Cell Free DNA and Other Immune Monitoring Techniques in Lung Transplantation – Are We There Yet?

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Disclosure

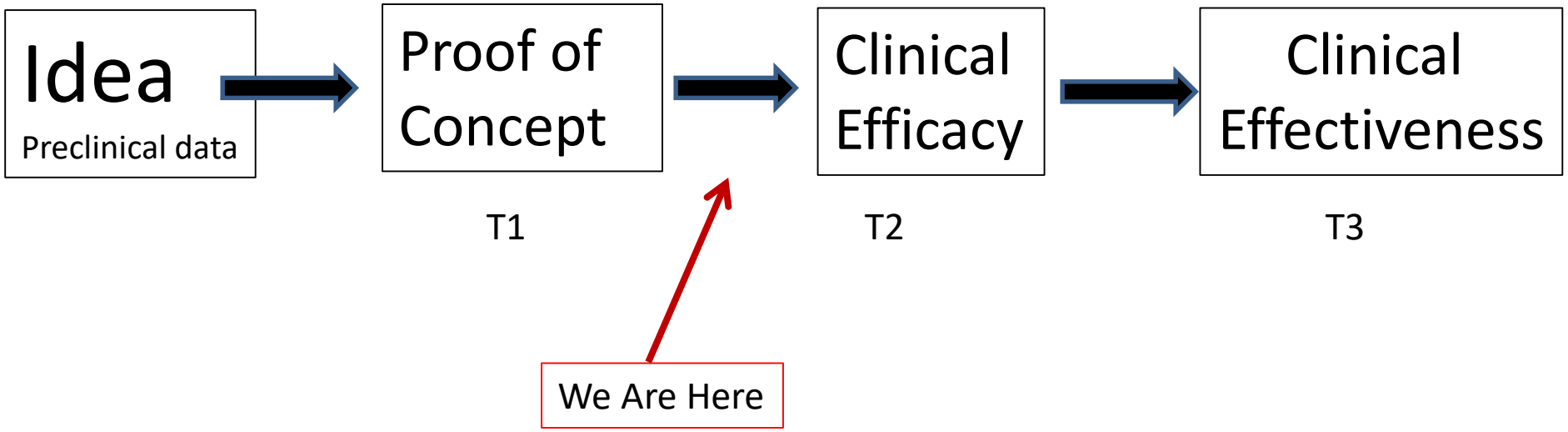
No Financial Disclosures

I will discuss cell free DNA assays which are commercially available, but not approved in lung transplantation

Learning Objectives

- Understand the principle of cell free DNA as a non-invasive test
- Review data from recent studies in lung transplant on the use of cell free DNA including test performance characteristics
- Recognize limitations of current technology and how findings in the field may impact new directions
- Review of additional non-invasive immune monitoring tools

Bridging the Translational Gaps



Immune Monitoring in Lung Transplantation: The Jump From T1-T2 has been hard

- T cell global activation assays (Immu-know)
- Antibody Mediated Rejection –
  - standardization of MFI cutoffs for HLA typing labs
  - Use of complement staining on histology
- Use of assays to detect anti Col V and anti ka-1 tubulin antibodies
- Cytokine and Chemokine assays of lung lavage*

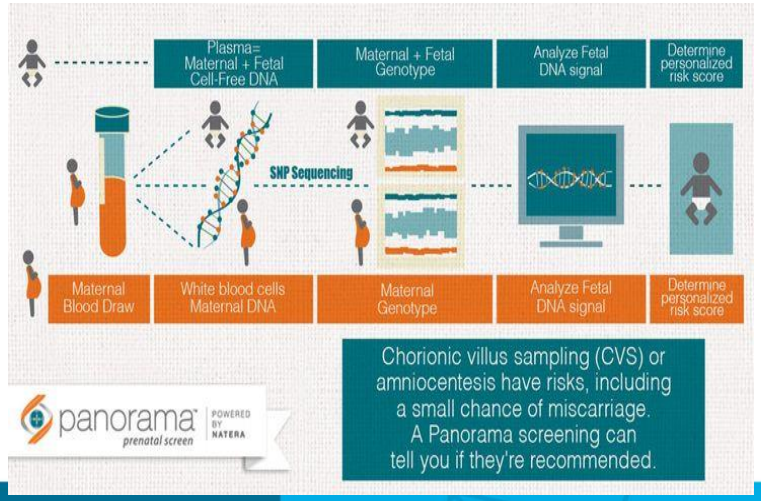
* See Mike Shino's Paper on CXCL9 plus A1 rejection AJT Jan 2018

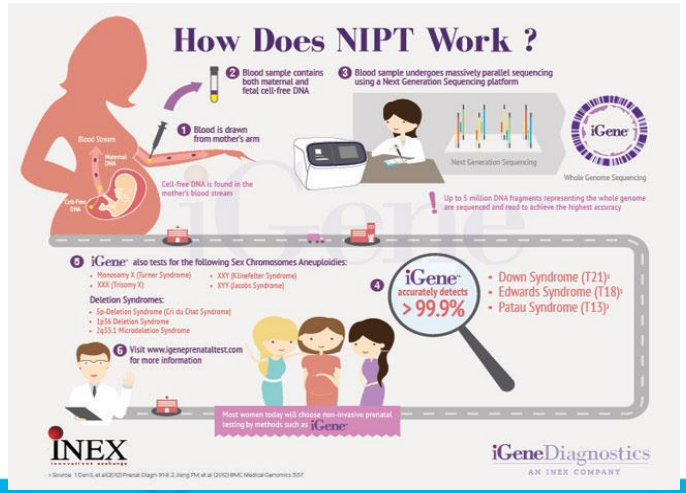
Cell Free DNA – conceptual

- Tissues injury (and turnover) leads to “spillage” of nuclear DNA into the circulation
- Pregnancy, Cancer, and Organ Transplantation are situations where there is >1 source of DNA in the circulation
- Quantifying Donor Derived Cell Free DNA (dcfDNA) gives an insight into the state of the graft –the “*liquid biopsy*”
- How would we use this technology in lung transplantation
  - Studies to date have focused on comparison of cfDNA to histology (gold standard with major caveats)
  - Fundamental unanswered question: Is low level injury meaningful?
  - Is finding “injury” sufficient – or do we need to have additional signals to tell us about mechanism?

Cell Free DNA is already “a thing” in medicine

- Experience in genetic testing for fetal chromosomal abnormalities has already dramatically reduced the need for amniocentesis in obstetrics.





Cell Free DNA – technical issues

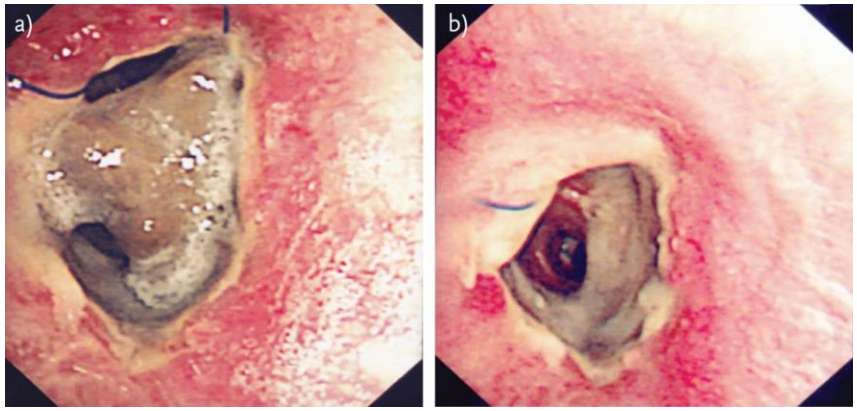
- Helpful to have a source of donor and recipient – SNP libraries
- Plasma recovered – DNA extracted.
- Libraries made, Next Gen Sequencing (50 -100 bp reads)
- Ultimate readout – percentage of total cell free DNA which is “foreign”

In house turn around time about 24 hours

Considerations

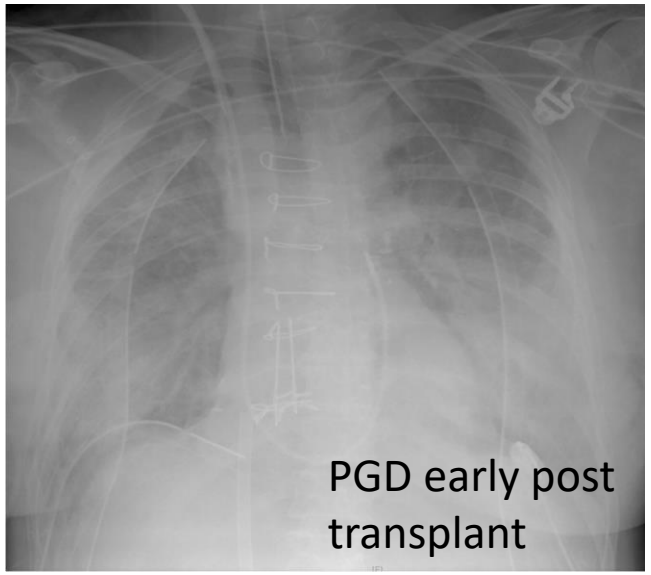
- Most cf DNA is nucleosomal – protected from degradation
- Read technology requires fragment lengths of 50+ bp
  - Small degraded pieces will not be picked up
  - Finding a specific sequence (donor HLA gene) may harder than a global measure of foreign DNA.

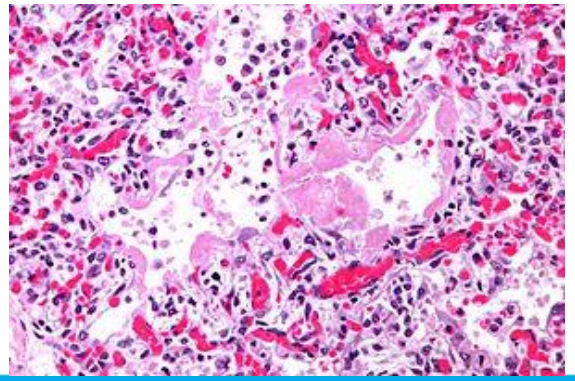
Lung Transplant = Lung Injury



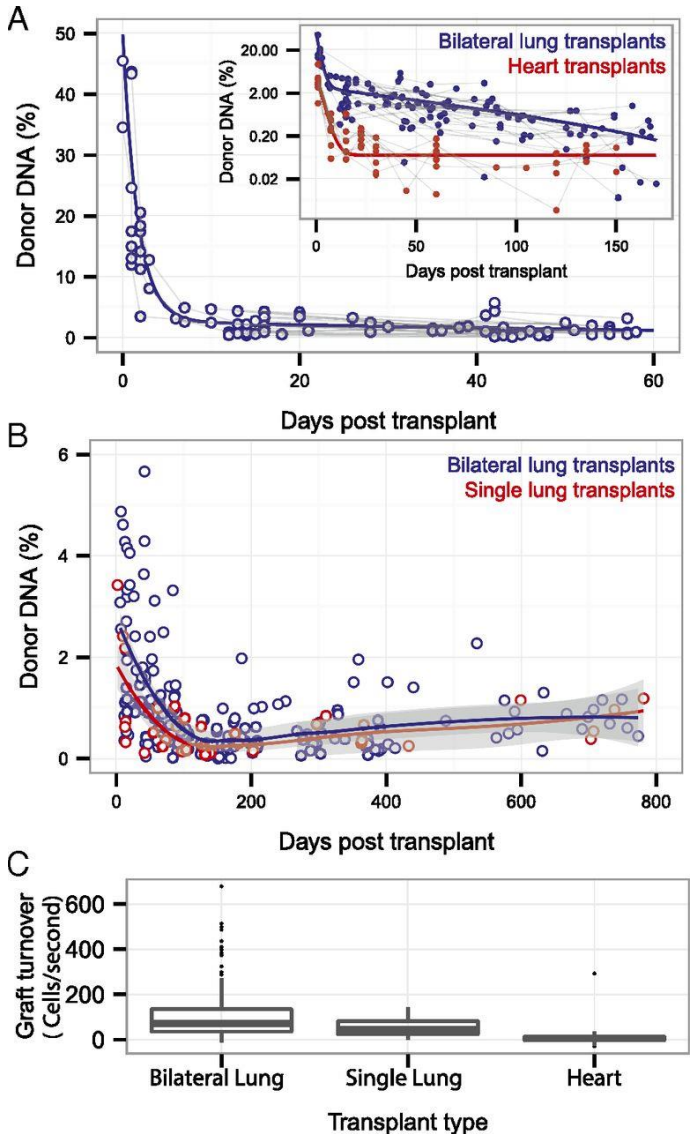
Mucosal sloughing anastomotic site

J. Fuller and A. Fisher ERS 2013





Resolving DAD



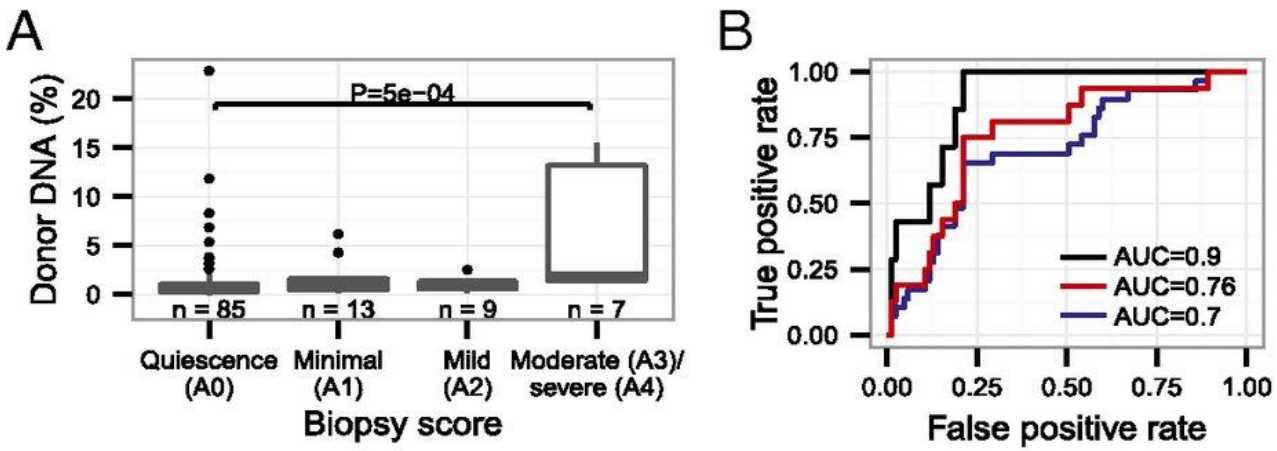
There is considerably more cfDNA in lung compared to heart transplant

It takes about 6 months to get to steady state

More cfDNA in bilateral transplants: we need to think about tissue "dose"

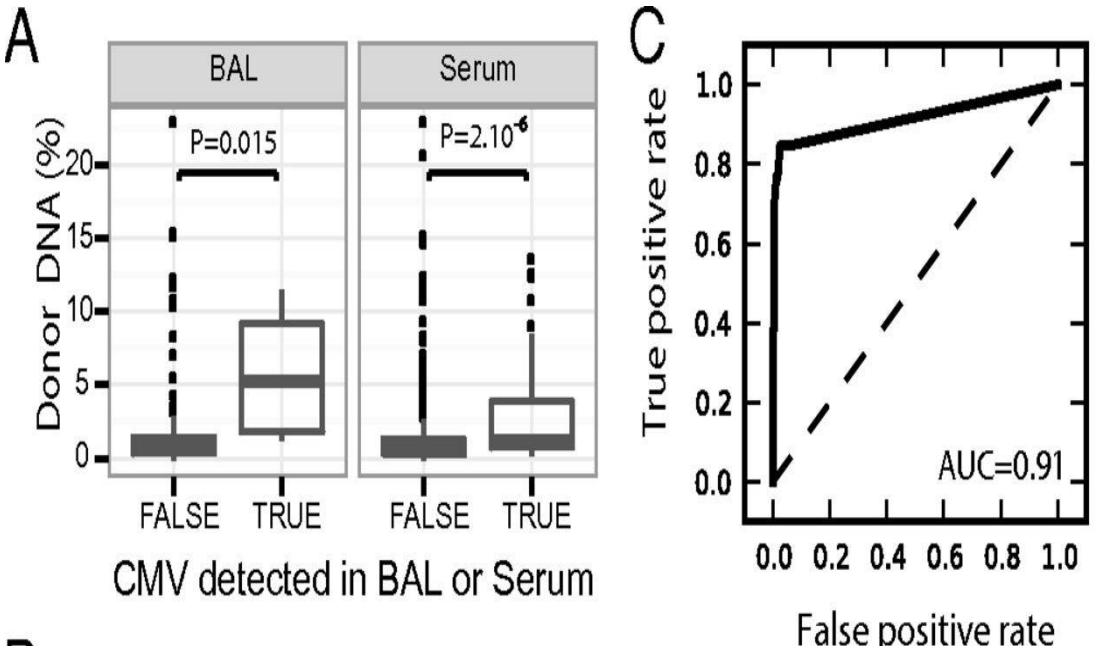
Iwijn De Vlaminck et al. PNAS 2015;112:13336-13341

Can we use dd cf DNA to inform us about rejection?



51 subjects, 114 biopsy procedures. All samples at least 6 months post transplant. They note an increase in dd cfDNA in rejection. Perhaps most importantly, increased dd cfDNA is most useful when comparing normal biospies to the highest grades of rejection.

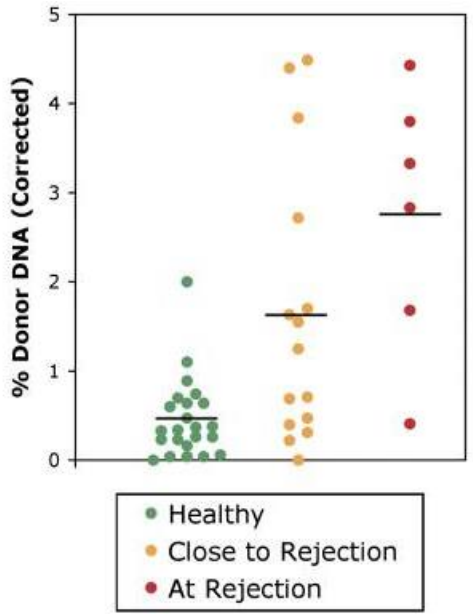
Does infection increase dd cfDNA?



CMV infection strongly associated with increased donor DNA in the recipient circulation. Hence the clinical utility of dd cfDNA is dependent on simultaneously ruling out concurrent infection

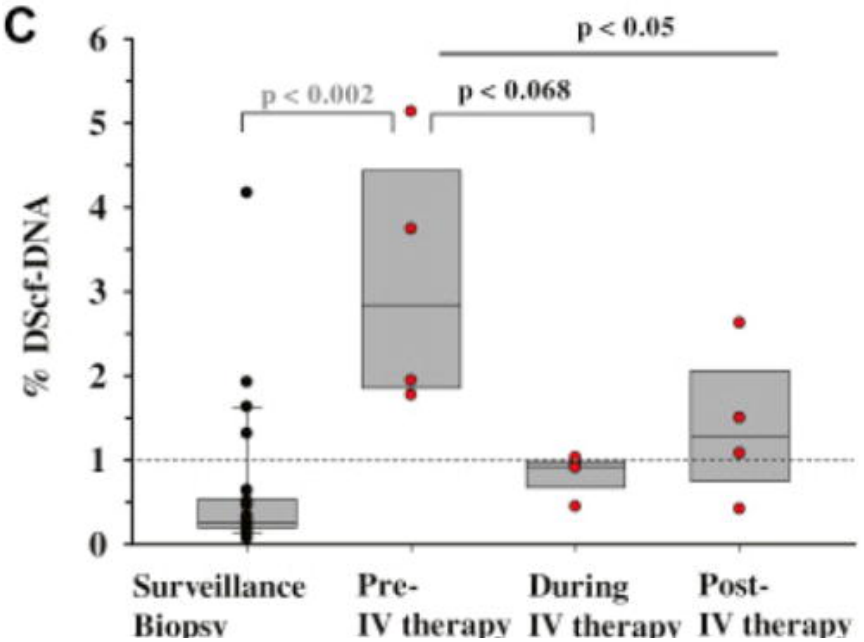
Iwijn De Vlaminck et al. PNAS 2015;112:13336-13341

Heart Transplantation and cfDNA



Adult heart transplant recipients and cf DNA. AUC =0.83, at 1% threshold Sensitivity 58%, Specificity 93%

TM Snyder et al. PNAS 2011; 108:6229-34

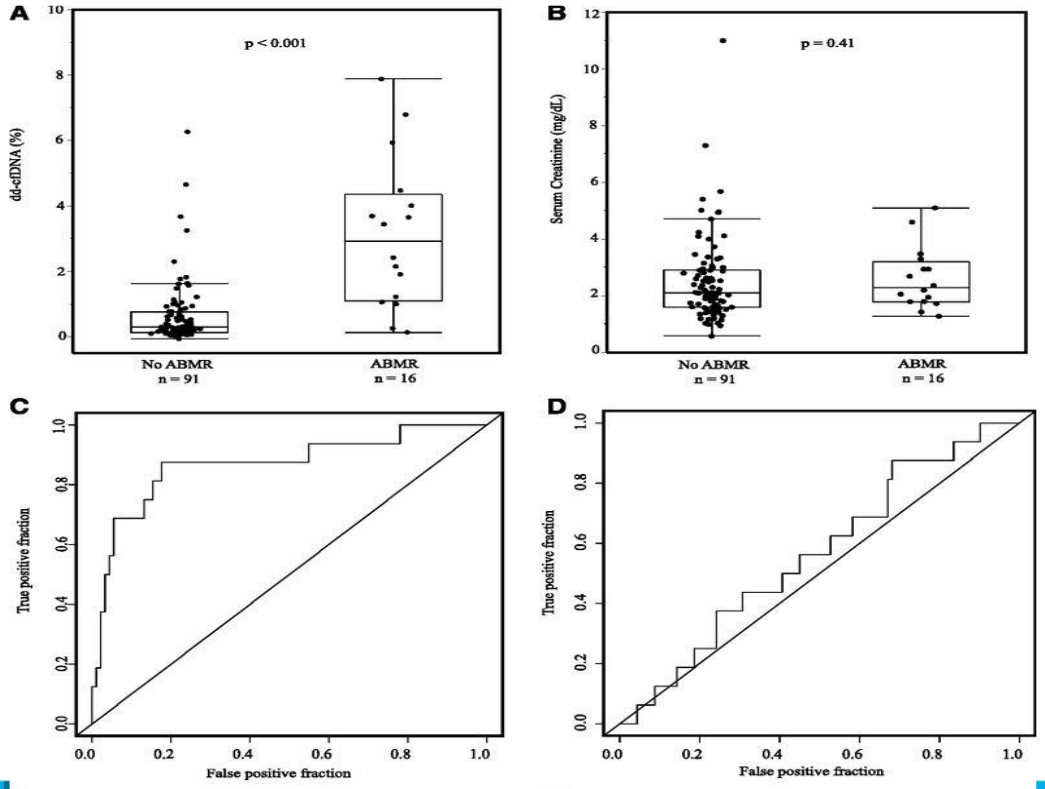


32 pediatric heart transplant patients. Cellular rejection had significant increased donor cf DNA and responds to therapy.

Mats Hidestrand et al. J Am Coll Cardiol 2014; 63:1224-26

Renal Transplantation

Cell free DNA%



Serum Creatinine

Prospective study from DART group (14 centers) in adult renal transplants. Rejection determined by renal biopsy and the performance of cfDNA vs. serum creatinine tested as biomarker. For ABMR the AUC of the ROC was .87.

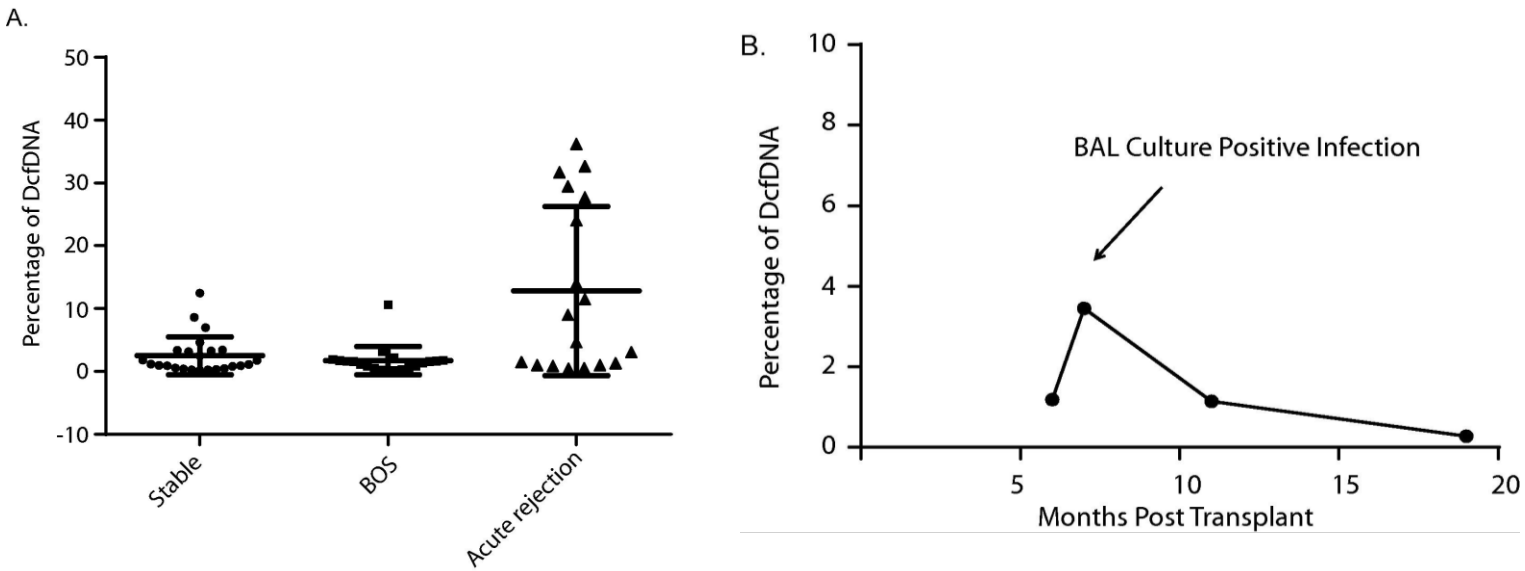
AUC of ROC for any rejection (including TCMR) was .74.

Roy Bloom et al. JASN 2017; 28:2221-32

What about using circulating specific donor DNA sequences?

- Concept is that with HLA specific differences there are specific sequences which can be probed in blood
- Computationally simplified
- Potentially cheaper
- Can be used in situations where “pure” donor material for SNP profiling not available

Detection of Donor HLA-DR DNA in serum



62 samples, 18 patients. At least 2 weeks post transplant. Serum pcr and sequencing of donor-HLA DR sequences.

"In our experiment, the measurement of cfDNA takes about 8h and the reagent costs less than 40 dollars per sample once the panel is established."

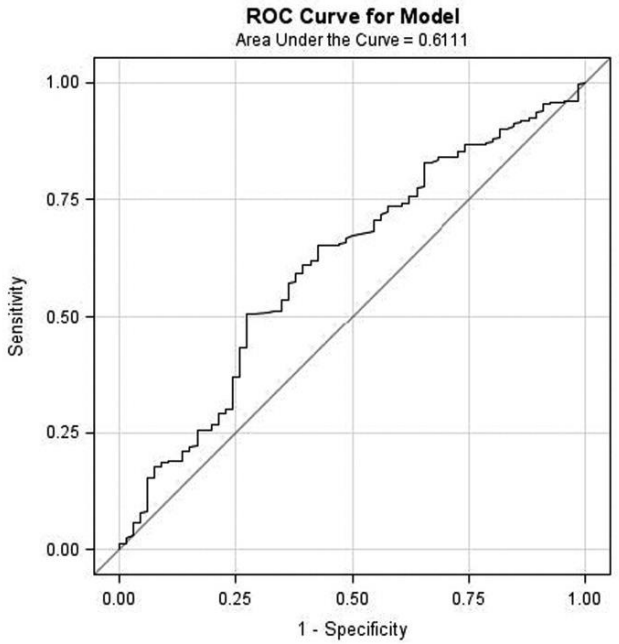
Jun Zou, T Mohanakumar et al. Human Immunology 2017; 78:342-9

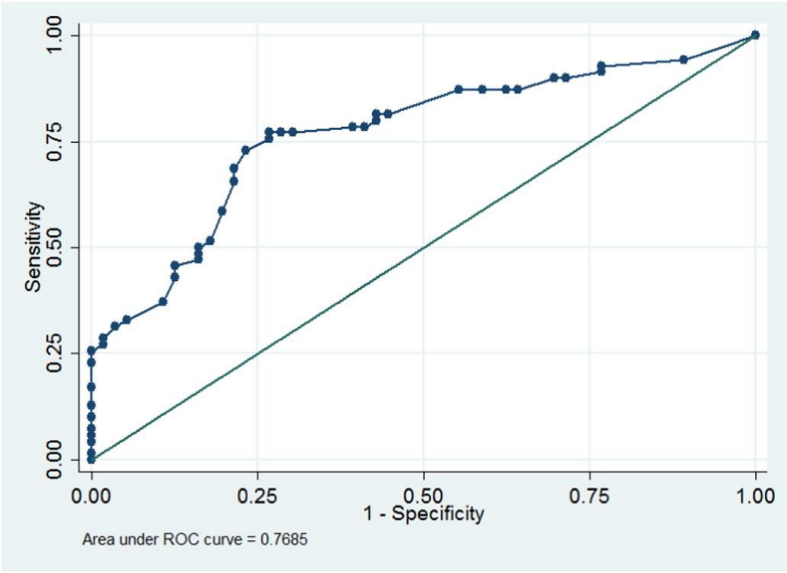
Immuno-Know: a cautionary tale

- Non-invasive test of blood that quantifies T cell activation
- Ultimately licensed by Cylex corporation
- Required outside testing, turn-around time was a major issue
- While there were differences among subjects with rejection, infection and quiescence, the degree of overlap limited clinical utility

Immuno-Know: in clinical practice not particularly helpful

B





ROC cure for use of high IMK (452) to detect cellular rejection. AUC =.61.

Mike Shino et al. JHLT 2012; 31:996-1002

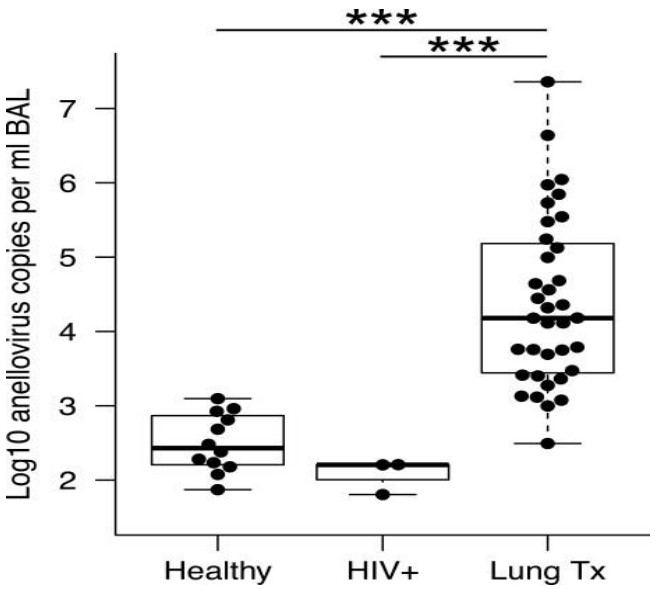
ROC curve for use of low value IMK <226 to detect ***infection***. AUC =.768.

Davide Piloni et al. Transplant Immunology 2016; 37:35-9

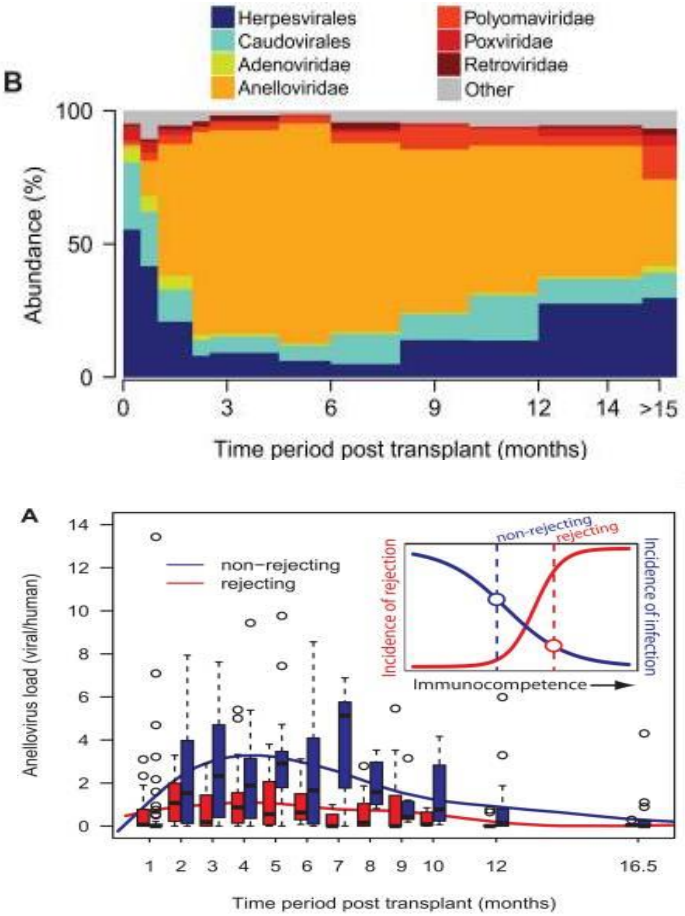
cfDNA and metagenomoics –tracking viral patterns over time

- Unbiased assessment of lung transplant patient's blood and BAL reveals a rich diversity of viral taxa (of unclear significance)
- Early studies have largely focused on Torque-Teno virus (aka anneloviridae) –ssDNA viruses
- Up to 80% of the viral DNA recovered does not correspond to annotated viruses –
  - Are these relevant pathogens for humans?
  - Possible contribution of bacteriophages

Viral metagenomics – a signal for immunosuppression



"Viral metagenomics reveal blooms of anelloviruses in the respiratory tract of lung transplant recipients" JC Young and Ron Collman AJT 2015; 15:200-9.



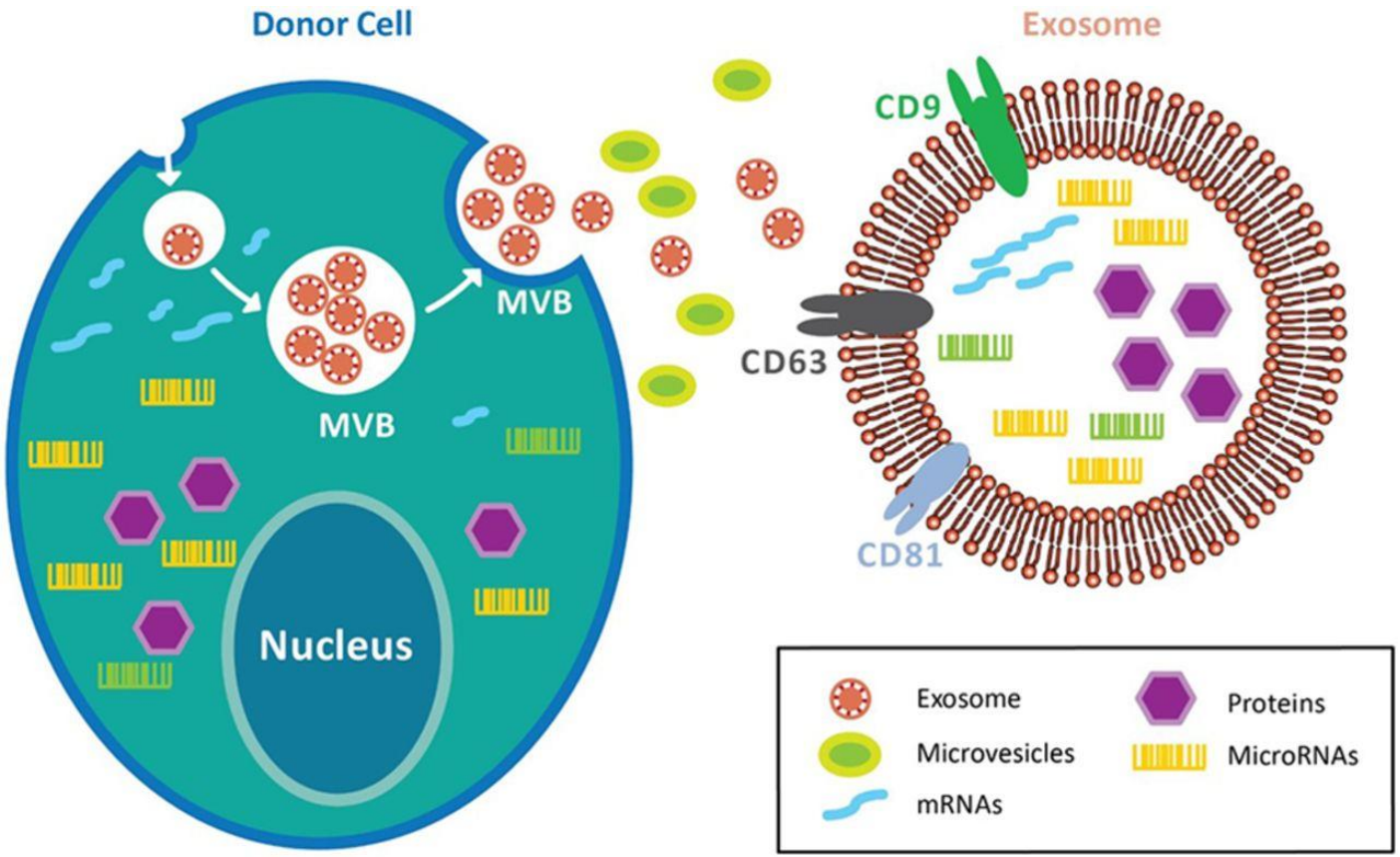
"Temporal response of the Human Virome to Immunosuppression and Antiviral Therapy". Iwijn De Vlaminck. Cell 2013; 21: 1178-87

Shotgun metagenomics is DIY – but available

Chiu Lab UCSF – they will run samples as a reference lab for metagenomics Next-Gen Sequencing (covers viral, fungal, protozoal sequences. Per website "…for research use only"

http://vddc.ucsf.edu/collab.html

Exosomes –a biomarker?



Ji-Hye Jung et al. Circulation Research 2017; 120:407-17

Exosomes/Microvesicles

- Previously felt to be inert cell fragments
- Microvessicles ranging between 30-300 nm
- Contain small bits of cargo
  - Cell surface molecules
  - RNAs including miRNAs
- Can reflect cell turnover for cells without great intrinsic process for protein breakdown
- May also reflect a way for cells to communicate – a Twitter for cells

Microvesicles as therapy

RESEARCH Open Access

Mesenchymal stem cells microvesicles stabilize endothelial barrier function partly mediated by hepatocyte growth factor (HGF)

Hualing Wang[1†], Ruiqiang Zheng[1†], Qihong Chen[2*], Jun Shao[2], Jiangquan Yu[2] and Shuling Hu[3]

Abstract

Background: Mesenchymal stem cells microvesicles (MSC-MVs) stabilize endothelial barrier function in acute lung injury (ALI); however, the detailed mechanism remains to be further defined. Hepatocyte growth factor (HGF), which is derived from MSC-MVs, might have a key role in the restoration of endothelial barrier function by MSC-MVs.

Methods: MSCs with lentiviral vector-mediated HGF gene knockdown (siHGF-MSC) were generated. A co-culture model of pulmonary microvascular endothelial cells and MSC-MVs collected from MSCs or siHGF-MSCs after 24 h of hypoxic culture was utilized. Then, endothelial paracellular and transcellular permeabilities were detected. VE-cadherin, and occludin protein expression in the endothelial cells was measured using Western blot. Endothelial cell proliferation was analysed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) assay. Endothelial cell apoptosis was analysed using TUNEL assay. Finally, IL-6 and IL-10 production was determined via an enzyme-linked immunosorbent assay (ELISA).

Results: Treatment with MSC-MVs significantly decreased LPS-induced endothelial paracellular and transcellular permeabilities, and the effect was significantly inhibited after HGF gene knockdown in MSC-MVs. Furthermore, treatment with MSC-MVs increased the expression of the endothelial intercellular junction proteins VE-cadherin and occludin. Treatment with MSC-MVs also decreased endothelial apoptosis and induced endothelial cell proliferation. Finally, the treatment reduced IL-6 production and increased IL-10 production in the conditioned media of endothelial cells. However, the effects of the treatment with MSC-MVs were inhibited after HGF gene knockdown.

Conclusions: MSC-MVs protect the barrier functions of pulmonary microvascular endothelial cells, which can be partly attributed to the presence of HGF in the MSC-MVs.

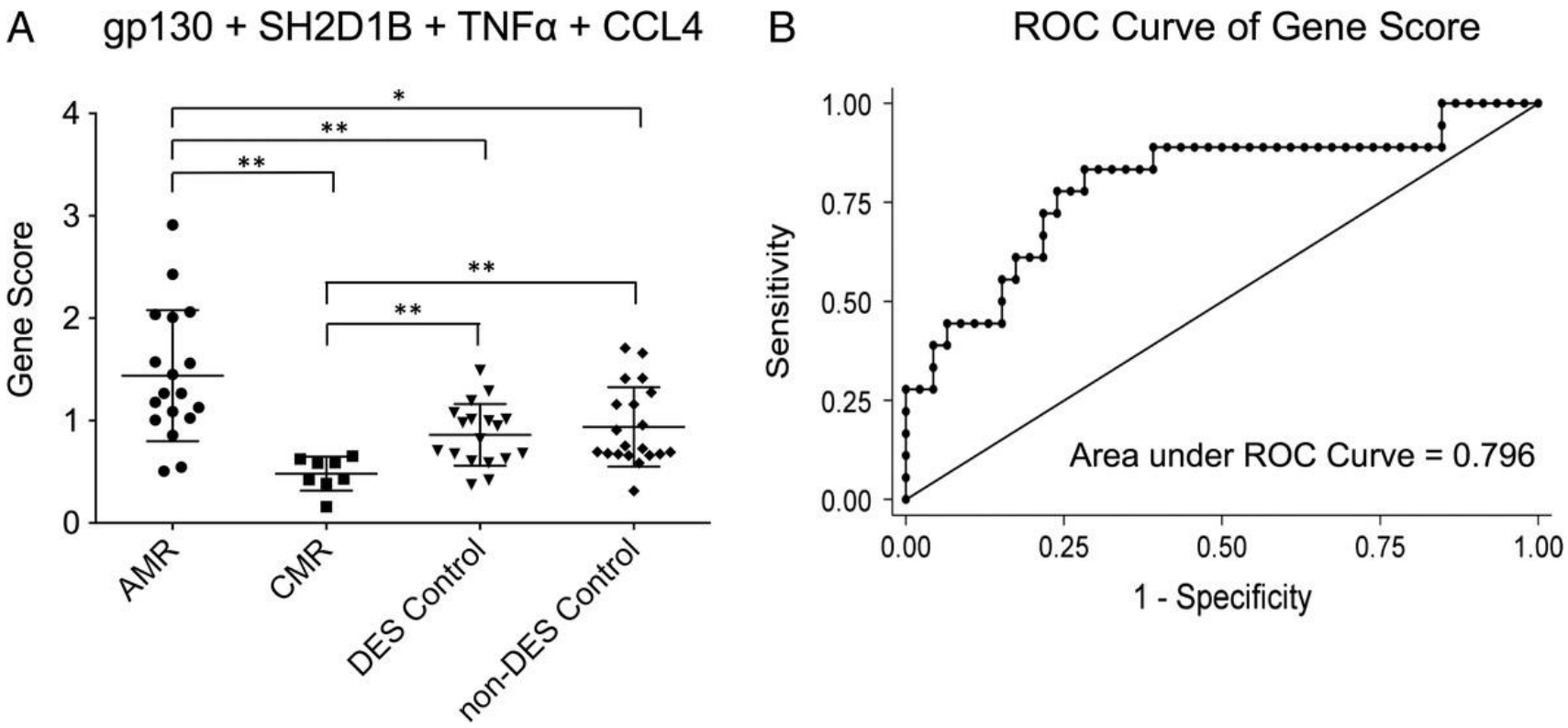
Keywords: Mesenchymal stem cells microvesicles, Hepatocyte growth factor, Endothelial permeability, Acute lung injury

Background

Acute lung injury (ALI) is characterized by increased lung permeability, pulmonary oedema and diffuse inflammation and results in the disruption of alveolar-bacterial lipopolysaccharides (LPS), lead to an increase in endothelial permeability by activating inflammatory responses, which contributes to the development of ALI. There are two pathways regulating permeability across

MSCs can mitigate against LPS induced ARDS (animals and ex-vivo lungs). MSC conditioned media had the same protective effect. One of the mechanisms is that MSCs secrete exosomes rich in factors that stabilize the endothelial barrier.

Exosome RNA Profiling For AMR in Kidney Transplants

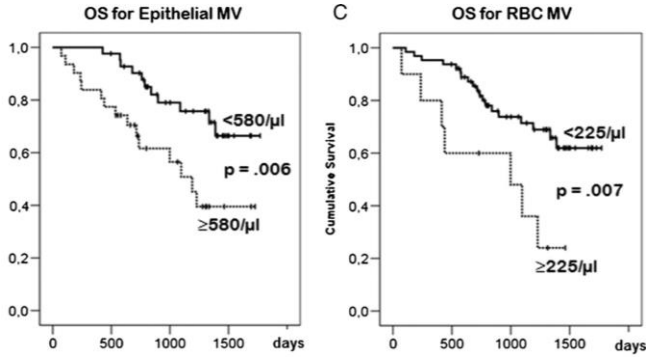


Plasma exosomal RNA gene transcripts from renal patients with AMR and controls. In final model a score based on 4 transcripts had a reasonable performance characteristic for non-invasively detecting AMR.

Hao Zhang et al. Transplantation 2017; 101:2419-28

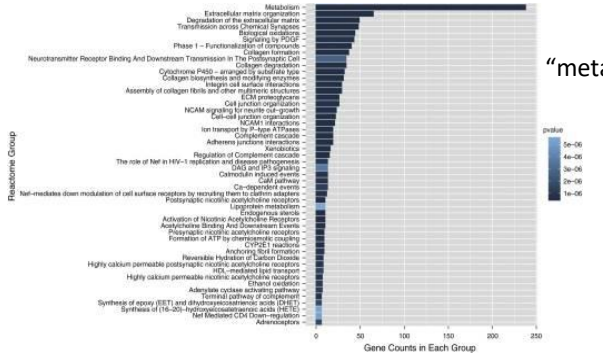
Microvesicles show promise in lung transplant monitoring

MP Antigen	Control Mean	Control 95% CI	CLAD Mean	CLAD 95% CI	*P* (*t* test)
Total (Annexin V)	4305	3670-4940	4668	2779-6556	0.71
Epithelial (EpCAM)	436	293-579	1533	597-2469	0.024
RBC (CD235a)	57	33-81	158	91-224	0.005
Platelet (CD41a)	46	32-61	65	38-93	0.21
HLA-II (HLA-DR/DQ)	1592	1255-1929	1712	944-2481	0.77
TLR-4 (CD284)	1803	1386-2220	2120	1496-2745	0.39



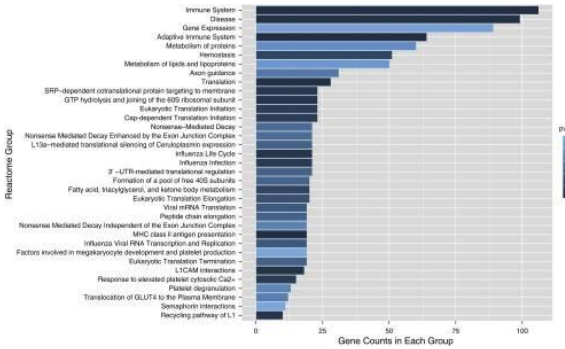
Anja Harms. Transplantation 2015; 99:2395-2400

Non-rejection



"metabolism genes" #1 hit

Rejection



"immune activation" #1 hit

Aric Gregson. AJRCCM 2015; 192:1290-1503

Key Points (are these technologies ready for prime time?)

Dad, are we there yet?

NO!

When will new technologies be “there”?

- Cell free DNA is a technology without a well formulated question…yet
  - Artifact of using an existing technology for a separate purpose
  - Longitudinal studies are key
- Viral next-gen sequencing
  - Needs to be paired with other measures to be informative
  - Cost needs to come down
- Microvesicles/Exosomes
  - Too early to tell, but hot topic
  - Lots of abstracts at ATC!

Thank You