

Rituximab: An emerging therapeutic agent for kidney transplantation

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Abstract: Rituximab (anti-CD20, anti-B-cell) is now emerging as an important drug for modification of B-cell and antibody responses in solid-organ transplant recipients. Its uses are varied and range from facilitating desensitization and ABO blood group-incompatible transplantation to the treatment of antibody-mediated rejection (AMR), post-transplant lymphoproliferative disorder (PTLD), and recurrent glomerular diseases in the renal allograft. Despite these uses, prospective randomized trials are lacking. Only case reports exist in regards to its use in *de novo* and recurrent diseases in the renal allograft. Recent reports suggests that the addition of rituximab to intravenous immunoglobulin (IVIG) may have significant benefits for desensitization and treatment of AMR and chronic rejection. Current dosing recommendations are based on data from United States Food and Drug Administration-approved indications for treatment of B-cell lymphomas and rheumatoid arthritis. From the initial reported experience in solid organ transplant recipients, the drug is well tolerated and not associated with increased infectious risks. However, close monitoring for viral infections is recommended with rituximab use. The occurrence of progressive multifocal leukoencephalopathy (PML) has been reported with rituximab use. However, this is rare and not reported in the renal transplant population. Here we will review current information regarding the effectiveness of rituximab as an agent for desensitization of highly human leukocyte antigen-sensitized and ABO-incompatible transplant recipients and its use in treatment of AMR. In addition, the post-transplant use of rituximab for treatment of PTLD and for recurrent and *de novo* glomerulonephritis in the allograft will be discussed. In summary, we will make recommendations based on existing literature and our extensive experience at Cedars-Sinai Medical Center for using rituximab in renal transplantation.

Keywords: kidney transplant, rituximab, desensitization, rejection, review

Introduction

Renal transplantation has long been recognized as the treatment of choice for end-stage renal disease (ESRD) as it offers improvements in quality and length of life.¹⁻³ Currently there are more than 80,000 ESRD patients awaiting transplant in the United States, with more than 30,000 new registrants each year.⁴ However, fewer than 18,000 kidney transplants are performed each year in the United States.⁴ A significant impediment to increasing organ transplantation rates is sensitization to human leukocyte antigen (HLA)-specific allo-antigen and ABO blood group incompatibility. The disparity in waiting time experienced by these patients is directly related to organ allocation policies adopted in the context of ongoing organ shortages which dictates that deceased donor recipients be blood type compatible and have a negative crossmatch with their donors. The scarcity of

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donor organs with these characteristics has contributed to the disenfranchisement of this group of highly HLA-sensitized (HS) ESRD patients and resulted in extensive wait time on dialysis. In addition, if transplanted these patients are at high risk for antibody-mediated rejection (AMR) and allograft loss.

Thus in an effort to optimize organ availability and expand the benefits of kidney transplantation to those patients, several transplant centers have developed protocols to overcome sensitization and blood group incompatibilities. Three desensitization protocols are currently utilized: low-dose intravenous immunoglobulin (IVIG) with plasmapheresis (PP), high-dose IVIG alone, and high-dose IVIG with rituximab.⁵⁻⁷ As a direct result of these efforts, it is now possible to perform successful renal transplantation in patients with blood group incompatibilities and a positive crossmatch (CMX).

For decades, the primary focus of transplant immunosuppressive agents was directed at T-cells. The importance of B-cells and antibodies with specificities for HLA in mediating acute rejection and preventing access to transplantation is now widely accepted.^{8,9} However agents specifically directed at modifying B-cell-mediated immune responses have not been available. Over the last several years clinical trials using IVIG have shown the ability to reduce anti-HLA antibodies and improve transplant rates.^{6,10,11} Recently, several investigators have also reported on the beneficial effect of rituximab (anti-B-cell, anti-CD20) in modification of allo-immune responses and treatment of AMR.¹²⁻¹⁴ Here we review current information regarding the effectiveness of rituximab as an agent for desensitization of HS and ABO-incompatible (ABOi) transplant recipients and its use in the treatment of AMR. In addition, the post-transplant use of rituximab for treatment of PTLD and for recurrent and *de novo* glomerulonephritis in the allograft will be discussed. In summary, we will make recommendations based on existing literature and our extensive experience at Cedars-Sinai Medical Center for using rituximab in renal transplantation.

Mechanism(s) of action

Rituximab is a chimeric anti-CD20 (anti-B-cell) monoclonal antibody that is approved for treatment of lymphoma. This antibody efficiently eliminates B-cells as the CD20 antigen is expressed early in B-cell ontogeny, but is absent on mature plasma cells.^{15,16} Rituximab has also been approved for use in rheumatoid arthritis and has demonstrated significant benefit in a number of autoimmune and inflammatory disorders.^{17,18} Of note, is the demonstrated benefit in vasculitic disorders that does not always correlate with reduction in

pathogenic antibody.^{18,19} Recent clinical data suggest that the beneficial effects of rituximab may be due to depriving T-cells of antigen presenting cell activity thus altering effector functions and inducing a regulatory profile.^{20,21} This data suggest that the beneficial effects of rituximab on autoimmune disease are more likely related to modification of dysfunctional cellular immunity rather than simply a reduction in antibody.

The variable region of rituximab binds to CD20 and marks the cell for destruction by three different mechanisms: antibody-dependent cell-mediated cytotoxicity (ADCC), complement-dependent cytotoxicity, and cell-mediated apoptosis via CD20 cross-linking.^{15,22} ADCC occurs by binding of the Fc portion of rituximab to Fc γ receptors on natural killer (NK) cells, macrophages and monocytes. These cells then act to destroy the B-cell bound by the monoclonal antibody. Complement dependent cytotoxicity is mediated by activation of the complement cascade by the Fc portion of anti-CD20 ultimately resulting in the assembly of the membrane attack complex and cell lysis. Finally, cross-linking of bound CD20 proteins causes an influx of calcium which leads to activation of caspases. This, in turn, causes apoptosis of the cell.

Effect on B-cell populations

Rituximab causes a profound and sustained depletion in the number of circulating B-cells. It also decreases B-cell populations in the lymph nodes and spleen. A recent study by Genberg and colleagues evaluated the pharmacodynamics after a single dose of rituximab (375 mg/m²) in renal transplant recipients.²³ Elimination of B-cells was rapid, and occurred over one to three days in the peripheral blood. It was also prolonged. B-cell populations did not begin to reemerge until after one year and remained suppressed for two years. This is longer than what is observed in patients with lymphoma or rheumatoid arthritis. It is notable that B-cell lymphopenia was present at baseline in the renal transplant population. It is possible that the delayed recovery of B-cells was related to the maintenance immunosuppression. Rituximab also leads to a significant reduction of B-cells in lymph nodes, although they were not completely eliminated. It is suggested that the densely populated lymph node is more difficult to penetrate and may require a higher dose of rituximab.

Elimination of some B-cell populations also occurs in the spleen, but not uniformly. This was demonstrated by Ramos and colleagues who determined the effect of rituximab in the spleens of individuals who underwent desensitization.²⁴ The investigators quantified B-cells in spleens removed from four groups of patients: those who underwent splenectomy for trauma (control group), those who underwent desensitization

with plasmapheresis (PP) and low-dose IVIG followed by subsequent splenectomy at the time of transplant (PP/IVIG group), those who underwent desensitization with PP, low-dose IVIG, and rituximab who also had a splenectomy at the time of transplant (PP/IVIG/rituximab group), and those who received rituximab, low-dose IVIG and rabbit antithymocyte globulin (rATG) but not splenectomy (combination group). Splenectomy in the combination group was done in the setting of refractory AMR and inadequate response to PP prior to transplant. Naïve B-cells (CD20⁺) were significantly lower in the spleens of those who received rituximab. There was no difference in CD20⁺ B-cell depletion between the two rituximab-treated groups. This suggests that the addition of rATG in the combination group did not have an effect on this population of cells. Plasma cells persisted despite treatment with rATG, maintenance immunosuppression, and rituximab.

The effect on memory-B-cells (CD27⁺) is of interest. In the above study by Ramos and colleagues, there was a trend toward a decrease in memory B-cells in the combination group compared to the IVIG/PP/rituximab group. From other studies, B-cell depletion with rituximab is often associated with delayed recovery of memory B-cells and a depletion of pathogenic B-cells.^{25,26} This may prevent development of a memory response to a novel antigen.

Desensitization

The transplantation of HS individuals poses a great challenge. HS individuals remain on the waiting list significantly longer than their nonsensitized counterparts leading to increased morbidity and mortality. When transplantation occurs, higher rates of rejection and lower rates of graft survival are seen.¹⁵ Various desensitization protocols have emerged to address this problem. The National Institutes of Health IGO2 trial was a prospective, randomized trial that showed decreases in panel-reactive antibodies (PRA) and improved transplantation rates in HS patients that received four monthly doses of IVIG (2 g/kg).⁶ There were more rejections in the group that received IVIG compared to placebo, however, three-year allograft survivals and mean serum creatinines were similar. Unfortunately, this process is lengthy and not uniformly effective. Desensitization protocols employing PP in combination with low-dose IVIG (100 mg/kg) have also had success.²⁷ This protocol is usually limited to live donor kidney transplantation because donor-specific antibody (DSA) will likely rebound days after discontinuing the therapy unless transplantation at the nadir of antibody is accomplished.

Rituximab is an attractive adjunct in desensitization protocols based on its success in the treatment of AMR and

the synergistic effect with IVIG observed in patients with autoimmune diseases. There are published protocols demonstrating the effectiveness of rituximab for desensitization.²⁸⁻³¹ Rituximab most commonly is used in combination with PP and/or IVIG. One center reported the efficacy of rituximab used alone for desensitization in five crossmatch (CMX) negative patients.³² Rates of acute rejection are high and vary from 20% to 100% in these series.

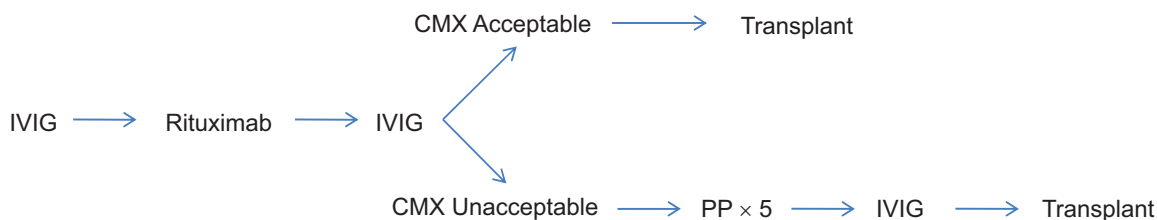
Our group recently reported on our experience using a combination of rituximab and IVIG for desensitization.⁷ In this trial, 20 patients underwent desensitization with IVIG (2 g/kg) followed by two weekly doses of rituximab (1 g). A final dose of IVIG (2 g/kg) was given one week after the last rituximab dose. Patients who subsequently showed a negative or acceptable CMX from a living or deceased donor received allografts. A CMX was deemed acceptable if the T-cell complement-dependent cytotoxicity (CDC) assay was negative at a 1:2 dilution and the T-cell flow cytometric donor-specific CMX had a mean channel shift of less than 250. Patients received alemtuzumab for induction at the time of transplant and tacrolimus, mycophenolate mofetil, and prednisone for maintenance immunosuppression.

Eighty percent of patients were successfully transplanted with this protocol. Those who were awaiting a deceased donor had an average wait time of 144 months prior to treatment and were transplanted, on average, five months after treatment. There was a 50% rate of acute rejection, however patient and allograft survival at one year were 100% and 94% respectively. This protocol has since been modified to include only one dose of rituximab (1 g) between the two doses of IVIG (see Figure 1). This was changed due to the long-term B-cell depletion seen with a single dose of rituximab.

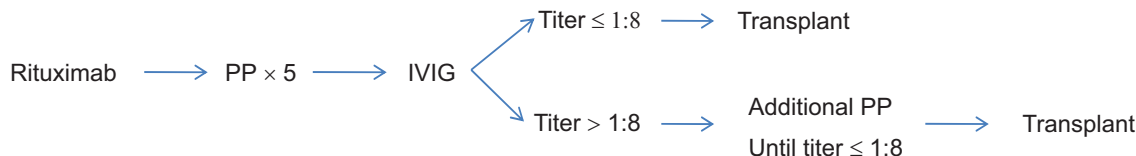
In summary, rituximab with PP and/or IVIG facilitates desensitization and allows for transplantation across a positive CMX. Protocols that involve PP are best suited for living donor transplantation. We have found our protocol to be effective for living and deceased donor transplantation while saving time and costs. More recently we have seen low rates of AMR (23%) in patients treated with the modified protocol. One-year patient and graft survival rates were 96% and 94%, respectively.³³

Concerns regarding rituximab interference with crossmatch results

The use of rituximab interferes with the B-cell CDC and B flow cytometric CMX. Rituximab activates complement, which results in a false positive B-cell CMX.³⁴ One can



CSMC HLA desensitization protocol



CSMC ABOi preconditioning protocol

Figure 1 Cedars-Sinai Medical Center (CSMC) protocols.

Abbreviations: ABOi, ABO incompatible; CMX, crossmatch; IVIg, intravenous immunoglobulins; HLA, human leukocyte antigen; MMF, mycophenolate mofetil; PP, plasmapheresis.

disregard the B-cell CMX in rituximab-treated individuals; however this may lead to severe early AMR in HS patients with HLA class II antibodies. Pronase is a proteolytic enzyme that removes the Fc receptors from the surface of B- and T-cells, and improves the sensitivity and specificity of the CMX. CD20 is removed by pronase treatment as well. Pronase treatment was shown to reduce or eliminate the effect of rituximab on the B-cell flow cytometric CMX.³⁴ Our experience with this technique is evolving and, as yet, is not always reproducible.

ABO-incompatible transplantation

The scarcity of organ donors, both living and deceased, contributes to the ever growing transplant wait list. Kidney transplantation across blood group barriers provides a means to expand the donor pool. Early experience involved removing anti-A/B antibodies by PP followed by three-drug immunosuppression and splenectomy to prevent severe AMR.³⁵ ABOi transplantation has since been used, especially in Japan, as a way to expand the donor pool. Sawada and colleagues were the first to report on the successful use of rituximab to lower anti-blood group antibodies in preparation for ABOi transplantation.³⁶

Rituximab has now largely replaced splenectomy in ABOi preconditioning protocols. In 2003, ABOi transplants were successfully performed in four patients without splenectomy under a preconditioning regimen consisting of a

single dose of rituximab, antigen-specific immunoadsorption, and three-drug immunosuppression.³⁷ Sonnenday and colleagues subsequently reported successful ABOi transplantation in six patients that received multiple sessions of PP followed by CMV immune globulin and a single dose of rituximab given one to two days prior to transplant.⁵ Tacrolimus and mycophenolate mofetil were started at the initial PP and the patients received induction with steroids and daclizumab. Excellent results were achieved after twelve months of follow-up. Patient and allograft survival were 100% with no episodes of AMR. There was one episode of cell-mediated rejection (CMR) discovered by protocol biopsy at three months post-transplant. Short-term data from multiple medical centers show similar excellent results with good allograft survival and low rates of rejection utilizing different protocols that include rituximab.^{38–40}

There are now long-term data supporting the efficacy of rituximab in ABOi preconditioning protocols. Genberg and colleagues reported their three-year experience with ABOi transplantation using rituximab instead of splenectomy.⁴¹ The study was a comparative, retrospective design that compared ABOi to ABO-compatible recipients who were transplanted during the same time period. There were 15 ABOi and 30 ABO-compatible adult recipients. There was no difference in patient survival, allograft survival, or rates of rejection between the two groups. There was also no

difference in serum creatinine, glomerular filtration rate, and proteinuria between the two groups at three years. Recently, Montgomery and colleagues reported on the Johns Hopkins University experience with ABOi transplantation.⁴² The one-, three-, and five-year graft survival rates were 98.3%, 92.9%, and 88.7%, respectively. Only 15% of the patients had AMR episodes. The authors used three different protocols. The final did not include the use of B-cell depletion. However other investigators feel this is an important part of ABOi transplantation.

Our group has adopted a protocol that employs administration of rituximab (1 g) one week prior to initiating five sessions of PP followed by IVIG (2 g/kg) (see Figure 2). Our goal is to reduce the anti-blood group titer to $\leq 1:8$. This regimen has yielded excellent results with 18 patients transplanted to date, 66% of whom have had one-year follow-up. There is 100% patient survival and 94% graft survival after one year.⁴³

Effect of anti-CD20 therapy in a mouse model of sensitization

Recently our group reported on development of a mouse model of HLA sensitization that was used to study the effectiveness of anti-CD20 treatment on B-cell depletion and anti-HLA antibody responses.⁴⁴ This model utilized skin grafts from C57BL/6 mice to

C57BL/6 mice. Immunization with the skin grafts from the HLA A2.1 transgenic animals resulted in a robust production of anti-HLA immunoglobulin M (IgM) and IgG antibodies with specificities to HLA A2.1 and multiple HLA class 1 targets, including A1, A3, A25, A26, A29, and A30. These antibodies also were cytotoxic to HLA A2-expressing target cells. Administration of two doses of a novel mouse anti-mouse rituximab (development in CD20^{-/-} mice) significantly suppressed levels of anti-HLA IgG2a antibodies, prolonged survival of secondary skin grafts, and reduced IgG deposits and C4d staining of these grafts. Administration of this antibody was also associated with a significant depletion of B220⁺/CD5⁻ B-cells. Overall this experimental data suggests that rituximab may have an important role in desensitization therapies and in the treatment of AMR.

Treatment of antibody-mediated rejection

AMR remains a significant problem and is a detriment to long-term renal allograft survival. The rate of AMR in patients undergoing desensitization is currently 19%.⁴⁵ However, the rates of AMR are much higher in PP-based protocols.^{46,47} Rituximab was initially used for the treatment of refractory rejection based on the recognition of intrarenal B-cell infiltrates in both CMR and AMR.⁴⁸ The presence of

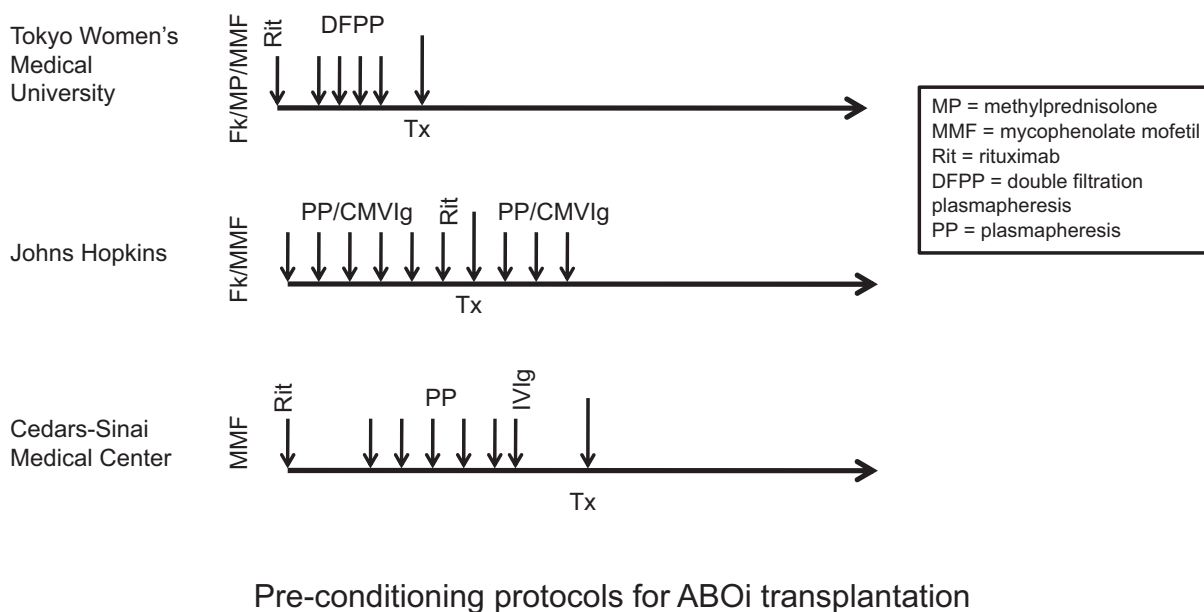


Figure 2 Preconditioning protocols for ABOi transplantation.

Abbreviations: ABOi, ABO incompatible; DFPP, double filtration plasmapheresis; IVlg, intravenous immunoglobulins; MMF, mycophenolate mofetil; MP, methylprednisolone; RIT, rituximab; PP, plasmapheresis.

B-cell infiltrates in acute CMR is a risk factor for steroid resistance and is associated with a worse prognosis.^{48,49} It is unclear if the B-cells present within the allograft function as plasma cell precursors, antigen presenting cells, and/or provide co-stimulatory signals to T-cells. B-cells also produce inflammatory cytokines that may directly injure the allograft.

The effect of rituximab on B-cell infiltration was reported in two studies. Zarkhin and colleagues prospectively studied pediatric renal transplant recipients who had biopsy proven CMR with B-cell infiltrates.¹² Patients were randomized to receive four weekly doses of rituximab (375 mg/m²) plus standard treatment versus standard treatment alone. The B-cell infiltrate was abolished in all cases after rituximab treatment with improvement in renal function. Steinmetz and colleagues identified nine patients with vascular allograft rejection and B-cell clusters present in biopsy specimens.⁵⁰ Patients who received one dose of rituximab (375 mg/m²) in

addition to conventional treatment had complete resolution of the B-cell infiltrate. Both studies were small and no differences were seen in clinical outcomes between the groups. It is notable that complete resolution of the B-cell infiltrate was seen whether patients received one dose of rituximab or four doses.

Many recent reports support the clinical efficacy of rituximab for the treatment of AMR (Table 1). Becker and colleagues initially reported the benefit of rituximab in the treatment of refractory rejection.⁵¹ The investigators evaluated 27 patients who received rituximab for refractory AMR diagnosed by the presence of thrombotic microangiopathy (TMA) or endothelitis in renal allograft biopsies. Patients received treatment with steroids, PP, and/or antithymocyte globulin (ATG) without improvement in creatinine prior to receiving a single dose of rituximab (375 mg/m²). There were three graft losses. The 24 successfully treated patients had good allograft function at the time of discharge. Other

Table 1 Overview of rituximab for AMR

Author	Study type	Subjects	Protocol	Results
Becker et al 2004	Case series	N = 27 Adults Rejection (TMA or endothelitis without cellular infiltrate) refractory to steroids or ATG/PP	Single dose of RIT (375 mg/m ²)	89% Graft survival Average Cr 0.95 at discharge
Wade et al 2006	Case series	N = 3 Adults AMR	RIT given with various combinations of PP, steroid, OKT3, and IVIG	1 of 3 responded to treatment
Faguer et al 2007	Case series	N = 8 Adults AMR	RIT (375 mg/m ² weekly × 4) with steroids and PP + various additional treatments	10 m average follow-up Graft survival 75% Cr improved (P = 0.04)
Zarkin et al 2008	Randomized, prospective trial	N = 20 Pediatrics CD20 ⁺ rejection	Patients randomized to standard care or standard care plus RIT 375 mg/m ² × 4 doses	12 m Follow-up Improved Cr in RIT group (P = 0.026)
Mulley et al 2009	Pilot study	N = 7 Adults AMR	Single-dose rituximab (500 mg) for AMR refractory to PP/low dose IVIG	20 m average follow-up Cr improved (P = 0.49) 100% allograft survival
Kasposztas et al 2009	Retrospective case-control	N = 54 Adults AMR	PP plus RIT ± IVIG vs PP alone ± IVIG	24 m follow-up Graft survival 90% (ritux) vs 60% (control) (P = 0.005) Mean GFR no change (P = 0.42)
Tanriover et al 2008	Pilot study	N = 7 Adults AMR	RIT (375 mg/m ²) with IVIG (2 g/kg)	24 m follow-up 86% one-year allograft survival 58% two-year allograft survival
Billing et al 2008	Pilot study	N = 6 Pediatric (one adult) Chronic AMR	IVIG (1 g/Kg) weekly × 4 doses followed by RIT 375 mg/m ² × 1 dose	12 m follow-up GFR stabilized in 4/6 patients

Abbreviations: AMR, antibody-mediated rejection; ATG, antithymocyte globulin; Cr, creatinine; IVIG, intravenous immunoglobulins; m, month; PP, plasmapheresis; RIT, rituximab; TMA, thrombotic microangiopathy.

recent reports also document a beneficial effect of rituximab on refractory AMR.^{13,52,53}

Kaposztas and colleagues reported two-year outcomes in their recent retrospective study looking at 54 patients treated for AMR.¹³ Group A had 26 patients that underwent treatment with PP and rituximab and Group B had 28 patients who received PP without rituximab. Patients that had low serum IgG levels also received IVIG. Two-year graft survival was significantly better in the group that received rituximab (90% versus 60%) with the difference attributed to rituximab. A trend toward improved graft survival was also seen in those that received IVIG ($P=0.050$). This study has one of the largest cohorts reported to date and supports the use of rituximab for the treatment of AMR with good short-term allograft survival. However, the study was retrospective with many patient variables that were not consistent between the groups.

Approaches to the treatment of AMR include high-dose IVIG, PP combined with lower dose IVIG, or rituximab.⁵⁴ The effectiveness of rituximab is reported in combination with IVIG, PP, and/or steroids.^{13,55–57} The ameliorative effects of rituximab on AMR are likely multifactorial. In addition to B-cell depletion and reduction in DSA, disruption of T-cell co-stimulator and antigen-presenting cell activities mediated by B-cells are likely altered and result in diminished T-cell effector functions.⁵⁴ Optimal treatment of AMR probably requires a combination of rituximab with PP and low-dose IVIG or with high-dose IVIG (1–2 gm/kg).⁵⁴ This is related to the inability of rituximab to deplete CD20 negative plasma cells that continue to produce DSA and mediate graft injury.

Our center has extensive experience with HS patients who received kidney transplants after desensitization with IVIG and rituximab.⁷ Recently, we evaluated 123 HS patients transplanted after desensitization (7/06–2/09). Twenty-two patients developed AMR post-transplant, usually within the first month. All were treated with a combination of steroids (10 mg/kg daily \times 3), IVIG 2 gm/kg (maximum dose 140 g \times 1), and rituximab (375mg/m² \times 1). Some patients also received PP and two underwent splenectomy. Six of twenty-two patients (27%) lost their allograft to severe AMR, usually within one month. Thus, a 73% survival rate for severe AMR was seen in this high-risk group.³³

Chronic antibody-mediated rejection (CAMR) is defined by criteria set forward in Banff in 2005.⁵⁸ CAMR has a poor prognosis with no currently well defined treatment protocol.⁵⁹ Billing and colleagues conducted a pilot study to investigate the use of IVIG with rituximab in six patients (aged 10–26 years) that had evidence of CAMR.⁶⁰ The patients received four weekly doses of IVIG (1 g/kg) followed by one

dose of rituximab (375 mg/m²). All patients showed greater than 40% positive C4d staining of peritubular capillaries. All biopsies had varying degrees of CD20⁺ infiltrates. Allograft function stabilized or improved in four patients after one year of observation. There was one graft loss at 18 months and one patient did not respond. Five out of six patients had complete depletion of B-cells in the peripheral blood. The two patients that did not respond had the highest degree of transplant glomerulopathy.

In summary, reports have demonstrated the efficacy of rituximab for treating refractory rejection, rejection that contains B-cell infiltrates, AMR, and CAMR. The available evidence suggests an improvement in creatinine and allograft survival in the short- and medium-term. The efficacy of rituximab is mostly seen when it is used in combination with other therapies for rejection, such as steroids, IVIG, or PP. Prospective randomized trials are now needed to validate the existing data and to clarify the dosing regimen.

Post-transplant lymphoproliferative disorder (PTLD)

The incidence of PTLT varies from 1.3% in kidney transplant recipients to 8.2% in lung transplant recipients.⁶¹ The incidence is higher in the pediatric population. Risk factors for PTLT include Epstein–Barr virus (EBV) status, type of organ transplanted and intensity of immunosuppression. EBV positivity has ranged from 29% to 100% in patients who developed PTLT.⁶² The majority of these tumors are CD20⁺ B-cell clones; mostly diffuse large cell lymphomas. Poor prognostic indicators include EBV-naïve recipients, advanced age, elevated lactate dehydrogenase (LDH), and an increased number of involved sites. Initial management has been reduction in immunosuppression, but response to this is reported to be variable and low.⁶³ Additional treatment strategies include antiviral therapy, interferon alpha, EBV-specific T-cell infusion, chemotherapy, and rituximab.^{62–65}

The use of rituximab to treat PTLT, including central nervous system involvement, was described in various reports and retrospective studies with response rates ranging from 44% to 65%.^{62,66} There have since been three prospective trials evaluating the safety and efficacy of rituximab used as a single agent to treat PTLT.^{67–69} Patients were initially treated with reduced immunosuppression. This was followed by four weekly doses of rituximab (375 mg/m²) if there was no response to initial reduction in immunosuppression. Rituximab infusion was well tolerated and only two of 52 adverse events were related to rituximab in the largest study.⁶⁷ EBV positivity and a normal LDH level predicted the response

to rituximab therapy. The patients that did not respond to rituximab in one study ($n = 7$) had late onset, EBV-negative PTLD.⁶⁸ Based on these studies, rituximab is useful to treat low risk, EBV-positive tumors. However chemotherapy, with or without rituximab, should be considered in patients with EBV-negative tumors, elevated LDH or late onset PTLD.

Multiple studies have also evaluated the efficacy of rituximab treatment in combination with chemotherapy.^{70–72} Orjuela and colleagues conducted a pilot study in a small group of pediatric solid organ transplant recipients.⁷¹ This group of patients usually has more aggressive PTLD. They were treated concurrently with rituximab, cyclophosphamide, and prednisone. Five out of the six patients achieved complete remission during the follow-up period, eight to 29 months. A large, retrospective study evaluated solid organ transplant recipients that received rituximab alone or chemotherapy with or without rituximab after failing reduction of immunosuppression for PTLD.⁷² The response rate for single-agent rituximab was 68%. There was a 74% response rate in those that received chemotherapy with or without rituximab. However, significant toxicity was associated with chemotherapy. The authors concluded that it would be reasonable to consider rituximab for treatment of PTLD if reduction in immunosuppression fails. Chemotherapy should be reserved for those individuals who fail rituximab treatment alone, those who need a rapid response, or those with EBV-negative disease.

Surveillance for EBV viremia is one measure that can be taken to prevent development of PTLD. This has been studied in patients that have undergone hematopoietic stem cell transplantation. Meerbach and colleagues screened 123 patients weekly after transplantation for EBV reactivation.⁷³ Twenty-five percent of patients developed EBV reactivation, 19.5% subclinical EBV viremia, and three patients developed PTLD. EBV levels normalized after reduction in immunosuppression. Four patients received pre-emptive therapy with cidofovir and rituximab because of a high viral load. The high EBV viral load was reduced in all patients with this treatment. Pre-emptive therapy was also explored in 27 pediatric patients who underwent stem cell transplantation.⁷⁴ EBV viral DNA was eliminated in eight patients who received rituximab; however four of these patients had a secondary increase in EBV viral load coincident with the emergence of CD20⁺ B-cells.

We believe monitoring for EBV post-kidney transplant is a reasonable approach to identify and prevent PTLD in renal transplant recipients. Reduction in immunosuppression may be of more benefit if it is initiated earlier, before PTLD

occurs, as suggested above. Single-agent rituximab therapy should be given in those with an elevated EBV viral load despite reduction in immunosuppression. Rituximab is also helpful for treating PTLD with low-risk features and EBV positivity. Other therapies such as interferon alpha and chemotherapy should be used in cases of PTLD that are refractory to rituximab or have poor prognostic risk factors such as EBV negativity or elevated LDH level.

Recurrent and *de novo* diseases in the renal allograft

Rituximab has been used for the treatment of various immune mediated glomerular diseases in the native kidneys. Most of the experience in treating recurrent disease in renal allografts is limited to case reports. In 2006, Becker and colleagues cited only three case reports of rituximab being used for recurrent disease in the renal allograft.⁶⁵ Since then, there have been numerous case reports describing the effectiveness of rituximab for various recurrent or *de novo* glomerular diseases including focal segmental glomerulosclerosis (FSGS), membranous nephropathy (MN), antineutrophil cytoplasmic antibodies (ANCA)-associated vasculitis, mixed cryoglobulinemia (MC), and TMA. We are unaware of any case reports discussing the use of rituximab for membranoproliferative glomerulonephritis (MPGN) type 1 or 2 despite the high recurrence rate of these diseases. Controlled trials examining the benefit of rituximab in these diseases are difficult to conduct considering the rarity of recurrent glomerular diseases in renal allografts. However, the mounting case reports provide a framework for treatment of recurrent and *de novo* diseases in the renal allograft that have traditionally had few therapeutic options.

Idiopathic FSGS recurs in up to 30% of initial renal transplants and as high as 80% in second transplants.⁷⁵ It is manifested by proteinuria, often nephrotic range, with or without allograft dysfunction. Allograft survival is poor without any treatment. It is hypothesized that the pathogenesis involves a circulating permeability factor. Treatment has consisted of PP, cyclophosphamide, high-dose cyclosporine, and renin-angiotensin system blockade. Multiple case reports have documented improvement in proteinuria after rituximab treatment in combination with PP or immunoadsorption.^{76–81} Remission was sustained from six months to over two years in some cases.

However, failure of rituximab to improve nephrotic syndrome in four consecutive cases of recurrent FSGS was reported by Yabu and colleagues.⁷⁵ The patients studied in this report had recurrent FSGS that either did not respond or

relapsed after PP therapy. They were subsequently treated with multiple doses of rituximab. Rituximab alone was unable to improve proteinuria in this case series. It is unclear what factors are responsible for individual patient responses. There may be differences in the underlying pathological processes among individuals causing FSGS. It appears that the patients who responded to rituximab therapy were treated early in the post-transplant period.

MN has a much lower rate of recurrence in renal allografts compared to FSGS. However, 25% of patients with recurrent MN lose their allografts to the disease. Recurrent disease usually occurs within the first year post-transplant. In contrast, *de novo* MN in the renal transplant, the most common *de novo* glomerulopathy in renal transplant recipients, occurs years after transplant. Rituximab was reported to improve proteinuria in patients with native kidney MN.⁸² It is possible that B-cell depletion decreases antibody deposition onto the podocyte membrane and eliminates damage. Based on this, there are case reports of rituximab treatment for recurrent MN in kidney transplant recipients.^{83–86} In these cases, all patients had biopsy-proven recurrent MN diagnosed within one year of transplant. Rituximab was given initially in four weekly doses in all cases with additional doses every four months up to one year in the other two cases. Improvement in proteinuria and nephrotic syndrome was seen in all cases for the duration of follow-up, 24 to 42 months.

One case report demonstrated improvement of nephrotic syndrome in a patient with *de novo* MN diagnosed 30 months after transplantation.⁸⁶ *De novo* MN is often associated with CAMR, and indeed, this patient had a PRA of 80% prior to transplant. His biopsy showed a mild plasma cell infiltrate. He was treated with pulse steroids, renin–angiotensin system blockade and one dose of rituximab. His nephrotic syndrome resolved and renal function was stable with minimal proteinuria 18 months after treatment.

The evidence for rituximab therapy in the treatment of ANCA-associated vasculitis is limited. ANCA-associated vasculitis is a group of diseases characterized by necrotizing glomerulonephritis with a paucity of immune complex deposition on renal biopsy. Renal survival was only 72% in one series in patients with Wegener's granulomatosis (WG) or microscopic polyangiitis (MPA).⁸⁷ Renal transplant is successful in patients with renal vasculitis with an overall recurrence rate of 8% to 17%.^{88,89} Rituximab is reported to induce remission in cases of ANCA-associated vasculitis refractory to treatment with cyclophosphamide.^{90,91} B-cells are precursors to ANCA-producing plasma cells and their depletion may induce remission.

There are a few case reports of recurrent ANCA vasculitis in renal transplant recipients that resolved after treatment with rituximab. One report discussed nonrenal recurrence of WG in a renal transplant recipient successfully treated with rituximab.⁹² Geetha and colleagues reported two cases of recurrent ANCA vasculitis with renal manifestations.⁸⁸ Recurrent disease was diagnosed by biopsy in both cases. Microscopic hematuria and dysmorphic red blood cells were seen in one case, and microscopic hematuria with proteinuria and increased creatinine was noted in the other. Rituximab was given in four weekly doses to each patient after they failed to respond to steroid therapy. Standard therapy with cyclophosphamide was not used in the first case due to concerns for infertility and avoided in the second case because of a history of intolerance. Remission was achieved in both patients with resolution of hematuria and improvement in creatinine.

Rituximab has also been used to treat MC in renal transplant recipients.^{93–96} The use of rituximab for the treatment of MC was based on reports from nonimmuno-compromised patients.^{97,98} Its use was first reported in three renal transplant patients that acquired MC *de novo* after transplantation.⁹³ One patient had chronic hepatitis C virus (HCV). Rituximab resolved proteinuria and cryoglobulins in all three patients, but two patients suffered serious infectious complications. The same authors reported improvement in nephrotic syndrome, renal function, and cryoglobulins in a subsequent case series.⁹⁴ This series consisted of seven renal transplant recipients, five of whom had chronic HCV, who received multiple doses of rituximab.

TMA occurs in the renal allograft as *de novo* or recurrent disease. Nondiarrhea-associated hemolytic uremic syndrome (HUS), demonstrated by TMA in the renal allograft, can recur in up to 33% to 56% of adults and 21% to 28% of children depending on the mutation responsible for the disease.⁹⁹ *De novo* TMA is rare, but the incidence can be as high as 20% in some medical centers.¹⁰⁰ Risk factors for *de novo* TMA in the renal allograft include treatment with calcineurin inhibitors or mTOR inhibitors, AMR, viral infection, or ischemia reperfusion injury. There are few case reports discussing the efficacy of rituximab for treating TMA. A single case report describes a case of recurrent HUS that was refractory to over 40 PP treatments.¹⁰¹ Multiple doses of rituximab were subsequently administered with stabilization of the disease. Some cases of HUS are caused by anti-factor H autoantibodies which provide a rationale for rituximab treatment.

Only anecdotal evidence exists describing the success of rituximab for *de novo* TMA. A primary cause of TMA

in native kidneys is the presence of autoantibodies to ADAMTS-13 (a disintegrin-like and metalloproteinase domain with thrombospondin repeats).¹⁰² Patients with genetic deficiency of or autoantibodies to this enzyme cannot degrade ultra heavyweight von Willebrand Factor (UHWVWF) and are predisposed to TMA after vascular injury. There are case reports describing the efficacy of rituximab for treatment of TMA. This efficacy is likely due to reduction or elimination of circulating auto-antibodies to ADAMTS-13.^{103–105}

Rituximab has shown efficacy in treating recurrent and *de novo* glomerular diseases in renal allografts. However evidence is limited to case reports. Prospective, randomized trials will likely not take place due to the rarity of these diseases. The decision to use rituximab for glomerular diseases in the renal allograft should be based on the safety and efficacy of the current standard therapies including a discussion of the risks and benefits of alternative therapies with the patient.

Infectious complications

The risk for infectious complications following rituximab administration is concerning. A trend toward an increased rate of infections was reported in a small group of patients ($n = 21$) that received rituximab for ABOi transplantation or a positive CMX.¹⁰⁶ There was no difference in patient or graft survival. Patients treated with rituximab in this series also received ATG. Types of infections included blood stream infection, pneumonia, esophagitis, peritonitis, colitis, and skin and soft tissue infection. Cryptococcal infection and disseminated herpes simplex virus 2 were also reported in three patients that received rituximab for the treatment of MC.⁹³ Furthermore, three bacterial infections and two fungal infections were reported in eight patients that received rituximab for the treatment of AMR.¹⁴

Several studies have reported viral infections following rituximab administration^{39,40,107,108} (Table 2). In a literature review conducted by Aksoy and colleagues, there were 64 serious viral infections identified in a group of patients, not primarily transplant recipients, treated with rituximab for lymphoma.¹⁰⁸ They found the most common viral infections to be hepatitis B virus (HBV), cytomegalovirus (CMV), and varicella zoster virus. A high rate of CMV viremia was also reported in a group of patients that received rituximab for ABOi preconditioning.^{39,40} This group of patients did not receive CMV prophylaxis, but underwent surveillance for CMV viremia. All were successfully treated with injectable ganciclovir administered at the first sign of viremia.

Table 2 Viral infections associated with rituximab

Cytomegalovirus
Polyomavirus BK
Polyomavirus JC
Herpes simplex virus
Varicella zoster
Parvovirus B19

The development of progressive multifocal leukoencephalopathy (PML) in patients treated with rituximab is of considerable concern. Safety alerts were issued by the US Food and Drug Administration, the European Medicines Agency, and the World Health Organization in 2006 and 2007, which described two cases of PML in patients with systemic lupus erythematosus treated with rituximab and other immunosuppressive agents. PML is a progressive neurological disorder first described in patients with non-Hodgkin's lymphoma and chronic lymphocytic leukemia.¹⁰⁹ It was an acquired immune deficiency syndrome (AIDS)-defining illness prior to the development of highly active antiretroviral therapy. It is caused by the reactivation of latent JC polyoma virus (JCV) as a result of defects in cellular immunity. A recent literature review identified 57 cases of PML in non-HIV infected individuals treated with rituximab.¹¹⁰ There were 52 cases in patients with B-cell lymphoproliferative disorders and five cases in patients treated for autoimmune diseases. There were no cases reported in renal transplant recipients. A case fatality rate of 90% was reported, with those diagnosed within three months of rituximab infusion having a 100% mortality rate.

Although PML is rare after rituximab infusion, the high fatality associated with it has led to consideration for JCV screening after treatment in patients with solid organ transplants. Kamar and colleagues screened 73 solid organ transplant recipients who received rituximab, 37 of whom received a kidney transplant, for JCV reactivation.¹¹¹ They assessed JCV DNA in whole blood by qualitative real-time polymerase chain reaction (PCR). Monitoring was done every three to four months after rituximab administration with an average follow-up of 13 months. Polyoma BK virus (BKV) was also assessed. JCV was detected in 5.5% of patients; one time in three patients and five times in a fourth patient. They were all in renal transplant patients who had also received T-cell-depleting agents. No cases of PML developed and all patients responded to a reduction in immunosuppression. BK virus-associated nephropathy, diagnosed by biopsy after a positive PCR, occurred in all four patients with JCV

reactivation. The authors concluded that monitoring for JCV is useful for identifying patients who are over immunosuppressed.

Reactivation of parvovirus B19 has also been documented after rituximab administration in two nontransplant patients.^{112,113} One patient developed pure red cell aplasia after being treated for lymphoma and another pancytopenia after treatment for refractory immune thrombocytopenic purpura. Both patients recovered. Although hematologic disturbances may be a side effect of rituximab, one must be aware that they are also a sign of reactivated viral infections such as CMV or parvovirus B19.

Despite the above findings, there are many reports that have demonstrated the safety of rituximab administration with infection rates similar to those seen in renal transplant recipients who did not receive rituximab.^{7,12,32,41,114} We reported a series of 20 HS patients who received IVIG and rituximab for desensitization followed by induction with the lymphocyte-depleting antibody alemtuzumab.¹¹⁴ There were no viral infections seen and seven bacterial infections, all related to asymptomatic urinary tract infection. All patients received CMV prophylaxis and prophylaxis for *Pneumocystis carinii* (*P. jirovecii*) pneumonia. PCR assays for CMV, EBV, parvovirus B-19, and polyomavirus BK were performed on whole blood specimens monthly for six months. We concluded that there is no increased risk of infectious complications with close monitoring and appropriate antimicrobial prophylaxis. Genberg and colleagues reported similar results in a group of patients that received rituximab for ABOi transplantation.⁴¹ There were no significant differences in infectious complications after three years of follow-up compared to patients that underwent ABO compatible transplants during the same time period.

In summary, serious infectious complications have been noted in the literature after rituximab administration. However, with appropriate surveillance, the risk can be minimized. Consideration should be given to monitoring JCV to prevent PML, especially in those patients who receive agents that alter T-cell function in addition to rituximab. The neurological status of patients who receive rituximab should also be monitored. One should consider an infectious etiology for hematologic disturbances acquired after rituximab therapy. Avoidance of over immunosuppression may reduce the risk of infectious complications. Monitoring T-cell function with specific assays, such as Cylex[®], is helpful for identifying individuals who are over immunosuppressed and will allow for proactive adjustment of the immunosuppression regimen.

Risks and side effects

The side effects associated with rituximab are mostly related to infusion reactions. Adverse reactions at the time of infusion include fever, chills, tachycardia, arthralgias, and hypotension.^{7,65} These reactions occur more frequently with the first dose. A cytokine release syndrome is described in patients being treated for lymphoma, especially if there is a large tumor burden. Prophylaxis is recommended by premedicating with antipyretics, steroids, and antihistamines. However, infusion reactions may still occur despite these measures.

Long-term risks of rituximab are related to infectious complications and hematologic abnormalities. Late onset neutropenia is well documented in the literature.^{14,40,115} It may be associated with or without leukopenia, and infections have not been reported. It tends to resolve without intervention. Anemia and thrombocytopenia can also occur. Finally, agammaglobulinemia is a very rare complication of rituximab therapy, but should be monitored¹¹⁶ (Table 3).

Dosing

The optimal dosing of rituximab for use in renal transplantation is unknown. Dosing based on body surface area (BSA) and fixed dosing have both been used for desensitization, ABOi preconditioning and treatment of AMR. The dose used for B-cell lymphomas is typically 375 mg/m² BSA given weekly for four weeks. The high cumulative dose is likely needed because of the tumor burden. However, a single dose of rituximab (375 mg/m²) in renal transplant recipients effectively depletes B-cells from the peripheral blood and significantly decreases their numbers in lymphoid organs and allografts.^{23,50} Doses as low as 20 mg/m² can completely eliminate B cells from the circulation in renal transplant recipients.¹¹⁷ In addition, a dose as low as 10 mg/m² was able to reduce the number of B-cells in the spleen.¹¹⁷

Dosing based on BSA may not be necessary. No improvement in the predictability of the area under the curve (AUC) was seen with rituximab dose based on BSA

Table 3 Immediate and long-term side effects of rituximab administration

Infusion-related adverse reactions	Long-term side effects
Fever	Leukopenia
Chills	Neutropenia
Tachycardia	Agammaglobulinemia
Hypotension	
Arthralgias	

compared to a fixed dose of rituximab in rheumatoid arthritis patients.¹¹⁸ Fixed doses of rituximab are now used to treat renal transplant recipients in a variety of situations. A single low-dose of rituximab, 500 mg, was sufficient to successfully treat seven patients with refractory AMR.⁵³ A low, fixed dose of rituximab has also been used successfully in a preconditioning regimen for ABOi transplantation.⁴⁰ At Cedars-Sinai Medical Center, we use a fixed dose of rituximab, 1 g, as part of our ABOi preconditioning regimen and desensitization protocol.^{7,45}

Several dosing regimens are reported for use in transplant recipients. A fixed-dose regimen seems appropriate given the inability of normalization to BSA to predict the AUC. A fixed dose would also facilitate administration and help reduce drug-dosing errors. The dose used may depend on the underlying condition being treated in this population. Patients with PTLD may require the standard weekly dosing compared to those with AMR who may be treated with a fixed, single dose. Prospective, randomized trials will need to be done to examine the efficacy of different dosing regimens on the various treatment indications.

On the horizon

There are a number of new agents now under development that could have applications for prevention and treatment of antibody-mediated conditions in transplantation. The most notable at this time is the proteasome inhibitor bortezomib. Bortezomib is currently approved for the treatment of multiple myeloma, however its plasma cell-depleting properties make it an attractive agent for use in desensitization protocols and treatment of AMR. This agent has the ability to eliminate DSA and treat both CMR and AMR.^{119,120} Clinical trials are needed to confirm these findings. Emerging biologic agents that are similar to rituximab include epratuzumab which binds to CD22 present on mature B-cells and therefore may deplete B-cells bound for memory or antibody production without depleting the whole population of cells. Two agents, belimumab and atacicept, inhibit the B lymphocyte stimulator/B-cell activating factor (BLyS/BAFF) pathway which plays a role in the development of B-cells and plasma cells. These agents are in development for the treatment of multiple myeloma, systemic lupus erythematosus, and rheumatoid arthritis. The anti-complement C5 inhibitor eculizumab shows promise for prevention and treatment of AMR.^{46,121} It is currently approved for the chronic treatment of paroxysmal nocturnal hemoglobinuria. It prevents cellular necrosis by inhibiting the membrane attack complex from forming. This may allow a window of opportunity for antibody altering

therapies to take effect. Finally, several humanized and human anti-CD20 biologics are under investigation for treatment of hematologic malignancies and autoimmune disorders. These include ocrelizumab, veltuzumab, and ofatumumab. Potential advantages of these agents include shorter infusion time, differing CDC and ADCC, less infusion reactions, and subcutaneous administration. However, approval of these drugs for use in transplantation will likely not be obtained and their use in kidney transplantation has not been reported.

Conclusion

Rituximab now plays a prominent role in the field of renal transplantation. Its uses are varied and range from facilitating desensitization and ABOi transplantation to the treatment of AMR, PTLD, and recurrent diseases in the renal allograft. Its effect on these processes is evident through its depletion of B-cells both in the peripheral blood, lymph tissues, and renal allograft. The most evidence exists for its use in PTLD. It has proven efficacy for desensitization, ABOi transplantation, and treatment of AMR, but prospective randomized trials are lacking. Only case reports exist in regards to its use in *de novo* and recurrent diseases in the renal allograft. The optimal dosing has not been determined and may depend on the specific indication it is being used for. Infectious complications are noted throughout the literature and surveillance for viral disease, especially CMV, is recommended in renal transplant recipients. The occurrence of PML is rare and not reported in the renal transplant population, but patients should be monitored closely for signs and symptoms of this disease. New biologic agents are under investigation for the treatment of antibody-mediated conditions, however their use in kidney transplantation, although promising, is still experimental.

Disclosures

The authors report no conflicts of interest in this work.

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