

## Full Reviews

# Antibody-incompatible kidney transplantation in 2015 and beyond

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### ABSTRACT

Rejection caused by donor-specific antibodies (principally ABO and HLA antibodies) has become one of the major barriers to successful long-term transplantation. This review focuses on clinical outcomes in antibody-incompatible transplantation, the current state of the science underpinning clinical observations, and how these may be translated into further novel therapies. The clinical outcomes for allografts facing donor-specific antibodies are at present determined largely by the use of agents developed in the 20th century for the treatment of T-lymphocyte-mediated cellular rejection, such as interleukin-2 agents and anti-thymocyte globulin. These treatments are partially effective, because acute antibody-mediated rejection is mediated to a considerable extent by T lymphocytes. However these treatments are essentially ineffective in chronic antibody-mediated rejection. Future therapies for the prevention and treatment of antibody-mediated rejection are likely to fall into the categories of those that reduce antibody production, extracorporeal antibody removal and disruption of the effector arms of antibody-mediated tissue damage.

**Keywords:** ABO, antibody, antibody-mediated rejection, HLA, kidney transplant

### INTRODUCTION

Antibodies directed against transplants are becoming recognized as a critical barrier to further improvements in the access of patients to transplantation and in the survival of allografts. This review will summarize the current results of transplantation in the presence of donor-specific antibodies (DSA),

and the possible research pathways that will lead to the control and prevention of such antibodies and the effective treatment of antibody-mediated rejection (AMR), both acute (AAMR) and chronic (CAMR).

The modern era of renal transplantation began in the 1960s with the introduction of azathioprine. Within a few years the importance of blood group incompatibility and HLA-specific antibodies was recognized, and their presence essentially vetoed transplantation outside experimental settings [1]. A focus on T-lymphocyte-mediated cellular rejection over the next half century has resulted in a therapeutic toolkit that has eliminated the vast majority of graft losses from this cause in adherent patients. Ultimately the therapies required to prevent T-lymphocyte-mediated rejection proved relatively simple, namely effective multipoint targeting of the interleukin-2 pathway and lymphocyte deletion therapy. An international focus from clinicians, scientists and industry on developing new treatments for antibody-mediated rejection (AMR) arguably began only a decade ago, and we are currently in an exciting era characterized by a rapid series of new discoveries about anti-graft antibodies, their mechanisms of production and action and the treatment of antibody-mediated rejection.

### CURRENT CLINICAL OUTCOMES

#### HLA antibodies

Donor-specific HLA antibodies may be preformed or develop *de novo* after a transplant. The current status of transplantation across preformed HLA antibodies (HLAi transplantation) is that acceptable graft outcomes can be achieved in living donor transplantation, so long as the pre-transplant complement dependent cytotoxic (CDC) crossmatch is

negative [2]. However such transplants do require antibody screening and careful management. It is not enough simply to transplant across a negative CDC crossmatch without taking account of preformed HLA antibodies. In many patients with low pre-treatment levels of donor-specific HLA antibodies, successful engraftment may be achieved using standard immunosuppression of tacrolimus, mycophenolate, prednisolone and basiliximab. However, in cases with higher levels of DSA, for example where the flow cytometric (FC) crossmatch is positive, antibody removal and induction immunosuppression or therapies for antibody-mediated rejection are required [3–7].

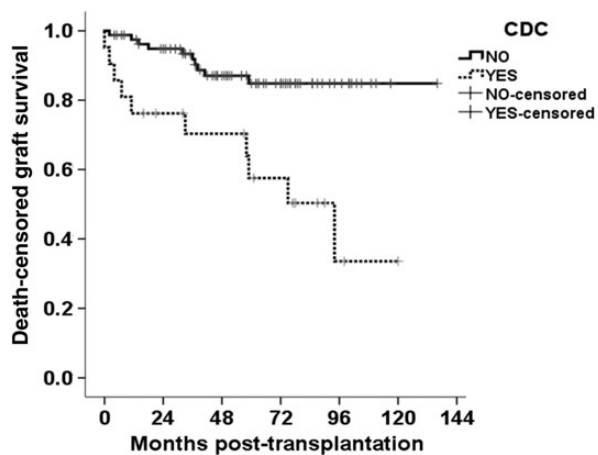
As a generalization, current clinical outcomes seem to indicate a mortality and graft loss rate about twice that of ‘antibody-compatible’ transplantation in the first year, unless the CDC is positive when the graft loss rate is higher, rising to 50% at 5 years using CDC methodology where there is no enhancement with anti-human globulin [2], and 30% graft loss when the more sensitive technique using AHG is used [7]. Other adverse prognostic features that can be identified pre-transplant are DSA that are combinations of Class I and Class II, and DSA that bind the complement component C1q in microbead assays [7, 8].

Therapies used in such clinical series include antibody removal pre-transplantation (plasma exchange, plasmapheresis or immunoadsorption), cellular depleting therapies (anti-thymocyte globulin, rituximab, alemtuzumab), intravenous immunoglobulins and proteasome inhibitor therapy (bortezomib), but there is no consensus on which of these approaches is most effective, and randomized trials are awaited. Transplantation across preformed HLA antibodies is best performed with living donors where there is time to achieve effective antibody removal, and possibly the graft is better equipped to cope with the rigours of early post-transplant antibody assault [2–9].

The outcomes in the face of CAMR due either to *de novo* HLA antibody production or persistent preformed DSA production are less encouraging [7–11]. For example one series showed a 10-year graft survival of <60% in those with *de novo* DSA, compared with >90% for those without DSA [12]. Rejection takes the form of glomerular basement membrane damage (transplant glomerulopathy), with proteinuria and progressive graft failure, usually over 2–3 years. There is no effective therapy for this condition, though we have seen it resolve durably if the DSA levels fall. Often transplant glomerulopathy may be associated with some active cellular infiltration in the peritubular capillaries and this may be temporarily amenable to therapy, but ultimately nearly every case of transplant glomerulopathy progresses to graft failure within 3–5 years (Figure 1).

### ABO antibodies

Transplantation across ABO incompatibility (ABOi) generally produces excellent outcomes, and the experience in Japan over a period of decades indicates that outcomes are equivalent to ABO-compatible transplantation [13]. However, in the USA and in the UK, ABOi renal transplantation seems to have a slightly increased risk of severe AAMR which may result in graft loss, with catastrophic rejection progressing over a period of hours. This rejection may occur without any warning, and may indeed occur in those with very low pre-transplant



**FIGURE 1:** Graft survival at University Hospitals Coventry and Warwickshire in HLA antibody incompatible transplantation, for patients with pre-treatment complement dependent cytotoxic (CDC) positive crossmatch ( $n = 21$ ) compared with those with DSA but with a negative CDC crossmatch (includes flow cytometric crossmatch positive and negative) ( $n = 91$ ). Five- and 10-year death-censored graft survivals were 84.8% for the CDC-negative group, 57.5 and 33.6% respectively for the CDC-positive group.

antibody levels [14]. Further multicentre studies of this rare phenomenon (2–4% of grafts) are required.

ABOi transplantation does differ from HLAi transplantation in that chronic antibody-mediated rejection is not attributable to ABO antibodies. Biopsies may be ongoing staining for C4d on the peritubular basement membrane suggesting that there is some form of inflammation but the clinical outcome is not adversely affected. The significance of peritubular C4d differs from the same finding in HLAi transplantation, since the C4d deposition is a sign of CAMR. When CAMR is reported in ABOi grafts, this seems to be due to the concurrent presence of HLA antibodies [15].

### Other antibodies

Non-HLA antibodies may be important players in AMR. Antibodies directed against the angiotensin receptor are of particular interest. These were first observed as a cause of sporadic AAMR associated with severe hypertension. Subsequent studies have extended their possible role to the causation of CAMR, and their presence has been associated with graft loss, independent of HLA antibodies [16, 17]. They may be auto- and allo-antibodies, and further work will define better the extent of their role in the causation of graft loss.

## THE BIOLOGY OF ANTIBODY PRODUCTION AND REJECTION

Decades of focus on T-cell-mediated rejection and the lack of a clinical model of antibody incompatible transplantation mean that our understanding of the biology of antibody production, and the mechanisms of AMR are at a relatively early stage, but knowledge is accumulating rapidly.

### Production of antibodies

Antibodies are synthesized by memory B lymphocytes and plasma cells. Both of these ultimately derive from immature cells of the B-lymphocyte lineage and receive T-cell help. It is possible that induction immunosuppression, enhanced beyond the level necessary to prevent most T-lymphocyte-mediated cellular rejection, may reduce the production of *de novo* HLA antibodies [18], and prospective and randomized studies are awaited.

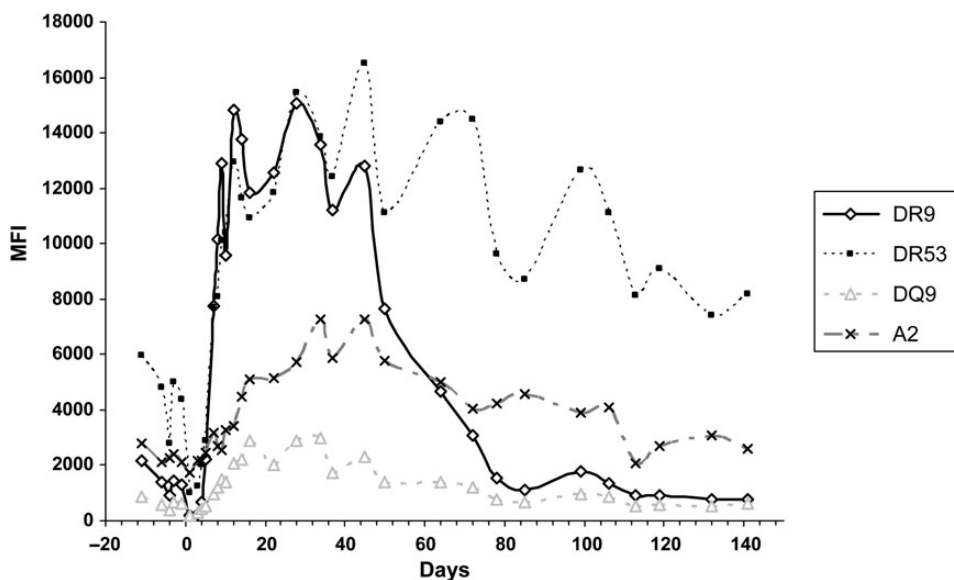
In HLA transplantation, pre-formed donor-specific HLA antibodies may have been present for many years before the transplant, and detailed monitoring of their levels in the early post-transplant period may show large changes in their levels, with both rises and falls. Figure 2 shows a case with a heterogeneous response, the rates of post-transplant increases in DSA, the pre- to peak variation and the rate of fall of DSA showing variation. The rises and falls in HLA antibody levels post-transplant are not uniform across a range of patients, and there is no clear pattern governing the responses, though HLA Class II antibodies may show greater responses than Class I, with the profiles of HLA DQ and DP similar to HLA DR [19].

It is likely that insights can be gained from more detailed studies of the characteristics of the antibodies, for example the classes and IgG subclasses of the responses (Table 1). New antibody production is initially of the IgM class, and IgM donor-specific HLA antibodies have been associated with AAMR in the absence of equivalent IgG antibodies [20] and reported in *de novo* HLA antibody production [21]. HLA

antibodies of the IgA class may also occur frequently, though their significance is not yet understood [26]. IgM antibody production may switch to IgG3 subclass, then to IgG1, IgG2 and ultimately IgG4. There is currently much interest in examining antibody subclasses in more detail [21], and we have observed some differences in subclass distribution, for example a higher incidence of IgG3 subclass in pregnancy-stimulated HLA antibodies and an association between pre-transplant IgG4 levels and subsequent AMR and graft survival [22].

One of the most remarkable observations in clinical practice is that DSA may disappear after transplantation. This has been observed in both ABO and HLA antibody-incompatible transplantation [13, 14, 19]. However, it seems likely that the mechanisms are different.

In HLA antibody-incompatible transplantation, many groups have noted that in some patients HLA antibodies may disappear after the transplant, even when sensitive microbead analysis is used. While some groups have attributed this to particular treatments administered, we have seen that this phenomenon is independent of any particular therapy over and above the standard immunosuppression of basiliximab, tacrolimus, mycophenolate and prednisolone [19]. The phenomenon does seem to be commoner when there is a vigorous antibody response post-transplant, and daily sampling shows that the fall in antibody levels occurs rapidly. This implies some form of 'active' removal of antibody from the circulation, and this is the subject of ongoing research. If it proved possible to induce the disappearance of DSA pre-transplant, that would be very useful. However,



**FIGURE 2:** HLA and ABO antibody levels pre- and post-transplant showing variation in antibody responses within the same patient, both in terms of rate of increase, magnitude of change from pre- to peak levels, and the rates of fall. The patient received a living donor transplant from his father. DSA levels were as shown in the legend. The CDC crossmatch was positive at a titre of 1 in 16, and there was additional ABO incompatibility, donor blood group A1 and recipient B. The recipient received plasma exchange pre-transplant, and post-transplant at Days 21 and 22 only. Immunosuppression was with prednisolone, tacrolimus, mycophenolate and basiliximab, with anti-rejection treatment including ATG (Days 1–15) and eculizumab (Days 24 and 31). The graft is still functioning at 5 years post-transplant, though with proteinuria and reduced function. DSA levels were measured by microbead assay (OneLambda). MFI levels were DR9—pre 2148, peak 14821 (Day 9), late 776; DR52—pre 5982, peak 12952 (Day 9), Day 141 8162; DQ9—pre 864, peak 1468 (Day 9), late 618; A2—pre 2774, peak 7258 (Day 45), late 2612. Follow-up at 2 years post-transplant showed little change in antibody levels from the Day 140 data shown here. Haemagglutination titres to blood group A1 were, IgG, pre 1, peak 128 (Day 10), late 4; IgM, pre 4, peak 128 (Day 10), late 32.

**Table 1. HLA characteristics of antibody and antigen that might affect clinical outcomes**

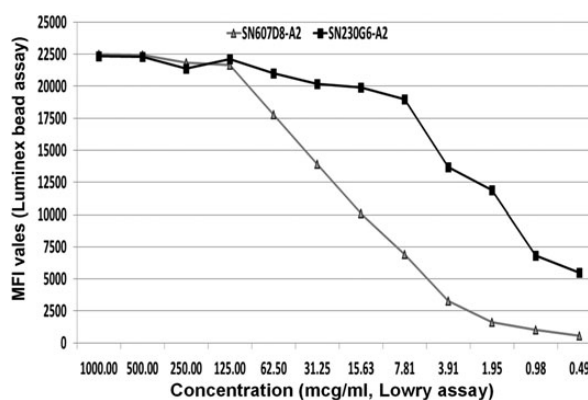
	Comment	Reference
<b>HLA antibody</b>		
Concentration	Not currently possible to measure antigen-specific concentration of antibody (see Figure 3)	
Antibody binding to HLA in solid phase assay (readout a combination of avidity and concentration)	Measureable by microbead assays	[2, 4]
Class	Mostly IgG, early reports of IgM mediated AMR and occurrence of IgA	[20, 21]
Subclass	Early studies show heterogeneity in responses	[21, 22]
Affinity	Measurement under investigation	
Glycosylation	Measurement under investigation	
Cellular binding	CDC and FC crossmatches Endothelial binding	[2, 23]
<b>Inhibitors</b>		
Soluble HLA	Early reports of methods to quantify	
HLA-E	Early reports of correlation with clinical outcomes	[24]
Idiotypic antibodies	Hard to measure with current tools but could be important	[25]
<b>Immune complexes</b>		
HLA-sHLA immune complexes	Not currently measureable	

it is not clear whether this requires exposure to donor HLA (and if so in what form of delivery), as well as immunosuppression.

In ABOi transplantation, successful engraftment is often followed by the disappearance of antibody, but biopsies of the graft show ongoing C4d staining in peritubular capillaries, as if there is some persistent immunological stimulation, making it possible that some antibodies are being produced and then absorbed by the graft. It will be interesting to see what happens to ABO antibodies in patients who later lose their grafts from causes other than ABO-related rejection, and then have graft nephrectomy—will their ABO antibodies reappear or not? ABOi heart transplants in neonates may result in long-term tolerance to the transplanted ABO, but this could be a special case owing to the neonatal timing of the transplant.

### Binding of antibodies to an allograft

There is much current research examining how antibody binds to antigen at a molecular level, and then how that response evolves into clinical rejection, since the simple act of antibody binding to antigen alone does not seem to cause rejection. Although the development of microbead assays have enabled far better measurement of antibody levels than was previously possible, they do not measure the concentration of antibody, even in a semi-quantitative manner, but measure a combination of concentration and affinity, as shown in Figure 3. Table 1 lists some of the antibody characteristics that



**FIGURE 3:** Dose response curve showing Luminex bead MFI value against concentrations of two human monoclonal HLA-A2-specific antibodies. The same concentrations of antibody may give markedly different MFI levels (e.g. <2000 and >10000, respectively, at 1.95 µg/mL).

could be associated with clinical outcomes; we are still at a preliminary stage of the understanding of which of these are important, and which could be manipulated for therapeutic benefit.

HLA is profoundly polymorphic, and the binding of antibody to an HLA molecule is determined not by the whole HLA molecule, but by small areas of polymorphism within the protein structure known as epitopes. An epitope may be determined by as little as a single amino acid substitution in a critical area of the molecule, even though the binding footprint of an antibody molecule is much larger. Different HLA molecules are defined by a series of epitopes, many of which are shared between different HLA specificities. This is why an antibody generated against one HLA allele will bind to many others [27].

A better understanding of exactly how the antibody-antigen reaction develops may allow for the development of therapies that disrupt this interaction. Hence, research groups are studying antibody affinity for antigen, the atomic structure and biophysical properties of HLA, especially in relation to glycosylation as well as the amino acid backbone.

Clinical studies of the mechanisms of rejection are hampered by the inability of current techniques usefully to measure the binding of antibody to the graft [15]. It is not clear why this is the case, but the clinical classifications of antibody-mediated rejection depend on the detection of effector responses such as complement and cells, and not on whether there is any donor-specific antibody in the graft. A test that gave an accurate readout of the levels of donor-specific antibody bound to graft endothelium would give immediate and important insights into AMR.

### Complement

It is widely acknowledged that activation of the complement system is a major driver of antibody-mediated tissue damage and subsequent transplant rejection. A good indicator of this is the CDC assay that correlates positively with risk of donor organ failure [2]. The presence of the complement protein fragment C4d in renal rejection is also a ‘smoking gun’

for activation of the complement cascade, which occurs downstream of C1q binding to clustered complexes of IgG and IgM antibodies on a cell surface. The antibody classes and subclasses typically found are IgG1 (HLA antibodies) and IgM (ABO antibodies), which are strong C1q binders and thus classical pathway activators. However, clinical investigation is made more difficult by the difficulties in measuring DSA in graft biopsies, and also by the observations that AAMR may occur in the absence of C4d deposition, and that C4d deposition may not reflect ongoing rejection; this could partly be due to endothelial synthesis of C4, so C4d should not necessarily be assumed to have been deposited on endothelium from circulating C4 [28].

### Cellular response

The cellular aspect of acute antibody-mediated rejection is interesting, because although hyperacute rejection looks more like an innate response, with neutrophils and macrophages predominately, AAMR is characterized by a marked T-lymphocyte infiltration into the graft, visible using immunohistochemistry, and indistinguishable from T-cell-mediated cellular rejection at a molecular level [29, 30].

### Response of endothelium

The predominant target of antibody-mediated rejection is vascular endothelium in the blood vessels (and glomeruli) of the allograft. Endothelial cells express ABO and HLA (classes I and II). Endothelium is not a passive victim of antibody binding and the rejection process, but has an adaptive response that may be protective to the graft. Indeed, clinical acute antibody-mediated rejection generally resolves in the presence of donor-specific antibody, and while this may be due to therapies which down-regulate the cellular responses, it is also possible that the graft becomes resistant to rejection [23, 28]. In the clinical setting, it is possible to measure vascular stress in the allograft by RNA analysis of renal biopsy material, the 'molecular microscope'. This approach can also help characterize the T- and B-cell activity within the allograft [31].

In some cases, the antibody binds to endothelium but does not cause rejection, for example in ABOi transplantation, where staining for complement C4d in the peritubular capillaries of well-functioning grafts seems to indicate accommodation of the graft to antibody. In HLAi transplantation, by contrast, longer term C4d staining is likely to indicate low grade-AMR, though in some cases there does seem to be DSA present for many years without causing rejection or C4d staining on biopsy.

## NEW THERAPIES

The successful treatment and ideally prevention of antibody-mediated rejection will require the development of new therapies that have specific new actions. It is not yet clear exactly which are the ideal targets, or whether there will be a solution as conceptually simple as the targeting of interleukin-2 in T-lymphocyte-mediated rejection. Therapies under consideration can be grouped into three main categories; (i) those that

target the cells responsible for antibody production; (ii) those that target mediators of antibody damage, including complement; (iii) more effective removal of antibody using extracorporeal techniques.

### Targeting the cells responsible for antibody production

Once the body has been programmed to produce the antibody in the long term, the cells responsible for this can be hard to target. For example, while bone marrow ablative therapy for haematological malignancies may appear to result in deletion of the host immune system, many antibody responses induced by prior vaccination may persist. We have seen HLA antibody production persist after chemotherapy and total lymphoid irradiation conditioning for cord cell transplantation.

There are some candidate therapies in use that could prove beneficial, but have not yet been tested in randomized trials. Rituximab (CD20 monoclonal antibody) and alemtuzumab (CD52 monoclonal antibody) are both capable of killing B lymphocytes, though do not have specific action against plasma cells, and both may spare memory B lymphocytes [18, 31, 32]. In the case of alemtuzumab, any benefits in clinical use might be due to deletion of effector leucocytes rather than effects on antibody production, and its use has even been reported to be associated with increases in the production of *de novo* HLA antibody levels in previously unsensitized transplant recipients [33]. In clinical use, neither agent can completely prevent increases in preformed HLA antibody levels after transplantation, though some data suggest that *de novo* HLA antibody production after transplantation may be reduced by prior administration of anti-CD20 therapy.

Anti-thymocyte globulin has potentially a very wide action, and may be particularly effective in dealing with cells in the effector arm of the rejection process, but again does not seem to completely prevent post-transplant synthesis of preformed DSA, although in a non-randomized study, its use was associated with less production of *de novo* HLA antibodies in the first 2 years post-transplant [33].

The cells that produce antibody could be targeted by means other than cytolytic therapies, and there are some candidate therapies available. Intravenous immunoglobulins (IVIg) have been used for some time. This product has a range of actions, but may down-regulate the production of HLA antibodies, and may be combined with anti-CD20 therapy [34]. Cytokine inhibition may be another route available, and studies are currently underway to evaluate the possible benefits of inhibitors of interleukin-6 and BAFF (B-cell activating factor). BAFF is an attractive target, as studies are emerging that associate BAFF with clinical outcomes in transplantation [35].

A further approach to reducing antibody production is targeting the protein synthetic capacity of plasma cells using proteasome inhibitors. Although these agents were initially conceived selectively to target malignant plasma cells, it is possible that they may impact on metabolically active cells producing DSA post-transplant, and uncontrolled studies have shown successful transplantation in the face of active immune responses [36, 37]. The effects of bortezomib on pre-transplant antibody production have been less encouraging, but randomized

trials and the introduction of second generation proteasome inhibitors are welcomed with great interest.

### Extracorporeal antibody removal

Most protocols for clinical antibody-incompatible transplantation involve extracorporeal antibody removal, at least in those with higher antibody levels. While the effects of antibody removal are partial and temporary with the current technology, it is generally believed to be beneficial [38, 39]. Randomized trials in the setting of preformed antibodies are lacking, but it does seem that removing antibodies to a level below the threshold of CDC crossmatch positivity prevents hyperacute rejection, and that plasma exchange is an effective therapy for AAMR.

Antibody removal therapies are constrained by several difficulties. These include the problem of providing long hours of therapy during times when there is a risk of bleeding; the pool of IgG outside the vascular compartment, which re-equilibrates only slowly with the vascular compartment; by rapid production of the antibody in the post-transplant period and by the inability of current therapies to selectively remove HLA antibodies, as all therapies remove total immunoglobulin, often together with other desirable blood components such as fibrinogen [39]. Similar constraints are also faced when total immunoglobulin removal methods are used in ABO-incompatible transplantation [40].

There is therefore a need for more selective therapies and modes of delivery that will allow removal of enough antibody at the times before and after transplant, and maybe soon after biopsy, and will impact on rejection at times when there is rapid antibody production (a doubling time of 12 h has been observed in some of our patients) [41]. The availability of large amounts of HLA protein has allowed the production of a selective HLA absorption column that indicates as proof of principle that HLA antibody removal therapy could be improved. Specific immunoadsorption therapy is already available to remove ABO antibodies [40, 42].

### Mediators of antibody damage

Inhibition and modulation of complement activation is an attractive therapeutic route, with FDA-approved biopharmaceuticals already in clinical use for rare conditions such as hereditary angioedema (HA) and paroxysmal nocturnal haematuria (PNH). These include the drugs cinryze (C1 esterase inhibitor) for HA and eculizumab (anti-complement C5 monoclonal antibody) for PNH [43, 44]. These two agents act at different points within the complement activation cascade with potential to protect cells and tissues directly from aggressive lytic mechanisms and also broader immune activation. C1 esterase inhibitor is a protein that blocks the enzymatic function of the complement C1s component. This results in the proteolytic activation cascade being arrested upstream of powerful inflammatory and cytotoxic mechanisms such as C3/C4 opsonisation, anaphylatoxin release (a powerful leucocyte chemoattractant mechanism) and assembly of the membrane attack complex.

Eculizumab acts further downstream through functional blockade of the complement component C5, resulting in

abrogated membrane attack complex formation and C5a anaphylatoxin release. Recruitment into an international randomized clinical trial of eculizumab in patients with pre-formed HLA antibodies has been completed, and results should be available in 2015. If a positive effect of this agent is shown, this will be pivotal in the future therapy of AMR, and possibly in the prevention of CAMR. If the randomized trial shows a marked reduction in AAMR rates in line with preliminary studies, there will be important questions to answer on the cost-effective application of the drug, and also whether its benefit will be extended to those with higher DSA levels who were not eligible for the current study.

### SUMMARY

Antibody-incompatible transplantation has become a clinical reality in the 21st century, though graft survival rates are still suboptimal. Many patients with very high levels of preformed DSA, both HLA and ABO, are considered untransplantable with current technology. Treatment for CAMR when it leads to transplant glomerulopathy is almost completely ineffective. The understanding of the drivers of antibody production and mechanisms of AMR is progressing rapidly, and it is likely that combinations of new therapies targeting antibody production, complement and extracorporeal antibody removal will improve graft survival rates in the coming decades.

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### CONFLICT OF INTEREST STATEMENT

The authors are co-researchers with Pure Transplant Solutions in production of immunoadsorption column to remove HLA antibodies.

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