Molecular Signals of Intragraft Rejection: Is INTERLUNG the Answer?

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and
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Edmonton, AB
The Molecular Microscope® Diagnostic System

Presenter: Phil Halloran

Our studies are supported in Mendez National Institute of Transplantation Foundation and by a licensing agreement with One Lambda/Thermo Fisher

- Phil Halloran
  - Has shares in Transcriptome Sciences Inc (TSI), a University of Alberta research company with an interest in molecular diagnostics
  - Has been a speaker in symposia for One Lambda/Thermo Fisher
  - Is a consultant to CSL

https://www.molecular-microscope.com/
http://transcriptome.com/
http://atagc.med.ualberta.ca/Services/MolecularMicroscopeSystem/
Learning Objectives: The INTERLUNG study

ClinicalTrials.gov: NCT02812290

MMDx-TBB, MMDx-3BMB

To understand:

1. The unmet need in lung transplant diagnostics
2. The principles of microarray analysis
3. Unsupervised and supervised analysis of high dimensionality data
4. The relationship of the MMDx-TBB diagnoses to histology diagnoses
5. The potential for changing care: MMDx-3BMB
MMDx-Lung: the TBB* project

INTERLUNG (and launching INTERLUNGEX)
ClinicalTrials.gov: NCT02812290

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*TBB = transbronchial biopsy
Molecular assessment of rejection and injury in lung transplant biopsies

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KEYWORDS:
- accuracy
- antibody-mediated rejection
- T-cell-mediated rejection
- outcome
- gene expression

BACKGROUND: Improved understanding of lung transplant disease states is essential because failure rates are high, often due to toxicologic lung allograft dysfunction. However, histologic assessment of lung transplant biopsies (TBBs) is difficult and often uninterpretable even with 10 pines.

METHOD: We prospectively studied whether microscopical assessment of single TBB pieces could identify disease states and reduce the amount of tissue required for diagnosis. By following strategies successful for heart transplants, we used expression of rejection-associated transcripts (annotated in kidney transplant biopsies) on unsupervised machine learning to identify disease states.

RESULTS: All 24 single-pie TBBs produced reliable transcript measurements. Paired TBB pieces available from 12 patients showed significant similarity but also showed some sampling variance. Alveolar content, as estimated by surfactant transcript expression, was a source of sampling variance. To offset sampling variation, for analysis, we selected 15 single-pie TBBs with high surfactant trancripts. Unsupervised hierarchical analysis identified 4 alveolar phenotypes (clusters) and several biopsies for their similarity to mult-normal, T-cell-mediated rejection (TCMR), T-cell transplant, antibody-mediated rejection (AMR), and inj (neutrophilic transtate). Molecular TCMR correlated with histologic TCMR. The relationship of molecular scores to histologic AMR could not be assessed because of the presence of AMR in this population.

CONCLUSIONS: Molecular assessment of single-pie TBBs can be used to classify lung transplant biopsies and correlated with microscopical histology. Ten of 3 pines for each TBB will probably be needed for offset sampling variance.


Molecular Assessment of Rejection and Injury in Lung Transplant Transbronchial Biopsies.
Histology of Transbronchial Lung Biopsies Has Poor Interobserver Agreement

A grade
Perivascular and interstitial mononuclear cell infiltration
Interobserver agreement = 0.18

B grade
Lymphocytic Bronchiolitis
Interobserver agreement = 0.035

Antibody mediated rejection
C4d straining?
Neutrophilic capillaritis/margination?
Interobserver agreement = ??

Methods

**Prospective TBB**
- Indication or surveillance
- All samples minus 1 sent for histology
- 1 TBB piece in RNALater sent for microarray

**Microarray analysis at the ATAGC**
- Quantitative expression of 453 rejection-associated transcripts (RATs)

**High Dimensionality Data Analysis**
- Principal components
  - Archetypal
Surfactant (SFT) transcript expression in 242 TBBs. The 50 highest variance probesets in 242 TBB were identified. From this, 11 probe sets representing four SFT genes were identified (11757270_x_at, 11763961_x_at, 11754641_x_at, 11742494_s_at, 11735664_s_at, 11764024_x_at, 11748373_s_at, 11734773_x_at, 11745166_x_at, 11763809_x_at, 11749911_x_at) and their geometric mean expression across 242 TBB samples was calculated. The samples were ordered by decreasing geometric mean. The 152 samples to the left of the dashed vertical line were deemed to have sufficiently high SFT expression (i.e. high alveolar content) to be used in subsequent analyses of the TBBs.

Developing MMDx-Lung (TBB): the same approach as for heart EMBs

Rejection transcript expression

Four-state (4 archetype) model:

- $S_{1_{\text{normal}}}$ = no rejection or injury
- $S_{2_{\text{TCMR}}}$ = TCMR (includes ABMR?)
- $S_{3_{\text{ABMR}}}$ = endothelial (not actually ABMR?)
- $S_{4_{\text{injury}}}$ = lung abnormalities not rejection
Expression of kidneys rejection transcripts in 256 high SFT TBBs indicates normal, TCMR, endothelial, and injury patterns similar to those in hearts.

### Summary of transcripts associated with archetype scores and principal components in TBBs

<table>
<thead>
<tr>
<th>Score*</th>
<th>Key cellular expression patterns of the top 20 transcripts correlated or anti-correlated with the molecular score</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>S1</strong>&lt;sub&gt;normal&lt;/sub&gt;</td>
<td>5 IFNG-inducible, 8 T/NK, 7 T/NK/MMDC</td>
<td>Absence of rejection and injury</td>
</tr>
<tr>
<td><strong>S2&lt;sub&gt;TCMR&lt;/sub&gt;</strong></td>
<td>15 T (5 T, 5 T/NK, 5 T/NK/MMDC), 3 IFNG-inducible, 2 MMDC</td>
<td>T cell-mediated rejection</td>
</tr>
<tr>
<td><strong>S3&lt;sub&gt;endothelial&lt;/sub&gt;</strong></td>
<td>17 HUVEC, 1 T/NK/MMDC, 1 MMDC, 1 Parenchymal</td>
<td>Endothelial</td>
</tr>
<tr>
<td><strong>S4&lt;sub&gt;injury&lt;/sub&gt;</strong></td>
<td>19 MMDC (16 MMDC, 3 T/NK/MMDC), 1 HUVEC</td>
<td>Macrophage infiltration (injury)</td>
</tr>
<tr>
<td><strong>PC1</strong></td>
<td>7 IFNG-inducible, 6 T/NK, 7 T/NK/MMDC</td>
<td>Rejection/inflammation</td>
</tr>
<tr>
<td><strong>PC2</strong></td>
<td>10 HUVEC, 2 IFNG-inducible, 5 MMDC, 1 Parenchymal, 2 unclear (low expression)</td>
<td>Endothelial</td>
</tr>
<tr>
<td><strong>PC3</strong></td>
<td>1 IFNG-inducible, 18 MMDC (14 MMDC, 4 T/NK/MMDC), 1 HUVEC</td>
<td>Macrophage infiltration (injury)</td>
</tr>
</tbody>
</table>

* 13 S1<sub>normal</sub>, 18 S2<sub>TCMR</sub>, 2 S3<sub>ABMR</sub>, 0 S4<sub>injury</sub>, 14 PC1, 3 PC2, and 1 of the PC3 top 20 transcripts were RATs

Abbreviations: IFNG – interferon gamma; T – effector T cells; NK – NK cells; MMDC – macrophages, monocytes, or dendritic cells; HUVEC – human umbilical vein endothelial cells; TCMR – T cell-mediated rejection; ABMR – antibody-mediated rejection

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Explaining the Molecular Microscope® report for transbronchial lung biopsies (MMDx-Lung)

Patient information
Date of transplant, date of biopsy, etc.

Clinical interpretation

- proportions: Normal, TCMR, ABMR, injury
- Visualization: Relationship of this biopsy to all others in the reference biopsies PC2 vs. PC1; PC2 vs. PC3

Clinical information
Time post-transplant; indication, DSA (if provided)

Additional detail
Rejection, injury-related transcript scores in this biopsy

Comparison to normal
Scores interpreted vs. relatively normal biopsies

Proportions
Normal, TCMR, ABMR, injury

Visualization
Relationship of this biopsy to all others in the reference biopsies PC2 vs. PC1; PC2 vs. PC3

Alveolar content
Too little makes interpretation difficult

This biopsy
Chronic lung allograft dysfunction (CLAD) is causing most lung transplants to fail prematurely (e.g. 5 years)

Histology cannot define CLAD
Can MMDx TBB define CLAD?
Relationships between molecular phenotype scores and chronic lung allograft dysfunction (CLAD) at biopsy. From left to right the $S_{\text{normal}}$, $S_{\text{TCMR}}$, $S_{\text{endothelial}}$, and $S_{\text{injury}}$ scores from the RAT-based archetype model and the principal component scores PC1, PC2, and PC3 from RAT-based principal component analysis in 152 TBBs (y axis) are plotted according to the CLAD status at biopsy (x axis). P-values of a Mann-Whitney U-test are reported at the top of each plot. N=36 CLAD, N=116 No CLAD.
MMDx-Lung: the mucosal biopsy (3BMB*) project

INTERLUNG (and launching INTERLUNGEX)
ClinicalTrials.gov: NCT02812290

*3BMB endobronchial mucosal biopsy from 3\textsuperscript{rd} bifurcation
The 3BMB study

Supplementary Table 1. Centers participating in the INTERLUNG 3BMB study

<table>
<thead>
<tr>
<th>Location</th>
<th>Principal Investigator(s)</th>
<th>Number of Biopsies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baltimore</td>
<td>Irina Timofte</td>
<td>46</td>
</tr>
<tr>
<td>Edmonton</td>
<td>Kieran Halloran, Philip Halloran</td>
<td>20</td>
</tr>
<tr>
<td>Melbourne</td>
<td>Gregory Snell, Glen Westall</td>
<td>52</td>
</tr>
<tr>
<td>San Antonio</td>
<td>Deborah Levine</td>
<td>8</td>
</tr>
<tr>
<td>St. Louis</td>
<td>Ramsey Hachem, Daniel Kreisel, Elbert Trulock</td>
<td>15</td>
</tr>
<tr>
<td>Toronto</td>
<td>Stephen Juvet, Shaf Keshavjee</td>
<td>56</td>
</tr>
<tr>
<td>Vienna</td>
<td>Peter Jaksch, Walter Klepetko</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>198</td>
</tr>
</tbody>
</table>
Mucosal biopsies: much safer than TBBs

- Prospective collection of **mucosal biopsies** from indication or surveillance bronchoscopies in lung transplant recipients
  - 3\textsuperscript{rd} airway bifurcation (3B-MB), typically between RLL and RML airway

- **1-2 pieces** for molecular analysis
  - Quantitative expression of **453 rejection-associated transcripts (RATs)**
    - Originally identified by association with kidney transplant rejection histology

- **No histology** component

Image courtesy of Olympus
The 205 3BMBs have variance that is compatible with TCMR. This could avoid TBBs in higher risk patients.
Figure 1. Principal component analysis (PCA) and archetypal analysis were performed based on rejection-associated transcript (RAT) expression in 198 3BMBs. The biopsies are plotted according to their PC1 and PC2 scores. Biopsies are colored according to their highest of three archetype scores (S1normal, S2TCMR, S3endothelial). Each score describes a biopsy’s similarity to each of the three archetypes (A1normal, A2TCMR, A3endothelial). The archetypes are represented by the enlarged points. Black – normal, red – TCMR, blue – endothelial.
Developing MMDx-Lung 3BMBs: the same approach as for heart EMBs, TBBs, livers

Rejection transcript expression

Four-state (4 archetype) model:

- $S_{1_{\text{normal}}}$ = no rejection or injury
- $S_{2_{\text{rejection}}}$ = TCMR (includes ABMR?)
- $S_{3_{\text{endothelial}}}$ = (??not actually ABMR?)
- $S_{4_{\text{injury}}}$ = lung abnormalities not rejection
### Table 4. Top transcripts correlated with S2\textsubscript{rejection} in 198 3BMBs

<table>
<thead>
<tr>
<th>Correlation</th>
<th>Gene Symbol</th>
<th>Gene Name</th>
<th>PBT Annotations</th>
<th>Mean Expression in biopsy groups</th>
<th>Primary Expression in Cell Panel</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.88</td>
<td>HLA-DMB</td>
<td>major histocompatibility complex, class II, DM beta</td>
<td>GRIT3, Rejection-RAT</td>
<td>276 861 366</td>
<td>B, DC, HUVEC (IFNG), MC, MP (IFNG)</td>
</tr>
<tr>
<td>0.87</td>
<td>GBP5</td>
<td>guanylate binding protein 5</td>
<td>GRIT3, ABMR-RAT, Rejection-RAT</td>
<td>74 422 107</td>
<td>HUVEC (IFNG), MP (IFNG), NK, T</td>
</tr>
<tr>
<td>0.86</td>
<td>PSMB9</td>
<td>proteasome subunit beta 9</td>
<td>GRIT3, ABMR-RAT, Rejection-RAT, TCMR-RAT, IFNG</td>
<td>873 2482 1102</td>
<td>DC, HUVEC (IFNG), MC, MP (IFNG), NK, T</td>
</tr>
<tr>
<td>0.86</td>
<td>IDO1</td>
<td>indoleamine 2,3-dioxygenase 1</td>
<td>GRIT3, ABMR-RAT, Rejection-RAT</td>
<td>141 1405 134</td>
<td>HUVEC (IFNG), MP (IFNG)</td>
</tr>
<tr>
<td>0.86</td>
<td>IFO30</td>
<td>interferon, gamma-inducible protein 30</td>
<td>GRIT3</td>
<td>903 3027 1066</td>
<td>DC, HUVEC (IFNG), MC, MP</td>
</tr>
<tr>
<td>0.85</td>
<td>FAM26F</td>
<td>family with sequence similarity 26, member F</td>
<td>GRIT3, ABMR-RAT, Rejection-RAT</td>
<td>145 678 208</td>
<td>MC, MP (IFNG)</td>
</tr>
<tr>
<td>0.85</td>
<td>CD53</td>
<td>CD53 molecule</td>
<td>GRIT3</td>
<td>169 630 268</td>
<td>B, DC, MC, MP (IFNG), NK, T</td>
</tr>
<tr>
<td>0.84</td>
<td>HLA-DRA</td>
<td>major histocompatibility complex, class II, DR alpha</td>
<td>GRIT3, ABMR-RAT, IRRAT950, Rejection-RAT</td>
<td>5636 11015 7149</td>
<td>B, DC, HUVEC (IFNG), MP (IFNG)</td>
</tr>
<tr>
<td>0.84</td>
<td>PSMB10</td>
<td>proteasome subunit beta 10</td>
<td>GRIT3, Rejection-RAT, TCMR-RAT</td>
<td>882 1448 829</td>
<td>DC, HUVEC (IFNG), MC, MP (IFNG), NK, T</td>
</tr>
<tr>
<td>0.84</td>
<td>PSMB8</td>
<td>proteasome subunit beta 8</td>
<td>GRIT3, Rejection-RAT</td>
<td>1171 2254 1192</td>
<td>DC, HUVEC (IFNG), MC, MP (IFNG), NK, T</td>
</tr>
<tr>
<td>0.84</td>
<td>IRF8</td>
<td>interferon regulatory factor 8</td>
<td>GRIT3</td>
<td>122 427 185</td>
<td>B, DC, MC, MP (IFNG)</td>
</tr>
<tr>
<td>0.84</td>
<td>LAPT M5</td>
<td>lysosomal protein transmembrane 5</td>
<td>IRRAT950</td>
<td>373 1117 539</td>
<td>B, DC, MC, MP, NK, T, M</td>
</tr>
<tr>
<td>0.84</td>
<td>HCLS1</td>
<td>hematopoietic cell-specific Lyn substrate 1</td>
<td></td>
<td>195 436 235</td>
<td>B, DC, MC, MP, NK, T, M</td>
</tr>
<tr>
<td>0.83</td>
<td>CD86</td>
<td>CD86 molecule</td>
<td>IRRAT950, QCMAT</td>
<td>52 162 73</td>
<td>DC, MC, MP, M</td>
</tr>
<tr>
<td>0.83</td>
<td>ARHGAP30</td>
<td>Rho GTPase activating protein 30</td>
<td></td>
<td>77 171 98</td>
<td>B, DC, MC, MP, NK, T, MMDC</td>
</tr>
<tr>
<td>0.83</td>
<td>EPSTI1</td>
<td>epithelial stromal interaction 1 (breast)</td>
<td>GRIT3</td>
<td>172 758 223</td>
<td>HUVEC (IFNG), MP (IFNG)</td>
</tr>
<tr>
<td>0.83</td>
<td>FYB</td>
<td>FYN binding protein</td>
<td></td>
<td>88 320 137</td>
<td>MC, MP, MMDC</td>
</tr>
<tr>
<td>0.83</td>
<td>HLA-DQA1</td>
<td>major histocompatibility complex, class II, DQ alpha 1; major histocompatibility complex, class II, DR alpha</td>
<td>GRIT3, IRRAT950, Rejection-RAT</td>
<td>1029 2845 1391</td>
<td>DC, MP (IFNG)</td>
</tr>
<tr>
<td>0.83</td>
<td>IRF1</td>
<td>interferon regulatory factor 1</td>
<td>GRIT3, ABMR-RAT, Rejection-RAT</td>
<td>199 482 225</td>
<td>HUVEC (IFNG), MC, MP (IFNG)</td>
</tr>
<tr>
<td>0.83</td>
<td>PTTPC</td>
<td>protein tyrosine phosphatase, receptor type, C</td>
<td>IRRAT30</td>
<td>216 825 354</td>
<td>B, DC, MC, MP, NK, T, MMDC</td>
</tr>
</tbody>
</table>

1 Spearman correlation
3 B – B cells, DC – Dendritic cells, HUVEC – Human umbilical vein endothelial cells, IFNG – IFNG-inducible, MP – Monocytes/macrophages, NK – NK cells, T – T cells, M – Monocytes/Macrophages

**Correlation of S\textsubscript{rejection} with time of biopsy post-transplant = +0.32**
## Table 6. Top transcripts correlated with PC1 in 198 3BMBs

<table>
<thead>
<tr>
<th>Correlation</th>
<th>Gene Symbol</th>
<th>Gene Name</th>
<th>PBT Annotations</th>
<th>Mean Expression in biopsy groups</th>
<th>Primary Expression in Cell Panel</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.93</td>
<td>LCP2</td>
<td>lymphocyte cytosolic protein 2</td>
<td>Rejection-RAT</td>
<td>A1</td>
<td>110</td>
</tr>
<tr>
<td>0.92</td>
<td>HLA-DMA</td>
<td>major histocompatibility complex, class II, DM beta</td>
<td>GRIT3, Rejection-RAT</td>
<td>A2</td>
<td>658</td>
</tr>
<tr>
<td>0.92</td>
<td>IRF8</td>
<td>interferon regulatory factor 8</td>
<td>GRIT3</td>
<td>A3</td>
<td>122</td>
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<tr>
<td>0.91</td>
<td>HCST</td>
<td>hematopoietic cell signal transducer</td>
<td>Rejection-RAT</td>
<td>B, DC, MC, MP, NK, T, MMDC</td>
<td></td>
</tr>
<tr>
<td>0.91</td>
<td>FYB</td>
<td>FYN binding protein</td>
<td></td>
<td>DC, MC, MP, NK</td>
<td></td>
</tr>
<tr>
<td>0.91</td>
<td>CD53</td>
<td>CD53 molecule</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.90</td>
<td>LCP1</td>
<td>lymphocyte cytosolic protein 1 (L-plastin)</td>
<td>IRITD5, IRRAT950</td>
<td>A1</td>
<td>474</td>
</tr>
<tr>
<td>0.90</td>
<td>TNFSF13B</td>
<td>tumor necrosis factor (ligand) superfamily, member 13b</td>
<td>GRIT3, TCMR-RAT</td>
<td>A2</td>
<td>66</td>
</tr>
<tr>
<td>0.90</td>
<td>GBP5</td>
<td>guanylate binding protein 5</td>
<td>GRIT3, ABMR-RAT, Rejection-RAT</td>
<td></td>
<td>74</td>
</tr>
<tr>
<td>0.90</td>
<td>LAPTMS</td>
<td>lysosomal protein transmembrane 5</td>
<td>IRRAT950</td>
<td>A1</td>
<td>539</td>
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<tr>
<td>0.90</td>
<td>DOCK2</td>
<td>dedicator of cytokinesis 2</td>
<td>IRRAT950</td>
<td>A2</td>
<td>60</td>
</tr>
<tr>
<td>0.90</td>
<td>PTPRCC</td>
<td>protein tyrosine phosphatase, receptor type, C</td>
<td>IRRAT30</td>
<td>A3</td>
<td>193</td>
</tr>
<tr>
<td>0.89</td>
<td>ARHGAP30</td>
<td>Rho GTPase activating protein 30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.89</td>
<td>CD86</td>
<td>CD86 molecule</td>
<td>IRRAT950, QCMAT, IRRAT950, QCMAT</td>
<td>A1</td>
<td>77</td>
</tr>
<tr>
<td>0.89</td>
<td>AOAH</td>
<td>acyloxyacyl hydrolase (neutrophil)</td>
<td>Rejection-RAT, TCMR-RAT, GRIT3, ABMR-RAT, TCMR-RAT</td>
<td>A2</td>
<td>52</td>
</tr>
<tr>
<td>0.89</td>
<td>PSMB9</td>
<td>proteasome subunit beta 9</td>
<td>GRIT3, ABMR-RAT, TCMR-RAT</td>
<td>A3</td>
<td>873</td>
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<tr>
<td>0.89</td>
<td>SASH3</td>
<td>SAM and SH3 domain containing 3</td>
<td>IRRAT950</td>
<td>A1</td>
<td>39</td>
</tr>
<tr>
<td>0.88</td>
<td>IL10RA</td>
<td>interleukin 10 receptor, alpha</td>
<td>IRRAT950</td>
<td>A2</td>
<td>144</td>
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<tr>
<td>0.88</td>
<td>CD48</td>
<td>CD48 molecule</td>
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<tr>
<td>0.88</td>
<td>HLA-DPA1</td>
<td>major histocompatibility complex, class II, DP alpha 1</td>
<td>GRIT3, ABMR-RAT, Rejection-RAT</td>
<td>A3</td>
<td>4037</td>
</tr>
</tbody>
</table>

1Spearman correlation
3B – B cells, DC – Dendritic cells, HUVEC – Human umbilical vein endothelial cells, IFNG – IFNG-inducible, MP – Monocytes/macrophages, NK – NK cells, T – T cells, MMDC –
### Pathway analysis

3BMB 198 AA3 **RAT** S2\textsubscript{rejection}, Top 100

**Table 9. Gene Ontology (GO) biological process terms associated with top 100 S2\textsubscript{rejection}-correlated transcripts**

<table>
<thead>
<tr>
<th>GO Term</th>
<th>Hits in Top 100</th>
<th>Fold Enrichment</th>
<th>P-Value</th>
<th>Corrected P-Value(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>interferon-gamma-mediated signaling pathway</td>
<td>14</td>
<td>35.2</td>
<td>8.5E-17</td>
<td>7.2E-14</td>
</tr>
<tr>
<td>immune response</td>
<td>23</td>
<td>9.8</td>
<td>8.8E-16</td>
<td>2.9E-13</td>
</tr>
<tr>
<td>\textit{T cell receptor signaling pathway}</td>
<td>15</td>
<td>18.1</td>
<td>7.5E-14</td>
<td>1.6E-11</td>
</tr>
<tr>
<td>positive regulation of \textit{T cell proliferation}</td>
<td>10</td>
<td>29.8</td>
<td>3.9E-11</td>
<td>6.3E-09</td>
</tr>
<tr>
<td>antigen processing and presentation of peptide or polysaccharide antigen via MHC class II</td>
<td>7</td>
<td>73.6</td>
<td>2.9E-10</td>
<td>3.8E-08</td>
</tr>
<tr>
<td>antigen processing and presentation</td>
<td>9</td>
<td>29.2</td>
<td>6.4E-10</td>
<td>7.0E-08</td>
</tr>
<tr>
<td>\textit{T cell costimulation}</td>
<td>9</td>
<td>20.6</td>
<td>1.1E-08</td>
<td>1.0E-06</td>
</tr>
<tr>
<td>innate immune response</td>
<td>16</td>
<td>6.6</td>
<td>1.3E-08</td>
<td>1.1E-06</td>
</tr>
<tr>
<td>inflammatory response</td>
<td>15</td>
<td>7.1</td>
<td>2.1E-08</td>
<td>1.5E-06</td>
</tr>
<tr>
<td>antigen processing and presentation of exogenous peptide antigen via MHC class II</td>
<td>9</td>
<td>17.5</td>
<td>4.1E-08</td>
<td>2.7E-06</td>
</tr>
</tbody>
</table>

\(^1\) Benjamini-Hochberg

\(S2\textsubscript{rejection} = \text{IFNG signaling, TCR signaling, presence of inflammation,}\)
3BMB Archetype Scores S1, S2, PC1 are associated with CLAD
Figure 2. Relationships between histology A/B lesions in paired TBBs, DSA, and clinical CLAD features and molecular scores.

Relationships between ISHLT A grade, B grade, DSA status at biopsy, CLAD status at biopsy, and molecular scores were examined in samples where the clinical data was available (see Tables 1 & 2). Samples are grouped according to whether they had the specified condition (x axis). Molecular scores are plotted on the y axis. PCA scores (PC1, PC2) are normalized so that they are on the same scale. Boxplots are overlaid on each plot. The p-values at the top of each plot are from two-sided Mann-Whitney U tests comparing molecular scores in the positive class versus the negative class. Bonferroni-corrected significance threshold is 0.01 (5 tests, uncorrected threshold 0.05)

3BMB: significant associations with B airway lesions, not A lesions (in paired TBB), with DSA, and with CLAD
Direct comparisons of TBB reports to 3BMB reports
Interesting case #1

TBB and 3BMB reports agreed and showed relatively healthy lung transplant (1 year and 330 days post Tx)
Relatively healthy lung transplant. Minimal ABMR-like changes. Minimal TCMR-like changes. Minimal parenchymal injury ( Injury score 0.11, IRRATs and QCMATs normal). (The relationship between the rejection archetype scores and actual rejection states is unclear and is the subject of the ongoing INTERLUNG study.)

<table>
<thead>
<tr>
<th>Proportion Rejection and Injury™</th>
<th>Relatively Normal</th>
<th>TCMR-Like</th>
<th>ABMR-Like</th>
<th>Lung Injury</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1 Relatively Normal</td>
<td>0.92</td>
<td>0.69</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>Model 2 Relatively Normal</td>
<td>0.90</td>
<td>0.69</td>
<td>0.06</td>
<td>0.11</td>
</tr>
</tbody>
</table>

Current Biopsy vs Reference Set: PC2 vs PC1
- A1: Relatively Normal
- A2: TCMR-Like
- A3: ABMR-Like
- A4: Lung Injury

Current Biopsy vs Reference Set: PC2 vs PC3
- A1: Relatively Normal
- A2: TCMR-Like
- A3: Endothelial
- A4: Lung Injury

Current Biopsy vs Reference Set: PC2 vs PC1
- A1: Relatively Normal
- A2: TCMR-Like
- A3: Endothelial
- A4: Lung Injury
## Molecular Phenotype

<table>
<thead>
<tr>
<th>Gene or Gene Set</th>
<th>Biopsy Score</th>
<th>Normal Limit</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABMR related</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ISA-selective transcripts (ISA-ST)</td>
<td>-0.14</td>
<td>&lt;0.01</td>
<td>Normal</td>
</tr>
<tr>
<td>Endothelial ISA-selective transcripts (eISA-ST)</td>
<td>-0.16</td>
<td>&lt;0.06</td>
<td>Normal</td>
</tr>
<tr>
<td>NK cell burster transcripts (KMB)</td>
<td>-0.06</td>
<td>&lt;0.06</td>
<td>Normal</td>
</tr>
<tr>
<td>T-cell burster transcripts (TCB)</td>
<td>-0.21</td>
<td>&lt;0.03</td>
<td>Normal</td>
</tr>
<tr>
<td>IFNα (Interferon alpha)</td>
<td>0.58</td>
<td>&lt;0.25</td>
<td>Normal</td>
</tr>
<tr>
<td>Cytotoxic T-cell transcripts</td>
<td>0.22</td>
<td>&lt;0.62</td>
<td>Normal</td>
</tr>
<tr>
<td>CD4 (Interferon gamma)</td>
<td>0.42</td>
<td>&lt;0.66</td>
<td>Normal</td>
</tr>
<tr>
<td>Other Rejection and Injury-related</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IFNB-inducible transcripts (IFNB-IT)</td>
<td>-0.10</td>
<td>&lt;0.04</td>
<td>Normal</td>
</tr>
<tr>
<td>Microporin transcripts (MCMP)</td>
<td>-0.21</td>
<td>&lt;0.44</td>
<td>Normal</td>
</tr>
</tbody>
</table>

## Local Histopathology Phenotype

### Acute Rejection
- ISHLT A Grade: NA
- ISHLT B Grade: NA
- ISHLT C Grade: NA
- C4d: Normal

### Airway Inflammation
- NA

### Chronic Airway Rejection
- NA

### Other
- NA

### Diagnosis
- NA

### Clinical Notes
- NA

## References

Interesting case #2

TBB and 3BMB reports agreed and showed minor abnormalities and low probability of rejection (279 days post Tx)
Lung Case #2

• Clinical information:
  ▪ Bx Indication: Surveillance
  ▪ preBx Treatment: Cyclosporine, Mycophenolate
  ▪ DSA Class II, PRA Class
  ▪ Primary Disease: Idiopathic Pulmonary Fibrosis
Lung Case #2

Redacted
# Lung Case #2

## Report Page 2

### Molecular Phenotype

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Biopsy score</th>
<th>Normal Limit</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMR-related</td>
<td>0.44</td>
<td>&lt;0.31</td>
<td>Slightly abnormal</td>
</tr>
<tr>
<td>Cytotoxic T-cell transcrs (CTC)</td>
<td>0.57</td>
<td>&lt;0.45</td>
<td>Normal</td>
</tr>
<tr>
<td>IFN-β (Interferon γ)</td>
<td>0.73</td>
<td>&lt;0.45</td>
<td>Abnormal</td>
</tr>
<tr>
<td>Rejection and injury-related</td>
<td>4.83</td>
<td>&lt;0.45</td>
<td>Normal</td>
</tr>
<tr>
<td>Injury-related</td>
<td>0.00</td>
<td>&lt;0.44</td>
<td>Normal</td>
</tr>
<tr>
<td>Other</td>
<td>0.11</td>
<td>&lt;0.30</td>
<td>Normal</td>
</tr>
<tr>
<td>Source of transcrs (Avascular content)</td>
<td>14.665</td>
<td>&gt;5267</td>
<td>Adequate</td>
</tr>
</tbody>
</table>

### Local Histopathology Phenotype

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Biopsy score</th>
<th>Normal Limit</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute Rejection</td>
<td>0</td>
<td>&lt;0.10</td>
<td>Normal</td>
</tr>
<tr>
<td>Airway inflammation</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic Airway Rejection</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>NA</td>
<td>&lt;0.60</td>
<td>Normal</td>
</tr>
</tbody>
</table>

### Clinical Notes

- No findings

### References

MMDx in TBBs, and 3BMBs

- TBBs: continuing to 1000 biopsies
  - Define ABMR and injury
  - Need more tissue: 2 bites (or more?)
- 3BMBs: highly promising – a change in care
  - Define ABMR and injury
  - Need more tissue: 2 bites (or more?)
Potential of molecular measurements to change care

Mechanisms (not just “biomarkers”)
Reclassify the disease states
New tests
International standard
Recalibrate conventional tests
Guide and monitor response to therapy
Empower clinical trials: new treatments
Study Team & Acknowledgments

Anna Hutton
Mido Qarni
Jessica Chang
Martina Mackova
Michael Parkes
Konrad Famulski
Rob Polakowski
Katelynn Madill-Thomsen
Jeff Reeve

Jeffery Venner (not shown)
Luis Hidalgo (not shown)
Kieran Halloran
Brendan Halloran

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Muttart Chair in Clinical Immunology
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  - Genome Canada
  - Roche Organ Transplantation Research Foundation
  - Canada Foundation for Innovation
  - University Hospital Foundation
  - Capital Health/Alberta Health Services
Thank you
A. Relationship between CLAD and molecular scores in 256 TBBs (55 CLAD, 175 No CLAD)

B. Relationship between CLAD and molecular scores in 198 3BMB (42 CLAD, 156 No CLAD)

The Molecular Features of CLAD in Transbronchial and Endobronchial Mucosa Biopsies

Parkes MD1, Halloran PF1, Chang J1, Famulski KS1, Reeve J1, Hachem R2, Jaksch P3, Juvet S4, Klepetko W3, Keshavjee S4, Kreisel D2, Levine D5, Roux A6, Snell GI7, Trulock E2, Timofte IL8, Westall GP7, Halloran KM1.