Reflections and Future Directions
HLA Testing in Transplantation

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Disclosure

• Nothing significant to disclose
Disclosure

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HLA class I and class II antigens

- Monomer with non-covalently associated subunit (β2m)
- Presents antigenic peptides to CD8+ T cells
- Expressed by all nucleated cells including endothelium

- Heterodimer
- Presents antigenic peptides to CD4+ T cells
- Restricted expression on antigen presenting cells (dendritic cells, B cells, macrophages)
- Inducible on other cells (endothelium and epithelium)
Polymorphism of the Major Histocompatibility Complex in humans - Human Leukocyte Antigen (HLA)
Approximately 25-30% chance of having an HLA matched sibling
Polymorphic residues on Class I HLA molecules (polymorphisms are concentrated around peptide binding groove)

Top view

Side view

HLA-A

α chain conserved aa

α chain polymorphic aa

Peptide

β2 microglobulin

HLA-B

HLA-C
HLA antibody development

Your ("self") HLA
HLA antibody development

Your ("self") HLA

Donor ("allo") HLA
Your ("self") HLA

Donor ("allo") HLA
HLA antibody development

Your (“self”) HLA

Donor (“allo”) HLA

Sensitizing events:
Pregnancy
Transplantation
Transfusion
Strategies used to avoid/minimize transplant rejection

- HLA typing and matching of recipient/donor pairs
- Detection of donor specific HLA antibodies.
Evolution of HLA testing

Past (2010)
- HLA typing
  - Serology
    - CDC
- HLA antibody screening
  - Lymphocyte panel
    - CDC
- Lymphocyte crossmatching
  - CDC

Present
- HLA typing
  - Molecular methods
    - IR SSOP, allele level
- HLA antibody ID
  - Solid Phase Assay
    - Virtual crossmatch
- Lymphocyte crossmatching
  - Flow cytometry
3-Color Flow Cytometric Crossmatch

Donor lymphocytes

Recipient serum

T cell

B cell

3-Color Flow Cytometric Crossmatch

3-Color Flow Cytometric Crossmatch

3-Color Flow Cytometric Crossmatch

3-Color Flow Cytometric Crossmatch

3-Color Flow Cytometric Crossmatch

3-Color Flow Cytometric Crossmatch

Detect fluorescent labels by flow cytometry

3-Color Flow Cytometric Crossmatch

Gating strategy

- T cell X-match
  - Negative
  - Weak positive
  - Strong positive

- B cell X-match
  - Negative
  - Weak positive
  - Strong positive

FITC-α-IgG
HLA antibody identification by LABScreen®
Single Antigen Luminex Assay

100 different types of beads
Each bead type coded with different red/infrared dye combination
Each bead type is coated with different recombinant HLA antigen

Detection with anti-IgG-PE

2 lasers

Classification laser (635nm)
Tells the instrument which bead is being examined

Reporter laser (532nm)
Tells the instrument how much PE dye is bound to the bead
HLA antibody identification by LABScreen® Single Antigen Luminex Assay
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Single Antigen Luminex Assay
Patient: A3,31 B7,60 DR1,14 (52) DQB5,6
Previous Donor: A1, B8 DR7,17 (53,52) DQB2
Unacceptable antigens: A1, A36, B8
Patient: A3,31 B7,60 DR1,14 (52) DQB5,6

Previous Donor: A1, B8 DR7,17 (53,52) DQB2

Unacceptable antigens: DR7, DR53, DQ2
## Canadian PRA Calculator

### Select Blood Group:
- [ ] O
- [ ] A
- [ ] B
- [ ] AB

### Select Region:
- [ ] Alberta
- [ ] Atlantic-Canada
- [ ] British-Columbia
- [ ] Manitoba
- [ ] Ontario
- [ ] Saskatchewan
- [ ] Quebec

### Check all unacceptable antigens:
- [ ] 1
- [ ] 2
- [ ] 3
- [ ] 11
- [ ] 23
- [ ] 24
- [ ] 25
- [ ] 26
- [ ] 29
- [ ] 30
- [ ] 31
- [ ] 32
- [ ] 33
- [ ] 34
- [ ] 36
- [ ] 43
- [ ] 46
- [ ] 66
- [ ] 68
- [ ] 69
- [ ] 74
- [ ] 80

### Check all B unacceptable antigens:
- [ ] 7
- [ ] 8
- [ ] 13
- [ ] 18
- [ ] 27
- [ ] 35
- [ ] 37
- [ ] 38
- [ ] 39
- [ ] 41
- [ ] 42
- [ ] 44
- [ ] 45
- [ ] 46
- [ ] 47
- [ ] 48
- [ ] 49
- [ ] 50
- [ ] 51
- [ ] 52
- [ ] 53
- [ ] 54
- [ ] 55
- [ ] 56
- [ ] 57
- [ ] 58
- [ ] 59
- [ ] 60
- [ ] 61
- [ ] 62
- [ ] 63
- [ ] 64
- [ ] 65
- [ ] 67
- [ ] 71
- [ ] 72
- [ ] 73
- [ ] 75
- [ ] 76
- [ ] 77
- [ ] 78
- [ ] 81

### Check all Bw unacceptable antigens:
- [ ] 4
- [ ] 6
- [ ] N/A

### Check all Cw unacceptable antigens:
- [ ] 1
- [ ] 2
- [ ] 3
- [ ] 4
- [ ] 5
- [ ] 6
- [ ] 7
- [ ] 8
- [ ] 9
- [ ] 10
- [ ] 11
- [ ] 12
- [ ] 13
- [ ] 14
- [ ] 15
- [ ] 16
- [ ] 17
- [ ] 18

### Check all DR unacceptable antigens:
- [ ] 1
- [ ] 2
- [ ] 3
- [ ] 4
- [ ] 5
- [ ] 6
- [ ] 7
- [ ] 8
- [ ] 9
- [ ] 10
- [ ] 11
- [ ] 12
- [ ] 13
- [ ] 14
- [ ] 15
- [ ] 16
- [ ] 17
- [ ] 18

### Check all DR51/52/53 unacceptable antigens:
- [ ] 51
- [ ] 52
- [ ] 53

### Check all DPA unacceptable antigens:
- [ ] 1
- [ ] 2
- [ ] 3
- [ ] 4

### Check all DPB unacceptable antigens:
- [ ] 1
- [ ] 2
- [ ] 3
- [ ] 4
- [ ] 5
- [ ] 6
- [ ] 7
- [ ] 8
- [ ] 9
- [ ] 10
- [ ] 11
- [ ] 12
- [ ] 13
- [ ] 14
- [ ] 15
- [ ] 16
- [ ] 17
- [ ] 18
- [ ] 19
- [ ] 20
- [ ] 21

### Check all DQA unacceptable antigens:
- [ ] 1
- [ ] 2
- [ ] 3
- [ ] 4
- [ ] 5
- [ ] 6

### Check all DQB unacceptable antigens:
- [ ] 2
- [ ] 4
- [ ] 5
- [ ] 6
- [ ] 7
- [ ] 8
- [ ] 9

[Calibrate] [Reset]
Canadian PRA calculator

Selected blood groups:
Selected regions:
Unacceptable antigens:
A: 1, 36,
B: 8,
BW:
CW:
DR: 7,
DRW: 53,
DPA:
DPB:
DQA:
DQB: 2, 7,

Calculated PRA: 85%
Calculated PRA (Class I): 34%
Calculated PRA (Class II): 81%
Total Number of Records Used for Calculation: 1708

Tinckam, Liwski, Pochinco, Mousseau, Grattan, Nickerson and Campbell, AJT 2015
Virtual Crossmatching

Correlation between donor HLA typing and recipient HLA antibody testing.

Presence/Absence of Donor Specific Antibodies (DSA)
Class I HLA antibody analysis

Recipient HLA typing (mother)
- A3,3
- B7,7
- Cw7,7
- DR4,15
- DQ6,7

Donor HLA typing (son)
- A1,3
- B7,8
- Cw7,7
- DR4,17
- DQ2,7

Donor specific antibodies: A1, B8
Class II HLA antibody analysis

Donor specific antibodies: DR17, DQ2?

Recipient HLA typing (mother)
A3,3  B7,7  Cw7,7  DR4,15  DQ6,7

Donor HLA typing (son)
A1,3  B7,8  Cw7,7  DR4,17  DQ2,7
Renal Transplant Patient Workup

- Sera collected monthly and after sensitizing event.
- Antibody identification by Luminex every 3 months.
- Unacceptable antigens and HLA typing are entered into MOTP database.

- Donor HLA typing, HDSSO, entered into MOTP database.
- Virtual crossmatch excludes potential recipients with unacceptable mismatches.
- Top 5 potential recipients are selected for FCXCM.
- Top 2 recipients with negative FCXCM proceed to transplantation.
- Post Transplant Antibody Monitoring (Day of Tx, 3 wk, 3/6/12 mo)
Renal Transplant Patient Workup
Cardiac and Liver Tx Patients

- Sera collected monthly and after sensitizing event.
- Antibody identification by Luminex every 3 months.
- Unacceptable antigens and HLA typing are entered into MOTP database.

- Donor HLA typing, HDSSO, entered into MOTP database.
- Virtual crossmatch excludes potential recipients with unacceptable mismatches.
- Top 5 potential recipients are selected for FCXM.
- Top 2 recipients with negative FCXM proceed to transplantation.
- Post Transplant Antibody Monitoring (Day of Tx, 3 wk, 3/6/12 mo)
## Virtual Crossmatch

> 200 patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>HLA typing</th>
<th>VXM</th>
<th>HLA antibodies identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>A1,3</td>
<td>B8,50</td>
<td>DR4,17</td>
</tr>
<tr>
<td>Patient 2</td>
<td>A2,3</td>
<td>B44,62</td>
<td>DR7,8</td>
</tr>
<tr>
<td>Patient 3</td>
<td>A3,11</td>
<td>B8,18</td>
<td>DR4,15</td>
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<td>A1,24</td>
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<td>A23,24</td>
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<td>DR10,16</td>
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<td>A1,30</td>
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<td>DR11,13</td>
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<td></td>
<td></td>
<td>A11,A24,A25,B18,B44,DR17</td>
</tr>
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<td>A2,3</td>
<td>B44,62</td>
<td>DR7,8</td>
<td></td>
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<td>A1,A26,A33,B52,DR15</td>
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<td>Patient 3</td>
<td>A3,11</td>
<td>B8,18</td>
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<td>A2,A31,A66,B7,B52</td>
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<td>A1,24</td>
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### HLA typing

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<th>A1,A2</th>
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</table>
Impact on crossmatch and recipient selection time

- Start
- Buffy coat ready
- DNA ready
- Gel ready
- Typing ready
- PCR/gel ready
- Cells ready
- Lympho/T quik
- CXM ready to read
- CXM ready to enter
- CXM reported

**Top 2 recipients called in based on virtual crossmatch results (7-8h)**

- SMM
- Plate setup
- FCXM
- FCXM

- CXM reading
- CXM entry

**12-15h**

**9-11h**

< 1% False Positive FCXM
Class I specificity
A1 A23 A24 A25 A32 A13 B27 B37 B38 B41 B44 B45 B47 B48 B49 B50 B51 B52 B53 B57 B58 B59 B60 B61 B63 B7 B76 B77 B8 B81 B82

cPRA = 96%

Patient typing  A*11,33  B*35,35  Cw*04,04  DRB1*04,13  DR52, 53  DQ*03(7),03(8)
Donor typing  A*11,03  B*35,62  Cw*04,10  DRB1*04,11  DR52, 53  DQ*03(7),03(8)
Access to transplants for highly sensitized patients

Deceased donor transplants (April 2011-October 2011)

- 37 transplants with PRA 0-79%
- 6 transplants with PRA 80-100%

14% of transplants fell in the 80-100% PRA range.
Virtual Crossmatching
National Impact

• Canadian Transplant Registry
  – Kidney Paired Exchange (KPD)
  – Highly Sensitized Patient Registry (HSP), cPRA ≥95%
KPD Data Review

KPD Match Cycles

Of the 425 Transplants completed to date:

- 42 from Paired Exchanges
- 282 from Domino Chains
- 101 from Closed Chains
- 84 domino chains of 1 to 6 transplants each
- 21 chains of 3 to 6 exchanges

Courtesy: Mr. Sean Delaney
Canadian Transplant Registry & Programs: KPD

Activity by Transplant Centre

Registered Transplant
Waitlist Transplant
Candidates Registered

Total Transplants in Province
% of National Total

Courtesty: Mr. Sean Delaney
Transplant Recipients by cPRA

<table>
<thead>
<tr>
<th>% of Candidate Population</th>
<th>% of Recipients (n=409*)</th>
<th>% of Candidates in cPRA Group Transplanted</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>27%</td>
<td>49%</td>
</tr>
<tr>
<td>1%-79%</td>
<td>43%</td>
<td>57%</td>
</tr>
<tr>
<td>80-94%</td>
<td>14%</td>
<td>65%</td>
</tr>
<tr>
<td>95-96%</td>
<td>4%</td>
<td>58%</td>
</tr>
<tr>
<td>≥97%</td>
<td>10%</td>
<td>16%</td>
</tr>
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</table>

*Includes both Registered and Waitlist Recipients. cPRA information for 6 Waitlist Recipients (1%) not available.

Courtesy: Mr. Sean Delaney
HSP Data Review

Tracking by Home Province

<table>
<thead>
<tr>
<th>Province</th>
<th>HSP Transplanted</th>
<th>Interprovincial Transplants</th>
<th>Intraprovincial Transplants</th>
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<td>12</td>
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<tr>
<td>AB</td>
<td>20</td>
<td>18</td>
<td>2</td>
<td>40</td>
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<tr>
<td>SK</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>MB</td>
<td>10</td>
<td>8</td>
<td>2</td>
<td>20</td>
</tr>
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<td>ON</td>
<td>95</td>
<td>33</td>
<td>62</td>
<td>190</td>
</tr>
<tr>
<td>QC</td>
<td>30</td>
<td>18</td>
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<td>60</td>
</tr>
<tr>
<td>NB</td>
<td>4</td>
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<td>3</td>
<td>11</td>
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<td>NS</td>
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<td>26</td>
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<td>3</td>
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<td>NL</td>
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<td>1</td>
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<tr>
<td>Total</td>
<td>194</td>
<td>107</td>
<td>87</td>
<td>388</td>
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</tbody>
</table>

Based on Recipient PHN Province

19 Tx + 2

Courtesy: Mr. Sean Delaney
HSP Data Review

HSP Transplants by cPRA and Proportion Transplanted

![Bar chart showing the proportion transplanted by cPRA percentage. The chart shows the following:
- cPRA 95%: 27, 59%
- cPRA 96%: 16, 62%
- cPRA 97%: 28, 57%
- cPRA 98%: 44, 56%
- cPRA 99%: 46, 29%
- cPRA 100%: 33, 5%

Proportion Transplanted (with count)

cPRA (%)

95 96 97 98 99 100

Proportion Transplanted (with count)

0% 10% 20% 30% 40% 50% 60% 70% 80% 90% 100%

Courtesy: Mr. Sean Delaney
HSP Data Review

HSP Transplants by cPRA and Proportion Transplanted

Proportion Transplanted (with count)
cPRA (%)

95: 27 (59%)
96: 16 (62%)
97: 28 (57%)
98: 44 (56%)
99: 46 (29%)
100: 33 (5%)

98.5 – 99.4
99.5 – 99.9

Courtesy: Mr. Sean Delaney
Canadian Experience with virtual crossmatching (as of March 2015)

- Live Donor Paired Exchange
  - 5 years
  - 249 transplants + 62
  - 276 negative virtual XM
  - 24 positive FCXM
    - 10 False pos (TX)
    - 14 True pos
      - 10, DQ/DP missing
      - 2, DSA not listed in error
      - 2, new DSA

- Highly Sensitized Patient Registry, cPRA ≥95%
  - 6 months
  - Transplanted 74/511 pt
    - 44 pt, cPRA > 97%
  - 3 FCXM pos

Halifax Lab (0 positive FCXM)
Flow Crossmatch Protocol

Incubate 5x10^5 cells (30 µl) and serum 30 µl (4°C)          30 min

Wash x3 (5 min/spin)                                    15 min

Incubate with 100 µl anti-IgG-FITC/CD3/CD19 (4°C)    30 min

Wash x2 (5 min/spin)                                     10 min

Total assay time                                        2 HOURS
                                                        1h 25 min
Rapid Optimized Flow Crossmatch Protocol

Halifax/Halifaster Protocol

Incubate $5 \times 10^5$ cells (30 µl) and serum 30 µl (4°C) 30 min

1.5$ \times 10^5$ cells (15 µl) 30 µl (RT) 20 min

Wash x3 (5 min/spin) 15 min

(1 min/spin) 3 min

Incubate with 100 µl anti-IgG-FITC/CD3/CD19 (4°C) (RT) 30 min

30 min

Wash x2 (5 min/spin) 10 min

(1 min/spin) 2 min

Total assay time 2 HOURS 1h 25 min

>70% Time Reduction 30 min
Going with the Flow, Canadian Crossmatch Standardization

Robert Liwski, Denise Pochinco, Kathryn Tinckam, Howard Gebel, Patricia Campbell and Peter Nickerson

On behalf of the Nationwide Laboratory Oversight Committee & Canadian Blood Services

Liwski et al ASHI 2012
FXM Result Variability (Cell 1 vs Serum 1)

- **T cell X-match**

- **B cell X-match**

Class I DSA: A33 (2.5K), B13 (7K)

Class II DSA: DP17 (5K)

Liwski et al ASHI 2012
T cell FXM comparison

Lab Method

Halifax Protocol

Delta MCF

Moderate class I HLA DSA

Liwski et al. ASHI 2012
FXM signal/noise ratio
Lab vs Halifax Protocol

Paired Student’s t-Test, 2 tail analysis

* p < 0.05, ** p < 0.01, *** p < 0.001

Liwski et al ASHI 2012
FXM Precision
Lab vs Halifax Protocol

Relative Standard Error (%)

<table>
<thead>
<tr>
<th>T cell XM</th>
<th>B cell XM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lab</td>
<td>Lab</td>
</tr>
<tr>
<td>7.0+/-1.3</td>
<td>7.4+/-2.2</td>
</tr>
<tr>
<td>vs</td>
<td>vs</td>
</tr>
<tr>
<td>4.0+/-1.5</td>
<td>5.9+/-2.2</td>
</tr>
</tbody>
</table>

p<0.001
p=0.058

Liwski et al ASHI 2012
FXM time
Lab vs Halifax Protocol

FXM time per lab

Average FXM time

Paired Student’s t-Test, 2 tail analysis
***p < 0.001

Liwski et al ASHI 2012
Brazilian FCXM PT Result correlation

Rob Liwski
on behalf of ABH
Significantly reduction in false pos/neg reactions. 2013 vs 2015

- 2013 Q2: 13 false reactions
- 2013 Q4: 13 false reactions
- 2015 Q2: 4 false reactions
- 2015 Q4: 5 false reactions
Santa Casa Lab, VXM vs FCXM Correlation
Standard vs Halifax Protocol

T cell (A and/or B)

B cell (A and/or B and/or DR)

Cumulative SAB MFI range

% Positive FCXM

- Standard (n=405)
  - FP rate = 3.3%

- Halifax (n=2483)
  - FP rate = 3.0%

Neumann and Liwski, manuscript in prep.
http://stats.stackexchange.com/questions/423/what-is-your-favorite-data-analysis-cartoon
Wait List 2016

• Impact of:
  – virtual XM
  – Local priority for cPRA >80%
  – KPD
  – HSP
Wait list patients by cPRA

- 99-100: 24%
- 95-98: 21%
- 80-94: 55%
- 70-79: 0%
- 60-69: 0%
- 50-59: 0%
- 40-49: 0%
- 30-39: 0%
- 20-29: 0%
- 1-19: 0%
- 0: 55%

N=279
Wait list patients by cPRA, female vs male

<table>
<thead>
<tr>
<th>cPRA Range</th>
<th>Female</th>
<th>Male</th>
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<tbody>
<tr>
<td>99-100</td>
<td>35.6%</td>
<td>15.8%</td>
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<tr>
<td>95-98</td>
<td></td>
<td>23.5%</td>
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<tr>
<td>80-94</td>
<td>23.5%</td>
<td>19.5%</td>
</tr>
<tr>
<td>70-79</td>
<td></td>
<td></td>
</tr>
<tr>
<td>60-69</td>
<td>40.9%</td>
<td>64.6%</td>
</tr>
<tr>
<td>50-59</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40-49</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30-39</td>
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<tr>
<td>10-19</td>
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</tr>
<tr>
<td>0-9</td>
<td></td>
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</tbody>
</table>
Wait list patients by cPRA
First Time Transplant Candidates

<table>
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<tr>
<th>cPRA Range</th>
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<th>Male</th>
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<tr>
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<td>60-69</td>
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<td>0</td>
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<td>60-69</td>
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<td>0-1</td>
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n=102
n=46

female
male

48.4/83.6
Wait list patients by cPRA, Previous Transplant Patients

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<th>cPRA Range</th>
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<tr>
<td>50-69</td>
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</tr>
<tr>
<td>20-49</td>
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<td>1-19</td>
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<tr>
<td>0</td>
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</tbody>
</table>

n=42

- n=20
- n=20
- n=42
Wait list patients by cPRA
First Time Transplant Candidates

Wait List
n=172
11/2014 - 10/2016

Transplanted
n=279

21.5%
13.5%

n=172
11/2014-10/2016
Wait list patients by cPRA
First Time Transplant Candidates

Advantaged National Priority
Advantaged Local Priority

n=279
n=172
11/2014-10/2016
Wait list patients by cPRA
First Time Transplant Candidates

Disadvantaged
Despite Priority

Advantaged
National Priority

Advantaged
Local Priority

n=172
11/2014-10/2016

n=279

%
Wait list patients by cPRA
First Time Transplant Candidates

Disadvantaged Despite Priority
Advantaged National Priority
Advantaged Local Priority
Disadvantaged?

n=279
n=172
11/2014-10/2016

Wait List
Transplanted
Average wait time by cPRA, wait list patients
Wait list patients by cPRA
Previous Tx vs First Time Tx Candidates

<table>
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<th>cPRA Range</th>
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<th>Previous Tx</th>
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<td>35.8%</td>
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<td>64.2%</td>
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</tr>
<tr>
<td>0</td>
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<td>96.7%</td>
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n=62
n=217
Disadvantaged patients

- cPRA 99-100
  - 27.5%: females, hx of pregnancy
  - 72.5%: re-transplants

  - Prevention
    - better HLA matching at time of first Tx
    - withdrawal of immunosuppression?

- Willing to cross DSA
  - Stratify 99-100% using decimal point cPRA
Better HLA matching at time of first transplant
Patients with previous transplants
cPRA vs degree of mismatch

# patients

<table>
<thead>
<tr>
<th>HLA identical</th>
<th>1/8</th>
<th>2/8</th>
<th>3/8</th>
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<th>5/8</th>
<th>6/8</th>
<th>7/8</th>
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<td>12</td>
<td>12</td>
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<td>4</td>
<td>4</td>
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</table>
Evolution and Clinical Pathologic Correlations of De Novo Donor-Specific HLA Antibody Post Kidney Transplant

C. Wiebe\textsuperscript{a,†}, I. W. Gibson\textsuperscript{b,c,†}, T. D. Blydt-Hansen\textsuperscript{d}, M. Karpinski\textsuperscript{e}, J. Ho\textsuperscript{e}, L. J. Storsley\textsuperscript{e}, A. Goldberg\textsuperscript{d}, P. E. Birk\textsuperscript{d}, D. N. Rush\textsuperscript{e} and P. W. Nickerson\textsuperscript{a,c,*}

Consecutive Transplants
(n=392)

Excluded (n=77)
- DSA pre transplant (n=30)
- Primary non-function (n=11)
- Moved (n=14)
- Death with function (n=22)

Study Patients
(n=315)

- dnDSA (n=47)
- No dnDSA (n=268)

Class II DSA
Mainly DQ specific
Evolution and Clinical Pathologic Correlations of De Novo Donor-Specific HLA Antibody Post Kidney Transplant

C. Wiebe^a, †, I. W. Gibson^b,c, †,
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D. N. Rush^e and P. W. Nickerson^a,c, *

Class II DSA
Mainly DQ specific

Wiebe et al. AJT 12:1157, 2012
Class II HLA Epitope Matching—A Strategy to Minimize *De Novo* Donor-Specific Antibody Development and Improve Outcomes

C. Wiebe\(^1,2\), D. Pochinco\(^3\), T. D. Blydt-Hansen\(^4\), J. Ho\(^1\), P. E. Birk\(^4\), M. Karpinski\(^1\), A. Goldberg\(^4\), L. J. Storsley\(^1\), I. W. Gibson\(^3,5\), D. N. Rush\(^1\) and P. W. Nickerson\(^1,2,3,*\)

Consecutive Transplants
(n=392)

Excluded (n=106)
- DSA pre transplant (n=30)
- Primary non-function (n=11)
- Moved (n=14)
- Death with function (n=22)
- No Sample (n=29)

Study Patients
(n=286)

HLA-DR/DQ *dnDSA* (n=45)
- HLA-DR (n=21)
- HLA-DQ (n=36)

No *dnDSA* (n=241)

Class II HLA Epitope Matching—A Strategy to Minimize *De Novo* Donor-Specific Antibody Development and Improve Outcomes

A

Locus-Specific Low-resolution Mismatch

- No (n=265) vs Yes (n=21): p=0.0015
- No (n=250) vs Yes (n=36): p=0.0022

B

Locus-Specific High-resolution Mismatch

- No (n=265) vs Yes (n=21): p=0.0033
- No (n=250) vs Yes (n=36): p=0.0351

C

Locus-Specific Epitope Mismatch

- No (n=265) vs Yes (n=21): p<0.0001
- No (n=250) vs Yes (n=36): p<0.0001

Wiebe et al. AJT 13:3114, 2013
Class II HLA Epitope Matching—A Strategy to Minimize De Novo Donor-Specific Antibody Development and Improve Outcomes

C. Wiebe¹,², D. Pochinco³, T. D. Blydt-Hansen⁴, J. Ho¹, P. E. Birk⁴, M. Karpinski¹, A. Goldberg⁴, L. J. Storsley¹, I. W. Gibson³,⁵, D. N. Rush¹ and P. W. Nickerson¹,²,³,∗

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and P. W. Nickerson\textsuperscript{1,2,3,*}

Disadvantaged patients

- cPRA 99-100
  - 27.5%: females, hx of pregnancy
  - 72.5%: re-transplants

  - Prevention
    - better HLA matching at time of first Tx
    - withdrawal of immunosuppression?

- Willing to cross DSA
  - Stratify 99-100% using decimal point cPRA
Willing to Cross DSA
Clinical Significance of Pretransplant Donor-Specific Antibodies in the Setting of Negative Cell-Based Flow Cytometry Crossmatching in Kidney Transplant Recipients

O. O. Adebiyi¹, J. Gralla², P. Klem³, B. Freed⁴, S. Davis¹, A. C. Wiseman¹ and J. E. Cooper¹, *

Retrospective single center study, 660 kidney Tx patients with negative pre-Tx FCXM. 162 patients with DSA by SAB. Treated with standard immunosuppression: Tac/Myc/Ster (Basiliximab or ATG) Impact on survival, AR and GFR
Clinical Significance of Pretransplant Donor-Specific Antibodies in the Setting of Negative Cell-Based Flow Cytometry Crossmatching in Kidney Transplant Recipients

O. O. Adebuiyi, J. Gralla, P. Klem, B. Freed, S. Davis, A. C. Wiseman and J. E. Cooper

A

<table>
<thead>
<tr>
<th>Pre-Tx DSA</th>
<th>No Pre-Tx DSA</th>
</tr>
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<tbody>
<tr>
<td>15.4%</td>
<td>11.4%</td>
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B

<table>
<thead>
<tr>
<th>AR at 1 year</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1000</td>
<td>12.3%</td>
</tr>
<tr>
<td>1000-2999</td>
<td>15.4%</td>
</tr>
<tr>
<td>≥3000</td>
<td>26.3%</td>
</tr>
<tr>
<td>No Pre-Tx DSA</td>
<td>11.4%</td>
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</tbody>
</table>

C

<table>
<thead>
<tr>
<th>Class</th>
<th>AR at 1 year</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class I</td>
<td>9.9%</td>
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<tr>
<td>Class II</td>
<td>22.0%</td>
<td></td>
</tr>
<tr>
<td>Mixed</td>
<td>19.4%</td>
<td></td>
</tr>
<tr>
<td>No Pre-Tx DSA</td>
<td>11.4%</td>
<td></td>
</tr>
</tbody>
</table>

Overall p-value: p=0.18

p=0.24

p=0.10
Clinical Significance of Pretransplant Donor-Specific Antibodies in the Setting of Negative Cell-Based Flow Cytometry Crossmatching in Kidney Transplant Recipients

O. O. Adebiyi¹, J. Gralla², P. Klem³, B. Freed⁴, S. Davis¹, A. C. Wiseman¹ and J. E. Cooper¹,*
Background

- Groups have published acceptable outcomes with crossing “low level/selected antibody”
- To increase access for the difficult to match group
- Suggest we consider identifying antibodies that we would be “willing to cross”
- Bead positive current Flow XM negative.

Courtesy: Dr. Trish Campbell
Crossing Antibodies

• Clinicians will identify suitable candidates for 'Willing to Cross'
• HLA Labs will determine what antibodies to cross and reason why
• HLA Committee will review WTC outcome data over time to inform future strategies
• HLA Committee recommend piloting 'Willing to Cross' for patients with cPRA > 98% in the KPD program
• Crossing antibodies for a patient would be a clinical and HLA Lab decision

Courtesy: Dr. Trish Campbell
Crossing Antibodies – Questions

• Which group of patients should be used for willing to cross?
  – KPD first then HSP
  – cPRA > 98%
  – Blood Group
  – Wait Times

• If a patient’s cPRA drops below 95%, is the patient still eligible for KPD cPRA points and does ranking apply to “Willing To Cross cPRA “ or original cPRA?

• Are programs open to proceeding with a Flow XM negative DSA positive result?

• Does the current and the historical crossmatch with the most sensitized sera need to be negative or just the current?

• Should we adopt uniform immunosuppressive protocols?

Courtesy: Dr. Trish Campbell
Willing to Cross DSA patient cases
Patient Case 1

- 47 yo female
- 100% cPRA (Cl/II: 98/98)
- 3 pregnancies, 11 transfusions
- Wait listed since June 2011
- On HSP registry
## Patient Case 1

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<thead>
<tr>
<th></th>
<th>Patient</th>
<th>Donor</th>
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<tbody>
<tr>
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<tr>
<td>B</td>
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<td>C</td>
<td>02</td>
<td>06</td>
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<td>04(53)</td>
</tr>
<tr>
<td>DQB1</td>
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</tr>
<tr>
<td>DQA1</td>
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<td>02</td>
</tr>
<tr>
<td>DPB1*</td>
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<td>02</td>
</tr>
</tbody>
</table>

**Weak DSA against HLA-DP2, 2000-3000**

**Husband was DP2 positive**
Class II pre-Tx
Class II pre-Tx

Negative FCXM
Class II 7 days post
Class II 12 days post
Class II 6 weeks post
Class II 5 months post
Patient Case 2

- 39 yo female
- Dilated Cardiomyopathy
- 100% cPRA (CI/II: 100/29)
- pregnancies, transfusions
- Wait listed since August 2014
## Patient Case 2

<table>
<thead>
<tr>
<th></th>
<th>Patient</th>
<th>Donor 1</th>
<th>Donor 2</th>
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<tbody>
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<tr>
<td>B</td>
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<tr>
<td>C</td>
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</tbody>
</table>

**Weak DSA against HLA-A1, 1500 MFI**

**Strong A1 DSA in historical sera, 20,000 MFI**
Class I, 1 year pre-Tx
Class I, Pre Tx
Class I, Pre Tx

Negative FCXM, current sera
Strong Positive FCXM, historical sera
Class I, 1 week post
Class I, 2 weeks post
Class I, 2 months post
Class I, 13 months post
Conclusions

• Continued improvement in HLA testing over the last few years
  • Implemented state of the art methodology
  • Allows more complete assessment of immunologic risk
  • Decreased TAT
  • Decreased cost
  • Allowed for our participation in LDPE and HSP program
  • Increased transplantation of Highly Sensitized patients

• Future Directions
  • Refinement of allocation strategy
  • Improve matching
    • epitope
  • Consider Willing to Cross DSA approach to improve transplantation of highly sensitized (99-100) patients.
Acknowledgements

• Halifax Lab Technologists
  – Geoff Adams
  – Geoff Peladeau
  – Kelly Heinstein

• National Collaborators
  – Kathryn Tinckam
  – Patricia Campbell
  – Peter Nickerson

• National PT Testing Committee

• Canadian Blood Services

• Labs (west to east)
  – Vancouver
  – Edmonton
  – Calgary
  – Saskatoon
  – Winnipeg
  – Toronto
  – Ottawa
  – Hamilton
  – London
  – Montreal
  – Laval
  – Quebec City
  – Halifax
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  - Halifax

Welcome to Dr. Anna Greenshields
ASHI Director-in-Training

- Peter Nickerson
- Hamilton
- London
- Montreal
- Laval
- Quebec City
- Halifax
CERTIFIES THAT

Queen Elizabeth II Health Science Center
The Capital District Health Authority HLA Laboratory

ASHI # 09-9-NS-01-1
UNDER THE DIRECTION OF

Robert Liwski, MD

HAVING MET ALL APPLICABLE STANDARDS
AND THE REQUIREMENTS OF THE SOCIETY,
IS GRANTED ACCREDITATION

From: 9/1/2014  
To: 8/31/2016

Assuming all interim requirements are met,
In the following areas:

HSC/BM Transplantation: Related Donor
Solid Organ Transplantation: Deceased Donor

HSC/BM Transplantation: Unrelated Donor
Solid Organ Transplantation: Live Donor

PRESIDENT

ACCREDITATION PROGRAM DIRECTOR

Accreditation does not automatically transfer when a change in ownership, director or location has occurred.