

Molecular Signals of Intragraft Rejection: Is INTERLUNG the Answer?



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Edmonton, AB



CUTTING EDGE of TRANSPLANTATION

TRANSPLANT SUMMIT 2019

NO SIZE FITS ALL: Uncovering the
Potential of Personalized Transplantation

Relevant Financial Relationship Disclosure Statement

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

In press JHLT Jan 2019

*TBB = transbronchial biopsy



ORIGINAL CLINICAL SCIENCE

Molecular assessment of rejection and injury in lung transplant biopsies

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KEYWORDS:
microarray;
antibody-mediated
rejection;
T-cell-mediated
rejection;
surfactant;
gene expression

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

METHODS: We prospectively studied whether microarray 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) in unsupervised machine learning to identify disease states.

RESULTS: All 242 single-piece 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 152 single-piece TBBs with high surfactant transcripts. Unsupervised archetypal analysis identified 4 idealized phenotypes (archetypes) and scored biopsies for their similarity to each: normal; T-cell-mediated rejection (TCMR; T-cell transcripts); antibody-mediated rejection (ABMR)-like (endothelial transcripts); and injury (macrophage transcripts). Molecular TCMR correlated with histologic TCMR. The relationship of molecular scores to histologic ABMR could not be assessed because of the paucity of ABMR in this population.

CONCLUSIONS: Molecular assessment of single-piece TBBs can be used to classify lung transplant biopsies and correlated with rejection histology. Two or 3 pieces for each TBB will probably be needed to offset sampling variance.

J Heart Lung Transplant ■■■■■

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**K. M. Halloran, M. D. Parkes, J. Chang, I. L. Timofte,
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Trulock, A. Roux, S. Juvet, S. Keshavjee, P. Jakob,
W. Klepetko, and P. F. Halloran.**

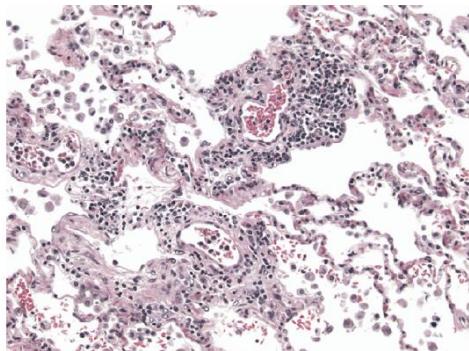
Molecular Assessment of Rejection and Injury in Lung Transplant Transbronchial Biopsies.

J Heart Lung Transplant in press 2019.

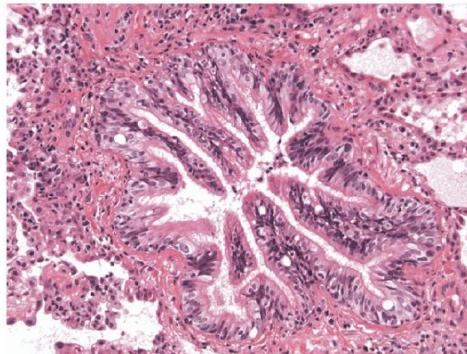
Lung transplantation plays a major role in management of end-stage lung disease, but outcomes are worse when compared with other organ transplants,^{1,2} with significant



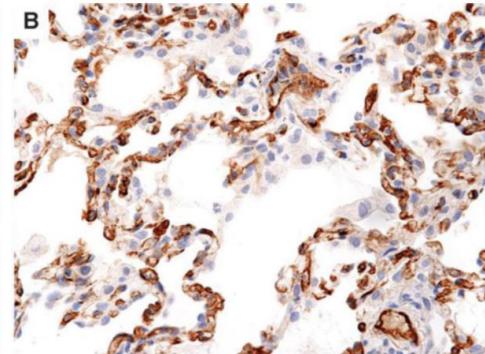
Histology of Transbronchial Lung Biopsies Has Poor Interobserver Agreement



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



B grade
Lymphocytic Bronchiolitis
Interobserver agreement = 0.035

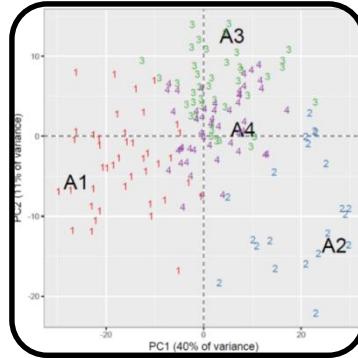
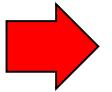
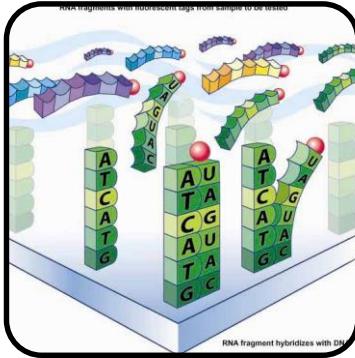
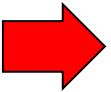
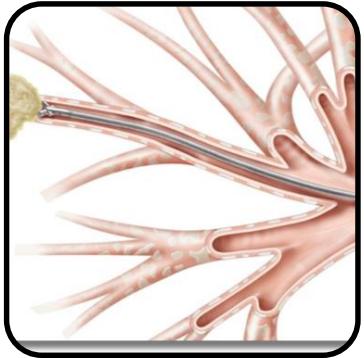


Antibody mediated rejection
C4d staining?
Neutrophilic
capillaritis/margination?
Interobserver agreement = ??

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Methods



Prospective TBB

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

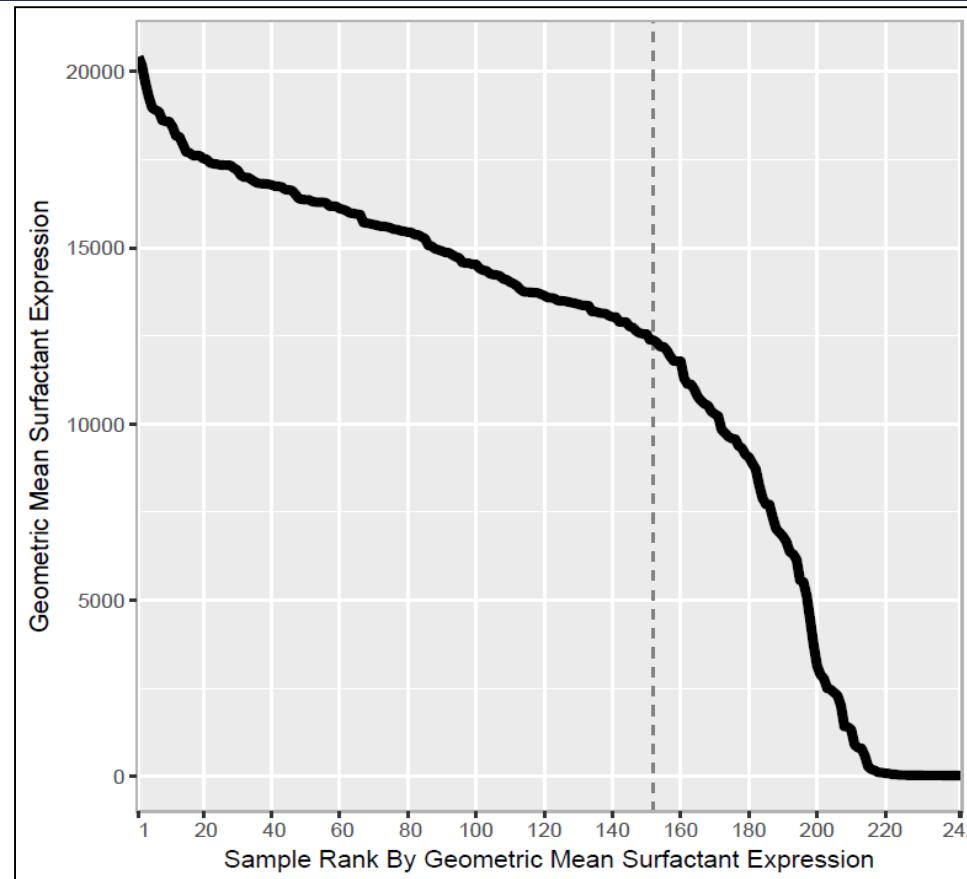
Microarray analysis at the ATAGC

- Quantitative expression of 453 rejection-associated transcripts (RATs)

High Dimensionality Data Analysis

- Principal components
 - Archetypal

Sample alveolar content



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

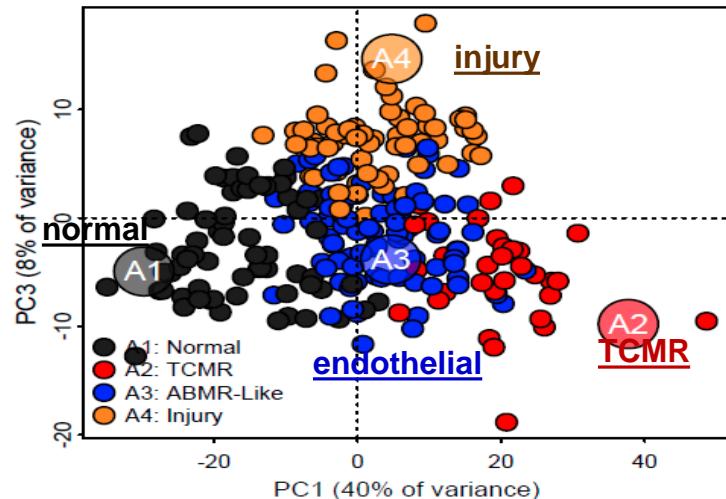
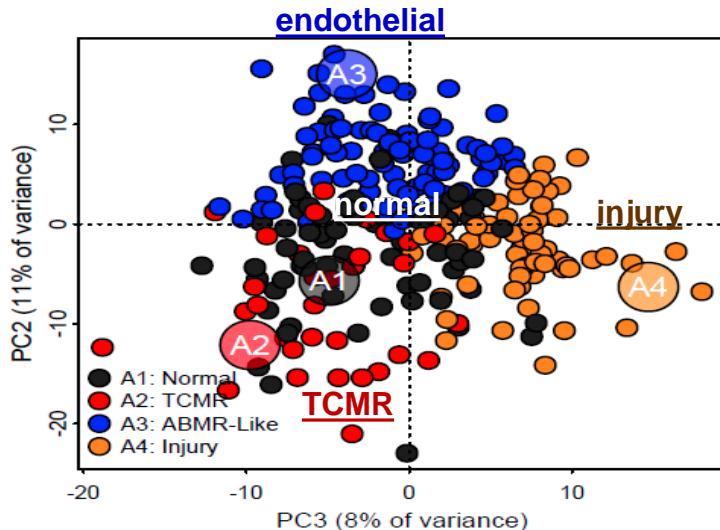
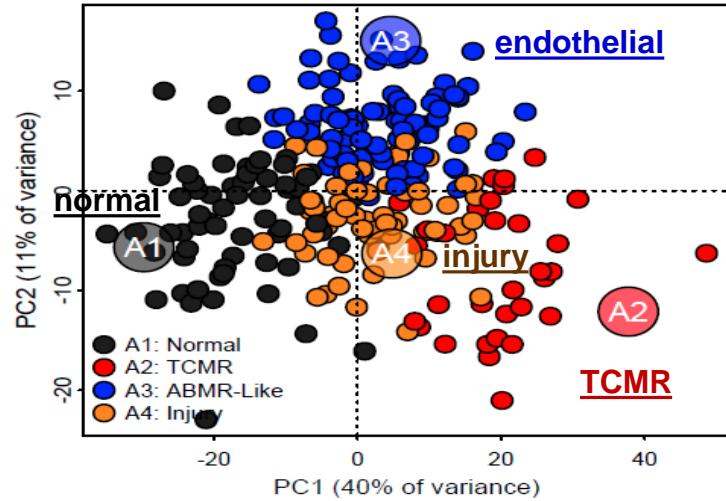
Four-state (4 archetype) model:

- $S1_{\text{normal}}$ = no rejection or injury
- $S2_{\text{TCMR}}$ = TCMR (includes ABMR?)
- $S3_{\text{ABMR}}$ = endothelial (not actually ABMR?)
- $S4_{\text{injury}}$ = lung abnormalities not rejection

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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

Score*	Key cellular expression patterns of the top 20 transcripts correlated or anti-correlated with the molecular score	Interpretation
S1 _{normal}	5 IFNG-inducible, 8 T/NK, 7 T/NK/MMDC	Absence of rejection and injury
S2 _{TCMR}	15 T (5 T, 5 T/NK, 5 T/NK/MMDC), 3 IFNG-inducible, 2 MMDC	T cell-mediated rejection
S3 _{endothelial}	17 HUVEC, 1 T/NK/MMDC, 1 MMDC, 1 Parenchymal	Endothelial
S4 _{Injury}	19 MMDC (16 MMDC, 3 T/NK/MMDC), 1 HUVEC	Macrophage infiltration (injury)
PC1	7 IFNG-inducible, 6 T/NK, 7 T/NK/MMDC	Rejection/inflammation
PC2	10 HUVEC, 2 IFNG-inducible, 5 MMDC, 1 Parenchymal, 2 unclear (low expression)	Endothelial
PC3	1 IFNG-inducible, 18 MMDC (14 MMDC, 4 T/NK/MMDC) 1 HUVEC	Macrophage infiltration (injury)

* 13 S1_{normal}, 18 S2_{TCMR}, 2 S3_{ABMR}, 0 S4_{Injury}, 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

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

Patient information

Date of transplant,
date of biopsy, etc.

Clinical interpretation

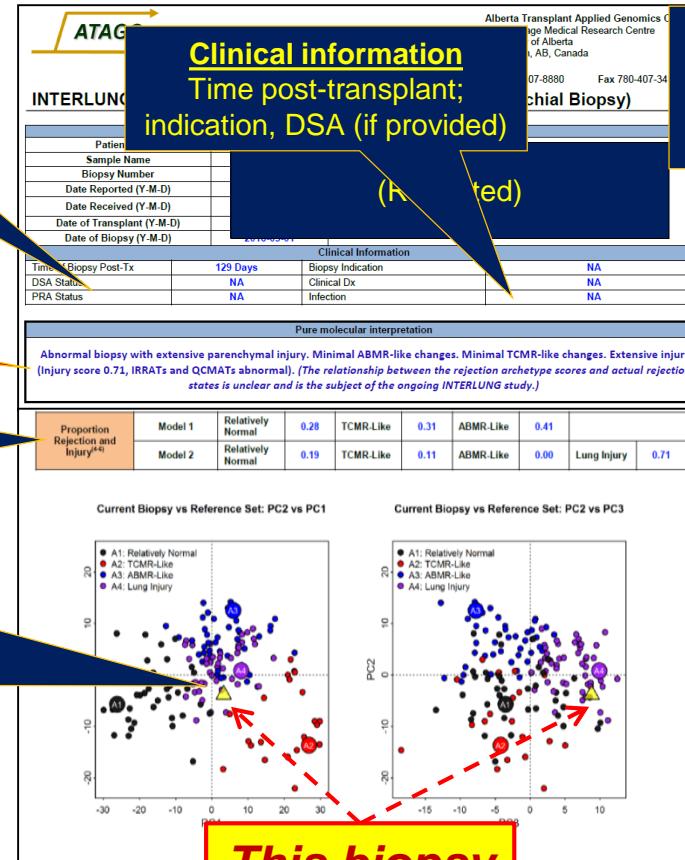
Proportions

Normal, TCMR,
ABMR, injury

Visualization

Relationship of this
biopsy to all others in
the reference biopsies

PC2 vs.PC1;
PC2 vs.PC3



	DSA-related	Endothelial transcripts (eDSAST)	<0.7	0.36	<0.4	Slightly abnormal
ABMR-related		NK cell transcripts (NKB)	0.86	<0.34	Normal	
		Cytotoxic T cell transcripts (QCAT)	0.70	<0.11	Abnormal	
		T cell bulk transcripts (TCB)	0.45	<0.45	Slightly abnormal	
		IFNG (Interferon gamma)	5.87	<5.38	Abnormal	
		CTLA4 (T cell checkpoint)	5.83	<4.63	Abnormal	
TCMR-related		IFNG-induced transcripts (GRIT)	0.48	<0.13	Abnormal	
		Injury transcripts (IRRAT)	0.84	<0.32	Abnormal	
		Macrophage transcripts (QCMAT)	0.80	<0.31	Abnormal	
Rejection and Injury-related		Surfactant transcripts (Aveolar content) [†]	15342	>8767	Adequate	
Injury-related						
Other						

*Normal range for all gene/genomic sets (except surfactant) includes values in the 90th percentile of biopsies with S1₉₀<0.7 (relatively normal biopsies). 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 gene sets.
‡Slightly abnormal – biopsy score is between the 90th and 99th percentiles of values in the biopsies with S1₉₀<0.7 (relatively normal biopsies). Abnormal – biopsy score exceeds values in the 99th percentile of biopsies with S1₉₀<0.7 (relatively normal biopsies).

	Local Histopathology Phenotype			
	Acute Rejection	Alway Inflammation	Chronic Al Rejection	Other
ISHLT A Grade	NA	ISHLT B Grade	NA	ISHLT C Grade
Diagnosis				
Clinical notes				
References				

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Alveolar content
Too little makes
interpretation difficult

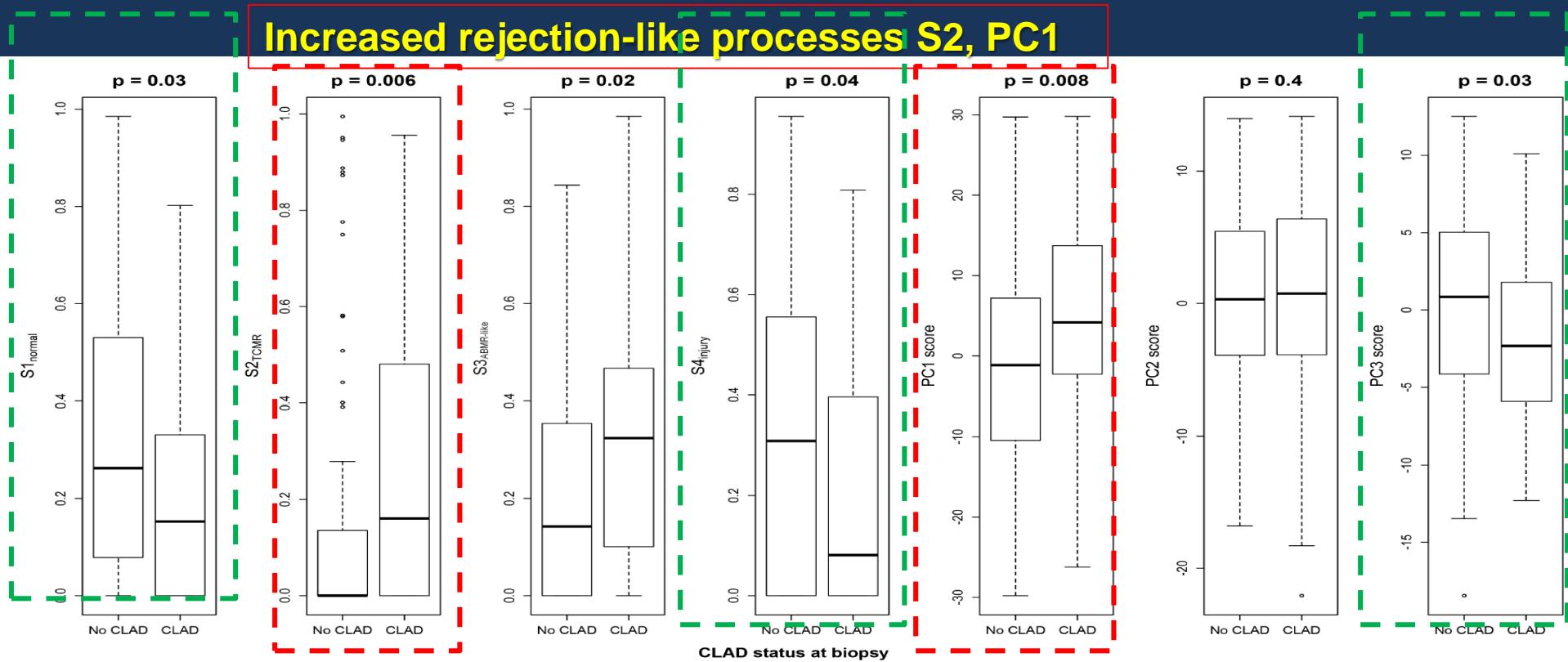
Chronic lung allograft dysfunction (CLAD) is causing most lung transplants to fail prematurely (e.g. 5 years)

Histology cannot define CLAD
Can MMDx TBB define CLAD?

Relating MMDx-Lung TBB scores to CLAD status

Decreased S4 S1 and PC3

Increased rejection-like processes S2, PC1



Relationships between molecular phenotype scores and chronic lung allograft dysfunction (CLAD) at biopsy. From left to right the $S_{1\text{normal}}$, $S_{2\text{TCMR}}$, $S_{3\text{endothelial}}$, and $S_{4\text{injury}}$ 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 3rd bifurcation

The 3BMB study

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

Location	Principal Investigator(s)	Number of Biopsies
Baltimore	Irina Timofte	46
Edmonton	Kieran Halloran, Philip Halloran	20
Melbourne	Gregory Snell, Glen Westall	52
San Antonio	Deborah Levine	8
St. Louis	Ramsey Hachem, Daniel Kreisel, Elbert Trulock	15
Toronto	Stephen Juvet, Shaf Keshavjee	56
Vienna	Peter Jaksch, Walter Klepetko	1
Total		198

Mucosal biopsies: much safer than TBBs

- Prospective collection of **mucosal biopsies** from indication or surveillance bronchoscopies in lung transplant recipients
 - **3rd 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

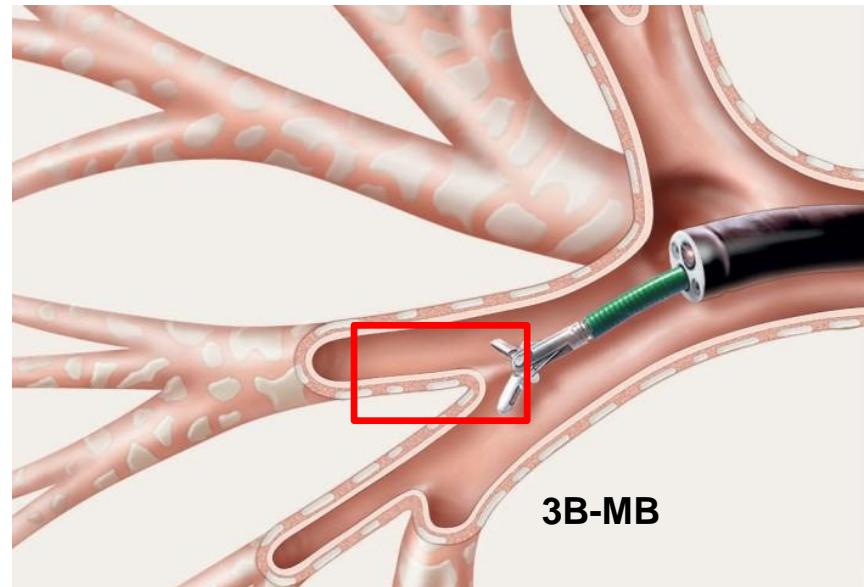


Image courtesy of Olympus

**The 205 3BMBs have variance
that is compatible with TCMR.
This could avoid TBBs in
higher risk patients**

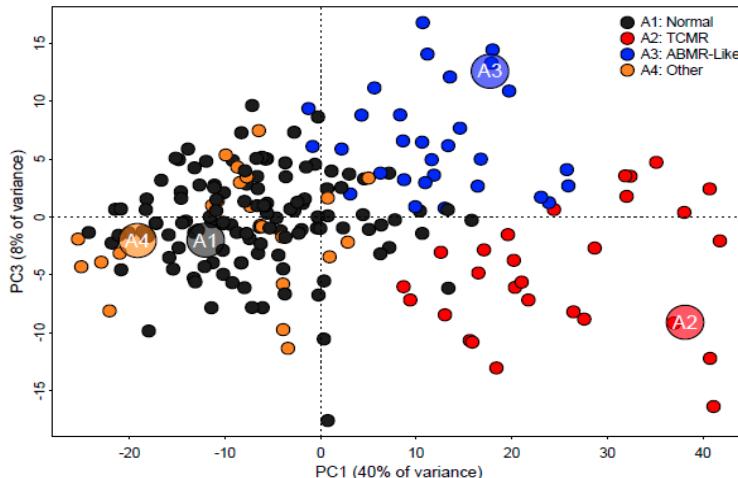
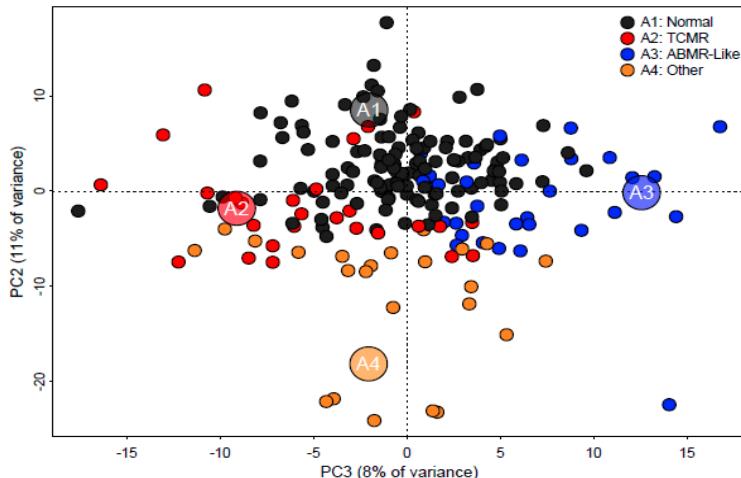
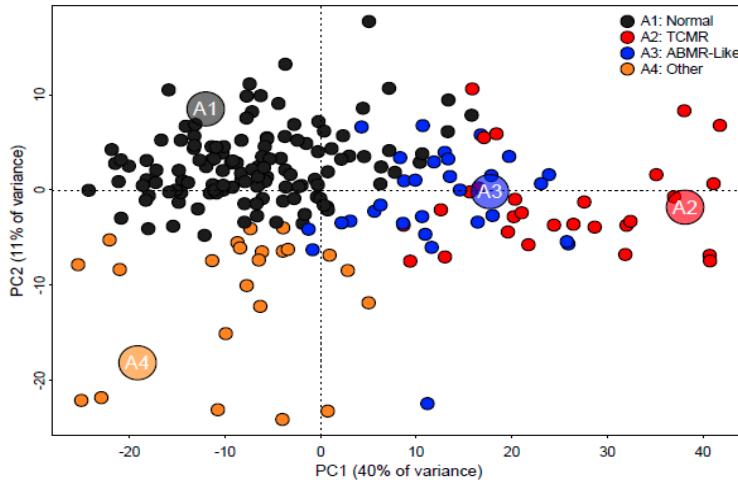
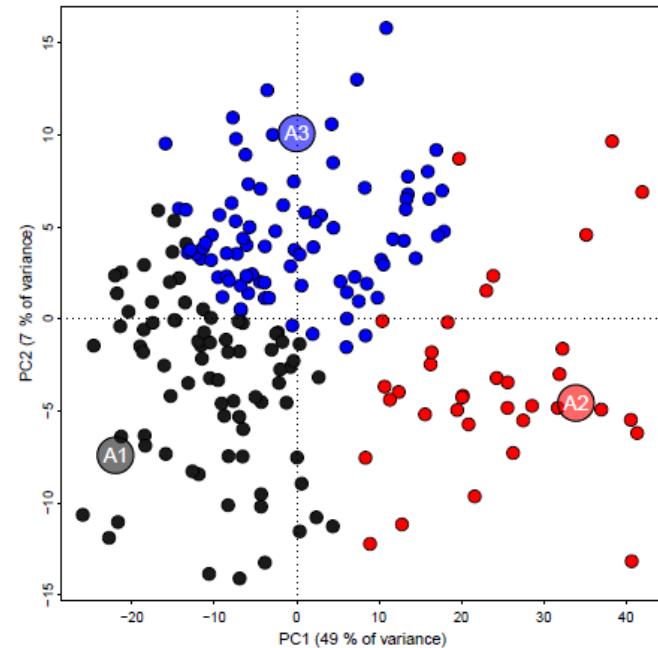


Figure 1 – 3BMB 198

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 ($S_{1_{\text{normal}}}$, $S_{2_{\text{TCMR}}}$, $S_{3_{\text{endothelial}}}$). Each score describes a biopsy's similarity to each of the three archetypes ($A_{1_{\text{normal}}}$, $A_{2_{\text{TCMR}}}$, $A_{3_{\text{endothelial}}}$). The archetypes are represented by the enlarged points. Black – normal, red – TCMR, blue – endothelial.



Developing MMDx-Lung 3MBBs: the same approach as for heart EMBs, TBBs, livers

Rejection transcript expression

Four-state (4 archetype) model:

- $S1_{\text{normal}}$ = no rejection or injury
- $S2_{\text{rejection}}$ = TCMR (includes ABMR?)
- $S3_{\text{endothelial}}$ = (??not actually ABMR?)
- $S4_{\text{injury}}$ = lung abnormalities not rejection



MB3198 AA3 **RAT S2_{rejection}** – Correlated Genes (Non-Redundant)

Table 4. Top transcripts correlated with S2_{rejection} in 198 3BMBs

Correlation ¹	Gene Symbol	Gene Name	PBT Annotations ²	Mean Expression in biopsy groups			Primary Expression in Cell Panel ³
				A1 _{normal}	A2 _{rejection}	A3 _{postinjury}	
0.88	HLA-DMB	major histocompatibility complex, class II, DM beta	GRIT3, Rejection-RAT	276	861	366	B, DC, HUVEC (IFNG), MC, MP (IFNG)
0.87	GBP5	guanylate binding protein 5	GRIT3, ABMR-RAT, Rejection-RAT	74	422	107	HUVEC (IFNG), MP (IFNG), NK, T
0.86	PSMB9	proteasome subunit beta 9	GRIT3, ABMR-RAT, Rejection-RAT, TCMR-RAT, IFNG	873	2482	1102	DC, HUVEC (IFNG), MC, MP (IFNG), NK, T
0.86	IDO1	indoleamine 2,3-dioxygenase 1	GRIT3, ABMR-RAT, Rejection-RAT	141	1405	134	HUVEC (IFNG), MP (IFNG)
0.86	IFI30	interferon, gamma-inducible protein 30	GRIT3	903	3027	1066	DC, HUVEC (IFNG), MC, MP
0.85	FAM26F	family with sequence similarity 26, member F	GRIT3, ABMR-RAT, Rejection-RAT	145	678	208	MC, MP (IFNG)
0.85	CD53	CD53 molecule	GRIT3, ABMR-RAT	169	630	268	B, DC, MC, MP (IFNG), NK, T
0.84	HLA-DRA	major histocompatibility complex, class II, DR alpha	GRIT3, ABMR-RAT, IRRAT950, Rejection-RAT	5636	11015	7149	B, DC, HUVEC (IFNG), MC, MP (IFNG)
0.84	PSMB10	proteasome subunit beta 10	GRIT3, Rejection-RAT, TCMR-RAT	882	1448	829	DC, HUVEC (IFNG), MC, MP (IFNG), NK, T
0.84	PSMB8	proteasome subunit beta 8	GRIT3, Rejection-RAT	1171	2254	1192	DC, HUVEC (IFNG), MC, MP (IFNG), NK, T
0.84	IRF8	interferon regulatory factor 8	GRIT3	122	427	185	B, DC, MC, MP (IFNG)
0.84	LAPTM5	lysosomal protein transmembrane 5	IRRAT950	373	1170	539	B, DC, MC, MP, NK, T, M
0.84	HCLS1	hematopoietic cell-specific Lyn substrate 1		195	436	235	B, DC, MC, MP, NK, T, M
0.83	CD86	CD86 molecule	IRRAT950, QC MAT	52	162	73	DC, MC, MP, M
0.83	ARHGAP30	Rho GTPase activating protein 30		77	171	98	B, DC, MC, MP, NK, T, MMDC
0.83	EPSTI1	epithelial stromal interaction 1 (breast)	GRIT3	172	758	223	HUVEC (IFNG), MP (IFNG)
0.83	FYB	FYN binding protein		88	320	137	MC, MP, MMDC
0.83	HLA-DQA1	major histocompatibility complex, class II, DQ alpha 1; major histocompatibility complex, class II, DR alpha	GRIT3, IRRAT950, Rejection-RAT	1029	2845	1391	DC, MP (IFNG)
0.83	IRF1	interferon regulatory factor 1	GRIT3, ABMR-RAT, Rejection-RAT	199	482	225	HUVEC (IFNG), MC, MP (IFNG)
0.83	PTPRC	protein tyrosine phosphatase, receptor type, C	IRRAT30	216	825	354	B, DC, MC, MP, NK, T, MMDC

¹Spearman correlation

²ABMR-RAT – ABMR-associated RATs, AMAT1 – Alternatively activated macrophage transcripts, BAT – B cell transcripts, DSAST – DSA-selective transcripts, ENDAT – Endothelial cell transcripts, IGT – Immunoglobulin transcripts, IRITD3 – Day 3 injury/repair transcripts (mouse), GRIT3 – IFNG-inducible transcripts, IRITD5 – Day 5 injury/repair transcripts (mouse), IRRAT30/IRRAT950 – Injury/repair-associated transcripts (human), Rejection-RAT – Rejection-associated RATs, QC MAT – Macrophage transcripts, TC MR-RAT – TC MR-associated RATs

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

Correlation of S2_{rejection} with time of biopsy post-transplant = +0.32

MB3198 RAT PC1 – Correlated Genes

Table 6. Top transcripts correlated with PC1 in 198 3BMBs

Correlation ¹	Gene Symbol	Gene Name	PBT Annotations ²	Mean Expression in biopsy groups			Primary Expression in Cell Panel ³
				A1 _{normal}	A2 _{rejection}	A3 _{endothelial}	
0.93	<i>LCP2</i>	lymphocyte cytosolic protein 2	Rejection-RAT	110	405	185	MC, MP, NK, T, MMDC
0.92	<i>HLA-DMB</i>	major histocompatibility complex, class II, DM beta	GRIT3, Rejection-RAT	658	1870	919	B, DC, HUVEC (IFNG), MC, MP (IFNG)
0.92	<i>IRF8</i>	interferon regulatory factor 8	GRIT3	122	427	185	B, DC, MC, MP (IFNG)
0.91	<i>HCST</i>	hematopoietic cell signal transducer	Rejection-RAT	137	340	194	DC, MC, MP, NK, T
0.91	<i>FYB</i>	FYN binding protein		88	320	137	MC, MP
0.91	<i>CD53</i>	CD53 molecule		169	630	268	B, DC, MC, MP (IFNG), NK, T
0.90	<i>LCP1</i>	lymphocyte cytosolic protein 1 (L-plastin)	IRITD5, IRRAT950	474	1609	755	DC, MC, MP, NK
0.90	<i>TNFSF13B</i>	tumor necrosis factor (ligand) superfamily, member 13b	GRIT3, TCMR-RAT GRIT3, ABMR-RAT, Rejection-RAT	66	224	101	DC, MC, MP
0.90	<i>GBP5</i>	guanylate binding protein 5	Rejection-RAT	74	422	107	HUVEC (IFNG), MP (IFNG), NK, T
0.90	<i>LAPTM5</i>	lysosomal protein transmembrane 5	IRRAT950	539	1506	797	B, DC, MC, MP, NK, T
0.90	<i>DOCK2</i>	dedicator of cytokinesis 2	IRRAT950	60	202	98	DC, MC, MP, NK, T
0.90	<i>PTPRC</i>	protein tyrosine phosphatase, receptor type, C	IRRAT30	193	691	314	B, DC, MC, MP, NK, T
0.89	<i>ARHGAP30</i>	Rho GTPase activating protein 30		77	171	98	B, DC, MC, MP, NK, T
0.89	<i>CD86</i>	CD86 molecule	IRRAT950, QC MAT IRRAT950,	52	162	73	DC, MC, MP
0.89	<i>AOAH</i>	acyloxyacyl hydrolase (neutrophil)	Rejection-RAT, TCMR-RAT GRIT3, ABMR-RAT, Rejection-RAT, TCMR-RAT	75	231	109	MC, MP, NK, T
0.89	<i>PSMB9</i>	proteasome subunit beta 9	Rejection-RAT, TCMR-RAT, Rejection-RAT, TCMR-RAT	873	2482	1102	DC, HUVEC (IFNG), MC, MP (IFNG), NK, T
0.89	<i>SASH3</i>	SAM and SH3 domain containing 3		39	110	56	DC, MC, MP, NK, T
0.88	<i>IL10RA</i>	interleukin 10 receptor, alpha		144	298	182	DC, MC, MP (IFNG), NK, T
0.88	<i>CD48</i>	CD48 molecule		87	316	154	B, MC, MP, NK, T
0.88	<i>HLA-DPA1</i>	major histocompatibility complex, class II, DP alpha 1	GRIT3, ABMR-RAT, Rejection-RAT	4037	9860	5853	B, DC, MC, MP, IFNG

¹Spearman correlation

²ABMR-RAT – ABMR-associated RATs, AMAT1 – Alternatively activated macrophage transcripts, BAT – B cell transcripts, DSAST – DSA-selective transcripts, ENDAT – Endothelial cell transcripts, IGT – Immunoglobulin transcripts, IRITD3 – Day 3 injury/repair transcripts (mouse), GRIT3 – IFNG-inducible transcripts, IRITD5 – Day 5 injury/repair transcripts (mouse), IRRAT30/IRRAT950 – Injury/repair-associated transcripts (human), Rejection-RAT – Rejection-associated RATs, QC MAT – Macrophage transcripts, TCMR-RAT – TCMR-associated RATs

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** -

Correlation with time of biopsy post-transplant = +0.37

Pathway analysis

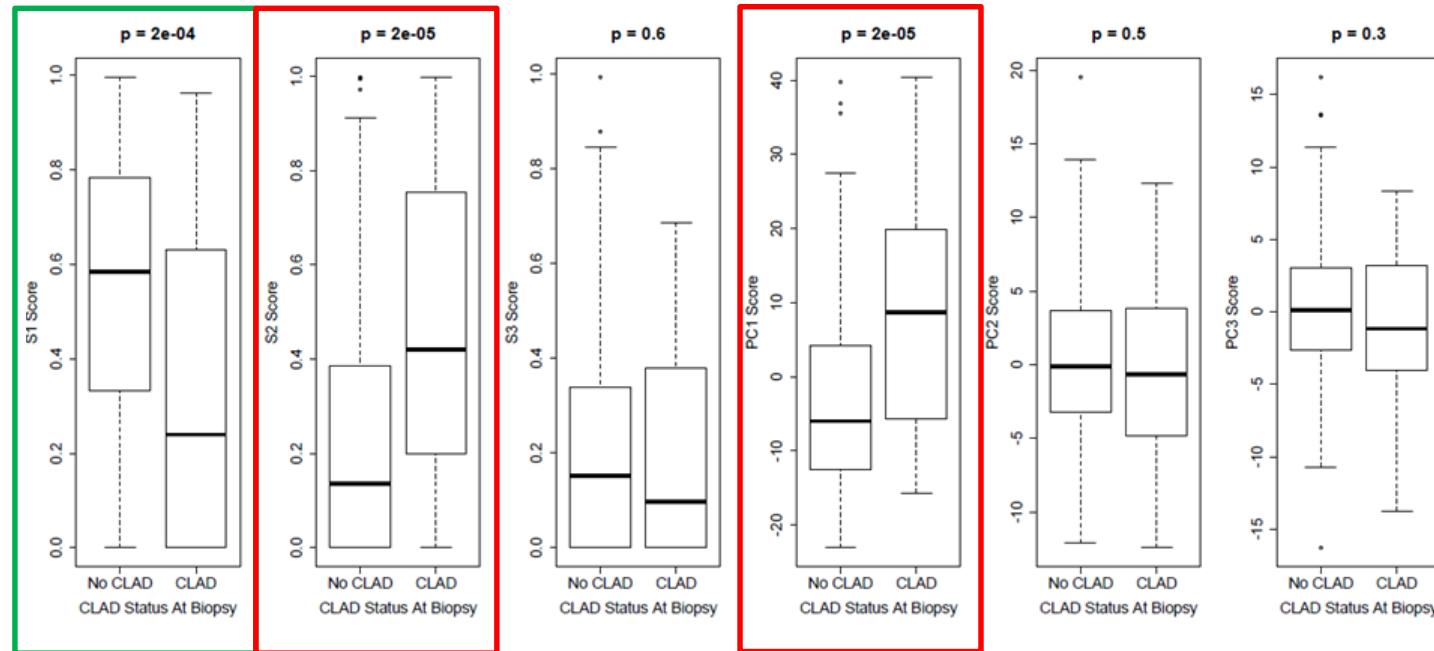
3BMB 198 AA3 **RAT S2_{rejection}**, Top 100

Table 9. Gene Ontology (GO) biological process terms associated with top 100 S2_{rejection}-correlated transcripts

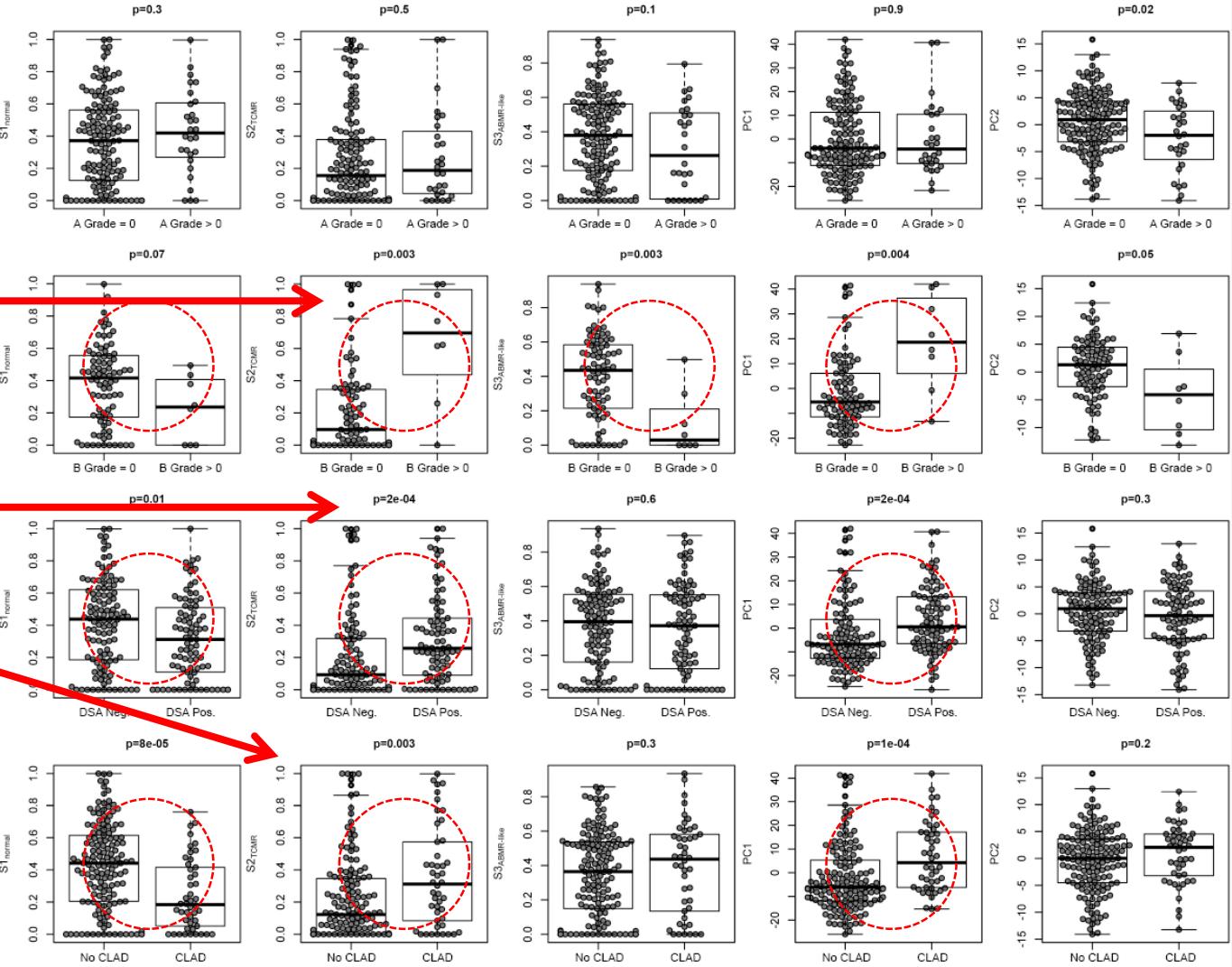
GO Term	Hits in Top 100	Fold Enrichment	P-Value	Corrected P-Value ¹
interferon-gamma-mediated signaling pathway	14	35.2	8.5E-17	7.2E-14
immune response	23	9.8	8.8E-16	2.9E-13
T cell receptor signaling pathway	15	18.1	7.5E-14	1.6E-11
positive regulation of T cell proliferation	10	29.8	3.9E-11	6.3E-09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

¹ Benjamini-Hochberg

3BMB Archetype Scores S1, S2, PC1 are associated with CLAD



3BMB: significant associations with B airway lesions, not A lesions (in paired TBB), with DSA, and with CLAD



Bonferroni-corrected significance threshold is 0.01 (5 tests, uncorrected threshold 0.05)

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)

Lung Case #1

Report – Page 1

TBB

3BMB

Redacted

General Information

General Information

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Lung Case #1

Report Page 2

3BMB

TBB

Molecular Phenotype				
	Gene/gene sets	Biopsy score	Normal Limit*	Interpretation†
ABMR-related	DSA-selective transcripts (DSAST)	-0.14	<0.31	Normal
	Endothelial DSA-selective transcripts (eDSAST)	-0.16	<0.46	Normal
	NK cell burden transcripts (NKB)	-0.06	<0.46	Normal
	Cytotoxic T cell transcripts (QCAT)	-0.01	<0.3	Normal
TCMR-related	T cell burden transcripts (TCB)	0.21	<0.45	Normal
	IFNG (Interferon gamma)	5.06	<5.42	Normal
	CTLA4 (T cell checkpoint)	4.55	<4.92	Normal
	IFNG-inducible transcripts (GRIT)	-0.10	<0.14	Normal
Rejection and injury-related	Injury transcripts (IRRAT)	-0.21	<0.44	Normal
	Macrophage transcripts (QCMAT)	0.11	<0.3	Normal
Other	Surfactant transcripts (Alveolar content)‡	9035	>9267	Slightly Low

*Normal range for all genes/gene sets (except surfactant) includes values in the 25th percentile of biopsies with $S_{1\mu g} \geq 0.7$ (relatively normal biopsies). The normal limit for surfactant includes values in the 25th percentile in all biopsies.

†Surfactant score is the geometric mean expression level calculated across all genes/gene sets.

‡Slightly abnormal – biopsy score is between the 90th and 99th percentile of values in the 99th percentile of biopsies with $S_{1\mu g} \geq 0.7$ (relatively normal biopsies).

§Abnormal – biopsy score exceeds values in the 99th percentile of biopsies with $S_{1\mu g} \geq 0.7$ (relatively normal biopsies).

TBB

Local Histopathology Phenotype					
Acute Rejection		Airway Inflammation		Chronic Airway Rejection	
ISHLT A Grade	NA	ISHLT B Grade	NA	ISHLT C Grade	NA
Diagnosis		NA			

Clinical notes					

References

- (1) Halloren PF, Potena L, Duong Van Huyen JP, Bruneval P, Leone O, Kim DH, et al. Building a tissue-based molecular diagnostic system in heart transplant rejection: the heart molecular microscope MMDx. J Heart Lung Transplant. 2017;36(11):1192-200.
- (2) Halloren PF, Farnulski KS, Reeve J. Molecular assessment of disease states in kidney transplant biopsy samples. Nature Reviews Nephrology. 2016;12(9):534-48.
- (3) Reeve J, Bohm GA, Eskandary F, Enecke G, Lefacheur C, Loupy A, et al. Assessing rejection-related disease in kidney transplant biopsies based on archetypal analysis of molecular phenotypes. JCI Insight. 2017;2(12).
- (4) Halloren K, Parkes MD, Chang J, Farnulski KS, Jimpte IL, Snell GI, et al. Molecular Features of Rejection and Injury in Lung Transplant Transbronchial Biopsies. 2018; [in preparation].
- (5) Halloren KM, Parkes MD, Chang J, Farnulski KS, Reeve J, Hachem R, et al. Molecular Diagnosis of Rejection Phenotypes in Lung Transplant Transbronchial Biopsies: Initial Findings of the INTERLUNG study. Journal of Heart and Lung Transplantation. 2018. RefType: Abstract
- (6) Halloren KM, Parkes MD, Chang J, Farnulski KS, Reeve J, Hachem R, et al. Molecular Detection of Rejection-like Changes in Proximal Bronchial Mucosal Lung Transplant Biopsies: Initial findings of the INTERLUNG study. Journal of Heart and Lung Transplantation. 2018. RefType: Abstract

Molecular Phenotype				
	Gene/gene sets	Biopsy score	Normal Limit*	Interpretation†
ABMR-related	DSA-selective transcripts (DSAST)	0.08	<0.19	Normal
	Endothelial DSA-selective transcripts (eDSAST)	0.05	<0.25	Normal
	NK cell burden transcripts (NKB)	0.31	<0.27	Slightly abnormal
	Cytotoxic T cell transcripts (QCAT)	0.22	<0.42	Normal
TCMR-related	T cell burden transcripts (TCB)	0.81	<0.44	Slightly abnormal
	IFNG (Interferon gamma)	4.62	<4.86	Normal
	CTLA4 (T cell checkpoint)	5.10	<5.24	Normal
	IFNG-inducible transcripts (GRIT)	-0.07	<0.12	Normal
Rejection and injury-related	Macrophage transcripts (QCMAT)	0.13	<0.32	Normal
	Surfactant transcripts (Alveolar content)‡	9035	>9267	Slightly Low

*Normal range for all genes/gene sets (except surfactant) includes values in the 25th percentile of biopsies with $S_{1\mu g} \geq 0.7$ (relatively normal biopsies). The normal limit for surfactant includes values in the 25th percentile in all biopsies.

†Slightly abnormal – biopsy score is between the 90th and 99th percentile of values in the 99th percentile of biopsies with $S_{1\mu g} \geq 0.7$ (relatively normal biopsies).

‡Abnormal – biopsy score exceeds values in the 99th percentile of biopsies with $S_{1\mu g} \geq 0.7$ (relatively normal biopsies).

3BMB

Local Histopathology Phenotype In Paired Transbronchial Biopsy						
Acute Rejection		Airway Inflammation		Chronic Airway Rejection		Other
ISHLT A Grade	NA	ISHLT B Grade	NA	ISHLT C Grade	NA	C4d Neutrophilic Capillitis and/or Margination
Diagnosis		NA				

Clinical notes

References

- (1) Halloren PF, Potena L, Duong Van Huyen JP, Bruneval P, Leone O, Kim DH, et al. Building a tissue-based molecular diagnostic system in heart transplant rejection: the heart molecular microscope MMDx. J Heart Lung Transplant. 2017;36(11):1192-200.
- (2) Halloren PF, Farnulski KS, Reeve J. Molecular assessment of disease states in kidney transplant biopsy samples. Nature Reviews Nephrology. 2016;12(9):534-48.
- (3) Reeve J, Bohm GA, Eskandary F, Enecke G, Lefacheur C, Loupy A, et al. Assessing rejection-related disease in kidney transplant biopsies based on archetypal analysis of molecular phenotypes. JCI Insight. 2017;2(12).
- (4) Halloren K, Parkes MD, Chang J, Farnulski KS, Jimpte IL, Snell GI, et al. Molecular Features of Rejection and Injury in Lung Transplant Transbronchial Biopsies. 2018; [in preparation].
- (5) Halloren KM, Parkes MD, Chang J, Farnulski KS, Reeve J, Hachem R, et al. Molecular Diagnosis of Rejection Phenotypes in Lung Transplant Transbronchial Biopsies: Initial Findings of the INTERLUNG study. Journal of Heart and Lung Transplantation. 2018. RefType: Abstract
- (6) Halloren KM, Parkes MD, Chang J, Farnulski KS, Reeve J, Hachem R, et al. Molecular Detection of Rejection-like Changes in Proximal Bronchial Mucosal Lung Transplant Biopsies: Initial findings of the INTERLUNG study. Journal of Heart and Lung Transplantation. 2018. RefType: Abstract

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

Report Page 1

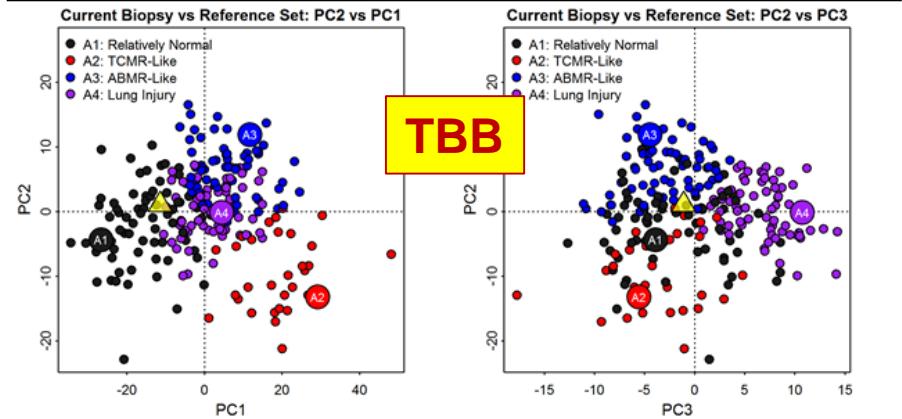
TBB

3BMB

Redacted

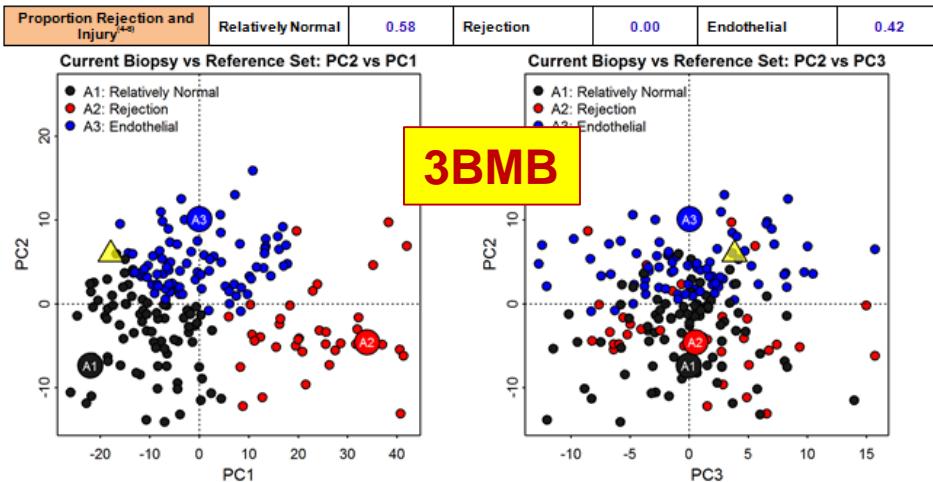
General Information										
Patient ID	LT00402	Name:								
Redacted										
Pure molecular interpretation										
Minor abnormalities. Mild ABMR-like changes of uncertain significance. Minimal TCMR-like changes. Minimal parenchymal injury (Injury score 0.021). (The relationship between the rejection archetype scores and actual rejection states is unclear and is the subject of the ongoing INTERLUNG study.)										

Proportion Rejection and Injury ⁽⁴²⁾	Model 1	Relatively Normal	0.56	TCMR-Like	0.00	ABMR-Like	0.44		
Proportion Rejection and Injury ⁽⁴²⁾	Model 2	Relatively Normal	0.56	TCMR-Like	0.00	ABMR-Like	0.23	Lung Injury	0.21

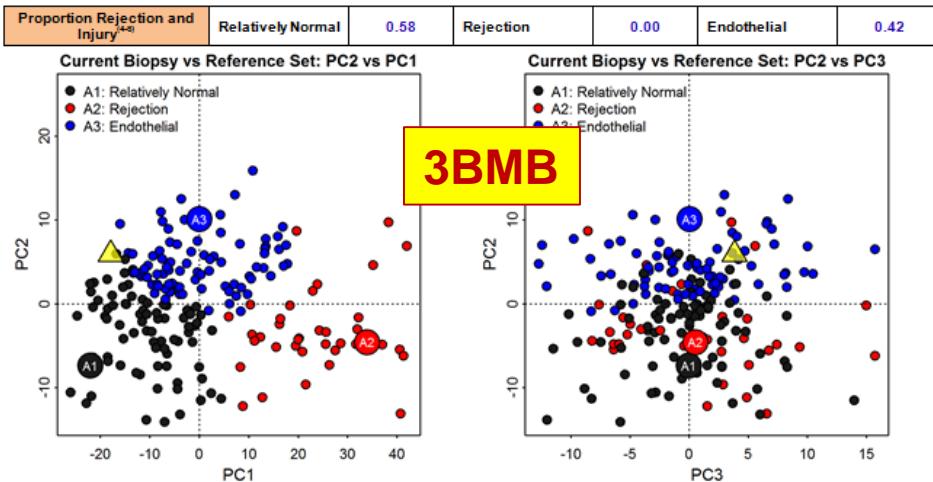


TBB

General Information										
Patient ID	LT00402	Name:								
Redacted										
Pure Molecular Interpretation										
Low probability of rejection. (The relationship between the rejection archetype scores and actual rejection states is unclear and is the subject of the ongoing INTERLUNG study.)										



3BMB



3BMB

Lung Case #2

Report Page 2

3BMB

TBB

Molecular Phenotype				
	Gene/gene sets	Biopsy score	Normal Limit*	Interpretation†
ABMR-related	DSA-selective transcripts (DSAST)	0.44	<0.31	Slightly abnormal
	Endothelial DSA-selective transcripts (eDSAST)	0.22	<0.46	Normal
	NK cell burden transcripts (NKB)	0.82	<0.46	Abnormal
TCMR-related	Cytotoxic T cell transcripts (QCAT)	0.66	<0.3	Abnormal
	T cell burden transcripts (TCB)	0.71	<0.45	Slightly abnormal
	IFNG (Interferon gamma)	5.73	<5.42	Abnormal
Rejection and injury-related	CTLA4 (T cell checkpoint)	4.83	<4.92	Normal
	IFNG-inducible transcripts (GRIT)	-0.01	<0.14	Normal
Injury-related	Injury transcripts (IRRAT)	0.08	<0.44	Normal
	Macrophage transcripts (QCMAT)	0.11	<0.3	Normal
Other	Surfactant transcripts (Alveolar content)†	14445	>9267	Adequate

*Normal range for all gene/gene sets (except surfactant). Includes values in the 90th percentile of biopsies with S_{1%}>0.7 (relatively normal biopsies). 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 99th percentile of biopsies with S_{1%}>0.7 (relatively normal biopsies).

§Abnormal – biopsy score exceeds values in the 99th percentile of biopsies with S_{1%}>0.7 (relatively normal biopsies).

TBB

Local Histopathology Phenotype						
Acute Rejection		Chronic Airway Rejection		Other		
ISHLT A Grade	NA	ISHLT B Grade	NA	ISHLT C Grade	NA	NA
					Neutrophilic Capillitis and/or Margination	NA
Diagnosis						
NA						
Clinical notes						

References

- (1) Halloran PF, Potens L, Duong Van Huyen JP, Bruneval P, Leone O, Kim DH, et al. Building a tissue-based molecular diagnostic system in heart transplant rejection: the heart molecular microscope MMDx. J Heart Lung Transplant. 2017;36(11):1192-200.
- (2) Halloran PF, Farnulski KS, Reeve J. Molecular assessment of disease states in kidney transplant biopsy samples. Nature Reviews Nephrology. 2016;12(9):534-48.
- (3) Reeve J, Bohmig GA, Eskandary F, Enecke G, Lefebvre C, Loupy A, et al. Assessing rejection-related disease in kidney transplant biopsies based on archetypal analysis of molecular phenotypes. JCI Insight. 2017;2(12).
- (4) Halloran K, Parkes MD, Chang J, Farnulski KS, Timofte IL, Snell GI, et al. Molecular Features of Rejection and Injury in Lung Transplant Transbronchial Biopsies. 2018;[in preparation].
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- (6) Halloran KM, Parkes MD, Chang J, Farnulski KS, Reeve J, Hachem R, et al. Molecular Detection of Rejection-like Changes in Proximal Bronchial Mucosal Lung Transplant Biopsies: Initial findings of the INTERLUNG study. Journal of Heart and Lung Transplantation. 2018. RefType: Abstract

	Gene/gene sets	Biopsy score	Normal Limit*	Interpretation†
ABMR-related	DSA-selective transcripts (DSAST)	0.44	<0.19	Abnormal
	Endothelial DSA-selective transcripts (eDSAST)	0.41	<0.25	Abnormal
	NK cell burden transcripts (NKB)	0.18	<0.27	Normal
TCMR-related	Cytotoxic T cell transcripts (QCAT)	-0.41	<0.42	Normal
	T cell burden transcripts (TCB)	-0.14	<0.44	Normal
	IFNG (Interferon gamma)	4.67	<4.86	Normal
Rejection and injury-related	CTLA4 (T cell checkpoint)	4.11	<5.24	Normal
	IFNG-inducible transcripts (GRIT)	-0.02	<0.12	Normal
	Macrophage transcripts (QCMAT)	0.03	<0.32	Normal

*Normal range for all gene/gene sets (except surfactant). Includes values in the 90th percentile of biopsies with S_{1%}>0.7 (relatively normal biopsies).

†Slightly abnormal – biopsy score is between the 90th and 99th percentile of values in the 99th percentile of biopsies with S_{1%}>0.7 (relatively normal biopsies).

‡Abnormal – biopsy score exceeds values in the 99th percentile of biopsies with S_{1%}>0.7 (relatively normal biopsies).

Bronchial Biopsy						
Local Histopathology Phenotype		Airway Inflammation		Other		
Acute Rejection	Airway Inflammation	ISHLT A Grade	ISHLT B Grade	ISHLT C Grade	Neutrophilic Capillitis and/or Margination	Not done
0	x			x	No findings	No
Diagnosis						Clinical notes

References

- (1) Halloran PF, Potens L, Duong Van Huyen JP, Bruneval P, Leone O, Kim DH, et al. Building a tissue-based molecular diagnostic system in heart transplant rejection: the heart molecular microscope MMDx. J Heart Lung Transplant. 2017;36(11):1192-200.
- (2) Halloran PF, Farnulski KS, Reeve J. Molecular assessment of disease states in kidney transplant biopsy samples. Nature Reviews Nephrology. 2016;12(9):534-48.
- (3) Reeve J, Bohmig GA, Eskandary F, Enecke G, Lefebvre C, Loupy A, et al. Assessing rejection-related disease in kidney transplant biopsies based on archetypal analysis of molecular phenotypes. JCI Insight. 2017;2(12).
- (4) Halloran K, Parkes MD, Chang J, Farnulski KS, Timofte IL, Snell GI, et al. Molecular Features of Rejection and Injury in Lung Transplant Transbronchial Biopsies. 2018;[in preparation].
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3BMB

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

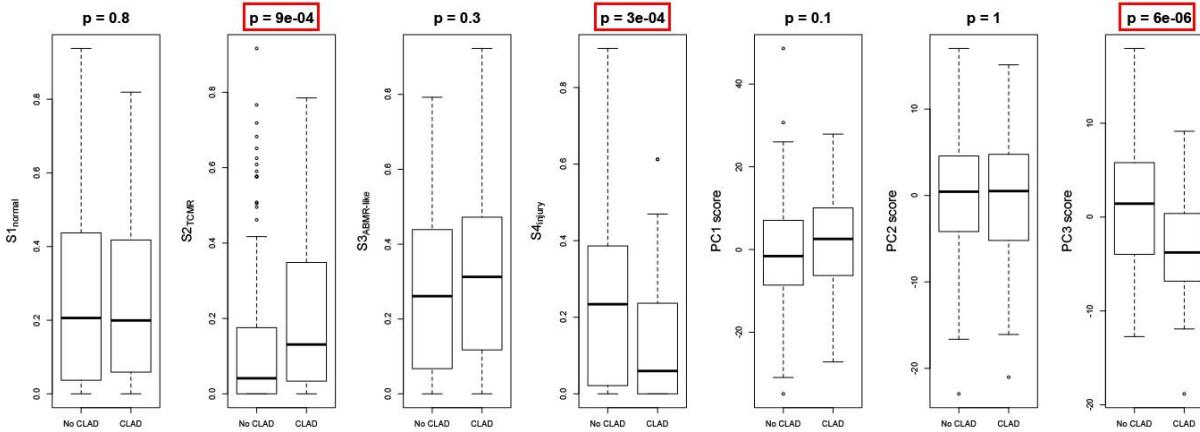


One Lambda/TMO licensing agreement
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Mutterm Chair in Clinical Immunology
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*Genome Canada
Roche Organ Transplantation Research Foundation
Canada Foundation for Innovation
University Hospital Foundation
Capital Health/Alberta Health Services*

Thank you

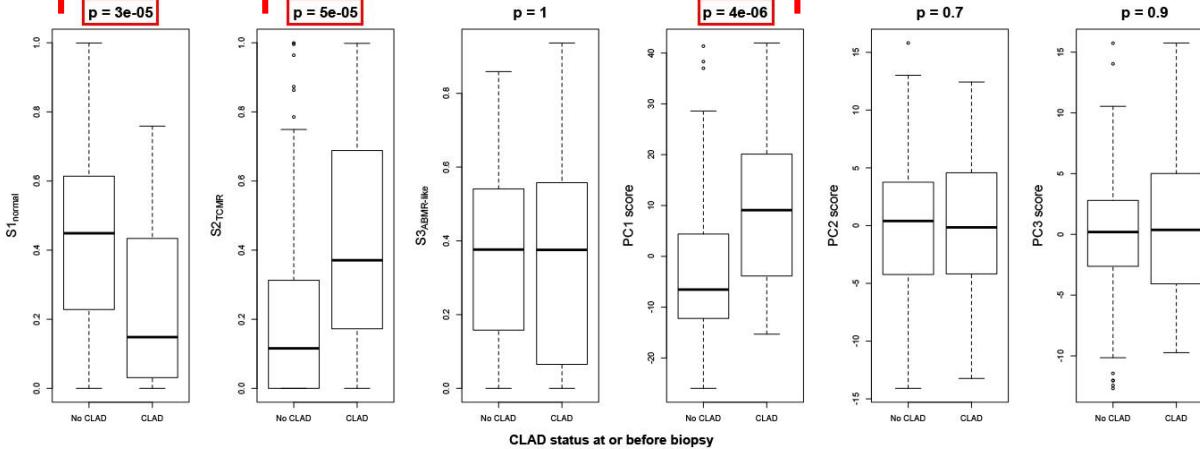
A. Relationship between CLAD and molecular scores in 256 TBBs (55 CLAD, 175 No CLAD)



TBB

The Molecular Features of CLAD in Transbronchial and Endobronchial Mucosa Biopsies

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



3BMB

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.

ATAGC