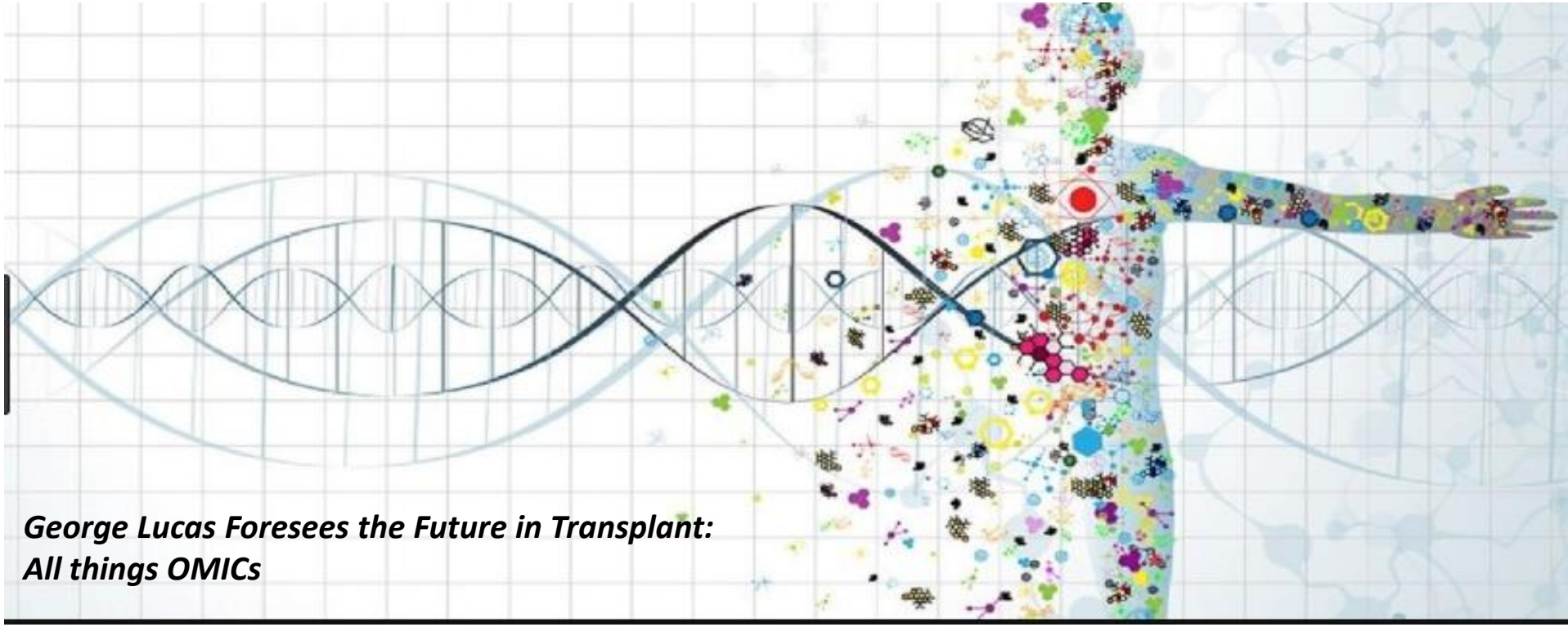
SHARK TANK: The FORCE is in the Blood

Minnie Sarwal

MD, PhD, MRCP, FRCP, DCH

Professor, Surgery, Medicine, Pediatrics
Director, Precision Transplant Medicine
University of California San Francisco, USA

Founder
Organ-I, USA
KITBio, USA



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CEOT, 2019, Arizona

UCSF

Disclosures

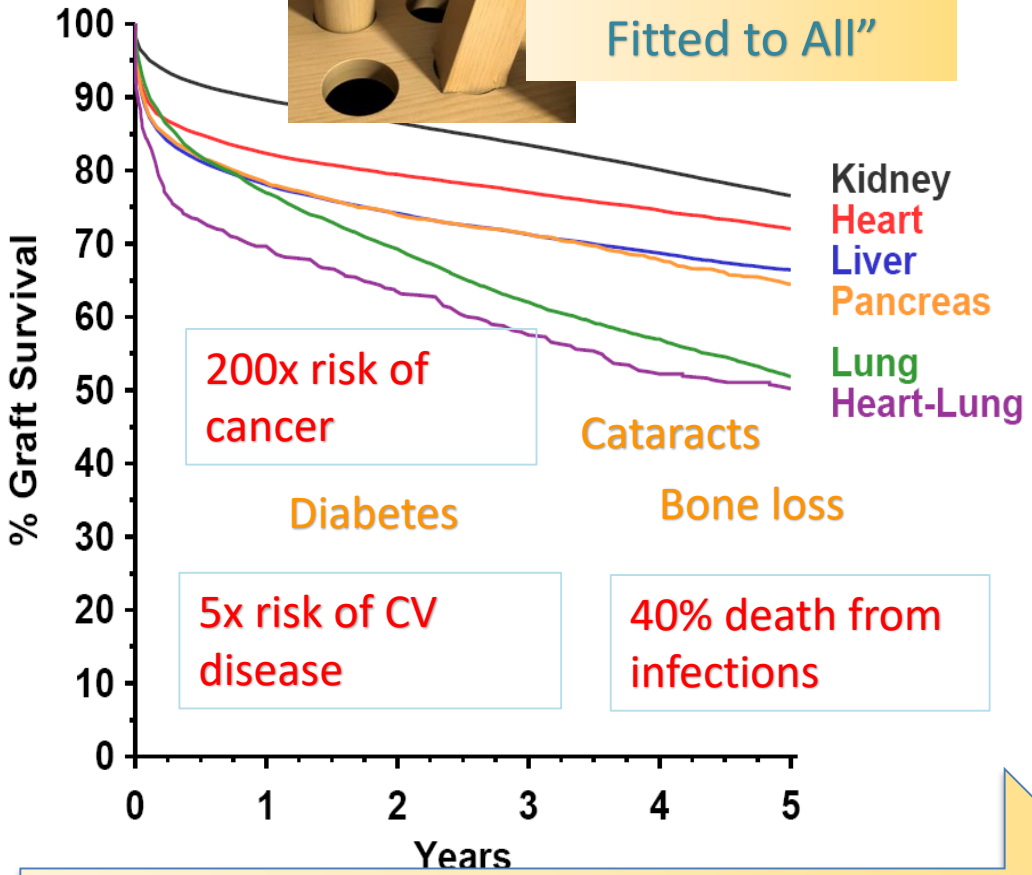
- **Scientific founder and advisory board memberships**
  - FDA Science Board Member
  - Terasaki Foundation
  - Founder, Organ-I, TRAP
  - Skyline Ventures, Mohr Davidow, Artiman Ventures

- **Past or present consultancy**
  - Bristol Meyers Squibb
  - Novartis
  - Roche
  - Astellas
  - Immucor
  - Jazz
  - Genentech 1
  - Genzyme
  - Ionis
  - Vitaeris

The Need : Precision Medicine in Tx

UNOS, SRTR

Rx: "One Size Fitted to All"



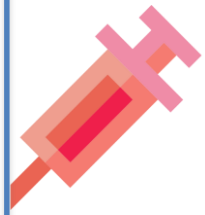
Rx: "One Size" *Cannot* be Fitted to All

Incomplete Donor/Recipient Matching

Archaic Diagnostic Tools for Rejection: LATE Detection

Serum creatinine

- Insensitive
- Not specific
- Late: 60% injured



Kidney biopsy

- Expensive
- Invasive
- Inter-operator variability

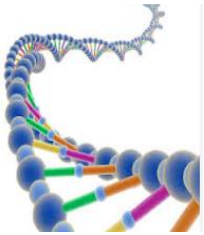
Acute Rejection

Untimely Tx Loss By ~10 yrs

Delivering the promise of precision medicine in transplantation by Blood Based OMICS

Pre-Transplant

Post-Transplant

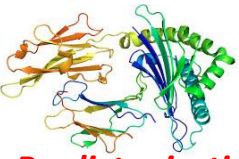


TRAP123

Transplant Risk Assessment Panel

Donor selection

Terasaki Foundation

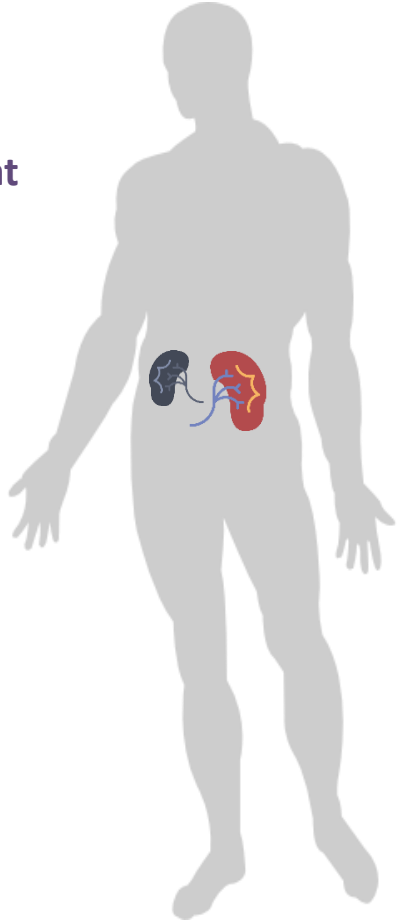


TxSeq

Transplant Immune Repertoire Sequencing

Predict rejection BEFORE tx

adaptive technologies



ddcfDNA

natera®

kSORT

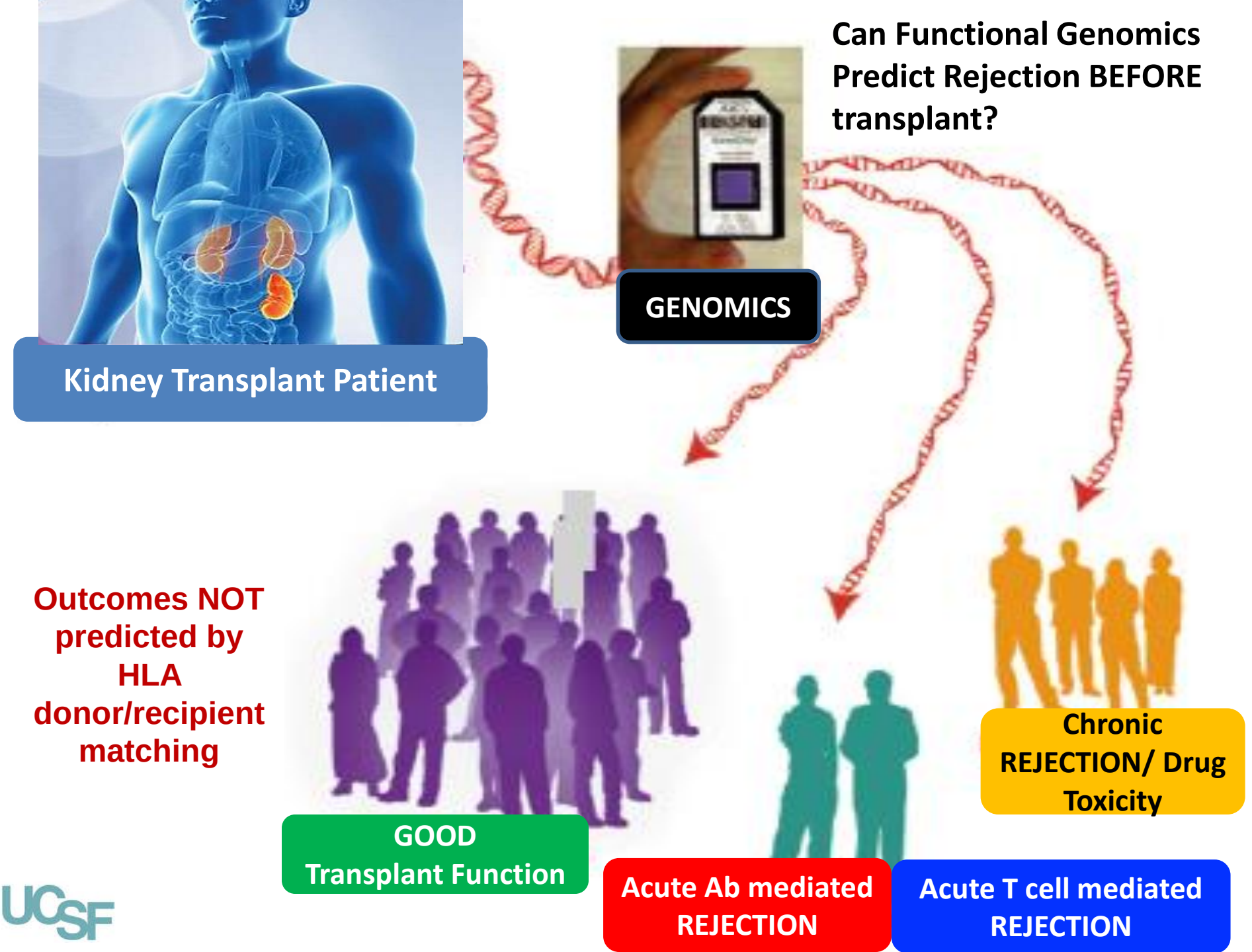
Kidney solid organ response test

kSORT KIDNEY SOLID ORGAN RESPONSE TEST

Immucor

UCSF IP

STANFORD SCHOOL OF MEDICINE IP


Can Functional Genomics Predict Rejection BEFORE transplant?
Kidney Transplant Patient
GENOMICS
Outcomes NOT predicted by HLA donor/recipient matching
GOOD Transplant Function
Acute Ab mediated REJECTION
Acute T cell mediated REJECTION
Chronic REJECTION/ Drug Toxicity
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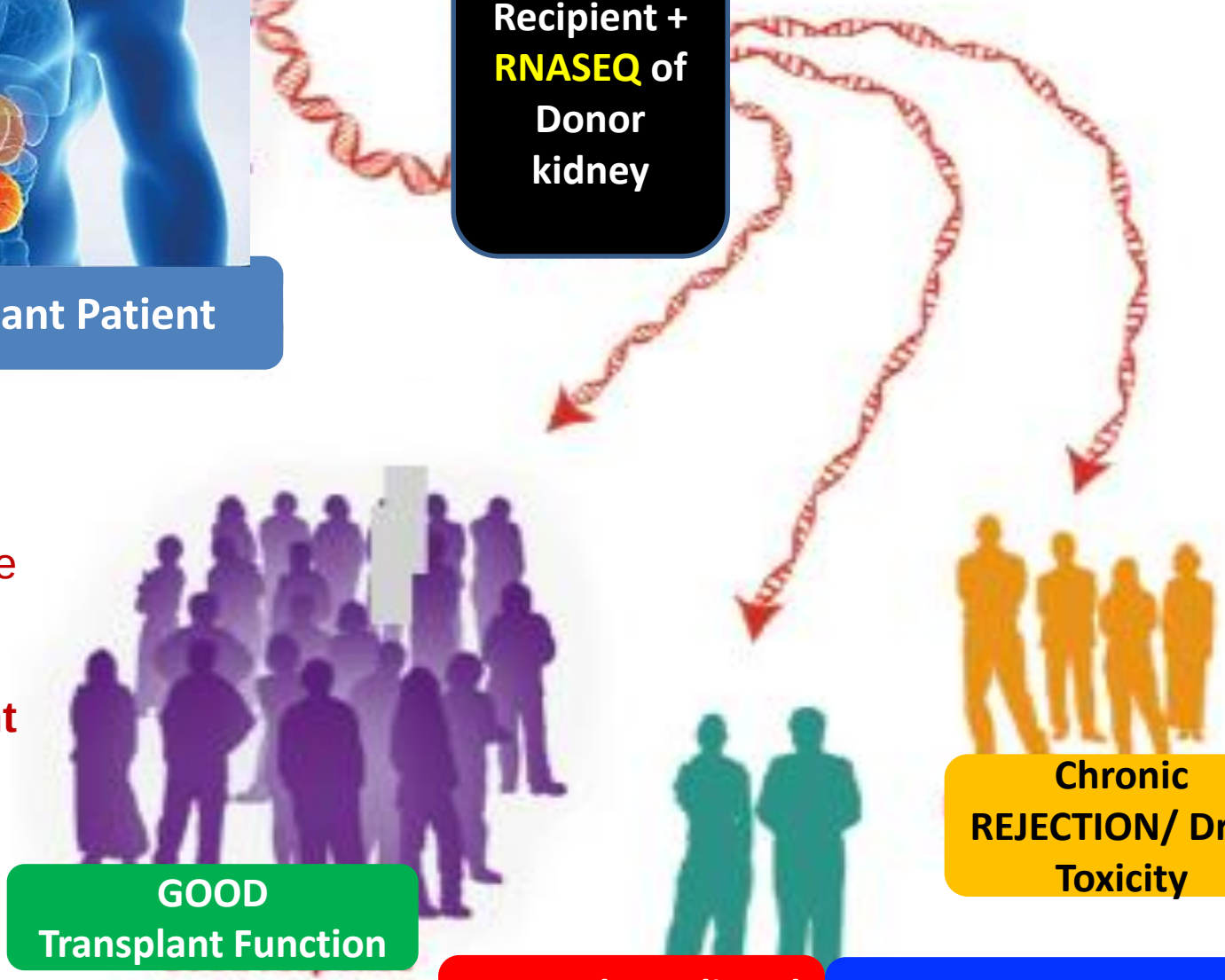
Kidney Transplant Patient

EXOME SEQ Of Donor/ Recipient + **RNASEQ** of Donor kidney

Can Functional Genomics Predict Rejection BEFORE transplant?

Interrogating the impact of **Non-HLA donor/recipient mismatches**



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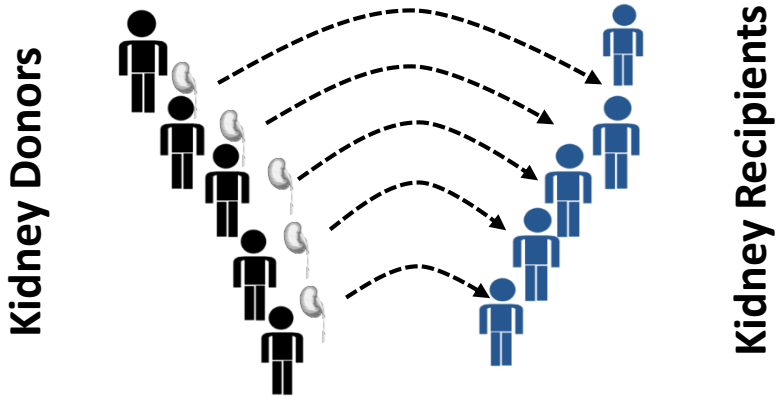
Predicting Rejection Immune Risk BEFORE transplant

Illumina HiSeq 2500



Rejection

Stable

EXOME SEQ
Donor Blood DNA

EXOME SEQ
Recipient Blood DNA

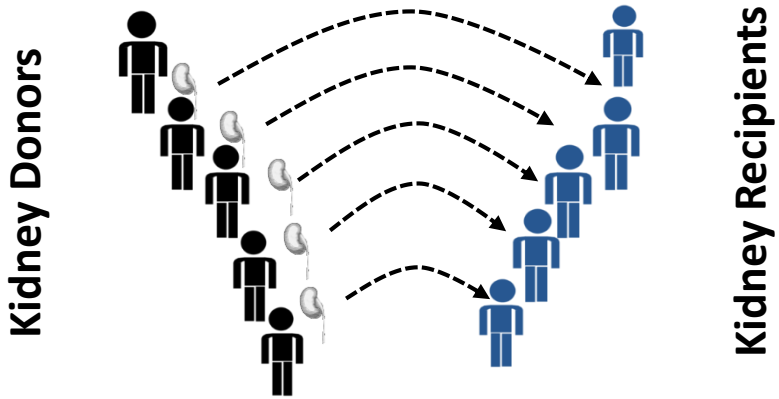
Antigens:
Expressed in donor
Negative in recipient

Drives Immune Response in Recipient = <u>Rejection</u>

Pineda et al, Frontiers in Immunology, 2018

Predicting Rejection Immune Risk BEFORE transplant

Illumina HiSeq 2500



Rejection

Stable

EXOME SEQ
Donor Blood DNA

EXOME SEQ
Recipient Blood DNA

Identification of Variants

Exome-Seq

Confirm expression in donor kidney, absent in recipient

RNA-Seq

Determine enrichment in antigenic locations
(Immune cells, endothelium, surface expressed variants)

Outcomes

Antibody Rejection

T-Cell Rejection

Stable

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Pineda et al, Frontiers in Immunology, 2018

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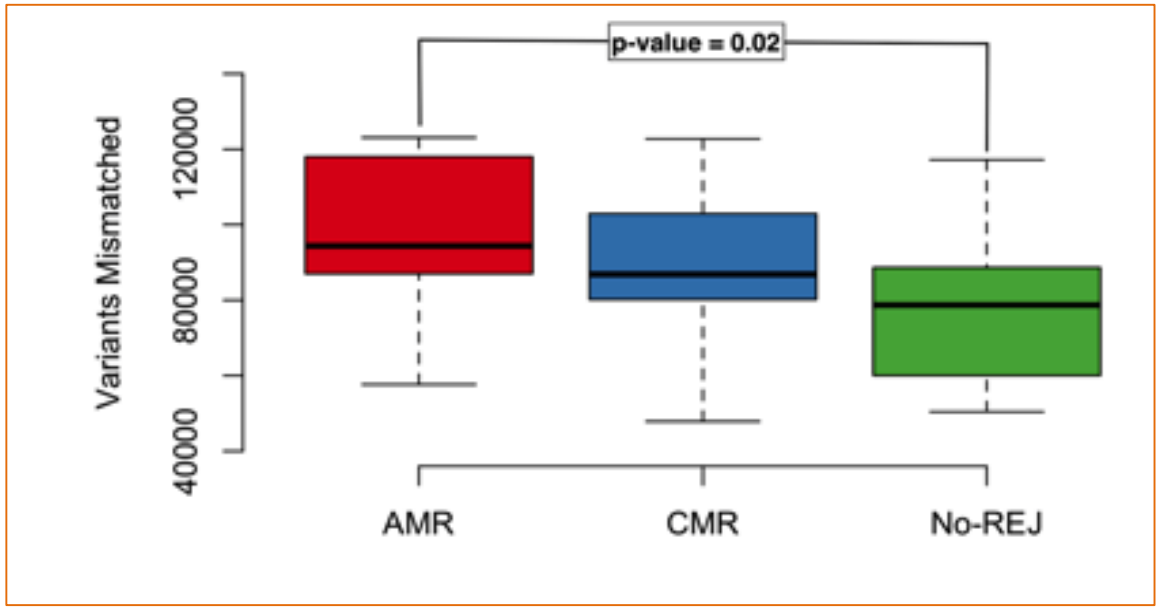
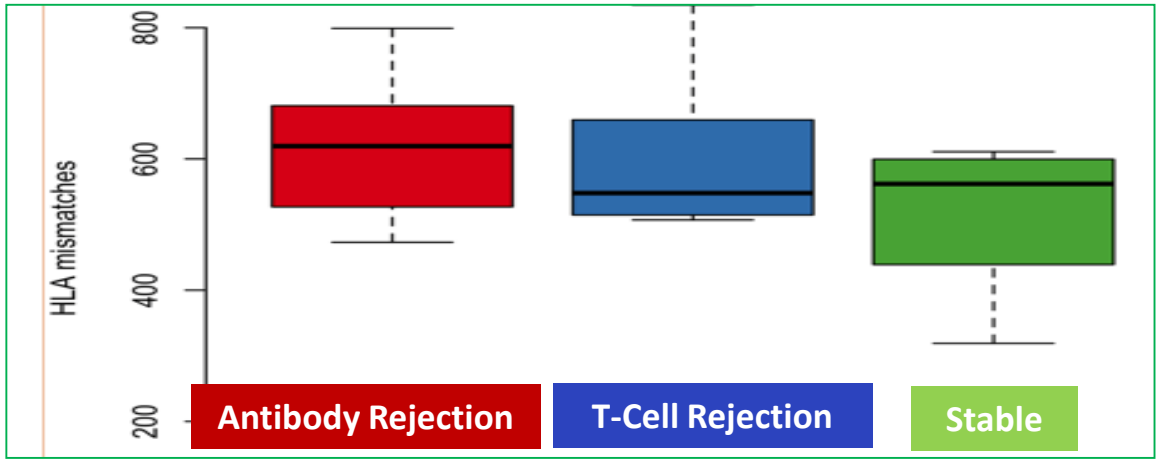
Higher Number of D/R Variants = Higher Risk of Rejection



Non-HLA

Currently not assessed

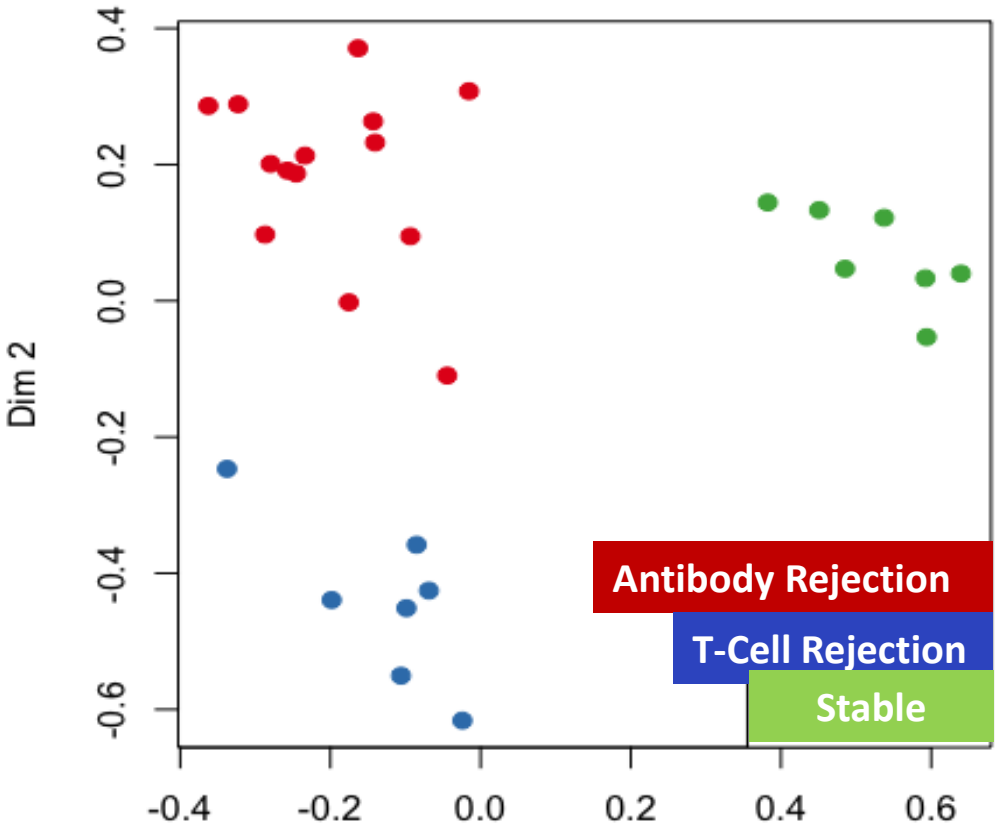


HLA

Mesnard et al, 2016; Pineda et al, 2018

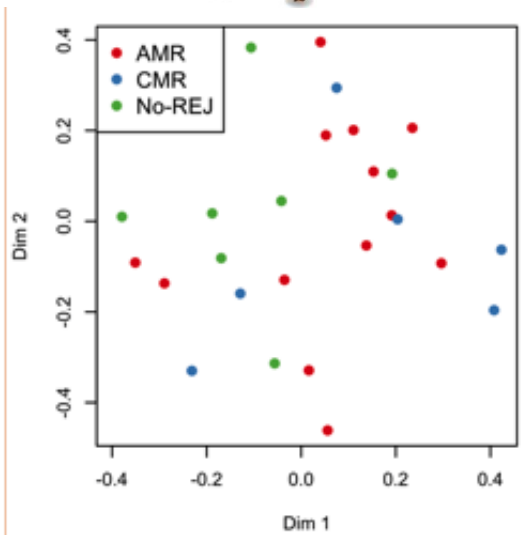
Transplant Risk Assessment Panel

Assessing Risk of AMR Before Doing the Transplant



Transplant Risk Assessment Panel

TRAP123



AMR prediction not possible with HLA variants

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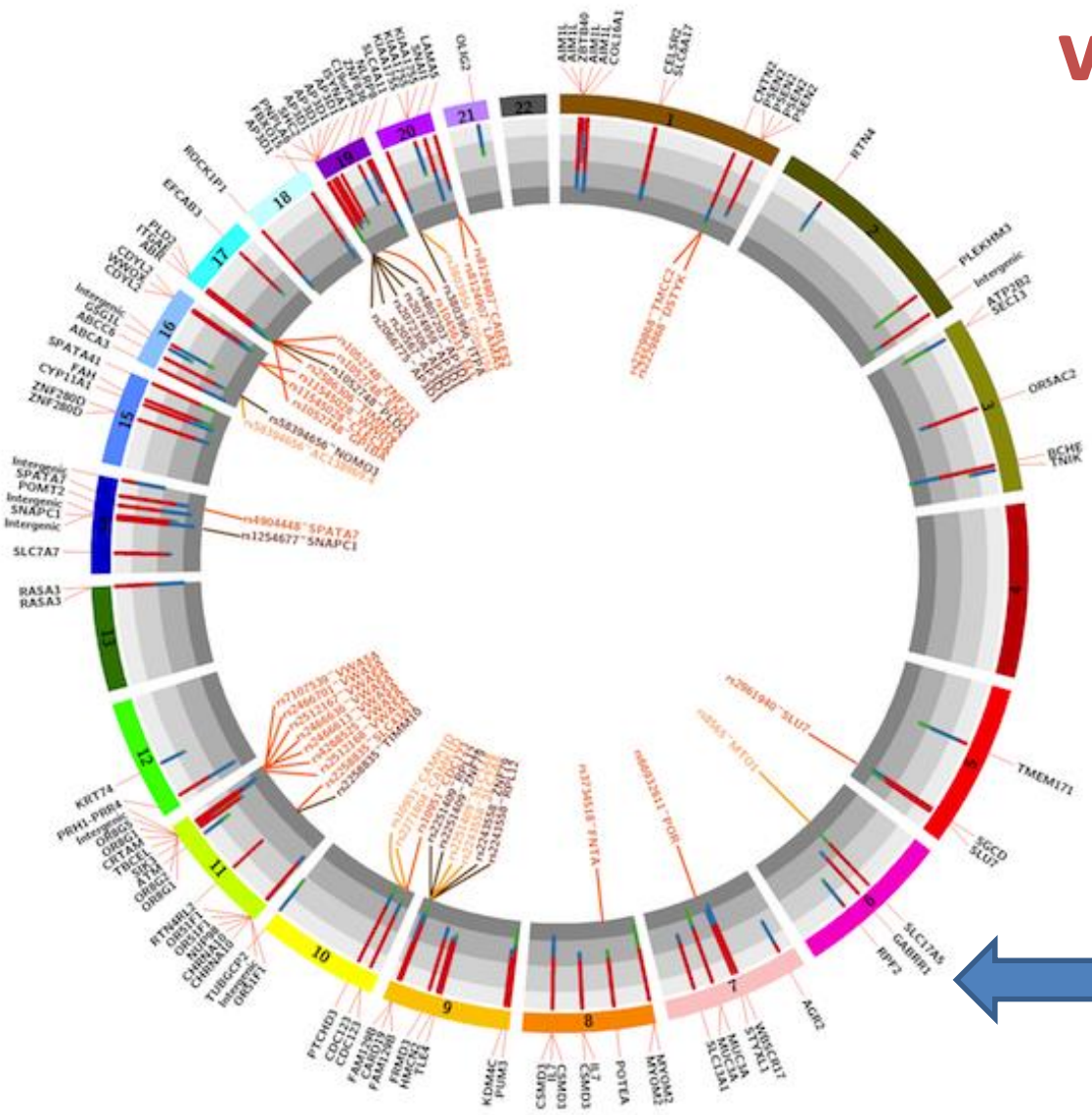
Non-HLA rejection variants span all 22 autosomes



HLA variants are only on chromosome 6

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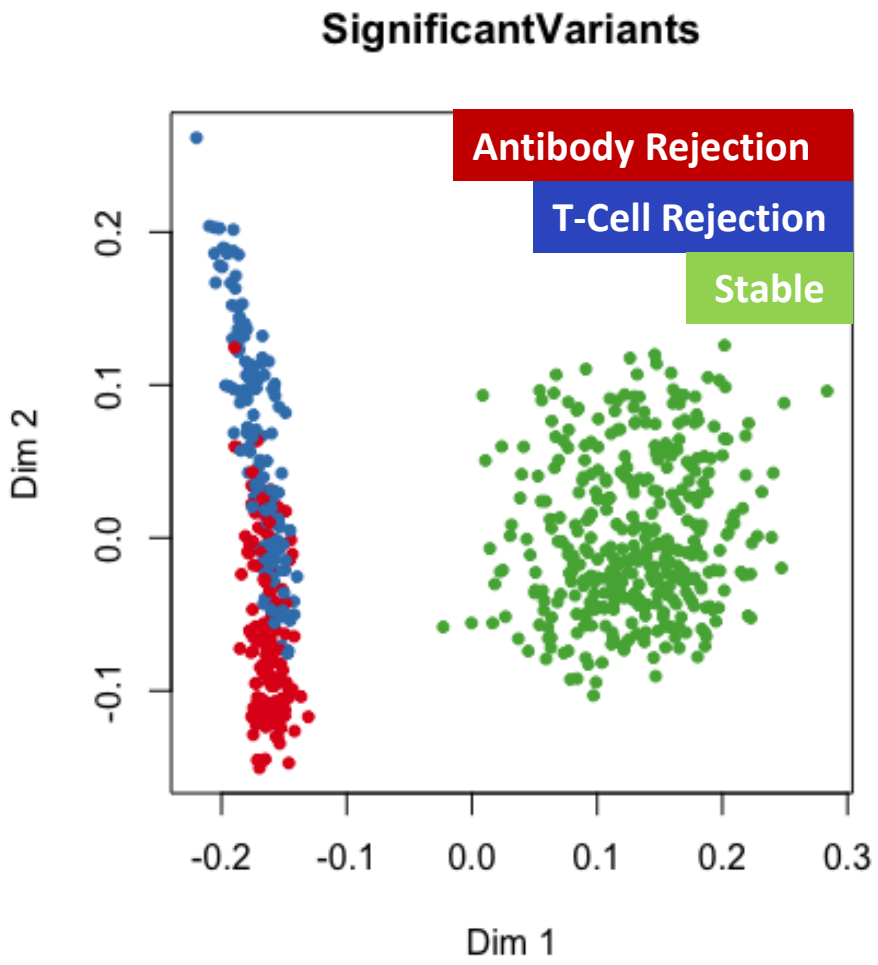
Validated in 123 nHLA variants for AMR prediction: GWAS data-set interrogation of 800 donor/recipient kidney transplant pairs



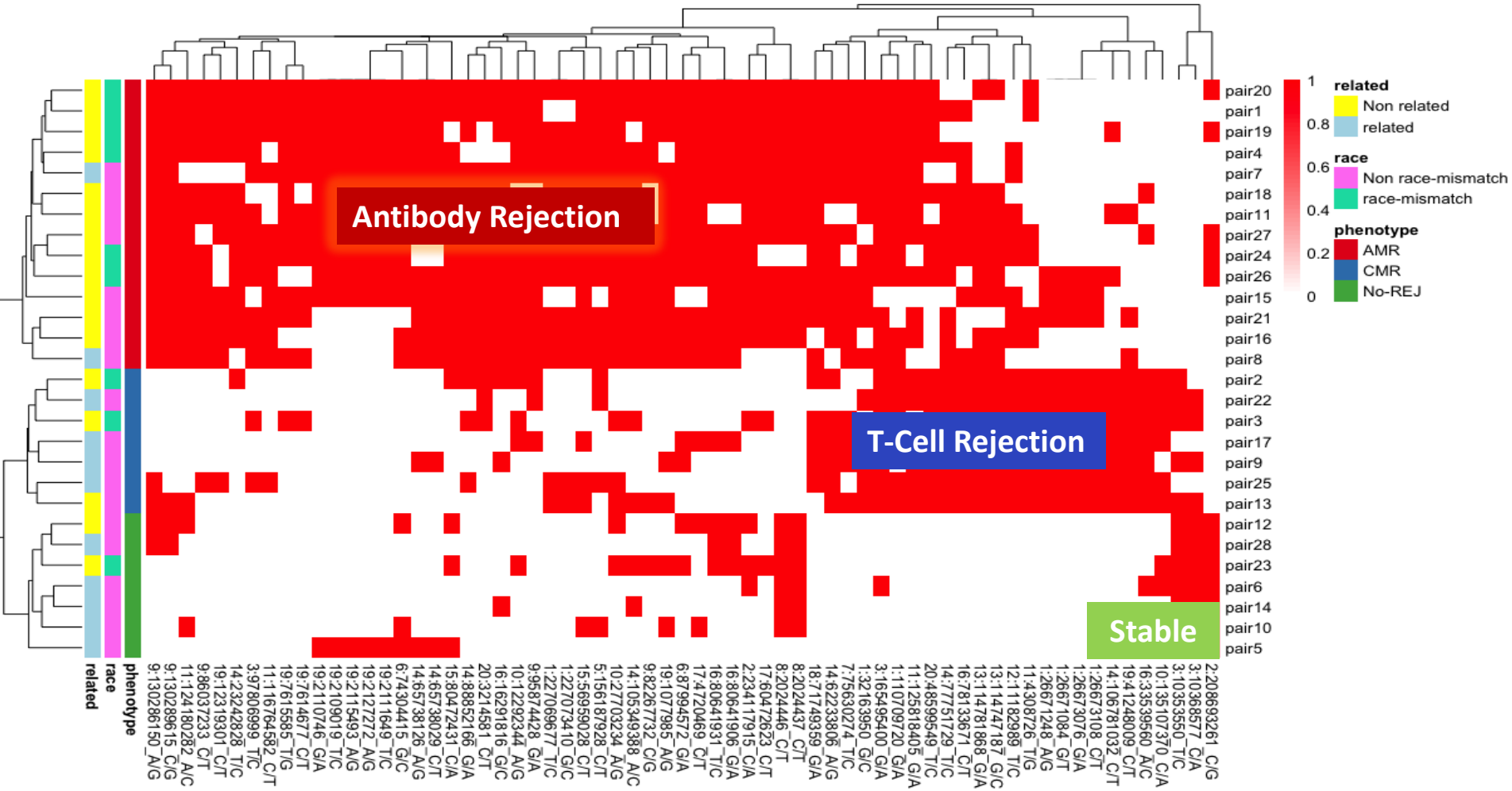
Sarwal, Sirota, Pineda, UCSF; unpublished

TRAP123

Transplant Risk Assessment Panel

Reduction to clinical practice:

Custom SNP Array



UCSF IP

Red= D/R variant mismatch; White= D/R variant match

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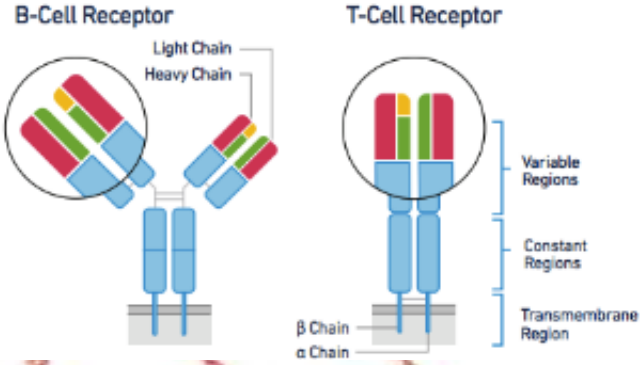
Kidney Transplant Patient

TCR and BCR Immuno Seq



Can variations in BCRseq Predict rejection?

GOOD Transplant Function

Acute Ab mediated REJECTION

Acute T cell mediated REJECTION

Chronic REJECTION/ Drug Toxicity

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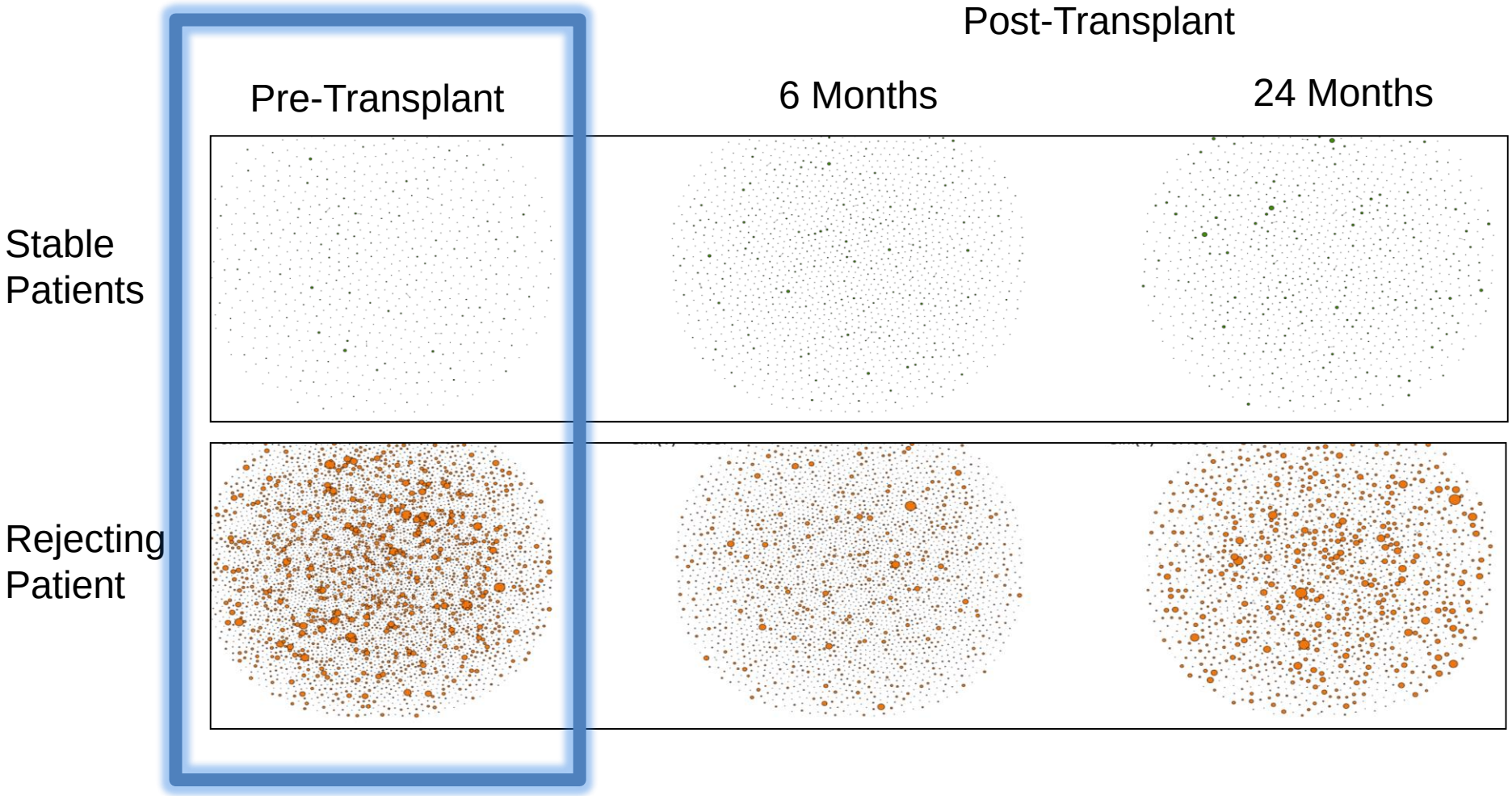
TxSeq

Network analysis of B-cell repertoires show greater pre-transplant diversity and clonal expansion in patients who will reject



Pineda et al, Nature Immunology, 2019, in press

Understanding pre-transplant risk

1. Select the donor-recipient pair with the lowest risk
2. Independently assess the recipient's risk of rejection (donor-agnostic)

Customize therapeutics to rejection risk

Post-Transplant monitoring for rejection should be non-invasive, predictive, specific, and sensitive

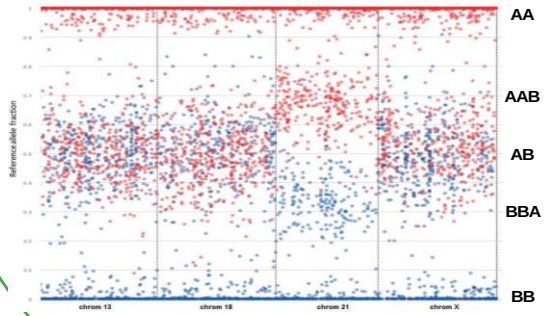
Natera's Technology Designed to Analyze Cell-Free DNA: mmPCR

- *10+ years of experience with cfDNA, over 1 million tests performed*
- *Single molecule sensitivity in a tube of blood*
- *COGS below $200 per sample*

UCSF/ Natera Collaborative Study

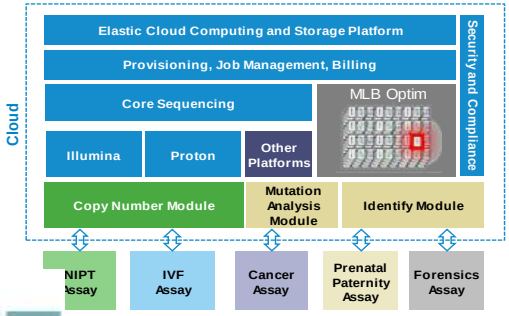
Proprietary Molecular

- >20,000 targets in one reaction, no custom equipment
- Low DNA input and high yield: as low as single cell (6pg)



Proprietary Bioinformatics

- Proprietary algorithms using HapMap, TCGA, Cosmic
- Cloud-based algorithms for signal processing on big data

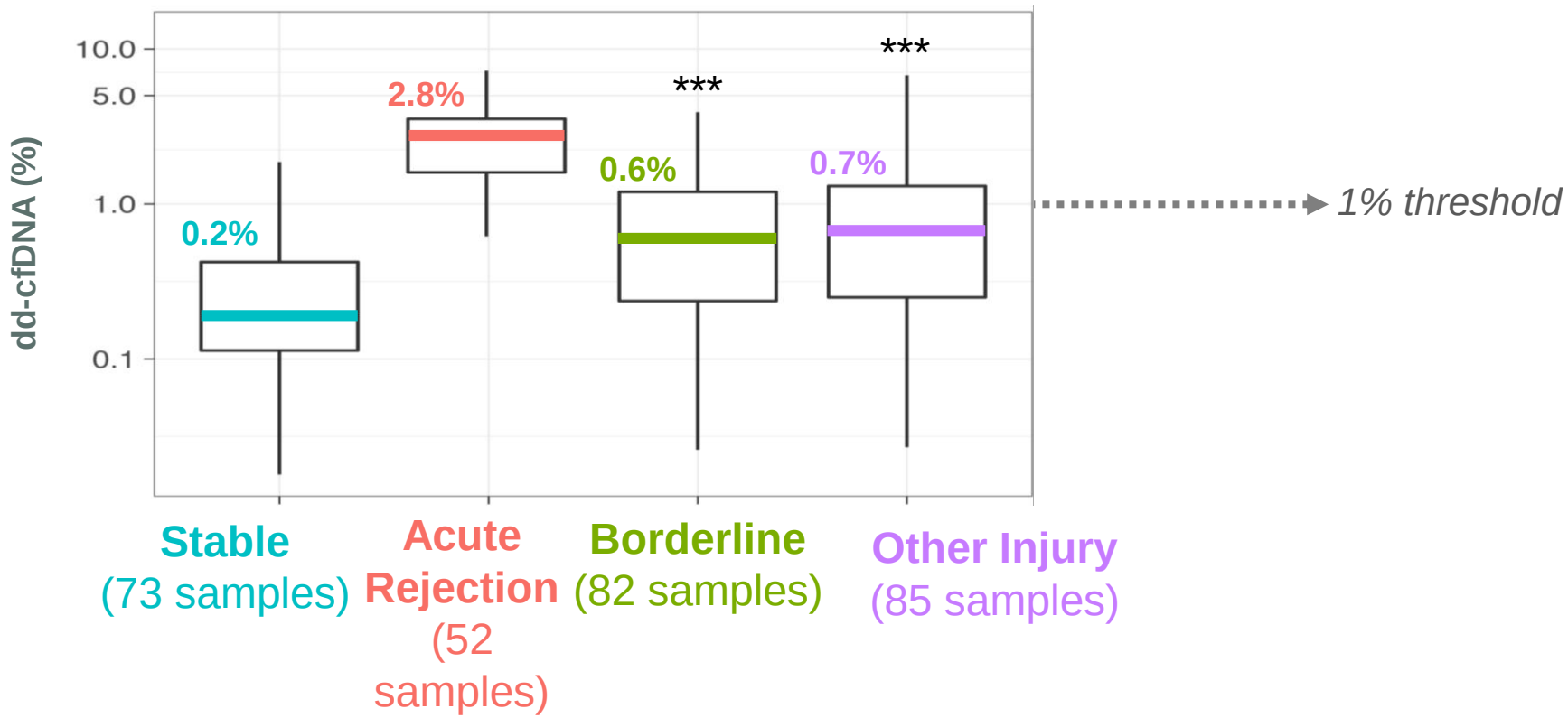


Custom Clinical Design

Approach does not use transplant-specific markers and does not require advance determination of donor or recipient genotypes

Increased ddcfDNA in Tx INJURY

Total 292 samples from 187 patients, Biopsy Matched



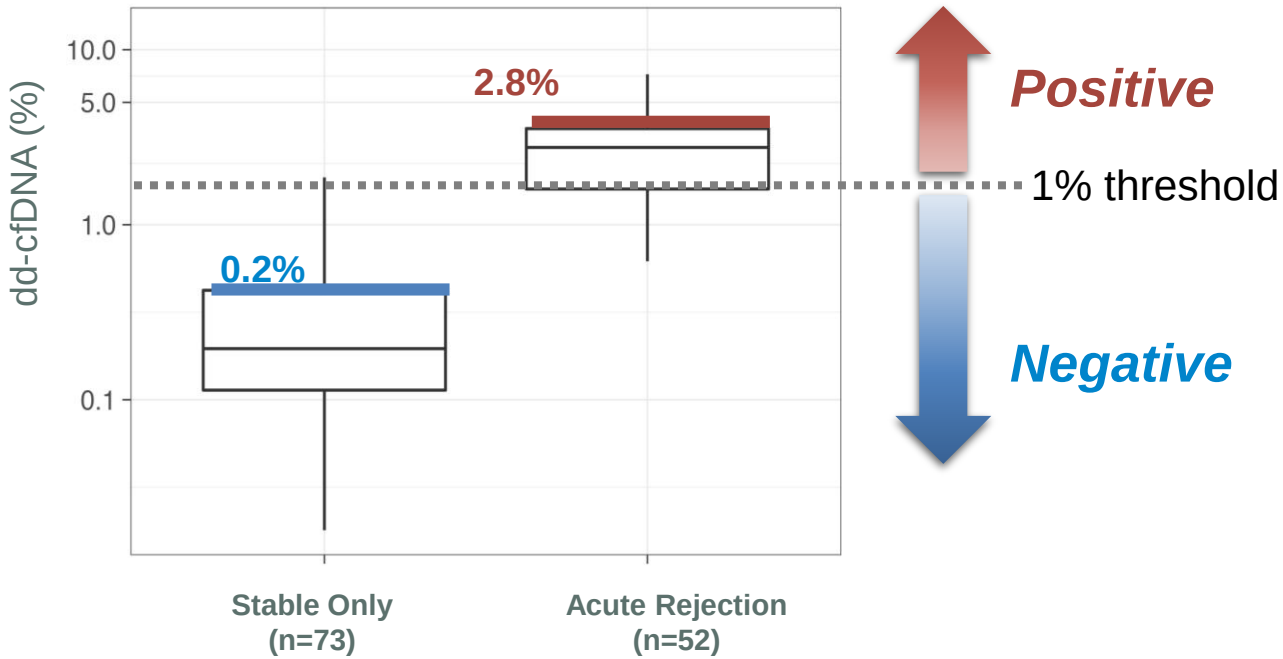
Specificity Among Stable Patients is HIgh

Sensitivity: 92.3%

Specificity: 93.2%

Area Under Curve (AUC): 0.95

Significance level p<0.0001



When dd-cfDNA >1%, less than 7% Were Stable

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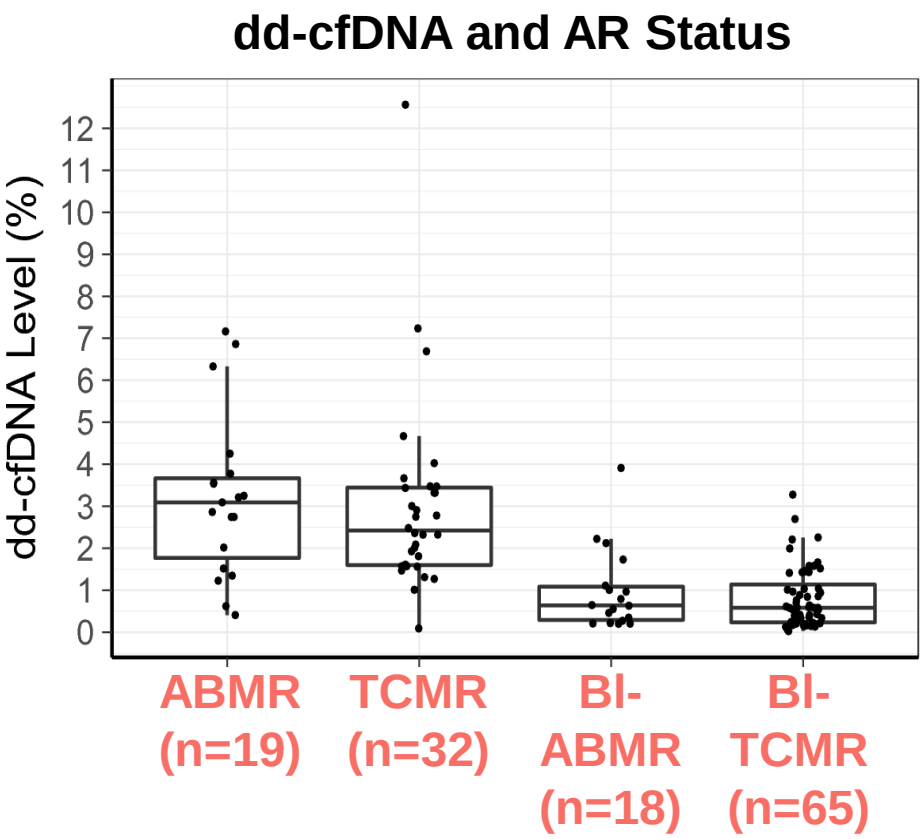
Assay Robust to Both ABMR and TCMR

- Of 52 AR samples: 19 were classified as antibody mediated rejection, 32 T-cell–mediated rejection, and 1 both

- **The fraction of dd-cfDNA did not differ significantly between ABMR and TCMR cohorts or between borderline cohorts**



Comparison of dd-cfDNA Assays

	Sigdel et al, 2019 (292 samples)	Bloom et al., 2017 (107 samples)
Performance Metrics		
Sensitivity	89% (n=52)	59% (n=27)
Specificity	73% (n=240)	85% (n=80)
AUC	0.87	0.74
Assuming 25% Prevalence of AR		
NPV	97%	84%
PPV	53%	61%
SNP density		
	13,392	266

Unbiased discovery of AR specific genes in peripheral blood:

Controlled for clinical, demographic and bx confounders

Li et al, AJT, 2012

Microarray discovery

Multi-Parameter Acute Rejection Biomarker Discovery

Affymetrix
Whole blood: 44 AR, 46 STA
FACS Purified Cell Subsets: 6 AR, 9 STA

Agilent
Whole blood: 15 AR, 11 STA

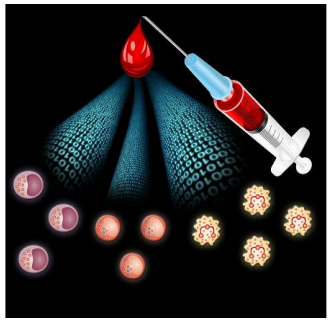
Lymphochip cDNA
Whole blood: 7 AR, 14 STA

SAM Analysis (FDR <0.05)

Selection Criteria (at least 2)

1. Identical fold change direction
2. AR/STA Classifier (2+ Datasets)
3. Statistical Deconvolution
4. Cell Specific Enrichment
5. Biologic Significance

17 locked genes



kSORT

Biomarker Discovery → Gene Selection → **Verification** → $p < 0.05$ → **Biomarker Selection**

Biomarker Definition
n = 177

Assess Performance

Biomarker Validation
n = 90

Cross-Validation

8 Center Adult Study
n = 558

12 Center Peds RCT
n = 367

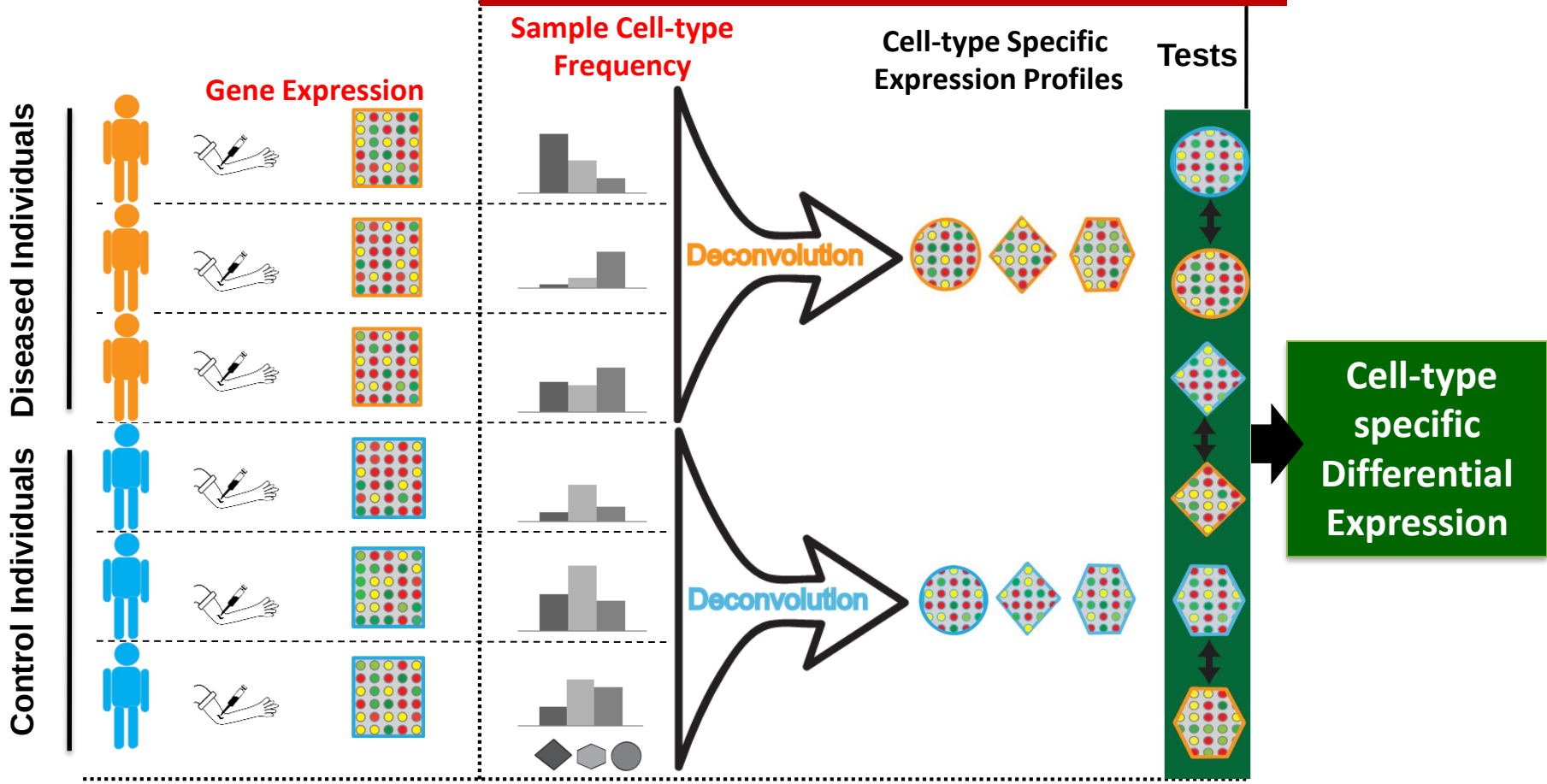
AART

SNSO1

Stanford Heart Adult Study
n = 140

Shai Shen Orr, Rob Tibshirani ...Sarwal M, Davis M, Butte A et al, Nature Methods, 2010

Cell-type specific Significance Analysis of Microarrays (csSAM)



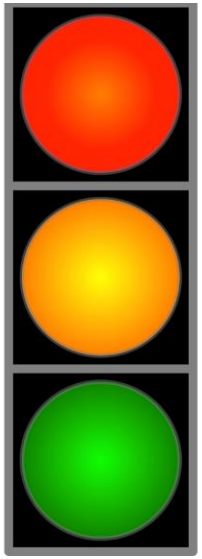
kSORT genes are from activated monocytes: TCMR+ ABMR

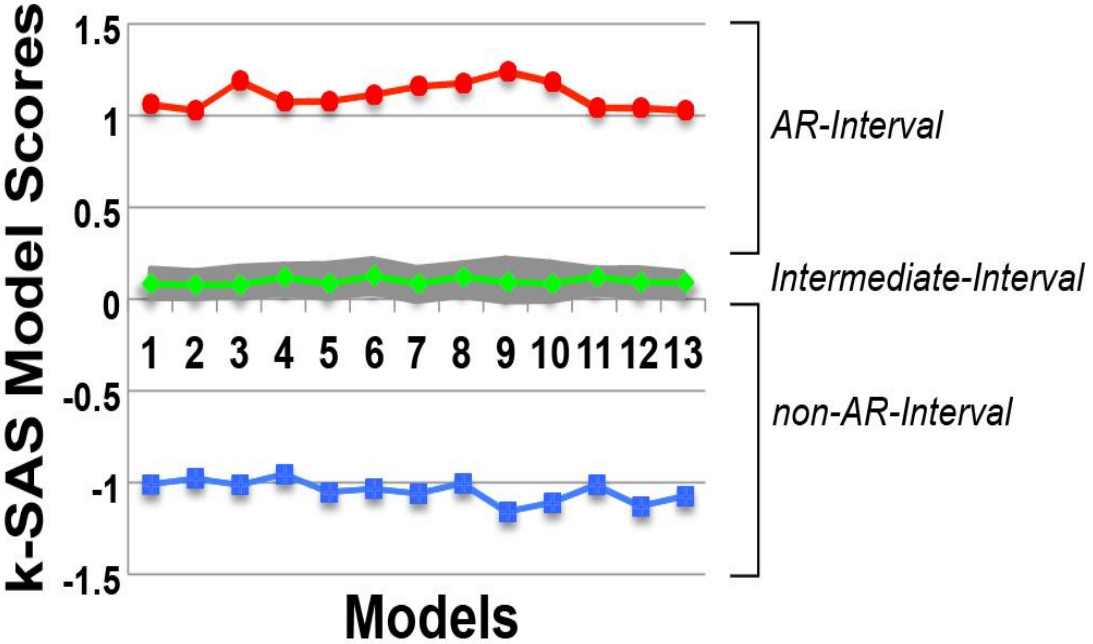
CFLAR, DUSP1, IFNGR1, ITGAX, MAPK9, NAMPT, NKTR, PSEN1,CEACAM4, EPOR, GZMK, RARA, RHEB, RXRA, SLC25A37, RNF130, RYBP

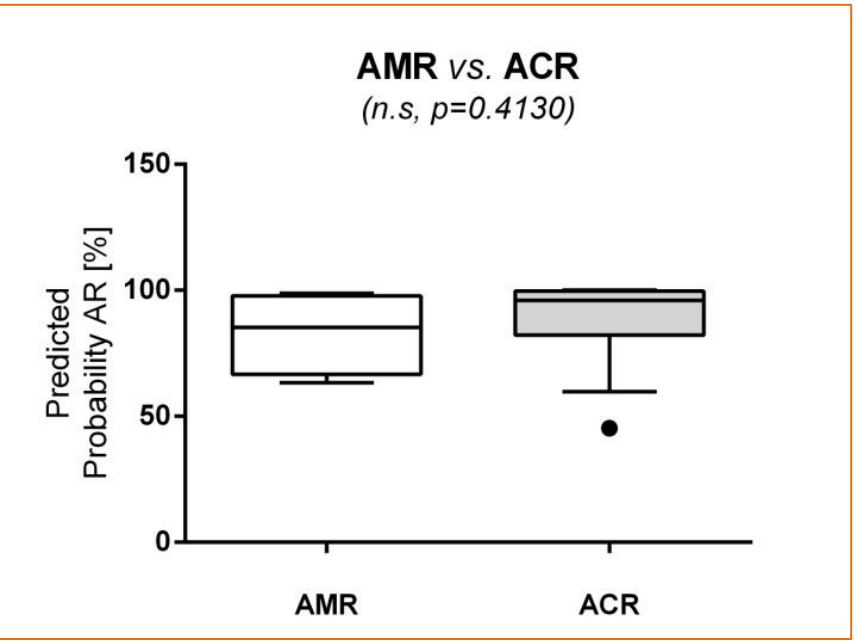
The expression of 17 genes in peripheral blood are put into an algorithm (kSAS) which results in a score within 3 possible intervals

Roedder, Sigdel, Salomonis, et al, Plos Medicine, 2014

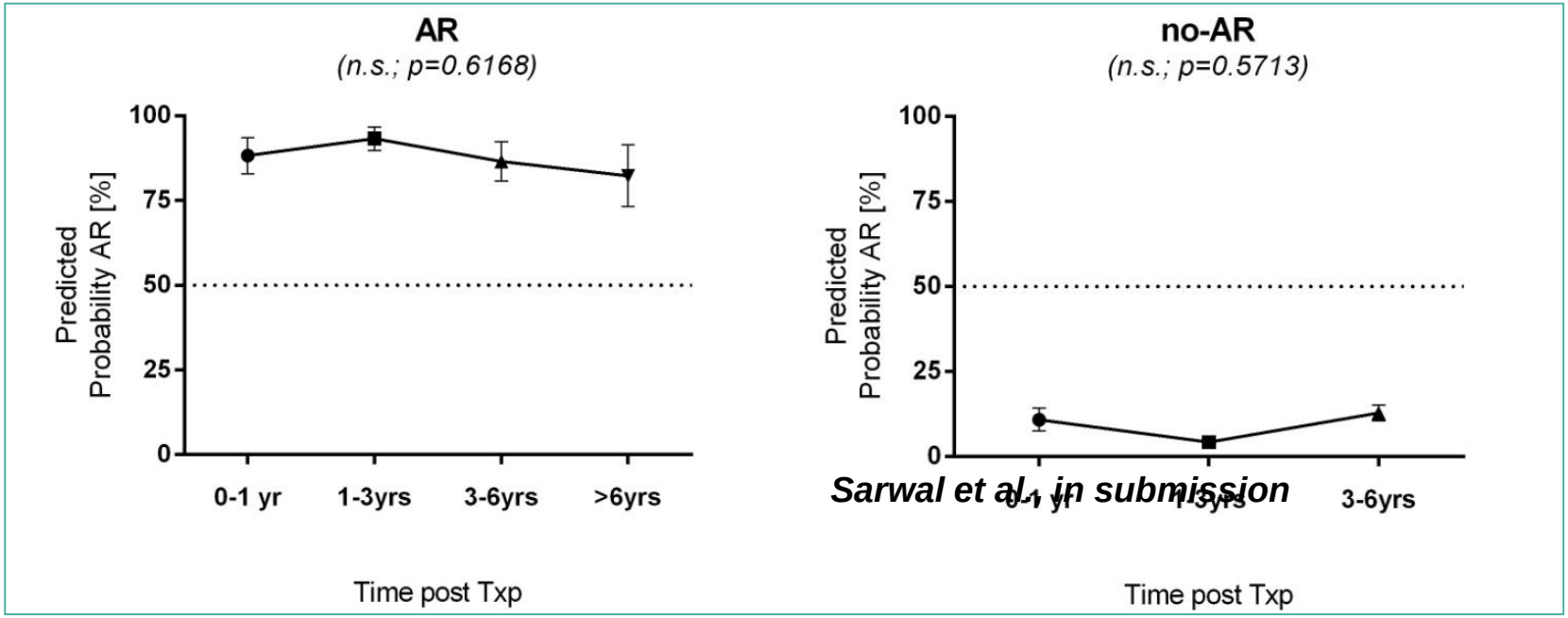
Σ_1^{13} [ρ(AR) – ρ(No-AR)] * 10 = kSAS Score







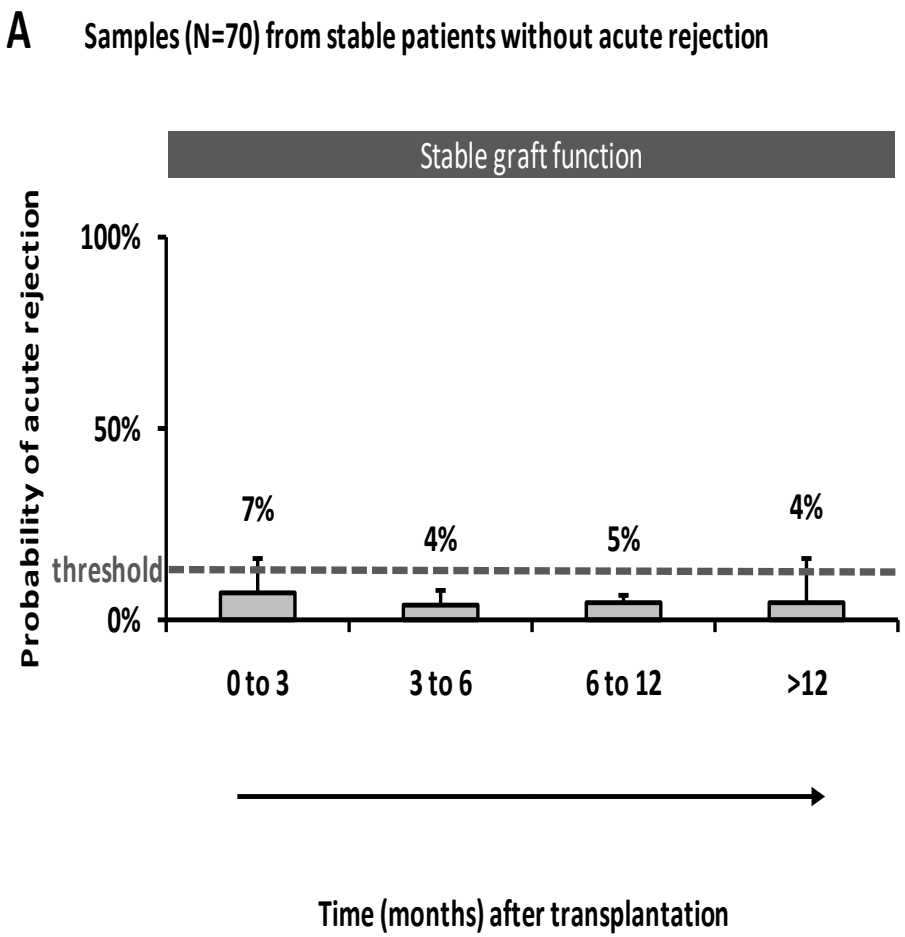
kSORT detects cellular and humoral rejection and is not confounded by time post-transplantation

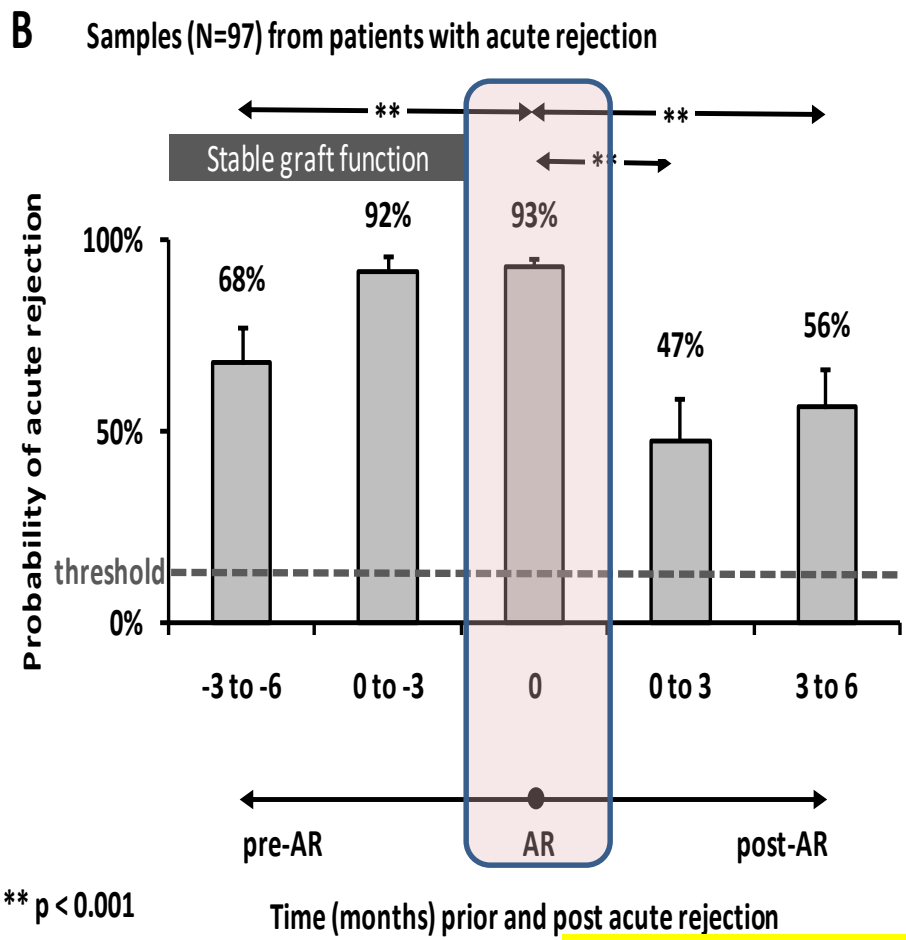


Sarwal et al., in submission

The Transcriptomic Window in Blood

kSORT detects pre-AR 3-4 months before rise in se. creatinine





kSORT is not impacted by time post-txp

kSORT can PREDICT AR

kSORT can monitor AR recovery

Sigdel, Sarwal, Clin Transplants, 2014

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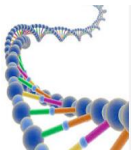
Blood Sampling: the only way to get to understanding all immune risk profiles

Pre-Transplant

Post-Transplant

TRAP123

Transplant Risk Assessment Panel



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Many thanks……

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