Calibration of Natera circulating donor-derived cfDNA against Molecular Microscope® Diagnostic System (MMDx) results in indication biopsies

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Molecular Microscope® Diagnostic System

https://www.molecular-microscope.com/

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

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MMDx

Relevant Financial Relationship Disclosure Statement

Presenter: Phil Halloran

The MMDx project is 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
  - Is a consultant to Natera

https://www.molecular-microscope.com/
http://transcriptome.com/
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MMDx

Contents

- Review MMDx
- First Trifecta study results (66 biopsies)

Unmet need

"…international variation in histologic grading is large, underrecognized, difficult to improve, and almost certainly of major clinical relevance. Urgent steps are needed to improve this area of practice."

P. N. Furness, N. Taub, K. J. Assmann, G. Banfi, J. P. Cosyns, A. M. Dorman, C. M. Hill, S. K. Kapper, R. Waldherr, A. Laurinavicius, N. Marcussen, A. P. Martins, M. Nogueira, H. Regele, D. Seron, M. Carrera, S. Sund, E. I. Taskinen, T. Paavonen, T. Tihomirova, and R. Rosenthal. Am J Surg Pathol. 27 (6):805-810, 2003.

MMDx

Unmet need

"…international variation in histologic grading is large, underrecognized, difficult to improve, and almost certainly of major clinical relevance. Urgent steps are needed to improve this area of practice."

P. N. … C. M.
Hill … s, M.
Nog… …onen,
… 3.

<u>What clinicians need in organ biopsy assessment:</u>
Precision: it is reproducible
Adequate representation of the organ
Accuracy: it reflects the true disease state
Standardization: it is the same in all centers
Personalization: it retains the uniqueness of each patient

MMDx

The Molecular Microscope® Diagnostic System (MMDx)

A central biopsy diagnostic system that uses microarrays to measure transcript changes (mRNA) compared to a Reference Set, using ensembles of up to 100 predefined machine-learning derived algorithms (classifiers), supervised and unsupervised:

1. Measure the molecular rejection and injury transcript changes.
2. Interpret the rejection and injury disease states and their severity.

Rejection states

- *T cell-mediated rejection (TCMR)*
- *Antibody-mediated rejection (ABMR): early-stage, fully-developed, late stage*
- *Recent under-immunosuppression/non-adherence*

Injury (wounding) states: response-to-wounding (RTW)

- *Measure recent parenchymal injury (prototype: AKI)*
- *Measure irreversible injury (atrophy-fibrosis)*
- *Predict risk of progression to failure*

MMDx

Liver core 5 mm: 10ug
Kidney core 3-5mm 3ug
1-2 EMB bites 1.4ug
1-2 TBB bites 1.4ug
1-2 3BMB bites 1.7ug

Collect Biopsy
Place immediately in RNALater

Shipping/Receiving
Room temperature

RNA Extraction, Clean-Up, and Quality Control

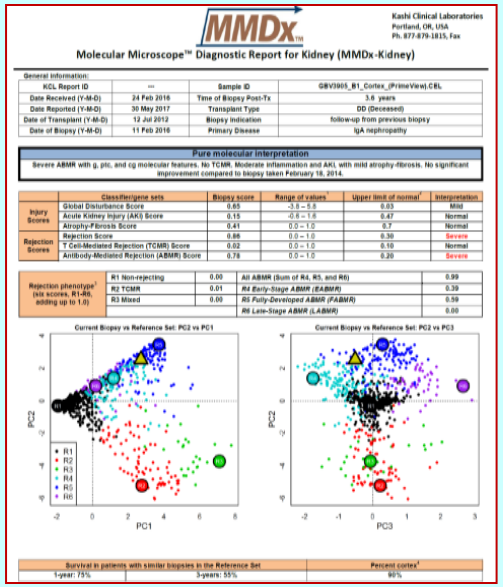
Molecular Microscope®
(MMDx-Kidney/Heart/Lung/Liver)

RNA Labeling

Hybridization

Wash & Stain

Scan Chips



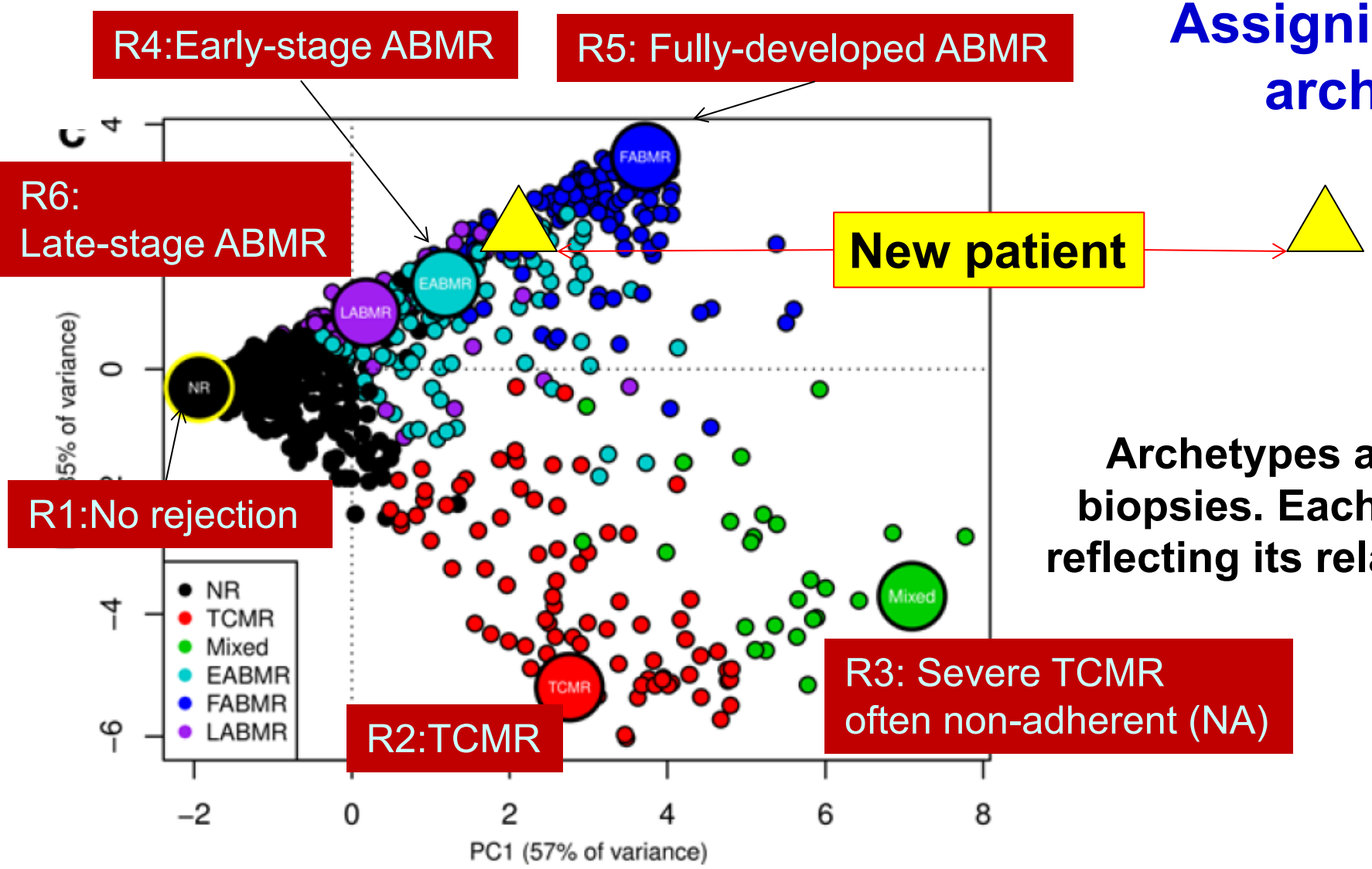
Key terms applied to MMDx

- Precision: reproducibility of the measurements in the same specimen
  - Microarray probe sets: >99% (Affymetrix data)
  - Computer algorithm scores: 99% [1,2]
  - Automated sign-out: in progress – will be 99% [1,2]
  - Human expert opinion: interpretation of boundary cases: possible vs. probable[1,2]
- Biological representation: adequacy of the specimen for the organ
  - MMDx needs much less than histology
  - Acknowledge structure e.g. renal medulla
  - Balance against risk of taking more pieces vs. ensuring adequacy
- Accuracy: ability of the signout to represent the true disease state
  - A never-ending quest for discovery in disease studies

1.J. Reeve, G. A. Bohmig, F. Eskandary, G. Einecke, K. Madill-Thomsen, M. Mackova, P. F. Halloran, and MMDx-Kidney study group. Generating automated kidney transplant biopsy reports using ensembles of molecular classifiers. *Am J Transplant 19 (10):2719-2731, 2019.*
2.Madill-Thomsen KS, Perkowska-Ptasinska A, Bohmig GA, Eskandary FA, Einecke G, Halloran PF, et al. Discrepancy Analysis Comparing Molecular and Histology Diagnoses in Kidney Transplant Biopsies. *Am J Transplant*. 2020;20(5):1341-50.

MMDx

Assigning unsupervised archetype scores



Archetypes are 6 theoretical idealized biopsies. Each real biopsy gets 6 scores reflecting its relationship to each archetype

Express the uniqueness of each new patient

The ***acute kidney injury*** ("AKI") landscape
Measuring the organ ***response-to-wounding***

IRRAT transcripts: AKI-selective transcripts mapped in biopsies of early kidney transplants with AKI/impaired GFR but no rejection [1]

Recent injury is not reliably detected by histology [1,2]

1. Famulski KS, de Freitas DG, Kreepala C, Chang J, Sellares J, Sis B, et al. Molecular phenotypes of acute kidney injury in human kidney transplants. Journal of the American Society of Nephrology. 2012;23(5):948-58.
2. G. Einecke, J. Reeve, G. Gupta, G. Bohmig, F. Eskandary, J. Bromberg, K. Budde, P. F. Halloran, and INTERCOMEX investigators. Factors associated with kidney graft survival in pure antibody-mediated rejection at the time of indication biopsy: Importance of parenchymal injury but not disease activity. *Undergoing revisions, May 2020.*

MMDx

Atrophy-fibrosis classifiers (ciProb, ctProb) trained on histologic findings give atrophy-fibrosis scores [1,2]

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

2.G. Einecke, J. Reeve, G. Gupta, G. Bohmig, F. Eskandary, J. Bromberg, K. Budde, P. F. Halloran, and INTERCOMEX investigators. Factors associated with kidney graft survival in pure antibody-mediated rejection at the time of indication biopsy: Importance of parenchymal injury but not disease activity. AJT undergoing revisions, May 2020.

MMDx

Clinical information
Time post-transplant: biopsy indications, DSA (if approved)

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

Sign-out
Interpretation of the molecular phenotype

Summary of binary molecular scores
(Injury, rejection)

Archetypal analysis
Rejection-related archetype scores (Normal, TCMR, ABMR)

Visualization
Principal component analysis (PCA) plots. Relationship of this biopsy to others in the reference set:
- PC2 vs PC1
- PC2 vs PC3

This biopsy
Yellow triangle

Percent cortex
Molecular estimate of the % of this biopsy core that is cortex

Summary of additional molecular scores
Rejection, injury-related binary classifiers (and AKI transcript set)

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

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

Characteristics of nearby biopsies
based on the nearest neighbors in 3-dimensional space from the PCA plots

MMDx

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

General Information:

Surname	(Redacted)
Date of Birth	
Date Received (Y-M-D)	
Date Reported (Y-M-D)	
Date of Transplant (Y-M-D)	
Date of Biopsy (Y-M-D)	

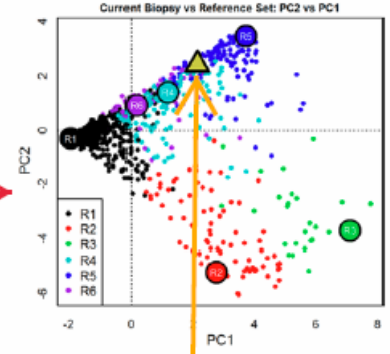
Pure molecular interpretation

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.

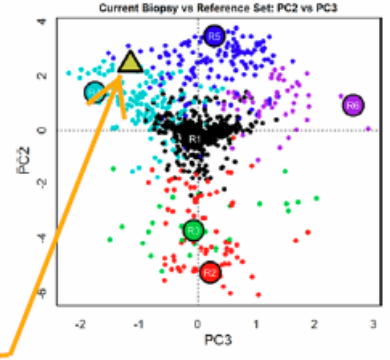
	Classifier/gene sets[1,2]	Biopsy	Range of values[A]	Upper limit of normal[B]	Interpretation
Injury Scores	Inflammation Score[3]	-0.32	-3.8 – 5.8	0.03	Mild
	Acute Kidney Injury (AKI) Score[4]	0.18	-0.6 – 1.6	0.38	Mild
	Atrophy-Fibrosis Score[5]	0.33	0.0 – 1.0	0.82	Mild
Rejection Scores	Rejection Score[6]	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[8,C] (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

Current Biopsy vs Reference Set: PC2 vs PC1



Current Biopsy vs Reference Set: PC2 vs PC3



Survival in patients with similar biopsies in the Reference Set		Percent cortex[B,E]
1-year: 92%	3-years: 76%	98%

Clinical Notes
—

A - The 2.5th to 97.5th percentiles in the entire Reference Set
B - 90th percentile in relevant Reference Set biopsies
C – Mean of scores from page 2
C - Scores from archetypal analysis
E - %cortex is a quality control measure

MMDx

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

	Classifier/Gene set	Biopsy score	Range of possible values[A]	Upper limit of normal[B]	Interpretation
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]	0.18	-0.6 – 1.6	0.38	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[9]	0.75	0.0 – 1.0	0.26	Severe
	Transplant glomerulopathy (cg) > 0 probability[9]	0.33	0.0 – 1.0	0.22	Mild
	Peritubular capillaritis (ptc) > 0 probability[9]	0.75	0.0 – 1.0	0.24	Severe
	DSA-positive probability	0.84	0.0 – 1.0	0.42	Moderate
	Interstitial inflammation (i) > 1 probability[9]	0.02	0.0 – 1.0	0.08	Normal
	Tubulitis (t) > 1 probability[9]	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 else, with BK/Borderline/Mixed withheld; ABMR-1 = ABMR vs everything else with TG/ABMR suspicious withheld; ABMR-2 = ABMR and Mixed vs everything else, with TG/ABMR suspicious withheld; ABMR-3 = ABMR vs everything else, with Mixed/TG/ABMR suspicious withheld.

Rank order of the most common histologic diagnoses in the 50 nearest molecular neighbors	Mean molecular scores in the 50 nearest molecular neighbors
ABMR: 64%	Rejection: 0.83
No Major Abnormalities (NOMOA): 12%	ABMR: 0.83
Transplant Glomerulopathy (TG): 8%	Atrophy-Fibrosis Score (oigt1): 0.28
Mixed Rejection: 6%	AKI Score (IRRATs): 0.20
ABMR Suspicious: 6%	TCMR: 0.02

References for the scores, classifiers, and archetypes

1. Halloran PF et al. Nature Reviews Nephrology 2016;12(9):534-48.
2. Halloran PF et al. Kidney Int 2014;(85):258-64.
3. Mueller TF et al. Am J Transplant 2007;7(12):2712-22.
4. Famulski K et al. JASN 2012; May;23(5):948-58.
5. Venner J et al. Journal of Clinical Investigation Insight 2016;1(1):e85323-doi:10.1172/jci.insight.85323.
6. Reeve J et al. Am J Transplant 2009 Aug;9(8):1802-10.
7. Reeve J et al. Am J Transplant 2013;13(3):645-55.
8. Sellares J et al. Am J Transplant 2013;13(4):971-83.
9. Reeve J et al. JCI Insight 2017;2(12):e94197-doi:10.1172/jci.insight.94197.
10. Madill-Thomsen K et al. Am J Transplant 2017 Feb 22;17(8):2117-28.
11. Einecke G et al. Am J Transplant 2017;18(4):821-831.

A - The 2.5th to 97.5th percentiles in the entire Reference Set
B - 90th percentile in relevant Reference Set biopsies
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C - Scores from archetypal analysis
E - %cortex is a quality control measure

Trifecta

An investigator-initiated international prospective study to calibrate circulating donor-derived cfDNA levels (dd-cfDNA%) (Prospera) at the time of indication biopsy to the MMDx findings in the biopsy (Goal: N=300).

(Also include DSA measurements by One Lambda.)

While the results are expressed as dd-cfDNA%, the detailed interpretation of the denominator (recipient cfDNA) is also an important question in relationship to the disease states in the biopsy

Trifecta Study ClinicalTrials.gov NCT04239703

MMDx

Trifecta study collaborators for first 66 biopsies

Trifecta Study Participating Center		
Name	**Center**	**Location**
Beata Naumnik	Medical University in Białystok	Białystok, Poland
Zbigniew Włodarczyk	University Hospital nr1 Bydgoszcz	Bydgoszcz, Poland
Ryszard Grenda	The Children's Memorial Health Institute	Warsaw, Poland
Alicja Dębska-Ślizień, Joana Konopa, Andrzej Chamienia	Medical University of Gdańsk	Gdańsk, Poland
Grzegorz Piecha	Medical University of Silesia	Katowice, Poland
Maciej Glyda, Katarzyna Smykal-Jankowiak	Wojewodzki Hospital	Poznan, Poland
Marek Myślak, Joanna Mazurkiewicz, Marta Gryczman	Pomeranian Medical University	Szczecin, Poland
Agnieszka Perkowska-Ptasińska, Dominika Dęborska-Materkowska, Michał Ciszek	Warsaw Medical University	Warsaw, Poland
Mirosław Banasik	Medical University of Wrocław	Wrocław, Poland
Andrew Malone, Tarek Alhamad	Washington University at St. Louis	St. Louis, MO, USA
Thomas Müller	University Hospital Zurich	Zurich, Switzerland

Trifecta Study ClinicalTrials.gov NCT04239703

MMDx

First 66 biopsies in the Trifecta study

for which cfDNA data and MMDx results are available and technically satisfactory (May 21/2020)

MMDx rejection case mix n=66

- **No rejection 29**
- **ABMR 24**
- Possible ABMR 5
- Mixed 2
- **TCMR 4**
- Possible TCMR 2

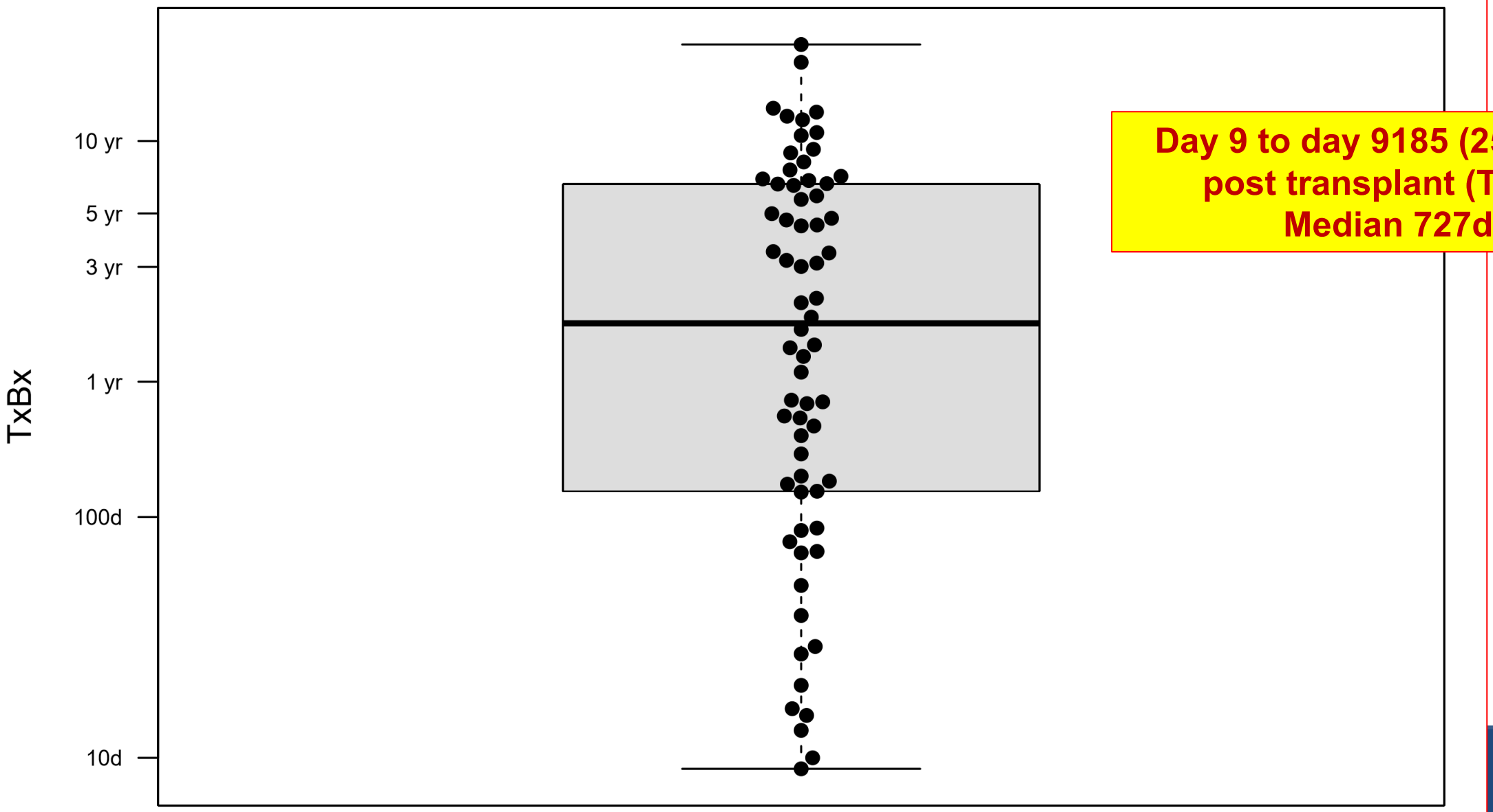
Histology case mix n=51+15 pending

- No rejection (18)
  - AKI: 3; BK: 1; DiabNeph: 1; IFTA: 6
  - “Normal” NOMOA: 4
  - Other: 3
- ABMR: 11 (+1 ABMR/BK +6 TG +2 Mixed = 20)
- ABMR/BK: 1
- ABMRsusp: 3
- TG: 6
- Mixed: 2
- TCMR: 6
- TCMR/BK: 1
- Borderline: 2
- GN: 1
- Missing or "No diagnosis": 15

Trifecta Study ClinicalTrials.gov NCT04239703

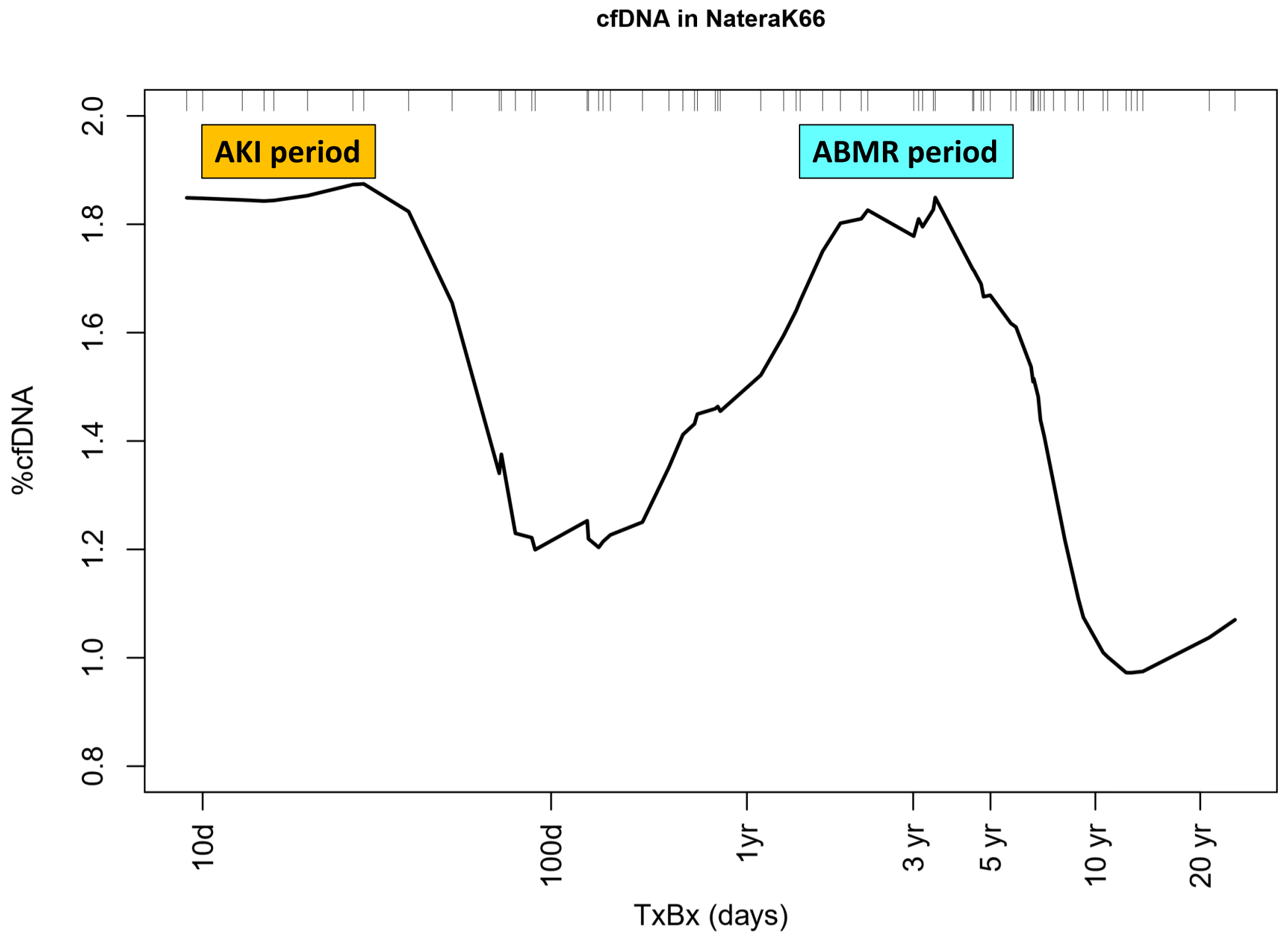
MMDx

Distribution of biopsies over time post transplant (TxBx)



Day 9 to day 9185 (25 years) post transplant (TxBx) Median 727d

MMDx


cfDNA in NateraK66
AKI period
ABMR period
%cfDNA
0.8
1.0
1.2
1.4
1.6
1.8
2.0
10d
100d
1yr
3 yr
5 yr
10 yr
20 yr
TxBx (days)

Relationship of circulating dd-cfDNA% to expression of individual genes in the biopsy

66 biopsies

The top 20/50000 probesets in biopsies correlating with dd-cfDNA%:

15 reflect all-rejection (green), 2 injury (orange), and 3 death domain agonist BID (white)

SYMBOL	Gene Name	Annotation (PBT)	Spearman corr coeff	P value
XCL1	chemokine (C motif) ligand 1 /// chemokine (C motif) ligand 2	All-rejection	0.644	5.3E-09
GNLY	granulysin	All-rejection	0.597	1.2E-07
PTPRE	protein tyrosine phosphatase, receptor type, E	injury (LivGST)	0.596	1.3E-07
GNLY	granulysin	All-rejection	0.596	1.3E-07
KLRD1	killer cell lectin-like receptor subfamily D, member 1	All-rejection	0.582	3E-07
SCIMP	SLP adaptor and CSK interacting membrane protein	All-rejection	0.576	4.2E-07
BID	**BH3 interacting domain death agonist**		0.571	5.4E-07
BID	**BH3 interacting domain death agonist**		0.568	6.5E-07
SLAMF7	SLAM family member 7	All-rejection	0.564	8.2E-07
CRTAM	cytotoxic and regulatory T-cell molecule	All-rejection	0.561	9.8E-07
CCL4	chemokine (C-C motif) ligand 4 /// chemokine (C-C motif) ligand 4-like 1 /// chemokine (C-C motif) ligand 4-like 2	All-rejection	0.559	1.1E-06
KLRD1	killer cell lectin-like receptor subfamily D, member 1	All-rejection	0.559	1.1E-06
CCL4	chemokine (C-C motif) ligand 4 /// chemokine (C-C motif) ligand 4-like 1 /// chemokine (C-C motif) ligand 4-like 2	All-rejection	0.557	1.2E-06
KLRD1	killer cell lectin-like receptor subfamily D, member 1	All-rejection	0.556	1.3E-06
EFHD2	EF-hand domain family member D2	Injury (LivGST)	0.555	1.4E-06
NKG7	natural killer cell granule protein 7	All-rejection	0.551	1.6E-06
BID	**BH3 interacting domain death agonist**		0.551	1.7E-06
LST1	leukocyte specific transcript 1	All-rejection	0.551	1.7E-06
CCL4	chemokine (C-C motif) ligand 4 /// chemokine (C-C motif) ligand 4-like 1 /// chemokine (C-C motif) ligand 4-like 2	All-rejection	0.548	1.9E-06
SLAMF7	SLAM family member 7	All-rejection	0.548	1.9E-06

MMDx

Relationship of circulating dd-cfDNA% to gene set and classifier scores in the biopsy: similar to single genes

66 biopsies

Correlation of rejection and injury scores with DD-cfDNA%

Gene sets (PBTs) and classifiers	cfDNA correlation with:	All N=66 biopsies	
		Spearman correlation coefficient	p-value
ABMR-related	ABMRProb	0.46	**1.08E-04**
	cg_{Prob}	0.43	**3.32E-04**
	g_{Prob}	0.47	**6.71E-05**
	ptc_{Prob}	0.55	**2.09E-06**
All-rejection-related	ABMR-RAT	0.56	**8.14E-07**
	Rej_{Prob}	0.49	**2.75E-05**
	GRIT3	0.47	**7.6E-05**
TCMR-related	QCAT	0.53	**5.08E-06**
	$TCMR_{Prob}$	0.23	0.07
	i_{Prob}	0.36	**0.003**
	t_{Prob}	0.33	**0.006**
Macrophage-related	QCMAT	0.39	0.001
	AMAT1	0.49	**3.61E-05**
AKI-related	IRRAT30	0.16	0.21
	IRITD3	0.28	0.02
Loss of parenchymal transcripts	KT1	-0.29	0.02
CKD-related	ci_{Prob}	0.15	0.22
	ct_{Prob}	0.13	0.31

Dd-cfDNA correlations with rejection and injury scores

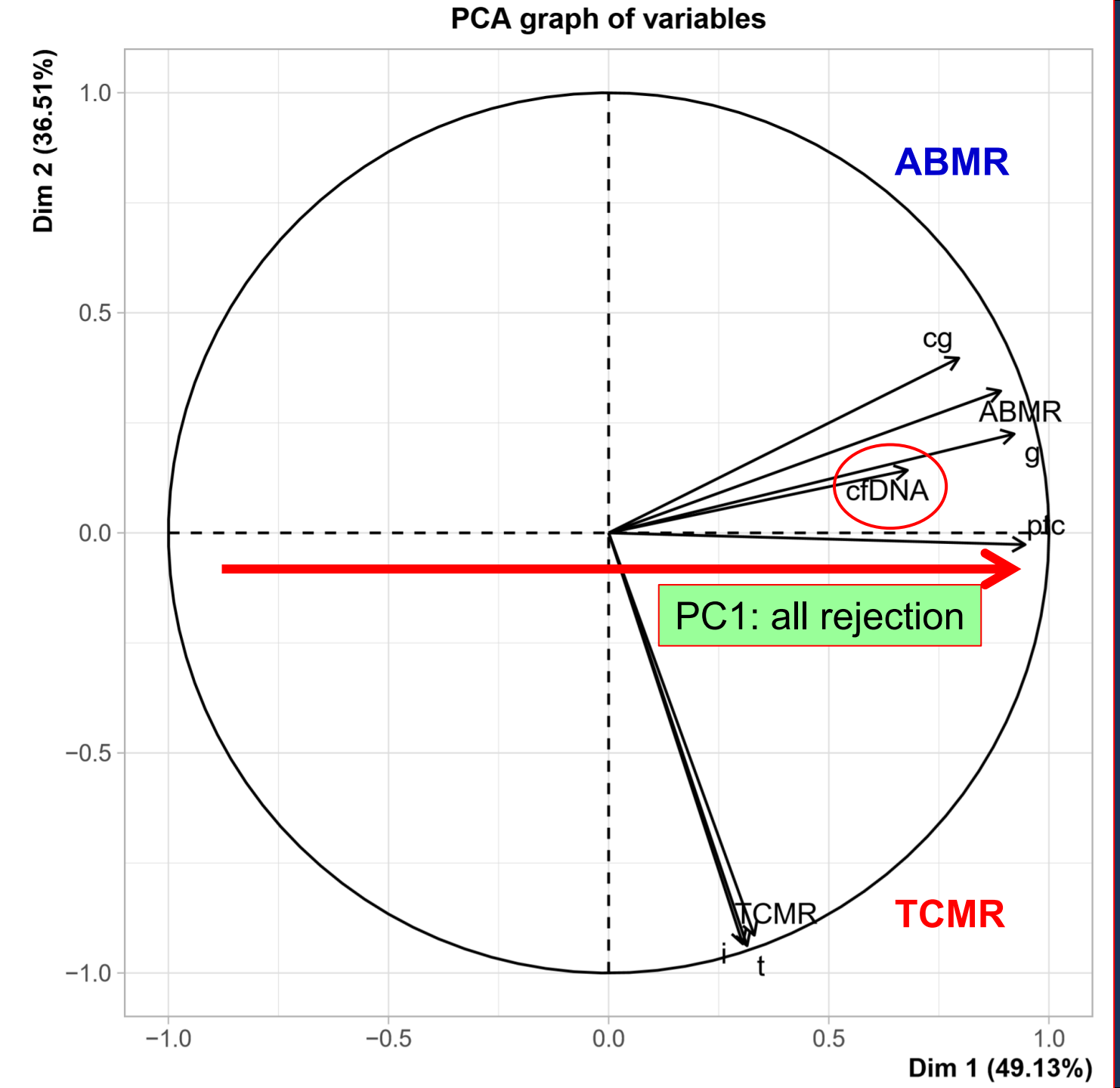
Overall strong correlation with rejection scores

Moderate with macrophage scores, especially alternative macrophage activation

Mixed with AKI scores

None with atrophy-fibrosis scores

In PCA, circulating dd-cfDNA% performs like a molecular rejection measurement when combined with molecular biopsy measurements



In these 66 biopsies, the actual dd-cfDNA scores are strongly related to the *rejection* scores when used like a biopsy rejection variable in PCA

Note: this is the same type of PCA we show on MMDx rejection report.

(Slight bias toward ABMR is expected given the case mix in these 66 biopsies)

MMDx

Dd-cfDNA% is highly associated with rejection and to a lesser extent AKI molecular changes in the biopsy

- Note constraints of case mix in interpretations
- Strongest: All-rejection and ABMR scores
- Effector T cell burden, some TCMR classifiers
  - Reflecting case mix
- Alternative macrophage activation
- Weakly with some AKI variables (IRITD3) not others (IRRAT)
- Not with atrophy-fibrosis (ci/ct classifiers)

Thank you

And special thanks to our wonderful investigators and collaborators and to Natera for supporting the study

MMDx