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Transplanting Against Histocompatibility Barriers

Dinesh Kannabhiran, Michelle L. Lubetzky and Darshana Dadhania

Additional information is available at the end of the chapter

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1. Introduction

Currently there are over 90,000 patients awaiting a kidney transplant in the United States. Despite the large number of wait-listed patients, just over 16,000 were transplanted in 2009 [1]. Mean waiting times for transplantation can range from 2 years to over 5 years depending on a variety of factors including blood type, sensitization status and deceased donor availability in the region. Despite the availability of a willing living donor, a patient may be forced to remain on the deceased donor waiting list because of ABO incompatibility (ABOi) and/or the presence of a positive crossmatch due to donor specific anti-HLA antibodies. Those with blood O and B tend to have the longest wait times (median waiting time for O:1852 days; A:1208 days; B:1937 days; AB: 855 days) and may also have difficulty finding a compatible living donor [2]. Sensitized patients are those with high levels of anti-HLA (Human Leukocyte Antigen) antibodies in their blood. These antibodies form as a result of exposure to foreign HLA during pregnancy, transfusions and exposure to previous organ transplants. Approximately 16% of patients on the waiting list are re-transplants. Patients who are sensitized (Panel Reactive Antibody, PRA 10% or greater) make up roughly 25% of the current waiting list, 16% of which have a PRA >80% [1]. In some cases, a highly sensitized patient can wait up to 10 years before being transplanted. These wait times a negative impact on patients as the morbidity and mortality of dialysis patients are higher compared to kidney transplant recipients[3]. In 2010 over 5,000 patients died while waiting to receive a kidney transplant [1]. Given the increased mortality associated with dialysis, renal transplantation remains the preferred treatment for patients with end stage renal disease [4].

Historically, ABO compatibility has been a requirement for renal transplantation due to high rates of rejection seen in ABO incompatible transplants. In blood group compatible transplants, a negative T cell CDC (complement dependent cytotoxicity) crossmatch with a donor is required for kidney transplantation. It is not only difficult to find a compatible

donor for the highly sensitized patients, but also difficult to achieve good long-term graft survival due to higher rates of acute and chronic rejection. In the last two decades, technology for assessment of histocompatibility barriers (ABO and anti-HLA antibodies) have advanced significantly. These new assays (advanced flow cytometry crossmatches, ELISA crossmatches, solid phase anti-HLA antibody assays) have improved our ability to determine the risk of acute rejection in the presence of these types of histocompatibility barriers. Complementary to these innovations, there have been great advancements in therapeutic options to remove and decrease production of anti-AB and anti-HLA antibodies for treatment of acute and chronic antibody mediated rejection and for reducing the risk of acute rejection episodes by providing treatments to modulate the immune response prior to transplantation. In this section, we will review the data on kidney transplantation in the ABO incompatible and crossmatch incompatible recipient-donor pairs.

2. Current therapies used for preparing patients for transplantation using donors with ABO and crossmatch incompatibility

In order to effectively reduce the immune response, a multi-faceted approach must be used for removal of antibodies prior to proceeding with an ABO or crossmatch incompatible transplant. For crossmatch incompatible patients, treatment protocols have been referred to as “desensitization” protocols. We will refer to these protocols as immunomodulatory or pre-transplant conditioning protocols since one is not actually changing the “sensitized” status of the patient. Figure 1 provides the basic mechanisms that are addressed to modulate the immune system of a potential recipient of an ABO or crossmatch incompatible organ. Splenectomy and cytotoxic therapies such as rituximab and bortezomib decrease the size of B and plasma cell clones. Physical removal of circulating antibodies is accomplished by procedures such as plasmapheresis or double filtration or immunoadsorption. Other additional immunosuppressive medications as well as IVIG via Fc receptor signaling impair the ability of cells to make antibodies. IVIG may also directly inhibit the function of circulating antibodies. A brief overview of the mechanisms is provided below and an in-depth review of immunosuppressive medications used in kidney transplantation is provided in Chapter 9.

2.1. Plasmapheresis (PP) and Immunoabsorption (IA)

Physical removal of antibodies can be accomplished by several different procedures such as conventional plasmapheresis, double-filtration plasmapheresis, semi-selective and antigen-specific immunoabsorption. Treatment with these procedures leads to serial reduction in antibody titer. However, without additional therapies to prevent production of these antibodies, the antibody titers rebound and return to baseline values once the treatments have stopped.

Plasmapheresis is the most commonly used method in the United States. It is a procedure in which the blood passes through a medical device that separates plasma from cellular

components of blood allowing for physical removal of plasma prior to returning the blood back to the patient. Patient's plasma volume that is removed is replaced with a replacement solution such as colloid solution (e.g. albumin and/or plasma) or a combination of crystalloid/colloid solution [5]. In Japan, double filtration plasmapheresis has been used. In this procedure, plasma is removed as for plasmapheresis and then passed through a second filter (plasma separator), whose smaller pore size traps only larger molecules like immunoglobulins. Thus, lower molecular weight plasma components can be passed back into the patient and less replacement fluid is needed. Although these procedures are not 100% efficient, sequential therapies allow for treatment of higher plasma volumes and greater reduction in antibody titers. These procedures are very effective for removal of antibodies against A and B blood group antigens and are associated with approximately 20% reduction in titer with each treatment. Common adverse reactions include hypocalcemia and pruritis seen in 5% of all patients [6].

Mechanisms Affected by Targeted Therapies:

1. Reduce the number of cells with potential for forming more antibodies



2. Physical removal of antibodies



3. Impair function of remaining antibodies



4. Impair the ability of antibody producing cells to make antibodies



Figure 1. Immune Modulation of ABO or Crossmatch Incompatible Recipients

Immunoabsorption is a procedure in which plasma of the patient, after separation from blood, passes through a column containing an active component that binds or removes immunoglobulins specifically [5]. A newer column for immunoabsorption was created by Glycores Transplantation AB (Lund, Sweden). This Glycosorb® ABO column has a matrix of sepharose beads coated with blood group A or B carbohydrate antigens that removes isohemagglutinins against the corresponding blood group. The Glycosorb® columns effectively remove anti-A or anti-B antibodies with approximately a 30% reduction in A/B

IgM and approximately a 20% A/B IgG levels after a single treatment. Importantly, titers of other antibodies, particularly those against *Pneumococcus*, *Hemophilus*, Diphtheria and tetanus seem largely unchanged [2, 7].

2.2. Splenectomy

Since the 1980s, splenectomy was performed in all successful ABOi kidney transplants to reduce the risk of rejection. The benefit for splenectomy was based on a small case series demonstrating a reversal of AMR (antibody mediated rejection) with splenectomy [8]. Since then, splenectomy had been a part of immunosuppressive regimen in most of the ABOi protocols and some early crossmatch incompatible protocols. The rationale was to physically remove the source of antibody producing cells and thus prevent rebound in antibody titer after plasmapheresis. In the past few years with the availability of new drugs such as rituximab, splenectomy is no longer a requirement for transplantation with incompatible donors.

2.3. Anti-CD20 antibody as surrogate for splenectomy

Rituximab, a monoclonal anti-CD20 antibody, was initially used for the treatment for Non Hodgkins lymphoma in 1997. Since then, it has been used in patients with immune complex mediated renal diseases and in kidney transplant recipients for treatment of rejection. It is a chimeric monoclonal antibody with human constant region and murine variable region that targets human CD20 molecule. CD20 is expressed on naive and mature activated B cells as well as some memory B cells. B cell depletion occurs via antibody dependent cytotoxicity and can be rapid, over 3-4 days and sustained, lasting for almost up to a year [9].

Tyden et al. [10] were first to demonstrate successful ABOi kidney transplantation in 4 patients with the use of single dose rituximab ($375\text{mg}/\text{m}^2$) in lieu of splenectomy. Since then, rituximab has been included in many immunosuppressive protocols for facilitating incompatible transplantation. The optimal dose of rituximab remains unknown. Toki [9] et al. studied the effect of B cell depletion with increasing doses of rituximab (10, 15, 35, 150, $300\text{ mg}/\text{m}^2$) in 5 patients. All but one dosage of rituximab ($10\text{ mg}/\text{m}^2$) was able to completely eliminate B cells from circulation in 30 days. However, depletion of circulating B cells may not correlate with depletion of B cells within the lymph nodes and/or spleen.

2.4. Proteasome inhibition to target plasma cells

Plasma cells produce antibodies within one week after antigen exposure in large volumes—approximately several thousand antibodies per second. The excess protein synthesis leads to increased number of misfolded protein accumulating in the endoplasmic reticulum and these proteins are naturally degraded by proteasomes. Degradation of these proteins via the ubiquitin-proteasome dependent pathways is important for maintaining cellular homeostasis. Bortezomib, the first drug of its kind, inhibits ubiquitin-proteasome pathway by binding to the 26S proteasome and promotes G2-M cell cycle arrest and cell apoptosis. Because of the potential for reducing antibody producing plasma cells, bortezomib has been

utilized with increased frequency for treatment of antibody mediated rejection and is being studied in conditioning regimens for recipients with incompatible donors [11, 12].

2.5. Intravenous immunoglobulin (IVIG)

IVIG is a commercial preparation of immune globulin which predominantly consists of intact IgG molecules and trace IgA obtained from pooled plasma from approximately 3000 to 10,000 healthy blood donors. These natural antibodies that are formed in the absence of immunization or foreign antigen exposure are thought to be essential in immunoregulatory effects. IVIG exerts its effect on immune systems through multiple mechanisms which include neutralization of circulating anti-HLA antibodies through anti-idiotypic antibodies, inhibition of complement activation, enhancing clearance of anti-HLA antibodies, negative signaling through Fc γ receptors, and selective down-regulation of antibody production. Two commonly used preparations are the pooled IVIG and the cytomegalovirus hyperimmune globulin (CMVIG). In the early 1990s there were both in vitro and in vivo studies that demonstrated high doses of IVIG could decrease PRA and increase rates of transplantation in highly sensitized individuals. IVIG is commonly administered following plasmapheresis to treat antibody mediated rejection and has been used in immunomodulatory therapies for ABOi and crossmatch incompatible transplants [13].

2.6. Other immunosuppressive therapies

Concurrent with these therapies, many protocols designed to precondition patients for incompatible transplants utilize standard maintenance immunosuppressive medications such as tacrolimus, mycophenolate mofetil and prednisone. The intention is to suppress the immune system and impair the ability of the immune cells to make more antibodies.

3. Renal transplantation with ABO incompatible donors

3.1. Patient assessment: ABO blood grouping

Blood group of an individual is defined by cell surface molecules that are present on all nucleated cells and platelets. Of the 30 blood group systems, the major blood group system is the ABO system, discovered by Karl Landsteiner in 1901. The blood group antigens consist of carbohydrates moieties attached to a glycosphingolipid and glycoprotein backbone. The major blood groups are A, B, AB and O and are defined by the type of molecules that are present on cell surface (A or B). Individuals develop naturally acquired antibodies against the molecules that are not present on their own cells. The antigenic stimulus for the development of these naturally occurring antibodies is believed to come from exposure to gut bacteria that express similar antigens.

These naturally occurring antibodies against A/B antigens are termed isohemagglutinins because of their ability to agglutinate cells (RBCs) that express the target molecules. This ability to cause agglutination in vivo results in acute thrombosis and inflammation when a recipient receives an ABO incompatible organ without any therapy to modulate the immune

response and reduce the number of circulating antibodies directed against the ABO group [14, 15]. In organ transplantation, ABO compatibility is the first step in determining the suitability of a donor for a particular recipient. Group A individuals express the A antigen on cell surface and produce antibodies against the non-expressed B antigen (anti B antibodies). Group B individuals express the B antigen on cell surface and produce anti A antibodies. Group AB individuals express both A and B antigens on cell surface and therefore do not produce either anti-A or anti-B antibodies. They are referred to as the “universal recipients” and can receive an organ from any individual. Individuals with blood group O express neither A or B antigen on cell surface and therefore produce antibodies against both A and B antigens. Given the lack of cell surface expression of A and B antigens, these individuals are considered “universal donors.” However because they express antibodies against both A and B antigens, they can only receive a donor from blood group O individuals.

Blood group A has two major subtypes, A1 and A2. A2 is expressed in approximately 20% of the US population and these individuals have lower cell surface expression of the blood group antigen compared to individuals with blood type A1. In addition, antibodies directed against the A antigen do not cause efficient agglutination of RBC from individuals with blood type A2. Given the lower cell surface expression of A2 antigen and less robust binding of anti- A to the A2 molecules resulting in less agglutination, donors with A2 subtype are considered to be less immunogenic. In fact, A2 donor kidneys have been transplanted to B recipients without the use of treatment protocols for immune modulation prior to transplantation [8], however, most patients do require some form of immunomodulatory therapy prior to transplantation [16]. The long-term outcomes were similar to that of ABO compatible transplantation [17].

3.1.1. Isohemagglutinin measurement

In addition to ABO grouping, the strength of the anti-A and anti-B titers need to be determined prior to initiating therapy for an ABOi kidney transplant. The presence of anti-A and anti-B causes hyperacute rejection within days to weeks in the setting of ABOi transplantation. These antibodies can be removed by plasmapheresis or immunoabsorption. Different centers use different goals for isohemagglutinin titers ranging from $<1/8$ to $<1/32$. Antibodies are measured using serial dilutions of a patient's sample, which are incubated for a period of time at 37°C with RBC aliquots with the appropriate blood group antigen. The sample is then checked for macroscopic agglutination of red blood cells (for IgM) or undergoes additional incubation with antihuman globulin to detect IgG agglutination [2]. The reagents for these tests are not standardized and therefore inter-laboratory variation does occur (up to 32-fold for IgM and 256-fold for IgG). ELISA and flow cytometry methods may be more accurate and reproducible [18].

3.2. Brief history of kidney transplantation with ABO incompatible donors

In 1954, Hume et al. [19] reported the first ABOi renal transplantation where the patient lost the graft on the 5th post-operative day. Though Starzl et al. [20] in 1964 reported 3 successful

kidney transplants across the ABO barrier, over the next 2 decades several case reports of unsuccessful attempts at kidney transplant across ABO antigens were published. Cooke et al. [21] reported a graft survival rate of 4% at 1 year in ABOi transplants. Therefore, ABO incompatibility generally was considered an absolute contraindication to kidney transplantation.

In 1981, Slapak et al. first reported the use of plasmapheresis (PP) as a tool to remove the anti-A and anti-B when a patient with blood group O inadvertently received a kidney from a donor with blood group A. Acute antibody mediated rejection was reversed with PP and the patient had normal renal function 20 months after transplantation. Six years later, Alexandre et al. [22] reported the successful outcome of ABOi transplantation in 26 patients with splenectomy and PP. The overall outcome of ABO incompatible transplants has improved over the years with the introduction of PP and more potent immunosuppressive therapies including the use of poly and monoclonal antibody therapies. As a result, splenectomy is no longer a required procedure for ABO incompatible kidney transplants. Today, ABO incompatible transplants are routinely performed in Japan where approximately 20% of living donor recipients receive a kidney from an ABO incompatible donor. The option of receiving an ABO incompatible transplant is being utilized more frequently in other countries as well.

3.3. Modulation of the immune response in recipients with ABO incompatible donors

A review of the major immunosuppressive protocols that have been studied to facilitate ABO incompatible transplants is summarized in Table 1. Most studies utilized some form of antibody removal via PP, double-filtration or immunoadsorption and the target anti-A/B titer was between 1:8 and 1:32. In the '80s and '90s, splenectomy was commonly performed in recipients of ABO incompatible transplants. In 2004, Squifflet et al. found that the outcome of splenectomized ABOi living related transplant recipients was similar to the outcome of ABO compatible living related transplant recipients maintained on cyclosporine based immunosuppression [23].

Overall, rejection rates were relatively high (29.3%- 58%), in patients who underwent splenectomy as part of the treatment protocol (Table 1- #1-3) [24-26]. With the introduction of rituximab, splenectomy was no longer required for successful transplantation with ABO incompatible donors and rituximab has been referred to as "medical splenectomy". Gloor et al. found that the incidence of antibody mediated rejection in the splenectomy group was 30 % compared to 18% in the rituximab group ($p=0.68$) [24]. Patient and graft survival were similar. Genberg et al. found that the combination of immunoadsorption and rituximab improved outcomes similar to that of ABO compatible living donor kidney transplants (graft survival was 86.7% in both the groups) [7].

More recently, Montgomery et al. [25] found that a conditioning regimen using PP and IVIG alone may be effective in successful ABOi transplantation. The group transplanted 28 ABOi patients who had received plasmapheresis and CMVIG alone without B cell ablative therapy

and followed them for 2 years. The incidence of humoral rejection was 17.8% which was similar to those patients who had splenectomy (n=17) or rituximab (n=15) in addition to PP and CMVIG [25]. In Japan where ABOi transplants are very common, acute rejection rates have decreased to less than 10%. In a series of 74 patients who received rituximab and PP for immunomodulation, the incidence of humoral rejection was only 6.7%. Patient survival was 100% and the graft survival was 97% [27].

Overall the goal of the pre-transplant conditioning regimen is to reduce the anti-A and/or anti-B titers to a low level. The acceptable titer for transplantation varies significantly among the centers, ranging from 1:32 to <1:8. Generally patients receive additional PP treatment in the early post-transplant period. The anti-A and anti-B titers are followed routinely post-transplant and a rise in the titer may be used as an indication to reinitiate PP and/or a biopsy procedure. For long term immunosuppression, these patients are usually maintained on triple drug therapy (calcineurin inhibitors, antimetabolites and steroids). Acute rejection episodes are treated as per center protocols.

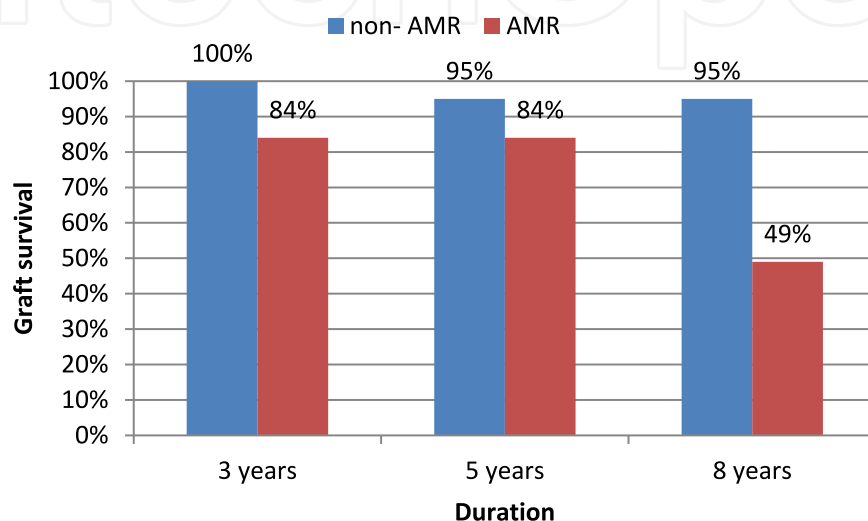
3.4. Clinical outcomes of recipients with ABO incompatible donors

The major goal of conditioning regimens for recipients of ABOi donors is to reduce the anti-A and/or anti-B titers to a level that allows for transplantation without hyperacute rejection and early graft loss. We reviewed some of the protocols that have led to successful transplantation using ABOi donors. However, these patients are still at risk for acute rejection episodes and acute rejection rates can be as high as 30% in some series. Acute antibody mediated rejection (AMR) has also been shown to affect graft survival in ABOi kidney transplants. Toki [28] et al. showed that the graft survival is much lower when patients have AMR compared to patients who do not experience AMR (5 year survival- 84% vs. 95%). Presence of AMR also correlates with the development of transplant glomerulopathy at 1 year (64% vs. 3%). Since any episode of AMR has a profound effect on the graft outcomes despite treatment, optimizing the evaluation and management of recipients with ABOi transplants is important to mitigate the risk of rejection episodes (Figure 2).

Most centers utilize some form of monitoring protocol that includes anti-A/anti-B titers as well as protocol biopsies. However, recent data does not indicate that titers are predictive of early acute rejection and/or poor allograft outcomes. In a study by Shimmura et al. pre-transplant anti-A/anti-B titers were not found to correlate with graft survival in the patients with anti A/B IgG titers [29]. However, the presence of donor specific anti-HLA antibodies did appear to have a more significant association with poor allograft outcomes than anti-blood group antibodies [28]. In another study, the authors found that the median anti-A/anti-B titer in those who had antibody mediated rejection was 16 (range 8-256). However, the positive predictive value of a high anti-A/B titers for AMR was poor (33.3%) [30]. Other studies have found that the absence of mycophenolate mofetil in the conditioning regimens was also associated with an increased risk of rejection [2, 28-30].

Acute antibody-mediated rejection requires morphologic evidence of acute tissue injury, circulating donor-specific alloantibodies, and immunological evidence of antibody-mediated process (particularly C4d positive staining). C4d is a degradation product of the

classic complement pathway. A unique feature of C4d is that it binds covalently to the endothelial and collagen basement membranes, thereby avoiding removal and raising the possibility of serving as an immunologic footprint of complement activation and antibody activity. Presence of C4d correlates with the presence of donor specific anti HLA antibodies and also poor graft survival. But the significance of C4d deposition is not clear. C4d deposition has been seen in up to 80% of protocol biopsies in ABOi transplantation without any sign of graft dysfunction. Platt et al. suggested that perhaps the binding of anti-donor antibodies and complement to the graft induces accommodation. So, the presence of C4d alone does not signify endothelial damage or rejection [31].



Difference in Graft Survival between AMR vs. Non-AMR at 5 years was significant, $P=0.009$
 AMR – antibody mediated rejection; Data adapted from Toki et al., AMJ Transplant, 2008

Figure 2. Graft survival rate influenced by antibody mediated rejection in recipients of ABOi kidney transplants.

Despite the acute rejection complication, long term outcomes for recipients of ABOi transplants are good and similar to the outcomes seen in ABO compatible transplantation. Results from Japan, where the largest number of ABOi transplants have been performed, are also promising. Ichimaru et al. [32] published a review of 1,012 ABOi transplants performed from 1989-2006 at 92 institutions. The 1-year, 3- year, 5- year and 10- year patient survival rates were 95%, 93%, 91% and 87% and the corresponding graft survival rates were 90%, 86%, 80% and 63%, respectively (Figure 3). Graft survival was significantly better in patients aged 15 or younger and in patients transplanted after 2001.

Futagawa and Terasaki [33] published an analysis of registry data of UNOS examining ABOI kidney recipients compared to ABO compatible transplantation. There was no significant difference in allograft survival at 1 and 5 years after transplantation (66.9 versus 66.7 %). These results were also validated in other studies (Figure 3). Genberg et al. [7] analyzed the protocol on 60 consecutive ABOi kidney transplants that included immunoadsorption (used primarily in Europe) instead of plasmapheresis. At 5- year follow-up, graft survival was 97% for the ABOi group vs. 95% for the ABO compatible recipients. Patient survival was identical (98%).

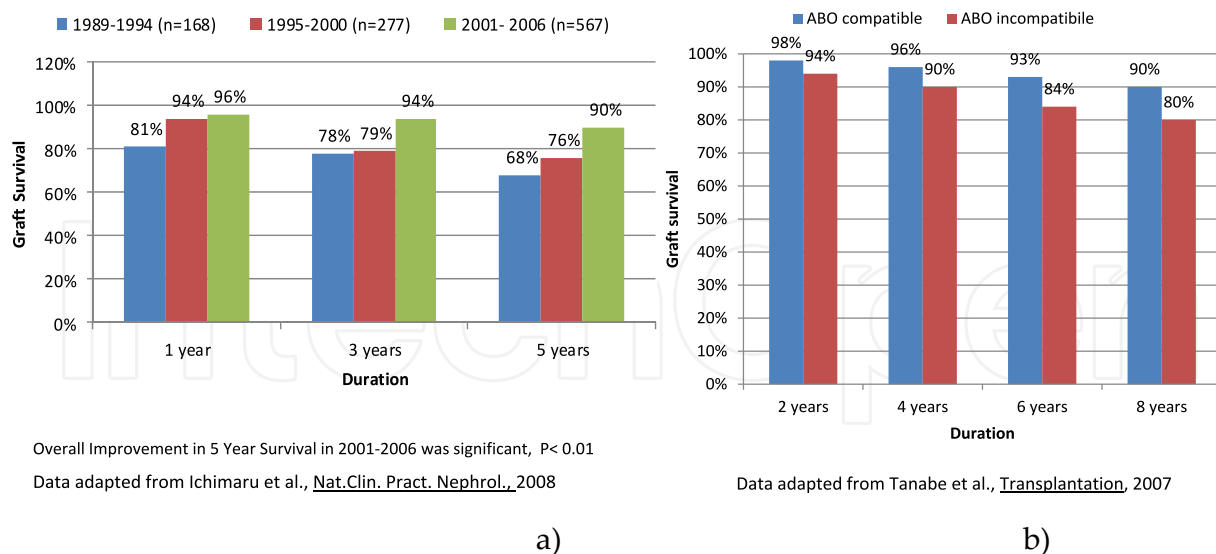


Figure 3. a) Trends in graft survival rates of ABOi kidney transplant recipients from 1989-2006
b) Comparison of graft survival rates in ABOi and ABO compatible kidney transplant recipients

3.5. Accommodation

After a successful ABOi transplantation, many allografts exhibit signs of “accommodation” which is defined as the absence of allograft injury in the presence of alloantibodies. Many of the recipients of ABOi kidney transplants demonstrate C4d deposition in protocol biopsies without any signs of acute or chronic rejection [34]. Haas and colleagues found that in ABOi graft recipients, individuals with diffuse C4d deposition without other signs of graft dysfunction did better than those without significant C4d deposition. Several experimental models have attempted to elucidate the mechanism of these findings. One explanation is that the binding of anti-A/B to the endothelium leads to upregulation of protective genes such as CD55 and CD59 which inhibit complement mediated cell injury and protects the graft acutely [35, 36]. It is postulated that reduction in cell surface expression of A /B antigens in the graft and development of endothelial chimerism may protect the graft long-term [37-39].

3.6. Future of transplantation with ABO incompatible donors

Using blood group frequencies in the US population, 30–35% of potential living donors will be blood group incompatible. Other than continuing on renal replacement therapy and waiting for a deceased donor transplant, the options for patients with ABOi donors are kidney paired donation or ABO incompatible transplantation. Not all recipient-donor pairs find a suitable donor exchange option quickly. Similar to waiting for a donor on the deceased donor list, blood type O recipients have an increased wait time on the kidney paired donation registry. This topic is covered in more detail in Chapter 9.

Research to facilitate ABOi transplants and reduce acute rejection rates in these individuals would be of value. Experimental therapies are being investigated to reduce the antibody

target on the endothelium. This is achieved either through the use of enzymes that cleave the carbohydrate antigen or by blocking antibody preventing isohemagglutinin binding. Kobayashi and others have shown that recombinant ABase infusion (an enzyme which removes A/B antigen from cell surface) in baboons leads to significant reduction in expression of blood group antigens on its kidneys. Hasegawa isolated a novel antibody (K7508) which targets blood group A antigen. They showed that group A red cells coated with the blocking antibody (K7508) were not recognized by other anti-A antibodies, indicating that antigen A was masked by K7508. Both these options reduce antibody-antigen binding in recipients of ABOi transplants and may improve our management of ABOi recipient-donor pairs [40, 41].

4. Renal transplantation with crossmatch incompatible donors

4.1. Patient assessment: Histocompatibility testing

The predominant method of evaluating a patient's sensitization is using the panel reactive antibody (PRA). Historically, this was done using a complement dependent cytotoxicity (CDC) assay. In this assay, the patient's serum is incubated with a panel of donor lymphocytes in the presence of rabbit complement which results in lymphocyte death in the presence of patient's antibodies binding to the cell surface and activating complement cascade. The percentage of lymphocytes giving a positive reaction (cell death) over the total number tested is the PRA of the patient. Sensitized patients who have been exposed to Class I and Class II HLA (human leukocyte antigens) via transfusions, pregnancies and previous transplants are likely to have PRA because they harbor many anti-HLA antibodies that react with larger pool of the donor leukocyte panel [42]. In this assay, it was difficult to determine the exact target of the anti-HLA antibodies since each lymphocytes of the donor would express more than one Class I and II HLA molecules. More recently, with the advent of solid phase assays (where HLA antigens are immobilized in a tray well or on a bead) using ELISA, flow-Cytometry, and Luminex technologies, we can be more precise about the specificity of the anti-HLA antibody when assessing the sensitization of a given patient [43]. We can also calculate the PRA (cPRA) based on the frequency of the HLA antigens in a larger pool of donors and perform a "virtual crossmatch" in which we try to predict the possibility of a positive crossmatch based on the semiquantitative strength of the anti-HLA antibodies present in the donor's serum and the HLA typing of the potential donor. These solid phase anti-HLA antibody assays allow each center to minimize the number of positive crossmatches for their recipients and assess relative risk of immunological complications early post-transplantation [44].

Prior to any kidney transplant, a prospective sensitive CDC (complement dependent cytotoxicity) crossmatch is required. The standard crossmatch at most centers is the AHG-CDC (anti-human immunoglobulin enhanced CDC) crossmatch). A positive donor T cell AHG-CDC is a contraindication to transplantation. In this crossmatch, the donor cells are mixed with the patient's serum and incubated with rabbit complement to evaluate if the recipient antibodies are able to elicit donor cell death. The strength of the reaction is graded

and any significant cell death above and beyond the negative control well is considered positive. If there is a positive reaction, it suggests that the patient is sensitized against the donor and is at high risk of hyperacute or accelerated rejection episode. The flow cytometry crossmatch is also utilized to identify any donor specific antibodies and it is considered to be a more sensitive crossmatch than the standard AHG-CDC crossmatch. The flow crossmatch is performed by incubating an individual's sera with donor lymphocytes (T & B) that are stained with a fluorochrome-conjugated anti-IgG. The fluorescence of the bound antibody is then detected using a laser and compared to a negative control. The difference in the signal between the donor crossmatch and the negative control is calculated and reported as a mean channel shift (MCS). The CDC crossmatch identifies complement activating antibodies but does not distinguish anti-HLA from non anti-HLA antibodies. Similarly the standard flow cytometry crossmatch detects both anti-HLA and non anti-HLA antibodies but does not distinguish between complement activating antibodies and non-complement activating antibodies. Both of these tests are used to assess donor-reactivity [45]. The association between positive flow cytometry crossmatch and acute rejection rates and graft survival is still being debated. Only one prospective blinded study has been performed and it showed that a positive flow cytometry crossmatch did not affect graft outcomes [46]. However, there are multiple other studies demonstrating increased acute rejection risk associated with a positive flow cytometry crossmatch. At some centers a positive flow cytometry crossmatch is considered a contraindication to transplantation while at other centers may utilize condition therapies or modify induction therapies to avoid early acute rejection episodes [47, 48].

To distinguish between anti-HLA and non anti-HLA antibodies, a series of solid phase antibody detection systems have been developed [49]. In these assays, the HLA antigens eluted from cell lines are immobilized on an artificial surface and patient's serum is incubated with the bound HLA antigens. If the patient has an anti-HLA antibody, it will bind to the antigen. The bound antibodies are detected using some type of fluorescence signal. Different platforms are used for solid phase antibody screening including ELISA, standard flow cytometer and the multiplexing assays on the Luminex® platform. At this time a solid phase antibody screening is required to list unacceptable antigen on the UNOS waiting list.

When performing histocompatibility testing, most centers are reporting DSA (donor specific antibodies) as part of the assessment. Although the term DSA can refer to any type of antibody directed against the donor, it commonly refers to the anti-HLA antibodies detected against the donor using solid phase antibody screening. The most commonly used platform, Luminex® platform, is able to provide the relative strength of the antibody in terms of MFI values (mean fluorescence intensity). However, it is not considered an absolute quantitative assay and the assay is not standardized across laboratories.. Therefore, MFI values obtained at one center cannot be compared directly to the MFI values obtained at another center. However, the relative strength (low, medium, strong) should be comparable [50].

Solid phase antibody assays are routinely used to report DSA and identify patients at risk for AMR (antibody mediated rejection) post-transplantation. Akalin and colleagues found in

their treatment protocols that AMR was observed only in patients with strong DSAs (MFI >6000 on Luminex® platform) [51]. Similarly Mayo clinic also found that the development of AMR was more likely in patients with strong DSAs and higher MCS on flow cytometry crossmatch [52]. Lefaucher et al. showed that patients with DSA MFI >6000 were 100 times more likely to develop AMR [53].

A prospective CDC crossmatch is performed on all patients. A positive donor CDC T cell crossmatch is considered to be a contraindication to transplantation in routine practice. However, other techniques are routinely used to measure the sensitization status of the patient, determine whether the antibody is directed against HLA or non-HLA, and assess risk for early acute rejection episodes [54]. These techniques also aid in measuring the response to immunomodulation treatment protocols and understanding when transplantation can safely be performed while minimizing the risk of acute rejection. New technology is continuously emerging and new assays to look at complement binding antibodies, IgG subtypes and endothelial antibodies are currently being studied [55-57].

4.2. History of kidney transplantation with crossmatch incompatible donors

In 1969, Patel and Terasaki were the first to demonstrate that the presence of pre-formed antibodies significantly affects transplant outcomes and that the crossmatch could help define who could be safely transplanted. Their pivotal paper showed that when transplanted with a positive crossmatch, 80% of patients would go on to lose their grafts, however with a negative crossmatch, only 4% of patients lost their grafts [58]. Since then our techniques for measuring antibodies and assessing sensitization have become quite sophisticated. Additionally, multiple studies have demonstrated that the presence of preformed antibodies predisposes patients to hyper-acute rejection as well as acute and chronic AMR. We now understand that simply the presence of preformed antibodies leads to decreased graft survival even in the absence of clinical signs of AMR [53].

Because of the shortage of available organs and the high percentage of sensitized patients on the wait-list for transplantation, many centers began looking into methods for immunomodulation to improve the rates of transplantation and outcomes in highly sensitized individuals since the early 90s. These early studies as well as many recent protocols involve the use of low or high dose IVIG and PP. The mechanism of action of these therapies was described earlier. More recently newer protocols have begun using rituximab, anti-CD20 antibody, bortezomib, the protease inhibitor and eculizumab, the anti-C5, terminal complement inhibitor.

4.3. Modulation of the immune response in recipients with crossmatch incompatible donors

Immunomodulation therapy can be given prior to transplantation to facilitate transplantation or at the time of transplantation to reduce acute rejection related complications. Because the earliest forms of immunomodulation focused on obtaining a negative CDC crossmatch to allow transplantation of sensitized patients, only individuals

with living donors were enrolled to determine if the treatment protocols utilizing PP and IVIG were successful in obtaining a negative crossmatch. With more experience, some centers have begun to treat patients who are on the waiting list for a transplant and suggested that PRA levels and time to transplantation can be decreased. Since 2000, there have been many studies describing protocols used for conditioning prior to transplantation [Table 2] as well as protocols administered peri-transplantation [Table 3] to reduce the risk of acute rejection in sensitized patients. We have divided data into two tables. Table 2 describes the protocols from 8 studies that predominantly focused on pre-transplant conditioning therapies to lower antibodies to a level that results in a negative crossmatch (some transplants occurred with persistent weakly positive crossmatches). The goal of these therapies was to reduce anti-HLA antibodies and allow for successful transplantation. If immunomodulation was unsuccessful, patients did not proceed to transplant. Three of the studies focused on use of high dose IVIG for immunomodulation [59-61]. In these studies rejection rates were 31-59% and patient and graft survival was 96-100% and 75-100% respectively. Four of the studies included rituximab as a part of their pre-transplant conditioning regimens [62-65]. Despite the addition of rituximab, AMR rates remained high, 37-50% and patient and graft survival were similar to the IVIG alone groups 86-100% and 79-94% respectively. Of these trials, only one was a randomized control trial and focused on treatment of patients awaiting a deceased donor transplant. Jordan et al published their data on the use of IVIG versus placebo. They found that transplantation rates improved and time to transplantation decreased significantly with the use of high dose IVIG. Additionally they demonstrated significant reductions in PRA after the use of IVIG ($p < 0.03$).

It is unclear whether high dose IVIG is an improved therapy over PP and low dose IVIG. The study in Table 2 by Stegall et al. compared PP/IVIG with IVIG alone using anti-thymocyte globulin (ATG) induction [66]. In their study they looked at transplantation outcomes in 3 treatment groups. The first group received high dose IVIG, the second received PP, low dose IVIG and rituximab, and the third received PP, low dose IVIG, rituximab with close post-transplant monitoring. All groups received anti-thymocyte globulin induction. In the first group only 5/13 (38%) had pre transplant tyhomglobulin a negative crossmatch while 84% in group 2 and 88% in group 3 ultimately had a negative crossmatch. AMR rates in the first group were quite high (80%), and significantly lower in group 2 (37%) and group 3 (29%). Additionally they noted that at baseline, patients with higher AHG titers were less likely to achieve a negative crossmatch at the time of transplant. Among the small number of patients that went on to receive a transplant with a persistently positive crossmatch, 70% developed AMR and 50% went on to lose their grafts.

Table 3 describes protocols that were used to reduce the risk of acute rejection episodes and graft loss in sensitized patients who had an acceptable crossmatch to proceed with transplantation. These patients did not have contraindications to transplantation but were sensitized and deemed to be at high risk for early acute rejection episodes based on the presence of donor specific antibodies. These patients were treated either prior to transplantation or at the time of transplantation (Table 3) [61-63, 65, 67-77]. In these studies, AMR rates were lower and improved in some studies compared to historical controls. It is

difficult, however, to compare one center's results to another's as there are no standardized methods to compare the strength of the antibodies in one group to another group at a different center. Acute rejection rates do appear to be lower compared to the studies in Table 2, a result attributable to the fact that this group's antibody titers were not high enough to cause a positive CDC crossmatch. Patient and graft survival ranged from 87-100% and 78-100% respectively.

The lowest rejection rates from Table 3 are seen in the data published by Mount Sinai using the addition of PP to IVIG/ATG pre-transplant conditioning protocols based on the intensity of DSA [51]. They studied a group of patients with CDC T cell negative crossmatch but T and/or B cell positive flow crossmatch. In their initial protocol patients were given low dose IVIG (300mg/kg) and ATG. However, they found with the initial 15 patients, 3 developed AMR so they increased their IVIG dosing to 2gm/kg. This still resulted in an AMR rate of 66% in patients with strong DSA and they altered their protocol to add PP in patients with strong DSA. They also noted that in the group of patients with weak DSA, there were no episodes of rejection. Once augmenting the protocol with PP, the AMR rate in the patients with strong DSA decreased to 7%. This study suggested that it was important to achieve MFI<6000 (at their center) to minimize the risk of acute rejection. Our center compared 33 flow XM positive patients treated with rituximab and IVIG prior to transplantation (living) or at the time of transplantation (deceased) to 16 flow crossmatch positive patients who had only received IVIG [78]. In our study cohort, use of rituximab was associated with a significant lower acute rejection rate at one year (16% vs. 45%; P=0.03). Despite these promising data, the majority of studies [Table 3] have much higher rates of AMR, significantly higher than the rejection rate of 13% reported across all transplants in the tacrolimus/MMF era of immunosuppression [79].

Not all studies have reported success when using IVIG and rituximab for immunomodulation. Recently Marfu et al. examined the use of IVIG (2g/kg) and rituximab (1 dose 375mg/m²) for immunomodulation of patients on the deceased donor waiting list [80]. They found that in patients with cPRA >50%, treatment with IVIG and rituximab did not increase rates of transplantation. Compared to the Cedars Sinai group, their subjects had higher cPRA values. Post conditioning therapy, there was no improvement in cPRA values, nor was there a significant reductions in DSAs. They also performed whole blood genome analysis on their desensitized patient and demonstrated reductions in some B cell transcripts. In particular they found that specific genes previously shown to be associated with tolerance were down-regulated in their patients treated with IVIG/rituximab [81]. It is unclear what effect these changes in B cell transcripts resulting from IVIG and rituximab therapy will have on long term graft survival.

Although there has been some success with IVIG, PP and rituximab based protocols, the rates of AMR are still quite high and the success of decreasing PRA on patients waiting to receive a transplant is marginal at best. Newer therapies are currently being evaluated to improve these results.

4.4. Clinical outcomes of recipients with crossmatch incompatible donors

While the initial studies on immunomodulation quoted AMR rates as high as 100%, more recent studies that incorporate the use of stronger induction therapy, as well as use of the strength of DSA to guide PP and higher dose IVIG, suggest lower AMR rates. Short term graft survival ranges from 78-96%. To date only a few studies have looked at long term graft outcomes. Haririan et al. looked at living donor transplantation against a positive crossmatch and compared outcomes to negative cross-match living donor controls [69]. They found that 1 and 5 year allograft survival rates were 90% and 69% in the positive crossmatch group as compared to 98% and 81% in controls. More recently Johns Hopkins published their outcomes data comparing desensitized patients (using IVIG and PP) with two groups, a dialysis only group and a dialysis or transplantation group [82]. They found that patients who underwent immunomodulation had a survival of 90.6% at 1 year, 85.7% at 3 years and 80.6% at 8 years as compared with 91.1%, 67.2% and 30.5% in the dialysis only group and 93.1%, 77% and 49.1% in the dialysis or transplantation group respectively ($P < 0.001$). While this group included only patients with live donors, there clearly remains a survival benefit to undergoing transplantation despite the need for immunomodulation. This survival benefit remained even in the group that was unable to obtain a negative crossmatch prior to transplantation. This data demonstrates a benefit to transplantation if a living donor is available over remaining on the wait list. However, it is unclear whether this can be extrapolated to those who received therapy prior to a deceased donor transplant.

Additionally there continues to be convincing evidence that the presence of DSA leads to poor graft outcomes, including increased incidence of chronic AMR and transplant glomerulopathy [83]. While there are no consensus guidelines as to the follow up of the sensitized patient post transplantation, it is clear that they should be followed more closely for monitoring of both acute and chronic AMR. In many centers this includes protocol biopsies and frequent monitoring of DSA. Any increase in serum creatinine or development of proteinuria should prompt repeat measurement of DSA and renal biopsy. Follow up should also include monitoring for sequelae of over-immunosuppression such as the development of BK viremia at regular intervals. At our center we currently monitor for DSA and BKV at 1 and 12 months in the highly sensitized patient.

Many studies have been done examining the use of protocol biopsies in transplanted patients, including those at high risk. The ability to detect and treat subclinical rejection at an early stage may have long term benefits on allograft survival [84, 85]. Persistent donor specific antibodies has been linked to the development of transplant glomerulopathy [86, 87]. Stegall and colleagues evaluated the incidence of transplant glomerulopathy in a large cohort of patients with protocol and diagnostic biopsies and found an incidence of 49% in well function renal allograft. The risk factors for transplant glomerulopathy included anti-HLA antibodies and history of prior acute rejection episode [88]. The implementation of protocol biopsies has been shown to increase detection of subclinical rejection and Rush et al. found that treatment of subclinical rejection reduced rates of early and late rejection as

well as improved graft survival at 2 years. Therefore it would be reasonable to perform protocol biopsies in a patient who undergoes immunomodulation and is at high risk for development of AMR and transplant glomerulopathy.

4.5. Future of crossmatch incompatible donors

While conditioning regimens for immunomodulation have shown some success in increasing transplantation rates in highly sensitized individuals, the rates of acute and chronic rejection remain high. Additionally, graft outcomes in patients with DSA are known to be worse than patients who are not sensitized to their donors [89, 90]. With the emergence of kidney paired donation programs (KPD), new options are now available for those patients who have a living donor but are highly sensitized. Given the growth and pool of donors in the current KPD programs, finding a compatible donor without the need for immunomodulation (prior to transplantation) is a more viable option. Segev et al. analyzed the benefits of a national KPD optimization scheme and found that highly sensitized patients would increase their rate of transplantation 6-fold (from 2.3% to 14.1%) [91]. Some highly sensitized patients may require immunomodulatory therapy even with the donors from the KPD program. However, data from more sensitive crossmatch techniques and solid phase antibody testing can be used to determine which donor would be associated with the the lowest relative risk of rejection for the recipient. Clearly this ability to assess relative risk with different donors will increase options for sensitized patients and allow us to optimize the donor selection process. Decision for whether or not to undergo pre-transplant conditioning therapy versus wait for a kidney to become available through KPD should be considered on a case by case basis.

Other novel therapies are currently being explored for immunomodulation. Bortezomib is a proteasome inhibitor that is FDA approved for the treatment of multiple myeloma. Its application in the field of transplantation is relatively new and based upon both in vitro and in vivo evidence of its activity against plasma cells. This agent is now being used to attempt reduction of DSA in sensitized patients [92, 93]. While many centers have incorporated this agent into treatment of AMR [94-96], its use for immunomodulation is limited to case reports. There have been 2 case reports that examined the use of bortezomib as a desensitizing agent pre-transplant. In the first, it was reported that one patient achieved a decrease in PRA from 57% to 31% and was able to be transplanted successfully [97]. In the other study, 2 patients received treatment with bortezomib and dexamethasone, and the effects were more modest, reduction of PRA from 87% to 80% and 37% to 13% [98]. Additionally at the 2010 American Transplant Congress, data was presented on the use of bortezomib in 6 patients for immunomodulation prior to transplantation. Compared to those treated with PP, 50% (3/6) received a transplant in the bortezomib group, while only 11% (1/9) in PP alone group [99]. There have also been small studies showing early treatment with bortezomib post-transplant can provide some modest reduction in DSAs that are detected during the early post-transplant period [100]. Whether bortezomib will become a meaningful agent for immunomodulation prior to transplantation remains to be determined.

Eculizumab is the newest agent to be considered for therapy of AMR and immunomodulation. It is a humanized monoclonal antibody against complement protein C5. It binds to C5 protein inhibiting its cleavage to C5a and C5b and preventing formation of the terminal complement complex C5b-9. It is FDA approved for paroxysmal nocturnal hemoglobinuria and for the prevention of atypical hemolytic uremic syndrome post-transplant [101]. More recently its use as an agent to prevent and treat AMR is being studied. An abstract presented at the 2010 American Transplant Congress from the Mayo Clinic showed that in 16 sensitized living donor transplant recipients treated with eculizumab, at the time of transplant, the AMR rate was as low as 6.25% [102]. Despite the reduced rates of AMR, 6 patients developed signs of chronic antibody mediated rejection. Again, this is a promising new agent whose role in the treatment of the sensitized patient is still being assessed.

5. Summary - Transplantation with incompatible donors

We have come a long way since the earliest studies in transplanting both the ABOi donor and the highly sensitized patient. Using blood group frequencies in the US population, 30–35% of potential living donors will be blood group incompatible. Other than continuing on renal replacement therapy and waiting for deceased donor transplant, the options for patients with ABOi donors are kidney paired donation (KPD) programs and ABO incompatible transplantation. Similar to compatible transplants, the waiting time for recipients with blood group O and B are longer in KPD programs (unless the donor is blood group O, universal donor). The mortality rate on dialysis while awaiting a transplant is very high (5-7 deaths per patient year) and therefore, for some individuals ABOi transplantation is not only a viable but a better option [2].

For the highly sensitized patient, sophisticated techniques to evaluate the level of sensitization and solid phase antibody screening tools can help to identify which antigens are unacceptable and likely to cause a positive crossmatch. With this information we can select recipient-donor combinations that would be amenable to immunomodulation and allow for successful transplantation with good long-term outcomes. For patients who have a living donor, KPD programs can be used to increase opportunities for the recipient and optimize chances for successful transplantation.

Conditioning therapies for immunomodulation do not come without a cost. AMR, chronic rejection and transplant glomerulopathy are frequent complications in recipients of incompatible donors. Patients must be monitored frequently for any signs of rejection as well as the infectious complications associated with high dose immunosuppressive therapies. The use of protocol biopsies should be considered and maintenance immunosuppression should be individualized. With the advent of non-invasive techniques for evaluating allograft function and the use of urinary biomarkers to detect early signs of graft dysfunction, monitoring of the highly sensitized patient will continue to evolve.

5.1. Economic considerations

The USRDS reports the annual cost of maintaining a patient on dialysis is \$70,000/year and the cost of an uncomplicated transplant is \$25,000 but improves to \$17,000/year when the graft is functioning well. Schwartz et al. performed a resource utilization study on 40 ABOi transplants and compared them with match ABO compatible transplants. The graft survival was similar in both the groups but, as expected, there was an increased rate of rejection in incompatible group. The average cost of an ABO-incompatible living donor kidney transplant is approximately \$38,000 more than that of conventional ABO-compatible living donor kidney transplant. The major areas contributing the high cost were nursing (due to increased length of stay), plasmapheresis treatments and pharmacy (rituximab dosing). However, this was much more cost effective when compared to long term maintenance hemodialysis [103]. The high costs of induction therapy, PP, and other immunomodulatory agents can significantly increase the cost of transplantation and must be considered when evaluating the cost-effectiveness of immunomodulation. A functioning graft over time will be more cost effective than remaining on hemodialysis but this needs to be further explored in the current era with expensive novel therapeutic options.

5.2. Recommendations and alternatives

Overall, if given the option, it would be best for a recipient to have an ABO and crossmatch compatible donor where additional therapies for immunomodulation are not required and the risk of acute and chronic rejection are lower. If a patient has an incompatible living donor, encouraging them to enroll in the KPD program can maximize their chances for a compatible donor. However, if the patient is not able to find a compatible donor within a reasonable time, histocompatibility data should be evaluated to identify options for transplantation with an incompatible donor given the benefits of transplantation over continuing dialysis therapy. Post transplantation, patients should be monitored closely for acute and chronic rejection using protocol biopsies as well as infectious complications. This type of approach is being utilized by many centers and we feel this approach will lead to the best outcome for a patient with an ABOi or crossmatch incompatible donor [104, 105].

Author details

Dinesh Kannabhiran, Michelle L. Lubetzky and Darshana Dadhania

Department of Transplantation Medicine and Extracorporeal Therapy, Division of Nephrology and Hypertension, Department of Medicine, NewYork-Presbyterian Hospital- Weill Cornell Medical College, New York, NY, USA

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Appendix

Renal Transplantation In ABO incompatible Kidney Transplant Recipients								
Study	n	Follow up Months	Conditioning regimen ^a	Target Anti-A/B Titer	Induction	Maintenance ^b	AR (%)	Survival (%)
Takahashi et al. AJT 2004 Japan[26]	441	108 (9yrs)	ImmunoAd (n=51)→ DF-PP ^{a,c} (n=390) + Splenectomy (n=433)	8-16 fold ↓	Varied by center	Anti-metabolite + Tacrolimus (36%) or cyclosporine (64%)	58 (overall)	3yr: P-87 3yr: G-71 9yr: P-84 3yr: G-59
Gloor et al. Txp. 2005 USA[24]	34	24	<u>Group1</u> (g1)n-23: PP with IVIG (0.1g/kg) ^a + MMF+ splenectomy <u>Group2</u> (g2)n-11: PP with IVIG (0.1 g/kg) ^a + MMF+ rituximab(375 mg/m ²)x1	≤1:8	rATG	MMF Tacrolimus	AMR g1- 30 g2- 18	P-96 / 91 G-87 / 82
Montgomery et al. Txp. 2009 USA[25]	57	42 (median)	<u>Group1</u> (g1)n-14: PP with CMVlg (0.1 g/kg) ^a + splenectomy <u>Group2</u> (g2)n-15: PP with CMVlg (0.1 g/kg) ^a + rituximab (375 mg/m ²)x1 <u>Group3</u> (g3)n-28: PP with CMVlg (0.1 g/kg) ^a (all 3 groups also received tacrolimus / MMF)	≤1:16	daclizumab	MMF Tacrolimus	AMR/ACR g1-36/ 14 g2-27/ 14 g3-29/ 14	(3 groups) 3yr: P-96 3yr: G-93
Genberg et al. Txp. 2008 Sweden[7]	15	36 (median)	rituximab (375 mg/m ²)x1+ Ag-ImmunoAd ^a + MMF/Tacrolimus/steroids IVIG (0.5 g/kg) x1	<1:8	None	MMF Tacrolimus	AMR- 0 ACR- 7	P-100 G-87
Wilpert et al. NDT 2010 Germany[106]	40	39 (median)	rituximab (375 mg/m ²)x1+ Ag-ImmunoAd ^a + MMF/tacrolimus/steroids IVIG (0.5 g/kg) x1	<1:4	basiliximab	MMF Tacrolimus	AMR- 5 ACR- 28	P-98 G-100
Fuchinoue et al. Txp. 2011 Japan[107]	113	60	All – PP or DF-PP ^a + <u>Group1</u> (g1)n-63: splenectomy <u>Group2</u> (g2)n-50: rituximab (dose varied)	<1:16	basiliximab	MMF Tacrolimus or cyclosporine	AMR/ACR g1: 16 /10 g2: 4 /4	P:100/100 G-90/100
Shirakawa et al. Clinical Txp. 2011 Japan[27]	74	>22	All- DF-PP ^a + MMF/tacrolimus/steroids+ rituximab <u>Group1</u> (g1)n-24: 0.5g vs. <u>Group2</u> (g2)n-24: 0.2g	<1:32	basiliximab	MMF Tacrolimus	AMR/ACR g1: 8 /16 g2: 6 /14	P-100/100 G-96/98

DF- Double filtration; Ag- Antigen specific; ImmunoAd- immunoabsorption; PP- Plasmapheresis; IVIG- Intravenous Immunoglobulin; MMF- Mycophenolate mofetil; rATG- rabbit anti-thymocyte globulin; CTX- cyclophosphamide; AR- Acute rejection (clinical & subclinical where reported) AMR-antibody mediated rejection; ACR- Acute cellular rejection; P- patient, G- graft;
^aNumber of treatments pre and post-transplant varied based on baseline titers; ^bEveryone received steroids; ^cPre- transplant treatment only

Table 1. Conditioning Regimens for Facilitating Renal Transplantation In Blood Type Incompatible Kidney Transplant Recipients

Crossmatch Incompatible Kidney Transplant Recipients								
Study	n	Follow Up Months	Conditioning regimen and Goal of Therapy	% Transplanted (txp/treated)	Induction	Maintenance ^a	AR (%)	Survival (%)
Schweitzer et al. Txp. 2000 USA[1]	11 LD	13	PP / IVIG (0.5g/kg) (# varied) MMF / Tacrolimus/steroids Goal: Neg. CDC XM	73% (11/15)	OKT3	MMF + tacrolimus	36	P- 100 G- 100
Jordan et al. Txp. 2003 USA[2]	42 (26 LD, 15 DD)	>24	LD- IVIG 2gm/kg x 1 DD – IVIG 2g/kg x 4 (monthly) Goal: Neg. CDC XM	LD-92% (24/26) DD-93% (14/15)	daclizumab	MMF + tacrolimus	31	P-98 G- 89
Jordan et al. JASN 2004[3] USA	DD RTC ^b 48 IVIG vs. 50placebo	30	IVIG 2gm/kg x 4 vs. Placebo x 4 (monthly) Goal: Neg. CDC XM	IVIG-35% (17/48) Placebo -20% (10/50)	Varied by center	Varied by center	IVIG 53 placebo 10	P-100 G-75 vs. 62
Gloor et al. AJT 2003 USA[4]	14 LD	15	PP/IVIG (0.1mg/kg) x 4 rituximab(375mg/m ²) x1 Goal: Neg. CDC XM	reviewed only those transplanted	rATG splenectomy	MMF + tacrolimus	43	P-86 G-79
Stegall et al. AJT 2006 USA[5]	61 LD (3 groups)	12	<u>Group1:</u> IVIG 2g/kg x1 <u>Group2:</u> PP/IVIG (0.1mg/kg) + rituximab (375mg/m ²) <u>Group3:</u> PP/IVIG (0.1mg/kg) + rituximab (375mg/m ²) + monitoring post-txp therapy	Group 1: 36%(5/13) Group 2: 84%(27/32) Group 3: 88% (14/16)	rATG	MMF + tacrolimus	Group 1: 80 Group 2: 37 Group 3: 29	P-93 G-82
Magee et al. Txp. 2008 USA[6]	29 LD	22	PP / IVIG (10g) (# varied) Tacrolimus. / MMF Goal:Neg. CDC T-cell XM	97% (28/29) 15 w/ weak Pos XM (3-Tcell XM, 12-Bcell XM)	rituximab (n=21) rATG OR basiliximab	MMF + tacrolimus	39	P-96 G- 89
Thielke et al. Txp. 2009 USA[7]	51 LD	23	PP/IVIG (0.1g/kg)x3-4 rituximab(375mg/m ²) x 1-2 Goal: Neg. T cell Flow XM	89% (51/57) 2 with Pos.Flow XM	rATG	MMF + tacrolimus	43	P-95 G- 93
Vo et al. NEJM 2008 USA[8]	16 (10 LD, 6DD)	12	IVIG 2gm/kg x2 monthly rituximab 1g x2 Goal ^c : Neg. CDC Tcell XM at 1:2 dilution & Flow Tcell XM -MCS<250	80% (16/20)	alemtuzumab	MMF + tacrolimus	50	P-100 G-94
Vo et al. Txp. 2010 USA[9]	76 (31 LD, 45 DD)	24	IVIG 2gm/kg (day 1 &30) Rituximab 1g (day 15) Goal ^c : Neg. CDC T cell XM at 1:2 dilution & Flow Tcell XM- MCS<250	reported only those transplanted	alemtuzumab or daclizumab or rATG	MMF + tacrolimus	37	P-95 G-84

Txp-Transplantation; LD- Living donor; DD-Deceased Donor; Neg.-negative; Pos.- positive; PP-plasmapheresis; IVIG- Intravenous Immunoglobulin; OKT3- Muromonab CD3; MMF- Mycophenolate mofetil; rATG- rabbit anti thymocyte globulin; PRA-panel reactive antibodies; AR-acute rejection; P- patient; G- graft; ^bEveryone received prednisone ^cOnly RTC (randomized controlled trial); ^dThese criteria for transplantation were not standard protocol but were specific for sensitized patients treated with the protocol.

Table 2. Conditioning Regimens for Facilitating Renal Transplantation and Prevention of Rejection In Crossmatch Incompatible Kidney Transplant Recipients

Prevention of Rejection in Sensitized Kidney Transplant Recipients								
Study	n	Follow Up Months	Conditioning regimen ^a	% Transplanted (txp/treated)	Induction	Maintenance ^b	AR (%)	Survival (%)
Montgomery et al. Txp. 2000 USA[10]	4 LD	6 (approx.)	PP / IVIG (0.1g/kg) (# varied)	all	daclizumab	MMF + tacrolimus	100	P-100 G-100
Glantz et al. AJT 2002 France[11]	13 (11 DD, 2LD)	>12	IVIG (2gm/kg) q monthly x 3 Goal: 50%↓ in PRA	87% (11/13)	rATG	MMF + tacrolimus	8	P-87 G-87
Akalin et al. CJASN 2008 USA[12]	21 LD 14 DD	18 (median)	<u>Group1</u> : LR- IVIG 2g/kg (peri-transplant divided doses) <u>Group2</u> : HR- above IVIG+ PP pre/post (# varied)	all	rATG	MMF + tacrolimus	Group 1: 29 Group 2: 7	P-100/ 93 G-89/ 86
Anglicheau et al. AJT 2007 France[13]	38 DD	>12	IVIG 2gm/kg on POD 0/ 21/ 42/63	all	rATG or basiliximab	MMF + tacrolimus or cyclosporin	26	P-97 G-95
Lefaucher et al. AJT 2007 France[14]	32 (16LD, 16 DD)	30 (mean)	Pre: IVIG 2gm/kg x3 (monthly) (pre or peri-transplant)	all	rATG	MMF + tacrolimus	41	P-95 G-78
Mai et al. Txp. 2009 USA[15]	20 (5 LD, 15 DD)	36	IVIG 500mg/kg x 3 (peri-transplant)	all	rATG	MMF + tacrolimus	50	P-94 G-89
Bachler et al. AJT 2010 Switzerland[16]	37 (11 LD, 26 DD)	>12	IVIG 2gm/kg total at time of induction	all	rATG	MMF + tacrolimus	38	P-95 G-92

PP- Plasmapheresis; LD- Living donor; DD-Deceased Donor; IVIG- Intravenous Immunoglobulin; OKT3- murineAnti-CD3; MMF- Mycophenolate mofetil; rATG- rabbit anti-thymocyte globulin; AR-acute rejection; P- patient; G- graft; LR-low risk; HR – high risk;
^aPatients were sensitized but they did not have a contraindication to transplantation; ^bEveryone received prednisone

Table 3. Conditioning Regimens for Prevention of Rejection in Sensitized Kidney Transplant Recipients

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