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Guideline

ASTCT Consensus Recommendations on Testing and Treatment of Patients with Donor-specific Anti-HLA Antibodies

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A B S T R A C T

Donor-specific anti-HLA antibodies (DSA) are an important cause of engraftment failure and may negatively impact survival outcomes of patients receiving allogeneic hematopoietic stem cell transplantation (HSCT) using an HLA-mismatched allograft. The incidence of DSA varies across studies, depending on individual factors, detection or identification methods and thresholds considered clinically relevant. Although DSA testing by multiplex bead arrays remains semiquantitative, it has been widely adopted as a standard test in most transplant centers. Additional testing to determine risk of allograft rejection may include assays with HLA antigens in natural conformation, such as flow cytometric crossmatch, and/or antibody binding assays, such as C1q testing. Patients with low level of DSA (<2,000 mean fluorescence intensity; MFI) may not require treatment, while others with very high level of DSA (>20,000 MFI) may be at very high-risk for engraftment failure despite current therapies. By contrast, in patients with moderate or high level of DSA, desensitization therapy can successfully mitigate DSA levels and improve donor cell engraftment rate, with comparable outcomes to patients without DSA. Treatment is largely empirical and multimodal, involving the removal, neutralization, and blocking of antibodies, as well as inhibition of antibody production to prevent activation of the complement cascade. Desensitization protocols are based on accumulated multicenter experience, while prospective multicenter studies remain lacking. Most patients require a full intensity protocol that includes plasma exchange, while protocols relying only on rituximab and intravenous immunoglobulin may be sufficient for patients with lower DSA levels and negative C1q and/or flow cytometric crossmatch. Monitoring DSA levels before and after HSCT could guide preemptive treatment when high levels persist after stem cell infusion. This paper aims to standardize current evidence-based practice and formulate future directions to improve upon current knowledge and advance treatment for this relatively rare, but potentially serious complication in allogeneic HSCT recipients.

1. INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) using human leukocyte antigen (HLA)-mismatched donors, particularly HLA-mismatched related (haploidentical, haplo) donors, is increasing used as standard practice worldwide. According to the Center for International Blood and Marrow Transplant Research (CIBMTR), HLA-mismatched transplants accounted for approximately 35% of all US allogeneic HSCT procedures performed in 2022, with haploidentical transplants surpassing the number of HLA-matched related donor transplants [1]. The continuous increase in number of haploidentical transplants is, at least in part, due to immediate donor availability and lower cost of donor cell acquisition, and, more importantly, improved transplant outcomes, now similar with HLA-matched donor transplantation [2-4].

Success of HLA-mismatched transplantation requires overcoming the donor-recipient HLA-barrier to mitigate risks of graft-versus-host disease (GVHD) and graft rejection, which constitute major causes of transplant-related morbidity and mortality. Several approaches have been developed, including administration of post-transplantation cyclophosphamide (PTCy) or selective α/β T-cell and B-cell depletion. However, graft failure, mediated by residual host T-cells or presence of donor-specific anti-HLA antibodies (DSA), remains a major obstacle, associated with high treatment-

related mortality (TRM) and poor survival in this setting [5-7].

Presence of preformed DSA at the time of transplantation correlates with humoral graft rejection in solid organ transplantation, and it has been increasingly recognized as a significant cause of immunologic allograft rejection after HLA-mismatched HSCT.

Over the last decade, our increased ability to detect anti-HLA antibodies in recipients' serum, distinguish their clinical significance, and desensitize patients with DSA before transplant, have decreased the incidence of DSA-induced engraftment failure and improved overall transplant outcomes. The European Blood and Marrow Transplant (EBMT) group has published guidelines for the detection and treatment of DSA during haploidentical HSCT [8]. However, emerging new evidence in this area means that updated clinical practice recommendations are needed. Therefore, the American Society of Transplantation and Cellular Therapy (ASTCT) Committee on Practice Guidelines undertook this project to formulate consensus evidence-based recommendations to address this unmet need, as well as to identify areas requiring further research.

2. EXPERT PANEL AND GRADING SYSTEM

The development of practice recommendations was approved by the ASTCT Committee on Practice Guidelines.

Transplant physicians and other subspecialty physicians, as well as scientists with experience in this field, were invited to participate in the review of published literature and provide recommendations regarding specific topics. Laboratory scientists were assigned to review the current methods of antibody detection and interpretation of results, while clinicians were tasked to review clinical data.

A standardized system for grading recommendations in evidence based guidelines was applied [9]. Studies were evaluated based on design, sample size, inclusion and exclusion criteria, laboratory methods, desensitization therapy and treatment outcomes.

The final draft was reviewed and approved by the DSA-specific expert panelists, members of the ASTCT Committee on Practice Guidelines and finally by the ASTCT Executive Committee.

3. LITERATURE SEARCH METHODOLOGY

The approach was adapted from the search methodology used for previous ASTCT evidence-based reviews. PubMed search terms included "donor-specific anti-HLA antibodies" AND "hematopoietic stem cell transplantation" The Advance search terms were "donor-specific" [tiab] AND "anti-HLA" [tiab] AND ("antibody" [tiab] OR "antibodies" [MeSH Terms] OR "antibodies" [tiab]) AND ("haematopoietic stem cell transplantation" [tiab] OR "hematopoietic stem cell transplantation" [MeSH Terms] OR ("hematopoietic" [tiab] AND "stem" [tiab] AND "cell" [tiab] AND "transplantation" [tiab]) OR "hematopoietic stem cell transplantation" [tiab]) NOT (kidney [ti] OR renal [ti] OR liver [ti] OR lung [ti] OR heart [ti] OR solid [ti] OR organ [ti]) AND English [lang].

The initial search identified 62 articles. Case reports and review articles were excluded. Moreover, we assessed articles beyond the search in accordance with laboratory methods to detect anti-HLA antibodies or DSA, non-HLA antibodies associated with stem cell transplantation and the advice of expert reviewers. As such, a total of 83 articles published between 2009 to 2023 were selected for this evidence-based review.

4. INCIDENCE AND RISK FACTORS FOR DEVELOPING DSA IN ALLOGENEIC HSCT WITH DIFFERENT DONOR TYPES

Exposure to non-self HLA antigens, through transfusion of multiple cellular blood products or intrauterine, has been known to trigger the developement of anti-HLA antibodies [6,10,11]. This phenomenon may be more relevant in haploidentical HSCT, particularly multiparous female recipients, as they are more likely to be allosensitized against their offspring's HLA antigens. The incidence of anti-HLA antibodies and DSA varies across studies mainly due to different donor types included as well as the lack of standardization of laboratory methodology and cutoff variations used to define clinically significant DSA.

4.1. Haploidentical donor transplantation

Incidence of DSA reported in haploidentical HSCT varies significantly, depending on the included proportion of high-risk scenarios and positive cutoff DSA levels. Using the mean fluorescence intensity (MFI) of 500, the reported incidence of anti-HLA antibodies and DSA ranged from 20-25% and 11-18%, respectively [10,12-15]. Other studies reported an incidence of anti-HLA antibodies and DSA ranging between 20-70% and 10-30%, respectively, when using MFI of $>1{,}000$[16-20]. Interestingly, up to 77% of children transplanted for non-malignant diseases were reported to have anit-HLA antibodies, among whom 27% had DSA [17,19]. For higer MFI levels of $>2{,}000$ or $>5{,}000$, the incidence of anti-HLA

antibodies was approximately 20%, and 4-14% were antibodies directed against the donor HLAs [5,21,22].

4.2. Unrelated donor and cord blood transplantation

For patients receiving unrelated donor transplantation, HLA-matching at least for HLA-A, -B, -C, and -DRB1 is preferred. However, recipients could potentially develop DSA against mismatches HLA-DQB1 and HLA-DPB1, which has been shown to significantly impact allograft outcome. [6] Approximately 20-40% of recipients of unrelated donor transplantation were found to have anti-HLA antibodies, while the incidence of DSA in this population ranged from 1-9% [6,23-25]. In cord blood transplantation (CBT), the reported incidence of anti-HLA antibodies was 20-25% with 3-7% of these recipients having DSA [7,26-29]. A summary of published studies reporting on the incidences of anti-HLA antibodies and DSA is presented in Table 1.

4.3. Risk factors for developing anti-HLA antibodies and DSA

Retrospective studies have shown a higher incidence of anti-HLA antibodies and DSA among female compared with male recipients [5,6,10,13,16,20,24,30]. Moreover, higher anti-HLA antibody levels have been noted in parous compared with nulliparous females, as pregnancy represents a strong risk factor for developing anti-HLA antibodies. This risk increases in the setting of child donor to mother recipient HSCT, where the mother could have been alloimmunized during pregnancy against her child donor's mismatched HLA, and the risk could be amplified by a higher number of pregnancies [6,10,11,16,20,31]. Moreover, female recipients were observed to have higher DSA levels compared to male recipients [13]. Two studies have shown that middle-age and older patients were more likely to have detectable anti-HLA antibodies compared with pediatric or younger patients [10,24]. This could be related to a higher life-time non-self antigen exposure among older recipients.

Exposure to foreign HLA antigens from transfusions of cellular blood products was also reported to increase risk of HLA alloimmunization, particularly in those receiving platelet and leukocyte containing products [6,12,32,33]. However, mature red blood cells express HLA class I at low level, as well as Bennett-Goodspeed antigens, HLA-identical antigens. This expression could potentially

Table 1
Incidence of anti-HLA antibodies and DSA in allogeneic hematopoietic stem cell transplantation

Study	Donor type	*N*	MFI cutoff	Incidence of anti-HLA antibodies	Incidence of DSA
Ciurea, et al. 2015 [13]	Haplo	122	> 500	NA	18%
Chang, et al. 2015 [14]	Haplo	345	> 500	25.2%	11.3%
Zhang, et al. 2020 [15]	Haplo	78	> 500	NA	11.5%
Ma, et al. 2022 [10]	Haplo	3,805	> 500	20.2%	NA
Gladstone, et al. 2013 [20]	Haplo	296	> 1,000	23%	14.5%
Bramanti, et al. 2019 [16]	Haplo	135	> 1,000	29.6%	14.1%
Lima, et al. 2021 [17]	Haplo	22	> 1,000	77.3%	27.3%
Carter, et al. 2022 [18]	Haplo	208	> 1,000	32.7%	11.1%
Lima, et al. 2022 [19]	Haplo	59	> 1,000	61%	18.6%
Liu, et al. 2023 [21]	Haplo	865	> 2,000	NA	3.8%
Yoshihara, et al. 2012 [5]	Haplo	79	> 5,000	20.2%	13.9%
Zhu, et al. 2023 [22]	Haplo	181	> 5,000	NA	14.3%
Ciurea, et al. 2011 [6]	UD	516	>500	19%	1.4%
Pan, et al. 2016 [24]	UD	123	>500	37.4%	6.5%
Lima, et al. 2023 [25]	UD	303	> 1,000	38.6%	3.6%
Spellman, et al. 2010 [23]	UD	115	>2,000	37%	8.7%
Takanashi, et al. 2010 [7]	CB	386	> 1,000	23.1%	5.2%
Ruggeri, et al. 2013 [26]	CB	294	> 1,000	21%	4.8%
Fuji, et al. 2020 [27]	CB	343	> 1,000	NA	7.3%
Jo, et al. 2023 [28]	CB	567	> 1,000	25.2%	3.5%

Abbreviation: CB: cord blood, DSA: donor-specific anti-HLA antibodies, Haplo: haploidentical donor, MFI: mean fluorescent intensity, NA: not available, UD: unrelated donor

cause HLA alloimmunization, especially in patients who received repeated red blood cell transfusions, as shown in a study of pediatric patients with sickle cell disease receiving allogeneic HSCT [34].

4.4. Summary

- *Incidence of DSA depends on type of transplant donor and MFI cutoff used, and ranges between 3 to 30% in haploidentical, 1 to 10% in unrelated, and 3 to 7% in cord blood transplantation.*
- *Risk factors for developing DSA include female sex, older age, multiparity and prior receipt of cellular blood product transfusions. (Levels of evidence 2+)*

5. IMPACT OF DSA ON TRANSPLANT OUTCOMES

5.1. Primary engraftment failure

The association of DSA with primary graft failure has been frequently demonstrated in HLA-mismatched related and unrelated donor HSCT, as well as in CBT [5,6,12-15,17,19,22,23,25,27,35]. A multivariable analysis in 79 recipients of unmanipulated haploidentical HSCT found that pre-transplant DSA levels >5,000 MFI was significantly associated with engraftment failure. [5] A study in 122 haploidentical HSCT recipients confirmed the observation of higher graft failure rate with DSA levels above 5,000 MFI [5,13]. Likewise, DSA were significantly associated with graft failure in a retrospective study of 592 unrelated donor HSCT recipients (odds ratio 21.3, P=0.0001) [6], In CBT, pre-transplant DSA exceeding1,000 MFI was significantly associated with low incidence of neutrophil engraftment (hazard ratio [HR] 0.49, P=0.011) [27]. Two systematic reviews and meta-analyses revealed that patients with pre-transplant DSA had 6 to 7-fold higher odds of primary graft failure compared to those without DSA. [36,37].

While the association of DSA with graft failure is recapitulated in multiple studies, the underlying mechanism remains unclear. The proposed mechanisms include antibody-dependent cell-mediated cytotoxicity [38] and complement-dependent cytotoxicity [13]. In animal models, the presence of preexisting anti-donor antibodies during stem cell infusion led to rapid graft rejection, highlighting the critical role of humoral immunity in graft rejection in allosensitized recipients [38,39].

5.2. Neutrophil and platelet recovery and graft function

Without desensitization therapy, patients with pretransplant DSA, in addition to engraftment failure, may experience delayed neutrophil and platelet recovery, and poor survival, as shown in retrospective studies of haploidentical, unrelated donor, and cord blood transplantation [5,7,19,25,26]. For example, in DSA positive vs. DSA negative haploidentical HSCT recipients, the 6-month cumulative incidence of donor neutrophil engraftment was 61.9% vs. 94.4% (P=0.026) and 28.6% vs. 79.6% (P=0.035) for donor platelet engraftment, respectively [5]. Similar findings have been reported after unrelated donor HSCT [25] and CBT [7], as well as in children who underwent haploidentical HSCT for non-malignant diseases [19].

The impact of DSA on graft function remains controversial. Primary poor graft function has been defined as frequent dependence on blood and/or platelet transfusions and/or growth factor support in the absence of other explanations, such as disease relapse, drugs, or infections, assuming that donor myeloid and lymphoid chimerism are within a desirable target level. [40] One study demonstrated that pre-transplant DSA were strongly associated with primary poor graft function after unmanipulated haploidentical HSCT [14]. However, another study failed to show any association between DSA and poor graft function when PTCy was used after haploidentical HSCT [16]. The lack of a standard definition of poor graft function makes it problematic to draw a definite conclusion on the impact of DSA on graft function as many other potential factors that could contribute to post-transplant cytopenias.

5.3. Impact of DSA on survival

Survival has also been negatively impacted by high DSA levels and their function. A retrospective study of haploidentical transplant recipients has shown that a very high initial DSA levels of >20,000 MFI and C1q positivity after desensitization were significantly associated with an increased non-relapse mortality (NRM), inferior overall (OS) and progression-free survival (PFS) than patients without DSA. In this study, 2-year OS was only 21% and 0% in patients with initial DSA >20,000 MFI and those with persistent C1q positive after desensitization, respectively, compared with 50% in patients without DSA [35]. Others have reported in pediatric patients who underwent HSCT for non-malignant diseases, that patients with, compared to those without DSA,

had worse event-free survival (EFS) (36.4% vs 77.1%; P<0.001) and OS (45.5% vs. 81.2%; P 0.003) [19]. A meta-analysis of 17 studies with positive DSA cutoffs ranging from 500 to 5,000 MFI showed that patients with DSA had inferior PFS (haploindentical HSCT: HR = 4.25, P=0.004; and for CBT HR = 4.83, P = 0.004) and OS (haploidentical HSCT: HR = 3.19, P < 0.0001; CBT: HR = 1.68, P = 0.03) [37].

5.4. Changes in DSA after desensitization

Multiple groups have attempted pre-HSCT desensitization in order to mitigate the negative effects on engraftment, and improve survival of patients with DSA (discussed in section 7). Almost all approaches have combined multiple strategies, focusing on blocking and removing DSA, as well as targeting antibody producing B-cells before transplant conditioning regimen begins. Treatment of patients with DSA levels up to 20,000 MFI has been effective in achieving engraftment and improving NRM and survival [35]. Another retrospective study found no difference in neutrophil recovery, EFS and OS when desensitized children with DSA transplanted for non-malignant diseases were compared to children without DSA [19]. Subsequent studies confirmed that DSA-desensitization therapy was able to overcome DSA barriers to engraftment and reduce NRM [21,22,41]. Levels of DSA tend to gradually decrease to zero over several weeks after treatment, while patients with persistently high DSA levels after desensitization are at highest risk of primary graft failure [13].

5.5. Summary and Recommendations

- *DSA are associated with primary graft failure and delayed engraftment, leading to inferior transplant outcomes in HLA-mismatched allogeneic HSCT.*
- *Anti-HLA antibodies testing should be part of the pre-HSCT work-up, performed within 1 month before starting conditioning regimen in all candidates of HSCT using related, unrelated or cord blood grafts with mismatched HLA antigens or alleles. (Grade of recommendation: B, Levels of evidence 2++)*
- *To optimize donor cell engraftment, graft function and survival, a donor without corresponding HLA is preferred for a recipient with anti-HLA antibodies, in addition to other donor-related factors affecting transplant outcomes. (Grade of recommendation: B, Levels of evidence 2++)*
- While the optimal strategy requires further study, DSA desensitization therapy is required to promote hematopoietic engraftment and may lead to improved survival for recipients with DSA, when a suitable alternative donor without the corresponding HLA is unavailable. (Grade of recommendation: C, Levels of evidence 2+)

6. NON-DONOR SPECIFIC HLA ANTIBODIES AND NON-HLA ANTIBODIES IN TRANSPLANTATION

The impact of non-donor specific anti-HLA antibodies on transplant outcomes is limited. A retrospective study of 592 unrelated HSCT recipients reported no difference in graft failure incidence for patients with and without non-donor specific anti-HLA antibodies [6]. This lack of correlation between non-donor specific anti-HLA antibodies and transplant outcomes was further demonstrated by other groups with respect to NRM, PFS, and OS, in addition to graft failure [11]. Similarly, after CBT, neutrophil engraftment was shown to be comparable for patients with and without non-donor specific anti-HLA antibodies [26]. In contrast, a multivariable analysis from one CBT study suggested that non-donor specific anti-HLA antibodies were associated with lower rate of neutrophil recovery, without significant effect on platelet recovery [7]. The difference in the intensity of the conditioning regimens and the number of cord blood units used for transplantation might explain the disparity in results between these two studies.

In addition to anti-HLA antibodies, evidence from published studies in the solid organ transplantation suggests that non-HLA antibodies may influence antibody-mediated allograft rejection [42,43]. These antibodies include anti-major histocompatibility complex class I-related chain A (MICA) antibodies, angiotensin II type 1 receptor activating autoantibodies (AT1R-AA), and anti-endothelial cell antibodies (AECA). However, so far, the impact of such antibodies on outcomes of allogeneic transplantation remains uncertain. Pre-transplant MICA antibodies have been reported to be associated with poor platelet recovery, high NRM and inferior OS [44-46] after HSCT. Whether there is a correlation between these antibodies and risk of GVHD remains to be clarified [45-47]. Few studies reported that the levels of AT1R-AA and AECA were increased in GVHD patients, but they were not correlated with graft failure, NRM, or relapse [48-50]. Poor graft function with megakaryocyte aplasia was also reported in a patient

with donor-specific human platelet antigen 15 after CBT [51].

6.1. Summary and Recommendatios

- *The impact of non-HLA antibodies on transplant outcomes is unclear.*
- *Available data suggest no deleterious impact of non-donor directed anti-HLA antibodies.*
- *Until new evidence emerges, pre-transplant desensitization is not recommended for recipients with non-donor specific anti-HLA antibodies and non-HLA antibodies. (Grade of recommendation: C, Levels of evidence 2-)*

7. CURRENT METHODS TO DETECT ANTI-HLA ANTIBODIES AND THEIR LIMITATIONS

The two main methods to identify anti-HLA antibodies are cell-based assays and solid-phase immunoassays.

7.1. Cell-based assays

Cell-based assays can be performed by complement-dependent cytotoxicity [52] or flow cytometric crossmatch [53]. In complement-dependent cytotoxicity, selected donor lymphocytes are incubated with recipient serum. If recipient DSA are present, antibody-antigen complexes form and fix complement, leading to complement-mediated cytotoxicity, which is then quantified based on the percentage of dead cells as determined by vital stain uptake (fluorescent or non-fluorecent) [52]. The sensitivity of this method can be improved by incubation with a secondary anti-human globulin, which enhances complement activation and allows detection of non-complement fixing antibodies [54]. Disadvantages of this method include its complexity, time-intensity, limited sensitivity and specificity, and the potential for interference with intravenous immunoglobulin (IVIG) or other therapeutic antibodies [52,55]. The flow cytometric crossmatch has higher sensitivity compared to complement-mediated cytotoxicity. It involves the incubation of fluorescent dye-labelled anti-human IgG antibodies that recognize DSA bound to donor lymphocytes after mixing with recipient serum. Antibodies are identified by fluorescence signal, which correlates semi-quantitatively with the level of antibodies. This process is independent from complement binding [56], although complement-binding methods have been described. Therefore, flow cytometric crossmatch can detect both lower complement-binding and non-complement-binding antibodies [55].

7.2. Solid-phase immunoassays

Solid-phase immunoassays test antibodies in recipient serum using purified HLA molecules, which conjugate to microtiter plates or to polystyrene beads [57-60]. After antibody-antigen complex formation, the solid plates or polystyrene beads are washed to remove unbound antibodies and conjugated secondary antibodies are added [60]. The strength of anti-HLA antibodies, reported as MFI, can be quantitatively analyzed using enzyme-linked immunosorbent assays or semi-quantitively by conventional flow cytometer or a fluoroanalyzer (Luminex™) [60]. The Luminex™ beads platform has 3 formats, including "mix or screen beads" as a screening test, "panel reactive antibody or phenotype" for screening and evaluating antibody activity and "single antigen" for determining the antigen specificity with the highest sensitivity [60]. This method is semi-quantitative (via antibody titers), convenient, and has the highest sensitivity and specificity compared to the other assays. Limitations of this method include the detection of bead-bound non-HLA antibodies, false positives resulting from manufacture-related conformational changes in HLA molecules ("cryptic epitopes"), and falsely low antibody levels or false negative results due to the "inhibition effect" (sometimes inaccurately referred to as "prozone effect"), a phenomenon that involves inhibition of binding of fluorescent-dye-conjugated secondary antibodies due to high levels of HLA antibodies [60-62]. An initial serum pretreatment step has been used to potentially eliminate test inhibitors and more consistenly achieve accurate MFI levels [35].

7.3. Summary and Recommendations

- *Among the two methods to identify anti-HLA antibodies in allogeneic HSCT:*
  - *Cell-based assays are now infrequently used due to limitations in both sensitivity and specificity, and the fact that they require viable donor lymphocytes.*
  - *Solid-phase immunoassays are increasingly utilized because they can reliably identify antibodies, even with HLA-allele specificity, including HLA-DQ and -DP specific antibodies. Solid phase assays are typically semiquantitative, unless titration is performed, and are limited by cost.*
- *We recommend routinely using solid-phase assays both for screening and HLA antibody identification. Cell-based assays are considered*

complementary (see Section 6). (Grade of recommendation: C Levels of evidence 2++)

8. DSA LEVELS ASSOCIATED WITH CLINICAL SIGNIFICANCE

8.1. Haploidentical transplantation

While different studies considered various DSA MFI levels to trigger desensitization treatment, early studies reported that pre-transplant IgG DSA ≥5,000 MFI on single-antigen bead assay was a significant risk factor for engraftment failure in haploidentical HSCT [5], and some reported that graft failure events occurred only in patients with DSA >5,000 MFI at the time of HSCT [13]. Subsequent studies showed that very high pre-transplant DSA levels >20,000 MFI were very difficult to decrease to safe levels using current desensitization methods, and were significantly associated not only with engraftment failure, but also with poor platelet engraftment, higher NRM, and inferior survival [35,63], while lower level DSA appear to be less deleterious [13,35]. In one prospective study of haploidentical transplant patients, DSA >2,000 MFI correlated with primary poor graft function (HR 10.56, P=.005) and DSA >10,000 MFI with primary graft rejection (HR 71.56, P<.001) [14]. A 2,000 MFI cutoff was also proposed recently because no engraftment failures were observed when DSA were below 2,000 MFI in a recent larger case-controlled retrospective study [35]. In patients with DSA >2,000 MFI, the risk for engraftment failure appears to progressively increase with higher DSA levels, and if antibodies bind complement [13].

8.2. Unrelated donor and cord blood transplantation

It has been shown that the median DSA level was significantly higher among patients with graft failure after unrelated donor HSCT compared to those without graft failure (10,334 MFI vs. 1,250 MFI, P=0.006) [25]. Similar results were observed after CBT, with median DSA for patients with graft failure of 7,750 MFI vs. 2,474 MFI in those who achieved engraftment (P=0.004) [26]. A retrospective study showed pre-transplant DSA with MFI ≥1,000 was associated with an increased risk of graft failure after single CBT (relative risk 1.77, P=0.02) [27].

8.3. Summary and Recommendations

- *Current evidence suggests that DSA <2,000 MFI in haploidentical HSCT and <1,000 MFI in single CBT have minimal impact on graft outcome and desensitization is not required. (Levels of evidence 2+)*
- *We recommend repeated DSA testing within 2 weeks of starting conditioning regimen in transplant recipients with previous history of low level DSA (<2,000 MFI in haploidentical HSCT and <1,000 MFI in single CBT) who have high risk of increased DSA levels such as receiving multiple units of blood transfusion or granulocyte infusion. (Grade of recommendation: C, Levels of evidence 2-)*
- *Higher DSA levels are associated with poor graft function, graft failure, and worse survival. A DSA level >1,000 MFI for CBT and >2,000 MFI for haploidentical and unrelated HSCT might serve as a threshold to initiate desensitization. (Grade of recommendation: C, Levels of evidence 2+)*

9. IMPORTANCE OF ANTIBODY-BINDING ASSAYS

Widely used solid-phase IgG DSA detection/identification immunoassays cannot distinguish between complement fixing and non-complement fixing antibodies, unless IgG1-4 secondary antibodies are used. The classical complement pathway is initiated when antibodies bind with C1q, leading to cell death via the formation of membrane attack complex [64]. The Luminex–C1q assay is a single antigen bead assay that specifically identifies complement-binding antibodies via a PE-labeled anti-C1q second-step reagent [65]. Antibody-binding assays have also been developed for the C3d and C4d products of classical complement pathway activation [66,67].

Complement-binding DSA have been associated with graft rejection and graft failure, both in solid organ and stem cell transplantation [25,35,68]. In haploidentical HSCT recipients whose DSA bind C1q had higher median DSA levels (15,279 MFI) compared with patients without C1q binding (median DSA 2,471 MFI, P=0.016). In this study, patients who remained C1q positive at the time of transplant experienced primary graft failure. Conversely, patients who became C1q negative before transplant successfully engrafted (P=0.008) [13]. A subsequent study also demonstrated that the mean DSA level for patients with DSA bound C1q was significantly higher than in patients without C1q binding (19,490.7 MFI vs. 3,701 MFI, P<0.001); remaining C1q positive after desensitization was associated with lower neutrophil engraftment (subdistribution hazard ratio [SHR] 0.33, P=0.049), higher NRM (SHR 4, P=0.03), inferior PFS (HR 4.56, P=0.002), and worse OS (HR 5.82, P=0.001) [35].

In two pediatric studies, 11 patients who underwent haploidentical HSCT for nonmaligant diseases [19], and 11 patients who underwent unrelated donor HSCT for both malignant and nonmalignant diseases [25] were evaluated. Only one patient in haploidentical HSCT cohort with the highest DSA (24,000 MFI) was C1q positive [19]. In the unrelated donor cohort 3 patients tested positive for C1q, graft rejection was observed in 2 patients and primary poor graft function was observed in 1 patient [25]. In another retrospective study, 1 of 6 patients who were C3d-positive before desensitization experienced graft failure after haploidentical HSCT [69].

9.1. Summary and Recommendations

- *Complement-binding immunoassays detect anti-HLA antibodies or DSA that can activate the classical complement pathway. A positive C1q assay is associated with high levels of DSA, and signifies a higher risk of allograft failure and unfavorable transplant outcomes.*
- *Although C1q-binding correlates roughly to DSA levels (>10,000 MFI), it is unclear for intermediate levels (2,000-10,000 MFI) which antibodies bind complement and thus present a higher allograft risk. Therefore, we recommend C1q testing before and after desensitization for patients with DSA>2,000 MFI in haploidentical HSCT (or >1,000 MFI in single CBT). Alternatively, a flow cytometric crossmatch may be used. (Grade of recommendation: C, Levels of evidence 2-)*

10. CURRENT APPROACH TO TREATMENT FOR PATIENTS WITH DSA (DESENSITIZATION)

Owing to the increased understanding of how DSA influence HSCT outcomes, densitization attempts have been made to decrease antibody levels and improve engraftment. These can be classified in 5 main approaches:

1) Reducing circulating levels of antibodies using plasmapheresis or immunoadsorption,

2) Regulation of the B-cell pool and/or of antibody production by plasma cells using the directed therapy such as rituximab or proteasome inhibitors,

3) Depleting complement or adding anti-idiotype or neutralizing antibodies by IVIG,

4) Blocking DSA using donor-derived antigen from irradiated blood products in the form of a buffy coat infusion for anti-HLA antibodies class I and class II and transfusion, of platelets for patients with HLA class I DSA, and

5) Blocking the complement cascade [8].

Antibody re-equilibration can occur after plasmapheresis as only 45% of IgG distributes in the intravascular space [70], while recovery of plasma IgG levels occurs 1-2 weeks after procedure [71]. Moreover, removing a large amount of IgG may lead to a rebound phenomenon [72]. The administration of high-dose IVIG after plasmapheresis may restore total antibody levels and may help preventing antibody rebound. Therefore, most desensitization protocols usually consist of multiple approaches (IVIG, plasmapheresis, rituximab), and plasmapheresis should be followed promptly by conditioning for transplantation to eliminate the source of antibodies.

Neutralization of antibodies by administration of irradiated, DSA-corresponding HLA Ag platelets [5,15,22,41,69,73]or donor-derived buffy coat [13,35,69,74] has demonstrated efficacy in several studies. The main limitation of platelet transfusions is that it applies only to recipients with DSA against HLA class I antigens. In the largest experience reported to date with a multimodality treatment, which included alternate day plamsa exchange, rituximab and IvIg and incorporating a donor-derived irradiate buffy coat infusion in the desensitization regimen, compared with a control group of patients, Ciurea et al. showed efficacy in treating patients with up to 20,000 MFI and an increased likelihood of neutrophil engraftment with a buffy coat infusion (SHR 2.09, P=0.049), There were no significant differences in transplant outcomes, including NRM and survival compared to a control group of patients without DSA. As mentioned above, patients with DSA >20,000 MFI had a higher rate of graft failure, NRM, and worse survival [35], suggesting that additional agents and/or approaches are needed to overcome extremely high DSA levels, often against multiple HLA antigens.

Addition of immunosuppressive agents before the conditioning regimen (such as tacrolimus and mycophenolate mofetil) has also been proposed in some studies, yet efficacy of this approach remains unclear [69]. Bortezomib was evaluated prospectively in a desensitization protocol and demonstrated limited efficacy, leading to stopping the trial and needing for additional desensitization therapies [75,76]. Recently, several studies showed that a lower intensity desensitization

approach might be adequate for patients with lower levels of DSA [69,73,77,78]. A summary of desensitization methods across different studies is shown in Table 2.

10.1. Summary and Recommendations

- *Multimodality pretransplant desensitization should be used to decrease DSA* (Figure 1):
  - *For DSA up to 20,000 MFI, plasmapheresis, rituximab, IVIG and infusion of donor-derived HLA antigen (either irradiated buffy coat for the corresponding HLA class I and II or platelet transfusions for corresponding HLA class I only) are recommended.*
  - *For DSA >20,000 MFI, patients may require antibody titration (due to bead saturation), and an alternative donor without corresponding HLA should be selected, or else should be treated using an investigational approach.*
  - *For DSA 2,000-10,000 MFI in haploidentical HSCT or 1,000- 10,000 MFI in CBT, additional data are needed to determine that patients with these lower DSA levels can be treated with a lower intensity desensitization protocol, such as rituximab with IVIG.*
- *Post-desensitization DSA levels should be measured to monitor clearance; additional intervention may be needed if elevated levels persist before neutrophil recovery.*
- *A summary of most studied desensitization protocol is presented in Table 2. (Grade of recommendation: C, Levels of evidence 2+)*

11. TESTING CONSIDERATIONS FOR HIGHLY SENSITIZED PATIENTS

Patients with strong pre-transplant DSA levels >20,000 MFI have higher NRM due to engraftment failure and lower survival, despite intense desensitization therapy [35]. In clinical practice, the importance of this association can be confounded by high levels of DSA that sometimes interfere with the solid-phase immunoassay, resulting in falsely lower DSA levels, a phenomenon often called "prozone effect" [61,62]. One proposed mechanism for the "prozone effect" is an inhibition caused by high IgM and/or IgG antibody titers binding closely to the target antigen beads (hence a better term is "inhibitory effect"). As a result, C1 molecules produce steric interference, partially or completely blocking the fluorescent-dye-conjugated secondary antibodies, leading to falsely lower results [61,62]. Moreover, C1 complex formation can trigger the classical complement pathway, leading to the deposition of C3b/d and C4b fragments, which might further augment the steric interference with the secondary antibodies binding [79,80]

To correct these falsely lower results, Schnaidt et al. reported that dilution of the testing serum reduces the concentration of HLA antibodies and C1, leading to increased space between molecules of bead-bound antibodies. This resulted in no interference from C1 with the binding site of fluorescent-dye-conjugated secondary antibodies on HLA antibodies [61,62]. In addition, EDTA can overcome the false lower results by chelating calcium, which is essential for formation of the C1 complex [61,62]. Weinstock and colleagues reported that a level of DSA more than 12,000 MFI in EDTA serum had specificity for a clear inhibitory ("prozone") effect [62]. Adding EDTA to serum testing is recommended to avoid dilutional effects to low titers HLA antibodies [61].

Another proposed hypothesis is that falsely lower DSA results involve the competitive binding of IgM antibodies to the beads. This limitation can be addressed by incorporating hypotonic dialysis to separate IgM from IgG, or using dithiothreitol/heat inactivation to disrupt pentameric IgM [81,82]. However, explaining inhibitory effect through chelation of calcium with EDTA cannot be applied to IgM-related hypothesis, because IgM is not dependent on calcium [62]. Wang et al. reported that EDTA demonstrated superiority in reversing the falsely lower antibody detection compared to dithiothreitol (84% vs. 47%, $P<0.0001$) [83].

11.1. Summary and Recommendations

- *Solid-phase immunoassays have limited capability to detect very high levels of DSA due to interferences with the binding site of fluorescence-conjugated antibodies on HLA antibodies. This falsely lower anti-HLA antibody detection/identification related to as the inhibition effect (previously called "prozone effect") can be reversed by dilutions and/or by adding EDTA to the recipient serum. (Grade of recommendation: C, Levels of evidence 2-)*

12. ANTIBODY TESTING POST-TRANSPLANT

DSA monitoring after desensitization or stem cell infusion varied across different studies and currently lacks standardization. Most commonly, DSA were monitored after completing desensitization and one day before stem cell infusion (day -1) [13,16,20,84]. For example, one study monitored DSA at day -7 and day -1 to determine if there is DSA rebound during non-myeloablative

Table 2
Selected reports on pre-transplant desensitization protocols used to treat recipients with DSA, MFI cutoff levels and treatment outcomes

Study	Type of HSCT (N)	MFI cutoff for desensitization	Maximum DSA (MFI)	Desensitization protocol	Response of DSA (MFI)	Neutrophil engraftment
Yoshihara S, et al. 2012 [5]	Haplo (N=11) (5 received treatment)	> 5,000	18,474	- Varied between pts - N=1: R 375 mg/m^2 single dose + PEX D-11 - N=1: R 375 mg/m^2 single dose + PEX D-17,-15, -13 - N=2: HLA-selected PLT transfusion 40 U D-1 - N=1: B 1.3 mg/m^2 D−18,−15 + dexamethasone 20 mg D−18,−17,−15,−14	- R+PEX: rebound DSA - PLT: rapid reduction - B: moderate reduction	5/5 (100%)
Leffell MS, et al. 2015 [84]	Haplo (N=15)	> 1,000	Max DSA ~ 15,000	- PEX 1 TPV AD + IVIG 100 mg/kg + Tacrolimus 1 mg/day IV & MMF 1 g twice daily until D-1 - Start protocol 1-2 weeks prior to transplant - Number of PEX/IVIG depend on level of DSA & risk factors - One pt received B 4 doses 15.5 weeks prior to starting the desensitization protocol	- Mean reduction 64.4% - Failed: N=1	14/15 (93%)
Choe H, et al. 2019 [76]	Haplo (N=7), CBT (N=3), Haplo+CBT (N=4)	> 2,000	20,937	- B 1.3 mg/m^2 on day 1, 4, 11, 14 q 21 days for 1-2 cycles plus IVIG 1-2 g/kg per cycle - Additional treatments with IVIG, R, PEX were added to achieve engraftment	Median MFI reduced from 7,756 to 4,630 (40% reduction)	7 of 14 (50%)
Chang YJ, et al. 2020 [77]	Mismatched related (N=55)	>2,000	8,904	- R 375 mg/m^2 single dose 3 days prior to transplantation	Median DSA pre-tx - 4,791 MFI Post-tx - 0 MFI (at D +7)	52/55 (95%)
Zhang R, el al. 2020 [15]	Haplo (N=5)	> 500	14,873	- 2 U of donor apheresis PLT D-1 ± R 375 mg/m^2 single dose 1-2 weeks prior to conditioning regimen (if DSA class II positive)	Significant decrease in 5/5	5/5 (100%)
Bailén, et al. 2021 [69]	Haplo (N=19)	> 1,000	25,000	- Varied between pts; included R 375 mg/m^2, IVIG 0.4 mg/kg/day, MMF 5-10 mg/kg/bid, tacrolimus, PEX, PLT, buffy coat, steroids	Mean reduction 74%	17/19 (89%)
Ciurea S, et al. 2021 [35]	Haplo (N=37); controls (N=345)	> 1,000	32,588	- PEX 1-1.5 TPV QOD for 3 sessions, starting 1-week prior to conditioning regimen, + R 375 mg/m^2 single dose 1 day after PEX completion, + IVIG 1 g/kg 1 dose 1 day after R ± donor irradiated buffy coat on D-1	Mean pre tx DSA - 10,198, Mean DSA post-tx - 5,937 (42% reduction)	- 100% for MFI<5000 - 97% for MFI<10,000* - 95% for MFI<20,000 - 50% for MFI >20,000
Hashem H, et al. 2022 [78]	Haplo (N=8)	> 3,000	8,000	- DSA > 8000 MFI: IVIG + R + PEX - DSA 3000-8000 MFI: IVIG + R	NA	4/8 (50%)
Shen Y, et al. 2023 [41]	Haplo (N=13)	> 4,000	20,114	- IVIG 0.4 g/kg within 72 hours prior to stem cell infusion + R 375 mg/m^2 1-2 doses D-16, -9 ± HLA-selected PLT infusion 48 hours prior to stem cell infusion	DSA reduction 48.3%	13/13 (100%) NE
Zhu J, et al. 2023 [22]	Haplo (N=19)	> 5,000	Max DSA not available	- Varied between pts - N=12 IVIG 1 g/kg/day D-4,-3,-2,-1,+1 - N=5 IVIG + R 375 mg/m^2 for 2−4 doses - N=1 IVIG + R + HLA-selected PLT infusion 1-week prior to transplantation - N=1 IVIG + HLA-selected PLT 1-week prior to transplantation	NA	19/19 (100%)
Altareb M, et al. 2023 [74]	Haplo (N=5)	> 500	21,195	- PEX 1-1.5 TPV QOD for 3 sessions, starting 1-week prior to conditioning regimen, + R 375 mg/m^2 single dose 1 day after PEX completion, + IVIG 1 g/kg 1 dose 1 day after R ± donor irradiated buffy coat on D-1	Significant decreased in 3/3	5/5 (100%)
Liu L, et al. 2023 [21]	Haplo (N=33)	> 2,000	18,823	- Double filtration PEX 1.5 TPV 2 sessions AD 1 week prior to conditioning regimen + R 375 mg/m^2 single dose 1 day after completion of PEX	Mean DSA pre-tx -7,506 Mean DSA post-tx - 2,013 (73% reduction)	33/33 (100%)

* Not statistically different compared with controlsAbbreviations: N: number, AD: alternate day, B: bortezomib, CBT: cord blood transplantation, CI: cumulative incidence, D: day, DSA: donor-specific anti-HLA antibody, Haplo: haploidentical transplantation, IVIG: intravenous immunoglobulin, Max: maximum, MFI: mean fluorescence intensity, Min: minimum, NE: neutrophil engraftment; MMF: mycophenolate mofetil, MMUD: mismatched unrelated donor transplantation, NA: not available, PEX: plasma exchange, PLT: platelet, pts: patients, R: rituximab, SD: standard deviation, TPV: total plasma volume, tx: treatment, U: unit.

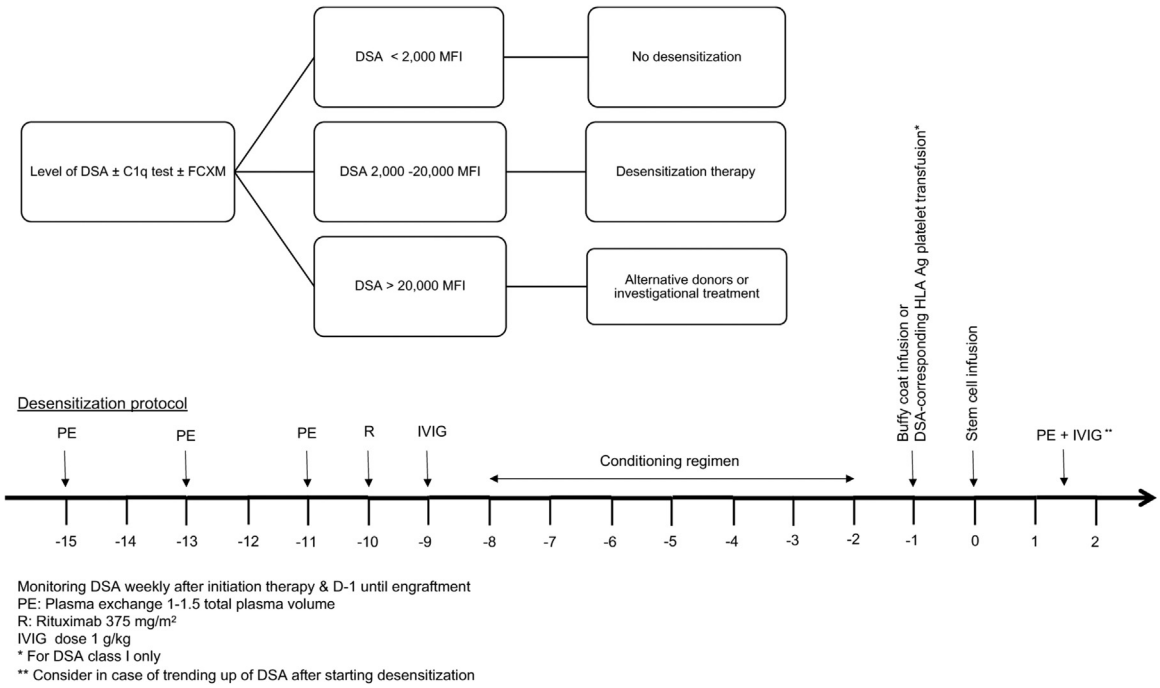

Figure 1. Multimodality pre-transplant DSA desensitization protocol.

conditioning. Two out of 15 (13.3%) patients experienced increased DSA levels with positive flow cytometric crossmatch at day -1. Patients received additional IVIG and plasmapheresis after stem cell infusion, resulting in achieved negative DSA levels. One patient had rebound DSA at day +4 after neutropenic fever, which may suggest an association with an infectious or inflammatory process [84].

At least weekly monitoring after starting desensitization or stem cell infusion for approximately one month, until antibody clearance or sucessfull engraftment, has been applied in some studies [69,75,77,84]. DSA above 1,000 MFI at day 7 was associated with primary poor graft function compared with DSA <1,000 MFI (33% vs. 0%, P = 0.040) [77]. Monthly monitoring after stem cell infusion was described in some studies, but its clinical relevance remains uncertain, because a great majority of patients will clear DSA if they do not experience engraftment failure. [15,24,41,85].

12.1. Summary and Recommendations

- *Monitoring DSA before and after desensitization, as well as after the stem cell infusion, is recommended to determine clearance of antibodies. Although frequency of DSA monitoring after stem cell infusion is unclear. We recommend testing at least weekly until engraftment and/or DSA <2,000 MFIs. Additional testing may be needed in patients with DSA with poor graft function or secondary graft failure (Grade of recommendation: C, Levels of evidence 2-)*
- *Additional desensitization may be needed in patients with persistenly high DSA or those experiencing increasing DSA levels after stem cell infusion. (Grade of recommendation: C, Levels of evidence 2-)*

13. FUTURE DIRECTIONS

Solid-phase immunoassays and C1q binding assay require 2-5 days for turnaround time, which limits timely intervention with additional therapies in highly sensitized patients or patients with rebound DSA. Highly sensitive and specific laboratory methods for detecting anti-HLA antibodies with optimal turnaround time and standardization across transplant centers would be ideal for managing recipients with DSA.

Currently, desensitization protocols vary across transplant centers with different DSA level cutoffs for initiating therapy. Most experience comes from retrospective studies with limited participant numbers. A large, prospective, multicenter study is necessary to establish a higher level of evidence and standard-of-care. Future studies will also have to evaluate individualized desensitization strategies, potentially tailored to the individual's DSA levels in order to decrease time to transplant and minimize costs. A novel method of antibody desensitization using antibody-cleaving

enzymes targeting IgG molecules has shown promise in rapidly degrading DSA in solid organ transplantations, and is currently undergoing investigation in HSCT.

DECLARATION OF COMPETING INTEREST

PK - none for this work; other COI - consulting for CareDx and reseach funding from Eurofins Viracor

PV - none declared

JZ - none declared

SS - none declared

BS - none declared

RMS - none declared

AV - none declared

JM - none for this work; other COI - honoraria from Kite, AlloVir, Bristol Myers Squibb, Novartis, CRISPR, Nektar Therapeutics, Caribou Bio, Sana Technologies, Legend Biotech and Cargo Therapeutics.

BRD - none declared

SRM – none declared

AED - none for this work; participated in advisory boards, and/or had a consultancy with and received honoraria from Celgene/BMS, Agios, Regeneron, Sobi, Novartis, Astellas, Gilead. AED served on clinical trial steering committees or DSMB for Novartis, Abbvie, Kura, Geron and Celgene/BMS.

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AB – none declared

SF – none declared

LC – none declared

JM – none declared

AR - none declared

RB - none for this work; travel & accommodation: Pfizer, Jazz Pharmaceuticals, Gilead Sciences, Sanofi. Research Funding: Jazz Pharmaceuticals. Speaker: Pfizer, Gilead Sciences.

TT – none declared

XH - none declared

CB – none declared

FA – none declared

KC – none declared

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MA – none for this work; director clinical services, Be The Match/National Marro Donor Program (NMDP), Minneapolis, MN

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AUTHORSHIP CONTRIBUTION

PK and PV wrote the first draft of the manuscript, reviewed and approved the manuscript. JZ, SS, BS, RMS, AV, JM, BRD, SRM, AED, NB, AB, SF, LC, JM, AR, RB, TT, HXJ, CB, FA, KC, PAC, MH, MA, MFV, AG reviewed, edited and approved the manuscript. SOC contributed with the concept, early draft writing, edited and approved the final version of the manuscript.

REFERENCES

1. Bolon YT, Atshan R, Allbee-Johnson M E-MN, Lee SJ. Current use and outcome of hematopoietic stem cell transplantation: CIBMTR summary slides. 2022.
2. Rashidi A, Hamadani M, Zhang MJ, et al. Outcomes of haploidentical vs matched sibling transplantation for acute myeloid leukemia in first complete remission. *Blood Adv*. 2019;3(12):1826–1836.
3. Meybodi MA, Cao W, Luznik L, et al. HLA-haploidentical vs matched-sibling hematopoietic cell transplantation: a systematic review and meta-analysis. *Blood Advances*. 2019;3(17):2581–2585.
4. Mehta RS, Marin D, Alousi A, et al. Haploidentical vs matched unrelated donors for patients with ALL: donor age matters more than donor type. *Blood Adv*. 2023;7(8):1594–1603.
5. Yoshihara S, Maruya E, Taniguchi K, et al. Risk and prevention of graft failure in patients with preexisting donor-specific HLA antibodies undergoing unmanipulated haploidentical SCT. *Bone Marrow Transplant*. 2012;47(4):508–515.
6. Ciurea SO, Thall PF, Wang X, et al. Donor-specific anti-HLA Abs and graft failure in matched unrelated donor hematopoietic stem cell transplantation. *Blood*. 2011;118(22):5957–5964.
7. Takanashi M, Atsuta Y, Fujiwara K, et al. The impact of anti-HLA antibodies on unrelated cord blood transplantations. *Blood*. 2010;116(15):2839–2846.
8. Ciurea SO, Cao K, Fernandez-Vina M, et al. The European Society for Blood and Marrow Transplantation (EBMT) Consensus Guidelines for the Detection and Treatment of Donor-specific Anti-HLA Antibodies (DSA) in Haploidentical Hematopoietic Cell Transplantation. *Bone Marrow Transplant*. 2018;53(5):521–534.
9. Harbour R, Miller J. A new system for grading recommendations in evidence based guidelines. *Bmj*. 2001;323(7308):334–336.

10. Ma N, Guo JP, Zhao XY, et al. Prevalence and risk factors of antibodies to HLA according to different cut-off values of mean fluorescence intensity in haploidentical allograft candidates: A prospective study of 3805 subjects. *Hla*. 2022;100(4):312–324.
11. Andolina JR, Walia R, Oliva J, et al. Non-donor specific anti-human leukocyte antigen (HLA) antibodies are not associated with poor outcome in hematopoietic stem cell transplant recipients. *Hum Immunol*. 2020;81(8):407–412.
12. Ciurea SO, de Lima M, Cano P, et al. High risk of graft failure in patients with anti-HLA antibodies undergoing haploidentical stem-cell transplantation. *Transplantation*. 2009;88(8):1019–1024.
13. Ciurea SO, Thall PF, Milton DR, et al. Complement-Binding Donor-Specific Anti-HLA Antibodies and Risk of Primary Graft Failure in Hematopoietic Stem Cell Transplantation. *Biol Blood Marrow Transplant*. 2015;21(8):1392–1398.
14. Chang YJ, Zhao XY, Xu LP, et al. Donor-specific anti-human leukocyte antigen antibodies were associated with primary graft failure after unmanipulated haploidentical blood and marrow transplantation: a prospective study with randomly assigned training and validation sets. *J Hematol Oncol*. 2015;8:84.
15. Zhang R, He Y, Yang D, et al. Combination treatment of rituximab and donor platelets infusion to reduce donor-specific anti-HLA antibodies for stem cells engraftment in haploidentical transplantation. *J Clin Lab Anal*. 2020;34(7):e23261.
16. Bramanti S, Calafiore V, Longhi E, et al. Donor-Specific Anti-HLA Antibodies in Haploidentical Stem Cell Transplantation with Post-Transplantation Cyclophosphamide: Risk of Graft Failure, Poor Graft Function, and Impact on Outcomes. *Biol Blood Marrow Transplant*. 2019;25(7):1395–1406.
17. Lima ACM, Bonfim C, Getz J, et al. The impact of donor-specific anti-human leukocyte antigen antibodies in salvage haploidentical hematopoietic cell transplantation with posttransplant cyclophosphamide in patients with nonmalignant disorders. *Hla*. 2021;97(6):493–504.
18. Carter M, Taniguchi M, Yang D, et al. Donor-specific HLA antibodies associate with chronic graft-versus-host disease in haploidentical hematopoietic stem cell transplantation with post-transplant cyclophosphamide. *Bone Marrow Transplant*. 2022;57(1):134–136.
19. Lima ACM, Bonfim C, Getz J, et al. Untreated Donor-Specific HLA Antibodies Are Associated With Graft Failure and Poor Survival After Haploidentical Transplantation With Post-Transplantation Cyclophosphamide in Pediatric Patients With Nonmalignant Disorders. *Transplant Cell Ther*. 2022;28(10):698.e691-698.e611.
20. Gladstone DE, Zachary AA, Fuchs EJ, et al. Partially mismatched transplantation and human leukocyte antigen donor-specific antibodies. *Biol Blood Marrow Transplant*. 2013;19(4):647–652.
21. Liu L, Ji X, Zhu P, et al. Double filtration plasmapheresis combined with rituximab for donor-specific antibody desensitization in haploidentical haematopoietic stem cell transplantation. *Br J Haematol*. 2023.
22. Zhu J, Wang Q, Liu Y, et al. High-Dose immunoglobulin Intervention as an effective and simple strategy for donor specific Anti-HLA antibody desensitization in haploidentical transplant. *Int Immunopharmacol*. 2023;120: 110299.
23. Spellman S, Bray R, Rosen-Bronson S, et al. The detection of donor-directed, HLA-specific alloantibodies in recipients of unrelated hematopoietic cell transplantation is predictive of graft failure. *Blood*. 2010;115(13):2704–2708.
24. Pan Z, Yuan X, Li Y, et al. Dynamic Detection of Anti-Human Leukocyte Antigen (HLA) Antibodies but not HLA-DP Loci Mismatches Can Predict Acute Graft-versus-Host Disease and Overall Survival in HLA 12/12-Matched Unrelated Donor Allogeneic Hematopoietic Stem Cell Transplantation for Hematological Malignancies. *Biol Blood Marrow Transplant*. 2016;22(1):86–95.
25. Lima ACM, Getz J, do Amaral GB, et al. Donor-Specific HLA Antibodies Are Associated with Graft Failure and Delayed Hematologic Recovery after Unrelated Donor Hematopoietic Cell Transplantation. *Transplant Cell Ther*. 2023;29(8):493.e491-493.e410.
26. Ruggeri A, Rocha V, Masson E, et al. Impact of donor-specific anti-HLA antibodies on graft failure and survival after reduced intensity conditioning-unrelated cord blood transplantation: a Eurocord, Société Francophone d'Histocompatibilité et d'Immunogénétique (SFHI) and Société Française de Greffe de Moelle et de Thérapie Cellulaire (SFGM-TC) analysis. *Haematologica*. 2013;98(7):1154–1160.
27. Fuji S, Oshima K, Ohashi K, et al. Impact of pretransplant donor-specific anti-HLA antibodies on cord blood transplantation on behalf of the Transplant Complications Working Group of Japan Society for Hematopoietic Cell Transplantation. *Bone Marrow Transplant*. 2020;55(4):722–728.
28. Jo T, Arai Y, Hatanaka K, et al. Adverse effect of donor-specific anti-human leukocyte antigen (HLA) antibodies directed at HLA-DP/-DQ on engraftment in cord blood transplantation. *Cytotherapy*. 2023;25(4):407–414.
29. Cutler C, Kim HT, Sun L, et al. Donor-specific anti-HLA antibodies predict outcome in double umbilical cord blood transplantation. *Blood*. 2011;118(25):6691–6697.
30. Detrait M, Dubois V, Sobh M, et al. Impact of anti-HLA antibodies on allogeneic hematopoietic stem cell transplantation outcomes after reduced-intensity conditioning regimens. *Exp Hematol*. 2012;40(10):792–799.
31. Morin-Papunen L, Tiilikainen A, Hartikainen-Sorri AL. Maternal HLA immunization during pregnancy: presence of anti HLA antibodies in half of multigravidous women. *Medical biology*. 1984;62(6):323–325.
32. Vichinsky EP, Earles A, Johnson RA, Hoag MS, Williams A, Lubin B. Alloimmunization in sickle cell anemia and transfusion of racially unmatched blood. *The New England journal of medicine*. 1990;322(23):1617–1621.
33. Ferrandiz I, Congy-Jolivet N, Del Bello A, et al. Impact of early blood transfusion after kidney transplantation on the incidence of donor-specific anti-HLA antibodies. *American journal of transplantation: official journal of the American Society of Transplantation and the American Society of Transplant Surgeons*. 2016.
34. McPherson ME, Anderson AR, Castillejo MI, et al. HLA alloimmunization is associated with RBC antibodies in multiply transfused patients with sickle cell disease. *Pediatr Blood Cancer*. 2010;54(4):552–558.
35. Ciurea SO, Al Malki MM, Kongtim P, et al. Treatment of allosensitized patients receiving allogeneic transplantation. *Blood Adv*. 2021;5(20):4031–4043.
36. Xie Y, Parekh J, Tang Z, Wu D, Wu X. Donor-Specific Antibodies and Primary Graft Failure in Allogeneic Hematopoietic Stem Cell Transplantation: A Systematic Review and Meta-Analysis. *Transplant Cell Ther*. 2021;27(8):687.e681-687.e687.
37. Huang Y, Luo C, Wu G, et al. Effects of donor-specific antibodies on engraftment and long-term survival after

allogeneic hematopoietic stem cell transplantation-A systematic review and meta-analysis. *Bone Marrow Transplant*. 2023;58(5):544–551.
38. Taylor PA, Ehrhardt MJ, Roforth MM, et al. Preformed antibody, not primed T cells, is the initial and major barrier to bone marrow engraftment in allosensitized recipients. *Blood*. 2007;109(3):1307–1315.
39. Xu H, Chilton PM, Tanner MK, et al. Humoral immunity is the dominant barrier for allogeneic bone marrow engraftment in sensitized recipients. *Blood*. 2006;108 (10):3611–3619.
40. Kharfan-Dabaja MA, Kumar A, Ayala E, et al. Standardizing Definitions of Hematopoietic Recovery, Graft Rejection, Graft Failure, Poor Graft Function, and Donor Chimerism in Allogeneic Hematopoietic Cell Transplantation: A Report on Behalf of the American Society for Transplantation and Cellular Therapy. *Transplant Cell Ther*. 2021;27(8):642–649.
41. Shen Y, Zhang L, Zhang X, et al. Treatment With the Combination of Rituximab and Intravenous γ Globulin Is Effective in Promoting Engraftment in Donor-Specific Antibody Positive Patients Receiving Myeloablative Conditioning Haploidentical Stem Cell Transplantation. *Transplant Cell Ther*. 2023;29(8):514.e511-514.e516.
42. Zhang Q, Reed EF. The importance of non-HLA antibodies in transplantation. *Nat Rev Nephrol*. 2016;12 (8):484–495.
43. O'Leary JG, Demetris AJ, Philippe A, et al. Non-HLA Antibodies Impact on C4d Staining, Stellate Cell Activation and Fibrosis in Liver Allografts. *Transplantation*. 2017;101(10):2399–2409.
44. Ansari M, Uppugunduri CR, Ferrari-Lacraz S, et al. The clinical relevance of pre-formed anti-HLA and anti-MICA antibodies after cord blood transplantation in children. *PLoS One*. 2013;8(8):e72141.
45. Fuerst D, Neuchel C, Niederwieser D, et al. Matching for the MICA-129 polymorphism is beneficial in unrelated hematopoietic stem cell transplantation. *Blood*. 2016;128(26):3169–3176.
46. Carapito R, Jung N, Kwemou M, et al. Matching for the nonconventional MHC-I MICA gene significantly reduces the incidence of acute and chronic GVHD. *Blood*. 2016;128(15):1979–1986.
47. Boukouaci W, Busson M, Peffault de Latour R, et al. MICA-129 genotype, soluble MICA, and anti-MICA antibodies as biomarkers of chronic graft-versus-host disease. *Blood*. 2009;114(25):5216–5224.
48. Chiron A, Bouaziz JD, Carmagnat M, et al. Anti-Angiotensin type 1 receptor antibodies in chronic graft-versus-host disease. *Transplantation*. 2014;98(4):470–474.
49. Vythoulkas D, Lazana I, Kroupis C, et al. Endothelial Injury Syndromes after Allogeneic Hematopoietic Stem Cell Transplantation: Angiopetin-2 as a Novel Predictor of the Outcome and the Role of Functional Autoantibodies against Angiotensin II Type 1 and Endothelin A Receptor. *International Journal of Molecular Sciences*. 2023;24(8):6960.
50. Yao J, Song A, Cao W, et al. Clinical significance of anti-endothelial cell antibody in allogeneic hematopoietic stem cell transplantation recipients with graft-versus-host disease. *Int J Hematol*. 2014;99(3):329–337.
51. Yasumi M, Yokota T, Endo T, et al. Relationship between donor-specific HPA-15 antibodies and poor graft function in HPA-15 mismatched cord blood transplantation. *Int J Hematol*. 2022;115(5):753–758.
52. Peña JR, Fitzpatrick D, Saidman SL. Complement-dependent cytotoxicity crossmatch. *Methods Mol Biol*. 2013;1034:257–283.
53. Bray RA. Lymphocyte crossmatching by flow cytometry. *Methods Mol Biol*. 2013;1034:285–296.
54. Zachary AA, Klingman L, Thorne N, Smerglia AR, Teresi GA. Variations of the lymphocytotoxicity test. An evaluation of sensitivity and specificity. *Transplantation*. 1995;60(5):498–503.
55. Altermann WW, Seliger B, Sel S, Wendt D, Schlaf G. Comparison of the established standard complement-dependent cytotoxicity and flow cytometric crossmatch assays with a novel ELISA-based HLA crossmatch procedure. *Histol Histopathol*. 2006;21(10):1115–1124.
56. Gebel HM, Bray RA, Nickerson P. Pre-transplant assessment of donor-reactive, HLA-specific antibodies in renal transplantation: contraindication vs. risk. *American journal of transplantation: official journal of the American Society of Transplantation and the American Society of Transplant Surgeons*. 2003;3(12): 1488–1500.
57. Zachary AA, Ratner LE, Graziani JA, Lucas DP, Delaney NL, Leffell MS. Characterization of HLA class I specific antibodies by ELISA using solubilized antigen targets: II. Clinical relevance. *Hum Immunol*. 2001;62(3):236–246.
58. Pei R, Lee J, Chen T, Rojo S, Terasaki PI. Flow cytometric detection of HLA antibodies using a spectrum of microbeads. *Hum Immunol*. 1999;60(12):1293–1302.
59. Pei R, Lee J-h, Shih N-J, Chen M, Terasaki PI. Single human leukocyte antigen flow cytometry beads for accurate identification of human leukocyte antigen antibody specificities. *Transplantation*. 2003;75(1): 43–49.
60. Peña JR, Saidman SL. Anti-HLA antibody testing in hematology patients. *Am J Hematol*. 2015;90(4):361–364.
61. Weinstock C, Schnaidt M. The complement-mediated prozone effect in the Luminex single-antigen bead assay and its impact on HLA antibody determination in patient sera. *Int J Immunogenet*. 2013;40(3):171–177.
62. Schnaidt M, Weinstock C, Jurisic M, Schmid-Horch B, Ender A, Wernet D. HLA antibody specification using single-antigen beads—a technical solution for the prozone effect. *Transplantation*. 2011;92(5):510–515.
63. Bailén R, Alenda R, Herruzo-Delgado B, et al. Results of haploidentical transplant in patients with donor-specific antibodies: a survey on behalf of the Spanish Group of Hematopoietic Transplant and Cell Therapy. *Front Immunol*. 2023;14: 1165759.
64. Walport MJ. Complement. *New England Journal of Medicine*. 2001;344(14):1058–1066.
65. Chen G, Sequeira F, Tyan DB. Novel C1q assay reveals a clinically relevant subset of human leukocyte antigen antibodies independent of immunoglobulin G strength on single antigen beads. *Human Immunology*. 2011;72 (10):849–858.
66. Sicard A, Ducreux S, Rabeyrin M, et al. Detection of C3d-binding donor-specific anti-HLA antibodies at diagnosis of humoral rejection predicts renal graft loss. *J Am Soc Nephrol*. 2015;26(2):457–467.
67. Mizutani K, Gotoh M. C4d binding correlated with strong HLA antibodies involved in graft failures. *Transplant Proc*. 2010;42(10):4021–4025.
68. Yabu JM, Higgins JP, Chen G, Sequeira F, Busque S, Tyan DB. C1q-fixing human leukocyte antigen antibodies are specific for predicting transplant glomerulopathy and late graft failure after kidney transplantation. *Transplantation*. 2011;91(3):342–347.
69. Bailén R, Vicario JL, Solán L, et al. Management of Donor-Specific Antibodies in Haploidentical Transplant: Multicenter Experience From the Madrid Group of

Hematopoietic Transplant. *Front Immunol*. 2021;12:674658.
70. Reverberi R, Reverberi L. Removal kinetics of therapeutic apheresis. *Blood Transfus*. 2007;5(3):164–174.
71. Orlin JB, Berkman EM. Partial Plasma Exchange Using Albumin Replacement: Removal and Recovery of Normal Plasma Constituents. *Blood*. 1980;56(6):1055–1059.
72. Reeves HM, Winters JL. The mechanisms of action of plasma exchange. *British Journal of Haematology*. 2014;164(3):342–351.
73. La Rocca U, Perrone MP, Piciocchi A, et al. Donor specific anti-HLA antibodies in hematopoietic stem cell transplantation, Single Center prospective evaluation and desensitization strategies employed. *Blood Transfus*. 2023.
74. Altareb M, Al-Awwami M, Alfraih F, et al. Incidence and significance of donor-specific antibodies in haploidentical stem cell transplantation. *Bone Marrow Transplant*. 2023;58(6):680–686.
75. Gergis U, Mayer S, Gordon B, et al. A strategy to reduce donor-specific HLA Abs before allogeneic transplantation. *Bone Marrow Transplant*. 2014;49(5):722–724.
76. Choe H, Gergis U, Hsu J, et al. Bortezomib and Immune Globulin Have Limited Effects on Donor-Specific HLA Antibodies in Haploidentical Cord Blood Stem Cell Transplantation: Detrimental Effect of Persistent Haploidentical Donor-Specific HLA Antibodies. *Biol Blood Marrow Transplant*. 2019;25(2):e60–e64.
77. Chang YJ, Xu LP, Wang Y, et al. Rituximab for desensitization during HLA-mismatched stem cell transplantation in patients with a positive donor-specific anti-HLA antibody. *Bone Marrow Transplant*. 2020;55(7):1326–1336.
78. Hashem H, Rihani R, Shanap MA, Khattab E, Tbakhi A, Sultan I. Novel conditioning regimen for upfront haploidentical hematopoietic cell transplantation in children with severe aplastic anemia and donor-specific anti-HLA antibodies. *Bone Marrow Transplantation*. 2022;57(2):304–305.
79. Schwaiger E, Wahrmann M, Bond G, Eskandary F, Böhmig GA. Complement component C3 activation: the leading cause of the prozone phenomenon affecting HLA antibody detection on single-antigen beads. *Transplantation*. 2014;97(12):1279–1285.
80. Visentin J, Vigata M, Daburon S, et al. Deciphering complement interference in anti-human leukocyte antigen antibody detection with flow beads assays. *Transplantation*. 2014;98(6):625–631.
81. Kosmoliaptsis V, Bradley JA, Peacock S, Chaudhry AN, Taylor CJ. Detection of Immunoglobulin G Human Leukocyte Antigen-Specific Alloantibodies in Renal Transplant Patients Using Single-Antigen-Beads is Compromised by the Presence of Immunoglobulin M Human Leukocyte Antigen-Specific Alloantibodies. *Transplantation*. 2009;87(6):813–820.
82. Zachary AA, Lucas DP, Detrick B, Leffell MS. Naturally occurring interference in Luminex® assays for HLA–-specific antibodies: Characteristics and resolution. *Human Immunology*. 2009;70(7):496–501.
83. Wang J, Meade JR, Brown NK, Weidner JG, Marino SR. EDTA is superior to DTT treatment for overcoming the prozone effect in HLA antibody testing. *Hla*. 2017;89(2):82–89.
84. Leffell MS, Jones RJ, Gladstone DE. Donor HLA-specific Abs: to BMT or not to BMT? *Bone Marrow Transplant*. 2015;50(6):751–758.
85. Wang L, Ji K, Chen L, et al. Posttransplant de novo DSA and NDSA affect GvHD, OS, and DFS after haplo-HSCT in patients without pre-existing HLA antibodies of hematological malignancies. *Front Immunol*. 2022;13:1047200.