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Cell-Free DNA and Transplantation

Jennifer Gray, PharmD

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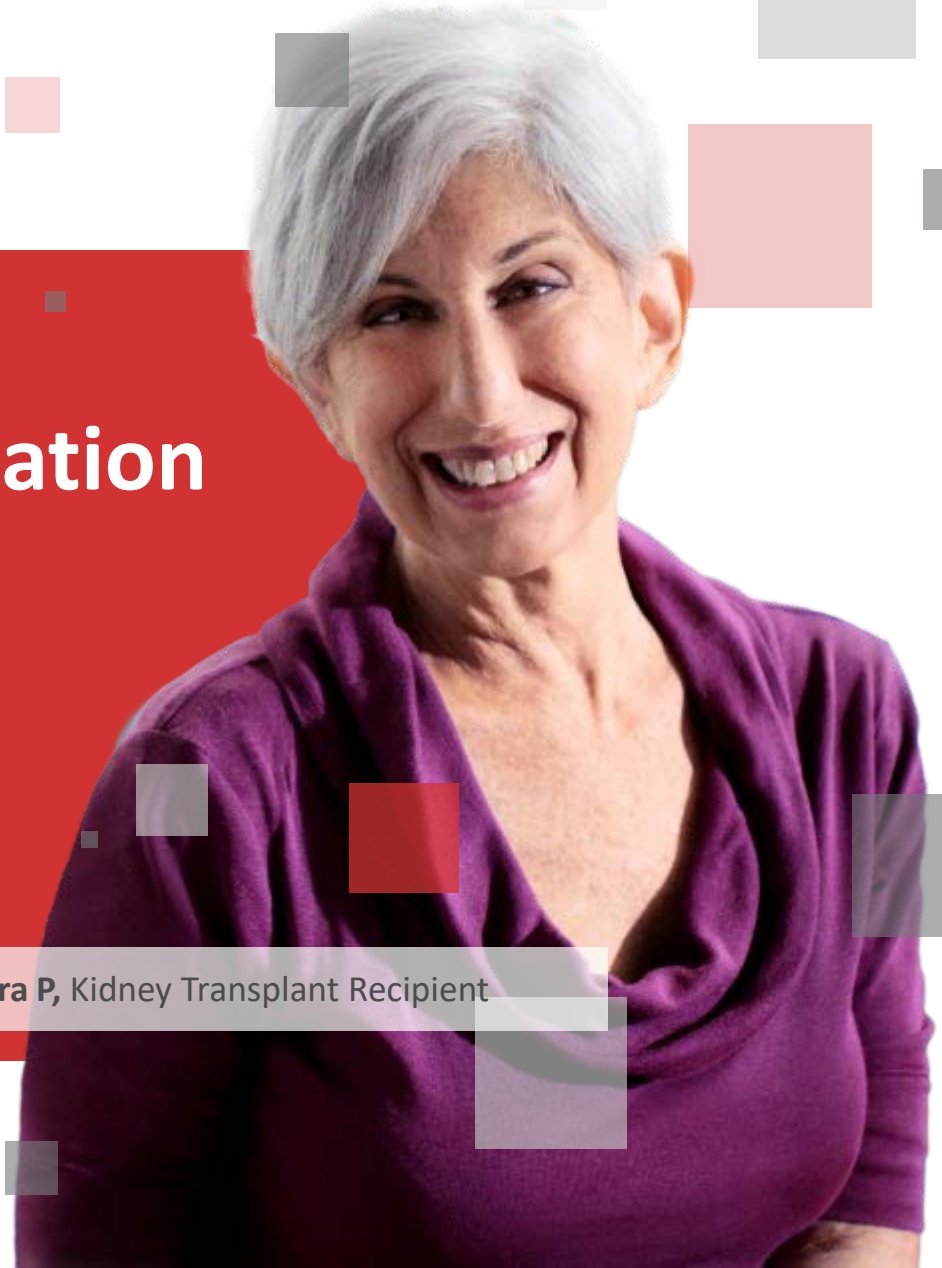

Barbara P, Kidney Transplant Recipient

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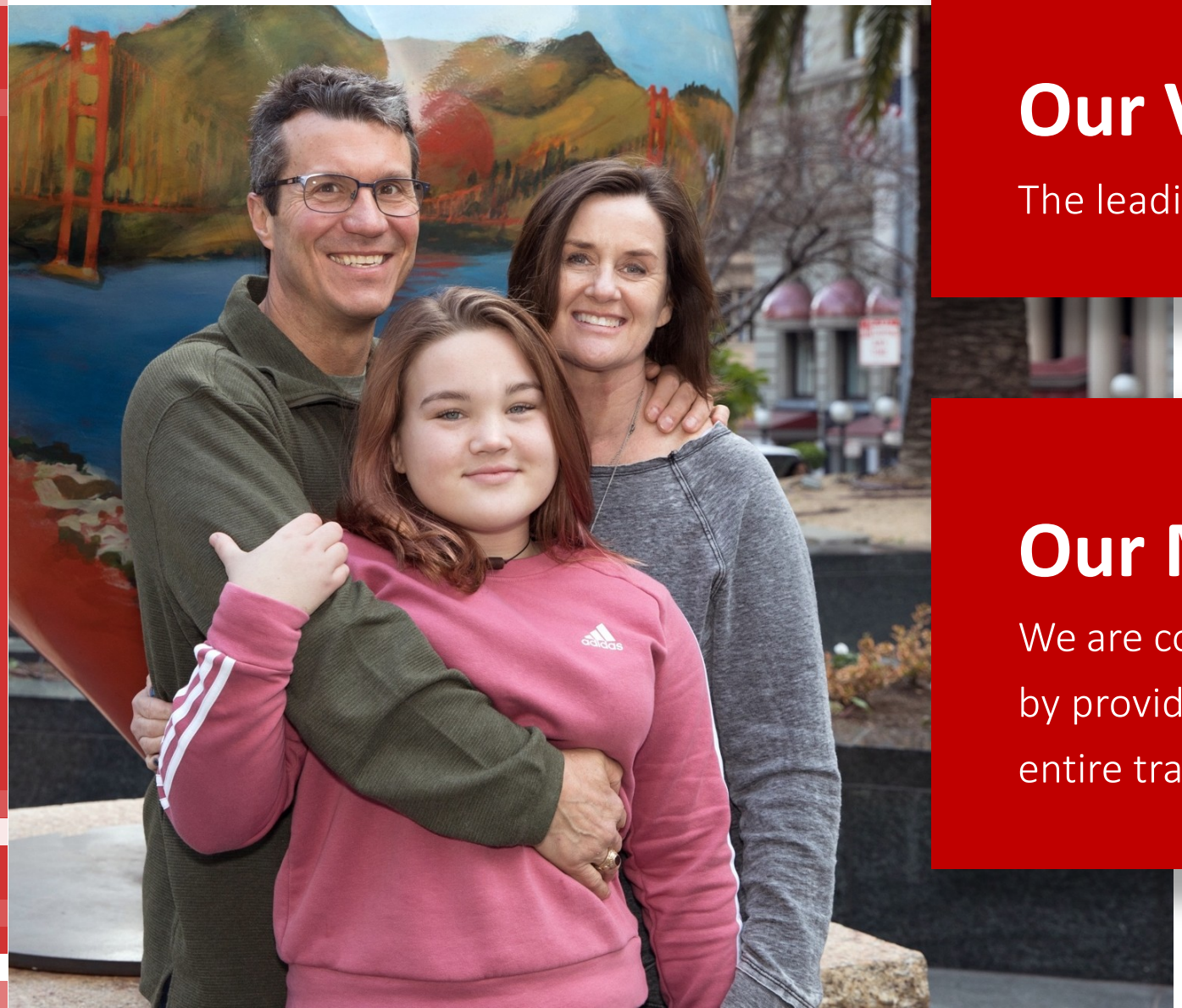
Your Partner in Transplant Care™



Our Vision

The leading partner for the transplant ecosystem

Our Mission

We are committed to improving long-term outcomes by providing innovative solutions throughout the entire transplant patient journey

Objectives for Today's Discussion

- Overview of Cell-Free DNA
  - Kidney Transplant Updates
  - Heart Transplant Updates
- Cell Free DNA in Lung Transplantation

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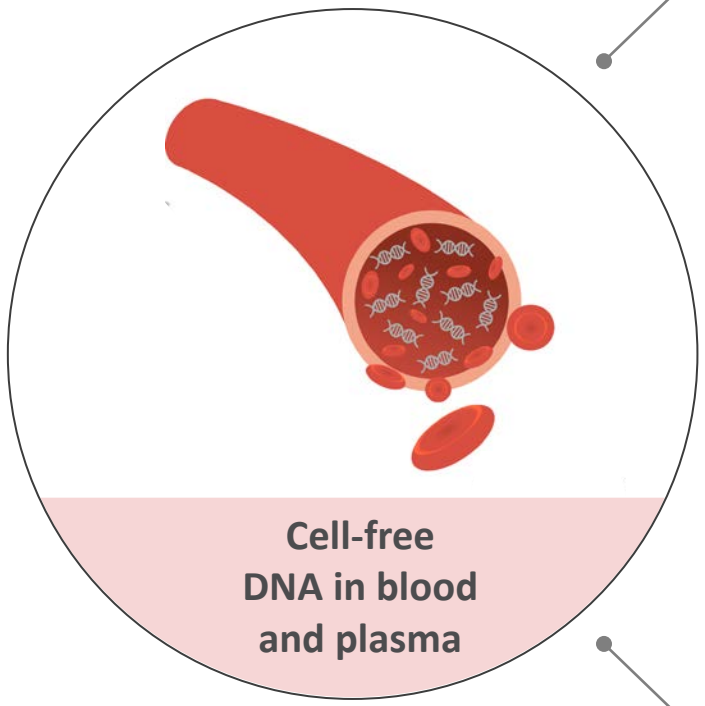
What is Donor-Derived Cell-Free DNA (dd-cfDNA)?



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

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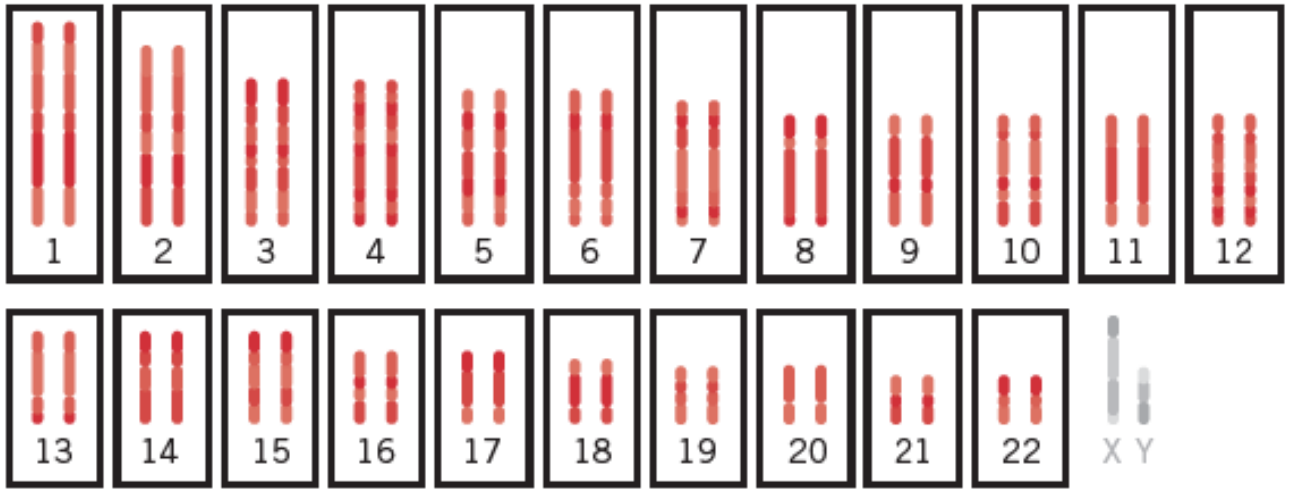
AlloSure® Analyzes Single Nucleotide Polymorphisms (SNPs) Across 100% of Somatic Chromosomes

KIDNEY AlloSure®

100% OF SOMATIC CHROMOSOMES



AlloSure® analyzes 405 SNPs, specifically selected across all 22 somatic chromosomes **to optimize the interrogation of donor from recipient DNA**

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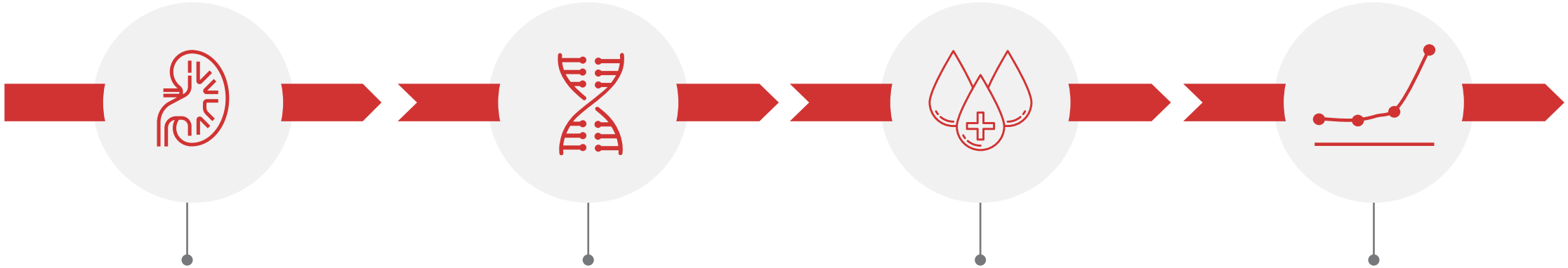
Donor-Derived Cell-Free DNA Levels Increase with Allograft Injury



Cell Injury
to the donor kidney

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

Measures
the level of dd-cfDNA shed from the donor kidney

A High AlloSure
level (dd-cfDNA) indicates potential kidney rejection or injury

When donor kidney injury occurs, the donor kidney cells release cell-free DNA into the plasma of the recipient resulting in increased levels of dd-cfDNA

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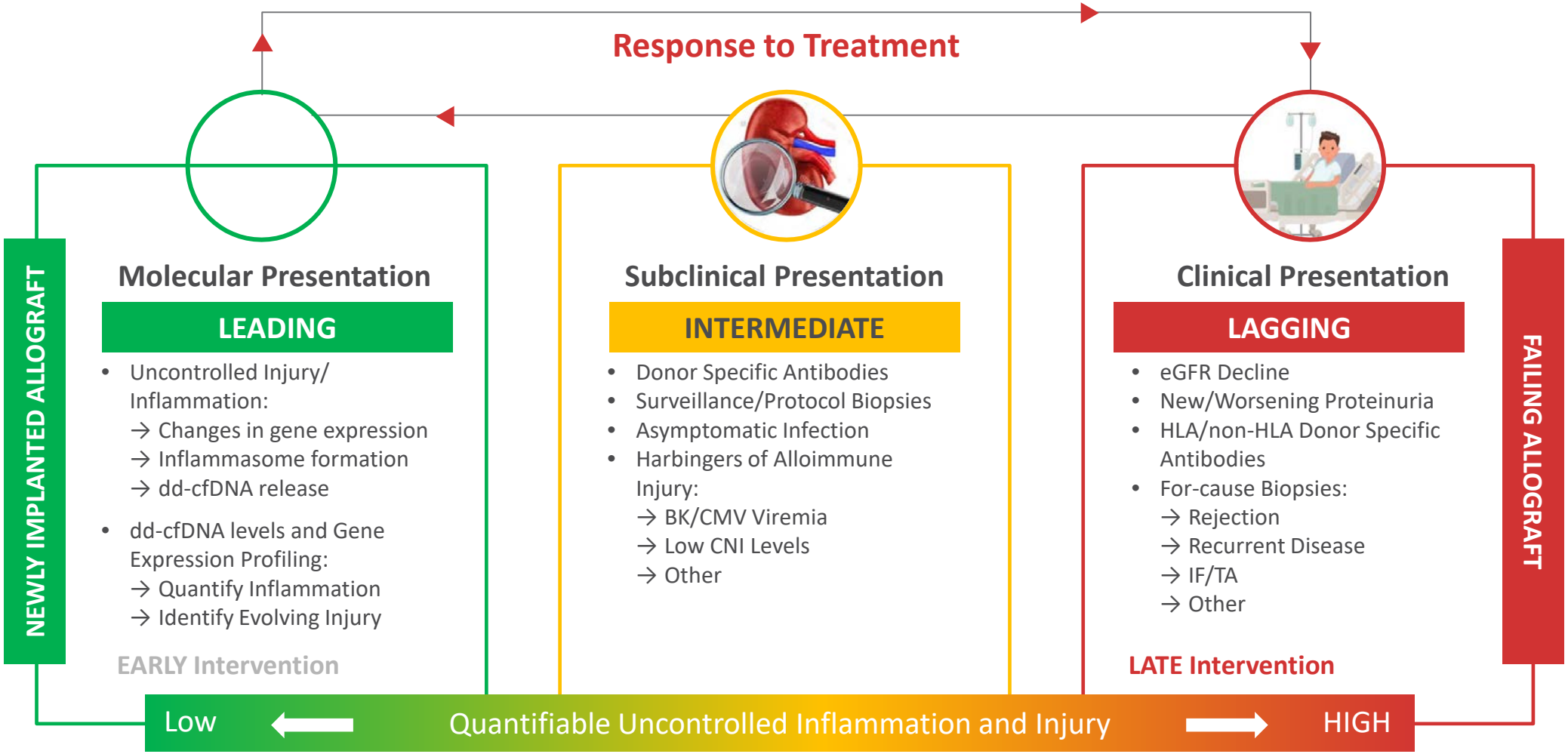
Power of Unlocking the Molecular Window Using Technology



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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

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The ADMIRAL Study: Overview and Results

Denice L, Kidney Transplant Recipient

AlloSure® Kidney: Evolving Clinical Insights

2017

DART Study[1]

AlloSure® Kidney **Outperforms Serum Creatinine**

AlloSure® Score **identifies rejection at 1%**

AlloSure® Kidney offers **Peace of Mind with a High NPV at a score of 0.21%**

(Bloom)

2017

Relative Change Value[2]

Defined Biological Variation of <61% between AlloSure® Scores

(Bromberg)

2020

RADAR Study[3]

Differentiation of **Ambiguous Rejection**

Introduction of AlloSure® Score at **0.5% as a new threshold**

(Stites)

2021

ADMIRAL[4]

Longitudinal Outcomes with dd-cfDNA Surveillance

(Bu)

1. *Bloom RD et al. J Am Soc Nephrol. 2017; 28.2221-2232*
2. *Bromberg et al. J Applied Laboratory Medicine; 2(3):309-321*
3. *Stites E, et al. Am J Transplant. 2020; 00:1-8*
4. *Bu L. et. al. ADMIRAL. Kidney Intl. Manuscript in publication.*

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Assessing Dd-cfDNA Monitoring Insights of Renal Allografts with Longitudinal Surveillance (ADMIRAL)

ADMIRAL: A New Landmark Study

Participating ADMIRAL Study Centers

1. Intermountain
2. Tampa General
3. University of Colorado
4. University of Maryland
5. University of Texas Health Science
6. Virginia Commonwealth University
7. Washington University (St. Louis, MO)

Bu L. et. al. ADMIRAL. Kidney Intl. Manuscript in publication.

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ADMIRAL Study Design

Multi-Center: 7 Major Kidney Transplant Centers Enrolled Patients

Robust Patient Cohort: 1,092 Patients with 3,965 AlloSure® Visits

Real-World Evidence: Centers Using AlloSure® Surveillance Protocol

Surveillance Testing: Median 6 AlloSure® Visits Per Patient

Long-Term Outcomes: Patients Monitored for up to 3 Years

Bu L. et. al. ADMIRAL. Kidney Intl. Manuscript in publication.

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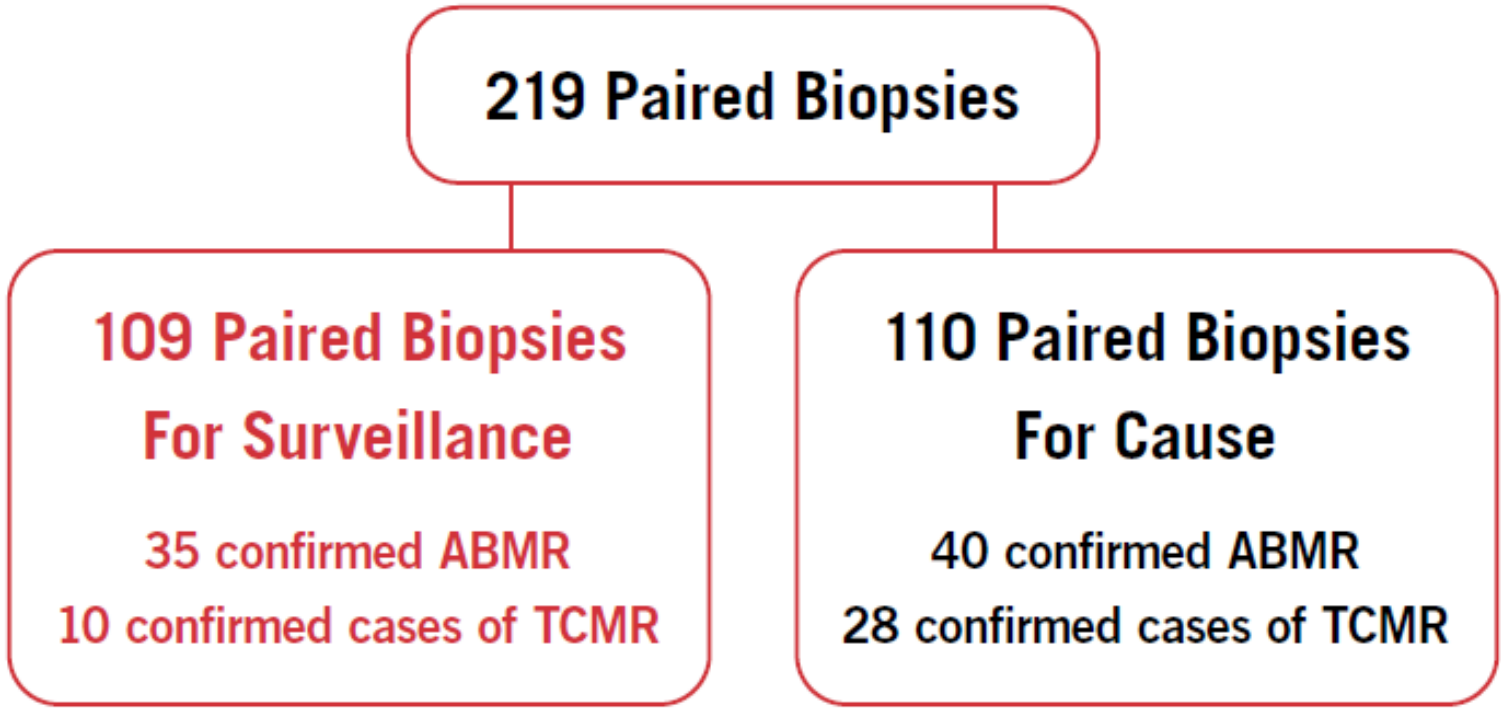
The ONLY dd-cfDNA Test Clinically Validated for Surveillance



The largest prospective cohort of kidney transplant recipients followed for three years with over 200 biopsy-paired dd-cfDNA results

Bu L. et. al. ADMIRAL. Kidney Intl. Manuscript in publication.

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The ONLY dd-cfDNA Service Clinically Validated for Surveillance

Additional Key Findings from ADMIRAL

De novo DSA

- **991 patients had DSA testing with AlloSure®**
- **44 patients with de novo DSA,** AlloSure® scores, and eGFR calculations

eGFR

- **All 1092 patients had eGFR calculations**
- **113 patients had biopsies with rejection,** AlloSure® scores, and eGFR calculations

Graft Injury

- **467 patients** with allograft injury and AlloSure® scores
- 180 patients with AlloSure® scores without injury

Bu L. et. al. ADMIRAL. Kidney Intl. Manuscript in publication.

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ADMIRAL Study Population Representative of US Transplant Patient Demographics

Characteristics		ADMIRAL	UNOS 2020/21
Sex	Female	40%	39%
	Male	60%	61%
Race	Caucasian	48%	55%
	African-American	28%	24%
	Hispanic	17%	14%
	Asian	5%	5%
	Other	2%	3%
Age at Tx (years)	Mean	49.5	46.7
	Min-Max Range	17-84	0-96
Re-transplant		8%	13%
Weight (kg)		84	77
Height (cm)		170	168
Median eGFR		69 ml/min/1.73m^2	73 ml/min/1.73m^2
Median Serum Creatinine		1.52 mg/dL	1.63 mg/dL
Calculated Panel Reactive Antibody (cPRA – sensitization of candidate)	Mean	34%	Not Available
	Range	1-96%	Not Available
Median AlloSure tests (n) per patient		6	Unknown
Deceased Donor*		94%	68%

*Statistically significant difference in deceased donor recipients in the ADMIRAL study compared with the UNOS registry (94% vs. 68%; p=0.04)

1. Bu L. et. al. ADMIRAL. Kidney Intl. Manuscript in publication.

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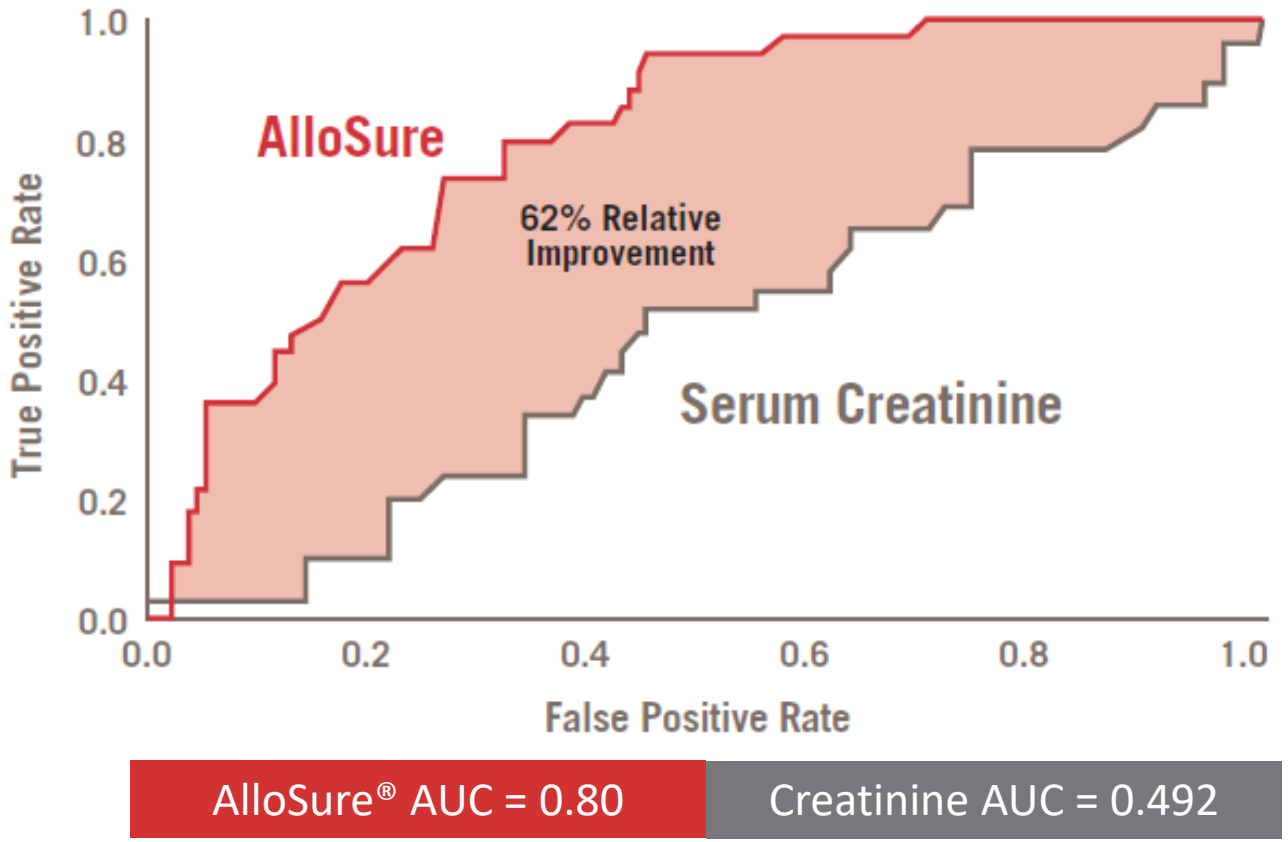
AlloSure® Identifies Subclinical and Clinical Rejection Better than Serum Creatinine

In the ADMIRAL study[1], **AlloSure® demonstrated 62% relative improvement over serum creatinine*** to discriminate **all rejection[1]**



AlloSure® AUC = 0.80 | Creatinine AUC = 0.492

*in AUC

1. Bu L. et. al. ADMIRAL. Kidney Intl. Manuscript in publication.

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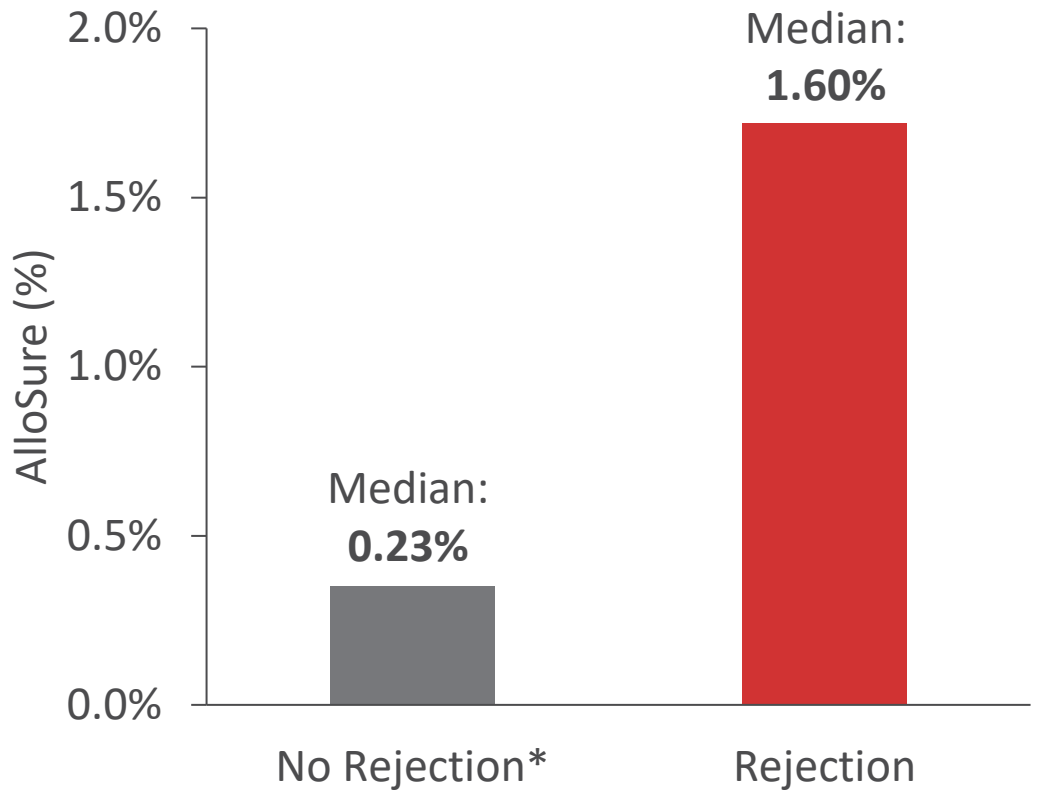
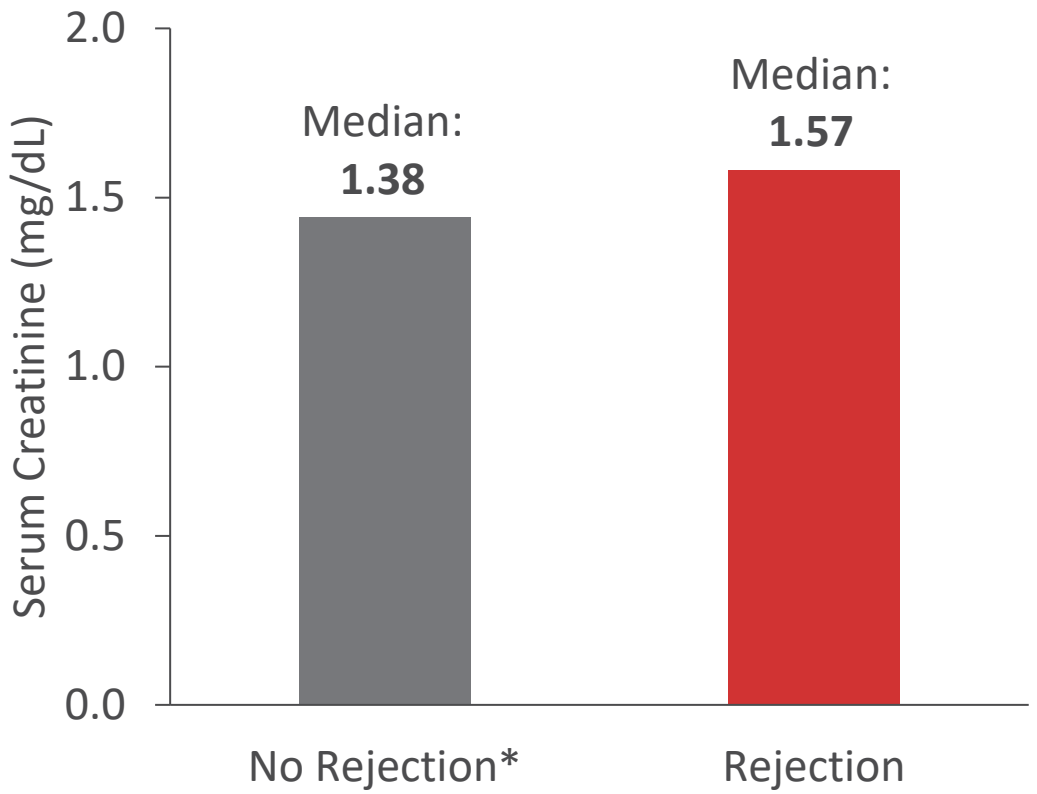
AlloSure® is Superior to Serum Creatinine in Detecting Biopsy-Confirmed Rejection

AlloSure p<0.0001



Serum Creatinine p=0.096



*Biopsies without rejection changes and other abnormalities except IFTA

1. Bu L. et. al. ADMIRAL. Kidney Intl. Manuscript in publication.

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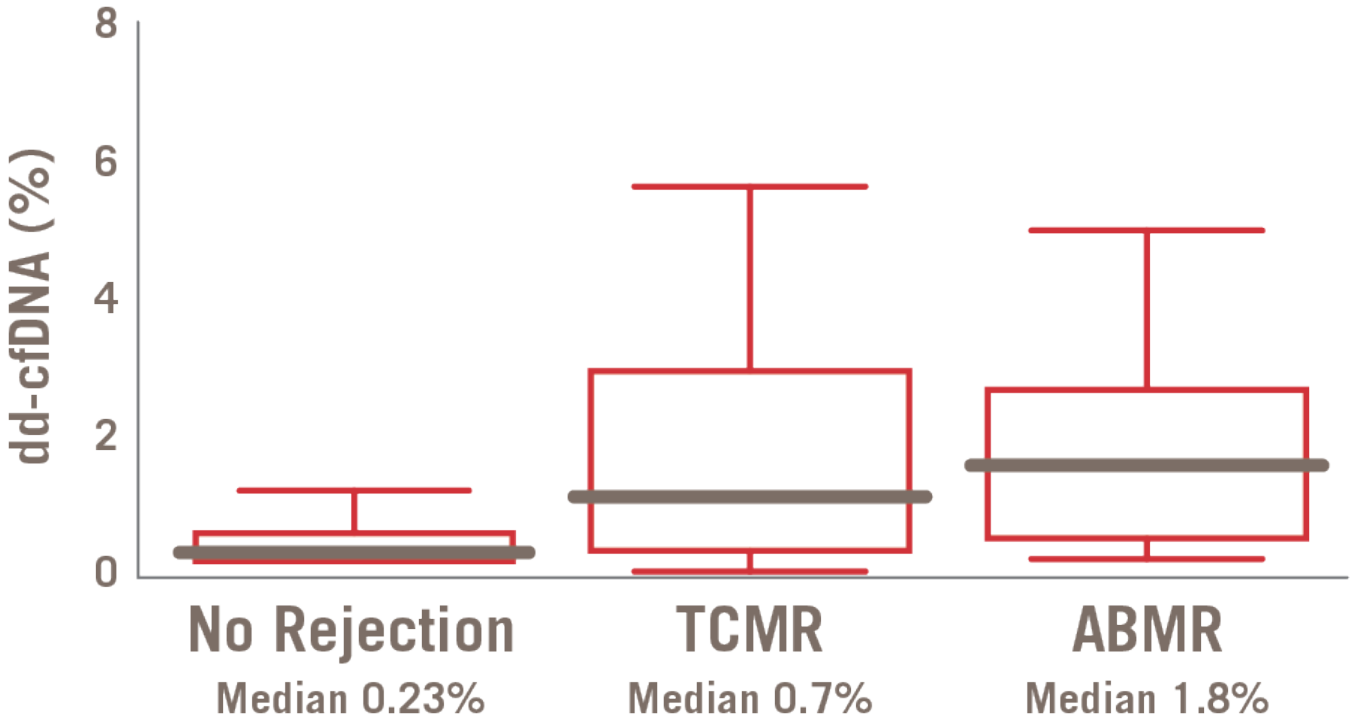
AlloSure® Detects Both TCMR and ABMR



AlloSure® Differentiates No Rejection* from ABMR and TCMR[1]

*Biopsies without rejection changes and other abnormalities except IFTA

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

1. Bu L. et. al. ADMIRAL. Kidney Intl. Manuscript in publication.

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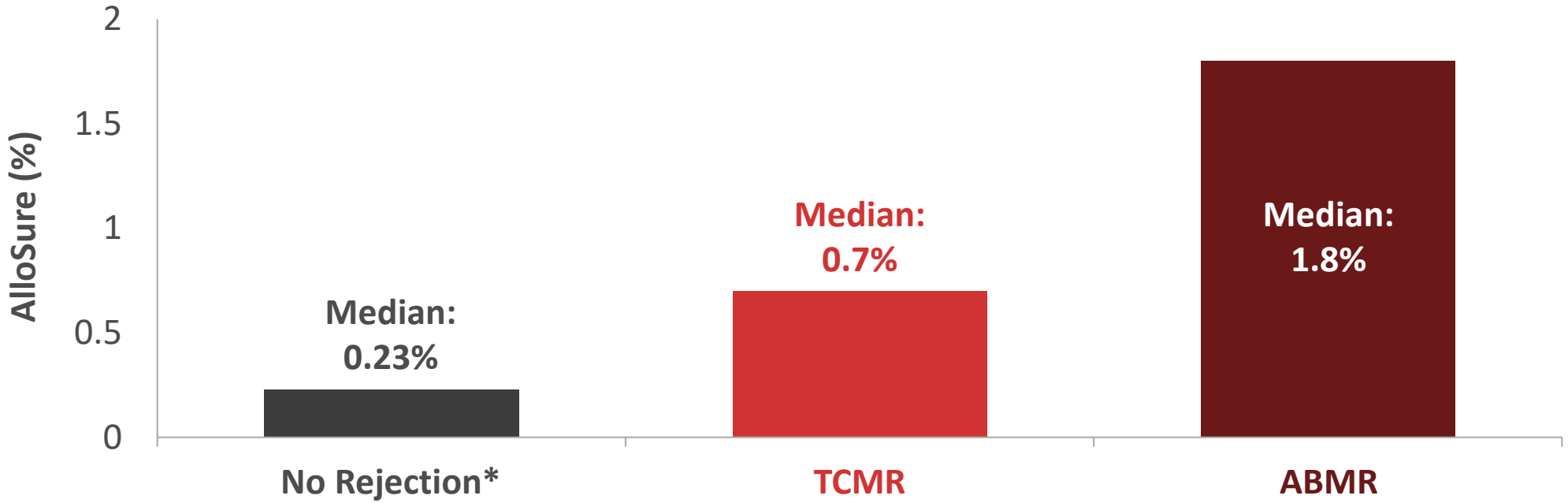
AlloSure® Detects Both TCMR and ABMR



AlloSure® Differentiates No Rejection* from ABMR and TCMR[1]

*Biopsies without rejection changes and other abnormalities except IFTA

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

1. Bu L. et. al. ADMIRAL. Kidney Intl. Manuscript in publication.

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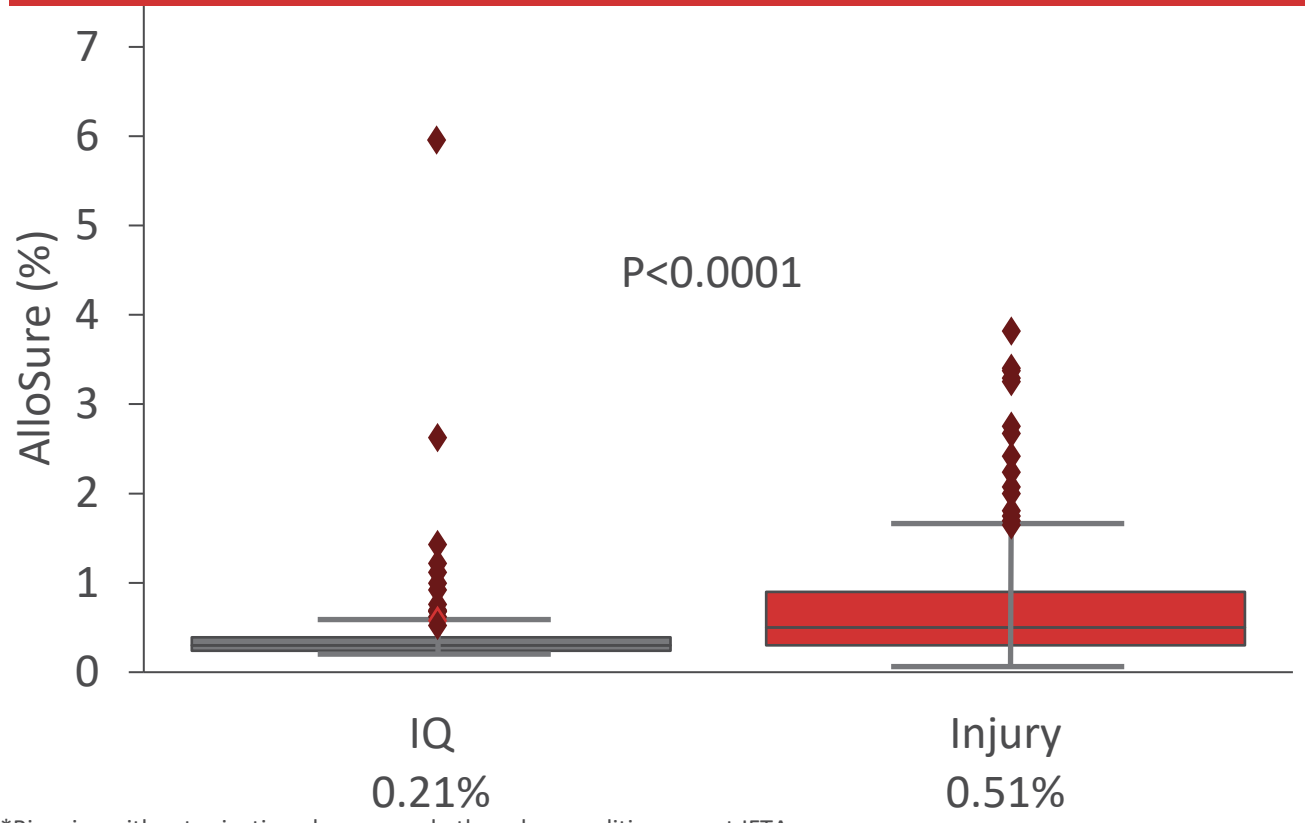
Unleash the Power to Predict Early Graft Injury*

Median AlloSure® scores of **0.21% seen in non-injury, or immunoquiescent (IQ)** patients and **0.51% in injury* patients.**



*Biopsies without rejection changes and other abnormalities except IFTA

1. Bu L. et. al. ADMIRAL. Kidney Intl. Manuscript in publication.

***What is Graft Injury?**

In ADMIRAL, injury is defined as:

- Out of range tacrolimus
- BK viremia
- dnDSA positive
- Urinary tract infection
- Proteinuria
- Allograft rejection
- Recurrent FSGS

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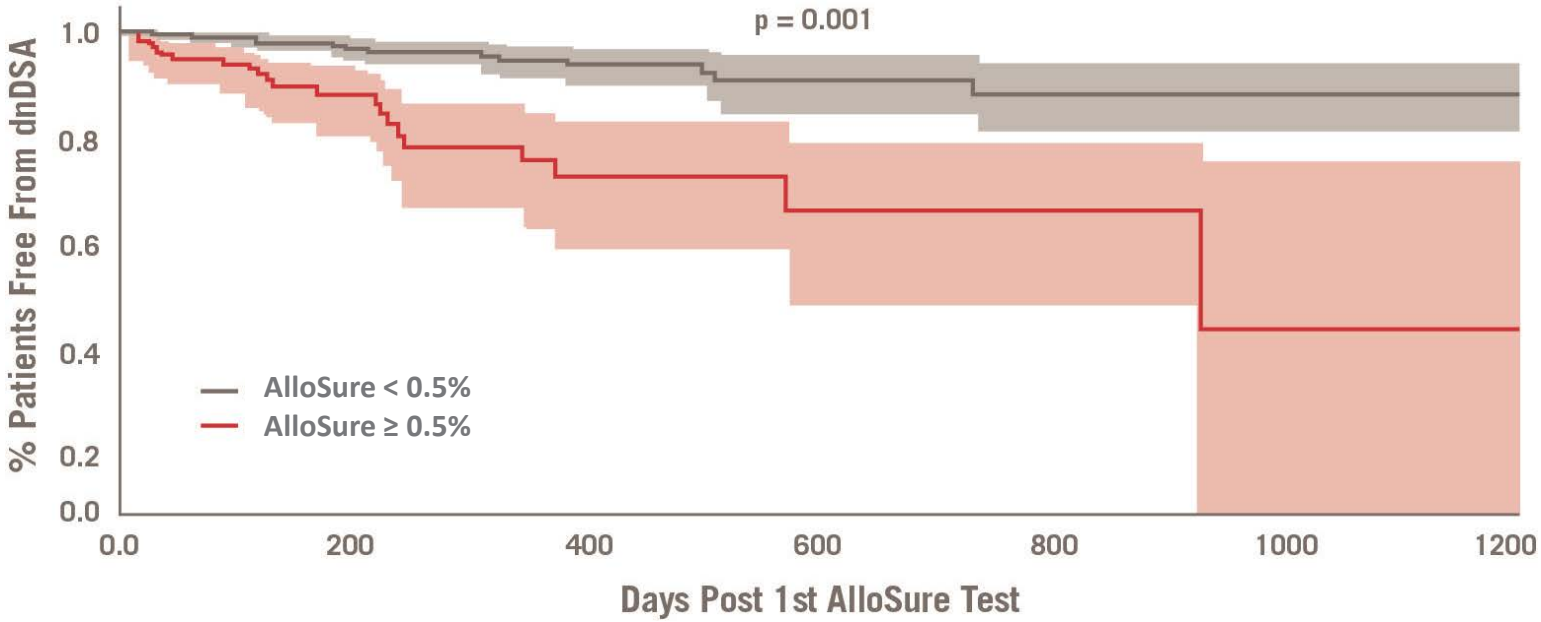
Unleash the Power to Predict dnDSA Detection

AlloSure® scores rose a median of 91 days before dnDSA detection[1]



AlloSure® elevations (≥0.5%) were associated with a **nearly 3-fold elevation* in the risk** of future dnDSA detection (p = 0.001)[1]

*in Hazard Ratio = 2.71

1. Bu L. et. al. ADMIRAL. Kidney Intl. Manuscript in publication.

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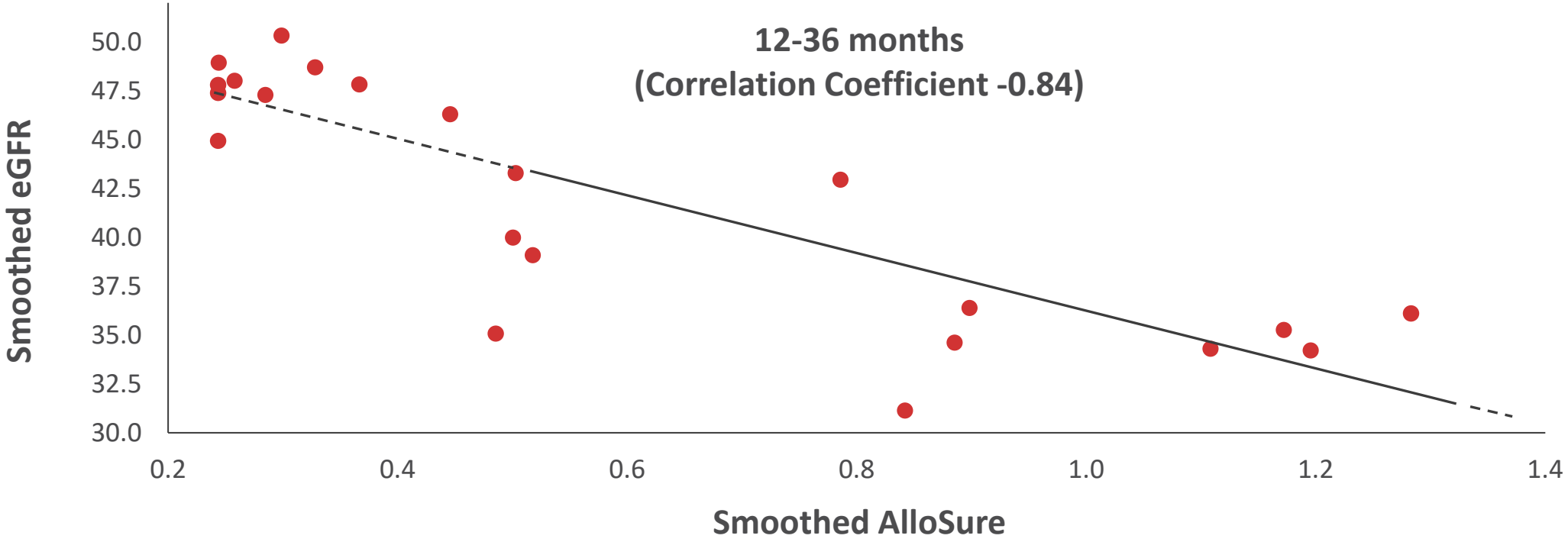
Higher AlloSure® Scores Correlated with eGFR Decline



Persistently elevated AlloSure® (> 1 result, ≥0.5%) has a **nearly 2-fold increase* in risk of > 25% decline in patients' eGFR over 3 years****[1]**

* Hazard Ratio = 1.97

**No statistical significance within first year

1. Bu L. et. al. ADMIRAL. Kidney Intl. Manuscript in publication.

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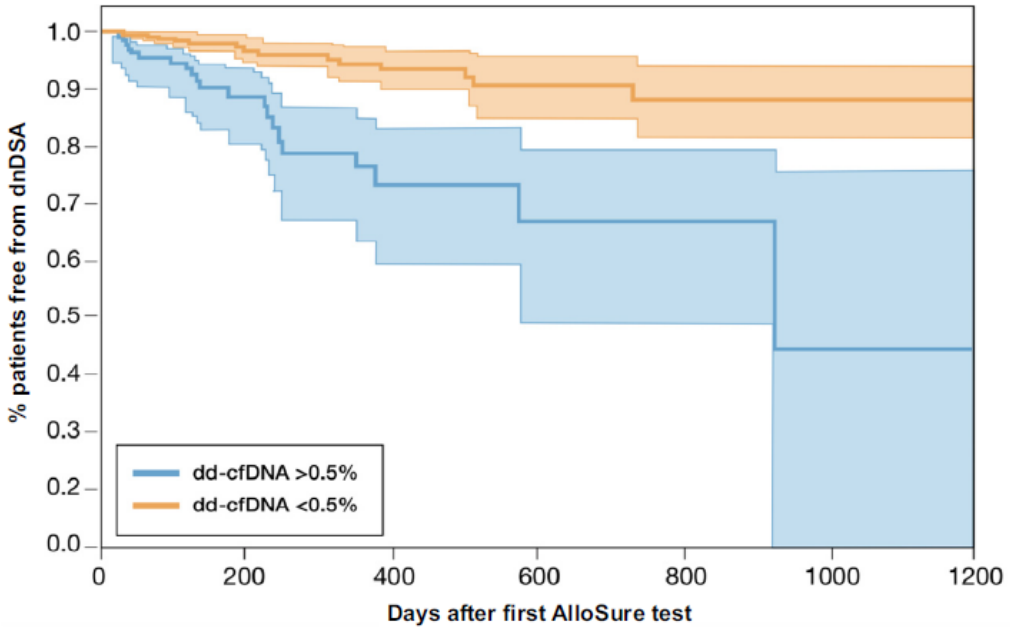
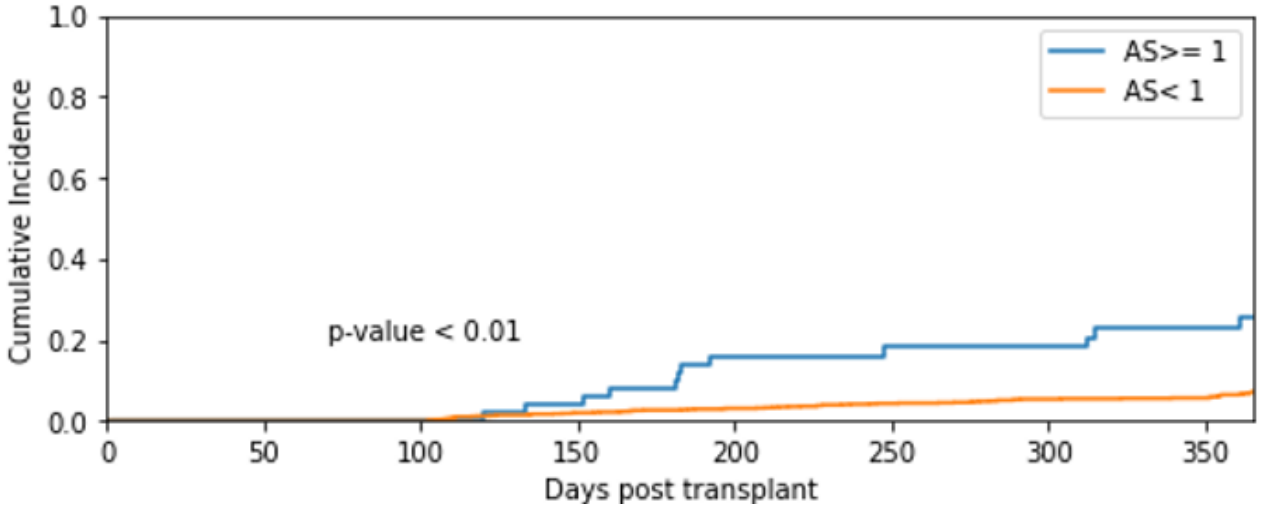
AlloSure Helps Identify High-Risk Patients



AlloSure® elevations (≥0.5%) in ADMIRAL were associated with a **nearly 3-fold elevation* in the risk** of future dnDSA detection (p = 0.001)[1]

Scores rose a median of **91 days before dnDSA were identified**[1]

Rejection, dnDSA, and Death-Censored Graft Loss after Day 100



In KOAR, AlloSure® elevations (≥1.0%) during the first 100 days post-transplant were associated **3-fold risk of adverse clinical outcomes** including **rejection**, **dnDSA**, and **death-censored graft loss** in the first year[2]

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*Hazard Ratio = 2.71

1. Bu, L., et al., Validation and clinical outcome in assessing donor-derived cell-free DNA monitoring insights of kidney allografts with longitudinal surveillance (ADMIRAL) study. Kidney Int, 2021.

2. Wojciechowski D, Patel A, Anand S, Klein J, Paramesh A, Sood P, Shekhtman G, Agrawal N, Fei M, Qu K, Brennan DC. Elevated Donor-Derived Cell-Free DNA (dd-cfDNA) in the Early Post-Transplant Period is Associated with an Increased Incidence of Adverse Clinical Outcomes in Kidney Transplant Recipients [abstract]. Am J Transplant. 2022; 22 (suppl 3)

The Predictive Power of AlloSure® Kidney

Key Takeaways from the ADMIRAL Study[1]

- The ONLY dd-cfDNA Service Clinically Validated for Surveillance in a Multicenter Study
- 62% Improvement Over Serum Creatinine in Identifying Subclinical and Clinical Rejection
- Be Empowered to Act with an Early Signal of Graft Injury, TCMR, and ABMR
- Unleash the Power to Detect Early Graft Injury and Predict dnDSA
- AlloSure® is the ONLY dd-cfDNA with Validated Longitudinal Management Using Relative Change Value

1. Bu L. et. al. ADMIRAL. Kidney Intl. Manuscript in publication.

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Heart Transplant Updates

Sam D, Heart Transplant Recipient

HeartCare™

AlloMap® HEART

The First Innovation in
Gene Expression Profiling

Immune Activity

AlloMap measures immune activity, identifying patients with stable allograft function and low probability of cellular rejection.

AlloSure® HEART

The First Innovation
in Donor-Derived Cell-Free DNA

Graft Injury

AlloSure measures donor-derived cell free DNA in the blood, which can be used as a molecular marker of allograft injury.

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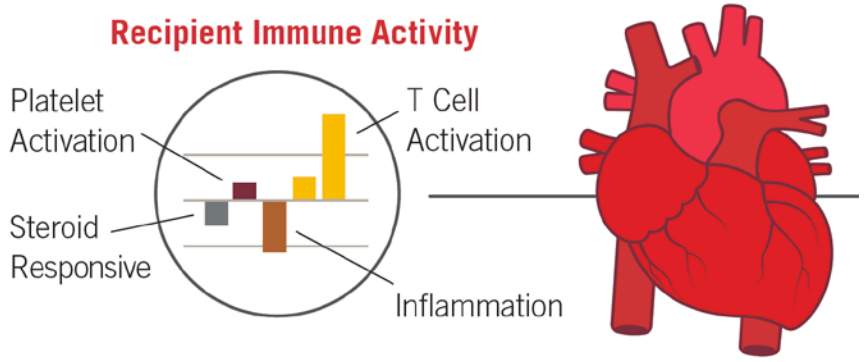
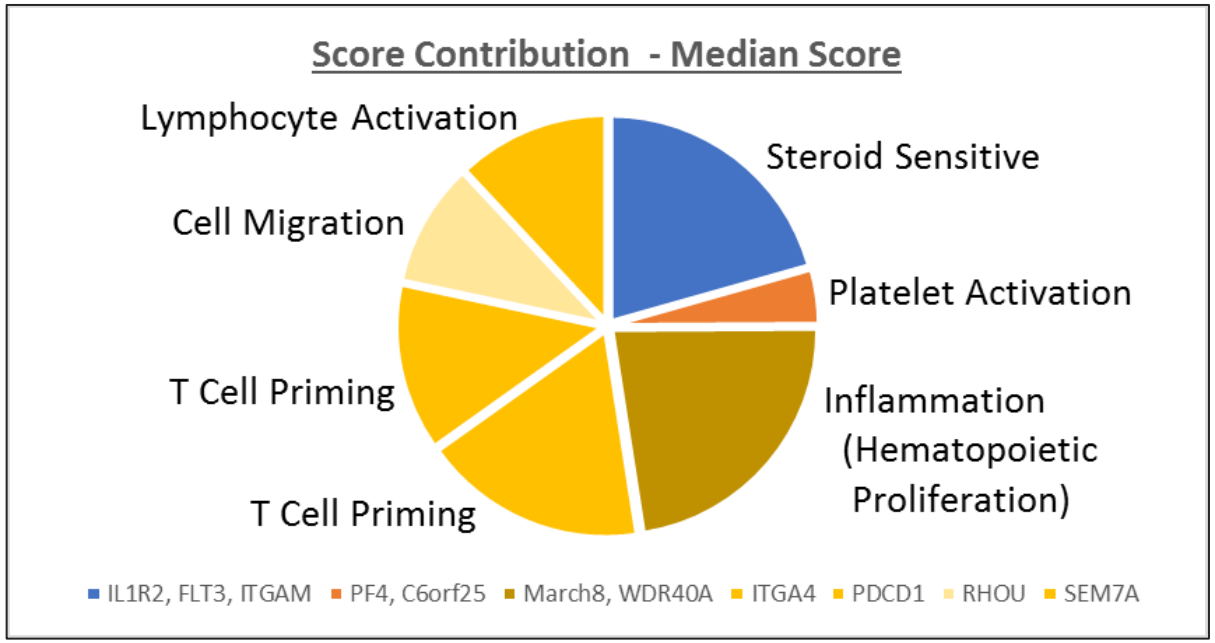
AlloMap Gene Expression Profiling (GEP) Measures Recipient Immune Activity



Genes in the AlloMap Signature

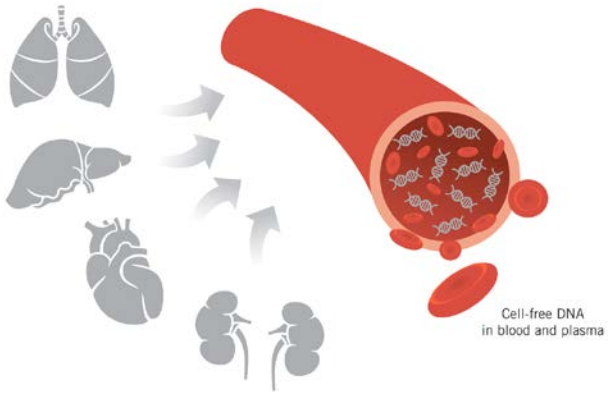
- Lymphocyte Activation: SEM7A
- Cell Migration: RHOU
- T Cell Priming: PDCD1, ITGA4
- Inflammation (Hematopoietic Proliferation): MARCH8, WDR40A
- Steroid Sensitive: IL1R2, FLT3, ITGAM
- Platelet Activation: PF4, C6orf25

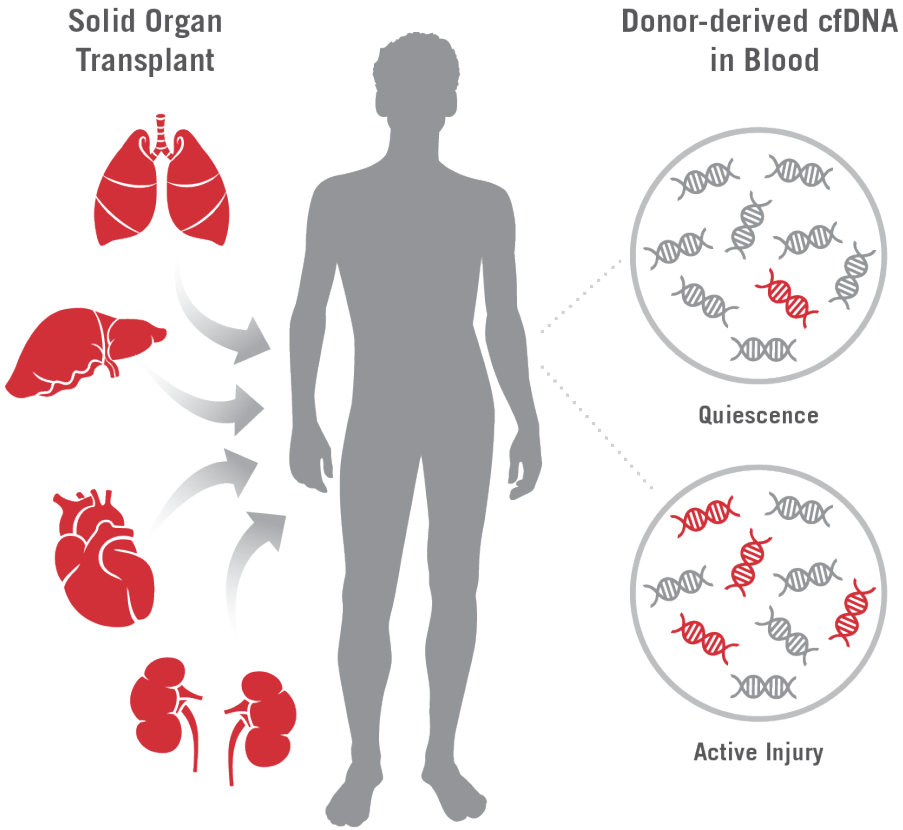


AlloSure® is an Indicator of Current Graft Status

Levels of dd-cfDNA can be measured in plasma, and dd-cfDNA (%) increases as cells from the transplanted organ are injured

<u>Does not require prior genotyping of the donor or recipient</u>





Interpreting HeartCare Results

LOW ALLOMAP / LOW ALLOSURE

56% of patients had this result in D-OAR study

High probability that the patient does not have acute rejection

- ACR>99% NPV
- AMR 98% NPV

Considerations

- Reduce frequency of scheduled biopsy
- Maintain HeartCare surveillance schedule
- If early post-transplant, review of current steroid medication may be indicated

HIGH ALLOMAP / LOW ALLOSURE

26% of patients had this result in the of D-OAR study

Potential reasons for high AlloMap result with low AlloSure-Heart

- Changes in immunosuppression or steroid dose use/adherence
- Early rejection
- Active CMV infection

Considerations

- Evaluate for suboptimal immunosuppression
- Check steroid dose and adherence
- Evaluate for active CMV infection
- Repeat HeartCare testing earlier than standard protocol

LOW ALLOMAP / HIGH ALLOSURE

11% of patients had this result in the D-OAR study

Potential reasons for high AlloSure-Heart result with low AlloMap

- Early cellular rejection
- Antibody-mediated rejection
- Non-rejection reasons such as infection or cardiac allograft vasculopathy (CAV) causing graft injury

Considerations

- Evaluate longitudinal AlloMap and AlloSure results to assess trends
- Donor-specific antibody testing
- Follow-up HeartCare, echocardiogram or EMB

HIGH ALLOMAP / HIGH ALLOSURE

6% of patients had this result in the D-OAR study

Relatively high probability that rejection injury is present (~20% PPV)

Considerations

Determine if rejection may be present

- Rejection workup including EMB with consideration for ABMR
- Donor-specific antibody testing
- Serial HeartCare testing

The table is provided for informational purposes only and is not intended as medical advice. A physician's test selection and interpretation, diagnosis, and patient management decisions should be based on his/her education, clinical expertise, current guidelines, and assessment of the patient.

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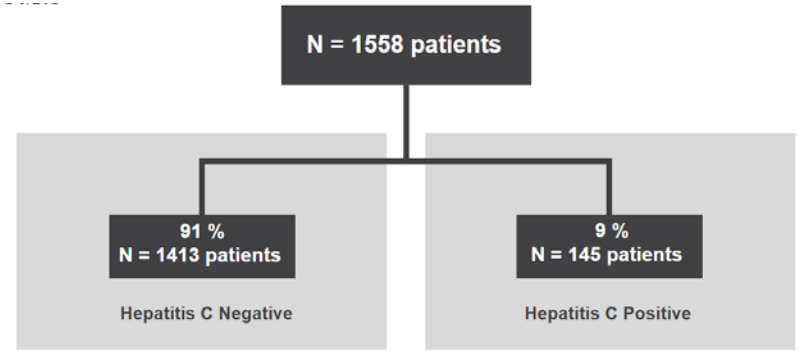
Latest Data and Publications

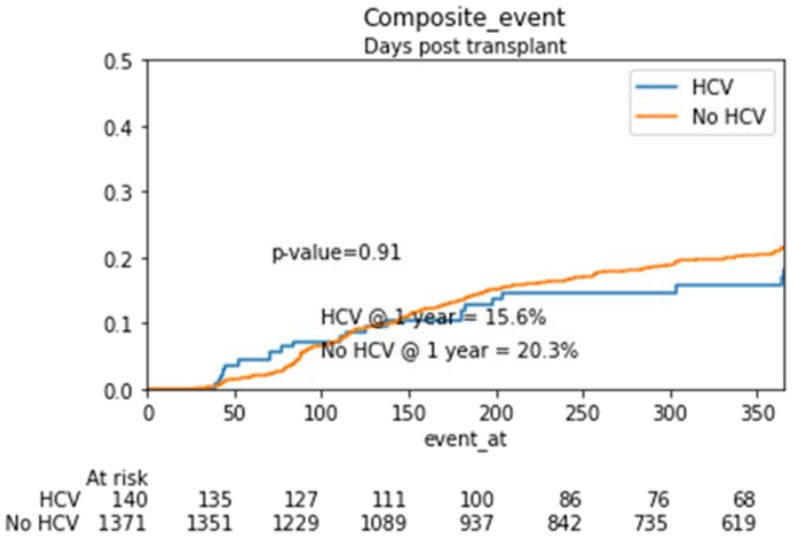
Sam D, Heart Transplant Recipient

Hepatitis C Donors Are Not Associated With Higher Rates Of Rejection After Cardiac Transplantation

SUMMARY

- Previous experience using HCV positive donors was a high rate of HCV infection and unacceptably poor outcomes in recipients
- The introduction of direct-acting antivirals (DAAs) that have a high cure rate of HCV3-4 has renewed interest in using organs from HCV-positive donors
- The aim of this study was to compare surrogate markers for outcomes, including HeartCare® multi-center SHORE registry
- Outcomes of interest were rejection defined as ISHLT ≥ 2R and/or AMR ≥ 1 and average AlloSure® levels during the first year. A composite outcome of de novo DSA, rejection, decline in LVEF, and/or development of CAV at one year was also assessed





The cumulative incidence of composite transplant outcomes for patients with donor hearts with HCV was not different than donor hearts without HCV

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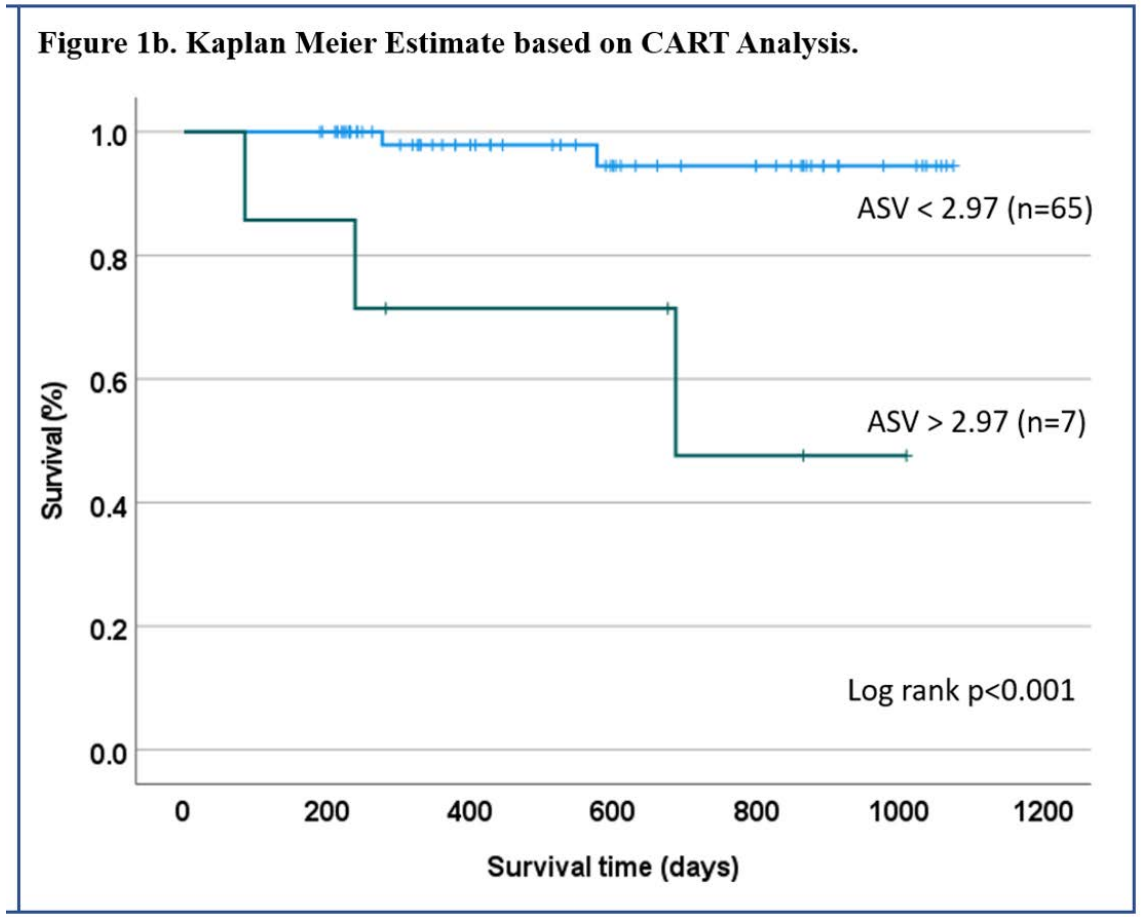
Patel, S et al., 2022 ISHLT Oral Presentation. – Montefiore

AlloSure® Variability Identifies Risk of Adverse Outcomes



SUMMARY

- 72 adult HTx patients from a single center monitored with AlloMap® and AlloSure®
- AlloSure® Variability (ASV) was defined as standard deviation of the 3 most recent (3-value) or all (all-value) sequential dd-cfDNA results and scaled x 10 for analysis (ASV*10)
- Used Cox proportional hazards model to assess the association between ASV and mortality and a survival classification and regression tree (CART) algorithm to define a threshold for increased mortality

Higher variability in dd-cfDNA over time was associated with an increased risk of mortality after OHTx

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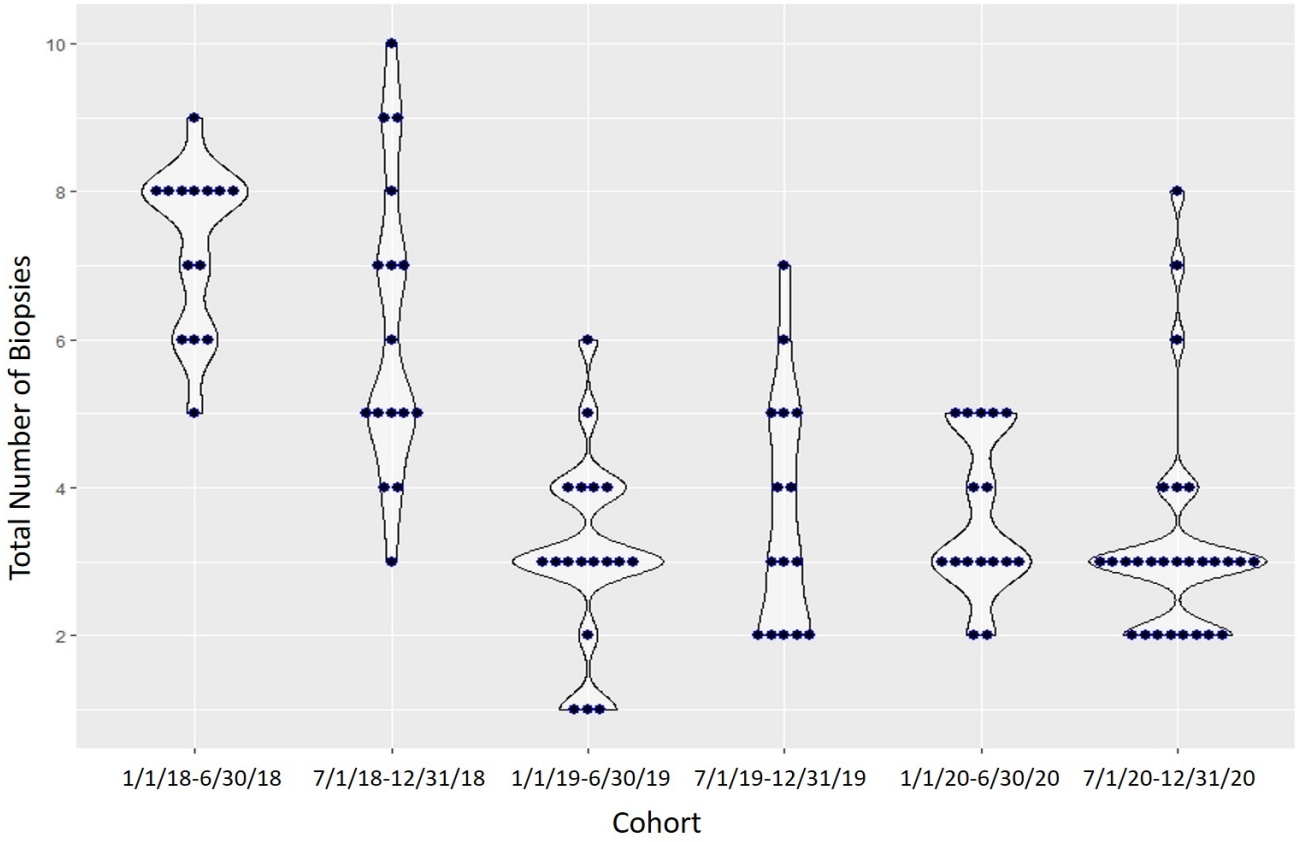
Kamath M, et al., 2022 ISHLT Abstract. - UCLA

Substantial Reduction in Biopsies with Initiation of Non-Invasive Rejection Surveillance at One Month Post-Transplant

Figure 1. Total Number of Biopsies Performed in the First 6 Months Post Transplant by Cohort



SUMMARY

- 106 HT recipients were included and divided into six cohorts
- Patients in later cohorts had fewer EMB in the first six months post transplant compared to those at the initiation of protocol ($p<0.01$)
- The July 2018 and July 2019 cohorts each had two patients expire, making their one-year survival 88% and 87% respectively
- All other cohorts had 100% survival. Baseline characteristics, immunosuppression and use of induction was similar between groups

Initiation of non-invasive surveillance at 28 days post-transplant significantly reduces the number of biopsies, with similar 1 year survival

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Henricksen, E et al., 2022 ISHLT Abstract. - Stanford

Should We Be Comforted By A “Negative” Endomyocardial Biopsy? Risk Of Future Events With Donor Derived Cell Free DNA In The Setting Of Histologic Quiescence

SUMMARY

- Retrospective analysis via the preliminary SHORE registry
- A total of 648 HT recipients with a mean age 57, 74% male, white 64%, 60% of which had PRA < 1% and had a total of 982 paired biopsies with a median dd-cfDNA of 0.05% for those with a Grade 0R and 0.06% for Grade 1R biopsy (Figure 1a)
- The dd-cfDNA was measured a median of 112 days post-transplant for Grade 0R and 109 days post-transplant for Grade 1R. Despite negative histology on EMB, those with a cfDNA >= 0.20% were at significantly higher risk for the development of significant rejection (14.3% v 5.2%, p<0.01) and dnDSA (11.3% v. 6.8%, p<0.01) over the subsequent year.

Figure 1a

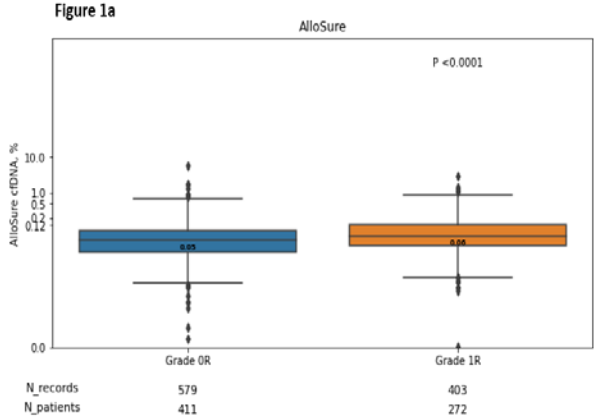


Figure 1b

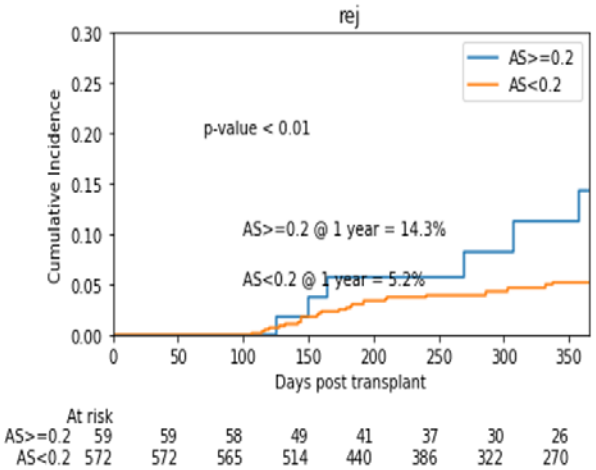
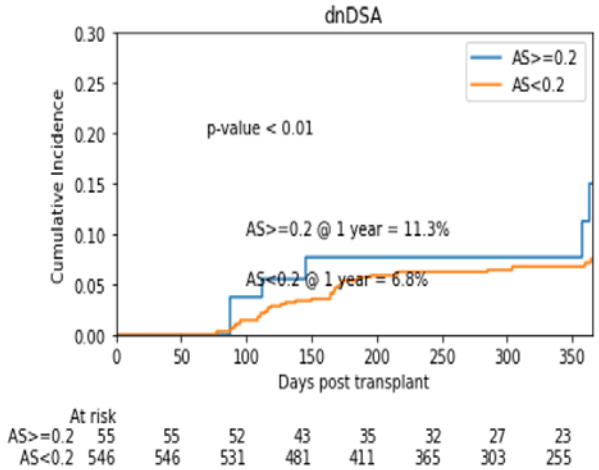


Figure 1c



The use of dd-cfDNA may be a better method to determine true quiescence and call into question the utility of the EMB as the gold standard for cardiac allograft monitoring

CareDx®

Donor-derived Cell-free DNA: A Useful Adjunct in the Lung Transplant Clinic

October 10, 2022

Anil J. Trindade, MD
Associate Medical Director, Lung Transplant Program
Assistant Professor of Medicine
Allergy, Pulmonary and Critical Care Medicine

VANDERBILT UNIVERSITY
MEDICAL CENTER

Disclosures and Funding

+ **CareDx, Inc.** →
    - PI on IIT “dd-cfDNA as a Biomarker for CLAD”
    - PI on IIT “dd-cfDNA to Assess Recovery from Acute Cellular Rejection”
    - Site PI and Steering Committee Member on TEAMMATE Study
    - Member, National Scientific Advisory Committee
    - Speaker fees

+ **Veloxis Pharmaceuticals** →
    - PI on IIT “Early Use Envarsus Post- Lung Transplant to Mitigate Side Effects.”

+ **NIAID 1U01AI167789-01** →
    - VUMC Site PI on “Comparison of High Dose vs. Standard Dose of Influenza Vaccines in Lung Allograft Recipients.”

Objectives

1) ALAD and CLAD limit lung allograft survival

2) dd-cfDNA: Evidence in lung transplantation &
How I use dd-cfDNA in the clinic

3) Future directions for dd-cfDNA in lung transplantation

Why Do We Perform Lung Transplants?

For Patients with Advanced Pulmonary Disease

1) Progressive lung disease despite optimal medical management
2) Expected two-year survival < 50%

Improved Survival

Improved Quality of Life

Survival Benefit of Solid Organ Transplantation for Wait-List Patients 1987- 2012

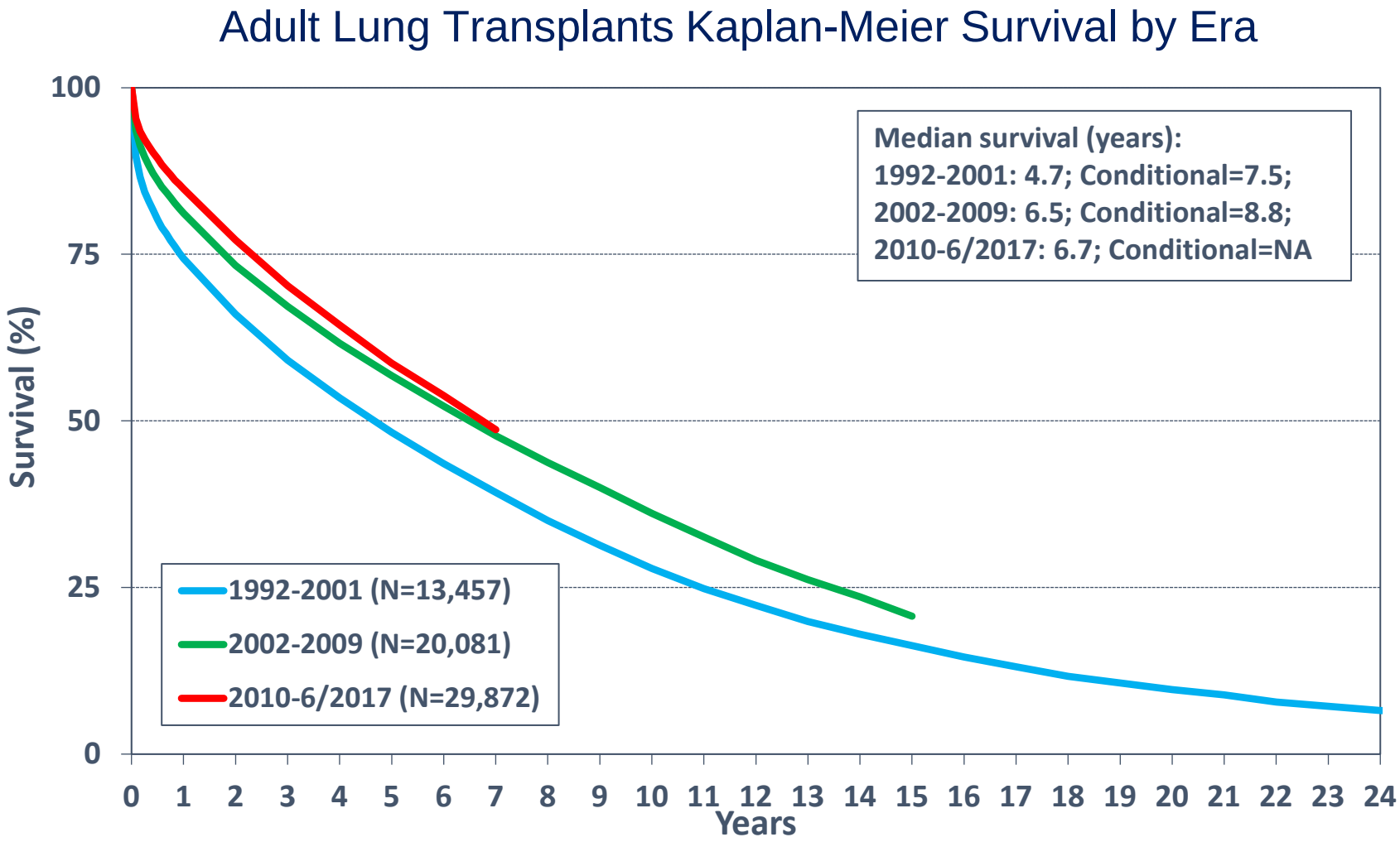
Table 1. Survival Benefit of Solid-Organ Transplant

Transplant Type and Patient Category	No. of Patients	No. of Life-years	Observed No. of Life-years Saved to Date	Observed No. of Life-years Saved per Patient to Date	Median Survival, y
Kidney					
Waiting list	355 189	987 009	...[a]	...	5.4
Transplant	314 561	2 246 383	1 372 969	4.4	12.4
Liver					
Waiting list	134 826	218 026	...	...	3.1
Transplant	112 319	659 637	465 296	4.3	11.6
Heart					
Waiting list	40 253	65 011	...	...	2.3
Transplant	54 746	358 555	269 715	4.9	9.5
Lung					
Waiting list	24 688	43 564	...	...	2.3
Transplant	26 943	116 301	64 575	2.6	5.2
Pancreas-kidney					
Waiting list	14 195	33 979	...	...	4.2
Transplant	16 995	119 620	79 198	4.6	14.5
Pancreas					
Waiting list	8568	26 733	...	...	8
Transplant	6177	34 193	14 903	2.4	13.3
Intestine					
Waiting list	1787	2086	...	...	1.8
Transplant	1588	6256	4402	2.8	5.1
Total					
Waiting list	579 506	1 376 408	...	...	...
Transplant	533 329	3 540 945	2 270 859	4.3	...

Organ Type	Median Survival (yrs)
Kidney	12.4
Liver	11.6
Heart	9.5
Lung	5.2
Pancreas-Kidney	14.5
Pancreas	13.3
Intestine	5.1

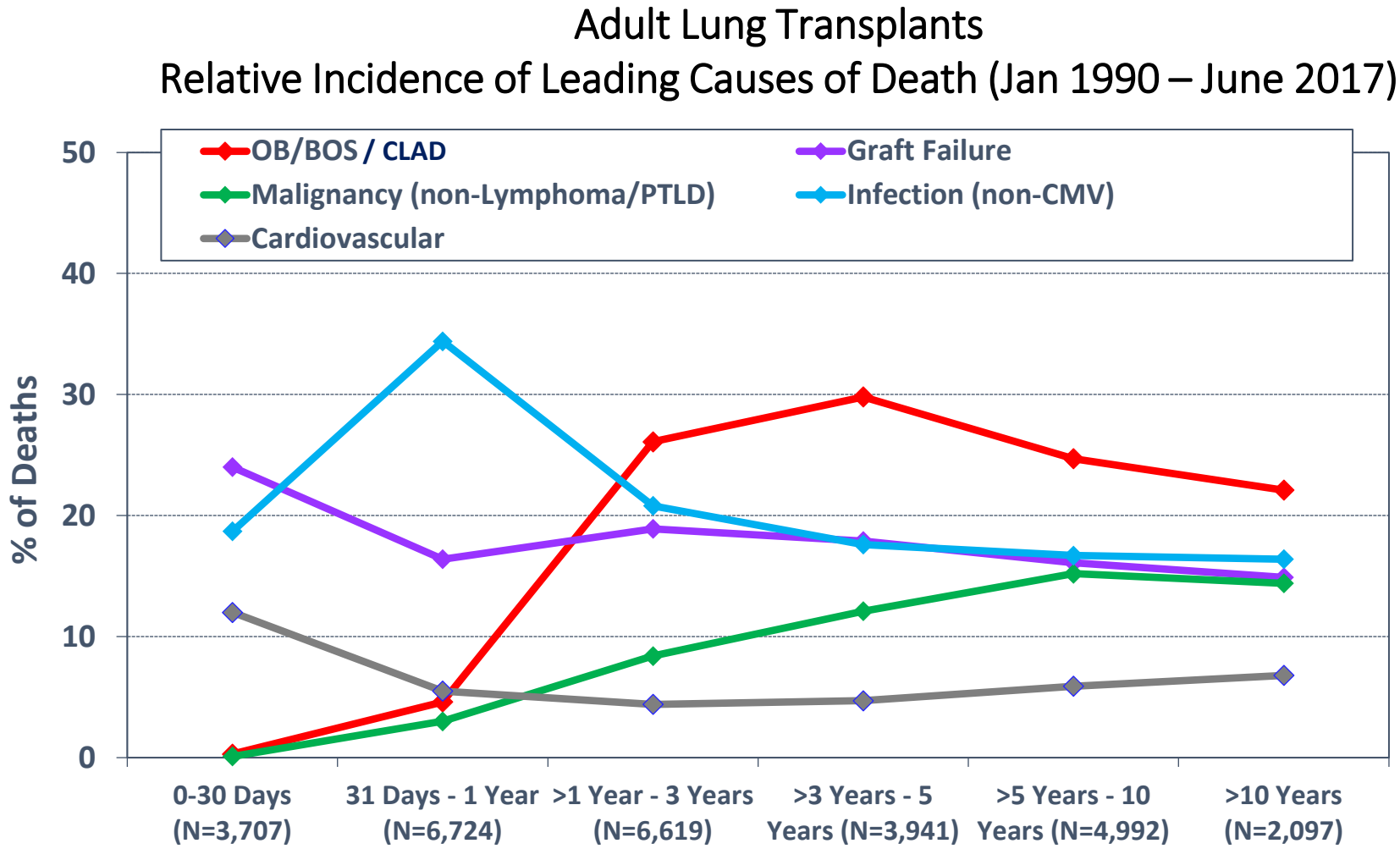
Rana JAMA Surg. 2015;150(3):252-259.

Median Survival for Lung Transplant Recipients Continues to Improve



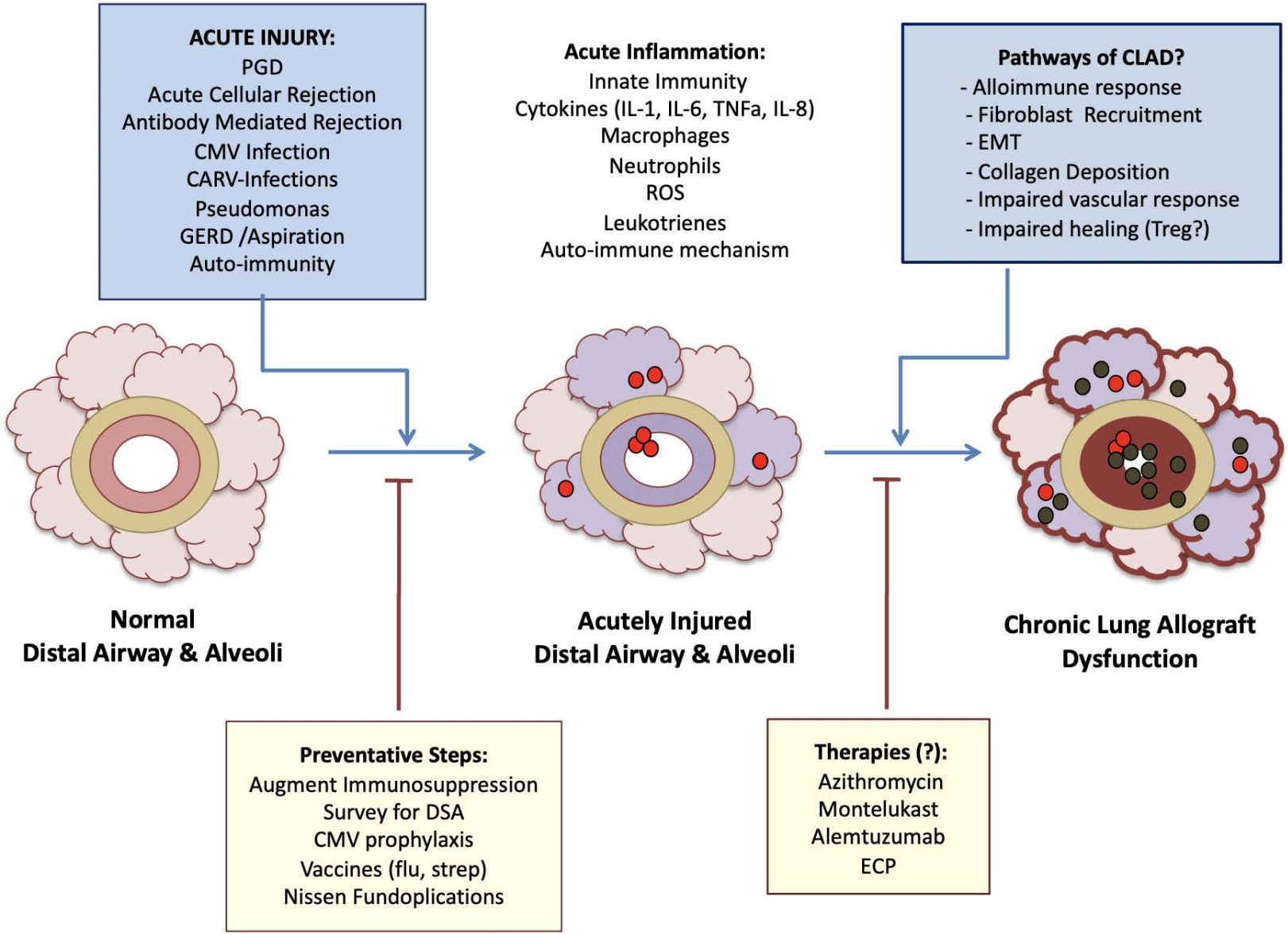
ISHLT International Registry JHLT 2019, Oct; 38(10): 1015-1066

Major Causes of Death Post- Lung Transplantation

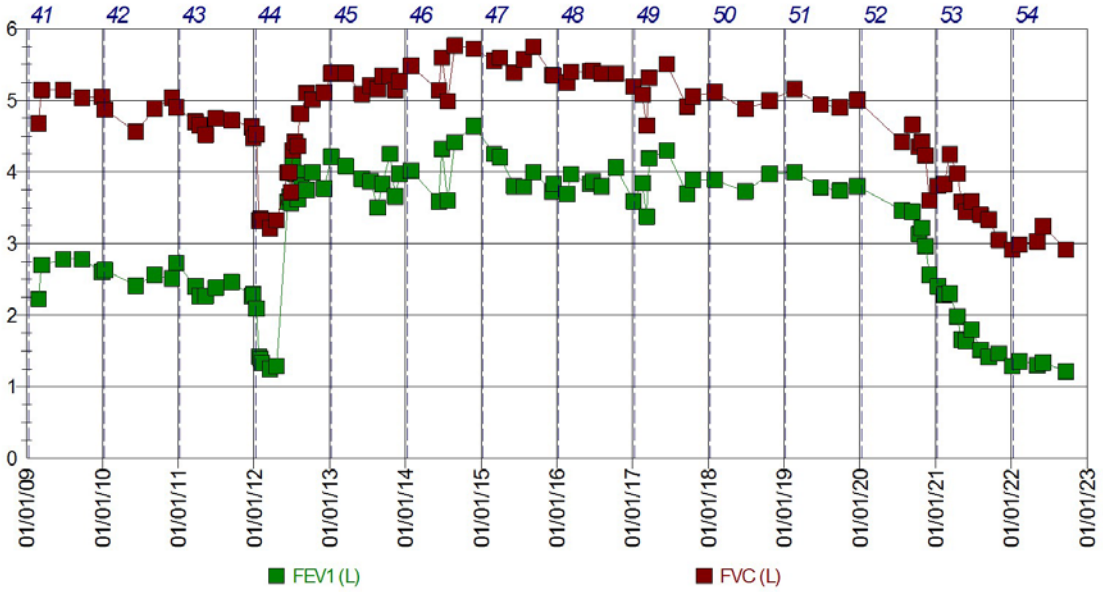


ISHLT International Registry JHLT 2018

Chronic Lung Allograft Dysfunction: An Accumulation of Injury



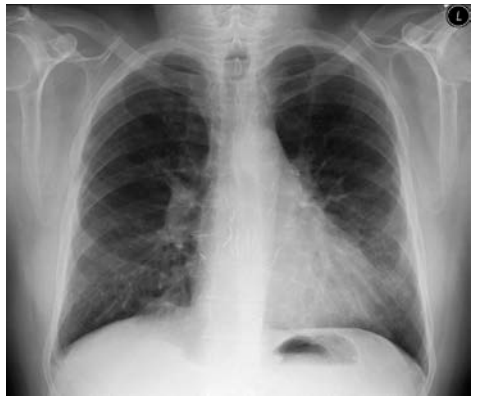
Spirometry is an Imprecise Tool for Assessing Lung Injury



Radiography

Spirometry

Bronchiolitis Obliterans Syndrome (BOS)

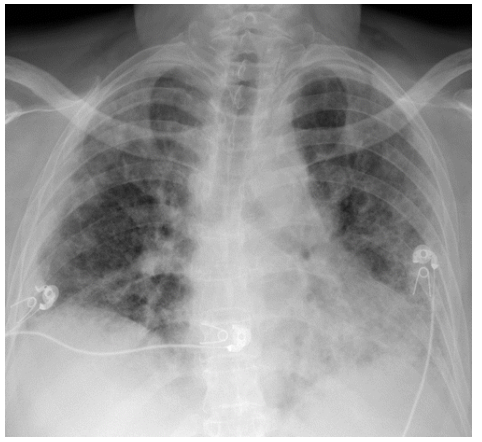


Hyperinflation

OBSTRUCTION Stages (Compare to Post-TXP baseline)

Stage 1: FEV1 < 80%
Stage 2: FEV1< 65%
Stage 3: FEV1 <50%
Stage 4: FEV1 <30%

Restrictive Allograft Syndrome (RAS)

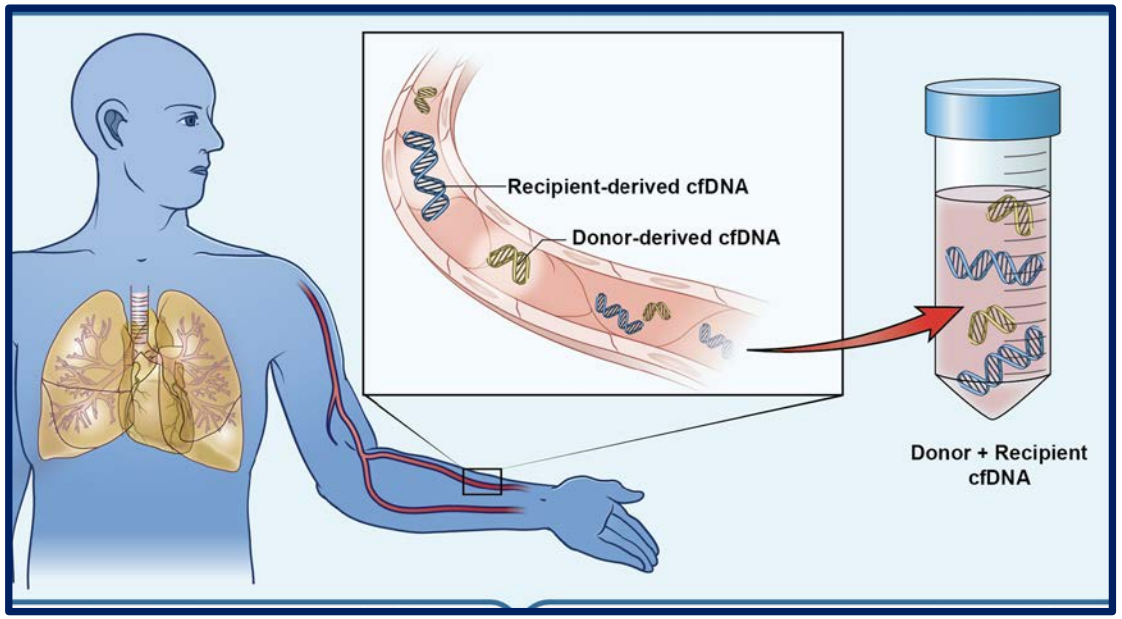


Reticular Opacities

RESTRICTION Stages (Compare to Post-TXP baseline)

Stage 1: FEV1 < 80%
Stage 2: FEV1< 65%
Stage 3: FEV1 <50%
Stage 4: FEV1 <30%

Dd-cfDNA is a Biomarker of Lung Allograft Injury



- DNA released into plasma by apoptotic cells
- dd-cfDNA (AlloSure®, CareDx, Inc)
  - Distinguishes individual cfDNA *without* genotyping either the donor or the recipient
  - Assesses differences in homozygosity in a standard SNP-set of 405 SNPs to differentiate donor and recipient

Grskovic et al., *J. Molecular Diagnostics* 2016
Dengu *Transplantation Reviews* 2020
Keller, Agbor-Enoh *Curr Transplant Reports* 2021

Objectives

1) ALAD and CLAD limit lung allograft survival

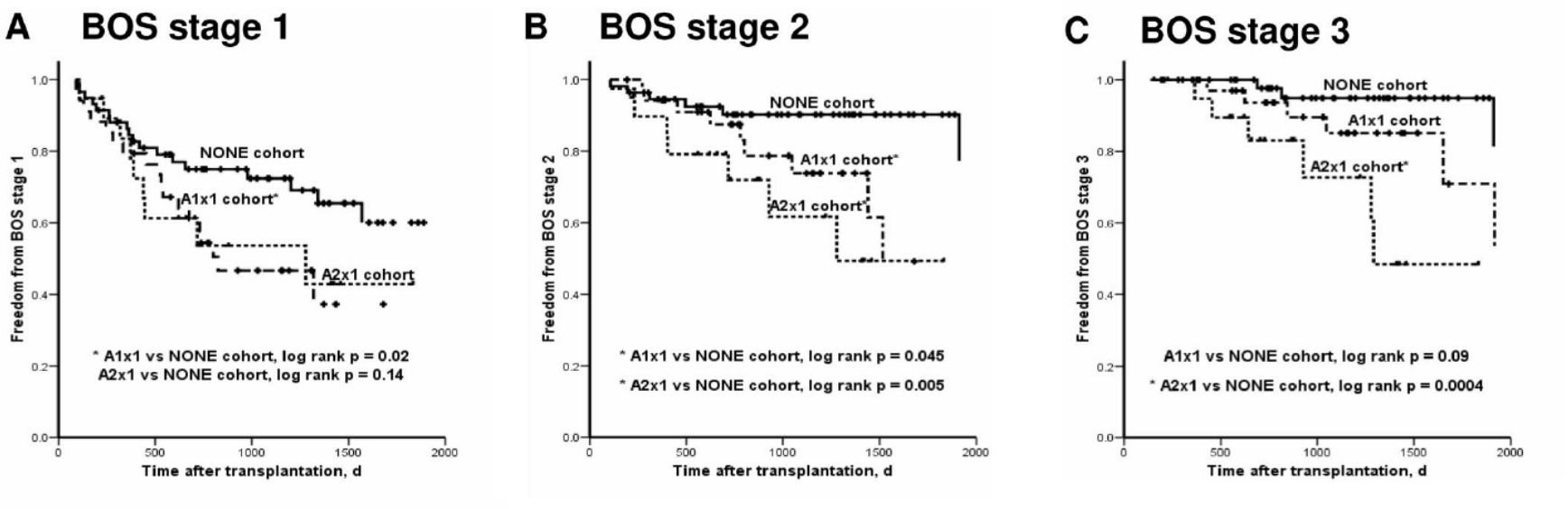
2) dd-cfDNA: Evidence in lung transplantation &
How I use dd-cfDNA in the clinic

3) Future directions for dd-cfDNA in lung transplantation

Case #1: A1 ACR- Of Minimal Importance?

- 57yo M with A1AT-deficiency s/p bilateral lung transplant 9-months ago. CMV D+/R+ s/p 6-months pre-emptive CMV prophylaxis with valganciclovir. Course c/b CMV viremia at 7-months, with improvement with valganciclovir.
- FEV1 stable (?) at 3.50L (88% of post-txp peak baseline, down 8% from prior).
- 9-month surveillance bronchoscopy A1B0.
- Donor-specific antibody screen negative
- No GERD, cultures negative, adherent with meds, non-smoker.
- **Do you augment immunosuppression?**

Acute Cellular Rejection (ACR) is a Major CLAD Risk Factor



+ Retrospective cohort of 259 patients at Barnes-Jewish (St. Louis, MO)

+ A1 and A2 ACR increases risk for BOS stage 1 and 2

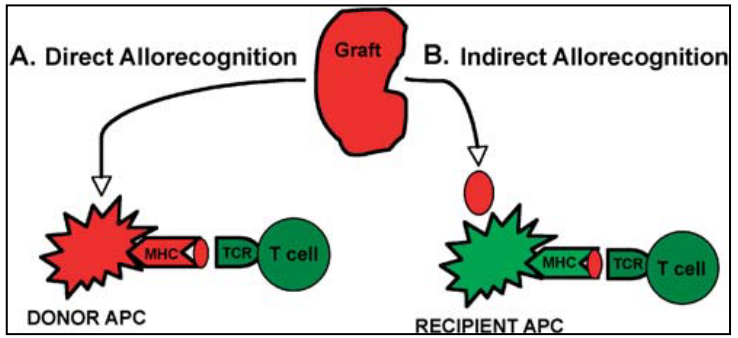
+ A2 ACR is a risk factor for stage 3 BOS

ACR is associated with decreased freedom from CLAD

Khalifah, Hachem Am J. Transplantation 2005

Why Does Acute Cellular Rejection Occur?

Acute Lung Injury Results In:



+ Graft injury and antigen / peptide shedding →
  PGD/ IRLI, GERD, Infection

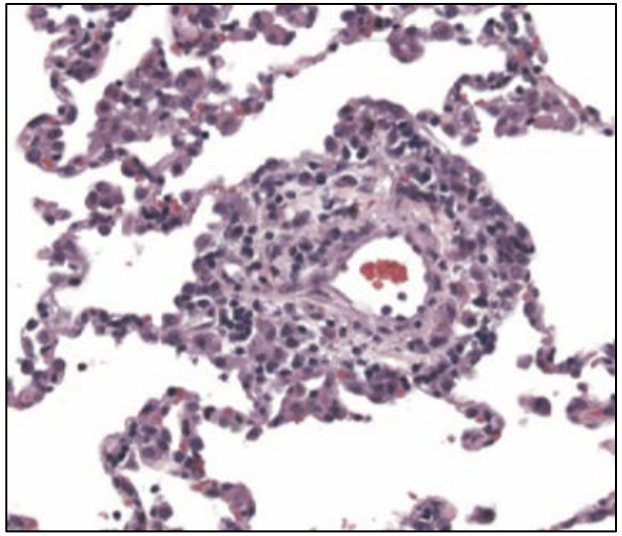
+ Presentation of donor peptides by antigen presenting cells

+ Activation of T lymphocytes due to recognition of self vs. not self
  Number of HLA mismatches is proportional to ACR risk

+ Binding of activated T cell receptor to allograft or APCs

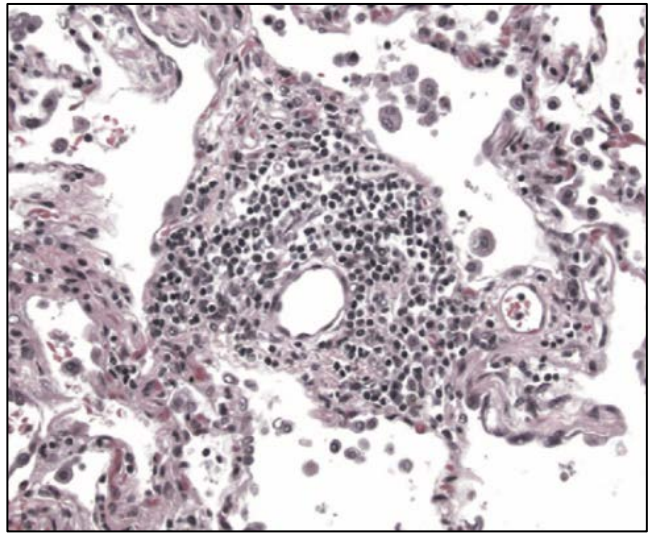
Acute Cellular Rejection is a Histologic Diagnosis

A1- Minimal



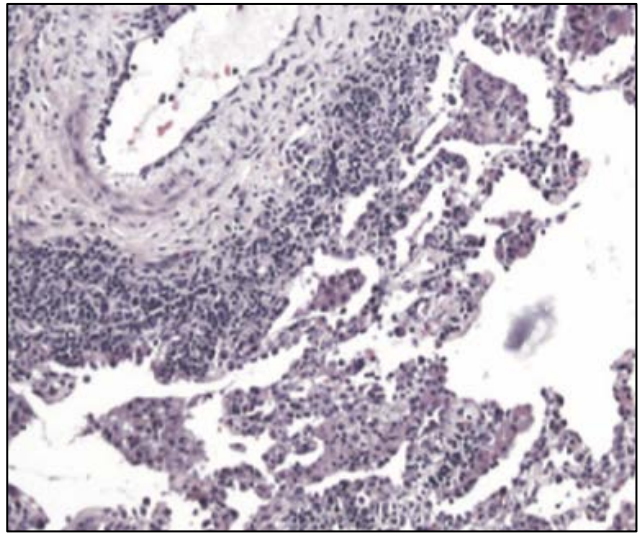
+ Infrequent perivascular lymphocytes
+ Adventitial cuffing 2-3 cells deep
+ Venules
+ No endothelialitis
+ Rarely seen on low power (40X),

A2- Mild



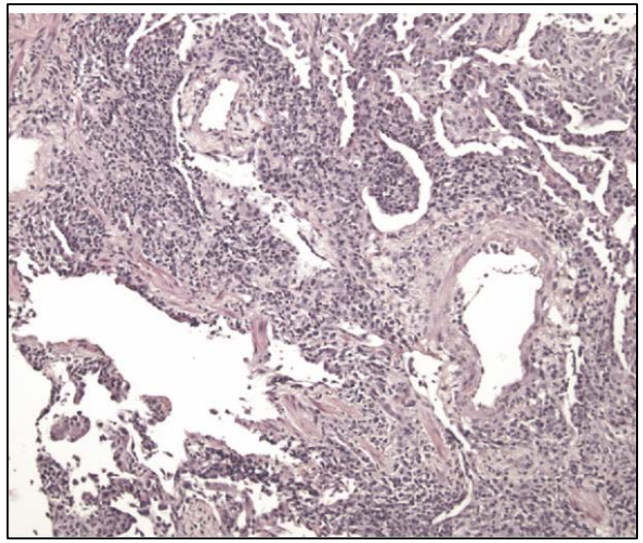
+ Frequent perivascular lymphocytes
+ Can see macrophages and eosinophils
+ Adventitial cuffing 2-3 cells deep
+ Venules and Arterioles involved
+ Endothelialitis can be appreciated
+ Can seen on low power (40X),

A3- Moderate



+ Frequent, dense perivascular lymphs that extends into the alveolar septae
+ Can also see macs, eos and pmns
+ Venules and Arterioles involved
+ Endothelialitis is more apparent
+ Can seen on low power (40X)

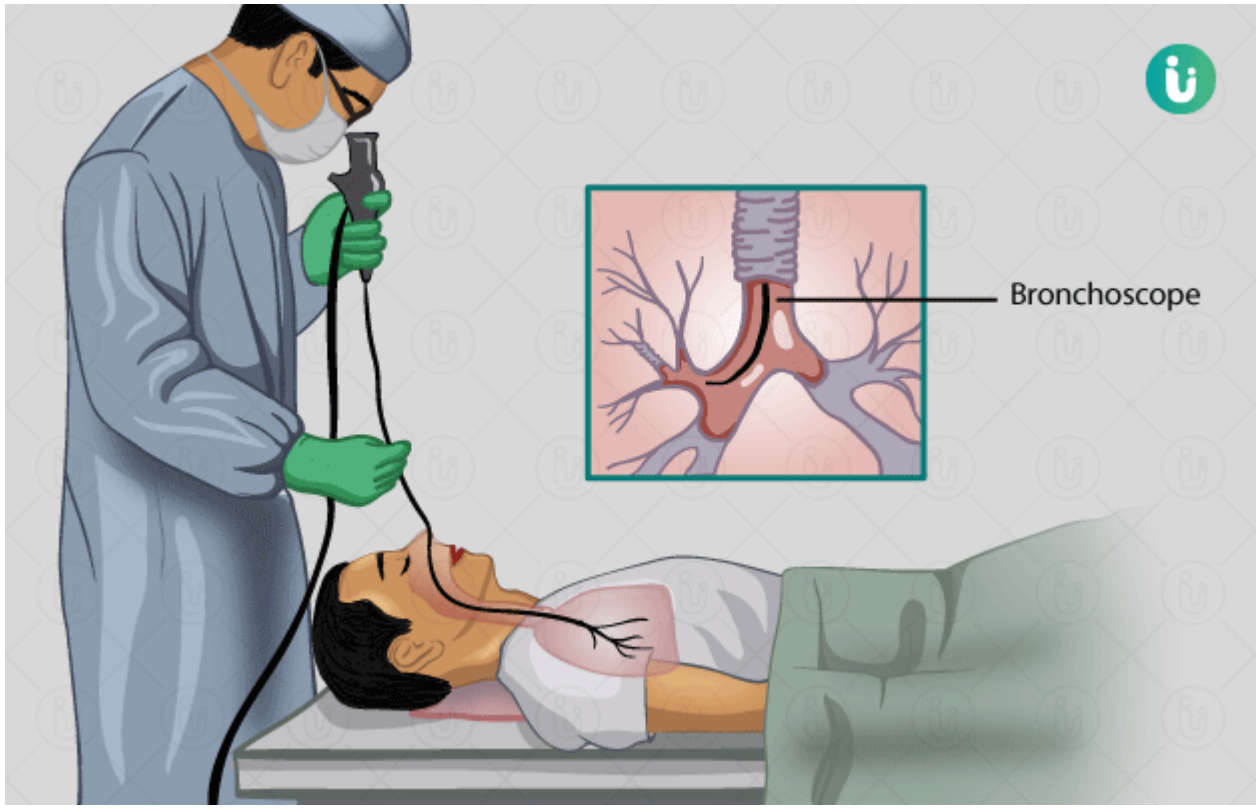
A4- Severe



+ Diffuse mononuclear infiltrate (vasculature, airways, interstitium
+ Can also see macs, eos and pmns
+ Diffuse alveolar damage
+ Can see areas of necrosis

Stewart, et al. JHLT 2007

Transbronchial Biopsies are NOT Benign



www.myupchar.com

TABLE III Complications of procedures

Complication	Percentage of biopsies
Pneumothorax	1.46
Bleeding >100 ml	4
Assisted ventilation	0.32
Arrhythmia	0.57
Death	0
Overall	6.35

Hopkins et al., JHLT 2002

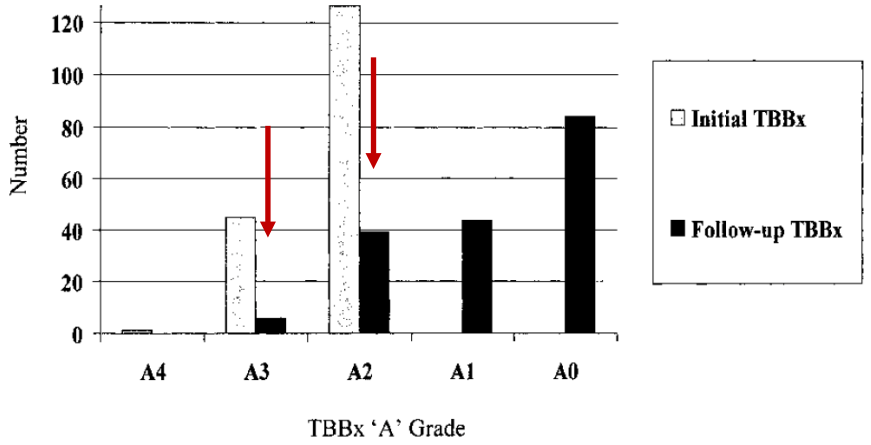
Acute Cellular Rejection- Treatment (and Efficacy)

There are limited studies regarding therapies for ACR in lung transplantation

Steroids:

Aboyoun, et al. AJRCCM 2001

+ Single-center retrospective study (Sydney, Australia)
+ 99 patients with ≥A2 ACR had follow-up TBBx within **45 days**.
+ Persistent ≥A2 ACR seen in 26%
+ Persistent ≥B2 ACR seen in 22%



There is limited evidence for **antithymocyte globulin**, **alemtuzumab**, or **extracorporeal photopheresis** for ACR treatment .

There are pros and cons to each of these therapies

ACR prevention is also encouraged (GERD, infection, smoking cessation, adherence)

Dd-cfDNA: A Biomarker of ACR in Lung Recipients

ELSEVIER

The Journal of Heart and Lung Transplantation
http://www.jhltonline.org

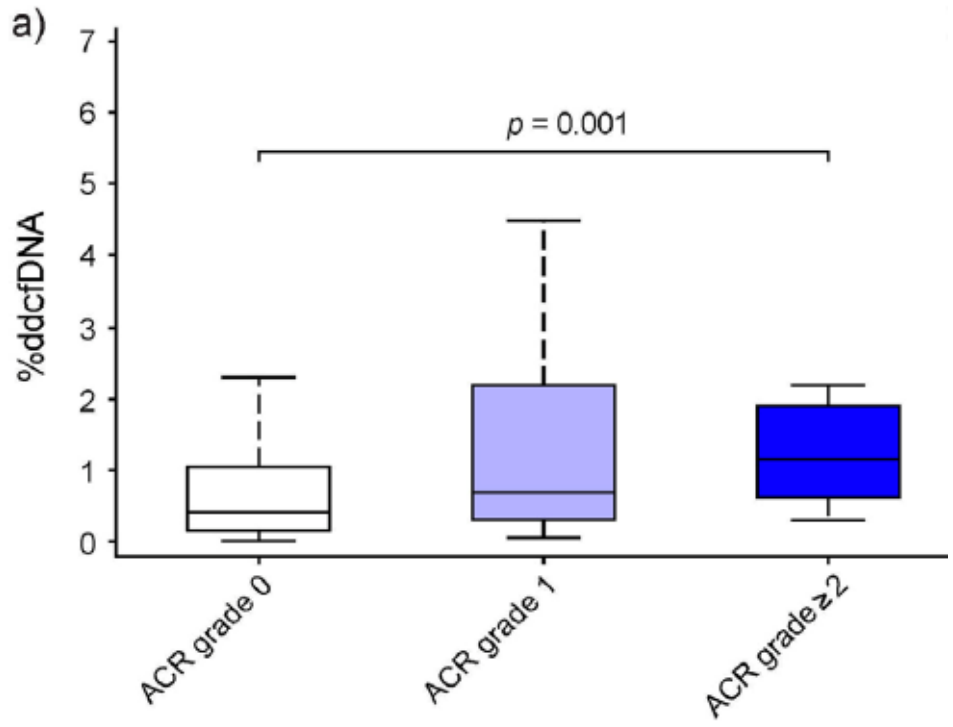
Donor-derived cell-free DNA accurately detects acute rejection in lung transplant patients, a multicenter cohort study

Moon Kyoo Jang, PhD,[a,b] Ilker Tunc, PhD,[b] Gerald J. Berry, MD,[a,c] Charles Marboe, MD,[a,e] Hyesik Kong, PhD,[a,b] Michael B. Keller,[a,b,d] Pali D. Shah, MD,[a,d] Irina Timofte, MD,[a,f] Anne W. Brown, MD,[a,g] Ileana L. Ponor, MDMBA,[h] Cedric Mutebi, BS,[i] Mary C. Philogene, PhD,[a,j] Kai Yu, PhD,[j] Aldo Iacono, MD,[a,f] Jonathan B. Orens, MD,[a,c] Steven D. Nathan, MD,[a,g] and Sean Agbor-Enoh, MDPhD[a,b,d]

+ Prospective, multicenter study of 148 lung transplant recipients followed for median 19.6 months post-txp.
+ TBBx samples compared to %dd-cfDNA
+ dd-cfDNA% identified using shotgun sequencing of donor and recipient SNPs
+ Single lung transplant values doubled
+ 30 episodes ACR with graft dysfunction
  - 7 episodes of A1 ACR
  - 23 episodes > A2 ACR

Jang, et al. J Heart Lung Transplant 2021

Dd-cfDNA: A Biomarker of ACR in Lung Recipients



Median % dd-cfDNA= 0.41 (0.15 – 1.06)

Median % dd-cfDNA= 0.68 (0.2 – 3.10)

Median % dd-cfDNA= 1.16 (0.5 – 2.04)

Comparing Clinical ACR vs No ACR:

median %dd-cfDNA=
1.23
(0.65 – 2.03)
($p < 0.01$)

For the threshold of >1% dd-cfDNA:

Sensitivity = 77%
Specificity = 84%
Positive Predictive Value =64%
Negative Predictive Value= 90%

Jang, et al. J Heart Lung Transplant 2021

Dd-cfDNA: A Biomarker of ALAD in Lung Recipients

ELSEVIER

The Journal of Heart and Lung Transplantation
http://www.jhltonline.org

ORIGINAL CLINICAL SCIENCE

Donor-derived cell-free DNA as a composite marker of acute lung allograft dysfunction in clinical care

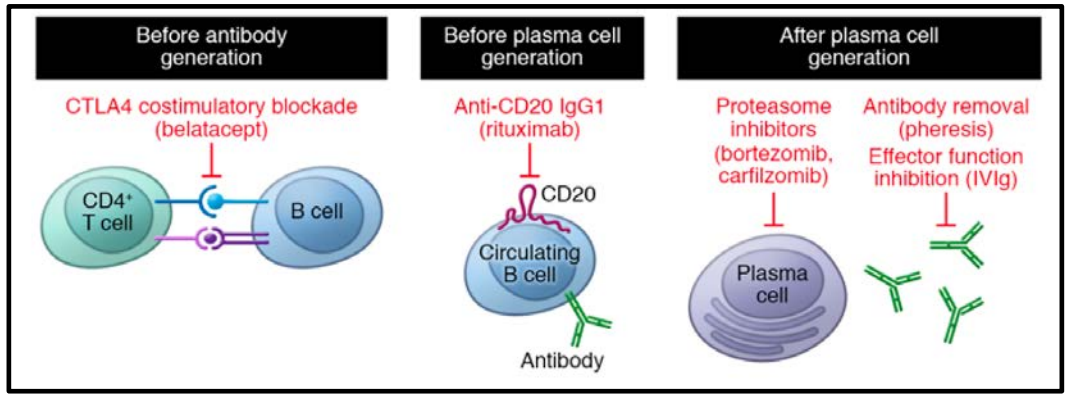
Michael Keller, M.D.,[a,b,c] Junfeng Sun, Ph.D.,[b] Cedric Mutebi, B.S.,[a] Pali Shah, M.D.,[c] Deborah Levine, M.D.,[d] Shambhu Aryal, M.D.,[e] Aldo Iacono, M.D.,[f] Irina Timofte, M.D.,[f] Joby Mathew, DPT,[c] Anu Varghese, MBBS,[f] Cassandra Giner, B.S.,[d] and Sean Agbor-Enoh, M.D., Ph.D.[a]

Negative Predictive Value of dd-cfDNA = 96.5%

Case #2: *de novo* DSA → Are You OK?

- 67yo F with ILD s/p right single lung transplant 15 months ago. Pre-transplant allo-sensitized with HLA class II DR52 Ab (crossmatch negative), though undetectable post-transplant.
- Post-transplant course is unremarkable.
- Developed COVID19 and treated with Remdesevir and Dexamethasone. Discharged to inpatient rehab due to deconditioning.
- Now returns to clinic for f/u. FEV1 and FVC are down 7% from prior. DSA screen with DR52 with MFI= 4800. Does not bind C1q.
- **Do you treat this DSA?**

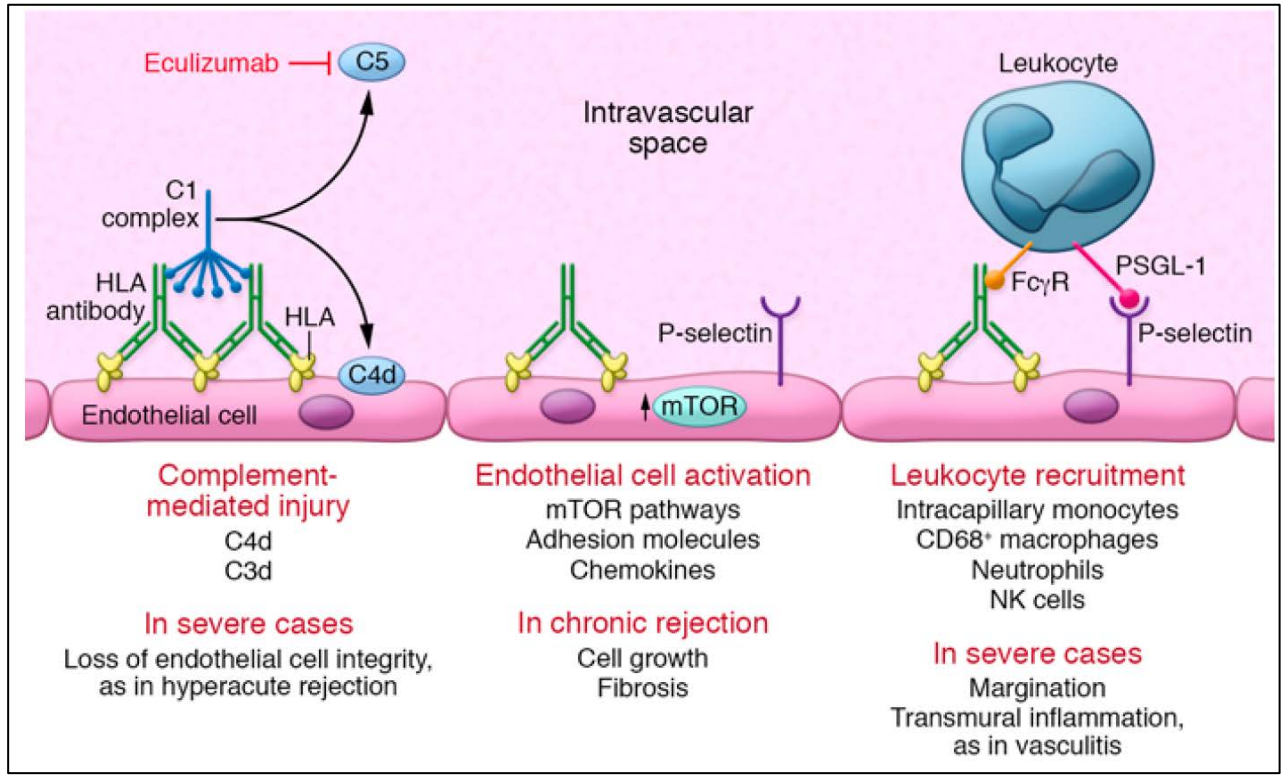
Antibody Mediated Rejection Results in Endothelial Damage



1) CD4-T cells activate immature B cells

2) Activated B-cells then differentiate into plasma cells

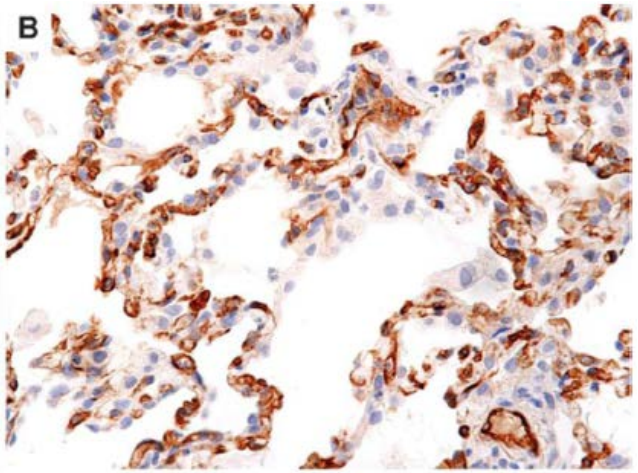
3) Plasma cells = “factories for Ab production”



Complement-binding Abs cause endothelial cell activation, leukocyte recruitment and binding and injury.

Valenzuela and Reed. JCI 2017

The Clinical Diagnosis of AMR in Lung is… Difficult



AMR Diagnosis Depends On:

1) Allograft Dysfunction
2) de novo DSA
3) Complement Activation (C4d)
4) Capillaritis

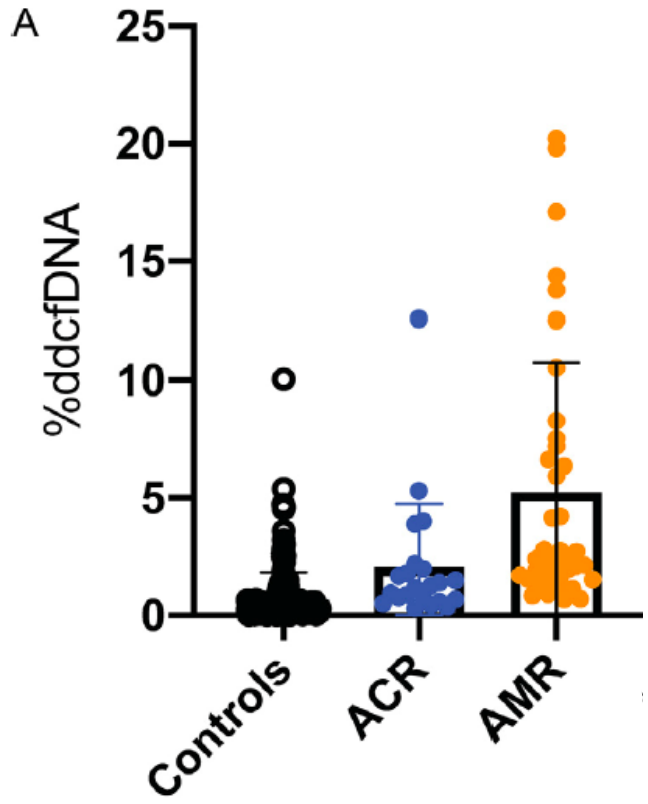
Table 1 Definition and Diagnostic Certainty of Clinical Pulmonary Antibody-mediated Rejection

	Allograft dysfunction	Other causes excluded	Lung histology	Lung biopsy C4d	DSA
Definite	+	+	+	+	+
Probable[a]	+	+	+	–	+
Probable	+	+	+	+	–
Probable	+	+	–	+	+
Probable	+	–	+	+	+
Possible	+	+	+	–	–
Possible	+	+	–	–	+
Possible	+	+	–	+	–
Possible	+	–	+	+	–
Possible	+	–	+	–	+
Possible	+	–	–	+	+

DSA, donor-specific antibodies; +, item present; –, item absent or missing.
[a]There is building evidence that antibody-mediated rejection can be diagnosed confidently in the absence of positive C4d staining, hence this group is recognized separately.

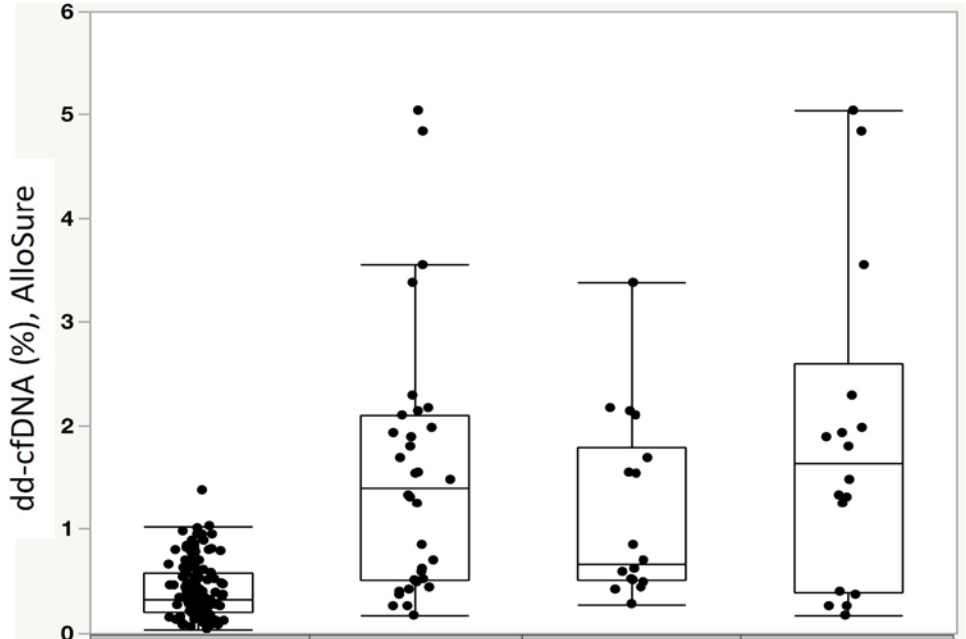
Levine, et al. JHLT (Consensus Statement) 2016

%dd-cfDNA Can Distinguish AMR Diagnostic Sub-Classes



Jang, et al. JHLT 2021

Figure 1B. Donor derived cell-free DNA Results by AMR Diagnostic Certainty



	Normal	Total AMR	Possible AMR *(subgroup)*	Probable AMR *(subgroup)*
	n = 148	n = 34	n = 18	n = 18
Median	0.32%	1.41%	0.66%	1.64%
IQR	0.21, 0.58	0.50, 2.11	0.51, 1.79	0.25, 2.61

Both *p*-values between possible and probable AMR versus normal were <0.0001

Levine DJ, et al. Am J. Transplant 2022;22 (suppl 3)

Case #3: When the Sniffles Can Kill

- 65yo F with ILD s/p left single lung transplant 6-months prior, undergoes for-cause bronchoscopy for fever, rhinorrhea, mild dyspnea. FEV1 unchanged.

- Bronchoscopy results:
  - Anastomoses intact
  - Thin airway secretions in periphery.
  - BALF= 80% macs, 3% pmns, 17% lymphs
  - AXB0 → No ACR on 4 pieces.

- The next day BAL fluid is positive for parainfluenza type III virus

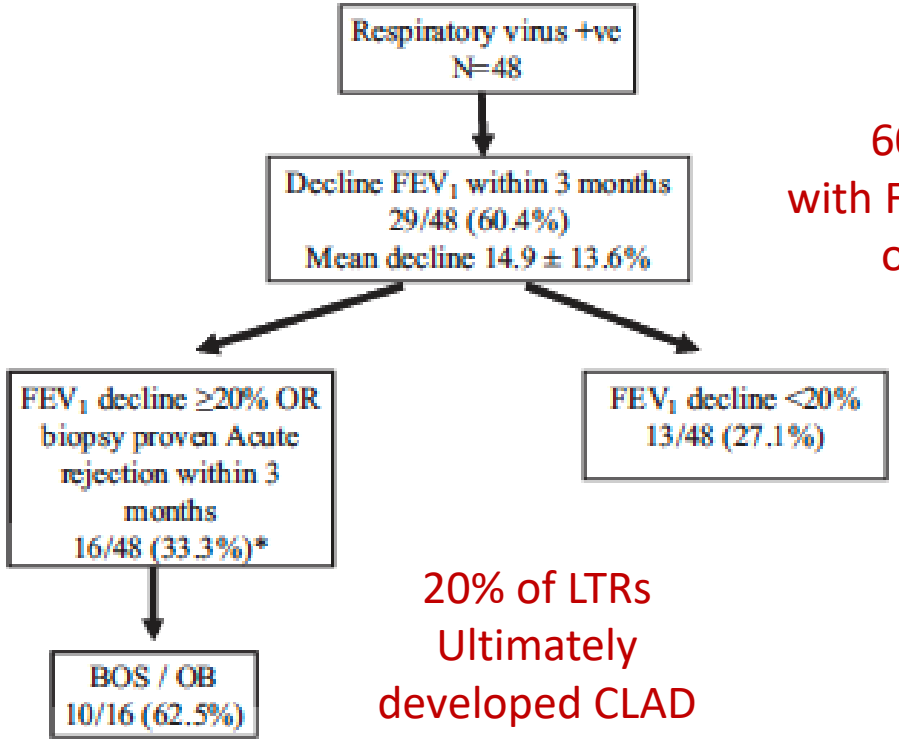
- How do you manage this patient?

Respiratory Virus Infection is a Risk Factor for Allograft Injury

A Prospective Molecular Surveillance Study Evaluating the Clinical Impact of Community-Acquired Respiratory Viruses in Lung Transplant Recipients

Deepali Kumar,[1,8] Shahid Husain,[2,8] Maggie Hong Chen,[3] George Moussa,[4] David Himsworth,[5] Oriol Manuel,[6] Sean Studer,[7] Diana Pakstis,[7] Kenneth McCurry,[7] Karen Doucette,[1] Joseph Pilewski,[7] Richard Janeczko,[5] and Atul Humar[1]

Transplantation 89(8), 2010.



60% LTRs with FEV1 decline of ~15%

20% of LTRs Ultimately developed CLAD

Clin Transplant 2013: 27: E64–E71 DOI: 10.1111/ctr.12054
© 2012 John Wiley & Sons A/S.
Clinical Transplantation

Rhinovirus and other respiratory viruses exert different effects on lung allograft function that are not mediated through acute rejection

Sayah, et al. Clinical Transplantation 2013

+ 59 lung transplant recipients at UCSF 6/2009-6/2011

+ 4-10 weeks following RVI had TBBx to assess for ACR

+ Incidence of post-CARV ACR= 8.9% (same as control)

+ CARV associated with -6.4% decline in FEV1.
  Rhinovirus = -4.6% decline
  Non-rhinovirus= -8.8% ($p < 0.05$)

%dd-cfDNA Can Predict Patients that Develop CLAD induced by CARV

ORIGINAL ARTICLE

AJT

Elevated cell-free DNA in respiratory viral infection and associated lung allograft dysfunction

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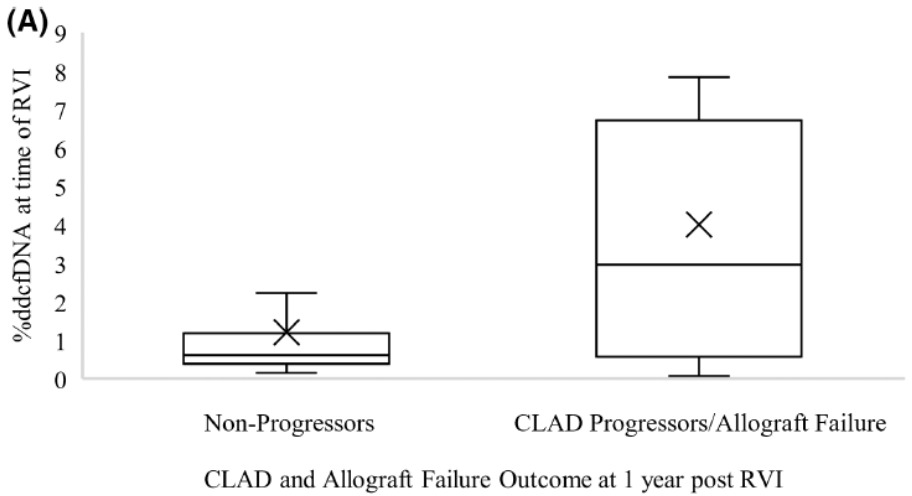
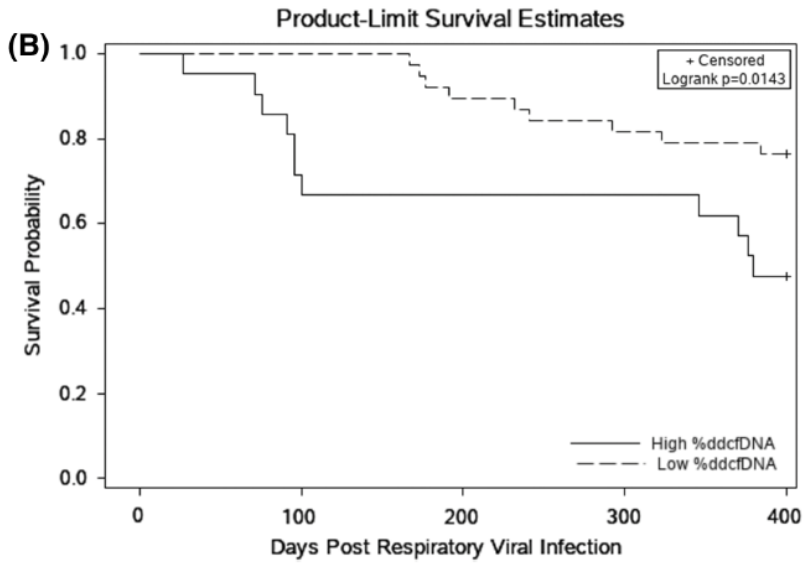
American J Transplantation 2022



CLAD and Allograft Failure Outcome at 1 year post RVI



+ 39 patients in GRAfT Cohort with RVI followed for 1 year

+ %dd-cfDNA assessed by shotgun sequencing within 7 days of infection

+ CLAD (spirometry) and allograft survival assessed by based on high (>1%) vs. low %dd-cfDNA (<1%)

Case #3: When the Sniffles Can Kill

How do you manage this patient?

Case #4: FEV1 is Declining → CLAD vs. LARD?

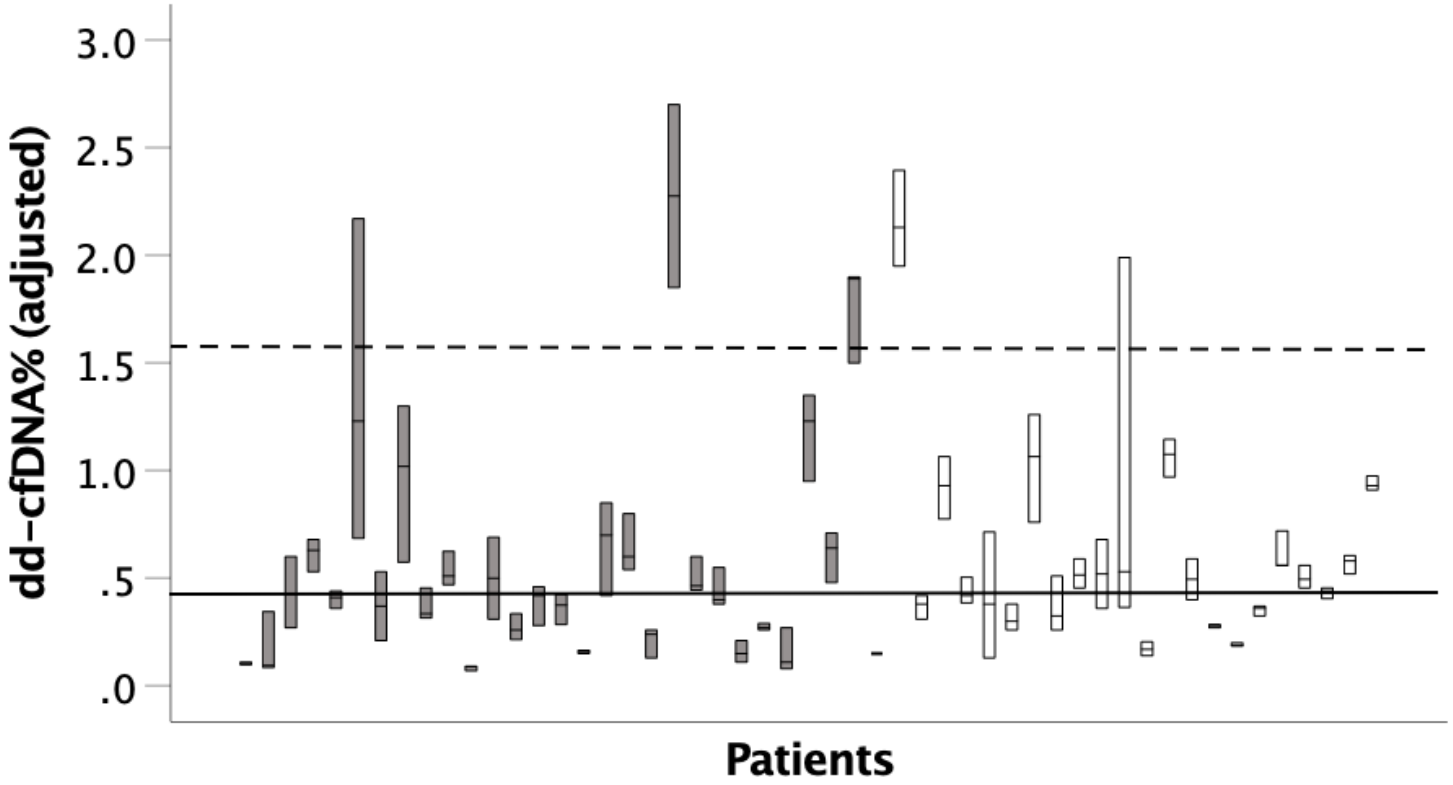
- 72yo M with COPD s/p bilateral lung transplant 6 years ago.
  With history of ACR x 2 (treated with pulse steroids), GERD s/p Nissen, and an episode of CMV viremia, which resolved with GCV. FEV1= 85% of post-transplant peak average (on Azithromycin).

- Has not been seen in past 1 year due to COVID pandemic and patient's anxiety.

- Home spirometry "stable". Patient feels "out of shape".

- Weight now increased by 20lbs. FEV1 and FVC have declined by 12%.
  CT chest with bibasilar atelectasis, otherwise no changes.

What Happens with dd-cfDNA Over Time?



In a prospective study of 51 patients > 2-years post-lung transplant that were clinically stable:

Median %dd-cfDNA=
0.45
(0.26 – 0.69)

95th percentile= 1.54%
CV_I= 26%
CV_G= 47%
Reference change value= 73%

Trindade, et al. Transplantation Direct 2022 (Accepted)

Can dd-cfDNA be a Biomarker for CLAD?

Objectives

1) ALAD and CLAD limit lung allograft survival

2) dd-cfDNA: Evidence in lung transplantation &
How I use dd-cfDNA in the clinic

3) Future directions for dd-cfDNA in lung transplantation

dd-cfDNA to Assess for ACR Recovery

Follow-Up Transbronchial Biopsy is Important to Assess Treatment Response

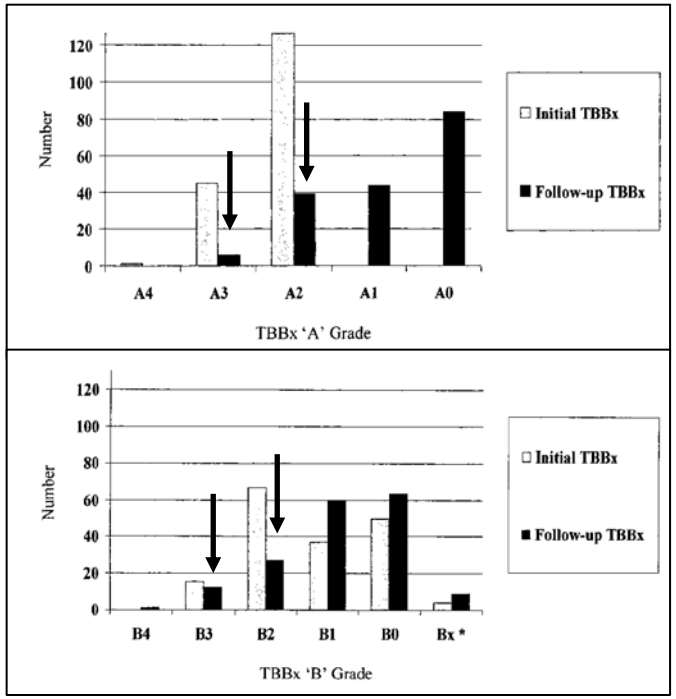
Index Diagnosis	Follow-up Diagnosis	Number
Acute cellular rejection Grade 2 (n = 96)	No infection or rejection	52
	Acute cellular rejection	29
	Grade 2	13
	Grade 3	10
	Grade 4	1
	With miscellaneous	4
	Clinical	1
	Chronic rejection	2
	Chronic and acute rejection	1
	Diffuse alveolar damage	3
	Lymphocytic bronchitis	3
	CMV pneumonia	2
	Miscellaneous	4

Guilinger, et al. AJRCCM 1995

+ Single-center retrospective study (Pittsburgh)
+ Bronchoscopy performed 2-6 weeks after therapy.
+ Incidence of persistent ACR was 30%.

Aboyoun, et al. AJRCCM 2001

+ Single-center retrospective study (Sydney, Australia)
+ 99 patients with ≥A2 ACR had follow-up TBBx within **45 days**.
+ Persistent ≥A2 ACR seen in 26%
+ Persistent ≥B2 ACR seen in 22%



The ~25% of patients with steroid-refractory ACR may have unmitigated inflammation for **4-6 weeks**

Transbronchial Biopsies are NOT Benign

TABLE III Complications of procedures

Complication	Percentage of biopsies
Pneumothorax	1.46
Bleeding >100 ml	4
Assisted ventilation	0.32
Arrhythmia	0.57
Death	0
Overall	6.35

Hopkins et al., JHLT 2002

2017 Medicare National Average Payment (www.cms.gov)

In-Office	In-Facility	Hospital Outpatient	ASC
$338	$163	$1,270†	$569
$359	$183	$2,431†	$1,117

Patient "Costs"

+ Travel Expenses
+ Lost time / revenue
+ Procedure Anxiety

Obviate the need for follow-up TBBx?

Can %dd-cfDNA be a biomarker of ACR resolution following therapy with steroids?

Allow for earlier detection of non-responders to ACR therapy (GC)?

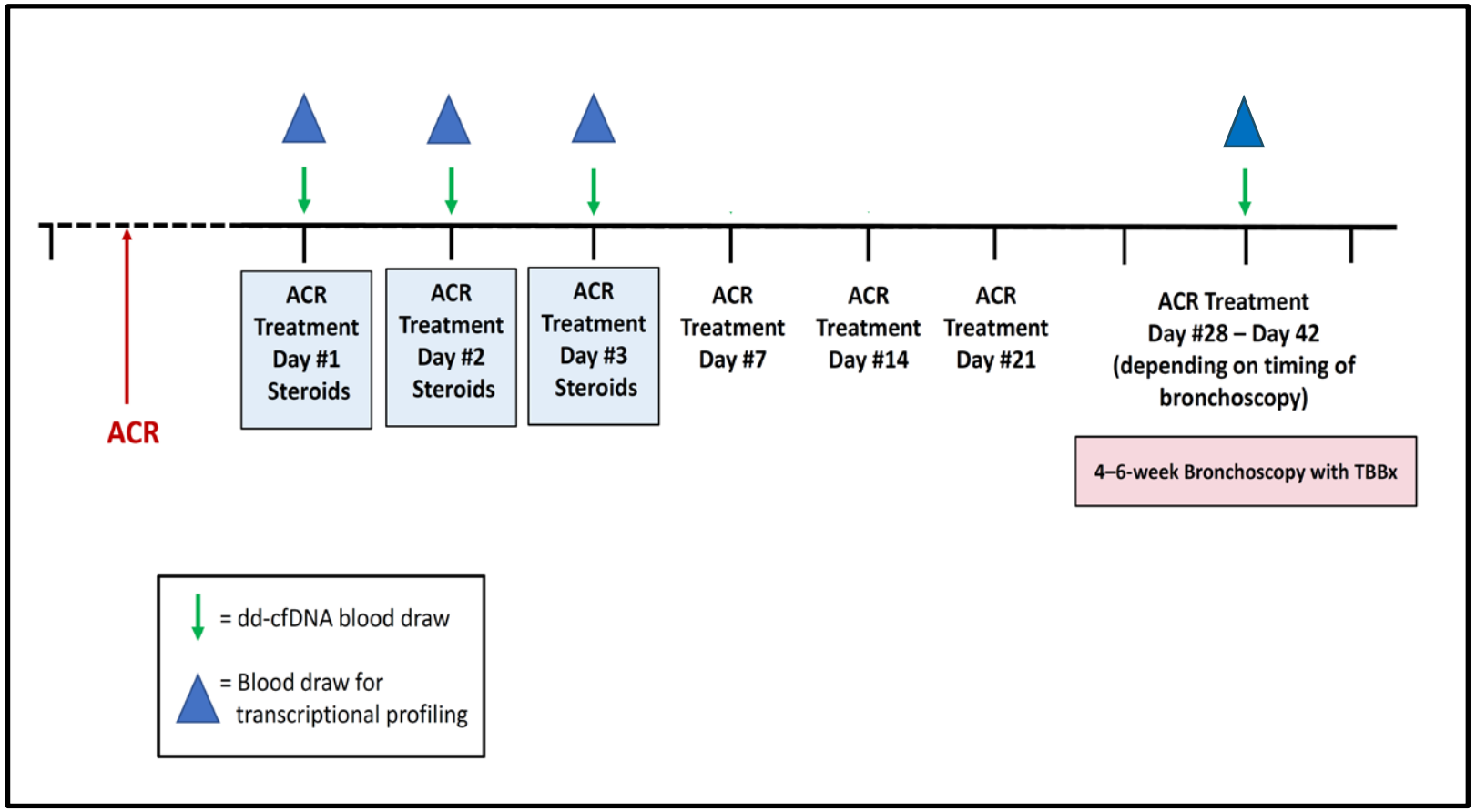
Specific Aims for Pilot Study:
dd-cfDNA as a Biomarker for ACR-recovery

1) To determine %dd-cfDNA levels in patients with histologic resolution of ACR.

2) To assess whether early dd-cfDNA kinetics can discriminate between glucocorticoid responders vs. non-responders following therapy for ACR.

3) Compare transcriptional (miRNA and mRNA) profiles between GC responders and non-responders within the first few days following ACR therapy.

Study Design:
dd-cfDNA as a Biomarker for ACR-recovery



Aim 1: dd-cfDNA as a Biomarker for ACR-Recovery

Diagnostic ability of 1% dd-cfDNA
as a threshold to exclude ACR

Assuming:

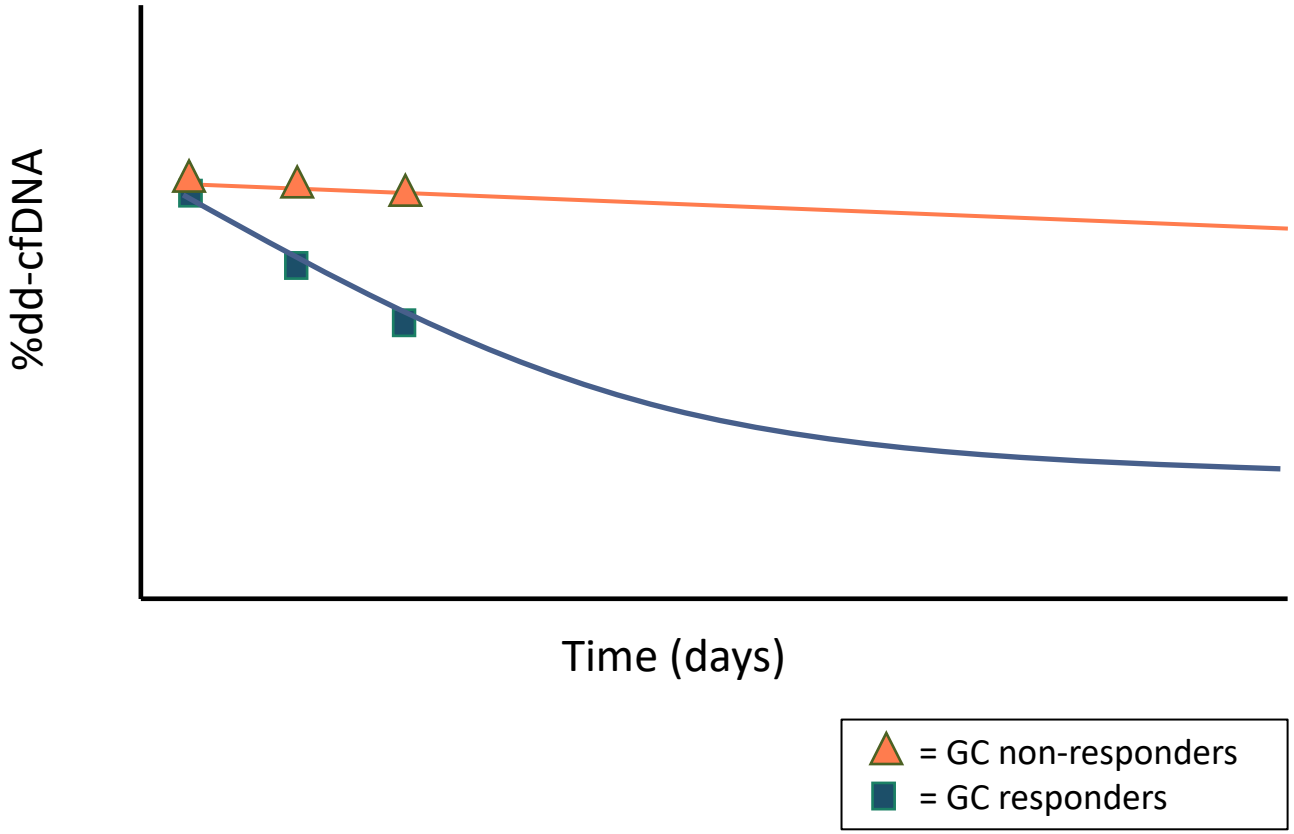
1) a 30% rate of GC-refractory ACR
2) %dd-cfDNA sensitivity for ACR of ~80%

Biopsy-Proven ACR	Dd-cfDNA < 1%	Dd-cfDNA > 1%	
Resolved	11	3	14
Unresolved	1	5	6
Total	12	8	20

Compare median %dd-cfDNA between biopsy resolved and unresolved ACR using a Wilcoxon Rank Sum test.

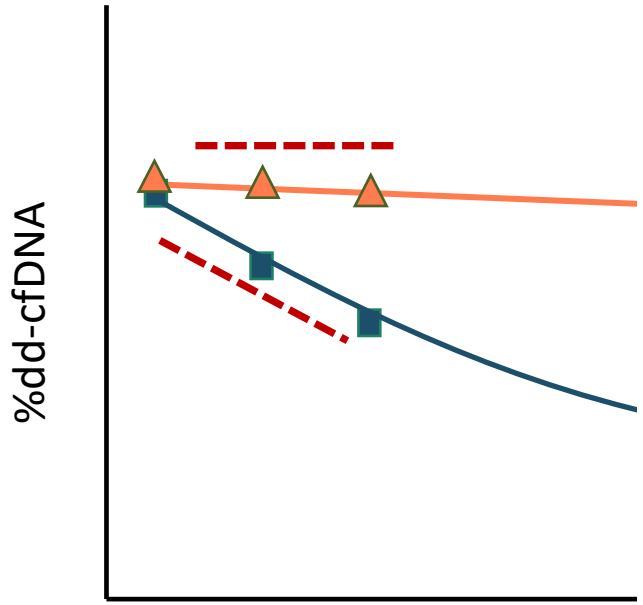
Aim 2: dd-cfDNA decline to Distinguish Early GC-Response

Slope of EARLY dd-cfDNA% Decay Between Glucocorticoid Responders and Non-responders



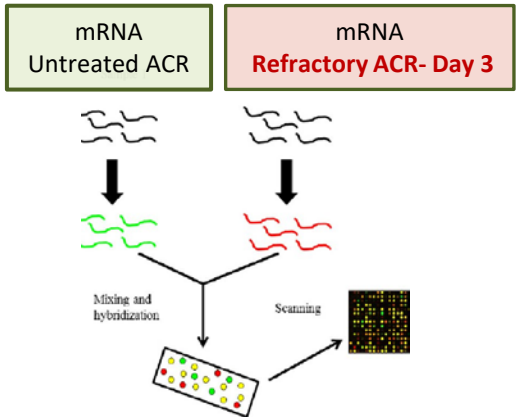
△slope within first 3-days of treatment (pulse steroids) will be compared between biopsy-proven GC responders and GC- non-responders.

Aim 3: Whole Blood Transcriptional Profiling to Identify Early Inflammatory Pathways Associated with (GC)-responsiveness in ACR

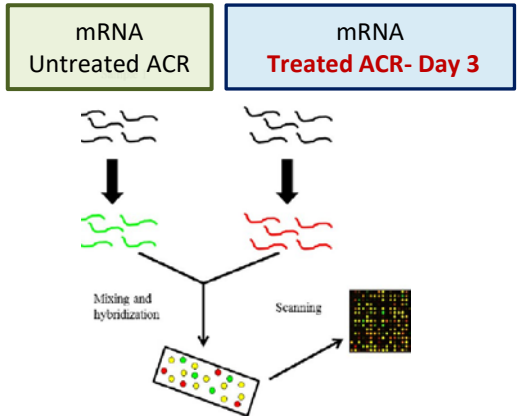


= GC- non-responders
= GC- responders

N =5



N =5



dd-cfDNA to Titrate Immunosuppression

THANK YOU!

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https://www.vanderbilthealth.com/program/lung-transplant