Cell-free DNA and Active Rejection in Kidney Allografts

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Disclosures

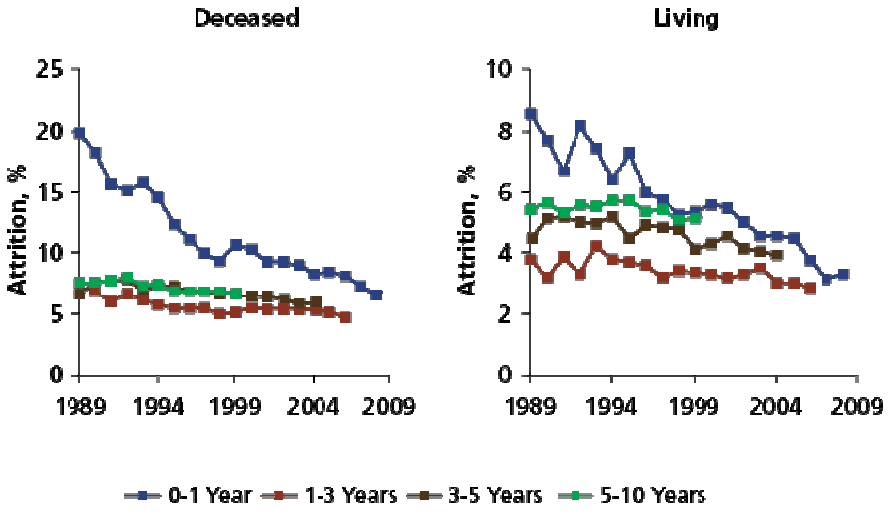
Jonathan S. Bromberg, MD, PhD

- **Research Contract with CareDx, Inc.**
- **Statements in this presentation include the speaker's own opinions and do not necessarily reflect the views of CareDx.**

Current Clinical Challenges

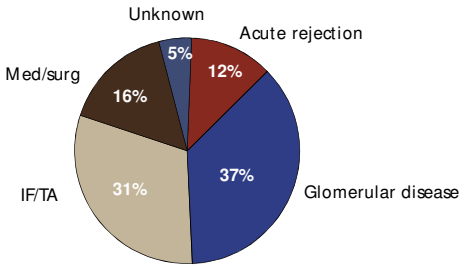
- Allograft loss is expensive, resulting in many major adverse outcomes for the patient, family, center, system, and US healthcare
- Current immunosuppression has not changed much in over 15 years
- Current outcomes have not changed much in over 15 years
- Current monitoring modalities have not changed much in over 15 years (Scr, U/A, viral NATs, DSA → DU/S, KBx) despite many attempts to define new invasive or non-invasive markers
- Current monitoring is often arduous, expensive, and inaccurate

Most Improvement in Graft Survival Is Due to Reduced Early Attrition



Lamb et al. *Am J Transplant.* 2011;11:450-462

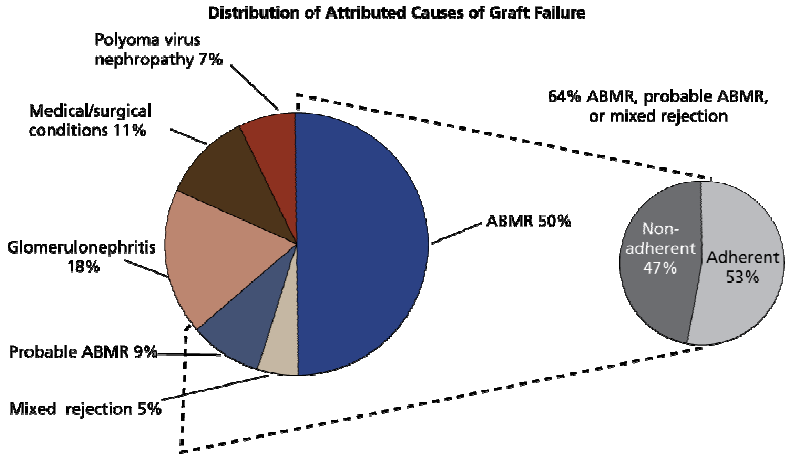
Causes of Allograft Failure



Death-censored graft loss after sequential surveillance biopsies

El-Zoghby ZM et al. *Am J Transplant.* 2009;9:527-535

Role of Antibody-Mediated Rejection and Nonadherence in Kidney Transplant Failure



Almost half of antibody-mediated rejection (ABMR) is due to nonadherence

Sellarés J et al. *Am J Transplant.* 2012;12:388-399

Recommended Monitoring for Rejection

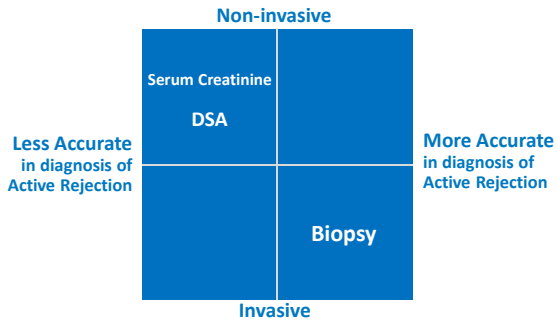
According to KDIGO clinical practice guidelines, early detection of allograft dysfunction is key

- Readily available serum creatinine is recommended, but an increase can be due to other conditions
- Scr is usually interpreted in the context of other tests and clinical events
- Scr is not sensitive or specific
- Expense, inconvenience, and risk of biopsies may outweigh the benefit of detecting rejection
- Surveillance biopsies have never been validated to actually improve outcomes (believers and non-believers)

Bia M, Adey DB, Bloom RD, et al. KDOQI US commentary on the 2009 KDIGO clinical practice guideline for the care of kidney transplant recipients. *Am J Kidney Dis.* 2010; 56(2):189-218. doi: 10.1053/j.ajkd.2010.04.010.

Current Surveillance Options have Limitations

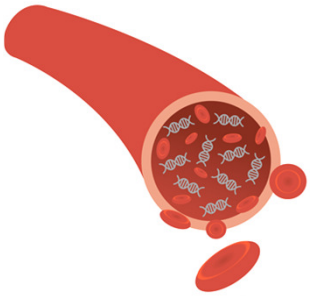
- Measuring serial serum creatinine levels is the most common approach to assess kidney function
  - Widely available, inexpensive, relatively non-invasive
  - Limitations: nonspecific, not sensitive, may be late signal
- Some programs use surveillance kidney biopsies
  - Limitations: high-cost, low-yield, sampling errors, inconvenience, risk to the patient, subjective interpretation



Nickerson P. Post-transplant monitoring of renal allografts: are we there yet? *Curr Opin Immunol.* 2009; 21(5) 563-568.

Cell-free DNA (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
in blood and plasma

cfDNA is an Established Biomarker for Prenatal Testing and Oncology

Widely recognized as a reliable biomarker to detect chromosomal abnormalities in prenatal testing[1]

The American College of Obstetrics and Gynocology includes fetal cfDNA testing as an option for prenatal screening to measure chromosomal abnormalities in the fetus such as trisomy 21 (Down syndrome)[1]

Used as a screening and prognostic tool for various cancers[2,3]

The FDA has approved a tumor cfDNA test (liquid biopsy) to identify EGFR mutations as a companion diagnostic for targeted therapies[3]

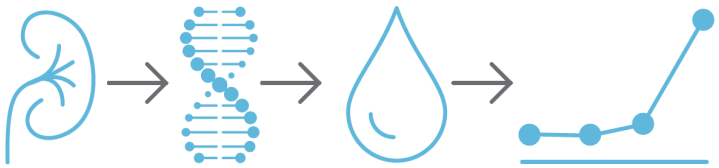
Kidney transplant rejection surveillance

Donor-derived cf DNA test validated for kidney transplant recipients[4, 5]

1. ACOG Practice Bulletin No. 163: Screening for fetal aneuploidy. *Obstet Gynecol*. 2016.
2. Schwarzenbach H, Hoon DSB, Pantel K. Cell-free nucleic acids as biomarkers in cancer patients. *Nat Rev Cancer*. 2011;426-437.
3. U.S. Department of Health and Human Services, U.S. Food & Drug Administration Website. https://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/DeviceApprovalsandClearances/Recently-ApprovedDevices/ucm519922.htm. Published September 9, 2016. Accessed April 10, 2017.
4. Bloom RD et al. Cell-free DNA and active rejection in kidney allografts. *J Am Soc Nephrol*. 2017. doi:10.1681/ASN.2016091034.
5. Bromberg JS et al. Biological Variation of Donor-Derived Cell-Free DNA in Renal Transplant Recipients: Clinical Implications. *J Appl Lab Med*. 2017;1(5).

Relationship of dd-cfDNA to Allograft Rejection

When organ injury occurs, cells release DNA into the plasma resulting in increased levels of dd-cfDNA



Cell Injury to the donor organ

DNA is Released into the recipient's plasma and becomes dd-cfDNA

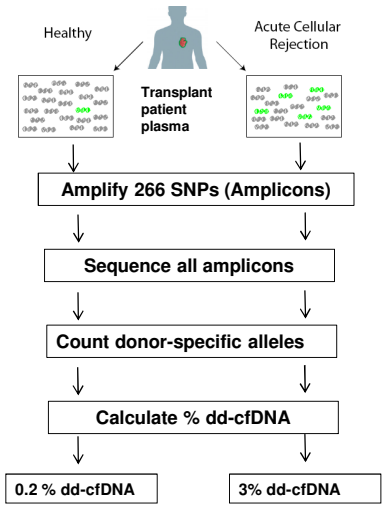
Measure the level of dd-cfDNA shed from the donor organ

A high level (dd-cfDNA) indicates potential organ rejection

Measuring Donor Derived (dd)-cfDNA in Transplant Patients

The method measures the small amount of dd-cfDNA from plasma:

- **Extract cfDNA**
  (Plasma collected in Streck BCT tubes)
- **Amplify 266 SNP loci selected to be sufficient to accurately measure dd-cfDNA. Amplify by concurrent multiplex PCR**
  (Access Array technology, Fluidigm)
- **Sequence amplified SNP loci**
  (MiSeq next-generation sequencing, Illumina)
- **Calculate the fraction of donor-specific nucleotides**

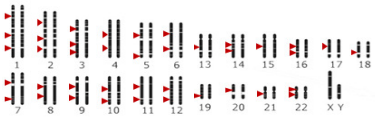


The Methodology does not Require Genotyping of the Donor or Recipient

dd-cfDNA is measured by determining the fraction of donor-derived nucleotides at 266 single-nucleotide polymorphism (SNP) location

- **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 found in plasma**
- **Next-Generation Sequencing (NGS) 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%**

SNPs selected from across the genome:



Grskovic M et al. Validation of a Clinical-Grade Assay to Measure Donor-Derived Cell-Free DNA in Solid Organ Transplant Recipients. *J Mol Diagn.* 2016;18(6):890-902.

Should Not be Ordered for

- **Recipients where a previous allograft is still in place**
- **Multi-organ recipients**
- **Recipients of a transplant from a monozyotic (identical) twin**
- **Recipients of allogeneic bone marrow transplant**
- **Pregnant recipients**
- **Under the age of 18 (not validated)**
- **Less than 2 weeks post transplant (likely to have resolving ATN with highly elevated dd-cfDNA)**

Multiple Studies Describe the Ability of dd-cfDNA to Identify Rejection in Organ Transplantation

Author	Organ	Description	Technology	Status
DeVlaminck, Valantine, Khush, Quake 2014	Heart	• dd-cfDNA diagnosis of acute rejection in Heart Tx patients • Sci Transl Med. 6(241):241	NGS shotgun, SNP detection	Research-grade
DeVlaminck, Valantine, Khush, Quake 2015	Lung	• dd-cfDNA diagnosis of acute rejection in Lung Tx patients • PNAS 112 (43): 13336	NGS shotgun, SNP detection	Research-grade
Grskovic et al 2016	Heart	• dd-cfDNA diagnosis of acute rejection in Heart tx patients • J Mol Diag 18(6):890-902	SNP targeted NGS	Clinical-grade
Bloom et al 2017	Kidney	• dd-cfDNA elevation in Kidney rejection • J Am Soc Nephrol	SNP targeted NGS	Clinical-grade
Bromberg et al 2017	Kidney	• dd-cfDNA reference range defined in Kidney transplant population • J Assoc Lab Med	SNPs targeted NGS	Clinical-grade
Schütz et al 2017	Liver	• dd-cfDNA elevation in Liver transplant rejection • PLoS Medicine	Digital PCR, SNP detection	Research-grade

Published Analytic and Clinical Evidence

Analytical Validity

Does the test accurately and robustly measure the biomarker?

Grskovic, M., et al. (2016). Validation of a Clinical-Grade Assay to Measure Donor-Derived Cell-Free DNA in Solid Organ Transplant Recipients. *J Mol Diagn* 18, 890-902.

Clinical Validity/Utility

How does the biomarker vary within the reference population?

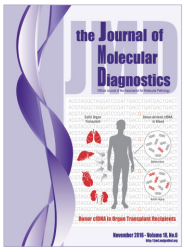
Bromberg, J.S., et al. (2017). Biological Variation of Donor-Derived Cell-Free DNA in Renal Transplant Recipients: Clinical Implications. *J Appl Lab Med* 2.

Are there differences in levels of the biomarker with organ rejection?

Bloom, R.D., et al. (2017). Cell-Free DNA and Active Rejection in Kidney Allografts. *J Am Soc Nephrol* 28.

Analytically Validated as a Sensitive, Accurate, and Precise Measurement of dd-cfDNA

Metric	AlloSure performance	Clinical applicability
Lower limit of quantification	0.20%	Results below 0.2% are not accurately quantified as different from zero and reported as less than 0.2%
Quantifiable range	0.20% -16%	• Results in kidney clinical validation studies range from 0% to 8% • Stable kidney recipient median = 0.21% • Critical decision point (Threshold) ~1%
Variability (CV)	6.8%	Excellent test reproducibility



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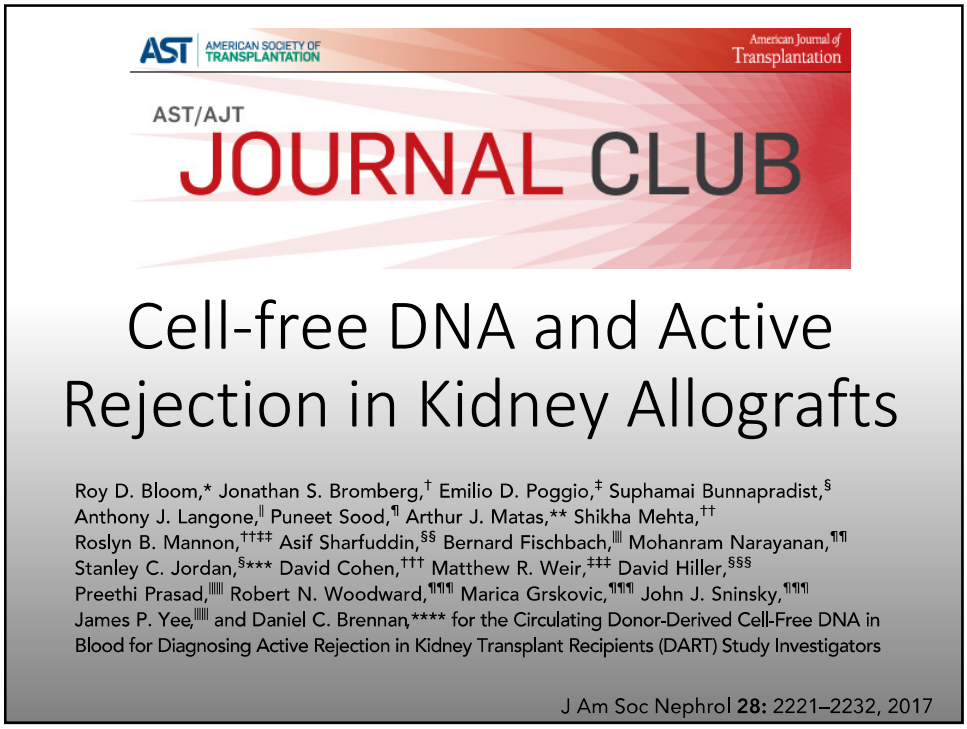
Clinical-grade test development

- **Analytical validation studies completed with reference materials validated by an orthogonal technology according to Clinical & Laboratory Standards Institute (CLSI)-recommended procedures**
- **Methods proficiency in accordance with standards for Next-Generation Sequencing**
- **Bioinformatics pipelines validated and locked**

Comparison to Common Clinical Analytes

Biomarker (Typical Value)	CV_A%	CV_I%	CV_G%	II	RCV_I%	RCV absolute	Reference
%dd-cfDNA (0.4)	6.8	21	37	0.57	61	61%	This study
Creatinine (100 µmol/l)	14	6.0	14.7	0.4	17.9	18 µmol/l	Omar
HbA1c (4%)	29	4.9	14	0.35	5.8	0.2%	Omar
Glucose (40 IU/l)	6.8	18	61	0.30	20.5	8.2 IU/l	Omar
Alanine aminostransferase (40 IU/l)		24.3	41.6	0.6	67.5	27	Omar

Biomarker (Typical Value)	CV_A%	CV_I%	CV_G%	II	RCV_I%	Monitoring Duration	Reference
%dd-cfDNA (0.4)	6.8	21	37	0.57	61	Monthly	This study
Creatine Kinase (174 IU/l)	14	22	42	0.52	72.2	Daily	Ross
Cardiac Troponin I (27 ng/l)	8.3	9.7	57	0.21	+46, log-normal increase	Hourly	Wu



Disclosures

Roy D. Bloom, MD

- Research funding, CareDx,
- Advisor, CareDx

Background

- Accurate and timely diagnosis of rejection and effective treatment is essential for long-term allograft survival
- Histological analysis is the "gold-standard" for distinguishing rejection from other causes of kidney allograft injury
  - Logistical challenges
  - Potential complications
  - Technical limitations
  - Patient inconvenience/discomfort

Background

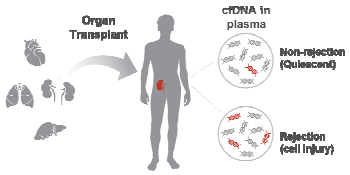
- Donor-derived cell-free DNA (dd-cfDNA)
  - Noninvasive test of allograft injury
  - May enable more frequent and quantitative assessment of allograft rejection and injury status
  - Safer
  - Simpler
  - Shown to discriminate rejection from no-rejection in single center studies previously
  - Least studied in kidneys

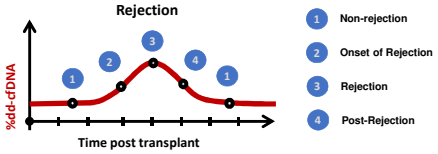
Donor-Derived Cell-Free DNA as a Biomarker in Transplantation

Cell-Free DNA (cfDNA)

- Fragments of DNA in the blood 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 short half-life of ~30 minutes

Donor-Derived Cell-Free DNA (dd-cfDNA)



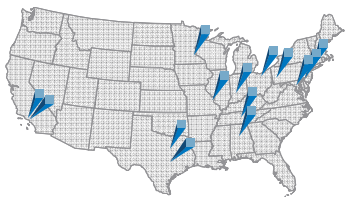


Key Results from Peer-Reviewed Publications:

- dd-cfDNA is very low in stable transplant recipients [1]
  - De Vlaminck STM 2014 (heart), Grskovic JMD 2016 (heart), Bromberg JALM 2017 (kidney), Schutz PLOS Med 2017 (Liver)
- dd-cfDNA is elevated at the time of rejection [2,3]
  - De Vlaminck STM 2014 (heart), Grskovic JMD 2016 (heart), Schutz PLOS Med 2017 (liver)
- dd-cfDNA decreases following successful treatment
  - De Vlaminck STM 2014 (heart), Grskovic JMD 2016 (heart)

DART Study

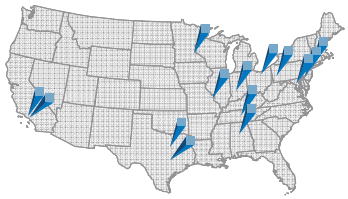
- The Circulating Donor-Derived Cell-Free **D**NA in Blood for Diagnosing **A**cute **R**ejection in Kidney **T**ransplant Recipients (DART)
- Investigated relationship of dd-cfDNA with active rejection



DART Study Design

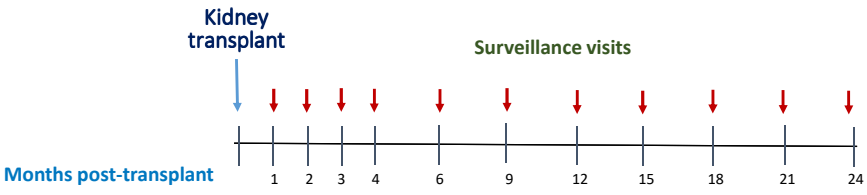
- The Circulating Donor-Derived Cell-Free **D**NA in Blood for Diagnosing **A**cute **R**ejection in Kidney **T**ransplant Recipients (DART)
- 14 centers, 384 patients, prospective observational study
- Enrolled within 1-3 months of transplant and followed longitudinally for 2 years

  OR
- Enrolled at time of clinical suspicion of rejection; blood draw at time of enrollment and longitudinal follow-up post-biopsy
- Allograft rejection reference cases met biopsy-based, histologic Banff WG 2013 criteria for TCMR or acute or chronic active ABMR

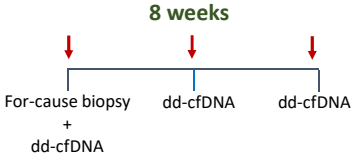


Two clinical scenarios of DART

1. Newly transplanted recipients with dd-cfDNA tests at 11 surveillance visits



2. Clinically indicated biopsy with dd-cfDNA tests at time of biopsy and 1-2 follow-up visits



Bloom RD et al. Cell-free DNA and active rejection in kidney allografts. *J Am Soc Nephrol*. 2017

Aims

Primary

- Determine the ability of dd-cfDNA to discriminate active rejection from no active rejection

Secondary

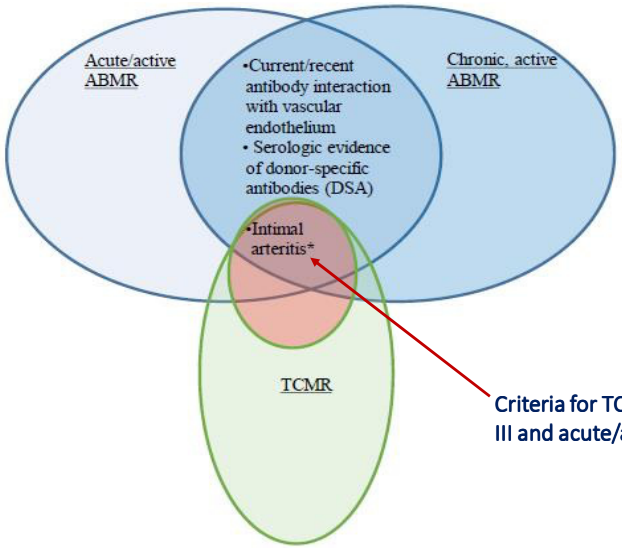
- Determine the ability of dd-cfDNA to discriminate ABMR from the absence of ABMR
- Compare the performance of dd-cfDNA to serum creatinine

Active Rejection

- T cell mediated rejection (TCMR)
- Acute/active antibody mediated rejection
- Chronic, active antibody mediated rejection

- Active rejection includes categorizations that all have pathology indicating active injury
  - BANFF 2007 for TCMR
  - BANFF 2013 for ABMR

Active rejection subclasses (acute ABMR, chronic ABMR, and TCMR) pooled in this study



- Share common histological criteria suggesting active cell injury

The Analysis Cohort Includes 102 Patients From DART, 27 with Active Rejection

384 patients:
- 1272 samples at time of data lock

→ 165 patients did not undergo biopsy:
- Patients and samples were not used in the main analysis of this study

219 patients underwent biopsy:
- 206 patients had 242 biopsies with pathological reports
  - 204 clinically indicated biopsies
  - 34 surveillance biopsies
  - 4 post-treatment of rejection

→ 117 patients excluded due to:
- 26 no for-cause biopsy
- 48 no blood sample with biopsy
- 6 Inadequate biopsy specimens

- interstitial fibrosis/tubular atrophy (IF/TA) grade I (21), grade II (8) grade III (3), grade not specified (1); IF/TA (grade I or II) and acute tubular necrosis (ATN, 12); IF/TA grade I and BKV (1)
- ATN (4); ATN, BKV, and IF/TA grade I or II (3); ATN and arteriosclerosis (1)
- CNI toxicity (1); CNI toxicity and IF/TA grade I (3); CNI toxicity, IF/TA, ATN (5)
- Glomerulonephritis (GN, 3); GN and IF/TA grade I (1); GN and CNI toxicity (1); ATN, GN, and IF/TA(1)
- no major findings (9)
- transplant glomerulopathy (1)
- cortical necrosis (1)

102 patients in main study cohor
- 107 biopsies and blood samples

27 patients in active rejection cohort:
- 27 biopsies and blood samples
- 10 chronic, active ABMR patients, 10 samples
- 6 acute/active ABMR patients, 6 samples
- 11 TCMR patients, 11 samples

ABMR = Antibody-mediated rejection
TCMR = T cell-mediated rejection

Bloom RD et al. Cell-free DNA and active rejection in kidney allografts. *J Am Soc Nephrol.* 2017. doi:10.1681/ASN.2016091034.

Patient Characteristics

Table 1. Patient characteristics

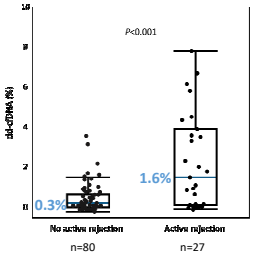
Clinical Characteristic	Active Rejection Group	No Active Rejection Group	*P* Value*
Number of patients	27	75	
Number of samples	27	80	
Race, *n* (%)			0.23
Black	13 (48)	23 (31)	
White	13 (48)	41 (55)	
Native Hawaiian or Other Pacific Islander	1 (4)	0 (0)	
Hispanic/Latino	0 (0)	4 (5)	
Asian	0 (0)	1 (1)	
Other	0 (0)	6 (8)	
Men, *n* (%)	16 (59)	45 (60)	>0.99
Age at enrollment, y	46±16	53±13	0.04
Post-transplant, d	968±1107	1189±1482	0.42
CMV serologic status, *n* (%)			0.15
D−/R+	4 (15)	13 (17)	
D+/R+	5 (19)	24 (32)	
D−/R−	3 (11)	16 (21)	
D+/R−	4 (15)	9 (12)	
Unknown	11 (41)	13 (17)	
Donor type, *n* (%)			0.03
Deceased donor	20 (74)	42 (56)	
Living unrelated	2 (7)	24 (32)	
Living related	5 (19)	9 (12)	
Child	2 (7)	3 (4)	
Sibling	2 (7)	4 (5)	
Parent	0 (0)	1 (1)	
Half-sibling	0 (0)	0 (0)	
Other biologic blood relation	1 (4)	1 (1)	
Creatinine	2.5±1.0	2.4±1.4	0.69
eGFR	32±12	36±21	0.21
HLA class 1 no. of mismatches (A, B)	2.7±1.4	2.6±1.4	0.59
HLA class 2 no. of mismatches (DR)	1.2±0.6	1.1±0.8	0.67
Weight, kg	85±19	84±21	0.73
Height, cm	170±10	171±8	0.58

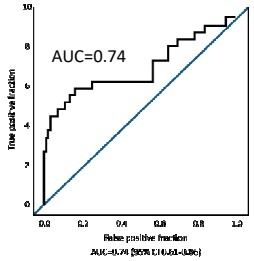
Data ranges are presented as mean±standard deviation. CMV, cytomegalovirus.
*The *P* values are the level of statistical significance in the differences of values found in the DART active rejection group and the no active rejection group. For continuous covariates, Wilcoxon rank sum test was used to generate the *P* values. For categoric covariates, Fisher exact test was used to generate the *P* values.

dd-cfDNA Discriminates Active Rejection from No Active Rejection in Clinical-Suspicion Setting

dd-cfDNA

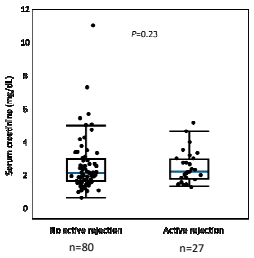
Median 5.3-fold higher in active rejection vs no active rejection. Receiver-Operator characteristics curve shows dd-cfDNA discriminates active rejection

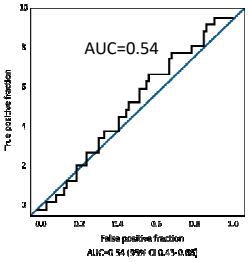




Serum creatinine

Does not discriminate active rejection from no active rejection.





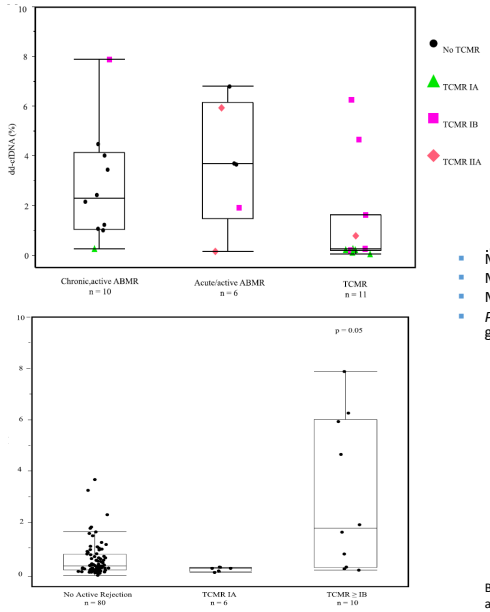
- Active rejection = Acute, active ABMR; Chronic/active ABMR; and TCMR, *n*=27 samples from 27 patients
- No active rejection, *n*=80 samples from 75 patients

Bloom RD et al. Cell-free DNA and active rejection in kidney allografts. *J Am Soc Nephrol*. 2017.

dd-cfDNA Provides Stratification With Higher Probability of Active Rejection at 1% dd-cfDNA Cutoff

Performance metric	AlloSure test performance at 1% threshold
ROC/AUC	0.74 (95% CI 0.61-0.86)
Sensitivity	85%
Specificity	59%
NPV	84%
PPV	61%

dd-cfDNA Levels are Higher in ABMR than TCMR

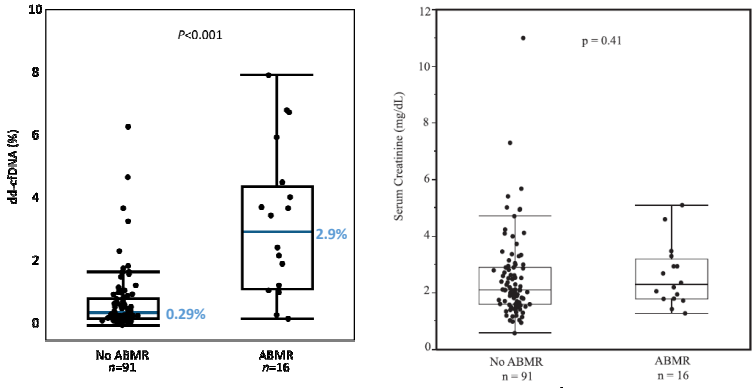


.87

- Median ABMR: 2.9%
- Median TCMR grade 1B and higher: 1.2%
- Median TCMR grade 1A: 0.2%
- *P*=0.05 for T-cell mediated rejection grades 1B and greater compared to controls

Bloom RD et al. Cell-free DNA and active rejection in kidney allografts. *J Am Soc Nephrol*. 2017.

dd-cfDNA is Very Sensitive for ABMR



Bloom RD et al. Cell-free DNA and active rejection in kidney allografts. *J Am Soc Nephrol.* 2017. doi:10.1681/ASN.2016091034.

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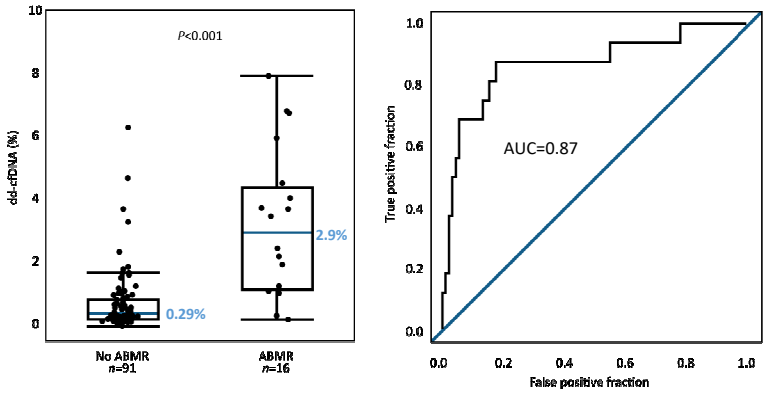

Bloom RD et al. Cell-free DNA and active rejection in kidney allografts. *J Am Soc Nephrol.* 2017. doi:10.1681/ASN.2016091034.

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Low dd-cfDNA (<1%) Has a Very Low Probability to be ABMR

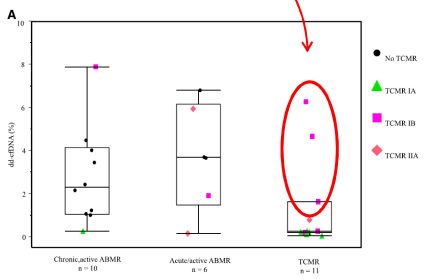
Performance metric	AlloSure test performance at 1% threshold
ROC/AUC	0.87 (95% CI 0.75-0.97)
Sensitivity	81%
Specificity	83%
NPV	96%
PPV	44%

Bloom RD et al. Cell-free DNA and active rejection in kidney allografts. *J Am Soc Nephrol.* 2017. doi:10.1681/ASN.2016091034.

Low dd-cfDNA (<1%) Has a Very Low Probability to be ABMR (high NPV)

Performance metric	AlloSure test performance at 1% threshold
ROC/AUC	0.87 (95% CI 0.75-0.97)
Sensitivity	81%
Specificity	83%
NPV	96%
PPV	44%

Low PPV due to the presence of TCMR IB with high dd-cfDNA in the "no ABMR" group



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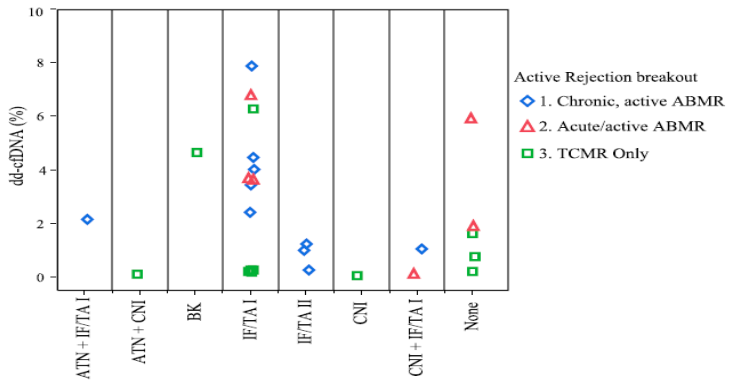
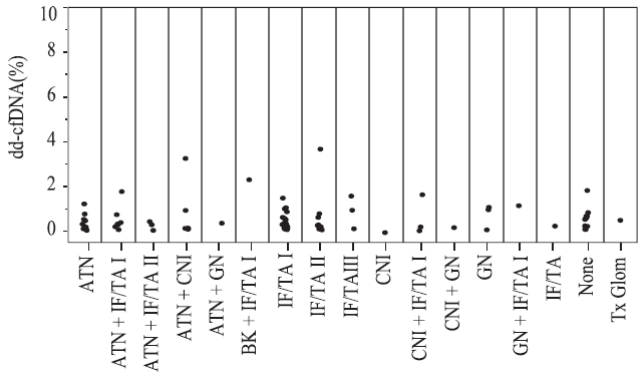
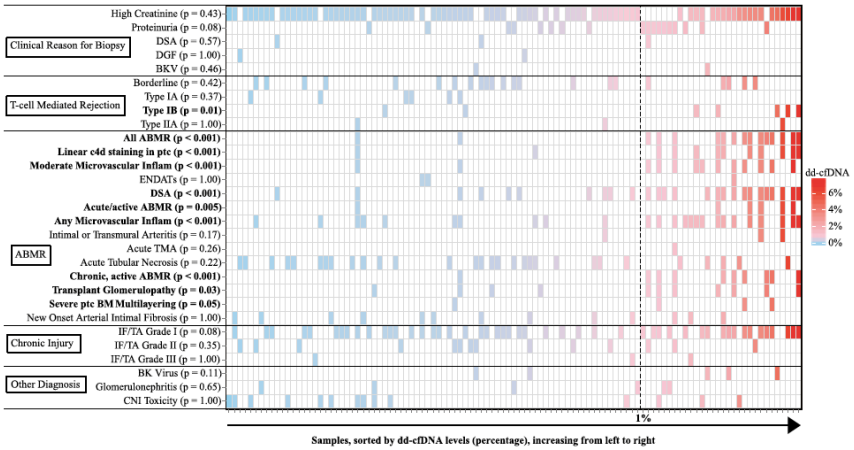
Dd-cfDNA in Patients with Active Rejection Does not Correlate with other Histopathological Findings



Dd-cfDNA in Patients without Active Rejection Does not Correlate with other Histopathological Findings



Correlation of BANFF Elementary Lesions and Clinical Features With dd-cfDNA Level



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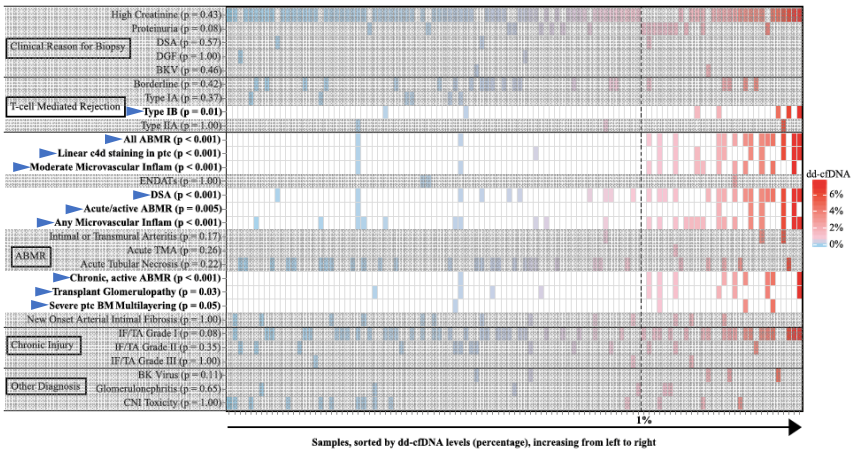
BANFF Elementary Lesions Correlate With dd-cfDNA Level



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Summary

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Summary

- dd-cfDNA may provide a quantifiable measurement of ABMR injury that can be established at baseline and potentially repeated at regular intervals
- dd-cfDNA may help ensure detection, diagnosis and definition of baseline status of ABMR
- dd-cfDNA could provide a more accurate means to assess response to anti-rejection therapy than biopsy or creatinine
- dd-cfDNA may be useful to assess clinical impact of dnDSA

Conclusions

- dd-cfDNA may be used to assess allograft rejection and injury; levels ≥1% indicate a high probability of antibody mediated rejection.
  - ABMR is associated with higher levels of dd-cfDNA than TCMR

Conclusions

- dd-cfDNA may be used to assess allograft rejection and injury; levels ≥1% indicate a high probability of antibody mediated rejection.
  - ABMR is associated with higher levels of dd-cfDNA than TCMR
- dd-cfDNA levels below 1% reflect absence of active antibody-mediated rejection.

Limitations

- Could not assess performance of dd-cfDNA in patients with subclinical rejection
  - only 1/34 pts with surveillance biopsies had active rejection
- Low # of active rejections
  - Demonstrated statistically significant performance characteristics
- Missing biopsy-matched blood samples
  - 77% center compliance
- Could injury have been unrelated to active rejection?
  - Possible but unlikely

Key Messages from DART

- **dd-cfDNA differentiates Active Rejection (Acute/active ABMR; Chronic, active ABMR; or TCMR) from No Active Rejection with high accuracy**
- **More accurate than Serum Creatinine in diagnosis of Active Rejection**
- **Sensitive in distinguishing ABMR from No ABMR**
- **Levels decrease following Rejection Treatment**

Uncertainties from DART

- How will this test perform over time as more data is accrued? Will the cut offs changes?
- What will be the gray zones for test cut offs?
- Will there be combinatorial data with other surveillance labs to more accurately diagnose or predict?

How will this be used in Real World Clinical Practice?

- Surveillance vs For Cause
- Early vs Late
- High risk vs Low risk
- Monitoring after rejection treatment, for late ABMR, immunosuppression weaning, immunosuppression compliance
- How will infection (CMV, EBV, BKV, UTI, pyelo) show up?
- Recurrent disease?
- How will obstruction show up?
- What will be the optimal monitoring schedule?