Statement of Disclosure

Mark Haas serves as a paid consultant on pathology adjudication committees for two industry-sponsored clinical trials:

- Shire ViroPharma – Treatment of Acute ABMR
- AstraZeneca – Treatment of Proliferative Lupus Nephritis

Neither represents a conflict of interest relevant to any of the material presented in this talk.
The Banff Classification for Diagnosis of Renal Allograft Rejection: Updates from the 2017 Banff Conference

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Updates to the Banff diagnostic criteria for TCMR
- i-IFTA and its association with graft loss
- Association of i-IFTA with TCMR
- Addition of chronic, active TCMR to the classification

Updates to the Banff diagnostic criteria for ABMR – use of surrogate markers to diagnose ABMR in the absence of detectable DSA
- C4d
- Molecular markers

Recommendations regarding use of molecular diagnostics in diagnosis of ABMR and TCMR
For Diagnosis of T Cell-Mediated Rejection (TCMR) Banff Does Not Score Inflammation in Scarred Areas (i-IFTA)
Inflammation in areas of tubular atrophy is strongly correlated with renal allograft loss
Mannon et al, Am J Transplant 10: 2066-73, 2010 (DeKAF Study)
The Banff 2015 (Loupy et al, AJT 17: 28-41, 2017) classification thus contains quantification for i-IFTA:

- **i-IFTA 0**: no inflammation or <10% scarred cortical parenchyma
- **i-IFTA 1**: inflammation in 10-25% of scarred cortical parenchyma
- **i-IFTA 2**: inflammation in 26-50% of scarred cortical parenchyma
- **i-IFTA 3**: inflammation in >50% of scarred cortical parenchyma
and also adds an updated description for chronic, active TCMR:

Chronic allograft arteriopathy (intimal fibrosis with mononuclear cell infiltration in fibrosis, formation of neointima); note that the such lesions may represent chronic, active ABMR as well as TCMR; the latter may also be manifest in the tubulo-interstitial compartment but does not actually define tubulo-interstitial criteria for diagnosing chronic, active TCMR
Effect of i-IFTA on 1 year protocol biopsy on subsequent graft survival
Lefaucheur, Loupy et al (Paris); AJT in press
Acute TCMR during first 12 months post-transplant is associated with i-IFTA on 1-year protocol biopsy
Lefaucheur, Loupy et al (Paris); AJT in press

<table>
<thead>
<tr>
<th>First-year T cell-mediated rejection</th>
<th>Number of patients</th>
<th>No. with i-IFTA @ 1 year</th>
<th>OR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>798</td>
<td>306</td>
<td>1</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>142</td>
<td>85</td>
<td>2.7</td>
<td>[1.87-3.97]</td>
<td>&lt;.001</td>
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</tbody>
</table>
Combined impact of i-IFTA and tubulitis at year one on subsequent graft survival
Lefaucheur, Loupy et al (Paris); AJT in press

8 year graft survival

i-IFTA, no tubulitis 88%
i-IFTA + tubulitis 78%

P = 0.07
Chronic, Active TCMR

Grade 1a  Interstitial inflammation involving >25% of sclerotic cortical parenchyma (i-IFTA2 or i-IFTA3) with moderate tubulitis (t2) involving 1 or more tubules, not including severely atrophic tubules

Grade 1b  Interstitial inflammation involving >25% of sclerotic cortical parenchyma (i-IFTA2 or i-IFTA3) with severe tubulitis (t3) involving 1 or more tubules, not including severely atrophic tubules

Grade 2  Chronic allograft arteriopathy (arterial intimal fibrosis with mononuclear cell inflammation in fibrosis and formation of neointima)
- May also be an manifestation of chronic, active ABMR or mixed ABMR/TCMR
Banff 2013 Classification of Antibody-Mediated Rejection (ABMR) in Renal Allografts

**Acute/Active ABMR; all 3 features must be present for diagnosis*\(^a\)**

1. **Histologic evidence of acute tissue injury, *including one or more of the following***:
   - Microvascular inflammation \((g > 0^b\) and/or \(ptc > 0\))
   - Intimal or transmural arteritis \((v > 0)^c\)
   - Acute thrombotic microangiopathy, in the absence of any other cause
   - Acute tubular injury, in the absence of any other apparent cause

2. **Evidence of current/recent antibody interaction with vascular endothelium, *including at least one of the following***:
   - Linear C4d staining in peritubular capillaries \((C4d2 \text{ or } C4d3 \text{ by IF on frozen sections, or } C4d > 0 \text{ by IHC on paraffin sections})\)
   - At least moderate microvascular inflammation \([g + ptc] > 2^d\)
   - Increased expression of gene transcripts in the biopsy tissue indicative of endothelial injury, *if thoroughly validated*

3. **Serologic evidence of donor-specific antibodies (HLA or other antigens)**

*\(^a\) These lesions may be clinically acute, smoldering, or subclinical. Biopsies showing two of the 3 features may be designated as “suspicious” for acute/active ABMR.

*\(^b\) Recurrent/de novo glomerulonephritis should be excluded

*\(^c\) These lesions may be indicated of ABMR, TCMR, or mixed ABMR/TCMR

*\(^d\) In the presence acute T cell-mediated rejection, borderline infiltrates, or evidence of infection, ptc \(> 2\) alone is not sufficient to define moderate microvascular inflammation and g must be \(\geq 1\).
Chronic, Active ABMR; all three features must be present for diagnosis

1. Morphologic evidence of chronic tissue injury, including 1 or more of the following:
   - Transplant glomerulopathy (cg >0), if no evidence of chronic TMA
   - Severe peritubular capillary basement membrane multilayering (requires EM)
   - Arterial intimal fibrosis of new onset, excluding other causes

2. Evidence of current/recent antibody interaction with vascular endothelium, including at least one of the following:
   - Linear C4d staining in peritubular capillaries (C4d2 or C4d3 by IF on frozen sections, or C4d > 0 by IHC on paraffin sections)
   - At least moderate microvascular inflammation ([g + ptc] > 2)
   - Increased expression of gene transcripts in the biopsy tissue indicative of endothelial injury, if thoroughly validated

3. Serologic evidence of donor-specific antibodies (HLA or other antigens)

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† In the absence of evidence of current/recent antibody interaction with the endothelium (those features in section 2), the term active should be omitted; in such cases DSA may be present at the time of biopsy or at any previous time post-transplantation.

g Includes GBM duplication by electron microscopy only (cg1a) or GBM double contours by light microscopy

h ≥7 layers in 1 cortical peritubular capillary and ≥5 in 2 additional capillaries, avoiding portions cut tangentially

i In the presence acute T cell-mediated rejection, borderline infiltrates, or evidence of infection, ptc > 2 alone is not sufficient to define moderate microvascular inflammation and g must be > 1.
Comparison of Predictive Value of Banff 2013 vs. Banff 2007 Criteria for Chronic, Active ABMR

123 patients, single center, indication bx Jan 2006 – Oct 2014
45 reached combined endpoint of graft loss or doubling of SCr

<table>
<thead>
<tr>
<th></th>
<th>Banff 2007</th>
<th>Banff 2013</th>
</tr>
</thead>
<tbody>
<tr>
<td>% with CAABMR</td>
<td>18%</td>
<td>36%</td>
</tr>
<tr>
<td>HR of CAABMR for</td>
<td>1.6 [0.7-3.8]</td>
<td>2.5 [1.2-5.2]</td>
</tr>
<tr>
<td>combined endpoint</td>
<td></td>
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</tr>
</tbody>
</table>
1. What to do with a biopsy showing (g + ptc) ≥1, C4d+, + TG, and NO Detectable DSA?

2. What to do with a biopsy showing (g + ptc) ≥2, C4d-, + TG, and NO Detectable DSA?

According to Banff 2013/2015, such biopsies were termed “suspicious” for ABMR, which resulted in some confusion as to whether or not to treat the patient for ABMR.
Microvascular Inflammation (MVI) is NOT Specific for Active ABMR

356 clinically indicated renal allograft biopsies.

209 with $\text{MVI} = 0$ (25% DSA+, 8% C4d+)
67 with $\text{MVI} = 1$ (36% DSA+, 15% C4d+)
80 with $\text{MVI} \geq 2$ (54% DSA+, 50% C4d+)

B Sis et al (Edmonton)
and Neither is Transplant Glomerulopathy (TG) Specific for Chronic ABMR - TG Has Multiple Etiologies

1. Chronic/Persistent Antibody-Mediated Rejection
   (73% of for-cause biopsies with TG at mean of 5.5 yrs post- transplant were C4d+, had concurrent DSA, or both; Sis et al, AJT 7: 1743-1752, 2007)

2. Hepatitis C
   - Need to differentiate from recurrent or de novo MPGN, using IF and/or EM
   - Possibly related to TMA associated with anti-cardiolipin antibodies

3. Other forms of TMA

4. Cell-Mediated Rejection (?)

What about C4d?
C4d Staining in Renal Allografts: correlation with donor-specific Ab

- **Collins et al, JASN 10: 2208-14, 1999**
  100% of AR with +DSA were C4d+
  No C4d in DSA- AR, CSA toxicity

- **Maueyyedi et al, JASN 13: 779-787, 2002**
  30% of early AR C4d+ - 90% had anti-donor antibody
  2 morphologic subtypes of AMR - capillary, arterial
  Arterial (fibrinoid necrosis) had worse outcome

- **Bohmig et al, JASN 13: 1091-9, 2002**
  21/24 C4d+ cases had DSA by flow cytometric XM
  50% of C4d- biopsies had DSA
  93% specificity, 31% sensitivity *(IHC on paraffin sections)*
Should DSA be required for ABMR diagnosis in C4d+ biopsies?

Gaston et al (DeKAF Study), Transplantation 90: 68-74, 2010
61 patients with late indication biopsy (median 79 mo), TG and MVI

45 C4d- and DSA- (‘isolated TG’)
14 C4d+ and DSA- (6) or C4d- and DSA+ (8)
12 C4d+ and DSA+
Influence of DSA and C4d on Outcomes in Chronic, Active ABMR with Transplant Glomerulopathy
Lesage et al (Quebec City), Transplantation 99: 69-76, 2015

Isolated TG vs. C4d and/or DSA

\[ P = 0.01 \]

Number at risk
- Isolated TG: 44
- TG+C4d or DSA: 14
- TG+C4d+DSA: 12
Given the high specificity of C4d for DSA and these outcomes data, can DSA requirement for ABMR diagnosis be waived in biopsies of ABO-compatible kidneys with MVI and C4d?

YES 53/65 (82%)
What to do with a biopsy showing \((g + ptc) \geq 2, \text{C4d-}, \pm \text{TG, and NO Detectable DSA}\)?

Test for non-HLA DSA
- Not all labs do such testing for all relevant non-HLA Abs
- In most labs, routine DSA testing does not include HLA-C and HLA-DP

Consider molecular testing
Molecular ABMR Classifier Score

Based on 30 non-redundant probes, selected from comparisons between biopsies + or - histologic ABMR (DSA+, C4d+ or C4d-)
Cell types of highest expression, based on literature and/or expression in cell cultures:
Endothelial cells – 17
NK cells – 5
Tubular epithelial cells – 4
T cells – 3
Macrophages – 2
IFN Gamma-induced - 2
Unknown cell type - 5
Association of molecular ABMR score with histologic diagnosis (mixed rejections excluded)

<table>
<thead>
<tr>
<th></th>
<th>ABMR</th>
<th>No ABMR</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;0.2</td>
<td>64</td>
<td>66</td>
<td>130;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>PPV=0.49</td>
</tr>
<tr>
<td>≤0.2</td>
<td>46</td>
<td>499</td>
<td>545;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NPV=0.92</td>
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<tr>
<td>Total</td>
<td>110;</td>
<td>565;</td>
<td>675;</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>accuracy=0.83</td>
</tr>
<tr>
<td>sensitivity</td>
<td>0.58</td>
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<td></td>
</tr>
<tr>
<td>specificity</td>
<td>0.87</td>
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P. Halloran et al, JASN 26: 1711-1720, 2015
One and Three Year Post-Biopsy Graft Survival As a Function of Microarray and Histologic Diagnosis of ABMR/Mixed Rejection


M+ = ABMR score >0.2; C+ = diagnostic or suspected ABMR C4d+ or C4d-
Can DSA requirement for ABMR diagnosis be waived in biopsies of ABO-compatible kidneys with MVI (g + ptc ≥2) and either C4d or a thoroughly validated molecular ABMR classifier?

YES 37/65 (57%)
# Recommended Indications for Use of Molecular Diagnostics in Renal Allograft Biopsy Diagnosis

<table>
<thead>
<tr>
<th>Histology/Serology</th>
<th>Differential Diagnosis</th>
<th>Possible Molecular Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>mild MVI ( (g + \text{ptc} = 1) )</td>
<td>ABMR vs. no ABMR</td>
<td>ABMR classifiers DSASTs</td>
</tr>
<tr>
<td>C4d negative, DSA positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>moderate to severe peritubular capillaritis but no glomerulitis</td>
<td>pure TCMR/borderline vs. mixed ABMR + TCMR/borderline</td>
<td>ABMR classifiers DSASTs</td>
</tr>
<tr>
<td>( (g = 0, \text{ptc} \geq 2) ); TCMR or borderline, C4d negative, DSA positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MVI ( (g + \text{ptc} \geq 2) )</td>
<td>ABMR vs. no ABMR</td>
<td>ABMR classifiers DSASTs</td>
</tr>
<tr>
<td>C4d negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>no identifiable anti-HLA DSA + non-HLA antibody</td>
<td></td>
<td></td>
</tr>
<tr>
<td>no MVI ( (g + \text{ptc} = 0) )</td>
<td>ABMR vs. no ABMR</td>
<td>ABMR classifiers DSASTs</td>
</tr>
<tr>
<td>C4d positive, ± DSA</td>
<td></td>
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<tr>
<td>ABO-compatible</td>
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### Recommended Indications for Use of Molecular Diagnostics in Renal Allograft Biopsy Diagnosis, Continued

<table>
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<tr>
<th>Histology/Serology</th>
<th>Differential Diagnosis</th>
<th>Possible Molecular Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>MVI (g + ptc &gt; 0)</td>
<td>ABMR vs. no ABMR</td>
<td>ABMR classifiers</td>
</tr>
<tr>
<td>C4d positive</td>
<td></td>
<td>DSASTs</td>
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<tr>
<td>no identifiable anti-HLA DSA</td>
<td></td>
<td></td>
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<tr>
<td>ABO-incompatible</td>
<td></td>
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<tr>
<td>TG (cg &gt; 0)</td>
<td>purely chronic ABMR or</td>
<td>ABMR classifiers</td>
</tr>
<tr>
<td>no/mild MVI (g + ptc ≤ 1)</td>
<td>no ABMR vs.</td>
<td>DSASTs</td>
</tr>
<tr>
<td>C4d negative, DSA positive</td>
<td>chronic, active ABMR</td>
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<tr>
<td>borderline infiltrate</td>
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<td>TCMR classifiers</td>
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<tr>
<td>BK virus nephropathy</td>
<td>BK virus nephropathy</td>
<td>TCMR classifiers</td>
</tr>
<tr>
<td>meets criteria for acute TCMR grade 1a or 1b</td>
<td>alone vs. BK virus nephropathy + TCMR</td>
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