Objectives

• Discuss the studies correlating the detection of non-HLA antibodies subsequent to cell death with graft outcome.
• Discuss the studies correlating the impact of anti-self and anti-GPCR antibodies acting alone or in concert with DSA to HLA.
• Describe immunological risk stratification using comprehensive antibody analysis: HLA and non-HLA specific antibody profiling.
Cellular Sublocalization of Non-HLA Antigens

Collagen-V: Col-V
- Minor sequestered fibrillar collagen, incorporated within Collagen 1 fibrils.
- An immunogenic extracellular matrix protein (lung, skin, placenta).
- Expressed in small airway epithelial cells, perivascular, peribronchial tissue.
- Found to induce humoral responses in lung facilitating BOS and fibrogenesis. (Goers, JI, 2008; Burlingham, JCI, 2007; Tiriveedhi, CEI, 2012; Hachem, AJT, 2012; Tiriveedhi, JHLT, 2013).

K-α-1-Tubulin: Kα1T

Non-HLA Specific Antigens: Autoantigens

Non-HLA Specific Antigens: Autoantigens

• Collagen-V: Col-V
• K-α-1-Tubulin: Kα1T
Non-HLA Specific Antigens: Autoantigens

- Collagen-V: Col-V
- K-α-1-Tubulin (Kα1T):
  - Common presence of both COL-V and Kα1T-abs in 67% of lung tx recipients (Hachem, AJT 2012).
  - 96% of pts with DSA had Col-V and Kα1T abs.
  - Col-V and Kα1T abs present in cardiac tx recipients with AMR and CAV (Nath, Transplantation, 2011).
  - Anti-Kα1T found in the absence of anti-HLA in lung pts with chronic rejection (Saini, JHLT, 2011; Goers, JI, 2008).

Non-HLA Specific Antigens: Autoantigens

- Collagen-V and Kα1 Tubulin
- Hachem et al AJT 12:2164, 2012
  - Studied ab to self ags before and after antibody directed therapy and correlated the results with development of BOS.
  - Found a correlation between the development of abs to self ags and DSA.
    - Those who cleared DSA but had persistent abs to self ags were significantly more likely to develop BOS than those who cleared these abs.

Conclusion: Antibodies to self antigens are an important risk factor for the development of BOS.

Non-HLA Specific Antigens: Autoantigens

- Collagen-V and Kα1 Tubulin
  - Determined the prevalence of pre-existing abs to self ags and the association between these abs and the development of PGD and DSA in chronic rejection.
  - In 317 lung recipients, 22.71% had abs to self ags; recipients with IPF and CF had the highest prevalence of abs to self ags.
  - The incidence of the following was higher in recipients with pre-existing abs to self ags.
    - PGD (88% vs 54%); DSA (70% vs 45%); and BOS (39% vs 30%).

Conclusion: Recipients with pre-existing abs to self antigens are at increased risk for development of PGD, DSA, and BOS.
Problem: clinically relevant humoral responses against many but not all of non-HLA antigens occur secondary to cell death

Clinical Impact of Non-HLA Specific Antibodies: Antibodies to G-Protein Coupled Receptors

Non-HLA Specific Antigens: GPCRs

- **Angiotensin Type 1 Receptor (AT₁R):**
  - G coupled protein. Abs associated with vascular remodeling and acute rejection independent of, and synergistic with, DSA-HLA.

- **Endothelin Receptor (ET₄R):**
  - G coupled protein. Abs induce endothelial cell activation and correlate with ACR, AMR and microvasculopathy.
Identification of AT₃R-agonistic IgG in patients with HLA-Ab negative vascular rejection

Bioassay for agonistic receptor-activity in cardiomyocytes

IgG agonistic effect is AT₃R specific

- Patients’ IgG
- AT₃R-IgG
- Ang II
- AT₃R-Ab

Vascular rejection: 5/16 C4d positive


AMR Associated with High Levels of AT₃R Specific Antibody in Patients with No Donor HLA Specific Antibodies

Raimondo et al., Transplantation 90:1473, 2010

<table>
<thead>
<tr>
<th>Acute Rejection</th>
<th>High anti-AT₃R (&gt;17 units) antibodies</th>
<th>Low anti-AT₃R (&lt;17 units) antibodies</th>
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<tr>
<td>AMR</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>CMR</td>
<td>0</td>
<td>9</td>
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</table>

- Total number of patients with no donor specific antibody to HLA or MICA is 45
- P < 0.001, Fisher’s Exact Test

Conclusions:
- These results provide insight into the AMR process in the absence of DSA-HLA and with no PTC C4d deposition.
- Assessing AT₃R antibody status along with the DSA –HLA provides additional information to determine the immune risk for recipients.


AT₃R – Abs target extracellular epitopes

Biacore: lower dissociation rates compared to natural agonist, Angiotensin II

Accelerated kidney transplant rejection and hypertensive encepha-lopahy in a pediatric patient associated with antibodies against AT₁R and HLA class II.

- Case study of a pediatric kidney recipient with a negative CDC crossmatch developed accelerated C4d positive rejection.
- Pretx AT₁R levels >40U/mL.
- Donor HLA specific class II antibody developed on day 6, perhaps a secondary response but no prior transfusions or sensitizing events.
- Perhaps the hypertension acted as a danger signal enhancing the expression of HLA class II antigens.

Hypothesis: There was a synergy of AT₁R and HLA class II antibodies contributing to the exceptionally rapid and severe onset of rejection.

Higher Risk of Kidney Graft Failure in the Presence of Anti-Angiotensin II Type-1 Receptor Antibodies
Taniguchi et al., AJT 13:2577, 2013

Graft Survival of the Abnormal Biopsy Group (ABG) Patients
Study of 351 kidney recipients

Increased Negative Impact of Anti-Angiotensin Type 1 Receptor Antibodies Together with De Novo Donor HLA Specific Antibodies on Graft Outcome in Heart Transplantation
Reinsmoen et al, Transplantation 97:595, 2014

- Sera from 200 heart recipients transplanted between May 2007 and August 2011 were tested for DSA by Luminex single bead antigen assays and for AT₁R-ab by ELISA.
- Clinical parameters included 5 year pAMR, pCMR, CAV and survival.
- Both pAMR and pCMR diagnoses (grades 2 and 3) were included since C4d negative AMR has been attributed to both DSA and AT₁R-ab. (Berry et al, JHLT, 2012).
Increased Negative Impact of Anti-Angiotensin Type 1 Receptor Antibodies Together with De Novo Donor HLA Specific Antibodies on Graft Outcome in Heart Transplantation

<table>
<thead>
<tr>
<th>FACTOR</th>
<th>HR</th>
<th>95% CI</th>
<th>P-VALUE</th>
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<tr>
<td>de novo DSA</td>
<td>19.2</td>
<td>5.8 – 63.8</td>
<td>&lt;0.0001</td>
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<tr>
<td>AT,R ≥ 12</td>
<td>7.1</td>
<td>2.5 – 20.0</td>
<td>&lt;0.0002</td>
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<td>AT,R ≥ 17</td>
<td>1.4</td>
<td>0.5 – 3.6</td>
<td>0.51</td>
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<td>de novo DSA + AT,R ≥ 12</td>
<td>10.5</td>
<td>3.7 – 30.0</td>
<td>&lt;0.0001</td>
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<tr>
<td>de novo DSA + AT,R ≥ 17</td>
<td>8.0</td>
<td>2.3 – 27.9</td>
<td>&lt;0.0001</td>
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</tbody>
</table>

Three Center Study Investigating the Clinical Impact of Antibodies to Non-HLA Antigens in Lung Transplantation
Transplantation, published ahead of print

- Cedars-Sinai Medical Center: Nancy L. Reinsmoen, James Mirocha and George Chaux.
- University of Pittsburgh Medical Center: Adriana Zeevi, Christopher Ensor, and Marilyn Marrari.
- Science Center, San Antonio: Deborah Levine.

Three Center Study Investigating the Clinical Impact of Antibodies to Non-HLA Antigens in Lung Transplantation
Reinsmoen et al, Transplantation, published ahead of print, 2017

- Aim: The aim of our study was to determine the clinical impact of these antibodies to non-HLA antigens on graft outcome in lung transplantation.
- Study: Pre and posttx sera from 162 lung recipients transplanted between January 1, 2011 and April 1, 2013 were tested for antibodies to AT,R, ET,R:
- Each center submitted complete HLA antibody profiles and clinical results: demographics, diagnosis, AMR, CMR, and BOS.
The Antibody Binding Levels to AT_R are Associated with the Antibody Binding Levels to ET_A

Freedom from AMR by AT_R, ET_A Strong Binding Status and De Novo DSA Status

Freedom from De Novo DSA by Pre-Transplant AT_R Antibody Binding Level
**Freedom from De Novo DSA by Pre-Transplant ETAR Antibody Binding Level**

Log-Rank P = 0.002
Wilcoxon P = 0.009
Log-Rank Trend Test P = 0.003 (2-sided)
P = 0.002 (1-sided)

**Freedom from De Novo DSA by Pre-Transplant HLA Antibody Status**

Log-Rank P = 0.063
Wilcoxon P = 0.043

HR = 1.69 with 95% CI = 0.97-2.97

**Freedom from De Novo DSA by Double Hit Status (Pre-tx HLA-Ab + and Strong AT_R Ab): Increased Negative Impact**

Log-Rank P = 0.004
Wilcoxon P = 0.001

HR = 2.26 with 95% CI = 1.28-4.00
Cox P = 0.005
Impact of Non HLA Specific Antibody in Lung Transplantation

- An increased negative impact on antibody mediated rejection and/or cellular mediated rejection and lung recipients with high pretransplant levels of antibodies to AT_R and ET_A.
- Impact of non HLA specific antibodies on the development of BOS is being investigated.


- CF patient with no preexisting DSAs to HLA underwent lung tx and developed a severe and intractable pulmonary hypertension associated with right ventricular dysfunction with rapid deterioration in hemodynamics leading to death on post op day 5.
- Post mortem samples were consistent with AMR, evidenced by diffuse capillaritis, blood extravasation, edema, and microthrombi with foci of ACR (A3).
- Pre-existing anti-AT_R and ET_A antibodies (potent vasoconstrictors) were detected and found to rise slightly post tx.
- Conclusion: Pre-existing anti-AT_R and ET_A antibodies may have contributed to the onset of AMR and to the catastrophic clinical course of this patient.
Immunological Risk Stratification by Assessing Both HLA and non-HLA Specific Antibodies

- The impact of GPCR-ab and other ab to self antigens, whether alone or in the presence of DSA-HLA point out the importance of investigating the presence of both antibodies to assess comprehensive immune status of the recipients.
- Pretx risk stratification includes: non-HLA ab levels, DSA strength and function, and HLA mismatch to identify recipients at higher risk for early graft dysfunction.
- Risk stratification, even in the absence of DSA-HLA, may help identify early time points for intervention before the immune response is sufficient to reach rejection status.
- The appearance of the antibodies may be secondary to immune activation through multiple mechanisms such as DSA-HLA, non-HLA-ab, stress, immune deregulation, or inflammatory events.

Conclusions

- Recipients may present with multiple antibodies: both HLA and non-HLA specific.
- The ability to reliably detect and define these antibodies will provide insight into the comprehensive immune risk assessment of the recipients including:
  - Immune mechanisms involved which could lead to more focused pharmacologic targeting of the non-HLA antibodies.
  - Modes of action of these antibodies.
  - Possible synergy.
  - Possible optimal interventions aimed at early treatment allowing for improved graft outcome.

Synergistic Impact of Donor HLA-Specific and Non-HLA Specific Antibodies on Graft Outcome