Molecular Signals of Intragraft Rejection: Is INTERHEART true NORTH?

Phil Halloran, MD PhD
Alberta Transplant Applied Genomics Center (ATAGC)
University of Alberta
and
Transcriptome Sciences Inc (TSI)
Edmonton, AB
Relevant Financial Relationship Disclosure Statement

The Molecular Microscope® Diagnostic System

Presenter: Phil Halloran

Our studies are supported in part 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-Behring

http://atagc.med.ualberta.ca/Services/MolecularMicroscopeSystem/
Learning Objectives: **The INTERHEART study** Clinicaltrials.gov NCT02670408

To understand:

1. The unmet need in heart transplant diagnostics
2. The principles of microarray analysis
3. Unsupervised and supervised analysis of high dimensionality data
4. The relationship of the MMDx diagnoses to histology diagnoses
5. The role of myocardial injury in heart transplant outcomes
<table>
<thead>
<tr>
<th>Center</th>
<th>Principal investigators</th>
<th>Number in 889 cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Coruña, Spain</td>
<td>Dra. Maria G. Crespo-Leiro</td>
<td>92</td>
</tr>
<tr>
<td>Bologna, Italy</td>
<td>Dr. Luciano Potena</td>
<td>201</td>
</tr>
<tr>
<td>Edmonton, Canada</td>
<td>Dr. Daniel Kim</td>
<td>113</td>
</tr>
<tr>
<td>France</td>
<td>Drs. Alex Loupy, P. Bruneval, and Xavier Jouven</td>
<td></td>
</tr>
<tr>
<td>Centre Hospitalier Universitaire de Bordeaux</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Centre Hospitalier Universitaire de Rouen</td>
<td></td>
<td>9</td>
</tr>
<tr>
<td>Hôpital Européen Georges-Pompidou</td>
<td></td>
<td>203</td>
</tr>
<tr>
<td>Centre Hospitalier Universitaire de Nantes</td>
<td></td>
<td>11</td>
</tr>
<tr>
<td>Hôpital Necker</td>
<td></td>
<td>7</td>
</tr>
<tr>
<td>Hôpital de la Pitié</td>
<td></td>
<td>24</td>
</tr>
<tr>
<td>Los Angeles, USA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cedars-Sinai Medical Center</td>
<td>Dr. Jon Kobashigawa</td>
<td>51</td>
</tr>
<tr>
<td>University of California Los Angeles</td>
<td></td>
<td>7</td>
</tr>
<tr>
<td>Sydney, Australia</td>
<td>Dr. Peter Macdonald</td>
<td>92</td>
</tr>
<tr>
<td>Vienna, Austria</td>
<td>Drs. Andreas Zuckermann, Arezu Aliabadi, and Johannes Goekler</td>
<td>76</td>
</tr>
<tr>
<td>Virginia, USA*</td>
<td>Dr. Keyur B. Shah</td>
<td>2</td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td>889</td>
</tr>
</tbody>
</table>

* Two biopsies from Virginia Commonwealth University were not formally part of the INTERHEART study but we included them on request of the center, with patient consent.
MMDx-Heart

INTERHEART
Clinicaltrials.gov NCT02670408

The problem: unreliable ("imprecise") histology diagnoses. We cannot train strong supervised classifiers on unreliable diagnoses.
Molecular phenotype of rejection in heart transplants is very similar to kidney rejection: permits kidney phenotype to guide development of MMDx-heart
Distinguishing a heart injury phenotype from rejection

889 EMBs from international centers

Local standard-of-care histology, DSA

Gene expression microarrays

New Archetypal Analysis - S1, normal; S2, toxic; S3, injury, S4, toxic, scores

Compare new four-archetype model vs. previous three-archetype model

Visualize archetypal analysis results in principal component analysis (PCA)

Identify top genes correlated with the archetype and PCA scores.

Perform pathway analysis using top transcripts.

Analyze relationships between archetype scores, PCA scores, pathogenesis-based transcript set scores, time of biopsy post-transplant

Relate molecular scores to left ventricular ejection fraction

Examines the effect of injury on histologic diagnoses of rejection

Table 2. Histology summary available in 889 EMBs

<table>
<thead>
<tr>
<th>Histology diagnoses* (% of known diagnoses)</th>
<th>All (889 biopsies)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Rejection</td>
<td>334 (38%)</td>
</tr>
<tr>
<td>TCMR Related</td>
<td>84 (9%)</td>
</tr>
<tr>
<td>pTCMR</td>
<td>273 (31%)</td>
</tr>
<tr>
<td>ABMR Related</td>
<td>9 (1%)</td>
</tr>
<tr>
<td>pABMR</td>
<td>71 (8%)</td>
</tr>
<tr>
<td>ABMR/TCMR (Mixed)</td>
<td>63 (7%)</td>
</tr>
<tr>
<td>pABMR/pTCMR</td>
<td>4 (0%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>DSA Status</th>
<th>All (454 patients)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>158 (37%)</td>
</tr>
<tr>
<td>Negative</td>
<td>267 (63%)</td>
</tr>
<tr>
<td>Not tested</td>
<td>29 (6%)</td>
</tr>
</tbody>
</table>

*Biopsies in the 889 cohort were labeled as follows:

- pAMR1
- pAMR1+ , pAMR1H+ ... Possible ABMR (pABMR)
- pAMR2, pAMR3
- TCMR80R
- TCMR10R
- pTCMR
- TCMR2R, TCMR3R
- TCMR

Biopsies in the 331 cohort were reclassified using the above criteria.

*The most recent DSA status at time of most recent biopsy was used, if known. DSA statuses dated more than 14 days after the biopsy were not considered. If the most recent DSA status at time of biopsy was not known, but the patient was most recently PRA negative, the DSA status was presumed negative. PRA statuses dated more than 14 days after the biopsy were not considered.

Figure 1. Overview of the work plan implemented in this investigation.

Developing the Molecular Microscope® system for EMBs (MMDx-Heart)

Rejection-associated transcripts

New four-state (4 archetype) model:

- $S_{1_{\text{normal}}} = \text{no rejection or injury}$
- $S_{2_{\text{TCMR}}} = \text{TCMR}$
- $S_{3_{\text{ABMR}}} = \text{ABMR}$
- $S_{4_{\text{injury}}} = \text{recent heart injury}$
Principal component analysis of 889 heart transplant biopsies based on their expression of rejection associated transcripts (RATs). Samples in A-C are colored according to their highest archetype score (white = S1-normal, red = S2-TCMR, blue = S3-ABMR, orange = S4-Injury) in the four-archetype model trained on RAT expression. The large A1-A4 text labels superimposed on plots A-C mark the position of the theoretical archetypes to which each sample is compared. Panels D-F are colored by ISHLT rejection grade cut-offs according to the key at the bottom of the figure.

Molecular classes of heart biopsies

Histology diagnoses: extensive disagreement, e.g. in “normal” and in “injury”
The response to injury dominates the first weeks after heart transplant and is sometimes confused with rejection.

Molecular Microscope® system for EMBs: correlations with depressed heart function (left ventricular ejection fraction, LVEF)

(Rejection/Injury Archetype) scores and LVEF:

- $S_{4\text{injury}}$ and $S_{2\text{TCMR}}$: low LVEF
- $S_{1\text{normal}}$: high LVEF
- $S_{3\text{ABMR}}$: little effect
Figure 4. Running average of LVEF vs. archetype scores. For each of the four archetype scores, the 606 biopsies with available LVEF data were sorted by the archetype score being plotted. Then a sliding window of size N=85 biopsies was used to plot the mean LVEF vs. mean archetype score. I.e., the first data point on the left on the A1 line corresponds to the mean LVEF and mean S1 of the 1st through 85th biopsies (sorted in ascending order of the 606 S1 scores), the second point to the 2nd through 86th biopsies, etc. The lines have different x-axis ranges because, e.g., the highest 85 S2 scores is ~0.4, while the highest 85 scores for each of S1, S3, and S4 are larger.
An integrated molecular diagnostic report for heart transplant biopsies using an ensemble of diagnostic algorithms

Michael D. Parkes, XX, Anzoo Z. Aliabadi, XX, Martin Cadeiras, XX, Marisa G. Crespo-Leiro, XX, Mario Deng, XX, Eugene C. Depasquale, XX, Johannes Goekler, XX, Daniel H. Kim, XX, Jon Kobashigawa, XX, Alexandre Loupy, XX, Peter Macdonald, XX, Luciano Potena, XX, Andreas Zuckermann, XX, and Philip F. Halloran, XX.

From the Alberta Transplant Applied Genomics Centre, Edmonton, Alberta, Canada; Medical University of Vienna, Vienna, Austria; Ronald Reagan UCLA Medical Center, Los Angeles, California, USA; Complejo Hospitalario Universitario A Coruña, A Coruña, Spain; Department of Medicine, University of Alberta, Edmonton, Alberta, Canada; Cedars-Sinai Medical Center, Beverly Hills, California, USA; The Victor Chang Cardiac Research Institute, Sydney, New South Wales, Australia; and the Cardiovascular Department, University of Bologna, Bologna, Italy.

BACKGROUND: We previously reported a microarray-based diagnostic system for heart transplant endomyocardial biopsies (EMBs), using either 3-arylethenyl (3AA) or 4-arylethenyl (4AA) unsupervised algorithms to estimate rejection. In the present study we examined the stability of machine-learning algorithms in new biopsies, compared 3AA vs 4AA algorithms, assessed supervised binary classifiers trained on histologic or molecular diagnostics, created a report combining many scores into an ensemble of estimates, and examined possible automated sign-outs.

METHODS: We studied 889 EMBs from 454 transplant recipients at 8 centres the initial cohort \(O = 331\) and a new cohort \(V = 558\). Published 3AA algorithms derived in Cohort 331 were tested in Cohort 558, the 3AA and 4AA models were compared, and supervised binary classifiers were created.

RESULTS: Algorithms derived in Cohort 331 performed similarly in new biopsies despite differences in case mix. In the combined cohort, the 4AA model, including a prenatalomaly score, retained correlations with histologic rejection and DSA similar to the 3AA model. Supervised molecular classifiers predicted molecular rejection (area under the curve [AUC] = 0.87) better than histologic rejection (AUC 0.78), even when trained on histology diagnoses. A report incorporating many AA and binary classifier scores interpreted by 1 expert showed highly significant agreement with histology \((p < 0.001)\), but with many discrepancies, as expected from the known noise in histology. An automated random forest score closely predicted expert diagnoses, confirming potential for automated signouts.

CONCLUSIONS: Molecular algorithms are stable in new populations and can be assembled into an ensemble that combines many supervised and unsupervised estimates of the molecular disease states. J Heart Lung Transplant 2019; 38: ___.

### Table 3. Ability of binary molecular classifiers trained in histology or molecular diagnoses to predict histologic or molecular diagnoses

<table>
<thead>
<tr>
<th>4AA Scores</th>
<th>Prediction tested</th>
<th>Areas under the receiver-operator characteristic curve (AUCs) for classifiers predicting the diagnosis of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>All Rejection*, † (ABMR, TCMR, Mixed)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$S_1_{\text{normal}}$, $S_2_{\text{TCMR}}$, and $S_3_{\text{ABMR}^*}$</td>
<td>Histologic diagnoses</td>
<td>0.73</td>
</tr>
<tr>
<td>Classifiers trained on Histologic diagnoses*, ‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Molecular diagnoses*</td>
<td>0.74</td>
</tr>
<tr>
<td></td>
<td>Molecular diagnoses*</td>
<td>0.74</td>
</tr>
</tbody>
</table>

* All rejection = ABMR, TCMR, Mixed (ABMR/TCMR), ABMR/pTCMR, and pABMR/TCMR vs all other biopsies. ABMR = ABMR, Mixed, and ABMR/pTCMR vs all other biopsies. TCMR = TCMR, Mixed, and pABMR/TCMR vs all other biopsies.
† Molecular scores derived from the RAT-based four-archetype model of rejection. For classification purposes, we used cut-offs of $S_2_{\text{TCMR}} \geq 0.3$ for TCMR, $S_3_{\text{ABMR}^*} \geq 0.5$ for ABMR, and either cut-off for all rejections.
‡ Genes used: top 20 transcripts associated with histologic diagnoses. TCMR included biopsies with histologic TCMR grades > 1R, ABMR included biopsies with histologic ABMR grades > 1, and either cut-off for all rejections.
§ Genes used: top 20 transcripts associated with molecular diagnoses based on the four-archetype model of rejection using cut-offs of $S_2_{\text{TCMR}} \geq 0.3$ for TCMR, $S_3_{\text{ABMR}^*} \geq 0.5$ for ABMR, and either cut-off for all rejections.

We trained (supervised) molecular classifiers using either molecular diagnoses or histology diagnoses.

Even the histology trained molecular classifiers agreed much better with MMDX diagnoses than histology diagnoses.
Figure 3. Molecular Microscope® Report for heart transplant biopsies (MMDx-Heart). The new biopsy is compared to the reference set of 889 endomyocardial biopsies and given a series of molecular scores culminating in the assignment of a molecular interpretation. This new biopsy was relatively normal with molecular features typical of well-differentiated parenchymal tissue with minimal injury or rejection. Patient information in the first table has been redacted. Archetype scores S1 (Normal), S2 (TCMR), S3 (ABMR), and S4 (Injury) from the 3-archetype model (3AA/model 1) or 4-archetype model (4AA/model 2) are given for the new biopsy in addition to corresponding binary classifier scores predicting the probability of molecular non-rejection, TCMR, and ABMR. The report provides a visualization of the new biopsy (yellow triangle) projected into the rejection-associated transcript-based principal component analysis of the 889 reference set biopsies. Biopsies in the reference set are colored according to their highest of four archetype scores in the 4AA model. Grey indicates that S1 (Normal) was the highest score, red corresponds to S2 (TCMR), blue to S3 (ABMR), and cyan to S4 (Injury). The right hand side of the report provides a table of additional molecular data including pathogenesis-based transcript (PBT) set scores and singular transcript expression scores relating to all rejections, ABMR, TCMR, and injury. Scores are represented as the log fold change in the new biopsy vs. normal biopsies (i.e. reference set biopsies with S1 (Normal) >0.7). For each score a normal limit is given, defined as the 95th percentile score in the normal biopsies. Scores in the 95th-99th percentile are labeled “slightly abnormal” and scores in the 99th percentile are labeled “abnormal.” The report also has space for additional clinical information if provided.

Automated ensemble sign-out for heart biopsies

A Molecular Analysis of Graft Survival in the INTERHEART Study:
The importance of parenchymal injury
Purpose: Rejection is a major cause of graft loss in heart and kidney transplants. The principal diagnosis associated with risk in kidneys is antibody-mediated rejection ‘ABMR’ (JCI Insight 2(12), 201710.1172/jci.insight.94197), and molecular rejection predicts graft failure better than histology (JASN 26(7):1711-1720, 2015). Similar comparisons in a heart transplant endomyocardial biopsy (EMB) population have not been performed.

Methods: The INTERHEART study population contains 1265 indication and protocol EMB single bite biopsies from 18 centers in Canada, the USA, Australia and Europe. Affymetrix microarrays analyzed gene expression. 948 biopsies from 483 transplants (478 patients) had follow-up time and graft status. We selected 1 random biopsy per transplant and analyzed 3-year post-biopsy survival. Median follow-up time in this subset was 394 days, and 51/483 hearts failed by 3 years post-biopsy. We analyzed rejection by unsupervised archetype analysis using kidney-derived rejection-associated transcripts ‘RATs’ (JHLT 36:1192-1200, 2017) and by our interpretation of ISHLT histologic diagnosis.

Results: Four clusters of biopsies were found by archetypal analysis: 1) Non-rejection (N=686), 2) TCMR (129), 3) ABMR (437), and 4) Injury (13), used for the Kaplan-Meier plot of survival analysis in 483 transplants (Figure 1). Because only 4 hearts from cluster 4 remained (too few to analyze as a group), and 1 failed, each was incorporated into the next most closely associated archetype (3 moved to non-rejection, 1 of which failed, and 1 to TCMR). TCMR was a greater hazard than ABMR, both by molecules and histology (Figure 1). The results using histologic diagnoses were similar to those from molecular archetypes, except that the separation between ABMR and non-rejection was not as distinct using histology. Eleven biopsies, including one that failed, lacked a histologic diagnosis and were not included in the histologic survival analysis.

In addition to belonging to a dominant archetype, each biopsy also has a score for each of the 4 archetypes, which permit the degree of molecular injury to be considered. These were used for multivariable Cox regression (Table 1). The only significant predictor of survival is degree of injury.

Conclusion: In our study population, graft loss within the first years after EMB is more highly associated with TCMR than with ABMR. However, the best predictor of graft loss, as in kidneys, is the extent of injury regardless of its cause. Presumably TCMR (and ABMR) produce graft loss via molecular injury to the parenchyma. ClinicalTrials.gov # NCT02670408
### Table 1. Hazard ratios (HR) for 3-year death-censored survival after biopsy

<table>
<thead>
<tr>
<th>Variable</th>
<th>HR</th>
<th>Lower/Upper limit</th>
<th>p-val</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular archetype scores:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non rejection score</td>
<td>Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ABMR score</td>
<td>0.85</td>
<td>0.26</td>
<td>2.8</td>
</tr>
<tr>
<td>TCMR score</td>
<td>2.32</td>
<td>0.77</td>
<td>7.0</td>
</tr>
<tr>
<td>Injury score</td>
<td>11.03</td>
<td>2.1</td>
<td>58.5</td>
</tr>
</tbody>
</table>

Reeve, J1; and Halloran, PF1 .and the INTERHEART Investigators
Unlike kidneys, short term graft loss (particularly within one year) after EMB is highly associated with TCMR but not ABMR. TCMR may reflect failure of immunosuppression or non-adherence. This difference between the heart and renal transplant populations raises the possibility that TCMR is relatively more destructive, and ABMR less destructive, in heart than in kidney transplants. ClinicalTrials.gov # NCT02670408
MMDx-Heart in EMBs

- Defined rejection in *unsupervised* analysis
- Defined parenchymal injury as a fourth archetype
- Added *supervised* analyses of TCMR and ABMR
- Showed that LVEF is depressed by TCMR and injury, not ABMR
- Found the early losses after biopsy often are related to TCMR and injury
- Continuing studies:
  - More bites, more events (survival)
  - Define CAV and fibrosis
  - Define effects of treatment
New INTERHEART extension (INTERHEARTEX) focus on Rx, late phenotypes, events

- Define fibrosis
  - MRI T1
  - Histology grades in EMB
- Define CAV suspected CAV
- Define survival events (death retx):
  - What did clinician suspect/ attribution? Classify deaths
    - Rejection
    - Non-adherence
    - Completely unexpected previously well
- Known or suspected dysfunction/rejection
  - HFLEF – heart failure low ejection fraction
  - New phenotype: HFPEF - heart failure/symptoms/dysfunction preserved ejection fraction
- Post Rx bx is standard of care
- Question: do MTORIs inhibit fibrosis/hypertrophy? If so do they preserve/improve LVEF? (Peter MacDonald)
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
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

One Lambda/TMO licensing agreement
Industrial Research Assistance Program (IRAP)
Muttart Chair in Clinical Immunology
Previous funding:
  Genome Canada
  Roche Organ Transplantation Research Foundation
  Canada Foundation for Innovation
  University Hospital Foundation
  Capital Health/Alberta Health Services
Thank you