The Force is in the Tissue

The tissue changes have the last word!

Michael Mengel and Phil Halloran

UNIVERSITY OF ALBERTA
EDMONTON·ALBERTA·CANADA

Alberta Transplant Applied Genomics Centre (ATAGC)

http://atagc.med.ualberta.ca/Services/MolecularMicroscopeSystem

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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
- Phil Halloran has been a symposium speaker for One Lambda/Thermo Fisher

https://www.molecular-microscope.com/
http://transcriptome.com/
http://atagc.med.ualberta.ca/Services/MolecularMicroscopeSystem/

Objectives

- To review the current standard of care for diagnosing rejection and tissue injury
- To understand the challenge of lacking a true ‘Gold Standard’ in diagnosing rejection
- To discuss the current gaps and needed steps in validating and calibrating non-tissue based diagnostics
  - cfDNA, DSA measurements

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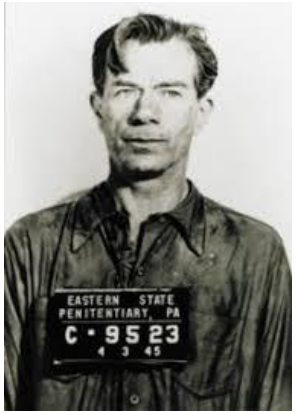
Follow Suttons Law
Go where the disease is – the tissue!

Willy Sutton
1901-1980

Medical Definition of **Sutton's law**: The principle of going straight to the most likely diagnosis.



Willy Sutton was asked why he robbed banks and replied: "because that is where the money is"

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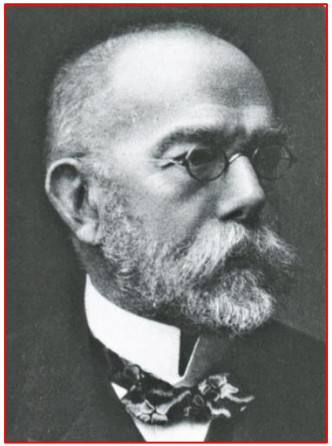
Two clinicians who pioneered disease classification

Robert Koch (1843-1910)



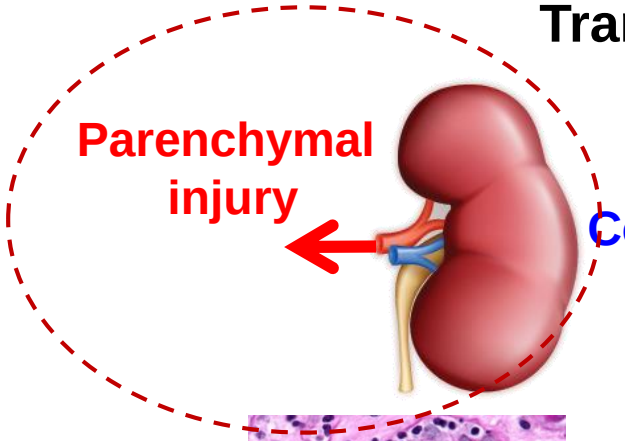
- A rigorous approach to studying etiology
- Koch's postulates

Rudolph Virchow (1821-1902)

- Microscopic examination of diseased tissue

Understanding of diseases involves many dimensions, particularly examination of the diseased tissue and search for etiology and mechanisms

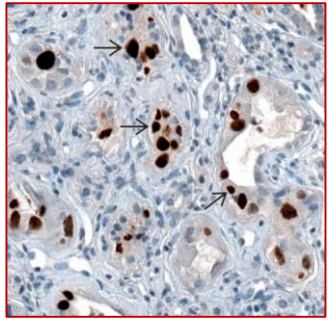
The changes in the tissue reflect rejection and injury



Transplantation

Parenchymal injury

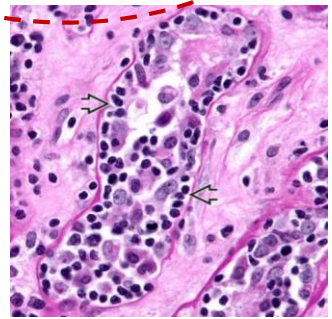
Compliance



Infection

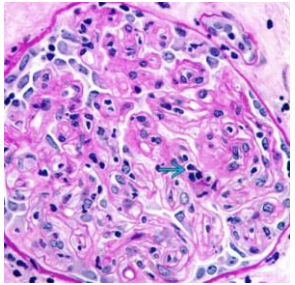
Rejection

Immunosuppression



TCMR

ABMR



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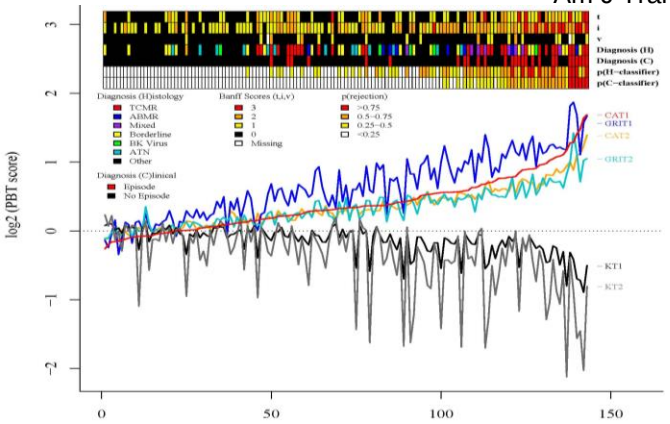
Microarray analysis of rejection in human kidney transplants using pathogenesis-based transcript sets.

Mueller TF, Einecke G, Reeve J, Sis B, Mengel M, Jhangri GS, Bunnag S, Cruz J, Wishart D, Meng C, Broderick G, Kaplan B, Halloran PF. Am J Transplant. 2007 Dec;7(12):2712-22.



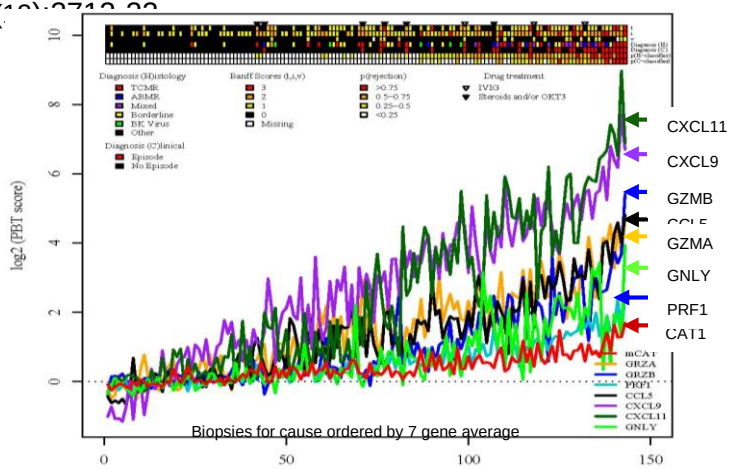


TABLE 4. Probability of upregulation of genes in pathogenesis based transcripts sets, compared by C4d staining status

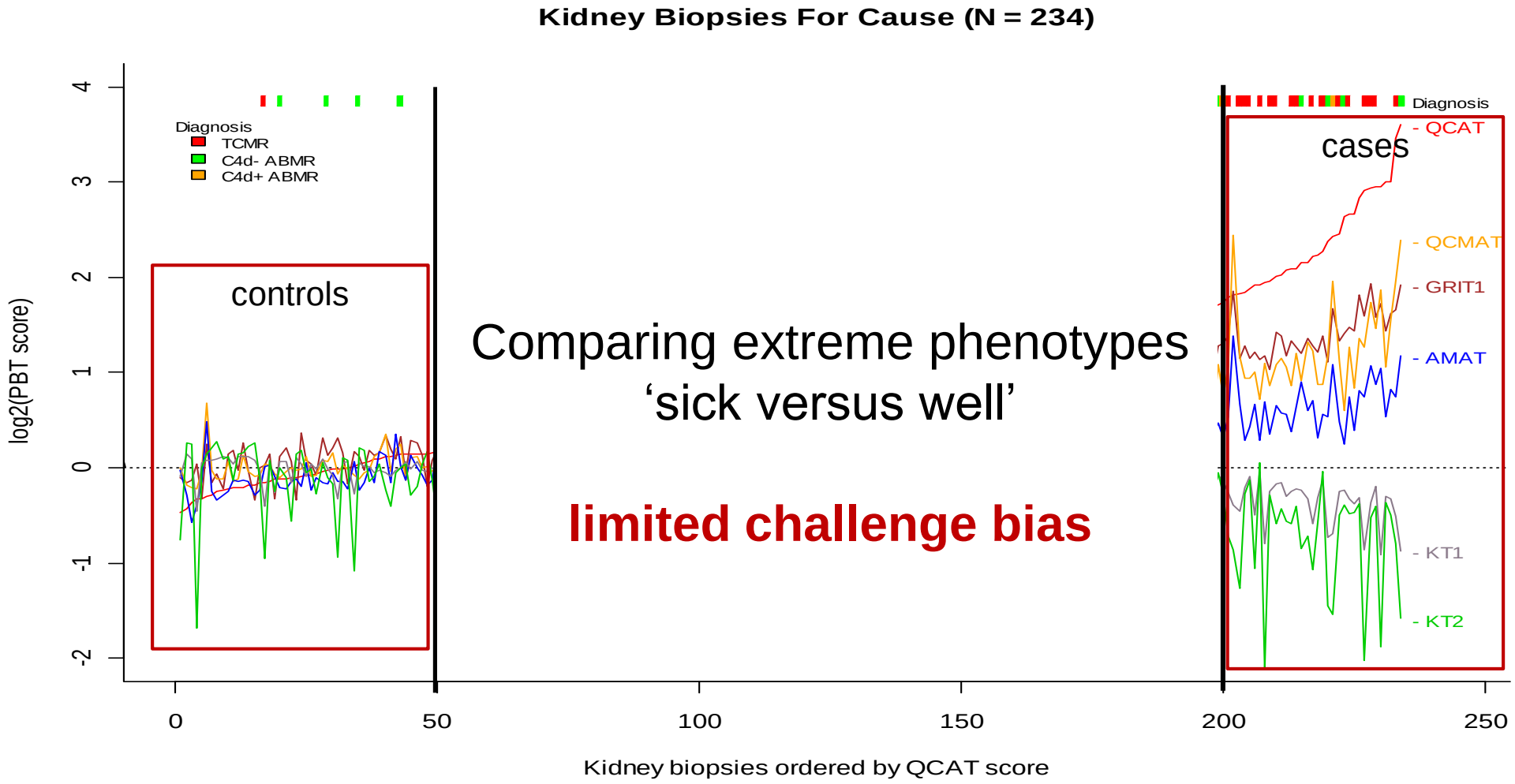
Groups*	KT	IRIT	GRIT	QCAT	CMAT	AMA	BAT	NKST	IGT	ENDAT
G2–G1	0.34	0.30	0.70	0.64	0.67	0.40	0.26	0.18	0.35	0.44
G2–G6	0.73	0.16	**0.02	**0.05	**0.05	0.07	0.07	0.06	**0.01	**0.04
G1–G3	0.38	0.48	**0.005	**0.02	**0.002	0.23	0.27	0.62	**0.007	0.11
G1–G4	0.86	0.25	**<0.001	**0.004	**0.002	0.16	0.12	0.52	**0.04	**0.03
G1–G5	0.76	0.40	**<0.001	**0.02	**0.01	0.20	0.31	0.43	0.09	0.13
G1–G6	0.91	0.36	**<0.001	**0.03	**0.01	0.17	0.20	0.44	**0.048	0.09
G3–G5	0.81	0.40	0.1	0.51	0.59	0.37	0.58	0.22	0.57	0.47
G4–G6	0.52	0.31	0.14	0.19	0.20	0.42	0.17	0.55	0.59	0.10

*Comparison made on the ratio of the first group to the second.
**P value for significance, <0.05.
KT, kidney transcripts; IRIT, injury and repair-induced transcripts; GRIT, gamma-interferon and rejection-induced transcripts; QCAT, quantitative cytotoxic T cell–associated transcripts; CMAT, quantitative constitutive macrophage-associated transcripts; AMA, alternative macrophage activation transcripts; BAT, B cell–associated transcripts; NKST, natural killer cell selective transcripts; IGT, immunoglobulin transcripts; ENDAT, endothelial cell–associated transcripts.

G1. Focal or diffuse PTC C4d+ (N=13)
G2. Minimal PTC C4d+ (N=4)
G3. Isolated glomerular C4d+ with glomerular disease (N=13)
G4. Isolated glomerular C4d+ staining without glomerular disease (N=15)
G5. C4d negative with glomerular disease (N=12)
G6. C4d negative biopsies without evidence of glomerular disease (N=25)

Hayde N, Bao Y, Pullman J, Ye B, Calder BR, Chung M, Schwartz D, Alansari A, de Boccardo G, Ling M, Akalin E. Transplantation. 2013 27;95(4):580-8.

All biopsies aligned by T cell burden (QCATs): Standardized with 8 control kidneys, PBTs from IQR filtered set

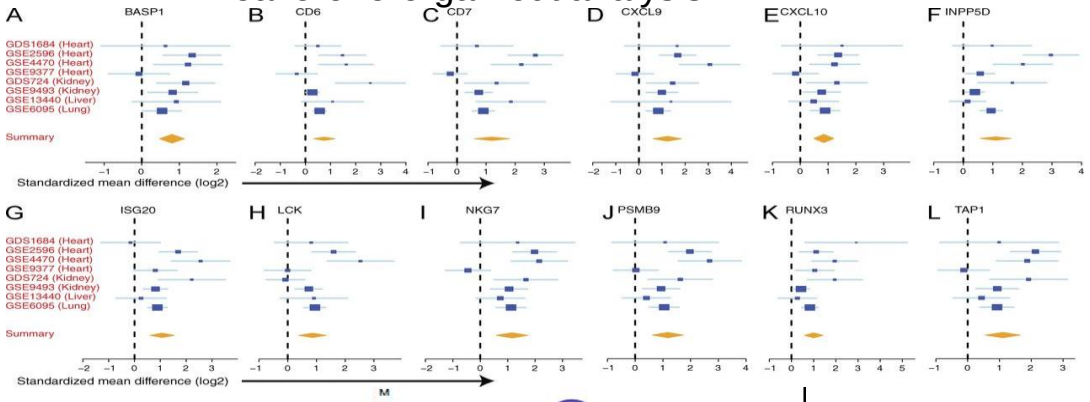


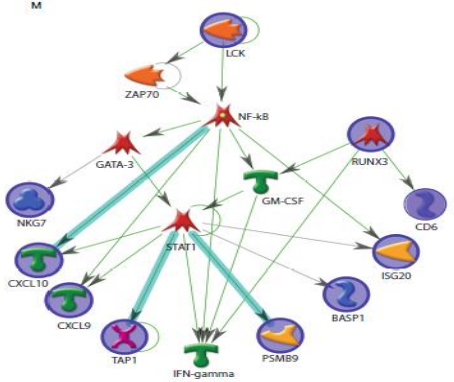
Comparing extreme phenotypes
'sick versus well'

limited challenge bias

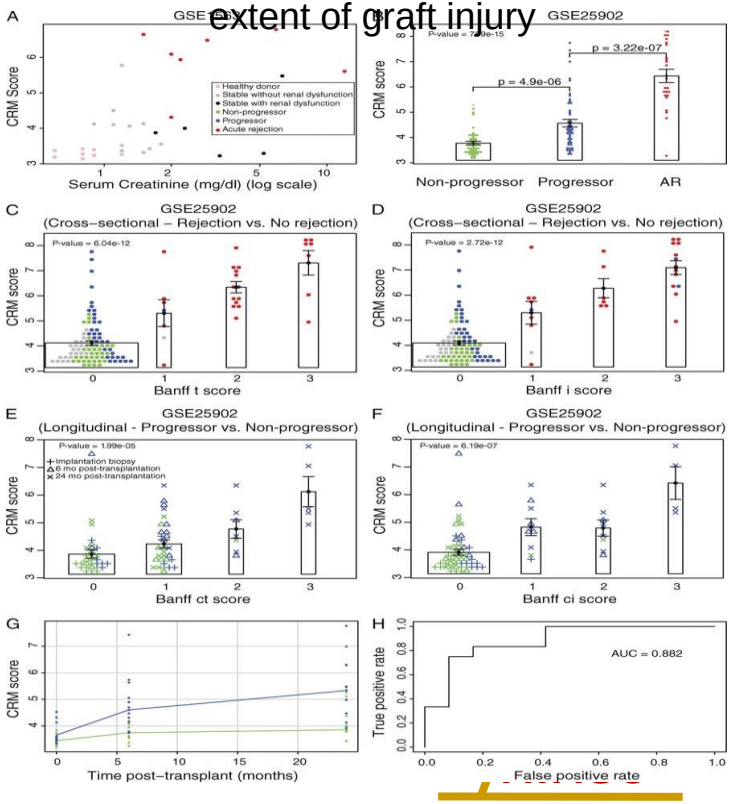
A **common rejection module** (CRM) for acute rejection across multiple organs identifies novel therapeutics for organ transplantation

Discovery of a CRM consisting of 12 genes by leave-one-organ-out analysis.





CRM score correlates significantly with extent of graft injury



Khatri P et al. J Exp Med 2013;210:2205-2221

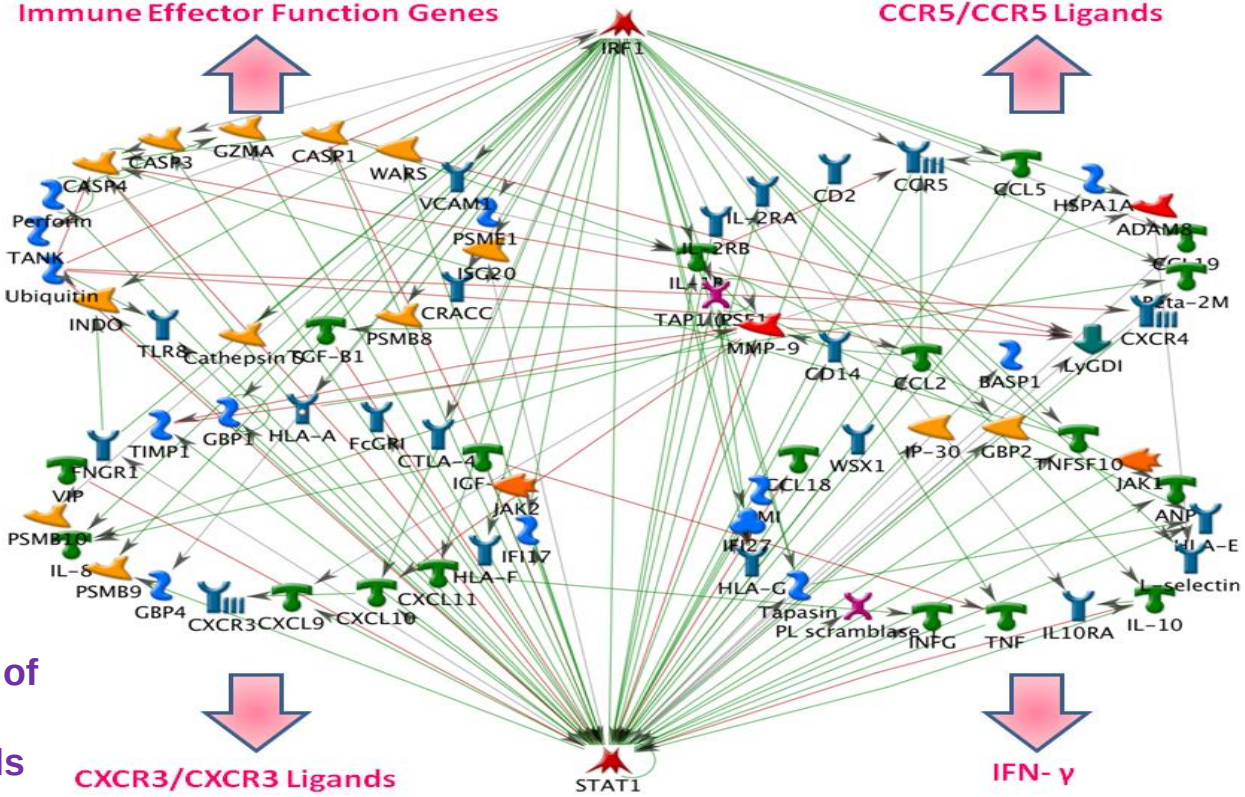
The immunologic constant of rejection: similar to autoimmunity, pathogen infection, and cancer

Activation of immune effector functions

Recruitment of cytotoxic immune cells

Recruitment of cytotoxic immune cells

Activation of interferon-γ stimulated transcripts



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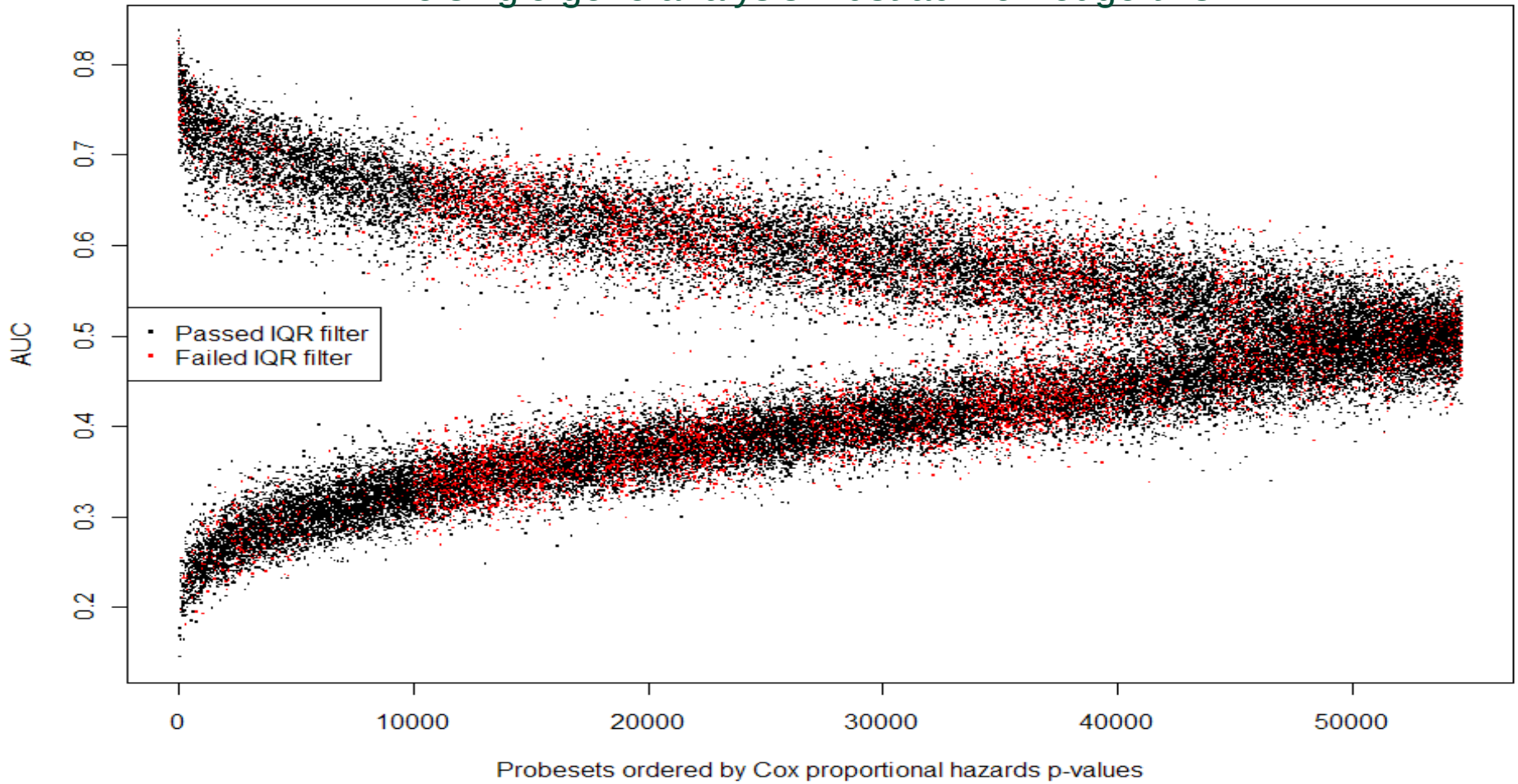
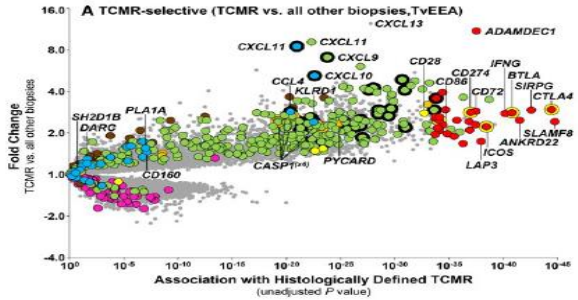
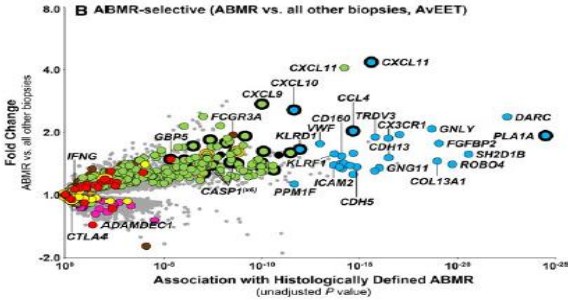
Spivey et al. The Journal of Translational Medicine 2011 Oct 12;9:174

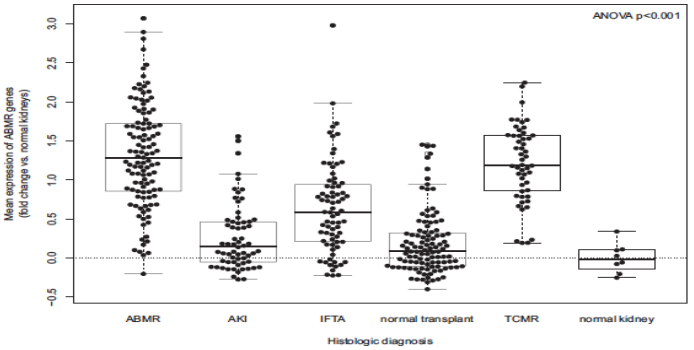
Tens of thousands of genes “predict” outcome!
The single gene analysis must acknowledge this

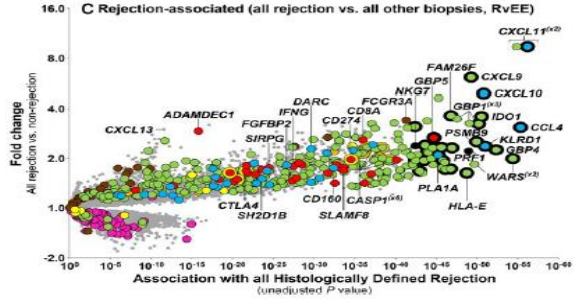


Significant overlap in the molecular phenotype between disease entities: **No transcript is specific**









Color key for probe sets:

- All 54,675 probe sets
- Top 30 TCMR-selective
- Top 30 ABMR-selective
- Top 30 Rejection-associated
- IFNG-inducible
- B7 family ligands and their receptors
- Inflammasome-related
- Acute kidney injury-associated (response to wounding)
- Kidney solute carriers (parenchymal function)

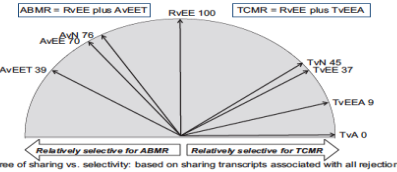
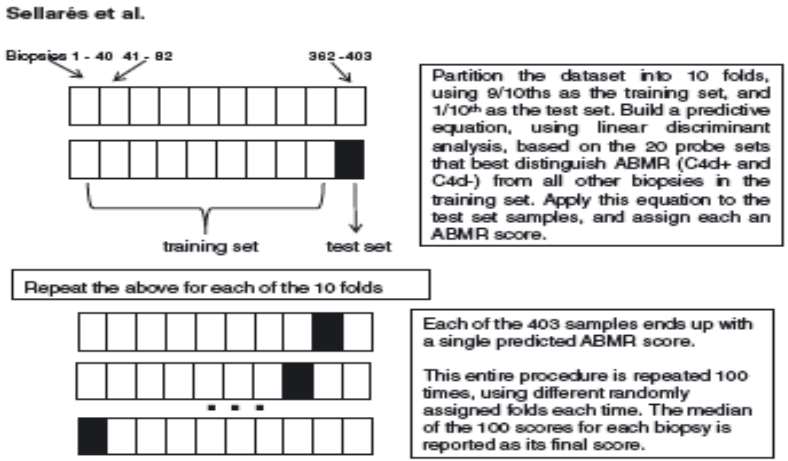
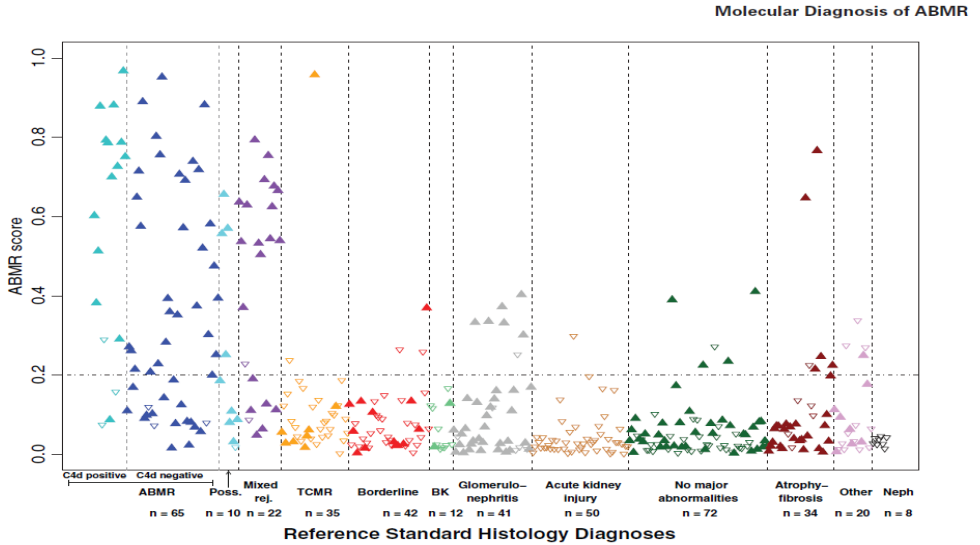


FIGURE 2 Illustration of the effect of changing the case mix in the positive and negative comparators on transcript sharing by ten alternative rejection-associated algorithms. Numbers indicate the non-redundant and annotated transcripts shared with rejection vs. everything else (RvEE) algorithm, based on the top 100 rejection-associated transcripts. TvN - TCMR vs. Nephrectomies, TvEE - TCMR vs. everything else (excluding ABMR), TvEET - TCMR vs. everything else including ABMR, TvA - TCMR vs. ABMR, AvEET - ABMR vs. everything else including TCMR, AvEE - ABMR vs. everything else (excluding TCMR), AvN - ABMR vs. Nephrectomies

Halloran et al. Review Am J Transplant. 2018 Apr;18(4):785-795

A molecular classifier for diagnosing AMR





Classifier score correlates with:

- Pathology (ptc, g, cg, I, cv, ah, ct, ci)
- Consensus amongst pathologists
- Presence of DSA
- outcome

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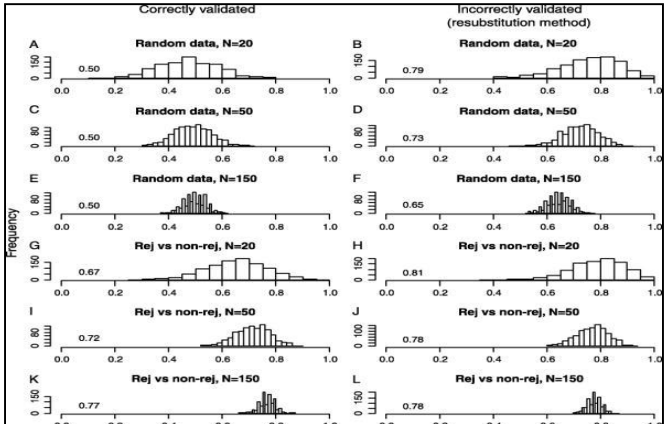
Sellares et al. Am J Transplant. 2013 Apr;13(4):971-83.

Potential sources of variance with diagnostic classifiers

1) Sampling variance (random splits)
2) Label assignment (Gold Standard!?!?)
3) Training set size (10-fold, 5-fold etc.)
4) Modelling strategy (which samples to exclude from the training sets)
5) Classifier type (LDA, SVM, etc)



Classifier accuracies using a linear discriminant analysis (LDA) classifier. The histograms show the distribution of test set accuracies based on 1000 random 50:50 training:test set splits of the data. The N for each training and corresponding test set was either 20, 50, or 150 as indicated. The phenotypes being classified were either random (A-F) or rejecting vs non-rejecting (G-L). Each LDA classifier used the top 10 genes by Bayesian t-test. The left panel shows the results from properly conducted analyses where the gene selection was restricted to the training sets, while the right

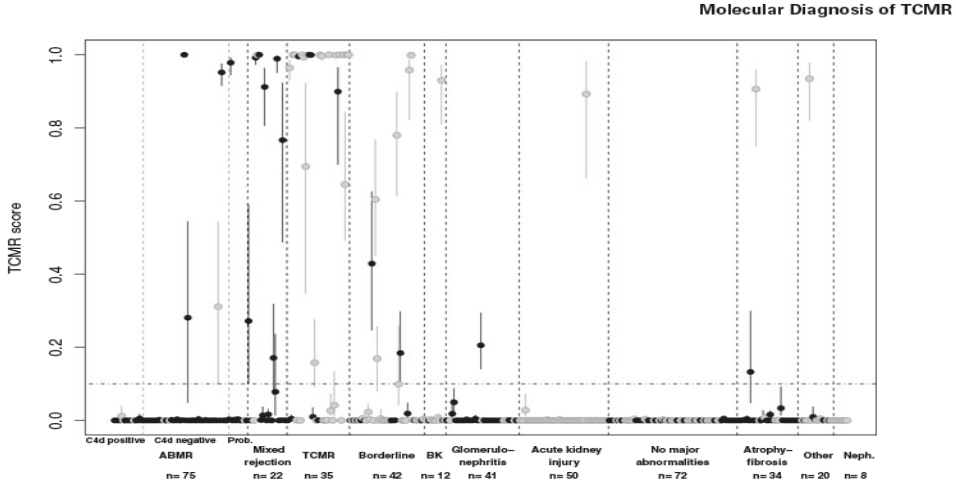


Figure 2: Relationship between the TCMR score and the histological reference standard diagnoses. Circles and solid vertical lines represent the median and interquartile range (IQR) of the TCMR score over the 100 classifier iterations. The biopsies are represented by their time period posttransplantation: early (<1 year: gray circles) and late (>1 year: black circles). Ordering within each histological stack is random. The horizontal line at 0.1 divides the samples into high and low TCMR scores -this threshold was used for the calculation of accuracy statistics. Neph=nephrectomies.

Reeve et al. Am J Transplant. 2013 Mar;13(3):645-55.

Common Errors in the Implementation and Interpretation of Microarray Studies.
Reeve, Jeff; Halloran, Philip; Kaplan, Bruce
Transplantation. 99(3):470-475, March 2015.

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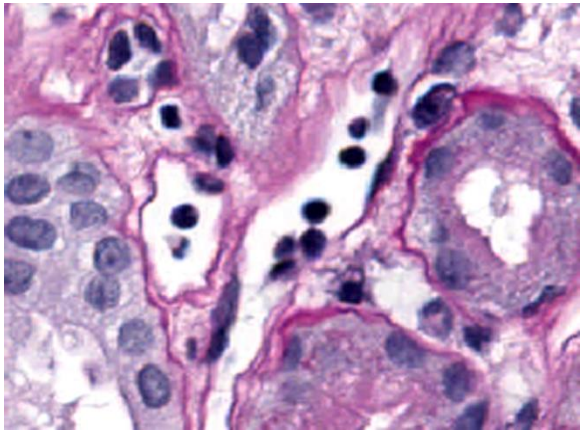
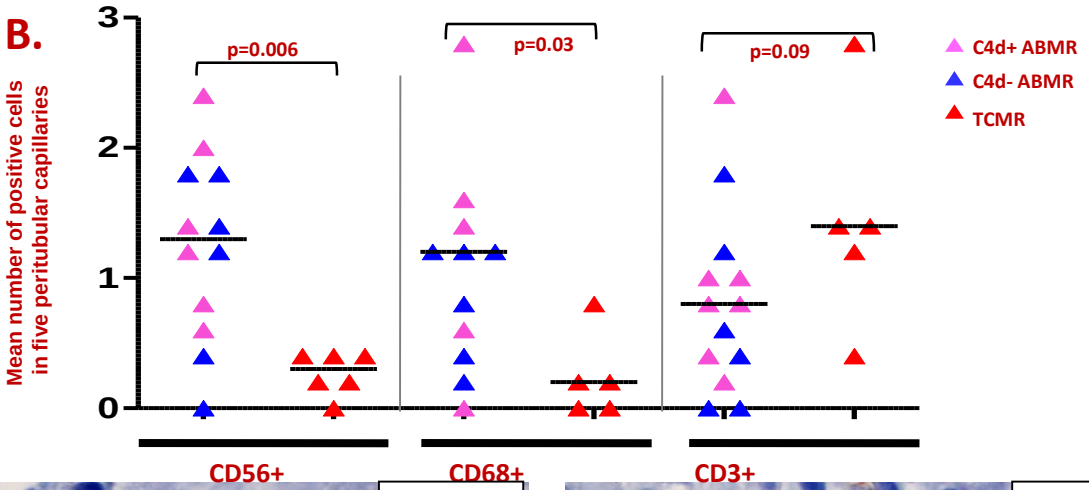
NK cells and macrophages in antibody mediated peritubular capillaritis

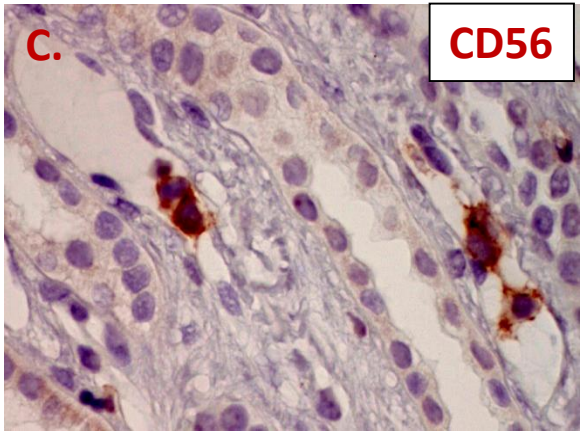
A.



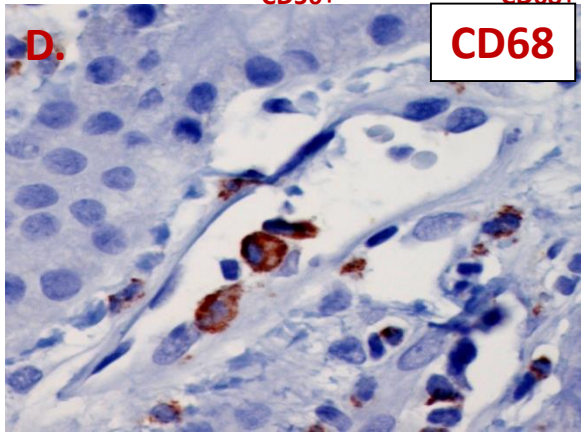
B.



C.



D.



E.

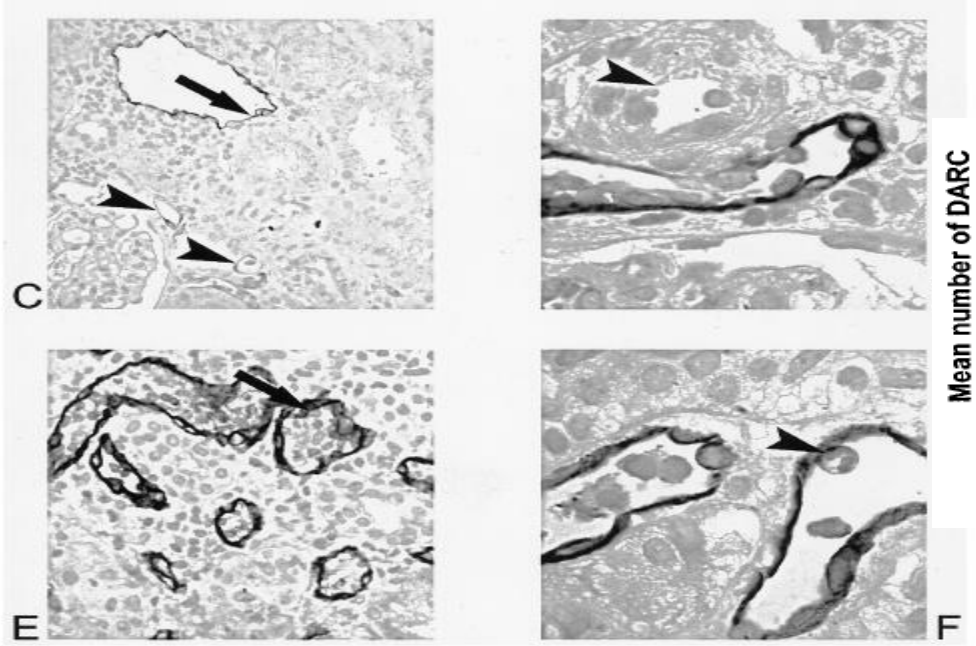
Hidalgo et al. AJT *2010; 10: 1812–1822*

0041-1337/03/7507-1030/0
TRANSPLANTATION

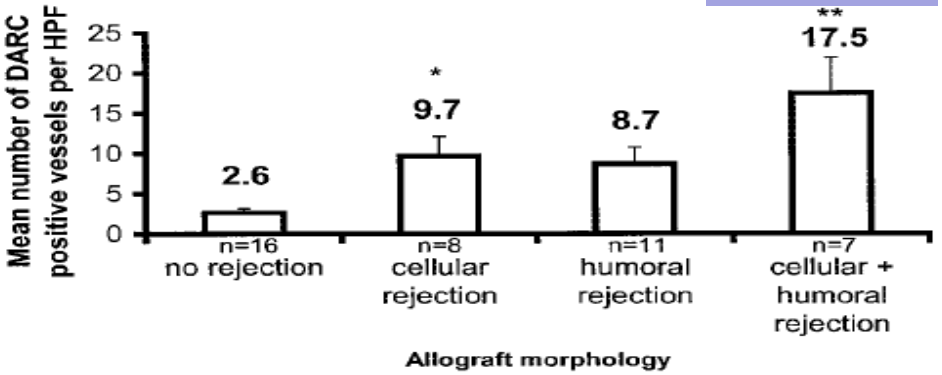
Vol. 75, 1030–1034, No. 7, April 15, 2003
Printed in U.S.A.

WHEN RENAL ALLOGRAFTS TURN DARC[1]

STEPHAN SEGERER,[2,7] GEORG A. BÖHMIG,[3] MARKUS EXNER,[4] YVES COLIN,[5] JEAN-PIERRE CARTRON,[5] DONTSCHO KERJASCHKI,[6] DETLEF SCHLÖNDORFF,[2] AND HEINZ REGELE[6]



C4d positive



Based on Banff 1997 classification using C4d but no DSA testing

More DARC in ptc in areas of inflammation, only very focal in glomeruli in sever ABMR

Kidney core 3-5 mm
1-2 EMB bites
1-2 TBB bites
Liver 3-5 mm

Collect Biopsy
Place immediately in RNALater

Shipping/Receiving
Room temperature

RNA Extraction, Clean-Up, and Quality Control

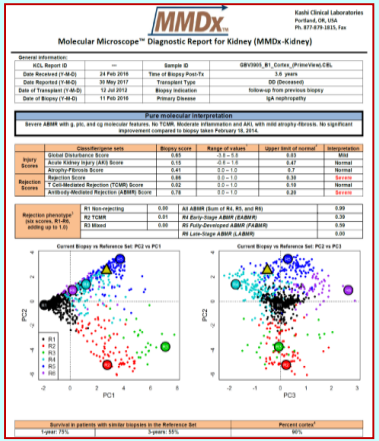
Molecular Microscope®
(MMDx-Kidney)

RNA
Labeling

Hybridization

Wash & Stain

Scan Chips



Evidence supporting the claim for superiority of molecular to histologic diagnosis

1. Histology relies on relatively few (6) canonical lesions, semi-quantitatively scored with considerable variability[1;2].
   MMDx uses hundreds of features (probe sets), measured on a continuous scale with high precision

2. When predicting a phenotype with a well-defined gold standard (survival), molecular measurements outperform histology[3-5].

3. MMDx outputs are continuous rather than semi-quantitative or binary, and can indicate when a biopsy has values near boundaries, allowing the observer to calibrate their diagnosis accordingly.

4. The MMDx supervised classifiers were trained on histology labels using microarray data, thereby combining information.

5. Many/most of the genes used by MMDx make biological sense.

6. Historically, the molecular findings have been used to update the Banff classification e.g. recognition of C4d- ABMR[6;7].

7. MMDx can assess recent injury and correlates with function better than histology[8-10]

8. Machine learning overcomes errors in sample labelling.

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Explaining the Molecular Microscope® report for core kidney transplant biopsies (MMDx-Kidney)

Clinical interpretation

Summary of molecular changes
(Injury, rejection)

Proportions rejection-related molecular changes
(Normal, TCMR, ABMR)

Visualization
Relationship of biopsy to others in reference set
PC2 vs. PC1

Visualization
PC2 vs. PC3

Survival of other kidneys like this one

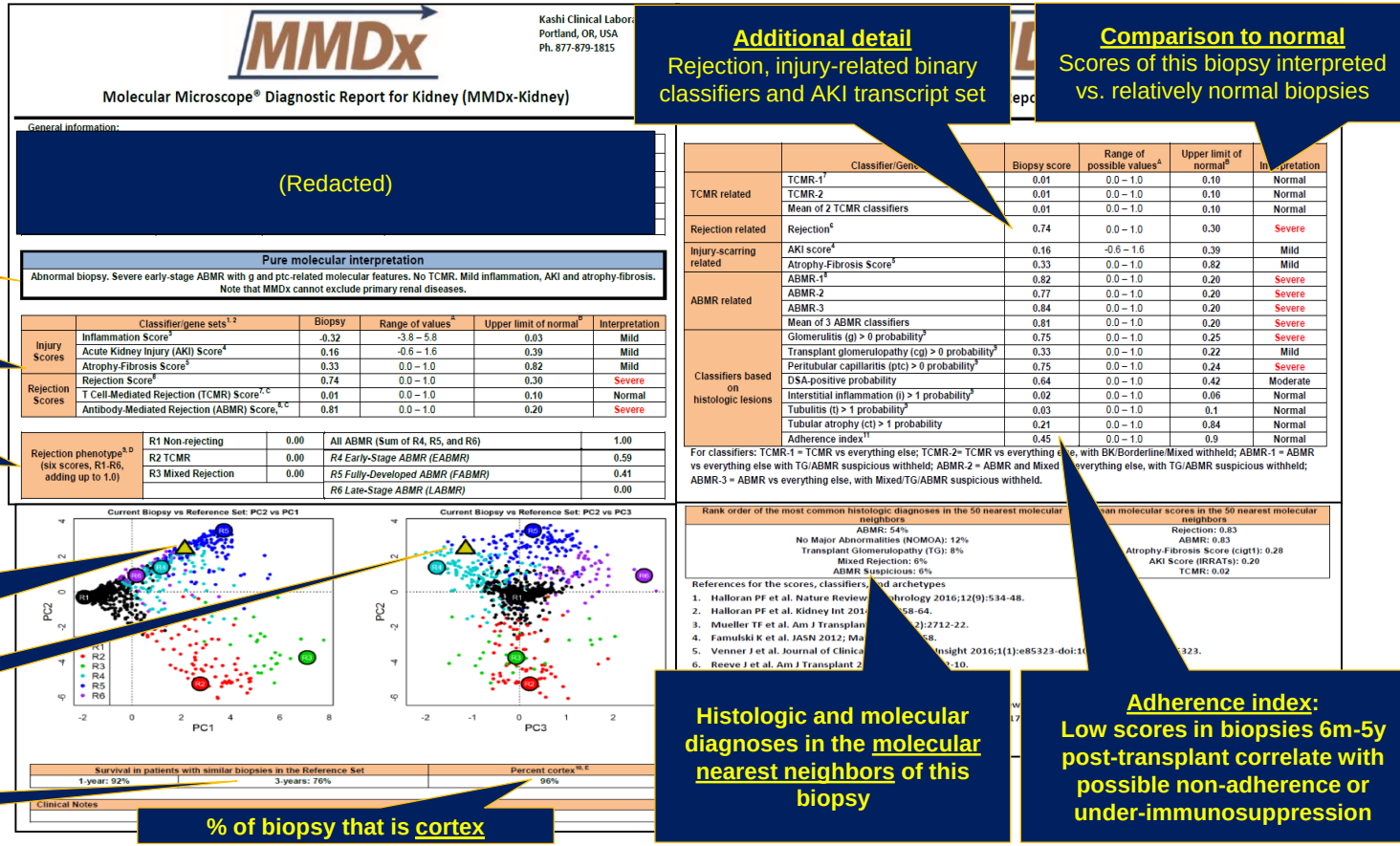
Additional detail
Rejection, injury-related binary classifiers and AKI transcript set

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 6m-5y post-transplant correlate with possible non-adherence or under-immunosuppression

% of biopsy that is cortex



MMDx

Kashi Clinical Labor…
Portland, OR, USA
Ph. 877-879-1815

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

General information:

(Redacted)

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.

	Classifier/gene sets[1,2]	Biopsy	Range of values[A]	Upper limit of normal[B]	Interpretation
Injury Scores	Inflammation Score[3]	-0.32	-3.8 – 5.8	0.03	Mild
	Acute Kidney Injury (AKI) Score[4]	0.16	-0.6 – 1.6	0.39	Mild
	Atrophy-Fibrosis Score[5]	0.33	0.0 – 1.0	0.82	Mild
Rejection Scores	Rejection Score[6]	0.74	0.0 – 1.0	0.30	Severe
	T Cell-Mediated Rejection (TCMR) Score[7,C]	0.01	0.0 – 1.0	0.10	Normal
	Antibody-Mediated Rejection (ABMR) Score,[8,C]	0.81	0.0 – 1.0	0.20	Severe

Rejection phenotype[9,D] (six scores, R1-R6, adding up to 1.0)				
	R1 Non-rejecting	0.00	All ABMR (Sum of R4, R5, and R6)	1.00
	R2 TCMR	0.00	*R4 Early-Stage ABMR (EABMR)*	0.59
	R3 Mixed Rejection	0.00	*R5 Fully-Developed ABMR (FABMR)*	0.41
			R6 Late-Stage ABMR (LABMR)	0.00

	Classifier/Gene…	Biopsy score	Range of possible values[A]	Upper limit of normal[B]	In…pretation
TCMR related	TCMR-1[7]	0.01	0.0 – 1.0	0.10	Normal
	TCMR-2	0.01	0.0 – 1.0	0.10	Normal
	Mean of 2 TCMR classifiers	0.01	0.0 – 1.0	0.10	Normal
Rejection related	Rejection[6]	0.74	0.0 – 1.0	0.30	Severe
Injury-scarring related	AKI score[4]	0.16	-0.6 – 1.6	0.39	Mild
	Atrophy-Fibrosis Score[5]	0.33	0.0 – 1.0	0.82	Mild
ABMR related	ABMR-1[8]	0.82	0.0 – 1.0	0.20	Severe
	ABMR-2	0.77	0.0 – 1.0	0.20	Severe
	ABMR-3	0.84	0.0 – 1.0	0.20	Severe
	Mean of 3 ABMR classifiers	0.81	0.0 – 1.0	0.20	Severe
Classifiers based on histologic lesions	Glomerulitis (g) > 0 probability[3]	0.75	0.0 – 1.0	0.25	Severe
	Transplant glomerulopathy (cg) > 0 probability[3]	0.33	0.0 – 1.0	0.22	Mild
	Peritubular capillaritis (ptc) > 0 probability[3]	0.75	0.0 – 1.0	0.24	Severe
	DSA-positive probability	0.64	0.0 – 1.0	0.42	Moderate
	Interstitial inflammation (i) > 1 probability[3]	0.02	0.0 – 1.0	0.06	Normal
	Tubulitis (t) > 1 probability[3]	0.03	0.0 – 1.0	0.1	Normal
	Tubular atrophy (ct) > 1 probability	0.21	0.0 – 1.0	0.84	Normal
	Adherence index[11]	0.45	0.0 – 1.0	0.9	Normal

For classifiers: TCMR-1 = TCMR vs everything else; TCMR-2= TCMR vs everything e…e, with BK/Borderline/Mixed withheld; ABMR-1 = ABMR vs everything else with TG/ABMR suspicious withheld; ABMR-2 = ABMR and Mixed …everything else, with TG/ABMR suspicious withheld; ABMR-3 = ABMR vs everything else, with Mixed/TG/ABMR suspicious withheld.

Current Biopsy vs Reference Set: PC2 vs PC1

Current Biopsy vs Reference Set: PC2 vs PC3

R1 Non-rejecting
R2 TCMR
R3 Mixed Rejection
R4 Early-Stage ABMR (EABMR)
R5 Fully-Developed ABMR (FABMR)
R6 Late-Stage ABMR (LABMR)

Rank order of the most common histologic diagnoses in the 50 nearest molecular neighbors
ABMR: 54%
No Major Abnormalities (NOMOA): 12%
Transplant Glomerulopathy (TG): 8%
Mixed Rejection: 6%
ABMR Suspicious: 6%

…an molecular scores in the 50 nearest molecular neighbors
Rejection: 0.83
ABMR: 0.83
Atrophy-Fibrosis Score (cigt1): 0.28
AKI Score (IRRATs): 0.20
TCMR: 0.02

References for the scores, classifiers, …d archetypes
1. Halloran PF et al. Nature Review… …hrology 2016;12(9):534-48.
2. Halloran PF et al. Kidney Int 201… …58-64.
3. Mueller TF et al. Am J Transplan… …2):2712-22.
4. Famulski K et al. JASN 2012; Ma… …68.
5. Venner J et al. Journal of Clinica… …nsight 2016;1(1):e85323-doi:10… …323.
6. Reeve J et al. Am J Transplant 2… …-10.

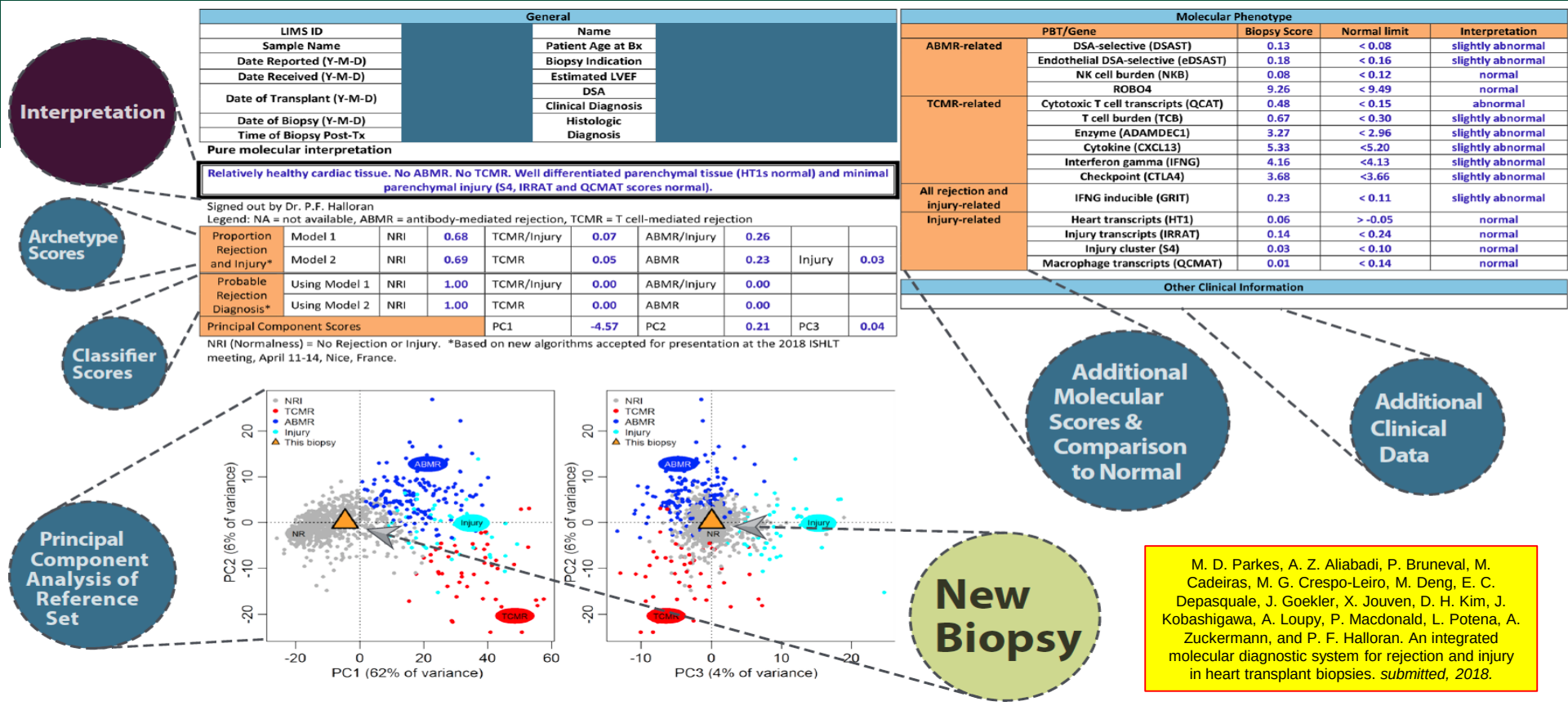
Survival in patients with similar biopsies in the Reference Set		Percent cortex[10,E]
1-year: 92%	3-years: 76%	96%

Clinical Notes

MMDx-Heart
Endomyocardial biopsies

Results on the first 1000 biopsies

INTERHEART ClinicalTrials.gov NCT02670408



M. D. Parkes, A. Z. Aliabadi, P. Bruneval, M. Cadeiras, M. G. Crespo-Leiro, M. Deng, E. C. Depasquale, J. Goekler, X. Jouven, D. H. Kim, J. Kobashigawa, A. Loupy, P. Macdonald, L. Potena, A. Zuckermann, and P. F. Halloran. An integrated molecular diagnostic system for rejection and injury in heart transplant biopsies. *submitted, 2018.*

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 $S1_{Normal}$ (NRI), $S2_{TCMR}$, $S3_{ABMR}$, 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 addition molecular data including pathogenesis-based transcript (PBT) set scores and singular transcript expression scores relating to all rejections, ABMR, TCMR, and injury. Score 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.

MMDx-Lung
Transbronchial biopsies

Results on the first 250 biopsies

INTERLUNG ClinicalTrials.gov: NCT02812290

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

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

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

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

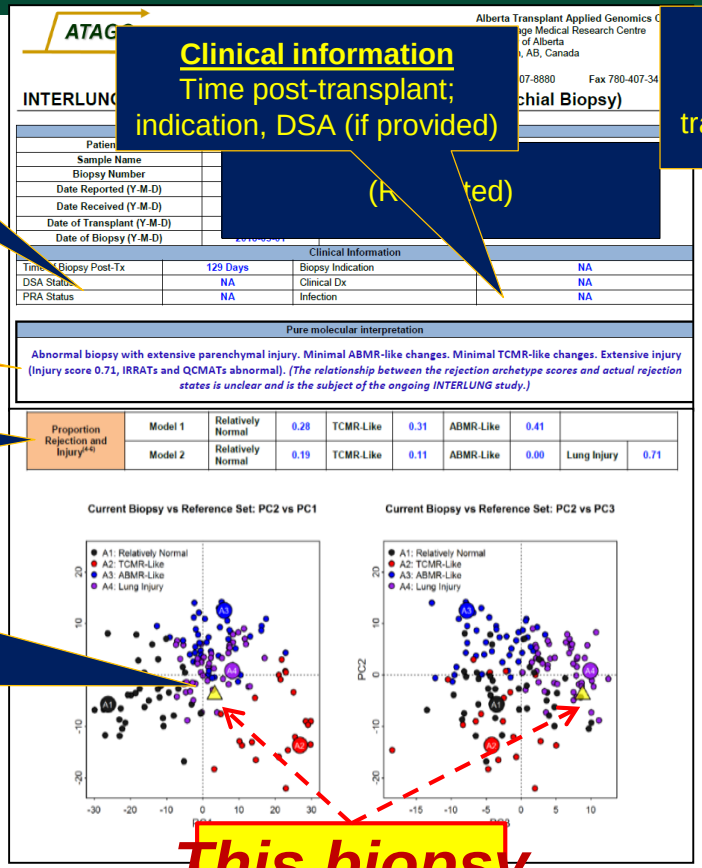
Comparison to normal
Scores interpreted vs. relatively normal biopsies

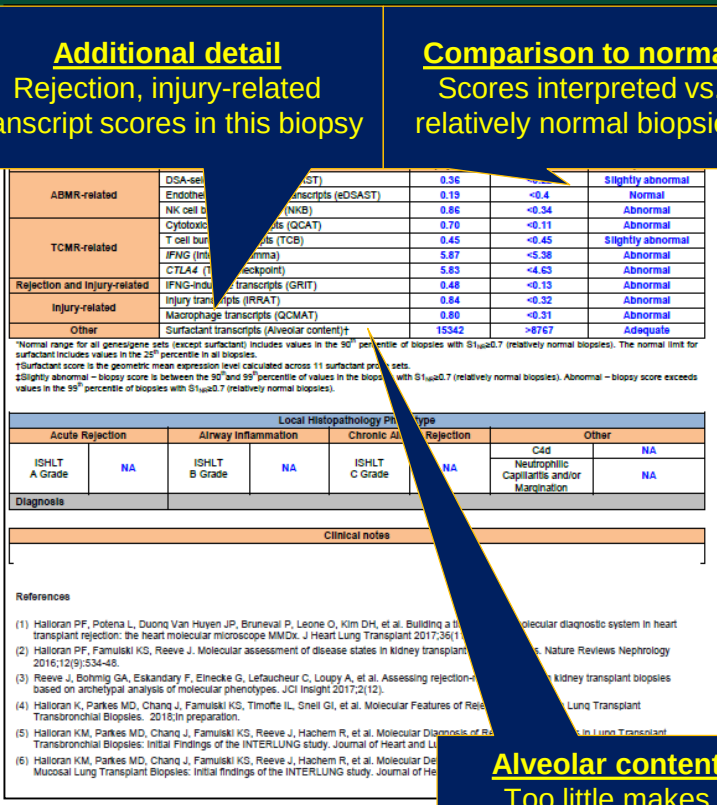
Clinical interpretation

Proportions
Normal, TCMR, ABMR, injury

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

Alveolar content
Too little makes interpretation difficult





This biopsy

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

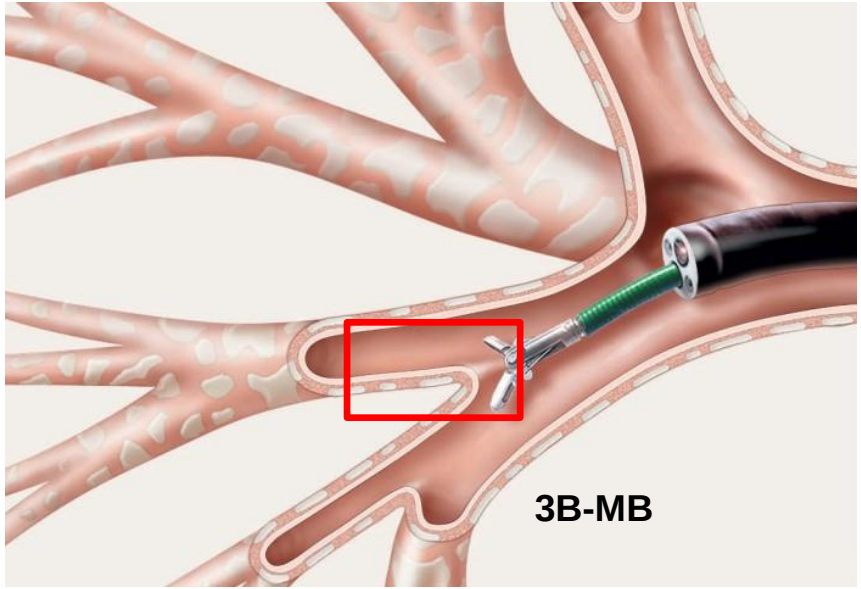


Image courtesy of Olympus

ATAGC

Lung Case #1

TBB Report – Page 1 3BMB

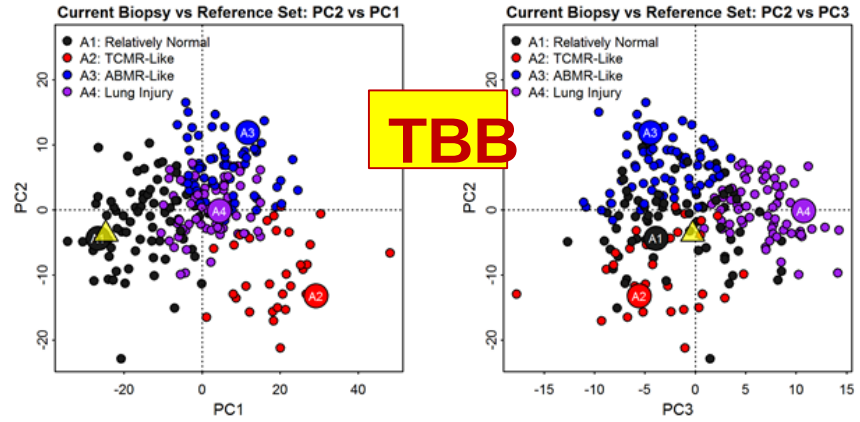
General Information

Redacted

Pure molecular interpretation

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.)*

Proportion Rejection and Injury[4-6]									
	Model 1	Relatively Normal	0.93	TCMR-Like	0.00	ABMR-Like	0.07		
	Model 2	Relatively Normal	0.90	TCMR-Like	0.00	ABMR-Like	0.00	Lung Injury	0.11

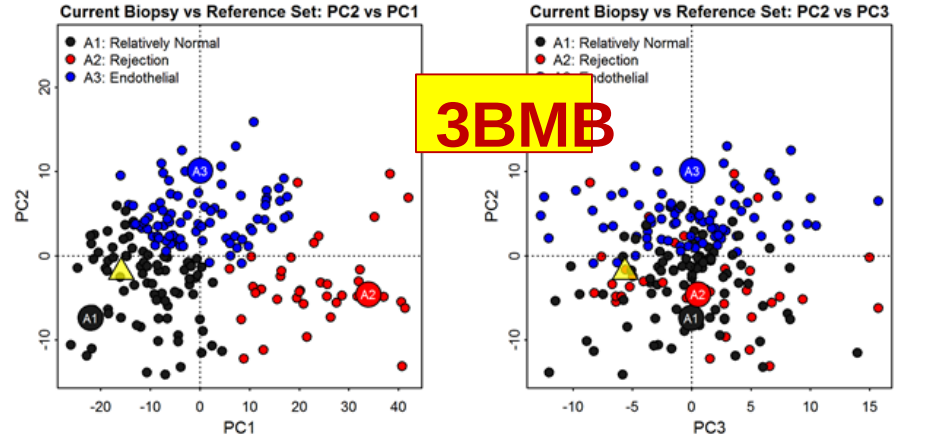


General Information

Pure Molecular Interpretation

Relatively healthy lung transplant. *(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[4-6]	Relatively Normal	0.70	Rejection	0.00	Endothelial	0.30



Lung Case #1

Report Page 2

TBB

3BMB

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
TCMR-related	Cytotoxic T cell transcripts (QCAT)	-0.01	<0.3	Normal
	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
Rejection and injury-related	IFNG-inducible transcripts (GRIT)	-0.10	<0.14	Normal
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 $S1_{NR}$≥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 a
‡Slightly abnormal – biopsy score is between the 90th and 99th percenti
values in the 99th percentile of biopsies with $S1_{NR}$≥0.7 (relatively norma

TBB

Local Histopathology Phenotype							
Acute Rejection		Airway Inflammation		Chronic Airway Rejection		Other	
ISHLT A Grade	NA	ISHLT B Grade	NA	ISHLT C Grade	NA	C4d	NA
						Neutrophilic Capillaritis and/or Margination	NA
Diagnosis	NA						

Clinical notes

References

(1) Halloran 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) Halloran PF, Famulski 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, Einecke G, Lefaucheur 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, Famulski KS, Timofte IL, Snell GI, et al. Molecular Features of Rejection and Injury in Lung Transplant Transbronchial Biopsies. 2018;In preparation.

(5) Halloran KM, Parkes MD, Chang J, Famulski 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) Halloran KM, Parkes MD, Chang J, Famulski 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
TCMR-related	Cytotoxic T cell transcripts (QCAT)	0.22	<0.42	Normal
	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
Rejection and injury-related	IFNG-inducible transcripts (GRIT)	-0.07	<0.12	Normal
	Macrophage transcripts (QCMAT)	0.13	<0.32	Normal

*Normal range for all genes/gene sets (except surfactant) includes values in
†Slightly abnormal – biopsy score is between the 90th and 99th percentile of v
values in the 99th percentile of biopsies with $S1_{NR}$≥0.7 (relatively normal biop

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	NA
						Neutrophilic Capillaritis and/or Margination	NA
Diagnosis	NA						

Clinical notes

References

(1) Halloran 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) Halloran PF, Famulski 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, Einecke G, Lefaucheur 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, Famulski KS, Timofte IL, Snell GI, et al. Molecular Features of Rejection and Injury in Lung Transplant Transbronchial Biopsies. 2018;In preparation.

(5) Halloran KM, Parkes MD, Chang J, Famulski 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

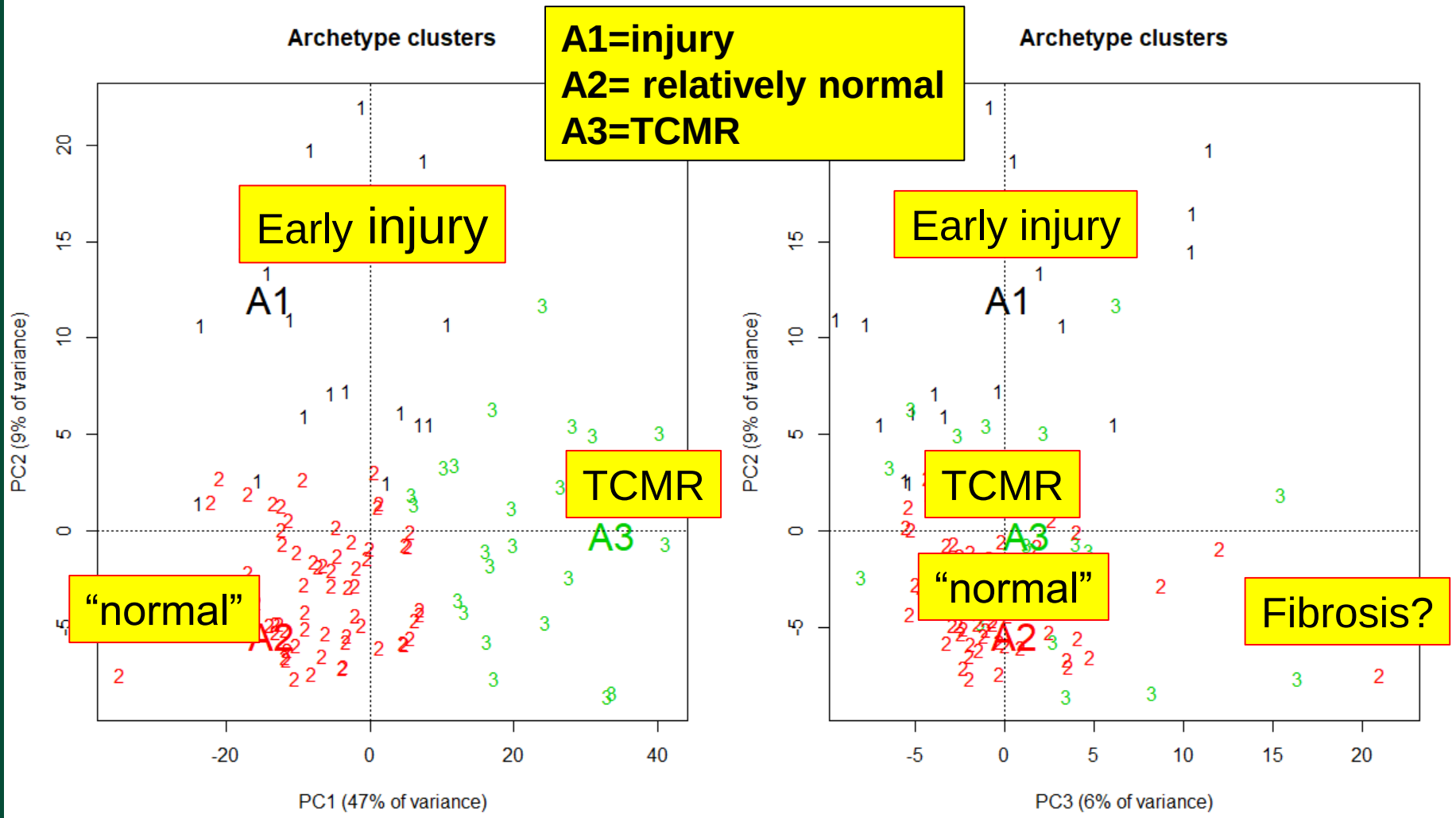
(6) Halloran KM, Parkes MD, Chang J, Famulski KS, Reeve J, Hachem R, et al. Molecular Detection of Rejection–like Changes in Proximal Bronchial Mucosal Lung Transplant Biopsies: Initial findings of the INTERLUNG study. Journal of Heart and Lung Transplantation. 2018. RefType: Abstract

MMDx-Liver

Molecular analysis of rejection and injury in human liver transplant biopsies:

First results of the INTERLIVER STUDY

INTERLIVER ClinicalTrials.gov NCT03193151


A1=injury
A2= relatively normal
A3=TCMR
Archetype clusters
Early injury
A1
TCMR
A3
“normal”
A2
PC2 (9% of variance)
PC1 (47% of variance)
Archetype clusters
Early injury
A1
TCMR
A3
“normal”
A2
Fibrosis?
PC2 (9% of variance)
PC3 (6% of variance)

Liver Case #1 B1 (596 days post-Tx) Report

Page 1

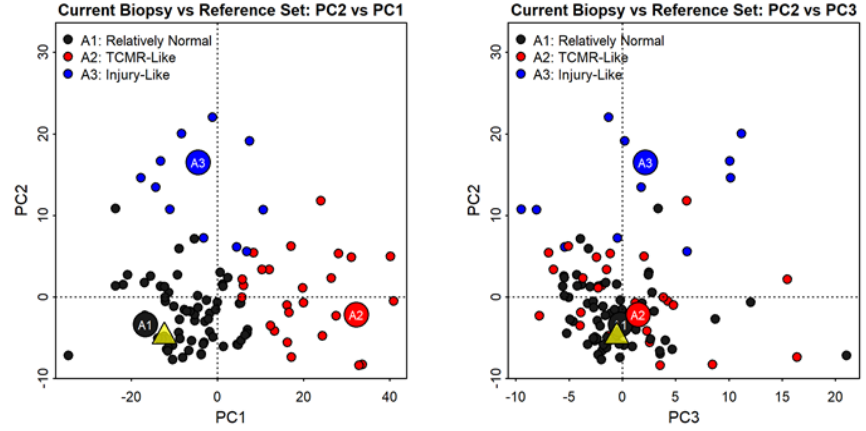
General Information			
Patient ID	BED0001	Name:	
Sample Name	BED0001		
Biopsy Number	B1		
Date Reported (Y-M-D)	2018-Dec-05	Patient Age at Biopsy:	
Date Received (Y-M-D)			
Date of Transplant (Y-M-D)	2016-May-02	NA	
Date of Biopsy (Y-M-D)	2017-Dec-19		

Clinical Information			
Time of Biopsy Post-Tx	596 Days	Biopsy Indication	For Cause
DSA Status	NA	Primary Disease	Cirrhosis, Nonalcoholic Steatohepatitis
PRA Status	NA		

Pure molecular interpretation

Relatively healthy liver transplant. Minimal TCMR-like changes. Minimal parenchymal injury (Injury score, IRRAT and QCMAT scores normal). *(The relationship between the rejection archetype scores and actual rejection states is unclear and is the subject of the ongoing INTERLIVER study.)*

Proportion Rejection and Injury[1-2]	Model 1	Relatively Normal	0.91	TCMR-Like	0.09	Injury-Like	0.00



Page 2

Molecular Phenotype				
	Gene/gene sets	Biopsy score	Normal Limit*	Interpretation†
ABMR-related	DSA-selective transcripts (DSAST)	-0.04	<0.18	Normal
	Endothelial DSA-selective transcripts (eDSAST)	-0.06	<0.2	Normal
	NK cell burden transcripts (NKB)	-0.07	<0.27	Normal
TCMR-related	Cytotoxic T cell transcripts (QCAT)	-0.04	<0.25	Normal
	T cell burden transcripts (TCB)	0.12	<0.4	Normal
	IFNG (Interferon gamma)	4.28	<4.55	Normal
	CTLA4 (T cell checkpoint)	3.20	<3.39	Normal
Rejection and injury-related	IFNG-inducible transcripts (GRIT)	-0.04	<0.13	Normal
Injury-related	Injury transcripts (IRRAT)	-0.16	<0.2	Normal
	Macrophage transcripts (QCMAT)	-0.09	<0.15	Normal

*Normal range for all genes/gene sets (except surfactant) includes values in the 90th percentile of biopsies with $S1_{Norm}$≥0.7 (relatively normal biopsies).
†Slightly abnormal – biopsy score is between the 90th and 99th percentile of values in the biopsies with $S1_{Norm}$≥0.7 (relatively normal biopsies). Abnormal – biopsy score exceeds values in the 99th percentile of biopsies with $S1_{Norm}$≥0.7 (relatively normal biopsies).

Pathology					
Acute Rejection					
Portal Vein Inflammation	0	Bile Duct Inflammation	0	Venous Endothelial Inflammation	0
Chronic Rejection					
Bile Duct Degeneration	0	Focal Obliteration	0	Cholestasis	0
Lumenal Narrowing	0	Mural Fibrosis	0	Arterial FIR	0
Other Disease					
Autoimmune Hepatitis	NA	Steatohepatitis Grading	NA	Fibrosis Grading	NA
Recurrent HCV	NA	Recurrent CMV Hepatitis	NA		

Clinical notes

References

(1) Halloran 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) Reeve J, Bohmig GA, Eskandary F, Einecke G, Lefaucheur 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).

Liver biopsy case 2 (751 days post-Tx)

Page 1

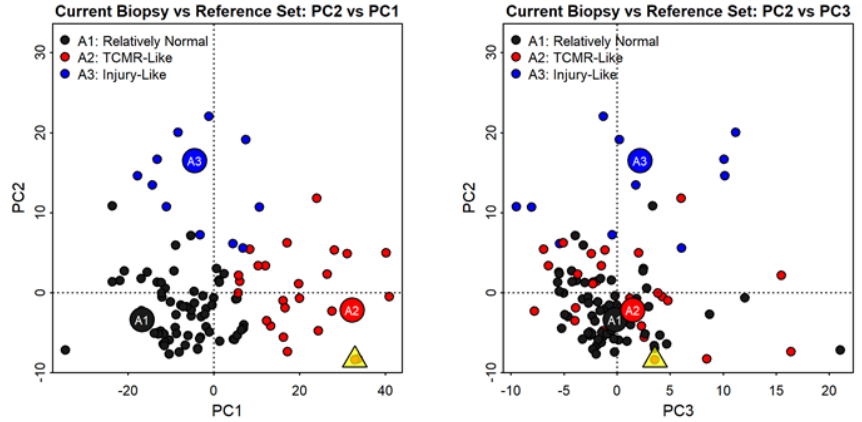
General Information			
Patient ID	BED0005	Name:	
Sample Name	BED0005		
Biopsy Number	B1		
Date Reported (Y-M-D)	2018-Dec-05	Patient Age at Biopsy:	
Date Received (Y-M-D)			
Date of Transplant (Y-M-D)	2016-Mar-01	NA	
Date of Biopsy (Y-M-D)	2018-Mar-22		

Clinical Information			
Time of Biopsy Post-Tx	751 Days	Biopsy Indication	For Cause
DSA Status	NA	Primary Disease	Primary Biliary Cholangitis
PRA Status	NA		

Pure molecular interpretation

Abnormal biopsy with extensive TCMR-like changes. Some parenchymal injury (IRRAT and QCMAT scores abnormal). *(The relationship between the rejection archetype scores and actual disease states is unclear and is the subject of the ongoing INTERLIVER study.)*

Proportion Rejection and Injury[1-2]	Model 1	Relatively Normal	0.00	TCMR-Like	1.00	Injury-Like	0.00



Page 2

Molecular Phenotype				
	Gene/gene sets	Biopsy score	Normal Limit*	Interpretation†
ABMR-related	DSA-selective transcripts (DSAST)	0.09	<0.18	Normal
	Endothelial DSA-selective transcripts (eDSAST)	-0.05	<0.2	Normal
	NK cell burden transcripts (NKB)	0.18	<0.27	Normal
TCMR-related	Cytotoxic T cell transcripts (QCAT)	1.63	<0.25	Abnormal
	T cell burden transcripts (TCB)	2.19	<0.4	Abnormal
	IFNG (Interferon gamma)	5.97	<4.55	Abnormal
	CTLA4 (T cell checkpoint)	4.62	<3.39	Abnormal
Rejection and injury-related	IFNG-inducible transcripts (GRIT)	0.67	<0.13	Abnormal
Injury-related	Injury transcripts (IRRAT)	0.38	<0.2	Abnormal
	Macrophage transcripts (QCMAT)	0.48	<0.15	Abnormal

*Normal range for all genes/gene sets (except surfactant) includes values in the 90th percentile of biopsies with $S1_{Normal} \geq 0.7$ (relatively normal biopsies).
†Slightly abnormal – biopsy score is between the 90th and 99th percentile of values in the biopsies with $S1_{Normal} \geq 0.7$ (relatively normal biopsies). Abnormal – biopsy score exceeds values in the 99th percentile of biopsies with $S1_{Normal} \geq 0.7$ (relatively normal biopsies).

Pathology					
Acute Rejection					
Portal Vein Inflammation	0	Bile Duct Inflammation	0	Venous Endothelial Inflammation	0
Chronic Rejection					
Bile Duct Degeneration	0	Focal Obliteration	0	Cholestasis	0
Lumenal Narrowing	0	Mural Fibrosis	0	Arterial FIR	0
Other Disease					
Autoimmune Hepatitis	NA	Steatohepatitis Grading	NA	Fibrosis Grading	NA
Recurrent HCV	NA	Recurrent CMV Hepatitis	NA		

Clinical notes

References

(1) Halloran 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) Reeve J, Bohmig GA, Eskandary F, Einecke G, Lefaucheur 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).

Complementary diagnostic tools in transplant pathology