Reading Human Biopsies Using mRNA Expression
MMDx uses predefined algorithms to assess the probability of disease states (rejection or injury) in the organ on the basis of the changes in gene expression in the biopsy...
Summary: The Molecular Microscope Diagnostic System (MMDx)

MMDx is a central diagnostic system that uses microarrays to measure mRNA levels in transplant biopsies. The biopsy is placed in RNAlater™ and shipped by courier at ambient temperature to the central laboratory. The mRNA levels are measured on a microarray and algorithms, based on a large Reference Set of biopsies, are used to generate an automated report in one to two business days. The system has been developed in kidney transplant biopsies (the INTERCOMEX study (1), ClinicalTrials.gov #NCT01299168) and heart endomyocardial biopsies (the INTERHEART study, ClinicalTrials.gov #NCT02670408). Furthermore, the system can now generate reports in lung transbronchial biopsy reports and will continue to evolve as the reference set (the INTERLUNG study, ClinicalTrials.gov #NCT02812290) increases.

Overview: The unmet need is PRECISION

Troubled Transplant Management
In managing a troubled transplant, the clinician must assess rejection and injury in the organ. MMDx uses predefined algorithms to assess the probability of disease states (rejection or injury), providing the following information to the clinician:

Rejection
• T cell-mediated rejection (TCMR)
• Antibody-mediated rejection (ABMR)
• Under-immunosuppression/non-adherence
• Guidance for therapy

Parenchymal Injury (Wounding) and Non-Rejection Diseases
• Acute parenchymal injury
• Irreversible injury (atrophy-fibrosis)
• Risk of progression to failure
• Potentially, probability of recurrent primary diseases or infections

Conventional diagnostic systems are subjective, opinion-based, and prone to inaccuracy. The error rates in conventional biopsy assessments can be estimated by the disagreement rate between pathologists in TCMR: when one diagnoses TCMR-like changes in a biopsy, a second will agree 50% of the time for kidney transplants (2), 28% for heart transplants (3), and 0-18% in lung transplants (4). We believe that the solution lies in centralized molecular analysis measuring mRNA levels, with automated analysis to interpret the results.

This will change care.

Advantages and Insights from Centralized Molecular Measurements
(see Appendix)
1. Requires less tissue than histology
2. User-friendly: simply put tissue into RNAlater at room temperature and place in provided return containers
3. Rapid: biopsy processing time is usually in one to two business days plus transit time
4. Provides objective, quantitative, and probabilistic assessment
5. Standard, worldwide assessment
6. Ability to correct errors in histology: recalibrate empirical histology systems
7. Ability to calibrate blood and urine biomarkers
8. Ability to identify mechanisms and druggable targets
9. Provides “theranostic” support for drug development and use

Challenges of Molecular Assessment
1. Potential sampling error: How much is needed to represent the organ? How variable is the tissue within the organ?
2. Highly focal diseases may be missed or overrepresented
3. Search for truth when conventional phenotyping is poor: no gold standard

What is a Microarray and How Does it Measure Gene Expression?
• Specific DNA sequences in about 500,000 tiny squares
• Patient sample RNA is extracted, mRNA amplified and labeled as cRNA (antisense)
• Labeled patient cRNA hybridizes to complementary DNA sequences in specific squares
• Intensity of label in square tells how much that gene is expressed
Total expression of > 20,000 protein coding genes is quantified

MMDx Project Highlights
• Reference sets of transplant biopsies: kidneys (>2400), hearts (>1000), lungs (transbronchial, >200), livers (in process)
• International network of Key Opinion Leaders (KOL) for interpretation
• Extensive peer-reviewed literature support (see Appendix)
• New understanding of rejection and injury mechanisms

The Reference Set
• Each new biopsy is compared to the molecular features of similar biopsies in the appropriate (kidney, heart, lung, liver) reference set.
• Reference sets are a sample of biopsies from international centers that represent the prevalent transplant population, whether early post-transplant or more than 30 years post-transplant.
Another advantage of MMDx is that it can read medulla and small samples, unlike histology (14), reducing the risk of an "inadequate" or "sub-optimal" biopsy sample and the need for another biopsy. The Molecular Microscope® system generates an automatic report which is then transmitted to the clinician. Biopsy processing time is usually in one to two business days plus transit time. Only molecular measurements and time of the biopsy post-transplant are used. The report uses the microarray measurements of the level of expression of selected genes or gene set measurements to assess the probability of TCMR and ABMR as well as the extent of acute and chronic injury. The possibility that recent non-adherence or under-immunosuppression has triggered rejection is raised by certain phenotypes (e.g. late-onset TCMR) and by the arteriolar hyalinosis (ah) classifiers (15;16). The current report format is shown below for two different biopsies, one with TCMR and one with ABMR. This page shows the key findings (Global Disturbance (inflammation), acute kidney injury and atrophy-fibrosis scores and different rejection classifier scores) as well as the overall molecular interpretation. The relationship of the new biopsy to its nearest molecular neighbors in the Reference Set estimates future graft survival. Many other classifiers (page 2 of the reports – not shown for simplicity) are also run to establish details of ABMR. For example, these estimate the probability of microcirculation inflammation (ptc- and g-changes) and glomerular double contours (cg) as an estimate of late-stage ABMR. These are high in the biopsy shown, indicating that this biopsy has fully-developed ABMR. The net result of all of these measurements is integrated by a novel method called archetype analysis, a form of cluster analysis (13).

Insights derived from this project have changed the understanding of mechanisms of rejection and the classification of transplant diseases.

The clinician-leaders in our trials indicate that when there is agreement between histology and the MMDx, the MMDx report gives them greater confidence in managing the patient.
Sample Reports (page 1) | Explanation

**Clinical Information**
- Time post-transplant, biopsy indications, DSA, (if approved)

**Patient Information**
- Date of transplant, date of biopsy, etc.

**Clinical Interpretation**
- Molecular phenotype
- Summary of molecular changes (Normal, TCMR, ABMR)

**Proportions**
- Rejection-related molecular changes (Normal, TCMR, ABMR)

**Visualization**
- Relationship of this biopsy to others in reference set:
  - PC2 vs PC1
  - PC2 vs PC3

**This Biopsy**
- Yellow Triangle

**Survival**
- Of other kidneys like this one

---

**Molecular Microscope® Diagnostic Report for Kidney (MMDx-Kidney)**

**Redacted**

**Sample Reports (page 2) | Explanation**

**Additional Detail**
- Rejection, injury-related binary classifiers (and AKI transcript set)

**Comparison to Normal**
- Scores of this biopsy interpreted vs. relatively normal biopsies

**Histologic and Molecular Diagnoses**
- in the molecular nearest neighbors of this biopsy

**Adherence Index**
- Low scores in biopsies 6 months to 5 years post-transplant correlate with possible non-adherence or under immunosuppression

---

**References for the scores, classifiers, and archetypes**

2. Famulski K et al. JASN 2012; May;23(5):948-58.
5. Famulski K et al. JASN 2012; May;23(5):948-58.
7. Famulski K et al. JASN 2012; May;23(5):948-58.
13. Famulski K et al. JASN 2012; May;23(5):948-58.
16. Famulski K et al. JASN 2012; May;23(5):948-58.
**General Information**

<table>
<thead>
<tr>
<th>Surname</th>
<th>First Name</th>
<th>Physician</th>
</tr>
</thead>
<tbody>
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<table>
<thead>
<tr>
<th>Date of Birth</th>
<th>Sample ID</th>
<th>Date Received (Y-M-D)</th>
<th>Time of Biopsy Post-Tx</th>
<th>9.6 years</th>
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<tr>
<td>Date Reported (Y-M-D)</td>
<td>Transplant Type</td>
<td>—</td>
<td>—</td>
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<tr>
<td>Date of Transplant (Y-M-D)</td>
<td>Biopsy Indication</td>
<td>—</td>
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<tr>
<td>Date of Biopsy (Y-M-D)</td>
<td>Primary Disease</td>
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**Biopsy Interpretation**

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>1.0</td>
<td>Normal</td>
</tr>
<tr>
<td>0.1</td>
<td>Mild</td>
</tr>
<tr>
<td>0.0</td>
<td>Severe</td>
</tr>
</tbody>
</table>

**Scores from archetypal analysis**

<table>
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<tr>
<th>Score</th>
<th>Description</th>
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</thead>
<tbody>
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<td>0.34</td>
<td>Mild</td>
</tr>
<tr>
<td>0.32</td>
<td>Severe</td>
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<tr>
<td>0.30</td>
<td>Normal</td>
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</table>

**Scores from page 2**

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.33</td>
<td>Mild</td>
</tr>
<tr>
<td>0.32</td>
<td>Normal</td>
</tr>
<tr>
<td>0.30</td>
<td>Severe</td>
</tr>
</tbody>
</table>

**References**

4. Famulski K et al. JASN 2012; May;23(5):948-58.

**Molecular Microscope® Diagnostic Report for Kidney (MMDx-Kidney)**

- **TCMR related**
  - TCMR-1: 0.01 (0.0 – 1.0)
  - TCMR-2: 0.01 (0.0 – 1.0)
- **ABMR related**
  - ABMR-1: 0.92 (0.0 – 1.0)
  - ABMR-2: 0.77 (0.0 – 1.0)
- **Injury related**
  - Injury Score: 0.10 (0.0 – 1.0)
  - Acute Injury Score: 0.10 (0.0 – 1.0)
  - Acute Inflammation Score: 0.10 (0.0 – 1.0)

**Rank order of the most common histologic diagnoses in the 50 nearest molecular neighbors**

<table>
<thead>
<tr>
<th>Classification</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tubulitis (t) &gt; 1</td>
<td>1.00</td>
</tr>
<tr>
<td>Tubular atrophy (ct) &gt; 1</td>
<td>0.90</td>
</tr>
<tr>
<td>Interstitial inflammation (i) &gt; 1</td>
<td>0.84</td>
</tr>
</tbody>
</table>

**Critical Notes**

- A: The 2.5th to 97.5th percentiles in the entire Reference Set
- B: 95th percentile in relevant Reference Set biopsies
- C: Mean of scores from page 2
- D: Scores from archetypal analysis
- E: Score in a quality control measure

---

**Survival in patients with similar biopsies in the Reference Set**

<table>
<thead>
<tr>
<th>Time</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-year: 92%</td>
<td></td>
</tr>
<tr>
<td>3-years: 76%</td>
<td></td>
</tr>
<tr>
<td>5-years: 56%</td>
<td></td>
</tr>
</tbody>
</table>

---

**Pure molecular interpretation**

Abnormal biopsy. Severe early-stage ABMR with g and ptc-related molecular features. No TCMR. Mild inflammation, AKI and atrophy-fibrosis. Note that MMDx cannot exclude primary renal diseases.

**Molecular Microscope® (MMDx-Kidney)**

- **ABMR related**
  - ABMR-1: 0.92 (0.0 – 1.0)
  - ABMR-2: 0.77 (0.0 – 1.0)
  - ABMR-3: 0.84 (0.0 – 1.0)
  - ABMR-4: 0.81 (0.0 – 1.0)

---

**Classifiers based on histologic lesions**

<table>
<thead>
<tr>
<th>Classifier</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCMR-1</td>
<td>0.01 (0.0 – 1.0)</td>
</tr>
<tr>
<td>TCMR-2</td>
<td>0.01 (0.0 – 1.0)</td>
</tr>
</tbody>
</table>

**For classifiers:**

- **TCMR-1**: TCMR vs everything else
- **TCMR-2**: TCMR vs everything else, with BK/Borderline/Mixed withheld
- **ABMR-1**: ABMR vs everything else
- **ABMR-2**: ABMR and Mixed vs everything else
- **ABMR-3**: ABMR vs everything else, with Mixed/TG/ABMR suspicious withheld
- **ABMR-4**: ABMR vs everything else, with Mixed/TG/ABMR suspicious withheld

**References for the scores, classifiers, and archetypes**

4. Famulski K et al. JASN 2012; May;23(5):948-58.

---

**Percent cortex**

<table>
<thead>
<tr>
<th>Age</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-year: 32%</td>
<td></td>
</tr>
<tr>
<td>2-years: 76%</td>
<td></td>
</tr>
<tr>
<td>5-years: 56%</td>
<td></td>
</tr>
</tbody>
</table>

---

**Notes**

A: The 2.5th to 97.5th percentiles in the entire Reference Set
B: 95th percentile in relevant Reference Set biopsies
C: Mean of scores from page 2
D: Scores from archetypal analysis
E: Score in a quality control measure
Scores from archetypal analysis

<table>
<thead>
<tr>
<th>Biopsy</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.12</td>
<td>0.66</td>
</tr>
<tr>
<td>0.03</td>
<td>Mean of scores from page 2</td>
</tr>
</tbody>
</table>

- 0.75
- 0.14
- 0.10

A  - The 2.5th to 97.5th percentiles in the entire Reference Set
C - Mean of scores from page 2
D - Scores from archetypal analysis
E - Percentile in relevant Reference Set Biop

References for the scores, classifiers, and archetypes
The Molecular Microscope Diagnostic System for Heart Transplant Biopsies

**INTERHEART Trial Summary**

(ClinicalTrials.gov NCT02670408)

The current standard for histologic diagnosis of heart transplant endomyocardial biopsies (EMBs) uses The International Society for Heart & Lung Transplantation (ISHLT) classification, which has limited interobserver reproducibility (3). To improve biopsy assessment, the Alberta Transplant Applied Genomics Centre (ATAGC) adapted the Molecular Microscope developed in kidney transplant biopsies to heart transplant endomyocardial biopsies (EMBs) in the INTERHEART study. The molecular phenotype of ABMR in heart transplants is remarkably similar to that in kidney transplants (17). This permits us to use kidney rejection-associated transcripts to classify heart transplant biopsies. The first generation Molecular Microscope system, evaluated in EMBs from Paris, Edmonton, and Bologna, has been published (18). Two major publications are under review (19;20).

**Study**

1. Distinguish injury from rejection.
2. Expand the Reference Set for heart transplants (EMBs) with molecular, histologic, DSA, and clinical data.
3. Develop the Molecular Microscope report for EMBs, incorporating rejection and injury transcripts discovered in kidney.
4. Validate and refine the system by reporting in real time (<48 hours from receiving the biopsy) 1000 new heart biopsies and obtain feedback from KOL clinicians. This involves unselected, prospectively collected, standard-of-care EMBs from North American, European, and Australian Centers.
5. Develop and optimize a transparent and user-friendly format to communicate this information to clinicians.
6. The MMDx-Heart report will express rejection as archetype scores for probability of ABMR, TCMR and non-rejection, with the scores adding up to 1.0 (see figure below).

**Visualization**

Relationship of this biopsy to others in reference set:  
- PC1 vs PC3  
- PC2 vs PC3

**The MMDx-Heart report: The scores in the new biopsy relative to those in the Reference Set colored by histologic diagnosis. This is incorporated into the report (page 1).**
Sample Reports (page 2) | Explanation

**Additional Detail**
Rejection, injury-related transcript set scores in this biopsy

**Comparison to Normal**
Scores of this biopsy interpreted vs. normal biopsies

---

**Molecular Diagnostic Report**

**Legend:** NA = not available, ABMR = antibody-mediated rejection, TCMR = T cell-mediated rejection

**Molecular Phenotype**

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Biopsy Score</th>
<th>Normal Score</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABMR-related</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DSA-selective (DOAS)</td>
<td>0.02</td>
<td>&lt; 0.08</td>
<td>abnormal</td>
</tr>
<tr>
<td>IFN-$\gamma$ inducible (GRIT) 1.14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NK cell burden (NKB)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Injury-related</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Injury cluster (S4)</td>
<td>0.16</td>
<td>&lt; 0.30</td>
<td>slightly abnormal</td>
</tr>
<tr>
<td>Injury transcripts (QCMATs)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Other Clinical Information**

<table>
<thead>
<tr>
<th>Diagnoses given without association</th>
<th>Model 1</th>
<th>Model 2</th>
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<tbody>
<tr>
<td>ABMR</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Injury</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

**Pure molecular interpretation**

Abnormal biopsy with severe ABMR. No TCMR. Some parenchymal dedifferentiation (HT1s slightly abnormal) with some parenchymal injury (S4 slightly abnormal and QCMATs abnormal). Considering the severity of the rejection changes the parenchyma is relatively well preserved.

Signed out by Dr. P.F. Halloran

---

**Additional Clinical Data**

A g. CAV if provided (not used for report)
### Severe ABMR

<table>
<thead>
<tr>
<th>Molecular Phenotype</th>
<th>PBT/Gene</th>
<th>Biopsy Score</th>
<th>Normal limit</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ABMR-related</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DSA-selective (DSAST)</td>
<td>1.02</td>
<td>&lt; 0.08</td>
<td>abnormal</td>
<td></td>
</tr>
<tr>
<td>Endothelial DSA-selective (eDSAST)</td>
<td>0.60</td>
<td>&lt; 0.16</td>
<td>abnormal</td>
<td></td>
</tr>
<tr>
<td>NK cell burden (NKB)</td>
<td>1.67</td>
<td>&lt; 0.12</td>
<td>abnormal</td>
<td></td>
</tr>
<tr>
<td>ROBO4</td>
<td>10.25</td>
<td>&lt; 9.49</td>
<td>abnormal</td>
<td></td>
</tr>
<tr>
<td><strong>TCMR-related</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytotoxic T cell transcripts (GQAT)</td>
<td>1.45</td>
<td>&lt; 0.15</td>
<td>abnormal</td>
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<tr>
<td>T cell burden (TCB)</td>
<td>1.26</td>
<td>&lt; 0.30</td>
<td>abnormal</td>
<td></td>
</tr>
<tr>
<td>Enzyme (ADAMDEC1)</td>
<td>1.99</td>
<td>&lt; 2.96</td>
<td>slightly abnormal</td>
<td></td>
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<tr>
<td>Cytokine (CXCL13)</td>
<td>4.91</td>
<td>&lt;5.20</td>
<td>normal</td>
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<tr>
<td>Interferon gamma (IFNG)</td>
<td>5.18</td>
<td>&lt;4.13</td>
<td>abnormal</td>
<td></td>
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<tr>
<td>Checkpoint (CTLA4)</td>
<td>3.94</td>
<td>&lt;3.66</td>
<td>slightly abnormal</td>
<td></td>
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<tr>
<td><strong>All rejection and injury-related</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>IFNG inducible (GRIT)</td>
<td>1.14</td>
<td>&lt; 0.11</td>
<td>abnormal</td>
<td></td>
</tr>
<tr>
<td><strong>Injury-related</strong></td>
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<td></td>
</tr>
<tr>
<td>Heart transcripts (HT1)</td>
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<td>slightly abnormal</td>
<td></td>
</tr>
<tr>
<td>Injury transcripts (IRB1T)</td>
<td>0.21</td>
<td>&lt; 0.24</td>
<td>normal</td>
<td></td>
</tr>
<tr>
<td>Injury cluster (S4)</td>
<td>0.17</td>
<td>&lt; 0.10</td>
<td>slightly abnormal</td>
<td></td>
</tr>
<tr>
<td>Macrophage transcripts (QCMAT)</td>
<td>0.70</td>
<td>&lt; 0.14</td>
<td>abnormal</td>
<td></td>
</tr>
</tbody>
</table>

### Other Clinical Information

- **Myasthenia gravis without exacerbation**, elevated antibody levels post IgG+ Rituxan late 2015, ongoing DQ2 at 150 µg at May 2016, IVIG + Roflumilast and Prednisone.

### The Molecular Microscope Diagnostic System for Lung Transplant Biopsies

**INTERLUNG Trial Summary**

**ClinicalTrials.gov NCT02812290**

The current standard for biopsy-based diagnoses of rejection of lung transplants is the transbronchial biopsy (TBB) interpreted by histology using the ISHLT guidelines. This system has many weaknesses and errors, and many TBBs cannot be assessed by histology. To improve diagnostics in lung transplant biopsies, the Molecular Microscope® developed in kidney transplant biopsies has been adapted to lung transplant TBBs. INTERLUNG will also explore the potential of mucosal biopsies (MB) from the third bronchial bifurcation (3B-MBs) to provide the same estimates as TBB, reducing the risks of biopsy complications. The first major publication is under review (21).

### Study

1. Distinguish rejection (TCMR, ABMR) from acute injury.
2. Define the molecular phenotype of CLAD.
3. Develop a reference set of >1000 TBBs with molecular, histologic, DSA, and clinical data.
4. Develop the Molecular Microscope system to report TBBs, incorporating rejection/injury principles discovered in kidney and heart.
5. Determine from experience how many TBB bites are needed to compensate for sampling error.
6. Develop an algorithm to determine from MB biopsies the potential of mucosal biopsies (3B-MB) to provide the same estimates as TBB.
7. Explore the potential for a safer biopsy format by testing whether mucosal biopsies from the third bronchial bifurcation (3B-MB) can provide similar assessments to TBB.

The provisional MMDx-Lung report for TBBs showing the relationship of the molecular findings in the new biopsy to those in the Reference Set. Molecular Scores will be summarized as Archetype Scores reflecting the probability of ABMR, TCMR and injury, with total adding to 1.0 (as shown). The A4 biopsies have increased expression of some endothelial transcripts but this may represent a form of lung injury.
**Clinical Information**
- Time post-transplant: indication, DSA (if provided)
- Patient Information: Date of transplant, date of biopsy, etc.
- Clinical Interpretation: Molecular phenotype
- Proportions: Normal, TCMR, ABMR, Injury
- Visualization: Relationship of this biopsy to others in reference set: PC2 vs PC1, PC2 vs PC3

**Sample Reports (page 1) | Explanation**

**Additional Detail**
- Rejection, injury-related transcript scores in the new biopsy
- Comparison to Normal: Scores of this biopsy interpreted vs. relatively normal biopsies
- Alveolar Content: Too little makes interpretation difficult
- Additional Clinical Data: e.g., histology, clinical notes, CLAD if provided

**References**
Alberta Transplant Applied Genomics Centre
250 Heritage Medical Research Centre
University of Alberta
Edmonton, AB, Canada
T6G 2S2
Ph. 780-407-8880
Fax 780-407-3417

INTERLUNG Study: Molecular Diagnostic Report (Transbronchial Biopsy)

DRAFT: FOR RESEARCH PURPOSES ONLY

*Normal range for all genes/gene sets (except surfactant) includes values in the 90th percentile of biopsies in the relatively normal group (archetype cluster 1). The normal limit for surfactant includes values in the 25th percentile in all biopsies.

†Surfactant score is the geometric mean expression level calculated across 11 surfactant probe sets.

‡Slightly abnormal – biopsy score is between the 90th and 99th percentile of values in the relatively normal reference biopsies (archetype cluster 1). Abnormal – biopsy score exceeds values in the 99th percentile of biopsies belonging to the relatively normal reference biopsies (archetype cluster 1).

Local Histopathology Phenotype

Acute Rejection
Airway Inflammation
Chronic Airway Rejection
Other

ISHLT A Grade
4
ISHLT B Grade
x
ISHLT C Grade
x
C4d
Neutrophil/ Capsule extent/ Margination
No

C4d

Diagnosis
ACR

References

Molecular Phenotype

<table>
<thead>
<tr>
<th>Gene Category</th>
<th>Genes/gene sets</th>
<th>Biopsy score</th>
<th>Normal Limit</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABMR-related</td>
<td>DSA-selective transcripts (DSAST)</td>
<td>0.95</td>
<td>&lt;0.38</td>
<td>Normal</td>
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<tr>
<td></td>
<td>Endothelial DSA-selective transcripts (eDSAST)</td>
<td>0.17</td>
<td>&lt;0.55</td>
<td>Normal</td>
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<tr>
<td></td>
<td>T cell burden transcripts (TBB)</td>
<td>0.53</td>
<td>&lt;0.55</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td>T cell transcripts (TCT)</td>
<td>1.41</td>
<td>&lt;0.4</td>
<td>Abnormal</td>
</tr>
<tr>
<td></td>
<td>T cell burden transcript (TBB)</td>
<td>1.61</td>
<td>&lt;0.48</td>
<td>Abnormal</td>
</tr>
<tr>
<td></td>
<td>IFN-Gamma transcripts (IFN-GRT)</td>
<td>0.24</td>
<td>&lt;0.23</td>
<td>Abnormal</td>
</tr>
<tr>
<td></td>
<td>Injury transcripts (IRNT)</td>
<td>0.31</td>
<td>&lt;0.28</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td>Microtranscript (QCMAT)</td>
<td>0.19</td>
<td>&lt;0.47</td>
<td>Normal</td>
</tr>
<tr>
<td>TCMR-related</td>
<td>Cytotoxic T cell transcripts (QCAT)</td>
<td>1.41</td>
<td>&lt;0.4</td>
<td>Abnormal</td>
</tr>
<tr>
<td></td>
<td>T cell burden transcripts (QCT)</td>
<td>1.61</td>
<td>&lt;0.48</td>
<td>Abnormal</td>
</tr>
<tr>
<td></td>
<td>IFN-Gamma transcripts (QIFN)</td>
<td>0.24</td>
<td>&lt;0.23</td>
<td>Abnormal</td>
</tr>
<tr>
<td></td>
<td>Injury transcripts (QIRNT)</td>
<td>0.31</td>
<td>&lt;0.28</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td>Microtranscript (QCMAT)</td>
<td>0.19</td>
<td>&lt;0.47</td>
<td>Normal</td>
</tr>
<tr>
<td>Other</td>
<td>Surfactant transcripts (Alveolar content)</td>
<td>1.37</td>
<td>&gt;0.7</td>
<td>Adequate</td>
</tr>
</tbody>
</table>

Normal range for all gene/gene sets (except surfactant) includes values in the 90th percentile of biopsies in the relatively normal group (archetype cluster 1). The normal limit for surfactant includes values in the 25th percentile in all biopsies.

Surfactant score is the geometric mean expression level calculated across 11 surfactant probe sets.

Slightly abnormal - biopsy score is between the 90th and 99th percentile of values in the relatively normal reference biopsies (archetype cluster 1). Abnormal - biopsy score exceeds values in the 99th percentile of biopsies belonging to the relatively normal reference biopsies (archetype cluster 1).

Molecular Phenotype

<table>
<thead>
<tr>
<th>Proportion Rejection and Injury</th>
<th>Model 1</th>
<th>0.00</th>
<th>TCMR/Injury</th>
<th>1.00</th>
<th>ABMR/Injury</th>
<th>0.00</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative Normal</td>
<td>0.00</td>
<td>TCMR</td>
<td>0.00</td>
<td>0.91</td>
<td>ABMR</td>
<td>0.05</td>
</tr>
<tr>
<td>Relative Abnormal</td>
<td>0.00</td>
<td>TCMR</td>
<td>0.95</td>
<td>0.09</td>
<td>Normal</td>
<td>0.90</td>
</tr>
</tbody>
</table>

Current Biopsy vs Reference Set: PC2 vs PC1

Current Biopsy vs Reference Set: PC2 vs PC3

ACR

References
The Molecular Microscope Diagnostic System for Liver Transplant Biopsies

INTERLIVER Trial Summary
(ClinicalTrials.gov NCT03193151)
The Molecular Microscope will be adapted to read liver transplant biopsies for injury and rejection in a new study (INTERLIVER), using the same strategies that have been used for heart biopsies. Sample collection has already begun at select centers and additional centers are joining the effort. The first generation report will be produced after approximately 100 biopsies have been collected in the Reference Set.

Insights Derived from Molecular Microscope Project
1. Mouse models of TCMR: CATs (24); GRITs (25); injury and dedifferentiation (26-29).
2. Inflammatory/injury disturbance in kidney transplant biopsies (30), in heart transplant endomyocardial biopsies (31), and in lung transbronchial biopsies (32) is stereotyped: molecules "travel in herds"
3. High frequency of C4d negative ABMR (33;34)
4. ABMR is the main cause of renal transplant loss and is often C4d negative (34;35)
5. High frequency of non-adherence in ABMR and graft loss (36)
6. Molecular classifier for all rejection (TCMR or ABMR) (37)
7. Mechanisms/landscape of TCMR: potential role of checkpoints (8;10)
8. Disappearance of TCMR despite persistence of ABMR (35)
9. TCMR does not program late graft loss (35)
10. Development of a molecular classifier for TCMR (2)
11. TCMR and ABMR share mechanisms (10;37)
12. Donor age is a risk factor in early ABMR (38)
13. Molecular classifier for ABMR (39;40)
14. Molecular Landscape of ABMR: potential role of NK cells and CD16a (9)
15. Molecular Landscape of ABMR in heart transplants (17)
16. Landscape of acute parenchymal injury (AKI) (7)
17. Mechanisms/landscape of atrophy-fibrosis: progression is due to injury, not autonomous fibrosis (5;41)
18. Risk score for graft failure involves AKI molecules (42)
19. Immunoglobulin transcripts (IGTs) (43) and mast cell transcripts (MCATs) (44) are features of atrophy-fibrosis
20. TCMR-like process in polyoma virus nephropathy (PVN) (2)
21. Lack of TCMR in many biopsies with isolated v lesions (37;45)
22. Regression equations improve histologic prediction of TCMR (46)
23. Regression equations improve conventional diagnosis of ABMR, even without DSA (47)
24. Sub-phenotypes of ABMR: early stage, fully-developed, and late-stage sub-phenotypes (11)
25. FOXP3 expression: associated with inflammation, not outcomes (48)
26. Lung transplants: first results with transbronchial biopsies (TBB) (21)
27. Lack of hyalinosis may indicate non-adherence (14)
28. MMDx-Kidney can read medulla (13)


Ordering Information
1. Go to www.molecular-microscope.com
2. Click "Order Now"
3. Complete ordering form and click "send"
4. Specimen Kit will be delivered to your center

Specimen Kit Includes:
- Instructions
- Specimen tube and labels
- Return containers and labels

Specimen Handling
- Unpack your Specimen Kit and instructions.
- Place biopsy (Kidney or Liver: 5 mm of biopsy or portion of core) immediately from the needle or bite (Heart or Lung: 2 bites) in specimen tube provided in the kit. Washes would destroy the sample; please refrain from saline or any other washes.
- Pack and ship. Pack samples and ship to address on the label provided in the kit.

Biopsy Results
- Results are generally available within 1-2 days (28 hours for Kidney, 48 hours for Heart) of receiving the sample.
- An account will be created for you on our secure results portal upon receipt of your first sample.
- You will receive an email notification once the report is available on the portal.

Client Services Assistance
- Assist with result interpretation/consultation
- Specimen requirements and handling
- Requisition completion
- Report status
- Client supplies
- Cost and billing

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Website: www.molecular-microscope.com

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