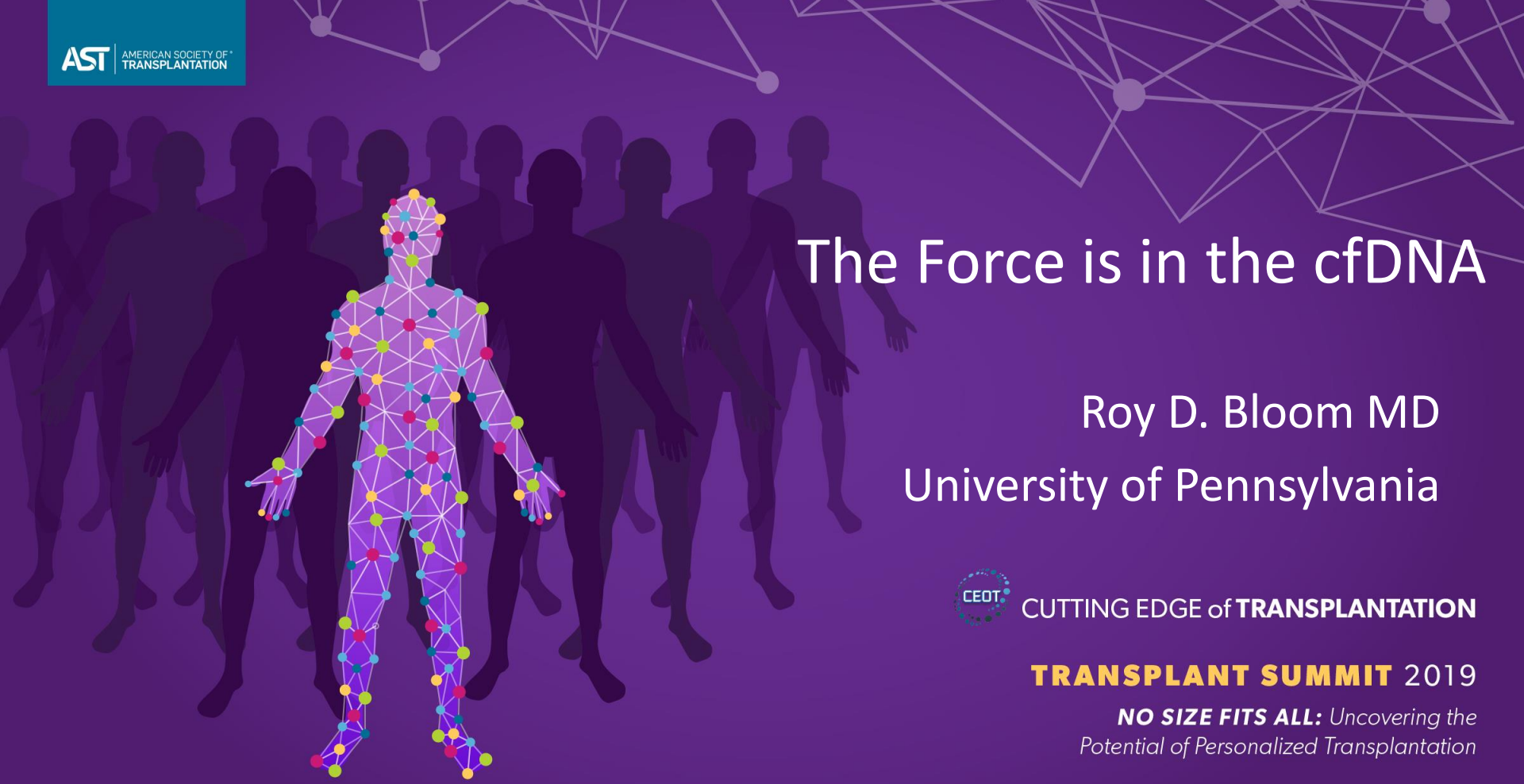
AST | AMERICAN SOCIETY OF TRANSPLANTATION



The Force is in the cfDNA

Roy D. Bloom MD

University of Pennsylvania

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

Disclosures

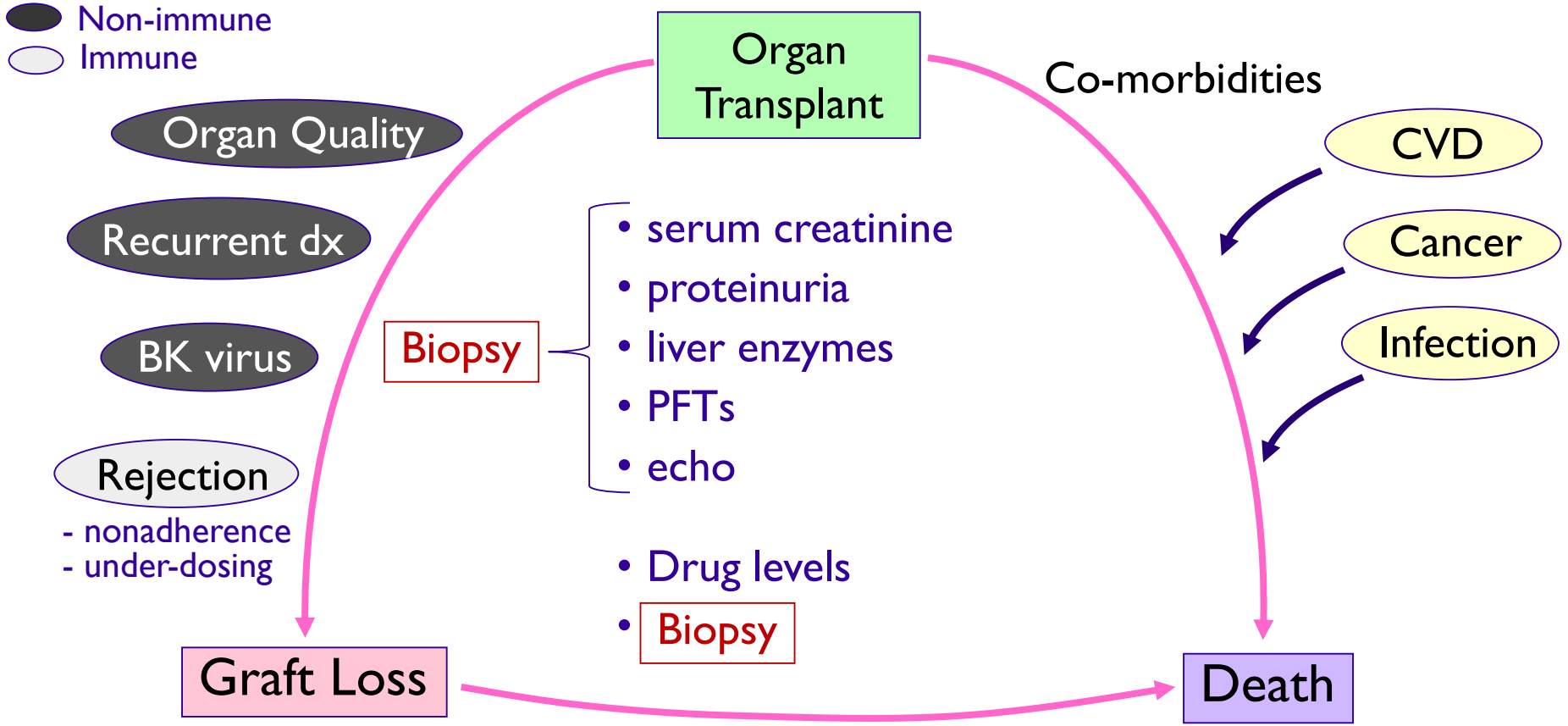
- Advisor: Veloxis, CSL Behring
- Royalties: UpToDate
- Research support: CareDx, Veloxis, Shire

- Nephrocentric presentation

Road Map

- What is donor-derived cell-free DNA?
- Why do we need it?
- What are we learning about it?
- What are its opportunities?
- How will donor-derived cell-free DNA be used in organ transplantation?

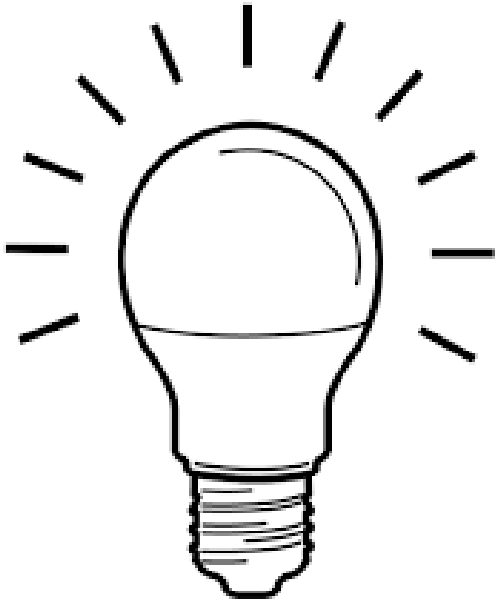
Contributors to Transplant Failure



Limitations of Current Surveillance

Unmet need in kidney transplantation

- Non-invasive accurate diagnostic test
- Safe, readily repeatable
- Informative
- Lead time vs to standard of care



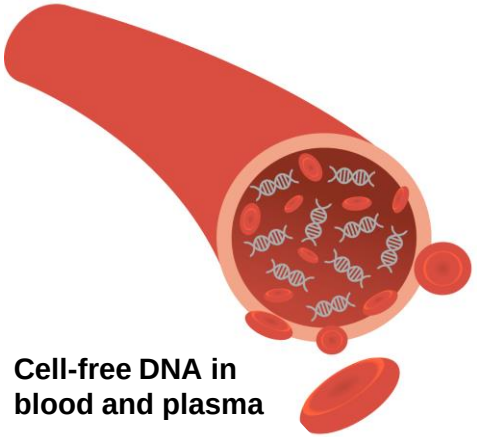
it?

Cell-free DNA

mic DNA in body fluids that
leath and injury

nucleosomal units of
bases

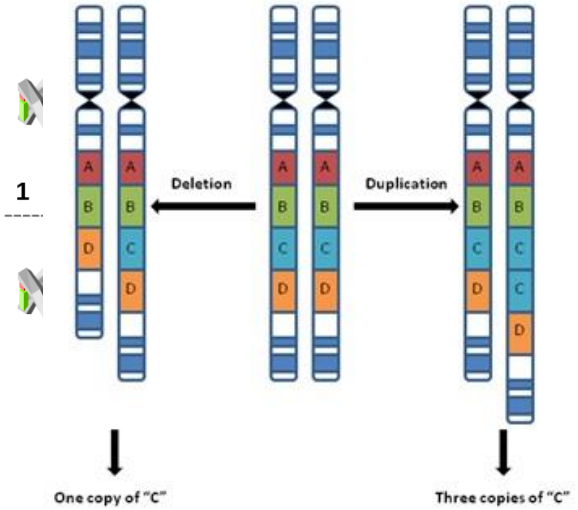
cleared by liver and kidney, $t_{½}$ 30 minutes



Cell-free DNA in blood and plasma

How it Works

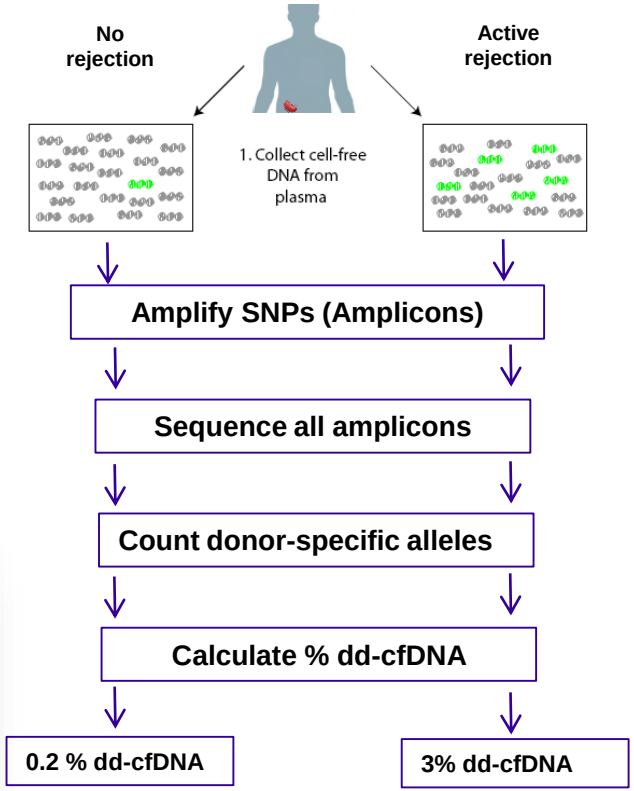
- Initial strategies
  - Interrogate both donor and recipient
  - Gender mismatched pairs
- Contemporary strategies
  - Population genomics
    - SNPs that have high allelic frequency
      - Homozygous in donor and recipient but differ
      - Distributed evenly in population
    - Copy number variation (CNV)



How it Works

1. Extract cfDNA
2. PCR
3. NGS

- Graft fraction (dd-cfDNA)
- Total graft cfDNA



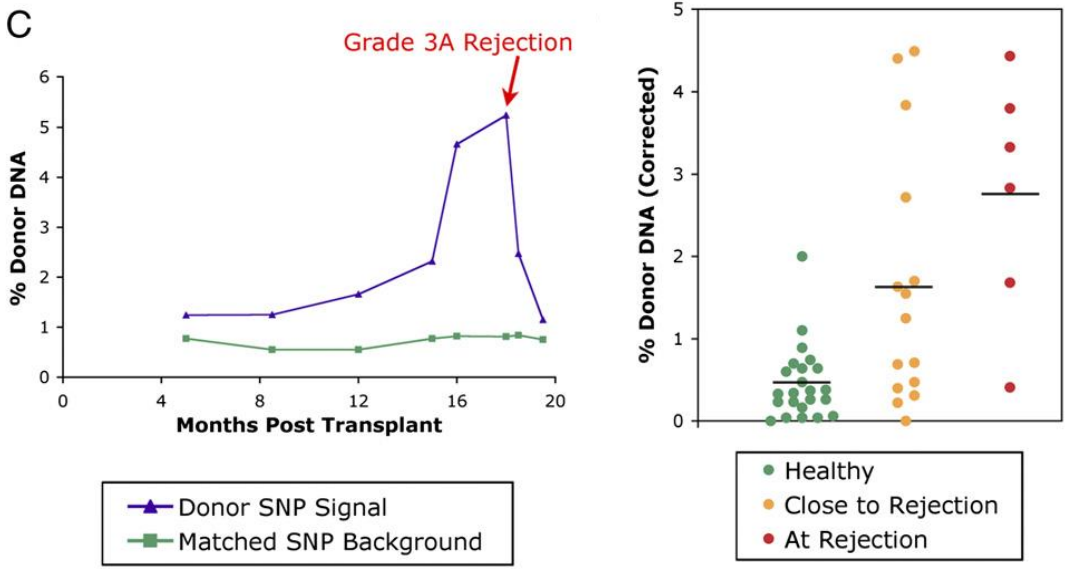
Modified from Snyder, PNAS 2011

Universal noninvasive detection of solid organ transplant rejection

Thomas M. Snyder[a,b], Kiran K. Khush[c], Hannah A. Valantine[c,1], and Stephen R. Quake[a,b,1]

[a]The Howard Hughes Medical Institute and [b]Departments of Applied Physics and Bioengineering, Stanford University, Stanford, CA 94305; and [c]Division of Cardiovascular Medicine, Stanford University School of Medicine, Stanford, CA 94305

Edited* by Leonard A. Herzenberg, Stanford University, Stanford, CA, and approved February 24, 2011 (received for review September 15, 2010)

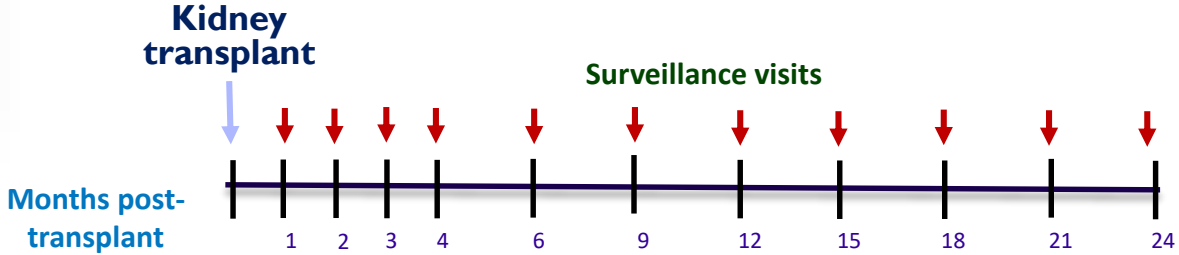


- Cf-DNA may provide signal prior to biopsy
- Cf-DNA may decline after treating rejection

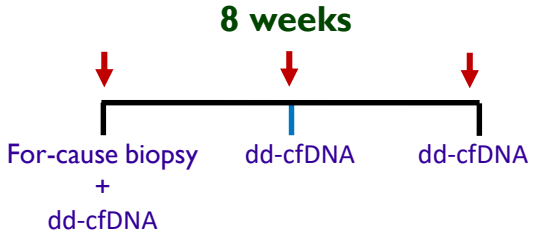
Dd-cfDNA and Rejection: DART

- 14 centers, n=384, prospective observational study, 2 scenarios:

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

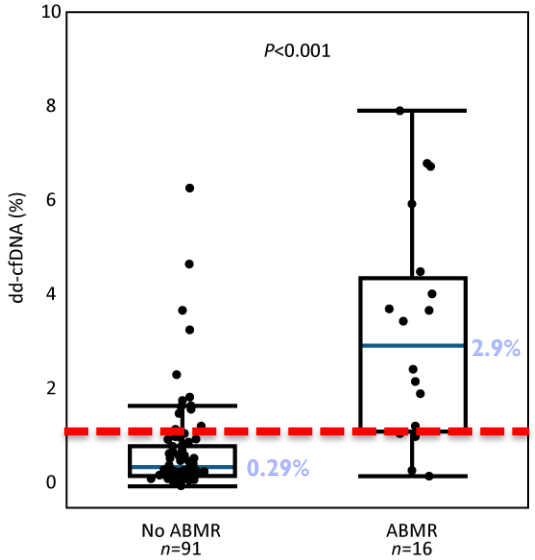


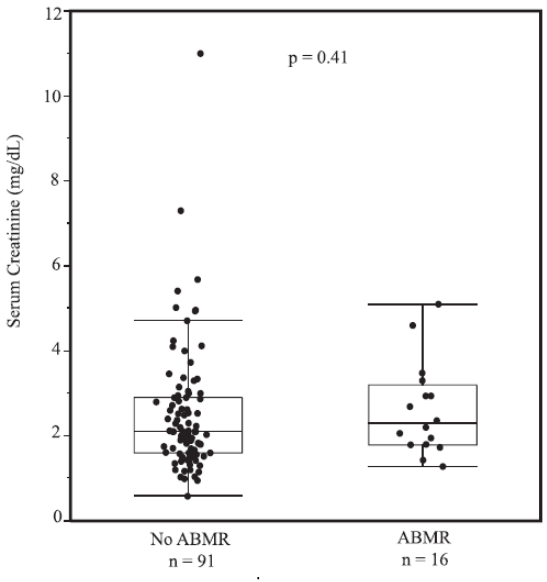
- Rejection based on Banff 2013 criteria

*Donor-Derived Cell-Free DNA in Blood for Diagnosing Acute Rejection in Kidney Transplant Recipients

Bloom RD *et al*, J Am Soc Nephrol. 2017

Dd-cfDNA is Sensitive for ABMR; Serum Creatinine is not

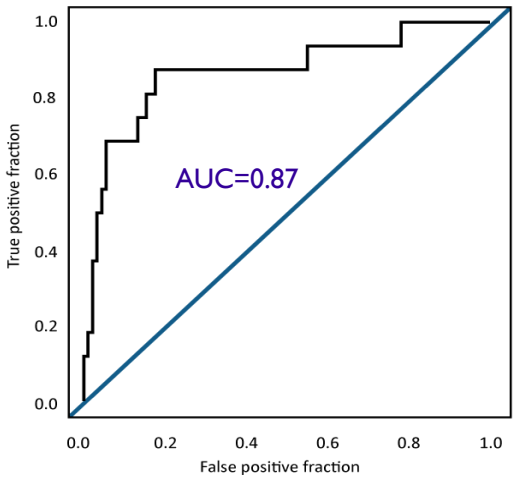




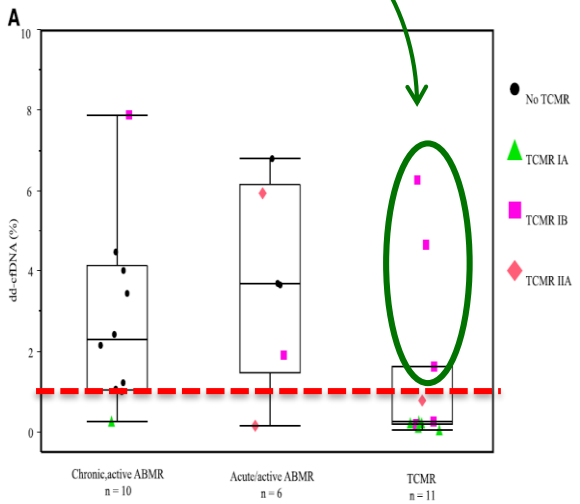
Bloom RD et al. Cell-free DNA and active rejection in kidney allografts. *J Am Soc Nephrol.* 2017. doi:10.1681/ASN.2016091034.

Strong NPV for ABMR

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



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%



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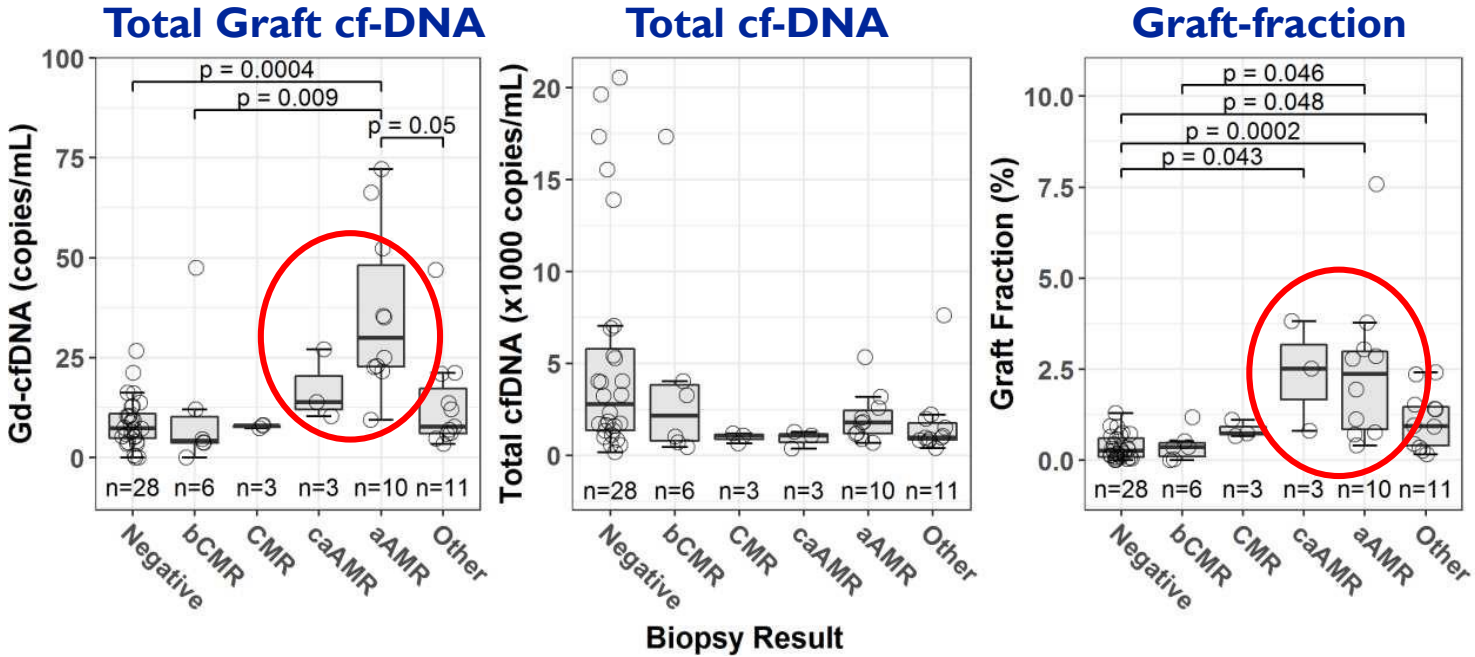
Higher dd-cfDNA With ABMR

Other Quantification Methods

- **n=55, for-cause bx**



bCMR=borderline cell-mediated rejection; CMR=cell-mediated rejection ; aAMR: acute antibody mediated rejection; caAMR=chronic active antibody mediated rejection; gd-cfDNA=graft-derived cell-free DNA; Graft Fraction=graft derived cell-free DNA/total cell-free DNA

Whitlam et al. *Am J Transplant 2018*.

Dd-cfDNA and ABMR

ABMR based on DSA

Study	Banff Criteria	Diagnostic cut-off (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Bloom, 2017	2013	1	81	83	44	96
Jordan, 2018 (with DSA)	2013	1	81	82	81	83
Whitlam, 2018	2013	0.75	85	75	48	95
Huang, 2019	2013	0.74	100	72	69	100
Sigdel, 2019	2017	1				

Received: 5 November 2017 | Revised: 6 December 2017 | Accepted: 7 December 2017
DOI: 10.1111/ajt.14625

MEETING REPORT

AJT

The Banff 2017 Kidney Meeting Report: Revised diagnostic criteria for chronic active T cell–mediated rejection, antibody-mediated rejection, and prospects for integrative endpoints for next-generation clinical trials

M. Haas[1] | A. Loupy[2] | C. Lefaucheur[3] | C. Roufosse[4] | D. Glotz[3] | D. Seron[5] | B. J. Nankivell[6] | P. F. Halloran[7] | R. B. Colvin[8] | Enver Akalin[9] | N. Alachkar[10] | S. Bagnasco[11] | Y. Bouatou[2,12] | J. U. Becker[13] | L. D. Cornell[14] | J. P. Duong van Huyen[2] | I. W. Gibson[15] | Edward S. Kraus[16] | R. B. Mannon[17] | M. Naesens[18] | V. Nickeleit[19] | P. Nickerson[20] | D. L. Segev[21] | H. K. Singh[19] | M. Stegall[22] | P. Randhawa[23] | L. Racusen[11] | K. Solez[24] | M. Mengel[24]

Challenges with ABMR

- ABMR diagnostic criteria are imprecise

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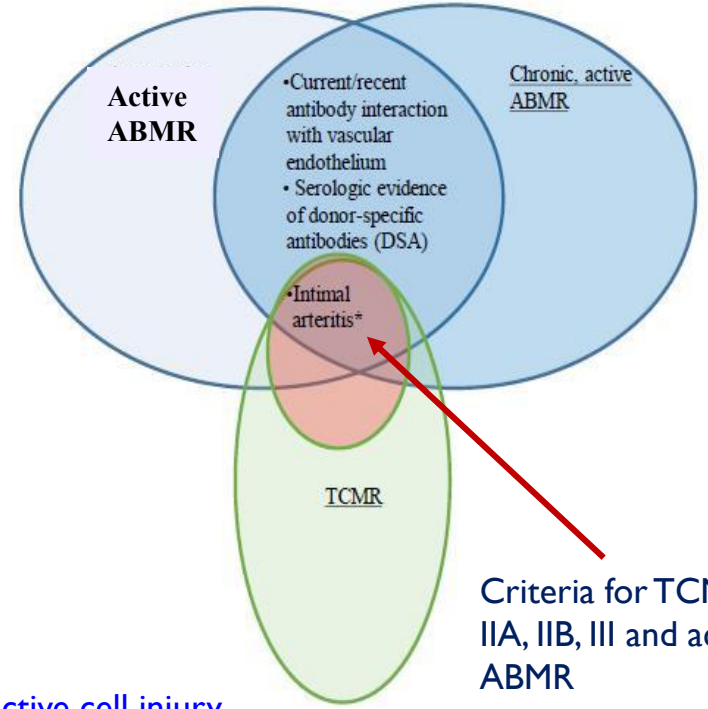
Challenges with ABMR

- Overlap in microvascular injury phenotypes
- Detectable DSA not required
- Molecular alternatives to DSA acceptable
  - "If thoroughly validated"
  - Tests have limitations
  - Not yet approved by regulators



- Share common histological criteria suggesting active cell injury

Dd-cfDNA and ABMR

Huang, 2019	2013	0.74	100	72	69	100

Dd-cfDNA and ABMR

Huang *et al*, 2019

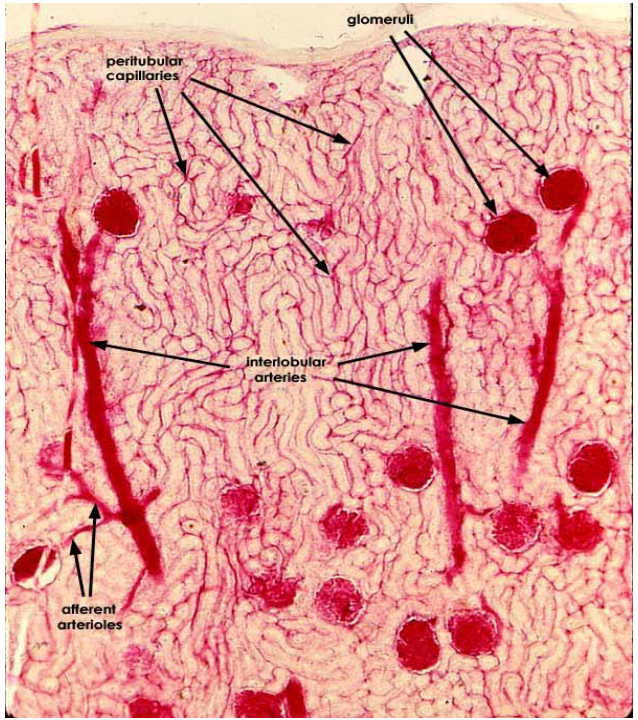
Whether Dd-cfDNA is considered a valid marker of endothelial injury/ABMR has major implications

- Reclassify as ABMR, Banff 2017

PPV=true POS/(true POS + false POS)

Why Does dd-cfDNA Better Discriminate ABMR than TCMR

- ABMR: Direct proximity of damaged endothelial cells to circulation following microvascular injury



http://www.siumed.edu/~dking2/crr/rnguide.htm#rnii

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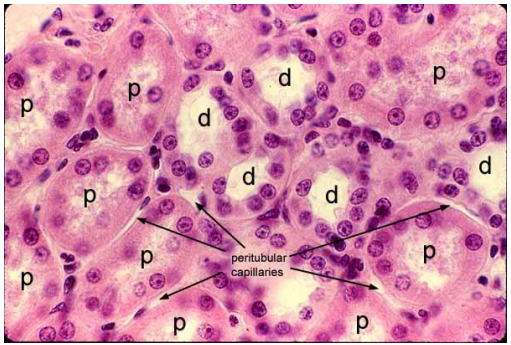
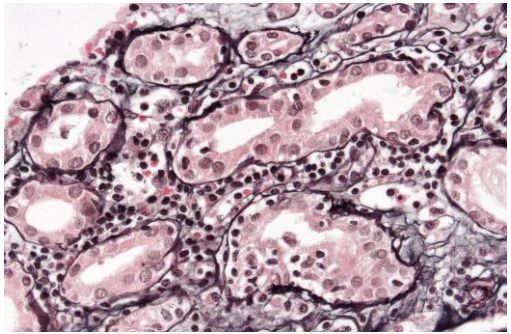


- TCMR: tubular and interstitial injury predominate (tubulointerstitial compartment)



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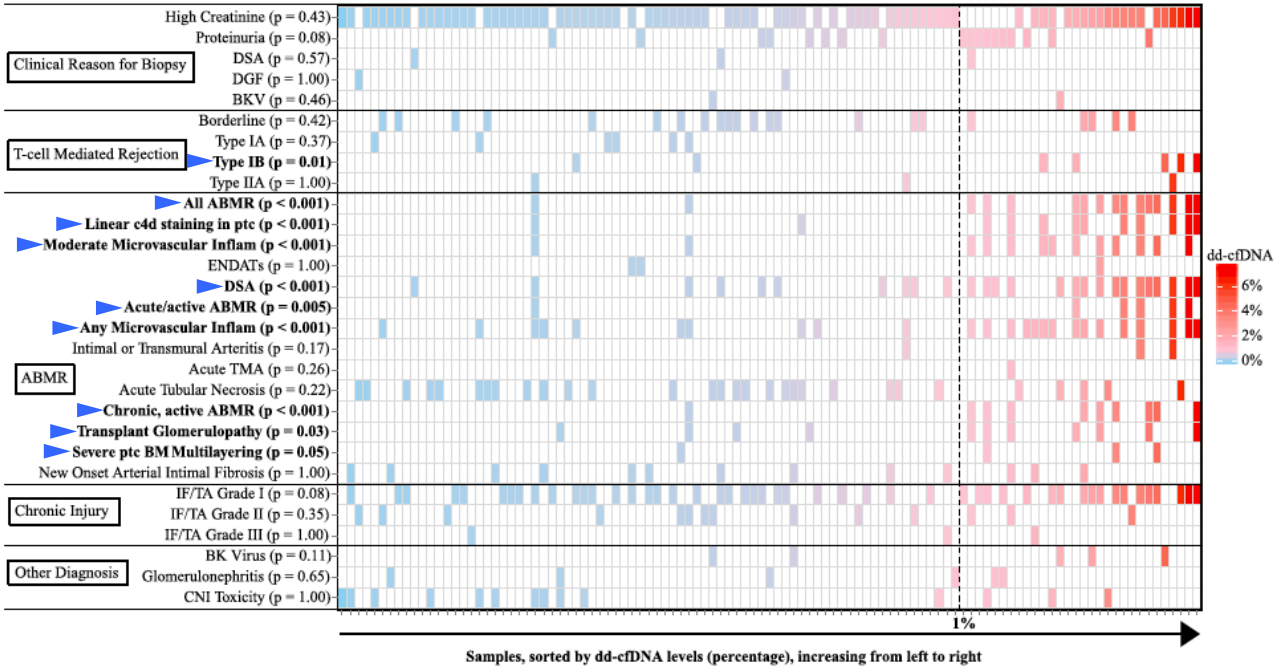
Correlation of Banff Elementary Lesions with dd-cfDNA



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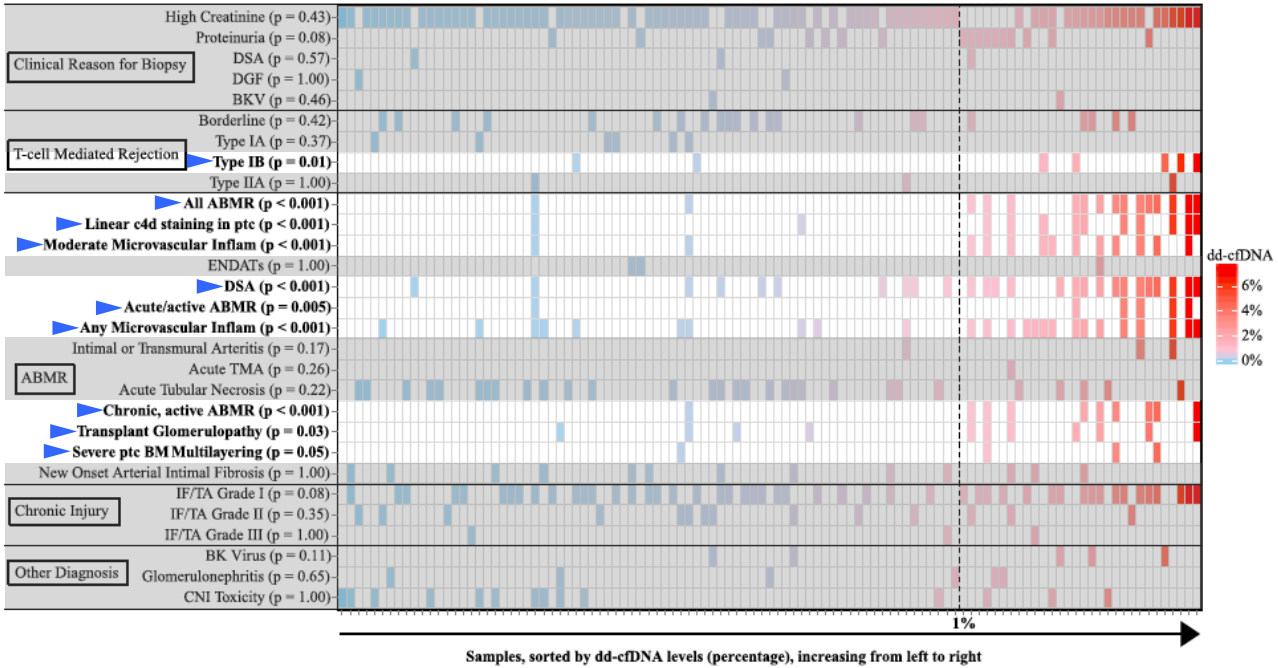
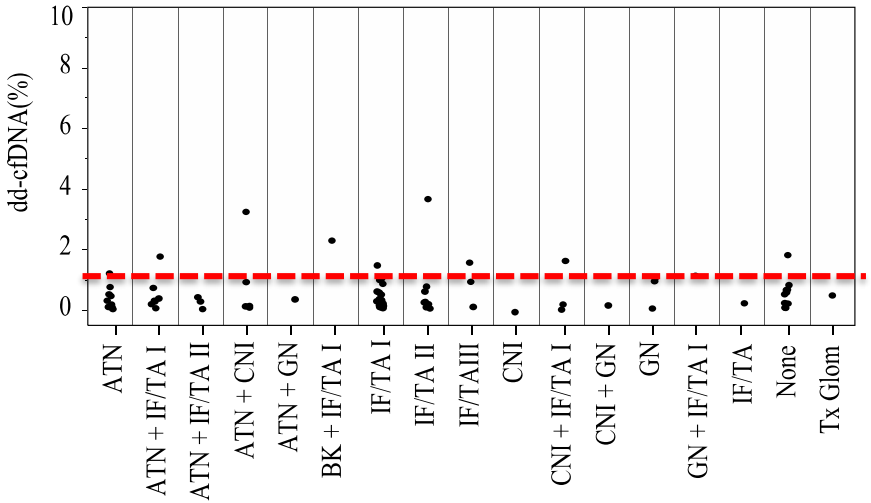


Bloom RD et al. Cell-free DNA and active rejection in kidney allografts. *J Am Soc Nephrol.* 2017. doi:10.1681/ASN.2016091034.

Cell-free DNA and Diagnoses Other Than Rejection

- n=80



- BK viremia, n= 14

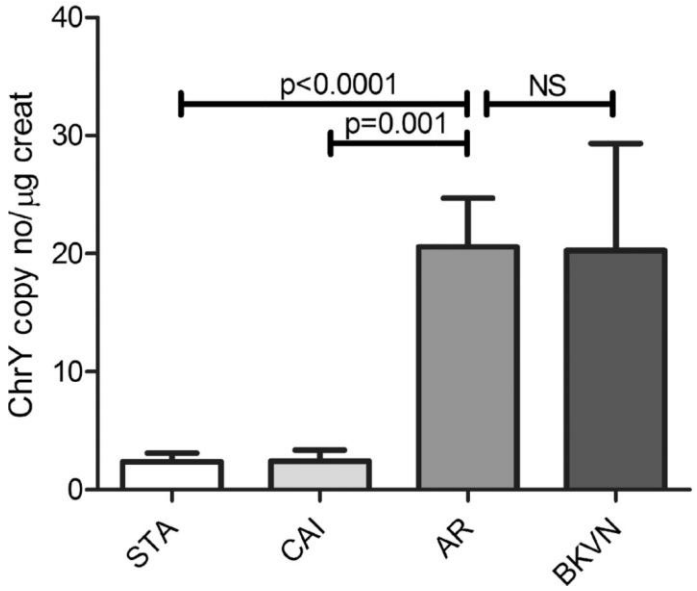
Biopsy	n	Median dd-cfDNA (%)	
No rejection	7	0.58	0.29-1.77
Rejection	7	3.38	1.22-4.65

- More info needed
  - BKVN
  - DSA

Bloom *et al*, J Am Soc Nephrol 2017

Urinary Cell-Free DNA

- n= 63, chromosome Y specific dd-cfDNA



Sigdel *et al*, Transplant 2013

nature COMMUNICATIONS

ARTICLE

DOI: 10.1038/s41467-018-04745-0 OPEN

Urinary cell-free DNA is a versatile analyte for monitoring infections of the urinary tract

Philip Burnham[1], Darshana Dadhania[2,3], Michael Heyang[1], Fanny Chen[1], Lars F. Westblade[4,5], Manikkam Suthanthiran[2,3], John Richard Lee[2,3] & Iwijn De Vlaminck[1]

- Microbiome
- Infectome
  - Growth rates
  - Antibiotic resistome profiling
  - Host response
  - Monitoring for infection
- dd-cfDNA

Many Knowledge Gaps

- Performance vs DSA?
- Discrimination of diagnoses other than active rejection?
- Optimal use and testing frequency?
  - Screening
  - In conjunction with/instead of DSA
  - With for-cause and/or protocol biopsy
- Define meaningful changes/implications
- How do different methods compare?
- Use with other biomarkers?

Conclusion

- Novel diagnostic in transplantation
- Versatility in blood and urine
- Early studies show immense promise
  - ABMR>TCMR
- Opportunity to
  - Harness the power of genomics to individual patients
  - Redefine how we manage transplant recipients

- SHARKS, THIS IS A KEEPER, CARPE DIEM