The Molecular Microscope Diagnostic System (MMDx®) and its implications for organ transplantation

Rejection

Injury

Progression

We are currently completing a licensing agreement with One Lambda/Thermo Fisher
to commercialize MMDx® as a centralized diagnostic service for biopsies:
service laboratories in USA, Europe.

Anna Hutton

Konrad Famulski

Luis Hidalgo

Vido Ramassar

Jessica Chang

Michael parkes

Michelle Ryan

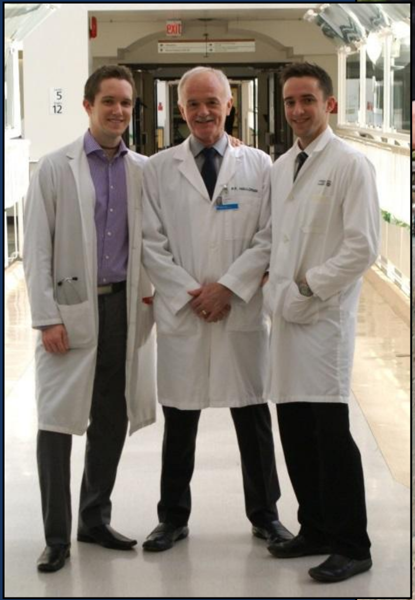
Rob Polakowski

Jeff Reeve

Katelynn Madill-Thomsen

Kieran Halloran

Brendan Halloran



Thank you to the current/recent MMDx collaborators

Jessica Chang
Gunilla Einecke
Konrad Famulski
Gaurav Gupta
Luis Hidalgo
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Roche Organ Transplantation Research Foundation
Genome Canada
Canada Foundation for Innovation
Novartis
Stromedix
University Hospital Foundation
Capital Health/Alberta Health Services
Astellas Pharma Inc.
Roche Molecular Systems, Roche Canada
Alberta Advanced Education and Technology
Alberta Heritage Foundation for Medical Research
Muttart Chair in Clinical Immunology

Special thanks to:
clinical collaborators and
the patients

>100 investigators in 3 international teams:
INTERCOMEX Clinicaltrials.gov NCT01299168
INTERHEART Clinicaltrials.gov NCT02670408
INTERLUNG ClinicalTrials.gov: NCT02812290

Contents

- The unmet need
- What is MMDx®?
- Mechanisms of rejection: TCMR, ABMR
- Injury, atrophy-fibrosis, progression
- MMDx® for heart transplants
- MMDx® for lung transplants

The unmet need:

Accurate assessment of rejection and injury in troubled transplants

Organ transplantation is a major force in health care

1,000,000 existing organ transplants

120,000 new transplants each year

Unmet needs and opportunities

Failures

Deaths

Complications

Sub-optimal function

ATAGC

What the clinician needs to know about a troubled transplant

Rejection

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

Parenchymal wounding (and diseases)

- Acute parenchymal injury (AKI)
- Irreversible injury (atrophy-scarring)
- Risk of progression to failure

Problems with histology (and other opinion-based diagnostics)

- Empirical: No external gold standard
- Variability between observers
  - Estimate of intrinsic error rate
- Based on non-specific lesions
- Requires empirical guidelines (algorithms)
- Experts interpret guidelines: “It depends…”
- Dichotomous (“0 vs. 1”) vs. continuous
- Continually changing: literature challenge

Histology: limited inter-observer agreement provides an estimate of “noise”

- “normal vs. abnormal”: good
- diagnosis in abnormal: not good
- for T cell-mediated rejection
  - – kidney transplant: 50%
    - Reeve et al. *Am J Transplant 13:645, 2013.*
  - – heart transplant: 28%
    - Crespo-Leiro et al *Transplantation 94:1172, 2012.*
  - – lung transplant: 0-18%
    - Arcasoy et al *Am.J.Transplant.* 11:320, 2011.
  - – liver transplant?
- histology does not correlate with function

Why study molecules?

Disease classification

Mechanistic understanding

Improved diagnostic accuracy

Understand injury and progression

Continuous automated measurements

Approaches to high dimensionality data

Platform: Discovery/development vs. application

Analysis: Supervised vs. unsupervised

Approaches to high dimensionality data

Platform: Discovery/development vs. application

Affymetrix microarrays

Analysis: Supervised vs. unsupervised

Both

What is MMDx®?

The Molecular Microscope Diagnostic System (MMDx®)

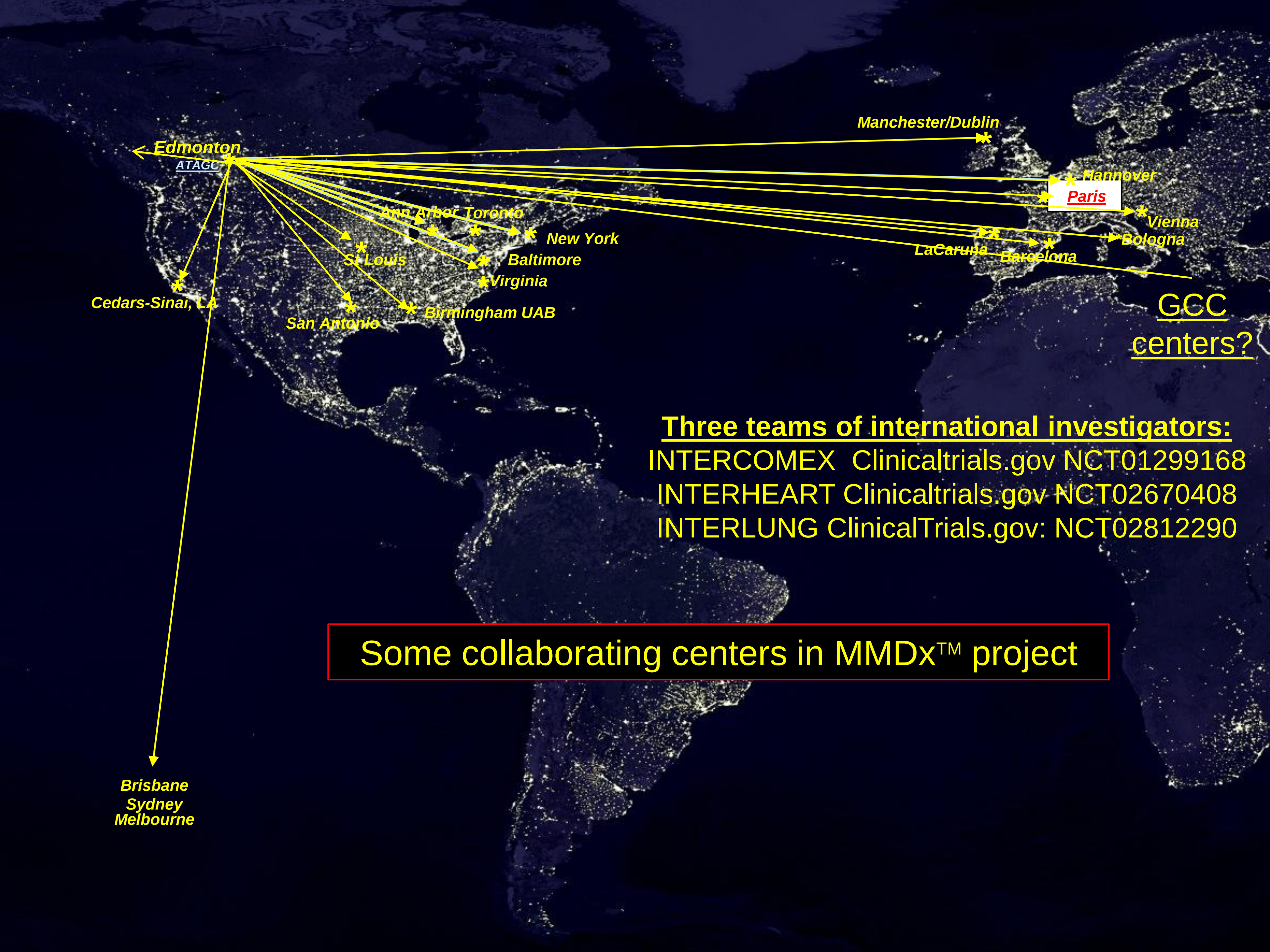
A central diagnostic system that receives biopsies (stabilized in RNAlater shipped Fedex room temp.), uses microarrays to measure transcript changes, and applies equations to compare each new biopsy to a Reference Set, generating an automated report in 29 hours.

Predefined algorithms (classifiers) assign scores that reflect probabilities:

- **Rejection Score (TCMR and/or ABMR)**
- **T cell-mediated rejection (TCMR) Score**
- **Antibody-mediated rejection (ABMR)**
- **Acute kidney injury (AKI) Score**
- **Extent of irreversible damage (Atrophy-scarring Score)**
- **Risk Score for progression to failure**
- **Probability of non-adherence: MH/time score**

Molecular Microscope™

Some collaborating centers in MMDx™ project



Three teams of international investigators:

INTERCOMEX Clinicaltrials.gov NCT01299168
INTERHEART Clinicaltrials.gov NCT02670408
INTERLUNG ClinicalTrials.gov: NCT02812290

Advantages and insights from molecular measurements: accuracy and safety

- ***Less tissue: safer*** (if histology can be dropped e.g. TBB)
  - Kidney: one 3mm core: can read medulla (INTERCOMEX)
  - Heart: 1-2 bites? (INTERHEART)
  - Lung 1-2 bites: potentially mucosal biopsy (INTERLUNG)
  - Liver: INTERLIVER is in planning
- Objective, quantitative, probabilistic
- Standardized assessment: international
- Correct errors in histology: C4d, v-lesions, need for DSA
- Calibrate blood and urine biomarkers
- Mechanisms and druggable targets
- "Theranostic" support: drug development

Challenges of molecular assessment

- How much tissue is needed to represent the organ? (chip actually needs very little)
- Highly focal diseases
- Search for truth when conventional phenotyping is poor (the “fool’s gold standard”)

Why do we use RNAlater? Why not formalin-paraffin (FFPE)

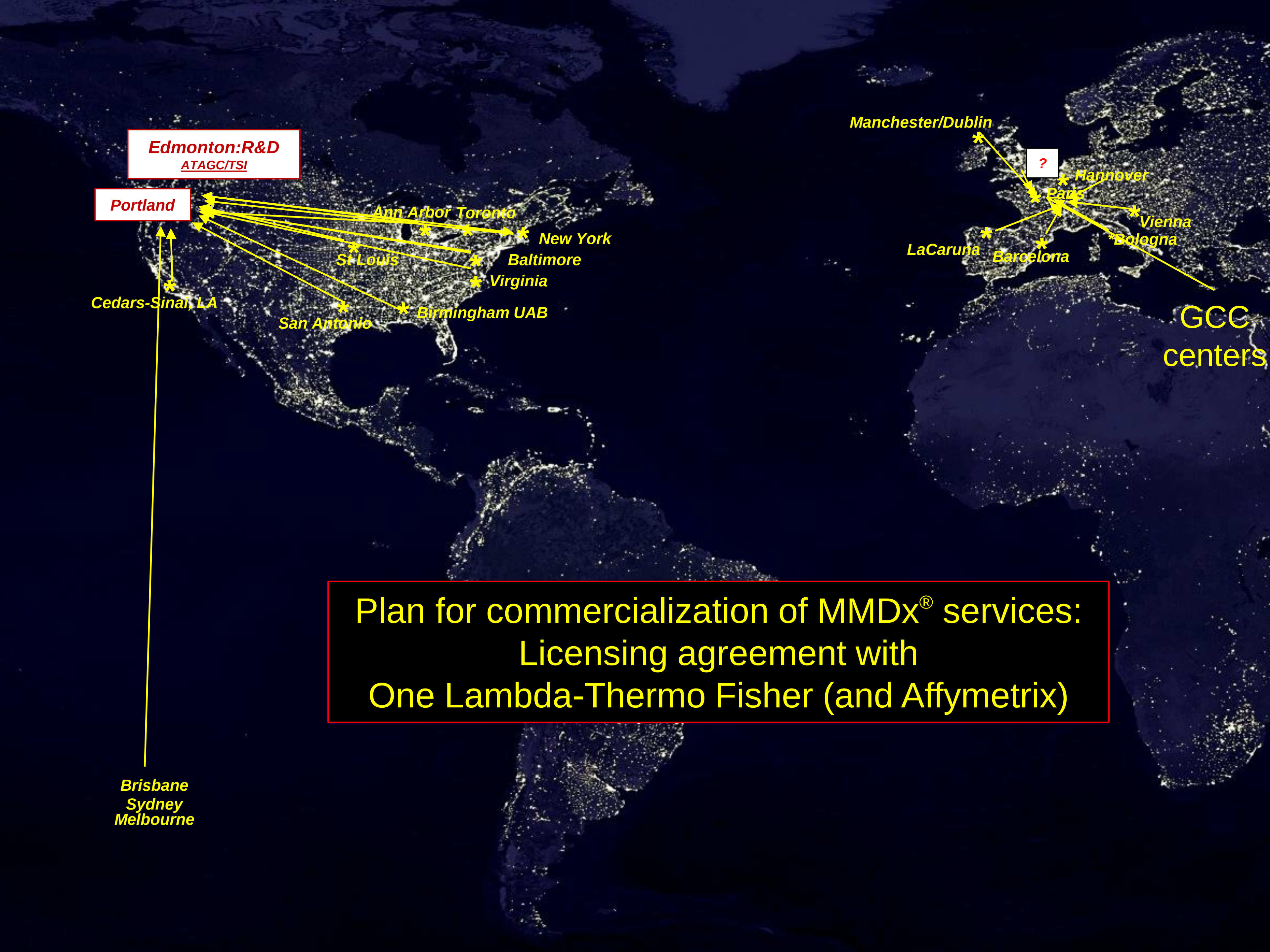
FFPE yields only 10% of RNA

Sampling error

FFPE damages the RNA:

False positives, false negatives

FFPE is very much second best



Plan for commercialization of MMDx® services:
Licensing agreement with
One Lambda-Thermo Fisher (and Affymetrix)

MMDx for kidney transplants

Using rejection-associated transcripts (RATs)

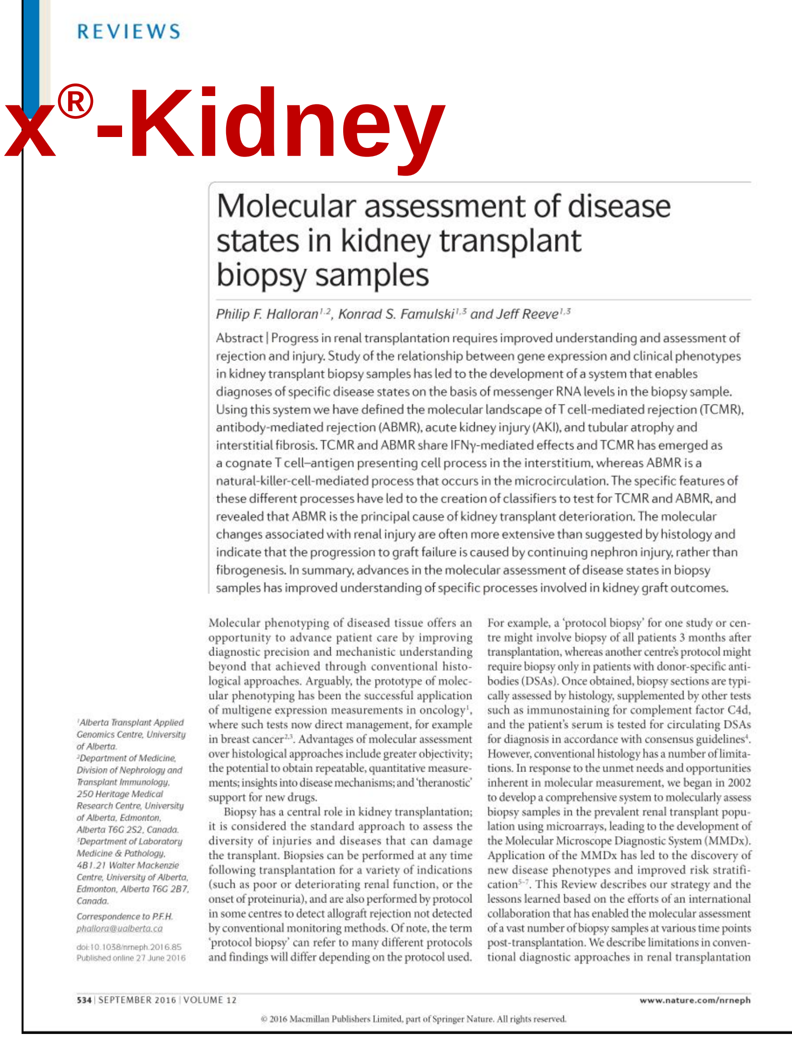
Developing the kidney diagnostic system

A supervised approach

(because kidney histology classes are “pretty good”

Introducing MMDx®-Kidney

- international collaboration of Canadian, European, and USA centers
    - Genome Canada
    - INTERCOM and INTERCOMEX Clinicaltrials.govNCT#01299168.
- This required reclassifying all the disease states since so many errors were found
- *Can read medulla and cortex*
- MMDx® is now ready for commercialization



Molecular assessment of disease states in kidney transplant biopsy samples

Philip F. Halloran[1,2], Konrad S. Famulski[1,3] and Jeff Reeve[1,3]

Abstract | Progress in renal transplantation requires improved understanding and assessment of rejection and injury. Study of the relationship between gene expression and clinical phenotypes in kidney transplant biopsy samples has led to the development of a system that enables diagnoses of specific disease states on the basis of messenger RNA levels in the biopsy sample. Using this system we have defined the molecular landscape of T cell-mediated rejection (TCMR), antibody-mediated rejection (ABMR), acute kidney injury (AKI), and tubular atrophy and interstitial fibrosis. TCMR and ABMR share IFNγ-mediated effects and TCMR has emerged as a cognate T cell–antigen presenting cell process in the interstitium, whereas ABMR is a natural-killer-cell-mediated process that occurs in the microcirculation. The specific features of these different processes have led to the creation of classifiers to test for TCMR and ABMR, and revealed that ABMR is the principal cause of kidney transplant deterioration. The molecular changes associated with renal injury are often more extensive than suggested by histology and indicate that the progression to graft failure is caused by continuing nephron injury, rather than fibrogenesis. In summary, advances in the molecular assessment of disease states in biopsy samples has improved understanding of specific processes involved in kidney graft outcomes.

Molecular phenotyping of diseased tissue offers an opportunity to advance patient care by improving diagnostic precision and mechanistic understanding beyond that achieved through conventional histological approaches. Arguably, the prototype of molecular phenotyping has been the successful application of multigene expression measurements in oncology[1], where such tests now direct management, for example in breast cancer[2,3]. Advantages of molecular assessment over histological approaches include greater objectivity; the potential to obtain repeatable, quantitative measurements; insights into disease mechanisms; and 'theranostic' support for new drugs.

Biopsy has a central role in kidney transplantation; it is considered the standard approach to assess the diversity of injuries and diseases that can damage the transplant. Biopsies can be performed at any time following transplantation for a variety of indications (such as poor or deteriorating renal function, or the onset of proteinuria), and are also performed by protocol in some centres to detect allograft rejection not detected by conventional monitoring methods. Of note, the term 'protocol biopsy' can refer to many different protocols and findings will differ depending on the protocol used. For example, a 'protocol biopsy' for one study or centre might involve biopsy of all patients 3 months after transplantation, whereas another centre's protocol might require biopsy only in patients with donor-specific antibodies (DSAs). Once obtained, biopsy sections are typically assessed by histology, supplemented by other tests such as immunostaining for complement factor C4d, and the patient's serum is tested for circulating DSAs for diagnosis in accordance with consensus guidelines[4]. However, conventional histology has a number of limitations. In response to the unmet needs and opportunities inherent in molecular measurement, we began in 2002 to develop a comprehensive system to molecularly assess biopsy samples in the prevalent renal transplant population using microarrays, leading to the development of the Molecular Microscope Diagnostic System (MMDx). Application of the MMDx has led to the discovery of new disease phenotypes and improved risk stratification[5–7]. This Review describes our strategy and the lessons learned based on the efforts of an international collaboration that has enabled the molecular assessment of a vast number of biopsy samples at various time points post-transplantation. We describe limitations in conventional diagnostic approaches in renal transplantation

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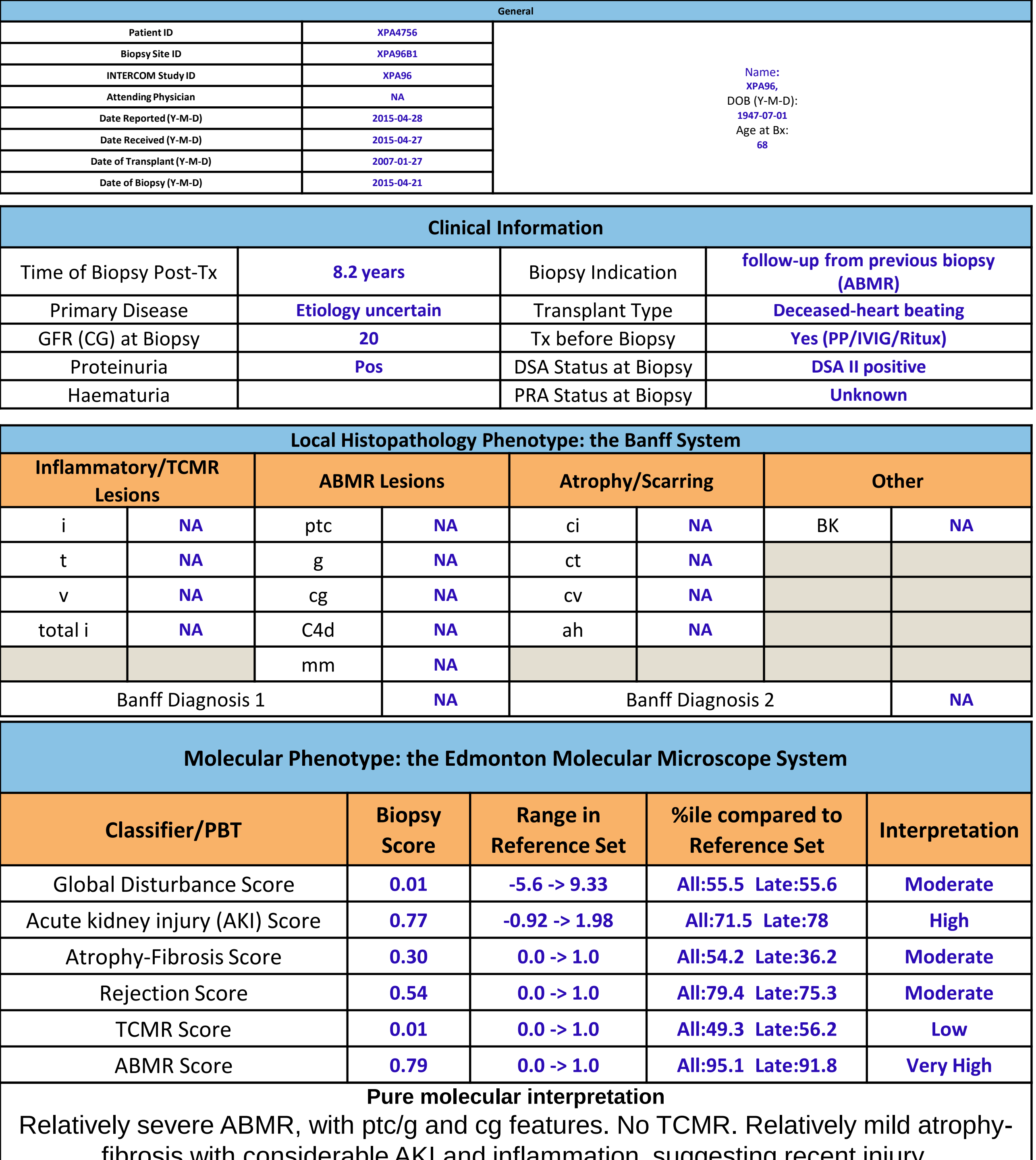
doi:10.1038/nrneph.2016.85

Reviewed in *Nature Reviews Nephrology 12 (9):534-548, 2016.*

The current MMDx report page 1



General

Patient ID	XPA4756	Name: XPA96, DOB (Y-M-D): 1947-07-01 Age at Bx: 68
Biopsy Site ID	XPA96B1	
INTERCOM Study ID	XPA96	
Attending Physician	NA	
Date Reported (Y-M-D)	2015-04-28	
Date Received (Y-M-D)	2015-04-27	
Date of Transplant (Y-M-D)	2007-01-27	
Date of Biopsy (Y-M-D)	2015-04-21	

Clinical Information

Time of Biopsy Post-Tx	8.2 years	Biopsy Indication	follow-up from previous biopsy (ABMR)
Primary Disease	Etiology uncertain	Transplant Type	Deceased-heart beating
GFR (CG) at Biopsy	20	Tx before Biopsy	Yes (PP/IVIG/Ritux)
Proteinuria	Pos	DSA Status at Biopsy	DSA II positive
Haematuria		PRA Status at Biopsy	Unknown

Local Histopathology Phenotype: the Banff System

Inflammatory/TCMR Lesions		ABMR Lesions		Atrophy/Scarring		Other	
i	NA	ptc	NA	ci	NA	BK	NA
t	NA	g	NA	ct	NA		
v	NA	cg	NA	cv	NA		
total i	NA	C4d	NA	ah	NA		
		mm	NA				
Banff Diagnosis 1			NA	Banff Diagnosis 2			NA

Molecular Phenotype: the Edmonton Molecular Microscope System

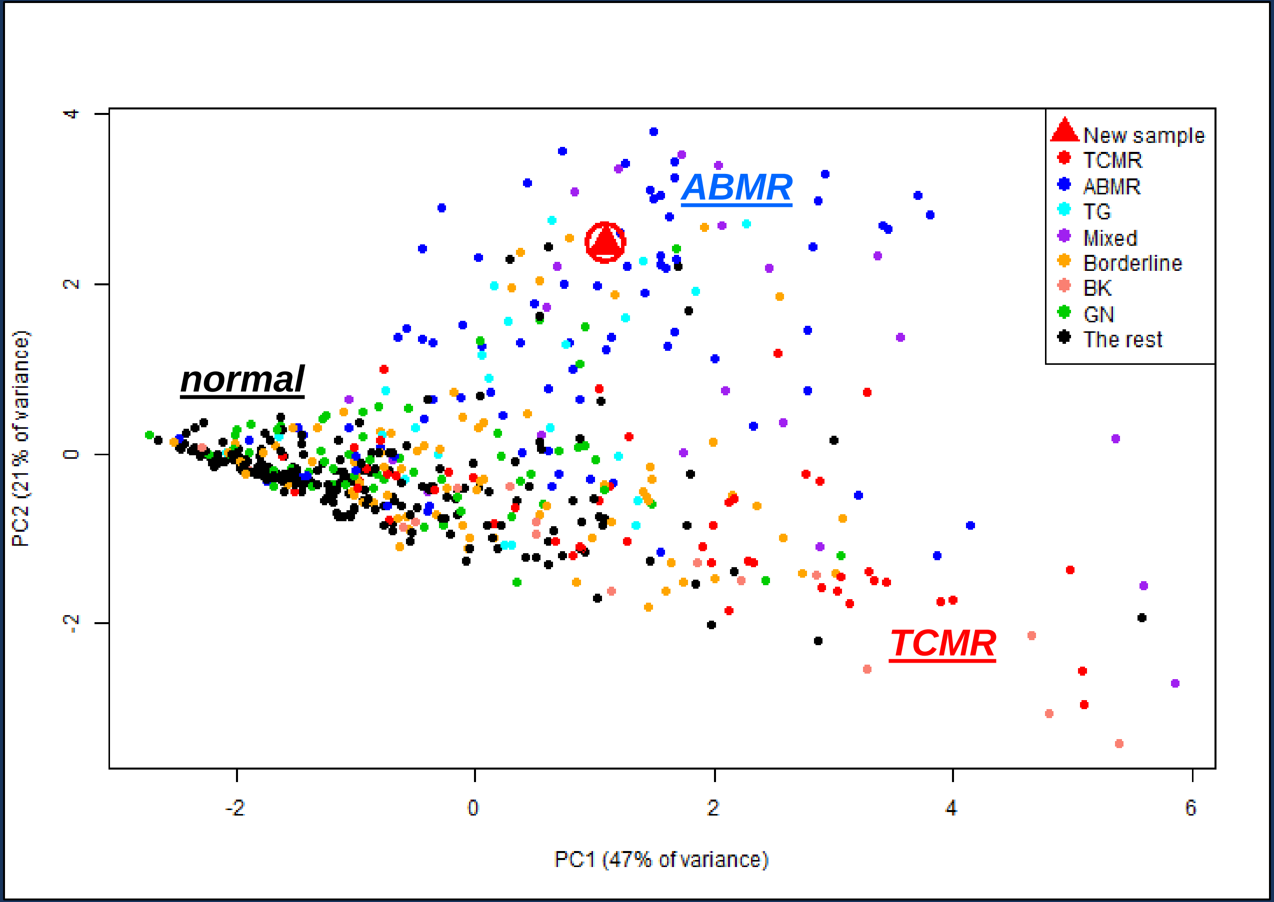
Classifier/PBT	Biopsy Score	Range in Reference Set	%ile compared to Reference Set	Interpretation
Global Disturbance Score	0.01	-5.6 -> 9.33	All:55.5 Late:55.6	Moderate
Acute kidney injury (AKI) Score	0.77	-0.92 -> 1.98	All:71.5 Late:78	High
Atrophy-Fibrosis Score	0.30	0.0 -> 1.0	All:54.2 Late:36.2	Moderate
Rejection Score	0.54	0.0 -> 1.0	All:79.4 Late:75.3	Moderate
TCMR Score	0.01	0.0 -> 1.0	All:49.3 Late:56.2	Low
ABMR Score	0.79	0.0 -> 1.0	All:95.1 Late:91.8	Very High

Pure molecular interpretation

Relatively severe ABMR, with ptc/g and cg features. No TCMR. Relatively mild atrophy-fibrosis with considerable AKI and inflammation, suggesting recent injury

Integrated interpretation incorporating histo-clinical variables can follow as data is available

The MMDx-Kidney report compares each new biopsy with solved biopsies in the reference set



Nearest neighbors (NN) analysis

MMDx-Kidney
The INTERCOMEX study

Reading kidney biopsy core portions (average 3mm) by their mRNA expression

Bottom line for INTERCOMEX (kidney biopsies MMDx-Kidney)

- Clinician feedback indicated that MMDx-kidney agreed with their clinical assessment significantly more frequently than did the local histology assessments.
- Clinicians frequently disagree with their own local histology assessments (20%).

"Real Time Assessment of Kidney Transplant Indication Biopsies by Microarrays: First Results of the INTERCOMEX Study"
manuscript in preparation

Mechanisms of rejection:

T cell-mediated rejection (TCMR)

Antibody-mediated rejection (ABMR)

TCMR landscape and classifier

J. M. Venner et al. Molecular landscape of T cell-mediated rejection in human kidney transplants: Prominence of CTLA4 and PD ligands. *Am J Transplant* 14 (11):2565-2576, 2014.

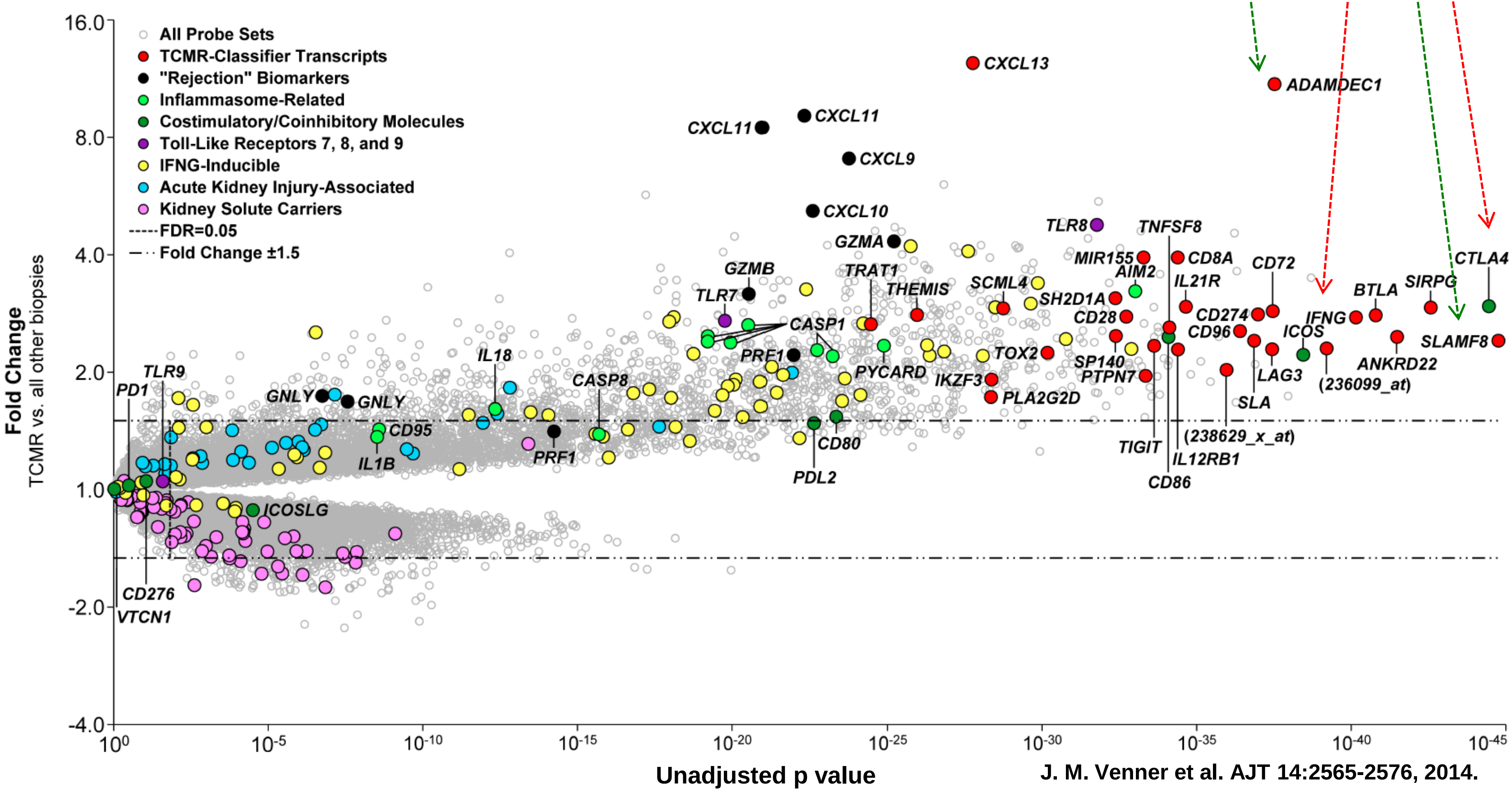
TCMR landscape

top TCMR transcripts (red) in relationship to IFNG effects, the inflammasome, and parenchymal injury and dedifferentiation changes

Top 30 TCMR transcripts

e.g. Activated effector T cells: IFNG, CTLA4

APCs: ADAMDEC1, SLAMF8



J. M. Venner et al. AJT 14:2565-2576, 2014.

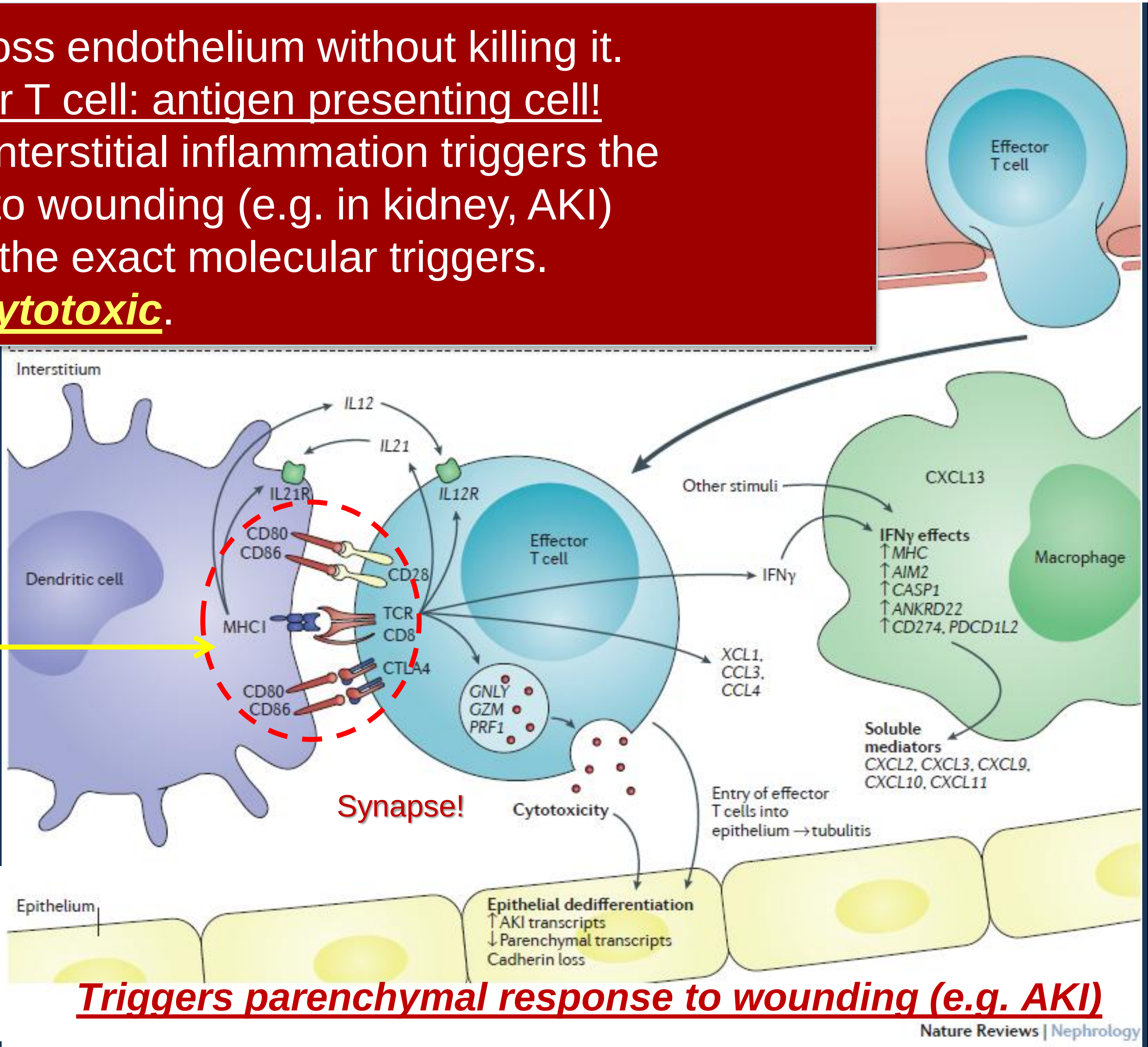
Strength of association with TCMR phenotype vs. Everything else

TCMR: Effector T cell crosses microcirculation to synapse with APC

(TCR-MHC) Dendritic cell? Macrophage?

- Effector T cell cross endothelium without killing it.
- Synapse: effector T cell: antigen presenting cell!
- T cell-mediated interstitial inflammation triggers the organ response to wounding (e.g. in kidney, AKI)
- We do not know the exact molecular triggers.
- ***Unlikely to be cytotoxic***.

Synapse!



Synapse!

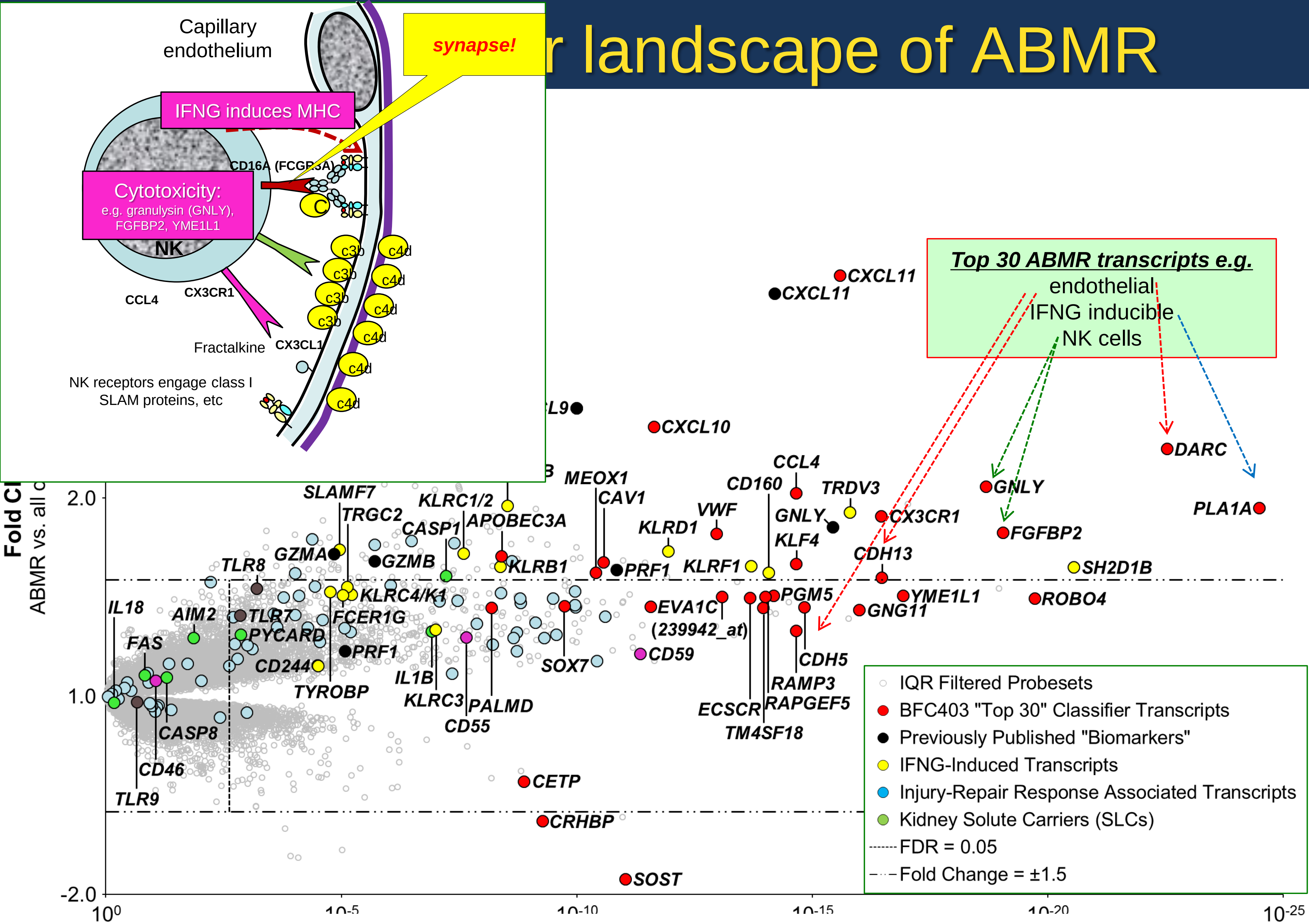
Triggers parenchymal response to wounding (e.g. AKI)

P. F. Halloran, K. S. Famulski, and J. Reeve. Molecular assessment of disease states in kidney transplant biopsies. *Nature Reviews Nephrology doi:10.1038/nrneph.2016.85. Published online 27 June 2016.:1-16, 2016.*

ABMR Landscape and Classifier

J. M. Venner, L. G. Hidalgo, K. S. Famulski, J. Chang, and P. F. Halloran. The molecular landscape of antibody-mediated kidney transplant rejection: Evidence for NK involvement through CD16a Fc receptors. *Am J Transplant* 15:1336, 2015

r landscape of ABMR



Top 30 ABMR transcripts e.g.
endothelial
IFNG inducible
NK cells

- ○ IQR Filtered Probesets
- ● BFC403 "Top 30" Classifier Transcripts
- ● Previously Published "Biomarkers"
- ● IFNG-Induced Transcripts
- ● Injury-Repair Response Associated Transcripts
- ● Kidney Solute Carriers (SLCs)
- ------- FDR = 0.05
- –·–·– Fold Change = ±1.5

Strength of association with ABMR phenotype vs. Everything else

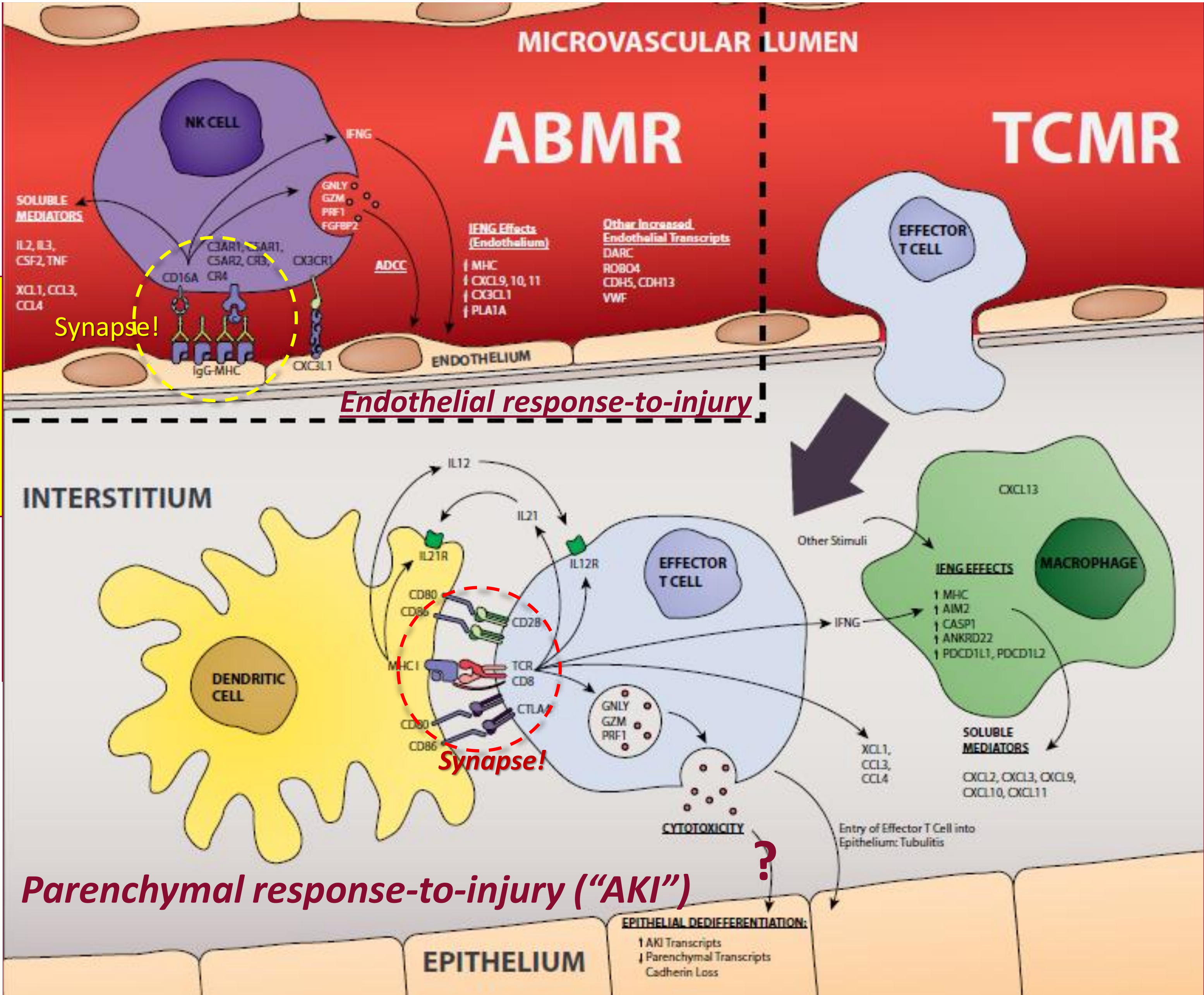
Parallels between antigen-specific events in TCMR and ABMR: The synapse and its consequences

ABMR

CD16-Activated NK cell is very similar to TCR-activated effector T cell

TCMR

P. F. Halloran, K. S. Famulski, and J. Reeve. Molecular assessment of disease states in kidney transplant biopsies. *Nature Reviews Nephrology doi:10.1038/nrneph.2016.85. Published online 27 June 2016.:1-16, 2016.*



Synapse!

Endothelial response-to-injury

Synapse!

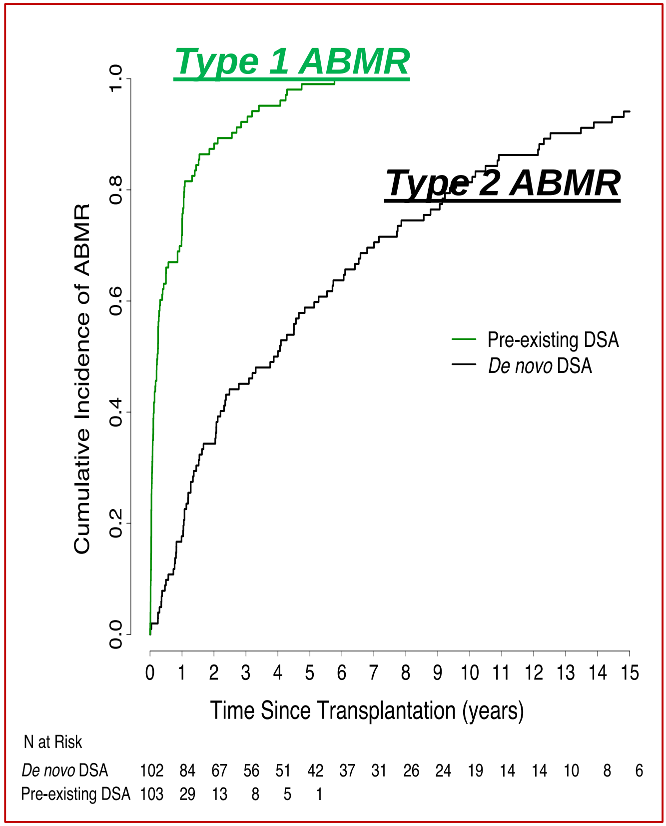
?

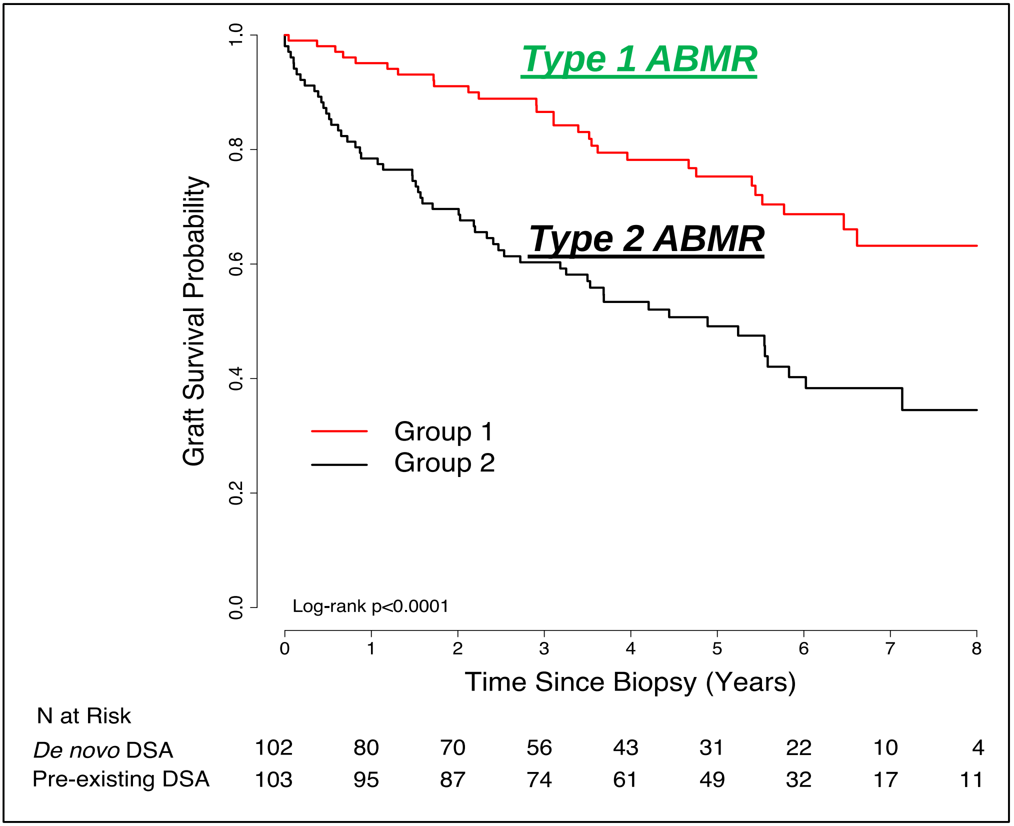
Parenchymal response-to-injury ("AKI")

Olivier Aubert et al JASN in press 2017

ABMR type 1: pre-existing donor-specific antibody

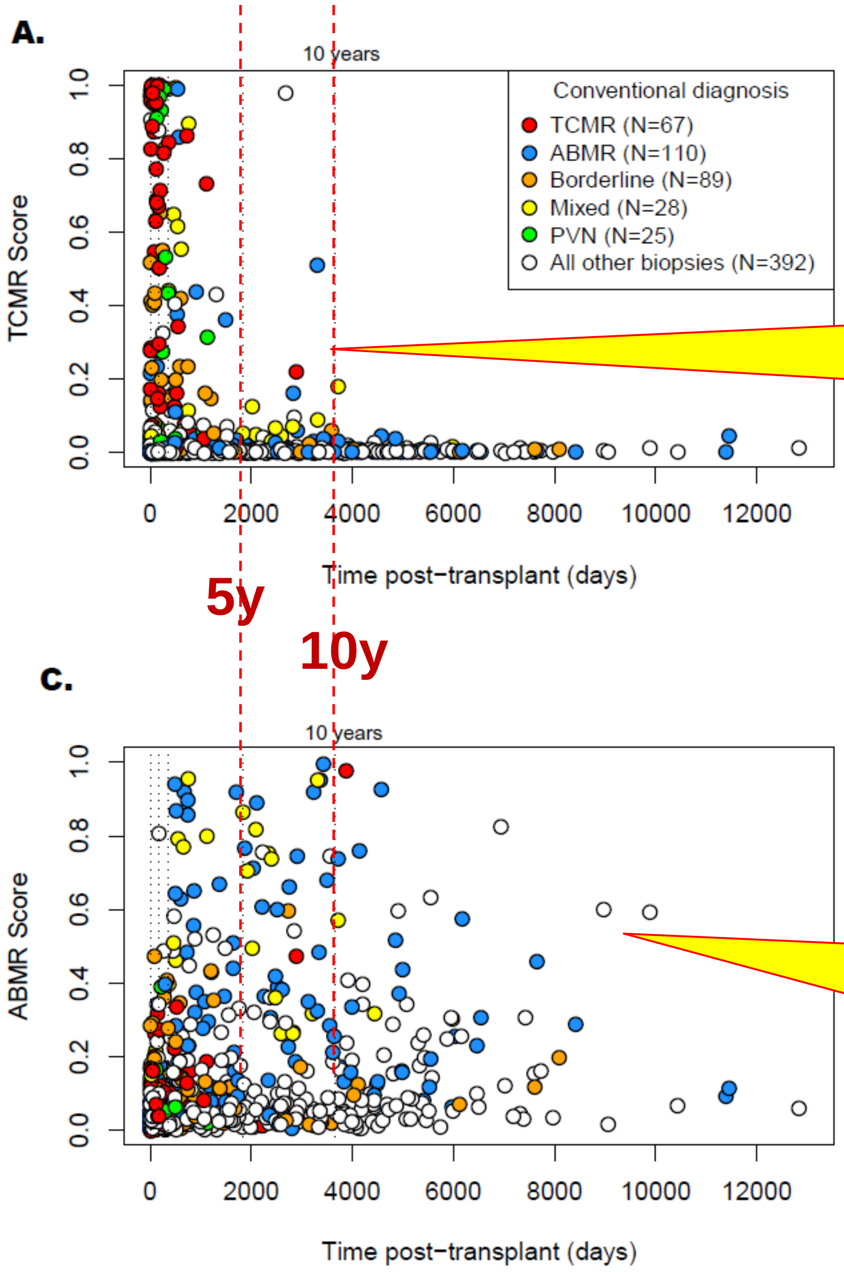
ABMR type 2: new onset donor specific antibody





These studies of the natural history of ABMR are essential for planning new clinical trials of therapy

P. F. Halloran et al. Disappearance of T cell-mediated rejection despite continued antibody-mediated rejection in late kidney transplant recipients. *JASN Published online 2015 doi: 10.1681/ASN.2014060588, 2015*



TCMR becomes rare after 5 years post transplant, and virtually never occurs after 10 years post transplant

Why?

In patients transplanted with no DSA, ABMR cases begin to present about one year post transplant and continue to present even 30 years post transplant

Why does TCMR stop appearing in unselected indication biopsies?
Mechanisms of adaptive T cell tolerance

Clonal deletion/abortion

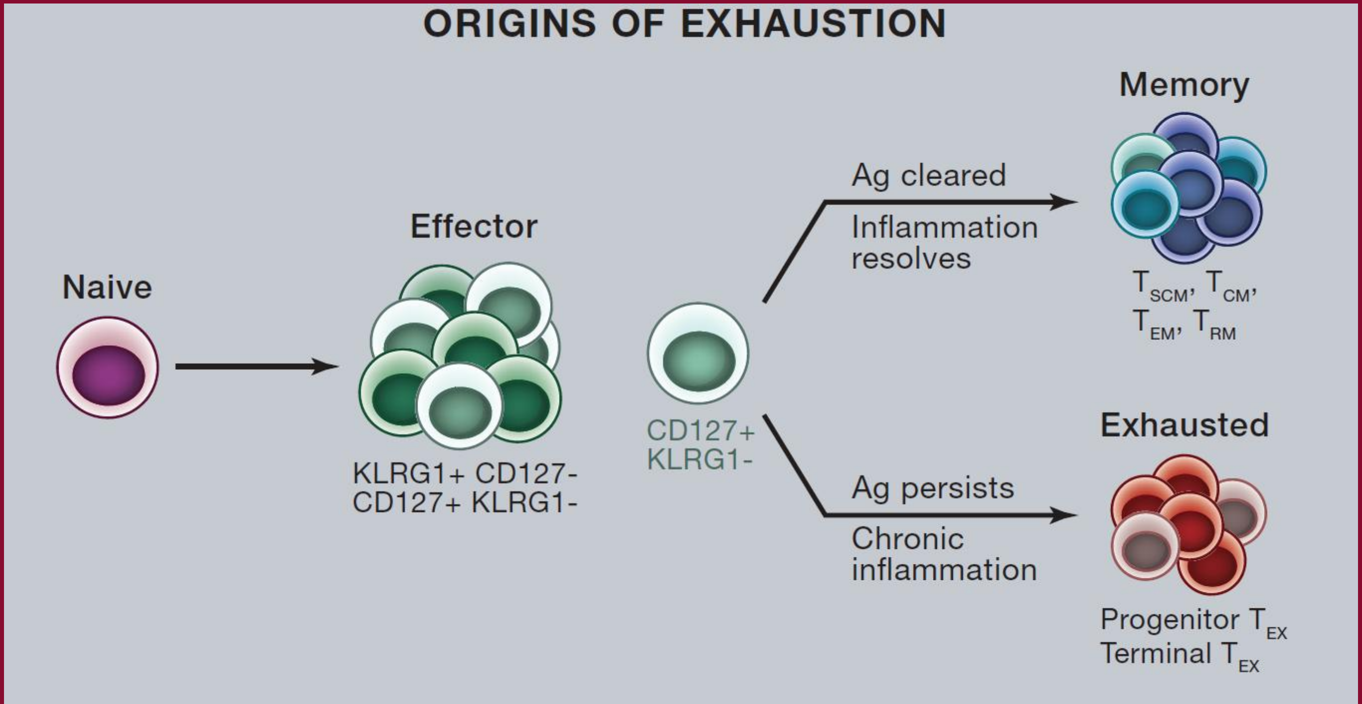
Clonal anergy

T regulatory cells

T cell exhaustion

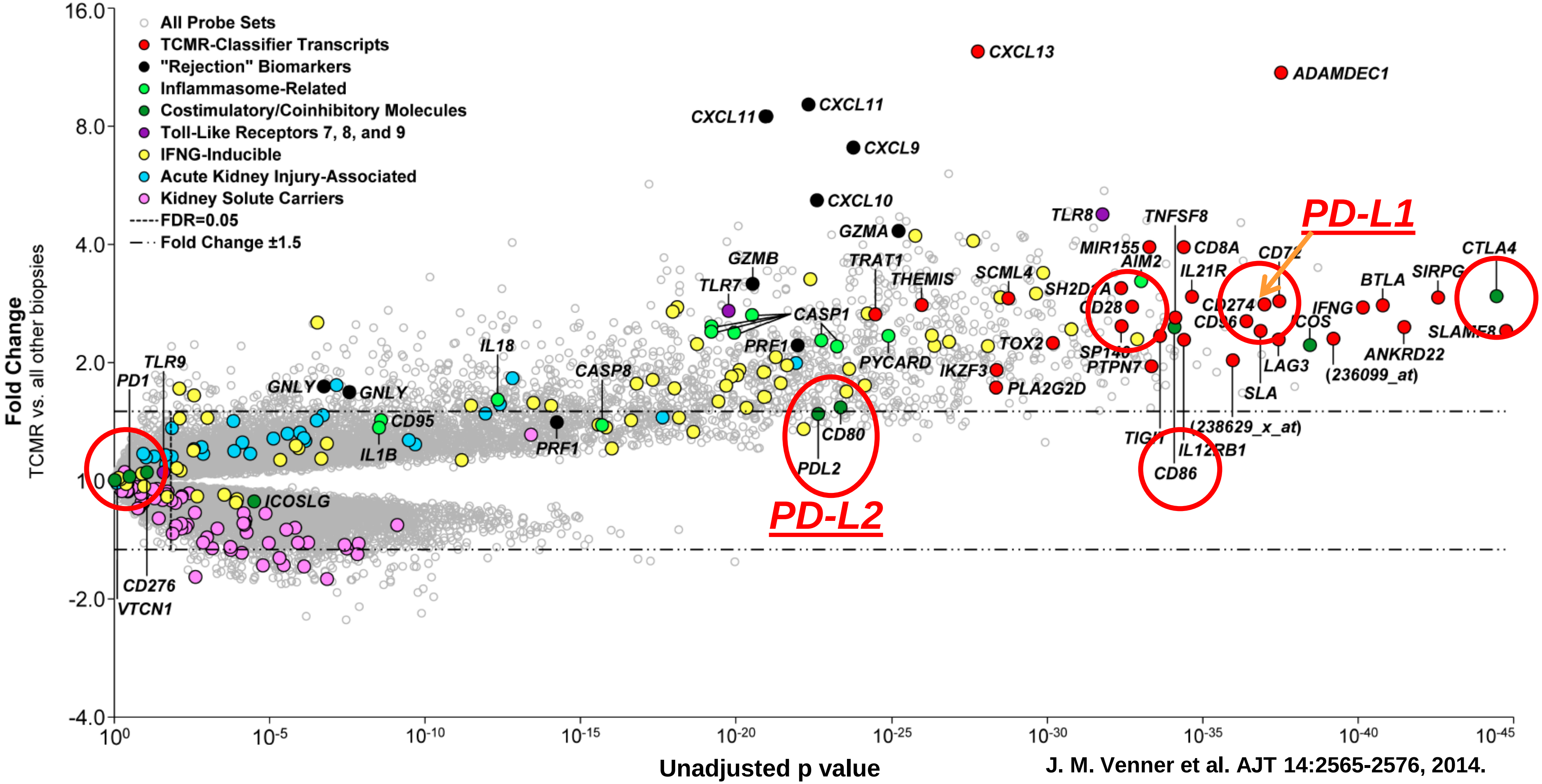
Immune checkpoints: PD1, CTLA4

Why does TCMR cease to occur?
Persistence of Ag leads to T cell exhaustion



K. E. Pauken and E. J. Wherry. SnapShot: T Cell Exhaustion. *Cell 163 (4):1038, 2015.*

TCMR expresses known checkpoints for T cell responses PD1-PD1LG/PD2LG, CTLA4-B7-1/B7-2, ICOS-ICOSLG



J. M. Venner et al. AJT 14:2565-2576, 2014.

Strength of association with TCMR phenotype vs. Everything else

Do all biopsies with TCMR need treatment?

Maybe some in protocol biopsies reflect exhausted T cells

What would happen if we treated a stable kidney transplant with checkpoint inhibitor anti PD1?

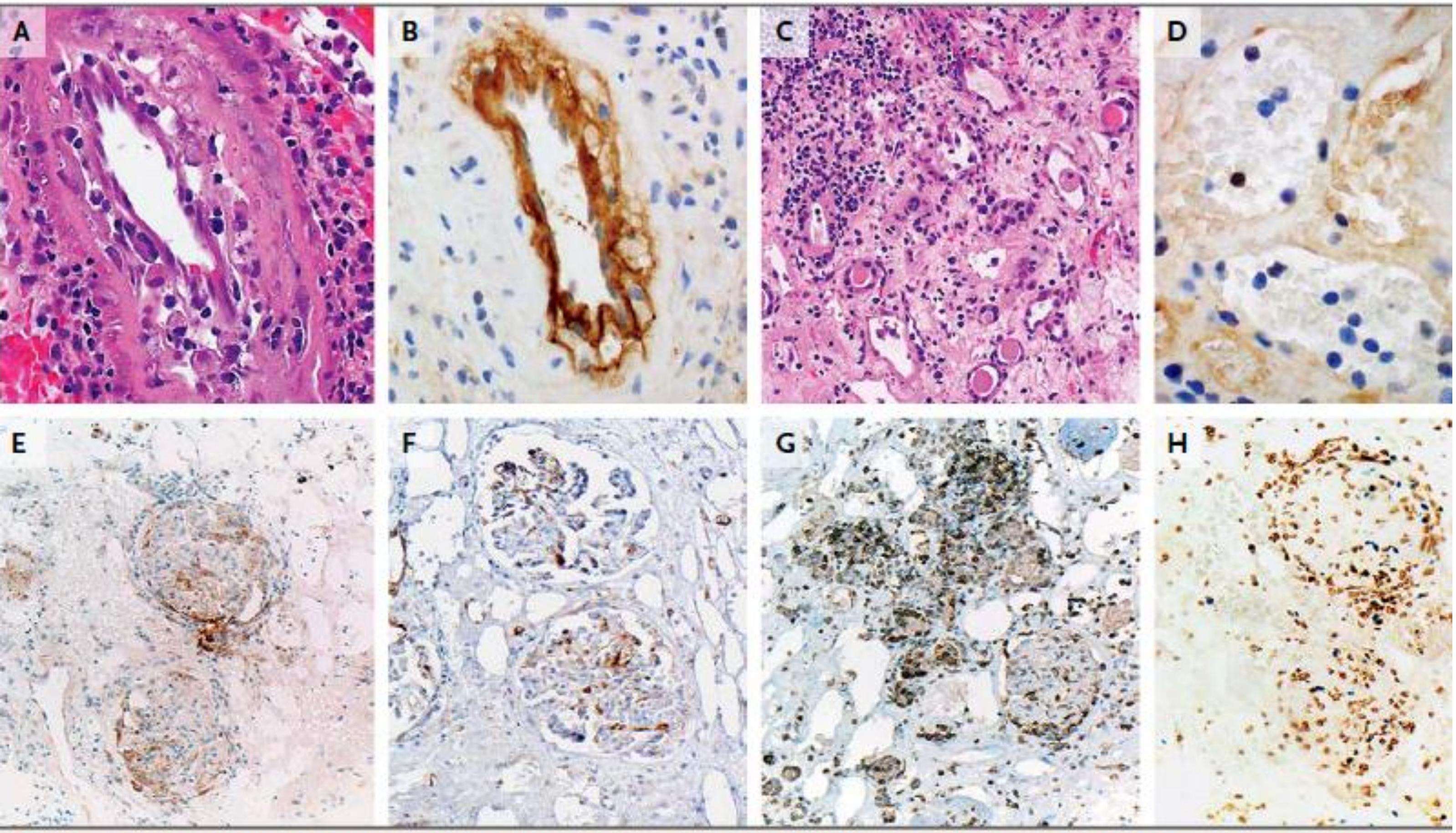


Figure 1. **Explanted Renal Allograft Showing Evidence of Rejection and Expression of PD-1 Pathway Molecules after Administration of Antibody.**

In Panel A (hematoxylin and eosin), arteries show intimal arteritis and focal intimal foam cells, findings consistent with chronic vascul thy in the allograft. In Panel B, strong immunostaining for C4d (a product of the classical complement pathway) is present in the a endothelium. Panel C (hematoxylin and eosin) shows glomerulitis, severe tubular loss, tubulitis, interstitial edema, and interstitial infl mation. In Panel D, peritubular capillaries show capillaritis but negative C4d immunostaining. In Panels E and F, immunostaining for grammed death 1 ligand 1 (PD-L1) and programmed death 1 ligand 2 (PD-L2), respectively, show that these molecules are present endothelial cells and infiltrating immune cells associated with glomeruli. In Panel G, infiltrating T cells expressing programmed de (PD-1) are associated with cells expressing PD-L1 and PD-L2. In Panel H, the infiltrating immune cells are predominantly CD8-posi and are coexpressed with Ki-67, findings consistent with an activated cytotoxic T-cell phenotype; shown are the results of double imm staining for CD8 (brown chromagen) and Ki-67 (blue chromagen).

Pure resistant TCMR induced by anti PD1 in kidney transplant stable for 28 years

E. J. Lipson, S. M. Bagnasco, J Moore Jr., S. Jang, M. J. Patel, A. A. Zachary, D. M. Pardoll, J. M. Taube, and C. G. Drake. Tumor Regression and Allograft Rejection after Administration of Anti–PD-1. *N Engl J Med 378 (9):896-898, 2016.*

The six rejection-related states

How many distinct rejection phenotypes are there in organ transplants?

Applying archetype analysis to kidney
1208 biopsies

Fig 2B

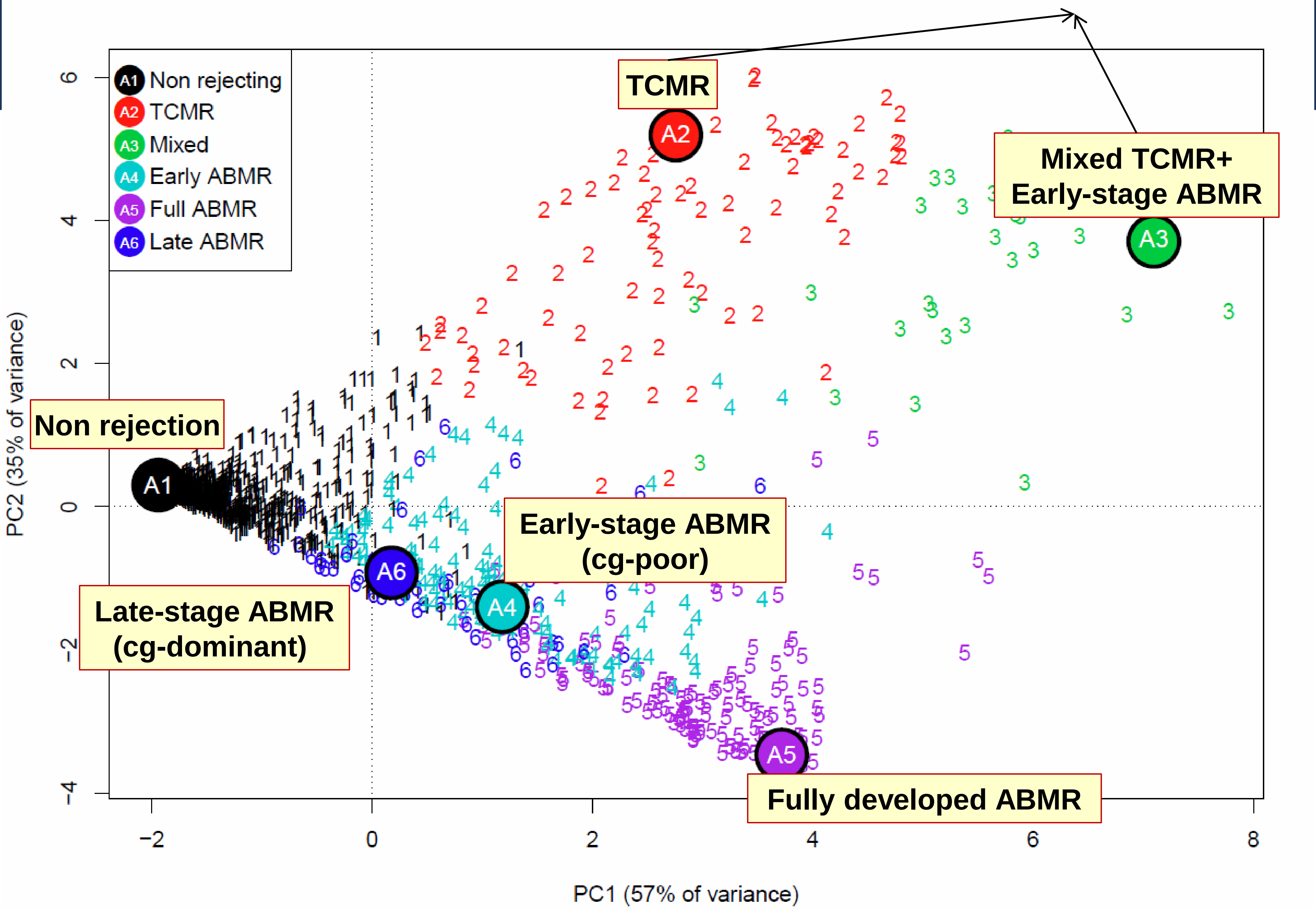
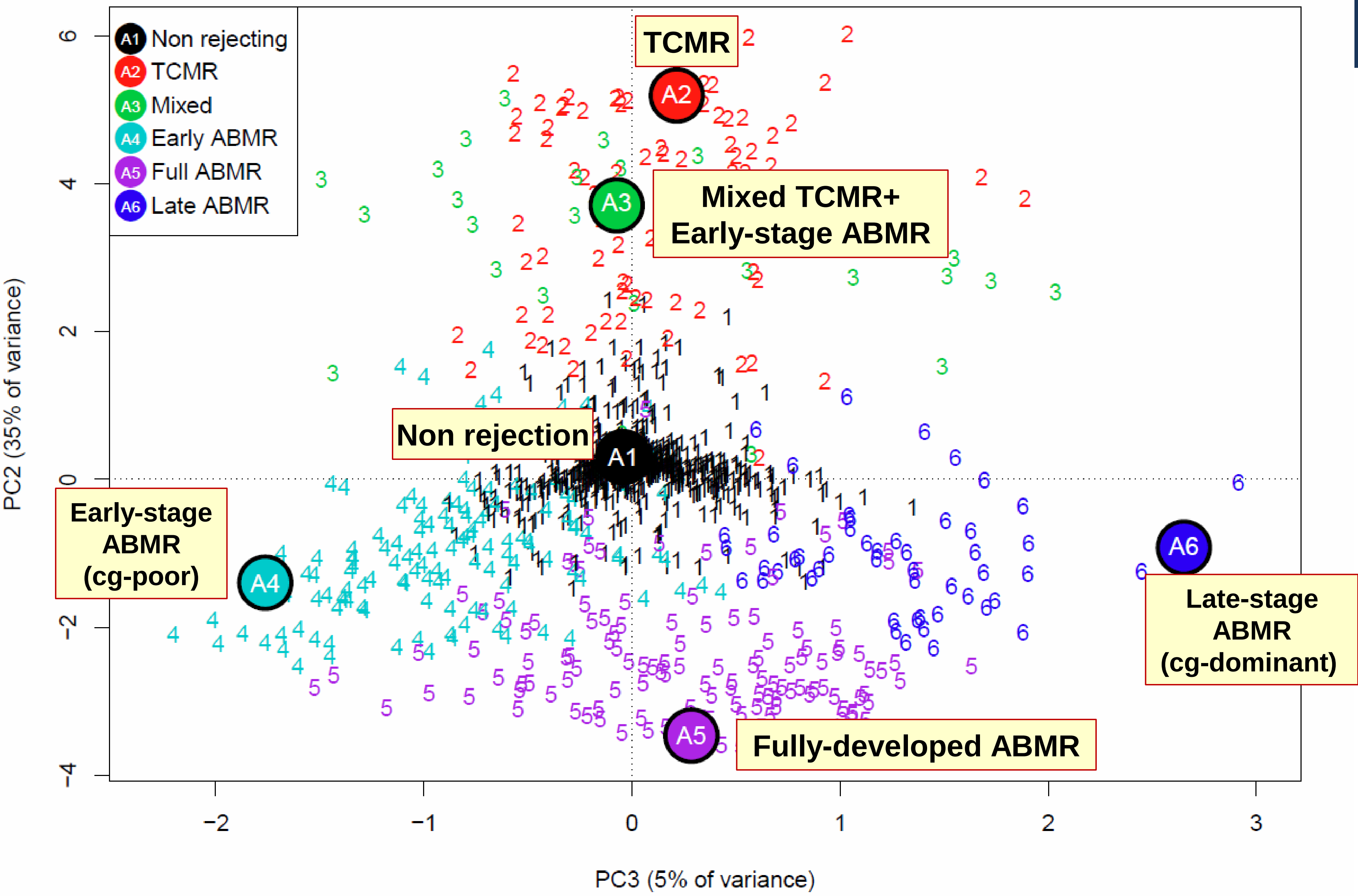
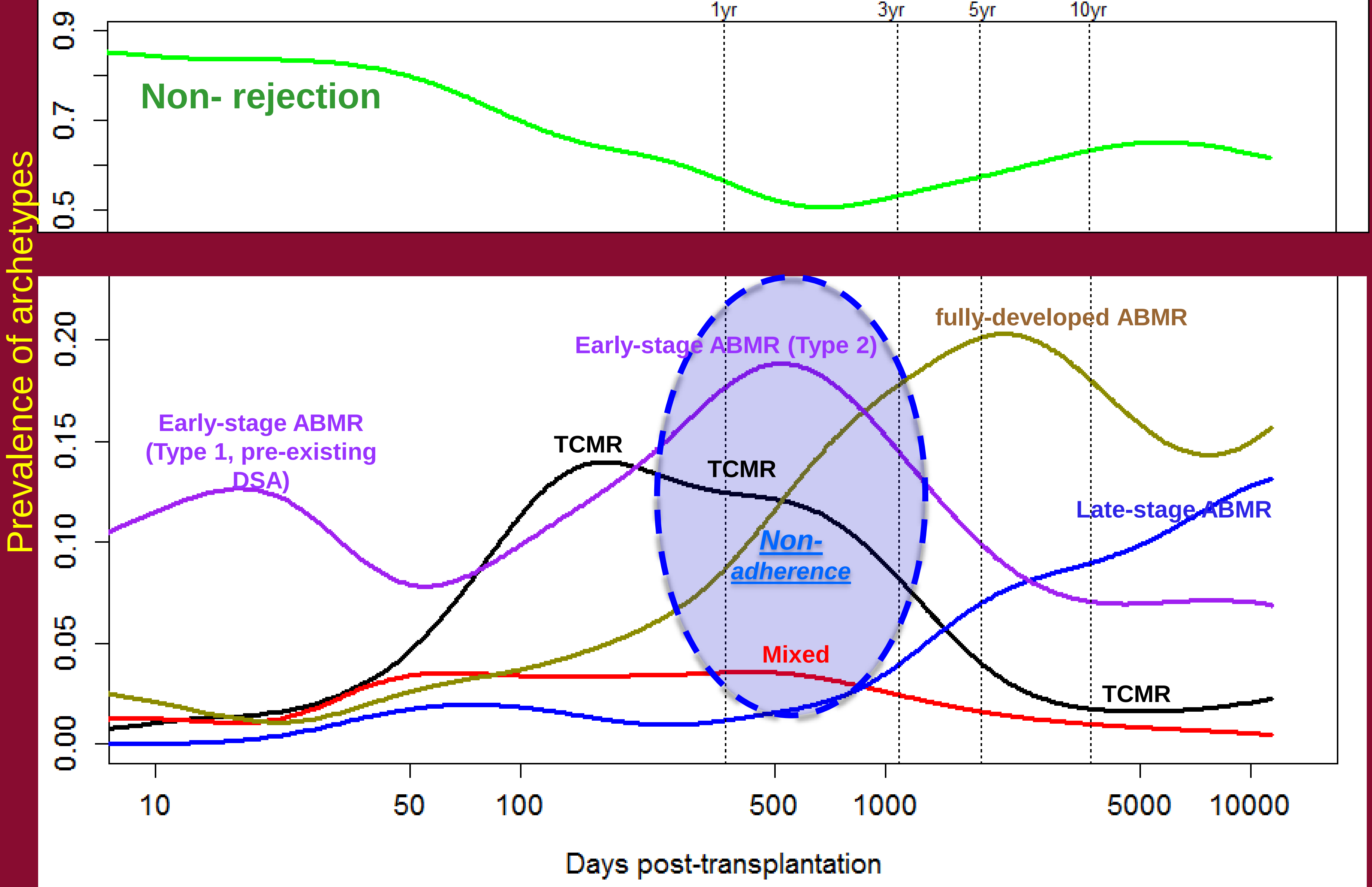

A1 Non rejecting
A2 TCMR
A3 Mixed
A4 Early ABMR
A5 Full ABMR
A6 Late ABMR
TCMR
Mixed TCMR+ Early-stage ABMR
Non rejection
Early-stage ABMR (cg-poor)
Late-stage ABMR (cg-dominant)
Fully developed ABMR
A1
A2
A3
A4
A5
A6
PC1 (57% of variance)
PC2 (35% of variance)
−2
0
2
4
6
8
−4
−2
0
2
4
6

Fig 2C

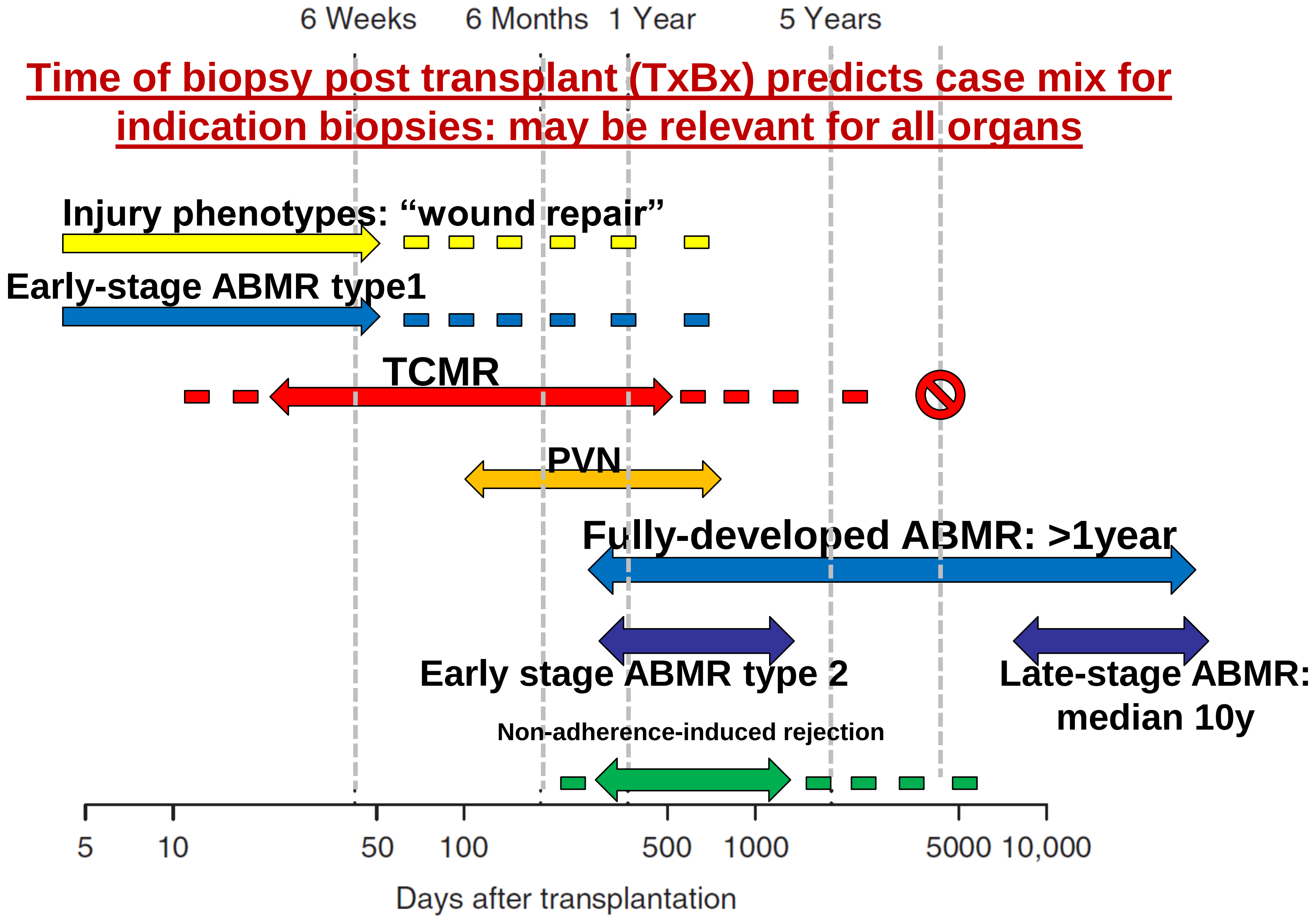

A1 Non rejecting
A2 TCMR
A3 Mixed
A4 Early ABMR
A5 Full ABMR
A6 Late ABMR
TCMR
Mixed TCMR+ Early-stage ABMR
Non rejection
Early-stage ABMR (cg-poor)
Late-stage ABMR (cg-dominant)
Fully-developed ABMR
A1
A2
A3
A4
A5
A6
PC2 (35% of variance)
PC3 (5% of variance)
6
4
2
0
−2
−4
−2
−1
0
1
2
3

Time course of rejection phenotypes in indication biopsies (archetypes)

Prevalence of the 6 archetypes over time post-Tx



Time of biopsy post transplant (TxBx) predicts case mix for indication biopsies: may be relevant for all organs



P. F. Halloran et al. Antibody-mediated rejection, T cell-mediated rejection, and the injury-repair response: new insights from the Genome Canada studies of kidney transplant biopsies. *Kidney Int (85):258-264, 2014.*

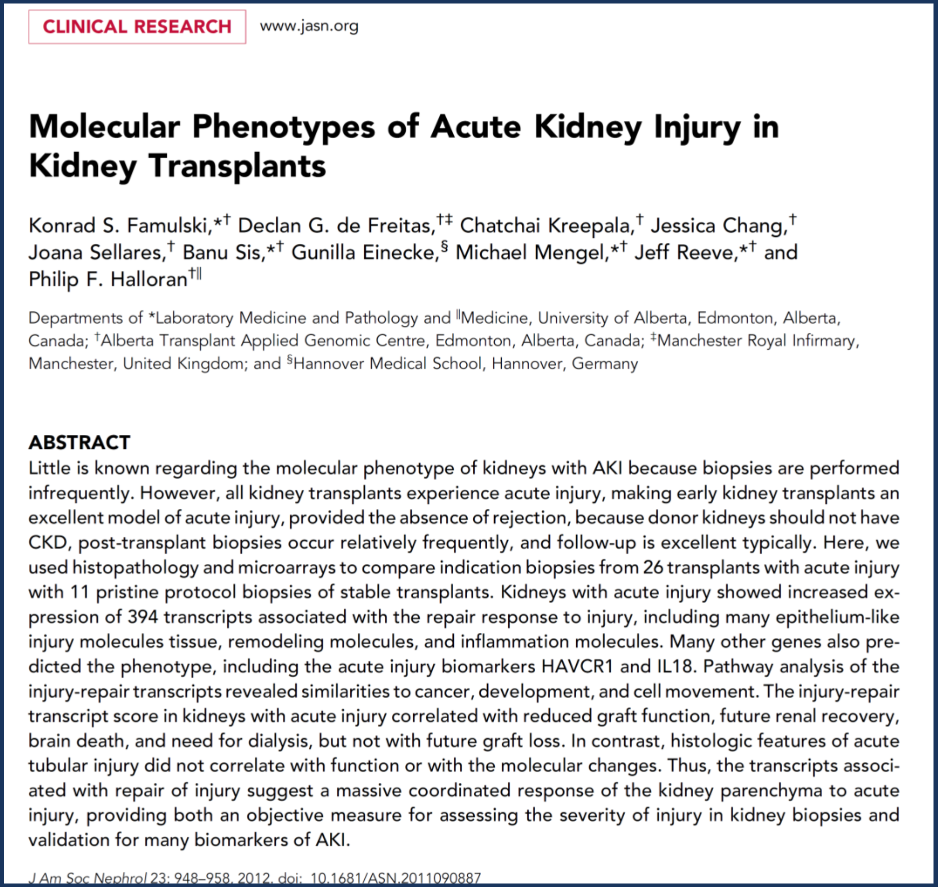
Injury, atrophy-fibrosis, progression

Lessons in kidney

(Lessons for heart, lung, liver?)

Molecules can assess acute injury: histology cannot

JASN 23:948, 2012



CLINICAL RESEARCH www.jasn.org

Molecular Phenotypes of Acute Kidney Injury in Kidney Transplants

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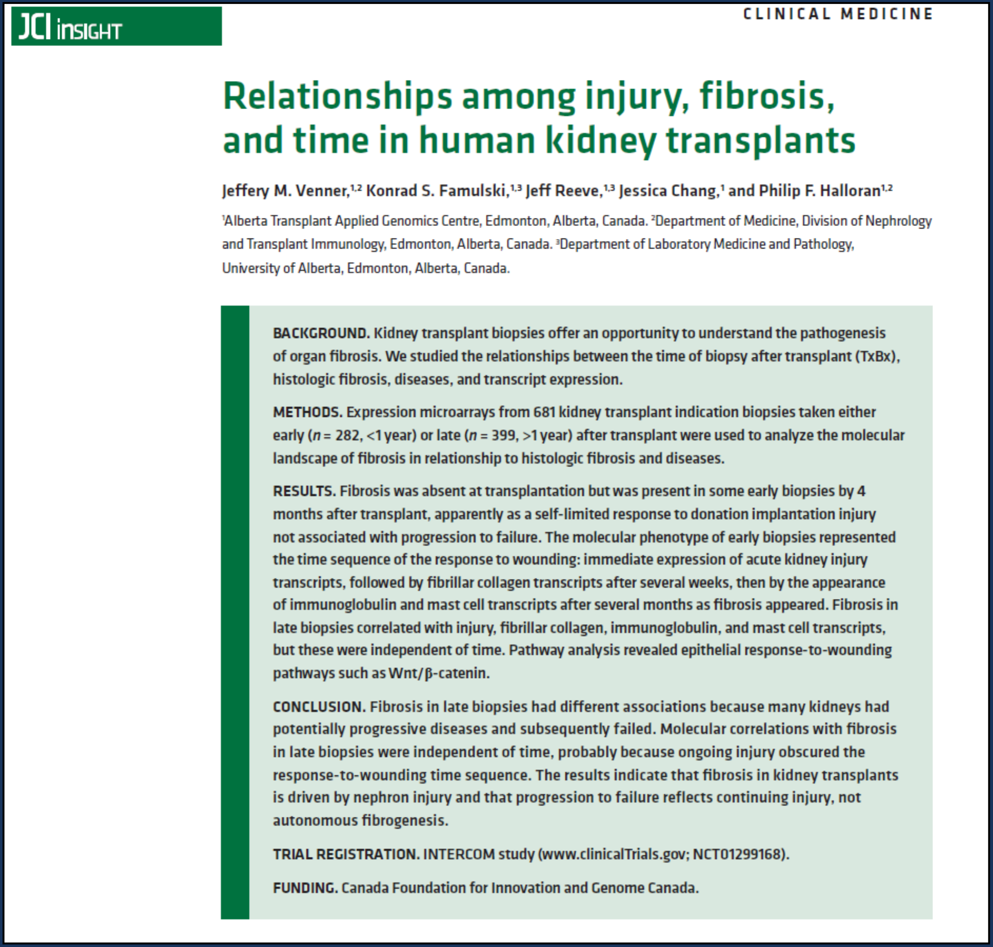
ABSTRACT

Little is known regarding the molecular phenotype of kidneys with AKI because biopsies are performed infrequently. However, all kidney transplants experience acute injury, making early kidney transplants an excellent model of acute injury, provided the absence of rejection, because donor kidneys should not have CKD, post-transplant biopsies occur relatively frequently, and follow-up is excellent typically. Here, we used histopathology and microarrays to compare indication biopsies from 26 transplants with acute injury with 11 pristine protocol biopsies of stable transplants. Kidneys with acute injury showed increased expression of 394 transcripts associated with the repair response to injury, including many epithelium-like injury molecules tissue, remodeling molecules, and inflammation molecules. Many other genes also predicted the phenotype, including the acute injury biomarkers HAVCR1 and IL18. Pathway analysis of the injury-repair transcripts revealed similarities to cancer, development, and cell movement. The injury-repair transcript score in kidneys with acute injury correlated with reduced graft function, future renal recovery, brain death, and need for dialysis, but not with future graft loss. In contrast, histologic features of acute tubular injury did not correlate with function or with the molecular changes. Thus, the transcripts associated with repair of injury suggest a massive coordinated response of the kidney parenchyma to acute injury, providing both an objective measure for assessing the severity of injury in kidney biopsies and validation for many biomarkers of AKI.

J Am Soc Nephrol 23: 948–958, 2012. doi: 10.1681/ASN.2011090887

JCI Insight 2016



JCI insight

CLINICAL MEDICINE

Relationships among injury, fibrosis, and time in human kidney transplants

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BACKGROUND. Kidney transplant biopsies offer an opportunity to understand the pathogenesis of organ fibrosis. We studied the relationships between the time of biopsy after transplant (TxBx), histologic fibrosis, diseases, and transcript expression.

METHODS. Expression microarrays from 681 kidney transplant indication biopsies taken either early ($n = 282$, <1 year) or late ($n = 399$, >1 year) after transplant were used to analyze the molecular landscape of fibrosis in relationship to histologic fibrosis and diseases.

RESULTS. Fibrosis was absent at transplantation but was present in some early biopsies by 4 months after transplant, apparently as a self-limited response to donation implantation injury not associated with progression to failure. The molecular phenotype of early biopsies represented the time sequence of the response to wounding: immediate expression of acute kidney injury transcripts, followed by fibrillar collagen transcripts after several weeks, then by the appearance of immunoglobulin and mast cell transcripts after several months as fibrosis appeared. Fibrosis in late biopsies correlated with injury, fibrillar collagen, immunoglobulin, and mast cell transcripts, but these were independent of time. Pathway analysis revealed epithelial response-to-wounding pathways such as Wnt/β-catenin.

CONCLUSION. Fibrosis in late biopsies had different associations because many kidneys had potentially progressive diseases and subsequently failed. Molecular correlations with fibrosis in late biopsies were independent of time, probably because ongoing injury obscured the response-to-wounding time sequence. The results indicate that fibrosis in kidney transplants is driven by nephron injury and that progression to failure reflects continuing injury, not autonomous fibrogenesis.

TRIAL REGISTRATION. INTERCOM study (www.clinicalTrials.gov; NCT01299168).

FUNDING. Canada Foundation for Innovation and Genome Canada.

Fibrosis does not cause failure: it reflects injury – “wounding”.

Injury at the time of transplantation causes development of some fibrosis but this is just wound repair and is not progressive.

Progression is due to ongoing injury from rejection (especially ABMR) and disease

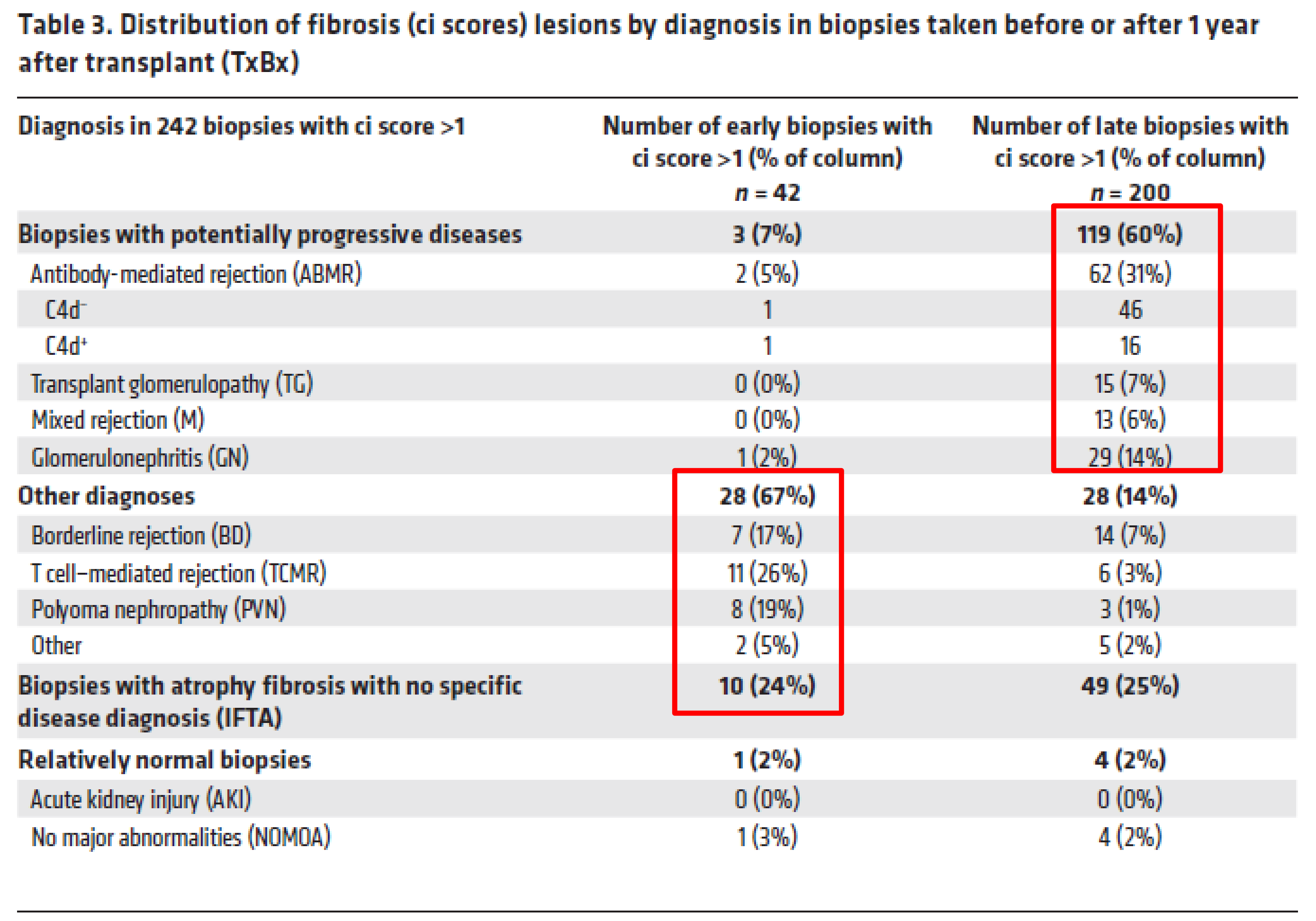
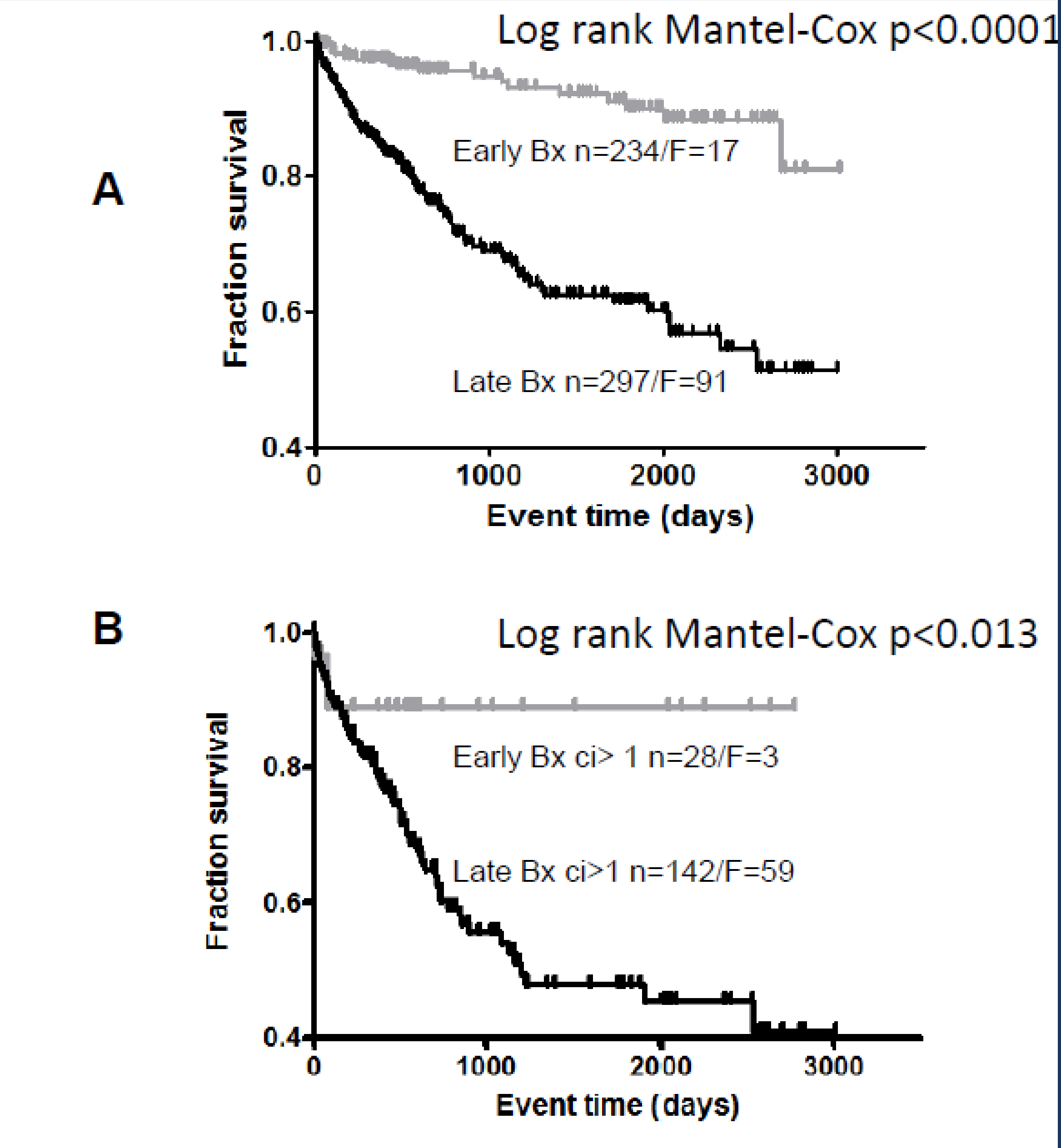


Table 3. Distribution of fibrosis (ci scores) lesions by diagnosis in biopsies taken before or after 1 year after transplant (TxBx)

Diagnosis in 242 biopsies with ci score >1	Number of early biopsies with ci score >1 (% of column) *n* = 42	Number of late biopsies with ci score >1 (% of column) *n* = 200
Biopsies with potentially progressive diseases	**3 (7%)**	**119 (60%)**
Antibody-mediated rejection (ABMR)	2 (5%)	62 (31%)
C4d$^-$	1	46
C4d$^+$	1	16
Transplant glomerulopathy (TG)	0 (0%)	15 (7%)
Mixed rejection (M)	0 (0%)	13 (6%)
Glomerulonephritis (GN)	1 (2%)	29 (14%)
Other diagnoses	**28 (67%)**	**28 (14%)**
Borderline rejection (BD)	7 (17%)	14 (7%)
T cell–mediated rejection (TCMR)	11 (26%)	6 (3%)
Polyoma nephropathy (PVN)	8 (19%)	3 (1%)
Other	2 (5%)	5 (2%)
Biopsies with atrophy fibrosis with no specific disease diagnosis (IFTA)	**10 (24%)**	**49 (25%)**
Relatively normal biopsies	**1 (2%)**	**4 (2%)**
Acute kidney injury (AKI)	0 (0%)	0 (0%)
No major abnormalities (NOMOA)	1 (3%)	4 (2%)

Among indication biopsies with atrophy-fibrosis (ci>1), early biopsies usually show non-progressive conditions; late biopsies show progressive diseases (ABMR, GN)

J. M. Venner, K. S. Famulski, J. Reeve, J. Chang, and P. F. Halloran. Relationships among injury, fibrosis, and time in human kidney transplants.
Journal of Clinical Investigation Insight 1 (1):e85323-doi:10.1172/jci.insight.85323., 2016.



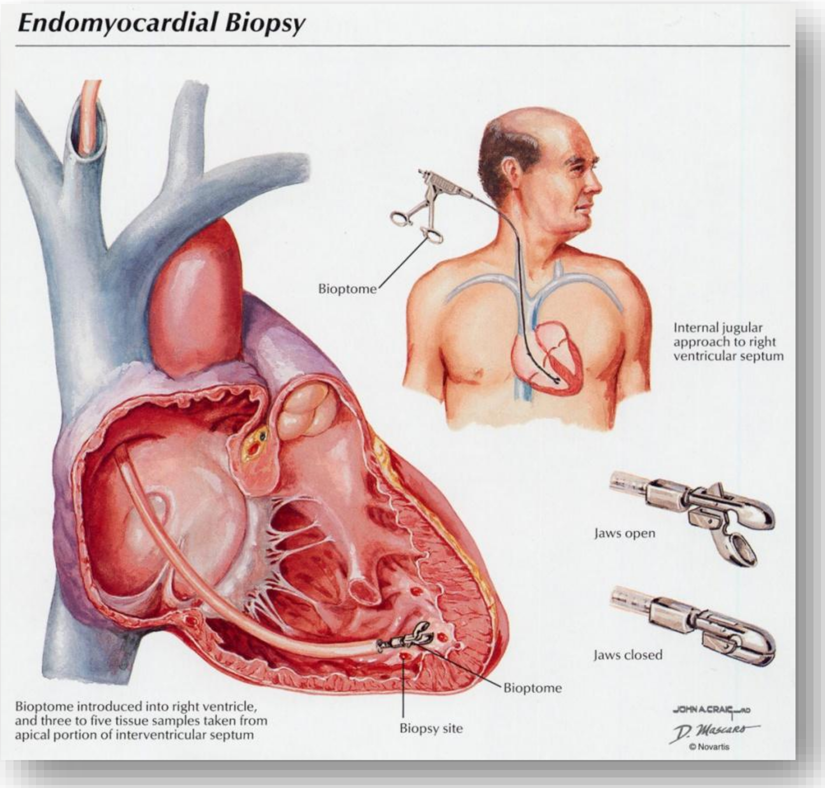
J. M. Venner, K. S. Famulski, J. Reeve, J. Chang, and P. F. Halloran. Relationships among injury, fibrosis, and time in human kidney transplants.
Journal of Clinical Investigation Insight 1 (1):e85323-doi:10.1172/jci.insight.85323., 2016.

MMDx for heart transplants

Using rejection-associated transcripts (RATs)

Endomyocardial biopsy

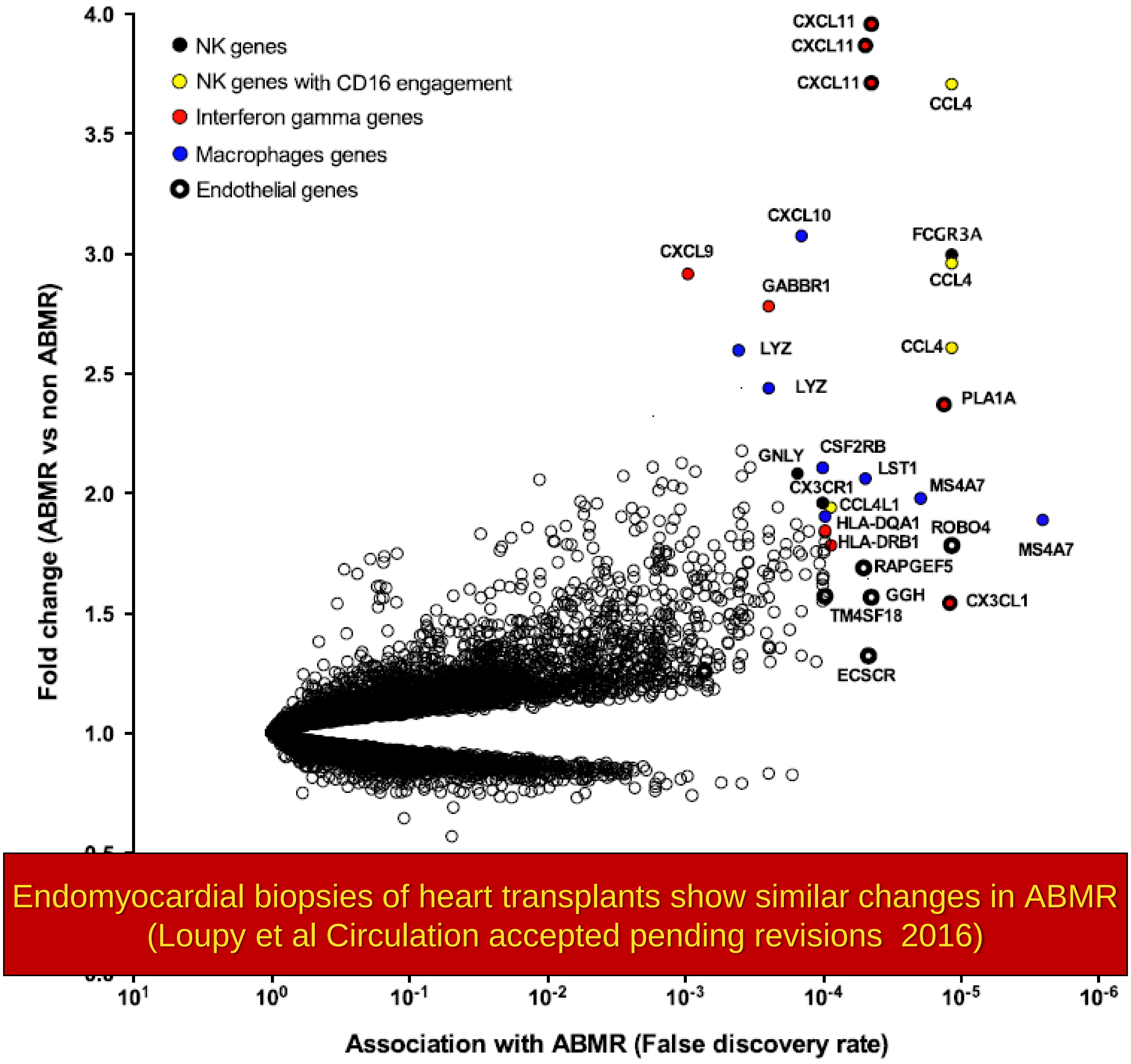
- Drs. Caves and Billingham developed the concept of scheduled endomyocardial biopsies to diagnose TCMR before the onset of graft dysfunction



JAMA. 1973 JUL 16;225(3):288-91;
LANCET. 1974 MAY 4;1(7862):821-6.

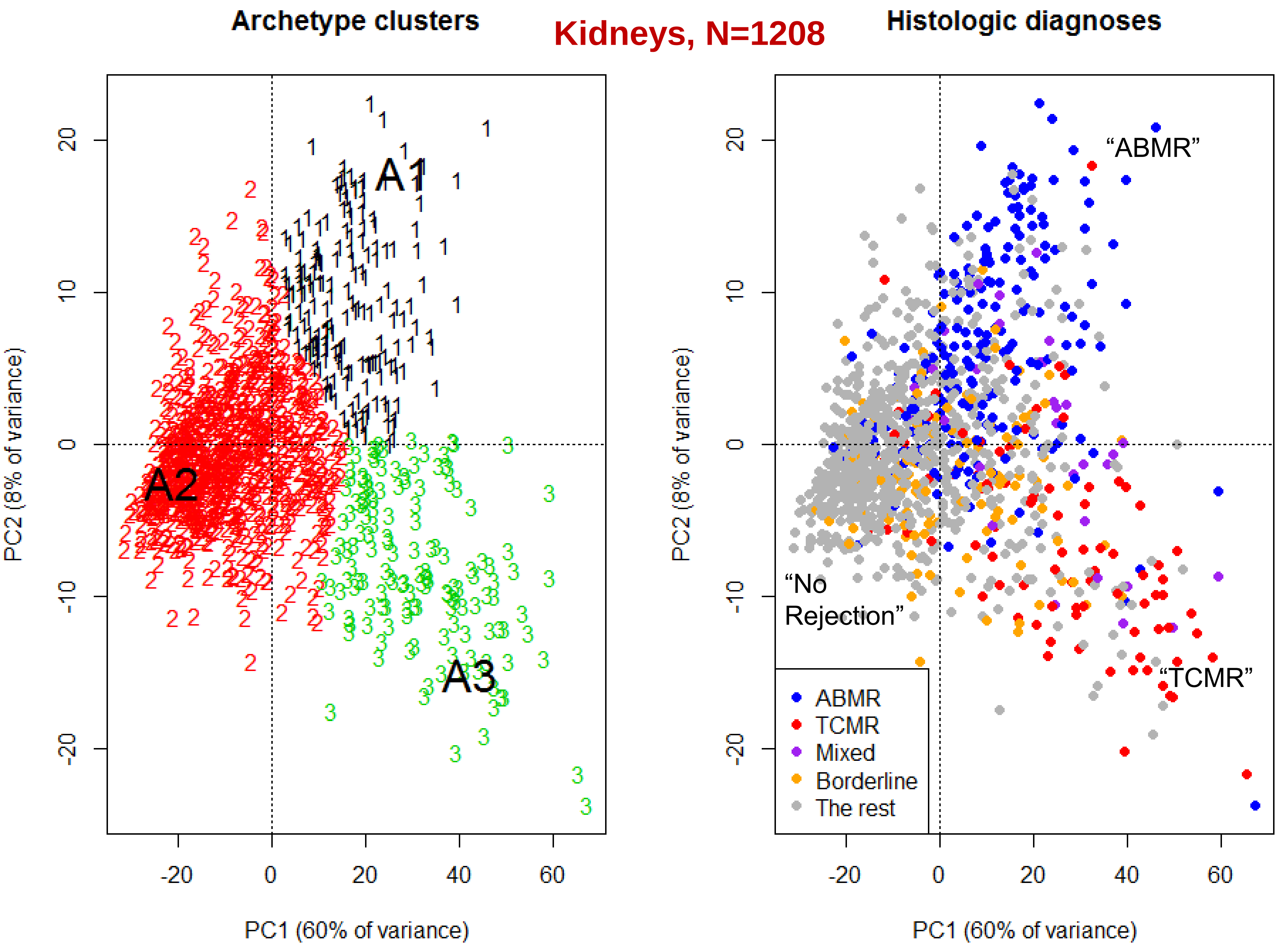
MMDx-Heart
The INTERHEART study

Reading human EMBs
by their mRNA expression

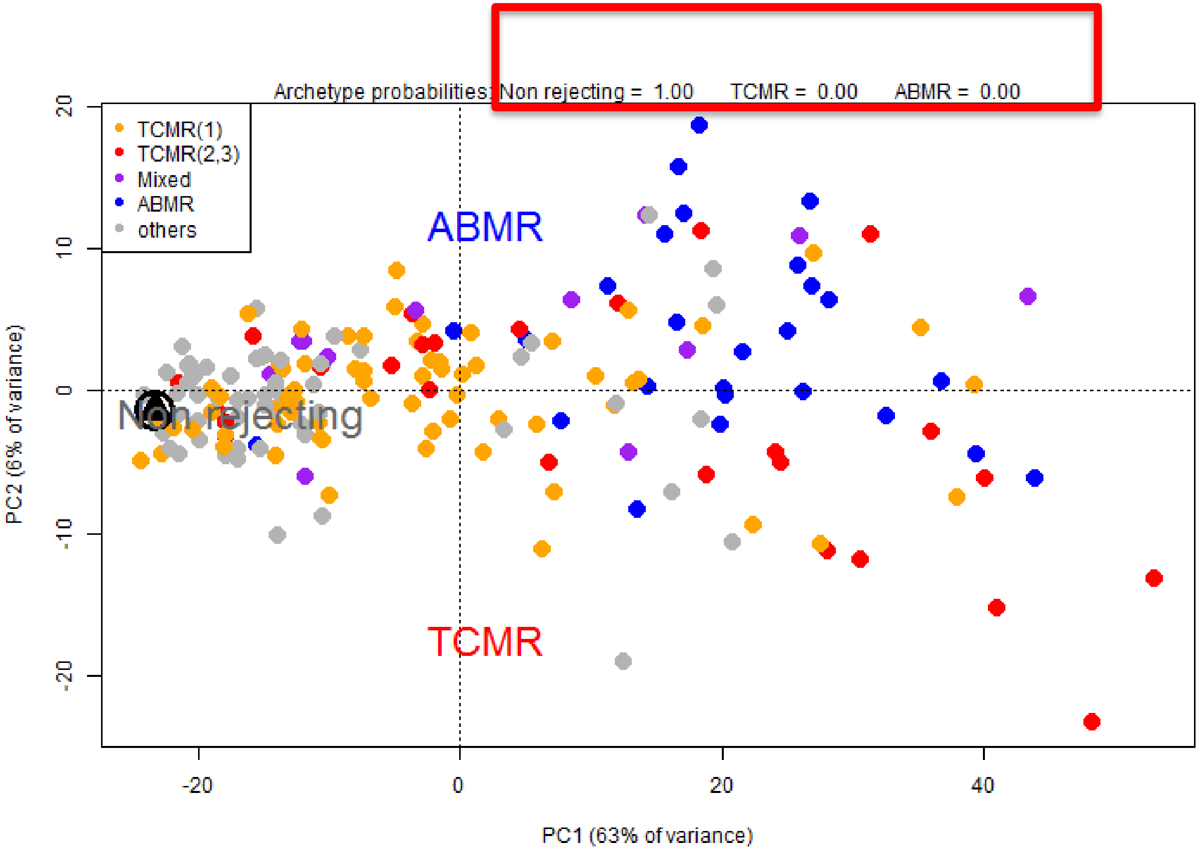


Endomyocardial biopsies of heart transplants show similar changes in ABMR
(Loupy et al Circulation accepted pending revisions 2016)

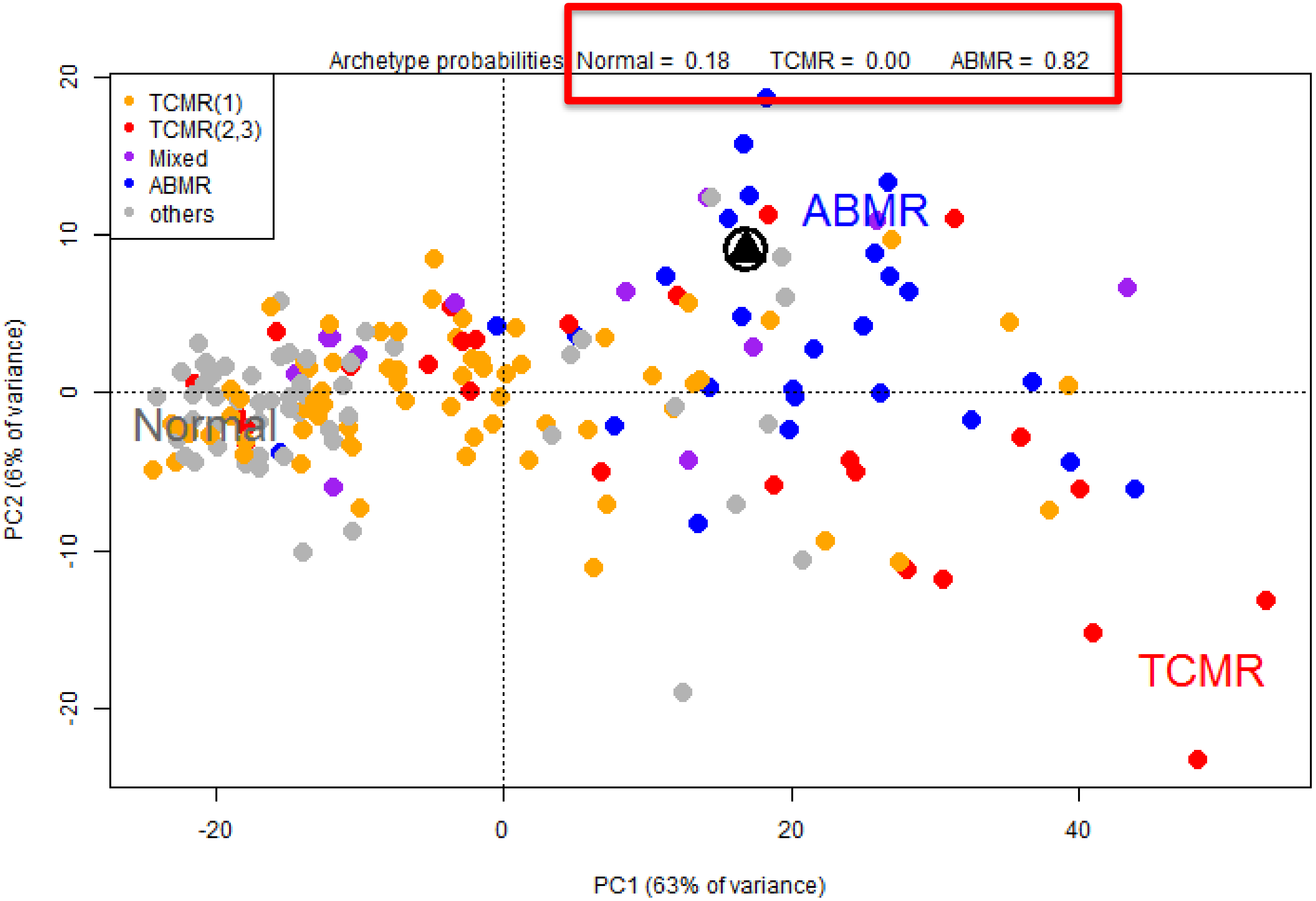
Kidneys, N=1208



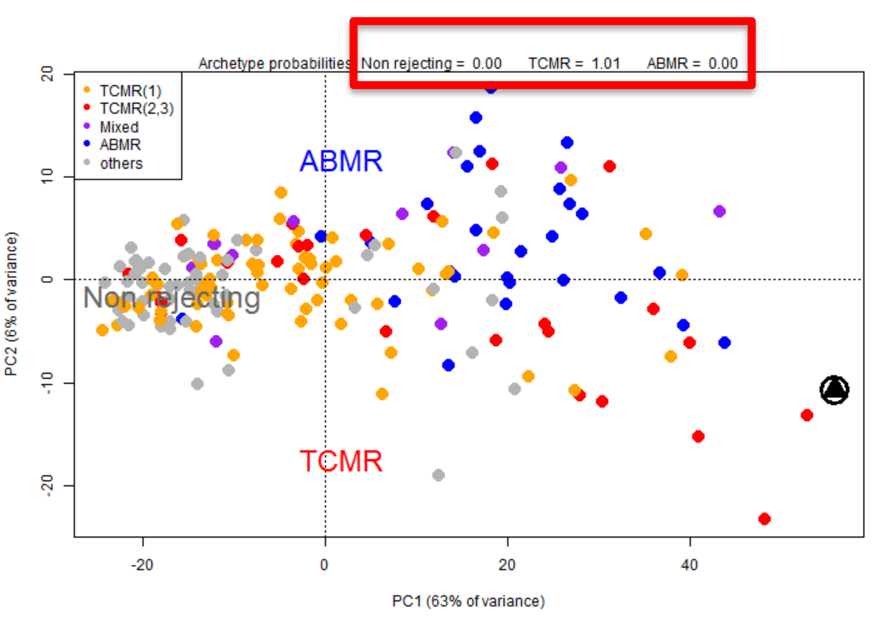
A normal heart transplant endomyocardial biopsy (EMB)



A heart transplant biopsy with probable antibody-mediated rejection

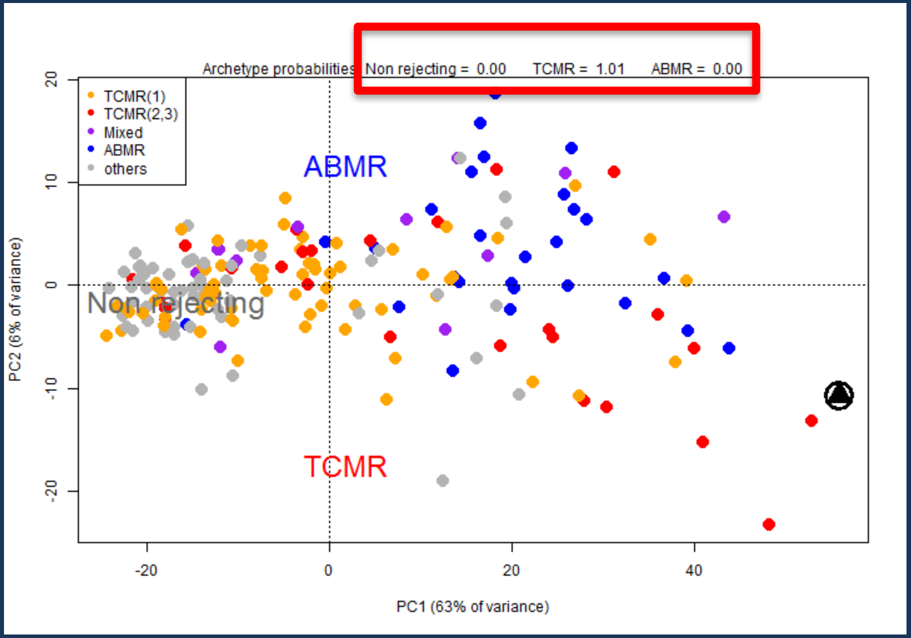


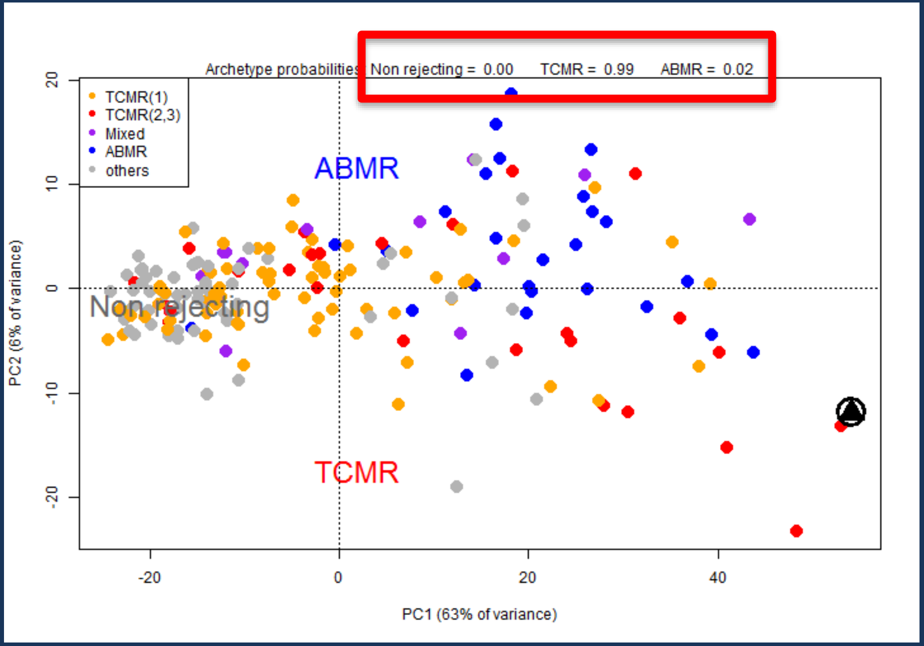
MMDx-heart report for an endomyocardial biopsy with TCMR



ATAGC

Reproducibility of MMDx in heart transplant EMB bites





EMB bite 1 vs. EMB bite 2

Preliminary insights from the INTERHEART study:

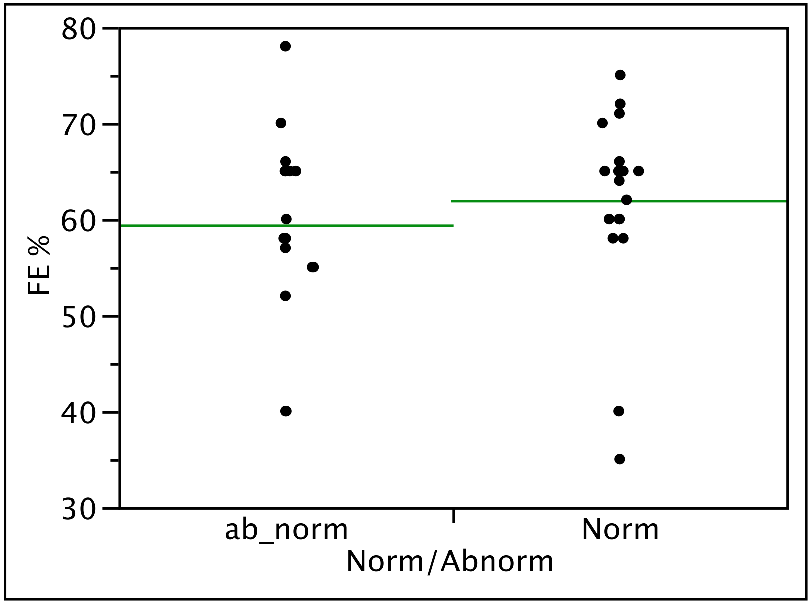
prospective validation and clinical correlates of the Molecular Microscope Diagnostic System in heart transplant biopsies.

Laura Borgese[1], Agnese Gaudenzi[1], Valentina Manfredini[1],
Ornella Leone[1], Valentina Agostini[1], Jeff Reeve[2], Marco Masetti[1],
Francesco Grigioni[1], Philip Halloran[2], Luciano Potena[1].

[1] Heart Failure and Heart Transplant Unit - S.Orsola-Malpighi Hospital- University of Bologna (Italy)
[2] ATAGC - University of Alberta (Canada)

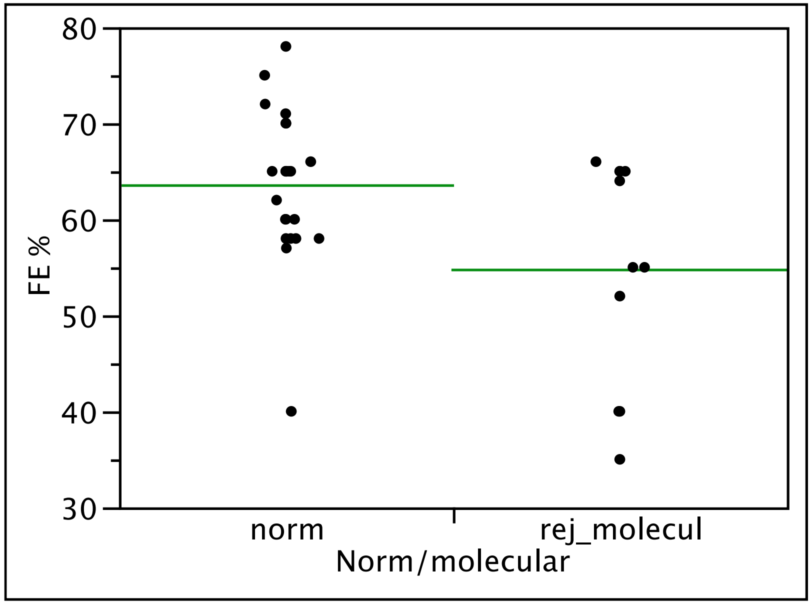
Ejection Fraction

Histology



P=0.4

Molecular microscope



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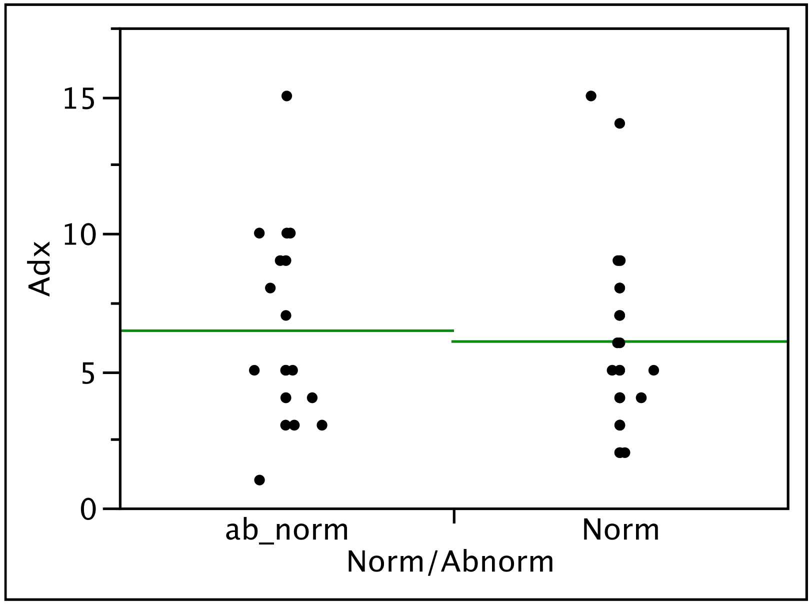
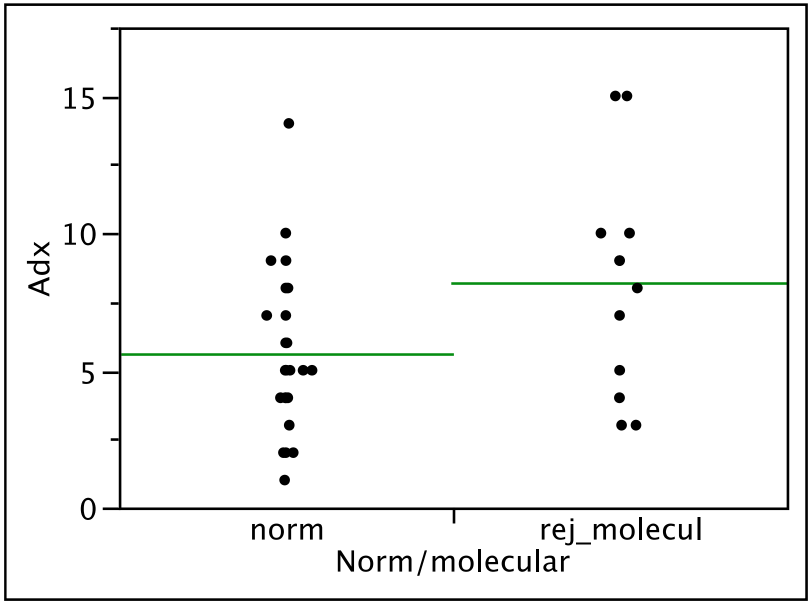
Right atrial pressure

Histology



P=0.7

Molecular microscope MMDx-Heart

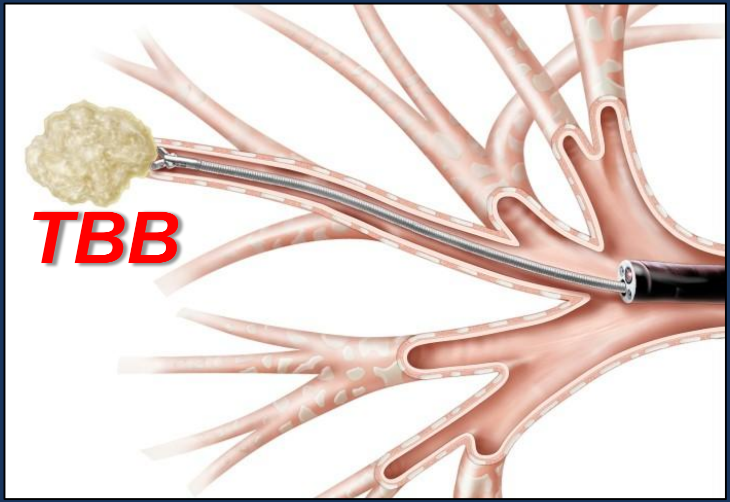


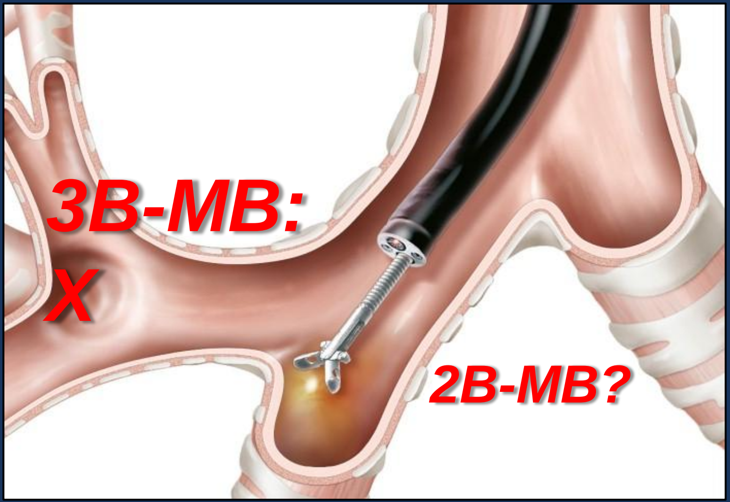
P = 0.03

MMDx®
for lung transplants
The INTERLUNG study

Using rejection-associated transcripts (RATs)

INTERLUNG Study





Transbronchial biopsy
High complications
Histology requires 10 bites
MMDx: 1-2 bites?

Mucosal biopsy

First generation TBB biopsy report (3B-MB to follow)

Although the reference set is small we can use kidney and heart experience to generate a report

FIGURE 1

Separation of lung rejection phenotypes based on kidney rejection-associated transcripts

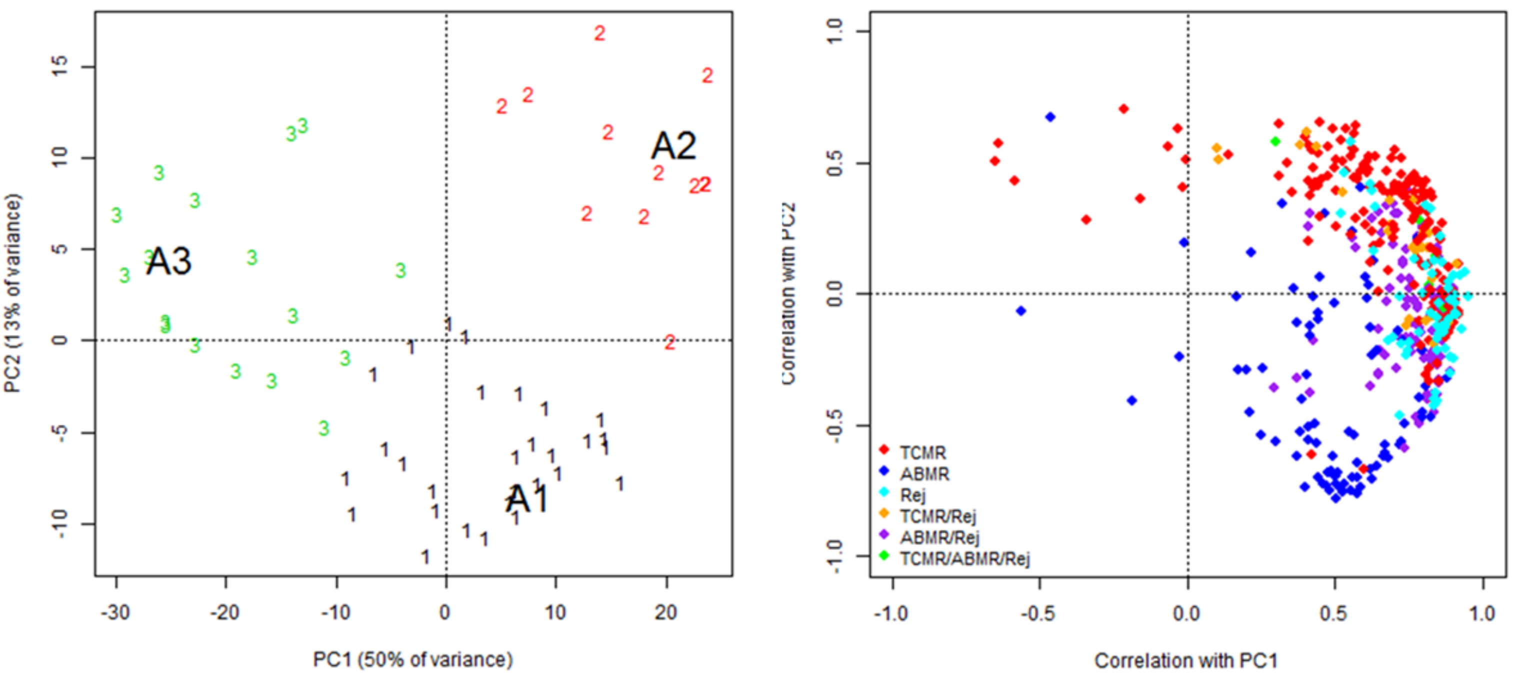
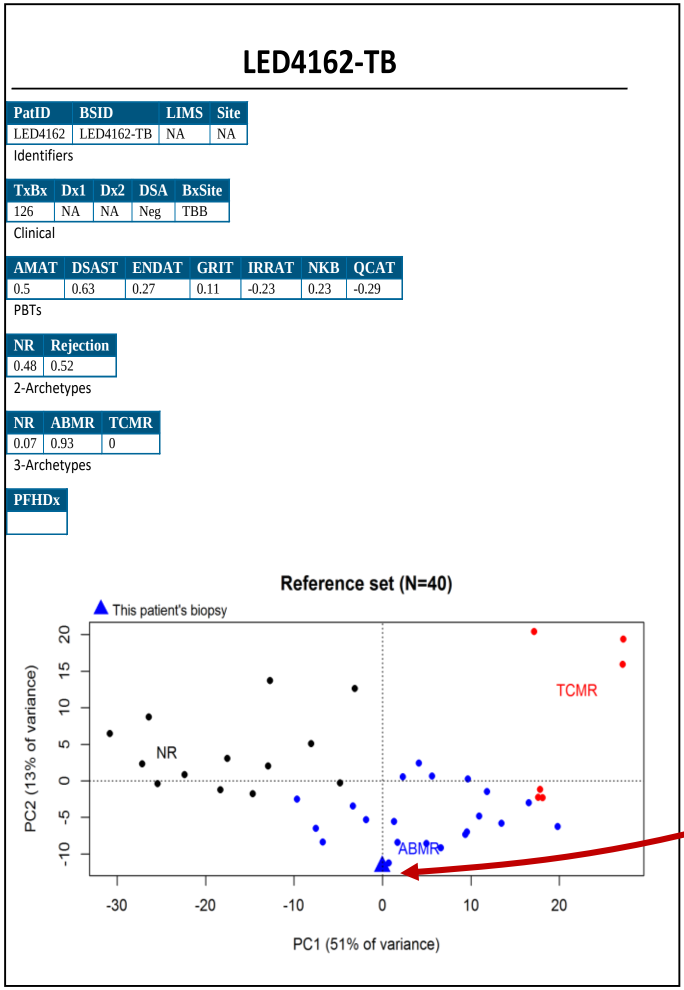


Figure 1. Left: Separation of transbronchial biopsies into molecular rejection phenotypes based on expression of 453 rejection-associated transcripts (RATs) derived from kidneys. A1 is ABMR, A2 is TCMR, and A3 is non-rejection. Right: correlation of the 453 RATs with rejection (PC1) and ABMR/TCMR (PC2) in N=58 transbronchial biopsies. RATs are colored by their associated kidney rejection phenotypes.

The first TBB biopsy reported in the MMDx-lung system



LED4162-TB

PatID	BSID	LIMS	Site
LED4162	LED4162-TB	NA	NA

Identifiers

TxBx	Dx1	Dx2	DSA	BxSite
126	NA	NA	Neg	TBB

Clinical

AMAT	DSAST	ENDAT	GRIT	IRRAT	NKB	QCAT
0.5	0.63	0.27	0.11	-0.23	0.23	-0.29

PBTs

NR	Rejection
0.48	0.52

2-Archetypes

NR	ABMR	TCMR
0.07	0.93	0

3-Archetypes

PFHDx

Reference set (N=40)

The blue triangle indicates that this biopsy has rejection genes suggesting ABMR-like changes

What we have covered:

- What is MMDx®?
- Mechanisms of rejection: TCMR, ABMR
- The six rejection-related states
- Injury, atrophy-fibrosis, progression
- MMDx® for heart transplants
- MMDx® for lung transplants
- MMDx® for liver transplants: beginning
- Plan: commercial availability in 2017

Thank you!