# Kidney Transplantation in Sensitized Patients

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# 1 Introduction to Kidney Transplantation in sensitized patients

Duck Jong Han

## Definition of Sensitization

Renal transplantation is widely acknowledged as the best therapeutic treatment modality for patients requiring renal replacement therapy.

For the majority of patients with end-stage renal disease, kidney transplantation provides significant benefits compared with dialysis in terms of improved patient survival, better quality of life, and lower ongoing cost after the first year [1–6].

Sensitization to human leukocyte antigens remains one of the major clinical challenges for successful kidney transplantation [7].

Contributing to the reduction in posttransplant acute and chronic rejection is the recognition of the importance of the role of pretransplant alloimmune sensitization to HLA antigen [8].

The definition of sensitization is variable, but the general consensus is a panel-reactive antibody (PRA) value of greater than 20%. In further characterizing a kidney transplant recipient to be highly sensitized, the level is even more variable, with prior literature starting at a PRA of 80% and above [9].

Despite the long history of use, there is no universally agreed PRA threshold to define sensitization. In theory, a non-sensitized patient has no antibodies and their PRA titer should be 0%. Accordingly, all patients with at least one potentially harmful HLA-specific antibody are sensitized. In practice, only patients with a PRA >5% are traditionally considered as sensitized. Highly sensitized patients are the proportion with the highest and multiple antibody titers, and are defined to having a PRA of >85% (Eurotransplant criteria) or >80% (US criteria) [10].

The highly sensitized patient remains particularly challenging. Generally this has been defined as patients with a calculated panel-reactive antibody (CPRA) greater than 95% [11].

## Donor-Specific Antibody (DSA)

Donor-specific antibody identified before kidney transplantation (preformed antibody) can cause early rejection, such as hyperacute rejection, accelerated acute rejection, early acute antibody rejection, and graft loss.

Alternatively, de novo developed DSA after transplant are associated with late onset acute antibody-mediated rejection, chronic antibody rejection, and transplant glomerulopathy. However there are also “benign” DSA that may not be clinically relevant, because they are not associated with antibody-mediated rejection or graft failure [12–15].

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Not all DSA leads to AMR, and the mere presence of DSA does not necessarily portent unfavorable outcomes [16].

The strongest independent predictor for ABMR and death censored graft survival was pretransplant DSA. cPRA was not predictive for ABMR, TCMR, or death censored graft survival. It can be concluded that with current DSA assignment, the broadness of sensitization measured by cPRA does not imply an immunologic risk [17].

DSA at the time of transplantation in immunologically unmodified patients had the increased risk of acute rejection. Posttransplant incidence of acute rejection was significantly greater in the MFI ≥1,000 group (35%:8/22) compared to the MFI <1,000 group (7%: 2/28) ($p < 0.001$) [18].

All assessment pretransplant aims to provide a risk profile for transplantation but clinical risk assessment in transplant, as with other areas of medicine, is never absolute. A very-low-risk transplant may still be acutely rejected, while a high-risk transplant in a highly sensitized patient across DNA with augmented immunosuppression and desensitization may fare well. No single result absolutely contraindicates transplantation [19].

Preformed DSA is typically unacceptable for deceased donors, as there is no time to desensitization pretransplant, but lower strength DSA can often be managed with treatment that begins posttransplant [20].

Assessing complement fixation can identify a subclass that poses a particular risk for rejection, such as IgG1 and IgG3 [21].

## Non-HLA Antibody

The non-HLA antibodies are directed against antigens that are expressed on endothelial cells, epithelial cells, keratinocyte, dendritic cell, and monocytes, but not on peripheral blood lymphocytes. They include antibodies against MHC class I chain-related protein A (MICA) and MHC class I chain-related protein B (MICB) which are encoded by genes within the MHC and are genetically linked to HLA-B. They also include anti-platelet, anti-endothelial cell, anti-angiotensin 2 receptor, and anti-basal membrane antibodies [22].

Among the patients with DSA, proximately 75% anti-HLA and 25% anti-MICA antibodies are reported [23].

In contrast with anti-HLA antibodies, anti-MICA antibodies are neither easily nor routinely evaluated in most transplant centers.

## Histocompatibility Testing and Virtual Crossmatching

Cell-based assay, either complement-dependent cytotoxicity crossmatch or flow cytometric crossmatch test has been the cornerstone of compatibility testing. Solid phase immunoassays use antigen-coated beads to identify HLA antibodies of known specificity in recipient serum, which enables determination of a virtual crossmatch (VXM) in deceased donor organ transplantation [20].

The virtual crossmatch is an extension of traditional crossmatching and relies on having current and carefully interpreted HLA antibody test results along with an accurate and comprehensive assessment of the donor HLA antigens [24].

Now UNOS policy mandate that complete donor typing (A, B, BW4, BW6, C, DR, DRBI, DRB3–5, DQA1, DQB1, DPB1) is reported which increased VXM accuracy. Additionally, most donor typing centers also test for HLA-DPA1 [20, 24].

The technology used to detect PRA has progressed from complement-dependent cytotoxicity assay to more modern purified HLA antigen coupled to specialized microparticles (luminex, flow cytometry). The reactive antigens are then assigned as unacceptable antigens [25].

## Epitope

The targets for antibodies directed against HLA molecule are known as epitope. An epitope typically but not always consists of a 3 amino acid sequence on the HLA molecules that is expressed on the exterior of the molecule [26].

Each HLA antigen is seen as patches of polymorphic amino acid residuals (eplet), which

constitutes the essential components of HLA epitopes [27].

HLA antibodies specifically recognize a wide range of epitope present on HLA antigens and molecularly defined high-resolution alleles corresponding to the same low-resolution antigen can possess different epitopes repertoires. Hence the determination of HLA compatibility at the allele level represents a more accurate approach to identify suitable donors for sensitized patients, and this is what is referred to as high-resolution typing [25].

Each HLA molecule has multiple antibody-binding sites, and different polymorphisms of the HLA molecule may share epitopes, permitting cross-reactivity between HLA types [28].

All specific HLA antibodies might cross-react with other HLA specificities.

This is due to the fact that all HLA antibodies are not against the complete HLA molecule but are directed against distinct epitopes on HLA molecules that are shared with other HLA antigens. This explains the sensitization against other non-donor-directed HLA molecules [29].

## Prevalence of Sensitization in Kidney Transplantation

Approximately 15% of wait-list candidate have some degree of sensitization [3]. Today 25% of the patient wait-listed for renal transplantation in the USA have a PRA of >10% while in the Eurotransplant zone, 14% have a PRA of >5% [10].

Sensitized kidney transplant candidates comprise approximately 30% of the deceased donor waiting list and have the longest wait times because of difficulty in finding a compatible donor [30, 31].

Currently 35% patients on transplant waiting list in the United States are sensitized with panel-reactive antibody (PRA) level >0% and 15% patients are highly sensitized with PRA level >80% [32].

The percentage of patient with >80% PRA in the USA has also been increasing in the last decade. For recipients of deceased donor kidney the proportion with a PRA level of 0% at the time of transplant have declined from 72.9% in 1998 to 59.7% in 2009. Over the same period, the proportion with a PRA of 80–100% has increased from 2.2 to 8.1% [10].

## Causes of Sensitization

Sensitization is caused by previous exposure to HLA antigens, usually through organ transplant, pregnancy, or blood transfusion [10, 33–35].

Particularly relevant is the exposure of a woman to her partner's HLA during pregnancy. This results in direct sensitization against the partner, potentially making the partner and/or her child an unsuitable donor [15, 36].

Rare causes of sensitization can occur without these and thought to be due to cross-reactive antigens from other exposures, such as viruses [28].

An adjuvant H1N1 influenza vaccine was found to be associated with the development of DSA [36, 37].

In retransplant patients, repeated HLA Ag mismatch may be the risk in patient who underwent graft nephrectomy of the first graft [38].

Anti-HLA sensitization after renal allograft nephrectomy was illustrated as such that at baseline, anti-HLA sensitization was significantly lower in the early and late asymptomatic groups than in the group of graft intolerance syndrome, but increased considerably within the 3 months following allograft nephrectomy. All patients undergoing a clinically indicated allograft nephrectomy become highly sensitized within 12 months after surgery [39].

It is obvious that mere HLA mismatch will inevitably lead to a higher potential for sensitization. They might be particularly important in patient with a long life expectancy because of the high likelihood of needing a second transplant during their life [10].

Many chronic graft losses are a direct consequence of chronic AMR: even after a very short engraftment under heavy immunosuppression, DSA and non-DSA appeared in more than 60% of patients secondary to the loss of the "sponge effect" and stopping immosuppression [40–42].

Stopping immunosuppression also could contribute to 47.6% of emerging of DSA without nephrectomy. However continuation of immunosuppressant need to be weighed against the risk of infection and is only appropriate in a few selected HLA-antibody negative patients while only retransplants are scheduled [43].

The 10-year actuarial graft survival for highly sensitized recipient was 43.9% compared with 52.4% for non-sensitized patients ($p < 0.01$). The combination of being highly sensitized by either pregnancy or blood transfusion increased the risk of graft loss by 23% (HR:1.230), and the combination of being highly sensitized from a prior transplant increased the risk of graft loss by 58.1% (HR 1.581). As a result the mode of sensitization predicts graft survival in highly sensitized kidney recipients (>98%). Patients who are highly sensitized from re-transplant have inferior graft survival compared with patients who are highly sensitized from other mode of sensitized [44].

## Approach to the Highly Sensitized Candidate

### Allocation System

The new US national KAS of the organ procurement and transplantation network effective as of December 2014, was designed to improve the chances of transplanting the most highly sensitized patients in the waiting list, and these were designed a patients with a calculated PRA value of 98%, 99% and 100% [45].

Theoretical number of potential donor offers needed to have a high probability of an acceptable match can be determined using the following equation [46].

$$\text{Probability of finding an acceptable match} = 1 - (\text{cPRA})n$$

$n$ = number of potential donor.

For the purpose of listing and allocation, cPRA is considered as rounded integer value. Estimated number of match runs needed to have a 95% probability of finding an acceptable donor based on cPRA is illustrated as 300 match in 99%, 3,000 match in 99.9%, and 300,000 match in 99.999% (Table 1.1) [28]

**Table 1.1** Estimated number of match runs needed to have a 95% probability of finding an acceptable donor based on candidate cPRA

| cPRA, % | Theoretical number of match runs to have a 95% chance of finding an acceptable donor |
|---|---|
| 10 | 2 |
| 20 | 2 |
| 30 | 3 |
| 40 | 4 |
| 50 | 5 |
| 60 | 6 |
| 70 | 9 |
| 80 | 14 |
| 85 | 19 |
| 90 | 29 |
| 95 | 59 |
| 99 | 300 |
| 99.5 | 600 |
| 99.9 | 3,000 |
| 99.99 | 30,000 |
| 99.999 | 300,000 |

cPRA, calculated panel-reactive antibody

To improve transplant rates among highly sensitized patient, the organ procurement and transplant network (OPTN) implemented key changes to the kidney allocation system in which candidates with cPRA scores of 98%, 99%, and 100% receive 24.4, 50.1, and 202.1 points, respectively (Fig. 1.1) [28].

In Italy, a nationwide hyperimmune program was begun in February 2011. All available kidneys are primarily proposed to highly sensitized patients with a panel-reactive antibody above 80% [47].

### Acceptable Mismatch Program

HLA antibody detected at a level anticipated to result in a high rate of rejection in the serum of candidates are designated as unacceptable [28].

The sweet spot for designating acceptable antigens is to set the level just low enough to avoid positive crossmatches but high enough to allow the candidate to receive as many organs as possible. At the University of Virginia the designate

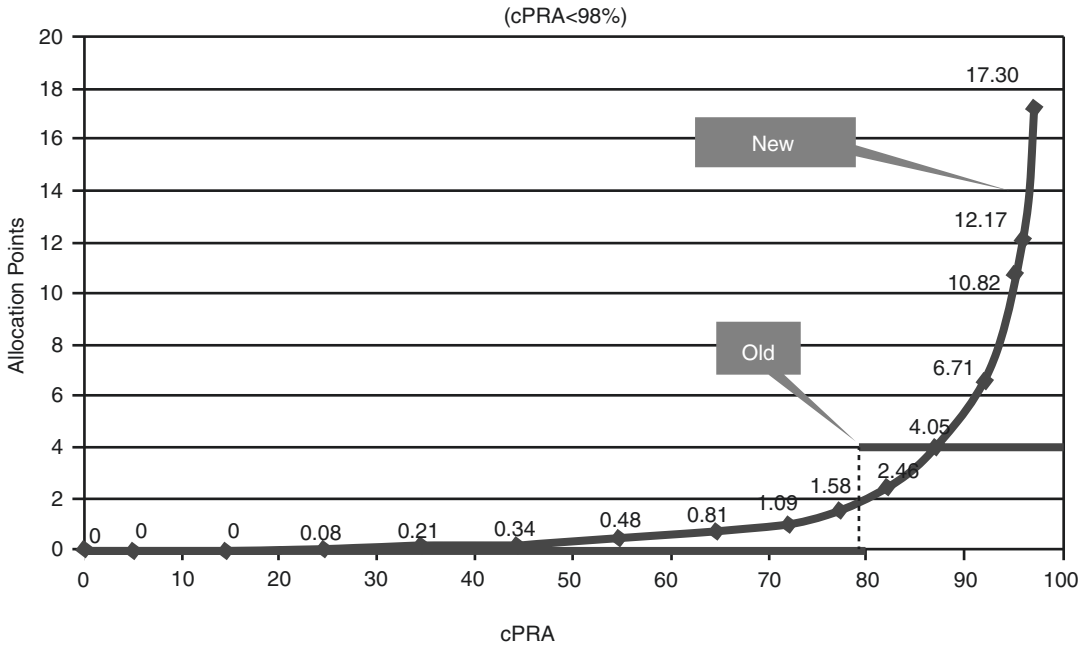


**Fig. 1.1** Allocation points by calculated panel-reactive antibody (cPRA) in the old versus new kidney allocation system

value of unacceptable antigens at MFI values exceeding 4,000. This allows for a virtual crossmatch to predict crossmatch results during organ allocation in USA [28].

The Eurotransplant Acceptable Mismatch (AM) program was launched in 1989 for highly sensitized patients (PRA >85%) awaiting a renal transplantation within Eurotransplant region. When a blood group compatible organ become available within the Eurotransplant region that matched the AM patient's own HLA plus acceptable antigens, this organ was mandatorily shipped to this patient [48].

All patients with an historical or current PRA of 85% or more for three consecutive months are eligible for the Eurotransplant AM program, where HLA antigen toward which the patient never formed allo-antibodies are defined.

A patient will not produce antibodies against self-antigens or closely related HLA antigens that share multiple epitope with the recipient, and these antigens can be defined as "acceptable" HLA antigens [10].

The methods for detection of acceptable HLA antigens are single Ag expressing cell line, solid phase technique, CDC, and HLA matchmaker algorithm to evaluate the role of HLA matching at the amino acid sequence level. Each HLA antigen is seen as patches of polymorphic amino acid residuals (eplet), which constitutes the essential components of HLA epitopes. Non-inherited maternal HLA antigens are often acceptable mismatches [27].

For high-risk sensitized patients there are two different strategies:

As organisatory measure, patient are given additional score points for organ allocation and enrolled into special program, such as acceptable mismatch program of Eurotransplant or paired kidney donation scheme in living donor kidney transplantation. Another is desensitization.

However either approach alone will not be successful and that a combination of measures, such as inclusion in special allocation program plus desensitization, will be necessary to finally allow transplantation [49].

## Desensitization

The options available to sensitized candidate are greater if patient has a living donor, even if the donor is incompatible.

In the highly sensitized recipient, an organ with a negative crossmatch may not be easily attainable and desensitization to lower the level or preformed antibodies to prevent rejection may be the only feasible option for transplant [50].

While hyperacute rejection is rare in the desensitized candidates, the rate of AMR and graft loss are high [51].

Despite the less than ideal allograft outcomes in desensitized recipients, they are cost effective and have a survival advantage with transplant compared with dialysis in most cases [52].

Desensitization procedures may be undertaken to increase access to either living or deceased donor transplantation, and in some situations may also be employed to facilitate participation in a kidney exchange, when the immunological barrier to overcome is low and desensitization would allow paired transplant to proceed [7].

A multicenter study from the USA reported that there was a strong survival benefit for sensitized patients undergoing desensitization followed by HLA incompatible living donor kidney transplantation compared with those remaining on the waiting list [53].

Desensitization with IV Ig and rituximab in broadly HLA-sensitized living donor transplant recipient has good long-term results with graft outcome similar to non-HLA-sensitized patients despite higher immunologic risk [54].

Agents for desensitization are depicted such as intravenous immunoglobulin (IVIg), rituximab (Rituxan), bortezomib (Velcade), and Eculizumab (C5 inhibitor) in clinical study and interleukin-6 receptor antibody (Tocilizumab), IgG endopeptidase, Obinutuzumab (Gazyva), Belimumab, and C1 esterase inhibitor (Beninert) in early clinical basis [31, 55].

However another study reported no significant survival advantage for desensitized patients compared with similar patients remaining on the waiting list in the United Kingdome [56].

Comparison of the studies is complicated by the different definitions of desensitization, different matching methods employed, the different population, and the observation that survival of US dialysis patients is worse than that of patients in the UK and Europe [7].

## Kidney Paired Exchange

When there is incompatibility between transplant candidates and their potential living donor, which is estimated to be 35% of all declined potential donor/recipient pairs, a donor exchange program offers an opportunity to receive a compatible kidney for the recipient. Started in south Korea and the Netherlands, the first US KPD exchange took place in the year 2000. Since then it has gained popularity in the USA since 2005 with the increasing public interest and need for kidney transplants [10].

The USA is unique in that in addition to a government-funded KPD registry managed by UNOS in 2010, there are also multiple single and multiple center registries that operate independently of each other [9].

A better immunological match by KPD will result in improved long-term graft survival and less economical strain with the use of less immunosuppression [11].

Donor exchange program can be simple 2 pair exchange, more complicated domino exchanges, or chain donation. For these programs to be successfully carried out, collaborations among centers are key. Another innovative aspect was the utilization altruistic donor for those highly sensitized recipients with an available but immunologically unsuitable living donor [57].

## Conclusion

In deceased donor transplantation to highly sensitized patients, acceptable mismatch program will be a good strategy without the long waiting time of this situation.

Donor exchange programs for living donor transplants would facilitate the process of find-

ing a suitable match with minimal cold ischemic time. Strategies such as desensitization can be used as a second option for those cases where no matched donor can be found [10, 55].

The first step toward transplanting highly sensitized living donor patients is to optimize local and national kidney exchange programs to find out a suitable match. Next is to apply desensitization that will allow transplantation and reduce to incidence of AMR [10, 55].

Many sensitized patients have willing live donors but are unable to use them because of HLA incompatibility. The options for these patients include remaining on the deceased donor list, entering a kidney-paired donation scheme, undergoing desensitization (high-dose IV of or p/p and low-dose IV Ig), or hybrid modality utilizing desensitization after identifying a more immunologically favorable donor is KPD (either HLA or ABO incompatibility) [11, 57, 58].

KPD and desensitization are not competitive strategy. Combining desensitization with KPD as a complimentary modality will increase the chances of finding a compatible donor.

Thereafter it is recommended to perform posttransplant protocol biopsies either routinely or in the case of positivity to enhance graft survival under the condition of high incidence of antibody-mediated rejection and graft loss.

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# 2 Preoperative Evaluation of Sensitized Patients

Soo-Kyung Kim and Hyosang Kim

**Q: What kinds of laboratory tests should be performed for characterization of patient sensitization?**

- HLA typing
- HLA antibody test for complement dependent cytotoxicity crossmatch, flow cytometry crossmatch and Luminex single antigen bead assay

## HLA Typing

**Q: Which HLA locus should be typed before renal transplantation?**

HLA typing can be divided into serologic typing and molecular typing. Molecular typing can be classified into high-resolution typing and low-resolution typing depending on the reported range. Low-resolution typing can distinguish allele groups to antigenic equivalent and results are reported up to the first field in the DNA-based nomenclature. High-resolution typing can resolve alleles to protein level, which encodes the antigen-binding site of the HLA molecule [1].

According to Eurotransplant guideline, donor and recipient should be typed for HLA-A, -B, -C, -DR, and -DQ. For HLA-A and -B, serological and DNA typing is accepted. For HLA-C, -DR, and -DQ, DNA typing must be performed. The minimum requirement for HLA typing for donors and recipients is at the serological split level [2].

According to Organ Procurement and Transplantation Network (OPTN) policies, deceased donor should be typed for HLA-A, -B, -C, -DRB1, -DRB3/4/5, -DQA1/DQB1, and -DPB1 with molecular typing method and should be reported at the level of serological splits. For transplantation candidates, HLA-A, -B, and -DR should be typed [3].

Recently, Sensitization in Transplantation: Assessment of Risk (STAR) 2017 Working Group recommended that both donor and recipient HLA-A, -B, -C, -DRB1, -DRB3/4/5, -DQA1/DQB1, and DPA1/DPB1 needs to be typed with molecular methods [4]. Anti-HLA antibodies recognize epitopes rather than whole HLA antigen, and there are some opinions that high-resolution typing is needed especially for sensitized patients [5, 6].

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## Serologic Typing

Serologic typing method use antisera with well-characterized anti-HLA antibody specificity. Patient's lymphocytes are separated and reacted with various sera with known anti-HLA antibody specificity in a tray well. Complement and vital dye is added, followed by incubation. If the HLA antigens expressed on patient's lymphocytes react with anti-HLA antibodies in the serum, complement cascade will occur and the vital dye is taken up into the cells via membrane attack complex. Tray well is read under the phase contrast microscope [7].

Serologic typing has advantages of getting rapid results, and it can discriminate null alleles, which do not express HLA molecules on cell surface. However, high-quality serum is needed and discrimination between alleles with small amino acid difference can be difficult [7]. These days serologic typing is replaced by molecular typing in clinical laboratories.

## Molecular Typing

For molecular typing, sequence-specific primer (SSP), sequence-specific oligonucleotide (SSO), reverse sequence-specific oligonucleotide (rSSO), and sequence-based typing (SBT) methods can be used.

For SSP typing, pre-made primer sets are used to amplify patient DNA. Patient DNA is amplified with each primer sets in each well of PCR tray. Amplification is detected by electrophoresis. By reading which primer set amplified DNA or not, patient's HLA type can be determined [8]. Advantage of SSP is that results can be obtained rapidly, within 4 h. However, it is not suitable for large volume of specimens, and additional step might be needed to resolve ambiguity.

For SSO typing, patient DNA is extracted and amplified according to HLA gene locus. Amplified DNA is blotted on solid support such as nylon membrane. Various sequence-specific oligonucleotide probes are hybridized and detected. SSO is suitable for large number of samples, 88–184 samples can be easily typed. However, ambiguity is often, and it takes almost 2 days to get results [8].

The rSSO is a widely used method with Luminex technology. Patient DNA is amplified using locus-specific primer and denatured. Microbeads attached to allele or group-specific oligonucleotide probe is reacted with patient sample. Patient DNA is bound to complementary DNA sequences on microbead and read on Luminex platform. Low to intermediate resolution is available with rSSO [9].

With SBT, patient DNA is amplified and sequenced by Sanger sequencing. High-resolution typing is available, but ambiguity can still happen and additional allele or group-specific PCR might be needed in that case. Drawbacks of SBT are that running cost is expensive and is time-consuming. With Next Generation Sequencing (NGS) high-resolution typing is available and it is relatively simple and cost-effective. However, since *HLA* is highly polymorphic, generation of consensus sequences is difficult and genotype discordance can occur due to incomplete reference typing, allele imbalance, or software error [10].

# HLA Antibody Testing

**Q: What kind of HLA antibody test can be done in sensitized patients?**

Prevention and early diagnosis of antibody-mediated rejection (ABMR) is critical in renal transplantation. For successful renal transplantation, various tests are performed. In pretransplant phase, complement-dependent cytotoxicity crossmatch (CDC), flow cytometry crossmatch (FCXM), and solid phase immunoassays are done to evaluate the existence of preformed HLA antibody, and to determine acceptable levels of donor-specific antibody (DSA) that allow for successful transplantation [11]. These assays differ in sensitivity and types of antibodies detected. Generally,

combination of these tests are performed to increase sensitivity, and to determine antibody characteristics.

## Complement-Dependent Cytotoxicity Crossmatch

**Q: What is the condition of false positive CDC?**

CDC has been the basic test to perform before transplantation [12]. If there is sufficient amount of antibodies, which can bind to donor antigen and induce complement cascade, cytotoxic response will occur. Various techniques are used to increase the sensitivity of the test such as extended incubation time, addition of washing steps prior to addition of complement, or addition of anti-human globulin (AHG) [13].

### Test Principle and Methods

CDC is performed by incubating donor cells expressing HLA antigens and recipient serum with the addition of complement and dyes. If antibody in patient serum can bind to donor lymphocyte and induce complement cascade, the donor cell is killed and the vital dye is taken up so dead cells can be identified by reading with phase contrast microscope [7].

CDC can be performed with unseparated lymphocytes, or T lymphocytes or B lymphocytes with viability more than 80%. Commonly used methods are NIH-CDC, long incubation, AHG-CDC (anti-human globulin augmented), and Amos wash method.

The standard CDC, also known as NIH-CDC, is the least sensitive method. Patient serum is serially diluted and dispersed on Terasaki tray. Donor lymphocytes with $2 \times 10^6$ cells/mL is added 1 μL in each well and incubated for 30 min at room temperature. Rabbit complement 5 μL is added and incubated for 60 min at room temperature. After that, vital dyes such as eosin, trypan blue, acridine orange, and ethidium bromide is added and read under phase contrast microscope [13]. OPTN requires to use more sensitive crossmatching assay than basic NIH-CDC.

AHG-CDC is a widely used method to increase sensitivity. Before addition of complement, 1 μL AHG is added and incubated for 2 min at room temperature. AHG allows the antibody Fc portion to be in closer proximity to activate complement [14]. AHG is used to detect low-level anti-HLA antibody and non-complement-fixing antibodies. Long incubation method is basically similar to NIH-CDC, but after addition of complement, incubation is extended to 120 min. For Amos wash method, washing steps are introduced before addition of complement. By this method, probable anti-complementary factors that can prevent complement fixing can be removed [15]. To differentiate IgG and IgM antibodies, pretreatment of patient sera with heat inactivation or treatment with dithiothreitol (DTT) or dithioerythritol (DTE) can be done.

Each HLA laboratory will adopt appropriate method and lymphocyte preparation, incubation time, incubation temperature, AHG treatment, staining, washing, etc. can vary from laboratory to laboratory.

### Interpretation

After neglecting the proportion of dead lymphocytes in the negative control well, more than 20% of the dead cells are interpreted as positive. Maximum dilution titer with positive crossmatch is reported.

T cells express HLA class I antigen and B cells express HLA class I and II antigen. Therefore, if both T cell CDC and B cell CDC are positive, it can be concluded that both HLA class I and class II antibodies are present, or that only class I antibodies are present.

In case of negative T cell CDC and positive B cell CDC, HLA class II antibody alone may be considered. In rare cases, non-HLA antibodies or low-level HLA class I antibodies may be considered. Therefore, it is recommended that solid phase immunoassay should be performed for exact identification of anti-HLA antibodies.

Since CDC is the reaction between the patient's serum and donor cell, the reaction does not only occur by anti-HLA antibodies. CDC results may be affected by the drug or patient's underlying disease. Rarely, positive CDC can be due to autoantibodies, such as anti-Lewis antibody. These autoantibodies are usually IgM, and the interference can be reduced by pretreatment of serum with DTT or DTE [16, 17]. In patients who had undergone rituximab (anti-CD20) or basiliximab/daclizumab (anti-CD25) or anti-thymocyte globulin or 6-mercaptopurine treatment, there is a possibility of false positives CDC. In patients who were treated with rituximab, B cell CDC may have a false-positive result over 3 months, since rituximab can be present in the patient's serum for that period [18]. In patients who were treated with these drugs, follow-up with solid phase immunoassay is helpful.

## Flow Cytometry Crossmatch

**Q: What factors can affect flow cytometry crossmatch?**

FCXM is more sensitive than CDC to detect anti-HLA antibodies [19, 20]. Although positive FCXM is associated with poor clinical outcome, it is not an absolute contraindication of transplantation.

### Principle

FCXM is similar to CDC in that patient's serum and donor lymphocytes are reacted. Unlike CDC which detects cytotoxic response, fluorochrome-conjugated antibodies are added and the signal is read by flow cytometer.

### Methods

In addition to patient serum and donor lymphocyte, negative control serum and positive control serum should be prepared. Negative control serum can be purchased by commercially available human AB serum drawn from blood group AB male donor and should be noncytotoxic. Positive control serum is made by pooling PRA positive sera. Negative control serum and positive control serum is tested in the same way with patient serum.

Donor lymphocytes are isolated and its viability should be more than 80%. Patient's serum and donor lymphocytes are incubated 20–30 min at room temperature. Although the number of lymphocytes and volume of serum varies according to laboratories, ASHI suggest using 500,000 cells and 30 μL of serum to be used [21].

Some laboratories treat lymphocytes with pronase to remove Fc receptor and CD20 on surface of B cells. Since B cells express Fc receptors on cell surface, non-HLA antibodies can bind and cause background noise. In patients desensitized with rituximab, pronase can remove CD20 of lymphocytes and can reduce the rituximab effect on B cell FCXM [22]. There are no standard protocol, but most of laboratories use 0.5–2.0 mg/mL pronase. If higher concentration of pronase is used, cell surface HLA molecules can also be removed, causing reduced HLA antibody reactivity. When pronase is treated in T cell FCXM, sensitivity can be increased, but cryptic epitope of HLA molecule can be exposed, causing nonspecific reactions [23, 24].

After incubating cell and serum, fluorescence-conjugated [$F(ab')_2$] antihuman IgG (Fc specific) is added. Fluorescence-conjugated anti-human CD3 is added to detect T cell reactivity and fluorescence-conjugated anti-human CD19 or anti-human CD20 is added to detect B cell reactivity. Flow cytometry acquisition should be gated at least 5,000–15,000 lymphocytes.

The result of FCXM is reported with median fluorescent intensity (MFI) ratio or median channel shift (MCS). MFI ratio is calculated by patient serum MFI divided by negative control serum MFI, and it is useful when dealing with log scale data. MCS is calculated by subtracting negative control serum value from patient serum value, and is useful with data on linear scale of 256 or 1,024 channels [21].

### Interpretation

HLA laboratories should establish their own FCXM cutoff. Since there are many variable factors in FCXM, such as flow cytometers, fluorochromes, reagents, cell-to-serum ratio, and incubation condition, it is difficult to standardize FCXM [25].

FCXM results should be interpreted in context with CDC results (Table 2.1). Low-titer class I DSA can cause negative T cell CDC and positive T cell FCXM. Negative T cell FCXM and positive B cell FCXM can be resulted from HLA class II antibodies, low-titer HLA class I antibodies, non-HLA antibodies such as autoantibodies and MICA antibodies, or nonspecific antibodies [26, 27]. Patients who are treated with rituximab can show false positive B cell FCXM for 3 months after injection [18, 28]. In these patients, monitoring with solid phase immunoassay would be more suitable. Antithymocyte globulin (ATG) can also affect cell based HLA antibody assay. The results of T cell FCXM after ATG treatment can be falsely positive. Moreover, ATG-treated patients can produce false positive result for B cell FCXM in spite of negative B cell CDC [29].

**Table 2.1** Interpretations of crossmatch results

| CDC | | FCXM | | |
|---|---|---|---|---|
| T cell | B cell | T cell | B cell | Interpretation |
| + | + | + | + | High-titer anti-class I antibodies with or without anti-class II antibodies. |
| + | – | + | – | Probably not anti-class I antibodies since B cell crossmatch is negative. Further tests needed. |
| – | + | + | + | Low-titer anti-class I antibodies with or without anti-class II antibodies. |
| – | – | + | + | Low-titer anti-class I antibodies with or without anti-class II antibodies |
| – | + | – | + | Anti-class II antibodies and/or low-titer class I antibodies. |
| – or + | + | – | – | IgM autoantibody possible. DTT treatment would be helpful. |
| – | – | – | – | HLA antibodies not detectable with CDC, FCXM. SAB assay may detect low-level antibodies. |

*CDC* complement-dependent cytotoxicity; *FCXM* flow cytometry crossmatch

## Solid Phase Immunoassay

**Q: 1. What are the factors affecting SAB assay?**
**2. What is the limitation of SAB assay?**

Before development of solid phase assay, cell-based panel-reactive antibody (PRA) tests were used. Nowadays, solid-phase assay such as ELISA, flow cytometry, or Luminex platform has been widely used. For luminex platform, pooled antigen panel, phenotype panel, and single antigen beads (SAB) panel can be applied. Pooled antigen panel consists of microbeads coated with HLA class I or class II molecules from multiple donors. Phenotype panel consists of microbeads that are coated with HLA class I or HLA class II molecule derived from a single individual. SAB, each bead is coated with only one kind of HLA allelic antigen. Among various PRA methods, Luminex SAB is the most sensitive and specific method for DSA detection. For HLA sensitized patients, it is recommended that SAB assay be performed at least once before transplantation [25]. Although SAB assay has increased sensitivity and specificity to detect HLA antibodies, it still has limitations. Many factors affect PRA results making inter-laboratory and intra-laboratory MFI results less reproducible. Interference can occur due to serum factors or drugs. Prozone phenomenon and shared epitope phenomenon can also occur. Here, subsequent discussion will be restricted to Luminex SAB assay.

### Principle

Luminex SAB assay uses multiplex bead assay technology. It has 5.6 μm diameter microbeads which are impregnated with two fluorescent dyes (classifier signal) [25, 30]. Every beads are uniquely color coded by combination of these two fluorescent dyes. Purified HLA molecules are immobilized to microbeads and up to 100 beads with a unique HLA antigen can be identified. By using phycoerythrin (PE)-conjugated

secondary antibody (reporter signal), specific HLA antibody in patient serum can be detected using dual-laser instrument [25].

## Methods

For Luminex SAB assay, patient serum is incubated with purified HLA molecules attached on microbeads and fluorescent-conjugated anti-human IgG. If there are anti-HLA antibodies in patient serum, it will bind to HLA molecules on microbead, and fluorescent-conjugated antihuman IgG will subsequently bind to patient's anti-HLA antibodies. After these steps, sample is analyzed on Luminex platform.

To improve sensitivity and to reduce interference, some laboratories modify the manufacturer methods. With hypotonic dialysis, DTT, ethylenediaminetetraacetic acid (EDTA), or heat inactivation, interference of C1 complex and IgM antibodies can be reduced.

With hypotonic dialysis of serum, IgM antibodies are precipitated, therefore reducing IgM interference. Procedure of hypotonic dialysis is simple and improves PRA specificity, but it is time consuming since it needs overnight incubation [31].

DTT disrupts disulfide bonds of IgM antibodies, therefore reducing IgM interference. 0.05 mol/L DTT 10 μL is added to 90 μL of serum and incubated at 37 °C for 30 min, and then centrifuged for 10 min before testing. DTT pretreatment is simple and fast, but there are some reports that DTT can also increase negative control beads' reactivity [31]. Some laboratories adjust DTT-to-serum ratio to reduce the effect of DTT on control beads.

Complement component C3 can also bind to bead, blocking anti-HLA antibody binding [32]. Heat inactivation of serum at 56 °C for 30 min or pretreatment of serum with EDTA can destroy complement activity [33].

Very high level anti-HLA antibodies can induce prozone effect. Very high level anti-HLA antibodies bind to HLA antigen coated beads, leading to tightly packed antibodies which might interfere binding of the detection antibodies, resulting in falsely low MFI [34]. In this case, serum dilution can be helpful.

Results are presented as antibody specificities and MFI value. One Lambda LABScreen® Single Antigen offers baseline normalized MFI value, which raw MFI of test serum is adjusted with negative control bead and negative control serum.

## Interpretation

SAB assay need comprehensive approach, considering patient's disease status, sensitization events, desensitization protocol used, recipient and donor's HLA type, and other related HLA antibody test results [35]. MFI represents degree of saturation, which is degree of antibody binding among total antigens expressed in beads [25]. Although the cutoff MFI 1,000–1,500 is generally used, MFI threshold may be modified on the basis of patient history, different HLA loci, or epitope/antigen groups [4]. De novo DSA after transplantation has clinical implication in relatively low MFI, and HLA-C and -DP antibodies are known to have clinical impact in higher MFI.

It is important to understand that SAB assay is not a quantitative assay, and MFI results are semiquantitative at best [35]. In FDA guideline for the qualification as a quantitative test, acceptable range of coefficient of variance (CV) is 15–25%. However, current CV for MFI variation is mostly more than 25% [36]. Therefore, smaller increases such as 1,000 MFI might not represent a real increase in antibody strength, instead it can be due to day-to-day variability [37]. STAR 2017 group recommended that differences of less than 25% in MFI values should not be interpreted as clinically meaningful [4].

With SAB assay result, calculated PRA (cPRA) can be derived. The cPRA is the percentage of donors with unacceptable HLA to which the patient has been sensitized [38]. The cPRA value is critical when counseling the wait time to highly sensitized patients waiting for deceased donor [39].

By comparing SAB assay and HLA typing results, virtual crossmatch is available. Donors without unacceptable antigens can be identified before having real crossmatch [39, 40].

## Limitations

Although SAB assay has revolutionized HLA antibody test, it still has limitations. Various factors can affect PRA result. Vendor, lot-to-lot variability, instrument type, operator, and reagents can all affect MFI value, causing low inter- and intra-laboratory reproducibility [41, 42].

False positive and false negative PRA results are possible. Since HLA molecule is artificially attached to microbead, hidden epitope can be exposed, resulting in false positive results [43, 44]. Or immunologically relevant epitope can be concealed, causing false negative results [35]. SAB assay does not represent every frequent alleles in every ethnicity, it can only include less than 100 HLA alleles. Therefore, antibodies against alleles which are not included in SAB assay cannot be detected. Because of the semi-quantitative nature of SAB assay, careful understanding should be needed when interpreting it. If the concentration of anti-HLA antibody is higher than the range measurable by SAB assay, MFI value does not change even if the concentration of anti-HLA antibody increases. Figure 2.1 shows an example of two HLA antibody titers with similar undiluted initial MFI value. Serum A contains excessive antibodies and remains saturated even with repeated dilution. However, serum B has antibodies enough to saturate bead initially, but not excessive. It means that there may be upper limitation for SAB assay, indicating high MFI value does not quantify how much antibody is present.

Unlike CDC, serum dilution is not routinely performed for FCXM and SAB assay; therefore prozone effect can occur [45]. This phenomenon manifests a strong positive cell based crossmatch in the absence of significant DSA in SAB assay. Figure 2.2 shows how the MFI values change with titration. Generally, antibody titer decreases as the serum is diluted. However, in this figure, some MFI values can increase with repeated dilution. Similarly, some patients with very high anti-HLA antibodies can show similar or increased SAB MFI after plasma exchange, due to prozone phenomenon. It is suggested that low MFI titer does not guarantee low level of anti-HLA antibody and neat MFI does not always reflect antibody strength. Various factors, such as IgM antibody, C1 complex, and IVIG, can also interfere PRA assay. To reduce these interferences, laboratories modify the manufacturer method as mentioned above.

Antibodies against public epitope may show low MFI, because these antibodies can react to multiple beads presenting HLA antigens with shared epitope.

SAB assay is a highly sensitive method to detect anti-HLA antibodies. Not all patients with DSA detected with SAB assay undergo ABMR or have poor graft survival [46]. Therefore, modified SAB assays which can

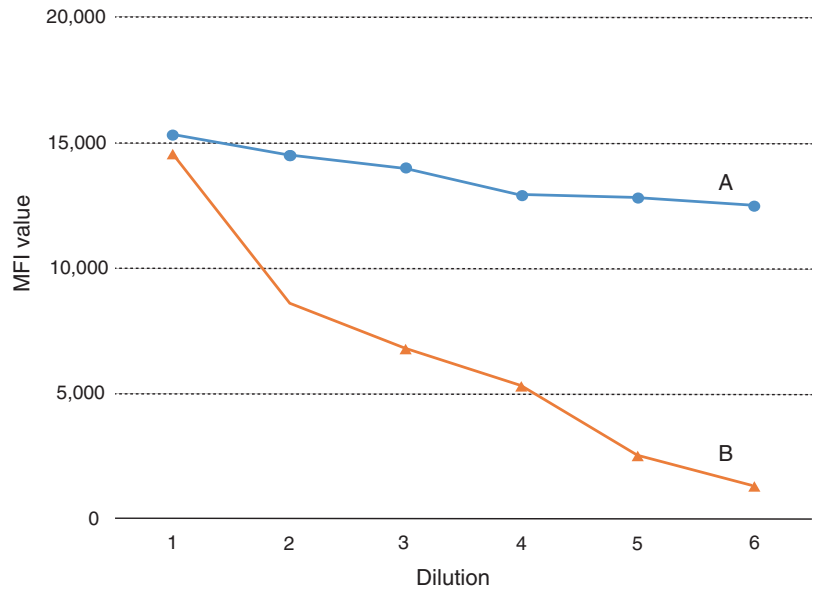


**Fig. 2.1** Difference of MFI values with dilution in SAB assay. A: Oversaturated; B: Not excessively saturated

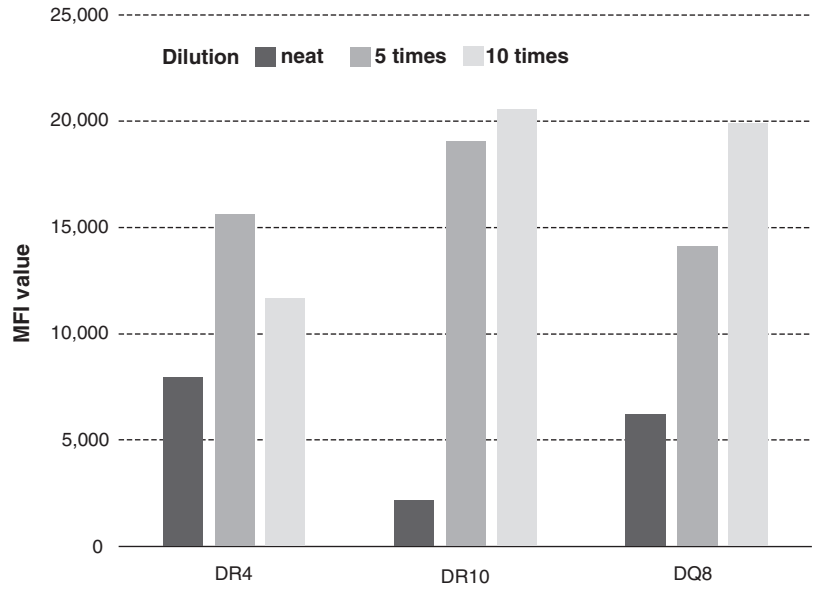


**Fig. 2.2** An example of prozone effect

selectively detect anti-HLA antibodies capable of fixing C1q or C3d were introduced.

## C1q SAB Assay

**Q: 1. What is the rationale of C1q-binding SAB assay?**
**2. Is this test positively correlated with the clinical outcome?**

Since standard IgG SAB assay cannot distinguish cytotoxic antibodies from noncytotoxic antibodies, C1q SAB assay has been developed to detect antibodies which can fix complements. C1q is the first component protein to constitute the complement pathway. Number of studies reported that C1q-binding anti-HLA antibodies are associated with high risk of ABMR and kidney allograft loss [47–49]. C1q SAB assay can be used for antibody screening and desensitization monitoring.

### Principle

C1q SAB assay is similar to IgG SAB assay except that it uses PE-conjugated anti-C1q antibody instead of PE-conjugated anti-human IgG antibody. C1q is spiked to patient serum and reacted. If the patient has sufficient anti-HLA antibody and C1q is bound to anti-HLA antibodies, PE-conjugated anti-C1q antibodies are subsequently bound and it is detected by reading the fluorescent.

### Methods

Patient sample is heat inactivated (56 °C for 30 min) to remove endogenous C1q. After heat inactivation, the serum is added to well plate and human complement C1q and beads expressing HLA antigens are added. After incubation, PE-conjugated anti-C1q is added and incubated. After washing steps, well plate is read with Luminex instrument.

Like IgG SAB assay, HLA laboratories treat serum with various methods to eliminate possible interference in C1q SAB assay. Some laboratories add AHG to increase sensitivity [50].

### Interpretation

Like IgG SAB assay, results are shown as antibody specificity and MFI. With manufacturer provided program, raw MFI and normalized MFI value (normalized with negative control serum and negative control bead) is available. Laboratories set their own cutoffs as MFI 300, 500, 1,000, or some use individual cutoff by adding 1,000 to the lower MFI showing increase of

300 MFI for the first time after sorting MFI from lowest to highest [51].

### Limitations

Clq binding to the antigen/antibody complex is the first step in the classical pathway, but anti-HLA antibodies capable of C1q fixing does not necessarily mean that subsequent complement cascade will occur leading to cell lysis [52]. Some argue that the relationship between C1q assay and clinical outcome may be affected by IgG subclass or titer [51]. There are some reports that C1q SAB results are associated with high IgG SAB MFI when interference is reduced through serum pretreatment [50, 53].

Later-developed C3d assay detects the later part of the complement cascade and thus theoretically better reflects the occurrence of the complement cascade.

## Epitope Analysis

**Q: What is the rationale of epitope analysis in pre-op sensitized renal transplant patients?**

Epitope is a part of the antigen that contacts with complementary determining region of an antibody. In the center of the structural epitope lies functional epitope that determines the specific binding of antibody. Duquesnoy et al. introduced concept of eplet which is functional epitope of HLA within radius of 3.0–3.5 Å [54].

The basic concept of HLA epitope analysis is that anti-HLA antibodies are directed against epitopes rather than whole antigen. And it is assumed that patient's anti-HLA antibodies do not react with the self-epitope to cause immune response. With patient and donor's high-resolution HLA type and SAB assay results, epitope matching can be done. It can be analyzed using a free software, HLA-Matchmaker (http://www.epitopes.net). However, the eplets provided by the HLA-Matchmaker are the theoretical epitopes, including those that are not actually confirmed to produce an antigen-antibody reaction. HLA epitope registry (http://www.epregistry.com.br) database offers a list of antibody-verified epitopes.

There may be mismatch at the antigen level, but at the epitope level there may be no mismatch. Epitope matching is especially helpful for highly sensitized patients waiting for a deceased donor to reduce transplantation waiting time and to improve clinical outcome [55]. Eurotransplant conduct Acceptable Mismatch program for highly sensitized (cPRA ≥85%) patients and employs HLA-Matchmaker to define acceptable mismatch [56].

Since there are less than 100 alleles in SAB assay, not every HLA alleles can be analyzed with present SAB assay. Some insist that by using epitope matching, unacceptable epitope can be found and HLA alleles carrying the same epitope could be defined as unacceptable [57].

## Correlation of Each Methodology

**Q: How can we interpret the various test results?**

When interpreting HLA antibody test results, related HLA tests (donor/recipient HLA typing, CDC, FCXM, SAB) should be interpreted together, considering patient's disease status, desensitization protocol used, and sensitizing history such as previous transplantation, pregnancy, and transfusion history.

CDC, FCXM, and SAB assay detect different types of antibodies and have difference in sensitivity. In addition, since these tests have inherent variability and are tested with modifications, cut-offs and correlation of each test may vary between laboratories.

CDC can detect not only HLA antibodies but also non-HLA antibodies. In this case, auto-control or pretreatment of serum to remove IgM antibodies can help. FCXM is more sensitive

**Table 2.2** Risk assessment of various test results

| CDC | FCXM | SAB | Sensitization history | HLA molecular MM | Risk assessment |
|---|---|---|---|---|---|
| + | + | + | | | Active memory, at risk for hyperacute rejection |
| – | + | + | | | Active memory, at risk for ABMR and TCMR |
| – | – | + | | | Active memory, at risk for ABMR and TCMR |
| – | – | – | Pregnancy or prior transplant with repeat MM | | At risk for latent memory with a recall B and T cell response |
| – | – | – | – | High | Increased risk for de novo alloimmunization |
| – | – | – | – | Low | Baseline risk for de novo alloimmunization |
| – | – | – | – | 0 | Low risk for de novo alloimmunization |

*CDC* complement-dependent cytotoxicity; *FCXM* flow cytometry crossmatch; *SAB* single antigen bead assay; *MM* mismatch. Modified and reprinted with permission by Tambur AR, Campbell P, Claas FH et al. from American Journal of Transplantation [4].

than CDC, but it cannot distinguish between HLA and non-HLA antibodies, and cytotoxic and noncytotoxic antibodies. IgG SAB is the most sensitive to detect HLA antibodies, and clinically insignificant low-level HLA antibodies may also be detected. IgG SAB cannot distinguish between complement-fixing and non-complement-fixing antibodies. C1q SAB can detect HLA antibodies which can fix complement, but it does not mean that it is necessarily cytotoxic (Table 2.2) [4].

## Cases of Sensitized Patients

### A Case with Low-Level Anti-Class I and -Class II HLA Antibodies

56-year-old female patient with previous history of pregnancy was evaluated for renal transplantation from living related donor. CDC was negative, T cell FCXM and B cell FCXM was positive. SAB assay showed she had B54 (MFI 6,228), DQ6 (2,510), and DR15 (1,941) DSAs. She was treated with rituximab 200 mg and went through four sessions of plasmapheresis. After desensitization, T cell FCXM became negative and SAB assay showed DSA B54 (1,792). Since she was treated with Rituximab, CDC and B cell FCXM were not evaluated. After one more session of plasmapheresis, renal transplantation was done. Creatinine was normalized on the second postoperative day (POD). At POD 4, follow-up HLA assays were done and T cell FCXM was negative and SAB assay showed no DSAs. At POD 36, DSA B54 (3,334) was detected but her creatinine level was normal and stable, below 0.7 mg/dL. Her creatinine level was 0.64 mg/dL at POD 8 months and stable up until now.

### A Case with High-Level Anti-Class II HLA Antibodies

49-year-old female patient with previous history of pregnancy was evaluated before renal transplantation. AHG T cell CDC and long incubation T cell CDC was negative, but B cell CDC was positive up to 1:2 dilution. SAB IgG assay showed DSA as B61 (5,716), DR8 (13,286), and DQ6 (11,869) with SAB C1q assay DSA DR8 (5,857). She was desensitized with rituximab 200 mg, five sessions of both plasmapheresis and IVIG 200 mg/kg. After desensitization, follow-up SAB IgG and SAB C1q assay revealed IgG DSA B61 (1,911), DR8 (9,089), and DQ6 (5,527) with negative C1q DSA. After one more session of plasmapheresis, renal transplantation was done. Her creatinine level normalized since POD 2. Follow-up SAB assay done at POD 4 revealed DSA DR8 (2,615) and DQB1∗06:01 (1,386). Another follow-up SAB assay done at POD 11

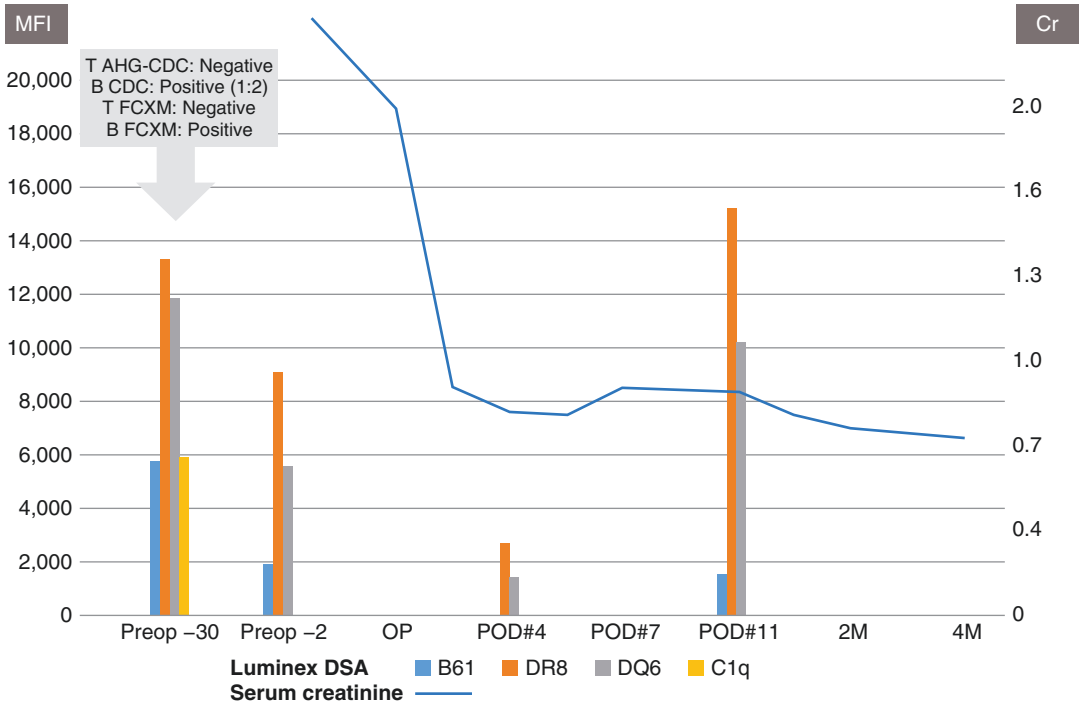


**Fig. 2.3** Pre- and post-desensitization in patient two

showed DSA B61 (1,534), DR8 (15,238), and DQ6 (10,196). Although high-level DSA was present, her creatinine level has been stable below 0.8 mg/dL for 4 months post-transplantation up until now (Fig. 2.3).

## A Case with CDC(+) and C1q(+) Anti-HLA Antibodies but with Stable Postoperative Course

57-year-old female patient with history of previous pregnancy and renal transplantation was evaluated for second renal allograft from her son. Preoperative AHG T cell CDC was positive (1:1) and B cell CDC was positive (≥1:32). T and B cell FCXM were both positive. SAB IgG showed A24(9,782), DR52(3,328), DQ5(7,666) DSAs, and SAB C1q assay detected DQ5(20,801).

Desensitization with rituximab (500 mg), plasmapheresis (21 sessions), and one cycle of bortezomib was done. Follow-up study showed negative T cell FCXM and DSA as A24 (10,574), DQ5(12,429), and C1q DQ5(22,635). Following renal transplantation, urine output was normal and serum creatinine showed 0.93 mg/dL at POD 3.

Postoperative T cell and B cell FCXM were still positive, and SAB IgG DSA was A24(6,209), DQ5(8,153), and C1q as DQ5(25,290) at POD 4. Even though postoperative DSA persisted, her renal function was good with serum creatinine as 0.67 mg/dL. Renal biopsy at postoperative 2 years and 4 months showed suspicious TCMR with g1, cg0, pct0, c4d0 (0%) and trivial CAN (chronic allograft nephropathy). She was treated with steroid pulse (1.5 g), plasmapheresis (5 sessions), and rituximab (200 mg). PRA IgG and C1q DSAs were persistent even under normal renal function.

At 4 years and 5 months after transplantation, second renal biopsy showed suspicious chronic active ABMR with g1, cg1, ptc1 (focal), c4d0 (0%), and mild CAN. She was treated with steroid pulse (1.5 g), plasmapheresis (4 sessions), IVIG (200 mg/kg ×4), and rituximab (100 g). Follow up PRA IgG and C1q assay were done every two to three month, and DQ DSA MFI remained over MFI 10,000 and

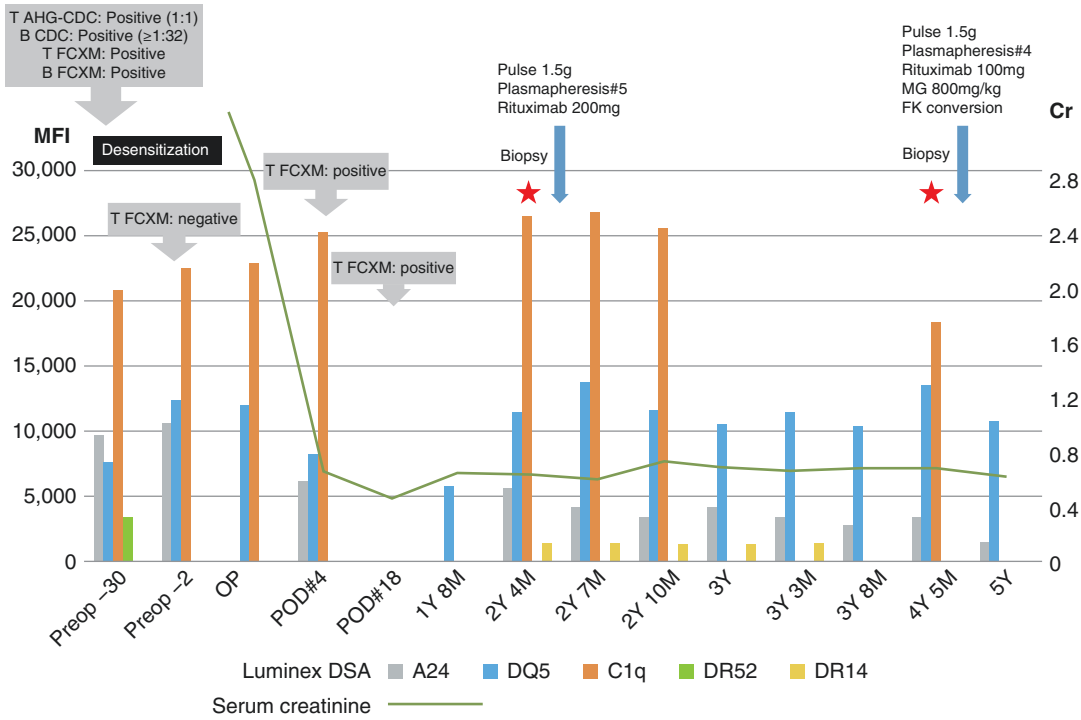


**Fig. 2.4** Pre- and post-desensitization in patient three

creatinine level has been stable below 0.8 mg/dL for five years post transplantation up until now (Fig. 2.4). It is known that HLA-DQ is not always expressed on kidney endothelium but its expression is induced by inflammatory cytokines [58]. Therefore, it needs longer time and higher MFI to induce graft dysfunction compared to non-DQ DSA. For de novo DQ DSA, average time to graft dysfunction after its detection was 11 months and MFI over 5,000-10,000 is associated with ABMR [59–62]. ABMR due to preformed DSA occurs earlier compared to de novo DSA ABMR, treated more aggressively and shows better graft survival [63].

## A Case with High-Level Anti-Class II HLA Antibodies with Postoperative DSA(−) ABMR

62-year-old female patient with diabetes was evaluated for renal allograft from her daughter. Preoperative CDC and T cell FCXM was negative but B cell FCXM was positive. SAB IgG assay showed B∗13;02(1,242), DR7(13,202), DQ2(2,609) DSA, and SAB C1q assay was negative.

Desensitization with rituximab (500 mg), plasmapheresis (4 sessions), and IVIG (300 mg/kg ×4) were done. Due to persistent DR7(7,662), three more sessions of plasmapheresis and IVIG were treated.

Following renal transplant, immediate postoperative course was not eventful. But at POD 4, creatinine elevated from 1.11 to 2.74 mg/dL. SAB DSA was negative. But under impression of ABMR, steroid pulse (1 g), one cycle of bortezomib, plasmapheresis (9 sessions), and IVIG were treated but without response. At POD 29 under hemodialysis, biopsy showed ATN, suspicious TCMR, and C4d(−) ABMR. DR7(1,316) DSA persisted. Treatment was restarted with steroid pulse (1 g), bortezomib, plasmapheresis (4 sessions), and IVIG. After then renal function recovered with increased urine flow. At POD 45, she was discharged with creatinine 1.30 mg/dL. Her renal function is stable with creatinine 0.81 mg/dL with no DSA for POD eight months up until now.

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# Preoperative Management (Desensitization)

3

Chan-Duck Kim

**Q: What kinds of desensitization protocols and agents can be used to overcome the HLA sensitization barrier in highly HLA-sensitized patients?**

1. Overview of desensitization protocol
2. Plasmapheresis or immunoadsorption for desensitization
3. IVIG for desensitization
4. Rituximab for desensitization
5. Proteasome inhibitors for desensitization
6. Complement inhibitors for desensitization
7. Anti-IL-6 receptor blockers for desensitization
8. IgG-degrading enzyme of *Streptococcus pyogenes* (IdeS) for desensitization

## Overview of Desensitization Protocol

Kidney transplantation is considered the treatment of choice for patients with end-stage kidney disease, owing to prolonged survival and improved quality of life [1]. The determination of suitable donor kidneys for transplant candidates become sensitized against human leukocyte antigen (HLA), which occurs mainly through blood transfusion, pregnancy, and previous organ transplantation, is a major challenge and sensitized patients have a reduced chance of receiving a crossmatch-negative organ [2]. Increasing degrees of HLA sensitization give rise to a higher chance of a positive crossmatch to potential donor kidneys. This is improper since transplantation with an HLA-incompatible donor is related with a higher rate of rejection [3] and higher rates of posttransplant graft loss and death [4]. However, desensitization appears to be a survival benefit after kidney transplantation from an HLA-incompatible donor compared with remaining on the transplant waiting list, and many HLA-sensitized patients either suffer more time on dialysis while waiting or are unlikely to find a compatible donor [5]. Desensitization protocols started to emerge in the mid-1990s. The goals of HLA desensitization therapies are to remove the presence of circulating HLA alloantibody and to prevent antibody-mediated rejection (ABMR). Desensitization therapies may be performed for a

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potential recipient of a living-donor allograft with defined HLA antibody against the donor HLA in an attempt to reduce the level of those donor-specific antibodies (DSA). It can also be used for an individual on the deceased-donor allograft waiting list broadly sensitized to HLA antigens, which usually mean panel-reactive antibody (PRA) ≥30%, with repeated positive results of crossmatch. The goals of desensitization are comparable in both situations, but the approach may be slightly different because of the unpredictable timing of deceased-donor kidney transplantation with reference to starting of desensitization therapy. Various desensitization protocols have been employed to facilitate HLA-incompatible transplantation and are diverse at different transplant centers depending on clinical experience and preference. Although there are no high-quality data in forms of randomized controlled trials comparing existing desensitization approaches and no uniformly accepted HLA desensitization protocol, desensitization regimens that involve plasmapheresis or immunoadsorption followed by low-dose (100 mg/kg) or high-dose (2 g/kg) intravenous immunoglobulin (IVIG) and depletion of the B cell population responsible for anti-HLA antibody production, most commonly with the anti-CD20 agent rituximab, have become the standard of care.

## Plasmapheresis or Immunoadsorption for Desensitization

**Q: Dose plasmapheresis or immunoadsorption effectively remove anti-HLA antibodies?**

Plasmapheresis or immunoadsorption have been used for desensitization therapy. They are a procedure that can eliminate immunoglobulin from a patient's sera and has been shown to lower HLA-specific alloantibody levels in many clinical settings [6]. Current methods for the elimination of HLA antibodies before transplantation are standard plasmapheresis, double-filtration plasmapheresis, and immunoadsorption. Desensitization is usually practiced by plasmapheresis in the USA and immunoadsorption is widely performed in the Europe. Plasmapheresis is not specific for alloantibody elimination and results in a lowering of all plasma proteins, including many clotting factors, and needs replacement with albumin or fresh frozen plasma. Immunoadsorption offers the opportunity for selective depletion of immunoglobulins without a need for substitution with fresh frozen plasma or albumin. There are two types of clinically available columns. One column contains highly purified protein A (isolated from *Staphylococcus aureus*) bound to a silica matrix (Immunosorba, Fresenius Medical Care, Bad Homburg, Germany). The other column, the Globaffin (Fresenius Medical Care) column, is a synthetic broadband immunoadsorber that contains a synthetic peptide (GAM) as a ligand. Both columns have a high affinity with a Fc segment, especially for IgG class antibodies [7]. The advantages of immunoadsorption over plasmapheresis are specificity, a greater amount of antibody removal, and the elimination of the requirement to replace large volumes of fresh frozen plasma. One treatment course with plasmapheresis or immunoadsorption results in a 15–20% reduction and three to six courses of treatment result in greater than 90% reduction in plasma alloantibody levels. However, anti-HLA alloantibody titers rebound and go back to baseline levels within a few weeks after the completion of plasmapheresis or immunoadsorption [8]. Moreover, compared to plasmapheresis, immunoadsorption is associated with better tolerability and a lower likelihood of allergic reactions, and therefore allows the treatment of larger plasma volumes with higher antibody reduction rates. Peritransplant immunoadsorption as a strategy enabling transplantation in highly sensitized crossmatch-positive cadaveric kidney allograft recipients was mainly investigated by a group from Vienna, Austria [9]. This group recently reported on a series of 101 DSA-positive recipients of deceased-donor kidney transplant who were subjected to immunoadsorption-based desensitization [10]. All patients received an immunoadsorption session before transplant and immunoadsorption was performed on a daily

basis postoperatively to decrease MFI to <3,000. The authors compared these results with those from a control group consisting of 513 DSA-negative kidney transplant recipients treated during the same period. The 3-year graft-survival rate in DSA-positive patients was significantly lower compared with those of DSA-negative patients (79 and 88%, respectively, $P < 0.01$). One-third of DSA-positive patients experienced acute ABMR and their DSA MFI value was significantly associated with the development of acute ABMR, showing 20 vs. 71% ABMR incidence with a MFI of <5,000 compared with >15,000 peak MFI, respectively. The authors concluded that immunoadsorption-based desensitization failed to prevent ABMR in one-third of DSA-positive patients in the setting of deceased-donor kidney transplant and that assessing the DSA MFI could help stratify for risk of rejection. In the study of Klein et al. [11], they reported on a series of 23 desensitized DSA-positive recipients of living-donor kidney transplant where the goal was to maintain MFI at <1,000. Patients received a median of eight apheresis treatments before and five treatments after transplantation. The induction therapy was based on either ATG or basiliximab. The 2-year graft survival rate was 100%, with excellent renal function (median serum creatinine level of 1.42 mg/dL) and protein-to-creatinine ratio of 0.12. Acute T cell mediated rejection was diagnosed in one patient (4%), and antibody-mediated changes were found in five patients (22%). The authors suggest that their treatment algorithm for desensitization of living donor kidney transplant recipients with DSA using immunoadsorption results in good graft outcomes with a low rate of side effects.

## IVIG for Desensitization

**Q: 1. Are there randomized trials comparing the use of plasmapheresis followed by low-dose IVIG with high-dose IVIG for desensitization?**
**2. Is desensitization protocol using plasmapheresis followed by low-dose IVIG successful?**

IVIG is a complex preparation derived from the gamma globulin fraction of pooled human plasma applied to treat hypogammaglobulinemia and various autoimmune disorders. It modulates the immune response via diverse mechanisms and broad mechanisms have been proposed in which IVIG inhibits the immune response at multiple pathway [12]. These mechanisms include neutralization of circulating antibodies, alteration of cytokine production, inhibition of B and T cell proliferation via interactions with Fc receptors, and downregulation of complement [13, 14]. Studies from humans receiving high-dose IVIG are strongly supportive of an interaction of IVIG with macrophages and dendritic cells which suppresses dendritic cells maturation [15]. IVIG infusions result in an upregulation of the Th2 cytokine (IL-4 and IL-13) and these Th2 cytokines decrease expression of the inflammatory FcγRIIA resulting in a net upregulation of the inhibitory receptor FcγIIB. FcγIIB is the only FcR on B cells and plasma cells. These actions results in plasma cell apoptosis and reduced B cell activation and antigen-presenting cell activity [16]. It therefore has powerful immunomodulatory effects and is now widely used for desensitization.

There are no randomized trials comparing the use of plasmapheresis followed by low-dose IVIG with high-dose IVIG for desensitization. One retrospective study has directly compared three different desensitization regimens in living-donor kidney transplant recipients (KTRs) with a positive T cell complement-dependent cytotoxicity (CDC) crossmatch [17]. In this study, the following three protocols were employed: high-dose IVIG (2 g/kg) given 1–3 days prior to transplant; plasmapheresis, low-dose IVIG (100 mg/kg), and rituximab 375 mg/m$^2$; and plasmapheresis, low-dose IVIG (100 mg/kg), and rituximab 375 mg/m$^2$ with pretransplant antithymocyte globulin (ATG) and intensive posttransplant DSA monitoring. Achieving a negative CDC crossmatch was significantly more likely with both plasmapheresis protocols versus high-dose IVIG (84, 88, and 38% for plasmapheresis/low-dose IVIG/rituximab, plasmapheresis/low-dose IVIG/rituximab/ATG with intensive posttransplant DSA monitoring, and high-dose IVIG groups, respectively). Patients with low baseline antibody titers

<1:4 were successfully desensitized by any of the three protocols. In patients with baseline titers of 1:8 and 1:16, the desensitization rate in the IVIG group was 33%. When the two plasmapheresis/low-dose IVIG groups were combined, the success of desensitization with baseline titers of 1:8 and 1:16 was 87%. Three of the eight patients not responding to the high-dose IVIG protocol did respond when switched to the plasmapheresis/low-dose IVIG protocol. Only one of ten patients with a baseline antibody titer >1:32 achieved a negative crossmatch regardless of the three desensitization protocols. Among patients who were transplanted, rates of ABMR were 80, 37, and 29% for patients receiving high-dose IVIG, plasmapheresis/low-dose IVIG/rituximab, and plasmapheresis/low-dose IVIG/rituximab/ATG with intensive posttransplant DSA monitoring, respectively. However, it should be noted that patients desensitized with high-dose IVIG did not receive rituximab or posttransplant administration of IVIG, which may have contributed to their higher rate of ABMR. These findings suggest that high-dose IVIG and plasmapheresis/low-dose IVIG/rituximab are similarly effective for desensitization in patients with a low titer of positive T cell CDC crossmatch, but patients with high titers do not respond well to either desensitization protocols. Given the high rates of ABMR with all three desensitization protocols, it seems reasonable to pay special attention when pursuing a transplant with a positive CDC crossmatch. In addition, whether or not the administration of rituximab or the routine posttransplant administration of IVIG would be of benefit in reducing the incidence of ABMR in a high-dose IVIG protocol was unclear at that time.

For desensitization prior to living-donor kidney transplantation, many transplant centers use a protocol that consists of plasmapheresis followed by low-dose IVIG. In this protocol, alternate-day plasmapheresis is performed prior to transplantation, and IVIG is used at a dose of 100 mg/kg after each session. Some centers used a modified protocol adding rituximab with varying degree of dose to plasmapheresis and low-dose IVIG. The number of pretransplant treatments is decided based upon the baseline DSA titer. Tacrolimus, mycophenolate mofetil, and steroid are typically started with the initiation of plasmapheresis, although this practice may different from center to center. If the DSA levels and pretransplant crossmatch results are considered acceptable at each transplant center, kidney transplantation will proceed. Some centers routinely perform plasmapheresis after kidney transplantation, while others only perform posttransplant plasmapheresis for the treatment of acute ABMR. Montgomery et al. [18] reported one of the first long-term experiences with desensitization using a regimen consisting of plasmapheresis and low-dose IVIG (100 mg/kg). In this high-volume single center study, living-donor kidney transplantation following the successful desensitization in the recipient ($n$ = 211) provided a statistically significant survival benefit compared with waiting for a compatible organ or remaining on dialysis (8-year survivals of 80.6, 49.1 and 30.5%, respectively; $P < 0.001$). These findings were corroborated in a large multicenter study that included patients with varying strengths of pretransplant DSA [5]. In a 22-center study, they estimated the survival benefit for 1,025 kidney transplants recipients from HLA-incompatible live donors who were matched with controls who remained on the waiting list or received a transplant from a deceased donor (waiting-list-or-transplant control group) and controls who remained on the waiting list but did not receive a transplant (waiting-list-only control group). Kidney transplants recipients from incompatible live donors who were undergoing perioperative desensitization therapy had a higher survival rate than either control group at 1 year (95.0 vs. 94.0% for the waiting-list-or-transplant control group and 89.6% for the waiting-list-only control group), 3 years (91.7 vs. 83.6% and 72.7%, respectively), 5 years (86.0 vs. 74.4 and 59.2%), and 8 years (76.5 vs. 62.9 and 43.9%) ($P < 0.001$ for all comparisons with the two control groups).

Our center also reported our experiences of living donor kidney transplantation using desensitization protocol of pretransplant plasmapheresis and low-dose IVIG with or without rituximab in highly sensitized patients [19]. Seven patients

with positive-crossmatch tests or high levels of panel-reactive antibody (PRA) were included. Six patients were crossmatch-positive, and one patient was crossmatch-negative but had high PRA levels. The mean follow-up period was 33.2 months after transplantation. The all patients showed no acute ABMR episodes for follow-up period and the patient and graft survival rates were 100%. The mean serum creatinine concentration at last follow-up was 0.92 mg/dL. Our experiences also suggest that the combination of plasmapheresis, low-dose IVIG with or without rituximab may prove effective as a desensitization regimen for positive-crossmatch and/or highly sensitized living donor renal transplant recipients. Further, we retrospectively analyzed our center's outcome of HLA incompatible kidney transplantation (HLAi-KT) from 2012 to 2018 (Fig. 3.1) [20]. HLAi-KT defines the recipients had positive CDC crossmatch or flow cytometry crossmatch (either T or B cell) before KT. There were 55 cases of HLAi-KT and 428 cases of HLA compatible KT. The mean follow-up duration was 42.5 months in HLAi group and 35.4 months in control group. Incidence of biopsy-proven acute rejection was 5.4% (3 of 55) in HLAi group and 7.5% (32 of 428) in control group ($P > 0.05$). All three HLAi-KTRs with rejection had ABMR, but ABMR rate was 37.5% (12 of 32) in control group. In addition, ABMR episodes within 1 year after transplantation were 3.6% in HLAi group (2 of 55), while 0.9% in compatible KTRs (4 of 428). The number of patients who underwent graft failure was 1 in HLAi group (1.8%) and 11 in control group (2.6%; $P > 0.05$). The comparison of consecutive estimated glomerular filtration rates showed no differences between groups except for the 14 days after transplantation. In summary, both sensitized and non-sensitized KTRs showed excellent outcomes in rejection rate, graft survival, and graft function. Our larger and longer follow-up data support the consistent effectiveness of plasmapheresis followed by low-dose IVIG as

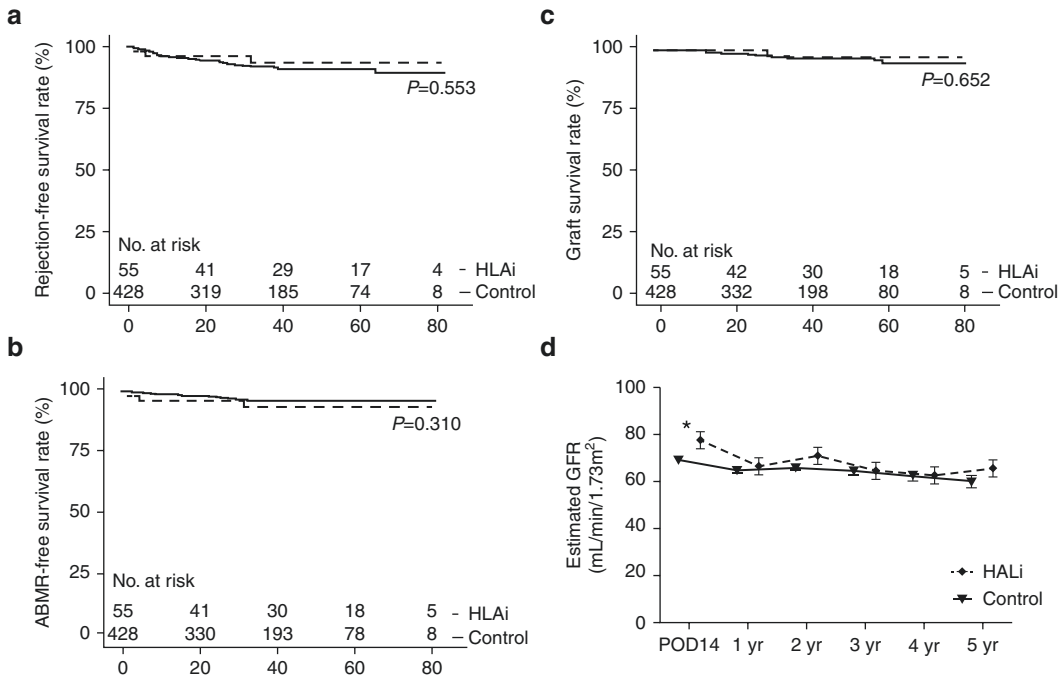


**Fig. 3.1** Comparisons of graft outcome between sensitized and non-sensitized kidney transplantation. (**a**) Biopsy-proven acute rejection free survival. (**b**) Biopsy-proven active antibody mediated rejection free survival. (**c**) Death-censored graft survival. (**d**) Serial changes of graft function. $*P < 0.05$ versus control. Abbreviations: *ABMR* antibody mediated rejection, *GFR* glomerular filtration rate, *HLAi* HLA incompatible

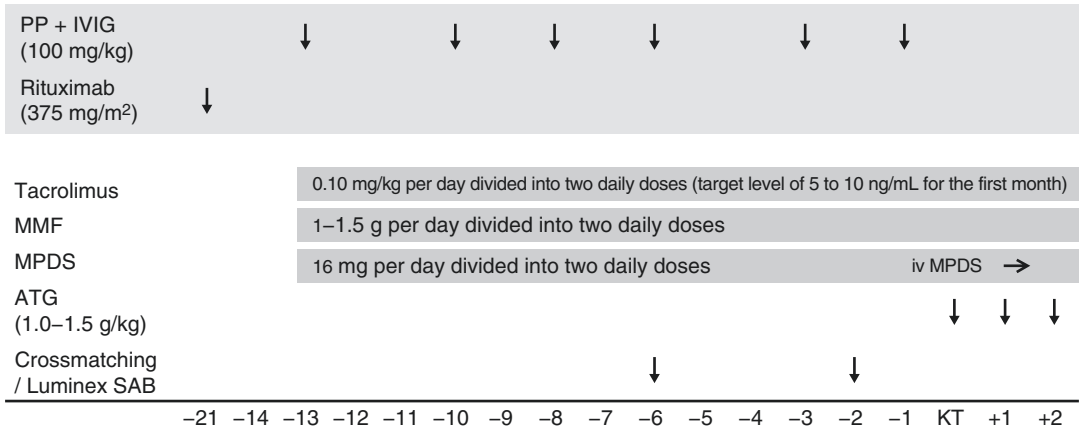


**Fig. 3.2** Desensitization protocol in Kyungpook National University Hospital. Abbreviations: *ATG* antithymocyte globulin, *IVIG* intravenous immunoglobulin, *MMF* mycophenolate mofetil, *MPDS* methylprednisolone, *PP* plasmapheresis, *SAB* single antigen bead

a desensitization regimen for positive-crossmatch. Desensitization protocol of our center is depicted in detail in Fig. 3.2. Briefly, the protocol included plasmapheresis, IVIG, and induction anti-thymocyte globulin (ATG). 375 mg/m$^2$ dose of rituximab was administered in 3 weeks before transplant. Plasmapheresis was initiated 10 to 14 days before KT and performed every other day. After each plasmapheresis, low-dose IVIG (0.1 g/kg) was injected. For induction immunosuppression, we used ATG (1.0–1.5 g/kg) for 3 days (day 0 to 2).

## Rituximab for Desensitization

**Q: Dose addition of rituximab to high-dose IVIG appears to be superior to high-dose IVIG alone for the desensitization?**

Rituximab is a chimeric murine-human monoclonal antibody that binds to CD20 [21]. CD20 is expressed early in B lymphocyte ontogeny in immature bone marrow-resident pre-B cells up to the point of initiation of plasma cell differentiation in secondary lymphoid tissues. This broad expression of CD20 in multiple immature and mature B cell populations results in a substantial elimination of pre-B and mature B lymphocytes by rituximab. It is the United States Food and Drug Administration (FDA) approved for treatment of B cell lymphomas and is also used for treatment of posttransplant lymphoproliferative disease (PTLD) and several autoimmune diseases. Rituximab has been used off label in desensitization protocols for HLA incompatible kidney transplantation or in the treatment of ABMR. Plasma cells and pro-B cells do not have surface CD20 expression, which decreases the effectiveness of rituximab treatment on inhibition of alloantibody production. Rituximab can be detected for months, and B cell recovery takes 6 to 12 months after the completion of the use of rituximab.

Investigators at Cedars Sinai Medical Center have reported that a combination of high-dose IVIG and rituximab produced better outcomes than high-dose IVIG alone for recipients of living-donor or deceased-donor kidney transplants [22]. In this trial, 20 highly sensitized patients received high-dose IVIG (2 g/kg on days 0 and 30) plus rituximab (1 g on days 7 and 22). After receiving this regimen, patients were eligible for kidney transplantation from either a living or deceased donor. With this approach, 16 of 20 patients were able to undergo kidney transplantation. At 1 year, the mean serum creatinine concentration was 133 μmol/L, and mean patient and allograft survival rates were 100 and 94%, respectively.

The combination protocol of high-dose IVIG and rituximab also decreased the PRA levels from 77% before treatment to 44% after treatment and lowered the mean time to transplantation from 144 months on dialysis prior to treatment to 5 months on dialysis after treatment. However, this protocol was also associated with an extremely high acute rejection rate of 50%, of which 31% were ABMR. However, most episodes were reversible. In addition, although the mean PRA decreased, 69 and 19% of patients had a positive flow cytometry crossmatch and a positive CDC crossmatch at the time of transplant, respectively. In a follow-up study of 76 highly sensitized patients who 75% of patients were PRA $\geq$ 80%, desensitization using high-dose IVIG (2 g/kg on days 1 and 30) and rituximab (1 g on day 15) reduced class I and II flow cytometry PRA levels and flow cytometry crossmatch median channel shifts (MCS), permitting transplantation in all patients [23]. They suggest that the addition of rituximab to high-dose IVIG appears to be more efficacious to high-dose IVIG alone for the desensitization of highly sensitized transplant candidates. One randomized study compared the use of high-dose IVIG plus rituximab with high-dose IVIG plus placebo for desensitization in highly sensitized kidney transplant candidates awaiting deceased-donor kidney transplantation [24]. Patients were randomly allocated to receive high-dose IVIG (2 g/kg, maximum dose 140 g) on days 1 and 20 with either rituximab (1 g) or placebo on day 15. The study intended to enroll 90 patients but was ceased early after only 15 patients were enrolled due to the development of three serious adverse events, all of which were occurrence of ABMR. Of the 15 enrolled patients, 13 received kidney transplants (six in the high-dose IVIG plus rituximab group and seven in the IVIG plus placebo group). The study revealed that all episodes of ABMR occurred among patients who received high-dose IVIG plus placebo and no DSA rebound was seen in those receiving high-dose IVIG plus rituximab. In addition, there were two cases of graft loss in the high-dose IVIG plus placebo group compared with none in the high-dose IVIG plus rituximab group. In the studies from Cedars Sinai Medical Center (Fig. 3.3) [22–24], they concluded that rituximab was an important adjunct for desensitization because of its ability to suppress memory B cell activation and DSA rebound. However, Marfo et al. conducted a prospective cohort study in highly sensitized kidney transplant candidates with a calculated panel-reactive antibody (cPRA) greater than 50% and on the deceased-donor waiting list for more than 5 years to investigate the effects of high-dose IVIG and rituximab treatment [25]. Desensitization protocol included two doses of high-dose IVIG (2 g/kg, max 120 g each dose) and a single dose of rituximab (375 mg/m$^2$). After a mean follow-up of 334 days, only two patients received a kidney

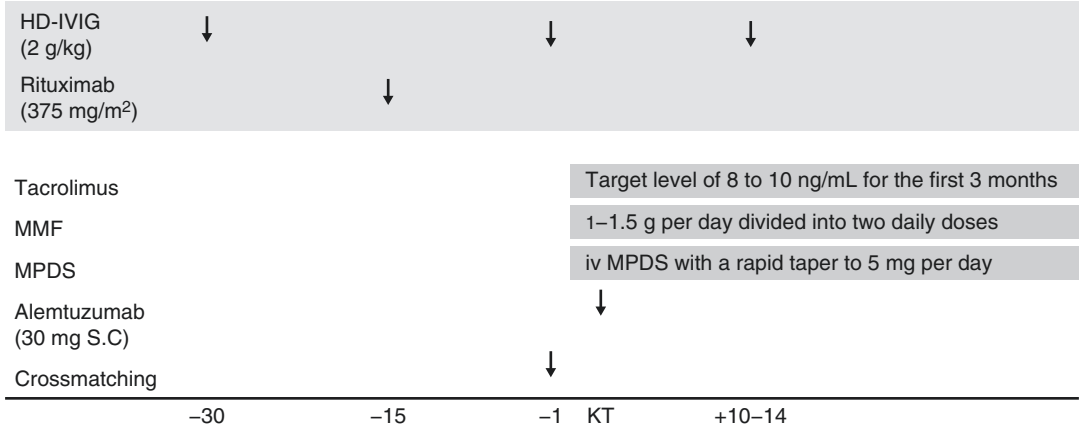


**Fig. 3.3** Desensitization protocol in Cedars-Sinai Medical Center. Abbreviations: *HD-IVIG* high-dose intravenous immunoglobulin, *MMF* mycophenolate mofetil, *MPDS* methylprednisolone

transplant compared with 14 patients in the non-desensitized group (18 vs. 52%). Desensitization treatment did not lead to any significant reduction in patients' class I and II cPRA level. There was also no change in the number of unacceptable antigens or their strength as measured by the mean fluorescence intensity (MFI). This study suggested that high-dose IVIG and a single dose of rituximab treatment in highly sensitized kidney transplant candidates at the top of the waiting list with cPRA levels greater than 90% was not successful in decreasing the strength and the levels of alloantibodies and increasing transplant rate, and future studies combining other anti-B lymphocyte and plasma cell agents are required to help such patients.

In the study of Van Den Hoogen et al. [26], the authors reported the result of a placebo-controlled trial of rituximab as an induction agent for KTRs. Two hundred and eighty patients were enrolled. One hundred thirty eight KTRs were randomized to rituximab and 142 KTRs were randomized to placebo. After 6 months, there was no difference in graft rejection rates. However, immunologically high-risk patients (PRA >6% or re-transplant) not receiving rituximab had a significantly higher incidence of rejection compared to those of rituximab-treated immunologically high-risk patients (38.2% vs. 17.9%, $p = 0.004$). The authors conclude that a single dose of rituximab given as an induction agent significantly reduces rejection rates in immunologically high-risk patients.

## Proteasome Inhibitors for Desensitization

**Q: Dose bortezomib-based desensitization protocol allowed kidney transplant candidates to achieve a negative crossmatch?**

Proteasome inhibitors such as bortezomib, a selective inhibitor of the 26S proteasome, was developed and approved by the FDA for the treatment of multiple myeloma and mantle cell lymphoma. Bortezomib inhibits antibody production from plasma cells, mediates apoptosis of this cell type, and decreases the number of bone marrow derived plasma cells through the mechanism of the disruption of the normal intracellular protein degradation process [27]. Therefore, it is anticipated to have strong suppressive effects on humoral immunity and may represent a promising desensitization agent. However, bone marrow derived long-lived plasma cells produce far less amount of protein than do malignant cells, and it would not necessarily anticipate equivalent response rates to proteasomal inhibition like as multiple myeloma [27]. Bortezomib has been used for the treatment of ABMR [28] and reported to provide significant and sustained reductions in anti-HLA antibody levels when used in combination with other therapies such as plasmapheresis and IVIG [29]. Woodle et al. performed a prospective iterative trial of bortezomib-based therapy for desensitization in highly sensitized patients [30]. Desensitization treatment included 6–8 bortezomib doses (1.3 mg/m$^2$/dose), plasmapheresis and one rituximab dose (375 mg/m$^2$, maximum dose 500 mg). Each bortezomib dose was preceded by intravenous methylprednisolone (100 mg for first two doses and 50 mg for following doses). HLA antibody reductions were observed in 38 of 44 (86%) patients and continued up to 10 months. Nineteen among 44 patients (43.2%) underwent kidney transplantation with low acute rejection rates (18.8%) and de novo DSA formation (12.5%). The authors suggest that bortezomib-based desensitization consistently and durably reduces HLA antibody levels providing an alternative to IVIG-based desensitization. In the study of Moreno Gonzales et al., however, bortezomib monotherapy given to a highly sensitized patient cohort is ineffective at lowering HLA antibody levels and this therapy was not well tolerated. The authors concluded that the modest reduction in antibody after the use of bortezomib did not appear to translate into important clinical outcomes such as negative crossmatch or even a reduction in cPRA. And so, they do not recommend bortezomib monotherapy routinely for desensitization

[31]. Plasma cell depletion due to bortezomib treatment induced germinal center B cell and follicular helper T cell expansion in the lymph nodes [32]. This compensatory mechanism may trigger increased antibody production and cause the modest efficacy in some highly sensitized or ABMR patients [31, 33]. Because the potential effect of bortezomib on desensitization remains interesting but still not valid, further well-designed studies are needed.

## Complement Inhibitors for Desensitization

**Q: Dose complement inhibitors can be used routinely as part of an HLA desensitization strategy?**

Although the important role of complement in the pathophysiology of antibody-mediated allograft injury or clinically apparent acute ABMR after kidney transplantation has been increasing, there are limited data for the use of complement inhibitors for HLA desensitization. Complement inhibitors targeting C5 and C1 esterase have now been studied for the prevention and treatment of AMR. Eculizumab is a humanized monoclonal IgG antibody that binds to the complement protein C5 with high affinity, inhibiting its cleavage to C5a and C5b and finally blocks the generation of membrane attack complex C5b-C9. It has been approved by the FDA for the treatment of paroxysmal nocturnal hemoglobinuria and primary atypical hemolytic uremic syndrome. It has been used primarily off-label in kidney transplantation to treat atypical hemolytic uremic syndrome, antiphospholipid syndrome, refractory ABMR, and thrombotic microangiopathy [34, 35]. One study examined outcomes in eculizumab-treated positive crossmatch kidney transplants compared to a historical control group [36]. Thirty living-donor KTRs with an initial positive B cell flow cytometry crossmatch MCS between 200 and 450 were enrolled and patients with a pretransplant MCS ≥300 received plasmapheresis prior to transplantation to achieve an MCS <300 by the day of transplant. Eculizumab dosing regimen was as follows: 1,200 mg immediately prior to transplant, 600 mg on postoperative day 1, and 600 mg weekly thereafter for 4 weeks. At week 4 post-transplant, patients with a B cell flow cytometry crossmatch MCS >200 received additional eculizumab (1,200 mg on week 5, then every 2 weeks thereafter until the MCS improved to <200). Outcomes were compared with those of a historical control group of 48 living-donor KTRs with a positive crossmatch who were desensitized with a similar protocol without the administration of eculizumab. Patients who administered eculizumab had a lower incidence of acute clinical ABMR compared with historical controls (6.7 vs. 43.8%, respectively). The percentage of patients who had a B cell flow cytometry crossmatch MCS >200 at 6 months was similar between eculizumab-treated patients and controls (46 vs. 41%), suggesting no additional benefit of eculizumab on the reduction of DSA compared with standard therapy. Among these patients, there was no difference in transplant glomerulopathy at 1 year (50 vs. 36%). The authors suggest that despite decreasing acute clinical ABMR rates, eculizumab treatment does not prevent chronic ABMR in KTRs with persistently high B cell flow cytometry crossmatch after positive crossmatch kidney transplants. This limitation of eculizumab treatment is further confirmed from recent study showing that eculizumab therapy reduced the rate of ABMR in patients with complement-activating DSA but not in those with non-complement-activating DSA [37]. C1-inhibitor (C1-INH), another complement inhibitor, is a serine protease inhibitor that inactivates both C1r and C1s and functionally blocks both the classical and lectin complement pathways. C1-INH which is approved for use by the FDA in the treatment of hereditary angioedema has been investigated in small pilot studies for ABMR treatment after kidney transplantation. One randomized, phase I/II placebo-controlled trial evaluated C1-INH (Berinert®) in sensitized renal transplant patients for the prevention of acute ABMR [38]. They enrolled

20 highly sensitized patients who desensitized with IVIG, rituximab, with or without plasmapheresis. The patients were randomized to receive plasma-derived human C1-INH (20 IU/kg/ dose) versus placebo intraoperatively, then twice weekly for 7 doses. None of the patients in the C1-INH treatment group and only one patient in the placebo group developed ABMR during the study period. The authors suggest that the addition of C1-INH to standard of care of HLA antibody reduction may prove useful in prevention of ABMR in highly sensitized KTRs. Further controlled studies are warranted. Further controlled studies to get more convincing results are warranted to use these expensive complement inhibitors as an agent for desensitization.

## Anti-IL-6 Receptor Blockers for Desensitization

**Q: Has blockade of IL-6 receptor shown promise in clinical trials of desensitization for highly HLA-sensitized patients?**

Interleukin-6 (IL-6) is a pleiotrophic cytokine that has been involved in inflammation. In transplantation, it is an attractive target as it promotes B cell differentiation to plasma cells, is important for immunoglobulin production, and induces Th17 cells [39]. Tocilizumab is an antagonist of the IL-6 receptor. Endogenous IL-6 is induced by inflammatory stimuli and mediates a variety of immunological responses. Inhibition of IL-6 receptors by tocilizumab leads to a reduction in cytokine and acute phase reactant production [40]. One phase I/II, single center, open label, pilot, exploratory study was conducted to examine the hypothesis that IL-6 is an important cytokine in the maintenance of anti-HLA antibody production in highly sensitized KTRs and that interruption of IL-6/IL-6 receptor interactions with tocilizumab would reduce or eliminate these antibodies and improve the chances of receiving a kidney transplant in highly sensitized patients who failed desensitization with high-dose IVIG and rituximab [41]. In this study, tocilizumab has been successfully used in combination with high-dose IVIG and rituximab to desensitize patients who failed standard of care desensitization therapy with good transplantation rates and low toxicity. The authors suggest that targeting the IL-6/IL-6 receptor pathway could provide a novel alternative for difficult to desensitize patients and larger controlled studies are essential to prove efficacy.

## IgG-Degrading Enzyme of *Streptococcus pyogenes* (IdeS) for Desensitization

**Q: Is IdeS safe and effective as novel agent for desensitization in highly HLA-sensitized patients?**

IdeS has a unique mechanism of action and efficiency that may represent a novel approach to desensitization in highly sensitized patients. IdeS is a secreted cysteine endopeptidase from the human pathogen *Streptococcus pyogenes* with an extraordinarily high degree of substrate specificity, catalyzing a single proteolytic cleavage at the lower hinge of human IgG. It rapidly cleaves human IgG at the hinge region, producing $F(ab')_2$ and Fc fragments and neutralizes all of the IgG in the body within 4 h of administration [42]. And so, Ides converts CDC crossmatch positive to negative and prevents complement-dependent and antibody-dependent cellular cytotoxicity [43]. The efficacy of IdeS as a novel desensitization agent was investigated in two independent phase I/II trial from the United States and Sweden that included 25 highly sensitized patients [44]. The median calculated PRA was 96 and 81% in the United States and Swedish studies, respectively. All enrolled patients administered IdeS intravenously 4–6 h before transplantation at a dose of 0.24 mg/kg in the United States and at a dose of 0.25 mg/kg or 0.50 mg/kg in the Swedish study. Treatment with IdeS revealed complete cleavage of IgG into $F(ab')_2$ and Fc fragments

within 6 h of infusion. Intact IgG remained absent for at least 7 days, and there was a persistent reduction in IgG levels at 28 days after administration. A total of 24 of 25 patients had perfusion of allografts after transplantation. Three of 11 patients (27%) in the Swedish study and 2 of 14 patients (14%) in the United States study developed ABMR at 2 weeks to 5 months after transplantation. All ABMR episodes resolved after treatment. One patient in the United States study had hyperacute rejection immediately after revascularization. This was thought to be mediated by non-HLA IgM antibody because the patient did not have detectable DSA after IdeS treatment. Although the results of this study are encouraging, the safety and efficacy of IdeS need to be validated in further studies. IdeS is not US FDA approved and is only available under research purposes.

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# 4 Induction and Maintenance Immunosuppressants in Sensitized Renal Allograft Recipients

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## Introduction

Immunologic rejection, antibody-mediated rejection (AMR) in particular, has higher incidence in sensitized patients, and graft loss secondary to acute or chronic rejection is also more frequent than in nonsensitized patients. Patient death, mostly due to infectious complications associated with overimmunosuppression, also has higher incidence in these recipients. Immunosuppressive regimens for sensitized patients, therefore, should be at a proper balance between graft protection from immune injury and the adverse consequences of overimmunosuppression.

Sensitized patients are a unique group of allograft recipients having memory B and T cells that elicit immunologic recall responses, which may lead to early and often severe AMR. Since donor-reactive cells rapidly expand by immunologic recall in a short timeframe of days to weeks after engraftment [1], the use of induction agents with cell-depleting capabilities appears to be a reasonable and efficient way to reduce the size of, albeit not eliminate, donor-specific clones, and improve graft outcomes in sensitized renal-allograft recipients. Currently available cell-depleting induction agents such as rabbit antithymocyte globulin (ATG, Thymoglobulin®), rituximab, a humanized anti-CD20 monoclonal antibody, alemtuzumab, and bortezomib, a proteasome inhibitor, will be reviewed. Maintenance immunosuppression suitable for sensitized patients will also be discussed.

## Induction Immunosuppressive Regimen

**Q: 1. Dose ATG have an advantage over IL2RA as an induction agent in sensitized kidney transplant patients?**
**2. Does rituximab or bortezomib have a role as an induction agent in sensitized patients?**
**3. What is the most appropriate induction regimen for sensitized kidney transplant patients?**

It is expected that those patients with preexisting donor-specific anti-HLA antibodies (DSA) will also harbor donor-specific memory B and T cells. Upon antigen re-exposure, memory B cells elicit a secondary immune response involving activation, clonal expansion, and differentiation into plasma cells or germinal center B cells, as well as functioning as antigen-presenting cells and

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directly facilitating T cell responses. Although these properties are shared by naive B cells, memory B cells exhibit faster kinetics with enhanced reactivation potential, and a recall response that is characterized by faster, higher-titer, and class-switched antibody production [2].

Reactivated memory T cells function as effector T cells, and also as follicular helper cells which are essential for optimal B cell responses and antibody generation. Since the memory B cells repertoire is broader than that of the plasma cell repertoire, a patient with a particular specificity of DSA before transplantation develops another specificity of DSA after transplantation, which was not detected before transplantation, by preexisting memory cells. Even patients with a complete absence of DSA by single-antigen bead (SAB) assay may develop DSA and AMR within days after transplantation by preexisting memory cells.

Since primary immune response initiated by naïve B cells occurs over a long timeframe of weeks to years, its time onset can hardly be predictable and thus early diagnosis and timely management is challenging. Conversely, the secondary immune response initiated by memory cells typically has an early and rapid onset, days to weeks after transplantation [1]. This short timeframe of immunologic response in sensitized patients offers a unique window of opportunity to make the peri-transplant administration of depleting-induction agents an efficient and relatively selective therapeutic tool for removing donor-reactive cells and reducing the size of donor-specific clones.

## Antithymocyte Globulin

ATG is a purified polyclonal immunoglobulin harvested from rabbits after immunizing them with a suspension of human thymic tissue. While it predominantly depletes T cells, ATG has numerous other immune modulatory actions, because this polyclonal antibody also contains antibodies against B cell antigens, plasma cell antigens, dendritic cells antigens, natural killer-cell antigens, adhesion molecules, and chemokine receptors [3].

Although in vitro studies have shown apoptosis of bone marrow resident plasma cells at clinically relevant concentrations of ATG, the depleting ability of ATG on plasma cells seems to be limited to circulating plasmacytes since in vivo ATG treatment is not associated with a reduction in splenic nor bone marrow plasma cells. Nevertheless ATG appears to reduce the risk of AMR in patients with preformed DSA, presumably by removing T cell help for alloreactive B cells and via B cell depletion by antibodies that directly bind B cells [4].

Given ATG induction, compared with no induction, is consistently associated with lower incidence of acute rejection (AR) [5], and interleukin-2 receptor antagonist (IL2RA) is the most popular induction agent in kidney recipients, it is reasonable to assess the efficacy of ATG by comparing ATG with IL2RA. There are several randomized controlled trials (RCTs) and retrospective observational analyses of ATG versus IL2RA in patients with either high immunologic risk or low/standard risk.

Brennan et al. conducted an international, multicenter RCT to compare ATG (five daily doses of 1.5 mg/kg) and basiliximab in renal recipients with high risk for AR or delayed graft function (DGF). The high immunologic risk for AR was defined as retransplantation or a panel-reactive antibody (PRA) value exceeding 20%. Maintenance immunosuppression consists of cyclosporine, mycophenolate mofetil, and prednisolone. The primary end point (a composite of AR, DGF, graft loss, or death at 12 months) did not reach significance. However, patients in the ATG group had fewer episodes of biopsy-proven AR (BPAR, 15.6% vs. 25.5%, $p = 0.02$), and the episodes were less severe (fewer required antibody treatment, 1.4% vs. 8.0%, $p = 0.005$) than those in basiliximab group. Because this study was conducted in the early 2000s, anti-HLA antibody was not measured, and information about whether ARs were antibody-mediated or T cell-mediated was not provided. Maintenance immunosuppression was cyclosporine-based, so the results of this study are not applicable to tacrolimus-treated patients.

There are only a few RCTs of ATG versus IL2RA in patients treated with tacrolimus and with high immunologic risk. Noel et al. reported a multicenter RCT comparing ATG with IL2RA (daclizumab) involving 227 patients with high immunologic risk [7]. The high risk was defined as current PRA >30%, peak PRA >50%, loss of kidney from rejection within 2 years of transplantation, or two or three previous grafts. ATG was administered daily between days 0 and 7 at a dose of 1.25 mg/kg/day. The maintenance immunosuppression comprised tacrolimus, mycophenolate mofetil, and steroids, and the primary endpoint was BPAR. The ATG group had a lower incidence of both BPAR (15.0% vs. 27.2%, $p = 0.016$) and steroid-resistant rejection (2.7% vs. 14.9%, $p = 0.002$) at 1 year. Cytomegalovirus (CMV) infection tended to be more frequent in the ATG group (18.6% vs. 10.5%, $p = 0.093$). Five-year follow-up data from these patients also showed similar results with lower AR rate and comparable patient and graft survival [8].

Pilch et al. conducted a single-center open-label RCT of ATG versus IL2RA in 200 patients with mixed immunologic risk [9]. The maintenance regimen was tacrolimus-based triple drug. AR at 1 year was similar between two groups (6% vs. 10%, in ATG and IL2RA groups, respectively). Thirty percent of patients had PRA exceeding 20%, and the incidence of AR in these high-immunologic-risk subgroups was 6 and 14%, in ATG- and IL2RA-treated patients, respectively ($p = 0.39$). The incidence of BK virus nephropathy was higher in the ATG group (9% vs. 1%, $p = 0.02$).

There have been four RCTs comparing ATG with IL2RA in low-immunologic risk patients [10–13]. Two of these were in cyclosporine-treated patients and two were in tacrolimus-treated patients. AR incidence was similar in ATG and IL2RA groups, with no difference in patient and graft outcome. CMV incidence was higher in the ATG group [10, 11] (Table 4.1). Several analyses of large registry data comparing ATG with IL2RA have also been published. Patient cohorts from registries were mixtures of both low and high immunologic risk (mostly low risk patients). Overall, AR rates were not different in entire cohorts or in the subgroup of low-risk patients. ATG was associated with lower rates of AR only in the subgroup of high-immunologic-risk patients. There was no difference in patient and graft survival [14].

These RCTs and retrospective analyses indicate that ATG is beneficial in preventing AR in high-immunologic-risk patients, whereas it has no benefit in low/standard-immunologic-risk patients. No differences in patient and graft survival, shown in RCTs despite reduced AR, in high-immunologic-risk recipients were likely due to a lack of statistical power and short follow-up. ATG is associated with increased infectious risk such as BK virus nephropathy and CMV infection in some, but not all, studies.

ATG may reduce the incidence of de novo DSA (dnDSA) in sensitized patients. Brokhof et al. reported a prospective cohort study of 114 moderately sensitized (positive DSA and negative flow crossmatch) patients followed for 3 years. Eighty-five patients received ATG (mean dose 5.0 mg/kg) as an induction and 29 received basiliximab. A tacrolimus-based triple drug maintenance regimen was used. DSA was serially measured with SAB for 1 year. The incidence of dnDSA (HR = 0.33) and AMR (HR = 0.9) was significantly lower in the ATG group [15].

Two retrospective cohort studies evaluated the optimal induction dose of ATG in high-immunologic-risk patients. Gurk-Turner et al. compared the outcome of an ATG dose of ≤7.5 mg/kg vs. >7.5 mg/kg in 96 adult patients with a regraft (85%) or PRA >40% (19%) [16]. The maintenance regimen consisted of tacrolimus, mycophenolate, and prednisolone. During the 25 ± 18 months follow-up, AR (9.5% vs. 8.8%, in lower and higher dose groups, respectively, $p = 0.9$), graft and patient survival did not differ. Klem et al. retrospectively analyzed outcomes of 83 high-immunologic-risk recipients who were treated with 4.5 mg/kg or 6 mg/kg ATG for induction [17]. High immunologic risk was defined as regraft (54%), African American (18%), or PRA ≥20% (76%). The maintenance regimen consisted of tacrolimus, prednisolone, and either mycophenolate (59%) or sirolimus (40%). Baseline PRA and tacroli-

**Table 4.1** Randomized controlled trials of ATG vs. IL2RA induction in kidney transplant patients

| Authors | N | Immunologic risk status | ATG dose | Maintenance immunosuppression | Follow-up duration (months) | Outcomes (ATG vs. IL2RA) | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Acute rejection (%) | Graft survival[a] (%) | CMV infection (%) |
| Lebranchu 2002 [10] | 100 | Low; first graft, PRA <25% | 1.0–1.5 mg/kg/d 6–10 d | Cyclosporine-based | 12 | 8.0 vs. 8.0 $p$ = n.s. | 100 vs. 96 $p$ = n.s. | 38 vs. 12 $p$ = 0.005 |
| Mourad 2004 [11] | 105 | Low; patients with a previous graft survival ≤1y excluded, PRA ≤20% | 1.0 mg/kg Mean 5.4 Infusions | Cyclosporine-based | 12 | 9.4 vs. 9.6 $p$ = n.s. | 98 vs. 98 $p$ = n.s. | 41.5 vs. 21.2 $p$ = 0.025 |
| Brennan 2006 [6] | 278 | High; Regraft, PRA > 20%, African American,higher DGF risk | 1.5 mg/kg/d Days 0–4 | Cyclosporine-based | 12 | 15.6 vs. 25.5 $p$ = 0.02 | 97.2 vs. 92.0 $p$ = n.s. | 7.8 vs. 17.5 $p$ = 0.02[b] |
| Abou-Ayache 2008 [12] | 109 | Low; first graft, PRA ≤20% | 1.0–1.5 mg/kg 4–9 infusions | Cyclosporine-based | 12 | 14.5 vs. 16.7 $p$ = n.s. | 96 vs. 97 $p$ = n.s. | 50.9 vs. 38.9 $p$ = n.s. |
| Noel 2009 [7] | 227 | High; Regraft, current PRA ≥30%, peak PRA ≥50% | 1.25 mg/kg/d Days 0–7 | Tacrolimus-based | 12 | 15.0 vs. 27.2 $p$ = 0.016 | 85.0 vs. 89.5 $p$ = n.s. | 18.6 vs. 10.5 $p$ = 0.093 |
| Ciancio[c] 2014 [13] | 85 | Low; PRA ≤5% in 95–98% of patients | 1 mg/kg/d Days 0–7 | Tacrolimus-based | 95 | 18.6 vs. 28.6 $p$ = n.s. | 88.4 vs. 88.1 $p$ = n.s. | 2 vs. 0 $p$ = n.s. |
| Pilch[d] 2014 [9] | 200 | Mixed; subgroup analyses of PRA >20% (high risk, $n$ = 63) and PRA <20% (low risk, $n$ = 137) | 1.5 mg/kg/d, 5 doses | Tacrolimus-based | 12 | High risk; 6 vs. 14 $p$ = 0.39 low risk; 6 vs. 9 $p$ = 0.74 | High risk; 6 vs. 7 $p$ = n.s. low risk; 6 vs. 7 $p$ = n.s. | 29 vs. 18 $p$ = 0.09 |

*ATG* rabbit antithymocyte globulin, *IL2RA* IL-2 receptor antagonist, *DGF* delayed graft function, *CMV* cytomegalovirus; Cyclosporine-based, cyclosporine, mycophenolate mofetil and prednisolone; Tacrolimus-based, tacrolimus and mycophenolate mofetil and prednisolone; *n.s.* not significant

[a]Death-censored graft survival

[b]The lower incidence of CMV infection in the ATG group may be due to the less prevalent D+/R- CMV serostatus in ATG group (15 vs. 23%)

[c]A three-arm study of ATG vs. alemtuzumab vs. IL2RA. Only data of ATG- and IL2RA-treated patients are shown here

[d]Significantly higher incidence of BK virus nephropathy in the ATG group (9% vs. 1%, $p$ = 0.02)

mus trough level during the follow-up were comparable. AR rates at 1 year were 10% and 11% in the lower and higher dose cohorts, respectively, with 100% patient and graft survival at 1 year in both cohorts. These results suggest that 3 daily doses of 1.5 mg/kg ATG provide good protection against AR, even in patients with increased immunologic risk, with the benefit of reduced cost and potentially decreased infection risk.

In conclusion, ATG appears to be beneficial in sensitized renal recipients, and should be used as a single or as a part of a combination of induction agents in these patients.

## Rituximab

Rituximab is a chimeric murine/human monoclonal antibody active against the cell membrane protein CD20, expressed on the surface of B-lymphocytes prior to their terminal differentiation into long-lived plasma cells. In addition to antibody-mediated immune responses, B cells are also involved in modulating T cell activities via antigen presentation and cytokine production. Rituximab is known to eliminate circulating CD20+ B cells and to reduce the numbers of these cells populating the spleen and lymph nodes [18].

In secondary immune responses seen in sensitized patients, memory B cells expand rapidly and generate a burst of plasma cells, mostly donor-specific, that peak on day 7 in peripheral blood, followed by secondary memory B cells that peak on 14 to 21 days. This plasma cell burst coincides with a sharp increase in serum antibodies that reach a plateau on day 10, indicating that the vast majority of the plasma cells generated are short-lived [1]. These short-lived plasmablasts and memory B cells, mobilized from the protective niche of bone marrow, are subject to depletion by rituximab. It is also suggested that memory B cells are mobilized from protective niches of secondary lymphoid organs during the peri-transplant period in response to inflammation associated with the surgical trauma of transplantation [19]. Peri-transplant administration of rituximab thus reduces the size of donor-reactive B cell lineage by depleting these young plasmablasts and memory B cells, although long-lived plasmacytes cannot be lysed by rituximab and the memory B cells are unlikely to be completely eliminated [20].

Zachary et al. demonstrated a preventive effect of rituximab on anamnestic responses in patients with cryptic sensitization to HLA antigen [19]. They identified HLA-specific B cells by staining with HLA tetramers. Twenty-six patients, who had tetramer-stained B cells and thus considered sensitized to HLA antigens, but had no detectable anti-tetramer antibody prior to transplantation, were identified. Of these patients, 16 were treated with rituximab, and 10 were not treated with rituximab. Posttransplant anti-tetramer antibody was detected in 13 of 16 patients not treated with rituximab, but none in treated patients. These results suggest that rituximab has the potential to control immunologic recall responses. The same group of investigators also looked at the impact of rituximab on HLA antibody rebound in 50 HLA-incompatible kidney transplant patients who were desensitized by plasmapheresis and IV immunoglobulin [20]. Patients had pretransplant DSAs with or without positive crossmatch. The 25 patients treated with rituximab (single dose, 375 mg/m$^2$) had a higher pretransplant PRA and more numbers of previous transplantation than 25 non-treated patients. A half of the patients also received ATG induction while the remainder received IL2RA. A significantly less HLA antibody rebound was observed in the rituximab-treated patients (7% DSAs and 33% non-DSAs) compared with the non-treated patients (32% DSAs and 55% non-DSAs). AMR rate and graft survival were comparable between the rituximab-treated and the untreated groups. In rituximab-treated patients, of the 39 HLA antibodies that increased posttransplant, 34 were specific for HLA mismatches present in previous allografts or pregnancies, implying a limited efficacy of rituximab in memory B cell depletion. Although rituximab-treated patients had a significantly greater reduction in the mean fluorescence intensity of DSAs, the rate of DSA persistence was not different between cohorts (52% in rituximab-treated vs. 40% in untreated cohorts).

The authors concluded that rituximab induction in HLA-incompatible recipients reduced the incidence and magnitude of HLA antibody rebound, but did not affect DSA elimination.

RCTs and retrospective analyses of the efficacy of rituximab as an induction agent have also been conducted. Tyden et al. reported a double-blind RCT in 140 low/standard-immunologic-risk patients [21]. The mean PRA was <5% in both groups. Ninety-six percent were first transplants. There was no significant difference in AR, patient or graft survival at 6 months between rituximab (375 mg/m$^2$) and placebo groups. Another double-blind single-center RCT in 280 kidney transplants compared rituximab induction with no induction [22]. Patients were stratified into immunologically high (PRA >6% or regraft) or low (PRA ≤6%) risk subgroups. The primary endpoint was the incidence of BPAR within 6 months. In high-risk subgroup ($n$ = 62), BPAR incidence was significantly lower in rituximab-treated patients compared with placebo-treated patients (18% vs. 38%, in rituximab and placebo groups, respectively). However, within low-risk subgroup, there was no difference in BPAR rate between rituximab and placebo groups.

A retrospective analysis of ABO-incompatible recipients with rituximab (200 mg/body) induction versus an ABO-compatible cohort without rituximab induction showed a decreased incidence of dnDSA (1.7% vs. 18.1%, with and without rituximab, respectively) and chronic AMR (3.5% vs. 28.8%) [23]. Approximately 30% of patients in these two cohorts had DSAs before transplantation. The maintenance regimen comprised tacrolimus, mycophenolate, and steroid. A similar retrospective study of ABO-incompatible patients with rituximab induction, versus ABO-compatible patients without rituximab was also published. But here, patients with preexisting DSA were excluded from analysis, and prevalence of dnDSA was not different (14.3% vs. 13.9%, in rituximab-treated and untreated cohort, respectively). Chronic AMR developed only in a minority of patients in each cohort [24] (Table 4.2).

These two RCTs and cohort studies suggested that rituximab is effective in preventing the development of dnDSA or AMR in sensitized patients, but has little benefit in immunologically low/standard risk patients.

In our institution, we give both ATG and rituximab for an induction for sensitized kidney transplant patients. To reduce infection risk, we use lower dose of each drug (3 or less daily dose of 1.5 mg/kg ATG and 200 mg/body rituximab). Although no randomized trials have evaluated this dual-drug induction, we think it is beneficial for sensitized patients by decreasing the number of donor-reactive immune cells via different mechanisms of each agent.

In summary, rituximab appears to be a very valuable induction agent for sensitized renal transplant patients, because it has the capability to mitigate the secondary immune response seen in these patients. However, there is a paucity of clinical data regarding the use of rituximab as an induction agent in these patients. Further experiences and well-designed RCTs on this issue are clearly needed.

## Bortezomib

Bortezomib is a selective inhibitor of the 26S proteasome, which is present in both the cytoplasm and the nucleus of eukaryotic cells. The 26S proteasome is the primary non-lysosomal pathway for intracellular protein degradation, but it is not a simple protein recycling pathway; it has the key role in the regulation of multiple cellular checkpoints, particularly by degradation of critical intracellular secondary messengers. For example, the 26S proteasome directly affects cell-cycle progression and apoptosis via degradation of cyclins and cyclin-dependent kinase inhibitors. The administered dose of bortezomib inhibits up to 80% of all proteasome activity, which is tolerated by most cells. Plasma cells in multiple myeloma, on the other hand, are rapidly dividing and produce immunoglobulin at a high rate. The disturbed degradation of misfolded antibodies by bortezomib in these plasma cells leads to greater endoplasmic stress and subsequent cell death. Actively proliferating cells are also more vulnerable to bortezomib because of its interfering action on cell cycle progression

**Table 4.2** Randomized trials and retrospective analyses of rituximab induction in kidney transplant patients

| Authors | Study type | Immunologic risk status | Rituximab dose | Maintenance immunosuppression | Follow-up duration | Outcomes (rituximab vs. controls) | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Rejection (%) | de novo DSA (%) | CMV infection (%) |
| Tyden 2009 [21] | RCT; rituximab ($n = 68$) vs. placebo ($n = 68$) | Low to moderate; mean PRA <5%, regraft 35% | 375 mg/ $m^2$ | Tacrolimus-based | 6 months | AR; 11.8 vs. 17.6 $p = 0.348$. | n.d. | 4.4 vs. 1.4 $p$ = n.s. |
| van den Hoogen[a]2015 [22] | RCT; rituximab ($n = 138$) vs. placebo ($n = 142$) | Mixed; high risk ($n = 62$, PRA >6% or regraft), low risk ($n = 218$, PRA ≤6% and first graft) | 375 mg/ $m^2$ | Tacrolimus-based | 12 months | AR; High risk; 17.9 vs. 38.2 $p = 0.004$ Low risk; 16.4 vs. 15.7 $p$ = n.s. | n.d. | 14.5 vs. 11.3 $p$ = n.s. |
| Kohei[b] 2012 [23] | Retrospective analysis; ABOi patients with rituximab induction ($n = 57$) vs. selected ABOc patients without rituximab induction ($n = 83$) | Moderate to high; pre-transplant DSA in 30% of patients, negative FCXM | 200 mg/ body | Tacrolimus-based | Up to 7 years | AMR at 6 months; 3.5 vs. 10.8 $p = 0.113$ CAMR at 2 years; 3.5 vs. 28.9 $p = 0.0001$ | 1.7 vs. 18.1 $p = 0.029$. | 3.5 vs. 10.8 $p = 0.113$ |
| Ashimine[c] 2014 [24] | Retrospective analysis; ABOi patients with rituximab induction ($n = 30$) vs. consecutive ABOc patients without rituximab induction ($n = 223$) | Low; patients with pretransplant DSA excluded | 200 mg/ body | 58% of rituximab patients and 19% of control patients were treated with tacrolimus. The remainder with cyclosporine. | Up to 5 years | AR; 3.3 vs. 7.0 $p$ = n.s. CAMR; 3.3 vs. 0.9 $p$ = n.s. | 14.3 vs. 13.9 $p$ = n.s. | 40 vs. 29 $p$ = n.s. |

*DSA* donor-specific anti-HLA antibody, *CMV* cytomegalovirus, *RCT* randomized controlled trial, *AR* acute rejection, *AMR* antibody-mediated rejection, *CAMR* chronic antibody-mediated rejection; Tacrolimus-based, tacrolimus, mycophenolate mofetil and prednisolone; *n.s.* not significant, *n.d.* no data, *ABOi* ABO-incompatible, *ABOc* ABO-compatible, *PRA* panel reactive antibodies, *FCXM* flow-cytometry crossmatch

[a]One rituximab-treated patient died of progressive multifocal leukoencephalopathy

[b]A three-arm analysis of ABO-i patients with splenectomy, ABO-i patients with rituximab induction and ABO-c patients without rituximab. The data of the second and the third arm are shown here

[c]A four-arm comparison analysis of ABO-c, ABO-i with splenectomy, ABO-i with rituximab, ABOi with neither splenectomy nor rituximab. The data of the first and the third arm are shown here

[25]. These pharmacodynamic insights may explain the favorable effect of bortezomib in early AMR, where rapidly dividing young plasmablasts are the target cells for bortezomib, but the lack of efficacy in late AMR or in desensitization settings, where long-lived plasmacytes are the target cells [26–28].

Bortezomib as an induction therapy in organ transplantation has not been adequately evaluated, although there are many reports of the use of this drug for the treatment of AMR and for desensitization. A pilot RCT evaluated safety/toxicity profiles of four B cell-targeted regimens as a prelude to a larger RCT [29]. Immunologically high-risk renal transplant recipients were randomized to induction with ATG, ATG + rituximab, ATG + bortezomib, or ATG + rituximab + bortezomib. Each treatment arm comprised ten patients. AR incidence was comparable between the arms with and without bortezomib. Half of the bortezomib-treated patients developed or experienced worsening of peripheral neuropathy.

Burghuber et al. evaluated combined use of bortezomib and belatacept in a sensitized nonhuman primate kidney transplant model [30]. The costimulation blockade was coadministered because the same group of investigators previously showed that bortezomib monotherapy reduced plasma cells, but DSA levels did not decrease, potentially due to humoral compensation by expansion of germinal center B cells and follicular helper T cells. Animals treated with combined use of bortezomib and belatacept showed a lower level of DSA, diminished bone marrow plasmacytes, and prolonged graft survival. The efficacy and tolerability of this dual regimen needs to be evaluated in the context of human transplantation.

Since sensitized kidney transplant patients develop secondary immune response characterized by rapidly proliferating donor-specific plasmablasts within days after transplantation, and these young plasma cell precursors appear to be susceptible to proteasome inhibitor-induced depletion, bortezomib might be a valuable option for reducing the number of donor-specific clonal cells. The efficacy and tolerability of bortezomib induction, alone or preferably in a combination with other agent(s), warrants investigation in sensitized kidney recipients.

## Alemtuzumab

Alemtuzumab is a humanized anti-CD52 panlymphocytic (both B and T cells) monoclonal antibody that is approved for treatment of chronic lymphocytic leukemia. Several RCTs and observational studies have compared alemtuzumab with ATG. One of concerns regarding alemtuzumab is that its benefits in reducing AR may decrease over time [31]. Other long-term outcomes, including graft and patient survival and development of chronic allograft nephropathy, may also be worse in patients receiving alemtuzumab compared with ATG. Thus, alemtuzumab may not be an appropriate induction agent for sensitized patients.

In a randomized trial, 139 high-risk patients (risk defined by regraft, PRA >20%, or black race) were treated with alemtuzumab or ATG, while 355 low-risk patients received alemtuzumab or basiliximab [31]. All patients received tacrolimus and mycophenolate mofetil and underwent early steroid withdrawal. In the high-risk cohort, there was no difference in AR between alemtuzumab and ATG groups at 12 months (10% vs. 13%, respectively). However, late acute rejection, defined as rejection that occurs between 12 and 36 months in patients who did not have AR in the first 12 months, was more common with alemtuzumab (10% vs. 2%), although this difference did not reach statistical significance. In the low-risk group, the AR rate was lower in patients who received alemtuzumab, but again, late rejection was more common in the alemtuzumab (8% vs. 3%, respectively). In post hoc analyses, C4d-positive AR was more prevalent in the alemtuzumab group. In a retrospective analysis of deceased donor kidney transplant recipients discharged on a steroid-free immunosuppression, alemtuzumab induction is associated with inferior graft survival when compared with ATG in the subgroup of PRA > 20% [32].

## Maintenance Immunosuppression

**Q: What is the most appropriate maintenance regimen for sensitized kidney transplant patients?**

A triple-drug regimen consisting of tacrolimus, mycophenolate, and prednisolone appears to be most appropriate in sensitized, high-immunologic-risk transplant patients. Alternative regimens that use cyclosporine in place of tacrolimus and sirolimus in place of mycophenolate have shown inferior outcomes such as higher incidence of AR and/or graft loss [33–35]. Steroid withdrawal or avoidance and calcineurin-inhibitor withdrawal or avoidance should also not be considered in sensitized patients since these regimens have shown to increase AR [36–38].

Two recent RCTs comparing belatacept, an inhibitor of the CD28-CD80/86 costimulatory pathway, with a tacrolimus-based regimen showed increased AR in the belatacept arm [39, 40]. A previous RCT where the comparator was cyclosporine-treated patients showed lower dnDSA in the belatacept arm [41], but this was not reproduced in subsequent studies with tacrolimus-treated controls [39, 40]. Besides, since memory T cells lack CD28 expression, belatacept appears to have limited efficacy in sensitized patients.

The optimal trough level of tacrolimus in sensitized patients has not been established. It is obvious that the higher the exposure to tacrolimus, the lower the risk of dnDSA and rejection, but higher trough level at the same time increases the risk of infection or nephrotoxicity. The optimal dose should be determined in the context of overall immunosuppression. In the Efficacy Limiting Toxicity Elimination (ELITE)-Symphony trial, "low-dose" tacrolimus, mycophenolate, and glucocorticoids (with daclizumab induction) produced superior allograft survival and fewer AR when compared to cyclosporine- and sirolimus-based regimens [35]. The immunologic-risk of enrolled patients was low/standard; those with a PRA >20%, a second renal transplant that the first allograft was lost owing to AR within the first year, or a positive crossmatch were excluded. The target trough level in the "low-dose" tacrolimus arm was 3–7 ng/mL. However, the achieved mean-trough level was 6.4 ng/mL in the first 12 months.

Wiebe et al. looked for the correlation between dnDSA and tacrolimus trough levels in a cohort of 596 primary kidney transplant patients without preexisting DSA, in the context of HLA-DR/DQ eplet mismatch [42]. HLA-DR/DQ eplet mismatch was an independent predictor of dnDSA development, and mean tacrolimus trough levels <5 ng/mL were significantly correlated with dnDSA. Mean trough levels in the 6 months before dnDSA development were significantly lower than the levels >6 months before dnDSA development in the same patients.

Davis et al. evaluated the association of mean tacrolimus trough levels and time in the therapeutic range, with the dnDSA risk in a cohort of 538 patients in the first year following kidney transplantation [43]. Patients at higher immunologic risk (calculated PRA > 20%, repeat transplant, African American race, cold ischemia time >24 h) received rATG induction and the majority of the remainder received no induction. The authors' target therapeutic-range was 6–9 ng/mL for months 0–3, and 5–8 ng/mL for months 4–12. A mean trough <8 ng/mL and time in the therapeutic range of <60% was associated with dnDSA. When patients were grouped according to mean trough levels during the first year, those groups with a mean trough of 6–7.9 ng/mL and ≥ 8 ng/mL developed similar incidences of dnDSA, while which were significantly lower than those in the groups with trough <6 ng/mL. These results indicate that higher trough levels are associated with lower incidence of dnDSA, and the minimal trough level to be maintained for the prevention of dnDSA is 6 ng/mL. The target level varies among centers, dependent on the patient population and immunosuppressive protocols including induction regimen.

The dose of mycophenolate and corticosteroid should be optimized and preferably individualized in the context of overall immunosuppression status, and patient's immunologic risk and general health. Mycophenolate mofetil (MMF) and enteric-coated mycophenolate sodium (EC-MPS) have equivalent efficacy. Some, though not all, reports suggest EC-MPS is associated with fewer gastrointestinal side effects. It is necessary to reduce the MMF dose in tacrolimus-treated patients, because tacrolimus, compared with cyclosporine, increases MMF exposure by 20–30% [44].

Intravenous methylprednisolone should be a part of initial immunosuppression since this has lymphocyte-depletive action and may help to diminish donor-reactive cells in the early post-transplant period. The initial dose and tapering protocol of prednisolone varies among institutions. In the absence of AR, tapering to 5 mg/day by 1–3 months is a common practice. Steroid withdrawal should not be attempted in sensitized patients since this increases the risk of AR.

## Conclusion

The secondary immune responses that are expected to occur in sensitized renal transplant recipients having memory cells are characterized by the development of proliferating donor-specific plasmablasts, followed by a rise in DSA and circulating secondary memory cells, typically during a short timeframe of days to weeks. Peri-transplant administration of depleting induction agents, such as ATG, rituximab, or bortezomib, therefore is a unique way to efficiently reduce the donor-specific clonal cells. Induction with depleting agent(s) should be implemented in sensitized patients, and to find an appropriate induction regimen for these patients, clinical trials evaluating the efficacy and adverse profile of a single or a combination of two or more of these drugs need to be conducted.

A triple-drug regimen consisting of tacrolimus, mycophenolate, and corticosteroid seems the most appropriate maintenance immunosuppression among the various regimens evaluated so far, for sensitized kidney recipients. The trough level of tacrolimus should be maintained at a minimum of 5 or 6 ng/mL. The dose of each drug in the maintenance regimen needs to be optimized in the context of overall status of immunosuppression.

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# 5 Postoperative Management

Chung Hee Baek

## Monitoring and Prevention of Infectious Disease

**Q: What kinds of infection should we monitor and manage after kidney transplant in sensitized patients?**

### Cytomegalovirus

**Q: 1. What are the risk factors for CMV infection following desensitized KT patients?**
**2. What are the conditions for preemptive anti-CMV treatment?**
**3. Is there any relation between CMV infection and graft rejection?**

Cytomegalovirus (CMV) is the most prevalent opportunistic infection after kidney transplantation [1]. Primary infection present as asymptomatic or self-limiting febrile disease, and CMV have latency in human cells. It can be reactivated in immunosuppressant patients. In addition, donor-derived transmission by allograft is possible, and de novo infection can also occur from the general population [1, 2]. The incidence of CMV infection varies depending on the CMV serostatus. It is 5–30% for recipients with CMV IgG, but the incidence is increased to 50% in recipients who were treated with T cell depletion therapy [3].

CMV infection has direct and indirect effects in kidney transplant recipients. As direct effects, CMV syndrome such as fever, fatigue, and myalgia and tissue-invasive CMV disease can be occurred. CMV infection can cause allograft rejection, chronic graft dysfunction, and graft failure. In the AST guideline [2], CMV infection is defined as presence of CMV replication regardless of symptoms, and CMV disease means CMV infection accompanied by clinical signs and symptoms.

The presence of CMV-specific antibody (serostatus) is important in the assessment of risk of CMV infection. Kidney transplants with CMV donor seropositive, recipient seronegative (D+/R−) have the highest risk of CMV infection. Cases with D+R+ and D−R+ have intermediate risk, and KT recipients with D−R− serostatus have the lowest risk of CMV infection [1]. In addition, because T cell is important in the host defense against CMV, lymphocyte-depleting agents such as thymoglobulin increase the risk of CMV infection. Overall immunosuppression state, host factors, and cold ischemic time are also risk factors of CMV infection [2].

For prevention of CMV disease, either antiviral prophylaxis or preemptive therapy can be

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considered. Antiviral prophylaxis is giving antiviral agents to all patients with risks for definite periods. Kidney transplant recipients with D+/R− are high-risk group, and have to receive antiviral prophylaxis. In a recent study [4] using United Network for Organ Sharing/Organ Procurement and Transplantation Network data, D+/R− was associated with a higher risk or graft failure (hazard ratio (HR) = 1.17, $P = 0.01$), all-cause mortality (HR 1.18, $P < 0.001$), and infection-related mortality (HR = 1.38, $P = 0.03$) compared with D−/R−. Recipients with D+/R− also have increased risk of all-cause mortality and infection-related mortality compared with recipients with D+/R+. Therefore, they concluded that CMV mismatch is still an independent risk factor for graft loss and patient mortality in the era of routing prophylactic and preemptive therapy. For preemptive therapy, CMV viral loads are monitored regularly and antiviral agents are initiated when the viral loads are higher than pre-set threshold. AST guidelines suggest to monitor CMV viral loads once weekly for 12 weeks after transplantation [2]. The therapy is discontinued when follow-up viral loads decrease below the lower limit of quantification.

The drugs of choice for CMV infection are valganciclovir and intravenous ganciclovir. Both drugs have the same efficacy and similar long-term outcomes [3]. However, intravenous ganciclovir is the recommended initial treatment for severe CMV disease and high viral loads [2]. In general, 5 mg/kg of intravenous ganciclovir every 12 h and 900 mg of valganciclovir twice daily are prescribed for treatment of CMV disease, but renal dose adjustment is necessary. If CMV is resistant to ganciclovir, foscarnet and cidofovir can be used for treatment, but data are insufficient. Immunosuppressive level should be reduced.

## BK Virus

**Q: 1. What are the risk factors for BK viremia or BK nephropathy following renal transplantation?**
**2. What is the preventive or treatment strategy for BK viremia?**

Primary infection with the BK virus usually occurs in the first decade of life, after which the BK virus establishes latency in the uroepithelium and renal tubular epithelial cells. Under conditions of immunosuppression, the virus reactivates and begins to replicate. The reactivation of BKV in the kidney allograft can lead to BK nephropathy in up to 10% of kidney transplant recipients. BK nephropathy affects graft function and increases the risk of graft loss.

BK nephropathy has limited treatment options, and hence the screening of kidney transplant recipients for BK virus replication is highly recommended. The American Society of Transplantation (AST) Infectious Community of Practice recommended that screening for BK virus replication should be performed at least every 3 months during the first 2 years posttransplant, and then annually until the fifth year posttransplant [5]. Screening for BKV replication can be done either by testing urine for high-level BK virus viruria/decoy cells or by testing plasma for BK virus viremia. In addition, Haufen in electron microscopy and urine mRNA can be used for BKV screening. BK virus monitoring and management protocol of our center is summarized in Fig. 5.1.

If the blood BK virus PCR levels were more than 4 $\log_{10}$ copies/mL, presumptive diagnosis of BK nephropathy could be made in the absence of pathologic findings. The definitive diagnosis of BK nephropathy (proven BK nephropathy) should be demonstrated by BK virus cytopathic changes in allograft tissue and immunohistochemistry or in situ hybridization [5].

Aggressive immunosuppressant reducing strategies were suggested for BK nephropathy. In addition to reducing calcineurin inhibitor or antimetabolites such as mycophenolate, switching from tacrolimus to low-dose cyclosporine or sirolimus is another strategy. KDIGO guidelines recommend reduction in tacrolimus trough levels to less than 6 ng/mL and mycophenolate doses of less than 1 g/day [6]. The AST consensus guidelines recommend the calcineurin inhibitor dose reduction by 25–50% followed by a 50% reduction in the antiproliferative agent or vice versa [5]. Then, if further reduction was neces-

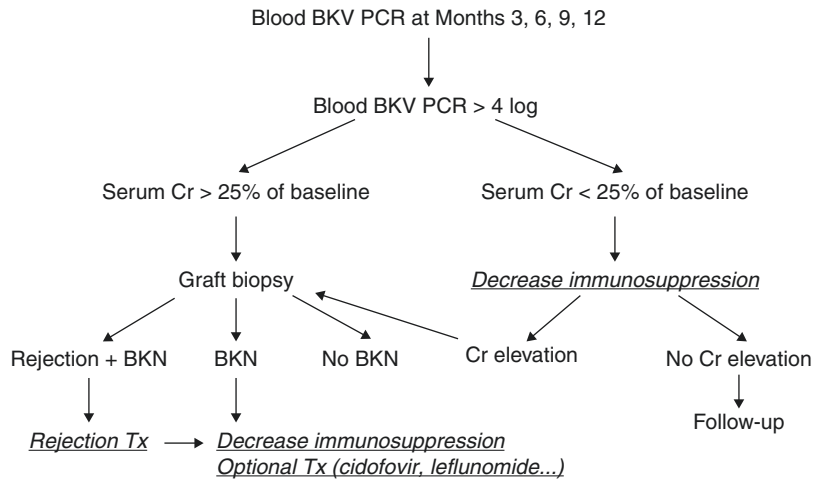


**Fig. 5.1** BK virus monitoring and management protocol of Asan Medical Center

sary, the antiproliferative agent is discontinued subsequently. However, acute rejection is always worrisome when reducing immunosuppression. In a retrospective study of our center [7], among 79 kidney transplant recipients reduced immunosuppressants due to BK viremia and 17 patients experienced acute rejection after immunosuppression reduction. Kidney transplant recipients who experienced acute rejection after immunosuppression reduction exhibited poorer graft function than patients without acute rejection. Moreover, the rejection group showed lower tacrolimus levels than that in the no rejection group, although the mycophenolate doses were not lowered in the rejection group. A greater number of patients in the rejection group exhibited calcineurin inhibitor level reduction by >20% at 1 month after the initial BK virus detection. The peak BK virus PCR level, mycophenolate discontinuation or reduction, and calcineurin inhibitor level reduction by >20% were significantly associated with acute rejection.

High-dose steroid is sometimes given for clinically suspected allograft rejection before testing for BK viremia is completed. In addition, steroids can be given when the pathologic findings of acute cellular rejection are observed together with those BK nephropathy at the same time. However, high-dose steroids induced BK viral activation and subsequently resulted in poor long-term graft function and early failure in patients with BK viremia [8]. Therefore, monitoring BK virus and preemptive modification of immunosuppressant may be important.

For sustained BK nephropathy after immunosuppressant reduction, several medications can be tried. The results of cidofovir, leflunomide, intravenous immunoglobulin, and fluoroquinolones were reported in small studies or case reports. However, no randomized controlled trials showed these agents are more effective than reduction of immunosuppression [5] and the results of previous studies are controversial. In a recent report of New England BK consortium [9], 8 (61.5%) of 13 centers report using leflunomide for persistent viremia/BK nephropathy, 4 (30.8%) centers consider using cidofovir for worsening viremia, and 8 (61.5%) centers consider using intravenous immunoglobulin (IVIG) for persistent viremia, or cellular/antibody-mediated rejection with BK viremia/BK nephropathy. None (0%) utilized fluoroquinolones for management of BK viremia in any of their patients. Further studies for refractory BK nephropathy are necessary.

## Management of Hyperglycemia

**Q: 1. What is the risk factor for post-transplant diabetes mellitus?**
**2. How can we diagnose posttransplant diabetes mellitus?**
**3. How can we manage hyperglycemia after transplantation?**

About 80% of kidney transplant recipients experience hyperglycemia after operation. Hyperglycemia may be temporary in the first 3 months after transplant. However, transient posttransplant hyperglycemia is related to a risk of posttransplant diabetes mellitus (PTDM) [10]. The 1-year incidence of PTDM is estimated at 10–20% in kidney transplant recipients [11]. Recipients with PTDM have increased risk for cardiac events, with similar incidence rates to patients who had diabetes before transplantation [12]. In addition, PTDM is associated with mortality and graft failure [11].

Risk factors of PTDM include obesity, old age, family history of diabetes, male gender, non-Caucasian ethnicity, high pretransplant triglyceride levels, hyperuricemia, hypomagnesemia, and vitamin D deficiency. Polycystic kidney disease, hepatitis C, cytomegalovirus infection, steroid pulse therapy have also been reported as risk factors of PTDM [12]. Glucocorticoids have diabetogenic effects via leading to ß-cell dysfunction and insulin resistance. Calcineurin inhibitors are also risk factors of PTDM, and tacrolimus suppresses insulin secretion stronger than cyclosporine. Mammalian target of rapamycin inhibitors have also been reported to be associated with PTDM. However, mycophenolate mofetil, azathioprine, and belatacept have not been associated with PTDM [12].

The peak plasma glucose levels occur 7–8 h after administration of moderate-dose steroids [10]. In addition, as postprandial hyperglycemia is a typical presentation of PTDM, oral glucose tolerance test (OGTT) is better to detect PTDM than fasting blood glucose (FBG). HbA1c can be also used for diagnosis of PTDM more conveniently in clinical practice. In a systemic review and meta-analysis [13], HbA1c cutoff point of 6.5% in kidney transplant recipients showed sensitivity of 0.48 (95% CI 0.31–0.65) and specificity of 0.96 (95% CI 0.95–0.97). HbA1c cutoff point of 6.2% had sensitivity of 0.76 (95% C 0.49–0.91) and specificity of 0.89 (95% CI 0.86–0.92). Therefore, HbA1c cutoff points of 6.5% and 6.2% resulted in high specificity and low/moderate sensitivity to diagnose PTDM. For the diagnosis of PTDM, diagnostic criteria of the American Diabetes Association for general population are generally used.

Lifestyle modification, oral anti-diabetic therapy, and then insulin are appropriate steps for management of late-PTDM. However, reverse management is recommended for immediate posttransplant hyperglycemia by International Consensus Meeting on PTDM [14]. Insulin is effective for glucose control in early posttransplant with high glucocorticoid doses. In a randomized trial [15], the treatment group assigned to immediate-postoperative basal insulin had 73% lower odds of PTDM than the control group assigned to short-acting insulin and/or oral antidiabetic agents. One year after transplantation, all patients in the treatment group were insulin-independent, but 7 of 25 controls required antidiabetic agents.

Oral anti-diabetic agents are diverse including newer agents such as sodium-glucose cotransporter type 2 (SGLT2) inhibitors. However, the International consensus group agreed that there were inadequate data to recommend a strategy of anti-glycemic agents [14]. Metformin is excreted by the kidney, and lactic acidosis can be occurred in patients with decreased renal function. Therefore, metformin use was discouraged in kidney transplant recipients in the past. However, the European Medicines Agency lowered the renal function threshold which metformin is contraindicated after reviewing evidences, and no drug interactions were reported between metformin and immunosuppressive agents [12]. Metformin can be considered for treatment of PTDM if there is no contraindication. Dipeptidyl peptidase-4 (DDP4) inhibitors enhance glucose-dependent insulin secretion and improve glycemic control. DPP4 inhibitors except linagliptin are cleared by the kidneys. Sitagliptin increases cyclosporine trough levels and vildagliptin decrease in tacrolimus trough levels. Therefore, monitoring of CNI levels after initiation of DPP4 inhibitors is necessary.

GLP-1 receptor agonists have protective effects on beta-cell, enhance glucose-dependent insulin secretion, suppress appetite, and delay gastric emptying [12]. Most GLP-1 receptor agonists except liraglutide are cleared by kidneys.

Although there are insufficient data in PTDM, no serious adverse events and drug interaction with tacrolimus were reported in liraglutide treatment. Sodium-glucose cotransporter type 2 (SGLT2) inhibitors lower plasma glucose levels by increasing urinary glucose excretion. SGLT2 inhibitors were reported to have protective effects on renal function. In a randomized trial, empagliflozin was associated with slower progression of nephropathy. It reduced doubling of the serum creatinine level by 44% and renal-replacement therapy by 55% [16]. However, no data of SGLT2 inhibitors on PTDM are available, and acute kidney injury and increased risk of genitourinary tract infection were reported in SGLT2 inhibitors treatment. Therefore, further studies of SGLT2 inhibitor in the management of PTDM are necessary for safety and efficacy.

## Management of Hypertension

**Q: 1. What is the target blood pressure after kidney transplantation?**
**2. What kind of antihypertensive drug can be used?**
**3. Can we use renin-angiotensin-aldosterone system blocker early after kidney transplantation?**

Cardiovascular disease is the leading cause of death in not only chronic kidney disease patients but also kidney transplant recipients [17]. Transplant recipients have several risk factors of hypertension. Diabetes, old age, obesity, smoking, male, and reduced renal function are traditional risk factors [18]. In addition, donor factors such as donor age, hypertension, obesity, and expanded criteria donors can affect posttransplant hypertension [17, 19]. Immunosuppressants are also associated with hypertension in various mechanisms. Corticosteroids can cause sodium retention and increased pressor response [20]. Low-dose prednisolone (5 mg/day) seems to have no effect on hypertension, but higher dose increase the risk of hypertension [17]. Calcineurin inhibitors induce hypertension by renal vasoconstriction and renal hypoperfusion. Activation of sodium-chloride cotransporter in the distal convoluted tubules is also reported as a mechanism of calcineurin inhibitor induced hypertension [17]. In the BENEFIT study [21], belatacept showed better blood pressure control than cyclosporine.

Target blood pressure in kidney transplant recipients is not determined. There is no prospective randomized study about optimal blood pressure in kidney transplant recipients [18]. American Herat Association (ACC/AHA) 2017 hypertension guidelines recommended to treat hypertension for target blood pressure <130/80 mmHg in patients with chronic kidney disease [22]. The 2018 European Society of Hypertension/European Society of Cardiology (ESH/ESC) guidelines recommended target blood pressure 130–139/70–79 mmHg for patients with chronic kidney disease [23]. These guidelines did not mention target blood pressure for kidney transplant recipients. Only 2012 KDIGO guidelines suggested to maintain blood pressure consistently ≤130 mmHg systolic and ≤ 80 mmHg diastolic, irrespective of the level of urine albumin excretion [24]. However, recommendation level is 2D with very low quality of evidence.

KDIGO guidelines suggest to choose antihypertensive medication after taking into account the time after kidney transplantation, use of calcineurin inhibitors, albuminuria, and other comorbidity [24]. In common with target blood pressure, there are no randomized controlled trials for optimal antihypertensive treatment in kidney transplant recipients. Calcium channel blockers decrease renal vascular resistance. Therefore, this class drugs can counteract the vasoconstrictive effect of calcineurin inhibitors. Some studies reported that calcium channel blocker had better renal function and blood pressure control [25, 26]. However, in other studies, calcium channel blockers were not superior to other class antihypertensive drugs [27, 28]. Non-dihydropyridine calcium channel blockers (verapamil, diltiazem) inhibit cytochrome P450 and increase blood levels of calcineurin inhibitors

and mTOR inhibitors [18]. Therefore, non-dihydropyridine calcium channel blocker should be used with caution and close monitoring of the drug levels is necessary.

Renin-angiotensin-aldosterone system (RAAS) blocker can slow the progression of chronic kidney disease in proteinuric patients [17]. However, the benefit of RAAS blocker in kidney transplant recipients is controversial. In a systematic review (21 trials with 1,549 patients) by Hiremath et al. [29], angiotensin-converting enzyme (ACE) inhibitor or angiotensin receptor blocker (ARB) significantly reduced proteinuria, hematocrit level, and glomerular filtration rate in kidney transplant recipients. However, data were insufficient to determine the effect on graft and patient survival. A recent double-blind, placebo-controlled, randomized trial compared Ramipril and placebo in kidney transplant recipients with an estimated glomerular filtration rate (eGFR) $\geq$ 20 mL/min/1.73m$^2$ and proteinuria $\geq$0.2 g/day [30]. Ramipril did not significantly reduce doubling of serum creatinine (the primary outcome), end-stage renal disease, or death in kidney transplant with proteinuria. Hiremath et al. conducted a systematic review and meta-analysis again and reported the results in 2016 [31]. Eight trials (1,502 patients) were included in the review. RAAS blockers did not significantly reduce all-cause mortality (risk ratio [RR], 0.96; 95% confidence interval [CI], 0.62–1.51), transplant failure (RR, 0.76; 95% CI, 0–49-1.18), or creatinine level doubling (RR, 0.84; 95% CI, 0.51–1.39). In addition, risk of hyperkalemia was significantly increased with RAAS blocker (RR, 2.44; 95% CI, 1.53–3.90). Therefore, clinicians should use RAAS blocker in kidney transplant recipients considering each patient's medical condition until more definitive evidence is reported.

The appropriate time to start RAAS blocker is unknown. Formica et al. [32] performed a randomized trial comparing losartan with amlodipine as initial therapy for hypertension in the early posttransplant period (day 0–30). Losartan use did not affect renal function, but hyperkalemia was the major complication. In a literature review [33], 7 studies that examined the early (within 12 weeks) initiation of RAAS blocker were reviewed. The authors concluded that early initiation of RAAS inhibitors seemed to be safe in posttransplant patients with functioning grafts, but data are insufficient to recommend these drugs in patients with early graft dysfunction. Glicklich et al. [34] reported results of a randomized, double-blinded placebo-controlled safety study for angiotensin converting enzyme inhibitor (enalapril 5 mg) use soon after kidney transplantation (1 month after kidney transplantation). Patients on ACE inhibitor had higher potassium and higher BUN levels at 6 months. However, serum creatinine was not different. Therefore, early posttransplant period is not contraindication of the use of RAAS blocker, but hyperkalemia should be cautioned.

Beta blockers are commonly used for blood pressure control and cardiovascular disease in chronic kidney disease and kidney transplant patients. Suwelack et al. [35] conducted a prospective study to compare antihypertensive potential of quinapril with atenolol in hypertensive kidney transplant recipients. Both agents decreased systolic and diastolic blood pressures (BP) to a similar extent (systolic BP change: $-8 \pm 3$ mmHg in quinapril group and $-5 \pm 3$ mmHg in atenolol group; diastolic BP change: $-5 \pm 2$ mmHg in quinapril group and $-4 \pm 2$ mmHg in atenolol group). Serum creatinine and Cockcroft-Gault clearance were not changed significantly in both groups after 5-year study period. However, urinary protein excretion remained stable in quinapril group, but it was significantly increased in atenolol group ($0.52 \pm 0.08$ to $0.54 \pm 0.14$ g/24 h in quinapril group; $0.34 \pm 0.03$ to $0.72 \pm 0.13$ g/24 h, $P < 0.02$). In recent retrospective study [36], use of a beta blocker ($P = 0.04$) and angiotensin-converting enzyme inhibitor or receptor blocker ($P = 0.03$) was associated with better survival in kidney transplant recipients. This effect was observed across all major clinical subgroups and it was also supported by propensity score analysis.

## Management of Dyslipidemia

**Q: 1. What is the characteristics of dyslipidemia after kidney transplantation?**
**2. What kinds of medication can be used for dyslipidemia in kidney transplant recipients?**

Renal dysfunction affects level and quality of lipids with more atherogenic profile [37]. Chronic kidney disease is associated with hypertriglyceridemia, LDL cholesterol elevation, and low HDL levels [38]. Dyslipidemia is also highly prevalent in kidney transplant patients. Hyperlipidemia is estimated to exist as high as 80% in kidney transplant recipients [39]. Several factors such as immunosuppression, weight gain, new onset diabetes after transplantation, and advancing age may contribute to the development of dyslipidemia in kidney transplant recipients [39].

Among various factors, contribution of immunosuppressant agents to dyslipidemia is significant. Corticosteroids are known to increase the activity of 3-hydroxy-3-methylglutary coenzyme A (HMG-CoA), the rate-limiting step in cholesterol synthetic pathway [39]. In addition, corticosteroids-induced insulin resistance increases hepatic VLDL synthesis and reduces lipoprotein lipase, which reduces triglyceride clearance. LDL is also increased with corticosteroid use due to downregulation of LDL receptor expression and increased conversion of VLDL to LDL cholesterol. Cyclosporine increases LDL cholesterol levels with decline in LDL clearance by interfering with binding of LDL cholesterol to the LDL receptor. Cyclosporine also leads to LDL receptor downregulation by interfering with bile acid synthesis. Tacrolimus showed less hyperlipidemia than cyclosporine, but the mechanisms remain unclear. Sirolimus may affects dyslipidemia by inhibiting lipoprotein lipase and decreasing lipolysis. Sirolimus also alters insulin signaling and increases the tissue lipase activity.

Habbig et al. [40] studied dyslipidemia in 386 pediatric renal transplant recipients. The prevalence of dyslipidemia was 88% at 1 year post-transplant. The use of tacrolimus and mycophenolate was associated with significantly lower concentrations of all lipid parameters compared to regimens containing cyclosporine and mTOR inhibitor.

The benefits of statins, HMG-CoA reductase inhibitors, are well established in patients with normal renal function. The Assessment of Lesocol in Renal Transplantation (ALERT) study was the first large randomized controlled trials which investigated effects of fluvastatin on cardiac and renal outcomes in 2,102 kidney transplant recipients [41]. In the ALERT study, fluvastatin lowered LDL cholesterol levels by 32%, after a mean follow-up period of 5.1 years. Fluvastatin did not significantly reduced the risk of a major adverse cardiac events (MACE), the primary endpoint [risk ratio 0.83 (95% CI 0.64–1.06), $P = 0.139$]. Cardiac deaths or non-fatal myocardial infarction occurred less frequently in fluvastatin group [70 vs. 104, 0.65 (0.48–0.88), $P = 0.005$]. The ALERT extension study was an open-label, 2-year extension study of previous ALERT study with fluvastatin XL 80 mg/day [42]. Total 1,652 patients were included, and mean total follow-up duration was 6.7 years. Fluvastatin group had a reduced risk of MACE (HR 0.79, 95% CI 0.63–0.99, $P = 0.036$) and cardiac death or non-fatal myocardial infarction (HR 0.71, 95% CI 0.55–0.93, $P = 0.014$). Mortality and graft failure was not significantly different between groups. In a Cochrane review which included 16 studies (3,229 patients) [43], statins significantly improved hyperlipidemia and showed a trend of reducing cardiovascular events in kidney transplant recipients. However, statin had no significant effect on mortality outcomes and the risk of acute rejection. The results were similar to ALERT study, because most of the data of this review was derived from ALERT study.

Fish oil (omega-3 fatty acids) reduces triglyceride levels in a dose-dependent manner with little effects on the levels of LDL and HDL cholesterols even in patients with chronic kidney disease and end stage renal disease [37]. A Cochrane review searched effect of fish oil on kidney transplant recipients of 15 studies (733

patients) [44]. Fish oil group had significantly lower serum creatinine levels [5 studies, 237 participants: mean difference (MD) −30.63 μmol/L, 95% CI −59.74 to −1.53; $I^2$ = 88%] and lower diastolic blood pressure (4 studies, 200 participants: MD −4.53 mmHg, 95% CI −7.60 to −1.45) than placebo group. Patients taking fish oil for more than 6 months experienced a modest increase in HDL cholesterol levels (5 studies, 178 participants: MD 0.12 mmol/L, 95% CI 0.03 to 0.21; $I^2$ = 47%). However, graft survival, acute rejection rates, and calcineurin inhibitor toxicity were not significantly different between fish oil and placebo groups.

Fibrates decrease triglyceride levels and increase HDL cholesterols levels by reducing hepatic VLDL cholesterol synthesis and increasing lipoprotein lipase activity [39]. Data of fibrates use in kidney transplant are rare. Because fibrates may increase creatinine levels especially in patients with eGFR <30 mL/min/1.73 $m^2$ and fibrates are metabolized by the kidneys [37], cautions are necessary in fibrates use in patients with low renal function.

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# Complication from Desensitization

6

Duck Jong Han

**Q: What kinds of complication can be occurred from various desensitization medication or method?**

1. **Medical complication**
   - Complication of rituximab (anti-CD20 mAb)
   - Complication of bortezomib
   - Complication of IVIg
   - Complication of eculizumab
   - Complication of C1 esterase inhibitor
   - Complication of IgG endopeptidase
   - Complication of anti-IL-6R mAb
   - Complication of plasmapheresis
2. **Surgical complication**

## Complication from Rituximab

**Q: 1. What kind of side effect doses the rituximab have?**
**2. Regarding the infection following the rituximab, is it related with the dose of rituximab?**

Rituximab is a mouse-human chimeric antibody that targets CD20, and recognized as a useful drug for the treatment of B cell non-Hodgkin's lymphoma and its use has been extended to such diseases as idiopathic thrombocytopenic purpura, thrombotic thrombocytopenic purpura, chronic rheumatoid arthritis, and ANCA-associated vasculitides. Recently, B cell depletion with rituximab has been an important component of desensitization for ABO incompatible or positive crossmatch kidney transplantation [1]. However rituximab has diverse side effects at immediate after use or even up to a year.

### Infection

Common side effects of rituximab are infusion-related reactions and infections. Rituximab has been associated with serious infections, including *Pneumocystis jirovecii* pneumonia (PJP) and the reactivation of hepatitis B virus (HBV) and tuberculosis (TB). The risk of infection appears to be the result of a variety of mechanisms, including prolonged B cell depletion, B-cell-T-cell crosstalk, panhypogammaglobulinemia, late-onset neutropenia, and blunting of the immune response after vaccination. Particular attention should be given to strategies to minimize the risk of infectious complications, including vaccinating

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against bacterial and viral pathogens, monitoring white cell count and immunoglobulin levels, prophylaxis against PJP, and screening for HBV and TB [2].

Chung et al. [3] reported that the overall prevalence of infection was significantly higher, and the infection-free survival rate was lower, in the group of combined rituximab, plasmapheresis, and IVIg (RPI), compared with the rituximab alone or control groups ($P < 0.05$). A trend toward more severe bacterial infections was seen in the RPI group compared with the other groups, and fungal infections developed only in the RPI group.

Therefore the use of combined RTX and PP therapy pretransplant significantly increases the risk for posttransplant infection.

Regarding the rituximab dose effect on infection, Nakao [4] reported in ABO mismatched patients that an initial rituximab dosage of 100 mg/body (for titers below 64) or 200 mg/body (for titers above 128) was administered 2 weeks before transplantation. If the percentage of peripheral B lymphocytes remained greater than 0.5%, additional rituximab (100 or 200 mg) was administered. Nine patients received rituximab 100 mg/body (low-dose rituximab [LDR] group). Overall survival and graft survival rates did not differ significantly between the LDR group and other cases. The incidences of myelosuppression and viral infection were lower in the LDR group than the other cases.

One serious complication associated with rituximab use is the reactivation of hepatitis B virus, and once reactivated, HBV may lead to death due to hepatitis in some patients. Rituximab alone without chemotherapy can induce HBV reactivation.

Yang et al. [5] also reported that rituximab increases the chance of HBV reactivation more than chemotherapy alone, and identified risk factors for HBV reactivation were being male, a lack of anti-HBs antibodies, HBV-DNA level, presence of lymphomas, anthracycline/steroid use, second-/third-line anticancer treatment, and youth.

Huang et al. [6] reported that the lack of entecavir administration is the most important factor of HBV reactivation in rituximab-associated therapy. This report concluded that the most important treatment to prevent HBV reactivation was the prophylactic administration of nucleoside analog therapy, not only for HBe antigen (−), HBs antigen (−), and anti-HBc-positive cases but also for anti-HBs-positive cases.

By the same token, Loomba et al. [7] reported that preventive nucleoside analog approaches as lamivudine or entecavir administration are recommended when a combination of lamivudine and chemotherapy has been suggested.

Patients with so-called "resolved hepatitis B virus infection" (HBsAg-negative/cAb-positive) may also be at risk. Mozessohn et al. [8] performed a systematic review of the English and Chinese language literature to estimate the risk of hepatitis B viral reactivation in HBsAg-negative/cAb-positive patients receiving rituximab for lymphoma. Data from 578 patients in 15 studies were included. "Clinical HBV reactivation" was estimated at 6.3% (I(2) = 63%, $P = 0.006$). Significant heterogeneity was detected. Reactivation rates were higher in prospective vs. retrospective studies (14.2% vs. 3.8%; OR = 4.39, 95% CI 0.83–23.28). Exploratory analyses found no effect of HBsAb status on reactivation risk (OR = 0.083; $P = 0.151$). Their meta-analysis confirms a measurable and potentially substantial risk of HBV reactivation in HBsAg-negative/cAb-positive patients exposed to rituximab. Preemptive use of nucleoside analogues such as lamivudine can largely prevent HBV reactivation in patients with chronic HBV.

Lee et al. [9] analyzed the risk of HBV reactivation in hepatitis B surface antigen (HBsAg)-negative/hepatitis B core antibody (anti-HBc)-positive kidney transplant patients receiving rituximab desensitization. Patients were divided into rituximab ($n = 49$) or control ($n = 123$) groups. All patients were observed for HBV reactivation, which was defined as the reappearance of hepatitis B surface antigen or HBV DNA. During the follow-up period (median, 58 months; range, 4–95 months), five patients (10.2%) in the rituximab group and two patients (1.6%) in the control group experienced HBV reactivation ($P = 0.003$). In the rituximab group, two patients experienced HBV-related severe

hepatitis, and one patient died due to hepatic failure. The median time from rituximab desensitization to HBV reactivation was 11 months (range, 5–22 months). By contrast, no patients in the control group experienced severe hepatitis. The status of hepatitis B surface antibody was similar between groups. Rituximab desensitization [hazard ratio (HR), 9.18; 95% confidence interval (CI), 1.74–48.86; $P = 0.009$] and hepatitis B surface antibody status (HR 4.74; 95% CI 1.05–21.23, $P = 0.04$) were significant risk factors for HBV reactivation. Rituximab desensitization for incompatible kidney transplantation significantly increased the risk of HBV reactivation in HBsAg-negative/anti-HBc-positive patients. Therefore, close monitoring of HBV DNA is required in these patients.

B cell depleting anti-CD20 monoclonal antibody therapies are being increasingly used as long-term maintenance therapy for neuroinflammatory disease compared to many non-neurological diseases where they are used as remission-inducing agents. While hypogammaglobulinemia is known to occur in over half of patients treated with medium to long-term B cell-depleting therapy (in our cohort IgG 38, IgM 56 and IgA 18%), the risk of infections it poses seems to be under-recognized. Tallantyre [10] report five cases of serious infections associated with hypogammaglobulinemia occurring in patients receiving rituximab for neuromyelitis optica spectrum disorders.

Healy [11] reported a case of myofasciitis and meningitis with deafness caused by systemic enterovirus infection in the setting of hypogammaglobulinemia induced by rituximab.

A biopsy of the left vastus lateralis showed macrophages infiltrating the epimysium and perimysial septa, with accompanying perivascular lymphocytic cuffs. The pathology was of myofasciitis. PCR was performed on the serum and CSF, demonstrating high copy numbers of enterovirus, sequenced as echovirus type 9.

Shah [12] also observed the low immunoglobulin (Ig) levels in rituximab treatment.

Their cohort of 30 patients had a mean age of 63 (SD 7) years, 23 were women, 16 had granulomatosis with polyangiitis and 13 were PR3 ANCA positive. Nine patients received concomitant cyclophosphamide. In this cohort, 20 patients had low serum IgG levels (<750 mg/dL) following RTX treatment. During the follow-up period, four individuals developed infections requiring hospitalization. In unadjusted logistic regression analysis, an IgG level $\leq$ 375 mg/dL was associated with 23 times higher odds of hospitalized infection. Low IgA was also associated with an increased risk of infections requiring hospitalization after adjusting for age, race, and eGFR [OR 24.6 (95% CI 1.5–799.5) $P = 0.03$]. Low IgM was not associated with a higher risk of infections requiring hospitalization.

Severe hypogammaglobulinemia was associated with increased odds of infection requiring hospitalization in this cohort. Further investigation is warranted given their study is limited by small sample size, concomitant cyclophosphamide use, and variable timing of Ig measurement.

The effectiveness of desensitization with rituximab in ABO-incompatible renal transplantation (ABO-I) has been widely reported. However, ABO-I outcomes are still worse than those of ABO-identical or ABO-compatible renal transplantation.

Okada [13] reported ABO-I cases treated with rituximab (RIT, $n = 131$), splenectomy (SPX, $n = 21$), or neither because of low anti-A/B antibody titers (NoR/S, $n = 53$). Graft survival, infection, and de novo HLA antibody production were compared for ABO-I and ABO-Id/C, followed by stratification into RIT and NoR/S groups. Propensity score-based methods were employed to limit selection bias and potential confounders. Overall graft survival for ABO-I was significantly lower than that for ABO-Id/C. Graft loss due to infection with ABO-I was significantly more frequent than that with ABO-Id/C, whereas acute antibody-mediated rejection (AMR) caused no graft failure in ABO-I recipients. Stratified analysis demonstrated significantly higher infection risk with RIT than with NoR/S. Safe reduction or avoidance of rituximab in desensitization protocols might contribute to further improvement of ABO-I outcome.

Morath et al. [14] observed a higher incidence of BK virus replication and BK virus-associated nephropathy. A higher frequency of viral infections such as CMV, HSV, VZV, and BK virus, as well as *P. jirovecii* pneumonia, wound, and severe urinary tract infections have been described. In the CTS and the Heidelberg cohort, an increased risk for early severe infections was observed, resulting in approximately one additional patient death in 100 ABOi kidney transplant recipients during the first year after surgery.

Transplantation in the presence of major ABO incompatibility, however, places the patient at a somewhat higher risk of early rejection, infection, and infection-associated death. Therefore, whenever possible, ABOc procedures should be preferred.

In Morath's report [15], 20 patient deaths were recorded in living donor kidney transplant recipients, with a 3.2% death rate (one of 31) in ABO and HLA incompatible kidney transplant recipients, a 1.4% rate (two of 144) in HLA incompatible patients and a 2.4% rate (six of 248) in ABO incompatible patients; these rates are higher than the 0.7% rate (11 of 1,541) in standard risk recipients. The increased death rate in their cohort of desensitized patients was most likely explainable by the higher rate of infection-associated death. Although the numbers in each group are small, more than 80% of patient deaths in the ABO and HLA incompatible, groups were due to infection, whereas infection was the cause in only 27.3% of the deaths in standard risk recipients. In the multivariate analysis, pretransplant desensitization with plasmapheresis and rituximab was identified as the driving cause of infection-associated death (HR = 3.40, $P = 0.002$).

Habicht [16] retrospectively analyzed 21 consecutive recipients who underwent ABOi renal transplantation. Pretransplant desensitization included administration of rituximab (375 mg/m$^2$), mycophenolate mofetil (MMF), tacrolimus, and prednisolone 4 weeks prior to scheduled transplantation as well as IA and IVIG. The intensified desensitization was associated with an increased risk of infectious complications. This observation prompted them to briefly escalate the desensitization protocol in ABOi kidney recipients in their centre.

A multivariate analysis revealed that infections including viral [CMV, HSV, VZV, and polyomavirus (BKV)], as well as bacterial infections leading to hospitalization such as urosepsis and pneumonia were significantly more common in the ABOi group (50%) than in the ABOc group (21%) ($P = 0.038$). CMV infections occurred in 14% of ABOi recipients as compared to 6.3% of ABOc recipients. In the ABOi group, 3 of 21 patients developed HSV or VZV eruptions while only 2 HSV infections were noted in 47 ABOc recipients despite a similar seroprevalence of >95% for HSV IgG and VZV IgG pretransplant in both groups. Interestingly, the most common viral infection was BK viremia leading to BKN in 5 of 20 ABOi grafts (25%) and 4 of 47 (8.5%) ABOc grafts.

In Lentine report [17], recipients of ABOi transplants experienced significantly ($P < 0.05$) higher incidence of wound infections (12.7% vs. 7.3%), pneumonia (7.6% vs. 3.8%), and urinary tract infections (UTIs) or pyelonephritis (24.5% vs. 15.3%) in the first 90 days compared with ABO-compatible recipients. In adjusted models, ABO incompatibility was associated with twice the risk of pneumonia (adjusted hazard ratio [aHR], 2.22; 95% confidence interval [CI], 1.14–4.33) and 56% higher risk of UTIs or pyelonephritis (aHR 1.56; 95% CI 1.05–2.30) in the first 90 posttransplantation days, and 3.5 times the relative risk of wound infections in days 91 to 365 (aHR 3.55; 95% CI 1.92–6.57). ABOi recipients, 19% of whom underwent pre- or peritransplant splenectomy, experienced twice the adjusted risk of early hemorrhage. ABOi transplantation offers patients with potential live donors an additional transplant option but with higher risks of infectious and hemorrhagic complications. Awareness of these complications may help improve protocols for the management of ABOi transplantation.

The single-center retrospective study by Naciri [18] assessed posttransplantation complications in 44 ABO-i versus 44 matched ABO-c patients. All patients were comparable at baseline except that ABO-i patients had greater immuno-

logical risks. ABOi patients have a significantly increased incidence of BK-virus (BKV) infection, as well as BKV-associated nephropathy.

Infection-related mortality following desensitization with rituximab is a problem. Reduced death-censored graft survival was reported in patients who were transplanted after desensitization for HLA antibodies, most likely as a consequence of an increased rate of antibody-mediated rejection episodes. Opelz [19] suggests that patient survival after ABO (and HLA) incompatible kidney transplantations is reduced due to infectious complications and infection associated death.

Higher rates of infection-associated deaths have also been identified in several other recent studies: Barnett and colleagues [20] reported on reduced patient survival 1 and 3 years after ABO incompatible kidney transplantation with four deaths in 62 desensitized patients (log rank $P = 0.018$ for patient survival) [1]. One patient death was due to sepsis, while the other three patient deaths were attributable to Pneumocystis jirovecii pneumonia (PcP).

### AMC Case of Hepatitis in Anti-CD20

In our single center (Asan Medical Center) analysis [21–23], ABOi KT was performed In 500 patients and a total 152 patients underwent FCXM positive KT. A total of 65 patients who underwent ABOi and FCXM positive KT were excluded. The remaining 435 patients in the ABOi KT group were divided into era 1 (2009–2012) and era 2 (2012–2018) by desensitization protocol. A total of 1,209 patients who underwent ABO compatible and FCXM negative KT from January 2012 to February 2018 served as a control group. In our initial desensitization protocol (era 1), patients received a single dose of rituximab (500 mg) 1 week before plasmapheresis.

After we experienced lethal infectious complications, a modified immunosuppression protocol was applied from January 2012 (era 2). We reduced the dose of rituximab from 500 to 200 mg in ABOi patients unless patients showed positive FCXM. Tacrolimus was given at an initial level of 8 ng/mL and reduced to 3–8 ng/mL 1 week after transplantation. The dose of MMF was reduced from 1.5 to 1 g/day after the 7th postoperative day.

The subgroup analysis of ABOi KT comprised the control group of 1,209, the era 1 group of 64, and the era 2 group of 371. All patients underwent ABOi KT under the condition that the IgG and IgM Ab titers against blood groups A or B were below 1:8.

The group of patients in the era 1 showed a significantly decreased patient survival in comparison with the era 2 and control groups during the 5-year follow-up (era 1 vs. era 2 vs. control group; 92.4% vs. 98.2% vs. 99.0%, $P < 0.01$) (Fig. 6.1). The overall graft survival rates in era1 group showed inferior outcomes than era 2 or control group at 5-year follow-up (era1 vs. era 2 vs. control group; 89.3% vs. 95.4% vs. 96.9%, $P = 0.03$).

To evaluate infectious complications according to rituximab dose, 303 patients who received an ABO i KT in the period from February 2009 to July 2016 in our center were retrospectively reviewed. The patients in era 1 showed a higher rate of infectious complications, such as CMV infection and pneumonia, compared to the patients in the era 2 or control groups (Table 6.1).

Recent years have seen increasing use of rituximab (RTX) for various types of primary and secondary glomerulopathies. However, there are no studies that specifically address the risk of infection related to this agent in patients with these conditions.

Trivin et al. [24] reviewed the outcomes of all patients who received RTX therapy for glomerular disease between June 2000 and October 2011 in eight French nephrology departments. Among 98 patients treated with RTX, 25 presented with at least one infection. They report an infection rate of 21.6 per 100 patient-years. Five patients died within 12 months following an RTX infusion, of whom four also presented with an infection. The median interval between the last RTX infusion and the first infectious episode was 2.1 months (interquartile range 0.5–5.1). Most infections were bacterial (79%) and pneumonia was the most frequent infection reported (27%). The presence of diabetes mellitus ($P = 0.006$), the

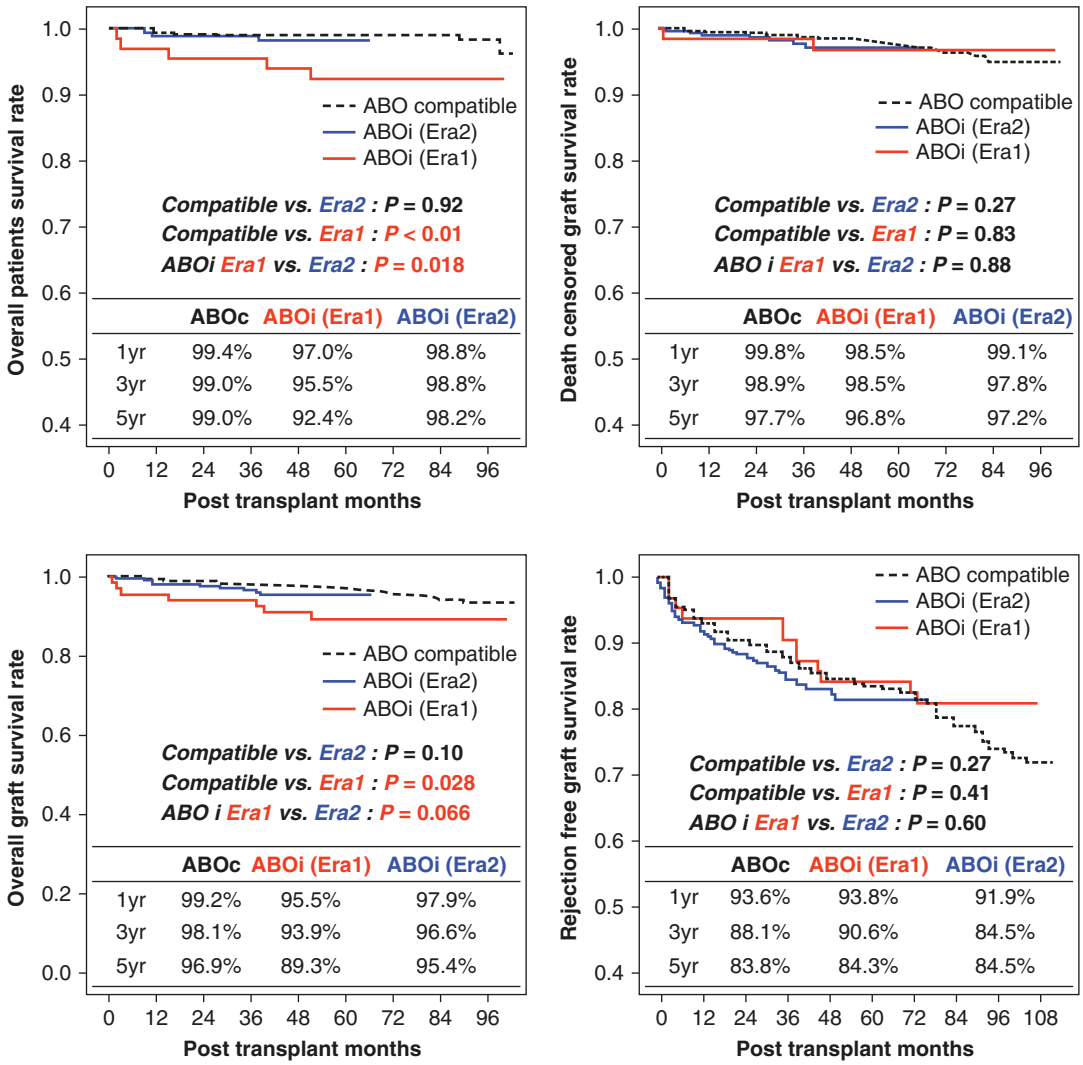


**Fig. 6.1** Long-term patient and graft survival stratified by Era and ABO incompatibility

cumulative RTX dose ($P = 0.01$), and the concomitant use of azathioprine ($P = 0.03$) were identified as independent risk factors. Renal failure was significantly associated with an increased infection risk by bivariate analysis ($P = 0.03$) and was almost significant by multivariate analysis ($P = 0.05$).

Schrezenmeier [25] reported a retrospective observational registry study (German Registry on Autoimmune Diseases) comprising a total of 681 patients was conducted. The data of 63 adult kidney transplant recipients who received rituximab between 2006 and 2013 were used in this analysis. At least one severe infection occurred in 57% of patients. The median time between the first rituximab infusion and the first infection was 4 (1–48) months. Of the overall 88 infections, 74 were severe bacterial infections, 5 were severe viral infections, 3 were severe fungal infections, 2 were combined severe bacterial and fungal infections, and 4 were combined severe viral, fungal, and bacterial infections. Seven patients died during the observational period, two of them due to infectious complications. In the observational period, one case of squamous cell carcinoma but no other malignancies were observed.

**Table 6.1** List of infections between ABO compatible and ABO incompatible KT

| Variables | ABO compatible ($N = 1,019$) Number (%) | ABOi Era 2 ($N = 239$) Number (%) | ABOi Era 1 ($N = 64$) Number (%) | $p$-Value |
|---|---|---|---|---|
| BK virus PCR- positive | 243 (23.8) | 62 (25.9) | 23 (36.9) | 0.09 |
| BK virus ≥4 logs | 86 (8.4) | 27 (11.3) | 10 (15.6) | 0.08 |
| BK nephropathy | 11 (1.1) | 8 (3.3) | 3 (4.7) | 0.007 |
| CMV antigenemia positive | 376 (36.9) | 72 (30.1) | 41 (64.1) | <0.001 |
| CMV antigenemia ≥50 | 88 (8.6) | 11 (4.6) | 11 (17.2) | 0.004 |
| Bacterial pneumonia | 40 (3.9) | 17 (7.1) | 2 (3.1) | 0.09 |
| PCP pneumonia | 11 (1.1) | 0 (0.0) | 3 (4.7) | 0.005 |
| Viral pneumonia | 13 (1.3) | 3 (1.3) | 3 (2.7) | 0.08 |
| Aspergillosis pneumonia | 1 (0.1%) | 0 (0.0) | 0 (0.0) | – |
| Varicella zoster | 65 (6.4%) | 14 (5.9%) | 4 (6.3%) | 0.96 |
| Influenza | 17 (1.7%) | 5 (4.5%) | 3 (4.7%) | 0.22 |
| Bacterial UTI | 105 (10.3%) | 17 (7.1%) | 5 (7.8%) | 0.28 |
| BK virus ≥4 logs + CMV antigenemia ≥50 + pneumonia + UTI + other viral infection + fungal infection | 333 (32.7) | 81 (33.9) | 31 (48.4%) | 0.035 |

A high incidence of infections was observed after rituximab treatment in kidney transplant recipients. Most infections occurred within 6 months after rituximab initiation. With more than 3 years of follow-up, they were able to document a low incidence of secondary malignancies after rituximab with only one case in their cohort.

## Rejection

Unexpectedly rituximab induced high prevalence of early acute rejection was reported by Clatworthy [26]. They conducted an open-label, randomized, controlled trial comparing rituximab, a B cell depleting, chimeric, anti-CD20 monoclonal antibody, with an anti-CD25 monoclonal antibody (daclizumab) as induction therapy in patients undergoing renal transplantation. They planned to recruit 120 patients, but the study was suspended after recruitment of the first 13 patients, owing to an excess incidence of acute cellular rejection in the rituximab group. Five of six patients (83%) who received rituximab had an episode of biopsy-confirmed cute rejection in the first 3 months after transplantation, as compared with one of seven patients (14%) in the daclizumab group ($P = 0.01$) (Table 6.1 and Fig. 6.1). All the episodes of rejection responded to intravenous methylprednisolone, and allograft function was similar in the two groups at 12 months. After rituximab treatment, peripheral B cells were undetectable in all patients. Serum cytokines, including tumor necrosis factor α, interleukin-6, and interleukin-10, were increased after transplantation, as compared with baseline values.

Their findings are surprising; patients who received rituximab had a rate of acute rejection that was not only higher than the rate in the control group (83% vs. 14%) but also was higher than that previously observed among patients who have not received induction therapy (35%). One possible explanation may be that proinflammatory cytokine release associated with B cell depletion might prime antigen-presenting cells. A short-lived cytokine-release syndrome often occurs after administration of the first dose of rituximab [27].

Although B cells may enhance immune responses, some B cells have immunoregulatory properties. Similarly, depletion of immunoregulatory B cells

may have contributed to the increased rejection in the rituximab-treated patients.

## Lung Disease

Pulmonary toxicity is a rare complication of rituximab therapy. Although rituximab is relatively safe and can be administered in an outpatient setting, rituximab-associated lung disease has been reported and may cause mortality despite early detection. Typically the pulmonary toxicity occurs at around the fourth cycle of rituximab. High index of suspicion is crucial and other concurrent pathology such as infective causes should be excluded. Radiological imaging and histological confirmation should be obtained and early treatment with corticosteroid should be initiated. Patients should receive counselling regarding respiratory symptoms and possible pulmonary toxicity.

Liote et al. [28] reported that post marketing surveillance detected anaphylactic shock and acute respiratory distress syndrome, fatal in 0.04–0.07%. Rituximab-induced lung injury can be subdivided to hyperacute early (within day 1), acute and subacute (7–21 days), and chronic (>28 days) from the last administration of rituximab.

Zaidi et al. [29] reported a 49-year-old man who was diagnosed with stage IVB plasmablastic lymphoma with jaundice, night sweats, and bilateral cervical swelling for 3 weeks.

Bilateral cervical lymphadenopathy, autoimmune hemolytic anemia with left internal jugular and brachial vein thrombosis were detected and his chest CT was normal. He received two cycles of R-hyperCVAD (rituximab, cytoxan, doxorubicin, vincristine, dexamethasone) and two cycles of R-CHOP (rituximab, cytoxan, doxorubicin, vincristine, prednisolone) with intrathecal chemoprophylaxis during every cycle. One week prior to the fourth cycle he developed type 1 respiratory failure. On day seven post fourth cycle R-CHOP, he developed neutropenic sepsis with neutropenia. On day 14 he complained of difficulty in breathing especially on talking and exertion. Chest showed bilateral opacities. Initial bronchoalveolar lavage were negative. Therefore the most likely cause for the acute respiratory distress syndrome (ARDS) was rituximab-induced lung disease. And he later succumbed in the intensive care unit. This patient fits into the subacute onset group, which is known to be the commonest form, usually occurring around the fourth cycle of rituximab. The underlying process probably reflects hypersensitivity reaction to the immunogenic chimeric anti-CD20 antibody. Common radiological findings in rituximab-induced lung injury are diffuse pulmonary infiltrates, ground glass opacification, pulmonary fibrosis, alveolar hemorrhage, and consolidative changes.

In Hadjnicolaou's [30] report, a total of 121 cases of potential rituximab (RTX) associated interstitial lung disease (ILD) were identified from 21 clinical studies/trials, 30 case reports, and 10 cases series. The most common indication for RTX was diffuse large B cell lymphoma. RTX-ILD occurred more frequently in male patients and was most common during the fifth and sixth decades of life. In most cases, RTX was part of combination chemotherapy, but in 30 (24.7%) cases it was given as monotherapy. The mean and median number of cycles of RTX before disease onset was four, but cases following the first cycle or as late as the 12th cycle were also identified. The mean time of onset, from the last RTX infusion until symptom development or relevant abnormal radiological change was 30 days (range 0–158 days). Abnormal radiological findings were similar in all patients, with diffuse bilateral lung infiltrates apparent on chest radiographs and/or thoracic CT. Hypoxemia was seen in all cases and pulmonary function tests were uniformly abnormal with a characteristic diffusion capacity deficit and restrictive ventilatory pattern. RTX-ILD was fatal in 18 cases.

ILD is a rare but potentially fatal complication of RTX therapy. This diagnosis should be considered in any patient who develops respiratory symptoms or new radiographic changes while receiving this biologic agent.

Child [31] report the case of an 84-year-old man with refractory immune thrombocytopenia

purpura (ITP) who was treated with rituximab and subsequently developed severe interstitial lung disease. There has been increasing use of rituximab in the treatment of ITP with success rates of up to 62% in adult patients with recurrent ITP. Interstitial lung disease is a rare but recognized complication of rituximab but has been rarely reported in the setting of ITP.

Aquaporin-4 immunoglobulin G (IgG)-positive neuromyelitis optica spectrum disorder (NMOSD-AQP4) is an inflammatory disease of the central nervous system (CNS) that predominantly affects the optic nerves and spinal cord.

Rituximab (RTX)—a monoclonal antibody to CD20 in B cells—is effective in the treatment of NMOSD. However, interstitial lung disease (ILD) is a very rare yet potentially fatal complication of RTX treatment. Ahn [32] detail the first reported case of RTX-induced ILD in a patient with NMOSD-AQP4. ILD should be suspected in patients with NMOSD undergoing RTX treatment who present with dyspnea and/or cough without any signs or symptoms of infection. Common side effects of RTX include infusion-related reactions, fever/chills, infection, and respiratory complications.

## Cardiac Disease

Life-threatening cardiac complication is reported but quite rarely.

A 62-year-old male underwent treatment of non Hodgkin's lymphoma with bone marrow involvement with thrombocytopenia was reported by Verma [33]. After 15 min of starting of IV infusion of rituximab, he started having severe retrosternal chest pain, diagnosed as acute ST elevation from inferior wall MI. Patient was preloaded with dual anti-platelets. Coronary angiogram showed 100% occlusion of proximal RCA. Thrombosuction of this culprit right coronary artery revealed underlying 90% stenosis. After that, balloon angioplasty of RCA was done. The procedure was terminated in the view of successful balloon angioplasty with good flow. He was kept on dual antiplatelet therapy for 1 month with regular platelet monitoring. With the growing increasing global use of rituximab for various oncological and immunological diseases, this complication of myocardial infarction should be kept in mind. They recommend that patients with known cardiovascular risk factors should be assessed for ischemic heart disease before treatment and be carefully monitored during and after treatment especially during first infusion when tumor burden is highest with a slow initial infusion rate, followed by increasing the rate in 30-min increments as tolerated. This life-threatening cardiovascular complication should also be kept in mind while using rituximab in patients without cardiovascular risk factors. The mechanism by which rituximab elicits infusion reactions remains unclear, although the symptoms associated with the reactions are thought to be related to the release of inflammatory cytokines. The incidence of infusion reactions was highest during the first infusion (77%) and decreased with each subsequent infusion. Adverse events can include urticaria, hypotension, angioedema, hypoxia, pulmonary infiltrates, acute respiratory distress syndrome, myocardial infarction, ventricular fibrillation, or cardiogenic shock. The majority of severe reactions occur approximately 30–120 min after starting the first infusion.

## Others

### Acute Thrombocytopenia

Rituximab-induced acute thrombocytopenia (RIAT), a rare complication of rituximab administration, is reported by Ureshino [34]. A 65-year-old man received rituximab for the treatment of high tumor burden follicular lymphoma in the leukemic phase. The next day, his platelet count abruptly dropped from 85,000 to 5,000/μL, which spontaneously recovered in a few days without specific treatment. They speculate that the occurrence of infusion-related cytokine release syndrome in rituximab-sensitive high tumor burden follicular lymphoma contributed to the development of RIAT. The patient is scheduled to receive cyclophosphamide (CHOP) chemotherapy (750 mg/$m^2$ cyclophosphamide, 50 mg/$m^2$ doxorubicin and

1.4 mg/m$^2$ vincristine on day 1, and 1 mg/kg prednisolone on days 1–5) followed by rituximab. On day 19 of CHOP chemotherapy, the patient received rituximab 375 mg/m$^2$ intravenously over 4 h. Within 2 h, he developed infusion-related hypersensitivity reactions consisting of a fever, chill, and nausea, which were resolved by 125 mg methylprednisolone. The next day (day 20), his platelet count abruptly dropped to 5,000/μL, which was verified on a peripheral smear and repeat complete blood count. The WBC also dropped to 2,200/μL.

## Complication from Bortezomib

**Q: 1. What kind of side effects does bortezomib have?**
**2. Are the side effects related with the dose of bortezomib?**

Proteasomes are located in the cell nucleus and cytoplasm and are the primary proteolytic mechanism in eukaryotic cells. The 26S proteasome specifically degrades ubiquitinated proteins and eliminates unnecessary, misfolded, and malfunctioning proteins, in addition to proteins involved in cell cycle regulation and oncogenesis. Bortezomib, a modified dipeptidyl boronic acid, is a reversible inhibitor of the 26S proteasome. Its ability to trigger apoptosis of bone marrow-derived plasma cells has led to its approved and widespread use in multiple myeloma. Bortezomib also shows several additional immunological effects that have resulted in its increasing utility in AMR and desensitization protocols. Bortezomib mediates apoptosis of activated T ells by preventing degradation of IkB and blocking nuclear factor-kB and subsequent transcription of interleukin-1, interleukin-6, and tumor necrosis factor-a. Specifically, bortezomib induces the loss of mitochondrial membrane potential, which translocates proapoptotic proteins and enhances caspase activities. Bortezomib suppresses the maturation of dendritic cells, which present antigens to T cells including the HLA antigens. Bortezomib inhibits angiogenesis by reducing VEGF and interleukin-release by tumor cells and prevents adaptation to hypoxia. In chronic allograft injury, a lymphangiogenesis process is involved so this inhibition could further disrupt rejection from taking place [35]. Bortezomib is a proteasome inhibitor that downregulates the T cell immune response and is increasingly being used for kidney transplant rejection.

### GI Toxicity

Prospective open labeled clinical trial using bortezomib in sensitized renal transplant was performed by Jeong [36]. The desensitization regimen consisted of 2 doses of IVIG (2 g/kg), a single dose of rituximab and 4 doses of bortezomib. There were 19 highly sensitized patients who received desensitization and 17 patients in the control. Deceased donor kidney transplantation was successfully performed in 8 patients (42.1%) in the desensitization group versus 4 (23.5%) in the control group. Desensitization was well tolerated, and acute rejection occurred only in the control group. Desensitization protocol using bortezomib, high-dose IVIg, and rituximab increased the DDKT rate in highly sensitized, wait-listed patients. One patient received only two doses of bortezomib because of neutropenia and abdominal pain after the second dose of bortezomib. Regarding adverse events, gastrointestinal toxicity was the most common adverse event (21%), followed by opportunistic infection (15.8%), and thrombocytopenia.

### Peripheral Neuropathy

Based on the few clinical trials that investigate its safety and efficacy, bortezomib appears to be successful as a desensitization agent. Notably, Shah [37] observed that 50% of the patients on bortezomib experienced a worsening or increase

in peripheral neuropathy during the first year of transplantation, although no severe cases were reported.

Gonzales [38] suggest that 32 doses of bortezomib monotherapy was not well tolerated and resulted in only a modest reduction in anti-HLA antibodies. Bortezomib was given in cycles (4 doses = 1 cycle). The initial dose for all patients was 1.3 mg/m$^2$ body surface area (BSA) intravenously on days 1, 4, 8, and 11 with at least 10 days in between the last dose of a cycle and the first dose of the next cycle. Five patients (50%) completed the 32-dose regimen without dose reduction or discontinuation. Dose reduction was required in two patients who developed severe peripheral neuropathy but eventually completed the 32-dose course. The severe neuropathy was manifested by left scapular myofascial pain and upper extremity edema. The other patient experienced progressive severe bilateral lower extremity neuropathy, anorexia, and insomnia. The other patient developed disseminated varicella zoster, severe local herpes recurrence, peritonitis in the setting of peritoneal dialysis, encephalopathy, ataxia, and visual hallucinations.

## Hematologic Toxicity

No systemic trial has been undertaken to support its use in ABMR. In randomized, placebo-controlled trial (the Bortezomib in Late Antibody-Mediated Kidney Transplant Rejection [BORTEJECT] Trial), Eskandari [39, 40] investigated whether two cycles of bortezomib (each cycle: 1.3 mg/m$^2$ intravenously on days 1, 4, 8, and 11) prevent GFR decline by halting the progression of late donor-specific antibody (DSA) positive ABMR. Forty-four DSA positive kidney transplant recipients with characteristic ABMR morphology. Patients were randomly assigned to receive bortezomib ($n$ = 21) or placebo ($n$ = 23). They detected no significant differences between bortezomib- and placebo-treated groups in median measured GFR at 24 months, 2-year graft survival (81% vs. 96%; $P$ = 0.12), urinary protein concentration, DSA levels, or morphologic or molecular rejection phenotypes in 24-month follow-up biopsy specimens. Bortezomib, however, associated with gastrointestinal and hematologic toxicity. The grades of anemia, thrombocytopenia, and leukocytopenia were significantly higher in the bortezomib group.

## Compensatory Humoral Reaction

In Kwun study [41] bortezomib treatment given intravenously twice weekly for 1 month (1.3 mg/m$^2$ per dose) clearly reduced the numbers of antibody-producing cells and CD38 + CD19 + CD20 – plasma cells in the bone marrow ($P < 0.05$), but donor-specific alloantibody levels did not decrease. They observed a rapid but transient induction of circulating IgG + B cells and an increased number of proliferating B cells in the lymph nodes after 1 month of treatment. Notably, bortezomib treatment induced germinal center B cell and follicular helper T cell expansion in the lymph nodes. These data suggest that bortezomib-induced plasma cell depletion triggers humoral compensation. On the basis of the effect of bortezomib on PC and in vivo compensation, they suggest that bortezomib should not be used alone for desensitization, because bortezomib depletion of PCs will lead to a compensatory response, which is likely to be deleterious to an allograft. They suggest that desensitization regimens incorporating the unique effect of bortezomib should be designed on the basis of its mechanistic effects and limitations and that adjuvant B cell depletion, blocking of GC initiation, or strategies to prevent GC compensation should be considered.

## Others

In a study of bortezomib in a new player in pre- and posttransplant desensitization [42], mean drug-related adverse events that were reported in more than 15% of patients enrolled in those studies consisted of asthenic condition (fatigue, weakness, and malaise), GI disorders, pyrexia,

thrombocytopenia, neutropenia, peripheral neuropathy, psychiatric disorders, and anorexia.

## Complication from IVIg

**Q: 1. What kind of side effect doses IVIg have?**
**2. Is it related with the dose of IVIg injection?**

Intravenous Ig (IVIg) was initially used to treat primary immune deficiencies and then for the treatment of various autoimmune, inflammatory, and infectious diseases.

Intravenous IVIg is applied to desensitize highly HLA-sensitized patients and to treat antibody mediated rejection (AMR). It is also used in the treatment of polyomavirus and parvovirus disease.

Some common side effects of IVIg infusion include pyrexia, rigors, and headache. Rare, but significant, adverse events include acute kidney injury related to sucrose-induced osmotic nephrotoxicity, hypersensitivity reaction, and vascular thrombosis [43].

### Thromboses

It is reported that life-threatening thromboses in pulmonary, coronary, cerebral, and peripheral vessels are associated with high-dose intravenous immunoglobulin (IVIg) therapy that is generally considered safe [44].

Sin [45] experienced a patient with a renal graft rupture that developed after high-dose IVIg was administered for desensitization. A needle biopsy performed 4 days prior to the rupture revealed the presence of glomerular thrombosis and mesangiolysis. The ruptured nephrectomy specimen contained renal infarction around the hemorrhagic segment and arterial wall thickening with intimal fibrosis. This might have contributed to rupturing associated with small arterial and glomerular arteriolar thrombi. The mechanisms underlying thrombosis development are IVIg-induced platelet activation, increased plasma viscosity, and coagulation factor XI contamination. She also received two rounds of high-dose IVIg (1 g/kg per day for 2 days) due to 100% PRA (panel reactive antibody) against class I and 92% against class I IHLA antigens as well as positive crossmatch test results against T cells. Fourteen days after surgery, IVIg was administered at a dose of 1 g/kg per day for 2 days to further reduce. Two days later, the creatinine levels had increased to 2.2 mg/dL. A biopsy showed that thromboembolisms had formed in the glomeruli along with focal segmental mesangiolysis. Four days later, the patient experienced severe graft pain. At the operation field a large perirenal hematoma with graft rupture was observed. The patient subsequently underwent hemodialysis.

In general, high doses of IVIG are relatively safe. However, serious side effects have been reported including acute renal dysfunction likely related to high osmotic load, thrombotic events with rapid infusions, and aseptic meningitis [46]. Slowing the infusion rate and using iso-osmolar preparations can reduce the risk of side effects [47].

### Hemolysis

Kahwaji [48] report the experience with IVIg-induced hemolytic anemia (IH) in ESRD patients receiving IVIG for desensitization or treatment of AMR. High cumulative doses were administered, ≥2 g/kg, in most cases. Markers of hemolysis, including direct antiglobulin tests, were recorded. There were 18 cases of IH in 16 patients. All patients developing hemolysis were non-O blood types. Isohemagglutinin titers ranged from 1:2 to 1:64 in the various IVIg products. Acute IH is a significant complication of high-dose IVIg infusion. Identified risk factors include non-O blood type of the recipient and administration of liquid IVIg preparations with high-titer anti-A/B IgG antibodies. They recommend monitoring hemoglobin 48–72 h

after IVIG infusion. If the hemoglobin decreases, a hemolytic workup is recommended. Hemolysis could be avoided in at-risk patients by choosing a low-titer product. However, other complications such as acute renal failure or thrombosis may be seen because the low-titer products are usually hyperosmotic.

Intravenous immunoglobulin (IVIg) is used for the treatment of a number of inflammatory conditions. Hemolysis due to passive transfer of blood group antibodies is a well-recognized complication of IVIg therapy. Therapy is largely supportive and consists of blood product support and hemodialysis. Welsh [49] report the use of therapeutic plasma exchange (TPE) as adjunct therapy for three patients with complications attributed to IVIg. Two patients had hemolysis attributed to IVIg; one patient was blood group A and the other blood group O.

TPE may be useful therapy for patients with severe hemolysis caused by IVIg or at risk for tissue damage by blood group antibodies.

### Myocardial Infarction

In Stenton report [50], an 81-year-old Vietnamese man was prescribed IVIG for treatment of toxic epidermal necrolysis secondary to allopurinol. Thirty minutes following the start of the IVIG infusion, the patient developed crushing retrosternal chest pain and shortness of breath. The pain improved upon discontinuation of IVIG infusion but recurred when IVIG was restarted. The troponin level reached 140 microg/L, and a persantine sestamibi stress test (MIBI) indicated anterolateral ischemia. The patient was diagnosed with non-ST-elevation MI. Although an association between IVIG administration and MI has not been demonstrated in clinical trials, accumulating clinical experience suggests that a relationship between IVIG and myocardial ischemia exists. Twenty published case reports were identified. Risk of acute MI seems to be increased with use of high-dose IVIG and in older individuals, especially those with at least one cardiovascular risk factor, such as ischemic heart disease or hypertension.

### Others

In Kakuta's report [51] 17 patients, each showing positive T cell FCXM (median ratio $\geq 1.4$) after 2 rounds of double-filtration plasmapheresis, received 4-day regimens of IVIG (1 g/kg per day) over 1-week periods. T cell and B cell FCXM determinations were obtained after every IVIG dose and again up to 4 weeks after initiating IVIG to ascertain negative conversion of T cell FCXM (median ratio $< 1.4$). The T cell FCXM-negative conversion rates after cumulative doses of 1, 2, 3, and 4 g/kg IVIG were 29.4%, 35.3%, 56.3%, and 46.7%, respectively. No deaths occurred during the study period. Adverse events, moderate or mild (total of 52), were observed in 13 patients (76.5%), and side effects (total of 38) were recorded in 11 patients (64.7%). The chief side effects included headache (29.4%), hepatic dysfunction (17.6%), rash (17.6%), and nausea (11.8%). Four patients (23.5%) suffered serious adverse events (total of 6), and serious side effects (total of 5) were recorded in 3 patients (17.6%). Serious side effects included leukopenia (5.9%), neutropenia (5.9%), thrombocytopenia (5.9%), headache (5.9%), and aggravation of renal function (5.9%), all of which were predictable. The protocol was discontinued in 2 patients after the third dose of IVIg. One became cytopenic (leukopenia, neutropenia, thrombocytopenia), without need of granulocyte colony-stimulating factor or platelet transfusions, and the other experienced headaches. In another patient, hemodialysis was initiated for the first time and continued until the point of transplantation to address deteriorating renal function after IVIg administration. There was no thromboembolism which is a concern due to the large dose of IVIg.

## Complication from Eculizumab

**Q: 1. What kind of side effect does eculizumab have?**
**2. What kind of vaccination is needed for the prevention of lethal infection?**

The effector functions of complement are centered on the formation of the C5 convertases that cleave the C5 to C5a and C5b. C5a as well as C3a which are anaphylatoxins involved in inflammation amplification and antigen presentation. C5b leads to formation of C5b-9 membrane attack complex, which can lyse nonnucleated cells (RBC, bacteria) or cause cellular activation and tissue injury when binding to nucleated cells. At the level of the kidney, primary dysregulation of the complement cascade is associated with thrombotic microangiopathy or C3 glomerulopathy. Eculizumab (Soliris) is a humanized monoclonal antibody, which blocks the cleavage and the activity of complement factor 5.

Humanized monoclonal antibody that binds C5 and blocks the enzymatic effect of C5 convertase thus preventing the creation of the membrane attack complex. The blockade is predominantly due to steric hindrance. In addition to a decrease in membrane attack complex formation via C5b, eculizumab blocks the release of C5a, a potent anaphylatoxin. C5a has been associated with the recruitment of phagocytes and upregulation of proinflammatory cytokines [52].

Clinical applications of eculizumab have been extended to AMR and prevention of delayed graft function in kidney transplantation. Duration and monitoring of treatment with eculizumab for complement defects is unclear. In AMR, it is used as a preventive measure following desensitization protocols or as salvage attempts in refractory AMR.

Limited data are available for the use of eculizumab in desensitization and AMR treatment. Its applications are complementary to desensitization protocols or to commonly used approaches for AMR treatment like plasma exchange, IVIg, and rituximab.

## Infection

The largest and most systematic clinical experience with eculizumab use for prevention of AMR was reported by Cornell, Stegall [53, 54] in which outcomes beyond 1 year in eculizumab-treated (EC) positive crossmatch kidney transplants (+XMKTx) was compared to a historical control group. +XMKTx received desensitization with either plasma exchange (PE) alone ($N$ = 48) or PE and EC ($N$ = 30). EC, given for at least 1 month, was continued in the setting of persistently high DSA (B flow cytometric crossmatch [BFXM] >200) including: 4 weeks ($n$ = 14); 9 weeks ($n$ = 6), 6 months ($n$ = 2), and 12 months ($n$ = 8). All patients had at least 2 years follow-up. The EC dosing regimen consists of 1,200 mg immediately prior to transplantation, 600 mg on postoperative day 1, and 600 mg weekly thereafter for 4 weeks.

In patients with persistently high DSA (BFXM >200), EC treatment continued (1,200 mg week 5, and then every 2 weeks). DSA assessments were performed at weeks 4, 9, 26, and 39. Eculizumab was discontinued at those time points if the B flow crossmatch channel shift was <200. Thirty patients received eculizumab from June 2008 to October 2011. Forty-eight historical controls were transplanted using a similar PE- and IVIG-based "desensitization."

Patients underwent induction therapy with anti-thymocyte globulin; and maintenance immunosuppression consisted of tacrolimus, mycophenolate mofetil, and prednisone.

The incidence of acute clinical ABMR was lower in the EC group than controls (6.7% vs. 43.8% $p < 0.01$). Death-censored allograft survival was similar between groups.

Despite decreasing acute clinical ABMR rates, eculizumab treatment does not prevent chronic ABMR in recipients with persistently high BFXM after +XMKTx.

In the eculizumab group. There was one episode of subclinical acute cellular rejection. There was one wound infection in the eculizumab group. One patient in the eculizumab group developed Burkitt's lymphoma 2.5 years after transplantation and died with a functioning graft.

West-Thielke [55] reported four patients underwent living donor kidney transplant from ABOi donors who were treated with a 9-week eculizumab course without therapeutic plasma exchange, intravenous immunoglobulin, or splenectomy. All patients had successful transplants and have normal graft function at the time of last follow-up. There were no cases of AMR or acute cellular rejection.

Patients received vaccination against Haemophilus influenzae, *Streptococcus pneumoniae*, and Neisseria meningitidis. Patients were immunized with conjugated, quadrivalent meningococcal vaccine (Menactra, Sanofi Pasteur, Lyons, France; Menveo, Novartis Vaccines and Diagnos tics, Cambridge, MA) protecting against strains A, C, Y, and W135 at least 2 weeks prior to receiving TPEX. In addition, patients were maintained on chemoprophylaxis with penicillin 500 mg every 12 h (levo-floxacin was utilized in patients allergic to penicillin) starting at time of eculizumab administration and continuing for 4 weeks after therapy with eculizumab was discontinued. One patient developed nausea, shoulder pain, and chest heaviness during the infusion of eculizumab on POD 49, which required early discontinuation of therapy.

Most serious infectious concerns are related to an increased risk of infections with encapsulated bacterial strains [56], Neisseria meningitides most notable, where complement activity plays an important role in the immune responses. Life-threatening infections with N. meningitides have been reported, and vaccination is recommended by the Advisory Committee on Immunization Practices.

Current recommendations for adult patients receiving eculizumab treatment include two doses of MenACWY at least 2 months apart with booster vaccinations every 5 years.

MenB vaccination is now recommended as well for patients >10 years of age. Vaccination is recommended at least 2 weeks prior to initiation of treatment with eculizumab.

Their protocol of eculizumab use posttransplantation includes vaccination with both conjugate tetravalent and MenB vaccination and 4–8 weeks of antibiotic prophylaxis with penicillin V or ciprofloxacin.

## Others

The efficacy of eculizumab for desensitization and treatment of AMR is unclear as complexed by patient characteristics and other therapeutic modalities [56].

Eculizumab safety data monitoring is ongoing and its availability is restricted by the FDA under a Risk Evaluation and Mitigation Strategy. Safety monitoring is ongoing; so far, most notable is the expected increase in infection risk with encapsulated organisms.

In general, eculizumab infusions are well tolerated by most patients with minimal adverse reactions noted. When adverse events occur, those commonly reported are: diarrhea, nausea, hypertension, headache, and vomiting. Additionally, anemia, leukopenia, urinary tract infections, and viral infections have been reported.

In Kulkarni report [57], 15 participants (five control, 10 treatment) with DSA and deteriorating renal function were enrolled. The treatment group received 6 months of eculizumab followed by 6 months of observation, whereas controls were observed.

The treatment group had an improved eGFR trajectory versus control, based on their predetermined two-sided 0.10 significance level ($p = 0.09$). Within-subject analysis of treated participants at 6-month intervals did not show significant change ($p = 0.60$).

Modeling C1q status showed that C1q-positive patients had significantly higher mean eGFR than patients with negative C1q ($p = 0.04$). Biopsies revealed elevated renal endothelial cell-associated transcripts (ENDATs), ENDATs in most participants, but ENDATs were not reduced with complement inhibition.

There were no significant differences in adverse events between the two arms of the study. There were no deaths or episodes of biopsy-proven acute rejection.

## Complication from C1 Esterase Inhibitor (C1 INH)

**Q: 1. What kind of side effect dose the C1 INH have?**
**2. What is the difference of side effect between eculizumab and C1 INH?**

C1-INH, which is a member of the serpin family of pro-tease inhibitors, inactivates C1r and C1s and is the only plasma protease that regulates the classic complement pathway.

Administration of exogenous human C1 esterase inhibitor (C1 INH) may provide prolonged protection from complement-mediated damage. Recombinant C1 INH prevented acute AMR in a primate model [58]. CINRYZEâ (Shire ViroPharma Incorporated, Lexington, MA) is a human plasma-derived C1 INH that is approved for use in patients with hereditary angioedema.

Up until now there is not a report on the prophylactic use of C1 esterase inhibitor in sensitized renal transplant recipients.

## Infection

Jordan et al. [59, 60] investigate the ability of C1 esterase inhibitor (C1INH) to prevent IRI/DGF in kidney transplant recipients. Seventy patients receiving deceased donor kidney transplants at risk for DGF were randomized to receive C1INH 50 U/kg ($n$ = 35) or placebo ($n$ = 35) intraoperatively and at 24 h. The primary end point was need for hemodialysis during the first week posttransplant. Assessments of glomerular filtration rate and dialysis dependence were accomplished.

C1INH did not result in reduction of dialysis sessions at 1 week posttransplant, but significantly fewer dialysis sessions ($P$ = 0.0232) were required 2–4 weeks posttransplant. Patients at highest risk for DGF (Kidney Donor Profile Index ≥85) benefited most from C1INH therapy.

There were no differences in patient and graft survival or graft losses at 1 year. One patient was removed from study analysis due to development of a significant posttransplant bleeding that led to acute kidney injury in a well function allograft. This was not considered to be related to the C1NH. C1NHY level were significantly higher in the treatment group, and this persisted at 1 week posttransplant.

Twenty patients (28.6%) experienced SAE. Ten from C1NH and 10 from the placebo group. All SAE resolved with treatment and were deemed not to be related to C1NH. Additional safety concerns with C1NH were risk of meningococcal infection and venous thromboembolism. At the 1-year assessment, there were two graft losses: one in the placebo group (antibody mediated rejection) and one in the C1NH group (surgical complication). The graft loss in the C1NH group was due to repeated retroperitoneal hematomas with acute kidney injury due to bleeding from a pseudoaneurysm of the iliac artery and renal artery anastomosis site. Significantly better renal function was seen at 1 year in C1INH patients ($P$ = 0.006). No significant adverse events were noted with C1INH.

## Others

Montgomery [61] report a phase 2b, multicenter double-blind randomized placebo-controlled pilot study to evaluate the use of human plasma-derived C1 esterase inhibitor (C1 INH) as add-on therapy to standard of care for AMR. Eighteen patients received 20,000 units of C1 INH or placebo (C1 INH $n$ = 9, placebo $n$ = 9) in divided doses every other day for 2 weeks. No discontinuations, graft losses, deaths, or study drug-related serious adverse events occurred.

C1 INH group demonstrated a trend toward sustained improvement in renal function. Six-month biopsies performed in 14 subjects (C1 INH = 7, placebo = 7) showed no transplant glomerulopathy (TG) (PTC + cg ≥ 1b) in the C1 INH group, whereas 3 of 7 placebo subjects had TG. Endogenous C1 INH measured before and after PP demonstrated decreased functional C1 INH serum concentration by 43.3%.

This new finding suggests that C1 INH replacement may be useful in the treatment of AMR.

Seven (78%) of nine subjects receiving C1 INH and six (67%) of nine receiving placebo had resolution of their AMR with a median time from first dose until AMR recovery of 20.0 days (range 19–86 days) in the C1 INH group and 20.5 days (range 20–22 days) in the

placebo group. Among the 6-month biopsies performed, no C1 INH subjects showed TG (PTC + cg ≥ 1b), whereas three of seven placebo subjects had TG. In the three placebo-treated subjects whose biopsies showed TG, there was evidence of ongoing chronic AMR.

Among 18 patients, 15 subjects (83%) had one or more AE during the study, which included nine subjects (100%) in the C1 INH group and six subjects (67%) in the placebo group. Only one patient (11%) in the C1 INH group had an AE (mild blurred vision) that was considered by the investigator to be related to study drug.

The most frequently reported AE was diarrhea, reported by three (33%) subjects in the C1 INH group and one (11%) patient in the placebo group. Peripheral edema ($n$ = 3), dyspepsia ($n$ = 2), pruritus ($n$ = 2), and urinary tract infection ($n$ = 2) occurred in subjects in the C1 INH group but not in subjects in the placebo group.

Viglietti [62] performed a prospective, single-arm pilot study to investigate the potential effects and safety of C1 inhibitor (C1-INH). Berinert (C1-INH) added to high-dose intravenous immunoglobulin (IVIG) for the treatment of acute ABMR that is nonresponsive to conventional therapy. Kidney recipients with nonresponsive active ABMR and acute allograft dysfunction were enrolled between April 2013 and July 2014 and received C1-INH and IVIG for 6 months (six patients). The primary end point was the change in eGFR at 6 months after inclusion (M + 6). Secondary end points included the changes in histology and DSA characteristics and adverse events as evaluated Until 6M.

No death or allograft loss was observed in C1-INH patients. One serious adverse event, i.e., gastrointestinal bleeding, occurred in one patient. The event was not considered to be related to the study drug. One episode of deep venous thrombosis of a lower limb occurred 5 months after inclusion in the study (adverse event), which led to Berinert discontinuation in this patient. This episode was caused by local venous compression due to a popliteal cyst.

## Complication from IgG Endopeptidase (IdeS)

**Q: 1. What kind of side effect does IdeS have?**
**2. What is the rebound of DSA?**
**3. What is the preventive measure for rebound DSA?**

The IgG-degrading enzyme derived from *Streptococcus pyogenes* (IdeS; GenBank accession number, ADF13,949.1) is a recombinant cysteine protease of *S. pyogenes* produced in *Escherichia coli* that cleaves all four human subclasses of IgG with strict specificity by hydrolyzing human IgG at Gly236 in the lower hinge region of the IgG heavy chains and cleaving human IgG into F(ab')2 and Fc fragments inhibiting complement-dependent cytotoxicity and antibody-dependent cellular cytotoxicity, which suggests that IdeS might be useful for desensitization. IdeS also cleaves B cell receptors from circulating B cells, with the resultant inhibition of antigen specific B cell IgG responses in vitro [63]. Within minutes after dosing, plasma IgG was converted into scIgG, and within a few hours after IdeS treatment, plasma IgG was cleaved into $F(ab')_2$ and Fc fragments with no intact IgG [64].

## Rebound Antibody Reaction

Jordan et al. [65] administered IdeS to 25 highly HLA-sensitized patients (11 patients in Uppsala or Stockholm, Sweden, and 14 in Los Angeles) before the transplantation of a kidney from an HLA-incompatible donor.

Patients in the US study also received intravenous immune globulin and rituximab after transplantation to prevent antibody rebound. Eligible patients were awaiting kidney transplantation on either the United Network for Organ Sharing waiting list (in the United States) or the Scandia transplant waiting list (in Sweden) with panel-

reactive antibody level of 95% (range, 22–100). The acceptance criteria for HLA-incompatible organs in recipients in the United States include a negative complement-cytotoxicity crossmatch, a negative flow-cytometric crossmatch, or a positive T cell and B cell flow-cytometric crossmatch with approximately 250 channel shifts or less and usually donor-specific antibody positivity.

In Sweden, patients were eligible if they had at least two anti-HLA antibodies with a mean fluorescence intensity of 3,000 or more.

Patients who underwent transplantation received IdeS at a dose of 0.24 mg per kilogram of body weight (in the United States) or at a dose of 0.25 or 0.50 mg/kg. Horse antithymocyte globulin is not susceptible to digestion by IdeS (unpublished data). Patients in the U.S. cohort received induction with alemtuzumab, at a dose of 30 mg administered subcutaneously 4 days after transplantation. and received IdeS over a period of 15 min approximately 4–6 h before the receipt of a kidney transplant from an incompatible donor. By 6 h after the start of the infusion, all the IgG molecules are completely cleaved into Fc and F(ab′)2 fragments, which probably reduces their pathogenicity. All the IgG molecules are inactivated for approximately 1–2 weeks, when new IgG synthesis is detected. There was a significant reduction in the total IgG level that persisted for 28 days. Briefly, near-complete inhibition of C1q-binding HLA antibodies was seen 1 h after treatment. The levels of all HLA antibodies were significantly reduced at 6 h after treatment. Rebound occurred in Swedish. In contrast, the U.S. cohort had fewer patients with rebound and lower levels of HLA antibodies after treatment with IdeS.

One patient in the US study had hyperacute rejection immediately after revascularization. This event was unexpected, since the tests for crossmatches and donor-specific antibodies were negative after IdeS treatment and before transplantation. Extensive investigations after the rejection showed high-titer IgM and IgA antibody reactive with donor-allograft endothelium, which they speculate was probably responsible for the immediate graft loss.

Thus, the antibody appears to be a non- HLA antibody that cannot be cleaved by IdeS.

There were 13 infectious complications that generally responded to treatment. However, in the Swedish study, one patient had prolonged parvovirus B19 viremia and one had persistent myalgias after the IdeS infusion. A total of 38 serious adverse events occurred in 15 patients (five events were adjudicated as being possibly related to IdeS). At transplantation, total IgG and HLA antibodies were eliminated. A total of 24 of 25 patients had perfusion of allografts after transplantation. Antibody-mediated rejection occurred in ten patients (seven patients in the U.S. study and three in the Swedish study) at 2 weeks to 5 months after transplantation; all these patients had a response to treatment. One graft loss, mediated by non-HLA IgM and IgA antibodies, occurred. The use of intravenous immune globulin and rituximab after transplantation probably prevents rebound donor-specific–antibody responses to some extent.

A subsequent multicenter, multinational, phase 2 trial of IgG endopeptidase for desensitization [66] is currently ongoing (NCT02790437). The immediate effect of IgG endopeptidase on donor-specific antibodies in the preliminary study was profound, with near or complete reduction of HLA antibodies at 6 h. Suppression of HLA antibodies was durable among patients treated with intravenous immunoglobulin and rituximab in the United States.

## Immunogenicity of IdeS

Lonze et al. [67] present Phase II, single-arm open label trial to assess the efficacy of IdeS to convert a positive crossmatch test to negative prior to transplantation with either a deceased or living donor kidney namely seven highly sensitized (cPRA98–100%) kidney transplant candidates who had DSA resulting in positive crossmatches with their donors (5 deceased, 2 living).

All pre-IdeS crossmatches were positive and would have been prohibitive for transplantation.

All crossmatches became negative post-IdeS and the patients underwent successful transplantation.

All received IdeS as monotherapy for desensitization and underwent successful transplantation within 24 h of IdeS administration. IdeS (0.25 mg/kg, intravenously) was given followed by repeat crossmatch tests 2- and 6-h postdose. A second dose of IdeS (0.25 mg/kg, intravenously) was administered if the 2-h crossmatch did not convert to negative. The transplant operations were performed as soon as a negative crossmatch result was obtained. In all cases, the transplant operation took place within 24 h of the first IdeS dose.

Induction immunosuppression consisted of high-dose corticosteroids (methylprednisolone 1,000 mg, intravenously), given in the operating room. Intravenous corticosteroids were tapered over 5 days and then converted to prednisone (30 mg, orally). Alemtuzumab (Campath, Millennium and ILEX Partners LP, Cambridge, MA, 20 mg, intravenously or subcutaneously) was administered on postoperative day (POD) 4. Two immunomodulatory therapies intended to minimize antibody rebound posttransplant, were given. Intravenous immune globulin (IVIG, Gamunex-C, Grifols, ResearchTriangle Park, NC, 2,000 mg/kg total dose) was given either as a single dose on POD7, or in two divided doses on POD7±1, and Rituximab (Rituxan, Genentech, San Francisco, CA, 1,000 mg, intravenously) was given on POD9. Three patients had DSA rebound and antibody-mediated rejection, which responded to standard of care therapies Three patients had delayed graft function, which ultimately resolved. No serious adverse events were associated with IdeS. All patients have functioning renal allografts at a median follow-up of 235 days. Three patients developed biopsy proven AMR. The earliest was observed on POD8 and the latest on POD27. Other patient developed a severe early AMR in the setting of exceptionally high-level DSA. The histopathological features of AMR were very advanced in this case, with extensive acute glomerulitis/capillaritis, inflammatory interstitial infiltrate, and interstitial hemorrhage. Following treatment with plasmapheresis, eculizumab, bortezomib, and splenic embolization, the DSA strengths decreased and the patient's renal function recovered.

IdeS as an alternative to pretransplant plasmapheresis could provide a major benefit both to patients in terms of time saved, and also to insurers as the costs of days to weeks of plasmapheresis would be exchanged for the costs of a single infusion.

Sensitized patients in need of a heart or lung transplant currently have no option but to hope for a compatible deceased donor. Next, as xenotransplantation moves toward clinical reality, one can envision a role for IdeS in eliminating antibody to nonimmunodominant epitopes that have not been eliminated by gene editing strategies. And finally, the main limitation of IdeS due to Immunogenicity, namely that it presently remains limited only to 1–2 doses, may be able to be overcome by reengineering IdeS to make it less immunostimulatory.

IdeS originates from *Streptococcus pyogenes* and is consequently expected to be recognized as foreign to the human immune system. The fact that all patients had detectable anti-IdeS IgG before dosing implies that the patients had been preexposed to IdeS, undoubtedly [64].

However, in the phase 1 study, it was demonstrated that after 6–12 months, anti-IdeS antibodies had returned to normal range. Thus, retreatment after this period may be possible and requires further investigation.

## Infection

A phase 2 clinical study on the safety, immunogenicity, pharmacokinetics, and efficacy of the IgG-degrading enzyme of *Streptococcus pyogenes* (IdeS [imlifidase]) were assessed in a single-center, open-label ascending-dose study in highly sensitized patients with chronic kidney disease [64]. Section of Transplant Surgery at Uppsala University Hospital, were eligible for the study if they had ≥2 identified HLA antibodies of which ≥1 was >3,000 MFI in single antigen bead analysis on ≥2 separate occasions. All eligible patients were pre-

screened and tested negative for the presence of IgE antibodies to IdeS.

Eight patients with cytotoxic PRAs (median cytotoxic PRAs of 64%) at enrollment received 1 or 2 intravenous infusions of IdeS on consecutive days (0.12 mg/kg body weight ×2 [$n$ = 3]; 0.25 mg/kg × 1 [$n$ = 3], or 0.25 mg/kg × 2 [$n$ = 2]). IgG degradation was observed in all subjects after IdeS treatment, with <1% plasma IgG remaining within 48 h and remaining low up to 7 days. Mean fluorescence intensity values of HLA class I and II reactivity were substantially reduced in all patients, and C1q binding to anti-HLA was abolished. IdeS also cleaved the IgG-type B cell receptor on $CD19^+$ memory B cells. Anti-IdeS antibodies developed 1 week after treatment, peaking at 2 weeks. A few hours after the second IdeS infusion, 1 patient received a deceased donor kidney offer. At enrollment, the patient had a positive serum crossmatch (HLA-B7), detected by complement-dependent cytotoxicity, flow cytometry, and multiplex bead assays. After IdeS infusion (0.12 mg/kg × 2) and when the HLA-incompatible donor ($HLA\text{-}B7^+$) kidney was offered, the HLA antibody profile was negative. The kidney was transplanted successfully. The percentage of both T cell and B cell PRAs was reduced in all patients within 1 h after IdeS treatment.

The total number of AEs reported during the study was 76, of which 27 were classified as related. There were five serious AEs (SAEs) reported. Four of these were reported as related. Three related SAEs were classified as infections and infestations: pneumonia and suspected upper respiratory infection. Myalgia was reported in two patients, 1 SAE grade 2. One patient experienced a suspected infusion reaction and dosing was interrupted. All symptoms were grade 1 (flushing, hypertension, hot flashes, sinus tachycardia, dyspnea, scleral hemorrhage, visual impairment) and resolved 11 min after the infusion was stopped. Two patients had increased levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) reported as AEs, both of which were classified as related.

The data obtained in this phase 2 clinical study demonstrate that IdeS treatment in sensitized patients with CKD is not only effective but also safe and well tolerated and has the potential to generate a window of 7 days after treatment when HLA antibodies are reduced below the threshold level, enabling the patient to be a candidate for transplant with an organ from an HLA-incompatible donor.

## Complication from Anti-IL-6R mAb (Tocilizumab: TCZ)

**Q: 1. What kind of side effect does the tocilizumab have?**
**2. What is the rebound effect following tocilizumab?**

Interleukin-6 is a pleiotropic cytokine with a significant array of biologic effects on numerous cell types which include B cells, T cells, plasma cells, hepatocytes, and endothelial cells.

Interleukin-6 is not expressed in normal individuals but contributes to the clinical manifestations of inflammatory and infectious diseases. Interleukin-6 is critical for induction of T follicular cells, and B cell differentiation to plasmablast. Interleukin (IL)-6 is responsible, in conjunction with other cytokines, for normal antibody production.

IL-6 is an important mediator of inflammation that is critical to shaping T cell immunity and inhibiting Tregs while increasing T helper 17 cell (Th17) populations. IL-6 is also critical for the progression of naıve B cells to plasmablasts and mature plasma cells. Plasmablasts also produce copious amounts of IL-6. Critical role for IL-6 produced by endothelial cells as a major factor responsible for intimal proliferation. It is accepted that IL-6 drives CD4 T cells toward Th17 phenotype while negating Treg differentiation. Recent evidence also indicates that IL-6 triggers IL-21 production by Tfh, driving B cell maturation to plasma cells during antibody responses. Thus, IL-6 shapes T cell immunity and is a powerful stimulant for pathogenic IgG production and chronic antibody mediated rejection.

Dysregulation of IL-6 production can occur in a number of disease states that ultimately are characterized by lymphadenopathy, excessive and unregulated antibody production, autoimmunity and is also associated with a deviation of T cell responses from Treg to Th17 with attendant inflammation and tissue injury [68].

Tocilizumab (TCZ, Actemra) is a first-in-class humanized monoclonal antibody directed at the IL-6 receptor and has been approved by FDA in the United States and other countries around the globe for the treatment of moderate to severe rheumatoid arthritis (RA) and idiopathic juvenile arthritis. Emerging data from patients with autoimmune diseases treated with TCZ suggest a potent reduction in disease targeted autoantibodies and memory B cells. In addition, recent data also suggest TCZ reduces Th17 cells and deviates naive T cells to Tregs. Reports have shown that tocilizumab also reduces antibody-producing cells, diminishes inflammatory markers, and improves clinical symptomatology in a number of other autoimmune diseases. Anti–IL-6R monoclonal antibody results in significant reductions of alloantibodies, antibody production by splenic and bone marrow plasma cells, direct inhibition of plasma cell anti-HLA antibody production, and induction of T regulatory cells (Tregs) with inhibition of T follicular helper cells (Tfh).

## Infection

Choi [69] identified 36 renal transplant patients with cAMR plus DSAs and TG who failed standard of care treatment with IVIg plus rituximab with or without plasma exchange. Patients were offered rescue therapy with the anti-IL-6 receptor monoclonal antibody (tocilizumab) with monthly infusions and monitored for DSAs and long-term outcomes. Most patients showed progressive renal dysfunction and had failed treatment with IVIg plus rituximab with or without plasma exchange.

Between 2011 and 2016, 36 patients were offered treatment with tocilizumab (8 mg/kg monthly, maximal dose 800 mg for 6–25 months), based on insurance approval.

The median follow-up was 3.26 years (IQR 1.82–3.81) with a maximal follow-up of 8 years.

The patients treated with tocilizumab exhibited good allograft survival with a graft survival probability of 80% at 6 years post-cAMR diagnosis. Tocilizumab-treated patients with TG exhibited good allograft survival with a graft survival probability of 77% at 6 years post cAMR. The patient survival was good at 6 years with a survival probability of 91%.

However, eGFRs for both cohorts remained stable during the study period. Regarding the impact of tocilizumab on Immunodominant (iDSA) levels, which are defined as the strongest DSAs detected in the patients' sera, declined significantly beginning at 24 months.

Among the 36 recipients treated with tocilizumab, only four had a graft loss (11.1%), which were due to cAMR.

Of interest, tocilizumab was discontinued for medical reasons in one patient and for financial reasons in the other three about 6 months before all graft losses were seen. Although it is uncertain, we must consider the possibility that rebound in IL-6–IL-6R signaling after cessation of tocilizumab is responsible for the initiation of alloimmune response and allograft loss.

Thirteen patients had infectious AEs: a total of five patients had cytomegalovirus infection, two patients had polyoma BK infection that resolved with treatment, and one patient was diagnosed with trichodysplasia spinulosa (a benign skin condition related to polyoma virus) that resolved 1 month after the completion of tocilizumab therapy. Seven patients had bacterial infections, which resolved with treatment, usually without the need for cessation of tocilizumab therapy. All infectious events resolved with directed treatment and without the need to stop tocilizumab therapy. One patient had transient visual disturbance with resolution. Eight patients developed hypogammaglobulinemia defined by IgG <600 mg/dL during tocilizumab therapy.

No significant adverse events or severe adverse events were seen. Tocilizumab provides good long-term outcomes for patients with cAMR and

TG, especially compared with historical published treatments.

Patel [70] reported that since 2015, 20 pts. received TCZ 8 mg/kg with >3 months follow-up added to tacrolimus/mycophenolate/pred for cAAMR refractory to treatment. Mean age at KTx was 37 ± 11.5 years, most were female [13], and received live donor KTx [11]. All patients had prior AMR with DSA—Class I = 4, Class II = 9, Class I and II = 7—that persisted despite plasmapheresis [6], IVIG at 2 g/kg [8], rituximab [2]. Sixteen patients also had prior ACR (borderline = 9, 1A = 3,1B = 3, 2A = 1). TCZ was started an average 1,648 ± 1,420 days after transplant, with a starting creatinine- = 2.35 ± 0.95, and given an average 323 ± 281 days. In the 3 months prior to initiation of TCZ, eGFR declined by 3.9 cc/min each month, compared to 0.05 cc/min each month on TCZ ($p = 0.008$). Proteinuria also stabilized on TCZ- initial urine protein:creatinine ratio of 1.01(±1.1) vs. 0.80 (±1.1) at f/u. Stabilization was not dependent on level or type of DSA, and nor did DSA change significantly during follow-up. There were 3 ACRs (2-borderline and 1B = 1) and one patient with recurrent AMR after stopping TCZ that responded to re-initiation. There were 2 cases of BK viremia (0 nephropathy), 1 EBV viremia, and 1 hospitalization for pneumonia. Only one patient stopped due to infusion-related reaction.

## Others

Vo et al. [71, 72] reported on the efficacy of tocilizumab in reducing anti-HLA antibodies and improving transplant rates in highly HLA-sensitized patients who were resistant to other desensitization strategies. This, phase I/II single center open label pilot exploratory study was conducted at Cedars-Sinai Medical Center, in which from July 2012 to November 2013, ten patients unresponsive to desensitization (DES) with IVIg + rituximab were treated with IVIg + TCZ. Patients received IVIg on days 0 and 30 at 2 g/kg and TCZ 8 mg/kg on day 15 then monthly for 6 months. If transplanted, patients received IVIg once and TCZ monthly for 6 months. Patients received alemtuzumab 30 mg subcutaneously as induction and were maintained on triple regimen with tacrolimus MMF and prednisone taper.

Five of 10 patients were transplanted. Mean time to transplant from first DES was 25 ± 10.5 months but after TCZ was 8.1 ± 5.4 months. Six-month protocol biopsies showed no antibody-mediated rejection. However, 1 patient showed mild ABMR on 12 months for cause biopsy, 6 months after completion of the TCZ dosing. This patient responded well to ABMR treatment with IVIg + rituximab. Donor-specific antibody strength and number were reduced by TCZ treatment. Renal function at 12 months was 60 ± 25 mL/min.

Tocilizumab and IVIg appear to be safe. From this pilot trial, they are cautiously optimistic that targeting the IL-6/IL-6R pathway could offer a novel alternative for difficult to desensitize patients. The adverse effects included nausea, abdominal pain with normal amylase and lipase, itching, fatigue, blurred vision, anemia, thrombocytopenia, liver function test abnormalities 1.5 or less normal, elevated blood pressures, and minimal infusion-related reactions to IVIg + TCZ.

In the transplanted group: 1 patient developed infective colitis with colonic perforation required bowel resection (possibly related to study drug) and 1 patient developed Bell Palsy (possibly related to study drug). The clinical correlates include an association with amyloid A amyloidosis, increased risk for development of cardiovascular complications, and the anemia of chronic disease associated with hepcidin elevation.

They also saw significant reductions in T follicular cell populations and increases in Treg cells after anti–IL-6R therapy. Enhanced Treg responses have also been reported in humans treated with TCZ. They tested the hypothesis that IL-6 is an important cytokine in the maintenance of anti-HLA antibody production in highly sensitized patients.

Choi et al. [73] report on their extended experience with TCZ for CABMR+TG. Methods: Since 4/2011 they identified 65 patients including those with CABMR+TG, DSA+, and/or

AT1R ab+. TCZ treatment was pursued after other treatments had failed. Briefly, after diagnosis of CABMR, patients received TCZ 4-8 mg/kg monthly for 3–37 doses and were followed up to 6 years from TCZ initiation.

Immunodominant (iDSA) levels tended to decrease after therapy (t0: 12,967 ± 20,000, t12 M: 9,180 ± 6,682,t36 M: 3,829 ± 6,001MFI) ($p$ = NS). Mean eGFRs were 53.18 ± 34.61 mL/min at 0 M vs. 50.43 ± 36.37 mL/min at 24 M. Graft survival was compared to a standard (SOC) group (39-non concurrent CABMR patients) treated with IVIg + rituximab ± PLEX. At 6 years, 92.6% of TCZ patients have functioning grafts v. 53.3% in SOC ($p$ = 0.0005). Two deaths in the TCZ group. Pre- and post-TCZ biopsies at mean of 29.5 ± 18.7 M from pre-biopsy showed significant reductions in g + ptc scores compared to biopsy at diagnosis.

## Complication from Plasmapheresis

**Q: 1. What kind of side effect does plasmapheresis have?**
**2. Are the complication from plasmapheresis preventable?**

Szczeklik [74] analyzed adverse effects of 370 plasmapheresis procedures in 54 patients in intensive care unit with disease included myasthenia gravis (33.3%), Guillain-Barre syndrome, Lyell's syndrome (9.3%), SLE (7.4%), and PTT (7.4%).

The adverse side effects observed most frequently during plasma filtration were: fall in arterial blood pressure, arrhythmias, sensations of cold with temporarily elevated temperature, and paresthesias.

Severe and life-threatening episodes, i.e., shock, fall in arterial blood pressure requiring pressor amines, persistent arrhythmias and hemolysis, developed in 2.16% of procedures.

The adverse-side effects are associated with large vessel catheterization, clotting disorders, septic complications resulting from impaired immunity caused by the removal of antibodies during the procedure, catheter-associated infections, and those related to transfusion of blood products. Moreover, life-threatening fall in arterial blood pressure, cardiac arrhythmias and water-electrolyte imbalance are likely to develop. Less severe reactions and symptoms are more common, e.g., urticaria, pruritus, limb paresthesias and pains, muscle contractions, dizziness, nausea, vomiting, transiently elevated temperature, shivers, seizures, head and chest pains. Reduced levels of hemoglobin, thrombocytopenia, hypokalemia, and reduced concentrations of fibrinogen were developed. The total incidence of complications is estimated at 25–40%. Life-threatening and non-life-threatening complications were developed.

Zhang et al. [75] analyzed the 28 enrolled patients diagnosed serologically and pathologically with anti-GBM disease from 2003 to 2013 in whom 16 treated with DFPP and 12 with IA,

A double volume of plasma was processed, and each patient received a 30–40 g human albumin supplement during each session. IA consisted of 10 cycles per session, with 8–10 sessions performed daily or every other day and each session regenerating 30–60 L of plasma.

Six patients had pulmonary hemorrhage and 18 had serum creatinine concentrations >500 umol/L. All patients showed progressive increases in serum creatinine and required CRRT during the course of disease. Efficacy of clearing anti-GBM antibody was similar in the two groups. One patient each had a pulmonary hemorrhage and a subcutaneous hemorrhage during treatment, but there were no other serious complications. At the end of follow-up, patient survival and renal survival were similar in the DFPP and IA groups. DFPP plus immunosuppressive therapy efficiently and safely removed anti-GBM antibodies. The fewer plasma-associated side effects and reduced loss of IgG suggest that DFPP may be a better treatment choice for anti-GBM disease, especially in patients with insufficient plasma.

## Surgical Complication

**Q: 1. What kind of surgical complications develop following desensitization?**
**2. What are the strategies for their prevention?**

### Lymphocele

Zschiedrich et al. [76] analyzed 100 ABOi KTx and 248 ABOc KTx in observational, single center study. Preoperative desensitization were single dose of rituximab (375 mg/m$^2$) 30 days before operation, and immunoadsorption. Seven days before surgery, oral immunosuppression with tacrolimus and MMF and prednisone was initiated followed by basiliximab induction.

In this study postoperative lymphoceles occurred more frequently in ABOi KTx.

### Bleeding

A single-center retrospective study by Naciri et al. [18] assessed posttransplantation complications in 44 ABO-i versus 44 matched ABO-c patients. All patients were comparable at baseline except that ABO-i patients had greater immunological risks.

Preoperative desensitization were single dose of rituximab (375 mg/m$^2$) 30 days before operation, and immunoadsorption. Twelve days before surgery, oral immunosuppression with tacrolimus and MMF and prednisone was initiated followed by basiliximab induction.

During the 6-month posttransplant period, more ABO-i patients presented with postoperative bleeds, thus requiring significantly more blood transfusions.

Habicht [16] retrospectively analyzed 21 consecutive recipients who underwent ABOi renal transplantation. Pretransplant desensitization included administration of rituximab (375 mg/m$^2$), mycophenolate mofetil (MMF), tacrolimus and prednisolone 4 weeks prior of scheduled transplantation as well as IA and IVIG.

Hemorrhagic complications occurred in 9.5% of the ABOi recipients as opposed to 2% in the ABOc group. The risk of lymphoceles requiring drainage or surgical repair and major wound-healing problems was increased in the ABOi group as compared to the ABOc group (19% vs. 6.4%). Proximal ureteral stenosis developed and required surgical repair 2 months after transplantation. Extensive histologic and immunohistochemical workup revealed CMV uretritis as the cause of stenosis. Interestingly, the most common viral infection was BK viremia leading to BKN in 5 of 20 ABOi grafts (25%) and 4 of 47 (8.5%) ABOc grafts.

Szezeklik [74] reported that during the 6-month posttransplant period, more ABO-i patients presented with postoperative bleeds, thus requiring significantly more blood transfusions.

Patient- and graft-survival rates, and kidney-function statuses were similar between both groups at 6 months posttransplantation.

It is reported that ABOi patients have more bleeding episodes either during transplantation or in the immediate posttransplant period [5–9]. This has been largely ascribed to apheresis session(s).

Significantly more lymphoceles (which require specific therapies) as well as significantly more wound dehiscences have been reported in ABOi patients.

Lentine [17] reported that recipients of ABOi transplants experienced significantly ($P < 0.05$) higher incidence of wound infections (12.7% vs. 7.3%), pneumonia (7.6% vs. 3.8%), and urinary tract infections (UTIs) or pyelonephritis (24.5% vs. 15.3%) in the first 90 days compared with ABO-compatible recipients. In adjusted models, ABO incompatibility was associated with twice the risk of pneumonia (adjusted hazard ratio [aHR], 2.22; 95% confidence interval [CI], 1.14–4.33) and 56% higher risk of UTIs or pyelonephritis (aHR 1.56; 95% CI 1.05–2.30) in the first 90 posttransplantation days, and 3.5 times the relative risk of wound infections in days 91 to 365 (aHR 3.55; 95% CI 1.92–6.57). ABOi recipients, 19% of whom underwent pre- or peritransplant splenectomy, experienced twice the adjusted risk of early hemorrhage.

ABOi transplantation offers patients with potential live donors an additional transplant

option but with higher risks of infectious and hemorrhagic complications. Awareness of these complications may help improve protocols for the management of ABOi transplantation.

In our center (Asan Medical Center) ABOi KT was performed on 276 patients (2009–2015) and a total 96 patients underwent FCXM positive KT. The overall patient survival rate in patients who underwent FXCM postivie KT was not significantly different from that of the control group during the 3-year follow-up ($P = 0.34$).

After desensitization with plasmapheresis and rituximab the FXCM positive group showed a higher rate of surgical complications—including hematoma (3.7% vs. 20.0%, $P < 0.001$), bleeding requiring operation (1.0% vs. 6.7%, $P = 0.002$), and lymphocele (2.7% vs. 8.9%, $P = 0.020$)—than the FXCM negative group. Infectious complications, however, demonstrated no significant differences [66].

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# 7 Treatment of Rejection in Desensitized KT Patients

Joong-Kyung Kim

## Overview of Treatment for Rejection in Desensitized KT Patients

**Q: What kinds of medications can be used to treat ABMR?**

- Plasmapheresis
- IV Ig
- Rituximab
- Corticosteroid
- Bortezomib
- Eculizumab
- C1 esterase inhibitor
- Ig G endopeptidase
- Tocilizumab

Many cases of ABMR in renal allografts associated with de novo DSAs can present as mixed ABMR and TCMR. When antibody-mediated rejection is diagnosed, pathologically T cell-mediated rejection is accompanied or antibody-mediated rejection is processed causing acute cellular rejection [1]. It is well known that CD+ 4 helper T cells can activate B cells [2].

In 2009, the KDIGO guidelines are to use more than one of corticosteroids, plasma remover, IVIG, anti-CD20 antibody, and lymphocyte-depleted antibodies in treatment of ABMR [3].

The purpose of the antibody-mediated rejection treatment is to remove the donor-specific antibody, suppress the donor-specific antibody production in B cell or plasma cells, and suppress the body response to suppress inflammation in the implantation body. Plasma exchange and IVIG remove antibody and have immunomodulation. Rituximab and splenectomy inhibit B cell activation. Bortezomib inhibit antibody producing from plasma cell. Eculizumab inhibit antibody-induced terminal complement activation. But regimens are not well studied. However, according to a study by Burton et al. in 2015, plasmapheresis and intravenous immunoglobulin are basically used in the treatment of acute antibody-mediated rejection, and rituximab is added depending on the situation [4].

## Plasmapheresis and IVIG

**Q: Is the combination therapy of plasmapheresis and IVIG the best way of treatment for antibody-mediated rejection?**

The most commonly used treatment methods for acute antibody-mediated rejection are plasmapheresis and IVIG.

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Therapeutic apheresis selectively removes cells or other targeted abnormal substances from circulation. Plasmapheresis is selective removal of plasma. Plasma can be separated from blood using centrifugation or filtration. Therapeutic plasma exchange, also called plasma exchange or therapeutic plasmapheresis, involves removal of patient plasma and replacement with fresh frozen plasma or isotonic albumin. Plasma filtration is a technique of separating plasma from cellular components by a highly permeable filter using a dialysis or hemofiltration machine. Traditionally, therapeutic plasma exchange was performed with centrifugation devices used in blood blanking procedures. Therapeutic plasma exchange is also possible using NxStage and Prismaflex continuous renal replacement therapy machines. Immunoadsorption is the further development of plasma exchange by which plasma is first removed and then passed over columns with specific ligands to remove specifically immunoglobulins. The adsorbed plasma is then reinfused into the patient. Advantageously, immunoadsorption does not remove other plasma components such as fibrinogen, and compounds of the coagulation cascade, which reduces adverse effects. Immunoadsorption is more selective modality to remove antibody, and is faster to remove donor-specific antibody and plasmapheresis [5]. By removing the HLA-specific antibody, the plasmapheresis removes the circulatory donor-specific antibodies.

During acute antibody-mediated rejection, donor-specific antibody generation is increased because of B cell clonal expansion. IVIG has been used to suppress alloantibody and modulate immune responses.

For a plasmapheresis, 1–1.5 plasma volume exchanged, 60–70% of plasma is removed [6]. The plasma volume is calculated as total blood volume × (1 − hematocrit). The total volume of replacement fluid is usually 1 plasma volume (40 mL/kg) or 1.5 plasma volumes (60 mL/kg). Plasmapheresis is performed four to six times, and sometimes performed three or seven times. If necessary, additional plasmapheresis may be performed [4]. Therapeutic plasma exchange and IVIG eliminate rate 60–75% of HLA antibodies [7].

Because antibody-mediated rejection was not clear, early studies of plasmapheresis were limitations in the treatment of antibody-mediated rejection. But there were benefits in the treatment in five random controlled trials [8].

IVIG preparations comprise pooled IgG antibodies from the serum of between 1,000 and 15,000 donors and were initially used as an IgG replacement therapy in immunocompromised patients [9]. The mechanisms of IVIG involved in reduction of antibody levels are multifactorial and complex. It neutralizes circulating anti-HLA antibodies through anti-idiotypic antibodies. It inhibits complement activation by binding C3b and C4b and neutralization of C3a and C5a. It blocks immune activation and enhancing the clearance of anti-HLA antibodies by competing for activating FcRs. It inhibits the expression CD19 on activated B cells and induces apoptosis of B cells. It induces the expression of FcIIB, which is a negative regulatory receptor on immune cells. It has inhibitory effects on cellular immune responses and nonspecific inhibitory effects on the immune system by binding to Fc receptors on macrophages, neutrophils, platelets, mast cells, and natural killer cells, and it also inhibits cytokine, chemokine, adhesion molecules, and endothelial cell activity [10, 11].

The dosage of IVIG has not been determined. Two general treatment protocols have been developed utilizing IVIG. The first is the use of high-dose IVIG (2 mg/g) alone and the second is to combine lower-dose IVIG with plasmapheresis. But 100 mg/kg/day of IVIG is the most common dose.

In general, high doses of IVIG are relatively safe. However, serious side effects have been reported including acute renal dysfunction likely related to high osmotic load, thrombotic events with rapid infusions, and aseptic meningitis [12]. Slowing the infusion rate and using iso-osmolar preparations can reduce the risk of side effects [10]. IVIG has the potential benefit of replacing antibodies lost during plasmapheresis [13].

In various reports, IVIG is administered after plasmapheresis for patients who have received a living donor or deceased kidney donor transplant

and who have acute antibody-mediated rejection to improve graft survival. Slatinska et al. reported that in plasmapheresis with IVIG group, graft survival and patient survival were better than plasmapheresis alone group [14]. Lefaucheur et al. reported that high-dose IVIG alone was inferior to plasmapheresis/IVIG/anti-CD20 as therapy for antibody-mediated rejection and donor-specific antibody [15]. Another study of Lefaucheur showed that graft survival, graft function, and donor-specific antibody levels could be improved along with bortezomib and high-dose IVIG treatment [16]. High-dose IVIG resulted in modest donor-specific antibody median fluorescence intensity reductions in patients with previous graft damage, with a larger effect occurring in class I donor-specific antibody in patients with a previous acute antibody-mediated rejection. There was no clinical treatment benefit in patients with ongoing chronic graft damage, whereas high-dose IVIG may reduce the risk of chronic graft dysfunction in those with an acute antibody-mediated rejection events [17].

## Rituximab

**Q: Is rituximab really useful for treatment for ABMR?**

Rituximab is a chimeric anti-CD20 monoclonal antibody on the surface of B lymphocyte. CD20 is expressed on pre-B and mature B lymphocytes throughout the antigen-independent stage of development until early stages of antigen-dependent B cell activation.

Rituximab causes a reduction in B cells in the peripheral blood within 1–3 days of administration, and complete B cell depletion in the majority of patients within 1–6 week. Single-dose rituximab in kidney transplant recipients evokes a long-term elimination of B cells in peripheral blood as well as within the kidney transplanted. The effect continues beyond the expected 3–12 months [18]. Single-dose rituximab ablate B cell particularly memory B cells, was long-lasting, lagging repopulation by CD5+ B cells [19]. As B lymphocytes also function as antigen presenting cells, rituximab is also likely to indirectly suppress T lymphocyte activity [20].

In many studies, doses of rituximab 375 mg/m$^2$ for antibody rejection therapy was commonly used. But low dose of rituximab (200 mg) is the sufficient dose in kidney transplantation [21]. Research of rituximab treatment for acute antibody rejection is in insufficiency. The treatment of rituximab in two random controlled trials did not show a good result. In the RITUX ERAH study, 40 people were enrolled, performed with 0.1 mg/kg of IVIG, six times of plasmapheresis, and corticosteroid treatment, but rituximab did not show a benefit [22]. Zarkhin reported no gains when mixed acute antibody-mediated rejection and chronic antibody-mediated rejection patients were injected with rituximab after administration of methylprednisolone and antithymocyte globulin [23]. Other studies on rituximab are retrospective. Kaposztas reported the rituximab with plasmapheresis group had a higher graft survival than plasmapheresis group [24].

In the chronic antibody-mediated rejection, only retrospective study of rituximab was investigated, and most studies did not favor both graft and graft function [25]. Currently, it is considered to use rituximab after administration of plasmapheresis and IVIG, because retrospective study showed a positive effect in rituximab although random controlled trial did not show a good result.

## Corticosteroid

**Q: Is there any benefit or rationale for the use of corticosteroid in treatment of ABMR?**

It is reported that corticosteroid was used in early 1960 to treat acute rejection [26]. Glucocorticoids are used to suppress inflammatory and immune response in the kidney transplant setting [27].

Corticosteroids inhibit T cells, so it can be considered primarily in acute T cell-mediated injection. Corticosteroids inhibit cytokine transcription and production, with multiple downstream effects on lymphocyte function, decreasing inflammation caused by donor-specific antibodies in graft. Thus, in the treatment of acute antibody-mediated rejection, the use of corticosteroids as well as IVIG and plasmapheresis is possible.

In the acute rejection, methylprednisolone is used at a dose of 0.5–1 g per day for 3–5 days. After the steroid pulse, Gray et al. [28] reduce the corticosteroids and keep dosage higher than before acute rejection. Intravenous steroid has similar efficacy, but it has fewer side effects [28].

## Bortezomib

**Q: 1. Is bortezomib better than rituximab?**
**2. Is there any synergistic effect of bortezomib with rituximab?**

Bortezomib, a selective inhibitor of the 26S proteosome, is mainly used for the treatment of multiple myeloma (a clonal B cell malignant tumor characterized by abnormal plasma cell expansion in bone marrow and myeloid malignant cells). The anti-plasma cell activity of this drug is derived from several mechanisms including inhibition of the NF-κB pathway, induction of caspase 8/9 mediated apoptosis, cleavage of DNA repair enzymes, and blockade of IL-6 production [29]. Because of this effect, bortezomib has been proposed as a candidate for ABMR treatment [30–32]. Under physiological conditions, the NF-κB pathway is constitutively active only in some types of cells, including neurons, B cells, and thymocytes, and is always inactive in all other cell types [33]. However, the dysregulation of NF-κB signaling is primarily associated with inflammatory diseases and cancer. Blockade of NF-κB signaling thus provided a therapeutic strategy in autoimmune diseases and cancer [34, 35]. Bortezomib mediates the apoptosis of activated T cell by blocking the degradation of IκB, blocking nuclear factor-κB, and activating transcription of interleukin-1, interleukin-6, and tumor necrosis factor-α.

The drug was administered as an intravenous bolus injection over 3–5 s. Bortezomib should be given 1.3 mg/m$^2$ on days 1, 4, 8, and 11. There should be at least 72 h of rest between consecutive doses [36].

Perry et al. showed that successfully treated two positive cross matching kidney recipients for early acute AMR at four courses of 1.3 mg/m$^2$ bortezomib in addition to daily plasma separation and IVIG. They have demonstrated a transient decrease in bone marrow-derived plasma cells in vivo. They also found that serum HLA alloantibody titers and specificity, as confirmed by Luminex, were much lower at 1-year follow-up than at the time of transplantation before bortezomib [37].

In Everly's study, six kidney recipients who developed biopsy proven mixed ABMR and TCMR received 1.3 mg/m$^2$ of four courses of bortezomib to resist classical therapies such as rituximab, IVIG, and ATG. Bortezomib was observed at a median of 743 days after transplantation. Median follow-up period was 7 months. With the exception of one patient, bortezomib caused an immediate reversal of ABMR and TCMR. Five out of six patients with ABMR and TCMR showed a 50% reduction in DSA regardless of the specificity of HLA class I or II. This was observed immediately after the first bortezomib dose regardless of the amount of DSA. During treatment with bortezomib, the patient was well tolerated [38].

In a study by Walsh et al., plasmapheresis, methylprednisolone, and single dose rituximab therapy with bortezomib were used to treat ABMR in the first 2 weeks after transplantation. Within 14 days of bortezomib-based therapy, patients experienced rapid ABMR reversal and removal of detectable DSA. Normal renal function and normal range of proteinuria were observed up to 6 months after the ABMR episode [39]. Since bortezomib was administered as part of multidrug therapy, the bortezomib's own effects could not be reliably established from previous studies. In fact, it is reasonable to assume that other desensitization therapies have enhanced the bortezomib effect by other mechanisms. Inadequate studies of bortezomib make it diffi-

cult to include this drug in successful desensitization protocols or ABMR treatment protocols.

Recently, Eskandary et al. reported results of the BORTEJECT Study (the Bortezomib in Late Antibody-Mediated Kidney Transplant Rejection [BORTEJECT]), a randomized, placebo-controlled trial to investigate the effect of bortezomib on the course of late ABMR. This study investigated whether two cycles of bortezomib (each cycle: 1.3 mg/m$^2$ intravenously on days 1, 4, 8, and 11) prevent GFR decline by halting the progression of late donor-specific antibody (DSA)-positive ABMR. Patients were randomly assigned to receive bortezomib ($n$ = 21) or placebo ($n$ = 23). This trial failed to show that bortezomib prevents GFR loss, improves histologic or molecular disease features, or reduces DSA, despite significant toxicity [40].

## Eculizumab

**Q: 1. Does eculizumab have any beneficial effect in desensitization?**
**2. Is eculizumab effective for treatment of ABMR?**

Eculizumab is a monoclonal antibody that targets the complement component C5 and has been approved for the treatment of two complement-mediated diseases, paroxysmal nocturnal hemoglobinuria and atypical hemolytic uremic syndrome [41, 42].

Eculizumab inhibits the cleavage of C5 by C5 convertases (C4bC2aC3b for the classical pathway and C3bBbC3b for the alternative pathway) into C5a and C5b. C5b binds the terminal pathway components C6, C7, C8, and C9 to nonenzymatically assemble the membrane attack complex [43]. Recently, several cases of treating refractory ABMR and thrombotic microangiopathy with eculizumab were reported [44–46]. In these cases, eculizumab was used alone or as a bridging treatment for B cell depletion, proteasome inhibition, or as a combination of plasmapheresis and/or IVIG.

Recently, however, failure of eculizumab has been reported as a remedy for C4d-negative ABMR of complement-independent antibody-induced injury [47]. Furthermore, other studies have shown that eculizumab has limited efficacy in early ABMR with severe oliguria [48].

If administered before transplantation, eculizumab is administered intravenously 1,200 mg before going to the operating room and 600 mg consecutively after surgery. For rejection therapy, 900–1,200 mg of eculizumab is administered intravenously every 2 weeks until the rejection is reversal.

Data on desensitization and ABMR therapy by eculizumab are inadequate. A more diverse and randomized controlled study is needed. Two preliminary RCT studies are underway; randomized, open-label, multicenter phase 2 study to determine the safety and efficacy of eculizumab in the prevention of ABMR (ClinicalTrials.gov Identifier: NCT01399593) and efficacy and safety of eculizumab for the treatment of antibody-mediated rejection following renal transplantation (ClinicalTrials.gov NCT01895127). In both studies, eculizumab showed no significant differences in the occurrence of ABMR and the therapeutic effect (transplant glomerulopathy progression, graft loss, and patient death) compared with the control group using standard therapy.

In the future, eculizumab is more likely to be used as potential co-therapy to reduce DSA levels than eculizumab alone.

## C1 Esterase Inhibitor (C1-INH)

**Q: 1. Does C1 esterase inhibitor (C1-INH) have a benefit for the treatment of ABAR?**
**2. What other effect does C1-INH have?**
**3. Is it effective to use C1-INH with other anticomplement agent?**
**4. Is C1-INH effective alone or recommended to use with other agents?**

Complement activation is the major pathway for allograft inflammation and injuries. Extensive data on animal models and recent human experiments suggest that C1-INH improves ischemia-

reperfusion injury [49]. C1-INH may be helpful in the treatment of ABMR. In a placebo-controlled, randomized study, C1-INH was found to be safe for use in kidney transplant patients. Treatment with C1-INH is likely to reduce ischemia-reperfusion injury and result in a significant reduction of C1q (+) HLA antibodies. The major pathway that causes allograft inflammation and injury is Combination complement activation. This system is particularly important for ABMR/cABMR as well as ischemia-reperfusion injury/delayed transplantation functions. The latter is becoming increasingly recognized as the leading cause of late graft loss and there are not many effective treatments available. The C1 inhibitor (C1-INH) regulates several pathways that contribute to acute and chronic transplantation of antibody reduction with IVIG, rituximab and C1-INH may be useful in the prevention of ABMR [50, 51].

In a phase 2, multicenter, randomized, double-blind, placebo-controlled study by Montgomery et al., patients received either C1INH or placebo ($N$ = 18) as an adjuvant therapy for ABMR. C1INH 20,000 units was administered or placebo for 2 weeks. Transplant glomerulopathy was not observed in the C1INH group ($N$ = 7) while 3 out of 7 in placebo group showed transplant glomerulopathy in 6-month biopsies [52]. Viglietti et al. conducted a prospective, single-arm study to investigate the efficacy and safety of C1-INH and high-dose IVIG treatment for acute ABMR therapy that did not respond to conventional therapies. Renal transplant recipients with ABMR and acute allograft dysfunction received C1-INH and IVIG for 6 months. In all patients, eGFR was improved between inclusion and 6 month after (from 38.7 ± 17.9 to 45.2 ± 21.3 mL/min/1.73 $m^2$, $p$ = 0.028), with no change in histologic characteristics except for decreased C4d deposition. C1q(+)DSA was significantly reduced in C1-INH-treated patients ($p$ = 0.026) [51].

However, these studies also have limitations in the small number of patients. In the future, a large-scale RCT for the C1-INH effect for the treatment of ABMR is needed, and a conclusion can be drawn based on these results.

## Ig G Endopeptidase (IdeS)

**Q: 1. Does IdeS have any beneficial effect on ABMR?**
**2. How about the antibody rebound after IdeS?**
**3. What is the strategy for antibody rebound?**

IdeS is an immunoregulatory enzyme that cleaves all four IgG antibody subclasses into F (ab') 2 and Fc fragments at the lower hinge regions with high specificity. IdeS, a cysteine protease, was discovered and purified from *S. pyogenes*. IdeS has a unique specificity for IgG and is a potent virulence factor produced by *S. pyogenes,* because removing the Fc region of host IgG essentially ablates humoral immunity given that cleaved IgG can no longer activate complement or mediate antibody-dependent cellular cytotoxicity [53]. Other immunoglobulins, including IgA, IgM, IgE, and IgD, are not affected by the administration of IdeS [54].

Patients who underwent transplantation received IdeS at a dose of 0.24 mg/kg of body weight (in the United States) or at a dose of 0.25 or 0.50 mg/kg (in Sweden; both doses were investigated in a dose-finding study). IdeS was administered intravenously on day 0, usually 4–6 h before transplantation.

IdeS cleaves human and rabbit IgG at hinge sites within 4–6 h after administration. The half-life of IdeS is about 8–12 h, but the IgG degradation capacity can last for 4 days.

In recent time, phase I/II studies of IdeS for desensitization were studied in 25 highly sensitized living donor and deceased donor kidney transplant patients with DSA in Sweden and the United States. All patients received IdeS infusion prior to transplantation. The purpose of this study was to assess the ability of Ides to remove DSA from patients who were positive at pretransplantation crossmatch. Patients in the United States were treated with desensitization using IVIg 2 g/kg and rituximab, but Sweden arm did not. Of the

25 patients who received IdeS, 24 were successfully transplanted, and ABMR occurred in three patients with Swedish arm due to DSA rebound at 2 weeks posttransplant. At 2 months and 5 months after transplantation in the United States, two patients developed ABMR and DSA intensity was increased and resolved with treatment. The difference in rebound time appears to reflect the use of IVIG and rituximab after transplantation in the United States patients. The long-term outcomes for these patients were generally good [55].

However, studies using IdeS to treat ABMR are currently lacking. As a result, the IdeS trial is being developed. In the future, more research on IdeS single therapy or other standard therapies and combination therapies for ABMR treatment will be needed.

## Tocilizumab

**Q: 1. Does tocilizumab have beneficial effect on ABMR as desensitization treatment?**
**2. Is tocilizumab effective as a treatment of ABMR?**

Tocilizumab is the first monoclonal antibody to IL-6R. Tocilizumab reduced peripheral pre- and post-switch B cells, IgG+ and IgA+ B cells, IgG and IgA, and significantly reduced B cell hypersensitivity in rheumatic arthritis patients. Interleukin-6 was initially identified as B cell stimulatory factor-2. Interleukin-6 is a pleiotropic cytokine with a significant array of biologic effects on numerous cell types which include B cells, T cells, plasma cells, hepatocytes, and endothelial cells [56]. Interleukin-6 has also been recognized as an important mediatory of allograft rejection [57].

Ashley et al. published a study on HLA-sensitized patients who failed to desensitize with IVIG and rituximab in a single center, Phase I/II open-label study. All patients received IVIG on days 1 and 30 and tocilizumab 8 mg/kg on day 15 and monthly for 6 months. Five out of ten patients underwent kidney transplant operation.

Mean time to transplant from first desensitization was 25 ± 10.5 months but after tocilizumab was 8.1 ± 5.4 months. Six-month protocol biopsies showed no antibody-mediated rejection. Donor-specific antibody strength and number were reduced by tocilizumab treatment. Renal function at 12 months was 60 ± 25 mL/min [56].

In another single center, open label study showed 36 renal transplant patients with cAMR plus DSAs and transplant glomerulopathy who failed standard treatment with IVIG plus rituximab with or without plasma exchange. Patients were offered rescue therapy with the anti-IL-6 receptor monoclonal tocilizumab with monthly infusions and monitored for DSAs and long-term outcomes. Tocilizumab-treated patients demonstrated graft survival and patient survival rates of 80% and 91% at 6 years, respectively. Significant reductions in DSAs and stabilization of renal function were seen at 2 years. No significant adverse events were seen. Tocilizumab provides good long-term outcomes for patients with cAMR and transplant glomerulopathy, especially compared with historical published treatments. Inhibition of the IL-6 receptor pathway has the potential for a novel approach to stabilize allograft function and prolong patient survival [58].

When various large-scale RCTs are announced in the future, various tocilizumab-based treatments can be developed. This could be used as an alternative treatment for ABMR that resists conventional therapy.

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# Pathology of the Allograft Kidney

8

Yong-Jin Kim

## Introduction to Banff Classification

**Q: 1. What are histopathologic findings of allograft dysfunction?**
**2. What is the Banff classification?**
- Antibody-mediated rejection (ABMR)
- T cell-mediated rejection (TCMR)
- Mixed ABMR and acute TCMR
- Differences in pathologic features in sensitized recipients
- Calcineurin-inhibitor toxicity
- BK virus nephropathy
- Recurrent diseases

**3. Molecular diagnostic**

The Banff Classification of Allograft Pathology is an international consensus classification for the reporting of biopsies from solid organ transplants. Since its initial conception in 1991 for renal transplants, it has undergone review every 2 years, with attendant updated publications.

Until the early 1990s, rejection of the renal allograft was classically classified into the following four types: hyperacute, acute, accelerated acute, and chronic rejection. However, there was considerable heterogeneity among pathologists in classification. Hence, it was felt that standardization of renal allograft biopsy was necessary to guide therapy. Hence, a group of pathologists, nephrologists, and transplant surgeons met in Banff Canada from 2 to 4 August 1991, to formulate a schema for nomenclature and classification of renal allograft pathology. The Banff classification has since been further strengthened by evidence-informed biannual updates elaborated during open international expert meetings. As a result, the Banff Classification of Allograft Pathology has become the predominant classification system used worldwide.

Table 8.1 is the summary of 2017 revised classification. For detail coding system, refer the "A 2018 Reference Guide to the Banff Classification of Renal Allograft Pathology" published by Roufosse et al. [1].

## Antibody-Mediated Rejection

**Q: 1. What is the pathognomonic histologic finding of antibody-mediated rejection?**
**2. What about the positivity of C4d staining in antibody-mediated rejection?**

ABMR arises in three major forms, hyperacute, acute, and chronic rejection, and often coincides with acute or chronic TCMR. Pathogenesis is

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**Table 8.1** Revised Banff 2017 classification of antibody-mediated rejection (ABMR) and T cell-mediated rejection (TCMR) in renal allografts [2]

| | |
|---|---|
| Category 1: | Normal biopsy or nonspecific changes |
| Category 2: | Antibody-mediated changes |
| | Active ABMR |
| | Chronic active ABMR |
| | C4d staining without evidence of rejection |
| Category 3: | Borderline changes |
| | Suspicious for acute TCMR |
| Category 4: | TCMR<br>Acute TCMR; Grade IA, IB, IIA, IIB, III<br>Chronic Active TCMR: Grade IA, IB, II |
| Category 5: | Interstitial fibrosis and tubular atrophy (IFTA) |
| | Grade I. Mild IFTA (≤25% of cortical area) |
| | Grade II. Moderate IFTA (26–50% of cortical area) |
| | Grade III. Severe IFTA (>50% of cortical area) |
| Category 6: | Other changes not considered to be caused by acute or chronic rejection |
| | BK virus nephropathy |
| | Posttransplant lymphoproliferative disorders |
| | Calcineurin inhibitor nephrotoxicity |
| | Acute tubular injury |
| | Recurrent disease |
| | De novo glomerulopathy (other than transplant glomerulopathy) |
| | Pyelonephritis |
| | Drug-induced interstitial nephritis |

related to donor-specific antibodies (DSA) to HLA or non-HLA antigens.

## Hyperacute Rejection

Hyperacute rejection is a variant of acute ABMR and refers to immediate rejection of the kidney upon perfusion with recipient blood, typically within 60 minutes. DSA titers are sufficient at the time of transplantation. Fortunately, this is now very rare, due to effective crossmatch screening. Light microscopically (Fig. 8.1), it looks like severe changes of TMA and sometimes totally necrotic. Widespread of microthrombi are in the glomeruli and arterioles. C4d is deposited in the PTC and glomeruli and intensity is stronger than usual acute ABMR.

## Active (Acute) Antibody-Mediated Rejection

In 2017 Banff meeting [2], they made consensus to erase the term "acute" from "acute/active ABMR." Because, clinically this microvascular inflammation (MVI) would occur not only in acute stage but also in smoldering, subclinical, or chronic stage. The word "active" would be more

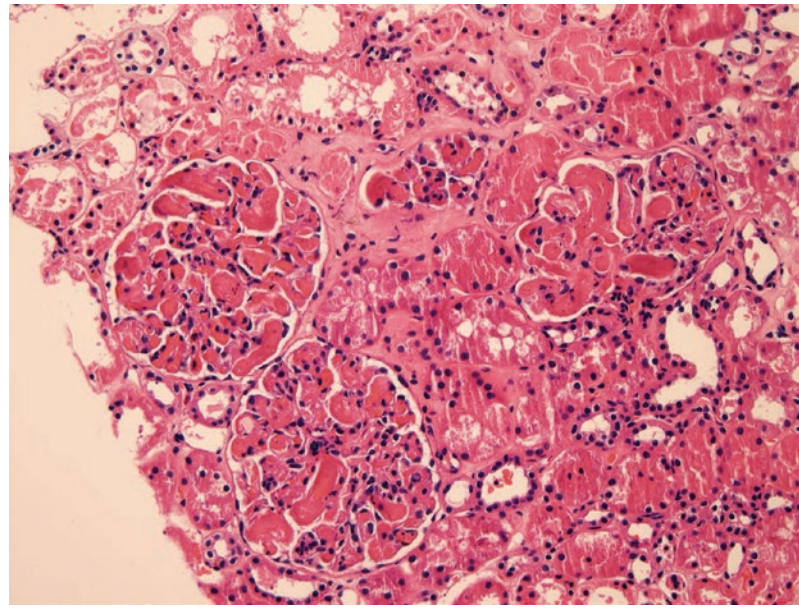

**Fig. 8.1** Hyperacute rejection 1 h after transplantation, due to preexisting high titer of DSA. Glomerular capillaries are occluded by large thrombi. Interstitium is edematous. (H&E, original magnification ×200)

**Table 8.2** 2017 Banff criteria for active ABMR [2]

| |
|---|
| 1. Histologic evidence of acute tissue injury, including one or more of the following: |
| – Microvascular inflammation ($g > 0$ and/or ptc > 0), in the absence of recurrent or de novo glomerulonephritis, although in the presence of acute TCMR, borderline infiltrate, or infection, ptc ≥ 1 alone is not sufficient and g must be ≥1 |
| – Intimal or transmural arteritis ($v > 0$) |
| – Acute thrombotic microangiopathy, in the absence of any other cause |
| – Acute tubular injury, in the absence of any other apparent cause |
| 2. Evidence of current/recent antibody interaction with vascular endothelium, including one or more of the following: |
| – Linear C4d staining in peritubular capillaries (C4d2 or C4d3 by IF on frozen sections, or C4d > 0 by IHC on paraffin sections) |
| – At least moderate microvascular inflammation ([g + ptc] ≥2) in the absence of recurrent or de novo glomerulonephritis, although in the presence of acute TCMR, borderline infiltrate, or infection, ptc ≥ 2 alone is not sufficient and g must be ≥1 |
| – Increased expression of gene transcripts/classifiers in the biopsy tissue strongly associated with ABMR, if thoroughly validated |
| 3. Serologic evidence of donor-specific antibodies (DSA to HLA or other antigens). C4d staining or expression of validated transcripts/classifiers as noted above in criterion 2 may substitute for DSA; however thorough DSA testing, including testing for non-HLA antibodies if HLA antibody testing is negative, is strongly advised whenever criteria 1 and 2 are met |

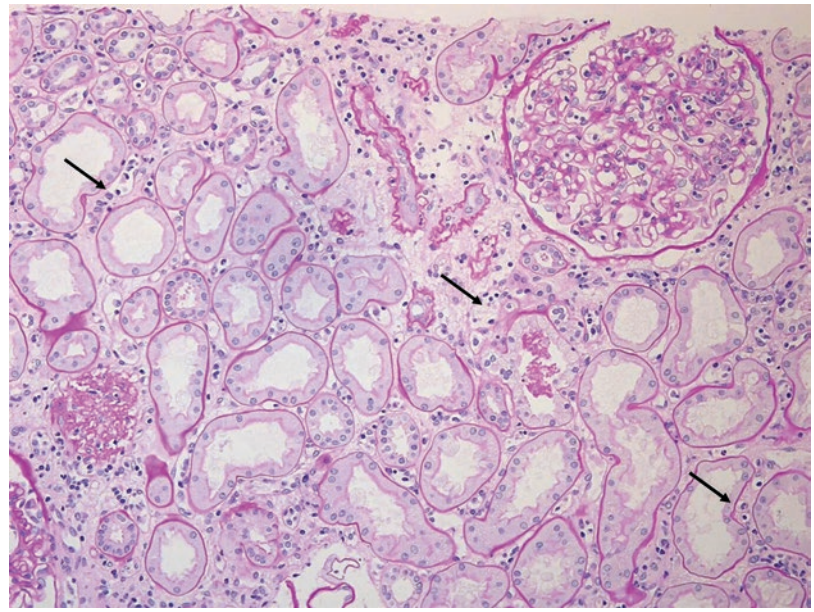

**Fig. 8.2** Active ABMR with microvascular inflammation; 2 months after transplantation, DSA (+). C4d (+). Glomerulus is infiltrated by mononuclear cells, neutrophils and reactive endothelial cells. The PTC are markedly dilated and infiltrated by mononuclear cells (arrows). In the edematous interstitium, mononuclear cells are scattered, but not enough to the degree of TCMR. (PAS, original magnification ×200)

reasonable in the pathology report indicates ongoing disease activity highlighted by MVI with or without concurrent chronic changes. However, true acute ABMR would be acute or early graft dysfunction in highly sensitized graft recipients (Table 8.2).

Histologically, acute ABMR has been divided into four types, based on light microscopy: (1) microvascular inflammation (MVI, Figs. 8.2 and 8.3) with neutrophils and mononuclear cells infiltration in glomeruli and peritubular capillaries (PTC), (2) intimal or transmural arteritis, (3) acute thrombotic microangiopathy (TMA), and (4) acute tubular injury in the absence of any other cause.

The criteria of glomerulitis is based on numbers of leukocytes (≥5) per glomerulus. In addition to this, endothelial swelling and capillary occlusion were adopted as a definition of transplant

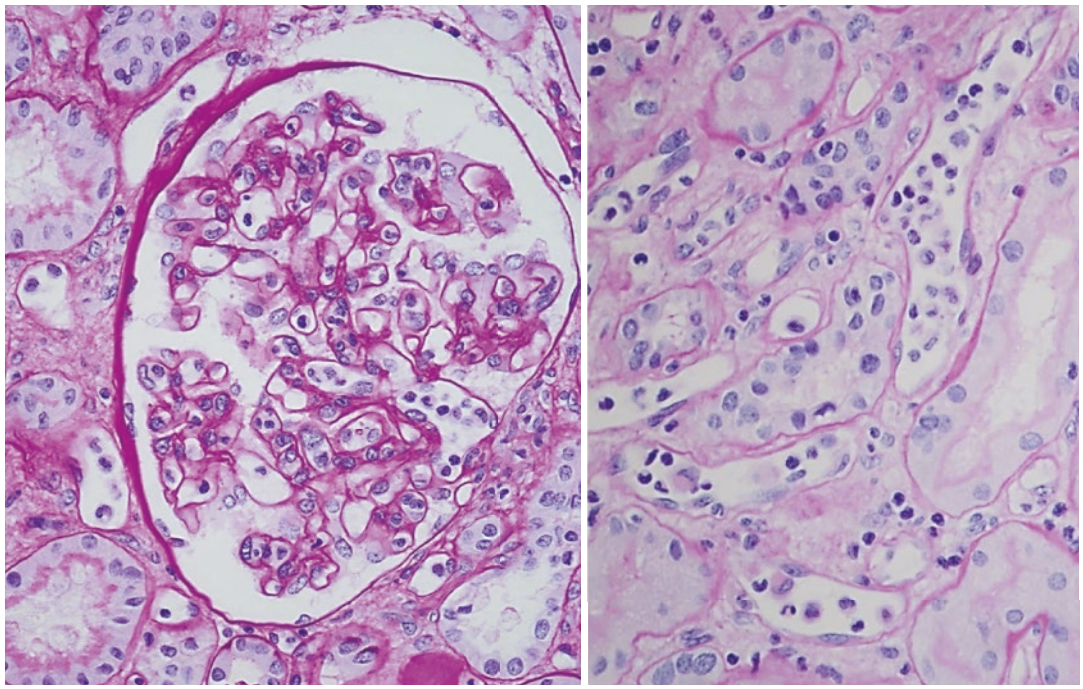

**Fig. 8.3** High power views of glomerulitis (left) and peritubular capillaritis (right). (PAS, original magnification ×400)

glomerulitis at the Banff 2013 meeting [3]. The determination of the numerical transplant glomerulitis, "g score" was still based on the percentage of glomeruli involved: 1–25, 26–50, and > 50% for g1, g2, and g3, respectively. The peritubular capillaries are often markedly dilated and infiltrated by mononuclear cells and/or neutrophils.

Determination of the "ptc" score is based on the number of inflammatory cells per cross section in more than 10% of PTCs in non-atrophic cortex: 3–4, 5–10, and > 10 for ptc1, ptc2, and ptc3, respectively.

Intimal or transmural arteritis (Fig. 8.4) is defined by the infiltration of mononuclear cells under arterial endothelial cells. Detection of even a single lymphocyte in the arterial intima is sufficient to diagnose. It has been scored according to the degree of luminal narrowing: <25%, ≥25%, and transmural necrosis for v1, v2, and v3, respectively. Arteritis or transmural inflammation are in severe cases of acute ABMR, but also in severe cases of TCMR.

Interstitial edema with scattered mononuclear cells may be present, but is not sufficient for the diagnosis of TCMR.

– **C4d staining** (Fig. 8.5)
C4d is detected in linear pattern; usually widespread, uniform distribution. But focal in some cases. Medullary vessels are typically positive. In immunohistochemistry by using paraffin sections, C4d has a similar pattern in the PTC wall, although the intensity typically is weak. Glomerular staining for C4d is considered non-diagnostic.

C4d-negative with acute ABMR histology pattern cases have been accepted as ABMR [3] (Fig. 8.6). In presensitized patients and ABOi cases, C4d became positive. Haas et al. [4] reported that C4d deposition can precede histologic evidence of acute ABMR with protocol biopsies.

– **ABO-incompatible grafts**
A big difference between ABOi and compatible grafts is the common presence of C4d in the PTC in the absence of ABMR histology or graft dysfunction. The frequency of transplant glomerulopathy is higher in ABOi graft compared with compatible graft [5].

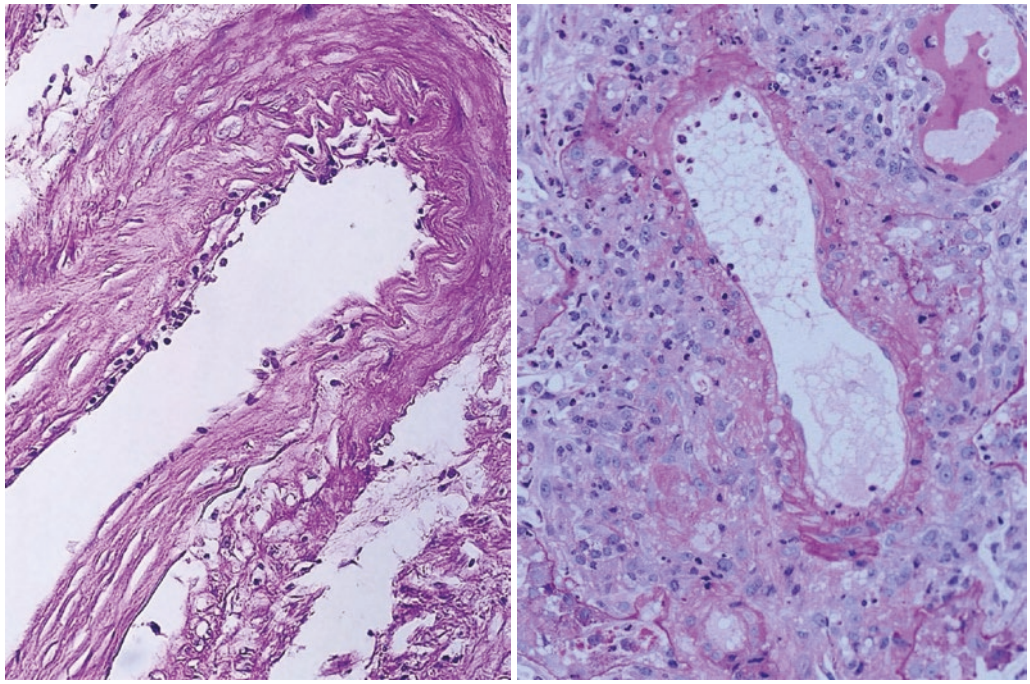

**Fig. 8.4** Arteritis. (Left) Mononuclear cells are underneath the endothelium (v1). (Right) Arterial wall is necrosis and infiltrated by mononuclear cells (v3). (PAS, original magnification ×200)

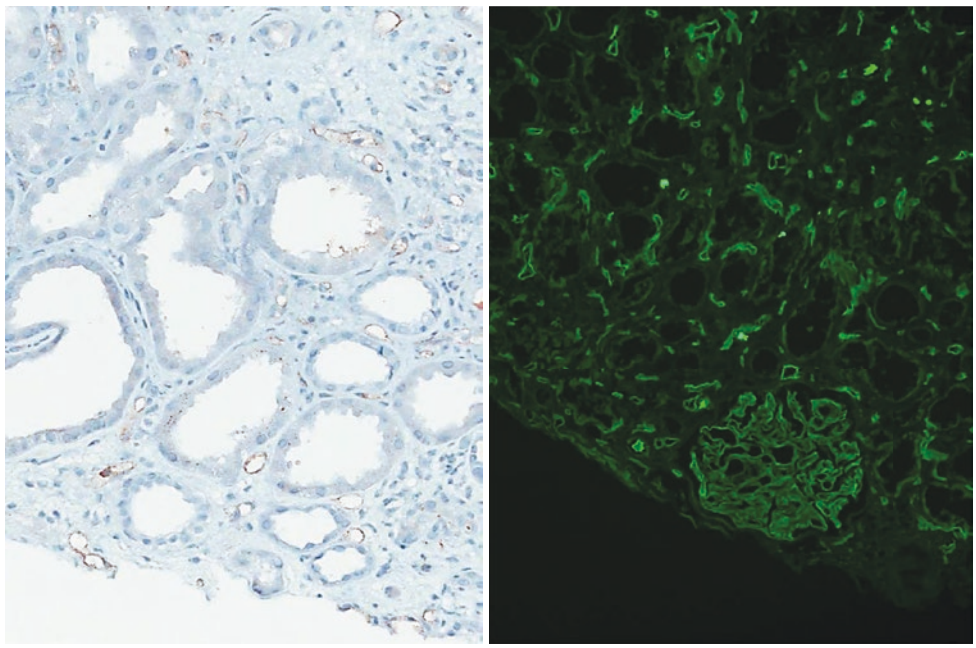

**Fig. 8.5** C4d stain positive in ABMR. (Left) Active ABMR in 4 week posttransplantation. Immunohistochemical staining of C4d is positive at peritubular capillary wall. (original magnification ×200) (Right) Chronic active ABMR in 2.5 years after transplantation. Immunofluorescent stain of C4d is positive at peritubular capillary as well as glomerular capillary wall. (original magnification ×100)

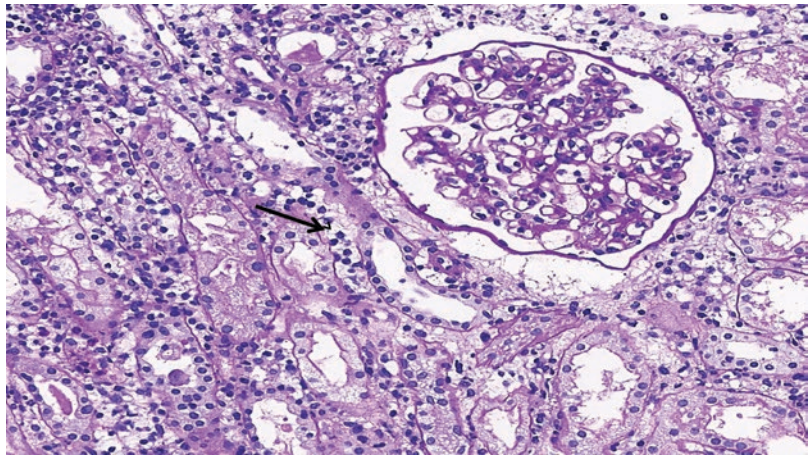

**Fig. 8.6** C4d negative ABMR, A 61 year-old woman received kidney from a deceased donor. DSA was positive. Serum creatinine reached 1.8 mg/dL on 13th day after transplantation. Several mononuclear cells are present in glomerular capillaries (g2) and peritubular capillaries (ptc2, arrow). These changes are not different from those of C4d positive ABMR cases. (PAS, original magnification, ×200)

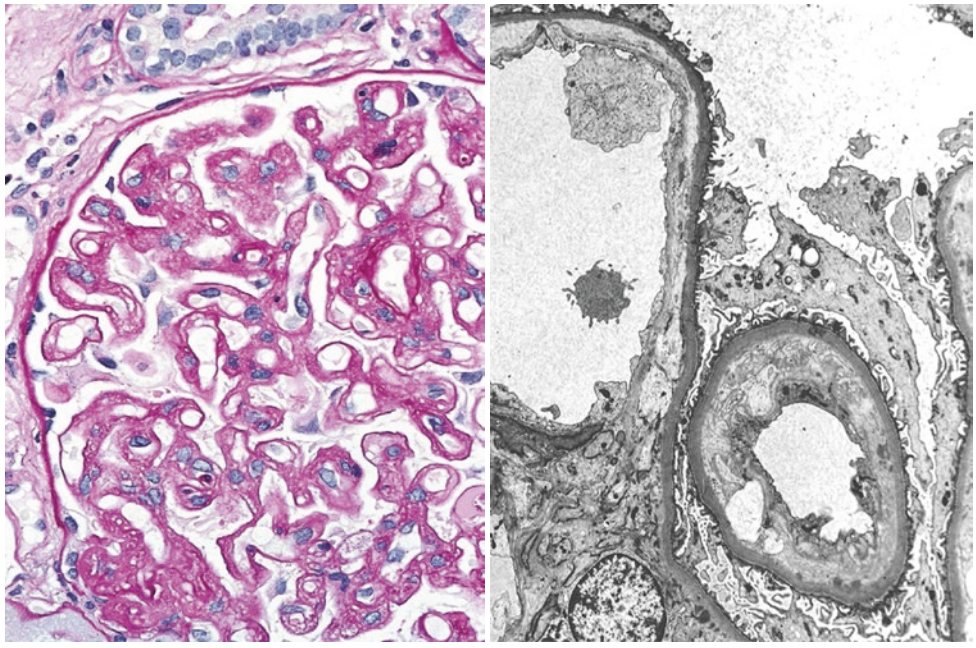

**Fig. 8.7** Transplant glomerulopathy in 4 years after transplantation. (Left) diffuse duplication of glomerular basement membrane affecting greater than 90% of capillaries. (PAS, original magnification ×400) (Right) Glomerular basement membrane is separated by loose matrix and some cell debris. The podocytes and foot processes are preserved well. (EM, original magnification ×20,000)

## Chronic Antibody-Mediated Rejection

Chronic ABMR is distinguished from acute ABMR by chronic injury pattern; transplant glomerulopathy, multilayering of PTC basement membrane, and arterial intimal fibrosis of new onset type.

The most characteristic change is transplant glomerulopathy (TG. Fig. 8.7), defined by duplication of GBM in the absence of specific de novo or recurrent glomerular disease or evidence of

TMA. In active cases, mononuclear cell infiltration and endothelial swelling are present (transplant glomerulitis).

The severity of TG is using "cg" score. The Banff cg0 was defined as no double contours by light microscopy or EM. "cg1" has two subcategories: cg1a indicates double contours associated with subendothelial widening detected only by EM, whereas cg1b corresponds to one or more glomerular capillaries with double contours in non-sclerotic glomeruli, observed by light microscopy [2]. The "cg" score is still based on the most severely affected glomeruli: 1–25, 26–50, and > 50% for cg1, cg2, and cg3, respectively. The risk of TG is increased by the presence of higher levels of class II DSA [6], particularly those reactive to HLA-DQ which was refractory to conventional therapy [7, 8]. A history of acute ABMR and presensitization also increases the risk [7].

In active cases, PTCs are dilated inflammatory cell infiltration and deposition of C4d as they are in active ABMR (Fig. 8.3). PTC basement membrane is thickened and multilayered, which should be evaluated by EM (as judged by ≥7 circumferential layer in one PTC and greater than five layers in two additional PTC) (Fig. 8.8). Liapis et al. [9] showed in approximately 30% of late biopsies with acute ABMR severe PTC multilayering. These are predictable signs of chronic rejection by electron microscopy before they become prominent and detectable by light microscopy.

Arterial intimal fibrosis is a typical feature of late allograft (Fig. 8.9), thought to be caused by antibodies or T cells. Intimal fibrous thickening without prominent elastic fiber accumulation is a

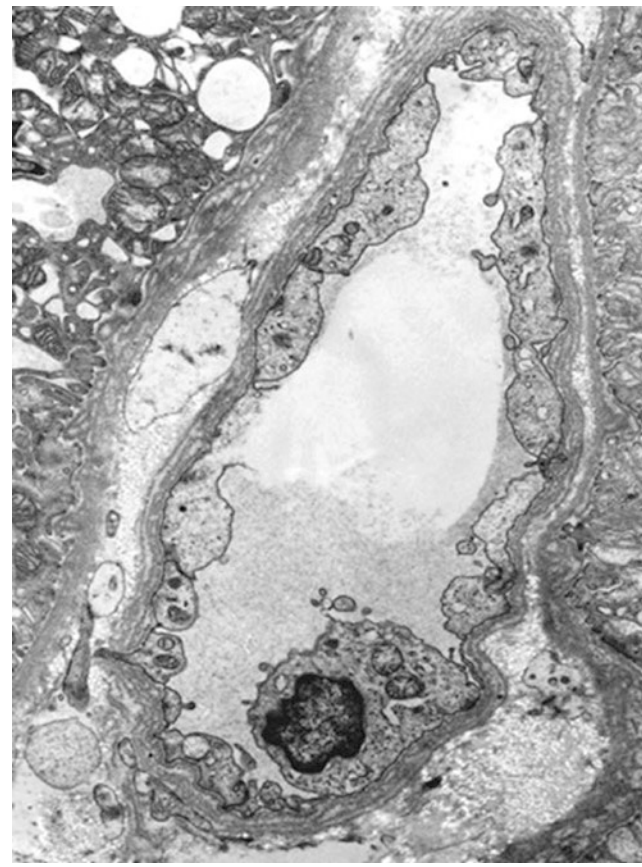

**Fig. 8.8** Multilayering of peritubular capillary basement membrane in 4 years after transplantation. Endothelial cell and its cytoplasm is edematous. Left side of PTC shows a part of tubular epithelial cells have numerous mitochondria. (EM, original magnification ×20,000)

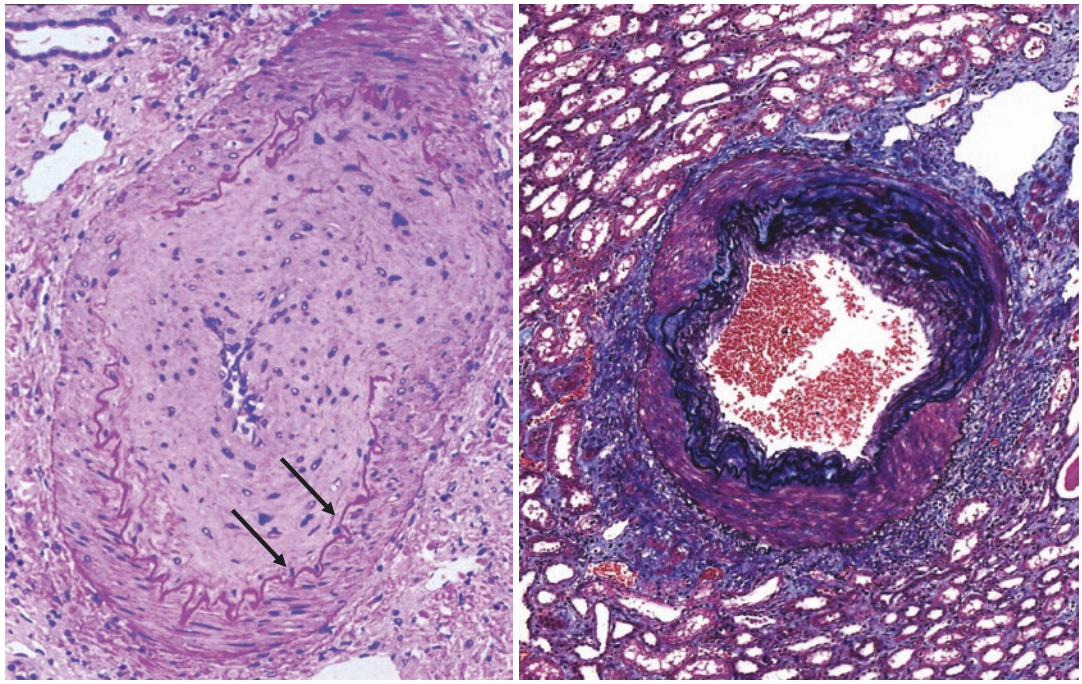

**Fig. 8.9** Comparison of chronic allograft arteriopathy and hypertensive arteriopathy. (Left) Artery from chronic TCMR shows thick intimal fibrosis (neointima) and no duplication of elastic lamina (arrows). (PAS, original magnification ×200) (Right) Artery from native kidney of hypertension shows neointima with marked duplication of elastic fibers. (Trichrome-elastic, original magnification ×200)

characteristic feature, in contrast to hypertensive artery, which shows the multilayering of elastic fibers. Arterial intimal thickening (cv) scores are still based on the most severely affected artery: 1–25, 26–50, and > 50% for cv1, cv2, and cv3, respectively (Table 8.3).

**Table 8.3** 2017 Banff criteria for Chronic active ABMR [2]

| Chronic active ABMR; all three criteria must be met for diagnosis |
|---|
| 1. Morphologic evidence of chronic tissue injury, including one or more of the following: |
| – Transplant glomerulopathy (cg >0) if no evidence of chronic TMA or chronic recurrent/de novo glomerulonephritis; includes changes evident by electron microscopy alone (cg1a) |
| – Severe peritubular capillary basement membrane multilayering (requires EM) |
| – Arterial intimal fibrosis of new onset, excluding other causes; leukocytes within the sclerotic intima favor chronic ABMR if there is no prior history of TCMR, but are not required |
| 2. Identical to criterion 2 for active ABMR, above |
| 3. Identical to criterion 3 for active ABMR, above, including strong recommendation for DSA testing whenever criteria 1 and 2 are met |

## T Cell-Mediated Rejection

**Q: 1. What is the pathognomonic finding of T cell-mediated rejection?**
**2. What affects the clinical outcome in T cell-mediated rejection?**

### Acute TCMR

The major finding in acute TCMR is infiltration of lymphocytes and some macrophages into a mildly edematous interstitium (i) and into the tubules (tubulitis, t). If both are not significant (i2, t2) or only one of these features is present, the diagnosis is made of borderline rejection. The borderline category exists only in TCMR.

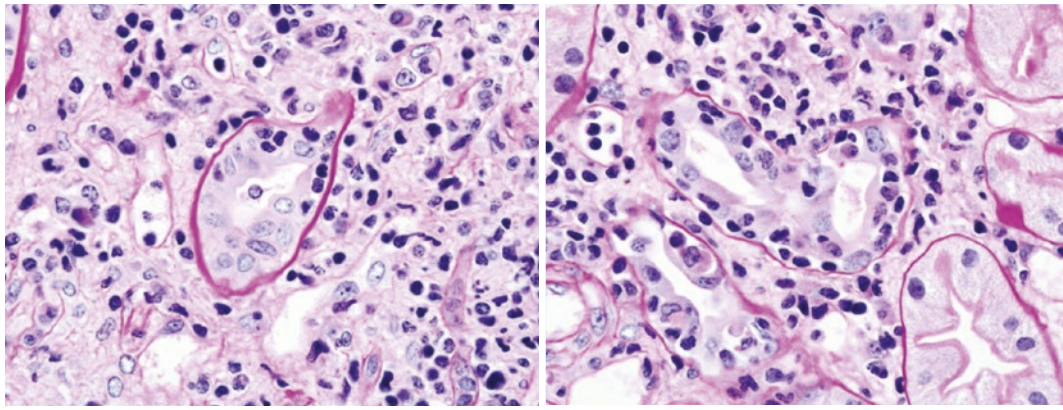

**Fig. 8.10** Acute TCMR with tubulitis from 2 weeks after transplantation. (Left) Five to six lymphocytes are inner aspect of tubular basement membrane, t2. (Right) More than ten lymphocytes are in tubular epithelium, t3. (PAS, original magnification ×400)

Tubulitis (Fig. 8.10) is infiltration of lymphocytes or macrophages along the inner aspect of the tubular basement membrane (TBM), recognized by small, dark nuclei and occasionally surrounded by small clear halos. In the Banff classification, determination of the numerical tubulitis (t) score is based on the maximum number of mononuclear cells in the most affected tubuli: 1–4, 5–10, and > 10 for t1, t2, and t3, respectively. Tubulitis in atrophic tubules (<50% of the original diameter and markedly thickened TBMs) is currently considered to be a non-diagnostic sign of parenchymal scarring.

Interstitial inflammation (i) was defined by a polymorphic interstitial infiltrate of mononuclear cells (lymphocytes, macrophages) and occasionally scattered polymorphonuclear leukocytes. "i" score is based on the parenchymal area affected by inflammatory cells: <10–25, 25–50, and > 50% for i1, i2, and i3, respectively. The diagnosis of acute TCMR grade I and II is using these "t" and "i" combination scores,

Lymphocytes also infiltrate arteries (v) in severe TCMR cases (Fig. 8.4). It should be noted that because arterial lesions may be indicative of ABMR, TCMR, or mixed ABMR/TCMR, all cases should be analyzed for C4d deposition for differentiation. TCMR requires C4d in PTC to be negative. If C4d is present, an additional diagnosis of concurrent ABMR should be considered. On occasion, this vasculitis can occur as an isolated event without tubulointerstitial changes. If biopsy samples are small and do not contain arteries, then transplant vasculitis may remain undetected.

– **Plasma cell-rich acute rejection (PCAR)**
  PCAR is a morphological type of acute rejection with prominent plasma cells, which normally account for >10% of interstitial mononuclear cells [10]. In previous studies, the response to antirejection therapy in PCAR, such as steroids, was less than satisfactory, with poor graft survival rates [11]. Some reports support the hypothesis that an antibody-mediated component participates in the graft injury of PCAR because it can be associated with both C4d staining and DSAs [12].

  Due to the rarity of PCAR, its incorporation into the Banff classification is still awaited. The differential diagnosis of PCAR includes BK virus nephropathy, posttransplant lymphoproliferative disorders, and cytomegalovirus infection (Table 8.4).

## Chronic Active TCMR

In Banff 2017 meeting, interstitial inflammation in fibrotic or scarred area (i-IFTA) was accepted

**Table 8.4** Banff criteria for Acute T cell-mediated rejection [2]

| |
|---|
| Suspicious (borderline) for acute TCMR |
| – Foci of tubulitis ($t > 0$) with minor interstitial inflammation (i0 or i1), or moderate-severe interstitial inflammation (i2 or i3) with mild (t1) tubulitis; retaining the i1 threshold for borderline with $t > 0$ is permitted although this must be made transparent in reports and publications |
| – No intimal or transmural arteritis ($v = 0$) |
| Acute TCMR |
| Grade 1A |
| Interstitial inflammation involving >25% of nonsclerotic cortical parenchyma (i2 or i3) with moderate tubulitis (t2) involving one or more tubules, not including tubules that are severely atrophic |
| Grade 1B |
| Interstitial inflammation involving >25% of nonsclerotic cortical parenchyma (i2 or i3) with severe tubulitis (t3) involving one or more tubules, not including tubules that are severely atrophic |
| Grade IIA |
| Mild to moderate intimal arteritis (v1), with or without interstitial inflammation and/or tubulitis |
| Grade IIB |
| Severe intimal arteritis (v2), with or without interstitial inflammation and/or tubulitis |
| Grade III |
| Transmural arteritis and/or arterial fibrinoid necrosis of medial smooth muscle with accompanying mononuclear cell intimal arteritis (v3), with or without interstitial inflammation and/or tubulitis |

as feature of chronic active TCMR in addition to chronic allograft arteriopathy [2], which is related to under immunosuppression [13]. Thus, tubulointerstitial lesions of chronic active TCMR (grade I, Fig. 8.11) is a combination of i-IFTA and tubulitis involving all but severely atrophic tubules, with moderately high thresholds for both (i-IFTA2–3; t2–3), a requirement for inflammation involving >25% of the total cortex (ti) present, and other differential diagnoses known to be associated with i-IFTA (e.g., chronic pyelonephritis, BK nephropathy, ABMR, recurrent glomerulonephritis, and obstruction) being ruled out. Inflammation in non-IFTA and IFTA areas can coexist in the same biopsy specimen. Such biopsy specimens should still be diagnosed as chronic active TCMR and not labeled acute plus chronic active TCMR, because the latter already addresses the acute/active component in the rejection process [2]. However, biopsies with chronic active TCMR can have an additional diagnosis of ABMR. And, there is no borderline or suspicious category for chronic active TCMR. In the study of Lefaucheur et al. [13], low levels of i-IFTA (i-IFTA 1) and mild tubulitis within foci of IFTA were not correlated with graft survival.

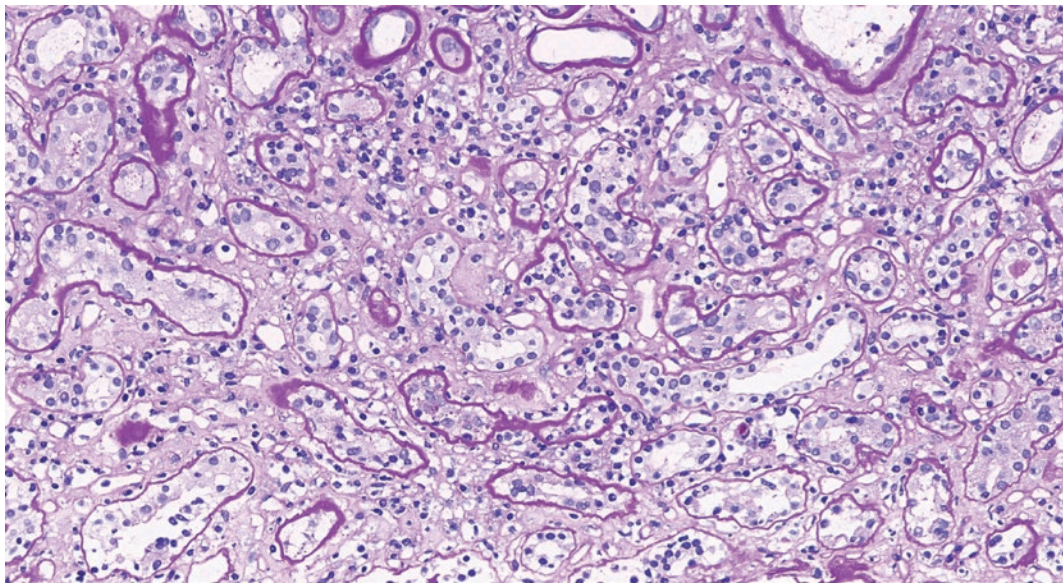

**Fig. 8.11** Chronic active TCMR grade Ib from 2.5 years after transplantation. Diffuse infiltration of mononuclear cells are in the area of interstitial fibrosis with tubular atrophy area (i-IFTA 2) and tubulitis in not severely atrophic tubules are associated. (PAS, original magnification ×200)

**Table 8.5** Banff criteria for Chronic Active TCMR [2]

| |
|---|
| Grade IA |
| Interstitial inflammation involving >25% of the total cortex (ti2 or 3) and > 25% of the sclerotic cortical parenchyma (i-IFTA score 2 or 3) with moderate tubulitis (t2) involving one or more tubules, not including severely atrophic tubules; other known causes of i-IFTA should be ruled out |
| Grade IB |
| Interstitial inflammation involving >25% of the total cortex (ti score 2 or 3) and > 25% of the sclerotic cortical parenchyma (i-IFTA score 2 or 3) with severe tubulitis (t3) involving one or more tubules, not including severely atrophic tubules; other known causes of i-IFTA should be ruled out |
| Grade II chronic allograft arteriopathy |
| Chronic allograft arteriopathy (arterial intimal fibrosis with mononuclear cell inflammation in fibrosis and formation of neointima) |

The traditional chronic allograft arteriopathy (Fig. 8.9) is classified as grade II lesion of chronic T cell-mediated rejection. It is characterized by intimal widening due to de novo accumulation of collages without elastic fiber proliferation, which is a feature of hypertensive arteriopathy. Varying degree, from absent to prominent, of intimal inflammatory cell infiltration is associated (Table 8.5).

## Mixed ABMR and Acute TCMR

**Q: 1. What is the incidence of mixed ABMR and TCMR?**
**2. How about the prognosis of mixed ABMR and TCMR?**

The incidences of mixed rejections were not known but not infrequent. These are not well categorized in the currently employed Banff classification. Clinically it has been manifested as the steroid resistant rejection, more severe and constitute an independent risk factor for graft failure [3].

## Differences in Pathologic Features in Sensitized Recipients

**Q: 1. What is the difference of pathologic finding between sensitized and nonsensitized patients?**
**2. Is there a difference of histologic finding between the preexisting DSA and de novo DSA?**

Although ABMR is a major complication in sensitized recipients, there were no specific or characteristic histopathologic changes for this. However, close follow-up study of Baganasco et al. [14] found that TG was preceded by glomerulitis in more than 90% of cases, with a median time interval of 12 months. MVI, particularly glomerulitis, irrespective of C4d and detectable posttransplantation DSA were associated with a high risk of development of TG in sensitized patient [14]. Haas et al. [4] compared ABMR resulted from preexisting DSA (type 1) and ABMR associated with de novo DSA (type 2). Both types of ABMR were more often associated with DSA against Class II HLA, and was associated with more interstitial fibrosis/tubular atrophy and more frequent cell-mediated rejection, although these did not differ with respect to C4d positivity. By univariate analysis, graft survival was lower with type 2 than type 1 ABMR with borderline significance. And moderate interstitial fibrosis/tubular atrophy and failure of the DSA relative intensity scale score were predictive to decrease in response to therapy.

Another comparison study [15] showed that patients with de novo DSA ABMR displayed increased proteinuria, more transplant glomerulopathy lesions, and lower glomerulitis, but similar levels of peritubular capillaritis and C4d deposition. De novo DSA ABMR was characterized by increased expression of IFNγ-inducible, natural killer cell, and T cell transcripts, but less expression of AKI transcripts compared with preexisting DSA ABMR. The preexisting DSA ABMR had superior graft survival compared with the de novo DSA ABMR (63% vs. 34% at 8 years after rejection, respectively; $P < 0.001$).

## CNI Nephrotoxicity

**Q: 1. Is CNI nephrotoxicity a true finding?**
**2. Is CNI nephrotoxicity a reversible condition?**

Calcineurin inhibitors are immunosuppressants and can cause renal toxicity. CNI nephrotoxicity also affects recipients with non-renal organ transplantation. For kidney transplantation, the actual occurrence rates at 5 and 10 years after kidney transplantation were 66 and 100%, respectively [16]. However, end-stage renal failure caused by CNIs is uncommon [17].

The histological features can be divided into two types, acute and chronic nephrotoxicity. Acute CNI nephrotoxicity include functional afferent arteriolar vasoconstriction and isometric vacuolization of tubular epithelium, usually the straight portions of proximal tubules (Fig. 8.12, left). In some severe cases, TMA can be developed. Chronic CNI nephrotoxicity includes arteriolar hyaline thickening, and interstitial fibrosis with tubular atrophy as a skip lesion (Fig. 8.12, right). The hyaline changes of arterioles are nodular shape, which are replaced by the necrotic smooth muscle cells of the media of vessel, is characteristic feature of chronic CNI toxicity [18]. This narrowing of arterioles may cause IFTA.

If serum CNI concentrations was controlled to lower levels to reduce CNI nephrotoxicity, the morphology of acute change could be reversible. But such methodologies may induce rejection episodes.

## BK Virus Nephropathy

**Q: 1. Is there a pure BK nephrotoxicity without rejection?**
**2. Is there any criteria for the mixed form of BK nephrotoxicity with rejection?**

This polyomavirus allograft nephropathy is caused by re-activation of latent intragraft polyomaviruses, typically associated with BK virus under immunosuppression status. The histological features (Fig. 8.13) are intranuclear inclusion

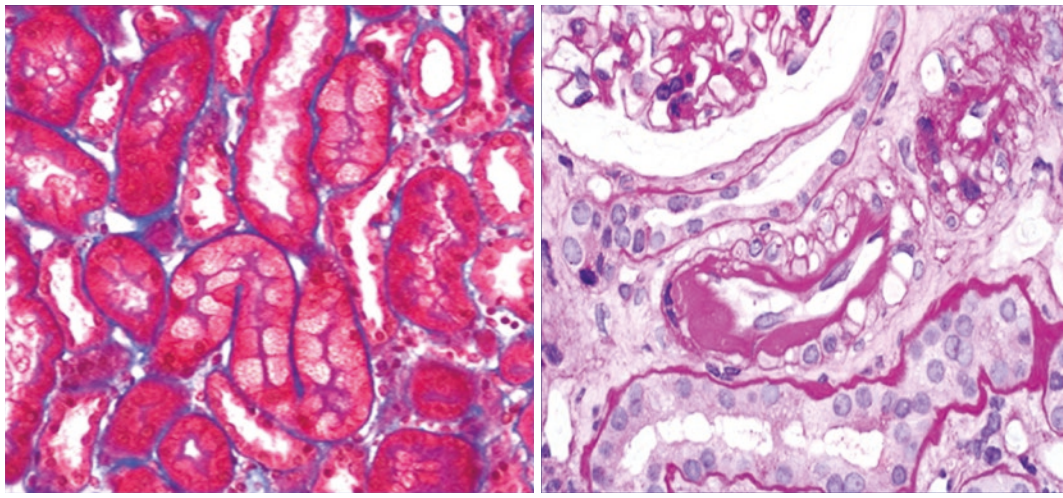

**Fig. 8.12** Calcineurin inhibitor toxicity. (Left) Isometric vacuolization of distal tubules are seen in the case of allograft failure in 2 weeks after transplantation. (trichrome, original magnification ×400) (Right) Nodular hyalinization of the arterioles and vacuolization of vascular smooth muscle cells in chronic calcineurin-inhibitor toxicity case, 3 years after transplantation. (PAS, original magnification ×400)

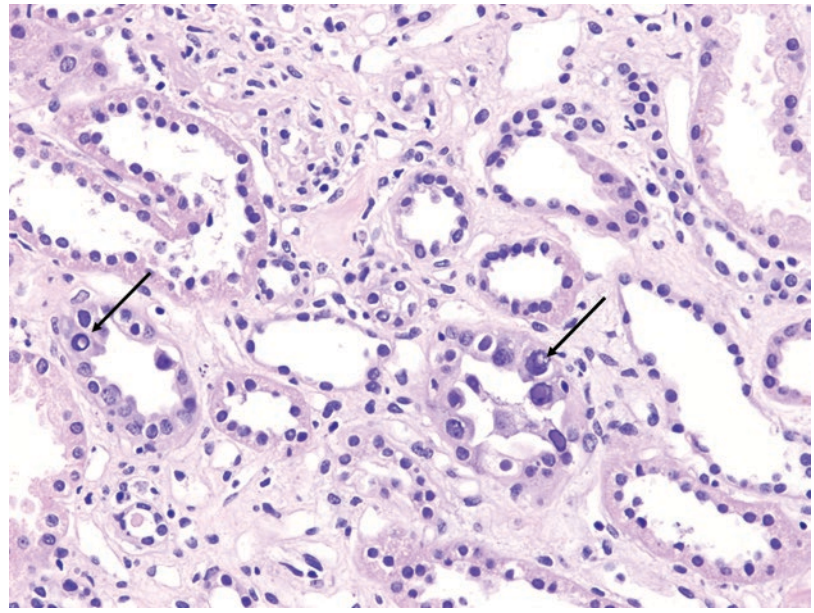

**Fig. 8.13** BK virus associated nephropathy. Damaged tubular epithelial cells with intranuclear inclusions (arrows) are detached from their basement membrane. Interstitium is infiltrated by lymphocytes. (H&E, original magnification ×200)

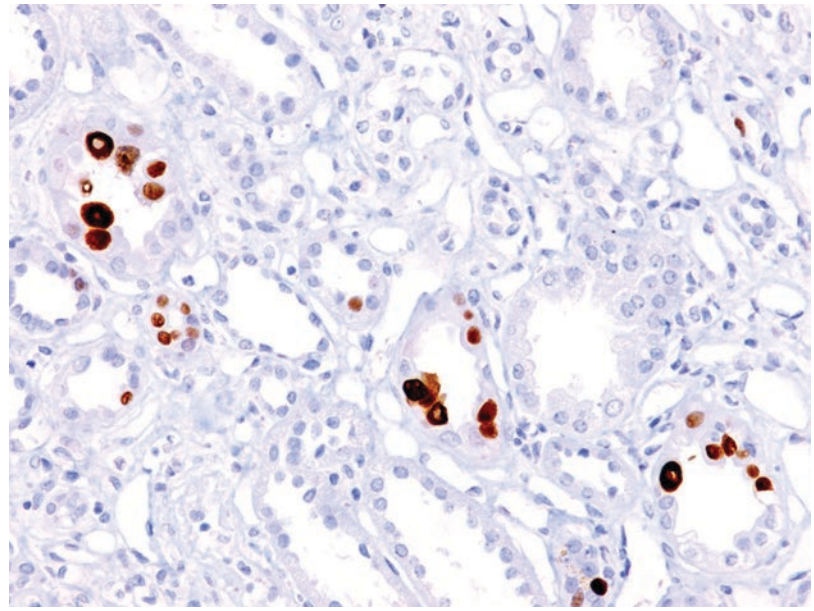

**Fig. 8.14** BK virus associated nephropathy. Viral inclusion cells are positive staining to SV40 antibody immunohistochemistry. (original magnification ×400)

body, tubular epithelial cell lysis, necrosis, shedding into the tubular lumen, interstitial lymphocytic inflammation, tubulitis. In late sage, interstitial fibrosis with tubular atrophy was developed. These findings are mixed with variable proportions in disease progress. To find out viral inclusions is sometimes difficult light microscopically. SV40 immunohistochemistry staining is very useful (Fig. 8.14).

Those pathological features of BKVN are similar to those of TCMR and CMV or other viral infections. And histologic diagnosis had a high rate of false-negative biopsies if specimens were obtain from only upper cortex. Because

BKVN started usually from medullary area, biopsy specimen should include some portion of medulla. And because of the focal nature of BKVAN, two cores biopsy is recommended.

Banff Polyomavirus Working Group has performed multicenter retrospective study to develop the histological staging system of this disease. AST (American Society of Transplantation) staging system focuses on interstitial inflammation and fibrosis [19]. In 2013, Banff Working Group proposed a new staging system consists of in situ viral load (pvl score) and interstitial fibrosis and now under consideration to incorporate official Banff criteria [17].

Questions on the development of pure BKVN without rejection and coexistence of rejections have been still in debate. But if BKVN features are associated with ABMR histology and DSA positive, the diagnosis of coexistence could be made. Even the diagnosis of acute TCMR, if the location of lesion is far from BKVN area, could be made.

Specific antiviral drugs for polyomavirus infections are not yet available; thus, patient screening and early diagnosis remain important. Therapeutic methods consist primarily of reduced maintenance immunosuppression proposed in AST guideline [19]. However, this reduction of immunosuppression may cause acute rejection.

## Recurrent Diseases

**Q: 1. Is it possible to differentiate the de novo vs. recurrent glomerular diseases?**
**2. Is it possible to differentiate the primary vs. secondary FSGS?**

Currently, glomerular diseases account for approximately 10–20% of cases of ESRD undergoing transplantation, and overall approximately 20% of these patients experience recurrence [20]. The same disease can also occur as de novo disease in the transplanted kidneys. Disease characteristics of the recurrent disease are similar to those of the original disease, but are usually mild in nature. This may be due in part to the use of immunosuppressive agents in the transplant patients. De novo diseases generally occur later than the recurrent diseases. Almost all diseases that occur in the native kidneys can occur de novo in transplant kidneys. However, the two most common diseases are membranous glomerulonephritis and focal segmental glomerulosclerosis (FSGS) [20].

To diagnose recurrent disease, we should confirm the diagnosis of the native kidney biopsy together with the kidney allograft biopsy to differentiate the de novo from the recurrent glomerular disease. The reported recurrence rates of IgAN after transplantation vary between 30 and 35%. IgAN recurrence occurs typically more than 3 years after transplantation [17].

The reported risk of recurrence of FSGS in the first graft ranges from 30 to 60%, whereas the rate approaches 100% in subsequent grafts [21]. Clinical features of FSGS recurrence include the early and acute onset of massive proteinuria in hours to days after transplantation. This immediate recurrence suggested the existence of humoral factors in recipients. Circulating urokinase receptor (suPAR), which has been reported as a cause of FSGS, may also be a predictor of FSGS recurrence [22]. In addition, novel candidates such as CLC-1, anti-CD40 Ab, and vasodilator-stimulated phosphoprotein are proposed [21]. In the late period of allograft showed frequently FSGS change, which was thought as not the recurrent FSGS but secondary FSGS caused by chronic CNI toxicity, hypertension and IFTA etc. Histologic changes were very similar to those of idiopathic FSGS.

Recurrence rates of membranous nephropathy after kidney transplantation have been reported to be 30–45% [24]. The disease usually occurs 2–3 years after transplantation with a 10–50% rate of graft loss at 10 years [24].

IgG4 and phospholipase A2 receptor (PLA2R) staining is helpful to differentiate de novo and recurrent membranous nephropathy [25]. Recurrent membranous nephropathy is usually positive for IgG4 and PLA2R staining [26].

## Recommendations for Use of Molecular Diagnostics

**Q: 1. Is there any correlation between molecular diagnostics and histology?**
**2. Is there any correlation between molecular diagnostic and clinical outcome?**

Molecular diagnostics were first introduced into the Banff classification in 2013, although this was limited to ABMR and they recommended for sampling of biopsy tissue for molecular studies [3].

The Alberta Transplant Applied Genomics Center (ATAGC) team at the University of Alberta developed a "molecular microscope" approach to kidney transplant biopsies and has provided a system for distinguishing TCMR from ABMR by the expression of activated ENDATs. They proposed new rules to integrate molecular tests and histology into a precise diagnostic system that can reduce errors, ambiguity, and interpathologist disagreement [26, 27].

A holistic molecular approach using machine learning and classifiers has been done in recent years and has provided valuable information for improving the classification and prognostic assessment of TCMR and ABMR [2, 28]. The ultimate goals are not only to improve our ability to predict graft outcomes but also to better guide therapy, including in those cases where histology and serology alone cannot optimally do so, leading to improved patient outcomes compared with the current standard of care.

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# Postoperative Results

9

Beom Seok Kim, Juhan Lee, and Kyu Ha Huh

## HLA-Incompatible Kidney Transplant vs. Compatible Kidney Transplant

**Q: 1. Does HLA-incompatible kidney transplant increase the risk of graft loss and death compared to compatible kidney transplant?**
**2. If so, what are the causes of inferior graft survival?**

Patients with donor-specific antibody (DSA) undergoing various desensitization therapies and subsequent kidney transplant (KT) have had promising short-term outcomes. HLA-incompatible living donor kidney transplant (HLAi LDKT) is now an important part of the landscape of KT. Although it is clear that desensitization increases access to transplantation and reduces the waiting time among sensitized patients, data on long-term outcomes are limited.

Single-center reports suggest that HLAi LDKT outcomes are not as good as compatible living donor KT. Mayo Clinic group reported a 5-year death-censored graft survival rate of 70.7% in 102 HLAi LDKT patients, compared to 88.0% in 204 compatible KT patients matched for age and sex [1]. At baseline, 41 had positive complement-dependent cytotoxicity (CDC) crossmatch (XM) and 61 had negative CDC XM, but positive flowcytometric (FC) XM. Compared to compatible KT patients, HLAi LDKT patients had longer duration of dialysis and higher proportion of re-transplant. Patient survival at 5 years was also lower in positive HLAi LDKT compared to compatible KT (83.5% vs. 92.5%). Haririan et al. compared 41 HLAi LDKT patients (positive FC XM) to compatible KT patients matched on gender, race, age, prior kidney transplant, and year of KT [2]. HLAi LDKT patients had significantly longer duration of dialysis than compatible KT patients. Graft survival rates at 1 and 5 years were 89.9% and 69.4% for HLAi LDKT group and 97.6% and 80.6% for the controls, respectively ($P = 0.04$). The 5-year patient survival rates were comparable between two groups (85.4% vs. 90.2%).

Multicenter study from 22 United States transplant centers reported that graft and patient survivals were significantly lower in XM positive

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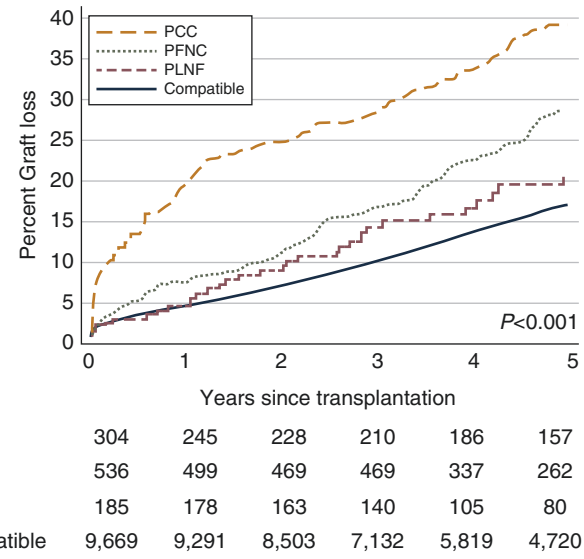



| | | | | | | |
|---|---|---|---|---|---|---|
| PCC | 304 | 245 | 228 | 210 | 186 | 157 |
| PFNC | 536 | 499 | 469 | 469 | 337 | 262 |
| PLNF | 185 | 178 | 163 | 140 | 105 | 80 |
| Compatible | 9,669 | 9,291 | 8,503 | 7,132 | 5,819 | 4,720 |

**Fig. 9.1** Graft survival by antibody strength. *PCC* positive cytotoxic crossmatch, *PFNC* positive flow, negative cytotoxic crossmatch, *PLNF* positive luminex, negative flow crossmatch. (Adapted from Orandi BJ, Chow EH, Hsu A, et al. Quantifying renal allograft loss following early antibody-mediated rejection. Am J Transplant 2015;15:489–498)

KT than in compatible KT [3]. They also found that increased DSA strength was associated with worse graft outcomes and higher mortality following HLAi LDKT (Fig. 9.1). CDC and FC XM positive patients continued to have elevated risk of graft loss and death after the first year post-transplant compared to compatible patients. However, it should be noted that there were significant differences in recipient characteristics, including dialysis duration and insurance type, between ILDKT and compatible KT groups.

Recent national registry data showed that 5-year transplant survival for HLAi LDKT (71%) was not as good as for "standard" living donor KT in highly sensitized patients (87%), but was closer to the outcomes of "standard" deceased donor KT in highly sensitized patients (73%) [4]. The risk of transplant loss was associated with increasing number of DSAs, center performing the transplant, and the XM status at the time of the transplant, rather than the pretreatment XM status.

HLAi LDKT carries an increased risk of antibody-mediated rejection (AMR) and transplant glomerulopathy jeopardizing the long-term success of transplantation [1, 5]. However, data from these studies are difficult to generalize because of heterogeneity among desensitization strategies, XM techniques, and DSA levels at baseline and at the time of transplant. In addition, it is difficult to know whether poor long-term outcomes of HLAi LDKT is due to antibody-mediated injury or other unfavorable baseline characteristics. ILDKT patients are more likely to have confounding risk factors such as cardiovascular risk and metabolic alteration due to long-term dialysis.

It is important to consider when deciding whether to desensitize is expected waiting time based on local donor pool and allocation system. In countries with low organ donation rates and limited size of kidney paired donation (KPD) pool, desensitization might be the only available option for highly sensitized patients [6–8]. Patients and clinicians tend to consider HLAi LDKT earlier instead of waiting for a deceased donor. These patients might be less likely to have comorbidities than those transplanted after prolonged dialysis vintage. In contrast to the results from Western countries, two single-center studies from Asia with relatively short period of dialysis reported comparable outcomes between HLAi LDKT and compatible living donor KT [9, 10].

Between 2006 and 2017, 95 patients underwent HLAi LDKT at our center after desensitization (38 CDC XM, 57 FCXM). We compared clinical outcomes between HLAi LDKT and compatible living donor KT ($n$ = 1,150). Mean dialysis duration was 17.2 months for HLAi LDKT and 17.5 month for compatible control, respectively. The 1- and

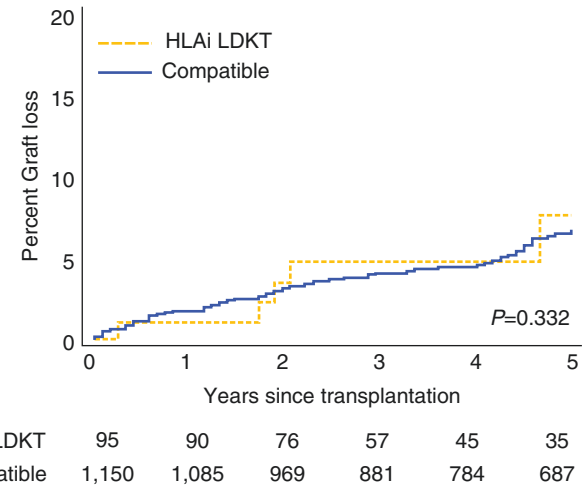



**Fig. 9.2** Graft survival in HLAi LDKT compared with compatible KT

5-year overall graft survival rates were 98.9% and 92.3% for patients with positive XM and 98.3% and 93.5% with negative XM, respectively (Fig. 9.2). Patient survival rate at 5-year was comparable between two groups (97.6% vs. 97.8%). During the study period, 29 HLAi LDKT patients experienced biopsy-proven rejection (30.5%, unpublished data). Earlier referral to an experienced center for desensitization treatment to reduce length of time on dialysis might yield better transplant outcomes [11, 12].

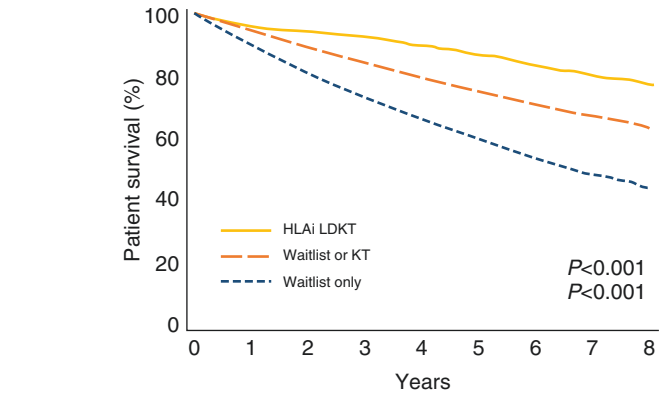
## HLAi LDKT vs. Wait-List

**Q: Does HLAiLDKT offer a better survival benefit than waiting compatible deceased donor in sensitized patient?**

Over the past decade, advances in KPD programs and allocation systems have improved access to KT for highly sensitized candidates [6, 13, 14]. However, the effectiveness of allocation system depends largely on the size of the donor pool and the breadth of an individual patient's sensitization. Even under optimal circumstances, hard-to-match patients are more likely to remain on the wait-list [15]. The benefit from paired donation may be limited to candidates with easy-to-match blood types and antibody profiles [16, 17]. Although there are some differences to some extent depending on the healthcare system, the choice for highly sensitized patients is not between an incompatible and compatible transplant, but rather, it is between an HLAi LDKT and remaining on the wait-list [12, 18]. In this regard, HLAi LDKT, including in combination with KPD, will remain a major treatment modality for hard-to-match patients. Therefore, it may be more logical to compare survival of HLAi LDKT patients to those remaining on the waiting list rather than to those compatible KT [12, 18, 19].

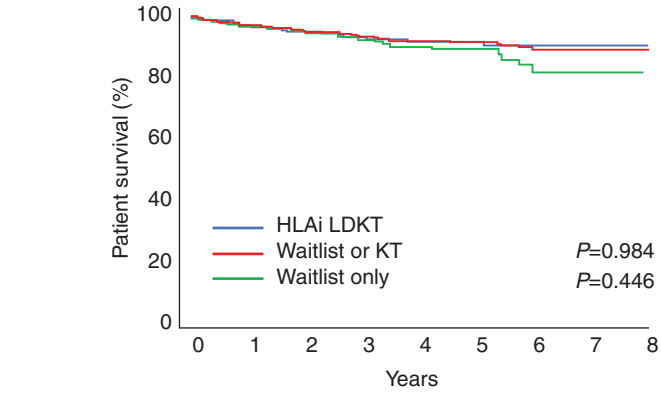
In a matched-control analysis, Johns Hopkins group demonstrated 8-year patient survival among HLAi LDKT recipients was 80.6% compared to 30.5% and 49.1% for matched-control patients remaining on dialysis (dialysis only group) or eventually receiving compatible KT (dialysis or transplant group), respectively ($P < 0.001$ for both comparisons) [12]. In the first year, there was no significant difference in the survival rate between the HLAi LDKT group and either control group. However, after the first year, the survival rate of the HLAi LDKT group was significantly higher. This clear survival benefit of HLAi LDKT was observed even among patients with positive CDC XM.

These findings were confirmed in a large multicenter study that included 1,025 HLAi LDKT patients with varying strengths of DSA [18]. Controls were patients who were on a waiting list




| | | | | | |
|---|---|---|---|---|---|
| HLAi LDKT | 1,025 | 958 | 832 | 584 | 327 |
| Waitlist or KT | 5,125 | 4,546 | 3,673 | 2,493 | 1,414 |
| Waitlist only | 5,125 | 4,141 | 3,024 | 1,810 | 916 |

**Fig. 9.3** Survival benefit of HLAi LDKT. (Adapted from Orandi BJ, Luo X, Massie AB, et al. Survival benefit with kidney transplants from HLA-incompatible live donors. N Engl J Med 2016;374:940–950)




| | | | | | |
|---|---|---|---|---|---|
| HLAi LDKT | 213 | 178 | 115 | 48 | 0 |
| Waitlist or KT | 852 | 519 | 276 | 112 | 0 |
| Waitlist only | 852 | 340 | 120 | 37 | 0 |

**Fig. 9.4** Patient survival in HLAi LDKT compared with a matched cohort. (Adapted from Manook M, Koeser L, Ahmed Z, et al. Post-listing survival for highly sensitised patients on the UK kidney transplant waiting list: a matched cohort analysis. Lancet 2017;389:727–734)

for compatible deceased donors but were otherwise similar to the transplanted patients. One-year, 5-year, and 8-year survival rates in HLAi LDKT patients were 95%, 86%, and 77%, respectively (Fig. 9.3). Survival rates were significantly lower in either control group (94%, 74%, and 63%, respectively, for patients in the dialysis or transplantation group, 90%, 59%, and 44%, respectively, for patients in the dialysis only group). In this study, survival benefit was seen across all DSA levels. In addition, the results were similar in an analysis that excluded patients from the highest-volume center.

In contrast, a similar study from the United Kingdom showed no survival benefit for HLAi LDKT over wait-list and subsequent KT [19]. They conclude that desensitization has no detrimental influence on patient survival, but does not offer a survival benefit (Fig. 9.4). The reasons for these discrepant findings were analyzed in detail and included differences in definitions of sensitization, control groups, and inferior dialysis outcomes in the United States compared with many other developed countries [20, 21]. However, these findings should be interpreted with caution, as two-thirds of control patients had less than 3 years of follow-up. Although relatively healthy candidates were enrolled on the waiting list, the survival rate of the control groups in this study was significantly higher than the survival rate reported in the United Kingdom renal registry [22].

Whether desensitization confers a survival benefit in HLAi LDKT, compared with remaining on dialysis for a potentially prolonged period while waiting for a compatible deceased donor KT, remains a matter of debate. Anticipated waiting time for a compatible deceased donor and dialysis outcomes as well as transplant outcomes affect the expected survival benefit [23]. Therefore, clinical decision should be made considering each individual's DSA profile, overall dialysis outcome, and transplant access based on the local donor pool and allocation system [8].

## HLAiLDKT Outcomes According to DSA Strength

**Q: 1. Can DSA characteristics including strength predict long-term outcomes?**
**2. Which DSA at baseline or at transplant predict the graft survival better?**

Since the landmark studies of Patel and Terasaki, positive CDC XM has been considered to be a contraindication to transplantation [24]. Concern that relevant DSAs were not being detected led to the development of more sensitive XM techniques [25]. In addition, solid-phase assay has allowed detection and characterization of the relevant DSA.

Early studies demonstrated an association between the baseline DSA strength (based on XM results) and response to desensitization, risk of AMR, and graft survival. Johns Hopkins group reported number of treatments required to reach a negative XM depends on baseline XM results; a patient with low levels of DSA detected by FC XM may be desensitized after two cycles of treatment, whereas a patient with a positive CDC XM usually requires many more cycles to convert to a negative XM [26]. Mayo Clinic group also found that the baseline DSA level correlates with response to desensitization, risk of AMR, and graft survival [27, 28]. Recent multicenter study confirmed that baseline XM results were associated with increased graft loss and mortality [3].

However, clinicians cannot perfectly predict response to desensitization therapy based on baseline DSA levels. Even in patients with similar DSA level, DSA is completely removed in some patients, and DSA is incompletely removed in most patients. Therefore, centers have their own transplantable criteria based on XM and solid-phase assay results at the time of transplant [1, 12, 29]. National registry data from the United Kingdom showed that XM status at transplant were more strongly associated with transplant survival than the XM status at baseline [4].

The development of solid-phase assays has significantly improved the sensitivity and precision of DSA. Serum mean fluorescence intensity (MFI) values have been used as a quantitative assessment of DSA strength by clinicians. However, MFI value alone, which only offers a semiquantitative measured of DSA level at best, is not entirely reliable for predicting immune response. The DSAs that bind on beads (in vitro) may not bind on the corresponding antigen of endothelial cells (in vivo) [30]. Therefore, it is difficult to determine MFI cutoff value suitable for proceeding to KT [31, 32]. A single result either before or after desensitization cannot perfectly predict all future events. In order to decide whether to proceed with KT, it should be based on changes in MFI levels before and after desensitization rather than a single test result.

In addition to DSA strength, technological advances in solid-phase assay have enabled the identification of various characteristics of DSA, such as complement-binding capacity and IgG subclass [33]. A modified single antigen bead assay detecting C1q binding as a surrogate for complement-binding capacity of DSA has been proposed as a way of determining which DSAs are deleterious [34]. However, these technologies are not well utilized in the final decision. If clinicians integrate the C1q-binding assays into risk assessment and add disappearance of C1q-binding DSA as a goal of desensitization protocols, then the risk of AMR and graft loss may be significantly reduced [35]. Recently, we added disappearance of C1q-binding DSA as a goal of desensitization protocols (Fig. 9.5). After confirming a negative conversion

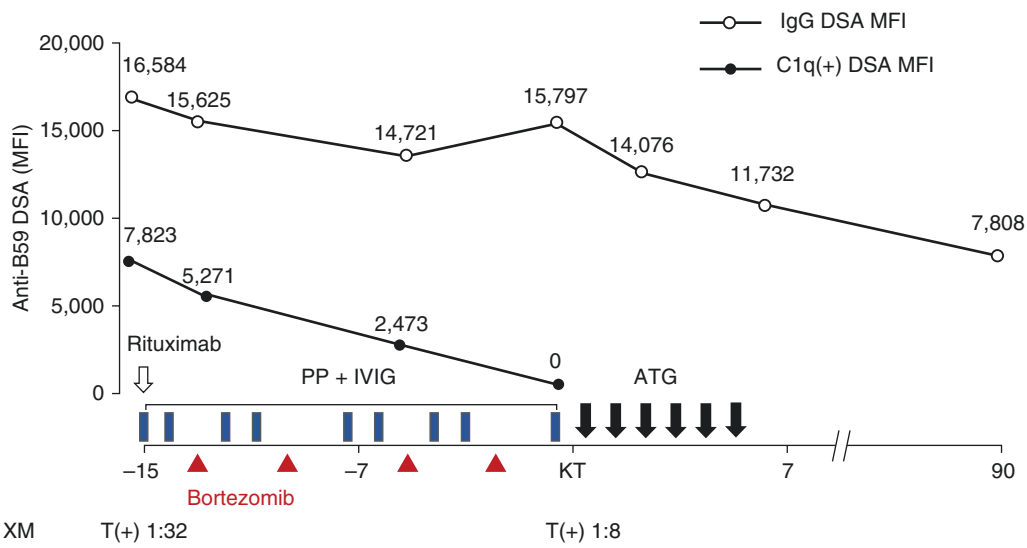



**Fig. 9.5** Bortezomib-assisted desensitization protocol and trends in DSA. *AHG* anti-human globulin, *ATG* anti-thymocyte globulin, *CDC XM* complement-dependent cytotoxicity crossmatch, *DSA* donor-specific antibodies, *IVIG* intravenous immunoglobulin, *KT* kidney transplantation, *MFI* mean fluorescent intensity, *PP* plasmapheresis. (Adapted from Lee J, Park BG, Jeong HS, et al. Successful kidney transplantation across a positive complement-dependent cytotoxicity crossmatch by using C1q assay-directed, bortezomib-assisted desensitization: A case report. Medicine (Baltimore) 2017;96:e8145)

in the C1q DSA, we decided to perform KT accepting a positive CDC XM of 1:8 at the time of transplant [36]. The patient did not receive post-transplant desensitization therapy, and his postoperative course was uneventful. The protocol biopsy performed at postoperative month 3 showed no evidence of antibody-mediated injury.

No single result absolutely contraindicates transplantation, rather the risks need to be weighed against the potential for benefit to be attained, in the context of individual patient. Therefore, the results of XM and solid-phase assays must be interpreted in the context of the individual patient.

## Transplant Outcomes According to Desensitization Protocol

**Q: Which desensitization protocols carry better transplant outcomes?**

1. Plasmapheresis + IVIG
2. High-dose IVIG
3. Proteasome inhibitors
4. Complement inhibitors
5. Immunoglobulin-G degrading enzyme of *Streptococcus pyogenes*

Various desensitization protocols that deplete DSAs have been employed to overcome HLA barriers and facilitate ILDKT. Current desensitization protocols using a combination of high-dose or low-dose intravenous immunoglobulin (IVIG), plasmapheresis (PP), and rituximab have proven beneficial to allow for successful transplantation [12, 37]. Achieving negative XM and short-term outcomes are primary determinants of desensitization in early reports. However, despite acceptable short-term recipient and graft outcomes, increased rates of AMR have raised concerns about the long-term success of desensitization protocols [38]. Recently, novel agents including proteasome inhibitors, complement inhibitors, IL-6 or IL-6 receptor blockers and immunoglobulin-G degrading enzyme of Streptococcus pyogenes are being tested to improve the efficacy of desensitization [39–42].

## Plasmapheresis and Intravenous Immunoglobulin

**Q: Is combined use of plasmapheresis and IVIG effective for most of the sensitized patients?**

The PP and low-dose IVIG protocol was first utilized in 1998 at Johns Hopkins Hospital in ILDKT patients [26]. The numbers of treatments (PP + IVIG) cycles were determined on the basis of baseline DSA titers. Patients received KT if the XM became negative. However, in a few cases, the strength of XM plateaued at a low level of reactivity (titer, <8) on the CDC assay, and they proceeded with KT. For desensitization, patients were treated with a mean of 4 ± 4 PP sessions before KT and 5 ± 4 sessions after KT. Patients with CDC negative and FC positive XM are usually preconditioned by receiving 2 PP sessions prior to transplant and then have an additional 2 posttransplant. Desensitization was attempted in 215 patients, and 211 underwent transplantation (98.1%). Montgomery et al. compared 8-year patient survivals in these patients [12]. In positive CDC XM group, patient survival rates were 87.7% at 1 year, 82.0% at 3 years, 78.0% at 5 years, and 78.0% at 8 years. In positive FC XM group, patient survival rates were 92.0% at 1 year, 85.5% at 3 years, 79.7% at 5 years, and 79.7% at 8 years. HLA-incompatible KT across all levels of DSA provided a significant survival benefit compared with waiting for a compatible organ or remaining on dialysis.

Riella et al. at Brigham and Women's Hospital reported long-term outcomes of 39 ILDKTs across CDC XM [43]. PP + IVIG treatments were performed before KT to achieve negative CDC XM. Following transplantation, routine PP was performed for 2 weeks with a frequency dependent on the strength of baseline XM. Twenty-five patients received rituximab. Patient survival was 95% at 1 year, 95% at 3 years, and 86% at 5 years. Death-censored graft survival was 94% at 1 year, 88% at 3 years, and 84% at 5 years. The majority of graft losses (four of six) was a result of chronic AMR. During the follow-up, 24 developed AMR after KT (61%) and 9 had T cell-mediated rejection (23%).

University of Illinois described the outcomes of ILDKT in 51 patients using similar desensitization protocol [44]. All patients undergoing the desensitization protocol were positive by T cell FCXM before starting the protocol (14 CDC, 37 FC XM positive). PP + IVIG treatments were performed before KT to achieve negative FC XM. Of them, 49 patients (86%) converted to negative XM, both by FC and CDC assay, and successfully underwent KT. Two patients were transplanted, despite persistent positive XM. Postoperatively, the recipients continued to receive PP + IVIG every other day for a week posttransplant. The use of rituximab was discontinued after higher incidence of infection was noted to occur. Mean follow-up was 23 months and 36 patients have more than 1-year follow-up. Patient survival was 95% at 1 year, 91% at 2 years. Death-censored graft survival rates at 1 and 2 years were 93% and 81%, respectively. There were 25 episodes of biopsy-proven or clinically presumed rejection in 22 patients in the first year. Of the 17 biopsy-proven episodes, 12 were AMR and 5 were T cell-mediated rejection.

University of Maryland reported its experience with 41 patients with flow XM positive and 41 matched controls [2]. Sensitized patients were desensitized using PP + IVIG before KT. After each treatment flow XM was repeated and if the median channel shift was reduced to within three standard deviations from the mean, transplant surgery was scheduled for the following day. Twenty patients remained positive flow XM after desensitization, despite reduced median channel value. Patients did not have additional PP + IVIG after KT and did not receive rituximab unless they developed AMR. Graft survival rates at 1 and 5 years were 89.9% and 69.4% for XM positive group and 97.6% and 80.6% for the controls, respectively. XM positivity after desensitization was an independent predictor of poor transplant outcomes. AMR was diagnosed in 5 patients in XM positive group within the first 10 days after

KT compared to none in the control group; two lost their grafts as a result.

## High-Dose IVIG

**Q: 1. Is high-dose IVIG alone effective as desensitization?**
**2. Which is better desensitization protocol between low-dose IVIG and plasmapheresis versus high-dose IVIG?**

Cedars-Sinai group developed high-dose IVIG therapy to desensitize sensitized patients and increase their chances of successful KT [37, 45]. In early experience, patients with positive CDC XM were desensitized using high-dose IVIG (2 g/kg) without rituximab [29]. Monthly IVIG treatments were performed to achieve negative CDC XM or acceptable XM (FC XM positive at a flow channel shift of <200). However, there were a group of patients who were noted to be CDC XM positive at the time of transplant. The 2-year graft survival was 84% in the daclizumab group and 90% in the anti-thymocyte globulin group, whereas the acute rejection rate was 36% (22% AMR) and 31% (21% AMR), respectively. Graft survival was significantly associated with XM results at time of transplant. They modified desensitization protocol by using alemtuzumab induction and added rituximab to their protocol in an attempt to decrease acute rejection rate [46]. Single dose of rituximab was given between the two pretransplant IVIG infusions. An additional dose of IVIG is administered 1 week after transplant. PP was added prior to KT in patients who had strong DSA and unacceptable XM after administration of high-dose IVIG with rituximab. The high-dose IVIG with rituximab showed excellent long-term outcomes. Of 66 sensitized patients (53 FC XM), death-censored graft survival was 87.9% and patient survival was 93.9% over a mean follow-up period of more than 5 years [47]. During the follow-up, AMR developed in 15 patients and T cell-mediated rejection occurred in five patients.

A similar desensitization protocol was used in 29 patients at Vanderbilt University. Patients received two doses of IVIG, the first dose was given 5 days before transplant and a second dose was given 7 days after transplant. A single dose of rituximab was given 1 day after transplant. Although the study was conducted in patients with relatively low level of DSA (baseline FC XM titer <1:32), 3-year patient and graft survivals were 95% and 90%. In addition, high-dose IVIG with rituximab results in early and sustained DSA removal over a 3-year posttransplant period.

Stegall et al. at Mayo Clinic compared the efficacy of single high-dose IVIG to two PP + IVIG based regimens in 61 positive CDC XM patients [28]. Thirteen patients received high-dose IVIG (group I); 32 patients received PP + IVIG with rituximab (group II); and 16 patients received PP + IVIG, rituximab, and posttransplant DSA monitoring (group III). Patients in group II and group III received additional PP + IVIG after transplant. The overall success rate of desensitization was 36% (5/13) in the high-dose IVIG group, 84% in group II (27/32) and 88% (14/16) in group III. The 8 patients unresponsive to high-dose IVIG subsequently underwent PP + IVIG with rituximab and 3 of the 8 patients (those with baseline titers 1:8, 1:16 and 1:256) achieved a negative XM. The acute AMR rate was 80% in group I and 37% and 29% in groups II and III, respectively.

Previous studies have shown that high-dose IVIG is effective when used in multiple doses [37, 45]. Comparing single dose of IVIG without rituximab to multiple PP treatments with rituximab could unfairly favor the PP-based protocol. It is therefore difficult to draw conclusions on the superiority between PP + IVIG and high-dose IVIG [48]. More importantly, patients with high titers (baseline CDC XM >1:32) do not respond well to either regimen.

## Bortezomib (Proteasome Inhibitor)

**Q: 1. Which is more effective between bortezomib and rituximab in terms of antibody response?**
**2. How about the combined use of bortezomib and rituximab?**

Bortezomib, a proteasome inhibitor, exerts an inhibitory effect directly on antibody-secreting

plasma cells. As plasma cells produce antibodies, they are the most obvious target for DSA suppression [39]. There are few studies in humans regarding desensitization and bortezomib, and long-term results are inconclusive.

Cincinnati group reported that bortezomib-based desensitization consistently and durably reduces DSA, which may allow for increased transplantability [40]. A Korean group reported similar results in deceased donor KT setting [49]. However, it remains unclear how much of the treatment effect was from bortezomib or the other therapies used (i.e., high-dose IVIG, PP, and rituximab). Recent clinical studies indicated that bortezomib monotherapy is ineffective at lowering DSA levels in sensitized patients [50, 51]. However, bortezomib was used in these patients because the baseline DSA titer was so high that it was difficult to achieve acceptable XM with the conventional desensitization protocols [36]. In addition, germinal center compensation induced by plasma cell depletion may limit bortezomib desensitization therapy [52].

## Eculizumab (C5 Inhibitor)

**Q: Is eculizumab alone effective for desensitization?**

Eculizumab is a monoclonal antibody that binds protein C5 of the complement cascade, inhibiting its cleavage to C5a and C5b and formation of membrane attack complex C5b-9. Stegall at Mayo Clinic reported significantly decreased incidence of early AMR in 26 highly sensitized recipients with a positive XM after treatment with eculizumab [53]. The incidence of AMR at 3 months was 7.7% (2/26) in the eculizumab group compared to 41.2% (21/51) in the historical control group who received similar PP based protocol without eculizumab to achieve acceptable XM. Eculizumab treatment also simplified the posttransplant management by decreasing the need posttransplant PP. However, recent update tempered the enthusiasm about the long-term benefit of eculizumab [54, 55]. Despite a marked decrease of early AMR episodes, incidence of neither transplant glomerulopathy nor subclinical microvascular inflammation was different between the eculizumab-treated and control groups at 1 and 2 years after transplant. Long-term graft survival of eculizumab group was similar to that of control group (positive XM, PP-based desensitization). Patient survival was similar among positive XM (eculizumab or XM control) and negative XM KT groups. Death-censored allograft survival rates at 5 and 7 years were 80.9% and 76.8% in the eculizumab group, 84.3% and 70.5% in the positive XM control group, and 95.9% and 91.6% in the negative XM control group. Most death-censored graft failures resulted from chronic AMR in the positive XM groups (100% of eculizumab, 85.7% of positive XM control group).

## IdeS (Immunoglobulin-G Degrading Enzyme of *Streptococcus pyogenes*)

**Q: 1. Is it possible to use IdeS as a monotherapy for desensitization?**
**2. How about the antibody rebound in IdeS?**
**3. What is the strategy to decrease antibody rebound?**

IdeS is an IgG endopeptidase that rapidly cleaves human IgG at the hinge region, producing F(ab')2 and Fc fragments [56]. It reliably converts a positive CDC XM to a negative result within 4 hours of administration. The drug seems to immediately subvert humoral immune responses and reduces the risk of hyperacute rejection in sensitized patients undergoing living or deceased donor KT with a positive XM [41, 57].

After a single dose of IdeS, DSA begins to reappear in the circulation within 7–10 days and can return to pretreatment levels by 14 days. This period of antibody elimination provides a window for immunomodulation during which the graft is quiescent, but the

subsequent rebound in DSA levels that often occurs can be associated with AMR. A phase I/II trial reported one case of hyperacute rejection resulting in graft loss and ten cases of AMR [41]. IgG endopeptidase is immunostimulatory, and inactivating anti-IdeS antibodies can appear after one or two doses, limiting the utility of subsequent posttransplant dosing to control rebound.

Ultimate goal of desensitization is to maximize the opportunity for transplant, with improved mortality and quality of life compared with that associated with dialysis. ILDKT through desensitization protocols has been shown to improve survival for some patients compared to remaining on dialysis therapy [12, 18]. Despite reports of improved short-term graft survival, long-term success remains elusive. The persistence of DSA posttransplant can result in chronic AMR that rapidly dissipates allograft function resulting in graft failure [3, 55]. Many centers have modified their desensitization protocol by using novel agents in an attempt to decrease chronic antibody-mediated injury and side effects. No current protocol is perfect for desensitization and prevention of AMR.

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