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KIRAN Personalized Treatment in Heart Transplantation

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Abstract

Purpose of review—We are entering the era of personalized medicine, in which pharmacogenomics and biomarker-based assays can be used to tailor diagnostic tests and drug therapies to individual patients. This new approach to patient-specific care offers the potential to maximize the efficacy of available medical treatments while reducing the incidence of adverse side effects. Here we present approaches to personalize the care of heart transplant recipients.

Recent findings—Four strategies for personalized post-transplant care are described, including (1) use of pharmacogenomic data to individualize the use of immunosuppressive drugs, (2) immune monitoring to prevent acute rejection while reducing the long-term consequences of over-immunosuppression, (3) non-invasive surveillance for acute rejection, and (4) targeted prophylaxis against opportunistic infections.

Summary—The long-term survival of heart transplant recipients is limited by side effects of immunosuppressive drugs, including infectious complications, renal dysfunction, and malignancy. We discuss strategies to maximize the benefits of immunosuppressive and prophylactic therapies while minimizing their long-term toxicities.

Keywords

heart transplantation; pharmacogenomics; cell-free DNA; gene expression profiling; immune monitoring

Introduction

Over two thousand years ago, Hippocrates stated that “it is more important to know what person the disease has than what disease the person has”. Modern medicine is finally fulfilling this prophecy, with a renewed focus on personalized medicine—a novel model in which medical decisions and practices are tailored to individual patients. There is perhaps no field in medicine that stands to benefit more from personalized medicine than transplantation. In the case of heart transplantation, early mortality has decreased significantly in the past two decades(1); long-term survival has not improved appreciably, however, and late mortality is often attributed to side effects of immunosuppressive therapy, such as infectious complications, renal failure, and malignancy.(1) There is now substantial

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evidence that genomic variation in the host leads to differences in the immune response and susceptibility to adverse outcomes.(2) We know, for example, that most drugs are either ineffective or incompletely effective in 30–60% of patients (3, 4) which is of critical importance to transplant recipients, who are treated with a large number of medications to suppress the immune response and prevent post-transplant complications. The ability to tailor medical therapy offers the potential to increase treatment efficacy, improve long-term outcomes, reduce adverse events, and improve patient satisfaction and compliance. This article reviews recent advances in personalized therapies after heart transplantation.

Pharmacogenomic approaches to personalized post-transplant care

The medications used for maintenance of immunosuppression in heart transplant recipients include long-term use of a calcineurin inhibitor (tacrolimus or cyclosporine) in almost all cases, an antimetabolite (usually mycophenolate mofetil), and in some cases a proliferation signal inhibitor (sirolimus or everolimus). There is now substantial evidence for individual variability with respect to drug pharmacokinetics and side effects to these powerful and often toxic drugs. It is estimated that genetics can account for 20% to 95% of variability in drug disposition and side effects.(5)

Tacrolimus is inactivated in the liver by cytochrome P450 enzymes in the 3A family (CYP3A). Polymorphisms in the *CYP3A5* gene have been found to correlate with tacrolimus dose requirements. Patients with the *CYP3A5*1* polymorphism have lower tacrolimus trough levels despite higher doses.(6) This *CYP3A5*1* polymorphism is present in 65% of African-Americans, compared with 8% of non-African-Americans, which explains why most African-American patients require higher tacrolimus doses to achieve target trough levels, and may contribute to the increased risk of allograft rejection observed in this patient group. Similarly, tacrolimus is a substrate for the drug transporter p-glycoprotein, encoded by the gene *ABCB1*. Homozygosity for the C allele of the synonymous rs1045642 variant has been associated with lower serum tacrolimus levels.(7) In contrast to tacrolimus, studies linking genetic polymorphisms in CYP3A and P-glycoprotein to drug concentrations have not shown an association with cyclosporine dose requirement.(8, 9) Despite this potential advantage, use of cyclosporine is decreasing due to data indicating a higher incidence of acute rejection with cyclosporine, compared to tacrolimus.(10)

Rather than adopting a blanket protocol for use of a particular calcineurin inhibitor (usually tacrolimus)(1) for all patients, transplant centers may do well to tailor the selection of an individual agent based on the patient's medical history and rejection risk profile. For example, the incidence of hypertension and hyperlipidemia appears to be higher with cyclosporine than tacrolimus, while this drug confers a lower risk of new-onset diabetes mellitus, tremor, and gastrointestinal effects.(2) In addition, cyclosporine, unlike tacrolimus, may cause gingival hyperplasia and hirsutism, which may represent a significant barrier to its use.

Similarly, the side effects caused by mycophenolate mofetil (MMF), such as diarrhea and leukopenia, are common and lead to dose reduction or discontinuation of therapy in

approximately 15% of treated patients.(11) Genetic predisposition has been shown to influence MMF adverse events.(12, 13)

Given these examples, and others, it is increasingly clear that some of the disparity seen in transplant outcomes may be attributed to biological factors conferred by genetic differences. Compared to white recipients, for example, black recipients have a higher burden of genetic variants predisposing to inflammation, which may require more intensive maintenance immunosuppression.(2, 14) Use of predictive models that incorporate clinical and genetic risk factors to personalize therapy therefore hold particular promise for post-transplant care.

Post-Transplant Immune Monitoring

At present, immunosuppressive treatment is mainly protocol-driven, and is not selected on the basis of individual immune alloreactivity. Similarly, immunosuppressive drug doses are guided mainly by the achievement of target drug concentrations in peripheral blood and by the development of side effects. Blood levels of calcineurin inhibitors are measured; however, their primary value is for preventing drug toxicity, and not in determining the immune response state. MMF blood levels are not routinely assessed, and there is no reliable method to assess the immunosuppressive influence of corticosteroids on the individual patient. Furthermore, there is no tool that can accurately assess the additive effects of different medications on the overall immune response of an individual patient. Our current strategy may therefore lead to either under-immunosuppression, resulting in acute rejection, or over-immunosuppression, resulting in opportunistic infections, malignancy, renal dysfunction, and other toxicities. Thus, a comprehensive immune function assay is greatly needed for immune monitoring, regardless of the immunosuppressive regimen adopted.

Such an immune monitoring (IM) assay (ImmuKnow®; Viracor Eurofins) has been approved by the US Food and Drug Administration for quantification of cell-mediated immunity in immunosuppressed populations.(15) IM measures adenosine triphosphatase (ATP) release from activated lymphocytes and correlates with the overall level of immune responsiveness. In a prospective study, 296 heart transplant recipients had a total of 864 IM assays performed at 2 weeks to 10 years post-transplant that were correlated with infection and rejection events within one month after IM testing. There were 38 infections and 8 rejection events, and the mean IM score was significantly lower during infection than at steady state (187 vs 280 ng ATP/ml, $p<0.001$). The average IM score was not significantly different during rejection, when compared to steady state, perhaps due to the small number of rejection events. Overall, IM monitoring offers several advantages over other measures of immune function. For example, although IM scores can predict the risk for developing infection in transplant recipients, there was little correlation between IM scores and immunosuppressive drug levels, due to the fact that each patient has their own unique response to immunosuppression. The same tacrolimus level of 8 ng/ml in two heart transplant recipients might reflect IM scores of 200 and 400 ng ATP/ml, respectively, illustrating the patients' different immune responses to the drug.

Another novel approach to assessment of the overall immune response is measurement of torque teno virus (TTV) load, quantified via sequencing of cell-free DNA from the blood of transplant recipients. A recent study quantified the relative genomic abundance of viral DNA

fragments in the plasma of heart and lung transplant patients.(16*) By far, the most abundant component of the plasma virome consisted of Torque Teno viruses, members of the *Anelloviridae* family. TTV infections are nearly ubiquitous in humans, with primary infection early in childhood.(17) Infection is usually asymptomatic and does not result in any known, clinically-significant disease. For both heart and lung transplants, a striking positive relationship was observed between the abundance of TTV sequences in plasma and overall level of immunosuppression: TTV viral loads increased dramatically during the first 6 months post-transplant and subsequently declined with weaning of immunosuppressive therapy. This observation raises the question of whether TTV load can be used to gauge immunocompetence. Consistent with this hypothesis are recent studies demonstrating the association between TTV viral loads and rejection (16*, 18*) (Figure 1) as well as opportunistic infection.(19)

More recently, a proof-of-concept study was performed in which a molecular barcode-based immune repertoire sequencing assay that sensitively and accurately measures the isotype and clonal composition of the B-cell antibody repertoire was used to monitor pharmacologically induced immunosuppression in a small cohort of heart transplant recipients.(18*) The antibody heavy chain IGH transcript is unique as its expression changes in abundance and in sequence when a B cell is activated. Activated B cells express high levels of mutated antibodies of the IgG and IgA isotypes, while naïve B cells express non-mutated IgM antibodies at low levels. A lower abundance of class-switched sequences indicates a lower number of activated B cells and, consequently, lower activity of the adaptive immune response. After analysis of 130 plasma samples from 12 patients, with an average follow-up period of 15 months, Vollmers et al. concluded that immune repertoire sequencing enables the measurement of a patient's net state of immunosuppression (correlation with tacrolimus level, $r=-0.87$) as well as the diagnosis of acute allograft rejection, which is preceded by increased immune activity, with a sensitivity of 71% and a specificity of 82%.

These studies illustrate the use of novel assays for assessment of the aggregate impact of multiple immunosuppressive medications. Such knowledge will enable assessment of an individual patient's immune response, in order to better predict the risk of acute rejection, opportunistic infection, or other drug toxicities, and will pave the way toward personalized immunosuppressive therapies.

Non-Invasive Assessment of Acute Rejection

Enhanced detection of acute rejection through non-invasive means has the potential to dramatically improve post-transplant care, both by increasing patient satisfaction and by avoiding invasive procedures.(20) A major step in the development of a noninvasive test for ACR was the multicenter CARGO study (21*) which used iterative validation steps involving hundreds of patient blood samples and corresponding biopsies to identify 11 genes expressed by peripheral blood mononuclear cells that reliably distinguished ACR from immunologic quiescence. The assay employs an algorithm that yields an expression score between 0 and 40, with higher scores (> 34) indicating a higher likelihood of acute rejection. This gene expression test, known commercially as the AlloMap[®] Molecular Expression test (CareDx, CA, USA) became available for clinical use in January 2005 for use in stable

patients who were at least 2 months post-transplant. Subsequently, the IMAGE clinical trial was launched to evaluate patient outcomes using the AlloMap[®] test as the primary means of ACR surveillance.(22**) In this multi-center study, 602 patients were randomized to surveillance using AlloMap[®] or endomyocardial biopsy, with a primary outcome of first occurrence of rejection with hemodynamic compromise, graft dysfunction, death or retransplantation. The trial investigators found that the incidence of the primary outcome was similar in the AlloMap[®] and biopsy groups (14.5% and 15.3%, respectively). Notably, the AlloMap[®] group had 67% fewer biopsies and higher patient satisfaction. Since publication of IMAGE, many heart transplant centers in the United States have adopted AlloMap[®] testing as their primary means of ACR surveillance. However, significant criticisms of the AlloMap[®] assay remain, including its low positive predictive value and the fact that it is contraindicated for use in patients deemed to be at high risk of adverse events.

Since publication of IMAGE, there has been interest in the use of within-patient variability of AlloMap scores to predict future clinical events and thereby personalize post-transplant care. The “AlloMap score variability (AMV)” was defined as the standard deviation of four AlloMap scores collected 315 days post-transplant. In a retrospective analysis of the CARGO II trial,(23) the negative predictive value for an adverse event (death, retransplant, or graft failure) with an AMV score of 0.6 was 97% (95 % CI 91.4–100.0), while the positive predictive value for an AMV score of 1.5 was 35.4% (95 % CI 13.5–75.8).(23) Use of the AMV assay for individualized care has not been widely adopted, however, due to the need for at least four AlloMap scores collected 315 days post-transplant, at a time when routine acute rejection surveillance is performed infrequently, and the fact that there is a long lag time between the calculation of an AMV score and a potential clinical event.

More recently, cell-free DNA has emerged as a promising means to interrogate the health of the allograft, thereby providing a direct, sensitive means to assess for allograft injury. In 2011, a multi-disciplinary group at Stanford University introduced a method to quantify donor-derived cell-free DNA (dd-cfDNA) in the circulation of a transplant recipient. This approach takes advantage of single-nucleotide polymorphisms (SNPs) distributed across the genome to discriminate donor and recipient DNA molecules. The concept was first successfully demonstrated in a retrospective study in heart transplantation, where increased levels of donor-derived DNA were shown to correlate with acute cellular rejection (ACR) events as determined by endomyocardial biopsy.(24)

The utility of dd-cfDNA in monitoring acute rejection was subsequently tested in a prospective study performed at Stanford in 65 *de novo* heart transplant recipients.(25*) Pre-transplant SNP genotyping and post-transplant shotgun sequencing of cfDNA were used to quantify donor and recipient specific SNPs. The investigators demonstrated that dd-cfDNA is markedly elevated on post-operative day 1 (up to 10%), reflecting early ischemia-reperfusion injury after transplant surgery, and subsequently falls to a very low baseline level (<0.1%) within several days, whereupon this low baseline is maintained in the absence of rejection. Comparison to endomyocardial biopsy showed that dd-cfDNA was significantly elevated at the time of acute rejection (Figure 2). Analysis of the performance of dd-cfDNA in distinguishing moderate-to-severe rejection from quiescence yielded an Area Under the Curve (AUC) of 0.83 (sensitivity of 58%, specificity of 93% at a threshold of 0.25%). This

study also demonstrated the potential for early diagnosis of rejection via cfdDNA monitoring: the level of cfdDNA was significantly elevated several weeks to months prior to rejection diagnosis by biopsy.

Of great importance in the era of personalized medicine is the fact that dd-cfDNA is a quantitative marker that can be trended over time.(26) This feature may be used to guide therapeutic decisions. For example, the level of dd-cfDNA may be used to inform medication dose titration. In the case of acute rejection, immunosuppression may be augmented when initial signs of graft injury appear, indicated by an early rise in dd-cfDNA levels, thereby potentially preventing the occurrence of a clinical rejection event. Furthermore, close monitoring of dd-cfDNA during immunosuppressive dose reduction may enable safe weaning of therapy in stable patients, thereby preventing the consequences of long-term patient over-immunosuppression. Finally, dd-cfDNA may be used to assess the response to treatment for acute rejection, thus enabling early assessment of treatment efficacy and reducing the need for follow-up biopsies.

Significant progress has already been made with respect to cell-free DNA testing, due to widespread interest in this technique: alternative measurements of dd-cfDNA based on targeted sequencing(27, 28) and digital PCR(29) have been introduced that offer the promise of lower assay cost and faster turnaround time. CareDx, Inc. has developed AlloSure®—a dd-cfDNA assay that uses a panel of 266 highly polymorphic SNPs to distinguish donor from recipient cell-free DNA without requiring whole genome sequencing. The performance of this assay in the clinical setting is currently being tested in 23 heart transplant centers in the United States in a CareDx-sponsored registry study.

Personalized Prophylaxis against Opportunistic Infections

Spanish heart transplant centers have led the way in developing protocols for targeted opportunistic infection (OI) prophylaxis. This approach is important due to the relatively low incidence of several OIs, drug-related toxicities and interactions, high medication costs, and potential emergence of drug resistance. In a prospective cohort study, Muñoz et al selectively administered antifungal prophylaxis to only 10% of their *de novo* heart transplant recipients, based on the presence of risk factors for invasive aspergillosis (IA): re-operation, post-transplant hemodialysis, cytomegalovirus (CMV) disease, and a case of IA in their program within the prior 2 months. This group demonstrated high efficacy and safety of targeted prophylaxis, with a reduction in the incidence of IA and Aspergillus-related mortality.(30) Another Spanish center retrospectively analyzed their clinical outcomes using a preemptive strategy of routine CMV monitoring followed by initiation of antiviral therapy when viral replication was first detected, rather than universal anti-CMV prophylaxis, and reported a low incidence of CMV disease (4.6%) in their patient cohort.(31) While there are no data available from randomized clinical trials to support the use of preemptive therapy or universal prophylaxis, studies such as this suggest that individualized prevention strategies may play an important role in the care of heart transplant recipients.

Conclusion

In the 19th century, Sir William Osler (1849–1919) of Johns Hopkins Hospital (Baltimore, MD), dedicated his life's work toward making the correct diagnosis, and the right choice and dose of drugs to benefit the patient as an individual. As he famously said, "If it were not for the great variability among individuals, medicine might well have been a science and not an art".(32) Unfortunately, our current healthcare model continues to be expensive, reactive, inefficient, and focuses largely on "one-size-fits-all" treatments. Adopting therapeutic plans that account for pharmacogenomics as well as individual patient risk factors for adverse events (such as age,(33) sex, degree of pre-transplant sensitization, and medical co-morbidities) will enable us to derive the maximum benefit from available therapies while avoiding their adverse consequences. Moreover, research in the "-omic" sciences has greatly improved understanding of the relationships between genes, proteins, and disease, providing important tools for personalized medicine. This collection of biologic information, as applied to understanding transplantation, has been coined "transplantomics"(34) and offers the potential to select optimal therapies, prescribe safer dosing options, improve patient safety, reduce inappropriate testing and procedures, decrease health care costs, and most importantly, increase patient awareness and empowerment, with the ultimate goal of providing personalized post-transplant care.

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Abbreviations

MMF	mycophenolate mofetil
ISHLT	International Society for Heart and Lung Transplantation
AR	acute rejection
IM	immune monitoring
TTV	torque teno virus
dd-cfDNA	donor-derived cell-free deoxyribonucleic acid
IGH	immunoglobulin H
CARGO	Cardiac Allograft Rejection Gene Expression Observational study
IMAGE	Invasive Monitoring Attenuation by Gene Expression Profiling
AMV	AlloMap [®] variability

AUC	area under the curve
OI	opportunistic infection
IA	invasive aspergillosis
CMV	cytomegalovirus

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Key points

1. Clinical and genetic risk factors can be used to select the optimal immunosuppressive drugs for an individual patient, rather than using a protocol-driven approach.
2. Assays such as ImmuKnow®, Torque Teno Virus load, and B-cell antibody repertoire sequencing can be used to measure the net state of immunosuppression in a given patient.
3. Gene expression profiling and measurement of donor-derived cell-free DNA can be used for non-invasive acute rejection surveillance.
4. Targeted prophylaxis against opportunistic infections can prevent the toxicities and drug resistance associated with universal use of antimicrobial agents.

Figure 1. Lower anellovirus load in heart transplant recipients with acute rejection

Time dependence of anellovirus load in the subgroup of patients who experience a moderate-severe acute cellular rejection event (biopsy grade 2R/3A, red data) and in the subgroup of patients with a rejection-free post-transplant course (biopsy grades <2R/3A, blue data). The inset shows the inverse relationship between the level of immunosuppression and the incidence of rejection and infection.

Source: De Vlaminc I, et al. Temporal Response of the Human Virome to Immunosuppression and Antiviral Therapy, *Cell*, 2013 November 21; 155(5) 1178–1187.

Figure 5A from Cell paper

Figure 2. Performance of donor-derived cell-free DNA as a marker for heart transplant rejection

Box plots of the fraction of donor-derived cell-free DNA for stable heart transplant recipients (biopsy grade 0), recipients diagnosed with mild rejection (1R/1A grade < 2R/3A), and recipients diagnosed with moderate-to-severe rejection (grade ≥ 2R/3A or AMR)

Source: De Vlaminc, I, et al. Circulating Cell-Free DNA Enables Noninvasive Diagnosis of Heart Transplant Rejection, *Science Translational Medicine*, 2014 June 18; 6(241) 241ra77.

Figure 5A from Science Translational Medicine paper