Immunologic Risk Prediction Model for Kidney Graft Function

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Christina Diane Bishop

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ABSTRACT

Clinicians lack appropriate non-invasive methods to be able to predict, diagnose, and reduce the risk of rejection in the years following kidney transplantation. Protocol biopsies and monitoring of serum creatinine levels are the most common methods of monitoring graft function after transplant; however, they have several negative aspects. Use of traditional factors regarding donors and recipients such as Human Leukocyte Antigen (HLA) DNA typing, pre-transplant anti-HLA antibody levels, and basic demographics (age, ethnicity/race, gender), has proved inadequate for post-transplant graft monitoring past the first few years. We propose that by utilizing immunologic factors available to clinicians across the United States, development of a non-invasive model for predicting renal graft outcome will provide a useful tool for post-transplant patient monitoring. We advocate an expanded model which incorporates both the traditional factors, as well as new factors, which have shown promise in predicting kidney outcome and are widely available for testing using commercial kits. These additional factors include major histocompatibility complex class I chain-related gene A (MICA) typing of donor and recipient, degree of matching for killer cell immunoglobulin-like receptors (KIRs) between donor and recipient, detection of MICA antibodies, and soluble CD30 level (sCD30). This proposed graft-function prediction model is the first to include all of these factors.

Using multi-center data from adult recipients of standard-criteria deceased-donor (SCD) kidneys, we were able to construct models, containing the traditional factors only, for prediction of outcome at 1 year and 3 years post-transplant. Using single-center data from adult recipients of standard-criteria deceased-donor kidneys, we developed comparison models containing traditional factors only, as well as, expanded models containing the new suggested variables for prediction of outcome post-transplant. These additional variables, when incorporated into the expanded models provided greater positive predictive values, greater negative predictive values, and lower false negative rates for graft outcome at 1 year and at 3 years post-transplant than the

models utilizing traditional factors only. Our results indicate that evaluation of sCD30, MICA and KIR as part of routine protocol testing, is helpful to clinicians for predicting risk of kidney graft rejection.

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CHAPTER I: INTRODUCTION

The current need for donated human organs for transplantation greatly exceeds the available supply. This unfortunate reality drives both legislation and research focused on finding a solution. Despite efforts to increase access to kidney transplantation, thousands of patients remain on waiting lists around the United States. According to the 2009 Organ Procurement and Transplantation Network (OPTN) and the Scientific Registry of Transplant Recipients (SRTR) Annual Report, the total number of candidates on the wait-list at the end of the year for a kidney transplant has risen by 86% in the last ten years, from 43,632 (1999) to 80,972 (2008).[1] Laws passed to regulate transplantable organs and to improve the national system, both ethically and medically, aim to provide equitable access for kidney transplant candidates to deceased donor kidneys and improve the outcomes of these transplants. As a result of these laws and regulations, several utility-based kidney allocation systems have been proposed which include various concepts such as Life Years From Transplant (LYFT) as a measure of transplant utility, Donor Profile Index (DPI) as a measure of donor quality, dialysis time as a measure of equity, and the Kidney Allocation Score (KAS) as an attempt to balance equity and utility. However, these measures have not assisted the transplant community in solving the original problem: available kidneys are a scarce resource.

Available Organs/ Deceased Donors

While the wait-list continues to grow, the lack of available organs is proving difficult to overcome. In 2008, there were only 10,101 kidney transplants from deceased donors (a +0.2% change from 2007) and 5,966 kidney transplants from living donors (-1.2% change from 2007).[1] To put this in perspective, one must consider that if no new candidates were added to the wait-list and that approximately 16,000 kidneys become available in a year's time (based on 2008 data), then it would take just over 5 years to

transplant all the candidates that existed on the wait-list in 2008. Of course, new patients will continue to be added to the list and the gap between candidates on the list and number of transplants will continue to widen, just as it has over the last decade.

There are several potential reasons for the shortage of organs.

- Perhaps the most common reason is that people are hesitant to donate organs. Often people are fearful that if they sign an organ donor card then emergency medical services (EMS) will not “try as hard”. They envision a medical technician or emergency room doctor throwing their organs into a cooler and rushing off to a waiting helicopter. Organs are not collected roadside or stolen from potential donors lying in a bathtub of ice. These irrational fears are caused by works of fiction and exacerbated by circulating urban legends.
- Physicians and nurses may not ask family members whether they would consent to donating organs when their loved one dies.
- Some caregivers, especially in rural areas, may not know how to contact an organ procurement organization (OPO).
- In other cases, the deceased's wishes to donate his or her organs may not be known by those in the position to act on those wishes.
- Family members may object to the procurement of organs from their deceased loved one, regardless of the deceased's wishes to the contrary.

If the pool of available organs from deceased donors is not expanded, then other sources must be explored. Current research is focused on two main goals: Increasing the availability of organs (e.g. xenotransplantation, social programs) or improving long-term survival rates of transplanted organs. Currently, the one year, unadjusted, national kidney graft survival rate is 91.0% for deceased donors (DD) and is 80.1% for deceased donor kidney allografts at three years.[1] Despite more powerful immunosuppressive drugs, chronic rejection continues to be a problem as the five and ten year graft survival rates drastically drop to 69.3% and 43.3% respectively.[1] The following sections

provide an overview of contemporary approaches for increasing the number of available kidney allografts and improving graft survival.

Available Organs/ Living Donors

One of the most successful strategies to reduce waiting time and improve post-transplant outcome is utilization of available living donor (LD) kidneys for transplantation. Kidneys from LDs have several advantages over their DD counterparts, including reduced waiting times, shorter and less expensive hospital stays, greater opportunity for pre-emptive transplantation, and improved post-transplant outcome. [2,3,4] Unfortunately, there has been a steady decrease in the use of LD kidney transplantation since 2004. There are various factors, which could be responsible for this decline.

- Economy--Changes in economic factors may increase reluctance to donate. If a donor is not financially sound, adequately covered by insurance, has a fastidious employer, or is unemployed, they might not feel secure in electing to undergo a non-emergency surgery.
- Older recipient population--The recipient population as it ages may have a more limited number of potential donors. As the potential transplant candidate ages, so does their friends and family. It is often more difficult for a 65 year old to obtain a LD than it is a 15 year old.
- Decline in emphasis on recruitment.
- Changes in donor/ recipient relationships--There has been a shift in the relationship of living donors and their recipients since 1999, which may be partially attributed to improved access to deceased donor kidneys for pediatric patients. The most common relationships in 1999 were full sibling (35%), followed by parent (17%) and offspring (16%).[1] However, in 2008 unrelated, non-spousal relationships such as friends, in-laws, or anonymous donors were

most common (26%), followed by full sibling (24%) and offspring (17%).[1] Parents only accounted for 10 percent of living kidney donors in 2008.

- Part of this increase in unrelated donors may be a result of the emergence of kidney chains in the last few years. Kidney chains are a product of kidney paired donation (KPD) programs, where two or more incompatible pairs exchange donors such that compatible transplants result.[5,6,7]
- These multiple links of recipient-donor pairs may span across the country, through several transplant centers. This has been made possible by mathematical optimization[8] and by replacing donor travel with organ transport.[9,10]

Efforts must continue to increase the feasibility of kidney transplantation by LD. The transplant community continues to focus on strategies to improve access to living donation for recipients of all races, ethnicities, and socioeconomic status.[11] Areas of current interest include transplantation across immunological incompatibility (e.g. positive-crossmatch (posXM) desensitization),[12,13] ABO incompatible (ABOi) transplantation,[14,15,16,17] use of laparoscopic donor nephrectomy to reduce donor recovery time, and a development of a national program offering economic support to donors.

Immunosuppressant Drugs

With limited donor populations, the transplant community is forced to explore alternate means of improving both availability and usage of kidneys. The long-term survival rate of transplanted organs desperately needs to be improved. The survival rate for a kidney transplant drastically drops after 5 years. While immunosuppressive drugs have improved over the last decade, their role in transplant success still comes with drawbacks. The drugs used to maintain allograft survival are not specific for the graft; they broadly suppress the immune system. While these drugs have allowed for excellent short term graft survival, they do not seem to have much effect on long-term

survival and often come with serious complications. Current immunosuppressive treatment does not always prevent acute and chronic rejection, and often has serious side effects that can lead to other complications, even increased mortality rates. Immunosuppressive drugs also have side effects that can compound the problem of kidney disease (e.g. cyclosporine [CsA] nephrotoxicity).[18] It is now widely accepted that patients would benefit from reduction of immunosuppression provided that graft rejection is prevented; but careful monitoring is essential.

Tolerance Induction

While pharmaceutical companies attempt to improve immunosuppressant drug treatments, other possible pathways to improved long-term transplantation are being explored. One of the major goals in transplantation is the induction of tolerance (i.e. indefinite allograft survival without the need for continued immunosuppression by medications). It is possible that induction of graft tolerance, by other mechanisms which are “more natural” to the host, may decrease the need for immunosuppressant drugs. Tolerance is specific in nature, inhibiting lymphocytes responsible for the rejection of the organ, but allowing the immune system as a whole to function normally. Central tolerance results from thymic deletion of T cells with high avidity for thymically expressed antigens. Regulatory T cells (Tregs) have been identified to play a critical role in the control of transplant tolerance. [19] Tregs suppress the proliferation and IL-2 production of cells in a dose-dependent fashion. Tregs constitutively express the high affinity IL-2 receptor, and therefore, have the ability to outcompete other cells for secreted IL-2.[20] Without stimulation by IL-2, lymphocytes do not undergo growth and differentiation. By defining the stimuli that determine the balance of effector and regulatory T cells, it may be possible to reduce the effect of IL-2 on development of pathogenic effector cells.[21]

Studies have also shown that dendritic cells (DCs) have a key role in the immune response and are required for full tolerance induction.[22] The principal function of

dendritic cells is to present antigens, but they also play a role in central tolerance. Only dendritic cells are capable of inducing a primary immune response in resting naïve T lymphocytes. Dendritic cells express various receptors (e.g. ILT3) involved in immune regulation of antigen presenting cells (APCs) and Tregs. Dendritic cells are abundantly found in the thymus, where they contribute to the negative selection of T cells; T cells that respond to DCs carrying self-peptides are destroyed. Dendritic cells could contribute to peripheral tolerance by inducing apoptosis of T cells with high avidity for certain MHC/peptides, by inducing anergy in T cells with low avidity for certain MHC/peptides (antigen-specific tolerance), and/or by producing IL-10 (which stimulates Tregs). It is not surprising that DC-based therapies for inducing tolerance are of foremost interest. Tolerance induction, using DCs primed with graft MHC/peptides, could prevent organ rejection and thus make possible the increase of available organs by decreasing the number of re-transplants. By inducing cell-specific or antigen-specific tolerance, it may be possible to reduce or eliminate the use of harsh immunosuppressant drugs (operational tolerance).

Hypotheses and Theoretical Framework

Until a broader understanding of tolerance mechanisms and improved immunosuppressant drug protocols are available, other solutions to the lack of available kidneys must be explored. As the ethical and statistical debates continue regarding utility-based kidney allocation, we approached the issue from the opposite angle. Rather than focusing on allocation and direct expansion of the donor pool, we concentrated on development of a post-transplant monitoring tool and retention of transplanted organs, as an indirect mechanism for increasing the number of available kidneys. Maximizing the life of kidney allografts post-transplantation is one way of reducing the strain on limited resources. If a transplanted patient looses their first kidney allograft, they are more often than not relisted for a subsequent transplant. The first graft is wasted, and now that patient stands to be transplanted with a second kidney

that could have been allocated to someone else. Through careful post-transplant monitoring perhaps that patient would have retained their allograft and there would not have been need for a second graft transplant, allowing the opportunity for others to receive a kidney.

An immunological risk scoring system would allow surgeons and nephrologists to predict graft loss earlier, possibly avoiding further renal damage and subsequent graft loss, while potentially allowing for reduction of immunosuppression. Unfortunately, when acute rejection (AR) is suspected, based on lab test results (i.e. elevated serum creatinine), damage to the kidney allograft is nearly established. A definite diagnosis can only currently be made by renal biopsy, an invasive procedure that is often associated with severe complications.[23] Even though improved immunosuppressant protocols have reduced the rate of AR, AR is still a major cause of graft dysfunction and later loss.[24] We lack appropriate non-invasive methods to be able to predict, diagnose, and reduce risk of rejection during the first year of kidney transplantation.[25,26] Utilizing immunologic factors that are available to clinicians across the United States, we developed a model to predict graft function at 1 year and at 3 years post-transplant. These models include the traditional factors: the degree of matching of human leukocyte antigen (HLA) typing for each donor and recipient pair, pre-transplant anti-HLA antibody levels, and demographics including ethnicity, gender, and age. We also develop an expanded model that includes the factors listed above, as well as additional factors, which we deem advantageous to more precise prediction of kidney graft outcome at 1 year and at 3 years. These supplementary factors include: degree of matching of major histocompatibility complex class I chain-related gene A (MICA) between donor and recipient, degree of matching for killer cell immunoglobulin-like receptor (KIR) typing between donor and recipient, identification of MICA antibodies, and sCD30 level. Testing kits for these additional factors are available through multiple vendors, and are simple to use. The hypothesis is that incorporating these additional tests into routine testing for donors and recipients will allow clinicians to use the expanded scoring models as a post-transplant tool. This tool will predict risk of kidney graft outcome and allow for monitoring of changes in immunosuppressant

therapies, more accurately, less invasively, and more timely than the current methods. This model is one of very few available non-invasive post-transplant monitoring tools, and to our knowledge, this is the first time a single model has been proposed utilizing MICA, KIR, sCD30, and traditional HLA testing on the same individual study population.

CHAPTER II: REVIEW OF LITERATURE

In order to develop a model for predicting risk of graft function, one must understand the principal mechanisms of kidney rejection. While many aspects of the human immune system continue to be a mystery, there are immunologic factors which have been proven through years of research to be key players in the process of kidney transplant rejection. For each recipient and potential donor, these conventional factors are assessed during evaluations which include laboratory testing and collection of patient history. This chapter seeks to outline the established central factors affecting renal transplant immunology, as well as, elucidate several supplementary tests, which may have a correlation with kidney graft outcomes.

Conventional Elements of Renal Transplantation

Major Histocompatibility Complex (MHC) —Human Leukocyte Antigens (HLA)

Transplanted organs express two types of alloantigens: major and minor. Major alloantigens include MHC class I and class II molecules.[27,28] Minor histocompatibility antigens (mH-Ags) are peptides, derived from polymorphic proteins, and indirectly presented to host T cells by MHC molecules.[29] These peptide/MHC complexes are recognized by host T-cells as “foreign” antigens, and activate a T-cell response, which may destroy the graft.[30] The activation of T cells by mH-Ags has been implicated in bone marrow (BM) and hematopoietic stem cell (HSC) transplants as a cause of graft-versus-host (GvH) disease.[31,32] Activation of T cells by mH-Ags does not result in a significant immunologic attack against kidney allografts, and therefore, identification of mH-Ags is not performed during routine testing of transplant patients and donors.[31]

The major MHC antigens, however, were first discovered because of the fundamental role they play in transplant rejection. MHC molecules, referred to as human leukocyte antigens (HLA) in humans, are routinely identified by DNA testing in both transplant recipients and donors. Whenever possible, recipients with a certain HLA type are matched with a potential donor of the same HLA type, because despite advances in immunosuppressant therapy, HLA matching continues to have a considerable effect on graft survival.[33] The number of disparities in HLA types between recipients and donors (referred to as mismatches) has been shown to be a risk factor for first-year acute rejection, and that these first-year acute rejection episodes are associated with decreased graft survival at 5 years after transplantation (n = 208).[34]

The polymorphic genes that encode the MHC are found on chromosome 6 in humans and are split into two classes: MHC class I (HLA-A, -B, -C) and MHC class II (HLA-DR, -DQ, -DP). The primary function of these antigens is to serve as recognition molecules in the initiation of an immune response. HLA antigens on specialized immune cells present peptides to effector cells, which set into motion the cellular and humoral arms of the immune response. The branch of the immune response activated depends upon the class of the presenting MHC molecule.

MHC class I molecules are found on all nucleated cells and act as indicators of the cell's health. MHC class I molecules bind peptides generated through an endogenous pathway, drawing their peptides from within the host cell. If a cell is genetically deficient (mutant) or infected (e.g. virus), it does not generate its normal assortment of peptides (including self-peptides).[27,28] MHC class I interaction with effector cells initiates the cellular immune response. When there is a reduced cell surface amount of self MHC class I molecules, or a foreign MHC/peptide complex is displayed on the cell surface, the affected cell becomes "flagged" as defective (the "missing-self" hypothesis).[35] CD8+ cytotoxic T cells (CTLs) and natural killer (NK) cells are able recognize different levels of MHC class I expression and foreign MHC/peptide complexes via their receptors.[36,37] If CTLs and NK cells do not recognize the appropriate level of self MHC class I on a cell's surface, they initiate destruction of that cell via apoptosis.

MHC class II molecules are found on antigen presenting cells (APCs) and bind peptides generated through an exogenous pathway. APCs continuously sample the extracellular environment by an endocytic process. External macromolecules are taken into an endosomal or lysosomal compartment where they are degraded. The resulting peptide fragments are co-assembled with MHC class II molecules and then delivered to the cell surface for expression and interaction with CD4+ T helper cells, which activates the humoral immune response.[36,37] The humoral response is a result of cytokine secretion by type 1 T helper cells (IL-2, IFN-γ, and TNF-α) and type 2 T helper cells (IL-4, IL-5, IL-15, and TGF-β). These cytokines drive proliferation and differentiation of T and B lymphocytes, including B cell isotype switching which results in production of antibodies that mediate opsonization, complement fixation, and Antibody-mediated cell-mediated cytotoxicity (ADCC).

MHC is the driving force behind the ability of the immune system to detect genetic polymorphisms between members of the same species in a process referred to as allorecognition.[38] In renal transplantation, there are two main mechanisms of allorecognition, direct and indirect. In direct allorecognition, host T cells interact with donor peptides presented by donor APCs. This occurs when donor “passenger leukocytes” migrate out of the graft to the host’s lymphoid tissues. The direct pathway leads to the early sensitization of host to donor antigens, which can lead to acute rejection.[39] This response diminishes over time regardless of whether or not chronic rejection occurs.[40] Indirect allorecognition occurs when host APCs migrate into the graft, take in and process donor antigens, and present these processed donor peptides to host T cells.[41,42] In contrast to direct allorecognition, the response generated by the indirect pathway can persist and has been correlated with chronic rejection.[40]

With regard to transplantation, HLA antigens play a major role in the rejection of foreign tissue. Before the recent era of new immunosuppressive drugs, patients who were well matched for HLA loci had better graft survival than recipients who were not well matched.[43,44] The HLA system is the most polymorphic system in the human body and not all antigens are represented with the same frequency in all ethnic groups.

Some antigens only occur in certain ethnic groups and have very low frequency within a given population. Patients with rare or uncommon HLA phenotypes are at a disadvantage in regards to finding a good match.[45,46] New and more powerful immunosuppressive treatments have increased the survival for poorly matched recipients, and driven organ allocation in the United States away from 'matching' and more towards equality in allocation and overall survival benefit.[46] However, mismatches still present a risk for development of antibodies and subsequent graft rejection. How well matched an organ is for its recipient should be considered when altering immunosuppressant regimens.

Panel Reactive Antibodies (PRA)

The percent PRA value is a measure of a patient's level of sensitization to donor antigens. It is the percentage of cells from a panel of blood donors against which a potential recipient's serum reacts. The PRA reflects the percentage of the general population that a potential recipient makes antibodies (is sensitized) against. The higher the PRA, the more sensitized a patient is to the general donor pool, and thus the more difficult it is to find a suitable donor. Antibodies against HLA antigens are not naturally occurring and arise following "sensitizing events," such as pregnancy, transfusion, or previous transplantation. Finding an acceptable graft for a patient who is highly sensitized depends on correctly identifying all HLA antibodies present in patient's sera. A potential allograft recipient with a high percentage (>80%) of preexisting antibody against HLA antigens (PRA) is often at a higher risk of a positive crossmatch, which implies a high risk of rejection.[47] Since crossmatching was established as a pre-transplant screening method, hyperacute rejections have been almost eliminated.[48,49]

There is a lack of standardization in regards to PRA levels between laboratories, due to the diverse methodologies currently performed by the individual HLA laboratories. Despite the increased sensitivity of the current methods for detecting PRA, there are

some HLA antigens and alleles not represented in the panels used by labs. Many labs run a battery of different panels in an attempt to avoid overlooking any donor specific antibodies (DSA) present. As the sensitivity of testing increases, so does the debate regarding the clinical significance of the antibodies detected. Antibodies detected using the most sensitive techniques (i.e. solid-phase single-antigen testing), may not be detected by other methods (i.e. antibody screens or multi-antigen phenotypes) and may not always result in a positive crossmatch. Which leads to the question: Are these antibodies clinically significant? In other words, are these antibodies "strong" (immunogenic) enough to initiate a rejection response? How each lab interprets their testing panels, in response to this question, determines what PRA is reported to the transplant center. Thus, achieving standardization between labs continues to be a goal among several histocompatibility societies.

Antibodies against donor HLA antigens are considered contraindicative to transplantation, and the antigens they react against are called "unacceptable antigens." These unacceptable antigens are listed for each recipient on the national waitlist. The United Network for Organ Sharing (UNOS), the organization that manages the United State's organ transplant system, implemented new policies in 2007 which deemed that PRA percentages be calculated based on antigens that are assigned as unacceptable in the UNOS database system and the frequencies of those antigens in the donor population.[50,51] The calculated PRA computes the PRA percentage using both anti-HLA class I and class II antibody specificities, assigned to each patient as unacceptable, and knowledge of the frequency of the assigned unacceptable HLA antigens in a representative population. This has alleviated some of the problems in standardization of PRA reporting.

While acute rejection (AR) of kidney allografts primarily occurs through a T cell mediated mechanism, several studies have shown that antibodies are involved in some acute and possibly chronic rejections.[52,53] Antibody-mediated acute rejection (AMR), also called acute humoral rejection (AHR), occurs in 20-30% of AR episodes.[54,55] Diagnosis of AMR relies on detection of circulating donor-specific antibodies and

histological evidence (i.e. C4d staining of graft biopsies).[56,57] The presence of DSA (antibodies that react with a donor's specific HLA antigens) before and after kidney transplantation is associated with rejection episodes and graft loss (n = 29).[58] Patients with high percent PRA are at greater risk of rejection.[59,60,61,62] During the first year post-transplant, presence of either class I or II DSA has been associated with poor graft survival, even when C4d staining of biopsy was not indicative of AMR.[52,53] This same study found that after the first year, those with class II DSA and C4d positive had worse graft outcomes (n = 297).[52] These studies reaffirm the need to better define which diagnostic criteria are useful for AMR and serve as a reminder that the time post-transplant should be taken into consideration when interpreting C4d staining.

In summary, antibodies against a donor's HLA antigens present before transplantation are associated with reduced graft survival and are contraindicative to transplantation.[63] Those antibodies found post-transplant, but before graft failure is detected (e.g. increase serum creatinine levels), are worth monitoring as they are likely the most significant cause of later graft failure. Antibodies developed post-transplant may take years to destroy the graft and the graft may function well for years despite the presence of antibodies (n = 948).[64] The time between transplantation and antibody development is under investigation to determine if it affects graft survival.

Affects of Age, Sex, and Ethnicity on Graft Outcome

Age

In addition to the immunological factors routinely tested for by histocompatibility labs, factors such as age, sex, and ethnicity can also be risk factors for graft failure (n = 125).[65] In elderly kidney transplant recipients, the most frequent cause of graft loss is death with a functioning graft due to infectious disease or cardiovascular causes (n = 38,836).[66] Recipients over the age of 60 have an increase risk of dying due to infection after transplant and are considered poor immune responders. Older recipients

who experience an acute rejection episode often have shorter graft and patient survival.[67,68] Compared to those under age 50, an increased risk for graft loss has been associated with donors and recipients over age 50, and the combination of a donor and recipient both over age 50 may act synergistically to affect graft survival [69,70,71]. This has led to a selection bias for allocating kidneys from old donors to older recipients in an attempt to optimize the usage of kidneys. In his paper, *The Impact of Age on Rejection in Kidney Transplantation*, de Fijter outlines the premises behind this:[67]

> "1. Elderly recipients are less likely to mount an acute rejection response.
> 2. The functional capacity of a kidney graft allocated to an elderly recipient may be limited as elderly recipients have diminished metabolic demands.
> 3. Older recipients have a shorter life expectation and can therefore receive grafts with a limited long-term prognosis.
> 4. Kidneys from old donors are more vulnerable to delayed graft function than kidneys from younger donors.
> 5. Kidneys from old donors are more likely to reject.
> 6. Kidneys from old donors are less likely to mount an adequate repair response following injury."[67]

However, several registry data studies and single-center studies do not support the hypothesis that older recipient age is a risk factor for chronic allograft nephropathy (CAN), but do not refute that acute rejection episodes are associated with an increased risk of CAN.[72,73] Alloantigen-independent factors, which may be recipient age-dependent, such as hyperlipidemia and hypertension have been associated with increased risk of CAN, and should be taken into consideration in management of older transplant recipients .[74,75]

Gender

One of the most common sensitizing events leading to development of HLA Abs in women is pregnancy. Women who have been sensitized to paternal HLA antigens through pregnancy are at risk for graft rejection if those same paternal HLA antigens are shared by the donor organ. In cases, where antibodies have been formed, primed cytotoxic T lymphocytes (CTLs) directed against the same paternal HLA antigens can persist for more than 10 years after pregnancy.[76,77]

Until recent years, women who had received a transplanted graft were strongly discouraged from becoming pregnant. It was feared that gestation would pose a threat to the graft, mother, and child. However, female transplant recipients who are carefully monitored by transplant nephrologists and high-risk obstetricians can have successful pregnancies if the mother maintains a stable creatinine level, insignificant proteinuria, and normal blood pressure. Pregnancy does put both mother and child at a higher risk, but when these factors are maintained within normal limits, pregnancy is not necessarily detrimental to a previous graft transplant.[78,79]

Donor gender has been shown to have a correlation with renal allograft survival (n = 124,911).[80] Several studies have shown that kidneys from female donors have a higher frequency of rejection than kidneys from male donors.[81,82] Recent attention has been focused on examination of this gender disparity through the evaluation of several different hypotheses. One hypothesis states that the size of the female donor kidney, relative to the male recipient, may induce nephron overload resulting in hyperfiltration-mediated injury and that this is responsible for reduced graft survival.[80] Simply put, that some female kidneys are simply too small (and thus have fewer nephrons) to meet the needs of a larger male body. A second hypothesis is that female donor grafts are more susceptible to cyclosporine nephrotoxicity or differences in the therapeutic response to cyclosporine.[81] This second hypothesis takes into account that there are not only physical size differences in kidneys, but perhaps female body

chemistry (e.g. hormones) reacts differently with traditional immunosuppressants making them more toxic to the graft or reducing their therapeutic effectiveness. To the contrary however, one study found that female to female transplants after the first year have similar graft survival to that of male donor grafts (to any recipient), and had significantly higher survival rates than female to male transplants (n = 170).[82] The higher frequency of rejection of female donor kidneys may be due to a combination of immunologic and non-immunologic factors which are affected by the gender of the recipient.

Despite the poor functional prognosis of female grafts and the increased possibility of sensitizing events (pregnancies), kidney transplants fare better in female than in male recipients overall.[83] Male recipients have a greater risk for kidney graft loss than females.[83] Zeier *et al* also found that the gender effect was more pronounced for younger (16 to 45yr) compared with older (>45 yr) donors (n = 124,911).[80] Perhaps the influence of non-HLA factors, such as hormonal status, should be considered when evaluating donors and recipients. Studies have shown that the immune system undergoes intense stimulation in a high-estrogen environment.[84,85] Females have a higher risk of acute rejection, yet have a decreased risk of graft loss due to chronic allograft failure (n = 73,477).[86] These studies indicate that there are important, gender-related differences in the immune response that require further investigation.

Ethnicity

In the genetics era, there is debate among scholars about the significance of race and ethnicity in terms of describing human genetic variation. While geographic location may be the best single explanation for human genetic variation,[87] it does not explain those differences between two groups living in the same area. Race has been used in medicine and other fields as a way to categorize both genetics and "lived experience."[88] Goodman's theory implies that what appears to be resulting genetic differences may actually be due to social interactions ("lived experience"); emphasizing

that human biology and health are linked to social, cultural, political, and economic processes.[88] While the variation in human biology is too complex to be reduced to race alone, information gathered based on race could be reflective of overall health in certain populations. There is substantial evidence that racial and ethnic health disparities are strongly associated with social factors, such as lack of access to resources, and also racial discrimination. [89] While race may appear to many as simply a social construction, inequalities still persist amongst ethnic groups. It would be ill-advised for healthcare providers to abandon the concept of race altogether. In transplantation, each of these processes is evaluated for the recipient and donor in an attempt to clarify their overall state of health and their available health resources, and the designation of race or ethnicity is used to indirectly to describe the human variation of that population.

A regional retrospective study of 20,240 transplants in the southeastern United States, found that even though patient survival rates at 1 year do not appear to be significantly different among different ethnic groups, ethnicity does play a role in allograft survival. In that study, African American recipients had poorer graft survival than their Caucasian counterparts.[90] Nationally, the 1 year kidney graft survival rates in the United States is greatest for Asians, followed in decreasing order by Hispanics/Latinos, Caucasians, and African Americans.[1] Donor kidneys from African Americans have lower graft survival when transplanted into Caucasian or African American recipients, and are associated with lower patient survival (at 5 years) (n = 72,495) and the highest risk of graft loss (after the first year) (n = 77,689) in African American recipients.[91,92] Graft and patient survival for Asian and Latino/Hispanic recipients, while only slightly better than graft survival for Caucasian recipients, is independent of donor ethnicity.[91,92] However, the limited number of Hispanic and Asian donors and recipients in the U.S. population bring these statistics under scrutiny. To our knowledge, no recent study addressing race/ethnicity and kidney allograft outcome has been reported solely specific for the state of Tennessee.

National studies indicate that compared to their Caucasian counterparts, young African American recipients (aged 18-49) have a higher risk of acute rejection (which affects

long-term graft survival) and graft loss (n = 112,120).[93] African American recipients appear to be more prone to graft loss because of chronic allograft nephropathy (CAN, formerly chronic rejection), and major etiologic factors for end-stage renal disease (type 2 diabetes mellitus and hypertension) are more prevalent in African Americans than in the general population (n= 3706).[94] It is possible that these etiologic factors, which were responsible for the original kidney damage, are contributing to the destruction of the kidney allograft leading to CAN.

Recent studies have shown that ethnicity and gender cannot be treated as totally independent risk factors for allograft outcome. When stratified by ethnicity and gender, black females had significantly reduced graft survival (n = 805).[95] However, when African American females were excluded from multivariate analyses, allograft survival between African American males and all Caucasians were similar, implying that gender may play the critical role in outcome rather than ethnicity.[95] These studies reinforce the need to expand our limited understanding of the human immune response, and while research may focus on minute parts of human physiology, their findings contribute to the broader comprehension of a person's overall biological-social health state.

Novel Supplementary Elements of Renal Transplantation

Major Histocompatibility Complex Class I Chain-Related Gene A (MICA)

The major histocompatibility complex (MHC) class I chain related gene A (MICA) is possibly the most polymorphic non-HLA antigenic system capable of inducing antibody responses involved in hyperacute, acute, and chronic graft loss. MICA encodes proteins that have a similar sequence to MHC class I molecules, are structurally similar to MHC class I, and function in natural killer (NK) cell recognition like other MHC class I

molecules.[96,97] MICA is highly polymorphic and located close to the classical HLA-B locus on chromosome 6.[98,99] MIC molecules show high homology with HLA molecules, but they do not bind β2 microglobulin nor do they bind conventional class I peptides.[100,101] MIC acts as a ligand for natural killer (NK) cells, γδ T cells, and αβ CD8+ T cells expressing the activating receptor NKG2D.[98,102] MICA gene expression can be induced by cellular stress such as heat, viral infection, inflammation, and DNA damage.[101] In epithelial cells, expression of MICA proteins on the cell surface serve as markers for immune surveillance and can activate NK cells and T cells, via their NKG2D receptors, to destroy the affected epithelial cell.[101] Ischemia-reperfusion injury (IRI) initiates inflammatory changes in vascular endothelial and tubular epithelial cells, leading to upregulation of the expression of adhesion molecules and MICA.[103] MICA proteins have been found in various cells/tissues, including all major organs except the brain;[98] however, they are mainly expressed in gastrointestinal epithelium, endothelial cells, and fibroblast.[104] MICA proteins have been observed in tissue samples from kidney allografts in both acute and chronic rejection.[105] While there is no pharmacological way of preventing kidney injury caused by IRI, reducing the role of MICA through MICA matching of recipients and donors (and subsequent prevention of the development of MICA DSA), may improve kidney transplant outcomes.

Sensitizing events for MICA antibodies are the same as for HLA antibodies.[106] Mismatched MICA donor antigens encountered after kidney transplantation may result in antibodies against MICA alleles. There is evidence that MICA antibodies, in addition to HLA antibodies, are associated with graft failure [107,108,109,110] and these antibodies have been suggested as a cause of acute and chronic rejection when HLA antibodies are not present.[102,111,112] Most studies to date have only performed MICA genotyping or checked for presence of MICA Abs, not both. If antibody testing is not performed, establishing if MICA genotype mismatching between donor and recipient elicits a clinically significant antibody response becomes problematic. Likewise, if genotyping is not performed on the donor-recipient pair, one is unable to definitively classify MICA Abs as DSA allo-antibodies or auto-antibodies against self MICA Ags. As

with antibodies to self-HLA antigens, it is unlikely that antibodies against self-MICA molecules would cause kidney loss, but donor-specific MICA antibodies from pre-sensitizing events may cause allograft rejection. Another limitation to current MICA studies is the lack of pre-transplant testing for MICA antibodies and post-transplant following of MICA antibody development. Without being able to determine if antibodies are against self-antigens or donor antigens (MICA genotyping of both recipient and donor) and when these antibodies developed (pre- and post-transplant MICA antibody testing), one cannot make an accurate interpretation of the role that MICA antibodies play in rejection.

Killer-cell immunoglobulin-like receptor (KIR)

Killer-cell immunoglobulin-like receptors (KIRs) were first identified as receptors found on natural killer (NK) cells that could induce cytolysis.[113,114] KIR can be expressed by NK cells and CD8+ T cells.[115] NK cell function depends on the interaction of KIR ligands with inhibitory and/or activating KIRs. The interaction of KIRs with their ligands is determined by the KIR cytoplasmic tail.[116,117] Long intracellular tails (designated "L") give inhibitory signals due to the immunoreceptor tyrosine-based inhibition motifs (ITIMs) in the tail. Where short tails (designated "S",) transmit activating signals through immunoreceptor tyrosine-based activation motifs (ITAMs).[116,118]

KIR ligands are HLA class I molecules. Inhibitory KIRs have a high affinity for different subsets of HLA class I molecules.[119,120] HLA-C and HLA-Bw4 are ligands for inhibitory KIR2DL and KIR3DL respectively.[121,122,123] All NK cells can express several KIRs, but at least one of these is inhibitory under normal conditions. Down-regulated expression of MHC class I expression in infected or transformed cells can result in destruction by NK cells, termed the "missing-self hypothesis".[35] Under normal conditions, cells express normal levels of HLA class I molecules. When these cells are damage, the surface expression of HLA is decreased. NK cells interact with these transformed cells and do not receive the appropriate "self HLA" inhibitory signal.

NK cells then induce apoptosis of these transformed cells. The same self/non-self recognition by NK cells mediates NK alloreactivity in renal allograft transplantation, and the missing expression of self HLA molecules on donor cells can be potential targets of NK cell killing. This “missing ligand” hypothesis states that for each KIR expressed in the recipient, the inhibitory ligand (HLA-associated epitope) needs to be present on (or be “matched” to) the donor cells to avoid NK cell alloreactivity.[124,125]

It has been suggested that some specific recipient receptor--donor ligand combinations might be associated with acute rejection episodes in renal transplantation with KIR/HLA incompatibility and that an imbalance of activating and inhibitory receptors may contribute to poor allograft outcome.[126] A higher number of inhibitory receptors in the recipient's genotype and matching for the receptors KIR2DL2 and KIR2DS2, has been observed in patients with stable kidney graft function (n= 224).[126] Another study of 12 HLA-matched sibling recipient-donor pairs found that when both genotypes lack KIR2DL2 and KIR2DS2, there was a higher risk for poor graft function.[127] These studies are based on the theory that recipient NK cell cytotoxicity against donor cells is the result of the recipient’s lack of the correct inhibitory receptors, or an overabundance of activating KIRs.[128] Such imbalances could result in excessive activation of NK cell alloreactivity against the donor graft cells. One study evaluated whether acute rejection in kidney transplants (occurring after immunosuppression was reduced) was associated with the predicted NK cell alloreactivity based on KIR gene and ligand analysis. In that study, HLA and KIR genotypings of 69 recipient-donor pairs were used to analyze the presence of KIR genes, and to predict NK cell alloreactivity based on the "missing self" and "missing ligand" hypothesis.[125] While the findings were solid, the study population was limited. In another study of 2,757 transplants, kidney recipient-donor pairs were separated according to their HLA-Cw alleles and HLA-Bw4 specificity (which are ligands for KIR) and NK cell alloreactivity was predicted based on the missing ligand hypothesis.[129] However, this study did not perform typing of KIR genes. Neither study could identify a significant relationship between KIR ligand matching and renal graft survival.

Very little is known about the expression of inhibitory and activating KIRs on T-cell subsets, and to our knowledge, there is currently no data on T-cell expression of KIRs in the post-transplant period. One study by Henel *et al* found evidence that inhibitory KIRs can change the T-cell effector profile by altering transcription of T cell receptor genes, without affecting granule release and cytotoxicity.[130] While these studies demonstrate a potential role of certain KIR/HLA ligand interactions in renal transplantation, the biological and functional aspects of KIR receptors still need to be explored in a clinical setting. Analysis of KIR expression on graft infiltrating NK cells and T cells, as well as their interactions with ligand-expressing cells, may provide valuable insight.

Soluble CD30 (sCD30)

The CD30 molecule was originally discovered as an antigen on the surface of Hodgkin and Reed Sternberg cells.[131] CD30 is a member of the tumor necrosis factor/ nerve growth factor receptor superfamily and is expressed on T cells that produce Th2-type cytokines.[132,133,134,135] During immune responses that activate these T cells, a soluble form of CD30 (sCD30) is released into the blood.[135] An association between CD30 expression and Th1 cytokine production has also been suggested.[136] It has also been hypothesized that CD30 functions as a co-stimulatory molecule, acting as a marker for the balance between Th1(cellular) and Th2 (antibody-mediated) responses.[137] While the exact role of CD30 is unclear, elevated pre- and post-transplant sCD30 levels have been associated with an increase risk of rejection and graft loss.[138,139,140]

Biopsy results were unavailable in many of the early studies regarding sCD30. Therefore, they were unable to determine whether patients with a high sCD30 were more prone to antibody-mediated or cell-mediated rejection. Studies comparing pre- and post-transplant serum levels of sCD30 have been performed, but their focus was limited to acute rejection episodes in the first post-transplant year.[140,141,142,143] In

the *American Journal of Transplantation* in 2005, Rajakariar *et al,* using 108 renal transplant recipients, correlated the levels of sCD30 at time of transplantation with the grade of rejection utilizing standard C4d biopsy immunostaining, but did not perform any post-transplant sCD30 testing and were limited to a maximum 5 year post-transplant follow-up period for determining graft outcome.[144] Poorer graft outcomes have been observed in patients with both PRA and high sCD30 serum levels before transplant, and high levels of pre-transplant sCD30 have been associated with lower five-year graft survival in kidney transplant patients.[145,146] High pre-transplant levels (>100 U/ml) of sCD30 have been linked to acute allograft rejection;[140,146,147] however, other studies indicate that pre- and post-transplant sCD30 levels are not predictors for acute kidney rejection, nor are they useful for monitoring during reduction of immunosuppressive drugs.[148,149]

Very few of these studies took into consideration other factors (those not specific to transplantation) which could also lead to increased sCD30 in transplant recipients. Interpretation as to the true relationship of sCD30 and acute rejection must be done carefully, with knowledge of the patient's entire immune status. Increased levels of sCD30 have been noted in various conditions such as virus infections, autoimmune disorders, pregnancies, and some tumors.[136,150,151,152] Since elevated sCD30 is not only associated with graft rejection, but also with states of increased immune reactivity, general classification of sCD30 levels (low to high) and interpretation of their relevance, should be done with caution. An increased level of sCD30 in a transplant recipient with rheumatoid arthritis may not be indicative of increased risk of kidney allograft rejection, but rather a reflection of increased Th2 activity due to the arthritis. Monitoring patient's sCD30 levels before and after transplantation, and then evaluating the change in concentrations, rather than categorizing levels as low to high, may provide useful insight into the correlation of sCD30 to graft function and survival.

Summary

Prediction of graft outcome proves to be a multi-facetted problem. There are numerous immunological and non-immunological factors that can contribute to graft loss, and until we can elucidate tolerance mechanisms and ameliorate immunosuppressant drug protocols, we should utilize our current knowledge to advance graft function. We propose to assess each of the factors outlined in this chapter for both individual, as well as collective risk effect on graft outcome on the same individual study population. These factors include the traditional factors (HLA matching for each donor and recipient pair, pre-transplant PRA levels, and standard population demographics) and supplementary factors (MICA matching between donor and recipient, KIRmatching between donor and recipient, identification of MICA antibodies, and sCD30 level). Testing kits for each of these factors are available to laboratories across the United States, and therefore, can easily be obtained for testing at other centers. After evaluation of each factor, we anticipate that we will be able to provide clinicians with a model that will aid them in the prediction of kidney graft outcome in the years after transplant and thus allow them to alter individual patient's immunosuppressive protocols accordingly. This novel tool will predict risk of kidney graft outcome and allow for monitoring of changes in immunosuppressant therapies, more accurately, less invasively, and more timely than the current methods.

CHAPTER III: SUBJECTS AND METHODS

Definitions

Traditional Risk Score / Model

Currently, kidney transplant surgeons and nephrologists across the United States have access to a "standard" group of variables used not only for optimal allocation of organs, but also to predict graft outcome. These variables include the recipient's PRA, recipient and donor ethnicity, gender, age, HLA typing of A, B, DR, and DQ loci. While each of these variables has been shown to have an effect on graft outcome, they are insufficient for accurate prediction of graft function. We used these factors to develop a model, which may be used by clinicians to help gauge the risk of graft outcome after kidney transplantation for a specific donor-recipient pair. This model was termed "Traditional Risk Score."

Expanded Risk Score / Model

Addition of other variables may increase the predictive ability of the scoring system. After consideration of current literature and availability of reagent test kits, we selected additional variables for evaluation: MICA typing, MICA Ab testing, KIR typing, and sCD30 level monitoring. Each variable was evaluated for inclusion in a second scoring system, termed "Expanded Risk Score." This expanded risk model included all the variables in the traditional risk model and additional test variables: MICA matching between recipient and donor, KIR matching between recipient and donor, pre-transplant level of sCD30 in the recipient, and detection of pre-transplant MICA antibodies.

Study Subjects

The United Network for Organ Sharing (UNOS) and the University of Tennessee Medical Center in Knoxville collect extensive data on transplant patients both pre- and post-transplant. At the time of transplant, these reports include recipient-donor pair demographic characteristics such as age, gender, ethnicity, PRA, HLA matching.

Statewide Population

The statewide study population consisted of 694 adult (age range 19-78 years) kidney-only, standard criteria deceased donor transplant recipients in Tennessee (excluding those from the University of Tennessee Medical Center, Knoxville, TN [TNUK]) from 2005 to 2008. A description of the study population is shown in Table 1(See APPENDIX). Of these 694 recipients (465 men and 229 women), 624 patients (89.9%) were reported to have a functioning graft at 1year post-transplant as indicated to UNOS on the Transplant Recipient Follow-Up Form (Table 3. See APPENDIX). Their corresponding deceased donors ranged from 1year to 59 years of age (446 men and 248 women). Ethnicity of recipients included 389 Caucasian and 305 African American. Ethnicity of donors included 587 Caucasian and 107 African American. Other ethnicities made up less than 4% of the study population and were therefore excluded from the study. Of the 455 recipients not lost to follow-up, 373 had a functioning graft at 3 years post-transplant (82.0%) (Table 3. See APPENDIX). Recipient and donor data were obtained from UNOS using an encrypted study identification number to protect confidentiality of the subjects. Since samples were not available, no additional testing was performed on this population for the purposes of this study.

TNUK Population

All adult (age range 20-74) kidney-only, standard criteria deceased donor transplant recipients at the University of Tennessee Medical Center (Knoxville) [TNUK] from 2005 to 2008 were evaluated for retrospective analysis (N=113). The cohort study subjects (recipient-donor pairs) were enrolled into this study if they had stored genomic DNA and stored pre-transplant sera from day of transplant or within 3 months prior to transplant. Stored genomic DNA of the corresponding deceased donor was also required. A description of the TNUK study population is shown in Table 2 (See APPENDIX). Previously stored samples were available on 64 of the recipient-donor pairs. These were tested for MICA, KIR, and sCD30. Of these 64 pairs, 62 had a functioning graft at their 1-year post-transplant follow-up (96.9%) (Table 3. See APPENDIX). A non-functioning graft was defined as the need for or return to dialysis treatments. Of the remaining 62 patients, 58 had a functioning graft at their 3-year post-transplant follow-up (93.5%) (Table 3. See APPENDIX). The recipient age range was from 20 to 74 years (45 men and 19 women). The donor age range was from 12 to 66 years (39 men and 25 women). Ethnicity of recipients included 61 Caucasian and 3 African American. Ethnicity of donors included 59 Caucasian and 5 African American. Other ethnic groups made up less than 2% of the study population and were therefore excluded from the study. Each donor-recipient pair was given a unique, numerical identifier to ensure patient confidentiality. The study was approved by the Institutional Review Board of the University of Tennessee, Knoxville (# 8159B) and by the Institutional Review Board of the University of Tennessee, Graduate School of Medicine (# 2988).

Additional testing: MICA, KIR, sCD30

Commercially available kits were used for all additional testing to ensure that these tests could be performed by HLA laboratories throughout the United States, thus allowing clinicians to utilize the expanded scoring system.

DNA samples from recipients and donors, previously collected during routine testing, were stored at -20°C until used. MICA genotyping was performed with LABType SSO MICA (One lambda Inc.), according to the manufacturer's instructions. KIR genotyping was performed using LABType SSO KIR (One lambda Inc.), according to the manufacturer's instructions. A match for MICA tying was evaluated the same as traditional HLA matching: a direct comparison of genotype. KIR matching was evaluated by comparison of the KIR receptor typing of the recipient to the presence or absence of its corresponding HLA ligand in the donor. Utilizing the classification scheme developed by Holm *et al*, each recipient KIR-donor HLA pair was classified according to the expected NK cell response: balanced, excess inhibition, excess activation, or undetermined.[153]

Pre-transplant recipient sera, collected on day of transplant or within 3 month prior to transplant, was stored at -20°C until use. Soluble CD30 levels were obtained from sera using Human sCD30 Flow Cytomix Simplex (eBioscience) according to the manufacturer's instructions. MICA antibody identification was performed using LABScreen MICA Single Antigen kits (One Lambda, Inc.) according to the manufacturer's directions.

Statistical Analysis

Characteristics of the study populations were summarized using descriptive statistics, including absolute numbers and percentages for categorical variables, and means and

standard deviations for continuous data. The predictors of interest were assessed by univariate analysis, and then in multivariate models adjusting for potential confounders.

Variables

Outcomes:

- Categorical outcomes: Graft functioning or graft not functioning. A graft was considered non-functioning if the patient required dialysis. This was measured at one year post-transplant and at three years post-transplant for both the standard and expanded model populations.
- Continuous outcomes: Serum creatinine levels (mg/dL) at 12 months post-transplant were evaluated in the expanded model population as an alternate measure of outcome at 1 year post transplant.

Predictor variables:

- Age (continuous-scale)
- Ethnicity/Race (categorical-nominal)—Caucasian/White or African American/Black
- Sex/Gender (categorical-nominal)
- PRA (categorical-nominal)—PRA between: 0 – 19%, 20 – 39%, 40 – 79%, and above 80% (divisions based on what is widely accepted as zero to very low, low, moderate, and high levels of PRA)
- HLA matching (categorical-nominal)—Class I HLA: AB loci match or AB loci mismatch; Class II HLA: DRDQ loci match or DRDQ loci mismatch. While other HLA antigens have proven valuable for prediction of graft outcome, they are not uniformly or routinely tested for by all HLA laboratories in the United States.
- KIR matching (categorical-nominal)—balanced, excess activation, excess inhibition, or undetermined. Note: The study population only had balanced or excess inhibitory receptor matches.

- MICA matching (categorical-nominal)—MICA match or MICA mismatch
- Soluble CD30 (pg/mL) (continuous-scale)
- MICA antibodies (categorical-nominal)—present or absent. Note: Presence of DSA and auto-Ab analysis was examined, but not included in the statistical development of models due to the small number of cases with DSA or auto-Abs.
- Serum creatinine (mg/dL) (continuous-scale) at 1 month, 6 months, and 12 months post-transplant. These were evaluated as predictors for the functioning/non-functioning outcomes.

Univariate Analysis and Descriptive Statistics

Continuous Variables

Descriptive statistics were calculated on all continuous variables. Continuous variables were assessed for normality (Shapiro-Wilk test, skew, and kurtosis) and for homogeneity of variance (Levene's test and Moses Extreme Reactions). Data transformations (log transformation and square root transformation) were used to correct for non-normality and unequal variances without success. For the 12 month creatinine outcome, all non-parametric continuous variables were tested for correlation using Kendall's tau (2-tailed). For the functioning/not functioning outcome at 1 year and 3 years post-transplant for both the traditional and expanded model populations, continuous variables were analyzed using logistic regression. Statistical significance was set at $p \leq 0.05$.

Nominal Variables

Frequencies were calculated for all nominal variables. Independent samples tests were performed for nominal variables and the 12 month creatinine outcome using Kruskal-Wallis, Mann-Whitney, Kolmogorov-Smirnov Z, and Jonckheere-Terpstra test for ordered alternatives. Cramer's V, Pearson chi-square test, and the likelihood ratio were calculated for all nominal variables and the functioning/not functioning outcome at 1

year and 3 years post-transplant for both the traditional and expanded model populations. Statistical significance was set at $p \leq 0.05$.

Generation of Models and Appropriateness of Design

Twelve total models were generated to assess outcome at 1year post-transplant and at 3 years post-transplant for the standard and expanded model populations. Binary logistic regression was used to generate both the traditional scoring system and expanded scoring system models using SPSS software (version 19; SPSS Inc.). Independent risk factors for graft failure were identified using Backward Stepwise (Likelihood Ratio) selection from multivariable logistic regression.

Each model was assessed for goodness of fit using the model chi-square statistic and the -2 log-likelihood (*-2LL*). The overall significance of each model was measured by Cox and Snell's R^2_{cs} and Nagelkerke's R^2_N. Statistical significance was set at $p \leq 0.05$.

The predictive ability of each model was evaluated for sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), false positive rate (FP), and false negative rate (FN). It is acceptable for these models to have a higher type I error and more false positives than false negatives. This way a patient's immunosuppressive medication levels are not erroneously reduced in someone who is indeed at a greater risk for rejection.

Diagnostic statistics were run for all models to evaluate outliers (standardized residuals) and influential cases (Cook's distance, leverage/hat values, and standardized DFBeta). In order to confirm that the assumptions of the logistic regression model were not violated, each model was checked for linearity, independence of errors, and multicollinearity. The assumption of linearity was tested by looking at whether the interaction term between the predictor and its log transformation was significant. Using the deviance statistic, the assumption of independence was evaluated for over-dispersion and under-dispersion. Multicollinearity was checked with tolerance and

variable inflation factor (VIF) statistics, eigenvalues, condition indexes, and variance proportions.

Each predictor variable and its interactions were assessed for contribution to the logistic regression model using the Wald statistic and likelihood ratio. Effect size for each predictor was measured using the odds ratio or coefficient. As is common in small samples, the Wald chi-square did not match with the overall test of the model. Interactions were not significant and were excluded from the models. However, all variables have been widely accepted as clinically important in the transplant community. Therefore, coefficients were calculated for each variable regardless of the significance of the Wald statistic. Each full model (all variables entered) for the statewide population was compared to the corresponding full model for the TNUK population. This allowed us to compare the full model across both population sets and different measures of outcome for the traditional scoring system. The best fit models, as determined by backward stepwise logistic regression are also listed with each full model for traditional and expanded scoring.

Summary List of Model Comparisons:

- Statewide—1 year—Function/Non-Function—Traditional
    - TNUK—1 year—Function/Non-Function—Traditional
- Statewide—3 year—Function/Non-Function—Traditional
    - TNUK—3 year—Function/Non-Function—Traditional
- TNUK—1 year—Function/Non-Function—Traditional
    - TNUK—1 year—Function/Non-Function—Expanded
- TNUK—3 year—Function/Non-Function—Traditional
    - TNUK—3 year—Function/Non-Function—Expanded
- TNUK—12 mo Creatinine—Traditional
    - TNUK—12mo Creatinine—Expanded

CHAPTER IV: RESULTS AND DISCUSSION

Univariate Analysis

Univariate analysis of the statewide population indicated that only two predictor variables in the traditional scoring system model were statistically significant for the 1 year and 3 years outcomes (Table 4. See APPENDIX). Univariate analysis indicated that donor grafts from women were significantly more likely to be non-functioning at 1 year (53%) than were donor grafts from men (47%), $x^2(1, N = 694) = 9.94, p < 0.05$) (Table 5. see APPENDIX). However, at 3 years post-transplant, kidney grafts that were mismatched for the DRDQ loci were more likely to be non-functioning (82%) than were DRDQ matched grafts (18%), $x^2(1, N = 455) = 4.41, p < 0.05$) (Table 6. See APPENDIX).

Univariate analysis of the TNUK population data for both the traditional scoring system variables and the expanded scoring system variables are listed in Table 7 (See APPENDIX). No significant associations were found between 1 year outcome and the predictor variables for the TNUK population. However, evaluation of the likelihood ratios (exact 2-tailed) of the TNUK population revealed a significant relationship between PRA, $\Lambda (3, N = 62) = 6.78, p < 0.05$) and KIR matching, $\Lambda (1, N = 62) = 7.01, p < 0.05$) with function at 3 years. Of the 58 functioning grafts at the 3 years follow-up, 72% had a PRA between 0-19% (Table 8. See APPENDIX) and 60% of the 58 grafts had excess inhibitory KIR receptors (Table 9. see APPENDIX). None of the patients with excess inhibitory KIR receptors had a non-functioning graft at 3 years.

Since sCD30 levels, recipient age, donor age, and 12 month creatinine levels were not normally distributed (Shapiro-Wilk test $p < 0.05$), Kendall's tau (2-tailed) was used to assess their relationships. Recipient age was significantly correlated to 12 month creatinine level in the TNUK population, $\tau = - 0.32, p < 0.05$ (Table 10. see APPENDIX). Creatinine levels at 12 months post-transplant ranged from 0.82-2.50 mg/dL with a mean of 1.46 mg/dL (± 0.43 mg/dL). Creatinine levels were analyzed for each group

within the variables using Mann-Whitney and Jonckheere-Terpstra Test (2-tailed) (Tables 11-12. see APPENDIX). Creatinine levels in male recipients (1.53 ± 0.45 mg/dL) were significantly different from female recipients (1.30 ± 0.35 mg/dL), U = 276.00, z = -2.03, $p < 0.05$, r = - 0.26 (Table 13. See APPENDIX). Creatinine levels were also significantly different for patients with a DRDQ mismatch (1.54 ± 0.44 mg/dL) than for those with a DRDQ matched kidney (1.21 ± 0.31 mg/dL), U = 182.50, z = -2.81, $p < 0.05$, r = - 0.36 (Table 13. See APPENDIX). Creatinine levels were higher on average for patients with MICA Abs present (1.73 ± 0.46 mg/dL) than levels were in those who did not have MICA Abs (1.42 ± 0.41 mg/dL), U = 336.00, z = 1.96, p = 0.050, r = 0.25) (Table 13. See APPENDIX).

Model Validity and Reliability

While the occurrence of kidney graft rejection is not a rare event, it was limited in the populations. For the state of Tennessee, 10% had a non-functioning graft at 1 year and 12% had a non-functioning graft at 3 years. The TNUK population was even more limited with 3% non-functioning at 1 year and 6% non-functioning at 3 years. These limited occurrences cause sampling distribution errors. This lack of variation in the dependent variable made it difficult to estimate a model. Summaries of the model goodness of fit (Table 14) and predictive abilities (Table 15) are given in the Appendix.

Goodness of Fit

The traditional scoring model 1 (full model) for non-functioning at 1 year, using data from the statewide population, was not statistically better at predicting outcome than the model with only the constant included (Table 14. See APPENDIX). However, donor gender was a significant variable (Table 16. See APPENDIX), which was in agreement with the likelihood ratio statistic from the univariate analysis. Backward stepwise selection produced a significant model (model 2), but of the three predictor variables in the model, only donor gender was significant (Table 17. See APPENDIX)

Using the statewide population data, neither the traditional scoring model 3(full model) for non-functioning at 3 years (Table 18. See APPENDIX), nor the backward stepwise model 4 (Table 19. See APPENDIX) contained any statistically significant predictor variables. While DRDQ matching was significant in univariate analysis, it did not reach statistical significance in either model. The full model 3 (full model) was not better at predicting the outcome than the model with only the constant included (Table 14. See APPENDIX). Backward stepwise selection (model 4) did produce a significant model overall which included DRDQ matching (Table 19. See APPENDIX).

For the TNUK population, neither model 5 nor model 6 for outcome at 1 year was statistically significant, nor did they contain any significant predictor variables (Tables20-21. See APPENDIX). This agreed with the univariate analysis for 1 year outcome of the TNUK population.

The traditional scoring model 7 (full model) for non-functioning at 3 years, using data from the TNUK, was not statistically better at predicting outcome than the model with only the constant included (Table 14. See APPENDIX). It did, however, contain one significant predictor variable, PRA 20-30%, which was in agreement with the univariate analysis of 3 year outcome (Table 22. See APPENDIX). The backward stepwise model 8 contained the PRA 20-30% $p < 0.05$, as well as the other PRA categories and DRDQ matching p = ns (Table 23. See APPENDIX).

The expanded scoring model 9 (full model) for outcome at 1 year (TNUK population) was not statistically better for prediction of outcome than the constant only model (Table 14. See APPENDIX) and did not contain any significant predictor variables (Table 24. See APPENDIX). The backward stepwise model 10, while significant (Table 14. See APPENDIX), did not contain any significant predictor variables (Table 25. See APPENDIX). Again, these models agreed with univariate analysis of TNUK data at 1 year outcomes.

Both the expanded scoring models for outcome at 3 years (TNUK population) were statistically significant (Table 14. See APPENDIX). Neither the full model nor the backward stepwise model contained significant predictor variables (Table 26-27. See

APPENDIX). This is in contrast to the 3 year TNUK univariate analysis that indicated KIR matching and PRA were significant.

Predictive Ability

All models had 100% sensitivity, except for model 8 TNUK backward stepwise standard scoring, which also had the highest false negative rate (Table 15. See APPENDIX). All TNUK expanded models (models 9-12) had 100% sensitivity and 100% specificity. These models also had 100% positive predictive values and 100% negative predictive values. They also had 0 % false positive and false negative rates. It is most interesting that of these models, the 3 year outcome models (models 11-12) were statistically significant and had the highest chi-square values. They also had the highest Cox & Snell R Square and Nagelkerke R Square values, as well as the lowest -2LL values. All standard models (statewide and TNUK) had false positive rates greater than 1.6% and specificities of 0% or 50%.

Summary

These models were designed to help clinicians adjust immunosuppressant levels (maintain lower therapeutic levels for patients' with lower risk); therefore, we focused on the following criteria when evaluating each model: chi-square goodness of fit, R square, -2LL, sensitivity, negative predictive value, and false negative rate. Using these criteria to evaluate the models, we ranked the significant models ($p < 0.05$), from most clinical relevance to least relevant. The last three models were so poor, that they were ranked based on R square and -2LL.

Model 12: TNUK expanded 3 yr (stepwise) $x^2(11) = 29.66$, $p < 0.01$, $R^2_{CS} = 0.38$, $R^2_N = 1.00$, -2LL = 0, 100% sensitivity, 100% NPV, 0%FN.

Model 11: TNUK expanded 3 yr (full) $x^2(15) = 29.66$, $p < 0.05$, $R^2_{CS} = 0.38$, $R^2_N = 1.00$, -2LL = 0, 100% sensitivity, 100% NPV, 0%FN.

Model 10: TNUK expanded 1 yr (stepwise) $x^2(7) = 17.80$, $p < 0.05$, $R^2_{CS} = 0.24$, $R^2_N = 1.00$, -2LL = 0, 100% sensitivity, 100% NPV, 0%FN.

Model 8: TNUK standard 3 yr (stepwise) $x^2(4) = 10.15$, $p < 0.05$, $R^2_{CS} = 0.15$, $R^2_N = 0.40$, -2LL = 19.51, 95.6% sensitivity, 50% NPV, 50%FN.

Model 2: State standard 1yr (stepwise) $x^2(3) = 16.80$, $p < 0.01$, $R^2_{CS} = 0.02$, $R^2_N = 0.05$, -2LL = 427.62, 100% sensitivity, NPV—unable to calculate, FN—unable to calculate.

Model 4: State standard 3 yr (stepwise) $x^2(1) = 4.11$, $p < 0.05$, $R^2_{CS} = 0.01$, $R^2_N = 0.02$, -2LL = 417.88, 100% sensitivity, NPV—unable to calculate, FN—unable to calculate.

In consideration of overall model statistics, the TNUK expanded models for outcome at 3 years (models 11-12) are the most helpful in prediction of graft loss. These models were not subject to undue influence by individual cases (Cook's, Leverage, standardized DFBeta), unacceptable levels of error (standardized residuals), or multicollinearity (VIF, tolerance, etc.). However, over-dispersion was present in both models (deviance statistic). This was most likely due to the poor variability in the outcome probabilities of the TNUK population or a result of other data complexities that are not understood. Unsystematic variation in the TNUK population (e.g. different types and dosage of immunosuppressant drugs) cannot be controlled for in such a limited population, therefore, no over-dispersion correction was performed. It is interesting that the backward stepwise selection Model 12 retains the proposed expanded testing variables, and drops the traditionally accepted factors of recipient gender and ethnicity, donor age, and HLA matching of AB and DRDQ.

CHAPTER V: CONCLUSIONS

Conclusions, Implications, and Recommendations

Clinicians lack appropriate non-invasive methods to be able to predict, diagnose, and reduce the risk of rejection in the years following kidney transplantation. Protocol biopsies and monitoring of serum creatinine levels are the most widely utilized approaches to monitoring graft function after transplant. However, biopsies come with some risks, as do all invasive procedures, and monitoring serum creatinine does not provide enough forewarning of rejection. Instead, we recommend the use of a post-transplant monitoring tool as an indirect approach for increasing the number of available kidneys by predicting risk of kidney graft outcome and for use in adjustment of post-transplant immunosuppression treatment.

Utilizing immunologic factors that were available to clinicians across the United States, we present a model for predicting graft function at 3 years post-transplant. This model includes the traditional factors, as well as additional factors, which other studies have indicated as predictors for renal allograft function. Traditional factors (HLA matching for each donor and recipient pair, pre-transplant PRA levels, and standard population demographics) and supplementary factors (MICA matching between donor and recipient, KIRmatching between donor and recipient, identification of MICA antibodies, and sCD30 level) were evaluated for incorporation into the model. Testing kits for the additional factors are available through multiple vendors, and are simple to use. Therefore, other centers should be able to incorporating these additional tests into their routine testing for donors and recipients and allow the center to develop a similar expanded scoring model for post-transplant monitoring of renal allografts. To our knowledge, this is the first time a single model has been proposed utilizing MICA, KIR, sCD30, and traditional HLA testing on the same individual study population.

Using multi-center data from adult recipients of SCD kidneys, we were able to construct models, containing the traditional factors only, for prediction of outcome at 1 year and 3

years post-transplant. Using single-center data from adult recipients of SCD kidneys, we developed comparison models containing traditional factors only, as well as, expanded models containing the new suggested variables for prediction of outcome post-transplant. These additional variables, when incorporated into the expanded models provided greater positive predictive values, greater negative predictive values, and lower false negative rates for graft outcome at 1 year and at 3 years post-transplant than the models utilizing traditional factors only

We suggest that either of the expanded models (models #11 and #12) for predicting kidney graft outcome at 3 years would be appropriate for use by clinicians in evaluating adjustment of post-transplant immunosuppression regimens. However, these models should be validated with a larger data set and evaluated for utility in the course of various immunosuppressant drugs before implementation in the clinic. These models contain both the traditionally accepted risk factors for graft rejection as well as the new proposed expanded testing variables. Both models were statistically significant, with excellent specificity, false negative rate, and negative predictive value. Individual variables and interaction terms were not significant in these logistic regression models, but univariate analysis indicated that 3 year outcomes may be related to KIR matching and percent PRA. While we are pleased that our 3 year rejection rates at TNUK and throughout Tennessee are low, it does create obstacles for research. The lack of variation in the outcome variables (because of the low percent of rejection in the TNUK population) created difficulties in developing appropriate models, and is the most likely explanation for the inability of this study to obtain similar significant findings observed in other studies.

The 3 year expanded model (#12 backward-stepwise) is valid for the TNUK population, and may not reflect other centers' population demographics and compliance, immunosuppressive protocols, rescue therapy for rejection episodes that do not result in return to dialysis, and numerous other factors that could influence a patient's immunologic status. It is interesting that removal of the traditional factors of HLA AB and DRDQ typing, recipient gender and ethnicity, and donor age from full 3 year expanded model results in the statistically significant model 12. However, it would be

unwise to disregard such important factors in transplant outcomes. We encourage other transplant centers to participate in additional testing of sCD30, MICA and KIR, and attempt to cross-validate this model or develop similar models utilizing a larger data set.

The TNUK population was not reflective of the statewide population, and therefore, comparisons between TNUK models and statewide population models could not be made. One of the most obvious differences in the statewide population and the TNUK population was the ethnicity of recipients and donors. TNUK has a predominantly Caucasian donor population and Caucasian recipient population, whereas statewide, the recipient ethnic groups are more even and the percentage of African American donors is twice as high as that of TNUK. We plan to investigate this and other socio-economic disparities across the state in the future. Because the two study populations were so dissimilar in their basic demographics, traditional TNUK models could not be compared to traditional statewide models, and subsequent conclusions regarding the efficacy of the TNUK expanded models could not be drawn for the statewide population. We advocate the use of the 3 year expanded model (model #12) as a secondary, non-invasive tool for use by TNUK clinicians for prediction of renal allograft outcome. We predict that a future investigation, utilizing various immunosuppression regimens, will contribute to the validity of this model as a "tailor-made" approach to graft maintenance.

Further study is needed to determine the lack of significant predictors in the model. Samples should continue to be collected both pre- and post-transplant for validation of this 3 year model, for monitoring of sCD30 level changes, PRA changes, and MICA antibody changes post-transplant, and for future development of a 5 year outcome model. Continued collection of DNA and serum samples will provide a greater pool for subsequent studies which may expound upon our understanding of each variable's veritable role in graft outcome.

While these models were designed to be based on immunologic risk factors, additional risks may develop (due to patient's or clinician's actions) which are not encompassed in the models and may shift the patient from being in the non-rejection to the rejection categorization. Social and economic factors contribute considerably to an individual's

collective health state. It is unrealistic to expect a single model to incorporate every minute aspect of each individual's immune status. Nevertheless, this general model of important transplant factors is a propitious tool in predicting risk for individuals in the TNUK population. This 3 year expanded model solely addresses immunologic factors, not ethical considerations, and is not intended for use as an allocation algorithm, but rather a tool to aid clinicians in the post-transplant care management of their patients.

Creatinine level is an established measure of graft function and is widely used to monitor effectiveness of treatment for rejection episodes. However, using creatinine levels alone to monitor grafts is unwise because elevated serum creatinine occurs after damage to the kidney allograft is nearly established. Our univariate analysis of factors and the 1 year function outcome in the TNUK population did not reveal a significant relationship. However, analysis of 12 month creatinine and these factors suggests that some factors warrant further consideration when formulating a predictive model. Male recipients, patients with a DRDQ mismatch, patients with MICA antibodies present, and surprisingly, younger recipients, had higher 12 month creatinine levels than their counterparts. These correlations agree with other studies linking male recipients, DRDQ mismatching, and presence of MICA antibodies to poorer graft outcome (reflective here as increased creatinine levels). It is unclear why younger recipients had significantly higher creatinine levels. Perhaps it is simply because the TNUK recipients were on average older ($\mu = 54.0 \pm 12.6$ years) than the statewide population ($\mu = 49.4 \pm 12.0$ years), and that "younger" recipients in the TNUK population are not as "young" as those in the statewide population. Further study is need to determine if higher creatinine levels in younger recipients were the result of greater metabolic demands in younger persons, non-compliance, or another factor not encompassed by this study such as extended cold-times. All of the 12 month creatinine correlations should be evaluated with caution, as they are reflective of the TNUK study population. It is not advisable to extrapolate these correlations to other populations, but rather they lay foundation for investigation of creatinine levels in subsequent case-controlled studies.

This study provided a limited opportunity for recipient-donor pair to pair comparisons. We did examine the 6 cases with failed grafts and contrasted them with functioning

cases matched for recipient age, donor age, recipient ethnicity, donor ethnicity, recipient gender, donor gender, and PRA level in various combinations. For example, a 47 year old female Caucasian recipient with greater than 80% PRA who received a kidney allograft from a 35 year old female Caucasian recipient had a non-functioning graft at 3 years post transplant. Compare this to a 47 year old female Caucasian recipient with greater than 80% PRA who received a kidney allograft from a 28 year old female Caucasian who still had a functioning graft at 3 years post-transplant. Based on the data we collected, the only difference in these cases was that the functioning graft pair was a match for AB, DRDQ, and MICA. Other comparisons of donor-recipient pairs have indicated that patients with MICA Abs and excess inhibitory KIR were more likely to have functioning grafts at 3 years. In fact, none of the patients with excess inhibitory KIR had a non-functioning graft at 1 year or 3 years. While these "findings" are intriguing and require further consideration, the predictor variables were not individually statistically significant within the model. Just as higher average creatinine levels were observed in patients with certain characteristics in the TNUK population, these case-wise evaluations do not provide a definitive connection between these variables, as they too are limited by the size of the study population. Individual immune statuses differ given a variety of biological, environmental, social, and economic factors, and have diverse responses to immunological challenge. Therefore to make any valid claims regarding a valid relationship between these characteristics at the population level, we would need to examine an immense number of recipient-donor pairs.

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APPENDIX

Table 1: Tennessee Statewide Study Population

Characteristic	Number of Recipients (% of Total Population)	Number of Donors (% of Total Population)
Gender		
Male	**465 (67%)**	**446 (64%)**
Female	**229 (33%)**	**248 (36%)**
Ethnicity/Race		
Caucasian/ White	**389 (56%)**	**587 (85%)**
African American/ Black	**305(44%)**	**107 (15%)**
Mean Age (Standard Deviation)	**49.4 (± 12.0 years)**	**32.0 (± 14.6 years)**

Table 2: TNUK Study Population

Characteristic	Number of Recipients (% of Total Population)	Number of Donors (% of Total Population)
Gender		
Male	**45 (70%)**	**39 (61%)**
Female	**19 (30%)**	**25 (39%)**
Ethnicity/Race		
Caucasian/ White	**61 (95%)**	**59 (92 %)**
African American/ Black	**3 (5%)**	**5 (8%)**
Mean Age (Standard Deviation)	**54 (± 12.6 years)**	**34 (± 14.2 years)**

Table 3: Population Outcome Comparison

Population	Year of Measure	Number of Functioning Grafts (% of Total Population)	Number of Non-Functioning Grafts (% of Total Population)
Tennessee	1 year	624 (90%)	70 (10%)
Tennessee	3 years	373 (82%)	82 (18%)
TNUK	1 year	62 (97%)	2 (3%)
TNUK	3 years	58 (94%)	4 (6%)

Table 4: Statewide Population: Univariate Analysis of Relationship Between Categorical Predictor Variables and Graft Function at 1 and 3 years Post-Transplant

Predictor Variable	Year Outcome Measured (Non-Functioning)	Pearson chi-squared (x^2)	Likelihood Ratio (Λ)	Cramer's V	Exact Sig. (2-sided)
PRA	1 year	2.141*	1.923	0.056	0.166
PRA	3 years	2.008*	1.895	0.067	0.150
Recipient Gender	1 year	3.423	3.300	0.070	0.081
Recipient Gender	3 years	0.214	0.212	0.022	0.698
Recipient Ethnicity	1 year	1.158	1.150	0.041	0.310
Recipient Ethnicity	3 years	0.669	0.669	0.038	0.464
DRDQ loci	1 year	3.218	3.460	0.068	0.090
DRDQ loci	3 years	4.414	4.721	0.098	0.041
AB loci	1 year	0.952	1.010	0.037	0.341
AB loci	3 years	0.895	0.947	0.044	0.389
Donor Gender	1 year	9.939	9.530	0.120	0.002
Donor Gender	3 years	1.468	1.448	0.057	0.258
Donor Ethnicity	1 year	0.178	0.173	0.016	0.727
Donor Ethnicity	3 years	0.249	0.255	0.023	0.630

Note: * more than 20% of cells had expected counts less than 5. Chi-square not valid.

Table 5: Frequency of Graft Outcomes at 1 year for Donor Gender (Statewide)

		Donor Gender		
		Male	**Female**	**Total**
Functioning at 1 Year	Count	413	211	624
	Expected Count	401.0	223.0	624.0
	% within Function 1 yr	66.2%	33.8%	100.0%
	% within Donor Gender	92.6%	85.1%	89.9%
	% of Total	59.5%	30.4%	89.9%
	Std. Residual	0.6	-0.8	
Non-functioning at 1 Year	Count	33	37	70
	Expected Count	45.0	25.0	70.0
	% within Function 1 yr	47.1%	52.9%	100.0%
	% within Donor Gender	7.4%	14.9%	10.1%
	% of Total	4.8%	5.3%	10.1%
	Std. Residual	-1.8	2.4	
Total	Count	446	248	694
	Expected Count	446.0	248.0	694.0
	% within Function 1 yr	64.3%	35.7%	100.0%
	% within Donor Gender	100.0%	100.0%	100.0%
	% of Total	64.3%	35.7%	100.0%

Table 6: Frequency of Graft Outcomes at 3 years for DRDQ matching (Statewide)

		DRDQ		
		mismatch	**match**	**Total**
Functioning at 3 years	Count	262	111	373
	Expected Count	269.7	103.3	373.0
	% within Function 3 yr	70.2%	29.8%	100.0%
	% within DRDQ	79.6%	88.1%	82.0%
	% of Total	57.6%	24.4%	82.0%
	Std. Residual	-0.5	0.8	
Non-functioning at 3 years	Count	67	15	82
	Expected Count	59.3	22.7	82.0
	% within Function 3 yr	81.7%	18.3%	100.0%
	% within DRDQ	20.4%	11.9%	18.0%
	% of Total	14.7%	3.3%	18.0%
	Std. Residual	1.0	-1.6	
Total	Count	329	126	455
	Expected Count	329.0	126.0	455.0
	% within Function 3 yr	72.3%	27.7%	100.0%
	% within DRDQ	100.0%	100.0%	100.0%
	% of Total	72.3%	27.7%	100.0%

Table 7: TNUK population: Univariate Analysis of Relationship Between Categorical Predictor Variables and Graft Function at 1 and 3 years Post-Transplant

Predictor Variable	Year Outcome Measured (Non-Functioning)	Pearson chi-squared (x^2)	Likelihood Ratio (Λ)	Cramer's V	Exact Sig. (2-sided)
PRA	1 year	4.175*	2.802a	0.255	0.141
PRA	3 years	9.522*	6.784b	0.392	0.010
Recipient Gender	1 year	0.872*	1.436	0.117	0.576
Recipient Gender	3 years	0.034*	0.035	0.023	1.000
Recipient Ethnicity	1 year	0.102*	0.195	0.040	1.000
Recipient Ethnicity	3 years	0.217*	0.411	0.059	1.000
DRDQ loci	1 year	0.632*	1.088c	0.099	1.000
DRDQ loci	3 years	1.365*	2.303d	0.148	0.564
AB loci	1 year	0.295*	0.543	0.068	1.000
AB loci	3 years	0.633*	1.145	0.101	0.644
Donor Gender	1 year	1.323*	2.022	0.144	0.516
Donor Gender	3 years	0.417*	0.442	0.082	0.642
Donor Ethnicity	1 year	5.102*	2.685	0.282	0.151
Donor Ethnicity	3 years	1.654*	1.153	0.163	0.292
MICA Abs	1 year	0.338*	0.617	0.073	1.000
MICA Abs	3 years	0.379*	0.327	0.078	1.000
KIR	1 year	2.654*	3.390	0.204	0.187
KIR	3 years	5.543*	7.011	0.299	0.031
MICA matching	1 year	0.277*	0.260	0.066	1.000
MICA matching	3 years	0.150*	0.158	0.049	1.000

Note: Exact Sig. (2-sided): a) p = 0.149; b) p = 0.012; c) p =0.635; d) p = 0.361**;** * more than 20% of cells had expected counts less than 5. Chi-square not valid.

Table 8: Frequency of Graft Outcomes at 3 years for PRA (TNUK)

		PRA 0-19%	PRA 20-39%	PRA 40-79%	PRA > 80%	Total
Functioning at 3 years	Count	42	4	6	6	58
	Expected Count	40.2	5.6	5.6	6.5	58.0
	% within Function 3 yr	72.4%	6.9%	10.3%	10.3%	100.0%
	% within PRA	97.7%	66.7%	100.0%	85.7%	93.5%
	% of Total	67.7%	6.5%	9.7%	9.7%	93.5%
	Std. Residual	0.3	-0.7	0.2	-0.2	
Non-functioning at 3 years	Count	1	2	0	1	4
	Expected Count	2.8	0.4	0.4	0.5	4.0
	% within Function 3 yr	25.0%	50.0%	0.0%	25.0%	100.0%
	% within PRA	2.3%	33.3%	0.0%	14.3%	6.5%
	% of Total	1.6%	3.2%	0.0%	1.6%	6.5%
	Std. Residual	-1.1	2.6	-0.6	0.8	
Total	Count	43	6	6	7	62
	Expected Count	43.0	6.0	6.0	7.0	62.0
	% within Function 3 yr	69.4%	9.7%	9.7%	11.3%	100.0%
	% within PRA	100.0%	100.0%	100.0%	100.0%	100.0%
	% of Total	69.4%	9.7%	9.7%	11.3%	100.0%

Table 9: Frequency of Graft Outcomes at 3 years for KIR (TNUK)

		KIR		
		Balanced	Excess Inhibitory	Total
Functioning at 3 years	Count	23	35	58
	Expected Count	25.3	32.7	58.0
	% within Function 3 yr	39.7%	60.3%	100.0%
	% within KIR	85.2%	100.0%	93.5%
	% of Total	37.1%	56.5%	93.5%
	Std. Residual	-0.4	0.4	
Non-functioning at 3 years	Count	4	0	4
	Expected Count	1.7	2.3	4.0
	% within Function 3 yr	100.0%	0.0%	100.0%
	% within KIR	14.8%	0.0%	6.5%
	% of Total	6.5%	0.0%	6.5%
	Std. Residual	1.7	-1.5	
Total	Count	27	35	62
	Expected Count	27.0	35.0	62.0
	% within Function 3 yr	43.5%	56.5%	100.0%
	% within KIR	100.0%	100.0%	100.0%
	% of Total	43.5%	56.5%	100.0%

Table 10: TNUK population: Correlation of 12 month Creatinine Level with Soluble CD30 level, Recipient Age, and Donor Age (Kendall's tau test)

		sCD30	Recipient Age	Donor Age	12 mo Creatinine
sCD30	Correlation Coefficient	1.000	-0.110	-0.016	-0.029
	Sig. (2-tailed)	.	0.208	0.857	0.746
	N	64	64	64	62
Recipient Age	Correlation Coefficient	-0.110	1.000	0.019	-0.216*
	Sig. (2-tailed)	0.208	.	0.830	0.018
	N	64	64	64	62
Donor Age	Correlation Coefficient	-0.016	0.019	1.000	0.173
	Sig. (2-tailed)	0.857	0.830	.	0.056
	N	64	64	64	62
12 mo. Creatinine	Correlation Coefficient	-0.029	-0.216*	0.173	1.000
	Sig. (2-tailed)	0.746	0.018	0.056	.
	N	62	62	62	62

Note: * Correlation is significant at the 0.05 level (2-tailed).

Table 11: TNUK population: Comparison of 12 month Creatinine Levels Across Different Categories of Nominal Variables (Mann-Whitney and Kolmogorov-Smirnov Z Tests)

Grouping Variable	Mann-Whitney U	Mann-Whitney U Sig.	Kolmogorov-Smirnov Test	Kolmogorov-Smirnov Sig.
PRA	*NA*	*NA*	*NA*	*NA*
Recipient Gender	276.00	0.042*	1.00	0.266
Recipient Ethnicity	91.00	0.934	0.54	0.929
DRDQ loci	182.50	0.005*	1.24	0.091
AB loci	165.00	0.282	0.84	0.476
Donor Gender	480.00	0.801	0.61	0.857
Donor Ethnicity	122.00	0.863	0.72	0.683
MICA Abs	336.00	0.050	1.00	0.264
KIR	417.00	0.465	0.75	0.632
MICA matching	507.50	0.186	0.75	0.636

Note: *Significant at the 0.05 level (2-tailed).

Table 12: TNUK population: Comparison of 12 month Creatinine Levels Across Different Categories of Nominal Variables (Jonckheere-Terpstra and Kruskal-Wallis Tests)

Grouping Variable	Jonckheere-Terpstra Test (standardized)	Jonckheere-Terpstra Sig.	Kruskal-Wallis Test	Kruskal-Wallis Sig.
PRA	-0.39	0.742	4.26	0.235
Recipient Gender	-2.03	0.042*	4.13	0.042*
Recipient Ethnicity	0.08	0.934	0.01	0.934
DRDQ loci	-2.81	0.005*	7.88	0.005*
AB loci	-1.08	0.282	1.16	0.282
Donor Gender	0.25	0.801	0.06	0.801
Donor Ethnicity	0.17	0.863	0.03	0.863
MICA Abs	1.96	0.050	3.83	0.050
KIR	-0.73	0.465	0.53	0.465
MICA matching	1.32	0.186	1.75	0.186

Note: *Significant at the 0.05 level (2-tailed).

Table 13: Twelve Month Creatinine Levels for Each Category of Nominal Variables

Predictor Variable/ Group	Number of Patients in Group (n)	Mean Creatinine level (mg/dL)	SD	95% C.I.
PRA 0-19%	44	1.45	0.39	1.33-1.57
PRA 20-39%	5	1.80	0.61	1.04-2.56
PRA 40-79%	6	1.55	0.53	0.99-2.11
PRA > 80%	7	1.23	0.37	0.88-1.58
Male Recipient	43	1.53	0.45	1.40-1.67
Female Recipient	19	1.30	0.35	1.13-1.46
Caucasian Recipient	59	1.46	0.44	1.35-1.58
African American Recipient	3	1.43	0.29	0.72-2.15
DRDQ mismatch	47	1.54	0.44	1.41-1.67
DRDQ match	15	1.21	0.31	1.04-1.38
AB mismatch	54	1.49	0.43	1.37-1.60
AB match	8	1.30	0.40	0.97-1.64
MICA Abs absent	53	1.42	0.41	1.30-1.53
MICA Abs present	9	1.73	0.46	1.38-2.09
KIR balanced	26	1.54	0.52	1.33-1.74
KIR inhibitory	36	1.41	0.35	1.29-1.53
Male Donor	37	1.45	0.43	1.31-1.59
Female Donor	25	1.48	0.44	1.30-1.66
Caucasian Donor	58	1.46	0.44	1.35-1.59
African American Donor	4	1.45	0.31	0.96-1.94
MICA mismatch	42	1.42	0.42	1.29-1.55
MICA match	20	1.56	0.46	1.34-1.77

Table 14: Model Goodness of Fit Summary

Model	Model df	Model Chi-square	Model Sig	-2 Log likelihood	Cox & Snell R Square	Nagelkerke R Square
1	11	19.159	0.058	425.252	0.027	0.058
2	3	16.796	0.001**	427.616	0.024	0.051
3	11	9.174	0.606	412.813	0.020	0.033
4	1	4.105	0.043**	417.882	0.009	0.015
5	11	8.689	0.651	9.111	0.127	0.523
6	1	-2.658	0.103	17.800	0.000	0.000
7	11	13.094	0.287	16.569	0.190	0.501
8	4	10.151	0.038**	19.512	0.151	0.397
9	15	17.800	0.273	0.000	0.243	1.000
10	7	17.800	0.013**	0.000	0.243	1.000
11	15	29.663	0.013**	0.000	0.380	1.000
12	11	29.663	0.002**	0.000	0.380	1.000

Note: **Significant at the 0.05 level (2-tailed).

Table 15: Model Predictive Ability Summary

Model	Sensitivity %	Specificity %	Positive Predictive Value %	Negative Predictive Value %	False Positive Rate %	False Negative Rate %
1	100	0	90.2	NA**	9.8	NA**
2	100	0	90.2	NA**	9.8	NA**
3	100	0	82.3	NA**	17.7	NA**
4	100	0	82.3	NA**	17.7	NA**
5	100	50	98.4	100	1.6	0
6	100	0	96.9	NA**	3.1	NA**
7	100	50	96.7	100	3.3	0
8	95.6	50	95.6	50	3.4	50
9	100	100	100	100	0	0
10	100	100	100	100	0	0
11	100	100	100	100	0	0
12	100	100	100	100	0	0

Note: ** Unable to calculate. Zero denominator.

Table 16: Model 1: Traditional Scoring Model (Full Model) for Non-Functioning at 1 year. Statewide Population.

	B	S.E.	Wald	df	Sig.	Odds Ratio	95% C.I.for Odds Ratio Lower	Upper
PRA			1.480	3	0.687			
pra 20-39%	0.036	0.571	0.004	1	0.950	1.036	0.338	3.173
pra 40-79%	0.469	0.410	1.311	1	0.252	1.599	0.716	3.568
pra > 80%	0.289	0.519	0.310	1	0.578	1.335	0.483	3.692
Recipient Age	-0.002	0.011	0.019	1	0.890	0.998	0.977	1.021
Recipient Female	0.421	0.278	2.295	1	0.130	1.523	0.884	2.625
Recipient African American	0.142	0.280	0.257	1	0.612	1.152	0.666	1.994
DRDQ match	-0.454	0.364	1.560	1	0.212	0.635	0.311	1.295
AB match	-0.177	0.414	0.182	1	0.670	0.838	0.372	1.886
Donor Age	0.005	0.009	0.278	1	0.598	1.005	0.987	1.023
Donor Female	0.817*	0.264	9.582	1	0.002	2.265	1.350	3.800
Donor African American	0.186	0.356	0.273	1	0.601	1.205	0.599	2.422
Constant	-2.846*	0.700	16.512	1	0.000	0.058		

R^2= 0.03(Cox & Snell), 0.06(Nagelkerke). Model $x^2(11) = 19.16$, $p = 0.058$. * $p < 0.05$

NOTE: B is the coefficient of the variable. S.E. is the standard error of the coefficient. Wald is the Wald statistic value. d.f. is the degrees of freedom. Sig. is the significance for the variable (p value).

Table 17: Model 2: Traditional Scoring Model (Backwards Stepwise) for Non-Functioning at 1 year. Statewide Population.

	B	S.E.	Wald	df	Sig.	Odds Ratio	95% C.I.for Odds Ratio Lower	Upper
Recipient Female	0.480	0.264	3.314	1	0.069	1.616	0.964	2.709
DRDQ match	-0.558	0.325	2.952	1	0.086	0.572	0.303	1.082
Donor Female	0.835	0.259	10.395	1	0.001	2.305	1.387	3.830
Constant	-2.629	0.223	139.149	1	0.000	0.072		

R^2= 0.02(Cox & Snell), 0.05(Nagelkerke). Model $x^2(3) = 16.80$, p = 0.001. * p < 0.05
NOTE: B is the coefficient of the variable. S.E. is the standard error of the coefficient. Wald is the Wald statistic value. d.f. is the degrees of freedom. Sig. is the significance for the variable (p value).

Table 18: Model 3: Traditional Scoring Model (Full Model) for Non-Functioning at 3 years. Statewide Population.

	B	S.E.	Wald	df	Sig.	Odds Ratio	95% C.I.for Odds Ratio Lower	Upper
PRA			1.986	3	0.575			
pra 20-39%	0.252	0.542	0.216	1	0.642	1.287	0.445	3.723
pra 40-79%	0.454	0.391	1.346	1	0.246	1.575	0.731	3.392
pra > 80%	0.509	0.553	0.847	1	0.357	1.664	0.563	4.918
Recipient Age	0.002	0.011	0.041	1	0.839	1.002	0.981	1.024
Recipient Female	0.020	0.282	0.005	1	0.945	1.020	0.586	1.774
Recipient African American	0.165	0.271	0.371	1	0.542	1.180	0.693	2.007
DRDQ match	-0.604	0.362	2.783	1	0.095	0.547	0.269	1.111
AB match	-0.011	0.465	0.001	1	0.981	0.989	0.397	2.462
Donor Age	0.005	0.009	0.342	1	0.559	1.005	0.988	1.023
Donor Female	0.319	0.255	1.562	1	0.211	1.375	0.834	2.266
Donor African American	-0.156	0.360	0.187	1	0.665	0.856	0.423	1.732
Constant	-1.961*	0.681	8.293	1	0.004	0.141		

R^2= 0.02(Cox & Snell), 0.03(Nagelkerke). Model $x^2(11) = 9.17$, $p = 0.606$. * $p < 0.05$
NOTE: B is the coefficient of the variable. S.E. is the standard error of the coefficient. Wald is the Wald statistic value. d.f. is the degrees of freedom. Sig. is the significance for the variable (p value).

Table 19: Model 4: Traditional Scoring Model (Backward Stepwise) for Non-Functioning at 3 years. Statewide Population.

	B	S.E.	Wald	df	Sig.	Odds Ratio	95% C.I.for Odds Ratio Lower	Upper
DRDQ match	-0.598	0.308	3.772	1	0.052	0.550	0.300	1.006
Constant	-1.394*	0.139	101.197	1	0.000	0.248		

R^2= 0.01(Cox & Snell), 0.02(Nagelkerke). Model $x^2(1) = 4.11$, $p = 0.043$. * $p < 0.05$
NOTE: B is the coefficient of the variable. S.E. is the standard error of the coefficient. Wald is the Wald statistic value. d.f. is the degrees of freedom. Sig. is the significance for the variable (p value).

Table 20: Model 5: Traditional Scoring Model (Full Model) for Non-Functioning at 1 year. TNUK Population.

	B	S.E.	Wald	df	Sig.	Odds Ratio	95% C.I.for Odds Ratio Lower	Upper
PRA			0.261	3	0.967			
pra 20-39%	0.963	1.884	0.261	1	0.609	2.619	0.065	105.212
pra 40-79%	-17.345	14036.550	0.000	1	0.999	0.000	0.000	.
pra > 80%	-16.206	12470.524	0.000	1	0.999	0.000	0.000	.
Recipient Age	-0.046	0.078	0.347	1	0.556	0.955	0.821	1.112
Recipient Female	-17.842	6939.659	0.000	1	0.998	0.000	0.000	.
Recipient African American	-18.347	21721.312	0.000	1	0.999	0.000	0.000	.
DRDQ match	-18.076	7839.011	0.000	1	0.998	0.000	0.000	.
AB match	-15.834	9733.612	0.000	1	0.999	0.000	0.000	.
Donor Age	0.003	0.074	0.001	1	0.971	1.003	0.867	1.159
Donor Female	-17.072	6408.956	0.000	1	0.998	0.000	0.000	.
Donor African American	3.030	2.731	1.231	1	0.267	20.700	0.098	4367.788
Constant	-0.700	3.627	0.037	1	0.847	0.497		

R^2= 0.13(Cox & Snell), 0.52(Nagelkerke). Model $x^2(11) = 8.69$, p = 0.651. * p < 0.05
NOTE: B is the coefficient of the variable. S.E. is the standard error of the coefficient. Wald is the Wald statistic value. d.f. is the degrees of freedom. Sig. is the significance for the variable (p value).

Table 21: Model 6: Traditional Scoring Model (Backward Stepwise) for Non-Functioning at 1 year. TNUK Population.

	B	S.E.	Wald	df	Sig.	Odds Ratio	95% C.I.for Odds Ratio Lower	Upper
Constant	-3.434*	0.718	22.848	1	0.000	0.032		

R^2= 0.00(Cox & Snell), 0.00(Nagelkerke). Model $x^2(1)$ = -2.67, p = 0.103. * p < 0.05
NOTE: B is the coefficient of the variable. S.E. is the standard error of the coefficient. Wald is the Wald statistic value. d.f. is the degrees of freedom. Sig. is the significance for the variable (p value).

Table 22: Model 7: Traditional Scoring Model (Full Model) for Non-Functioning at 3 years. TNUK Population.

	B	S.E.	Wald	df	Sig.	Odds Ratio	95% C.I.for Odds Ratio Lower	Upper
PRA			5.971	3	0.113			
pra 20-39%	3.466*	1.425	5.913	1	0.015	32.000	1.959	522.756
pra 40-79%	-17.488	15867.422	0.000	1	0.999	0.000	0.000	.
pra > 80%	2.079	1.510	1.895	1	0.169	8.000	0.414	154.429
DRDQ match	-19.301	9259.240	0.000	1	0.998	0.000	0.000	.
Constant	-3.466*	1.016	11.647	1	0.001	0.031		

R^2= 0.19(Cox & Snell), 0.50(Nagelkerke). Model $x^2(11)$ = 13.1, p = 0.287. * p < 0.05
NOTE: B is the coefficient of the variable. S.E. is the standard error of the coefficient. Wald is the Wald statistic value. d.f. is the degrees of freedom. Sig. is the significance for the variable (p value).

Table 23: Model 8: Traditional Scoring Model (Backward Stepwise) for Non-Functioning at 3 years. TNUK Population.

	B	S.E.	Wald	df	Sig.	Odds Ratio	95% C.I.for Odds Ratio Lower	Upper
PRA			5.971	3	0.113			
pra 20-39%	3.466*	1.425	5.913	1	0.015	32.000	1.959	522.756
pra 40-79%	-17.488	15867.422	0.000	1	0.999	0.000	0.000	.
pra > 80%	2.079	1.510	1.895	1	0.169	8.000	0.414	154.429
DRDQ match	-19.301	9259.240	0.000	1	0.998	0.000	0.000	.
Constant	-3.466*	1.016	11.647	1	0.001	0.031		

R^2= 0.15(Cox & Snell), 0.40(Nagelkerke). Model $x^2(4)$ = 10.2, p = 0.038. * p < 0.05
NOTE: B is the coefficient of the variable. S.E. is the standard error of the coefficient. Wald is the Wald statistic value. d.f. is the degrees of freedom. Sig. is the significance for the variable (p value).

Table 24: Model 9: Expanded Scoring Model (Full Model) for Non-Functioning at 1 year. TNUK Population.

	B	S.E.	Wald	df	Sig.	Odds Ratio	95% C.I.for Odds Ratio Lower	Upper
PRA			0.000	3	1.000			
pra 20-39%	-30.566	10480.299	0.000	1	0.998	0.000	0.000	.
pra 40-79%	-1.725	14071.026	0.000	1	1.000	0.178	0.000	.
pra > 80%	-25.760	25933285.283	0.000	1	1.000	0.000	0.000	.
Recipient Age	0.227	1458.146	0.000	1	1.000	1.254	0.000	.
Recipient Female	-58.708	55179.497	0.000	1	0.999	0.000	0.000	.
Recipient African American	0.003	23068.797	0.000	1	1.000	1.003	0.000	.
DRDQ match	-2.975	14548.576	0.000	1	1.000	0.051	0.000	.
AB match	53.322	25933175.662	0.000	1	1.000	1.44E23	0.000	.
Donor Age	-0.070	552.888	0.000	1	1.000	0.933	0.000	.
Donor Female	-35.155	12903.481	0.000	1	0.998	0.000	0.000	.
Donor African American	60.141	38418.279	0.000	1	0.999	1.32E26	0.000	.
sCD30	0.002	13.211	0.000	1	1.000	1.002	0.000	1.76E11
MICA abs present	-1.882	11688.414	0.000	1	1.000	0.152	0.000	.
KIR inhibitory	-101.391	22567.298	0.000	1	0.996	0.000	0.000	.
MICA match	35.629	16043.016	0.000	1	0.998	2.98E15	0.000	.
Constant	-30.459	83087.442	0.000	1	1.000	0.000		

R^2= 0.24(Cox & Snell), 1.00(Nagelkerke). Model $x^2(15) = 17.8$, $p = 0.273$. * $p < 0.05$
NOTE: B is the coefficient of the variable. S.E. is the standard error of the coefficient. Wald is the Wald statistic value. d.f. is the degrees of freedom. Sig. is the significance for the variable (p value).

Table 25: Model 10: Expanded Scoring Model (Backward Stepwise) for Non-Functioning at 1 year. TNUK Population.

	B	S.E.	Wald	df	Sig.	Odds Ratio	95% C.I.for Odds Ratio Lower	Upper
Recipient Age	2.122	169.802	0.000	1	0.990	8.349	0.000	2.87E145
Recipient Female	-20.262	10431.010	0.000	1	0.998	0.000	0.000	.
AB match	-40.176	14005.141	0.000	1	0.998	0.000	0.000	.
Donor Female	-48.844	5706.225	0.000	1	0.993	0.000	0.000	.
Donor African American	51.074	5060.138	0.000	1	0.992	1.52E22	0.000	.
KIR inhibitory	-129.173	9050.949	0.000	1	0.989	0.000	0.000	.
MICA match	83.081	5882.552	0.000	1	0.989	1.21E36	0.000	.
Constant	-161.625	11885.134	0.000	1	0.989	0.000		

R^2= 0.24(Cox & Snell), 1.00(Nagelkerke). Model $x^2(7) = 17.8$, $p = 0.013$. * $p < 0.05$
NOTE: B is the coefficient of the variable. S.E. is the standard error of the coefficient. Wald is the Wald statistic value. d.f. is the degrees of freedom. Sig. is the significance for the variable (p value).

Table 26: Model 11: Expanded Scoring Model (Full Model) for Non-Functioning at 3 years. TNUK Population.

	B	S.E.	Wald	df	Sig.	Odds Ratio	95% C.I. for Odds Ratio Lower	Upper
PRA			0.001	3	1.000			
pra 20-39%	94.909	3192.120	0.001	1	0.976	1.65E41	0.000	.
pra 40-79%	212.096	15978.321	0.000	1	0.989	1.29E92	0.000	.
pra > 80%	253.078	7945.731	0.001	1	0.975	8.14E109	0.000	.
Recipient Age	7.983	273.906	0.001	1	0.977	2929.718	0.000	4.13E236
Recipient Female	39.662	3266.925	0.000	1	0.990	1.68E17	0.000	.
Recipient African American	282.608	21371.097	0.000	1	0.989	5.43E122	0.000	.
DRDQ match	-11.588	14000.454	0.000	1	0.999	0.000	0.000	.
AB match	-461.494	21563.194	0.000	1	0.983	0.000	0.000	.
Donor Age	-1.503	73.209	0.000	1	0.984	0.222	0.000	4.60E61
Donor Female	-118.864	4167.370	0.001	1	0.977	0.000	0.000	.
Donor African American	102.272	4001.572	0.001	1	0.980	2.61E44	0.000	.
sCD30	-0.079	2.733	0.001	1	0.977	0.924	0.004	195.739
MICA abs present	-14.305	1884.269	0.000	1	0.994	0.000	0.000	.
KIR inhibitory	-325.162	10160.988	0.001	1	0.974	0.000	0.000	.
MICA match	194.282	6411.107	0.001	1	0.976	2.38E84	0.000	.
Constant	-370.636	13479.376	0.001	1	0.978	0.000		

R^2= 0.38(Cox & Snell), 1.00(Nagelkerke). Model $x^2(15)$ = 29.7, p = 0.013. * p < 0.05
NOTE: B is the coefficient of the variable. S.E. is the standard error of the coefficient. Wald is the Wald statistic value. d.f. is the degrees of freedom. Sig. is the significance for the variable (p value).

Table 27: Model 12: Expanded Scoring Model (Backward Stepwise) for Non-Functioning at 3 years. TNUK Population.

	B	S.E.	Wald	df	Sig.	Odds Ratio	95% C.I.for Odds Ratio Lower	Upper
PRA			0.002	3	1.000			
pra 20-39%	104.615	2737.293	0.001	1	0.970	2.71E45	0.000	.
pra 40-79%	184.231	7782.417	0.001	1	0.981	1.02E80	0.000	.
pra > 80%	336.529	8543.255	0.002	1	0.969	1.42E146	0.000	.
Recipient Age	6.549	175.970	0.001	1	0.970	698.268	0.000	4.27E152
AB match	-540.569	15533.469	0.001	1	0.972	0.000	0.000	.
Donor Female	-148.864	4204.860	0.001	1	0.972	0.000	0.000	.
Donor African American	179.077	4649.711	0.001	1	0.969	5.92E77	0.000	.
sCD30	-0.108	2.871	0.001	1	0.970	0.897	0.003	249.438
MICA abs present	-37.458	1643.244	0.001	1	0.982	0.000	0.000	.
KIR inhibitory	-313.185	8619.757	0.001	1	0.971	0.000	0.000	.
MICA match	212.493	5609.826	0.001	1	0.970	1.93E92	0.000	.
Constant	-329.339	9187.545	0.001	1	0.971	0.000		

R^2= 0.38(Cox & Snell), 1.00(Nagelkerke). Model $x^2(11) = 29.7$, p = 0.002. * p < 0.05
NOTE: B is the coefficient of the variable. S.E. is the standard error of the coefficient. Wald is the Wald statistic value. d.f. is the degrees of freedom. Sig. is the significance for the variable (p value).

VITA

Christina Bishop graduated from the University of Tennessee, Knoxville, in 2001 with a Bachelors of Science degree in Medical Technology. She was certified as a medical technologist (MT)/ clinical laboratory scientist (CLS) by both the American Society of Clinical Pathologist (ASCP) and the National Credentialing Agency for Laboratory Personnel (NCA) in 2001. In October 2003, she became a Certified Histocompatibility Technologist (CHT) (The American Board of Histocompatibility and Immunogenetics--ABHI) and joined the American Society of Histocompatibility and Immunogenetics (ASHI), of which she is still a member. Christina was the recipient of the 2004 ASHI/ SEOPF J. Marilyn MacQueen Award for outstanding CHT in the field less than 3 years. She became a Tennessee state licensed laboratory supervisor in 2004. In 2007 she accepted a graduate research assistantship at the University of Tennessee, Knoxville, in the Comparative and Experimental Medicine Program, Department of Surgery, Transplant Lab. She is currently employed as Associate Director by Dialysis Clinics, Inc, for whom she has worked full-time since 2002. Christina graduated with a Doctor of Philosophy degree in Comparative and Experimental Medicine in August 2011. She is continuing her career in transplant immunology and will serve as Director of the DCI Transplant Lab upon completion of her ABHI Director's certification.