

## Comprehensive Review

# Diagnosis and Management of Antibody-Mediated Rejection: Current Status and Novel Approaches

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**Advances in multimodal immunotherapy have significantly reduced acute rejection rates and substantially improved 1-year graft survival following renal transplantation. However, long-term (10-year) survival rates have stagnated over the past decade. Recent studies indicate that antibody-mediated rejection (ABMR) is among the most important barriers to improving long-term outcomes. Improved understanding of the roles of acute and chronic ABMR has evolved in recent years following major progress in the technical ability to detect and quantify recipient anti-HLA antibody production. Additionally, new knowledge of the immunobiology of B cells and plasma cells that pertains to allograft rejection and tolerance has emerged. Still, questions regarding the classification of ABMR, the precision of diagnostic approaches, and the efficacy of various strategies for managing affected patients abound. This review article provides an overview of current thinking and research surrounding the pathophysiology and diagnosis of ABMR, ABMR-related outcomes, ABMR prevention and treatment, as well as possible future directions in treatment.**

**Keywords:** Antibody-mediated rejection, complement C4d, donor-specific antibodies, phenotype

**Abbreviations:** ABMR, antibody-mediated rejection; ALG, antilymphocyte globulin; APC, antigen-presenting cell; ARR, absolute risk reduction; ATG, anti-human thymocyte globulin; ATN, acute tubular necrosis; BAFF, B cell activating factor; CI, confidence interval; DSA, donor-specific antibodies; ENDATs, endothelial activation and injury transcripts; Fc $\gamma$ Rs, Fc gamma; FDA, US Food and Drug Administration; HR, hazard ratio; IF, immunofluorescence; IFTA, interstitial fibrosis and tubular atrophy; IHC, immunohistochemistry; IVIG, intravenous immunoglobulin; KTR, kidney transplant recipient; MICA, major histocompatibility complex class I-related chain A antibody; NK, natural killer; OR, odds ratio; PKE, paired kidney exchange; Poly, polymorphonuclear cell; PP, plasmapheresis; PRA, panel reactive antibody; PTC, peritubular capillary; rATG, rabbit anti-human thymocyte globulin; RCT, randomized controlled trial; RRI, relative risk increase; RRR, relative risk reduction; TCMR, T cell-mediated rejection; TG, transplant glomerulopathy; TMA, thrombotic microangiopathy

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## Introduction

The widespread use of potent and specific immunosuppressive agents has significantly reduced acute cellular rejection rates and substantially improved 1-year graft survival following renal transplantation. Substantial improvement of long-term (10-year) outcomes, however, has not been realized (1–4). A recent analysis of more than 250 000 North American renal transplant recipients showed that despite modest improvements in long-term graft survival between 1989 and 2005 (5), and improvements in graft half-life in the past decade for both living and deceased donor transplants (6), high attrition rates persist that stubbornly limit recent progress (5).

The ongoing therapeutic challenge is to achieve effective and safe immunosuppression and avoid unwanted toxicities to produce enduring renal allograft function (7–9). The incidence of hyperacute rejection caused by preexisting anti-HLA donor-specific antibodies (DSA) has been nearly eliminated by crossmatch and compatibility matching

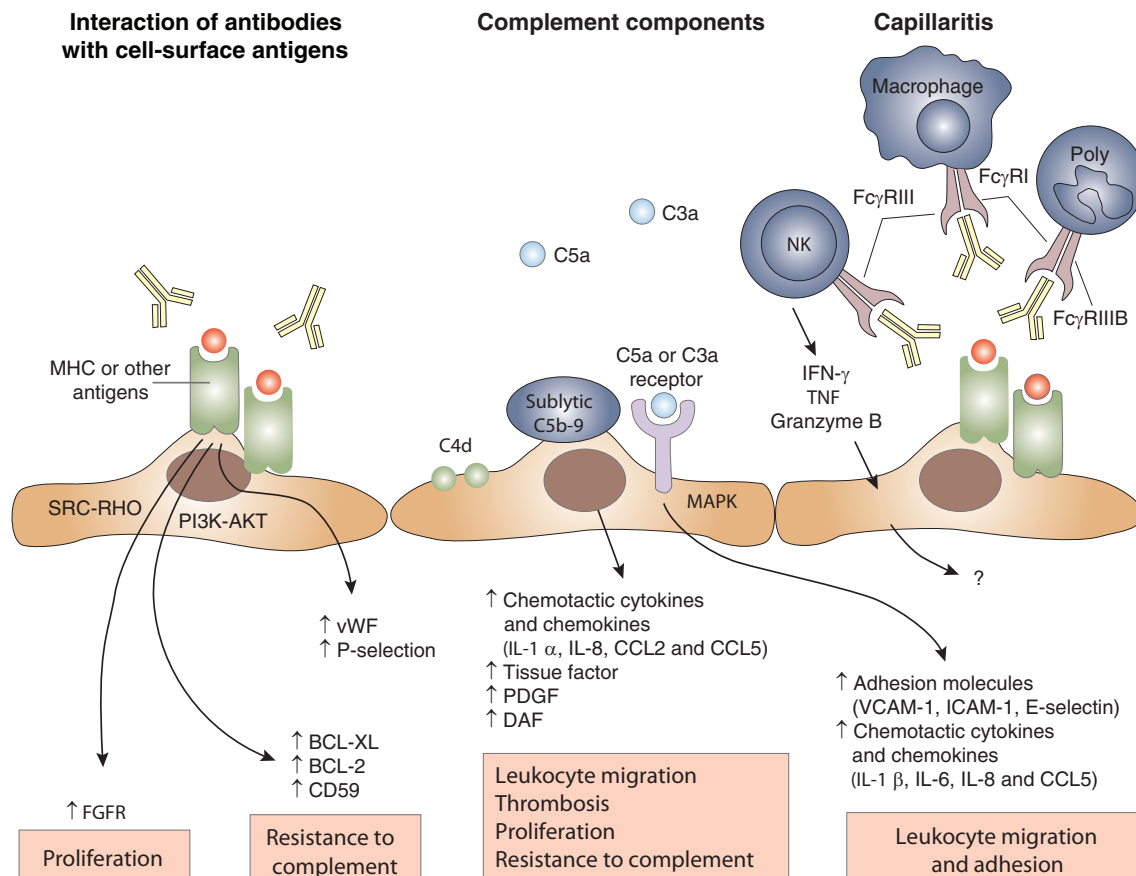
strategies. Similarly, the incidence of acute T cell-mediated injury has been significantly reduced with the effective multimodal application of immunosuppressive agents. However, acute and chronic antibody-mediated rejection (ABMR) are playing an increasingly critical role in kidney allograft loss and are considered among the most important barriers that limit long-term outcomes (10–14).

Although the cellular and molecular pathways that regulate ABMR are still under investigation, new knowledge of humoral immunobiology indicate that B cell and plasma cell activation results in the generation of DSA, which bind to HLA or non-HLA molecules on the endothelium (15,16). Antibody binding to endothelium and subsequent cellular activation involving complement-dependent and -independent pathways leads to the recruitment of natural killer (NK) cells, polymorphonuclear neutrophils and macrophages, which contribute to capillaritis and eventual tissue injury (Figure 1) (15–17). The morphologic nature of endothelial cell injury in acute ABMR demonstrates platelet aggregation, thrombotic microangiopathy (TMA) and neutrophilic accumulation, resulting in an early pattern of cellular

necrosis and a relatively rapid decline in allograft function. Chronic ABMR results from a repetitive pattern of chronic thrombotic events and inflammatory changes, which result in cellular injury and repair. It manifests as late transplant glomerulopathy (TG) and results in a decline in renal function (18). In addition to pathology mediated directly by antibodies, recent evidence suggests that B cells and plasma cells may themselves influence rejection or tolerance (19,20). The clinical picture of ABMR has become increasingly complex, with questions abounding regarding its classification, the precision of diagnostic approaches, and the efficacy of various therapeutic strategies for safely and effectively managing affected patients (21). This article provides an overview of current progress in clinical and translational research surrounding ABMR pathophysiology and ABMR-related outcomes, prevention, treatment and future directions.

**Defining and diagnosing ABMR**

The first description of acute ABMR identified two distinct features: neutrophils in peritubular capillaries (PTCs) and



**Figure 1: Mechanisms of donor-specific antibody-mediated endothelial injury in renal allografts.** Anti-MHC antibodies may either result in direct injury to the capillary endothelium or in indirect injury via complement fixation or recruitment of inflammatory cells with Fc receptors. In cases with donor-specific antibodies that lack C4d deposition, endothelial injury and cellular recruitment could be important mediators. Poly, polymorphonuclear cell. Reproduced with permission from Farkash and Colvin (15).

*de novo* antidonor HLA class I antibodies (22,23). Around the same time, C4d, a degradation product of the complement pathway that binds covalently to the endothelium, was identified as a stable marker of antidonor humoral activity (24). Subsequently, the correlation between DSA, histologic findings of microcapillary injury and diffuse (>50%) C4d deposits in the PTCs were described in acute ABMR (25). C4d and DSA were also linked to the histopathologic features of chronic ABMR (26,27). Since 2003, the Banff Working Group classification system for renal allograft biopsies has differentiated T cell-mediated rejection (TCMR) from ABMR (28,29). The most recent Banff 2013 diagnosis of ABMR, published in this issue of the journal, requires histologic evidence of acute or chronic tissue injury, evidence of current/recent antibody interaction with vascular endothelium and serologic evidence of the presence of circulating DSA (30). Importantly, C4d staining is no longer a requirement for the diagnosis of ABMR (Table 1).

### **C4d and the diagnosis of ABMR**

C4d is a split product of C4 activation and has no known biological action. It may be activated by the classical and lectin complement pathways and serves as a footprint of antibody-antigen interactions on the surface of endothelial cells (31). Although useful, C4d has significant limitations for the diagnosis of ABMR, not least because of methodological issues (immunoperoxidase vs. immunofluorescence, frozen vs. paraffin), poor understanding of the meaning of minimal and focal staining, and its waxing and waning deposition. Staining depends on the density of the capillary network, with poor sensitivity in chronic settings, and C4d positivity has been reported in the absence of other evidence of graft injury (21). Furthermore, C4d staining may not be associated with measurable DSA in the case of non-HLA antibodies or antibodies absorbed by the allograft (31).

Overall, the sensitivity of C4d is low, and its expression depends on the density of PTCs. In this regard, a number of studies have established the concept of C4d-negative acute and chronic ABMR (32–35). Loupy et al (33) reported that C4d staining waxed and waned and was not a sensitive indicator of parenchymal disease in the first year after transplant. In this study, 55% of C4d-negative biopsies with ABMR had evidence of concomitant capillary inflammation (33). Sis et al described that 60% of kidneys with high endothelial activation and injury transcripts (ENDATs) and chronic ABMR or graft loss were C4d negative (34). Findings were confirmed by another study in which 63% of late kidney failures after biopsy were attributable to ABMR, but many were C4d negative (35). A recent microarray study from Sellarés et al (36) concluded that changes in ABMR-associated gene expression (mostly in endothelial or NK cells) correlated with the presence of capillary lesions or DSA and may predict graft failure independent of C4d staining. Together, these observations point to the low sensitivity of C4d for the diagnosis of

humoral rejection and support the addition of novel biomarkers of capillary inflammation and endothelial injury, including NK cells and macrophages to the diagnosis algorithm of ABMR (33–38). This recommendation was officially acknowledged at the 11th Banff Conference on Allograft Pathology (Figure 2) (21) and was incorporated into the new Banff 2013 diagnostic criteria for ABMR (30) (Table 1).

### **DSA and the diagnosis of ABMR**

Terasaki et al identified HLA antibodies in the serum of patients after transplantation nearly 45 years ago (39). However, the importance of a low-strength antibody that is undetectable by cell-based methodology was not recognized until studies from the same group, three decades later, discovered a strong association between HLA antibodies detected by solid-phase assays and graft failure (40).

DSA may be directed against HLA or other endothelial cell antigens, and its presence is required for the diagnosis of acute and chronic active ABMR (21,37,41). In addition, there is growing evidence supporting the roles of preformed and *de novo* DSA as independent risk factors for acute and chronic ABMR and graft loss (14,41–51). A recent systematic review and meta-analysis demonstrated that the presence of DSA before transplantation was associated with a twofold greater risk of acute rejection and a 75% greater risk of graft loss (46). Despite these findings, our understanding of the biological relevance of DSA remains limited. *In vitro* studies suggest that anti-HLA class I alloantibodies result in endothelial cell injury and activation through both complement-dependent and complement-independent pathways (52,53). However, little is known about signal transduction in response to class II antibodies or the pathogenesis of DSA-induced renal allograft injury in actual patients. It is important to note that not all DSA fix complement or cause ABMR and, conversely, not all episodes of acute graft injury with capillary inflammation and C4d deposition are associated with DSA being detectable with standard assays. In fact, the majority of patients with DSA maintain normal kidney function for years and have long-term outcomes similar to nonsensitized patients (14,42,48).

Another important limitation is that currently available HLA antibody tests are qualitative and have not been cleared by the US Food and Drug Administration (FDA) for quantitative measurements (54). More studies are needed to identify risk stratification strategies on the basis of semiquantitative measures of DSA and calculated panel reactive antibody (PRA), subclasses of immunoglobulin G anti-HLA antibodies, and C1q complement-fixing DSA (44,55,56). Pending the results of collaborative standardization studies (57), consensus guidelines on the testing and clinical management issues associated with HLA and non-HLA antibodies in transplantation were recently published (58). These recommendations are intended to provide guidance on the

**Table 1:** Revised (Banff 2013) classification of antibody-mediated rejection (ABMR) (30)

Acute/active ABMR; all three features must be present for diagnosis<sup>1,2</sup>

1. Histologic evidence of acute tissue injury, including one or more of the following:
  - Microvascular inflammation ( $g > 0^3$  and/or  $ptc > 0$ )
  - Intimal or transmural arteritis ( $v > 0^4$ )
  - Acute thrombotic microangiopathy (TMA), in the absence of any other cause
  - Acute tubular injury, in the absence of any other apparent cause
2. Evidence of current/recent antibody interaction with vascular endothelium, *including at least one of the following*:
  - Linear C4d staining in peritubular capillaries (C4d2 or C4d3 by IF on frozen sections, or C4d  $> 0$  by IHC on paraffin sections)
  - At least moderate microvascular inflammation ( $[g + ptc] \geq 2^5$ )
  - Increased expression of endothelial activation and injury transcripts (ENDATs) or other gene expression markers of endothelial injury in the biopsy tissue, if thoroughly validated
3. Serologic evidence of donor-specific antibodies (HLA or other antigens)

Chronic, active ABMR; all three features must be present for diagnosis<sup>1,6</sup>

1. Morphologic evidence of chronic tissue injury, including one or more of the following:
  - Transplant glomerulopathy ( $cg > 0$ ),<sup>7</sup> if no evidence of chronic TMA
  - Severe peritubular capillary basement membrane multilayering (requires electron microscopy [EM])<sup>8</sup>
  - Arterial intimal fibrosis of new onset, excluding other causes<sup>9</sup>
2. Evidence of current/recent antibody interaction with vascular endothelium, *including at least one of the following*:
  - Linear C4d staining in peritubular capillaries (C4d2 or C4d3 by IF on frozen sections, or C4d  $> 0$  by IHC on paraffin sections)
  - At least moderate microvascular inflammation ( $[g + ptc] \geq 2^5$ )
  - Increased expression of endothelial activation and injury transcripts (ENDATs) or other gene expression markers of endothelial injury in the biopsy tissue, if thoroughly validated
3. Serologic evidence of donor-specific antibodies (HLA or other antigens)

C4d staining without evidence of rejection; all three features must be present for diagnosis<sup>10</sup>

1. Linear C4d staining in peritubular capillaries (C4d2 or C4d3 by IF on frozen sections, or C4d  $> 0$  by IHC on paraffin sections)
2.  $g = 0$ ,  $ptc = 0$ ,  $cg = 0$  (by light microscopy [LM] and by EM if available),  $v = 0$ ; no TMA, no peritubular capillary basement membrane multilayering, no acute tubular injury (in the absence of another apparent cause for this)
3. No acute cell-mediated rejection (Banff 1997 type 1A or greater) or borderline changes

<sup>1</sup>For all ABMR diagnoses, it should be specified in the report whether the lesion is C4d-positive (C4d2 or C4d3 by IF on frozen sections; C4d  $> 0$  by IHC on paraffin sections) or without evident C4d deposition (C4d0 or C4d1 by immunofluorescence [IF] on frozen sections; C4d0 by IHC on paraffin sections).

<sup>2</sup>These lesions may be clinically acute, smoldering or subclinical. Biopsies showing two of the three features, except those with donor-specific antibodies (DSA) and C4d without histologic abnormalities potentially related to ABMR or T cell-mediated rejection (TCMR) (C4d staining without evidence of rejection; see footnote 10) may be designated as "suspicious" for acute/active ABMR.

<sup>3</sup>Recurrent/*de novo* glomerulonephritis should be excluded.

<sup>4</sup>It should be noted that these arterial lesions may be indicative of ABMR, TCMR or mixed ABMR/TCMR. "v" lesions are scored in arteries having continuous media having two or more smooth muscle layers.

<sup>5</sup>In the presence of acute TCMR, borderline infiltrates or evidence of infection,  $ptc \geq 2$  alone is not sufficient to define moderate microvascular inflammation and  $g$  must be  $\geq 1$ .

<sup>6</sup>Lesions of chronic, active ABMR can range from primarily active lesions with early transplant glomerulopathy (TG) evident only by EM ( $cg$  1a) to those with advanced TG and other chronic changes in addition to active microvascular inflammation. In the absence of evidence of current/recent antibody interaction with the endothelium, the term active should be omitted; in such cases DSA may be present at the time of biopsy or at any previous time posttransplantation.

<sup>7</sup>Includes glomerular basement membrane (GBM) duplication by EM only ( $cg$ 1a) or GBM double contours by LM.

<sup>8</sup> $\geq 7$  layers in one cortical peritubular capillary and  $\geq 5$  in two additional capillaries, avoiding portions cut tangentially.

<sup>9</sup>While leukocytes within the fibrotic intima favor chronic rejection, these are seen with chronic TCMR as well as chronic ABMR, and are therefore helpful only if there is no history of TCMR. An elastic stain may be helpful as absence of elastic lamellae is more typical of chronic rejection and multiple elastic lamellae are most typical of arteriosclerosis, although these findings are not definitive.

<sup>10</sup>The clinical significance of these findings may be quite different in grafts exposed to anti-blood-group antibodies (ABO-incompatible allografts), where they do not appear to be injurious to the graft and may represent accommodation, and anti-HLA antibodies where more clinical outcome data are needed.

use and clinical application of contemporary methods for HLA antibody detection.

### **ABMR classification and phenotypes**

The 2011 Banff meeting report and a 2010 workshop held by the FDA both noted the confusion generated by reports on acute and chronic ABMR, and emphasized the importance of correctly defining ABMR phenotypes (21,54). In the Banff

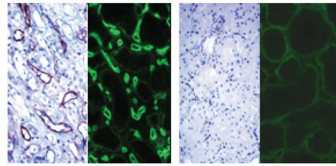
report, two principal phenotypes of acute ABMR were defined: (1) ABMR phenotype 1 in the presensitized patient, occurring early posttransplant; and (2) ABMR phenotype 2, which develops from the emergence of *de novo* DSA in the late posttransplant period and is thought to be mostly related to nonadherence or inadequate immunosuppression (12,59,60). However, additional characteristics—including the nature of the antibody; the significance of C4d; the severity of microcapillary injury, gene transcripts,

**C4d-Positive  
ABMR**

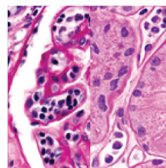
**Serologic Evidence**  
• DSA present



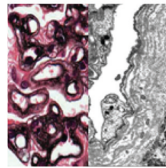
**Immunopathologic Evidence**  
• IF: Diffuse-positive C4d in PTC  
• IHC: Diffuse- or focal-positive C4d in PTC



**Histopathologic Evidence ACUTE**  
• ATN like changes, and/or  
• Peritubular capillaritis, and/or  
• Glomerulitis, and/or  
• Arterial fibroid necrosis, and  
• No evidence for chronic capillary injury (reduction and/or multilayering of glomerular and peritubular capillary basement membranes)



**Histopathologic Evidence CHRONIC**  
• Transplant glomerulopathy, and/or  
• PTC basement membrane multilamination, and/or  
• IFTA, and/or  
• Fibrous intimal thickening of arteries  
• May be accompanied by glomerulitis and/or capillaritis



**C4d-Negative  
ABMR**

Proposed criteria under discussion  
by Banff working group

**Serologic Evidence**  
• DSA present



**Immunopathologic Evidence**  
• Negative C4d staining; and  
• Endothelial activation, detected by increased mRNA expression of endothelial genes, such as W/F, PECAM, SELE, etc; and/or  
• Evidence for glomerular and/or capillary endothelial cycling (CD31+Ki67+ cells lining the microcirculation)



**Histopathologic Evidence ACUTE**  
• Peritubular capillaritis, and/or  
• Glomerulitis, and/or  
• Thrombotic microangiopathy, and/or  
• Arterial fibroid necrosis, and  
• No evidence for chronic capillary injury (reduction and/or multilayering of glomerular and peritubular capillary basement)



**Histopathologic Evidence CHRONIC**  
• Transplant glomerulopathy, and/or  
• PTC basement membrane multilamination, and/or  
• Fibrous intimal thickening of arteries  
• May be accompanied by glomerulitis and/or capillaritis

**Figure 2: Acute and chronic definitions of ABMR based on C4d positivity.** ABMR, antibody-mediated rejection; ATN, acute tubular necrosis; DSA, donor-specific antibodies; IF, immunofluorescence; IFTA, interstitial fibrosis and tubular atrophy; IHC, immunohistochemistry; PTC, peritubular capillary. Reproduced with permission from Mengel et al (21).

molecular and cellular signatures; and the pathology and function of the allograft—are relevant, were included in the Banff 2013 criteria, and may subsequently affect the design of clinical trials of patients with ABMR (16,21,30,34,36) (Table 1).

**Predictors of poor outcome related to antibody-mediated injury**

Acute and chronic ABMR are associated with poor outcomes after kidney transplantation. Specifically, patients with acute ABMR are at greater risk for subsequent rejection, chronic ABMR and graft loss (10,14,33,42,61). Similarly, those with chronic ABMR are at increased risk for

graft loss (12,13,35,60,62,63). However, not all ABMR phenotypes have poor outcomes, and many patients maintain stable graft function for years after treatment of the initial injury. We will review the independent roles of C4d, circulating antibodies, B cells and plasma cells, microcirculation injury/inflammation, subclinical ABMR and novel biomarkers to predict outcomes in patients with acute and chronic ABMR.

**C4d and microvascular injury**

C4d and microcirculation inflammation are independent biomarkers of subsequent rejection, chronic ABMR and graft loss in patients with acute ABMR (24,33,48,62). Loupy et al (33) demonstrated that higher increments of C4d Banff

scores predict greater microvascular inflammation at both 3 months and 1 year after transplant, as well as worse TG and higher levels of class II DSA. The extent of microvascular injury was similar between biopsies with focal and diffuse C4d. However, the presence of microcirculation inflammation and class II DSA at 3 months was related to a fourfold increased risk of chronic ABMR independent of C4d (33). We recently demonstrated that focal C4d staining in postreperfusion biopsies was a significant predictor of subsequent ABMR in sensitized patients (48). Despite the important diagnostic and prognostic roles of C4d and microcirculation inflammation in acute ABMR, prospective studies are required to determine whether the treatment of isolated C4d staining or microcirculation inflammation in patients with DSA improves outcomes.

C4d and endothelial injury are also associated with poor outcomes in chronic ABMR (12,13,35,60,63–65). In support of this observation, most graft losses in the current era of immunosuppression have evidence of chronic ABMR with positive C4d staining (12,13,35). In the Deterioration in Kidney Allograft Function (DeKAF) study, patients with new-onset kidney allograft dysfunction underwent a biopsy at a mean time of 7.3 years after transplant (60). Most biopsies had some evidence of antibody-mediated injury (C4d or DSA), and the risk of subsequent graft failure was significantly increased in the presence of C4d (60). Other studies have confirmed the association of both focal and diffuse C4d staining in chronic ABMR with graft loss (64,65). Microcirculation injury defined as microcirculation inflammation (PTC and g) or microcirculation deterioration (cg and PTC multilayering) was also an important predictor of graft loss in late biopsies (>1 year after transplant), independent of C4d staining (35,63). As a result, the new Banff 2013 criteria further characterize ABMR based on current/recent antibody interactions with vascular endothelium including C4d staining, at least moderate microvascular inflammation ( $[g + ptc] \geq 2$ ), increased expression of ENDATs or gene expression of other validated markers of endothelial injury in the biopsy tissue (Table 1) (30).

In summary, there is a clear and independent association between C4d and microcirculation injury with poor outcomes in kidney transplant recipients (KTRs) with acute or chronic ABMR. Standardized risk stratification strategies are needed to better define preventive and treatment approaches for each ABMR phenotype.

**Donor-specific antibodies**

Preexisting (49,55) or *de novo* circulating antibodies (14,63) have been shown to compromise renal allograft survival. These antibodies may be directed against HLA or non-HLA molecules on endothelial cells, including major histocompatibility complex class I-related chain A antibody (MICA), and angiotensin type 1 receptor (37,41,66). In support of this observation, among recipients of HLA-identical sibling

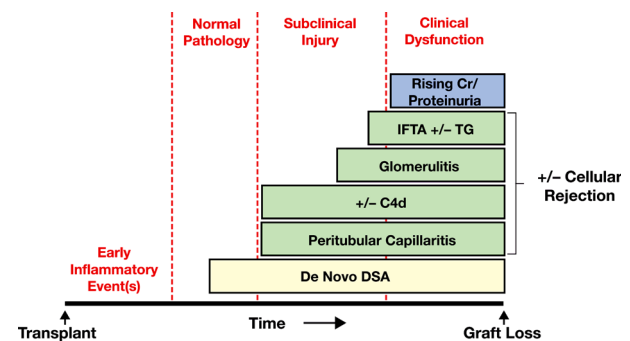
transplants, patients with no PRA had significantly higher 10-year graft survival than patients with PRA >1%, suggesting that non-HLA immunity has an important role in clinical transplantation and chronic graft loss (37).

In sensitized patients with preexisting anti-HLA-DSA, 8-year graft survival rates were significantly worse than in sensitized patients without HLA-DSA or nonsensitized patients (55). Peak HLA-DSA strength was related to the risk of acute ABMR (49,55) and graft loss (55). Conversely, in renal transplant recipients without preexisting DSA, 10-year graft survival was significantly lower for patients who developed *de novo* HLA-DSA (14). Histopathology of ABMR phenotype 2 could be observed in the absence of graft dysfunction. Similar findings were reported earlier, where patients with *de novo* DSA at the time of biopsy had worse graft survival than those with preexisting DSA, indicating that patients with ABMR phenotype 2 have worse graft outcomes than those with ABMR phenotype 1 (63). *De novo* DSA are predominantly directed at class II donor HLA mismatches and are associated with non-adherence and cellular rejection (Figure 3) (14). Although the reason for this is unclear, it appears that class I antibodies are associated with early ABMR, whereas class II antibodies are more commonly associated with late ABMR and graft failure (14,35,48–51,63).

In summary, although DSA are important risk factors for graft loss, the majority of patients with DSA have stable allograft function and experience no rejection. It is therefore important to determine the pathogenic role and specificity of anti-class I and class II HLA and non-HLA-DSA and to better understand the effect of *de novo* DSA compared with preexisting DSA.

**B cells and plasma cells**

The relationship between the presence of circulating DSA and the development of antidonor B cell responses in allograft rejection and tolerance is currently under active



**Figure 3: The natural history of phenotype 2 ABMR.** ABMR, antibody-mediated rejection; DSA, donor-specific antibodies; IFTA, interstitial fibrosis and tubular atrophy; TG, transplant glomerulopathy. Reproduced with permission from Wiebe et al (14).

investigation (19,20). While the absence of circulating DSA may indicate graft tolerance, it is also possible that antibodies produced as a result of a B cell response are not detectable because of binding/absorption by the graft itself (20,67). To investigate donor-directed B cell responses, a recent study (19) used donor-derived fibroblasts as targets to quantify DSA-secreting cells isolated from peripheral blood of KTRs before and after transplantation. Even in the absence of circulating DSA (with no evidence of rejection), the number of DSA-secreting cells was increased posttransplant in all patients, suggesting a greater role for B cells and plasma cells in posttransplant immune regulation than previously thought (19).

B cells may also contribute to posttransplant immune regulation through its antigen-presenting function (20). In addition to pathways used by other antigen-presenting cells (APCs), B cells can present antigen via antigen binding to the clonotypic B cell receptor (68). In turn, B cells (unlike other APCs) undergo clonal expansion, which may contribute to rejection by amplifying antidonor B cell responses (20).

### **Subclinical ABMR**

Subclinical ABMR is defined as immunohistological evidence of ABMR in KTRs with normal renal allograft function. Evidence suggests that untreated subclinical ABMR is an important predictor of poor renal allograft outcomes (69,70). At 1-year posttransplant, those with subclinical ABMR at 3 months had more interstitial fibrosis and tubular atrophy (IFTA) and TG compared with patients who did not have subclinical ABMR at 3 months (69). Similarly, patients with C4d-negative subclinical ABMR at 3 months (defined as PTC + g > 0) had more PTC and IFTA and a lower GFR at 1 year relative to those without subclinical lesions at 3 months (69). These findings were in agreement with earlier observations that demonstrated a strong association between subclinical rejection (70) and chronic allograft nephropathy in 83 patients who received HLA-incompatible renal allografts and support the indication of protocol biopsies in sensitized recipients. Protocol biopsies can further identify subclinical TG, which is considered an important risk factor for chronic injury and graft dysfunction (69,71). As a result, it is recommended that high-risk patients (i.e. desensitized or DSA-positive/crossmatch-negative) should be monitored by protocol biopsies in the first 3 months after transplantation. Protocol biopsies may also be conducted after ABMR to determine the effectiveness of therapy and to identify prognostic indicators of outcome (58).

### **Novel biomarkers**

New assays and molecular tests may be considered as diagnostic and prognostic tools in patients with ABMR. There is evidence that ENDATs and DSA-selective transcripts are indicators of active ABMR damage and worse

graft outcomes (17,34,72). The expression of these transcripts in biopsies may provide a new tool for understanding the pathogenesis of late kidney graft loss and ABMR, as well as for predicting graft outcomes and defining ABMR even in C4d-negative biopsies in patients with antibodies (17,34). Differentially expressed microRNAs and their predicted targets identified by deep sequencing may also be candidates for further investigation to understand the mechanism and management of kidney allograft fibrosis in patients with ABMR (73). The C1q assay is another test that is designed to distinguish complement-fixing from non-complement-fixing antibodies (56). Recent studies indicate that a positive C1q assay for *de novo* DSA correlates with acute rejection and long-term graft loss after kidney transplantation (74–76). Other investigators have found no significant difference in graft survival between patients with or without preformed C1q-fixing DSA (77), suggesting that additional studies are needed to clarify the role of this assay in clinical transplantation. Finally, C4d-fixing luminex-binding antibodies have been reported to predict graft failure in heart transplantation, but the role of this assay in kidney transplantation is being debated (78,79). In summary, while new assays with potential diagnostic and prognostic value are being developed in the area of ABMR, these tools need to be validated by larger studies.

## **Preventing ABMR**

More than 20 000 patients awaiting kidney transplantation in the United States are sensitized (typically owing to blood transfusion, pregnancy or previous transplants) to HLA class I and/or class II antibodies (80). Until recently, transplantation was routinely avoided in sensitized patients, at the expense of prolonging waiting time for suitably HLA-matched organs. However, with the advent of virtual crossmatch, desensitization protocols and paired kidney exchange (PKE) programs, timely kidney transplantation has become a reality for many of these high-risk patients (81,82) (Table 2). Highly sensitized patients may be able to participate in special programs such as the Eurotransplant Acceptable Mismatch Program (83), in which the HLA typing of panel donors with negative reactions is determined during screening if PRA is below 100%; alternatively, selection and crossmatching of blood donors with a single HLA mismatch to the patient's phenotype can be undertaken (84). It has been argued that implementation of these programs may lead to similar graft survival rates to those observed in nonsensitized patients (84).

### **Prevention of acute ABMR phenotype 1**

Only one randomized controlled clinical trial (RCT) has been conducted to lower allosensitization prior to transplantation (85). In this study, 101 adult patients with a PRA  $\geq$ 50% were enrolled in a trial sponsored by the National Institutes of Health. Patients received intravenous immunoglobulin

**Table 2:** Strategies to prevent ABMR

1. Do not transplant highly sensitized patients
2. Avoid blood transfusion
3. Paired kidney exchange
4. In sensitized patients, precise characterization of their alloantibodies and exact HLA typing of the donor at the time of transplantation
5. Participation in special programs (such as the Eurotransplant Acceptable Mismatch Program)
6. Removal of DSA (plasmapheresis, immunoadsorption)
7. Direct or indirect inhibition of DSA production
  - a. Anti-B cell agents (rituximab<sup>1</sup>)
  - b. Anti-plasma cell agents (proteasome inhibitors, e.g. bortezomib<sup>1</sup>)
  - c. Rabbit anti-human thymocyte immunoglobulins (e.g. thymoglobulin?)
  - d. Costimulation blockade (e.g. belatacept)?
8. Inhibition of complement cascade (eculizumab<sup>1</sup>)
9. Intravenous immunoglobulin<sup>1</sup>
  - e. Neutralizing DSA: anti-idiotypic activity
  - f. Inhibiting complement activation by binding C3b, C4b
  - g. Inhibiting activation of macrophages, neutrophils by binding FcγRs
  - h. Apoptosis of B cells (inhibits CD19 expression)
10. Splenectomy

ABMR, antibody-mediated rejection; DSA, donor-specific antibodies; FcγRs, Fc gamma.

<sup>1</sup>These drugs are used off-label in solid organ transplantations.

(IVIg) 2 g/kg monthly for 4 months or an equivalent volume of placebo with additional infusions at 12 and 24 months after entry if not transplanted while on IVIg or placebo. IVIg significantly reduced PRA levels in study subjects compared with placebo, and more patients in the IVIg arm were transplanted (35% vs. 17%). Seven graft failures occurred (four IVIg, three placebo) among adherent patients with similar 2-year graft survival rates (80% IVIg, 75% placebo). The investigators concluded that IVIg is better than placebo in reducing anti-HLA antibody levels and improving transplantation rates in highly sensitized patients with end-stage renal disease. In a follow-up study by the same group, the combination of B cell depletion therapy and high-dose IVIg was shown to be effective in reducing PRA from  $77 \pm 18\%$  to  $44 \pm 30\%$  at the time of transplantation (86). However, recent studies have not been able to reproduce these data, specifically in patients with PRA >80% (87–89).

Two randomized clinical trials have examined the role of rabbit anti-human thymocyte globulin (rATG) as induction therapy in sensitized kidney transplants based on current or peak PRA levels (90,91). The use of rATG was associated with a significant reduction in the incidence of acute rejection and improved 1-year survival, specifically in patients who remained rejection-free, suggesting that ATG induction may be associated with better outcomes in sensitized patients (90,91). Compared with anti-IL-2 therapy (basiliximab), induction with rATG in moderately sensitized KTR was associated with reduced incidence of *de novo* DSA and ABMR (92). In contrast, outcomes from the CTOT02 study, including nonsensitized adults and children, found anti-IL-2 induction to be protective against the development of anti-HLA antibodies; however, no difference in allograft survival was associated with anti-HLA antibody development (93,94). Although these studies support the use of ATG and IL-2 blockade in sensitized and low-risk patients, respectively, the effects of induction with

rATG or anti-IL-2 therapy on *de novo* DSA or long-term outcomes remain largely unknown.

Nonrandomized clinical observations suggest that a combination of plasmapheresis and low-dose IVIg combined with IL-2 blockade or rATG for induction has become the standard of care for the treatment of sensitized patients (11,46,95). Using this approach, desensitization was associated with improved patient survival compared with chronic dialysis (95). Despite these promising findings, long-term outcomes for crossmatch-positive living-donor kidney transplantation are generally inferior to nonsensitized KTRs (51,96), suggesting that better immunomodulatory strategies are required.

Alemtuzumab, a lymphocyte-depleting, CD52-specific monoclonal antibody, is increasingly used as induction therapy in renal transplantation. A recent review and meta-analysis of 10 RCTs (enrolling more than 1200 patients), as well as studies specifically in highly sensitized patients, concluded that alemtuzumab induction is associated with a comparable or lower risk of biopsy-proven acute rejection compared with rATG or IL-2 receptor antibodies (97–99). In contrast, other studies have demonstrated potential negative effects of alemtuzumab on the regulation of humoral immunity, including unexpectedly high rates of ABMR (100) and high rates of circulating alloantibody and intragraft C4d at 1-year posttransplant (100). Increased risk for ABMR with alemtuzumab may be partly mediated by dysregulation of B cell activating factor (BAFF), as an increase in BAFF mRNA expression was observed in monocytes of alemtuzumab-treated patients (101,102).

The anti-CD20 agent rituximab may also have utility as an induction agent for renal transplant recipients, although its efficacy is yet to be proven and it is not currently licensed in this setting (103). An RCT (ClinicalTrials.gov number



NCT00565331) presented at the 2013 American Transplant Congress evaluated a single dose of rituximab as induction therapy added to standard concomitant immunosuppression (i.e. tacrolimus, mycophenolate mofetil and steroids) in patients with PRA >6% and re-transplants. The results showed that rituximab was significantly more effective than placebo at preventing biopsy-proven acute rejection within the first 6 months posttransplant, though this study did not report the effect of rituximab induction on the incidence of ABMR (104). In a retrospective study of patients receiving ABO-incompatible KTRs, rituximab induction inhibited the development of *de novo* DSA and reduced the rate of chronic ABMR (relative to splenectomy) in this subset of patients (105). Further induction trials of rituximab are ongoing (103), including the Rituximab Induction in Renal Transplantation (ReMIND) trial (Clinical-Trials.gov number NCT01095172).

Several observational studies have used bortezomib and eculizumab in their desensitization protocols. Bortezomib is a proteasome inhibitor that acts on plasma cells and is effective in removing preformed DSA when combined with plasmapheresis (106,107). It is also associated with durable reductions in DSA and stable allograft function in *de novo* DSA-positive renal transplant recipients (108). The efficacy of the humanized anti-C5 antibody eculizumab in the prevention of ABMR was also recently assessed in renal transplant recipients with a positive crossmatch (109). The incidence of ABMR was 8% with eculizumab compared with 41% in the control group, and the rate of TG at 1 year was also significantly lower with eculizumab (109). There is an ongoing, multicenter, international, randomized trial testing the role of eculizumab plus conventional treatment (or conventional treatment alone) that may clarify its utility (NCT00670774) (110). However, these published single-center observations have not yet been confirmed by larger studies, and none of these drugs is approved by the FDA for the prevention or treatment of ABMR. Furthermore, these protocols are associated with costs that may not be covered by insurance.

A common approach to ABMR prevention has been to avoid transplanting highly sensitized patients. However, avoiding transplant renders chronic dialysis the only option, with implications for patient health and quality of life, as well as healthcare costs. Long-term survival in posttransplant patients has been improved considerably by desensitization, and the enrollment of patients in special programs to optimize matching can lead to timely transplants with better outcomes. A recent study examined both the efficacy and cost-effectiveness of desensitization using IVIG and rituximab in 146 patients who were originally DSA-positive (PRA >80%) and transplanted with an acceptable crossmatch (111). The patient survival rate at 3 years was 96.6% in the desensitization arm compared with 79.0% for patients remaining on dialysis. Each patient treated with desensitization was estimated to save the US healthcare system \$18753. These data suggest that survival and

financial gains can be achieved by a desensitization approach; however, this was a relatively small study, and the extent of the relative benefits of desensitization over dialysis will ultimately be determined by drug cost.

A growing option for the prevention of ABMR in highly sensitized patients is the use of PKE transplant programs, such as the National Kidney Registry, the Alliance for Paired Donation and the United Network for Organ Sharing Kidney Paired Donations Pilot Program (112,113) (see Table 2). Such programs enable sensitized patients with immunologically incompatible living donors to be transplanted with high-quality grafts from other living donors in similar situations who were willing to exchange organs. Although cost has been a concern for kidney exchange registries in the United States, it seems that the PKE could help participating centers avoid complex desensitization protocols while improving long-term outcomes. Furthermore, mathematical modeling predicts that an optimized matching algorithm and a national PKE program would improve outcomes and reduce healthcare costs for highly sensitized patients (114). With the rising number of highly sensitized patients in PKE programs, some centers combine desensitization and paired exchange options.

### **Prevention of acute ABMR phenotype 2**

Nonadherence and the choice of maintenance immunosuppression may influence the development of ABMR after transplant (13,14,115). For example, calcineurin inhibitor minimization or withdrawal strategies may increase the incidence of *de novo* DSA and ABMR (13,14,115). Analyses from two prospective randomized clinical trials demonstrated that the conversion of cyclosporine to everolimus at 3–4.5 months after transplant was associated with significantly higher rates of *de novo* DSA (10.8% vs. 23%,  $p=0.04$ ) and ABMR (3% vs. 13%,  $p=0.03$ ) (116). Whereas avoiding calcineurin-based regimens may be advantageous in KTRs by reducing the potential risks of nephrotoxicity and other adverse events after transplant, the intentional or unintentional reduction of immunosuppression increases the risk of ABMR and graft loss (12,13,117). Treatment with belatacept, a selective costimulation blocker that targets CD80/CD86-CD28 interaction to prevent T cell activation, was associated with a low rate of *de novo* DSA over 3 years of treatment in phase III trials, although this was not an initial end point of the studies and requires confirmation (118,119). This observation is supported by experimental data demonstrating that belatacept inhibits primary T cell-dependent antibody responses and the generation of DSA in primates (120). A phase II clinical study in which conversion from a calcineurin inhibitor-based regimen to belatacept had no effect on the incidence of *de novo* DSA or ABMR despite higher rates of cellular rejection (7% vs. 0%) (117).

In summary, notwithstanding the advent of novel immunosuppressive agents, the ideal regimen for the prevention of

ABMR phenotypes 1 and 2 in sensitized KTRs remains unknown.

## Treatment of ABMR

### Acute ABMR

The primary aims of therapeutic modalities for ABMR are to remove existing antibodies and inhibit their redevelopment. The management of ABMR is challenging and is associated with poorer outcomes compared with traditional anti-T cell rejection therapy for pure cell-mediated rejection (121). A recent systematic review of treatments for acute ABMR in renal allografts found 10388 citations but only five small randomized and eight non-RCTs (Table 3) (122). Of these trials, benefit was found in five studies evaluating plasmapheresis or immunoadsorption, and small, nonrandomized controlled studies suggested benefit from rituximab or bortezomib (33,120,122,124–128,130–134). An evaluation of a small group of patients from a randomized trial of kidney allograft recipients suggested that immunoadsorption (received by five patients and compared with the outcomes of five controls) was effective in reversing severe C4d-positive ABMR (123). However, it is important to note that immunoadsorption is not practiced in the United States.

Ironically, there are no randomized controlled studies that support the benefits of IVIG in acute ABMR, despite its common use in this context (122). Only one randomized controlled study has found plasmapheresis to be beneficial (125); two controlled studies found no benefit (124,127) and one found potential harm (126), indicating that the role of plasmapheresis for the treatment of acute ABMR remains under debate. Uncontrolled or controlled nonrandomized studies support a role for rituximab, bortezomib, plasmapheresis and IVIG (45,128–134). However, the relative importance of these therapies is difficult to assess because treatment strategies were not standardized, doses and frequencies were not similar, and the specific drugs were combined with other agents.

One-year results were recently reported from a phase III, multicenter, randomized, placebo-controlled trial (RITUX ERAH) that examined the effect of rituximab (combined with plasmapheresis, IVIG, corticosteroids, tacrolimus and mycophenolate mofetil) on a composite measure of graft loss or absence of improvement of renal function at day 12, in patients with biopsy-proven acute ABMR. ABMR occurred after a median of 35.5 days, with no advantage of rituximab over control for the graft loss or renal function outcome (135).

Eculizumab was recently used for the treatment of multidrug-resistant ABMR (136), but there are no randomized controlled studies to confirm the efficacy of this expensive drug. In summary, efficacy data for the treatment of acute ABMR are of very low quality, and

larger RCTs and dose–response studies are needed to fully evaluate therapies in this setting (122). In the absence of strong evidence to support consensus guidelines for the treatment of ABMR, the Kidney Disease: Improving Global Outcomes Transplant Work Group recommends the use of corticosteroids, plasmapheresis, IVIG, anti-CD20 antibodies and lymphocyte-depleting antibodies alone or in combination (137) (Figure 4).

### Chronic ABMR

Chronic ABMR is a more difficult condition to treat because irreversible tissue damage has occurred in the setting of severely compromised graft survival (138). A small-scale retrospective study of rituximab combined with standard maintenance immunosuppression (including prednisone, mycophenolate mofetil and calcineurin inhibitors) in 31 patients with chronic ABMR had encouraging results, with partial therapeutic response and an increase in median graft survival in the rituximab-treated group compared with the control group (685 days vs. 439 days, respectively). The outcomes within the rituximab group were dichotomous, with significantly different median survival time in responders compared with nonresponders and control patients, though there were no pathologic parameters that distinguished any subset of patients (139).

Clinical trials of rituximab for the treatment of chronic ABMR are ongoing or recruiting patients (NCT00476164 [RituxiCAN-C4] in the United Kingdom and NCT00307125 in the United States).

### New directions and future perspectives

Despite the important role of ABMR in patient morbidity and mortality after renal transplantation, our current understanding of the pathogenesis and pathologic phenotypes of ABMR is limited. Evidence supports an important role for DSA in acute and chronic ABMR. However, not all DSA detected by current assays cause injury in the allograft and not all ABMR phenotypes cause rapid allograft failure. Similarly, C4d has significant limitations as a biomarker of ABMR. It will therefore be essential to determine risk stratification strategies for DSA, C4d and ABMR phenotypes to guide preventive and therapeutic approaches, including plasmapheresis, IVIG and anticomplement and anti-B/plasma cell therapies.

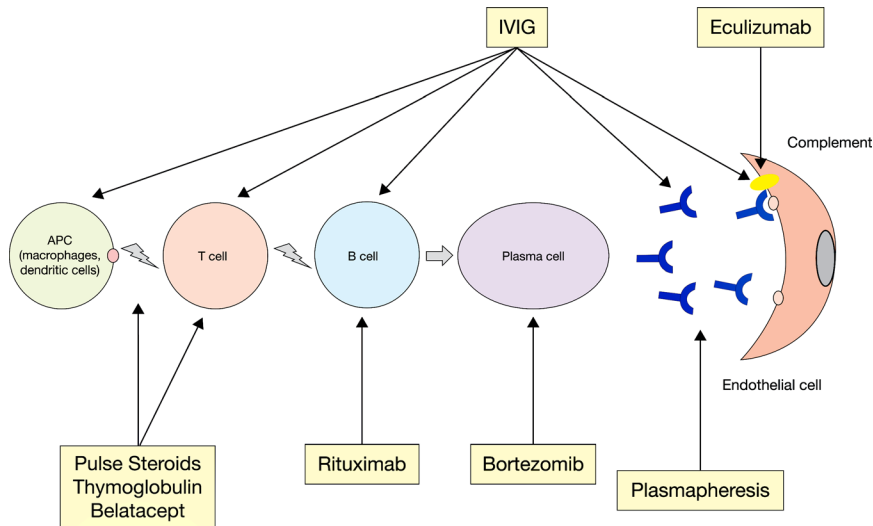
Treatment options for ABMR are being informed by growing awareness of the complex role played by B cells in acute ABMR and chronic allograft dysfunction and the underlying biological processes. B cell lineages are now known to have multiple negative effects on the alloimmune response, including antigen presentation to T cells, the production of cytokines supporting T cell activation, antibody production and tertiary lymphoid organ and lymphatic vessel formation (140). Donor-specific B cells can be detected in peripheral blood using HLA-binding

**Table 3:** Summary of controlled trials from a systematic review assessing treatment strategies for ABMR<sup>1</sup>

Refs.	ABMR definition	Trial design and intervention (N)	Patients with hemodialysis dependency or graft loss (intervention vs. control)
Böhmgig et al (123)	Banff 1997	Stratified RCT; 9–14 sessions of immunoadsorption (protein A)	Treatment benefit observed: 0 vs. 4 at 3 weeks ARR = 0.8 (95% CI, 0.2–0.9)
Blake et al (124)	Vascular	Stratified RCT; 5 PP treatments	No treatment benefit: 4 vs. 6 at 6 months; RRR = 0.3 (95% CI, 0.001–0.8)
Bonomini et al (125)	Vascular, MP-resistant	RCT; 3–7 PP treatments	10 vs. 13 at 5 years; RRR = 0.2 (95% CI, 0.001–0.5)
Kirubakaran et al (126)	Vascular	RCT; 8 PP treatments	Treatment benefit observed: 7 vs. 17 at 2 weeks; RRR = 0.6 (95% CI, 0.3–0.8)
Allen et al (127)	Vascular, MP-resistant	RCT; 6 PP treatments	Trend to harm: 6 vs. 3 at 1 month; RRI = 0.5 (95% CI, 0.001–0.8)
Franco et al (128)	Vascular, MP-resistant	Historical control; 6 PP treatments	No treatment benefit (trend to harm at 220 days): 3 vs. 4 at 6 days; RRR = 0.2 (95% CI, 0.001–0.8)
Lefaucheur et al (129)	Banff 1997	Historical control; 4 PP treatments; 2 rituximab doses	11 vs. 9 at 220 days; RRI = 0.2 (95% CI, 0.001–0.5)
Kaposztas et al (130)	ALG-resistant	Historical control; rituximab	Treatment benefit observed: 6 vs. 13 at 3 months; OR = 0.4 (95% CI, 0.1–1.3)
Vangelista et al (131)	Vascular, anti-HLA	Nonrandomized case-controlled; 4–5 PP treatments	Treatment benefit observed: 1 vs. 6 at 3 years; OR = 0.1 (95% CI, 0.008–0.9)
Macaluso et al (132)	ABMR (no other details)	Nonrandomized, case-controlled; 4 doses of bortezomib, 1 dose of rituximab, 5 PP treatments	Treatment benefit observed: 2 vs. 8 at 2 years; OR = 0.2 (95% CI, 0.04–1.09)
Loupy et al (45)	Vascular with DSA	Nonrandomized, case-controlled; OKT3 vs. IVIG vs. PP and rituximab	Treatment benefit observed: 1 vs. 3
Lubetzky (133)	With DSA	Nonrandomized, case-controlled; rituximab vs. bortezomib	Treatment benefit observed: 1 vs. 10 at 3 months; OR = 0.1 (95% CI, 0.01–0.9)
Waiser et al (134)	With DSA	Historical cohort; 4 bortezomib doses vs. 1 rituximab dose, 6 PP treatments and 30 g IVIG	Treatment benefit observed for PP and rituximab: HR = 0.19 (vs. OKT3) HR = 0.11 (vs. IVIG)
			Treatment benefit observed for bortezomib: 3 vs. 1 at 6 months; OR = 5.3 (95% CI, 0.5–59.3) No treatment benefit: 2 vs. 3 (at 6 months); OR = 0.5 (95% CI, 0.06–4.0)

ABMR, antibody-mediated rejection; ALG, antilymphocyte globulin; ARR, absolute risk reduction; CI, confidence interval; DSA, donor-specific antibodies; HR, hazard ratio; IVIG, intravenous immunoglobulin; MP, methylprednisolone; OR, odds ratio; PP, plasmapheresis; RCT, randomized controlled trial; RRI, relative risk increase; RRR, relative risk reduction; RRI, relative risk increase.

<sup>1</sup>Adapted with permission from Roberts et al (122).



**Figure 4: Therapeutic modalities for ABMR.** ABMR, antibody-mediated rejection; APC, antigen-presenting cell; IVIG, intravenous immunoglobulins.

tetramers (141,142), and these tetramers may also represent a potential therapeutic agent to deplete donor-specific B cells. Strategies currently used in transplantation to deplete B cells or inhibit B cell activation are rATG, alemtuzumab and rituximab. However, despite the short-term depletion of B cells, alemtuzumab is associated with altered phenotypic and functional properties of the repopulated cells (143), which may contribute to increased rates of ABMR (144,145). The maintenance immunosuppressant belatacept may provide indirect inhibition of B cells through costimulatory blockade of CD80 and CD86, as this disables the stimulation of CD28, a mediator of antibody production by B cells and B cell proliferation (146). However, belatacept is not under evaluation as a treatment for ABMR.

Limited clinical trial evidence suggests that the proteasome inhibitor bortezomib (which induces plasma cell apoptosis) may be useful in combination with plasmapheresis to reduce anti-HLA antibodies in sensitized patients and to treat ABMR following renal transplantation (138,140,147). Other investigational B cell-depleting therapies include potent anti-CD20 antibodies (e.g. ofatumumab and ocrelizumab) and an anti-CD22 antibody (epratuzumab) (138,140,148). Agents targeting the BAFF pathway, which costimulates B cell survival and expansion, are also in clinical development (e.g. atacicept and belimumab) (140,149). The inhibition of antibody effector function is another interesting area of research, and some promise has already been shown by eculizumab, an anti-C5 antibody, in the prevention and treatment of ABMR (140,149).

Many of the potential treatment options for ABMR have been imported from other areas of medicine, without appropriate clinical trials in kidney transplantation; hence,

there is a need for well-designed clinical trials that use standardized and contemporary diagnostic, monitoring and therapeutic strategies for ABMR. There are challenges in organizing multicenter, prospective clinical trial study groups aimed at developing agents for DSA reduction and treatment of ABMR. There is also a bias toward developing B cell/antibody-targeting drugs for indications outside of transplantation (such as oncology or rheumatology), and the FDA has highly stringent requirements for the approval and labeling of new agents in the transplantation arena. Before novel and more effective treatments become available, the close monitoring of high-risk patients and an emphasis on adherence to well-tolerated maintenance immunosuppressants are recommended to minimize the risk of ABMR.

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