MOLECULAR DIAGNOSTICS FOR ALLOGRAFT INJURY

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Disclosure

Grant Support: NIH, CareDx, Immucor
Advisory Board and Consultant: CareDx, Immucor, Transplant Genomics

CURRENT CLINICAL PRACTICE IN KIDNEY TRANSPLANTATION

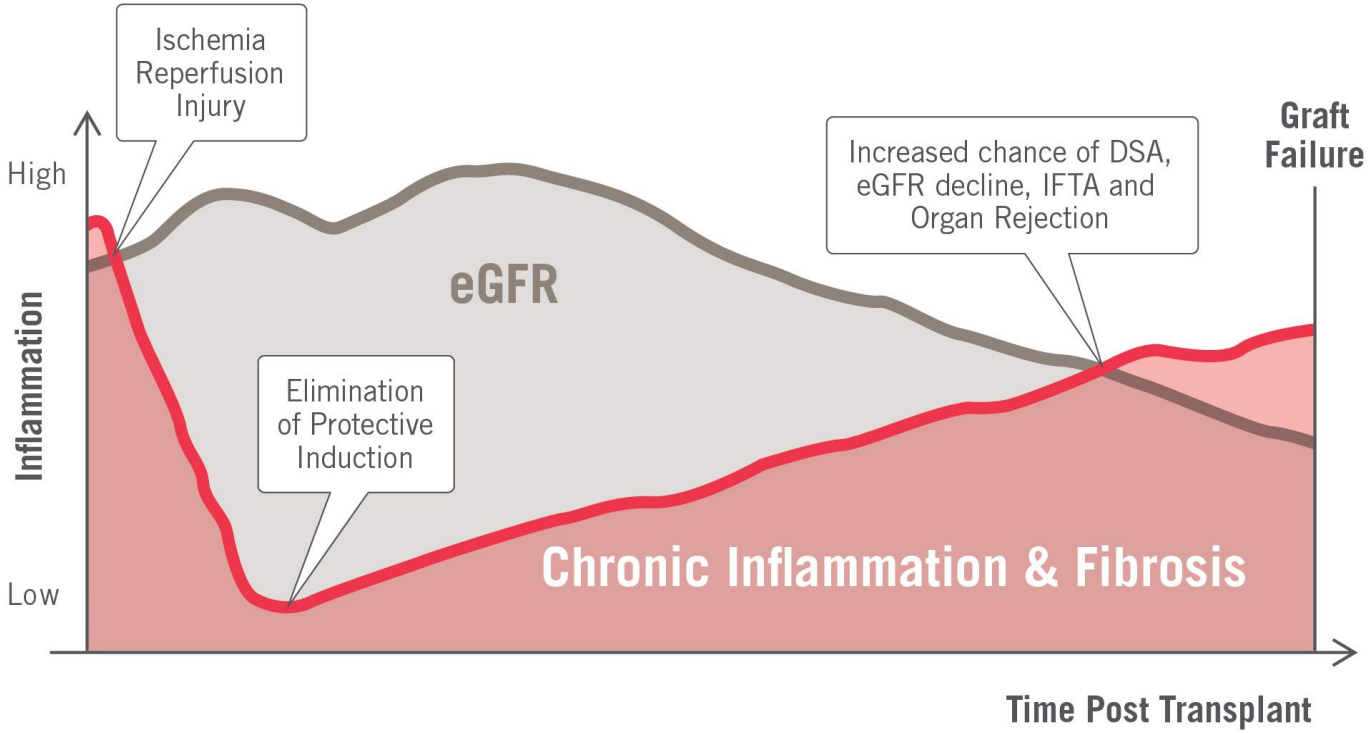
IMMUNOSUPRESSION

- **INDUCTION**
  - Anti-thymocyte globulin or Alemtuzumab in immunologically high-risk patients
  - Basiliximab (IL2-R antagonist) or no induction in low-risk patients
  - IVIG, plasmapheresis, rituximab, eculizumab, or bortezomib in desensitization protocols for CXM positive patients with DSA
- **MAINTENANCE**
  - Tacrolimus
  - Mycophenolate mofetil or Mycophenolic acid
  - Prednisone (25-30% are on rapid steroid withdrawal protocol)

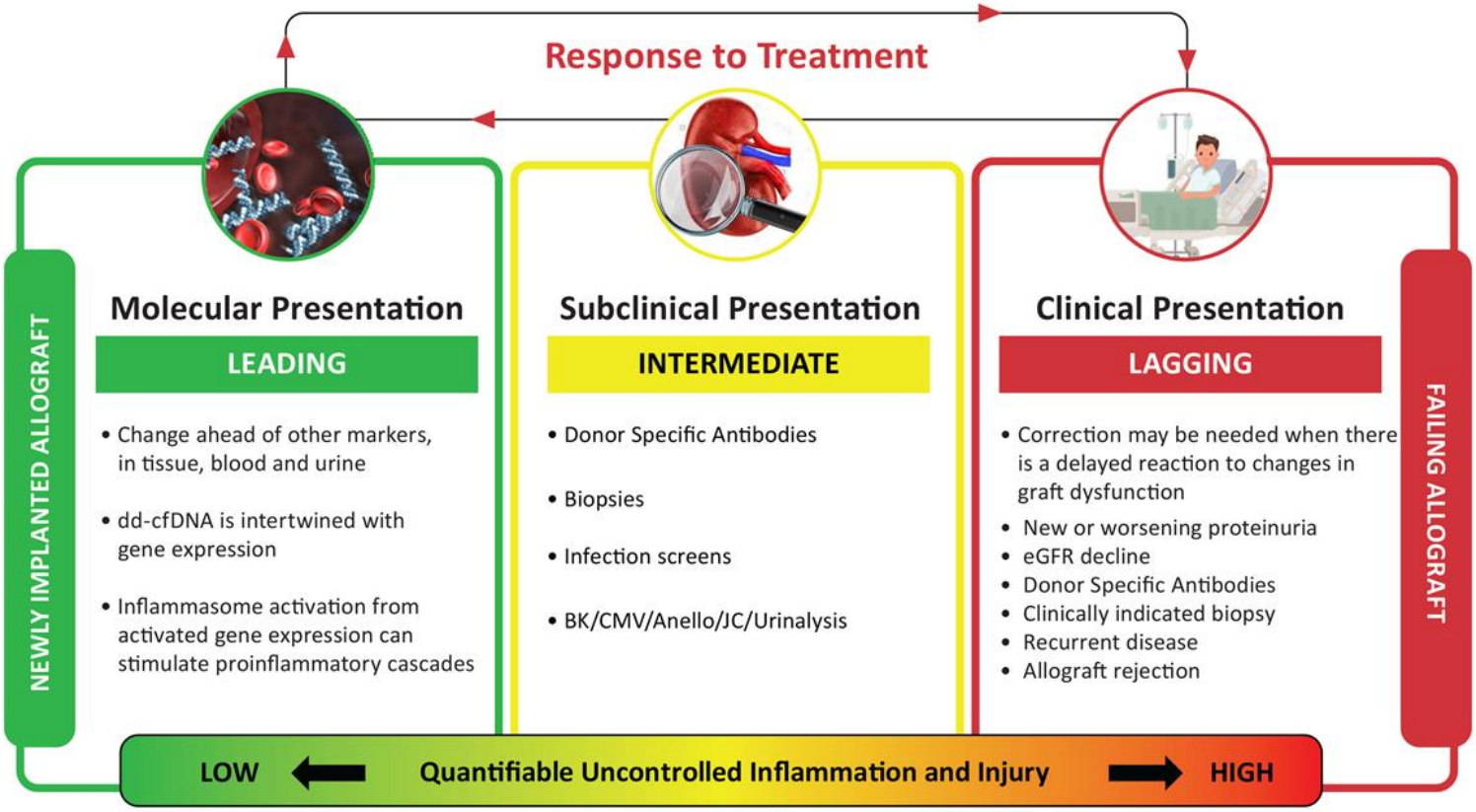
CLINICAL MONITORING

- Serum creatinine and tacrolimus levels
- Spot urine protein/creatinine ratio
- BKV viremia
  - Once a month first 6 months, at 9 and 12 months and then annually
- Luminex single antigen beads
  - 1, 3 and 12 months and then annually
- Protocol biopsy (10-15% of transplant centers)
  - 3-6 months and 12 months

What Biomarker Can Allow for Early Intervention?



Power of Unlocking the Molecular Window Using Technology

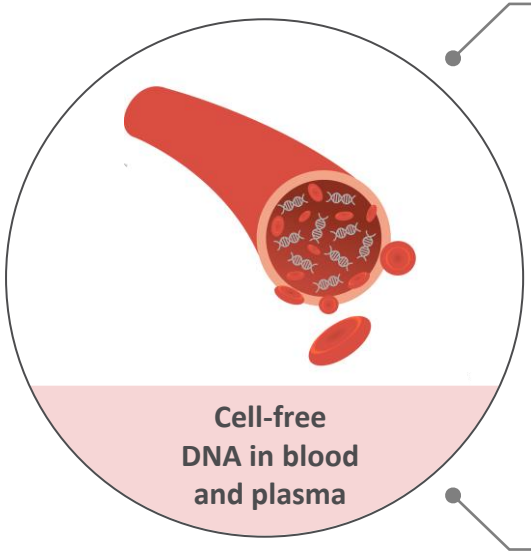


Fehr T., Cohen C. Predicting an allograft's fate. Kidney Int 2011;80:1254–1255.
Naesens M. et al. Progressive histological damage in renal allografts is associated with expression of innate and adaptive immunity genes. Kidney Int 2011;80:1364–76

NOVEL BIOMARKERS

- **Cell-free DNA**
  - Allosure (CareDx)
  - Prospera (Natera)
  - TRAC (Transplant Genomics)
- **Gene transcripts**
  - Allomap (CareDx)
  - TRUGRAF (Transplant Genomics)
  - kSORT (Immucor)

What is Donor-Derived Cell-Free DNA (dd-cfDNA)?



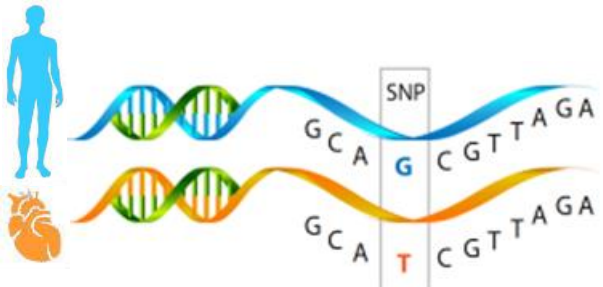
Cell-free DNA refers to fragments of DNA in the bloodstream that originate from cells undergoing cell injury and death

DNA degrades into nucleosomal units consisting of ~166 bases

cfDNA is cleared from the blood by the liver and kidney, and has a half-life of ~30 minutes

CELL-FREE DNA METHODOLOGY

- dd-cfDNA is measured by determining the fraction of donor-derived nucleotides at single-nucleotide polymorphism (SNP) location
- The method does not require prior genotyping of the donor or recipient:
  - SNPs are chosen that each have two alleles, distributed equally in the population
  - The SNP regions are amplified from the low levels of dd-cfDNA
  - Next-Generation Sequencing is used to count each allele
  - Example: If we detect 99 counts of allele A, and 1 count of allele B:
    - Infer Recipient is homozygous for allele A
    - Infer Donor has B allele, estimate dd-cfDNA≈1%



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

J Am Soc Nephrol 28: 2221–2232, 2017. doi: https://doi.org/10.1681/ASN.2016091034

D DNA in Blood for Diagnosing

A Acute

R Rejection in Kidney

T Transplant Recipients

14 DART study centers nationwide in the US

US Renal demographic represented

384 Patients were enrolled

102 & 27 For cause biopsy cohort: 102 patients (107 samples with both biopsy and AlloSure), 27 with active rejection

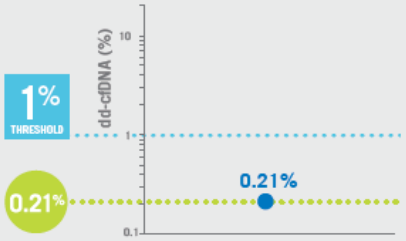
AlloSure Performance Characteristics from DART

96% of AlloSure results from healthy stable recipients were below 1% threshold

50% of AlloSure results from healthy stable recipients were below 0.21%

Active Rejection

ALLOSURE CAN RULE OUT REJECTION



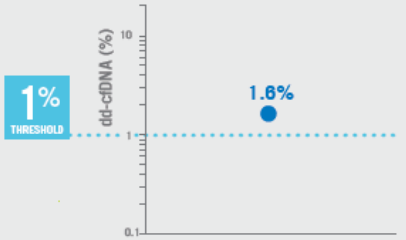
95% NPV for Active Rejection*

Sensitivity: 85%
Specificity: 33%
Prevalence: 10% †

} at 0.21% dd-cfDNA

0.21% is the median from DART healthy stable recipients

ALLOSURE HAS HIGH SPECIFICITY FOR REJECTION DETECTION



44% PPV for Active Rejection*

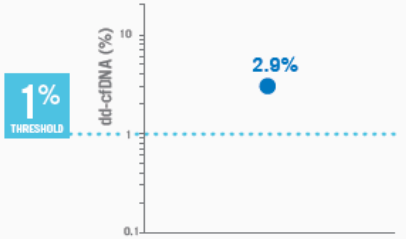
Sensitivity: 52%
Specificity: 93%
Prevalence: 10% †

} at 1.6% dd-cfDNA

1.6% is the median from DART active rejection

ABMR in DSA Positive Patients

ALLOSURE HAS HIGH PPV FOR ABMR IN DSA POSITIVE PATIENTS



85% PPV for ABMR in DSA+ Patients

Sensitivity: 50%
Specificity: 94%
Prevalence: 40% ≈

} at 2.9% dd-cfDNA

2.9% is the median from DART ABMR

*Active Rejection = acute/active ABMR; chronic, active ABMR; and TCMR IA and greater

† Prevalence of rejection within the first year post-transplant

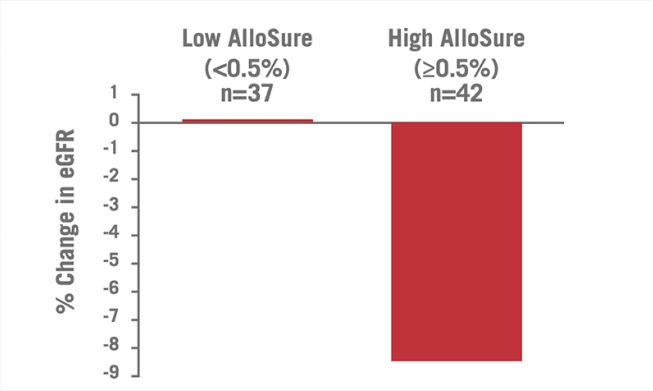
≈ Prevalence of ABMR in DSA positive patients

RADAR Study (Resolution by AlloSure Differentiates Ambiguous Rejection)

Am J Transp 2020

1

Patients with High AlloSure (≥0.5%) were at Increased Risk of eGFR Decline



2

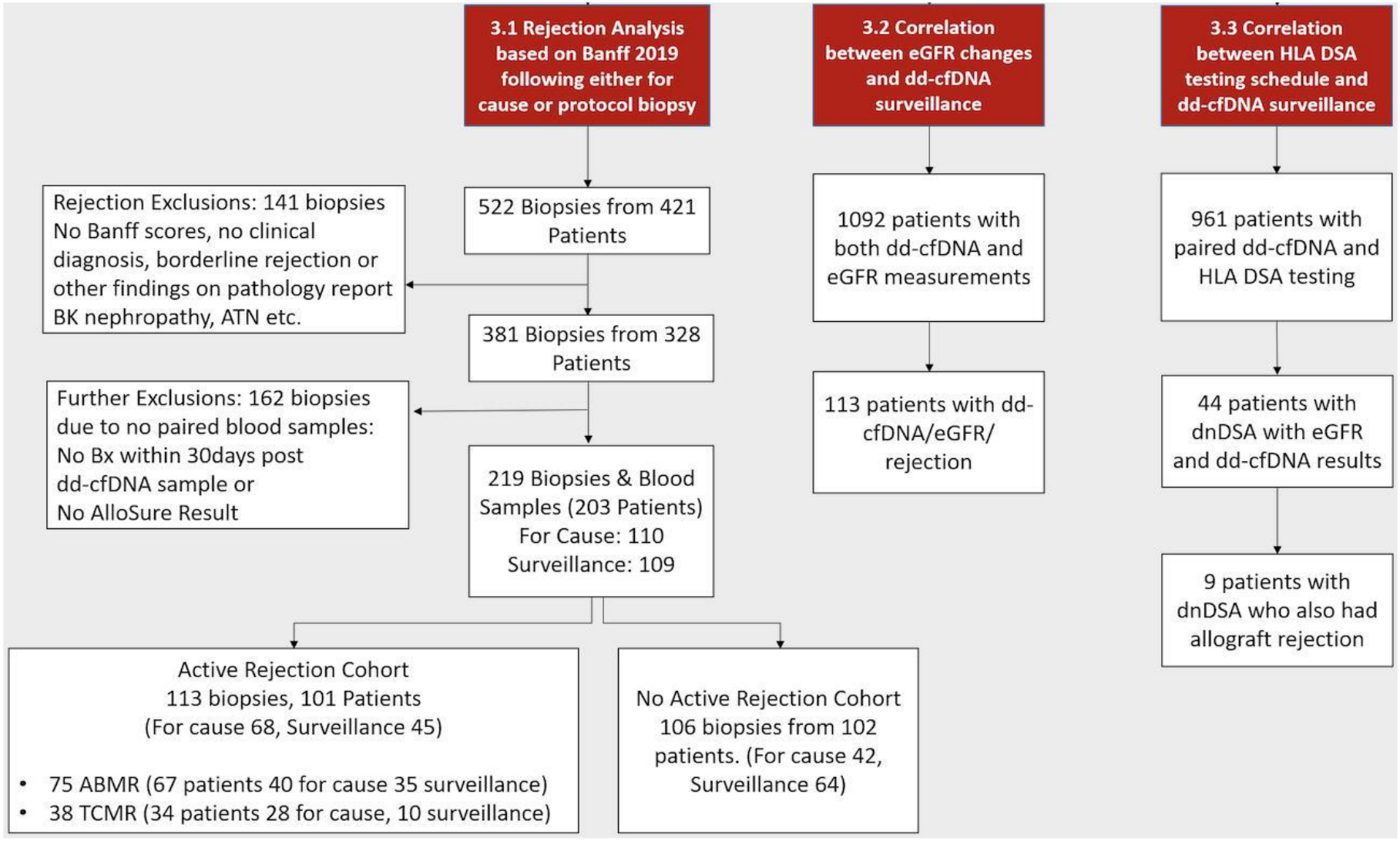
Patients with High AlloSure (>0.5%) had greater presence of dnDSAs and Recurrent Rejection

	Low AlloSure (dd-cfDNA <0.5%) N=37	High AlloSure (dd-cfDNA ≥0.5%) N=42	p-value
% Change in eGFR Median (SD)	**-0.00** (18.149)	**-8.50** (14.98)	0.0040
Presence of DSAs	**2.7%** 1/37	**40.5%** 17/42	<0.0001
Recurrent Rejection	**0.0%** 0/37	**21.4%** (9/42)	0.0028

79 patients across 11 transplant centers with TCMR 1A (n=52) or Borderline Rejection (n=27) (Banff 2017 criteria) were assessed

ADMIRAL – Independent Multicenter Validation of AlloSure

Bu et al. Kidney Int. 2022 Apr;101(4):793-803



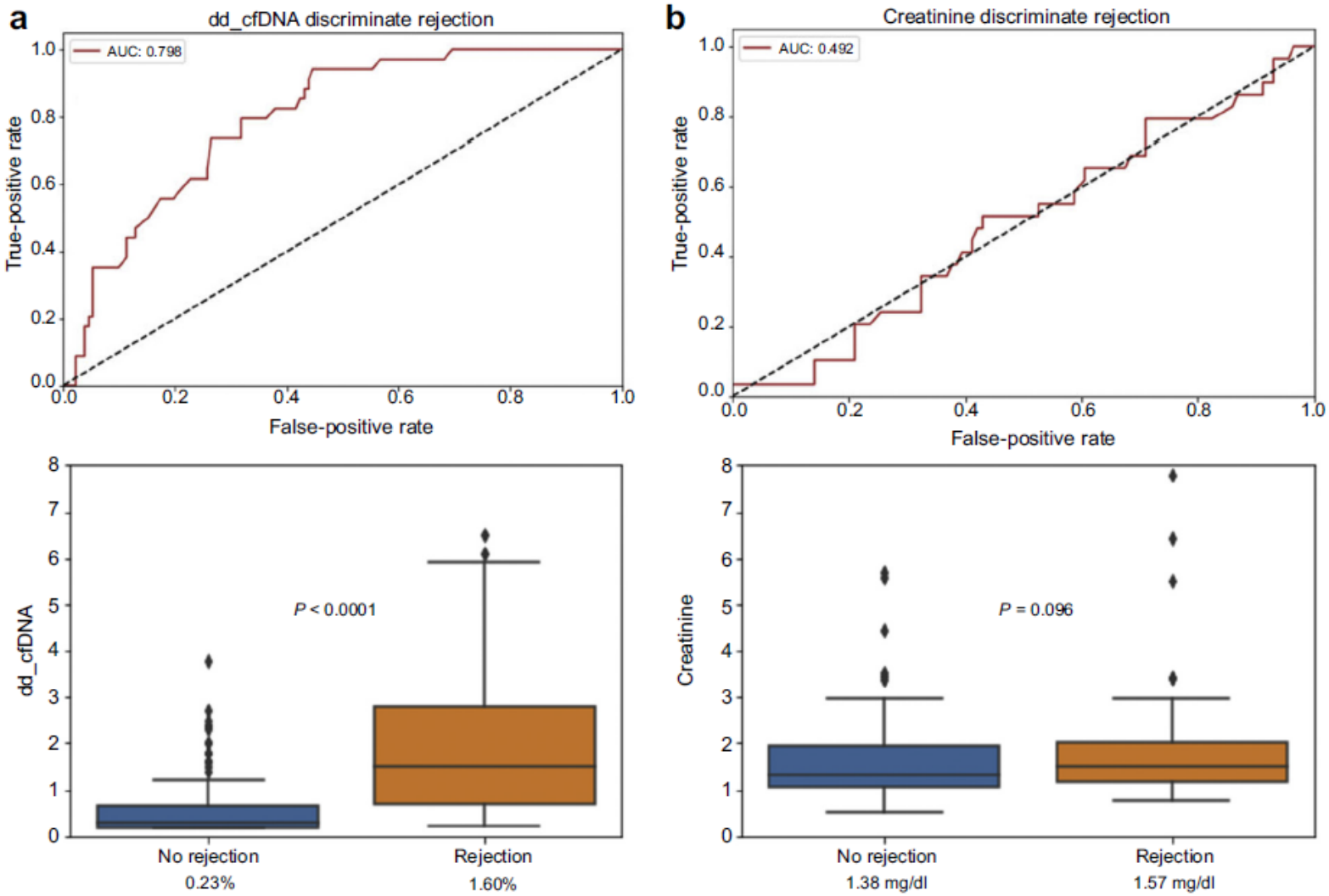
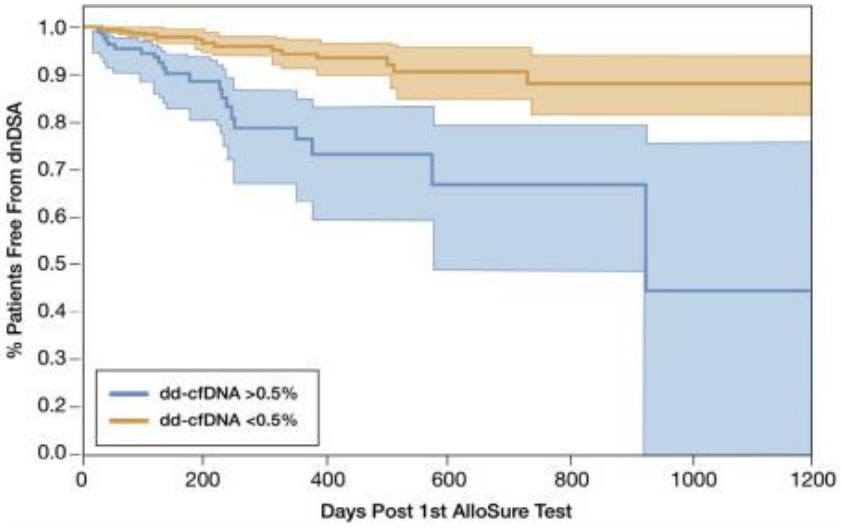


Figure 2 | Box and whisker plot showing the median donor-derived cell-free DNA (dd-cfDNA) and creatinine levels observed in patients with and without allograft rejection. (**a**) The ROC analysis for dd-cfDNA: area under the receiver-operating characteristic curve (AUROC) 0.798, with a median of 0.23% seen in patients with no rejection and 1.6% in patients with allograft rejection; $P < 0.0001$. (**b**) The ROC analysis for creatinine: AUROC 0.492, with a median creatinine of 1.38 mg/dl in patients with no rejection versus 1.57 mg/dl in patients with allograft rejection; $P = 0.096$.

Elevations in dd-cfDNA > 0.5% associated with Detection of *de novo* DSA

- dd-cfDNA > 0.5% was associated with nearly a 3-fold risk of subsequent dnDSA (HR 2.71, p<0.001).
- After multivariate adjustment, every 1% increase in AS was associated with a ~20% increase in dnDSA risk (HR 1.19, p = 0.004).
- Elevation of dd-cfDNA occurred a median of 91 [30 – 125] days before dnDSA detection with a median increase of 121% [69 – 183] from the prior value.

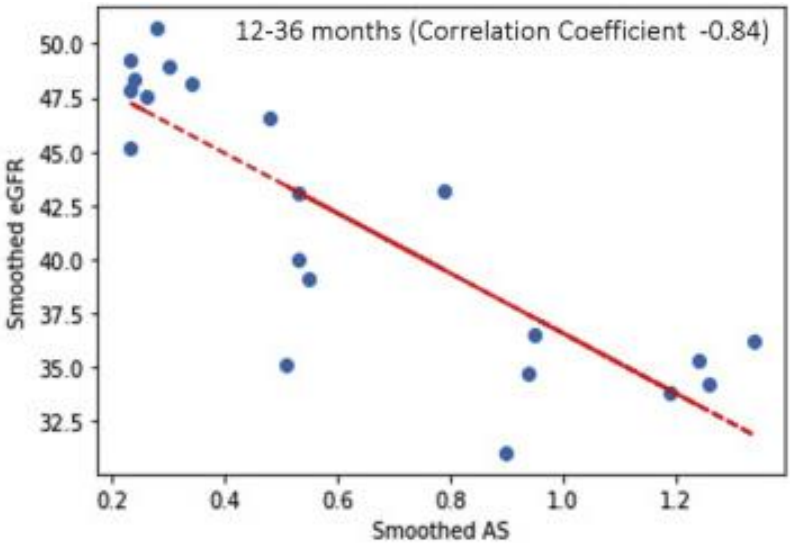


Bu et al. Kidney Int. 2022 Apr;101(4):793-803

Elevations in dd-cfDNA Identify High Risk Patients

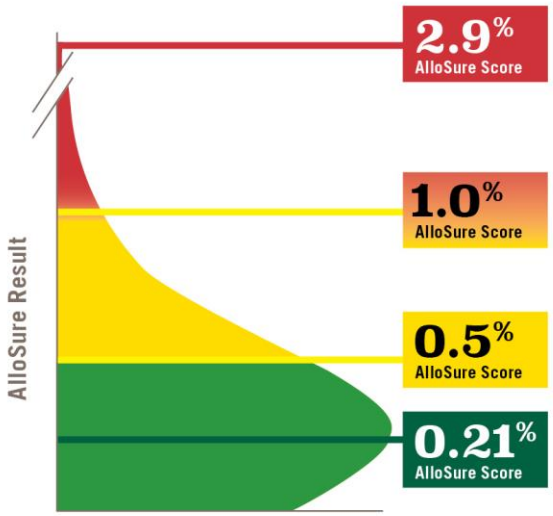
dd-cfDNA > 0.5% Associated with Clinically Significant eGFR Decline

- K-means clustering, an unsupervised machine learning algorithm, used to investigate association between AlloSure scores & eGFR decline.
- Elevations in dd-cfDNA (≥0.5%) were associated with eGFR decline between 12 – 36 months after transplant.
- Persistently elevated dd-cfDNA (>1 result ≥0.5%) doubled the risk of a 25% decline in eGFR (HR 1.97, p = 0.041).



Bu et al. Kidney Int. 2022 Apr;101(4):793-803

AlloSure Clinical Interpretation



89% PPV for ABMR in DSA** positive patients[4]

Threshold for active rejection[5]

Delineation of biopsy-confirmed TCMR1A*** /Borderline rejections associated with adverse outcomes[6]

95% NPV**** for active rejection[7]

High Risk of ABMR for DSA+ Patients

Likely Risk for Active Injury

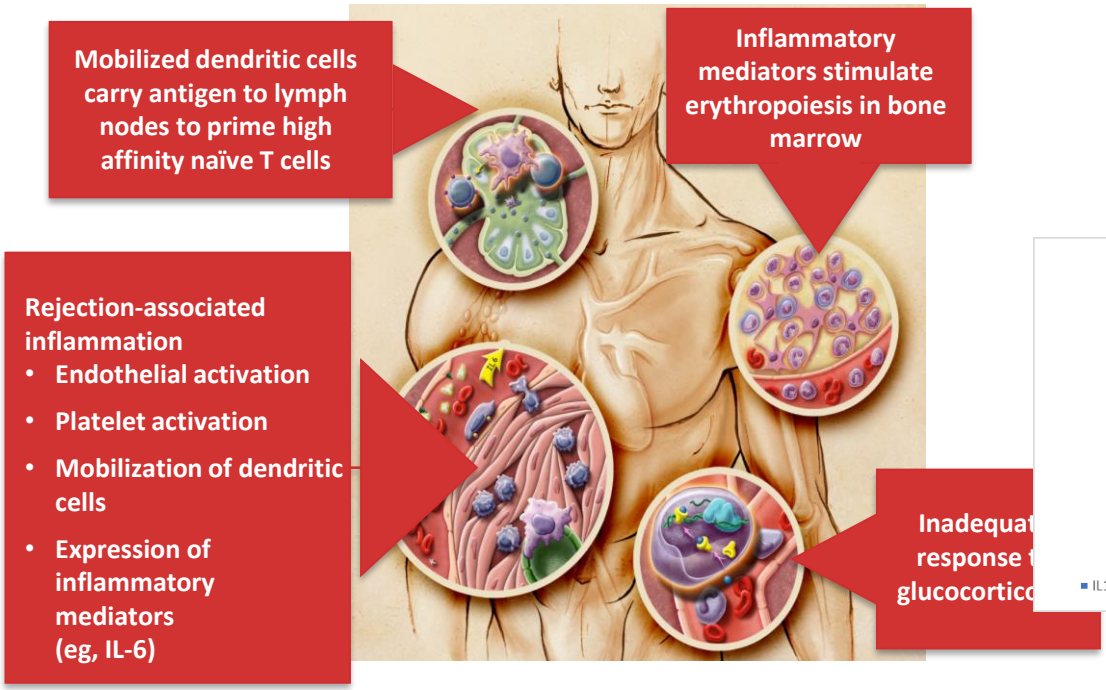
Likely Risk for poorer graft prognosis with biopsy-confirmed TCMR1A / Borderline cases

Low Risk: "Peace of Mind" Score

[1]*Jordan SC et al. Transplant Direct. 2018; 4:e379*
[2]*Bloom RD et al. J Am Soc Nephrol. 2017; 28.2221-2232*
[3]*Stites E, et al. Am J Transplant. 2020; 00:1-8*
[4]*Bloom RD et al. J Am Soc Nephrol. 2017; 28.2221-2232*

*ABMR = Antibody-Mediated Rejection
**DSA = Donor-Specific Antibodies
***TCMR = T Cell-Mediated Rejection
****NPV: Negative Predictive Value

AlloMap Heart Gene Expression, a 11-Gene Transcript Profiling Measures Recipient Immune Activity



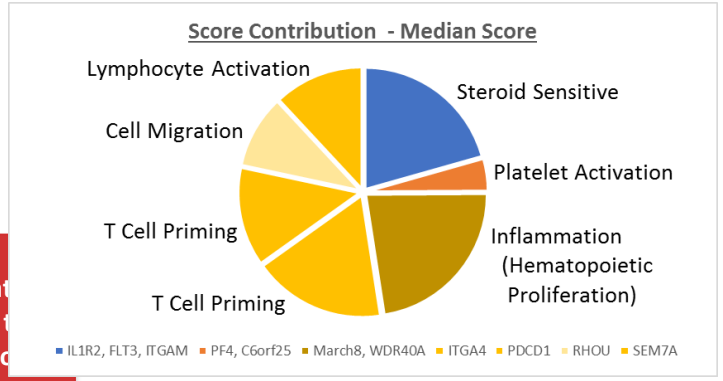


IMAGE study demonstrated non-inferiority of clinical outcomes in patients with AlloMap surveillance compared to patients with biopsy surveillance (6 months to 5 years post transplant)

Pham MC, New Engl J Med 2010; 362:1890

The International Society of Heart and Lung Transplantation (**ISHLT**) guidelines for care of heart transplant recipients recommend that the AlloMap Heart test can be used as a non-invasive method for ruling out moderate to severe acute cellular rejection in asymptomatic patients

Costanzo MR, J Heart Lung Transplant 2010;29:914

AlloMap Kidney Clinical Validation

Original Investigation

Kidney360

Clinical Validation of an Immune Quiescence Gene Expression Signature in Kidney Transplantation

Enver Akalin,[1] *Matthew R. Weir*,[2] *Suphamai Bunnapradist*,[3] *Daniel C. Brennan*,[4] *Rowena Delos Santos*,[5] *Anthony Langone*,[6] *Arjang Djamali*,[7] *Hua Xu*,[8] *Xia Jin*,[8] *Sham Dholakia*,[9] *Robert N. Woodward*,[8] *and Jonathan S. Bromberg*[10]

Key Points

- AlloMap Kidney is a gene expression profile developed using candidate genes from the AlloMap assay broadly used in heart transplantation.
- AlloMap Kidney was validated to differentiate quiescence from rejection in two independent sample sets using a quantitative scale.
- Blood cell gene expression and donor-derived cell-free DNA contribute independent signals and inform on different aspects of allograft rejection.

Kidney360. 2021 Sep 28;2(12):1998

Validation of a Blood Gene Expression Classifier to Differentiate Immune Quiescence from Rejection

Kidney360

DCAF12
MARCH8
FLT3
IL1R2
PDCD1

5 gene classifier (Developed on 56 peripheral blood samples)

AlloMap Kidney

PRIMARY Validation set

Q samples 98
R samples 18
7 TCMR
10 ABMR
1 Mixed

SECONDARY Validation set

Q samples 8
R samples 11
7 TCMR
2 ABMR
2 Mixed

Q, quiescence; R, rejection

AlloMap Kidney Classifier Scores

	PRIMARY Validation	SECONDARY Validation
Quiescence	**9.49** (7.68-11.53) *Median*	The cohorts were statistically different and the medians were similar to the primary validation set
Rejection	**13.09** (11.25-15.28) ***Median***	
	p<0.001	***p=0.028***
AUC for discriminating rejection	**0.786**	**0.800**

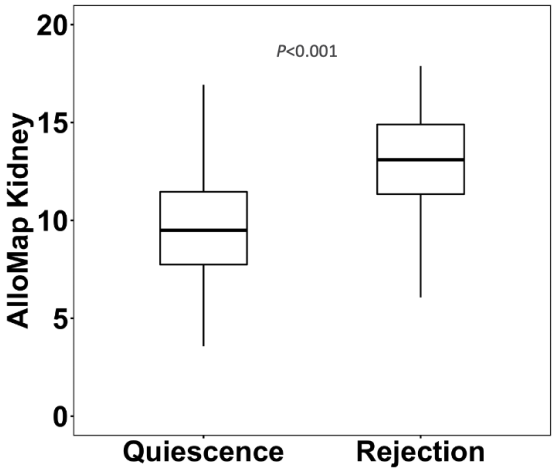
0.894
AUC

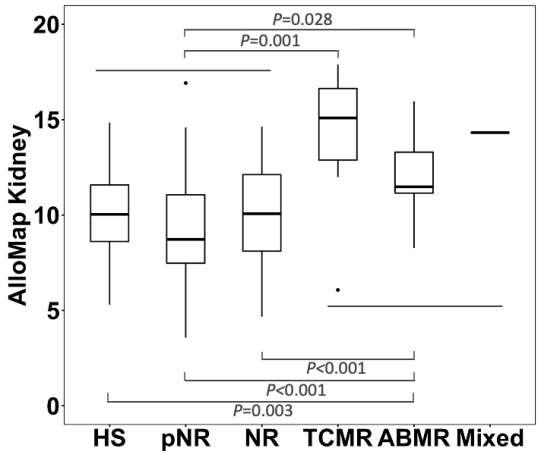
The ability to discriminate rejection from quiescence was improved when AlloSure and AlloMap Kidney were used together

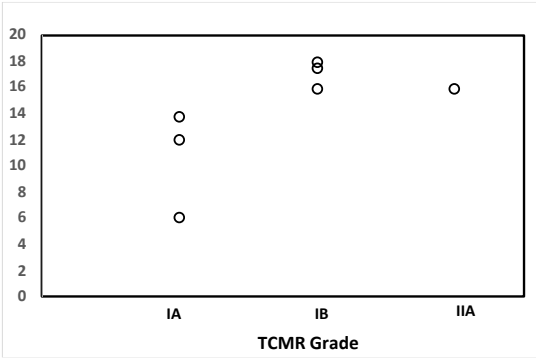
Conclusions Validation of AlloMap Kidney demonstrated the ability to differentiate between rejection and immune quiescence using a range of scores. The diagnostic performance suggests that assessment of the mechanisms of immunological activity is complementary to allograft injury information derived from AlloSure dd-cfDNA.

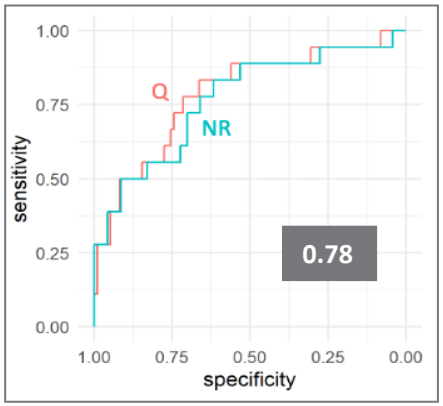
Enver Akalin, Matthew R. Weir, Suphamai Bunnapradist, et al. ***Clinical Validation of an Immune Quiescence Gene Expression Signature in Kidney Transplantation.*** *Kidney360*. DOI: 10.34067/KID.0005062021
Visual Abstract by Edgar Lerma, MD, FASN

DART Validation Data Differentiates Quiescence from Rejection



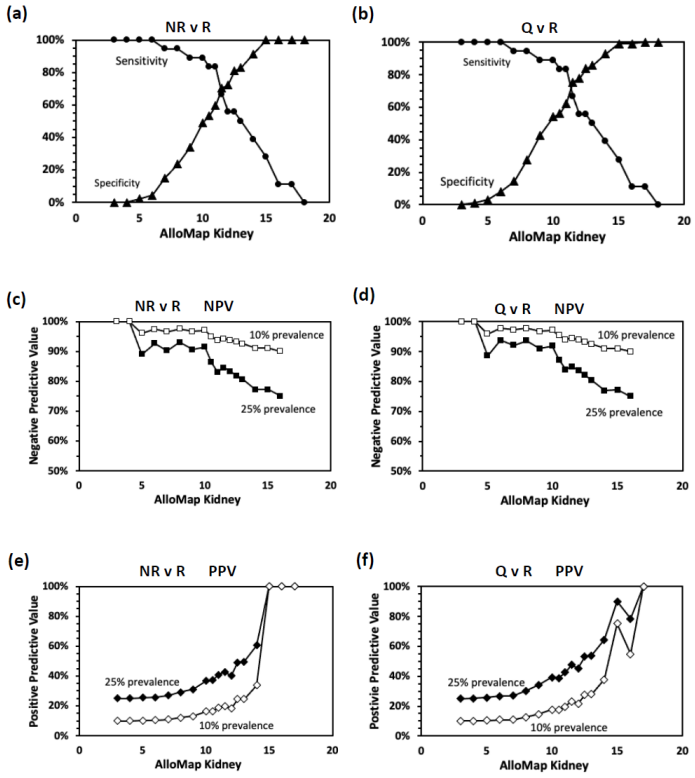






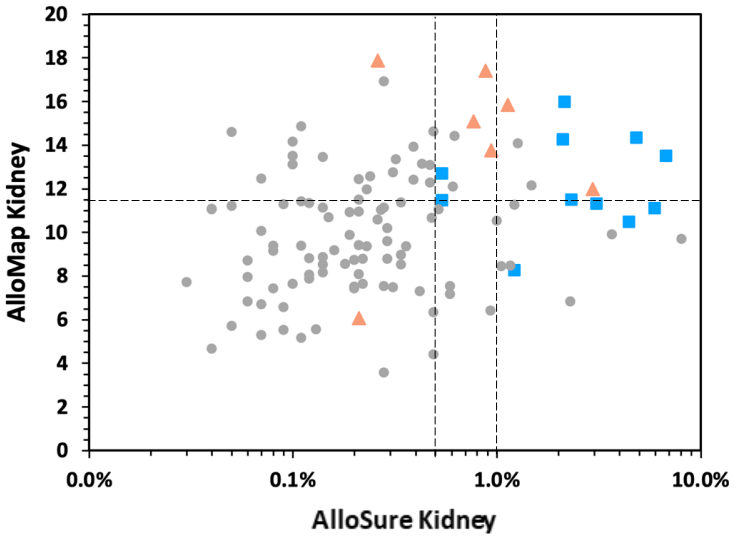
- All three defined groups of quiescence had significantly lower scores compared to the rejection cohort
- Each defined type of rejection had elevated scores compared to quiescence cohort, trend for TCMR higher than ABMR

AlloMap Kidney score of 11.5 demonstrates a high Negative Predictive Value

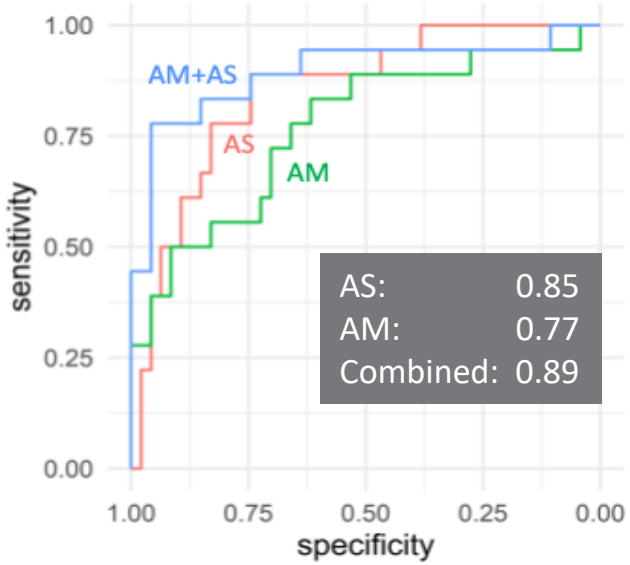


At a score of 11.5, AlloMap Kidney had a PPV of 23.2% and an NPV of 95.3% at 10% prevalence and a PPV of 47.6% and an NPV of 87.2% at 25% prevalence to discriminate rejection from quiescence

Combination of AlloMap Kidney and AlloSure Improves Ability to Discriminate Rejection from Quiescence



Triangles = TCMR
Squares = ABMR

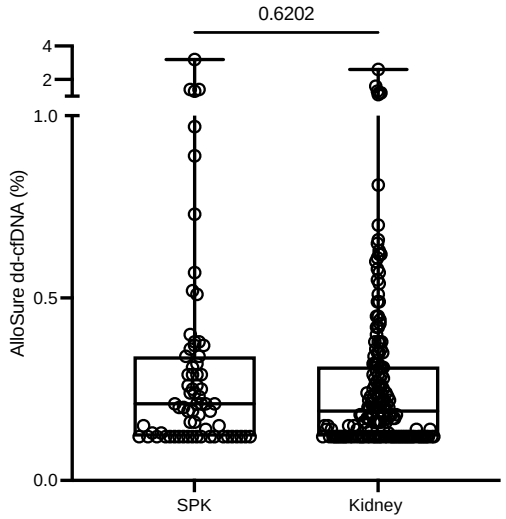


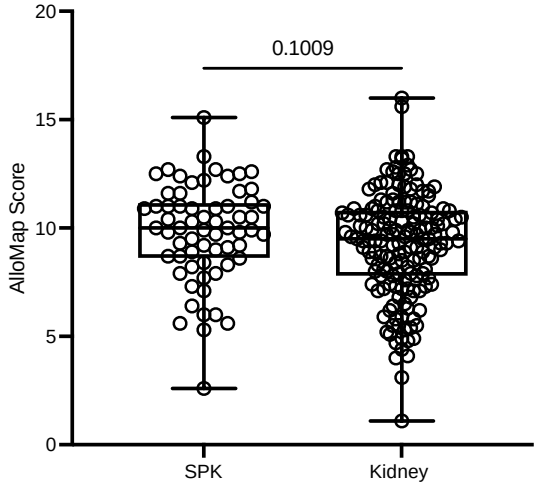
- **Simple 'combined score' yields higher AUC**
- More complex methods improves performance even further, but will need to be validated

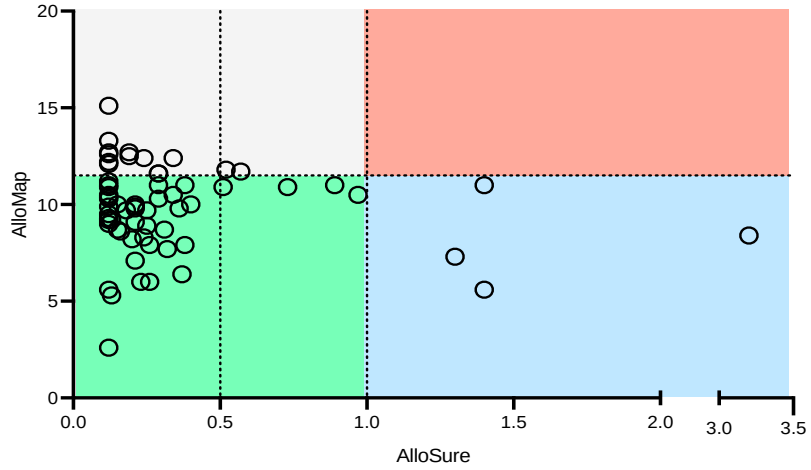
ALLOSURE AND ALLOMAP IN KIDNEY AND PANCREAS TRANSPLANT RECIPIENTS

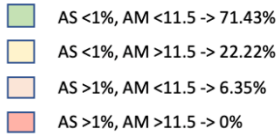
(Akalin et al. American Transplant Congress 2023)

- This is a non-randomized, non-interventional, prospective pilot cohort study to monitor kidney/pancreas transplant patients post-transplant to determine if non-invasive measures using dd-cfDNA (Allosure) and AlloMap can predict and confirm the development of allograft injury and rejection in either organ.
- 26 kidney/pancreas transplant recipients was enrolled at the time of transplantation or anytime within 3 years after transplantation starting at least 1 month after transplantation. Patients had blood samples drawn at the time enrollment and at 3, 6, 9, and 12 months after enrollment and at the time of clinically indicated kidney and/or pancreas transplant biopsy.
- Molecular profiles of SPK recipients were compared to 166 kidney transplant recipients enrolled in the OKRA registry (NCT03326076) undergoing longitudinal surveillance with dd-cfDNA (Allosure) and blood GEP (Allomap)


0.6202
AlloSure dd-cfDNA (%)
4
2
1.0
0.5
0.0
SPK
Kidney


0.1009
AlloMap Score
20
15
10
5
0
SPK
Kidney


AlloMap
20
15
10
5
0
0.0
0.5
1.0
1.5
2.0
3.0
3.5
AlloSure


AS <1%, AM <11.5 -> 71.43%
AS <1%, AM >11.5 -> 22.22%
AS >1%, AM <11.5 -> 6.35%
AS >1%, AM >11.5 -> 0%

TRUGRAF

Received: 25 April 2018 | Revised: 12 June 2018 | Accepted: 3 July 2018

DOI: 10.1111/ajt.15011

ORIGINAL ARTICLE

AJT

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

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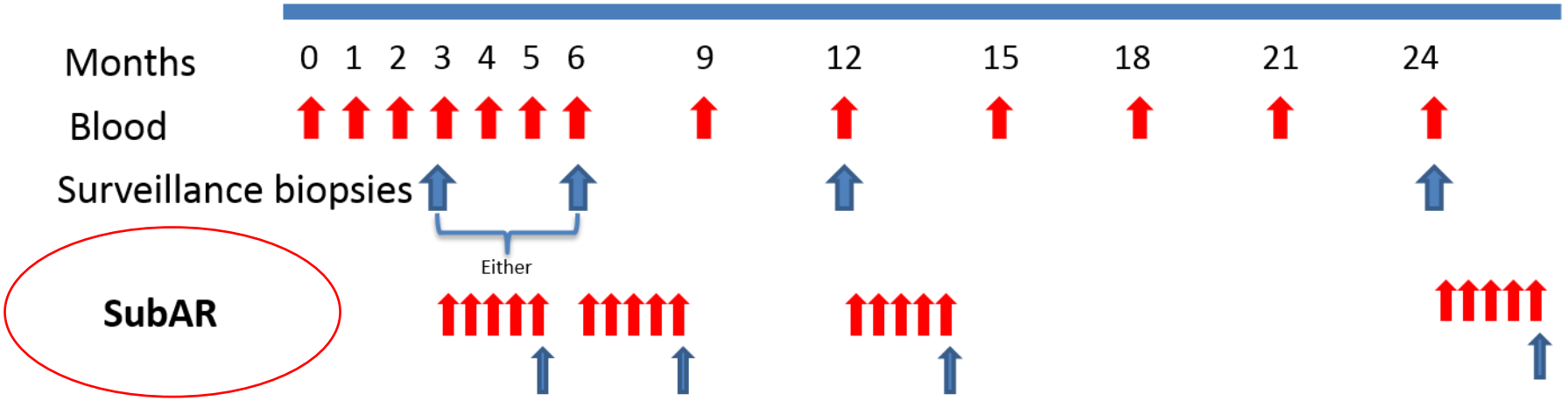
CTOT 08 Trial - 24-month Multi-Center Observational Study – 5 Centers - Surveillance Biopsies at 2-6, 12 and 24 months

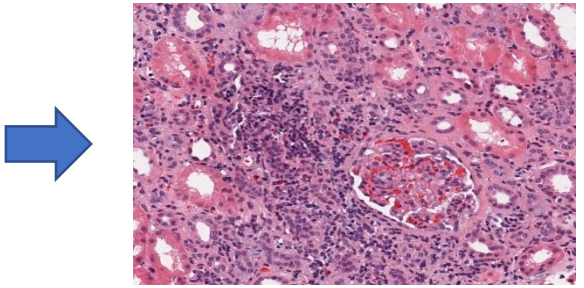


SubAR: histology on a surveillance biopsy

acute rejection (≥ Banff borderline cellular rejection and/or antibody mediated rejection)

AND stable renal function,

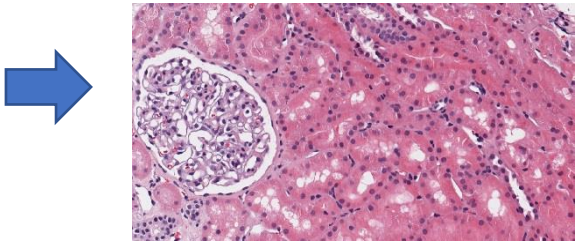
serum creatinine <2.3 mg/dl and <20% increase in creatinine compared to a minimum of 2-3 prior values over a mean period and range of 132 and 75-187 days, respectively



Transplant eXcellence (**TX**): normal histology on surveillance biopsy

(no evidence of rejection - Banff i=0 and t=0, g=0, ptc=0; ci=0 or 1 and ct=0 or 1)

AND stable renal function as defined above. Surveillance biopsies were performed on all subjects at 2-6, 12 and 24 months following transplantation



Test Performance by Locked Threshold Probability (subAR positive test)

Dataset	Paired samples	TX:subAR (% subAR prevalence)	Prob. Thresh	% Neg (Spared biopsy)	NPV	True Neg	False Neg	% Pos (pick up subAR)	PPV	True Pos	False Pos
Discovery set	N=530	400:130 (24.5%)	0.375	74.7%	88%	349	47	25.3%	61%	83	51
Validation set #1	N=138	96:42 (30.4%)	0.375	71.7%	78%	77	22	28.3%	51%	20	19
Validation set #2	N=129/138	93:36 (27.9%)	0.375	72.1%	80%	74	19	27.9%	47%	17	19

72-75% of patients would have a negative test and could therefore be spared a surveillance biopsy by ruling out the presence of subAR with 78-88% NPV.

The remaining 25-28% would have a positive test and would therefore be at higher risk harboring subAR with 47-61% PPV.

Combining Blood Gene Expression and Cellfree DNA to Diagnose Subclinical Rejection in Kidney Transplant Recipients

Sookhyeon Park,[1,2] *Kexin Guo*,[1,3] *Raymond L. Heilman*,[4] *Emilio D. Poggio*,[5] *David J. Taber*,[6] *Christopher L. Marsh*,[7] *Sunil M. Kurian*,[8] *Steve Kleiboeker*,[9] *Juston Weems*,[9] *John Holman*,[10] *Lihui Zhao*,[1,3] *Rohita Sinha*,[9] *Susan Brietigam*,[1] *Christabel Rebello*,[1] *Michael M. Abecassis*,[11,12] *and John J. Friedewald*[1,2]

CJASN 16: 1539–1551, 2021. doi: https://doi.org/10.2215/CJN.05530421

We, therefore, undertook an analysis to describe the performance of the TruGraf gene expression profile individually and combined with measurements of plasma donor-derived cfDNA to complement the diagnostic accuracy of either test alone to monitor stable kidney transplant recipients for subclinical rejection.

Of 428 samples, 76% (n=325) and 24% (n=103) were classified no rejection and subclinical rejection, respectively, by histologic phenotypes.

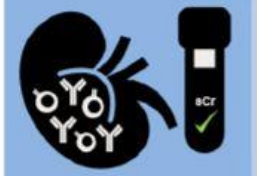
Can blood gene expression assays and donor-derived cellfree DNA be used to diagnose subclinical rejection?

CJASN
Clinical Journal of the American Society of Nephrology

Methods

- Post hoc analysis
  Clinical Trials in Organ Transplantation 08
- Surveillance biopsies
  2 to 6, 12, and 24 months post-transplant
- 208 subjects
  428 biopsy samples
- 2011 – 2014

Results



Diagnosis of Subclinical Rejection	Gene Expression Profile Assay (95% CI)	Donor Derived cfDNA (95% CI)	Combined Tests (95% CI)
Positive Predictive Value	47% (0.35- 0.59)	56% (0.44-0.67)	81% (0.63-0.95)
Negative Predictive Value	82% (0.78-0.86)	84% (0.80-0.88)	88% (0.84-0.92)
AUROC	0.75	0.72	0.81

Gene expression profile better at detecting cellular rejection

AUROC 0.80 vs. 0.62, P=0.001

Donor Derived cfDNA better at detecting antibody rejection

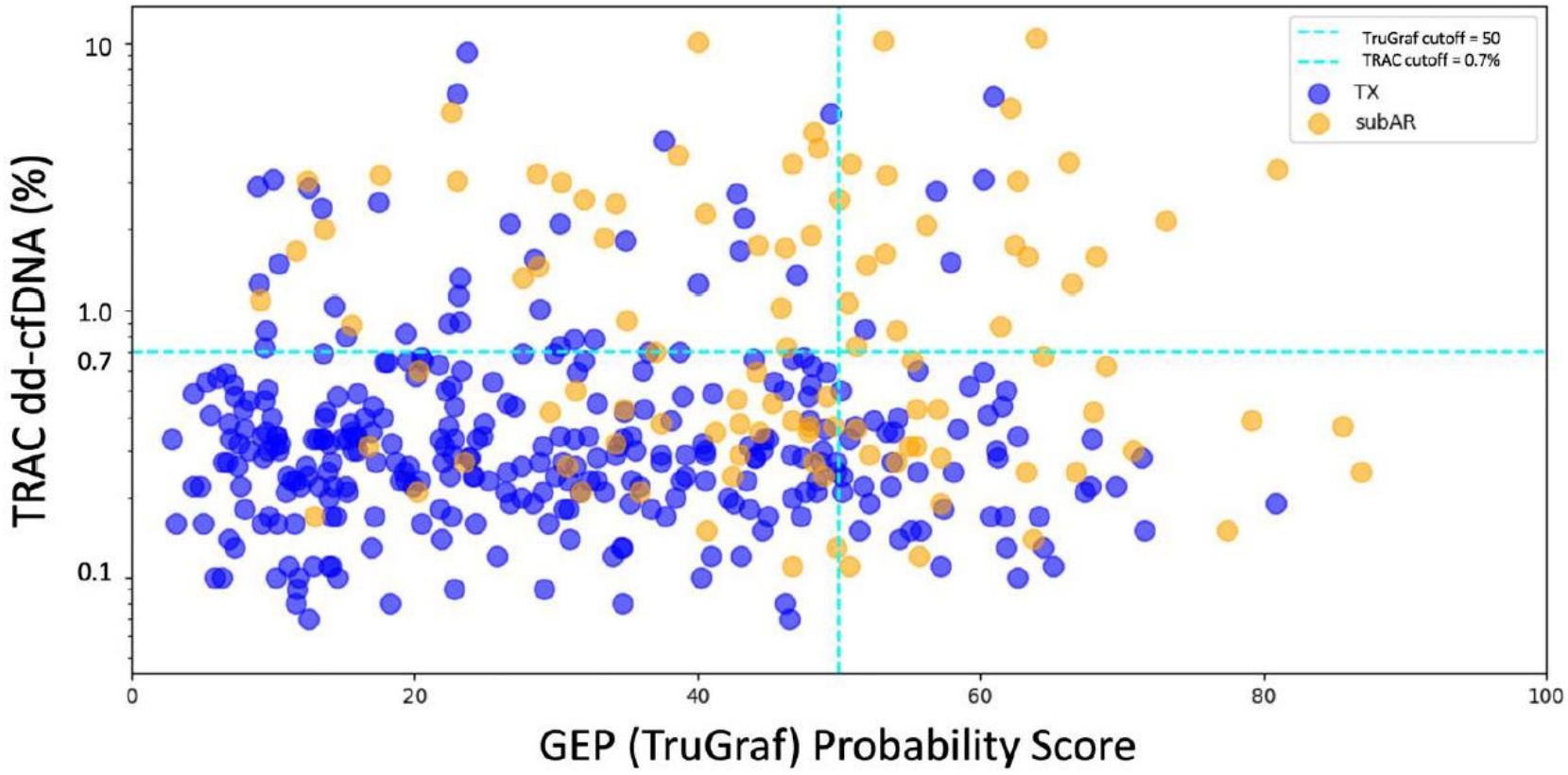
AUROC 0.84 vs. 0.71, P=0.003

Conclusions Donor-derived cellfree DNA and gene expression profiles provide a less invasive monitoring strategy for subclinical rejection, with different detection of antibody- and cell-mediated rejection.

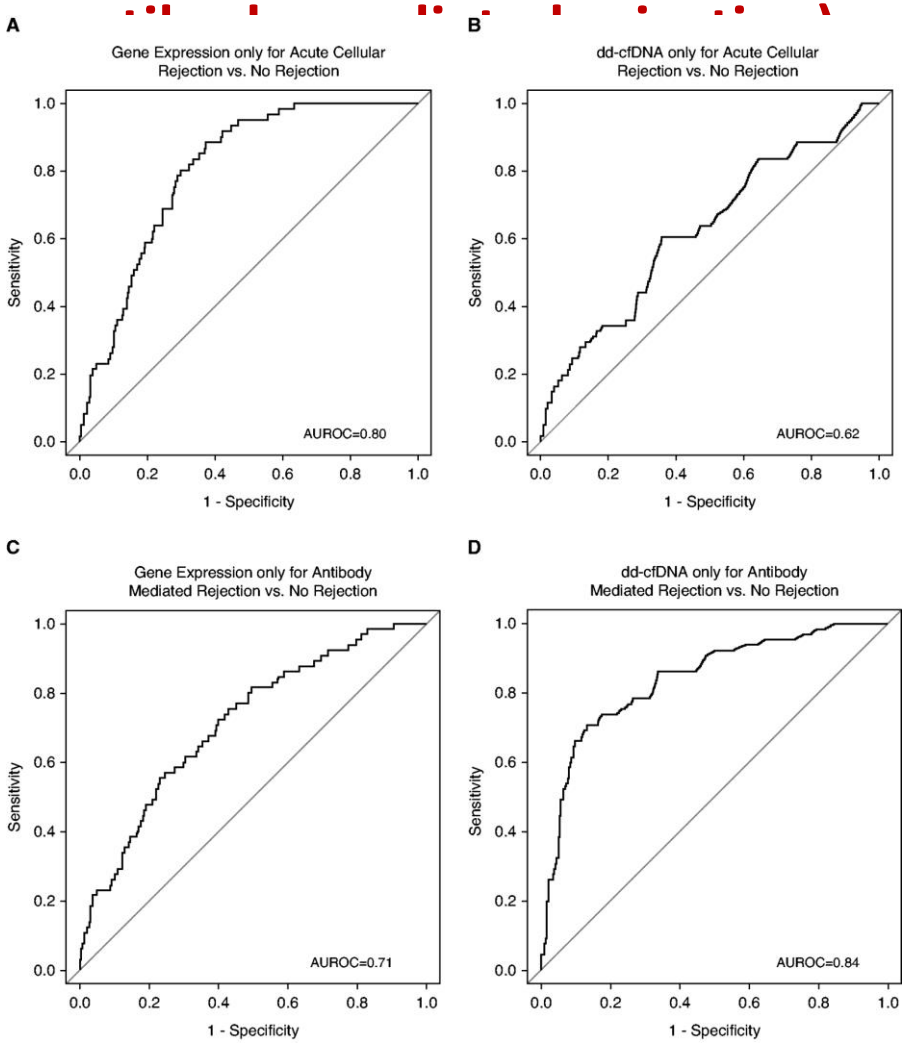
Sookhyeon Park, Kexin Guo, Raymond L. Heilman, et al. ***Combining Blood Gene Expression and Cell-Free DNA to Diagnose Subclinical Rejection in Kidney Transplant Recipients***. CJASN doi: 10.2215/CJN.05530421. **Visual Abstract by Sinead Stoneman, MB BCh BAO, MRCPI**

Supplemental Figure 1

Distribution of Samples by Clinical Phenotype, Gene Expression Profile Probability Score, and % donor derived-cfDNA



Differential performance of the gene expression profile and donor-derived cfDNA based on rejection type (acute cellular versus acute



Sookhyeon Park et al. CJASN 2021;16:1539-1551

CJASN
Clinical Journal of the American Society of Nephrology

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PLOS | MEDICINE

The kSORT Assay to Detect Renal Transplant Patients at High Risk for Acute Rejection: Results of the Multicenter AART Study

Silke Roedder[1☯], Tara Sigdel[1☯], Nathan Salomonis[2☯], Sue Hsieh[1], Hong Dai[3¤a], Oriol Bestard[4], Diana Metes[5], Andrea Zeevi[5], Albin Gritsch[6], Jennifer Cheeseman[7], Camila Macedo[5], Ram Peddy[3], Mara Medeiros[8], Flavio Vincenti[1], Nancy Asher[1], Oscar Salvatierra[9], Ron Shapiro[5], Allan Kirk[7¤b], Elaine Reed[6], Minnie M. Sarwal[1]*

Abstract

Background: Development of noninvasive molecular assays to improve disease diagnosis and patient monitoring is a critical need. In renal transplantation, acute rejection (AR) increases the risk for chronic graft injury and failure. Noninvasive diagnostic assays to improve current late and nonspecific diagnosis of rejection are needed. We sought to develop a test using a simple blood gene expression assay to detect patients at high risk for AR.

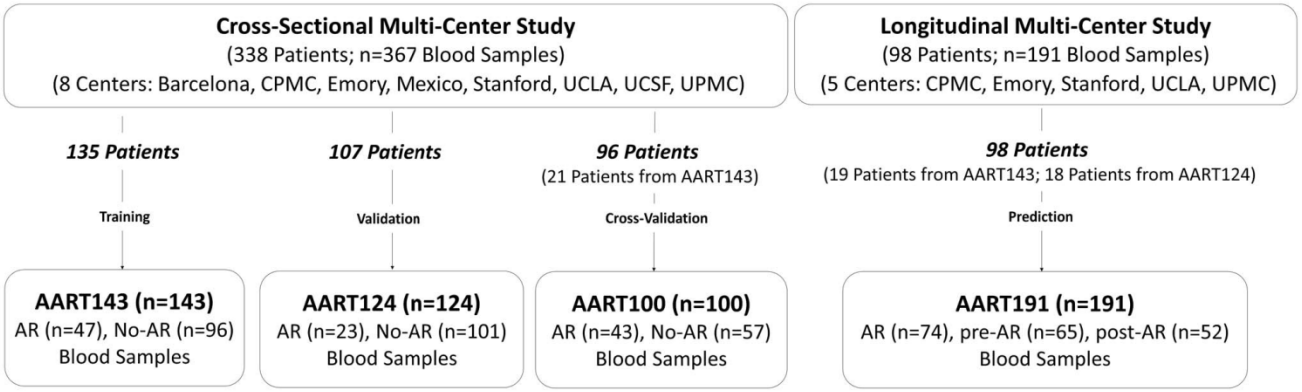
Methods and Findings: We developed a novel correlation-based algorithm by step-wise analysis of gene expression data in 558 blood samples from 436 renal transplant patients collected across eight transplant centers in the US, Mexico, and Spain between 5 February 2005 and 15 December 2012 in the Assessment of Acute Rejection in Renal Transplantation (AART) study. Gene expression was assessed by quantitative real-time PCR (QPCR) in one center. A 17-gene set—the Kidney Solid Organ Response Test (kSORT)—was selected in 143 samples for AR classification using discriminant analysis (area under the receiver operating characteristic curve [AUC] = 0.94; 95% CI 0.91–0.98), validated in 124 independent samples (AUC = 0.95; 95% CI 0.88–1.0) and evaluated for AR prediction in 191 serial samples, where it predicted AR up to 3 mo prior to detection by the current gold standard (biopsy). A novel reference-based algorithm (using 13 12-gene models) was developed in 100 independent samples to provide a numerical AR risk score, to classify patients as high risk versus low risk for AR. kSORT was able to detect AR in blood independent of age, time post-transplantation, and sample source without additional data normalization; AUC = 0.93 (95% CI 0.86–0.99). Further validation of kSORT is planned in prospective clinical observational and interventional trials.

Conclusions: The kSORT blood QPCR assay is a noninvasive tool to detect high risk of AR of renal transplants.

17 GENE SET:

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

Assessment of 558 Samples from 436 Patients in the **AART Study** (Patient/Sample Flow):



Statistics	kSORT Predictions					
	AART143 (Training Set)		AART124 (Validation Set)		AART100 (Cross-Validation Set)	
	AR	No-AR	AR	No-AR	AR	No-AR
Real Results						
AR	39	8	21	2	36	3
No-AR	9	87	1	100	3	43
Sensitivity (95% CI)	82.98% (69.19%–92.35%)		91.30% (71.96%–98.93%)		92.31% (79.13%–98.38%)	
Specificity (95% CI)	90.63% (82.95%–95.62%)		99.01% (94.61%–99.97%)		93.48% (82.1%–98.63%)	
PPV (95% CI)	81.25% (68.06%–89.81%)		95.46% (78.20%–99.19%)		93.21% (79.68%–97.35%)	
NPV (95% CI)	91.58% (84.25%–95.67%)		98.04% (93.13%–99.46%)		93.48% (82.45%–97.76)	
AUC (95% CI)	0.94 (0.91–0.98)		0.95 (0.88–1.00)		0.92* (0.86–0.98)	

Diagnostic performance of kSORT, a blood-based mRNAassay for noninvasive detection of rejection after kidney transplantation: A retrospective multicenter cohort study

Elisabet Van Loon[1,2] | **Magali Giral**[3,4,5,6] | **Dany Anglicheau**[7,8] | **Evelyne Lerut**[9] | **Valérie Dubois**[10] | **Maud Rabeyrin**[11] | **Sophie Brouard**[3,4,5,6] | **Silke Roedder**[12] | **Michael G. Spigarelli**[12] | **Marion Rabant**[13] | **Kris Bogaerts**[14,15] | **Maarten Naesens**[1,2] | **Olivier Thaunat**[16,17,18]

***Am J Transplant*. 2020**

The primary objective was to determine the diagnostic performance of the kSORT assay to detect AR (T cell–mediated and/or antibody-mediated rejection) as compared to a concomitant renal biopsy.

AR was reported on the concomitant biopsy in 188 of 1763 (10.7%) blood samples and any rejection (including borderline changes) in 614 of 1763 (34.8%) blood samples.

The kSORT assay had no diagnostic value for AR (area under the curve [AUC] 0.51, 95% confidence interval [CI] 0.50-0.56; *P* = .46) overall, or when considering indication biopsies (N = 487) and protocol-specified biopsies (N = 1276) separately (AUC of 0.53, 95% CI 0.50-0.59, *P* = .44 and 0.55, 95% CI 0.50-0.61, *P* = .09, respectively).

This large retrospective study utilizing samples obtained under real-world clinical conditions, was unable to validate the kSORT assay for detection of AR in the first year after transplantation.

Performance and Advancement of the Kidney Solid Organ Response Test

Joshua Lee, MD,[1] Mariel Barbachan e Silva, BSc,[2] Yi Bao, MS,[3] Ryan Whitmarsh, PhD,[1] Sukanta Banerjee, PhD,[1] Jeannine O'Conner,[1] Jeffery Holbert, MD,[1] Tiffany K. Bratton, PhD,[1] Pilib Ó. Broin, PhD,[2] and Enver Akalin, MD, FAST, FASN[3]

(*Transplantation* 2023;107: 2271–2278).

- Kidney transplant recipients enrolled in IRB-approved "Immune monitoring study" and had blood samples collected in RNA-Paxgen tube at the time of transplantation or clinically indicated biopsy were included in this study
- 95 blood samples analyzed (18 patients had blood samples before transplant and 77 patients after transplant)
- 65 patients had clinically indicated biopsies at the time of sample collection.
  - 15 biopsies showed acute rejection (9 T-cell mediated rejection (TCMR) and 6 active antibody-mediated rejection (ABMR)
  - 16 chronic active ABMR, 3 chronic inactive ABMR,
  - 18 normal biopsies and 13 interstitial fibrosis and tubular atrophy without rejection
- 31 patients with rejection compared to remaining 64 patients without rejection.

Performance and Advancement of the Kidney Solid Organ Response Test

Joshua Lee, MD,[1] Mariel Barbachan e Silva, BSc,[2] Yi Bao, MS,[3] Ryan Whitmarsh, PhD,[1] Sukanta Banerjee, PhD,[1] Jeannine O'Conner,[1] Jeffery Holbert, MD,[1] Tiffany K. Bratton, PhD,[1] Pilib Ó. Broin, PhD,[2] and Enver Akalin, MD, FAST, FASN[3]

(*Transplantation* 2023;107: 2271–2278).

TABLE 3.

Prediction of rejection by kSORT score

	kSORT Score >9		kSORT Score >5	
Statistic	**Value, %**	**95% CI**	**Value, %**	**95% CI**
Sensitivity	55.88	37.89% to 72.81%	64.71	46.49% to 80.25%
Specificity	73.77	60.93% to 84.20%	73.77	60.93% to 84.20%
PPV	54.29	41.48% to 66.55%	57.89	45.76% to 69.15%
NPV	75.00	66.64% to 81.84%	78.95	69.90% to 85.83%
Disease prevalence	35.79	26.21% to 46.28%	35.79	26.21% to 46.28%

CI, confidence interval; kSORT, kidney solid organ response test; NPV, negative predictive value; PPV, positive predictive value.

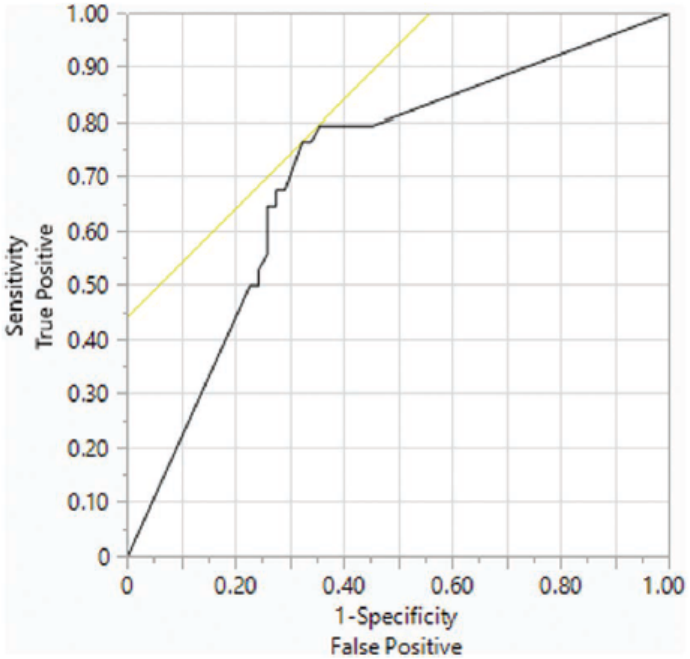


FIGURE 1. ROC curve for kSORT detection of biopsy-proven rejection with a cutoff of ≥9 as positive. The AUC is 0.71. AUC, area under the curve; kSORT, kidney solid organ response test; ROC, receiver operating characteristic.

CONCLUSION

- Cell-free DNA tests have good negative predictive value (> 90%) indicating immune quiescence for significant rejection but low positive predictive value (~40-50%). PPV increases with DSA and kidney dysfunction to 80-90%
- Gene transcripts: Allomap and TRUGRAF has good NPV > 90% but low PPV 40-60% and PPV value increases with combining cell-free DNA
- kSORT assay has different NPV, PPV and AUC in published 3 studies

LIMITATIONS

- In order to use those biomarkers as a standard of care to monitor all kidney transplant recipients, it should be proven to be cost effective by decreasing number of clinically indicated biopsies and improving graft survival
- It may take 48-72 hours to receive the results and could not be used in decision making for differential diagnosis of acute renal failure
- Decreased PPV especially in borderline and mild TCMR (Ia) and can miss those rejections
- It might lead to unnecessary biopsies in patients with false positive results

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