Expert Opinion

Antibody-mediated rejection in kidney transplantation: an update

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Introduction: Acute antibody-mediated rejection (AMR) in renal-transplant recipients is generally less responsive to conventional antirejection therapy and has a worse prognosis than acute cellular rejection.

Areas covered: This review provides a broad understanding of the pathogenesis of AMR, recent advances in its therapy, and future directions. Conventional therapeutic approaches to AMR have minimal impact on mature plasma cells, the major source of antibody production. Emerging therapies include bortezomib, a proteasome inhibitor, and eculizumab, an anti-C5 antibody. In several reports, bortezomib therapy resulted in prompt reversal of rejection, decreased titers of donor-specific antibodies (DSA), and improved renal allograft function. Eculizumab also reversed AMR and prevented its development in patients with high post-transplantation DSA levels.

Expert opinion: Despite the small sample size and lack of controls, these studies are encouraging, and although larger studies and long-term follow-up are needed, bortezomib and eculizumab may play a major future role in AMR therapy.

Keywords: complement inhibition, donor-specific antibody, humoral rejection, proteasome inhibition

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1. Introduction

Kidney transplant rejections are classified into T-cell-mediated (acute cellular rejection; ACR) and antibody-mediated (humoral) rejection (AMR). AMR occurs in up to 20 – 30% of all acute rejection episodes following kidney transplantation and can co-exist with cellular rejection. The term 'AMR' defines all allograft rejections caused by antibodies directed against donor-specific human leukocyte antigen (HLA), blood group antigen (ABO), or endothelial cell antigens [1]. Alloantibodies preferentially attack the peritubular and glomerular capillaries, in contrast to T cells, which characteristically infiltrate tubules and arterial endothelium. Acute AMR has a worse prognosis than ACR and is generally less responsive to conventional antirejection therapy [2]. The 1-year graft loss rate following AMR varies from 15 – 20%, despite intensive conventional immunosuppressive therapy. Approximately 30% of the patients on the transplant wait list are sensitized to HLA. Immunologic memory and preformed anti-HLA antibodies pose a powerful barrier towards successful transplantation. Desensitization protocols have improved both the rate and long-term outcome of transplantation in high immune-risk patients, such as those who are highly sensitized and those with ABO blood group incompatibilities. Nearly

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Article highlights.

- Acute antibody-mediated rejection (AMR) has a worse prognosis than acute cellular rejection (ACR), which highlights the need for developing novel strategies for its early diagnosis and therapy.
- AMR is classified into hyperacute, acute, and chronic.
- C4d deposition in peritubular capillary (PTC) with either morphologic evidence of tissue injury or circulating donor-specific antibodies (DSA) confirms a diagnosis of AMR.
- Therapeutic options for AMR are evolving.
- PP/immunoadsorption remove circulating antibodies; intravenous immunoglobulin (IVIg)/mycophenolate mofetil (MMF) inhibits them; steroid/rituximab/antithymocyte globulin (ATG)/splenectomy cause B-cell depletion; and MMF/ATG/calcineurin inhibitors suppress T cells.
- Proteasome inhibitors, such as bortezomib, cause apoptosis of mature plasma cells, the major source of DSA.
- Eculizumab inhibit terminal complement activation.
- Bortezomib and eculizumab are the emerging therapies for AMR.

This box summarizes key points contained in the article.

30% of such patients can develop AMR. This underscores the importance of developing novel strategies for both early diagnosis and therapy of AMR. The current review aims to focus on the treatment of acute AMR following kidney transplantation with special emphasis on emerging treatments.

2. Classification of antibody-mediated rejection

The classification of AMR is based on clinical setting, underlying pathophysiology, and temporal relationship to transplantation. The three types of AMR are: i) hyperacute; ii) acute; and iii) chronic.

2.1 Hyperacute antibody-mediated rejection

Hyperacute AMR is caused by preformed donor-specific antibodies (DSA). It is rarely seen nowadays due to the routine use of pretransplantation cross-matching. It usually manifests shortly after the vascular anastamoses are established but it can be delayed up to 3 days. Clinically, it is characterized by widespread vascular thrombosis and the kidney turns cyanotic and flaccid, requiring immediate removal of the allograft. Histologically, the major findings associated with hyperacute AMR are neutrophil and platelet margination in glomerular and peritubular capillaries, red blood cell stasis, fibrin deposition and thrombosis within the microvasculature, acute tubular injury and widespread hemorrhagic cortical necrosis. These changes depend on the interval between transplantation and biopsy or removal of the graft [3,4]. Immunofluorescence (IF) studies demonstrated IgG in glomerular and peritubular capillaries.

2.2 Acute antibody-mediated rejection

The reported incidence of acute AMR varies in different centers depending on protocols for performing transplantation in highly sensitized patients and the methods used to detect DSA. Patients with acute AMR present with sudden onset of graft dysfunction that often arises in the first few weeks after transplantation. Presensitization is a major risk factor but most patients with AMR had a negative cross-match. There are three types of acute AMR: type I is acute tubular necrosis (ATN) like, type II is glomerular type, resembling thrombotic microangiopathy, and type III is vascular type with arterial inflammation.

The more frequent glomerular form of acute AMR is characterized by diffuse peritubular capillary (PTC) staining for the complement component C4d. The histological appearance may show scattered glomerular, PTC and tubulointerstitial neutrophils or monocyte–macrophages. The vascular/arterial type is characterized primarily by necrotizing arteritis, with mural fibrinoid necrosis and variable inflammation in the artery wall, including lymphocytes, monocytes and neutrophils along with luminal thrombosis. This lesion typically results in cortical infarction with focal interstitial hemorrhage. In the vascular form of antibody-mediated rejection, IgG and occasional IgM accompanied by C3 can be found in the walls of arteries. Rafiq *et al*. observed 17 patients prospectively to look for clinical outcomes of three different histopathologic types of acute AMR [5]. None of the patients with types II and III acute AMR responded to treatment and lost their allografts earlier, or later due to transplant glomerulopathy. All patients with type I AMR had good responses to the treatment, indicating a milder form of injury and pathologic process susceptible to current therapeutic modalities.

2.3 Chronic antibody-mediated rejection

Chronic AMR is a slow, progressive loss of graft function that usually develops > 1 year after transplantation. Several studies have shown that circulating anti-HLA class I or II antibodies, either donor reactive/*de novo* or non-donor reactive, are found in a substantial fraction of renal allograft recipients, and these are associated with later graft loss. Transplant glomerulopathy and arteriopathy are the pathologic features that are usually attributed to alloimmune mechanisms. Despite the successful treatment, more than 40% of patients with AMR will develop transplant glomerulopathy – the major chronic histologic lesion associated with chronic antibody-mediated damage [6]. Transplant glomerulopathy carries one of the worst prognoses of all chronic histological changes with 5-year graft survival rates less than 50% from the time of diagnosis. The mechanism and the treatment of chronic antibody-mediated damage remain unclear.

3. Diagnosis of antibody-mediated rejection

More detailed pathologic classification of AMR was outlined at the 2001 Banff meeting. This replaced the original category

2 of the Banff 97 classification [7]. Criteria for acute AMR in renal allografts include three cardinal features [7]:

1) Morphologic evidence of acute tissue injury, such as: i) acute tubular injury, ii) neutrophils and/or mononuclear cells in PTC and/or glomeruli, and/or capillary thrombosis; or iii) intimal arteritis/fibrinoid necrosis/ intramural or transmural inflammation in arteries.
2) Immunopathologic evidence for antibody action, such as: i) C4d and/or (rarely) immunoglobulin in PTC or ii) immunoglobulin and complement in arterial fibrinoid necrosis.
3) Serologic evidence of circulating antibodies to donor HLA or other antidonor endothelial antigens (DSA).

C4d deposition in PTC along with one of two remaining criteria clinches a diagnosis of AMR [7].

3.1 C4d staining

C4d is a fragment of C4b, an activation product of the classic complement pathway. Splitting of C4 into C4a and C4b is triggered by antidonor antibodies. C4b (and C4d) contain an occult sulfhydryl group that forms a covalent, thioester bond with nearby proteins on activation by antibody and C1 [8]. No functional role of C4d *per se* has been reported. C4d acts as an immunologic foot print of complement activation and antibody activity. PTC deposition of C4d is strongly associated with circulating antibody to donor HLA class I or class II antigens and is currently the best single marker of complement-fixing circulating antibodies to the endothelium [9]. Tissue deposition of C4d can be detected either by monoclonal antibody and IF in frozen section or by polyclonal antibody and immunohistochemistry on formalin-fixed paraffin tissue section (**Figure 1**).

PTC staining for C4d – but not its deposition in glomerular capillaries, arteries or arterioles – is a marker of AMR. C4d staining can be diffuse or focal. Feucht *et al.* reported capillary deposition of C4d that binds covalently to the capillary wall and therefore persists in graft tissue, in 51 of 93 biopsies from allografts with early graft dysfunction [10]. In renal allografts with AMR, C4d deposits are detected on the luminal surface of PTC endothelial cells or between endothelial cells and the PTC basement membrane [11]. C4d was found to be 95% sensitive and 96% specific for the presence of DSA in one study [12]. Occasionally, C4d staining can be detected as an isolated finding in the absence of DSA and graft dysfunction. This may represent a state of accommodation (growing resistance of endothelial cells against humoral effectors) or presence of harmless antibodies [13].

3.2 Donor-specific antibodies

Antibodies to donor HLA class I or II antigens (DSA) are present in 88 – 95% of patients who have C4d deposition and acute graft dysfunction versus < 10% in C4d-negative acute rejection. Antibodies to donor ABO antigens show a similar association. DSA positivity in patients at transplantation is a significant risk factor for AMR compared to patients without DSA [14]. In sensitized patients, pretransplant DSA against class 1 HLA predicted subsequent AMR and reduced graft function in a recent study [15]. Mature plasma cells are the major source of DSA production. C4d deposition without detectable circulating DSA could result from antibody levels below the detection threshold due to immunoadsorption by the graft.

The advent of solid-phase antibody testing has greatly enabled the characterization of the HLA-specific antibodies and has largely replaced cell-based antibody testing methods that require viable cells. Solid-phase antibody testing employs either an enzyme-linked immunosorbent assay (ELISA)-based system or a color-coded bead-based fluorometric assay. The latter is more sensitive and employs soluble HLA antigen-coated beads that can be detected by flow cytometer or by the Luminex technology (LABScreen, One Lambda, Inc., Canoga Park, CA). Fluorometric-bead-based assay is less affected by prior therapy with agents such as antithymocyte globulin (ATG), rituximab or intravenous immunoglobulin (IVIg). Several studies have documented poor long-term allograft function in patients who developed anti-HLA antibodies [16-19]. Recently, major histocompatibility-complex class I chain-related gene A (MICA) antibodies and non-HLA antibodies to endothelial targets such as angiotensin II type 1 receptor (AT1-R) have been found to be associated with AMR [20,21]. Activating IgG antibodies targeting the AT1-R were detected in the serum from patients presenting with refractory vascular rejection, absent anti-HLA DSA and malignant hypertension [22]. AT1-R blockade with losartan was found to be beneficial in such patients. At present, there is no consensus on when to test for DSA, especially in the absence of allograft dysfunction; this is a subject of ongoing prospective studies. The clinical relevance of low levels of DSA detected by newer, highly sensitive assays is unclear but characteristics such as antigen specificity and binding strength may be useful in assessing clinical relevance of such DSA.

4. Therapeutic strategies in antibody-mediated rejection

Knowledge of the mechanism of injury in AMR has provided insights to therapeutic interventions. AMR involves the production of high levels of DSA by plasma cells. The plasma cells could be pre-existing (prior to transplant) or newly created from memory or naïve B cells. The main mechanism of injury involves antibody-dependent activation of complement cascade with resultant capillaritis and glomerulitis, although evidence of a complement-independent mechanism has been reported [23]. T cells are vital for the initiation of primary and memory-B-cell responses that result in generation of plasma cells. Therapeutic approaches to AMR are based on the following concepts:

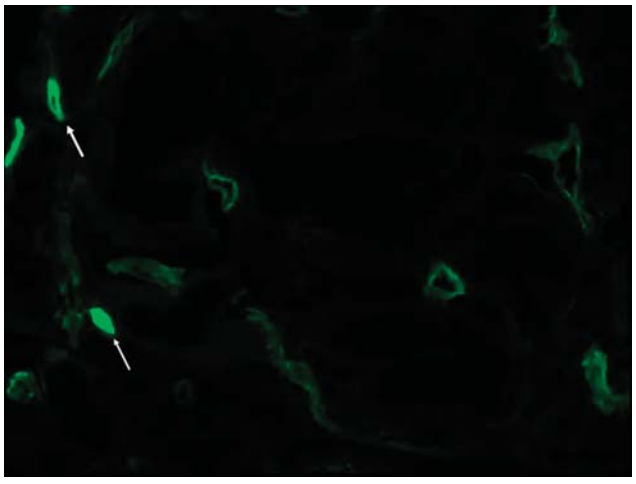

Figure 1. Immunoflurescence staining of a renal biopsy specimen in a patient with AMR. Showing C4d deposition along the peritubular capillaries ×20 (arrow pointing towards peritubular capillary C4d deposit).

- Circulating antibody removal: plasmapheresis (PP), immunoadsorption.
- Residual antibody inhibition: IVIg, mycophenolate mofetil (MMF).
- Suppression of antibody production or B-cell depletion: steroids, rituximab, ATG, splenectomy.
- Suppression of T-cell response: MMF, ATG, calcineurin inhibitors.
- Plasma-cell apoptosis/depletion: Proteasome inhibitor, e.g., bortezomib
- Inhibition of terminal complement activation: anti-C5 antibody, e.g., eculizumab. Emerging therapies target the last two mechanisms. Multiple interventions are usually applied simultaneously in AMR. Details of individual therapies are described below.

4.1 Plasmapheresis

Plasmapheresis (PP) removes alloantibodies from the circulation. It is the fastest and most effective method for the elimination of DSA. PP modalities include plasma exchange, double filtration PP and immunoadsorption. Plasma exchange has been the preferred method in the United States because of cost and ease of the procedure [24]. Because plasma exchange is the most commonly used method, the term PP is synonymous with plasma exchange. The usual prescription includes 1.0 – 1.5 volume exchange using albumin solution daily or on alternate days, continued until serum creatinine falls within 30% of previous baseline values [25].

Although PP is effective in removing DSA from circulation, it does not suppress antibody synthesis and rebound in circulating DSA after PP has been documented [26]. PP is therefore commonly used with agents that neutralize antibodies (e.g., IVIg) or suppress antibody production (e.g., calcineurin inhibitors, MMF or rituximab). Pascual *et al.* reported the successful treatment of five patients with refractory AMR using a combination of PP and rescue immunosuppression with tacrolimus and MMF [27]. They reported 100% graft survival at 19.6 months mean follow-up. An observational study of 18 patients with AMR treated with PP and intensification of immunosuppression reported 1-year and 5-year graft survival rates to be 86 and 78%, respectively [28].

The adverse effects of PP include volume contraction, bleeding diathesis, allergic reaction, blood-borne pathogen transmission and antigen sensitization. Most of these reactions can be minimized by the avoidance of FFP use in favor of 5% albumin.

4.2 Intravenous immunoglobulin

IVIg is a commercially prepared product from pooled human plasma of 50,000 – 100,000 or more screened, healthy donors. It is composed of more than 90% intact IgG, a few dimers, fragments of Fabs (fragment antigen binding fragments) and traces of IgM and IgA [29].

The mechanism of action of IVIg is unclear. The proposed mechanisms of action include suppression of immunoglobulin synthesis, anti-idiotypic activity against DSA (with resultant neutralization of DSA), blockade of the Fc receptor, inhibition of complement activation, and anticytokine activity [29,30].

IVIg is usually used in combination with PP but some studies employed IVIg alone (usually in high dose 1 – 2 g/kg). The Cedars-Sinai transplant program used high-dose IVIg plus pulse steroids in seven renal and three cardiac allograft recipients with refractory AMR [31]. IVIg was effective in reversing rejection within 2 – 5 days of infusion with no recurrence in kidney transplant recipients; however, recurrence occurred in two heart transplant recipients. At a mean follow-up of 23 months, all renal allografts were functioning with a mean serum creatinine of 1.4 mg/dl. Rocha *et al.* found similar 1-year graft survival (81 vs 84%, p = ns) in AMR patients treated with IVIg, PP and pulse steroids combined and in patients with ACR treated with pulse steroids alone or with antilymphocytic therapy [26].

In a non-randomized control trial, Lefaucheur *et al.* treated 12 patients with AMR with high-dose IVIg alone (control) and 12 with PP + IVIg + rituximab. At 36-month follow-up, graft survival rate was 50% in the control group and 91.7% in the treatment group [32]. Beneficial effects of combined PP plus IVIg were reported in other retrospective studies [33,34].

One potential benefit of IVIg is its ability to replenish gammaglobulin lost during PP, hence decreasing infection risk. Serious adverse effects from IVIg are rare but include aseptic meningitis (which occurs within 48 – 72 h of administration and is self-limiting), acute renal failure (osmotic injury, especially with high-dose IVIg), thrombotic events and severe anaphylactic reactions (associated with IgA sensitization in

patients with IgA deficiency, can be avoided by the use of IVIg with low IgA content) [24]. The severity of common first-dose reactions such as headache, fever, chills, myalgias and hypotension/hypertension can be reduced by slowing the infusion rate. Severe adverse effects can be minimized by the use of isosmolar preparation, sterile water as diluent, and avoidance of IVIg concentrations > 5% [29]. The usual recommended dose is 100 mg/kg of IVIg after each PP session and 300 – 400 mg/kg for 1 – 2 days after last PP with a cumulative dose of 1000 mg/kg [27]. However, various dosing schedules are currently in use and the optimal dose is poorly defined.

4.3 Immunoadsorption

In immunoadsorption (IA), plasma is processed through an adsorbent column and re-infused into the patient. As there is no loss of volume, no replacement fluid is needed. There are two immunoadsorption columns: a protein A adsorption column that adsorbs immunoglobulin and an ABO antigen column that adsorbs specific anti-A or anti-B antibodies regardless of immunoglobulin class or subclass [35]. In a randomized, controlled trial, Bohmig *et al*. treated five patients (test group) with IA using protein A and another five patients without IA with the option of IA rescue after 3 weeks [36]. Both groups received tacrolimus conversion and if indicated, anticellular treatment. All IA treated patients responded to treatment within 2 weeks whereas four of the control patients were dialysis-dependent despite rescue IA. The same group, in a non-randomized trial has previously reported the beneficial effects of IA in AMR [37]. Min *et al*. treated six patients with AMR employing IA using staphylococcal protein A plus tacrolimus/MMF combination therapy [38]. They reported 100% patient and graft survival in the IA group with mean serum creatinine of 1.2 mg/dl after 18months mean follow-up.

Immunoadsorption is an attractive strategy for efficient and highly specific antibody depletion. However, because of the cost, membrane unavailability and the relative ease of using PP, IA is not commonly used for AMR treatment.

4.4 Antilymphocyte therapy

ATG is a polyclonal preparation generated from the immunization of rabbits with human thymus. Mechanisms of ATG action include abrogation of T-cell help by elimination of $CD4^+$ T-cell and B-cell interaction, direct B-cell toxicity and modulation of alloantibody production. It has also been shown to induce apoptosis [39]. Many studies have used ATG as part of AMR treatment especially when both cellular and humoral features are seen in biopsy.

Shah *et al*. used ATG 0.75 mg/kg/day for 5 – 10 days in combination with plasma exchange in seven patients with AMR [39]. Reversal of AMR occurred in 85% with mean serum creatinine of 1.4 mg/dl at 1-year follow-up and no difference in graft survival in the treated patients compared with those without AMR. ATG can be administered in three or four divided doses to a cumulative dose of 6 mg/kg. The platelets and white blood count should be monitored with dose adjustments as needed.

4.5 Steroid

Most patients presenting with clinical acute allograft rejection receive pulse methylprednisolone therapy empirically or based on allograft biopsy findings of ACR, AMR or a combination of the two. Steroids help to treat the cell-mediated component. Steroids also work by down-regulating the B-cell response through decreased activity of T helper cells, which indirectly suppresses AMR. A commonly used dosing schedule is methyl prednisone 250 – 500 mg/day intravenously for 3 – 5 days followed by a prednisone taper.

4.6 Mycophenolate mofetil

The mechanism of action of MMF involves blockade of lymphocyte-specific isoforms of inosine monophosphate dehydrogenase. MMF inhibits *in vitro* antibody production and reduces *in vivo* humoral response in transplant recipients. When used in combination with tacrolimus, it limits B-cell response in renal allograft recipients with AMR [40]. Cyclosporine appears to interfere with the metabolism of MMF, which may decrease the biological effects of this drug on alloantibody production [41].

4.7 Deoxyspergualin

Deoxyspergualin (DSG) is an analogue of spergualin and shows antiproliferative action against interleukin (IL)-2 stimulated maturation of T cells. It also blocks B-cell differentiation, proliferation as well as inhibit cytotoxic T-cell differentiation. There are very few reports of its use in AMR treatment. Nojima *et al*. reported on the use of DSG and PP in five living donor kidney recipients with AMR. There was resolution of AMR in four out of five patients (80%) [42]. The dose of DSG was 3 mg/kg/day for 10 days with PP of 1 – 9 sessions depending on treatment outcome. All the patients received pulse steroids. DSG is not a well recognized therapy for AMR, and only a few case reports exist.

4.8 Splenectomy

The spleen is the largest lymphoid organ in the body and it plays an important role in alloantibody generation. Splenectomy reduces the B-cell immune response and the numbers of precursor and mature plasma cells. Splenectomy has been used as part of pretransplantation desensitization protocols, especially in highly sensitized patients and ABO mismatch. However, there are case reports on the use of splenectomy for treatment of refractory AMR. Kaplan *et al*. reported four cases of severe AMR (within 2 weeks of transplantation, in highly sensitized patients) who failed 10-day treatment with standard therapy including steroids, plasma exchange, IVIg, ATG and rituximab [43]. With persistent deterioration of renal function, laparoscopic splenectomy was done as a rescue therapy with 100% graft survival after 8 months of follow-up with a mean serum creatinine of 1.3 mg/dl. Locke *et al*. had

similar reports on five patients with severe AMR treated with PP, IVIg and rituximab followed by splenectomy within 48 h of diagnosis [44]. They reported resolution of AMR with 100% patient and graft survival after 18 months of follow-up and no significant postsplenectomy infectious complications. All patients received pneumococcal, meningococcal and *Haemophilus* influenza vaccines. The disadvantages of splenectomy include a lifelong risk of sepsis with encapsulated organisms, permanent effects on the immune system, a lack of independent reduction in DSA titers, and surgical risks. For these reasons splenectomy is not favored as a conventional treatment for AMR.

4.9 Rituximab

Rituximab is a chimeric anti-CD 20 (anti-B-cell) monoclonal antibody that is approved by the US Food and Drug Administration (FDA) for the treatment of lymphoma. The CD 20 antigen is expressed early in B-cell ontogeny but it is absent on mature plasma cells [45]. The variable region of rituximab binds to CD 20 through three different mechanisms and marks the cell for destruction, thereby leading to a profound and sustained depletion in the number of circulating B cells. The three mechanisms of action of rituximab include antibody-dependent cell cytotoxicity, complement-dependent cell killing and induction of apoptotic cell death [24].

Genberg *et al*. examined the pharmacodynamics after a single dose of rituximab given to renal transplant recipients. They demonstrated that B-cell elimination was rapid and occurred in the peripheral blood over 1 – 2 days [46]. The effect on the B-cell population was also prolonged and B cells did not re-emerge for 1 year and remained suppressed for 2 years. Rituximab has demonstrated benefits in renal transplantation and is being used in some pretransplantation desensitization protocols, post-transplant lymphoproliferative disorders, allograft *de novo* or recurrent glomerulonephritis as well as in the treatment of AMR.

The initial report of using rituximab to effectively treat AMR came from Becker *et al*. who evaluated 27 patients with refractory rejection who received a single dose of rituximab [47]. Three grafts were lost but the 24 surviving grafts had good function at the time of discharge. Kaposztas *et al*. reported a retrospective study of 54 patients with AMR [48]. Patients in group A (n = 26) were treated with PP and rituximab and group B patients (n = 28) received PP without rituximab. Patients with low serum IgG levels also received IVIg. Two-year graft survival was significantly better in the rituximab group (90 vs 60%, p = 0.005) with the difference attributed to rituximab. Mulley *et al*. reported a case series of seven patients with refractory AMR who responded to treatment with a single low dose of rituximab (500 mg) [49]. All patients recovered renal function with 100% patient and graft survival at 21 months mean follow-up. Three patients had significant viral infections but recovered fully. Several other recent reports support the utility of rituximab in treating acute AMR [50-53]. Some of the studies are limited by incomplete criteria for AMR diagnosis (especially older studies before the advent of DSA and C4d) and inconsistent patient variables. All cases in which rituximab has shown efficacy have received IVIg, PP and/or steroids. The beneficial effects of rituximab in this setting are likely multifactorial. In addition to depleting B cells and reducing DSA, rituximab has been shown to disrupt T-cell co-stimulator and antigen-presenting-cell activities mediated by B cells, thereby diminishing T-cell effector functions.

The optimal dosing and length of therapy for rituximab is unclear. It is also unclear whether multiple doses of rituximab would yield better depleting activity and antibody reduction than a single dose. Some individuals have been reported with pre-existing antichimeric antibodies; others rapidly develop such antibodies *de novo*. This leads to decreased efficacy of rituximab. Although useful as a part of combination therapy, the major limitation of rituximab has been its inability to deplete CD 20 negative plasma cells that continue to produce DSA and mediate graft injury.

Common adverse reactions (≥ 25%) reported with the use of rituximab in clinical trials of lymphoid malignancies included infusion reactions, fever, lymphopenia, neutropenia, chills, infection and asthenia [54]. The adverse events reported at ≥ 10% in clinical trials of rheumatoid arthritis included upper respiratory tract infections, nasopharyngitis, urinary tract infections and bronchitis. Activation of viral infections such as progressive multifocal leukoencephalopathy and hepatitis B also has been reported with rituximab therapy [54].

4.10 Bortezomib

Experts and researchers in the field of AMR have long recognized the potential utility of an antihumoral agent with the ability to directly target plasma cells. Traditional modalities have been able to successfully remove antibodies, inhibit antibody activity and even suppress antibody production but none have been shown to affect mature antibody-producing plasma cells. In theory, and now in clinical experience, the proteasome inhibitor bortezomib has been shown to cause plasma-cell apoptosis resulting in the reduction and elimination of circulating DSA levels in patients with acute AMR [55,56].

Bortezomib (Velcade, Millenium Pharmeceuticals Cambridge, MA) is a first-in-class proteasome inhibitor that is approved by the US FDA for the treatment of multiple myeloma (a plasma-cell neoplasm) [57]. It is a modified dipeptidyl boronic acid that is available for intravenous injection use only. Bortezomib is a reversible inhibitor of the chymotrypsin-like activity of the 26S proteasome in mammalian cells. The 26S proteasome is a large complex that degrades ubiquitinated proteins. This ubiquitin–proteasome pathway plays an essential role in regulating the intracellular concentration of specific proteins, thereby maintaining homeostasis within cells. Bortezomib inhibition of the 26S proteasome prevents this targeted proteolysis, which can affect multiple signaling cascades within the cell. Specifically,

activation of the transcriptional activator nuclear factor kappa B (NF-κB) is prevented, leading to unregulated accumulation of unfolded proteins and defective ribosomal products within the endoplasmic reticulum. This can disrupt the normal cell homeostasis, thus resulting in plasma-cell apoptosis [58]. A schematic representation of bortezomib action is shown in **Figure 2**.

The distinct pathological changes of AMR are caused by high levels of DSA, which are produced by plasma cells (either from those that existed pretransplant or from those newly created from memory or naïve B cells). By targeting plasma cells, bortezomib may directly destroy the source of this damaging DSA. In some desensitization protocols, it has been observed that among patients with similar DSA levels at baseline, some developed AMR whereas others did not. This made it difficult to define the exact relationship between the two. However, Burns *et al*. sought to examine the natural history of AMR in highly sensitized patients undergoing positive cross-match kidney transplantation [59]. In this study, a high DSA level after kidney transplantation (particularly within the first month) was the major risk factor for the development of AMR. There also seemed to be little correlation between baseline DSA levels and post-transplant DSA levels.

Bortezomib is a cytotoxic agent the utility of which was first recognized in the treatment of cancer; it has also been shown to suppress T-cell function [60]. The recommended dose of bortezomib is 1.3 mg/m^2 given as a 3 – 5 sec bolus injection. The mean half-life after first dose ranged from 9 to 15 h at doses ranging from 1.45 to 2.00 mg/m^2 in patients with advanced malignancies, but the pharmacokinetics of the drug as a single agent have not been fully characterized at the recommended dose in myeloma patients (the same dose that has been used in the treatment of AMR). The binding of bortezomib to human plasma proteins averaged 83% in the original study population and the metabolism occurs in the liver via cytochrome P450 enzymes, 3A4, 2D6, 2C19, 2C9, and 1A2 [57]. No pharmacokinetic studies were conducted with bortezomib in patients with hepatic or renal impairment. However, the drug is metabolized by liver enzymes and therefore its clearance may decrease in patients with hepatic impairment. Also, no clinical information is available on the use of bortezomib in patients with creatinine clearance values < 13 ml/min and patients on dialysis.

The safety and efficacy of bortezomib was initially established in a study by Richardson *et al*., in which the drug was given to 202 patients with refractory multiple myeloma [61]. The most commonly reported adverse events included asthenic conditions (fatigue, malaise, and weakness: 65%), nausea (64%), diarrhea (51%), anorexia (43%), constipation (43%), thrombocytopenia (43%), neutropenia (11%), peripheral neuropathy (37%), pyrexia (36%), vomiting (36%) and anemia (32%) [54]. More recently, a prospective study looking at the toxicity profile of bortezomib in 50 renal transplant candidates and recipients found that hematologic (anemia, thrombocytopenia), gastrointestinal (GI: nausea, diarrhea) problems and mild peripheral neuropathy are common but generally mild and transient [62].

Bortezomib is contraindicated in patients with hypersensitivity to bortezomib, boron or mannitol. Currently available studies are summarized in **Table 1**. The drug is pregnancy category D and is considered unsafe for nursing mothers. Also, its safety and efficacy in children has not been established [61].

At present, our knowledge of bortezomib as a therapy for acute AMR is based on clinical experience. At the University of Cincinnati, Everly *et al*. treated six kidney transplant recipients with refractory AMR and concomitant ACR with bortezomib at labeled dosing (1.3 mg/m^2/dose × 4 doses). They found that in each case this therapy provided: i) prompt rejection reversal; ii) marked and prolonged reductions in DSA levels; iii) improved renal allograft function; and iv) suppression of recurrent rejection for at least 5 months [55]. Immunodominant DSA (iDSA) levels were decreased by more than 50% within 14 days and remained substantially suppressed for up to 5 months. In addition, one or more additional DSAs were present at lower concentrations (non-iDSA) in each patient and were also reduced to undetectable levels. Two grafts were lost, one of which was attributed to non-compliance with immunosuppressive medications and the other was lost in the absence of any acute rejection on biopsy for unknown reasons. The same group subsequently reported the outcome of AMR in two patients who received a bortezomib-based regimen as primary therapy [63]. Both patients experienced prompt AMR reversal and DSA elimination within 14 days. In another study, five patients with mixed ACR and AMR were given four doses of bortezomib [64]. There was prompt AMR and ACR reversal in all patients and significant reduction in DSA in four patients. Commonly recognized bortezomib-related toxicities (GI toxicity, thrombocytopenia and paresthesias) occurred in some of the patients but all were transient [62].

Sberro-Soussan *et al*. explored the utility of bortezomib as sole desensitization agent in four patients with subacute AMR and persistent DSA; they did not see any decrease in DSA titers after one cycle of bortezomib [65]. Lack of adjunctive steroid use, a critical component for enhancing the pro-apoptotic effect of bortezomib, was described as a possible reason for the observation. Trivedi *et al*. reported the first clinical experience with using bortezomib in patients with stable renal allograft function [66]. Eleven recipients of living donor kidney transplantation on clonal stimulation-deletion protocol with elevated anti-HLA antibodies (> 1000 mean fluorescence intensity) were treated with 1.3 mg/m^2 of bortezomib together with methylprednisolone 250 mg on days 1, 4, 8 and 11 followed by two to four sessions of PP. Six of the patients were also given a dose of rituximab. Bortezomib effectively reduced the level of both DSA and non-DSA anti-HLA antibodies although four patients had persistent elevation or reappearance of anti-HLA antibodies, suggesting that more than one cycle of bortezomib treatment may be

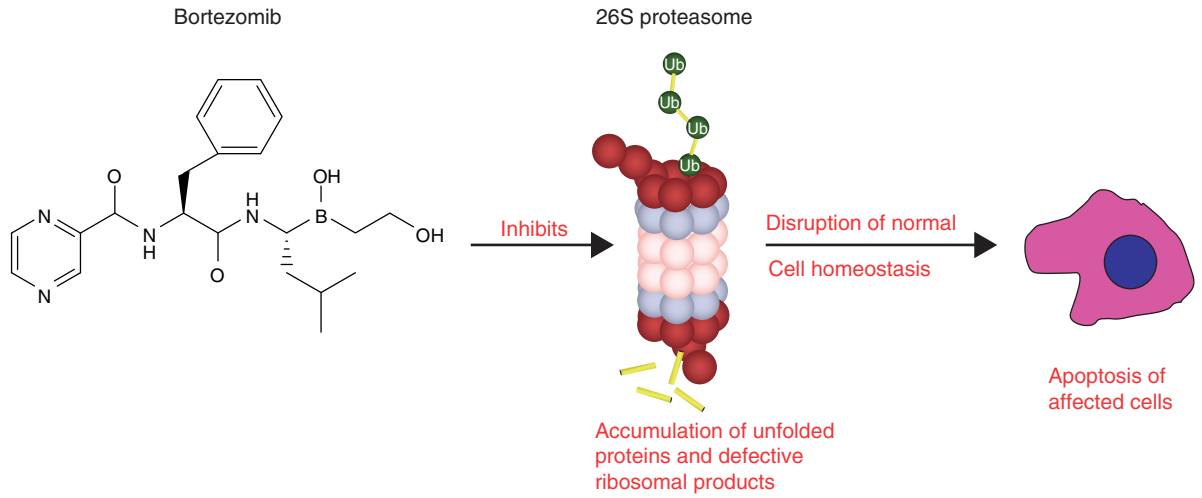

Figure 2. A schematic representation of bortezomib action.

required in some cases. Minimal adverse effects such as diarrhea and thrombocytopenia were observed in the study. A small study comparing the effect of bortezomib on early (< 6 months post-transplant) versus late AMR found greater improvement in early rejection in terms of graft function, histology and DSA levels [67].

Pretransplant HLA-antibody screening may be able to predict therapeutic response to bortezomib in early AMR. Woodle *et al*. observed a hierarchy of response to bortezomib, showing that HLA antibody reductions were greater in DSA than non-DSA and that *de-novo* DSA was more responsive than preformed DSA [68]. This difference in response is probably attributable to the susceptibility of the particular type of plasma cell responsible for producing the respective antibodies to bortezomib.

Given the potential that bortezomib has shown so far as a desensitizing agent and AMR treatment modality in renal allograft recipients, more studies on this agent are sure to arise in the near future. Currently, a trial looking at the impact of *in vivo* treatment of bortezomib on anti-HLA production by normal antibody secreting cells in sensitized renal transplant candidates is recruiting participants (www.ClinicalTrials.gov identifier NCT00722722).

4.11 Eculizumab

C4d staining of peritubular capillaries has been a valuable instrument in the tissue diagnosis of AMR and serves as evidence that early complement activation occurs almost invariably in the course of AMR. Eculizumab is a monoclonal antibody directed against the complement protein C5, it can therefore block the activation of terminal complement [69]. This prevents the generation of C5a anaphylatoxin and the formation of C5b-C9 membrane attack complex. Eculizumab is currently approved by the US FDA for the treatment of paroxysmal nocturnal hemoglobinuria. Locke *et al*. reported the successful treatment of a patient with refractory AMR using eculizumab [70]. At the Mayo Clinic, Stegall *et al*. used eculizumab at the time of transplant to demonstrate that incomplete complement activation and blockade of terminal complement generation by eculizumab prevented the development of AMR in patients who developed high levels of DSA post-transplant [71]. No significant adverse effects were reported. These early clinical experiences suggest that blockade of terminal complement may prove a very effective therapeutic approach in AMR.

5. Conclusion

AMR after kidney transplantation has a worse prognosis than ACR. C4d staining of PTC and detection of DSA has improved our ability to diagnose AMR. Conventional therapeutic approaches to AMR include PP, IVIg, steroids, MMF, rituximab and calcineurin inhibitors. None of these agents has a significant effect on the major source of antibody production: namely, the mature plasma cells. There is now emerging evidence that proteasome inhibition with bortezomib can target mature plasma cells, inducing apoptosis with reversal of AMR, decrease in DSA titer and improved graft function. Preliminary evidence also shows that complement protein C5 antagonism with eculizumab, a monoclonal antibody, can successfully treat AMR. These studies are encouraging but are limited by small sample sizes and lack of control groups. Larger studies and long-term follow-up are needed. Bortezomib and eculizumab may play a major future role in AMR therapy.

6. Expert opinion

In the last couple of years, there has been a significant stride towards a broader understanding of the pathogenesis of AMR and its therapeutic strategies. Still more extensive studies and follow-up are needed to determine whether these

Table 1. Published studies of the use of bortezomib in AMR.

Study[‡]	*N*	Patients	Treatment	Outcome
Everly, M *et al*. 2009 [64]	5	Renal transplant recipients with mixed AMR and ACR	Bortezomib 1.3 mg/m^2/dose × 4	Prompt AMR and ACR reversal significant decrease in DSA levels
Everly, M *et al*. 2008 [55]	6	Kidney/kidney–pancreas transplant patients with mixed AMR and ACR, elevated DSA who failed conventional therapy (PP, IVIG, rituximab)	Bortezomib 1.3 mg/m^2/dose × 4	Prompt rejection reversal marked and prolonged reductions in DSA levels improved renal allograft function suppression of recurrent rejection for at least 5 months > 50% decrease in iDSA levels within 14 days and suppression for up to 5 months
Perry, D *et al*. 2009 [56]	2	positive cross-match renal transplant recipient with AMR	Bortezomib 1.3 mg/m^2/dose on days 1, 4, 8, 11 daily PP, IVIg	Decrease HLA allospecificities Decrease in number of plasma cells in bone marrow
Sberro-Soussan R *et al*. 2010 [65]	4	Renal transplant recipients sub-acute AMR with persistently elevated DSA	Bortezomib 1.3 mg/m^2/dose × 4 as sole desensitizing agent	No significant decrease in DSA within 150 days post-treatment
Trivedi H, *et al*. 2009 [66]	11	Living donor renal transplant patients with anti-HLA alloantibodies	Bortezomib 1.3 mg/m^2/dose with Methylprednisolone 250 mg on days 1, 4, 8, 11 2 – 4 sessions of PP 1 dose rituximab (in 6 patients)	Reduced DSA and non-DSA levels Stable graft function 4 months post-treatment
Walsh, R *et al*. 2010 [63]	2	Living donor renal transplant recipients	*Patient 1:* PP, rituximab Bortezomib with methylprednisolone pretreatment PP × 3 on alt. days, starting 72 h after bortezomib dose *Patient 2:* PP, rituximab Bortezomib second course of rituximab and bortezomib (PP before each dose) followed by 3 PP treatments post-bortezomib	*Patient 1:* DSA levels became undetectable Serum creatinine returned to baseline *Patient 2:* DSA levels became undetectable Serum creatinine reached a new post-transplant nadir

[‡]All studies were retrospective case reports.

ACR: Acute cellular rejection; AMR: Antibody mediated rejection; DSA: Donor-specific antibody; iDSA: Immunodominant DSA; IVIg: Intravenous immunoglobulin; PP: Plasmapheresis.

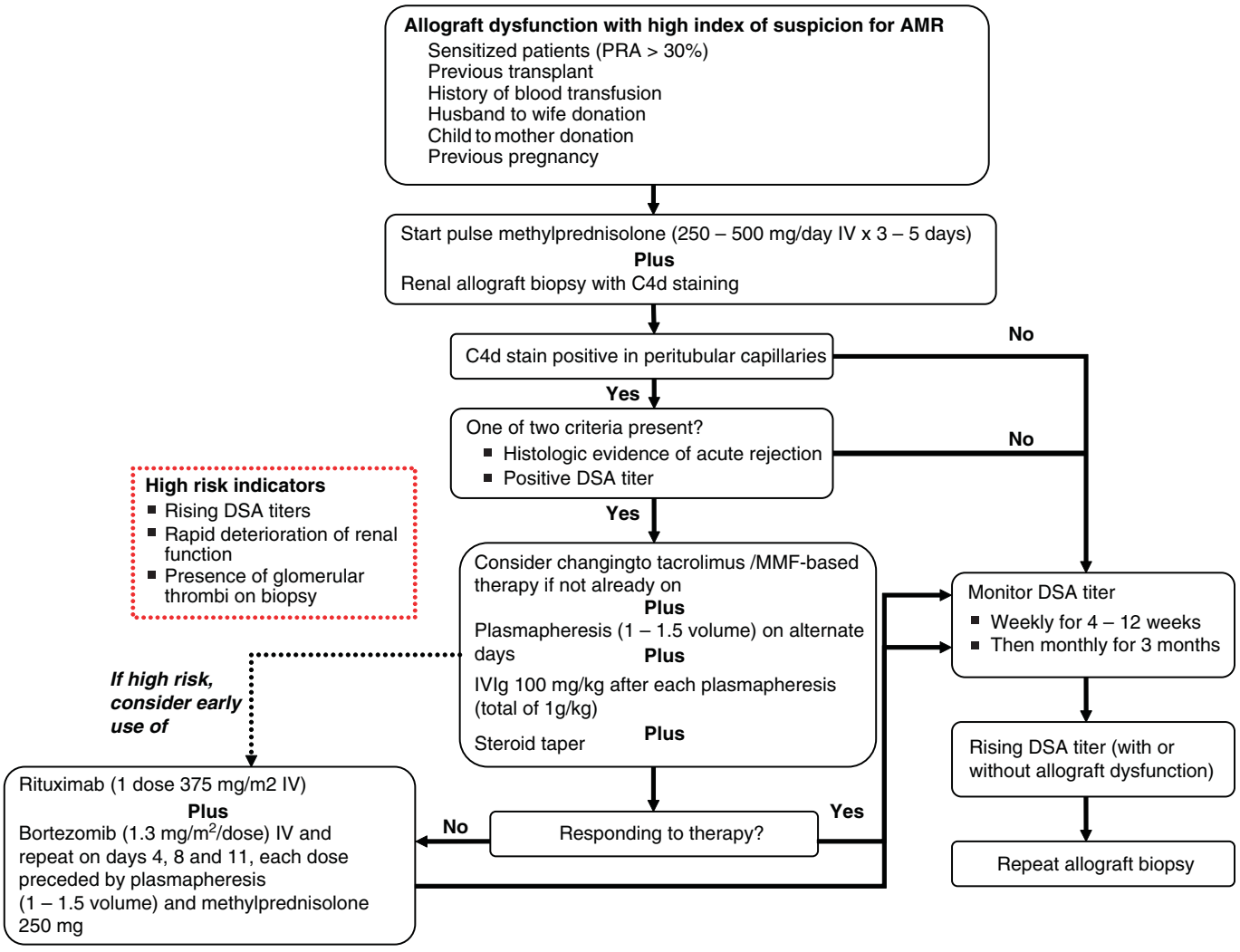

Figure 3. Therapeutic approach to acute antibody-mediated rejection (AMR).

advances will translate into improved outcomes. AMR is generally associated with an aggressive clinical course that is less responsive to conventional antirejection therapies, with significant adverse impact on allograft outcome. With the increasing use of desensitization protocols and transplantation of high-immune risk recipients, we are likely to see a higher incidence of AMR.

C4d immunostaining of PTC is the cornerstone for diagnosing AMR and should be incorporated into all biopsies obtained for allograft dysfunction. Serum DSA level after kidney transplantation is a major determinant of AMR. There is evidence that the development and persistence of DSA even in the absence of graft dysfunction is associated with adverse long-term graft outcome. The immunologic mechanism initiating the development of high levels of DSA is still unclear. Mature plasma cells are the major source of DSA production.

Antihumoral therapies that provide a prompt and significant reduction of DSA titer in AMR result in long-term graft outcome comparable to ACR episodes with absent DSA [72]. Traditional treatments of AMR such as PP, IVIg, rituximab and ATG deplete predominantly immature B cells but not the plasma cells [73]. The proteasome inhibitor bortezomib has been shown to induce significant apoptosis of human plasma cells, preventing alloantibody production *in vitro* [56]. There is now emerging evidence that bortezomib-based antihumoral therapy is effective in reversing AMR and controlling DSA levels [55,63,66]. Pretreatment with PP and rituximab is recommended: the former will remove preformed antibodies and the latter may potentate bortezomib efficacy by reducing plasma-cell generation from the memory-B-cell population [63]. These early studies using bortezomib are encouraging but are limited by small sample size and the lack of controls, which makes it difficult to assess

true efficacy. Larger studies and long-term follow-up are needed but the preliminary evidence cautiously suggests that proteasome inhibition with bortezomib may play a major future role in AMR therapy.

The therapeutic approach to AMR is summarized in **Figure 3**. Pulse methylprednisolone at a dose of 250 – 500 mg IV should be administered for 3 – 5 days, followed by steroid taper in all patients presenting with acute renal allograft rejection. Steroid helps to treat any concomitant ACR and can exert a favorable effect on AMR by down-regulating the B-cell response. Maintenance immunosuppression should be switched to tacrolimus/MMF combination if the patient is not on this; the tacrolimus/MMF dose should be augmented if the patient is already on it. PP should be initiated on an alternate-day schedule using 1.0 – 1.5 volume exchanges. An alternate-day regimen should enable enough recovery of coagulation factors so that albumin replacement can be used and FFP use minimized to reduce the risk of antigen sensitization. Typically, PP is continued until serum creatinine reaches within 30% of previous baseline value. IVIg is administered after each PP session initially at a dose of 100 mg/kg with higher dose for couple of days after final PP aiming for a cumulative target dose of 1000 mg/kg. ATG therapy can be considered in patients with severe concurrent ACR.

Patients who fail to respond to this approach should be offered further therapy with rituximab and bortezomib. A single dose of rituximab (375 mg/m^2) is recommended followed by bortezomib 1.3 mg/m^2/day. The bortezomib dose should be repeated on days 4, 8 and 11. Each dose of bortezomib should be preceded by PP and methylprednisolone pretreatment. Earlier switch to this second-line approach should be strongly considered in high-risk candidates such as those with rising DSA titers, rapidly deteriorating renal function and biopsy evidence of glomerular thrombi.

Even when AMR is treated successfully, patients need to be monitored closely. Renal function should be evaluated twice a week for 1 month and weekly for 3 months. Careful monitoring of DSA titers is also important. A 50% reduction in DSA level generally favors improved graft survival. The suggested interval for DSA monitoring is weekly for 4 – 12 weeks and monthly for 3 months, using either ELISA or the more sensitive single antigen bead Luminex assay. The significance of a persisting low DSA level is unclear but a rising DSA titer despite stable allograft function should prompt repeat biopsy.

AMR remains a formidable challenge in kidney transplantation, especially in high-immune-risk recipients. There has been significant progress in our understanding of this entity but several important issues regarding AMR remain. Optimal therapy is yet to be defined and a better understanding of the relative pathogenic contributions of memory B cells versus mature plasma cells is crucial in this regard. It is not clear if success in the treatment of acute AMR is to be followed only by the hurdle of chronic antibody-mediated injury. The novel mechanisms of action and preliminary results with agents such as bortezomib and eculizumab are encouraging but require more studies and long-term follow-up.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

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