Unacceptable HLA antigens:  
The key to finding a compatible donor for your patient

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J. Michael Cecka, Ph.D.  
Facilitator: Ruthanne L Hanto RN, MPH

Who is in the room today?

- Histocompatibility Lab Director
- Other Lab Personnel
- Physician or Surgeon
- Transplant Coordinator
- Mixed group
- Other

What is your level of understanding of Donor Antigens and Candidate Unacceptables in KPD?

- 1 Clueless
- 2 Novice
- 3 Basic understanding
- 4 Good at some stuff
- 5 Expert – ask me anything
Goal

- Provide an understanding of the importance of defining unacceptable antigens for successful KPD matches.
- Provide an understanding of the importance of donor antigen reporting in KPD paired donors

OPTN KPDPP Match Offer Refusal Reasons

- 20% Data are incomplete
- 40% other matches in the exchange fell through

- Of the remaining (40%) of refused matches:
  - 33% refused due to positive crossmatch or unacceptable antigens
  - 7% due to “candidate involved in a pending exchange”
  - 60% due to a variety of other donor and candidate reasons
Objectives

- Define appropriate candidate unacceptable antigens for successful KPD transplants.
- Accurately report donor antigens for successful KPD donation.
- Demonstrate data entry into the KPD application of UNet™

Unacceptable HLA Antigens:
The key to finding a compatible donor for your patient in KPD

Donor Antigens

- J. Michael Cecka, Ph.D., Professor Emeritus
  UCLA Immunogenetics Center

UNOS Kidney Paired Donation Recommendations

- Laboratory recommendations
  - Molecular HLA typing HLA-A,B,C,Bw4,Bw6, DRB1-5, DQA, DQB
  - DQA required for donors only
  - Solid-phase tests for antibody (single antigen)
  - Two levels of stringency - Unacceptable antigens + All others
  - Update antibody profile whenever
    - Antibodies change on quarterly screens
    - A sensitizing event occurs
    - A patient is inactive >3 mo
    - An unexplained positive crossmatch occurs
  - Histocompatibility Committee review of positive crossmatches in real time
Virtual Crossmatch

Critical Elements of the virtual crossmatch

- Accurate and realistic identification of patient antibodies that define unacceptable HLA antigens
- Accurate and complete identification AND reporting of donor HLA types
- If the donor antigens don’t match the patient’s antibody specificities there will be mistakes
- If the donor HLA type is not complete, there will be mistakes

Most virtual crossmatch failures are due to:

a. Inability to identify all unacceptable HLA antigens
b. Lack of allele-level HLA typing of the donor
c. Data entry errors
d. Failure to type the donor for HLA-DP
e. Sleeping during the webinar
HLA antigens are complex

**HLA Antigens and Alleles**

<table>
<thead>
<tr>
<th>Locus</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>DR</th>
<th>DQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antigens</td>
<td>26</td>
<td>47</td>
<td>17</td>
<td>15</td>
<td>7</td>
</tr>
<tr>
<td>Alleles</td>
<td>965</td>
<td>1,543</td>
<td>626</td>
<td>762</td>
<td>107</td>
</tr>
</tbody>
</table>

Some HLA antigens are common

Some HLA antigens have a different distribution
Some HLA antigens are rare and limited

HLA Antigens were defined by antibodies: Parent Antigens and Splits

Antigens

- HLA-A9
- HLA-A23, HLA-A24

- HLA-B14
- HLA-B64, HLA-B65

Alleles

- A*23:01 A*24:02
- A*23:02 A*24:03
- A*23:03 A*24:04
- A*23:04 A*24:06
- B*14:01 B*14:02
- B*14:03 B*14:04
- B*14:05

Don’t report broad HLA Antigens or allele groups

<table>
<thead>
<tr>
<th>Broad Ag</th>
<th>Antigen</th>
<th>Allele Groups</th>
<th>Antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td>A9</td>
<td>23,24</td>
<td>B14</td>
<td>64,65</td>
</tr>
<tr>
<td>A10</td>
<td>25,26,34,66</td>
<td>B15</td>
<td>62, 63, 71, 75, 76, 77</td>
</tr>
<tr>
<td>A19</td>
<td>29-33,74</td>
<td>-</td>
<td>60,61</td>
</tr>
<tr>
<td>A28</td>
<td>68,69</td>
<td>-</td>
<td>9,10</td>
</tr>
<tr>
<td>B12</td>
<td>44,45</td>
<td>-</td>
<td>17,18</td>
</tr>
<tr>
<td>B16</td>
<td>38,39</td>
<td>DRB1*03</td>
<td>5,6</td>
</tr>
<tr>
<td>B17</td>
<td>57,58</td>
<td>DQB1*01</td>
<td>7,8,9</td>
</tr>
<tr>
<td>B21</td>
<td>49,50</td>
<td>DQB1*03</td>
<td></td>
</tr>
<tr>
<td>B22</td>
<td>54,55,56</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Some common uncommon alleles

- A*11:01
- A*11:02 (~10% of 11s)
- A*30:01
- A*30:02 (~50% of 30s)
- A*66:01
- A*66:02 (~20% of 66s)
- B*27:05
- B*27:08 (~1% of 27s)
- B*15:02 (75)
- B*15:11 (~5% of 75s)

The promiscuous habits of HLA-DQ alpha

DQA1*02:01 DQB1*02:01 DQA1*02:01 DQB1*02:01 DQA1*02:01
DQ81*02:01 DQ81*02:01 DQ81*03:03 DQ81*04:01
DQA1*02 associated with DR7...Surrogate CPRA=23%

HLA-DP B1 Allele Frequencies

98% of 12,000 unrelated HSCT donors and recipients had common HLA-DP alleles (>2%)
75% both common 23% one common

Petersdorf, E. Blood 2007; 110(13)4560
Based on the following HLA profile, what would you enter into the KPD database?

- A 2,11
- B 12, 35
- Bw 4 pos; Bw6 pos
- Cw 5,4
- DR 4, 103
- DR51 neg; DR52 neg; DR53 pos
- DQ 5, 7
- DQA 01, 03
- DP undetermined

**Base on the B 12, 35 HLA profile, you would enter B 12 and B 35 into the KPD database?**

- True
- False

**Based on the following HLA profile, what would you enter into the KPD database?**

- A 2,11
- B 12, 35
- (Split B12 to B44)
- Bw 4 pos; Bw6 pos
- Cw 5,4
- (Report DR1 DR4)
- DR 4, 103
- DR51 neg; DR52 neg; DR53 pos
- DQ 5, 7
- DQA 01, 03
- DP undetermined
- (Perform DP typing)
Summary

- Make a close friend in the HLA lab...preferably the director
- Check the donor HLA type for accuracy, correctness and completeness prior to activating the pair...then have the lab check again
- Do not enter antigens that are not entered as unacceptable antigens (Parent, alleles)
- Do not forget Bw4.6 and DR51-53 DQ alpha
- Type donors for DP even though it is not required
- Use the preselect option...review with the laboratory
Why is the definition of unacceptable antigens so important for KPD?

- Candidates who come to KPD are usually sensitized (>60% have CPRAs >80)
- Compatible KPD matches are based on the presence of no or low levels of donor specific antibody (DSA)
- Unacceptable antigens (UAs):
  - Antigens considered a contraindication to Tx
  - Usually based on antibodies to potential donors
- Accurate UA definition optimizes planning for compatible exchanges
- Failure to accurately assess unacceptable antigens (UAs) disrupts exchanges

What are the effects of inaccurately entering unacceptable antigens on exchanges?

a. There are no effects
b. Your individual match is declined but the rest of the exchange can still move forward
c. The entire exchange could be terminated

Steps for UA Definition

- Development of center specific criteria for acceptable risk for KPD and/or KPD with desensitization
- Correlation of solid phase antibody assays (SPI) with crossmatch tests and center’s risk criteria
- Analysis of the antibody specificities for candidates to define corresponding UAs
- Re-evaluation of antibodies periodically and following any sensitizing event
**Center Specific Criteria**

- Dependent upon clinical protocols and the level, if any, of donor specific antibody (DSA) that is acceptable
- Factors to consider:
  - Are any repeat mismatches acceptable?
  - Are DSA levels that are XM - acceptable?
  - Is desensitization an option?
- These factors determine the stringency that must be applied to UA definition

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**Correlation of SPI with Crossmatch Results**

**T-CDC XM vs. Phenotype Panel MFI**

<table>
<thead>
<tr>
<th>MFI</th>
<th>Negative</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>500</td>
<td></td>
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</tr>
<tr>
<td>1000</td>
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<td></td>
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<tr>
<td>1500</td>
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<tr>
<td>2000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2500</td>
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</tr>
</tbody>
</table>

Cutoff: 9,000 MFI, correlation: $r = 0.92$, correct prediction: 96%

Zachary AA, et al. Hum Imm. 70: 574, 2009

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**T cell FCXM vs. Phenotype Panel**

<table>
<thead>
<tr>
<th>MFI</th>
<th>Negative</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>500</td>
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<td></td>
</tr>
<tr>
<td>1000</td>
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<td>4500</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5000</td>
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</tr>
</tbody>
</table>

Cutoff: 5300 MFI, correlation: $r = 0.87$, correct prediction: 98%

Zachary AA, et al. Hum Imm. 70: 574, 2009
**Considerations for SPI: Crossmatch Correlations**

- Correlations must be assay and antigen specific
  - HLA-Cw, DQ, DP concentrations are enhanced compared to HLA-A,B,DR (Zachary, et al. Meth Mol Biol 2012; Zachary, Rainesmoen, Cur Opin Organ Transplant, 2011).
  - Individual HLA-A,B,DR concentrations may vary
- Correlations should be on-going to account for:
  - Lot-to-lot variability
  - Changes in phenotypes and/or single antigen panels
Considerations for Individual Candidate’s Antibody Analysis

- Define the level of DSA that represents a contraindication or UA for each candidate –
  - Any DSA?
  - DSA+, but “virtual” CDC XM or FCXM negative?
  - Depends upon your center’s criteria
- Should account for day-to-day assay variability
- Should consider possible test interference

“Cut Off” MFI Values for Positive Antibody Levels

- There are no generally accepted “cut off” MFI values
- Reported MFI “cut offs” range from 500 to 3000
- Often what is considered “positive” is lower than what is unacceptable
- Values should be antigen specific
  - E.g., DQ values are higher than for DR UAs

High Risk vs. Low Risk UAs

- High risk – when DSA is at a level that is absolute contraindication at your center
  - List as UA
- Low risk – DSA is present but at lower levels and could be positive in combination with other donor antigens or when target antigen is homozygous
  - Try to identify antigen combinations that could reach unacceptable risk level
  - Consider these combinations in evaluating prospective KPD exchanges
Interpretation of Results: Positive Control Variation

SPI: Test Interference

- Substances inherent to the serum
  - High IgM levels
  - Immune complexes
  - Antibody to plastic

- Extrinsic factors
  - Therapeutic Abs: thymoglobulin, eculizumab, high dose IVIg
  - Bortezomib
**Methods to Deal with Interference and/or prozone**

- Dilution
- Ethylenediaminetetraacetic acid (EDTA) (Schnaidt M, et al. Transplantation. 2011)

**Frequency of Antibody Testing**

- Once at initial evaluation is NOT enough
- Specificity should be confirmed on at least two samples, preferably three to assess antibody trend
- Regular testing is necessary while the candidate is on the wait list to be sure UAs are current
- Test after any sensitizing event
  - Transfusions
  - Infection, other inflammatory events

• 65 renal transplant candidates with proinflammatory event c/in 1 month of aby testing
  A. 35 with culture+ infection; 97.1% had significant increase in aby
  B. 30 with proinflamm. events
    - Surgery
    - Myocardial infarction
    - AV line placement
**Recommended Best Practices**

*(Guidelines from the OPTN/UNOS Histocompatibility Committee and a recent national KPD Consensus Conference)*

- UAs should be based on Center specific risk criteria
- Correlation of antibody assays with risk criteria is essential
- Antibody definition should be done by at least two methods, one of which should be a solid phase assay
  - Crossmatch confirmation should be cell based
- Specificity should be confirmed using a single antigen assay for:
  - HLA-A,B,C, DRB1, DRB3-5, DQA1, DQB1, DPB1
- Antibody interpretation may require high resolution typing of both recipient and donor alleles

**Best Practices (con’t.)**

- Antibodies and corresponding UAs should be confirmed on at least two samples, updated at least quarterly, after any sensitizing event, and after any unexpected positive crossmatch
- Both high and low risk UAs should be considered
- Labs should achieve $\geq 95\%$ accuracy in virtual crossmatch prediction
- Histocompatibility evaluation should be used for preapproval of exchange matches

**Effective UA Definition:**

- **Recipient DSA Analysis**
- **UA Definition**
- **Potential Donor Mismatches**
  - High Risk Unacceptable
  - Low Risk Possibly Acceptable
  - Center Decision
  - No Risk Acceptable
- Preliminary - Final XM
Given the following results, what would you list for UAs?

### HLA Class I Abs

<table>
<thead>
<tr>
<th>Normalized MFI</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>16,952</td>
<td>A2</td>
</tr>
<tr>
<td>16,582</td>
<td>A69</td>
</tr>
<tr>
<td>12,951</td>
<td>A68</td>
</tr>
<tr>
<td>4364</td>
<td>B57</td>
</tr>
<tr>
<td>3108</td>
<td>A1</td>
</tr>
<tr>
<td>3102</td>
<td>B58</td>
</tr>
</tbody>
</table>

### HLA Class II Abs

<table>
<thead>
<tr>
<th>Normalized MFI</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>22,025</td>
<td>DR1</td>
</tr>
<tr>
<td>19,248</td>
<td>DR51 (B5*02:02)</td>
</tr>
<tr>
<td>18,564</td>
<td>DR9</td>
</tr>
<tr>
<td>17,438</td>
<td>DR51 (B5*01:01)</td>
</tr>
<tr>
<td>14,800</td>
<td>DQ6</td>
</tr>
<tr>
<td>12,204</td>
<td>DQ5</td>
</tr>
</tbody>
</table>

Specificity A68 with a normalized MFI 12,951 would be listed as an UA?

- True
- False

Answer: At JHMI, UAs are based on Abs that are CDC XM-:

- Class I: A2, A69, A68
- Class II: DR1, DR51, DR9
Only unacceptable antigens that are highly likely to cause a positive crossmatch
All other antigens to which the candidate has antibodies, not including the unacceptable antigens

Summary

- Accurate identification of both donor HLA and candidate unacceptable antibodies is critical to successful matching
- Transplant centers and HLA labs must work together to identify, enter, and verify appropriate data into the KPD system
- Declined Matches affect all other Matches in an Exchange

OPTN
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