The ABC’s of HLA: Beginners to Advanced

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Every cell expresses a HLA to present antigens to T lymphocytes

Consequences of Pre-formed Donor-Specific HLA Antibodies

- Hyperacute rejection
- Delayed graft function
- Accelerated acute rejection
- Chronic rejection
- Prolonged waiting times
- No transplantation

HLA is the Challenging Barrier to Transplantation

<table>
<thead>
<tr>
<th>HLA mismatched Allograft</th>
<th>Induction Therapy</th>
<th>Maintenance Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recipient</td>
<td>Lymphocytes Depletion</td>
<td>Immunosuppression</td>
</tr>
<tr>
<td></td>
<td>Antibody Depletion</td>
<td>Cyclosporine</td>
</tr>
<tr>
<td></td>
<td>Antibody Blocking</td>
<td>MMF</td>
</tr>
<tr>
<td></td>
<td>IVIG</td>
<td>Steroids</td>
</tr>
<tr>
<td></td>
<td>Anti-CD5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anti-CD2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anti-CD4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anti-CD8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Unacceptable Antigens</td>
<td></td>
</tr>
</tbody>
</table>

Recipient Rejection

HLA antibodies
- Transplantation
- Pregnancy
- Transfusion
**Means of HLA Antibody-Mediated Rejection**

1. Activation of complement cascade
   - C1q

2. Antibody-dependent Cell-mediated cytotoxicity (ADCC)
   - FcR
   - NK Cell

3. Opsonization & increased antigen presentation
   - FcR
   - APC
   - APC

4. Activation of Endothelial Cell
   - Organ Allograft Endothelium
   - HLA Class I
   - HLA Class II

**Histocompatibility Testing for Solid Organ Transplantation**

**Recipient**
- HLA Typing
- HLA Antibodies

**Donor**
- HLA Typing
- HLA Mismatch
- Preformed-DSA

**Pre-Transplant**
- Serum
- Crossmatch
- Compatibility

**Post-Transplant**
- HLA Antibodies
- Donor-specific Antibodies (DSA)

**Complement Dependent Cytotoxicity (CDC) Crossmatch**

**The New England Journal of Medicine**

**Abstract**

Crossmatch tests of the prospective kidney-transplant donor's lymphocytes with the serum of the prospective recipient in 225 transplants showed that eight of 186 with negative crossmatch failed to function immediately, in contrast to patients receiving secondary transplants. The effect was not a nonspecific one, for more immediate failures occurred among transplants from unrelated than among those from related donors. The corresponding frequency of positive crossmatch was 24 of 30 serologically positive and 8 of 187 negative.

<table>
<thead>
<tr>
<th></th>
<th>CDC xM</th>
<th>Hyperacute or Accelerated Rejection</th>
<th>Functional Graft</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive (n=225)</td>
<td></td>
<td>24</td>
<td>6</td>
</tr>
<tr>
<td>Negative (n=195)</td>
<td></td>
<td>8</td>
<td>187</td>
</tr>
</tbody>
</table>

**Specificity Problem**
Flow Cytometry Crossmatch

- Median Channel Shift (MCS) – a quantitative readout (Ag+Ab)
- Detects only IgG antibodies
- Non-specific reactivity can be reduced by Pronase digestion

Measure FITC intensity by flow cytometry

T cell MCS > 50
B cell MCS ≥ 120

Virtual Flow Cytometry Crossmatch

- Median Channel Shift (MCS) – a quantitative readout (Ag+Ab)
- Detects only IgG antibodies
- Non-specific reactivity can be reduced by Pronase digestion

T cell MCS > 50
B cell MCS ≥ 120

Flow Crossmatch - problems

- ~8% of flow crossmatches are false positive – unnecessary exclusion
- ~7% of flow crossmatches are false negative – risk to patient
Virtual Crossmatch - Essentials

Donor Recipient

- Buccal swab
- Serum
- DNA
- HLA Antibody Testing
  - Anti HLA-A2 antibodies
- HLA Typing
  - A2, A24, B7, B18, DR1, DR4

Virtual Crossmatch - Advantages

- Eliminates the physical crossmatch
  - Saves 4-6 hours – cuts down cold ischemic time
  - No samples required
  - Reduces laboratory & OPO workload
  - Reduces laboratory, OPO, and Tx program cost
- Adds precision to actual crossmatch
  - CDC/flow XM prediction
  - DSA identification
- Improves allocation efficiency
- Increased rate of transplantation for sensitized patients
- Risk of memory response can be accounted:
  - Previous transplants & pregnancies

Crossmatch Methods

<table>
<thead>
<tr>
<th>Crossmatch method</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Cost (US $)</th>
<th>Turnaround time</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDC</td>
<td>Low</td>
<td>Low</td>
<td>600</td>
<td>3.5 hours</td>
</tr>
<tr>
<td>Flow</td>
<td>Intermediate</td>
<td>Intermediate</td>
<td>600</td>
<td>5 hours</td>
</tr>
<tr>
<td>Pronase</td>
<td>&gt;Intermediate</td>
<td>&gt;intermediate</td>
<td>600</td>
<td>6.5 hours</td>
</tr>
<tr>
<td>Virtual</td>
<td>100%</td>
<td>100%</td>
<td>0</td>
<td>10 min</td>
</tr>
</tbody>
</table>

Virtual crossmatch by listing Unacceptable Antigens in UNet

- Unacceptable Antigens
  - Previous transplants & pregnancies
  - DSA identification
**Unacceptable HLA Antigens & Virtual Crossmatch**

<table>
<thead>
<tr>
<th>Candidate</th>
<th>Potential Donors, &gt;12,000</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>anti-A2</td>
<td>48% cPRA</td>
</tr>
<tr>
<td>+ anti-DR4</td>
<td>61% cPRA</td>
</tr>
<tr>
<td>+ anti-DQ5</td>
<td>76% cPRA</td>
</tr>
</tbody>
</table>

**KAS: Major Allocation Components**

- Increase priority for sensitized candidates/CPRA sliding scale
- Replace SCD/ECD with KDPI
- Add longevity matching
- Include pre-registration dialysis time
- Incorporate A2/A2B to B
- Base pediatric priority on KDPI
- Remove payback system
- Remove variances

**Priority points for CPRA>19%**

<table>
<thead>
<tr>
<th>CPRA (%)</th>
<th>KAS Priority Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–19</td>
<td>0</td>
</tr>
<tr>
<td>20–29</td>
<td>0.08</td>
</tr>
<tr>
<td>30–39</td>
<td>0.21</td>
</tr>
<tr>
<td>40–49</td>
<td>0.34</td>
</tr>
<tr>
<td>50–59</td>
<td>0.48</td>
</tr>
<tr>
<td>60–69</td>
<td>0.81</td>
</tr>
<tr>
<td>70–74</td>
<td>1.09</td>
</tr>
<tr>
<td>75–79</td>
<td>1.58</td>
</tr>
<tr>
<td>80–84</td>
<td>2.46</td>
</tr>
<tr>
<td>85–89</td>
<td>4.05</td>
</tr>
<tr>
<td>90–94</td>
<td>6.71</td>
</tr>
<tr>
<td>95</td>
<td>10.82</td>
</tr>
<tr>
<td>96</td>
<td>12.17</td>
</tr>
<tr>
<td>97</td>
<td>17.3</td>
</tr>
<tr>
<td>98</td>
<td>24.4</td>
</tr>
<tr>
<td>99</td>
<td>50.09</td>
</tr>
<tr>
<td>100</td>
<td>202.1</td>
</tr>
</tbody>
</table>
Sequence-Specific Oligonucleotide (SSO) Hybridization Method

Distribution of CPRA scores in UCSF Kidney Transplant Waitlist (n=5461)

<table>
<thead>
<tr>
<th>CPRA</th>
<th>Points</th>
<th>#Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-19%</td>
<td>0</td>
<td>3974</td>
</tr>
<tr>
<td>20-84%</td>
<td>0.08-2.46</td>
<td>867</td>
</tr>
<tr>
<td>85-97%</td>
<td>4.05-17.3</td>
<td>273</td>
</tr>
<tr>
<td>98%</td>
<td>24.4</td>
<td>46</td>
</tr>
<tr>
<td>99%</td>
<td>50.09</td>
<td>85</td>
</tr>
<tr>
<td>100%</td>
<td>202.1</td>
<td>216</td>
</tr>
</tbody>
</table>

- 100 Color-coded Polystyrene beads using a blend of different fluorescent intensities of two dyes
- Each bead is conjugated with oligonucleotide probe specific for a HLA allele(s)

Polystyrene Microspheres

Luminex technology

Luminex: rSSO Method

Hybridization

Detection & Interpretation
Single Antigen Bead-based HLA Antibody Testing: Luminex Technology

**Detection & Interpretation**

- **Patient's serum** + **Single Antigen beads**
- **A2**, **B55**, **A66**
- **Detection & Interpretation**

**HLA class I antibody test results: Antibodies to A2 CREG**

- Specificities: A2, A68, A69, B57, B58
- **CPRA:** 62%
- **One Antibody**
Public and Private Epitopes (antigenic determinants)

Public Epitopes
- A2
- A68
- A69
- B57
- B58

Private Epitopes

Specificities:
- A2, A68, A69, B57, B58

CPRA:
- 62%

One Antibody CPRA:
- 62%

HLA class I antibody test results:
- Antibodies to A2 CREG

No antibodies to self-HLA are made.
Individuals alloimmunized by a specific HLA type can make antibodies to many HLA types.
### Cross-REactive Groups (CREG)

<table>
<thead>
<tr>
<th>CREG</th>
<th>HLA Specificities</th>
<th>CPRA value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>A1, A3, A11, A29, A30, A31, A32, A80</td>
<td>65%</td>
</tr>
<tr>
<td>A2</td>
<td>A2, A23, A24, A48, A49, B57, B58</td>
<td>75%</td>
</tr>
<tr>
<td>A10</td>
<td>A25, A26, A32, A33, A34, A43, A66, A74</td>
<td>22%</td>
</tr>
<tr>
<td>Bw4</td>
<td>A23, A24, A25, A32, Bw4</td>
<td>74%</td>
</tr>
<tr>
<td>B5</td>
<td>B18, B35, B46, B49, B50, B51, B52, B63, B66, B71, B72, B73, B75, B76, B77, B78</td>
<td>56%</td>
</tr>
<tr>
<td>Bw6</td>
<td>Bw6</td>
<td>85%</td>
</tr>
<tr>
<td>B7</td>
<td>B7, B8, B13, B27, B41, B42, B47, B48, B54, B55, B56, B59, B60, B61, B67, B81, B82</td>
<td>59%</td>
</tr>
<tr>
<td>B8</td>
<td>B8, B18, B38, B59, B64, B65, B67</td>
<td>36%</td>
</tr>
<tr>
<td>B12</td>
<td>B13, B37, B41, B44, B45, B47, B49, B50, B60, B61</td>
<td>48%</td>
</tr>
<tr>
<td>C1</td>
<td>Cw1, Cw7, Cw9, Cw10, Cw12, Cw14, Cw16, B46, B73</td>
<td>77%</td>
</tr>
<tr>
<td>C2</td>
<td>Cw2, Cw4, Cw5, Cw6, Cw7, Cw8, Cw9, Cw10, Cw12, Cw14, Cw16, B46, B73</td>
<td>66%</td>
</tr>
<tr>
<td>DR1</td>
<td>DR1, DR10, DR13</td>
<td>21%</td>
</tr>
<tr>
<td>DR51</td>
<td>DR51, DR15, DR16</td>
<td>29%</td>
</tr>
<tr>
<td>DR52</td>
<td>DR52, DR11, DR12, DR13, DR14, DR17, DR18</td>
<td>62%</td>
</tr>
<tr>
<td>DR53</td>
<td>DR53, DR4, DR7, DR9</td>
<td>50%</td>
</tr>
<tr>
<td>DQ1</td>
<td>DQ5, DQ6</td>
<td>64%</td>
</tr>
<tr>
<td>DQ2</td>
<td>DQ2</td>
<td>37%</td>
</tr>
<tr>
<td>DQ3</td>
<td>DQ7, DQ8, DQ9</td>
<td>56%</td>
</tr>
<tr>
<td>DQ4</td>
<td>DQ4</td>
<td>10%</td>
</tr>
<tr>
<td>DP1c</td>
<td>DP2, DP3, DP4, DP6, DP9, DP10, DP11, DP14, DP17, DP18, DP20, DP28</td>
<td>---</td>
</tr>
<tr>
<td>DP2c</td>
<td>DP1, DP5, DP13, DP15, DP19, DP23</td>
<td>---</td>
</tr>
</tbody>
</table>

**Bw6 Antibodies**

Spouse HLA
- A2-B61(Bw6)-DR4
- A2-B39(Bw6)-DR4

Self HLA
- A2-B44(Bw6)-DR4
- A2-B52(Bw6)-DR4

Women alloimmunized by Bw6 motif can make antibodies to 2/3 of HLA-B types

Bw6 Antibodies – Risk of memory response

CPRA=85%
Bw4 Antibodies

A | B | Cw | DR | DR | DQ
---|---|---|----|----|---
2  | 35(Bw6) | 4 | 4 | 53 | 8
31 | 35(Bw6) | 4 | 11 | 52 | 7

CPRA=61%

Antibodies to all HLA except to self-HLA

Bw4 & Bw6 Antibodies

| A | B | Cw | DR | DR | DQ |
---|---|---|----|----|---|
2  | 46 | 1  | 9  | 53 | 9 |
2  | 46 | 1  | 14 | 52 | 5 |

Allele-specific Antibodies
HLA lab updates VXM & PXM qualification weekly

- Antigen Report (HLA, Tissue/Apex)
- Excel file of waiting list

- Coordinator
- On-call staff
- Surgeon
- Physician

 HLAXpress™ Pipettor

 HLA Antibody Report

Probabilities:
- Probability of matching:
  - Full match: 1%
  - Partial match: 0%

Patient HLA Types:
- A1, A2, A3, B1, B2, B6, Cw1, Cw2, Cw3, DRB1, DRB3, DQA1, DQB1, DPA1

Prior Allografts:
- Date of Transplant: 2012
- Donor Name: Maria
- HLA Type: A2, B7, Cw1, Cw2, Cw3, DRB1, DRB3, DQA1, DQB1, DPA1

This candidate does NOT qualify for virtual crossmatch and requires physical flow cytometry crossmatch.

VXM or PXM

HLA Lab

9/30/2016
Antibody Binding Sites on HLA (epitopes)

Conformational epitope

Peptide+HLA epitope

Peptide epitope

Linear epitope

Problems with Single Antigen Assay

(False Positive/Negative Reactions)

- Protein Miss fold
- Denatured Antigens
- Cryptic Epitope
- Loss of Epitope
- High Sensitivity
- Variable Densities
- Not all Alleles are Covered

HLA Antibody Profile in Kidney Waiting List Candidates (n=5281)

- HLA Antibody Screen by Mixed Beads/Phenotype Beads
  - Negative (n=1182)
  - Positive (n=4099)
  - Female – 1st Tx (n=364)
  - Female – Re-Tx (n=26)
  - Male – 1st Tx (n=751)
  - Male – Re-Tx (n=41)

- HLA Antibody Testing by Single Antigen Beads
  - 4099 candidates X 123 antibodies = 504,177 antibodies with MFI
HLA Expression Variation

- Locus-specific (<Cw)
- Allele-specific (<B44)
- Tissue-specific (<neuronal tissue)
- Cytokine-induced (IFN-γ)
- Down regulation by viral infection and tumor transformation.

Single Class I DSA MFI vs. T cell Crossmatch MCS

Single Class II DSA MFI vs. B cell Crossmatch MCS

HLA lab Protocol for Deceased Donor Kidney Transplantation

- Single antigen bead HLA antibody identification (At least 2 sera are tested that are drawn within a year)

- HLA Antibodies Negative
  - Few / well defined HLA-A,B,C, DR, DQB and/or DQA Antigens only

- HLA Antibodies Positive
  - Well defined antibodies and/or
    - DPβ, DPα Ab
    - Allele-specific Ab
    - Unstable Ab
    - Too many weak Ab
    - Typically >1000 MFI

- Unacceptable HLA Antigens
  - None
  - - CREG with any MFI
  - - Typically >2000 MFI

Crossmatch

- VXM (DSA)
- VXM (DSA)
- PXM - Call Lab/ Director

All VXM are retrospectively confirmed by FXM
9/30/2016

New Kidney Allocation System

Waiting List (n=5416)

- New Candidate
- HLA Typing & Antibodies by Single HLA Beads
- Virtual XM candidate
- Physical XM candidate
- Tray List: quarterly sera
- List Unacceptable Antigens; Receives points per CPRA

<20% CPRA

100% CPRA

CPRA

0-79% (n=1159)
80-94% (n=151)
95-98% (n=120)
99% (n=80)
100% (n=209)

% of candidates

Antibody profile of candidates with total points ≥7 (n=1719)

Most transplants are performed using VXM approach in new KAS era

Re-Tx candidates with total points ≥7 (n=1719)

- FXM
- VXM

FXM

14.6% (n=64)

VXM

85.4% (n=108)

Male

Female

5.5%

17.9%

31.7%

28.8%

61.2%

63.3%
Pre- vs. Post-KAS: Transplant rate

![Graph showing transplant rate comparison between Pre-KAS (1/1/2014 to 12/3/2014) and Post-KAS (12/4/2014 to 7/31/2015).]

CPRA 99-100% recipient “bolus effect”

- Transplants to CPRA 99-100% patients rose sharply after KAS but have been tapering over time, likely due to a bolus effect.

Frequency of CREG Antibodies in Kidney waitlist candidates with different CPRA Groups

![Bar charts showing the frequency of antibodies in candidates with different CPRA groups.]

Most 100% CPRA candidates are sensitized to large number of HLA antigens

- **Candidate#1:**
  - DR: 4 7 8 11 12 13 14 15 16 17 18 103
  - DRw: 51 52
  - DQ: 6 7 8 9

- **Candidate#2:**
  - A: 1 2 11 24 25 26 29 30 31 32 33 34 36 43 66 68 69 74
  - B: 13 18 27 37 38 39 41 42 44 45 46 47 49 51 52 53 54 55 56 57
  - Cw: 1 2 5 6 9 10 12 14 15 16 18
  - DR: 1 4 7 8 9 10 11 12 13 15 16 103 14:02
  - DR: 51 53
  - DQ: 4 6 7 8 9
  - DQA: 02 03
  - DP: 2 3 6 9 10 14 17 18 20 28 04:02
CIBMTR monitoring of 1-year Overall Survival for First Allogeneic HCT (performed 2011-2013) suggests that, based on the complexity of the HCTs performed at UCSF:

Our predicted OS rate should be 78.9% (95% CI: 71.5-86.6%)
Our actual OS was 85.4% (N = 103)