Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

20 Other Forms of Immunosuppression

BEN SPRANGERS, JACQUES PIRENNE, CHANTAL MATHIEU, and MARK WAER

CHAPTER OUTLINE

Small Molecules

LEFLUNOMIDE AND MALONONITRILAMIDES

Leflunomide, initially developed as an agriculture herbicide, was explored as an immunosuppressant because of its ability to inhibit the enzyme dihydroorotate dehydrogenase.[1] The potential of leflunomide as an immunosuppressant in the field of transplantation was extensively demonstrated in various experimental studies, but its long half-life (several days) poses the problem of potential overimmunosuppression in transplant patients. Analogs of the active metabolite of leflunomide have been developed and are called malononitrilamides (MNAs). FK778 (also known as MNA715 or HMR1715) is the best studied synthetic MNA, and as it has a much shorter half-life than leflunomide (6–45 hours vs. 15–18 days) it was believed to represent an attractive alternative to leflunomide for application in organ transplantation.[2]

Chemical Structure and Pharmacology

Leflunomide (*N*-(4)) trifluoro-methylphenyl-5-methyl-isoxawol-4-carboximide) is a prodrug and is rapidly converted to its biologically active metabolite teriflunomide (A771703). Serum levels of teriflunomide are referred to as leflunomide levels. The half-life of teriflunomide is long in humans (approximately 15 days). The drug enters the enterohepatic recirculation and is excreted by the intestinal and urinary systems in equal proportions. Leflunomide is insoluble in water and is suspended in 1% carboxymethylcellulose for oral administration.

The MNAs are designed to be structurally similar to A771726. Oral bioavailability of FK778 is not substantially affected by food, and no gender effect on pharmacokinetics was observed in phase I studies.

Mechanism of Action

Leflunomide and its analogs have strong antiproliferative effects on both T lymphocytes and B lymphocytes, thus limiting the formation of antibodies.[3,4] Inhibition of pyrimidine synthesis is the most important mechanism of action as leflunomide directly inhibits the enzyme dihydroorotate dehydrogenase.[5] Lymphocytes rely entirely on the de novo pathway of pyrimidine biosynthesis and cannot use another, the so-called "pyrimidine salvage pathway." Dihydroorotate dehydrogenase inhibition leads to depletion of the nucleotide precursors uridine triphosphate and cytidine triphosphate, which are necessary for the synthesis of RNA and DNA, and hence strongly suppress DNA and RNA synthesis.

The in vivo mechanism of action of leflunomide may depend on factors such as drug levels, disposable uridine pools, and the immune activation pathway involved. Studies have indicated that, in addition to inhibition of dihydroorotate dehydrogenase, leflunomide and the MNAs may act through inhibition of tyrosine kinases. Phosphorylation of the epidermal growth factor receptor of human fibroblasts has been shown to be inhibited by leflunomide.[6] It was shown that leflunomide directly inhibited the interleukin (IL)-2-stimulated protein tyrosine kinase activity of p56lck and p59fyn,[6] which is associated with activation through the T cell receptor/CD3 complex. At higher concentrations, A771726 also inhibited IL-2-induced tyrosine phosphorylation of Janus kinase (JAK)1 and JAK3 protein tyrosine kinases.[7] Leflunomide analogs have also been shown to possess strong inhibitory activity on the antiapoptotic tyrosine kinase Bruton's tyrosine kinase, a key factor for T cell-independent antibody formation.[8] The hypothesis that leflunomide may exhibit more than one mechanism of action in vivo was further illustrated in mice where uridine restored proliferation and IgM production by lipopolysaccharide-stimulated B cells, whereas suppression of IgG production was not reversed. This phenomenon correlated in a dose-dependent manner with tyrosine phosphorylation of JAK3 and STAT6 proteins, known to be involved in IL-4-induced signal transduction pathways.[4] This double in vivo mechanism of action was confirmed in rats, in which xenoreactivity was counteracted by the administration of uridine, whereas alloreactivity was not.[9]

Inhibition of various macrophage functions by leflunomide and MNAs has also been described; in particular, inhibition of the production of oxygen radicals,[10–12] the inhibition of IgE-mediated hypersensitivity responses,[13] the expression of IL-8 receptor type A, as well as tumor necrosis factor (TNF)-mediated nuclear factor kappa B (NF-κB) activation.[14] Tacrolimus also inhibits maturation of dendritic cells by preventing upregulation of activation markers and IL-12 production, and this phenomenon was not reversible by exogenous uridine. FK778 has equivalent or stronger immunosuppressive activity than leflunomide, both in vitro and in vivo.[2] The immunosuppressive effect is synergistic with that of calcineurin inhibitors (CNI) and mycophenolate mofetil (MMF).[15,16]

Interestingly, FK778 and leflunomide have been shown to possess antiviral effects, although the precise mechanism is unclear: inhibition of viral replication of members of the herpesvirus family by preventing tegument acquisition by viral nucleocapsids during the late stage of virion assembly has been implicated.[17,18] Leflunomide is effective against multidrug-resistant cytomegalovirus (CMV) in vitro,[19] although this in vitro activity is modest and the selectivity index is low.[20] This anti-CMV effect of leflunomide and FK778 was confirmed in a rat model of heterotopic heart transplantation.[21,22] Another interesting feature is that both leflunomide and FK778 have vasculoprotective effects, independent of the inhibition of dihydroorotate dehydrogenase.[23,24]

Experimental Experience

In various rodent transplantation studies, leflunomide was shown to be at least equally potent as cyclosporine,[1] and able to synergize with cyclosporine to induce tolerance.[25] Specific characteristics of leflunomide-mediated immunosuppression in rats were its ability to interrupt ongoing acute rejections,[26,27] and its efficacy in preventing and treating chronic vascular rejection.[28]

One of the most attractive characteristics of leflunomide and the MNAs is their strong capacity to delay xenograft rejection and to induce partial xenograft tolerance.[29] This may be related to the strong suppressive effects of leflunomide on T cell-independent xenoantibody formation, and on its capacity to induce natural killer (NK) cell nonresponsiveness and to modulate xenoantigen expression.[30]

Monotherapy with FK778 in rats,[31] and its combination with microemulsified cyclosporine in dogs[32] or tacrolimus in nonhuman primates,[33] reduced chronic allograft nephropathy[31] and significantly prolonged renal allograft survival.[31–33]

Clinical Experience

The main role of leflunomide in renal transplantation nowadays is the treatment of BK virus nephropathy (BKVN), although its efficacy has never been documented in trials.[34–38] Based on the in vitro effective anti-BK concentration, an in vivo target level of 50 to 100 mg/mL has been proposed. In a prospective study, 26 renal transplant recipients with biopsy-proven BKVN were treated with leflunomide in combination with discontinuation of MMF and reduction of tacrolimus to a 4 to 6 ng/mL range.[38] Although the leflunomide levels were in the lower range (on average 50 mg/mL), a significant reduction in serum and urine BK virus (BKV) titers was obtained, allograft function stabilized, and the overall graft loss rates because of BKV were only 15%.[35] Less encouraging results were obtained in another prospective open-label study in which viral clearance was only obtained in 40% of patients with significant toxicity, resulting in discontinuation of the drug in 17% of patients.[39] The contribution of reduction of immunosuppressive agents and leflunomide to the efficacy of BKVN treatment is unclear at this moment.[40–42] Based on recent in vitro data, it has been suggested that the combination of mammalian target of rapamycin (mTOR) inhibition with leflunomide might be an effective treatment approach.[43] Treatment with leflunomide in kidney transplant recipients with BK viremia was able to prevent the development of

BKVN.[44] A study from Jaw et al. reported on three kidney transplant recipients with BKVN who were treated with a combination of leflunomide and everolimus; all patients experienced significant reductions in viral loads (one with complete resolution) and two patients had preserved allograft function at the end of follow-up.[45] In case reports, patients with resistant/refractory CMV infections,[46–50] extensive cutaneous warts,[51] and a patient with Kaposi's sarcoma[52] have been successfully treated with leflunomide. FK778 has also been studied in the context of BKVN, but although it was able to decrease BK viral load, FK778 treatment was associated with more acute rejections, decreased renal function, and more adverse events compared with reduction of immunosuppression.[53]

In animal studies, leflunomide was able to reverse acute and chronic rejection. Two clinical studies reported that leflunomide was capable of stabilizing allograft function in patients with worsening allograft function due to chronic allograft dysfunction.[54]

A phase II multicenter study was performed with FK778 involving 149 renal transplant patients,[55] where FK778 was combined with tacrolimus and corticosteroids. The patients receiving FK778 experienced a reduced number of acute rejections, but there was no effect on graft survival at week 16.[55] The reduction of acute rejection episodes was most pronounced in the subgroup in which target levels were obtained in the second week. Of note, mean total and low-density lipoprotein cholesterol levels were 20% lower in the FK778 group versus the placebo group.[55] The validity of these results was hampered by the design of the study, and, at this time, the development of FK778 in the field of organ transplantation has ceased.

Toxicity

Although rats tolerate leflunomide well, dogs readily develop anemia and gastrointestinal ulcerations. Reportedly, the most frequent side effects in arthritis patients receiving long-term leflunomide treatment were diarrhea (17%), nausea (10%), alopecia (8%), and rash (10%), leading to a dropout rate of ± 5%.[56] Recently, thrombotic microangiopathy attributed to leflunomide was reported in patients treated for BKVN.[54] In the phase II study mentioned previously, involving FK778, there was a dose-dependent increase in side effects, including anemia, hypokalemia, symptomatic myocardial ischemia, and esophagitis.[55] Other reported side effects are pneumonitis and peripheral neuropathy.[57,58] Leflunomide has teratogenic effects in both animals and humans, and a washout period with cholestyramine is advised for both women and men before considering conception.[59] Combining leflunomide with methotrexate might increase the risk for bone marrow suppression and liver toxicity.[60,61] Furthermore, rifampin accelerates the conversion of leflunomide to teriflunomide, and might increase the levels. Combination with warfarin potentially increases the international normalized ratio.

Conclusion

The role of leflunomide in renal transplantation is limited to the treatment of patients with BKVN and some promising results have been reported in this respect. The MNAs, because of their shorter half-life, were considered a promising class of immunosuppressants but results in randomized clinical trials have been disappointing, and the development of these agents in organ transplantation has been halted.

FTY720 OR FINGOLIMOD

Chemical Structure and Pharmacology

FTY720 or 2-amino-2-[2-(4-octylphenyl)ethyl]-1,3-propanediol hydrochloride is a synthetic structural analog of myriocin, a metabolite of the ascomycete *Isaria sinclairii,* a type of vegetative wasp.[62–64] Maximal concentration and area under the curve are proportional to the dose, indicating that the pharmacokinetic profile of FTY720 is linear. The volume of distribution is largely superior to the blood volume, indicating a widespread tissue penetration. FTY720 undergoes hepatic metabolism and has a long half-life (around 100 hours). ASP0028 is a newly developed $S1P_1/S1P_5$-selective agonist in Astellas Pharma Inc.

Mechanism of Action

FTY720 has a unique mechanism of action as it mainly affects lymphocyte trafficking.[30,65–67] FTY720 acts as a high-affinity agonist of the sphingosine 1-phosphate receptor-1 (S1PR1 or Edg1). Binding of its receptor results in internalization of S1PR1, rendering lymphocytes unable to respond to the naturally occurring gradient of S1P (low concentrations in thymus and secondary lymphoid organs, high concentrations in lymph and plasma) retaining lymphocytes in the low-S1P environment of lymphoid organs.[67,68] After FTY720 administration in mice, B and T cells immediately leave the peripheral blood and migrate to the peripheral lymph nodes, mesenteric lymph nodes, and Peyer's patches. The cells return to the peripheral blood after withdrawal of the drug without undergoing apoptotic death.[69] This altered cell trafficking is accompanied by a reduction of lymphocyte infiltration into grafted organs.[69–71] Interestingly, lymphocytes treated ex vivo with FTY720 and reintroduced in vivo similarly migrate to the peripheral lymphoid tissues, indicating that FTY720 acts directly on lymphocytes. This process of accelerated homing was completely blocked in vivo by coadministration of anti-CD62L, anti-CD49d, and anti-CD11a monoclonal antibody.[30] In vitro, FTY720 in the presence of TNF-α increases the expression of certain intercellular adhesion molecules on human endothelial cells.[72] Thus alteration of cell trafficking by FTY720 may result not only from its direct action on lymphocytes, but also from an effect on endothelial cells.

Interestingly, it has been suggested that $CD4^+CD25^+$ regulatory T cells are differently affected by FTY720 compared with T-effector cells.[73] $CD4^+CD25^+$ regulatory T cells express lower levels of $S1P_1$ and $S1P_4$ receptors and, hence, show reduced response to FTY720. Furthermore, in vitro FTY720-treated $CD4^+CD25^+$ T-regulatory cells possess an increased suppressive activity in an antigen-specific proliferation assay.[73,74]

Unlike CNI, FTY720 is a poor inhibitor of T cell function in vitro.[75] In particular, FTY720 does not influence antigen-induced IL-2 production. In vitro exposure to high FTY720 concentrations (4×10^{-6}) induces chromatin

condensation, typical DNA fragmentation, and formation of apoptotic bodies. Whether administration of FTY720 in vivo is also associated with significant apoptosis is a matter of debate.[30,76]

S1PR are also present on murine dendritic cells. Upon administration of FTY720, dendritic cells in lymph nodes and spleen are reduced, the expression of CD11b, CD31/PECAM-1, CD54/ICAM-1, and CCR-7 is downregulated, and transendothelial migration to CCL19 is diminished.[77] In a recent study it was demonstrated that FTY720 inhibited lymphangiogenesis and thus prolonged allogeneic islet survival in mice.[78]

Experimental Experience

FTY720 given daily by oral gavage has marked antirejection properties in mice, rats, dogs, and monkeys.[75,76,79,80] FTY720 (0.1–10 mg/kg) prolongs survival of corneal and skin allografts in highly allogeneic rodent models.[81,82] In a DA to LEW rat combination, a short course of peritransplant oral FTY720 (5 mg/kg; days −1 and 0) prolongs cardiac allograft survival and is as efficient as a 10-day posttransplant treatment with tacrolimus at 1 mg/kg.[83] Cardiac and liver allograft survival is prolonged in the August and Copenhagen Irish (ACI) rat to Lew rat model by either induction or maintenance treatment with FTY720.[84] Even delayed administration of FTY720 interrupts an ongoing allograft rejection, suggesting a role for FTY720 as a rescue agent.[85,86] FTY720 blocks not only rejection but also graft-versus-host disease after rat intestinal transplantation.[87] FTY720 may also protect from ischemia-reperfusion injury, partially through its cytoprotective actions.[88–91]

Both small- and large-animal models provide evidence that FTY720 acts in synergy with CNI, and that this benefit does not result from pharmacokinetic interactions.[85] An induction course with FTY720 acts in synergy with posttransplant tacrolimus in prolonging cardiac allograft survival in rats.[86] A similar phenomenon has been observed when FTY720 is used posttransplant in combination with cyclosporine in rat skin and heart allografts.[85,92] FTY720 shows synergistic effect with CNI in heart and liver transplant in the ACI to Lew rat model.[80] FTY720 shows synergy with cyclosporine in dog kidney (0.1–5 mg/kg/day) and monkey kidney (0.1–1 mg/kg/day) transplantation.[75] FTY720 (0.1 mg/kg) synergizes with CNI in dog liver transplantation.[93] Synergy between FTY720 and rapamycin was also observed in rat cardiac transplantation.[94] In a murine lung transplant model, FTY720 attenuated ischemia-reperfusion injury.[95] In a sensitized murine cardiac transplant model, FTY720 in combination with CTLA4-Ig resulted in inhibition of alloantibody production, reduction of donor-specific IFN-γ-producing T cells and prolonged allograft survival.[96]

KRP-203 or 2-amino-2-(2-[4-3(-benzyloxyphenylthio)-2-cholorophenyl]ethyl)-1,3-propanediol hydrochloride has a similar molecular structure as FTY720. KRP-203 alone or in combination with low-dose cyclosporine or MMF prolonged skin, heart, and renal allograft survival.[97–99] A short course of KRP203 in BALB/c mice receiving C57BL/10 islet allografts resulted in significantly prolonged islet allograft survival.[100] Additional injection of intragraft regulatory T cells allowed for prolonged drug-free graft survival, suggesting tolerogenic effects.[100]

In rats ASP0028 at a dose of 1.0 mg/kg level significantly decreased the number of peripheral lymphocytes. In addition, heart transplant studies in rats indicated that ASP0028 combined with suboptimal-dose of tacrolimus significantly prolonged allograft survival, comparable to that of FTY720 in combination with suboptimal-dose of tacrolimus with a wider margin of safety than FTY720, in terms of side effects of macular edema and bradycardia.[101] In a study using a cynomolgus monkey renal transplantation model, ASP0028 was evaluated in combination with suboptimal-dose of tacrolimus. In the animals receiving ASP0028 allograft median survival time was significantly prolonged.[101]

Clinical Experience

Stable renal transplant patients maintained on cyclosporine tolerate well one oral dose of FTY720 (0.25–3.5 mg). Similarly to its effect in animals, single doses of FTY720 cause a lymphopenia that is dose-dependent in intensity and duration, and that equally affects CD4 cells, CD8 cells, memory T cells, naïve T cells, and B cells.[102]

In phase II and III studies in de novo renal transplantation, it was shown that 2.5 mg FTY720 in combination with full-dose cyclosporine and steroids is as effective as MMF in combination with full-dose cyclosporine and steroids, although the FTY720-treated patients had lower creatinine clearance at 12 months.[103,104] FTY720 5 mg did not allow a 50% reduction in cyclosporine exposure.[103,105] FTY720 2.5 mg in combination with reduced-dose cyclosporine resulted in underimmunosuppression.[106] Also in combination with tacrolimus, FTY720 2.5 mg was not superior to MMF in a recent study in de novo renal transplant recipients.[107] Recently, it was reported that FTY720 in combination with everolimus was not beneficial with regard to prevention of acute rejection and preservation of allograft function in renal transplant recipients at high risk for delayed graft function.[108]

Toxicity

The side effects of FTY720 are in general similar to those of other immunosuppressants, with hypertension, anemia, constipation, and nausea most commonly reported. Side effects specific for FTY720 are bradycardia, macular/retinal edema, dyspnea, and a transient rise in liver function tests.[109,110] Although it is considered a main impediment of further clinical development, reduction of heart rate after the first dose of FTY720 is transient and does not persist in the maintenance phase.[109,110] Importantly, typical side effects of CNI, such as nephrotoxicity, neurotoxicity, and diabetogenicity, have not been observed with FTY720.

Conclusion

FTY720 has a unique mechanism of action. The available clinical studies show that FTY720 is not superior to standard care and therefore its future in organ transplantation is uncertain.

BREDININ OR MIZORIBINE

Chemical Structure and Pharmacology

Bredinin, 4-carbamoyl-1-β-D-ribofurano-syhmidazolium-5-olate, is a nucleoside analog that is structurally similar to

ribavirin. It was first isolated from the culture media of the ascomycetes *Eupenicillium brefeldianum* harvested from the soil of Hachijo Island (Japan, 1971). It has weak antibiotic activity against *Candida albicans.*[111]

Mechanism of Action

Mizoribine exerts its immunosuppressive function through selective inhibition of the enzymes inosine monophosphate dehydrogenase and guanosine monophosphate synthetase, both of which are required for the generation of guanosine monophosphate from inosine monophosphate in the de novo pathway. In contrast to azathioprine, mizoribine is not incorporated into nucleic acids in the cells, resulting in fewer side effects, such as myelosuppression and hepatotoxicity. Mizoribine was found to inhibit both humoral and cellular immunity by selectively inhibiting lymphocyte proliferation.[112]

Experimental and Clinical Experience

In a canine model of renal transplantation, mizoribine prolonged graft survival. In humans, compared with azathioprine, mizoribine showed equally potent immunosuppressive activity and fewer adverse effects.[113–116] As expected based on its similarity in structure to ribavirin, mizoribine exhibits in vitro antiviral activity against CMV, respiratory syncytial virus, measles, hepatitis C, corona virus, parainfluenza, and influenza virus.[117–120] In a clinical study, mizoribine was substituted for MMF in patients with BKV in their urine. BKV DNA in the urine became negative in five out of seven patients.[121] In the remaining two patients, there was a significant decrease in urinary BKV DNA. No acute rejection or deterioration of graft function occurred during the study period.[121] Mycophenolate substitution with mizoribine has also been shown to be able to prevent and reverse gastrointestinal symptoms.[122,123] Japanese studies have suggested different combinations including mizoribine (with cyclosporine/basiliximab/corticosteroids or with everolimus/tacrolimus) in living donor kidney transplantation.[124,125]

Toxicity

The principal adverse reactions associated with the use of mizoribine were leukopenia, abnormal hepatic function, rash, increased levels of uric acid, and vomiting. Hyperuricemia can result in acute renal failure in renal transplant recipients treated with high-dose mizoribine.[126]

Conclusion

Mizoribine has mainly been used in Japan and is infrequently used elsewhere. As a consequence, experience with mizoribine is limited, but results show that it is a safe and effective immunosuppressant in human kidney transplantation. Because of its antiviral activity, mizoribine might be yet another drug to be evaluated in the setting of BKVN.

JAK3 INHIBITORS: TOFACITINIB

Several JAK3 inhibitors have been developed (e.g., tyrphostin AG-490, PNU156804, dimethoxyquinazoline compounds [WHI-P131], CP-690,550 [tofacitinib], and Mannich base NC1153), and several of them have been shown to possess immunosuppressive properties.[127–130] Given the lymphocyte-restricted role of JAK3, JAK3 inhibitors are considered an interesting novel class of immunosuppressive drugs.

Chemical Structure and Pharmacology

Of the multiple potential candidate compounds, one has entered clinical trials: the ATP congener tofacitinib (Pfizer, NJ), which binds to the ATP catalytic site on JAK molecules. It has been proposed that analysis of P-STAT5 at the cellular level could be an adequate means to monitor the immunomodulatory effect of tofacitinib.[131] The oral bioavailability of tofacitinib is above 70% and tofacitinib has a volume of distribution of 87L. Tofacitinib is metabolized primarily by CYP3A4 and to a much lesser extent by CYP2C19, and its metabolites are largely inactive. Due to its metabolism through the CYPA4 pathway, drug interactions in solid-organ transplant recipients can be expected and dose adjustments are necessary in the presence of azole antifungals and rifamycins. Dose adjustments are recommended in the context of renal and hepatic failure.

Mechanism of Action

JAK3 is a tyrosine kinase essential for the signal transduction from the common gamma-chain of the cytokine receptors for IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21 to the nucleus. As the expression of the receptors is restricted to immune cells, this makes them an attractive target for new immunosuppressants. Signal transduction mediated by JAK3 is obligatory for lymphocyte activation, differentiation, and homeostasis, as evidenced by the finding that deficiency in JAK3 results in severe combined deficiency syndrome.[132–135] A possible detrimental effect of interference with IL-2 signaling relates to the fact that tolerance induction is essentially dependent on the IL-2 pathway.[136–138] Thus the challenge for immunosuppressive drug design has been to achieve selective inhibition of JAK3 versus JAK2 activities. Tofacitinib was initially claimed to be a selective JAK3 inhibitor that was found to have 20- to 100-fold less activity to both JAK1 and JAK2 based on inhibitory concentration 50% (IC_{50}) values; however, numerous other laboratories published inconsistent IC_{50} results at the various JAK domains. Tofacitinib inhibits JAK1 and JAK2 to a greater degree than was initially thought and also inhibits Tyk2 marginally.[139–144]

Experimental Experience

Tofacitinib is the most potent (inhibitory potency of 1 nM) and selective JAK3 inhibitor developed to date. In both rodents and nonhuman primates, tofacitinib exerted strong suppression of immune reactions and prolonged the survival of cardiac and renal allografts. In monotherapy it significantly delayed the onset of rejection in kidney allografts.[145–147] In nonhuman primates, tofacitinib significantly reduced IL-2-enhanced IFN-γ production and CD25 and CD71 expression by T cells. Furthermore, tofacitinib inhibited cellular alloimmune responses in vitro.[147,148] Administration in vivo resulted in a reduction of NK cell and T cell numbers, whereas CD8 effector memory T cell levels were unaltered.[148,149] Recently, it was shown that tofacitinib selectively inhibits T cell effector function while preserving the suppressive activity of $CD4^+CD25^{high}$ regulatory T cells.[150]

Clinical Experience

Tofacitinib was evaluated as induction and maintenance therapy in a phase IIA, multicenter, open-label, randomized controlled trial in kidney transplant recipients.[151] All patients received induction therapy, MMF, and steroids. Patients in group 1 received tacrolimus, and patients in groups 2 and 3 were administered tofacitinib 15 mg and 30 mg twice per day, respectively.[151] Because of the high incidence of BKVN in the tofacitinib groups, the protocol was adjusted to stop MMF and decrease the steroid exposure. Compared with tacrolimus, both tofacitinib groups showed similar rates of acute rejection episodes and allograft function, but experienced greater incidences of hyperlipidemia and infections (suggestive of additional JAK2 inhibition).[151] A phase IIB trial compared cyclosporine at standard dosage versus tofacitinib 15 mg twice per day for 6 months then 10 mg twice per day, or tofacitinib 15 mg twice per day for 3 months then 10 mg twice per day. In addition, all patients received induction therapy, MMF, and steroids. Unexpectedly, the lower tofacitinib dosage arm showed a trend toward the lowest rejection rate and a superior allograft function. However, in this study there were important adverse events, including serious infections and posttransplant lymphoproliferative disease in both tofacitinib arms.[152] In a post hoc analysis of this study patients were reclassified based on median tofacitinib exposure (2 h postdose concentration more [above-median exposure] or less [below-median exposure] than 122 mg/mL).[153] The incidences of biopsy-proven acute rejection were noninferior compared with the cyclosporine A group. However, serious infections and CMV disease were only significantly increased in the above-median exposure group, and posttransplant lymphoproliferative disorder (PTLD) cases only occurred in the above-median exposure group.[153] Serious adverse events due to leukopenia and neutropenia remained significant in both the tofacitinib groups compared with the cyclosporine group.[153]

Toxicity

In a dose escalation study, the most frequent adverse events were infection and gastrointestinal side effects. Tofacitinib 15 mg and 30 mg twice per day were associated with a mean decrease in hemoglobin from baseline of 11%.[154] There was in addition a decrease in NK cells and B cells. There were no changes in the number of neutrophils, total lymphocytes, platelets, CD4 T cells, or CD8 T cells.[154]

Conclusion

In summary, combination of tofacitinib with MMF resulted in acceptable rates of acute rejection with evidence of over-immunosuppression when tofacitinib 30 mg twice per day was combined with MMF. Tofacitinib 15 mg twice per day coadministered with MMF resulted in similar outcomes while associated with modest lipid elevations and a higher rate of viral infections. In our opinion, considering the known side effect profile of CNI, further evaluation of tofacitinib is warranted. Given the side effects reported with the use of tofacitinib, the challenge remains to develop immunosuppressive drugs with truly selective inhibition of JAK3 versus JAK2 activities. The lack of success in developing a selective antagonist of JAK3 probably relates to the focus on chemical analogs of ATP whereas more success is to be expected from developing molecules displaying allosteric inhibition via binding of noncatalytic sites. Time-weighted 2 h postdose concentration therapeutic drug monitoring in tofacitinib-treated group is potentially interesting to relaunch tofacitinib as an immunosuppressive drug for kidney transplant recipients, especially in patients with refractory acute rejection where blockade of IL-15 and IL-7 can prevent the immunologic skewing toward memory cells.[155,156]

AEB071 OR SOTRASTAURIN

Chemical Structure and Pharmacology

Sotrastaurin (AEB071) is a low-molecular-weight, synthetic compound that potently and reversibly inhibits all 10 isoforms of protein kinase C (PKC), most importantly, PKC-θ PKC-α, and PKC-β, with lesser activity on PKC-λ.[157] Sotrastaurin is primarily metabolized through hepatic CYP3A4 into inactive metabolites and *N*-desmethyl-sotrastaurin, which has similar potency as sotrastaurin and is present at low blood concentrations (less than 5% of the parent exposure). Renal excretion of sotrastaurin is negligible and only a small amount is excreted in the bile (1% of the dose). The elimination half-life of sotrastaurin averages 6 hours. In clinical trials, it is recommended that patients administer sotrastaurin consistently either with or without food to avoid food-related fluctuations in drug exposure over time.[157]

Clinical drug interaction studies to date have demonstrated that sotrastaurin increases the area under the concentration–time curve of everolimus (1.2-fold) and of tacrolimus (2-fold).[158] Sotrastaurin increased tacrolimus concentration inasmuch as the tacrolimus dose needed to achieve a given C_0 was up to 47% lower when combined with sotrastaurin versus MMF.[158] Conversely, sotrastaurin area under the concentration–time curve is increased up to 1.8-fold by cyclosporine and 4.6-fold by ketoconazole.[159]

Mechanism of Action

PKC is a family of serine/threonine-specific protein kinases involved in diverse signal transduction pathways that modulate a whole range of cellular processes, including activation, proliferation, differentiation, apoptosis, and autophagy.[160] PKC-θ has been shown to be essential in the T cell receptor/CD3 signal transduction pathway. PKC-α modulates Th1 responses, including IFN-γ production. PKC-β controls B cell receptor-induced NF-κB transactivation and T-independent B cell responses. Finally, PKC-ε influences macrophage function.[161,162]

Experimental Experience

Of note, sotrastaurin was demonstrated to be nontoxic when added to human islet cultures.[163] These results suggest AEB071 to be an appropriate immunosuppressive candidate for clinical trials in islet transplantation. Recently it was shown in nonhuman primates that sotrastaurin in combination with cyclosporine at subtherapeutic doses resulted in markedly prolonged renal allograft survival, indicating synergistic immunosuppressive effects.[164]

Clinical Experience

In a phase II randomized controlled trial, sotrastaurin with standard or reduced tacrolimus was compared with standard tacrolimus alone, in addition to induction therapy and steroids.[165] Three months posttransplant, stable patients on

sotrastaurin were switched from tacrolimus to MMF. The three arms showed equal efficacy up to 3 months; at the end of the study there was no difference in allograft function, although the incidence of acute rejection was significantly higher in the standard tacrolimus with sotrastaurin arm.[165] Because of lack of efficacy, this study was prematurely discontinued. In another recent trial in de novo renal transplant recipients with immediate graft function, study subjects were randomized to sotrastaurin or tacrolimus.[166] All patients received basiliximab, MMF, and steroids. This study demonstrated a lower degree of efficacy but better renal function with the CNI-free regimen of sotrastaurin with MMF versus the tacrolimus-based control.[166] The combination of sotrastaurin with everolimus was evaluated in a two-stage Phase II study of de novo kidney transplant recipients evaluating sotrastaurin, in phase 1, 131 patients were randomized 2:1 to sotrastaurin 300 mg or cyclosporine A (CsA) and in phase II, 180 patients were randomized 1:1:1 to sotrastaurin 300 or 200 mg or CsA. All patients received basiliximab, everolimus (EVR), and prednisone.[167] Composite efficacy failure rates (treated biopsy-proven acute rejection, graft loss, death, or lost to follow-up) at 12 months were higher in sotrastaurin arms (Stage 1: 16.5% and 10.9% for sotrastaurin 300 mg and CsA; Stage 2: 27.2%, 34.5%, and 19.4% for sotrastaurin 200 mg, 300 mg, and CsA) and eGFR was significantly better in sotrastaurin groups versus CsA at most time points, except at 12 months.[167] In another randomized controlled trial 298 patients were randomized 1:1:1:1 to receive sotrastaurin 100 mg or 200 mg twice per day plus standard tacrolimus (sTAC; 5–12 ng/mL), or sotrastaurin 300 mg (n = 75) twice per day plus reduced tacrolimus (rTAC; 2–5 ng/mL), or enteric-coated mycophenolic acid (MPA) plus sTAC; all patients received basiliximab and corticosteroids.[168] The sotrastaurin 100 mg group was prematurely discontinued because of higher composite efficacy failure (treated biopsy-proven acute rejection ≥ grade IA, graft loss, death, or loss to follow-up) versus the MPA group. Sotrastaurin 200 and 300 mg had comparable efficacy to MPA in prevention of rejection with no significant difference in renal function between the groups.[168]

Toxicity

In the clinical trial mentioned previously, there was a 12% incidence of tachycardia and 18% incidence of serious infections.[165,166] A dose-dependent chronotropic effect has been observed in preclinical and phase I sotrastaurin studies; therefore the higher heart rate and tachycardia observed with the sotrastaurin in combination with MMF were not unexpected. All tachycardia adverse events were mild, and the majority occurred soon after transplantation. In the study of Tedesco-Silva et al., gastrointestinal and cardiac adverse events were more frequent with sotrastaurin whereas higher treatment discontinuation, deaths, and graft losses occurred with sotrastaurin 300 mg.[167] In the study of Russ et al., mean heart rates, gastrointestinal adverse events, and discontinuation due to adverse events were more frequent with sotrastaurin, whereas leukopenia was more frequent with MPA.[168]

Conclusion

Sotrastaurin blocks PKC-mediated early T cell activation, providing a new approach for immunosuppression distinct from CNI. However, the efficacy results of this phase II study do not support the combination of sotrastaurin 300 mg twice per day with MMF as a CNI-free regimen.[167] In addition, sotrastaurin should be evaluated in nonrenal transplant recipients as data suggest it is nonnephrotoxic.[169,170]

BORTEZOMIB

Chemical Structure and Pharmacology

Bortezomib is a proteasomal inhibitor approved by the Food and Drug Administration for the treatment of multiple myeloma. Bortezomib is increasingly used in the setting of solid-organ transplantation. Bortezomib was administered in four doses of 1.3 mg/m^2 intravenously in 3- to 5-minute infusions over an 11-day period in kidney transplant recipients. Before bortezomib administration, patients need to be premedicated with methylprednisolone. Peak serum concentration is reached at 30 minutes and the drug is cleared within 1 hour.[171]

Mechanism of Action

Bortezomib is a selective inhibitor of the 26S proteasome, preventing the activation of NF-κB.[172] It induces apoptosis of rapidly dividing, metabolically active cells with extensive protein synthesis. The ability of bortezomib to target plasma cells spurred interest as a new therapeutic approach in the treatment or prevention of alloantibody formation in organ transplantation.

Experimental Experience

In vitro, bortezomib was associated with a reduction in the number of bone marrow-derived plasma cells and attenuated alloantibody production.[173] Bortezomib primarily acts through plasma cell depletion, resulting in reduced antibody production to T-dependent antigens. As marginal zone B cells are resistant to bortezomib-mediated effects, T-independent type 2 responses are less affected.[174] In a mouse model of lupus, bortezomib depleted both short- and long-lived plasma cells, resulting in a reduction of antidouble-stranded DNA antibody production.[175] Vogelbacher et al. recently established that bortezomib alone or in combination with sirolimus can also prevent alloantibody formation in a rat model of kidney transplantation.[176]

Clinical Experience

Several clinical trials have been reported and are ongoing to evaluate bortezomib in the prevention and treatment of acute humoral rejection.[177] However, mixed results have been reported in this context.[178–180] Everly et al. administered bortezomib to six patients with antibody-mediated rejection with concomitant acute cellular rejection refractory to currently available therapies.[179] Bortezomib administration resulted in resolution of antibody-mediated rejection in all six patients, a decrease of donor-specific antibody levels, and improvement of allograft function.[179] In another study, Walsh et al. reported the results of bortezomib treatment in 28 patients with antibody-mediated rejection.[181] Bortezomib treatment was associated with variable results, with better responses in early (occurring in the first 6 months after transplantation) versus late (occurring 6 months or more after transplantation) antibody-mediated rejection.[181] Although some studies have reported bortezomib-based

desensitization induced long-term decrease in donor-specific antibodies,[182] in the study of Sberro-Soussan et al., bortezomib treatment did not result in a decrease of donor-specific antibody mean fluorescence intensity when used as sole desensitization therapy in four renal transplant recipients with subacute antibody-mediated rejection with persistent donor-specific antibodies.[180] As the densitization procotols also include IV Ig, rituximab, and plasmapheresis, it is impossible to attribute the reduction in donor-specific antibodies solely to bortezomib. Moreover, in a report from the Mayo Clinic only a modest reduction in HLA antibody titers and no effect on cPRA or flow crossmatch was observed.[183] A recent report in nonhuman primates undergoing fully mismatched allogeneic skin transplant reported that bortezomib treatment was followed by a rebound effect characterized by increased circulating IgG^+ B cells, increased germinal center B cells, and follicular helper T cells in the lymph nodes.[184] This reaction was termed *humoral compensation* by the authors and shown to be driven by B cell activating factor of TNF family (BAFF).[184] These data suggest that future trials should combine bortezomib with agents inhibiting BAFF signaling such as belimumab and atacicept. Newer proteasome inhibitors such as carfilzomib result in irreversible inhibition whereas bortezomib only induces temporal inhibition of the proteasome.[185] Studies are ongoing evaluating carfilzomib in desensitization protocol in patients with preformed HLA antibodies (NCT02442648) and in the treatment of acute humoral rejection in lung transplantation (NCT02474927).

Toxicity

In general bortezomib is administered intravenously twice weekly for 1 month at 1.3 mg/m^2 per administration. The most common side effects associated with bortezomib treatment are gastrointestinal toxicity (including paralytic ileus), thrombocytopenia, and neuropathy.[79,186] These can be potentially severe and disabling. To date, no increase in rate of opportunistic infections has been reported. Importantly, although bortezomib is associated with substantial reductions in donor-specific antibody levels, it does not result in a decrease in protective antibody levels.[187]

Conclusion

Proteasome inhibitor therapy has potential in the treatment of antibody-mediated rejection in kidney transplant recipients both as primary and rescue therapy. Optimal responses with bortezomib are obtained when antibody-mediated rejection occurs early posttransplant and bortezomib treatment is initiated promptly. Recent data suggest that bortezomib should be combined with agents inhibiting BAFF signaling such as belimumab and atacicept to prevent rebound humoral immunity.

OTHERS

1,25-Dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) and some of its new synthetic structural analogs were considered promising immunomodulators, in addition to its well-known role in mineral and bone homeostasis, nonclassical effects of vitamin D such as immune regulation have increasingly been recognized.[188,189] A role for $1,25(OH)_2D_3$ in immune regulation was suggested by the presence of its receptor (vitamin D receptor, or VDR) in almost all immune cells[190,191] the control of VDR expression in immune cells by immune signals[192] and the presence of vitamin D metabolizing enzymes such as CYP27B1 in T and B lymphocytes, and CYP2R1 in dendritic cells,[193,194] which allows for the local conversion of $25(OH)D_3$ into $1,25(OH)_2D_3$.[195]

$1,25(OH)_2D_3$ results in more immature or tolerogenic phenotype of dendritic cells,[196–198] induction of $CD4^+CD25^{high}CD127^{low}$ regulatory T cells,[199–201] and B cell apoptosis.[202] A clear additive and even synergistic effect was observed between $1,25(OH)_2D_3$ or its analogs and other more classical immunosuppressants such as cyclosporine, sirolimus, or MMF, both in vitro and in several in vivo autoimmune disease models, such as autoimmune diabetes[203,204] and experimental autoimmune encephalomyelitis.[205–207] In transplant models, monotherapy, $1,25(OH)_2D_3$ and its analogs provoke only a modest prolongation of graft function.[196,204,208–216] Human data are still lacking that can sustain the proposed benefits of vitamin D supplementation for optimal immune function. In a randomized trial involving lung transplant recipients, once monthly oral vitamin D supplementation (cholecalciferol; 100,000 IU) did result in increased levels of 25-hydroxy vitamin D, but clinical outcomes were unaffected (chronic lung allograft dysfunction, overall survival, prevalence of acute rejection, lymphocytic bronchiolitis and infection, lung function, pulmonary and systemic inflammation, and bone mineral density).[217] A major concern remains the side effects of $1,25(OH)_2D_3$ on calcium and bone metabolism.

Apoptosis plays an important in the immune system as it controls lymphocyte deletion in primary and secondary lymphoid organs. Apoptosis also plays a critical role in the maintenance of immune tolerance. Two pathways of apoptosis (intrinsic and extrinsic apoptosis pathway) have been described, and agents that specifically influence the intrinsic apoptosis pathway have shown promise as immunosuppressive agents. The BH3-mimetic ABT-737 has been reported to inhibit allogeneic immune responses through a combination of lymphopenia, deletion of alloreactive T cells, and relative enrichment of regulatory T cells.[218,219] In vitro, ABT-737 inhibited allogeneic T cell activation, proliferation, and cytotoxicity. In vivo, a combination of ABT-737 with low-dose CsA resulted in long-term skin graft survival in a murine major histocompatibility complex I (MHC I) mismatch model.[218,220] Furthermore, ABT-737 in combination with low-dose CsA and anti-CD154 (costimulation blockade) induced stable mixed chimerism (and tolerance) in a MHC-mismatched murine bone marrow transplant model.[221] Interestingly, ABT-737 also induced apoptosis in memory T cells and prolonged skin allograft survival in sensitized mice.[222] Another Bcl-2 inhibitor, AB199, is currently being evaluated in a clinical trial concerning systemic lupus erythematosus.

Novel inhibitors of the mTOR pathway have been reported. mTOR is the kinase subunit of mTOR complex 1 and mTOR complex 2. Although rapamycin partially inhibits mTOR complex 1, it does not inhibit mTOR complex 2. Dual blockade of mTOR complex 1 and mTOR complex 2 has shown potential to abrogate chronic rejection in a rat cardiac allograft model.[223] ATP-competitive inhibitors compete with ATP and inhibit both mTOR complexes.[224] This new class of mTOR inhibitors has mainly been evaluated for anticancer properties and only AZD8055 has been

evaluated as an immunosuppressant. AZD8055 is a powerful inhibitor of T cell proliferation in vitro and a 10-day course of AZD8055 induced a prolongation of heart allograft survival in a murine MHC-mismatched heart transplant model.[225] AZD8055 is rapidly metabolized by human hepatocytes (resulting in a short half-life), and therefore, a novel compound has been developed with a longer half-life, AZD2014. AZD2014 is currently being evaluated in phase I and II trials in oncology.[226] AZD2014 is rapidly absorbed after oral intake with a median time to peak of 30 to 60 minutes. The elimination half-life is approximately 3 hours with important interpatient variability. The currently recommended dose of AZD2014 is 50 mg twice daily.[227]

Cladribine is an adenosine deaminase-resistant analog of deoxyadenosine and is used in the treatment of leukemia and lymphoma. A number of studies have explored the immunosuppressive capacity of cladribine. In vitro, cladribine inhibits both B and T cell proliferation.[228] In vivo, cladribine monotherapy was shown to prolong skin allograft survival in mice,[229] in combination with cyclosporine it prolonged liver and heart allograft survival in rats,[230] and was more effective than cyclosporine monotherapy in small-bowel allografts.[231] However, no clinical trials are published to date.

The farnesyltransferase inhibitor A-228839 was developed as an anticancer compound that inhibits Ras GTPases. A-228839 inhibited lectin-induced proliferation and antigen-presenting cell-induced T cell proliferation. The compound also inhibited lymphocyte Th1 cytokine production and promoted apoptosis in lectin-activated lymphocytes.[232] Another farnesyltransferase inhibitor, ABT-100 was shown in vitro to block the secretion of IFN-γ and IL-4 by naïve T cells and suppressed alloreactivity. In a rat heterotopic cardiac transplant model, ABT-100 alone or in combination with subtherapeutic dose of cyclosporine delayed the development of acute rejection.[233] Given its combined antirejection and antioncogenic effects, farnesyltransferase inhibitors could represent an attractive new class of immunosuppressants in malignancy-prone organ transplant recipients if future clinical trials confirm their efficacy.

When a T cell receptor recognizes its specific antigen the lymphoid cell-specific kinase lck is phosphorylated and, together with the receptor-associated CD3 complex, phosphorylates zinc-associated phosphorylase 70. These events trigger the downstream cascade that increases intracellular calcium, activating calcineurin. Inhibitors of lck have become available recently. A-770041 and structurally similar molecules have been shown to prolong the survival of heterotopic murine heart and renal subcapsular islet grafts as well as to blunt the production of immunoglobulin IgG2a. Emodin ($C_{15}H_{10}O_5$), the cyclic derivative from rhubarb root and rhizomes, has been shown to prolong murine skin graft survival and to dampen IL-2 production.[234] However, neither of these archetypal compounds shows sufficient specificity for lck versus other kinases to warrant clinical development.[233]

FR 252921, an immunosuppressive agent isolated from the culture of *Pseudomonas fluorescens,* inhibits AP-1 transcription activity, and acts predominantly against antigen-presenting cells. FR 252921 demonstrated synergy with tacrolimus in vitro and in vivo. In murine models of skin transplantation, compared with the optimal dose of tacrolimus alone, the combination of FR 252921 and tacrolimus prolonged graft survival.[235–237]

Brequinar sodium exerts its immunosuppressive effects through the inhibition of the enzyme dihydroorotate dehydrogenase, resulting in inhibition of both T and B lymphocytes. Although the characteristics of brequinar suggest that it would be an attractive immunosuppressant, the suboptimal pharmacologic profile jeopardizes its use in transplant patients. The future use of this drug in the field of transplantation will require the development of analogs exhibiting a shorter half-life and reduced toxicity.

Spergualin was originally isolated from the culture filtrate of *Bacillus laterosporus,* and explored as a new anticancer or antibiotic substance. Its analog 15-deoxyspergualin subsequently became widely known as a promising new immunosuppressant. The precise mode of action of 15-deoxyspergualin is largely unknown and, because of its poor oral bioavailability, 15-deoxyspergualin must be delivered parenterally, which hampers its widespread clinical use.[238] Its efficacy has been demonstrated in the treatment of kidney allograft rejection, ABO-incompatible kidney transplantation, and transplantation in sensitized patients.[239–241] Until analogs are developed that allow oral administration,[242] the major clinical indication of 15-deoxyspergualin is limited to the treatment of rejection crises where 15-deoxyspergualin may be an interesting alternative to steroids or antilymphocyte agents. The fact that it remains effective after recurrent administration is promising.

Upon cellular uptake, cyclophosphamide is extensively metabolized into its active compounds phosphoramide mustard and acrolein.[243,244] The reaction of the phosphoramide mustard with DNA results in cell death.[245] Because of its limited efficacy and multiple side effects, the only standard indication for cyclophosphamide in transplantation today is the desensitization of highly sensitized recipients before kidney transplantation.[246] Most of these protocols involve repeated plasmapheresis, in combination with cyclophosphamide, either with or without continuation of steroids, until a kidney transplant becomes available.

Total Lymphoid Irradiation

For several decades, total lymphoid irradiation (TLI) has been used for the treatment of Hodgkin's disease. The possibility of applying TLI as an immunosuppressive regimen rather than an anticancer treatment was discovered by investigators at Stanford University.[247]

PROCEDURE

TLI is delivered through two ports. A first so-called mantle port includes the lymph nodes of the neck, axilla, and mediastinum. The other port is called the "inverted Y" and encompasses aortic, iliac, and pelvic lymph nodes and the spleen. Usually, a total dose of 40 to 50 Gy (1 Gy = 100 rad) is administered in daily fractions of 1.5 to 2.5 Gy.

MECHANISMS OF ACTION

Much of the currently available experimental evidence on the immunologic mechanisms underlying TLI-induced tolerance points to the importance of suppressor cells. The

group of Strober-identified post-TLI suppressor cells as host-type NK T cells, as the protective effect of TLI against graft-versus-host disease was abrogated in mice with a CD1d-inactivated gene.[248] These host-type NK T cells produced IL-4 and stimulated donor-type cells to produce IL-4 also.[248,249] Definitive evidence of the functional importance and activity of these suppressor cells was delivered by the demonstration that they could prevent graft-versus-host disease in vivo.[250] Post-TLI attenuation of effector T-lymphocyte reactivity was equally proposed to be responsible for the observed immunosuppressed state after TLI.[251–253] This intrinsic T cell defect was dependent on the irradiation of both thymus and extrathymic tissues.[254] After TLI, anergized T cells were shown to be incapable of proliferating even in the presence of exogenous IL-2.[255] In other studies, TLI was shown to lead to thymic clonal deletion of donor- or host-reactive lymphocytes.[256] TLI-treated mice also exhibited decreased antidonor cytotoxic T cell precursor frequencies.[257] Finally, Strober's group showed that Th2 lymphocytes recover soon after TLI, whereas Th1 lymphocytes remain deficient for several months,[10] and showed that this defect can also be prevented by thymic shielding during irradiation.[251] This Th2 dominance after TLI has been confirmed by other groups in rodents[258] and in large animals.[259] Recently, Nador et al. demonstrated that tolerance induction after conditioning with TLI and antithymocyte globulin (ATG) depends on the ability of naturally occurring regulatory NK T cells and regulatory T cells to suppress the residual alloreactive T cells that are capable of rejecting the allograft.[260]

EXPERIMENTAL EXPERIENCE

TLI-treated BALB/c mice receiving fully allogeneic C57BL6 bone marrow and skin graft on the first day after TLI became stable hematopoietic chimeras without signs of graft-versus-host disease, and developed permanent donor-specific tolerance with preserved anti-third-party reactivity.[261] Tolerance induction was critically dependent on the width of the irradiation field, the time of transplantation after TLI, the total dose of TLI, and the absence of presensitization.[261–263]

Although bone marrow chimerism could easily be induced, tolerance to either heart[264] or kidney allografts[265] was not obtained, suggesting that TLI-induced bone marrow chimerism does not necessarily create tolerance toward organ-specific antigens.

The combination of TLI and low-dose cyclosporine was found to be effective and clinically safe in rats,[266] and TLI with postoperative ATG-induced permanent and specific transplantation tolerance toward heart allografts in about 40% of transplanted dogs.[267] These encouraging results led to a similar trial in clinical kidney transplantation. Myburgh et al. applied a modified TLI regimen in baboons, with low dosage and wide-field exposure, and showed that tolerance can be achieved in larger animals without concomitant bone marrow transplantation.[268]

Also, in heart or heart–lung transplantation experiments between xenogeneic nonhuman primate species, preoperative TLI, when administered in combination with cyclosporine and ATG,[269] cyclosporine and splenectomy,[270] or cyclosporine and medrol,[271] was more efficient than any other treatment regimen. Pretransplant TLI, combined with cyclosporine and methotrexate in a pig heart-into-baboon model, resulted in a graft survival time of more than 2 weeks. This regimen was able to inhibit xenoreactive natural antibody production but not the xenoreactivity of macrophages. In a pig islet-into-rat xenograft model, TLI in combination with deoxyspergualin was extremely effective,[272] and even in a discordant lamb-into-pig model, TLI synergized with cyclosporine and azathioprine to provoke a 30-fold increase of the mean xenograft survival time.[273]

The principal disadvantage for the clinical application of TLI is that the complete regimen of fractionated daily irradiations needs to be administered and completed before, and sufficiently close to, the moment of transplantation, and finding a suitable donor organ within such a restricted time frame is problematic. Investigators have therefore explored the possibility of using TLI after transplantation. In mouse and rat heart allograft models, posttransplant TLI significantly prolonged graft survival when combined with monoclonal anti-CD4 antibodies[274] or with infusion of donor-type dendritic cell precursors.[275]

CLINICAL EXPERIENCE

The first clinical kidney transplants utilizing TLI were performed at the University of Minnesota in 20 patients who had previously rejected a renal allograft.[276] Because similar results (an increase of about 30% 1-year graft survival compared with historical control data) were achieved in this patient population using cyclosporine, and because of the ease of administration, the investigators concluded to prefer cyclosporine over TLI.

In the 1980s, a controlled trial was performed at the University of Leuven, in which end-stage diabetic nephropathy patients received cadaveric kidney allografts, investigating the effect of pretransplant TLI (20 daily fractions of 1 Gy, followed by 1 weekly TLI dose until a suitable donor was found), followed by low-dose posttransplant prednisone maintenance treatment.[277] Long-term (8-year) follow-up revealed that rejection episodes were more frequent, and patient and graft survival were significantly inferior in the TLI-treated group.[278] The excess mortality in the TLI-treated patients was due to sepsis, resulting from high-dose steroid therapy needed to treat rejection crises. This clinical experience confirmed the animal data that also showed that TLI by itself is insufficient to provoke long-term graft survival or tolerance and that extra manipulations are needed.

In a study at Stanford University, 24 patients received a first, and one patient a second, cadaveric renal allograft using TLI and ATG.[279] The actuarial graft survival was 76% and 68% at 1 and 2 years, respectively. Ten of the 25 patients never had a rejection crisis despite an overall poor HLA-matching between donor and recipient. In follow-up studies, a specific antidonor mixed lymphocyte culture hypo- or nonresponsiveness was demonstrated[280] and, in some patients, all immunosuppressive drugs could be withdrawn.[281] An evaluation in a larger group of 52 patients treated with the same protocol at the same center showed a 3-year graft survival of about 50%, which is less than in cyclosporine-treated patients (around 75%).[279]

Posttransplant TLI in combination with anti-CD3 monoclonal antibodies, or with ATG and donor-specific blood transfusions, seemed very effective in a rat heart allograft

model. On the basis of these results, the efficacy of TLI was evaluated in heart transplant patients with therapy-resistant or early vascular rejection.[282–284] This resulted in a significant reduction of rejection recurrences, an effect which was maintained for at least 2 years. In the meantime, these favorable results have been confirmed by several other groups. Also, TLI-treated patients develop less coronary atherosclerosis than matched controls despite multiple rejection episodes.[285–289]

Scandling et al. have reported the use of TLI to induce tolerance in the setting of combined kidney/hematopoietic stem cell transplantation between HLA-matched donor/recipient pairs.[290,291] Patients received a conditioning regimen of 10 doses of TLI (80–120 cGy), five doses of rabbit ATG, MMF for 1 month, and cyclosporine for at least 6 months. Donor hematopoietic stem cells were injected intravenously on day 11 in the outpatient infusion center.[291] In a recent study, Scandling et al. reported on 38 patients undergoing HLA matched or mismatched combined kidney/hematopoietic transplantation.[292] Sixteen out of 22 matched patients had persistent chimerism for at least 6 months and were successfully withdrawn from all immunosuppression. Whether immunosuppression can be withdrawn in mismatched patients with mixed chimerism remains to be established at this time.[292]

CONCLUSION

TLI has been shown to be a safe immunosuppressive regimen. It has been abandoned in clinical practice for organizational reasons, except for the treatment of therapy-resistant rejection of heart or heart–lung transplant. However, its ability to induce tolerance in HLA-matched patients, in combination with ATG and hematopoietic stem cell transplantation, might renew interest in this treatment modality. To date, no evidence of radiation-related late effects has been documented with TLI.[293]

Photopheresis

Extracorporeal photopheresis is a technique in which leukocytes, removed from patients by leukopheresis, are exposed to 8-methoxypsoralen and ultraviolet A light. It was developed as an immunoregulatory treatment for erythrodermic cutaneous T cell lymphoma.[294] Subsequently, the procedure was shown to be safe as an alternative treatment for various human immune and autoimmune diseases.[295] Furthermore, in rats[296] and monkeys,[297] the regimen was shown to result in extended skin allograft and cardiac allograft and xenograft survival.

Different mechanisms have been shown to contribute to the immunomodulatory effect of photopheresis: selective inhibition of effector cells,[296,298] induction of a high rate of apoptosis,[299] increased capacity to phagocytose apoptotic T cells, resulting in the induction of anticlonotypic immune responses,[300] shift toward Th2 immune activation,[301] and induction of regulatory CD4 and CD8 cells.[302,303]

In clinical transplantation, photopheresis has been applied as both a therapeutic and prophylactic option. It has been applied in the treatment of recurrent or resistant acute rejection in renal transplant patients,[301,304–309] but the number of patients included in these studies is limited, and prospective randomized trials are needed. The safety and efficacy of photopheresis in the prevention of acute rejection of cardiac allografts have been evaluated in primary cardiac allograft recipients, randomly assigned to standard triple-drug immunosuppressive therapy (cyclosporine, azathioprine, and prednisone) alone or in conjunction with 24 photopheresis sessions performed during the first 6 months after transplantation. After 6 months of follow-up, photopheresis-treated patients developed significantly fewer multiple rejections, and there were no significant differences in the rates or types of infection. Although there was no significant effect on graft survival rates at 6 or 12 months, this study indicated that photopheresis may be an effective new immunosuppressive regimen in transplant recipients.[310] In patients with refractory bronchiolitis obliterans after lung transplantation, photopheresis resulted in a stabilization of graft function and/or in some of these patients in histologic reversal of rejection.[311,312]

Splenectomy

Pretransplant splenectomy in the recipient before transplantation was first proposed by Starzl et al. in 1963 as a means of improving graft survival.[313] Although splenectomy is a standard procedure for patients who develop hypersplenism or azathioprine-associated leukopenia, evidence on the role of splenectomy in enhancing graft survival is controversial.[313–316] A large prospective randomized trial in Minneapolis showed splenectomy improved graft survival significantly,[317] but longer-term follow-up showed loss of beneficial effects because of an increased infection-related mortality.[318] Several other single-center studies have shown an alarming risk of sepsis and death, nullifying any early benefits of splenectomy on graft survival,[319,320] and a multicenter analysis from the South-Eastern Organ Procurement Foundation confirmed a modest improvement in graft survival after splenectomy, but a relentless increase in patient mortality.[321]

Splenectomy has a place in the preparation of a recipient who is to receive an ABO-incompatible graft, a practice that is likely to become more widely employed in living related donor transplantation, where an ABO-incompatible but otherwise suitable donor is the only available donor. Alexandre et al. reported a series of 38 such ABO-incompatible living donor transplants in which the recipient was prepared by plasmapheresis, donor-specific platelet transfusion, and splenectomy.[322–324] Although the authors believe that the need for plasmapheresis and donor-specific platelet transfusion should be reevaluated, splenectomy was thought to be important, because 3 of 38 recipients who did not have a splenectomy lost their grafts from acute vascular rejection, in contrast to only 5 of 33 who did undergo splenectomy. A small-scale but successful experience with postsplenectomy ABO-incompatible living donor kidney transplantation has also been reported by Ishikawa et al. in Japan.[325] In the setting of ABO-incompatible kidney transplantation, antigen-specific immunoadsorption, rituximab, and bortezomib treatment have been developed as alternatives to plasmapheresis and splenectomy. This will further reduce the indications for splenectomy in organ transplantation.[326,327]

Splenectomy has also been applied in combination with eculizumab as salvage therapy in patients developing severe antibody-mediated rejection after HLA-incompatible kidney transplantation.[328] In a follow-up report, splenectomy was substituted for splenic irradiation.[329]

Plasmapheresis

Plasmapheresis is increasingly used to facilitate kidney transplantation in patients with high levels of anti-HLA antibodies or ABO incompatibility.[330–332] Furthermore, plasmapheresis has been used in the treatment of antibody-mediated allograft rejection.[333] Plasmapheresis is a component of several desensitization protocols applied in patients with living donors and an incompatible crossmatch because of donor-specific antibodies. Different combinations of plasmapheresis with rituximab, corticosteroids, IV IG, and bortezomib have been reported.[334] Plasmapheresis should be performed until crossmatch testing becomes negative typically on a daily basis or on alternate days, and the number of plasmapheresis sessions is dependent on the antibody levels and degree of mismatch. Transplantation should be performed within days of the last desensitization before rebound of anti-HLA antibody titers occurs. Encouraging early results of this approach have been reported, although they were associated with considerable morbidity.[335–337] Recent studies provided evidence of an important survival benefit for patients undergoing HLA-incompatible kidney transplantation after desensitization.[330,331] In an analysis by Segev and colleagues, 1025 HLA-sensitized patients from 22 centers undergoing desensitization before kidney transplant from a living donor between 1997 and 2011 were included. Different desensitization protocols were applied in this study. Compared with patients on the waiting list or receiving a deceased donor transplant or patients on the waiting list not receiving a transplant, the 8-year survival rates of HLA-sensitized recipients was 13.6 and 32.6 percentage points higher, respectively. Also the risk of death was significantly reduced after transplantation from an incompatible live donor after desensitization treatment. The most important adverse events associated with desensitization were anaphylaxis, hypotension, and infections.[331]

Current ABO-incompatible transplantation protocols also include plasmapheresis.[322–324] Brynger et al. have reported some successful ABO-incompatible grafts without prior plasmapheresis of the recipient,[338] and it was originally felt that splenectomy was a prerequisite for successful ABO-incompatible transplantation. However, it has been demonstrated that combining plasmapheresis and IV IG (without splenectomy) allows ABO-incompatible renal transplantation. Plasmapheresis aims to reduce ABO antibody titers below center-specific critical thresholds before transplantation, and plasmapheresis might be combined with rituximab.[339] Excellent outcomes have been reported in patients undergoing ABO-incompatible living kidney transplantation after desensitization with 5- and 10-year allograft survival of 95% and 90%, respectively.[334,340,341]

Plasmapheresis is also a component of the treatment of antibody-mediated allograft rejection. Although some initial reports suggested a beneficial effect,[342] controlled trials were unconvincing. Nojima et al. reported the successful treatment of antibody-mediated acute renal allograft rejection by combining plasmapheresis with 15-deoxyspergualin.[343] Antibody-mediated rejection is increasingly being recognized as a determinant of short-term and long-term allograft outcome.[344] The optimal treatment of antibody-mediated rejection is undetermined at this moment, and possible therapeutic approaches include combinations of plasmapheresis exchange, IV IG, and anti-CD20 antibody to remove donor-specific antibodies and inhibit antibody production. However, evidence on safety and efficacy is weak, and the optimal treatment protocol has yet to be determined.[345]

Immunoabsorption has been applied as an alternative to plasmapheresis and was found to be an equally efficient method.[326,346] Studies of this approach in highly sensitized candidate transplant recipients are continuing.

References

1. Bartlett RR, Dimitrijevic M, Mattar T, et al. Leflunomide (HWA 486), a novel immunomodulating compound for the treatment of autoimmune disorders and reactions leading to transplantation rejection. Agents Actions 1991;32:10–21.
2. Jin MB, Nakayama M, Ogata T, et al. A novel leflunomide derivative, FK778, for immunosuppression after kidney transplantation in dogs. Surgery 2002;132:72–9.
3. Chong AS, Gebel H, Finnegan A, et al. Leflunomide, a novel immunomodulatory agent: in vitro analyses of the mechanism of immunosuppression. Transplant Proc 1993;25:747–9.
4. Siemasko K, Chong AS, Jack HM, Gong H, Williams JW, Finnegan A. Inhibition of JAK3 and STAT6 tyrosine phosphorylation by the immunosuppressive drug leflunomide leads to a block in IgG1 production. J Immunol 1998;160:1581–8.
5. Williamson RA, Yea CM, Robson PA, et al. Dihydroorotate dehydrogenase is a high affinity binding protein for A77 1726 and mediator of a range of biological effects of the immunomodulatory compound. J Biol Chem 1995;270:22467–72.
6. Mattar T, Kochhar K, Bartlett R, Bremer EG, Finnegan A. Inhibition of the epidermal growth factor receptor tyrosine kinase activity by leflunomide. FEBS Lett 1993;334:161–4.
7. Elder RT, Xu X, Williams JW, Gong H, Finnegan A, Chong AS. The immunosuppressive metabolite of leflunomide, A77 1726, affects murine T cells through two biochemical mechanisms. J Immunol 1997;159:22–7.
8. Mahajan S, Ghosh S, Sudbeck EA, et al. Rational design and synthesis of a novel anti-leukemic agent targeting Bruton's tyrosine kinase (BTK), LFM-A13 [alpha-cyano-beta-hydroxy-beta-methyl-*N*-(2, 5-dibromophenyl)propenamide]. J Biol Chem 1999;274:9587–99.
9. Chong AS, Huang W, Liu Wet al. In vivo activity of leflunomide: pharmacokinetic analyses and mechanism of immunosuppression. Transplantation 1999;68:100–9.
10. Bass H, Mosmann T, Strober S. Evidence for mouse Th1- and Th2-like helper T cells in vivo. Selective reduction of Th1-like cells after total lymphoid irradiation. J Exp Med 1989;170:1495–511.
11. Karaman A, Fadillioglu E, Turkmen E, Tas E, Yilmaz Z. Protective effects of leflunomide against ischemia-reperfusion injury of the rat liver. Pediatr Surg Int 2006;22:428–34.
12. Manna SK, Mukhopadhyay A, Aggarwal BB. Leflunomide suppresses TNF-induced cellular responses: effects on NF-kappa B, activator protein-1, c-Jun N-terminal protein kinase, and apoptosis. J Immunol 2000;165:5962–9.
13. Jarman ER, Kuba A, Montermann E, Bartlett RR, Reske-Kunz AB. Inhibition of murine IgE and immediate cutaneous hypersensitivity responses to ovalbumin by the immunomodulatory agent leflunomide. Clin Exp Immunol 1999;115:221–8.
14. Manna SK, Aggarwal BB. Immunosuppressive leflunomide metabolite (A77 1726) blocks TNF-dependent nuclear factor-kappa B activation and gene expression. J Immunol 1999;162:2095–102.
15. Bilolo KK, Ouyang J, Wang X, et al. Synergistic effects of malononitrilamides (FK778, FK779) with tacrolimus (FK506) in prevention of acute heart and kidney allograft rejection and reversal of ongoing heart allograft rejection in the rat. Transplantation 2003;75:1881–7.

16. Deuse T, Schrepfer S, Reichenspurner H. Immunosuppression with FK778 and mycophenolate mofetil in a rat cardiac transplantation model. Transplantation 2003;76:1627–9.
17. Evers DL, Wang X, Huong SM, Huang DY, Huang ES. 3,4',5-Trihydroxy-trans-stilbene (resveratrol) inhibits human cytomegalovirus replication and virus-induced cellular signaling. Antiviral Res 2004;63:85–95.
18. Knight DA, Hejmanowski AQ, Dierksheide JE, Williams JW, Chong AS, Waldman WJ. Inhibition of herpes simplex virus type 1 by the experimental immunosuppressive agent leflunomide. Transplantation 2001;71:170–4.
19. Waldman WJ, Knight DA, Lurain NS, et al. Novel mechanism of inhibition of cytomegalovirus by the experimental immunosuppressive agent leflunomide. Transplantation 1999;68:814–25.
20. Farasati NA, Shapiro R, Vats A, Randhawa P. Effect of leflunomide and cidofovir on replication of BK virus in an in vitro culture system. Transplantation 2005;79:116–8.
21. Chong AS, Zeng H, Knight DA, et al. Concurrent antiviral and immunosuppressive activities of leflunomide in vivo. Am J Transplant 2006;6:69–75.
22. Zeng H, Waldman WJ, Yin DP, et al. Mechanistic study of malononitrileamide FK778 in cardiac transplantation and CMV infection in rats. Transplantation 2005;79:17–22.
23. Deuse T, Schrepfer S, Schafer H, et al. FK778 attenuates lymphocyte-endothelium interaction after cardiac transplantation: in vivo and in vitro studies. Transplantation 2004;78:71–7.
24. Savikko J, Von WE, Hayry P. Leflunomide analogue FK778 is vasculoprotective independent of its immunosuppressive effect: potential applications for restenosis and chronic rejection. Transplantation 2003;76:455–8.
25. Lin Y, Vandeputte M, Waer M. A short-term combination therapy with cyclosporine and rapamycin or leflunomide induces long-term heart allograft survival in a strongly immunogenic strain combination in rats. Transpl Int 1996;9(Suppl. 1):S328–30.
26. Sun Y, Chen X, Zhao J, et al. Combined use of rapamycin and leflunomide in prevention of acute cardiac allografts rejection in rats. Transpl Immunol 2012;27:19–24.
27. Williams JW, Xiao F, Foster P, et al. Leflunomide in experimental transplantation. Control of rejection and alloantibody production, reversal of acute rejection, and interaction with cyclosporine. Transplantation 1994;57:1223–31.
28. Xiao F, Shen J, Chong A, et al. Control and reversal of chronic xenograft rejection in hamster-to-rat cardiac transplantation. Transplant Proc 1996;28:691–2.
29. Lin Y, Goebels J, Xia G, Ji P, Vandeputte M, Waer M. Induction of specific transplantation tolerance across xenogeneic barriers in the T-independent immune compartment. Nat Med 1998;4:173–80.
30. Chiba K, Yanagawa Y, Masubuchi Y, et al. FTY720, a novel immunosuppressant, induces sequestration of circulating mature lymphocytes by acceleration of lymphocyte homing in rats. I. FTY720 selectively decreases the number of circulating mature lymphocytes by acceleration of lymphocyte homing. J Immunol 1998;160:5037–44.
31. Pan F, Ebbs A, Wynn C, et al. FK778, a powerful new immunosuppressant, effectively reduces functional and histologic changes of chronic rejection in rat renal allografts. Transplantation 2003;75:1110–4.
32. Kyles AE, Gregory CR, Griffey SM, et al. Immunosuppression with a combination of the leflunomide analog, FK778, and microemulsified cyclosporine for renal transplantation in mongrel dogs. Transplantation 2003;75:1128–33.
33. Qi S, Zhu S, Xu D, et al. Significant prolongation of renal allograft survival by delayed combination therapy of FK778 with tacrolimus in nonhuman primates. Transplantation 2003;75:1124–8.
34. Hirsch HH, Randhawa P. BK polyomavirus in solid organ transplantation. Am J Transplant 2013;13:179–88.
35. Josephson MA, Gillen D, Javaid B, et al. Treatment of renal allograft polyoma BK virus infection with leflunomide. Transplantation 2006;81:704–10.
36. Krisl JC, Taber DJ, Pilch N, et al. Leflunomide efficacy and pharmacodynamics for the treatment of BK viral infection. Clin J Am Soc Nephrol 2012;7:1003–9.
37. Kuypers DR. Management of polyomavirus-associated nephropathy in renal transplant recipients. Nat Rev Nephrol 2012;8:390–402.
38. Williams JW, Javaid B, Kadambi PV, et al. Leflunomide for polyomavirus type BK nephropathy. N Engl J Med 2005;352:1157–8.
39. Faguer S, Hirsch HH, Kamar N, et al. Leflunomide treatment for polyomavirus BK-associated nephropathy after kidney transplantation. Transpl Int 2007;20:962–9.
40. Egli A, Kohli S, Dickenmann M, Hirsch HH. Inhibition of polyomavirus BK-specific T-Cell responses by immunosuppressive drugs. Transplantation 2009;88:1161–8.
41. Johnston O, Jaswal D, Gill JS, Doucette S, Fergusson DA, Knoll GA. Treatment of polyomavirus infection in kidney transplant recipients: a systematic review. Transplantation 2010;89:1057–70.
42. Topalis D, Lebeau I, Krecmerova M, Andrei G, Snoeck R. Activities of different classes of acyclic nucleoside phosphonates against BK virus in primary human renal cells. Antimicrob Agents Chemother 2011;55:1961–7.
43. Liacini A, Seamone ME, Muruve DA, Tibbles LA. Anti-BK virus mechanisms of sirolimus and leflunomide alone and in combination: toward a new therapy for BK virus infection. Transplantation 2010;90:1450–7.
44. Zaman RA, Ettenger RB, Cheam H, Malekzadeh MH, Tsai EW. A novel treatment regimen for BK viremia. Transplantation 2014;97:1166–71.
45. Jaw J, Hill P, Goodman D. Combination of leflunomide and everolimus for treatment of BK virus nephropathy. Nephrology (Carlton). 2017;22:326–9.
46. Ciszek M, Mucha K, Foroncewicz B, Chmura A, Paczek L. Leflunomide as a rescue treatment in ganciclovir-resistant cytomegalovirus infection in a seronegative renal transplant recipient—a case report. Ann Transplant 2014;19:60–3.
47. El Chaer F, Mori N, Shah D, et al. Adjuvant and salvage therapy with leflunomide for recalcitrant cytomegalovirus infections in hematopoietic cell transplantation recipients: a case series. Antiviral Res 2016;135:91–6.
48. Goldsmith PM, Husain MM, Carmichael A, Zhang H, Middleton SJ. Case report: multidrug-resistant cytomegalovirus in a modified multivisceral transplant recipient. Transplantation 2012;93:e30–2.
49. Miszewska-Szyszkowska D, Mikolajczyk N, Komuda-Leszek E, et al. Severe cytomegalovirus infection in a second kidney transplant recipient treated with ganciclovir, leflunomide, and immunoglobulins, with complications including seizures, acute HCV infection, drug-induced pancytopenia, diabetes, cholangitis, and multi-organ failure with fatal outcome: a case report. Ann Transplant 2015;20:169–74.
50. Morita S, Shinoda K, Tamaki S, et al. Successful low-dose leflunomide treatment for ganciclovir-resistant cytomegalovirus infection with high-level antigenemia in a kidney transplant: a case report and literature review. J Clin Virol 2016;82:133–8.
51. Nguyen L, McClellan RB, Chaudhuri A, et al. Conversion from tacrolimus/mycophenolic acid to tacrolimus/leflunomide to treat cutaneous warts in a series of four pediatric renal allograft recipients. Transplantation 2012;94:450–5.
52. Basu G, Mohapatra A, Manipadam MT, Mani SE, John GT. Leflunomide with low-dose everolimus for treatment of Kaposi's sarcoma in a renal allograft recipient. Nephrol Dial Transplant 2011;26:3412–5.
53. Guasch A, Roy-Chaudhury P, Woodle ES, Fitzsimmons W, Holman J, First MR. Assessment of efficacy and safety of FK778 in comparison with standard care in renal transplant recipients with untreated BK nephropathy. Transplantation 2010;90:891–7.
54. Leca N. Leflunomide use in renal transplantation. Curr Opin Organ Transplant 2009;14:370–4.
55. Vanrenterghem Y, van Hooff JP, Klinger M, et al. The effects of FK778 in combination with tacrolimus and steroids: a phase II multicenter study in renal transplant patients. Transplantation 2004;78:9–14.
56. Smolen JS, Kalden JR, Scott DL, et al. Efficacy and safety of leflunomide compared with placebo and sulphasalazine in active rheumatoid arthritis: a double-blind, randomised, multicentre trial. European Leflunomide Study Group. Lancet 1999;353:259–66.
57. Chikura B, Lane S, Dawson JK. Clinical expression of leflunomide-induced pneumonitis. Rheumatology (Oxford) 2009;48:1065–8.
58. Kho LK, Kermode AG. Leflunomide-induced peripheral neuropathy. J Clin Neurosci 2007;14:179–81.
59. Ostensen M. Disease specific problems related to drug therapy in pregnancy. Lupus 2004;13:746–50.
60. Curtis JR, Beukelman T, Onofrei A, et al. Elevated liver enzyme tests among patients with rheumatoid arthritis or psoriatic arthritis treated with methotrexate and/or leflunomide. Ann Rheum Dis 2010;69:43–7.

61. Suissa S, Ernst P, Hudson M, Bitton A, Kezouh A. Newer disease-modifying antirheumatic drugs and the risk of serious hepatic adverse events in patients with rheumatoid arthritis. Am J Med 2004;117:87–92.
62. Fujita T, Inoue K, Yamamoto S, et al. Fungal metabolites. Part 11. A potent immunosuppressive activity found in Isaria sinclairii metabolite. J Antibiot (Tokyo) 1994;47:208–15.
63. Fujita T, Inoue K, Yamamoto S, et al. Fungal metabolites. Part 12. Potent immunosuppressant, 14-deoxomyriocin, (2S,3R,4R)-(E)-2-amino-3,4-dihydroxy-2-hydroxymethyleicos-6-enoic acid and structure-activity relationships of myriocin derivatives. J Antibiot (Tokyo) 1994;47:216–24.
64. Sasaki S, Hashimoto R, Kiuchi M, et al. Fungal metabolites. Part 14. Novel potent immunosuppressants, mycestericins, produced by Mycelia sterilia. J Antibiot (Tokyo) 1994;47:420–33.
65. Halin C, Scimone ML, Bonasio R, et al. The S1P-analog FTY720 differentially modulates T-cell homing via HEV: T-cell-expressed S1P1 amplifies integrin activation in peripheral lymph nodes but not in Peyer patches. Blood 2005;106:1314–22.
66. Mandala S, Hajdu R, Bergstrom J, et al. Alteration of lymphocyte trafficking by sphingosine-1-phosphate receptor agonists. Science 2002;296:346–9.
67. Matloubian M, Lo CG, Cinamon G, et al. Lymphocyte egress from thymus and peripheral lymphoid organs is dependent on S1P receptor 1. Nature 2004;427:355–60.
68. Pappu R, Schwab SR, Cornelissen I, et al. Promotion of lymphocyte egress into blood and lymph by distinct sources of sphingosine-1-phosphate. Science 2007;316:295–8.
69. Yuzawa K, Stephkowski SM, Wang M, Kahan BD. FTY720 blocks allograft rejection by homing of lymphocytes in vivo. Transplant Proc 2000;32:269.
70. Habicht A, Clarkson MR, Yang J, et al. Novel insights into the mechanism of action of FTY720 in a transgenic model of allograft rejection: implications for therapy of chronic rejection. J Immunol 2006;176:36–42.
71. Zhang Q, Chen Y, Fairchild RL, Heeger PS, Valujskikh A. Lymphoid sequestration of alloreactive memory CD4 T cells promotes cardiac allograft survival. J Immunol 2006;176:770–7.
72. Li XK, Enosawa S, Kakefuda T, Amemiya H, Suzuki S. FTY720, a novel immunosuppressive agent, enhances upregulation of the cell adhesion molecular ICAM-1 in TNF-alpha treated human umbilical vein endothelial cells. Transplant Proc 1997;29:1265–6.
73. Sawicka E, Dubois G, Jarai G, et al. The sphingosine 1-phosphate receptor agonist FTY720 differentially affects the sequestration of CD4+/CD25+ T-regulatory cells and enhances their functional activity. J Immunol 2005;175:7973–80.
74. Zhou PJ, Wang H, Shi GH, Wang XH, Shen ZJ, Xu D. Immunomodulatory drug FTY720 induces regulatory CD4(+)CD25(+) T cells in vitro. Clin Exp Immunol 2009;157:40–7.
75. Troncoso P, Stepkowski SM, Wang ME, et al. Prophylaxis of acute renal allograft rejection using FTY720 in combination with subtherapeutic doses of cyclosporine. Transplantation 1999;67:145–51.
76. Masubuchi Y, Kawaguchi T, Ohtsuki M, et al. FTY720, a novel immunosuppressant, possessing unique mechanisms. IV. Prevention of graft versus host reactions in rats. Transplant Proc 1996;28:1064–5.
77. Lan YY, De CA, Colvin BL, et al. The sphingosine-1-phosphate receptor agonist FTY720 modulates dendritic cell trafficking in vivo. Am J Transplant 2005;5:2649–59.
78. Yin N, Zhang N, Xu J, Shi Q, Ding Y, Bromberg JS. Targeting lymphangiogenesis after islet transplantation prolongs islet allograft survival. Transplantation 2011;92:25–30.
79. Suzuki S, Kakefuda T, Amemiya H, et al. An immunosuppressive regimen using FTY720 combined with cyclosporin in canine kidney transplantation. Transpl Int 1998;11:95–101.
80. Yamashita K, Nomura M, Omura T, et al. Effect of a novel immunosuppressant, FTY720, on heart and liver transplantations in rats. Transplant Proc 1999;31:1178–9.
81. Chiba K, Hoshino Y, Suzuki C, et al. FTY720, a novel immunosuppressant possessing unique mechanisms. I. Prolongation of skin allograft survival and synergistic effect in combination with cyclosporine in rats. Transplant Proc 1996;28:1056–9.
82. Gao M, Liu Y, Xiao Y, et al. Prolonging survival of corneal transplantation by selective sphingosine-1-phosphate receptor 1 agonist. PLoS ONE 2014;9e105693.
83. Xu M, Pirenne J, Antoniou EA, Afford SC, D'Silva M, McMaster P. Effect of peritransplant FTY720 alone or in combination with post-transplant tacrolimus in a rat model of cardiac allotransplantation. Transpl Int 1998;11:288–94.
84. Suzuki S, Enosawa S, Kakefuda T, Amemiya H, Hoshino Y, Chiba K. Long-term graft acceptance in allografted rats and dogs by treatment with a novel immunosuppressant, FTY720. Transplant Proc 1996;28:1375–6.
85. Suzuki S, Enosawa S, Kakefuda T, et al. A novel immunosuppressant, FTY720, with a unique mechanism of action, induces long-term graft acceptance in rat and dog allotransplantation. Transplantation 1996;61:200–5.
86. Xu M, Pirenne J, Antoniou S, Gunson B, D'Silva M, McMaster P. FTY720 compares with FK 506 as rescue therapy in rat heterotopic cardiac transplantation. Transplant Proc 1998;30:2221–2.
87. Mitsusada M, Suzuki S, Kobayashi E, Enosawa S, Kakefuda T, Miyata M. Prevention of graft rejection and graft-versus-host reaction by a novel immunosuppressant, FTY720, in rat small bowel transplantation. Transpl Int 1997;10:343–9.
88. Delbridge MS, Shrestha BM, Raftery AT, El Nahas AM, Haylor JL. Reduction of ischemia-reperfusion injury in the rat kidney by FTY720, a synthetic derivative of sphingosine. Transplantation 2007;84:187–95.
89. Fuller TF, Hoff U, Kong L, et al. Cytoprotective actions of FTY720 modulate severe preservation reperfusion injury in rat renal transplants. Transplantation 2010;89:402–8.
90. Man K, Ng KT, Lee TK, et al. FTY720 attenuates hepatic ischemia-reperfusion injury in normal and cirrhotic livers. Am J Transplant 2005;5:40–9.
91. Suleiman M, Cury PM, Pestana JO, Burdmann EA, Bueno V. FTY720 prevents renal T-cell infiltration after ischemia/reperfusion injury. Transplant Proc 2005;37:373–4.
92. Hoshino Y, Suzuki C, Ohtsuki M, Masubuchi Y, Amano Y, Chiba K. FTY720, a novel immunosuppressant possessing unique mechanisms. II. Long-term graft survival induction in rat heterotopic cardiac allografts and synergistic effect in combination with cyclosporine A. Transplant Proc 1996;28:1060–1.
93. Suzuki T, Jin MB, Shimamura T, et al. A new immunosuppressant, FTY720, in canine kidney transplantation: effect of single-drug, induction and combination treatments. Transpl Int 2004;17:574–84.
94. Wang ME, Tejpal N, Qu X, Yu J, Okamoto M, Stepkowski SM, et al. Immunosuppressive effects of FTY720 alone or in combination with cyclosporine and/or sirolimus. Transplantation 1998;65:899–905.
95. Stone ML, Sharma AK, Zhao Y, et al. Sphingosine-1-phosphate receptor 1 agonism attenuates lung ischemia-reperfusion injury. Am J Physiol Lung Cell Mol Physiol 2015;308:L1245–52.
96. Khiew SH, Yang J, Young JS, Chen J, Wang Q, Yin D, et al. CTLA4-Ig in combination with FTY720 promotes allograft survival in sensitized recipients. JCI. Insight 2017;2:92033.
97. Fujishiro J, Kudou S, Iwai S, et al. Use of sphingosine-1-phosphate 1 receptor agonist, KRP-203, in combination with a subtherapeutic dose of cyclosporine A for rat renal transplantation. Transplantation 2006;82:804–12.
98. Shimizu H, Takahashi M, Kaneko T, et al. KRP-203, a novel synthetic immunosuppressant, prolongs graft survival and attenuates chronic rejection in rat skin and heart allografts. Circulation 2005;111:222–9.
99. Suzuki C, Takahashi M, Morimoto H, et al. Efficacy of mycophenolic acid combined with KRP-203, a novel immunomodulator, in a rat heart transplantation model. J Heart Lung Transplant 2006;25:302–9.
100. Khattar M, Deng R, Kahan BD, et al. Novel sphingosine-1-phosphate receptor modulator KRP203 combined with locally delivered regulatory T cells induces permanent acceptance of pancreatic islet allografts. Transplantation 2013;95:919–27.
101. Dun H, Song L, Ma A, et al. ASP0028 in combination with suboptimal-dose of tacrolimus in cynomolgus monkey renal transplantation model. Transpl Immunol 2017;40:57–65.
102. Budde K, Schmouder L, Nashan B, et al. Pharmacodynamics of single doses of the novel immunosuppressant FTY720 in stable renal transplant patients. Am J Transplant 2003;3:846–54.
103. Tedesco-Silva H, Pescovitz MD, Cibrik D, et al. Randomized controlled trial of FTY720 versus MMF in de novo renal transplantation. Transplantation 2006;82:1689–97.

104. Tedesco-Silva H, Szakaly P, Shoker A, et al. FTY720 versus mycophenolate mofetil in de novo renal transplantation: six-month results of a double-blind study. Transplantation 2007;84:885–92.
105. Salvadori M, Budde K, Charpentier B, et al. FTY720 versus MMF with cyclosporine in de novo renal transplantation: a 1-year, randomized controlled trial in Europe and Australasia. Am J Transplant 2006;6:2912–21.
106. Mulgaonkar S, Tedesco H, Oppenheimer F, Walker R, Kunzendorf U, Russ G, et al. FTY720/cyclosporine regimens in de novo renal transplantation: a 1-year dose-finding study. Am J Transplant 2006;6:1848–57.
107. Hoitsma AJ, Woodle ES, Abramowicz D, Proot P, Vanrenterghem Y. FTY720 combined with tacrolimus in de novo renal transplantation: 1-year, multicenter, open-label randomized study. Nephrol Dial Transplant 2011;26:3802–5.
108. Tedesco-Silva H, Lorber MI, Foster CE, et al. FTY720 and everolimus in de novo renal transplant patients at risk for delayed graft function: results of an exploratory one-yr multicenter study. Clin Transplant 2009;23:589–99.
109. Ettenger R, Schmouder R, Kovarik JM, Bastien MC, Hoyer PF. Pharmacokinetics, pharmacodynamics, safety, and tolerability of single-dose fingolimod (FTY720) in adolescents with stable renal transplants. Pediatr Transplant 2011;15:406–13.
110. Oppenheimer F, Mulgaonkar S, Ferguson R, et al. Impact of long-term therapy with FTY720 or mycophenolate mofetil on cardiac conduction and rhythm in stable adult renal transplant patients. Transplantation 2007;83:645–8.
111. Mizuno K, Tsujino M, Takada M, Hayashi M, Atsumi K. Studies on bredinin. I. Isolation, characterization and biological properties. J Antibiot (Tokyo) 1974;27:775–82.
112. Ichikawa Y, Ihara H, Takahara S, et al. The immunosuppressive mode of action of mizoribine. Transplantation 1984;38:262–7.
113. Aso K, Uchida H, Sato K, et al. Immunosuppression with low-dose cyclosporine combined with bredinin and prednisolone. Transplant Proc 1987;19:1955–8.
114. Kokado Y, Ishibashi M, Jiang H, Takahara S, Sonoda T. A new triple-drug induction therapy with low dose cyclosporine, mizoribine and prednisolone in renal transplantation. Transplant Proc 1989;21:1575–8.
115. Takeuchi N, Ohshima S, Matsuura O, et al. Immunosuppression with low-dose cyclosporine, mizoribine, and steroids in living-related kidney transplantation. Transplant Proc 1994;26:1907–9.
116. Tanabe K, Tokumoto T, Ishikawa N, et al. Long-term results in mizoribine-treated renal transplant recipients: a prospective, randomized trial of mizoribine and azathioprine under cyclosporine-based immunosuppression. Transplant Proc 1999;31:2877–9.
117. Hosoya M, Shigeta S, Ishii T, Suzuki H, De CE. Comparative inhibitory effects of various nucleoside and nonnucleoside analogues on replication of influenza virus types A and B in vitro and in ovo. J Infect Dis 1993;168:641–6.
118. Naka K, Ikeda M, Abe K, Dansako H, Kato N. Mizoribine inhibits hepatitis C virus RNA replication: effect of combination with interferon-alpha. Biochem Biophys Res Commun 2005;330:871–9.
119. Saijo M, Morikawa S, Fukushi S, et al. Inhibitory effect of mizoribine and ribavirin on the replication of severe acute respiratory syndrome (SARS)-associated coronavirus. Antiviral Res 2005;66:159–63.
120. Shigeta S. Recent progress in antiviral chemotherapy for respiratory syncytial virus infections. Expert Opin Investig Drugs 2000;9:221–35.
121. Funahashi Y, Hattori R, Kinukawa T, Kimura H, Nishiyama Y, Gotoh M. Conversion from mycophenolate mofetil to mizoribine for patients with positive polyomavirus type BK in urine. Transplant Proc 2008;40:2268–70.
122. Liu H, Wang Y, Fan B, et al. Improvement in severe mycophenolic acid-associated gastrointestinal symptoms after changing enteric-coated mycophenolate sodium to mizoribine in renal transplant recipients: two case reports. Intern Med 2016;55:2005–10.
123. Shi Y, Liu H, Chen XG, Shen ZY. Comparison of mizoribine and mycophenolate mofetil with a tacrolimus-based immunosuppressive regimen in living-donor kidney transplantation recipients: a retrospective study in China. Transplant Proc 2017;49:26–31.
124. Ushigome H, Uchida K, Nishimura K, et al. Efficacy and safety of high-dose mizoribine combined with cyclosporine, basiliximab, and corticosteroids in renal transplantation: a Japanese multicenter study. Transplant Proc 2016;48:794–8.
125. Yoshimura N, Nakao T, Nakamura T, et al. Effectiveness of the combination of everolimus and tacrolimus with high dosage of mizoribine for living donor-related kidney transplantation. Transplant Proc 2016;48:786–9.
126. Akioka K, Ishikawa T, Osaka M, et al. Hyperuricemia and acute renal failure in renal transplant recipients treated with high-dose mizoribine. Transplant Proc 2017;49:73–7.
127. Behbod F, Erwin-Cohen RA, Wang ME, et al. Concomitant inhibition of janus kinase 3 and calcineurin-dependent signaling pathways synergistically prolongs the survival of rat heart allografts. J Immunol 2001;166:3724–32.
128. Kirken RA, Erwin RA, Taub D, et al. Tyrphostin AG-490 inhibits cytokine-mediated JAK3/STAT5a/b signal transduction and cellular proliferation of antigen-activated human T cells. J Leukoc Biol 1999;65:891–9.
129. Kudlacz E, Perry B, Sawyer P, et al. The novel JAK-3 inhibitor CP-690550 is a potent immunosuppressive agent in various murine models. Am J Transplant 2004;4:51–7.
130. Stepkowski SM, Kao J, Wang ME, et al. The Mannich base NC1153 promotes long-term allograft survival and spares the recipient from multiple toxicities. J Immunol 2005;175:4236–46.
131. Quaedackers ME, Mol W, Korevaar SS, et al. Monitoring of the immunomodulatory effect of CP-690,550 by analysis of the JAK/STAT pathway in kidney transplant patients. Transplantation 2009;88:1002–9.
132. Macchi P, Villa A, Giliani S, et al. Mutations of Jak-3 gene in patients with autosomal severe combined immune deficiency (SCID). Nature 1995;377:65–8.
133. Roberts JL, Lengi A, Brown SM, et al. Janus kinase 3 (JAK3) deficiency: clinical, immunologic, and molecular analyses of 10 patients and outcomes of stem cell transplantation. Blood 2004;103:2009–18.
134. Russell SM, Johnston JA, Noguchi M, et al. Interaction of IL-2R beta and gamma c chains with Jak1 and Jak3:implications for XSCID and XCID. Science 1994;266:1042–5.
135. Russell SM, Tayebi N, Nakajima H, et al. Mutation of Jak3 in a patient with SCID: essential role of Jak3 in lymphoid development. Science 1995;270:797–800.
136. Kundig TM, Schorle H, Bachmann MF, Hengartner H, Zinkernagel RM, Horak I. Immune responses in interleukin-2-deficient mice. Science 1993;262:1059–61.
137. Malek TR, Bayer AL. Tolerance, not immunity, crucially depends on IL-2. Nat Rev Immunol 2004;4:665–74.
138. Malek TR, Yu A, Vincek V, Scibelli P, Kong L. CD4 regulatory T cells prevent lethal autoimmunity in IL-2Rbeta-deficient mice. Implications for the nonredundant function of IL-2. Immunity 2002;17:167–78.
139. Jiang JK, Ghoreschi K, Deflorian F, et al. Examining the chirality, conformation and selective kinase inhibition of 3-((3R,4R)-4-methyl-3-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)piperidin-1-yl)-3-oxopropanenitrile (CP-690,550). J Med Chem 2008;51:8012–8.
140. Karaman MW, Herrgard S, Treiber DK, et al. A quantitative analysis of kinase inhibitor selectivity. Nat Biotechnol 2008;26:127–32.
141. Meyer DM, Jesson MI, Li X, et al. Anti-inflammatory activity and neutrophil reductions mediated by the JAK1/JAK3 inhibitor, CP-690,550, in rat adjuvant-induced arthritis. J Inflamm (Lond). 2010;7:41–7.
142. Soth M, Hermann JC, Yee C, et al. 3-Amido pyrrolopyrazine JAK kinase inhibitors: development of a JAK3 vs JAK1 selective inhibitor and evaluation in cellular and in vivo models. J Med Chem 2013;56:345–56.
143. Thoma G, Nuninger F, Falchetto R, et al. Identification of a potent Janus kinase 3 inhibitor with high selectivity within the Janus kinase family. J Med Chem 2011;54:284–8.
144. Williams NK, Bamert RS, Patel O, et al. Dissecting specificity in the Janus kinases: the structures of JAK-specific inhibitors complexed to the JAK1 and JAK2 protein tyrosine kinase domains. J Mol Biol 2009;387:219–32.
145. Borie DC, Larson MJ, Flores MG, et al. Combined use of the JAK3 inhibitor CP-690,550 with mycophenolate mofetil to prevent kidney allograft rejection in nonhuman primates. Transplantation 2005;80:1756–64.
146. Borie DC, O'Shea JJ, Changelian PS. JAK3 inhibition, a viable new modality of immunosuppression for solid organ transplants. Trends Mol Med 2004;10:532–41.

147. Changelian PS, Flanagan ME, Ball DJ, et al. Prevention of organ allograft rejection by a specific Janus kinase 3 inhibitor. Science 2003;302:875–8.
148. Paniagua R, Si MS, Flores MG, et al. Effects of JAK3 inhibition with CP-690,550 on immune cell populations and their functions in nonhuman primate recipients of kidney allografts. Transplantation 2005;80:1283–92.
149. Conklyn M, Andresen C, Changelian P, Kudlacz E. The JAK3 inhibitor CP-690550 selectively reduces NK and CD8+ cell numbers in cynomolgus monkey blood following chronic oral dosing. J Leukoc Biol 2004;76:1248–55.
150. Sewgobind VD, Quaedackers ME, van der Laan LJ, et al. The Jak inhibitor CP-690,550 preserves the function of CD4CD25FoxP3 regulatory T cells and inhibits effector T cells. Am J Transplant 2010;10:1785–95.
151. Busque S, Leventhal J, Brennan DC, et al. Calcineurin-inhibitor-free immunosuppression based on the JAK inhibitor CP-690,550: a pilot study in de novo kidney allograft recipients. Am J Transplant 2009;9:1936–45.
152. Vincenti F, Tedesco SH, Busque S, et al. Randomized phase 2b trial of tofacitinib (CP-690,550) in de novo kidney transplant patients: efficacy, renal function and safety at 1 year. Am J Transplant 2012;12:2446–56.
153. Vincenti F, Silva HT, Busque S, et al. Evaluation of the effect of tofacitinib exposure on outcomes in kidney transplant patients. Am J Transplant 2015;15:1644–53.
154. van GE, Weimar W, Gaston R, et al. Phase 1 dose-escalation study of CP-690 550 in stable renal allograft recipients: preliminary findings of safety, tolerability, effects on lymphocyte subsets and pharmacokinetics. Am J Transplant 2008;8:1711–8.
155. Baan CC, Kannegieter NM, Felipe CR, Tedesco Jr SH. Targeting JAK/STAT signaling to prevent rejection after kidney transplantation: a reappraisal. Transplantation 2016;100:1833–9.
156. Moore CA, Iasella CJ, Venkataramanan R, et al. Janus kinase inhibition for immunosuppression in solid organ transplantation: Is there a role in complex immunologic challenges? Hum Immunol 2017;78:64–71.
157. Kovarik JM, Slade A. Overview of sotrastaurin clinical pharmacokinetics. Ther Drug Monit 2010;32:540–3.
158. Kovarik JM, JU Steiger, Grinyo JM, et al. Pharmacokinetics of sotrastaurin combined with tacrolimus or mycophenolic acid in de novo kidney transplant recipients. Transplantation 2011;91:317–22.
159. Kovarik JM, Stitah S, Slade A, et al. Sotrastaurin and cyclosporine drug interaction study in healthy subjects. Biopharm Drug Dispos 2010;31:331–9.
160. Spitaler M, Cantrell DA. Protein kinase C and beyond. Nat Immunol 2004;5:785–90.
161. Mecklenbrauker I, Saijo K, Zheng NY, Leitges M, Tarakhovsky A. Protein kinase C-delta controls self-antigen-induced B-cell tolerance. Nature 2002;416:860–5.
162. Tan SL, Parker PJ. Emerging and diverse roles of protein kinase C in immune cell signalling. Biochem J 2003;376:545–52.
163. Merani S, McCall M, Pawlick RL, Edgar RL, Davis J, Toso C, et al. AEB071 (sotrastaurin) does not exhibit toxic effects on human islets in vitro, nor after transplantation into immunodeficient mice. Islets 2011;3:338–43.
164. Bigaud M, Wieczorek G, Beerli C, et al. Sotrastaurin (AEB071) alone and in combination with cyclosporine A prolongs survival times of non-human primate recipients of life-supporting kidney allografts. Transplantation 2012;93:156–64.
165. Budde K, Sommerer C, Becker T, et al. Sotrastaurin, a novel small molecule inhibiting protein kinase C: first clinical results in renal-transplant recipients. Am J Transplant 2010;10:571–81.
166. Friman S, Arns W, Nashan B, et al. Sotrastaurin, a novel small molecule inhibiting protein-kinase C: randomized phase II study in renal transplant recipients. Am J Transplant 2011;11:1444–55.
167. Tedesco-Silva H, Kho MM, Hartmann A, et al. Sotrastaurin in calcineurin inhibitor-free regimen using everolimus in de novo kidney transplant recipients. Am J Transplant 2013;13:1757–68.
168. Russ GR, Tedesco-Silva H, Kuypers DR, et al. Efficacy of sotrastaurin plus tacrolimus after de novo kidney transplantation: randomized, phase II trial results. Am J Transplant 2013;13:1746–56.
169. Pascher A, De SP, Pratschke J, et al. Protein kinase C inhibitor sotrastaurin in de novo liver transplant recipients: a randomized phase II trial. Am J Transplant 2015;15:1283–92.
170. Trotter JF, Levy G. Sotrastaurin in liver transplantation: has it had a fair trial? Am J Transplant 2015;15:1137–8.
171. Everly JJ, Walsh RC, Alloway RR, Woodle ES. Proteasome inhibition for antibody-mediated rejection. Curr Opin Organ Transplant 2009;14:662–6.
172. Sunwoo JB, Chen Z, Dong G, et al. Novel proteasome inhibitor PS-341 inhibits activation of nuclear factor-kappa B, cell survival, tumor growth, and angiogenesis in squamous cell carcinoma. Clin Cancer Res 2001;7:1419–28.
173. Perry DK, Burns JM, Pollinger HS, Amiot BP, Gloor JM, Gores GJ, et al. Proteasome inhibition causes apoptosis of normal human plasma cells preventing alloantibody production. Am J Transplant 2009;9:201–9.
174. Lang VR, Mielenz D, Neubert K, et al. The early marginal zone B cell-initiated T-independent type 2 response resists the proteasome inhibitor bortezomib. J Immunol 2010;185:5637–47.
175. Neubert K, Meister S, Moser K, et al. The proteasome inhibitor bortezomib depletes plasma cells and protects mice with lupus-like disease from nephritis. Nat Med 2008;14:748–55.
176. Vogelbacher R, Meister S, Guckel E, et al. Bortezomib and sirolimus inhibit the chronic active antibody-mediated rejection in experimental renal transplantation in the rat. Nephrol Dial Transplant 2010;25:3764–73.
177. Shah N, Meouchy J, Qazi Y. Bortezomib in kidney transplantation. Curr Opin Organ Transplant 2015;20:652–6.
178. Diwan TS, Raghavaiah S, Burns JM, Kremers WK, Gloor JM, Stegall MD. The impact of proteasome inhibition on alloantibody-producing plasma cells in vivo. Transplantation 2011;91:536–41.
179. Everly MJ, Everly JJ, Susskind B, et al. Bortezomib provides effective therapy for antibody- and cell-mediated acute rejection. Transplantation 2008;86:1754–61.
180. Sberro-Soussan R, Zuber J, Suberbielle-Boissel C, et al. Bortezomib as the sole post-renal transplantation desensitization agent does not decrease donor-specific anti-HLA antibodies. Am J Transplant 2010;10:681–6.
181. Walsh RC, Everly JJ, Brailey P, et al. Proteasome inhibitor-based primary therapy for antibody-mediated renal allograft rejection. Transplantation 2010;89:277–84.
182. Woodle ES, Shields AR, Ejaz NS, et al. Prospective iterative trial of proteasome inhibitor-based desensitization. Am J Transplant 2015;15:101–18.
183. Moreno Gonzales MA, Gandhi MJ, Schinstock CA, et al. 32 Doses of bortezomib for desensitization is not well tolerated and is associated with only modest reductions in anti-HLA antibody. Transplantation 2017;101:1222–7.
184. Kwun J, Burghuber C, Manook M, et al. Humoral compensation after bortezomib treatment of allosensitized recipients. J Am Soc Nephrol 2017;28:1991–6.
185. Kortuem KM, Stewart AK. Carfilzomib. Blood 2013;121:893–7.
186. De Sousa-Amorim E, Revuelta I, Diekmann F, et al. High incidence of paralytic ileus after bortezomib treatment of antibody-mediated rejection in kidney transplant recipients: report of 2 cases. Transplantation 2015;99:e170–1.
187. Everly MJ, Terasaki PI, Hopfield J, Trivedi HL, Kaneku H. Protective immunity remains intact after antibody removal by means of proteasome inhibition. Transplantation 2010;90:1493–8.
188. Bikle D. Nonclassic actions of vitamin D. J Clin Endocrinol Metab 2009;94:26–34.
189. Verstuyf A, Carmeliet G, Bouillon R, Mathieu C. Vitamin D: a pleiotropic hormone. Kidney Int 2010;78:140–5.
190. Provvedini DM, Tsoukas CD, Deftos LJ, Manolagas SC. 1,25-dihydroxyvitamin D3 receptors in human leukocytes. Science 1983;221:1181–3.
191. Takahashi K, Nakayama Y, Horiuchi H, et al. Human neutrophils express messenger RNA of vitamin D receptor and respond to 1alpha,25-dihydroxyvitamin D3. Immunopharmacol Immunotoxicol 2002;24:335–47.
192. Baeke F, Korf H, Overbergh L, et al. Human T lymphocytes are direct targets of 1,25-dihydroxyvitamin D3 in the immune system. J Steroid Biochem Mol Biol 2010;121:221–7.
193. Overbergh L, Decallonne B, Valckx D, et al. Identification and immune regulation of 25-hydroxyvitamin D-1-alpha-hydroxylase in murine macrophages. Clin Exp Immunol 2000;120:139–46.
194. Stoffels K, Overbergh L, Bouillon R, Mathieu C. Immune regulation of 1alpha-hydroxylase in murine peritoneal macrophages: unravelling the IFN-gamma pathway. J Steroid Biochem Mol Biol 2007;103:567–71.

195. Dusso AS, Kamimura S, Gallieni M, et al. gamma-Interferon-induced resistance to 1,25-(OH)2 D3 in human monocytes and macrophages: a mechanism for the hypercalcemia of various granulomatoses. J Clin Endocrinol Metab 1997;82:2222–32.
196. Gregori S, Casorati M, Amuchastegui S, Smiroldo S, Davalli AM, Adorini L. Regulatory T cells induced by 1 alpha,25-dihydroxyvitamin D3 and mycophenolate mofetil treatment mediate transplantation tolerance. J Immunol 2001;167:1945–53.
197. Penna G, Adorini L. 1 Alpha,25-dihydroxyvitamin D3 inhibits differentiation, maturation, activation, and survival of dendritic cells leading to impaired alloreactive T cell activation. J Immunol 2000;164:2405–11.
198. van Halteren AG, van EE, de Jong EC, Bouillon R, Roep BO, Mathieu C. Redirection of human autoreactive T-cells Upon interaction with dendritic cells modulated by TX527, an analog of 1,25 dihydroxyvitamin D(3). Diabetes 2002;51:2119–25.
199. Baeke F, Takiishi T, Korf H, Gysemans C, Mathieu C. Vitamin D: modulator of the immune system. Curr Opin Pharmacol 2010;10:482–96.
200. Jeffery LE, Burke F, Mura M, et al. 1,25-Dihydroxyvitamin D3 and IL-2 combine to inhibit T cell production of inflammatory cytokines and promote development of regulatory T cells expressing CTLA-4 and FoxP3. J Immunol 2009;183:5458–67.
201. Tang J, Zhou R, Luger D, et al. Calcitriol suppresses antiretinal autoimmunity through inhibitory effects on the Th17 effector response. J Immunol 2009;182:4624–32.
202. Chen S, Sims GP, Chen XX, Gu YY, Chen S, Lipsky PE. Modulatory effects of 1,25-dihydroxyvitamin D3 on human B cell differentiation. J Immunol 2007;179:1634–47.
203. Casteels KM, Mathieu C, Waer M, et al. Prevention of type I diabetes in nonobese diabetic mice by late intervention with nonhypercalcemic analogs of 1,25-dihydroxyvitamin D3 in combination with a short induction course of cyclosporin A. Endocrinology 1998;139:95–102.
204. Gysemans C, Waer M, Laureys J, Bouillon R, Mathieu C. A combination of KH1060, a vitamin D(3) analogue, and cyclosporin prevents early graft failure and prolongs graft survival of xenogeneic islets in nonobese diabetic mice. Transplant Proc 2001;33:2365.
205. Branisteanu DD, Mathieu C, Bouillon R. Synergism between sirolimus and 1,25-dihydroxyvitamin D3 in vitro and in vivo. J Neuroimmunol 1997;79:138–47.
206. Branisteanu DD, Waer M, Sobis H, Marcelis S, Vandeputte M, Bouillon R. Prevention of murine experimental allergic encephalomyelitis: cooperative effects of cyclosporine and 1 alpha, 25-(OH)2D3. J Neuroimmunol 1995;61:151–60.
207. van EE, Branisteanu DD, Verstuyf A, Waer M, Bouillon R, Mathieu C. Analogs of 1,25-dihydroxyvitamin D3 as dose-reducing agents for classical immunosuppressants. Transplantation 2000;69:1932–42.
208. Bertolini DL, Araujo PR, Silva RN, Duarte AJ, Tzanno-Martins CB. Immunomodulatory effects of vitamin D analog KH1060 on an experimental skin transplantation model. Transplant Proc 1999;31:2998–9.
209. Hullett DA, Cantorna MT, Redaelli C, et al. Prolongation of allograft survival by 1,25-dihydroxyvitamin D3. Transplantation 1998;66:824–8.
210. Johnsson C, Tufveson G. MC 1288—a vitamin D analogue with immunosuppressive effects on heart and small bowel grafts. Transpl Int 1994;7:392–7.
211. Lemire JM, Archer DC, Khulkarni A, Ince A, Uskokovic MR, Stepkowski S. Prolongation of the survival of murine cardiac allografts by the vitamin D3 analogue 1,25-dihydroxy-delta 16-cholecalciferol. Transplantation 1992;54:762–3.
212. Pakkala I, Taskinen E, Pakkala S, Raisanen-Sokolowski A. MC1288, a vitamin D analog, prevents acute graft-versus-host disease in rat bone marrow transplantation. Bone Marrow Transplant 2001;27:863–7.
213. Raisanen-Sokolowski AK, Pakkala IS, Samila SP, Binderup L, Hayry PJ, Pakkala ST. A vitamin D analog, MC1288, inhibits adventitial inflammation and suppresses intimal lesions in rat aortic allografts. Transplantation 1997;63:936–41.
214. Redaelli CA, Wagner M, Gunter-Duwe D, et al. 1alpha,25-dihydroxyvitamin D3 shows strong and additive immunomodulatory effects with cyclosporine A in rat renal allotransplants. Kidney Int 2002;61:288–96.
215. Redaelli CA, Wagner M, Tien YH, et al. 1 alpha,25-Dihydroxycholecalciferol reduces rejection and improves survival in rat liver allografts. Hepatology 2001;34:926–34.
216. Veyron P, Pamphile R, Binderup L, Touraine JL. New 20-epi-vitamin D3 analogs: immunosuppressive effects on skin allograft survival. Transplant Proc 1995;27:450.
217. Vos R, Ruttens D, Verleden SE, et al. High-dose vitamin D after lung transplantation: a randomized trial. J Heart Lung Transplant 2017;10.
218. Cippa PE, Kraus AK, Edenhofer I, et al. The BH3-mimetic ABT-737 inhibits allogeneic immune responses. Transpl Int 2011;24:722–32.
219. Gabriel SS, Bon N, Chen J, et al. Distinctive expression of Bcl-2 factors in regulatory T cells determines a pharmacological target to induce immunological tolerance. Front Immunol 2016;7:73.
220. Cippa PE, Kamarashev J, Chen J, et al. Synergistic Bcl-2 inhibition by ABT-737 and cyclosporine A. Apoptosis 2013;18:315–23.
221. Cippa PE, Gabriel SS, Chen J, et al. Targeting apoptosis to induce stable mixed hematopoietic chimerism and long-term allograft survival without myelosuppressive conditioning in mice. Blood 2013;122:1669–77.
222. Cippa PE, Gabriel SS, Kraus AK, et al. Bcl-2 inhibition to overcome memory cell barriers in transplantation. Am J Transplant 2014;14:333–42.
223. Zhang L, You J, Sidhu J, et al. Abrogation of chronic rejection in rat model system involves modulation of the mTORC1 and mTORC2 pathways. Transplantation 2013;96:782–90.
224. Pike KG, Malagu K, Hummersone MG, et al. Optimization of potent and selective dual mTORC1 and mTORC2 inhibitors: the discovery of AZD8055 and AZD2014. Bioorg Med Chem Lett 2013;23:1212–6.
225. Rosborough BR, Raich-Regue D, Liu Q, Venkataramanan R, Turnquist HR, Thomson AW. Adenosine triphosphate-competitive mTOR inhibitors: a new class of immunosuppressive agents that inhibit allograft rejection. Am J Transplant 2014;14:2173–80.
226. Powles T, Wheater M, Din O, et al. A randomised phase 2 study of AZD2014 versus everolimus in patients with VEGF-Refractory Metastatic Clear Cell Renal Cancer. Eur Urol 2016;69:450–6.
227. Basu B, Dean E, Puglisi M, et al. First-in-human pharmacokinetic and pharmacodynamic study of the dual m-TORC 1/2 inhibitor AZD2014. Clin Cancer Res 2015;21:3412–9.
228. Gorski A, Grieb P, Korczak-Kowalska G, Wierzbicki P, Stepien-Sopniewska B, Mrowiec T. Cladribine (2-chloro-deoxyadenosine, CDA): an inhibitor of human B and T cell activation in vitro. Immunopharmacology 1993;26:197–202.
229. Gorski A, Grieb P, Makula J, Stepien-Sopniewska B, Mrowiec T, Nowaczyk M. 2-Chloro-2-deoxyadenosine—a novel immunosuppressive agent. Transplantation 1993;56:1253–7.
230. Schmid T, Hechenleitner P, Mark W, et al. 2-Chlorodeoxyadenosine (cladribine) in combination with low-dose cyclosporin prevents rejection after allogeneic heart and liver transplantation in the rat. Eur Surg Res 1998;30:61–8.
231. Oberhuber G, Schmid T, Thaler W, et al. Evidence that 2-chlorodeoxyadenosine in combination with cyclosporine prevents rejection after allogeneic small bowel transplantation. Transplantation 1994;58:743–5.
232. Si MS, Ji P, Tromberg BJ, et al. Farnesyltransferase inhibition: a novel method of immunomodulation. Int Immunopharmacol 2003;3:475–83.
233. Si MS, Ji P, Lee M, et al. Potent farnesyltransferase inhibitor ABT-100 abrogates acute allograft rejection. J Heart Lung Transplant 2005;24:1403–9.
234. Liu YX, Shen NY, Liu C, Lv Y. Immunosuppressive effects of emodin: an in vivo and in vitro study. Transplant Proc 2009;41:1837–9.
235. Fujine K, Abe F, Seki N, Ueda H, Hino M, Fujii T. FR252921, a novel immunosuppressive agent isolated from Pseudomonas fluorescens no. 408813 II. In vitro property and mode of action. J Antibiot (Tokyo) 2003;56:62–7.
236. Fujine K, Tanaka M, Ohsumi K, et al. FR252921, a novel immunosuppressive agent isolated from Pseudomonas fluorescens no. 408813. I. Taxonomy, fermentation, isolation, physico-chemical properties and biological activities of FR252921, FR252922 and FR256523. J Antibiot (Tokyo) 2003;56:55–61.
237. Fujine K, Ueda H, Hino M, Fujii T. FR252921, a novel immunosuppressive agent isolated from Pseudomonas fluorescens no. 408813 III. In vivo activities. J Antibiot (Tokyo) 2003;56:68–71.
238. Thomas FT, Tepper MA, Thomas JM, Haisch CE. 15-Deoxyspergualin: a novel immunosuppressive drug with clinical potential. Ann NY Acad Sci 1993;685:175–92.

239. Amemiya H, Koyama I, Kyo M, et al. Outline and long-term prognosis in 15-deoxyspergualin-treated cases. Japan Collaborative Transplant Study Group of NKT-01. Transplant Proc 1996;28:1156–8.
240. Groth CG. Deoxyspergualin in allogeneic kidney and xenogeneic islet transplantation: early clinical trials. Ann NY Acad Sci 1993;685:193–5.
241. Takahashi K, Tanabe K, Ooba S, et al. Prophylactic use of a new immunosuppressive agent, deoxyspergualin, in patients with kidney transplantation from ABO-incompatible or preformed antibody-positive donors. Transplant Proc 1991;23:1078–82.
242. Lebreton L, Annat J, Derrepas P, Dutartre P, Renaut P. Structure-immunosuppressive activity relationships of new analogues of 15-deoxyspergualin. 1. Structural modifications of the hydroxyglycine moiety. J Med Chem 1999;42:277–90.
243. Boddy AV, Yule SM. Metabolism and pharmacokinetics of oxazaphosphorines. Clin Pharmacokinet 2000;38:291–304.
244. de Jonge ME, Huitema AD, Rodenhuis S, Beijnen JH. Clinical pharmacokinetics of cyclophosphamide. Clin Pharmacokinet 2005;44:1135–64.
245. de Jonge ME, Huitema AD, van Dam SM, Rodenhuis S, Beijnen JH. Population pharmacokinetics of cyclophosphamide and its metabolites 4-hydroxycyclophosphamide, 2-dechloroethylcyclophosphamide, and phosphoramide mustard in a high-dose combination with Thiotepa and Carboplatin. Ther Drug Monit 2005;27:756–65.
246. Alarabi A, Backman U, Wikstrom B, Sjoberg O, Tufveson G. Plasmapheresis in HLA-immunosensitized patients prior to kidney transplantation. Int J Artif Organs 1997;20:51–6.
247. Fuks Z, Strober S, Bobrove AM, Sasazuki T, McMichael A, Kaplan HS. Long term effects of radiation of T and B lymphocytes in peripheral blood of patients with Hodgkin's disease. J Clin Invest 1976;58:803–14.
248. Lan F, Zeng D, Higuchi M, Higgins JP, Strober S. Host conditioning with total lymphoid irradiation and antithymocyte globulin prevents graft-versus-host disease: the role of CD1-reactive natural killer T cells. Biol Blood Marrow Transplant 2003;9:355–63.
249. Lan F, Zeng D, Higuchi M, Huie P, Higgins JP, Strober S. Predominance of NK1.1+TCR alpha beta+ or DX5+TCR alpha beta+ T cells in mice conditioned with fractionated lymphoid irradiation protects against graft-versus-host disease: "natural suppressor" cells. J Immunol 2001;167:2087–96.
250. Hertel-Wulff B, Palathumpat V, Schwadron R, Strober S. Prevention of graft-versus-host disease by natural suppressor cells. Transplant Proc 1987;19:536–9.
251. Bass H, Strober S. Deficits in T helper cells after total lymphoid irradiation (TLI): reduced IL-2 secretion and normal IL-2 receptor expression in the mixed leukocyte reaction (MLR). Cell Immunol 1990;126:129–42.
252. Field EH, Becker GC. The immunosuppressive mechanism of total lymphoid irradiation. I. The effect on IL-2 production and IL-2 receptor expression. Transplantation 1989;48:499–505.
253. Field EH, Becker GC. Blocking of mixed lymphocyte reaction by spleen cells from total lymphoid-irradiated mice involves interruption of the IL-2 pathway. J Immunol 1992;148:354–9.
254. Palathumpat VC, Vandeputte MM, Waer M. Effects of thymus irradiation on the immune competence of T cells after total-lymphoid irradiation. Transplantation 1990;50:95–100.
255. Field EH, Steinmuller D. Nondeletional mechanisms of tolerance in total-lymphoid irradiation-induced bone marrow chimeras. Transplantation 1993;56:250–3.
256. Salam A, Vandeputte M, Waer M. Clonal deletion and clonal anergy in allogeneic bone marrow chimeras prepared with TBI or TLI. Transpl Int 1994;7(Suppl 1):S457–61.
257. Florence LS, Jiang GL, Ang KK, Stepkowski SM, Kahan BD. In vitro analysis of T cell-mediated cytotoxicity displayed by rat heart allograft recipients rendered unresponsive by total-lymphoid irradiation and extracted donor antigen. Transplantation 1990;49:436–44.
258. Field EH, Rouse TM. Alloantigen priming after total lymphoid irradiation alters alloimmune cytokine responses. Transplantation 1995;60:695–702.
259. Stark JH, Smit JA, Myburgh JA. Nonspecific mixed lymphocyte culture inhibitory antibodies in sera of tolerant transplanted baboons conditioned with total lymphoid irradiation. Transplantation 1994;57:1103–10.
260. Nador RG, Hongo D, Baker J, Yao Z, Strober S. The changed balance of regulatory and naive T cells promotes tolerance after TLI and anti-T-cell antibody conditioning. Am J Transplant 2010;10:262–72.
261. Strober S, Slavin S, Gottlieb M, et al. Allograft tolerance after total lymphoid irradiation (TLI). Immunol Rev 1979;46:87–112.
262. Waer M, Ang KK, Van der Schueren E, Vandeputte M. Allogeneic bone marrow transplantation in mice after total lymphoid irradiation: influence of breeding conditions and strain of recipient mice. J Immunol 1984;132:991–6.
263. Waer M, Ang KK, Van der Schueren E, Vandeputte M. Influence of radiation field and fractionation schedule of total lymphoid irradiation (TLI) on the induction of suppressor cells and stable chimerism after bone marrow transplantation in mice. J Immunol 1984;132:985–90.
264. Gottlieb M, Strober S, Hoppe RT, Grumet FC, Kaplan HS. Engraftment of allogeneic bone marrow without graft-versus-host disease in mongrel dogs using total lymphoid irradiation. Transplantation 1980;29:487–91.
265. Howard RJ, Sutherland DE, Lum CT, et al. Kidney allograft survival in dogs treated with total lymphoid irradiation. Ann Surg 1981;193:196–200.
266. Rynasiewicz JJ, Sutherland DE, Kawahara K, Najarian JS. Total lymphoid irradiation: critical timing and combination with cyclosporin A for immunosuppression in a rat heart allograft model. J Surg Res 1981;30:365–71.
267. Strober S, Modry DL, Hoppe RT, et al. Induction of specific unresponsiveness to heart allografts in mongrel dogs treated with total lymphoid irradiation and antithymocyte globulin. J Immunol 1984;132:1013–8.
268. Myburgh JA, Smit JA, Stark JH, Browde S. Total lymphoid irradiation in kidney and liver transplantation in the baboon: prolonged graft survival and alterations in T cell subsets with low cumulative dose regimens. J Immunol 1984;132:1019–25.
269. Sadeghi AM, Laks H, Drinkwater DC, et al. Heart-lung xenotransplantation in primates. J Heart Lung Transplant 1991;10:442–7.
270. Bollinger RR, Fabian MA, Harland RC, et al. Total lymphoid irradiation for cardiac xenotransplantation in nonhuman primates. Transplant Proc 1991;23:587–8.
271. Panza A, Roslin MS, Coons M, et al. One-year survival of heterotopic heart primate xenografts treated with total lymphoid irradiation and cyclosporine. Transplant Proc 1991;23:483–4.
272. Thomas F, Pittman K, Ljung T, Cekada E. Deoxyspergualin is a unique immunosuppressive agent with selective utility in inducing tolerance to pancreas islet xenografts. Transplant Proc 1995;27:417–9.
273. Tixier D, Levy C, Le Bourgeois JP, Leandri J, Loisance D. [Discordant heart xenografts. Experimental study in pigs conditioned by total lymphoid irradiation and cyclosporine A]. Presse Med 1992;21:1941–4.
274. Trager DK, Banks BA, Rosenbaum GE, et al. Cardiac allograft prolongation in mice treated with combined posttransplantation total-lymphoid irradiation and anti-L3T4 antibody therapy. Transplantation 1989;47:587–91.
275. Hayamizu K, Huie P, Sibley RK, Strober S. Monocyte-derived dendritic cell precursors facilitate tolerance to heart allografts after total lymphoid irradiation. Transplantation 1998;66:1285–91.
276. Najarian JS, Ferguson RM, Sutherland DE, et al. Fractionated total lymphoid irradiation as preparative immunosuppression in high risk renal transplantation: clinical and immunological studies. Ann Surg 1982;196:442–52.
277. Waer M, Vanrenterghem Y, Roels L, et al. Total lymphoid irradiation in renal cadaveric transplantation in diabetics. Lancet 1985;2:1354.
278. Waer M, Leenaerts P, Vanrenterghem Y, et al. Factors determining the success rate of total lymphoid irradiation in clinical kidney transplantation. Transplant Proc 1989;21:1796–7.
279. Levin B, Hoppe RT, Collins G, et al. Treatment of cadaveric renal transplant recipients with total lymphoid irradiation, antithymocyte globulin, and low-dose prednisone. Lancet 1985;2:1321–5.
280. Chow D, Saper V, Strober S. Renal transplant patients treated with total lymphoid irradiation show specific unresponsiveness to donor antigens the mixed leukocyte reaction (MLR). J Immunol 1987;138:3746–50.
281. Strober S, Dhillon M, Schubert M, et al. Acquired immune tolerance to cadaveric renal allografts. A study of three patients treated with total lymphoid irradiation. N Engl J Med 1989;321:28–33.
282. Hunt SA, Strober S, Hoppe RT, Stinson EB. Total lymphoid irradiation for treatment of intractable cardiac allograft rejection. J Heart Lung Transplant 1991;10:211–6.
283. Levin B, Bohannon L, Warvariv V, Bry W, Collins G. Total lymphoid irradiation (TLI) in the cyclosporine era—use of TLI in resistant cardiac allograft rejection. Transplant Proc 1989;21:1793–5.

284. Salter SP, Salter MM, Kirklin JK, Bourge RC, Naftel DC. Total lymphoid irradiation in the treatment of early or recurrent heart transplant rejection. Int J Radiat Oncol Biol Phys 1995;33:83–8.
285. Asano M, Gundry SR, Razzouk AJ, et al. Total lymphoid irradiation for refractory rejection in pediatric heart transplantation. Ann Thorac Surg 2002;74:1979–85.
286. Chin C, Hunt S, Robbins R, Hoppe R, Reitz B, Bernstein D. Long-term follow-up after total lymphoid irradiation in pediatric heart transplant recipients. J Heart Lung Transplant 2002;21:667–73.
287. Madden BP, Barros J, Backhouse L, Stamenkovic S, Tait D, Murday A. Intermediate term results of total lymphoid irradiation for the treatment of non-specific graft dysfunction after heart transplantation. Eur J Cardiothorac Surg 1999;15:663–6.
288. Pelletier MP, Coady M, Macha M, Oyer PE, Robbins RC. Coronary atherosclerosis in cardiac transplant patients treated with total lymphoid irradiation. J Heart Lung Transplant 2003;22:124–9.
289. Valentine VG, Robbins RC, Wehner JH, Patel HR, Berry GJ, Theodore J. Total lymphoid irradiation for refractory acute rejection in heart-lung and lung allografts. Chest 1996;109:1184–9.
290. Scandling JD, Busque S, Dejbakhsh-Jones S, et al. Tolerance and chimerism after renal and hematopoietic-cell transplantation. N Engl J Med 2008;358:362–8.
291. Scandling JD, Busque S, Shizuru JA, Engleman EG, Strober S. Induced immune tolerance for kidney transplantation. N Engl J Med 2011;365:1359–60.
292. Scandling JD, Busque S, Shizuru JA, et al. Chimerism, graft survival, and withdrawal of immunosuppressive drugs in HLA matched and mismatched patients after living donor kidney and hematopoietic cell transplantation. Am J Transplant 2015;15:695–704.
293. Lim TS, O'Driscoll G, Freund J, Peterson V, Hayes H, Heywood J. Short-course total lymphoid irradiation for refractory cardiac transplantation rejection. J Heart Lung Transplant 2007;26:1249–54.
294. Edelson R, Berger C, Gasparro F, et al. Treatment of cutaneous T-cell lymphoma by extracorporeal photochemotherapy. Preliminary results. N Engl J Med 1987;316:297–303.
295. Perotti C, Torretta L, Viarengo G, et al. Feasibility and safety of a new technique of extracorporeal photochemotherapy: experience of 240 procedures. Haematologica 1999;84:237–41.
296. Perez MI, Edelson RL, John L, Laroche L, Berger CL. Inhibition of antiskin allograft immunity induced by infusions with photoinactivated effector T lymphocytes (PET cells). Yale J Biol Med 1989;62:595–609.
297. Pepino P, Berger CL, Fuzesi L, et al. Primate cardiac allo- and xenotransplantation: modulation of the immune response with photochemotherapy. Eur Surg Res 1989;21:105–13.
298. Perez M, Edelson R, Laroche L, Berger C. Inhibition of antiskin allograft immunity by infusions with syngeneic photoinactivated effector lymphocytes. J Invest Dermatol 1989;92:669–76.
299. Yoo EK, Rook AH, Elenitsas R, Gasparro FP, Vowels BR. Apoptosis induction of ultraviolet light A and photochemotherapy in cutaneous T-cell Lymphoma: relevance to mechanism of therapeutic action. J Invest Dermatol 1996;107:235–42.
300. Rook AH, Suchin KR, Kao DM, et al. Photopheresis: clinical applications and mechanism of action. J Investig Dermatol Symp Proc 1999;4:85–90.
301. Baron ED, Heeger PS, Hricik DE, Schulak JA, Tary-Lehmann M, Stevens SR. Immunomodulatory effect of extracorporeal photopheresis after successful treatment of resistant renal allograft rejection. Photodermatol Photoimmunol Photomed 2001;17:79–82.
302. Gatza E, Rogers CE, Clouthier SG, et al. Extracorporeal photopheresis reverses experimental graft-versus-host disease through regulatory T cells. Blood 2008;112:1515–21.
303. Griffith TS, Kazama H, VanOosten RL, et al. Apoptotic cells induce tolerance by generating helpless CD8+ T cells that produce TRAIL. J Immunol 2007;178:2679–87.
304. Dall'Amico R, Murer L, Montini G, et al. Successful treatment of recurrent rejection in renal transplant patients with photopheresis. J Am Soc Nephrol 1998;9:121–7.
305. Genberg H, Kumlien G, Shanwell A, Tyden G. Refractory acute renal allograft rejection successfully treated with photopheresis. Transplant Proc 2005;37:3288–9.
306. Horina JH, Mullegger RR, Horn S, et al. Photopheresis for renal allograft rejection. Lancet 1995;346:61.
307. Kumlien G, Genberg H, Shanwell A, Tyden G. Photopheresis for the treatment of refractory renal graft rejection. Transplantation 2005;79:123–5.
308. Sunder-Plassman G, Druml W, Steininger R, Honigsmann H, Knobler R. Renal allograft rejection controlled by photopheresis. Lancet 1995;346:506.
309. Wolfe JT, Tomaszewski JE, Grossman RA, et al. Reversal of acute renal allograft rejection by extracorporeal photopheresis: a case presentation and review of the literature. J Clin Apher 1996;11:36–41.
310. Barr ML, Meiser BM, Eisen HJ, et al. Photopheresis for the prevention of rejection in cardiac transplantation. Photopheresis Transplantation Study Group. N Engl J Med 1998;339:1744–51.
311. O'Hagan AR, Stillwell PC, Arroliga A, Koo A. Photopheresis in the treatment of refractory bronchiolitis obliterans complicating lung transplantation. Chest 1999;115:1459–62.
312. Salerno CT, Park SJ, Kreykes NS, et al. Adjuvant treatment of refractory lung transplant rejection with extracorporeal photopheresis. J Thorac Cardiovasc Surg 1999;117:1063–9.
313. Starzl TE, Marchioro TL, Waddell WR. Human renal homotransplantation in the presence of blood group incompatibilities. Proc Soc Exp Biol Med 1963;113:471–2.
314. Opelz G, Terasaki PI. Effect of splenectomy on human renal transplants. Transplantation 1973;15:605–8.
315. Pierce JC, Hume DM. The effect of splenectomy on the survival of first and second renal homotransplants in man. Surg Gynecol Obstet 1968;127:1300–6.
316. Stuart FP, Reckard CR, Ketel BL, Schulak JA. Effect of splenectomy on first cadaver kidney transplants. Ann Surg 1980;192:553–61.
317. Fryd DS, Sutherland DE, Simmons RL, Ferguson RM, Kjellstrand CM, Najarian JS. Results of a prospective randomized study on the effect of splenectomy versus no splenectomy in renal transplant patients. Transplant Proc 1981;13:48–56.
318. Sutherland DE, Fryd DS, Strand MH, et al. Results of the Minnesota randomized prospective trial of cyclosporine versus azathioprine-antilymphocyte globulin for immunosuppression in renal allograft recipients. Am J Kidney Dis 1985;5:318–27.
319. Alexander JW, First MR, Majeski JA, et al. The late adverse effect of splenectomy on patient survival following cadaveric renal transplantation. Transplantation 1984;37:467–70.
320. Peters TG, Williams JW, Harmon HC, Britt LG. Splenectomy and death in renal transplant patients. Arch Surg 1983;118:795–9.
321. Lucas BA, Vaughn WK, Sanfilippo F, Peters TG, Alexander JW. Effects of pretransplant splenectomy: univariate and multivariate analyses. Transplant Proc 1987;19:1993–5.
322. Alexandre GP, Squifflet JP, De BM, et al. Present experiences in a series of 26 ABO-incompatible living donor renal allografts. Transplant Proc 1987;19:4538–42.
323. Reding R, Squifflet JP, Pirson Y, et al. Living-related and unrelated donor kidney transplantation: comparison between ABO-compatible and incompatible grafts. Transplant Proc 1987;19:1511–3.
324. Squifflet JP, De MM, Malaise J, Latinne D, Pirson Y, Alexandre GP. Lessons learned from ABO-incompatible living donor kidney transplantation: 20 years later. Exp Clin Transplant 2004;2:208–13.
325. Ishikawa A, Itoh M, Ushlyama T, Suzuki K, Fujita K. Experience of ABO-incompatible living kidney transplantation after double filtration plasmapheresis. Clin Transplant 1998;12:80–3.
326. Tyden G, Kumlien G, Genberg H, Sandberg J, Lundgren T, Fehrman I. ABO incompatible kidney transplantations without splenectomy, using antigen-specific immunoadsorption and rituximab. Am J Transplant 2005;5:145–8.
327. Tyden G, Kumlien G, Genberg H, Sandberg J, Lundgren T, Fehrman I. ABO-incompatible kidney transplantation and rituximab. Transplant Proc 2005;37:3286–7.
328. Orandi BJ, Zachary AA, Dagher NN, et al. Eculizumab and splenectomy as salvage therapy for severe antibody-mediated rejection after HLA-incompatible kidney transplantation. Transplantation 2014;98:857–63.
329. Orandi BJ, Lonze BE, Jackson A, et al. Splenic irradiation for the treatment of severe antibody-mediated rejection. Am J Transplant 2016;16:3041–5.
330. Montgomery RA, Lonze BE, King KE, et al. Desensitization in HLA-incompatible kidney recipients and survival. N Engl J Med 2011;365:318–26.
331. Orandi BJ, Luo X, Massie AB, et al. Survival benefit with kidney transplants from HLA-incompatible live donors. N Engl J Med 2016;374:940–50.
332. Takahashi K, Saito K. ABO-incompatible kidney transplantation. Transplant Rev (Orlando) 2013;27:1–8.

333. Clark WF, Huang SS, Walsh MW, Farah M, Hildebrand AM, Sontrop JM. Plasmapheresis for the treatment of kidney diseases. Kidney Int 2016;90:974–84.
334. Inui M, Miyazato T, Furusawa M, et al. Successful kidney transplantation after stepwise desensitization using rituximab and bortezomib in a highly HLA-Sensitized and ABO incompatible high titer patient. Transplant Direct 2016;2:e92.
335. Allen NH, Dyer P, Geoghegan T, Harris K, Lee HA, Slapak M. Plasma exchange in acute renal allograft rejection: a controlled trial. Transplantation 1983;35:425–8.
336. Kirubakaran MG, Disney AP, Norman J, Pugsley DJ, Mathew TH. A controlled trial of plasmapheresis in the treatment of renal allograft rejection. Transplantation 1981;32:164–5.
337. Taube DH, Williams DG, Cameron JS, et al. Renal transplantation after removal and prevention of resynthesis of HLA antibodies. Lancet 1984;1:824–8.
338. Brynger H, Rydberg L, Samuelsson B, Blohme I, Lindholm A, Sandberg L. Renal transplantation across a blood group barrier—'A2' kidneys to 'O' recipients. Proc Eur Dial Transplant Assoc 1983;19:427–31.
339. Schwartz J, Padmanabhan A, Aqui N, et al. Guidelines on the use of therapeutic apheresis in clinical practice-evidence-based approach from the Writing Committee of the American Society of Apheresis: the seventh special issue. J Clin Apher 2016;31:149–338.
340. Takahashi K, Saito K, Takahara S, et al. Results of a multicenter prospective clinical study in Japan for evaluating efficacy and safety of desensitization protocol based on rituximab in ABO-incompatible kidney transplantation. Clin Exp Nephrol 2017;21(4):705–13. Available online at: https://doi.org/10.1007/s10157-016-1321-5.
341. Tanabe K, Ishida H, Inui M, et al. ABO-incompatible kidney transplantation: long-term outcomes. Clin Transpl 2013:307–12.
342. Cardella CJ, Sutton DM, Falk JA, Katz A, Uldall PR, deVeber GA. Effect of intensive plasma exchange on renal transplant rejection and serum cytotoxic antibody. Transplant Proc 1978;10:617–9.
343. Nojima M, Yoshimoto T, Nakao A, et al. Combined therapy of deoxyspergualin and plasmapheresis: a useful treatment for antibody-mediated acute rejection after kidney transplantation. Transplant Proc 2005;37:930–3.
344. KDIGO clinical practice guideline for the care of kidney transplant recipients. Am J Transplant 2009;9:S1–155.
345. Roberts DM, Jiang SH, Chadban SJ. The treatment of acute antibody-mediated rejection in kidney transplant recipients-a systematic review. Transplantation 2012;94:775–83.
346. Palmer A, Taube D, Welsh K, Bewick M, Gjorstrup P, Thick M. Removal of anti-HLA antibodies by extracorporeal immunoadsorption to enable renal transplantation. Lancet 1989;1:10–2.