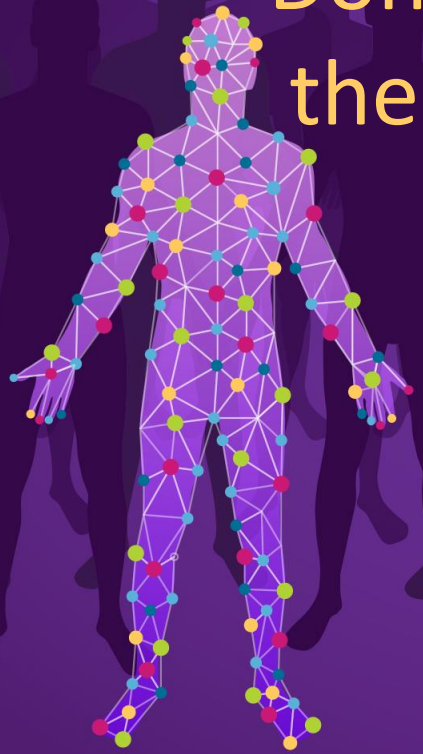
AST | AMERICAN SOCIETY OF TRANSPLANTATION

Donor-derived cell-free DNA: Has the answer been there all along?



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Stanford University

CEOT CUTTING EDGE of TRANSPLANTATION

TRANSPLANT SUMMIT 2019

NO SIZE FITS ALL: *Uncovering the Potential of Personalized Transplantation*

FEBRUARY 21–23, 2019 • ARIZONA BILTMORE • PHOENIX, AZ

Disclosure

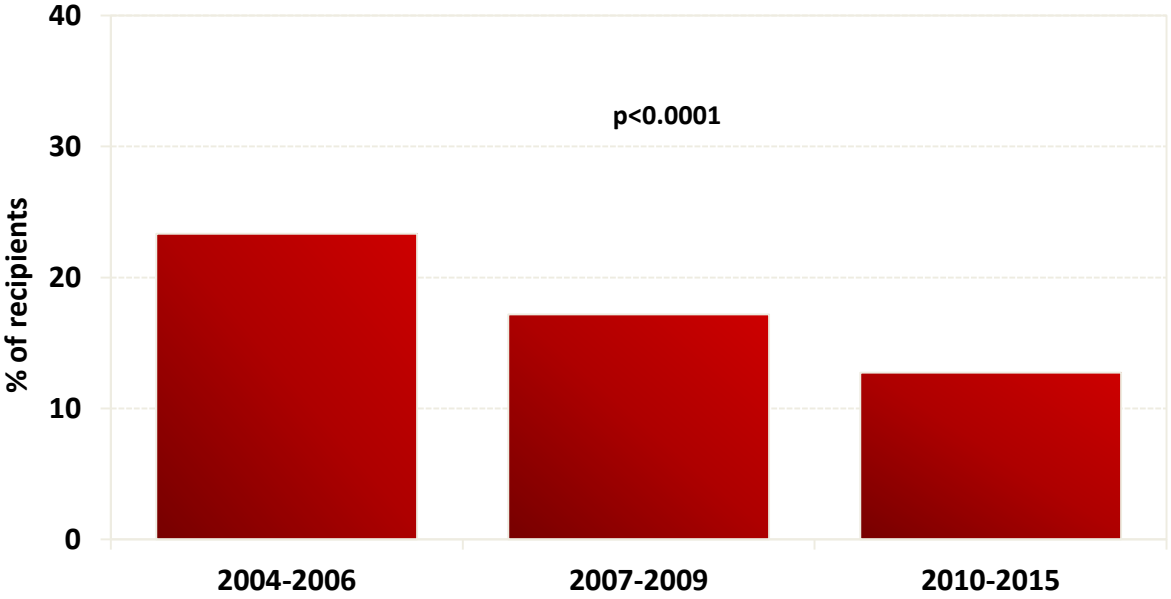
CareDx, Inc: scientific advisor, speakers' bureau, research grant recipient, steering committee

Learning Objectives

- To appreciate the need for non-invasive biomarkers for surveillance of graft health
- To understand the principles of cell-free DNA testing
- To review major studies to-date of donor-derived cell free DNA testing for acute rejection monitoring after heart transplantation
  - Stanford shotgun sequencing (GTD- Genome Transplant Dynamics)
  - Targeted sequencing
    - AlloSure®
    - myTAI$_{HEART}$
  - One genome method

Need for acute rejection surveillance

Adult Heart Transplants
% of Recipients Experiencing ***Treated*** **Rejection** Between Transplant Discharge and 1-Year Follow-Up by Era

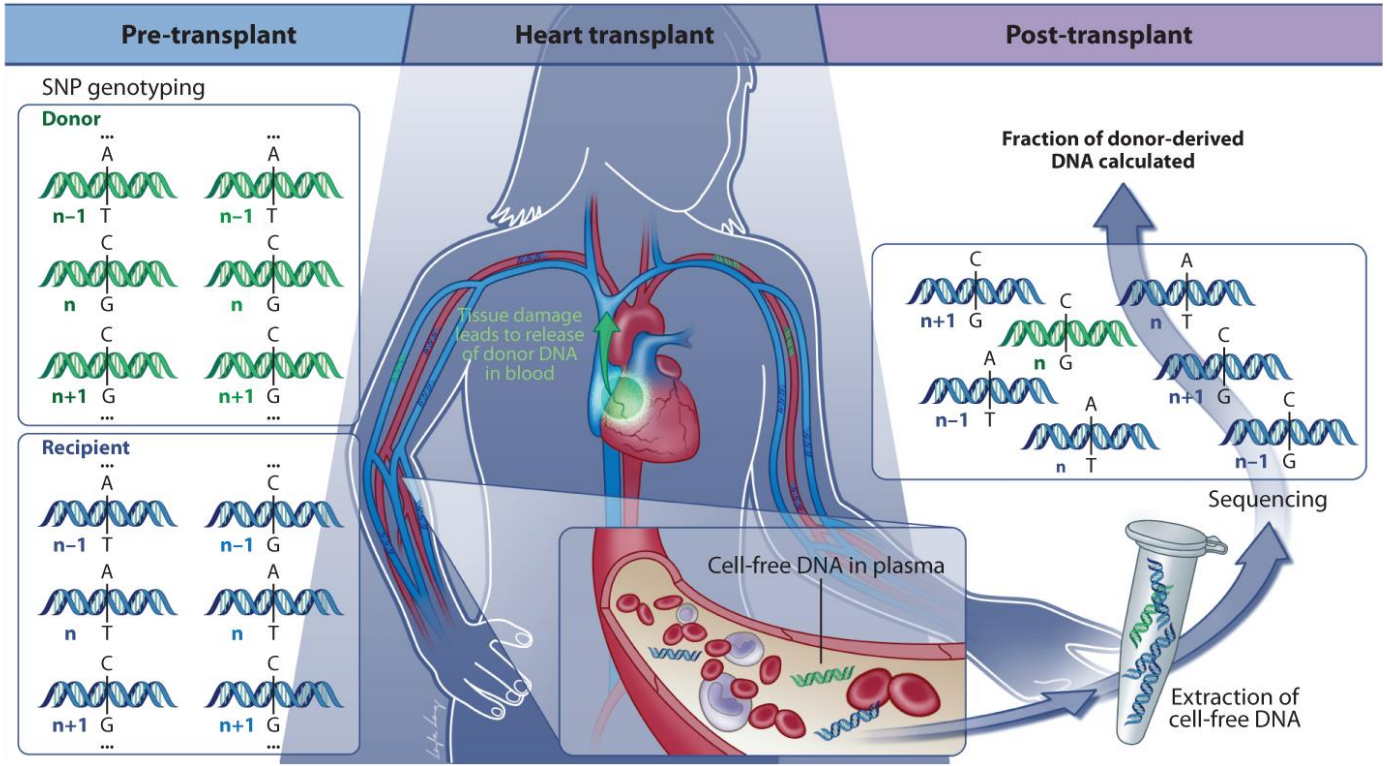


Treated rejection = Recipient was reported to (1) have at least one acute rejection episode that was treated with an anti-rejection agent; or (2) have been hospitalized for rejection.

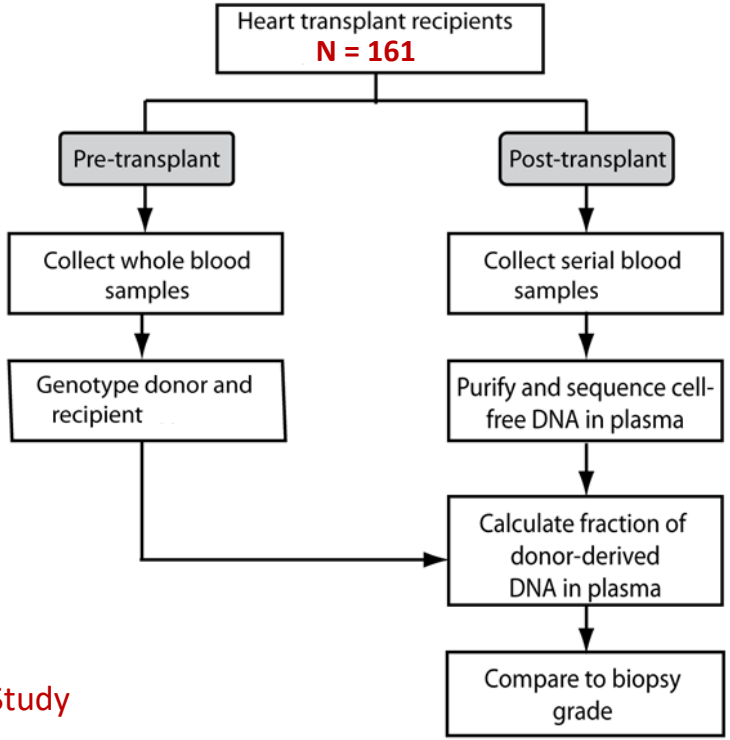
Non-Invasive Markers of Rejection

- Electrocardiogram
  - Surface
  - Intramyocardial
- Cardiac Imaging
  - Echocardiography
  - Magnetic Resonance Imaging
  - Nuclear Imaging
- Biomarkers
  - B-type Natriuretic Peptide (BNP)
  - Troponin (TnI, TnT)
  - High-sensitivity CRP
- Genomic Markers
  - Gene Expression Profiling
  - Cell-free DNA
  - mRNA/miRNA
  - Proteomics

Cell-Free DNA: a promising post-transplant biomarker

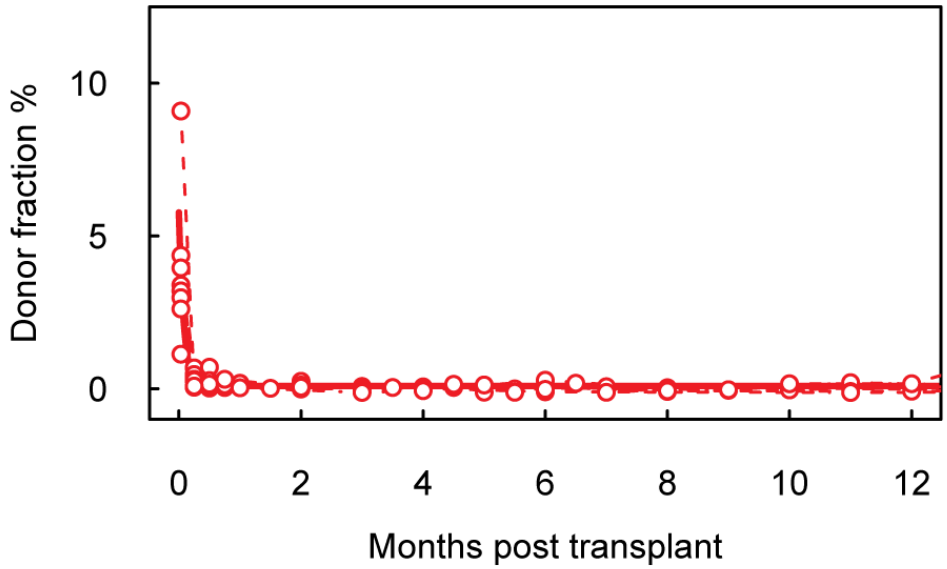


cfDNA prospective study design



Genome Transplant Dynamics Study
NIH 1RC4AI092673

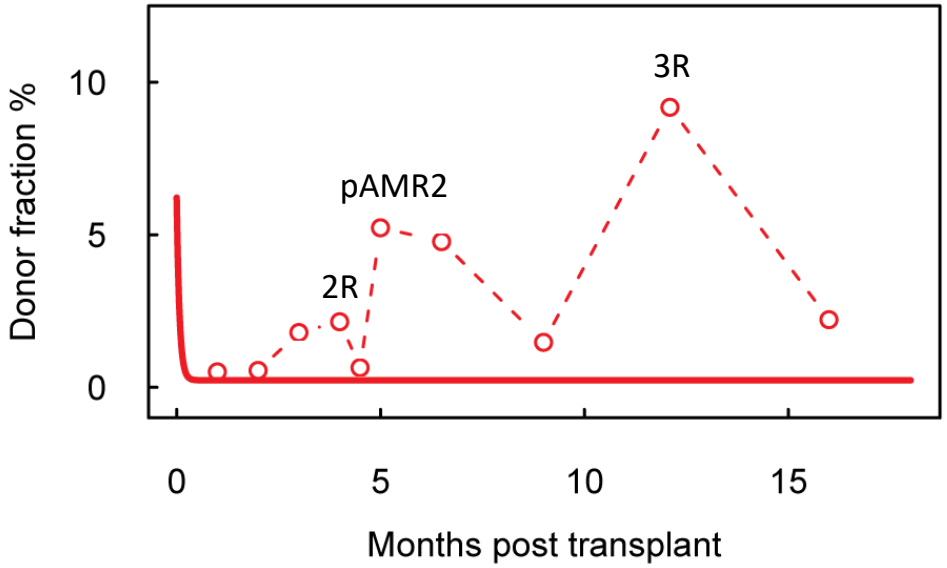
dd-cfDNA signal in absence of rejection



Elevated signal immediately post transplant followed by a quick decay (2.4 days) to a low baseline level

De Vlaminck, Sci Trans Med, 2014

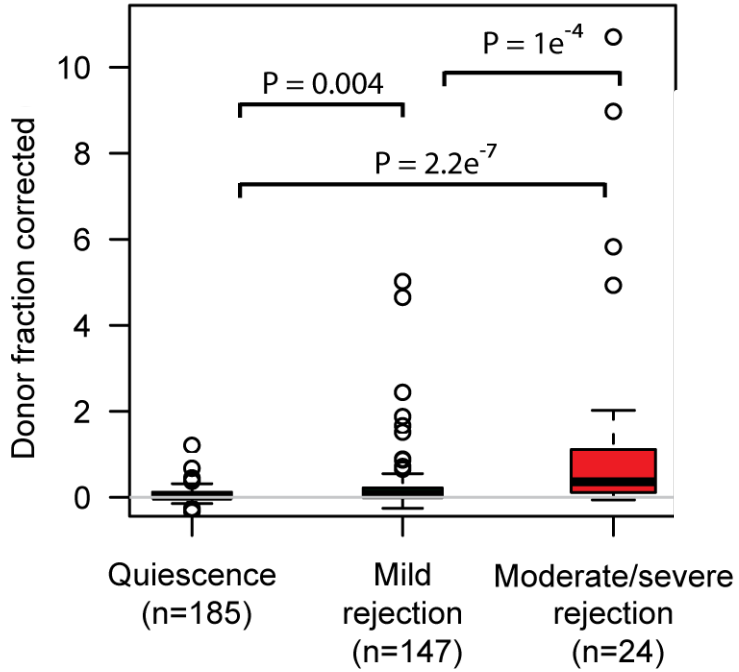
dd-cfDNA signal at time of acute rejection



Elevated donor DNA at time of rejection

De Vlaminck, Sci Trans Med, 2014

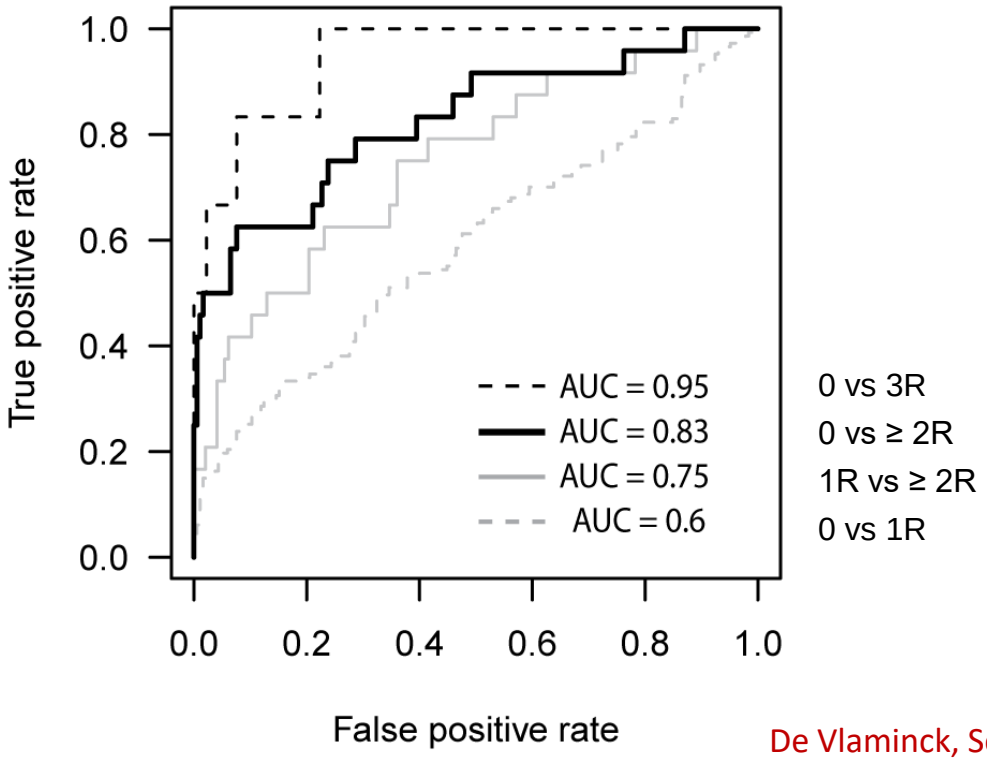
Comparison of dd-cfDNA levels: no rejection vs rejection



Significant increase in fraction of donor-derived DNA at rejection

De Vlaminck, Sci Trans Med, 2014

Test performance: Detection of acute rejection



De Vlaminck, Sci Trans Med, 2014

dd-cfDNA: A Rapidly Evolving Technology

- Technology used in previous studies to measure SNP alleles:
  - Shotgun sequencing methods (Stanford) (1)
  - Targeted amplification (Wisconsin) (2)
  - Both requiring recipient AND donor genotypes

- New approaches (AlloSure, myTAI$_{HEART}$, one genome method) have been developed to discriminate donor from recipient after sequencing cfDNA from a recipient blood sample only (3, 4)

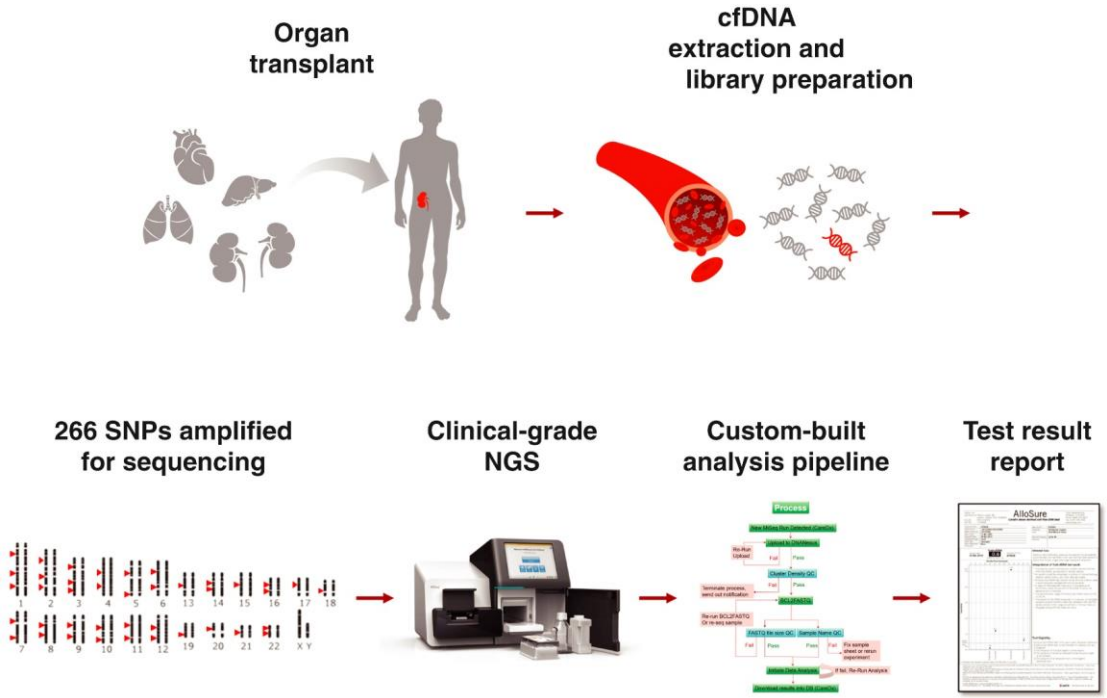
(1) Snyder et al., PNAS, 2011
De Vlaminck et al., Sci Transl Med, 2014

(2) Beck et al., Clin Chem, 2013
Hidestrand et al., JACC, 2014

(3) Sharon E et al. PLoS Comput Biol, 2017

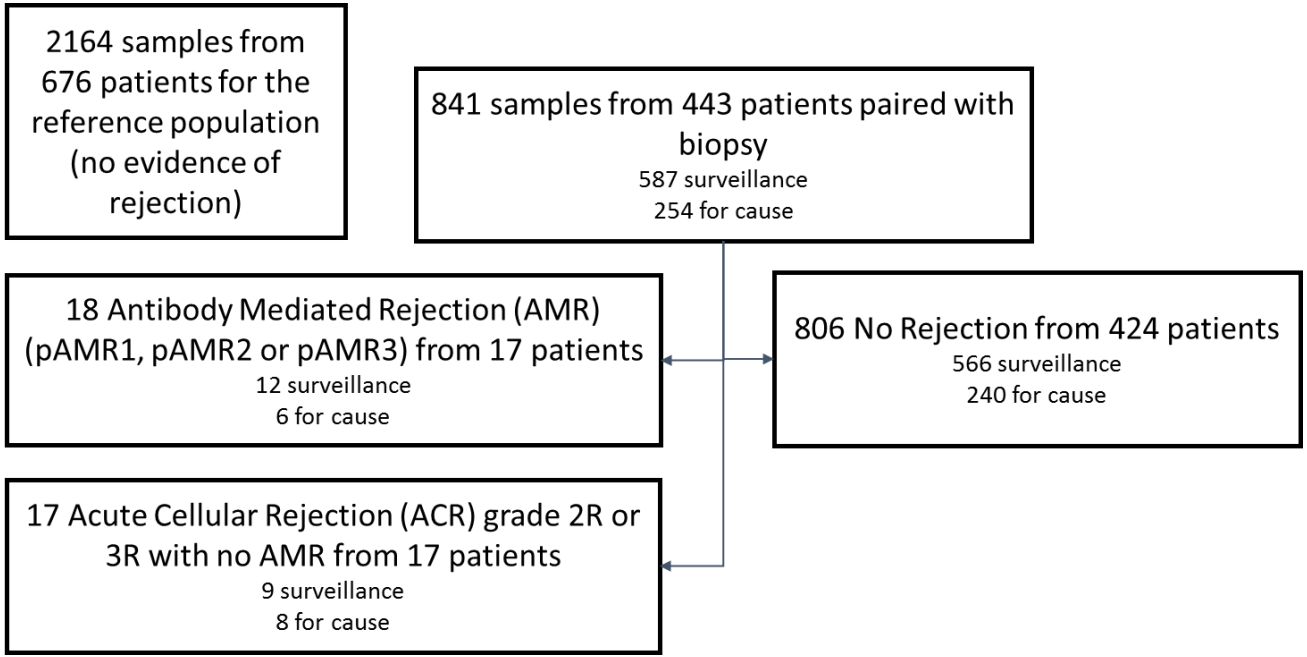
(4) Grskovic et al, J Mol Diagnostics, 2016

AlloSure® dd-cfDNA assay



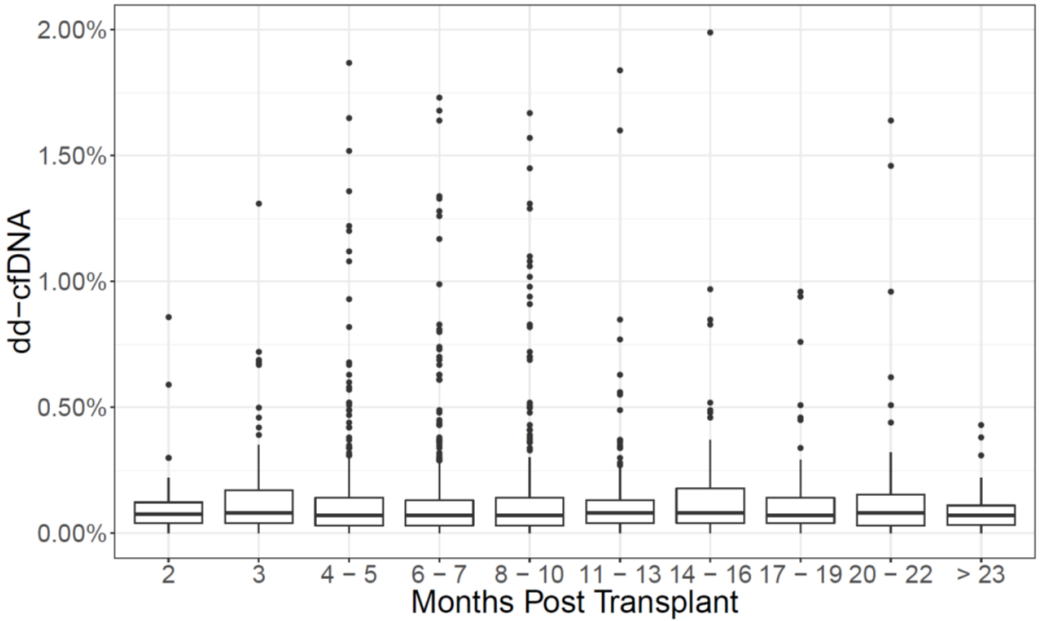
Grskovic, M. *J Molecular Diagnostics,* 2016

Prospective AlloSure® study in heart transplantation: D-OAR Registry



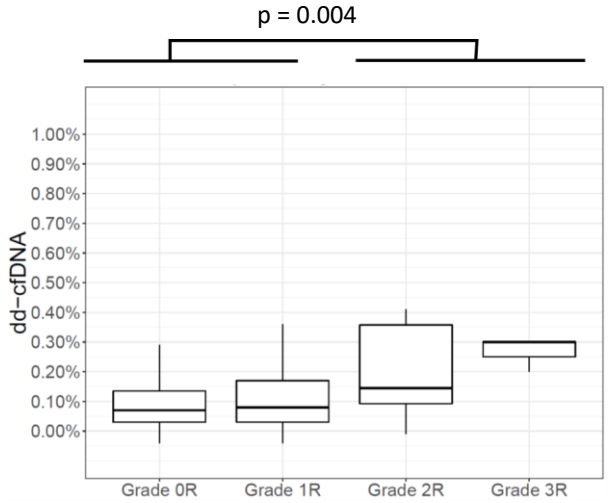
Khush K, *submitted for publication*

D-OAR Study: dd-cfDNA levels post-transplant (no rejection)

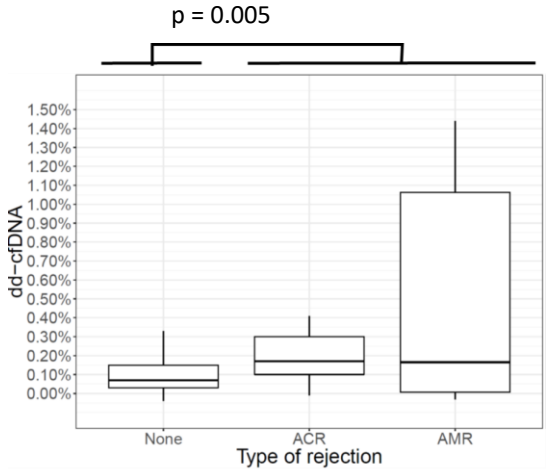


Khush K, *submitted for publication*

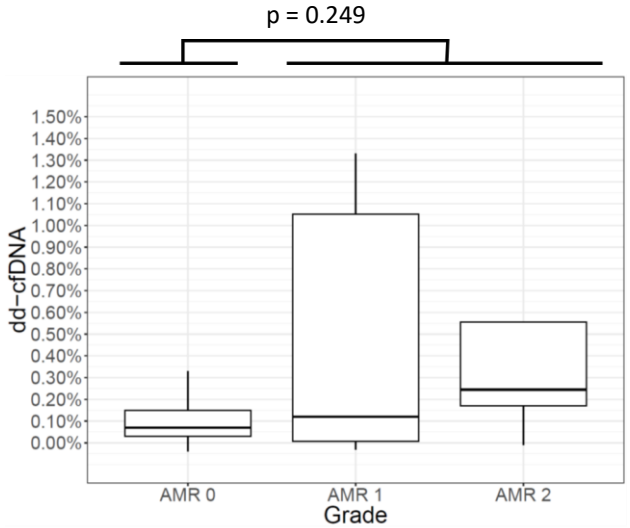
D-OAR Study Results



	Grade 0R	Grade 1R	Grade 2R	Grade 3R
N:	451	355	14	3
Med:	0.07%	0.08%	0.15%	0.30%



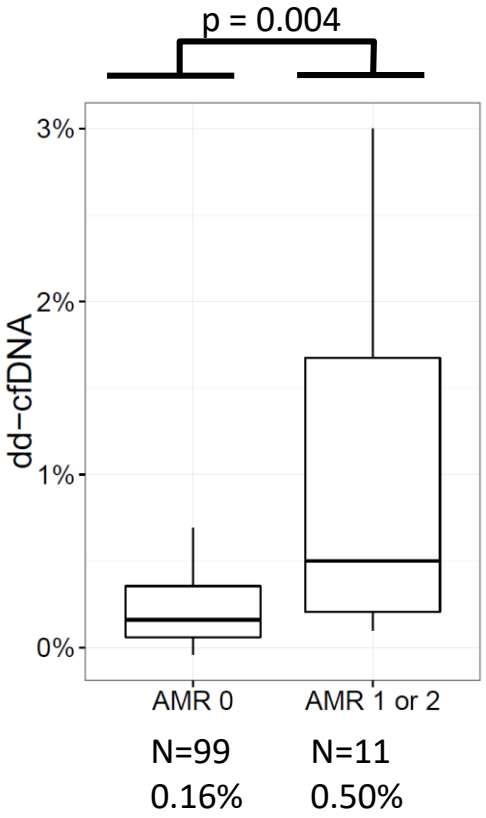
	None	ACR	AMR
N:	806	17	18
Med:	0.07%	0.17%	0.17%



	AMR 0	AMR 1	AMR 2
N:	676	14	4
Med:	0.07%	0.12%	0.25%

Khush K, *submitted for publication*

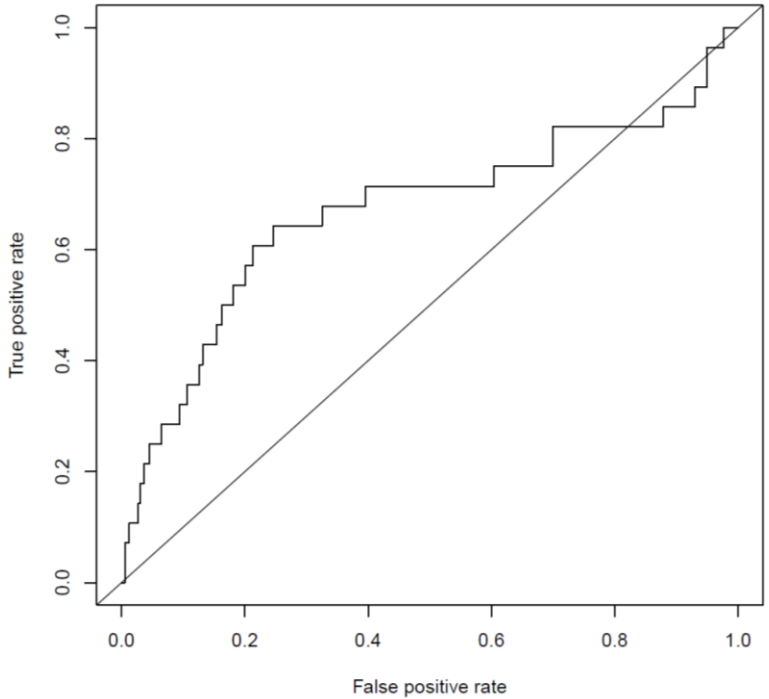
D-OAR Study Results: Cedars Sinai cohort



33 patients, 110 samples

Khush K, *submitted for publication*

AlloSure test performance



Threshold 0.2%

AUC 0.64

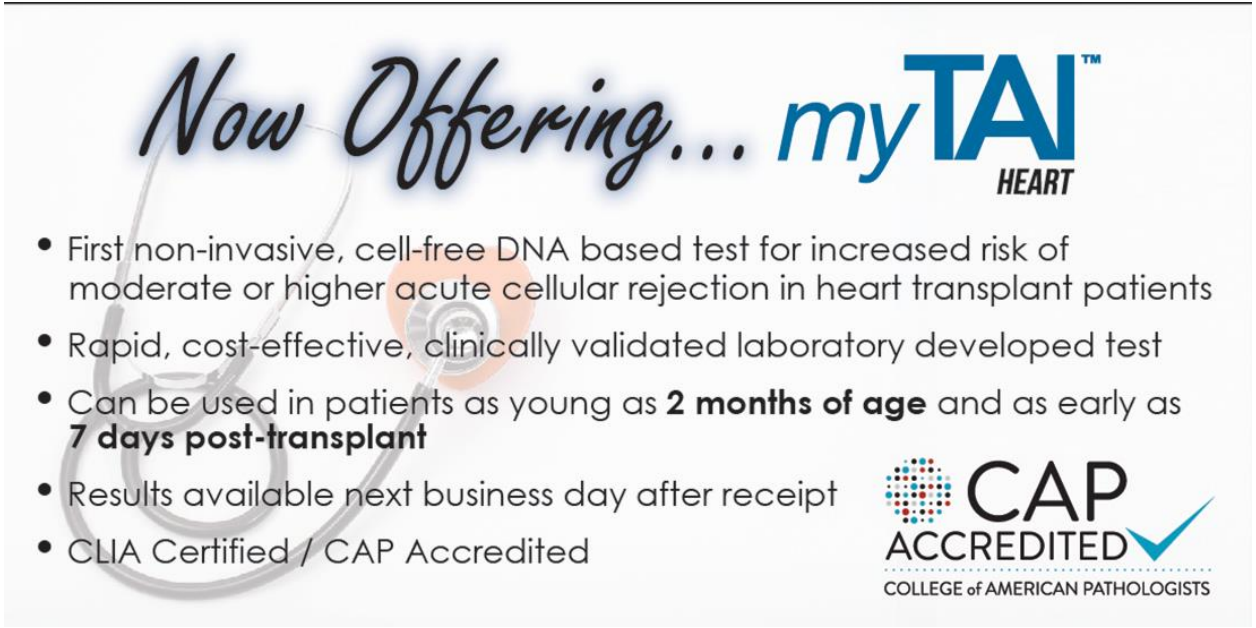
Sensitivity 44%

Specificity 80%

PPV 9%

NPV 97%

Khush K, *submitted for publication*


Now Offering... myTAI™ HEART
• First non-invasive, cell-free DNA based test for increased risk of moderate or higher acute cellular rejection in heart transplant patients
• Rapid, cost-effective, clinically validated laboratory developed test
• Can be used in patients as young as 2 months of age and as early as 7 days post-transplant
• Results available next business day after receipt
• CLIA Certified / CAP Accredited
CAP ACCREDITED
COLLEGE of AMERICAN PATHOLOGISTS

myTAI$_{HEART}$

- Targeted sequencing “relies on selected highly-informative genomic regions” [Digital Analysis of Selected Regions (DANSR)]
- Method 1: donor and recipient genotyped
- Method 2: recipient genotyping only
- 88 pediatric heart transplant patients, mean age 13 years (0.1-30 years)
- 158 blood samples paired with biopsy

Hidestrand M, et al. JACC, 2014
Ragalie W, et al. JACC, 2018

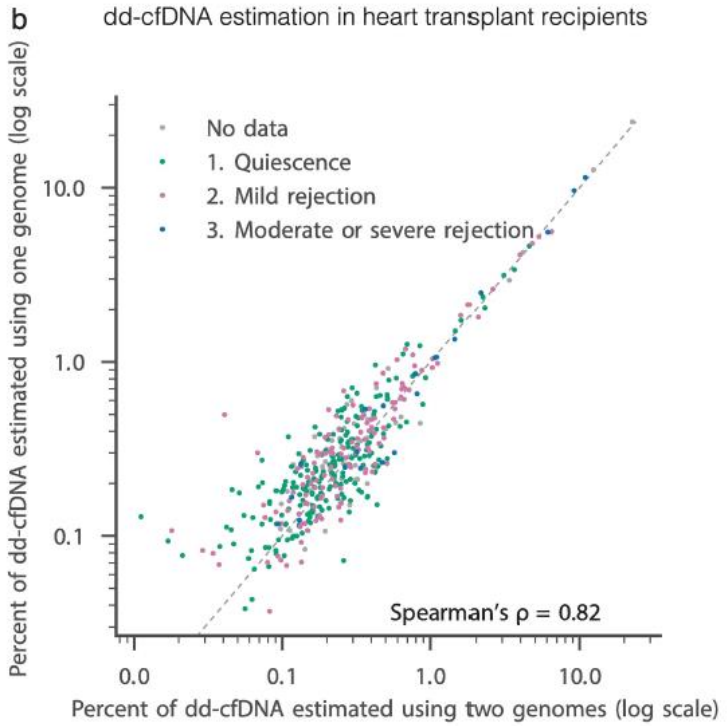
myTAI$_{HEART}$

Biopsy Grade	N	% dd-cfDNA (threshold 0.2%)	
		Method 1	Method 2
0R	134	0.11%	0.25%
1R	21	0.37%	0.89%
2R	3	0.97%	1.22%
3R	0	-	-

Method	Comparison	P-value	AUC
1	0R vs 1R/2R	0.02	0.78
2	0R vs 1R/2R	<0.001	0.84

“One genome” method

- Shotgun sequencing of total cfDNA
- Computational approach to estimate dd-cfDNA levels in the absence of a donor genotype



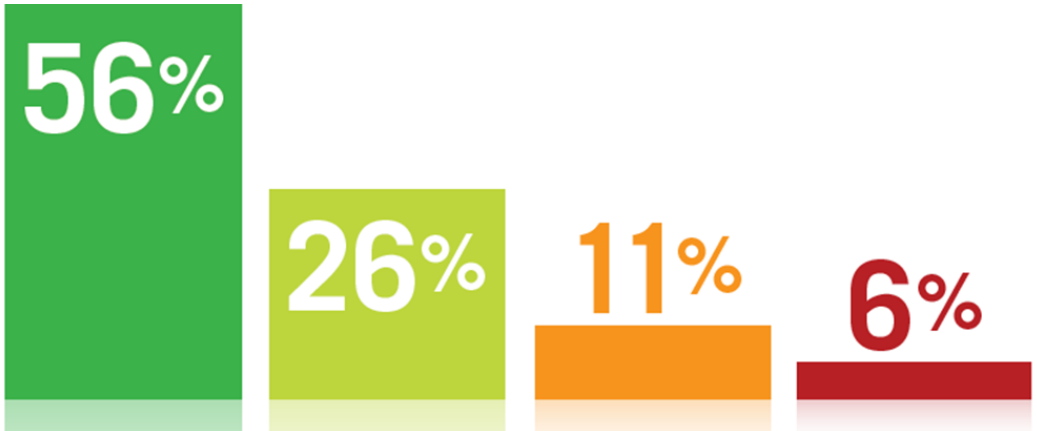
Sharon E, et al. PLOS Computational Biology, 2017

SHORE Study: Surveillance HeartCare Outcomes Registry

- HeartCare = AlloMap + AlloSure

- Hypothesis:
  - AlloMap (gene expression test) detects immune activation and is clinically validated for ACR monitoring
  - AlloSure (cfDNA assay) detects graft injury and is validated for ACR and AMR monitoring
  - Perhaps a COMBINED AlloMap + AlloSure monitoring approach will be better than either test alone

SHORE Study: Surveillance HeartCare Outcomes Registry



	56%	26%	11%	6%
AlloMap	Low	High	Low	High
AlloSure-Heart	Low	Low	High	High

SHORE study design

- 5 year study
- 35 sites in US
- 1,600 patients

- **Primary objective**: To assess the clinical utility of surveillance using HeartCare testing, in association with the clinical care of heart transplant recipients

- **Endpoints**: deaths, number of biopsies, number of rejection events, measures of graft function

Heart Allograft Routine Testing Schedule (HARTS)

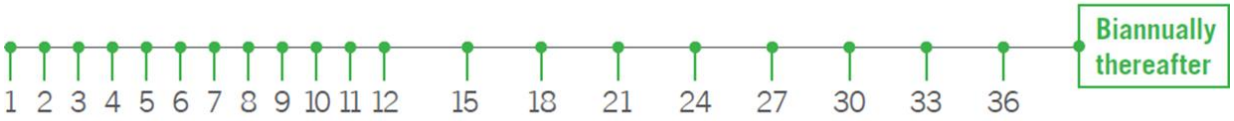
A blood test can be administered more frequently and conveniently than an invasive procedure, providing for more continuous rejection surveillance.

monthly
First 1 – 12 months

quarterly
2nd – 3rd Year

biannually
4th – 5th Year +

HARTS TIMELINE



Courtesy of CareDx, Inc.

Conclusions: dd-cfDNA for heart transplant monitoring

- We have come a long way in the quest for non-invasive ways to assess graft health after transplant
- Fewer biopsies are being performed than ever before
- Fewer procedural complications, more satisfied patients
- AlloSure has been tested in prospective registry study (D-OAR)
  - Detects both ACR and AMR at a threshold of 0.2%
- Upcoming SHORE study (CareDx): Combined AlloMap + AlloSure for heart transplant monitoring
- $MyTAI_{HEART}$ is available for use (pediatric patients)
- “One genome method” is being studied for clinical use

THANK YOU