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REVIEW

Prevention of antibody-mediated kidney transplant rejection

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Summary

There is increasing evidence that antibody-mediated rejection is the major cause of late kidney graft failure. Prevention of antibody-mediated allograft damage has therefore become an important issue in kidney transplantation. Such prevention starts already before transplantation with the avoidance of sensitizing events. When a patient is already sensitized, precise characterization of alloantibodies and exact HLA typing of the donor at the time of transplantation are mandatory. To ensure timely and successful transplantation of highly sensitized patients, desensitization, and inclusion in special programs such as the Eurotransplant Acceptable Mismatch Program should be considered. After transplantation, close monitoring of kidney function, testing for the *de novo* development or changing characteristics of alloantibodies, and attention to non-adherence to immunosuppression is obligatory. In the current overview, we discuss the currently available measures for the prevention of antibody-mediated kidney graft rejection.

Sensitization as a major obstacle in kidney transplantation

Although overall graft survival rates have improved in recent years, sensitization of the recipient at the B-cell level remains a major obstacle. In the US, about 40% of kidney transplant candidates are sensitized or highly sensitized [1]. Although the fraction of sensitized patients appears to be lower in the Eurotransplant area, still 12% of patients on the kidney waiting list are sensitized, e.g., they possess a panel-reactivity (PRA) of >5% and <85%, and 2% have a PRA of ≥85% and thus are considered highly sensitized [2]. Sensitized kidney transplant candidates have two major disadvantages: (i) They are less likely to receive a transplant because the antibodies often cause a positive pretransplant crossmatch result against a potential kidney donor. These patients therefore may experience prolonged waiting times and consequently an increased rate of death on the waiting list [3]. (ii) Even if the pretransplant crossmatch result is negative and a sensitized patient eventually is transplanted, there is an increased risk for antibody-mediated allograft injury and graft loss [4,5]. This is attributed to the presence of donor-specific HLA antibodies (DSA) that are not detected in the current crossmatch procedure. It is also discussed that the presence of high PRA may be an indicator for generally increased alloreactivity of the recipient.

Overlooked pre-existing DSA can cause damage in the graft directly after transplantation via antibody-mediated allograft injury. Patients may also experience an early post-transplant rebound of pre-existing DSA that may have decreased by the time of transplantation, or they

may develop *de novo* DSA against the graft later post-transplant. Persistent DSA that were present already pretransplant and especially *de novo* DSA are currently discussed as main contributors to chronic allograft injury and late graft failure [6,7].

Prevention of antibody-mediated allograft injury by avoidance of sensitization

Avoidance of pretransplant sensitization is able to reduce the rate of antibody-mediated allograft injury after transplantation (Fig. 1). An Irish study showed that with the introduction of recombinant human erythropoietin, the rate of blood transfusions and the level of sensitization in hemodialysis patients decreased significantly [8]. Two patient cohorts were compared, one cohort awaiting transplantation before the introduction of erythropoietin (n = 205; waiting list of 1989) and a second cohort awaiting transplantation after the introduction of erythropoietin (n = 126; waiting list of 1994). A 34% reduction of blood transfusions was noted in hemodialysis patients during the study period. Sensitization as a consequence of blood transfusions decreased from 63% in the earlier cohort to 28% in the latter group.

Meier-Kriesche *et al.* reported that patients experience a negative impact from poor HLA matching of a first kidney transplant that failed when they need a second graft [9]. They examined 15 980 kidney transplant candidates from the Scientific Renal Transplant Registry in the US who were relisted after loss of a primary kidney allograft. With increasing number of HLA mismatches of the first transplant a steady increase in PRA was seen at relisting. Although the adjusted mean change in PRA level from initial transplant to relisting was only 0.8% after the failure of a 0 HLA-ABDR mismatched transplant, the rate was 22% after failure of a first transplant with six HLA mismatches.

Especially in young patients who may require retransplantation during their lifetime, sensitizing events, such as the administration of blood transfusions or poor HLA matching, should therefore be avoided. To further reduce sensitizing events, it is planned within the Eurotransplant area to replace conventional matching for the HLA-A and -B loci by more precise matching for HLA epitopes that are responsible for antibody induction (Fig. 1).

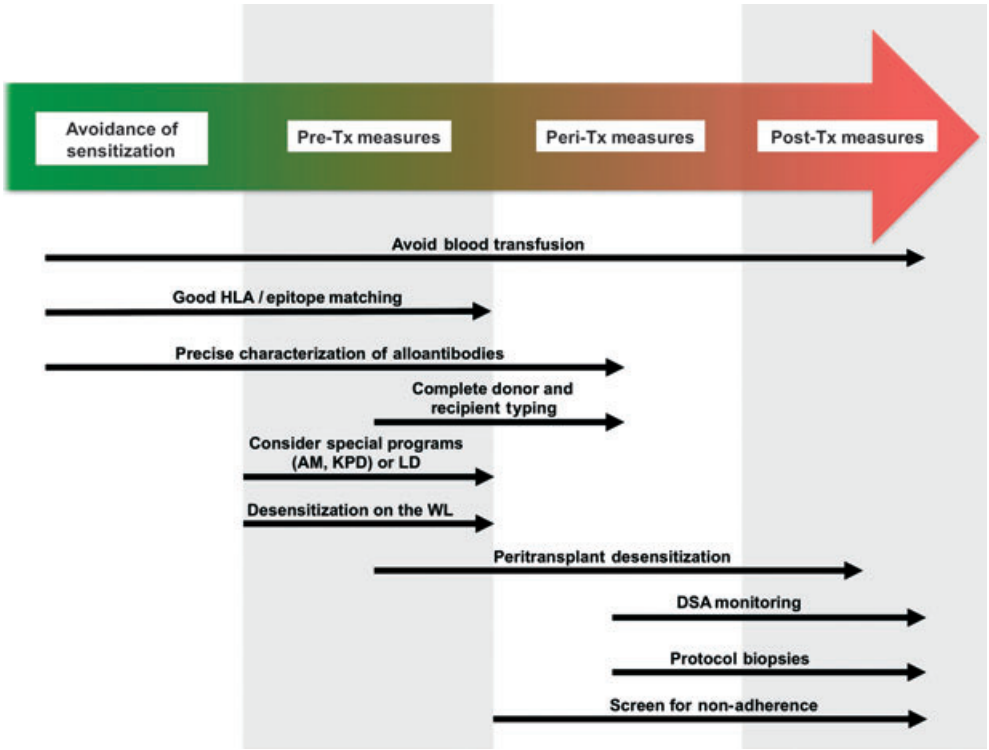

Figure 1 Measures for prevention of antibody-mediated allograft injury. Measures for prevention of antibody-mediated allograft injury include prevention of sensitization, exact knowledge on the patient's HLA alloantibody status and donor HLA antigens at the time of grafting, inclusion of patients into special programs, such as Eurotransplant Acceptable Mismatch (AM) Program, kidney-paired donation (KPD) or living donation (LD), desensitization for timely and successful transplantation (Tx), and post-transplant monitoring especially for the recognition of *de novo* donor-specific antibodies (DSA) and subclinical rejection. WL, waiting list.

Precise characterization of the patient's alloantibodies before transplantation for prevention of early antibody-mediated allograft injury

Even in the absence of a positive crossmatch, pretransplant donor-directed HLA alloantibodies are believed to have a negative impact on kidney graft survival. However, it is a matter of debate whether or not relatively weak pretransplant HLA alloantibodies that are exclusively detected by single-antigen flow beads (SAFB) are associated with an increased risk of graft loss. It is well recognized that false positive or negative antibody results may occur with current detection technology. Even in patients without sensitizing events, seemingly HLA-specific auto- and alloantibodies that react with denatured HLA molecules on SAFB have been described [10]. Such reactions have been shown to complicate the clinical interpretation of positive SAFB results. Varying antigen density on the beads, prozone effects in sera with high antibody reactivity, and differences in kits obtained from the two different vendors are additional technical difficulties that are associated with the quantification of antibodies detected with the SAFB technique.

Van den Berg-Loonen found that there was no significant difference in graft survival when highly sensitized patients from the Eurotransplant Acceptable Mismatch (AM) Program were compared based on whether they had additional SAFB-detected DSA or not [11]. Other groups reported that pretransplant SAFB-detected DSA were associated with higher rates of antibody-mediated allograft rejection or graft loss, however, precise evaluation of the predictive value of DSA exclusively detected by sensitive SAFB testing as compared with DSA that were found with less sensitive techniques, such as complement-dependent lymphocytotoxicity (CDC) or ELISA, was usually not performed [12]. In a detailed comparative study, Lefaucheur *et al.* analyzed the relationship between pretransplant HLA alloantibodies detected by SAFB and acute antibody-mediated rejection after transplantation [13]. Peak pretransplant SAFB-detected DSA had not only the highest sensitivity but also the lowest specificity for acute antibody-mediated allograft rejection as compared to other tests such as CDC or ELISA. Of note, peak pretransplant SAFB-detected DSA were analyzed in this study only in sera selected for the highest CDC-PRA reactivity, which might have biased the results. In an analysis of CDC- as well as ELISA-negative sera from the prospective Collaborative Transplant Study Serum Project, a total of 118 patients who lost their graft during the first 3 years after transplantation showed no higher incidence of SAFB-detected DSA in their pretransplant sera than 118 matched control patients with functioning grafts, indicating that DSA detected exclusively by SAFB may not necessarily be clinically relevant [14]. It follows that, in the case of deceased-donor kidney transplantation, the definition of unacceptable HLA antigens before transplantation based on the specificity of antibodies against HLA detected by SAFB may put the kidney transplant candidate at an undue disadvantage. Lefaucheur *et al.* recently published an algorithm for the transplantation of sensitized kidney recipients who possess HLA alloantibodies [15]. In this algorithm, the definition of unacceptable HLA antigens was based on results of pretransplant SAFB measurements (cutoff 1000 MFI). With this approach, more than 50% of highly sensitized patients (defined by a peak calculated PRA of >85%) were calculated to have a low frequency of potentially matched donors ascribable to an unfavorable HLA antigen/HLA alloantibody constellation, and these patients were proposed for additional measures such as desensitization. In contrast, ELISA-based risk estimation was found to be relevant in two independent large series and is therefore used by us as an indicator of increased risk of rejection for patients on the kidney waiting list in a donor-independent manner [5,16].

In summary, we believe that CDC and ELISA methods should be considered for the purpose of organ allocation, whereas SAFB testing may guide peri- as well as post-transplant desensitization treatment but not the definition of unacceptable antigens.

Measures for successful transplantation of sensitized patients

Methods are available for the timely transplantation of sensitized patients that are associated with low rates of antibody-mediated rejection and good allograft outcome. In the Eurotransplant area, highly sensitized kidney transplant candidates may be included in the AM Program. More recently, comparable programs were introduced by other organ procurement organizations [17,18]. In the case of living-donor kidney transplantation, alternative solutions such as kidney-paired donation programs exist [19]. With these approaches, one tries to avoid the hurdle of recipient sensitization by finding a suitable donor against which the recipient possesses no alloantibodies. Another approach is to overcome this hurdle by desensitization. In the case of deceased-donor kidney transplantation, the patient may be desensitized either immediately before transplantation, at the time of an organ offer, or already in advance while he is on the waiting list to increase the chance of a crossmatch-negative organ. In the case of living-donor kidney transplantation, the patient may be desensitized before transplantation until all crossmatches and antibody screenings become negative. Table 1 details the approaches to recipient desensitization by different groups with the respective outcomes.

Table 1. Selected studies on crossmatch-/DSA-positive kidney transplantation [modified after Süsal and Morath (65)].

Reference	No. of patients	Transplant period	XM/DSA positivity	Desensitization method	Donor type	Follow-up (months)	Graft survival (%)	AMR rate (%)
Higgins *et al.* (34)	13	–	CDC; FCXM	IA	DDK	26 (median)	53.8	–
Glotz *et al.* (25)	13	–	PRA 64% CDC (*n* = 2)	High-dose IVIg	DDK (*n* = 11) LDK (*n* = 2)	12	84.6	–
Jordan *et al.* (66)	45*	–	CDC	High-dose IVIg	LDK (*n* = 28) DDK (*n* = 15)	24	89.1	–
Stegall *et al.* (24)	61	2000–2005	CDC	(1) PPh + low-dose IVIg + anti-CD20 (2) High-dose IVIg	LDK	12	82	(1) 29–37 (2) 80
Higgins *et al.* (67)	24	2003–2006	CDC (*n* = 8) FCXM (*n* = 9) DSA (*n* = 7)	PPh	LDK (*n* = 21) DDK (*n* = 3)	3	87.5	41.7
Vo *et al.* (68)	16	2005–2007	PRA 77%	High-dose IVIg + anti-CD20	LDK (*n* = 10) DDK (*n* = 6)	12	94	31
Thielke *et al.* (69)	51	2001–2007	FCXM	PPh + low-dose IVIg	LDK	12	93	24
Haririan *et al.* (70)	41	1999–2006	FCXM	PPh + low-dose IVIg	LDK	12 60	89.9 69.4	12 (up to day 10 only)
Bartel *et al.* (27)	68	1999–2008	PRA >40% CDC (*n* = 21) DSA (*n* = 30)	IA	DDK	60	63	24–30
Morath *et al.* (64)	34	2006–2009	CDC and/or ELISA (*n* = 17) DSA (*n* = 10)	IA + anti-CD20 (LDK) PPh + anti-CD20 (DDK)	LDK (*n* = 6) DDK (*n* = 28)	12	100 (LDK) 92.4 (DDK)	9
Stegall *et al.* (51)	26	2008–2010	FCXM	(PPh) + anti-C5 antibody	LDK	12 (mean)	100	8 (at 3 months)
Montgomery *et al.* (32)	211	1998–2009	CDC (*n* = 74) FCXM (*n* = 95) Multiplex bead (*n* = 42)	PPh + low-dose IVIg	LDK	12	n.a. (patient survival 90.6)	n.a.

AMR, antibody-mediated rejection; CDC, complement-dependent cytotoxicity crossmatch; DDK, deceased-donor kidney; DSA, donor-specific antibody; ELISA, enzyme-linked immunosorbent assay crossmatch; FCXM, flow-cytometry crossmatch; IA, immunoadsorption; IVIg, intravenous immunoglobulins; LDK, living-donor kidney; Multiplex bead, positive bead crossmatch; n.a., not applicable; PPh, plasmapheresis; PRA, panel-reactive antibody; XM, crossmatch.
*Including two heart transplants.

In some highly sensitized patients with a broad range of HLA alloantibodies, timely and successful transplantation can only be achieved by a combination of all available options, including special programs, such as the Eurotransplant AM Program and recipient desensitization [20].

Desensitization for prevention of antibody-mediated allograft injury

Alloantibodies may be removed from the patient's circulation by either plasmapheresis or immunoadsorption, and alloantibody responses may be suppressed by treatment with immunoglobulins (IVIg) [21–27]. Newer therapeutic strategies combine these measures with the application of the anti-CD20 antibody rituximab or the proteasome inhibitor bortezomib, based on the rationale that depletion of B-lymphocytes or plasma cells may reduce the *de novo* production of DSA.

An alternative and more recently introduced approach is the blockage of the formation of the terminal complement complex by administration of the complement C5-inhibitor eculizumab. Eculizumab prevents severe antibody-mediated allograft injury by preventing complement-dependent lysis of graft cells that carry alloantibody on their surface.

IVIg-based desensitization

The IVIg-based desensitization of kidney transplant recipients was introduced by Glotz *et al.* and Jordan *et al.* already in the 1990s [28,29]. Proposed effects of IVIg include the neutralization of HLA antibodies by anti-idiotypic reactivity, inhibition of complement activation, release of anti-inflammatory cytokines, inhibition of B-cells by interaction with Fc-receptors, and inhibition of maturation and function of dendritic cells; however, the exact mechanism of action is still not understood [30].

The IVIg-based desensitization protocol consists of monthly administration of 2 g/kg body weight of IVIg (high-dose IVIg) until crossmatches become negative (living-donor kidney transplantation) or a decrease of PRA is achieved (deceased-donor kidney transplantation). The multicenter NIH IG02 trial investigated the effectiveness of 4-monthly infusions of high-dose IVIg as compared to placebo in deceased and living-donor kidney transplant recipients with a PRA of >50% [23]. As compared with placebo-treated patients, IVIg-treated patients had a moderate and transient reduction of PRA and a higher transplantation rate (35% vs. 17%). Acute rejection episodes were observed more often in the IVIg group, and graft survival after 2 years was not significantly different between the two groups.

Meanwhile, several studies confirmed that reduction of DSA by IVIg is mild and of short duration only, and most authors add plasmapheresis or immunoadsorption to recipient desensitization protocols when strong DSA are detectable in the patient's serum. DSA reduction by plasmapheresis or immunoadsorption is far more pronounced and more predictable than desensitization with IVIg.

Plasmapheresis-based desensitization

In contrast to IVIg-based desensitization protocols, plasmapheresis aims at the removal of DSA from the patient's circulation at the time of transplantation. The Johns Hopkins protocol for the transplantation of sensitized kidney graft recipients is based on a combination of plasmapheresis and low-dose IVIg (CMV-Ig; 100 mg/kg body weight), together with potent immunosuppression including either IL2-receptor antibody or thymoglobulin. Although this protocol has been used at Johns Hopkins for more than a decade, and more than 200 patients have been treated accordingly, up to now no detailed analysis on graft survival is available. In a review article, 1- and 3-year graft survival rates in 62 living-donor kidney recipients transplanted against a positive flow cytometric or anti-human globulin-enhanced CDC crossmatch were reported not to be different compared to rates in non-sensitized recipients of live donor kidneys [31]. More recently, a detailed analysis only on patient survival in sensitized living-donor kidney transplant recipients was published from the same group [32]. A total of 211 patients with either an initially positive CDC crossmatch with their donor ($n = 74$), or a negative CDC but positive flow cytometric crossmatch result ($n = 95$), or negative crossmatch results but DSA that were detectable by SAFB ($n = 42$) were analyzed. Although desensitized living-donor kidney transplant recipients showed impaired 1-year patient survival of about 90%, they had a clear survival benefit in the long-term: patient survival at 8 years was 80.6% compared to 30.5% for 1050 matched control patients in the 'dialysis-only' group and 49.1% for 1040 matched control patients in the 'dialysis-or-transplantation' group ($P < 0.001$ for both comparisons). The authors argued that desensitization for incompatible living-donor kidney transplantation should be preferred over waiting for a compatible deceased-donor organ without the need for desensitization. Although this assumption may be true for some of the patients, other patients may benefit from an inclusion into special programs for sensitized patients, such as the Eurotransplant AM program, or desensitization either on the waiting list (when an organ is available in due time) or desensitization immediately pretransplant. Therefore, although an important study, the results may not easily be extrapolated to

the Eurotransplant area where special programs for highly sensitized patients exist.

Immunoadsorption-based desensitization

First reports on the efficacy of immunoadsorption for the removal of HLA alloantibodies were published more than 20 years ago. Palmer *et al.* showed that 8 of 10 highly sensitized patients who were waitlisted for a deceased-donor kidney could be transplanted after successful desensitization by staphylococcal protein A immunoadsorption [33]. In 1996, Higgins *et al.* reported on 13 deceased-donor kidney transplant recipients in whom a single pretransplant immunoadsorption treatment was capable of rendering a positive pretransplant CDC crossmatch negative [34]. However, graft survival rates in both cohorts were rather low: in the study by Palmer *et al.* only six of eight patients had a functioning graft after 1 year, and Higgins *et al.* observed a 54% graft survival rate after a median follow-up of 26 months. More recently, the Vienna group published long-term results of a protocol for the transplantation of sensitized patients using immunoadsorption in combination with potent immunosuppression including anti-lymphocyte antibody therapy [27,35,36]. Between 1999 and 2008, 68 patients with a CDC-PRA of ≥40% were transplanted, including 21 patients with an initially positive CDC crossmatch, 30 patients with DSA that were detected by SAFB, and 17 patients that were sensitized against third party HLA only [27]. After a single pretransplant immunoadsorption treatment, crossmatch results were negative. Immunoadsorption was continued after transplantation until a stabilization of graft function was noted. In contrast to the studies by Palmer *et al.* and Higgins *et al.*, repeated post-transplant immunoadsorption treatments may have prevented the deleterious effects of a rebound of DSA. The overall 5-year graft survival rate was 63%, and antibody-mediated rejection episodes occurred in 24–30% of patients.

As of November 18, 2011, 24 kidney transplant candidates who were sensitized against their living-donor were treated at our center by a combination of repeated pre- and post-transplant immunoadsorption and rituximab. More than 50% of these patients had positive CDC and/or ELISA crossmatch results, and all patients were desensitized successfully and transplanted. At the time of transplantation, CDC and ELISA crossmatch results as well as ELISA-detected DSA were negative and remained negative in the majority of patients also in the post-transplant phase. More recently, SAFB-detected DSA below 1000 MFI was considered a prerequisite in these patients before transplantation. Results of a first cohort of 10 patients with a positive crossmatch result with their living donor were published recently, showing a 100% 2-year graft survival rate. Acute T-cell mediated rejection occurred in two patients and antibody-mediated changes were found in three patients. Last serum creatinine in this cohort was 1.6 mg/dl with a urinary protein to creatinine ratio of 0.1 [37].

Comparison of different desensitization strategies

Randomized controlled trials that compare the clinical efficacy of different desensitization strategies are not available. The Mayo group performed an uncontrolled comparison of three different desensitization strategies in transplantations performed between 2000 and 2005 [24]. A total of 61 patients with a positive anti-human globulin-enhanced CDC T-cell crossmatch with their potential living donor were analyzed. Patients were desensitized either by plasmapheresis, low-dose IVIg and rituximab (n = 32; 2000–2003), high-dose IVIg only (n = 13; 2003–2004), or plasmapheresis combined with low-dose IVIg and rituximab and DSA monitoring (n = 16; 2004–2005). A negative crossmatch was achieved in 85% of patients treated with plasmapheresis, low-dose IVIg and rituximab, compared with 36% in patients treated with high-dose IVIg only. Acute antibody-mediated rejection occurred in 80% of patients in the high-dose IVIg group, whereas the rate was 29% and 37% in patients desensitized by plasmapheresis, low-dose IVIg and rituximab depending on whether they had or had not DSA monitoring, respectively. The overall graft survival for all groups was 82% at 1 year.

To remove HLA alloantibodies during a short time period immediately before transplantation, the administration of IVIg alone may not be sufficient. HLA alloantibodies need to be removed by plasmapheresis or immunoadsorption. Although no direct comparison of these two antibody-elimination strategies exists, there are some theoretical advantages of immunoadsorption over plasmapheresis that deserve mention. Immunoadsorption allows the treatment of multiple plasma volumes without the need for substitution of plasma components. With the treatment of 2.5 plasma volumes, 87% of IgG may be removed and multiple treatments allow the near complete elimination of IgG or a specific antibody [38–42]. Disadvantages of immunoadsorption may be a more variable reduction of antibodies of the IgG3 and IgM isotypes, and, most importantly, the fact that immunoadsorption is not routinely available everywhere in the world, including the US.

Anti-CD20 therapy

In highly sensitized kidney transplant recipients, targeting B-cells by anti-CD20 therapy with e.g., rituximab as an

induction therapy is thought to prevent *de novo* alloantibody formation (Table 1). B-cells are important antigen presenting cells and, in addition, are critical for T-cell activation and the development of T-cell memory during alloimmune responses. Despite a lack of effect against long-lived plasma cells, in some reports, anti-CD20 therapy was associated with a reduction of DSA reactivity [43]. Rituximab may prevent the generation of antibody-producing cells from the naïve B-cell pool, and may target short-lived plasma cells that express CD20 on their surface. In addition, anti-CD20 therapy may deplete B-cell aggregates within allografts [44].

A recent study by Kohei *et al.* investigated the effect of rituximab on the development of DSA and chronic antibody-mediated rejection when comparing ABO-incompatible kidney transplants (with rituximab induction therapy; performed during the years 2005–2009) to ABO-compatible transplants (without rituximab therapy; transplant years 2001–2007) [45]. Chronic antibody-mediated rejection rates 2 years after transplantation were 3.5% and 28.9%, respectively. *De novo* DSA occurred in 1.7% and 18.1% of patients, respectively, after ABO-incompatible or ABO-compatible kidney transplantation, indicating that targeting B-cell immunity at the time of transplantation may reduce antibody-mediated allograft injury during the subsequent course.

Proteasome inhibition

Proteasome inhibition has recently been propagated as a promising tool for the treatment of antibody-mediated allograft rejection after transplantation [46,47]. Results in desensitization of patients with this agent before transplantation, however, are less consistent. Wahrmann *et al.* observed no significant decrease of circulating HLA alloantibodies in two sensitized dialysis patients treated with two cycles of bortezomib [48], indicating that bortezomib may not be able to eliminate long-lived plasma cells. *In vitro* studies indicate that contact with alloantigen enhances the susceptibility of plasma cells to proteasome inhibition-mediated apoptosis, which might serve as an explanation for the observed differences in the effectivity of bortezomib in the pre- and post-transplant phase [49].

In the pretransplant phase, a combination of bortezomib and the elimination of DSA by apheresis might be an effective approach to target those antibodies.

Complement inhibition

A completely new concept in the prevention of antibody-mediated allograft injury is the blockage of complement activation. The monoclonal antibody eculizumab binds to complement factor C5 and prevents generation of the pro-inflammatory peptide C5a and assembly of the membrane attack complex C5b-9 [50]. Unlike in other desensitization strategies, alloantibodies are not removed or modulated by eculizumab. However, lysis of the cells following deposition of alloantibodies is prevented by inhibition of the formation of the membrane attack complex. Stegall *et al.* recently reported on results obtained in a first series of patients in whom the use of eculizumab was tested for the prevention of antibody-mediated rejection in crossmatch-positive living-donor kidney transplant recipients [51]. Twenty six flow cytometric crossmatch-positive kidney transplantations realized under the usage of eculizumab were compared with a historic control group of 51 transplantations where desensitization had been performed without eculizumab. In addition, patients with T- and B-flow crossmatch channel shifts exceeding 300 received pretransplant plasmapheresis, corresponding to 69% of patients in both groups. Acute antibody-mediated rejection was observed in only 7.7% of patients treated with eculizumab as compared to a 41.2% rate in the historic control group. Although the patients were not at very high immunologic risk - none of the patients in the eculizumab group and only eight patients in the historic control group had a positive anti-human globulin enhanced T-cell CDC crossmatch – transplant glomerulopathy occurred in 7% and 36% of patients after 1 year, respectively.

The concept of complement inhibition together with recipient desensitization extends the armamentarium for the prevention of antibody-mediated allograft injury. Studies are underway to further investigate this substance in living and deceased-donor kidney transplantation, and in patients with positive CDC crossmatches and high immunologic risk.

Eculizumab may also be a promising strategy for the induction of accommodation after kidney transplantation. Accommodation, which is frequently observed after ABO-incompatible kidney transplantation, implies the resistance of a vascularized transplant to antibody-mediated damage. It has been suggested that interaction of the graft endothelium during the early post-transplant period with low titer alloantibodies in the absence of strong complement activation may be an approach to achieve accommodation. Such an interaction is thought to lead to induction of complement inhibitors on allograft tissue, conferring resistance to complement-mediated severe allograft damage [52].

Special programs for prevention of antibody-mediated allograft injury

Eurotransplant acceptable mismatch program

The Eurotransplant AM Program enables transplantation of highly sensitized kidney patients within a relatively

short time period with good graft outcome. Patients with historic or current CDC-PRA ≥85% are accepted for this program. More recently, the actual PRA was replaced by a virtual PRA based on HLA alloantibody specificities in the patient's serum in relation to the HLA antigen distribution in the donor population. In the Eurotransplant AM Program, HLA antigens are defined toward which the highly sensitized patient never formed antibodies. These antigens are defined as acceptable HLA mismatches. Since the number of acceptable antigens in any given patient usually is low, highly sensitized patients are given the highest priority when a compatible donor organ becomes available. Approximately 60% of highly sensitized patients can be transplanted via the AM Program within 2 years [17,18]. Unfortunately, ascribable to an unfavorable HLA antigen/HLA antibody constellation, approximately 40% of highly sensitized patients do not receive an organ offer via this program and accumulate on the waiting list. These patients need additional measures such as desensitization at the time of an organ offer immediately before transplantation, desensitization in advance on the waiting list to increase the chance of a crossmatch-negative organ, or a special program in which they immediately receive an organ offer after the accomplishment of desensitization (Fig. 2). It is often criticized that a majority of highly sensitized (often retransplant) patients in the AM Program are transplanted faster than non-sensitized patients on the regular waiting list, and Eurotransplant intends to eliminate this imbalance by the introduction of the 'donor incidence' score for the individual patient. Hereby, the chance of the patient to receive an organ will be calculated based on the patient's computer-registered unacceptable HLA antigen mismatches and the frequency of organ donors not carrying these antigens.

Meanwhile, other procurement organizations have implemented comparable special programs for transplantation of highly sensitized kidney patients, e.g., the 'Permissible Antigen Program' in France. In the US, on October 1, 2009, the OPTN replaced the measured PRA by the calculated PRA. Laboratories are required to enter unacceptable HLA antigens into the OPTN computer system (a measure which was implemented in the Eurotransplant region already some 25 years ago), and the calculated PRA is determined based on HLA frequencies

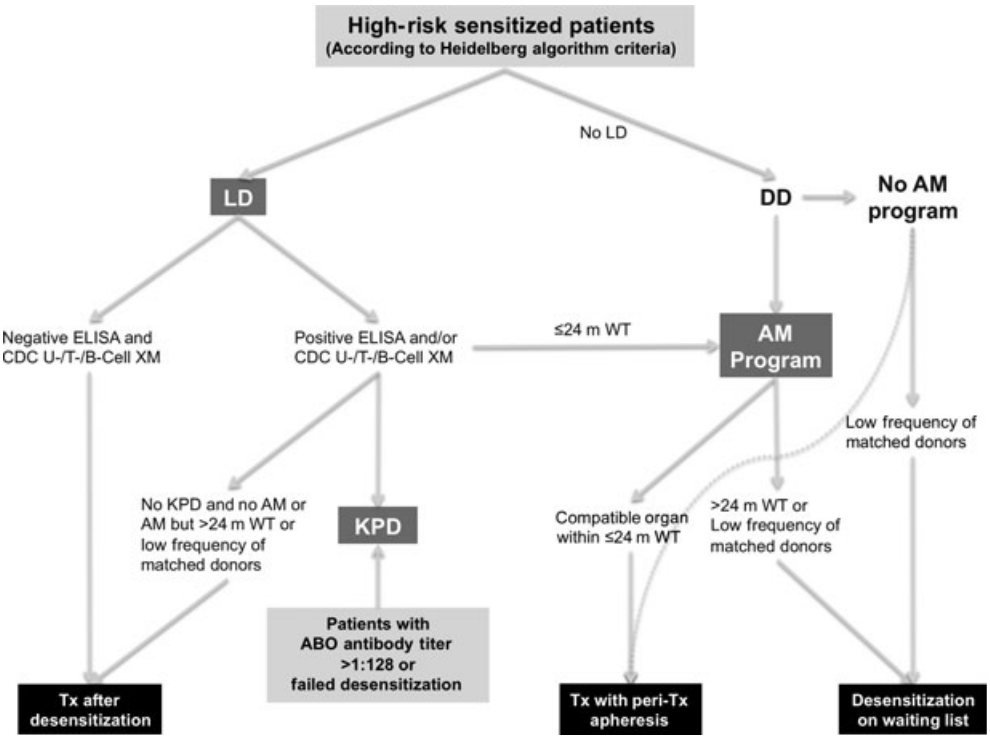

Figure 2 Options for transplantation of high-risk sensitized patients. Patients with a live kidney donor (LD) and HLA alloantibodies but negative ELISA and complement-dependent cytotoxicity (CDC) crossmatch (XM) results with unseparated (U) cells, T cells and B-cells may easily be transplanted after desensitization. When crossmatch results are positive, alternatives, such as kidney-paired donation (KPD) and the Eurotransplant Acceptable Mismatch (AM) Program should also be considered. Patients waiting for a deceased-donor organ (DD) may best be transplanted within the AM Program with peritransplant apheresis to diminish the effect of undetected or overlooked donor-specific antibodies. When there is a low frequency of matched donors, or when the AM waiting time (WT) is exceeding more than 24 months, then, desensitization on the WL should be considered.

derived from the US donor population. This change in allocation policy is reported to have led to an 83% reduction of kidney offers referable to positive crossmatch results, and more than a doubling of transplants performed in highly sensitized patients [53].

Kidney-paired donation

To avoid transplantation over positive crossmatch barriers or in patients who possess DSA or anti A/B-antibodies against their living-donor, kidney-paired donation has been introduced, i.e., in the Netherlands and in the US. With this approach, kidney recipients with an incompatible donor are matched to another incompatible donor-recipient couple to obtain a compatible constellation. Kidney-paired donation may further be facilitated by the introduction of altruistic donors, by donor chains, or even by the introduction of altruistic unbalanced paired kidney exchange [19,54]. Unbalanced paired kidney exchange utilizes compatible donor-recipient couples who voluntarily participate in kidney-paired donation to improve transplantation rates. In addition, kidney-paired donation may be combined with recipient desensitization to further optimize the number of successful transplants (Fig. 2). Blood group as well as HLA incompatible donor-recipient couples may be transplanted this way, however, matching options for blood group type O recipients are limited. It is also important to know that donor exchange in CDC crossmatch-positive patients is only practically feasible when patients with blood group incompatibility to their donors are also included. Otherwise the donor pool is too small and it is not possible to find a sufficiently large fraction of matching couples. This is a critical issue because, currently, the results of transplantations performed in blood group incompatible pairs using column adsorption of ABO antibodies are, without the need for excessive immunosuppression or interventions like splenectomy, not different from outcomes in blood group compatible transplantation [55]. Only patients with very strong ABO antibody titers or those patients who failed desensitization may require kidney-paired donation (Fig. 2). Meanwhile, legislation in some countries such as Germany prohibits living-donor kidney transplantation in persons who are not closely emotionally related, thus restricting kidney-paired donation to only few patients.

Post-transplant HLA alloantibody monitoring for the prevention of antibody-mediated allograft injury

It is still not clear whether the excellent short term results that were recently reported in desensitized patients will result in equally good long-term graft survival [56]. Current desensitization protocols do not eliminate antibody-producing plasma cells and therefore desensitized patients remain at risk of antibody-mediated allograft injury during long-term follow-up.

Lachmann *et al.* investigated the influence of DSA on graft outcome in a cross-sectional manner [57]. About 10% of patients had DSA after a median of 5 years after transplantation, and these patients had a significantly lower graft survival rate of 49% after the next 5.5 years compared to an 83% rate in patients without HLA alloantibodies and a 70% rate in patients who possessed HLA alloantibodies that were not donor-directed. In this study, however, DSA were measured at different time points and no differentiation was made between *de novo* DSA and DSA that were existing already at the time of transplantation. Hidalgo *et al.* found DSA in 37% of patients who had an indication biopsy 7 days to 31 years post-transplant [6]. Especially *de novo* DSA, which made up 60% of all DSA, that were directed against HLA class II antigens were associated with strongly impaired graft survival. Whereas in this study HLA antibodies were measured at the time of significant allograft dysfunction, Gill *et al.* investigated in 70 patients whether the screening for HLA alloantibodies during the first year after transplantation may help to predict the development of antibody-mediated rejection [58]. Of the 11 patients who developed *de novo* DSA, clinically overt rejection occurred in all 11 concomitantly or even before the detection of DSA.

Although it is currently a matter of debate whether or not alloantibodies should be monitored routinely in all transplanted patients, it seems to be justified in immunologically high-risk patients, desensitized patients, patients with suspected rejection, and during therapy of antibody-mediated rejection if one wants to recognize allograft injury in its early stages to prevent its translation to chronic rejection.

Prevention of non-adherence and insufficient immunosuppression as additional important issues in the prevention of antibody-mediated allograft injury

Until only recently, chronic allograft injury was considered to be a multifactorial event mainly attributable to non-immunologic causes, such as hypertension or calcineurin-inhibitor toxicity [59]. Today, most authors believe that HLA alloantibodies are responsible for the great proportion of late graft losses [7,60]. In this context, we believe that insufficient immunosuppression and avoidance of non-adherence to immunsuppressive medication will in future be issues of major interest.

An analysis of more than 25 000 kidney transplant recipients from the Collaborative Transplant Study showed that reduction or discontinuation of cyclosporine, tacrolimus or mycophenolate mofetil after the first post-transplant year in patients with good graft function was associated with significantly reduced subsequent kidney graft survival [61]. In a recent publication, Sellarés *et al.* attributed 64% of graft losses in a selected patient cohort with indication biopsies to (antibody-mediated) rejection [62]. Importantly, about half of the patients with rejection-associated loss of the allograft were identified as non-adherent.

We believe that insufficient immunosuppression and non-adherence contribute significantly to premature graft loss. Patients at high risk for non-adherence are young adults who are in the transition phase from pediatric to adult renal services. Insufficient immunosuppression may also occur during immunosuppressive minimization (tapering) or calcineurin-inhibitor-avoidance trials. A recent publication from Liefeldt *et al.* backs up this contention [63]. Fourteen of 61 patients (23%) that were converted from cyclosporine to everolimus at 3–4.5 months after transplantation developed DSA, compared with only 7 out of 65 patients (11%) who continued on cyclosporine. Eight patients on everolimus but only two patients on cyclosporine developed antibody-mediated rejection. Therefore, these patient cohorts should be screened rigorously for the occurrence of alloantibodies and antibody-mediated allograft injury.

Integrated algorithms for the transplantation of sensitized patients

A considerable fraction of patients on the waiting list is broadly sensitized and may only be transplanted by the combination of all available measures including the Eurotransplant AM Program, living-donor kidney transplantation and desensitization (Figs 1 and 2). An integrated algorithm that combines seven independent measures for the timely and successful transplantation of high-risk sensitized patients was recently published by our group [64]: (i) Pretransplant identification of high-risk patients based on ELISA screening and CDC crossmatch results in deceased-donor kidney transplantation, and also SAFB in living-donor kidney transplant recipients, (ii) a good HLA match in the case of deceased-donor kidney transplantation, (iii) inclusion of eligible patients in the Eurotransplant AM Program, (iv) pretransplant desensitization by apheresis and rituximab, (v) post-transplant apheresis, (vi) protocol biopsies on days 7 and 90, and (vii) monitoring of HLA antibodies by ELISA and SAFB.

In the period between April 1, 2006 and November 18, 2011, 79 high-risk sensitized patients were transplanted at our center using this algorithm. Death-censored graft survival for this actual high-risk cohort is given in Fig. 3. Results obtained in 28 patients after deceased-donor and six patients after living-donor kidney transplantation have been published recently [64]. A total of 27 of the 34 patients had donor-directed antibodies and 17 had a positive crossmatch result. Death-censored graft survival in this series was 96.4% and 100%, respectively. The incidence of delayed graft function was 46% and 17%, respectively, and antibody-mediated rejection episodes occurred in two patients and in one patient after deceased or living-donor transplantation, respectively.

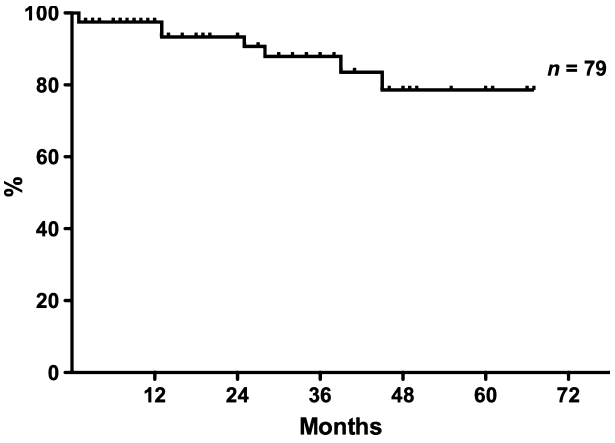

Figure 3 Death-censored graft survival in 79 patients treated according to the 'Heidelberg Algorithm' as of November 2011. A total of 55 patients desensitized by plasmapheresis and 24 patients desensitized by immunoadsorption received a deceased or living-donor kidney transplant, respectively. Part of this work was recently published [37,64,71].

Summary and conclusions

Prevention of antibody-mediated allograft injury should be initiated early and already before the kidney patient receives a transplant. Preventive measures include avoidance of sensitization by limitation of blood transfusions and poor HLA matching during first transplantations. New measures, such as matching for antibody epitopes in addition to whole HLA alleles may further help preventing sensitization. When the patient is already sensitized, antibody-mediated allograft injury can best be prevented by transplantation of the patient with an organ toward which he never developed HLA alloantibodies. This may be achieved by inclusion of patients in special programs such as the Eurotransplant AM program or by kidney-paired donation. Most patients, however, need desensitization in combination with other measures, for getting successfully transplanted. After transplantation, insufficient immunosuppression and non-adherence may lead in

patients who are not prone to tolerance first to a cellular rejection and then, via development of *de novo* DSA to antibody-mediated rejection and graft loss. The exact knowledge on the patient's alloantibodies before and after transplantation is a prerequisite for early diagnosis of allograft pathology and early and targeted treatment to prevent antibody-mediated rejection and to ensure long-term allograft survival. Protocol biopsies may further help in guiding post-transplant therapy in these high-risk recipients, although the final proof for their usefulness is currently lacking.

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References

1. 2009 Annual Report of the U.S. Organ Procurement and Transplantation Network and the Scientific Registry of Transplant Recipients: Transplant Data 1999–2008. U.S. Department of Health and Human Services, Health Resources and Services Administration, Healthcare Systems Bureau, Division of Transplantation, Rockville, MD.
2. *Eurotransplant International Foundation Annual Report 2010*. Edited by Arie Oosterlee and Axel Rahmel. Central Office. Leiden. The Netherlands.
3. Fuggle SV, Martin S. Tools for human leukocyte antigen antibody detection and their application to transplanting sensitized patients. *Transplantation* 2008; **86**: 384.
4. Collaborative Transplant Study, University of Heidelberg, Germany, Website. Available at: http://www.ctstransplant.org. Accessed November 21, 2011.
5. Süsal C, Döhler B, Opelz G. Presensitized kidney graft recipients with HLA class I and II antibodies are at increased risk for graft failure: a Collaborative Transplant Study report. *Hum Immunol* 2009; **70**: 569.
6. Hidalgo LG, Campbell PM, Sis B, *et al.* De novo donor-specific antibody at the time of kidney transplant biopsy associates with microvascular pathology and late graft failure. *Am J Transplant* 2009; **9**: 2532.
7. Einecke G, Sis B, Reeve J, *et al.* Antibody-mediated microcirculation injury is the major cause of late kidney transplant failure. *Am J Transplant* 2009; **9**: 2520.
8. Vella JP, O'Neill D, Atkins N, Donohoe JF, Walshe JJ. Sensitization to human leukocyte antigen before and after the introduction of erythropoietin. *Nephrol Dial Transplant* 1998; **13**: 2027.
9. Meier-Kriesche HU, Scornik JC, Susskind B, Rehman S, Schold JD. A lifetime versus a graft life approach redefines the importance of HLA matching in kidney transplant patients. *Transplantation* 2009; **88**: 23.
10. Morales-Buenrostro LE, Terasaki PI, Marino-Vazquez LA, Lee JH, El-Awar N, Alberu J. ''Natural'' human leukocyte antigen antibodies found in nonalloimmunized healthy males. *Transplantation* 2008; **86**: 1111.
11. van den Berg-Loonen EM, Billen EV, Voorter CE, *et al.* Clinical relevance of pretransplant donor-directed antibodies detected by single antigen beads in highly sensitized renal transplant patients. *Transplantation* 2008; **85**: 1086.
12. Amico P, Honger G, Mayr M, Steiger J, Hopfer H, Schaub S. Clinical relevance of pretransplant donor-specific HLA antibodies detected by single-antigen flow-beads. *Transplantation* 2009; **87**: 1681.
13. Lefaucheur C, Loupy A, Hill GS, *et al.* Preexisting donor-specific HLA antibodies predict outcome in kidney transplantation. *J Am Soc Nephrol* 2010; **21**: 1398.
14. Süsal C, Ovens J, Mahmoud K, *et al.* No association of kidney graft loss with human leukocyte antigen antibodies detected exclusively by sensitive Luminex single-antigen testing: a Collaborative Transplant Study report. *Transplantation* 2011; **91**: 883.
15. Lefaucheur C, Antoine C, Suberbielle C, Glotz D. Mastering the risk of HLA antibodies in kidney transplantation: an algorithm based on pretransplant single-antigen flow bead techniques. *Am J Transplant* 2011; **11**: 1592.
16. Süsal C, Opelz G. Kidney graft failure and presensitization against HLA class I and class II antigens. *Transplantation* 2002; **73**: 1269.
17. Doxiadis II, Smits JM, Persijn GG, Frei U, Claas FH. It takes six to boogie: allocating cadaver kidneys in Eurotransplant. *Transplantation* 2004; **77**: 615.
18. Claas FH, Rahmel A, Doxiadis II. Enhanced kidney allocation to highly sensitized patients by the acceptable mismatch program. *Transplantation* 2009; **88**: 447.
19. Montgomery RA. Renal transplantation across HLA and ABO antibody barriers: integrating paired donation into desensitization protocols. *Am J Transplant* 2010; **10**: 449.
20. Morath C, Zeier M, Süsal C. Desensitization of HLA-incompatible kidney recipients. *N Engl J Med* 2011; **365**: 1644; author reply.
21. Zachary AA, Montgomery RA, Ratner LE, *et al.* Specific and durable elimination of antibody to donor HLA antigens in renal-transplant patients. *Transplantation* 2003; **76**: 1519.
22. Montgomery RA, Zachary AA, Racusen LC, *et al.* Plasmapheresis and intravenous immune globulin provides effective rescue therapy for refractory humoral rejection and allows kidneys to be successfully transplanted into cross-match-positive recipients. *Transplantation* 2000; **70**: 887.
23. Jordan SC, Tyan D, Stablein D, *et al.* Evaluation of intravenous immunoglobulin as an agent to lower allosensitization and improve transplantation in highly sensitized adult patients with end-stage renal disease: report of the NIH IG02 trial. *J Am Soc Nephrol* 2004; **15**: 3256.
24. Stegall MD, Gloor J, Winters JL, Moore SB, Degoey S. A comparison of plasmapheresis versus high-dose IVIG desensitization in renal allograft recipients with high levels

of donor specific alloantibody. *Am J Transplant* 2006; **6**: 346.
25. Glotz D, Antoine C, Julia P, *et al.* Desensitization and subsequent kidney transplantation of patients using intravenous immunoglobulins (IVIg). *Am J Transplant* 2002; **2**: 758.
26. Beimler JH, Morath C, Schmidt J, *et al.* Successful deceased-donor kidney transplantation in crossmatch-positive patients with peritransplant plasma exchange and Rituximab. *Transplantation* 2009; **87**: 668.
27. Bartel G, Wahrmann M, Regele H, *et al.* Peritransplant immunoadsorption for positive crossmatch deceased donor kidney transplantation. *Am J Transplant* 2010; **10**: 2033.
28. Tyan DB, Li VA, Czer L, Trento A, Jordan SC. Intravenous immunoglobulin suppression of HLA alloantibody in highly sensitized transplant candidates and transplantation with a histoincompatible organ. *Transplantation* 1994; **57**: 553.
29. Glotz D, Haymann JP, Sansonetti N, *et al.* Suppression of HLA-specific alloantibodies by high-dose intravenous immunoglobulins (IVIg). A potential tool for transplantation of immunized patients. *Transplantation* 1993; **56**: 335.
30. Watanabe J, Scornik JC. IVIG and HLA antibodies. Evidence for inhibition of complement activation but not for anti-idiotypic activity. *Am J Transplant* 2005; **5**: 2786.
31. Montgomery RA, Zachary AA. Transplanting patients with a positive donor-specific crossmatch: a single center's perspective. *Pediatr Transplant* 2004; **8**: 535.
32. Montgomery RA, Lonze BE, King KE, *et al.* Desensitization in HLA-incompatible kidney recipients and survival. *N Engl J Med* 2011; **365**: 318.
33. Palmer A, Taube D, Welsh K, Bewick M, Gjorstrup P, Thick M. Removal of anti-HLA antibodies by extracorporeal immunoadsorption to enable renal transplantation. *Lancet* 1989; **1**: 10.
34. Higgins RM, Bevan DJ, Carey BS, *et al.* Prevention of hyperacute rejection by removal of antibodies to HLA immediately before renal transplantation. *Lancet* 1996; **348**: 1208.
35. Lorenz M, Regele H, Schillinger M, *et al.* Peritransplant immunoadsorption: a strategy enabling transplantation in highly sensitized crossmatch-positive cadaveric kidney allograft recipients. *Transplantation* 2005; **79**: 696.
36. Haas M, Bohmig GA, Leko-Mohr Z, *et al.* Peri-operative immunoadsorption in sensitized renal transplant recipients. *Nephrol Dial Transplant* 2002; **17**: 1503.
37. 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.
38. Gjorstrup P, Berntorp E, Larsson L, Nilsson IM. Kinetic aspects of the removal of IgG and inhibitors in hemophiliacs using protein A immunoadsorption. *Vox Sang* 1991; **61**: 244.
39. Belak M, Borberg H, Jimenez C, Oette K. Technical and clinical experience with protein A immunoadsorption columns. *Transfus Sci* 1994; **15**: 419.
40. Morath C, Becker LE, Leo A, *et al.* ABO-Incompatible Kidney Transplantation Enabled by Non-Antigen-Specific Immunoadsorption. *Transplantation* 2012; **93**: 827.
41. Goldammer A, Derfler K, Herkner K, Bradwell AR, Horl WH, Haas M. Influence of plasma immunoglobulin level on antibody synthesis. *Blood* 2002; **100**: 353.
42. Biesenbach P, Schmaldienst S, Smolen JS, Horl WH, Derfler K, Stummvoll GH. Immunoadsorption in SLE: three different high affinity columns are adequately effective in removing autoantibodies and controlling disease activity. *Atheroscler Suppl* 2009; **10**: 114.
43. Fehr T, Rusi B, Fischer A, Hopfer H, Wuthrich RP, Gaspert A. Rituximab and intravenous immunoglobulin treatment of chronic antibody-mediated kidney allograft rejection. *Transplantation* 2009; **87**: 1837.
44. Steinmetz OM, Lange-Husken F, Turner JE, *et al.* Rituximab removes intrarenal B cell clusters in patients with renal vascular allograft rejection. *Transplantation* 2007; **84**: 842.
45. Kohei N, Hirai T, Omoto K, Ishida H, Tanabe K. Chronic antibody-mediated rejection is reduced by targeting B-cell immunity during an introductory period. *Am J Transplant* 2012; **12**: 469.
46. Everly MJ, Everly JJ, Susskind B, *et al.* Bortezomib provides effective therapy for antibody- and cell-mediated acute rejection. *Transplantation* 2008; **86**: 1754.
47. Perry DK, Burns JM, Pollinger HS, *et al.* Proteasome inhibition causes apoptosis of normal human plasma cells preventing alloantibody production. *Am J Transplant* 2009; **9**: 201.
48. Wahrmann M, Haidinger M, Kormoczi GF, *et al.* Effect of the proteasome inhibitor bortezomib on humoral immunity in two presensitized renal transplant candidates. *Transplantation* 2010; **89**: 1385.
49. Cascio P, Oliva L, Cerruti F, *et al.* Dampening Ab responses using proteasome inhibitors following *in vivo* B cell activation. *Eur J Immunol* 2008; **38**: 658.
50. Thomas TC, Rollins SA, Rother RP, *et al.* Inhibition of complement activity by humanized anti-C5 antibody and single-chain Fv. *Mol Immunol* 1996; **33**: 1389.
51. 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.
52. Dorling A. Transplant accommodation-are the lessons learned from xenotransplantation pertinent for clinical allotransplantation? *Am J Transplant* 2012; **12**: 545.
53. Cecka JM, Kucheryavaya AY, Reinsmoen NL, Leffell MS. Calculated PRA: initial results show benefits for sensitized patients and a reduction in positive crossmatches. *Am J Transplant* 2011; **11**: 719.

54. Ratner LE, Rana A, Ratner ER, *et al.* The altruistic unbalanced paired kidney exchange: proof of concept and survey of potential donor and recipient attitudes. *Transplantation* 2010; **89**: 15.
55. Tyden G, Kumlien G, Genberg H, Sandberg J, Lundgren T, Fehrman I. ABO incompatible kidney transplantations without splenectomy, using antigen-specific immunoadsorption and rituximab. *Am J Transplant* 2005; **5**: 145.
56. Stegall MD, Dean PG, Gloor J. Mechanisms of alloantibody production in sensitized renal allograft recipients. *Am J Transplant* 2009; **9**: 998.
57. Lachmann N, Terasaki PI, Budde K, *et al.* Anti-human leukocyte antigen and donor-specific antibodies detected by luminex posttransplant serve as biomarkers for chronic rejection of renal allografts. *Transplantation* 2009; **87**: 1505.
58. Gill JS, Landsberg D, Johnston O, *et al.* Screening for de novo anti-human leukocyte antigen antibodies in nonsensitized kidney transplant recipients does not predict acute rejection. *Transplantation* 2010; **89**: 178.
59. Nankivell BJ, Alexander SI. Rejection of the kidney allograft. *N Engl J Med* 2010; **363**: 1451.
60. Terasaki PI. Humoral theory of transplantation. *Am J Transplant* 2003; **3**: 665.
61. Opelz G, Döhler B. Effect on kidney graft survival of reducing or discontinuing maintenance immunosuppression after the first year posttransplant. *Transplantation* 2008; **86**: 371.
62. 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.
63. Liefeldt L, Brakemeier S, Glander P, *et al.* Donor-specific HLA antibodies in a cohort comparing everolimus with cyclosporine after kidney transplantation. *Am J Transplant* 2012; **12**: 1192.
64. Morath C, Beimler J, Opelz G, *et al.* An integrative approach for the transplantation of high-risk sensitized patients. *Transplantation* 2010; **90**: 645.
65. Süsal C, Morath C. Current approaches to the management of highly sensitized kidney transplant patients. *Tissue Antigens* 2011; **77**: 177.
66. Jordan SC, Vo A, Bunnapradist S, *et al.* Intravenous immune globulin treatment inhibits crossmatch positivity and allows for successful transplantation of incompatible organs in living-donor and cadaver recipients. *Transplantation* 2003; **76**: 631.
67. Higgins R, Hathaway M, Lowe D, *et al.* Blood levels of donor-specific human leukocyte antigen antibodies after renal transplantation: resolution of rejection in the presence of circulating donor-specific antibody. *Transplantation* 2007; **84**: 876.
68. 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.
69. Thielke JJ, West-Thielke PM, Herren HL, *et al.* Living donor kidney transplantation across positive crossmatch: the University of Illinois at Chicago experience. *Transplantation* 2009; **87**: 268.
70. Haririan A, Nogueira J, Kukuruga D, *et al.* Positive crossmatch living donor kidney transplantation: longer-term outcomes. *Am J Transplant* 2009; **9**: 536.
71. Morath C, Schmidt J, Opelz G, Zeier M, Süsal C. Kidney transplantation in highly sensitized patients: are there options to overcome a positive crossmatch? *Langenbecks Arch Surg* 2011; **396**: 467.