

# cfDNA in Heart Transplantation: A Powerful Biomarker

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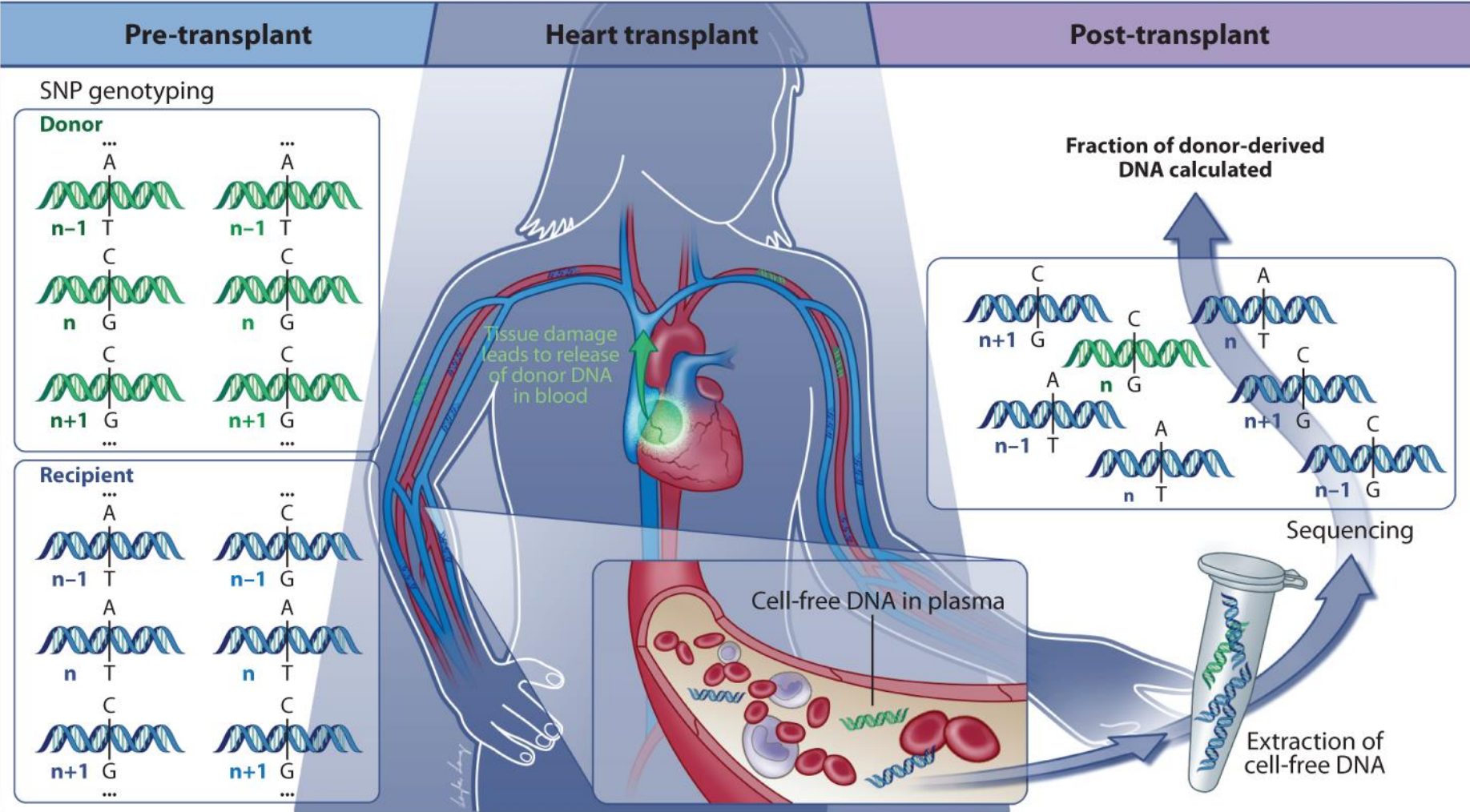
# Faculty / Presenter Disclosure

- Faculty: **Kiran K. Khush, MD, MAS**
- Relationships with commercial interests:
  - Consulting Fees: **CareDx, Inc.**

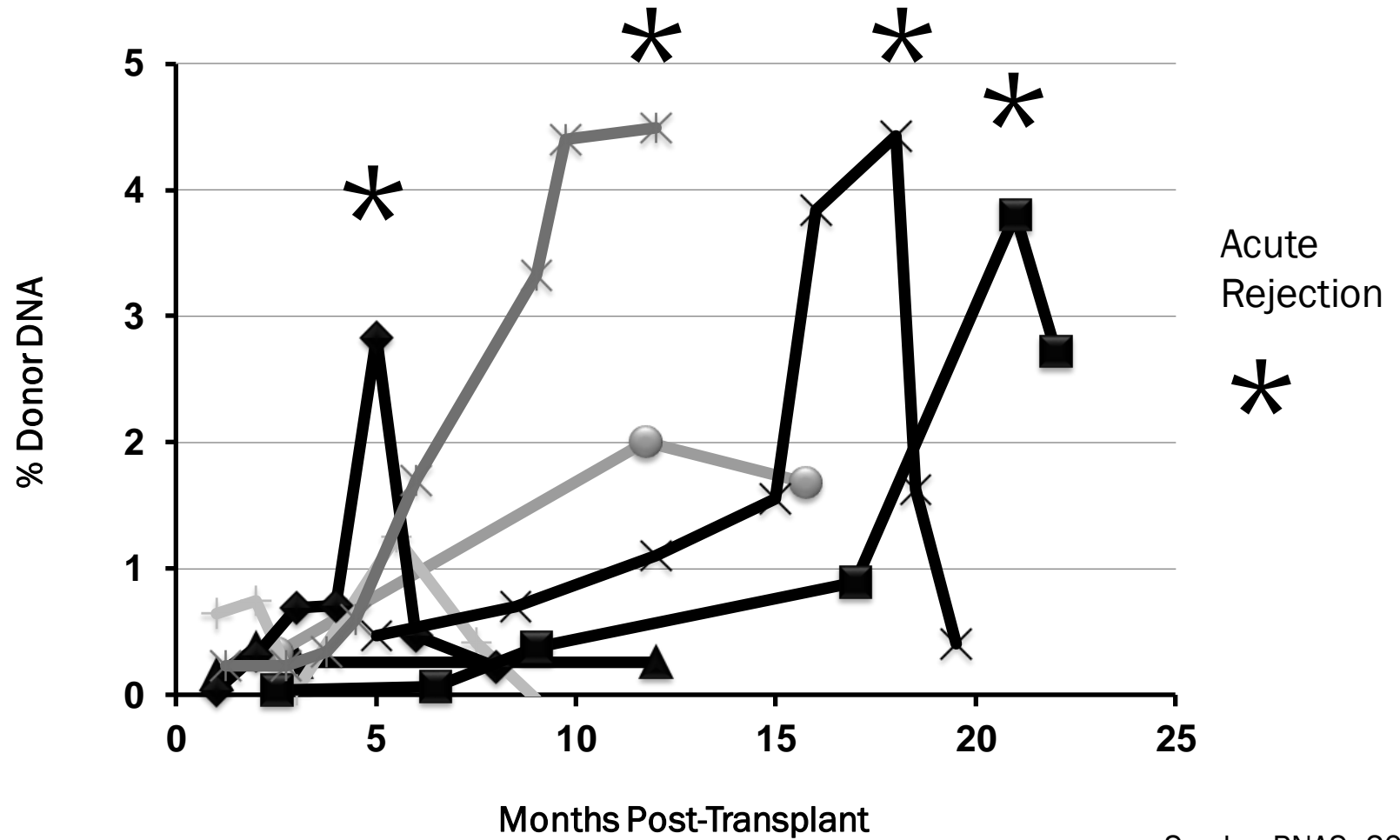
# Mitigating Potential Bias

- Only work that has been published and/or discussed at scientific meetings will be presented.

# Cell free donor DNA as a marker of acute rejection

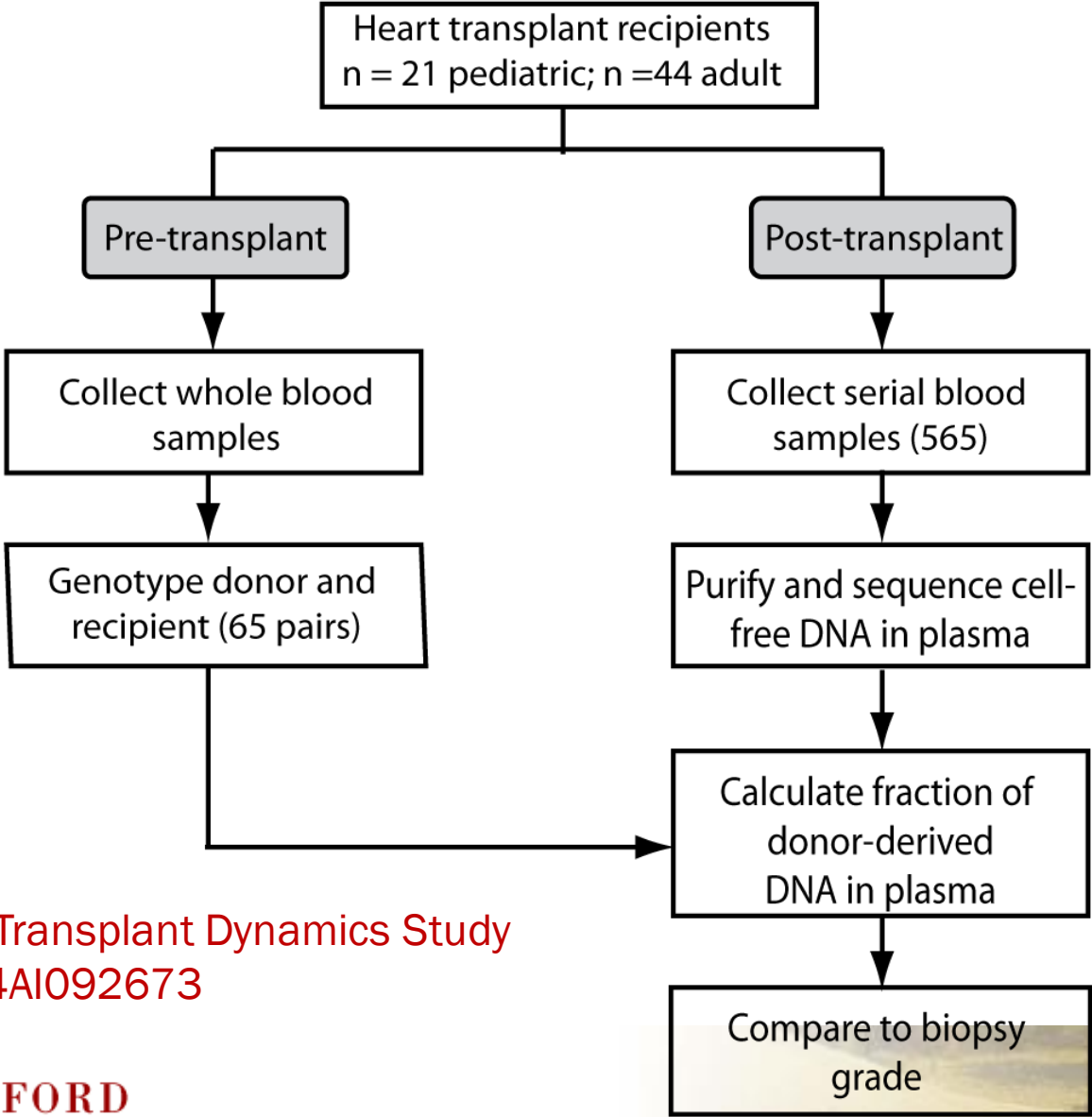


# Preliminary Results: Heart Transplant



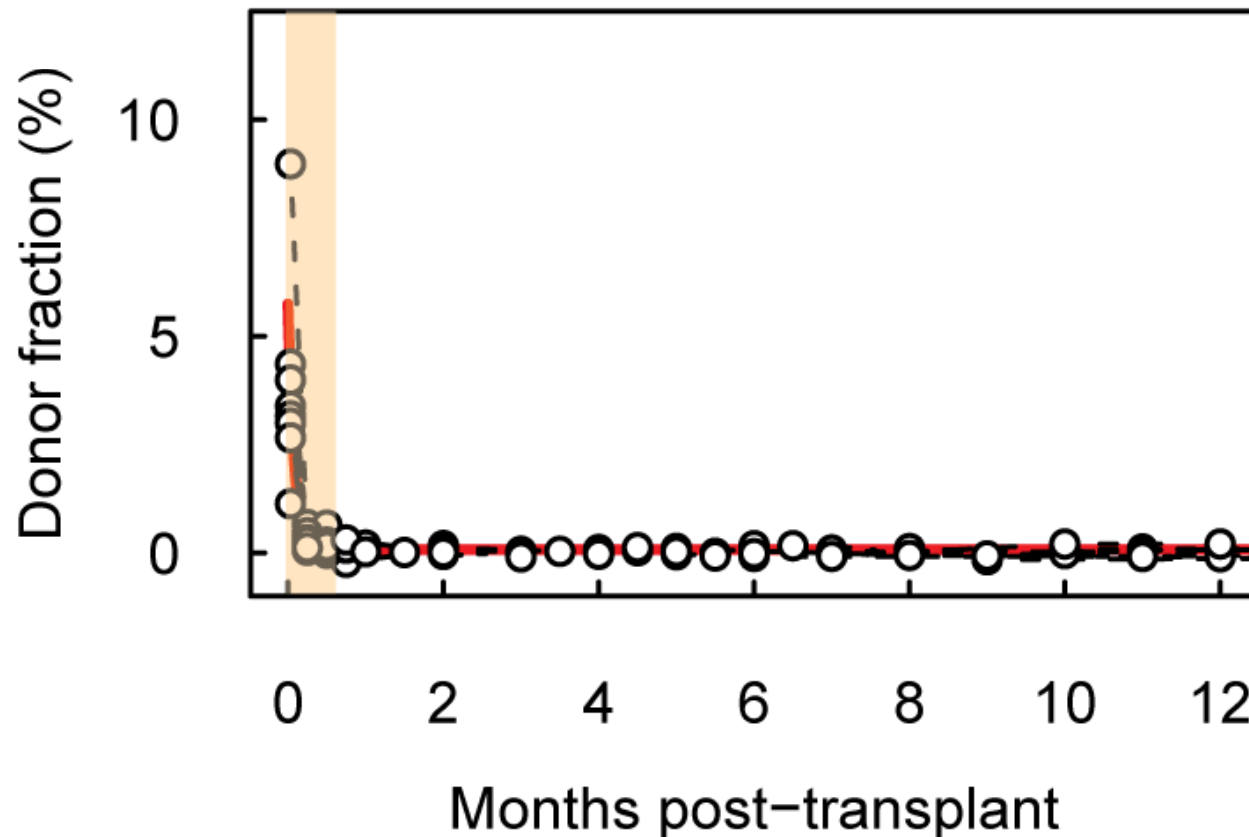
Snyder, PNAS, 2011

# Heart transplant: prospective study design and numbers



Genome Transplant Dynamics Study  
NIH 1RC4AI092673

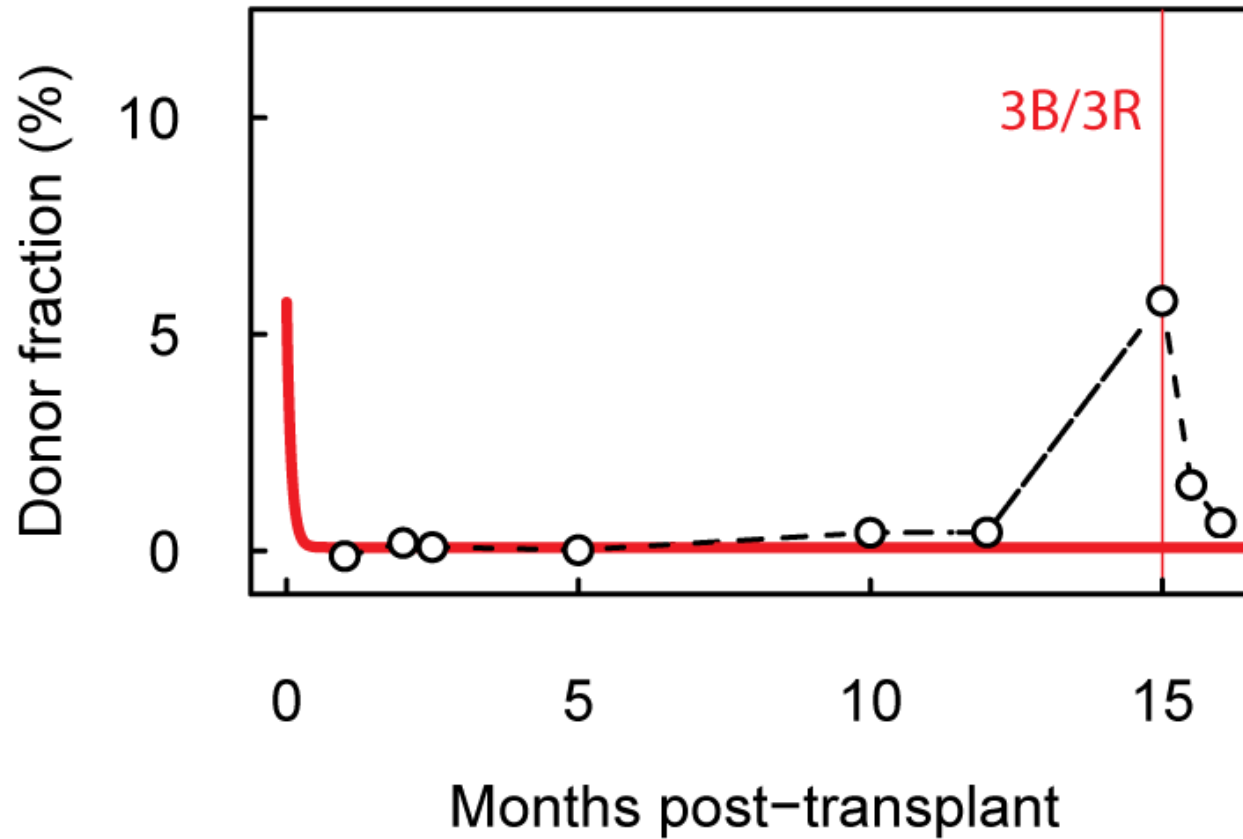
## Dd-cfDNA in the absence of rejection



Elevated signal immediately post transplant followed by a quick decay (decay time 2.4 days) to a low baseline level

DeVlamick, Science Translational Medicine (2014)

## Dd-cfDNA at the time of acute cellular rejection

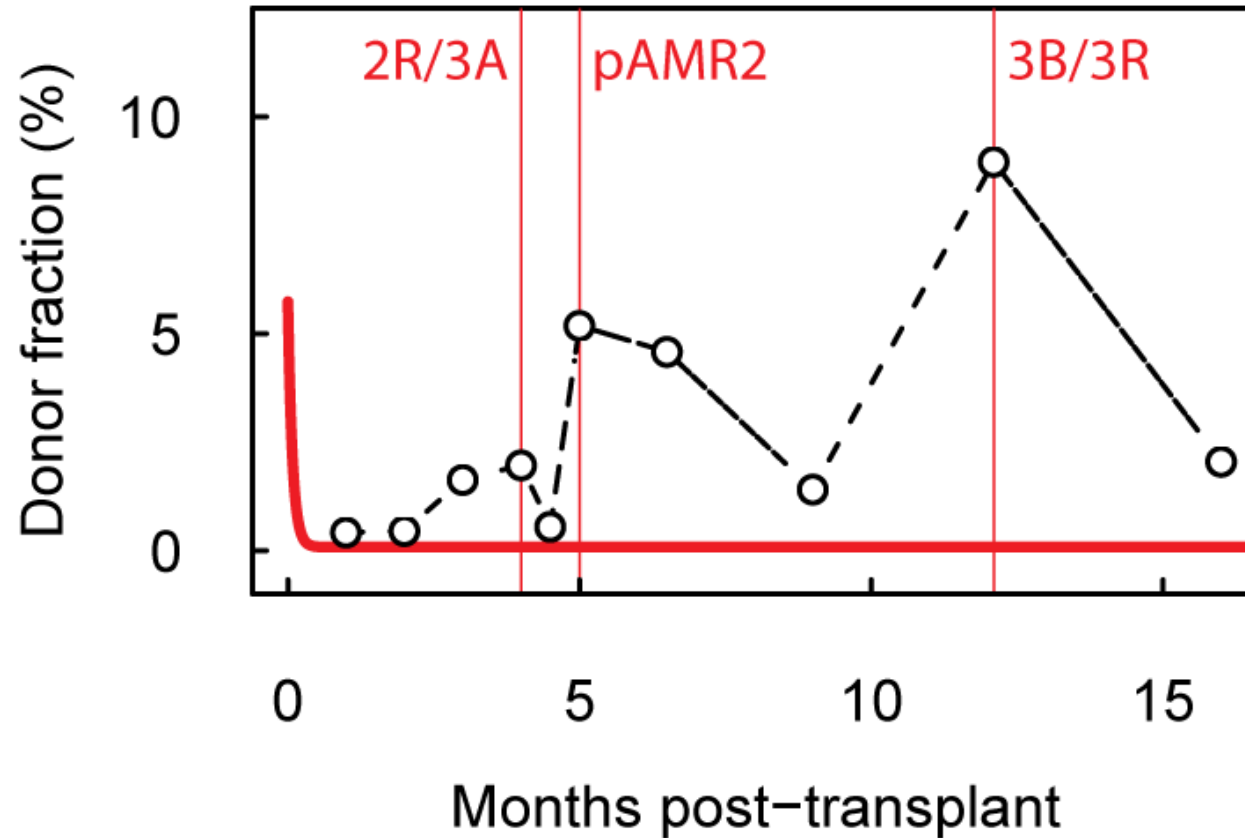


DeVlamick, Science Translational Medicine (2014)



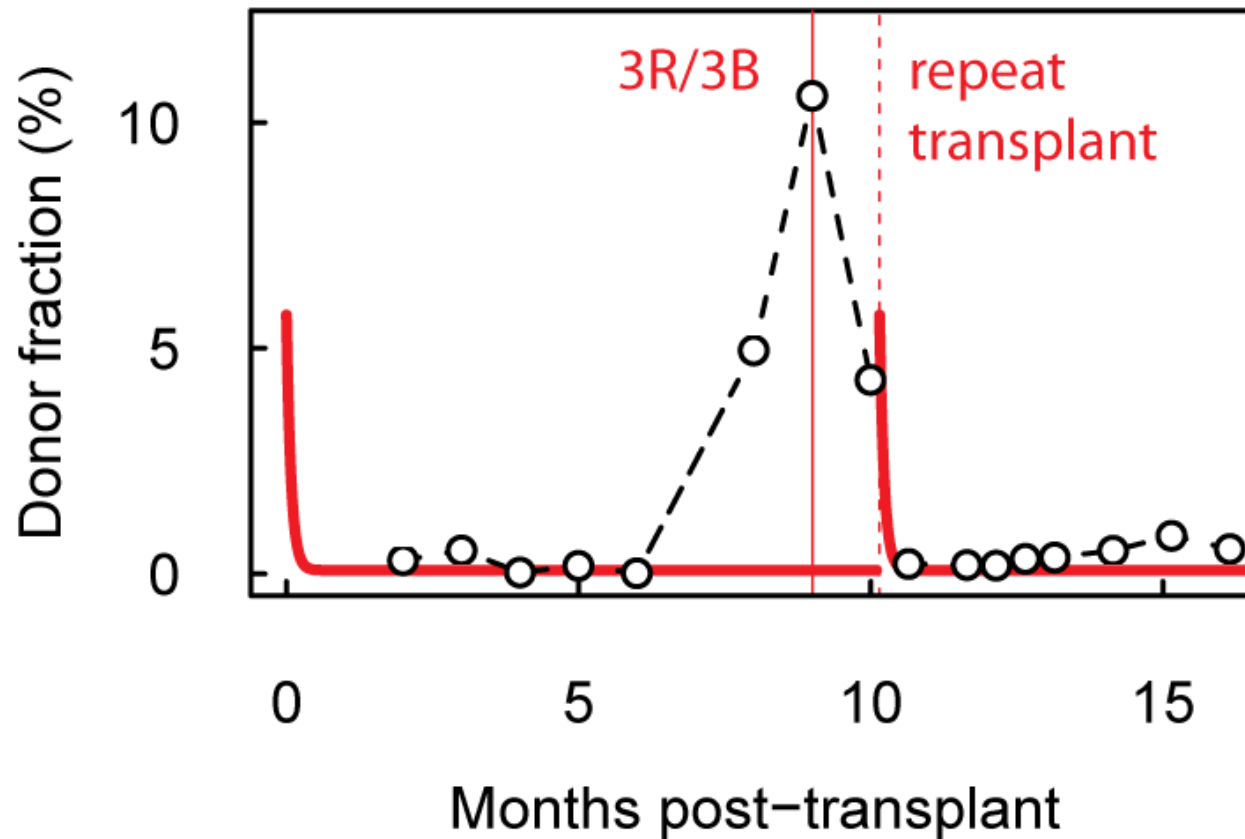


# Dd-cfDNA at the time of acute cellular and antibody-mediated rejection



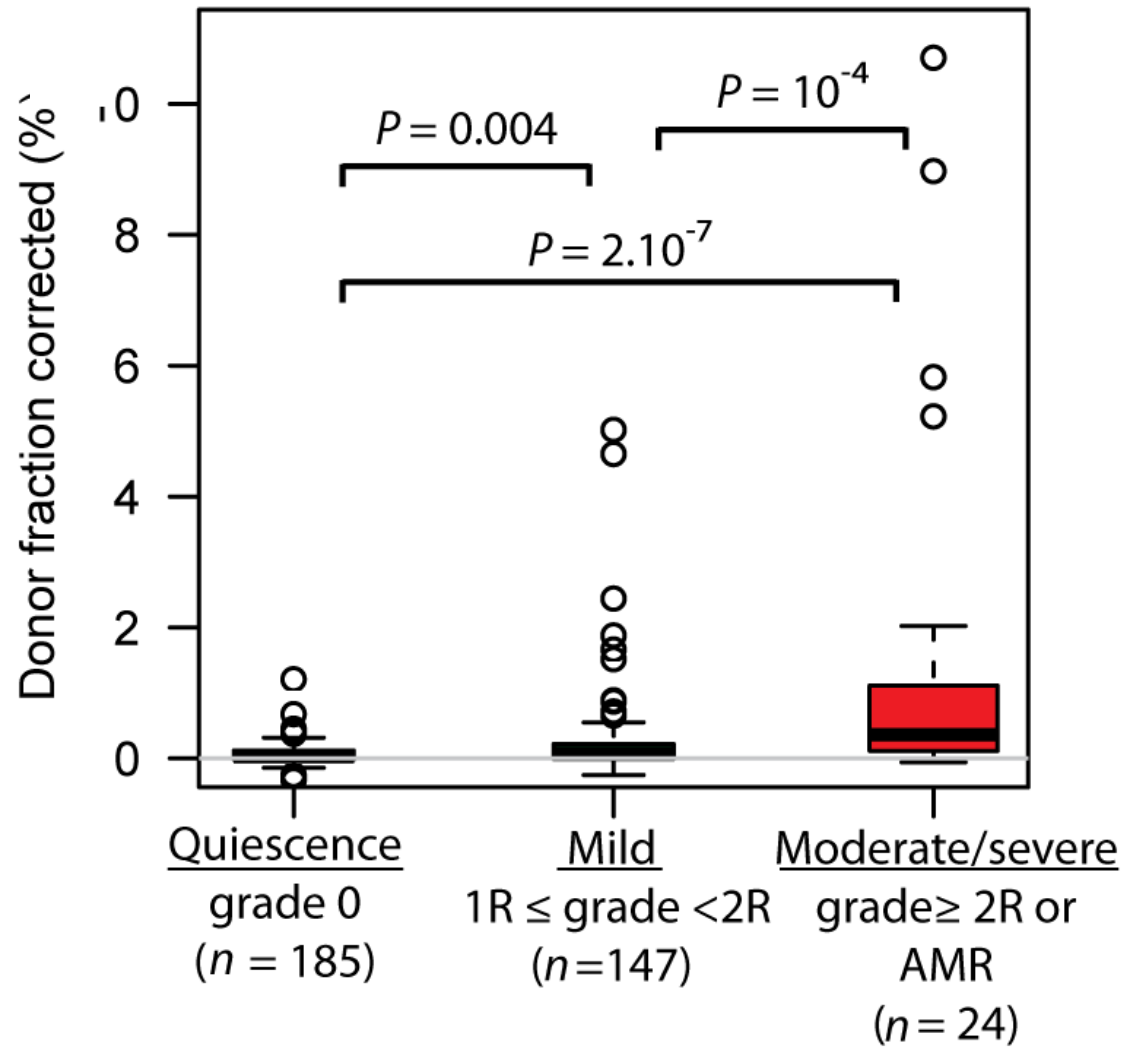
DeVlamick, Science Translational Medicine (2014)

# Dd-cfDNA in a case of graft loss and re-transplantation



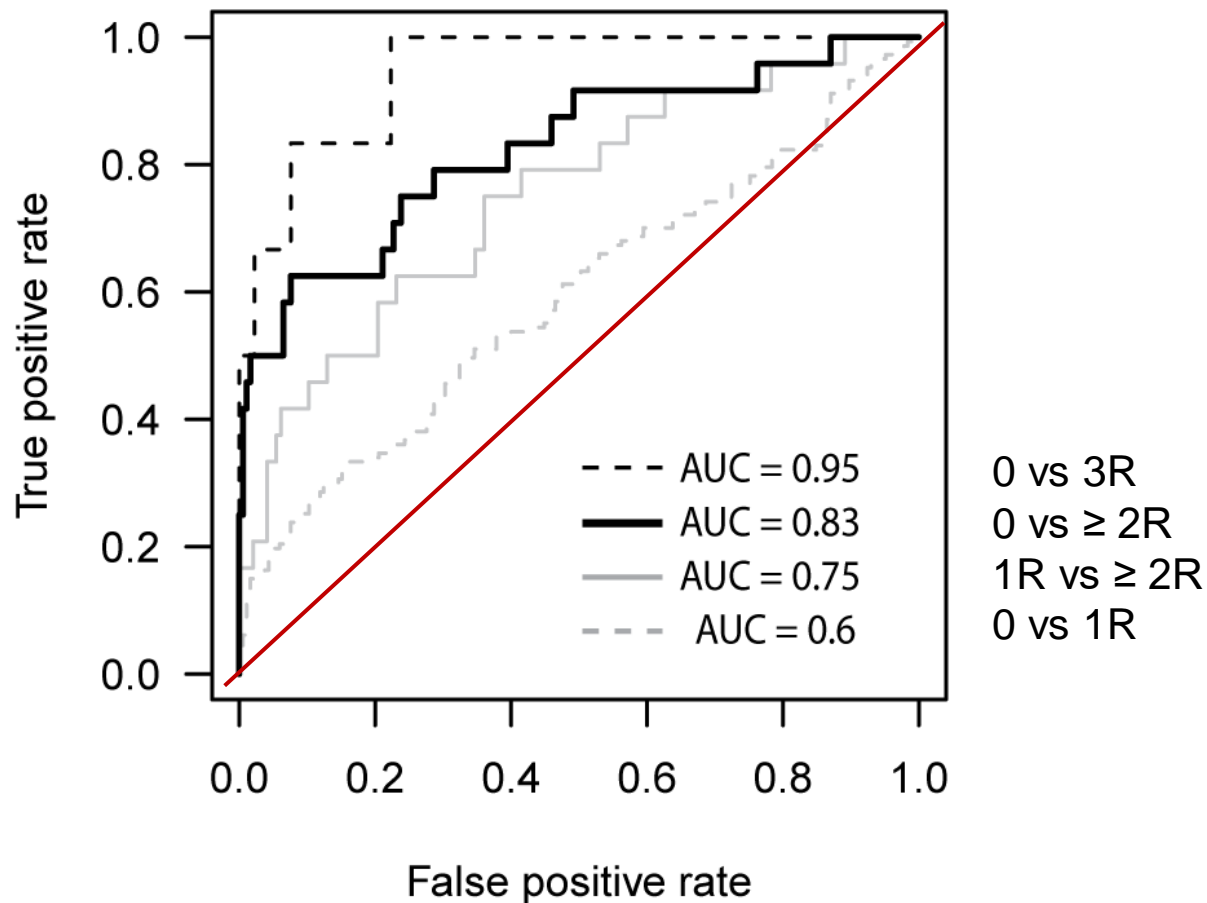
DeVlamick, Science Translational Medicine (2014)

# Dd-cfDNA: analysis of diagnostic performance



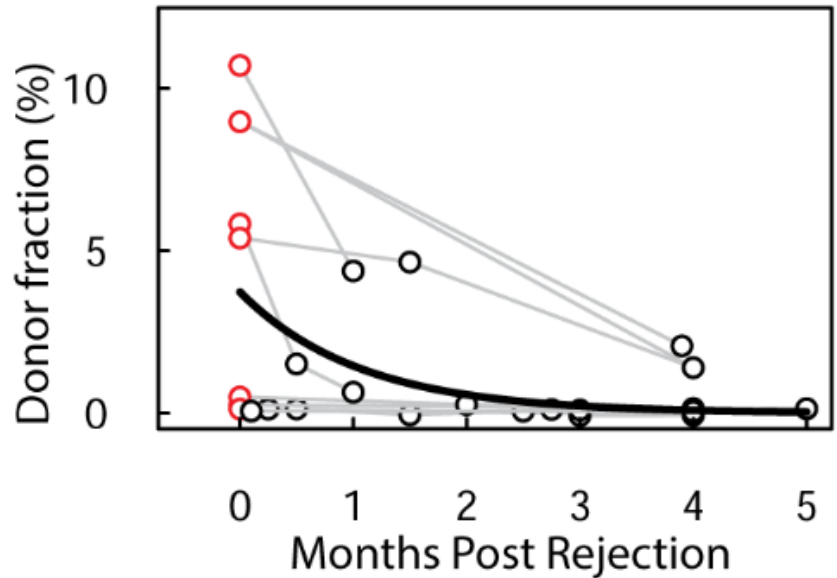
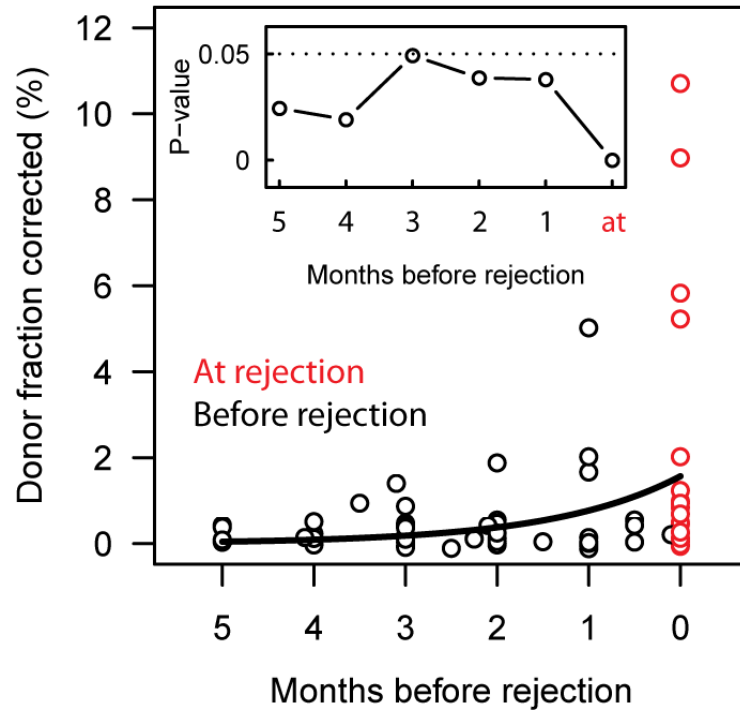
DeVlamick, Science Translational Medicine (2014)

# Analysis of diagnostic performance



DeVlamick, Science Translational Medicine (2014)

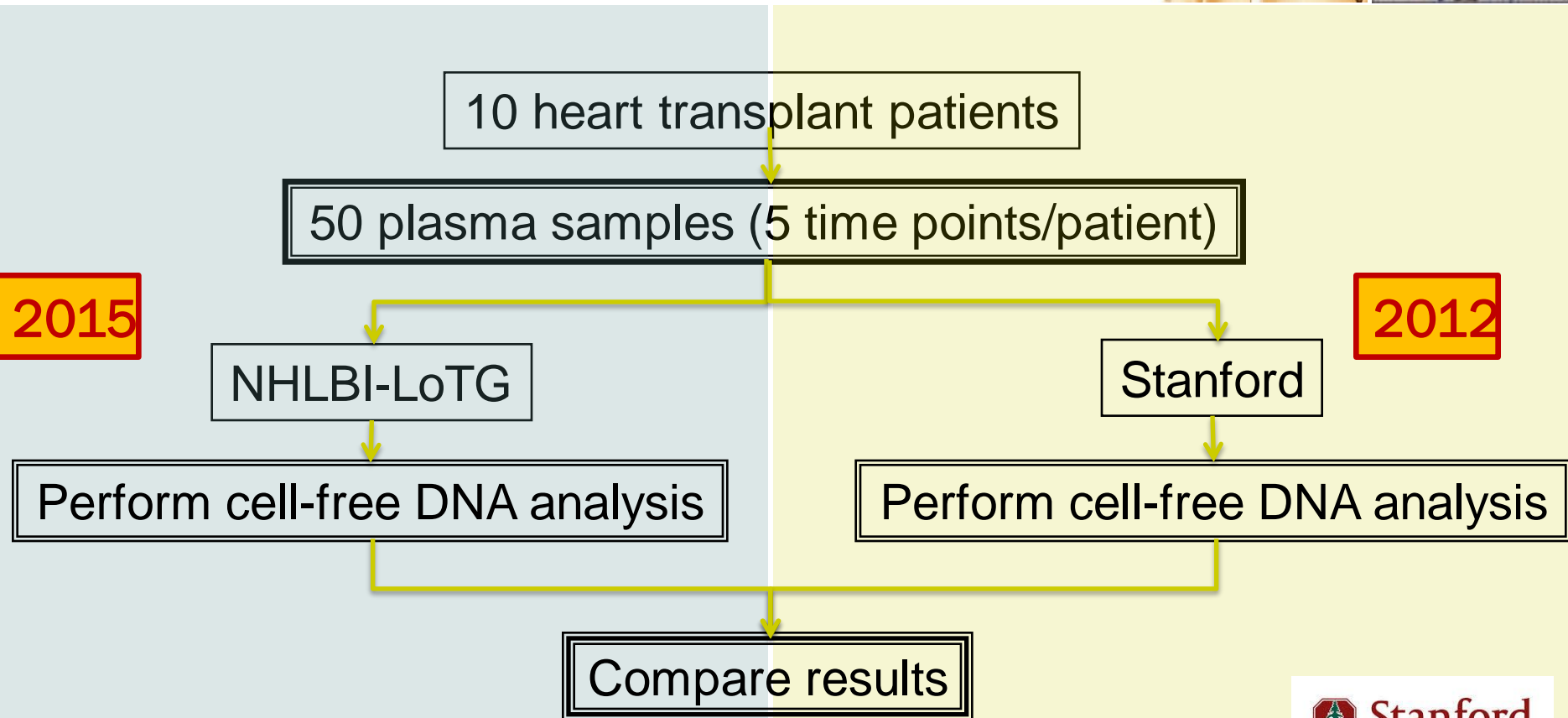
# Early diagnosis and monitoring of therapeutic response



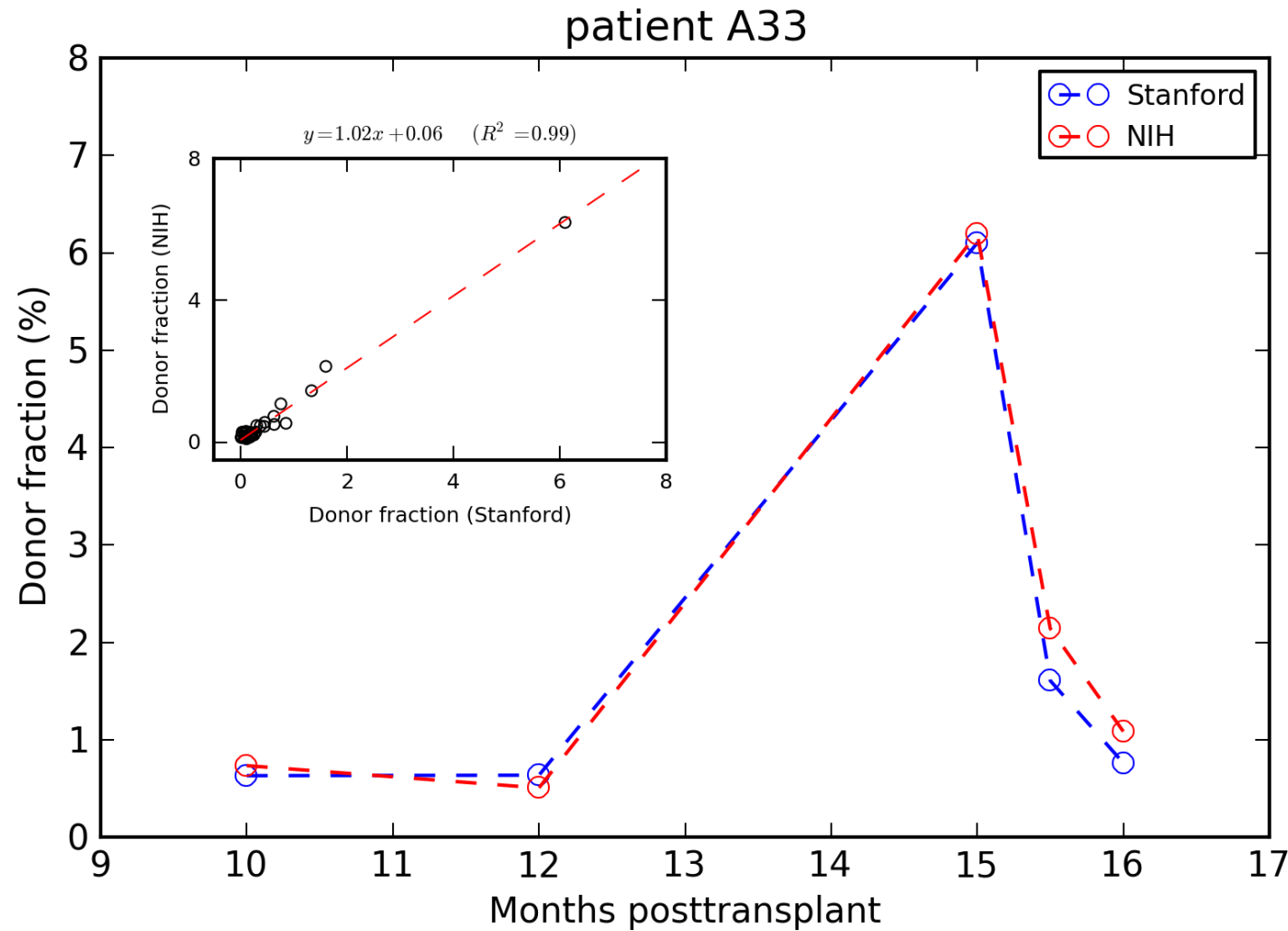
DeVlamick, Science Translational Medicine (2014)

# Replication/Reproducibility Studies

NHLBI - Genomic Research Alliance for Transplantation (GRAfT)



# Replication/Reproducibility Studies



Data from Sean Agbor-Enoh, NIH

# dd-cfDNA: A Rapidly Evolving Technology

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- Technology used in previous studies to measure SNP alleles:
  - Shotgun sequencing methods (Stanford) (1)
  - Targeted amplification (Wisconsin, Chronix) (2)
  - Both requiring recipient AND donor genotypes
- A new approach has been developed (CareDx, Inc: **AlloSure™**) with targeted amplification of SNPs (n=266) that **DOES NOT require genotyping of the donor or recipient** (3)
- “One genome” informatics algorithm (4)

(1) Snyder et al., PNAS 108(15):6229, 2011  
De Vlamincx et al., Sci Transl Med. 6(241):241, 2014

(2) Beck et al., Clin Chem 59:12, 2013  
Hidestrand et al., JACC 63:1224, 2014

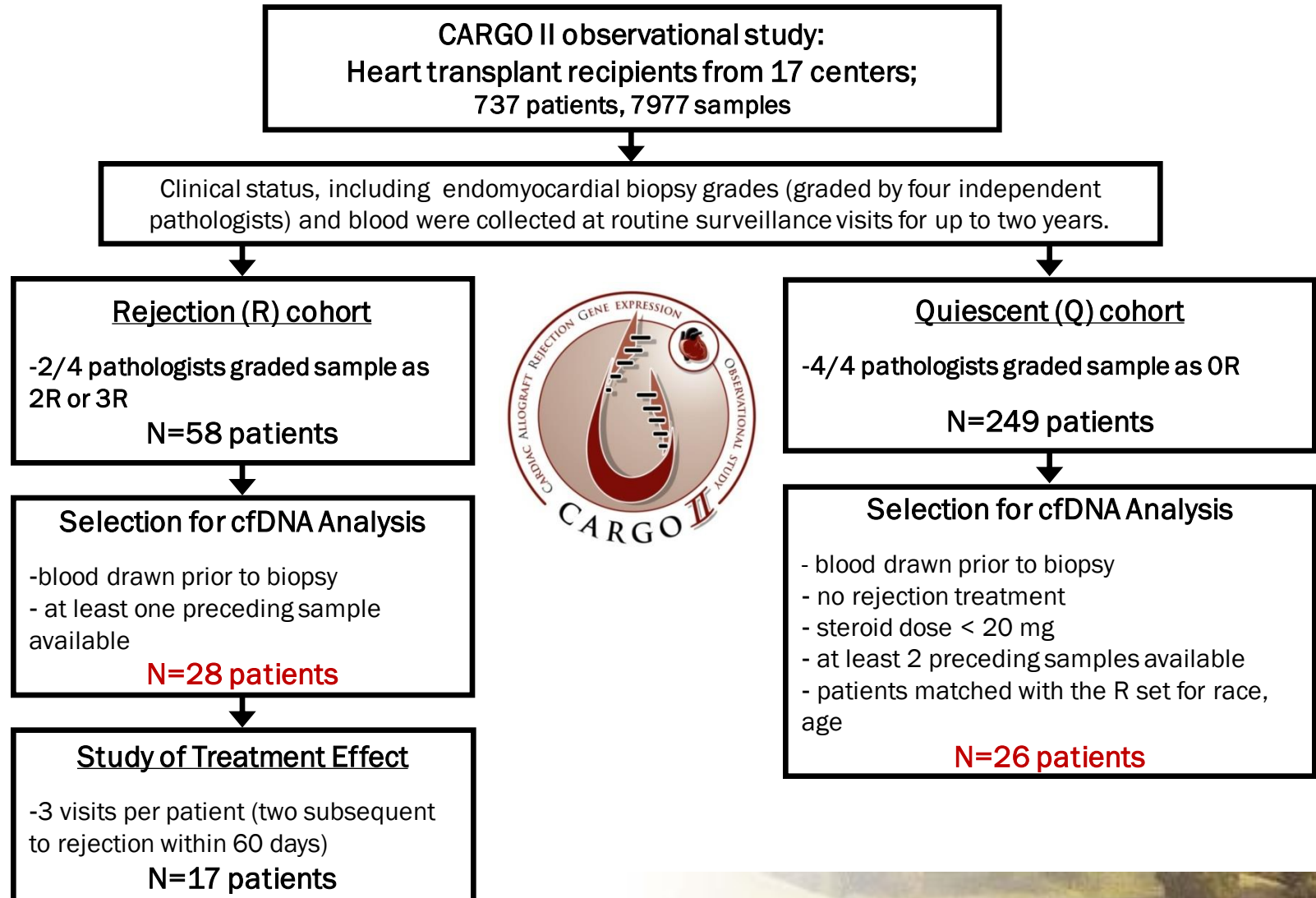
(3) Grskovic et al, Jol Mol Diagnostics, Nov 2016

(4) Sharon et al. *Submitted for publication*

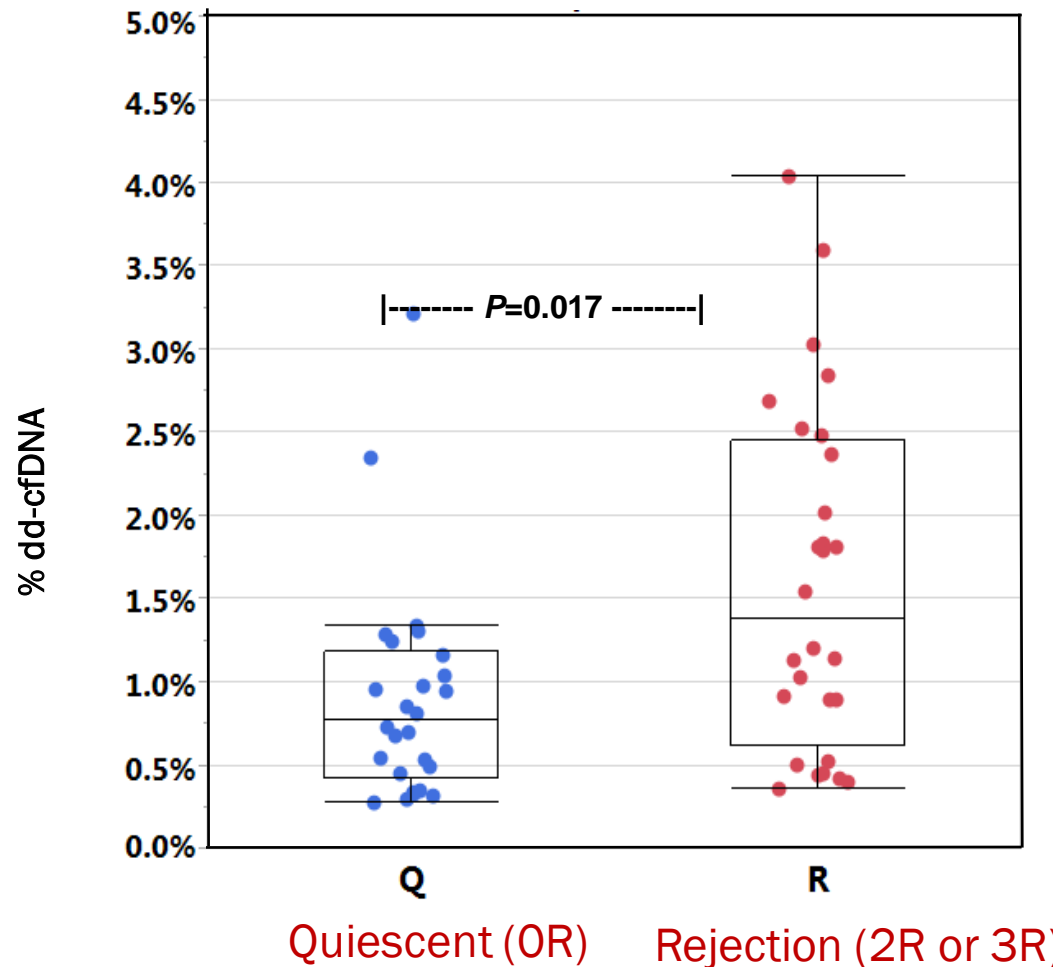




# CARGO II: Retrospective Analysis of dd-cfDNA (AlloSure™) in acute heart transplant rejection

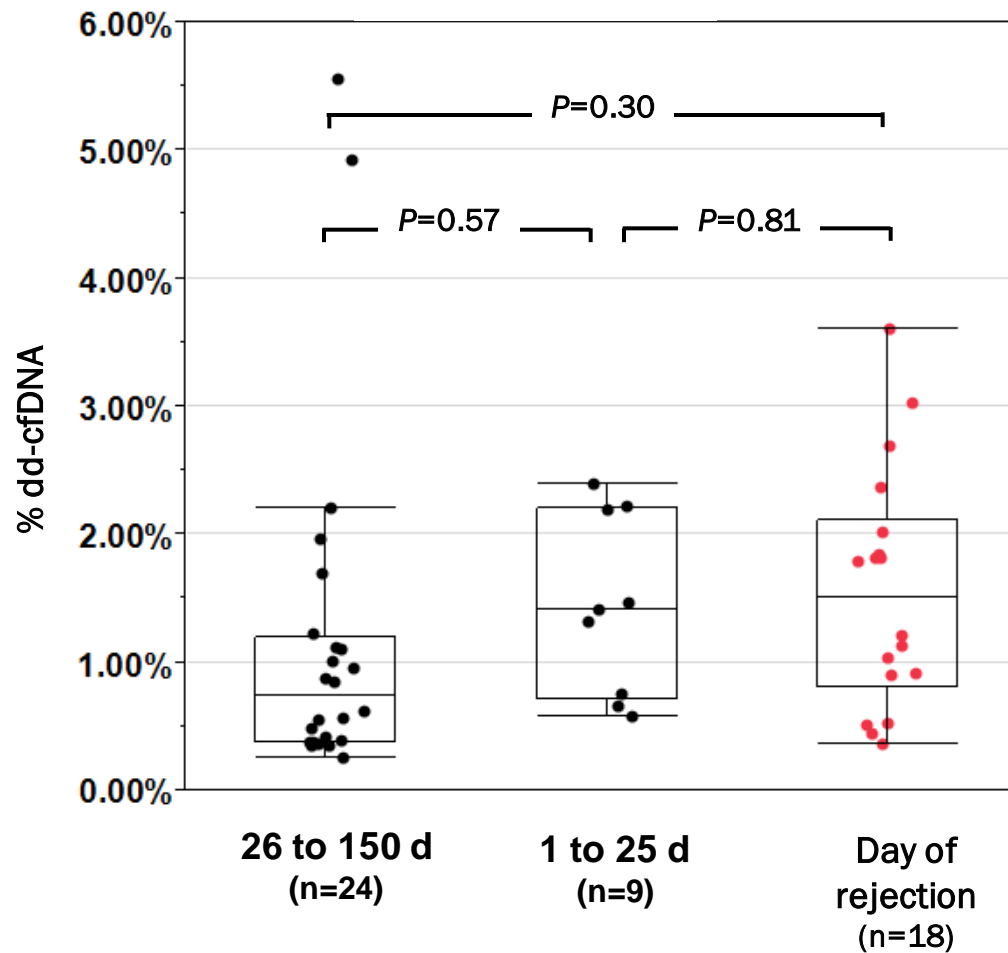


# Increased dd-cfDNA Levels Correlate with Acute Rejection in Heart Transplant Recipients



Crespo-Leiro, ISHLT 2015

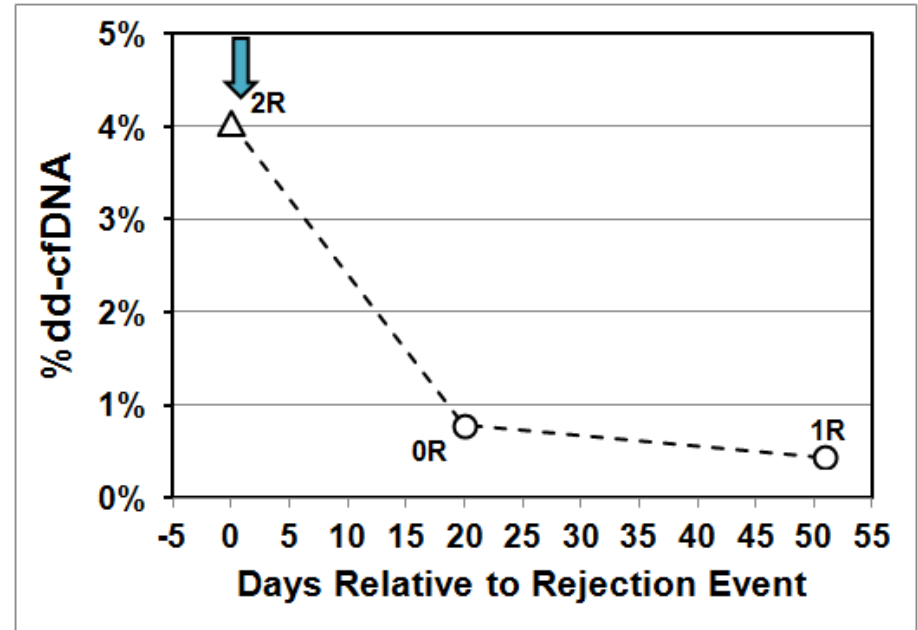
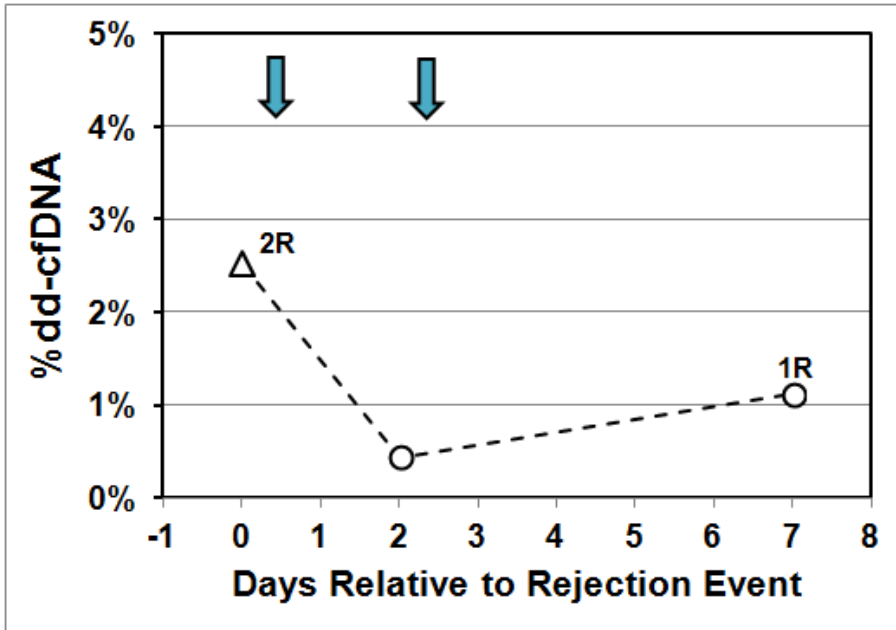
# dd-cfDNA tends to increase within one month prior to acute rejection



Days prior to rejection

# dd-cfDNA Levels Decrease Following Rejection Treatment

↓ = Rejection treatment



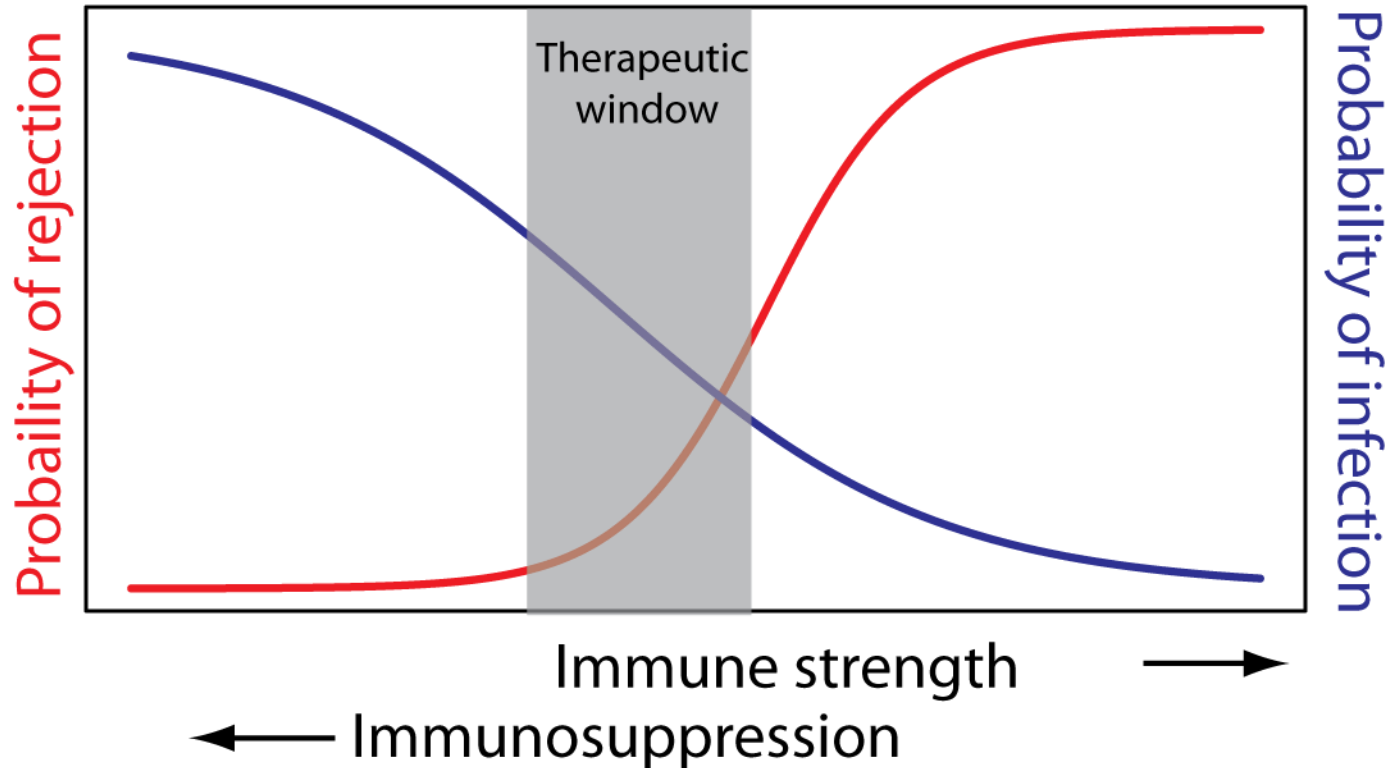
# Conclusions

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- Donor-derived cell-free DNA is an informative **pan-organ non-invasive marker of acute rejection after solid organ transplantation**.
- Dd-cfDNA may enable clinicians to non-invasively **distinguish acute rejection** from other post-transplant complications
- Measurement of serial dd-cfDNA levels may permit **earlier detection of acute rejection**, before graft damage/dysfunction occurs.
- dd-cfDNA levels reliably **fall after treatment** of acute rejection
- Early measurements of dd-cfDNA may identify transplant recipients at risk of **chronic graft injury**



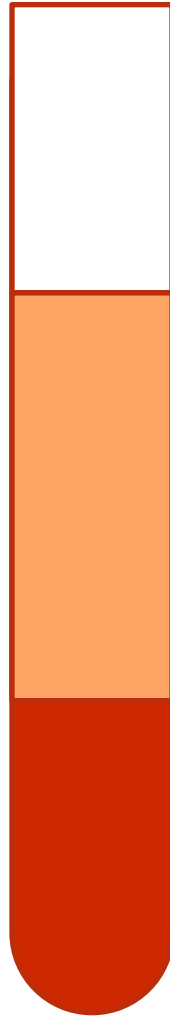
# The challenge of post-transplant therapy



The therapeutic window is narrow, and can **vary between patients**. Sometimes rejection and infection can present in similar ways.

# Non-human DNA is also present in plasma

PERIPHERAL BLOOD FROM  
TRANSPLANT PATIENTS



PLASMA  
CELL-FREE DNA

BLOOD CELLS

DNA FROM THE PATIENT

DONOR DNA

NON-HUMAN DNA

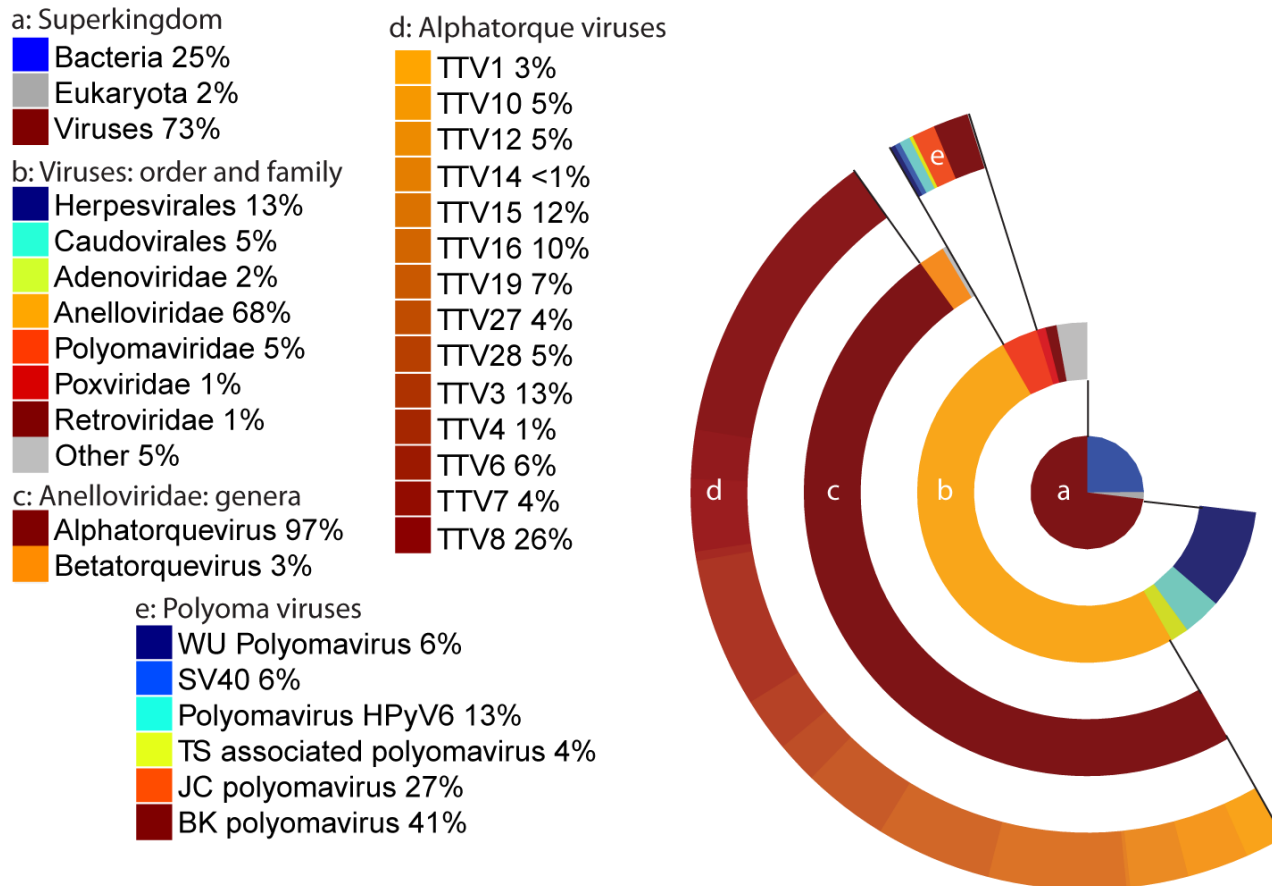
MICROBES

VIRUSES

FUNGI



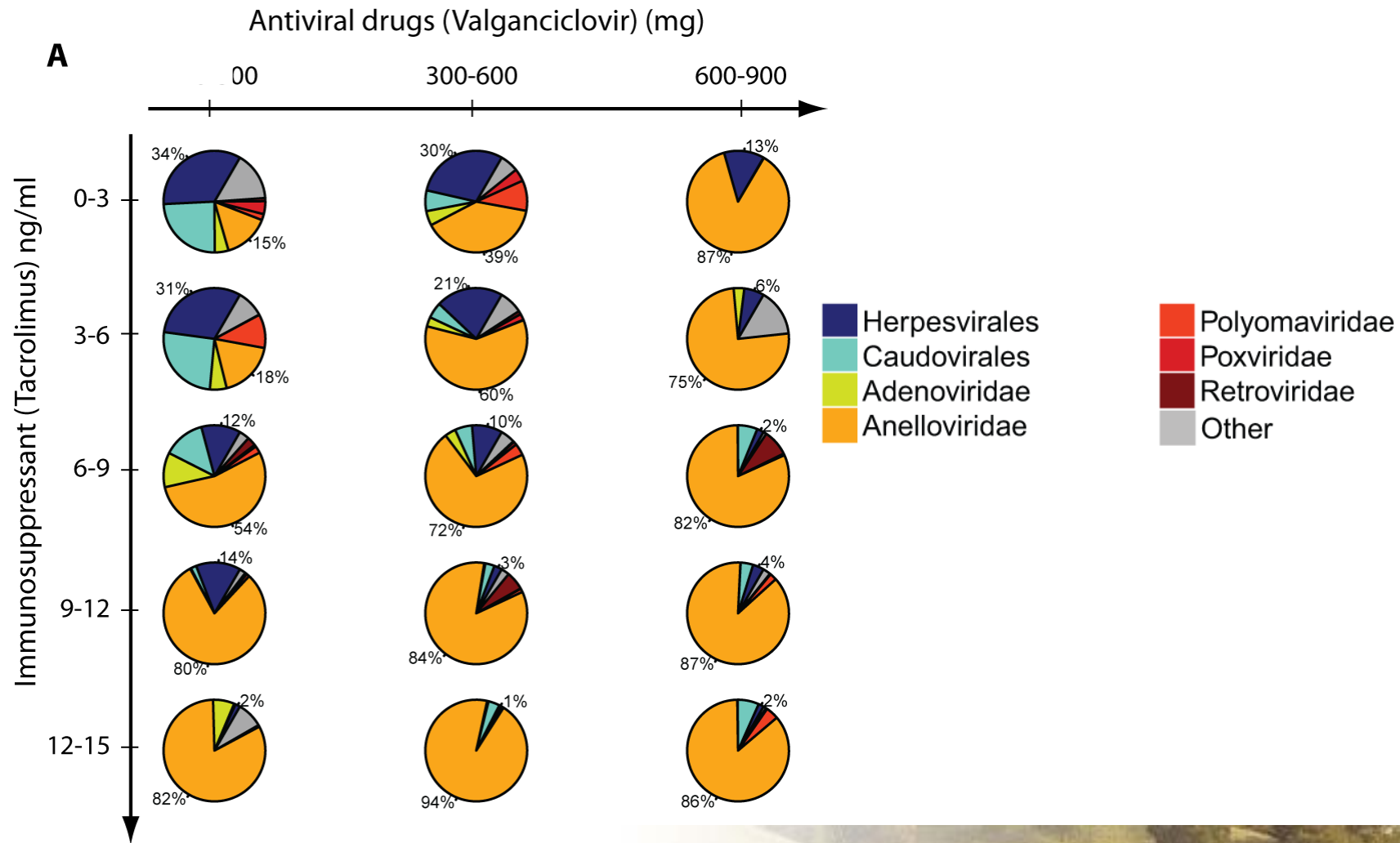
# Relative genomic abundance



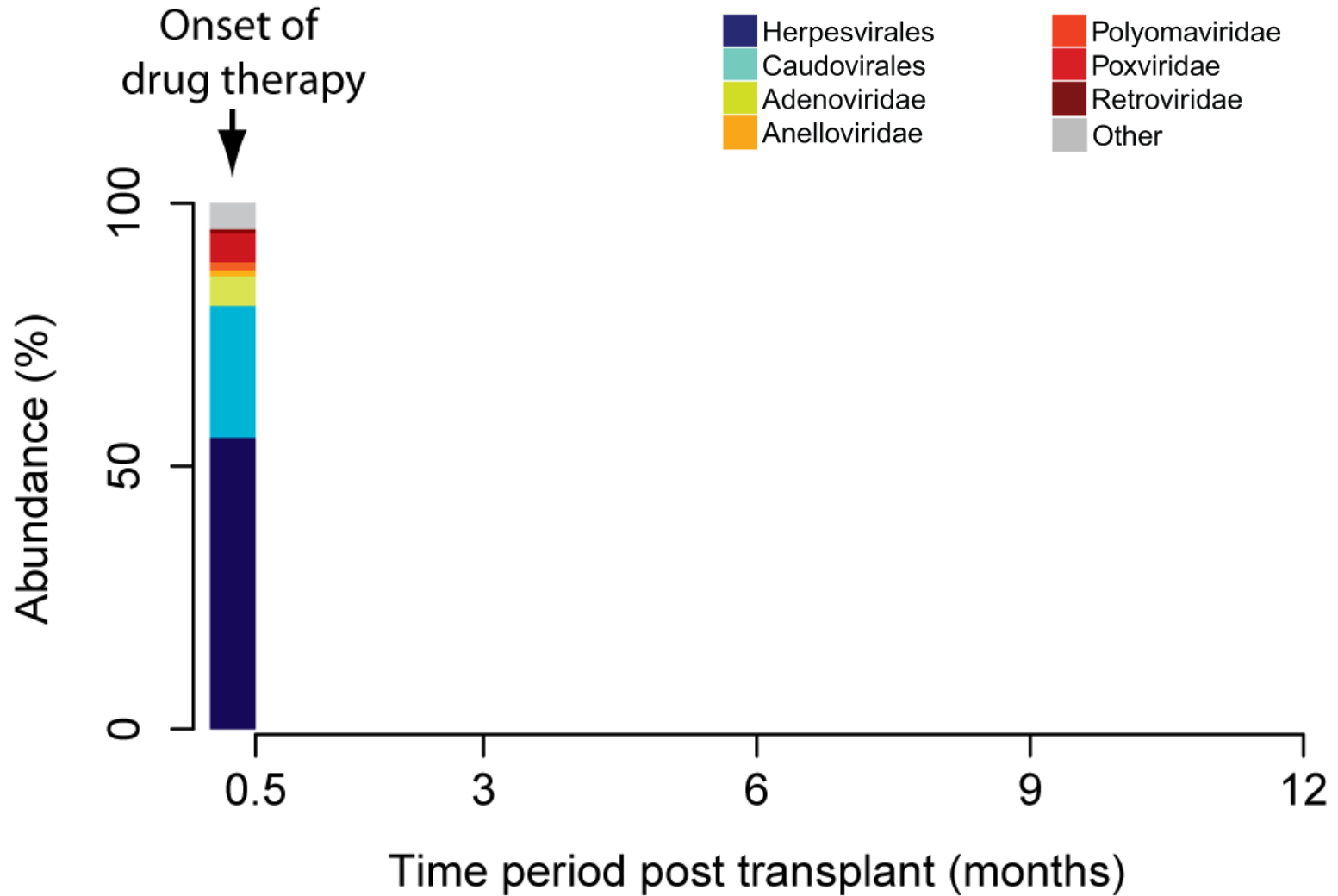
The anelloviridae fraction is primarily composed of viruses from the alphatorque genus.



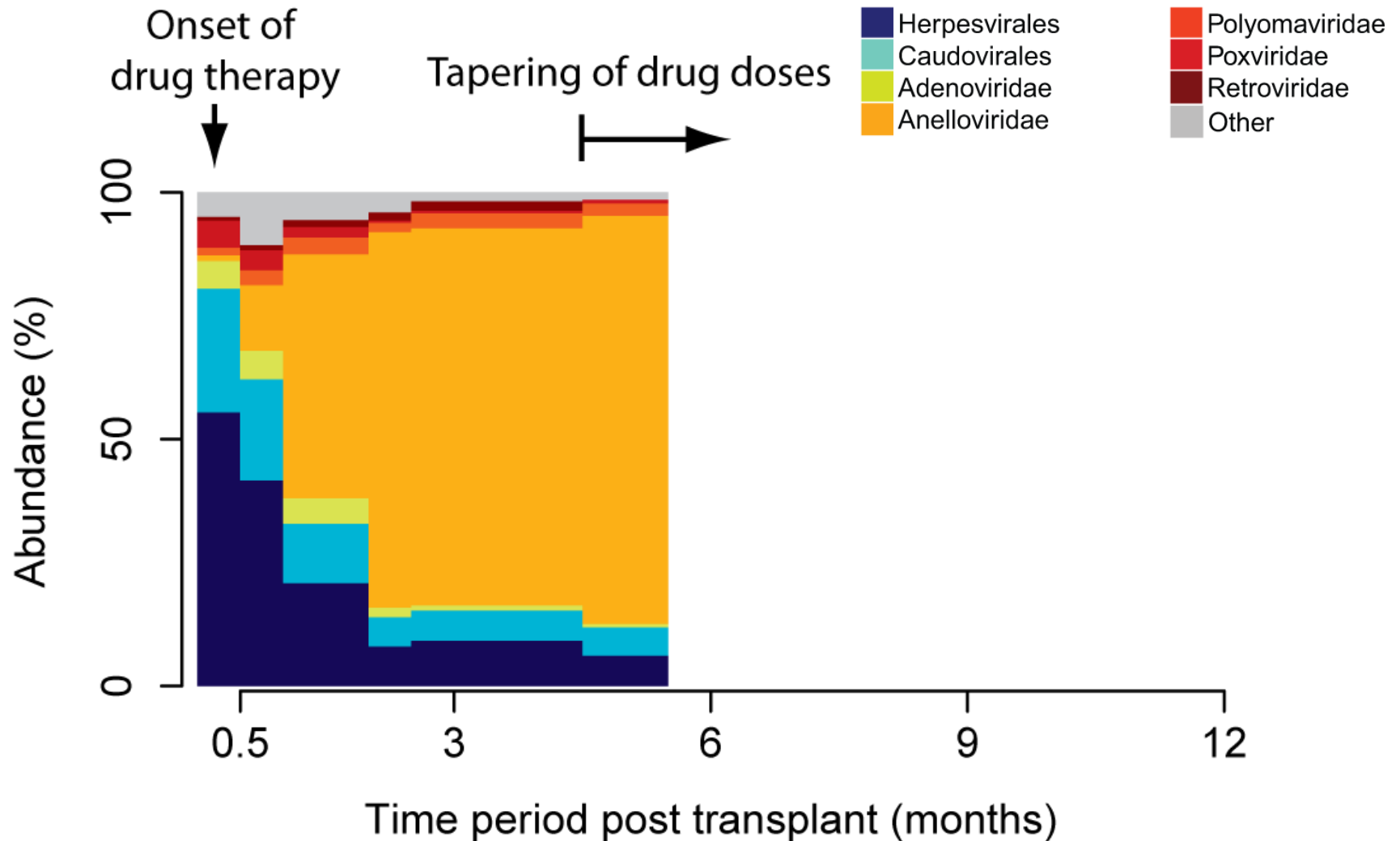
# Immunosuppressants and antivirals alter structure of the virome



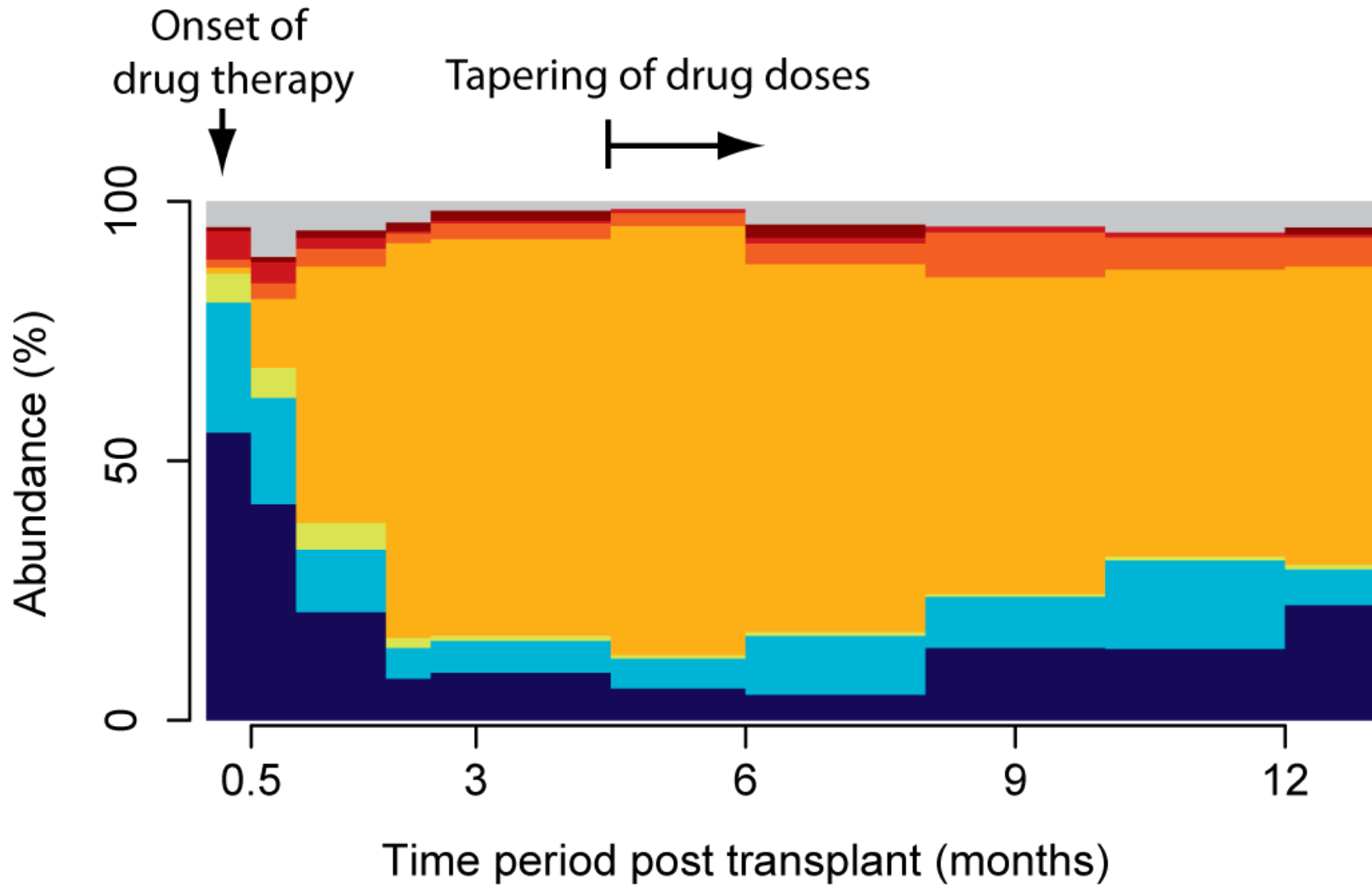
# Virome temporal dynamics



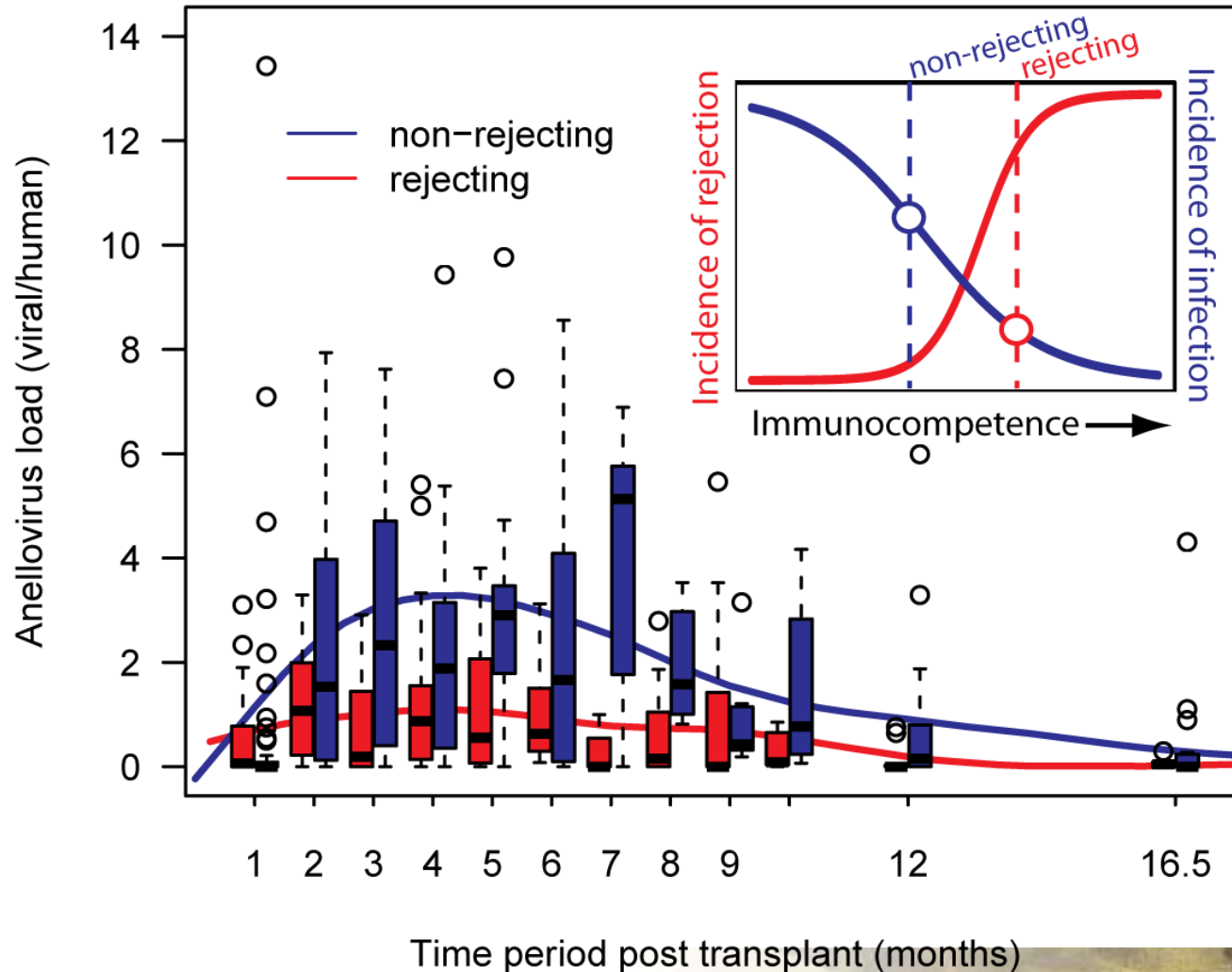
# Virome temporal dynamics



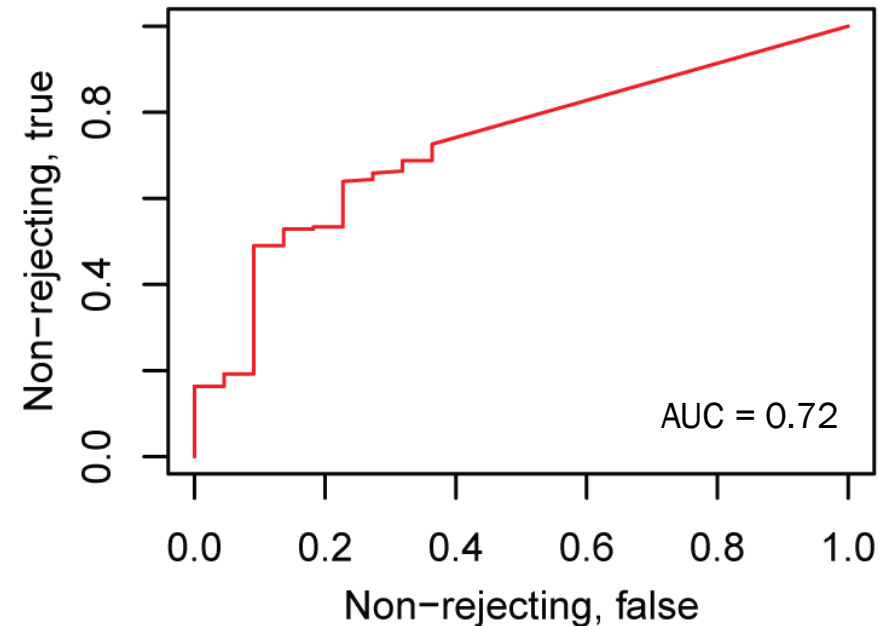
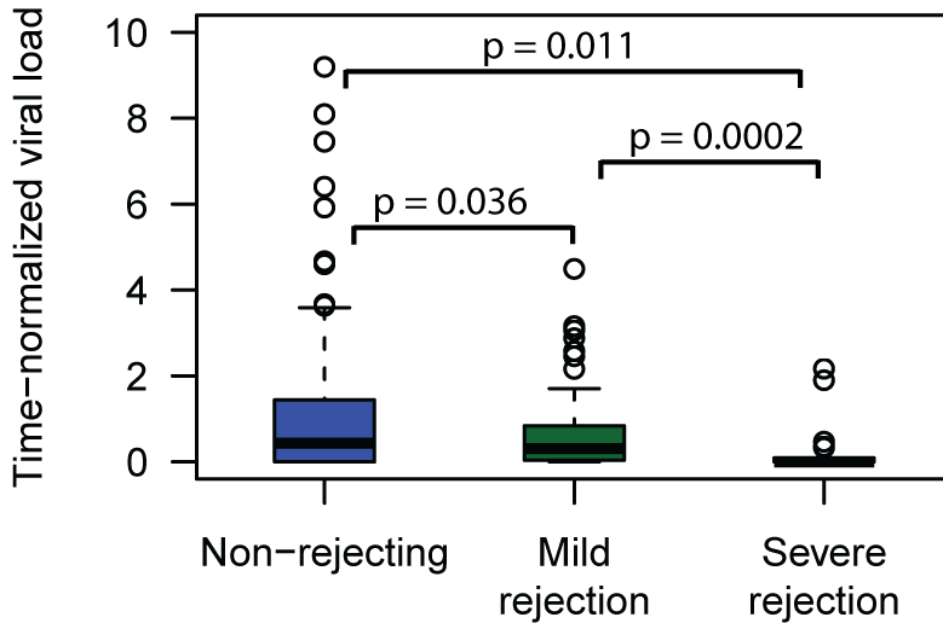
# Virome temporal dynamics



# Anellovirus load for rejecting vs non-rejecting recipients

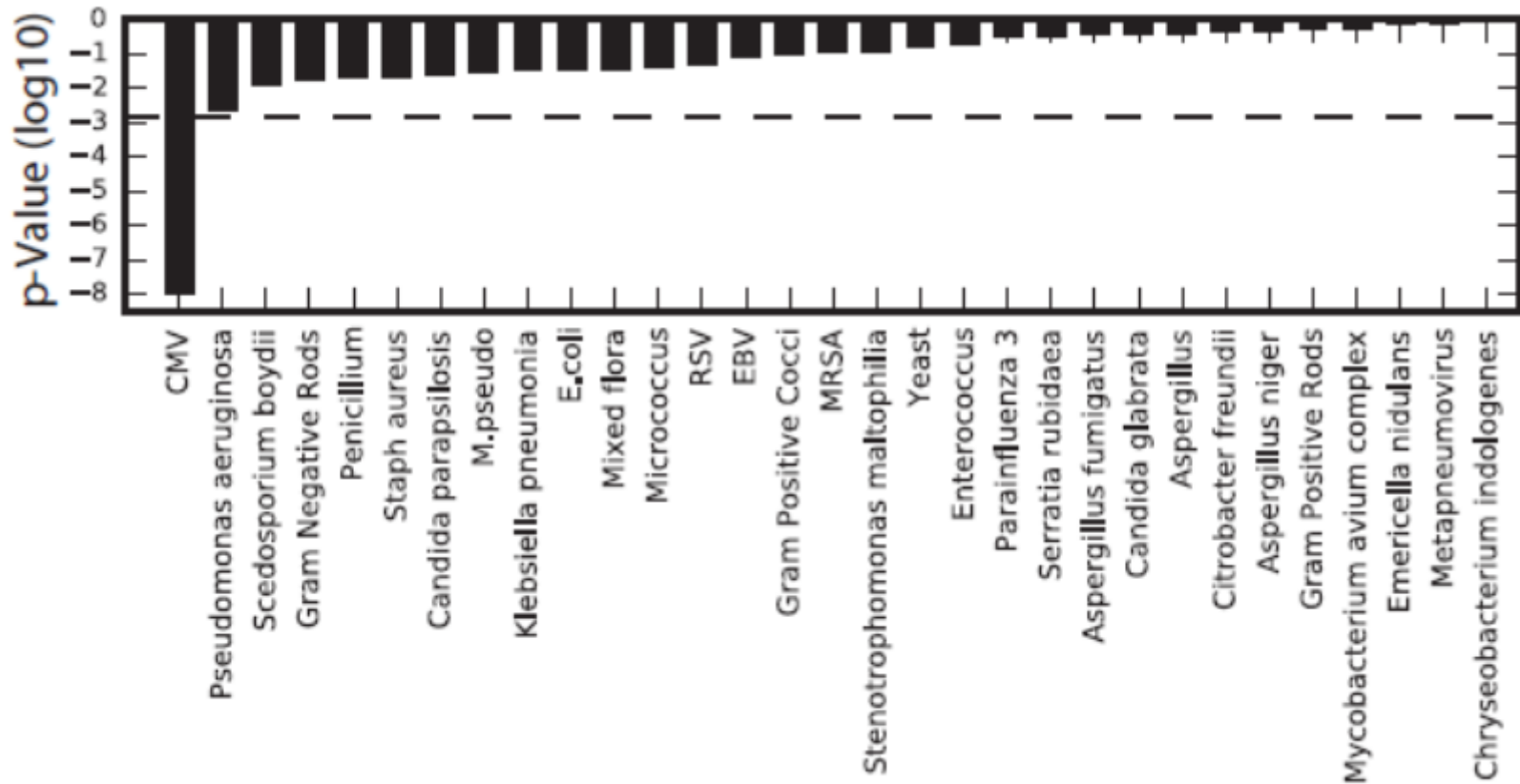


# Anellovirus load for rejecting vs non-rejecting recipients

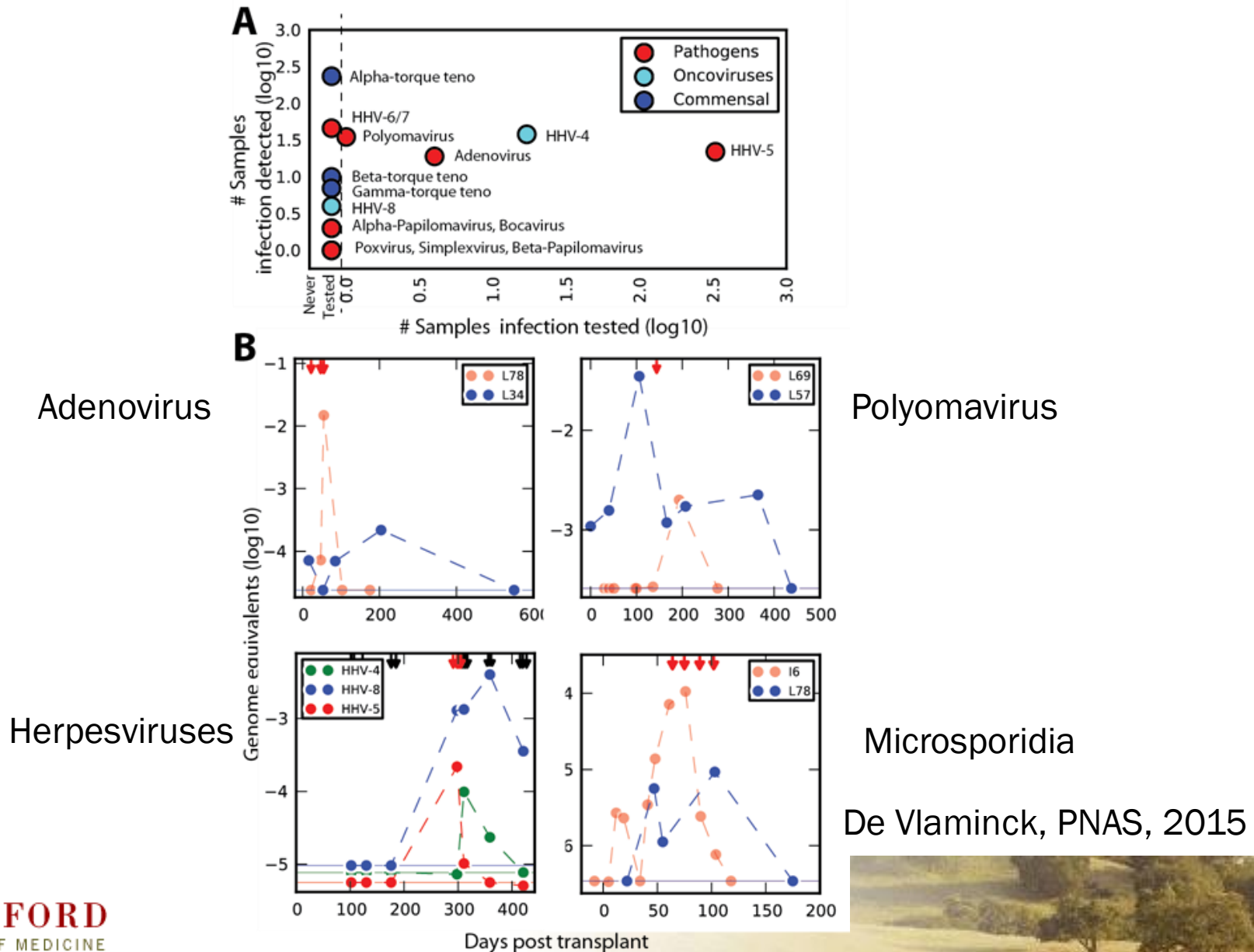


Can anellovirus load be used as a marker of a patient's net state of immunosuppression?

# Correlation between shotgun sequencing and clinical lab results



# Non-Biased Detection of Specific Pathogens





# Conclusions

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- cfDNA sequencing can be used to study the microbiome, and changes over time
- Structure of the virome is strongly affected by immune modulation and antivirals.
- The total viral load increases markedly at the onset of immunosuppressive therapy.
- Anellovirus load allows stratification of rejecting and non-rejecting recipients.
- Non-biased sequencing of the virome may enable diagnosis of infectious complications



# Future Directions

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- D-OAR: Prospective registry study of AlloSure™ assay to study test performance
  - 23 heart transplant centers
  - ~700 study subjects and ~2500 samples collected as of this week
  - Transition from research-grade to clinical-grade testing will facilitate adoption for patient management



# Future Directions

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- **Mitochondrial cfDNA** as a marker of acute rejection after transplantation (DeVlamick, Cornell)
- **Other sample types**
  - Fingertprick blood samples – point of care diagnostics
  - cfDNA in urine to monitor infections and rejection
- Identifying the **tissues of origin** of cell-free DNA
  - Genome-wide methylation patterns  
K. Sun, D. Lo, PNAS, 2015
  - Patterns of nucleosome and transcription factor occupancy  
M. Snyder, J. Shendure, Cell, 2016



# Acknowledgments

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