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Sensitization in Transplantation: Assessment of Risk (STAR) 2017 Working Group Meeting Report

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Abbreviations
cPRA – Calculated Panel Reactive Antibody
DSA – Donor Specific Antibody
MFI – Mean Fluorescent Intensity
STAR – Sensitization in Transplantation: Assessment of Risk

ABSTRACT

The presence of pre-existing (memory) or *de novo* donor specific HLA antibodies (DSA) is a known barrier to successful long-term organ transplantation. Yet, despite the fact that laboratory tools and our understanding of histocompatibility have advanced significantly in recent years, the criteria to define presence of a DSA and assign a level of risk for a given DSA varies markedly between centers. A collaborative effort between ASHI and the AST provided the logistical support for generating a dedicated multidisciplinary working group, which included experts in histocompatibility as well as renal, liver, heart and lung transplantation. The goal was to perform a critical review of biologically driven, state-of-the-art, clinical diagnostics literature; and to provide clinical practice recommendations based on expert assessment of quality and strength of evidence. The results of the STAR (Sensitization in Transplantation: Assessment of Risk) meeting are summarized here, providing recommendations on the definition and utilization of HLA diagnostic testing, and a framework for clinical assessment of risk for a memory or a primary alloimmune response. The definitions, recommendations, risk framework, as well as highlighted gaps in knowledge are intended to spur research that will inform the next STAR working group meeting in 2019

Introduction

The presence of pre-existing (memory) or *de novo* donor specific HLA antibodies (DSA) is a known barrier to successful long-term organ transplantation (1). Yet, the criteria to define and assign a level of risk for a given DSA varies markedly between centers, despite the fact that available laboratory tools and our understanding of histocompatibility have advanced significantly in recent years. Unfortunately, much of our current clinical practice is based on transplant survival studies that were designed in the time of older technologies, confounding our ability to interpret and implement those results into current clinical practice or to design new clinical studies. Consequently, there is a need to update guidelines for antibody testing and patient risk assessment in order to enable clinical programs to design personalized immunosuppression protocols.

A collaborative effort between ASHI and the AST provided the logistical support for generating a dedicated multidisciplinary working group which included experts in histocompatibility as well as renal, liver, heart and lung transplantation. The goal was to perform a critical review of biologically driven, state-of-the-art, clinical diagnostics literature where comprehensive account of methodology was provided; and to provide clinical practice recommendations based on expert assessment of the strength of evidence (Table 1). A complete list of publications that were reviewed by the working groups is provided in supplement table 1.

Participants were divided into smaller discussion groups based on their clinical expertise and were tasked with critical (albeit not systematic) review of the literature. The literature search focused on clinical diagnostics of circulating HLA antibodies. For the purposes of the first STAR working group report non-HLA antibodies were considered out of scope. Moreover, histologic diagnostics were not considered as this falls within the purview of the Banff Foundation for Allograft Pathology. To supplement the literature review, a survey was conducted of both clinical and laboratory programs supporting organ transplantation.

Subgroups were charged with providing educational primers on (i) the definition and utilization of HLA diagnostic testing, and (ii) the biological basis of immunological naïveté verses memory. Organ specific groups were tasked to establish criteria to assess patients' risk in the context of a naïve vs. memory immunological response, and to use this distinction to inform HLA diagnostic utilization pre- and post-transplantation. Initial recommendations were formulated followed by face to face deliberations of the full group on February 26, 2017, at the Biltmore hotel, Arizona. Importantly, subgroups were asked to identify key knowledge gaps that if addressed could significantly advance clinical practice. Representatives from the FDA, NIH and UNOS attended as observing stakeholders. It should be noted that the STAR working group refrained from specific recommendations for therapeutic protocols for two major reasons: (i) high quality evidence is lacking to support one approach over another; (ii) for a given patient, the risk of memory or *de novo* alloimmunity, and the requirements for risk mitigation therapies, varies significantly based on the target organ (most notably in the case of liver transplants).

Defining the Presence/Absence of an HLA Antibody

Solid phase single antigen bead (SAB) technology revolutionized HLA diagnostics in the last 15 years by detecting very low level antibodies in patients' sera using a mean fluorescent intensity (MFI) readout (2). There has been much discussion as to the ability to set an MFI cut-off for determining the presence or absence of an HLA antibody. An objective difficulty lies in the relatively high coefficient of variation (CV) for the assay – a point documented clearly in the CTOT-ARRA funded HLA antibody standardization study (3). While emphasizing that SAB MFI cannot be used as a quantitative assay, the study did determine that "MFI positive cutoffs ranging from values 1000-1500 yielded a high level of agreement (>90%) in antigen specificity assignment. The MFI cut-off of 1400 units was found to optimize the correct classification rates for both class I and II kits" – see caveats below. The ability to set such a cut- off value, to define the presence or absence of an HLA antibody, is critical to improving the quality of clinical trials in the field and allowing for comparability between studies, a point specifically emphasized by the Federal agency observers during the STAR working group meeting.

Terminology

The survey results, combined with reviews of the relevant literature, made it evident that there is confusion with terminology in the community. It was agreed there must be clarity and consistent use of terms to allow comparison between research studies, as well as clinical outcomes, and to facilitate improvement in practice guidelines and health system policy.

Specifically, the following major terminology and misuse of terms were identified:

- **Mean Fluorescence Intensity (MFI) ≠ Titer.** A high MFI value is often referred to as a high titer antibody but only rarely indicates the actual testing of serum by dilution studies. Some antibodies with relatively high MFI values may dilute rather quickly and therefore do not qualify as high titer antibodies (4). Moreover, HLA Antibody Single Antigen Bead (SAB) MFI assessment is not licensed by the FDA as a quantitative assay. Determination of antibody titer is important as it is likely to have implications on the injurious qualities of that antibody and a reference point for determining efficacy of desensitization therapy (5).
- **0% calculated Panel Reactive Antibody (cPRA) ≠ Immunologically Naïve.** The fact that a patient has no detectable HLA antibodies does not infer they are immunologically naïve with regard to HLA antigens. It is entirely possible that a non-transplanted patient has been exposed and responded to an allo-HLA antigen through pregnancy or transfusion and yet they do not have a detectable HLA antibody in the current sera.
- **Acceptable HLA mismatch ≠ Immunologically Naïve.** The term "unacceptable HLA antigens" is used in the context of listing a patient's HLA antibody specificities in UNet to avoid donor offers that

the clinical program is not willing to cross due to the risk associated with a memory response. Not uncommonly it is assumed that the remaining “acceptable antigens” infers that there is no immune memory or that there is no HLA antibody specific for the “acceptable antigen”. This is entirely a false premise – in many instances just because a DSA MFI is below the program’s “risk threshold” does not mean the antibody does not exist and that the recipient is immunologically naïve to that mismatch and therefore at no or minimal risk.

- **Pre-Transplant DSA Titer ≠ Post-transplant Memory Response.** It is often inferred that the amount of antibody pre-transplant can be used to predict the risk and intensity of the post-transplant recall response. This is completely without basis – at present we have no tools to determine if a low titer antibody will remain low or rapidly increase in titer.
- **Complement (C’) Binding Activity *In Vitro* ≠ *In Vivo* C’ Binding Activity.** While certain antibody sub-classes do have higher affinity for C1q binding, complement activation is largely a consequence of a high concentration of DSA (4, 6, 7). Indeed, it has been shown that activation of C1q requires the presence of 6 antibody molecules in close proximity (8) . Consequently, C1q positive DSA in the serum, similar to IgG DSA in the serum should be considered in the context of gradations of the antibody’s levels rather than yes/no responses. Moreover, patients with a C1q negative DSA in the serum can frequently have C4d+ ABMR in the tissue indicating that a negative C1q assay does not infer the DSA is incapable of activating complement *in vivo* (9). Therefore, while emerging data suggests C1q positive DSA may indicate a potential risk for adverse graft outcomes, more research in this regard is required to clearly demonstrate its distinct utility (10).
- **Eplet ≠ Epitope.** A commonly misused term is epitope instead of eplet. Epitope refers to the complete contact area between an antibody and an antigen. Eplet is a portion of the epitope that in theory forms the third complementarity determining region of the immunoglobulin variable heavy chain (CDR H3) antibody binding site, defined purely based on amino-acid mismatching between donor and recipient (i.e. represents the potential functional epitope of the antibody determining specificity, whereas the entire structural epitope, comprised of the binding by all 6 CDRs, determines antibody avidity) (11, 12). Currently, only a subset of the theoretical eplets have been proven to be antigenic.

Quality and Comprehensive use of HLA Diagnostics

It was identified that the quality and comprehensive use of HLA diagnostics varies greatly in the published literature. This is in part related to the retrospective nature of many reports that examine longitudinal outcomes in cohorts prior to 2010, after which UNOS started to mandate more comprehensive HLA loci typing as well as solid phase testing for HLA antibodies. Key gaps remain that need to be considered in interpreting the literature:

- **Lack of donor HLA loci typing does not equal absence of a DSA directed to that HLA loci.** Presence of HLA-DSA is (at times) determined in the absence of comprehensive donor HLA typing. The corollary is that absence of DSA for a given loci cannot be rigorously determined if the donor typing at that loci is not available.
- **Lack of high resolution typing cannot be substituted by statistical assumptions of the missing data**. Given the complexity of HLA genetics, and its polymorphism in different ethnic groups, imputation of missing HLA data may introduce substantial bias, and may lead to false conclusions, especially with regards to HLA-Class II antigens. Currently available frequency–tables may support clinical consultation for individual patient management, but in the vast majority of the cases is not sufficient for clinical trials adjusting for the confounding effect of HLA mismatching on outcomes.
- **Failure to routinely use methods to rule out serum inhibitors in HLA antibody assays leads to under recognition of DSA.** Inhibitors, such as endogenous C' activation *in vitro*, can block the ability of secondary antibodies to appropriately recognize DSA binding to the SAB, producing low MFI readings and an inaccurate interpretation that a DSA is absent, or at a low level (4, 13).
- **Failure to consider shared epitopes between solid phase beads leads to under recognition of DSA.** While the aforementioned MFI cut-off between 1000-1500 units is generally optimal for recognition of a DSA specificity, it is nonetheless a guideline and <u>not an absolute</u>. When a number of beads share the same DSA epitope it is entirely possible to have a DSA with the MFI <1000 on all beads. Knowledge of shared epitopes is therefore essential for proper interpretation of SAB assays.

In order to improve "precision" and "personalized" medicine the consensus was that comprehensive HLA diagnostics must become the standard of care and most certainly have to be imbedded in clinical trial research going forward. The STAR working group recommendations for HLA antigen typing and antibody testing are summarized in Table 2.

Immune Memory

Immunological memory - the ability of the immune system to respond rapidly and with vigor upon re-encounter with the same antigen. Modern immunology now demonstrates that infection or vaccination results in the generation of long-lived subsets of phenotypically, functionally, and metabolically distinct B and T cells. Memory T and B cells are the progeny of antigen-specific naïve cells that have been clonally expanded in the course of an immune response that survive even after antigen has been eliminated. They reside in specific anatomic locations, have distinct phenotypes and are uniquely poised to confer immediate protection and generate secondary responses that are more rapid and of higher magnitude as compared to primary responses against the same antigen (14). In transplant recipients, donor-reactive memory T and B cells can arise from prior exposure to foreign HLA via prior blood transfusion, transplantation, or pregnancy. Additionally, heterologous immune mechanisms, whereby T cell responses elicited by infectious pathogens are cross-reactive with donor antigens, provide another potential source of alloreactive memory T cells in transplant recipients (15, 16). Given that immune memory is a known barrier to graft survival (17), though its impact can vary by organ type, the STAR working group recommendations are aimed at detecting and evaluating the immune status of the patient. That said, it is important to recognize, at least as currently measured, that immune memory's *in vitro* assessment has severe limitations and gaps that fail to incorporate aspects of well described immunobiology.

Clinical Measurement of Immune Memory

Currently classification of patients as "sensitized" or "naïve" is strongly influenced by the most recent circulating HLA antibody test – percent panel reactive antibody (PRA) and specific HLA antibody identification. While this information is beneficial to predict lymphocyte crossmatch results, it does not provide complete and accurate information regarding the patient's sensitization history and their likelihood to have a recall memory response against the transplanted organ. Specifically, patients with 0% PRA in a current serum sample may have had historic HLA antibodies after a sensitizing event that may or may not be apparent to the clinician based on, availability of sera, frequency and length of historic HLA antibody testing. Moreover, recent literature demonstrates that HLA specific B-cell memory may be present even in the absence of detectable HLA antibody (18, 19). The meeting highlighted that our current "memory assays" are limited to detecting circulating HLA antibodies at a specific time-point (i.e. Flow PRA and the SAB assays) and thus focus on only a small portion of the memory alloimmune response. Clearly, we are only beginning to scratch the surface of detecting donor specific B- and T-cell memory (e.g. IFN-γ ELISPOT assay) pre-transplant (20). The STAR working group developed definitions for alloimmune memory (Table 3) and recommendations to evaluate a patient's potential for alloimmune memory (Table 4).

Primary (naïve or *de novo*) Alloimmune Response

It is difficult to document that a patient is truly "naïve" for a given mismatched alloantigen, rather it is generally expressed in terms of relative risk for a memory response to that alloantigen on the basis of patient history and HLA antibody testing (vida supra). Confounding the definition of "naïve", the STAR working group found the transplant literature inconsistent in comprehensively assessing the presence of pre-transplant alloimmune memory, a requirement if concluding that a post-transplant alloimmune response is *de novo*. Key questions thus arise: (i) Can one be assured an observed alloimmune response is *de novo* versus memory; (ii) Does post-transplant distinction between memory vs. primary alloimmunity have clinical implications; and (iii) Can one assess an individual's risk for a primary immune response to a given mismatched alloantigen?

The confidence in assigning an alloimmune response as *de novo* versus memory is not difficult when comprehensive state-of-the-art assessment fails to detect DSA pre-transplant <u>and</u> TCMR or ABMR occurs for the first time late (i.e. >6-months) post-transplant. The challenge resides when these requirements are not met. Moreover, at least 4 other parameters further confound classification: (i) immunogenicity of a given mismatched alloantigen; (ii) immunogenicity of a given transplanted organ (e.g. kidney >> liver); (iii) immune responsiveness of the individual (e.g. younger >> older); and (iv) the adequacy of immunosuppression given (i) to (iii). Emerging literature is bringing all of these into focus. For example, recent studies, excluding pre-transplant DSA using state-of-the-art HLA diagnostics, where target tacrolimus trough levels were between 8-12 ng/ml in the first 3 months and 7-12 ng/ml for the first year did not report a new DSA on serial post-transplant screening prior to 6 months. In comparison, if the target tacrolimus trough level was between 6-9 ng/ml during the first 3 months, a new DSA incidence of 7.4% at 1 month has been observed (21-23). Given this complexity the STAR working group concluded that in general a new DSA observed in the first 2-weeks post-transplant likely represents a memory response. Between 2 weeks and 3 months, as immunosuppression is weaned and cells are repopulated from depletion therapy (when used), then both memory and *de novo* alloimmunity may emerge. After 3 months, the later the onset of a new DSA the more likely it is related to *de novo* alloimmunity. Clearly, these are broad guidelines and represent an area for further study and refinement to determine the relative contribution of memory and primary alloimmunity early (i.e. <6-months) post-transplant. However, the distinction may prove very relevant as literature is reporting differences in outcomes related to memory vs. *de novo* DSA associated ABMR (24, 25). Similarly, whether treatment protocols are equally effective for both memory and primary alloimmunity requires further research.

Literature rigorously defining an alloimmune response as *de novo* has reported that the level of HLA whole antigen mismatch does not accurately reflect the immunogenic risk of a given donor to elicit a *de novo* alloresponse (26). Indeed, for a given level of HLA serologic antigen mismatch a donor-recipient pair, at the molecular level, maybe very similar to one another or quite disparate. New computational tools are emerging that allow accurate quantitation at the HLA molecular mismatch level (e.g. in terms of amino acid polymorphisms or differences in electrostatic charge) for any donor-recipient combination, which may allow more accurate assessment of a patient's risk for a *de novo* alloimmune response post-transplant (27, 28). While the optimal computational methods and threshold values to assign risk are yet to be determined and validated, especially in diverse genetic backgrounds and across all organ transplants, the STAR working group saw this area as one holding great promise for the field requiring immediate investment. In particular, it may allow for personalized immunosuppression and in particular minimization to avoid unwanted side-effects.

Alloimmune Risk Assessment

Based on the aforementioned discussion of the biology of memory and primary alloimmune responses, the STAR working group constructed a general framework for assigning risk independently for memory and primary alloimmune responses at the time of transplant. Summarized in Table 5, the framework proposes that risk can be broadly assigned using currently available state-of-the-art HLA diagnostics. The novel aspect of this framework is the assignment of two types of risk (e.g. one for memory and one for *de novo* alloimmunity). While the *de novo* risk assignment on the basis of molecular HLA mismatch is yet to be optimized the STAR working group saw the creation of the framework as critical in order to foster research in the field of HLA immunogenicity and to ultimately define immunodominant HLA epitopes driving TCMR and ABMR. The utility of the framework is seen as allowing individual transplant programs to first and foremost define the memory and primary alloimmune risks present for a given patient and organ transplant type, and then to either avoid the risk or develop tailored induction and maintenance immunosuppressive therapies to address the risk. As stated at the outset, protocols vary widely across clinical programs and the literature does not currently have high quality evidence to recommend one protocol over another. It is hoped that this framework will drive clinical research to address this gap.

Organ Specific HLA Diagnostic Assessment Guidelines

There was broad consensus amongst the organ specific groups for the recommendations contained in Tables 1 to 3. However, immediate pre-transplant evaluation and post-transplant assessment varied amongst the organ specific groups and these are reflected in Table 6, mainly to do with grade and strength of the recommendations. Of note, while there was general agreement in regard to the need for post-transplant DSA monitoring, especially in the context of memory, the lack of high quality evidence preclude the STAR working group from making any specific recommendations as to the frequency and duration – at this point it should be a program specific decision.

Key Knowledge Gaps

The STAR working group identified general as well as organ specific gaps in the current knowledge that should be addressed within the following broad categories:

- **Risk Stratification for Memory and Primary Alloimmune Responses**

The literature review, as well as the survey, elucidated the lack of integration between HLA antibody information and current knowledge of immunobiological processes as a tool to guide clinical practice. In general, HLA antibodies are evaluated as present/absent rather than evaluating the patient's immune-sensitization status; the type of immunization (i.e. pregnancy vs. transfusion vs. prior transplant); strength of antibodies; and trajectory of antibody responses. While this is a gap in education, a more fundamental deficit relates to the absence of tests to detect the potential presence of immune memory in the absence of circulating HLA antibodies. Development of robust high-throughput tools to identify and quantify T cell and B cell memory are required for pre-transplant risk assessment and tailoring of immunosuppression protocols pre- and post-transplant.

Improved matching algorithms, beyond pre-transplant crossmatch and the current HLA-A, -B, -DR matching scheme, are required. This will help minimize the risk of *de novo* HLA antibodies post-transplantation and lead to improved graft survival. Similarly, research to define the effects of different immunosuppression regimens on the likelihood of developing *de novo* HLA DSA and/or TCMR should lead to more individualized treatment protocols.

- **Desensitization / Crossing HLA Antibody Barriers**

Multiple desensitization protocols are currently available (29-32). However, it is not clear how to determine the optimal patient population that may respond to each of these approaches, or to predict whether the response will be sufficient to bridge those patients to transplantation, or if a specific protocol is even required in the case of liver transplants. Assays to monitor the efficacy of these treatments are lacking and thus the ability to compare between the different protocols is limited. Moreover, the effectiveness of desensitization in targeting memory (especially B-cells) is completely unknown. It is currently not clear whether some DSA attributes are more detrimental than others and what is the relationships between these characteristics (e.g. complement binding antibodies, titers, antibody sub-classes, the dynamics of isotype switching over time, the impact of FcγR genotypes, etc.)(33).

- **Post-Transplant Monitoring**

Determining the utility of regular screening for DSA, the frequency and the associated cost-benefit is required for both memory and *de novo* alloimmune monitoring in all organs. While the epidemiology of memory and *de novo* alloimmunity and their natural history is becoming clearer, especially for kidney, there is a need for their further evaluation in all organs especially in the context of non-Caucasian genetics to determine risk factors and rates of progression – critical for the future design of prevention and intervention trials. The utility of HLA diagnostics in monitoring response to treatment is also in its infancy. As more therapeutic agents become available, defining a non-invasive tool (e.g. DSA attributes, other novel assays) that correlate with effective therapy will be required.

Call for Immediate Action

Two key themes that emerged from the working group are the following: (1) Currently there are no minimal guidelines for the details of information required for publications related to HLA antibodies in the context of solid organ transplantation. The lack of sufficient details prohibits in-depth understanding of the differences and similarities between studies and results in confusion. This can be resolved by requiring minimal criteria for publication; and (2) There is a pressing need to create centralized registries for highly sensitized patients and HLA-incompatible transplants. This is especially true for those transplanted with living donors in kidney paired exchange programs as well as with deceased donors when prioritized by KAS; Registries should also be created for patients who experience ABMR post-transplant. There registries should collect HLA antibody and typing information in a streamlined fashion; and house data defining treatment protocols and transplant outcome in a constant and consistent manner. These data registries could also be mined for epidemiological information (for example, race specific outcome).

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Disclosure

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Supporting Information

Additional Supporting Information is available online in the supporting information tab for this article.

Table S1: Complete list of references reviewed for STAR 1 recommendations

Table 1:

Strength of Recommendation		Patients	Clinicians	Policy
1	**Recommend**	Most would want	Most would do	Supports policy
2	**Suggest**	Majority would want but many would not	Different choices for different patients	Substantive debate to follow
3	**Do not Recommend**			

Quality of Evidence

A	**High**	RCT or Very strong evidence of association with no confounders
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, or
	There is no specific evidence to address recommendation, however it aligns with standard of care and would be agreed by a majority of experts that no specific evidence on the topic needs to be generated, nor would it be expected to be generated

Table 2: Recommendations for HLA Typing and Antibody Diagnostic Testing

HLA Typing

- **Should be "comprehensive"** requiring information regarding all major HLA loci – HLA-A, -B, -C, -DRB1, -DRB3/4/5, -DQA1/DQB1, and -DPA1/DPB1 in both donor and recipient **[2B]**
- **Should be performed using molecular methods** and, at least when determination of DSA is required, antigens with more than one allele common in the donor population, should be assessed at high-resolution (e.g. resolved to at least the common well defined (CWD) alleles) **[1A]**

HLA Antibody Assessment

- **Should be performed by solid phase assays and should include information regarding all major HLA loci** [HLA-A, -B, -C, -DRB1, -DRB3/4/5, -DQA1/DQB1, -DPA1/DPB1] **[2B]**. If possible antibody information should be captured at the **allele level** (*in fact, the software provided by the manufacturer already provides the information at the allelic level, in addition to the serologic level that is currently used*).
- **Measures to remove inhibition <u>must</u> be put in place [1A].** Verified methods include EDTA and/or titration studies. Other methods such as dialysis, DTT treatment or heat-inactivation have been reported. All approaches should be further optimized.
- **Mechanism should be put in place to detect phenomenon of potential "epitope sharing"** (such as stacking of antibodies against members of a known CREG) **[EO]**. Methods to test for this hypothesis should be sought when possible (e.g., performing surrogate XM if possible), or as minimal practice alert the clinicians of the potential presence of such phenomenon. In such

instances one cannot rely on the use of virtual crossmatch (vXM) and a physical/lymphocyte crossmatch must be performed.

- **A MFI between 1000 to 1500 may be used as a universal cut-off values** for multi-center clinical trials **[2B]**[Note: a cut-off of 1400 may have the best performance attributes (i.e. optimal cut-off for the correct classification using both class I and II beads in the CTOT standardization project)]. Caveats:
    - o Specificities below the MFI cut-off may be considered a “false negative” and assigned when the antigen/allele in question belongs to a CREG group or shares epitope(s).
    - o Specificities above the MFI cut-off may be considered a “false positive” where reactivity is suspected or confirmed to be:
        - Directed against denatured/cryptic epitope(s).
        - Part of a non-specific pattern (e.g. “hot beads”).
        - Directed against an auto-antigen/allele.
- **Differences of <25% in MFI values should not be considered clinically meaningful**, even in a very rigid standard operating procedure environment. In more relaxed situations, differences of <50% are likely meaningless **[2B]**.

Table 3: Working Definitions for Alloimmune Memory Responses

- **Latent Potential for an Alloimmune Memory Response:** One or more of,
    - A history of a sensitizing event;
    - Non-DSA HLA antibody detected at one or more time points prior to transplant;
    - Non-DSA HLA antibody detected at the time of transplant.
- **Active Potential for an Alloimmune Memory Response:** Donor specific antibody (DSA) are present at the time of transplant or in a historical serum sample tested, representing a risk for DSA associated injury.
- **Alloimmune Memory Response:** The development post-transplant at any time of an antibody that was detected prior to transplant and/or the development of a new DSA in the first 2 weeks post-transplant. Caveat to consider:
    - Development of a new DSA between 2 weeks and 3 months may still represent memory.

Table 4: Recommendations for evaluating a patient’s potential for alloimmune memory

- An accurate patient history must be obtained and shared with the histocompatibility laboratory, on an on-going basis **[1A]**. Specifically, the clinical program needs to document:

    I. HLA sensitizing events:

        - Pregnancies
        - Transfusions
        - Previous transplant
        - Implants (VADs, homografts, etc.)

    II. Inflammatory events that may boost pre-existing alloimmune memory:

        - Major surgeries
        - Major infections
        - Recent vaccinations

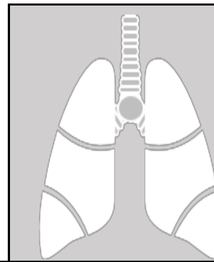
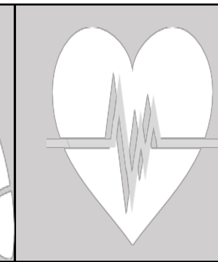
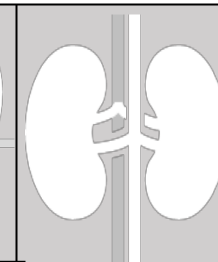
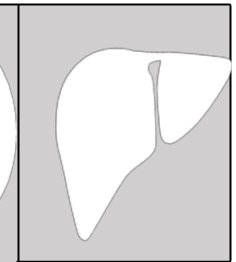
- Only patients without HLA sensitizing events may be considered immunologically low risk for alloimmune memory **[EO]**. All other patients should be categorized as having latent potential or active potential for an alloimmune memory response.
- The patient's alloimmune status should be used for risk stratification and informing frequency of pre- and post-transplant testing **[EO]**.
- The patient's immunization to alloantigens is dynamic. Re-evaluation of this status is required pre- and post-transplantation to assess whether management and monitoring protocols should be adjusted **[EO]**.

Note: While it is important to continually reassess the patient's alloimmune sensitization pre-transplant, the workgroup recognized there is insufficient data to recommend the frequency of testing in the absence of a sensitizing event.

Table 5: HLA Diagnostic Approach to Assign a Patient's Risk for Memory or Naïve Alloimmune Response

Pre-Transplant Donor - Recipient HLA Laboratory Evaluation						Immune Risk Assessment
CDC Crossmatch	Flow Crossmatch	Single Antigen Bead	History of Sensitization	HLA Molecular MM	HLA Identical	
DSA Positive	DSA Positive	DSA Positive				**Active memory and at risk for hyperacute rejection**
Negative	DSA Positive	DSA Positive				**Active memory and at risk for ABMR and TCMR**
Negative	Negative	DSA Positive				**Active memory and at risk for ABMR and TCMR**
Negative	Negative	Negative	Pregnancy or Prior Transplant with repeat MM			**At risk for latent memory with a recall B- and T- cell response**
Negative	Negative	Negative	cPRA with unknown repeat MM			**Potential risk for latent memory with a recall B- and T- cell response**
Negative	Negative	Negative	No	High		**Increased risk for *de novo* alloimmune response**
Negative	Negative	Negative	No	Low		**Baseline risk for *de novo* alloimmune response**
Negative	Negative	Negative	No	0	Yes	**Low risk for *de novo* alloimmune response**

Table 6 Organ Specific Recommendations

				
Peri-Transplant Evaluation (Cross-match)				
A Virtual and or Prospective Crossmatch between Donor and Recipient should be performed prior to allocation ideally and at a minimum prior to transplant	1C	1C	1C	
Avoiding HLA antibody is the preferred strategy	2C	2C	2C	
Post-Transplant Assessement of HLA Antibody				
Stable Grafts: Memory				
Early in paitents with active memory or at risk for latent memory	2C	2B	1A*	
Frequency depends on number and strength of pre-tx DSA	2C	2C	1A	
Not routine in liver				1D
Guided by non-liver organ in combined liver - other organ				1A
Stable Grafts: Naïve				
At intervals post-transplant	2C	2B	EO	EO
After modifications of immunosuppression or CNI avoidance protocols	EO	EO	1A**	
Suspected or documented non-adherence	EO	EO	1A***	
Graft Dysfunction: Memory and Naïve				
As part of investigation of acute and chronic graft dysfunction	1B	1B	1B	
If there are histologic features of graft injury	1B	1B	1B	
In liver, test patients w/ steroid resistant rejection and chronic rejection or those w/ clinical or histologic features of acute or chronic AMR				EO
Ancillary HLA Diagnostic Assays				
Complement or Isotype assays				
May be done but the role must be determined at the center level	2C		2C	

* (34)

** (21,35,36)

*** (22)

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