

Novel therapeutic and diagnostic management of heart transplant patients

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ABSTRACT

Heart transplantation was performed for the first time 40 years ago and it is now universally considered the “gold standard” treatment for individuals suffering from end-stage heart failure. The increased understanding of the molecular mechanisms and of the role of the immune system in allograft rejection led to an overall improvement of graft survival, which is now around 10 years. The introduction of novel immunosuppressive drugs reduced the rate of acute allograft rejection but did not improve significantly the long-term graft survival. In addition, adverse effects (e.g. infections, cancer and renal failure) associated with immunosuppressive drugs are increasing over time and may affect post-transplantation outcomes. An immunosuppression-free protocol based on tolerance induction is the Holy Grail for heart transplant recipients, but it is still far beyond our reach. In this review, we discuss the landscape of immunological challenges that heart transplanted individuals face and we critically review the novel immunological approaches available to overcome these remaining issues. Some of the novel approaches, successfully tested in preclinical and clinical models, may lead to a prolongation of patient's and heart allograft survival.

Keywords: heart transplantation, tolerance, allograft survival, immunosuppression, chronic rejection, chronic allograft vasculopathy.

INTRODUCTION

The International Society for Heart and Lung transplantation recently reported that around 3,500-4,000 heart transplants are performed annually (2,200 of which only in the United States) with an average survival of 90 % at 1 year, 70 % at 5 years and 50 % at 10 years (1) thanks to the rapid improvement of immunosuppressive regi-

mens (Table 1). Nevertheless, long-term drug toxicity and inadequate control of chronic immune-mediated graft injury are still a major challenge in the management of heart transplant recipients. In this paper, we provide a perspective on the state of the art of heart transplantation, particularly focusing on novel immunological approaches and challenges that, if overcome, may result in the permanent acceptance of heart allograft survival, also known as tolerance.

Current issues with heart transplantation

The primary responsible issues for allograft loss and patients death faced nowadays by

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clinicians when dealing with heart transplanted patients are summarized in *Table 2*. Even with the improvement in immunosuppressive regimens, antibody mediated

rejection (AMR) may affect up to 15% of the recipients within the first year, worsening their graft survival (2). Even though endothelium has been considered the main

Table 1 - List of indications and most important side effects of current treatment in heart transplantation: induction therapy and maintenance therapy with CNIs, antiproliferative drugs and mTOR inhibitors.

	Agents	Indication	Side effects
Induction therapy	Steroids Daclizumab Alemtuzumab Basilizumab	Lower acute rejection compared to induction free therapy; used in a high number of patients in order to delay and reduce the need for a CNI-induced renal dysfunction.	High rate of infections and malignancies.
Maintenance therapy	Tacrolimus and Cyclosporine (CNIs)	Tacrolimus has become the first choice with lower rejection at 6 and 12 months post transplantation; encouraging results in combination with mTOR inhibitors in patients with CAV or malignancies.	Hypertension, gingival hyperplasia, cholelithiasis, hirsutism for cyclosporine; Tacrolimus is associated with high rate of new onset diabetes; both cause serious nephrotoxic effects.
	Mycophenolate Mofetil and Azathioprine (Antiproliferative drugs)	MMF has synergic effects with Tacrolimus and caused fewer side effects than Aza.	Myelosuppression.
	Everolimus (mTOR inhibitors)	Reduces progression of malignancies, CAV and other cardiac events; in combination with MMf allows CNI-free regimen and thus better renal outcome.	Anemia, early pericardial effusion and dyslipidemia, lower limb edema.

CAV = Coronary Allograft Vasculopathy; MMF = Mycophenolate mofetil.

Table 2 - List of the main current issues in heart transplantation.

Current Issues	Time of onset	Description
Antibody mediated rejection (AMR)	May affect up to 15% of the donor recipients within the first year.	Mainly mediated by alloantibody against donor antigens targeting capillary endothelium.
Coronary Allograft Vasculopathy (CAV)	Most common cause of late graft failure and most important cause of death beyond the first year after malignancy.	Accelerated coronary artery disease
Drug adverse effect	Dependent on the immunosuppressive regimen employed.	Adverse effects occur both during induction and maintenance therapy, affecting long-term graft survival.
Infection	< 1 month: nosocomial. 1-6 months: opportunistic and/or activation of latent infections. > 6 month: community acquired infections	Majority of infections are bacteria and virus related.
Cancer	Leading cause of death in long-term survivors, affecting 50% of recipients by 15 years.	Skin cancers, particularly squamous cell carcinoma, are the most frequent (about 60% of all cancers), followed by non-skin cancers (35%) and lymphoproliferative disorders (10-15%).

Table 3 - Overview of post-transplant infections and available therapies in heart-transplanted patients.

Infection	Frequency	Agent (% of infection)	Treatment
Bacterial	0.6 IE/patient	GRAM+ (37.7%) GRAM- (62.3%)	Removal of removable possible foci of infection, draining focal collection if possible and arranging a therapy guided by susceptibility testing. Treatment of Multi Drug Resistant GRAM- bacteria: Carbapenemase or high-dose Trimethoprim-Sulfamethoxazole.
Viral	0.16 IE/patient	CMV (34%) VZV (27%) HSV (19%) EBV (4%) Others (16%)	CMV: IV ganciclovir (5 mg/kg, 2 times/day) or oral valganciclovir (900 mg, 2 times/day) for 2-4 weeks; Disseminated, visceral or extensive cutaneous or mucosal HSV disease: IV Acyclovir (5-10 mg, every 8 hours); Herpes Zoster localized: Acyclovir (800 mg, 5 times/day); Herpes Zoster disseminated or Invasive disease or Herpes zoster ophthalmicus or Herpes Zoster oticus: IV Acyclovir (10 mg/kg, every 8 hours).
Fungal	0.08 IE/patient	<i>Aspergillus</i> (46%) <i>Candida</i> (38%) Others (16%)	Aspergillosis: Voriconazole (6 mg/kg IV every 12 h for 1 day, followed by 4 mg/kg IV every 12 hours). Invasive candidiasis: IV deoxycholate preparation of Amphotericin B (0,5-0,7 mg/daily).
Pneumocystis carini	< 0.01 IE/patient		Pneumocystis Pneumonia: IV Trimethoprim-Sulfamethoxazole (15-20 mg/kg/day often associated with corticosteroids).
Parasitic	< 0.01 IE/patient	<i>Toxoplasma</i> (few cases) <i>Giardia</i> (rare)	Toxoplasmosis: oral Pyrimethamine (200 mg/day, then 75 mg/day), oral sulfadiazine (1-1.5 g every 6 hours) and folinic acid (10-20 mg/day) for 4-6 weeks followed by Trimethoprim-Sulfamethoxazole; Giardiasis: oral Tinidazole (2 g/day) and oral Nitazoxanide (500 mg for 3 days).

IE = episode of infection; CMV = cytomegalovirus; VZV = varicella zoster virus; HSV = herpes simplex virus; EBV = virus Epstein Barr; IV = intravenous.

target of humoral injury because it releases pro-coagulants and pro-inflammatory factors (2), treatment of AMR that targets immune system and coagulation cascade have been thus far disappointing.

This left the transplant community with high-dose intravenous immunoglobulin or plasmapheresis as the only available options to treat patients with alloantibodies in order to prevent AMR development.

Cardiac allograft vasculopathy (CAV) is the major cause of graft loss and accounts for 30% of all deaths (3). It is a form of accelerated coronary artery disease, with an intimal concentric proliferation and luminal stenosis of both epicardial vessels and microcirculation (4).

Clinical manifestations are characterized mostly by progressive graft dysfunction and

sudden death, and less commonly by typical acute coronary syndromes with plaque rupture (3, 4). In fact, allograft denervation, which causes silent myocardial ischemia, may further complicate the diagnosis of CAV, which is based on coronary angiography or intravascular ultrasound (IVUS) (5).

The only definitive treatment for CAV is retransplantation (6). Other therapeutic approaches to CAV consist on diet restriction, control of blood pressure, statins and percutaneous revascularization, while the switch of immunosuppressive therapy to mTOR inhibitors is still uncertain (7, 8).

Immunosuppression is associated with various drugs adverse effects (Table 1 and 2). Cyclosporine is associated with hypertension, gingival hyperplasia and hirsut-

ism, while Tacrolimus has been correlated with new onset diabetes (9-10). However, the attempt of withdrawing CNI's showed controversial results (11).

Conversely, administration of mTOR inhibitors showed encouraging results in reducing rejection rate but treated individuals exhibited several drug-related side effects (i.e. anemia, dyslipidemia and renal dysfunction) to a higher degree as compared to both Azathioprine or Mycophenolate mofetil (9).

Development of opportunistic infections has been associated with an increased mortality rate and the development of accelerated CAV, acute rejection and post-transplant lymphoproliferative disorders (Table 3).

Bacteria (Gram-negative bacilli and *Staphylococcus* species) and viruses (particularly cytomegalovirus) are the most common infectious agents in heart-transplanted patients (12) (Table 3).

Finally, malignancy has to be mentioned as a major issue in the management of heart transplanted patients, occurring in nearly 50% of them in the long-term follow up (after 15 years). Skin cancers are the most frequent (60% of all cancers), followed by non-skin cancers (prostate, breast, bladder, kidney, colon, Kaposi Sarcoma) accounting

for 35% of cases, and lymphoproliferative disorders (10-15%) (13).

Novel strategies tested in preclinical setting (Table 4)

Preventing Ischemia Reperfusion (IR). IR damages the heart graft through two processes:

- 1) hypoxia, which causes depletion of ATP, electrolyte disturbances, cytotoxic enzymes activation and increase in cell membranes permeability, all resulting in cell death and apoptosis (14);
- 2) reperfusion, which induces an inflammatory response mediated by Reactive Oxygen Species (ROS) (15), with complement activation, cytokine/chemokine release and leukocyte infiltration. As a result, IR injury causes early graft dysfunction and contributes to the onset of chronic graft failure (16).

Different strategies have been tested to prevent IR damage, among them: targeting chemokine receptors (17), facilitating the conversion from ATP to adenosine, activating adenosine receptor (18) and targeting miRNA involved in IR etiology (19). Another possible treatment is the use of solution of carbon monoxide (CO) to preserve the graft. CO has anti-thrombotic, anti-

Table 4 - List of novel preclinical strategies with their mechanism of function and their targets: the ischemia-reperfusion (IR) injury, the alloimmunity damage represented by P2X7R and selectins activation and the regulatory T-cells (Tregs).

Target	Notes	Experimental therapies	Mechanism of function
IR-injury	It's the principle cause of rejection within the first month.	Use of CO and biliverdin in preservation solution.	Cause anti-thrombotic, anti-apoptotic and vasodilatation effects.
Alloimmunity	T-cell mediated damage is the leading cause of graft rejection in the short- and long- term in heart transplantation.	Inhibitor of P2X7R.	P2X7R is upregulated during rejection and renders APCs more responding to the damaging effect of ATP.
		Selectins inhibition.	Selectins promote T-cell activation and migration into allograft.
Tregs	Critical role in achieving tolerance.	Take advantage of immunomodulatory role.	Promote tolerance through the inhibition of effector T-cells and suppression of dendritic cells.

apoptotic, anti-inflammatory and a vasodilatory effect (20).

Targeting emerging pathways of alloimmune response. Targeting the purinergic system of ionotropic purinergic receptor 7 (P2X7) represents a novel strategy to block alloimmune response. ATP, released at high concentrations by damaged/dead cells, is sensed by P2X receptors (21), which activates T cells (22). Recent studies showed that targeting P2X7 with oATP prolonged graft survival in murine models of heart, lung and islet transplantation (23, 24).

Selectins have been reported to have a relevant role in heart allograft rejection as well by facilitating T cell activation and migration into the graft. Izawa et al. (25) assessed the specific role of donor and recipient's selectins in murine cardiac allograft rejection. Despite a protection on allograft rejection, mice deficient in all E, P and L selectins experienced cardiac allograft rejection because of a compensating effect of other adhesion molecules such as integrins (25). In a model of chronic rejection (MHC class II single mismatch), the lack of selectins in the recipient did not protect against graft rejection, while targeting donor selectins provided a significant improvement of graft survival with less luminal stenosis of coronary arteries and less mononuclear cells infiltration.

Use of regulatory T cells (Tregs). Tregs promote a state of tolerance through different mechanisms of action: directly through CTLA4 (26) or perforine and granzyme (27) and indirectly through cytokines like IL-10 and TGF-beta (28), which inhibit alloimmune responses. Isolated Tregs co-injected during bone marrow transplantation promoted engraftment and ameliorated graft versus host disease (GVHD) (29) and promoted heart allografts survival in MHC-mismatched murine transplantation (30). Although encouraging results have been generated, hurdles are still present includ-

ing the low frequency of Tregs in peripheral blood (1-3 % of blood CD4⁺ cells) (31) and the lack of specific surface markers, which made the isolation of a pure Treg population difficult.

Novel strategies tested in the clinical setting (Table 5)

Targeting the ischemia reperfusion injury. Currently two clinical trials (PROCEED II and PROTECT) offered to use a new organ care system (OCS) to preserve the graft. The first one (PROCEED II) is a global clinical trial, which demonstrated that the OCS is as safe and effective as the current standard of care in preserving standard donor hearts for transplantation. The second one (PROTECT) is a non-randomized multi-center European study, which showed a great reduction in rejection rate in individuals who received the heart preserved with OCS (32, 33).

Targeting AMR. A new prospective randomized multicenter trial (NCT01769443) is evaluating the use of Bortezomib, along with plasmapheresis, in reducing AMR in sensitized patients candidate. Bortezomib, a proteasome inhibitor used primarily for treatment of multiple myeloma, is active against normal alloantibody producing plasma cells (34). Bortezomib also reduces donor-specific antibodies (DSA) with resolution of AMR in renal transplant patients. A second trial (NCT01556347) offered a multi-drug regimen (Bortezomib, Thymoglobulin, Plasmapheresis) to eliminate or at least reduce alloantibody levels in sensitized individuals. However, the use of Bortezomib as well as of Thymoglobulin should be carefully evaluated considering their toxic effects.

Targeting immune-mediated and metabolic pattern of CAV. Two ongoing trials are testing if the B cells depleting agent Rituximab (NCT01278745) or Thymoglobulin (NCT01157949), as induction agent and

Table 5 - List of main novel experimental strategies in clinical setting and their targets: the IR-injury, alloantibodies-mediated response, CAV (with their ongoing clinical trials) and stem cells.

Target	Notes	Experimental Therapies
IR-injury	It is the principal cause of rejection within first month.	PROCEED II trial compares the rejection rate in recipients of a heart preserved on ice with heart preserved using the OCS.
B cells and alloantibodies	Associated with chronic allograft rejection and the development of CAV.	A randomized multicenter trial evaluating Bortezomib plus plasmapheresis in order to reduce sensitization in patients before transplantation (NCT01769443); another trial (NCT01556347) evaluating the effect of multi drug desensitization therapy on alloantibodies level.
Cardiac allograft vasculopathy (CAV)	Accelerated form of coronary disease associated with poor heart transplant function and graft survival.	Prevent CAV by testing Rituximab's ability in decreasing B cells number (NCT01278745), ACE inhibitors's effect on the atherosclerotic plaque (NCT01078363) and early induction with Thymoglobulin (NCT01157949).
Mesenchymal stem cells (MSCs)	Induce a shift from Th1 to Th2, suppress T cell effectors and promote Tregs generation.	Utilize their immunomodulatory and anti-inflammatory abilities.
Hematopoietic stem cells (HSCs)	Documented immunomodulatory abilities; differentiation into competent cardiomyocytes.	Utilize their immunomodulatory and cell differentiation abilities.

administered early on after transplant, can prevent the development of CAV (35).

Furthermore, preliminary data suggest that angiotensin converting enzyme inhibitors (ACE-i) delays the onset of the atherosclerotic plaque, probably by increasing the number of circulating endothelial progenitor cells and by reducing inflammation and fibrosis. Complete data will be available in 2015 (NCT01078363). This may reinforce the use of ACE-i as a potential therapy against CAV.

Use of stem cells. To date, stem cells represent a highly promising treatment for cardiac regeneration and tissue repair, but are still very far from clinical use in transplantation. However, stem cells could also exert immune-regulatory properties and may thus function as an additional immunosuppressive regimen (36, 37). Mesenchymal Stem Cells (MSCs) have been studied as a new therapeutic option for transplantation and autoimmune disease (37). Derived from many tissues including bone marrow

and adipose tissue, MSCs are multi-potent non-hematopoietic progenitor cells recently employed in the treatment of several immune-mediated diseases (38). In vitro data suggest that MSCs induce a shift from a pro-inflammatory T-helper 1 (Th1) profile towards an anti-inflammatory T-helper 2 (Th2) cell profile, suppress T cell cytotoxic effects and promote generation of Regulatory T cells (Tregs) (39). Moreover, MSCs express chemokine receptors on their surface and can be recruited into inflammatory sites where they exert their immune-modulatory properties (40). Indeed, the infusion of donor-derived MSCs improved allograft survival in a semi-allogeneic murine model of heart transplantation by favoring Tregs expansion and abrogating anti-donor Th1 activity (41).

The future of Heart Transplantation

As chronic allograft loss in heart transplantation represents the major late complication affecting long-term survival, its

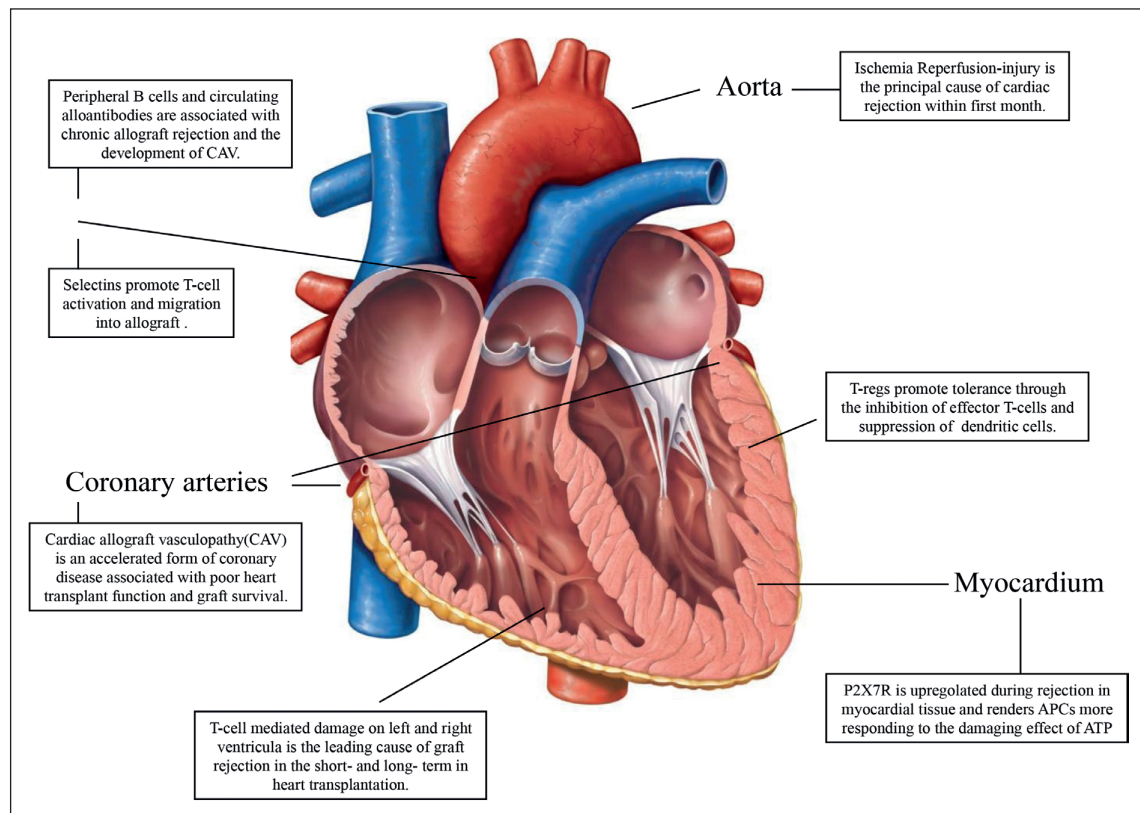


Figure 1 - Novel immunological pathways and therapeutic targets in cardiac allograft vasculopathy.

early detection and diagnosis has become a cornerstone in the management of heart transplant recipients. While coronary angiography and myocardial biopsy remain the standard procedure, new non-invasive methodologies have been explored and established in order to facilitate and accelerate the identification of chronic graft failure in heart transplanted patients.

Phosphorous-31 magnetic resonance spectroscopy (³¹P-MRS) (42). Left ventricular (LV) high-energy phosphates (HEPs) *in vivo* in humans can be studied using localized ³¹P-MRS and represent a non-invasive way to assess myocardial function and dysfunction (43, 44). Phosphocreatine (PCr)/adenosine triphosphate (ATP) ratio was the most promising index for the evaluation

of myocardial metabolism (45). While the benefits of this method have been already demonstrated in a group of diabetic subjects prone to severe cardiac vasculopathy, more rigorous clinical data are required before making final recommendations on the use of this technique.

Targeting new pathways/hormonal axis in allograft failure. Immunophenotyping of transplanted patients have been suggested in the last decade as the least invasive and more informative method to monitor the immune state (46). This non-invasive technique may provide useful information on the state of antigen-specific alloreactivity in transplant recipients and it shed the light on potential immune target for therapeutic purposes. In particular, the hunt for novel

biomarkers to be employed in immune assays and to be searched because of their association with the graft immune response locally, lead to the discovery of novel pathways and hormonal axes potentially involved in mediating acute rejection and chronic allograft dysfunction (47). Of note, there is a growing evidence that hormonal axes may play a role in modulating immune response (48).

High-throughput molecular screening methods. With the advancement of high throughput “omic” methods such as genomics, metabolomics, transcriptomics and proteomics, new efforts have been made to identify potential mechanisms of graft injury and to develop novel biomarkers to diagnose chronic allograft dysfunction (49, 50). Identification of biomarkers that may overcome the clinical variables associated with differences in immunosuppressive drug response, variations in recipient and donor gender, age, HLA match, ischemia time for the organ, donor source, recipient hematocrit, recipient white blood cells counts, recipient concomitant infection, etc. is highly desired in the field of organ transplantation and may represent a novel standardized approach capable of detecting graft injuries (49). This may also apply to heart transplantation, where early and non-invasive diagnostic tools are needed because this is a life-saving procedure for the recipient, and in case of failure, the only viable solution is re-transplantation.

CONCLUSION

The development of novel therapeutic approaches represents an important goal to improve long-term graft survival in heart transplantation. Several agents have been already tested in animal models and in *in vitro* assays providing numerous strategies potentially effective in improving heart transplant survival (Table 4).

However, experimental results also suggest the urgent need of more clinical trials to test this large amount of agents that will be available in the next years, in order to discover which immunotherapeutic strategy would be more successful and/or feasible and which target would offer the best therapeutic response in clinical practice (Table 5).

The unveiling of novel immunological pathways involved in the development of CAV (Figure 1) may open the path to defeat one of the major hurdles faced by heart transplanted patients.

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