The new Banff vision of the role of HLA antibodies in organ transplantation:
Improving diagnostic system and design of clinical trials

Carmen Lefaucheur
1. Banff 2015: Integration of HLA-Ab for improving diagnosis

2. Banff 2017: HLA-Ab for surrogate endpoint in clinical trials
Criterion 3 for diagnosis of ABMR in the kidney allograft: requirement of serologic evidence of DSAs against HLA or other antigens

Can DSA be waived for the diagnosis of ABMR in biopsies showing both morphologic evidence of acute or chronic tissue injury and C4d staining?

Opinion of the majority of experts at Banff 2015: «Biopsies meeting histologic criteria of ABMR and showing diffuse or focal linear peritubular capillary C4d staining on frozen on paraffin sections are associated with a high probability of ABMR and should prompt expedited DSA testing»

Potential role of DSAs currently not tested for in many centers (HLA DP, non-HLA antigens)
« The vital role and importance of serologic data in the overall assessment of the patient is heavily underscored »

« DSA testing shows outstanding sensitivity and negative predictive value for biopsy-diagnosed AMR »

« Quantitative DSA should be an essential component in the surveillance for AMR »

« Investigators have raised the issue of reintroducing HLA DSA testing information for use in the diagnosis of AMR and for risk assessment of persistent AMR and chronic allograft vasculopathy (CAV) »
## Implementing HLA-Ab detection into the AMR classification: Question identified and recommendations

<table>
<thead>
<tr>
<th>Question</th>
<th>Recommendations</th>
<th>Definitions</th>
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</table>
| What is the optimum timing of DSA testing post-transplantation?         | Stratify the patients based on risk for AMR and monitor:  
  - High and intermediate risk with each biopsy early post-transplant, 3, 6, 9, 12 months first year and yearly if no clinical indication.  
  - Low risk minimum 3,6,12 months, yearly after and anytime clinically indicated | - High Risk: presence of DSA at the time of transplant  
  - Intermediate Risk: presence of DSA in historical samples |
Importance of post-transplant DSA monitoring

**De novo DSA**

- **1st Month:**
  - Cooper: 15.6%
  - DeVos: 8.0%
  - Heilman: 8.2%
  - Everly: 3.0%
  - Wiebe: 0.0%

- **1st Year:**
  - Cooper: 27.0%
  - DeVos: 20.0%
  - Heilman: 17.6%
  - Everly: 11.0%
  - Wiebe: 2.0%

- **>1st Year:**
  - Cooper: 0% yr 2
  - DeVos: 5.0%/yr
  - Heilman: n.a.
  - Everly: 2.3%/yr
  - Wiebe: 2.0%/yr

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De novo DSA

- **20% 1st year**
- **5% per year**
- **2% per year**
Post-Tx DSA monitoring improves risk stratification for allograft loss

Value of Donor–Specific Anti–HLA Antibody Monitoring and Characterization for Risk Stratification of Kidney Allograft Loss

Denis Viglietti,*† Alexandre Loupy,†‡ Dewi Vernerey,§ Carol Bentlejewski,‖ Clément Gosset,§
Olivier Aubert,† Jean-Paul Duong van Huyen,** Xavier Jouven,† Christophe Legendre,†‡
Denis Glotz,*† Adriana Zeevi,‖ and Carmen Lefaucheur*†

Donor-specific HLA alloantibodies: Impact on cardiac allograft vasculopathy, rejection, and survival after pediatric heart transplantation

Andrew Tran, MD, a David Fixler, MD, a Rong Huang, MS, b
Tiffany Meza, MBA, MHS, c Chantale Lacelle, PhD, d and Bibhuti B. Das, MD a
DYNAMIC MODELING TO ASSESS IMPROVEMENT IN RISK PREDICTION ACCORDING TO DSA MONITORING AND CHARACTERIZATION

Model 1
Reference Model
- Donor characteristics
- Recipient characteristics
- Transplant characteristics
- Immunological Anti-HLA DSA characteristics

Model 2
= Reference Model + DSA detection Post transplant

Model 3
= Model 2 + DSA characteristic 1 Post transplant

Model 4
= Model 2 + DSA characteristic 2 Post transplant

Post-TX prospective anti-HLA DSA monitoring strategy
Day 0 Year 1 Year 2

Kidney allograft survival

NGO in official relations with WHO
www.tts.org

The Transplantation Society
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| When DSA should be treated?                                              | • Increased level (Titer and MFI) of persistent DSA should be biopsied to rule out subclinical rejection  
• Strong correlation of persistent DSA with graft dysfunction             | • Level DSA levels assessed by MFI strength and/or titration of sera  
• Persistent DSA: presence of DSA in serial samples  
• Transient DSA: presence of DSA only in one sample                         |
Major impact of subclinical AMR on allograft outcomes

Loupy et al., JASN (2015)

Loupy et al., AJT (2016)
Implementing HLA-Ab detection into the AMR classification: Question identified and recommendations

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<th>Recommendations</th>
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</thead>
<tbody>
<tr>
<td>Should DSA testing be performed with diagnosis of pAMR?</td>
<td>Testing for DSA presence and level (HLA and non HLA) should be performed to:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>a) correlate with severity of pAMR</td>
<td></td>
</tr>
<tr>
<td></td>
<td>b) assess efficacy of treatment</td>
<td></td>
</tr>
</tbody>
</table>
Post-Tx DSA level correlate with the severity of allograft injury and the risk of allograft loss

Lefaucheur et al., JASN (2010)
SAB assays to assess antibody removal by PP/IVIG/Rituximab

Tambur et al, Hum Immunol (2016)
<table>
<thead>
<tr>
<th>Problem</th>
<th>Interpretation</th>
<th>Resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-Ab to denatured antigens</td>
<td>False positive results: HLA-Ab to cryptic epitopes, clinically irrelevant</td>
<td>Repeat testing after acid treatment of SAB; surrogate crossmatch</td>
</tr>
<tr>
<td>Intrinsic and extrinsic factors inhibiting the SAB assay</td>
<td>False low MFI or negative results: due to inhibition of SAB assay</td>
<td>Dilution of sera pre-testing, adsorption, inhibition of C1q, addition of EDTA, heat treatment</td>
</tr>
<tr>
<td>Low MFI on SAB resulting in higher reactivity using cellular targets</td>
<td>False low MFI: DSA to a shared target present on multiple beads</td>
<td>Adequate analysis of specific DSA epitope</td>
</tr>
<tr>
<td>Using MFI to evaluate level and strength of DSA for risk stratification</td>
<td>Low or high MFI level of DSA may not correlate with risk of AMR, or response to treatment following antibody removal therapies</td>
<td>Modified SAB assay to distinguish between C’ and non-C’ binding DSA and determining titer of DSA (serial dilutions)</td>
</tr>
</tbody>
</table>
BANFF/ASHI HLA panel experts

Integrative assessment of DSA

- C’ binding
- Preformed
- De novo
- MFI levels
- Eplets
- IgG Subclass
- Titration

Graft failure
Disease progression
Risk prediction
Response to therapy

- Tambur et al, AJT (2015)
- Wiebe et al, AJT (2016)
- Loupy et al, NEJM (2013)
- Tait et al, Transplantation (2013)
« Accumulating evidence supports the concept that not all DSA are equivalent and that DSA properties (ability to bind complement or IgG subclass) beyond simple positivity and mean MFI are associated with distinct outcomes and injury phenotypes »

« These distinct DSA properties and their relationship with distinct allograft injury patterns is also increasingly demonstrated in other solid organ transplants such as liver and heart. »
# Biological rationale:
**Effects of complement-activating IgG subclasses**

<table>
<thead>
<tr>
<th>Cell Expression</th>
<th>abundance</th>
<th>complement activation</th>
<th>FcγRI</th>
<th>FcγRIIA-H131</th>
<th>FcγRIIA-R131</th>
<th>FcγRIIB</th>
<th>FcγRIIIA-F158</th>
<th>FcγRIIIA-V158</th>
<th>FcγRIIB NA1</th>
<th>FcγRIIB NA2</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG1</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>IgG2</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>--</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>IgG3</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>IgG4</td>
<td>+</td>
<td>-</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Valenzuela et al, Transplantation (2016)
Animal model of ABMR:
C' activation by DSA induces distinct allograft injury phenotype

![Image showing histological changes](image)

- **C’ DSA** vs **Control**
  - Endothelial Gene Expression
    - P = 0.002
    - P = 0.033
    - P = 0.020
  - Serum C3a concentration (ng/mL)
    - P = 0.014
  - Microvascular injury sum score
    - P = 0.026
  - CD68+ macrophage infiltrate per whole kidney cross-section
    - P = 0.402

Sis et al. (submitted)
Clinical correlations in kidney transplant patients: HLA-DR and -DQ Eplet mismatches and transplant glomerulopathy

<table>
<thead>
<tr>
<th>Odds Ratio of Developing TG based upon Total Eplet Threshold</th>
<th>Univariate</th>
<th>Multivariate **</th>
</tr>
</thead>
<tbody>
<tr>
<td>DR + DQ: ≥36 vs. &lt;36</td>
<td>2.01 [1.01-4.01]</td>
<td>3.21 [1.26-7.56]</td>
</tr>
<tr>
<td>DQ: ≥18 vs. &lt;18</td>
<td>1.50 [0.75-3.00]</td>
<td>2.42 [1.03-5.70]</td>
</tr>
<tr>
<td>DR: ≥15 vs. &lt;15</td>
<td>2.44 [1.16-5.12]</td>
<td>3.64 [1.42-9.37]</td>
</tr>
</tbody>
</table>

** Model includes Eplet exposure, recipient age, sex, peak PRA, race, induction and donor type.

Sapir-Pichhadze et al. AJT (2015)
Clinical correlations in kidney transplant patients: DSA C'-binding capacity and kidney allograft injury phenotype

Loupy et al. NEJM (2013)
Clinical correlations in kidney transplant patients: DSA IgG subclasses and kidney allograft injury phenotype

Lefaucheur et al. JASN (2016)
Gene expression profiling to define subtypes of ABMR

Cohort of interest with interrogation of the reference set (n=590)
1. Banff 2015: Integration of HLA-Ab for improving diagnosis

2. Banff 2017: HLA-Ab for surrogate endpoint in clinical trials
Criteria for validating surrogate variables

- Biological plausibility of the relationship
- Demonstration of the prognostic value of the surrogate for the clinical outcome
- Evidence that treatment effects on the surrogate correspond to effects on the clinical outcome

ICH Guideline E9 (1998)
DSA: 50 years of biological plausibility

**HLA antibodies**

**Hyperacute rejection**

With antibodies, CXM pos = 24/30 immediate failure

Patel & Terasaki, NEJM (1969)

**HLA antibodies**

**Acute rejection**

Feucht et al, KI (1993)

**HLA antibodies**

**Graft survival**

Terasaki, 15th Workshop

**From kidney to other transplants**

Heart

Liver

Lung
Class II HLA epitope matching and development of \textit{de novo} DSA

Wiebe et al., AJT (2015)
**DSA: biological gradient**

### Risk of ABMR

<table>
<thead>
<tr>
<th>DSA MFI_{max}</th>
<th>HR</th>
<th>P</th>
<th>[95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 500</td>
<td>1</td>
<td>&lt; 0.001</td>
<td>[4.6 – 134.8]</td>
</tr>
<tr>
<td>500 – 1500</td>
<td>24.8</td>
<td>&lt; 0.001</td>
<td>[4.6 – 134.8]</td>
</tr>
<tr>
<td>1500 – 3000</td>
<td>23.9</td>
<td>0.001</td>
<td>[3.5 – 160.8]</td>
</tr>
<tr>
<td>3000 – 6000</td>
<td>61.3</td>
<td>&lt; 0.001</td>
<td>[11.5 – 327]</td>
</tr>
<tr>
<td>&gt; 6000</td>
<td>113</td>
<td>&lt; 0.001</td>
<td>[30.8 – 414]</td>
</tr>
</tbody>
</table>

**Max DSA MFI > 500**

### Risk of graft loss

- **MFI ≤ 460**
- **460 < MFI ≤ 3000**
- **3000 < MFI ≤ 6000**
- **MFI > 6000**

Lefaucheur et al., JASN (2010)
**DSA removal: Beneficial effect**

Lefaucheur et al, AJT (2009)

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>N</th>
<th>Graft survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>OKT3</td>
<td>1993</td>
<td>43</td>
<td>57%</td>
</tr>
<tr>
<td>IVIg</td>
<td>2007</td>
<td>21</td>
<td>70%</td>
</tr>
<tr>
<td>PP/IVIg</td>
<td>2003</td>
<td>16</td>
<td>81%</td>
</tr>
<tr>
<td>PP/Ritux</td>
<td>2007</td>
<td>8</td>
<td>75%</td>
</tr>
<tr>
<td>PP/IVIg/Ritux</td>
<td>2009</td>
<td>12</td>
<td>91.3%</td>
</tr>
<tr>
<td>PP/Ritux/Bortezomib</td>
<td>2011</td>
<td>107</td>
<td>81%</td>
</tr>
</tbody>
</table>
Post-therapy drop of MFI correlates with improved graft survival independently of graft function and histology

N=278, median FU=3.5 yrs

<table>
<thead>
<tr>
<th>Multivariate Predictors</th>
<th>HR</th>
<th>95%CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>eGFR at ABMR diagnosis</td>
<td>0.93</td>
<td>[0.90-0.95]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IF/TA at ABMR diagnosis</td>
<td>2.44</td>
<td>[1.36-4.37]</td>
<td>0.003</td>
</tr>
<tr>
<td>Change in eGFR after SOC</td>
<td>0.24</td>
<td>[0.16-0.35]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Change in ptc Banff grade after SOC</td>
<td>1.50</td>
<td>[1.16-1.93]</td>
<td>0.002</td>
</tr>
<tr>
<td>Change in DSA IgG MFI after SOC</td>
<td>1.30</td>
<td>[1.11-1.52]</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Viglietti et al., ATC 2016
DSA as a surrogate endpoint for interventions in clinical trials

Occurrence of *de novo* DSA
- Efficacy of novel agents for baseline immunosuppression
- Safety of minimization strategies

Change in DSA level/C’-binding capacity
- Therapy efficacy in desenzitization
- Therapy efficacy in ABMR
- Post-Tx prophylaxis protocols in HLA-incompatible patients

Enrichment strategies based on DSA to increase endpoint frequency

Targeted population for graft loss
- High level DSA
- C’-binding DSA

Targeted population for occurrence of *de novo* DSA
- High class II epitope mismatch load
Thank you for your attention