Donor-specific HLA Antibodies in Solid Organ Transplantation: Clinical Relevance and Debates

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Abstract

Early outcomes following solid organ transplantation have markedly improved in recent years. Antibody-mediated rejection caused by donor specific anti-human leukocyte antigen antibodies (HLA-DSA) is widely recognized to be a risk factor for rejection episode, graft loss and decreased graft survival. The presence of HLA-DSA before transplantation and the appearance of these antibodies after transplantation can induce a wide spectrum of allograft injuries, ranging from the absence of allograft lesions with normal biopsy histopathologies to indolent subclinical processes to acute rejection with early allograft loss. However, the interpretation of the current DSA results is not easy and has led to many discussions and controversies. Current challenges exist in identification of pathologic DSA, monitoring and diagnostic algorithms, appropriate risk stratification, minimization for preformed or de novo DSA by proper use of immunosuppression. This article summarizes recent advances concerning the impact of preformed and de novo DSA in solid organ transplantation, with a focus on the clinical significance of DSA and available treatment modalities. Areas requiring further investigation are also identified.

Introduction

Despite all advances in the development of effective immunosuppressive regimens in transplantation, graft rejection plays an increasingly critical role in graft dysfunction and eventual loss of the allograft. The contribution of both acute cellular rejection and antibody-mediated rejection (AMR) episodes has been increasingly recognized. The pathologic impact of donor-specific antibodies (DSA); alloantibodies specifically directed against donor human leukocyte antigens (HLA)) was subsequently widely recognized not only in kidney but also in heart, liver, pancreas, intestine and lung transplantation.⁵ Presence of HLA-DSA before transplantation and the marked increase over time in detection of DSA post-transplantation are significantly associated with acute or chronic graft rejection as well as active pathological findings for antibody-mediated injury.⁶,⁷ As a result, monitoring of DSA is becoming increasingly accepted as standard-of-care in transplant recipients.⁸,⁹ Effective strategies have been adopted to minimize the deleterious effects of AMR by prevention of DSA production and reasonable selection of adequate immunosuppressive regimens.

The aim of the present review is to describe the incidence of anti-HLA-DSA after solid transplantation, and to illustrate their clinical significance and pathologic correlations by exploring recent improvements and controversies. Insights into the management of patients with preformed or post-transplant de novo DSA and recommendations for future research of DSA in transplantation are also within the scope of this review.

Both preformed and de novo DSA represent a significant obstacle to transplantation

Preformed DSA before transplantation

Exposure to “non-self” HLA molecules, as after blood transfusion, pregnancy or sensitization events, can lead to the development of preformed anti-HLA antibodies.¹⁰ Transfusion avoidance or the use of HLA-matched blood may reduce this risk and improve outcomes.¹¹ Pre-existing sensitization to HLA-DSA may be a contraindication to transplantation due to the increased risk of acute rejection, delayed graft function, and decreased graft survival.¹²,¹³ In support of this observation, pre-transplant DSA significantly
increased the risk for AMR and kidney allograft failure by 76%, despite a negative flow cytometry crossmatch result.16

Musat et al.17 investigating 113 consecutive adult liver transplant recipients, found an association between pretransplant DSA and AMR (anti-class-I, hazard ratio (HR) = 2.7, p < 0.01; anti-class-II, HR = 6.0, p < 0.01). Also, another study showed that in kidney transplant recipients with negative cytotoxicity crossmatches, the presence of class II DSA at the time of transplantation, especially with high strength, was associated with an augmented risk of AMR occurrence.18 An analysis of 1,270 liver transplant recipients showed an association between pretransplant DSA intensity and recipient survival.19 Strong HLA-DSA levels at baseline were found to be associated with worse allograft outcomes even after successful desensitization.20

Although a definite clinical relevance of DSA has been found, unfavorable clinical outcomes are confirmed in some but not all DSA-positive patients. In a large retrospective study, AMR occurred in only 2% kidney recipients, who had at least one strong DSA with a mean fluorescence intensity (MFI) value greater than 6,000.21 Tamer et al.25 found that preformed DSA disappeared in 85% of liver transplant recipients after transplantation, and only persistent DSA with high MFI values were responsible for AMR, suggesting that preformed DSA are deleterious to graft survival only when they persist after transplantation.23 Thus, some researchers dispute that preformed DSA are likely problematic only when these antibodies remain positive after transplantation and above a certain threshold.24 Regardless, the occurrences of AMR, once happened, most often lead to graft loss or serious consequences. Thus, it is important to detect the presence and levels of HLA-DSA before transplantation for accurate assessment of a patient’s immunologic risk.

De novo DSA post-transplantation

The antibodies that do not preexist but develop after transplantation and are directed against foreign graft HLA are considered as de novo anti-HLA DSA. Extensive studies in the past have evaluated the prevalence of DSA after transplant and their clinical relevance in terms of risk of acute or chronic rejection. The average annual incidence of de novo DSA varies in a wide range post-transplant. Approximately 10–30% of heart transplant recipients developed de novo DSA (predominantly anti-HLA class II) after transplantation.25 Everly et al.26 reported that 11% of the patients without DSA at the time of renal transplantation would have detectable DSA 1 year later, and the incidence of de novo DSA would increase to 20% over the next 4 years. Wiebe et al.27 found that the mean time to appearance of de novo DSA was 4.6 years post-transplant in 15% of renal recipients. In a retrospective analysis of 505 patients, Willicombe et al.25 reported a rate of de novo production of DSA in 18.2% of patients, with the mean time to detection of DSA of 9.98 ± 12.48 months after kidney transplantation. Likewise, 32% of previously non-sensitized kidney recipients developed de novo DSA as reported in the study by Gingu et al.29

Once de novo DSA appears, the probability of graft loss within 3 years in primary kidney transplantation is 24%.26 The independent risk factors that have been identified to develop de novo DSA have included female sex of the recipient, young age of the recipient, viral infection (especially cytomegalovirus and Epstein-Barr virus), class II HLA mismatching, prior cellular rejection, sensitizing events (blood transfusion, retransplantation, pregnancy, etc.) and non-adherence to immunosuppressant medication.25,26,28 Also, nephrectomy is considered as a factor that facilitates production of DSA; while tacrolimus concentration ≥3 ng/mL is protective against the development of allosensitization and can facilitate retransplantation.38

Development of de novo DSA post-transplant has been reported to be associated with AMR, increased risk of graft loss and poor transplant outcomes.36-37 A recent study showed that the production of de novo DSA after liver transplantation was an independent risk predictor (HR = 1.85, p = 0.01) of graft loss in a multivariable model.38 Piazza et al.39 prospectively screened 120 non-presensitized kidney transplant recipients, and 24.2% developed DSA at 1 year following transplantation. Patients with positive HLA-DSA had a higher incidence of acute rejection episodes (62% vs. 13%, p < 0.001), greater probability of allograft failure (34% vs. 1%, p < 0.001), and higher serum creatinine levels (2.5 ± 1.3 vs. 1.7 ± 0.5 mg/dL, p = 0.04) at 2 years after transplantation compared with those patients without DSA. O’Leary et al.40 reported that, in 749 liver transplant recipients without any preformed DSA, the formation of de novo DSA led to a higher risk of death (HR = 1.8, p = 0.007). Support for these findings came from a study by Wiebe et al.,27 which showed that patients who developed de novo DSA were twice as likely to have clinical rejection within the first 6 months post-kidney transplant, which in turn preceded de novo DSA development.

A recent study of 315 low-risk renal transplant recipients demonstrated that appearance of de novo DSA was the strongest predictor of graft loss (odds ratio (OR) = 6.4, p < 0.01), resulting in a 40% decrease in 10-year graft survival.27 For heart transplantation, circulating class II DSA after transplantation increased the risk of future pathologic AMR (HR = 2.97).40 Also, heart transplant recipients with de novo DSA had a three-fold increased risk of mortality.41 Clinical manifestations vary among organs and include acute/chronic graft dysfunction arising from microvascular injury and/or progressive tissue fibrosis. Biopsies, even in stable grafts with DSA, reveal microvasculature injury.42 De novo DSA dramatically accelerate post-transplant progression of arteriosclerosis, roughly three times faster, in DSA-positive patients compared with DSA-negative patients.43 DSA have been reported to play a role in inflammation and fibrosis formation.44-46 Miyagawa-Hayashino et al.46 evaluated 79 pediatric liver transplant recipients with protocol liver biopsies and detected DSA in 32 individuals (48%); these patients had a higher frequency of bridging fibrosis or cirrhosis (88%) than the DSA-negative patients (17%) did.

It seems that the earlier de novo DSA appear, the worse the outcome is. Kasahara et al.48 demonstrated that once liver transplant recipients developed DSA detected by flow cytometry crossmatch within the first month after transplant, 100% experienced acute rejection episodes, as compared to an incidence of only 17.4% in DSA-negative recipients. It is reported that antibodies which are developed within a year after kidney transplantation can result in graft failure in a mean time of 5.1 years. In contrast, antibodies, which are formatted after the first year, are associated with a slow rate of failure, and 80% of patients have functional grafts one decade after transplantation.51 It is possible that antibodies formed within the first year react rapidly on the endothelium, initiating a cascade of events that lead to rejection. In the light of these observations, protocol biopsies are helpful to facilitate an early diagnosis for chronic AMR, at least in patients who develop DSA within the first post-transplant year. Interestingly, the presence of de novo DSA within the first post-transplant year but cessation of their production in the subsequent years predicts a good prognosis regarding long-term allograft function and survival.43

Taken together, surveillance for preformed and de novo DSA may predict graft dysfunction and clinical outcomes even in those low immunologic risk patients. From this point, it seems that all
patients need to be monitored for DSA to identify the time, interval of DSA onset, DSA strength or clearance.52

Mechanisms by which DSA induce graft damage

The main mechanisms by which DSA mediate graft damage include:53–58; (1) complement activation via the classical pathway and the resultant formation of the membrane attack complex, as evidenced by the presence of C1q, C4d and C3d at the site of complement activation59; (2) direct and indirect damage to vascular endothelium through their interactions with HLA and/or non-HLA antigens expressed on cell surface; and (3) activation of proinflammatory cells such as natural killer (NK) cells, macrophages and neutrophils, which are involved in inducing injury of vascular endothelium (Fig. 1).60,61

The classical complement pathway is activated by binding of the globular domains of C1q with immunoglobulin (Ig)G or IgM bound to antigen epitopes on the graft endothelium.62 Once activated by C1q, the classical complement cascade leads to the generation of the key effector molecules of the complement system, the terminal membrane attack complex which causes cell lysis. As complement activation initiated by C1q crosslinking of IgG bound to the allograft, it is hypothesized that DSA with high capability of binding C1q may confer the highest risk of graft injury.

Yabu et al.63 were the first to report the clinical significance of C1q-positive de novo DSA in adult kidney transplant recipients. They found that DSA testing with C1q assay had higher levels of specificity for transplant glomerulopathy and graft loss than testing with the standard IgG DSA assay. The appearance of C1q-positive DSA was associated with substantially worse pathology and clinical outcomes in comparison with C1q-negative DSA.64,65 In a study of 1,016 renal transplant recipients, patients with C1q-positive DSA had the poorest 5-year graft survival after transplantation (54%), as compared with patients with non-C1q-positive DSA and patients without DSA (93% and 94%, respectively).66 Also, patients who developed de novo C1q-binding DSA after transplantation had the highest risk of graft loss.67 A study has shown that circulating C1q DSA are a strong and independent predictor of response to treatment in kidney recipients with AMR.68 Thus, patients with strong DSA and C1q-positive DSA are considered at greatest risk of graft loss and should be closely monitored for DSA persistence after transplantation.68

C4d is an important component of the complement cascade, and thus is considered as a marker of complement regulation. Detection of DSA together with typical C4d deposition along renal peritubular capillaries was thought of as the gold standard technique to detect complement activation.69 The presence of DSA in serum samples obtained at the time of for-cause biopsies is strongly correlated with pathological findings, including microvascular inflammation, intragraft C4d staining, transplant glomerulopathy, interstitial inflammation and tubulitis.70 Thus, appearance of DSA, C4d deposit, and microvascular injury may represent great risk for hemodynamic instability, graft dysfunction, and ultimately graft failure.

Despite the widespread use of C4d staining in the clinical management of transplant recipients, over time it has been realized that C4d is neither completely specific nor sufficiently sensitive for the diagnosis of AMR.71 C4d-negative kidneys may share features of antibody-mediated injury. A study showed that, of the biopsies, 55% of C4d-negative biopsies had evidence of concomitant microvascular injury.72 Data have revealed a high endothelial-specific gene expression in biopsies from kidney transplant recipients with DSA but who are negative for C4d, indicating ongoing antibody-mediated injury even without impact of C4d activation.73 Despite C4d staining indeed a useful tool in identifying patients at great risk of unfavorable clinical outcomes, it alone may not be sensitive enough to establish a diagnosis of acute AMR.

In addition to complement-dependent mechanisms, experimental evidence has demonstrated that antibody-mediated damage against the graft may occur in the absence of complements through antibody-dependent cell cytotoxicity and/or direct activation of endothelial cells.74–76 Antibody-dependent cell cytotoxicity involving γδ T cells or NK cells contributes to DSA-mediated graft injury.77 It is known that inflammatory events may lead to increased interferon levels, which induce an up-regulation of MHC expression on endothelial cells and stimulate B-cells and plasma cells to produce DSA. DSA are capable of inducing injury of vascular endothelium through recruitment of neutrophils, macrophages, or NK cells. Circulating DSA, even at a low concentration or undetectable by the single-antigen bead (SAB) assay, might be retained on the graft endothelium and stimulate NK cells via CD16.78

Activated endothelial cells, through production of cytokines and chemokines like interleukin (IL)-1β, IL-8, and monocyte chemotactic protein-1 (commonly known as MCP-1) to recruit neutrophils and monocytes to the graft site, initiate and promote graft rejection together with their interactions with platelet and mononuclear cells. Interestingly, CD4+CD25high cells (Tregs) display a prote-
tive role against DSA development during the first post-transplant year in kidney transplant recipients. Alberu et al. prospectively studied a cohort of unsensitized renal transplant recipients before transplant and quantified the numbers of peripheral blood Tregs at different time points during the first post-transplant year. The temporal relationship was observed between peripheral Treg number and production of de novo DSA. DSA-negative patients with better outcomes had significantly higher numbers of Tregs at 12 months after kidney transplantation, reflecting better immune acceptance of the graft. Other experimental data has also shown that a small amount of rapamycin could synergize with alloantigen to activate Tregs, permitting the achievement of specific clinical transplantation tolerance and long-term heart graft survival in an immunocompetent mouse. A further understanding of the underlying mechanisms of DSA in AMR episodes is, thus, of key importance for improving risk stratification and therapeutic strategies.

Detection of DSA with novel techniques

Patel and Terasaki published their landmark study in 1969, which revealed the detrimental effect of preformed DSA on short-term allograft survival, as detected by complement-dependent cytotoxicity (CDC) crossmatch with a limited sensitivity. Since that time, more sensitive solid-phase assays based on enzyme-linked immunosorbent assay (commonly known as ELISA), flow cytometry and Luminex® platforms have been introduced for detection and specification of DSA. Flow cytometric crossmatch (referred to FCXM) is a cell-based assay for detecting antibodies that bind to the surface of donor lymphocytes, and this technique is more sensitive than CDC. It is sensitive but cannot differentiate complement fixing antibodies from non-complement fixing antibodies. SAB technology enables the identification of antibodies that bind to individual HLA allelic variants with high sensitivity and specificity. But this method is less predictive of transplant outcome and less clinically relevant, since it detects both complement-binding (often regarded as pathogenic antibodies) and non-complement fixing anti-HLA antibodies. The reported MFI values determining the border between positive and negative reactions differs between centers and amongst studies. It has been indicated that transplantation with a threshold of MFI of 750 or less is of the greatest benefit and cost-saving in those without preformed DSA. Increasing the threshold to an MFI of 1,000–5,000 may provide an acceptable balance for improving transplant eligibility of the listed patients but incurs increased risk of acute rejection and long-term graft failure, even if the CDC crossmatch is negative. In view of the different methods with their own advantages, a reasonable method of risk stratification prior to transplant has been suggested as the following sequence of immunologic testing: CDC crossmatch followed by Luminex® for DSA identification and then using flow crossmatch testing only if further stratification for risk of AMR is desired.

Specifity and strength of DSA

Specificty of DSA

The main effect of DSA ultimately depends on its specificity and strength. Class I and II DSA have their own features in timing and frequency of appearance and clinical significance. It has been reported that HLA class I DSA are produced sooner (median time to detection 6.6 months) and are associated with rapid graft loss; while, class II DSA occur later (median time to detection 12.5 months) and may be associated with chronic transplant glomerulopathy. In renal transplantation, DSA against HLA class II antigens are more common than class I and represent 95% of all de novo DSA, demonstrating the importance of HLA class II incompatibility. Patients with both class I and II DSA or even class II alone are at the greatest risk for chronic AMR, with anti-DQ de novo DSA being the predominant specificity in kidney, liver, heart and lung transplant patients. Most of the performed class I DSA (94%) change to negative at 1 year after kidney transplantation. Unlike class I DSA, preformed class II DSA with higher MFI are more commonly persistent after transplantation (77%).

O’Leary et al. reported that strong preformed class I DSA (MFI > 5,000) remained positive in only 5% of liver transplant recipients after transplantation and were not associated with rejection episodes. In contrast, strong preformed class-II DSA (MFI > 5,000) remained persistent in 23% of patients and were an independent predictor for early rejection. Most patients with preformed class I DSA alone at low or moderate levels are unlikely to have severe short- or long-term consequences in liver transplantation. The liver is capable of eliminating or neutralizing HLA antibodies, particularly class I antibodies, which may in part account for its tolerogenic properties. Thus, DSA class II-incompatible graft transplantation needs careful monitoring and should be avoided in those patients with high immunological risk. It is necessary to bring class II matching to a higher level such as epitope matching, in order to further minimize de novo DSA development. Antigen mismatches are associated with anti-HLA-DQ DSA. DQ molecules are the principal stimulators of de novo DSA in non-sensitized renal transplant recipients and these antibodies may contribute to inferior graft outcomes. Previous studies have demonstrated that donor-specific HLA-DQ antibodies were the most commonly detected type, which may be associated with unfavorable outcomes after renal and cardiac transplantation. DQ antibodies individually, particularly those expressed at higher MFIs (>13,000), have been shown to result in inferior graft outcomes, as compared with results from patients without DSA, suggesting that patients with de novo DQ-DSA development may require similar interventions as those with de novo-A, -B and -DR antibodies.

Willcombe et al. demonstrated a strong association between mismatching at the DR/DQ loci and subsequent risk for DQ-DSA development, AMR, and graft loss. To be noted, most clinical studies have focused on antibodies against class I HLA-A and -B and class II HLA-DR and -DQ rather than against class I HLA-C and class II HLA-DP. The clinical implications of anti-HLA-C and -DP antibodies in the context of transplantation are less significant because these antigens are expressed at lower levels than other HLA antigens. In fact, patients with preformed HLA-C and -DP DSA appear to be at high risk for the occurrence of AMR. Screening of these antibodies is therefore necessary, and modulation of immunosuppression is perhaps required in cases of positivity.

Strength of DSA

High-MFI has been suggested as a determinative factor for worse graft outcomes. Preformed DSA with a high MFI that persist after liver transplantation are associated with severe early rejection and graft loss. A multivariate model has shown that in comparison with no or weak DSA (MFI < 1,000), preformed class I and/or
II DSA with an MFI of 5,000 in liver transplantation are independently correlated with risk of death (HR = 1.51, p = 0.02).19 Strong HLA-DSA level (MFI > 10,000) at baseline has been reported to be predictive of an increased risk of AMR and decreased long-term allograft survival in liver transplantation.103 In a receiver operating characteristic curve analysis, the cut-off MFI value of 4,487 for class II DSA at the time of kidney transplantation predicted the occurrence of AMR with high sensitivity (100%) and specificity (87.0%).18

Findings in our center also demonstrated that DSA-positive renal transplant recipients who developed an acute rejection episode had a higher cumulative MFI value (8,118.3 ± 5,287.4; range, 1,785–14,985) than patients who did not develop an acute rejection episode (3,283.7 ± 2,601.0; range, 786–8,113; p < 0.05), suggesting that patients with de novo DSA at high strength might suffer a high risk of developing an acute rejection episode.104 A study predicting the outcome in kidney transplant recipients showed that 8-year graft survival rate decreased progressively with increasing peak HL-A-DSA level, being 82.5% in patients with MFI < 465, 78.4% with MFI 466–3,000, and 60.6% with MFI > 3,000 (p < 0.001).105 Also, patients with MFI > 6,000 had > 100-fold higher risk of AMR than patients with MFI < 465 (relative risk = 113; 95% confidence interval: 31–414).

However, controversies still exist regarding the strength of DSA and clinical significance. A few studies have found no association between the level of DSA and the occurrence of AMR.106,107 In support of this view, Amico et al.108 found that the number, class, and cumulative strength of HLA-DSA determined by SAB were not predictive for the occurrence of AMR. To be noted, since MFI is a semiquantitative way of describing the “strength” of DSA and that it can also be affected by dilutions with EDTA in the process of SAB analysis, the results of these studies on DSA strength should be generalized with caution.

Several studies have been carried out to clarify the meaning of low MFI and postoperative changes in DSA. The clinical relevance of DSA at low levels is still being debated. The presence of weakly reactive DSA of peak value has been shown to be associated with AMR and poor kidney graft survival.109 On the contrary, in another study, compared with DSA-negative patients, patients with weak pretransplant DSA (<3,000 MFI) did not have increased risk of AMR, cellular rejection, or graft loss, suggesting that preformed weak DSA in the setting of a negative FCXM confers minimal immunologic risk.109 Thus, it seems that pretransplant DSA at very low level does not necessitate desensitization therapy and should not represent a barrier to renal transplant. Findings from other studies support that under close monitoring and proper use of an immunosuppression regimen, the patients with weak DSA are allowed to be transplanted with outcomes equivalent to those without DSA.110

The cut-off MFI value of Luminex assay for predicting the development of acute AMR with acceptable sensitivity and specificity is identified to fall within a wide range of 3,000 to 10,000, according to findings from among various reports.19,108,111 These discrepancies may be partially caused by different techniques for antibody detection used by the various investigators, different methods of DSA calculation, different immunosuppression protocols, sizes and types of the patient populations, and timings and frequencies of sample detection. On the other hand, the difference in clinical impact between a single DSA and multiple DSA remains unclear. Some investigators have used the sum of MFIs but others have used single-peak MFI. Further efforts are needed to standardize these sources of variability in an attempt to resolve the challenges of clinical interpretation when DSA levels are measured.

**DSA desensitization**

The early outcomes of recipients with DSA have been satisfactory by sufficient desensitization before transplantation.112 Even a case with DSA can be transplanted safely by proper desensitization therapy. The type of treatment in the presence of DSA post-transplantation largely depends on clinical manifestations and histological presentations. In patients with acute or chronic allograft dysfunction, various protocols are used to reduce the amount of circulating DSA. The commonly used protocols include (1) removal of harmful antibodies by plasmapheresis or immunoabsorption and (2) treatment modalities such as intravenous immunoglobulin (IVIG), antithymocyte globulin, anti-CD20 antibody (i.e. rituximab), anti-C5 antibody (i.e. eculizumab), and proteasome inhibitor (i.e. bortezomib), along with calcineurin inhibitor (CNI) and/or mycophenolate mofetil.113–117 On the contrary, there is no consensus regarding the treatment of clinically stable transplant recipients with circulating de novo DSA. It is largely unknown how to treat these patients. A closer monitoring of these patients, in addition to maintaining use of immunosuppressive therapy which typically includes tacrolimus and mycophenolate mofetil, is generally suggested.

Plasmapheresis and immunoabsorption techniques have been used to remove various types of alloantibodies. However, repeated treatments or an additional inhibitor of antibody production is usually required, due to the tendency of DSA to rebound. Therapeutic plasma exchange followed by low-dose IVIG has been reported to decrease circulating DSA for pretransplant desensitization, in an attempt to increase donor availability.118,119 The treatment reduces DSA levels by 25.3% and 35.5% after three and six plasma exchange procedures, respectively.119 HLA class I DSA are removed slightly more than class II DSA, with the least reduction rate corresponding to DR-DSA.120

In maintenance regimens, early CNI withdrawal is not advisable, especially in those who have absence of depleting induction, which will then cause increased risk of de novo DSA production.121 A high variability of tacrolimus level, which reflects exposure to low immunosuppressive drug levels, is reported to be a strong risk factor for de novo DSA development.122,123 Interestingly, cyclosporine is considered to be associated with an increased rate of DSA production.38 These evidence support strict adherence to immunosuppression and use of tacrolimus rather than cyclosporine for preventing de novo DSA formation. Few data have demonstrated a consistent effect of mycophenolic acid on de novo DSA production. A low dose of mycophenolic acid may be associated with the formation of DSA, and a minimum exposure of 1.3 mg/L may prevent the formation of DSA.124

Early conversion to mammalian target of rapamycin (referred to as mTOR) inhibitor has been reported to increase the risk of developing class II DSA, especially in the presence of DQ mismatches.125 In a retrospective study performed by Kamar et al.126 a substantially increased incidence of DSA was observed in patients on a CNI-free everolimus-based immunosuppression regimen. A multivariate regression model revealed everolimus, >3 mismatches and living donor as significant risk factors for production of DSA. Thus, mTOR inhibitor monotherapy administered early after transplant is not recommended. Additionally, conversion to a CNI-free, everolimus-based regimen should be carefully implemented only in immunologically low-risk patients and closely monitoring is needed.127

Induction therapy with newer agents, like rituximab or bortezomib, may be of interest for patients at immunological risk. However, the exact benefits are unproven in adequately powered

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studies. Rituximab induction has been reported to be beneficial to desensitize or abrogate rebound antibodies in patients undergoing desensitization for AMR. Desensitization with IVIG and rituximab resulted in early and sustained DSA removal and a low incidence of acute rejection over a 3-year post-transplant period in living donor kidney transplant recipients with pretransplant DSA and a positive crossmatch. Stable lung transplant recipients with early de novo DSA that were treated with IVIG and rituximab achieved similarly good early survival, as compared with their counterparts without DSA. Bortezomib has been demonstrated to reduce DSA levels by depleting plasma cells in highly sensitized transplant candidates. In a series of living donor kidney transplants that developed de novo DSA, Evert et al. demonstrated complete DSA removal in 18 of 26 patients and a 50% reduction in DSA in an additional seven recipients treated preemptively with bortezomib, but 56% of the patients with a complete response relapsed after a median of 3.8 months. Using bortezomib in combination with plasmapheresis and rituximab, Woodle et al. recently found antibody reductions in 86% of patients, persisting up to 10 months. However, Guthoff et al. reported that bortezomib alone did not result in a sustained reduction in DSA in sensitized patients awaiting transplant. Controversy also exists over the use of alemtuzumab (i.e. Campath-1H) in risk of DSA formation. A few studies have demonstrated that alemtuzumab induction immunosuppression is associated with reduced incidence of de novo DSA formation. However, accumulating evidence shows that alemtuzumab-induced lymphocyte depletion, especially in a CNI-free immunosuppressive regimen, may result in the activation of the humoral response toward alloantigens. Thus, alemtuzumab treatment is associated with an increased risk for the development of de novo DSA and inferior graft function, with an excess risk for early AMR. Targeting the B-cell compartment by intervention of B-cell-activating factor/proliferation-inducing ligand (commonly known as BAFF/APRIL) pathway appears to be a novel strategy and has gained considerable interest. Kwun et al. demonstrated that simultaneous blockade of BAFF and APRIL using a fusion protein (commonly known as TACI-IgFc; atacicept) that might prevent early DSA production and AMR, in a depletion-induced preclinical AMR model. In addition, novel costimulatory blockers, such as T-cell-specific surface glycoprotein CD28 (commonly known as CD28) and cytotoxic T-lymphocyte protein 4 (commonly known as CTLA4-Ig; belatacept) that prevent T-cell help to B-cells may represent an important adjunct to prevent de novo DSA.

### Outlook

DSA have been proven to be associated with acute and chronic allograft rejection. Criteria for acute AMR are nearing consensus agreement, but detection of chronic AMR will likely require additional studies. Chronic AMR is a challenge for diagnosis, because the disease progresses slowly by years, with fluctuating pathology and variable clinical manifestations. Also, not all cases have detectable C4d or DSA at any particular time. So, how to specify single, peak or accumulated DSA strength in combination with detection of complement fixation using Clq, C4d, or C3d assays in the attempt to improve predictive accuracy for silent chronic AMR deserves further attention from the transplant community. Although accumulating extensive experimental and clinical evidence shows an association between circulating DSA and potential pathologic manifestations, causation largely remains unclear. HLA-DSA can induce a wide spectrum of allograft injuries, ranging from the absence of allograft lesions with normal biopsy histopathologies to indolent subclinical processes to acute AMR with early allograft loss. Considering that some patients can do quite well even after developing de novo DSA, it is unclear whether every de novo DSA has equal pathogenicity or some of these antibodies are simply acting as “innocent bystanders”. Also, how to differentiate pathogenic versus inert antibodies in different individuals remains unknown. Of course, some detrimental consequences of DSA may be subclinical or remain clinically silent over long periods of time.

It is possible that antibody class, strength, specificity, complement-binding capacity, functional properties, and antigen density and location are determinants for the ultimate clinical outcome. While some DSA+/AMR− biopsies retain normal histopathologies, they do show increased levels of rejection-associated transcripts (whole-blood gene expression), including those related to interferon, T-cell, B-cell, NK cell and macrophage function. Thus, specific prospective studies addressing immunological risk stratification are required to define the clinical benefit and cost-effectiveness of the comprehensive assessment of HLA-DSA before implementation in current clinical practice.

Long-term transplant outcomes may benefit from routine monitoring for DSA. The challenge, therefore, is to develop a cost-effective DSA monitoring algorithm. However, currently, there is no standard or consensus follow-up protocol for de novo DSA after transplantation. Ideally, all allograft recipients should be tested for DSA prior to transplantation, and positive patients should be retested on a monthly basis post-transplantation to determine persistence. To be noted, some non-HLA DSA, like, angiotensin-II type-1 receptor (commonly known as AT1R), anti-glutathione-S-transferase T1 (commonly known as GSTT1), MHC class-I related chain A (commonly known as MICA) antibodies, can also induce acute and chronic graft dysfunction, all of which deserve additional attention. Future research is required in the exploration of mechanisms to identify pathologic DSA, development of monitoring and diagnostic tools, appropriate risk stratification, and minimization for de novo DSA by proper use of immunosuppression.

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### Conflict of interest

The authors have no financial interests or any conflict of interests to disclose.

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