جامعة قطر
QATAR UNIVERSITY

9. National
Congress of Turkish Transplantation Immunology and Genetics Society
18-21 April 2024

Next-Gen ABMR Diagnostics: Unveiling the Potential of cfDNA

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Professor of Immunology, College of Medicine, Qatar University

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Clinical Professor, Department of Medical Education, Texas A&M College of Medicine

Director of Clinical Services, Be The Match / National Marrow Donor Program (NMDP)

Outline

- A glance at some modalities of transplant management and risk stratification tools
- Emerging modalities in diagnosis of antibody mediated allograft rejection
  - Intragraft transcription profile
  - Peripheral blood transcription profiles
  - Donor derived cell free DNA (ddcfDNA)

A glance at the present

transplant international

WILEY

ORIGINAL ARTICLE | Full Access

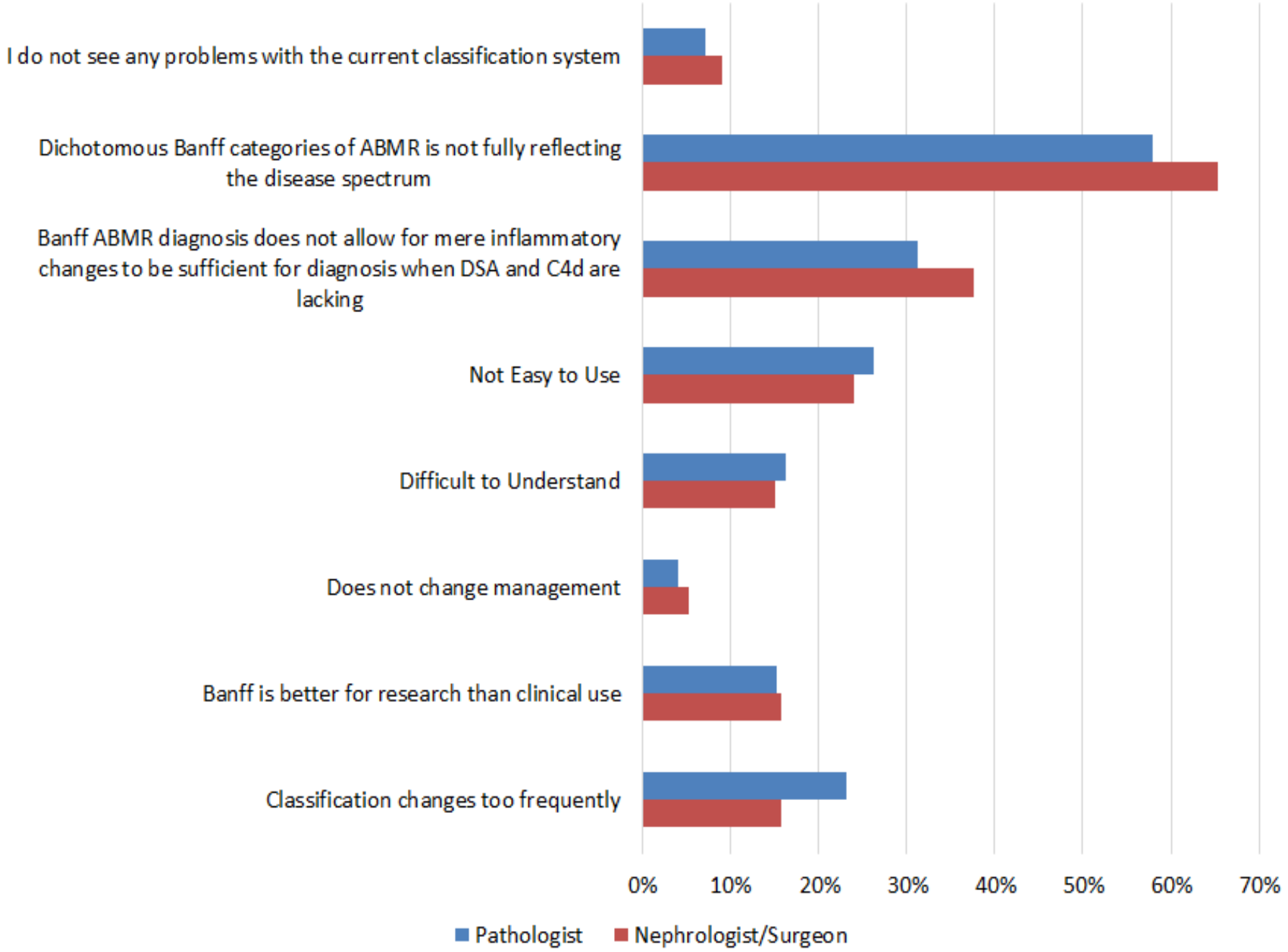
A 2020 Banff Antibody Mediated Injury Working Group examination of international practices for diagnosing antibody mediated rejection in kidney transplantation

Carrie A. Schinstock, Medhat Askar, Serena M. Bagnasco, Ibrahim Batal, Laurine Bow, Klemens Budde, Patricia Campbell, Robert Carroll ... See all authors

First published: 10 January 2021 |
https://doi-org.srv-proxy2.library.tamu.edu/10.1111/tri.13813

Texas A&M

Figure 4. Limitations of the current ABMR Banff Classification



A Glance At The Present

- There is unmet need in transplantation to create an objective diagnostic test for all forms of allograft rejection
- The pathology of rejection are not consistent
- Nonetheless, genomic based detection of rejection is promising tool

Intragraft Transcription Profiles (Transcriptome)

Received: 13 September 2017 | Revised: 31 October 2017 | Accepted: 17 November 2017

DOI: 10.1111/ajt.14600

MINIREVIEW

AJT

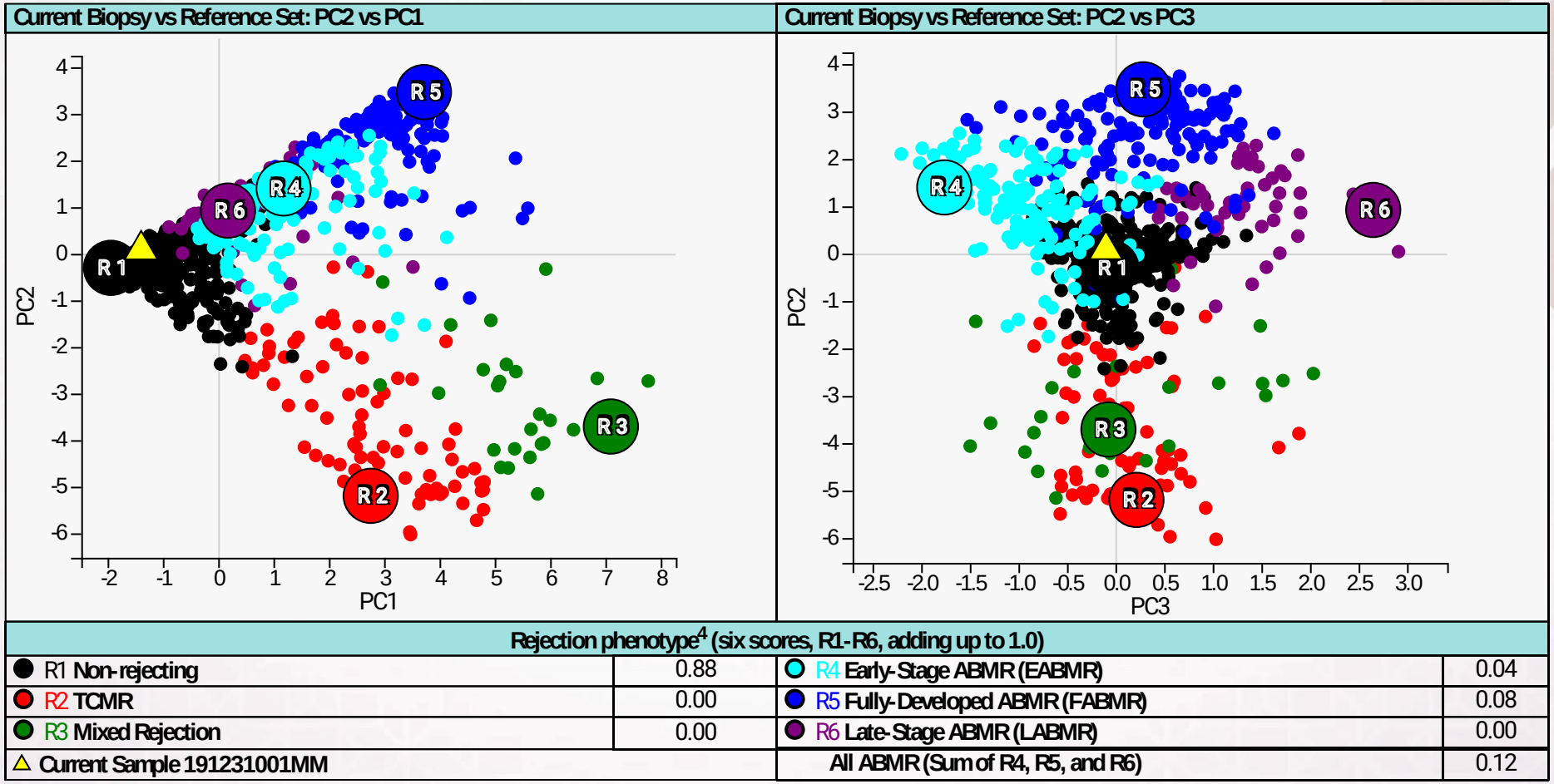
Review: The transcripts associated with organ allograft rejection

Philip F. Halloran[1,2] | Jeffery M. Venner[1] | Katelynn S. Madill-Thomsen[1,2] | Gunilla Einecke[3] | Michael D. Parkes[1] | Luis G. Hidalgo[4] | Konrad S. Famulski[1,4]

Biopsy Transcriptome

The MMDx Process

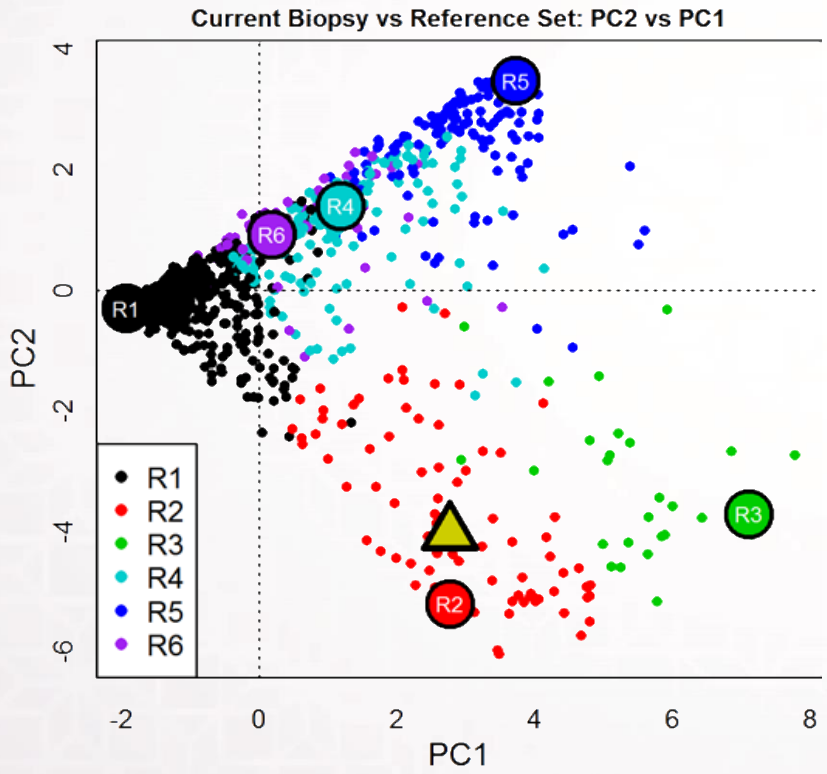
Biopsy Transcriptome

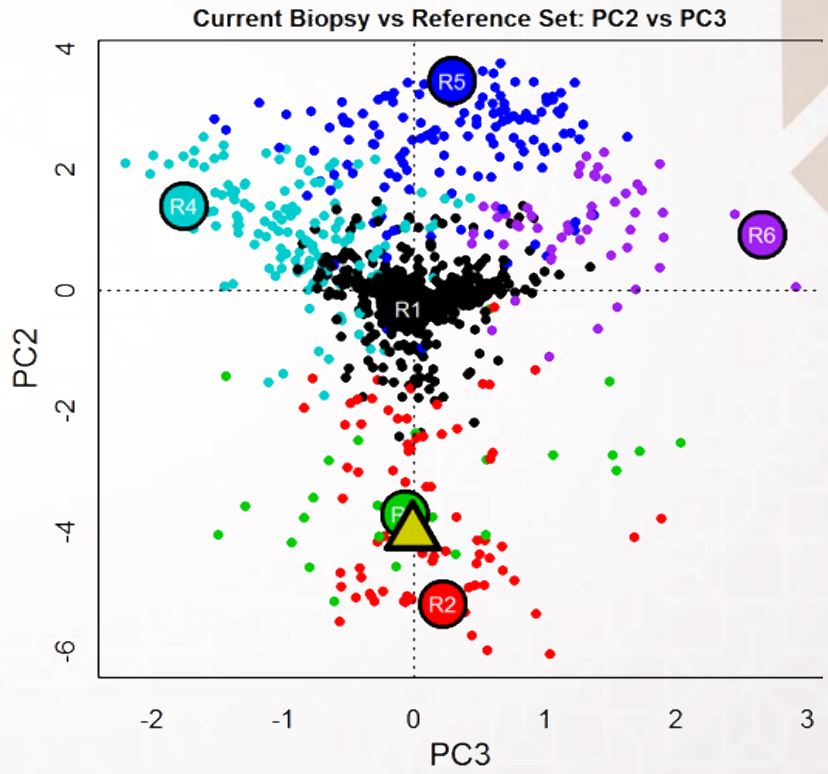


Rejection phenotype[4] (six scores, R1-R6, adding up to 1.0)			
R1 Non-rejecting	0.88	R4 Early-Stage ABMR (EABMR)	0.04
R2 TCMR	0.00	R5 Fully-Developed ABMR (FABMR)	0.08
R3 Mixed Rejection	0.00	R6 Late-Stage ABMR (LABMR)	0.00
△ Current Sample 191231001MM		All ABMR (Sum of R4, R5, and R6)	0.12

Biopsy Transcriptome

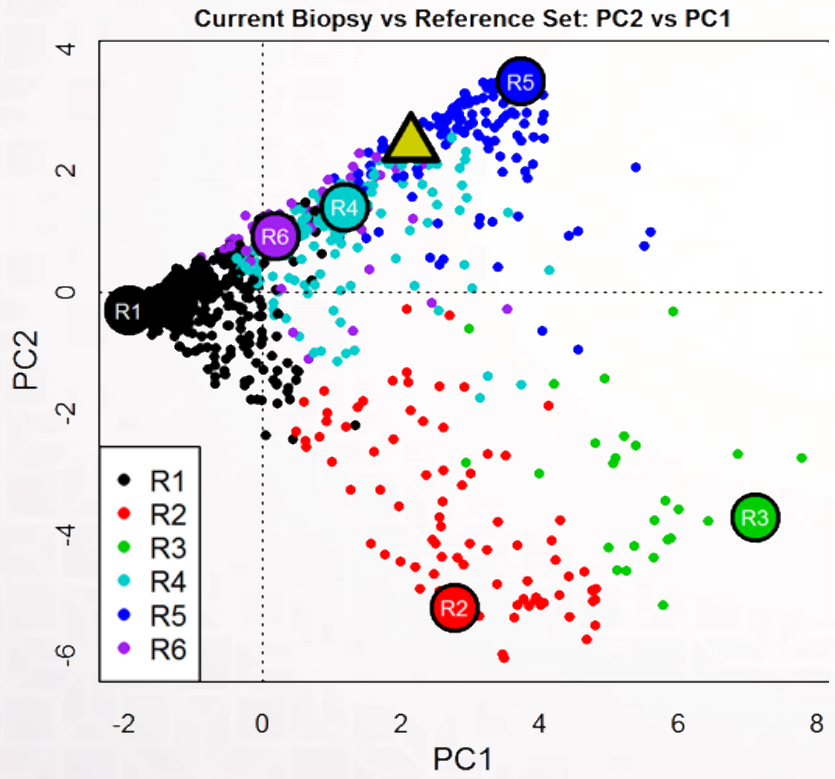
Rejection phenotype[9, D] (six scores, R1-R6, adding up to 1.0)	**R1 Non-rejecting**	**0.01**	**All ABMR (Sum of R4, R5, and R6)**	**0.16**
	R2 TCMR	**0.75**	***R4 Early-Stage ABMR (EABMR)***	**0.14**
	R3 Mixed Rejection	**0.08**	***R5 Fully-Developed ABMR (FABMR)***	**0.00**
			R6 Late-Stage ABMR (LABMR)	**0.02**

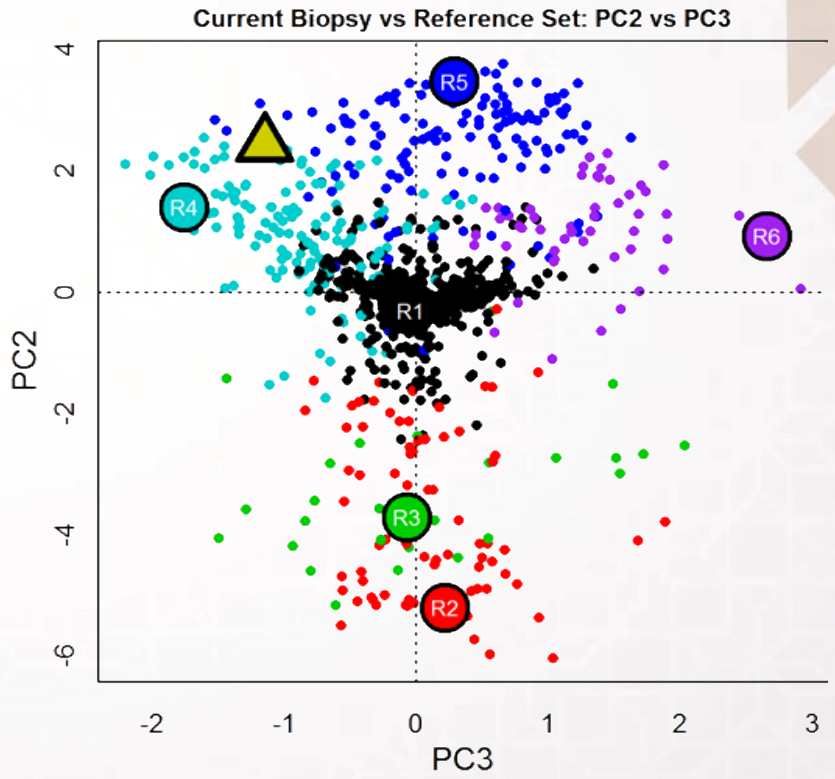




Biopsy Transcriptome

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**





Biopsy Transcriptome

ELSEVIER

The Journal of Heart and Lung Transplantation

http://www.jhltonline.org

Building a tissue-based molecular diagnostic system in heart transplant rejection: The heart Molecular Microscope Diagnostic (MMDx) System

Philip F. Halloran, MD, PhD,[a,b] Luciano Potena, MD, PhD,[c] Jean-Paul Duong Van Huyen, MD,[d] Patrick Bruneval, MD,[d,e] Ornella Leone, MD,[c] Daniel H. Kim, MD,[f] Xavier Jouven, MD,[d,g] Jeff Reeve, PhD,[h] and Alexandre Loupy, MD, PhD[d]

Biopsy Transcriptome

ELSEVIER

The Journal of Heart and Lung Transplantation

http://www.jhltonline.org

FEATURED PAPERS

Molecular assessment of rejection and injury in lung transplant biopsies

Kieran M. Halloran, MD, MSc,[a] Michael D. Parkes, MSc,[b] Jessica Chang, BSc,[b] Irina L. Timofte, MD,[c] Gregory I. Snell, MD,[d] Glen P. Westall, MD, PhD,[d] Ramsey Hachem, MD,[e] Daniel Kreisel, MD, PhD,[e] Elbert Trulock, MD,[e] Antoine Roux, MD, PhD,[f] Stephen Juvet, MD, PhD,[g] Shaf Keshavjee, MD, MSc,[g] Peter Jaksch, MD,[h] Walter Klepetko, MD,[h] and Philip F. Halloran, MD, PhD[a,b]

Biopsy Transcriptome

Received: 24 November 2019 | Revised: 7 February 2020 | Accepted: 9 February 2020

DOI: 10.1111/ajt.15828

Check for updates

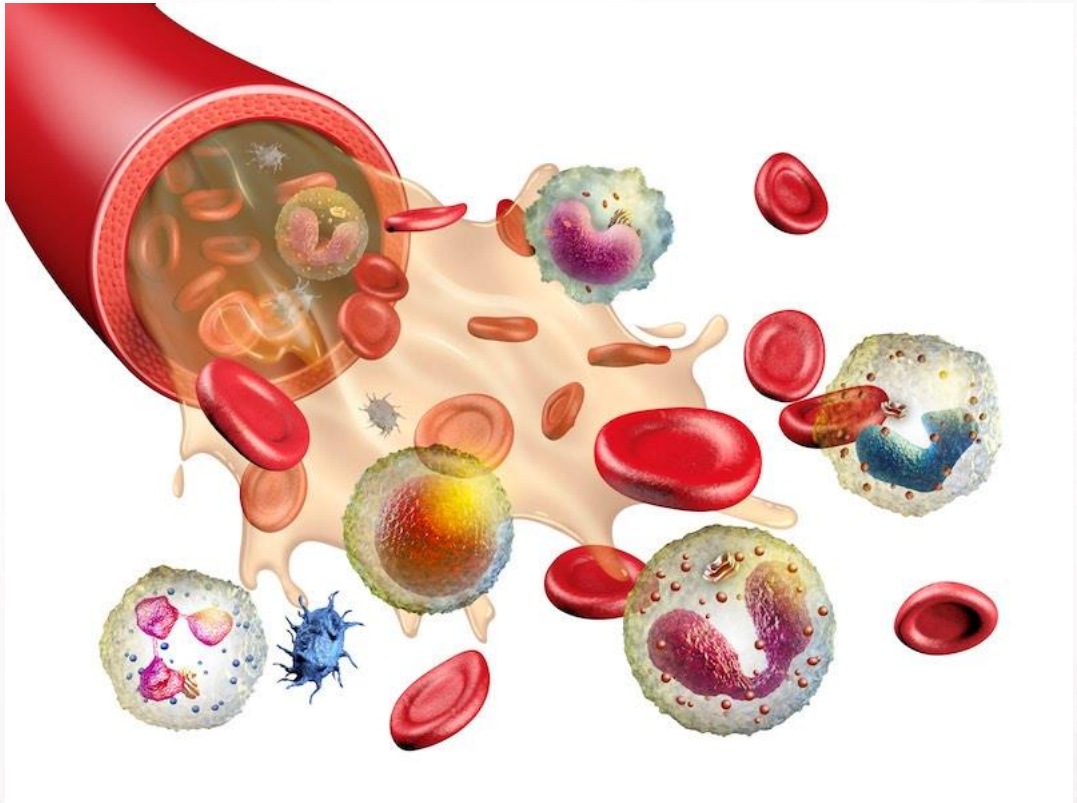
AJT

ORIGINAL ARTICLE

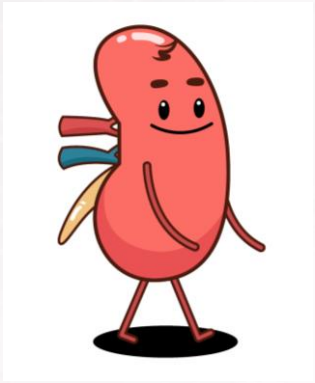
The molecular diagnosis of rejection in liver transplant biopsies: First results of the INTERLIVER study

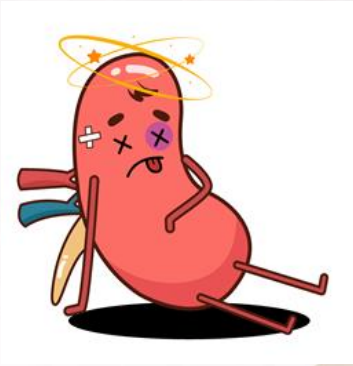
Katelynn Madill-Thomsen[1] | Marwan Abouljoud[2] | Chandra Bhati[3] | Michał Ciszek[4] | Magdalena Durlik[5] | Sandy Feng[6] | Bartosz Foroncewicz[4] | Iman Francis[2] | Michał Grąt[7] | Krzysztof Jurczyk[8] | Goran Klintmalm[9] | Maciej Krasnodębski[7] | Geoff McCaughan[10] | Rosa Miquel[11] | Aldo Montano-Loza[12] | Dilip Moonka[2] | Krzysztof Mucha[4] | Marek Myślak[13] | Leszek Pączek[4] | Agnieszka Perkowska-Ptasińska[5] | Grzegorz Piecha[14] | Trevor Reichman[3] | Alberto Sanchez-Fueyo[11] | Olga Tronina[5] | Marta Wawrzynowicz-Syczewska[8] | Andrzej Więcek[14] | Krzysztof Zieniewicz[7] | Philip F. Halloran[1,12]

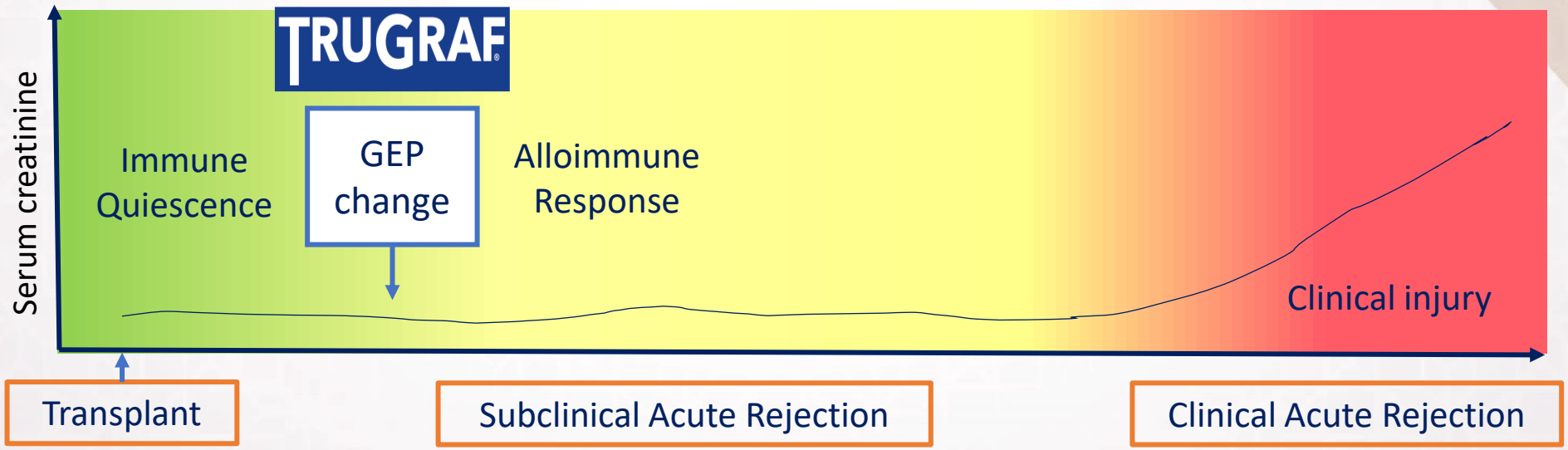
Peripheral Blood Transcriptome



PB Transcriptome







PB Transcriptome

DOI: 10.1111/ajt.15011

AJT

ORIGINAL ARTICLE

Development and clinical validity of a novel blood-based molecular biomarker for subclinical acute rejection following kidney transplant

John J. Friedewald[1] | Sunil M. Kurian[2] | Raymond L. Heilman[3] | Thomas C. Whisenant[4] | Emilio D. Poggio[5] | Christopher Marsh[2] | Prabhakar Baliga[6] | Jonah Odim[7] | Merideth M. Brown[7] | David N. Ikle[8] | Brian D. Armstrong[8] | jane I. charette[1] | Susan S. Brietigam[1] | Nedjema Sustento-Reodica[1] | Lihui Zhao[1] | Manoj Kandpal[1] | Daniel R. Salomon[2,†] | Michael M. Abecassis[1] | for the Clinical Trials in Organ Transplantation 08 (CTOT-08)

Received: 13 July 2020 | Revised: 30 October 2020 | Accepted: 3 November 2020

DOI: 10.1111/ctr.14145

Clinical TRANSPLANTATION
The Journal of Clinical and Translational Research
WILEY

ORIGINAL ARTICLE

Avoiding surveillance biopsy: Use of a noninvasive biomarker assay in a real-life scenario

Audrey Ang[1] | Courtney Schieve[2] | Stanley Rose[2] | Clifton Kew[1] | M. Roy First[2] | Roslyn B. Mannon[1]

	Previous study (n = 99)*	Previous study UAB (n = 25)*	Current study (n = 90)
Specificity	74%	57%	78%
Sensitivity	71%	50%	38%
NPV	89%	78%	81%
PPV	48%	28%	35%

Ang et al, 2020

Original Article

Transl Androl Urol 2022;11(10):1399-1409

Identification of a novel peripheral blood signature diagnosing subclinical acute rejection after renal transplantation

Yue Xu[1,2#], Hao Zhang[1,2#], Di Zhang[1,2], Yuxuan Wang[1,2], Yicun Wang[1,2], Wei Wang[1,2], Xiaopeng Hu[1,2]

		Group	
		subAR	non-subAR
Risk group	High risk	10	11
	Low risk	2	42
	Sensitivity	0.833	
	Specificity		0.792

Xu et al, 2022

What is cfDNA?

- Circulating free DNA (cfDNA) are degraded DNA fragments released to the blood plasma

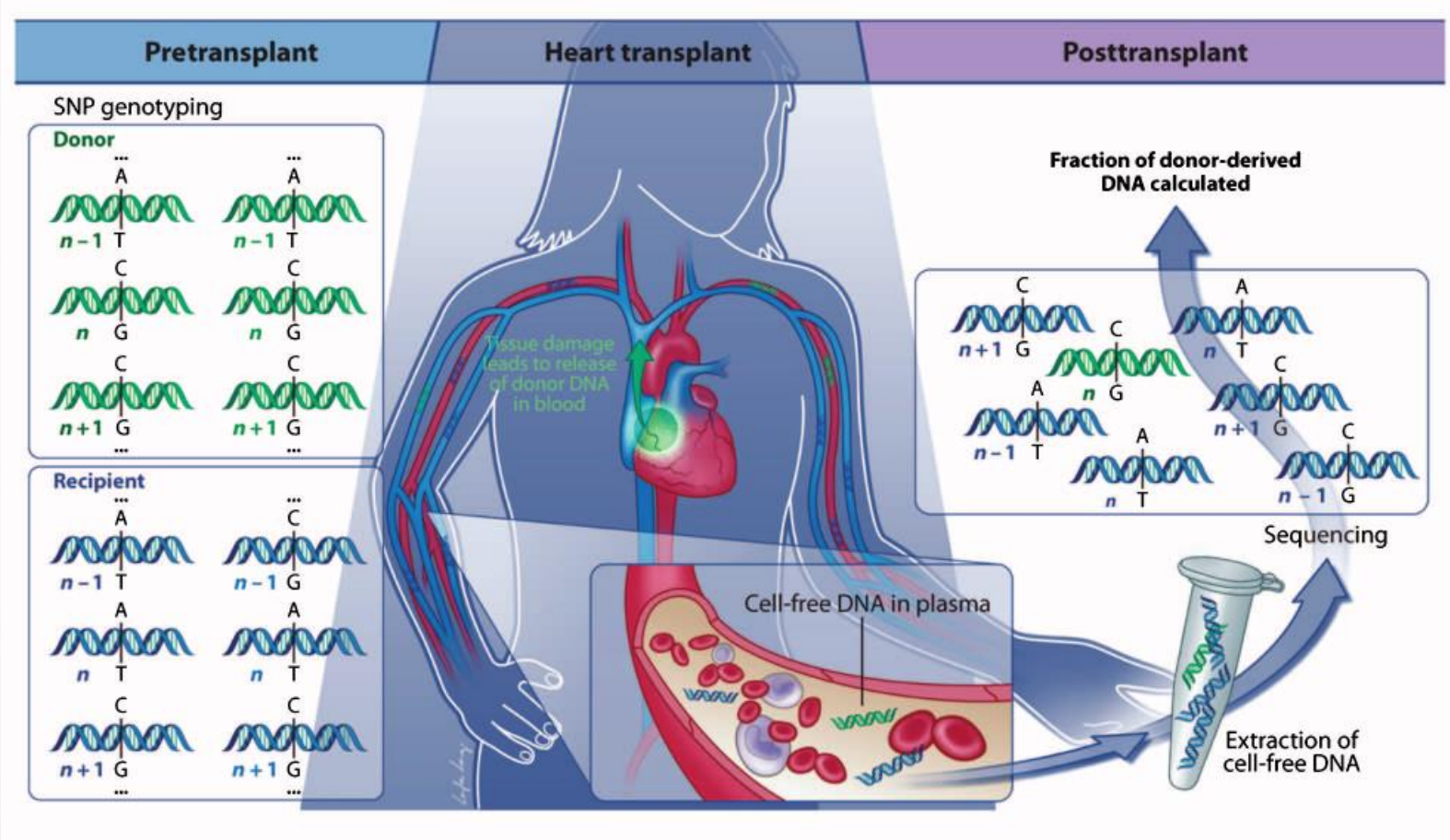
- cfDNA is used to describe various forms of DNA freely circulating the bloodstream, including tumor DNA (ctDNA) and cell-free fetal DNA (cffDNA) and donor derived DNA (ddcfDNA)

Relevance of elevated cfDNA?

Elevated levels of cfDNA are observed in

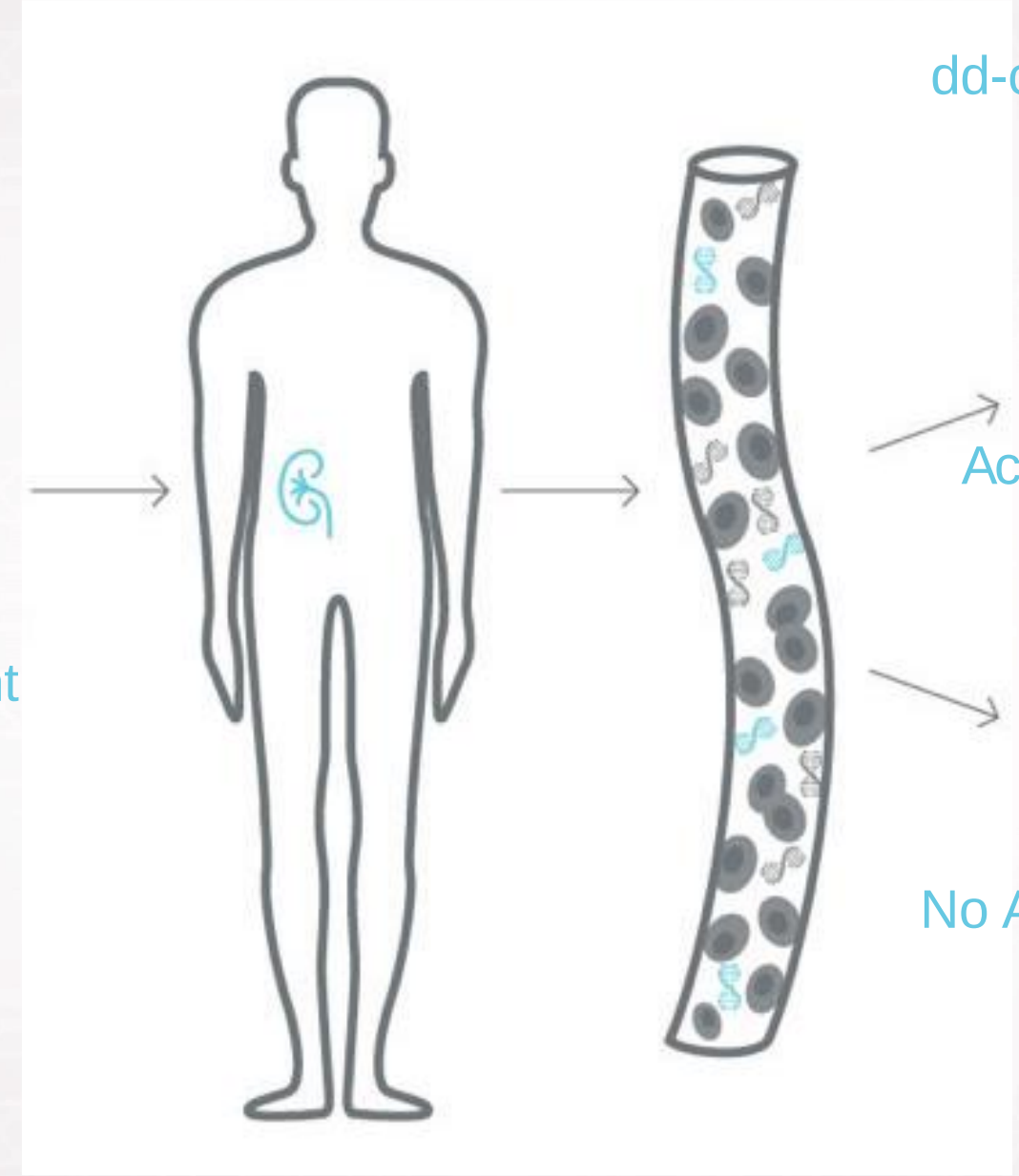
- Congenital fetal malformation
- Cancer, especially in advanced disease
- Allograft rejection

cfDNA



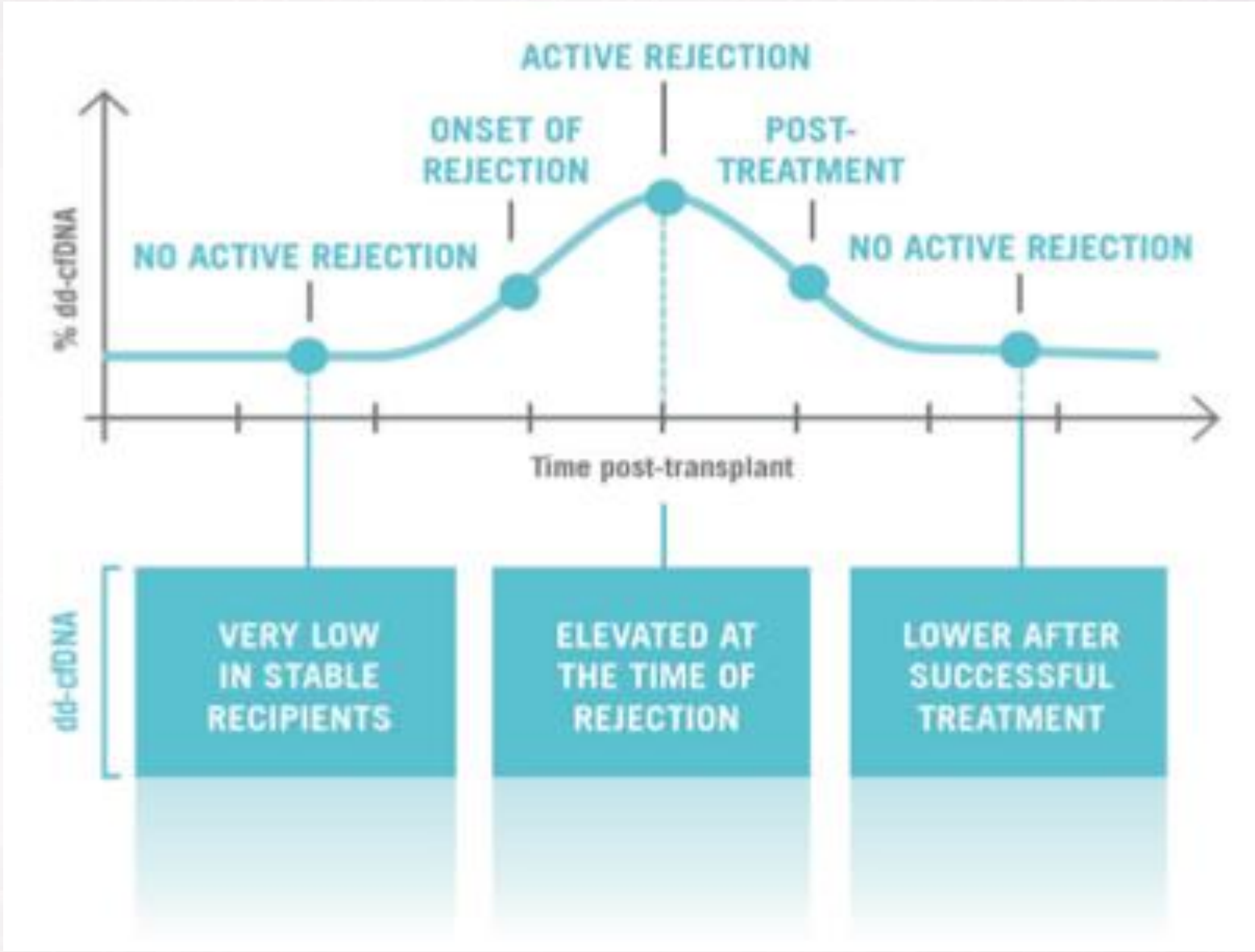
De Vlaminck et al, 2014

cfDNA



https://caredx.com/

cfDNA



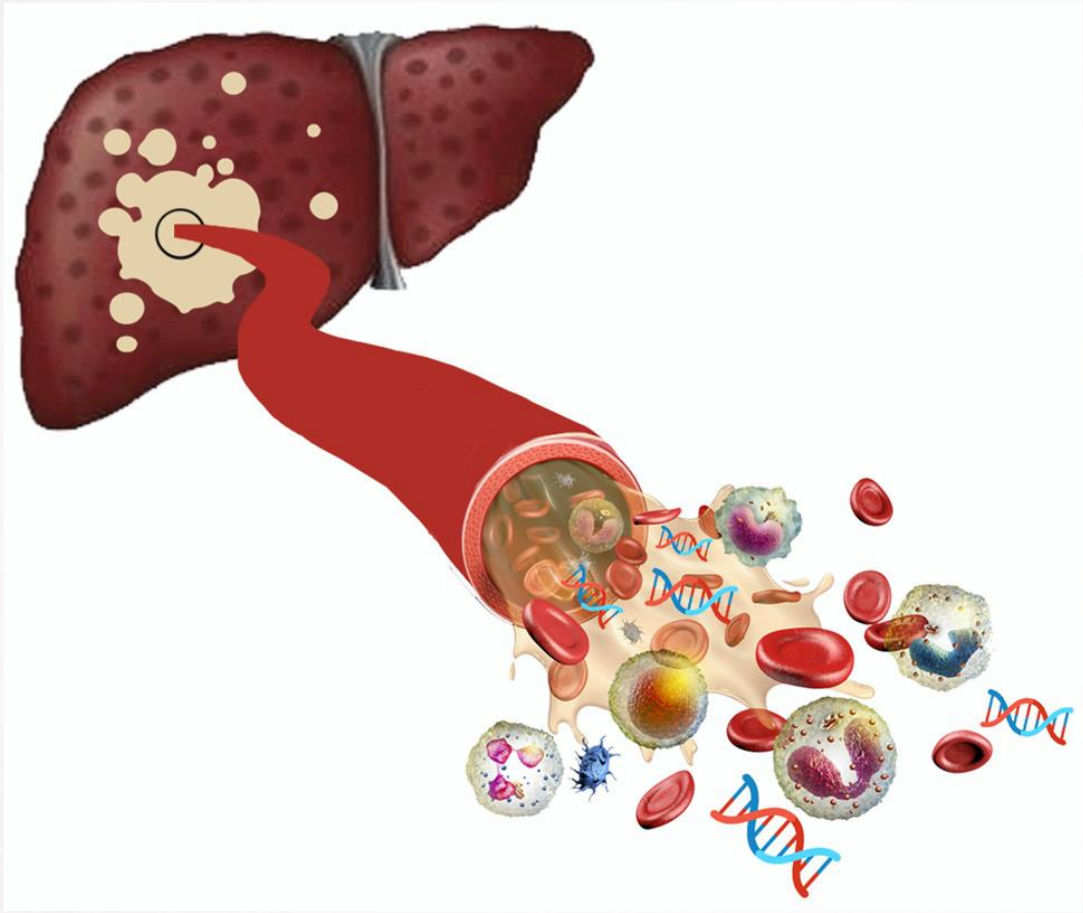
https://caredx.com/

Next Generation Allograft ABMR Diagnostics

A test to diagnose all forms of allograft antibody mediated rejection (ABMR) that is:

- Non-invasive
- Objective
- Cost effective

cfDNA As A Liquid Biopsy Strategy



Pelizarro et al, 2021

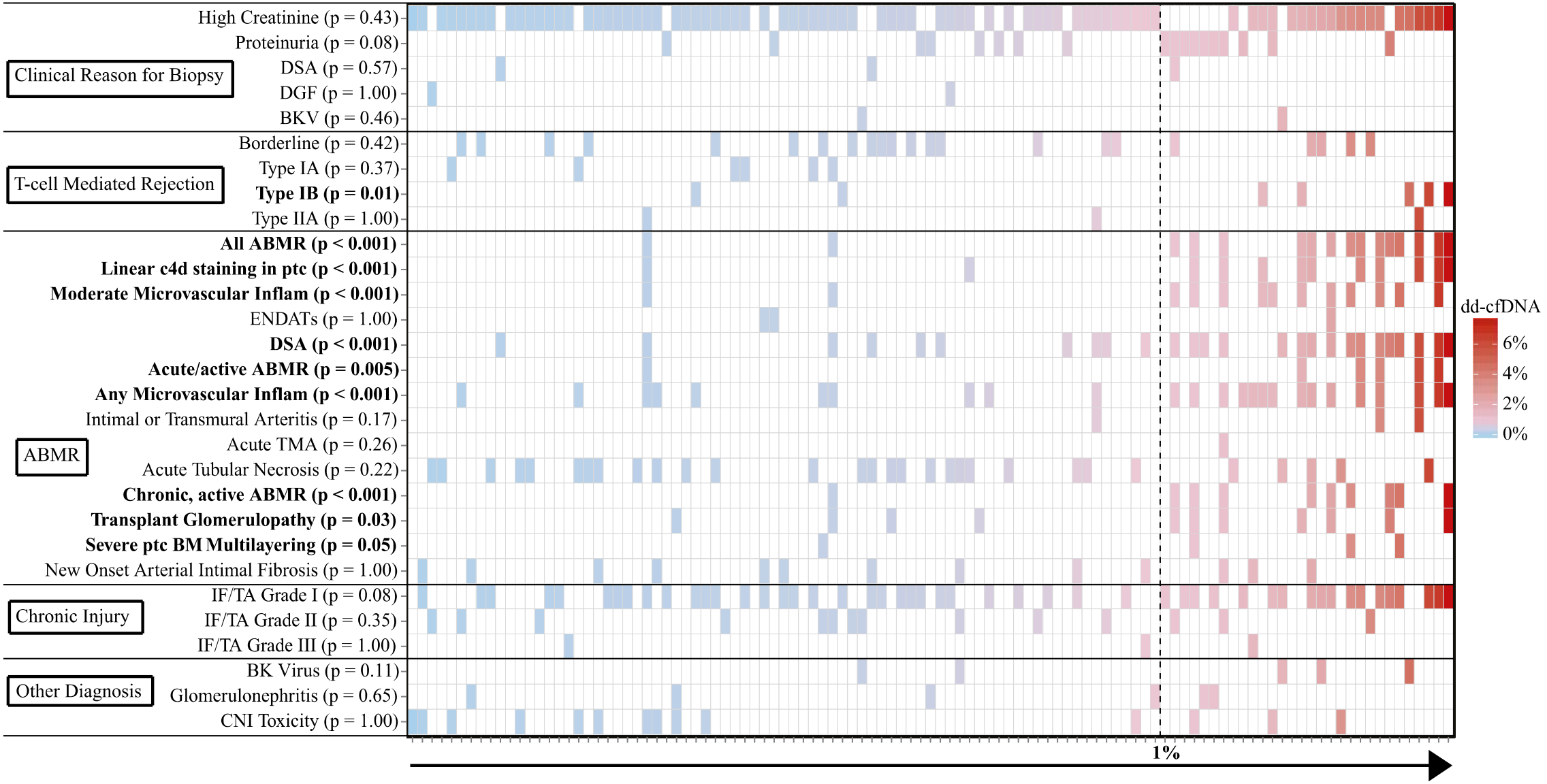
Liquid vs. Tissue Biopsy

	Tissue Biopsy	Liquid Biopsy
Specimen	Allograft biopsy (invasive)	Peripheral blood (less invasive)
Representation	Sampling error, single (limited number of samples)	On demand
Treatment monitoring	Not possible unless with another biopsy	Real time
Cost	High	Relatively low

Cell-Free DNA and Active Rejection in Kidney Allografts

Roy D. Bloom,* Jonathan S. Bromberg,† Emilio D. Poggio,‡ Suphamai Bunnapradist,§ Anthony J. Langone,‖ Puneet Sood,¶ Arthur J. Matas,** Shikha Mehta,†† Roslyn B. Mannon,††‡‡ Asif Sharfuddin,§§ Bernard Fischbach,‖‖ Mohanram Narayanan,¶¶ Stanley C. Jordan,§*** David Cohen,††† Matthew R. Weir,‡‡‡ David Hiller,§§§ Preethi Prasad,‖‖‖ Robert N. Woodward,¶¶¶ Marica Grskovic,¶¶¶ John J. Sninsky,¶¶¶ James P. Yee,‖‖‖ and Daniel C. Brennan,**** for the Circulating Donor-Derived Cell-Free DNA in Blood for Diagnosing Active Rejection in Kidney Transplant Recipients (DART) Study Investigators

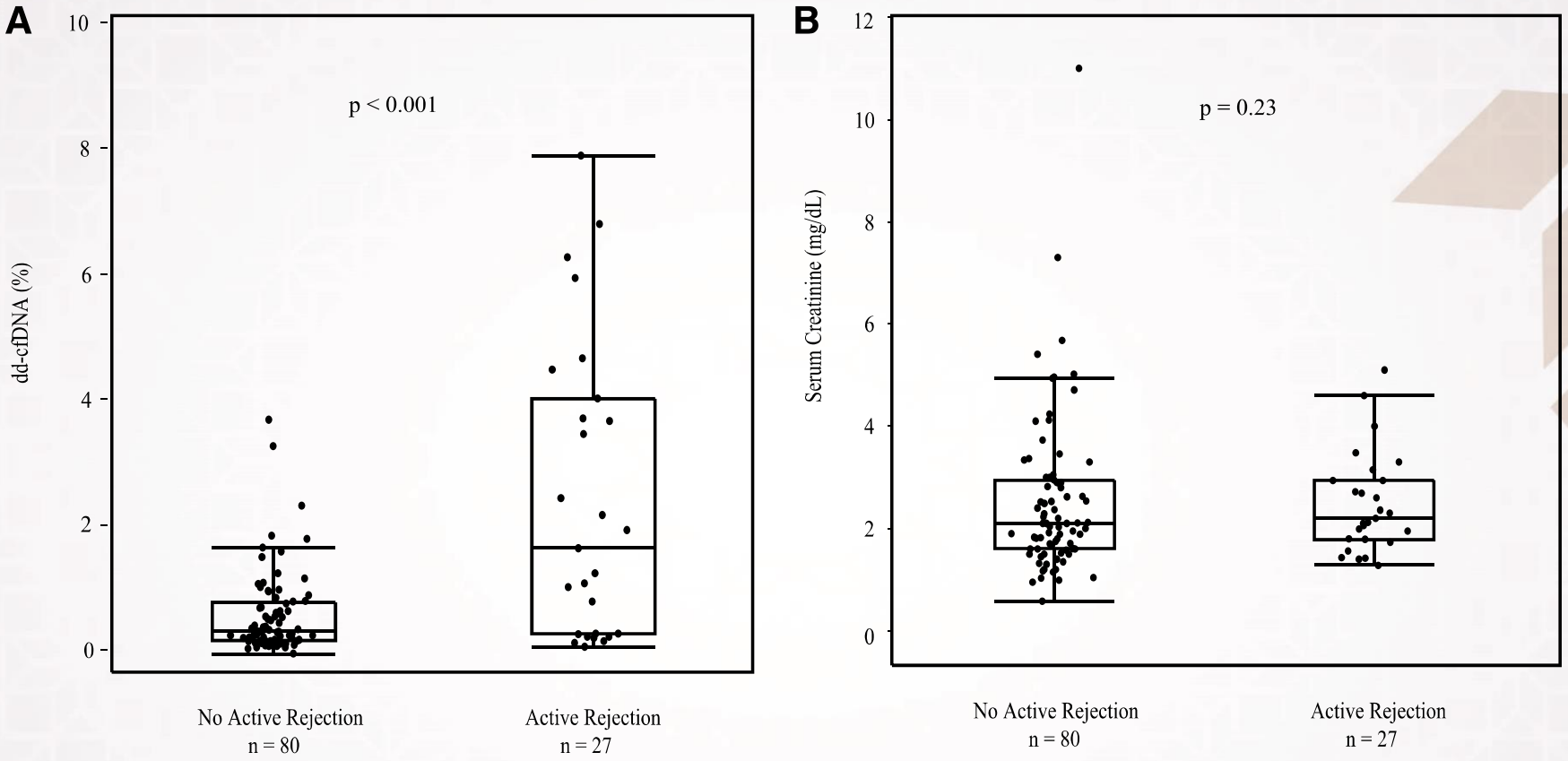
*Department of Medicine, University of Pennsylvania, Perelman School of Medicine and Penn Kidney Pancreas Transplant Program, Philadelphia, Pennsylvania; †Department of Surgery and Department of Microbiology and Immunology and ‡‡‡Division of Nephrology, Department of Medicine, University of Maryland School of Medicine, Baltimore, Maryland; ‡Department of Nephrology and Hypertension, Cleveland Clinic, Cleveland, Ohio; §Department of Medicine, David Geffen School of Medicine at the University of California Los Angeles, Los Angeles, California; ‖Department of Medicine, Vanderbilt University Medical Center, and Medical Specialties Clinic, Veteran Affairs Hospital Renal Transplant Program, Nashville, Tennessee; ¶Thomas Starzl Transplant Institute, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania; **Division of Transplantation, Department of Surgery, University of Minnesota, Minneapolis, Minnesota; ††Division of Nephrology, Department of Medicine, and ‡‡Division Transplantation, University of Alabama School of Medicine, Birmingham, Alabama; §§Division of Nephrology and Transplant, Department of Medicine, Indiana University School of Medicine, Indianapolis, Indiana; ‖‖Baylor Research Institute, Dallas Texas; ¶¶Division of Nephrology & Hypertension, Texas A&M Health Science Center College of Medicine, Temple, Texas; ***Division of Nephrology, Cedars-Sinai Medical Center, Los Angeles, California; †††Department of Surgery, Columbia University Medical Center, New York, New York; §§§Biostatistics, ‖‖‖Clinical Research, ¶¶¶Research and Development, CareDx, Inc., Brisbane, California; and ****Washington University School of Medicine, St. Louis, Missouri



Bloom et al, 2017

Performance Metric	Performance at 1% Threshold
ROC/AUC	0.74 (95% CI 0.61-8.85)
Sensitivity	85%
Specificity	59%
NPV	84%
PPV	61%

Bloom et al, 2017



Bloom et al, 2017

Science Translational Medicine
AAAS

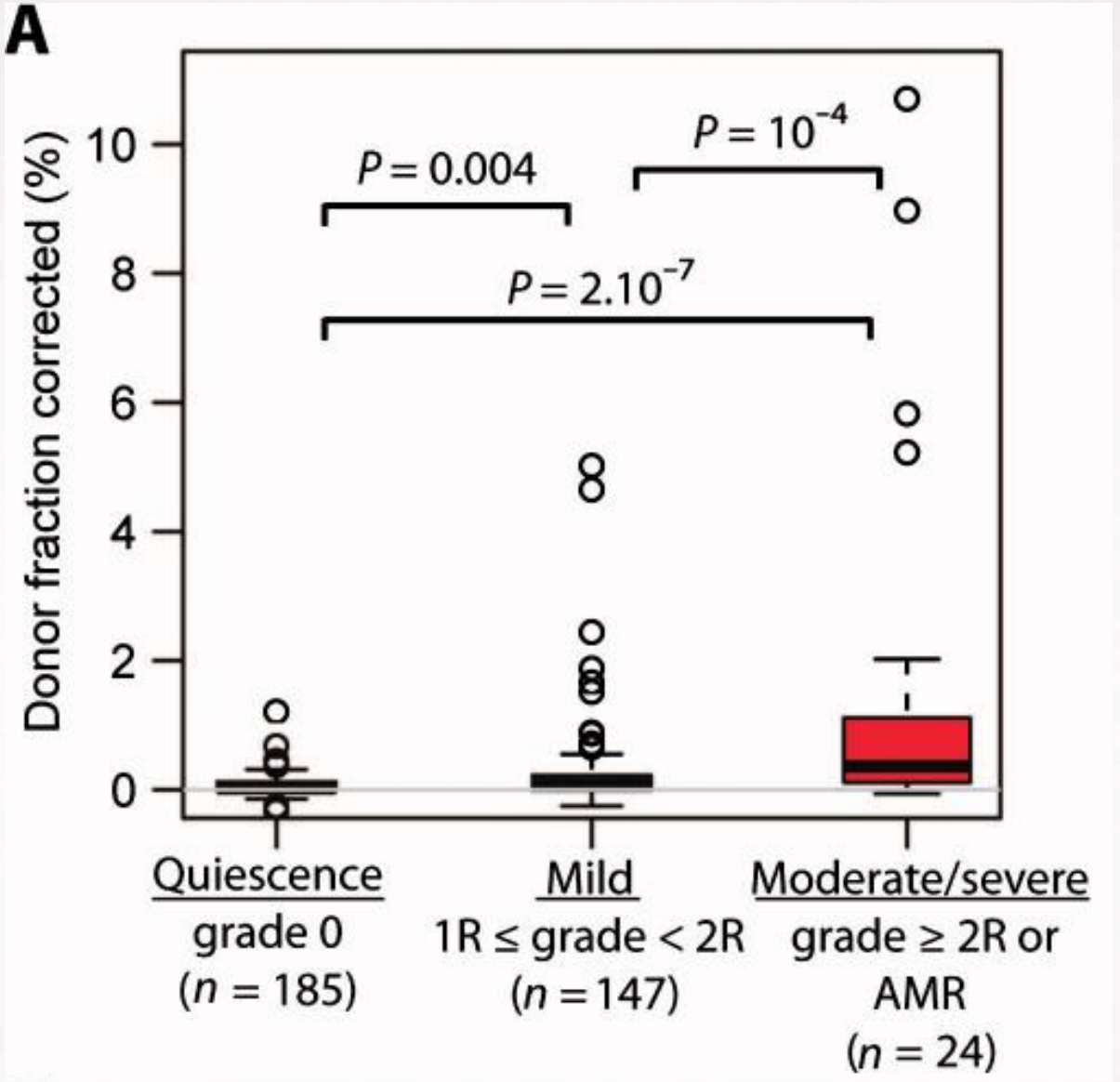
RESEARCH ARTICLE

GENOMICS

Circulating Cell-Free DNA Enables Noninvasive Diagnosis of Heart Transplant Rejection

Iwijn De Vlaminck,[1,2] Hannah A. Valantine,[3] Thomas M. Snyder,[1,2] Calvin Strehl,[3] Garrett Cohen,[3] Helen Luikart,[3] Norma F. Neff,[1,2] Jennifer Okamoto,[1,2] Daniel Bernstein,[4] Dana Weisshaar,[5] Stephen R. Quake,[1,2]* Kiran K. Khush[3]*

18 June 2014 Vol 6 Issue 241 241ra77



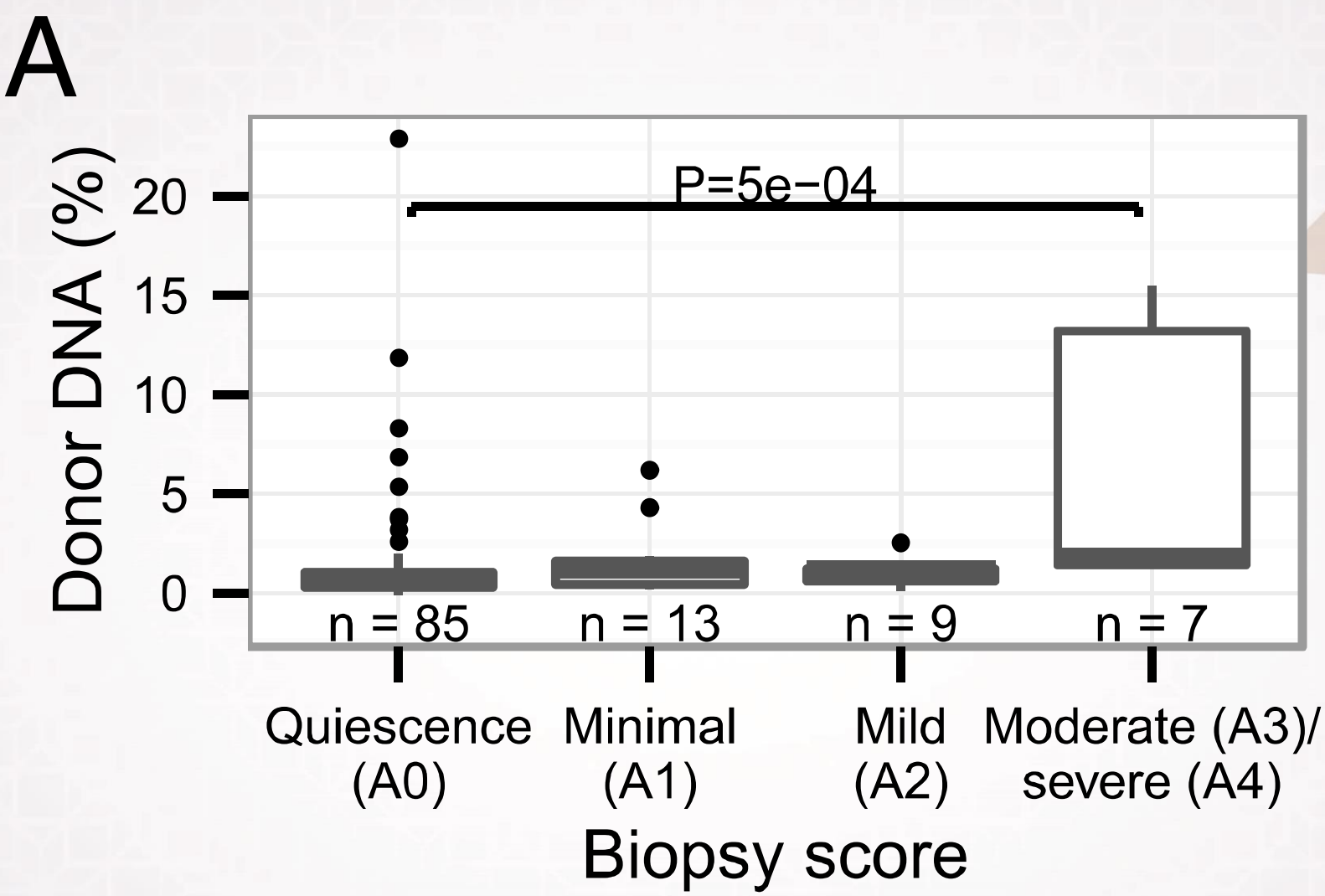
De Vlaminck et al, 2014

Noninvasive monitoring of infection and rejection after lung transplantation

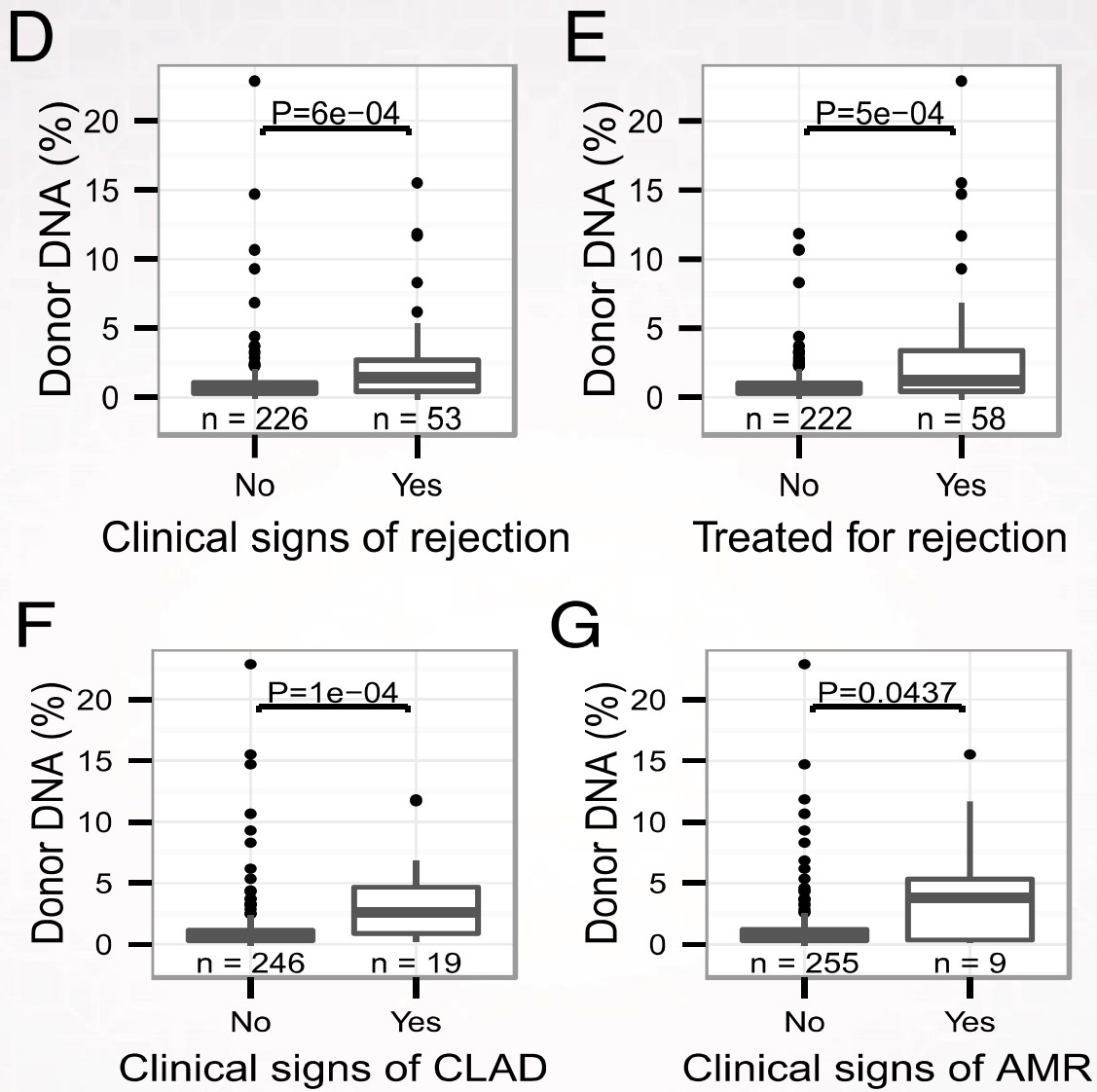
Iwijn De Vlaminck[a,b,c,1], Lance Martin[a,b,c,1], Michael Kertesz[a,b,c,2], Kapil Patel[d], Mark Kowarsky[a,b,c], Calvin Strehl[e], Garrett Cohen[e], Helen Luikart[e], Norma F. Neff[a,b,c], Jennifer Okamoto[a,b,c], Mark R. Nicolls[d], David Cornfield[d], David Weill[d], Hannah Valantine[e], Kiran K. Khush[e], and Stephen R. Quake[a,b,c,3]

[a]Department of Bioengineering, Stanford University, Stanford, CA 94305; [b]Department of Applied Physics, Stanford University, Stanford, CA 94305; [c]Howard Hughes Medical Institute, Stanford University, Stanford, CA 94305; [d]Division of Pulmonary and Critical Care Medicine, Stanford University School of Medicine, Stanford, CA 94305; and [e]Division of Cardiovascular Medicine, Stanford University School of Medicine, Stanford, CA 94305

13336–13341 | PNAS | October 27, 2015 | vol. 112 | no. 43



De Vlaminck et al, 2015



De Vlaminck et al, 2015

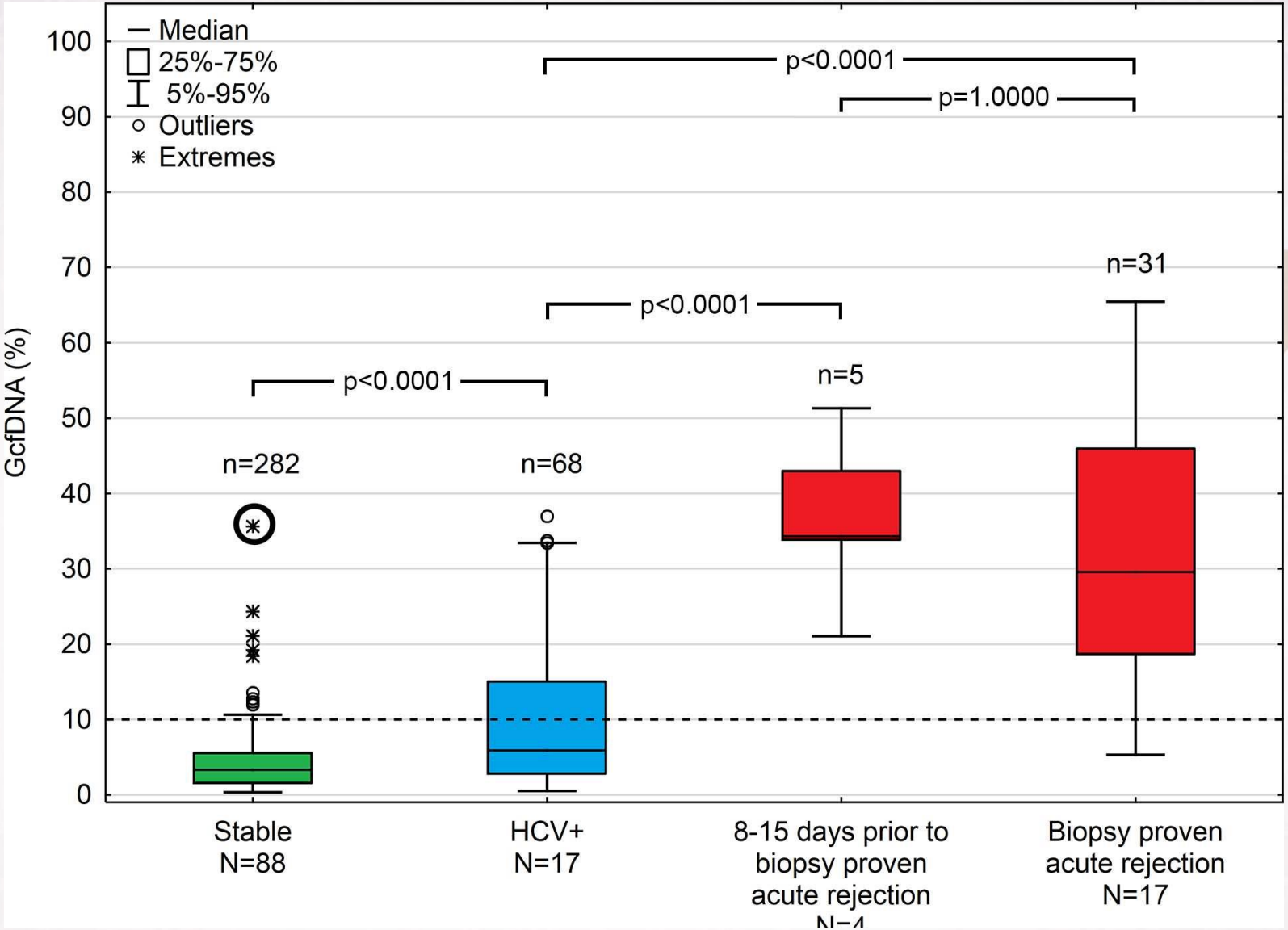
PLOS MEDICINE

RESEARCH ARTICLE

Graft-derived cell-free DNA, a noninvasive early rejection and graft damage marker in liver transplantation: A prospective, observational, multicenter cohort study

Ekkehard Schütz[1], Anna Fischer[2], Julia Beck[1], Markus Harden[3], Martina Koch[4], Tilo Wuensch[5], Martin Stockmann[5], Björn Nashan[4], Otto Kollmar[6], Johannes Matthaei[2], Philipp Kanzow[2], Philip D. Walson[2], Jürgen Brockmöller[2], Michael Oellerich[2]*

1 Chronix Biomedical, Göttingen, Germany, **2** Department of Clinical Pharmacology, University Medical Center Göttingen, Göttingen, Germany, **3** Department of Medical Statistics, University Medical Center Göttingen, Göttingen, Germany, **4** Department of Hepatobiliary Surgery and Transplantation, University Medical Center Hamburg-Eppendorf, Hamburg, Germany, **5** Department of Surgery, Charité–Universitätsmedizin Berlin, Berlin, Germany, **6** Department of General, Visceral and Pediatric Surgery, University Medical Center Göttingen, Göttingen, Germany



Schütz et al, 2017

ORIGINAL ARTICLE

Serial perioperative cell-free DNA levels in donors and recipients undergoing living donor liver transplantation

K. Prakash[1], S. Aggarwal[2], S. Bhardwaj[2], G. Ramakrishna[2] and C. K. Pandey[1]

[1]Department of Anaesthesiology and Critical Care, Institute of Liver and Biliary Sciences, Vasant Kunj, New Delhi, India
[2]Department of Molecular and Cellular Medicine, Institute of Liver and Biliary Sciences, Vasant Kunj, New Delhi, India

Acta Anaesthesiologica Scandinavica **61** (2017) 1084–1094

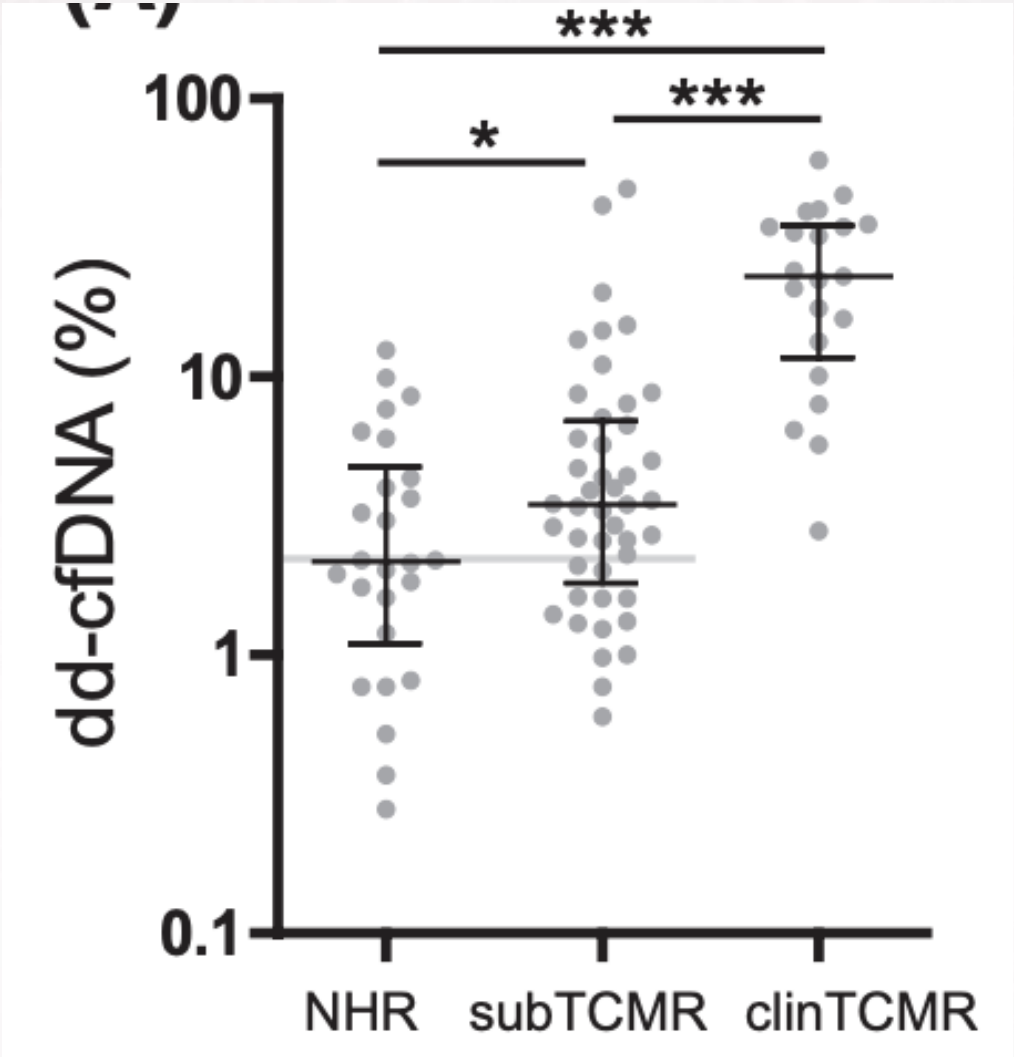
Received: 12 November 2021 | Accepted: 5 April 2022

DOI: 10.1002/lt.26479

ORIGINAL ARTICLE

Elevated fractional donor-derived cell-free DNA during subclinical graft injury after liver transplantation

Anna K. Baumann[1] | Julia Beck[2] | Theresa Kirchner[1] | Björn Hartleben[3] | Ekkehard Schütz[2] | Michael Oellerich[4] | Heiner Wedemeyer[1] | Elmar Jaeckel[1] | Richard Taubert[1]



Baumann et al, 2021

Received: 3 February 2023 | Revised: 21 March 2023 | Accepted: 25 March 2023

DOI: 10.1111/ctr.14984

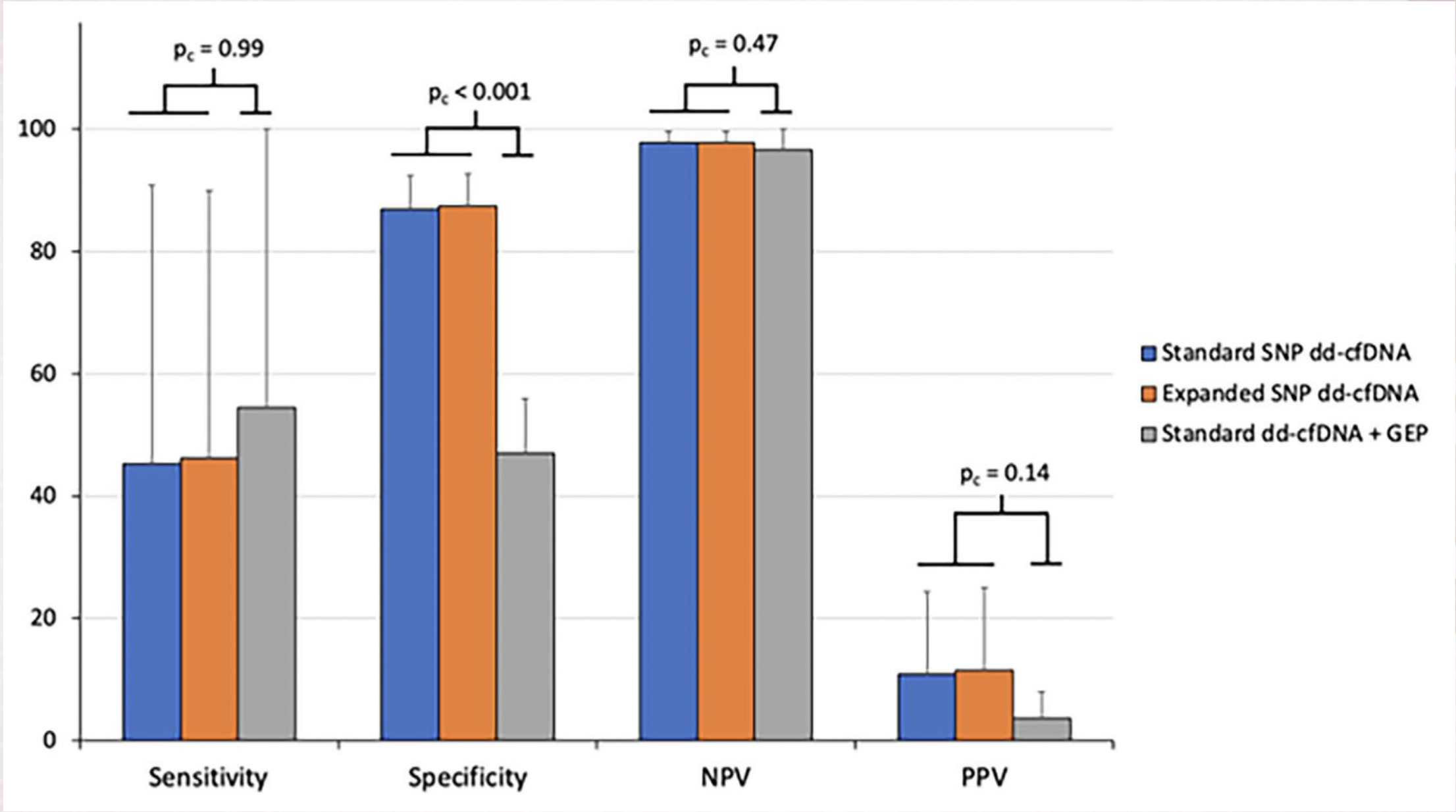
Clinical TRANSPLANTATION
The Journal of Clinical and Translational Research

WILEY

ORIGINAL ARTICLE

Comparison of two donor-derived cell-free DNA tests and a blood gene-expression profile test in heart transplantation

Nicholas Rodgers[1] | Bryn Gerding[1] | Vincenzo Cusi[1] | Florin Vaida[2] | Yuko Tada[1] | Gerald P. Morris[1] | Eric D. Adler[1] | Josef Stehlik[3] | Paul J. Kim[1]


p_c = 0.99
p_c < 0.001
p_c = 0.47
p_c = 0.14
100
80
60
40
20
0
Sensitivity
Specificity
NPV
PPV
Standard SNP dd-cfDNA
Expanded SNP dd-cfDNA
Standard dd-cfDNA + GEP

Implementation

Human Immunology 82 (2021) 838–849

Contents lists available at ScienceDirect

journal homepage: www.elsevier.com/locate/humimm

Research article

A practical guide to chimerism analysis: Review of the literature and testing practices worldwide

Amanda G. Blouin [a], Fei Ye [a], Jenifer Williams [b], Medhat Askar [a,b,c,*]

[a] Department of Laboratory Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, United States
[b] Department of Pathology & Laboratory Medicine, Baylor University Medical Center, Dallas, TX, United States
[c] Department of Pathology and Laboratory Medicine, Texas A&M Health Science Center College of Medicine, United States

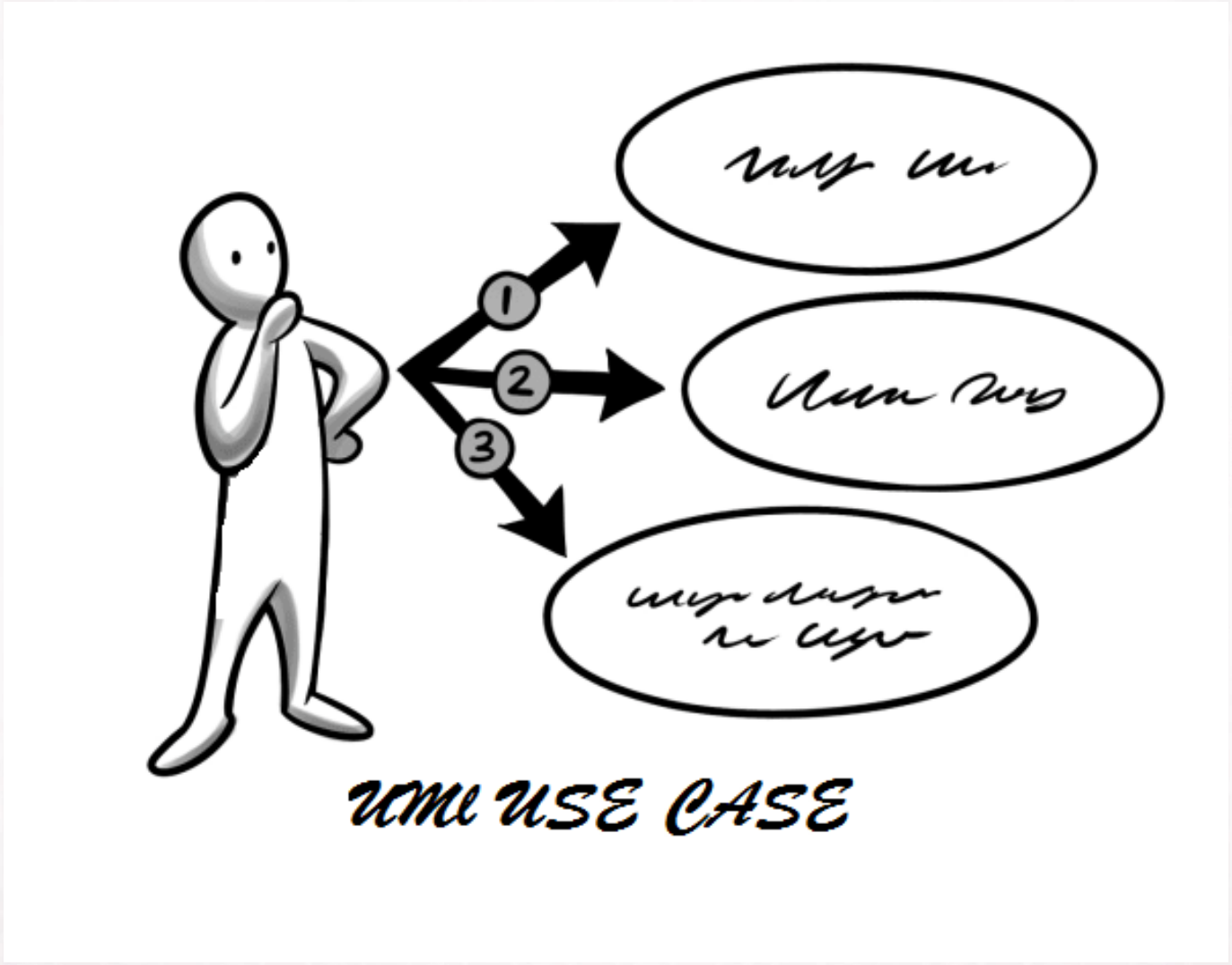
Implementation

- Same performance characteristics as NGS based chimerism analysis
- Collection tube to preserve cfDNA integrity (.e.g., Cell-Free DNA BCT STRECK, PAXgene Blood ccfDNA Tube, …)
- cfDNA extraction to produce enough yield and integrity (e.g., Maxwell® RSC ccfDNA LV Plasma Kit)

Implementation

%	V1 + V2	V3+V4
0.5	0.32%	0.52%
1	0.93%	0.98%
2	2.06%	2.33%
5	4.76%	5.40%
10	9.19%	10.12%

Use Case

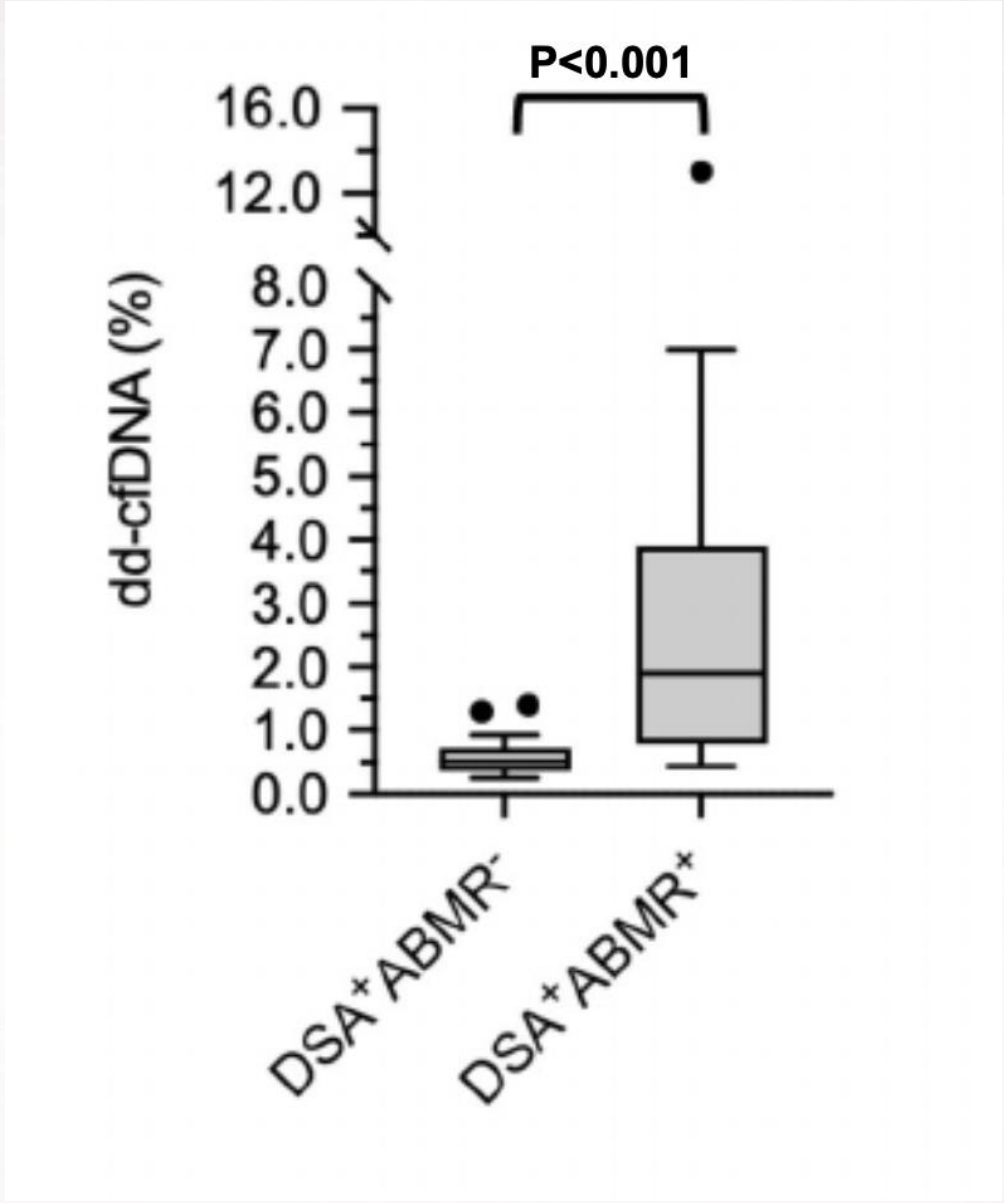


Transplant International

ORIGINAL ARTICLE

Diagnostic value of donor-derived cell-free DNA to predict antibody-mediated rejection in donor-specific antibody-positive renal allograft recipients

Katharina A. Mayer[1], Konstantin Doberer[1], Amanda Tillgren[2], Thierry Viard[2], Susanne Haindl[1], Sebastian Krivanec[1], Roman Reindl-Schwaighofer[1], Michael Eder[1], Farsad Eskandary[1], Silvia Casas[2], Markus Wahrmann[1], Heinz Regele[3] & Georg A. Böhmig[1]



Mayer et al, 2021

Non-Invasive Testing

- Transplant date: 23/09/2021
- Elevated creatinine: 15/4/2022
- DSA: 17/4/2022 (DR13: 2000 MFI)
- cfDNA: 17/4/2022 (4.65%)

Biopsy

Diagnosis:

- Acute T-cell mediated rejection BANFF Grade IB

- Acute antibody mediated rejection (C4d minimally positive)

- No viral inclusions identified (SV-40 negative)

Follow Up Non-Invasive Testing

- DSA: 28/4/2022 (DR13: 1000 MFI)
- cfDNA: 28/4/2022 (0.81%)

$DSA^+/cfDNA^+$ vs. $DSA^+/cfDNA^-$

Date	Transplant Date	Sample Date	Donor %	DSA (MFI)
Case #1	23-Sep-2021	17-Apr-2022	4.68%	DR13 (2,000)
		28-Apr-2022	0.81%	DR13 (1,000)
Case #2	3-Apr-2022	21-Apr-2022	0.46%	DR53 (2,000)
		16-May-2022	0.15%	DR53 (1,400)
Case #3	4-Apr-2022	21-Apr-2022	0.99%	None
		16-May-2022	0.40%	None

Summary

- Advances in molecular transplant diagnostics are biologically plausible and less subjective
- They offer the promise to standardize diagnosis and treatment of rejection
- They offer a promising non-invasive diagnostic and monitoring tool for early detection of ABMR, monitoring response to treatment as well as distinguishing a population of immune quiescent who may benefit from immunosuppression minimization

Stay Tuned

Thank you

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