Membership and Professional Standards Committee (MPSC) peer-review process flags transplant programs for further review using one of two methods. Programs performing at least 10 transplants during at 2.5 year cohort are flagged based on the comparison of observed to expected event counts (death or graft failure) and the corresponding p-value (<0.05). Programs performing 9 or fewer transplants are flagged if they have any adverse events. This leads to different flagging rates for programs of different sizes. The p-value has low sensitivity to identify poorly performing programs with a “moderate” number of transplants (10 to 20) whereas flagging every event results in a high false positive rate for “small” centers with <10 transplants.

During the July 2007 review, 27% of “small” centers and 7% of centers with 10+ transplants were flagged for review (all organs). The Scientific Registry of Transplant Recipients (SRTR) has developed alternative approaches for flagging centers with more consistent flagging rates.

Methods: The new method would allow for different choices of sensitivity (RR) and specificity (p-value) for different purposes and would flag program if either {p-value < .05 (a different value could be chosen)} or {observed / expected > RR}. The resulting false negative rate is less than 50% for centers with the given RR. This approach avoids an arbitrary definition of small versus large programs, and has sensitivity (or power) > 50% to flag programs with the selected RR, regardless of size.

Results: The following table shows the number of programs that would have been flagged during the July 2007 review using a p-value of 0.05 for several different values of RR:

Program Size (# transplants)	N	Current flag	RR ≥ 1.5	RR ≥ 2	RR ≥ 2.5	RR ≥ 3
≤ 9	212	58	46	41	37	32
10 - 20	99	2	33	21	14	8
≥ 21	491	39	100	63	43	41
Total	802	99	179	125	94	81

Discussion: This approach gives a more balanced distribution of flagging across programs of different sizes and has sensitivity >50% for transplant programs of all sizes. With the choice of RR=2.5, the overall number of centers flagged would be nearly unchanged from current methods.

Abstract# 330

Transplant Characteristics Are Associated with Low-Performance Ratings: Is It Time for Stratified Evaluations To Avoid Disincentives To Perform Higher-Risk Transplants? Jesse D. Schold,¹ Richard J. Howard,² Herwig-Ulf Meier-Kriesche,¹ ¹Medicine, University of Florida, Gainesville, FL; ²Surgery, University of Florida, Gainesville, FL.

Introduction

Center performance ratings are of increasing importance to the transplant community with the introduction of the CMS final rule. Ongoing debate exists regarding how

much center ratings are directly a reflection of quality of care or whether ratings can be substantially influenced by exogenous factors.

Methods

The study examined data from the national SRTR database from 2000-2007. Centers’ semi-annual graft and patient survival were calculated along with a comparison with expected outcomes adjusted for covariates used by the SRTR. Centers meeting three criteria for poor performance were categorized within each cohort. Patient characteristics at each center were compared with performance evaluations.

Results

Overall, half of transplant centers met criteria for low performance in at least one of the semi-annual intervals. Several center factors investigated were not significantly associated with the likelihood to meet low performance criteria including the proportion of older, obese and privately insured patients. In contrast, centers with higher levels of ECD transplants (most ECDs=70% vs least ECDs=40%), African American recipients (most AAs=75% vs least AAs=35%) and patients with low albumin level (lowest albumin =60% vs highest albumin =32%) were more likely to meet low performance criteria. Approximately half the centers that initially met criteria for low performance no longer met criteria when excluding ECD transplants and African American recipients from the performance assessment.

Conclusions

Given the extreme implications of performance ratings for transplant centers including possible loss of funding to centers with low performance, it is critical that we recognize potential weaknesses and biases of performance ratings.

Our results are important towards understanding factors related to performance ratings and raising questions as to whether risk adjustment techniques are adequate for fair transplant center performance evaluations. Rather than only risk adjustment, stratified evaluations may be a partial solution to remove disincentives to performing higher risk transplants. It is also important to recognize factors not associated with low performance such that centers do not unnecessarily limit access to groups based on perceived deleterious impact on ratings.

Joint Plenary Session III

Abstract# 331

A Prospective Study of CD8+ T-Cell Mediated Immunity to Cytomegalovirus (CMV) as a Predictor of CMV Disease. Deepali Kumar,¹ Susan Chernenko,³ George Moussa,³ Jutta Preiksaitis,² Karen Doucette,¹ Oriol Manuel,² Isabel Cobos,¹ Atul Humar.¹ ¹Transplant Infectious Diseases, University of Alberta, Edmonton, Canada; ²Provincial Public Health Lab, University of Alberta, Edmonton, Canada; ³University of Toronto, Toronto, Canada.

Background: Solid organ transplant (SOT) recipients at high-risk for CMV are commonly given prolonged antiviral prophylaxis. However, CMV disease occurring after the discontinuation of prophylaxis is a major problem and methods to predict late-onset disease are needed. We prospectively evaluated the use of serial CMV specific CD8+ T-cell response measurements for predicting the risk of late-onset CMV disease.

Methods: SOT patients at high risk for CMV disease (D+/R-, induction therapy, lung transplant recipients) were enrolled. Cell-mediated immunity (CMI) was determined by stimulation of whole blood with a synthetic 21-peptide pool containing CMV epitopes restricted through various HLA class I alleles, followed by measurement of interferon-γ production (QuantiFERON® CMV-assay). CMI testing was done at baseline, 1, 2, and 3 months post-transplant. The primary endpoint was development of CMV disease in the first 6-months post-transplant.

Results: There were 108 patients who were enrolled and completed all assessments. Transplant types were lung (n=48), liver (n=19), kidney (n=32) and other (n=9). 32.4% were CMV D+/R- upon enrolment. CMV disease developed in 19/108 (17.6%) patients within the first 6-months. At the end of prophylaxis (usually 3-months post-transplant), CMI was detectable (interferon-γ ≥ 0.2 IU/ml) in 29/108 (26.9%) patients. CMV disease occurred in 1/29 (3.4%) patients with detectable CMV-CMI and 18/79 (22.8%) patients with no detectable CMI at the end of prophylaxis; p=0.04. In a time-to-event analysis, a lack of detectable CMI at the end of prophylaxis was a significant predictor of subsequent CMV disease (p=0.025). In the subgroup of D+/R- patients (n=35), the optimal cut-off for the assay appeared to be 0.1 IU/ml of interferon-γ. With this cut-off, CMV disease occurred in 1/10 (10%) patients with a positive CMI and 10/25 (40%) patients with a negative CMI at the end of prophylaxis; p=0.12.

Conclusion: A lack of detectable cell mediated immunity to CMV at the end of prophylaxis was predictive of subsequent CMV disease. CMI testing may be clinically useful for identifying patients that could benefit from more prolonged prophylaxis.

Abstract# 332

Role of TIM3-Galectin-9-Pathway in Regulation of T Cell-Mediated Alloimmune Responses in a Murine Vascularized Cardiac Transplant Model. Olaf Boenisch,¹ Takaya Muramatsu,¹ Francesca D'Addio,¹ Robert Padera,¹ Hideo Yagita,² Nader Najafian.¹ ¹*Transplantation Research Center, Brigham and Women's Hospital and Children's Hospital, Boston, MA;* ²*Immunology, Juntendo University of Medicine, Tokyo, Japan.*

T cell immunoglobulin and mucin domain (TIM)-3, a molecule expressed on terminally differentiated Th1 cells, is an important regulator of Th1 autoimmunity and in induction of transplantation tolerance. Its functions in alloimmune responses during acute and chronic rejection are unknown.

TIM 3-23, a novel blocking antibody of TIM-3, was administered to recipients in various donor-recipient strain combinations on days 0 (500ug), 2, 4, 6, 8 and 10 (250ug) after transplantation. The frequency of IFN γ -, IL-4-, and Granzyme B-producing splenocytes was measured by ELISPOT. The frequency of CD44high CD62Llow effector T cells and CD4+CD25+FoxP3+ regulatory T cells (Tregs) was assessed by Flow cytometry.

TIM 3-23 did not affect graft survival in B6 recipients of fully MHC-mismatched BALB/c allografts (MST 7 days vs. 7, n=6). B6 CD28KO (MST 10 days, n=9 vs. 16, n=12, p=0.001) and B7-1/2 DKO (MST 52 days, n=3 vs. >100, n=4, p=0.01) recipients, both lacking CD28-B7 costimulation, promptly rejected BALB/c allografts after TIM-3 blockade when compared to controls. Accordingly, grafts of treated animals displayed severe necrosis, cellular myocarditis and vasculitis. Accelerated rejection in TIM 3-23 treated CD28KO mice was accompanied by a decline of Tregs (2.90 $\pm$ 0.13 vs. 3.81 $\pm$ 0.19%, p=0.03, n=6) but increased frequencies of IFN- γ (256 $\pm$ 141 spots/500.000 responder cells vs. 66 $\pm$ 21, n=4), IL-4- (93.3 $\pm$ 12.8 vs. 33.5 $\pm$ 6.9 cells, n=4) and granzyme-B- (178.3 $\pm$ 41.1 vs. 69.8 $\pm$ 7.2 cells, n=4) producing alloreactive T cells. Allografts mismatched at only MHC class I (bm1 into B6) or class II alleles (bm12 into B6) survive long-term, although they do develop chronic allograft vasculopathy (CAV). Targeting TIM-3 resulted in accelerated graft rejection of MHC class II- (MST 29.5 days, n=5 vs. >56, n=4, p=0.01) but not class I- (MST >56 days in treated and control mice) mismatched hearts, mainly due to accelerated and pronounced CAV in the former group.

These data establish the regulatory functions of TIM-3 in alloimmune responses in solid organ transplantation models. The inhibitory actions may be secondary to modulation of effector or regulatory T cells and appear to dominate in conditions of low levels of T cell activation, due to a restricted degree of allogeneic mismatch or absence of CD28 costimulation.

Abstract# 333

The Effect of Pre-Transplant Ablation Therapy on Post-Transplant Outcome in Liver Recipients with Hepatocellular Carcinoma. Erick B. Edwards,¹ Ann M. Harper,¹ Richard B. Freeman.² ¹*Research Department, United Network for Organ Sharing, Richmond, VA;* ²*Division of Transplant Surgery, New England Medical Center, Boston, MA.*

Background. Since the inception of the MELD-based system for deceased donor liver allocation in the U.S. in 2002, patients with hepatocellular carcinoma (HCC) can receive extra priority (exception points) for transplant. In order to receive the extra priority, the OPTN requires transplant centers to submit justification data (tumor size, number, ablation data, etc.) for each HCC candidate. Candidates who receive ablation therapy (AT) continue to receive priority even though their tumor sizes may no longer meet the criteria necessary for the exception. We questioned whether HCC recipients receiving AT had better post-transplant outcomes than HCC recipients not receiving AT.

Methods. Based on OPTN justification data, we identified 1782 adult liver transplants for HCC between 4/30/2003 and 8/31/2006. Univariate post-transplant survival was estimated using the Kaplan-Meier method, and multivariable post-transplant outcomes were analyzed with Cox regression models. In the first model, we tested for the overall effect of AT. In the second model, we tested for the individual effect of each type of AT. In each model, the recipient factors included age, ethnicity, gender, pre-transplant listing stage of HCC, number of tumors, total tumor size, biopsy, AFP, serum creatinine, bilirubin, INR, and several pre-transplant co-morbidity factors. Donor factors included age, gender, ethnicity, height, weight, cold ischemia time, cause of death, and donation after cardiac death. **Results.** We identified 859 recipients who received AT prior to transplant. The majority received either TACE alone or RFA alone. Unadjusted graft survival at 1 year and 2 years was 87.4% and 78.0% for recipients who received AT and 83.8% and 74.8% for recipients that did not receive AT. In the first Cox model, the adjusted relative risk (RR) of graft failure associated with AT was 0.785, p=0.014. In the second model, TACE (RR=0.791), and RFA (RR=0.725) were each associated with a statistically significant reduced risk of graft failure. **Conclusions.** AT appears to improve significantly the post-transplant graft survival rates among recipients with HCC. The use of TACE or RFA is responsible for the majority of this effect. Further analyses are necessary to determine how much of this effect is attributable to the therapy itself and how much is due to careful patient selection.

Abstract# 334

Th1 Immune Response and Ischemia-Reperfusion (I-R) Injury in Renal Graft Recipients with Delayed Graft Function (DGF). M. Taveri,¹ A. Loverre,¹ R. Verrienti,¹ G. Zaza,¹ M. Rossini,¹ R. Melchiorre,¹ G. Castellano,¹ P. Dittono,² M. Battaglia,² F. P. Selvaggi,² A. Crovace,³ F. P. Schena,¹ G. Grandaliano.¹ ¹*Nephrol Dial Transpl Unit DETO, Univ Bari, Italy;* ²*Urol Transpl Unit, Univ Bari, Italy;* ³*Veter Unit, Univ Bari, Italy.*

I-R injury in transplanted kidney is a major cause of DGF, an event associated with an increased risk of acute rejection. Adaptive immunity was suggested to play a role in the pathogenesis of renal I-R injury, although the influence of the Th1/Th2 bias in this scenario is still debated. Thus, the aim of the present study was to evaluate the features of T cell response during I-R injury at the peripheral and tissue level in renal graft recipients with DGF.

The mRNA levels of specific Th1 (T-bet) and Th2 (GATA-3) transcription factors were evaluated in circulating lymphomonocyte of kidney transplant recipients with early graft function (EGF) (n=10) and DGF (n=10), before (T0) and 24 hours after transplantation (T24) by real time PCR. Infiltrating lymphocytes were characterized in graft biopsies of patients with DGF (n=40) and in a control group of patients with tubular damage by acute CNi toxicity (n=10) by immunohistochemistry. In addition, we evaluated the Th1/Th2 bias at the renal level in a pig model of I-R injury.

T-bet/GATA-3 mRNA ratio was similar in the 2 groups of patients at T0. At T24 the DGF group presented a significantly higher increase of T-bet/GATA-3 ratio compared with the EGF group (798 $\pm$ 346 vs 288 $\pm$ 147% of T0, p<0.001). Moving to the tissue level, DGF patients presented a number of interstitial CD4⁺ (8.0 $\pm$ 5.1 vs 2.6 $\pm$ 2.1, p=0.04) and CD8⁺ (10.8 $\pm$ 6.7 vs 4 $\pm$ 2, p=0.02) T cells significantly higher compared to the control group, while no significant differences were observed in CD20⁺ cells number between the two groups. Also at the tissue level the ratio between T-bet⁺ and GATA-3⁺ cells was significantly higher in the DGF compared with the control group (3.5 $\pm$ 1.8 vs 1.1 $\pm$ 0.9, respectively, p=0.005). To confirm that these changes were due to I-R, we investigated the presence of T-bet⁺ and GATA-3⁺ cells in a pig model of I-R injury. Interestingly, the ratio was significantly increased after 24 hours of reperfusion (basal 2.5 $\pm$ 0.9 vs 24 hours 8.1 $\pm$ 3.6; p=0.02).

In conclusion, our results suggest that kidney transplant recipients with DGF present a bias toward a Th1-driven immune response both at the peripheral and at the tissue level. This event, due to the I-R process, as suggested by the animal model, may represent a link between DGF and acute graft rejection.

Abstract# 335

Preemptive Kidney Transplantation in Minorities Not Associated with Patient Survival Benefit: A Biologic Phenomenon or Selection Bias? Douglas S. Keith.¹ ¹*Medicine, Division of Nephrology, McGill University, Montreal, QC, Canada.*

Minority kidney transplant recipients have more exposure to dialysis prior to transplantation than Caucasians due to delayed listing and longer waiting times after listing. The purpose of this study was to determine if minorities have the same patient and graft survival benefit of preemptive transplantation as Caucasians. All first time deceased donor kidney transplant alone recipients registered in the UNOS database between 1997 and 2004 were included in the study. Minorities were defined as any racial or ethnic group including Hispanics other than Caucasian. Cox proportional hazard modeling was used to determine the hazard ratios of graft and patient survival based on the duration of dialysis and minority status. In contrast to Caucasians, the negative association of duration of dialysis with graft survival was attenuated in minority recipients.

	Adjusted Patient and Graft Survival		Adjusted Hazard Ratios for Patient Survival (95% Confidence Intervals)	
	Ref. Group Preemptive Tx	Ref. Group Preemptive Tx	Ref. Group Preemptive Tx	Ref. Group Preemptive Tx
Duration of Dialysis Pretransplant	Caucasians N=32,866	Minorities N=27,091	Caucasians N=32,866	Minorities N=27,091
12 Months or Less	1.14 (1.04-1.25)	1.13 (0.97-1.32)	1.07 (0.94-1.21)	0.80 (0.64-1.00)
13 to 24 Months	1.21 (1.10-1.32)	1.09 (0.95-1.26)	1.23 (1.09-1.38)	0.80 (0.65-0.97)
25 to 36 Months	1.25 (1.14-1.37)	1.12 (0.97-1.29)	1.37 (1.22-1.55)	0.79 (0.65-0.96)
37 to 48 Months	1.29 (1.17-1.49)	1.13 (0.97-1.30)	1.35 (1.21-1.55)	0.92 (0.75-1.12)
49 to 60 Months	1.32 (1.17-1.49)	1.21 (1.04-1.40)	1.53 (1.32-1.78)	0.96 (0.79-1.18)
Greater than 60 Months	1.43 (1.28-1.60)	1.27 (1.10-1.46)	1.61 (1.39-1.86)	1.10 (0.91-1.33)

As expected a strong negative association between duration of dialysis and patient survival was seen in Caucasian recipients. In contrast the relationship between patient survival and duration of dialysis was U shaped in minorities with the worst patient survivals seen among preemptive transplants and those patients with over 60 months of dialysis. The difference in outcomes between Caucasians and minorities could be related to the biologic differences in the effect of dialysis on subsequent transplantation or to a differential selection bias introduced by duration of dialysis in the two populations.

Abstract# 336

Identification of a Novel B Cell Population in Spleen That Produces Anti-Carbohydrate Natural Antibodies. Lorenzo Benatui,¹ Jonathan G. Godwin,¹ Shamik Gosh,¹ John Iacomini.¹ ¹*Transplantation Research Center, Brigham and Women's Hospital & Children's Hospital Boston - Harvard Medical School, Boston, MA.*

Background. We constructed immunoglobulin gene knock-in mice lacking expression of the α Gal epitope that carry rearranged VH and VL genes encoding antibodies specific for the carbohydrate antigen Gal α 1-3Gal β 1-4GlcNAc-R (α Gal). Here, we describe two novel populations of B cells in the spleen of these M86VHVLGT⁰ mice and their role in the production of α Gal specific antibodies.

Methods. M86VHVLGT⁰ mice were sacrificed and single cell suspensions from spleen, bone marrow, peripheral lymph nodes and peritoneal cavity were prepared and stained with different antibodies and with fluorescently labeled α Gal-BSA for flow cytometric analysis. Using multiparameter cell sorting, we purified marginal (MZ) and follicular (FO) zone B cells, in addition to two novel splenic B cell populations. Cells were adoptively transferred into B cell deficient μ MT/GT⁰ double knock-out mice. Serum samples were collected, and production of α Gal specific IgM antibodies was assessed by ELISA.

Results. Cytometry analysis revealed 2 novel B cell populations found in the spleen of the M86VHVLGT⁰ mouse, characterized by decreased expression of IgM (unknown 1, Uk1) or IgD (unknown 2, Uk2). These populations lay parallel to MZ and FO populations, respectively, when analyzed in terms of IgM and IgD expression. MZ, Uk1 and Uk2 populations exhibited binding of α Gal. ELISPOT analysis determined that α Gal secretion was limited to MZ and Uk1 B cells. Naïve or LPS-activated Uk2 and FO B cells do not produce α Gal specific antibodies. Additionally, restoration of α Gal specific antibody production in μ MT/GT⁰ mice was only found in recipients of MZ or Uk1 cells. Histological analysis showed that both Uk1 and Uk2 B cells are located in the marginal zone of the spleen, but only Uk1 B cells are capable of circulating to lymph nodes.

Conclusions. We have identified two novel B cell populations (Uk1 and Uk2) that reside in the splenic marginal zone of the M86VHVLGT⁰ mouse. These populations differ from MZ and FO B cells in their expression levels of IgM and IgD, and from each other both in their capacity to migrate to the lymph nodes and produce α Gal specific antibodies. Uk1 and MZ B cell populations are responsible for the production of α Gal specific antibodies.

Abstract# 337

Mechanisms of B Cell Tolerance in ABO-Incompatible Pediatric Liver Transplantation. Yuko Igarashi,¹ Toshimitsu Irei,¹ Masayuki Shishida,¹ Yuko Tanaka,¹ Toshimasa Asahara,¹ Hideki Ohdan.¹ ¹*Department of Second Surgery, Hiroshima University, Hiroshima, Japan.*

B cell tolerance refers to a phenomenon in which unresponsiveness of B cells that recognize blood group A/B antigens is induced without continuous immunosuppressive therapy. We have recently demonstrated that B cells responding to blood group A antigens develop a tolerant state following blood group A-to-O pediatric liver transplantation. To investigate these B cell tolerance mechanisms, we employed mice with naturally occurring antibodies (Abs) against human blood group A carbohydrates in their sera and possessing B cells with receptors for blood group A determinants. B cells with receptors for A carbohydrates in mice belong to the CD5⁺ B-1 subset, with phenotypic properties similar to those of human B cells. When these cells were temporarily eliminated by injecting synthetic A carbohydrates, subsequent treatment with cyclosporin A or tacrolimus, which blocks B-1 cell differentiation, completely inhibited the reappearance of B cells with receptors for A carbohydrates in mice. It is probable that calcineurin inhibitors used for preventing T cell-mediated rejection simultaneously suppress B-1 cell differentiation. However, despite a very limited dose of calcineurin inhibitors, the B cell tolerance toward blood group A antigens was persistently maintained in the blood group A-to-O liver transplant recipient. B cell tolerance after ABO-incompatible transplantation might be a consequence of presentation of blood group carbohydrate antigens by cells in the engrafted liver. Immune fluorescence staining of the human liver reveals that blood group antigens are predominantly expressed on the liver sinusoidal endothelial cells (LSECs). We have previously proven that LSECs, which constitutively express Fas-L and PDL-1 have the capacity to tolerate alloreactive T cells. Taken together, we hypothesize that blood group antigen-reactive B cells are also tolerated through the interaction with the LSECs. In order to address this possibility, we used α -1,3-galactosyltransferase-deficient mice. When the α -Gal-expressing LSECs isolated from wild-type mice were adoptively transferred via the portal vein into the splenectomized congenic α -Gal-deficient mice, these mice lost the ability to produce anti- α -Gal Abs (n = 4). This finding suggests that the LSECs expressing blood group carbohydrates play a pivotal role in the tolerization of newly developed B cells specific for the corresponding carbohydrate antigens after ABO-incompatible liver transplantation.

Abstract# 338

Allo-Specific Memory B Cell Development. Kate M. Smith,¹ Jason Xenakis,¹ Ali H. Charafeddine,¹ Neal N. Iwakoshi.¹ ¹*Emory Transplant Center Department of Surgery, Emory University, Atlanta, GA.*

Success in kidney transplantation has resulted from control of T-cell-mediated acute rejection. However, little has been done to improve the fate of patients who possess pretransplant donor-specific antibody (DSA), and no proven therapies exist to specifically prevent DSA formation post-transplant. While methods to detect and characterize DSAs are clearly useful, the B-cell subsets that produce DSAs or more importantly sustain their production are poorly characterized. This study set out to investigate the fundamental mechanisms determining the formation of donor-specific memory B cells and plasma cells in novel mouse models of DSA formation. We have developed two complementary and novel systems to track the phenotypic and functional properties of polyclonal donor-specific B-cells. The first system involves allosensitization between normal C57BL/6 and Balb/c mice. We used advanced flow cytometric methods to track donor-specific B cells with H-2k^b or H-2k^d tetramers. Tetramers are comprised of four identical H-2 molecules bound to a fluorescently labeled streptavidin molecule that is able to bind the donor-specific B-cell antigen receptor (surface immunoglobulin). In our second system, we use donor mice that constitutively express full-length membrane-bound chicken ovalbumin (mOVA) protein in all tissues. Analogously, Ova specific-B cells can be analyzed flow cytometrically with the use fluorescently-labeled ovalbumin. Both systems allow us to identify donor-specific memory B cells (7AAD⁺CD4⁺CD8⁺Ova⁺tetramer-IgD⁺B220⁺CD138⁺) and plasma cells (7AAD⁺CD4⁺CD8⁺Ova⁺tetramer-IgD⁺fb220⁺CD138⁺) during skin graft rejection (day 14 post transplant). We were also able to track the development of a stable memory cell population in the bone marrow and spleen for > 90 days cells. For the Ova system quantitative ELISA and ELISOT measurements for serum DSA and DSA-secreting cells, respectively, correlated with the development of donor-specific memory B cells. Using these assays we will evaluate polyclonal donor-specific B memory subsets under multiple conditions of immunity and tolerance and for the first time, characterize their functional properties and migration patterns. These experiments will provide new information on the basic biology of the memory B-cell response to allografts in mice and facilitate the development of these methods in sensitized patients that may lead to critical therapeutic opportunities for DSA production.

Abstract# 339

Inhibitory Effects of Atacicept (TACI-Ig) on Circulating Antibodies, B Cells and Plasma Cells in Allosensitized Cynomolgus Monkeys. Svyetlana Boskovic, Neal R. Smith, Tatsuo Kawai, Joren C. Madsen, Ognjenka Nadazdin, David H. Sachs, Antony B. Cosimi, Robert B. Colvin. *Departments of Surgery and Pathology, Massachusetts General Hospital, Boston, MA.*

Purpose: To evaluate the effects of atacicept (TACI-Ig; Transmembrane Activator and Calcium-modulator and cyclophilin ligand Interactor -immunoglobulin) treatment on B cell/plasma cell function in sensitized cynomolgus monkeys.

Background: Organ transplantation for sensitized patients has been limited by the lack of specific therapy to control alloantibody production and plasma cell function. Atacicept is a recombinant human soluble receptor that acts as an antagonist to BAFF (B cell Activation Factor of TNF Family) and APRIL (A Proliferation-Inducing Ligand), two stimulatory ligands responsible for B cell maturation and survival. In mice, TACI-Ig inhibits antibody response, survival of plasma cells and development of autoimmune disease.

Methods: Cynomolgus monkeys that developed donor specific alloantibodies (DSA) after rejecting heterotopic heart allografts received 8 doses of atacicept at 10 mg/kg S.C., twice a week. Recipients were monitored over 3 months for levels of DSA, antibodies to ABO blood group and gal[α 1,3 gal] antibodies, and peripheral B cells. Spleen biopsy was performed at one month to evaluate splenic plasma cells by staining for immunoglobulin light chains via in situ hybridization.

Results: All three recipients demonstrated a reduction in DSA levels by 36.3 $\pm$ 11% in anti-T and 24.3 $\pm$ 11% in anti-B cell DSA, compared to pre-treatment levels. Similarly, gal[α 1,3 gal] xenogeneic antibody levels decreased by 55 $\pm$ 4.4% in IgG and by 60.7 $\pm$ 27.5% in the IgM fraction. Antibody levels to ABO blood group antigens tested on two subjects with blood group A also showed marked reduction in IgM (from 1:32 to 1:4 and from 1:64 to 1:16) and in IgG antibodies (1:16 to 1:8, 1:64 to 1:32). Two of three subjects showed significant reduction of CD20⁺CD3⁺ B cells transiently, with nadir one month after last dose (83% and 73%). Splenic plasma cells were decreased in all three subjects by 75% (mean of 103 $\pm$ 45 cells/mm²) when compared to controls (mean of 293 $\pm$ 21).

Conclusions: Atacicept decreased B cell and plasma cell numbers and reduced alloantibody levels, as well as gal[α 1,3 gal] xenogeneic antibodies and anti-ABO blood group antibodies, in sensitized cynomolgus monkeys. Further testing of the efficacy of atacicept to lower pathogenetic antibodies in transplantation is warranted by the encouraging results of this pilot trial.

Abstract# 340

In Vivo Neutralization of the B-Lymphocyte Survival Factor, BLyS/BAFF, Abrogates Alloantibodies and Promotes Long-Term Islet Allograft Survival. Ming Yu,¹ Armen Badkerhanian,¹ Thi S. Migone,² Ghazal Zekavat,¹ Matthew Deasey,¹ Susan Y. Rostami,¹ Ali Naji,¹ Hooman Noorchashm.¹
¹*Surgery, University of Pennsylvania School of Medicine, Philadelphia, PA;*
²*Clinical Immunology, Human Genome Sciences Inc., Rockville, MD.*

The TNF-related B-lymphocyte survival factor, BLyS/BAFF, is critical for primary follicular (FO) and marginal zone (MZ) B-cell survival. In vivo neutralization of BLyS/BAFF, using a newly developed monoclonal antibody, depletes the FO and MZ B-cell compartments. Here, we hypothesized that targeting B-lymphocytes, via the BLyS/BAFF pathway, could promote humoral tolerance to islet allografts. Cohorts of STZ-diabetic C57BL/6 mice were transplanted with isolated islets from BALB/c donors. Treatment with anti-BLyS/BAFF alone (100mcg/mse x2 doses+15mcg/wk/mse) did not protect islet allografts from acute rejection (MST=14d; n=4). On the other hand, a 14-day course of Rapamycin (1mg/kg) prevented acute allograft rejection (MST=35d; n=9). When Rapamycin was combined with the anti-BLyS regimen, islet allograft survival was markedly prolonged (MST>150d; n=4). Importantly, islet allograft survival was coincident with the absence of detectable serum alloantibodies and blunted donor specific T-cell responses. Following treatment with anti-BLyS, B-lymphocyte compartment reconstitution was detectable at 30 days following treatment and was characterized by stringent selection at the transitional→FO B-cell tolerance checkpoint. Overall, these data indicate that the BLyS/BAFF pathway may be a logical target of immunotherapy for the achievement of humoral transplantation tolerance.

Abstract# 341

Expression of IL-21 Receptor on B Lymphocytes of Highly Sensitized Patients Is Up-Regulated: A Potential Target for B-Cell IgG Production. Reza Tavana, Lui Shuquing, Jean Tchervakov. *Surgery, McGill University, Montreal, QC, Canada.*

Background: Highly sensitized patients' ability to be transplanted is severely compromised because of high level of antibodies against various HLA antigens. Interleukin-21 is a type I cytokine that signals through a receptor composed of the IL-21R and the common cytokine receptor γ -chain (γ_c). It is produced by T-cells and has been shown to be the contributing factor in terminal differentiation of memory B-cell to Anti-body producing B-cell and plasma cells.

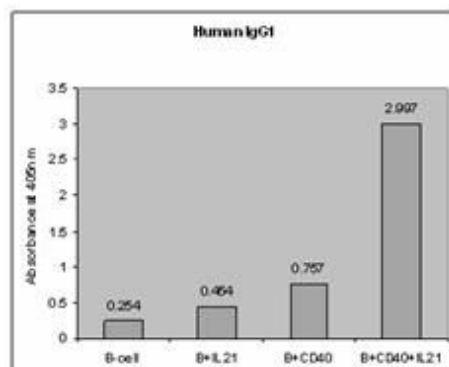
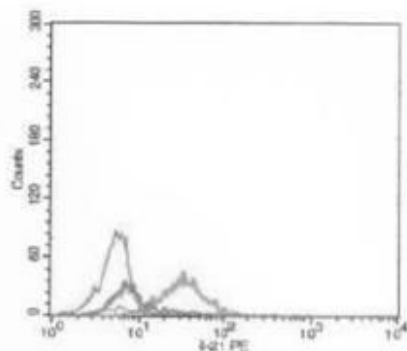
Objective: We evaluated the effect of IL-21 Co-stimulation on Antibody production capacity of B-cells and also measured the expression of IL-21R on B lymphocytes of highly sensitized patients compared to Non-sensitized patients on the kidney transplant list.

Methods: Patients with a PRA level >20% (sensitized) were compared to Non-sensitized patients from the transplant waiting list. After consent was obtained, peripheral blood was taken from patients before initiating hemodialysis. Leukocytes were labelled with anti-CD19, anti-IL-21 antibodies and paraffin fixed after RBC lysis. IL-21R Expression was measured by Flow-cytometry over FACSscan machine on the same day.

Results:

The Expression of the IL-21R is significantly higher on B-lymphocytes of highly sensitized patients (HSP) compared with non sensitized patients (NSP) (Fig 1. p<0.05). In-vitro Co-stimulation of Isolated peripheral B-cell with IL-21 and anti-CD40 results in higher IgG1 production compared with Anti-CD-40 or IL-21 stimulation alone (Fig 2).

Conclusions: IL-21 is an important cytokine in B-lymphocyte stimulation and increases IgG1 production. IL-21 Receptor on B-lymphocytes is up-regulated in sensitized kidney transplant recipients. IL-21 to IL-21R interaction between T and B-lymphocytes may be an important pathway in antibody production in highly sensitized renal transplant recipients.

**Abstract# 342**

Antigen Presentation by B Cells Promotes Heart Allograft Rejection and Development of Alloreactive Memory T Cells. Yue-Harn Ng,¹ Qi Li,¹ Autumn Marlowe,¹ Rosemary Hoffman,¹ Geetha Chalasani,¹
¹*Medicine (Renal-Electrolyte), Immunology and Surgery, Thomas E. Starzl Transplantation Institute, University of Pittsburgh, Pittsburgh, PA.*

B cells and memory T cells contribute to treatment-resistant acute and chronic rejection that result in allograft loss. It is possible that B cells contribute to allograft rejection by mechanisms other than alloantibody production since donor specific antibodies often precede cellular rejection. Our previous studies show that B cells promote proliferation and differentiation of alloreactive T cells into memory T cells. We tested whether antigen presentation by B cells promotes generation of alloreactive memory T cells.

Methods and Results: We created mixed bone marrow chimeras in irradiated μ MT (B cell-deficient) recipients by transplantation of syngeneic bone marrow from μ MT, wild-type (wt) and MHCko (lack MHC I & II expression on all cells) mice. μ MT+MHCko chimeras lack expression of MHC I & II on B cells but not other professional APCs such as DCs hence antigen presentation specifically by B cells is disrupted. Allograft rejection and development of alloreactive memory T cells in μ MT+MHCko chimeras was compared to μ MT+wt chimeras that had intact antigen presentation by both B cells and APCs. Skin allograft rejection was comparable between μ MT+MHCko and μ MT+wt chimeras (MST = 17 and 16 days, respectively, p = 0.6, n = 5/grp). However, heart allograft rejection was significantly delayed in the μ MT+MHCko chimeras compared to μ MT+wt chimeras (MST = 23 and 15 days, respectively, p=0.03, n = 5/grp). Development of alloreactive memory T cells was assessed in chimeras at 8 weeks after skin allograft rejection by quantitation of antigen-specific IFN γ producing T cells. Alloreactive CD4 and CD8 memory T cells were significantly fewer in μ MT+MHCko chimeras (7-fold fewer CD4, p = 0.02, and 5-fold fewer CD8, p = 0.003, n = 5/grp) than in μ MT+wt chimeras. These results show that the disruption of antigen presentation by B cells significantly delays heart but not skin allograft rejection. Development of alloreactive CD4 and CD8 memory T cells is significantly impaired in the absence of antigen presentation by B cells.

Conclusions: Antigen presentation by B cells accelerates heart allograft rejection and leads to development of alloreactive memory T cells. These findings emphasize antibody-independent functions of B cells in promoting alloimmune responses and highlight the need for B-cell targeted therapies to improve long-term allograft survival.

Abstract# 343

Addition of Anti-CD20 to Therapeutic Anti-CD154 or CsA in Monkeys Attenuates Alloantibody Production and Severity of Cardiac Allograft Vasculopathy (CAV). Shahrooz S. Kelishadi,¹ Tianshu Zhang,¹ Tiffany Stoddard,¹ Mitch Higuchi,¹ Emily Welty,¹ Christopher J. Avon,¹ Amal Laaris,¹ Xiang-Fei Cheng,¹ Agnes M. Azimzadeh,¹ Richard N. Pierson III.¹
¹*Surgery, University of Maryland School of Medicine and Baltimore VAMC, Baltimore, MD.*

Purpose: To test whether depletion of CD20+ B cells at the time of engraftment alters the prevalence of anti-donor alloantibody (Ab) or severity of CAV in the context of therapeutic immunosuppression with (CsA) or high dose CD154 inhibition.

Methods: Forty-five MLR-mismatched heterotopic cardiac cynomolgus allograft recipients were treated with high dose anti-CD154 monotherapy (α CD154; n=14, 5 with ATG induction) or α CD154 with additional anti-CD20 therapy (rituximab 20mg/kg q wk for 4 weeks: α CD154 + α CD20; n=18, 11 with ATG). Thirteen other animals received therapeutic CsA (target trough >500ng/ml), five of which received additional α CD20. Graft survival was censored at 90 days. Ab was deemed (+) if present (by flow cytometry) around time of explant.

Results: 12 animals died with beating grafts, mainly with ATG-associated lung pathology or infectious etiologies and are excluded from this analysis. 3 animals that had sustained α CD154 levels <100 μ g/ml after protocol day 30 were considered "subtherapeutic" for this reagent and also excluded from further consideration. Graft survival with α CD154 + α CD20 (median >90d) and proportion of grafts surviving to 90 days (6/6) was significantly increased relative to α CD154 alone (median 43d, p=0.007;

4/14 >90d). Graft survival with CsA + α CD20 (median >90d) and proportion of grafts surviving to 90 days (4/4) was increased relative to CsA alone (median 66d, 3/6 >90d). With therapeutic α CD154 (trough level >100 μ g/ml until graft explant), 12/13 (92%) developed Ab vs. 1/6 with α CD154 + α CD20 (17%) ($p=0.006$). Average CAV score for the α CD154 group was 2.44 ± 0.44 vs. 0.7 ± 0.8 in the α CD154 + α CD20 group ($p=0.006$ using unpaired t test). Preliminary CAV scoring suggests that added α CD20 inhibited CAV (scores ranging 0.0-0.1) relative to CsA alone (median 2.1; range 1.5-2.4); Ab analysis is in progress for these groups.

Conclusions: Using α CD20 is associated with significant attenuation of CAV when used with either "therapeutic" α CD154 or CsA. Our findings demonstrate for the first time that α CD20 reduces the severity of CAV in conjunction with both conventional immunosuppression and costimulation pathway blockade. Mechanisms include inhibition of Ab production, and perhaps others that remain to be defined.

Abstract# 344

Subsaturating Concentrations of Anti-HLA Antibody (Ab) Activates the MAPK, Hedgehog and Wnt Signaling Pathway in Endothelial Cells (ECs) resulting in Accommodation. S. Ramachandran,¹ N. Fukami,¹ G. Simon,¹ M. Koch,³ S. Shenoy,¹ M. Jendrisak,¹ W. Chapman,¹ T. Mohanakumar.^{1,2} ¹Surg, WUSM; ²Path & Immunol, WUSM; ³Int Med-Renal, Washington Univ Sch Med, St. Louis, MO.

Sensitized recipients having donor specific anti-HLA Abs have been successfully transplanted following a conditioning regimen employing plasmapheresis with or without pooled human immunoglobulin. In vitro studies have shown that exposure of ECs to subsaturating concentrations (SSC) of anti-HLA Ab (priming) followed by subsequent exposure to high concentrations (HC) of anti-HLA induces the expression of protective genes, Bcl2 and HO-1 and confers protection to complement mediated lysis of ECs. However, the molecular events following priming with exposure to SSC of anti-HLA Ab still remains undefined. To determine the priming events and to define the kinetics of protective gene expression, we exposed human aortic ECs to SSC of anti-HLA Ab for 72 hrs and re-exposed them to saturating concentrations of anti-HLA Abs. ECs were collected at 24, 48, and 72 hrs after exposure to SSC as well as 24 hrs after exposure to HC. Expression profile of signaling intermediates (MAPK, WNT, NF- κ B, Hedgehog, PI3kinase, stress pathway, TNF family) in the ECs were analyzed by gene array. Analysis of the Bcl2 and HO-1 expression showed no significant increase in expression following exposure to SSC of w/32 (priming) or control Ab alone at any of the time points (24, 48 & 72 hrs). However, subsequent exposure of the ECs to saturating concentrations of anti-HLA Ab resulted in a 2.3 fold increase in the expression of Bcl2 and 2.6 fold increase in the expression of HO-1. A significant increase in expression of MAPK1 (9.1 fold; MAPK/ERK pathway), TANK (4.83 fold), WNT2 (4.59 fold) and BMP4 (11.31 fold) was observed in ECs primed with SSC of anti-HLA Ab compared to control Ab. Further, a significant decrease in the expression of e-selectin, p-selectin, PECAM1, ICAM-1, VACM-1 was also observed in primed ECs compared to controls. These results demonstrate that priming of the ECs with SSC of anti-HLA Abs result in the activation of the MAPK, Hedge Hog and Wnt pathway which can lead to cell survival signals and decreased expression of the adhesion molecules and chemotactic ligands. A second stimulus of the primed ECs with high concentration of HLA leads to Bcl2 and HO-1 upregulation and decreased expression of adhesion molecules that can lead to decreased alloimmune responses resulting in the observed prolongation of the accommodated allografts.

Ischemia/Reperfusion Injury: New Therapeutic Concepts

Abstract# 345

Thrombomodulin Protects Against Hepatic Ischemia Reperfusion Injury in the Rat. Masashi Kishiwada,¹ Shuji Isaji,¹ Naohisa Kuriyama,¹ Ichiro Ohsawa,¹ Takashi Hamada,¹ Masanobu Usui,¹ Hiroyuki Sakurai,¹ Masami Tabata,¹ Tatsuya Hayashi,² Esteban C. Gabazza,³ Koji Suzuki.² ¹Hepatobiliary Pancreatic Surgery, Mie University Graduate School of Medicine, Tsu, Japan; ²Immunology, Mie University Graduate School of Medicine, Tsu, Japan; ³Molecular Pathobiology, Mie University Graduate School of Medicine, Tsu, Japan.

Background/aims: Microcirculation disturbance, endothelial injury and cytokine overproduction are implicated in the pathophysiology of hepatic ischemia reperfusion injury (IRI). Thrombomodulin (TM) is a membrane-bound endothelial thrombin receptor that accelerates thrombin-catalyzed protein C activation, inhibits thrombin-induced fibrin formation, and also regulates inflammation. In the present study, we investigated the effects of recombinant human soluble TM (rhsTM) on hepatic IRI in the rat.

Methods: Wistar Hannover rat was used for preparing ischemia/reperfusion model. Hepatic IRI was induced by subjecting 70% area of the rat liver to 90 minutes of ischemia followed by 6 h of reperfusion. The rats were randomly assigned to a group receiving an intravenous injection of rhsTM (3 mg/kg body weight) and to a group treated with saline 60 minutes prior to the beginning of reperfusion. Sinusoidal endothelial cells (SECs) and Kupffer cells (KCs) were isolated using centrifugal elutriation. The plasma levels and the concentration in cultured cell supernatant of IL-6 and TNF α were measured by specific enzyme immunoassays.

Results: The plasma ALT, AST and hyaluronic acid levels were significantly decreased, and the histological damage of the liver was attenuated in rhsTM-treated rats as compared to control rats. Using Laser Doppler flow-meter we found that rhsTM treatment improved hepatic microcirculation. The intrasinusoidal fibrin deposition, injury of SECs and liver dysfunction during hepatic IRI were weaker in rhsTM-treated rats than in control rats. TM activity in SECs was significantly recovered, and plasma IL-6 and TNF α were significantly decreased in rhsTM-treated rats as compared to control rats. Further, IL-6 and TNF α production in isolated KCs was also significantly decreased in rhsTM-treated rats as compared to control rat.

Conclusion: The present results suggest that rhsTM is useful for the prevention of SECs damage and KCs activation induced by IRI. Our present study also suggests that disturbance of hepatic microcirculation is induced in part by intrasinusoidal microthrombus formation and by locally released inflammatory cytokines from KCs.

Abstract# 346

Protective Effects of Preservation Solution Including Activated Protein C in Small-for-Size Liver Transplantation in Rats. Naohisa Kuriyama,¹ Shuji Isaji,¹ Takashi Hamada,¹ Masashi Kishiwada,¹ Ichiro Ohwsawa,¹ Masanobu Usui,¹ Hiroyuki Sakurai,¹ Masami Tabata,¹ Koji Suzuki.² ¹Hepatobiliary Pancreatic Surgery, Mie University Graduate School of Medicine, Tsu, Mie, Japan; ²Molecular Pathobiology, Mie University Graduate School of Medicine, Tsu, Mie, Japan.

Background: Small-for-size liver graft is a serious obstacle of partial orthotopic liver transplantation (OLT). However, various therapeutic strategies including surgical innovations, pharmacological agents and gene therapies to protect small-for-size liver graft have not yet been developed.

Aims: Activated protein C (APC) is known to have cell protective properties via its anti-inflammatory and anti-apoptotic activities. This study aimed to examine the cytoprotective effects of preservation solution containing APC on OLT using small-for-size rat liver graft (20% partial liver).

Methods: Liver grafts were assigned to two groups: in the control group, the grafts were flushed and stored in Histidine-tryptophan-ketoglutarate (HTK) solution alone for 6 h; in the APC group, in HTK solution containing APC for 6 h.

Results: The APC group significantly increased 7-day graft survival from 60% to 100%, decreased levels of transaminase, and improved histological features of hepatic IRI compared to the control group. Myeloperoxidase activity demonstrated that the APC group markedly suppressed the infiltrations of neutrophil. Hepatic expressions of tumor necrosis factor- α and IL-6 of the APC group were remarkably decreased. The APC group significantly reduced serum hyaluronic acid levels, indicating attenuated sinusoidal endothelial cell injury. Moreover, the APC group markedly increased hepatic levels of nitric oxide caused by upregulated endothelial nitric oxide synthesis (NOS) together with downregulated inducible NOS, and decreased hepatic levels of endothelin-1. Finally, hepatocellular apoptosis of the APC group was remarkably suppressed by downregulated hepatic caspase-8 and caspase-3 activities.

Conclusions: Preservation solution containing APC inhibited pro-inflammatory cytokine synthesis, which leads to hepatocellular apoptosis and liver injury. One of the cytoprotective effects of the APC treatment was to upregulate hepatic eNOS, followed by increased expression of hepatic NO, and to decrease expression of hepatic ET-1, resulting in the prevention of microcirculatory disturbance. Preservation solution containing APC is a potential novel and safe product for small-for-size liver transplantation to improve liver graft function and animal survival.

Abstract# 347

Deletion of CD39 on Natural Killer Cells Attenuates Hepatic Ischemia/Reperfusion Injury. Guido Beldi,^{1,2} Yara Banz,¹ Alexander Kroemer,¹ Annette Pexa,¹ Yan Wu,¹ Keiichi Enjoji,¹ Daniel Candinas,² Xiang Li,¹ Simon C. Robson.¹ ¹Transplant Centers, BIDMC, HMS, Boston, MA; ²Transplant Surgery, Bern University, Bern, Switzerland.

Background: CD39 (nucleoside triphosphate diphosphohydrolase (NTPDase)-1) is an ectonucleotidase, expressed by the vasculature and defined immune cell subsets involved in liver ischemia reperfusion injury (IRI) e.g. natural killer NK and NKT cells. CD39, in tandem with CD73/ecto-5'-nucleotidase, modulates purinergic signaling via nucleotide P2-receptors and also generates extracellular adenosine. Pharmacological agonists for the adenosine-2A receptors upon NKT cells are thought to block interferon-gamma (IFN γ) release and provide protective effects post-warm partial liver IRI.

Aim: To examine consequences of CD39 expression on NK or NKT cells and study effects on hepatic IRI.

Methods and Results: CD39 is the dominant ectonucleotidase in both NK and NKT cells. NK cells lack expression of CD73, unlike NKT cells that express both ecto-enzymes. Effects of CD39 on liver injury were evaluated in a model of partial warm hepatic ischemia. CD39 and IFN γ null mice are protected equivalently against early biochemical manifestations of liver injury seen with warm partial hepatic IRI in wild type mice. Adoptive transfers were also performed into Rag2/common gamma null mice (deficient in T cells, B cells and NK cells) using NK and NKT cells of wild type and mutant mice with targeted deletion of either CD39 or IFN γ . Our data show that relative protective effects of CD39 deletion are seen with transfer of NK but not NKT cells. The improvements in IRI outcomes with adoptive transfer of IFN γ null NK cells are comparable to CD39 null NK cells; both are associated with decreased neutrophil

infiltration and tissue injury. Deletion of CD39 results in aberrant NK cell responses in vitro with decreased secretion of IFN γ in response to exogenous IL-12 and IL-18. Furthermore, secretion of IFN γ from CD39 null NK cells and cytotoxic activity can be significantly decreased by the non-hydrolysable ATP γ S, are unaffected by adenosine and restored by bypass of P2Y-receptor responses in vitro.

Conclusion: NK and NKT cells both express CD39 but differentially express CD73. CD39 modulates NK numbers and their function in vitro. Importantly, CD39 deletion on NK cells is protective in hepatic IRI. Disordered purinergic signaling with P2Y-receptor desensitization results in decreased IFN γ secretion by CD39 null NK cells with less injury post hepatic IRI.

Abstract# 348

Interleukin-10 Delivery Via Mesenchymal Stem Cells (MSC): A Novel Approach To Prevent Lung Ischemia/Reperfusion Injury. E. Manning,¹ S. Pham,¹ S. Li,¹ R. Vazquez-Padron,¹ J. Mathew,¹ P. Ruiz,¹ S. Salgar.¹ ¹*Surgery, University of Miami, Miami, FL.*

Aim: About 15-30% of patients develop significant Ischemia/Reperfusion (I/R) injury and immediate graft dysfunction & loss after lung transplantation. MSC have immunosuppressive properties and interleukin-10 (IL-10) is a potent anti-inflammatory cytokine. In this study, autologous MSC & IL-10 engineered MSC were tested for their ability to prevent lung I/R injury. **Methods:** MSC (plastic adherent) isolated from Lewis rat bone marrow were transduced with rVIL-10-retrovirus & selected on neomycin. Following 120 min of left lung ischemia, Group A, rats received viral IL10 (vIL10) transduced MSC ($\sim 15 \times 10^6$; i.v.); Group B, received empty vector engineered MSC; Group C, received MSC; and Group D, received saline. Group E received no ischemia or MSC. Left lung ischemia was induced by clamping left pulmonary artery/vein/bronchus at end inspiration. At 4h, 24h, 72h, & 7 days following ischemia, blood was collected from left & right pulmonary veins. **Results:** Mean blood oxygenation (PaO $_2$ /FiO $_2$ ratio, mmHg) was reduced ($P < 0.05$) at 24h post-IR injury in Group B (138 ± 86 ; $n=9$) and Group D (152 ± 51 ; $n=10$), compared to MSC-vIL10 (353 ± 105 ; Group A; $n=10$) treated animals. By days 3 & 7 following IR injury with MSC-vIL10 therapy oxygenation levels approached normal (475 ± 55 & 435 ± 33 ; $n \geq 9$) but empty vector control was low (238 ± 164 & 217 ± 158 ; $n \geq 9$). As early as 4h following IR injury PaO $_2$ /FiO $_2$ was 319 ± 94 in MSC-vIL10 group ($n=7$) compared to untreated controls (63 ± 19 mm Hg; $n=6$). MSC therapy increased PaO $_2$ /FiO $_2$ (275 ± 139 Vs 187 ± 150 in untreated controls, 7 days post-IR; $n \geq 9$). Extravasation of Evans blue dye (as a measure of capillary permeability/damage) was lower ($P < 0.5$) in MSC-vIL10 treated animals. Engineered MSC produced ~ 5 ng/ml vIL-10 *ex vivo*. Bronchoalveolar lavage from IR injured lung at 24h post-MSC-vIL10 therapy demonstrated reduced ($P < 0.05$) inflammatory cells ($\times 10^6$) compared to untreated controls (Neutrophils, 2.8 Vs 5.4; Macrophages, 3.3 Vs 2.9; CD4 T cells, 0.6 Vs 2.2; CD8 T cells, 1.1 Vs 3.1). Inflammatory mediators IL-1a, MCP-1a, MIP-1a, & IL-1b protein levels were markedly increased in the injured lung compared to sham operated controls; their analysis & histopathology analysis in MSC-vIL10 treated & untreated groups are underway. *Ex vivo* expanded MSC were CD34 $^{+}$, CD45 $^{+}$ (5%), CD29 $^{+}$ (92%), CD80 $^{+}$ (10%), CD90 $^{+}$ (>90%), MHC Class I ^{low} & MHC Class II $^{-}$. **Conclusion:** Interleukin-10 delivery via MSC to prevent lung transplant IR injury seems promising.

Abstract# 349

A20 Promotes Hepatocyte Proliferation by Enhancing IL-6 Signaling: A Mean To Improve Success of Small-for-Size Liver Grafts. Cleide G. Da Silva,¹ Haley E. Ramsey,¹ Christopher Longo,¹ Eva Csizmadia,¹ Christiane Ferran.¹ ¹*Surgery and Medicine, BIDMC - Harvard Medical School, Boston, MA.*

Living donor liver transplantation (LDLT) has emerged as a solution to ease organ shortage in orthotopic liver transplantation. However, LDLT is often complicated by small-for-size liver graft that is highly susceptible to injury and shows decreased liver regeneration. Suppression of liver regeneration in small-for-size grafts correlates with impaired priming as a result of limited NF- κ B activation and decreased production of the priming cytokines TNF and IL-6. We have shown that the hepatoprotective protein A20 promotes liver regeneration, partly through blockade of the cyclin dependent kinase inhibitor p21. However the impact of A20 expression in livers on IL-6 production and/or signaling were still unknown.

In this study we demonstrate that secretion of IL-6 (ELISA) following treatment with LPS or TNF was moderately lower in hepatocytes transduced with a recombinant A20 adenovirus (rAd) as compared to non-transduced or rAd. β -galactosidase transduced cells. This indicates that IL-6 production in hepatocytes is not solely NF- κ B dependent (not totally blocked by the NF- κ B inhibitor A20). Despite similar or lower IL-6 levels, IL-6 signaling as evaluated by phosphorylation of STAT3 (Western blot; WB) was enhanced in A20 expressing hepatocytes. This was confirmed in experiments showing that A20 increases STAT-3 phosphorylation in response to exogenous human IL-6. Accordingly, hepatocyte proliferation was significantly higher in rAd.A20 transduced hepatocytes as opposed to controls. The pro-proliferative function of A20 mapped to the 7Zn domain. Since the balance of IL-6 signaling in hepatocytes is finely regulated through a negative feed-back loop provided by the IL-6/STAT-3 dependent induction of suppressor of cytokine signal-3 (SOCS3), we investigated whether A20 affects IL-6 signaling by modulating SOCS3 expression. Our results indicate that A20 indeed decreased IL-6 mediated upregulation of SOCS3 (WB). This later result was confirmed

in vivo. Improved regeneration and survival in A20 treated livers correlated with a substantial decrease in SOCS3 levels before and 24 hours following extended (78%) liver resection in mice. These results suggest that A20 enhances priming of hepatocytes by IL-6 likely through down-regulation of SOCS3. This added to the effect of A20 on p21 would further enhance its pro-proliferative function in hepatocytes to benefit survival and function of small-for-size liver grafts.

Abstract# 350

A Novel siRNA-Based Therapy for Preventing Ischemia-Reperfusion Injury by Coronal Artery Infusion of Donor Organs. Xiufen Zheng,¹ Dong Chen,¹ Xusheng Zhang,¹ Motohiko Suzuki,¹ Norihiko Kubo,¹ Marianne Beduhn,¹ Dameng Lian,¹ Jifu Jianh,¹ Bertha Garcia,¹ Anthony M. Jevnikar,¹ Wei-Ping Min.¹ ¹*University of Western Ontario, London, ON, Canada.*

Background: We have previously demonstrated that silencing inflammatory, apoptosis and complement genes can prevent ischemia-reperfusion (I/R) injury occurring in heart transplantation. However, the method for efficiently delivering siRNA into donor organ has not been established. This study was designed to develop a new method to induce gene silencing by coronal artery infusing with siRNA solution for prevention I/R injury in heart transplantation.

Methods: Multiple siRNAs that specifically target TNF α , Caspase 8, and C5a receptor (C5aR) genes were generated and selected. siRNA protection of donor organs was evaluated in a rat heart transplantation model. Heart grafts from Lewis rats were infused with siRNA solution via coronal artery and preserved in HKT solution at 4 $^{\circ}$ C for 18 hrs, and subsequently transplanted into syngeneic Lewis rats. Cardiac functions were assessed by heart beating rate. Gene expression at mRNA level was determined by qPCR. The I/R injury was assessed by immunohistochemistry.

Results: After donor heart perfusion with siRNA solution, siRNA was found to enter the myocardial cells, indicated by the fluorescence emitted from the dye labeled siRNA. The levels of TNF α , caspase 8 and C5aR genes were significantly up-regulated in the grafts after *ex vivo* preservation for 18 hrs. These up-regulated TNF α , caspase 8, and C5aR genes were significantly knocked down by siRNA infusion. Using a siRNA infused organ as a donor, the graft survival was significantly prolonged in heart transplantation. While siRNA solution-treated heart grafts retained strong heartbeat up to the end point of observation (>10 days), the control grafts lost function within 3 days. In addition, an improved cardiac function was observed in the graft preserved in siRNA solution. The protection of graft by siRNA solution is associated with prevention of I/R injury. siRNA solution-treated organs exhibited almost normal histological structures as well as less neutrophil and lymphocyte infiltration, compared with control solution-treated organs.

Conclusions: This study developed a novel *ex vivo* siRNA delivery system using coronal artery infusion, which can effectively silencing genes in donor hearts and prevent cardiac I/R injury.

Abstract# 351

Inhibition of Nuclear Factor (NF- κ B) by Bcl-xL Gene Transfer Ameliorates Liver Injury Induced by Ischemia/Reperfusion. Bibo Ke,¹ Xiu-Da Shen,¹ Feng Gao,¹ Seiichiro Tsuchihashi,¹ Bo Qiao,¹ Ronald W. Busuttil,¹ Thomas Ritter,² Jerzy W. Kupiec-Weglinski.¹ ¹*The Dumont-UCLA Transplant Center, David Geffen School of Medicine at UCLA, Los Angeles, CA;* ²*Institute of Medical Immunology, Humboldt University, Berlin, Germany.*

Background: Ischemia/reperfusion (I/R) insult is a prime factor leading to liver dysfunction. Apoptosis plays key role in the early graft loss following orthotopic liver transplantation (OLT). Bcl-xL has been showed to exert an anti-apoptotic function both in vitro and in vivo. This study was designed to evaluate potential cytoprotective effects and mechanisms for Bcl-xL in liver I/R injury by Ad-Bcl-xL gene transfer. **Methods:** A mouse model of partial 90 min warm hepatic ischemia followed by 6 h of reperfusion was used. Balb/c wide-type (WT) mice ($n=6$ /gr) were injected with Ad-Bcl-xL or Ad- β -gal reporter gene (2.5×10^9 pfu, i.p. at day -2). Sham control WT mice underwent the same procedure, but without vascular occlusion. Mice were sacrificed after 6 h of reperfusion; liver tissue and blood samples were collected for future analysis. **Results:** Ad-Bcl-xL treated mice showed significantly lower sGOT levels (IU/L), as compared with Ad- β -gal or WT controls (509 ± 361 vs. 2819 ± 706 , and 2212 ± 841 , respectively; $p < 0.005$). These correlated with histologic Suzuki's grading of hepatic I/R injury, with WT/Ad- β -gal controls showing significant edema, sinusoidal congestion/cytoplasmic vacuolization, and severe hepatocellular necrosis. In contrast, WT mice treated with Ad-Bcl-xL revealed minimal sinusoidal congestion without edema/vacuolization or necrosis. Ad-Bcl-xL gene transfer significantly reduced local neutrophil accumulation and apoptosis (3.8 ± 2.9 of TUNEL+ cells in Ad-Bcl-xL vs. 21.5 ± 5.3 and 23.5 ± 6.5 of TUNEL+ cells in WT or Ad- β -gal treated mice; $p < 0.01$). Unlike in controls, intragraft expression of mRNA coding for TNF- α , E-selectin/ICAM-1, and IP-10/MCP-1 remained depressed in the Ad-Bcl-xL group. Ad-Bcl-xL gene transfer markedly depressed the activation of NF- κ B, Caspase-3, and increased HO-1, A20, and Bcl-2/Bcl-xL expression, as compared with WT/Ad- β -gal controls. **Conclusion:** This study demonstrates that inhibition of NF- κ B activation contributes to the cytoprotective effects after Bcl-xL gene transfer in hepatic I/R injury. The induction of anti-oxidant HO-1 and anti-apoptotic A20, Bcl-2 by Bcl-xL gene transfer exerts synergistic cytoprotective effect against antigen-independent hepatic inflammatory injury induced by I/R.

Abstract# 352

Hepatocyte Growth Factor Upregulates A20 in Renal Proximal Tubular Epithelial Cells: A Potential Protective Preconditioning Regimen in Kidney Transplantation. Elizabeth R. Maccariello,^{1,2} Cleide G. Da Silva,¹ Daniel Soizic,¹ Scott M. Damrauer,¹ Lynn Y. Choi,¹ Jeffrey J. Siracuse,¹ Elzbieta Kaczmarek,¹ Christiane Ferran.¹ ¹*Surgery and Medicine, BIDMC, Harvard Medical School, Boston, MA;* ²*Nephrology, Universidade Federal Fluminense, Rio de Janeiro, Brazil.*

Background: Primary graft non-function (PNF) affects survival and function of renal allografts. PNF, secondary to the ischemic and inflammatory injury in the peri-transplant period, leads to acute tubular necrosis and predisposes to acute rejection. Defining new preconditioning regimens to reduce PNF are desirable. A20 is part of a negative anti-inflammatory loop aimed at inhibiting NF- κ B in renal proximal tubular epithelial cells (RPTEC). Hepatocyte growth factor (HGF) is a pleiotropic growth factor upregulated in acute kidney injury and acute rejection likely to modulate inflammation and promote repair. In the present study we evaluated the effect of HGF on RPTEC and hypothesized that some of its protective functions may relate to the upregulation of A20.

Methods and Results: Treatment of RPTEC with HGF (50ng/ml) led to a 2.4 ± 0.8 ($n=4$; $p=0.04$) fold increase in A20 mRNA (real time-PCR), which translated into a significant 3.0 ± 1.5 fold increase ($n=5$; $p=0.04$) in A20 protein by 6h, as shown by Western blot (WB). Two lines of evidence suggested that upregulation of A20 by HGF was NF- κ B-independent. HGF did not degrade I κ B α in RPTEC (WB) nor upregulated the NF- κ B dependent molecule ICAM-1, as shown by flow cytometry analysis (FACS). Further, A20 was still upregulated in RPTEC expressing the NF- κ B inhibitor I κ B α , both at the mRNA and protein levels. Upregulation of A20 by HGF protected RPTEC from a subsequent inflammatory insult, here mimicked by the addition of TNF. Pretreatment of RPTEC with HGF for 6 hours blunted TNF-induced (10U and 25U/ml) upregulation of ICAM-1, as analyzed by FACS.

Conclusion: To our knowledge this is the first demonstration that A20 could be upregulated in RPTEC, in a non-NF- κ B dependent manner. Further studies are carried out to elucidate the transcription factors involved in HGF-induced upregulation of A20. From a clinical standpoint, these results highlight the unique ability of HGF to protect RPTEC from inflammation by inducing the anti-inflammatory protein A20, remarkably without triggering other pro-inflammatory signals. We propose that HGF-based therapies could serve in preconditioning regimens to prevent ischemia/reperfusion injury and reduce PNF and acute rejection in renal transplantation.

Abstract# 353

Protection of Liver Ischemia Reperfusion Injury by Silencing of TNF- α and Complement 3 Genes. Roberto Hernandez-Alejandro,¹ Xusheng Zhang,² Dong Chen,² Xiufen Zheng,² Hongtao Sun,² Weihua Liu,² Marianne Beduhn,² Aminah Shunnar,² Motohiko Suzuki,² Norihiko Kubo,² Bertha Garcia,² Anthony Jevnikar,^{1,2} William Wall,^{1,2} Douglas Quan,^{1,2} Wei-Ping Min.^{1,2} ¹*Multi-Organ Transplant Program, London Health Sciences Centre, London, ON, Canada;* ²*Department of Surgery, Pathology, and Medicine, University of Western Ontario, London, ON, Canada.*

Background. Liver ischemia reperfusion injury (IRI) is one of the main causes of graft dysfunction and rejection in liver transplantation. It has been documented that IRI is associated with inflammatory and complement pathway activation. This study was designed to investigate the efficacy of small interfering RNA expression vector (shRNA) targeting TNF- α and complement 3 (C3) genes in the protection of mouse liver IRI.

Methods. shRNA expression vectors were constructed for TNF- α and C3 genes. Mice received shRNA by hydrodynamic injection prior to IRI, which consisted of interrupting blood supply to the left lateral and median lobes of the liver for 45 minutes followed by reperfusion. IRI was evaluated using liver histopathology, as well as levels of serum alanine transferase (ALT) and aspartate transaminase (AST). Neutrophil accumulation was determined by a myeloperoxidase (MPO) assay. Lipid peroxidation was assessed by malondialdehyde (MDA) levels. Realtime PCR was used to test gene silencing efficacy in vitro and in vivo.

Result. We demonstrated that IRI is associated with an increase in TNF- α and C3 mRNA levels in liver tissue 6 hours after reperfusion. shRNA-treatment effectively down-regulated TNF- α and C3 expression in IRI livers. In comparison with vehicle control, the serum levels of ALT (9843.31 ± 2610.66 U/L vs 1960.00 ± 1361.98 U/L) and AST (7188.25 ± 3295.99 U/L vs 1405.13 ± 774.65 U/L), were significantly reduced in mice treated with TNF- α and C3 shRNA. Additionally, the neutrophil accumulation and lipid peroxidase-mediated tissue injury, detected by MPO and MDA respectively, were improved after shRNA treatment. Tissue histopathology showed an overall reduction of injury area in shRNA-treated mice.

Conclusion. This is the first demonstration that liver IRI can be prevented through gene silencing of inflammatory genes and complement genes, showing potential for shRNA-based clinical therapy.

Abstract# 354

The Natural History of Antibody-Mediated Rejection in Kidney Transplantation: A Prospective Observation Based on Desensitized Recipients. Qiquan Sun,¹ Ronald Pelletier,¹ Patrick Adams,² Tibor Nadasdy,³ Gregg Hadley,¹ Ronald Ferguson.¹ ¹*General Surgery, Ohio State University, Columbus, OH;* ²*Tissue Typing Laboratory, Ohio State University;* ³*Pathology, Ohio State University.*

Background: The low incidence of Antibody-mediated Rejection (AMR) has made it difficult to observe the process from its beginning, so the natural history of AMR remains obscure. Cross-match positive, desensitization recipients have high incidence of early AMR development after renal transplantation.

Methods: With the aim of outlining the natural history of AMR, in this 4 year prospective study, we monitored 38 cases of desensitization recipients (36 cross-match positive and 2 ABO incompatible) with sequential biopsy and donor-specific antibody (DSA) evaluation. Standard desensitization treatment included plasmapheresis combined with IVIG and mycophenolate mofetil. Transplantation was performed after achieving a negative AHG-CDC T cell cross-match. Allograft biopsies were taken on day 0, day 4, day 8, day 11, and 1 year post-transplantation and whenever AMR (Banff 2001 criteria) occurred.

Results: Of 38 recipients, 21 had demonstrable DSA and were C4d+ on biopsy after transplantation, with 15 developing AMR. Seven recipients had DSA and were C4d negative on biopsy, 5 had no DSA and were C4d+ on biopsy, and 5 were negative for both. The main findings are: (1) DSA caused AMR in 54% cases; (2) in the first three days after transplantation, a temporary decrease in DSA was observed in all AMR cases, and all of them quickly rebounded thereafter; (3) C4d can be detected very early (can be seen on day 1, 89% in day 4 protocol biopsies, frequently in the absence of graft dysfunction, and 100% in index biopsies); (4) the pathologic changes observed in sequential biopsies were C4d deposition followed by acute tubular injury, then interstitial inflammation and peritubular capillary margination seen in index biopsies; (5) pure AMR occurred early (100% at day 3), usually evolving into mixed AMR with accompanying cellular rejection (87% in index biopsies); (6) Most recipients (87%) had initial graft function before developing AMR; (7) when graft dysfunction occurred, severe histological damage was already evident.

Conclusion: Post-transplant DSA usually, but not always, causes AMR. Biopsies performed at different stages of AMR development show varying histology. Recognition of the early histologic changes will facilitate timely therapeutic intervention in patients who develop AMR.

Abstract# 355

Long-Term Impact of Antibody-Mediated Rejection in ABO-Incompatible Renal Transplantation. Daisuke Toki,¹ Hideki Ishida,¹ Shimizu Tomokazu,¹ Hiroki Shirakawa,¹ Yutaka Yamaguchi,² Kazunari Tanabe.¹ ¹*Department of Urology, Tokyo Women's Medical University, Tokyo, Japan;* ²*Department of Pathology, Jikei Medical University Kashiwa Hospital, Chiba, Japan.*

Background: Antibody-mediated rejection (AMR) has been recognized as a major problem in ABO-incompatible (ABO-I) renal transplantation (RTX). However, little is known about the long-term impact of AMR in the ABO-I renal transplant setting, especially after the introduction of a tacrolimus (FK)-based immunosuppressive regimen. The aim of this study was to assess the long-term impact of AMR on the clinical and pathological outcomes in ABO-I RTX.

Methods: Fifty-eight patients who underwent ABO-I RTX at our institution between March 1999 and December 2004 under an FK-based immunosuppressive regimen were enrolled in this study. Protocol biopsies were performed regardless of renal function at one month and one year after RTX. Fifty-six of the 58 patients received the biopsy at one month and 43 of 56 patients underwent biopsy at one year posttransplant. AMR was diagnosed by morphological features based on the Banff '05 update and other characteristic findings for AMR previously reported, such as mesangiolysis, interstitial hemorrhage, and cortical infarction. We evaluated graft survival, incidence of chronic rejection characterized by transplant glomerulopathy (TGP) at one year posttransplant, and renal function using serum creatinine at three years posttransplant according to the incidence of AMR at one month posttransplant.

Results: The overall graft survival rate at 3, 5, and 8 years after RTX was 93%, 87%, and 63%, respectively. The incidence of AMR at one month was 34% (19/56). The graft survival rate of the patients with AMR was significantly lower than that of the patients without AMR ($p<0.01$, 3 years: 79% VS 100%, 8 years: 38% VS 94%). The incidence of TGP in the patients with AMR was significantly higher than that of the patients without AMR ($p<0.001$, 57% VS 7%). The serum creatinine concentration at three years after RTX was significantly higher in the patients with AMR than in those without AMR ($p<0.001$, 1.95 mg/dl VS 1.32 mg/dl).

Conclusion: In this study, we revealed that AMR in ABO-I RTX is associated with not only graft loss but also the progression of chronic renal impairment, functionally as well as pathologically, even after the introduction of an FK-based immunosuppressive regimen. Further studies are needed to establish a more effective immunosuppressive regimen, such as rituximab induction therapy; against AMR in ABO-I RTX.

Abstract# 356

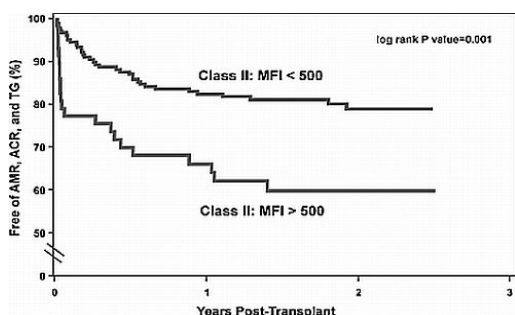
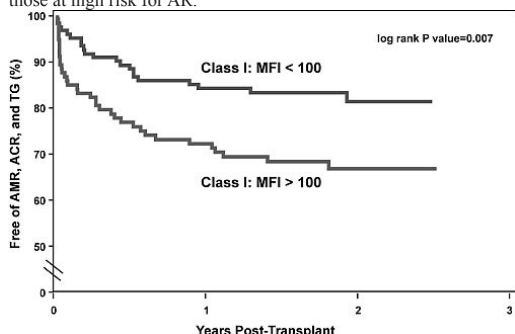
Donor Specific Antibodies (DSA) Detected by Single Antigen Bead Assay (Luminex) as Predictors of Kidney Transplant (KT) Outcomes. N. Singh, J. Pirsch, D. Lorentzen, G. Levenson, B. Voss, J. Torrealba, A. Djamali, J. Pascual, R. Hoffman, Y. Baker, J. Odorico, L. Fernandez, H. Sollinger, M. Samaniego. *University of Wisconsin, Madison, WI.*

Luminex is the most sensitive assay for detection of DSA. To determine if low levels of DSA detected by Luminex correlate with KT outcomes, we studied the association of DSA with graft and patient (Pt) survival, and combined incidence of cellular rejection, antibody-mediated rejection and transplant glomerulopathy (ACR+AMR+TG). Pre-KT sera from DDKT or SPK with a negative (-) NIH crossmatch (Xmatch) performed between 1/05-7/06 (n=237) were retested for DSA using T cell-AHG and Luminex. To investigate if a strength of DSA correlated with outcomes, we set cutoff values of 100, 500 and 1000 max. mean fluorescence intensity (MFI) for each locus (A, B, DR, DRB and DQ), and the sum of MFI values by locus and by class (Class I vs. II). Outcomes were compared in Pts with and without DSA.

Of Pts with (-) NIH Xmatch, 6.7% had a (+) T-cell-AHG, and 89.8% had a (+) DSA by Luminex. There was no difference in outcomes between AHG (-) and AHG (+) Pts.

Pts with class I DSA sum >100 MFI had a higher incidence of ACR+AMR+TG (33.3% vs. 18.8% at 2 yrs, $P=0.007$) than those with class I DSA sum <100 MFI. Pts with class II DSA sum >500 MFI had a higher incidence of combined ACR+AMR+TG (40.2% vs. 21.2% at 2 yrs, $P=0.001$) than those with class II DSA sum <500 MFI (See figures). Both, the sum or a max. MFI of 100 for A, B, DR and DR-B, and 500 for DQ were significantly related to the episodes of acute rejection (AR) and TG.

A DSA MFI of 1000 against DR or DR-B was associated to lower Graft and Pt survival, respectively. T-cell AHG had no incremental value over a (-) NIH Xmatch in predicting outcomes. Pre-KT DSA testing with Luminex in Pts with a (-) NIH Xmatch identifies those at high risk for AR.



Abstract# 357

De Novo Donor Specific Antibodies during Acute Rejection: An Independent Predictor of Graft Loss. M. Everly,¹ J. Everly,¹ P. Brailey,¹ B. Susskind,¹ R. R. Alloway,¹ E. S. Woodle.¹ *U of Cincinnati.*

New methods for measuring anti-HLA antibodies have improved detection of humoral responses in kidney transplant (KTx) patients (pts). Conflicting data exists regarding anti-donor specific HLA antibodies (DSA) as a risk factor for graft loss, therefore we analyzed risk factors for graft loss including de novo DSA detected (by new technologies) at the time of acute rejection (AR).

Methods: KTx pts with AR by biopsy (Bx) between 1/1/2003 and 8/1/2007 were evaluated for DSA [Luminex platform/Labscreen single antigen beads]. All DSA were de novo, ie, not detected before KTx. AR was defined by Bx and Banff '97 criteria (update 2003). Univariate analysis defined variables with a $p<0.25$, which entered the multivariate analysis (MVA). MVA was performed by stepwise logistic regression (STATA/SE v.9.2).

Results: Of 90 pts with AR, 48 (53%) had DSA at diagnosis. Median follow-up was 23.1 ± 18.5 months post AR. Univariate analysis of risk factors for graft loss showed recipient age and gender, donor age and race, transplant (txp) type, AB mismatch, time to bx, AR reversal rate, and recurrent AR to be insignificant. African American (AA) race, delayed graft function (DGF), mean baseline serum creatinine above 2mg/dL,

DSA, and repeat transplants (txp) entered the MVA. In the MVA, DSA and repeat txp were significant. An interaction was noted between DSA and repeat txp (75% of the repeat txps had de novo DSA). Thus, two MVA models were analyzed with Model 1 removing and Model 2 including repeat txp. MVA results: Model 1 – graft loss was 5.5 times more likely in primary txps with de novo DSA ($p=0.047$); Model 2 – repeat txps with de novo DSA were 16 fold increased graft loss risk ($p=0.035$).

Risk Factors for Graft Loss: MVA Results

Variables	Odds Ratio	95% Confidence Interval
AA recipient race	4.8	0.82 – 28
DGF	7.8	0.89 – 68
Repeat transplant recipient	30	2.5 – 360
Baseline Serum Creatinine >2mg/dL	5.7	0.63 – 51
De novo DSA in Primary Txp recipient	5.5	1.02 – 29
De novo DSA in Repeat Txp	16	1.22 – 209

Conclusions: De novo DSA in AR is an independent predictor of graft loss and its degree of influence is comparable to other established risk factors (AA race, DGF, increased baseline creatinine). Additional studies are warranted to: 1) confirm the predictive ability of DSA and 2) determine whether reduction/elimination of DSA will allow improvements in graft survival.

Abstract# 358

High Incidence of Humoral Rejection in Recipients with De Novo Donor-Specific Anti-HLA Antibodies after Living Related Kidney Transplantation. Hideki Ishida,¹ Li Xiaobei,¹ Hiroki Shirakawa,¹ Tomokazu Shimizu,¹ Kiyoshi Setoguchi,¹ Daisuke Toki,¹ Kazuya Omoto,¹ Miyuki Furusawa,¹ Kazunari Tanabe.¹ *Urology, Tokyo Women's Medical University, Tokyo, Japan.*

Objective There are very few detailed reports until date about newly developed anti-HLA antibodies (*de novo* Abs) after living related kidney transplantation (LRKT). We retrospectively investigated the relationship between the *de novo* development of anti-HLA Abs and the outcome of LRKT.

Methods The subjects included 87 patients who received living kidney allografts at our institution between Jan., 2000 and Jul., 2004. Panel reactive antibody assay (Flow-PRA) was performed in all the recipients before and six months after LRKT. Graft biopsies were performed as well within and after six months of the transplantation (Tx). All the data of 87 recipients were collected prospectively during the period of follow-up. Humoral rejection rate, donor specificity, and time of appearance of the *de novo* Abs were retrospectively studied.

Results Among the 87 LRKT recipients, 47 (54%) showed negative/negative results, 15 (17%) showed positive/positive results, 12 (14%) showed positive/negative results, and 13 (15%) showed negative/positive results (*de novo* Abs) in the pre-/post-transplant Flow-PRA analysis. Among the 13 cases with *de novo* Abs, 5 (38%) had donor-specific Abs (DSA) and the remaining 8 (62%) had non-donor specific Abs (NDSA) as determined by LAB single antigen analysis. Four of the five recipients (80%) with DSA showed evidence of both vascular and humoral rejection in the graft biopsies performed within 6 months of the transplantation, while one of the eight recipients (13%) with NDSA showed evidence of cellular rejection during the same period. A 5-year graft survival rate of the recipients with *de novo* Abs was 69%, compared with 96%, 88% and 93% in other groups without *de novo* Abs ($P=0.009$).

Conclusions LRKT recipients with developing *de novo* Abs has much higher incidence of humoral rejection and worse prognosis, especially those with donor-specific *de novo* Abs. Cautious monitoring for the appearance of anti-HLA antibodies should be adopted after transplantation, even in patients without anti-HLA Ab prior to the transplantation.

Abstract# 359

Pre-Transplant (TX) Allo- and Donor-Specific (ALLO/DO) Reactivity Detected by Intracellular Cytokine Flow Cytometry (CFC) Assesses the Degree of Sensitization and Predicts Antibody Mediated Rejection (AMR) in Highly Sensitized Patients (HS). Miekko Toyoda,¹ Andy Pao,¹ Ashley Vo,¹ Marina Lukovsky,¹ Raju Radha,¹ Tetsu Sado,¹ Shili Ge,¹ Ning-Ning Chai,¹ Anna Petrosyan,¹ Edwina Skinner,¹ Stanley C. Jordan.¹ *Transplant Immunology Laboratory and Comprehensive Transplant Center, Cedars-Sinai Medical Center, Los Angeles, CA.*

Background: We have previously reported in a cohort of 15 HS that allo/DO B cell numbers as analyzed by CFC are elevated in many HS, but not normal controls (NC). HS w/ high(+) CFC may likely develop AMR. Here, we report on pre-Tx allo/DO reactivity in a cohort of 55 HS and confirm the utility of this novel assay in the management of HS. **Methods:** Samples obtained in 14 NC and pre-Tx in 55 HS enrolled in the NIH IG03 study were submitted for CFC. Whole blood from HS or NC was incubated w/ irradiated peripheral blood mononuclear cells (PBMCs, stimulator) from DO, 3rd party w/ or w/o ABO compatibility, or from HS, DO or ABO incompatible 3rd party, respectively. IFN γ + cells in CD3- cells were enumerated and results were expressed as a ratio against IFN γ + cells w/o stimulation. A ratio >5 represents high(+) response that might be associated with AMR. **Results:** Significantly more HS had high(+) CFC responses against at least one of 3 stimulators compared to NC (35/55 [64%] vs. 1/14 [7%], $p<0.001$). HS w/ high(+) responses were primarily female (30/41 [73%] vs. 5/14 [36%], $p<0.02$). 0/4 males w/ Tx (0%) showed high(+) response, while 5/10 males w/o

Tx (50%) did (1.5±1.1 vs. 7.3±8.5, p<0.05). No male w/ Tx developed AMR. Many females showed high(+) response regardless of Tx status (5/8 [63%], 13.1±20.0 in Tx vs. 24/33 [74%], 9.2±10.3 in no Tx, NS). However, all 3 females w/ AMR showed extremely high(+) response (25.1±6.2 vs. 6.2±6.2 in 5 females w/o AMR, p<0.03). Despite significantly higher response than the 4 males w/o AMR (p<0.03), the other 5 females did not experience AMR. Conclusions: 1) CFC is a novel assay to measure allo/DO CD3- cell responses, assess the degree of sensitization, and predict AMR in HS, 2) Allo/DO CD3- cell numbers are elevated in many HS, but not NC, 3) HS w/ high(+) CFC are at increased risk for AMR and may need additional pre-Tx desensitization, 4) Allo/DO reactivity are higher in HS females, which may explain their higher rate of AMR, 5) CFC cut off levels for AMR prediction may be higher in females than males, 6) Monitoring HS using the CFC pre- and post- desensitization may help determine the efficacy of desensitization and risk for AMR.

Abstract# 360

Pre-Transplant Anti-Endothelial Cell Antibody Levels Are Not Predictive of Acute Antibody Mediated Rejection in Renal Allograft Recipients. Robert W. Chang,¹ Alice Peng,¹ Rafael Villicana,¹ Ashley A. Vo,¹ Anna Petrosyan,¹ Nancy Reinsmoen,¹ Stanley C. Jordan,¹ Miekio Toyoda.¹ ¹Comprehensive Transplant Center, Cedars-Sinai Medical Center, Los Angeles, CA.

Background: Anti-endothelial cell antibodies (AECAs) have a reported association with antibody mediated rejection (AMR) and graft loss, although specific targets are not defined. AMR is generally due to antibodies (Abs) directed at HLA or ABO antigens. However, non-ABO and non-HLA Abs such as AECAs may induce AMR. Correlations between AECAs and AMR in cardiac allograft recipients, and a higher proportion of highly sensitized (HS) patients (Pts) with AECA positivity compared to non-HS Pts, have also been noted. Here, we examine the relationship between the presence of pre-transplant (Tx) AECAs and acute cellular rejection (CMR) or AMR in both HS and non-HS renal-Tx Pts.

Methods: Pre-Tx AECAs were measured by cellular ELISA and graded as (-), (+), or (++) in 258 adult kidney ABO compatible-Txs, 114 HS and 144 non-HS, from 2004-2007. These results were correlated with CMR or AMR episodes.

Results: 35/114 (31%) and 14/155 (10%) of the HS and non-HS Pts, respectively, exhibited AECA positivity (p<0.001). Of the HS Pts, nine (8%) had AMR and 18 (16%) had CMR. No correlation between AECA levels and AMR was noted. AMR developed in 10% and 11% of Pts with (-) and (++) AECA levels, respectively. CMR was seen in 17%, 12%, and 11% of Pts with (-), (+), or (++) AECA levels, respectively. Of the non-HS Pts, 4 (3%) p=0.06 vs. HS) and 26 (18%) had AMR and CMR, respectively. Again, no correlation between AECA levels and AMR or CMR was noted. A subgroup analysis of Pts with pre-existing systemic vasculitis (SV) where AECA positivity has been described showed 1/15 (7%) and 4/15 (27%) of HS Pts with SV had AMR and CMR, respectively. AECA levels were not significantly elevated in either HS or non-HS Pts with SV. No correlation between AECA levels and AMR or CMR was noted in this subgroup analysis.

Conclusion: Although significantly more HS Pts exhibit AECA positivity, this does not correlate with subsequent development of AMR or CMR. The rates of CMR are similar in both HS and non-HS Pts, while the rates of AMR are higher in HS Pts (8%) vs. non-HS Pts (3%). HS Pts with SV exhibit higher rates of both CMR and AMR compared with non-HS Pts with SV regardless of AECA status. Pts in this subgroup may benefit from closer monitoring. Overall, pre-Tx AECA monitoring does not offer significant benefits in predicting AMR or CMR episodes.

Abstract# 361

Focal vs. Diffuse C4d Staining in Acute Antibody-Mediated Rejection (AMR) of the Kidney Allograft: Is There Any Difference in Outcomes? J. Pascual, J. Torrealba, A. Djamali, G. Leverson, N. Singh, J. Odorico, S. Knechtel, H. Sollinger, J. Pirsch, M. Samaniego. *University of Wisconsin, Madison, WI.*

The clinical significance of **focal** deposition of C4d in peritubular capillaries (PTC) in patients (Pts) with kidney transplant (KT) dysfunction and light microscopic features of AMR is not well known. As some studies have reported a lack of impact on graft survival (GS), Pts with KT dysfunction and **focal** C4d staining are not treated for AMR as opposed to those with **diffuse** staining. We performed a retrospective analysis to study the impact of **focal** C4d deposition in Pts with KT dysfunction and light microscopic features of AMR according to the Banff 2005 schema.

From 2002-2005, 829 adult KT alone were performed at our institution. Alemtuzumab induction was used in 613 Pts (602 had a functional KT at discharge). In addition to MMF and steroids, immunosuppression was CNI-based in 481 Pts [CsA (n=294) or TAC (n=187)] or CNI-free in 121 Pts.

C4d staining of <25% of PTC was diagnosed as **focal**, while C4d staining in >25% of PTC was diagnosed as **diffuse**. During 2.5 yrs of mean follow-up, C4d-positive AMR was diagnosed in 128 Pts (21.2%), 58 early (before day 90th, E-AMR) and 70 late (after day 90th, L-AMR). Among the 58 cases of E-AMR, 49 (84%) had **diffuse** C4d staining and 9 were **focal** (16%) while among the L-AMR cases, 54 (77%) were **diffuse** and 16 (23%) were **focal**. Cases with **focal** C4d staining were not treated for AMR, and only Pts with **diffuse** C4d deposition received AMR specific protocols. E-AMR cases

were treated with plasmapheresis, IVIg and rituximab, and Pts with L-AMR received IVIg and rituximab.

The 2-year GS post-AMR in Pts with E-AMR and **focal** C4d staining was 33% vs. 50% in Pts with **diffuse** staining; while cases of L-AMR with **focal** C4d deposition had a GS of 55% vs. 60% in cases with L-AMR and **diffuse** staining. The number of cases with **focal** staining was low, and the numerically evident differences were not statistically significant (log-rank p=NS).

Notably, when losses due to death with a functional graft were censored, post-AMR GS was significantly lower in Pts with E-AMR and **focal** staining than in their counterparts with **diffuse** C4d deposition (41% vs. 67%, log-rank p=0.04).

In this retrospective single center study, **focally positive** C4d AMR carries a worse prognosis than previously thought, and causes a significant reduction of GS. Whether any degree of C4d staining in the context of KT dysfunction should be treated as AMR remains a pending question.

Abstract# 362

Association between C4d Staining in Kidney Transplant (KT) Biopsies and Donor Specific Antibody (DSA). Boonsong Kiangkitiwan,¹ Cynthia Drachenberg,² Matthew Cooper,³ Marcela Riveros-Angel,² Debra Kukuruga,² Heather Hurley,³ Marcy Kennedy,² David Klassen,¹ Abdolreza Haririan.¹ ¹Depts of Medicine; ²Pathology; ³Surgery, University of Maryland, Baltimore, MD.

We are increasingly understanding the role of Ab-mediated injury after KT. However, the available tools that help to recognize this injury are not optimal. We sought to evaluate the correlation between the presence of PTC C4d staining in biopsies with immunohistochemistry and the results of simultaneous anti-HLA DSA testing using single antigen microbead assay. We identified 297 cases with 400 biopsy/DSA samples. Patient and transplant-related characteristics are summarized in table 1.

Patient and transplant-related characteristics			
Age (yr)	50.4±14.3	P-PRA	16.7±30.5
Male (%)	68.6	C-PRA	9.1±22.9
AA (%)	48.4	α-CD25/ATG/α-CD52 (%)	41/36/23
Deceased donor (%)	78.1	Cr at Bx (mg/dl)	4.1±2.6
HLA-MM	4.0±1.7	DSA+ (%)	33.3

Time of biopsy was 29.1±43.0 mo after KT. However, 172 cases were biopsied in the 1st year posttransplant. The extent of C4d staining was graded as <10% (0), 10-50% (1), and ≥50% (2) of PTC, and the intensity was graded as none (0), light (1), and strong (2) staining. Table 2 shows the percent of biopsies with positive DSA for each grade for all biopsies and those performed during 1st year.

Percent DSA positivity with C4d staining for all biopsies and those performed during 1st year			
% PTC grade	DSA (all/1-yr)	Class I (all/1-yr)	Class II (all/1-yr)
0	26.0/22.0	15.7/16.3	18.8/13.8
1	41.5/26.9	14.6/11.5	36.6/23.1
2	72.7/65.2	60.6/56.5	54.5/43.5

These findings demonstrate the significant discordance between detection of DSA and C4d, which is a relatively specific histological evidence of Ab-mediated injury. This factor should be taken into account when clinical decisions for treatment of patients with either C4d or DSA positivity are made. The observed discrepancy could be partially due to the technique used for staining of the biopsy specimens, inability to detect anti-HLA DSA with the available technology, or non-HLA DSA. Long term follow up data are needed to evaluate the impact of these markers on graft outcomes.

Kidney: Chronic Rejection

Abstract# 363

Early Expression of Epithelial to Mesenchymal Transition Markers Is Predictive of Long-Term Renal Graft Function. Alexandre Hertig,¹ Dany Anglicheau,² Jerome Verine,³ Maxime Touzot,¹ Laurent Mesnard,¹ Nicole Brousse,⁴ Denis Glotz,⁵ Christophe Legendre,² Eric Rondeau,¹ Yi-Chun Xu-Dubois.¹ ¹Urgences Nephrologiques et Transplantation Renale, APHP, Hopital Tenon, Paris, France; ²Transplantation Renale, APHP, Hopital Necker, Paris, France; ³Anatomo-Pathologie, APHP, Hopital Saint Louis, Paris, France; ⁴Anatomo-Pathologie, APHP, Hopital Necker, Paris, France; ⁵Transplantation Renale, APHP, Hopital Saint Louis, Paris, France.

Introduction Epithelial to mesenchymal transition (EMT) is a potential mechanism of tissue fibrogenesis. In a previous study, we had reported that the early expression of EMT markers was associated with the progression of renal grafts towards interstitial fibrosis and tubular atrophy (IF/TA). Here, we report the long-term follow-up of this cohort, paying a special attention to the evolution of graft function.

Patients and Methods 83 patients engrafted with a kidney from a cadaveric (n=63) or a living (n=20) donor, and in whom sequential protocol biopsies had been performed at 3 and 12 months, were included. The phenotype of epithelial cells was studied at three months according to the expression of vimentin (an intermediate filament normally expressed by fibroblast-like cells) and to the cellular localization of β-catenin. Grafts in which these two markers were abnormally expressed by more than 10% of tubular cells were considered as EMT+ grafts. Serum creatinine and creatinine clearance (estimated

by Gault and Cockcroft index) were collected from 12 to 24 months post-transplant and compared according to the EMT status of the graft.

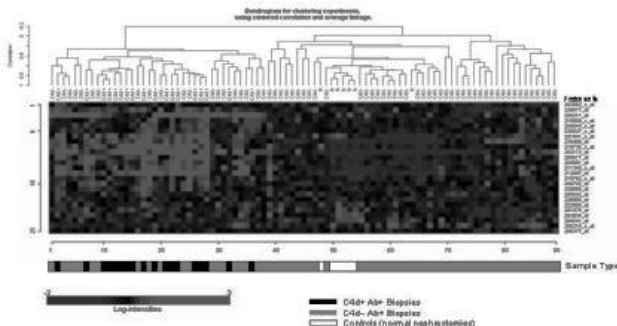
Results Multivariate analysis demonstrated that the early expression of EMT markers was an independent risk factor of the progression of graft fibrosis between 3 and 12 months. More importantly, these early phenotypic changes were associated with a progressive and sustained deterioration of the graft function: EMT+ patients had a statistically higher serum creatinine from twelve months after transplantation, and a significantly lower creatinine clearance from 18 months after transplantation (EMT+ 49.4±4.5 ml/min vs EMT- 61.1±2.3 ml/min, $p=0.01$). The difference was persistent at 24 months.

Conclusion The expression of EMT markers by tubular epithelial cells at an early time point post-transplant (three months) is highly suggestive of an ongoing fibrogenic process, and has repercussions on the long-term graft function. Therefore, these epithelial phenotypic changes are relevant and promising biomarkers for an early detection of IF/TA.

Abstract# 364

Microarrays Detect Deteriorating C4d(-) Human Renal Allografts with Ongoing Antibody-Mediated Injury. Banu Sis,¹ Kara Allanach,¹ Sakarn Bunnag,¹ Thomas F. F. Mueller,¹ Philip F. Halloran.¹ ¹*Medicine, University of Alberta, Edmonton, AB, Canada.*

We recently reported that 73% of transplant glomerulopathy (TG) has evidence of alloantibody-mediated injury in biopsies for cause. We found that 1/3 of TG is C4d+Ab+ and 1/3 is C4d-Ab+ suggesting that C4d staining is not sensitive enough to detect all biopsies with antibody-mediated rejection (ABMR) which are missed by C4d. Using Affymetrix microarrays, we analyzed gene expression in 173 renal allograft biopsies for cause. We previously reported that both ABMR and T cell-mediated rejection (TCMR) biopsies show increased expression of transcript sets associated with cytotoxic-T cells (CATs) and gamma-interferon effects (GRITs) compared to biopsies without rejection ($p<0.05$). However, ABMR biopsies were discriminated by a selective increased expression of 25 "endothelial cell-associated transcripts" (ENDATs). These genes included established endothelial markers such as VWF, PECAM1, SELE, CD34, and cadherin 5, which are involved in endothelial-cell activation. Hierarchical clustering of 82 biopsies with Ab+ using ENDATs identified a group of C4d-Ab+ biopsies (n=20) clustered with C4d+ Ab+ biopsies (n=18).



Thus 25% of biopsies with antibody (20 of 82) had increased ENDAT-scores despite being negative for C4d. These C4d-Ab+ biopsies with high ENDATs, had higher scores for CATs and GRITs, increased incidence/severity of TG, tubular atrophy/interstitial fibrosis, and worse future graft function ($p<0.05$), but similar incidence of TCMR or borderline lesions, in comparison to C4d-Ab+ biopsies with no increase in ENDATs. The C4d-Ab+ cases with endothelial activation show extensive inflammation in the allograft, as measured by the gene sets, which is similar to C4d+ ABMR. There are a significant number of cases with alloantibody and no C4d that show increased expression of endothelial genes. Thus the transcriptomics detects deteriorating C4d- allografts with ongoing alloantibody mediated injury. We conclude that increased expression of endothelial genes provides a new feature of ABMR, and can be used as a new diagnostic test to detect and treat C4d- ABMR.

Abstract# 365

Probabilistic (Bayesian) Modeling of Gene Expression in Transplant Glomerulopathy. Jason S. Hawksworth,¹ Trevor S. Brown,² David B. Leiser,¹ Alexander Stojadinovic,¹ Doug K. Tadaki,² Orlena Cheng,³ Roz B. Mannon,³ Eric A. Elster.^{2,4} ¹*Surgery, Walter Reed Army Medical Center, Washington, DC;* ²*Regenerative Medicine, Naval Medical Research Center, Silver Spring, MD;* ³*NIDDK, National Institute of Health, Bethesda, MD;* ⁴*Surgery, National Naval Medical Center, Bethesda, MD.*

Introduction: Transplant glomerulopathy (TG) is a distinctive lesion identified on allograft biopsy associated with rapid decline in glomerular filtration rate and poor outcome. Recent evidence increasingly supports an immunologic pathogenesis of TG, however the molecular pathways involved have not been elucidated. Furthermore,

use of gene expression data to reliably predict TG has not been previously published.

Methods: Retrospective review of 963 renal transplant core biopsies from 166 patients identified TG in 20 biopsies from 18 (10.8%) patients. A cohort of patients (n=32) with stable function (SF) allografts were studied for comparison. TG was graded by the current Banff classification and 44/52 samples were stained for C4d. The biopsies were analyzed for transcript expression of 87 genes with quantitative RT-PCR. Data was evaluated using Bayesian modeling software, and dependence relationships between the transcript expression and TG were established. Next, a probabilistic Bayesian model was designed and validated to predict histopathology based on gene expression signatures. **Results:** The mean grade (±SD) of TG was 2.65 ±0.49. Peritubular capillary C4d deposition was identified in 15/18 (83.3%) of the biopsies with TG and 8/26 (30.8%) with SF ($p<0.001$). The Bayesian network model was analyzed and, interestingly, CD86 was critically related to TG, suggesting a B-cell mediated process. TG was also predicted by upregulation of CCL2, CCL3, CCL5, CXCL9, IL-8, IL-10, and ICAM gene expression. Ten percent of the samples were excluded randomly from the initial model, and subsequently used for cross validation. In the validation analysis, the model effectively predicted TG (AUC of 0.92, 90% PPV) and SF (AUC of 0.866, 83% PPV). **Conclusions:** This study provides a compelling and clinically relevant example of the combination of quantitative gene expression with probabilistic Bayesian modeling to predict renal allograft pathology. Potentially important molecular pathways associated with transplant glomerulopathy were also identified. The application of this integrated approach has broad implications in the field of transplant diagnostics and interpretation of large data sets.

Abstract# 366

Impact of Pre-Transplant Donor Specific Antibodies (DSA) Detected by Single Antigen Bead Assay (Luminex) on Transplant Glomerulopathy (TG). N. Singh, J. Pirsch, D. Lorentzen, G. Levenson, J. Torrealba, A. Djmal, J. Pascual, R. Hoffman, Y. Becker, J. Odorico, L. Fernandez, H. Sollinger, M. Samaniego. *University of Wisconsin, Madison, WI.*

Transplant Glomerulopathy (TG) is a prominent feature of chronic rejection characterized by double contours of the glomerular capillaries, and is associated with poor kidney transplant (KT) survival. Although TG can develop in the absence of HLA sensitization, anti-HLA antibodies are known risk factors for TG (Sis B et al, AJT 2007). The aim of this study was to evaluate the correlation of TG with DSA detected by Luminex in pre-KT sera.

Pre-KT sera samples from simultaneous pancreas and kidney (SPK) and deceased donor KT recipients transplanted between 1/2005-7/2006 (n=237) were retested for DSA using Luminex. To determine the strength of DSA that correlated with the finding of TG in indication biopsy specimens, we established cutoff values of 100 and 500 mean fluorescence intensity (MFI).

Patients (pts) were followed for a median period of 1.84 yrs (range 0.05-2.49 yrs). During this interval, 89 Pts had indication biopsies performed. TG was identified in 8 of these Pts, and 6 out of 8 (75 %) had positive C4d staining. Of the 237 Pts, 159 were found to have pre-KT DSA >100 MFI. 43 pts had class I and 116 pts had class II DSA. Class II DSA but not class I DSA were found to be significantly related to TG (see table). Among class II DSA, only locus DR and DR-B 3, 4 and 5 were found to be significantly related to TG.

We conclude that KT recipients with pre-KT Class II DSA detected by Luminex, especially those against loci DR and DR-B, are at high risk to develop TG and hence need close post-KT monitoring.

DSA	MFI	TG	P-VALUE
CLASS I	> 100	6.7%	P = 0.11
	< 100	1.7%	
	> 500	5.4%	P = 0.65
	< 500	3.8%	
CLASS II	> 100	7.6%	P = 0.02
	< 100	0.9%	
	> 500	9.6%	P = 0.008
	< 500	2.3%	

Abstract# 367

Five-Year Outcome with Tacrolimus (TAC) and Mycophenolate Mofetil (MMF) Versus TAC and Sirolimus (SLR) without Chronic Steroid Therapy Monitored by Surveillance Biopsy in Kidney Transplantation. Mysore S. Anil Kumar,¹ Muhammad I. Saeed,¹ Alexander E. Poor,¹ Nedjema Sustento-Reodica,² Gregory E. Malat,¹ Karthik Ranganna,¹ Arjun M. S. Kumar.¹ ¹*Surgery/Division of Transplantation, Drexel University/Hahnemann University Hospital, Philadelphia, PA;* ²*Pathology, Drexel University/Hahnemann University Hospital, Philadelphia, PA.*

This prospective study compared 5-year outcome in 150 kidney recipients without chronic steroid therapy transplanted between October 2001 and June 2004; 75 patients each in TAC/MMF and TAC/SLR groups. Serial surveillance biopsies were performed to evaluate sub clinical acute rejection (SCAR) and chronic allograft injury (CAI). Patient demography was comparable. Biopsy proven clinical acute rejection was 12%vs.8%, 3%vs.3%, 1%vs.1%, 1%vs.0% and 2%vs.0% in TAC/MMF vs. TAC/SLR groups at 1, 2, 3, 4 and 5 years respectively. SCAR was 17%vs.17%, 8%vs.6%, 15%vs.4%, 10%vs.5%, 5%vs.4%, 4%vs.4% and 2% vs.2% at 1, 6, 12, 24, 36, 48 and 60 months respectively.

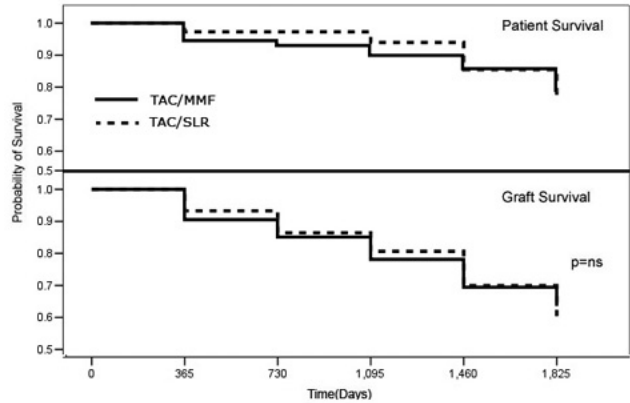
KIDNEY: CHRONIC REJECTION

The daily dose and blood levels of TAC were significantly lower in TAC/SLR group compared to TAC/MMF group. Table shows CAI in 2 groups at 5 years.

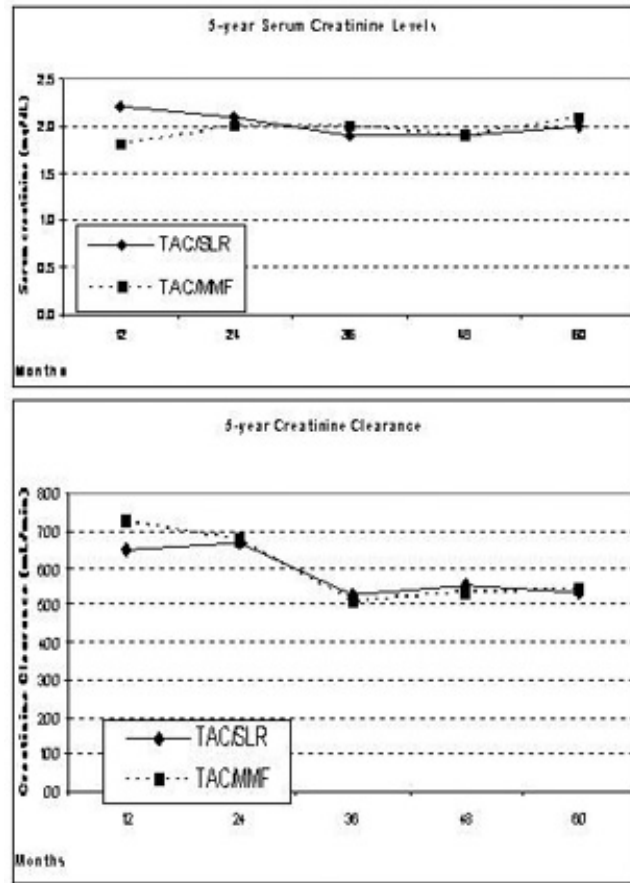
CAI at 5 years in TAC/MMF and TAC/SLR groups

Cumulative findings	TAC/MMF	TAC/SLR	p value
CAI:hypertension	45%	32%	0.05
CAI:calcineurin toxicity	21%	9%	0.02
CAI:viral infections	4%	3%	1.0
CAI:obstruction	4%	4%	1.0
CAI:pyelonephritis	4%	5%	0.99
CAI:Chronic active antibody mediated rejection	8%	9%	0.99
CAI:Chronic active T-cell mediated rejection	11%	13%	0.9
CAI:interstitial fibrosis/ tubular atrophy	55%	21%	0.002
Recurrent disease	3%	3%	1.0

Patient and graft survival is shown.



Renal function is shown.



Despite lower prevalence of CAI in TAC/SLR group long-term graft function and patient and graft survival are comparable between TAC/MMF and TAC/SLR groups.

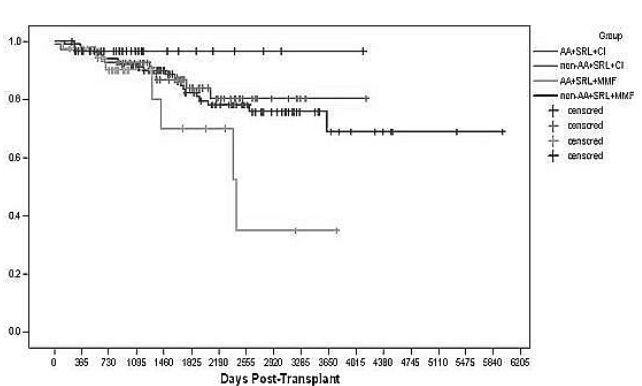
Abstract# 368

Differences in Kidney Allograft Outcomes between Ethnicities When Converting to Sirolimus Based Immunosuppression. N. Patel,¹ D. J. Taber,¹ N. A. Weimert,¹ J. N. Fleming,¹ F. M. Egidi,² H. Ayiku,² J. McGillicuddy,³ C. F. Bratton,³ A. Lin,³ P. K. Baliga,³ K. D. Chavin.¹ *Dept of Pharmacy; ²Division of Nephrology; ³Division of Transplant, MUSC, Charleston, SC.*

Objective: The aim of this study was to determine if ethnicity impacts graft outcomes in kidney transplant patients converted to sirolimus (SRL) and either maintained on calcineurin inhibitors (CI) or mycophenolate (MMF) with steroids. **Methods:** This was a retrospective analysis of all kidney transplants converted to SRL and transplanted from 7/91 to 4/07. Patients were divided into 4 groups: Group 1: AAs converted to SRL + continued on CI; group 2: non-AAs converted to SRL + continued on CI; group 3: AAs converted to SRL + continued on MMF; group 4: non-AAs converted to SRL + continued on MMF. Pediatrics and multiorgan transplants were excluded. **Results:** A total of 257 patients were included (61% AA). Demographics, baseline immunosuppression, and reason for SRL conversion were similar between groups. Table 1 displays characteristics and outcomes. Patients converted to SRL+CI regimens had higher rates of acute rejection before SRL conversion (p<0.02), but equal rates after conversion. Development of proteinuria was similar across groups. Figure 1 displays the graft survival rates for each group. AA patients converted to SRL+MMF tended to have poorer outcomes compared to AA patients converted to SRL+CI. Non-AA patients converted to SRL+MMF tended to have better graft outcomes compared to non-AA patients converted to SRL+CI, although this did not reach statistical significance (p=0.186). **Conclusion:** AAs converted to SRL may benefit from continued CI, while non-AAs converted to SRL appear to have better outcomes with MMF. Further prospective studies are warranted to confirm these findings.

Characteristics	AA SRL+CI (n=113)	AA SRL+MMF (n=42)	non-AA SRL+CI (n=70)	non-AA SRL+MMF (n=32)	p-Value within Ethnicity
Living Donor	8%	2%	34%	38%	NS
Re-Transplant	14%	12%	16%	3%	NS
Delayed Graft Fxn	18%	21%	10%	15%	NS
SCR at SRL Conversion	2.48	2.14	2.08	2.19	NS
Acute Rej before SRL	43%	21%	40%	9%	<0.02
Acute Rej after SRL	7%	7%	6%	3%	NS
Proteinuria > 500 mg/day	29%	36%	34%	22%	NS

Kaplan-Meier Analysis for Graft Survival



Abstract# 369

Correlation of Allograft Failure in Recipients of Kidneys from the Same Deceased Donor. Daniel Louvar,¹ Bertram Kasiske,² Na Li,³ Jon Snyder,⁴ Yi Peng,⁴ Ajay Israni.² *¹Renal, U. of MN, Minneapolis, MN; ²Nephrology, Hennepin County Medical Center, Minneapolis, MN; ³Biostatistics, U. of MN, Minneapolis, MN; ⁴Minneapolis Medical Research Foundation, Minneapolis, MN.*

There are no large registry studies evaluating the correlation of allograft failure for recipients of kidneys from the same deceased donor. We examined outcomes in such recipient pairs using data from the United States Renal Data System.

Methods: We studied the correlation of graft failure events within 19,461 pairs of same-donor recipients transplanted during 1995 through 2003. Analyses were limited to patients with functioning grafts 3 months post-transplant (tx) and adjusted for known donor, recipient, and tx management factors. We estimated odds ratios to measure the increased risk for 1, 2, and 3-year graft failure and death-censored graft failure when the contralateral kidney had such an event. We also evaluated the effect of recipient pairs transplanted at the same center vs different centers.

Results: There is a strong correlation in outcomes for 2 recipients with the same donor (table). The correlation was stronger within pairs transplanted at the same center than for those transplanted at different centers. Differences in the correlation of graft failures within pairs transplanted at the same versus separate centers diminished over 2 and were absent by 3 years post-tx. Results for death-censored allograft failure were similar.

Conclusion: Unmeasured donor factors contribute significantly to the correlated graft failure outcomes in paired recipients of deceased donor kidneys and need further study.

Kidney tx outcomes in the first year may be affected by differences in management between transplant centers, more so than in subsequent years post-tx.

	Death-censored graft failure	Graft failure
Year post-tx	Odds Ratio ¹ (95%CI)	Odds Ratio ¹ (95%CI)
Y1 S	3.46 (2.06-5.82)	2.57 (1.85-3.50)
Y1 D	2.58 (1.54-4.33)	1.83 (1.35-2.42)
Y2 S	2.25 (1.59-3.18)	1.84 (1.50-2.25)
Y2 D	2.02 (1.47-2.76)	1.68 (1.41-1.98)
Y3 S	1.79 (1.36-2.36)	1.62 (1.37-1.91)
Y3 D	1.85 (1.45-2.36)	1.59 (1.39-1.82)

¹Odds ratio of death-censored graft failure and graft failure in recipients of a donor pair, given that the outcome occurred in the recipient of the contralateral kidney, with both recipients being transplanted at the same (S) center or at different (D) centers. All odds ratios significant with $p < 0.001$.

Abstract# 370

A Cross-Sectional Description of the Pathologic Features of Troubled Kidney Transplants: The DeKAF Study. Sita Gourishankar,¹ D. Rush,⁴

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The Deterioration of Kidney Allograft Function (DeKAF) study is a NIH-funded multicenter observational study of late allograft (ktx) loss. The study examines two cohorts: a "long-term cohort" (LTC) of prevalent ktx with SCr < 2.0 mg/dL with deterioration of function (25% increase in SCr or proteinuria) and a "prospective cohort" (PC) of incident ktx developing a persistent >25% increase in SCr. We examined the pathologic features of the first renal biopsy (bx) obtained for new onset deterioration in each cohort. All bx were read and scored centrally using a modified Banff schema.

	PC (n=46;%)	LTC (n=110;%)
time from transplant to biopsy (months)	5.8	74.9
SCr at biopsy	2.41	2.65
Proteinuria	19.23	54.17
CI≥2	13.04	33.64
CI≥2	8.70	32.73
I≥2	28.26	30.00
T≥2	36.96	30.91
tubular atrophy >25%	28.89	34.54
tubulitis >25%	47.82	49.09
CV≥2	10.87	15.46
AH≥2	4.35	30
PTC inflamm ≥2	5.46	15.26
PTC PMN≥2	5.46	18.64
CG>0	4.35	27.27

On average, bx were obtained at 6 (PC) and 75 (LTC) months post-tx. Mean SCr was similar, but more patients (pts) in LTC had proteinuria. Moderate to severe interstitial fibrosis and tubular atrophy (TA) were more prevalent in LTC than PC (33 vs 13% ci score ≥2; 33 vs 9% for TA). The rate of vascular sclerosis >25% was similar (10.87% PC vs 15.46% LTC); however, hyaline arteriolar sclerosis >25% was more common in the LTC (30 vs 4.35%). Interstitial inflammation (i) and tubulitis (t) scores were similar in both cohorts. However, more pts in LTC had peritubular capillary infiltrates (>5 cells) and evidence of tx glomerulopathy.

While rates of interstitial inflammation and tubulitis sufficient to warrant diagnosis of acute rejection are similar in the prospective and long-term cohorts, long term cohort pts had more proteinuria, interstitial fibrosis, tubular atrophy, hyaline arteriolar sclerosis, and transplant glomerulopathy. Analyses of histologic findings and renal outcome are ongoing.

Abstract# 371

Immunohistochemical Characterization of Glomerular (G) and Tubulointerstitial (TI) Infiltrate in Renal Transplant Patients (Pts) with Chronic Allograft Dysfunction (CAD). M. Rossini, C. Divella, A. Loverre, A. Schena, A. Maiorano, V. Gesualdo, G. Zaza, G. Grandaliano, F. P. Schena.

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The term chronic allograft nephropathy (CAN) has been abolished by the last Banff Meeting Report (Am J Transplant, 2007) and 2 categories have been introduced for chronic changes: chronic active T cell-mediated rejection and chronic active humoral rejection (CAHR). Aim of the study was to review all cases of CAN diagnosed in the last 4 years and to identify immunohistochemical markers of chronic rejection.

A cohort of 79 CAD pts with biopsy-proven CAN was analyzed. Each case was reviewed and assigned into 3 groups according to Banff 2005 criteria: chronic rejection (CR), chronic calcineurin toxicity (CNIT) or chronic lesions not otherwise specified (NOS). CD4+, CD8+, CD20+, CD68+ cells and C4d deposits were assessed by immunohistochemistry.

Twenty-eight pts were classified as CNIT, 34 as CR, 19 of which were CAHR, and 17 as NOS. Serum creatinine and 24h proteinuria at renal biopsy, extent of interstitial fibrosis and glomerulosclerosis were not significantly different among 3 groups (Table1). The number of CD4+ cells was higher at TI level in CR compared to CNIT (Table1; *p=.05).

CD8+ cells were higher at TI and G level in CR compared to CNIT (Table1; *p=.05). TI and G CD20+ cells were not different among the 3 groups (Table1). The number of G CD68+ cells was increased in CR compared to CNIT and NOS (Table1; *p=.05). No significant difference in CD20, CD4, CD8, CD68 expression was found at TI and G level between C4d+ and C4d- cases of CR. CD68, CD8, CD4 but not CD20 expression at TI level correlated with TI fibrosis ($R^2 = .105, .077, .156$, respectively, $p < .05$) at the univariate analysis. Only TI CD4+ cells independently correlated with fibrosis at multiple regression analysis.

In conclusion, our data suggest that: 1. infiltrating CD8+ cells may differentiate CR from CNIT; 2. infiltrating cell characterization might improve diagnostic/prognostic features of graft biopsy in CAD.

	CR	CNIT	NOS
SCr(mg/dl)	2.7±1.4	2.5±1.1	2.1±.8
24hprot(g/24h)	2.1±2.7	2.1±6.2	1.5±1.5
TI Fibr(%)	37±18.7	33±12.7	33.2±28.6
TICD4(cell/hpf)	15.5±13.9*	10.2±6.2*	11.4±12.1
GCD4(cell/glom)	5±1.4	3±.5	4±.9
TICD8(cell/hpf)	31.02±18.7*	19.6±12.9*	24.6±17.7
GCD8(cell/glom)	1.05±1.5*	2±.3*	6±1.1
TICD20(cell/hpf)	9.7±10.8	8.6±9	7.9±9.7
GCD20(cell/glom)	1±.3	1±.2	0.3±.07
TICD68(cell/hpf)	15.5±13.9	10.2±6.2	11.4±12.1
GCD68(cell/glom)	1.7±1.9*(**)	1±1.3*	4±.5**

Kidney: Complications IV

Abstract# 372

Is Gastric Bypass a Viable Strategy for Weight Reduction among Kidney Transplant Candidates and Recipients? Krista L. Lentine,¹ Paolo R. Salvalaggio,¹ Huiling Xiao,¹ Mark A. Schnitzler,¹ Ralph J. Graff,¹ Daniel C. Brennan,² Kevin C. Abbott,³ Kian Modanlou.¹

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Morbid obesity limits access to kidney transplantation and predicts adverse transplant outcomes. There are limited data on the safety and efficacy of gastric bypass (GB) as a weight reduction therapy among transplant candidates and recipients.

Methods: We examined USRDS registry data to identify Medicare-insured kidney transplant candidates and recipients with billing claims for GB procedures. GB were categorized according to occurrence before listing, on the waitlist, or after transplant. We studied the clinical characteristics of GB-treated patients, and subsequent outcomes including progression from listing to transplant and 30-day mortality. USRDS surveys BMI data at dialysis start, waitlist entry, transplant, and transplant anniversaries. We computed changes between most recently reported body mass index (BMI) values preceding and following GB, when available.

Results: We identified 72 transplant candidates treated with GB before listing, 29 who underwent GB on the waitlist, and 87 GB cases after transplant. Patients treated with GB were most commonly female, white race, and without diabetic or hypertensive renal failure (Table). 30-day mortality after GB, calculable for listed and transplanted patients, was 3.4% and 3.4%, respectively. 1 transplant recipient experienced graft failure within 30 days of GB. Of 29 patients treated with GB on the waitlist, 20 proceeded to transplant.

Post-GB weight loss was detected for 60% with GB pre-listing, 70% with GB on the waitlist, and 90.7% with GB after transplant. Among patients listed for transplant in the same era and BMI >35 at first dialysis who were not treated with GB, 22% had lost weight between dialysis start and listing.

(Figure on Page 278)

A. Characterization of Kidney Transplant Candidates & Recipients who underwent Gastric Bypass (1991-2004)

Characteristic	Before Listing (N= 72)	On the Waitlist (N= 29)	Post Transplant (N= 87)
	n (%)	n (%)	n (%)
Age at GB (yrs)	42.3 ± 10.8	47.0 ± 11.1	45.2 ± 11.3
Female sex	46 (63.9)	17 (58.6)	52 (59.8)
Race			
White	41 (56.9)	18 (62.1)	67 (77.0)
Black	29 (40.3)	9 (31.0)	20 (23.0)
Other	2 (2.8)	2 (6.9)	0 (0)
Cause of ESRD			
Diabetes	21 (29.2)	9 (31.0)	27 (31.0)
Hypertension	12 (16.7)	6 (20.7)	9 (10.3)
Other	39 (54.1)	14 (48.3)	51 (58.7)
Time (months)			
GB to Listing	16.3 ± 13.0	—	—
GB to TX (if TX)	—	15.6 ± 11.0	—
Transplant to GB	—	—	4.3 ± 2.6
Mortality			
Within 30d of GB	—*	1 (3.4)	3 (3.4)
Within 60d of GB	—*	0 (0)	1 (1.1)

TX, transplant. Continuous variables expressed as mean ± SD

*Sampled from Waitlist, and thus known to survive to Listing

B. Reported BMI data among Kidney Transplant Candidates & Recipients who underwent Gastric Bypass[†]

BMI	Before Listing (N= 29)	On the Waitlist (N= 10)	Post Transplant (N= 43)
	Mean ± SD	Mean ± SD	Mean ± SD
Most recent pre-GB	38.1 ± 12.4	38.0 ± 9.0	45.9 ± 7.1
Most recent post-GB	35.2 ± 6.3	33.2 ± 7.8	39.5 ± 7.9
Change in BMI			
Mean ± SD	-2.8 ± 12.2	-3.0 ± 11.6	-6.4 ± 5.1
Median, IQR [‡]	-6.6(-12.3, 8.8)	-2.5(-13.0, 0)	-7.0(-10.6, -1.6)
% with BMI decrease after GB	18 (62.1)	7 (70.0)	39.0 (90.7)

[†]Limited to patients with BMI data at reporting time preceding and following GB

[‡]IQR, inter-quartile range

Conclusions: GB has been performed in small numbers of kidney transplant candidates and recipients, and is followed by weight-loss in the majority of cases. Peri-operative mortality is comparable to reports in patients without kidney disease. GB warrants prospective study as a strategy for reducing complications of obesity in ESRD.

Abstract# 373

Clinical and Genetic Risk Factors for Posttransplant Diabetes Mellitus in Adult Renal Transplant Recipients Treated with Tacrolimus and Mycophenolate Mofetil. Shigeru Satoh,¹ Kazuyuki Numakura,¹ Mitsuru Saito,¹ Takamitsu Inoue,¹ Kazuyuki Inoue,² Masatomo Miura,² Hideaki Kagaya,² Hiroshi Tsuruta,¹ Takashi Obara,¹ Teruaki Kumazawa,¹ Takeshi Yuasa,¹ Norihiko Tsuchiya,¹ Toshio Suzuki,² Tomonori Habuchi.¹ ¹Urology, Akita University School of Medicine, Akita, Japan; ²Pharmacy, Akita University Hospital, Akita, Japan.

INTRODUCTION: The present study investigated the incidence of posttransplant diabetes mellitus (PTDM) and calculated the risk of developing PTDM under a tacrolimus and mycophenolate mofetil (MMF)-based immunosuppression based on clinical characteristics, tacrolimus-pharmacokinetics, and genetic polymorphisms related to tacrolimus-pharmacokinetics, cytokines and diabetes mellitus.

METHODS: Seventy-one non-diabetic adult kidney recipients (male 37, female 34) were studied. Patients with continuous high plasma glucose levels, over 6.5mg/dl of hemoglobin A1c, or requiring insulin and/or oral anti-diabetic agents for more than 3 months after transplantation at 1-year after transplantation were diagnosed as having PTDM. Fifteen genomic polymorphisms were assessed.

RESULTS: One year after transplantation, 21 recipients (29.6%) developed PTDM. Positive risk factors were age (p=0.028) and body mass index (p=0.048). There were no significant differences in acute rejection rate, total steroid doses, tacrolimus-pharmacokinetics or its related to genetic polymorphisms between the two groups. The frequencies of PTDM were significantly higher in patients with adiponectin T45G TT genotype than in those with the G allele (p=0.034), and in patients with glucocorticoid receptor (NR3C1) Bcl I CC genotype than in those with the G allele (p=0.004).

CONCLUSIONS: The incidence of PTDM at 1-yr after transplantation was 29.3% in our cohort. Elder or obese patients were risky for the development of PTDM. The presence of the adiponectin T45G TT or NR3C1 Bcl I CC genotype may also be risk factors for PTDM, suggesting that insulin and glucocorticoid sensitivity-related genes are associated with the development of PTDM. Analysis of these genotypes is a possible method of predicting a patient's risk for developing PTDM and would be a valuable asset in selecting appropriate immunosuppressive regimens for individuals.

Abstract# 374

Pharmacokinetics and Pharmacodynamics of Cinacalcet in Patients with Hyperparathyroidism after Renal Transplantation. Andreas L. Serra,¹ Natascia Corti,² Stefan Russmann,² Simone C. Braun,¹ Rudolf P. Wuthrich.¹ ¹Clinic for Nephrology, University Hospital, Zurich, Switzerland; ²Division of Clinical Pharmacology and Toxicology, University Hospital, Zurich, Switzerland.

Persistent hyperparathyroidism (HPT) with hypercalcemia is common after renal transplantation. Studies have shown that treatment with cinacalcet corrects hypercalcemia and lowers PTH levels in these patients. So far cinacalcet's steady-state pharmacokinetics and their correlation with pharmacodynamics (PK/PD) have only been studied in hemodialysis patients, but not in renal transplant recipients with persistent HPT. To gain further insight into cinacalcet's effects on calcium-phosphate homeostasis, we determined its steady-state pharmacokinetics and pharmacodynamic effects in these patients.

In a prospective, single center, open label study we examined the effect of a 2-week treatment with 30 mg and subsequent 2-week treatment with 60 mg cinacalcet daily on calcium-phosphate homeostasis over 24 hours and determined the steady-state pharmacokinetics of cinacalcet in stable renal allograft recipients. The urinary calcium excretion was determined in timed urine samples.

Median AUC₀₋₂₄ was 784.8 ng*h/ml and C_{max} was 68.5 ng/ml for 60 mg cinacalcet which is higher, and oral clearance (CL/F) was 76.9 l/h which is lower in renal transplant recipients compared to previously published data of hemodialysis patients (50 mg cinacalcet AUC₀₋₂₄ 179, C_{max} 17.2, CL/F 279). We also observed a non-proportional increase of AUC₀₋₂₄ after doubling of the cinacalcet dose. The once daily administration of cinacalcet dose-dependently reduced iPTH and serum calcium. Cinacalcet and parathyroid hormone (PTH) concentrations showed an inverse correlation and were fitted to a simple Emax model (E_{max}=80 % reduction vs. baseline, EC₅₀=13 ng/ml). The 8-hour fractional urinary excretion of calcium was increased after 60 mg cinacalcet (baseline 0.85±0.17 %, 30 mg 1.53±0.35 %, 60 mg 1.92±0.37 %). Renal function remained stable.

Cinacalcet's higher and non-proportional increase of AUC₀₋₂₄ in transplant recipients compared to hemodialysis patients evokes the possibility of a pharmacokinetic interaction with concomitant cyclosporine treatment. Cinacalcet effectively corrected the biochemical abnormalities of persistent HPT. The transient calciuria could potentially favor nephrocalcinosis and reduce bone mineral density, suggesting that higher doses of cinacalcet need to be used with caution in renal transplant recipients with severe persistent hyperparathyroidism.

Abstract# 375

Screening for Proteinuria in the Kidney Transplant Clinic. Bryce A. Kiberd,¹ Romuald Panek.¹ ¹Dalhousie University, Halifax, NS, Canada.

Proteinuria is a predictor of progression in kidney disease. It is not clear whether measuring albuminuria will have greater clinical utility over measurement by dipstick or total proteinuria in kidney transplant recipients. There has also been a trend away from using 24 hour collections to using spot urine albumin/creatinine (AC) and protein/creatinine (PC) ratios. We compare the prevalence of proteinuria estimated by dipstick, AC and PC in prevalent patients (>6 months post transplant) in the kidney transplant clinic.

Significant albuminuria defined as ≥30 mg/g was present in 52% (211/403). Albuminuria was seen in 29% (70/245) with negative and 67% (34/51) with trace dipstick proteinuria. Significant predictors of albuminuria in a multivariate logistic analysis were eGFR (OR 0.977 per ml/min/1.73m², 95% CI 0.965-0.989 p=0.001), diastolic BP (OR 1.028 per mmHg, 95% CI 1.008-1.048 p=0.007), and MMF use (OR 0.50, 95% CI 0.32-0.77 p=0.002). Macroalbuminuria (AC>299 mg/g) was seen in 17.4% (70/403) and significant predictors in a multivariate logistic analysis were lower eGFR and higher systolic BP. Sirolimus use was associated with more macroalbuminuria and MMF use with less macroalbuminuria.

In a subset of patients followed for >2 years prior GFR loss was considerably greater (p=0.021) in patients with albuminuria (-0.88 ml/min/1.73m²/year) compared to those without (-0.15 ml/min/1.73m²/year). However other measures of proteinuria were also significantly (p for trend) associated with prior GFR loss (ml/min/1.73m²/year) as shown in the Table.

Mean GFR loss/year by Proteinuria Grade				
Dipstick (n)	Grade 1 Neg (n=227)	Grade 2 Trace/0.3g/L (n=100)	Grade 3 > 0.3g/L (n=47)	Prob.
Δ eGFR/year	-0.36	-0.56	-1.38	0.054
AC mg/g (n)	<30 (n=176)	30-299 (n=133)	>300 (n=64)	
Δ eGFR/year	-0.15	-0.84	-0.98	0.029
PC g/g (n)	<0.13 (n=185)	0.13-0.49 (n=109)	>0.50 (n=80)	
Δ eGFR/year	-0.33	-0.26	-1.40	0.02

*A PC cut point of 0.129 g/g had a sensitivity and specificity for albuminuria > 30 mg/g of 87% and 88% respectively (c=0.92, 95% CI 0.89-0.95), and a PC cut point of 0.49 g/g had a sensitivity and specificity for macroalbuminuria >300 mg/g of 100% and 94% respectively (c=0.98, 95% CI 0.97-0.99).

AC may be more sensitive and therefore have more clinical utility than other measures of proteinuria for progression. However prospective follow up of renal function change and CV outcomes is required.

Abstract# 376

Detection of Changes in Glomerular Filtration Rate in Renal Transplant Recipients: Serum Creatinine Versus Serum Cystatin C. Christine A. White,¹ Ayub Akbari,² Steve Doucette,³ Dean Fergusson,³ Nasser Hussain,² Laurent Dinh,² Guido Filler,⁴ Nathalie LePage,⁵ Greg Knoll.² ¹Department of Medicine, Queen's University, Kingston, ON, Canada; ²Department of Medicine, University of Ottawa, Ottawa, ON, Canada; ³Clinical Epidemiology Program, Ottawa Health Research Institute, Ottawa, ON, Canada; ⁴Department of Pediatrics, University of Western Ontario, London, ON, Canada; ⁵Department of Laboratory Medicine, Children's Hospital of Eastern Ontario, Ottawa, ON, Canada.

Serum creatinine is a crude marker of GFR in renal transplant recipients and changes in GFR are frequently not accompanied by commensurate changes in serum creatinine concentration. Serum cystatin C and estimates of GFR (eGFR) based on cystatin C have been shown to be more accurate than serum creatinine and creatinine-based eGFR in renal transplant recipients. The purpose of this study was to determine whether the Filler, Lebricon and Rule cystatin C-based eGFR equations were better able to detect changes in true GFR than the MDRD and Cockcroft Gault creatinine-based eGFR equations. We performed two measures of ^{99m}Tc-DTPA GFR, serum creatinine and serum cystatin C on each of 183 stable renal transplant recipients at least 6 months apart. We calculated and compared the percent annual change in the measured GFR and the estimated GFR using the various GFR estimation equations. We also determined the sensitivity, specificity, positive predictive value and negative predictive value of each prediction equation for the detection of decline in measured GFR. Results are presented below:

	Mean annual % change	Sensitivity to detect fall in GFR (%)	Specificity to detect fall in GFR (%)	PPV to detect fall in GFR (%)	NPV to detect fall in GFR (%)
DTPA GFR	-1.9 ± 45.0				
Filler eGFR	4.9 ± 46.5	57	72	74	54
Lebricon eGFR	3.7 ± 37.1	57	72	74	54
Rule eGFR	5.2 ± 48.4	57	72	74	54
Cockcroft Gault eGFR	-2.1 ± 28.0	62	58	69	53
Abbreviated MDRD eGFR	-1.1 ± 31.1	59	60	68	52

The cystatin C and creatinine-based eGFR equations all demonstrated poor sensitivity and diagnostic performance to detect a decline in GFR. Novel equations derived and validated in the transplant population are needed to accurately assess kidney function over time.

Abstract# 377

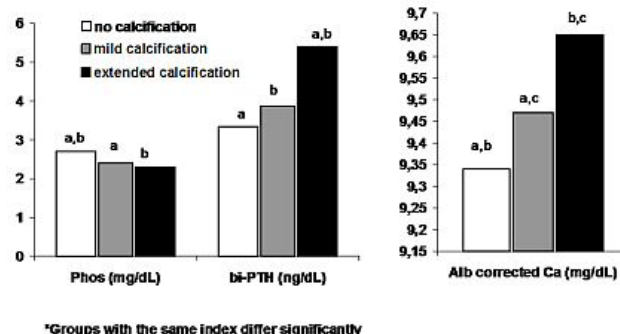
Microcalcifications in the Renal Graft Are Highly Prevalent in the Early Posttransplant Period and Are Related to a Disturbed Mineral Metabolism. Pieter Evenepoel,¹ Maarten Naesens,¹ Evelyne Lerut,¹ Hylke De Jonge,¹ Bert Bammens,¹ Kathleen Claes,¹ Boudewijn Van Damme,¹ Yves Vanrenterghem,¹ Dirk Kuypers.¹ ¹University Hospitals Leuven, Leuven, Belgium.

Background: Hypercalcemia, hypophosphatemia and renal phosphate wasting are common after kidney transplantation and are related to persistent hyperparathyroidism and hyperphosphatoninism. Animal data suggest that these alterations in mineral metabolism may contribute to nephrocalcinosis and progressive graft dysfunction. Supporting clinical data are limited.

Aim: To test the hypothesis that nephrocalcinosis is highly prevalent in the early posttransplant period and is related to a disturbed mineral metabolism.

Methods: Biomarkers of mineral metabolism (including albumin-corrected serum calcium [Ca_a], serum phosphorus [P], bioactive PTH, calcidiol, calcitriol and alkaline phosphatase) and renal calcium and phosphorus excretion parameters were prospectively assessed in 201 renal transplant recipients (62% male, mean age 55 ± 14 yrs) at the time of their 3-month protocol biopsy. These protocol biopsies were screened for the presence of microcalcifications. Intratubular, interstitial and/or cytoplasmic microcalcifications were observed in 30.4% of biopsies. Calcifications were more prevalent in recipients of a living related donor as compared to cadaveric donor. High serum Ca_a levels, high serum PTH levels, a high urinary Ca×P product and high fractional excretion of P and low serum P levels were significantly associated with renal microcalcifications (see figure below). Microcalcifications were not related to the fractional excretion of Ca, use of diuretics, immunosuppressive regimen, serum alkaline phosphatase level and history of delayed graft function. The extent of microcalcifications correlated significantly with the severity of mineral metabolism disturbances.

Conclusion: Our data demonstrate that nephrocalcinosis is highly prevalent in the early posttransplant period and suggest that a disordered mineral metabolism is implicated in its pathogenesis.



Abstract# 378

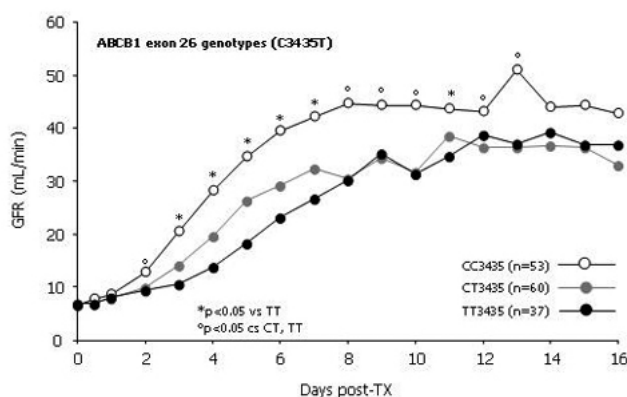
Polymorphism in ABCB1, the Gene Encoding for P-Glycoprotein, Predicts Recovery of Graft Function Early after Kidney Transplantation. Dario Cattaneo,¹ Sara Baldelli,¹ Alessandra Bitto,¹ Norberto Perico,¹ Eliana Gotti,¹ Silvio Sandrini,² Maurizio Salvadori,³ Paolo Rigotti,⁴ Giuseppe Remuzzi.¹ ¹Mario Negri Institute for Pharmacological Research, Bergamo, Italy; ²Spedali Civili, Brescia, Italy; ³Azienda Ospedaliera Careggi, Florence, Italy; ⁴Ospedale Giustiniano, Padua, Italy.

The pharmacokinetics of cyclosporine (CsA) is characterized by wide inter-individual variability. This might be particularly relevant in the early post-transplant period, due to the detrimental effects of the drug on the kidney. P-glycoprotein (P-gp), the product of the ABCB1 gene, plays a key role in the distribution of CsA at cellular level. Single nucleotide polymorphisms (SNPs) of ABCB1 might potentially influence the response of patients to CsA. In particular, the SNP in position 3435 of the exon 26, despite its silent nature, has been recently associated with altered specificity for its ligand, such as CsA (Kimchi-Sarfaty et al, Science 2007, 315:525). Whether P-gp pharmacogenetics would help to guide CsA treatment early post-transplant remains ill defined.

We sought to evaluate the effects of the SNPs in the exon 26 on the rate of recovery of graft function, as estimated GFR early postoperatively, in 150 kidney transplant patients given CsA as part of their immunosuppressive regimen. The frequency of DGF (as need for post-operative dialysis) among the different ABCB1 genotypes was also estimated.

Of the 150 kidney transplant recipients, 35% had the ABCB1 wild type (C/C) genotype in exon 26, 40% were heterozygous (C/T) and 25% were homozygous (T/T) for the polymorphic variant in position 3435. GFR values were significantly lower in patients carrying one of the two mutant alleles than in the wild type (Figure). The frequency of DGF was 15%, 28% and 30% in patients with the CC, CT and TT genotypes, respectively.

These findings demonstrate that in patients carrying the CT or TT mutant alleles in exon 26 of the ABCB1 gene and given CsA, the recovery of graft function is less prompt, and the risk to develop DGF higher than in wild-type CC genotype. Pre-transplant screening for ABCB1 polymorphism would help to identify patients who may safely receive CsA early post kidney transplantation.



Abstract# 379

Cigarette Smoking Deteriorates Renal Allograft Function. Patricia Delgado,¹ Esteban Porrini,¹ Alejandra Alvarez,¹ Luis Hortal,² Dolores Checa,³ Roberto Gallego,² Javier Garcia,⁴ Adelaida Morales,⁵ Marian Cobo,¹ Lourdes Perez,¹ Domingo Hernandez,¹ Eduardo Salido,¹ Armando Torres.¹
¹Research and Nephrology Section, Hospital Universitario de Canarias, Tenerife, Spain; ²Hospital U. Doctor Negrin, Gran Canaria; ³Hospital U. Insular, Gran Canaria; ⁴Hospital U. NS Virgen de La Candelaria, Tenerife; ⁵Hospital General de Lanzarote.

Cigarette smoking has shown to reduce graft and patient survival in renal transplant patients. However, whether it could directly produce allograft dysfunction has not been investigated. The aim of this study was to assess the smoking influence on renal graft function.

We studied a cohort of 827 adult renal transplant patients, transplanted from Jan/96 to Dec/05 and followed until Dec/06. Smoking habits were recorded at the time of transplant (never, former, current smoker). During summer of 2007, a telephonic survey allowed us to obtain complete information about smoking habits in 642 patients (aged 47.6 ± 13, 65% male): status (never, former, current), years of habit, years of quit, and number of cigarette smoked per day. Number of "pack-years" was calculated. Renal function was measured by inverse serum creatinine at 3rd month and then annually. Time to decline a thirty percent in inverse serum creatinine was registered. Patients were divided in two groups: those who always smoked during all transplant period (smokers, n=94) and those who did not (n=546).

Results: Surveyed and not surveyed patients were comparable. Smokers were younger than non smokers (43.8 ± 11.2 vs 48.3 ± 13.2, P=0.001), without differences in donor age, sex, dialysis duration, cold ischemia time, % diabetics, pretransplant hypertension, pretransplant LVH and cardiovascular events. Kaplan-Meier survival curves, censoring at time of death, graft loss or at the end of follow-up, showed that smokers presented a shorter period to achieve a 30% decline in inverse serum creatinine (log rank 5.737, P=0.017), and that patients who had quit smoking at the time of transplantation showed a K-M curve similar to those who had never smoked. By multivariate Cox regression analysis, smoking during transplant (HR 1.98, CI95% 1.338-2.947, P=0.001), acute rejection (HR 1.52, CI95% 1.064-2.195, P=0.022) and systolic blood pressure above 140 mmHg at first year (HR 1.48, CI95% 1.054-2.099, P=0.024) were risk factors for allograft dysfunction. Conclusion: Cigarette smoking during renal transplantation is an important and modifiable contributor to early graft dysfunction.

Abstract# 380

Kidney Damage According to Histological Findings after Extrarenal Transplantation. Anke Schwarz,¹ Hermann Haller,¹ Michael Mengel,² Verena Broecker,² ¹Nephrology, Hannover Medical School, Hannover, Germany; ²Pathology, Hannover Medical School, Hannover, Germany.

Renal insufficiency occurs frequently after extrarenal transplantation as a result of acute tubular necrosis at transplantation, high blood pressure, and CNi toxicity. We performed 56 renal biopsies in 54 patients after heart (4), lung (20), liver (22), bone marrow (9), and cornea (1) transplantation since 2000. The time from transplantation to biopsy was 49±53 months in general and was longest after liver (60±61 months) and shortest after bone marrow transplantation (37±43 months). The histologic changes were: tubular atrophy/interstitial fibrosis of ≥20% in 52% of biopsies (heart biopsies 75%, lung 70%, liver 46%, bone marrow 22%); acute tubular changes in 54% (heart 25%, lung 65%, liver 46%, bone marrow 56%); arteriolar hyaline sclerosis in 41% (heart 75%, lung 65%, liver 23%, bone marrow 22%); arteriosclerosis in 41% (heart 50%, lung 50%, liver 36%, bone marrow 22%); glomerular sclerosis of ≥20% in 21% (heart 50%, lung 25%, liver 18%, bone marrow 22%); glomerulonephritis in 13% (heart 25%, liver 18%, bone marrow 22%); that means IgA-nephropathy after heart and liver, immune complex nephritis twice after liver, MPGN after liver, and membranous glomerulonephritis and minimal changes after bone marrow transplantation; thrombotic microangiopathy in 7% (lung 10%, liver 5%, bone marrow 11%); finally one case of polyoma nephritis after lung transplantation. Among 1702 heart and lung transplantations, 13 patients needed kidney transplantation (0.8%) after 96±40 months; and among 2221 liver transplantations, 11 needed kidney transplantation (0.5%) after 112±65 months (sign. later, p=001). Conclusion: As expected, most histologic changes were those of CNi toxicity and hypertension. Surprising is the high number of glomerulonephritis under immunosuppression. Thrombotic microangiopathy without the typical clinical signs were interpreted as CNi-related toxicity and seems to occur more often after extrarenal than after renal transplantation. Patients with heart and lung transplantation reach end-stage renal failure more often and earlier than patients with liver transplantation.

Kidney: Living Donor Issues II

Abstract# 381

The Effect of State and National Legislation on Living Kidney Donation Rates in the United States: A National Study. L. Ebony Boulware,¹ Misty U. Troll,¹ Neil R. Powe.¹ ¹Department of Medicine, Johns Hopkins School of Medicine, Baltimore, MD.

Context: Living kidney transplantation, a superior therapy to deceased donor kidney transplantation, is underutilized. States have enacted legislation and the federal

government has launched initiatives to compensate living organ donors, but the effect of policy on improving living kidney donation rates in the United States is unknown.

Objective: To determine whether public policies are associated with changes in living kidney donation rates in the continental U.S.

Design, setting, and study subjects: Series of cross-sectional analyses using records of state legislatures in 48 continental states and living kidney donation rates from the United Network for Organ Sharing.

Main Outcome Measures: Living kidney donation rate during each year from 1988-2006 and change in donation rates before and after legislation enactment in each state and launch of federal initiatives.

Results: From January 1990 through December 2005, 28 states enacted legislation for living donors (24 mandating paid leave, 8 tax deductions, 3 unpaid leave, 2 encouraging paid leave). Few states (n=5) enacted legislation prior to 1999. There was a steady increase in the mean living kidney donation rate in the continental U.S. during the study period (mean (standard deviation) annual increase in donations 1.5 (0.04) donations per 1,000,000 population). In analyses accounting for length of time state legislation had been enacted, the types of legislation enacted, and the incidence and prevalence of ESRD in each state, there was a slightly (but not statistically significantly) greater average annual increase in donations after compared to before state legislation enactment (annual increase in donations per 1,000,000 population [95% confidence interval (CI)] 1.85 [1.40-2.30] compared to 1.62 [1.20-2.03] respectively, p>0.05). In similar analyses among states not enacting their own legislation, there was a slightly (but not statistically significantly) greater average annual increase in donations after compared to before the launch of federal initiatives in 1997 (annual increase in donations per 1,000,000 population [95% CI] 1.31 [0.98-1.74] compared to 1.0 [0.53-1.46] respectively, p>0.05), but federal initiatives had no effect on donation rates for states enacting legislation.

Conclusion: Existing state and federal policies may serve to support living donors but have not substantially improved donation behaviors.

Abstract# 382

Novel Model To Estimate GFR in Living Kidney Donors Using Renal Volumes Measured by 3-D CT Scans. Emilio D. Poggio,¹ Nidhi Sharma,² Michael Leiber,³ Maxime Freire,² Brian R. Herts,² ¹Nephrology, Cleveland Clinic; ²Radiology, Cleveland Clinic; ³Quantitative Health Sciences, Cleveland Clinic.

Introduction: Accurate and precise renal function assessment is essential in the evaluation of prospective kidney donors. While direct measurement of GFR is the "gold standard", it is not widely available. Moreover, creatinine (SCr)-based estimation equations are suboptimal to assess kidney function in this setting. CT scans are increasingly being used to study renovascular anatomy in donors and has replaced angiographic exams in many institutions. 3D imaging reconstruction allows for kidney volumes (KV) measurements which have been shown to highly correlate with measured GFR in this population. Thus, the purpose of this study was to develop a model to estimate measured GFR that not only incorporates SCr and demographic data but also KV as measured by 3D CT scans.

Methods: 244 individuals who underwent donor evaluation were identified. An automated segmentation algorithm was used to measure renal parenchymal volume from preoperative abdominal CTs. Patient demographics and SCr values were obtained from the medical records. GFR (normalized for BSA) was measured by I¹²⁵ iothalamate renal clearances (iGFR). An analysis of covariance model was created to correlate measured iGFR with KV, patient age, sex, race, weight, height and SCr. Pearson's correlation coefficient was calculated for each variable.

Results: KV (p<0.001), age (p<0.001), SCr (p<0.001) and weight (p<0.001) significantly correlated with iGFR. Sex (0.60), race (0.90) and height (0.76) were not statistically significant. The new fitted regression model is: KV-eGFR (ml/min/1.73 m²) = 70.77 - (0.444*age) + (0.366*weight) + (0.200*volume) - (37.317*SCr). We then compared the performance of the KV-eGFR model to the re-expressed MDRD equation using calibrated SCr assay. The R² was 0.61 vs 0.50, respectively; signed median % difference was +1.8% vs -12.7%, respectively (% difference b/w estimated GFR and iGFR); and accuracy within 30% (% of estimated GFR values that fall within 30% of iGFR) of 96.3% vs 89.8%, respectively. Finally, the KV-eGFR model was closer to iGFR (in absolute values) than the MDRD eq. in 177/244 (70.5%) cases vs 72/244 (29.5%) cases, respectively.

Conclusions: Kidney volumes highly correlate with iGFR and the proposed GFR estimation model outperforms the MDRD equation in potential living kidney donors. The KV-eGFR model could be used to estimate donor GFR in lieu of I¹²⁵ iothalamate GFR which is less clinically available.

Abstract# 383

Time 0-Renal Biopsy in Living Kidney Donor: A Valuable Opportunity To Correlate Predonation Clinical Data with Histological Features.

Eduardo Mancilla,¹ Roxana M. Rodriguez,² Salvador Magana,¹ Mario Vilatoba,² Bernardo Gabilondo,² Salvador Aburto,¹ Luis E. Morales,² Carmen Avila-Casado,¹ Norma Uribe,² Francisco Rodriguez,¹ Fernando Magana,¹ Josefina Alberu,² ¹Nephrology, INCIC, Mexico City, Mexico; ²Transplant, INCMNSZ, Mexico City, Mexico.

Living kidney donation is rapidly increasing worldwide to offer a partial (?) solution for the numerous ESRD wait-listed pts. In spite of properly followed guideline criteria

for donor selection, current reports identify unsuspected renal pathology by time 0-biopsy. **Aim:** To explore whether the findings at time 0-renal biopsy (bx) correlates with pre-donation clinical data including renal function. **Methods:** KT databases from 2 institutions were reviewed. Time 0-renal bx are routinely performed from the upper pole during back-table and evaluated by 2 nephropathologist for interstitial fibrosis (IF), tubular atrophy (TA), arteriolar hyalinosis (AH), mesangial increase (MI), and glomerulosclerosis (GS). Pre-donation data gathered from the donors were demography, body weight, BMI, systolic/diastolic BP, SCr, proteinuria, and eGFR by Levey equation. **Results:** 231 time 0-bx were performed; 219/231 (94.8%) were adequate for analysis; 100 (45.6%) were normal. Findings for the remaining 119 bx are shown in the table.

Table 1. N=119 cases

Findings	N	%
IF	64	53.7
TA	29	24.3
MI	26	21.8
AH	23	19.3
GS	23	19.3

Clinical data is summarized in the table.

Table 2

Age	35.4±10
Gender F/M	127 (56.7%)
Weight (Kg)	66.27±10.14
BMI	25.53±2.99
SBP (mmHg)	115.46±9.2
DBP (mmHg)	73.5±6.7
SCr (mg/dl)	0.91±0.25
Proteinuria (mg/24h)	70.25±62.8
eGFR (ml/min)	96±16.65

On bivariate analysis, IF was significantly correlated to age ($r=0.22$, $p=.001$) and SCr ($r=0.19$, $p=.005$); TA to diastolic BP ($r=0.15$, $p=.03$) and proteinuria ($r=0.20$, $p=.009$); AH to SCr ($r=0.15$, $p=.02$) and eGFR ($r=-0.16$, $p=.018$); MI to BMI ($r=0.13$, $p=.047$); and GS showed no correlation. Multivariate analysis failed to sustain the significant associations found on bivariate analysis, most likely due to a low event/parameter relation. **Conclusions:** A significant correlation was observed between time 0-bx findings and clinical pre-donation parameters. Whether these histological findings at the time of kidney donation represent a higher burden/risk for the remaining kidney ought to be evaluated during follow-up. In an era where living donation is increasing, we should advise a closer surveillance of these donors in order to modify risk factors that participate in kidney damage progression.

Abstract# 384

Predictors of Poor Early Graft Function Following Laparoscopic Donor Nephrectomy (LDN). Matthew Cooper,¹ Abdolreza Haririan,¹ Stephen Jacobs,¹ Michael Phelan,¹ Benjamin Philosophie,¹ Stephen Bartlett,¹ Joseph Nogueira,¹ ¹Dept of Surgery, Urology, and Medicine, University of Maryland, Baltimore, MD.

LDN has become the standard of care in many transplant centers. Poor early graft function remains an important complication. We conducted a retrospective study to evaluate the risk factors for slow or delayed graft function following LDN

METHODS: Donor and recipient records from the first 1000 LDN were reviewed (1996-2005).

RESULTS: Slow graft function (SGF) was defined as $Cr \geq 3.0$ mg/dl at POD5 and DGF as the need for dialysis within the first week following transplantation. Donor variables examined included age, sex, race, BMI, eGFR, and number of renal arteries. Recipient variables included age, sex, race, BMI, prior tx, pre-tx DM, history of smoking and drug usage. Additional variables evaluated included degree of relationship (LURT), HLA mismatch, antilymphocyte induction, de novo CNi usage, R v. L nephrectomy, WIT, total OR time, and performance of simultaneous deceased donor pancreas tx (SPLK). Univariate analysis was performed with significance defined as $p < 0.05$. Significant variables were then included in the multivariate analysis.

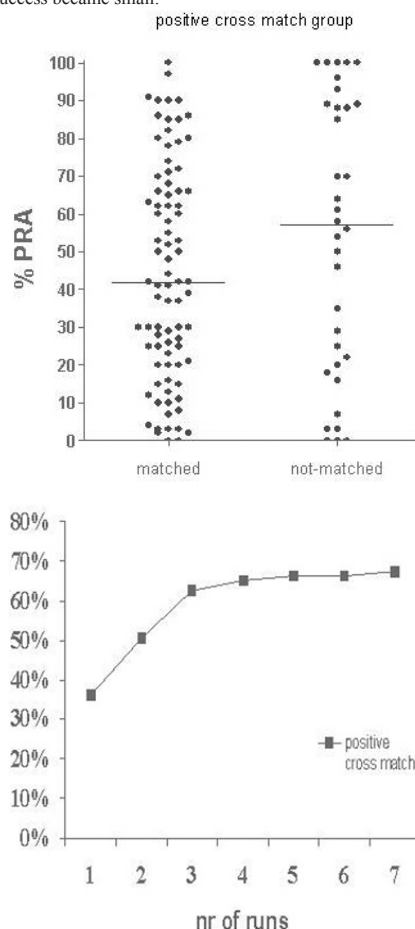
Parameter	Univariate (OR, 95%CI)	p-value	Multivariate (OR, 95%CI)	p-value
Male Recip	1.48 (0.92-1.18)	0.04	1.49 (0.95-2.32)	0.08
AA Recip	1.68 (1.17-2.42)	0.005	1.34 (0.49-3.62)	0.57
Donor Age/ decade	1.19 (1.02-1.39)	0.02	1.32 (1.08-1.60)	0.005
AA Donor	1.63 (1.12-2.37)	0.01	1.18 (0.43-3.23)	0.75
Prior Tx	0.36 (0.13-1.00)	0.05	0.74 (0.24-2.22)	0.59
LURT	1.47 (1.02-2.11)	0.04	0.97 (0.58-1.65)	0.92
SPLK	0.33 (0.13-0.83)	0.02	0.41 (0.14-1.19)	0.10
Recip BMI/ 5kg/m2	1.49 (1.30-1.72)	0.000	1.49 (1.26-1.78)	0.000
HLA MM	1.18 (1.05-1.32)	0.003	1.14 (0.97-1.34)	0.10
R Kidney	2.52 (1.12-5.69)	0.03	2.87 (1.17-7.06)	0.02
WIT	1.00 (1.02-1.28)	0.02	1.16 (1.02-1.33)	0.03
Recip Smoke	1.48 (1.04-2.12)	0.03	1.40 (0.92-2.15)	0.12

CONCLUSION: Our results suggest that donor age, recipient BMI, male recipient, WIT and right lap nephrectomies are independent risk factors for SGF/DGF following LDN. Several are modifiable variables and worthy of intervention.

Abstract# 385

A Kidney Exchange Program Is Especially Indicated for Immunized Patients. M. Klerk de,^{1,2} M. Witvliet,³ E. Buijzer de,² B. Haase,² F. Claas,³ W. Weimar.^{1,2} ¹Internal Medicine, ErasmusMC, Rotterdam, Netherlands; ²Dutch Transplant Foundation, Leiden, Netherlands; ³National HLA Reference Laboratory, LUMC, Leiden, Netherlands.

Background: A kidney exchange program is the logistic solution for patients with positive cross match (X^+) or ABO incompatible donors. A major problem in all kidney transplantation programs is the sensitized recipient. We analyzed the success rate for immunized recipients in the Dutch kidney exchange program. **Methods:** From January 2004 till December 2007 242 donor-recipient pairs were registered. There were 117 couples in the X^+ group while 125 pairs were ABO incompatible. In the X^+ group the median PRA was 46% (2-100%). To create new combinations a match program was run 16 times (every 3 months) with a median of 46 (16-66) participating couples. Allocation criteria included bloodtype (first identical, then compatible), HLA match probability within the actual exchange donor pool to ensure that highly sensitized recipients have the best chance to receive a kidney, and the waittime on dialysis. Cross matches between new donor and recipient were performed centrally in our Reference laboratory with CDC-tests. **Results:** After 16 match runs, we found matching couples for 83/117 (71%) X^+ pairs, for 21/87 (24%) ABO incompatible pairs with O recipients and for 29/38 (76%) ABO incompatible pairs with non-O recipients. Median PRA of the 83 recipients in the X^+ group was 42% (2-100%). After 3 match runs chances for success became small.



Conclusion: The overall success rate for ABO incompatible and X^+ pairs in the Dutch kidney exchange program after 4 years is 55%. However, the success rate for immunized patients in the X^+ group is significantly higher (71%) as our match program gives priority to those recipients with the smallest chance of finding a compatible donor in each match run. Thus our kidney exchange program is especially suited for immunized patients.

Abstract# 386

Clinical Outcome of Multicenter Domino-Kidney Transplantation. Yu-Ji Lee,¹ Dae Joong Kim,¹ Sam Uel Lee,² Sang Young Chung,³ Baik Hwan Cho,⁴ Jin Young Kwak,⁵ Chong Myung Kang,⁶ Jin Tak Park,⁷ Duck Jong Han.⁸ ¹Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea; ²Department of Surgery, Kangdong Sacred Heart Hospital, Hallym University College of Medicine, Seoul, Korea; ³Department of Surgery, Chonnam National University Medical School, Gwangju, Korea; ⁴Department of Surgery, Chonbuk National University Medical School, Jeonju, Korea; ⁵Department of Surgery, Hanyang University College of Medicine, Seoul, Korea; ⁶Department of Internal Medicine, Hanyang University College of Medicine, Seoul, Korea; ⁷Korean Organ and Tissue Donor Program, Seoul, Korea; ⁸Department of Surgery, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea.

Although paired kidney donation (PKD) program is an established method to overcome incompatibilities between kidney donor-recipient pairs (DRP), significant proportion of the incompatible DRP participating in such program could remain unmatched. Domino-kidney transplantation (KT) in which altruistic living non-directed donor kidney (LNDK) is offered to a pool of incompatible DRP, and is used to initiate a chain of PKD transplants, could provide more opportunities of kidney transplantation to DRP in PKD program. We introduce our experience of multicenter domino KT for the last 7 years. Sixteen hospitals participated in the domino-kidney transplantation between February, 2001 and July, 2007. 181 domino-kidney transplants were performed with 69 domino-KT chains initiated by altruistic LNDK. 2-pair chains were 43, 3-pair chains 14, 4-pair chains 9, 5-pair chains 2, and 7-pair chains 1. Mean age of enrolled donors and recipients were 42.9±8.2 and 42.4±8.4 years, respectively. Male to female ratio of enrolled donors and recipients were 86:95 and 111:70, respectively. The types of donors were altruistic living non-directed donors (39.8%), spouse (47%), parent-child (4.4%), sibling (5.5%), and unrelated (3.3%). The distribution of ABO blood type was A: 30.9%, B 31.5%, AB 12.7%, and O 24.9% in donors and 30.9%, 29.8%, 16.0%, and 23.2% in recipients. Mean HLA mismatch was 3.3±0.9. 1-year and 5-year graft survival rates were 98.6% and 87.5%, respectively. 1-year and 5-year patient survival rates were 98.5% and 93.4%, respectively. Domino-KT could provide additional opportunities of transplantation to incompatible DRP, especially to disadvantaged DRP such as O recipients or AB donors. According to our experience, the outcome of domino-KT was comparable to that of conventional living donor KT.

Abstract# 387

The Development of a Multi-Regional Kidney Paired Donation Program. Ruthanne Hanto,¹ Francis Delmonico,¹ William Reitsma.² ¹NEPKE, NEOB, Newton, MA; ²MAPEP, NJSN, Springfield, NJ.

Using an optimization matching algorithm the New England Program for Kidney Exchange (NEPKE) efficiently matches incompatible donor/recipient pairs. In 2006, the Mid-Atlantic Paired Exchange Program (MAPEP) and other individual centers began sharing data with NEPKE. This is a report of the success of two regional programs working together to increase the probability of participants in both programs finding a compatible match.

Methods: Incompatible pairs and non-directed donors (NDD) are referred to NEPKE through transplant centers. Donor and recipient ABO, HLA and recipient HLA antibody screening are entered into the computer database. Utilizing the optimization program, searches for compatible matches are conducted every 30 to 45 days. The program identifies potential 2 and 3-way matches, NDD chains, and list exchange chains. Following the determination of compatibility NEPKE notifies transplant centers involved of the potential match and centers accept or decline the offer. Transplant centers notify their pairs and preliminary crossmatches are performed. A conference call is scheduled to coordinate simultaneous donor nephrectomies and recipient transplants. Surgeons speak prior to incision to ensure simultaneous donation.

Results: From July 2005 to December 2007, 186 pairs and 10 NDDs have entered NEPKE. During this time frame over one thousand possible matches were identified, with the majority of these matches involving the same pairs in multiple matches. After "optimizing" and eliminating multiple matches 13 two-way exchanges; 15 three-way exchanges; 7 three-way list chain exchanges; and 25 NDD chain matches were offered to transplant centers as possible matches. Most common reason offers were declined include: positive crossmatch (21.6%); donor factors (20%), and recipient inactive or transplanted (20%). Four offers occurred in MAPEP alone pairs, 47 in NEPKE, and 14 offers were cross-regional. Three matches are pending for 9 additional transplants. One previous and one pending transplant are the result of cross-regional exchanges. Five transplants performed and 6 pending involve NDD chains. One pending match involves a list exchange chain.

Conclusion: Using a computerized optimization algorithm to match 2 and 3 way exchanges, NDD and list exchange chains has lead to a substantial increase in the number of KPD matches and transplants performed. Cross-regional coordination is feasible and expands the number of transplants performed beyond the ability of individual exchange programs.

Abstract# 388

Short-Term Complications in Recent Living Kidney Donors. Jennifer L. Wainright,¹ Connie L. Davis.² ¹Department of Research, United Network for Organ Sharing (UNOS), Richmond, VA; ²Department of Medicine, University of Washington, Seattle, WA.

Background

As living donors become an increasingly important source of life-saving organs, there is growing concern about the lack of comprehensive research on donor outcomes. In addition to possible long term consequences, there is a risk that donors will experience complications during or following surgery. The purpose of the current research is to evaluate the level of this risk for recent living kidney donors.

Methods

The cohort included all living kidney donors reported to the OPTN in 2005-2006 (n=13,000). Rates of complications reported on the Living Donor Registration (LDR) form were calculated.

Donor Demographics	
Gender (% female)	59.2%
Ethnicity (% African American)	12.9%
Age (median)	41 years
BMI >= 30	22.8%
History of Hypertension	1.6%
Planned Laparoscopic	86.8%
Converted to Open Surgery	1.1%
No Health Insurance	12.2%

Results

Of the 13,000 living kidney donors in 2005-2006, none died during surgery, and 0.5% (n=68) needed blood transfusions during surgery.

In the six weeks following donation, 3.9% (n=512) had at least one serious adverse event (SAE): 1.7% (n=220) needed readmission following initial discharge, 0.6% (n=72) needed an interventional procedure, 0.5% (n=61) needed re-operation, 0.3% (n=41) had vascular complications, and 2.1% (n=274) had other complications. When all SAEs were considered in combination, the rate was 3.9%. Because over 50% of LDR forms were submitted by transplant centers fewer than 6 weeks post-donation, all complication rates should be considered minimum estimates. One donor was reported to have died from donation-related causes within 6 weeks of donation.

The number of living donor kidney transplants performed by a transplant center in 2005 - 2006 ranged from 1 to 362 transplants. Additionally, the risk of donor complications is not equal across transplant centers. For example, there was a significant correlation between the number of living donor kidney transplants performed at a transplant center and the percentage of that center's patients who were readmitted within 6 weeks of donation, with greater donor volume associated with a lower rate of readmission.

Conclusions

Living kidney donation is relatively safe, but prospective donors should be made aware that there is a non-trivial risk (3.9%) of short-term complications post-donation. As with many other major surgical procedures, complication rates are lower, on average, at institutions that perform a larger number of these procedures.

Abstract# 389

Expression Profiles in Donor Kidneys: Distinctive Gene Patterns Associated with Age and Graft Function. Daniel G. Maluf, Valeria R. Mas, Kellie J. Archer, Kenneth Yanek, Eric Gibney, Anne King, Adrian H. Cotterell, Robert A. Fisher, Marc P. Posner. *Hume-Lee Transplant Center, Surgery-Biostatistics, VCUHS, Richmond, VA.*

Background. Graft survival after kidney transplantation (KTx) is related to donor organ quality and factors such as donor age influence the long-term graft function. We studied the molecular profiles associated with donor age and graft function at 1-year post-KTx.

Patients and Methods. Kidney biopsies from 30 deceased donor kidneys (DDK) were evaluated using microarray. Donor groups were classified by age as <25, 25-50, >50 and a Jonckheere-Terpstra test for trend was applied to identify probe sets (Pset) with a significant monotonic trend with age. Pset specific linear models were fit and the significance of the estimated slope parameter was obtained. The False Discovery Rate (FDR) method was used to adjust for multiple comparisons and Psets. Expression profiles from allografts with normal function versus impaired function at 1 yr after KTx were also compared. Linear models were also used to examine whether there was any significant association between GFR and gene expression. Differential expression profiles were analyzed using Interaction Networks and Functional Analysis.

Results. Donor characteristics included 66% Caucasian, 62% male, and median age: 43 (range 16, 67). Eighty-one Psets were significant associated with age (p<0.001) and 145 Psets were found differentially expressed in aged kidneys using a trend analysis (p<0.001). Gene ontology terms classified the over expressed genes as related to inflammatory response, apoptosis, cell adhesion, extracellular matrix structural constituent, and B cell differentiation and proliferation. Under expressed genes were related to ion transport, lipid transport and metabolic process, and protein binding. The top identified canonical pathways were B cell receptor (p=2.67E-03), Glucocorticoid receptor (p=8.22E-03), VEGF (p=9.42E-3), and IL-6 (p=1.11E-02) signaling. When comparing patients with GFR <45 (N=12) vs. GFR ≥45 (N=17) ml/min/1.73 m² there were 285 probe sets significantly differentially expressed (FDR<0.15) and 60 Psets were significant associated with graft function when the linear model was fit (FDR<0.15).

These Psets were related to cell-to-cell signaling and interaction, cell growth and proliferation, and immune response.

Conclusions. A distinctive gene expression pattern was identified in aged DDK. A set of differentially expressed genes was associated with graft function at 1-year post-KTx.

Kidney: Polyoma

Abstract# 390

Analysis of OPTN/UNOS Data for Rate of Treatment for, and Risk Factors for, BK Virus Nephropathy (BKVN) in Kidney Transplant Recipients. Vikas Dharmidharka,¹ Wida Cherikh.² ¹University of Florida; ²Research Department, UNOS/OPTN.

Data for BKVN, a recently emerged graft-threatening complication of kidney transplantation, are from single center series. The OPTN/UNOS in the USA collected data on BKVN development and treatment on their follow up forms. In this study, we determined the rates of BKVN treatment by post-transplant time and elucidated the risk factors for BKVN from this large national transplant database.

We included all primary and solitary kidney transplant recipients transplanted between January 1, 2003 and December 31, 2006 from the database (N=48,178). Cumulative incidence of BKVN over time was calculated by Kaplan-Meier method. A Cox proportional hazards regression model was fitted to determine risk factors for development of BKVN within 2 years of transplant.

The cumulative incidence of BKVN rose over time post-transplant, from 0.70% at 6 months to 2.18% at 1 year and 3.74% at 2 years. The table below depicts risk factors significantly associated with BKVN treatment. BKVN rates were higher in more recent years post-transplant, which is a concern. Risk for BKVN was higher with certain immunosuppressive regimens that included Thymoglobulin or tacrolimus/mycophenolate combinations. High center volume and living kidney donation exerted a protective effect.

Variable	AHR	P-value
Thymoglobulin vs. No induction	1.32 [1.15, 1.52]	<.0001
Discharge Immunosuppression: CsA+MMF vs. TAC+MMF	0.52 [0.43, 0.63]	<.0001
CsA vs. TAC+MMF	0.50 [0.34, 0.75]	0.0008
TAC vs. TAC+MMF	0.73 [0.60, 0.89]	0.0019
TOR Inhibitor Use Yes vs. No	0.60 [0.44, 0.80]	0.0006
Delayed Graft Function: Yes vs. No	0.82 [0.70, 0.97]	0.0225
Recipient Female vs. Male	0.65 [0.58, 0.73]	<.0001
Recipient Black vs. White	1.21 [1.06, 1.37]	0.0038
Recipient Hispanic vs. White	0.61 [0.50, 0.74]	<.0001
Living vs. Deceased Donor	0.85 [0.75, 0.95]	0.0055
Transplant Year: 2004 vs. 2003	1.77 [1.47, 2.13]	<.0001
Transplant Year: 2005 vs. 2003	2.70 [2.25, 3.23]	<.0001
Transplant Year: 2006 vs. 2003	5.58 [4.62, 6.74]	<.0001
Center Volume >=100	0.85 [0.76, 0.95]	0.0033

Abstract# 391

Intensive Versus Routine Care Screening for Polyoma Viral Reactivation (Cluster Randomization) and a Road Map for Stepwise Modification of Immunosuppression Therapy (IT). R. K. Wali,¹ C. Drachenberg,² H. Hirsch,³ A. Joshi,⁴ R. Munivenkatappa,² M. Portocarrero,¹ M. Weir,¹ M. Cooper,⁵ S. Bartlett,⁵ E. Ramos.¹ ¹Med., Univ of Maryland Sch Med, Baltimore, MD; ²Pathology, Univ of MD Sch Med, Baltimore, MD; ³Medicine, Univ of Basel, Basel, Switzerland; ⁴Univ of MD, Baltimore County, MD; ⁵Surgery, Univ of MD Sch Med, Baltimore, MD.

We sought to determine if intensive screening improves detection of polyomaviral reactivation in asymptomatic patients and pre-emptive stepwise modification can improve outcome of polyomaviral nephropathy (PVN).

Methods: This is a prospective single center study. We randomly assigned *de novo* KT (cluster randomization) to Intensive Screening (IS; n=648) and Routine Care (RC; n=260) for the detection of decoy cells. IS was initiated at week-4 of KT and RC at the time of increase in serum creatinine. This was complemented with urine and blood nucleic acid testing. All patients had biopsies performed for detection of polyoma nephritis (PVN). Both groups were treated with pre-specified stepwise modification of IT based on cell cytology and viremia (Step 1: decrease dose of cellcept by 50%, Step 2: decrease dose of tacrolimus by 50%, or switch to sirolimus therapy, Step 3: discontinue cellcept). Primary outcome included persistence of decoy cells/viremia following each step in modification of IT every three months and secondary outcomes included acute rejection, graft function and graft loss.

Results: Polyomaviral reactivation developed in 11.7% in IS group and 40% had PVN without changes in serum creatinine. The estimated cumulative rate of primary outcome in the IS versus RC groups, 3-months (51% vs. 79%) relative risk (RR), 0.47; 95%CI, 0.20-1.08; p=0.051; 6-months (20% vs. 52%) (RR=0.67; 95%CI, 0.38-1.20; p=0.009); and 12-months (2% vs. 33%) (RR=0.88; 95%CI, 0.41-1.43; p=0.003). Secondary outcomes: despite similar degrees of Step-Wise IS modification, rate of acute rejection non-significant (p=0.25), but 25% of patients in RC lost the graft vs no graft loss in IS group. Patients who continue to remain on tacrolimus vs. those who were switched to sirolimus therapy had persistent viremia (OR=10.25; 95%CI, 1.2-92.0; p=0.003).

Conclusion: IS for polyomaviral reactivation allows early detection of PVN in the presence of stable graft function. Stepwise modification in IT resulted in early resolution of decoy cells and viremia in both groups, albeit slowly in RC group, and it did not prevent the graft loss in RC group.

Abstract# 392

BK Virus Non-Coding Control Region (NCCR) Variants in Healthy Seropositive Transplant Recipients. Aaron T. Dall,¹ Lisa Jurgens,¹ Sundaram Hariharan,¹ Orentas Rimas.² ¹Medicine, Medical College of Wisconsin, Milwaukee, WI; ²Pediatrics, Children's Hospital of Wisconsin, Milwaukee, WI.

BACKGROUND: DNA sequencing of the BK viral (BKV) genome Non-Coding Control Region (NCCR) from individual patient isolates demonstrate divergent sequence and alterations in the arrangement of modularly conserved sequence blocks (P-Q-R-S) ranging in size from 39 to 68 base pairs.

AIM: Primary aim to molecularly clone and analyze patient-derived BK virus NCCR sequence variants. Our secondary aim is to determine if reporter gene constructs of patient-derived NCCR variants differ in their promoter activity upon transfection into a mammalian cell line (Vero) and a human primary tubular epithelial cell line.

METHODS: BKV DNA was amplified and sequenced from blood and urine samples of 18 renal transplant recipients. Via sequence alignment, unique NCCRs were determined. These sequences were PCR amplified and cloned into reporter plasmids containing the Renilla Luciferase gene. Promoter activity was measured via luminometer 24 hours after transfection into Vero cells and human tubular epithelial cells.

RESULTS: Variation in naturally occurring BKV NCCR promoter regions exist as single basepair insertions and deletions, and insertions or deletions of partial sequence blocks (P-Q-R-S). Single basepair substitutions were most commonly seen (46% of analyzed samples). Promoter activity within Vero cells ranged from 188% to 39% as compared to NCCR activity of an archetypal strain (WWb). Low promoter activity (<50%) was seen in isolates with duplications of the P block and large deletions of the R block.

CONCLUSION: These sequence blocks are rich in regulatory elements and control the expression of both BKV structural and regulatory genes. Variation in these cis-acting eukaryotic transcriptional promoter binding sites corresponds to differential promoter activity in naturally occurring BKV isolates. Current plans include repeating the promoter activity studies in human primary tubular epithelial cells.

Abstract# 393

Humoral and Cellular Immunity to Polyomavirus BK Large T and VP1 Antigens after Pediatric Kidney Transplantation. Patrizia Comoli,¹ Sabrina Basso,¹ Hans H. Hirsch,² Alberta Azzi,³ Iris Fontana,⁴ Michela Cioni,⁵ Gerardo Botti,⁵ Valeria Cappelli,⁵ Franco Locatelli,¹ Fabrizio Ginevri.⁵ ¹Pediatric Hematology/Oncology, Fondazione IRCCS Policlinico S. Matteo, Pavia, Italy; ²Division of Infectious Diseases, University Hospitals Basel, Basel, Switzerland; ³Public Health Dept., University of Firenze, Firenze, Italy; ⁴Dept. Transplant Surgery, S. Martino Hospital, Genova, Italy; ⁵Pediatric Nephrology, Istituto G. Gaslini, Genova, Italy.

Polyomavirus BK-associated nephropathy (BKVN) has emerged as a cause of graft failure after kidney transplantation (KTx). In a cohort of 37 pediatric renal recipients undergoing prospective three-monthly monitoring for BK by blood and urine Q-PCR, we evaluated antibody response, measured by enzyme immunoassay using BK VLP, and cellular immune response, reported as frequency of IFN γ -secreting cells in a ELISPOT assay after 9-day stimulation with BKV large T (LT) and VP1 peptides. We could not observe any influence of recipient pre-transplant BKV-specific IgG or T-cell levels, which were generally low, on BKV infection after allografting. After transplantation, both specific IgG levels, and frequency of BKV-specific T cells increased according to the degree of viral exposure. In detail, BKV-seropositive patients who never reactivate the virus (group 1, n=10) did not show significant increase in IgG levels (from a median OD of 0.37 at month +1 to 0.39 at the end of follow-up), while patients with urinary shedding alone (group 2, n=14) or with viremia (group 3, n=13) increased from a median OD of 0.3 to 1.2 (p=0.09), and 0.26 to 2.8 (p>0.0005). In the case of cellular immunity, VP1-specific T-cells increased in the three groups. Conversely, LT-specific T-cells, which were high (median 71 SFU/10⁵ cells) and remained unchanged throughout the follow-up period in group 1 patients, had a significant increase in recipients belonging to both group 2 and 3. Interestingly, patients with urinary shedding who do not progress to viremia show a median 3-fold increase in LT-specific T-cell levels at peak viremia, compared to no increase observed in patients who develop viremia. The latter group mount a significant response to LT only after therapeutic reduction of immunosuppression. At peak viremia, viremic patients already show a 8-fold rise in specific IgG compared to the 1.5 increase observed in group 2 recipients. Our data suggest that inability to reach protective levels of BKV LT-directed T cells, rather than specific IgG, predispose KTx recipients to BKV replication.

Abstract# 394

Mapping of HLA-A Restricted BK Virus T-Antigen Epitopes Using ELISPOT Assay Guided by a Bioinformatics Approach. Bala Ramaswami,¹ Iulia Popescu,² Diana Metes,² Camila Macedo,² Ron Shapiro,² Parmjeet Randhawa.¹ ¹Pathology, Univ Pittsburgh, Pittsburgh; ²Surgery, Univ Pittsburgh, Pittsburgh, PA.

BACKGROUND: A detailed understanding of the cell mediated immune response to BK virus infection is needed for vaccine design, and to facilitate development of assays that will help predict clinical outcome in individual patients with active viral infection.

METHODS: Peptides derived from the BK viral Large T-antigen (LT-Ag) sequence (accession # P03071) were analyzed for predicted ability to bind to HLA0201, A0101, A0301, and A2402 alleles using three predictive algorithms, SYFPEITHI, BIMAS and IEBD MHC-I binding predictions. The entire BKV LT-Ag amino acid sequence was prescreened as 9-mers against the aforementioned alleles. For non-HLA-A02 targets, MHC peptide binding studies on SYFPEITHI predicted sequences were carried out in 96 well based in-solution assays using a labeled antibody to the correctly folded MHC-peptide complex. Immune recognition of these peptides by human peripheral blood mononuclear cells (PBMC) from healthy controls and kidney transplant (KTx) patients was evaluated using a functional IFN γ secretion based ELISPOT assay.

RESULTS: Autologous dendritic cells (DC) or T2 cell based ELISPOT assays on 5/6 healthy individuals and 5/7 kidney transplant recipients confirmed immune recognition of HLA-A02 epitopes at positions 351-450 and 533-626, which represent two different p53 binding domains. For HLA A01 restricted peptides 3/4 healthy and 1/4 KTx patients have been provisionally identified to have Large T Ag memory CD8+ T cell responses, while for HLA A03 allele 1 healthy control and 2/5 HLA-A03 individuals appeared to show memory responses exceeding the background (no-peptide or mock lysate treated cells). Additional experimental controls are needed for definitive assignment. These MHC class I restricted peptides of interest include P77, P235, P270 & P604 (HLA-A01 restriction), and P156, P159, P172, P208, P394, P414, & P506 (HLA-A03 restriction).

CONCLUSIONS: The BKV large T-antigen contains multiple putative 9-mer epitopes that can act as targets for class I MHC restricted cell mediated immunity. These epitopes are conserved across different BKV genotypes, and localize to Large T-antigen p53 binding domains, chains A-F, helicase, DNA binding and N terminal J domains. A high degree of sequence homology with the corresponding JC virus and SV40 sequences suggests that immunity to BKV will result in cross protection against infection by other Polyoma virus species.

Abstract# 395

BK Virus VP-1 Protein Capsids Contain Conformational Epitopes That Can Elicit Virus Neutralizing Antibodies. Parmjeet S. Randhawa,¹ Cathy Huang,¹ Raphael Viscidi,² Ron Shapiro,³ Neil Christenson.⁴ ¹Pathology, Division of Transplantation Pathology, University of Pittsburgh Medical Center, Pittsburgh, PA; ²Pediatrics, The Johns Hopkins Hospital, Baltimore, MD; ³Surgery, University of Pittsburgh Medical Center, Pittsburgh, PA; ⁴Pathology, Jake Gittlen Cancer Research Foundation, Pennsylvania State University, Hershey, PA.

Introduction: The antibody response to human BKV virus (BKV) is incompletely characterized. Antibody responses to the VP-1 protein have been detected in kidney transplant patients, but it is not known if these have virus neutralizing activity.

Methods: Recombinant BK, JC, and SV40 virus like particles were used to produce a panel of 20 monoclonal antibodies. These antibodies were characterized for isotype and for ability to bind the respective antigens in ELISA assays. To test neutralizing activity, BKV Gardner strain (ATCC# VR837) viral particles were incubated with the corresponding antibodies for 2 hours at 37 degrees C, and used to infect WI 38 cells. BKV infection was monitored by quantitative real time PCR using primers directed against the VP-1 gene. Neutralizing activity was defined as greater than 95% inhibition of viral yield.

Results: The monoclonal antibodies were of the IgG 2a or IgG 2b class with the exception of one IgG 1 and one IgM antibody. All 12 anti-BKV monoclonal antibodies bound to BKV capsids in-vitro in ELISA assays. This binding affinity was species specific, as only 1 antibody showed weak binding activity to JCV and SV40 capsids. Neutralization of infectious BK virus was shown for 10/12 antibodies. Denaturation of capsid proteins indicated that the monoclonal antibodies recognized primarily conformational epitopes, with only monoclonal antibody appearing to have a linear component. Paucity of linear epitopes was further suggested by lack of reactivity of sera from BKV seropositive subjects in ELISA assays based on genotype-specific short peptide sequences derived from the BKV VP-1 loop region. Four monoclonal antibodies each generated from JCV capsids and SV40 capsids did not show any BKV neutralizing activity.

Conclusions: BKV VP-1 protein capsids contain species specific conformational epitopes which can elicit virus neutralizing and non-neutralizing antibody responses. Measurement of these antibodies in renal transplant recipients may have diagnostic and prognostic applications. BKV specific monoclonal antibodies deserve further study as potential therapy of acute infections in the viremic phase.

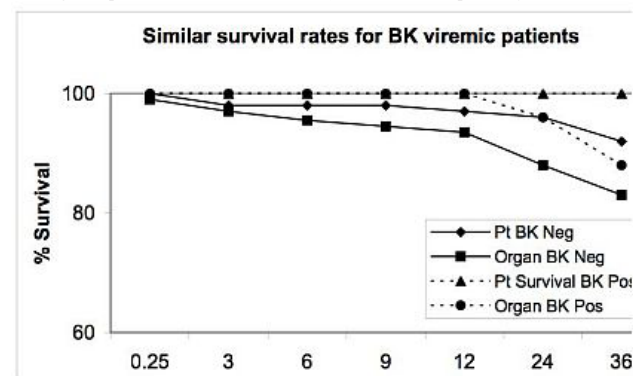
Abstract# 396

Impact of Immunosuppression Reduction in BK Viremic Patients: 3 Year Follow-Up. S. Kuppachi,¹ A. Guasch,¹ C. P. Larsen,¹ K. E. Kokko.¹ ¹Transplant Center, Emory University, Atlanta, GA.

Background: Development of BK nephropathy is a risk factor for allograft loss. BK viremia (BKV) precedes the development of BK nephropathy. It has been reported with 1-year follow-up that reduction of immunosuppression leads to control of BKV. Here, we report our 3-year follow-up experience of BKV patients that were identified by a prospective screening protocol and managed by sequential reduction of immunosuppression.

Methods: All kidney or kidney-pancreas transplant recipients at Emory University between 5/03-5/04 were screened prospectively for BKV by real time PCR during follow-up visits (1-6, 9, 12, 24, 36). Patients with a BK viral load of greater than 10,000 copies/ml blood received a kidney biopsy to screen for BK virus nephropathy by immunohistochemistry. BKV without nephropathy resulted in reduced immunosuppression by a 50% reduction in mycophenolate dose. BKV with nephropathy resulted in discontinuation of mycophenolate. All identified BK patients were monitored every 2-4 weeks until viral load was below 10,000 copies/ml. Immunosuppression was further reduced if viral loads failed to decrease.

Results: 135 recipients were followed over a 36-month period. 24 patients had received simultaneous kidney and pancreas, 2 a liver and kidney and the rest kidney alone. 27 of 135 (20%) patients developed BKV within a year from time of transplantation. Average time to diagnosis of BKV was 4.7 months. Average dose of mycophenolate at 3 years was 1.3 g/d in the BKV negative population as compared to 0.56 g/d in the BKV positive population. Average 12 hour trough blood level of tacrolimus at 3 years was 9.1 ng/ml in the BKV negative population as compared to 7.4 ng/ml in the BKV positive population. Survival rates for both patient and organ were comparable at 3 years in BK viremic vs BK negative patients (100% vs 92%) and (88% vs 83%) respectively.



Conclusions: While BK viremia is a historic risk factor for organ loss, prospective monitoring and reduction of immunosuppression is associated with comparable 3 year patient and organ survival to patients that never develop BK viremia.

Abstract# 397

A Molecular Modeling Approach To Develop Compounds with Anti-BK Virus Activity. Marta Bueno,¹ Carlos J. Camacho,¹ Parmjeet S. Randhawa.² ¹Department of Computational Biology, University of Pittsburgh, Pittsburgh, PA; ²Division of Transplant Pathology, University of Pittsburgh Medical Center, Pittsburgh, PA.

Background: There are currently no BK virus (BKV) specific therapies available for clinical use. This study evaluates viral large T antigen as a potential target for drug development, since (a) this is a key molecule that participates in several different stages of viral replication, (b) has no homologous human protein, and (c) offers multiple functional domains for chemical binding, particularly the ATP binding site, the DNA binding site, the hexamerization surfaces, and the hinge region.

Methods: Virtual screening and protein modeling techniques were applied to BKV large T antigen using a model developed from the known crystal structure of SV40 large T antigen. Two different structural states of large T antigen (monomer and dimer structure) in three different states (with the nucleotide pocket empty, with bound ADP and bound ATP), were evaluated for a total of 6 large T antigen receptor conformations.

Results: A computational solvent mapping analysis of small molecular probes allowed identification of multiple functional sites, which represent potential drug binding pockets on the large T antigen molecule. It was possible to classify 1200 molecular conformations centered on the ATP binding site, hexamerization surface and hinge region of the viral protein. We docked 1209 medium sized fragments (<100 Da) to confirm the results obtained by computational solvent mapping, and further characterize chemical properties of the ATP binding site. In another approach, known chemical structures of Hsp90 and Rho-kinase inhibitors were used to search compound databases and obtain a subset of 8294 compounds (mean size of 350 Da) capable of docking large T antigen. Cross-referencing the top solutions obtained by energy ranking, we were able to identify 50 compounds that bind large T antigen in all 6 conformational states. A subset of 5 compounds simultaneously binds the ATP binding site, hexamerization surface and hinge region of BKV large T antigen.

Conclusions: Virtual screening and three dimensional homology modeling technology has allowed us to identify compounds that can bind multiple sites on the large T antigen. These compounds are predicted to preferentially inhibit viral replication without the toxicity expected from simultaneous inhibition of host cell kinases.

Abstract# 398

Repression of BK Virus Infection of Human Proximal Tubular Epithelial Cells by Pravastatin. Andrey Sorokin,¹ Takahito Moriyama,¹ ¹Department of Medicine, Division of Nephrology, Kidney Disease Center, Medical College of Wisconsin, Milwaukee, WI.

BK Virus (BKV), a human polyomavirus, causes BKV nephritis, which often leads to graft loss after renal transplantation. Currently, the only efficient therapy against BKV nephritis appears to be a reduction/change of immunosuppressive agents, and this may increase the inherent risk of rejection. Since human renal proximal tubular epithelial cells (HRPTEC) represent a main natural target of BKV nephropathy, the analysis of BKV infection of HRPTEC is likely to provide necessary additional insight into BKV biology and contribute to the development of strategies for treatment of BKV nephritis. Here we report the ability of 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) reductase inhibitor pravastatin, which is routinely used to treat hypercholesterolemia, to repress BK virus entry pathways in HRPTEC and, correspondingly, prevent BKV infection. The percentage of HRPTEC infected with BKV was assessed by immunofluorescent analysis in the absence and presence of pravastatin. Both, the percentage of BKV infected cells and the intensity of BKV infection, assessed by western blotting using antibodies against large T antigen, were significantly decreased in HRPTEC treated with pravastatin. It is likely, that pravastatin's inhibitory effect is explained by depletion of caveolin-1, a critical element of caveolae. We demonstrate that BKV enters HRPTEC by caveolar-mediated endocytosis and disruption of Caveolin 1 mRNA and protein inhibits BKV infection of HRPTEC. We provide evidence that pravastatin dramatically decreased caveolin-1 expression in HRPTEC and interfered with internalization of labeled BKV particles. Our data suggest that pravastatin, acting via depletion of caveolin-1, prevented caveolar-dependent BKV internalization and repressed BKV infection of HRPTEC. Our data represent the first report of inhibitory action of statins upon BKV infection.

Liver: Complications and Recurrent Disease

Abstract# 399

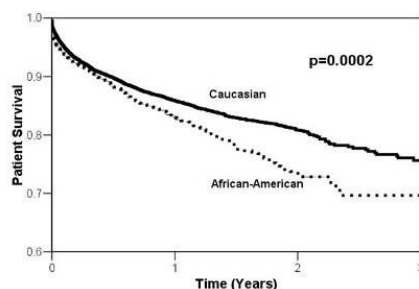
Survival after Orthotopic Liver Transplantation (OLT) in the United States: Race Still Matters in the MELD Era. N. S. Becker,¹ C. A. O'Mahony,¹ O. A. Taylor,¹ J. Suarez,¹ N. L. Sussman,¹ J. M. Vierling,¹ R. J. Stribling,¹ J. A. Goss,¹ ¹Michael E DeBakey Department of Surgery, Baylor College of Medicine, Houston, TX.

Introduction: Previous studies have reported survival differences after orthotopic liver transplantation (OLT) for minority racial groups. The current study examines outcomes following OLT performed after implementation of the Model for End-Stage Liver Disease (MELD) allocation system.

Methods: Data on 17,649 adult OLTs performed from 2/2002-12/2005 were extracted from the United Network for Organ Sharing (UNOS) database. Patient survivals stratified by racial group were calculated and compared. Demographic and clinical variables were compared and univariate and multivariate analyses of patient survivals were conducted.

Results: 74.6% of patients were Caucasian, 12.2% Hispanic, 8.9% African-American and 4.4% Asian. Overall 1- and 3-yr patient survivals were 85% and 77%. Stratified by race, only African-American survivals differed from Caucasian (3-year survivals of 71% vs. 77%; Figure 1). Compared to Caucasians, African-American patients were younger (48yrs $\pm$ 11.5 vs. 52yrs $\pm$ 9.9), were more likely to be Status 1 (10.3% vs. 5.1%), to have a serum creatinine $\geq$ 1.5mg/dL (37% vs. 31%), to receive an multi-organ transplant (8% vs. 5%), to have fulminant hepatic failure (12% vs. 7%), to have a higher MELD score (22.9 $\pm$ 10.4 vs. 20.1 $\pm$ 9.4), to be in the ICU (16% vs. 11%), and to be ventilated pre-transplant (8% vs. 5%); all p-values $<$ 0.001. After adjustment for each of these variables, race was still an independent predictor of mortality (p=0.01, HR: 1.22, CI: 1.048-1.425). Multivariate analysis of the African-American group (n=1481) alone revealed that a BMI greater than 40 (p=0.001, HR: 2.5, CI: 1.49-4.17), a creatinine greater than 1.5 mg/dL (p=0.002, HR: 1.6, CI: 1.18-2.10), and ICU admission pre-transplant (p=0.018, HR: 1.5, CI: 1.07-2.17) are independent predictors of mortality in this subset of patients.

Conclusion: In the current MELD era, race is still a predictor of worse outcomes, even after adjustment for multiple clinical variables. Further work is necessary to elucidate why this disparity exists.



Abstract# 400

Outcomes of Subsequent Pregnancies in Female Liver Transplant Recipients. Michael Schiraldi,¹ Lisa A. Coscia,¹ Serban Constantinescu,²

Nicole M. Sifontis,³ Peter Axelrod,² John A. Daller,¹ Michael J. Moritz,⁴ Vincent T. Armenti,⁵ ¹Surgery, Temple University School of Medicine, Philadelphia, PA; ²Medicine, Temple University School of Medicine, Philadelphia, PA; ³Pharmacy Practice, Temple University School of Pharmacy, Philadelphia, PA; ⁴Surgery, Lehigh Valley Hospital, Allentown, PA; ⁵Surgery, Thomas Jefferson University, Philadelphia, PA.

The purpose of this study was to analyze the effects of successive pregnancies in female liver transplant recipients on newborn and maternal outcomes. Data were collected from the National Transplantation Pregnancy Registry via questionnaires, phone interviews and hospital records. Analyses for linear trends (proportions and continuous variables) were done by Chi square and least squares regression. There were 217 outcomes of 213 pregnancies, including twins. Of the 125 liver recipients who had a first pregnancy, 61 had between one and four subsequent pregnancies. There were no significant differences in the variables analyzed as noted in the table.

	1st Pregnancy	2nd Pregnancy	3rd Pregnancy	4th Pregnancy	5th Pregnancy
No. of Recipients	125	61	21	4	2
Age at conception	27 $\pm$ 5	29 $\pm$ 6	29 $\pm$ 5	31 $\pm$ 1	32 $\pm$ 1
Pregnancy outcomes*	127	62	21	5	2
Livebirths	75%	73%	67%	80%	100%
Spontaneous abortions	17%	19%	24%	20%	0
Therapeutic abortions	5%	5%	10%	0	0
Stillbirths	3%	0	0	0	0
Mean Gestational age (wks)	36 $\pm$ 4	38 $\pm$ 3	37 $\pm$ 3	36 $\pm$ 4	37 $\pm$ 2
Prematurity (< 37 wks)	41%	27%	36%	50%	50%
Mean birthweight (g)	2562 $\pm$ 832	2902 $\pm$ 634	2735 $\pm$ 718	2325 $\pm$ 829	2849 $\pm$ 751
Low birthweight (<2500 g)	40%	27%	29%	50%	50%
Neonatal death	2%	0	0	0	0
Rejection during pregnancy	11%	3%	14%	0	0
Graft loss within 2 yrs of delivery	7%	5%	10%	0	0

*includes twins

CONCLUSIONS: Successive pregnancies in liver transplant recipients are not associated with adverse fetal outcomes and/or increased maternal graft loss. Female liver recipients with excellent allograft function without significant recurrent disease or chronic rejection who wish to have more than one pregnancy should not be discouraged to conceive.

Abstract# 401

A Randomized Trial of Intra-Operative Biliary Stent at Orthotopic Liver Transplantation. Marylise Boutros,¹ Amy Neville,¹ Dionisios

Vrochides,¹ Steven Paraskevas,¹ Prosanto Chaudhury,¹ Peter Metrakos,¹ Jean Tchervenkov,¹ Myriam Fernandez,¹ Marcelo Cantarovich,² Marc Deschenes,² Peter Ghali,² Philip Wong,² Jeffery S. Barkun,¹ ¹Division of General Surgery, Department of Surgery, McGill University Health Centre, Montreal, QC, Canada; ²Multi-Organ Transplant Program, Department of Medicine, McGill University Health Centre, Montreal, QC, Canada.

Purpose:

Biliary complications remain one of the most problematic issues in liver transplantation. Following a published series of cases, we hypothesized that placement of a biliary endoprosthesis at the time of orthotopic liver transplantation (OLT) will decrease the rate of early biliary complications (BC) and the need for biliary interventions after OLT.

Methods:

We conducted a randomized trial of direct operative placement of a biliary endoprosthesis in the choledcho-choledochal anastomosis (CCA) at the time of OLT. There was no difference in the post-operative management between stented and non-stented patients, except that stented patients underwent an ERCP at 6 weeks to remove it. BC were

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recorded and categorized in a blinded fashion. Univariate, multivariate and survival analyses were performed.

Results:

Over a 4.5-year period (2002-2007), 148 OLT recipients were randomized to receive a CCA with biliary stent (n=76) or CCA alone (n=72). Patients with hepatic artery thrombosis (n=8; 5.7%) were excluded. There was no significant difference in demographic or graft-related variables. The mean age at transplant was 55 years, 82% were male, the mean MELD was 21 and 23% had hepatitis C. The mean donor age was 46 years, 59% of donors were male, and the mean cold ischemia time was 8.7hrs. The only complication related to the biliary stent was one occlusion. The rate of overall BC in the stented patients was 24.7% vs. 32.8% in non-stented patients, (p=NS). However, stented patients had significantly less BC in the first 60-days post-OLT (6.8% vs. 21.0%, p<0.02) and significantly less anastomotic leaks (2.4% vs. 9.6%, p<0.05). Over the year following OLT, stented patients also required less biliary therapeutic interventions (mean 1.8 vs. 0.9 interventions/patient, p<0.04) and fewer readmissions (mean 2.8 vs. 1.6 readmissions/patient, p<0.01). We also observed improved late graft survival (>6mo) in the stented group.

Conclusions:

Intraoperative stenting of the CCA at OLT does not appear to reduce the long-term rate of BC but it decreases the incidence of biliary leaks and significantly improves many facets of early patient management.

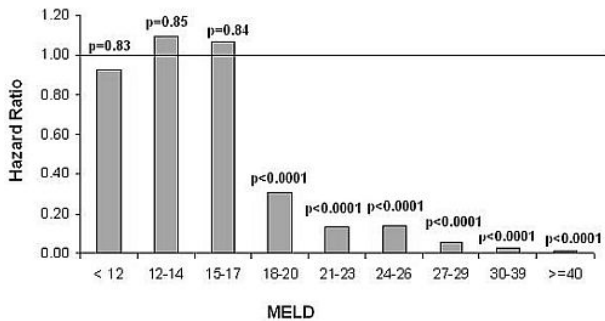
Abstract# 402

Is There Survival Benefit of Retransplantation of the Liver? A 23-Year Experience at a Single Center. Johnny C. Hong,¹ R. Mark Ghobrial,¹ Douglas G. Farmer,¹ Junming Xu,¹ Hasan Yersiz,¹ Jonathan R. Hiatt,¹ Ronald W. Busuttil.¹ *Surgery, University of California Los Angeles, Los Angeles, CA.*

Background: Long-term outcomes after retransplantation of the liver (re-OLT) is inferior compared to primary OLT. However, the survival benefit of re-OLT based on Model for End Stage Liver Disease (MELD) is not known.

Methods: A single-center analysis of 421 adult patients who underwent re-OLT between February 1984 to February 2007 was performed. Survival benefits, at a given MELD score, were calculated by comparing re-OLT survival at 3 months to expected 3-month survival without retransplantation

Results: Of 421 pts, 380 underwent re-OLT, and 41 received 3 transplants. The figure shows re-OLT survival benefit at any MELD score with increased significance in patients with MELD scores ≥ 18. Although MELD scores 30-40 predicted the highest mortality after re-OLT, they also demonstrated survival benefit. Multivariate Cox regression identified cold ischemia time >10 hrs (RR 1.8, P 0.001), MELD 30-40 (RR 2.0, P 0.04), time from first OLT (8 days - 1 year, RR 1.8, P 0.02) and third transplant (RR 1.7, P 0.03) as independent predictors for mortality following re-OLT.



Conclusions: Re-OLT should be considered in all patients even with low MELD scores. Although patients with high MELD scores (30-40) exhibit poor survival outcomes, a survival benefit is achieved. Survival benefit that considers both probability of death without re-OLT and expected survival with re-OLT, should be used for selection of retransplantation candidates.

Abstract# 403

Advancing Donor Age Does Not Predict Increased Length of Stay (LOS) in a Multivariate Analysis of Complications after Liver Transplantation. Edmund Q. Sanchez,¹ Srinath Chinnakotla,¹ Linda W. Jennings,¹ Marlon F. Levy,¹ Robert M. Goldstein,¹ Goran B. Klintmalm.¹ *Baylor Regional Transplant Institute, Dallas/Ft. Worth, TX.*

This study analyzed posttransplant complications, MELD score, and donor ages and their effect on length of stay (LOS).

Methods: This IRB approved retrospective review of our prospectively maintained database included 1427 liver transplant recipients transplanted between 1999-2006. LOS <14 days, 14-30d, and >30d were analyzed. Primary analysis to look at LOS, MELD, and donor age was performed using Wilcoxon two-sample test. Complication groups were analyzed using logistic regression and odds ratio estimates were determined. Kaplan-Meier for patient survival for the LOS groups was performed.

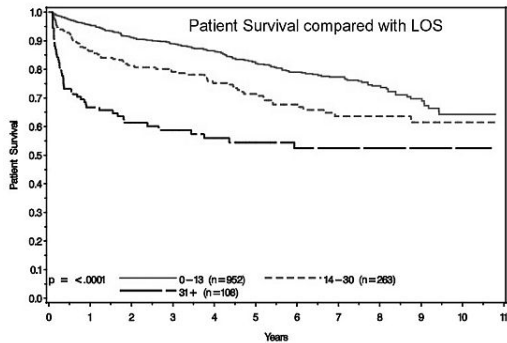
Results:

LOS group compared with MELD and Donor Age				
LOS	Median MELD (n)	Median MELD with donor age 51-60	Median MELD with donor age 61-70	Median MELD with donor age >70
<14 days	15 (973)	16 (245)	16 (119)	17 (34)
14-30 days	19 (285)	18 (98)	19 (56)	16 (15)
>30 days	22 (124)	23 (41)	22 (22)	23.5 (8)
P value	<0.0001	<0.0001	0.0001	0.2206

Results of Logistic Regression Analysis					
LOS >14 days c=0.836			LOS >30 days c=0.924		
Complication	Odds Ratio Estimate	95% confidence limits	Complication	Odds Ratio Estimate	95% confidence limits
Sepsis	5.647	2.686-11.874	Sepsis	6.454	3.605-11.556
Neurologic	5.456	3.301-9.020	Pulmonary	5.371	3.203-9.005
Biliary	4.440	2.68-7.356	Biliary	4.684	2.550-8.604
Other	4.422	3.015-6.846	Abdominal	3.375	2.201-6.475
Abdominal (other than liver)	3.451	2.192-5.435	Neurologic	3.372	1.931-5.889
Pulmonary	2.430	1.701-3.470	Other	2.713	1.603-4.591
Cardiac	2.335	1.207-4.518			
Vascular complication of liver allograft	1.839	1.096-3.088			
Endocrine	1.675	1.171-2.397			

Univariate analysis: Allograft dysfunction, vascular complication of liver allograft, intra-abdominal (other than liver), biliary, cardiac, pulmonary, neurologic, sepsis, renal, and endocrine were statistically significant (p<0.001) using Fisher exact test.

Multivariate analysis: Using logistic regression, significant complications were analyzed to determine the complications linked with LOS >14d and >30d.



Conclusions: Analysis of 1427 liver transplants demonstrated increasing LOS with increasing MELD in each donor age group (50-60, 61-70) p<0.0001. For donor age >70, this relationship was not significant (p=0.2206). Multivariate analysis using logistic regression determined odds ratio estimates for each complication resulting in LOS >14 days and >30 days. LOS >14 days and LOS >30 days were associated with different complications (as shown above). Allograft dysfunction (including PNF) and renal complications were not significant factors in multivariate analysis. Patient survival significantly decreased (p<0.001) with increased LOS >14 days.

Abstract# 404

Recurrent Primary Biliary Cirrhosis after Liver Transplantation at a Center Practicing Corticosteroid Minimization. J. Campsen,¹ M. Zimmerman,¹ J. Trotter,¹ M. Wachs,¹ T. Bak,¹ T. Steinberg,¹ M. Kaplan,¹ I. Kam.¹ *Transplant Surgery, University of Colorado, Aurora, CO.*

Introduction: Some patients with primary biliary cirrhosis (PBC) may require long-term corticosteroid (CS) therapy following liver transplantation (OLT) due to recurrent inflammation in the graft. Our center has attempted to minimize CS use in all of our OLT recipients. We reviewed our experience in this cohort of patients to determine 1) patient outcome including recurrent disease and 2) long-term requirement for CS use in PBC patients. **Methods:** From 1988 to 2006, 1,102 OLTs were performed in 1,032 adults at the University of Colorado of which 70 patients (6.8 %) with PBC received 74 allografts. Recurrence was defined by characteristic histologic changes on biopsy. Bivariate and multivariate analyses were used to evaluate predictors of CS withdrawal. 13 potential predictors of CS discontinuation were considered: age, gender, BMI, race, presence of inflammatory bowel disease (IBD), type of graft (cadaver or living donor (LD)), recurrence of AIH, warm ischemia time, follow up time (time since transplant), and immunosuppressant (IS). **Results:** Overall survival at 5 years was 85%. The 1, 5 and 10 year recurrence-free survival was 90, 72, and 54%, respectively. Disease recurred in 18 patients (25.7%). Of these 18 patients, none received a second transplant because of recurrent disease. CS was withdrawn in 73% of patients at time of review. Independent predictors of CS discontinuation are age (>median) (p = 0.0029) and LD graft type (p=0.0018). Conversely, Cyclosporine (CSA) (p=0.0012), female gender (p=0.0197), and BMI > 31 (p=0.0306) were negatively associated with CS withdrawal. Interestingly, CS withdrawal did not influence PBC recurrence. **Conclusions:** 1) Long-term outcomes in PBC patients are favorable and disease recurrence can be managed medically without

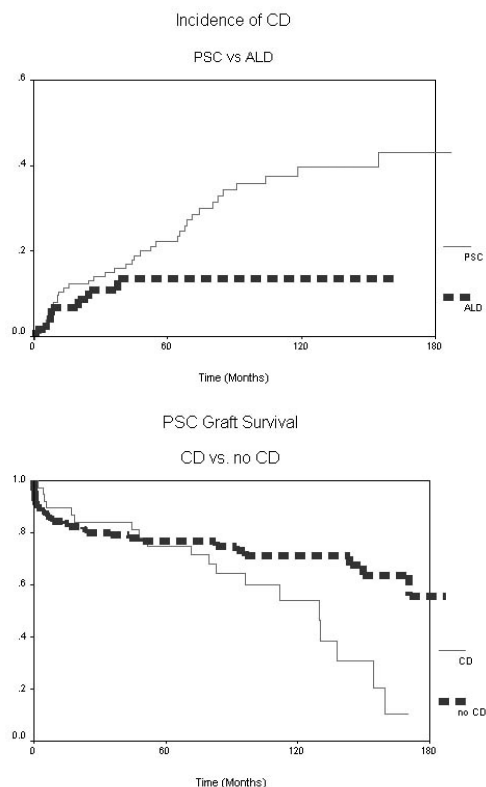
re-transplantation. 2) Using an aggressive CS minimization approach, almost 3/4 of the patients were CS-free at the time of last follow-up. 3) Increasing age and LD grafts were associated with successful CS withdraw; while CSA, female gender, and increasing BMI were associated with unsuccessful CS withdraw.

Abstract# 405

Incidence and Outcome of Cholestatic Disease Following Liver Transplantation for Primary Sclerosing Cholangitis. Philippe M. Garzon,¹ Preeti Dhar,¹ Shah A. Shimul,¹ Sean Cleary,¹ Gary A. Levy,¹ Lilly B. Les,¹ Grant R. David,¹ Catral S. Mark,¹ Ian McGilvary,¹ Greig D. Paul.¹ *Multi-Organ Transplant, University Health Network, University of Toronto, ON, Canada.*

Cholestatic disease (CD), either chronic rejection or recurrent primary sclerosing cholangitis (PSC), post liver transplantation (LT) occurs in 5-35% of PSC grafts. The study objectives were to evaluate the incidence and long-term outcome of CD. From 1985 to 2006, 141 grafts in 125 consecutive PSC patients and 85 grafts in 79 concurrent alcoholic liver disease (ALD) patients were compared. CD was diagnosed by biliary imaging and/or histology. Median follow-up was 57 months. The groups were similar including MELD score and cold ischemic time; however, roux-en-Y biliary anastomosis was more common in the PSC group (95% vs. 13%, $p<0.01$). The PSC group had more CMV hepatitis (22% vs. 6%, $p<0.01$) and acute rejection (60% vs. 30%, $p<0.01$), and fewer biliary anastomotic strictures (7% vs. 16%, $p<0.01$). CD occurred in 38 PSC grafts and 7 ALD grafts ($p<0.01$). The incidence of CD was greater in the PSC group ($p=0.02$, Fig. 1). No significant risk factors for CD were identified. In the PSC group the graft survival was lower in the CD group ($p=0.04$, Fig. 2); however, patient survival at 10 and 15 years was not affected by CD: 73% and 67% vs 75% and 60% because the re-LT rate was greater in this group (26% vs. 6%, $p<0.01$). At 15 years, patient survival in the PSC group was better than for ALD (64% vs 22%, $p<0.01$).

Long-term outcome post LT for PSC is good and better than for ALD; however, CD continues to develop beyond the first 5 years with a prevalence of 43% at 15 years. Graft loss in patients with CD is high, but with re-Tx, patient survival is similar to non-CD patients. Further studies to distinguish chronic rejection vs. recurrent PSC may provide insight into the prevention of CD following LT for PSC.



Abstract# 406

Comparative Outcomes of Patients Undergoing Deceased/Living Donor Transplantation for Autoimmune and Cholestatic Liver Diseases. Randeep Kashyap, Parvez S. Mantry, Adel Bozorgzadeh. *Division of Solid Organ Transplantation, Strong Memorial Hospital, Rochester, NY; Division of Gastroenterology and Hepatology, Strong Memorial Hospital, Rochester, NY.*

Introduction: Autoimmune Hepatitis (AIH), Primary Sclerosing Cholangitis (PSC), and Primary Biliary Cirrhosis (PBC) all progress to end stage liver disease and share etiopathogenesis. There are no comparisons of the outcomes of patients undergoing liver transplantation for each these autoimmune liver diseases and no comparative data for LDLT vs DDLT.

Methods: We analyzed the surgical registry of liver transplants (SRTR) and reviewed the outcomes of all patients transplanted for AIH, PSC and PBC from 1995 to 2006. Survival analyses were performed using SPSS vs.14

Results: 6763 patients underwent adult liver transplantation for these 3 conditions between Jan.1995 and Oct. 2006. Of these 6261 were DDLT and 502 were LDLT. AIH, PSC, PBC comprised of 24%, 41 % and 35 % respectively of all DDLTs and 12%, 57% and 31% of all LDLTs. The mean age at transplant was 46 ± 13 yrs in AIH, 54 ± 9 yrs in PBC and 47 ± 12 yrs in PSC recipients for DDLT and 42 ± 13 , 53 ± 18 and 44 ± 12 for AIH, PBC and PSC respectively. The mean MELD score of pts at the time of transplant with AIH, PBC and PSC was 20, 17 and 17 respectively (only available for transplants done after 2002) for DDLT and 14, 20 and 12 for LDLT. Overall, for patients who underwent LDLT- survival was 91.8% compared to DDLT- survival 82% (p value-0.01) at the end of the study period. AIH patients had the worst survival compared to PSC and PBC after DDLT (p -0.02). PBC had a worse survival as compared to PSC and AIH after LDLT.

Time period	liver transplant survival data		
	1yr(DDLT/LDLT)	3yrs(DDLT/LDLT)	5yrs(DDLT/LDLT)
AIH	88.7%/84.7%	83%/84.7%	62.6%/77.6%
PBC	92.2%/81%	87.8%/76.6%	73.4%/66.4%
PSC	95%/87.4%	92%/86.8%	79.2%/74.3%

Discussion: Our analysis showed that patients with AIH have a worse long term survival compared to PBC and PSC after DDLT. This may be explained by possibly more advanced disease at the time of presentation or more septic complications due to pre-transplant salvage therapy with immunosuppressants. Also, PBC had the worst relative outcome after LDLT in this group-possibly explained by the older age of the recipients. Overall, LDLT offered better outcomes than DDLT in terms of survival in patients with AIH, PBC and PSC. This study highlights an important and previously unvisited aspect of transplantation for autoimmune and cholestatic liver diseases.

Abstract# 407

Inflammatory Bowel Disease Course in Patients Transplanted for Primary Sclerosing Cholangitis. Ariana Wallack,¹ Joel S. Levine,² Lisa Forman.² *Internal Medicine, Univ. of Colorado HSC, Aurora, CO; Gastroenterology and Hepatology, Univ. of Colorado HSC, Aurora, CO.*

Purpose

The natural history of IBD following liver transplant (LT) for PSC is unknown. Prior studies have not shown factors consistently associated with disease activity, but were limited by small sample sizes. The aim of the study is to describe our experience with IBD post-LT in patients with PSC and determine factors predictive of disease activity.

Methods

A survey was mailed to 115 liver recipients transplanted for PSC between 1988 and 2006 asking about medications, IBD activity and quality of life after LT. Responses were linked to our LT database.

Results

88 (77%) recipients responded. 76% were male with a median of 82 (7-215) months since transplant. 93% were Caucasian with a median transplant age of 46 (16-71) years. 16% developed recurrent PSC post-LT. 74% had a diagnosis of IBD (63% UC). Immunosuppression included tacrolimus in 84% and cyclosporine in 11%. 71% experienced at least one episode of acute rejection. 73% rated their quality of health 4-5 on a scale of 1-5. There was no significant difference in demographic variables between IBD and non-IBD cohorts.

66% of respondents had IBD pre-LT. Of the 30 patients without pre-existing IBD, 7 developed IBD post-LT. Of the *de novo* IBD cohort, 43% were male, median transplant age was 45 years (39-57), and 86% were Caucasian. IBD developed in 71% at more than 3 years post-LT. There was no statistical difference between the *de novo* IBD cohort and those with pre-existing IBD except for IBD type (57% UC vs 88%, $p=0.008$).

Significantly more patients were not on any IBD medications post-LT compared to pre-LT (75% vs 91%, $p=0.02$). Fewer post-LT patients were on aminosaliclates (63% vs 79%, $p=0.03$) and prednisone (14% vs 31%, $p=0.03$) compared to pre-LT. Post-LT recipients developed fewer IBD flares requiring hospitalization (27% vs 45%, $p=0.004$).

38% reported improvement in IBD activity post-LT. 23% and 33% reported no change and worsening activity, respectively. There was no significant difference between reported disease activity and immunosuppression, CMV status, rejection rate, recurrent PSC, transplant age, gender or race.

Conclusion

1) *De novo* IBD occurs in 23% post-LT, with less UC compared to pre-LT. 2) Post-LT, IBD activity improves clinically. 3) No factors were associated with development of *de novo* IBD or disease activity. 4) Prospective multi-center studies are needed to further characterize the natural history of IBD post-LT and identify predictive factors.

Non-Organ Specific: Economics,
Public Policy, Allocation, Ethics II

Abstract# 408

Implementation of an Abbreviated Pre-Emptive Monitoring Strategy for the Prevention of CMV Infection: A Pharmacoeconomic Analysis. N. A. Weimert,¹ D. J. Taber,¹ D. Wray,² A. Lin,³ C. F. Bratton,³ J. McGillicuddy,³ M. Egidi,² H. Ayiku,² P. K. Baliga,³ K. D. Chavin.³ ¹Dept of Pharmacy, MUSC; ²Dept of Medicine, MUSC; ³Dept of Surgery, MUSC, Charleston, SC.

Background: Symptomatic CMV continues to be a significant problem. The costs associated with its development remain high without an optimal method for prevention. The aim of this study was to measure the pharmacoeconomic impact of implementing an abbreviated pre-emptive monitoring strategy versus valganciclovir (VGC) prophylaxis in a large teaching hospital. **Methods:** Costs for this analysis were based on a societal perspective, including drug, personnel, hospital, outpatient infusion and monitoring costs related to resources needed to perform pre-emptive monitoring and treatment of CMV related events. Time per hr costs were nurse/data coordinator \$45, physician \$200, and PharmD \$52. CMV PCR cost was \$203. VGC cost per mg was calculated for prophylaxis, treatment and 30 days of consolidation as needed based on an AWP (\$0.09 per mg). Estimated cost of CMV syndrome was based on cost of PICC line placement, drug, personnel and supply costs based on 21 days of therapy and was estimated to be \$11,885.50. Inpatient admission cost per day were \$3727. **Results:** A total of 119 patients were included in this analysis. Baseline and transplant demographics were well matched. Table 1 displays the total direct and indirect costs accumulated for each group. CMV syndrome occurred in three patients for each group. There was no CMV disease in either group. 27% of patients in the pre-emptive group had DNAemia, but only 4 patients required oral anti-viral therapy. Provider time and lab monitoring costs were significantly higher in the preemptive group, while direct medication cost was significantly higher in the prophylactic group. **Conclusions:** Frequency of disease severity and outcomes were equal in each group. Although the overall costs between strategies is equivocal, allocation of resources to provide pre-emptive monitoring places the burden of disease prevention on the health care system versus the patient necessitating further abbreviation of these strategies.

	Pre-emptive	Prophylactic	p-value
Total	\$4266±8326	\$4102±6698	0.9
VGC	\$493±1285	\$4788±2063	<0.001
Physician Time (hr)	\$362±209	\$70±58	<0.001
Other Provider Time (hr)	\$78±45	\$61±26	0.01
CMV PCR	\$1,256±600	\$80±237	<0.001
CMV Syndrome	\$19,140±12564	\$15,512±6282	0.04

Abstract# 409

The Never-Ending Altruistic Donor. Michael A. Rees,¹ J. E. Kopke,² G. Hil,³ W. Reitsma,⁴ O. G. Pankewycz,⁵ A. L. Miller,¹ A. M. Hawxby,⁶ R. A. Montgomery,⁷ D. Abraham,⁸ T. Sandholm,⁸ A. R. Roth,⁹ T. Somnez,¹⁰ M. U. Unver.¹¹ ¹Urology, University of Toledo Medical Center, Toledo, OH; ²Institute for the Study of Health, Univ of Cincinnati, Cincinnati, OH;

³National Kidney Registry, Babylon, NY; ⁴NJ Sharing Network, Springfield, NJ; ⁵Medicine, Buffalo General Hospital, Buffalo, NY; ⁶Surgery, Univ of Mississippi, Jackson, MS; ⁷Surgery, Johns Hopkins Univ., School of Medicine, Baltimore, MD; ⁸Computer Sciences, Carnegie Mellon Univ, Pittsburgh, PA; ⁹Economics, Harvard Univ. & Business School, Cambridge, MA; ¹⁰Economics, Boston College, Chestnut Hill, MA; ¹¹Economics, Univ of Pittsburgh, Pittsburgh, PA.

INTRODUCTION: We propose a non-simultaneous form of kidney paired donation that starts with a living, non-directed donor (LND).

METHODS: A paired donation matching algorithm was developed to allow for LNDs to start potentially Never-Ending Altruistic Donor (NEAD) chains in addition to closed loops of 2-, 3- and 4-way exchanges.

RESULTS: In July 2007, a LND from Michigan traveled 1500 miles to donate a kidney to a woman whom he had never met in Arizona. The following week, the Arizona recipient's husband donated one of his kidneys to a 32-year-old woman in Ohio. The following month, the mother of this recipient traveled to a city 3 hours away to donate her kidney to a patient whose incompatible donor simultaneously gave a kidney to the fourth patient in the chain. The incompatible donor for this fourth recipient is now slated to give her kidney to a patient in Maryland. Over the past 9 months, 60 transplant programs have partnered to perform 10 paired donation transplants. Demonstrating the advantage of NEAD chains over classic paired donation, 8 of 10 transplants resulted from altruistic donor chains.

CONCLUSION: In order to fully realize the potential of the above approach, one must be willing to supplant two prevalent ideas: 1) that kidneys from altruistic donors

should be given to the top candidate on the deceased donor waiting list, and 2) that paired exchanges must be done simultaneously. While there are certain pitfalls to using chains of donors as opposed to traditional "swaps" (i.e. the possibility that a donor could renege, or the accumulation of type AB donors who are not likely to be able to begin another chain), this proposed paradigm shift could result in a very significant increase in both the number and quality of paired donation kidney transplants.

Abstract# 410

Communication Skills Training (CST) of Surgical Residents Enhances Performance in End of Life Simulations and Improves Donation Rates. A. Goldberg,¹ R. Milner,¹ C. Fisher,¹ J. Gaughan,¹ J. Travaline,¹ M. Jacobson,¹ M. Seamon,¹ D. Dempsey,¹ M. Harrison,² P. Mulvania,² G. George,² H. Nathan.² ¹Temple University Hospital, Philadelphia, PA; ²Gift of Life Donor Program, Philadelphia, PA.

Purpose: Medical literature and national best practices correlate families' understanding of brain death with organ donation rates. Analysis of hospital data demonstrated variability in family communication. Although surgical residents frequently interact with family members of potential donors and play a critical role in the donation process, they receive no formal CST. We sought to determine if pre-training residents improved performances in CST involving explanation and notification of brain death to family members and aided efforts to achieve the national donation rate goal of 75%.

Methods: In collaboration with a regional organ procurement organization (OPO), an educational model for end of life CST was developed. 17 surgical residents were divided into 2 groups. The first group (n=9) attended a 3-hour didactic session at the OPO that included role-playing exercises explaining brain death to families. OPO staff, trained to serve as family role-players and skills station coaches, debriefed residents after each simulation. The second group (n=8) received no specialized training. Six weeks later both resident groups participated in formal videotaped family communication simulations. Independent observers (hospital faculty and senior OPO staff), blinded to training, evaluated residents' communication skills using a 12-parameter assessment tool. All residents reviewed their videotaped performances and evaluations, then repeated the simulations after six months.

Results: During the study period, the pre-trained resident group assessment scores increased by 25% (p=0.0001), while the untrained group increased by 39% (p<0.0001). While evidence of improvement existed in both groups, pre-trained residents consistently scored higher when compared to untrained (80% vs. 71%, p=0.037). During this same time period, donation rates in the Surgical Intensive Care Unit (SICU) increased by 21%.

Conclusion: Our educational model demonstrated effective training in communication skills during end of life discussions. Although a direct relationship cannot be established, donation rates in the SICU increased after resident training. Incorporation of this training program into resident education has the potential to improve donation rates and increase the number of organs available for transplantation.

Abstract# 411

A Model Hospitalist Program for a Large Solid Organ Transplant Program. Saverio Mirarchi,¹ Graeme N. Forrest,¹ Benjamin Philosophie.² ¹Medicine, University of Maryland, Baltimore, MD; ²Surgery, University of Maryland, Baltimore, MD.

Objective: To improve the efficiency of care for solid organ transplant patients by having internists work in conjunction with transplant surgeons to manage transplant patients admitted to the hospital more than 30 days post organ transplant. This includes patients who have undergone kidney, pancreas, and liver transplants which our center transplants over 300 of these organs/year. In 2002, the organ transplant program was divided into a surgical transplant service (STS) and medical transplant hospitalist service (MTHS). The MTHS consists of four full time physicians, one part time physician, and one nurse practitioner with daily rounds from a transplant surgeon on a weekly rotating schedule.

Methods: We analyzed our data from the past five years since the inception of the MTHS at a large university medical center. The length of stay (LOS) for the MTHS and STS were compared to data from the previous combined program as well as data from the University Health Consortium (UHC). In addition, we looked at the cost of care to determine if there were any savings related to reduced LOS and adjusted for the combined salary of the MTHS.

Results: In the review period, the total admissions for both the MTHS and STS averaged 1450 admissions/year. Over the past five years the MTHS showed a major decrease in the LOS index, defined as the ratio between the observed LOS and the expected LOS based on UHC data. This index dropped from 1.27 to 0.91 for patients on the MTHS. There was also a parallel drop in the STS LOS index from 1.53 to 1.05. This was associated with a significant cost savings for the hospital. On average, the program has realized an average savings of approximately \$1,000,000 per year. When accounting for the combined salary for the MTHS group which is 625,000 dollars/year, the total savings over the 5 year period this amounts to \$1.9 million.

Conclusion: The creation of a MTHS working within an organ transplant program at a large university center has been able to demonstrate a major decrease in LOS for transplant patients which has resulted in decreased costs and improved efficiency. This has also allowed more focused care for a complex medical population. We believe that our program can serve as a model for similar programs at other busy transplant sites.

Abstract# 412

Organ Procurement Travel Practices and Risks: A Survey of Members of the American Society of Transplant Surgeons (ASTS). Michael J. Englesbe,¹ Robert M. Merion,¹ Jennifer Sinibaldi,¹ Elaine Berg,² Shawn J. Pelletier,¹ Christopher J. Sonnenday,¹ Jeffrey D. Punch.¹ ¹*Surgery - Transplantation, University of Michigan, Ann Arbor, MI;* ²*New York Organ Donor Network.*

Introduction: Although surgeons spend considerable time and resources traveling for organ procurement procedures, little is known about travel practices, risks, and concerns of surgeons regarding these activities.

Methods: We sent an email to all members of the ASTS requesting completion of a 20-item web-based questionnaire. A multivariable logistic regression model was fitted to assess factors associated with surgeon reports of travel-related accidents.

Results: The survey response rate was 38% (281/747). Most respondents (90%) practiced in the US or Canada; median time in transplant practice was 13.2 years (IQR 8.1, 18.8). Procurement procedures constituted a median of 4.2% (IQR 0, 10.0) of income. Modes of transportation included: fixed wing aircraft (21%), personal car (16%), car with professional driver (16%), ambulance (14%), OPO-owned vehicle (10%), and helicopter (5%). Many respondents (40%) were unsure who provided life or disability insurance for them while on organ procurement travel. Involvement in ≥ 1 procurement-related travel accident was reported by 15% of respondents. These accidents occurred while traveling by: fixed wing aircraft (41%), private car with professional driver (18%), ambulance (14%), helicopter (11%), other (11%), and personal car (5%). Multivariable analysis, adjusted for years in practice and number of donor operations, revealed the following significant risk factors for involvement in a procurement-related travel accident: thoracic (vs. abdominal) surgeon (OR 2.91; 95% CI 1.04-8.16) and percent of travel by air (fixed wing/helicopter) (OR 1.01; 95% CI 1.00-1.02). One-third of respondents reported that personnel at their center had been involved in an accident (122 accidents and 68 injuries). There were 10 fatalities reported. Respondents reported being in dangerous situations not resulting in an accident a median of 2.3 times (IQR 0.2, 4.7), and reported feeling less than "very safe" 84% of the time. Categories of travel worries (ordered from more to lesser importance) included: weather, significant other worrying about them, driver fatigue, mechanical failure, pilot/driver failure, and pilot fatigue.

Conclusion: Organ procurement travel practices are associated with important, previously unquantified risks and concerns for surgeons. National standards for organ procurement travel are needed.

Abstract# 413

Transplant Tourism in the US: A Single Center Experience. Jagbir Gill,¹ Gerald Lipshutz,¹ David Gjertson,¹ Suphamai Bunnapradist,¹ Bhaskara Madhira,¹ Alan Wilkinson,¹ Michael Cecka,¹ Gabriel Danovitch.¹ ¹*UCLA, LA, CA.*

With increasing demand for kidney transplants (Tx), more patients are opting to travel outside the US to obtain transplantation. We describe the characteristics and outcomes of 32 kidney Tx recipients followed at our center who traveled abroad for a kidney Tx between 1995 and 2007.

Methods: Data were obtained via chart review. We compared demographics to all Tx recipients at our center during the same period and compared post-Tx outcomes to a cohort of patients transplanted at our center matched for age, race, Tx year, dialysis time, prior Tx, and donor type. Median follow-up time was 487 days (range 31-3056).

Results: Demographics are outlined in Table. Patients transplanted abroad were more likely to be Asian and had shorter dialysis times. Most patients were transplanted in China (44%) followed by Iran (16%), the Philippines (13%), and India (9%). Living unrelated Tx were most common. All patients were discharged on a calcineurin inhibitor, pred, and either MMF (90%), AZA (7%), or rapamycin (3%). 7 patients received induction. Only 4 patients received CMV prophylaxis. The median duration of hospitalization was 15 days.

The median time post-tx to initial visit at our center was 35 days. 4 patients required urgent admission to hospital, 3 of whom lost their grafts. 17 patients (53.2%) had at least 1 infectious complication including CMV and VRE. 7(40%) required hospitalization and 1 died from hepatic failure after contracting hepatitis B from the donor. 4 patients (12.5%) had surgically related complications.

Demographics			
	Tx Abroad	Tx local	P value
N	32	2863	
Mean Age (y)	47.2 (+/-11.0)	43 (+/-16.7)	0.15
Race			<0.001
Asian	62.5%	10.5%	
White	28.1%	34.9%	
Black	3.1%	15.5%	
Other	6.3%	39.1%	
% Re graft	6.3%	8.6%	0.94
Pre-tx dialysis	87.5%	88.3%	0.62
Med dialysis time (d)	530.8 (0-1800)	929 (0-14,403)	<0.001
Donor Source			<0.001
Living Unrelated	59.4%	8.7%	
Living Related	12.5%	27.6%	
Deceased	28.2%	63.7%	

Graft survival at 1-year was 96% for both "tourists" and matched recipients at our center. Median time to graft loss was 500 (31-2832) days. Mean SCr and rejection

1-year post Tx was not significantly different between recipients transplanted abroad and at our center.

Conclusion: Compared to patients transplanted locally, patients transplanted abroad had similar graft survival and renal function post Tx, but had a high incidence of infectious complications.

Abstract# 414

Comparison of Patient Information in the Adult-to-Adult Living Donor Liver Transplantation Cohort Study (A2ALL) and the Scientific Registry of Transplant Recipients (SRTR) Database. B. W. Gillespie,¹ R. M. Merion,¹ A. O. Ojo,¹ A. Shaked,¹ R. S. Brown,¹ C. A. Nielsen,¹ C. L. Berg,¹ E. Ortiz-Rios,¹ M. M. Abecassis,¹ A. S. Ashworth,¹ C. E. Freise,¹ R. M. Ghobrial,¹ J. F. Trotter,¹ J. E. Everhart.¹ ¹*A2ALL Study Group, NIH-NIDDK.*

Background: Although the SRTR database includes data submitted to the Organ Procurement and Transplantation Network (OPTN), audits of OPTN data have rarely been performed. The aim of this study was to compare data submitted to the OPTN with data from detailed medical record extraction by trained A2ALL coordinators.

Methods: OPTN/SRTR data elements (n=44) for 785 liver transplant candidates who had a living donor evaluated between 1/1/98 and 2/28/03 at the 9 A2ALL centers, and 12 data elements for 386 living liver donors, were compared with A2ALL chart review data performed independently of OPTN data submission.

Results: Values were present and identical $\geq 90\%$ of the time in both databases for 9 of 24 baseline recipient elements (sex, birth date, race, ethnicity, ABO, weight, previous transplant, medical condition, on ventilator), 2 of 20 recipient transplant or follow-up elements (variceal bleed, waitlist removal reason), and 6 of 12 donor elements (sex, race, ethnicity, ABO, relationship to recipient, state of residence). Values present but discrepant in the two sources were uncommon (<9%). Values were often missing in OPTN/SRTR and present in A2ALL (Table). Conversely, variables with values present in OPTN/SRTR but missing in A2ALL were less common (education, cold ischemia time and HCV RNA; ~6% each).

Conclusion: Many variables studied had values present and identical in the A2ALL and OPTN/SRTR databases. However, variables with missing data were more prevalent in the OPTN/SRTR database and may be associated with bias or reduced power when used in OPTN/SRTR analyses.

Variable	% Missing in OPTN/SRTR, present in A2ALL
Recipient baseline elements (n=24) (785 subjects):	
Height, education, functional status, encephalopathy, ascites, variceal bleed, diabetes, previous upper abdominal surgery, spontaneous bacterial peritonitis, dialysis, angina/CAD, drug-treated hypertension	10-12%
Recipient elements at transplant or follow-up (n=20) (594 subjects):	
HCV-RNA (among 269 with HCV)	37%
Cause of death (among 107 deaths)	28%
Weight, encephalopathy, dialysis, INR, ALT SGPT, treated rejection during first year post-transplant	14-17%
HCC recurrence, history of TIPSS, functional status	10-12%
Living Donor elements (n=12) (386 subjects):	
Height, weight, CMV IgG, education	20-28%

Abstract# 415

Economic Impact of Tolerance Induction by Combined Kidney and Bone Marrow Transplantation. Tatsuo Kawai,¹ David H. Sachs,² Thomas Spitzer,³ Nina Toloff-Rubin,¹ Mary Lin Farrell,¹ Megan Sykes,² Robert B. Colvin,⁴ A. Benedict Cosimi.¹ ¹*Transplant Unit, Massachusetts General Hospital, Boston, MA;* ²*Transplant Biology Research Center, Massachusetts General Hospital, Charlestown, MA;* ³*Hematology, Massachusetts General Hospital, Boston, MA;* ⁴*Pathology, Massachusetts General Hospital, Boston, MA.*

[Background] The primary benefits anticipated following successful induction of allograft tolerance are avoidance of the complications of long-term immunosuppression and prevention of chronic rejection. We have previously reported successful induction of renal allograft tolerance in recipients of HLA mismatched combined kidney and bone marrow transplantation (CKBMT). No evidence of chronic rejection has been observed in these recipients after immunosuppression-free periods of 1 to 4 years. In the current study, we have evaluated the longer-term economic impact of this approach.

[Method] The conditioning regimen for CKBMT included cyclophosphamide, thymic irradiation, anti-CD2 mAb, and a calcineurin inhibitor, which was discontinued after 9-12 months. 18 stable renal transplant recipients receiving ongoing triple or double drug immunosuppressive therapy with compatible follow up times were compared with the four tolerant recipients. Tolerant patients and stable patients on maintenance immunosuppression were also comparable with respect to their age, their original disease and donor-recipient histocompatibility.

[Results] The perioperative charges for CKBMT were approximately \$90,000 higher than those for conventional living donor kidney transplantation. After 9-12 months, continuing medications in CKBMT recipients included only occasional over-the-counter analgesics. In contrast, the stable conventionally treated recipients were taking an average of 8 pills daily. These included treatments required for de novo diabetes (11%), hypertension (67%), hyper lipidemia (22%) and gastro-intestinal symptoms/

prophylaxis (78%) in addition to their maintenance immunosuppression. These needs resulted in annual maintenance charges of over \$15,000/year/allograft recipient and do not include unmeasured costs related to issues such as quality of life or absences from employment.

[Conclusion] Even with this admittedly expensive approach to tolerance induction, the overall medical costs for conventional kidney transplantation with ongoing immunosuppression and treatment for complications will exceed the cost of tolerance after approximately 5 years.

Abstract# 416

Increasing African American Donation Rates in a Midwest Metropolitan Community. Susan Gunderson,¹ Susan Mau Larson,¹ David M. Radosevich,² Clarence Jones,³ Bill Tendle,³ Tiffany Scott.¹ ¹LifeSource, St. Paul, MN; ²Transplant Information Services, University of Minnesota, Minneapolis, MN; ³Southside Community Health Services, Minneapolis, MN.

Purpose: African Americans are underrepresented in deceased organ donation in this +2 million community. Historically minimal educational outreach had occurred and this study was designed to understand the community's disposition toward donation and to increase support for donation.

Methods: Television, newspaper, and radio advertising aired over a 24 month period beginning in 2004 using the nationally produced Donate Life-African American campaign as the primary intervention. Other components included related faith-based and community outreach. The OPO partnered with a minority focused community health clinic to survey African Americans pre- and post- intervention. A mailed, self-administered survey was sent to a sample drawn from organizational lists with a high likelihood of including African American households. For the evaluation, the sample was separated into 1) community members and 2) church members exposed to faith-based campaigns.

Results: African Americans in this community have a sophisticated understanding of the importance of donation (average 12 of 15 knowledge questions scored correctly) in pre-intervention survey. In both community and church groups media exposure and donation knowledge increased after the media campaign ($p<0.001$ and $p=0.004$ respectively). Similarly, donor designation rates for the entire population on the drivers licenses increased (33.0% versus 40.1%, $p=0.119$). Among all African Americans the propensity to donate was, however, unchanged following the campaign. Propensity to donate increased ($p=0.034$) in the community sample whereas there was a reduced propensity to donation ($p=0.092$) in the church sample.

During the study time period the OPO also experienced significant increases in donation authorization rates among African-Americans, increasing from 37% to 75%.

Authorization Rate				
	2003	2004	2005	2006
African American	37%	29%	52%	75%
Caucasian	76%	72%	80%	87%
Total Population	69%	66%	70%	78%

Summary: A media based campaign combined with grassroots outreach is an effective tool to increase knowledge and awareness. Partnership between the OPO and community and faith based leadership was a significant positive byproduct of the project and should be included in future outreach efforts.

Regulation, Tolerance and Intragraft Markers in Human Transplantation

Abstract# 417

Anti-Thymocyte Globulin Induction Therapy Favors the Generation of Memory CD4⁺FoxP3⁺CD127^{-low} Regulatory T Cells in Patients after Kidney Transplantation. V. D. K. D. Sewgobind,^{1,2} M. M. L. Kho,¹ L. J. W. van der Laan,² T. van Dam,¹ H. W. Tilanus,² J. N. M. IJzermans,² W. Weimar,¹ C. C. Baan.¹ ¹Internal Medicine; ²Surgery, Erasmus Medical Center, Rotterdam, Netherlands.

Background: It has been hypothesized that the clinical benefits of Anti-Thymocyte Globulin (ATG) induction therapy do not completely result from immunodepletion, but may also result from induction of immunoregulatory T-cells (Treg). In this prospective, controlled study we investigated the effect of ATG-induction therapy on the frequency and phenotype of peripheral CD4⁺FoxP3⁺ CD127^{-low} T-cells in kidney transplant patients.

Methods: After transplantation, 16 patients received ATG-induction therapy (Thymoglobulin®) and triple therapy consisting of tacrolimus, MMF and steroids. The control group (n=18) received triple therapy only. By flow cytometry, T-cells were analyzed for markers associated with immune regulation: CD25, FoxP3 and CD127. Within the FoxP3⁺ T-cell population, the CD45RO (memory) and CCR7 (homing receptor) markers were characterized.

Results: Pre-transplant levels of CD4⁺FoxP3⁺CD127^{-low} T-cells in all patients were 3-17% (median 6%) of CD4⁺ T-cells. One wk post ATG induction therapy, no measurable numbers of Treg were present. At 12 wks post ATG-induction therapy, a higher proportion of the first detectable T-cells expressed FoxP3 compared to the control-group (ATG vs. control-group; 7 vs. 4%, median, respectively, $p=0.03$) and then returned to pre-transplant levels at 26 wks. This increased proportion of CD4⁺FoxP3⁺CD127^{-low}

^{low} T-cells resulted in a significantly higher FoxP3⁺/FoxP3^{neg} ratio than in the control-group at 12 wks; (ATG vs. control-group; 0.074 vs. 0.046, median, $p=0.02$). At 26 wks, we found a decline in the proportion of naive FoxP3⁺ Treg (CD45RO^{neg}CCR7⁺ pre-transplant vs. post-transplant; 21 vs. 9%, median, $p=0.02$) which was associated with a rise in the proportion of memory FoxP3⁺ Treg (CD45RO⁺ pre-transplant vs. 26 wks post-transplant; 65 vs. 82%, $p=0.02$). Moreover, the proportion of memory T-cells exceeded that in the control-group at 26 wks (ATG vs. control-group; 82% vs. 67%, median, $p=0.05$) which was mainly due to an increase in the proportion of effector memory FoxP3⁺ Treg (CD45RO⁺CCR7^{neg}).

Conclusion: After ATG-induced immune cell depletion, a shift towards CD4⁺FoxP3⁺CD127^{-low} peripheral regulatory T-cells with the memory phenotype was measured in kidney transplant patients. This finding suggests that ATG treatment triggers the generation of de novo peripheral regulatory T-cells by homeostatic proliferation.

Abstract# 418

Kidney Transplant Recipients Readily Develop Donor-Specific CD4⁺CD25^{bright}+FoxP3⁺ Regulatory T Cells in the First Year after Transplantation. Thijs Hendriks,¹ Eveline van Gurp,¹ Wendy Mol,¹ Wenda Schoordijk,¹ Mariska Klepper,¹ Annemarie Geel,¹ Jan IJzermans,² Willem Weimar,¹ Carla Baan.¹ ¹Internal Medicine, Erasmus MC, Rotterdam, Netherlands; ²Surgery, Erasmus MC, Rotterdam, Netherlands.

Introduction In a prospective study, we investigated whether donor-specific regulatory CD4⁺CD25^{bright}+FoxP3⁺ T cells develop in kidney transplant patients.

Methods We analyzed the percentage and function of peripheral regulatory CD4⁺CD25^{bright}+FoxP3⁺ T cells of 51 patients before, 3, 6 and 12 months after kidney transplantation. The immune regulatory capacities of CD4⁺CD25^{bright}+FoxP3⁺ T cells were assessed by their depletion from PBMC and reconstitution to CD25^{neg}dim responder T cells at a 1:10 ratio in the MLR.

Results In the first year after transplantation, peripheral CD4⁺CD25^{bright}+FoxP3⁺ T cells decreased from $8.7\% \pm 3.0$ pre-transplantation to $6.1\% \pm 1.8$ at 12 months ($p<0.001$). While MLR reactivity to 3rd party-Ag (3rd P) significantly improved ($p<0.05$), the reactivity against donor antigens remained low. Functional analysis demonstrated potent donor-specific regulatory activities by CD4⁺CD25^{bright}+FoxP3⁺ T cells after transplantation. Depletion of CD4⁺CD25^{bright}+FoxP3⁺ T cells from PBMC resulted into increased proliferation upon stimulation by donor antigens ($p<0.01$). Upon reconstitution, the capacity of CD4⁺CD25^{bright}+FoxP3⁺ T cells to control the proliferation of anti-donor reactive CD25^{neg}dim T cells increased over time: from 58% (median) pre-transplant to 85% post-transplant at month 12 ($p<0.01$). Moreover, the anti-donor regulatory activities by the CD4⁺CD25^{bright}+FoxP3⁺ T cells were significantly more vigorous than those controlling 3rdP-Ag stimulated responder T cells (65%, $p<0.05$).

Conclusion The generation of potent donor-specific regulatory CD4⁺CD25^{bright}+FoxP3⁺ T cells in the periphery of kidney transplant patients prevents the development of adequate alloreactivity.

Abstract# 419

Recruitment of FOXP3 Expressing Regulatory T Cells into Tolerant Graft of Human Liver Transplantation. Ying Li,¹ Xiangdong Zhao,¹ Hironori Haga,² Tatsuaki Tdsuruyama,³ Kathryn J. Wood,⁴ Shimon Sakaguchi,⁵ Shinji Uemoto,⁶ Takaaki Koshiba.¹ ¹Innovation Center for Immunoregulation Technologies and Drugs, Transplant Tolerance Unit, Graduate School of Medicine, Kyoto University, Kyoto, Japan; ²Diagnostic Pathology, Graduate School of Medicine, Kyoto University, Kyoto, Japan; ³Pathology and Biology of Disease, Graduate School of Medicine, Kyoto University, Kyoto, Japan; ⁴Nuffield Department of Surgery, University of Oxford, John Radcliffe Hospital, Oxford, United Kingdom; ⁵Experimental Pathology, Institute for Frontier Medical Science, Kyoto University, Kyoto, Japan; ⁶Surgery, Graduate School of Medicine, Kyoto University, Kyoto, Japan.

(Background) Experimental transplantation (Tx) models demonstrated that recruitment of regulatory T cells (Tregs) at the graft site was crucial for the development of tolerance (Tol). However, this has never been examined in clinical Tx. **(Methods)** The expressions of innate and adaptive immunities-associated markers, including FOXP3, were systemically quantified with real-time PCR in the grafts of liver Tx patients, who discontinued immunosuppression completely (Group-Tol). By immunohistochemistry and immunofluorescent staining, the number of FOXP3 protein expressing cells (FOXP3⁺ cells) and their distribution were examined in relation to CD4⁺ and CD8⁺ cells. The results were compared with those of grafts which removed due to chronic rejection (Group-CR) and the normal liver tissues (Group-NL). **(Results)** The expressions of CD4, CD8, CD25, CD19, Vα24 and FOXP3 mRNA were higher in Group-CR than those in Group-NL (CD19: $p=0.084$ the others: $p<0.01$), whereas in Group-Tol, only FOXP3 and CD19 mRNA were up-regulated in the absence of all other markers up-regulation (CD19: $p=0.055$ FOXP3: $p<0.01$ the others NS). Unlike FOXP3 mRNA, FOXP3⁺ cells were readily detectable in the portal area (PA) in Group-Tol (12.1cells/10PAs), while virtually no FOXP3⁺ cells were detectable in Group-CR (0cells/10PAs) or Group-NL (0.8cells/10PAs) ($p<0.001$ Group-Tol vs. Group-CR, -NL). In Gr-Tol, 92% of FOXP3⁺ cells was CD4⁺ and 8% was CD8⁺ and FOXP3⁺ cells accumulated within clusters of CD4⁺ and CD8⁺ cells. **(Conclusions)** This is the first report providing evidence that

CD4⁺FOXP3⁺ Tregs accumulate in the tolerant graft and suppress rejection in a cell-cell-contact-dependent manner in human liver transplantation. Intra-graft expression of FOXP3 protein, but not *FOXP3* mRNA expression, can be an informative biomarker in distinguishing tolerance and rejection.

Abstract# 420

Anti-Donor Alloimmunity in Long-Term "Stable" HLA-Identical LRD-Kidney Transplant Patients Monitored by Multiple *Ex Vivo* Assays.

James M. Mathew,^{1,2} Teresa Vallone,² Anne Rosen,^{1,2} Bonnie B. Blomberg,² Laphalle Fuller,² George W. Burke,² Gaetano Ciancio,² Violet Esquenazi,² Joshua Miller.^{1,2} ¹*Surgery - Transplant, Northwestern University & Jesse Brown VA Med Center, Chicago, IL;* ²*Surgery - Transplant, University of Miami & VA Med Center, Miami, FL.*

Purpose: The most tolerated clinical renal transplants are presumed to be between HLA-identical (HLA-id) siblings. However, even in this group of patients the 10-year actual graft survival is only 67% with a slope that has a late downward curve. We hypothesize that this is partly due to adaptive anti-donor immune responses that can be detected even among "stable" long-term recipients by using multiple *ex vivo* assays.

Methods: We have monitored the anti-donor immune responses between 44 and 114 months post-operatively in 17 HLA-id and 10 haploidentical LRD-kidney transplant patients, who had no significant increase in their creatinine levels. Six pre-transplant HLA-id recipients and 4 non-transplant HLA-id normals were also included for comparison. The assays used were MLR, micro-CML, ELISPOT assays for IFN- γ and granzyme-B producing cells, Transvivo-DTH (Tv-DTH), and microcytotoxicity and luminex for antibody specificities.

Results: It was observed that 9/17 HLA-identical transplant recipients remained specifically unresponsive to the donor in all 6 assays. However, 8/17 developed reactivity to the donor in one or more of these assays, as was also seen in all 10 haploidentical controls. This was in contrast to the total unresponsiveness in the HLA-id recipients pre-transplantation and in HLA-id normals. All individuals were reactive to third party or to appropriate positive controls in all assays at all times tested. None, however, developed anti-donor antibodies. Among the HLA-id post-transplant reactors, positive responses were observed in MLR, IFN- γ and granzyme-B production, and Tv-DTH (Table 1).

Anti-donor reactivity in HLA-identical and haploidentical LRD-renal transplant patients in *ex vivo* assays

	HLA-Identical			Haploidentical		
	Total	Reactors	Non-Reactors	Total	Reactors	Non-Reactors
MLR	17	2	15	10	7	3
Micro-CML	16	0	16	10	4	6
IFN- γ	17	5	12	7	3	4
Granzyme-B	13	5	8	8	2	6
Tv-DTH	9	4	5	7	7	0
Anti-donor Abs	17	0	17	10	0	10

Conclusions: These results indicated that anti-donor responses could be detected even in a significant proportion of "stable" long-term HLA identical kidney transplant recipients. Speculatively this may be the cause of late graft failures in this group of patients.

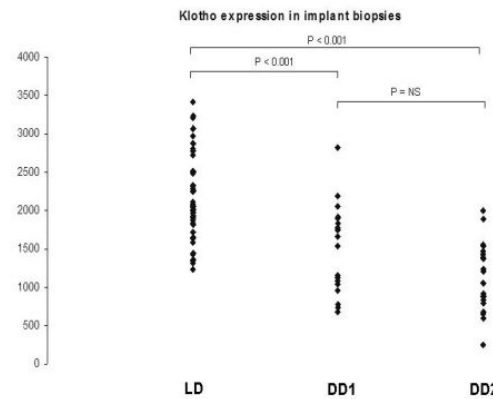
Abstract# 421

Expression of Renal Klotho Is Decreased in Deceased Donor Kidneys.

Thomas F. Mueller,¹ Philip F. Halloran,¹ Valerie A. Luyckx.¹ ¹*Medicine, University of Alberta, Edmonton, AB, Canada.*

Klotho is a gene almost exclusively expressed in renal distal tubules and loss of expression is associated with accelerated aging. In various models of acute and chronic renal injury, and with normal aging, renal klotho expression has been found to decrease. Increased donor age is associated with poorer long term allograft function. We hypothesized that klotho mRNA expression in renal implant biopsies would correlate with donor kidney quality, as determined by donor chronologic age and peri-transplant renal injury.

Our recent unsupervised microarray analysis of 87 renal implant biopsies revealed a continuum of organ quality across all samples ranging from the best living donor (LD) kidneys at one end to the worst performing deceased donor (DD) kidneys at the other (Am J Transpl 2007, Nov 16, epub). Three predominant groups were identified of LD, DD1 kidneys with low risk (9.5%) of delayed graft function (DGF) and DD2 kidneys with high risk (35%) of DGF ($p < 0.05$). Analysis of klotho gene expression among these groups also revealed a spectrum of expression from highest levels in LD to lowest in DD2 kidneys, especially those who developed DGF. Differences were highly significant among LD vs DD kidneys ($p < 0.001$), although donor age was not different between these groups.



Klotho transcript levels were significantly different among kidneys that developed DGF compared to those with immediate graft function (IGF) ($p < 0.05$).

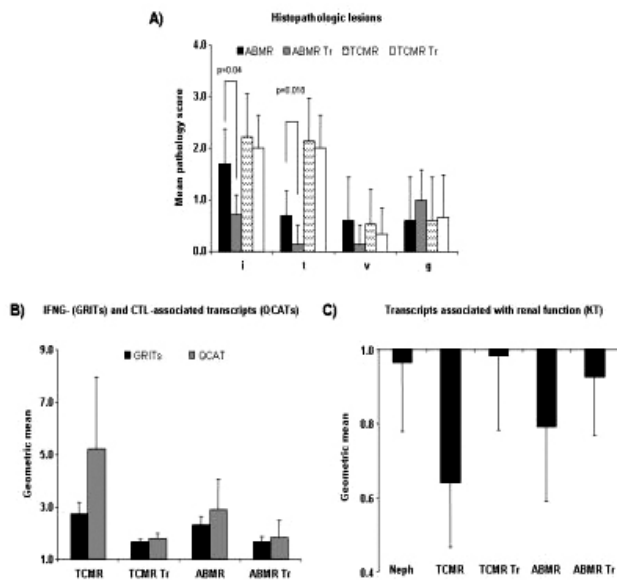
In conclusion, reduced klotho gene expression is associated with grafts at risk of poorer function and appears to be independent of donor age. Decreases in klotho expression likely reflect other factors impacting the renal tissue, which may impact potential for repair and ultimately allograft function.

Abstract# 422

Anti-Rejection Treatment before Biopsy Is Associated with Extensive Normalization of Transcript Changes Despite Persistence of Histopathology Lesions.

Luis G. Hidalgo,¹ Sakarn Bunnag,¹ Thomas Mueller,¹ Phil F. Halloran.¹ ¹*Medicine, University of Alberta, Edmonton, AB, Canada.*

Renal allograft rejection episodes produce a stereotypical response in the allograft characterized by infiltration by cytotoxic T lymphocytes (CTL), potent IFN response by donor and recipient cells, and decreased transcripts associated with the epithelium. We previously identified pathogenesis based transcript sets (PBTs) that reflect the disturbance in rejecting allografts (QCATs - CTL, GRITs - IFN response, KTIs - decreased function, AJT 7:2712, 2007). We hypothesized that anti-rejection treatment would reverse the transcriptome changes of rejection more than histopathologic lesions. Using microarray analysis we measured expression of these PBTs in 18 antibody mediated rejection (ABMR) (11 untreated, 7 treated) and 31 T-cell mediated rejection (TCMR) (25 untreated, 6 treated) biopsies, normalized to nephrectomy samples. Histopathology scoring of the primary rejection lesions were analyzed; interstitial inflammation(i), tubulitis(t), intimal arteritis(v), and glomerulitis(g) (Fig 1A). Of these, only i and t differentiated between ABMR and treated ABMR ($p < 0.05$) yet none differentiated between TCMR and treated TCMR. PBTs on the other hand differentiated treated from untreated for both ABMR ($p < 0.04$) and TCMR ($p < 0.005$) with all 3 PBTs (Fig 1B/C). The changes in transcript expression in treated cases were dramatic. Particularly impressive was the consistent correction of the disturbances in PBT expression since values in treated cases approached those of nephrectomy samples. Thus, histologic rejection lesions persist following anti-rejection treatment whereas transcript disturbances of rejection are greatly reduced compared to untreated cases. This study suggests that assessment of anti-rejection treatment is more sensitively monitored by transcript expression than histopathology.

**Abstract# 423**

Blood and Intra-Graft Toll-Like Receptor 4 mRNA Profiles in Tolerance and Chronic Rejection in Kidney Transplant Patients. Joanna Ashton-Chess,¹ Cécile Braudeau,¹ Magali Giral,¹ Emilie Dugast,¹ Stephanie Louis,¹ Annaick Pallier,¹ Christophe Braud,¹ Anne Moreau,¹ Karine Renaudin,¹ Jean-Paul Souillou,¹ Sophie Brouard.¹ ¹INSERM U643; CHU Nantes; Institut de Transplantation et de Recherche en Transplantation; Université de Nantes, Nantes, France.

Deciphering the mechanisms of tolerance and chronic immune-mediated rejection remains a major goal in transplantation. Data in rodents suggests that Toll-Like-Receptors (TLR), regulators of innate immune responses, play a role in determining graft outcome. However, few studies have focused on TLR in human kidney transplant recipients. We addressed this issue by analyzing the peripheral blood (n = 75) and graft biopsies (n = 26) of renal transplant patients and healthy volunteers. We analyzed, for the first time, the expression of TLR4 in PBMC from kidney recipients with contrasted situations: operational tolerance and chronic immune-mediated rejection (Banff 2005), compared to patients with normal histology and stable graft function, non transplant patients with renal failure and healthy volunteers. We found that MyD88 and TLR4 were significantly contrasted in the PBMC, and in particular in monocytes, of patients with chronic immune-mediated rejection vs. operational tolerance. Chronic rejection patients had significantly increased TLR4 and MyD88 compared to operationally tolerant patients, who resembled healthy volunteers and non transplant patients with renal failure. Interestingly, analysis of TLR4 transcripts in graft biopsies from patients with normal histology or chronic immune-mediated rejection reflected the blood findings, with a significant increase of TLR4 in chronic immune-mediated rejection. Thus, we provide data to support a link between TLR4 expression and long-term graft outcome. The role of TLR and their endogenous ligands in mediating allograft rejection or acceptance therefore warrants further investigation and could give rise to new strategies of therapeutic intervention. Our results suggest that peripheral blood TLR4 shows potential as a biomarker of chronic immune-mediated rejection and that measuring blood TLR4 levels may help to identify patients experiencing chronic rejection who require a biopsy. Moreover, our data suggest that absence of TLR signaling may be a feature of operational tolerance to kidney grafts.

Abstract# 424

Identification of Immune Tolerance in Renal Transplants. Maria Hernandez Fuentes,¹ B. Sawitzki,² E. Perucha,¹ P. Sagoo,¹ P. Miqueu,³ S. Chapman,⁴ D. Stephens,⁵ L. Craciun,⁶ S. Tomiuk,⁷ S. Brouard,³ M. Giral,³ U. Janssen,⁷ M. Goldman,⁶ K. Wood,⁴ A. Warrens,⁸ H.-D. Volk,² J.-P. Souillou,³ R. Lechler.¹ ¹King's College London, United Kingdom; ²Charité University Medicine, Berlin, Germany; ³ITERT-INSERM U643, Nantes, France; ⁴University of Oxford, United Kingdom; ⁵McGill University, Canada; ⁶Université Libre Bruxelles, Belgium; ⁷Miltenyi Biotec GmbH, Cologne, Germany; ⁸Imperial College London, United Kingdom.

Identification of immunological tolerance is an important prerequisite in order to establish an individually-tailored approach to the post-transplant management of allograft recipients. It will also provide new insight into the mechanism underlying the balance between tolerance and rejection. Here we present data from a multi-centre study aimed at identifying tolerance to renal allografts. We have collected samples from five selected groups of renal transplant recipients: drug-free tolerant patients

that were functionally stable despite remaining immunosuppression-free for more than one year; functionally stable patients on minimal immunosuppression (<10 mg/day prednisone); stable patients maintained with calcineurin inhibitors (CNI); stable patients maintained on CNI-free immunosuppression regimen; and patients showing signs of chronic rejection. A group of age and sex matched healthy volunteers was also included as control.

Several biomarkers and bioassays, were combined to provide an immunological 'fingerprint' of the tolerant state.

Immunophenotype showed a selective expansion of peripheral blood B and NK lymphocytes in drug-free tolerant patients. This group of patients was also characterized by the absence of anti-donor specific antibodies. The differential expression of several immune relevant genes and a high ratio of foxp3/ α -1,2-mannosidase expression in these patients was observed. TCR landscape analysis highlighted differences between the V β repertoires of drug-free tolerant recipients and chronic rejection patients. Additionally, direct pathway donor-specific hyporesponsiveness by IFN γ ELISpot and lack of indirect pathway anti-donor responses assessed by *trans-vivo* DTH were detected in drug-free patients.

Conclusions: The diagnostic capabilities of the combined results of several of the above mentioned biomarkers and bioassays are as follows: specificity 0.964, sensitivity of 0.933 and a positive predictive value of 82.4%. These biomarkers could be used to inform drug weaning protocols of kidney transplant recipients.

Abstract# 425

Bile Acid Aspiration Stimulates Lung Allograft Immunity. Frank D'Ovidio,¹ Marco Mura,² Thomas K. Waddell,² Lianne G. Singer,² Cecilia Chaparro,² Micheal Hutcheon,² Andrew Pierre,² Martin Post,² Mingyao Liu,² Shaf Keshavjee.² ¹Columbia University; ²University of Toronto.

Bile acids detected in the broncho alveolar lavage (BAL) as a marker of aspiration has been associated in a dose dependent fashion to earlier development of bronchiolitis obliterans syndrome. We sought to study the relationship between bile acids and active immune molecules as detected in the BAL.

Methods: BAL collected prospectively from lung transplant recipients at routine surveillance bronchoscopies were assayed for bile acids. Samples were then assayed by Luminex for cytokines (TNF- α , IL-1 β , IL-12, IFN- γ , IL-10, IL-6, IL-15, IL-8, MCP-1), and by ELISA for pulmonary collectins (SP-A, SP-D). Results were analyzed according to levels of bile acids as per ROC testing for accuracy for bronchiolitis obliterans syndrome diagnosis (high levels ≥ 3.5 μ mol/L).

Results: We prospectively examined 120 lung transplant recipients and a total of 271 BAL samples were collected. In 114 no bile acids were detected, low levels were present in 110, and high levels were detected in 47 samples. Samples with high bile acids had significantly greater innate (TNF- α , IL-1 β , IL-12, IL-10, IL-6) and adaptive (IFN- γ , IL-2) cytokines as well as greater chemokines (MCP-1, IL-8) compared to the other samples. In contrast pulmonary collectins SP-A and SP-D were significantly reduced in samples with high bile acids. The figures shows the median and interquartile range for each molecule according to bile acid levels.

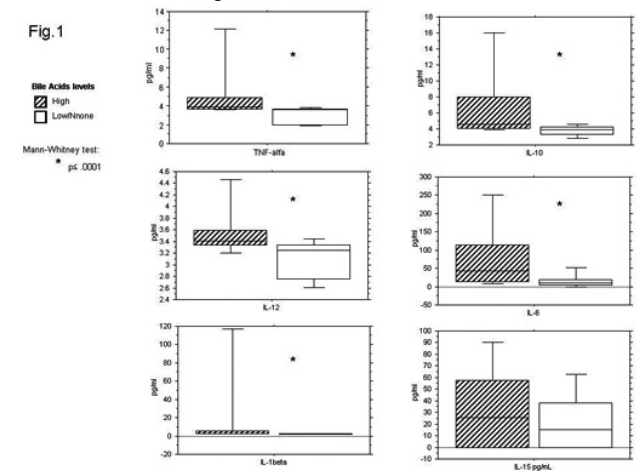
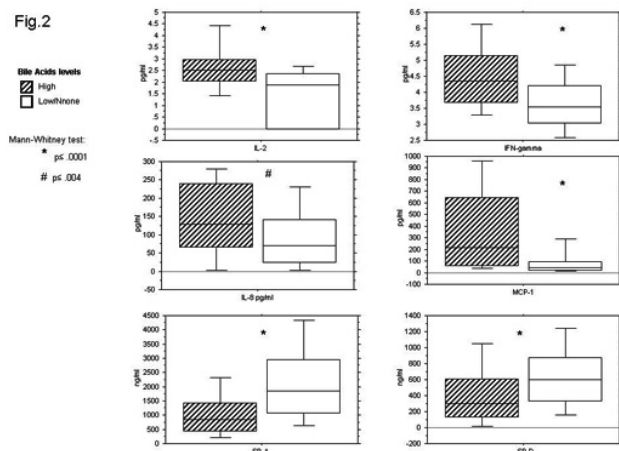


Fig.2



Conclusion: Bile acids detected in the BAL as markers of aspiration stimulate the lung allograft immunity in a dose dependent fashion. In particular high levels of bile acids are associated with an impaired lung specific innate defense system provided by the pulmonary collectins and with a broncho-alveolar district "cytokine storm".

Tolerance/Immune Deviation III

Abstract# 426

CD4⁺ T Cell Converted Double-Negative Regulatory T-Cells: A Novel Cell-Based Therapeutic Approach To Prevent and Cure Autoimmune Type I Diabetes in NOD Mice. Dong Zhang,¹ Wensheng Zhang,¹ Yan Tian,¹ XinXiao Zheng,¹ ¹*Surgery, Starzl Transplantation Institute, University of Pittsburgh School of Medicine, Pittsburgh, PA.*

Recently we have reported that CD4⁺ T-cells could convert to CD4-CD8-CD3⁺ (DN) T-cells after either allogeneic or syngeneic DC triggered proliferation, and IL-2 and IL-15 enhanced the conversion. Converted DN T-cells were resistant to AICD and expressed unique cell markers and gene profile. Moreover, converted DN T-cells were more potent in suppressing alloantigen triggered proliferation of naïve CD4⁺CD25⁺ T-cells than naïve and proliferated CD4⁺CD25⁺ Tregs in vitro.

In this study we further test the function of the converted DN T-cells in autoimmune diabetic NOD models. Adoptive transfer of T-cells from diabetic NOD mice precipitated a rapid onset of diabetes in all NOD/SCID recipients. In contrast, NOD/SCID recipients received DN T-cells(converted from CD4⁺ T-cells of diabetic NOD mice) alone remain diabetic free more than 30 weeks. The co-transfer of DN T-cells with diabetogenic T-cells at 1: 1 ratio significantly delay the onset of diabetes in NOD/SCID mice (P=0.0012). Thus, DN T-cells converted from diabetogenic CD4⁺ T-cells from diabetic NOD mice were capable of suppressing autoimmunity. Interestingly, the addition of GAD65 peptide, a beta-cell specific antigen, in the conversion further enhanced the suppressive function of DN T cells.

In a prediabetic NOD model, the adoptive transfer of 2 x 10⁶ DN T cells (converted from NOD CD4⁺ T-cells by co-culture with NOD DC plus rIL-15 and GAD65 in MLR) into 5 weeks old prediabetic NOD female mice resulted in significantly delay of onset of diabetes. Only 25% mice became diabetes at 22 weeks after DN T cell transfer compare with 75% mice became diabetic in control group. Moreover, a single transfer of 4 x 10⁶ DN T-cells reversed new onset of diabetes, albeit transient, in autoimmune NOD mice. We then combined rapamycin with DN T-cells in a MHC mismatched islet allograft model in spontaneous autoimmune diabetic NOD recipients. The single transfer of 5 x 10⁶ DN T-cells plus 14 days low dose rapamycin treatment significantly prolonged islet allograft survival in comparison with untreated group (MST 40 days vs. 6 days) and rapamycin alone treated group MST (40 days vs. 8 days).

The results of using ex vivo CD4⁺ T-cells converted DN T-cells in NOD mouse models support the concept and the feasibility of potentially utilizing this novel cell-based therapeutic approach clinically for the treatment of autoimmune type I diabetes.

Abstract# 427

A Novel Approach To Protect Islet Allografts by Co-Transplant with Hepatic Stellate Cells Via Promoting Infiltrating CD8⁺ T Cell Apoptosis. Horng-Ren Yang,¹ Goupeng Jiang,¹ John J. Fung,¹ Shiguang Qian,¹ Lina Lu,¹

¹*Immunology and General Surgery, Cleveland Clinic, Cleveland, OH.*

Liver transplant tolerance was recognized by spontaneous acceptance of liver allograft in many species. The underlying mechanism remains unclear. Interestingly, although liver allografts are accepted, hepatocyte transplants in the same combination are promptly rejected, indicating a crucial role of liver tissue cells in immune suppression. We have demonstrated a profound T cell inhibitory activity of hepatic stellate cells (HpSC), which are known to participating in repairing and fibrosis during liver injury. Addition of activated (a) HpSC, but not quiescent HpSC, significantly inhibited allo-

DC induced-T cell proliferative responses (MLR) in a dose dependent manner, which was associated with enhanced T cell apoptosis (TUNEL). Neutralization of B7-H1 by anti-B7-H1 mAb significantly reduced the HpSC-induced T cell apoptosis and reversed the inhibition of T cell proliferation, suggesting a key role of B7-H1. To evaluate this in vivo, BALB/c islets (300) were co-transplanted with 3 x 10⁵ activated HpSC (B6) into STZ-induced diabetic B6 recipients. Co-transplant with HpSC effectively protects islet allografts from rejection. This was associated with reduction of graft infiltrating T cells and enhancement of apoptotic activity. Co-transplant with HpSC from B7-H1^{-/-} livers markedly lost their islet graft protective capacity, associated with less apoptosis of infiltrating cells. To determine the subsets of apoptotic T cells, T cells were isolated from spleen, draining lymph nodes, and grafts for phenotype and function analyses. On POD 8, a marked reduction of graft infiltrating CD4⁺ (30.7 %) and CD8⁺ T cells (31.3 %) was seen in HpSC co-transplant group, as compared to islets alone, which was further progressed thereafter. CD8 T cells dropped ~7 folds on POD 140. CD4⁺/CD8⁺ ratio was increased from 0.5 at the early POD to 2.0 in the long-term survival grafts. Adoptive transfer of CFSE-labeled DES transgenic T cells was used to track the response and fate of antigen-specific CD8⁺ T cells. The results showed active division of DES⁺ cells within the allograft in both the islet only and HpSC co-transplantation groups. However, accumulation of these DES⁺ cells was significantly lesser in the HpSC co-transplantation group as compared to that in the islet only group (1.9x10³ vs. 10x10³ cells per graft). These findings suggest that HpSC induce antigen-specific CD8⁺ T cell death, and may not inhibit their activation.

Abstract# 428

CD8⁺ Memory T Cells Specific for a Single Surrogate Minor Antigen Are Sufficient To Inhibit Transplant Tolerance Induced by CTLA-4 Ig and Anti-CD154. Mandy L. Ford,¹ Craig M. Steeds,¹ Maylene E. Wagener,¹ Allan D. Kirk,¹ Christian P. Larsen,¹ ¹*Emory Transplant Center, Emory University, Atlanta, GA.*

Donor-specific memory T cells are potent mediators of allograft rejection due to their ability to proliferate and give rise to cytotoxic and inflammatory cytokine-secreting effectors within hours of stimulation. Furthermore, memory T cells have been shown to be relatively resistant to the effects of many tolerance-induction protocols, including blockade of the CD28 and CD154 pathways. While seminal studies have shown that donor-reactive memory cells, generated through pre-sensitization with donor tissue or infection with pathogens with cross-reactive epitopes, contribute to costimulation blockade-resistant rejection of fully MHC disparate allografts, the role of memory T cells specific for minor antigens in costimulation blockade-resistant rejection has not been well studied. We addressed the ability of memory T cells specific for a single donor-derived class I epitope to mediate this process. TCR transgenic T cells (OT-I) specific for SIINFEKL/Kb were adoptively transferred into naïve B6 recipients, which were then infected with ovalbumin- (SIINFEKL) expressing Listeria monocytogenes (LM-OVA). At memory, mice received a skin graft expressing ovalbumin (mOVA), and therefore the SIINFEKL epitope. Results showed that the transferred OT-I T cells proliferated in response to LM-OVA, but not in response to a control infection with wild-type Listeria (LM). At day 9, OT-I T cells comprised ~15% of the total CD8⁺ T cell compartment. During memory, the SIINFEKL-specific cells comprised ~1-2% of the total CD8⁺ T cell compartment. Engraftment of mOVA skin on LM-OVA memory recipients resulted in rejection with accelerated kinetics relative to LM infected controls (MST=13d vs 20d). Following treatment with CTLA-4 Ig and anti-CD154, 9/10 LM-OVA infected recipients experienced costimulation blockade-resistant rejection, while LM-infected controls went onto long-term graft survival (p<0.001). LM-OVA-infected recipients also resisted the engraftment of mOVA-expressing donor bone marrow following a tolerance induction protocol containing busulfan, CTLA-4 Ig, and anti-CD154. These results suggest that memory T cells specific for a single surrogate minor antigen are sufficient to induce costimulation blockade-resistant rejection, and support the feasibility of using this model to study the specific requirements for donor-reactive memory T cell activation and tolerance induction during transplantation.

Abstract# 429

The Zinc Finger Transcription Factor Ikaros Is a Master Regulator of Th1, Th2 and Th17 Cell Differentiation. Andrew D. Wells,¹ Justin J. Taylor,² Edward J. Pearce,² Rajan M. Thomas,¹ ¹*Pathology, University of Pennsylvania, Childrens Hospital of Philadelphia, Philadelphia, PA;* ²*Pathobiology, University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA.*

During an immune response, CD4 helper T cells can be instructed by non-antigen-specific signals to differentiate into functionally distinct subsets with mutually exclusive patterns of cytokine production. We find that Ikaros, a zinc finger transcription factor required for lymphocyte development, is crucial for the development of polarized T helper subsets. In the absence of Ikaros DNA binding activity, CD4 T cells induced to undergo Th2 differentiation in vitro or in vivo produce high levels of IL-4, but fail to silence expression of IFN-gamma and IL-17, cytokines that contribute to inflammatory disease processes such as autoimmunity and organ transplant rejection. Similarly, Ikaros is required for repression of IL-4 and IL-17 expression by Th1 cells, and inhibition of IFN-gamma and IL-2 production by Th17 cells. Our results show that Ikaros controls the expression of transcription factors such as GATA-3, c-Maf, Stat-6, and Runx3, and in polarized Th2 cells, Ikaros inhibits IFN-gamma gene expression through direct

repression of the T-bet locus. These studies place Ikaros, a DNA binding protein previously recognized only as a regulator of lymphocyte development, as a master regulator of peripheral T cell differentiation and function.

Abstract# 430

Defective Viral Clearance with Aging Leads to the Generation of Alloreactive IL-17 Producing T Cells. Bethany M. Tesar,¹ Heather Stout-Delgado,¹ Wendy E. Walker,¹ Hua M. Shen,¹ Daniel R. Goldstein.¹

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Introduction As the fastest growing subpopulation seeking organ transplantation is >65 yrs of age, it will be imperative to discern how aging impacts the acquisition of transplantation tolerance. Prior work has demonstrated that viral infections induce the development of alloreactive T cells, which impede the induction of transplantation tolerance. In this study, we tested the hypothesis that aging alters host defense against viruses leading to the development of cross-reactive T cells, which impair transplantation tolerance induction.

Methods + Results We first examined how aging modifies the function of plasmacytoid DCs (pDCs), as IFN α production by pDCs is essential for control of viral infections. Using both in vitro and in vivo murine systems, we found that aged pDCs produced lower levels of IFN α in response to TLR9 activation with CpG sequences or herpes simplex (HSV)-2 virus (ELISA). Aged mice (18-20 mths) failed to clear this virus as effectively as young (2-4 mths) mice. This was associated with increased liver inflammation, the release of systemic Th17-skewing cytokines, IL-6 and IL-21, and augmented splenic IL-17 levels in aged mice (ELISA). Prior to transplantation, unmanipulated aged mice (B6 or CBA background) produced more donor-specific IL-17 effector-memory T cells as compared to unmanipulated young mice (ELISPOT). Furthermore, MLR assays demonstrated that aged memory CD4⁺ T cells from unmanipulated mice produced significantly more IL-17 in response to donor antigen compared to young T cells (ELISA). To determine if aged recipients manifest an altered response to therapies that prolong allograft survival, aged and young B6 mice received BALB/c skin allografts and perioperative anti-CD45 and anti-CD154. We found that aged mice rejected their allografts significantly faster (median survival 20 days) than young mice (MST, 76 days, $p < 0.01$). Similar results were noted when we employed perioperative treatment with anti-CD154 + DST and when we altered the donor-recipient (B6 to CBA) strain combination. Pre-treating aged mice with an anti-IL-17 mAb improved the efficacy of the graft-prolonging therapy compared to aged mice that received control mAb ($p = 0.04$).

Conclusion Our results suggest that impaired control of viral infections with aging leads to the generation of alloreactive IL-17 producing T cells that impair therapies that may induce transplantation tolerance.

Abstract# 431

Prevention of Type 1 Diabetes by Thymus Genetic Modification with Protective MHC Class II Molecules. Jesus R. Paez-Cortez,¹ Michela Donnarumma,¹ Chaorui Tian,¹ John Iacomini.¹ *¹Transplantation Research Center, Brigham and Women's Hospital & Children's Hospital – Harvard Medical School., Boston, MA.*

Introduction. Susceptibility to type 1 diabetes is determined by multiple genetic factors, among the strongest of which is the inheritance of at-risk genes that lead to disease development. Here we examined whether diabetes can be prevented by providing protective MHC class II genes through directly infecting the thymus of diabetes prone NOD mice.

Methods. After direct exposition of the thymus, lentiviruses encoding control (pHAGE-CMV-DsRed-IRES-ZsGreen-W) or protective MHC class II I α β^4 (pHAGE-CMV-I α β^4 -IRES-ZsGreen-W) genes were injected in a single thymic lobe of 3 to 4 week old female euglycemic NOD mice. Gene expression was determined by microscopic examination at different time points after injection and blood glucose levels were monitored weekly to examine whether this approach prevents the development of diabetes. The presence of diabetogenic CD8⁺ T cells were detected by flow cytometry via tetramer staining of splenocytes. Pancreatic islet integrity and insulin production was assessed by immunohistochemistry.

Results. Viral gene expression was observed exclusively in thymic epithelial cells beginning at 5 days post-injection in both groups. NOD mice injected with control lentivirus developed diabetes by 22 weeks post injection, similar to non-treated animals (18-20 weeks). In contrast, pHAGE-CMV-I α β^4 -IRES-ZsGreen-W injected animals remained normoglycemic at 12 months post-injection. Diabetogenic CD8⁺ T cell population were not detected in splenocytes of I α β^4 injected animals using MHC class I tetramers. Islet integrity and insulin production was preserved in the treated group, in marked contrast to controls, which exhibited characteristic lymphocytic infiltration of islet cells and low insulin storage.

Conclusions. Thymic genetic modification with protective a MHC class II I α β^4 molecule can be used to prevent diabetes in NOD mice. Central deletion of diabetogenic T cell populations may be involved in prevention of autoimmunity in these animals.

Abstract# 432

Role of Invariant NKT Cells in Liver Sinusoidal Endothelial Cell-Induced Immunosuppression of T Cells with Indirect Allospecificity. Masayuki Shishida, Hideki Ohdan, Yuka Tanaka, Masataka Banshodani, Yuka Igarashi, Toshimasa Asahara. *Surgery, Hiroshima University, Hiroshima, Japan.*

We have reported that liver sinusoidal endothelial cells (LSECs) endocytose portally injected allogeneic splenocytes and can negatively regulate T cells with indirect allospecificity via the Fas/FasL pathway. As a result of *in vitro* transmigration across the LSECs from BALB/c mice treated with a portal injection (PI) of B6 MHC class II-deficient (C2D) splenocytes, the naive BALB/c CD4⁺ T cells lost their responsiveness to the stimulus of BALB/c splenic antigen presenting cells (APCs) that endocytosed the donor-type alloantigens. However, they maintained a normal response to the stimulus of BALB/c APCs that endocytosed third-party C3H alloantigens. In the present study, we examined whether invariant NKT (iNKT) cells influence the ability of LSECs to endocytose irradiated allogeneic cells. BALB/c wild-type (WT) mice or BALB/c CD1d-deficient (*CD1d*^{-/-}) mice that lacked iNKT cells were portally injected with 30×10^6 irradiated B6 C2D splenocytes labeled with PKH-26. Only $3.56 \pm 3.19\%$ LSECs endocytosed the labeled splenocytes in the BALB/c *CD1d*^{-/-} mice at 12 h after PI, whereas $16.82 \pm 2.69\%$ LSECs endocytosed the labeled splenocytes in the WT control mice ($P < 0.001$, $n = 4$ each). When BALB/c WT mice intraperitoneally received $4 \mu\text{g}$ α -GalCer, before PI of B6 C2D splenocytes, the expression of MHC class II on LSECs and endocytic activity of LSECs were enhanced. Thus, we found that the endocytic activity of LSECs was regulated by the iNKT cells. Intraportal adoptive transfer of LSECs isolated from BALB/c WT mice, treated with a PI of B6 C2D splenocytes, into BALB/c mice significantly prolonged the survival of subsequently transplanted heart allografts ($n = 4$) as compared to the adoptive transfer of LSECs isolated from BALB/c *CD1d*^{-/-} mice, treated similarly, into the BALB/c mice ($n = 4$). However, intraportal adoptive transfer of LSECs isolated from BALB/c WT mice, which received $4 \mu\text{g}$ α -GalCer intraperitoneally prior to PI of B6 C2D splenocytes, into BALB/c mice did not result in a further prolonging of the effect ($n = 4$). These findings indicate that iNKT cells are required for such LSEC-induced immunosuppression of T cells with indirect allospecificity; however, α -GalCer-induced activation of iNKT cells does not promote such suppressive effects on these T cells. In conclusion, naive iNKT cells play a pivotal role in the LSEC-induced immunosuppression of T cells with indirect allospecificity.

Abstract# 433

Pre-Transplant Infusion of Mesenchymal Stem Cells Induces Tolerance to Semi-Allogeneic Heart Transplant through the Generation of Regulatory T Cells. Federica Casiraghi, Nadia Azzollini, Barbara Imberti, Marina Morigi, Daniela Cugini, Paola Cassis, Regiane Cavinato, Marta Todeschini, Norberto Perico, Giuseppe Remuzzi, Marina Noris. *Transplant Research Center, Mario Negri Institute, Ranica, Bergamo, Italy.*

Notwithstanding the considerable amounts of in-vitro data supporting the non-immunogenicity and immunomodulatory effects of mesenchymal stem cells (MSC), scanty and conflicting data are available on their in-vivo immunomodulatory capacities.

In this study we formally investigated whether MSC had immunomodulatory properties in solid organ transplantation, using a semi-allogeneic heterotopic heart transplant mouse model, and studied the underlying mechanism(s). MSC, isolated from bone marrow by adherence, were depleted from CD45+CD11b+ cells before injection. Either single (portal vein day -7, $n=12$) or double (portal vein day -7 and i.v. day -1, $n=14$) pre-transplant infusions of donor B6C3 MSC (500,000) in B6 recipients induced T cell hyporesponsiveness and prolonged B6C3 cardiac allograft survival (median, untreated: 10 d $n=7$; single MSC infusion: 41 d; double MSC infusion: 26 d, $p < 0.05$ vs untreated). The effect was abrogated when MSC were injected with Lin-cKit+ hematopoietic stem cells (HSC, 8 d, $n=5$) suggesting that HSC impaired MSC immunomodulatory properties. Both the induction (pre-transplant) and the maintenance phase (>100 d post-transplant) of MSC-induced tolerance were associated with CD4+CD25+Foxp3+Treg expansion in lymphoid organs and in the graft. Consistently either total splenocytes or purified CD4+CD25+ cells from MSC-infused tolerant mice prolonged survival of a donor allograft when adoptively transferred into naive mice, whereas third-party allografts were rejected. Infusion of recipient-derived B6 MSC prolonged a B6C3 cardiac allograft as well (median 63 d, $n=8$ $p < 0.05$ vs untreated) and expanded B6C3 specific Treg. A double i.v. pre-transplant infusion of recipient MSC had the same tolerogenic effect as intraportal/i.v. infusions, which makes the protocol applicable in clinical setting. Single peritransplant or post transplant MSC infusions were less effective. Both donor and recipient MSC were unable to induce tolerance to fully MHC-mismatched heart transplant.

Altogether these findings indicate that MSC-immunomodulatory properties require HSC-removal, pre-transplant infusion, partial sharing of MHC antigens between the donor and the recipient and are associated with expansion of donor-specific Treg.

Abstract# 434**Hematopoietic Progenitor Cells Derived from Embryonic Stem Cells Induce Transplantation Tolerance Via Non-Deletional T Cell Regulation.**Sabrina Bonde,^{1,2} Kun-Ming Chan,^{1,2} Nicholas Zavazava,^{1,2} ¹University of Iowa, Iowa City, IA; ²Veterans Administration Hospital, Iowa City, IA.

Bone marrow-induced hematopoietic mixed chimerism is the most robust mechanism for the induction of transplantation tolerance. However, immunogenicity of bone marrow cells requires harsh immunosuppressive regimens that can lead to severe side-effects including death. Here, we examined whether embryonic stem (ES) cells can be successfully coaxed to form hematopoietic progenitor cells (HPC) which potentially could be less immunogenic than bone marrow cells. Here, we transduced ES cells with HOXB4, a hematopoietic transcription factor that confers self-renewal properties to hematopoietic cells and differentiated them into hematopoietic cells. Transduced cells had a 100-1000fold greater proliferation capacity than controls. At the end of the differentiation procedure, most cultures were >80 % CD45⁺. HPCs were purified using immunomagnetic bead separation. The separated cells were further characterized for leukocyte markers and showed a high percentage of CD34, CD117, CD31 and low class I, but no class II expression. Further, they poorly express co-stimulatory molecules such as CD80 and CD86. When transplanted in Rag2^{-/-}γc^{-/-} mice, HPCs fully reconstituted bone marrow, forming multi-lineage hematopoietic cells. To now determine whether these cells engraft in allogeneic recipients, the cells were transplanted in syngeneic and allogeneic MRL mice. All transplanted animals became chimeric (n>30), reaching 20-30 % after 28 days. Thereafter donor cells declined as a result of out-competition by resident bone marrow cells. This pattern was identical in both syngeneic and allogeneic recipients. Unexpectedly, allogeneic chimeric mice became tolerant to donor-type cardiac allografts as monitored over 100 days. Grafts showed no mononuclear cell infiltration or signs of chronic rejection. Interestingly, the T cells in tolerant animals showed responses to MRL alloantigen similar to that of controls, suggesting that our protocol was likely non-deletional, but could involve regulatory T cells. Indeed, when stained for CD25⁺FoxP3⁺ cells, the allografts showed a high percentage of these cells, but not in controls, confirming our hypothesis. Thus, these data show for the first time the potential of ES-derived HPCs to regulate engraftment of allografts, providing an alternative approach for the induction of transplantation tolerance.

Chronic Rejection/Injury: Novel Pathways and Therapies

Abstract# 435**A20 Prevents Transplant Arteriosclerosis in a Model of Aortic Allograft.**Jeffrey J. Siracuse,¹ Mark D. Fisher, Eva Csizmadia, Scott M. Damrauer, Lynn Y. Choi, Cleide G. Da Silva, Karam Moon, Elizabeth R. Maccariello, Soizic Daniel, Christiane Ferran. ¹Department of Surgery, Division of Vascular Surgery and the Transplant Center, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA.

We had previously shown that A20 is part of the regulatory atheroprotective response of endothelial (EC) and smooth muscle (SMC) cells to injury. A20 is a NF-κB dependent gene with potent anti-inflammatory effects in EC and SMC, through blockade of NF-κB. A20 also serves an anti-proliferative function in SMC and opposite anti-apoptotic or pro-apoptotic functions in EC and neointimal SMC. Based on these functions, A20 would be a good candidate to prevent transplant arteriosclerosis (TA) and chronic rejection in vascularized organ grafts. This is supported by A20 expression in EC and SMC correlating with the absence of TA in rat kidney allografts and long-term functioning human kidney allografts.

Fully mismatched C57BL/6 (H2^b) and BALB/c (H2^d), were used as donors and recipients of an aortic to carotid allograft. In this combination, TA lesions start at 4 weeks and become occlusive by 8 weeks when left without immunosuppression. A20 expression in the graft was achieved by recombinant adenoviral (rAd) mediated gene transfer prior to retrieval. Control mice were infused with saline or control rAd beta-galactosidase. The grafts were harvested at 4 weeks and analyzed for TA lesions by measuring intima to media ratios (I/M) and for markers of inflammation and of the immune response by immunohistochemistry. A20 expressing vessels were significantly protected from intimal hyperplasia with I/M reaching 0.4± 0.09 as compared to saline (1.62±0.17) and beta-Gal (1.86±0.06) treated vessels. This effect of A20 did not associate with a decrease in infiltrating CD3, CD4 or CD8 T cells in A20 vessels as compared to controls. A possible modification of the phenotype of these T cells (T-regs vs. effector T cells) is being explored. Rather, protection from TA correlated with increased expression of endothelial and inducible nitric oxide synthases (NOS) in EC and SMC of A20 expressing vessels, suggesting that this effect was, at least in part, related to increased *in situ* production of nitric oxide.

In conclusion, we present the first direct evidence that expression in the vessel wall of the anti-inflammatory and atheroprotective protein A20 prevents transplant arteriosclerosis through a mechanism implicating increased expression of NOS.

Abstract# 436**Carbon Monoxide Inhalation Reverses Established Chronic Allograft Nephropathy through the NO Pathway.** G. Faleo, A. Nakao, J. Kohmoto, R. Sugimoto, K. Tomiyama, A. Ikeda, M. A. Nalesnik, D. B. Stolz, N. Murase. *Thomas E Starzl Transplantation Institute, University of Pittsburgh, Pittsburgh, PA.*

Chronic allograft nephropathy (CAN) is the most common cause of graft loss; however an established therapeutic strategy in preventing/treating CAN is not yet available. We have previously shown that carbon monoxide (CO) effectively inhibits CAN development. Here, we examine the mechanisms of CO in overturning CAN through vascular endothelial cell protection.

Methods: Orthotopic kidney transplantation (KTx) was performed in Lewis to b6h6 recombinant BN rats under brief tacrolimus (0.5 mg/kg, d0-6, im). By d60 after KTx, BN developed CAN with decreased creatinine clearance (CCr, 0.58 ±0.1 ml/min), significant proteinuria (92.8 ±30.5 mg/24h), and increased Banff scores for intimal arteritis, interstitial fibrosis, and tubular atrophy. Recipients were then treated with inhaled CO 20 ppm from d60 to d150.

Results: Inhaled CO effectively reversed the severity of CAN and markedly improved renal function at d90 (table) and recipient survival (>150d vs. 81d air control). CO treatment resulted in reduced cytokine mRNA levels (TNF-α, IFN-γ) and improved Banff scores compared to untreated controls. In untreated allografts, CD31 expression on peritubular capillaries (PTC) was markedly diminished, while CO-treated grafts showed normal CD31 expression, suggesting significant improvement in maintaining PTC integrity with CO. Interestingly, eNOS and iNOS protein expression was significantly upregulated in untreated grafts, while it was maintained at steady levels in CO-treated grafts. Immunohistochemistry revealed eNOS expression on vascular endothelial cells while iNOS on infiltrates. Further, serum nitrate/nitrite levels were significantly higher in untreated than in CO-treated recipients. Elevated levels of MDA, a marker for oxidative stress, in air control group were accordingly reduced in CO-treated grafts. Renal cortical blood flow data showed a better perfusion in CO-treated group at 90d (69.2 ±5.1 vs. 45.4 ±7.2 ml/min/100g).

Conclusions: CO reverses CAN progression and restores vascular integrity. Results suggest CO provides vascular protection by inhibiting eNOS/iNOS upregulation and subsequent eNOS uncoupling and ROS generation.

	CCr (d90)	Proteinuria (d90)	CD31 stain (d90)	eNOS protein (d90)	iNOS protein (d90)	Nitrate/Nitrite	MDA
Isograft	1.58	<5	+++	+	+	10.2	307.1
AIR	0.32	357.3	+	+++	++	41.7	436.4
CO	0.85*	155.9*	+++	+	+	19.1*	336.9

*p<0.05 vs. air

Abstract# 437**Non-Invasive Detection of Chronic Rejection in Rat Cardiac Allograft: In Vivo MR Imaging of Immune Cells Accumulation as a Marker for Late Allograft Outcome.** Qing Ye,¹ Yijun L. Wu,¹ Lesley M. Foley,¹ T. K. Hitchens,¹ Haosen Zhang,¹ Chien Ho,¹ ¹NMR Center, Carnegie Mellon University, Pittsburgh, PA.

INTRODUCTION: We have been developing a non-invasive means for detecting allograft rejection with MR imaging (MRI) techniques (1). With cellular MRI, we can monitor the infiltration of immune cells labeled with iron particles (2). In this study, we used MRI to monitor the immune cells accumulation in the heart allograft to assess the status of chronic cardiac allograft rejection (CCAR) by detecting immune cells *in situ* labeled with micrometer-sized paramagnetic iron oxide (MPIO).

METHODS: A heterotopic working heart model was employed using PVG.1U to PVG.R8 rat pairs as allograft (n= 38) and PVG.R8 to PVG.R8 pairs as isograft (n=16). Macrophages were *in situ* labeled via i.v. injection of MPIO particles encapsulated with dragon green fluorescence at a dose of 3 mg Fe/kg. The heart grafts were then imaged by *in vivo* MRI at 4.7 Tesla at different time points. At the endpoint of the study, grafts were harvested for high-resolution 3D *ex vivo* MRI at 11.7 Tesla. Immunofluorescence staining with anti-rat ED1 antibody and Avindin D Texas red was used to confirm MPIO cell labeling, and histology was used to verify MRI results.

RESULTS: Macrophage infiltration associated with CCAR can be detected by MRI during CCAR from post-operation day (POD) 20 to beyond POD 100. An *in vivo* MR image of an allograft on POD 112 is shown in Fig. 1A. Very distinct, dark spots of hypointensity are observed on different myocardial regions of the graft. This data is confirmed with *ex vivo* MRI (Fig. 1B) and these MRI detected dark spots were caused by macrophages (Tensar red, Fig. 1C) labeled with MPIO particles (Dragon green, Fig. 1D). Fig. 1E shows macrophages (with immunohistochemical staining with anti-rat ED1 antibody, brown, ED1+ cells) have accumulated in the graft, which co-localized with CCAR indicated by H&E staining (Fig. 1F).

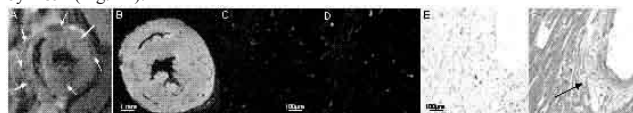


Figure 1. Representative T2*-weighted *in vivo* MR image of a POD 112 allograft in Fig. 1A, very distinct, dark spots of hypointensity are exhibit on the different regions of the graft (white arrow), which represent MPIO labeled macrophages accumulated in the rejecting graft. This MRI data are confirmed with *ex vivo* high-resolution MRI (Fig. 1B) and these MRI detected dark spots were caused by macrophages (Tensar red, Fig. 1C) labeled with MPIO particles (Dragon green, Fig. 1D). Fig. 1E shows macrophages (with immunohistochemical staining with anti-rat ED1 antibody, brown, ED1+ cells) have accumulated in the graft, which co-localized with CCAR indicated by H&E staining (Fig. 1F).

CONCLUSIONS: We detected a progression of macrophage infiltration as the CCAR progresses using MRI. This study demonstrates the feasibility of non-invasive detection of chronic rejection in rat cardiac allograft using *in vivo* MRI.

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Abstract# 438

Preventing Kidney Injury by Perfusing Donor Organ with siRNA-Solution. Xiufen Zheng,¹ Jifu Jiang,¹ Xusheng Zhang,¹ Marianne Beduhn,¹ Aminah Shunner,¹ Hongtao Sun,¹ Weihua Liu,¹ Motohiko Suzuki,¹ Norihiko Kubo,¹ Bertha Garcia,¹ Anthony M. Jevnikar,¹ Wei-Ping Min.¹ ¹University of Western Ontario, London, ON, Canada.

Background: Renal ischemia-reperfusion (I/R) injury occurs in kidney transplantation, and has no current clinical cure. Gene silencing using siRNA to specifically knock down I/R-associated genes, such as inflammatory, complement and apoptosis genes, may effectively prevent I/R injury. This study was designed to prevent renal I/R injury by perfusing donor kidney organs using siRNA solution.

Methods: C57/BL6 kidneys were excised and perfused with 200 µl of HTK that contains 100 µg/ml siRNAs targeting Fas, Rel B, and complement 3 (C3) genes. siRNA-solution-perfused kidneys were implanted into syngenic recipients in a life-supporting kidney transplant model. Forty-eight hours after transplantation, the grafted kidneys were harvested. The I/R injury was assessed by renal function, histopathology and immunohistochemistry.

Results: Perfusion procedure resulted in effective incorporation of siRNA into kidney tissues, demonstrated by fluorescence-labelled siRNA that was observed 30 min after perfusion. Gene expression of Fas, Rel B, and C3 was significantly knocked down 24 hours after perfusion with siRNA solution. Perfusion with siRNA prior to transplantation remarkably reduced BUN and serum creatinine levels. Furthermore, protection of I/R injury by perfusion with siRNA was demonstrated by improved histopathology, attenuated apoptosis, and decreased inflammatory cytokine secretion.

Conclusion: This is the first demonstration that perfusing donor organs with siRNA solution effectively delivers siRNA and induces gene silencing in kidney. Silencing Fas, Rel B, and C3 using siRNA solution prevented renal I/R injury, highlighting the promise of clinical application using siRNA-based therapy.

Abstract# 439

In Situ Targeting of Recipient's Dendritic Cells for Therapy of Chronic Allograft Vasculopathy. Zhiliang Wang,¹ William J. Shufesky,¹ Angela Montecalvo,¹ Sherrie Divito,^{1,2} Adriana T. Larregina,^{2,3} Adrian E. Morelli.^{1,2} ¹T.E. Starzl Transplantation Institute and Dept. of Surgery; ²Immunology; ³Dermatology, University of Pittsburgh, Pittsburgh, PA.

Chronic allograft vasculopathy (CAV) is a component of chronic rejection and a major cause of graft loss. Non-immunologic factors and indirect allorecognition participate in the pathogenesis of CAV. New therapies with tolerogenic dendritic cells (DCs) are based on *in situ*-delivery of alloAg to "quiescent" DCs of the recipient's lymphoid organs via apoptotic cells, vesicles or particles. We have shown that the ability of apoptotic cells to deliver alloAg and an inhibitory signal to DCs down-regulates the indirect alloresponse and prolongs allograft survival in mice. **Aims:** to test if targeting of recipient's DCs *in situ* with donor apoptotic cells ameliorates CAV by down-regulating indirect pathway allo-immunity. **Methods:** We performed functional aortic (abdominal) transplantation in mice [BALB/c→C57BL/6 (B6)]. B6 mice were injected *i.v.* with 10⁷ BALB/c UVB-induced early apoptotic splenocytes (d-7). Sixty days later, grafts were evaluated in sections with H&E, vanGieson's (elastic fibers) and Masson's (collagen) techniques. CFSE-labeled 1H3.1 TCRtg CD4 T-cells specific for IA^b (B6) loaded with IEα₃₂₋₄₈ (BALB/c) were used to evaluate the indirect pathway T-cell response. **Results:** PKH67⁺ BALB/c apoptotic cells injected (*i.v.*) in B6 mice were captured by splenic CD8⁺ and CD8α⁺ DCs, but not plasmacytoid DCs. Splenic DCs with apoptotic cells remained quiescent *in vivo* (MHC-I/II⁺, CD80/86⁺, ICOSL⁺, PDL-1/2⁺) and were unable to up-regulate MHC-II and CD86 upon culture with GM-CSF. Injection of donor apoptotic cells induced defective activation and deletion of indirect pathway 1H3.1 CD4 T-cells. Therapy with donor apoptotic splenocytes reduced intimal thickness in aortic allografts (100±21 vs. 196±28mm in controls; *p*<0.001) and proliferation of α-smooth muscle cells and collagen deposition. The effect of apoptotic cells was allospecific, superior than that of cells alive, and depended on the physical properties of apoptotic cells, since necrotic cells did not achieve the effect. Treatment with donor apoptotic cells decreased significantly the indirect pathway T-cell response (assessed by ELISPOT for IFN-γ) and reduced the level of circulating alloAb. **Conclusion:** *In situ*-targeting of recipient's DCs with (early) apoptotic cells carrying donor alloAg is a novel approach to prevent CAV by down-regulating the indirect pathway alloresponse.

Abstract# 440

The Antifibrotic Agent, Pirfenidone, Has Direct Inhibitory Effects on T Cell Activation, Proliferation, and Cytokine and Chemokine Production, Leading to Suppression of Host Alloresponses. Gary A. Visner,¹ Fengzhi Liu,¹ Hanzhong Liu,¹ Liqing Wang,² Wayne W. Hancock.² ¹Medicine, Children's Hospital Boston, Boston, MA; ²Pathology, Children's Hospital of Philadelphia, Philadelphia, PA.

There is an urgent need to develop new therapies effective against the fibrotic and other complications of chronic allograft rejection. While pirfenidone (PFD) is an established anti-fibrotic agent, we previously showed that PFD treatment reduced acute rejection in a rat lung transplant model suggesting that it might have direct immune modulating properties. Accordingly, in this study, we tested the effects of PFD on T cell responses. We first evaluated whether PFD alters T cell proliferation and cytokine release in response to T cell receptor (TCR) activation *in vitro*. Since PFD can inhibit TGF-β by mononuclear cell fractions, we also examined whether PFD affects the suppressive effects of regulatory T cells (CD4⁺CD25⁺). The effects of PFD on alloantigen-induced T cell proliferation *in vivo* were then assessed by adoptive transfer of CFSE-labeled T cells across a parent->F1 MHC mismatch, as well as by using a murine heterotopic cardiac allograft model (BALB/c→C57BL/6). PFD was found to significantly inhibit TCR-stimulated CD4⁺ T proliferation *in vitro* (*p*<0.01), whereas CD8⁺ T cell proliferation was not significantly affected. While the beneficial effects of PFD were not associated with increased CD4⁺ T cell apoptosis, PFD use inhibited TCR-induced production of multiple cytokines and chemokines, including IFN-γ, TNF-α, IL-1β, IL-4, IL-17, FGF-β, IP-10, MIP-1 and Mig (*p*<0.01). Interestingly, there was no change on TGF-β production by purified T cells, and PFD also had no effect on the suppressive properties of naturally occurring regulatory T cells. Similar to the *in vitro* studies, PFD inhibited allo-antigen-induced T cell proliferation *in vivo* (parent->F1 model), and showed synergistic effects with low dose rapamycin in this model. Lastly, though PFD alone did not affect the tempo of acute cardiac allograft rejection across a full MHC mismatch, use of PFD plus a subtherapeutic regimen of rapamycin significantly prolonged allograft survival (*p*<0.05), decreased mononuclear cell infiltration and prevented development to chronic rejection, including arteriosclerosis and myocardial fibrosis. We conclude that PFD may be an important new agent in transplantation, with particular relevance to combating chronic rejection by inhibiting both fibroproliferative and alloimmune responses.

Islet Transplantation: Experimental

Abstract# 441

Composite Islet-Kidneys Reverse Diabetes across a Fully Allogeneic Barrier in Nonhuman Primates. Hideyuki Iwaki, Atsushi Hirakata, Adam Griesemer, Vaja Tchipashvili, Yoshinori Ishikawa, Akira Shimizu, David Sachs, Kazuhiko Yamada. Transplantation Biology Research Center, Massachusetts General Hospital, Boston, MA.

We have previously reported studies in miniature swine showing that transplantation (Tx) of prevascularized donor islets as part of composite Islet-Kidney (I-K) reversed diabetic hyperglycemia across fully allogeneic barriers, while free islets did not. In order to test the potential clinical applicability of this strategy, we have extended it to a fully allogeneic nonhuman primate model. **Methods:** Two diabetic baboons received composite I-Ks and one diabetic baboon received free islets across fully allogeneic barriers. **(1) I-K preparation in donors:** Two I-Ks were prepared by isolating islets from 60% partial pancreatectomies and injecting them under the autologous renal capsule, allowing for vascularization before allogeneic Tx. These I-Ks were harvested at days 203 and 102 for allogeneic I-K Tx. **(2) Induction of insulin-dependent diabetes (IDDM) and allogeneic I-K or free islet Tx:** All recipients received streptozotocin at 2000 mg/m² x (Body Surface Area). IDDM was induced successfully in two animals, while one animal required total pancreatectomy to induce IDDM. After confirming IDDM by fasting blood sugar (FBS) >300mg/dl for 3 consecutive days, either I-Ks or free islets were transplanted (single donor to each recipient) with ATG (day -3) followed by MMF and low-dose tacrolimus. Free islets were injected into the liver through the ileocolic vein. Islet function was assessed by FBS and renal function was assessed by serum creatinine. Immunologic status was examined by CML/MLR assays. **Results:** All three recipients had strong CTL/MLR responses to donors preTx, indicative of a fully allogeneic combination. FBS decreased immediately after I-K Tx and no insulin therapy was required throughout the experimental period (days 225 and 279). BS levels averaged 82.2±15.6 mg/dl in the first baboon and 125.4±42.7 mg/dl in the second. Normal creatinine levels (<1.0mg/dl) were maintained by these life-supporting I-K grafts. In contrast, the recipient of allogeneic free islets had unstable BS levels and required insulin from day 60 (BS 400 at day 60). **Conclusions:** Life-supporting I-Ks from single donors achieved glucose regulation without insulin therapy and maintained normal renal function. These results demonstrate the feasibility of composite I-K Tx in a non-human primate allogeneic model, with possible clinical applicability for the cure of diabetic nephropathy.

Abstract# 442**Donor Cell Infusion without Immunosuppression as a Novel Therapy for Induction of Donor-Specific Tolerance in Islet Cell Transplantation.**

Xunrong Luo,¹ Kathryn Pothoven,¹ Derrick McCarthy,² Matthew DeGutes,² Aaron Martin,² Xiaomin Zhang,³ Guliang Xia,³ Dixon Kaufman,³ Stephen Miller.² ¹Medicine, Northwestern University; ²Microbiology and Immunology, Northwestern University; ³Surgery, Northwestern University, Chicago, IL.

Background In autoimmune models, peptide-pulsed splenic antigen presenting cells that are chemically fixed with ethylcarbodiimide (ECDI) have been used as a powerful and safe method to induce antigen specific T cell tolerance. ECDI fixed donor cells for allo-antigen specific transplant tolerance has not been well studied. **Material and Methods** C57BL/6 mice were rendered diabetic by STZ. Kidney subcapsular allogeneic islet transplant was performed 10 days after diabetes stabilization (day 0). 1×10^8 ECDI-treated donor splenocytes were injected i.v. either once (day -7) or twice (day -7 and day +1). Animals were analyzed for graft outcome. **Results** Control mice receiving islet graft alone rejected the graft between day 11 to day 18 (MST = 14 days, N=8). Mice receiving one dose of ECDI-treated donor splenocytes (on day -7) showed similar graft survival as controls (MST = 15 days, N=5). In contrast, mice receiving 2 doses of ECDI-treated donor splenocytes (on day -7 and day +1) showed significant prolongation of graft survival with 72.7% functional grafts at day 60 (N=11), and some remained functional >100 days. This protection is donor-specific as mice receiving ECDI-treated SJL cells rejected Balb/c islet grafts as controls (MST = 18 days, N=4). Immunohistochemistry of protected grafts showed positive insulin staining within well-defined islet architecture. Peri-islet infiltrates were composed of CD4+, CD8+, and CD11c+ cells, with occasional Foxp3+ cells. Anti-donor antibody production (IgG1, G2a, b, G3) was completely abolished in long-term graft survivors. This tolerance was undisturbed by anti-CD25 antibody treatment during maintenance stage, but could not be established if treatment was given around the time of the first donor cell infusion. In addition, lack of PD-L1 also impaired tolerance induction evidenced by using PD-L1^{-/-} as recipients. **Conclusion** 1. Multiple infusions of ECDI-treated donor splenocytes significantly prolonged allo-graft survival in the islet transplant model. 2. The protective effect is donor-specific and is dependent on regulatory T cells as well as the PD-L1 signaling pathway. Therapy with ECDI-treated donor cells may emerge to be a novel and potent agent for induction of donor-specific transplant tolerance.

Abstract# 443**Increased Expression of Hypoxia Inducible Factor 1 α (HIF 1 α) Markedly Improves Outcomes Following Transplantation of Human Islets into Mice.** Rebecca Stokes,¹ K. Cheng,¹ C. Scott,¹ W. Hawthorne,² P. O'Connell,² J. E. Gunton.¹ ¹Garvan Institute, Darlinghurst, NSW, Australia; ²NPTU, Westmead, NSW, Australia.

The aim was to investigate the effects of increasing HIF-1 α protein in human islets upon islet-transplant outcomes.

HIF-1 α is a transcription factor which co-ordinates a program of cellular responses to stressors including hypoxia. In other cell-types HIF-1 α improves survival following hypoxic-challenge. HIF-1 α functions as a heterodimer with ARNT which we have shown to be important for normal β -cell function (1). Islet transplantation subjects islets to hypoxia. It is thought up to 70% of islets die within 1 week of transplantation and this is at least partly due to hypoxia. The role of HIF-1 α in β -cell function is unknown.

We hypothesized that increasing levels of the protective factor HIF-1 α in islets before transplantation would improve survival and engraftment and thus improve islet transplant outcomes.

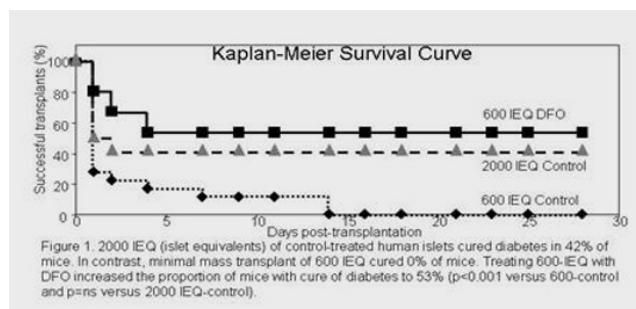
Isolated human pancreatic islets from 9 separate donors were cultured overnight in control media, or media supplemented with desferrioxamine (DFO), a small molecular stimulator of HIF-1 α protein. Islets were transplanted into diabetic SCID mice.

There were 3 transplant groups, with mice receiving:

1. Supra-physiological-mass transplant of 2000 control-cultured IEQ (islet equivalents)
2. Minimal-mass-transplant of 600 control-cultured IEQ, or
3. Minimal-mass-transplant of 600 IEQ cultured with DFO.

For each human donor, at least 1 of each of the 3 transplant groups was performed to avoid the confounder of inter-donor variability. Recipients of 2000 control IEQ cured in 42% of cases. Minimal-mass-transplantation was ineffective: 600 IEQ cured 0% mice at 28-days. However, minimal-mass-transplant of DFO treated islets had 53% success ($p < 0.001$ vs group 2 and $p = ns$ vs 2000-control-IEQ). Blood glucose levels were markedly improved in the 600 DFO treated group compared to 600 IEQ control group ($p < 0.0001$) and equivalent to 2000 control IEQ transplants ($p = ns$).

This data demonstrates increasing HIF-1 α in human islets prior to transplantation markedly improves islet transplant outcomes. HIF-1 α and DFO may have a therapeutic role in human islet transplantation.

**Abstract# 444****Long-Term Disappearance of Neovascularization of Transplanted Islets.**

Eba Hathout, Nathaniel Chan, Annie Tan, John Chrisler, John Hough, Naoaki Sakata, John Mace, Ricardo Peverini, Richard Chinnock, Lawrence Sowers, Andre Obenaus. Loma Linda University, Loma Linda, CA.

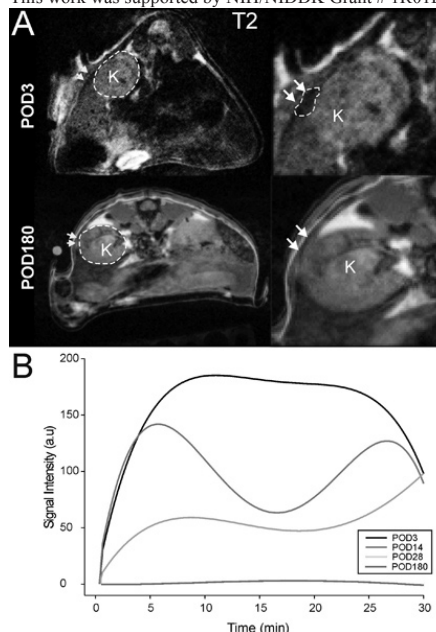
We recently reported an in vivo time-line for neovascularization of transplanted islets using dynamic contrast enhanced (DCE) magnetic resonance imaging (MRI) over a 14-day period. However, vascularization of transplanted islets must be maintained for extended periods to provide long-term function.

In this dataset, we investigated whether vascularization was maintained in transplanted Feridex-labeled syngeneic murine subcapsular islets (400 IEQ per kidney) using DCE imaging on an 11.7T MR scanner and subsequent immunohistochemistry over 180 days.

Sub-capsular transplants could be visualized at post-transplant days 3 and 14 using T2 weighted imaging. However, the islets could not be seen on MRI at post-transplant day 180. Injection of the contrast agent gadolinium (Gd)-DTPA for DCE at 3, 14 and 28 days showed increased signal in the transplant area. At 180 days, there was no change in signal intensity after contrast injection during DCE. Immunohistochemistry confirmed MRI and DCE findings.

These results suggest that islet neovascularization occurs early after transplantation but is likely not maintained for the 180-day duration of our experiments.

This work was supported by NIH/NIDDK Grant # 1R01DK077541.



A) T2 imaging at day 3 clearly identifies iron-labeled islets (arrows) in the subcapsular region. No iron-labeled islets are observed at day 180 (arrows).

B) DCE imaging for neovascularization of transplanted islets in the subcapsular region demonstrates a temporal decline in signal intensity.

Abstract# 445**Oleanolic Acid, a Natural Triterpenoid, Significantly Improves Islet Survival and Function Following Transplantation.** N. Angaswamy,¹ D. Saini,¹ S. Ramachandran,¹ N. Benshoff,¹ W. Liu,¹ N. Desai,¹ W. Chapman,¹ T. Mohanakumar.^{1,2} ¹Surg, WUSM; ²Path & Immunol, Washington Univ Sch Med, St. Louis, MO.

Oleanolic acid (OA), a triterpenoid in medicinal herbs, is an integral part of normal human diet. OA has anti-oxidant, anti-inflammatory properties (inhibits iNOS &

COX2) & lowers plasma glucose levels. We hypothesize that these properties of OA will prevent early islet cell loss following transplantation & also benefit long term function of allograft. C57BL/6 mice, made diabetic by streptozotocin (200mg/kg) were transplanted with 500 BALB/c islets (isolated by collagenase digestion) under kidney capsule. OA (0.5mg/day) was administered i.p. in 100 µl of PBS (with 6M DMSO) or PBS-DMSO as vehicle control daily from day -1 onwards. Blood glucose was monitored daily. Immunohistochemical analyses of grafts were performed for CD4 & CD8 markers. Cellular immune responses to donor antigens & cytokines produced by cells and in sera were measured using ELISPOT & Luminex assays. Effect of OA on function of transplant with suboptimal dose of islets (100-250) was also analyzed. Optimal dose of islets (500) transplanted into diabetic BL/6 mice administered with OA significantly reduced time taken to reverse diabetes following transplantation ($<2 \pm 1$ vs 4 ± 2 days, $p=0.003$). Further, OA treatment reversed diabetes even with suboptimal dose (200) of islets while untreated animals did not achieve normoglycemia. As expected, control diabetic mice rejected on 6 ± 2 days whereas, OA administration alone prolonged islet allograft survival to 23 ± 3 days ($p<0.001$). OA treatment resulted in >3 fold increase in serum KC, IL-10 & VEGF ($p<0.0003$) & 2 fold decrease in MCP-1, IP-10 & IL-4 ($p<0.005$) in Luminex assay. Stimulation of splenocytes from OA treated mice with donor BALB/c cells resulted in significantly reduced IFN γ (4.5 fold), IL-4 (3.5), IL-2 (2.3) & IL-17 (4). In addition, proliferation in MLR was also reduced 2.5 fold. Immunohistochemical analysis of grafts showed significant reduction in cellular infiltration in OA treated animals with reduction in both CD4 and CD8 T cells. Daily administration of OA markedly improved islet engraftment & function with reversal of diabetes even when suboptimal dose of islet were transplanted. Further, OA treatment allowed significant long term survival of allograft with no other immunosuppression. We demonstrate that prevention of inflammatory signaling cascades by OA resulted in marked reduction of cellular infiltration into graft allowing long term function of allograft.

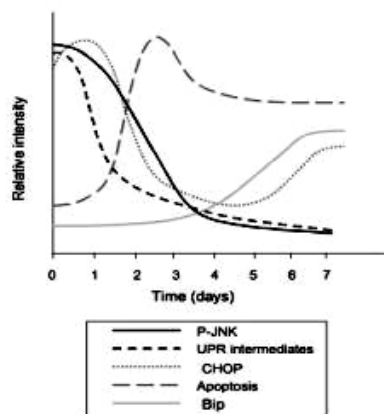
Abstract# 446

Endoplasmic Reticulum Stress May Be an Important Cause of Cell Loss after Human Islet Isolation. Soon Hyang Park,¹ Michel Tremblay,² Steven Paraskevas.¹ ¹*Surgery, McGill University Health Center, Montreal, QC, Canada;* ²*McGill Cancer Center, McGill University, Montreal, QC, Canada.*

Purpose: To evaluate the presence of endoplasmic reticulum (ER) stress, induced by conditions to which islets are subjected during isolation (ischemia, nutrient deprivation, thermal stress and cytokine release) in human islets and to determine if this leads to the Unfolded Protein Response (UPR), which could alter cell survival.

Methods: Human islets were purified from cadaveric pancreata by collagenase dissociation and continuous density gradient purification. Islet preparations were cultured in serum-free medium and sampled at the end of isolation and daily thereafter. Total mRNA was purified and gene expression evaluated by RT-PCR. Activity in UPR signaling pathways was evaluated by immunoblot. Apoptosis was measured by a caspase-3 activity assay. Representative trends observed in >5 isolations are described.

Results: Following isolation, a rapid increase in UPR signaling was observed in the PERK and IRE-1 modules of the UPR. These include the phosphorylation of PERK target eIF2 γ and splicing of mRNA for the transcription factor XBP-1. These changes occurred concurrently with a rapid spike in JNK activity and a rise in expression of the UPR target gene CHOP. After these signals peaked, caspase-3 activity increased with time (apoptotic cells), as did expression of ER chaperone Bip (surviving cells).



Conclusion: We consistently observed UPR activation in human islets. ER stress and the UPR may be one important and unrecognized cause of apoptosis in this context. Current investigations focus on UPR modification and determination of a causal relationship with apoptotic cell death.

Abstract# 447

Immunosuppression and the Risk of Renal Transplant Failure Due to Recurrent Glomerulonephritis. Atul Mulay,¹ Carl van Walraven,¹ Greg Knoll.¹ ¹*The Ottawa Hospital, Ottawa, ON, Canada.*

Glomerulonephritis (GN) is the most common cause of end-stage renal disease among those who undergo kidney transplantation. Recurrent GN is a major cause of kidney transplant failure. Immunosuppressive medication is used to treat GN in the native kidney prior to the development of end-stage renal disease but the impact of different immunosuppression on recurrent GN post-transplantation is unknown.

We used the United States Renal Data System to determine the association of routine post-transplantation immunosuppressant use with time to renal allograft failure due to recurrent GN. Immunosuppressants were treated as time-varying covariates. The study-cohort included patients with kidney failure due to GN who received first kidney transplant between 1990 and 2003.

The study cohort included 41,272 patients with a median follow-up of 51 months. Ten-year overall graft survival (including death as graft loss) and death-censored graft survival was 56.2% and 70.5% respectively. Use of cyclosporine (hazard ratio 0.93; 95% CI 0.62-1.41), tacrolimus (hazard ratio 1.03; 95% CI 0.67-1.60), azathioprine (hazard ratio 0.88; 95% CI 0.68-1.13) or mycophenolate mofetil (hazard ratio 1.10; 95% CI 0.85-1.40) was not associated with risk of graft failure due to recurrent GN after adjusting for important covariates. There was no difference of recurrent GN causing graft failure between cyclosporine and tacrolimus ($P=0.4$) or between azathioprine and mycophenolate mofetil ($P=0.1$). However, change in any immunosuppressant during follow-up was independently associated with graft loss due to recurrence (HR 1.31, 95% CI 1.07-1.60, $P=0.01$). When we restricted the analysis to patients who had no change in immunosuppression during follow-up we again found no association between any of the immunosuppressive medications and the risk of graft loss due to recurrent GN.

Despite the increased use of tacrolimus, cyclosporine and mycophenolate mofetil to treat GN in native kidney disease, the use of these medications following kidney transplantation had no impact on the risk of graft loss due to recurrent GN.

Abstract# 448

Rituximab Induction in High Risk Kidney Transplant Recipients May Decrease the Incidence and Severity of Recurrence of Focal Segmental Glomerulosclerosis. Junichiro Sageshima,¹ Gaetano Ciancio,¹ Alessia Fornoni,¹ Linda Chen,¹ Carolyn Abitbol,¹ Jayanthi Chandar,¹ Warren Kupin,¹ Giselle Guerra,¹ David Roth,¹ Sherry Shariatmadar,¹ Gaston Zilleruelo,¹ George W. Burke III.¹ ¹*University of Miami Miller School of Medicine, Miami, FL.*

BACKGROUND: Disease recurrence is a major obstacle of kidney transplant for focal segmental glomerulosclerosis (FSGS). Anti-CD20 antibody (rituximab) has been used for nephrotic syndrome of native kidney. The significant reduction of proteinuria in transplant recipients with FSGS recurrence was also reported after rituximab use for posttransplant lymphoma. We hypothesized that rituximab induction could alter the posttransplant course of FSGS recipients, particularly in those patients with rapid progression to end-stage renal disease who are higher risk of recurrence.

METHODS: We compared the outcome of transplants for primary FSGS treated with and without rituximab. From Jan. 2000 to Dec. 2003, 11 FSGS patients (6-21 yo) received renal allografts along with our "standard" immunosuppressive protocol, consisting of tacrolimus, mycophenolate, corticosteroids, antithymocyte globulin and/or daclizubab. From Jan. 2004 to Dec. 2007, 18 other FSGS recipients (7-24 yo) received rituximab in addition to the "standard" immunosuppression. Posttransplant proteinuria was treated with plasmapheresis (PP) and maintenance angiotensin blockade (AB).

RESULTS: There was no adverse event related to rituximab infusion. The overall incidence of posttransplant proteinuria was significantly lower in recipients with rituximab induction ($p < 0.05$). Four recipients treated with "standard" immunosuppression developed massive proteinuria (u-protein/creat. > 10) immediately following transplantation; they responded poorly to PP and AB. Four other recipients had moderate proteinuria. In contrast to this, of the 18 patients induced with rituximab, only 2 had massive proteinuria and 4 had mild to moderate proteinuria which responded well to PP and AB. With a median follow-up of 26 months, there was no significant difference of graft survival between 2 groups (2-year survival: 81% without rituximab vs. 84% with rituximab). A half of the graft loss was related to non-compliance.

CONCLUSION: While the mechanism of action is unclear, our observation indicates that rituximab induction may decrease the incidence and severity of recurrence of FSGS following kidney transplantation. A larger-scale study is desirable to confirm this observation.

Abstract# 449

Mesangial Chimerism in Recurrent IgA Nephropathy. Geoffrey Talmon,¹ Dylan Miller.¹ ¹*Department of Pathology and Laboratory Medicine, Mayo Clinic, Rochester, MN.*

Background

IgA nephropathy (IN) is the most common primary glomerulonephritis and nearly 50% of patients who undergo a renal transplant for IN recur. Data support that bone

marrow-derived cells are capable differentiating into various mesenchymal cells within the kidney. The extent to which this phenomenon versus proliferation of resident mesenchymal cells is involved in populating mesangium is not well understood. The mesangial injury and/or hypercellularity seen in IN provides a robust in vivo model for determining if this phenomenon is prevalent in human kidneys.

Design

Follow-up biopsies from male patients receiving female renal allografts for IN that showed recurrent disease were selected. Fluorescent in-situ hybridization and immunofluorescent staining was performed on unstained slides from the paraffin-embedded tissue for smooth muscle actin, X, and Y chromosome centromeres. Cells within nonsclerotic glomeruli with triple positivity (Y+) were assumed to be mesangial cells derived from the recipient.

Results

Four cases of recurrent IN with nonsclerotic glomeruli were obtained, each displaying at least minimal mesangial proliferation by light microscopy (one "minimal", two "mild", one "moderate"). Mesangial cells with Y chromosome centromeric material were observed in each case (100%). Between 6 and 34 mesangial cells were present in each glomerulus (mean 16.45) with no to three Y+ cells seen (mean 1.8). These accounted for between 8% and 16% of mesangial cells in individual glomeruli. The ratio of Y+ to total mesangial cells in each case ranged from 1:7.8 to 1:11.3 (mean 1:9.35). The case exhibiting minimal mesangial hypercellularity had a ratio of 1:9.6, those with mild had ratios of 1:7.8 and 1.8.7, and that with moderate 1:11.3.

Conclusions

Recipient-derived mesangial cells make up a fraction of the population of glomerular cells in renal allografts affected by recurrent IN. Although the number of cases is small, the number of recipient-derived cells does not seem to be directly related to the degree of mesangial hypercellularity seen by light microscopy. The consistent presence of these "colonizing" cells in patients with recurrent IN does, however, suggest that there may be a role for targeted therapy directed against circulating recipient cells.

Abstract# 450

Recurrent Lupus Nephritis after Renal Transplantation: A Single Center Experience. Scott A. Kendrick,¹ William T. Kendrick,¹ William J. Cook,² Bruce A. Julian,¹ Clifton E. Kew II,¹ ¹Medicine, University of Alabama at Birmingham, Birmingham, AL; ²Pathology, University of Alabama at Birmingham, Birmingham, AL.

In the mid 1970's, patients developing ESRD secondary to systemic lupus (SLE) were deemed to be poor transplant candidates because of concern for early recurrent lupus nephritis (RLN) leading to allograft loss. Subsequently, RLN was considered an unusual complication of kidney transplantation, occurring in <4% of allografts. However, over the last decade, several reports have shown the frequency of RLN to range from 8-30%. We sought to determine the frequency of RLN at our center and to identify any clinical variables associated with RLN.

Between 6/1977-11/2005, 172 allografts in 166 patients with ESRD due to SLE functioned for more than 90 days after engraftment. Immunosuppression consisted of Azathioprine (AZA), or Cyclosporine (CSA) and AZA, or Mycophenolate (MMF) and CSA, or Tacrolimus and MMF depending on the date of transplant. All received steroids. Proteinuria was defined as 2+ on dipstick or urine protein/creatinine ratio >0.5. Medical charts were reviewed.

We found pathologic evidence of RLN in 18 (24%) of 75 patients who underwent biopsy due to allograft dysfunction or proteinuria, comprising 10% of all patients transplanted for SLE. Characteristics of these patients are shown below:

	RLN n=18	No RLN n=154	
	Mean±SD (%)	Mean±SD (%)	p Value
Age at transplant (yr)	34±10	36±9	NS
Duration on dialysis (mo)	33±26	38±36	NS
Interval to diagnosis (mo)	54±41	N/A	NS
Interval to follow-up (mo)	69±42	84±65	NS
Female	13 (72)	126 (81)	NS
Black	14 (78)	94 (61)	NS
Hemodialysis pre-transplant	13 (72)	111 (72)	NS
Living donor transplant	7 (39)	58 (38)	NS
DGF	1 (6)	11 (7)	NS
Antibody induction	15 (83)	127 (83)	NS
Acute rejection	8 (44)	72 (47)	NS
Systemic symptoms SLE	5 (28)	5 (5)	<0.05
Graft loss	8 (44)	65 (42)	NS
On AZA at transplantation discharge	3 (17)	84 (59)	<0.05
Biopsied %	100	37	
Proteinuria %	83	56	<0.1
Interval to proteinuria (mo)	30±36	33±53	NS

In conclusion, the rate of RLN after transplantation is comparable with that recently reported. Because our center does not perform protocol biopsies, we may have underestimated the rate of recurrence. Patients with RLN did not differ significantly on a demographic basis from those without RLN, except that they were less frequently discharged on AZA after transplantation and more frequently manifested clinical lupus.

Abstract# 451

Recurrent Focal Segmental Glomerulosclerosis after Kidney Transplantation. Li Ping Tan,¹ Sanjeev Akkina,¹ Teddy Eastlund,¹ Aleksandra Kukla,¹ Kasiske Bertram.^{1,2} ¹Renal Diseases and Hypertension, University of Minnesota, Minneapolis, MN; ²Department of Medicine, Hennepin County Medical Center, Minneapolis, MN.

Introduction: Risk factors and treatment for recurrent Focal Segmental Glomerulosclerosis (FSGS) are not well established in the adults. **Methods:** We retrospectively analyzed 143 patients with adult onset FSGS who were transplanted at the University of Minnesota from 1974 to 2006. **Results:** For patients with FSGS, the probability of developing recurrent disease was not altered by transplant type, number of HLA mismatches, immunosuppression protocol or race by Cox proportional hazards. Graft survival at 1 and 5 years was no different between FSGS and recurrent FSGS patients. Of the 23 cases of recurrent FSGS, 8 received plasmapheresis. Patients receiving plasmapheresis were on average 47 years old at the time of transplant. Time to diagnosis of recurrence varied widely, with a median of 4.5 days post transplant (range 3-2880 days). Mean proteinuria was 15.2g/g Cr at time of diagnosis. An median of 10 plasma exchanges were administered. 87.5% (7 of 8 patients) had remission. A median of 4 sessions (range 1-10 sessions) were needed to decrease proteinuria by 50%. 7 of 8 treated with plasma exchange continue to have functioning grafts while 1 returned to dialysis. Median creatinine at last follow up was 2.75mg/d (range 1.3-7.5). Median follow up was 56.5 months (interquartile range 40-71.8). **Discussion:** Recurrence of disease is common. Being African-American and receiving a living donor kidney have been considered risk factors in predisposing to recurrence, however this was not seen in our analysis. Plasmapheresis was effective in reducing proteinuria. Overall graft survival was not worsened with recurrence. More studies are needed to guide management during disease recurrence.

Abstract# 452

Columbia Classification of Focal and Segmental Glomerulosclerosis (FSGS) and the Risk of Recurrence after Transplantation. Guillaume Canaud,¹ Laure-Hélène Noel,² Daniel Dion,² Julien Zuber,¹ Rebecca Sberro,¹ Renaud Snanoudj,¹ Eric Thervet,¹ Marie-France Mamzer,¹ Frank Martinez,¹ Christophe Legendre.¹ ¹Department of Kidney Transplantation, Necker, Paris, France; ²Department of Pathology, Necker, Paris, France.

Introduction: Recurrence of primary Focal Segmental Glomerulosclerosis (FSGS) after renal transplantation is associated with a poor graft survival. Identification of risk factors for recurrence is therefore an important issue, which remains partially answered. In 2004, the Columbia University proposed an histological classification which included the 5 following variants: collapsing (COL), cellular (CELL), tip (TGL), perihilar (PH) and not otherwise specified (NOS). This classification is helpful to predict prognosis and define therapeutic options on native kidneys. However whether the Columbia classification would predict or not the risk and forms of recurrence on renal allograft is unknown. The aim of this study was to address this important issue.

Material and methods: During the 1996-2006 period, 24 patients with biopsy-proven primary FSGS on native kidneys received a renal transplant, and underwent at least one graft biopsy within the post-transplant course. Among them, 12 recipients experienced FSGS recurrence as defined by unexplained post-transplant proteinuria. Biopsies were assessed according to Columbia classification.

Columbia variants and FSGS recurrence			
	Natives kidney	First transplantation	Second transplantation
Patient 1	CELL	MCD	
Patient 2	NOS	MCD	
Patient 3	CELL	NOS	
Patient 4	COL	MCD	
Patient 5	CELL	TIP then PH	
Patient 6	TIP	NOS	
Patient 7	PH	COL	TIP
Patient 8	NOS	PH	
Patient 9	CELL	MCD	
Patient 10	NOS	PH	MCD
Patient 11	PH	CELL	
Patient 12	COL	TIP	

Conclusion: Our study raised the following points, though conclusions were limited by the small population-size: i) the frequency of FSGS recurrence as well as the post-transplant histological form is independent of the variant on native kidney ii) early post-transplant biopsies for FSGS recurrence disclosed only minor podocytes injuries in 33.3 % of the cases (minimal change disease (MCD)), iii) all FSGS variants are seen in case of post transplant recurrence. We thus inferred that the value of Columbia University Classification of FSGS should be considered carefully in the field of renal transplantation. This issue warrants further studies in a larger cohort.

Kidney Immunosuppression: Steroid Minimization – Avoidance Protocols

Abstract# 453

A Randomized Double Blind, Placebo-Controlled Trial of Early Corticosteroid Cessation Versus Chronic Corticosteroids: Five Year Results. E. Steve Woodle,¹ Astellas Steroid Withdrawal Group.² ¹Univ Cinc; ²APUS.

In five decades of renal transplantation, only three double blind trials of corticosteroid withdrawal (CSWD) have been conducted. These trials, however, evaluated late CSWD (ie, beyond three months), with short blinding periods (≤ 1 yr), and most patients did not receive induction therapy. The final, five year results of a fourth double blind, randomized placebo controlled trial in kidney transplant patients (pts) with 1) early CSWD (7 days posttransplant), 2) maintenance of double blind for five years, and 3) induction therapy in all patients, are presented.

METHODS: CSWD (191 pts) was compared to chronic corticosteroid therapy (CCS 195 pts) under tacrolimus /mycophenolate therapy with either thymoglobulin (Thymo) or IL-2R antibody (Ab) induction. Prednisone dose in CCS pts was reduced to 5mg/day at 6 months. The composite primary endpoint included death, graft loss or severe acute rejection (AR) defined as AR requiring antibody treatment or Banff 95 Grade ≥ 2 B or Banff 97 Grade ≥ 2 A.

RESULTS: Data are presented as CCS/CSWD. No differences occurred in demographic or immunologic risk data, or Thymoglobulin induction 69.7%/65.4% of pts. No differences noted at 5 yr in primary endpoint (13.3%/15.7%); death (6.2%/5.2%), death censored graft loss (3.6%/6.3%), or severe acute rejection (5.1%/7.9%). Death to infection was higher with CCS 2.6%/ 0%, $p=0.06$. Biopsy confirmed AR approached significance (10.8%/17.8%, $p=0.06$). No differences in renal function occurred: serum creatinine (1.5 $\pm$ 0.8 / 1.6 $\pm$ 0.7 mg/dl); calculated creatinine clearance (60.2 $\pm$ 20.5 / 57.1 $\pm$ 20.2 ml/min; proportion of pts. with creatinine clearance <40 ml/min (7.4% / 10.8%). An absolute increase in chronic allograft nephropathy (CAN) of 5.8% occurred with CSWD (4.1% / 9.9%, $p=0.03$), however, overall graft survival was not different. CSWD provided cardiovascular risk/metabolic benefits: new onset diabetes requiring insulin (13.5% / 2.1%, $p=0.005$); change in triglycerides (in pts without hyperlipidemia at baseline +26.1 / -69.0 mg/dl, $p=0.001$), and bone disorders (fractures/avascular necrosis) (11.3%/4.7%, $p=0.02$).

CONCLUSIONS: To our knowledge, the blinding period is the longest of any trial to date in transplantation. Results indicate that early CSWD provides cardiovascular risk and bone disease benefits without significant differences in allograft survival or function at five years. It is important to note that these differences were observed despite the low prednisone dose in CCS pts after 6 months (5 mg/day).

Abstract# 454

Long Term Follow-Up of >1000 Kidney Transplant Recipients on Prednisone (P)-Free Maintenance Immunosuppression (IS). Abhinav Humar,¹ Raja Kandaswamy,¹ William Payne,¹ David Sutherland,¹ Bertram Kasiske,² Hassan Ibrahim,² Kristen Gillingham,¹ Arthur Matas.¹ ¹Surgery, University of Minnesota, Minneapolis, MN; ²Medicine, University of Minnesota, Minneapolis, MN.

Randomized studies have shown little or no increase in AR in kidney tx recs on P-free IS; but concern remains about long-term outcome. We present 8-yr f/u of a protocol incorporating rapid (<6 days) discontinuation of P, with now over 1000 patients transplanted using this protocol. Between 1/1999 and 10/2007, 1003 adult tx recs were treated with thymoglobulin (5 doses)(extended in DGF), P (5 days), a CNI, and either MMF or SRL. Of these, 658 were LD (260 LURD); 345 DD. Of the 1003, 41% were female; 88% white; mean recipient age was 47 $\pm$ 14 years and mean donor age was 39.7 $\pm$ 13.2 years. Diabetes was present in 34.3% of the recipients and 12.5% of the transplants were retransplants. The peak PRA was $>10\%$ in 20% of recipients; 14% had tx PRA $>10\%$.

Table 1 shows actuarial survival rates. Graft survival rates were significantly better in LD vs DD transplants ($p=0.002$) and acute rejection rates lower ($p=0.04$). Compared to national data from SRTR, overall outcomes were not significantly different. With mean follow-up of 3.5 years, a total of 110 (11%) recipients have died - the most common cause being cerebrovascular accident (14%) followed by malignancy (9%). There were only 5 (4.5%) patient deaths due to cardiac causes. Of 195 (19.4%) graft losses, 86 (44%) were from DWF (death with function); 43 (10%) from CR/CAN. Renal function has been stable with mean serum Cr of 1.7 mg/dl at 3 and 5 years posttransplant with creatinine clearance of 63 and 60 respectively. At 5 years posttx, compared to pretransplant values, recipients showed a 9.6% increase in weight, a 3% decrease in serum cholesterol, and a 10.8% decrease in serum lipid values. 84% of the kidney recs remain P-free; the most common reason for restarting P was acute rejection (AR).

Year Post-tx	n (1003)	% Patient	% Graft	% DC-Graft	% AR-free	% CR-free
1	885	96%	94%	97%	82%	93%
2	676	94%	89%	94%	77%	87%
3	503	91%	84%	91%	76%	82%
4	345	89%	80%	88%	74%	77%
5	215	88%	77%	85%	72%	72%
6	97	82%	72%	84%	70%	70%
7	23	82%	70%	82%	69%	68%

Conclusion: Short-term data suggests kidney tx recs do well with rapid discontinuation of P. Our intermediate-term data suggests that patient and graft survival rates remain good and renal function remains stable. Ongoing long-term follow-up is necessary.

Abstract# 455

Implementation of a Steroid-Free, Rapamycin and Neoral Maintenance Immunosuppression Regimen Reduces Acute Cellular Rejection (ACR) but Not Acute Humoral Rejection (AHR). Ronald Pelletier,¹ Anjali Satoskar,² Qiquan Sun,¹ Gyongyi Nadasdy,² Tibor Nadasdy,² Amer Rajab,¹ Ronald Ferguson.¹ ¹General Surgery, Ohio State University, Columbus, OH; ²Pathology, Ohio State University, Columbus, OH.

Background. Since 4/02 our program has employed a steroid-free, Rapamycin and Neoral maintenance immunosuppression regimen for kidney transplant recipients. Prior to that time recipients were treated with prednisone, MMF and Neoral. We noted a significant reduction in acute cellular rejection (ACR) after implementing this regimen. This retrospective analysis was performed to examine the impact, if any, on the incidence, character, and outcomes of early AHR.

Results. This study includes 1611 consecutive kidney recipients transplanted between 1/99 and 12/06. There were 717 recipients in the prednisone, MMF, and Neoral era (Grp 1) and 894 in the steroid-free, Rapamycin and Neoral era (Grp 2). Recipient age, gender, African-American race, AB and DR mismatch, and frequency of PRA $>10\%$ was not statistically significantly different between the 2 groups. However, 43% of Grp 1 pts vs 63% of Grp 2 pts received a living donor kidney due to a more recent volume increase in this procedure ($p<0.001$). There were a total 722 kidney biopsies in 466 pts performed in the first 6 months post-transplant; 478 in 287 pts when excluding pre-perfusion biopsies. Nineteen percent (54/287) of these showed $>50\%$ peritubular capillary (PTC) C4d deposition. Comparison of Grp 1 to Grp 2 demonstrated the following: 1) The incidence of clinical acute rejection in the first 6 months was 12.7% (91/717) in Grp 1 and 5.6% (50/894) in Grp 2 ($p<0.001$), 2) The overall incidence of a C4d+ biopsy was similar in the 2 groups (3.3% (n=24) vs 3.4% (n=30), $p=ns$), 3) Two of 24 (8.3%) Grp 1 pts with a C4d+ biopsy demonstrated no evidence of concomitant ACR versus 13/30 (43.3%) pts in Grp 2 ($p=0.006$). The 1 year graft survival is 68% for C4d+ pts versus 91.5% for all others ($p<0.001$). However, the 1 year graft survival for C4d+ pts was similar in Grp 1 and Grp 2 (67% and 70% respectively, $p=ns$).

Conclusions. Despite a significant reduction in the incidence of acute rejection using our newer, steroid-free immunosuppression protocol, there has been no reduction in the incidence of early (0-6 months) AHR evidenced by C4d+ kidney biopsy. However, the percentage of AHR unassociated with ACR has significantly increased. The poor graft survival in pts with early AHR has not improved with our newer immunosuppression regimen.

Abstract# 456

Risk Factors for Acute Rejection (AR) and Graft Loss (GL) after Kidney Transplantation with Rapid Discontinuation of Prednisone (RPD). E. K. Gross,¹ M. Hughes,¹ M. Hill,¹ T. Jie,¹ K. Gillingham,¹ R. Kandaswamy,¹ A. Humar,¹ T. Dunn,¹ A. J. Matas.¹ ¹Department of Surgery, University of Minnesota, Minneapolis, MN.

Early prednisone withdrawal IS protocols aim to minimize steroid toxicity while maintaining low rates of AR and GL. The purpose of this study is to identify patient groups at high risk for AR and GL with RPD.

Methods: From 1999 to 2007, 1002 transplants were performed within our RPD protocol with a 3-month minimum follow-up. IS consisted of induction with polyclonal antibody, a six day prednisone taper, and dual agent maintenance with a CNI and MMF or SRL. A multivariate analysis (MVA) using Cox regression identified risk factors for AR and GL. Factors included for the analysis were: donor source (LD vs. DD), first vs. retx, 3-6 vs. 0-3 HLA MM, gender (F vs. M), AA vs. non-AA race, pre-tx type 1 DM (Y vs. N), age <18 vs. 18-50, age >50 vs. 18-50, DGF (Y vs. N), and PRA >50 vs. <50 . Additional factors considered for GL were: -DGF/+AR vs. -DGF/-AR, +DGF/-AR vs. -DGF/-AR, and +DGF/+AR vs. -DGF/-AR.

Results: By MVA, significant risk factors for AR included: retx (vs. primary) ($p<.001$), AA race (vs. non-AA) ($p<.05$), age 18-50 (vs. >50) ($p<.0001$) and PRA >50 (vs. <50) ($p.0027$).

Risk Factor	HR	P Value
ReTx vs. Primary	2.0	<0.0001
AA vs. non AA	1.7	0.045
Age >50 vs. 18-50	0.51	<0.0001
PRA >50 vs. <50	2.0	0.0027

Risk factors for GL included: pre-tx T1 DM ($p<.01$), ≥ 1 AR episode ($p<.0001$) and DGF ($p<.0001$). When we repeat the MVA omitting AR and DGF, donor source (DD vs. LD) becomes a significant risk factor ($p<.01$).

Risk Factor	HR	P Value
PreTx T1 DM	1.5	0.0069
LD vs. DD*	0.67	0.0093
AR, no DGF	3.8	<0.0001
DGF, no AR	3.7	<0.0001
DGF, AR	7.3	<0.0001

* AR and DGF omitted

Conclusions: Four risk factors for AR were identified in the RDP study population: retx, AA race, age 18-50 (vs. >50) and PRA >50 (vs. <50). Four risk factors for GL were identified: pre-tx T1 DM, AR, DGF and DD tx (when AR and DGF omitted). These risk factors for AR and GL are the same as we observed in prednisone-containing protocols. Additionally, many of these factors are not modifiable. Identification of high risk groups allows for individualization of IS. Increasing LDs and utilization of IS protocols to decrease or minimize DGF and AR are goals for improving graft outcome.

Abstract# 457

Effect of Steroid Free Maintenance Immunosuppression Protocol on Progression of Chronic Allograft Nephropathy. N. Nainani, N. Patel, M. Rahman, N. Tahir, B. Murray, A. Gundroo, G. Blessios, K. Tornatore, R. Venuto. *Transplant Unit, Erie County Medical Center, SUNY, Buffalo, NY.*

Aim: The impact of steroid free maintenance immunosuppression on chronic allograft nephropathy (CAN) is unclear. The aim of this study is to evaluate the progression of CAN using Chronic Allograft Damage Index (CADI score) in renal transplant recipients (RTR), given no maintenance prednisone.

Methods: We retrospectively studied 89 RTR from Sep 2004 to Sep 2006. All patients were induced with 2 doses of Alemtuzumab. Maintenance immunosuppression consisted of tacrolimus with the target trough level of 5-8 ng/ml and mycophenolate sodium with a maximum dose of 720 mg BID. GFR(MDRD) was measured at 3, 6, 9, 12 and 24 months with surveillance biopsies(bxs) scheduled at 3, 12 and 24 months. Patients were divided into low grade (CADI < 4) and high grade (CADI ≥ 4) groups. Clinical rejection was defined as biopsy(bx) proven rejection with increase in serum creatinine and subclinical rejection as bx evidence with no decline in renal function.

Results: No rejection was noted in 62 patients. Out of these, 60(97%) had a CADI score <4 at 3 month bx and only 2 (3%) had a CADI ≥4. At 12 month bx, 56 out 60 (90%) had a CADI still <4, and 6 (10%) had a CADI ≥4. More than 90% of all bxs at 3 and 12 months showed a CADI <2. To date, 16 patients have had the 24 month bx and only 1 showed increased injury as compared to 12 month. No difference was noted between mean GFR (ml/min/1.73m²) at 3(52.7±2.3), 6(52.4±2.6), 9(52.5±2.48), 12(54±2.7) and 24(51±3.2) months.

CADI SCORE	3 month biopsy	12 month biopsy	p value
< 4	60 (97%)	56 (90%)	0.27
≥ 4	2 (3 %)	6 (10 %)	

Eight of 27 remaining RTR showed subclinical rejection on first or second bx. Seven of these patients had a CADI <4 at first bx and 1 had a CADI ≥4. At 12 months, 6 of these continued to have a CADI <4. One progressed from a low to higher CADI score at 12 months. The patient who had a CADI ≥4 continued to exhibit this level of injury at 12 months. The final 19 RTR were excluded due to the effect of their clinical rejection on CADI score.

Conclusion: Alemtuzumab induced steroid free, low dose calcineurin maintenance immunosuppression regimen exhibits no significant progression of CAN as assessed by CADI scored bxs up to 24 months post transplant. This study suggests that quantitatively assessed surveillance bxs should help determine the risk-benefit ratio of steroid free maintenance immunosuppression regimens.

Abstract# 458

Randomized, Prospective Prednisone (P) Withdrawal Trial in Kidney Transplant Patients Treated with Sirolimus (S) vs. Microemulsified Cyclosporine (CsA) Based Regimens. Uday S. Nori,¹ Todd E. Pesavento,¹ Elizabeth A. Davies,² Jon VonVisger,¹ Becky S. Miller,² Ronald M. Ferguson.¹ *Division of Nephrology, The Ohio State University Medical Center, Columbus, OH; ²Division of Transplantation, The Ohio State University Medical Center, Columbus, OH.*

Many studies have previously reported safe withdrawal of prednisone (PW) late after kidney transplantation (Ktx). To determine the best immunosuppression regimen during the PW, we performed a prospective trial with stable KTx patients randomized to either CsA or 'S' based regimen. **Methods:** All patients received antibody induction therapy at the time of Rtx and maintained on CsA, P and Cellcept®. Patients excluded if they had >1 acute rejection, >1 gm/d proteinuria or serum creatinine >2.5 mg/dl. 161 patients were enrolled and data presented for 141 patients with >52 weeks follow-up (f/u) with mean f/u of 112.2±29.2 weeks. No differences observed in baseline characteristics in both groups. All patients then randomized to either CsA (n=69) or 'S' (n=72) and Cellcept® converted to equivalent dose of MS. CsA dosed by C2 level (2-hour) with goal level of 600 ng/ml. Sirolimus target level was 8 ng/ml. **Results:** 5 patients withdrew from study, 12 patients on S returned to CsA regimen because of side effects. 5 patients in the CsA group and 1 patient in 'S' group had AR (3 of them due to drug non-compliance). Death censored graft survival was 100%. Mean CsA drug level achieved was 664±188 ng/ml and 'S' drug level was 8.2±1 ng/ml. No significant differences noted in hematological values or BP measurements.

Characteristic	CsA (n=69)	S (n=72)	p value
S creat start	1.6±0.4	1.5±0.4	NS
S creat final	1.7±0.4	1.4±0.4	0.0027
Weight loss, kg	5.2±6.0	6.5±5.6	NS
Cholesterol final	184±35	195±62	NS
Triglycerides final	154±88	220±80	<0.05
HbA1c final, %	6.0±0.7	7.2±1.1	<0.01

All values in mg/dl unless specified

Conclusions: Late PW, with either CsA or 'S', can be accomplished safely, with a low rate of AR (4%) or side-effects. Renal function was significantly worse in patients on CsA. Longer-term follow-up of enrolled patients is planned.

Kidney: Complications V

Abstract# 459

Unforeseen Consequence of the De-Emphasis of HLA-Matching in Kidney Transplantation. Herwig-Ulf Meier-Kriesche,¹ Brian Susskind,² Juan Scornik,¹ Shezhad Rehman,¹ Jesse D. Schold.¹ *¹Medicine, University of Florida, Gainesville, FL; ²Medicine, University of Cincinnati, Cincinnati, OH.*

Introduction

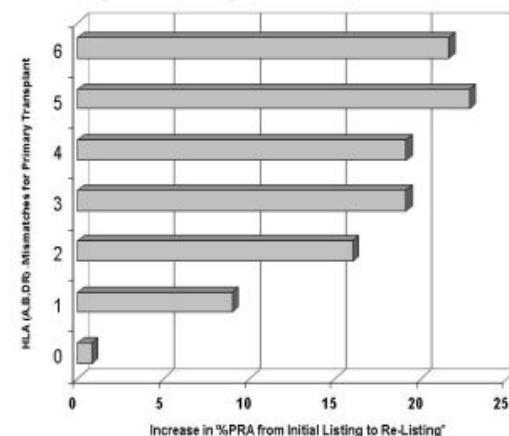
With the advent of more potent immunosuppression HLA matching has been de-emphasized in the allocation of deceased donor kidneys due to the limited impact on acute rejection and graft survival. An unforeseen consequence of poorer matching could be an increase in sensitization of patients in need for a repeat transplant.

Methods

Our study examined candidates listed in the US from 1988-2007 from the SRTR database that were re-listed following loss of a primary kidney transplant (n=19,827). The primary outcome of the analysis was change in PRA from the listing prior to recipient's initial transplant to the subsequent listing. Absolute change in peak and current PRA levels were examined in general linear models as well as the proportion of patients with a rise in PRA level in a logistic model.

Results

HLA(A,B,DR)-matching in the primary transplant was strongly associated with change in PRA level (p<.001, Figure 1). Among recipients with 6-HLA MM, over 50% had a rise in PRA at re-listing as compared to 25% of 0-HLA MM recipients. Younger recipient and donor age, males, deceased donor transplants and African American recipients were also significantly associated with elevation in PRA. In addition, the effect was apparent stratified by primary donor type.



* Adjusted for recipient and donor age, donor type, gender, and race

Conclusions

While there might be a limited impact of HLA matching on graft survival, many patients might be negatively impacted from poor HLA matching from their first transplant when needing a second transplant. As high PRA is one of the strongest risk factors for not getting transplanted, this should be taken into account when evaluating the impact of HLA matching in kidney transplantation. This might be particularly important in younger patients and in patients with a long life expectancy in general because of the high likelihood of needing a second transplant during their lifetime.

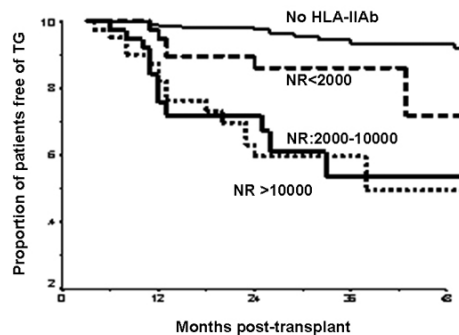
Abstract# 460

The Risk of Transplant Glomerulopathy (TG) and Its Prognosis Relate to the Characteristics of Anti-HLA Class II Antibodies. Naim Issa,¹ Fernando G. Cosio,¹ Sanjeev Sethi,¹ Mark D. Stegall,¹ James M. Gloor.¹ *¹William J von Liebig Transplant Center, Mayo Clinic, Rochester, MN.*

Background: Previous studies showed that the risk of developing TG is strongly associated with anti-HLA class II antibodies (HLA-IIAb), particularly when those antibodies (Ab) have donor specificity (DSA). The goal of this study was to assess whether the characteristics of HLA-IIAb relate to the incidence and prognosis of TG. **Methods:** HLA-IIAb were measured in pre-transplant sera of 598 kidney transplant recipients with a negative cytotoxic crossmatch at the time of transplantation. Single HLA antigen-coated flow beads were used to determine: 1) presence of HLA-IAb and/or HLA-IIAb; 2) Ab normalized ratio (NR, a measure of antibody activity); 3) DSA; 4) HLA-IIAb subclass. All patients had protocol biopsies at one year and as clinically indicated. TG was diagnosed by protocol or clinical biopsies.

Results: Among the 598 patients, 235 (40%) had detectable HLA-Ab: HLA-IAb (12%), HLA-IIAb (11%) and both (17%). Seventy three patients (12%) developed TG within

53.5±19 months of follow up. By multivariate Cox analysis the risk of TG related to the presence of HLA-IIAb [HR=7.77, CI: (4.54-13.2), $p<0.0001$] and a history of antibody-mediated acute rejection [3.11 (1.86-5.19), $p<0.0001$]. In addition, both the presence of DSA [HR=2.05 (1.03-4.03), $p=0.041$] and higher NR [1.07 (1.00-1.13), $p=0.03$] (Figure) were independent correlates of TG.



In contrast, the presence of HLA-IAb and the antigen subclass of the HLA-IIAb (60% anti-DR and 40% anti-DQ) did not relate to TG risk. Death censored graft loss occurred in 4.6% of patients without TG and in 38.4% of patients with TG ($p<0.0001$). By multivariate analysis, reduced survival of grafts with TG related to higher HLA-IIAb NR [2.12 (1.4-3.2), $p<0.0001$], lower graft function [4.55 (2.1-9.7), $p<0.0001$] and higher proteinuria at diagnosis [1.47 (1.2-1.7), $p<0.0001$].

Conclusions: HLA-IIAb are present in 28% of conventional transplants. HLA-IIAb are associated with higher risk of TG and reduced graft survival. Furthermore, the risk of TG and its prognosis relate to the level of HLA-IIAb quantitated in a solid phase assay.

Abstract# 461

Clinical Significance of C4d Deposition in Stable Renal Allografts in Early Posttransplantation Period. Hye Eun Yoon,¹ Su Hyun Kim,¹ Jin Young Kim,¹ Sang Ju Lee,¹ Sun Cheol Park,² In Sung Moon,² Yong Soo Kim,¹ Suk Young Kim,¹ Byung Kee Bang,¹ Chul Woo Yang.¹ ¹Internal Medicine, The Catholic University of Korea, Seoul, Korea; ²Surgery, The Catholic University of Korea, Seoul, Korea.

Purpose: The clinical significance of C4d positivity in patients with acute rejection is well defined but its significance in stable graft function is undetermined. This study was performed to evaluate the clinical outcome of protocol biopsy-proven C4d positive renal transplants with stable graft function in the early posttransplantation period.

Methods: 151 renal allograft biopsies were included. Protocol biopsies (n=79) were performed from stable allografts on day 14 posttransplantation, and indication biopsies (n=72) were performed from dysfunctioning allografts. Incidence of C4d positivity was compared between protocol and indication biopsies. Clinical characteristics, biopsy findings, graft function, acute rejection episodes, and graft survival rates were compared between the C4d-positive and C4d-negative grafts in each group.

Results: C4d deposition in protocol biopsies was detected in 4 of 79 biopsies (5.1%), whereas 9.7% (7 of 72 biopsies) in indication biopsies. The histological findings of C4d-positive protocol biopsies were minimal inflammation of tubulointerstitium. On the other hand, those of C4d-positive indication biopsies were various including acute humoral rejection, acute cellular rejection, acute tubular necrosis and calcineurin inhibitor toxicity. In the protocol biopsy group, graft function during 1 year after biopsy, acute rejection rate, and cumulative graft survival did not differ between the C4d-positive and C4d-negative grafts. All C4d-positive allografts maintained stable graft function without any antirejection therapy. In the indication biopsy group, graft function during 1 year after biopsy and acute rejection rate did not differ between the C4d-positive and C4d-negative grafts. However the cumulative graft survival rate was worse in the C4d-positive grafts than the C4d-negative ones ($p=0.008$).

Conclusion: C4d positivity associated with allograft dysfunction indicates a poor graft outcome. However, C4d-positive allografts with stable graft function in the early posttransplantation period take an indolent course.

Abstract# 462

Are Donor Specific Antibodies and Diffuse C4d Staining Independently Helpful in Stratifying Risk of Graft Failure in Patients with Kidney Transplants? Sarah de Freitas,¹ Robert Vaughan,² Nizam Mamode,¹ Steven H. Sacks,¹ Ross Nortley,¹ Robert Hangartner,³ Jennifer E. Else,⁴ Olivia J. Shaw,² Patrick J. O'Donnell.³ ¹Department of Transplantation, Guy's and St Thomas' NHS Foundation Trust, London, United Kingdom; ²Clinical Transplantation Laboratory and St Thomas' NHS Foundation Trust; ³Department of Histopathology, Guy's and St Thomas' NHS Foundation Trust; ⁴Department of Histopathology, St George's Health Care Trust, London.

Purpose: To look at the impact of diffuse (>50%) C4d staining detected on Transplant Kidney biopsy, with and without associated serum Donor Specific HLA Antibody (DSA) detection, on Kidney Transplant outcome.

Methods: This was a retrospective single centre study reviewing all the adult patients who had a kidney transplant biopsy between April 2003 and October 2006 at Guy's Hospital.

Results: 45 patients had diffuse (>50%) C4d staining out of 228 who had kidney transplant biopsies in this time and had been followed up within the centre. Of these 45 patients, 20 also had DSA prior or at the time of biopsy. The fall in eGFR in this group a year post biopsy was greater than those with diffuse staining for C4d but no DSA. The mean change in eGFR from the day of biopsy at a year was -3.1 ml/min/1.73m2 (+/-12.1) in those with DSA compared with +17.7 ml/min/1.73m2 (+/-23.2) for those with C4d but without DSA. The changes in eGFR from the pre-biopsy baseline at one year showed a fall in eGFR in both groups but this was greater in those with DSA (-21.5 compared with -9.6 ml/min/1.73m2).

Of 179 patients who never had diffuse or focal C4d staining on biopsy, only 81 had had DSA tested. Of these only 8 (10%) had a positive DSA result. From these eight, four had features of rejection and four did not. One person in each of these groups is dialysis dependant and one person in the rejection group has eGFR <15 ml/min/1.73m2. Although small numbers, this outcome appears to be worse than that of C4d negative patients with no DSA but features of rejection who in fact showed an improvement in eGFR from the day of biopsy by 14.4 ml/min/1.73m2 or an improvement from their pre-biopsy baseline of 1.3 ml/min/1.73m2 at one year.

Conclusion: DSA is of additional value in evaluating risk of graft failure. This appears to be of value in those with and without diffuse C4d staining on biopsy.

Abstract# 463

Utility of Post-Transplantation Flow Cytometry Crossmatching in Predicting Graft Outcomes. Michelle Willicombe,¹ Graham Shirling,² Ray Fernando,² Henry Stephens,² Paul Sweny,¹ Peter J. Dupont.¹ ¹Department of Renal Medicine, Royal Free Hospital, London, United Kingdom; ²Histocompatibility Laboratories, Anthony Nolan Trust, London, United Kingdom.

Background:

De novo development of donor-specific anti-HLA antibodies after renal transplantation may be associated with increased rejection and decreased graft survival. Flow-cytometry crossmatches (FCXM) have been suggested as method of screening for development of donor-specific HLA antibodies post-transplantation, but interpretation of crossmatch results can be confounded by antibodies directed against antigens other than HLA. We assessed the impact of developing a positive FCXM post-transplantation on clinical outcomes in a cohort of live donor renal allograft recipients.

Methods:

29 patients were studied. 23/29 (79%) received tacrolimus-based and 6/29 (21%) ciclosporin-based immunosuppression. Median follow-up was 24 months. All patients had negative complement-dependent cytotoxic (CDC) T cell crossmatches pre-transplantation.

Results:

Following transplantation, 8/29 patients (28%) developed a new positive FCXM. 5/8 (63%) developed a positive T cell FCXM, 2/8 (25%) a positive B cell FCXM, and 1/8 (12%) both T and B cell positive FCXM. Only 3/8 patients (38%) with a positive FCXM had detectable anti-HLA antibodies by Luminex screening and none had antibodies directed against mismatched donor HLA antigens. Clinical outcomes were similar in patients with a new positive FCXM post-transplantation compared with those with a negative FCXM. 4/8 (50%) in the group with a positive FCXM had an acute rejection episode in the first 12 months compared with 10/21 (41%) in the group with a negative FCXM ($p=NS$). Graft function at 12 months was not different between the groups (positive FCXM - median creatinine 137mmol/L; negative FCXM - median creatinine 141mmol/L; $p=NS$). 2/8 grafts (25%) were lost within the first year in the positive FCXM group compared with 1/21 (5%) in the group with negative post-transplant FCXM ($p=NS$).

Conclusions:

The development of a positive FCXM post-transplantation alone is not predictive of adverse clinical outcomes. This may be explained by the poor correlation between a positive FCXM and the presence of antibody directed against mismatched donor HLA antigens. Surveillance for development of donor-specific anti-HLA antibodies after transplantation may be best performed using high-resolution bead technologies rather than FCXM. Use of the FCXM alone, without establishing antibody profiles, is of limited predictive value.

Abstract# 464

Incidence and Risk Factors for Wound Complications in Patients Undergoing Positive Cross Match Kidney Transplantation. David J. Rea,¹ Harrison S. Pollinger,¹ Mark D. Stegall,¹ James M. Gloor.¹ ¹William von Liebig Transplant Center, Mayo Clinic, Rochester, MN.

Introduction. Desensitization protocols have allowed for successful kidney transplantation in patients with a positive crossmatch against their living donor (+XM KTx). However, these protocols also have led to increased complications in recipients. The aim of this study was to examine the incidence of and risk factors for wound complications +XM KTx.

Methods. We retrospectively analyzed 170 patients (59 men, 110 women) undergoing +XM KTx between 4/2000 and 9/2007 at our institution. The median follow-up period

was 1082 days (81 -2777 days). Wound complications (<3 months) after KTx were categorized using standard CDC criteria. The risks factors analyzed included: age, body mass index (BMI), diabetes (DM), number of pre- and post-KTx PP treatments, rituximab, high dose IVIG (2mg/kg) and acute humoral rejection (AHR). rATG induction therapy, tacrolimus, MMF and prednisone maintenance regimens and selective plasmapheresis (PP) for high levels of alloantibody were utilized in our patients. Data were compared using the Fisher's exact and Chi-square tests.

Results. The overall incidence of wound complications was 34.5% (23% superficial, 9% deep and 8% organ space) compared to 7% in our conventional transplants. Risk factors which achieved statistical significance with respect to superficial wound infection were BMI>30, DM and the use of high dose IVIG. Deep wound infection was significantly related BMI >30, DM, and development of AHR. Incisional hernia was statistically more likely for patients who received > 16 PP treatments.

Wound Complications & Risk Factors

Variable (% pts.)	Superficial Wound Infection	Deep Wound Infection	Organ/Space Infection	Incisional Hernia
BMI>30 (27%)	41%*	20%*	9%	17%
BMI≤30 (73%)	16%	4%	7%	11%
PP> 16 (16%)	25%	14%	14%	25%*
PP≤ 16 (84%)	23%	7%	6%	11%
(+) DM (21%)	40%*	20%*	3%	17%
(-) DM (79%)	19%	5%	9%	12%
(+) AHR (31%)	31%	15%*	12%	17%
(-) AHR (69%)	19%	5%	6%	11%
(+) HD-IVIg (25%)	40%*	10%	0%	12%
(-) HD-IVIg (75%)	17%	8%	10%*	13%

*p<0.05

Conclusions. +XM KTx recipients have a higher rate of wound complications than conventional transplants. While common risk factors prevail (DM and obesity), the development of AHR and prolonged PP significantly increased the incidence of wound complications. A lower wound complication rate in patients without AHR suggests that uncomplicated +XM KTx may not be associated with higher wound complication rates.

Kidney: Deceased Donor Issues III

Abstract# 465

Long Term Graft and Patient Outcomes in Highly-HLA Sensitized (HS) Deceased Donor (DD) Kidney Transplant (KT) Recipients Desensitized with High Dose (HD) IVIG. Alice Peng,¹ Ashley Vo,¹ Rafael Villicana,¹ Donald Dafeo,¹ Nancy Reinsmoen,¹ Stanley Jordan.¹ ¹*Comprehensive Transplant Center, Cedars Sinai Medical Center, Los Angeles, CA.*

Introduction: The development of desensitization protocols has allowed successful transplantation of crossmatch (CMX) (+) organs. Experience with desensitization of HS DD KT recipients is limited, as most centers consider desensitization for LD recipients only. Our center has extensive experience with the use of HD IVIG in DD kidney transplantation. For HS pts, recent data shows an increased acute rejection rate but acceptable 1 yr graft survival. Questions remain regarding the long-term durability of HLA-incompatible transplants, especially with increasing evidence of the negative impact of donor specific antibodies (DSA). Here, we examined the long-term graft & patient survivals for HS DD KT recipients. **Patients & Methods:** Between Jan 1994 and Dec 2006, 71 HS patients underwent desensitization and DD transplantation using combinations of HD IVIG and rituximab. FCMXs often remained (+) at transplant. The average PRA was 58%; 46% were retransplants. We examined the 1, 3, and 5-yr graft and patient survival rates, mean SCR of the functioning grafts, and causes of graft loss.

Results: Of the 71 HS DD patients, 60 patients (88.2% death-censored graft survival) had functioning grafts at 1-yr. 8 grafts had failed, 1 was lost to follow-up (LTFU), and 2 had death with functioning grafts (DWFG). Of 27 patients with 3 yrs of follow-up, 20 patients had functioning grafts (83.3%), 4 grafts had failed, 1 was LTFU, and 2 had DWFG. At 5 yrs, 9 of 17 patients had functioning grafts (69.2%), 4 had failed, none were LTFU, and 4 had DWFG. 3 of the 4 transplants lost at 5 yrs were lost within the 1st yr of their transplant due to rejection. The 4th failed graft was lost at 15 mo due to noncompliance. The average SCR of the functioning grafts at 1, 3, and 5 yrs were 1.39 ± 0.56 mg/dl, 1.29 ± 0.60 mg/dl, and 1.67 ± 1.26 mg/dl respectively. Patient survivals were 97.1%, 92.3%, and 76.5% at 1-, 3-, and 5-yrs respectively. **Conclusions:** The 1, 3, and 5-yr outcomes in our desensitized HS DD KT recipients were 88.2%, 83.3%, and 69.2%. The majority of our failed grafts were lost to early acute rejection. Late failures were due to noncompliance or DWFG. Overall, desensitization therapy with HD IVIG offers HS patients an opportunity for successful DD KT and long term graft survival.

Abstract# 466

Amount of Antibody, as Expressed by Fluorescence Intensity, Does Not Correlate nor Is Predictive of Crossmatch Outcome. Ronald H. Kerman,¹ Stephen M. Katz,¹ Jacqueline Lappin,¹ Eva McKissick,¹ Kathleen Hosek,¹ Noriel Acorda,¹ Barry D. Kahan,¹ Charles T. Van Buren.¹ ¹*Surgery-Organ Transplantation, University of Texas Medical School, Houston, TX.* UNOS has replaced panel reactive antibody (PRA) with a calculated PRA (CPRA). The CPRA should result in a more standardized definition of patient sensitization. Each transplant center can set criteria for indentifying and listing unacceptable antigens either

based upon the amount of antibody (Ab) measured by the titer of donor specific HLA antibodies, DSA, or the fluorescence intensity (FI) of the donor specific single antigen bead. It is unclear whether Abs indentified by sensitive single antigen bead and solid phase assays (Flow PRA and Luminex) correlate with and are predictive of a clinically relevant end-point (a + FCXM).

We evaluated the PRA, DSA, bead specific Ag FI and FCXM reactivity of pre-transplant (pre-Tx) sera from 219 recipients of a deceased donor renal allograft to determine whether amount of Ab (measured by FI) predicts a (+) FCXM.

Patients with a (+) DSA, a (+) FCXM and pre-Tx class I PRA ≥ 80% (N = 103, mean PRA of 91 ± 6%) when compared to patients with pre-Tx PRA < 80% (N = 58, mean PRA 53 ± 21%) had comparable mean FIs (7,407 ± 4,305 vs 7,483 ± 5,380), FI ranges (1,000 – 20,243 vs 1,552 – 17,153) and median FIs (5,681 vs 5,670). The class II comparisons were of the same pattern. Surprisingly, patients with a (+) DSA, a (-) FCXM and pre-Tx class I PRA ≥ 80% (N = 22, mean 90 ± 6%) compared to patients with pre-Tx PRA < 80% (N = 36, mean PRA 50 ± 18%) had comparable mean FIs (6,758 ± 4,841 vs 6,412 ± 3,844), FI ranges (1,656 – 16,596 vs 2,162 – 11,637) and median FIs (5,924 vs 5,627). Again, the class II PRA comparisons were of the same pattern. Of great interest is that the mean FI, FI range and median FI of DSA (+)/FCXM (+) and DSA (+)/FCXM (-) are not statistically different.

Because the mean FIs, FI ranges and median FIs of patients who are DSA (+) and either FCXM (+) or (-) are not statistically different from each other the amount of antibody (expressed as FI) can not be used to predict a (+) FCXM. Other factors such as Ab avidity for the antigen epitope and consideration of cross reactive Abs may contribute to predicting crossmatch outcome. Therefore, since FI is not predictive of crossmatch results it should not be an acceptable criteria for identifying clinically relevant donor specific HLA Abs or used for listing unacceptable antigens as they relate to donor allocation schemes.

Abstract# 467

Strategies To Improve the Quality of Organs from DCD: Pre-Treat the Donor with Erythropoietin and Treat the Recipient with 5-Aminoisoquinolinone. Rui Maio,¹ Nuno Figueiredo,¹ Isa Santos,¹ Bruno Sepodes,² Helder Mota-Filipe,² Christoph Thiemermann,³ Paulo Costa.¹ ¹*Clinica Universitaria Cirurgia I, Hospital Santa Maria, Lisboa, Portugal;* ²*Unidade Farmacologia e Farmacotoxicologia, Faculdade Farmacia Lisboa, Portugal;* ³*William Harvey Research Institute, Royal London School of Medicine, United Kingdom.*

Background: We have recently showed that pre-treatment of the donor with EPO causes a substantial reduction of the dysfunction and injury associated with the transplantation of kidneys recovered after cardiac death. 5-aminoisoquinolinone (5-AIQ) a potent water soluble PARP inhibitor has proven to reduce renal ischemia-reperfusion (I/R) injury. The aim of our study was to determine the effects in the graft and in the receptor of the pre-treatment of the donor with EPO and treatment of the recipient with 5-AIQ, in a porcine model of DCD kidney transplantation.

Material/Methods: 24 Landrace pigs were killed by lethal injection; their kidneys were subjected to 30 min of warm ischemic time (WIT) and then transplanted after 24 h of cold storage in Celsior. In the pre-treated group, donors received a single dose of EPO (1000 IU/kg) 30 min before cardiac arrest. In the treated group, recipients received a continuous dose of 5-AIQ (5mg/kg/h) 10 minutes before reperfusion and maintained during 60 minutes. Blood, urine and renal tissue samples were collected at the end of the experiment for biochemical, histological and immunohistochemistry (PARS, iNOS and COX-2) evaluation. Data analysis performed with Graph Pad Prism Statistical Package; p<0.05 considered statistically significant.

Results: Transplantation of kidneys from DCD resulted in: a significant rise of the levels of creatinine, N-acetyl-b-D-glucosaminidase, glutathione-S-transferase, AST, LDH, ALT, fractional excretion of Na⁺, interleucine 1 and 6, malondialdehyde levels and myeloperoxidase activity (p<0.05); a significant reduction in urine flow and creatinine clearance, disturbances in the histological and immunohistochemistry pattern. Administration of EPO before ischemia and 5-AIQ before reperfusion reduced significantly the biochemical (p<0.01), histological and immunohistochemical evidence of glomerular dysfunction and tubular injury. They also reduced systemic injury, inflammatory response and oxidative stress.

Conclusions: Pre-treatment of the donor with EPO and treatment of the recipient with 5-AIQ causes a substantial reduction of the dysfunction and injury associated with the transplantation of kidneys recovered after cardiac death.

Abstract# 468

US Results of Mismatching Donor and Recipient Age after Kidney Transplantation. Maureen A. McBride,¹ Ciara J. Gould,¹ Wida S. Cherikh,¹ Francis L. Delmonico,² Timothy L. Pruett.³ ¹*United Network for Organ Sharing, Richmond, VA;* ²*Massachusetts General Hospital, Boston, MA;* ³*University of Virginia HSC, Charlottesville, VA.*

Background. As the transplant community considers the concepts inherent in a new deceased donor kidney allocation system, the question arises to why a new system is needed. Anecdotally, there are many reports that "old" kidneys are going into "young" patients leading to early graft failures, and that "young" kidneys are going into "old" patients leading to increased incidence of death with a functioning graft. We set out

LIVER: EXTENDED CRITERIA DONOR, DCD, ECD

to determine if the age distribution of donors and kidney recipients affected transplant outcomes to determine whether these anecdotes are true.

Methods. Comparisons of donor and recipient demographics and outcomes were performed on 22,236 deceased donor, kidney-alone transplants performed between 2002 and 2004. Recipients with any previous organ transplant were excluded. The numbers of kidneys that failed within 3 years and the number of recipients who died with a functioning graft within 3 years of transplant were tabulated.

Results. There were 2,721 transplants performed in recipients aged 18-34; 6,339 ages 35-49; 9,046 ages 50-64; and 3,217 ages 65+. However, 1,363 (43.6%) of kidney transplants from pediatric donors (0-17) were performed in recipients age 50 or older, and 126 (13.1%) of kidneys from donors aged 65 or greater were transplanted into recipients between the ages of 18-49. Death with a functioning graft occurred within 3 years for 1,682 (7.6%) recipients. The majority (78%) of these deaths occurred among recipients age 50 or older (55% of the transplants); and among these older recipients, 381 (28.9%) had received kidneys from donors aged less than 34. Among the recipients aged 18-34 who received an organ from a donor over age 50, 94 (22.3%) failed within 3 years and 61 (65%) of these patients were relisted for a second transplant by November 30, 2007.

Conclusions. There are significant numbers of older kidneys allocated to younger recipients and younger kidneys allocated to older recipients. This does appear to be resulting in excess numbers of deaths with functioning grafts among older patients and excess early graft failures among younger recipients. These results justify the need for a revision to the current allocation system to more appropriately allocate deceased donor kidneys.

Abstract# 469

Underutilization of Kidneys from Deceased Donors Older Than Age 70. Tariq Shah,^{1,3,4} Suphamai Bunnapradist,² Jagbir Gill,² Steven K. Takemoto.¹
¹National Institute of Transplantation, Los Angeles; ²Medicine, UCLA; ³MOTC, St Vincent Medical Center, LA, CA; ⁴USC Medicine & Urology, LA, CA.

Since 2000, despite the shortage of kidneys from deceased donors for transplantation, only about 100 kidney transplants each year were performed with donors older than age 70. Kidneys from these older donors represent only 6% of those allocated in the United States as “expanded criteria donors”. In this study, we examine how these kidneys might be optimally utilized.

Methods: This retrospective examination of 9,580 recipients of expanded criteria donor (ECD) kidney-only transplants performed during 2000-2007 and reported to the Organ Procurement Transplant Network excluded those with dual and en bloc procedures. Outcomes for recipients of donors age 50-59 and ≥70 compared to a reference group of donors age 60-69 were stratified by recipient age and expressed as hazard ratios (HR) after adjusting for recipient and donor covariates using Cox proportional hazards.

Results: 26% of ECD kidneys were transplanted to recipients younger than age 51, 31% to age 51-60, 18% to 61-65 and 25% to recipients older than age 65. The table below shows the hazard of graft loss (GL), functional graft loss (FGL) censoring death, and death (Dth). Compared to kidneys from donors age 60-69, recipients younger than 51 were about 25% less likely to die or lose an ECD kidney from a donor aged 50-59 and 70% more likely with kidneys from donors older than 69. A similar trend was seen with older recipients, but the risks seemed to be lower.

Logistic regression indicates recipients older than age 65 were 5 times more likely to have a donor older than age 70 than recipients younger than 50, hypertension and creatinine >1.5 were less likely in older donor kidneys. Interestingly, the use of these kidneys relative to the total number of ECD kidneys has decreased since 2002 (Odds ratio=0.63, 0.53-0.76, P<0.001).

Conclusion: An increase in the supply of kidneys might be achieved with increased utilization from deceased donors older than age 70. Outcomes were similar to those from donors age 60-69 in recipients that were older than age 50.

Donor Age*		<=50		51-60		Recipient Age 61-65		>=65	
		HR, 95% CI, P		HR, 95% CI, P		HR, 95% CI, P		HR, 95% CI, P	
GL	50-59	0.77, 0.65-0.90, 0.002		0.95, 0.80-1.11, 0.50		0.84, 0.68-1.03, 0.10		0.92, 0.77-1.09, 0.34	
	>=70	1.79, 1.24-2.59, 0.002		1.17, 0.82-1.66, 0.40		1.32, 0.96-1.82, 0.09		1.12, 0.89-1.40, 0.33	
FGL	50-59	0.80, 0.66-0.95, 0.013		1.01, 0.84-1.22, 0.13		0.72, 0.54-0.96, 0.02		0.93, 0.73-1.20, 0.59	
	>=70	1.91, 1.28-2.84, 0.002		1.23, 0.80-1.89, 0.34		1.29, 0.84-1.98, 0.24		0.85, 0.59-1.21, 0.35	
Dth	50-59	0.74, 0.57-0.98, 0.034		0.90, 0.73-1.10, 0.31		0.99, 0.77-1.27, 0.93		0.86, 0.70-1.05, 0.14	
	>=70	1.69, 0.93-3.07, 0.088		1.22, 0.80-1.86, 0.36		1.23, 0.82-1.84, 0.32		1.16, 0.90-1.50, 0.26	

* Donor age reference 60-69

Abstract# 470

Outcomes of Kidney Transplantation from Cardiac Death Donors with Rising Serum Creatinine. Tamotsu Tojimbara,¹ Kazuhiro Iwadoh,¹ Koji Nanmoku,¹ Kotaro Kai,¹ Akihito Sannomiya,¹ Ichiro Koyama,¹ Kazunari Tanabe,² Ichiro Nakajima,¹ Shohei Fuchinoue,¹ Satoshi Teraoka.¹ ¹Surgery III, Tokyo Women's Medical University, Tokyo, Japan; ²Urology, Tokyo Women's Medical University.

Background: To alleviate chronic shortage of renal graft, donation after cardiac death (DCD) donors have been accepted as the organ resources in Japan. We have used DCD

donors with progressively rising creatinine (Cr) at time of nephrectomy. In this study, the outcomes of kidney transplantation (KTx) from DCD donors, according to the terminal renal function of the donors, were retrospectively analyzed.

Methods: Between 1995 and 2003, 103 KTx from DCD donors were performed at our institute. Four of the 103 patients were excluded because of incomplete data. Mean Cr level of the donors at time of organ procurement was 2.4 ± 2.3 mg/dl (0.06 – 11.0). Mean age of the donors and recipients were 45 ± 17 years and 42 ± 15 years, respectively. Recipients were stratified into 3 groups according to the terminal Cr of the donors: Cr < 2.0 mg/dl (Group 1), 2.1 mg/dl < Cr < 4.0 mg/dl (Group 2), Cr > 4.1 mg/dl (Group 3); and evaluated for outcomes. The recipients were followed up for an average of 5.9 ± 2.7 years postoperatively.

Results: The data in each group are shown in Table.

	Group 1 (n=58)	Group 2 (n=22)	Group 3 (n=19)	p value
Donor terminal Cr (mg/dl)	1.0 ± 0.5	2.8 ± 0.6	6.3 ± 2.4	< 0.0001
Donor age (year)	44 ± 19	44 ± 16	51 ± 14	0.3633
Pre-arrest cannulation (%)	51 (88)	15 (68)	13 (68)	0.0582
WIT (min)	6.3 ± 10.6	7.9 ± 9.4	5.7 ± 7.1	0.1695
TIT (hr)	10.3 ± 5.2	11.7 ± 6.8	11.7 ± 4.9	0.5130
Recipient age (year)	40 ± 16	45 ± 12	46 ± 12	0.3609
DGF (%)	41 (71)	20 (91)	18 (95)	0.0164
PNF (%)	2 (4)	1 (5)	0 (0)	
Post-op dialysis (day)	8.1 ± 8.9	8.8 ± 6.6	9.6 ± 5.0	0.1818
Cr at one month (mg/dl)	2.0 ± 1.2	2.6 ± 1.3	2.4 ± 1.3	0.0489

There were significant differences in donor terminal Cr, incidence of delayed graft function (DGF) and Cr level one month after KTx between the groups (Kruskal-Wallis). Overall patient survival rates at 1, 5 and 10 years were 100%, 96% and 96%, respectively. Overall graft survival rates at 1, 5 and 10 years were 91%, 88% and 77%, respectively. Graft survival rates were similar among the groups.

Conclusion: Our data demonstrated that kidneys from DCD donors with a progressively rising Cr could be used safely with promising long-term outcomes.

Liver: Extended Criteria Donor, DCD, ECD

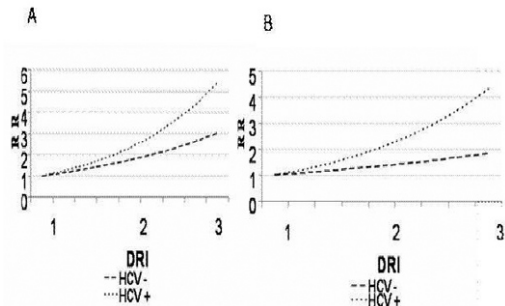
Abstract# 471

Impact of the Donor Risk Index in the Outcome of HCV+ Liver Transplant Recipients. Daniel G. Maluf,¹ Erick B. Edwards,² Todd R. Stravitz,¹ Myron H. Kauffman.² ¹Hume Lee Transplant Center, Virginia Commonwealth University, Richmond, VA; ²United Network for Organ Sharing, Richmond, VA.

Background: The use of extended criteria donors (ECD) is widely accepted across the USA. We have investigated the impact of ECD liver allografts on the outcome of hepatitis C virus (HCV)-infected patients undergoing LTx.

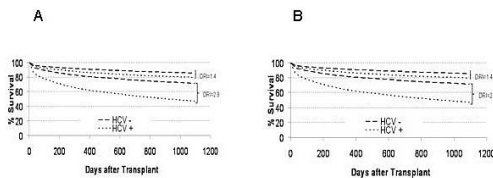
Methods: An analysis of 9901 liver transplant recipients / donors was performed from the Organ Procurement and Transplantation Network database transplanted between June, 2002 and June, 2006. The Donor Risk Index (DRI) was calculated as described by Feng, et al. MELD exceptions were excluded from the analysis. Relative risk (RR) estimates of patient and graft loss were derived from Cox regression models.

Results: Recipients were 75.9% Caucasian, 67.3 % male, 52 years old (median), and mean follow-up time was 712 days (range: 0 – 1906). Based on hepatitis C serology, 44.6 % (n= 4414) of LTx recipients were HCV(+). The median DRI was 1.3 (range: 0.77 to 4.27). Increasing DRI was associated with a statistically significant increase in the relative risk of graft failure and patient death for both HCV(+) and HCV(-) recipients as showed. However, HCV(+) recipients demonstrated a much greater increase in RR of patient and graft loss as a function of the DRI than HCV(-) subjects, even after adjustment for recipient MELD, age, gender, ethnicity, year of transplant, diabetes, dialysis, portal vein thrombosis, previous abdominal surgery, and previous malignancy (p= 0.001).



Moreover, graft and patient survival rates were also negatively affected by the interaction between DRI and HCV serology (p= 0.001)

Figure 2.



Conclusion: An additive, if not synergistic, adverse interaction between donor DRI and recipient HCV status exists, suggesting that HCV infected patients should not receive liver grafts from higher DRI donors.

Abstract# 472

Utilization, Outcomes, and Retransplantation of Liver Allografts from Donation after Cardiac Death: Implications for Further Expansion of the Cadaver Donor Pool. Frederic W. Selck,¹ Eric B. Grossman,¹ Lloyd E. Ratner,² John F. Renz.² ¹New York Organ Donor Network, New York, NY; ²College of Physicians and Surgeons of Columbia University, New York-Presbyterian Hospital, New York, NY.

OBJECTIVE: Donation after cardiac death (DCD) for liver transplantation (LTX) has significant potential but application remains infrequent. This investigation examines DCD utilization, outcomes, and retransplantation to identify mechanisms to optimize DCD application.

METHODS: Retrospective analysis of the Scientific Registry of Transplant Recipients database from 01/02 through 04/07 identified 845 DCD and 21,441 non-DCD adult, initial, whole-organ, liver-only LTX. Demographic and physiologic (Model for End-Stage Liver Disease [MELD]) data were compared. Outcome measures were graft survival determined as death or retransplantation and listing for retransplantation within one-year post-LTX.

RESULTS: DCD donors were younger ($p<0.001$), with fewer African-American and non-caucasian race ($p<0.001$), and fewer deaths secondary to stroke ($p<0.001$). DCD recipients were older ($p<0.001$), with lower MELD ($p<0.001$), and less likely in ICU ($p=0.02$) or high-urgency status ($p<0.001$). DCD allografts were more frequently imported from another allocation region (12% vs 7% [$p<0.001$]). Cox regression analysis of time to DCD graft failure demonstrates higher DCD graft failure within the first 180 days (20.5% DCD vs 11.5% non-DCD, [$p<0.001$]), but approximates non-DCD survival thereafter. DCD listing for retransplantation and graft failure progressed continuously over 180 days versus 20 days in non-DCD. When retransplanted, DCD recipients waited longer and received higher risk allografts ($p=0.039$) more often from another region. More DCD recipients remain waiting for retransplantation with fewer removed for death, clinical deterioration, or improvement.

CONCLUSIONS: DCD utilization is impeded by early outcomes and a temporally different failure pattern that limits access to retransplantation. Allocation policy that recognizes these limitations and increases access to retransplantation is necessary for expansion of this donor population.

Abstract# 473

Orthotopic Liver Transplantation with Allografts from DCD Donors. Roberto C. Lopez-Solis,¹ Eghesad Bijan,¹ Tom Kusum,¹ Sharma Vivek,¹ Patel Sunil,¹ Marsh Wallis,¹ Fontes Paulo,¹ Marcos Amadeo,¹ Michael E. de Vera.¹ ¹Division of Transplant Surgery, University of Pittsburgh Medical Center, Pittsburgh, PA.

BACKGROUND: In the current era of liver transplantation, organ shortage continues to be a significant problem. The use of extended criteria allografts from donation after cardiac death (DCD) donors to increase transplantation rates is widely practiced. This study is a review of one of the largest single center experiences utilizing DCD donors in the world with a follow-up of almost 15 years.

METHODS: From 03/01/1993 to 10/31/2007, 3,431 liver transplants were performed at our institution, 146 (4.2%) of which were liver allografts from DCD donors. Patient and donor demographics, recipient and graft survival, and the incidence of primary non function, hepatic artery thrombosis, retransplantation, and bile duct complications were analyzed for this subset of recipients.

RESULTS: Kaplan Meier analysis showed a 1- and 5-year patient and graft survival of 81% and 71%, and 71% and 59%, respectively. The mean age of recipients was 52 ± 10 years with an average MELD score of 18.7 ± 9.3 (range, 6 to 40), and there were 101 male patients (69%). Donor mean age was 36 ± 16 years and cold ischemia time was 646 ± 173 minutes. One hundred and three patients (70.5%) are alive and 24 (16.4%) underwent retransplantation. The incidence of primary non-function was 11.6% (17 patients) and hepatic artery thrombosis was 4.1% (6 subjects). Biliary complications consisting of bile cast syndrome, intrahepatic strictures, and anastomotic stenoses occurred in 40 patients (27.4%). No difference was found in the survival rate of patients who received grafts from donors > 60 years old compared to those who received grafts from younger donors. However, 37% of patients with donor grafts > 60 years old underwent retransplantation and the incidence of bile duct complications in this group was 50%.

Conclusion: DCD donors are a viable source of liver allografts for transplantation. Patients who receive DCD livers have outcomes comparable to subjects who receive grafts from brain dead donors. Use of DCD livers from donors over 60 years of age is accompanied by a higher incidence of retransplantation and biliary complications.

Abstract# 474

Machine Preservation of Liver Allografts for Transplantation: Updated Results of a Phase I Clinical Trial. James V. Guarrera,¹ Ben Arrington,¹ Benjamin Samstein,¹ John F. Renz,¹ Lloyd E. Ratner,¹ Robert S. Brown,¹ H. Thomas Lee,² Jean C. Emond.¹ ¹Center for Liver Disease and Transplantation, Columbia University Medical Center, New York, NY; ²Department of Anesthesiology, Columbia University Medical Center, New York, NY.

Background: Hypothermic machine perfusion (HMP) is in its infancy in liver transplantation (LTx). Potential benefits include diminished reperfusion injury and improved early function. **Methods:** The study was designed as a Phase I trial of liver HMP. Exclusion criteria included: multiple organ recipients, MELD>35, ICU patients, and patients >65 years of age. Donor livers >65 years, biopsy with >30% macrosteatosis and DCD were also ineligible for HMP. Seventeen patients were enrolled transplanted with livers that underwent HMP for 4-7 hours using dual centrifugal perfusion with Vasosol solution at 4-6°C. Patient, operative and early outcome variables were recorded. We compared outcomes to 17 matched cold stored (CS) controls from the same era. **Results:** All 17 HMP grafts functioned immediately by usual clinical criteria with intraoperative bile production. Results are summarized in Table 1.

Characteristics of HMP LTx patients compared to CS controls

	HMP (n=17)	CS (n=17)	p value
Donor age	40.8±11.7	44.6±10.2	NS
Recipient age	55.5±6.7	51.8±9.1	0.18
MELD score	18.7±7.3	18.3±4.8	NS
CIT (hours)	9.6±2.1	9.0±2.7	NS
WIT (minutes)	45.9±5.7	46.3±6.4	NS
Perfusion time (hours)	4.5	N/A	N/A
Primary Nonfunction	0	0	NS
Initial Poor Function	0	2	NS
Vascular Complications	0	0	NS
Biliary Complications	1	3	NS
Peak AST (IU/L)	1336 (r, 229-3461)	3397 (r, 525-12896)	0.015
Peak Scr (mg/dL)	1.7 (r, 1.0-3.4)	2.6 (r, 0.6-7.6)	0.055
LOS (days)	12.2±6.6	15.1±4.6	0.17

In the HMP group perfusate AST levels strongly correlated with recipient Peak AST by linear regression ($p<0.001$). **Conclusions:** HMP of liver grafts provides safe and reliable preservation in our pilot series. Perfusate AST may allow pretransplant prediction of reperfusion injury. A larger randomized trial will be necessary to demonstrate the magnitude of benefits of HMP over CS in LTx.

Abstract# 475

Allograft Quality and Recipient Risk Status in Liver Transplantation. Murat Akyol,¹ John O'Neill,² Kerri M. Barber,² David Collett.² ¹Liver Transplant Unit, Royal Infirmary of Edinburgh, Edinburgh, United Kingdom; ²Statistics and Audit Directorate, UK Transplant, Bristol, United Kingdom.

Background: The relationship between allograft quality and recipient risk status was investigated in relation to patient survival 1 year after liver transplantation.

Data: The classification for deceased donor livers and patients on the liver transplant list was derived from a cohort of 723 first adult elective liver only transplants performed in the UK 2000-2001, using livers from deceased heartbeating donors. A similar cohort of 1,615 liver transplants performed between 2002-2006 was then analysed to determine whether the survival of liver recipients depends on the matching of allograft quality to recipient risk status.

Results: An iterative technique using logistic regression was applied to the 2000-2001 cohort to investigate several donor and recipient factors which allowed us to define poor vs good quality allografts and high vs low risk patients. Kaplan-Meier (K-M) survivor functions obtained from analysing the 2002-2006 cohort revealed differences between the 4 groups ($p=0.08$). The worst outcome was observed when poor quality allografts were transplanted to high risk recipients (86% survival). Low risk recipients who received poor quality allografts had poorer survival (89%) than either high risk or low risk recipients of good quality allografts (91% survival in both groups). A Cox regression analysis was then performed to assess the combined effect of liver quality and cold ischaemia time (CIT) on patient death within 1 year post-transplant. The analysis showed that although poor quality livers were associated with significantly worse patient survival than good quality livers, the difference could be explained by the inferior survival associated with the poor quality livers with long CITs (>12hrs). Patients who receive poor quality livers with CITs ≤12 hours have comparable survival to those who receive good quality livers. A comparison of the K-M survivor functions for the poor quality long CIT group (85% survival) and the group containing all other livers (90% survival) revealed a statistically significant difference ($p=0.02$).

Conclusion: Allograft quality allied with CIT is of prime importance in determining the outcome of patients following liver transplantation. Poor quality allografts with CIT >12hrs are associated with the lowest patient survival one year after transplantation. Current policy of matching poor quality allografts to low risk patients is justified if the CITs can be kept below 12hrs.

Abstract# 476**Liver Discard Probability Cannot Be Reliably Predicted Prior to Organ Recovery.** Eric B. Grossman,¹ Frederic W. Selck.¹ ¹*New York Organ Donor Network, New York, NY.*

Purpose: Liver discard rates have increased in a large, urban organ procurement organization from 8 % to 24 %. The reason is likely the result of increased transplant surgeon willingness to consider organs close to the margin of clinical acceptability. However, the costs associated with recovering these organs are high if the liver is discarded. We endeavored to determine whether a model based on pre-recovery data could predict liver discard.

Method: Retrospective analysis of 374 liver donors recovered between January of 2006 to May of 2007. The outcome measure was the liver being discarded, which occurred in 60 (16.04 %) of donors. A univariate analysis identified those donor characteristics correlated with discard. Covariates significant at the 15 percent level were included in a multivariate model. The multivariate model was assessed for both sensitivity and specificity to the outcome measure.

Results: Variables available for assessment included age, sex, race, body-mass-index, blood type, length of stay, cause of death, serologies, and laboratory values. Univariate analysis revealed positive correlations between donor age ($P<.036$), final sodium ($P<.031$), mean AST ($P<.044$), initial GGT ($P<.021$), mean/final bilirubin ($P<.02$), direct bilirubin ($P<.05$), initial albumin ($P<.05$), RPR positivity ($P<.026$), and HCV ($P<.06$) with liver discard. A multivariate model was constructed by including all donor characteristics that were significant at the 15 percent level ($P\leq.15$). A final model, consisting of donor age, final sodium, mean AST, final bilirubin, final creatinine, mean direct bilirubin, initial albumin, RPR positivity, HCV positivity, donor blood type, and cause of death was estimated. Receiver operating characteristic analysis yielded an AUC of .7427, suggesting that model sensitivity and specificity is too low to reliably forecast liver viability.

Conclusion: Using available pre-recovery data does not provide a reliable means of predicting liver discards. This is in contrast with a similar discard analysis for kidneys reported for this OPO. In fact, our experience suggests that pre-recovery liver biopsy may be effective in decreasing the post-recovery incidence of liver discard.

Discard Rate by Predicted probability of discard

Predicted Probability of Discard (%)	0 to 19	20 to 39	40 to 59	60 to 79	80 to 99
Transplanted (%)	89.8	73.7	57.9	20.0	n / a
Discarded (%)	10.2	16.3	42.1	80.0	n / a

No predictions above 79 % were observed

Liver: Immunosuppression

Abstract# 477**Sirolimus and Hepatic Artery Complications: Reassessing the Black Box Warning ?** Greg J. McKenna,¹ Edmund Q. Sanchez,¹ Tariq Khan,¹ Dmitry Nikitin,¹ Sugam Vasani,¹ Srinath Chinnakotla,¹ Henry B. Randall,¹ Richard Ruiz,¹ Nicholas Onaca,¹ Marlon F. Levy,¹ Robert M. Goldstein,¹ Goran B. Klintmalm.¹ ¹*Baylor Regional Transplant Institute, Dallas/Ft. Worth, TX.***Introduction**

The FDA warns against using sirolimus (SRL) in liver transplants, reporting increased hepatic artery thrombosis (HAT), excess mortality and graft loss when SRL is used as initial immunosuppression (IS) with calcineurin inhibitors. We report the largest experience to date of patients with SRL used as initial IS, assessing hepatic artery complications and survival outcomes.

Materials and Method

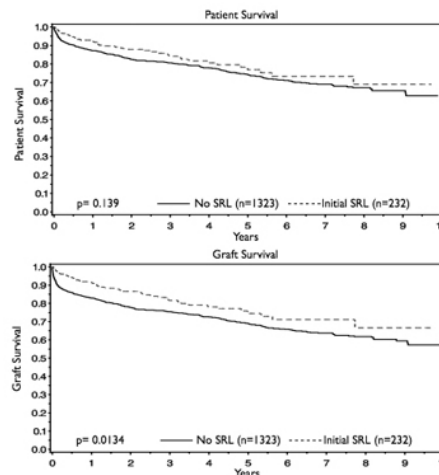
All 1554 OLT pts from 1998-2007 were reviewed. Those using SRL as initial IS were identified, and the remaining OLT pts from that time period were used as controls. Ultrasound assessed graft vascular status and any issues were verified by angiogram. Hepatic artery complications and survival outcomes were assessed.

Results

SRL was used as initial IS in 232/1554 (14.9%) OLT pts (81.5% SRL/CYA, 15.9% SRL/FK, 2.6% SRL/MMF). 13/232 (5.6%) used aortic conduits for hepatic artery inflow. During the study period, initial SRL dose was reduced from 5mg (1998-1999) to 2mg (2000-2007) aiming for serum drug levels of 4-10ng/ml.

8/232 (3.4%) SRL pts had HAT vs 6.8% in non-SRL controls ($p=0.31$). Only 3/232 (1.3%) SRL pts developed HAT while actually on SRL (5/232 pts had late HAT, mean 322d post OLT, when SRL had been d/c prior for various reasons). All 3 HAT occurred prior to Jan 1999, and no HAT have occurred in last 207 pts treated with initial SRL. Hepatic artery stenosis (HAS) was 5.6% vs 12.7% ($p=0.02$) in SRL pts vs non-SRL controls.

Patient survival with SRL was similar to non-SRL (84% vs 80% 3-yr pt survival $p=0.13$). However graft survival was improved in those pts using SRL (82% vs 75% 3-yr graft survival, $p=0.01$)

**Conclusion**

In contrast to the FDA warning, our experience shows that using sirolimus as initial immunosuppression in OLT pts does not increase HAT or patient mortality, and results in lower HAS rates and improved graft survival. Since these FDA warnings affect drug insurance/medicare coverage, new trials should be performed to reassess the validity of this FDA warning for using sirolimus in liver transplantation.

Abstract# 478**Impact of Sirolimus on HCV Recurrence Rates after Liver Transplantation.** Sonal Asthana,¹ Christian Toso,¹ Glenda Meeberg,¹ Toshiyasu Kawahara,¹ David Bigam,¹ A. J. Shapiro,¹ Andrew Mason,¹ Norman Kneteman.¹ ¹*Multiorgan Transplant Unit, University of Alberta Hospital, Edmonton, AB, Canada.*

Background: Liver transplantation for hepatitis C virus (HCV)-induced cirrhosis is associated by high rates of post-transplant HCV recurrence. Several factors have been shown to predict recurrence, but there is limited data about the impact of sirolimus (SRL) on post-transplant HCV. Two cases have been reported, who cleared serum HCV RNA without antiviral treatment after conversion to sirolimus (Somanakis et al. J Hepatol 2005). The present cohort study specifically addressed the impact of SRL therapy on HCV recurrence.

Methods: All patients undergoing first liver transplant between January 1998 (when the first HCV patient was treated with SRL) to December 2006 were studied. Data was collected on a number of variables including transplant era, donor and recipient ages, MELD, cold ischemia time, immunosuppressive drugs, steroid-treated rejection. Biopsy-proven HCV recurrence was the primary outcome studied. Statistical analysis was performed using chi-square and 't' tests as appropriate, while survival and multivariate analysis was carried out using the Kaplan-Meier and Cox proportional hazards model.

Results: 157 patients were transplanted for HCV in this period. Among them, 97 patients received SRL, including 39 with hepatocellular carcinoma. Patients with or without sirolimus immunosuppression had similar characteristics, including MELD, recipient and donor ages, duration of follow-up. On Kaplan-Meier univariate analysis, patients on sirolimus therapy demonstrated a trend towards more recurrence, but this was statistically not significant (5 years: 70% with SRL vs 54% without, log-rank: $p=NS$). Patient survival rates were comparable (5 years: 72% with SRL vs 74% with no SRL, log-rank: $p=NS$). On multivariate Cox analysis no parameter reached significance in predicting HCV recurrence. A high MELD score ($p=0.03$), steroid-treated rejection ($p=0.02$) and tacrolimus therapy ($p=0.03$) were associated with worse patient survival.

Conclusion: While stronger prospective studies are still required, this cohort data suggest that SRL does not significantly impact on post-transplant HCV recurrence.

Abstract# 479**Calcineurin Inhibitor-Free Immunosuppressive Protocol with Everolimus Monotherapy in De Novo Liver Transplantation.** Michele Masetti,¹ Roberto Montalti,¹ Gianluca Rompianesi,¹ Antonio Romano,¹ Mario Spaggiari,¹ Nicola De Ruvo,¹ Giorgio E. Gerunda.¹ ¹*Liver and Multivisceral Transplantation Center, University of Modena, Modena, Italy.***AIMS**

We carried out a prospective, randomized, open-label trial comparing two immunosuppressive protocols consisting of Everolimus (Ev) monotherapy vs Cyclosporine (Cs) with the aim of evaluating the safety and efficacy of Ev monotherapy in *de novo* liver transplantation (LT).

MATERIAL and METHODS

From 10-2006 we enrolled 50 pts (Ev n= 34; Cs n= 16). There were no significant difference in demographics variables, LT indication or pre-LT MELD score between the two gr. Mean follow-up was 6.8±3.9 months. All the enrolled pts were treated with

an initial dose of Cs of 2mg/kg/day, to target 100ng/ml for the first 10 days. Pts were randomized on day 10 into one of the two following gr on a 2:1 basis.

Ev gr: initial dose of Ev was 2 mg/day, to reach blood level of 8 ng/mL. The dose was increased on day 30, when Cs was discontinued, in order to reach an Ev blood level between 10 and 12 ng/mL.

Cs gr: after the 10th post-operative day the dose of Cs was adjusted to a target level of 250 ng/ml until day 30, then to 200 ng/ml until the end of month 6.

All pts received basiliximab induction on day 0 and 5 after LT. Pts were weaned off prednisone by 5 weeks.

RESULTS

Pt survival at 6 and 12 months was similar in the Ev and Cs gr (95.2% and 86.6% vs 87.5% and 87.5% respectively; $p=ns$). Causes of death were sepsis (1), HCV recurrence (1), pulmonary embolism (1) in the Ev gr, and sepsis (1), rupture of splenic artery aneurysm (1) in the Cs gr. Rejection rate was 16.5% vs 25.1 ($p=ns$) in the Ev and Cs gr respectively. Creatinine clearance and eGFR are summarized in the table.

Parameters and Timing	Ev gr	Cs gr	p
Creatinine clearance (mL/min)			
Pre-LT	92.2±38	76.2±25	ns
3 months	99.2±40	64.5±39	ns
6 months	97.8±39	61.7±29	.04
eGFR (mL/min/1.73 m ²)			
Pre-LT	76.3±30	60.8±31	ns
3 months	90.4±31	54.4±33	.02
6 months	77.0±25	53.8±16	.04

The overall incidence of infection episodes was comparable between two gr (5.9% Ev gr vs 12.5% Cs gr; $p=ns$). Cholesterol but not triglycerides increased in the Ev gr compared with the Cs gr ($p<.05$); Ev dose reduction decreased such parameters without the need for statin implementation.

CONCLUSION

Ev monotherapy in *de novo* LT showed similar patient survival and incidence of morbidity compared to a Cs immunosuppressive protocol. The primary endpoint was achieved inasmuch as renal function was statistically better in the Ev gr.

Abstract# 480

Adverse Effects and Poor Tolerance of Sirolimus (SIR) in Liver Transplant (LT) Recipients. T. A. Shaikh,¹ R. K. Sterling,¹ M. L. Shiffman,¹ R. A. Fisher,¹ A. H. Cotterell,¹ D. G. Maluf,¹ M. P. Posner,¹ A. Ashworth,¹ M. Akyeampong,¹ R. T. Stravitz.¹ *Hume-Lee Transplant Center, Virginia Commonwealth University, Richmond, VA.*

Background: SIR is a potent immunosuppressive agent that inhibits T-cell activation and proliferation. In LT recipients, SIR has primarily been used as a renal-sparing agent, but its toxicity and tolerability in this population has not been well defined. **Aims:** To identify the adverse effects and predictors of discontinuation of SIR in LT recipients.

Methods: Records from 327 adult LT recipients transplanted between 1/2000 and 12/2006 were reviewed. Reasons for starting and discontinuing SIR were captured, as were all significant adverse effects and laboratory abnormalities. Factors predicting SIR discontinuation in univariate analysis were further analyzed by multivariable logistic regression (MLR). **Results:** Mean age of the study group was 50 ± 11 years, and 75% were male. Underlying liver disease was HCV ± alcohol in 56%, 24% had hepatocellular carcinoma, and 14% received living donor grafts. Calcineurin inhibitors (CNI) were started post-operatively in 91% (85% tacrolimus/15% cyclosporine), with or without mycophenolate and prednisone. 179 patients (54%) started SIR a median of 15 days (IQR: 4-138) post-LT primarily for renal insufficiency (76%) or CNI neurotoxicity (7%). SIR was overlapped with tacrolimus and cyclosporine in 65% and 16%, respectively. Prior to starting SIR, total cholesterol was 139 ± 69 mg/dL, LDL-cholesterol 90 ± 49, triglycerides 193 ± 126. Peak lipids after SIR were 263 ± 97, 138 ± 81, and 475 ± 456 mg/dL, respectively, despite lipid-lowering therapy. Serum creatinine was 2.05 ± 1.04 and 1.08 ± 0.5 mg/dL before and after SIR, respectively. Before SIR, 70% of patients had no proteinuria, but only 36% had no proteinuria after SIR. High range proteinuria (>300mg/dL) was noted in 3% before and 16% after SIR. Finally, SIR was discontinued in total of 81 (45%) patients, for indications of cytopenias (20%), hyperlipidemia (19%), mouth ulcers (11%), sepsis (7.5%), skin reactions (7.5%), nephrotic syndrome (6%), GI intolerance (4%), pneumonitis/BOOP (2.5%), myopathy (2.5%), and combinations of above (20%). MLR failed to identify any pretreatment predictors of discontinuation. **Conclusions:** Immunosuppression with SIR improves azotemia at the expense of considerable hematologic, metabolic, dermatologic, renal, pulmonary and muscle toxicity. Considering the high incidence of proteinuria after SIR treatment, the use of SIR as a less nephrotoxic agent must be re-considered.

Abstract# 481

Six Year Experience with Campath-1H (C1H) Induction in Adult Liver Transplantation. Panagiotis Tryphonopoulos, Debbie Wepler, Seigo Nishida, David Levi, Jang Moon, Gennaro Selvaggi, Tomoaki Kato, Nikolaos Pararas, Amutha Sornaraj, Ronald Posner, Alfredys Berlanga, Maria Torres, Leopoldo Arosemena, Eddie Island, Phillip Ruiz, Andreas Tzakos. *Miami Transplant Institute, University of Miami, Miller School of Medicine, Miami, FL.*

Introduction: We report our 6 years experience with the use of Campath-1H (C1H) in adult liver transplantation.

Materials and methods: From December 2001 until July 2007 we administered C1H induction with low dose maintenance tacrolimus immunosuppression to 166 adult recipients of a liver allograft. Most common primary diseases were Laennec (n=60), cryptogenic cirrhosis (n=31) and autoimmune: PSC (n=17), PBC (n=16) and AIH (n=14). The first dose of C1H was administered immediately before (n=74) or after (n=92) the transplant procedure. Follow up was until September, 2007.

Results: Five year patient and graft survival was 90% and 85% respectively. There were 14 deaths due to stroke (n=2), chronic rejection (n=2), failure to thrive/pneumonia (n=3), sepsis (n=2), hepatic artery thrombosis, HCC, prostate cancer, graft lymphoma and non-compliance (one each). Seven patients were retransplanted, for primary non function (n=2), portal vein thrombosis (n=1), hepatic artery thrombosis (n=1), hepatitis B (n=1) and chronic rejection (n=2).

Thirty six patients had biopsy proven rejection episodes: mild (n=28), moderate (n=9) or severe (n=3). The average tacrolimus 12 hour trough levels were 6.6, 5.2 ng/ml and 2.78 ng/ml for the 1st, 3rd and 5th year post-transplantation, respectively. There was no significant difference in the outcome of the transplant so far, between patients that received C1H before or after the transplant procedure.

Immunosuppression-related complications included A). Opportunistic infections: most common were Herpes Zoster (n=19), CMV (n=3), and Herpes Simplex (n=3), B). Neoplasms: skin cancer (n=3), Kaposi sarcoma (n=1), lymphoma (n=2) and C). Nephrotoxicity: Five patients received a kidney graft for diabetic nephropathy (n=2), nephrotic syndrome (n=1) and calcineurin nephrotoxicity (n=2).

Conclusion: The use of C1H induction with half the usual dose of tacrolimus is an effective regimen in adult liver transplantation. The timing of C1H administration does not seem to affect the clinical outcome so far.

Abstract# 482

Benefit of Mycophenolate Mofetil, Delayed and Reduced Tacrolimus and Daclizumab in De Novo Liver Transplantation – Supportive Evidence from the 1-Year Per Protocol Population and Additional Analyses. A. David Mayer,¹ James M. Neuberger.¹ *The Liver Unit, Queen Elizabeth Hospital, Birmingham, United Kingdom.*

Introduction: In the prospective ReSpECT study, primary liver transplant patients were randomised to 1 of 3 groups: A) standard-dose tacrolimus (target trough level > 10 ng/ml) for the 1st month; B) 2 g mycophenolate mofetil (MMF) IV until at least day 5, 2 g PO thereafter + reduced-dose tacrolimus (target trough level ≤ 8 ng/ml); and C) MMF as in Group B + reduced-dose tacrolimus introduced on day 5 (target trough level ≤ 8 ng/ml) + daclizumab on days 1 and 7. Steroids were given in all groups according to local centre protocol. Results at 1 year showed that 2 g MMF + delayed and reduced tacrolimus + daclizumab is associated with significantly less impairment of renal function compared with standard treatment. Here we present the results of the per protocol (PP) population.

Methods: The PP population, which was defined prior to the sub-group analysis, consisted of patients from the full analysis set who had no inclusion/exclusion criteria violation, had at least one creatinine clearance (CrCl) value beyond 6 months, were treated according to the protocol and did not receive any prohibited medication during the first 14 days and for less than 1 week at any time during the study. A composite endpoint comprising freedom from renal dysfunction (≥ 20% decrease from baseline in calculated CrCl), acute rejection, graft loss or death was also investigated.

Results: The full analysis set included 181, 168 and 168 patients, whereas the PP population only included 69, 67 and 67 patients in Groups A, B and C, respectively. The mean difference in calculated CrCl from baseline to 1 year was significantly smaller in Group C compared with Group A (-10.7 ml/min vs -22.0 ml/min, $p = 0.038$), but was not significantly different between Groups A and B (-20.50 ml/min). The incidences of death ($n = 2, 0, 1$) and graft loss ($n = 1, 0, 0$) were similar in all 3 groups. The incidence of the composite endpoint at 1 year was in both the full analysis set and the PP population significantly lower in Group C compared with Group A (PP population: 70% vs 93%, $p < 0.0001$), but was not significantly different between Groups A and B (85%).

Conclusions: The PP analysis confirms the results from the full analysis set that 2 g MMF + delayed and reduced tacrolimus + daclizumab is associated with less impairment of renal function compared with standard treatment with no negative effect on death and graft loss.

PTLD/Malignancies: All Topics

Abstract# 483

IL-27 Produced by LCL Is Required for Type-1 EBV-Specific CD8⁺ T Cell Priming in EBV-Seronegative Pediatric Tx Recipients. Iulia Popescu,¹ Camila Macedo,¹ Yun Hua,¹ Michael Green,² David Rowe,² Louise Smith,² Walter Storkus,³ Steve Webber,² Diana Metes.¹ *¹Thomas E. Starzl Transplantation Institute; ²Children Hospital; ³Dermatology, University of Pittsburgh, Pittsburgh, PA.*

Cellular immunotherapy implementing EBV specific CTLs represents an effective strategy to restore T cell immunity in Tx patients with post-transplant lymphoproliferative disorders (PTLD). However, the ability to generate EBV-specific CTLs from EBV-naïve pediatric Tx patients has proven challenging. Our goal was to test different methods of EBV-Ag presentation using selected cytokines required for Type-1 T cell commitment,

and to identify the most effective protocol. Peripheral blood was obtained from 4 EBV-seronegative and 4 EBV-seropositive pediatric heart (H) Tx patients. LCL vs DC-based methods were compared as follows: (i) LCL (ii) LCL + IL-12 (iii) Type-1 polarized DC (treated with IL-1-b, TNF-a, IL-6 and IFN-g) loaded with MHC class I-restricted EBV-peptide pool (DC/pep.) and (iv) DC/pep. + IL-12. The EBV-specific CD8⁺ T cell phenotype and function were screened using flow cytometry, IFNγ ELISPOT and cytotoxicity assays. The yields and the functional activities of *in vitro* co-cultures differed based on the induction method employed, and on the EBV status of the patients tested. For the EBV-seropositive pediatric HTx patients, all four methods resulted in the successful expansion of functional Type-1 EBV-specific CD8⁺ T cells, suggesting that memory CD8⁺ T cell are readily reactivated *in vitro*. For the EBV-seronegative pediatric Tx patients however, only the LCL+IL-12 approach resulted in significant augmentation of Type-1 EBV-specific CTLs that were competent to secrete IFN-γ (400±50/10⁵ cells) and to kill (300±170 LU/10⁵ cells) EBV⁺ targets. We found that IL-27 secreted by LCL (and not by DC) was critical in triggering expression of IL-12Rb2 on naïve CD8⁺ T cells, and rendering these cells responsive to IL-12p70. Further addition of exogenous IL-12p70 (which is generally not produced by LCL) proved to be essential for effective Type-1 priming. However, blocking IL-27 during EBV-priming has abolished IL-12Rb2 expression and subsequent IFNγ production. These results demonstrate that the inducible expression of IL-12Rb2 on naïve CD8⁺ T cells was dependent on IL-27, and support the critical early role of EBV infected B cells in the *in vivo* priming of naïve precursors into potent EBV Type-1 CD8⁺ T cell in children.

Abstract# 484

EBV-Specific CD8⁺ T Cells from Pediatric Heart Transplant Patients Carrying Chronic High EBV Loads Display Contrasting Features: Activated Phenotype and Exhausted Function. Camila Macedo,¹ Steve Webber,² Albert Donnenberg,³ Iulia Popescu,¹ Yun Hua,¹ Louise Smith,² Michael Green,² David Rowe,² Diana Metes,¹ ¹Thomas E. Starzl Transplantation Institute; ²Children's Hospital; ³Pittsburgh Cancer Institute, University of Pittsburgh, Pittsburgh, PA.

Serial EBV load monitoring in pediatric heart transplant patients (PHTx) has identified a group of asymptomatic children that exhibit persistently high EBV loads in peripheral blood (≥16,000 copies/ml on at least 50% samples over a period of at least 6 months). These patients have a high rate of progression to late PTLD. Our goal is to characterize the deficiency of EBV-specific CD8⁺ T-cell immunity that allows this state to occur and be maintained. Twenty-one stable EBV⁺ PHTx patients were categorized as follows: Group 1 (n=6) no detectable viral load; Group 2 (n=12) low viral load (≤16,000 copies/ml); Group 3 (n=4) high viral load (≥16,000 copies/ml). Twelve healthy subjects were recruited as controls. Flow cytometric analysis with HLA-A2 EBV-tetramer (TMR) probes in conjunction with mAbs against memory/activation markers was performed on peripheral blood CD8⁺ T cells, and their EBV-specific IFN-γ production was measured by ELISPOT. EBV-"latent" specific CD8⁺ T cells in G2 patients were mostly CD62L⁺/CD45RO⁺ (central memory) and expressed heterogeneous levels of PD-1 and high CD127 (IL-7 receptor α), the EBV-lytic-specific CD8⁺ T cells were more frequent, and biased toward CD62L⁺/CD45RO⁺ (effector memory) and CD62L⁺/CD45RA⁺ (stable effector memory), corresponding to terminally differentiated memory compartments. This cell population also expressed heterogeneous levels of PD-1 and down-regulated CD127. In contrast, both EBV-lytic and -latent specific TMR⁺ CD8⁺ T cells from G3 patients were homogeneously CD62L⁺/CD45RO⁺ (effector memory), CD38⁺ and CD127⁺, suggestive of "recently activated" phenotype. Interestingly, although patients in groups G2 and G3 had high frequencies of EBV-specific TMR⁺ CD8⁺ T cells (G2 0.65%±1.5, G3 1.9%±3.5, p=0.3), only G2 patients exhibited a direct correlation between TMR⁺ CD8⁺ T cells and EBV-specific IFN-γ production. These results demonstrate that different levels of chronic EBV-antigenic pressure trigger significant differences in the phenotypic and functional features of EBV-specific CD8⁺ T cells from PHTx, suggesting that the immunologic characterization of high EBV load carrier state is a combined "activated" phenotype with "exhausted" function of EBV-specific CD8⁺ T cells.

Abstract# 485

EBV-Encoded LMP1 Indirectly Activates the Jak/STAT Pathway through Induction of IFNγ. Maria Vaysberg,¹ Stacie L. Lambert,¹ Sheri M. Krams,¹ Olivia M. Martinez.¹ ¹Surgery Division of Transplantation, Stanford University, Stanford, CA.

Background and Aims: Epstein Barr virus (EBV) is associated with multiple human malignancies including post-transplant lymphoproliferative disease (PTLD) B cell lymphomas. LMP1 is an EBV-encoded protein expressed in PTLD tumors that can drive tumor growth through constitutive activation of NFκB, Erk, p38, Akt and JNK. We have shown that EBV⁺ B cell lines from PTLD patients express LMP1 and have a constitutively active Jak/STAT pathway. Here we determine the role of the viral protein LMP1 in STAT activation.

Methods: Direct interaction of LMP1 with JAK3 was analyzed by immunoprecipitation of PTLD cell line lysates with anti-LMP1 antibody and Western blotting for JAK3. NGFR-LMP1 chimeric proteins were expressed in the EBV Burkitt's lymphoma cell line BL41 to permit inducible LMP1 signaling. Gel shift assays were performed with whole cell extracts from NGFR-LMP1 BL41 cells after 0-24h of LMP1 activation. Additionally lysates were resolved on SDS-PAGE and Western Blotting was performed with pSTAT1, STAT1, pSTAT3 and STAT3 antibodies. Cytokine secretion was assayed

by pan-cytokine luminex kit and ELISA. IFNγR1 and IFNγ specific antibodies were used to block IFNγ signaling in cells prior to pSTAT1/STAT1 WB.

Results: Although JAK3 could associate with the common γ chain in EBV⁺ B cell lines derived from PTLD tumors and in the BL41 B cell lymphoma line transformed with the B95.8 strain of EBV, no interaction of LMP1 with JAK3 was observed. STAT/DNA complexes were detected in NGFR-LMP1 BL41 clones after 24 hours of LMP1 signaling but not after 20-30 min, suggesting that STAT activation is indirect. STAT1 was the predominant STAT activated by LMP1. Inhibition of cytokine secretion with brefeldin A prior to LMP1 activation abolished STAT binding indicating that a secreted protein is responsible for STAT activation. IFNs are known inducers of the STAT pathway and pan-cytokine luminex assay revealed that LMP1 can induce IFNγ but not IFNα. Moreover, IFNγ receptor blockade prior to LMP1 activation dramatically decreased STAT1 activation.

Conclusion: Together, these data demonstrate that LMP1 does not activate the Jak/STAT pathway through direct interaction with JAK3 but rather through an indirect pathway involving IFNγ induction. Thus the oncogenic protein LMP1 of EBV usurps cellular cytokine pathways to augment tumor growth. Understanding the viral-host cell interactions that promote tumorigenesis will aid in development of novel strategies for treatment of PTLD.

Abstract# 486

PTLD Derived Tumor Variants of EBV Latent Membrane Protein 1 Induce Sustained ERK Activation and cFos Induction. Maria Vaysberg,¹ Stacie L. Lambert,¹ Sheri M. Krams,¹ Olivia M. Martinez.¹ ¹Surgery Division of Transplantation, Stanford University, Stanford, CA.

Aims: Post transplant lymphoproliferative disorder (PTLD) is a serious complication of solid organ transplantation that is closely associated with Epstein Barr Virus (EBV) infection. EBV⁺ PTLD lymphomas express several latent viral genes including latent membrane protein 1 (LMP1), a proven oncogene that is essential for human B cell transformation. LMP1 is able to activate Erk, JNK, p38, NFκB and PI3K. The aim of this study is to determine whether LMP1 isolated from PTLD tumors differs in signaling ability from LMP1 derived from the B.95 strain of EBV, originally isolated from a patient with infectious mononucleosis.

Methods: LMP1 variants isolated from a panel of EBV⁺ PTLD-associated B cell lines were cloned and sequenced. Inducible chimeric constructs containing the LMP1 C-terminus and NGFR transmembrane domain were created for each tumor variant and expressed in the Burkitt's B lymphoma cell line BL41. LMP1 signaling in BL41 clones was induced by crosslinking of NGFR. Activation of p38, Erk, Akt and JNK was assayed by Western Blotting (WB) with phospho-specific antibodies. NFκB activation was assayed by WB for IκB and cFos induction was analyzed by WB and the TransAM cFos binding assay.

Results: All three tumor variants of LMP1, as well as the B.95 LMP1 isoform, were able to induce p38 activation within 30min of NGFR crosslinking while Akt and JNK were activated within 10min. All variants showed similar ability to activate NFκB. However, tumor LMP1 variants induced prolonged Erk activation (up to 3hrs) while the B.95 LMP1 variant induced a transient response. cFos is induced only during the sustained phase of Erk activation. Indeed, the tumor variants of LMP1, but not B.95 LMP1, were able to induce cFos protein. Similarly, cFos binding to the AP1 consensus site was only observed in tumor LMP1-induced nuclear lysates. Two mutations in the C-terminus- aa212 (S vs G) and aa366 (T vs S)-are conserved in the tumor variants LMP1 compared to B.95 LMP1. Point mutation of either of these amino acids from the B.95 to tumor variant version allowed for sustained activation of Erk and subsequent cFos induction and binding to the AP1 site.

Conclusion: Tumor-derived LMP1 has enhanced ability to induce the cFos oncogene and this property can be localized to two amino acids in the C terminus. These findings suggest that these specific amino acid residues of LMP1 are important in determining whether EBV infection is benign or results in PTLD.

Abstract# 487

Anti-CD20 Monoclonal Antibody (Rituximab) for Refractory Post-Transplant Lymphoproliferative Disorders (PTLD) in Children: Final Results of a Phase II Multi-Center Clinical Trial. Steven A. Webber,¹ William Harmon,² Betsy Blume,² Albert Faro,³ Robert Hayashi,³ Charles Canter,³ Stuart Sweet,³ Jay Fricker,⁴ Minnie Sarwal,⁵ Ronald Jaffe,¹ Richard Fine.⁶ ¹Pediatrics, Children's Hospital of Pittsburgh, Pittsburgh, PA; ²Pediatrics, Children's Hospital Boston, Boston, MA; ³Pediatrics, St. Louis Children's Hospital, St. Louis, MO; ⁴Pediatrics, Univ. of Florida, Gainesville, FL; ⁵Pediatrics, Stanford University, Palo Alto, CA; ⁶School of Medicine, SUNY, New York, NY.

Background: Our initial registry data suggested that rituximab is effective in two-thirds of children with refractory PTLD. No prospective clinical trials have confirmed this.

Methods and Results: We report the final results of a small prospective phase II multi-center non-randomized clinical trial of rituximab in pediatric solid organ recipients with refractory PTLD (no response by 28 days, progressive disease or relapse after reduced immunosuppression; or PTLD with rejection). Treatment comprised 375 mg/m²/dose x 4 (if complete response or no response) with 4 additional doses if partial response. Primary outcome was proportion alive with functioning graft at 2 years and with no

evidence of PTLTD. 15 children were enrolled at 5 sites. Mean age at transplant 7.1 years, mean time to PTLTD 43 months (4-153). Organs transplanted were lung 6, heart 5, kidney 4. All PTLTD were of B cell origin and expressed CD20 and all were EBV positive. Histology was: polymorphic 11, monomorphic 3, Hodgkins-like 1. 7 patients received 4 doses, 7 patients 8 doses and 1 patient 7 doses. Treatment was associated with minimal side effects in 12, mild-moderate infusion related reactions in 2 and moderate reaction in 1. No patient had treatment discontinued because of side effects. Twelve patients (80%) showed complete response after 4-8 doses, 2 had progressive disease and one had stable disease. At 24 months, 10 (67%) were alive with one graft loss (kidney) and none with residual disease. At latest follow-up (mean 60.4 months, 42-82), 10 remain alive with 1 further graft loss (lung) and no PTLTD. The 5 deaths occurred between 2.5 and 13 months and were associated with progressive disease (2), chronic rejection (2), and complications of elective surgery (1). **Conclusions:** These findings support prior registry data and suggest that rituximab without chemotherapy is a successful second line treatment in approximately two-thirds of children with refractory PTLTD.

Abstract# 488

Cancer after Organ Transplantation in France. Jean Michel Rebibou,¹ Fabienne Pessione,¹ Francois Aubin,¹ Bernard Loty.¹ *¹Agence de la Biomedecine, France.*

Cancer prevention appears as a major challenge in transplantation. Estimating cancer frequency is a major step toward designing prevention policy.

We report on patterns of cancer incidence among 47000 transplantations registered in the French data base.

Transplantations performed in France between 1990 and 2005 were analysed. Liver transplantation indicated because of liver malignancy. Post-transplant cancers are recorded either as posttransplant complication or as cause of death. Non melanoma skin cancers were not included. Incidence of cancer in French general population was estimated according to the data of the study Incidence of cancer in France performed by the national institute for sanitary survey (INVS). Excess risk of cancer was evaluated by using the method of indirect standardisation adjusted on age and gender (Standardized incidence Ratio). A Cox model was performed to estimate the impact of recipient age and gender, transplant organ and period of the study.

Analysed transplantations included 29 389 kidney, 9 644 liver, 6458 heart and 1489 lung transplantations. Compared to general population the SIR of cancer in transplanted patients was 2.6 IC_{95%} [2.5-2.7]. This risk appeared higher in thoracic organ recipients, RR 5.5 IC_{95%} [4.5-6.8] and 3.2 IC_{95%} [3-3.5] for lung and heart transplantations respectively. Analysis according to the cancer type displayed an increased risk of all type of cancer, but the amount of the increase varies with the type of cancer and with the transplanted organ. Non Hodgkin lymphoma (NHL) risk was very increased (RR=14.4 IC_{95%} [13.3- 15.5]) particularly in lung transplantation (RR=79.2 IC_{95%} [49-90]). The risk of lung cancer is higher for recipients of a thoracic organ and the risk of kidney cancer appeared higher for kidney recipients. Ten year cumulative incidence was 8.6% for all cancer and transplantation types, 1.7% for NHL. Multivariate analysis demonstrated that cancer risk increased with recipient age ($p<10^{-3}$), 10 year cumulative incidence was 15% for recipients older than 60 year. It was higher in male ($p<10^{-3}$) and in thoracic organ recipients when compared with kidney recipients ($p<10^{-3}$). Cancer incidence did not vary according to the transplantation period (1995-1999 vs 2000-2005, RR=0.95 $p=0.34$) and NHL risk was significantly lower during the period 2000-2005 (RR=0.8, $p=0.02$).

This work does not report any increase in cancer incidence among transplant recipients while cancer incidence increased in the general population. The observed decrease of NHL risk is of particular interest.

Regulatory T Cells: Costimulation and Parenchymal Control

Abstract# 489

The Function of the Novel Costimulatory Molecule B7-H4 in Alloimmune Responses *In Vivo*. Kazuhiro Yamaura,^{1,2} Olaf Boenisch,¹ Takaya Murayama,¹ Joyce Popoola,¹ Miyuki Azuma,³ Nader Najafian,¹ Mohamed H. Sayegh.¹ *¹Transplantation Research Center, Brigham and Women's Hospital, Children's Hospital Boston, Harvard Medical School, Boston, MA; ²Department of Surgery, Shinshu University School of Medicine, Matsumoto, Japan; ³Department of Molecular Immunology, Tokyo Medical and Dental University, Tokyo, Japan.*

Both costimulatory and co-inhibitory signals are delivered by B7 ligands through the CD28 family of receptors on T lymphocytes determining the ultimate immune responses. Although B7-H4, a recently discovered member of the B7 family, is known to negatively regulate T cell immunity in autoimmunity and cancer, its role in transplantation rejection and tolerance has not been established. To study its role in physiologic rejection processes, we first treated B6 WT recipients of BALB/c hearts with a blocking mAb against B7-H4 or isotype IgG control and found no difference in graft survival (MST 7 days, $n=8$ vs. 7, $n=10$). However, B7-H4 blockade resulted in accelerated allograft rejection in CD28 deficient B6 recipients (MST 9.5 vs. 19, $n=8$, $p=0.003$), indicating that B7-H4 signaling can mediate negative regulation in the absence of CD28 costimulation. We next studied B7-1/B7-2 double deficient (DKO) B6 recipients of BALB/c heart allografts, as these mice are truly independent of CD28/CTLA-4:B7 signals. While cardiac allografts were accepted in control DKO recipients (MST>100, $n=5$), blocking

B7-H4 precipitated rejection (MST 45, $n=5$, $p=0.01$) demonstrating non-redundant functions of these two negative pathways. Based on these results, we next evaluated the role of B7-H4 in acquired transplantation tolerance by blocking CD28:B7 using CTLA4-Ig. B7-H4 blockade abrogated prolongation of allograft survival by CTLA4-Ig (250 μ g on day 2) in the fully MHC-mismatched cardiac allograft model (MST 13 vs. 46.5, $n=8$, $p=0.0002$). We conclude that the novel B7-H4 molecule can regulate alloimmune responses independent of an intact CD28/CTLA-4:B7 costimulatory pathway. The interplay between positive (stimulatory) and negative (regulatory) costimulatory signals is an important determinant of the outcome of the alloimmune response and could be exploited to induce tolerance.

Abstract# 490

Specific Inhibition of Effector T Cells by Myeloid Derived Suppressor Cells (MDSC) in Tolerant Recipients of Rat Kidney Allografts. Anne-Sophie Dugast,¹ Michele Heslan,¹ Claire Usal,¹ Helga Smit,¹ Bernard Vanhove.¹ *¹ITERT, UMR 643 INSERM, Nantes, France.*

In a model of MHC-mismatched kidney allograft in the rat, treatment with anti-CD28 antibodies induced a form of tolerance independent of Treg cells but associated with a two-fold accumulation of MDSC in the blood. To further characterize these cells, we analyzed their phenotype and mechanism of action by flow cytometry, western blotting and suppression assays. MDSC expressed CD80 and CD86, NKRP-1, CD172a (SIRPa), CD11a, CD11b, HIS48 and for a fraction of them CD4 but did not express MHC class II molecules. MDSC dose-dependently suppressed the proliferation of T cells in MLR and after stimulation with anti-CD3 + anti-CD28 antibodies. Although detected in blood, bone marrow, spleen and lymph nodes, MDSC were only suppressive in blood and bone marrow. This suppression was lost after physical separation from the responding T cells by semi-permeable membranes in transwell assays, as well as after addition of L-NMMA, a selective inhibitor of inducible nitric oxide synthase (iNOS), suggesting a role for NO in the suppression. Western blot analyses revealed that iNOS was expressed only after contact between MDSC and activated CD4⁺CD25^{high} effector T cells and to a much lesser extent after contact with activated CD4⁺CD25^{high} T reg cells. MDSC affected the viability of stimulated CFSE-labeled effector T cells by blocking their proliferation but not their activation. In contrast, MDSC did not block the response of T reg cells stimulated by anti-CD3 + anti-CD28 antibodies. This selective suppression of effector but not of regulatory T cells was confirmed by cytokine profile analyses. *In vivo*, the expression of iNOS was higher in the blood of tolerant recipients, as well as in the graft, as compared with isografted recipients. In addition, the injection in stable tolerant animals of aminoguanidine, which inhibits iNOS, induced graft rejection within 3 weeks. In conclusion, these results suggest that MDSC, accumulated in the blood of tolerant recipients of kidney allografts, release high levels of NO after contact with activated effector T cells and specifically control their proliferative response.

Abstract# 491

Liver Non-Parenchymal Components Inhibit Dendritic Cell Differentiation and Maturation. Ching-Chun Hsieh,¹ Horng-Ren Yang,¹ Guoping Jiang,¹ John J. Fung,¹ Shigunag Qian,¹ Lina Lu.¹ *¹Immunology and General Surgery, Cleveland Clinic, Cleveland, OH.*

The inherent tolerogenicity of liver allografts could be due to comparatively large numbers of potentially tolerogenic antigen presenting cells, in particular dendritic cells (DC). It is not clear whether the unique antigen presenting function of liver DC is intrinsic, or is altered by the microenvironmental factors within the liver. In the present study, we investigated the effect of hepatic stromal cells (HpSC), a unique tissue cells in the liver which were actively expanded in the liver allografts, on generation and function of bone marrow derived DC (BM DC). We hypothesize that liver HpSC may modulate immune response via inhibition of liver dendritic cells (DC) which are known of bone marrow (BM) origin. In this study, DC were propagated from B6 BM cells with GM-CSF for 5 days. Irradiated HpSC isolated from B6 mouse liver were added at the beginning into the culture at HpSC: BMDC progenitor ratio of 1:20. The differentiation, maturation and function of propagated DC were determined by characterizing their surface molecule expression and functions of instructing T cell activation / differentiation. The results showed that addition of HpSC markedly blocked the differentiation of DC from BM precursors (most cells remained in CD11b⁺CD11c⁺ precursors stages). The incidence of CD11c⁺ cells was 7% vs. 41.6% in normal BM DC culture (without HpSC). The presence of HpSC also prevented maturation of CD11c⁺ DC, as evidenced by low expression of CD40, CD80 and CD86, but high of B7-H1. The inhibitory effect appeared to be mediated by soluble factor (s) produced by HpSC, since addition of the HpSC culture supernatant or transwell culture provided comparable inhibitory activity. Culture of allogeneic CD4⁺ T cells with HpSC-DC elicited poor proliferative response in a 3d MLR assay, with low IL-2 and IFN- γ production. Three color staining of T cells stimulated by HpSC-DC showed that CD25⁺Foxp3⁺ T cells were preferentially expanded, suggesting that HpSC-DC were capable of inducing Treg. In contrast, BM DC induced vigorous CD4⁺ T cells proliferation, most of activated CD4⁺ T cells were CD25⁺Foxp3⁺ associated with high levels of IFN γ and IL-2 in the culture, indicating induction of Th1 cells. In conclusion, liver tissue cells, such as HpSC markedly inhibit DC differentiation and maturation, suggesting that the tolerogenic property of liver DC may not be intrinsic, but is altered by the microenvironmental factors in the liver.

Abstract# 492

Donor-Specific Memory CD4 T Cells Provide Help for Alloantibody Production in the Absence of CD40/CD154 Interactions and Germinal Center Formation. Anna Valujskikh,¹ Marion Rabant,¹ Victoria Gorbacheva.¹ *¹Immunology, Cleveland Clinic, Cleveland, OH.*

Background. It is generally believed that CD40 signaling is essential for immunoglobulin class switching in response to T-dependent antigens. Our previous findings in a model of murine heart allograft rejection indicate that donor-specific memory CD4 T cells provide help to B cells and induce alloantibody isotype switch despite treatment of the recipients with donor-specific cell transfusion and anti-CD154 Ab MR1. We investigated whether memory CD4 T cell help can be delivered in the absence of CD40/CD154 interaction and whether this help involves ICOS/B7RP-1 costimulatory pathway.

Methods. TCR transgenic Marilyn (Mar) memory CD4 T cells were generated through in vitro stimulation with specific male antigen-derived HY_{day} peptide followed by 4 weeks of in vivo rest. Wild type B6 (H-2^b) or congenic CD40^{-/-} female mice containing naïve or memory Mar cells received heterotopic heart transplants from male C3H (H-2^k) donors. Donor-specific alloAb serum titers were determined by flow cytometry on d 7, 14, 21, 28 and 60 post transplant.

Results. Monotherapy with MR1 Ab (1mg i.v. on d -1) inhibited alloAb production in wild type B6 recipients injected with naïve CD4 T cells. In contrast, memory CD4 T cells were resistant to the effects of MR1 treatment and induced high titers of donor-specific IgG Ab. To test whether help by memory CD4 T cells can be genuinely CD40/CD154-independent, we performed adoptive transfer of naïve and memory CD4 T cells into CD40^{-/-} mice. Memory, but not naïve Mar cells provided help to CD40-deficient B cells and induced production of donor but not third party-reactive IgG₁, IgG_{2a}, IgG_{2b} and IgG₃ as early as 1 week post transplant with serum concentration of alloAb peaking at 2 weeks post transplant. Despite high titers of serum IgG, PNA and B220 staining revealed no germinal center formation in the spleens of CD40^{-/-} recipients containing memory CD4 T cells. AlloAb production was not affected by the treatment of CD40^{-/-} recipients with MR1 Ab or anti-ICOS mAb, indicating that the memory CD4 T cell help to CD40^{-/-} B cells was independent of CD154 engagement by an alternative ligand or ICOS/B7RP-1 pathway.

Conclusion. Unlike naïve CD4 T cells, donor-specific memory CD4 T cells can initiate alloAb response in CD40-deficient heart allograft recipients. Our results suggest that therapies interrupting CD40/CD154 signaling may fail to efficiently block de novo alloantibody production in T cell sensitized transplant patients.

Abstract# 493

Fibroblastic Reticulum Cells of the Lymph Node Modulate Immune Responses by Reversing Dendritic Cell Maturation. Robert S. Liwski,¹ Konrad Kronenberger,¹ Kenneth A. West.¹ *¹Departments of Pathology and Medicine, Dalhousie University, Halifax, NS, Canada.*

Fibroblastic reticulum cells (FRC) of the lymph node (LN) are known to influence the migration of naïve T cells and Dendritic cells (DC) through the formation of a stromal FRC network and the production of chemokines. However little is known about FRC interactions with DC and T cells and their impact on DC and T cell activation. We have evaluated the interaction between DC, FRC and T cells in an *in vitro* model.

For the generation of FRC, lymph nodes of BALB/c mice were isolated, cultured *ex vivo* and characterized by FACS analysis. After co culture with FRC, DC survival rate, maturation state and expression of cell surface molecules were evaluating using FACS. The ability of DC to induce T cell proliferation was evaluated using the D011.10 TCR transgenic system and CFSE labeling. To assess the production of soluble cytokines by FRC, DC and T cells ELISA, CBA and Luminex multiplex assays were used. Real time PCR was used to evaluate the production of cytokines and growth factors.

Our *in vitro* culture method leads to a highly homogeneous FRC population that expresses gp38, ER-TR 7, CD44 and V-CAM but is negative for MHC II. Confocal microscopy demonstrated that DC migrated to FRC where they made physical contact leading to stable associations *in vitro* as well as *in vivo* in the LN. coinubation with FRC resulted in a prolongation in DC survival. When DC that had been matured with LPS were incubated with FRC it resulted in a profound down regulation of CD40, MHC II and costimulatory molecules on the DC. FRC supernatant was also able to downregulated DC maturation. FRC were found to produce several downregulatory growth factors including hepatocyte growth factor, IL-6 and IL-11. FRC conditioned DC had a strongly diminished ability to induce activation of naïve T cells. The phenotype and cytokine profile of these T cells are under current investigation.

Our results demonstrate that LN FRC provide a milieu that induces reversal of DC maturation and downregulation of T cell activation. These DC may have tolerogenic properties that would useful in modifying alloresponses.

Abstract# 494

Paradoxical Role of B7-CD28 Costimulation in Fully Allogeneic Versus Class II Mismatched Vascularized Cardiac Allografts. Jun Yang, Joyce Poopola, Kazuhiro Yamaura, Mohamed H. Sayegh, Anil Chandraker. *Transplantation Research Center, Brigham and Women's Hospital & Children's Hospital Boston, Harvard Medical School, Boston, MA.*

The T cell B7-CD28 co-stimulatory pathway plays an important role in T cell activation, differentiation and proliferation. Our group and others have demonstrated that in full

MHC mismatched transplant models CD28-deficient recipients have significantly delayed cardiac allograft rejection compared to WT recipients and that B7-1/B7-2 double knockout (B7DKO) recipients are unable to reject cardiac allografts. In this study, we investigated the role of the B7-CD28 pathway in the bm12 into B6 MHC class II mismatched cardiac transplant model.

In this model, though cardiac allografts have long-term survival they develop severe transplant arteriosclerosis within 8 weeks of transplantation. The hearts from bm12 mice were transplanted into B6 WT and B6 background CD28KO, B7-1, B7-2 single knockout and B7DKO mice. Bm12 cardiac grafts transplanted into a B6 WT recipient had a median survival time (MST) of more than 56 days. Bm12 cardiac grafts transplanted into single B7-1 or B7-2 KO recipients had comparable survival times to WT recipients. Unexpectedly, allograft rejection was remarkably accelerated when bm12 hearts were transplanted into B7DKO recipients (MST 7 days). Allograft rejection was also significantly accelerated when bm12 hearts were transplanted into CD28KO recipient (MST 14 days). To investigate the mechanisms of these findings, lymphocytes harvested at day 10 post-Tx from spleens and regional lymph nodes were assessed by flow cytometry for phenotypic differentiation of the activation status, regulatory T cell markers and effector cell generation. The ratio of effector to regulatory cells was 1.4: 3.8: 5.2 in the WT, CD28KO and B7DKO recipients, respectively. Cytokine production was evaluated by ELISPOT using donor specific antigen stimulation of recipient splenocytes. The mean IFN- γ production was 173 spots per 500,000 splenocytes in WT, 206 in CD28KO and 702 in B7DKO.

This study for the first time demonstrates the paradoxical role of B7-CD28 co-stimulation in fully allogeneic and class II mismatched grafts and proposes a possible mechanism through the re-alignment of the effector to regulatory T cell ratio in favor of a highly alloreactive immune response. These data suggest that B7 blockade may have a paradoxical effect in recipients of MHC class II mismatched grafts, an issue that should be explored in ongoing clinical trials with LEA29Y.

Joint Plenary Session IV

Abstract# 495

Kidney Donors Outcome in the Fourth and Early Fifth Decades after Donation. Hassan N. Ibrahim,¹ Mark J. Hill,² Robert F. Bailey,² John S. Najarian,² Arthur J. Matas.² *¹Medicine, University of Minnesota, Minneapolis, MN; ²Surgery, University of Minnesota, Minneapolis, MN.*

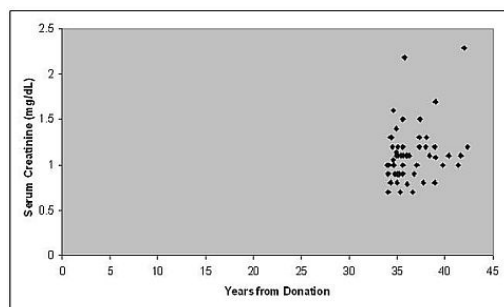
The major concern regarding kidney donation has been whether the occurrence of hyperfiltration, on the background of increasing prevalence of hypertension with aging and the decline in glomerular filtration rate (GFR) noted in some people as they get older, may put donors at a higher risk for progressive kidney disease. We have previously reported on donors > 20 years after donation and found them to incur no excessive risk of hypertension or kidney disease.

Herein, we report on renal and non-renal outcomes on the world's largest experience of kidney donors to date (n=302) who donated more than 35 years ago.

Methods: Kidney donors were asked to fill out a survey detailing their medical history since donation and obtain a physical exam and laboratory testing by their local physician. Results are expressed as mean $\pm$ standard deviation (SD). Age at donation was 34.3 $\pm$ 10.9 years and current age was 62.1 $\pm$ 11.6 years. Main results are shown in the table.

Outcomes of Kidney Donors in the 4-5 Decades After Donation		
n		302
Vital status	Alive	194/302 (64%)
	Deceased	102/302 (34%)
	Unknown	6/302 (2%)
Cause of Death	Unknown	69/102 (60%)
	Cardiac/CVA	16/33
	Cancer	12/33
	Respiratory	3/33
	Bleeding	2/33
Age at death		73.2 $\pm$ 12.7 years
Time from donation to death		27.8 $\pm$ 8.8 years
Serum creatinine at 20 years after donation (n=142)		1.20 $\pm$ 1.30mg/dL
Serum creatinine at 34-42 years after donation (n=59)		1.16 $\pm$ 0.52mg/dL
Alive and taking antihypertensive medications		107
Documented dipstick positive proteinuria		15

The graph below depicts the cross-sectional distribution of last serum creatinine available 35 years or more after donation regardless whether the donor is presently alive or not though the majority is from currently alive donors.

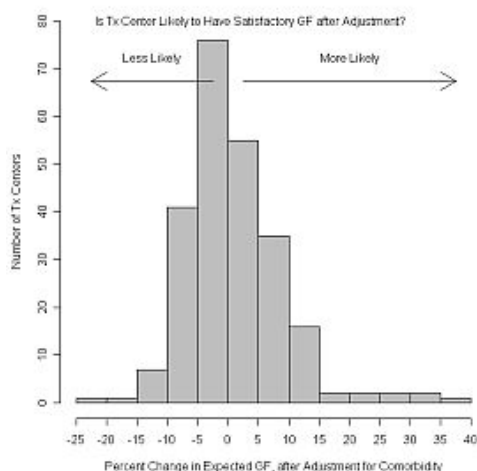


Conclusion: In the longest follow-up of kidney donors to date, this data indicates that 4-5 decades of living with one kidney has no serious adverse renal effects and a prevalence of hypertension that is probably similar to the general population considering the age of these donors.

Abstract# 496

Centers for Medicare and Medicaid Services Outcome Requirements for Transplant Centers and the Importance of Proper Adjustment for Comorbidities. Eric D. Weinhandl,¹ Jon J. Snyder,¹ Ajay K. Israni,² Bertram L. Kasiske.^{1,2} ¹United States Renal Data System, Minneapolis Medical Research Foundation, Minneapolis, MN; ²Medicine, Hennepin County Medical Center, Minneapolis, MN.

The Centers for Medicare and Medicaid Services (CMS) have announced that 1-year graft survival at a kidney-only transplant (KTx) center will be considered unacceptable if (a) the difference between observed and expected graft failures (GF) exceeds 3, (b) the ratio of observed to expected GF exceeds 1.5, and (c) the ratio is statistically significant ($p < 0.05$). Implicit in implementation of this ruling is accurate calculation of the expected number of GF at each center. Pre-existing recipient comorbidity may vary across centers, and estimates of expected outcomes that do not adjust for comorbidity may be problematic. We conducted an analysis of 25,991 first-time, KTx recipients from 2000 to 2004, who carried Medicare coverage during the 6 mo before KTx. Using USRDS and UNOS data, we assessed 11 comorbid conditions from Medicare claims during the 6 mo before KTx, and estimated expected GF during the first year following KTx with a Cox model that included donor and recipient characteristics, with and without adjustment for pre-existing comorbidity.



During the 5-year era, 241 centers performed KTx (median = 87 KTx/center). Unadjusted 1-year graft survival was 87.9% and 92.8% among deceased- and living-donor recipients, respectively. Changes in expected GF per center, after adjustment for comorbidity, ranged from -21.3% to 37.1% (SD = 8.1%); 9 centers (3.7%) had expected GF decline by >10%, while 25 centers (10.4%) had expected GF rise by >10% (figure). Before and after adjustment for comorbidity, 19 (7.8%) and 18 (7.5%) centers, respectively, met all criteria for review, but only 16 met criteria for review both before and after comorbidity adjustment. We conclude that failure to adjust for pre-existing recipient comorbidity results in grossly inaccurate estimation of expected GF per KTx center, more often resulting in expected GF that is too low. Using data that are not adjusted for comorbidity to judge the quality of Tx programs could encourage denial of access to high-risk patients.

Abstract# 497

Constitutive OX40 Costimulation Converts Foxp3+ Tregs to Foxp3+ Inflammatory Cells That Mediate Prompt Graft Rejection and Autoimmunity. Xiang Xiao,¹ Alexandar Kroemer,¹ Wenda Gao,¹ Gulcin Demirci,¹ Xian C. Li.¹ ¹Transplant Research Center, Beth Israel Deaconess Med Ctr, Boston, MA.

Background: Regulation is central to the induction of transplant tolerance. CD4⁺CD25⁺ regulatory T cells (Tregs) are a highly specialized T cell subset in regulation and are believed to be "hardwired" to Foxp3. However, it is not clear whether Foxp3⁺ Tregs are fully committed to suppression in their entire life span. As manipulation of Foxp3⁺ Tregs is of considerable scientific and clinical interests, a clear understanding of this question is an important and clinically relevant issue.

Methods: We have developed a new model where the endogenous Foxp3 gene is genetically linked to the reporter protein EGFP (foxp3gfp.KI). We then crossed the foxp3gfp.KI mice with OX40 KO mice and OX40Ltg mice and generated foxp3gfp.KI mice that are either deficient for OX40 or overexpress the ligand for OX40. We used these new foxp3gfp knockin models to critically study the role of OX40 in regulating Foxp3⁺ Treg homeostasis *in vivo*.

Results: We found that transgenic expression of the ligand for OX40 *in vivo* promotes a massive expansion of Foxp3⁺ Tregs, which accumulate in large numbers in both the lymphoid and non-lymphoid sites in OX40L transgenic mice. The expansion of Foxp3⁺ Tregs *in vivo* appears to be mediated by increased survival of Tregs in OX40Ltg mice. Surprisingly, Foxp3⁺ Tregs expanded by OX40 *in vivo* completely lack of suppressor functions despite constitutive expression of Foxp3 at high levels. Instead, OX40 reprograms Foxp3⁺ Tregs into a new type of Foxp3⁺ effector cells that mediate prompt skin allograft rejection and autoimmunity. Thus, Foxp3⁺ Tregs are not a fixed cell type; they can be readily reprogrammed to become cytotoxic T cells. Importantly, OX40 plays a critical role in uncoupling Foxp3 and Treg functions and then reprogramming them to act as T effector cells.

Conclusions: OX40 controls the *in vivo* homeostasis and regulatory functions of Foxp3⁺ Tregs. Our data also suggest that the effector programs of Foxp3⁺ Tregs are extremely flexible. This finding may have important implications for therapeutic manipulation of Foxp3⁺ Tregs in the clinic.

Abstract# 498

Emergence of a Volume-Outcomes Relationship for Lung Transplantation Is Associated with Regionalization of the Procedure to High-Volume Centers: An Analysis of 12,603 Procedures. John E. Scarborough,¹ Robert D. Davis,¹ Carmelo A. Milano,¹ Kyla M. Bennett,¹ Paul C. Kuo,¹ Theodore N. Pappas.¹ ¹Surgery, Duke University Medical Center, Durham, NC.

Introduction: The purpose of our study was to examine temporal trends in the regionalization patterns and center volume-outcomes relationship for lung transplantation in the United States over the past decade.

Methods: A retrospective analysis of all adult single-organ lung transplants included in the Scientific Registry of Transplant Recipients for three consecutive time periods between 1997 and 2006 was performed. For each time period, lung transplant centers were divided into three groups based on each center's annual volume of the procedure (low-volume group = 1-17 procedures per year, medium-volume group = 18-30 procedures per year, high-volume group = greater than 30 procedures per year). One-year observed-to-expected patient death ratios were then calculated and compared for each group in each time period. A temporal analysis of the percentage of transplants being performed relative to center volume was also performed. Statistical comparisons were made using chi square testing.

Results: A total of 12,603 lung transplant procedures were included in the analysis. In Period 1, there was not a significant difference in the one-year observed-to-expected patient death ratio of low-volume lung transplant centers when compared to high-volume centers (ratio 1.15 for low-volume centers vs. 0.79 for high-volume centers, $p = 0.07$). By Period 3, however, a significant relationship between center volume and outcomes had emerged (ratio 1.30 for low-volume centers vs. 0.79 for high-volume centers, $p = 0.006$). Over this same time period, the percentage of lung transplants within the United States that are performed at low-volume centers has decreased significantly (from 43.3% of all lung transplants in Period 1 to 22.7% in Period 3, $p < 0.0001$), while the percentage being performed at high-volume centers has increased significantly (from 20.5% of all lung transplants in Period 1 to 51.3% in Period 3, $p < 0.0001$).

Conclusions: A significant relationship between center volume and patient outcomes has emerged for lung transplantation over the past decade. At the same time, the percentage of these procedures being performed at high-volume centers has increased. These findings suggest that regionalization patterns for a given procedure may be influenced by the presence or absence of a volume-outcomes relationship for that procedure.

Abstract# 499

Clinical Efficacy of Adoptive Antitumor Immunotherapy with Activated NK Cells Extracted from Donor Liver Graft Perfusate in Liver Transplant Recipients with HCC. Kohei Ishiyama, Hideki Ohdan, Masahiro Ohira, Daskali Marlen, Yuka Tanaka, Masayuki Shishida, Toshimitsu Irei, Hiroyuki Tahara, Masataka Banshodani, Yuka Igarashi, Nabin Basnet, Toshimasa Asahara. *Department of Surgery, Hiroshima University, Hiroshima, Japan.*

Liver transplantation (LT) has emerged as one of the few curative treatment modalities for patients with hepatocellular carcinoma (HCC). However, the increase in the incidence of HCC recurrence due to immunosuppressants administered after LT is a serious issue. We have recently proposed a novel strategy of adjuvant immunotherapy for preventing the recurrence of HCC after LT: intravenous administration of IL-2-stimulated natural killer (NK) cells extracted from donor liver graft to liver transplant recipients. Since the immunosuppressive regimen currently used after LT reduces the adaptive immune components but well maintains the innate components of cellular immunity, the augmentation of NK cells response might be a promising immunotherapeutic approach. We confirmed that the IL-2-stimulated donor liver NK cells exhibited a significantly high level of TRAIL and showed vigorous cytotoxicity against an HCC cell line without cytotoxicity against normal cells. After obtaining approval from the ethical committee of our institute, we successfully applied this therapy to 13 cirrhotic patients with HCC from January 2006. The average number of NK cells that had been administered to LT recipients at 4 days after LT was $304.2 \pm 225.5 \times 10^6$ cells/body. The LT recipients were categorized as follows: (1) based on the Milan criteria, 8 recipients met the criteria while 5 did not, and (2) based on the TNM stage, 2 recipients were categorized as pathological TNM stage I; 6, stage II; 4, stage III; and 1, stage IV. In our institute, the 2-year recurrence-free survival rates of the LT recipients treated with and without this therapy were 100% and 71.3%, respectively. Kinetic studies revealed that in the early postoperative period, the peripheral blood obtained from the treated LT recipients exhibited a significant improvement in cytotoxicity against HCC cell line as compared to the untreated LT recipients ($p < 0.01$). Furthermore, flow cytometric analyses revealed that the frequency of TRAIL⁺ NK cells increased remarkably in the peripheral blood of the treated LT recipients ($p < 0.05$). In conclusion, the administration of IL-2-stimulated donor liver NK cells contributes to the promotion of host anticancer activity and has the potential to regulate HCC recurrence after LT.

Acute Rejection

Abstract# 500

KDR: A Novel T Cell Receptor Expressed on Graft Infiltrating Lymphocytes. Monika Edelbauer,¹ David M. Briscoe.¹ *¹Division of Nephrology, Children's Hospital Boston, Boston, MA.*

VEGF, a well-established angiogenesis factor, is expressed within allografts at high levels in association with acute and chronic rejection. In previous studies, we have reported that VEGF possesses potent proinflammatory properties in part via its ability to mediate leukocyte trafficking into allografts. Recently, we discovered that VEGF mediates CD4⁺ and CD8⁺ T cell migration via interaction with its receptor KDR (also called VEGFR2). Blockade of T cell KDR significantly inhibits transendothelial migration. These observations suggest that KDR may be a novel T cell receptor for allogeneic lymphocyte recruitment. Here, we first examined the expression of KDR on peripheral human CD4⁺ and CD8⁺ T cells by FACS analysis. We found that $\leq 1\%$ of circulating T cells express KDR, and its expression was at low levels on individual unactivated T cells. Further, we found that the expression of KDR on T cells increased markedly following activation with mitogen and following interactions with activated allogeneic endothelial cells. Induced expression of KDR on CD4⁺ and CD8⁺ T cells was at a similar level as that observed on endothelial cells. Therefore, KDR appears to be selectively expressed on T cells, that traffic into allografts. To test the pathophysiological significance of these observations, we analyzed the expression of KDR in a total of 19 cardiac, and 5 renal, human allograft biopsies. We correlated the expression of KDR with CD3⁺ T cell infiltrates; and by double immunofluorescence staining, we determined co-expression of KDR on individual CD3⁺ T cell infiltrates. In cardiac allografts we found that KDR was expressed throughout the endomyocardium, and was most notable on endothelial cells in all biopsies examined. By grid counting of 3-4 areas of each biopsy, we found that the mean number of CD3⁺ T cell infiltrates ranged from 4 to 79 cells/hpf ($\times 600$ mag.). By double staining, we noted that KDR was expressed on $29 \pm 4\%$ (mean $\pm$ SEM) of these CD3⁺ T cell infiltrates. Similarly, we found that KDR was co-expressed on CD3⁺ T cells within renal allografts. While infiltrates were more focal, again $30 \pm 2\%$ (mean $\pm$ SEM) of graft infiltrating T cells expressed KDR. Collectively, these observations for the first time identify KDR as a novel receptor on allogeneic T cells. We suggest that intra-graft VEGF may interact with T cell KDR to facilitate homing and recruitment of allospecific lymphocytes into allografts.

Abstract# 501

Interaction of Infiltrating CD8⁺ T Cells and Tissue Cells in Tolerant Liver Allografts: Using TCR Transgene Approach. Guoping Jiang,¹ Qiwei Zhang,¹ Horng-Ren Yang,¹ Kathleen Brown,¹ John J. Fung,¹ Lina Lu,¹ Shiguang Qian.¹ *¹Immunology and General Surgery, Cleveland Clinic, Cleveland, OH.*

The liver allografts are accepted without requirement of immunosuppression in mice. The underlying mechanisms are not completely understood. We hypothesized that it resulted from an abortive T cell response within the liver due to hyporesponsiveness or apoptosis. To test this, we examined the activation and fate of allo-Ag specific CD8⁺ T cells following liver transplants (LTX) compared with heart transplants (HTX) that were acutely rejected. Following transplantation [B10 (H2^b) $\rightarrow$ C3H (H2^k)], CFSE labeled CD8⁺ T cells (10×10^6) from Des TCR Tg mice (H2K^b specific TCR) were adoptively transferred into recipients. Animals were sacrificed two days following Des T cell administration for analyses of T cells in the grafts or draining lymph nodes (D-LN). Host CD45⁺ leukocytes were quickly infiltrated following LTX. Among CD3⁺ population $\sim 48\%$ were CD4⁺ and $\sim 52\%$ CD8⁺. CD8⁺ T cells were further increased thereafter in grafts and D-LN, associated with high INF-g production. CD8⁺ T cells in the liver grafts rapidly reduced to 36% by POD 14, and to 12% by POD 40. However, the incidence of CD4⁺ T cells remained high. CFSE dilution assay and ELISPOT showed an active division of Des⁺ T cells in liver allografts either on POD 7 or 40. These Ag-specific CD8⁺ T cells functioned well evidenced by INF-g production in response to allo-Ag. However, compared to HTX, the accumulation of Des⁺ cells in grafts was significantly lower in LTX [9.6% (17×10^4 /heart), and 0.59% (6.14×10^4 /liver)] on POD 7, and further dropped to 0.14% (3.14×10^4 /liver) on POD 40. The expended cohorts of adoptively transferred cells followed by their elimination suggested elimination of Ag-specific CD8⁺ cells. To examine the role of liver environment, graft CD45⁺ non-parenchymal cells (NPC) were isolated tested for regulatory effect on Des⁺ T cell response. Liver allografts showed significantly expansion of CD45⁺ NPC, which were donor MHC class I⁺ (H2b⁺), B7-H1⁺, TRAIL⁺ and low for CD40 and CD86. These cells did not inhibit Des⁺ T cell proliferation in response to B10 spleen stimulation, but significantly enhanced their death, which was dependent on B7-H1/TRAIL. This was confirmed by using CD45⁺ NPC from B7H1^{-/-} or TRAIL^{-/-} mice. In conclusion, activated T cells in liver grafts may stimulate tissue cells to express inhibitory and death inducing molecules, resulting in T cell death and graft acceptance.

Abstract# 502

FOXP3⁺ Graft-Infiltrating Lymphocytes from Acutely Rejecting Renal Allografts Are Recently-Activated T Cells. John H. Fechner,¹ Debra D. Bloom,¹ Zhen Davis,¹ Qingyong Xu,¹ Stuart J. Knechtle.¹ *¹Surgery, University of Wisconsin, Madison, WI.*

FOXP3⁺ graft-infiltrating lymphocytes (GIL) have been detected in the rejecting allografts of transplant patients. FOXP3 is a marker for regulatory T cells (T_{reg}). Published reports suggest that human FOXP3 can be upregulated following TCR stimulation without induction of regulatory function. To investigate whether FOXP3⁺ GIL during acute rejection are T_{reg}, we analyzed the phenotype of GIL harvested from acutely-rejected non-human primate kidney allografts.

METHODS: Renal allografts with histologically-confirmed acute rejection were harvested at the time of necropsy from macaques that had undergone experimental transplantation. Following digestion of kidney fragments using collagenase, GIL were isolated using Lymphocyte-Separation Media and cryopreserved. Axillary lymph nodes (Ax LN) were also isolated either at the time of necropsy or by biopsy. For SEB-stimulated lymphocytes, Ax LN cells were stimulated for 5 days in the presence of 25 ng/ml SEB for 5 - 6 days. To perform intracellular interferon-gamma (IFN γ) analysis, cells were stimulated with PMA and ionomycin in the presence of Brefeldin A for 5 - 6 hours at 37°C. Samples were prepared for flow cytometry by first staining for extracellular antigens. Cells were then fixed and permeabilized (kit from eBiosciences) prior to intracellular staining for FoxP3, Ki67 and IFN γ .

RESULTS: The percentage of FOXP3⁺ in CD3⁺ GIL was similar to that found in Ax LN (Table 1). The majority of these cells were CD4⁺. While 80% of the CD3⁺/CD4⁺/FOXP3⁺ population of both Ax LN and GIL were CD25⁺, a significantly higher frequency of CD39⁺ and Ki67⁺ cells were found in GIL ($p < 0.01$; Student's t-test). Similar levels of CD39 and Ki67 expression were found in SEB stimulated lymphocytes. Unlike the SEB-stimulated lymphocytes, few IFN γ -producing cells were demonstrated following PMA/ionomycin stimulation of GIL.

CONCLUSION: Our current data indicates that the majority of FOXP3⁺ GIL from acutely rejecting renal allografts are recently activated CD4 T cells that lack effector function. This suggests that FOXP3 T cells within rejecting allografts may indeed be T_{reg}.

Table 1. Flow Cytometric Analysis of FoxP3⁺ T Cells

	% of CD3 ⁺ (Ab Clone)	% of CD3 ⁺ /CD4 ⁺ /FoxP3 ⁺ (Ab Clone)		
	FoxP3 (259D)	CD39 (A1)	Ki67 (B56)	IFN γ (45.B3)
Ax LN (n = 5)	2.8 $\pm$ 1.9	23.2 $\pm$ 16.2	22.8 $\pm$ 3.1	0.8 $\pm$ 0.7
GIL (n = 5)	4.3 $\pm$ 2.0	72.9 $\pm$ 11.2	69.8 $\pm$ 16.9	0.2 $\pm$ 0.4
SEB (n = 2)	16.5 $\pm$ 2.6	65.8 $\pm$ 7.2	71.0 $\pm$ 15.8	16.5 $\pm$ 3.5

Abstract# 503**Induction of Long-Term Survival of Cardiac Allografts in Wild-Type Mice by Alloantigen (alloAg)-Specific FOXP3⁺CD4⁺CD25⁺ Natural Regulatory T (nT_{reg}) Cells.** Guliang Xia,¹ Jie He,¹ Joseph R. Leventhal.¹¹*Surgery - Organ Transplantation, Northwestern University, Chicago, IL.***Aims:** Current study was aimed at preventing heart allograft rejection in wild-type mice by alloAg-specific Foxp3⁺CD4⁺CD25⁺ nT_{reg}.**Methods:** Fresh naive CD4⁺CD25⁺ nT_{reg} were isolated from congenic B6.PL mice via autoMACS and enriched for alloAg specificity by in vitro culture with either anti-CD3/CD28-coated Dynabeads (d0-11), then donor bone marrow-derived dendritic cells (DC) d11-20 (Beads/DC-expansion) or first with donor DC (d0-8) then anti-CD3/CD28-coated Dynabeads (d8-16) (DC/Beads-expansion) in the presence of interleukin(IL)-2, IL-2/transforming growth factor(TGF)-β or IL-2/TGF-β/retinoic acid(RA).**Results:** The % of intracellular Foxp3⁺ nT_{reg} quickly decreased from 96.2% (fresh) to 7.1% (d+18) during Beads/DC-expansion or to 6.2% (d+16) during DC/Beads-expansion with IL-2 alone. Addition of TGF-β (2.5 ng/mL) significantly rescued the loss with Foxp3 expressed at 23.9% (d+18) for Beads/DC-expanded nT_{reg} and at 66.8% (d+16) for DC/Beads-expanded nT_{reg}, while total fold of expansion of nT_{reg} remained similar (24.8–30.2 for Beads/DC- or 5.2–7.1 for DC/Beads-expansion) regardless of the presence or absence of TGF-β. Introducing RA (100 nM) into Bead/DC-based, TGF-β/IL-2-conditioned culture resulted in marginal improvement with 28.9% (d+18) nT_{reg} being Foxp3⁺. In MLR assays, nT_{reg} expanded with TGF-β/IL-2 exerted more potent suppression than cells conditioned with IL-2 alone. *In vivo*, Beads/DC-expanded, TGF-β/IL-2-conditioned nT_{reg} synergized with transient host T cell-depletion (anti-Thy1.2 mAb i.p. 50 μg at d-3 & 25 μg at d+2) in C57BL/6 mice to suppress BALB/c heart allograft rejection with 57.1% (n=7) and 100% (n=10) allografts surviving over 100 days when 4x10⁷ or 8x10⁷ cells/mouse were injected immediately post-transplant, respectively. Anti-Thy1.2 treatment alone led to only 18.8% long-term survival. Infused nT_{reg} survived long-term (5.8 % circulating T cells (8x10⁷ cell dose) or 1.2 % (4x10⁷ cell dose) at d+7 post-transplant) and expressed high level Foxp3 (40–60%) *in vivo*. Long-term surviving allografts showed characteristics of 'acquired immune privilege' with cellular infiltrates that were Foxp3⁺, TGF-β⁺, IL-10⁺ and indoleamine 2,3-dioxygenase (IDO)⁺, although signs of mild to moderate chronic rejection were still evident.**Conclusions:** AlloAg-specific Foxp3⁺CD4⁺CD25⁺ nT_{reg} synergize with peritransplant host T cell-depletion to induce long-term survival of allografts in wild-type mice.**Abstract# 504****Heart Transplantation Outcomes in Outbred Mice.** Qi Li,¹ Dawn K. Reichenbach,¹ Daniel Zecher,¹ Amanda L. Williams,¹ Rosie A. Hoffman,¹ A. Jake Demetris,¹ Fadi G. Lakkis.¹ ¹*Starzl Transplantation Institute, University of Pittsburgh, Pittsburgh, PA.*

Findings in mouse models of transplantation often fail to translate well in humans. Three variables may account for the discrepancy: (1) evolutionary divergence between mice and humans, (2) influence of infection history on alloimmunity, and (3) use of highly inbred strains of laboratory mice. Here, we investigated whether the use of inbred mouse strains skews the rejection phenotypes and their response to treatment due to decreased genetic diversity and/or fixation of undesirable genetic loci, known as inbreeding depression. We examined heterotopic cardiac allograft survival in outbred and inbred mouse populations in the presence or absence of immunosuppression. In the absence of immunosuppression, heart transplantation within or between outbred stocks of mice (n = 35) resulted in three distinct rejection phenotypes that resemble accelerated (1–4 days), acute (8–25 days), and chronic rejection (> 75 days), respectively. In contrast, all fully allogeneic grafts transplanted between inbred mice (n = 12) were rejected acutely (7–13 days) as were historical controls (n > 50). The accelerated phenotype, present in 29% of outbred to outbred transplantations, was characterized by extensive hemorrhagic necrosis of the heart with thrombosis, neutrophil margination and neutrophilic arteritis, and did not correlate with donor/recipient MHC II disparity. Immunosuppression with T cell costimulation blockade did not prevent accelerated rejection (incidence = 27% in the treated group, n = 26) but did convert the acute rejection phenotype into longterm allograft survival in all groups studied. The same accelerated phenotype was observed if transplantation was performed from outbred to inbred mice (incidence = 39%; n = 23) but could not be duplicated if inbred to outbred transplantation was performed (n = 20). Finally, C3 depletion with cobra venom factor abrogated the accelerated rejection phenotype in outbred to outbred transplantations (n = 16), suggesting a role for complement in the pathogenesis of this phenotype. In summary, our data (1) indicate that the use of outbred mouse stocks may uncover clinically-relevant rejection phenotypes not observed in inbred mouse strains, and (2) underscore the importance of the donor background in determining the phenotype of rejection. Outbred mouse stocks may provide a platform to uncover MHC-unlinked genetic loci that play an important role in the outcome of solid organ transplantation.

Abstract# 505**Th17 Are Limited in Their Ability To Reject Allografts.** Yu Yang,^{1,2} Yanyan Niu,^{1,2} Jiangnan Xu,^{1,2} Yaozhong Ding,^{1,2} Jonathan S. Bromberg.^{1,2}¹*Recanati/Miller Transplantation Institute, Mount Sinai School of Medicine;*²*Department of Gene and Cell Medicine, Mount Sinai School of Medicine, New York, NY.***Background:** Transplant rejection involves networks of T cell interactions. Th1, Th2, and Th17 subsets are important mediators of immune responses and inflammation. T-bet is the master transcription factor for Th1 development, while Stat6 plays a critical role for Th2 differentiation. Here, we generated Stat6 and T-bet double deficient (DKO) mice so that both Th1 and Th2 development are blocked, leaving Th17 and Treg pathways intact, to determine their roles in allograft rejection and survival.**Methods:** Stat6 KO and T-bet KO (all on BALB/c background) mice were crossed and DKO selected. T cells were analyzed by flow cytometry for cell surface marker expression. Wild type, single KO, and DKO CD4 T cells were positively selected; stimulated with plate-bound anti-CD3ε and anti-CD28 mAbs, or with T cell depleted splenocytes; and IL-4, IFNγ and IL-17 production examined by flow cytometry. Vascularized cardiac transplants were performed in wild type BALB/c, single KO, and DKO mice with C57BL/6 donors.**Results:** T cells from Stat6/T-bet DKO mice had normal CD4 and CD8 T cell distribution; normal CD44, CD69, and CD62L expression; and normal populations of CD4⁺CD25⁺ Foxp3⁺ natural Treg. Anti-CD3 plus anti-CD28 or alloantigenic stimulation induced little IFNγ or IL-4, showing that the Th1 and Th2 pathways were blocked. There were no differences in Th17 generation after stimulation, indicating that blocking Th1 and Th2 did not autoregulate or result in default generation of Th17. However, IL-6 plus TGFβ treatment of DKO induced a very high percentage of Th17, suggesting Th1 and Th2 pathways both independently suppressed Th17 development. While wild type and single KO mice rejected cardiac allografts within 9 days, Stat6/T-bet DKO mice had prolonged graft survival (>30 days), suggesting that Th17 alone did not mediate acute allograft rejection.**Conclusion:** Th1 and Th2 cells and cytokines are sufficient for normal graft rejection and inhibit the development of Th17. Stat6/T-bet DKO mice have impaired development of Th1 and Th2, but not Th17 or Treg. DKO recipients have prolonged allograft survival, suggesting that Th17 cells by themselves are not easily induced to mediate early acute rejection, but that rejection requires Th1 or Th2 cells and cytokines for rejection. These findings have important implications for devising suppression and tolerance protocols.**Abstract# 506****Increased Recipient Age Impairs the CD4⁺ Effector T-Cell Response While Regulatory T-Cell Functions Remain Preserved.** Christian Denecke,¹ Irene Kim,¹ Daman Bedi,¹ Xupeng Ge,¹ Anke Jurisch,¹ Anne Weiland,¹ Steven McGuire,¹ Johann Pratschke,² Stefan G. Tullius.¹ ¹*Div. of Transplant Surg, BWH, Transplant Surg Res Lab;* ²*Dept. of Surgery, Charite Berlin, Germany.*

Elderly recipients represent the most rapidly growing segment of patients on the waiting list. However, little is known about age-dependent alterations of the immune response in organ transplantation. We examined age dependent T-cell functions in a transgenic mouse transplant model.

Effector T-cell phenotype, -function, cytokine production and regulatory T-cell function were analyzed in 3 and 18mths old B6 mice. In an *in vivo* transplant model, BL/6 nude mice were reconstituted with 2x10⁶ young or old transgenic alloantigen-specific CD4⁺ T-cells and engrafted with bm12 skin grafts. T-cell phenotype and cytokine secretion were sequentially analyzed in all lymphatic compartments.Splenocytes of naive old B6 mice contained significantly higher frequencies of T-cells with an effector/memory phenotype (CD4⁺CD44^{high}CD62L^{low} and CD8⁺CD44^{high}CD62L^{low}; p<0.005). *In vitro* proliferation and IFNγ-production were significantly reduced in aged mice indicating an impaired T-cell response with increasing age as assessed by MLR (p<0.005) and ELISPOT (p<0.001). In parallel, regulatory functions remained age-independent as alloantigen-specific CD4⁺CD25⁺FoxP3⁺ T-cells isolated from sensitized old mice demonstrated a dose-dependent well preserved suppressor function. Next, we tested the age-dependent alloantigen -specific CD4⁺ T-cell function in a transgenic skin transplant model: Age did not significantly impact rejection kinetics (young vs. old: 10.1 vs. 14.3days, n.s.) However, T-cell migration and activation were significantly different: Fewer numbers of activated CD4⁺CD25⁺ and effector/memory phenotype T-cells (CD4⁺CD44^{high}CD62L^{low}) were found in recipient spleens (p<0.05) and draining lymph nodes (dln) (p<0.05) after transfer of old T-cells. Chemokine receptor staining revealed less CXCR3⁺ and CCR7⁺ T-cells in dln following the transfer of old T-cells (total cell numbers x104: CXCR3⁺: 10.9±4.2 vs. 0.95±0.2, CCR7⁺: 4.7±1.0 vs. 0.35±0.2, p<0.05). This was paralleled by reduced intra-graft T-cell infiltration as observed by immunohistochemistry.In summary, native elderly mice showed an increased frequency of effector memory T-cells but an overall impaired T-cell response. Regulatory T-cell function remained preserved. *In vivo* allospecific CD4⁺ T-cell activation and migration was impaired in elderly transplant recipients.

Abstract# 507

The Critical Role of CD4 T Cells in CD154 Blockade-Resistant Memory CD8 T Cell Activation and Allograft Rejection in Sensitized Recipients.

Yue Wang, Zheng Wu, Ronald W. Busuttil, Yuan Zhai, Jerzy W. Kupiec-Weglinski. *Surgery, Dumont-UCLA Transplant Ctr, Los Angeles, CA.*

Background: The sensitized transplant recipients may undergo an "accelerated" form of rejection, which is mediated by T cell-dependent mechanisms. These patients often experience increased rate of early rejection episodes, which are difficult to control with currently used immunosuppressive agents. **Methods:** In our model of cardiac graft rejection in sensitized recipients, B6 mice are first challenged with B/c skin, followed 40-60 days later by B/c heart transplant (HTx). Unlike in naive hosts, HTx rejection and alloreactive CD8 activation in this model are CD154 blockade-resistant. We first performed systemic analysis at the intragraft transcriptional level by microarray to identify disparities in local immune responses in naive vs. sensitized hosts. Aiming to improve the efficacy of costimulation blockade in the sensitization settings, we then determined the role of CD4 T cells in costimulation blockade-resistant alloimmune response by using CD4 depleting (GK1.5) vs. CD4 blocking (YTS177) Ab, in conjunction with CD154 blockade (MR1). **Results:** HTx harvested from groups of naive (day 4-6), sensitized (day 2-4), control Ig or MR1 Ab treated mice (n=3/gr) were subjected to microarray analysis. MR1 treatment suppressed HTx expression of pro-inflammatory genes (IL-1 β , IL-6, TNF- α), and T cell-targeted chemokines (RANTES, Mig, CXCL10) early after HTx in naive, but not sensitized recipients. Five groups of sensitized mice treated at the time of HTx with: (1) control Ig; (2) MR1 [day 0]; (3) GK1.5 [day -1/+1]; (4) MR1 [day 0] + GK1.5 [day -1/+1]; (5) MR1 [day 0] + YTS177 [day -1/+1] were contrasted. HTx were rejected promptly in <5 days in Gr. (1), (2) & (3), but survived ca. 30 days in Gr. (4) & (5). CTL activation was determined by FACS phenotyping at day 10 and 30. The simultaneous blockade of CD154 costimulation and CD4 help, but not a single blockade with MR1 Ab or anti-CD4 Ab, was required to inhibit peripheral alloreactive CD8 activation in sensitized mice. Additionally, CD8 activation in the absence of CD4 help showed defective cytotoxic molecule profile, with suppressed perforin but upregulated granzyme B expression at the graft site. **Conclusion:** CD154 blockade-resistant CD8 activation is critically dependent on CD4 T cells. This study provides novel immunological basis to study the potential synergy between adjunctive CD4 and CD154 targeted therapies to control accelerated graft rejection in sensitized hosts.

Abstract# 508

Epithelial Deterioration during Mouse and Human Renal Allograft Rejection Can Be Reproduced In Vitro by Soluble Inflammatory Mediators. G. Einecke, L. G. Hidalgo, P. F. Halloran. *Department of Medicine, University of Alberta, Edmonton, Canada.*

The hallmark of T cell mediated rejection (TCMR) are interstitial inflammation and tubulitis. The mechanisms of tubulitis and epithelial deterioration during TCMR are unknown. We previously showed that tubulitis in mouse allografts is independent of cytotoxic molecules (GzMA/B, Prf) and is preceded by molecular changes with loss of epithelial genes, reflecting epithelial dedifferentiation. Human TCMR is associated with loss of the same epithelial genes and re-expression of embryonic pathways (Wnt, Notch). We hypothesized that TCMR is mediated through soluble factors released by effector T cells or macrophages in the interstitium, and that supernatants of effector T cells would simulate these changes in epithelial cultures.

We established an *in vitro* model in which cultured primary human renal epithelial cells are incubated with supernatants from effector T cell/monocyte co-cultures. The transcript changes in this model, analyzed by microarrays, closely simulated those in human and mouse TCMR (Fig1), with loss of epithelial transcripts, activation of Wnt/Notch pathways, and increased expression of Ifng-inducible and injury-related transcripts previously defined in our mouse model. Some of these changes were reproduced by incubation of epithelial cells with Ifng or Tgfb. The *in vitro* model identified additional epithelial transcript changes not previously identified *in vivo* (not affected by Ifng or ischemic injury, not expressed in T cells, macrophages, B cells, or NK cells). Expression of these transcripts (n = 305) was highly altered in human TCMR compared to non-rejecting biopsies of 177 human renal allograft biopsies and distinguished TCMR from antibody-mediated rejection in a hierarchical cluster analysis.

Thus we have established an *in vitro* model that closely simulates the epithelial events during human TCMR and confirms that these changes are independent of direct contact with inflammatory cells, supporting the hypothesis that interstitial effector T cells mediate allograft deterioration by soluble mediators. Together with previous mouse and human data these results provide the first *in vitro* model of the epithelial consequences of TCMR.

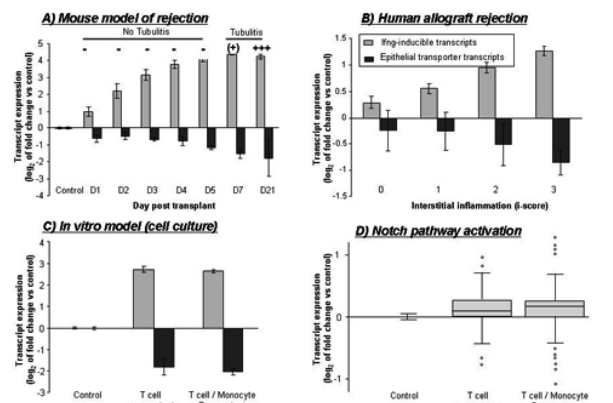


Figure 1. Epithelial gene expression during rejection. Renal allograft rejection is characterized by loss of transcripts encoding epithelial transporters (solute carriers (Slc)) and re-expression of embryonic pathways such as Wnt and Notch pathways. A) In a mouse model of rejection, loss of Slc transcripts begins at day 1. Transcript changes precede the development of histologic lesions, including tubulitis, and deteriorate with time. B) In human allograft rejection, loss of Slc transcripts correlates with interstitial inflammation. C) and D) The epithelial changes, including loss of Slc and activation of embryonic pathways, can be simulated in an *in vitro* cell culture model, in which primary human renal proximal tubular epithelial cells are incubated for 48h with supernatants from effector T cells or from effector T cell/monocyte co-cultures.

Humoral Sensitization and B Cell Biology

Abstract# 509

Complement-Fixing Anti-HLA Alloreactivity in the Absence of Early Graft Dysfunction Is Not Associated with Inferior Long-Term Kidney Allograft Function. Gregor Bartel,¹ Markus Wahrmann,¹ Heinz Regele,² Nicole Hutter,² Markus Exner,³ Georg A. Boehmig.¹ ¹Medicine 3, Medical University of Vienna, Vienna, Austria; ²Pathology, Medical University of Vienna, Vienna, Austria; ³Laboratory Medicine, Medical University of Vienna, Vienna, Austria.

Objective. C4 split product deposition to HLA antigen-coated microparticles ([C4d] FlowPRA) was previously shown to be a specific marker of C4d-positive antibody-mediated rejection (AMR). The objective of this study was to assess the predictive value of [C4d]FlowPRA reactivity in a cohort of non-biopsied patients with stable graft function during the first year.

Methods. A total of 133 kidney transplant recipients were enrolled (inclusion criteria: functioning graft at 12 months; prospective collection of sera taken before and at 1-3, 6, and 12 months after transplantation). Included patients were serially screened for humoral panel reactivity applying [IgG] and [C4d]FlowPRA screening.

Results. Fifty-four of the included 133 recipients had stable graft function within the first year and were not subjected to diagnostic renal biopsy. In this particular patient group, detection of complement-fixing HLA reactivity tended to be less frequent than in the 79 patients with biopsied graft dysfunction ($\geq 10\%$ [C4d]FlowPRA before transplantation: 9% vs. 18% of recipients, $P=0.2$; $\geq 10\%$ [C4d]FlowPRA after transplantation: 11% vs. 24%, $P=0.06$). In line with our previous results, within the group of biopsied patients, pre- and/or post-Tx [C4d]FlowPRA reactivity was tightly associated with the immunohistochemical detection of peritubular capillary C4d deposition ($P=0.005$) reflecting ongoing AMR. Remarkably, in initially stable patients, detectable [C4d]FlowPRA reactivity was not associated with inferior long-term outcomes. Within this patient group, recipients with and without (pre- and/or post-transplant) C4d-fixing anti-HLA reactivity did not differ with respect to 4 yr allograft survival ($P=0.4$), 4 yr serum creatinine levels (1.5 vs. 1.5 mg/dl; $P=0.7$), and proteinuria at 4 yrs (0.2 vs. 0.18 g/24h; $P=0.8$). Similar results were obtained for a comparison of [IgG]FlowPRA positive vs. negative subjects.

Conclusion. Our data suggest that a considerable number of patients with initially stable graft function may have excellent long-term graft function despite serologically detectable levels of (complement-fixing) alloreactivity. For these antibody-positive recipients, a potential role of graft accommodation can be speculated.

Abstract# 510

The Immunoproteasome Subunit Beta 10 as a Novel Peripheral Blood and Intra-graft Biomarker of Chronic Antibody Mediated Allograft Rejection in Clinical Transplantation. Joanna Ashton-Chess,¹ Vojislav Jovanovic,¹ Yohann Foucher,¹ Emilie Dugast,¹ Magali Giral,¹ Karine Renaudin,¹ Anne Moreau,¹ Anne-Sophie Dugast,¹ Maud Racapé,¹ Claire Usal,¹ Helga Smit,¹ Jean-Marie Heslan,¹ Jean-Paul Soullillou,¹ Sophie Brouard.¹ ¹INERM U643; CHU Nantes; Institut de Transplantation et de Recherche en Transplantation; Université de Nantes, Nantes, France.

In an attempt to identify non-invasive biomarkers of specific histological scarring, we compared publicly available gene sets derived from microarray studies of human renal transplant biopsies published in the literature with our own microarray data derived from studies of rat heart allografts. In this way we identified an immunoproteasome subunit (proteasome subunit beta 10 -PSMB10) as a potentially interesting candidate. PSMB10 is one of three members of the immunoproteasome that are induced by

interferon gamma.

Messenger RNA profiling in renal transplant biopsies (n = 52) with normal histology, interstitial fibrosis and tubular atrophy, calcineurin inhibitor toxicity, transplant glomerulopathy or chronic antibody-mediated rejection (Banff 2005) revealed PSMB10 to be strongly and significantly increased in chronic antibody mediated rejection vs. the other three histological diagnoses. Receiver Operator Characteristic (ROC) curve analysis showed that PSMB10 mRNA could diagnose chronic antibody-mediated rejection with an AUC of 0.99, a sensitivity of 1.0 and a specificity of 0.95. Moreover, PSMB10 mRNA was significantly increased in the PBMC (n = 24) of patients with chronic antibody-mediated rejection compared to those with normal histology. ROC analyses revealed an impressive AUC of 1.0 with all patients being correctly classified. Similar results were also observed in a rat allograft model where PSMB10 was significantly increased at day 100 post transplantation in both the heart allograft and the PBMC of animals presenting chronic transplant vasculopathy vs. syngeneic grafts. Moreover, inhibition of the proteasome by administration of Velcade® at 0.1 mg/kg every other day for the first 20 days post transplantation significantly and dose-dependently prolonged allograft survival (MST 31.7 days in velcade-treated vs. 6.3 days in untreated animals). Together our data point towards PSMB10 as a blood and intra-graft biomarker of chronic antibody-mediated rejection as well as a potential therapeutic target. Furthermore, our results suggest that using a threshold of PSMB10 in the blood could help in guiding the decision to biopsy in the clinic.

Abstract# 511

BAFF Monitoring after B-Cell Depletion Therapy for Acute Renal Transplant Rejection. Valeriya Zarkhin,¹ Snehal Mohile,¹ Li Li,¹ Jonathan Martin,¹ Minnie Sarwal.¹ ¹Pediatrics, Stanford University, Stanford, CA.

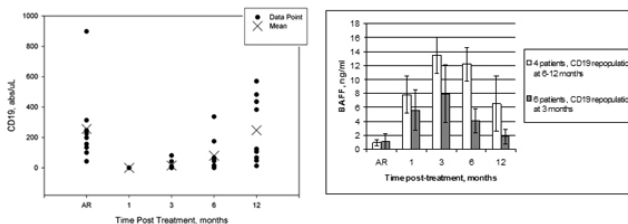
Introduction: The objective of this study was to investigate the interaction between B-cell activation factor of the TNF family (BAFF) level and circulating B-cell repopulation in pediatric patients with acute kidney transplant rejection treated with the B-cell-depleting agent Rituximab.

Methods: 10 pediatric patients (3-23 yrs) with biopsy proven B-cell positive AR were treated with steroids and Rituximab (4 x 375 mg/m²/dose/week). All patients were followed up for 12 months. Peripheral blood CD19 cells and donor specific antibodies (DSA) were monitored monthly. Serum level of BAFF was measured by ELISA at AR, 1, 3, 6, and 12 months post-AR treatment and correlated with clinical outcomes.

Results: Complete depletion of circulating and intra-graft B-cells was observed with Rituximab, with improvement in AR grade in all patients. The median time of peripheral B-cells repopulation was 5 months (range 3-12 months, Fig. 1A). No correlation was found between pre-treatment peripheral B-cell number and the B-cell repopulation time (r=0.59, p=0.09). BAFF levels rose significantly with B-cell depletion with maximum values at 3 months post-treatment (7.5 fold increase, p=0.0001) and returned to pre-treatment levels, with B-cell recovery, at 12 months (Fig. 1B). Serum BAFF levels correlated positively with B-cell depletion >6 months (r=0.91, p=0.004, Fig 1B). A lack of depletion of DSA I, but not DSA II correlated with higher BAFF levels (r=0.99, p=0.007).

Fig. 1A. Circulating B-cell depletion after Rituximab treatment.

Fig. 1B. Increase in serum BAFF during circulating B-cell depletion.



Conclusions: The timing of B-cell repopulation and depletion of DSA I may be dependant on serum BAFF level. Anti-BAFF treatment may be considered in addition to Rituximab or standard immunosuppressive treatment protocols in patients with persistent and/or antibody mediated rejection.

Abstract# 512

The Development of Donor Specific Antibodies in Renal Allograft Recipients Is Associated with Immunosuppression Reduction. Lorita M. Rebollato,¹ Paul I. Terasaki,² Mikki Ozawa,² Kimberly P. Briley,¹ Paul Catrou,¹ Carl E. Haisch.³ ¹Pathology, The Brody School of Medicine at ECU, Greenville, NC; ²The Terasaki Foundation, Los Angeles, CA; ³Surgery, The Brody School of Medicine at ECU, Greenville, NC.

Background: The development of donor specific HLA antibodies (DSA) post-transplant has been associated with graft failure. We have shown in a longitudinal study that increases in DSA may precede rejection by months. This retrospective analysis evaluates changes in maintenance immunosuppression (MI) and appearance of DSA in stable transplant recipients.

Methods: Sera from stable renal transplant recipients were collected at 4-6 month intervals and tested for the presence of DSA. The types and doses of immunosuppression were correlated with the appearance of DSA. Two hundred eighty stable renal transplant recipients who received either a deceased or a living donor kidney were monitored post-transplantation for the development of DSA. Patients have been followed for 1

to 7 years and had a minimum of 4 serum samples analyzed. All recipients received anti-lymphocyte induction therapy. Maintenance immunosuppression (MI) consisted of a calcineurin inhibitor, prednisolone, and mycophenolic acid. HLA single antigen beads analyzed in the Luminex instrument were used to establish donor specificity of the antibodies. A chart review was undertaken to determine the doses of the MI post-transplantation. All MI was managed by the transplant team and changed according to clinical indications without regard to DSA.

Results: Of the 280 patients monitored 37 developed DSA post-transplantation with a functioning graft. DSA was against HLA-Class II antigens in 26 of 37 (70%); Class I antigens in 8 of 37 (22%); and against both Class I and II antigens in 3 of 37 (8%). DSA against Class II was against DQ in all except one case. In the majority of the recipients the appearance of DSA was preceded with dose reduction of the MI, either calcineurin inhibitor or mycophenolic acid or both.

Conclusions: Our data show that DSA developed predominantly against HLA-class II antigens and that the appearance of DSA was often preceded with reduction of one or more of the MI. This data shows the importance of monitoring DSA with MI decreases in a stable allograft recipient.

Abstract# 513

Antibody Production and Antigen Presentation Are Directly Inhibited by Mycophenolate Mofetil. Anat R. Tambur,¹ Joe Leventhal,¹ Nancy D. Herrera,¹ Joshua Miller.¹ ¹Division of Organ Transplantation, Northwestern University, Chicago, IL.

Immunosuppressive medications are primarily designed to target T cell proliferation. Mycophenolate Mofetil (MMF) exerts its effect by inhibiting *de-novo* synthesis of Guanine, a DNA building block.

We, and others, have previously shown that MPA (active metabolite of MMF) affects the differentiation of monocytes into dendritic cells (DC). We further demonstrated that cell-surface receptors associated with antigen up-take and antigen-processing and presentation (CD83 and CD205) are down regulated when cells are matured in the presence of MPA. This phenotype translated into a decreased uptake of alloantigens and reduced stimulation of T cells. We concluded that MMF inhibits also cell functions requiring mRNA synthesis.

We now present data regarding the role of MPA in maturation and function of B-lineage cells. PBMCs from 10 subjects were cultured in the presence of CpG, IL-2, IL-10 and CD40L for 5 days to induce memory B and plasma cell maturation in-vitro. Cultures were performed in the presence or absence of MPA (50 ugr/ul) for the length of the incubation period.

In-vitro stimulation of B cells increased the memory population (CD19+ CD27+) from 2.8 +/- 1.1% to 7 +/- 2%. Similarly, plasma cells were increased from 4.4 +/- 1.3% to 6.4 +/- 1.8%. The addition of MPA to the culture inhibited stimulation for both memory and plasma cells (4.7 +/- 3% p=0.01; and 4.7 +/- 2.3% p=NS, respectively).

We have further analyzed the effects of MPA on antibody secretion using an ELISA (measuring soluble antibodies) as well as a B-cell ELISPOT assay (assessing the number of B cells that produce antibodies). While an expected increase in OD values was observed for stimulated samples compared with non-stimulated samples, a significant decrease was observed when stimulation occurred in the presence of MPA. Non-stimulated cells: 0.249 +/- 0.28; Stimulated cells: 0.391 +/- 0.33; MPA treated stimulated cells: 0.279 +/- 0.43; p<0.0005. The number of antibody producing cells was also significantly lowered when cultures were done in the presence of MPA (a mean of 106 cells were counted for the stimulated cells compared with 1-2 cells for non-stimulated and MPA-treated-stimulated cells).

To our knowledge this is the first time where in-vitro experimental data document the inhibitory effect of MPA on B lineage cells and antibody secretion, although clinically known for some time. These results confirm our previous observations regarding the effects of MMF on non-proliferating immune cells.

Abstract# 514

A Non-Allogeneic Stimulus Triggers the Production of De Novo HLA and MICA Antibodies. Luis E. Morales-Buenrostro,^{1,2} Lluvia A. Marino-Vazquez,¹ Anh Nguyen,³ Paul I. Terasaki,² Josefina Alberu.¹ ¹Nephrology and Transplantation., Instituto Nacional de Ciencias Medicas y Nutricion Salvador Zubiran, Mexico City, DF, Mexico; ²Terasaki Foundation Laboratory, Los Angeles, CA; ³One Lambda Inc., Canoga Park, CA.

Background: In a previous study, we found that healthy people developed HLA Abs after immunization against hepatitis B virus. The aim of this prospective study was to establish if stimulation with influenza vaccine is capable of triggering the production of HLA and MICA Abs.

Methods: We determined the presence of HLA and MICA Abs (de novo and preformed Abs) in 3 groups of patients vaccinated against influenza: A) 42 healthy adults, B) 40 ESRD patients, and C) 25 TR. Additionally, we followed 22 healthy unvaccinated people without exposure to sensitizing factor: D) control group. Sera samples were collected at baseline (pre influenza shot), at 1 week, and monthly up to 6 months after immunization. HLA Abs were assessed with Labscreen Single Antigen beads for Luminex. All samples of each patient were tested simultaneously. A luminescence value higher than 500 was considered positive only if it was 3 times the baseline value. We analyzed the data using Chi square, one way ANOVA test, and Logistic regression.

Results: The table shows the types of Abs in each group.

Table. Distribution of antibodies by group.

Antibodies	Group A N=42	Group B N=40	Group C N=25	Group D N=22
HLA class I	0	3 (7.5 %)	2 (8.0 %)	0
HLA class II	1 (2.4 %)	0	1 (4.0 %)	0
MICA	0	4 (10.0 %)	1 (4.0 %)	0
HLA I & HLA II	0	0	1 (4.0 %)	0
Any De Novo**	1 (2.4 %)	7 (17.5 %)	5 (20.0 %)	0
Preformed HLA I*	21 (50.0 %)	24 (60.0 %)	17 (68.0 %)	2 (9.1 %)
Preformed HLA II*	8 (19.1 %)	9 (22.5 %)	16 (64.0 %)	1 (4.5 %)
Preformed MICA	17 (40.5 %)	11 (27.5 %)	6 (24.0 %)	5 (22.7 %)
Any Preformed*	28 (66.7 %)	31 (77.5 %)	22 (88.0 %)	6 (27.3 %)

**p=0.016 *p<0.001

Interestingly, we found preformed Abs across all four groups, including the control group (which is free of any known sensitizing factors). The proportion of de novo Abs was higher in the group B and C. Multivariate analysis shows that the only independent factor associated with development of de novo Abs was the presence of preformed Abs.

Conclusions: We observed a nonspecific immunologic response triggered by external stimulus and was not necessarily associated to the vaccine in people previously sensitized.

Abstract# 515

A Unique B Cell Signature Associated with Operational Tolerance.

Kenneth A. Newell,¹ Adam Asare,² Allan D. Kirk,¹ Trang Gisler,² Manikkam Suthanthiran,³ William Burlingham,⁴ William Marks,⁵ Laurence Turka,⁶ Vicki Seyfert-Margolis.² ¹*Surgery, Emory University, Atlanta, GA;* ²*University of California, San Francisco, San Francisco, CA;* ³*Medicine, Cornell University Medical Center, New York, NY;* ⁴*Surgery, University of Wisconsin, Madison, WI;* ⁵*Surgery, Swedish Medical Center, Seattle, WA;* ⁶*Medicine, University of Pennsylvania, Philadelphia, PA.*

Introduction. Decisions about the minimization and ultimate withdrawal of immunosuppression (IS) would be facilitated by the identification of biomarkers associated with operational tolerance (OT). **Methods.** As part of an ITN/NIH supported study tolerant kidney transplant recipients (off all IS for > 1yr with stable function, N=22) were compared to recipients with stable function on IS (SIS, N=34), recipients with chronic allograft nephropathy (CAN, N=20), and healthy volunteers (HV, N=18). PBMC, whole blood total RNA, and urine samples from each group were examined using flow cytometry, microarrays, and RT-PCR respectively. **Results.** Analysis of microarrays revealed significantly higher expression of B cell differentiation genes in tolerant recipients compared to the SIS and CAN groups. Consistent with this finding, tolerant recipients also displayed higher numbers of naïve B cells in peripheral blood and increased expression of CD20 in urine relative to the SIS and CAN groups. No differences in Treg or genes associated with regulatory cells were observed in tolerant recipients relative to other groups. These analyses failed to demonstrate significant differences between tolerant recipients and HVs although support vector machine learning methods suggested potential differences in a number of genes including NFAT and calcineurin. Finally, relative to tolerant patients, those with CAN showed decreased numbers of T and NK cells and expressed lower levels of genes associated with immune cell activation in peripheral blood. **Conclusions.** Differences in B cell numbers may be useful in identifying tolerant renal transplant recipients or those predisposed to developing tolerance and could potentially provide insights into the mechanisms of tolerance.

Abstract# 516

Erythrocyte Bound Complement, E-C4d, as a Marker for Humoral Rejection in Lung Transplant (LTx) Recipients.

A. Golocheikine,¹ D. Saini,¹ S. Ramachandran,¹ D. Phelan,³ A. Aloush,⁵ E. Trulock,⁴ G. A. Patterson,⁵ T. Mohanakumar.^{1,2} ¹*Surg, WUSM;* ²*Pathol & Immunol, WUSM;* ³*HLA Laboratory, Barnes-Jewish Hospital;* ⁴*Intern Med-Pul, WUSM;* ⁵*Cardio Surg, Washington Univ Sch Med, St. Louis, MO.*

Development of antibodies (Abs) to mismatched donor HLA antigens has been associated with acute and chronic rejection. Complement activation, and C4d deposition, has been correlated with humoral rejection of allografts. However, utility of C4d staining in LTx has been controversial. A recent study (Arthritis Rheum. 2004 Nov;50(11):3596-604) shows a strong correlation between erythrocyte bound C4d (E-C4d) in diagnosis and monitoring of SLE. Goal of our study is to determine the utility of measuring E-C4d in the diagnosis of humoral rejection following human LTx. 26 LTx recipients were analyzed post-LTx for E-C4d using FACS of RBCs incubated with anti-C4d (Quidel) followed by FITC- goat anti-mouse. 10 normals were also analyzed. The serum was analyzed for development of anti-HLA Abs by solid phase assays and for the presence of autoAbs to K α 1 tubulin and collagenV (ELISA). Biopsies from 11 patients were stained immunohistochemically for C3d deposition. Summary of results are presented in Table 1.

Table 1:

# of Patients	Avg % of E-C4d + MFI	Anti-HLA Abs/DSA or both	Presence of autoAbs	Infection/AR
16	E-C4d=21.73% MFI=7	Anti-HLA=11/16 DSA=8/11	n=13/16 CollV=9 K α 1 tubulin=11	Infection=5/16 AR=4/16
10	E-C4d=4.89% MFI=2.39	Anti-HLA=4/10 DSA=2/4	(n=4) CollV=2 K α 1 tubulin=3	Infection=2/10 AR=0/10

% E-C4d in normals- 10.35%; MFI in normals- 5.46; MFI=Mean Fluorescence Intensity;

AR=Acute Rejection; DSA=Donor Specific Abs

16 out of 26 patients show significant increase in the % bound E-C4d (P<0.05) as compared to controls. 11/16 had anti-HLA and 13/16 had autoAbs which were significantly different from those with low E-C4d (P= 0.005). Staining of the biopsies showed C3d deposition in 6 recipients with increased E-C4d. All 4 patients with acute humoral rejection had elevated E-C4d. We conclude that there is a significant correlation between increase in % E-C4d in LTx recipients and development of Abs to either HLA antigens or auto-antigens during the post-LTx period. Biopsies from patients with increased E-C4d showed deposition of C3d in the allografts. Preliminary data suggest that measurement of E-C4d using a non-invasive method of flow cytometry may be of value in monitoring LTx patients for humoral rejection.

Innate Immunity: Chemokines, Cytokines

Abstract# 517

Systemic Activation of Dendritic Cells Induced by Local Surgical Trauma in a Mouse Skin Transplant Model.

Harras B. Zaid,¹ Kym Garrod,¹ Lee Reinhardt,¹ Richard M. Locksley,² Qizhi Tang,¹ Sang-Mo Kang.¹ ¹*Dept. of Surgery, Division of Transplantation, UCSF, San Francisco, CA;* ²*HHMI and Dept. of Medicine and Microbiology & Immunology, UCSF, San Francisco, CA.*

Innate immunity is emerging as an important initiator and modulator of the adaptive immune response. In the setting of transplantation, ischemia-reperfusion injury and tissue trauma appear to potentiate the alloimmune response. One of the mechanisms through which the innate immune system modulates an adaptive immune response is dendritic cells (DC). In this study, we examined DC activation in a mouse model of skin transplantation by monitoring the expression of MHC II and p40 chain of the proinflammatory cytokine IL-12. The p40 expression was detected in live cells using the Yef40 reporter mouse, in which a transgene for yellow fluorescent protein (YFP) was placed downstream of the endogenous IL-12p40 gene, thus faithfully "reporting" p40 expression. Skins from C57BL/6 or BALB/c donors were grafted on the dorsal thorax of C57BL/6 Yef40 mice. Draining and non-draining lymph nodes (LN) were harvested at 24 hours and examined for YFP expression by fluorescent microscopy. A marked increase in the numbers of YFP-expressing DC was observed in draining LN in both syngeneic and allogeneic graft recipients. Surprisingly, YFP-expressing DC also increased in non-draining LN when compared to non-transplanted controls. Similarly, DC in draining and non-draining LN showed higher MHC II expression than those in non-transplanted controls. Upregulation of MHC II was highest at 24 hours and decreased significantly by 48-72 hours. To assess whether DC activation in non-draining LN was functionally significant, we monitored the activation of adoptively transferred ovalbumin-specific OT-II TCR transgenic T cells in response to footpad antigen challenge in mice with or without a syngeneic skin transplant on the contralateral upper thorax. OTII T cells in transplanted animals proliferated approximately 5- to 10-fold better and a higher percentage of the OTII cells produced IL-2 than those from non-transplanted animals. Thus, local surgical trauma results in a widespread, time-limited, functional activation of DC that appears to act as a partial adjuvant for T cell responses. Together, these data suggest that surgical trauma may incite a systemic barrier to transplantation via the activation of DC and therapeutic interventions that reduce the surgical trauma and DC activation may help to improve survival of transplanted grafts.

Abstract# 518

Divergent Role of Tissue/Donor Dendritic Cells in Rejection Versus Tolerance of Cardiac Allografts: Studies with CX3CR1-Deficient Mice.

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Although donor/tissue dendritic cells (dDC) have long been known to play a key role in mounting alloimmune responses, however, their generation, trafficking and role in tolerance have not rigorously examined. We have used B6.FVB-Tg (Itgax-DTR/EGFP) 57 mice which has a GFP linked to the CD11c promoter. Using these mice as the donors of heart allograft transplantation provided us a unique model to study dDC post-transplantation. Our trafficking data indicate there is a rapid migration of dDC into spleen (3 hours post transplantation) but not lymph nodes and that dDC were unexpectedly detected in the spleen of the recipients long after rejection of heart allografts suggesting dDC could escape from the immunosurveillance of host immune system. Our data also show that dDC proliferate in the lymphoid tissue of the recipients and co-express Class II molecule of the recipients. We then show that CX3CR1 pathway regulates generation

of heart tissue DC constitutively. As compared to WT hearts, CX3CR1^{-/-} hearts contain lower number of DC and transplanting CX3CR1^{-/-} donor hearts into WT BALB/c mice led to significant prolongation of allograft survival without immunosuppression (MST of 8 vs. 17 days, respectively). Increasing CX3CR1^{-/-} heart DC by implanting donors with FLT3-producing hybridoma cells has restored the time of rejection. Unexpectedly, induction of long-term survival with anti-CD154 blockade (MR1) and CTLA-4 Ig (but not low dose Rapamycin) was abrogated when CX3CR1^{-/-} hearts were used as donors, with concomitant lesser Tregs in the CX3CR1^{-/-} heart allografts as compared to WT. Furthermore, co-transplanting hearts from WT and CX3CR1^{-/-} into the same recipient treated with MR1 resulted in significant prolongation of CX3CR1^{-/-} heart allograft survival. Depleting the dDC of heart donors prior to transplantation with diphtheria toxin also worsened markedly chronic rejection in the recipients at day 100 post-transplantation. Our data indicate that, in contrast to the widely accepted dogma, the presence of donor DC in graft tissue is not only central to allograft rejection but also is necessary for the induction and maintenance of peripheral tolerance.

Abstract# 519

Local C5a Interaction with C5aR on DCs Modulates DC Function, Subsequently Up-Regulating Allospecific T Cell Responses. Qi Peng, Ke Li, Steven H. Sacks, Wuding Zhou. *MRC Centre for Transplantation, Department of Nephrology and Transplantation, King's College London, Guy's Campus, London, United Kingdom.*

The innate system of immunity plays an important role in ischemia-reperfusion injury and allograft rejection. The early stages of inflammatory processes are accompanied by complement activation. One biological consequence of this activation is the release of potent inflammatory anaphylatoxins, C3a and C5a, which have been reported to regulate a range of inflammatory responses. We previously reported that DCs express C3aR and C5aR, and C3a-C3aR interaction has a positive impact on murine BM DCs, in terms of activation phenotype and capacity for Ag uptake and allostimulation. However, the role of C5a in modulating DC function remains unclear. The aim of this study is to investigate the role of local C5aR signalling in modulating murine BM DC function and subsequent regulation of the allospecific T cell response. We first evaluated if C5a-C5aR interaction could result from local expression of factors. Our results showed that C5aR mRNA was detected in WT DCs at different stage of DC culture by RT-PCR, and C5a was detected by ELISA in the culture supernatants from different stages of DC culture. We next determined if C5a-C5aR interaction modulates DC function in allospecific T cell stimulation *in vitro* and *in vivo*. We found that BM DCs cultured from C5aR^{-/-} mice or treated with C5aR antagonist (C5aRa, W54011) exhibited a less activated phenotype (producing significantly less IL-12 and more IL-10, in response to LPS stimulation); both C5aR^{-/-} and antagonist-treated DCs (LPS stimulated) showed reduced capacity to stimulate naïve alloreactive T cells, as measured by IFN- γ production and thymidine uptake. As regards interaction *in vivo*, following i.p. administration of the C5aRa-treated DCs into allogeneic mice for 10 days, *ex vivo* mixed lymphocyte reaction showed that CD4⁺ T cells from those recipients have reduced thymidine uptake, but increased IL-4 production compared to that with untreated DCs. Conversely, DCs treated with C5aR agonist (C5a) exhibited a more activated phenotype (producing more IL-12 and less IL-10) and were more potent in allospecific T cell stimulation. Our findings demonstrate that murine BM DCs can express C5aR and C5a can be generated locally; C5a-C5aR interaction up-regulates murine BMDC activation and their allostimulatory capacity. Thus, targeting C5a-mediated signal may be able to prevent allograft injury.

Abstract# 520

Role of TNF α in Early Chemokine Production and Leukocyte Infiltration into Heart Allografts. Daisuke Ishii,¹ Austin D. Schenck,¹ Robert L. Fairchild,¹ ¹*Immunology, Glickman Urological and Kidney Institute., Cleveland Clinic, Cleveland, OH.*

OBJECTIVES: The acute phase cytokines IL-6 and TNF α are produced early during inflammatory processes, including wound healing and ischemia/reperfusion. The goal of this study was to investigate the role of these cytokines in the induction of early chemokine production and leukocyte infiltration into heart allografts.

METHODS: C57BL/6 (H-2b), BALB/c (H-2d), and BALB/c.IL-6^{-/-} mice received vascularized syngeneic or complete MHC mismatched, A/J (H-2a), cardiac grafts. Grafts were retrieved at different time points and total RNA and tissue protein were prepared and analyzed by quantitative RT/PCR and ELISA to test expression levels of TNF- α , IL-6, CXCL1/KC, CXCL2/MIP-2, and CCL2/MCP-1 in the grafts. Anti-TNF α mAb (500 ug) was given at the time of transplantation with or without anti-CD154 mAb (200 ug on days 1 and 2). Infiltration of CD4⁺, CD8⁺ T cells, neutrophils and macrophages was assessed by flow cytometry and immunohistochemistry. Donor-reactive T cell priming to IFN- γ producing cells in the recipient spleen was measured by ELISPOT.

RESULTS: Expression of TNF α and IL-6 mRNA reached an initial peak at 3 hrs post-transplant and a second peak at 9-12 hrs with equivalent levels in both iso- and allo-grafts. The neutrophil and macrophage chemoattractants CXCL1/KC, CXCL2/MIP-2 and CCL2/MCP-1 reached peak levels at 9 hrs post-transplant in both sets of grafts and then declined to background levels. IL-6 deficiency in the recipient or the cardiac allograft did not prolong allograft survival. In untreated mice, heart allografts were rejected at 8.6 $\pm$ 0.6 days after transplantation. Anti-TNF α mAb decreased neutrophil and macrophage chemoattractant levels 50% at 9 hrs post-transplant and subsequent neutrophil, macrophage and CD8⁺ cell infiltration into the allografts as well

as extended graft survival to 14.1 $\pm$ 0.8 days. Anti-TNF α mAb also decreased the number of donor-reactive IFN- γ producing CD8 T cells almost 90% on day 7 post-transplant. Whereas anti-CD154 mAb prolonged survival to day 21, administration of anti-TNF α and anti-CD154 mAb delayed rejection to day 32 and resulted in the long-term (> 80 days) survival of 40% of the heart allografts.

CONCLUSIONS: These data indicate that anti-TNF α antibodies can delay donor-reactive CD8 T cell priming and leukocyte infiltration into heart allografts rejection. As a conjunctive therapy, TNF α antibodies can promote long-term survival of the allografts.

Abstract# 521

Synergy between IL-6 and TNF α Promotes T Cell Alloreactivity and Impairs the Graft-Prolonging Effects of Costimulatory Blockade. Hua Shen,¹ Bethany M. Tesar,¹ Wendy E. Walker,¹ Daniel R. Goldstein,¹ ¹*Internal Medicine, Yale University, New Haven, CT.*

Introduction Recent evidence indicates that inflammation impairs immune regulation, yet the mechanisms behind this effect are not clear, in particular in regards to transplantation tolerance. In this study, we investigated the role of inflammatory cytokines, IL-6 and TNF α , in transplantation tolerance induction.

Methods + Results We first examined the impact of IL-6 + TNF α on *in vitro* T cell alloimmune responses. T cells that were stimulated by allogeneic APCs in conditioned media harvested from LPS-activated DCs proliferated more than APC-stimulated T cells that were cultured in conditioned media derived from non-LPS-activated DCs. The ability of CD4 and CD8 T cells to respond to allogeneic APCs in the LPS-activated conditioned media was significantly impaired by the addition of either anti-IL-6 or anti-TNF α mAbs. The addition of both mAbs further diminished T cell proliferation, indicating that IL-6 and TNF α synergize to augment *in vitro* T cell allostimulation. In support of these findings, we noted reduced T cell proliferation during the MLR when T cells were cultured in conditioned media derived from either IL-6^{-/-} or TNF α ^{-/-} LPS-activated DCs. Furthermore, these diminished responses was restored by the addition of recombinant IL-6 or TNF α , respectively. To examine the *in vivo* implications of these findings, we employed a murine skin allograft model, with recipients that were either B6 wild type, IL-6^{-/-} or TNF α ^{-/-}. These groups were transplanted with a BALB/c skin graft and treated with (or without) perioperative costimulatory blockade (CTLA4 Ig and anti-CD154). In the absence of immune modulation, all groups rejected BALB/c skin allografts at a similar tempo (<12 days). In the presence of costimulatory blockade, IL-6^{-/-} recipients (median survival time, MST = 22 days, p=0.05) rejected their allografts at a slower tempo compared to WT (MST = 16 days) or TNF α ^{-/-} recipients (MST = 18 days). However, this response in IL-6^{-/-} recipients was further delayed by administering a TNF α inhibiting mAb (MST = 60 days), indicating that synergy between IL-6 and TNF α occurs *in vivo* and prevents the ability of costimulatory blockade to delay the onset of allograft rejection.

Conclusions We conclude that synergy between IL-6 and TNF α augments T cell alloimmune responses and impairs the effects of costimulatory blockade to delay allograft rejection.

Abstract# 522

A Novel Role of Th17 Cells in Allograft Rejection and Vasculopathy. Francesca D'Addio,¹ Jesus Paez-Cortez,¹ M. Javed Ansari,¹ Laurie Glimcher,² John Iacomini,¹ Mohamed Sayegh,¹ Xueli Yuan,¹ ¹*Transplantation Research Center, Renal Division, Brigham and Women's Hospital, Boston, MA;* ²*Harvard School of Public Health, Boston, MA.*

Introduction: Transcription factor T-bet plays a crucial role in Th1/Th2 development. Here, we investigated the role of T-bet in Th17 differentiation and function of Th17 cytokines in allograft rejection using an MHC class II mismatched model of cardiac allograft vasculopathy.

Methods/Results: Cardiac allografts from bm12 mice were transplanted into wild-type as well as T-bet and IFN- γ deficient C57BL/6 recipients. T-bet^{-/-} mice showed significantly accelerated allograft rejection (MST=14.75 $\pm$ 0.96 days). However, as previously reported, all IFN- γ ^{-/-} and majority of the C57BL/6 mice accepted grafts for greater than 60 days. Upon *in vitro* stimulation of recipient splenocytes by irradiated donor cells, T-bet^{-/-} and INF- γ ^{-/-} lymphocytes produced significantly less INF- γ and more Th2 cytokines. Interestingly, production of the proinflammatory cytokines IL-17 and IL-6 was significantly higher in T-bet^{-/-} (337 $\pm$ 94.4 and 339 $\pm$ 10.5 pg/ml) than C57BL/6 (135.1 $\pm$ 68.3, 75.3 $\pm$ 25.9 pg/ml, P=0.0399 and 0.0001 compared to T-bet^{-/-}) and INF- γ ^{-/-} (95.8 $\pm$ 29.8, 20844.5 pg/ml, P=0.0135 and 0.0093 compared to T-bet^{-/-}) mice. *In vivo* administration of IL-17 neutralizing antibody (mab421) significantly prolonged survival of bm12 hearts (MST>30 days, p<0.01 compared to the 15.3 $\pm$ 2.6 days of the control IgG group) in T-bet^{-/-} mice. Immunofluorescence staining of bm12 hearts harvested from T-bet^{-/-} recipients indicated that both CD4 and CD8 infiltrating lymphocytes produced IL-17. However, T-bet-CD4 double knockout mice did not reject bm12 heart grafts, nor did the grafts exhibit chronic vasculopathy. In contrast, T-bet-CD8 double knockout mice rejected (MST: 18.9 $\pm$ 3.2 days). Splenocytes from T-bet-CD4 knockouts produced significantly lower IL-6 (12.9 $\pm$ 3.8) and IL-17 (7.9 $\pm$ 4.6 pg/ml) than observed in T-bet knockouts (940 $\pm$ 176.3 and 210.2 $\pm$ 73.4 pg/ml) and T-bet-CD8 knockouts (1240.5 $\pm$ 215.6 and 113.1 $\pm$ 61.2 pg/ml respectively) recipients when re-stimulated with donor cells, while there was no significant difference in INF- γ production.

Conclusion: T-bet deficiency results in up-regulation of IL-17 expression in addition to Th2 associated cytokines resulting in acceleration of chronic rejection despite profound deficiency of IFN- γ . T-bet deficiency may contribute to the alloimmune responses independent of IFN- γ , mediated predominantly by the novel proinflammatory Th17 CD4 T cells.

Abstract# 523

The Toll-Interleukin-1 Receptor Member TIR8/SIGIRR Negatively Regulates Adaptive Immune Response to Kidney Transplantation. Marina Noris, Paola Cassis, Nadia Azzollini, Regiane Cavinato, Daniela Cugini, Federica Casiraghi, Sistiana Aiello, Mauro Abbate, Susanna Tomasoni, Alberto Mantovani, Giuseppe Remuzzi. *Transplant Research Center, Mario Negri Institute, Ranica, Bergamo, Italy; Istituto Clinico Humanitas, Milan, Italy.*

Evidence is accumulating that TLRs/IL-1R are activated by I/R injury after transplantation (tx), leading to adaptive immune response and influencing graft function and survival. We tested the hypothesis that a negative regulator of TLRs/IL-1R, toll-IL-1R8/SIGIRR (TIR8) modulates inflammation in the kidney graft and prevents the activation of the adaptive immune response.

Studies were done in the mouse model of C57x129 (H-2^b) to DBA/2 (H-2^d) fully mismatched kidney tx. As reported (J Immunol, 2001, 167:4821), most (83%) wt allografts were spontaneously accepted without immunosuppression (median survival >60 days, n=12). By in situ hybridization, TIR8 mRNA level was higher (P<0.05) in cortical tubuli, glomeruli, perivascular and peritubular areas of kidney grafts at 1, 3 and 6-7 d post-tx, than in naive kidneys. To assess how local expression of TIR8 affects the outcome of kidney grafts, we transplanted TIR8^{-/-} B6x129 kidneys into DBA/2 mice. Most (83%) recipients of TIR8^{-/-} kidneys rejected their grafts with a median survival of 9.5 d (n=12, P<0.05 vs wt) and had more severe graft dysfunction (BUN levels) at day 1, 3 and 6-7 days post-tx, than recipients of a wt allograft (P<0.05). Histological analysis at 6-7 days of TIR8^{-/-} grafts revealed typical cell-mediated rejection, with widespread tubulitis and tubular necrosis, whereas no lesions were found in wt grafts.

A prominent and more abundant interstitial neutrophil infiltration was found at 1 and 3 d post-tx in TIR8^{-/-} than in wt grafts (P<0.05). Higher numbers (P<0.05) of CD11c⁺ cells and a higher % of CD11c⁺ cells expressing MHCII were found in TIR8^{-/-} than in wt grafts at 3 and 6 d post-tx. At the same time points recipients of a TIR8^{-/-} graft had increased frequency of anti-donor IFN- γ -producing splenocytes (P<0.05), than naive mice and recipients of wt allografts, whereas anti-third party alloreactivity was comparable in the 3 groups. On the other hand, recipients of a TIR8^{-/-} kidney had lower % of CD4⁺FoxP3⁺ Treg in splenocytes and in the graft than wt kidney recipients (P<0.05).

In summary, TIR8 deletion in the kidney graft exacerbated the post-tx inflammatory response, increased T cell alloreactivity, blocked the formation of Treg and ultimately prevented the development of tolerance.

Abstract# 524

The Absence of Interferon Regulatory Factor-3 (IRF-3) Confers Protection Against the Liver Ischemia and Reperfusion Injury through an IL-10 Independent Pathway. Elizabeth R. Benjamin,¹ Xiu-Da Shen,¹ Feng Gao,¹ Yuan Zhai,¹ Genhong Cheng,¹ Ronald W. Busuttil,¹ Jerzy W. Kupiec-Weglinski.¹ *¹Surgery, Dumont-UCLA Transplant Center, Los Angeles, CA.*

PURPOSE:

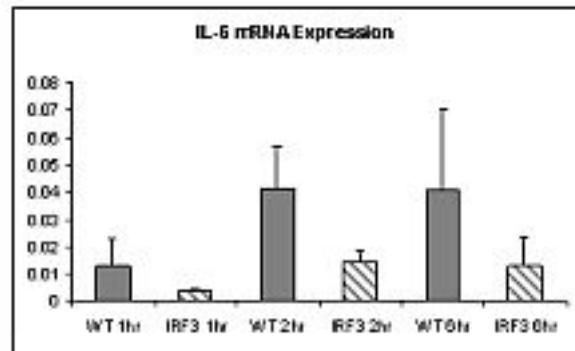
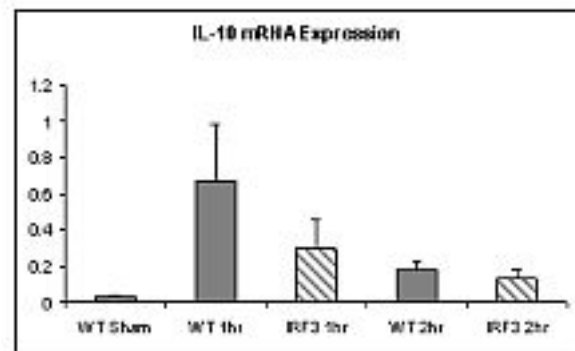
Toll-like receptor 4 (TLR4) mediated liver reperfusion damage after warm ischemia requires signaling through the MyD88-independent, IRF3-dependent pathway with CXCL-10 (IP-10) playing a central role in the injury development. Studies using CXCL-10 KO mice have shown that these mice are protected through an IL-10 dependent mechanism. We chose to investigate IRF3, upstream of CXCL-10, to further characterize its role in the injury progression, and to better understand the involvement of IL-10 in this pathway.

METHODS:

We used IRF3 KO mice and their WT counterparts in a model of partial hepatic warm ischemia with 1, 2, and 6h of tissue reperfusion (n=2 KO, WT at 1 and 6h; n=3 KO, WT at 2h). WT bone marrow derived macrophages were generated and stimulated with LPS to determine the kinetics of IL-10 production. Tissue was analyzed for histology and mRNA levels were measured by qPCR.

RESULTS:

Kinetic studies showed peak IL-10 production at 1 and 2h post-reperfusion (PR). On pathology, IRF3 KO mouse livers were protected from IR injury both early PR, at 1 and 2h, and at 6hrs PR when compared to WT. Consistent with these data, IL-6 mRNA induction was decreased in IRF3 KO, as compared with WT at 1, 2, and 6h. Although IL-10 induction was maintained in the CXCL-10 KO mice, the IRF3 KO mice showed decreased levels of IL-10 at 1h PR. By 2h, IL-10 levels were normalized to WT.



CONCLUSION:

IRF3 KO mice are protected from liver IR injury with evidence of this protection as early as 1h PR. Although CXCL-10 KO mice are protected from IR injury with maintained IL-10 expression, the absence of the upstream molecule, IRF3, confers protection in an IL-10 independent manner. These data suggest a novel mechanism of IR injury mediated by IRF3 in the liver.

Abstract# 525

Adoptive Transfer of Donor Double Negative (DN) T-Reg Cells Suppresses NK Cell-Mediated Bone Marrow Rejection. Zhu-Xu Zhang,^{1,2} Kathy M. He,¹ Yuexia Ma,¹ Xuyan Huang,¹ Anthony M. Jevnikar.^{1,2} *¹Multi Organ Transplantation Program, Lawson Health Research Institute, LHSC., London, ON, Canada; ²Medicine, University of Western Ontario, London, ON, Canada.*

Background: Bone marrow (BM) transplantation may induce donor-specific tolerance to prevent rejection of allogeneic solid organs while maintaining immunity against infections and tumors. Currently allogeneic BM transplantation is limited by donor T cell mediated graft-versus-host disease (GVHD), as well as a variable requirement for recipient marrow ablation and high numbers of donor BM cells. Furthermore, sustained macro-chimerism has not yet been easily or predictably achieved in partially ablated patients or large animals. While rejection of allografts is mediated primarily by recipient T cells, recent studies have demonstrated the capacity of NK cells to reject allogeneic BM and to prevent long-term mixed chimerism. Thus, NK cells represent a barrier to long term BM engraftment even with T cell tolerance. We have previously identified a novel type of regulatory T (Treg) cell with a "double negative" (DN) phenotype (TCRab⁺CD3⁺CD4⁺CD8⁻). DN-Treg cells can effectively suppress anti-donor T and B cell responses and prolong graft survival in allo- and xenotransplantation models. We therefore tested the capacity of DN-Treg to alter NK cell function.

Methods: C57BL/6 BM cells were i.v. injected into sub-lethally-irradiated (6.5 Gy) CB6F1 (H-2b/d) in a "parent to F1" model, or into allo-disparate BALB/c mice. BM cells were co-transplanted with various numbers of C57BL/6 DN-Treg cells or CD4⁺ or CD8⁺ T cells as controls. Recipient spleen cells were collected 7 days after to detect donor progenitors in a colony-forming-unit (CFU) assay. Mice then received cardiac (n=8) or skin transplants (n=8) to confirm tolerance.

Results and Conclusion: We found that donor-derived DN-Treg cells suppress NK cell-mediated allogeneic BM graft rejection in both "parent-to-F1" and fully MHC-mismatched BM transplantation models. Adoptive transfer of DN-Treg cells with donor BM cells promoted the establishment of stable mixed chimerism and donor specific tolerance to BM donor cardiac and skin grafts (MST>160 days), without inducing GVHD in sub-lethally irradiated mice. Perforin deficient DN-Treg cells were unable to efficiently inhibit NK cell function, and donor BM did not engraft. These results demonstrate a potential approach to control innate immune responses and promote allogeneic BM engraftment and donor specific tolerance through the use of DN-Treg cells.

Kidney Immunosuppression: CNI Minimization/Avoidance Protocols

Abstract# 526

Opticcept Trial: Efficacy and Safety of Monitored MMF in Combination with CNI in Renal Transplantation at 12 Months. R. Gaston,¹ B. Kaplan,² H.-U. Meier-Kriesche,³ L. Shaw,⁴ T. Shah,⁵ D. Patel,⁶ R. Bloom.⁴ ¹UAB, Birmingham, AL; ²Univ of Ill, Chicago, IL; ³Univ of FL, Gainesville, FL; ⁴UPenn, Philadelphia, PA; ⁵NIT, Los Angeles, CA; ⁶Roche, Nutley, NJ.

Opticcept is a 2-year open-label, prospective, randomized, multicenter study involving 720 single-organ renal allograft recipients administered either A) concentration-controlled MMF and reduced level CNI (MMF_{CC}/CNI_{RL}) B) concentration-controlled MMF and standard level CNI (MMF_{CC}/CNI_{SL}) or C) fixed-dose MMF (1 g BID) and standard level CNI (MMF_{FD}/CNI_{SL}). Trough-based dose adjustments were made in the MMF_{CC} arms. Antibody induction and/or corticosteroids were administered according to center practice. Primary endpoints were the proportion of patients with treatment failure (biopsy-proven acute rejection [BPARG], graft loss, death), and mean percent change in calculated glomerular filtration rate (GFR; Nankivell equation) at 12 months. Safety endpoints were incidences of adverse events (AEs) and serious AEs. Non-inferiority ($\alpha=0.05$) of A vs. C (MMF_{CC}/CNI_{RL} vs. MMF_{FD}/CNI_{SL}) for treatment failure was tested.

Baseline characteristics did not differ among treatment groups with living donors accounting for approximately 50% of grafts. 82% received tacrolimus (Tac) and 18% cyclosporine (CyA); CNI doses and levels were significantly lower in Group A. MMF doses were greater in CyA-treated subjects in all groups.

	A. (MMF _{CC} /CNI _{RL})	B. (MMF _{CC} /CNI _{SL})	C. (MMF _{FD} /CNI _{SL})
N	243	237	240
Treatment failure	50 (20.6%)*	68 (28.7%)	64 (26.7%)
BPARG*	15 (6.2%)	23 (9.7%)	23 (9.6%)
Graft loss*	5 (2.1%)	4 (1.7%)	4 (1.7%)
Death*	5 (2.1%)	2 (0.8%)	5 (2.1%)
Lost to f/u*	25 (10.3%)	36 (15.2%)	32 (13.3%)

*Events are mutually exclusive because only 1st event counted/patient

Serum creatinine tended to be lower at 12 months in the Tac vs. CyA treated patients and in Group A ($p=0.08$); stability of renal function over time was greatest in Group A. Despite higher MMF doses in Group A ($p<0.001$) at most time points, significantly fewer MMF withdrawals occurred in Group A vs. Groups B and C.

Conclusions: A concentration-controlled MMF and reduced level CNI regimen is not inferior to that of fixed-dose MMF and standard-dose CNI as regards BPARG and other end points. This regimen facilitated higher MMF dosing without an overall increase in adverse effects, and with a trend toward preservation of kidney function versus standard-dose CNI regimens. Longer follow-up may provide additional insights into the impact of these short-term findings.

Abstract# 527

Comparison at One Year of Interstitial Fibrosis (IF) by Automatic Quantification in Renal Transplant Recipients with Cyclosporine (CsA) Discontinuation and Sirolimus (SRL) Introduction. Audé Servais,¹ Vannary Meas-Yedid,² Yvon Lebranchu,³ Isabelle Etienne,⁴ Guy Touchard,⁵ Christophe Legendre,¹ Pierre Francois Westeel,⁶ Olivier Toupance,⁷ Bruno Hurault de Ligny,⁸ Christophe Olivo-Marin,² Eric Therivet.¹ ¹Transplantation, Hôpital Necker, Paris, France; ²Analyse Image Quantitative, Institut Pasteur, Paris, France; ³Nephrologie, CHU, Tours, France; ⁴Nephrologie, CHU, Rouen, France; ⁵Nephrologie, CHU, Tours, France; ⁶Nephrologie, CHU, Amiens, France; ⁷Nephrologie, CHU, Reims, France; ⁸Nephrologie, CHU, Caen, France.

Introduction: We previously reported the clinical results of a multicentric study showing that CsA conversion to SRL at week (W) 12 is associated with a significant improvement in renal function. Using routine renal biopsy (RB) performed at W52 during this study, we investigated the effect of the conversion on interstitial fibrosis (IF).

Methods: This multicenter, prospective, randomized trial included 193 renal recipients randomized at W12 to be switched from CsA to SRL or to continue CsA. Routine RB was performed at W52. For each RB, a section was imaged using a colour video camera and analyzed by a program of colour segmentation which automatically extracts green colour areas characteristic of IF. Results were expressed as percentage of IF and grade according to Banff classification.

Results: Male donor gender was associated with higher IF (30±2% vs. 23±2%, $p=0.03$). IF was numerically higher in patients who had experienced acute rejection (33±4%, $n=18$ vs. 26±1%, $n=99$, $p=0.06$). There was a positive correlation between renal function and the percentage of IF on RB ($p=0.004$). Despite significant improvement of renal function at W52 in the SRL group, we observed no difference of IF. Higher than grade I IF was diagnosed in more than 50 % of patients.

Intent to treat (n=117)	Mean IF (%)	Grade I (%)	Grade II (%)	Grade III (%)
Sirolimus (n=58)	27±15	47.5%	45.8%	6.8%
Cyclosporine (n=59)	27±15	44.8%	48.3%	6.9%

Conclusion: Despite significant improvement in renal function after CsA to SRL conversion at 3 months, we found no difference of IF on RB at W52. The observed

improvement of renal function may be due to a hemodynamic effect. A longer delay may be necessary to observe histological improvement. The higher IF score than the one previously reported by others may be explained by the use of expanded criteria donors.

Abstract# 528

Effects of CNI or MMF Withdrawal on Carotid Intima Media Thickness in Renal Transplant Recipients. J. S. Mourer,¹ E. J. P. de Koning,¹ S. P. Berger,¹ J. Op't Roodt,¹ M. Spaans,¹ A. J. Rabelink,¹ J. W. de Fijter.¹ ¹Nephrology, Leiden University Medical Center, Leiden, Netherlands.

Introduction: carotid intima media thickness (IMT) is a strong predictor of cardiovascular events, the main cause of mortality in renal transplant recipients. Calcineurin inhibitors (CNI) have an unfavourable cardiovascular risk profile. The aim of this study is to assess the impact of concentration-controlled withdrawal of either mycophenolate mofetil (MMF) or CNI on repeated IMT measurements.

Methods: We included 119 stable renal transplant patients on CNI-based immunosuppression, including steroids (10 mg/d) and MMF (2g/d), who were randomized to MMF-withdrawal (group A: CsA-AUC 3000 ng·h/ml) or CNI-withdrawal (group B: AUC-MPA 75 µg·h/ml). Patients were treated for traditional risk factors according to stringent predefined targets. Ambulatory bloodpressure (ABPM), lipids, estimated creatinine clearance (MDRD) and IMT were measured at baseline and after 12 months.

Results: Groups were comparable with respect to demographic characteristics, immunological profile, renal function, systolic and diastolic bloodpressure and lipids. Mean duration of follow-up was 17.4±5.5 months. Only 1 patient (1.3%) in group B and 3 patients (3.8%) in group C experienced acute rejection despite adequate exposure ($p=0.31$). IMT did not change in patients who continued CNI (0.615 ± 0.097 to 0.610 ± 0.100 mm; $p=0.43$), whereas it significantly decreased after withdrawal of CNI (0.638 ± 0.105 to 0.614 ± 0.095 mm; $p=0.01$). No differences in blood pressure (Group B $126\pm10/77\pm7$ vs group C $125\pm12/73\pm7$ mmHg) or cholesterol (Group B 2.6 ± 1.0 mmol/l vs Group C 2.5 ± 0.5 mmol/l) were present between the 2 groups at the end of the study. At one year MDRD clearance was better after CNI withdrawal; 64.5 ± 21.4 vs 56.1 ± 16.3 ml/min ($p=0.04$). There was no correlation between improvement in MDRD and IMT in the CNI withdrawal group ($p=0.35$).

Conclusion: CNI withdrawal improves IMT irrespective of changes in lipids or blood pressures (and MDRD).

Abstract# 529

Calcineurin Inhibitor Effects on Glucose Metabolism and Endothelial Function Following Renal Transplantation. Anders Åsberg,¹ Karsten Midtvedt,² Monica H. Voytovich,² Pål-Dag Line,³ Janicke Narverud,² Anna V. Reisæter,² Lars Mørkrid,⁴ Trond Jenssen,² Anders Hartmann.² ¹School of Pharmacy, University of Oslo, Norway; ²Medical Department, Rikshospitalet Medical Center, Norway; ³Surgical Department, Rikshospitalet Medical Center, Norway; ⁴Department of Clinical Chemistry, Rikshospitalet Medical Center, Norway.

Background: Calcineurin inhibitors (CNI) are involved in the development of post transplant diabetes mellitus (PTDM). Changes in insulin secretion and sensitivity are central mechanisms involved in the development of PTDM. In addition alterations in endothelial function seem to be involved. The present study investigated the effect of CNI's on these factors.

Methods: In a predefined sub-study of a previously published randomized trial it was aimed to compare the effect of CNI treatment ($n=27$) with complete CNI-avoidance ($n=27$) on insulin secretion and sensitivity as well as endothelial function. An oral glucose tolerance test and endothelial function investigation with laser Doppler flowmetry was performed in 44 patients, 10 weeks and 12 months following transplantation.

Results: Insulin sensitivity differed already 10 weeks posttransplant and was significantly better after 12 months in patients never treated with CNI drugs ($P=0.043$). Endothelial function was significantly correlated with insulin sensitivity ($N=27$, $R^2=0.22$, $P=0.013$) at 10 weeks posttransplant, but not after 12 months ($P=0.54$). Insulin secretion tended to be higher in CNI treated patients both at week 10 and month 12 ($P=0.068$).

Conclusions: Findings in the present study indicate that long-term CNI treatment reduce insulin sensitivity which was associated with impaired endothelial function. In response to this peripheral insulin resistance a tendency towards a compensatory increase in insulin secretion was seen. These effects combined may indicate a future risk for premature cardiovascular disease in CNI treated renal transplant recipients, but this hypothesis needs further study.

Abstract# 530

Final Renal Function Outcomes from the Spare-the-Nephron (STN) Trial: Mycophenolate Mofetil (MMF)/Sirolimus (SRL) Maintenance Therapy and CNI Withdrawal in Renal Transplant Recipients. S. Mulgaonkar,¹ T. C. Pearson,² A. Patel,³ J. Scandling,⁴ H. Shidban,⁵ M. Weir,⁶ D. Patel.⁷ ¹St. Barnabas Med Ctr; Livingston, NJ; ²Emory Univ Hospital, Atlanta, GA; ³Henry Ford Hospital, Detroit, MI; ⁴Stanford Univ, Palo Alto, CA; ⁵NIT, Los Angeles, CA; ⁶Univ of Maryland, Baltimore, MD; ⁷Roche, NJ.

Purpose: To compare the effect on renal function of maintenance immunosuppression with MMF and SRL to that of MMF and a CNI in renal allograft recipients.

Methods: In a 2-year open-label, prospective, randomized, controlled, multicenter study, 305 subjects maintained on MMF and a CNI were randomized 30-180 days post-transplantation to either MMF (1-1.5 g BID) plus SRL (2-10 mg followed by ≥ 2 mg/day; trough 5-10 ng/mL) and to discontinue the CNI (MMF/SRL) or to continue their current regimen containing either tacrolimus (Tac) or cyclosporine (CsA; MMF/CNI). Antibody induction and/or corticosteroids were administered according to individual center practice. The primary endpoint was the percent change in measured glomerular filtration rate (GFR; cold iohalamate) at 12 months post-randomization. Additional renal endpoints included calculated GFR (Nankivell equation), serum creatinine (Scr), and calculated creatinine clearance (CrCl; Cockcroft-Gault) at 12 and 24 months.

Results: Outcomes of the first 123 subjects receiving MMF/SRL and 126 receiving MMF/CNI (Tac, n=98; CsA, n=28) completing 1 year of follow-up will be reported here. Final outcomes of all 305 subjects will be presented at the congress. Mean time from transplant to randomization in both groups was 117 days. Groups were similar at baseline for all reported renal function endpoints. Measured GFRs were 60.6±24.7, 60.0±28.8 and 62.1±29.6 at baseline and 66.3±26.1, 60.8±38.4 and 58.7±33.2 mL/min/1.7m² at month 12 for the MMF/SRL and MMF/CNI treatment groups, and the subset receiving MMF/TAC, respectively.

Mean Percent Change (n) in Renal Function From Randomization (Baseline) to Month 12

	MMF/SRL	MMF/CNI	MMF/TAC
Measured GFR	25.8±75.0 (96)	11.3±61.5 (100)	4.7±54.4 (77)
Calculated GFR	7.1±23.0 (97)	1.4±19.8 (104)	-0.4±19.1 (79)
Scr	-1.8±20.9 (99)	6.8±33.0 (105)	9.2±35.1 (80)
Calculated CrCl	5.6±22.4 (99)	-0.5±23.2 (105)	-2.1±23.3 (80)

Conclusions: After 12 months of therapy, maintenance immunosuppression with MMF/SRL after CNI withdrawal appears to preserve renal function when compared with MMF/CNI-containing regimen. Final results will be presented at the congress.

Abstract# 531

Improved Outcomes after De Novo Renal Transplantation: 2-Year Results from the Symphony Study. H. Ekberg,¹ H. Tedesco-Silva,² A. Demirbas,³ S. Vitko,⁴ J. Klempnauer,⁵ A. Gurkan,⁶ R. Margreiter,⁷ C. Hugo,⁸ J. Grinyo,⁹ U. Frei,¹⁰ Y. Vanrenterghem,¹¹ P. Daloze,¹² P. Halloran.¹³ ¹Lund Univ, Sweden; ²Federal Univ Sao Paulo, Brazil; ³Akdeniz Univ, Turkey; ⁴IKEM, Czech Republic; ⁵Medizinische Hochschule Hannover, Germany; ⁶SSK Tepecik Hospital, Turkey; ⁷Universitaetsklinik Innsbruck, Austria; ⁸FAU Erlangen-Nuernberg, Germany; ⁹Ciutat Univ de Bellvitge, Spain; ¹⁰Virchow-Klinikum, Germany; ¹¹KU Leuven, Belgium; ¹²CHUM Montreal, Canada; ¹³Univ Alberta, Canada.

Introduction: Results from the 1-year Symphony core study showed that a regimen with 2g mycophenolate mofetil (MMF)+low-dose tacrolimus (3-7ng/ml)+daclizumab+steroids results in lower acute rejection rates and improved renal function compared with 2g MMF+steroids and either standard-dose cyclosporine (CsA), low-dose CsA (50-100ng/ml)+daclizumab, or low-dose sirolimus (4-8ng/ml)+daclizumab.

Methods: 968 patients were included in an optional 1-year observational follow-up study (61% of the core ITT population). Glomerular filtration rate (GFR) data from 84% of patients were available at 2 years.

Results: At inclusion into follow-up, 47% of patients received a regimen containing standard- or low-dose CsA, 34% tacrolimus and 17% sirolimus, whereas about 25% were included in each group at randomization, indicating that follow-up patients are likely to have been switched to tacrolimus, especially from sirolimus. Most patients were maintained on MMF over the study period: 96% and 85% at years 1 and 2. The incidences of biopsy-proven acute rejection (2-4%) and graft loss (1-3%) were low in year 2 in all groups. At 2 years, the rate of uncensored graft loss was lowest in patients receiving tacrolimus (8% vs 10-13% in other groups; Kaplan-Meier estimates). GFR at the end of the core study was slightly better in the follow-up ITT patients (65-70ml/min) than in the core study ITT patients (57-65ml/min), suggesting inclusion of better-performing patients in the follow-up. Renal function was generally stable over year 2. A slight improvement in GFR in the sirolimus group (+1.6ml/min) was observed, whereas the tacrolimus group still had superior GFR (69 vs 65-67ml/min in other groups).

Conclusions: In follow-up patients, renal function was stable during the second year and GFR differences were less marked than at 1 year. The favorable outcomes of a regimen consisting of MMF+low-dose tacrolimus+daclizumab+steroids on renal function, acute rejection and graft loss were confirmed by this follow-up study.

Abstract# 532

A Prospective Randomized Study of Alemtuzumab vs Rabbit Anti-Thymocyte Globulin Induction in Kidney and Pancreas Transplantation. A. C. Farney,¹ R. Singh,¹ J. Rogers,¹ E. Ashcroft,³ E. Hartmann,² L. Hart,¹ W. Doares,³ M. Gautreaux,¹ S. Iskandar,⁴ P. Adams,² R. Stratta.¹ ¹Surgery; ²Medicine; ³Pharmacy; ⁴Pathology, WFUBMC, Winston-Salem, NC.

Alemtuzumab (Alem) and rabbit anti-thymocyte globulin (rATG) are the most commonly used T-cell depleting induction agents in kidney (K) and pancreas (P) transplantation (tx). **Methods:** We conducted an IRB approved prospective randomized study comparing single dose Alem (30mg) to alternate day rATG induction in Ktx and Ptx patients (pts). Pts received FK and MMF. A risk stratification (PRA>20%, re-tx, African Americans <40 years old) defined high immunologic risk and determined steroid maintenance or elimination. Expanded criteria donors (ECD) were included. Results: Between 2/1/05 and 8/24/07 225 pts enrolled and 222 pts were transplanted. Of 222 pts, 180(81%) had Ktx alone, 38(17%) KPTx, and 4(2%) PAKtx. Of 180 Ktx alone, 152(84%) were deceased donor, and 61(34%) were ECDs. Recipient age, race, re-tx, HLA match, PRA titer, ECDs, CMV status, and immunologic risk were similar for Alem (n=113) and rATG (n=109) groups.

Group(n)	Ktx	Ptx(KPtx)	ECD	Re-tx	PRA(>20%)	Race(AA)	Low Immunologic Risk
Alem(113)	85	28(24)	33	12	34	34	72
rATG(109)	95	14(14)	28	10	32	36	71

Overall pt, Ktx, and Ptx survival are 96, 92, and 86% at 16 months median follow-up. Actuarial survival rates, initial length of stay, delayed graft function, steroid free rates, major infection, and incidence of PTLD (1 rATG pt) were similar for Alem and rATG groups, but treated acute rejection (AR) occurred in 17(15%) Alem pts compared to 29(27%) rATG pts (p=0.03) and biopsy proven rejection (BPAR) in 13(12%) Alem pts compared to 23(21%) rATG pts (p=0.04).

Group	1,2 yr Ptx Survival	1,2 yr Ktx Survival	1,2 yr Ptx Survival	Treated AR	BPAR	CAN
Alem	96, 94%	93, 91%	80, 80%	17(15%)	13(12%)	19(17%)
rATG	99, 98%	95, 91%	90, 90%	29(27%)	24(22%)	27(25%)

Only 1 Alem BPAR has occurred after 12 months. Total daily MMF doses were similar for Alem and rATG groups at 3 months (1567±457mg vs 1670±508mg). Neupogen use was greater in the Alem group, 26(23%), than in the rATG group (14(13%), p=0.03). Excluding PAK, chronic allograft nephropathy (CAN) was observed in 19(17%) Alem pts and 27(25%) rATG pts. (p=0.14). Conclusions: Alem and rATG induction both provide excellent 1 and 2 yr pt, Ktx, and Ptx survival. Alem is associated with lower acute rejection rates and perhaps less CAN, but requires increased Neupogen administration to help maintain MMF dosing.

Abstract# 533

Thymoglobulin Dosing Intensity and Density: Effects on Induction Efficacy Ruth-Ann M. Lee,¹ Adele H. Rike,¹ Rita R. Alloway,¹ Jason Everly,¹ Amit Govil,¹ Stefanie Young,¹ Michael Cardi,² E. Steve Woodlee.¹ ¹Surgery, U of Cincinnati; ²Christ Hospital.

Thymoglobulin (TMG) induction reduces acute rejection (AR) risk in kidney transplantation (KTx). However, inadequate data exists regarding dosing strategies (fixed versus CD3 based), intensity, and density of dosing on TMG efficacy. The purpose of this study was to evaluate TMG dosing intensity and density on AR.

Methods: Data was collected prospectively in 426 pts who received TMG induction. Variables with a p<0.25 entered the multivariate (MVA) stepwise logistic regression model. TMG dose was 1.5 mg/kg; TMG regimens were: fixed dosing (days 0,2,4,6) CD3 based (dose given when CD3+ cells > 25/uL).

Results: Data is presented as (CD3 dosing/fixed dosing). Total # pts 223 / 203, mean follow-up (days) 1431±761/963±702 (p<0.0001). AR rate was higher with CD3 based dosing (16% / 9%, p<0.005). To determine whether this was due to an independent effect of CD3 based dosing, rigorous MVA was performed. Univariate analysis (UVA) of AR risk factors (gender, AA recipient race, PRA, DGF, DR mismatches, diabetes, repeat transplant) and TMG dosing data (# doses, total dose (mg/kg), # days CD3 suppression, dosing interval, dosing regimen type (fixed v CD3-based)) was performed. Significant UVA factors that entered the MVA model included: AA race, repeat txp, current PRA>25%, CD3 based dosing, total TMG dose (<4, 4-5, 5-6, and > 6 mg/kg) (Table). In the final MVA model, the only significant risk factor for AR was the CD3-based dosing regimen. This observation was further confirmed by MVA analysis that controlled for DGF by excluding DGF pts. Mean total TMG dose effect was controlled by grouping pts according to total dose magnitude in MVA.

UVA for AR	Odds Ratio	95% Confidence Interval
African American	1.8	0.93 - 3.6
Current PRA>25%	2.8	0.87 - 8.82
Fixed dosing regimen	0.5	0.24 - 0.92
CD3 -based dosing regimen	2.1	1.08 - 4.06
MVA Final Model		
CD3-based dosing	3.38	1.11 - 10.2

Conclusion: CD3-based TMG dosing is an independent RF for AR in KTx pts receiving TMG induction. Rigorous examination by multivariate analysis adds to the robustness of this observation. This may be an effect of TMG dosing density as CD3-based dosing

increased the interval between TMG doses compared to the fixed dose group. This observation argues for adoption of fixed dose TMG regimens for induction.

Abstract# 534

Patterns of Acute Rejection in Kidney Transplant Recipients Treated with Alemtuzumab Induction and Steroid-Free Maintenance Therapy.

Adam W. Bingham,¹ Juan Palma-Vargas,¹ Jorge Ortiz,¹ Preston Foster,¹ Francis Wright.¹ ¹Texas Transplant Institute, San Antonio, TX.

Background: Alemtuzumab (Campath 1H) has been used as induction therapy for kidney transplant recipients with acute rejection rates reported by us and others of 7 to 20% at one year. The histologic type of rejection and the time frame for occurrence after treatment with alemtuzumab have not been well established. This study is a retrospective single center review of acute rejection episodes of kidney transplant recipients treated with alemtuzumab induction with respect to the kinetics and histologic patterns of acute allograft rejection.

Methods: From 11/01/03 to 10/31/06, 416 kidney transplants were done meeting the inclusion criteria for this review. All patients had negative T and B cell flow cytometric crossmatches and received induction therapy with alemtuzumab 30mg IV intra-operatively, methylprednisolone 500 -750 mg during the first 24 hours, and were then maintained on tacrolimus (target level 5-7) and mycophenolate mofetil without steroids. All episodes of biopsy-proven acute rejection (AR) were reviewed. Patients in pre-transplant desensitization protocols or with documented non-adherence with medications were excluded from the analysis.

Results: A total of 44 of 416 patients (10.6%) experienced AR during the study period, with a mean follow up of 28 months (range 14- 48 months). Of the AR episodes, 19 (43%) occurred within the first 3 months post-transplant, 13 (30%) occurred between 3-6 months, 5 (11%) occurred between 6-9 months, 1 (2%) occurred between 9-12 months, and 6 (14%) occurred more than one year post-transplant. Of the rejection episodes within the first 3 months post-transplant, 9/19 occurred within the first 30 days. Histologic analysis showed that 9/19 rejection episodes (47%) within the first 3 months included an antibody mediated component (6/9 within the first 30 days.) In contrast, 3/25 rejection episodes (12%) which occurred greater than 3 months post-transplant were antibody mediated. Of the 12 patients with antibody mediated rejection, only 3 patients had panel reactive antibody (PRA) levels > 25% at the time of transplant.

Conclusion: This large experience with alemtuzumab induction therapy with a steroid-free maintenance protocol, demonstrates that the majority of rejection episodes occur within the first 6 months post-transplant, with the largest fraction in the first 3 months. A significant number of early rejection episodes are antibody mediated and occur in unsensitized recipients.

Abstract# 535

20 Year Follow-Up Results of a Randomized Controlled Trial Comparing Anti-Lymphocyte Globulin-Induction to No Induction in Renal Transplant Patients.

M. Cantarovich, A. Durrbach, M. Ladouceur, Ch. Hiesse, G. Benoit, B. Charpentier. *McGill University Health Center, Montréal, QC, Canada; Hôpital de Bicêtre, Université Paris-Sud, Le Kremlin Bicêtre, France; Hôpital de Bicêtre, Université Paris-Sud, Le Kremlin Bicêtre, France; Hôpital de Bicêtre, Université Paris-Sud, Le Kremlin Bicêtre, France; Hôpital de Bicêtre, Université Paris-Sud, Le Kremlin Bicêtre, France.*

Background: There is a need to report on long-term follow-up results of randomized controlled trials in renal transplantation (Tx). **Purpose:** To determine the impact of ALG-induction on long-term outcomes post-renal Tx. **Methods:** Between 01/85 and 01/86, 123 consecutive adult pts received a deceased donor renal Tx at a single institution. Pts were randomized into 2 groups (Gr.). Gr. 1: 63 pts (40±10 yr-old) received cyclosporine A (CsA), prednisone and azathioprine (1.5 mg/kg/d); Gr.2: 60 pts (36±9 yr-old) received ALG (3 vials (100 mg/vial)/day for 14 days), CsA, prednisone and delayed initiation (45 days post-Tx) and low-dose azathioprine (1 mg/kg/d). In both Gr., CsA was started prior to surgery at a dose of 4 mg/kg/iv. Thereafter, CsA was given orally and the dose was adjusted according to blood trough levels (150-250 ng/ml). Pts did not receive CMV prophylaxis. Cold ischemia time did not differ (Gr.1: 38±8 hr, Gr.2: 39±9 hr). There was no difference in HLA (A-B-DR) matching (Gr.1: 2.4±1.1, Gr.2: 2.6±1.2). **Results:** The incidence of acute rejection was lower in Gr.2 (28% vs. 75%, $P<0.0001$). The incidence of CMV infection was 10% in Gr.1 and 18% in Gr.2 ($P=NS$). The overall incidence of cancer was 22.2% in Gr.1 and 11.7% in Gr.2 ($P=NS$). The incidence of lymphoma was 3% in Gr.1 and 5% in Gr.2 ($P=NS$).

Short and long-term outcomes

	Patient survival*		Graft survival*		Serum creatinine (μmol/L)*	
	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2
1 year	100%	100%	79%	93%	155±45	153±73
5 years	91%	100%	67%	75%	144±44	150±51
10 years	83%	88%	56%	51%	190±163	140±34
15 years	71%	72%	43%	47%	146±46	151±56
20 years	64%	54%	38%	31%	128±47	144±47

* $P=NS$

Conclusion: The use of ALG-induction resulted in a lower incidence of acute rejection and improved graft survival during the first year post-renal Tx. Patient and graft survival at 20 year follow-up was not affected by ALG-induction. It remains to be

determined whether long-term graft survival may improve if current maintenance immunosuppressive strategies are used.

Abstract# 536

Thymoglobulin Has Its Greatest Efficacy in Recipients of Standard Criteria Donors and Donors without Hypertension.

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In a randomized, international, multicenter study, comparing the use of Thymoglobulin (TMG) and basiliximab (BAS) in recipients at high risk for delayed graft function (DGF) or rejection, TMG was associated with less acute rejection (15.6% vs 25.5%, $P=0.02$) and a lower triple endpoint (rejection, death or graft loss, 20.6% TMG vs 33.6% BAS, $P=0.02$) but not a significantly lower quadruple endpoint including DGF. The purpose of this study was to compare the efficacy of TMG and BAS for induction stratified by donor source: standard criteria donor (SCD), extended criteria donor (ECD) or donor with hypertension (HTN). **Methods:** Retrospective review of data collected in the original randomized trial. Data-capture limitations necessitated defining ECD as donor age > 60 or donor age between 50 and 60 with both a donor history of HTN and donor renal insufficiency (history of ATN or creatinine above 2.5 mg/dl during the 24 hours prior to organ recovery/start of cold ischemia time). **Results:** 75 recipients received ECD kidneys [TMG n=40 (28.4%), BAS n=35 (25.6%), $P=NS$]. Outcomes are presented below.

Table 1. Efficacy of TMG vs BAS Stratified by Donor Characteristics

Donor Characteristic	TMG n=40	BAS n=35	P	Odds Ratio
ECD				
Acute Rejection	10 (25.0%)	10 (28.6%)	0.73	0.87
Triple Endpoint	12 (30.0%)	12 (34.3%)	0.69	0.87
SCD				
Acute Rejection	12 (11.9%)	25 (24.5%)	0.02	0.48
Triple Endpoint	17 (16.8%)	34 (33.3%)	0.007	0.50
All non-HTN donors				
Acute Rejection	15 (14.0%)	27 (25.2%)	0.04	0.56
Triple Endpoint	19 (17.8%)	36 (33.6%)	0.008	0.53
SCD non-HTN donors				
Acute Rejection	9 (11.0%)	19 (22.9%)	0.04	0.47
Triple Endpoint	12 (14.6%)	28 (33.7%)	0.004	0.43
Death	1 (1.2%)	6 (7.2%)	0.047	0.17

There were no differences in the rates of DGF between the groups examined. **Conclusion:** Standard and non-HTN donor recipients had a tremendous benefit of TMG compared to BAS with less acute rejection and death. Contrary to the perceived niche of TMG in ECD recipients, TMG has its most beneficial effect in SCD recipients and recipients of donors without HTN at risk for acute rejection or DGF.

Abstract# 537

Evaluation of Immunosuppressive Regimens for ABO-Incompatible Living Kidney Transplantation. Splenectomy or Rituximab? In Combination with FK/MMF.

Hideki Ishida,¹ Tomokazu Shimizu, Hiroki Shirakawa, Kiyoshi Setoguchi, Daisuke Toki, Shouichi Iida, Kazuya Omoto. ¹Urology, Tokyo Women's Medical University, Tokyo, Japan.

We have changed our immunosuppressive protocol in ABO-incompatible kidney transplantations and attempted to determine whether the changes in agents have resulted in better outcomes.

We used tacrolimus(FK), mycophenolate mofetil(MMF) and methylprednisolone(MP) in immunologically high risk patients between 2000 and 2007. Moreover, we performed splenectomy at the time of the transplant surgery in 117 patients (group 1) with ABO-incompatibilities between 2000 and 2004, and administered rituximab as an alternative to splenectomy in 38 patients(group 2) with ABO-incompatibilities between 2005 and 2007. In this study, we compared the graft survival rates as well as the incidence of acute rejection in these two treatment eras.

The graft survival rate at one year was 94% in group 1 and 97% in group 2 ($P=NS$). The graft was lost in one case of the 38 cases of group 2 due to insufficient doses of the immunosuppressive drugs. The incidence rate of acute rejection was 15% (18/117) in group 1, and 11% (4/38) in group 2 ($P<0.01$). There were no significant differences in serum creatinine level one year after transplantation between two groups (1.5±0.3 in group 1 vs. 1.4±0.4mg/dl in group 2). No serious adverse events associated with rituximab or splenectomy were encountered in either groups.

In ABO-incompatible kidney transplantation, rituximab under FK/MMF combination as an alternative to splenectomy seems to yield an excellent result in terms of the incidence rate of acute rejection.

Abstract# 538

Single-Dose Induction with Rabbit Anti-Thymocyte Globulin (rATG) Safely Improves Renal Allograft Function and Reduces Chronic Allograft Nephropathy. R. Brian Stevens,¹ David Mercer,¹ Theodore Rigley,¹ Kathy Nielsen,¹ Megan Henning,¹ Anna Skorupa,¹ John Sandoz,¹ Anna Kellogg,¹ Vinaya Rao,¹ Clifford Miles,¹ Gerald Groggel,¹ Lucile Wrenshall.¹ ¹*Divisions of Transplantation and Nephrology, University of Nebraska Medical Center, Omaha, NE.*

We conducted a prospective, randomized trial in renal transplant recipients comparing two dosing protocols [single dose (6mg/kg) vs. divided doses (1.5mg/kg for 4 doses)] of rabbit anti-thymocyte globulin (rATG; Thymoglobulin®). We present herein the results of the first 142 patients [single dose (n=70), divided dose (n=72)]. After a mean follow-up of 16.2 ± 10.9 months, there were no significant differences between groups in adverse events related to rATG infusion, patient and graft survival, and biopsy-proven acute rejection. Estimated glomerular filtration rate (eGFR) on day 4 was significantly higher in the single-dose group than in the divided-dose group (50.0 ± 24.8 vs. 41.2 ± 25.7 ml/min/1.73, p = 0.05). Throughout the first 6 months post-transplantation, recipients of kidneys from non-marginal deceased donors derived the greatest benefit in renal function (eGFR) from the single-dose regimen (p = 0.006). The incidence of chronic allograft nephropathy (CAN) was also lower in the single-dose group, in both clinically-indicated and protocol biopsies combined (p = 0.045) and in 12-month protocol biopsies alone (p = 0.05). This study demonstrates that administration of 6mg/kg rATG as a single 24-hour infusion is safe and is associated with better renal function than conventional administration in divided doses.

Demographics	Single-dose rATG (n = 70)	Divided-dose rATG (n = 72)
Recipient Age	45.5 ± 12.4	49.3 ± 10.5
Males	66%	63%
Donors (LD/DD)	57%/43%	57%/43%
DD Cold Ischemic Time	15.1 ± 6.1	13.7 ± 7.1
HLA MM	3.4 ± 1.9	3.8 ± 1.8
High Risk (race, PRA)	8 (11%)	11 (15%)

Fig. 1 Renal Function after Transplantation for Non-marginal Deceased Donors

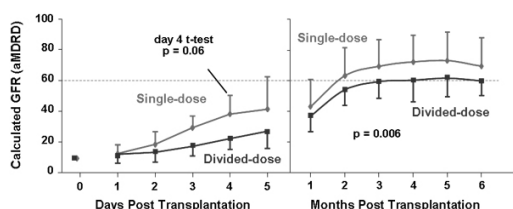
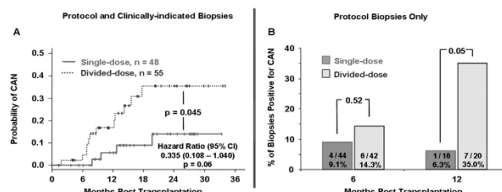


Fig. 2 Chronic Allograft Nephropathy after ATG Induction

**Abstract# 539**

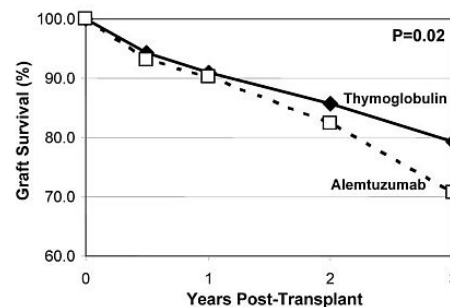
Induction in the Elderly Transplant Recipient: An Analysis of the OPTN/UNOS Database. Suphamai Bunnapradist,¹ Steven Takemoto,² Jagbir Gill,¹ Tariq Shah.² ¹*Medicine-Nephrology, UCLA, LA, CA;* ²*Medicine-Nephrology, National Institute of Transplantation, LA, CA.*

Introduction: The choice of immunosuppression in the elderly kidney transplant recipient remains unclear. The objective of this study was to compare outcomes with different T cell-depleting induction agents in the elderly.

Method: All solitary kidney transplant recipients over the age of 60 years that received induction therapy with either alemtuzumab or Thymoglobulin from 2002 to 2007 were included in this UNOS analysis. Overall graft survival, the risk of graft loss, and the risk of rejection were compared using Kaplan Meier, Cox proportional hazards, and logistic regression, respectively.

Results: Patients receiving alemtuzumab had a significantly lower 3-year graft survival (70.8%) than patients receiving Thymoglobulin (79.3%), p=0.02 (Figure). After adjusting for other risk factors, alemtuzumab had a higher risk of graft loss compared to patients given Thymoglobulin [HR 1.35 (1.09-1.68), p=0.007]. Compared to Thymoglobulin, the risk of acute rejection at hospital discharge was lower for alemtuzumab [OR 0.22 (0.10-0.50), p<0.001]; however, the risk of acute rejection was significantly higher with alemtuzumab at 6 months [OR 1.46 (1.05-2.02), p=0.023] and at 12 months post transplant [OR 1.86 (1.03-3.38), p=0.041].

Discussion: We found that elderly transplant recipients who received induction with alemtuzumab had a greater risk of late acute rejection and graft loss compared to those treated with Thymoglobulin.



Disclosure - Grant/Research Support: Genzyme

Abstract# 540

ATG Versus Basiliximab Induction Therapy in Kidney Allograft Recipients Receiving a Dual Immunosuppressive Regimen: Six-Year Results. Frank Ulrich,¹ Sebastian Niedzwiecki,¹ Andreas Pascher,¹ Peter Fellmer,¹ Sascha Weiss,¹ Sven Kohler,¹ Stefan G. Tullius,² Petra Reinke,³ Peter Neuhaus,¹ Johann Pratschke.¹ ¹*Dept. of General, Visceral and Transplantation Surgery, Charité, Universitätsmedizin Berlin, Berlin, Germany;* ²*Division of Transplant Surgery, Brigham and Women's Hospital, Boston, MA;* ³*Dept. of Nephrology and Medical Intensive Care, Charité, Universitätsmedizin Berlin, Berlin, Germany.*

Purpose: The aim of this prospective randomized study was the comparison of efficacy and incidence of adverse events in two induction therapy regimens (ATG versus basiliximab) in patients receiving a dual immunosuppression.

Methods: 120 recipients of first or second deceased donor kidney transplants were prospectively randomized to receive either ATG (Fresenius) or basiliximab (Novartis) as induction therapy. Dual immunosuppression consisted of tacrolimus (Astellas) and methylprednisolone. CMV prophylaxis was not applied on a regular basis. Statistical analysis was performed with Fisher's exact or chi-squared test, ANOVA or Mann-Whitney U test, Kaplan Meier curves and log-rank test.

Results: Patient characteristics of populations treated with ATG versus basiliximab were similar concerning average age (48 years), gender and dialysis time prior to transplantation (78 vs. 88 months). Average donor age and cold ischemia were also comparable (43 vs. 41 years and 833 vs. 852 minutes). The actuarial 5-year patient survival for the ATG subpopulation is 91.7% in comparison to 85% in the basiliximab group (n.s.). Analyzing graft survival after 5 years, rates of 88.3% in ATG patients compared to 75% in the basiliximab group can be observed (n.s.). The incidence of acute rejection episodes was similar in both groups (ATG: n=20 vs. basiliximab: n=16). 18 (30%) patients in the ATG group and 20 (33.3%) in the basiliximab group showed a delayed graft function. Serum creatinine was not significantly different at 1 and 5 years (ATG: 1.4±0.6 mg/dl and 1.4±0.8 mg/dl vs. basiliximab: 1.3±0.7 mg/dl and 1.5±0.9 mg/dl). Patients in the ATG group had a higher rate of CMV infections (n=13 vs. n=3; p=0.05), whereas patients treated with basiliximab had significantly more hematological complications like anaemia, leukopenia and thrombocytopenia.

Conclusion: Comparing induction therapy with ATG and basiliximab, our data shows similar patient and graft survival rates with slightly better results in the ATG group. Patients treated with ATG had a higher rate of CMV infections but less hematological complications.

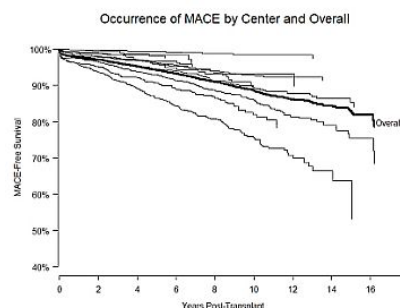
Kidney: Complications VI

Abstract# 541

Cardiac Events Post Kidney Transplantation: Interim Results from the Patient Outcomes in Renal Transplantation (PORT) International Transplant Study. Bertram L. Kasiske,^{1,2} Ajay K. Israni,^{1,2} Jon J. Snyder,¹ Melissa A. Skeans,¹ Eric D. Weinhandl,¹ Yi Peng.¹ ¹*Chronic Disease Research Group, Minneapolis Medical Research Foundation, Minneapolis, MN;* ²*Medicine, Hennepin County Medical Center, Minneapolis, MN.*

Predicting cardiovascular events (CVD) and the varied effects of immunosuppressive medication on CVD risk factors requires an understanding of how traditional and non-traditional risk factors impact CVD after kidney transplantation (KTx). Single-center studies have generally lacked statistical power and generalizability. Registry studies have lacked sufficient data on CVD risk factors. The PORT project is creating a multicenter international database of KTx recipients with the primary objective of developing risk prediction models for post-transplant CVD. As a preliminary data assessment, an analysis was done on 19,669 KTx from 1990-2007 from 11 transplant centers representing 5 European centers, 4 North American centers, and 2 centers from Asia/Oceania. All data were extracted from preexisting databases at each individual transplant center and processed into the consolidated PORT database. 1,131 major adverse cardiac events (MACE) were identified, defined as non-fatal or fatal myocardial infarction, cardiac

arrest, and sudden death. The MACE-free survival curves by participating center are shown in the figure.



In this preliminary analysis, the overall one-year cumulative incidence of MACE was 2.2%, ranging from 0.2% to 4.4% across centers; and the five-year cumulative incidence was 5.8%, ranging from 0.6% to 13.5%. The prevalence of diabetes pre-transplant varied from 10% to 47% across centers. In Cox proportional hazards models adjusted for age, gender, race, donor type, transplant center, and reported history of diabetes, hypertension (HTN), and AMI, patients with a reported history of AMI had a 112% increased risk (79%-152%, $p < 0.0001$) for MACE. For the final analysis, the definition of MACE will be expanded to include major revascularization events. The final PORT database will be used to develop and validate an equation to predict MACE and other health outcomes of interest, after accounting for differential CVD risk factors internationally.

Abstract# 542

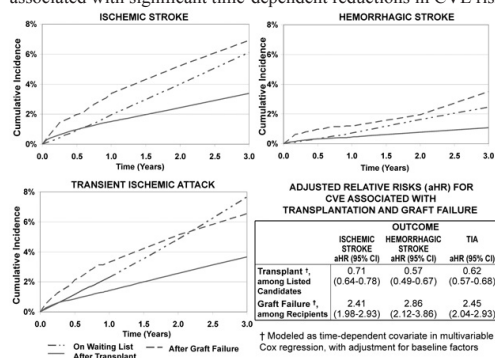
Maintenance of Allograft Function Reduces the Risk of Cerebrovascular Disease Events after Kidney Transplantation. Krista L. Lentine,¹ Lisa R. Rocca Rey,^{1,2} Mark A. Schnitzler,¹ Kevin C. Abbott,³ Daniel C. Brennan.⁴ ¹Saint Louis University Center for Outcomes Research; ²University of Milan; ³Walter Reed Army Medical Center; ⁴Washington University.

We examined the incidence and mortality implications of cerebrovascular events (CVE) after kidney transplant. We also compared variations in risk on the transplant waitlist and after allograft failure.

Methods: We used registry data from the US Renal Data System to retrospectively investigate ischemic stroke (IS), hemorrhagic stroke (HS) and transient ischemic attacks (TIA) among 29,614 adults who received kidney transplants in 1995-2002 with Medicare as primary payer. Patients with prior indications of CVE in the registry were excluded. We ascertained events from billing claims, and estimated incidence of first events by the product-limit method. At-risk time was censored at: loss of Medicare, 3yr transplant anniversary, non-CVE death or end of study (12/31/2002).

Cox regression was used to identify independent correlates of CVE, and to examine CVE events as time-dependent mortality predictors. We estimated CVE incidence after graft failure among patients without CVE diagnoses prior to graft loss ($n=2,599$), and among 51,501 Medicare-insured transplant candidates on the waitlist. Further, we modeled transplantation as a time-dependent predictor of CVE risk on the waitlist.

Results: Observed incidences of individual CVE types by kidney transplant status are shown below (Figure). In multivariate regression, allograft failure strongly predicted increased risk of subsequent CVE. Among listed candidates, receipt of a transplant was associated with significant time-dependent reductions in CVE risk (Table).



Adjusted for baseline factors, CVE after transplant predicted increased risk of subsequent mortality: HR 5.15 (CI 4.53-5.85) after IS, 10.56 (CI 8.89-12.53) after HS, 2.36 (CI 2.01-2.78) after TIA, and 4.67 (CI 4.22-5.18) for any CVE.

Conclusions: Cerebrovascular disease risk should be considered in the post-transplant monitoring of kidney transplant recipients. Along with known benefits for diverse complications of ESRD, maintenance of allograft function may reduce the risk of vascular disease in the cerebral circulation.

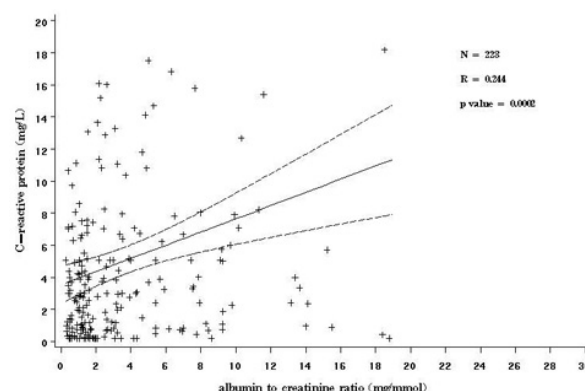
Abstract# 543

Microalbuminuria Post-Renal Transplantation Is Related to Inflammation and Cardiovascular Risk. G. V. Ramesh Prasad,^{1,2} Farah Bandukwala,¹ Michael Huang,² Jeffrey S. Zaltzman.^{1,2} ¹Division of Nephrology, Department of Medicine, University of Toronto; ²Renal Transplant Program, St. Michael's Hospital, Toronto, ON, Canada.

Background: Microalbuminuria predicts progressive renal damage and cardiovascular disease in the general population, while in renal transplant recipients (RTR) predicts graft loss and all-cause mortality. In the former it clusters with both traditional cardiovascular risk factors and elevated C-reactive protein (CRP). Our objective was to define the relationship between microalbuminuria and these risk factors in stable RTR.

Methods: Over one year, we identified 223 stable RTR who were at least 2 months post-transplant and provided 3 successive urine albumin-to-creatinine ratio (ACR) measurements, excluding those with recent illness and overt proteinuria. Microalbuminuria was defined as averaged ACR ≥ 2.0 in men and 2.8 in women (CDA 2003). Framingham-based traditional as well as novel cardiovascular risk factors associated with microalbuminuria were determined by univariate ($p < 0.20$), followed by stepwise backwards elimination ($p > 0.05$) multivariate logistic regression analysis.

Results: Overall prevalence of microalbuminuria was 48% (108/223). Averaged ACR correlated to CRP ($R=0.24$, $p=0.0002$, Fig 1).



Patients with microalbuminuria had higher CRP (7.01 ± 8 v 3.21 ± 3 mg/L, $p < 0.0001$) and systolic BP (129 ± 17 v 123 ± 12 mmHg, $p=0.004$). By multivariate logistic regression analysis, microalbuminuria was associated with increasing CRP (Odds Ratio 1.129 per 1 mg/L [95% CI 1.058-1.204], $p=0.0002$), time post-transplant (1.005 per year [1.001-1.009], $p=0.017$), SBP (1.248 per 10mmHg [1.023-1.522], $p=0.029$) and smoking (1.938 [1.023-3.672], $p=0.042$). Microalbuminuria did not correlate with prior acute rejection, delayed graft function, or any specific antihypertensive or immunosuppressive agents.

Conclusions: Post-transplant microalbuminuria is highly prevalent and is associated with elevated CRP, elevated BP, and smoking. Its relationship to these other factors suggests that it reflects an inflammatory state in otherwise stable patients and thus may indicate graft and patient health, making microalbuminuria a potential target for intervention.

Abstract# 544

Patient Survival during the First Two Years after Kidney Transplantation: Relationship with Cardiac Troponin T (cTnT). LaTonya J. Hickson, Fernando G. Cosio, Ziad M. El-Zoghby, Allan S. Jaffe, Matthew D. Griffin, Mark D. Stegall. William J. von Liebig Transplant Center, Mayo Clinic, Rochester, MN.

The first year after kidney transplantation (Tx) is associated with increased mortality relative to dialysis. Early post-Tx deaths are often cardiovascular (CV) and frequently occur after the first week post-Tx. cTnT is a sensitive and specific marker of myocardial injury. In this study we investigated whether cTnT relates to early post-Tx survival.

Methods: 396 patients received kidney Tx from 9/2004 to 12/2006, 74% from living donors. cTnT was measured during the pre-Tx workup and periodically while on the Tx waiting list. 261 patients (64%) had a dobutamine stress echo (DSE) and 103 (26%) had a coronary angiogram. The combined end point of the study was death or major cardiac events. Survival was censored for graft loss.

Results: Mean age was 51 ± 12 , 59% males. Pre-Tx cTnT level was elevated (>0.01 ng/ml) in 56% of patients, including 0.01-0.03 in 28%; 0.04-0.1 in 16%; and >0.1 ng/ml in 12%. During 21 ± 10 months of follow up (range: 0-46) 20 patients died (5%) and 27 (6.8%) reached the study endpoint. Elevated cTnT was associated with reduced survival [HR=2.25, CI (1.6-3.1), $p < 0.0001$]. Thus, 2 year cumulative survival was 98%, 95%, 84% and 77% in patients with pre-Tx cTnT levels: <0.01 (normal), 0.01-0.03, 0.04-0.1 or >0.1 ng/ml, respectively. In multivariate analyses, higher cTnT related to reduced survival independent of the following pre-Tx variables: longer time on dialysis [1.48 (1.28-2.11), $p=0.035$], low serum albumin [0.336 (0.129-0.876), $p=0.026$] and lower LV ejection fraction (EF) [0.96, (0.93-0.99), $p=0.013$]. Other DSE derived parameters did not relate significantly to survival. cTnT further stratified the risk associated with

other variables. Thus, among patients with EF<55%, 2 year survival was 97%, 88% and 60% (p=0.0003) in patients with cTnT <0.01, 0.01-0.03 and >0.03, respectively. Similarly, these cTnT ranges stratified risk in patients with low albumin (p=0.02) and in those who received pre-Tx dialysis (p=0.03).

Conclusion: An elevated pre-Tx cTnT is a strong and independent predictor of reduced early post-Tx survival. cTnT allows stratification of risk in patients who have other risk factors such as low EF, low serum albumin and dialysis>2 years. In all patients, independent of any other variables, a normal cTnT was an excellent predictor (97%) of survival. These results suggest that cTnT should be included in the evaluation of kidney transplant candidates.

Abstract# 545

Validation of Framingham Risk Assessment by Actual Cardiovascular Event Data in Renal Transplant Recipients. Adele H. Rike,¹ Jason Everly,¹ Rita R. Alloway,² Michael Cardi,³ Gautham Mogilishetty,² Shazad Safdar,³ Janet Durbin,² E. Steve Woodlee.¹ ¹Surgery, Univ. of Cincinnati; ²Nephrology, Univ. of Cincinnati; ³Christ Hospital.

Framingham risk score (FRS) predicts cardiovascular (CV) risk in the general population, but may underestimate CV risk in kidney transplant (txp) patients (pts). FRS has not previously been validated by prospective cardiovascular event (CVE) data collection in kidney txp pts. The purpose of this study was to validate FRS with actual observed CVE data in kidney txp pts.

Methods: CVE data was collected at routine intervals in our kidney txp pts and entered in a cardiovascular risk database. FRS was calculated from baseline to 7 yrs posttransplant (PTx). Individual FRS factors of age, sex, smoking, diabetes mellitus (DM), high-density lipoprotein (HDL), total cholesterol (TC), and blood pressure (BP) were evaluated for their ability to predict acutal CVE occurring after kidney txp. Pts with coronary artery disease (CAD) were excluded from the FRS analysis. CVE were defined as sudden death, myocardial infarction, angina, and cerebrovascular accident/transient ischemic attack. FRS factors were evaluated by cox proportional hazards in univariate (UVA) and multivariate (MVA) models.

Results: 693 renal txp pts were evaluated from 1997-2007. Demographics and FRS data are presented in Table 1. Mean follow-up was 1420 ± 958 days. FRS decreased significantly from preTx to 7 yrs PTx (p<0.05). FRS was significantly correlated with CVE (p< 0.01) in the UVA model. MVA results for individual FRS factors are in Table 2.

	n=693
Male	56%
African American	23%
Mean Age	47.9 ± 12.7
PreTx CAD	12.3%
PreTx FRS (% 10 Yr Risk)	8.1 ± 7.3
FRS 1 Yr (% 10 Yr Risk)	7.8 ± 7.2
FRS 2 Yr (% 10 Yr Risk)	8.2 ± 6.8
FRS 3 Yr (% 10 Yr Risk)	7.1 ± 6.1
FRS 5 Yr (% 10 Yr Risk)	7.6 ± 6.2
FRS 7 Yr (% 10 Yr Risk)	7.2 ± 5.8

MVA of CVE	Odds Ratio	95% Confidence Interval	p
SBP>160mmHg	4	1.03 - 15.4	0.04
DM	3.5	2.1 - 5.7	0.0001
Smoking	2.4	1.4 - 4.06	0.002
TC>240mg/dL	2.3	1.06 - 5.07	0.04
HDL>40mg/dL	0.6	0.34 - 0.93	0.03

Conclusions: Individual component risk factors of the FRS were each demonstrated to be statistically significant predictors of CVE in kidney txp pts. This study presents the first correlation of FRS with actual CVE data collected prospectively in kidney txp pts.

Abstract# 546

Inhibition of Rho-Kinase by Fasudil Effectively Lowers Peripheral and Central Blood Pressure in Hypertensive Renal Transplant Recipients. Eckhart Büsselmaier,¹ Catrin Palm,² Kay Herbrig,² Jens Passauer.² ¹Medizinische Klinik und Poliklinik D, Universitätsklinikum Münster, Münster, Germany; ²Medizinische Klinik III, Universitätsklinikum Carl Gustav Carus, Dresden, Germany.

Rho kinase (ROK) modulates calcium sensitivity of vascular smooth muscle cells and contributes to the regulation of peripheral vascular tone in man. In essential hypertension increased ROK-activity contributes to the generation of vascular resistance. Arterial hypertension is a common complication in renal transplant recipients. In this study we were interested in the role of ROK for systemic hemodynamics in hypertensive renal transplant recipients (TX).

We tested the specific inhibitor of ROK fasudil. 10 TX and 10 matched control subjects (C) received either fasudil (1600 g/min) or placebo over a period of 30 minutes intravenously. Peripheral blood pressure and heart rate were recorded every 5 min over a total of 120 minutes. Measurements for pulse wave analysis (SphygmoCor VT) were performed every 10 minutes during this period. Statistics by ANOVA for repeated measurements.

Compared to placebo fasudil significantly reduced peripheral mean arterial pressure (~ 8.71.4 mmHg, p<0.05; figure 1) and increased heart rate (+ 6.31.1 bpm, p<0.001) in TX but not in C. Likewise, central systolic pressure(p=0.006; (figure 2), augmented pressure and augmentation index were decreased in TX only.

Figure 1: Mean arterial pressure

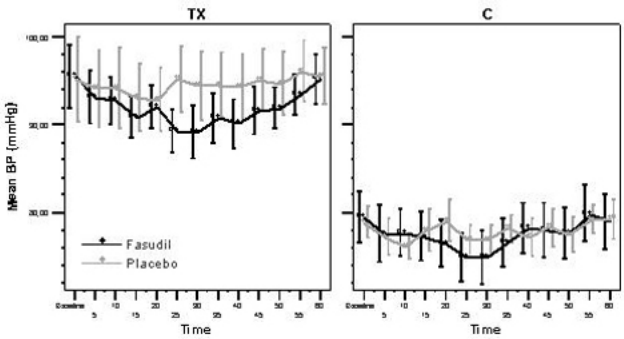
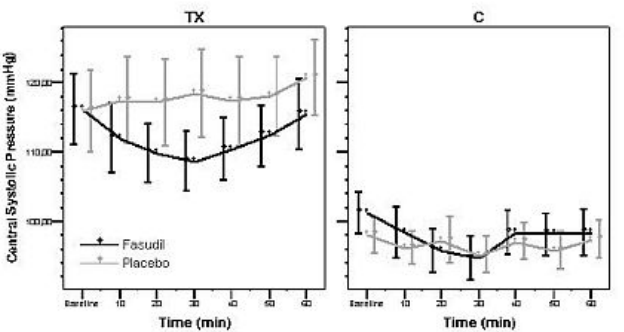


Figure 2: Central systolic pressure



We conclude that acute inhibition of ROK by fasudil consistently and effectively lowers blood pressure in TX with a calcineurin inhibitor-based immunosuppression. Interestingly, ROK-inhibition also reduces central blood pressure and arterial stiffness in these patients. Improvement of both these parameters has been linked to a reduction in cardiovascular morbidity and mortality in large trials. Hence ROK inhibition might prove beneficial for the treatment of hypertension in renal transplant recipients.

Abstract# 547

Use of Cardioprotective Medications in Kidney Transplant (KTx) Recipients: Observations from the Deterioration of Kidney Allograft Function (DeKaf) Study. Robert Gaston,¹ B. Kasiske,² R. Leduc,³ A. Fieberg,³ J. Cecka,⁴ F. Cosio,⁵ S. Gourishankar,⁶ P. Halloran,⁶ L. Hunsicker,⁷ D. Rush,⁸ A. Matas.³ ¹UAB, Birmingham, AL; ²HCMC, Minneapolis, MN; ³U Minn, Minneapolis, MN; ⁴UCLA, Los Angeles, CA; ⁵Mayo Clinic, Rochester, MN; ⁶U Alberta, Edmonton; ⁷U Iowa, Iowa City; ⁸U Manitoba, Winnipeg. Death with function causes half of late KTx failure, and cardiovascular disease (CVD) is the most common cause of death. Chronic kidney disease (CKD) is a CVD risk equivalent, justifying aggressive risk reduction with blood pressure (BP) control, statins, aspirin, and use of angiotensin converting inhibitors (ACEI) and angiotensin receptor blockers (ARB). DeKaf is an NIH-sponsored prospective observational study examining causes of KTx failure at 7 transplant centers in the US and Canada, with current enrollment of over 1800 subjects. We examined the use of cardioprotective medications among patients transplanted after 10/1/05 with at least 6 mos follow-up, focusing on subgroups with preexisting diabetes (DM) and/or CVD.

	All (n=433) %	DM (n=179) %	CVD ¹ (n=139) %
Mos after KTx	1	6	1
Any BP med	87	87	87
Calcium blocker (CCB)	51	44	40
β blocker	69	66	70
ACEI or ARB	13	26	12
Statin	35	46	54
Aspirin	43	45	60

¹Coronary disease, PVD, MI, stroke, CHF

Overall, 87% of all subjects were receiving antihypertensive therapy 30 days after transplantation, identical to the percentage on drug therapy at 6 months. Similar usage was noted among DM and CVD subjects, with wide variability in practice from center

to center. The most commonly used BP medications were beta-blockers, followed by CCBs, and both were used with the same frequency at 1 and 6 months. In contrast, the percentage of patients on an ACEI or ARB more than doubled between 1 and 6 months, suggesting reluctance to use these agents early after Tx (a practice not necessarily evidence-based); however, more than 70% of subjects did not receive ACEI/ARB therapy even at 6 months. Fewer than half of all patients received aspirin, including only 60% with DM and/or CVD. Similarly, only half with DM and/or CVD received a statin at 1 and 6 months. These data indicate current management of KTx recipients fails to utilize optimal CVD risk reduction measures in a timely fashion, perhaps missing an opportunity to reduce long-term morbidity and mortality from CVD in this at-risk population.

Abstract# 548

Cardiovascular Events and Mortality in Renal Transplantation: Comparison of Early Corticosteroid Withdrawal Regimens and Chronic Corticosteroid Therapy. Adele H. Rike,¹ Jason Everly,¹ Rita R. Alloway,² Michael Cardi,³ Gautham Mogilishetty,² Marketa Meyer,² E. Steve Woodle.¹
¹Surgery, Univ of Cincinnati; ²Nephrology, Univ of Cincinnati; ³Christ Hospital.

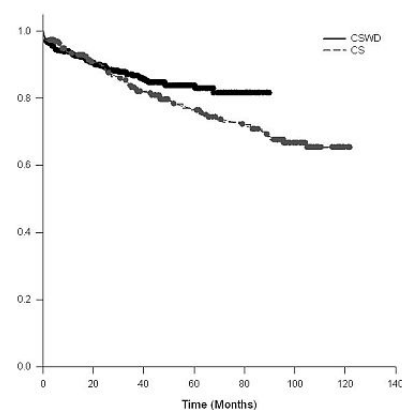
Background: Cardiovascular (CV) risk reduction has been a primary reason for pursuing early corticosteroid withdrawal (ECSWD). To date, actual cardiovascular event data (CVE) (rather than CV risk) has not been reported for ECSWD. Therefore, we analyzed and compared actual CV events (CVE) and CV-related survival in ECSWD (≤ 7 days) and chronic corticosteroid (CCS) pts.

Methods: CVE and heart failure (HF) data were prospectively collected. CVE were defined as sudden death, myocardial infarction, angina, and cerebrovascular accident/transient ischemic attack. HF events were defined as pulmonary edema or HF diagnosis. Statistical analyses included Student's t-test, Cox Proportional-hazards Regression, and Kaplan Meier (KM) survival estimates and time to CVE.

Results: Data on 693 RTx pts (471 ECSWD and 222 CCS pts) were analyzed. Demographic data and results are presented in Table 1. Mean follow-up in all pts was 1420 ± 958 days. No difference was observed in HF events between groups (5.7% ECSWD v 6.8% in CCS group, $p < 0.57$). CCS pts experienced more CVE (27.5% v 13.4%), despite a higher proportion of pre-existing cardiovascular disease (CVD) in ECSWD pts (7% v 15%). CVE-free survival was numerically greater in ECSWD pts and approached significance on KM analysis (Figure) ($p < 0.08$). A trend toward better pt survival by KM was observed for ECSWD ($p < 0.11$).

Demographics and Results

	CS Group (n=222)	CSWD group (n=471)	p
Male	49%	60%	0.007
African American	27%	21%	0.08
Mean Age (yrs)	46.4 ± 12	48.7 ± 12.9	0.03
PreTxp FRS	7.8 ± 6.7	8.2 ± 7.4	0.49
PreTxp CVD	7%	15%	0.003
PreTxp DM	26%	31%	0.14
% pts w/ CVE	27.5%	13.4%	< 0.0001
Mean #CVE/pt	1.6 ± 1	1.3 ± 0.6	< 0.0001



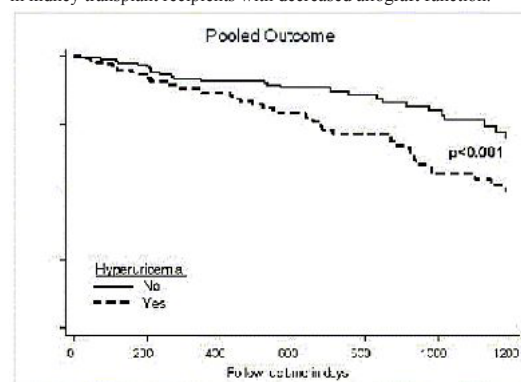
Conclusions: RTx recipients receiving ECSWD experienced: 1) fewer CVE and 2) a trend toward overall better pt survival. These differences in CVE and pt survival do not present until at least 3 yrs PTx, and therefore require long term followup to become evident.

Abstract# 549

The Association between Hyperuricemia at Six Months after Kidney Transplantation and the Development of New Cardiovascular Disease, Chronic Allograft Nephropathy, and Graft Loss. Enver Akalin,^{1,2} Sri V. Ganeshan,² Jonathan A. Winston,¹ Paul Munther.^{1,3} ¹Renal Division; ²Reagan Miller Transplantation Institute; ³Department of Community and Preventive Medicine, Mount Sinai School of Medicine, New York, NY.

We conducted a retrospective cohort study to assess the prevalence and the predictors for the development of hyperuricemia at 6 months after kidney transplantation and the

association between hyperuricemia and clinical outcomes including patient and graft survival, new cardiovascular events and chronic allograft nephropathy (CAN). Adult patients who underwent kidney transplantation at Mount Sinai Medical Center between 1.1.2001-12.30.2004 were included. Patients who died or lost the allograft within 6 months after transplantation were excluded from analysis. Of the 307 patients with a functioning allograft at 6 months after transplantation, 163 patients (53%) had normal uric levels and 144 patients (47%) had hyperuricemia. After age, race, sex adjustment, receiving a cadaveric kidney, having an eGFR < 50 ml/min, and taking diuretics and cyclosporine were associated with a higher odds ratio of hyperuricemia. Over a mean of 4.3 years of follow-up, 81 patients had one, or more, of the pooled outcomes; 40 had new cardiovascular events, 41 developed biopsy-proven CAN, 4 patients died, and 20 had graft failure. Kaplan-Meier survival curves demonstrated that the pooled outcomes of events occurred more frequently in hyperuricemic patients (Figure, $p < 0.001$). Due to association between low eGFR and hyperuricemia, we analyzed the clinical outcomes in patients with low and normal eGFR. While 44.7% of hyperuricemic patients with an eGFR < 50 ml/min had one of the pooled outcomes, it was 20.8% in patients with normal uric acid levels ($p = 0.038$). Among patients with an eGFR ≥ 50 ml/min, 19.4% of normouricemic and 25% of hyperuricemic patients had one of the events. These results suggest an important association between hyperuricemia at 6 months after transplantation and the new cardiovascular events, biopsy-proven CAN, and graft loss in kidney transplant recipients with decreased allograft function.



Liver: Pediatrics

Abstract# 550

Health-Related Quality of Life (HRQOL) for Children after Liver Transplantation: Impact of Time Since Transplant. Vicky Lee Ng,¹ Anthony R. Otley,¹ Rachel Taylor,¹ Anil Dhawan,¹ Susan Gilmour,¹ Amanda Hood,¹ David Nicholas.¹ ¹Paediatric Academic Multi-Organ Transplant (PAMOT) Program, Hospital for Sick Children, Toronto, ON, Canada.

Background: Long-term survival after liver transplantation (LT) is now the rule rather than the exception. Hence, assessment of outcomes for children after LT must consider not only the quantity, but also the quality, of life years survived and restored. **Aim:** To examine key HRQOL themes after pediatric LT raised by both recipients and their parent proxies, with evaluation by time (1-3 yrs, 3-5 yrs, 5-10 yrs, and > 10 yrs) since LT. **Methods:** Semi-structured 1:1 item generation interviews were conducted in person with children (C) and parents (P) at time of ambulatory LT follow-up at 4 pediatric LT programs in Canada and UK. All interviews were audio-taped, transcribed verbatim, and subjected to content analysis utilizing QSR NVIVO 2.0 software for HRQOL related theme generation. The participants interviewed were part of a larger research program aimed at developing a disease-specific instrument to assess HRQOL for children after LT. **Results:** Data representing 91 (47% male) pediatric LT recipients was obtained from a total of 146 (62 C, 84 P) item generation interviews. Median recipient age at LT was 2.4 (range, 0.05 to 17) yrs, for primary indications including biliary atresia (45%), fulminant liver failure (17.6%), metabolic liver disease (4.4%), malignancy (8.8%) and others (24.2%). Median patient age at time of interview was 9.9 (range, 1.1 to 18.8) yrs. Themes emerging at all time points post-LT included infection risks, limitations on physical activities, side effects from immunosuppression meds, educational supports, and ongoing bloodwork. Themes identified within the medium (1-5 yrs) follow-up included worries about rejection episodes, need for future re-transplantation, school absenteeism, and altered sibling and family dynamics. The impact of living with a surgical scar was a more frequent theme with recipients > 3 yrs from LT. As time from LT increased to > 10 yrs, themes suggest a focus on normalization and health promoting behaviours, along with expressed desires to be like healthy peers. **Conclusions:** Unique HRQOL themes emerged from item generation interviews not captured by currently available generic HRQOL tools. HRQOL themes identified after pediatric LT suggest the importance of considering time trajectories from LT, and a focus on elements of 'everyday life' apart from LT.

Abstract# 551

Excellent Outcome after Liver Transplantation in Children with Cystic Fibrosis. Christopher D. Anderson,^{1,2} Jeffery A. Lowell,^{1,2} Yumirle P. Turmelle,^{1,2} Ravinder Anand,⁴ Changhong Song,⁴ Ross W. Shepherd,^{1,2} Sue V. McDiarmid,^{1,2} The SPLIT Research Group.⁴ ¹*Departments of Surgery and Pediatrics, Washington University in St. Louis, St. Louis, MO;* ²*Liver Care Center, St. Louis Children's Hospital, St. Louis, MO;* ³*Department of Pediatrics and Surgery, University of California, Los Angeles, Los Angeles, CA;* ⁴*The EMMES Corporation, Rockville, MD.*

Introduction: Cirrhosis affects approximately 10% of patients with cystic fibrosis (CF). Some studies have reported benefits of liver transplantation (LT) in CF patients, but large outcome studies are not available. We report the outcomes of a large cohort of CF patients undergoing LT.

Methods: Pre and Post-LT patient characteristics, post-LT morbidity and mortality, and patient and graft survival were studied in patients enrolled in the Studies of Pediatric Liver Transplantation (SPLIT) registry.

Results: As of 6/1/2007, 2702 patients age < 18yr received a 1st isolated LT. 62 CF patients were listed for 1st LT, neither waitlist deaths nor the probability of death from time of listing was different from non-CF. 42 (1.6%) CF patients underwent LT with an average followup of 3yrs (0-10yrs). Patient characteristics at the time of transplant were: average PELD: 5.4 (86.5% had a PELD < 10), median age: 11.9 yrs (0.7 - 17.5), 81% caucasian, 59.5% male. Post-LT, the 1, 3, and 5 yr patient survival in CF patients was 92.9%, 88.6%, and 88.6% compared to 90.8%, 88.0%, and 86.0% in non-CF patients. Graft survival in CF patients was 85.0%, 81.2%, and 81.2% at 1, 3, and 5 yrs compared to 85.6%, 80.7%, and 78.1%. Rejection rates were not different (50.6% CF vs 56.4% non-CF @ 5 yrs.). Total reoperations (<30 day) were less (21.4% vs 38%; p=0.03). However, renal complications (<30 day) were increased (14.3% vs 6.1%; p=0.04), with 50% of these patients requiring dialysis. Standardized height and weight scores showed no improvement over 2 years followup in the CF patients (height Z -1.4 at Tx to -1.4 at 2 yrs., weight Z -1.2 to -1.5), but tended to improve in the non-CF group (height Z -1.3 at tx to -0.8 at 2 yrs., weight Z -1.0 to -0.1).

Conclusions: Patient and graft survival following LT for CF liver disease are equivalent to non-CF patients. In addition, death rates from time of listing are not increased compared to non-CF patients. These data support LT as a treatment for CF liver disease, but studies investigating the lack of growth improvement and increased renal complications in these patients may further improve outcomes.

Abstract# 552

Utilization of Grafts from Pediatric Cardiac Death Donors for Kidney and Liver Transplantation. Willem J. Van der Werf,¹ LeGrande Belnap,¹ Elizabeth S. Roach,² Susan L. Bratton,³ ¹*Division of Abdominal Transplant, Department of Surgery, Intermountain Medical Center, Salt Lake City, UT;* ²*Intermountain Donor Services, Salt Lake City, UT;* ³*Department of Pediatrics, Primary Children's Medical Center, Salt Lake City, UT.*

The shortage of cadaveric donors has led many transplant centers to expand their criteria for accepting life-saving organs. Utilization of donation after cardiac death (DCD) donors has been estimated to increase the number of cadaveric donors. We report our experience with a recently established DCD program at a pediatric hospital and the outcome with the transplanted grafts. **Methods:** In 2005 a protocol for DCD was established at a free standing pediatric hospital. From 2005 to 2006 all patients undergoing withdrawal of care were evaluated for DCD. Patients meeting criteria for DCD underwent withdrawal by the critical care team and organ retrieval was initiated if asystole was reached in less than 60 minutes. In addition, one DCD liver was imported and was included in the liver results. **Results:** During the 2 year study period 7 patients (25% of total donors) underwent DCD resulting in 14 organs (11 kidneys and 3 livers) transplanted. The 7 cases had a mean donor age of 9 yrs (range 3-16), WIT of 22 min (9-55), time sBP < 60 of 11 min (7-15), and time from asystole to aortic flush of 8 min (6-12). Four kidneys were transplanted locally with CIT 5-14 hrs, no DGF, and one month creat 1.2-1.7. The remaining 7 kidneys were exported for transplant. Four livers were transplanted locally with donor age 7 mth-16 yrs, WIT 12-28 min, recipient age 14 mth-61 yrs, CIT 6-15 hrs, AST peak 190-3630, AST day 7 33-44, INR peak 1.4-1.8, INR day 7 1.0-1.1, Total Bili one month 0.3-1.0, and graft survival 1.5-2.9 yrs. There were no vascular or biliary complications. **Conclusions:** A protocol for DCD at a pediatric hospital increased the number of pediatric donors by 25%. Liver and kidney grafts from pediatric DCD donors demonstrated excellent graft function and survival.

Abstract# 553

Liver Retransplantation in Children. A 21 Year Single Centre Experience. Christophe Bourdeaux, Andrea Brunati, Magda Janssen, Jean-Bernard Otte, Etienne Sokal, Raymond Reding. *Pediatric Liver Transplant Program, Université Catholique de Louvain, Saint-Luc University Clinics, Brussels, Belgium.*

When graft failure occurs in liver recipients, secondary transplantation represents the only chance of long-term survival. In such instance however, several surgical and immunological aspects should be carefully considered, with respect to their impact on final outcome.

In the present study, the epidemiology and outcome of graft loss following primary pediatric liver transplantation (LT) were analysed, with the hypothesis that early retransplantation (reLT) might be associated with lower immunological risks when compared to late reLT.

Between March 1984 and December 2005, 745 liver grafts were transplanted to 638 children at Saint-Luc University Hospital, Brussels. Among them, a total of 90 children (14%) underwent 107 reLT, and were categorized into two groups (early reLT, n=58; late reLT, n=32), according to the interval between both transplant procedures (< or > 30 days).

Ten-year patient survival rate was 85% in recipients with a single LT, versus 61% in recipients requiring reLT (p=0.001). Ten-year patient survival rates were 59% and 66% for early and late reLT, respectively (p=0.423), the corresponding graft survival rates being 51% and 63% (p=0.231). Along the successive eras, the rate of reLT decreased from 17% to 10%, whereas progressive improvement of outcome post-reLT was observed. No recurrence of chronic rejection (CR) was observed after reLT for CR (0/19). Two children developed a positive cross-match at reLT (2/10, 20%), both retransplanted late for CR secondary to immunosuppression withdrawal following a post-transplant lymphoproliferative disease.

In summary, the current need for reLT has been decreasing over years, with a parallel improvement of its outcome. The results presented could not evidence better results for early reLT when compared to late reLT. The latter did not seem to be associated with higher immunological risk, except for children with immunosuppression withdrawal following the first graft.

Abstract# 554

The Role of Parenteral Lipids in the Development of a Serum Conjugated Bilirubin Exceeding 100 umol/L in Neonates on Parenteral Nutrition: A Multiple Variable Analysis. Ivan R. Diamond,¹ Nicole de Silva,¹ Amarpreet Sandhu,¹ Paul B. Pencharz,¹ Jae H. Kim,¹ Aileen Moore,¹ Paul W. Wales.¹

¹*Group for Improvement of Intestinal Function and Treatment, The Hospital for Sick Children, Toronto, ON, Canada.*

Background: A serum conjugated bilirubin greater than 100 umol/L (CB100), in neonates who receive parenteral nutrition (PN), has been demonstrated to be a predictor of end-stage liver disease requiring transplantation. Given the recent interest in the role of omega-6 lipids in the development of Parenteral Nutrition Associated Liver Disease (PNALD), we sought to examine in a multiple variable model the role of days of maximal lipid (>2.5 g/kg/day), in the development of this outcome.

Method: Between 2003 and 2004, data were collected prospectively on all neonates undergoing an abdominal surgical procedure. Univariate logistic regression models for the prediction of CB100 were developed with the following predictors: gestational age, percentile weight, percent predicted small bowel and colonic length, resection of the ileocecal valve, presence of a stoma, post-operative enteral tolerance, number of septic episodes, days of PN amino acid > 2.5g/kg/day, days of PN lipid > 2.5g/kg/day, and total days of PN. Univariate predictors significant at the 0.2 level were entered into a backward stepwise multiple variable logistic regression.

Results: 152 infants received PN post-operatively, and 22 developed CB100. Predictors that met criteria for consideration in the multiple variable model were: age (p=0.079), weight (p=0.059), small bowel length (p=0.001), presence of a stoma (p=0.163), proportion of enteral feeds post-operatively (p=0.049), days of PN amino acid > 2.5g/kg/day (p=0.00), days of lipid > 2.5 g/kg/day (p=0.00), and total days of PN (p=0.00). The final multiple variable model which had a negative predictive value of 97.6% and positive predictive value of 52.6% is presented in the table below.

Multiple Variable Predictors of CB100	
	OR (95% CI)
Days of PN Lipid >2.5 g/kg/day	1.038 (1.004 - 1.074)
Number of Septic episodes	1.764 (1.038 - 2.875)

OR:- Odds ratio. 95% CI: 95% confidence interval

Conclusions: Our model suggests a key role of PN lipids and intercurrent septic events in the development of CB100 from PNALD. These data may provide targets, such as careful line care, reduction in maximal lipid dose, or the use of alternate lipids such as Omega-3 fatty acids, to prevent CB100 an identified marker for the need of subsequent liver transplantation in infants with PNALD.

Abstract# 555

Is Graft Fibrosis in Operationally Tolerant Patients after Pediatric Living-Donor Liver Transplantation Antigen-Dependent? Donghua Cheng,¹ Takaaki Koshiba,¹ Ying Li,¹ Xiangdong Zhao,¹ Mami Yoshitomi,¹ Hiromi Sakashita,² Hironori Haga,² Mikiko Ueda,³ Shinji Uemoto.³ ¹*Innovation Center for Immunoregulation and Technologies and Drugs, Graduate School of Medicine, Kyoto University, Kyoto, Japan;* ²*Diagnostic Pathology, Graduate School of Medicine, Kyoto University, Kyoto, Japan;* ³*Surgery, Graduate School of Medicine, Kyoto University, Kyoto, Japan.*

Background: Operational tolerance is defined as long-term acceptance of a transplanted organ after complete cessation of immunosuppression (IS). There are, however, no histological criteria of tolerance. **Methods:** Protocol biopsy was performed on the tolerant patients after pediatric living-donor liver transplantation (LTx) (Gr-Tol n=29). The graft was stained with hematoxylin and eosin (HE), Masson trichrome, and cytokeratin 7 and examined for cellular infiltrates, vessels damages, and/or fibrosis.

Immunohistochemistry was performed for CD4⁺ and CD8⁺ cells. Results were compared with those of the patients on maintenance IS (Gr-IS n=29) and the liver tissue from normal subjects (Gr-normal n=11). **Results:** The follow-up time in Gr-Tol was longer than that in Gr-IS. (Gr-Tol and Gr-IS: 121m and 52m, p<0.01) In Gr-Tol, typical features of neither acute nor chronic rejection were observed following Banff criteria. The extent of graft fibrosis in Gr-Tol, however, was greater, than those in Gr-IS and Gr-normal (Gr-Tol, Gr-IS and Gr-normal; 1.6, 0.9 and 0 (Ishak's modified staging) (Gr-Tol vs. Gr-IS, Gr-IS vs. Gr-normal p<0.01). Each number of CD4⁺ and CD8⁺ cells in graft infiltrates was increased in Gr-Tol, compared with that in Gr-normal, but equivalent with that in Gr-IS (CD4⁺ Gr-Tol, Gr-IS and Gr-normal; 14.6, 10.6 and 5.3 cells/field, Gr-Tol vs. Gr-normal p<0.05, Gr-Tol vs. Gr-IS NS / CD8⁺ Gr-Tol, Gr-IS and Gr-normal; 27.6, 26.3 and 10.5 cells/field Gr-Tol vs. Gr-normal p<0.01, Gr-Tol vs. Gr-IS NS). **Conclusions+Discussion:** In tolerant graft after pediatric living-donor Ltx, neither acute nor chronic rejection was observed, but fibrosis developed. Because of the similar extents of CD4⁺ and CD8⁺ cells infiltrates and different follow up time between tolerant and immunosuppressed patients, it remains questionable whether fibrosis in tolerant graft is antigen-dependent. Serial protocol biopsy before and after starting weaning IS will detect fibrosis early, and observing whether reintroduction of maintenance IS reverses fibrosis in that case will answer this question. Operational tolerance may not always guarantee intact graft morphology.

Abstract# 556

Development of "Operational Tolerance" after Pediatric Liver Transplantation Is Associated with the Absence of Early Rejection and the Presence of HLA-B Mismatch between Donors and Recipients. Mami Yoshitomi,¹ Takaaki Koshiba,¹ Shinji Uemoto.¹ *Innovation Center for Immunoregulation Technologies and Drugs, Graduate School of Medicine, Kyoto University, Kyoto, Japan.*

Background: In the setting of our pediatric living-donor liver transplantation (Ltx), 15% of all the patients (significantly higher proportion, compared with those of other transplant centers) achieved complete withdrawal of immunosuppression (IS), which is referred to as "operational tolerance". Nonetheless, some patients encountered rejection while they were undergoing weaning from IS. It is, therefore, essential to identify and characterize the differences that will enable patients in these two distinct populations to be distinguished reliably. **Methods:** The study groups consisted of group tolerance (Gr-Tol) in which 88 patients are successfully weaned off from IS, and group rejection (Gr-Rej) in which 22 patients experienced clinically evident rejection during or after weaning IS process. The correlation between the clinical outcome (success or failure of weaning IS) and following parameters was assessed: donor/recipient age, donor/recipient gender, ABO compatibility, HLA mismatch, graft size, early (<1month) rejection episode and initial immunosuppression. **Results:** There was no difference between Gr-Tol and Gr-Rej with respect to donor/recipient age (Gr-Tol and Gr-Rej; 32y and 32y NS/35m and 26m NS), or donor/recipient gender (Gr-Tol and Gr-Rej (female); 60% and 50% NS/60% and 73% NS). ABO compatibility did not differ between the two groups (Gr-Tol and Gr-Rej (i identical: compatible: incompatible); 63%:27%:10% and 55%:27%:18% NS). The presence of HLA-B mismatch was more frequent in Gr-Tol than that in Gr-Rej (Gr-Tol and Gr-Rej; 95% and 76% p<0.05), while the presence of HLA-A or DR mismatch did not affect success or failure of weaning IS (HLA-A Gr-Tol and Gr-Rej; 73% and 76% NS, HLA-DR; 83% and 82% NS). Graft size did not differ between the two groups (GBWR Gr-Tol and Gr-Rej; 2.9% and 3.2% NS). The patients in Gr-Rej experienced early rejection more frequently than those in Gr-Tol (Gr-Tol and Gr-Rej; 17% and 50% p<0.05). Mean trough level of tacrolimus within 7 days after Ltx was compatible between the two groups (Gr-Tol and Gr-Rej; 11ng/ml and 11ng/ml NS). **Conclusions:** Development of operational tolerance after pediatric Ltx was associated with the absence of early rejection and the presence of HLA-B mismatch between donors and recipients.

Abstract# 557

Auxiliary Partial Orthotopic Liver Transplantation (APOLT) in Children with Fulminant Hepatic Failure. Tomoaki Kato,¹ Genarro Selvaggi,¹ Hidenori Takahashi,¹ Eddie Island,¹ Erick Hernandez,¹ Lesley Smith,¹ David Levi,¹ Seigo Nishida,¹ Phillip Ruiz,² Andreas Tzakis.¹ *Miami Transplant Institute, University of Miami.*

Patients with fulminant liver failure (FHF) who undergo auxiliary partial orthotopic liver transplantation (APOLT) have a chance to come off immunosuppression (ISP) when the native liver regenerates. It may be most beneficial for children with FHF; however, the literature regarding its use in children has been limited. From the beginning of the pediatric liver transplant program at our institution, 31 patients underwent liver transplantation for FHF. Of those, 7 received APOLT and the remaining standard liver transplantation (OLT). Seven children (age 8 months to 8 years) who received APOLT (APOLT group) were compared to matched control group of 11 patients (OLT group). Since APOLT was offered routinely at our institution since 2005, 6 of APOLT cases were done since 2005. In APOLT group, either left lateral segment or left lobe graft was used. Recipients left lobe was removed in all cases. In OLT group, 7 received whole liver graft and 4 received partial liver graft. All native livers showed submassive to massive necrosis at the time of transplant in pathology. All children (100%) in APOLT group are currently alive with a median follow up of 761 days (range 116-4139 days) where 8 (72%) patients are alive in OLT group (median follow up 1724 days). Six of 7 children in APOLT group (87%) showed native liver regeneration. First

four APOLT recipients (57%) are currently off ISP with fully regenerated native liver. Two of those patients developed complete atrophy of the graft liver, one underwent graft removal due to sepsis caused by severe rejection. One remaining patient who is off ISP is displaying progressive atrophy of the transplant liver. Incidence of acute rejection was 57% (4/7) in APOLT group vs 30% (3/10) in OLT group. Other postoperative complications included hepatic artery thrombosis (HAT) (n=1), bile leak (n=1), biliary structure (n=1) and bowel obstruction (n=1) in APOLT group, HAT (n=1), bile leak (n=2), bowel perforation (n=2), chylothorax (n=1) and aplastic anemia (n=2). Median posttransplant length of stay was 15 days in APOLT and 20 days in OLT group. **Conclusions:** APOLT was safely performed in children with FHF. Significant proportion of recipients displayed native liver regeneration and came off immunosuppression.

Abstract# 558

Natural Killer Cell Dysfunction in Pediatric Acute Liver Failure. Nada Yazigi,¹ Greg Tiao,¹ Alexandra Filipovich,² John Bucuvalas.¹ *Pediatric Liver Care Center, Cincinnati Children's Hospital Medical Center, Cincinnati, OH; ²Division of Hematology/Oncology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH.*

In pediatric patients, indeterminate Acute Liver Failure (ALF) accounts for ~50% of all cases, and carries a particularly poor prognosis without transplantation. Evidence exists to suggest that acute liver failure may reflect a disproportionate immune response to a common stimulus. NK cells comprise a central component of the innate immune system.

We hypothesized that NK cell dysfunction (innate or secondary to an antigenic insult) plays a pathologic role in indeterminate ALF.

Methods

We reviewed peripheral NK cell function in a series of 15 consecutive children cared for at Cincinnati Children's Hospital, who met criteria for indeterminate ALF as defined by the Pediatric ALF Study Group. Peripheral blood testing was carried for NK cell number, cytolytic function and perforin and granzyme activity as part of our clinical ALF protocol.

Results

Seven of fifteen patients had NK cell dysfunction. Only the severity of cholestasis was statistically higher in the NK cell dysfunction group. There was no statistical difference between the 2 groups with respect to age, INR, or peripheral blood cell counts. 3 of seven patients with NK cell dysfunction died in contrast to none of eight in the normal NK cell group. Of the 3 patients with NK cell dysfunction who eventually received a liver transplant, 2 had severe early recurrence of chronic hepatitis in the graft at a year follow up. Those outcomes are in sharp contrast to the group with no NK cell dysfunction where 7 patients needed transplantation, but all had no complications both on short or long term follow up.

Conclusion

We documented NK cell dysfunction at the time of ALF diagnosis in 7/15 pediatric patients with indeterminate ALF. This subgroup of patients was found to have higher: mortality, risk of infections, as well as recurrent disease in the graft. Our findings suggest that NK cell dysfunction is involved in the pathogenesis of indeterminate ALF. As such, it could therefore be a prime target for therapeutic intervention, with goals to rescue the patient from liver failure and/or to improve post-transplantation outcomes.

Liver: Renal Complications

Abstract# 559

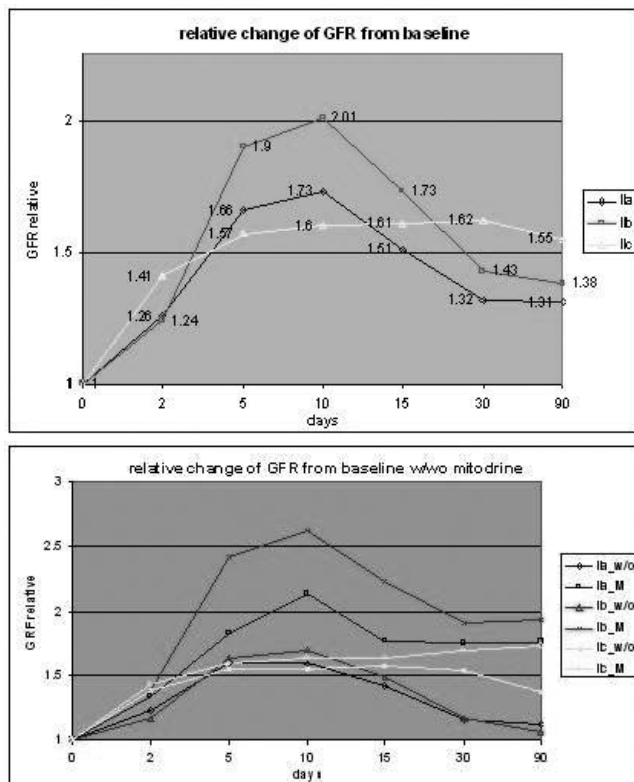
Treatment for Hepatorenal Syndrome in Patients Waiting for Liver Transplantation. Efficacy of Two Different Plasma Volume Expansion/Vasoconstriction Regimens Compared to TIPS. Lioba Schewior,¹ J. Klupp,² U. Frei,¹ P. Neuhaus,² G. Junge.² *¹Dept of Nephrology, Charité, Berlin, Germany; ²Dept of Surgery, Charité.*

Background HRS is a reversible renal failure which occurs in pts with advanced liver disease and portal hypertension and is characterized by a marked decrease in GFR and RPF in absence of other identifiable causes. Vasodilation theory is currently the most accepted hypothesis to explain the pathogenesis. In decompensated cirrhotics, probability of developing HRS is 8-20%/yr and increases to 40% at 5 yrs. Ideal treatment is LTx. However, there is an urgent need for effective alternative tx to increase surv chances for pts until LTx can be performed. Interventions that have shown some promise are vasoconstrictors in splanchnic circulation and TIPS. Main objective was to compare efficacy of two different regimens (albumin/terlipressin resp HES/terlipressin both w/ wo midotrine) against TIPS whereas GRF was considered as primary efficacy endpoint.

Pts/Tx Dx of HRS was based on criteria, as proposed by International Ascites Club. Only pts with ESLD on the waiting list for LTx were eligible to be enrolled. Pts were assigned to tx arms and randomized w/wo midotrine. Volume/vasoconstrictor tx lasted for 10d, midotrine was continued; follow up for 90d. **Results**

Demographics at baseline							
group	[n]	age [yrs]	MELD	crea [mg/dl]	GFR [ml/min]	MAD [mmHg]	COP [mmHg]
Ila	20	47.4	26	2.8	24	93	16
Ilb	15	48.2	27	3.1	21	94	15.8
Ilc	10	51.5	23	2.7	25	94	16.4

Ila (albumin/terlipressin); Ilb (HES/terlipressin); Ilc (TIPS)



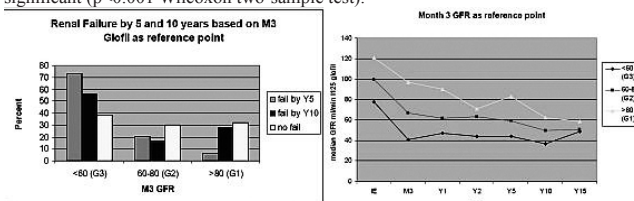
Discussion Combination of volume expansion/vasoconstriction improved effectively GFR in pts with HRS. Use of albumin shows no advantage compared to (cost effective) HES. Although a marked improvement was observed during iv-treatment, renal fct deteriorated upon treatment withdrawal whereas pts with continued mitodrine showed superior long term outcome.

Abstract# 560

Renal Failure after Liver Transplant (LT) Is Dependent on Month 3 GFR, a Long-Term Review of up to 15 Year GIoFil Data. Edmund Q. Sanchez,¹ Srinath Chinnakotla,¹ Henry B. Randall,¹ Greg J. McKenna,¹ Richard Ruiz,¹ Nicholas Onaca,¹ Marlon F. Levy,¹ Robert M. Goldstein,¹ Goran B. Klintmalm.¹ ¹Baylor Regional Transplant Institute, Dallas/Ft. Worth, TX. We analyze our single institution experience to quantify the long-term incidence of renal failure based on month 3 GFR compared to subsequent determinations.

Methods: This is an IRB approved retrospective review of the prospectively maintained database of LT recipients. Exclusion: patients on renal replacement therapy (RRT) at time of transplant, combined liver kidney, fulminant hepatic failure, and <1-year follow-up. GFRs (I¹²⁵ iothalamate GIoFil method) were measured at initial evaluation (IE), month 3 (M3), year 1 (Y1), 2 (Y2), 5 (Y5), 10 (Y10), and 15 (Y15). Patients were grouped by GFR >80 (G1), 60-80 (G2), and <60 (G3). IE and M3 GFR were used as starting points for longitudinal analyses. Paired data analysis for IE, M3, Y5 and Y10 was also performed. Renal failure was defined as GFR <30, received kidney transplant, on dialysis, or on kidney transplant list.

Results: 592 liver transplant patients were reviewed between 1985 and 1999. Paired GIoFil data was available for the Y5 and Y10 analysis in 114 patients. M3 GFR correlated more with long-term renal function ($p < 0.0024$). G1 demonstrated largest reductions in GFR over time. G2 and G3, when corrected for patients that got kidney transplantation and RRT, demonstrated progressive reduction in GFR. G3, G2, and G1 were statistically significant ($p < 0.001$ Wilcoxon two-sample test).



Conclusion: This study clearly demonstrates progressive decline in GFR continuing out to 15 years after liver transplantation. M3 GFR correlates better with long-term renal function compared to IE GFR (not truly reflective of renal function at time of LT). If M3 GFR <60, data showed a high rate of renal failure in our paired data analysis by

Y5 ($p < 0.0024$). By correcting for patients with renal failure, the previously reported stability in GFR between Y1 and Y5 is not seen. Analysis of grouping demonstrates that G1 patients at M3 have lower incidence of renal failure >10 years after LT. G2 and G3 patients will be at higher risk for renal failure each year after transplantation.

Abstract# 561

Evaluation of the Benefit on Renal Function of a Delayed Introduction of Tacrolimus in Liver Transplant Recipients: 2-Year Follow-Up

Results. Yvon Calmus,¹ Jean Gugenheim,² Christophe Duvoux,³ Christian Ducerf,⁴ Philippe Wolf,⁵ Didier Samuel,⁶ Claire Vanlemmens,⁷ Martine Neau-Cransac,⁸ Ephrem Salamé,⁹ Olivier Chazouillères,¹⁰ Nicole Declercq,¹¹ Georges-Philippe Pageaux,¹² Lionel Rostaing.¹³ ¹Paris; ²Nice; ³Créteil; ⁴Lyon; ⁵Strasbourg; ⁶Villejuif; ⁷Besançon; ⁸Bordeaux; ⁹Caen; ¹⁰Paris; ¹¹Lille; ¹²Montpellier; ¹³Toulouse, France.

Introduction: Calcineurin inhibitors have demonstrated efficacy in liver transplantation. However, they have a potential to impair renal function. Delayed tacrolimus (TAC) administration may reduce the risk of renal dysfunction.

Methods: A prospective study included liver transplant pts randomised to delayed introduction of TAC (Day 5) + Daclizumab (DAC) (Group A) or to immediate TAC administration (Group B). In both groups TAC T0 was 10-20 ng/ml until Week 4 and 5-15 ng/ml thereafter. MMF was given at 2 g/d for 2 months, and corticosteroids (CS) at standard doses. Pts with a serum creatinine (SCr) > 180 µmol/l at 12 hours (H12) were excluded. The primary endpoint was the rate of pts with a mean SCr > 130 µmol/l at Month 6. Month 24 results are presented.

Results: 207 pts were randomised. Baseline characteristics were similar. At Month 6, mean TAC T0 was 9.6 (Group A) and 11.2 ng/ml (Group B). At Month 24, patient survival was 85% (Group A) and 87% (Group B), graft survival was 91.6% and 89.4%. Incidence of BPAR was 24 % and 26 %. SCr at 2 year was 114.2 ± 37.3 and 116.5 ± 35.1 µmol/L.

At month 6	TAC delayed (N=98)	TAC immediate (N=101)	p
SCr > 130	22.4%	29.7%	0.24
GFR*	72 ± 29	66 ± 24	0.09
For pts with SCr > 100 at H12	64.5 ± 22	62 ± 25	0.67
For pts with SCr ≤ 100 at H12	76.5 ± 32	67.5 ± 23	0.06
At month 24	TAC delayed (N=93)	TAC immediate (N=93)	
SCr > 130	36.4%	43.5%	0.62
GFR*	75.7 ± 29	69.7 ± 24	
For pts with SCr > 100 at H12	71.4 ± 22	68.9 ± 28	
For pts with SCr ≤ 100 at H12	77.4 ± 32	70.0 ± 23	

* : Glomerular filtration rate (Cockcroft formula- ml/min)

At least one serious adverse event related to treatment was reported in 35.1% pts in Group A and 36.1% pts in Group B. Infectious and metabolic complications were similar in the 2 groups.

Conclusions: Delayed TAC administration with DAC and MMF is a safe immunosuppressive therapy for LTx pts with no increased BPAR. No difference regarding renal function at 2-year follow up was observed as compared with immediate introduction.

Abstract# 562

Long Term Renal Function of Liver Transplant Recipients Subjected to a Renal Protective Immunosuppression with Low-Dose CNI Combined with MMF Plus Steroids.

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Background: The withholding of CNIs and the early introduction of MMF to CNI based protocols has become widespread practice in liver transplant (LTx) recipients with early renal dysfunction. While there is a sound theoretical basis for this approach, there is little data on the long term results. **Methods:** We compared 195 LTx recipients, receiving a low-dose CNI immunosuppression (IS) combined with MMF (low CNI / MMF) and 441 LTx recipients receiving a standard full-dose CNI (full CNI) regime. We examined the renal function at 12, 24 and 36 months (m) as well as the slope of renal function ($\Delta m24-m6$) of both groups. **Results:** The mean age 52.5 ± 11.2 vs. 51.8 ± 9.7 (y) and observation time 41.9 ± 30.8 vs. 38.5 ± 24.7 (m) were similar between both groups. The gender distribution 71.2 vs. 60.6% male and the mean MELD scores of 21.2 ± 10.9 vs. 16.2 ± 9.1 were significantly different (low CNI/MMF vs. full CNI, $p < 0.05$). S-creatinine immediately after LTx (day 0) was 135.1 ± 95.4 µmol/l in the low CNI/MMF group and 103.5 ± 46.7 µmol/l in the full CNI group, respectively. However, at 12 m the low CNI/MMF group (70.73 ± 23.08 ml/min) with initially impaired renal function was similar to the full CNI group 117.3 ± 68.1 vs. 110.7 ± 48.8 µmol/l (MDRD-GFR 70.73 ± 23.08 vs. 73.21 ± 24.07 ml/min). At 2 and 3 years after transplantation, the renal function remained similar between the two groups (115.5 ± 67.4, 117.6 ± 55.5 full CNI vs. 116.0 ± 46.0, 119.3 ± 43.7 low CNI/MMF µmol/l). Renal function was significantly better at 12, 24 and 36 m when patients were continuously treated with MMF (114.7 ± 34.5, 118.8 ± 49.1, 119.4 ± 42.8 µmol/l) as compared to the patients where MMF (127.0 ± 95.6, 126.7 ± 65.4, 128.3 ± 55.7 µmol/l; all $p < 0.05$.) was stopped within the first year. The slope of renal function between 6 and 24 m after LTx in the low CNI/MMF group was superior to the

full CNI group. **Conclusion:** Compared to full CNI, low CNI/MMF a) allows renal function to recover in patients with impaired renal function at the time of LTx and b) preserves long term renal function. CNI sparing in combination with MMF may become standard in LTx to maintain long-term renal function.

Abstract# 563

Prospective Assessment of Renal Histopathological Lesions Prior to Liver Transplantation. Yvon Calmus,¹ Evangeline Pillebout,² Philippe Cluzel,³ Filomena Conti,¹ Olivier Scatton,¹ Olivier Soubrane,¹ Corinne Antoine,² Gary Levy,⁴ Denis Glotz,² Dominique Nochy,⁴ ¹Hopital Cochin; ²Hopital Saint-Louis; ³Hopital Pitié-Salpêtrière; ⁴Hopital Européen Georges Pompidou, Paris, France.

Terminal renal failure occurs in more than 10 % of liver transplant recipients after 10 years. We have previously shown that, beside renal toxicity of calcineurin inhibitors, renal lesions may be related to diabetes, arterial hypertension, accumulation of hydroxyethylstarch (ELHOES), and the etiology of the liver disease. We made the hypothesis that these lesions may be already present at the time of liver transplantation (LT), a finding that could lead to adapt the perioperative management. This work investigated prospectively whether renal histopathological lesions were present before LT by performing systematically a renal biopsy by endovenous route in 60 candidates to LT with end-stage liver disease. These patients were 58 ± 10 years old, 46 males; 10/60 had a diabetes, and 21 an arterial hypertension; the liver disease was related to alcohol in 32 cases, HCV in 12 cases, HBV in 5 cases, and to a cholestatic disease in 7 cases. At the time of the pre-LT workup, the biochemical parameters were: Child score 10 ± 2, MELD score 18 ± 4, prothrombin rate 50 ± 12, creatinin serum level 90 ± 6 µmol/L, proteinuria 0.12 ± 0.04 g/24h. Severe side effects related to the procedure were limited to 2 cases of macroscopic hematuria, lasting less than 24 hours. In 10 cases, the material obtained during the procedure did not allow the histological analysis. Among the 50 samples available, 21 were considered as normal; in 29 cases, lesions related to mesangial IgA glomerulonephritis (14 cases), diabetic glomerulosclerosis (12 cases), ELHOES accumulation (5 cases), thrombotic microangiopathy (1 case) were found, often associated; in 5 cases, the lesions were severe and lead to combined kidney/liver transplantation in 2 cases. In conclusion, significant renal lesions are detectable in more than 50 % of the candidates to LT. Interestingly, histological findings often combined lesions related to the liver disease and to an associated cause (diabetes, previous treatment by ELHOES or interferon). Results of histological analysis could help to decide either to perform a combined renal/liver transplantation, to adapt the immunosuppressive regimen, or to abandon the LT project.

Abstract# 564

Liver Graft Outcomes in Recipients with and without Combined Kidney Transplantation: A Propensity Analysis. Erick B. Edwards,¹ Ann M. Harper,¹ Richard B. Freeman,² ¹Research Department, United Network for Organ Sharing, Richmond, VA; ²Division of Transplant Surgery, New England Medical Center, Boston, MA.

Background. Since early 2002, deceased donor livers have been allocated in the U.S. according to the Model for End Stage Liver Disease (MELD). Because serum creatinine is one of the components of MELD, liver candidates with renal insufficiency have been transplanted in increasing numbers, with some candidates receiving a kidney along with the liver transplant. We aimed to compare the liver graft outcomes for liver alone (LTA) transplants with those from combined liver-kidney transplants (CLKT). A propensity score analysis was used to reduce the impact of selection bias in the comparison of outcomes in the two groups. **Methods.** Demographics, clinical factors and outcomes on LTA and CLKT recipients from 3/1/02 to 12/31/06 (N=10,388) obtained from the OPTN database were used for the analysis. Univariate post-transplant survival rates were estimated using Kaplan-Meier survival, and multivariable post-transplant outcomes were analyzed using a Cox regression model with and without stratification by categories of the propensity score. The propensity score (probability of receiving a CLKT) for each recipient was estimated using a logistic regression model. Several donor and recipient factors were included in both the Cox and logistic regression models. **Results.** We identified 739 CLKT and 9649 LTA in the 2002-2006 cohort. Unadjusted graft survival rates at 1 year, 2 years and 3 years were 82.2%, 76.6%, and 73.2% for LTA and 82.8%, 77.8%, and 74.1% for CLKT. The most significant factors associated with receiving a CLKT AND having post-transplant liver graft failure were serum creatinine, on dialysis prior to transplant, diabetes, Black ethnicity, and donor age. In the Cox model without propensity adjustment, the relative risk (RR) of graft failure for CLKT vs. LTA was 0.667, p<0.0001. In the Cox model with propensity adjustment, the RR of graft failure for CLKT vs. LTA was 0.697, p=0.0002. **Conclusions.** In this cohort, liver graft outcomes for CLKT were significantly better than those for LTA based on the multivariable analysis. The results were similar, although slightly less significant, when the model was adjusted for propensity. The superior outcomes of CLKT may be due to unobserved differences between these groups of recipients, reflecting data not currently captured by the OPTN.

Abstract# 565

Effect of Liver Transplantation on Allosensitization in Patients with Combined Liver and Kidney Failure. Lindsay J. Gage,¹ Richard K. Gilroy,² Clifford D. Miles,¹ Vinaya Rao,¹ Alan N. Langnas,³ Troy J. Plumb,¹ ¹Internal Medicine, University of Nebraska Medical Center, Omaha, NE; ²Internal Medicine, University of Kansas Medical Center, Kansas City, KS; ³Surgery, University of Nebraska Medical Center, Omaha, NE.

Kidney and liver failure not infrequently coexist in patients. Simultaneous liver and kidney transplantation (SLK) and kidney after liver transplantation (KAL) are two available options. Observational studies suggest that SLK offers improved outcomes, but this strategy may lead to unnecessary kidney transplants in patients who may recover renal function, thus depleting the kidney donor pool. While some patients should clearly be offered SLK, it is not uncommon to encounter clinical scenarios when the decision is less clear. The aim of this study was to determine if liver transplantation leads to allosensitization, which would be pertinent to decision-making in this setting.

Methods: We reviewed liver transplant recipients at our center who were also listed for kidney transplantation and had at least one measurement of PRA performed between May 1996 and November 2006. **Results:** Forty-two subjects evaluated for combined liver and kidney failure had PRA data available. There were 32 subjects in the KAL group who received an isolated liver transplant, 20 of whom subsequently received a kidney transplant. Ten subjects underwent SLK. The KAL group was more likely to have alcoholic or viral hepatitis as the cause of ESLD. Although the difference did not meet statistical significance, only patients who underwent KAL were allosensitized (25 percent of KAL vs. 0 percent of SLK). Additionally, there were 3 biopsy-proven kidney rejection episodes, all in KAL patients. **Conclusion:** In this study, only patients receiving KAL were noted to be allosensitized or experienced acute rejection in the transplanted kidney. Allosensitization following liver transplant may be an important consideration when determining whether patients receive a liver versus SLK.

Abstract# 566

Outcome of Combined Liver and Kidney Transplant in Hepatitis C Recipients: A Single Center Long-Term Follow-Up Experience. Ana Carolina Del Pozo,¹ Lou Mazzella,¹ Juan Del Rio Martin,¹ Rodriguez-Lai Gonzalo,¹ Mark Sturdevant,¹ Kishoer Iyer,¹ Tom Schiano,¹ Enver Akalin,¹ Barbara Murphy,¹ Jonathan Bromberg,¹ Scott Ames,¹ Susan Lerner,¹ Graciela de Boccardo,¹ ¹Recanati/Miller Transplant Institute, Mount Sinai Medical Center, New York, NY.

BACKGROUND: The prevalent liver disease for orthotopic liver transplant (OLT) in the US is hepatitis C related (HCV) cirrhosis. Candidates, who also have end stage kidney disease (ESKD) or hepatorenal syndrome (HRS) requiring prolonged dialysis, are elected for combined liver-kidney transplant (CLKT). **OBJECTIVE:** To analyze HCV recipients and donor demographics, rejection rates, patient and graft survivals, rate and severity of recurrence, long-term liver function and glomerular filtration rate (GFR). To compare survival of HCV recipients of isolated liver transplant with HCV recipients of CLKT. **DESIGN and METHODS:** Retrospective study of HCV adult patients who received CLKT in our center. **RESULTS:** From February 1992 to January 2007, 23 adult patients with HCV-cirrhosis received CLKT. 78% was male with a mean age of 61 years. The mean MELD was 26 (range: 21-34). The mean donor age was 35 years. The average pre-CLKT total bilirubin was 4.8, INR 1.5 and GFR 19. The average time on dialysis pre-transplant was 22 months. The prevalent kidney diseases were diabetes and prolonged HRS. Liver survival was 86%, 77%, 67% and 56% while kidney survival was 87%, 83%, 61% and 57%. The overall rejection rate was 26% for the liver and 4.3% for the kidney. Overall patient survival was 91%, 81%, 70% and 60% at 6 months, 1, 3 and 5 years for HCV recipients of CLKT; while it was 86%, 81%, 79% and 78% respectively for HCV recipient of isolated liver transplant (P=0.09). After 5 year-follow up of the CLKT the mean bilirubin was 0.5, platelet count was 183 and the mean INR was 1. The average GFR was 58, 61 and 52 at 6 months, 1 and 5 years after CLKT.

CONCLUSIONS: CLKT represents a proper therapeutic option for patients with both liver and renal failure with good long-term organ function. The simultaneous transplantation does not reduce the patient survival in HCV recipients. Although non statistical different, the better survival in CLKT HCV recipients may be related to the important influence of the renal function in the MELD score and the favorable as well as the younger donor age for CLKT recipients.

Abstract# 567

Liver Transplantation and Renal Insufficiency: TRY Study Interim Analysis. Nicolas Janus,¹ Vincent Launay-Vacher,¹ Faouzi Saliba,² Svetlana Karie,¹ Jerome Dumortier,³ Richard Lorho,⁴ Christophe Duvoux,⁵ Yvon Calmus,⁶ Gilbert Deray,¹ Georges-Philippe Pageaux,⁷ ¹Pitié-Salpêtrière, Paris; ²Paul Brousse, Villejuif; ³Edouard Herriot, Lyon; ⁴Pontchaillou, Rennes; ⁵Henri Mondor, Créteil; ⁶Cochin, Paris; ⁷Saint-Eloi, Montpellier, France.

Aims

- to determine renal function (RF) before liver transplantation (BT), at 1 month (1M), at 1 year (1Y) and at 5 years (5Y) after transplantation (Tx).
- to describe RF according to immunosuppressive therapy.

Methods: national, retrospective, multicenter, observational study.

Results (interim, 949 patients)

Median follow-up post-Tx was 8 years (5-21). Most frequent Tx indications were alcoholic (34%) and HCV (10%) cirrhosis. aMDRD Glomerular Filtration Rate (GFR) was < 60 mL/min/1.73m² in 11% (BT), 46% (1M), 48% (1Y) and 55% (5Y) of the patients. Changes in GFR were then compared according to the immunosuppressive protocol:

-group "CNI+MMF" = a calcineurin inhibitor (CNI) + mycophenolate mofetil (MMF).

-group "CNI" = a CNI without MMF. In this group, some patients received only CNI and some CNI + Azathioprine. There was no difference between those 2 sub-groups, neither on RF nor on CNI doses. All those patients were thus pooled.

In both groups, GFR decreased from BT: -14% in "CNI+MMF" vs -25% in "CNI" at 1M (p=0.06), -14% vs -29% at 1Y (p=0.03), and -12% vs -33% at 5Y (p=0.02). Although their mean GFR BT was lower (86 vs 97 mL/min/1.73m², p=0.0002), the decrease in RF in "CNI+MMF" patients was less severe. Table 1 compares the 93 patients who were on MMF + CNI at 1M and at 1Y and at 5Y with the 662 patients who were on CNI without MMF at each time.

		CNI+MMF (n=93)	CNI (n=662)	p
1M	GFR	-13%	-24%	NS
	Dose CsA	300.2 mg (n=29)	405.3 mg (n=303)	<0.0006
	Dose FK	7.2 mg (n=63)	9.8 mg (n=353)	<0.0001
1Y	GFR	-12%	-29%	0.05
	Dose CsA	226.2 mg (n=27)	260.8 mg (n=280)	NS
	Dose FK	5.1 mg (n=66)	5.4 mg (n=377)	NS
5Y	GFR	-12%	-33%	0.02
	Dose CsA	181.9 mg (n=25)	188.6 mg (n=265)	NS
	Dose FK	3.4 mg (n=63)	3.4 (n=386)	NS

Conclusion

Nearly 50% of the patients had renal insufficiency in the 5 years following liver Tx. The reduction in the GFR is less pronounced in patients treated with MMF even if they were significantly more at risk BT. Except at 1M, there was no difference in CNI doses between the 2 groups, suggesting that the sustained lower decrease in RF observed in "CNI+MMF" may not be only explained by a CNI dose reduction.

Non-Invasive Immune Monitoring in Transplantation

Abstract# 568

Cellular Islet Autoimmunity Influences Clinical Outcome of Islet Cell Transplantation. Volkert Huurman,¹ Robert Hilbrands,² Gabrielle Pinkse,³ Pieter Gillard,² Gaby Duinkerken,³ Pieter v.d.Linde,¹ Ellen v.d.Meer-Prins,³ Minke Versteeg-v.d.Voort Maarschalk,³ Koen Verbeeck,² Behrooz Alizadeh,³ Chantal Mathieu,⁴ Frans Gorus,² Dave Roelen,³ Frans Claas,³ Bart Keymeulen,² Daniel Pipeleers,² Bart Roep.³ ¹*Surgery, Leiden University Medical Center, Leiden, Netherlands;* ²*Diabetes Research Center, Brussels Free University, Brussels, Belgium;* ³*Immunohematology and Blood Transfusion, Leiden University Medical Center, Leiden, Netherlands;* ⁴*LEGENDO, University Hospital Gasthuisberg, Leuven, Belgium.*

Background: Islet cell transplantation can cure type 1 diabetes (T1D), but only a minority of recipients remains insulin-independent in the following years. We tested the hypothesis that allograft rejection and recurrent autoimmunity contribute to this progressive loss of islet allograft function.

Methods: Twenty-one T1D patients received cultured islet cell grafts prepared from multiple donors and transplanted under anti-thymocyte globulin (ATG) induction and tacrolimus plus mycophenolate mofetil (MMF) maintenance immunosuppression. Immunity against auto- and alloantigens was measured before and during one year after transplantation. Cellular auto- and alloreactivity was assessed by lymphocyte stimulation tests against autoantigens and cytotoxic T lymphocyte precursor assays, respectively. Humoral reactivity was measured by auto- and alloantibodies. Clinical outcome parameters remained blinded until their correlation with immunological parameters.

Results: All patients showed significant improvement of metabolic control and 13 out of 21 became insulin-independent. Multivariate analyses showed that presence of cellular autoimmunity before and after transplantation was associated with delayed insulin-independence (p=0.001 and p=0.01, respectively) and lower circulating C-peptide levels during the first year after transplantation (p=0.002 and p=0.02, respectively). 7/8 patients without pre-existent T-cell autoreactivity became insulin-independent, versus 0/4 patients reactive to both islet autoantigens GAD and IA-2 before transplantation. Autoantibody levels and cellular alloreactivity were not associated with outcome.

Conclusions: Cellular islet-specific autoimmunity affects clinical outcome of islet cell transplantation under ATG-tacrolimus-MMF immunosuppression. Tailored immunotherapy targeting cellular islet autoreactivity is required. Monitoring cellular immune reactivity can be useful to identify factors influencing graft survival and to assess efficacy of immunosuppression.

Abstract# 569

A Mechanistic Link between Acute Rejection and Epithelial to Mesenchymal Transition (EMT): BMP-7 Is Downregulated & TGFβ1 to BMP-7 Ratio Favors EMT during Acute Rejection of Human Renal Allografts. T. Muthukumar, R. Ding, C. Snopkowski, C. Chang, B. Li, S. Sharma, S. Seshan, D. Dadhania, M. Suthanthiran. *Nephrology, Cornell University Medical College, New York, NY.*

The mechanistic basis for acute rejection (AR) being a risk factor for chronic allograft damage has not been resolved. Epithelial to mesenchymal transition (EMT), a key event in fibrosis, is induced by TGFβ1 & reversed by bone morphogenetic protein-7 (BMP-7). In experimental models, exogenous BMP-7 reduces EMT and reverses renal interstitial fibrosis.

A plausible mechanistic link for the association between AR & the subsequent development of chronic allograft damage is that AR results in BMP-7 deficiency and the balance between TGFβ1 to BMP-7 is in favor of TGFβ1 & EMT.

Hypothesis: AR results in BMP-7 deficiency & the balance between TGFβ1 to BMP-7 is in favor of EMT.

We measured urinary cell levels of mRNA for BMP-7, TGFβ1, tubular markers E-cadherin, NKCC2, & cytotoxic T cell marker Granzyme B in urine samples collected from 78 human kidney transplant recipients. The samples were collected at the time of allograft biopsy from 28 subjects with biopsy confirmed AR & 50 subjects with stable graft function & normal allograft protocol biopsy. We isolated total RNA from urine cell pellets, reverse transcribed to cDNA & measured mRNA levels using gene specific primers & probes in the pre-amplification enhanced real time quantitative PCR assays. Transcript levels were expressed as copies/ ug total RNA.

Urinary cell BMP-7 mRNA levels were significantly lower in the 28 recipients with AR compared to 50 recipients with stable graft function and normal biopsy whereas mRNA levels of TGFβ1 and granzyme B were higher. mRNA levels of E-cadherin or NKCC2 were not different between the two groups.

mRNA	Acute Rejection (N=28) median copies/ ug total RNA	Normal Biopsy (N=50)	P
BMP-7	279	934	0.04
TGFβ1	726500	313000	0.01
E-cadherin	70900	45700	0.7
NKCC2	1985	1785	0.2
Granzyme B	62600	3180	0.001

The TGFβ1 to BMP-7 ratio was higher in the AR Group (median ratio: 1635) compared to recipients with stable graft function & normal biopsy (median ratio: 438, P=0.0002).

Our observations that BMP-7 is specifically down-regulated during an episode of AR and that the balance between TGFβ1 & BMP-7 is in favor of EMT advance a mechanism for the deleterious impact of AR on the long-term outcome of human renal allografts.

Abstract# 570

Allospecific CD154+ T-Cells Predict Rejection Risk and Measure Immunosuppressive Effect after Abdominal Organ Transplantation in 100 Recipients. Chethan Ashokkumar,¹ Anjan Talukdar,¹ Qing Sun,¹ Janine Janosky,¹ Patrick Wilson,¹ George Mazariegos,¹ Henkie Tan,¹ Ron Shapiro,¹ Sarangarajan Ranganathan,¹ Nydia Chien,¹ Angus Thomson,¹ Adriana Zeevi,¹ Rakesh Sindhi.¹ *Hillman Center for Pediatric Transplantation, Children's Hospital of Pittsburgh and The University of Pittsburgh, Pittsburgh, PA.*

Background: Immunosuppressant failures, e.g. rejection and toxicity, necessitate safe drug minimization to maintain post-transplant survival. Persistent donor-specific alloreactivity characterizes rejection-prone individuals (Rejectors), and can facilitate precise drug therapy, but cannot be measured easily or reliably with classical alloresponse assays in high-risk pediatric recipients. Antigen-specific T-cells express CD154 rapidly, but have not been used to measure alloimmunity. **Methods:** Allospecific CD154+T-cells were measured in <24 hours with polychromatic flow cytometry to identify Rejectors (who had experienced acute cellular rejection within 60 days post-transplantation) in single mixed leukocyte responses (MLR) from 100 cross-sectional recipients-88 children with liver or intestine allografts, and 12 adults with renal allografts. Where possible, results were correlated with proliferative alloresponses measured by CFSE-dye dilution (n=45), allograft biopsies (n=46), and expression of CTLA4, a negative T-cell costimulator, which antagonizes CD154-mediated effects (n=52). **Results:** In the first 33 children, logistic regression identified donor-specific, memory CD154+ T-cytotoxic cells (Tc) as enhanced among Rejectors, compared with Non-Rejectors (408±231 vs 90±36 per 10,000 cells, p=0.003), relatively drug-resistant (r with drug levels =-0.5, p=NS), with greatest sensitivity/specificity (>93%) for Rejectors. In the remaining 67 recipients, blinded prediction of Rejection status by memory CD154+Tc replicated sensitivity/specificity (>96%), and correlated with proliferative alloresponses (r=0.62, p=0.007), and biopsy data. Inverse correlation was seen with allospecific memory CTLA4+Tc cells (r=-0.556, p=0.0003). Allospecific CD154+T-helper cells showed concentration-dependent down-regulation by immunosuppressants (r=-0.85, p=0.007). **Conclusions:** Collectively, allospecific CD154+T-cells are viable clinical biomarkers for rejection-prone recipients, and immunosuppressive effect in abdominal organ transplantation. Clinical use may facilitate safe minimization of immunosuppression.

Abstract# 571**Noninvasively Developed Molecular Signatures Accurately Predict Acute Rejection of Human Renal Allografts.** B. Li,¹ R. Ding,¹ D. Dadhania,¹ C. Snopkowski,¹ T. Muthukumar,¹ C. Chang,¹ M. Lagman,¹ V. K. Sharma,¹ S. Seshan,¹ J. Schwartz,¹ M. Suthanthiran.¹ *Nephrology & Transplantation Medicine, Weill Cornell Medical College, New York, NY.*

Acute rejection is a complex biologic process involving multiple cell types, cytokines and chemokines/chemokine receptors. We hypothesized that an mRNA panel that included genes implicated in the anti-allograft response would distinguish allografts undergoing acute rejection from normal allografts with a high degree of accuracy. We tested this hypothesis by measuring levels of urinary cell mRNA and peripheral blood cell mRNA for cell surface proteins CD3, CD20, CD25, CD103, and CTLA4; chemokines/chemokine receptors IP10, MIG, CXCR3; cytotoxic attack molecules granzyme B (GB) and perforin, and immunoregulators Foxp3, TGF-beta1 and IL-10. Gene specific primer pairs and probes were used in pre-amplification enhanced real time quantitative PCR assays to measure mRNA and transcripts for 18s rRNA. For each cell source, we used logistic regression to identify a linear function of up to 5 log-transformed measures that would distinguish biopsies of AR patients from those of Stable transplant patients.

Molecular Signatures Distinguishing AR Biopsies from Normal Biopsies						
Molecular Signature	Number of Patients	Cell Type Profiled	mRNA Type	Sensitivity (%)	Specificity (%)	P-Value
1	22 AR, 19 Stable, 41 TOTAL	Urinary Cells	CTLA4, Foxp3, GB, CD3, MIG	100	100	<0.0001
2	22 AR, 33 Stable, 55 TOTAL	Peripheral Blood Cells	MIG, CD3, IP10, IL-10	82	88	<0.0001
3	17 AR, 18 Stable, 35 TOTAL	Urinary Cells	CTLA4	100	100	<0.0001
		Peripheral Blood Cells	CTLA4, CD103			

Our study demonstrates that molecular signatures developed using urinary cell levels of just 5 genes (Signature 1, urinary cell levels of mRNA for CTLA4, Foxp3, GB, CD3, and MIG), or a combination of 3 urinary and blood cell levels (Signature 2, urinary cell level of CTLA4 mRNA and peripheral blood cell levels of CD103 and CTLA4) differentiate AR from Stable biopsies with 100% sensitivity and 100% specificity. Blood cell levels alone are also informative, but less so. We conclude that molecular signatures, developed from noninvasively ascertained mRNA profiles of urinary cells/peripheral blood cells, predict acute rejection with extraordinary accuracy. Clinical Trials to validate the predictive value of these signatures are worthy of pursuit.

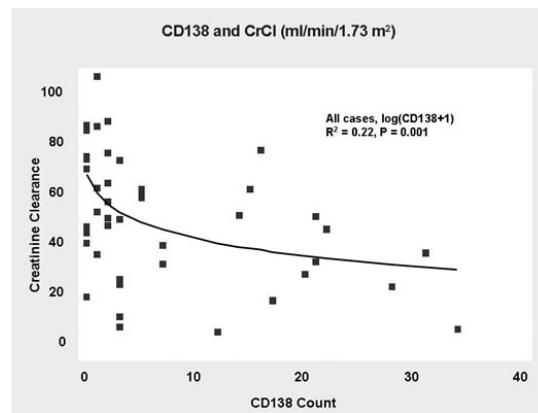
Abstract# 572**CD20+ and CD138+ Lymphocytes Are Associated with Acute Cellular Rejection and Graft Dysfunction in Pediatric Renal Recipients.** E. Tsai,¹ B. Cho,¹ D. Gjertson,¹ W. Wallace,¹ E. Reed,¹ R. Ettenger.¹ *Pediatrics, Mattel Children's Hospital UCLA, LA, CA.*

We have described the association of CD20+ B cell infiltrates in renal transplant (Tx) biopsies (bxs) with acute cellular rejection (ACR) and Tx dysfunction (dysfx). We have also found metabolically active plasma cells (Pcs) staining for S6 ribosomal protein (S6rp) within these Txs. Herein, we report the significance of CD138+ Pcs in rejection (rj) and evaluate the impact of CD20, CD138, and S6rp on long term Tx fx by calculated creatinine clearance (CrCl).

We studied 46 Tx bxs from 32 pediatric (ped) patients (pts) who were bxd for suspicion of rj from Nov 2001 to Nov 2004. Pts were given Daclizumab and maintained on prednisone, mycophenolate mofetil, tacrolimus or cyclosporine. Immunohistochemical staining and quantification for CD20, CD138, S6rp, and C4d were performed under 500x light microscopy. Bxs were classified by modified Banff 97 criteria. CrCl was followed 2 yr post-bx.

CD138+ Pcs were associated with C4d-negative ACR (p=0.0002) but not antibody mediated rj (AMR, p=0.82). ROC analysis confirmed >5 CD138+ cells/hpf strongly associated with ACR, yielding 89% sensitivity, 83% specificity, correctly classifying 84% and comprising total ROC area 0.92 (95% CI 0.82, 1). Higher CD138 counts at bx correlated with worse Tx fx (Fig 1). A univariate regression model showed that CD20, CD138, S6rp and time were associated with a decline in Tx fx at bx. Multivariate model showed that CD20, CD138, and time had the main effects on CrCl decline, with S6rp dropping out. All patients regardless of rj status had a 16 ml/min/1.73 m² CrCl decline exerted by time (p=0.004). Pts with CD20 had an additional sustained 19 ml/min/1.73 m² CrCl decline seen 2yrs post-bx (p=0.03). Pts with CD138 also had 19 ml/min/1.73 m² CrCl decline at bx (p=0.03), but there was an interaction between time and CD138 that negated a sustained effect (p=0.04).

This study identifies a numerical threshold of >5 CD138 cells/hpf that is associated with ACR and Tx dysfx. Infiltrating CD20 cells had the greatest, sustained effect on Tx dysfx. We conclude that cells of the B lineage, particularly CD20, play a key, but undefined, role in ACR.

**Abstract# 573****Intragraft Cytotoxic Effector and Regulatory Profiles Distinguish Chronic Antibody Mediated Rejection from Chronic Calcineurin Inhibitor Toxicity and Acute Rejection in Renal Transplantation.** Joanna Ashton-Chess,¹ Emilie Dugast,¹ Anne Moreau,¹ Karine Renaudin,¹ Magali Giral,¹ Robert B. Colvin,² Sophie Brouard,¹ Jean-Paul Soulillou.¹ *INSERM U643; CHU Nantes; ITRT, Nantes, France; ²Massachusetts General Hospital and Harvard Medical School, Boston.*

There is now evidence that FOXP3+ cells are not indicators of tolerance, since FOXP3 is also increased during acute rejection. However, it is unknown whether FOXP3+ cells are present during chronic antibody mediated rejection. Moreover, the relative balance of regulatory, effector and cytotoxic pathways in chronic vs. acute injury has yet to be explored. Here we addressed this issue. Intragraft regulatory, effector and cytotoxic transcriptional profiles were analysed within renal transplant biopsies (n = 43) classified (Banff 2005) as displaying normal histology, chronic calcineurin inhibitor toxicity (CNI-tox), chronic antibody mediated rejection (CAMR) and acute cellular rejection (ACR). Granzyme B, Tbet and FOXP3 mRNA were measured by quantitative PCR and FOXP3-positive cells were additionally quantified in graft biopsies by immunohistochemistry. Distinguishing mRNA profiles were analyzed in the peripheral blood (n = 26). Our data show that FOXP3 mRNA is increased not only in ACR (p<0.0001) but also in CAMR (p<0.05). Expression of FOXP3 mRNA correlated tightly with the density of FOXP3 protein-positive cells by immunohistochemistry (Spearman r = 0.71; p < 0.0001); FOXP3+ cells were found in aggregates and within tubules. Moreover, graft cytotoxic, effector and regulatory pathways were all found to be active in chronic as well as acute graft injury. Significant increases in Granzyme B, Tbet and FOXP3 mRNA were observed in CAMR, CNI-tox and ACR compared to normal histology (p<0.0001, p<0.001 or p<0.05). However, differences in the relative contribution of each pathway were evident, with significant accumulation of FOXP3 mRNA predominating in ACR and granzyme B predominating in CAMR. Thus, CAMR can be distinguished from both ACR and CNI-tox by an unfavorable intragraft Granzyme B/FOXP3 mRNA ratio (p<0.05). Interestingly, this ratio was reversed in the blood, suggesting different migratory patterns for regulatory and cytotoxic cells between the blood and the graft. Our data thus confirm that intragraft and peripheral blood FOXP3 accumulation is also a feature of CAMR of kidney grafts. Moreover, CAMR can be distinguished from other graft injury types based on its intragraft or blood cytotoxicity/regulatory profile.

Abstract# 574**Statistical and Non Statistical Analysis of Peripheral Blood Gene Expression of Operational Tolerance and Chronic Rejection in Human Renal Allograft Recipients.** Christophe Braud,¹ Victor Shivozhlezov,² Magali Giral,¹ Annaick Pallier,¹ Dominique Baeten,³ Luca Giacomelli,² Eugenia Pechkova,² Jean-Paul Soulillou,¹ Claudio Nicolini,² Sophie Brouard.¹ *INSERM U643, Nantes, France; ²Nanoworld Institute-CIRSDNNOB, Genoa, Italy; ³Clinical Immunology and Rheumatology, Amsterdam, Netherlands.*

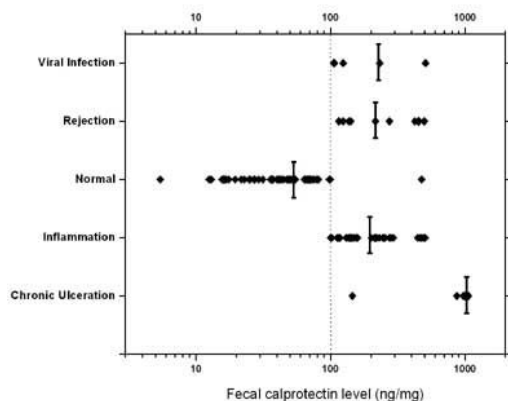
Survival of solid organ grafts depends on life long immunosuppression which results in increased rates of infection and malignancy. Induction of tolerance to allograft would represent the optimal solution for controlling both chronic rejection and side effects of immunosuppression. We previously showed that operational tolerance after kidney transplantation could occur in some patient. Here, the potential of high throughput microarray technology allowed us to study the peripheral blood gene expression profile associated to operational tolerance and chronic rejection in a cohort of human kidney graft recipients (n=26). Microarrays were used to compare the gene expression profile of PBMC from patients with chronic rejection and drug-free operationally tolerant recipients. Results have been treated using a classical statistical and a non-statistical analysis based on the identification of key leader genes associated respectively to chronic rejection and operational tolerance, either as those mostly changing their expression or having the strongest interconnections. 343 differentially expressed genes were identified between operational tolerant patients and patients with chronic rejection.

The non-statistical bioinformatic approach identified 68 leader genes which define the highest interaction genes derived from the 343 SAM-gene list. An interaction map between the genes identified has been calculated. This network is formed around 2 majors clusters: a network of interleukins and a network of signal transduction which allow us the identification of key genes such as BANK1, a negative modulator of CD40-mediated Akt activation, thereby preventing hyperactive B cell response in blood from patients with operational tolerance and IL7R, a specific marker absent on potentially regulatory CD4⁺CD25^{high} T cells in blood from patients with chronic rejection. We have identified by a non-statistical analysis of the peripheral blood gene expression in human kidney recipients a cluster of genes which are strongly interconnected and which could be a starting point for further analysis of the molecular mechanisms of kidney graft operational tolerance and chronic rejection.

Abstract# 575

Fecal Calprotectin Level as a Noninvasive Marker for Small Bowel Allograft Monitoring. Edip Akpinar,¹ Jacinto Vargas,¹ Tomaoki Kato,¹ Erick Hernandez,² Lesli Smith,² Gennaro Selvaggi,¹ Seigo Nishida,¹ Eddie Island,¹ Jang Moon,¹ David Levi,¹ Philip Ruiz,³ Andreas G. Tzakis.¹ ¹*Liver-GI Transplantation, University of Miami, Miami Transplant Institute, Miami, FL;* ²*Pediatric Gastroenterology, University of Miami, Miami, FL;* ³*Pathology, University of Miami, Miami, FL.*

Background: Protocol endoscopy with biopsy is currently the gold standard of small bowel transplant (SBTx) monitoring, however it is invasive, costly, needs skilled operator, may require anesthesia and may cause complications. Fecal calprotectin level (FCL) is a neutrophilic innate defense protein and its fecal level increases in inflammatory bowel problems. It might be a good candidate noninvasive marker for posttransplant monitoring in SBTx pts. **Methods:** A pilot study was performed to test the utility of FCL measurement in follow up of SBTx pts. Ileostomy effluents were collected at various postoperative days during endoscopy/biopsy procedures. FCLs were measured by an ELISA based commercial kit using a rabbit polyclonal antibody. A value of 100 ng/mg cut off level was selected. The results were retrospectively evaluated in combination with clinical, endoscopic and histopathological findings. FCL values are presented as median ng/mg. **Results:** FCLs were measured in 122 samples that were obtained from 29 pts at various periods after SBTx. Retrospective evaluation showed that 11 samples coincided with rejection episodes in 6 pts (FCL:217), 6 samples were coincided with viral enteritis in 3 pts (all Rotavirus) (FCL:125), 51 samples were coincided with nonspecific inflammation in 21 pts (FCL:207), 11 samples coincided with chronic intestinal ulceration in 2 pts (FCL:998), and finally 50 samples coincided with normal histopathological findings in 19 pts (FCL:43). No significant FCL difference was found between rejection and inflammation and viral infection. However FCL evolution in individuals showed that FCL can predict rejection days before histopathological diagnosis.



Conclusion: FCL is a sensitive test for ongoing organic intestinal allograft pathologies, including rejection. It might be useful as prescreening marker to avoid unnecessary endoscopies.

Abstract# 576

Algorithm Based on Multiparameter Mixed Lymphocyte Reaction Assay for Tailoring Maintenance Immunosuppressants after Living Donor Liver Transplantation. Yuka Tanaka, Hideki Ohdan, Toshimasa Asahara. *Department of Surgery, Hiroshima University, Hiroshima, Japan.*

Background: No reliable immunological parameters exist for identifying liver allograft recipients in whom immunosuppressants can be safely withdrawn. For minimizing maintenance immunosuppressants, we established an algorithm determining anti-donor alloreactivity based on multiparameter mixed lymphocyte reaction (MLR) assay, wherein the number and phenotype of alloreactive precursors can be quantified.

Methods: We enrolled 68 adults undergoing living donor liver transplantation (LT). The initial immunosuppressive regimen comprised tacrolimus/cyclosporine and methylprednisolone, which were gradually tapered off by 6 months after LT. Thereafter,

therapeutic adjustments were determined by a policy of slow tapering off in the case of normal liver function. MLR assay was performed at 6 month intervals to monitor immune status. In this assay, CFSE-labeled PBMCs from recipients were used as responders. Irradiated donor and third-party PBMCs were used as stimulators. After coculture, the responder cells were stained with CD4 or CD8 mAbs along with CD25 mAb, followed by FCM analyses.

Results: The proliferation and CD25 expression of CD4⁺ and CD8⁺ T cell subsets in response to anti-donor and anti-third-party stimuli were analyzed; the immune status of LT patients was categorized as hypo-response, norm-response, or hyper-response for CD4⁺ T cells and as hyper-response for CD8⁺ T cells. Of the 68 patients, 33 had normal liver function at >6 months after LT. We examined the fluctuation of immunosuppressants at 6 months after MLR in these patients. In patients whose immune status was categorized as hyper-response for CD4⁺ or CD8⁺ T cells (n=4), immunosuppressants had to be increased. In patients with norm-response immune status (n=7), immunosuppressant tapering was abandoned. Immunosuppressant therapy was successfully tapered off in patients with hypo-response immune status (n=22). Of 10 patients with hypo-response immune status at >3 years after LT, immunosuppressants were completely discontinued in 3. In these "operational tolerance" patients, the precursor frequency of anti-donor CD4⁺ T cells (mean=5.2±1.9%) was not reduced compared to that in non-tolerance patients, suggesting that donor-specific immune tolerance is maintained via inhibitory/suppressive mechanisms rather than via clonal deletion.

Conclusion: Multiparameter MLR assay can provide a clinically validated rule predicting the success of tailoring/weaning immunosuppression.

Psychosocial and Compliance Issues

Abstract# 577

Psychological Factors Associated with Non-Adherence among Adolescents before and after Kidney Transplant. Nataliya Zelikovskiy.¹

¹*Dept. of Pediatrics, Div. of Nephrology, The Children's Hospital of Philadelphia, Philadelphia, PA.*

Purpose: Little is known about psychosocial risk factors for poor adherence among pediatric transplant patients. Identification of variables that impact illness management can guide targeted interventions to improve adherence.

Methods: A longitudinal study was conducted to determine whether quality of life (PedsQL), family functioning (FAD), and parent adjustment (PIP) would predict adherence in adolescent transplant patients. Psychological questionnaires were administered prior to and 12 months after the transplant. Medical Adherence Measure (MAM), a semi-structured interview was used to assess adherence. Adherence was calculated as % missed and % late doses of those prescribed.

Results: 60 patients (M=14.32 years ±2.18, 75% male, 63% Caucasian) and their parents were evaluated at the time of listing for kidney transplant. The rate of non-adherence prior to transplant was high, with 90% of patients reporting some degree of non-adherence. Of these patients, 37% missed and 28% took late > 10% of prescribed doses. On the quality of life measure, behavior issues were associated with missed (r=-.42, p=.01) and late doses (r=-.39, p<.05), and mental health issues were associated with late doses (r=-.38, p<.05). Adolescent reports of problems in affective responsiveness among family members was associated with missed (r=.33, p=.04) and late (r=.51, p=.001) doses. Missed doses were also associated with mother reports of difficulties with overall family functioning (r=.34, p<.05), communication (r=.41, p<.01) and role definitions (r=.33, p<.05) among family members.

49 of the families were re-evaluated one year after the kidney transplant. 65% had been on dialysis prior to transplant and 42% received living-related transplants. 58% of the patients reported some degree non-adherence post-transplant, and using more stringent criteria, 17% of patients reported missing and 27% reported taking late >10% of prescribed doses. Worse quality of life such as limitations due to emotional problems (r=-.32, p<.05), behavioral problems (r=-.39, p<.01), and difficulties with family cohesion (r=-.32, p<.05) was related to worse adherence.

Discussion: Adolescent quality of life in behavioral and emotional domains, and family functioning play a significant role in adherence both before and after transplant. Programs to improve adherence among transplant patients should incorporate psychosocial supports and behavioral interventions to improve adjustment of patients and families.

Abstract# 578

Multi-Method Assessment of Adherence in Pediatric Kidney Transplantation. Nataliya Zelikovskiy,¹ Diana Shellmer,² JoAnn Palmer,³ Sonya Lopez,³ H. Jorge Baluarte,¹ Kevin Meyers.¹

¹*Department of Pediatrics, The Children's Hospital of Philadelphia, Philadelphia, PA;* ²*Department of Pediatrics, Pittsburgh Children's Hospital, Pittsburgh, PA;* ³*Department of Nursing, The Children's Hospital of Philadelphia, Philadelphia, PA.*

Purpose: There is no gold standard to measure adherence reliably. This study examined the rates of adherence with immunosuppressant medications for transplant recipients using a multi-method adherence battery, comparing the different detection methods.

Methods: Pediatric transplant recipients 1-3 years post transplant were recruited. Over a three-month period an adherence battery consisted of: consecutive blood serum levels, Medical Adherence Measure (MAM) a semi-structured interview, and Medication Event Monitoring System (MEMS) to track use of Prograf or Rapamune. Non-adherence was

defined as missing > 10% of prescribed doses on MAM and MEMS, and >2 SD among consecutive blood serum levels. **Results:** Participants were 28 transplant patients ($M = 14.11 \pm 3.38$ years old, 75% male, 71.4% Caucasian). On the MAM, 67.9% of the patients acknowledged some non-adherence but minimized how many doses they missed. Using MEMS technology, 90% had some non-adherence and specifically, 23.8% of the participants missed doses and only 56% of their doses were taken within the allowable time frame. Using ≥ 2 SD criteria for blood serum levels, 42% of the participants were considered non-adherent. Non-adherence worsened with years since transplant. More missed ($r = .59, p = .001$) and late doses ($r = .59, p = 0.04$) on the MAM and >2 SD among blood serum levels ($r = .83, p = .001$) was associated with higher incidence of acute rejections. Adherence data was examined for patients with documented acute rejections ($N = 12$). Sensitivity and specificity of each detection method was also examined. The MAM and SD detection methods each identified non-adherence in 67% of the patients with acute rejections; MEMS did not identify any additional non-adherent patients. Only 25% of the patients with acute rejections were identified consistently by all three adherence detection methods; all patients with acute rejections were identified by at least one method. **Discussion:** Non-adherence worsening with time since transplant and was associated with acute rejections. Since no single method of detecting adherence identified all the patients with acute rejections, multi-method adherence assessments should be used to accurately capture patients who are non-adherent.

Abstract# 579

Non-Adherence (NA) in a Population of Inner City Kidney Transplant Recipients (KTR's) Is Related to High Levels of Personal Control, Negative Perceptions Regarding Medication and Decreased Belief in the Power of Health Care Providers. Sima Terebello,¹ Mariana Markell.¹ ¹Medicine, SUNY Downstate Medical Center, Brooklyn, NY.

NA is a leading cause of allograft loss and results from multiple factors. Locus of control (LOC) and beliefs regarding health have been associated with adherence in other populations. 51 randomly chosen KTR's were interviewed using a confidential questionnaire administered by an outside investigator that included questions regarding LOC, health beliefs and self-efficacy. The population was 55% female, 92% Black, 15% Hispanic, 71% deceased donor kidney, 47% diabetic, 49% greater than High School education, 36% employed, 30% married or cohabiting, 63% income <20K per year, 65% insured by Medicaid. Mean age 47.8 ± 13.5 yrs, time on dialysis 72.8 ± 51 mos, months since transplant 23.9 ± 14.3 , total meds 7.3 ± 3.0 . By Pearson correlation, non-adherence (NA), defined as "having missed doses of immunosuppression over the preceding 3 months", was not correlated with race, gender, income, type of insurance, age, marital status, type of txp, mos on dialysis, time since transplant, or number of medications. NA was correlated with higher education level ($r = 0.4, p = 0.006$), current employment ($r = 0.3, p = 0.05$), and knowledge of most recent creatinine value ($r = 0.35, p = 0.015$). NA was associated with concerns regarding prednisone (long term effects, dependency) $r = 0.33, p = 0.018$, feelings of greater personal control over illness ($r = 0.36, p = 0.011$), and inversely correlated with powerful others LOC (feeling that one's health is dependent on other people), $r = -0.31, p = 0.029$ and belief in the necessity of medication for maintenance of transplant health, $r = -0.3, p = 0.031$. We conclude, in our population of inner-city patients: 1. NA is not associated with standard demographic factors including income, race and gender. 2. Contrary to findings in other populations, NA is associated with higher education and current employment. 3. NA is associated with knowledge about creatinine value, concern regarding long-term effects of prednisone and disbelief in the importance of transplant medications. 4. NA is associated with feelings of personal control and feeling that powerful others (e.g. health care providers) are not of high importance in the outcome of illness. 5. Education programs designed to address NA in this population should be targeted towards altering negative beliefs regarding medications and stress the importance of partnering with the transplant team for optimal long-term outcome.

Abstract# 580

Medication Adherence and Health-Related Quality of Life in Adolescent Solid Organ Transplant Recipients. Katie A. Devine,¹ Laura E. Simons,² Ronald L. Blount,¹ Laura Mee,³ Megan Ratcliff,³ Nicole Fenton,¹ Emily Osborn,¹ Sandra Amaral,³ Barry Warshaw,³ William Mahle,³ Robert Vincent,³ Alexandria Berg,³ Jill DePaolo.³ ¹Psychology, University of Georgia, Athens, GA; ²Children's Hospital Boston, Boston, MA; ³Children's Healthcare of Atlanta, Atlanta, GA.

Purpose: The present study aimed to prospectively examine the relationships among nonadherence, health-related quality of life (HRQOL), and family factors in adolescent kidney, liver, and heart transplant recipients.

Method: 68 adolescent transplant recipients aged 11 to 20 years ($M = 15.8, SD = 2.5$; 44% female; 57% kidney, 25% liver, 18% heart) and their parents participated. At baseline and 18-month follow-up assessments, adolescents and their parents independently completed phone interviews assessing self-/proxy- reported medication adherence, HRQOL, and family cohesion and conflict. Medical record reviews were conducted to obtain current medications, immunosuppressant drug assays, and clinical outcomes in the past year (i.e., rejection episodes, hospitalizations, graft loss).

Results: At baseline, adolescents classified as nonadherent based on self-report and Tacrolimus standard deviation (SD) reported significantly lower general health perceptions ($F(3, 64) = 3.63, p < .05$), self-esteem ($F = 4.58, p < .01$), mental health

($F = 3.09, p < .05$), and behavior HRQOL ($F = 2.75, p < .05$) compared to adolescents classified as adherent. Similarly, parents of adolescents classified as nonadherent reported significantly lower physical functioning ($F = 3.18, p < .05$), self-esteem ($F = 5.85, p < .01$), and behavior HRQOL ($F = 2.82, p < .05$) for their adolescents. Family conflict was correlated with adolescent report of behavior ($r = -.49, p < .01$), physical functioning ($r = -.33, p < .01$), self-esteem ($r = -.42, p < .01$), and mental health HRQOL ($r = -.34, p < .01$). Family conflict was correlated with parent report of behavior ($r = -.46, p < .01$) and physical functioning ($r = .24, p < .05$). Improvement and deterioration in HRQOL from baseline to 18-month follow-up is currently being examined. It is expected that increased family conflict and decreased medication adherence will be associated with deteriorations in HRQOL.

Conclusions: The interrelationships between medication adherence, family conflict, and HRQOL domains such as self-esteem and mental health suggest that interventions targeting these domains may result in improvements in medication adherence behavior.

Abstract# 581

CAM (Complementary & Alternative Medicine) Use in Kidney Transplant Recipients (KTR's) Is Associated with Non-Adherence (NA) to Medication and Unhappiness with the Transplant Experience. Mariana Markell,¹ Mariya Stratilatova,² Sima Terebello.¹ ¹Renal Medicine, SUNY Downstate Medical Center, Brooklyn, NY; ²Nephrology, NY Medical College, Valhalla, NY.

The use of CAM in general is associated with non-disclosure by patients to physicians. 51 randomly chosen KTRs were interviewed using a confidential questionnaire administered by an outside investigator, including questions on CAM usage, whether it was doctor-recommended, and whether the patient disclosed use. CAM was defined as ingestion of herbal or other preparations, use of mind-body techniques or manipulation of the body for healing by someone not an allopathic medical provider. Use of vitamins and spirituality were excluded. The population was 55% female, 92% Black, 15% Hispanic, 71% deceased donor kidney, 47% diabetic, 49% > high school education, 36% employed, 30% married or cohabiting, 63% income <20K per year, 65% insured by Medicaid. Mean age 47.8 ± 13.5 yrs, time on dialysis 72.8 ± 51 mos, months since transplant 23.9 ± 14.3 , total meds 7.3 ± 3.0 . 45% of patients ($n = 23$) used CAM. By Pearson r , CAM use was correlated with NA to immunosuppressants, $p = 0.041, r = 0.4$, blood sugar-lowering medications, $p = 0.003, r = 0.57$, and cholesterol lowering medications, $p = 0.0001, r = 0.84$, worries about long-term effects of medicines, $p = 0.02, r = 0.324$, belief that doctors place too much trust in medication, $p = 0.032, r = 0.3$, and that natural remedies are safer than medicines, $p = 0.034, r = 0.3$. CAM use was inversely related to belief that health depends on allopathic medicines, $p = 0.014, r = -0.341$, medicines protect from worsening disease, $p = 0.048, r = -0.28$, and that following doctors orders is the best way to stay healthy, $p = 0.04, r = -0.29$, and that having a kidney transplant makes them feel happy, $p = 0.005, r = -0.39$. We conclude, in our population of Inner-City patients: 1. Use of CAM is correlated with medication non-adherence. 2. Patients who use CAM are more worried about long term effects of medication, believe that natural remedies are safer than medications and that doctors place too much trust in medication. 3. Patients who use CAM do not believe that their health depends on allopathic medication, that medicines protect from worsening disease, or that following a doctor's orders is the best way to maintain optimal health. 4. Patients who use CAM are less happy with their kidney transplant. 5. Discussing CAM use and motivation for use is important in the Transplant Clinic and may alert the provider to possible risk for non-adherence.

Abstract# 582

Predicting Quality of Life Following Heart Transplantation: The Role of Attachment Style. Susan E. Abbey,¹ Susan Holtzman,² Donna E. Stewart,² Heather J. Ross.¹ ¹Multi Organ Transplant Program, Toronto General Hospital, University Health Network, Toronto, ON, Canada; ²Women's Health Program, Toronto General Hospital, University Health Network, Toronto, ON, Canada.

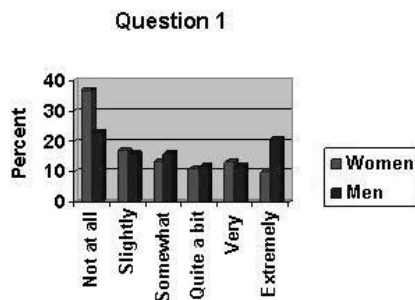
Background: According to attachment theory (Bowlby, 1977), individuals develop stable, enduring patterns of interpersonal behavior based on early childhood experiences with caretakers, and these patterns guide thoughts and expectations in relationships throughout the life span. Previous studies indicate that styles of attachment can impact physical and psychological well-being among individuals suffering from various chronic health conditions, including diabetes and cancer. However, the extent to which attachment may impact quality of life among heart transplant recipients remains unknown. **Method:** Ninety-one heart transplant recipients (74 male, 17 female), who were an average of 6 years post-transplant, completed a written questionnaire that included measures of attachment style (Relationship Questionnaire; Bartholomew and Horowitz, 1991), quality of life (SF-36; Ware et al., 2000, Illness Intrusiveness Rating Scale; Devins et al., 1983) and demographics. Linear and logistic regression analyses tested whether four styles of attachment (secure, fearful, preoccupied, dismissing) were related to post-transplant quality of life, after controlling for relevant demographic factors. **Results:** Patients high on fearful attachment (fearful of rejection, low-self-esteem) were significantly less likely to be working following transplantation. Patients high on preoccupied attachment (emotionally dependent on others) reported that their transplant interfered significantly more with various aspects of their life. Meanwhile, patients who scored high on secure attachment (trusting of others, feel worthy of others attention) reported significantly

greater emotional well-being (SF-36) and felt that their transplant interfered significantly less with various aspects of their life (IIRS). **Conclusions:** Findings highlight the potential utility of assessing attachment style in transplant populations, and tailoring clinical care based on these styles of interpersonal behaviour.

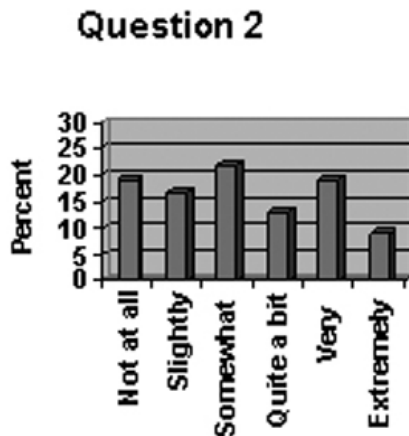
Abstract# 583

Sexual Concerns among Kidney Transplant Recipients. Rebecca J. Muehrer,¹ Dorothy M. Lanuza,¹ Arjang Djamali.² ¹*School of Nursing, University of Wisconsin Madison, Madison, WI;* ²*School of Medicine, Division of Nephrology, University of Wisconsin Madison, Madison, WI.*

Kidney transplantation leads to marked improvements in health, yet transplant (tx) recipients often have difficulty with sexual functioning, which can affect quality of life. Specific sexual concerns of tx recipients remain under investigated. The purposes of this study were to 1) further establish the psychometric properties of the sexual concerns questionnaire (SCQ) including reliability and preliminary construct validity and 2) identify the sexual concerns of kidney tx recipients. The SCQ was answered by 390 kidney tx recipients who rated each item on a 0 (not at all) to 5 (extremely) scale. A Cronbach's alpha correlation coefficient was calculated to determine the reliability of the SCQ. An alpha value of .83 was calculated for the questionnaire indicating it was reliable. Exploratory factor analysis (EFA) was performed to establish preliminary construct validity of the SCQ. As a result from the EFA, 14 items were dropped and a 5 factor structure was accepted. Examples of items and responses include Question 1: "How difficult is it for your vagina to get or stay wet or moist?" (for women) and "How difficult is it for you to get or keep an erection?" (for men)



and Question 2 "How comfortable are you talking about sexual concerns with your doctors and nurses?"



Participants were also asked to indicate how important their sexuality was to them on a 0 (not at all) to 6 (extremely) rating scale. Twenty-six percent of participants rated their sexuality as quite a bit important, 26% rated their sexuality as very important, and 20% rated their sexuality as extremely important. The findings provide evidence of a reliable questionnaire with evidence for preliminary construct validity. They also indicate that sexuality is an important issue for a majority of kidney tx recipients.

Abstract# 584

A Cross-Sectional Study of Fatigue before and after Liver Transplantation. James R. Rodrigue,¹ Timothy Antonellis,¹ David R. Nelson,² Alan I. Reed,³ Douglas W. Hanto,¹ Michael Curry.¹ ¹*The Transplant Center, Beth Israel Deaconess Medical Center, Boston, MA;* ²*Department of Medicine, University of Florida, Gainesville, FL;* ³*Department of Surgery, University of Iowa, Des Moines, IA.*

This study examined fatigue severity and sleep problems in pre- and post-LT patients and explored factors associated with fatigue severity.

Methods. 229 patients (123 pre-LT, 106 post-LT) at 2 U.S. transplant centers completed the Fatigue Symptom Inventory (FSI), Multidimensional Fatigue Symptom Inventory (MFSI), Pittsburgh Sleep Quality Index (PSQI), SF-36, and Profile of Mood States (POMS). Demographic characteristics, BMI, LT indication, MELD (pre-LT patients), waiting time (pre-LT patients), and survival days (post-LT patients) were gathered from medical records.

Results. Global scores of fatigue, sleep quality, and mood disturbance did not differ significantly between pre- and post-LT patients (all p 's > 0.05).

	Pre-LT (n=123)	Post-LT (n=106)	
Days of fatigue past wk (FSI)	4.4±2.2	4.2±2.0	p=0.56
Ave fatigue past wk (FSI)#	4.8±2.0	4.3±1.8	p=0.06
Total fatigue (MFSI)▲	18.5±20.4	18.2±17.0	p=0.92
Sleep quality (PSQI)▲	8.6±3.7	8.9±2.8	p=0.55
QOL – physical (SF-36)¶	38.6±10.5	43.1±10.2	p=0.001
QOL – mental (SF-36)¶	43.8±11.9	46.0±12.8	p=0.17
Mood disturbance (POMS)▲	17.1±18.7	16.4±16.5	p=0.78

1 (none) to 10 (extremely high); ▲ Higher score = more fatigue, poorer sleep quality, or more mood disturbance; ¶ Higher score = better QOL

One-third of pre-LT (32%) and post-LT (33%) patients reported severe fatigue. Poor sleep quality was reported by 68% and 79% of pre- and post-LT patients, respectively. Pre-LT fatigue was predicted by higher BMI ($\beta = 0.21$) and MELD ($\beta = 0.20$), depression ($\beta = 0.35$), and poor sleep quality ($\beta = 0.48$), adj $R^2 = 0.44$, $F = 10.72$, $p < 0.0001$. Post-LT fatigue was predicted by older age ($\beta = 0.19$), tension-anxiety ($\beta = 0.27$), anger-hostility ($\beta = 0.38$), and poor sleep quality ($\beta = 0.18$), adj $R^2 = 0.38$, $F = 7.45$, $p < 0.0001$. More fatigue was associated with lower SF-36 physical ($r = -0.34$) and mental ($r = -0.44$) QOL.

Conclusions. Fatigue and poor sleep quality are clinically significant problems for LT candidates and recipients. BMI, psychological functioning and sleep quality are modifiable variables that predict fatigue severity and should be targets of intervention when addressing fatigue symptoms.

Abstract# 585

Health Literacy in Kidney Transplant Recipients. Elisa J. Gordon,¹ Michael S. Wolf.² ¹*Medicine, Albany Medical Center, Albany, NY;* ²*Medicine, Northwestern University.*

Background: In order to successfully manage the transplant long-term, kidney recipients must have a basic understanding of key transplant-related concepts and terms indicative of their condition and treatment to properly communicate with health care providers and manage their health. Kidney recipients must also possess numeracy skills to enable proper medication-taking and to monitor serum creatinine levels, bodily temperature, etc. The objective of this study was to examine health literacy levels among kidney transplant recipients.

Methods: We surveyed 124 consecutive adult renal transplant recipients using the Test of Functional Health Literacy in Adults (S-TOFHLA), and a modified version of the Rapid Estimate of Adult Literacy in Medicine, called the "REALM-Transplant," which measured patients' knowledge of 69 kidney transplant-related terms that patients are expected to have familiarity with. Open-ended and multiple choice questions assessed numeracy related to kidney survival.

Results: Most kidney recipients (91%) had adequate health literacy (S-TOFHLA), but 81% were unfamiliar with at least 1 kidney transplant-related term (REALM-T). Patients who were less educated ($p < 0.0001$), had lower income ($p < 0.002$), and were single or without a partner ($p = 0.046$) had significantly lower health literacy levels (S-TOFHLA). Patients less familiar with transplant-related terms (REALM-T) had less education ($p < 0.0001$), lower income ($p < 0.0001$), and were nonwhites ($p = 0.033$). The five least familiar terms were: sensitization (50%), urethra (45%), trough level (41%), blood urea nitrogen (32%), and toxicity (31%). Sixteen percent wanted more information about their transplant. Numeracy levels varied: 21% knew the likelihood of 1-year survival; 29% knew that half of kidney recipients have problems with the transplant in the first 6 months; 86% knew the normal range of creatinine for kidney recipients; and 86% were aware of the risk of death within the first year of transplantation.

Conclusion: At this clinic, kidney transplant recipients generally had high levels of health literacy. However, most had difficulty recognizing frequently used transplant-related terms, which could impede their understanding of health information and self-care management. Greater efforts are needed to educate kidney recipients about transplant concepts, which may foster better self-care management, and ultimately transplant outcomes.

Late-Breaking Abstract Session

Abstract# LB01

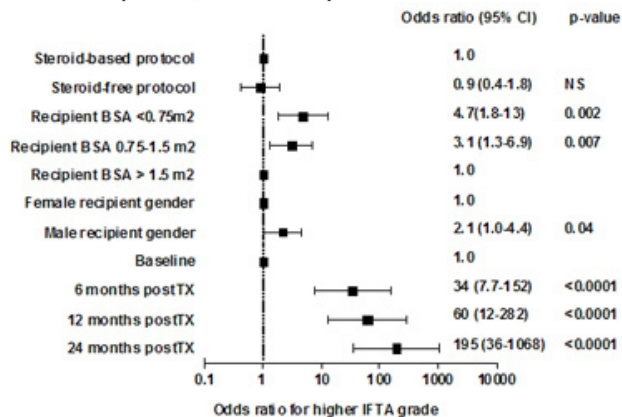
A Protocol Biopsy Analysis from an NIH Multicenter Pediatric Renal Transplant Trial Reveals No Adverse Effect of Steroid Avoidance on the Histological Evolution of Chronic Graft Injury. N. Kambham,¹ M. Naesens,¹ T. Sigdel,¹ J. Waskerwitz,¹ O. Salvatierra,¹ M. Sarwal.¹ ¹*Pediatrics and Surgery, Stanford University, CA.*

Aim: To assess the subclinical histological evolution in the first year of an open-label NIH-CCTPT multicenter trial of 130 pediatric renal transplant recipients, randomized to steroid-free (SF; n=60) or steroid-based (SB; n=70) immunosuppression.

Methods: 268 protocol biopsies performed at 0, 6 and 12 mo, were blindly analyzed

by a single pathologist using the Banff, CADI and CNIT classifications. All indication biopsies with clinical acute rejection (AR; 23.3% for SF and 21.4% for SB) were excluded from this analysis. The histological and clinical parameters were assessed using multivariate generalized-estimating-equations statistical analysis.

Results: Subclinical AR was present in 12.2% SF vs. 8.5% SB bx at 6 mo and 0% SF vs 5% SB bx at 12 mo (p=NS); borderline AR was seen in 2.4% SF vs. 8.5% SB bx at 6 mo and 10% SF vs. 5% SB bx at 12 mo (p=NS). Despite the pristine condition of the kidneys at implantation, regardless of steroid exposure, there was a significant trend increase (p<0.0001) in chronic tubulo-interstitial damage; 35% of 6 mo bx and 53% of 12 mo bx demonstrated IFTA; with moderate/severe changes (IFTA grade 2-3) in 6.8% and 10% of 6 and 12 mo bx respectively. The prevalence of biopsies with ischemic glomerular changes (p<0.0001), tubular microcalcifications (p=0.009), vascular intimal thickening (p=0.0008) and the number of sclerosed glomeruli (p<0.0001) increased over the first year after transplantation, without any difference between the SB and SF group. A critical risk factor for IFTA injury by multivariate analysis, independent of time after transplantation, was smaller recipient size.



Conclusion: In this first ever serial histological analysis, embedded in a randomized multicenter pediatric study of steroid avoidance, we found significant progression of chronic graft injury in the first year post-transplantation in both study arms. Small recipient size is the primary risk factor for tubulo-interstitial damage, likely related to vascular size discrepancies between recipient and the graft, resulting in chronic graft ischemia.

Abstract# LB02

Donor-Derived Disease Transmission Events in the United States: A Report from the OPTN/UNOS Diseases Transmission Advisory Group (DTAG). Michael Ison,¹ Joyce Hager,¹ Victoria McEwen,¹ Emily Blumberg,¹ Kevin Carney,¹ Jim Cutler,¹ Michael DiMaio,¹ Rick Hasz,¹ Lewis Teperman,¹ Amit Tevar,¹ Michael Nalesnik.¹ ¹OPTN/UNOS Diseases Transmission Advisory Group, OPTN/UNOS, Richmond, VA.

Background: Donor-derived disease transmission is increasingly recognized as a source of morbidity and mortality among transplant recipients. Policy 4.0 of the OPTN currently required reporting of donor-derived events.

Methods: All potential donor-derived transmission events (PDDTE) that were reported to OPTN/UNOS and the DTAG through the patient safety system were reviewed by the Group from January 1, 2006 – December 31, 2007. Summary data were prepared for presentation.

Results: Reports of PDDTE have increased from 7 in 2005, the first full year data was collected, to 60 in 2006, the first year that electronic submission was available, to 97 in 2007. During the 2006-2007 period, there were 52 reports of malignancy PDDTE with 9 (17%) documented transmissions and 3 attributable deaths (Table 1); there were 72 reports of infectious diseases PDDTE with 19 (26%) documented transmissions and 6 attributable deaths (Table 2). Many of the cases remain under investigation so documented transmission and deaths may increase.

Conclusions: PDDTE are being increasingly reported to DTAG. Documented transmissions occur in 17-26% of reports with 32% of confirmed transmissions resulting in patient deaths. This likely under represents the true incidence and impact of donor-derived infections.

Table 1: Potential Donor-Derived Malignancies

Malignancy	Reports in Donor	Confirmed Cases	Recipient Deaths
Breast	1	0	0
Colon	1	0	0
GBM	3	1	1
HCC	1	0	0
KS	1	0	0
Leukemia	1	0	0
Lung	7	2	2
Lymphoma	3	2	0
Melanoma	2	1	0
Myeloid Sarcoma	1	0	0
Ovarian	1	1	0
Pancreatic AdenoCarcinoma	1	0	0
Prostate	2	0	0
Renal Cell	24	2	0
Renal Papillary	1	0	0
Thyroid	2	0	0

Table 2: Potential Donor-Derived Infectious Diseases

Disease	Reports in Donor	Confirmed Cases	Recipient Deaths
Bacteremia	3	2	2
Meningitis	1	0	0
Candidemia	1	0	0
Chagas	4	3	2
EBV	3	0	0
HBV	6	0	0
HCV	9	4	0
HIV	7	4	0
Histoplasmosis	3	0	0
HTLV	1	0	0
Influenza	1	0	0
Legionella	1	0	0
Listeria	1	0	0
RMSF	1	0	0
Schistosomiasis	2	1	0
Syphilis	2	0	0
Toxoplasmosis	6	0	1
TB	8	3	1
WNV	5	0	0
Zygomycosis	1	0	0

Abstract# LB03

Prior Living Kidney Donors Who Were Subsequently Placed on the Waiting List: An Updated OPTN Analysis. Wida S. Cherikh,¹ Pang-Yen Fan,² Sarah E. Taranto,¹ Henry B. Randall,³ Carlton J. Young,⁴ ¹Research, UNOS, Richmond, VA; ²Univ. of Massachusetts Memorial Medical Ctr., Worcester, MA; ³Baylor Univ. Medical Ctr., Dallas, TX; ⁴Univ. of Alabama at Birmingham, Birmingham, AL.

Background. The safety of living donation, particularly with respect to individuals who have donated their organ and were subsequently placed on the waiting list due to end stage organ failure remains to be of great interest and importance. The present study uses the OPTN database to examine individuals who have donated their kidney (KI) and ended up on the wait list (WL) due to end stage kidney failure.

Data and Methods. We included KI candidates ever on the WL list during 1/1/1996-3/31/2007, and who were indicated as a previous donor, or had a prior living donation record in the OPTN living donor (LD) database as matched by SSN. Characteristics of the previous LDs were summarized. Relationship between donors and recipients were ascertained on previous LDs who were found through linking the OPTN LD and WL databases by SSN. Rates of being placed on the WL within 5 years of donation were computed for White and Black donors who donated during 1996-2001.

Results. There were 148 previous living KI donors who were on KI WL during 1/1/1996-3/31/2007. Sixty-two (42%) of them were White, 64 (43%) Black, 13 (9%) Hispanic, 5 (3%) Asian, and 4 (3%) other. Three most common WL diagnoses were glomerular diseases, hypertensive nephrosclerosis and diabetes. Majority of these donors donated between the age 18 and 34. Median and mean age at donation was 30 and 31.9 years, respectively. Majority of the donors donated to their full siblings and most have received a kidney transplant since placed on the WL.

Overall median and mean years from donation to listing were 20 and 18.7 years, respectively. Median number of years from donation to listing was 21 years for White and 16 years for Black donors. Two (0.01%) of the 19,918 White donors and 4 (0.10%) of the 3,777 Black donors who donated during 1996-2001 were placed on the KI WL within 5 years of donation.

Summary. Since the median time for previous living donors to be placed on the KI WL is 20 years, the number and the rate of being placed on the WL within 5 years of donation are very small. However, our findings on the relatively shorter time for Black donors to be placed on the WL and the higher rate of being on the WL within 5 years of donation experienced by Black donors are interesting and warrant further investigation.

Abstract# LB04

CNI Sparing in *De Novo* Renal Transplantation: 3-Year Results from the Symphony Study. H. Ekberg, H. Tedesco-Silva, A. Demirbas, S. Vitko, J. Klempnauer, A. Gurkan, R. Margreiter, C. Hugo, J. Grinyo, U. Frei, Y. Vanrenterghem, P. Daloze, P. Halloran. *Lund Univ, Sweden; Federal Univ Sao Paulo, Brazil; Akdeniz Univ, Turkey; IKEM, Czech Republic; Medizinische Hochschule Hannover, Germany; SSK Tepecik Hospital, Turkey; Universitaetsklinik Innsbruck, Austria; FAU Erlangen-Nuernberg, Germany; Ciutat Univ de Bellvitge, Spain; Virchow-Klinikum, Germany; KU Leuven, Belgium; CHUM Montreal, Canada; Univ Alberta, Canada.*

Introduction: In the 1-year Symphony core study, a regimen with 2g mycophenolate mofetil (MMF) + low-dose tacrolimus (3-7 ng/ml) + daclizumab + steroids resulted in less acute rejections and better glomerular filtration rate (GFR) compared with 2g MMF + steroids and either standard-dose cyclosporine (CsA), low-dose CsA (50-100 ng/ml) + daclizumab or low-dose sirolimus (4-8 ng/ml) + daclizumab.

Methods: 960 patients participated in an optional follow-up of 2 years. GFR data from 79% of included patients (48% of the core ITT population) were available at 3 years. Here we present results in the ITT population.

Results: At inclusion into follow-up 47%, 34% and 17% of patients received CsA, tacrolimus or sirolimus, and at 3 years 37%, 31% and 14%, respectively. Many follow-up patients had been switched to tacrolimus in the 1st year, including 24% of patients randomized to sirolimus. At 3 years 95% of patients were on MMF and 69% on steroids.

Over the 2nd and 3rd year all arms had a low rate of biopsy-proven acute rejection (BPAR; 2-4%) and of graft loss (3-5%). Low-dose tacrolimus remained clearly superior in terms of BPAR (13% vs. 26%-38% in the other arms). Uncensored 3-year graft survival was 89% with low-dose tacrolimus and low-dose CsA, 87% with standard-dose CsA and 85% with low-dose sirolimus ($p=0.19$). Patient survival was between 94% and 97% ($p=0.52$). In the four arms, the mean GFR change over the 2nd and 3rd year was between +1 and -3 ml/min and low-dose tacrolimus still had the highest GFR (69 vs 64-66 ml/min, $p=0.15$).

Conclusions: Observational follow-up results based on approximately half of the core Symphony population indicate that during the 2nd and 3rd year renal function was stable, BPAR and graft loss rates were low and many patients changed treatment regimen substantially. Still, the ITT arm with 2g MMF + low-dose tacrolimus + daclizumab + steroids was superior at 3 years with respect to renal function and graft loss but differences were less marked and statistically not significant.

Abstract# LB05

Discovering What the Eye Can't See: Gene Expression in Baseline Biopsies of Kidney Allografts. P. Cravedi,¹ O. Cheng,¹ A.D. Kirk,² D. Kleiner,³ R.B. Mannon.¹ ¹NIDDK, NIH, Bethesda, MD; ²NCI, NIH, Bethesda, MD; ³Division of Transplantation, Emory University Hospital, Atlanta, GA.

Histological evaluation of time zero donor kidney biopsies has not conclusively predicted graft outcome. We hypothesize that gene expression analysis could provide additional information to determine graft outcome in the first year of transplantation. To this end, we evaluated all 49 implantation biopsies obtained post reperfusion in 18 deceased donors (DD) and 31 living donors (LD) at our center. Biopsies were evaluated and scored using Banff criteria. Low density real time PCR arrays were utilized to measure intragraft expression of 95 genes associated with programmed cell death, fibrosis, innate and adaptive immunity, and oxidative stress signaling. Results of expression were defined as folds compared to a pool of 25 normal kidney biopsies. In DD, histological features of ATN were more common (44%) than in LD grafts (6%; $p<0.001$), whereas arteriosclerosis was infrequent in both groups (6% and 15%, respectively), as well as the extent of glomerular sclerosis (0% and 2%). There was no association between these histological features and renal function at 1 year post transplant. Not surprisingly, DD grafts displayed a pattern of gene expression remarkably different from LD, including an increased expression of complement protein C3 (10.8±1.9 vs. 0.5±0.1-fold, $p<0.001$), and the chemokines IL-8 (14.8±5.4 vs. 3.0±0.5-fold, $p=0.001$) CXCL1 (7.6±3.0 vs. 1.7±0.2-fold, $p<0.001$) and CXCL2 (10.2±2.1 vs. 3.1±0.3-fold, $p<0.001$), consistent with the proinflammatory setting of ischemia-reperfusion injury. Intriguingly, donor biopsies in recipients with serum creatinine <1.5 mg/dL at 1 year had significantly higher expression of the angiogenic peptide angiogenin (4.50.9 vs. 1.40.3 folds, $p<0.05$) and lower levels of apoptosis markers Fas ligand (1.00.5 vs. 1.30.4 folds, $p<0.05$) and clusterin (1.00.2 vs. 1.50.3 folds, $p<0.05$), stress marker heat shock protein 2 (1.10.2 vs. 1.70.4 folds, $p<0.05$), and cell surface receptor b2 integrin (0.70.1 vs. 1.00.1 folds, $p<0.01$), indicating a potentially reparative milieu associated with later improved function. Thus, gene expression analyses of post-reperfusion kidney graft biopsies may reflect intragraft events not detectable histologically. Recognizing and understanding these pathways may shape patient management and guide the design of targeted therapies to improve graft outcomes.

Abstract# LB06

ISA247: A Phase IIB Multicenter, Open Label, Concentration-Controlled Trial in *de novo* Renal Transplantation. A.O. Gaber,¹ S. Busque,² S. Mulgaonkar,³ R. Gaston,⁴ A. Jevnikar,⁵ H.-U. Meier-Kriesche,⁶ ¹The Methodist Hospital; ²Stanford School of Medicine; ³St. Barnabas Medical Center; ⁴University of Alabama Birmingham; ⁵London Health Sciences Centre; ⁶University of Florida.

Background: ISA247 is a novel calcineurin inhibitor (CNI), developed using a pharmacodynamic approach for use in autoimmune disease and solid organ transplantation. In moderate to severe plaque psoriasis, a Canadian Phase III trial has demonstrated that ISA247 is efficacious with minimal changes to renal function and a European trial is presently underway comparing ISA247 to cyclosporine A (CsA). In renal transplantation, a Phase IIa study comparing ISA247 to CsA in stable renal transplant recipients demonstrated ISA247 to be efficacious and well tolerated. A Phase IIb study in *de novo* renal transplant patients comparing ISA247 to tacrolimus is ongoing and final data will be available May 2008. We hypothesize that ISA247 is non-inferior to tacrolimus in terms of efficacy.

Methods: This is a 6 month, randomized, multicenter, open-label, concentration-controlled study comparing three oral ISA247 dosing groups (0.4, 0.6, or 0.8 mg/kg bid) to tacrolimus in 42 North American transplant centres. All CNI's were titrated to target trough concentrations. Inclusion criteria included males and (non-pregnant) females between the ages of 18-65 who were receiving a first deceased or living donor renal transplant. Cold ischemia times were to be ≤ 24 hours, and peak panel reactive antibodies ≤ 30%. The primary efficacy parameter of the trial is non-inferiority (in at least one dose group) in biopsy proven acute rejection (BPAR) at 6 months as compared to tacrolimus. Secondary objectives include: renal function; PK/PD relationships; patient and graft survival; and proportion of patients with hypertension, hyperlipidemia or new onset diabetes mellitus (NODM).

Results: Interim data, as previously presented at the 2007 ATC, demonstrated that ISA247 had rejection rates similar to tacrolimus (ISA247 0.4 mg/kg bid 11%, ISA247 0.6 mg/kg bid 8%, ISA247 0.8 mg/kg bid 3%, tacrolimus 9%) and confirmed previous results indicating an improved safety profile. 334 patients have now been enrolled between January 2006 and June 2007, with an optional extension to 12 months added to the trial. A recent approval by both FDA and Health Canada has allowed continued use of ISA247 in these patients until commercialization. The six month final results will be available for presentation at ATC 2008.