Sensitization in transplantation: Assessment of Risk 2022 Working Group meeting report

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American Journal of Transplantation 23 (2023) 133–149

Contents lists available at ScienceDirect

American Journal of Transplantation

journal homepage: www.journals.elsevier.com/american-journal-of-transplantation

Meeting report

Sensitization in transplantation: Assessment of Risk 2022 Working Group Meeting Report

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ARTICLE INFO

Keywords:
clinical research/practice
translational research/science
histocompatibility
solid organ transplantation
immunobiology
alloantibody
B-cell biology
desensitization
guidelines
T-cell biology

ABSTRACT

The Sensitization in Transplantation: Assessment of Risk workgroup is a collaborative effort of the American Society of Transplantation and the American Society of Histocompatibility and Immunogenetics that aims at providing recommendations for clinical testing, highlights gaps in current knowledge, and proposes areas for further research to enhance histocompatibility testing in support of solid organ transplantation. This report provides updates on topics discussed by the previous Sensitization in Transplantation: Assessment of Risk working groups and introduces 2 areas of exploration: non–human leukocyte antigen antibodies and utilization of human leukocyte antigen antibody testing measurement to evaluate the efficacy of antibody-removal therapies.

Abbreviations: ABMR, antibody-mediated rejection; AT1R, angiotensin II type 1 receptor; cPRA, calculated panel-reactive antibody; DSA, donor-specific antibody; EC, endothelial cell; ETAR, endothelin 1 type A receptor; GWAS, genome-wide association study; HLA, human leukocyte antigen; LIMS1, senescent cell antigen-like-containing domain protein 1; MFI, mean fluorescence intensity; MML, molecular mismatch load; NK, natural killer; SAB, single antigen bead; STAR, Sensitization in Transplantation: Assessment of Risk; TCMR, T-cell–mediated rejection.

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https://doi.org/10.1016/j.ajt.2022.11.009
Received 22 August 2022; Received in revised form 20 October 2022; Accepted 2 November 2022

1. Introduction

The Sensitization in Transplantation: Assessment of Risk (STAR) workgroup was formed to align knowledge between clinicians and histocompatibility professionals. The goal was to provide recommendations for clinical testing, highlight gaps in current knowledge, and propose further research to enhance histocompatibility testing in support of solid organ transplantation. The STAR 2017 workgroup[1] provided basic definitions to clarify the interpretation of human leukocyte antigen (HLA) typing, HLA antibody testing, and determination of donor-specific antibodies (DSAs). The STAR 2019 workgroup[2] built on this foundation, updating relevant sections as well as providing a review of the requirements for assays to meet analytical validation, clinical validation, and clinical utility standards before they can be incorporated into routine clinical practice.

To address the current needs of the field, 2 new topics were introduced as part of the STAR 2022 workgroup: evaluation of tests to detect non-HLA antibodies and measurement of HLA antibody levels to assess the effectiveness of antibody-removal therapies. In keeping with previous reports, each of these new sections includes an educational component followed by a review of the literature, recommendations for use, and highlights of gaps in the field. A review of recent publications led to updated recommendations for the previously discussed topics: naïve alloimmunity, memory alloimmunity, and attributes of HLA antibodies. The latter group proceeded to develop clinical recommendations and their findings are summarized in a companion manuscript (*in-submission*).

Two interactive webinars were presented in September 2021 under the auspices of the American Society for Transplantation to allow for real-time feedback from the transplant community. The (virtual) STAR meeting took place in conjunction with the Cutting Edge of Transplantation conference, on April 5, 2022. Feedback from the audience was discussed by the workgroup and responses were incorporated into this report.

2. The role of non-HLA antibody testing in solid organ transplantation

Immune responses against non-HLA targets leading to transplant rejection and graft loss have been postulated following the rejection of kidneys from HLA identical sibling donors.[3-7] Polymorphic areas residing outside the HLA region, as well as nonpolymorphic antigens, were shown to correlate with reduced allograft survival in recent genetic studies.[8-18] The identification of these potential targets led to the development of technologies to detect non-HLA antibodies. The presence of histologic lesions resembling those associated with HLA-DSA–induced antibody-mediated rejection (ABMR) in the absence of HLA-DSAs has further fueled the exploration of non-HLA antigens as targets of immune injury. Mechanistic studies utilizing animal models and in vitro experiments explored the pathogenicity of non-HLA antibodies directed at antigens expressed on the graft's endothelium (eg, angiotensin II type 1 receptor (AT1R), endothelin 1 type A receptor (ETAR), major histocompatibility complex class I related chain A, and the C-terminal laminin-like globular domain 3 of perlecan. These studies demonstrated an ability to induce pathologic changes, and potentially complement activation,[19,20] endothelial cell (EC) activation with upregulation of adhesion molecules, and inflammatory cytokine production.[16,21-25]

2.1. Overall comments on reviewed literature

Using the PubMed search tool with key terms "non-HLA antibody and transplantation" supplemented with ad hoc searches, and eliminating reviews, case studies, and off-topic papers, we reviewed papers published between 2010 and 2022 but included some of the original papers describing targets. Out of scope for this review were ABO antibodies and single nucleotide polymorphisms. We then concentrated our review on non-HLA targets studied in >1 cohort using commercial reagents and rigorously examined the efficacy of non-HLA antibody testing to improve diagnostics and patient care in the pretransplant and posttransplant clinic settings.

The technical characteristics of the current commercially available assays are summarized in Table 1. We identified 2 types of recurring limitations. From the methodologic perspective, we observed the absence of consistency in threshold of positivity (eg, ranging between 10 and 40 U/mL for AT1R antibody); lack of comprehensive HLA typing information across all loci, and lack of pretransplant and posttransplant Luminex single antigen bead (SAB) HLA antibody assessment to monitor for the presence of preformed or de novo HLA-DSAs posttransplant. From an analytic perspective, many of the studies did not use multivariate models to adjust for confounders that may affect rejection or graft survival (recipient age, living vs deceased donors, type of maintenance and induction therapies, etc). Papers evaluating the presence and potential effects of AT1R antibodies predominate the non-HLA literature because of the wide use of the commercially available kit that assesses for this antibody. Less common is literature describing the use of other tests, such as the EC crossmatch or others.[26-32]

To establish the clinical validity, it is important to assess the temporal correlation between test results and clinical findings such that causality can be differentiated from an association. Specifically, non-HLA antibodies can be detected: (1) pretransplant, (2) posttransplant but prerejection, (3) posttransplant at the time of rejection (documented by a biopsy finding), or (4) at some random point postrejection. To demonstrate causality and to dismiss the possibility that the antibodies were formed in response to the injury, it is imperative to demonstrate that the antibodies can be detected prior to tissue pathology and the absence of HLA-DSAs. The majority of published literature is focused on analyzing at, or after, rejection time points without longitudinal specimens. We further pose that attributing synergistic effects between non-HLA and HLA-DSAs require additional studies to first verify the independent risk attributable to the non-HLA antibodies, including the need for surveillance biopsies, to show an independent contribution of non-HLA antibodies to rejection lesions and transplant outcomes.

2.2. AT1R/ETAR

Most manuscripts assessing AT1R antibodies focus on kidney transplant recipients,[13,30,33-39] with limited studies for other organs.[40-45] Generally, study cohorts included both patients with and without HLA-DSAs and were either underpowered or not designed to assess the effects of pretransplant non-HLA antibodies.[13,33,35,36] There have been several small (n = 62-101) high-quality studies[12,38-40,44,46] that performed comprehensive HLA evaluation (typing and antibody testing) to study the impact of pretransplant AT1R antibodies. These studies did not demonstrate a significant independent effect of pretransplant AT1R antibodies on allograft survival. Several studies have reported an association with acute rejection, abnormal biopsy findings,[29] and an increased risk of poor outcomes when the patient is also sensitized to HLA.[47] Posttransplant, 1 large (n = 1845) study reported an independent association of AT1R antibodies on renal allograft survival; however, this study was limited by the lack of uniform testing time points or the availability of testing prior to dysfunction in those with rejection.[33] Several studies propose a synergistic or additive effect when HLA-DSAs and non-HLA antibodies are both present, correlating with increased risk of allograft loss,[12,33] de novo DSAs, or recipient survival in a liver transplant study.[42,43] HLA and non-HLA antibodies were suggested to have distinct mechanisms of vascular activation.[48,49] Nevertheless, given the limitations highlighted above, further studies are needed to confirm whether these observations truly reflect synergy rather than a marker of more severe graft injury.

Similar limitations exist in studies of liver or thoracic transplant recipients, with the added concern of having fewer publications. The association of pretransplant AT1R antibodies with inferior outcomes is

Table 1
Technical characteristics of non-HLA antibody detection assays.

Technical characteristics	AT1R antibody	ETAR antibody	MICA antibody	XM-ONE EC crossmatch (Tie-2+ precursor EC)	EC crossmatch (primary, mature EC cell lines)	VEGF-A, VEGF-C, LEPTIN, ET-1, tubulin, collagen	Polyreactive/ natural antibodies	Non-HLA/AEC antibody panels
Platform								
ELISA	√ [a]	√				√	√ [f]	
Luminex			√ [b]					√ [h]
Flow cytometry							√ [g]	
Cell-based				√	√			
Source								
Commercially developed	√	√	√	√ [c]		√ [e]		√
Laboratory developed					√ [d]		√	√
Specificity								
Monospecific	√	√				√	√ [f]	
Multiplex			√					√
Nonspecific				√	√		√ [g]	
Analytic validity	√	√	√	√	√	√	i	i
Research tool	√	√	√	√	√	√	√	√
Identifies donor specificity			√	√				

AEC, anti-endothelial cell; AT1R, angiotensin II type 1 receptor; EC, endothelial cell; ET-1, endothelin 1; ETAR, endothelin 1 type A receptor; HLA, human leukocyte antigen; MICA, major histocompatibility complex class I related chain A; VEGF, vascular endothelial growth factor.
[a] Lot to lot variability, difficult to quality control
[b] Requires MICA typing of patient and donor for interpretation
[c] May be difficult with deceased donors, as generally fresh cells are required
[d] Primary, surrogate EC cell lines—can be used posttransplant
[e] All are commercially developed except for tubulin and collagen ELISA platforms which are laboratory developed
[f] Reactivity to malondialdehyde
[g] Reactivity to apoptotic cells
[h] Some laboratory developed Luminex assays include beads for AT1R and ETAR antibody detection
[i] Lack of standardized reagents; positive threshold associated with outcome not defined

reported in high-risk patients bridged to heart transplant with mechanical circulatory devices[40] or in the presence of HLA-DSAs.[50] Additional publications studied the role of both AT1R and ETAR antibodies in solid organ transplantation; however, their analyses varied in study design and clinical cohorts. Moreover, studies that examined the role of ETAR indicated a strong correlation between ETAR and AT1R,[29,37,41,45,51] confounding the interpretation of their independent effects.

2.3. Other assays

Additional assays to detect non-HLA antibody targets are available on the Luminex platform from commercial vendors[38,52-57] or as laboratory developed reagents.[11,12,47,56,58,59] Initial reports proposed that non-HLA antibodies are significantly associated with early T-cell–mediated rejection (TCMR),[38,52] humoral rejection,[54,55,57] and graft loss.[12,53,58] Others reported a potential synergistic nature of non-HLA antibodies with HLA-DSAs when present.[52,59] Our review identified similar limitations to those highlighted for the AT1R and ETAR publications, including variation in cohort and study design, no unified threshold values of the Luminex assay, and lack of cross-validation studies. One large (n = 874) study that included protocol biopsies and routine HLA DSA monitoring (3 months and annually thereafter) reported an independent association between pretransplant non-HLA antibody breadth and/or strength with features of ABMR.[57] Nevertheless, it remains unclear if non-HLA Luminex detection panels identify antibodies that mechanistically contribute to graft injury and rejection or arise from other graft injury/inflammation processes that are not caused by the non-HLA antibodies (including infection, ischemic injury, or de novo/recurrent disease).

2.4. Defining non-HLA antibody-mediated pathology

The pathologic effects of non-HLA antibodies on allografts are not understood. Given various non-HLA targets and variable tissue expression patterns, there is no histopathologic feature or phenotype that is specific for non-HLA antibodies alone or in combination with HLA-DSAs. Commonly described are associations with microvascular inflammation or nonspecific chronic tissue injury similar to vasculopathy, chronic lung allograft dysfunction, and tissue fibrosis, whereas complement deposition (C4d positivity) is less frequently observed.[8,33,35] Using the same histologic criteria employed to diagnose ABMR, in the presence or absence of HLA-DSAs, results in an inherent bias toward unfounded conclusions regarding the independent pathogenicity of non-HLA antibodies. Clinical studies that parse out the strength and breadth of non-HLA antibodies correlating with acute and chronic injury and distinguishing between the effects of preformed versus de novo non-HLA antibodies in primary and retransplant patients are required to classify graft pathology attributable to non-HLA antibodies as distinct from HLA-DSAs.

2.5. Clinical utility

Clinical utility suggests a reliable contribution of test results in informing the medical management of a patient. To reach clinical utility, an assay should first demonstrate clinical validity, often requiring prospective randomized trials prior to widespread adoption. There have been no trials to date that study the impact of non-HLA antibodies alone on transplantation outcomes. Notably, 1 Australian center[39] has adopted routine pretransplant testing for AT1R with intervention in positive cases (plasmapheresis, angiotensin receptor blockade, and thymoglobulin induction) limited by the presence of concurrent HLA-DSAs. In the absence of a prospective randomized clinical trial, there is currently insufficient evidence to recommend this approach as standard of care. Considering this information, and reflecting on current uncertainty, our organ-specific recommendations for non-HLA antibody testing are presented in Table 2.

2.6. Gaps and recommendations

There is a great need for further validation of high-throughput Luminex platforms with standardized quality control and proficiency

Table 2
Organ-specific recommendations for non-HLA antibody testing.

Indication	Heart/lung		Kidney			Liver
	AT1R/ETAR	MICA	AT1R/ETAR	MICA	XM one	AT1R/ETAR
Pretransplant						
First transplant	**3C**[b]	**3D**	**3C**	**3D**	**3C**	**3D**
Special circumstances or at-risk categories[a]	**EO**	**IE**	**EO**	**3D**	**2D**	**EO/2D**
Posttransplant						
Stable graft	**3C**	**3D**	**3C**	**3D**	**IE**	**3D**
Immediate/early graft dysfunction no HLA DSA	**2C**	**IE**	**EO**	**EO**	**IE**	**IE**
Rejection TCMR on biopsy HLA DSA negative	**3C**	**IE**	**3C**	**3D**	**IE**	**IE**
Rejection TCMR on biopsy HLA DSA positive	**3D**	**IE**	**3D**	**3D**	**IE**	**IE**
Rejection ABMR on biopsy HLA DSA negative	**EO**	**EO**	**2B/C**	**2D**	**IE**	**EO**
Rejection ABMR on biopsy HLA DSA positive	**EO**	**IE**	**EO**	**3D**	**IE**	**3D**
Follow-up pretransplant non-HLA testing positive	**EO**[c]		**EO**[c]			**EO**[c]

There are no sufficient studies to grade intestinal, pancreas, or multivisceral transplants.
ABMR, antibody-mediated rejection; AT1R, angiotensin II type 1 receptor; DSA, donor-specific allo-HLA antibody; ETAR, endothelin 1 type A receptor; HLA, human leukocyte antigen; MICA, major histocompatibility complex class I related chain A; TCMR, T-cell–mediated rejection; XM, crossmatch.
[a] May include VAD, repeat transplant after graft loss of unknown cause, or HLA DSA+ transplant.
[b] Assessment of strength of evidence are from the STAR 2017 Working Group Meeting: 2, Suggest; 3: do not recommend; B: moderate (Strong evidence of association or evidence of a dose response gradient); C: low (Observational Study); D: very Low (other types of studies or serious limitations to study quality); EO: there is absence of evidence and/or the working group expert opinion only was used; IE: insufficient evidence for grading
[c] When injury on biopsy is more severe or inconsistent with low strength of HLA DSA.

reagents (that are currently lacking) to better assess the role of non-HLA antibodies for clinical decision-making. Cross-validation of these platforms with well-curated study populations, including longitudinal serum and biopsy samples, is critical to understand the correlation with graft pathology in different organs and with respect to transplant immune status. Some progress has already been made toward this effort because overlapping profiles of non-HLA antibodies were identified independently from separate cohorts.[20,52] Furthermore, cross-validation of platforms has been introduced as a project of the 18th International HLA & Immunogenetics Workshop, in collaboration with the respective vendors. The discovery of additional new targets will also be important with further development and refinement of new platforms.[9,60-62] Gaps in current knowledge and recommendations for the near future are summarized in Table 3.

3. Measuring levels of HLA antibodies to assess the efficacy of antibody-removal therapy

Accurate utilization of HLA antibody testing is critical pretransplant to determine the breadth of allosensitization and to assign presence of DSA both pretransplant and posttransplant (summarized in the STAR 2017 report[1]). A brief update on HLA antibody testing is presented in Supplementary Material. Here, we focus on those circumstances where a more precise measurement of antibody strength is of special value. Those include measurement of DSA strength when evaluating patient/living-donor candidacy for pretransplant desensitization and/or measuring the efficacy of desensitization protocols; measurement of DSA strength in transplant recipients with ABMR and/or assessing the efficacy of treatment; predicting the likelihood of success in desensitization of

Table 3
Non-HLA antibodies: gaps in current knowledge and recommendations for near future studies.

Gaps:

- Positive threshold:
  - Normal vs abnormal
  - Transplant vs "healthy patients; stable vs unstable graft
  - Intervention/risk stratification/treatment
- Testing protocols and testing approaches (monospecific vs multiplex)
  - Pretransplant
  - Posttransplant
  - Treatment
- Antigen expression across tissues and longitudinally
- Synergistic effects between HLA-DSAs and non-HLA antibodies
- Correlation with pathology
- Effects of therapy

Recommendations:

- **The STAR working group recommends a continued collaboration effort with industry partners in the development of:**

1) Standardized reagents; improve quality control, threshold metrics, reproducibility, proficiency testing, and standardized reporting between laboratories and studies

- **The STAR working group highlights the need to determine clinical utility of non-HLA antibody testing:**

2) Standardized thresholds for non-HLA antibodies for risk stratification across pathologies.
3) Well-annotated cohorts of patients with and without HLA-DSAs, complete HLA DSA reporting across all loci.
4) Serial time points or serum testing and detailed surveillance and for cause biopsies.
5) Sufficient sample size for robust multivariate models to show independence of effect.
6) Correlate standardized reagent results to noninvasive biomarkers of rejection/injury (establishes further diagnostic precision/sensitivity/specificity of allograft pathology).

- **The STAR working group supports the need for continued discovery and mechanistic studies:**

7) In animal and in vitro model systems
8) Antigen expression patterns of non-HLA targets across different organ types at the time of quiescence and dysfunction/rejection

DSA, donor-specific antibody; HLA, human leukocyte antigen; STAR, Sensitization in Transplantation: Assessment of Risk.

Table 4
Advantages and disadvantages of end points used to measure desensitization efficacy.

Characteristics	Advantages	Disadvantages
Mean fluorescence intensity	• Readily available • Easy to measure	• Subject to bead saturation and interference • Difficult to interpret among patients with multiple antibodies • The relevance of "immunodominant" antibody has not been shown
Number of unacceptable antigens	• Readily available • Easy to measure	• Can be subject to some of the same limitations as MFI • May underestimate the effect of antibody change among highly sensitized patients • May overestimate the effect of a treatment among patients with low levels of antibodies that are near the positive/negative threshold • Complex data that is difficult to interpret • Does not translate into "transplantability"
Transplantation	• Clinically meaningful outcome	• Influenced by factors other than antibody reduction including donor availability, center practices, medical comorbidities, etc.
Crossmatches	• Meaningful outcome among living donor candidates with a potential donor	• Only applicable if a donor is available • Multiple different crossmatch assays

patients on the deceased-donor waitlist; and quantifying and comparing efficacy between different investigational antibody-removal modalities.

3.1. Approaches to quantify HLA antibody strength

Because of known shortcomings of SAB assays,[63,64] several laboratories elected to test samples using ≥1 dilutions when there are concerns of misinterpreting results of neat (undiluted) sera.[65,66] The utility of this approach was demonstrated in a retrospective study evaluating recipients of living-donor kidney transplantation.[67] Specifically, it was shown that the efficacy of plasmapheresis/low-dose intravenous immunoglobulin desensitization diminishes after 4 to 5 cycles, and that the success/failure of desensitization could have been predicted prior to treatment initiation. Following this report, many laboratories have adopted testing of at least one serum dilution when antibodies of interest have mean fluorescence intensity (MFI) values >12 000 to 15 000 MFI units. More recently, Timofeeva et al.[68,69] reported a successful implementation of this approach in exploring transplantation of highly sensitized heart transplant candidates and guiding ABMR therapy in lung transplant recipients.

Neat cPRA	• Useful way to simplify complex information • cPRA is related to probability of receiving an organ	• Insensitive to changes in antibody among highly sensitized patients • A change in a common antibody, such as A2 might lead to a disproportionate change in cPRA • Can be unreliable if based on neat MFI
cPRA combined with titers	• Sensitive to changes in antibody among the patients who would most likely benefit from desensitization • Useful way to combine complex antibody information • cPRA is related to probability of receiving an organ	• A change in common antibodies might lead to a disproportionate change in cPRA • Expensive and labor intensive

cPRA, calculated panel-reactive antibody; MFI, mean fluorescence intensity.

Desensitization protocols are required also for patients awaiting deceased-donor organ offers. A common observation in prior studies was the unpredictable nature of individual patients' responses and therefore the inability to choose candidates with high likelihood of success. Addressing antibody strength in a cohort of highly sensitized patients (calculated panel-reactive antibody [cPRA] = 100%), Tambur et al.[70] demonstrated that cPRA is not a sufficient metric to capture the level of sensitization. Specifically, patients with the same cPRA value (100%) showed different patterns of antibody reduction because their serum was diluted; some patients demonstrated a reduction in cPRA values (to allow 10-fold increase in compatible donor offers) following 2- to 3-fold reduction of antibody titer, whereas others would not demonstrate reduction in their cPRA values even after 1000-fold decrease in antibody titer (thus, no increase in compatible donors[70]). Consequently, understanding antibody titer, beyond the mere breadth of HLA sensitization (cPRA value), is critical to determine which patients may benefit from waitlist desensitization.

The decision of whether a full titer analysis is required or whether 2 to 3 selected dilutions are sufficient to provide clinically useful information is dependent on the context of use and the specific readout required from these tests.[63] Specific recommendations are presented in the next sections. Note, different approaches to measure both antibody concentration and affinity suggest that an antibody's affinity for an antigen may be correlated with its pathogenicity.[71,72] Yet, these studies have not been validated and are therefore not yet translatable to diagnostic testing. Overall, the STAR group reiterates the recommendations provided in the STAR 2017 report.[1]

3.2. Quantifying HLA antibody pretransplant to evaluate the efficacy of desensitization

Desensitization therapies have been studied for the last 2 decades among patients with high levels of HLA antibodies and reduced access to transplantation. Measuring the efficacy and comparing the various treatment options has been challenging because many of the studies have included heterogeneous populations with varied levels of baseline sensitization and differing access to living donors.[73] Moreover, the efficacy of desensitization has been measured in multiple ways, including the rate of transplantation, change in MFI, decrease in number of unacceptable antigens, converting a crossmatch from positive to negative, and changes in cPRA.[74-88] Some of these variables were assessed as dichotomous parameters, for example, crossmatch positive or negative rather than quantifying changes in the positivity strength. Indeed, converting a complement-dependent cytotoxicity-positive crossmatch to only flow cytometry-positive crossmatch captures some of this information, yet the nuances that are captured by this change are very limited. Moreover, although crossmatch positivity is associated with antibody strength, a crossmatch cannot assess the contribution of individual antibodies in cases in which the patient has >1 DSA, nor can it assess differential responses that are not because of HLA antibodies (especially for B-cell reactivity). Many of the previously reported studies failed to follow the recommendation of utilizing means to eliminate or at least detect inhibition in the SAB assay, a phenomenon that was reported to affect at least some antibodies in approximately three-fourths of the highly sensitized patients.[89] Furthermore, studies have reported high MFI values without applying safeguards to determine bead saturation, which is also very common among the highly sensitized patients. The validity, reliability, and applicability of these end points are dependent on the assays themselves and whether the population has access to a living donor. The specific advantages and disadvantages of each of these end points must be considered when appropriately designing a desensitization clinical trial (Table 4).

3.3. Quantifying DSA posttransplant to evaluate the response to treatment

The detection of HLA-DSAs has been central to the diagnosis of ABMR since the inception of the ABMR classification system >2 decades ago.[90]

Table 5
Advantages and disadvantages of end points to measure posttransplant antibody-removal efficacy.

Characteristics	Advantages	Disadvantages
Nonantibody end points		
Graft survival	• Clinically meaningful outcome • Easily measured and compared	• Influenced by factors other than antibody reduction
Graft function	• Clinically meaningful outcome • Easily measured and compared	• Influenced by factors other than antibody reduction
Histology	• Readily available • Shows specificity for alloantibody damage	• Too invasive for multiple repeat measures • Ongoing issues with interrater variability • Subject to sampling error • Influenced by the coexistence of non-ABMR specific damage
Antibody quantification end points		
Median fluorescence intensity	• Readily available • Easy to measure	• Subject to bead saturation and interference, which can make the results unreliable • Difficult to interpret among patients with multiple antibodies
Immunodominant DSA	• Simplifies complex information	• Subject to the same technical considerations of using MFI • Unclear whether the "immunodominant" DSA contributes to the most pathogenicity • May change with serum dilution

Yet, outside of the very early posttransplant setting, in the context of early active ABMR (<30 days posttransplant),[91] the role of detectable DSA in long-term transplant outcome remains unclear. Studies[92-97] with heterogeneous patient populations, with preformed or de novo DSAs, with varying degrees of ABMR chronicity, changes in the Banff classification system,[98] and the evolution of antibody detection techniques, as have been delineated above, have all contributed to a lack of clarity regarding the impact of ABMR treatment and DSA quantity on meaningful clinical outcomes in all types of solid organ transplantation. The complexity is further increased by reports in which patients have multiple DSAs, potentially at varying MFI values. Hoping to "simplify" DSA reporting and provide some quantification, several investigators have used measures, including the MFI sum, "immunodominant" DSA, percent change in MFI, or other elaborate scoring systems. Although the desire to simplify the data and analysis is understandable, these measures have not been validated and can lead to magnification of unreliability and irreproducibility of the HLA antibody data. The lack of standardization in reporting DSA quantification methods limits the ability to understand the natural history of alloantibodies without intervention and to compare the efficacy of therapy when used.[73,99] The timing of therapy initiation for posttransplant DSAs appears to be associated with clinical response to treatment, with significantly higher incidence of early ABMR in patients

Crossmatches	• Simplifies complex information	• Only applicable if donor cells are available • Multiple different crossmatch assays
Single antigen bead titers	• Optimal test for quantification of individual DSA specificities	• Expensive and labor intensive • Unclear how to evaluate information among patients with multiple DSA specificities

ABMR, antibody-mediated rejection; cPRA, calculated panel-reactive antibody;DSA, donor-specific antibody; MFI, mean fluorescence intensity.

with preformed DSAs but worse long-term graft survival in patients with de novo DSAs.[100] Although early treatment, at the time of DSA detection, significantly prolonged graft survival compared with no treatment,[101] these grafts ultimately deteriorated because of chronic changes. The specific advantages and disadvantages of the different end points are summarized in Table 5.

Currently, there is insufficient evidence to support the use of any approach to measure HLA antibodies/HLA DSA reduction as a reliable surrogate outcome for treatment efficacy, ABMR status, graft survival, or graft function. Moreover, a review of the literature highlights the lack of standardized approaches to report antibody characteristics of the study population, specifically when considering the known limitation of the SAB assays. These limitations are applicable both for studies of desensitization and studies of ABMR treatment with ramifications on the ability to compare results from different centers using the same therapeutic approach or compare results between studies using different therapeutic modalities.

Given the stagnation in the development of new immunosuppressive drugs and other barriers within regulatory agencies and pharmaceutical industry, we must standardize approaches to characterize and report on HLA antibodies. These minimal standards will be applicable to evaluate response to treatment and natural history among patients with preformed DSAs, de novo DSAs, and ABMR. We further emphasize the need for reporting detailed and standardized information at baseline and during follow-up time points (Table 6). Importantly, the time of DSA detection does not mean the time of DSA appearance because patients may harbor the presence of DSAs for weeks or months prior to their detection in clinical cases. Thus, it is critical to disclose the interval between the latest DSA negative test, date of DSA detection, and initiation of treatment. Although dilution studies seem to provide valuable information, it is currently performed by very few centers. Most of the centers perform only 1 or 2 dilutions, rarely exceeding a dilution of 1:100, which does not reveal saturation of the beads. A significant resistance to adopting

Table 6
Recommended standards of reporting for desensitization trials, including patients without an approved living donor.

- The STAR working group **recommends** obtaining baseline cPRA at several dilutions to identify infliction points, in which a significant drop in antibody strength occurs, to measure the efficacy of desensitization therapy
  - Pretreatment values should be analyzed in relation to posttransplant values as well as a further time point (eg, 6 mos) to determine durability of response.
  - At minimum we recommend performing 2 dilutions (eg, 1:32 and 1:256 as 1:32 dilution approximates the point of complement binding and 1:256 typically indicates saturation of the beads).
- The STAR working group **recommends** recording the number of unacceptable antigens pretreatment and posttreatment
- The STAR working group **recommends** using cPRA per a few titers as an approach to simply complex data that is sensitive to changes in antibody quantity (per their frequency in the donor population) and therefore is directly related to a candidate's probability of receiving a transplant.
- The STAR working group **strongly recommends to NOT use** rate of transplantation as the primary end point for desensitization trials among patients without an approved living donor because of the potential for disparities in access to transplantation.

Recommended standards of reporting for desensitization trials including patients with an approved living donor:

- The STAR working group **recommends** evaluating DSA(s) strength at baseline by titer, comparing with end of transplant as well as posttransplant levels (if transplanting across residual DSAs).
- The STAR working group **recommends** recording the number of unacceptable antigens among patients with an approved living donor as well as information regarding their cPRA. We acknowledge that the cPRA per titer is likely not as relevant to an individual donor/recipient pair; however, the use of similar end points for desensitization trials will be useful for comparing the efficacy of various therapies among patients in all organ groups.
- When a specific living donor is available, the crossmatch (from positive to negative), change in DSA titer, or rate of transplantation can be used as secondary end points.

Recommended standards of reporting for presence of DSA and outcome in ABMR treatment trials:

- The STAR working group **recommends** detailed reporting of antibody characteristics
  - Recording the number, specificity, and relative strength of each of the DSAs (if MFI is >10,000, at least 1 dilution should be performed to determine whether antibody strength is affected by bead saturation)
  - Known duration of DSA presence (time between last sample with no DSA to DSA appearance, and to initiation of ABMR treatment)
  - Recording whether DSA was preformed or de novo (with time posttransplant to the first appearance of DSA in support of the de novo determination)
- The STAR working group **recommends** the use of standardized methods to follow up changes in DSA following treatment to evaluate efficacy of response
  - Use same testing methods to determine baseline levels as well as follow-up levels
  - Follow predetermined timeline of tests in relation to baseline (eg, up to 7 d prior to treatment initiation; 2 wks after treatment (±7 d); and 3, 6, and 12 mos following end of treatment to follow long-term efficacy—recommended at least for clinical trials)
  - Use of some dilutions if baseline DSA MFI >10,000 to follow responses (recommend adding dilutions until DSA levels fall <10,000)
- For clinical trials, the STAR working group **recommends** performing some serial dilution studies to allow comparison between results from different clinical trials and identifying the most efficacious approach to match different clinical scenarios.

ABMR, antibody-mediated rejection; cPRA, calculated panel-reactive antibody; DSA, donor-specific antibody; MFI, mean fluorescence intensity; STAR, Sensitization in Transplantation: Assessment of Risk.

dilution studies has been the perceived notion of exorbitant increased cost associated with this procedure. In reality though, because only 2 dilutions are recommended, the added cost is quite reasonable (adding twice the cost of the reagents but only about 10% of additional labor cost, which is the most expensive component of the overall fee for performing the test). On the other hand, many centers opted to add in vitro complement binding assays (C1q or C3d), which most often add a higher cost to the overall antibody evaluation. The 2 dilutions recommended in this report provide an estimate of in vitro complement binding as well as bead saturation and thus provide more information for a similar or even lower cost. Among the clinical benefits of such an approach is the ability to identify, prior to initiation of desensitization, those patients that are not likely to benefit from this treatment modality. Referring these patients to Kidney-Paired-Exchange programs will spare them the consequences associated with an unsuccessful desensitization protocol and may provide them better access to histocompatible transplantation within an overall lower financial burden at the societal level. For patients awaiting deceased-donor transplantation, it can allow the provider the informed ability to choose other modalities, such as destination ventricular assist device as the treatment of choice, etc. Beyond the immediate clinical benefits, the ability to categorize HLA antibodies as weak, moderate, strong, and very strong, in a reproducible manner, will allow comparison of efficacy results between different trials. It is imperative that additional studies be performed to validate the value of additional dilutions in patients with very strong HLA antibodies.

4. Primary alloimmune response

4.1. HLA molecular mismatch load analysis

A brief update on the different molecular mismatch load (MML) analysis tools that are currently available is presented in Supplemental Material. Here, we address the potential clinical utility of HLA-MML assessment. Recent work is aimed to evaluate whether MML can be used as a prognostic or predictive biomarker. In keeping with the US Food & Drug Administration requirements for biomarker qualifications, the evidentiary framework needed is dependent on the proposed context of use, taking into consideration the benefits and risks associated with biomarker implementation. In the context of transplantation, one may envision the use of HLA-MML for the following: (1) enrichment of population at risk when enrolling patients into clinical trials specific for transplant rejection, (2) management of immunosuppression when the patient is considered for drug minimization, (3) risk stratification for immunosuppression/induction therapy at the time of transplant, or (4) incorporation into organ allocation algorithms.

Recent retrospective studies demonstrate that class II (DR and/or DQ) eplet-MML is strongly correlated with TCMR, de novo DSA, ABMR, and graft loss.[102-105] Similar correlations were found using other approaches for calculating the HLA-MML. Retrospective studies in both observational cohorts and post hoc analysis of randomized clinical trials further showed correlation between patients with low levels of class II eplet-MML and the ability to tolerate less immunosuppression.[102,105-107] In general, the degree of class II eplet-MML exhibits a linear relationship with the risk of developing a primary alloimmune response.[103] In the context of biomarker-guided enrichment approach for clinical trials of drug development, thresholds are required to provide strata of risk.

An important caveat to keep in mind is that while empiric data from retrospective diverse cohorts support the notion that low HLA-MMLs tolerate less immunosuppression without developing alloimmunity, emerging data demonstrate empirically unsupported assumptions used by the HLAMatchmaker software.[108] Further studies demonstrated that the immunogenicity of certain mismatches can be higher than others,[109-111] thus questioning the rigor in assigning the same value to all mismatches. The use of HLA-MML versus the need to better understand immunogenicity and assigning permissible versus nonpermissible mismatches is a focus of the 18th International Histocompatibility & Immunogenetics Workshop. Further, there is an urgent need for prospective clinical trials prior to determining the value of HLA-MML utilization for clinical care. Technical requirements and recommendations for use of HLA-MML in studies are presented in Table 7. Gaps in knowledge and recommendations for studies required prior to implementation in clinical use are presented in Table 8.

4.2. Non-HLA mismatch

The STAR 2019 report[2] highlighted multiple genome-wide association studies (GWASs) that investigated genetic determinants of alloimmune responses. Although many potential associations have been reported,[112,113] findings are inconsistent, and studies are limited by the complexity of donor/recipient genetic interactions and center-specific confounding effects.

For example, single-gene association reports have identified potential variants that may influence tacrolimus metabolism and graft rejection,[114-116] yet these findings and other candidate genes influencing alloimmunity were not validated in larger, more diverse cohorts.[117,118] One promising study showed in a discovery and validation cohort that recipients homozygous for LIM zinc finger domain containing 1 (*LIMS1*) deletion who received a kidney from a donor with at least one copy of the *LIMS1* gene had ~60% higher risk of either TCMR or ABMR and were more likely to develop anti-LIMS1 antibodies but not more likely to experience graft failure.[60] Another validation study confirmed only the association with TCMR.[119] Recent work suggests that natural killer (NK) cell genetic compatibility may also impact alloimmune responses, because donor ECs that do not express HLA molecules capable of binding recipient inhibitory killer immunoglobulin receptors; this can lead to NK cell activation and an increased risk of chronic humoral rejection and graft loss.[120] Clearly, there is a need to better define the molecular mechanisms underlying non-HLA genetic effects and propose that NK cell/innate immunity should be a new topic in the next STAR workgroup.

Finally, assessing genetic differences at the genome-wide level shows promise, because GWASs comparing donor and recipient mismatches in polymorphic residues of transmembrane or secreted proteins correlated with 5-year allograft survival.[61] Another GWAS using data from the iGeneTrain consortium calculated a polygenic risk score that was associated with kidney function posttransplant,[121] and a study with patients from the GoCAR cohort calculated a percent identity by descent that was associated with early vascular fibrosis and overall graft survival.[122] However, further work is needed to expand these types of studies to larger, more diverse cohorts.

In summary, although the number of retrospective studies attempting to correlate genetic markers outside of the HLA region with alloimmunity continues to grow, there is still a dearth of clinical trials aimed at validating these findings in prospective and genetically diverse cohorts. As stated by Oetting et al,[123] caution must be exercised when citing reports that have not been validated in independent cohorts as this can lead to propagation of false positive results. Many of the associations identified in these studies may not be direct modulators of immune responses but rather are driven by differences in donor expression of non-HLA proteins.

5. Alloimmune memory

This report builds on the STAR 2019 publication[2] providing an overview of recent developments in quantifying donor-specific memory T- and B-cell frequencies to improve pretransplant risk assessment.

5.1. Alloreactive T cells in organ transplantation

Measuring alloreactive T cells is highly complex because of the following: (1) timing of assessment that is directly influenced by the type of donor-antigen presenting pathways: direct, indirect, or semidirect; (2)

Table 7
Technical requirements and recommendations for HLA molecular mismatch studies.

Definition of the software used to determine mismatch load	• Type of approach used (eg, HLA-Matchmaker; PIRCHE-II; EMMA, amino-acid counting) • Version of the software used (if applicable)
High resolution HLA typing	• Typing at two-field resolution • Definition of DQα/β and DPα/β heterodimers
Accurate definition of studied outcomes	• Prospective DSA monitoring • Definition of SAB MFI cut-off used for antibody detection (see recommendations from previous STAR reports) • Biopsy proven rejection and Banff schema used, incorporation of protocol biopsies • Adjustment for potential confounders (eg, immunosuppression regimen, patient adherence)
Accurate definition of HLA molecular mismatch assessment	• Single molecule HLA mismatch score (correlation with DSA against each specific HLA molecule; not combining both donor alleles into an allo-"universe")
Assessment of HLA molecular mismatch model accuracy	• Multivariable statistical analysis • Model discrimination ability and calibration performance • External validity of final model

DSA, donor-specific antibody; HLA, human leukocyte antigen; MFI, mean fluorescence intensity; SAB, single antigen bead; STAR, Sensitization in Transplantation: Assessment of Risk.

Table 8
Gaps in current knowledge and recommendations for near future studies.

Gaps:

- Determine accurate way to assess and correlate molecular mismatch load with clinical outcome
  - Can molecular mismatch scores can be combined across loci and class? (for association with rejection or graft survival)
  - Can (and how) effects of mismatches at different HLA loci be adjusted? (eg, for prediction of rejection or graft survival)
  - Can different algorithms for HLA molecular mismatch assessment be combined to assess B-cell and T-cell alloreactivity?

Recommendations:

- The STAR working group emphasizes the need for innovative studies to assess utility of HLA molecular mismatch load assessment for the purpose of the following:
  - Implementation in patient recruitment strategies to enrich population at risk for the purpose of clinical trials
  - Immunological risk stratification—in support of precision medicine (eg, immunosuppression management)
  - Utilization in organ allocation policies
    - Impact on equity of access to transplantation
    - Impact on longevity of organ and patient survival
    - Best utility of scare resources

HLA, human leukocyte antigen; STAR, Sensitization in Transplantation: Assessment of Risk.

assessing naïve/memory T-cell responses specific to donor antigens or to a panel of allo-antigens that may describe different biological features of the alloimmune response; and (3) peripheral blood assessment of T-cell alloreactivity may not fully illustrate global T-cell alloimmune response in secondary lymphoid organs.

Several in vitro assays have been developed showing relevant associations with transplant outcomes, although major concerns remain regarding assay reproducibility and reliability, the need for rapid turnaround times, and especially for clinical validation in interventional studies. Most studies assessing alloreactive T cells have focused on peripheral blood functional assays that mainly mimic directly primed alloreactive T-cell responses, either by measuring frequencies of donor-reactive interferon gamma-secreting memory T cells with ELIspot assays or by sequencing alloreactive T-cell clonotypes following short-term

mixed lymphocyte reaction.[124] Although lacking statistical power, a recent prospective interventional trial using pretransplant frequencies of donor-specific interferon gamma-secreting memory T cells to guide immunosuppression minimization showed the feasibility of implementing this assay in clinical transplantation and opened a door to design subsequent trials to prove its validity.[125] Nevertheless, such assays may be less predictive of pathologic immune responses that are likely mediated via indirect rather than direct presentation.[126]

Defining immunophenotypes of bulk (non–donor-specific) memory T-cell populations can assess a given patient's overall "memory T-cell

Table 9
Assays to quantify memory T cells and memory B cells.

T-cell assay	Pros & cons	Utility
Bulk T-cell immunophenotyping by flow cytometry	Pros: i. Rapid detection of bulk T-cell immunophenotypes that may correlate with overall immune risk ii. Technically feasible to be performed by clinical laboratory Cons: i. Assay does not provide information about the alloreactive T-cell compartment, thus limiting the overall sensitivity and specificity of the assay	Could be a near-term bridge for gross assessment of T-cell risk while antigen-specific assays are being developed
Panel-reactive T-cell (PRT) assay	Pros: i. Quantifies frequency of alloreactive (but not necessarily donor-reactive) T cells making IFN-gamma ii. Does not require the availability of donor tissue Cons: i. Does not directly quantify donor-specific T-cell frequency, thus has lower sensitivity and specificity relative to ELISpot/AIM ii. Only quantifies alloreactive cells making IFN-gamma	Useful for assessing prevalence of alloreactive T cells in situations where donor tissue is not available
IFN-gamma ELISpot/AIM assay	Pros: i. Quantifies frequency of donor-reactive T cells making IFN-γ (ELISpot) or expressing CD154 (AIM) ii. Increased sensitivity and specificity relative to PRT and bulk assessments Cons: i. May only measure direct alloreactivity ii. Takes 3-4 d to obtain results and is technically challenging iii. Requires the availability of donor tissue iv. Does not assess noncirculating T cells	Useful for determining the frequencies of circulating donor-specific T cells based on functionality
TCRβ CDR3 sequencing	Pros: i. Directly identifies donor-reactive T cells based on TCR sequence and therefore does not require the detection of functionality Cons: i. May only measure direct alloreactivity ii. Takes 3-4 d to obtain results and is technically challenging iii. Requires the availability of donor tissue iv. Does not assess noncirculating T cells	Useful for determining the frequencies of circulating donor-specific T cells regardless of functionality
B-cell assay	**Pros & Cons**	**Utility**
Flow cytometry to quantify the frequency and phenotype of HLA-binding B cells	Pros: i. Rapid detection of HLA-reactive B cells using HLA tetramers or single HLA-coated beads in a multiplex format. Cons: i. Sensitivity limited by low frequencies of donor-specific memory B cells; challenges in avoiding nonspecific binding to tetramers or HLA-coated beads in a multiplex format. ii. Assay does not provide information on the capacity of the memory B cells to differentiate into antibody-secreting cells.	If technical limitations can be overcome, this assay is most promising for adaption as a clinical assay to quantify frequencies and phenotypes of donor-HLA–specific B cells.
In vitro differentiation of memory B cells into ASCs; assessment of anti-HLA IgG in culture supernatant with Luminex SAB	Pros: i. Simple detection of anti-HLA IgG released into the culture supernatant using single HLA-coated beads in a multiplex format; sensitivity enhanced by concentrating culture supernatants Cons: i. Indirect quantification that assumes all ASC secrete the same amount of IgG, low sensitivity due to low frequency memory donor-specific B cells, requires 6-10 d in vitro culture.	Useful for proof-of-principle research studies to assess the functional importance of donor-specific memory B cells frequency in determining graft outcomes.
In vitro differentiation of memory B cells into ASCs; assessment of HLA-specific ASCs by ELISPOT or fluorospot assay	Pros: i. Enumerates the frequency of HLA-specific ASC, high sensitivity for low frequency memory B cells, provides a direct assessment of the frequency of memory HLA-specific B cells capable of differentiating into ASC. Cons: Requires 6-10 d culture, expensive and labor intensive, cannot test many different HLA Class I and class II molecules in a single ELISPOT assay.	Useful for proof-of-principle research studies to assess the functional importance of donor-specific memory B cells frequency in determining graft outcomes.
In vitro culture newly generated ASCs from blood; IgG assessment by ELISPOT or Luminex SAB assay	Pros: i. Eliminates the need for 6-10 d culture. Cons: i. ASCs may only appear transiently in the blood during acute rejection	Useful for proof-of-principle research studies to assess ongoing donor-specific B-cell responses

ASC, antibody-secreting cell; HLA, human leukocyte antigen; IgG, immunoglobulin G; IFN, interferon; SAB, single antigen bead; TCR, T cell receptor.

risk" for rejection. Recently, terminally differentiated effector memory $CD8^+$ T cells with the inhibitory FCGRIIB have been associated with immune protection in patients off tacrolimus,[127] whereas CD8 with the activating receptor FCGRIIIA is associated with higher rejection risk.[128] An important caveat of these studies is that the use of bulk memory T-cell immunophenotypic signatures does not provide information on the donor-specific compartment. Pros and cons of currently available assays to quantify memory T cells are presented in Table 9, and their implementation in clinical trials is summarized in Supplementary Table 1.

5.2. Alloreactive B cells in organ transplantation

Not all memory B cells are actively producing HLA antibodies, and thus they may evade detection by the Luminex-based SAB assay. Several assays exist to sample the peripheral alloreactive memory B-cell pool, with the most common being an in vitro differentiation of memory B cells into antibody-secreting cells, quantifying the frequency of HLA-specific immunoglobulin G producing B-cell frequencies using the ELISpot assay and distinct HLA detection systems. Improved sensitivity of detecting secreted HLA-specific antibodies after in vitro differentiation of polyclonally activated B cells using protein G affinity purification was demonstrated in a clinical pilot study documenting correlation between donor-specific memory B-cell frequency, Luminex detectable DSA in in vitro cultures, and an increased incidence of ABMR.[129-131]

Kramer et al[132] used HLA class II tetramers for the isolation and expansion of single HLA class II-specific memory B cells and sequencing of the V(D)J genes of the variable-heavy and variable-light domains, thus understanding of the breadth of humoral alloimmune responses. However, translation into a clinically viable assay for assessing HLA-reactive B cells remains challenging. High-dimensional flow cytometry and RNA sequencing to characterize circulating activated B cells during ABMR in kidney transplant recipients demonstrated increased frequencies of activated $CD19^+IgD^-$ B cells enriched for CD27, CD38, and Ki67.[133] However, it is not clear whether this approach can be used to detect similar memory B cells beyond the setting of active ABMR, especially in the pretransplant setting. Replicating conditions of bone marrow microenvironment in vitro allows for survival and maturation of early minted blood $CD19^{int}CD27^{hi}CD38^h$ antibody-secreting cells into long-lived plasma cells,[134,135] thus permitting the study of the frequency and biology of terminally differentiated long-lived plasma cells. Coupling this new system with known methods to assess HLA antibodies may allow for a more rapid assessment of ongoing antidonor B-cell responses by avoiding the need for a 7-day in vitro culture. Pros and cons of currently available assays to quantify memory T cells are presented in Table 9, and their implementation in clinical trials is summarized in Supplementary Table 1.

Using single-cell RNA sequencing followed by the expression of B-cell receptors cloned from graft-infiltrating $CD20^+$ and plasma cells, Asano et al[136] reported that these cells were not HLA-specific; rather, they were reactive to local renal-expressed antigens and the class-switched intrarenal B cells had unique proinflammatory signatures. This study raises the possibility that intragraft autoreactive B cells and antibodies may play critical roles, suggesting that analysis of B cells infiltrating rejecting allografts may provide new insights into their direct role in graft rejection.

Although the field of HLA-specific memory T and B-cell characterization is progressing, many of the gaps defined in the STAR 2019 report remain unresolved. Prospective clinical trials to assess the predictive value of circulating HLA-specific memory B cells are also urgently needed. Knowledge gaps and recommendation for studies to increase understanding and measuring of memory T and B cells are highlighted in Table 10.

6. HLA antibody attributes

HLA antibodies are potent mediators of the adaptive immune system that, if directed at donor-specific targets, represent an ongoing threat to graft integrity and function. Here, we review updates in assays to measure HLA attributes and discuss mechanistic studies that explore HLA antibody-induced effector function.

6.1. Assays to determine HLA antibody attributes

Complement binding in SAB assays, whether C1q or C3d, remains a focus of multiple studies.[24,137-144] Most studies confirmed a positive correlation between complement binding assays, high DSA MFI levels, and/or high DSA IgG titers with increased risk for ABMR or graft loss. Nevertheless, low levels (MFI) of HLA-DSAs are still a signature of risk for adverse outcomes. Although IgM antibodies are not typically associated with allograft injury, identification of IgM-producing B cells may suggest patients at risk of ABMR and allograft failure.[145] The association of IgG3 with acute rejection and IgG4 in patients with chronic rejection continue to be reported in kidney and liver transplantation.[146-149] Importantly though, concerns with assay specificity (ie, crossreactivity between IgG subclass reagents) hamper accurate detection of IgG subclasses, making clinical interpretation and recommendations questionable.[150] Work exploring the correlation between SAB MFI values and flow cytometry crossmatch using human HLA monoclonal antibodies and surface

Table 10
Gaps/need for understanding and measuring T-cell memory.

- Need for further clinical validation and standardization of current immune assays tracking circulating donor-reactive alloreactive T cells in interventional clinical trials
- Limited availability of donor-related biological samples to assess donor-specific T-cell responses
- Acceptable negative predictive values but low sensitivity and specificity of current assays based on short-term cocultures assessing cytokine-producing alloreactive T cells predicting allograft rejection
- Does peripheral blood accurately illustrate the global antidonor T-cell immune status, including that originated in secondary lymphoid organs?
- Biological difference and accuracy of tracking memory versus naïve and direct versus indirectly primed alloreactive T cells

Gaps/need for understanding and measuring B-cell memory

Need for a sensitive and antigen-specific test to quantify donor-specific memory B-cell frequency, especially for donor-specific memory B cells.

- Define the potential utility of week-long assays involving the in vitro differentiation of memory B cells into antibody-secreting cells, and then quantifying secreted IgG by solid phase or ELISPOT assays
- Compare the relative sensitivity of quantifying anti-HLA IgG in culture supernatants, or the ELISPOT assay for quantify the frequency of an anti-HLA IgG-secreting cells.
- Flow cytometry-based approach using HLA multimers can identify HLA-specific B cell; does the extremely low frequencies of HLA-specific memory B cells limit the clinical utility of this approach?
- Test if the quantification of memory donor-HLA specific B-cell frequency will complement DSA quantification to better identifying patients most likely to develop humoral responses and/or rejection.
- Does specific types of sensitizing events (eg, blood transfusions vs pregnancies vs graft rejection) result in differently persistent memory B cell and DSA responses?
- Is there a biologically significant role for non-HLA-specific, autoreactive B cells and antibodies, and a need for their quantification?
- Does the peripheral blood sufficiently reflect the HLA-specific memory B-cell compartment present in secondary lymphoid organs?

DSA, donor-specific antibody; HLA, human leukocyte antigen; IgG, immunoglobulin.

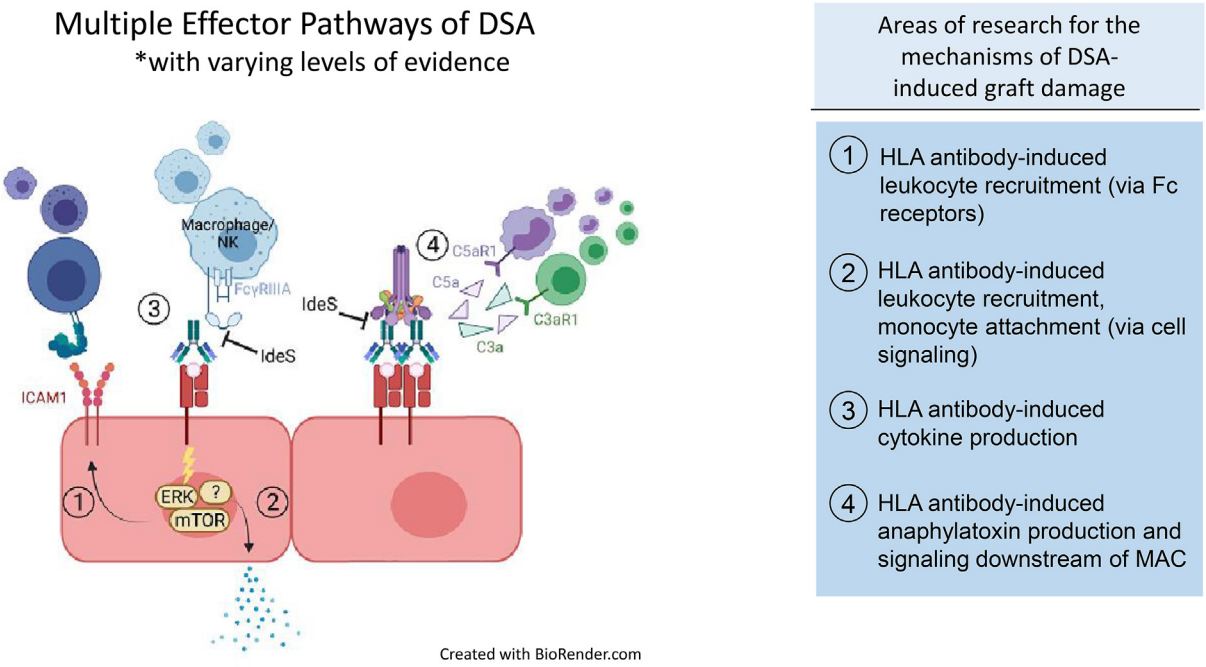

Figure 1. Schematic of the multiple cellular and environmental changes following HLA antibody ligation. Antibodies binding to HLA molecules can induce several downstream effects. (1) Endothelial signaling via ERK, mTOR, and likely other modalities yet to be identified that increase adhesion molecule upregulation, monocyte attachment, and endothelial cell proliferation and migration. (2) AKT signaling promotes interleukin 6 production in endothelial cells; several cytokine/chemokine signatures associate with ABMR. (3) FcR ligation on macrophages and natural killer cells that result in polarization, recruitment, and activation; glycosylation status could alter these outcomes. (4) C3a and C5a production that have potential to bind C3aR1 and C5aR1 and induce $CD4^+$, $CD8^+$, and myeloid effector functions. Areas in which Imlifidase is effective at preventing effector function are shown. Created with BioRender.com. DSA, donor-specific antibody; HLA, human leukocyte antigen, IdeS, Imlifidase; FcR, fragment crystalizable (region) receptor; mTOR, mammalian target of rapamycin; ERK, extracellular-signal regulated kinase.

plasmon resonance reported poor correlation, largely due to HLA amino-acid variations.[72,151]

6.2. *HLA antibody-induced effector function*

Mechanisms by which HLA antibodies elicit effector functions, mediating graft damage, are divided into Fc-dependent and independent effector functions (Fig.).

6.2.1. *Fc-dependent effector functions*

The glycosylation status of antibodies as a posttranslational modification may impact effector functions.[152,153] However, studies of DSAs in transplant recipients have been small and inconclusive concerning transplant outcomes.[154,155] An association of HLA-DSAs with leukocyte recruitment was reported[156] and proposed an Fc-dependent role of HLA antibodies on macrophage polarization.[157] Associations of *FCGR2A* and *FCGR3A* polymorphisms with graft injury[158,159] supports this

Table 11
HLA antibody attributes.

Gaps/needs:
- Analytically validated reagents to study different strengths/titers of IgG antibody subclasses in the context of commercially available HLA antibody testing platforms.
- New technologies able to assess the functional characteristics of HLA antibodies in complex biological fluids, such as serum, with a sensitivity comparable to the current HLA-SAB platforms.
- Additional studies to ascertain whether in vitro complement binding assays are surrogate markers for antibody effector function in vivo.

Recommendations for current implementation:
- The STAR working group continues to recommend not to consider absence of DSA complement binding antibodies as a lack of detrimental activity of DSA

Recommendations for further studies:
- The STAR working group strongly recommends development of analytically valid reagents to define IgG subclasses in human sera prior to translating these assays to the clinic.
- The STAR work group further emphasizes the need for development of assays to measure HLA antibody affinity and avidity to better understand the evolution of the humoral anti-HLA alloimmune response, and their possible pathogenicity.
- The STAR working group suggests studies to compare class I and class II glycosylation status and dynamic evolution with time of DSA.
- The STAR working group highlights the need for longitudinal analyses to follow changes in HLA antibody subclasses given dynamic biological responses
- The STAR working group recommend development of studies to decipher the mechanisms of action of different IgG subclasses on graft injury
- The STAR working group highlights the need to develop assays to detect complex mixtures of anti-HLA IgG subclasses in human sera competing for binding to the same epitope

HLA antibody effector function studies:
Multiple gaps require multiple future studies:
- Prospective trials to determine whether glycosylation status impacts ABMR, graft injury, or transplant outcome
- Mechanistic studies to assess the specific impact of the Fc region of HLA antibodies on leukocyte recruitment
- Comprehensive and long-term studies to assess FcR polymorphism and their impact on graft injury and function
- A systematic approach to identify key HLA class I and class II signaling pathways, including upstream and downstream targets of these pathways, recognizing the inherent redundancies of the signaling modalities
- Mechanistic studies assessing the link of HLA antibody-released anaphylatoxins and C3aR1/C5aR1
- Systematic approaches to determine whether therapeutics targeting cytokine/chemokine induction and maintenance pathways result in diminished graft injury
- Mechanistic studies defining the role of NK cells in acute and chronic rejection

ABMR, antibody-mediated rejection; DSA, donor-specific antibody; HLA, human leukocyte antigen; NK, natural killer; SAB, single antigen bead; STAR, Sensitization in Transplantation: Assessment of Risk.

hypothesis. In this regard, Imlifidase, a Streptococcal protease that cleaves the Fc region of IgG, has been shown to be safe and efficacious in diminishing DSA levels pretransplant and allowing transplantation across highly sensitized individuals.[160-162] Ongoing clinical trials were designed illuminate the long-term impact of Imlifidase on transplant outcomes.

6.2.2. *Fc-independent effector functions*

Recent evidence describes cellular signaling downstream of HLA antibody binding to HLA targets that promote the following: (1) adhesion molecule upregulation, proliferation, and migration of ECs[163]; (2) the endothelial to mesenchymal transition[164]; and (3) induction of dendritic cell maturation.[165] HLA antibody ligation of the major histocompatibility complex on ECs can also lead to anaphylatoxin production that can result in monocyte recruitment.[157] One proposed mechanism of recruitment is the cellular expression of anaphylatoxin receptors C3aR1 and C5aR1 on $CD4^+$ T cells, $CD8^+$ T cells, and myeloid cells that results in differential downstream effector functions once ligated.[166]

ABMR has been associated with cytokine and chemokine signatures unique from TCMR.[167,168] HLA class II antibodies were shown to impact interleukin 6 production via Akt signaling,[169] suggesting a mechanism by which antibodies in ABMR cases could lead to altered cytokine and chemokine secretion. Mouse model studies describe a requirement for NK cells in ABMR.[170] However, the impact of HLA antibodies on NK cell activation and function in acute and chronic settings remains unclear.[168,171-173] Table 11 summarizes current gaps, recommendations for current implementation, and the STAR group vision of studies needed to further our understanding of HLA antibody attributes that impact graft outcome.

7. Future Direction of STAR

The next iteration of STAR is planned for 2025. By then it is anticipated that the current topic areas outlined in this summary report will have seen significant progress. In addition, the steering committee will add new areas of focus that are currently emerging (eg, role of self/nonself mismatching driving innate alloimmunity[120]). One thing is abundantly clear: there is much to be learned in the field that has the potential to improve transplant patient outcomes.

Funding

This study was sponsored by the American Society of Histocompatibility and Immunogenetics, American Society of Transplantation, and the Paul Terasaki Research Fund.

Disclosure

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

Data availability

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

Acknowledgments

The authors thank the American Society of Transplantation for logistical assistance.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ajt.2022.11.009.

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