Cardiac Transplantation

Mark H. Drazner, M.D., M.Sc.

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This is to acknowledge that Dr. Drazner has disclosed a financial interest or other relationships with commercial concerns related directly or indirectly to this program. Dr. Drazner will be discussing "off-label" uses in his presentation.

Biographical Information

Mark Drazner, M.D., M.Sc.
Associate Professor
James M. Wooten Chair in Cardiology
Medical Director, Heart Failure/Cardiac Transplantation
Division of Cardiology
Department of Internal Medicine

Interests:

Cardiac transplantation
Heart failure
Left ventricular hypertrophy

It has been nearly 40 years since Dr. Christiaan Barnard performed the first human-to-human heart transplant. This procedure has become an accepted treatment for patients with End-Stage Heart Failure (ESHF), a subgroup of patients with advanced chronic heart failure (CHF) whom otherwise have a poor prognosis with few therapeutic alternatives. Chronic inotropic support is associated with a dismal prognosis (1). The current generation of left ventricular assist devices, though leading to improved survival as compared to optimal medical therapy remains flawed with pump malfunction and/or infectious complications resulting in a 2-year survival rate of only 23% (2). Ventricular assist device therapy in conjunction with pharmacological therapy to recover ventricular function has recently been described (3) but whether this treatment strategy can be duplicated, and be broadly applicable, needs further investigation. Cardiac repair with stem cell therapy remains investigational.

The objectives of this discussion are two-fold. First, to address questions common among physicians caring for patients with heart failure including which patients should be referred for consideration of heart transplantation. The second objective is to highlight recent advances in the field of cardiac transplantation focusing on advances in the diagnostic tools used to evaluate rejection and in the immunosuppressive regimens used to prevent rejection.

1. Who should be referred for consideration of cardiac transplantation?

Cardiac transplantation is constrained by availability of donor organs. The number of available organs in the United States has been decreasing. In 1995, 2,528 heart were transplanted and in the year 2005 only 2,063. This supply of donor hearts is not sufficient in relation to the estimated 40,000+ patients with ESHF who would be potential candidates for transplantation. This "supply-demand" mismatch results in the need to allocate judiciously the donated hearts in order to maximize the societal benefit achieved by these precious resources.

Although there are complex algorithms followed to identify potential cardiac transplant candidates, the process can be conceptually simplified into identifying patients who are sick enough to need a transplant but well enough to benefit from a transplant. By that I mean identifying individuals with a poor prognosis from their ESHF ("sick enough to need a transplant") but not having any other major co-morbid condition which would limit their ability to regain a high functional status following transplant or jeopardize their post-transplant survival ("well enough to benefit from a transplant").

1A. Assessing prognosis in Heart Failure

Accurately predicting the outcome for an individual patient with CHF is extremely difficult despite numerous known prognostic factors (see Table 1).

Table 1: Assessing Prognosis in Heart Failure

NYHA class	Repeated hospitalizations
Left ventricular diastolic dimension (8)	Circulatory-renal intolerance of ACE-inhibitors (9)
Elevated BNP/NT-proBNP	Inability to tolerate Beta-blockers (10)
Low peak oxygen consumption (4)	Elevated pulmonary capillary wedge pressure (11)
Elevated V_E/V_{CO2} (12)	Elevated heart rate
Ischemic etiology	Hyponatremia
Diabetes (13)	Elevated uric acid
Low systolic blood pressure (14)	Renal insufficiency (15)
Elevated JVP (16)	Third heart sound (16)
Prolonged QRS duration	

Peak oxygen consumption ($V0_2$) from a maximal cardiopulmonary stress test has emerged as a critical parameter in assessing severity of illness. In a now classical report, Dr. Donna Mancini initially demonstrated that patients with a peak $V0_2$<14 ml/kg/min had a significant survival benefit with transplantation (compared to those with the same peak $V0_2$ and did not undergo transplantation), though much of the discriminatory power came from individuals with a peak $V0_2$<10 ml/kg/min (4). It has been suggested that a peak $V0_2$ < 14 ml/kg/min in patients intolerant of beta-blockers and a peak $V0_2$<12 ml/kg/min in those on beta-blockers are reasonable threshold values for consideration of transplantation (5). Several caveats to these guidelines are the need to use higher threshold values for younger patients (since predicted $V0_2$ is age-based), and adjusting $V0_2$ to lean mass for obese individuals (since the above measures of $V0_2$ are based on total body weight, leading to a lower value in an obese individual who does the same amount of exercise as a lean person). Recently, the slope of the minute ventilation to carbon dioxide production relationship (V_E/V_{C02}) has emerged as a powerful prognostic variable in heart failure (6,7). This parameter has the advantage of being measurable at sub-maximal exercise. A value greater than 34 suggests an unfavorable prognosis.

Given the numerous risk factors available in CHF, attempts have been made to develop a multi-marker risk score. The first of these efforts to be widely considered by the transplant community was the Heart Failure Survival Score (17) which is calculated for a hypothetical patient below.

Table 2. Calculation of Heart Failure Survival Score (17) in a Hypothetical Patient

Clinical Characteristic	β-Coefficient	Hypothetical Patient Points	Product (β x Points)
Ischemic cardiomyopathy (Yes = 1)	+ 0.6931	1	+0.6931
Resting heart rate	+0.0216	100	+2.16
LVEF	-0.0464	17	-0.7888
Mean BP	-0.0255	80	-2.04
Interventricular conduction delay (Yes = 1)	+0.6083	0	0
Peak VO2	-0.0546	13	-0.7098
Serum Sodium	-0.0470	132	-6.204
Total			-6.89 Take absolute value

The absolute value of the sum of the products is taken and then categorized (see Table 3) as low, medium, or high risk. Risk categories were validated against outcomes (Figure 1). The hypothetical patient above (score = 6.89) would be considered high risk.

Table 3. HFSS Score and Risk Category

HFSS Score	Risk Category
< 7.2	High Risk
7.2 to 8.09	Medium Risk
≥8.1	Low Risk

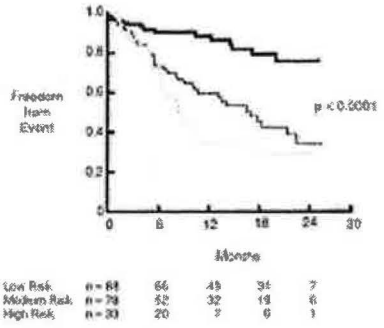


Figure 1. Validation of HFSS score in predicting freedom from death or urgent transplant. From Aaronson et. al. (17).

This score has been validated in other patient populations including those on beta-blockers (18,19). One limitation of this score is the complexity of calculation needed.

More recently, the "Seattle Heart Failure Model" has been developed. This model was developed from the dataset of a large randomized trial (PRAISE1, the Prospective Randomized Amlodipine Survival Evaluation trial) and then validated in other well known randomized clinical trials (ELITE2, RENAISSANCE, Val-HeFT), a tertiary heart failure clinic, and an Italian Heart Failure Registry (IN-CHF) (20). The unique features of this model are that it is publicly available at http://depts.washington.edu/shfm with an interactive entry screen (see Figure 2) and that it does not require any specialized testing such as right heart catheterization or cardiopulmonary stress testing, offering the opportunity to make it broadly applicable. However, the majority of the experience with the Seattle Heart Failure Model has been in patients with less severe CHF (NYHA II and III), leaving it uncertain how well it will operate in patients being considered for heart transplantation. Additionally, the model does not take advantage of the prognostic value of natriuretic peptides (either BNP or NT-proBNP).

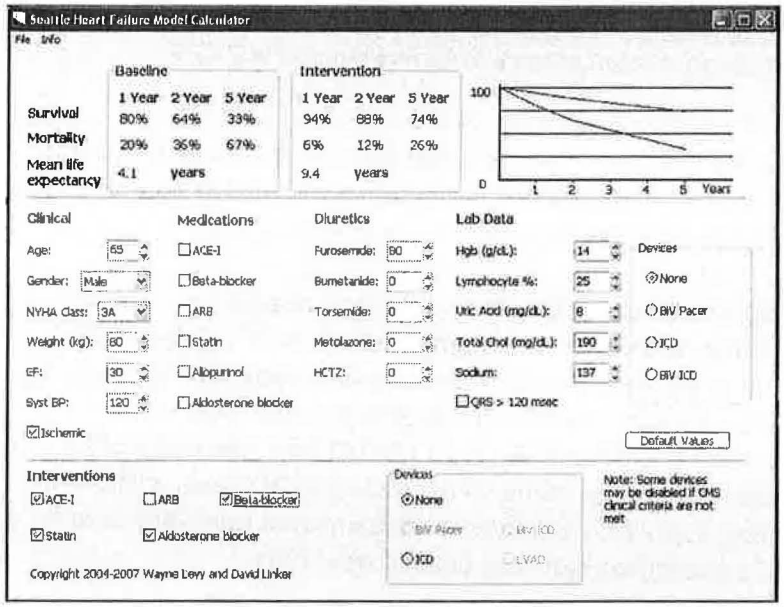


Figure 2. Seattle Heart Failure Model interactive data entry screen. Available at http://depts.washington.edu/shfm

Overall, development of a risk prediction score, such as the Heart Failure Survival Score or the Seattle Heart Failure Model, holds great potential in assessing whether severity of illness warrants transplantation. Certainly, there are some patients where this is readily apparent to all (e.g., inotrope dependence), but an accurate model may allow identification of patients earlier in their course and assist practitioners in triaging referrals to a transplant center. Incorporation of biomarkers (such as BNP) in future risk scores seems likely in the future.

1B. Absence of comorbidities

The second component when assessing the suitability of a patient as a transplant candidate is whether they have other major comorbid conditions which would impact unfavorably on the outcome of the transplant. Such conditions represent either relative or absolute contraindications to cardiac transplantation (Table 4).

Table 4. Comorbid conditions: relative and absolute contraindications to heart transplantation

1. Advanced age: > 65 – 70 years of age (consider alternate donor)
2. Obesity: BMI > 30 kg/m2 or >140% ideal body weight
3. Renal insufficiency: GFR < 40-50 ml/min (exclude hypoperfusion from CHF)
4. Diabetes mellitus with severe end-organ damage (nephrosis; extensive proliferative retinopathy)
5. Irreversible pulmonary parenchymal disease: FEV_1 < 50%; FEV_1/FVC < 40 to 50%
6. Cirrhosis
7. Cerebrovascular and peripheral vascular disease
8. Pulmonary emboli
9. Infection including HIV, HCV, Hepatitis B
10. Infiltrative disease likely to recur in allograft: sarcoid and amyloid
11. Pulmonary Hypertension: Fixed PVR > 5 Wood units; transpulmonary gradient >16 to 20 mm Hg; PA systolic > 60 mm Hg
12. Active pulmonary embolus/infarction
13. Active peptic ulcer disease
14. Severe osteoporosis
15. Progressive neurological disease
16. Malignancy if risk of recurrence is not low
17. Psychosocial: Inadequate resources or inability to comply with post-transplant lifestyle
18. Substance abuse (tobacco, alcohol, drugs). 6 month tobacco abstinence required at UTSW.
19. Medical noncompliance

The reader is referred to the recently published International Society for Heart and Lung Transplantation (ISHLT) guidelines for the evaluation of potential recipients (5) as well as two other frequently cited documents (21,22) for a detailed discussion of listing criteria.

Many of the criteria above (Table 4) are relative, not absolute, contraindications. There is ongoing debate about the merits of some of them. For example, the ethics of excluding HIV individuals from heart transplantation in the modern era of retroviral therapy has been questioned (23). A recent analysis from the UNOS database demonstrated that patients with sarcoid cardiomyopathy have good medium term outcomes (24). As a Doris Duke research fellow working with me, Dr. Louise King questioned the ethics of excluding individuals without insurance from transplantation, finding that 24% of donors were uninsured (and thus could donate a heart but not have received a transplant had they needed one) (25).

Overall, the decision as to whether a patient is a suitable candidate for cardiac transplantation represents a balance between the severity of their cardiac illness and the severity of their medical comorbid conditions.

2. How are donated hearts allocated?

The allocation of organs is under the auspices of the United Network for Organ Sharing (UNOS) which was awarded a federal contract to oversee the Organ Procurement and Transplantation Network (26). The present allocation system for hearts (27) categorizes active potential recipients into 1 of 4 statuses: UNOS 1A, 1B, and 2, while status 7 is inactive (Table 5). Hearts are allocated based on blood type (O to O or B; A to A or AB; B to B or AB; AB to AB), listing status, distance of recipient transplant center from donor hospital (nautical miles), and time accrued on the waiting list.

Table 5. Criteria for UNOS Listing Status.

UNOS Status			
1A	1B	2	7
Post-LVAD or RVAD when stable (30 days)	LVAD or RVAD	Does not meet criteria for 1A or 1B	Inactive
Total artificial heart	Continuous IV inotropes		
IABP			
ECMO			
VAD with device related complication (thromboembolism, infection)			
Continuous mechanical ventilation			
Swan + 1 high dose inotrope (Dbx ≥7.5 mcg/kg/min or Milrinone ≥0.5 mcg/kg/min)			
Swan + multiple intravenous inotropes			

The sequence of adult heart allocation is shown (Table 6).

Table 6. Allocation of adult hearts

1. Local 1A then 1B
2. Transplant center within 500 miles of donor hospital: 1A then 1B
3. Local 2
4. Transplant center between 500-1000 miles of donor hospital: 1A then 1B
5. Transplant center within 500 miles of donor hospital: Status 2
6. Transplant center between 500-1000 miles of donor hospital: Status 2
7. Transplant center between 1000-1500 miles of donor hospital: 1A then 1B then 2
8. Transplant center between 1500-2500 miles of donor hospital: 1A then 1B then 2
9. Transplant center >2500 miles of donor hospital: 1A then 1B then 2

In general, our experience at UTSW has been that an organ is available in < 2 months for Status 1A, 3-12 months for Status 1B, with longer waits for status 2 (which increasingly are not offered organs).

Note that a critical prioritization in the heart allocation system (UNOS 1A versus 1B) can be influenced by physician decision making such as insertion of a Swan-Ganz catheter, the use and type of inotropic support, and the threshold at which to implant a ventricular assist device. In contrast, allocation of other organs appears more objective, with liver allocation based on the MELD score (3 laboratory measurements: creatinine, Bilirubin, INR) and lungs by the Lung Allocation Score (largely comprised of lab values, results of 6-minute walk, PA and PCWP pressures, FVC, and disease diagnosis).

3. What happens after the transplant?

3A. Immunosuppression

Early efforts at cardiac transplantation were hampered by ineffective immunosuppressive therapy. Following Dr. Christiaan Barnard's historical cardiac transplant in Mr. Louis Washkansky in 1967, another ~100 transplants were performed in 1968. In this pre-cyclosporine era, immunosuppression consisted of azathioprine, steroids, and radiation. Only 40 patients were alive on the ninth postoperative day with 15% surviving more than 6 months (28) highlighting the ineffectiveness of that immunosuppressive approach. Fortunately, two years later (1970) the

fungus *Tolypocladium inflatum* was identified which led to the development of cyclosporine A, subsequently introduced to prevent rejection in cardiac transplant recipients in 1983 by Dr. Norman Shumway at Stanford. The modern era of cardiac transplantation had begun. The immunosuppressive therapies presently available can be grouped broadly as shown in Table 7.

Table 7. Categories and examples of immunosuppressives.

1. Calcineurin inhibitors: cyclosporine, tacrolimus
2. Anti-metabolites: azathioprine, mycophenolic acid
3. Proliferation signal inhibitors/TOR inhibitors: sirolimus, everolimus
4. Corticosteroids
5. Anti-lymphocyte antibodies ("cytolytic therapy"): ATGAM, thymoglobulin, OKT3
6. Anti-cytokine receptor antibodies: IL2 receptor antibodies such as basiliximab and daclizumab

A schematized cartoon of the events leading to acute cellular rejection, and the sites of action of immunosuppressives, is shown (Figure 3).

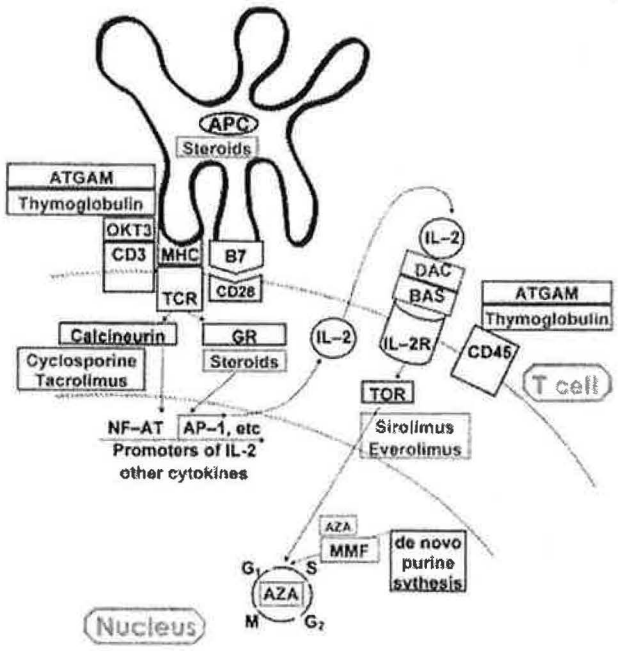


Figure 3. Events leading to rejection and the sites of action of immunosuppressives. The presentation by antigen presenting cells (APCs) of foreign (donor) antigen in conjunction with the major histocompatibility complex (MHC) to the recipient T-cell receptor (TCR), as well as costimulatory signals from the B7-CD28 interaction, triggers rejection. This signal leads to calcineurin activation, dephosphorylation of NFAT and NFAT translocation to the nucleus, where it binds to promoters of cytokines including interleukin 2 (IL2). IL2, via autocrine activation of the high affinity IL-2 receptor, activates T-cell proliferation through the target of rapamycin (TOR) as well as cyclin/cyclin-dependent kinases. The phases of the cell cycle are shown: G1 (first growth phase), S (synthesis of DNA), G2 (second growth phase), and M(cell division). GR=glucocorticoid receptor AZA=azathioprine MMF=Mycophenolate Mofetil. From (29).

3A. i. Induction therapy

Some cardiac transplant programs favored administering intensive immunosuppressive therapy immediately following transplant with the intent of inducing tolerance to the allograft, a strategy which has been termed "**induction therapy**." For this purpose, antibody-based therapy directed against lymphocytes traditionally has been administered, using either a polyclonal antibody (ATGAM [equine] or Thymoglobulin [rabbit]) which recognize numerous antigens on the T- and B-cell surfaces, or a monoclonal antibody directed against the CD3 surface antigen on the T-cell (muromonab, OKT3) (29). Such therapies have been shown to delay rejection in the early postoperative period, but have not led to tolerance and have been associated with numerous side effects including allergic reactions and an increased risk of infection and malignancy. OKT3, in particular, was associated with a worrisome side effect profile including aseptic meningitis, a capillary leak leading to pulmonary edema, lymphoproliferative disease, and development of human anti-mouse antibodies associated with delayed humoral rejection with hemodynamic compromise. As a result, the use of these agents for induction therapy, and in particular OKT3, has declined (30).

Recently, monoclonal antibodies to the high affinity IL2 receptor (CD25) expressed on activated T-cells (basiliximab and daclizumab) have been evaluated for induction therapy following cardiac transplantation. These agents are less potent immunosuppressives than the anti-lymphocyte antibodies described above. Daclizumab is a humanized monoclonal antibody which contains 90% human sequence (murine antigen binding sequence imposed on a human antibody). In contrast, Basiliximab is a chimeric antibody with murine variable regions imposed on the human IgG constant regions. From 1996 to 2004, induction therapy use increased from approximately 40% to 50% of patients in the ISHLT registry, despite a sharp decline in the use of OKT3, largely due to a rapid rise in the use of IL2R antagonism (see Figure 4).

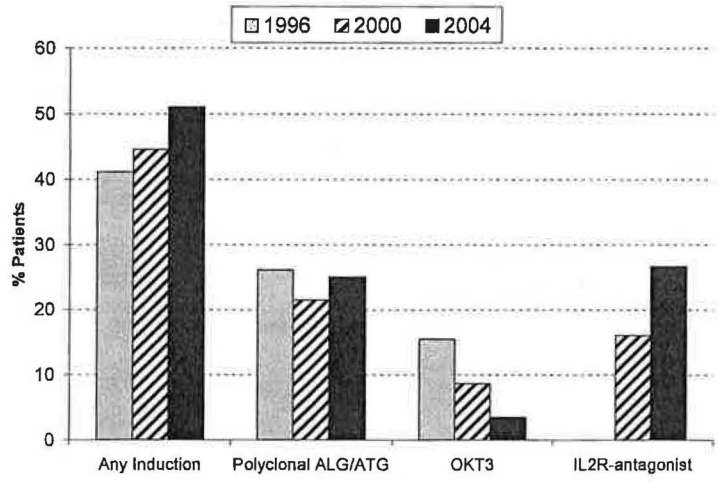


Figure 4. Temporal trends in induction therapy in ISHLT registry. Adapted from (31). Note that 50% of recipients receive induction therapy in 2004 despite a decline in OKT3 use, due to a rapid rise in use of IL2-receptor antagonists.

Several studies recently have compared the IL-2R antagonists with either polyclonal or monoclonal antibodies, or placebo, as induction therapy (see Table 8 below). The majority of these trials have demonstrated that IL2R antagonists have comparable efficacy to cytolytic therapy and better efficacy than placebo in preventing rejection, without the significant infectious complications associated with anti-lymphocyte antibodies including OKT3. However,

the largest trial assessing daclizumab (37) found an increased risk of infectious deaths when combined with cytolytic therapy. Although the protocol had specified that cytolytic induction therapy would not be used, randomization occurred within 12 hours of transplantation. When patients subsequently deteriorated, most often due to the development of renal insufficiency, calcineurin inhibitors were discontinued and cytolytic therapy was administered in their place. Of the 21 deaths in daclizumab arm, 8 had received concomitant cytolytic therapy within the first 30 days and 6 (of the 8) died. In contrast, of the 11 deaths in the placebo arm only 2 had received early (<30 day) cytolytic therapy and none died. Subsequently, an analysis of the Scientific Registry of Transplant Recipients from January 1998 through October 2003 addressed the safety of daclizumab (38). The 684 patients who did receive daclizumab were compared to the 2525 who did not. There was no increased risk of patient death including infectious death associated with daclizumab. As in the randomized trial, daclizumab patients had a lower risk of acute rejection both at 6 and 12 months.

Table 8. Randomized trials of IL2 antagonism as induction therapy in cardiac transplantation

Author	Year	Type/Length of follow-up	Comparator	**IL2 blocker associated with:**
Basiliximab trials				
Mehra (32)	2005	Randomized 1 year Pharmaco-kinetics assessed	Basiliximab (n=25) vs. Placebo (n=31)	1. Trend towards increased time to first rejxn grade ≥3A or with hemodynamic compromise: 74 vs. 40 days 2. Similar adverse events including infectious complications 3. CD25 saturation threshold averaged 38 ± 13 days 4. Increased death: 3 (12%) basiliximab vs. 1 (3.2%) placebo
Segovia (33)	2006	Randomized 1 year	Basiliximab (n=48) vs. OKT3 (n=51)	1. Less side effects (fever, hypotension, pulmonary edema) 2. No change grade 3A at 1 year (40%) 3. No change 1 year survival
Mattei (34)	2007	Randomized 6-months	Basiliximab (n=38) vs. Thymoglobuline (n=42)	1. Less side effects including infectious death (0 vs. 14%, =0.03) 2. Similar ≥1B rejection 3. Trend towards more ≥3A rejection: 18% vs. 7%, p=0.09
Carrier (35)	2007	Randomized 6 months	Basiliximab (n=17) vs. RATG (n=18)	1. More rejxn grade ≥3A (35% vs. 17%); noninferiority not reached. 2. Less CMV by quantitative PCR.
Daclizumab trials				
Beniaminiovitz (36)	2000	Randomized 1 year	Daclizumab (n=28) vs. Placebo (n=27)	1. Lower frequency of rejection, defined either as grade ≥2 or ≥3A 2. Same side effects 3. Same mortality: 2 deaths (Dac) vs 4 deaths (Placebo)
Hershberger (37)	2005	Randomized 1 year	Daclizumab (n=216) vs. Placebo (n=218)	1. Lower rejection at 6 months 2. Reduced primary composite endpoint at 6 months (p=0.007) and trend at 12 months (p=0.06). Endpoint includes: ≥3A rejxn, hemodynamic graft dysfxn, retransplant, death, loss to f/u 3. *Higher infectious death when combined with cytolytic therapy (15% vs. 0%)

Although the observational data (38) are reassuring for the use of IL2R antagonists overall, given the increased mortality seen in the large randomized trial (37), the combination of IL2R antagonism and cytolytic therapy should be avoided as induction therapy.

Another potential rationale for the use for IL2 receptor antibodies as induction therapy is to permit delayed introduction of calcineurin inhibition until later in the postoperative state, in the hopes that renal function will have stabilized at that point (39, 40). The published experience from UT Southwestern (39) reported that administration of basiliximab with delayed (post-operative day 5) introduction of cyclosporine to a cohort (n = 25) of subjects at increased risk of post-operative renal dysfunction (due to CrCl <50 ml/min, Cr > 2.5 mg/dL, CI < 2.2 l/min/m^2, intra-aortic balloon pump, or a ventricular assist device) was associated with a smaller increase in serum creatinine as compared to a comparable historical control who had received cyclosporine, with no apparent increased risk of infection or rejection. Further data are needed on the safety and efficacy of using IL2R blockers with delayed post-operative initiation of calcineurin inhibition.

3A. ii. Maintenance immunosuppressive therapy

The general principle of maintenance immunosuppressive protocols following cardiac transplantation is to administer higher-dose therapy early post-transplant at a time of an increased risk of rejection, and then wean down the intensity of immunosuppression over the first year.

Standard maintenance immunosuppression post-transplant uses 3 agents early (calcineurin inhibitor + anti-metabolite + steroids), with many programs aiming to wean steroids off over the first 6 – 12 months leaving the patient on 2 immunosuppressive medications lifelong. The mainstay of maintenance immunosuppressive therapy remains calcineurin inhibition, either cyclosporine or tacrolimus. Target levels of both are progressively lowered with time following transplant. The second component of the immunosuppressive regimen is an anti-metabolite. Initial experience was with azathioprine, and more recently with mycophenolate mofetil which is broken down to mycophenolic acid. The third component of the immunosuppressive regimen is corticosteroids, which are administered at higher doses early (e.g., preoperative solumedrol 500 mg and 125 mg q8 on postoperative day one, followed by prednisone 1 mg/kg/day) and then tapered down either to a low maintenance dose (e.g., 0.1 mg/kg/day) or entirely off over the next ~6-12 months. Those patients who can be successfully weaned off steroids (usually at least 50%) may enjoy better outcomes, as reported by us (41,42) and others [including (43-46)]. However, since these observations have come from non-randomized data, it remains inconclusive whether the discontinuation of steroids itself is associated with improved outcomes, or whether the ability to discontinue steroids successfully serves as a marker to identify healthier recipients (e.g., a more immunologically privileged recipient) who would have had a better outcome whether or not they had remained on steroids.

In the last 5 – 10 years, three major changes to standard triple maintenance immunosuppressive therapy have been considered. These will be addressed sequentially.

Potential change #1:
Should Tacrolimus replace Cylosporine as the standard calcineurin inhibitor?

Tacrolimus was first administered to human solid organ recipients in 1989 (47), 6 years following the introduction of cyclosporine. Three relatively small trials (n = 73, 75, 82) comparing cyclosporine to tacrolimus in cardiac transplant recipients were reported in 1998-1999 (48-50), which demonstrated relatively equivalent outcomes with these 2 agents. There have been reports demonstrating that tacrolimus can rescue cyclosporine-treated patients who have had severe rejection (51,52). In the last 5 years, 5 additional trials have been published to address this question (Table 9).

Table 9. Recent trials comparing Cyclosporine and Tacrolimus.

Study	N=	Other Rx	Endpoints
Single Center			
Mehra (53)	63	1. MMF 2. Steroid (taper off)	1. Among black recipients, those treated with TAC had less treated rejection at 1 year (TAC 36% vs. CYA 63%) 2. Among black recipients, TAC associated with improved 1 year survival: TAC 95% vs. CYA 73%
Kobashigawa (54)	67	1. AZA 2. Steroids	1. No difference 5-year survival, rejection (≥3A or treated), vasculopathy, DM. 2. Lower TGL and Cr (TAC)
Meiser (55)	60	1. MMF (dose adjusted) 2. Steroid (taper off)	1. No difference 1 year survival: TAC 93% vs. CYA 90% 2. Less rejection (≥ grade II or IB if rx'd) with TAC 3. Some assessments of 1-year vasculopathy better (TAC)
Multi-center			
Grimm (56)	314	1. Induction Rx (ALG/ATG or OKT3) 2. AZA 3. Steroids	1. No difference 1 year survival: TAC 93% vs. CYA 92% 2. Less 6-month rejection ≥ grade IB with TAC: TAC 54% vs. CYA 66% p = 0.03 (central adjudication) 3. Less 6-month rejection ≥ grade 3A with TAC: TAC 28% vs. CYA 42% p=0.01 4. TAC vs. CYA associated with more DM, less HTN and dyslipidemia
Kobashigawa (57)	230*	1. Induction Rx permitted 2. MMF (3 g/d) 3. Steroids	1. No significant difference 1 year survival: TAC 95% vs. CYA 90% 2. Primary endpoint (≥3A rejxn or HD compromise requiring Rx) at 6 months: TAC 22% vs. CYA 32%, P=NS 3. Composite endpoint at 1 year: TAC 23% vs. CYA 37% (p=0.03) 4. Any treated rejection: TAC 42% vs. CYA 59% 5. 1 year MPA levels: TAC 2.1 vs. CYA 1.6 (? P) 6. Cr and TGL lower in TAC 7. More insulin Rx >30 days among baseline nondiabetics (TAC)

*There was a third arm of this trial assessing Tacrolimus and Sirolimus (n=113).

Overall, 1 trial reported improved survival with tacrolimus (53) among African American recipients, while the other 4 trials did not although these trials were not powered for survival. The weight of the evidence does support a benefit of tacrolimus over cyclosporine in preventing rejection. One of the 2 multi-centered trials demonstrated that Tacrolimus reduced rejection at 6 months (56) when assessed by a central panel of pathologists, but not by local pathologists (the local interpretation was the primary endpoint of the trial (58)). However, this trial was conducted

on a background of Azathioprine therapy. Since most centers now use mycophenolate mofetil, the relevance of this trial is diminished. In the second multi-centered trial (57) which was conducted in the United States, rejection was less common at 12 months with tacrolimus, albeit this again was a secondary endpoint. Additionally, mycophenolate mofetil was not dose adjusted. Since mycophenolic acid levels are lower when administered with cyclosporine as compared with Tacrolimus, an observation confirmed in this trial (though statistical significance was not commented upon), there is a degree of uncertainty as the whether the higher mycophenolic acid levels may have skewed the results in favor of tacrolimus. Given this limitation, the European single center trial (55) which did adjust mycophenolate mofetil dose by trough levels becomes an important observation. This trial was strongly favorable for benefit of tacrolimus in reducing rejection lending support to the efficacy of tacrolimus over cyclosporine, though one needs to exercise caution noting this trial enrolled a total of 67 subjects. African American recipients may be a group that particularly enjoys an advantage from tacrolimus (53).

A limitation of these trials is the use of rejection demonstrated on biopsy as a primary endpoint, rather than survival. Although biopsy proven rejection is an unfavorable event in cardiac transplant recipients, 1 year survival rates were not different between tacrolimus and cyclosporine in 4 of the 5 trials and long term data are not available. Finally, when comparing cyclosporine to tacrolimus, one needs to consider side effect profiles. However, tacrolimus was better tolerated when assessed by some parameters (e.g., hypertension and dyslipidemia) but was more diabetogenic, again leaving doubt over which agent is superior.

Potential change #2:
Should Mycophenolate Mofetil replace Azathioprine as the standard anti-metabolite?

Both Azathioprine and Mycophenolate Mofetil inhibit progression through the cell cycle. Azathioprine is initially converted to 6-mercaptopurine and then thio-inosine-monophosphate, inhibiting the *de novo* purine and purine salvage pathways and blocking lymphocyte proliferation. Mycophenolate mofetil is hydrolyzed to mycophenolic acid, which is a noncompetitive inhibitor of inosine monophosphate dehydrogenase, thereby blocking *de novo* purine synthesis which proliferating lymphocytes are dependent upon.

A double blind, randomized, active-controlled trial of 650 subjects undergoing their first heart transplantation was conducted in 1994 – 1995 at 28 heart transplant centers in the United States, Europe, and Australia (59). Subjects were randomized to mycophenolate mofetil (3 grams/day) or azathioprine (1.5 – 3 mg/kg body weight daily) and followed initially for 1 year. Cyclosporine and prednisone tapered to off at 6 months were concomitant immunosuppressive therapy, and induction therapy was permissible (20% of patients received). **There was no difference in survival or rejection in enrolled subjects. However, 72 (11%) subjects withdrew from the study before they received any study drug.** At the time of this trial, an intravenous preparation of mycophenolate mofetil was not available and if subjects were unable to take oral medications by post-operative day 5, they were withdrawn per protocol. When the trial was reanalyzed using treated patients (n=588), mycophenolate mofetil was associated with reduced 1-year mortality (6.2% vs. 11.4%, p=0.03) and need for treatment of rejection (66% vs. 74%, p=0.03). More herpetic infections were seen in the mycophenolate mofetil arm (53% vs. 44%, p=0.03). Subsequently, a three-year analysis of this trail was published (60) and confirmed reduced mortality among the treated population randomized to mycophenolate mofetil as well as a strong trend to reduced progression of coronary artery intimal thickness, a marker of transplant

vasculopathy (see below). In a subsequent reanalysis using a more sophisticated intravascular ultrasound analytic technique ("site to site" analysis, matching sites between studies), subjects treated with mycophenolate mofetil did have less evidence of development of transplant vasculopathy as assessed by 1-year maximal intimal thickness $\geq$ 0.3 mm (23% vs. 43%, p=0.005) (61). Nevertheless, the need to analyze only treated patients and not all enrolled patients is a major limitation of these results.

Given these limitations of the randomized trial, an analysis of the ISHLT/UNOS registry was conducted (62). In this observational study, cyclosporine was given to 4942 with azathioprine and 657 with mycophenolate mofetil. There were differences in baseline characteristics between the two groups including recipient and donor age (older in MMF arm), higher VAD use in MMF arm, and more patients in ICU in azathioprine arm. One and 3 year survival were higher in the mycophenolate mofetil arm (96% vs. 93%; 91% vs. 86%, p=0.001) in actuarial survival, and this difference persisted in multivariable logistic regression when adjusted for known differences in baseline characteristics as well as center transplant volume.

In another analysis of the ISHLT database, mycophenolate mofetil as compared to azathioprine was associated with significantly reduced risk (RR 0.73, 95% CI 0.56 to 0.95) of incident malignancy (63). Malignancy plays a major role in limiting long term post-transplant survival, so preventative strategies are important (64). A recent review summarizes the clinical trial experience with mycophenolate mofetil in cardiac transplantation, as well as the potential benefit of monitoring its drug levels (65).

Despite the design flaw in the large randomized study (59), mycophenolate mofetil has largely replaced azathioprine in many centers (see Figure 5 below). A new formulation of mycophenolate sodium (enteric coated, Myfortic) is available, designed to more reliably deliver mycophenolate in the hopes that it will reduce side effects. In a randomized trial of 154 first time heart transplant recipients, this compound had similar efficacy to mycophenolate mofetil (66). Fewer patients with the enteric-coated formulation required 2 or more study medication dose reductions during the treatment period, suggesting it may be better tolerated than the older formulation. However, the total incidence of gastrointestinal adverse events reported was similar in the 2 arms (69% enteric vs. 62% mycophenolate mofetil) and the incidence of diarrhea (13% enteric vs. 22% MMF) did not differ significantly (p=0.12). Adverse events classified as "blood and lymphatic system disorders" also did not differ between the 2 arms (56% vs. 55%). There was no advantage as assessed by gastrointestinal tolerance of the enteric coated formulation in 2 randomized trials of renal transplant recipients as well (67,68).

Potential change #3: Should TOR inhibitors (proliferation signal inhibitors) such as everolimus and sirolimus be used following cardiac transplantation?

Two inhibitors of mTOR (mammalian target of rapamycin), have been evaluated in cardiac transplant recipients: everolimus (RAD, trade name Certican) and sirolimus (formerly rapamycin, trade name Rapamune). Sirolimus is FDA approved for use in renal transplant recipients. Everolimus has not yet been approved by the FDA for clinical use with its initial application for cardiac transplantation being denied (69).

There are several potential applications of TOR inhibitors in heart transplant recipients.

1. Replace anti-metabolite therapy (i.e., MMF or azathioprine) as part of maintenance immunosuppression (including cyclosporine) immediately post-transplant (57, 70-72).

Randomized trials have addressed this strategy both with everolimus (72) and sirolimus (57,71). Similar results were noted. In trials comparing TOR inhibitor to azathioprine, the TOR inhibitor was associated with reduced incidence of rejection and a reduced development of transplant vasculopathy. 2-year data from the everolimus trial have recently been reported showing persistent benefit (70). Renal function was significantly worse when the TOR inhibitor replaced azathioprine with background cyclosporine use. Viral infections were more common with azathioprine than with either TOR inhibitor.

There is a concern of chest complications including mediastinitis with early post-operative use of these agents as they are potent inhibitors of wound healing (71,73,74). These agents have led to bronchial anastomotic dehiscence following lung transplantation, and graft loss/hepatic artery thrombosis following liver transplantation.

Despite the evidence from the everolimus trial (72), the Circulatory and Renal Drugs Advisory Committee voted against the initial application of everolimus for use in heart transplant recipients citing several concerns (69). First, more subjects in the everolimus versus azathioprine arm had missed biopsies at various time points, a difference which may have resulted in fewer grade 3A rejections being diagnosed, and thereby influenced the primary endpoint. Second, the IVUS analysis was in a small substudy of the overall cohort without prespecified selection criteria leading to possible selection bias. Third, they cited concerns over the nephrotoxicity associated with everolimus. Further trials are ongoing.

2. Slow the development or progression of transplant vasculopathy (70-72,75)

The evidence suggesting that early post-transplant use of everolimus or sirolimus, as compared to azathioprine, prevents incident transplant vasculopathy is discussed immediately above.

A provocative study addressed the strategy of adding sirolimus and discontinuing either azathioprine or mycophenolate mofetil in cardiac transplant recipients once transplant vasculopathy was discovered (75). Cyclosporine was continued. Transplant vasculopathy was defined by epicardial stenosis >50%, IVUS intimal thickening >0.5 mm, or severe diffuse vessel tapering. This was a randomized, open-label study of 46 subjects. Subjects were followed for 2 years. There was a dramatic reduction in the primary composite endpoint (see Table) consisting of death, angioplasty, MI, CABG or a >25% increase in a catheterization score. The catheterization score was based on angiographic severity of epicardial stenoses in 17 segments, weighted for the impact of the segment on overall myocardial perfusion (76). The components of the endpoint each favored sirolimus vs. standard therapy.

Table 10. Number of subjects reaching components of primary endpoint at 2 years.

Endpoint	Control	Sirolimus
Death	4	1
PTCA	5	1
CABG	1	0
MI	7	1
> 25% increase catheterization score	8	2
Total	25	5

From Mancini (75).

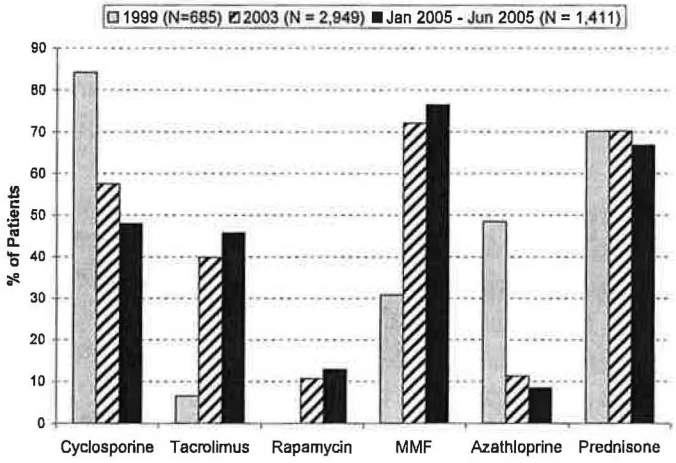
3. Replace calcineurin inhibition in the setting of renal dysfunction, in the hopes of rescuing renal function and avoiding progression to ESRD (77,78). These studies have demonstrated improvement of renal function.

Summary of findings regarding immunosuppression

As witnessed above by the flurry of recent randomized trials testing alternative immunosuppressive therapies in cardiac transplantation, the field is moving beyond anecdotal, single center observations. It is unfortunate that several of the large randomized trials conducted to date have had major limitations (as discussed above). These flaws may reflect the difficulties inherent in conducting trials in this patient population, or perhaps are part of the learning curve of randomized trials in transplant patients. The small number of overall transplants in the United States (~ 2000), conducted at many centers, also poses challenges. Nevertheless, it is clear that further efforts to conduct well designed randomized trials are necessary to optimize immunosuppressive therapy post-transplantation.

One way to gauge the strength of the evidence for the respective immunosuppressives as perceived by the transplant community is to look at utilization rates. Review of such data from the ISHLT registries from 1999 to 2005 (Figure 5) shows 4 observations:

Figure 5. Trends in maintenance immunosuppressive use at 1 year from 1999 to 2005. Adapted from (31).



1. Tacrolimus use has increased substantially with a concomitant decline in the use of cyclosporine, such that there is a nearly 50-50 split between the 2 calcineurin inhibitors.
2. Mycophenolate mofetil use has dramatically increased at the expense of Azathioprine.
3. Low level adoption of sirolimus (rapamycin) is emerging.
4. The majority of patients (65%) continue to be on corticosteroids 1 year post transplant.

3B. Rejection

Following cardiac transplantation, several types of rejection of the allograft can occur.

Table 11. Types of Rejection.

1. Hyperacute
2. Acute
   A. Cellular
   B. Noncellular (including humoral or antibody-mediated rejection)
3. Chronic ("Transplant vasculopathy")

3B. i. Hyperacute rejection. This catastrophic event occurs within minutes to hours of transplant due to recipient pre-formed antibodies to donor antigens (e.g., HLA or ABO). Fortunately this is a rare event with improved pre-transplant screening methods.

3B. ii. Acute rejection

In contrast to hyperacute rejection, acute rejection remains a common clinical event. It usually occurs in the first year post-transplant, often within the first 3 – 6 months. A review of recent randomized trials shows an incidence of treated rejection at 6 months ranging from 26% (37) to 48% (56) and at 1 year ranging from 21% (72) to 60% (57). Acute rejection can present in a number of ways (Table 12), including being asymptomatic and detected via surveillance endomyocardial biopsy (see Figure 6).

Table 12. Presentation of Acute Rejection

1. Surveillance endomyocardial biopsy
2. Graft dysfunction on echocardiography
3. Symptoms of heart failure (right or left-sided) including fatigue
4. Arrhythmias
5. Fever
6. Syncope
7. Loss of voltage on electrocardiogram
8. Death

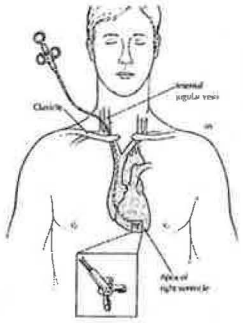


Figure 6. Endomyocardial biopsy via right internal jugular vein

Endomyocardial biopsy is the standard method to detect rejection. A bioptome is introduced either via the internal jugular vein or femoral vein to sample the right ventricle. The recent ISHLT grading system for rejection requires at least 3 samples with >50% myocardium to avoid potential sampling error (79). A recent case report documented an individual who died of fulminant acute cellular rejection evident at autopsy, despite repeated negative endomyocardial biopsies (80). In this case, the cellular infiltrate was only mild near the endocardium, the area sampled by endomyocardial biopsy, but was more intense in the mid-myocardium and extensive near the epicardium. Fortunately, such a gradient effect (infiltrate deeper in myocardium where bioptome doesn't sample) does not appear to be a common event. Earlier autopsy data showed involvement throughout the right and left ventricle (81). At UT Southwestern, in the first year following transplantation, a patient will undergo endomyocardial biopsy either 20 times (if they are being weaned off steroids entirely) or 12 times (if they are left on maintenance steroids). Both groups have biopsies q3 months during the second year and then annually. Given the relatively low frequency of rejection with increasing time, the utility of routine surveillance biopsy, in the absence of clinical or graft deterioration, past the first 6 months has been questioned (82).

An international grading system of cardiac biopsies was developed in 1990 (83) in an effort to standardize nomenclature. This grading system was revised in 2005 (79).

Table 13. Grading of Cellular Rejection using 2005 Revised Classification.

Grade	*Category*	*Finding*
0	Absent	No rejection
1R	Mild	Interstitial and/or perivascular infiltrate with up to 1 focus of myocyte damage
2R	Moderate	Two or more foci of infiltrate associated with myocyte damage
3R	Severe	Diffuse infiltrate with multifocal myocyte damage ± edema, ± hemorrhage ± vasculitis

It has become apparent that many cases of rejection occur in the **ABSENCE** of a cellular infiltrate. Paradoxically, this event (rejection without cellular infiltrate) is particularly common when there is significant allograft dysfunction (84). This syndrome has been termed **"acellular"** or **"noncellular" rejection**. As described above, this could be due to sampling error during endomyocardial biopsy. Other potential causes include ischemic injury to the allograft, development of transplant vasculopathy, recurrence of the native disease which had caused the underlying cardiomyopathy, or an infectious myocarditis (e.g., CMV). However, there has been increasing evidence that many cases of rejection in the absence of a cellular infiltrate are secondary to humoral or antibody mediated rejection. Noncellular rejection has been associated with markers of recipient antibody production, being present in individuals who had a positive retrospective crossmatch (crossmatch between donor serum and recipient lymphocytes after the transplant) (85,86) and recipients who develop donor specific antibodies (87).

The strongest evidence that antibodies directed against the donor represent the underlying pathophysiology of noncellular rejection comes from immunohistochemical and immunofluorescence studies. Using the endothelial marker CD34 and the macrophage marker CD68, much of the endothelial swelling noted on routine histology was found to be intravascular macrophages (88). **A major advance came from studies assessing the presence of capillary C4d** (89-92), the inactive fragment of C4b (part of the C3 convertase) generated during activation of the classical complement pathway. C4d has a thioester moiety and forms a covalent bound to the surrounding structures, and has been termed a "durable chemical marker of complement activation in the tissue" (93). C4d deposition has been associated with donor specific antibodies (94). In one study, 21/25 (84%) of biopsies from transplant recipients with anti-donor antibodies were C4d positive whereas only 7/60 (12%) biopsies from recipients without anti-donor antibodies were positive for C4d. Patients who had their native heart biopsied for non-transplant cardiac disorders also had a low prevalence of C4d staining (11%) (95). However, C4d is not always associated with anti-donor HLA antibodies, possibly because the antibodies bind to the graft and are not detectable in the serum, or because the antibodies are non-HLA directed (e.g., directed against vimentin, MICA, heat shock protein, myosin, or other endothelial proteins) (88). Recently, phosphorylated S6 ribosomal protein, a downstream target in the PI3 kinase/AKT/mTOR pathway, was shown to be a novel marker of antibody mediated rejection and correlated both with donor-specific antibodies and C4d staining (96). The revised 2005 classification grades antibody mediated rejection as shown below.

Table 14. Grading of Antibody Mediated Rejection (AMR) in the 2005 Revised Classification.

Grade	*Category*	*Criteria*
AMR 0	Negative	No histologic or immunopathologic features of AMR
AMR 1	Positive	Histologic features of AMR Positive immunofluorescence or immunoperoxidase staining for AMR (positive CD68, C4d)

The guideline committee suggested that each biopsy be evaluated histologically for AMR (capillary injury with endothelial swelling and intravascular marcrophages; edema and hemorrhage; intravascular thrombi and myocyte necrosis) and if present, then immunostaining should be performed (79). However, data have already emerged suggesting that histologic features are not sensitive enough, suggesting the need for routine immunohistochemistry (97).

The treatment of rejection, both cellular and noncellular, involves augmentation of immunosuppression which usually leads to its resolution. Therapeutic options for significant cellular rejection include pulse Solumedrol (e.g., 1 gram IV Q day for 3 days) and cytolytic therapy (ATGAM, OKT3). At UTSW, we administer cytolytic therapy for rejection refractory to Solumedrol or rejection with severe hemodynamic compromise. The management of acellular rejection is emerging. Steroids and/or cytolytic therapy are often administered if there is significant graft dysfunction. In addition, plasmaphoresis (to clear antibodies from the circulation), IVIG, photophoresis, total lymphoid irradiation, and rituximab have been used with some anecdotal success. Rituximab is an intriguing option (98-100) as it is a monoclonal antibody directed against the pan-B cell marker CD20, potentially useful in an entity dependent upon antibody production, though its sparing of plasma cells may be a limitation.

3B. iii. Molecular diagnosis of acute rejection

Endomyocardial biopsy is invasive and expensive, conveys risk including cardiac perforation, arrhythmia, and damage to the tricuspid valve, and requires expert interpretation. A noninvasive measure of rejection would be useful. Recently, the Cardiac Allograft Rejection Gene Expression Observational (CARGO) study (101) was conducted in an effort to develop a molecularly-based marker of rejection. Blood was collected from cardiac transplant recipients who were undergoing endomyocardial biopsy, and peripheral blood mononuclear cells were isolated. The hypothesis was that there would be a gene expression signature detectable in the mononuclear cells which correlated with immune activation/leukocyte trafficking. The study was conducted in three phases: a gene discovery phase, training and diagnostic and development phase, and a validation phase. In the gene discovery phase, 7370 genes were screened in 285 samples from 98 patients (either rejecting or not) using microarray technology and yielded 97 candidate target genes. Another 155 candidate genes were selected from literature review. Real time PCR assays were developed and tested from samples of individuals who were or were not having rejection. Ultimately, 11 genes best associated with rejection grade ≥3A formed the basis of a commercial diagnostic test termed "Allomap." The 11 genes are broadly categorized as being related to 1. Steroid responsiveness (ITGAM, FLT3, IL1R2) 2. Platelets (G6B, PF4) 3. Hematopoiesis (WDR40A, MIR) 4. T lymphocyte activation (PDCD1) 5. T lymphocyte migration (ITGA4) 6. T lymphocyte (SEMA7A) and 7. Unknown (ARHU). The assay was then tested in a unique set of samples (validation set) consisting of 63 samples, including 31 episodes of rejection. The area under the ROC curve for rejection was 0.72. Scores increased with time post transplant as maintenance steroids were weaned. The assay was then tested in subjects ≥6 months and ≥1 year post-transplant and had improved diagnostic accuracy (AUC 0.8). Finally, the assay was tested in a "prevalent population" sample consisting of 281 samples from 166 patients ≥1 year post-transplant. The prevalence of rejection was low (only 3.2% had grade ≥3A) as would occur in clinical practice at this late post-transplant time point. At a threshold of 30, the assay had an excellent negative predictive value (99.6%) with a low positive predictive value (6.8%). 68% of samples had a score <30, suggesting that 2/3 of patients could be spared biopsy.

A follow-up report was published of the initial experience based on 211 samples (102): 147 (70%) had an Allomap score < 34 and there were no cases of grade ≥2R rejection. The low positive predictive value for a score ≥34 (7.8%) mirrored that seen in the CARGO study. Algorithms have been developed suggesting that a negative Allomap test in recipients >6 months post-transplant means no biopsy is needed if an echocardiogram shows intact graft function and there is no change in clinical status.

Another major finding from the CARGO study was the poor agreement between pathologists on assigning ISHLT grade (103). In the CARGO study, there was a local pathologist who interpreted the biopsies, which then was re-interpreted (blinded to the local read) by a core center comprised of 3 experts (each of the 3 were blinded to the interpretations of the other core center experts). The concordance for grade 0 biopsies was good (87%) between the local pathologists and the expert panel. However, the concordance for either grade 2 (17%) or grade 3A/B (40%) was poor. Strikingly, even the 3 experts disagreed among themselves on grade 2 (concordance 60%) and Grade 3A/3B (58%), highlighting the subjectivity involved in interpretation of cardiac biopsies. For biopsies interpreted by the local pathologist as grade ≥3A, Allomap score increased as the number of central experts (i.e., from 1 expert pathologist interpreting as ≥3A to all 3 expert pathologists interpreting as ≥3A) suggesting that high molecular testing scores were associated with increasing certainty of pathological findings (104).

Although an exciting development, there are some concerns raised with the utility of the present assay. First, the assay has been tested only for cellular rejection. Since noncellular/humoral rejection is an emerging clinical entity, avoiding biopsy based on an Allomap score may lead to a missed diagnosis of asymptomatic humoral rejection. On the other hand, it is not clear what to do with a patient who has asymptomatic humoral rejection (i.e., intact graft function with positive C4d). Second, the algorithm for care incorporating the Allomap assay includes an assessment of graft function with echocardiography. However, an echocardiogram is not routinely performed during the routine clinic visits post-transplant, so it would lead to a substantial increase in the number of echocardiograms performed (and their associated cost). The cost of the Allomap itself is not dissimilar to the cost of the endomyocardial biopsy. Finally, the high negative predictive value of the Allomap score is in large part predicated on the low prevalence of rejection after 6 months. For example, it has been calculated that if you biopsy patients based on a coin flip (i.e., biopsy if heads, don't biopsy if tails), the coin flip also would have an excellent negative predictive value (98.6%) (105).

The promise of a noninvasive, molecularly-based diagnostic test is an exciting development in cardiac transplantation. Microarray analysis identified gene expression profiles associated with rejection or with *Trypanosoma cruzi* infection (106), suggesting that such tools ultimately may allow clinicians to tailor immunosuppression to individual patient characteristics. An assay of intracellular ATP levels in CD4+ cells (Cylex ImmunKnow™) is one such strategy. Here, low ATP levels suggest the intensity of immunosuppression is too high (increased risk of infection) while high intracellular ATP levels suggest inadequate immunosuppression (increased risk of rejection). There is limited experience as of yet with this assay (107,108).

3B. iv. Chronic rejection (transplant vasculopathy)

Transplant vasculopathy is an accelerated for of coronary artery disease which occurs post-transplantation and is a major obstacle to long-term survival. It initially presents as intimal thickening with sparing of the elastic lamina and progresses to luminal stenosis, occlusion of branch vessels ("pruning"), and myocardial infarction. There are differences in histopathology between typical coronary atherosclerosis and vasculopathy. Vasculopathy is typically a more concentric intimal process, involves the intramural arteries and even the coronary veins (109). Due to denervation, chest pain is not common. Clinical presentations of vasculopathy are listed.

Table 15. Clinical presentation of transplant vasculopathy

1. Abnormal ECG including ST elevation (AMI) or Q waves
2. Heart failure
3. Arrhythmias
4. Allograft dysfunction on echocardiogram
5. Enlarged cardiac silhouette on chest X-ray
6. Syncope
7. Changes on surveillance coronary angiogram
8. Chest pain (reinnervation)
9. Death

Both immunological and non-immunological factors are risk factors for the development of vasculopathy (110,111). The immunological factors include mismatching of HLA antigens DR between donor and recipient (112,113), previous episodes of rejection (114-116), especially antibody mediated rejection (117), and the presence of HLA antibodies in the recipient (118). These observations likely explain in part why HLA matching is associated with long-term survival following heart transplantation (119,120). The nonimmunological risk factors for vasculopathy include classical CAD risk factors such as deranged glucose homeostasis (121), advanced age, hypertension, and smoking (111), as well as other factors such as CMV infection (122-124) which has been shown to impair endothelial function (125). The mode of donor death is a risk factor for vasculopathy. The hearts from donors who die of intracranial hemorrhage (126) or "explosive brain death" (127) are at increased risk of later developing vasculopathy, believed to be a consequence of high catecholamine exposure during these types of death.

The diagnosis of transplant vasculopathy can be overlooked by routine coronary angiography ("luminogram") due to its concentric nature. Intravascular ultrasound which directly measures intimal thickness is the gold standard. Progression of intimal thickness from baseline (early post-transplant) to 1 year is an early marker of this disease, with a cutoff often set at >0.5 mm. Increased intimal thickness at 1 year has been associated with subsequent death and nonfatal MI in a single center study (128) and death in a multicenter study (129) establishing the prognostic value of this assessment. Progressive intimal thickness on IVUS is shown (Figure 7).

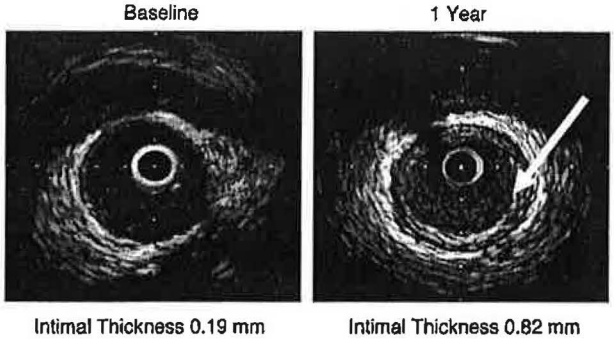


Figure 7. Increased intimal thickness after 1 year post-transplant as assessed by intravascular ultrasound. Arrow points to increased intimal thickness as compared to baseline seen on left panel. From (128)

The treatment of vasculopathy includes statins, an alteration of immunosuppression, conventional approaches towards revascularization (PCI or CABG), and re-transplantation.

The initiation of pravastatin (130,131) and simvastatin (132) early after transplant in randomized trials has reduced the development and progression of transplant vasculopathy and improved survival (Figure 8).

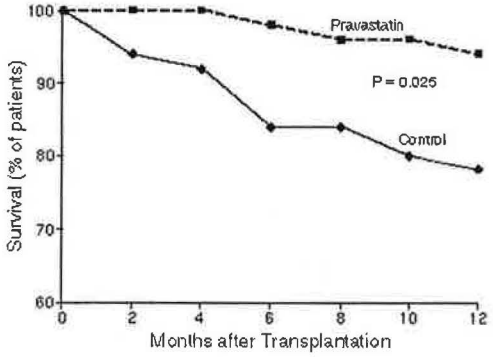


Figure 8. Pravastatin improves survival following heart transplantation. From (130)

Given the increased risk of rhabdomyolysis due to the interaction of cyclosporine and statin therapy, it is important to initiate statins at lower doses, e.g. simvastatin 5 mg and pravastatin 20 mg (i.e., do not resume pre-transplant doses). These can be uptitrated as tolerated.

In addition to statins, choice of immunosuppression influences vasculopathy. Specifically, as discussed above, mycophenolate mofetil (61), everolimus (72), and sirolimus (71) appear to be beneficial in terms of reducing the development and progression of vasculopathy as compared to azathioprine. The most convincing data, in my opinion, is for the use of sirolimus once vasculopathy is clinically evident (75). PCI and CABG can be performed depending upon anatomy, and can provide transient benefit. However, given the diffuse nature of the vasculopathic process, the prognosis usually remains poor following such interventions, especially when distal disease is present (133). Retransplantation is an option for select individuals (134) though this option does raises some ethical issues given the limited availability of donor organs and deaths on the waiting list.

3C. Other complications

3C. i. Cytomegalovirus (CMV)

CMV infection post-transplantation is a major cause of morbidity. Complications from infection can be classified as either direct (i.e., CMV syndrome or tissue-invasion such as pneumonitis or retinitis) or indirect (135). The latter reflects the effect of CMV on the immune system and leads to an increased risk of other bacterial or fungal infections, acute rejection, and vasculopathy. The highest risk for post-transplant CMV infection is when an organ from a CMV positive donor (D+) is implanted into a recipient who has not previously been exposed to CMV (R-). Intermediate risk groups for post-transplant CMV infection are those who are D+/R+ or D-/R+, whereas the lowest risk group is D-/R- (both donor and recipient have not had CMV).

There have been 2 strategies recommended to prevent CMV infection post-transplant, termed "prophylaxis" and "pre-emptive" (135). A variant of prophylaxis termed "targeted prophylaxis" (136) treats individuals deemed high risk for CMV immediately post transplant, while the pre-emptive strategy employs monitoring for CMV (either CMV antigenemia or quantitative PCR) and initiation of treatment when there is evidence of active infection. Prophylactic treatment for the highest risk group (D+/R-) employs CMV hyperimmune globulin in addition to IV ganciclovir or valganciclovir. There have been advocates of both the prophylactic (136) and pre-emptive (137,138) strategies. A recent committee of international experts favored prophylaxis (135). During prophylaxis, it is unusual to have breakthrough CMV infection. However, upon discontinuation of the prophylactic therapy, late onset CMV infection (i.e., > 100 days post transplant) can occur (139). It remains uncertain how long to continue prophylaxis therapy, though the lung transplant literature suggests that at least 180 days are needed (140).

3C. ii. Non-CMV complications

There are numerous other complications post-transplant, either secondary to the immunosuppressive side effects or the immunosuppressed state they induce. Such complications include non-CMV infections, hypertension, weight gain, osteoporosis, diabetes, gout, dyslipidemia, and post-transplant lymphoproliferative disease as well as other malignancies. Chronic renal failure post-transplant is an emerging concern (141).

3D. Outcomes

3D. i. Exercise capacity

Following cardiac transplantation, the exercise capacity of patients, albeit improved compared to pre-transplantation, does not usually return to normal. The majority of studies report a post-transplant peak $V0_2$ between 16 and 20 ml/kg/min (142). Increased aerobic capacity is noted by 6 months. Younger patients increase their exercise capacity to a greater extent than older patients (143). The mechanism for the persistently impaired aerobic capacity is multifactorial including 1. chronotropic incompetence secondary to denervation of the heart 2. diastolic dysfunction from either rejection or hypertension 3. recipient-donor size mismatch and 4. skeletal muscle abnormalities, either residual from the heart failure state or induced by the post-transplant steroids (144).

Despite these limitations, the vast majority (90%) of recipients in the ISHLT database report no activity limitations at years 1 through 7 (145). Further, there are case reports of individuals who do achieve superior aerobic capacity following transplantation. A 45 year old man who underwent transplant 2 decades prior was reported to have a peak $V0_2$ of 59 ml/kg/min, similar to endurance-trained age-matched male athletes, and he was able to complete half-ironman triathlons (416^{th} out of 611 competitors) (146). His peak heart rate on an exercise test was 177 beats per minute, similar to age matched controls, suggesting he had achieved cardiac reinnervation (146). Cardiac reinnervation is possible, as had been documented previously in studies which showed that recipients who had chest pain during an ischemic event had higher levels of cardiac norepinephrine, a marker of reinnervation (147). Subsequently, PET scanning

with the catecholamine analogue C-11 hydroxyephedrine, a more sophisticated marker of cardiac reinnervation, found that cardiac reinnervation was associated with a higher exercise peak heart rate and left ventricular ejection fraction (148). Reinnervation was more likely in younger recipients who did not have rejection (149), similar to the individual described above.

3D. ii. Survival

Although there have been no randomized trials of transplantation versus ongoing medical therapy, the outcomes of individuals following cardiac transplantation are far superior to those with true ESHF. Individuals who are inotrope dependent have a 1-year survival <25% (1,150). In contrast, the US Transplant - Scientific Registry of Transplant Recipients (151) reported that the unadjusted 1-year patient survival rate among the 2,191 adult recipients transplanted in 2006 was 88%. At UTSW, our one-year survival has ranged from 90-100% over the last decade. Data from the ISHLT (31) demonstrated that 50% of cardiac recipients will live 9.9 years. The conditional half-life (for those who survive the first year) was 13 years. There has been a significant increase in survival when comparing 4-year eras starting in 1982 (see Figure) largely due to improved survival in the first year. Following the first 6 months, survival declines in a nearly linear fashion with approximately 3.4% annual mortality, and the slope of the curve has not appreciably changed (31).

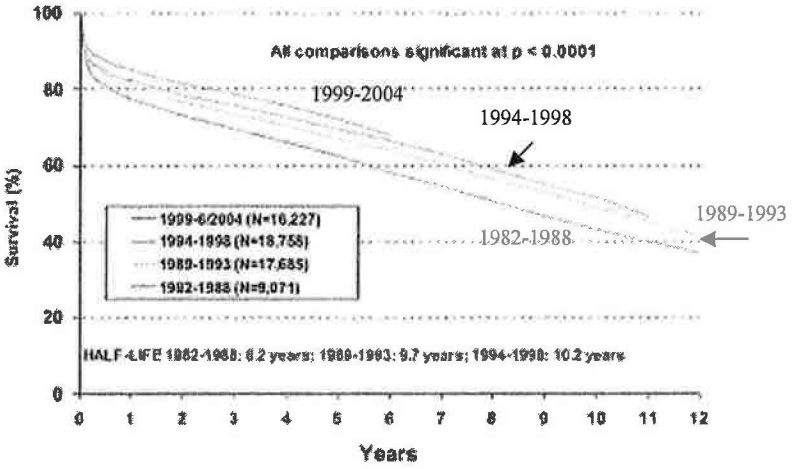


Figure 9. Improving survival post-transplant is largely due to gains in the first year. From (31)

Data from the Cardiac Transplant Research Database, a select group of 32 institutions voluntary participating in a registry, reported similar data with improving 5-year outcomes when comparing 3 eras of 1990-1994 to 1995-1999 and 2000-2004: 70% to 73% to 77%, respectively. These improvements in 5-year survival were mirrored by lower rates of rejection, CMV infection, and malignancy during the more recent era (but not early vasculopathy) (152).

References

1. Hershberger RE, Nauman D, Walker TL, et al. Care processes and clinical outcomes of continuous outpatient support with inotropes (COSI) in patients with refractory endstage heart failure. J Card Fail 2003;9:180-7.
2. Rose EA, Gelijns AC, Moskowitz AJ, et al. Long-term mechanical left ventricular assistance for end-stage heart failure. N Engl J Med 2001;345:1435-43.
3. Birks EJ, Tansley PD, Hardy J, et al. Left ventricular assist device and drug therapy for the reversal of heart failure. N Engl J Med 2006;355:1873-84.
4. Mancini DM, Eisen H, Kussmaul W, Mull R, Edmunds LH, Jr., Wilson JR. Value of peak exercise oxygen consumption for optimal timing of cardiac transplantation in ambulatory patients with heart failure. Circulation 1991;83:778-86.
5. Mehra MR, Kobashigawa J, Starling R, et al. Listing criteria for heart transplantation: International Society for Heart and Lung Transplantation guidelines for the care of cardiac transplant candidates--2006. J Heart Lung Transplant 2006;25:1024-42.
6. Arena R, Myers J, Abella J, et al. Development of a ventilatory classification system in patients with heart failure. Circulation 2007;115:2410-7.
7. Nanas SN, Nanas JN, Sakellariou D, et al. VE/VCO2 slope is associated with abnormal resting haemodynamics and is a predictor of long-term survival in chronic heart failure. Eur J Heart Fail 2006;8:420-7.
8. Lee TH, Hamilton MA, Stevenson LW, et al. Impact of left ventricular cavity size on survival in advanced heart failure. Am J Cardiol 1993;72:672-6.
9. Kittleson M, Hurwitz S, et al. Development of circulatory-renal limitations to angiotensin-converting enzyme inhibitors identifies patients with severe heart failure and early mortality. J Am Coll Cardiol 2003;41:2029-35.
10. Estep JD, Mehta SK, Uddin F, et al. Beta-blocker therapy in patients with heart failure in the urban setting: moving beyond clinical trials. Am Heart J 2004;148:958-63.
11. Stevenson LW, Tillisch JH, Hamilton M, et al. Importance of hemodynamic response to therapy in predicting survival with ejection fraction less than or equal to 20% secondary to ischemic or nonischemic dilated cardiomyopathy. Am J Cardiol 1990;66:1348-54.
12. Arena R, Myers J, Aslam SS, Varughese EB, Peberdy MA. Peak VO2 and VE/VCO2 slope in patients with heart failure: a prognostic comparison. Am Heart J 2004;147:354-60.
13. Dries DL, Sweitzer NK, Drazner MH, et al. Prognostic impact of diabetes mellitus in patients with heart failure according to the etiology of left ventricular systolic dysfunction. J Am Coll Cardiol 2001;38:421-8.
14. Rouleau JL, Roecker EB, Tendera M, et al. Influence of pretreatment systolic blood pressure on the effect of carvedilol in patients with severe chronic heart failure: the Carvedilol Prospective Randomized Cumulative Survival (COPERNICUS) study. J Am Coll Cardiol 2004;43:1423-9.
15. Dries DL, Exner DV, Domanski MJ, Greenberg B, Stevenson LW. The prognostic implications of renal insufficiency in asymptomatic and symptomatic patients with left ventricular systolic dysfunction. J Am Coll Cardiol 2000;35:681-9.
16. Drazner MH, Rame JE, Stevenson LW, Dries DL. Prognostic importance of elevated jugular venous pressure and a third heart sound in patients with heart failure. N Engl J Med 2001;345:574-81.
17. Aaronson KD, Schwartz JS, Chen TM, Wong KL, Goin JE, Mancini DM. Development and prospective validation of a clinical index to predict survival in ambulatory patients referred for cardiac transplant evaluation. Circulation 1997;95:2660-7.
18. Koelling TM, Joseph S, Aaronson KD. Heart failure survival score continues to predict clinical outcomes in patients with heart failure receiving beta-blockers. J Heart Lung Transplant 2004;23:1414-22.
19. Lund LH, Aaronson KD, Mancini DM. Validation of peak exercise oxygen consumption and the Heart Failure Survival Score for serial risk stratification in advanced heart failure. Am J Cardiol 2005;95:734-41.
20. Levy WC, Mozaffarian D, Linker DT, et al. The Seattle Heart Failure Model: prediction of survival in heart failure. Circulation 2006;113:1424-33.
21. Mudge GH, Goldstein S, Addonizio LJ, et al. 24th Bethesda conference: Cardiac transplantation. Task Force 3: Recipient guidelines/prioritization. J Am Coll Cardiol 1993;22:21-31.
22. Costanzo MR, Augustine S, Bourge R, et al. Selection and treatment of candidates for heart transplantation. A statement for health professionals from the Committee on Heart Failure and Cardiac Transplantation of the Council on Clinical Cardiology, American Heart Association. Circulation 1995;92:3593-612.
23. Jahangiri B, Haddad H. Cardiac transplantation in HIV-positive patients: are we there yet? J Heart Lung Transplant 2007;26:103-7.

24. Zaidi AR, Zaidi A, Vaitkus PT. Outcome of heart transplantation in patients with sarcoid cardiomyopathy. J Heart Lung Transplant 2007;26:714-7.
25. King LP, Siminoff LA, Meyer DM, et al. Health insurance and cardiac transplantation: a call for reform. J Am Coll Cardiol 2005;45:1388-91.
26. Hauptman PJ, O'Connor KJ. Procurement and allocation of solid organs for transplantation. N Engl J Med 1997;336:422-31.
27. http://www.unos.org/PoliciesandBylaws2/policies/pdfs/policy_9.pdf. Allocation of thoracic organs.
28. Meine TJ, Russell SD. A history of orthotopic heart transplantation. Cardiol Rev 2005;13:190-6.
29. Lindenfeld J, Miller GG, Shakar SF, et al. Drug therapy in the heart transplant recipient: part I: cardiac rejection and immunosuppressive drugs. Circulation 2004;110:3734-40.
30. Uber PA, Mehra MR. Induction therapy in heart transplantation: is there a role? J Heart Lung Transplant 2007;26:205-9.
31. Taylor DO, Edwards LB, et al. Registry of the International Society for Heart and Lung Transplantation: twenty-third official adult heart transplantation report--2006. J Heart Lung Transplant 2006;25:869-79.
32. Mehra MR, Zucker MJ, Wagoner L, et al. A multicenter, prospective, randomized, double-blind trial of basiliximab in heart transplantation. J Heart Lung Transplant 2005;24:1297-304.
33. Segovia J, Rodriguez-Lambert JL, Crespo-Leiro MG, et al. A randomized multicenter comparison of basiliximab and muromonab (OKT3) in heart transplantation: SIMCOR study. Transplantation 2006;81:1542-8.
34. Mattei MF, Redonnet M, Gandjbakhch I, et al. Lower risk of infectious deaths in cardiac transplant patients receiving basiliximab versus anti-thymocyte globulin as induction therapy. J Heart Lung Transplant 2007;26:693-9.
35. Carrier M, Leblanc MH, Perrault LP, et al. Basiliximab and rabbit anti-thymocyte globulin for prophylaxis of acute rejection after heart transplantation: a non-inferiority trial. J Heart Lung Transplant 2007;26:258-63.
36. Beniaminovitz A, Itescu S, Lietz K, et al. Prevention of rejection in cardiac transplantation by blockade of the interleukin-2 receptor with a monoclonal antibody. N Engl J Med 2000;342:613-9.
37. Hershberger RE, Starling RC, Eisen HJ, et al. Daclizumab to prevent rejection after cardiac transplantation. N Engl J Med 2005;352:2705-13.
38. Kobashigawa J, David K, et al. Daclizumab is associated with decreased rejection and no increased mortality in cardiac transplant patients receiving MMF, cyclosporine, and corticosteroids. Transplant Proc 2005;37:1333-9.
39. Rosenberg PB, Vriesendorp AE, Drazner MH, et al. Induction therapy with basiliximab allows delayed initiation of cyclosporine and preserves renal function after cardiac transplantation. J Heart Lung Transplant 2005;24:1327-31.
40. Delgado DH, Miriuka SG, Cusimano RJ, Feindel C, Rao V, Ross HJ. Use of basiliximab and cyclosporine in heart transplant patients with pre-operative renal dysfunction. J Heart Lung Transplant 2005;24:166-9.
41. Rosenbaum DH, Adams BC, Mitchell JD, et al. Effects of early steroid withdrawal after heart transplantation. Ann Thorac Surg 2006;82:637-44; discussion 644.
42. Olivari MT, Jessen ME, Baldwin BJ, et al. Triple-drug immunosuppression with steroid discontinuation by six months after heart transplantation. J Heart Lung Transplant 1995;14:127-35.
43. Kobashigawa JA, Stevenson LW, Brownfield ED, et al. Corticosteroid weaning late after heart transplantation: relation to HLA-DR mismatching and long-term metabolic benefits. J Heart Lung Transplant 1995;14:963-7.
44. Taylor DO, Bristow MR, O'Connell JB, et al. Improved long-term survival after heart transplantation predicted by successful early withdrawal from maintenance corticosteroid therapy. J Heart Lung Transplant 1996;15:1039-46.
45. Opelz G, Dohler B, Laux G. Long-term prospective study of steroid withdrawal in kidney and heart transplant recipients. Am J Transplant 2005;5:720-8.
46. Price GD, Olsen SL, Taylor DO, O'Connell JB, Bristow MR, Renlund DG. Corticosteroid-free maintenance immunosuppression after heart transplantation: feasibility and beneficial effects. J Heart Lung Transplant 1992;11:403-14.
47. Starzl TE, Todo S, Fung J, Demetris AJ, Venkataramman R, Jain A. FK 506 for liver, kidney, and pancreas transplantation. Lancet 1989;2:1000-4.
48. Meiser BM, Uberfuhr P, Fuchs A, et al. Single-center randomized trial comparing tacrolimus (FK506) and cyclosporine in the prevention of acute myocardial rejection. J Heart Lung Transplant 1998;17:782-8.
49. Reichart B, Meiser B, Vigano M, et al. European Multicenter Tacrolimus (FK506) Heart Pilot Study: one-year results--European Tacrolimus Multicenter Heart Study Group. J Heart Lung Transplant 1998;17:775-81.
50. Taylor DO, Barr ML, Radovancevic B, et al. A randomized, multicenter comparison of tacrolimus and cyclosporine immunosuppressive regimens in cardiac transplantation: decreased hyperlipidemia and hypertension with tacrolimus. J Heart Lung Transplant 1999;18:336-45.

51. Marcus N, Khaghani A, Burke M, Yacoub M, Banner N. Medium-term outcome of tacrolimus immunosuppression following rejection or graft dysfunction in heart transplant patients. Transplant Proc 1998;30:1134-5.
52. Mentzer RM, Jr., Jahania MS, Lasley RD. Tacrolimus as a rescue immunosuppressant after heart and lung transplantation. The U.S. Multicenter FK506 Study Group. Transplantation 1998;65:109-13.
53. Mehra MR, Uber PA, Scott RL, et al. Ethnic disparity in clinical outcome after heart transplantation is abrogated using tacrolimus and mycophenolate mofetil-based immunosuppression. Transplantation 2002;74:1568-73.
54. Kobashigawa JA, Patel J, Furukawa H, et al. Five-year results of a randomized, single-center study of tacrolimus vs microemulsion cyclosporine in heart transplant patients. J Heart Lung Transplant 2006;25:434-9.
55. Meiser BM, Groetzner J, Kaczmarek I, et al. Tacrolimus or cyclosporine: which is the better partner for mycophenolate mofetil in heart transplant recipients? Transplantation 2004;78:591-8.
56. Grimm M, Rinaldi M, Yonan NA, et al. Superior prevention of acute rejection by tacrolimus vs. cyclosporine in heart transplant recipients--a large European trial. Am J Transplant 2006;6:1387-97.
57. Kobashigawa JA, Miller LW, Russell SD, et al. Tacrolimus with mycophenolate mofetil (MMF) or sirolimus vs. cyclosporine with MMF in cardiac transplant patients: 1-year report. Am J Transplant 2006;6:1377-86.
58. Mehra MR, Uber PA, Kaplan B. Immunosuppression in cardiac transplantation: science, common sense and the heart of the matter. Am J Transplant 2006;6:1243-5.
59. Kobashigawa J, Miller L, Renlund D, et al. A randomized active-controlled trial of mycophenolate mofetil in heart transplant recipients. Mycophenolate Mofetil Investigators. Transplantation 1998;66:507-15.
60. Eisen HJ, et al. Three-year results of a randomized, double-blind, controlled trial of mycophenolate mofetil versus azathioprine in cardiac transplant recipients. J Heart Lung Transplant 2005;24:517-25.
61. Kobashigawa JA, Tobis JM, Mentzer RM, et al. Mycophenolate mofetil reduces intimal thickness by intravascular ultrasound after heart transplant: reanalysis of the multicenter trial. Am J Transplant 2006;6:993-7.
62. Hosenpud JD, Bennett LE. Mycophenolate mofetil versus azathioprine in patients surviving the initial cardiac transplant hospitalization: an analysis of the Joint UNOS/ISHLT Thoracic Registry. Transplantation 2001;72:1662-5
63. O'Neill JO, Edwards LB, Taylor DO. Mycophenolate mofetil and risk of developing malignancy after orthotopic heart transplantation: analysis of the transplant registry of the International Society for Heart and Lung Transplantation. J Heart Lung Transplant 2006;25:1186-91.
64. Hauptman PJ, Mehra MR. It is time to stop ignoring malignancy in heart transplantation: a call to arms. J Heart Lung Transplant 2005;24:1111-3.
65. Kobashigawa JA, Meiser BM. Review of major clinical trials with mycophenolate mofetil in cardiac transplantation. Transplantation 2005;80:S235-43.
66. Kobashigawa JA, Renlund DG, Gerosa G, et al. Similar efficacy and safety of enteric-coated mycophenolate sodium (EC-MPS, myfortic) compared with mycophenolate mofetil (MMF) in de novo heart transplant recipients: results of a 12-month, single-blind, randomized, parallel-group, multicenter study. J Heart Lung Transplant 2006;25:935-41.
67. Budde K, Curtis J, Knoll G, et al. Enteric-coated mycophenolate sodium can be safely administered in maintenance renal transplant patients: results of a 1-year study. Am J Transplant 2004;4:237-43.
68. Salvadori M, Holzer H, de Mattos A, et al. Enteric-coated mycophenolate sodium is therapeutically equivalent to mycophenolate mofetil in de novo renal transplant patients. Am J Transplant 2004;4:231-6.
69. Hiatt WR, Nissen SE. New drug application 21-628, Certican (everolimus), for the proposed indication of prophylaxis of rejection in heart transplantation: report from the Cardiovascular and Renal Drugs Advisory Committee, US Food and Drug Administration, November 16, 2005, Rockville, Md. Circulation 2006;113:e394-5.
70. Vigano M, Tuzcu M, Benza R, et al. Prevention of acute rejection and allograft vasculopathy by everolimus in cardiac transplants recipients: a 24-month analysis. J Heart Lung Transplant 2007;26:584-92.
71. Keogh A, Richardson M, et al. Sirolimus in de novo heart transplant recipients reduces acute rejection and prevents coronary artery disease at 2 years: a randomized clinical trial. Circulation 2004;110:2694-700.
72. Eisen HJ, Tuzcu EM, Dorent R, et al. Everolimus for the prevention of allograft rejection and vasculopathy in cardiac-transplant recipients. N Engl J Med 2003;349:847-58.
73. Kuppahally S, Al-Khaldi A, Weisshaar D, et al. Wound healing complications with de novo sirolimus versus mycophenolate mofetil-based regimen in cardiac transplant recipients. Am J Transplant 2006;6:986-92.
74. Zucker MJ, Baran DA, Arroyo LH, et al. De novo immunosuppression with sirolimus and tacrolimus in heart transplant recipients compared with cyclosporine and mycophenolate mofetil: a one-year follow-up analysis. Transplant Proc 2005;37:2231-9.
75. Mancini D, Pinney S, Burkhoff D, et al. Use of rapamycin slows progression of cardiac transplantation vasculopathy. Circulation 2003;108:48-53.

76. McGiffin DC, Savunen T, Kirklin JK, et al. Cardiac transplant coronary artery disease. A multivariable analysis of pretransplantation risk factors for disease development and morbid events. J Thorac Cardiovasc Surg 1995;109:1081-8; discussion 1088-9.
77. Bestetti R, Theodoropoulos TA, Burdmann EA, Filho MA, Cordeiro JA, Villafanha D. Switch from calcineurin inhibitors to sirolimus-induced renal recovery in heart transplant recipients in the midterm follow-up. Transplantation 2006;81:692-6.
78. Rothenburger M, Teerling E, Bruch C, et al. Calcineurin inhibitor-free immunosuppression using everolimus (Certican) in maintenance heart transplant recipients: 6 months' follow-up. J Heart Lung Transplant 2007;26:250-7.
79. Stewart S, Winters GL, Fishbein MC, et al. Revision of the 1990 working formulation for the standardization of nomenclature in the diagnosis of heart rejection. J Heart Lung Transplant 2005;24:1710-20.
80. Bhalodolia R, Cortese C, Graham M, Hauptman PJ. Fulminant acute cellular rejection with negative findings on endomyocardial biopsy. J Heart Lung Transplant 2006;25:989-92.
81. Nakhleh RE, Jones J, Goswitz JJ, Anderson EA, Titus J. Correlation of endomyocardial biopsy findings with autopsy findings in human cardiac allografts. J Heart Lung Transplant 1992;11:479-85.
82. Sethi GK, Kosaraju S, Arabia FA, Roasdo LJ, McCarthy MS, Copeland JG. Is it necessary to perform surveillance endomyocardial biopsies in heart transplant recipients? J Heart Lung Transplant 1995;14:1047-51.
83. Billingham ME, Cary NR, Hammond ME, et al. A working formulation for the standardization of nomenclature in the diagnosis of heart and lung rejection: Heart Rejection Study Group. The International Society for Heart Transplantation. J Heart Transplant 1990;9:587-93.
84. Miller LW, Wesp A, Jennison SH, et al. Vascular rejection in heart transplant recipients. J Heart Lung Transplant 1993;12:S147-52.
85. Hammond EH, Yowell RL, Nunoda S, et al. Vascular (humoral) rejection in heart transplantation: pathologic observations and clinical implications. J Heart Transplant 1989;8:430-43.
86. Michaels PJ, Espejo ML, Kobashigawa J, et al. Humoral rejection in cardiac transplantation: risk factors, hemodynamic consequences and relationship to transplant coronary artery disease. J Heart Lung Transplant 2003;22:58-69.
87. Costanzo-Nordin MR, Heroux AL, Radvany R, Koch D, Robinson JA. Role of humoral immunity in acute cardiac allograft dysfunction. J Heart Lung Transplant 1993;12:S143-6.
88. Reed EF, Demetris AJ, Hammond E, et al. Acute antibody-mediated rejection of cardiac transplants. J Heart Lung Transplant 2006;25:153-9.
89. Feucht HE, Felber E, Gokel MJ, et al. Vascular deposition of complement-split products in kidney allografts with cell-mediated rejection. Clin Exp Immunol 1991;86:464-70.
90. Behr TM, Feucht HE, Richter K, et al. Detection of humoral rejection in human cardiac allografts by assessing the capillary deposition of complement fragment C4d in endomyocardial biopsies. J Heart Lung Transplant 1999;18:904-12.
91. Duong Van Huyen JP, Fornes P, Guillemain R, et al. Acute vascular humoral rejection in a sensitized cardiac graft recipient: diagnostic value of C4d immunofluorescence. Hum Pathol 2004;35:385-8.
92. Crespo-Leiro MG, Veiga-Barreiro A, Domenech N, et al. Humoral heart rejection (severe allograft dysfunction with no signs of cellular rejection or ischemia): incidence, management, and the value of C4d for diagnosis. Am J Transplant 2005;5:2560-4.
93. Michaels PJ, Fishbein MC, Colvin RB. Humoral rejection of human organ transplants. Springer Semin Immunopathol 2003;25:119-40.
94. Rodriguez ER, Skojec DV, et al. Antibody-mediated rejection in human cardiac allografts: evaluation of immunoglobulins and complement activation products C4d and C3d as markers. Am J Transplant 2005;5:2778-85.
95. Smith RN, Brousaides N, Grazette L, et al. C4d deposition in cardiac allografts correlates with alloantibody. J Heart Lung Transplant 2005;24:1202-10.
96. Lepin EJ, Zhang Q, Zhang X, et al. Phosphorylated S6 ribosomal protein: a novel biomarker of antibody-mediated rejection in heart allografts. Am J Transplant 2006;6:1560-71.
97. Hammond ME, Stehlik J, Snow G, et al. Utility of histologic parameters in screening for antibody-mediated rejection of the cardiac allograft: a study of 3,170 biopsies. J Heart Lung Transplant 2005;24:2015-21.
98. Kaczmarek I, Deutsch MA, Sadoni S, et al. Successful management of antibody-mediated cardiac allograft rejection with combined immunoadsorption and anti-CD20 monoclonal antibody treatment: case report and literature review. J Heart Lung Transplant 2007;26:511-5.
99. Baran DA, Lubitz S, Alvi S, et al. Refractory humoral cardiac allograft rejection successfully treated with a single dose of rituximab. Transplant Proc 2004;36:3164-6.

100. Keren A, Hayes HM, O'Driscoll G. Late humoral rejection in a cardiac transplant recipient treated with the anti-CD20 monoclonal antibody rituximab. Transplant Proc 2006;38:1520-2.
101. Deng MC, Eisen HJ, Mehra MR, et al. Noninvasive discrimination of rejection in cardiac allograft recipients using gene expression profiling. Am J Transplant 2006;6:150-60.
102. Starling RC, Pham M, Valantine H, et al. Molecular testing in the management of cardiac transplant recipients: initial clinical experience. J Heart Lung Transplant 2006;25:1389-95.
103. Marboe CC, Billingham M, Eisen H, et al. Nodular endocardial infiltrates (Quilty lesions) cause significant variability in diagnosis of ISHLT Grade 2 and 3A rejection in cardiac allograft recipients. J Heart Lung Transplant 2005;24:S219-26.
104. Marboe C, Deng MC, Billingham M, et al. Increased molecular testing scores associated with agreement among cardiac pathologists for the diagnosis of ISHLT 3A and higher rejection. J Heart Lung Transplant 2006;25:S105-6.
105. Dev S, Lloyd-Jones DM. Problems associated with screening for asymptomatic cardiac transplant rejection. J Heart Lung Transplant In press:In press.
106. Morgun A, Shulzhenko N, Perez-Diez A, et al. Molecular profiling improves diagnoses of rejection and infection in transplanted organs. Circ Res 2006;98:e74-83.
107. Kowalski R, Post D, Schneider MC, et al. Immune cell function testing: an adjunct to therapeutic drug monitoring in transplant patient management. Clin Transplant 2003;17:77-88.
108. Kowalski RJ, Post DR, Mannon RB, et al. Assessing relative risks of infection and rejection: a meta-analysis using an immune function assay. Transplantation 2006;82:663-8.
109. Rahmani M, Cruz RP, Granville DJ, McManus BM. Allograft vasculopathy versus atherosclerosis. Circ Res 2006;99:801-15.
110. Mehra MR. Contemporary concepts in prevention and treatment of cardiac allograft vasculopathy. Am J Transplant 2006;6:1248-56.
111. Valantine H. Cardiac allograft vasculopathy after heart transplantation: risk factors and management. J Heart Lung Transplant 2004;23:S187-93.
112. Kaczmarek I, Deutsch MA, Rohrer ME, et al. HLA-DR matching improves survival after heart transplantation: is it time to change allocation policies? J Heart Lung Transplant 2006;25:1057-62.
113. Costanzo-Nordin MR. Cardiac allograft vasculopathy: relationship with acute cellular rejection and histocompatibility. J Heart Lung Transplant 1992;11:S90-103.
114. Mehra MR, Ventura HO, Chambers R, et al. Predictive model to assess risk for cardiac allograft vasculopathy: an intravascular ultrasound study. J Am Coll Cardiol 1995;26:1537-44.
115. Stoica SC, Cafferty F, Pauriah M, et al. The cumulative effect of acute rejection on development of cardiac allograft vasculopathy. J Heart Lung Transplant 2006;25:420-5.
116. Uretsky BF, Murali S, Reddy PS, et al. Development of coronary artery disease in cardiac transplant patients receiving immunosuppressive therapy with cyclosporine and prednisone. Circulation 1987;76:827-34.
117. Kfoury AG, et al. Impact of repetitive episodes of antibody-mediated or cellular rejection on cardiovascular mortality in cardiac transplant recipients: defining rejection patterns. J Heart Lung Transplant 2006;25:1277-82.
118. Tambur AR, Pamboukian SV, Costanzo MR, et al. The presence of HLA-directed antibodies after heart transplantation is associated with poor allograft outcome. Transplantation 2005;80:1019-25.
119. Hosenpud JD, Edwards EB, Lin HM, Daily OP. Influence of HLA matching on thoracic transplant outcomes. An analysis from the UNOS/ISHLT Thoracic Registry. Circulation 1996;94:170-4.
120. Opelz G, Wujciak T. The influence of HLA compatibility on graft survival after heart transplantation. The Collaborative Transplant Study. N Engl J Med 1994;330:816-9.
121. Valantine H, Rickenbacker P, et al. Metabolic abnormalities characteristic of dysmetabolic syndrome predict the development of transplant coronary artery disease: a prospective study. Circulation 2001;103:2144-52.
122. Gao SZ, Hunt SA, Schroeder JS, Alderman EL, Hill IR, Stinson EB. Early development of accelerated graft coronary artery disease: risk factors and course. J Am Coll Cardiol 1996;28:673-9.
123. Grattan MT, Moreno-Cabral CE, Starnes VA, Oyer PE, Stinson EB, Shumway NE. Cytomegalovirus infection is associated with cardiac allograft rejection and atherosclerosis. JAMA 1989;261:3561-6.
124. Fateh-Moghadam S, Bocksch W, Wessely R, Jager G, Hetzer R, Gawaz M. Cytomegalovirus infection status predicts progression of heart-transplant vasculopathy. Transplantation 2003;76:1470-4.
125. Petrakopoulou P, Kubrich M, Pehlivanli S, et al. Cytomegalovirus infection in heart transplant recipients is associated with impaired endothelial function. Circulation 2004;110:II207-12.
126. Yamani MH, Erinc K, Starling RC, et al. Donor intracranial bleeding is associated with advanced transplant coronary vasculopathy: evidence from intravascular ultrasound. Transplant Proc 2004;36:2564-6.

127. Mehra MR, Uber PA, Ventura HO, Scott RL, Park MH. The impact of mode of donor brain death on cardiac allograft vasculopathy: an intravascular ultrasound study. J Am Coll Cardiol 2004;43:806-10.
128. Tuzcu EM, Kapadia SR, Sachar R, et al. Intravascular ultrasound evidence of angiographically silent progression in coronary atherosclerosis predicts long-term morbidity and mortality after cardiac transplantation. J Am Coll Cardiol 2005;45:1538-42.
129. Kobashigawa JA, Tobis JM, Starling RC, et al. Multicenter intravascular ultrasound validation study among heart transplant recipients: outcomes after five years. J Am Coll Cardiol 2005;45:1532-7.
130. Kobashigawa JA, Katznelson S, Laks H, et al. Effect of pravastatin on outcomes after cardiac transplantation. N Engl J Med 1995;333:621-7.
131. Kobashigawa JA, Moriguchi JD, Laks H, et al. Ten-year follow-up of a randomized trial of pravastatin in heart transplant patients. J Heart Lung Transplant 2005;24:1736-40.
132. Wenke K, Meiser B, Thiery J, et al. Simvastatin initiated early after heart transplantation: 8-year prospective experience. Circulation 2003;107:93-7.
133. Halle AA, 3rd, DiSciascio G, Massin EK, et al. Coronary angioplasty, atherectomy and bypass surgery in cardiac transplant recipients. J Am Coll Cardiol 1995;26:120-8.
134. Radovancevic B, McGiffin DC, Kobashigawa JA, et al. Retransplantation in 7,290 primary transplant patients: a 10-year multi-institutional study. J Heart Lung Transplant 2003;22:862-8.
135. Fishman JA, Emery V, Freeman R, et al. Cytomegalovirus in transplantation - challenging the status quo. Clin Transplant 2007;21:149-58.
136. Snydman DR. The case for cytomegalovirus prophylaxis in solid organ transplantation. Rev Med Virol 2006;16:289-95.
137. Devyatko E, Zuckermann A, Ruzicka M, et al. Pre-emptive treatment with oral valganciclovir in management of CMV infection after cardiac transplantation. J Heart Lung Transplant 2004;23:1277-82.
138. Singh N. Antiviral drugs for cytomegalovirus in transplant recipients: advantages of preemptive therapy. Rev Med Virol 2006;16:281-7.
139. Paya C, Humar A, Dominguez E, et al. Efficacy and safety of valganciclovir vs. oral ganciclovir for prevention of cytomegalovirus disease in solid organ transplant recipients. Am J Transplant 2004;4:611-20.
140. Zamora MR, Nicolls MR, Hodges TN, et al. Following universal prophylaxis with intravenous ganciclovir and cytomegalovirus immune globulin, valganciclovir is safe and effective for prevention of CMV infection following lung transplantation. Am J Transplant 2004;4:1635-42.
141. Ojo AO, Held PJ, Port FK, et al. Chronic renal failure after transplantation of a nonrenal organ. N Engl J Med 2003;349:931-40.
142. Habedank D, Ewert R, Hummel M, Wensel R, Hetzer R, Anker SD. Changes in exercise capacity, ventilation, and body weight following heart transplantation. Eur J Heart Fail 2007;9:310-6.
143. Osada N, Chaitman BR, Donohue TJ, Wolford TL, Stelken AM, Miller LW. Long-term cardiopulmonary exercise performance after heart transplantation. Am J Cardiol 1997;79:451-6.
144. Mancini D. Surgically denervated cardiac transplant. Rewired or permanently unplugged? Circulation 1997;96:6-8.
145. Taylor D, Edwards L, Boucek M, et al. Registry of the International Society for Heart and Lung Transplantation: Twenty-third Official Adult Heart Transplantation report - 2006, 2006.
146. Haykowsky M, Tymchak W. Superior athletic performance two decades after cardiac transplantation. N Engl J Med 2007;356:2007-8.
147. Stark RP, McGinn AL, Wilson RF. Chest pain in cardiac-transplant recipients. Evidence of sensory reinnervation after cardiac transplantation. N Engl J Med 1991;324:1791-4.
148. Bengel FM, Ueberfuhr P, Schiepel N, Nekolla SG, Reichart B, Schwaiger M. Effect of sympathetic reinnervation on cardiac performance after heart transplantation. N Engl J Med 2001;345:731-8.
149. Bengel FM, Ueberfuhr P, Hesse T, et al. Clinical determinants of ventricular sympathetic reinnervation after orthotopic heart transplantation. Circulation 2002;106:831-5.
150. Stevenson LW, Miller LW, Desvigne-Nickens P, et al. Left ventricular assist device as destination for patients undergoing intravenous inotropic therapy: a subset analysis from REMATCH (Randomized Evaluation of Mechanical Assistance in Treatment of Chronic Heart Failure). Circulation 2004;110:975-81.
151. US Transplant - Scientific Registry of Transplant Patients. 2006. http://www.ustransplant.org
152. Taylor DO, Brown RN, Jessup ML, et al. Progress in heart transplantation: Riskier patients yet better outcomes: A 15-year multi-institutional study. J Heart Lung Transplant 2007;26:S61.