FDA 2017 AMR Workshop

Consideration of Quantitative Use of HLA Antibody Assays

And

Summary of the 2017 ASHI/AST STAR workgroup meeting

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DISCLOSURE:

Dr Tambur is a full time employee of Northwestern University, Chicago, Illinois.

Dr Tambur is a consultant to Astellas and direct the core lab for the Astound clinical trial.

Dr Tambur is a consultant to CSL Behring and received consultation fees.
Why do we need to quantify?

1. Predict XM results - Immediate
2. Project response to treatment? - Peri/post
4. Diagnostic aid to Bx/clinical presentation - Post
5. Monitor response to treatment (rejection) - Post
6. Predict long term outcome - Future
What is REALISTIC to EXPECT from the Single Antigen Bead Assay

One molecule of fluorochrome binds to one molecule of antibody

Logical to assume the assay will be quantitative

$\text{MFI} = \text{antibody strength}$
Why is it not working as expected?

Reagents Issues
- Amount of Ag on the beads
- 3D of the antigen
- Peptides lodged within the antigen
- etc.

Manufacturing issues
- 100 analytes per each class (thinking of increasing - XXX)
- Need to generate/procure the right cell lines
- Small market ~200 labs in the US
- Huge expense
Why is it not working?

Assay specific

Small volume - - - 20% variability (CTOT trial)
Automation? - - - did not seem to solve the issues

Reed E. et al. Comprehensive assessment and Standardization of Solid-Phase Multiplex-Bead Arrays for the Detection of Antibodies to HLA. AJT 2013

Serum specific

Inhibition
(Over)saturation

Shared epitope phenomenon

Lot 1 Lot 2 ASHI Lot 1 Lot 2 ASHI

%CV in MFI
Use of Titration Studies to Quantify Other Assays

- Agglutination assays to measure antibodies to Blood Group Antigens
- Antibodies to Autoantigens
- Antibodies in response to vaccination

Antibody Titer

- Antibody titre is the concentration of antibodies against a particular antigen
- Serology test is usually done using micro well plate.
- So that the test sample can be done in a very small sample.

https://i.ytimg.com/vi/otyp7xoVmSk/maxresdefault.jpg

http://www.microbiologybook.org/mayer/ab-ag-rx.htm
Labor intensive and expensive approach to remove inhibition
Titration reveals (over)saturation

| Titer | 65516 | 32768 | 16384 | 8192 | 4096 | 2048 | 1024 | 512 | 256 | 128 | 64 | 32 | 16 | 8 | 4 | 2 | 1 | N |
|-------|-------|-------|-------|------|------|------|------|-----|-----|-----|----|----|----|---|---|---|---|---|---|
| Median IgG MFI |       |       |       |      |      |      |      |     |     |     |    |    |    |   |   |   |   |   |   |
| A     | 19232 | 20281 | 14052 | 17873| 20532| 16789| 19132| 19115| 17307| 16540| 12786| 11078| 8459| 5746| 3288| 3496| 1307| 595 |
| B     | 15381 | 16508 | 17241| 20314| 19607| 16429| 16266| 17548| 14404| 11723| 7668 | 6132 | 3302 | 2424 | 1425 | 1294 | 1146 |
| C     | 20826 | 19193 | 17899| 15601| 15808| 15420| 14584| 11215| 11567| 5633 | 5020 | 2837 | 2273 | 1294 | 268  |
| DRB1  | 24272 | 22366 | 22068| 23131| 21672| 19041| 16641| 17709| 15110| 11994| 8871 | 5632 | 3068 | 2150 | 1311 | 585  |
| DQ    | 20155 | 21339 | 5685 | 16218| 14460| 15835| 18060| 16443| 16735| 16208| 12296| 8975 | 5445 | 3443 | 2414 | 1391 | 717  |
| DP    | 10765 | 10747 | 15802| 14639| 10563| 6880 | 5012 | 2956 | 2192 | 1433 | 12982| 7556 | 5814 | 3699 | 2553 | 1345 | 133  |
| DRB345| 23754 | 7100  | 23027| 20288| 20762| 21684| 19648| 18409| 17705| 17936|       |      |      |      |      |      |      |

Tambur AR & Wiebe C  Transplantation 2017, In Press

Critical information that is not appreciated otherwise

SIMILAR DATA OBTAINED FOR C1q ASSY
Is there an added value to titration studies over MFI?

N=48

Tambur AR & Wiebe C Transplantation 2017, In Press
Is there an added value to titration studies over MFI?

Tambur AR & Wiebe C. Transplantation 2017, In Press
Titration DOSE NOT resolve phenomenon of low MFI value/bead due to “Shared Epitope” recognition

Requires other approaches to resolve
Titration studies provide better tool to monitor antibody response to treatment

Tambur A et al. Human Immunology 2016
Titration studies provide better tool to monitor antibody response to treatment.
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**Decision Making/Monitoring of Responses to Ab Removal Rx**

- **Pre-RX 76487-1**
- **Pre-TX 76487-2**
- **Post-TX 76487-3**
- **F/U Post 76487-4**
- **IgG C1q Titer**

**IgG Titer**

- **Pre-TX 76487-1**
- **Pre-TX 76487-2**
- **Post-TX 76487-3**
- **F/U Post 76487-4**

**C1q Titer**

- **Pre-TX 76487-1**
- **Pre-TX 76487-2**
- **Post-TX 76487-3**
- **F/U Post 76487-4**
Correlation Between Antibody Titer and Response to PP/IVIg

**Class I**

- Log2 titer reduction vs Treatment cycles
- n=478
- Ranges: Rounds 1-2: 51, 24; Rounds 3-9: 11, 74, 51, 84, 133; Rounds 14: 50

**Class II**

- Log2 titer reduction vs Treatment Cycles
- n=223
- Ranges: Rounds 1-2: 24, 28; Rounds 3-9: 28, 45, 32, 49; Rounds 14: 17

_D Pinelli et al, abstract accepted for ATC 2017_

*20 patients for Class I, 17 patients for Class II (NW/JH)*
Adjust our expectation from the assay

Do not use strict MFI as cutoff

Use additional tools to assess presence and strength of antibodies

Remediation...
Moving forward

Provide vendors resources of patient samples to enable QC / improvement of assays
<table>
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<tr>
<td><strong>Co-Chair</strong></td>
<td>Peter Nickerson</td>
</tr>
<tr>
<td><strong>Steering Committee</strong></td>
<td>Frans Claas; Ron Gill; Denis Glotz; Jon Kobashigawa; Michael Mengel; Peter Nickerson; Parmjeet Randhawa; Steve Woodle</td>
</tr>
<tr>
<td><strong>Group Leads</strong></td>
<td>Frans Claas; Trish Campbell; Sandy Feng; Howie Gebel; Annette Jackson; Kathryn Tinckam; Roz Mannon; Elaine Reed</td>
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<td><strong>Administrative Support</strong></td>
<td>Victoria Converse; Anthony Celenza</td>
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<tr>
<td><strong>Stakeholders</strong></td>
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Goals

• Establishing criteria to assess whether immune memory exists, how much risk that memory represents, and practice guidelines to manage the risk before and after transplant

• Establishing criteria to assess a patient as immunologically naïve, how much risk a given donor represents, and practice guidelines for management both before and after transplant

Guiding Principles

• Biologically driven based on scientific data

• Focused on state-of-the-art clinical diagnostics

• Clinical practice recommendations based on “GRADE” and Strength of evidence
Deliverables

1. Primer/Technical
   - Definition of terms
   - Overview of clinical laboratory diagnostics and their strengths and limitations

2. Definition of Immunologically Naïve vs. Memory – Biologic Basis
   - Class I and Class II – which genetic loci are relevant?
   - What clinical diagnostic tools are available and required?
   - What quality assessment is required for these tools to be valid?
   - Future basic science and diagnostic needs?
Deliverables

3. Immunologic Memory – clinical application
   • Pre-transplant assessment/management – risk stratification/desensitization
     • Class I vs. Class II
     • Determination of antibody attributes that have clinical impact
   • Peri-operative assessment/management
   • Post-transplant diagnostic monitoring
   • Post-treatment ABMR diagnostic monitoring
   • Future needs?

4. Immunologically Naïve – clinical application
   • Post-transplant diagnostic monitoring based on risk stratification
   • Post-treatment ABMR diagnostic monitoring
   • Future needs?
Recommendations for HLA testing to support clinical decision making in solid organ transplantation

- Donor and Recipient HLA typing

  1. Should be “comprehensive” requiring information regarding all major HLA loci – HLA-A, -B, -C, -DRB1, -DRB3/4/5, -DQA1/DQB1, and -DPA1/DPB1

  2. Should be performed using molecular methods and, at least for antigens with more than one allele common in the donor population, should be given at high-resolution (e.g. resolving to at least CWD alleles)
Patient HLA antibody assessment

1. Should be performed by a solid phase assay and should include information regarding all major HLA loci – HLA-A, -B, -C, -DRB1, -DRB3/4/5, -DQA1/DQB1, -DPA1/DPB1. 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).

2. Measures to remove inhibition must be put in place. Verified methods include EDTA (25/60mM) and/or titration studies. Other methods such as dialysis, DTT treatment or heat-inactivation have been reported but should be further verified.

3. Mechanism should be put in place to detect phenomenon of potential “epitope sharing” (such as stacking of antibodies against members of a known CREG). 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 vXM and physical/lymphocyte XM must be performed.
Why is it not working?

HLA is complicated (expensive), so we tend to over-simplify things

Solving equation by one Blondie:

\[ \frac{1}{n} \sin x = ? \]

\[ \frac{1}{n} \sin x = \]

\[ \sin x = 6 \]

3. Find x.

Here it is
I don't have high resolution typing but I can impute...

Donor typed as DR13 DQ6

Which allele of DQ6 is DSA?

What is the donor is part EUR part AFA?

And somehow we know the HR typing for the DRB1?

Is it the

\[ \text{DRB1}^*13:02 \text{ w DQB1}^*06:04 \]

or

\[ \text{DRB1}^*13:02 \text{ w DQB1}^*06:09 \]
New End-Points for New Generation Clinical Trials - DSA?
Make Sure DSAs are Measured Appropriately

- HR typing of donors (at least “for cause”) for all HLA loci
- Assigned Abs based on HR (provided with the kits)
- Determine whether SAB-kit indeed includes reagents to test DSA
- Remove potential inhibitory factors from serum
- Account for potential “Shared-Epitope” reactions
- MFI should not be used as a strict cut-off value
- Monitoring efficacy of treatment is best done by dilution studies
SENSITIZATION IN TRANSPLANTATION: ASSESSMENT OF RISK (STAR)
NORTH AMERICAN 2017 WORKING GROUP

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