REVIEW

Complement inhibition as potential new therapy for antibody-mediated rejection

Farsad Eskandary[1], Markus Wahrmann[1], Jakob Mühlbacher[2] & Georg A. Böhmig[1]

1 Division of Nephrology and Dialysis, Department of Medicine III, Medical University Vienna, Vienna, Austria
2 Department of Surgery, Medical University Vienna, Vienna, Austria

Key words
antibody-mediated rejection, complement inhibition, eculizumab, kidney transplantation

Correspondence
Georg A. Böhmig MD, Division of Nephrology and Dialysis, Department of Medicine III, Medical University Vienna, Währinger Gürtel 18-20, A-1090 Vienna, Austria.
Tel.: +43 140400 43630;
fax: +43 1 40400 39302;
e-mail: georg.boehmig@meduniwien.ac.at

Conflicts of interest
The authors declare no conflicts of interest.

Received: 1 July 2015
Revision requested: 7 September 2015
Accepted: 13 October 2015
Published online: 10 November 2015

SUMMARY

Antibody-mediated rejection (ABMR) is a leading cause of kidney allograft failure. While the exact mechanisms contributing to donor-specific antibody (DSA)-triggered tissue injury are still incompletely understood, complement activation via the classical pathway is believed to be one of the key players. There is now growing interest in complement blockade as an antirejection treatment. One attractive strategy may be inhibition of terminal complex formation using anti-C5 antibody eculizumab. Anecdotal reports, case series, and a unique cohort of flow crossmatch-positive live donor kidney transplant recipients subjected to eculizumab-based desensitization have demonstrated successful prevention and reversal of acute clinical ABMR. Nevertheless, maybe due to complement activation steps proximal of C5 or even complement-independent mechanisms, subclinical rejection processes that might culminate in chronic injury were found to escape inhibition. Larger studies designed to clarify the actual clinical value of terminal complement inhibition as an antirejection treatment are currently underway. In addition, alternative concepts, such as therapies that target key component C1, are currently under development, and we will see in the near future whether new strategies in the pipeline will have the potential to beneficially impact clinical practice.

Transplant International 2016; 29: 392–402

General remarks

Antibody-mediated rejection (ABMR) is one of the cardinal causes of kidney allograft loss [1–3]. Despite major advances in the understanding of its pathophysiology and diagnosis, the clinical management of this rejection form has remained a big challenge [2,4,5]. A major achievement has been the definition of clear-cut criteria for its diagnosis and classification [6,7], a critical step towards a systematic development of therapeutic strategies applied to prevent or reverse rejection processes that otherwise would culminate in chronic tissue injury leading to graft dysfunction and loss.

Numerous anecdotal reports and uncontrolled series have proposed therapeutic efficacy of a variety of different strategies, which are commonly based on apheresis (plasmapheresis or immunoadsorption) for extracorporeal antibody depletion and measures that interfere with B-cell immunity, such as CD20 antibody rituximab, high-dose intravenous immunoglobulin (IVIG) or pro-

doi:10.1111/tri.12706

teasome inhibition [2,4,5,8]. A caveat is that published protocols are heterogeneous, and, even though therapeutic success has been reported in many cases, formal proof of efficiency is commonly lacking [9].

Many transplant centres worldwide have gathered experience in handling presensitized patients on the wait list, and several different desensitization strategies have now been published, both in the context of live and deceased donor transplantation [10–14]. Over the years, however, it has turned out that, despite improved overall patient survival [12], many desensitized high-risk recipients develop clinical and subclinical ABMR culminating in adverse average long-term allograft survival [15,16].

Disappointing long-term results may reinforce a need for further improvement of our currently available therapeutic repertoire. A more detailed understanding of the pathophysiology of ABMR may provide a clue for a more targeted and effective treatment. One attractive therapeutic target may be the complement system. Indeed, there are several lines of experimental and clinical evidence suggesting a critical role of complement as an important mediator of allograft injury [17,18].

Recent clinical experience with eculizumab, an innovative therapeutic tool that selectively blocks terminal complex formation, has added significantly to our understanding of the pathophysiology of ABMR and opened a new therapeutic perspective [17]. Eculizumab is a monoclonal antibody targeting complement component C5 that has already been approved for the treatment of two different complement-mediated diseases, paroxysmal nocturnal haemoglobinuria (PNH) and atypical haemolytic uraemic syndrome (aHUS) [19,20]. It was only a question of time that this agent was tested in the prevention or treatment of ABMR. This review provides a detailed discussion of terminal complement blockade as a new approach towards the treatment of ABMR.

Complement as a trigger of ABMR

From a theoretical point of view, it is likely that the manifold steps triggered upon activation of the complement cascade contribute significantly to the process of rejection. The release of anaphylatoxins, surface-bound cleavage products and the formation of the terminal complex may, among others, attract inflammatory cells, trigger the coagulation cascade, promote endothelial cell activation and, in a multifaceted crosstalk with components of specific immunity, enhance or modulate adaptive immunity [17,18,21]. There are several lines of clinical evidence supporting a key role of complement in the process of ABMR. For example, it has been recognized early that preformed complement-activating antibodies against donor antigens can result in hyperacute rejection [22]. Moreover, *in vivo* complement activation as reflected by capillary deposition of complement split-product C4d is well established to be associated with acute or chronic microcirculation inflammation and injury that may culminate in unfavourable graft survival [23–26]. Recent studies have shown that C4d may indicate a particularly severe form of ABMR posing an additive and independent risk of adverse allograft survival [27,28]. A relevance of DSA-mediated complement activation is further emphasized by recent studies suggesting that the *in vitro* detection of human leukocyte antigen (HLA) antibody-triggered complement deposition in modified solid-phase assays associates with ABMR occurrence and adverse graft survival [29,30].

Of course, associative clinical studies should be interpreted with caution as they do not permit the conclusion of a causative relationship. In addition, even though several experimental models may support a role of complement in rejection [18], one has to be aware that animal models may not adequately mirror the human situation. In this context, we want to highlight an elegant mouse heart transplant model of ABMR triggered by preformed DSA showing that a monoclonal antibody blocking complement component C5 was effective in preventing rejection [31]. Interestingly, in this model, C5 blockade allowed for the establishment of long-term stable graft function and maintenance of normal graft histology despite the presence of antibody and complement [31], a finding that could not be reproduced in clinical transplantation [32].

Over the last years, it has turned out that the situation is not as trivial as anticipated. For example, emerging evidence has revealed that ABMR does not necessarily associate with detectable capillary C4d deposits [33,34], and more recently, a phenotype of C4d-negative ABMR, which of course does not entirely exclude a causative role of complement activation, has entered the Banff 2013 classification as a separate entity [7]. In addition, some animal models were unable to definitely demonstrate a causal relationship between antibody-mediated complement activation and graft damage [35–37]. Moreover, clinicians are aware of an impressive phenomenon, namely, that in ABO-blood group-incompatible transplantation, isoagglutinins often trigger early steps of complement activation as indicated by intense capillary C4d deposition, but without any morphological and

clinical correlate of tissue injury [38]. This has evoked the hypothesis that early complement activation without evidence of tissue damage might even indicate a state of resistance to immunological injury [39].

On the basis of the afore-listed results of experimental and clinicopathological studies, an efficiency of targeted complement blockade cannot be unconditionally postulated. Even the more exciting are the results of the first studies that have adoptively transferred this concept into the context of ABMR.

Eculizumab for terminal complement blockade

Properties and mechanism of action

Eculizumab is a high-affinity monoclonal, humanized antibody directed against human complement component C5 [17,40]. The complementarity-determining regions in the variable chain of the Fab region are of murine origin and were cloned and grafted into human germline coded, heavy- and light-chain frameworks. To avoid unwanted proinflammatory effector functions, mediated by Fc receptor binding or complement activation, the constant heavy chains and hinge region of the human IgG subclasses two and four were fused together [17,40]. Eculizumab blocks the cleavage of C5 by C5 convertases (C4bC2aC3b for the classical pathway and C3bBbC3b for the alternative pathway) into the enzymatic split products C5a, a potent anaphylatoxin, and C5b. C5b successively binds the terminal pathway components C6, C7, C8, and C9 to nonenzymatically assemble the membrane attack complex (MAC) [17,40]. Essential steps of DSA-triggered complement activation and potential sites of intervention are illustrated in Fig. 1.

Monitoring of eculizumab therapy

One simple strategy to monitor C5 inhibition may be the use of a standard 50% haemolytic complement (CH50) assay using sensitized sheep erythrocytes. In a recent large pharmacodynamics study, 22 patients under long-term eculizumab therapy for treatment of PNH were carefully monitored for circulating free eculizumab levels and complement inhibition evaluated by CH50 assay [41]. A remarkable finding was a marked variability in the half-life and free eculizumab levels prior to re-injection, despite a uniform infusion schedule (900 mg every 2 weeks). Most importantly, the authors of this study reported the frequent finding of CH50 levels above a 10% threshold (49% of evaluated pre-

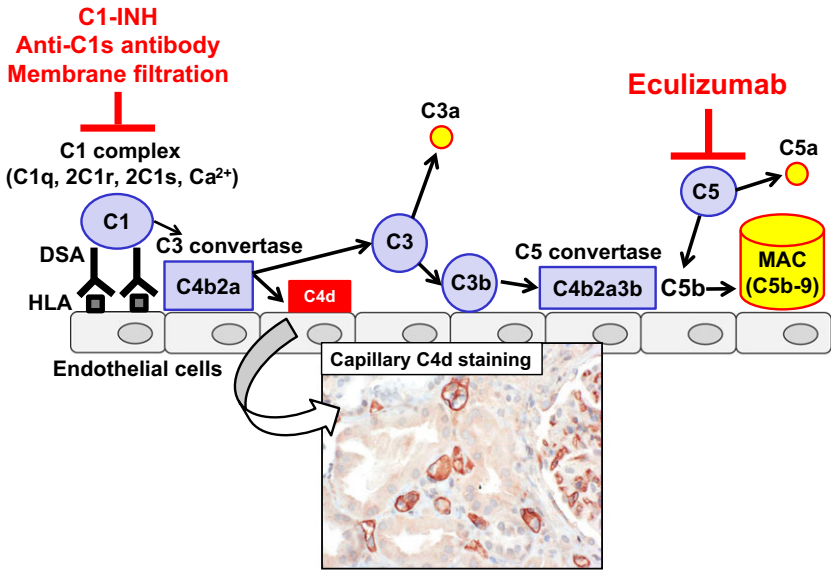

Figure 1 Schematic illustration of classical complement activation and therapeutic strategies targeting complement. In a first step of the classical cascade, antibodies bound to HLA expressed on donor endothelial cells interact with C1q and stabilize the formation of the C1 complex (Ca^{2+} dependent). Active C1 subunit C1s cleaves factor C4 into C4a and C4b, the latter providing a binding site for C2 facilitating its cleavage by C1s into C2a and C2b. Under the control of complement-regulatory proteins and Factor I, surface-bound C4b is degraded to C4d. Capillary C4d staining has proven to be a useful diagnostic marker. If strong activation overwhelms these control mechanisms, surface-bound C4b2a becomes a stable, active C3 convertase that cleaves C3 into the anaphylatoxin C3a and C3b which again covalently binds to the surface. The combination of C4b2a with C3b forms the active C5 convertase (C4b2a3b) that can now cleave C5 into the anaphylatoxin C5a and C5b. C5b is responsible for the assembly of the membrane attack complex (MAC) which is inserted in the cell membrane. By stably binding to C5 in a 0.5:1 molar ratio of antibody to C5, eculizumab hinders its cleavage.

Transplant International 2016; 29: 392–402

infusion samples), which were associated with low eculizumab levels, biochemical signs of haemolysis, and a more frequent need of transfusions. Interestingly, in some cases, residual C5 activity despite an excess of free eculizumab levels was detected, a finding which was not related to previously reported genetic variants of C5 that associate with a poor response to the antibody [42]. Such data suggest a need for a careful monitoring of underdosing to avoid a breakthrough of complement activation, which may be associated with disease activity [41]. In a study of sensitized kidney transplant recipients subjected to eculizumab-based desensitization, eculizumab pharmacokinetics were assessed by monitoring of free and C5-bound eculizumab and in parallel detection of complement activity using a haemolytic assay based on chicken erythrocytes. Interestingly, maybe as a result of a higher maintenance dosage (1200 mg every 2 weeks), the authors of this study reported on generally sufficient eculizumab levels and an efficient blockade of complement in most of their patients [32,43,44].

Infection prophylaxis

Late complement pathway deficiencies may predispose to infections, including meningococcal meningitis [45]. Accordingly, guidelines for the use of terminal complement blockade include vaccination to prevent infection with *Neisseria meningitidis*. Vaccination, however, may not always be successfully, also due to the fact that currently available vaccines do not cover the whole spectrum of pathogenic *Neisseria* serovars [46]. For example, in a study of long-term eculizumab treatment (79 patients with PNH), two cases of meningococcal infection were recorded, and additional antibiotic prophylaxis was recommended [47]. In a series of aHUS patients, which included ciprofloxacin prophylaxis in patients vaccinated shortly before treatment initiation, no such infections were reported [48]. Using eculizumab in transplant patients, one has also to take into account an insufficient response to meningococcal vaccines due to immunosuppressive therapy. For example, in a recent report, a case of meningococcal sepsis was described following eculizumab treatment for recurrent aHUS, obviously as a result of inadequate immunization [49]. Such observations strongly suggest that vaccination should be performed before initiation of immunosuppression and reinforce the use of chemoprophylaxis in immunosuppressed patients [49]. Importantly, in a study of eculizumab for the prevention of ABMR in sensitized recipients, who were already immunized 1 month before transplantation, no major infectious complications were reported [43].

Approved clinical indications

Eculizumab has proven to be highly effective in the treatment of two different complement-mediated diseases, namely PNH [19,50,51] and aHUS [20,48]. PNH is caused by somatic mutations in the phosphatidylinositol *N*-acetylglucosaminyltransferase subunit A (*PIGA*) gene, which lead to a reduced or absent expression of distinct membrane lipid bilayer-anchored proteins including complement-regulatory proteins CD55 and CD59, on the surface of hematopoietic cells. Altered complement regulation may pave the way to uncontrolled activation of the alternative complement pathway and subsequent red cell lysis [19]. Terminal complement blockade by eculizumab was shown to markedly reduce the need for red blood cell transfusions and to relief many of the symptoms and complications in patients suffering from PNH [19,50,51]. aHUS is caused by a dysregulation of the alternative pathway of the complement system, for example due to mutations in complement regulators (e.g., Factor H, Factor I, or membrane cofactor protein) which may result in uncontrolled complement activation and subsequent thrombotic microangiopathy in the kidneys and other organs [52]. In a large multicentre study, terminal complement inhibition was demonstrated to effectively reverse symptoms caused by thrombotic microcirculation damage and restore renal function [48], and such treatment was shown to be effective also in the treatment and prevention of recurrent aHUS [53].

Eculizumab for prevention of ABMR in patients at risk

Much has been learned from a unique uncontrolled trial investigating the use of eculizumab as a strategy for the prevention of ABMR in presensitized crossmatch-positive live donor kidney transplant recipients [32,43,44]. This seminal trial, which was performed at the Mayo Clinic, has now included a total of 30 sensitized patients exhibiting a positive B-cell flow-cytometric crossmatch (FCXM) within a defined range of channel shifts (<450 and ≥200). Patients showing initial channel shifts ≥300 were subjected to additional pretransplant plasmapheresis treatment to reduce the antibody burden. In the initial phase of the study, a few patients received post-transplant plasmapheresis in addition. All patients received antithymocyte globulin (ATG) induction therapy and tacrolimus-based baseline immunosuppression. The first intravenous dose of eculizumab (1200 mg) was administered shortly before transfer to the surgical ward and was followed by serial infusions

(600 mg) in the post-transplant period. The duration of therapy with eculizumab was based on the course of crossmatch intensity after transplantation. After 4 weeks, treatment was discontinued if B-cell FCXM channel shifts below 200 were recorded. If this was not the case, two-weekly eculizumab infusions (1200 mg) were continued. For comparative analysis of outcome results, the authors evaluated a historical cohort of patients ($n = 48$) desensitized according to centre standard of care using pre- and post-transplant plasmapheresis together with IVIG.

In 2011, Stegall *et al.* [43] reported short-term results obtained in the first 26 recipients. Transplant outcomes in the first 12 months were impressive and, even though there was no randomized controlled design, the main finding was that the frequency of clinically significant ABMR was markedly reduced: only two patients (7%), as compared to 22 of 48 patients (44%) in the historical cohort ($P < 0.01$), developed acute ABMR, and rejection episodes in these recipients promptly responded to plasmapheresis. A remarkable finding was that under continued complement blockade, many patients maintained high levels of DSA but did not show any morphologic signs of ABMR in protocol biopsies despite the frequent finding of C4d deposition as a sign of early complement activation. Because of potentially deleterious DSA persistence, two patients were maintained on therapy with eculizumab over a period of 12 months. In this first report, the authors documented only one case of chronic rejection and one patient death after >2 years due to Burkitt lymphoma [43].

Incomplete prevention of acute ABMR

In the Mayo Clinic study, three of the patients developed early clinical ($n = 2$) or subclinical ($n = 1$) ABMR despite eculizumab treatment [44]. A detailed serologic analysis including IgG subclass and IgM detection using single-bead arrays revealed the development of IgM-type DSA in all three rejecting patients, while this was the case in only one of the nonrejecting patients. It was hypothesized that IgM DSA could have effectively triggered C3 activation and the formation of proinflammatory C3 activation products, which was not prevented by terminal complement blockade [44].

Incomplete prevention of chronic ABMR

In a very recent analysis of the cohort, Cornell *et al.* [32] evaluated transplant outcomes and the evolution of morphological changes beyond 1 year of follow-up. This important analysis has contributed significantly to our understanding of the actual role of complement, in particular C5, in the apparently multifactorial process of human allograft rejection. A major finding was that allograft survival over 3 years was not different between eculizumab-treated patients and a historical control group of recipients subjected to plasmapheresis and IVIG. Remarkably, despite prolonged eculizumab treatment, the most frequent histologic abnormality prior to graft loss (six cases) was transplant glomerulopathy. While none of the patients who lost their transplant had clinical ABMR, all of them were positive for HLA class II DSA and showed peritubular capillaritis and advanced transplant glomerulopathy in prior biopsies. Maybe the most important observation was that in case of persistently high antibody levels, eculizumab failed to prevent the development of subclinical inflammation and chronic injury in the microcirculation (transplant glomerulopathy), despite prolonged application and effective prevention of early clinical rejection. Indeed, transplant glomerulopathy was found in as many as 50% of the patients with high B-cell FCXM channel shifts and anti-HLA class II DSA. These data were somewhat discouraging, especially given the high costs associated with long-term eculizumab treatment. At the same time, however, it became also evident that outcomes were favourable if post-transplant antibody levels were low. Interpreting these data, one may argue towards a dominant role of complement-independent mechanisms of antibody-mediated injury, which may culminate in the development of chronic injury. Of course, one has also to keep in mind that blocking terminal complex formation does not prevent earlier steps of complement activation, such as the release of C3a. Finally, it cannot be excluded that despite continuous eculizumab exposure, low levels of C5 activation have contributed to injury. However, using the read-out of a haemolytic assay based on chicken erythrocytes, complement was found effectively blocked in most of the studied patients [32]. Of course, it remains possible that this blood assay may not necessarily reflect the situation in the microenvironment of the transplanted tissue.

Treatment of refractory ABMR

For the treatment of refractory ABMR, which in some cases associated with thrombotic microangiopathy and/or a genetic background of altered complement regulation, a variety of anecdotal reports have been published in recent years [54–63]. As detailed in Table 1, eculizumab was given either alone, or as a bridging to initiation of B-cell depletion, proteasome inhibition, or in

Transplant International 2016; 29: 392–402

combination with other therapeutic measures such as plasmapheresis and/or IVIG. In many published anecdotal reports, eculizumab was shown to effectively reverse rejection (Table 1). This preliminary clinical experience with terminal complement blockade as an antirejection treatment also extends to other types of organ transplants, and there are several promising case reports of its use in severe rejection in lung [64], intestinal [65] and even full-face transplantation [66].

However, also failure of eculizumab as a salvage therapy has been reported, as, for example, in a report of two cases of C4d-negative ABMR, where complement-independent mechanisms of antibody-triggered injury may have been dominant [62]. Moreover, a recent study has revealed that eculizumab was of limited efficacy in severe oliguric cases of early ABMR [67]. In combination with splenectomy, however, terminal complement blockade was effective in reversing rejection and prevented irreversible microvascular damage in the long term. In this study of 267 sensitized recipients subjected to desensitization with PP, IVIG and IL-2 receptor antibody or ATG induction, 24 were reported to experience strong DSA rebound and early severe rejection after a median of 6 days. These patients were, in addition to plasmapheresis, either treated with splenectomy or eculizumab alone or a combination of both. While a considerable number of patients subjected to splenectomy or eculizumab as the sole treatment lost their allografts and/or developed chronic injury, all five recipients subjected to combined therapy had a favourable outcome and only one of them showed mild transplant glomerulopathy after 1 year. For the combined group, a 100% 1-year death-censored graft survival was reported, as compared to 78% (splenectomy only) and only 30% (eculizumab only) in the other groups [67]. Of course, as suggested by a high rate of noninfectious and infectious complications in this study, such intense treatment including heavy immunosuppression and major reoperation can be expected to take its toll and should thus not be intensely promoted before well-substantiated prospective data are available.

Outlook

The Mayo Clinic study has added significantly to our current understanding of the pathophysiology of ABMR and provided a first impression of the effects of complement blockage in this specific context [32,43,44]. However, interpreting study results, it has to be pointed out that this trial was primarily designed to evaluate clinical acute rejection as an early endpoint, and its nonrandomized methodology and a small sample size may preclude definitive conclusions. Nevertheless, reported data have highlighted a high potential of complement blocking strategies in transplant rejection and may provide a solid basis for future studies. Several trials planned or ongoing have now been registered in the public accessible database of the US National Institutes of Health. Five studies designed to investigate the use of eculizumab in organ transplantation, one of them terminated because of a low rate of patient recruitment, are described in Table 2. Recently, preliminary results of a randomized, open-label, multicentre phase 2 study to determine the safety and efficacy of eculizumab in the prevention of ABMR (ClinicalTrials.gov Identifier: NCT01399593; study initiation in 2011, estimated completion date: April 2016) were reported by Alexion Pharmaceuticals, Inc. (http://alexionpharma.com/). This trial included 102 sensitized live donor kidney transplant recipients at risk of ABMR. Patients were randomized to eculizumab or standard-of-care treatment (51 recipients in each group). Preliminary results were disappointing, as there were no significant differences in the primary composite endpoint evaluated at week 9 (biopsy-proven ABMR, graft loss, patient death, or loss to follow-up: 9.8% vs. 15.7%, $P = 0.554$). A remarkable finding was the considerably low rate of this endpoint also in the control arm, which may have perhaps been a result of less permissive criteria for DSA acceptance.

Interference with classical complement at the level of C1

A potential caveat of terminal complement blockade may be that despite efficient blockade of C5, critical complement activation steps preceding MAC formation, such as the delivery of early pro-inflammatory chemoattractant complement split products (e.g., C3a), could maintain microcirculation inflammation and injury. A promising approach, which may have the potential to handle refractory early ABMR, but could also counteract the transition to chronic ABMR, is the inhibition of classical complement at the level of C1.

Strategies targeting this key component are just moving to human trials. In a recently published small randomized controlled trial (phase I/II), Vo *et al.* [68] investigated the impact of C1 inhibitor (C1-INH) in highly sensitized kidney transplant recipients subjected to desensitization with IVIG plus rituximab with or without plasma exchange. In this placebo-controlled pilot trial (20 included patients), no differences with respect to adverse events were reported. However, there were also no differences between groups regarding

Table 1. Eculizumab for the treatment of ABMR – case reports.

Authors year (References)	No. of patients (Barrier)	Organ (Donor type)	Desensitization	ABMR phenotype	Eculizumab infusions	Additional treatment	Rejection reversal
Locke *et al.* 2009 [54]	1 (FCXM+, DSA+)	Kidney (LD)	PP, IVIG	C4d+, TMA	1×	PP, IVIG, Ritux	Yes*
Lonze *et al.* 2010 [55]	1 (FCXM+, DSA+)	Kidney (LD)	IVIG, rituximab, bortezomib, PP	C4d+	8×	–	Yes
Biglarnia *et al.* 2011 [56]	1 (ABOi)	Kidney/pancreas (DD)	IA, rituximab	C4d+	2×	–	Yes
Stewart *et al.* 2012 [57]	1 (ABOi)	Kidney (LD)	PP, IVIG, rituximab		7×	Splenectomy, PP/IVIG	Yes
González-Roncero *et al.* 2012 [58]	2 (none)	Kidney (LD, DD)	None	Case 1: C4d+ Case 2: C4d+, TMA	1×	PP, IVIG, ritux	Yes
Noone 2012 [59]	1 (DSA+)	Kidney (DD)	IVIG	C4d+, TMA (CFHR3/1 deletion)	2×	PP, IVIG, rituximab	Yes
Kocak *et al.* 2013 [60]	2 (DSA+)	Kidney (LD)	PP, IVIG	Case 1: C4d+, TMA Case 2: C4d+	Case 1: 5× Case 2: 4×	IVIG,PP, rituximab (Case 1: Alemtuzumab)	Yes
Ghirardo *et al.* 2014 [61]	1 (DSA+)	Kidney (DD)	PP, IVIG, rituximab	C4d+	15×	PP, IVIG	Yes
Burbach *et al.* 2014 [62]	2 Case 1: aHUS Case 2: DSA+	Kidney (DD)	Case 1: PP/IVIG Case 2: Bortezomib, IVIG, rituximab, IA	Case 1: C4d– Case 2: C4d–	Case 1: 2a† Case2: 7 months	Case 1: PP, IVIG, rituximab	No
Chehade *et al.* 2015, [63]	1 (DSA+)	Kidney, paediatric (DD)	IVIG	C4d+	2×	PP, ATG, IVIG	Yes

ABOi, ABO-blood group incompatible; ABMR, antibody-mediated rejection; ATG, antithymocyte globulin; DD, deceased donor; DSA, donor-specific antibody; FCXM, flow-cytometric crossmatch; IA, immunoadsorption; IVIG, intravenous immunoglobuline; LD, living donor; PP, plasmapheresis; aHUS, atypical haemolytic uraemic syndrome.

*The patient died from pulmonary haemorrhage

†Eculizumab was started months before the onset of ABMR because of aHUS recurrence and was administered continuously.

Table 2. Registered trials (ClinicalTrials.gov) designed to evaluate eculizumab in the prevention or treatment of ABMR.

Registry number	Centres	Number of patients	Organ	Eculizumab	Design	Inclusion	Comparator group	Primary endpoint
NCT01095887	Mayo Clinic	6*	Kidney	Peri-Tx	Uncontrolled, open label	ABOi Tx	–	ABMR within 3 months
NCT01327573	Yale University	20	Kidney	Post-Tx	Randomized, open-label	Late ABMR (>6 months)	Standard immunosuppression	eGFR over 6 months
NCT01399593	USA, Europe, Australia	102	Kidney	Peri-Tx	Randomized, open-label	Sensitized live donor Tx recipients	Standard of care	Composite endpoint after 9 weeks†
NCT01895127	Brigham and Women's Hospital	21	Kidney	Post-Tx	Randomized, open-label	Acute ABMR	Plasmapheresis/IVIG	eGFR over 3 months
NCT02013037	Cedars-Sinai Medical Center	20	Heart	Peri-Tx	Open-label, historical control group	≥70% PRA	–	ABMR & left ventricular dysfunction

ABOi Tx, ABO-blood group-incompatible transplantation; ABMR, antibody-mediated rejection; DSA, donor-specific antibody; eGFR, estimated glomerular filtration rate; IVIG, intravenous immunoglobulin; PRA, panel-reactive antibodies.

*The study was terminated after recruitment of six patients.

†This composite endpoint includes biopsy-proven ABMR, graft loss, patient death, or loss to follow-up.

ABMR occurrence (two versus three cases in the control arm). However, for this endpoint, the study was not adequately powered. An interesting finding was a somewhat lower rate of delayed graft function (one versus four cases in the control group), suggesting a beneficial effect on ischaemia–reperfusion injury [68].

Another promising innovation may be interference with C1s using an inhibitory anti-C1s antibody. In a recent *in vitro* study, a monoclonal anti-C1s antibody was demonstrated to effectively block HLA antibody-triggered complement activation [69]. Currently, a phase 1 trial evaluating the safety and activity of a humanized variant of this antibody (TNT009) in healthy volunteers is underway (ClinicalTrials.gov Identifier: NCT02502903).

Finally, a recent study demonstrated efficient C1 removal by modified extracorporeal treatment. In this randomized controlled cross-over trial, we evaluated the effect of a new apheresis concept by combining semiselective immunoadsorption with membrane filtration. We found a markedly enhanced elimination of C1q, on average by more than 80% upon a single apheresis session [70]. However, using this strategy, one has to consider the risk of altered plasma coagulation due to the concomitant removal of fibrinogen and other macromolecular coagulation factors [71]. An uncontrolled pilot study to evaluate this strategy in ABO-incompatible live donor kidney transplantation is currently recruiting patients (ClinicalTrials.gov Identifier: NCT02120482).

Future studies will clarify whether complement blockade upstream of C3 can efficiently counteract complement-dependent injury in the context of ABMR. However, one has to take into account that the pathogenesis of rejection is a complex process involving a variety of different pathogenetic mechanisms, which may necessitate targeted interference at various levels to efficiently treat or prevent irreversible injury to the graft. Accordingly, at the same time, other new strategies that could help control ongoing chronic rejection processes, such as proteasome inhibition to interfere with components of specific immunity including antibody-secreting cells [72], will have to be tested in systematic prospective trials.

Conclusion

Therapeutics targeting key steps of complement activation show great promise for use in ABMR prevention and treatment. Preliminary results have highlighted an efficacy of terminal complement blockade in preventing and reversing acute clinical ABMR. Nevertheless, the

results of a recent study suggest that prevention of chronic rejection processes may be incomplete, possibly a result of an activation of initial steps in the cascade or even complement-independent effects of alloantibodies. Considering a dominant role of chronic rejection as a cause of graft failure in the long term, there is urgent need for new strategies to better control chronic injury, which, in addition to a more selective interference with B-cell immunity, may include innovative therapies targeting proximal complement activation steps.

Funding

The authors declare no funding.

REFERENCES

1. Sellares J, de Freitas DG, Mengel M, *et al.* Understanding the causes of kidney transplant failure: the dominant role of antibody-mediated rejection and nonadherence. *Am J Transplant* 2012; **12**: 388.
2. Djamali A, Kaufman DB, Ellis TM, Zhong W, Matas A, Samaniego M. Diagnosis and management of antibody-mediated rejection: current status and novel approaches. *Am J Transplant* 2014; **14**: 255.
3. Halloran PF, Chang J, Famulski K, *et al.* Disappearance of T cell-mediated rejection despite continued antibody-mediated rejection in late kidney transplant recipients. *J Am Soc Nephrol* 2014; **26**: 1711.
4. Bartel G, Schwaiger E, Böhmig GA. Prevention and treatment of alloantibody-mediated kidney transplant rejection. *Transpl Int* 2011; **24**: 1142.
5. Fehr T, Gaspert A. Antibody-mediated kidney allograft rejection: therapeutic options and their experimental rationale. *Transpl Int* 2012; **25**: 623.
6. Racusen LC, Colvin RB, Solez K, *et al.* Antibody-mediated rejection criteria – an addition to the Banff 97 classification of renal allograft rejection. *Am J Transplant* 2003; **3**: 708.
7. Haas M, Sis B, Racusen LC, *et al.* Banff 2013 meeting report: inclusion of c4d-negative antibody-mediated rejection and antibody-associated arterial lesions. *Am J Transplant* 2014; **14**: 272.
8. Walsh RC, Alloway RR, Girnita AL, Woodle ES. Proteasome inhibitor-based therapy for antibody-mediated rejection. *Kidney Int* 2012; **81**: 1067.
9. Roberts DM, Jiang SH, Chadban SJ. The treatment of acute antibody-mediated rejection in kidney transplant recipients-a systematic review. *Transplantation* 2012; **94**: 775.
10. Vo AA, Lukovsky M, Toyoda M, *et al.* Rituximab and intravenous immune globulin for desensitization during renal transplantation. *N Engl J Med* 2008; **359**: 242.
11. Bartel G, Wahrmann M, Regele H, *et al.* Peritransplant immunoadsorption for positive crossmatch deceased donor kidney transplantation. *Am J Transplant* 2010; **10**: 2033.
12. Montgomery RA, Lonze BE, King KE, *et al.* Desensitization in HLA-incompatible kidney recipients and survival. *N Engl J Med* 2011; **365**: 318.
13. Morath C, Beimler J, Opelz G, *et al.* Living donor kidney transplantation in crossmatch-positive patients enabled by peritransplant immunoadsorption and anti-CD20 therapy. *Transpl Int* 2012; **25**: 506.
14. Woodle ES, Shields AR, Ejaz NS, *et al.* Prospective iterative trial of proteasome inhibitor-based desensitization. *Am J Transplant* 2015; **15**: 101.
15. Bentall A, Cornell LD, Gloor JM, *et al.* Five-year outcomes in living donor kidney transplants with a positive crossmatch. *Am J Transplant* 2013; **13**: 76.
16. Bentall A, Herrera LP, Cornell LD, *et al.* Differences in chronic intragraft inflammation between positive crossmatch and ABO-incompatible kidney transplantation. *Transplantation* 2014; **98**: 1089.
17. Stegall MD, Chedid MF, Cornell LD. The role of complement in antibody-mediated rejection in kidney transplantation. *Nat Rev Nephrol* 2012; **8**: 670.
18. Farrar CA, Sacks SH. Mechanisms of rejection: role of complement. *Curr Opin Organ Transplant* 2014; **19**: 8.
19. Parker C. Eculizumab for paroxysmal nocturnal haemoglobinuria. *Lancet* 2009; **373**: 759.
20. Zuber J, Fakhouri F, Roumenina LT, Loirat C, Fremeaux-Bacchi V. Use of eculizumab for atypical haemolytic uraemic syndrome and C3 glomerulopathies. *Nat Rev Nephrol* 2012; **8**: 643.
21. Kolev M, Le Friec G, Kemper C. Complement–tapping into new sites and effector systems. *Nat Rev Immunol* 2014; **14**: 811.
22. Patel R, Terasaki PI. Significance of the positive crossmatch test in kidney transplantation. *N Engl J Med* 1969; **280**: 735.
23. Feucht HE, Schneeberger H, Hillebrand G, *et al.* Capillary deposition of C4d complement fragment and early renal graft loss. *Kidney Int* 1993; **43**: 1333.
24. Böhmig GA, Exner M, Habicht A, *et al.* Capillary C4d deposition in kidney allografts: a specific marker of alloantibody-dependent graft injury. *J Am Soc Nephrol* 2002; **13**: 1091.
25. Mauiyyedi S, Colvin RB. Humoral rejection in kidney transplantation: new concepts in diagnosis and treatment. *Curr Opin Nephrol Hypertens* 2002; **11**: 609.
26. Regele H, Böhmig GA, Habicht A, *et al.* Capillary deposition of complement split product C4d in renal allografts is associated with basement membrane injury in peritubular and glomerular capillaries: a contribution of humoral immunity to chronic allograft rejection. *J Am Soc Nephrol* 2002; **13**: 2371.
27. Sapir-Pichhadze R, Curra SP, John R, *et al.* A systematic review of the role of C4d in the diagnosis of acute antibody-mediated rejection. *Kidney Int* 2014; **87**: 182.
28. Kikic Z, Kainz A, Kozakowski N, *et al.* Capillary C4d and kidney allograft outcome in relation to morphologic lesions suggestive of antibody-mediated rejection. *Clin J Am Soc Nephrol* 2015; **10**: 1435.
29. Loupy A, Lefaucheur C, Vernerey D, *et al.* Complement-binding anti-HLA antibodies and kidney-allograft survival. *N Engl J Med* 2013; **369**: 1215.
30. Sicard A, Ducreux S, Rabeyrin M, *et al.* Detection of C3d-binding donor-specific anti-HLA antibodies at diagnosis of humoral rejection predicts renal graft loss. *J Am Soc Nephrol* 2014; **26**: 457.
31. Wang H, Arp J, Liu W, *et al.* Inhibition of terminal complement components in presensitized transplant recipients prevents antibody-mediated rejection leading to long-term graft survival and accommodation. *J Immunol* 2007; **179**: 4451.

32. Cornell LD, Schinstock CA, Gandhi MJ, Kremers WK, Stegall MD. Positive crossmatch kidney transplant recipients treated with eculizumab: outcomes beyond 1 year. *Am J Transplant* 2015; **15**: 1293.
33. Loupy A, Hill GS, Suberbielle C, *et al.* Significance of C4d Banff scores in early protocol biopsies of kidney transplant recipients with preformed donor-specific antibodies (DSA). *Am J Transplant* 2011; **11**: 56.
34. Haas M. Pathology of C4d-negative antibody-mediated rejection in renal allografts. *Curr Opin Organ Transplant* 2013; **18**: 319.
35. Csencsits K, Burrell BE, Lu G, Eichwald EJ, Stahl GL, Bishop DK. The classical complement pathway in transplantation: unanticipated protective effects of C1q and role in inductive antibody therapy. *Am J Transplant* 2008; **8**: 1622.
36. Hirohashi T, Uehara S, Chase CM, *et al.* Complement independent antibody-mediated endarteritis and transplant arteriopathy in mice. *Am J Transplant* 2010; **10**: 510.
37. Hirohashi T, Chase CM, Della Pelle P, *et al.* A novel pathway of chronic allograft rejection mediated by NK cells and alloantibody. *Am J Transplant* 2012; **12**: 313.
38. Haas M, Rahman MH, Racusen LC, *et al.* C4d and C3d staining in biopsies of ABO- and HLA-incompatible renal allografts: correlation with histologic findings. *Am J Transplant* 2006; **6**: 1829.
39. Haas M, Segev DL, Racusen LC, *et al.* C4d deposition without rejection correlates with reduced early scarring in ABO-incompatible renal allografts. *J Am Soc Nephrol* 2009; **20**: 197.
40. Rother RP, Rollins SA, Mojcik CF, Brodsky RA, Bell L. Discovery and development of the complement inhibitor eculizumab for the treatment of paroxysmal nocturnal hemoglobinuria. *Nat Biotechnol* 2007; **25**: 1256.
41. Peffault de Latour R, Fremeaux-Bacchi V, Porcher R, *et al.* Assessing complement blockade in patients with paroxysmal nocturnal hemoglobinuria receiving eculizumab. *Blood* 2015; **125**: 775.
42. Nishimura J, Yamamoto M, Hayashi S, *et al.* Genetic variants in C5 and poor response to eculizumab. *N Engl J Med* 2014; **370**: 632.
43. Stegall MD, Diwan T, Raghavaiah S, *et al.* Terminal complement inhibition decreases antibody-mediated rejection in sensitized renal transplant recipients. *Am J Transplant* 2011; **11**: 2405.
44. Bentall A, Tyan DB, Sequeira F, *et al.* Antibody-mediated rejection despite inhibition of terminal complement. *Transpl Int* 2014; **27**: 1235.
45. Ram S, Lewis LA, Rice PA. Infections of people with complement deficiencies and patients who have undergone splenectomy. *Clin Microbiol Rev* 2010; **23**: 740.
46. Ali A, Jafri RZ, Messonnier N, *et al.* Global practices of meningococcal vaccine use and impact on invasive disease. *Pathog Glob Health* 2014; **108**: 11.
47. Kelly RJ, Hill A, Arnold LM, *et al.* Long-term treatment with eculizumab in paroxysmal nocturnal hemoglobinuria: sustained efficacy and improved survival. *Blood* 2011; **117**: 6786.
48. Legendre CM, Licht C, Muus P, *et al.* Terminal complement inhibitor eculizumab in atypical hemolytic-uremic syndrome. *N Engl J Med* 2013; **368**: 2169.
49. Struijk GH, Bouts AH, Rijkers GT, Kuin EA, ten Berge IJ, Bemelman FJ. Meningococcal sepsis complicating eculizumab treatment despite prior vaccination. *Am J Transplant* 2013; **13**: 819.
50. Hillmen P, Hall C, Marsh JC, *et al.* Effect of eculizumab on hemolysis and transfusion requirements in patients with paroxysmal nocturnal hemoglobinuria. *N Engl J Med* 2004; **350**: 552.
51. Brodsky RA, Young NS, Antonioli E, *et al.* Multicenter phase 3 study of the complement inhibitor eculizumab for the treatment of patients with paroxysmal nocturnal hemoglobinuria. *Blood* 2008; **111**: 1840.
52. Noris M, Remuzzi G. Atypical hemolytic-uremic syndrome. *N Engl J Med* 2009; **361**: 1676.
53. Zuber J, Le Quintrec M, Krid S, *et al.* Eculizumab for atypical hemolytic uremic syndrome recurrence in renal transplantation. *Am J Transplant* 2012; **12**: 3337.
54. Locke JE, Magro CM, Singer AL, *et al.* The use of antibody to complement protein C5 for salvage treatment of severe antibody-mediated rejection. *Am J Transplant* 2009; **9**: 231.
55. Lonze BE, Dagher NN, Simpkins CE, *et al.* Eculizumab, bortezomib and kidney paired donation facilitate transplantation of a highly sensitized patient without vascular access. *Am J Transplant* 2010; **10**: 2154.
56. Biglarnia AR, Nilsson B, Nilsson T, *et al.* Prompt reversal of a severe complement activation by eculizumab in a patient undergoing intentional ABO-incompatible pancreas and kidney transplantation. *Transpl Int* 2011; **24**: e61.
57. Stewart ZA, Collins TE, Schlueter AJ, *et al.* Case report: Eculizumab rescue of severe accelerated antibody-mediated rejection after ABO-incompatible kidney transplant. *Transplant Proc* 2012; **44**: 3033.
58. Gonzalez-Roncero F, Suner M, Bernal G, *et al.* Eculizumab treatment of acute antibody-mediated rejection in renal transplantation: case reports. *Transplant Proc* 2012; **44**: 2690.
59. Noone D, Al-Matrafi J, Tinckam K, *et al.* Antibody mediated rejection associated with complement factor h-related protein 3/1 deficiency successfully treated with eculizumab. *Am J Transplant* 2012; **12**: 2546.
60. Kocak B, Arpali E, Demiralp E, *et al.* Eculizumab for salvage treatment of refractory antibody-mediated rejection in kidney transplant patients: case reports. *Transplant Proc* 2013; **45**: 1022.
61. Ghirardo G, Benetti E, Poli F, *et al.* Plasmapheresis-resistant acute humoral rejection successfully treated with anti-C5 antibody. *Pediatr Transplant* 2014; **18**: E1.
62. Burbach M, Suberbielle C, Brocheriou I, *et al.* Report of the inefficacy of eculizumab in two cases of severe antibody-mediated rejection of renal grafts. *Transplantation* 2014; **98**: 1056.
63. Chehade H, Rotman S, Matter M, Girardin E, Aubert V, Pascual M. Eculizumab to treat antibody-mediated rejection in a 7-year-old kidney transplant recipient. *Pediatrics* 2015; **135**: e551.
64. Dawson KL, Parulekar A, Seethamraju H. Treatment of hyperacute antibody-mediated lung allograft rejection with eculizumab. *J Heart Lung Transplant* 2012; **31**: 1325.
65. Fan J, Tryphonopoulos P, Tekin A, *et al.* Eculizumab Salvage Therapy for Antibody-Mediated Rejection in a Desensitization-Resistant Intestinal Re-Transplant Patient. *Am J Transplant* 2015; **15**: 1995.
66. Chandraker A, Arscott R, Murphy GF, *et al.* The management of antibody-mediated rejection in the first presensitized recipient of a full-face allotransplant. *Am J Transplant* 2014; **14**: 1446.
67. Orandi BJ, Zachary AA, Dagher NN, *et al.* Eculizumab and splenectomy as salvage therapy for severe antibody-mediated rejection after HLA-incompatible kidney transplantation. *Transplantation* 2014; **98**: 857.
68. Vo AA, Zeevi A, Choi J, *et al.* A phase I/II placebo-controlled trial of C1-inhibitor for prevention of antibody-mediated rejection in HLA sensitized patients. *Transplantation* 2015; **99**: 299.

69. Thomas KA, Valenzuela NM, Gjertson D, *et al.* An Anti-C1s Monoclonal, TNT003, Inhibits Complement Activation Induced by Antibodies Against HLA. *Am J Transplant* 2015; **15**: 2037.
70. Eskandary F, Wahrmann M, Biesenbach P, *et al.* ABO antibody and complement depletion by immunoadsorption combined with membrane filtration–a randomized, controlled, cross-over trial. *Nephrol Dial Transplant* 2014; **29**: 706.
71. Biesenbach P, Eskandary F, Ay C, *et al.* Effect of combined treatment with immunoadsorption and membrane filtration on plasma coagulation-Results of a randomized controlled crossover study. *J Clin Apher* 2015; doi: 10.1002/jca.21399. [Epub ahead of print]
72. Eskandary F, Bond G, Schwaiger E, *et al.* Bortezomib in late antibody-mediated kidney transplant rejection (BORTEJECT Study): study protocol for a randomized controlled trial. *Trials* 2014; **15**: 107.