# The role of rituximab in kidney and other solid organ transplantation

**Rituximab is a genetically engineered, therapeutic, chimeric murine–human anti-CD20 monoclonal antibody.[1] It consists of regions derived from the murine anti-CD20 antibody, linked to a human immunoglobulin G (IgG) region,[1,2] with 95% of the resulting protein being of human origin.[2]**

The CD20 receptor is exclusively expressed on B cells and appears during the pre-B-cell stage, but its exact function is not completely understood.[2] Early studies by Deans *et al* demonstrated that cross-linking CD20 resulted in increases in intracellular calcium and showed that CD20 was associated with the Src family of tyrosine kinases, suggesting the involvement of CD20 in transmembrane signalling.[3]

Rituximab, used as an adjunct with chemotherapy in CD20-positive (CD20+) B cell malignancies, induces high response rates and long-term remission[4] and cure rates.[5] It is also being used in non-neoplastic conditions like hepatitis C virus-associated B cell proliferation[6] and autoimmune diseases.[7–10]

This paper reviews the use of rituximab mainly in kidney transplantation, focusing on its mechanisms of action and the clinical efficacy in different transplant settings. Its use in other solid organ transplants is also briefly reviewed.

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## Overview of mechanism of action

The therapeutic activity of rituximab is delivered through the combination of immunological mechanisms – namely, antibody-dependent cell-mediated cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC) and induction of apoptosis – as well as non-immunological mechanisms.

Structurally, rituximab consists of a human IgG1 and kappa chain constant (Fc) region and murine heavy and light chain variable (Fab) regions that bind with high affinity to CD20.[11,12] Retaining the human Fc portion of the structure reduces the generation of human anti-mouse antibody responses and prolongs the half-life of the antibody.[11] In addition, following binding of rituximab to CD20 through the Fab domain, the human Fc domain binds to human immune effector cells leading to tumour cell lysis through CDC and ADCC.[13]

Despite clear *in vivo* efficacy, the specific therapeutic mechanisms of action are not comprehensively established. ADCC results in the destruction of antibody-coated target cells. IgG binds to target cell antigen, with consequent recruitment, binding and activation of immune effector cells expressing Fcγ receptors, which are expressed by almost all cells of the immune system.[11,14]

Rituximab also binds to complement C1q, activating the complement cascade via the classical pathway.[2,12] An important synergism between CDC and ADCC has been demonstrated, which is related to the complement pathway's ability to promote inflammation and enhance the activation status of innate effectors.[2]

Besides an ability to trigger host cellular and humoral immune responses against tumour cells, rituximab is also able to induce apoptosis in target cells, through both caspase-dependant and -independent mechanisms, and to exert a synergistic effect with different chemotherapeutic agents.[15,16]

It also has non-immunological and direct effects on podocytes. These cells have been shown to express sphingomyelin phosphodiesterase acid-like 3b (SMPDL3b) molecules, which regulate sphingomyelinase activity, an important component that maintains the integrity of podocytes.[17] In transplant recipients with recurrent focal segmental glomerulosclerosis (FSGS) and experimental models of xenotransplantation, a reduction of SMPDL3b expression is observed, resulting in disruption of actin stress fibres, an integral part of podocytes. Rituximab has been shown to directly bind to SMPDL3b and stabilise filamentous actin and the actin cytoskeleton.[18]

## Clinical use of rituximab and kidney transplantation

Genberg *et al*[19] studied the effects of a single dose of rituximab, in combination with conventional triple immunosuppressive therapy, on the B cell population in peripheral blood and tissues of kidney transplant recipients. In 88% of the cases, complete depletion of B cells in peripheral blood was observed and, in the majority of patients, was still undetectable 15 months after treatment. B cells were also eliminated in renal tissue. The study concluded that a single dose of rituximab in kidney transplant recipients results in the long-term elimination of B cells in peripheral blood, as well as within the graft.[19] It is well known that B cells are effective antigen-presenting cells, capable of activating donor-specific T cells within peripheral lymph nodes.[19,20] However, the clinical significance of B cells within the transplanted organ and the therapeutic benefit of eradication has yet to be understood, although studies indicate that B cell infiltration during acute rejection is associated with poor clinical outcomes.[21]

By these findings, treatment with rituximab, both as an induction and an antirejection therapy, can be beneficial in the prevention of acute as well as chronic rejection.[19]

### Rituximab as induction therapy in antibody-compatible renal transplantation

The majority of immunosuppressive regimens in kidney transplantation currently include a biologic induction agent, given just before or soon after implantation in addition to routine maintenance therapy. An optimal induction agent should balance cost-effectiveness, safety and efficacy.

Takagi *et al* performed a retrospective analysis of the efficacy and safety of rituximab as an induction agent. In this study, the incidence of antibody-mediated

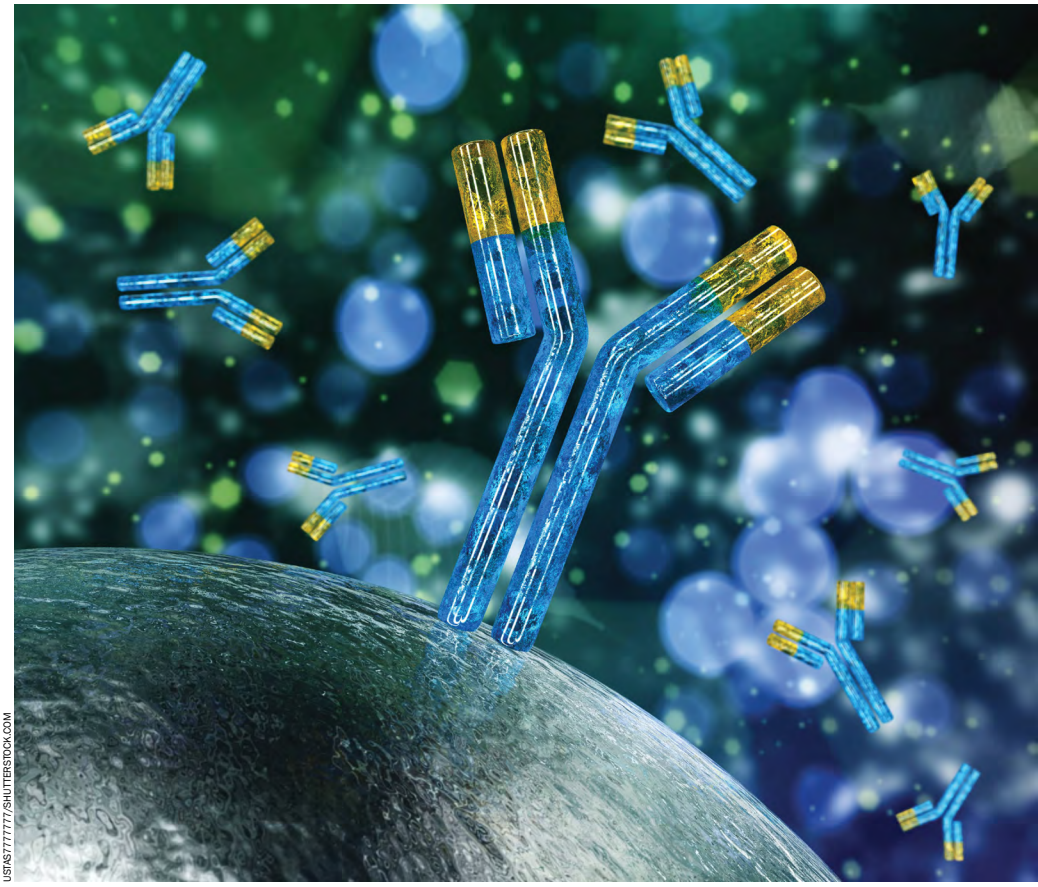
rejection (ABMR) was significantly lower in the rituximab group (8.2%) than in the non-rituximab group (23.3%) (p=0.05).[22] Tydén *et al* obtained similar results in a prospective, double-blind, randomised, placebo-controlled multicentre study,[23] which showed a trend towards fewer and milder rejections in the rituximab group compared with the placebo group (11.6% versus 17.6%) during the first six months, although the observed difference was not statistically significant (p=0.317). In contrast to these studies, Clatworthy *et al* reported the premature termination of a clinical trial due to acute rejection episodes in five of six patients treated with rituximab as B cell-depleting induction therapy after kidney transplantation.[24] In this study, daclizumab, an anti-CD25 monoclonal antibody that targets activated T cells, was compared with rituximab. The authors proposed that the increased incidence of rejection observed might be attributable to the systemic release of cytokines caused by B cell depletion, resulting in T cell activation and an increased risk of acute rejection.[24] In contrast to the Tydén trial, the patients in this trial were not treated with maintenance steroids.

Van den Hoogen *et al* randomly assigned kidney transplant recipients (n=280) in a double-blind fashion to either single-dose rituximab or placebo at transplantation. A single dose of rituximab did not reduce the overall incidence of biopsy-proven acute rejection at six months.[25] Another randomised clinical trial (NCT01095172: ReMIND) is currently recruiting patients to receive rituximab to assess if B cell depletion, rather than reducing acute rejection, will allow minimisation of immunosuppression, which may lead to better graft survival.

## Rituximab and desensitisation therapy in antibody-incompatible transplantation

### ABO-incompatible transplantation

ABO-incompatible (ABOi) kidney transplantation across the blood group antigen barrier has become an established procedure with acceptable results. Various desensitisation protocols have been developed[26–28] and, conventionally, recipients undergo sessions of pre-transplant plasmapheresis, double-filtration plasmapheresis (DFPP) or plasma immunoadsorption (IA) for removal of antibodies against the donor's blood type. To prevent re-emergence of the antibodies, apheresis followed by high-dose intravenous immunoglobulin (IVIG), which has an apoptotic effect on B cells, a mixture of immunosuppressive therapies and, in some cases, surgical splenectomy have been explored.



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With rituximab's potent ablation of B cell populations, several centres have established its use as a technique of 'pharmacological splenectomy' in preconditioning immunosuppressive therapy, which allows the humoral immune system to repair with an intact spleen.

The major objective of induction immunosuppression in ABOi kidney transplantation is to suppress the rapid production of anti-A/-B antibodies for at least one week after transplantation. This is achieved by desensitisation to A/B antigens and treatments include mycophenolate mofetil (MMF), tacrolimus and low-dose steroids for two or four weeks before transplantation,[27,29] in combination with of rituximab and anti-ABO antibody removal by plasmapheresis.[30]

Post-operative immunosuppression is based on a standard protocol, combining either anti-thymocyte globulin (ATG) or anti-CD25 monoclonal antibody with MMF, tacrolimus, and steroids.[27,31] An optimum protocol for blood group-incompatible transplantation has, however, not yet been defined and current protocols differ in techniques to remove ABO antibodies, titre targets and immunosuppression regimens.

Exact therapeutic dosages and the timing for administration of rituximab are currently not clear. Toki *et al*[32] indicated that a low dose of <375 mg/m$^2$ has a potent impact on the depletion of B cells in the spleen and peripheral blood. The same authors demonstrated that even a single dose of rituximab at 50 mg/m$^2$ depleted B cells from the peripheral blood.[32] Fuchinoue *et al*[33] showed that there was no difference in serum creatinine levels one year after transplantation, irrespective of rituximab dose, as well as showing that patients receiving rituximab induction had a lower incidence of acute ABMR and acute cellular rejection, and better graft survival at five years compared with ABOi transplant recipients who were not treated with rituximab.[33] The results of this study also suggested that rituximab-treated recipients tended to have fewer episodes of infections such as cytomegalovirus (CMV) infection. Kohei *et al*[34] reported the

long-term prevention of *de novo* donor-specific antibody (DSA) development and a reduction in chronic ABMR episodes in ABOi transplant patients treated with rituximab, compared with a cohort of ABO-compatible living donor transplant recipients not treated with rituximab and with kidney transplant recipients who underwent a splenectomy.

Lo *et al*[28] conducted a systematic review and meta-analysis in 2016, which identified all studies that described outcomes of adult living donor ABOi kidney transplantation using any form of preconditioning therapy. During a mean follow-up period of 28 months, the overall graft survival among those who received rituximab and those who underwent splenectomy, IA or apheresis, was 94.5% (95% confidence interval [CI] 91.6–96.5%), 79.7% (95% CI 72.9–85.1%), 94.1% (95%CI 88.2–97.1%) and 88.0% (95% CI 82.6–91.8%), respectively. Rituximab or IA appeared to be the most effective preconditioning strategies before ABOi kidney transplantation.[28]

### Anti-human leukocyte antigen antibody-incompatible transplantation

Desensitisation is a strategy of delivering immunomodulating therapy to highly sensitised transplant candidates as a means of eliminating or reducing anti-human leukocyte antigen (anti-HLA) antibody levels to enable HLA-incompatible (HLAi) transplantation.[35,36] In the context of desensitisation of recipients for transplantation, the use of rituximab would seem to be a logical strategy, since reduction or elimination of B cells that express CD20 and make anti-HLA antibodies should have a beneficial effect. This approach has inherent drawbacks, however. Anti-CD20 antibodies have no effect on plasma cells, which are the primary source of acute antibody production[37] and, consequently, rituximab has no immediate effect on circulating antibody levels.[37] Although rituximab has been used as an adjunct to conventional therapy in various small case series, its role as a sole agent is still unclear in highly sensitised transplant recipients.

In the last decade, various desensitising protocols using rituximab as part of combination therapy have been published. Vo *et al*[37] studied 20 highly sensitised patients, who underwent desensitisation with IVIG and rituximab. Of these 20 patients, 16 (80%) subsequently underwent successful transplantation, with six receiving a kidney from a deceased donor and ten receiving a kidney from a living donor. The remaining four patients all had panel-reactive antibody levels >50%.[37] Similarly, Morath *et al*[38] have shown the benefit of using a combination of rituximab induction with pre- and post-transplantation IA, which enabled successful transplantation in a crossmatch-positive living donor kidney transplant recipient group. After a median of ten IA sessions, this technique allowed the rapid elimination of DSAs, with good graft function.[38]

> *"With rituximab's potent ablation of B cell populations, several centres have established its use as a technique of 'pharmacological splenectomy' in preconditioning immunosuppressive therapy"*

In 2015, Ide *et al*[39] demonstrated successful kidney transplantation with immediate graft function and undetectable DSA and non-DSA levels, using a phased desensitisation protocol with rituximab and bortezomib in three highly sensitised patients, with no evidence of rejection after one year of follow-up. Conventional therapy, consisting of a single rituximab dose combined with plasmapheresis, had failed in these patients.[39]

Currently, it appears that the use of rituximab in conjunction with other modalities (such as plasmapheresis, DFPP, IA and IVIG) might constitute an improved approach for the management of allosensitisation in various non-randomised trials.[35,37,40]

## Rituximab as a treatment for ABMR

ABMR in kidney transplant recipients is a complicated process, which is still incompletely understood. The literature provides reasonable evidence that circulating B cells inflict injury on the allograft[41–43] and cause ABMR. Sarwal *et al* stained biopsies of patients with steroid-resistant rejection episodes and demonstrated unexpected, large aggregates of CD20+ B cells.[44] In the context of rejection, rituximab is a rational therapeutic choice to terminate B cell-mediated events and reverse the rejection, as it has a high affinity towards anti-CD20+ antibodies. It has been used with some success in the last decade in a number of small series of patients with severe, steroid-resistant ABMR[45] both with acute[46,47]and chronic clinical presentation.[48–50]

### Acute ABMR

The updated BANFF 2013 classification defined the requirement for three features –morphological, immunohistological and serological evidence – for a confirmed diagnosis of acute ABMR. Faguer *et al*[46] reported a pilot study on eight consecutive renal transplant patients presenting with acute ABMR, which assessed the efficacy of rituximab (375 mg/m$^2$ weekly for three to five consecutive weeks), in addition to plasmapheresis, steroids, MMF and tacrolimus. After a follow-up period of ten (range seven to 23) months, patient and graft survival were 100% and 75%, respectively. Renal function improved in six cases, with serum creatinine levels decreasing from 297±140 µmol/l to 156±53 µmol/l (p=0.015) in these patients; graft loss occurred in two patients.[46] Similar results were obtained in a small study reported by Rodriguez Ferrero *et al.*[47] In 2009, Lefaucheur C *et al* compared the efficacy of a combination of plasmapheresis, IVIG and rituximab (group B) against high-dose IVIG alone (group A) in the treatment of acute ABMR. Graft survival at 36 months was 50% in group A versus 91.7% in group B (p=0.02).[51]

RITUX ERAH, a multicentre, randomised, double-blind, placebo-controlled trial reported in 2016, suggested that rituximab does not provide any additional benefit to IVIG, plasmapheresis, and corticosteroids in patients with acute ABMR.[52] However, Macklin *et al*[53] systematically reviewed the use of rituximab for the treatment of ABMR in kidney transplantation. Of seven primary studies in the setting of acute ABMR, four reported increased graft survival and one reported improved graft function with rituximab.[53] The results of

these studies demonstrate some evidence of efficacy of rituximab in acute ABMR, but the evidence lacks high quality in the absence of randomised controlled trials.

### Chronic ABMR

A number of case series have suggested that rituximab might be a useful strategy for the treatment of chronic ABMR.[48–50] Fehr *et al*[48] reported on four kidney allograft recipients with chronic ABMR one to 27 years post-transplantation, who were treated with a combination of rituximab and IVIG. Kidney allograft function improved in all four patients, while DSAs were reduced in two of the four patients. One patient, however, experienced an acute rejection episode 12 months after this treatment, while another developed severe, possibly rituximab-associated lung toxicity.

Billing *et al*[54] demonstrated that chronic ABMR in paediatric renal transplant recipients can be treated successfully and safely with a combination of IVIG and rituximab. In contrast, of the seven studies included in a systematic analysis by Macklin *et al*,[53] one showed improved graft outcomes with a rituximab-based regimen, whereas three studies reported inferior results and three reported no difference. The authors concluded that rituximab did not appear to improve outcomes in chronic ABMR.[53]

A randomised controlled trial in chronic ABMR is currently ongoing in the UK (NCT00476164: RituxiCAN-C4) to determine whether anti-CD20 therapy with rituximab can stabilise or improve renal function and proteinuria in patients with C4d-positive chronic humoral rejection in whom standard therapy has failed.

Most published evidence for the use of rituximab in chronic ABMR has come from either retrospective studies or small case series. The establishment of its efficacy, dose duration and frequency, and safety profile will require randomised, multicentre studies. With the current state of the evidence, there is a clinical therapeutic equipoise on the use of rituximab in chronic ABMR.

## Rituximab in the management of recurrent kidney transplant glomerulonephritis

Glomerular disease that has caused end-stage renal disease has been shown to recur after renal transplantation at variable time periods. Both the US Renal Data System (USRDS) and Australian and New Zealand Dialysis and Transplant Registry (ANZDATA) indicate that recurrent disease is one of the common causes of graft failure, well ahead of the acute rejection.

> *Rituximab promises to be an important adjunct therapy in patients with recurrent transplant glomerulonephritis*

Rituximab has been used anecdotally as immunotherapy in the management of recurrent or *de novo* nephrotic syndrome after kidney transplantation. Focal segmental glomerulosclerosis (FSGS) has been shown to recur in around 30% of renal transplants, resulting in a high incidence of graft loss. The biomarkers B7.1 and soluble urokinase plasminogen activating receptor (SUPAR) have been incriminated as potential pathogenic factors related to FSGS.[17,55] Alachkar *et al* have shown that rituximab therapy in FSGS resulted in the reduction of mean proteinuria, an improvement in podocyte effacement and reduction in SUPAR levels.[56] In a multicentre retrospective review,[57] seven paediatric or young adult transplant recipients resistant to intensive plasmapheresis were treated with rituximab; three patients achieved complete remission and two a significant reduction in proteinuria.

The identification of an antibody to the M-type phospholipase A2 receptor (PLA2R) has improved the understanding of the pathogenesis of membranous nephropathy (MN).[58] A rise in anti-PLA2R antibodies correlates to disease activity and recurrence of MN in transplanted patients.[59] Recurrence occurs in 40% of transplanted patients, and rituximab has demonstrated effectiveness in reducing proteinuria and stabilising renal function in some studies,[60] with concomitant improvement in anti-PLA2R titres.[60,61]

Several individual cases on the use of rituximab in other forms of recurrent glomerulonephritis have been reported, including one with remission of disease achieved in a patient with recurrent membranoproliferative glomerulonephritis[62] refractory to steroids, MMF and plasmapheresis. When rituximab and cyclophosphamide were administered to a cohort of five renal transplant patients with biopsy-confirmed recurrent antineutrophil cytoplasmic antibody glomerulonephritis, four patients achieved remission and the fifth was refractory to treatment.[63]

Proliferative glomerulonephritis with monoclonal IgG deposits (PGNMID) is a monoclonal gammopathy of renal significance. Tsuji *et al* have reported a *de novo* case of PGNMID in a transplant where the patient received rituximab with subsequent reduction in proteinuria and serum creatinine levels.[64]

Rituximab promises to be an important adjunct therapy in patients with recurrent transplant glomerulonephritis; however, prospective multicentre trials are required to confirm its exact role and efficacy.

## Rituximab and post-transplant lymphoproliferative disease

Post-transplant lymphoproliferative disease (PTLD) is a recognised complication of immunosuppression following solid organ transplantation. Strong predisposing factors are primary infection or reactivation with the Epstein–Barr virus, young age, poor HLA mismatch, an antecedent history of CMV disease and the use of ATG.[65,66] Approximately 1–2% of kidney transplant recipients develop PTLD, whereas the incidence for other solid organs is higher, with PTLD reported in 1–2.8%, 6.3%, 10% and 20% in liver, heart, lung and small bowel transplant recipients, respectively.[67–69] The treatment remains controversial, as the spectrum of morphological, histological (monoclonal and polyclonal hyperplasia) and cytogenetic features is broad, and large multicentre, controlled trials and an understanding of the pathogenesis are lacking.

Rituximab is the mainstay of first-line therapy after reduction of immunosuppression and is administered either as monotherapy or combination therapy (for example, with cyclophosphamide, doxorubicin, vincristine and prednisolone [R-CHOP]), with significant efficacy and survival

benefit in B cell lymphomas.[70]

Evens *et al* retrospectively analysed a large (n=80) multicentre cohort and suggested a significant improvement with early rituximab-based treatment in PTLD, with progression-free survival rates of 70% and an overall survival of 73%, compared with 21% ($p<0.0001$) and 33% ($p=0.0001$), respectively, without rituximab.[71] In another retrospective study, Elstrom *et al* reported an overall response rate of 68% and an estimated overall survival of 31 months with rituximab treatment for PTLD.[72]

Rituximab as a single agent was evaluated in two Phase II PTLD studies in patients who did not respond to a reduction in immunosuppression.[73,74] The only factor predictive of a response at Day 80 was a normal lactate dehydrogenase level.[74] The need for a prognostic score that would determine the response to monotherapy was recognised, and Choquet *et al*[74] proposed a scoring system depending on age >60 years, high lactate dehydrogenase levels and an Eastern Cooperative Oncology Group performance status of 2–4, with patients in intermediate- to high-risk groups considered for rituximab with combination therapy.

Anthracycline-based chemotherapy in combination with rituximab (such as R-CHOP), which is currently used in patients with non-transplant diffuse large B-cell lymphoma, is now generally considered an effective treatment strategy that helps to achieve long-term disease-free survival in PTLD.[75]

## Role of rituximab in other solid organ transplantation

The use of rituximab, either as a single agent or in combination with other therapeutic agents, in other solid organ transplants is still undefined and detailed analysis is outside the scope of this review. Multiple cases of successful use of rituximab as salvage therapy for refractory ABMR after a failure of combination therapy with cytolytic antibodies, corticosteroids, plasma exchange and cyclophosphamide[76–78] have been reported in heart transplantation. Garrett *et al*[35] treated eight heart transplant patients for vascular ABMR with rituximab 375 mg/m$^2$ per week for four weeks. All patients had normalisation of left ventricular function with a complete histological resolution of ABMR. Melcher *et al* described the course of a patient who received a simultaneous kidney and pancreas transplant and subsequently developed clinical signs of acute kidney and pancreatic dysfunction secondary to ABMR. After treatment with plasmapheresis, IVIG and rituximab, serum creatinine, amylase and glucose levels returned to normal.[79] Kawagishi *et al* demonstrated that rituximab and infusion therapy might become a new strategy for ABOi liver transplantation.[80] Several major transplant centres have systematically employed rituximab during the perioperative period for ABOi liver transplantation, and outcomes have improved dramatically.[81–83]

## Morbidity associated with rituximab

The use of biological agents is associated with a number of short- and long-term risks, which need to be taken into consideration. Rituximab may cause hypogammaglobulinaemia, resulting from secondary antibody deficiency and causing opportunistic infections.[84] Regular monitoring of the B cell population, serum immunoglobulin levels and response to vaccines may help to detect patients with persistent hypogammaglobulinaemia.[84] Haematological complications of rituximab include thrombocytopenia and neutropenia.[85,86] Rituximab has also been associated with progressive multifocal leukoencephalopathy, with central nervous system demyelination predominantly observed in B cell lymphoproliferative disorders where rituximab is used with other immunosuppressive agents.[87]

### Key points

- **Rituximab is a genetically engineered chimeric murine–human anti-CD20 monoclonal antibody, increasingly being used in transplantation as therapy for B cell-mediated events.**
- **Rituximab has proved especially useful in acute antibody-mediated rejection and post-transplant lymphoproliferative disorders.**
- **It is used as an important adjunct therapy in desensitisation protocols for highly sensitised transplant recipients and recipients of ABO-incompatible transplants.**
- **In future, rituximab and newer B cell-depleting agents will contribute to the evolving strategies to prevent graft loss from immune-mediated events other than T cell-mediated rejection.**

## Future of B-cell-depleting agents in solid organ transplantation

Acute T cell-mediated events in kidney transplantation have been largely controlled. The current therapeutic focus is on an improvement of long-term outcomes, many of which are B cell-dependent and regulated by various pro-activation and survival signals like the B cell-activating factor of the tumour necrosis factor (TNF) family (BAFF).[88] These form potential targets for modulating B cell therapy in transplantation. There is evidence that increased BAFF levels are linked to an increased incidence of ABMR.[89] Promising agents like belimumab (a BAFF inhibitor) slow the development of B cells. Other potential agents include the anti-CD22 monoclonal antibody epratuzumab and inhibitors of costimulation interactions (anti-CD40L antibodies and CTLA4-Ig). Inhibition of these targets may result in a reduction of the B cell population.

In both murine and human models, a subset of B cells exhibit distinct immunoregulatory properties. In human peripheral blood, B cells are characterises by both pro-inflammatory (TNF α) and anti-inflammatory (interleukin [IL] 10) cytokine expression. Cherukuri *et al*[90] showed that a simple ratio of IL-10 to TNF-α best characterises B-cell immune regulatory function. Based on this ratio, transitional B cells (TrBs) exhibited an anti-inflammatory cytokine profile, and a reduced TrB IL-10/TNF-α ratio was predictive of a worse clinical outcome; hence, it will be interesting to determine how individual interventions affecting IL-10- and TNF-α-secreting TrB subsets correlate with clinical outcome.[91]

In conclusion, B cell-depleting agents,

used selectively and critically, promise to add to strategies developed to address immunological longevity of transplanted organs ■

**Declaration of interest**

[Please provide a declaration of interest]

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