

Donor Derived Cell Free DNA - Has the Answer Been There All Along?



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CUTTING EDGE of **TRANSPLANTATION**

TRANSPLANT SUMMIT 2019

***NO SIZE FITS ALL:** Uncovering the
Potential of Personalized Transplantation*

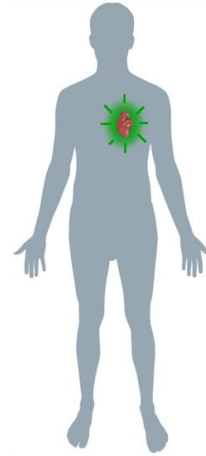
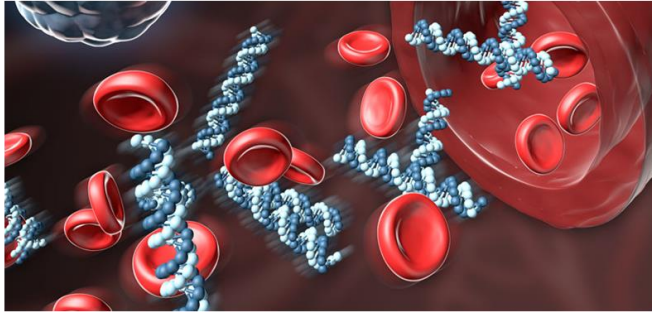
Disclosure

None

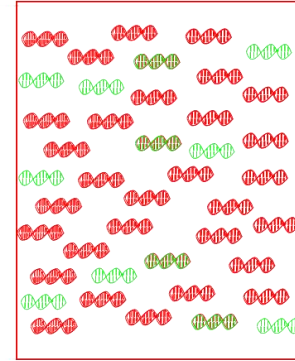
Learning Objectives

- To understand the concept of donor-derived cell-free DNA as a way of assessing damaged lung transplants and well as their infection status.
- To describe the special issues of noninvasive monitoring in lung transplant patients which distinguish these individuals from other solid organ recipients.
- To explain how cell-free DNA provides a window on the microbiome and virome in transplant patients and how this profile is impacted by anti-viral therapy and immunosuppression.
- Understand a new clinical study evaluating relationship between donor-derived cell-free DNA and lung allograft function over time.

Circulating cell-free DNA



→
Plasma
Sample

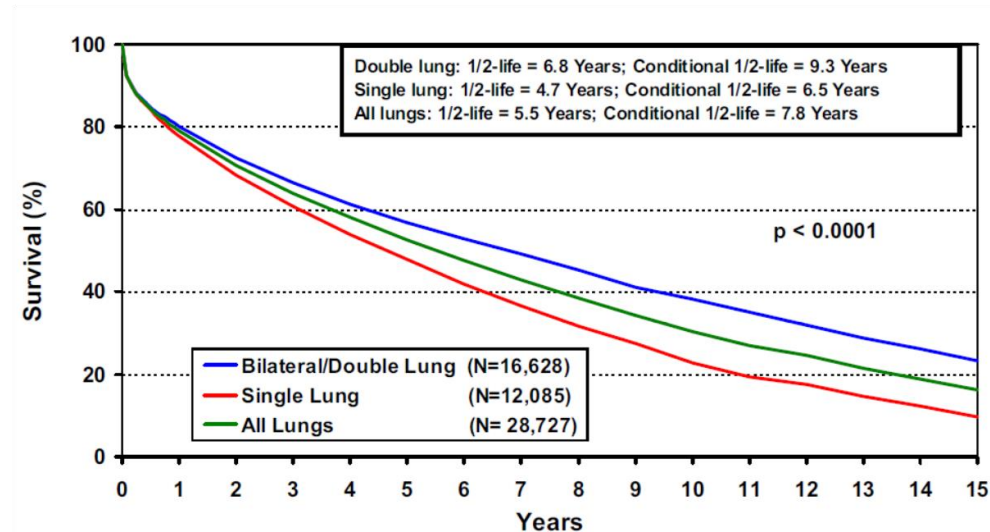


- Abundant in plasma: 1,000 – 10,000 genome copies per ml
=> up to 100 billion fragments of DNA per ml.
- Clearance rate: ~ 20 minutes. Turnover: ~ 10,000 cells per second or ~ 1 ml cell volume per hour
- Donor-derived DNA present in circulation of transplant recipients.
Y.M. Dennis Lo et al., the Lancet (1998).

T. Snyder, S.R. Quake, et al PNAS (2011)

I. De Vlaminck, S.R. Quake et al., Science Translational Medicine (2014)

Patient survival rates in lung transplantation



Major pulmonary complications following transplantation:

- Bronchial Obliterans Syndrome: progressive loss of lung function ~ chronic rejection
- Acute rejection
- Infections of the lung

ISHLT report, 30,1104 (2011)

Les acides nucléiques du plasma sanguin chez l'Homme.

par P. MANDEL et P. MÉTAIS.

Biologie Comptes Rendus (1948)

Sujet	Sexe	Age	Affection	P phospho- protéine mg.	P ribonu- cléique mg.	P desoxyri- bonu- cléique mg.	P total acides nucléiques mg.
1	F	42	Normal	0	5,0	1,2	6,2
2	F	22	»	0	4,0	0,4	4,4
3	H	24	»	0	5,2	1,3	6,5
4	F	27	»	0	4,7	0,3	5,0
5	F	20	»	0	3,7	0,8	4,5
6	H	48	»	0	4,6	1,3	5,9
7	H	45	»	0	4,5	0,6	5,1
8	F	26	»	0	5,0	0,2	5,2
9	F	37	»	0	4,8	0,6	5,4
10	H	39	»	0	5,0	0,9	5,9
11	H	62	Insuffis. card.	0	3,8	0,7	4,5
12	H	62	»	0	3,8	0,45	4,25
13	H	42	»	0	5,1	0,9	6,0
14	F	33	Endocard. maligne	0	3,35	0,65	4,0
15	H	19	»	0	3,5	0,8	4,3
16	F	5	Goutte	0	5,6	0,4	6,0
17	H	58	Basedow	0	3,6	0,3	3,9
18	H	61	Diabète	0	3,6	0,4	4,0
19	H	48	»	0	3,5	0,4	3,9
20	F	52	Cirrhose	0	5,3	1,2	6,5
21	F	48	Ictère	0	3,6	0,4	4,0
22	H	48	Goutte	0	3,5	1,0	4,5
23	H	33	»	0	2,66	0,8	3,46
24	H	33	Goutte	0	5,5	0,5	6,0
25	H	26	»	0	4,75	0,75	5,5
26	H	26	Néphrite	0	3,75	0,7	4,45
27	H	37	Tuberculose	0	3,5	0,45	3,95
28	F	23	Grossesse 7 ^e m.	0	7,65	1,35	9,0
29	H	»	»	0	7,25	1,00	8,25

The discovery of circulating cell-free DNA and RNA set the stage for the liquid biopsy field to emerge a half century later - but only after combining with next generation sequencing and some clever ideas!

Dans un cas de grossesse on trouve des valeurs plus fortes : 8,25 et 9 mg. de P nucléique p. litre.

Noninvasive diagnosis of fetal aneuploidy by shotgun sequencing DNA from maternal blood

H. Christina Fan*, Yair J. Blumenfeld†, Usha Chitkara†, Louanne Hudgins†, and Stephen R. Quake*[§] PNAS (2008)

*Department of Bioengineering, Stanford University and Howard Hughes Medical Institute, 318 Campus Drive, Clark Center, Room E300, Stanford, CA 94305; †Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, Stanford University, 300 Pasteur Drive, Room HH333, Stanford, CA 94305; and §Division of Medical Genetics, Department of Pediatrics, Stanford University, 300 Pasteur Drive, Stanford, CA 94305

Communicated by Leonard A. Herzenberg, Stanford University School of Medicine, Stanford, CA, August 22, 2008 (received for review July 13, 2008)

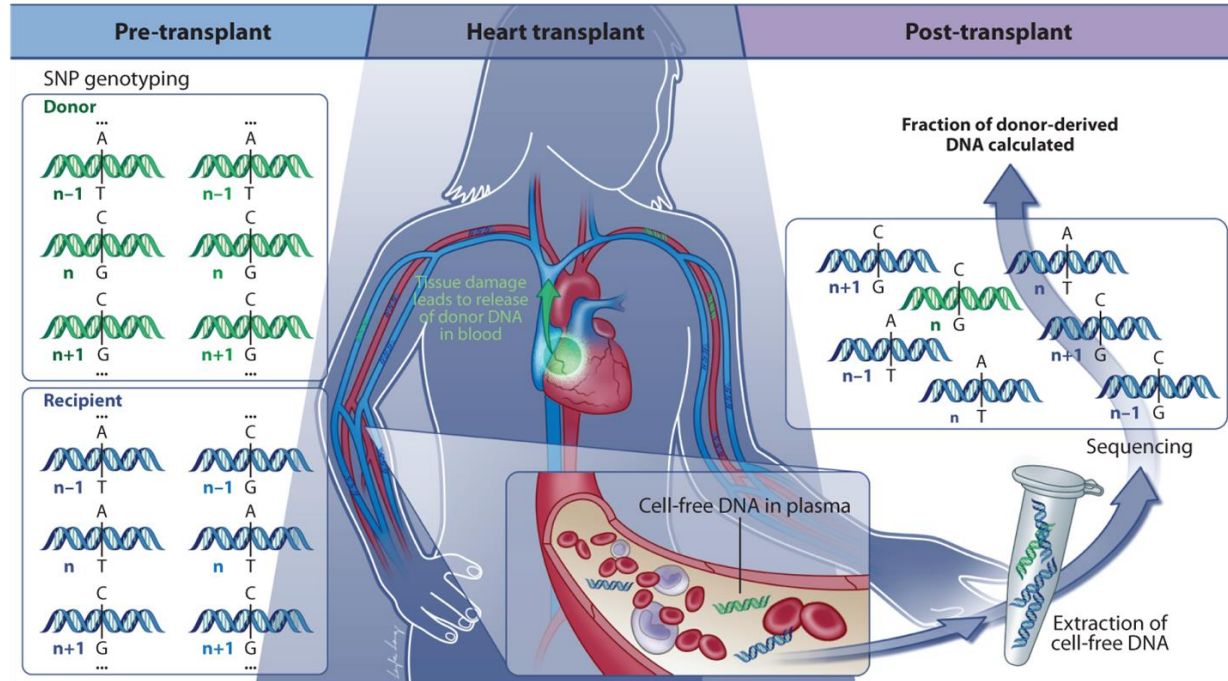
Non-invasive prenatal measurement of the fetal genome

H. Christina Fan^{1,†,*}, Wei Gu^{1,*}, Jianbin Wang¹, Yair J. Blumenfeld², Yasser Y. El-Sayed² & Stephen R. Quake^{1,3,4} Nature (2012)

The vast majority of prenatal genetic testing requires invasive sampling. However, this poses a risk to the fetus, so one must make a decision that weighs the desire for genetic information against the risk of an adverse outcome due to hazards of the testing process. These issues are not required to be coupled, and it would be desirable to discover genetic information about the fetus without incurring a health risk. Here we demonstrate that it is possible to non-invasively sequence the entire prenatal genome. Our results show that molecular counting of parental haplotypes in maternal plasma by shotgun sequencing of maternal plasma DNA allows the inherited fetal genome to be deciphered non-invasively. We also applied the counting principle directly to each allele in the fetal exome by performing exome capture on maternal plasma DNA before shotgun sequencing. This approach enables non-invasive exome screening of clinically relevant and deleterious alleles that were paternally inherited or had arisen as *de novo* germline mutations, and complements the haplotype counting approach to provide a comprehensive view of the fetal genome. Non-invasive determination of the fetal genome may ultimately facilitate the diagnosis of all inherited and *de novo* genetic disease.

3 million women tested in 2017, thousands of lives saved, amniocentesis rates plunged 70%

Cell free donor DNA as a marker for rejection



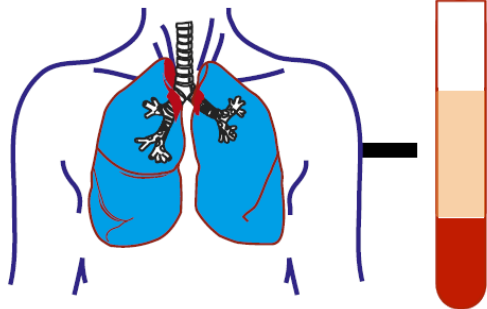
Pre-transplant genotyping, 1 Million or 2.5 Million SNP markers

Post-transplant sequencing of cell-free DNA

I. De Vlaminck, S.R. Quake et al., Science Translational Medicine (2014)

Patient Recruitment

**Collect plasma samples
post lung transplant
(51 patients, 398 samples)**



**Quantify cell-free
DNA (cfDNA) level**

**Correlate cfDNA
level with clinical tests**

Donor-derived
cfDNA

Rejection (Biopsy)

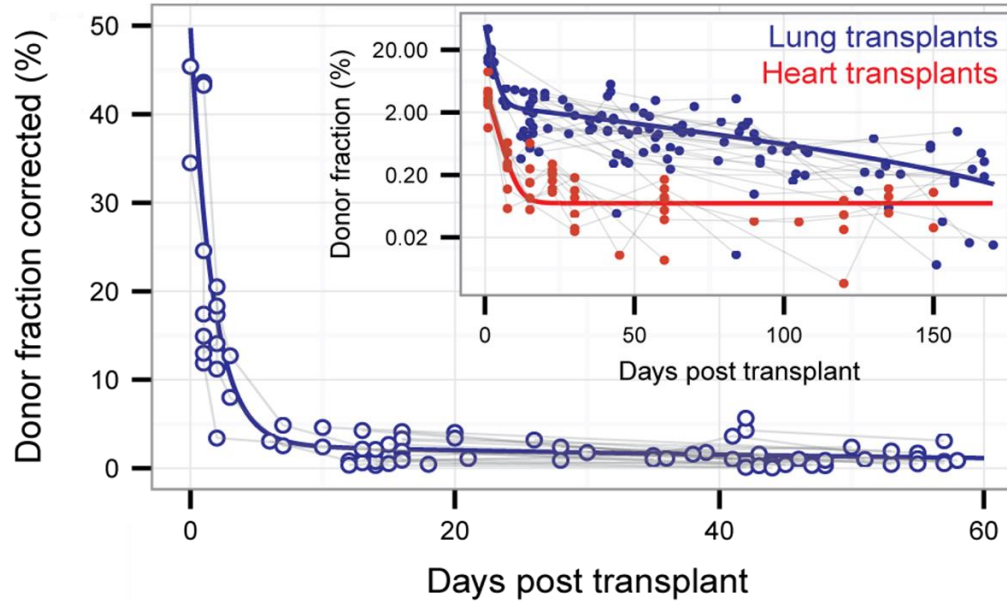
Clinical
tests

Infection-
derived cfDNA

Infection (Culture, qPCR)

De Vlamincx et al,
PNAS 2015; 112: 13336-41

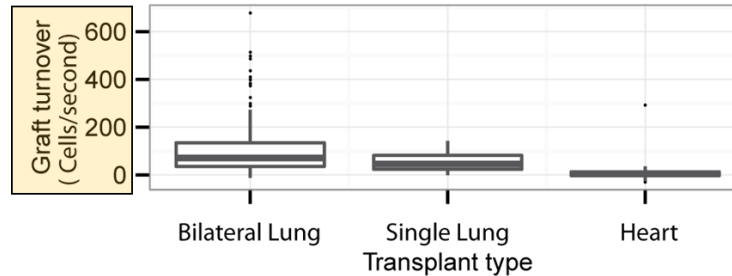
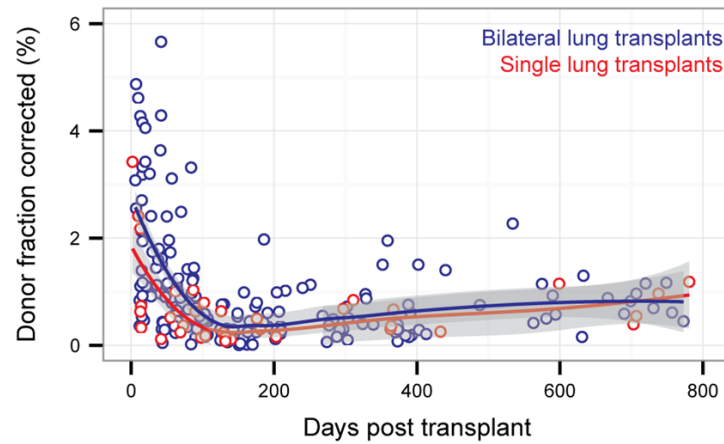
Lung transplants – signal in absence of rejection



Very high levels of donor DNA immediately post transplant
High background level compared to heart transplants

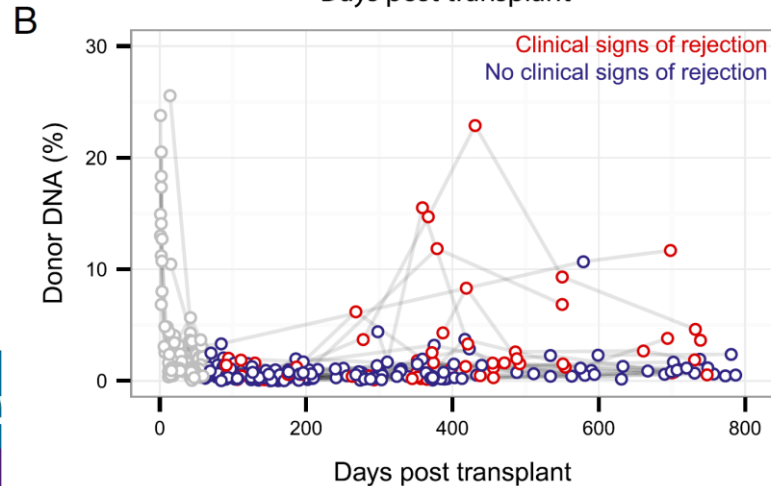
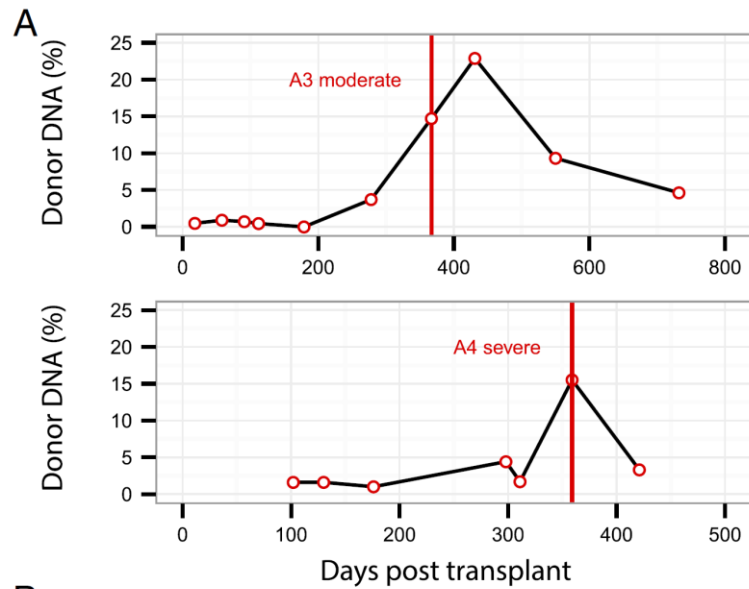
PNAS (2015)

Lung transplants – signal in absence of rejection



Evidence for continued tissue damage over the course of the transplant
In line with the clinical observations of progressive decay of lung function?
Patient-to-patient variability in early time behavior remains unexplained.

De Vlaminck et al,
PNAS 2015; 112: 13336-41



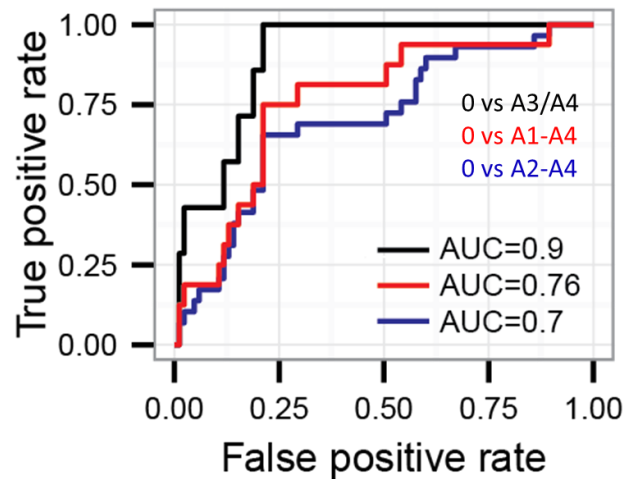
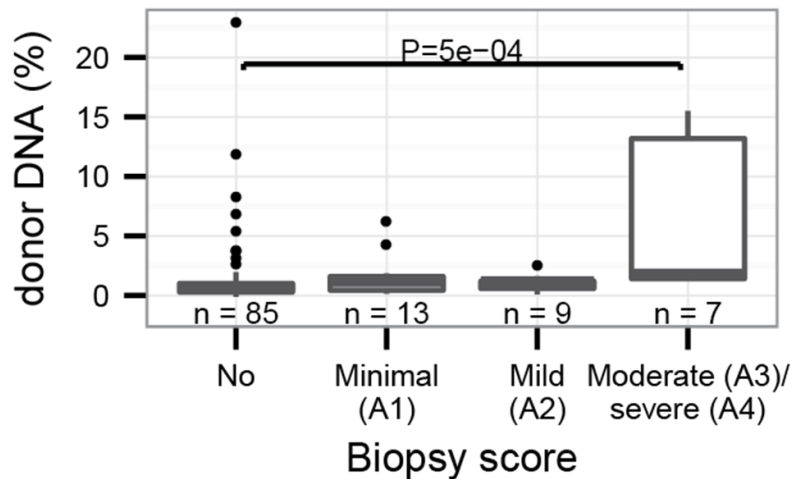
Cell-free donor DNA at the time of rejection

De Vlamincx et al,
PNAS 2015; 112: 13336-41



CUTTING EDGE of TRANSPLANTATION

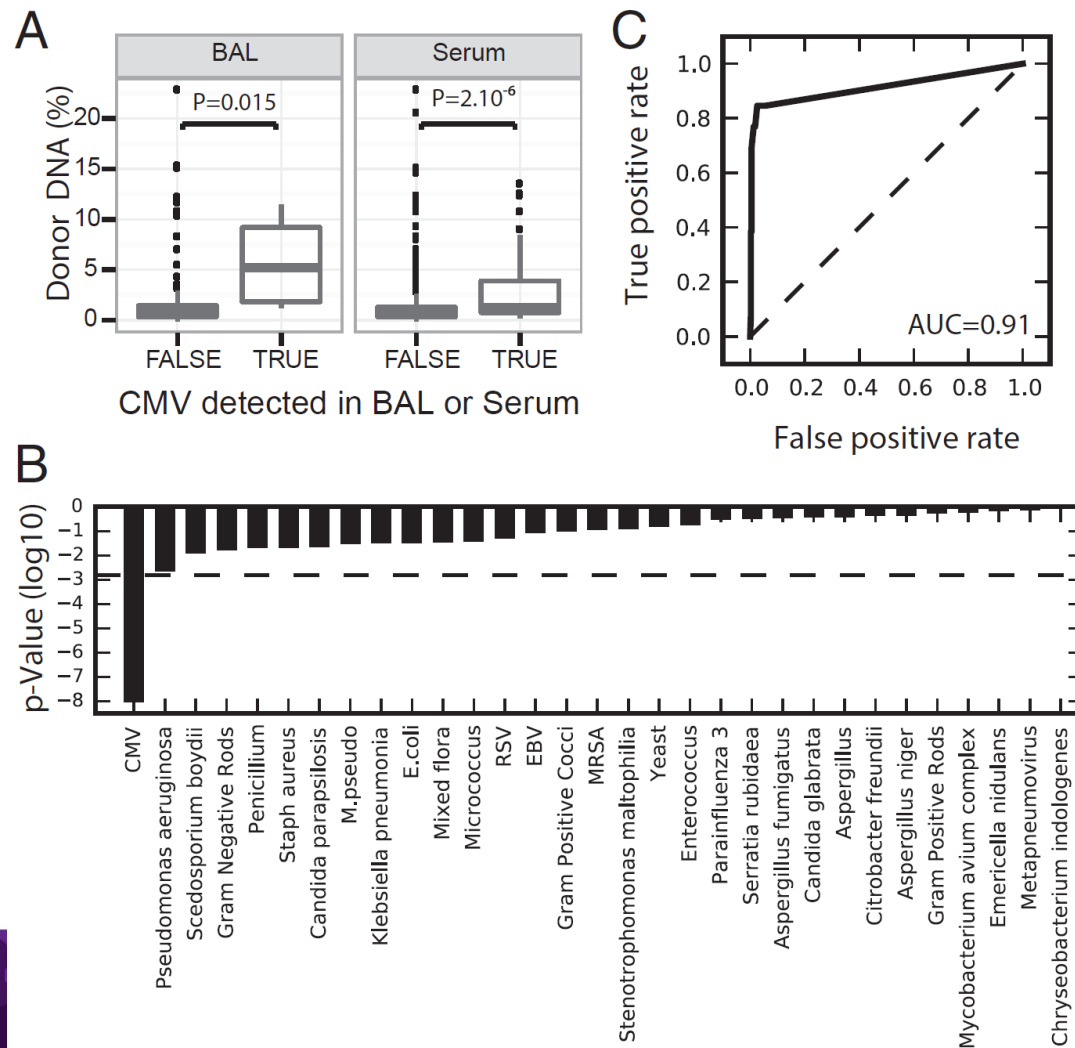
Analysis of performance against biopsy



Exclude samples collected within first 60 days
Account for volume difference for single/bilateral transplants

PNAS, 2015

CMV-infection induced allograft injury



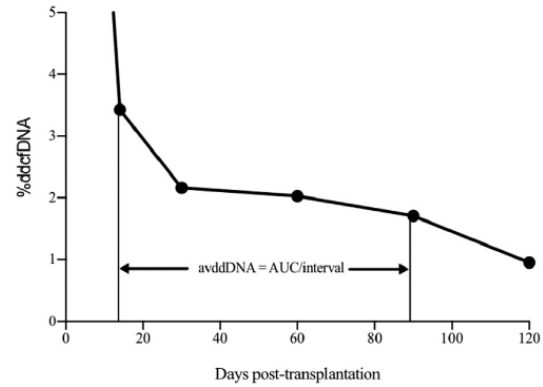
De Vlamincx et al,
PNAS 2015; 112: 13336-41

Donor-derived cfDNA (ddcfDNA) predicts allograft failure and mortality after lung transplantation.

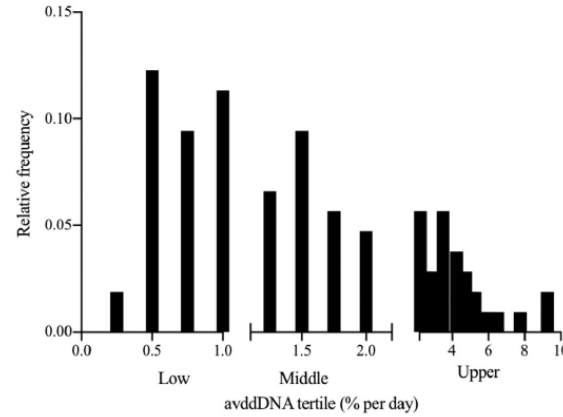
- Multi-center, prospective cohort study
- 106 lung transplant patients
- Monitored for allograft failure
 - CLAD, retransplantation, respiratory failure
 - Serial plasma collected in the 1st 3mos post-tp
 - Average %ddcfDNA and related to allograft failure

Agbor-Enob et al. *EBioMedicine* 2019

A

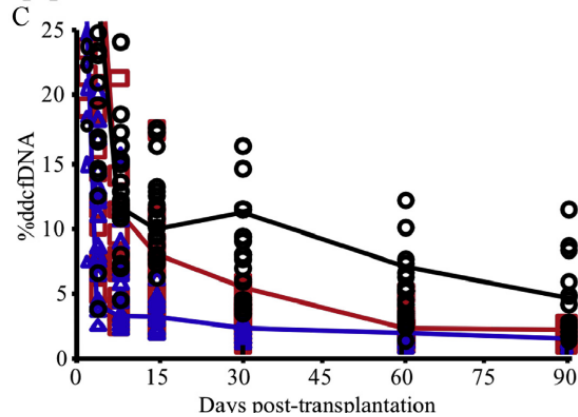


B

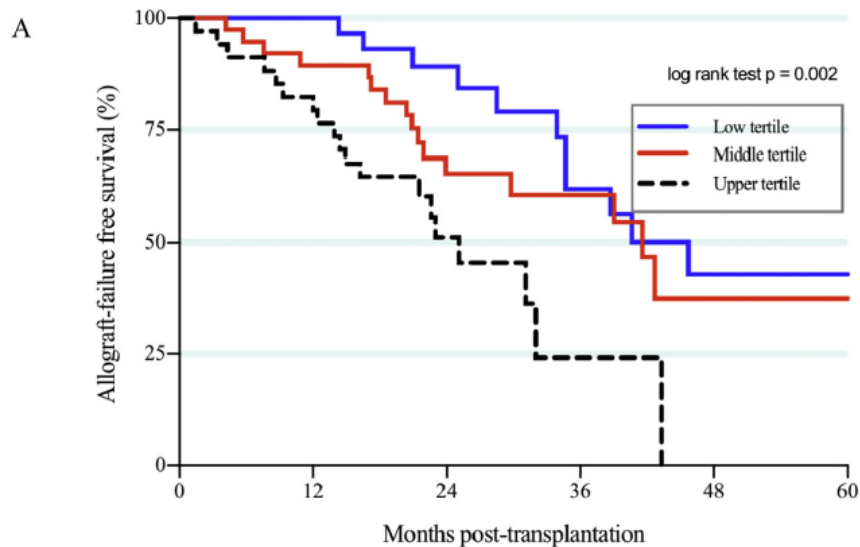


Results (1)

A



Agbor-Enob et al. *EBioMedicine* 2019

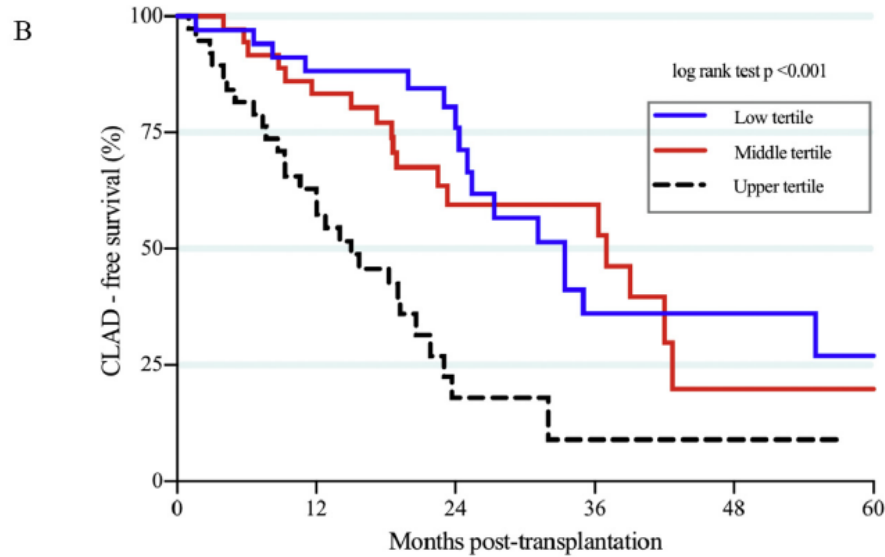


Results (2)

Month post-transplant	Number of subjects at risk at x-axis mark					
	0	12	24	36	48	60
avddDNA tertile						
Low	36	36	19	13	7	3
Middle	35	32	17	11	5	2
Upper	35	28	12	2	0	0

Agbor-Enob et al. *EBioMedicine* 2019

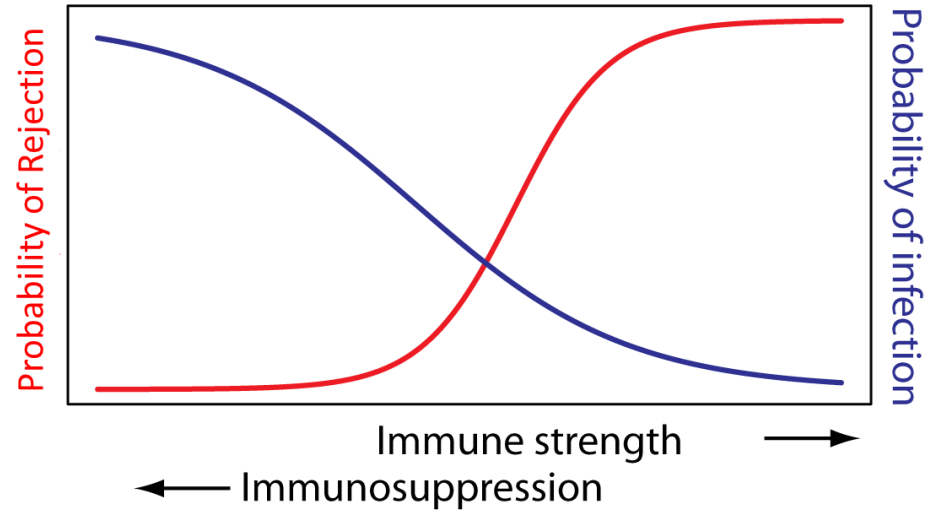
Results (3)



Month post-transplant	Number of subjects at risk at x-axis mark					
	0	12	24	36	48	60
avddDNA tertile						
Low	36	31	18	8	6	4
Middle	35	31	16	9	3	3
Upper	35	23	5	2	2	1

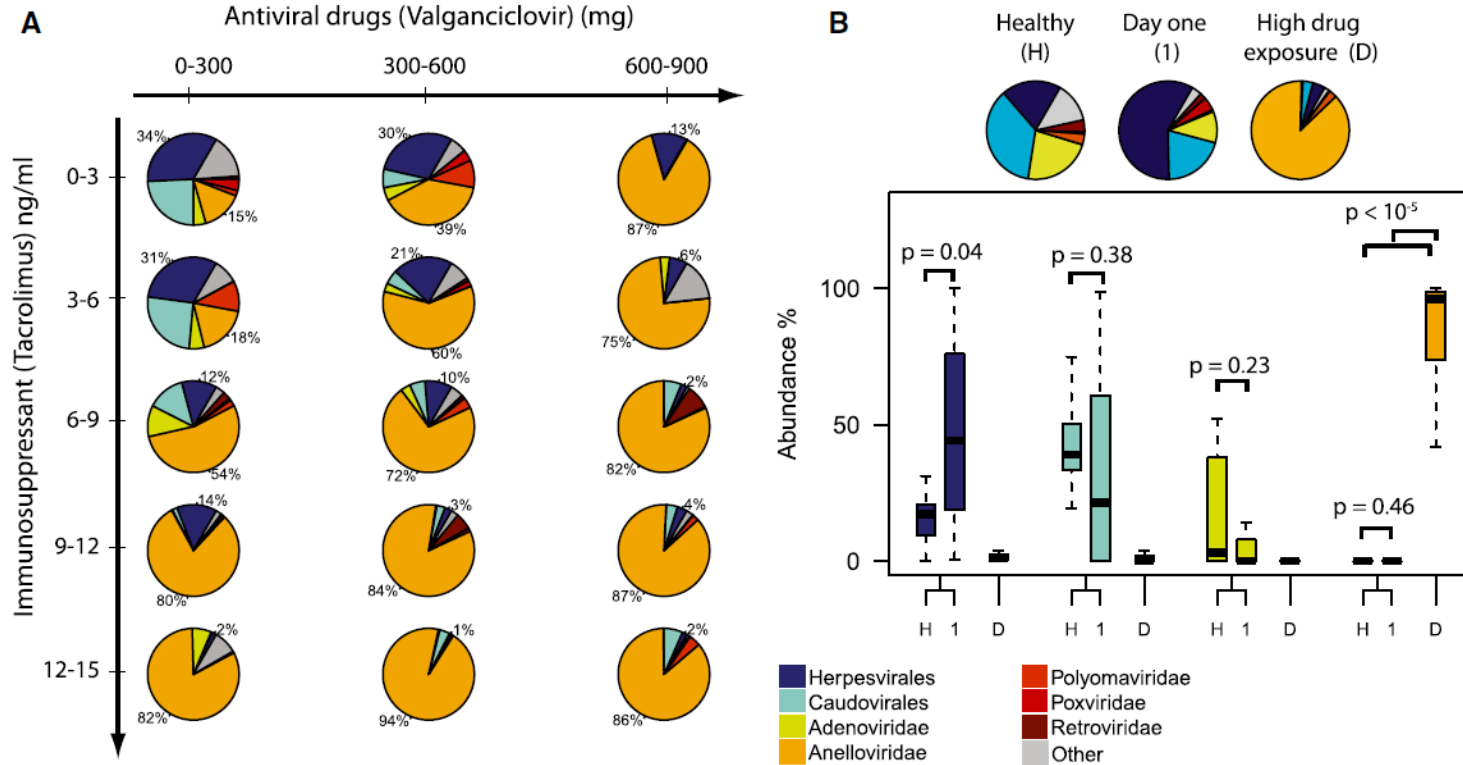
Agbor-Enob et al. *EBioMedicine* 2019

The challenge of post transplant therapy



The therapeutic window is small, in some cases non-existent.
Drug toxicity/intolerance further complicates the situation

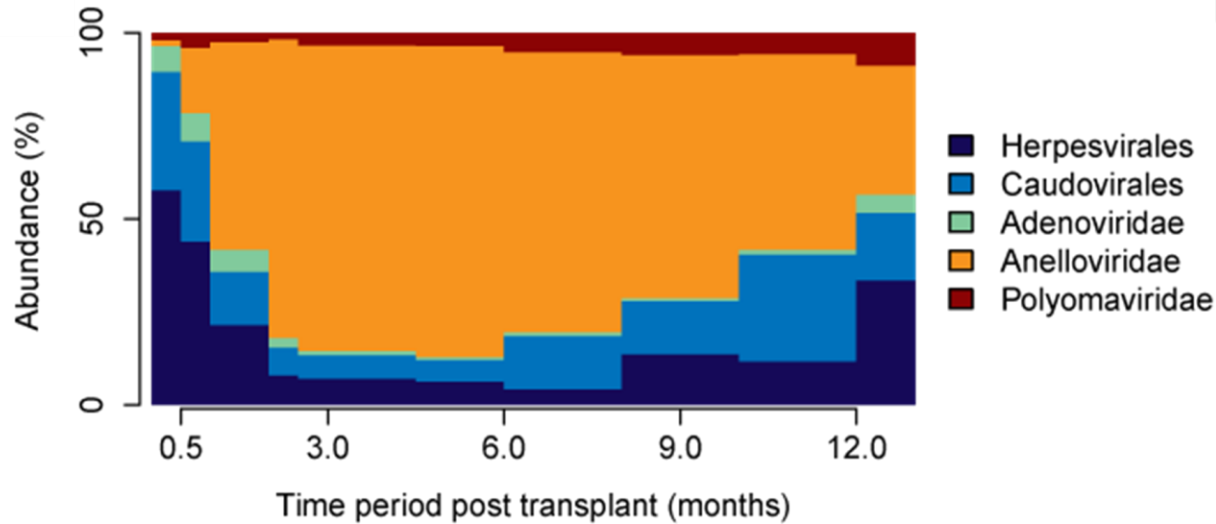
Relative viral genomic abundance as a function of drug dosing



De Vlamincx et al,
Cell 2013; 155:1178-87

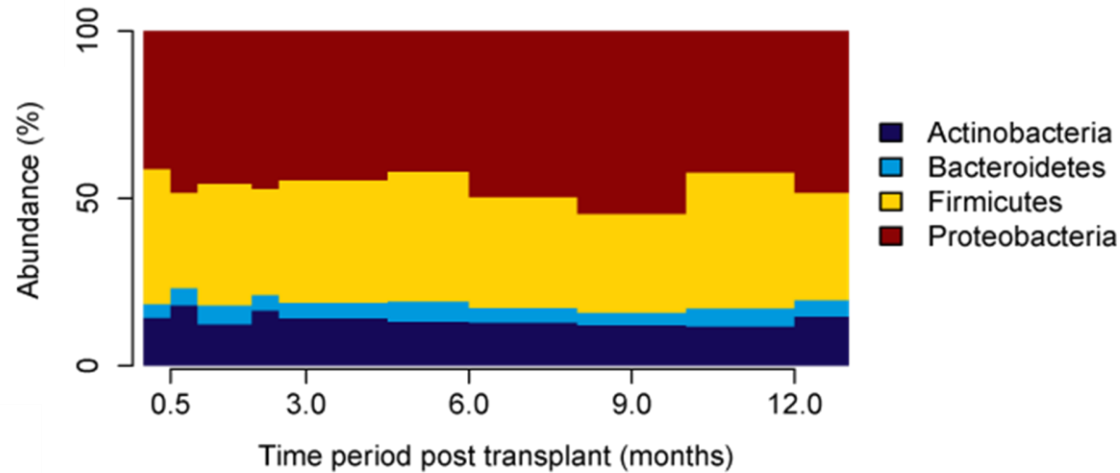
TRANSPLANTATION

Virome time dynamics



More detail on the time dynamics is observed when looking at the abundance at the family and order level of taxonomic classification

Bacterial phyla relative abundance: stable over time



In contrast to the virome dynamics; no appreciable changes observed in the relative abundance of bacterial phyla

Conclusions

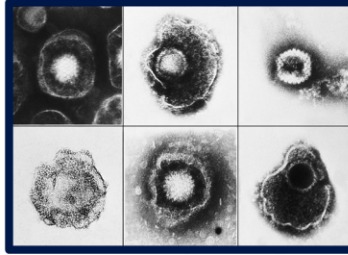
- Donor-derived cell-free DNA: an exciting approach for detecting allograft damage and infectious pathogens in lung transplant patients.
- Increased cell-free DNA can predate clinical signs and symptoms.
- New clinical study shows a correlation between the relative % of ddcfDNA, allograft injury and CLAD.
- Further study needed.

cfDNA is now used as a clinical test for organ transplant rejection

- **Publications**

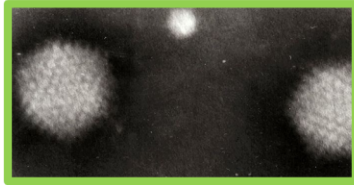
- Validation of a Clinical-Grade Assay to Measure Donor-Derived Cell-Free DNA in Solid Organ Transplant Recipients, JOURNAL OF MOLECULAR DIAGNOSTICS (2016)
- Biological Variation of Donor-Derived Cell-Free DNA in Renal Transplant Recipients: Clinical Implications, Journal of Applied Laboratory Medicine (2017)
- Cell-Free DNA and Active Rejection in Kidney Allografts, Journal of the American Society of Nephrology (2017)
- Clinical Trials (more information at [ClinicalTrials.gov](https://clinicaltrials.gov))
 - Non Invasive Blood Test To Diagnose Acute Rejection After Kidney Transplantation
 - Outcomes AlloMap Registry: the Long-term Management and Outcomes of Heart Transplant Recipients With AlloMap Testing

Herpesviruses



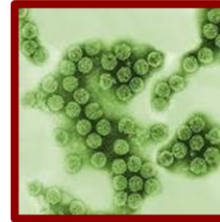
Large dsDNA genome 200-300 kb
Problematic in organ transplantation
Target of antiviral prophylaxis

Adenoviruses



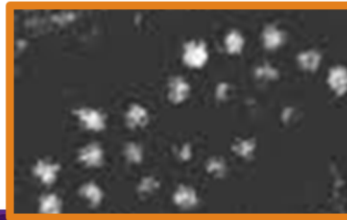
dsDNA genome
Infections of the respiratory tracts
Usually mild but can be
problematic in transplantation

Polyomaviruses



dsDNA 5 kb genome
Carcinogenic.

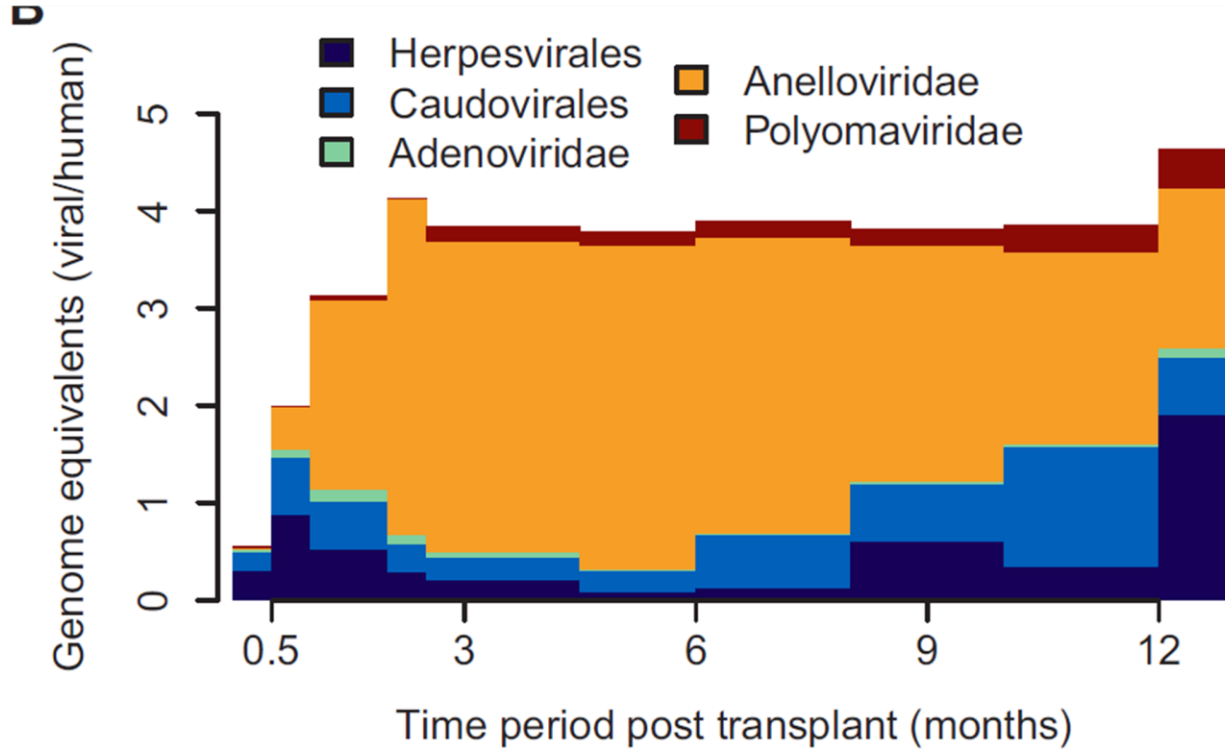
Anelloviruses



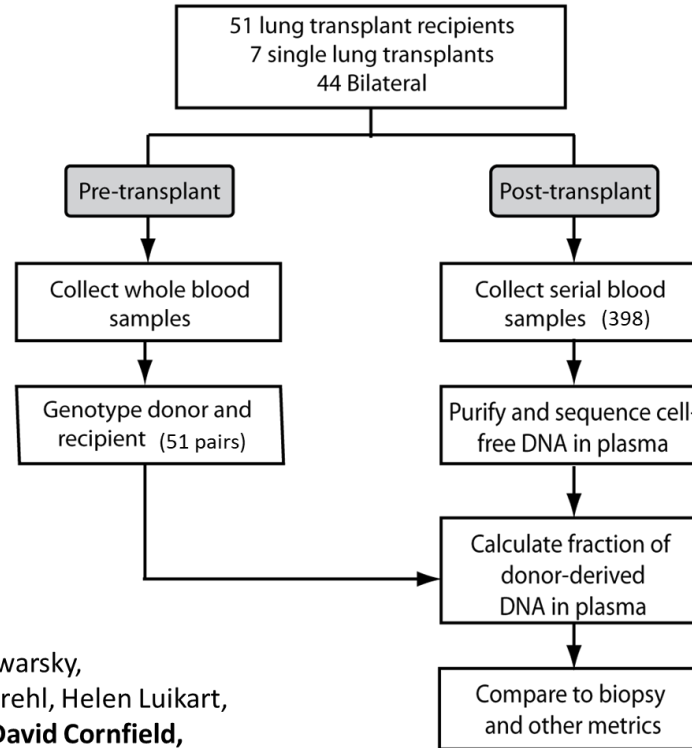
ssDNA 3 – 4 kb genomes
Almost ubiquitous but not
associated with any disease

Images from
wikipedia

Immunosuppressants and Antiviral

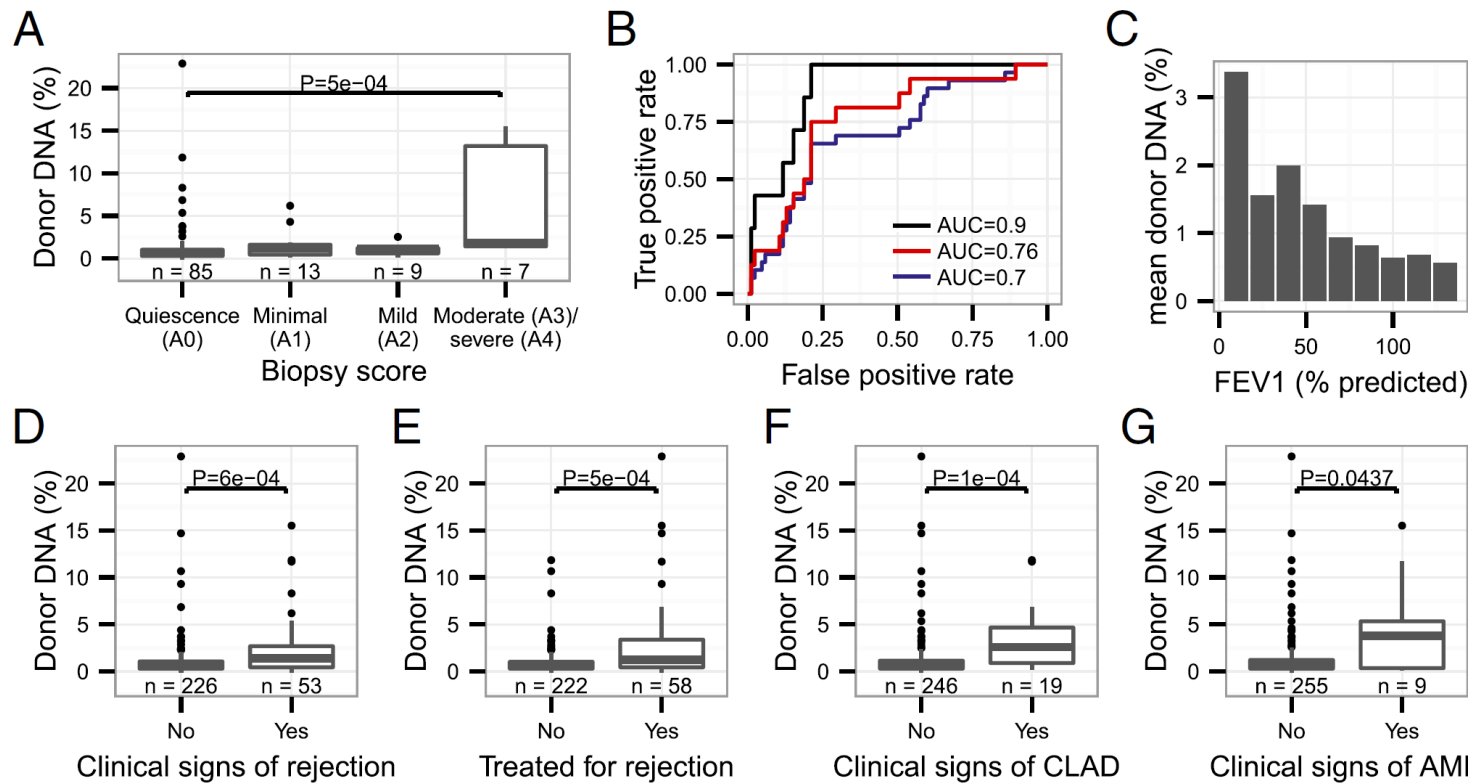


Study design



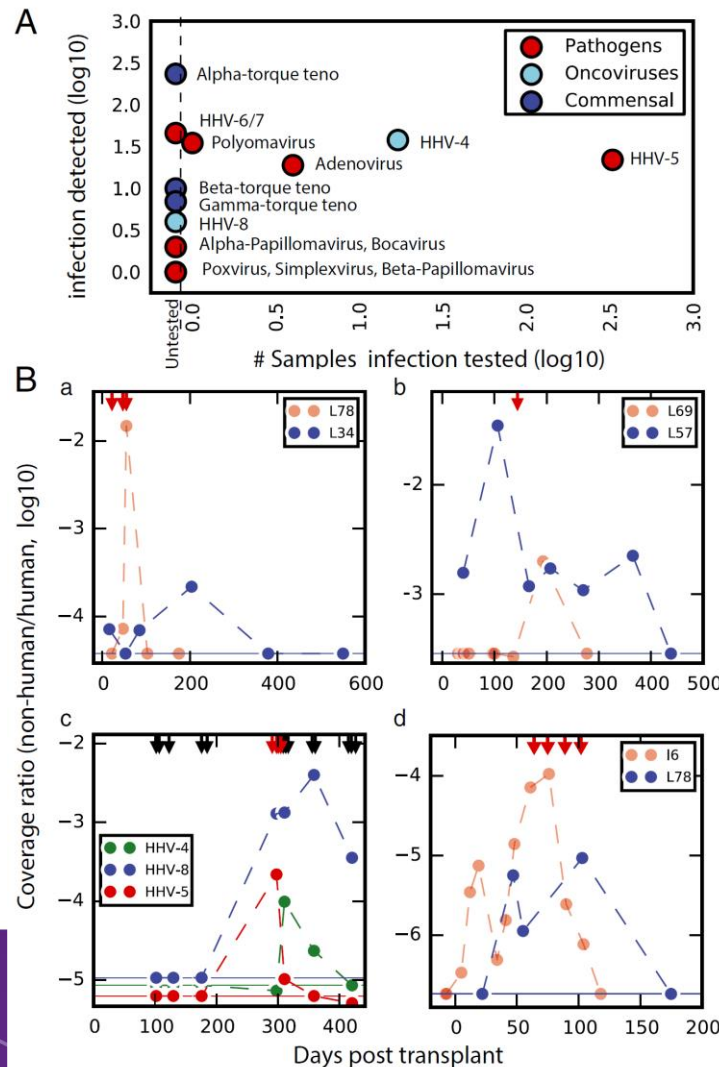
Lance Martin, Michael Kertesz, Mark Kowarsky,
Jennifer Okamoto, Norma Neff, Calvin Strehl, Helen Luikart,
Kapil Patel, David Weill, Mark Nicolls, David Cornfield,
Kiran Khush, Hannah Valentine, Stephen Quake

Analysis of the performance of cfdDNA as a marker of lung transplant rejection



De Vlamincx et al,
PNAS 2015; 112: 13336-41

Monitoring the 'Infectome'



De Vlamincx et al,
PNAS 2015; 112: 13336-41