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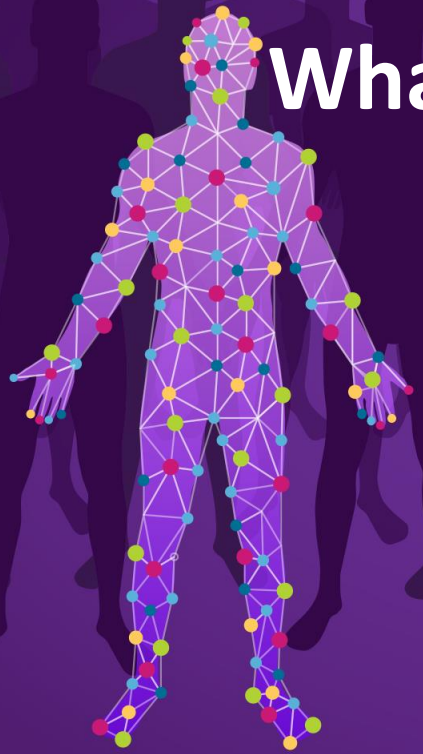
Measuring Immunoresponsiveness: What tools do we have in our arsenal?



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CEOT CUTTING EDGE of TRANSPLANTATION

TRANSPLANT SUMMIT 2019

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

FEBRUARY 21–23, 2019 • ARIZONA BILTMORE • PHOENIX, AZ

Disclosures

- No disclosures related to this discussion

Objectives

- 1. Review concept of measuring immunoresponsiveness
- 2. Discuss available tools that are or could be used for measuring immunoresponsiveness
- 3. Discuss potential future tools that could be used for measuring immunoresponsiveness

Immunoresponsiveness

- Ability to respond to foreign antigen
  - Must be aware of Ag presence (T cell mediated)
    - Direct pathway – T cells recognize intact allo-MHC molecules
    - Indirect pathway – T cells recognize processed alloantigens
  - Must be able to produce response

Patient Care Post-Transplant is Challenging

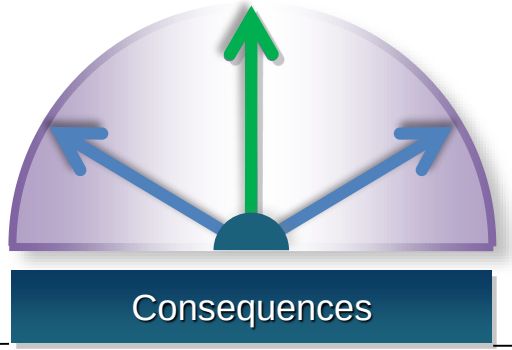
Patients are on life-long immunosuppressive drug therapy

Immunosuppression

TOO LITTLE

Increased risk of:

- Rejection ….leading to failure of the organ



TOO MUCH

Increased risk of:

- Infections
- Kidney failure
- Cancer
- Onset of Diabetes

Transplant Recipients are high value patients in the Health Care System
Median survival: Heart: 12 years; Kidney: 10 years

What We Have

ImmuKnow

- Quantification of cell-mediated immunity
- Measures adenosine triphosphatase (ATP) release from activated lymphocytes
- Overall level of immune responsiveness

The Benefit of Immune Monitoring (IM): A Review of 864 Immune Monitoring Assays in Heart Transplantation

- Between December 2000-July 2008, 864 IM assays from 296 patients were correlated to infection (requiring antimicrobial therapy) and treated rejection events within 1 month **after** IM testing.
- Of the 864 IM assays scores there were:
  - 38 subsequent episodes of infection
  - 8 subsequent episodes of treated rejection
- These were compared to 818 IM assays from stable patients without infection or rejection
- All patients were on tacrolimus, mycophenolate mofetil and +/- corticosteroids without induction therapy

- The average IM score was significantly lower in patients with infection vs. steady state patients:

  187 ± 126 vs. 280 ± 126, p<0.001

- The mean IM score was numerically higher in patients who developed rejection vs. steady state patients:

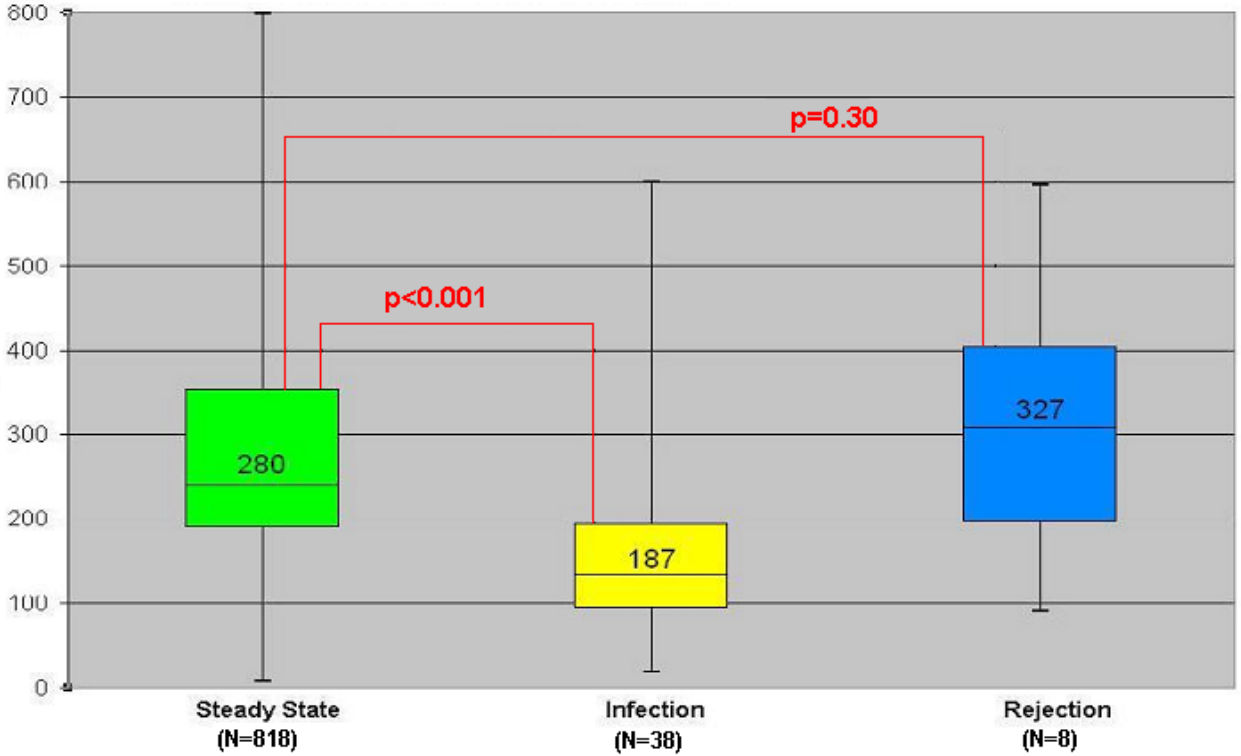
  327 ± 175 vs. 280 ± 126, p=0.30

- 3/8 rejection episodes had hemodynamic compromise and for these, the mean IM score was higher

  491 ± 121 vs. 280 ± 126

Kobashigawa, *J Heart Lung Transplant.* 2010 May;29(5):504-8.

Adult Heart Transplant Experience with Immune Monitoring



IM Study Conclusions

- The non-invasive IM test appears to predict infection in heart transplant patients
- The association between high IM scores and rejection is inconclusive due to the small number of rejection episodes

Kobashigawa, *J Heart Lung Transplant.* 2010 May;29(5):504-8.

The search for the perfect biomarker

IDEAL

- Noninvasive or minimally invasive
- High sensitivity and specificity
- Quick turnaround time
- Cost-effective
- Reproducible

LIMITATIONS

- Labor and complexity
- Lack of standardization
- Lack of cross-validation
- Time and cost-consuming
- Difficult to automate

BNP and immune monitoring in heart transplant

- 66 patients beyond 1st year post HTx were divided into: low (<250pg/ml) and high (≥250pg/ml) groups [1]
  - High BNP was an independent predictor of poor survival and was associated with allograft dysfunction and CAV
  - Lower BNP associated with 95% survival rate

- 146 primary Htx recipients retrospectively assessed with serial analysis of NT-pro BNP alongside biopsy schedule [2]
  - For a 2-fold increase in NT-proBNP, OR=2.9 for significant (≥2R) rejection
  - If 5-fold increase in NT-proBNP=9.1
  - A within-individual increase in NT-proBNP demonstrated a strong graded relationship with the odds of significant rejection independent of hemodynamic parameters.

1 Mehra et al., Am J Cardiol 2004;94:454–458
2 Kittleson et al., JHLT 2009;28:704–9

CRP and immune monitoring in heart transplant

- 210 patients assessed individual and combined value of NT-pro BNP and CRP assessed as markers of acute rejection, CAV and mortality.
  - Individually, increased NT-proBNP and CRP did not predict CAV
  - Combined elevation of the two identified patients at higher risk for CAV (HR 2.10) and mortality (HR 3.14)

Arora et al., Transplantation 2007

Troponins and immune monitoring

- 35 patients (422 samples) more than 3 months post-HTx assessed for troponin T serum concentrations compared to histological grade of acute cellular rejection [1]
  - Troponin T noted to increase in parallel with severity of graft rejection.
  - High negative predictive value for significant rejection (ISHLT grade 3/4) of 96.2% with cut-off of 15ng/L.

- Use of a novel high sensitivity Troponin I assay was retrospectively assessed in 98 post-transplant patients matched to endomyocardial biopsies [2]
  - cTnI concentrations were significantly higher in rejection (≥2R) samples versus non-rejection samples.
  - cTnI also increased in a graded manner with higher biopsy severity grades.
  - Cut-off point of 15ng/L- Sensitivity 94%, specificity 60%, negative predictive value 99%

1 Dengler et al., J Am Coll Cardiol 1998;32:405-12
2 Patel et al. Circ Heart Fail, 2014;7:463-469

Troponins and immune monitoring

- Troponin T measured in 90 recipients concurrent to endomyocardial biopsy who were 0-5 years post-transplant [1]
  - Only 1 of the 12 ISHLT grade 3 rejection specimens had corresponding elevated cTnT
  - Only 3 of the 29 ISHLT grade 2/3 specimens had elevated cTNT
  - Overall, very poor sensitivity shown by cTNT for ISHLT grade 2 or 3 rejection

- Prospective analysis comparing troponin I and T levels to biopsy results in 29 HTx recipients [2]
  - Only 2 rejection episodes (defined as ≥ISHLT grade 3), with no significant relationship between cTnT/cTnI and rejection.
  - Overall, troponins were not useful indicators of cardiac rejection

Alexis et al., JHLT 1998;17(4):395-8
Mullen et al., Eur J Cardiothorac Surg 2002;22;233-7

What We Could Have in Future

Biomarkers

- Pharmacokinetic
- Pharmacodynami ctivity, p7056 kinase
- Pharmacogenetic LR-4, TLR-9, TLR-2,-3,-5
- Markers of viral ir
- Immune markers
  - Soluble – cytok
  - T-cell activatior
  - T-cell proliferat
  - Intracellular ma ession, ATP levels in CD4+ cells

Barcelona Consensus Conference

- 19 experts in field of therapeutic drug monitoring of IS drugs and biomarkers in tx
- Reviewed all articles since 2000
- Three types of biomarkers
  - Those associated with risk of rejection (alloreactivity/tolerance)
  - Those reflecting individual response to IS
  - Those associated with graft dysfunction and injury

Biomarkers Associated with Risk of Rejection

- T-Cell IFN-gamma
- IL-2
- T-Cell Surface Antigens – no trials yet
- T-Cell Regulatory Populations – no trials yet

T cell IFN-gamma

- Literature:
  - Pleiotropic effect; can elicit inflammatory T-helper 1-driven immune responses or enable T-regulatory to control immune responses
  - It is the cell subpopulation that determines whether immune response will be effector or regulatory
  - Evaluated via ELISPOT assay
- Summary recs:
  - Monitoring intracellular or total IFN-gamma before and early after tx can help id high risk of acute rejection in kidney and liver tx
  - Monitoring production with donor-specific stim can help id pts who could get IS minimized
  - Ongoing trials

IL-2

- Literature:
  - Drives T-cell growth, induces T-reg differentiation, mediates activation-induced cell death
  - CD3, CD8 and CD69 cells most predictive
  - Evaluated via ELISPOT assay
- Summary recs:
  - Monitoring intracellular IL-2 before and early after tx can help id high risk of acute rejection in kidney and liver tx
  - IL-2 inhibition may reflect interindividual response to CNIs
  - Ongoing trials

Biomarkers Associated with Risk of Rejection

- Limitations of ELISPOT
  - Donor-specific cells not usually available
  - Impossible to simultaneously analyze different lymphocyte subsets and or effector/regulatory cytokines

Biomarkers that Reflect Pt response to IS

- Target Enzyme Activity
- Nuclear Factor of Activated T-Cell-Regulated Gene Expression
- Pharmacogenetic Markers

Target Enzyme Activity

- IMPDH
  - Inosine-monophosphate-dehydrogenase (IMPDH) is inhibited by MPA
  - Determination of IMPDH activity before tx might help id renal tx pts a higher risk of rejection or MPA-associated side effects
  - Monitoring IMPDH activity may complement MPA drug levels to better guide MPA therapy
  - Ongoing trials
- P-p70S6 kinase/pS6RP
  - Suppressed by mTORs
  - Assays sensitive to other IS drugs
  - Not ready for prime time

Nuclear Factor of Activated T-Cell-Regulated Gene Expression

- Real-time polymerase chain reaction technique allows rapid, highly reproducible tool
- Test semiautomated, standardized
- Low variability in individual pt
- Residual NFAT-regulated gene expression helps id renal tx pts at risk infections, malignancy, rejection, CV risk
- Monitoring residual NFAT-reg gene expression complements CNI trough levels
- Trials ongoing

Pharmacogenetic Markers

- Based on id of constitutive genetic markers located in genes influencing drug responses
- CsA – CYP3A4*22, donor ABCB1
- Tac – CYP3A5
- MPA – UGT1A9, IMPDH1
- mTORi – no validated PG biomarkers

- CYP3A5 genotype-based adjustment of Tac helpful
- No beneficial clinical outcomes trials yet

CPIC Guidelines:
CYP3A5 genotyping and tacrolimus

Table 2 Dosing recommendations for tacrolimus based on *CYP3A5* phenotype

CYP3A5 phenotype[a]	Implications for tacrolimus pharmacologic measures	Therapeutic recommendations[b]	Classification of recommendations[c]
Extensive metabolizer (CYP3A5 expresser)	Lower dose-adjusted trough concentrations of tacrolimus and decreased chance of achieving target tacrolimus concentrations.	Increase starting dose 1.5–2 times recommended starting dose.[d] Total starting dose should not exceed 0.3 mg/kg/day. Use therapeutic drug monitoring to guide dose adjustments.	Strong
Intermediate metabolizer (CYP3A5 expresser)	Lower dose-adjusted trough concentrations of tacrolimus and decreased chance of achieving target tacrolimus concentrations.	Increase starting dose 1.5–2 times recommended starting dose.[a] Total starting dose should not exceed 0.3 mg/kg/day. Use therapeutic drug monitoring to guide dose adjustments.	Strong
Poor metabolizer (CYP3A5 nonexpresser)	Higher ("normal") dose-adjusted trough concentrations of tacrolimus and increased chance of achieving target tacrolimus concentrations.	Initiate therapy with standard recommended dose. Use therapeutic drug monitoring to guide dose adjustments.	Strong

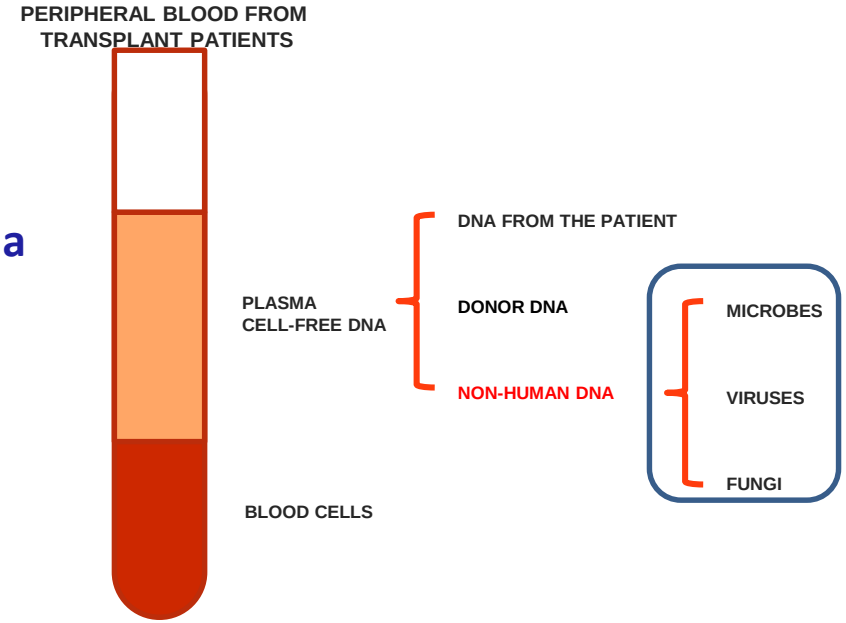
[a]Typically, with other CYP enzymes, an extensive metabolizer would be classified as a "normal" metabolizer, and, therefore, the drug dose would not change based on the patient's genotype. However, in the case of CYP3A5 and tacrolimus, a CYP3A5 expresser (i.e., CYP3A5 extensive metabolizer or intermediate metabolizer) would require a higher recommended starting dose and the CYP3A5 nonexpresser (i.e., poor metabolizer) would require the standard recommended starting dose. [b]This recommendation includes the use of tacrolimus in kidney, heart, lung, and hematopoietic stem cell transplant patients, and liver transplant patients in which the donor and recipient genotypes are identical. [c]Rating scheme is described in **Supplementary Data** online. [d]Further dose adjustments or selection of alternative therapy may be necessary because of other clinical factors (e.g., medication interactions, or hepatic function).

Biomarkers Associated with Graft Dysfunction or Injury

- Chemokines
  - Small molecular wt proteins secreted by many cells
  - Direct leukocyte navigation, associated with inflammatory and immune responses
  - CXCR-3, CXCL-9, CXCL-10 are abundant in rejection grafts
- Donor derived cell free DNA

Could a Virus be the Solution?

Non-human DNA is also present in plasma



Relative genomic abundance

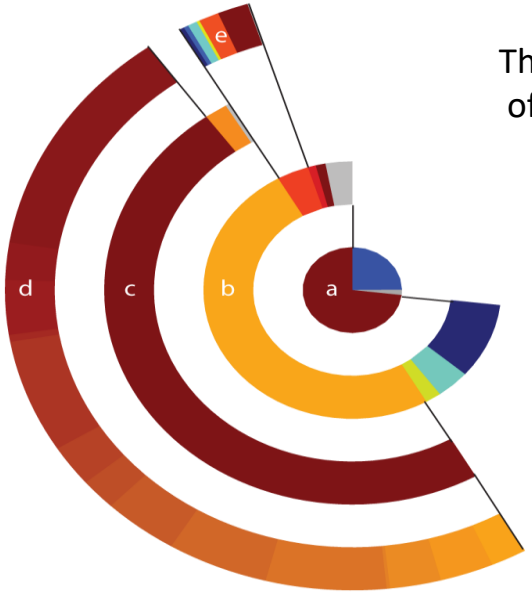
a: Superkingdom
- Bacteria 25%
- Eukaryota 2%
- Viruses 73%

b: Viruses: order and family
- Herpesvirales 13%
- Caudovirales 5%
- Adenoviridae 2%
- Anelloviridae 68%
- Polyomaviridae 5%
- Poxviridae 1%
- Retroviridae 1%
- Other 5%

c: Anelloviridae: genera
- Alphatorquevirus 97%
- Betatorquevirus 3%

d: Alphatorque viruses
- TTV1 3%
- TTV10 5%
- TTV12 5%
- TTV14 <1%
- TTV15 12%
- TTV16 10%
- TTV19 7%
- TTV27 4%
- TTV28 5%
- TTV3 13%
- TTV4 1%
- TTV6 6%
- TTV7 4%
- TTV8 26%

e: Polyoma viruses
- WU Polyomavirus 6%
- SV40 6%
- Polyomavirus HPyV6 13%
- TS associated polyomavirus 4%
- JC polyomavirus 27%
- BK polyomavirus 41%



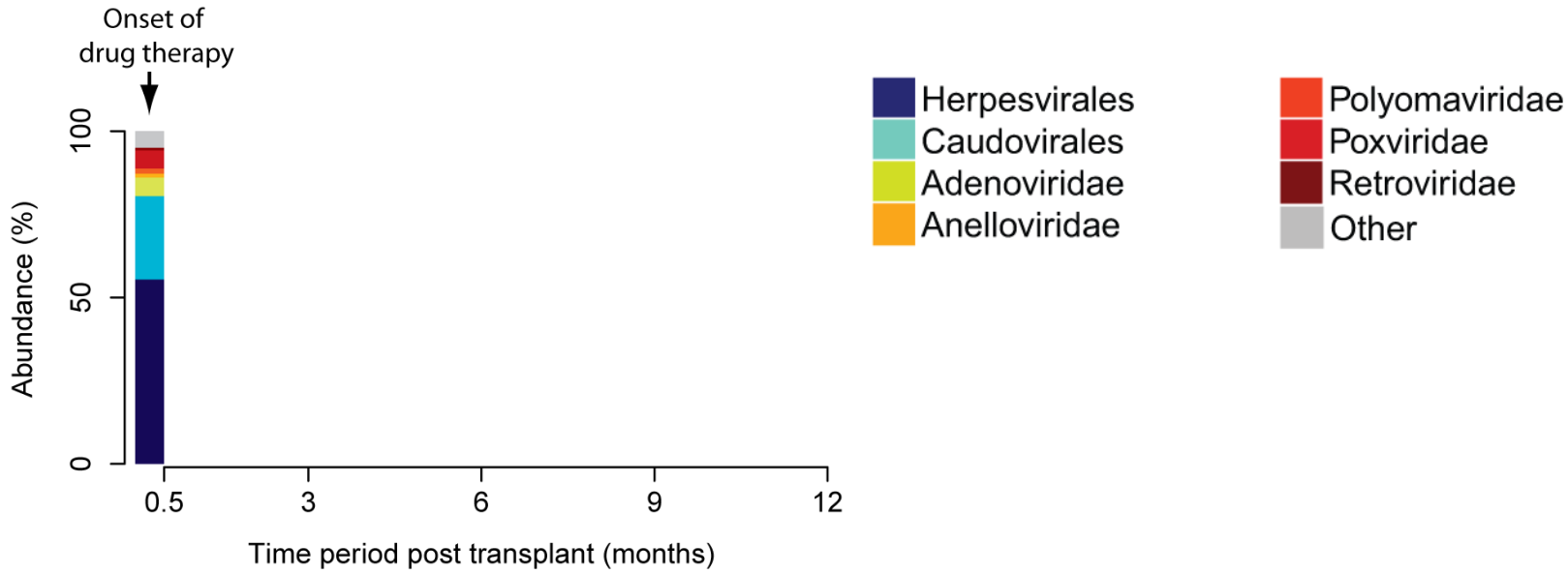
The anelloviridae fraction is primarily composed of viruses from the alphatorque genus.

De Vlaminck, Cell, 2013

Torque Teno Virus Load

- Quantified via sequencing cell-free viral DNA from recipient blood
- Most abundant member of the Anelloviridae
- Nearly ubiquitous in humans, asymptomatic infection in childhood
- Increase dramatically during first 6 months after tx then decline with weaning of IS meds

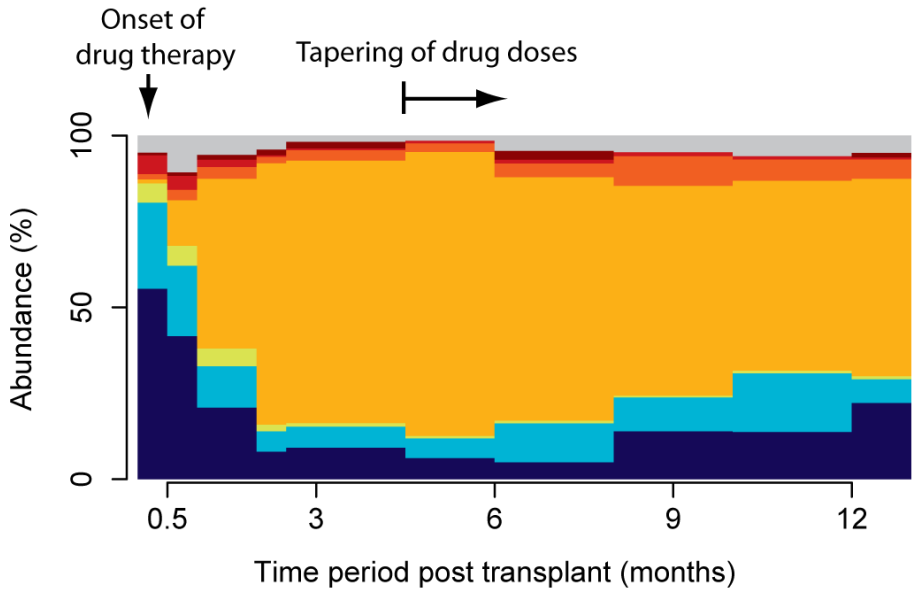
Virome temporal dynamics



De Vlaminck, Cell, 2013

96 patients; 656 samples

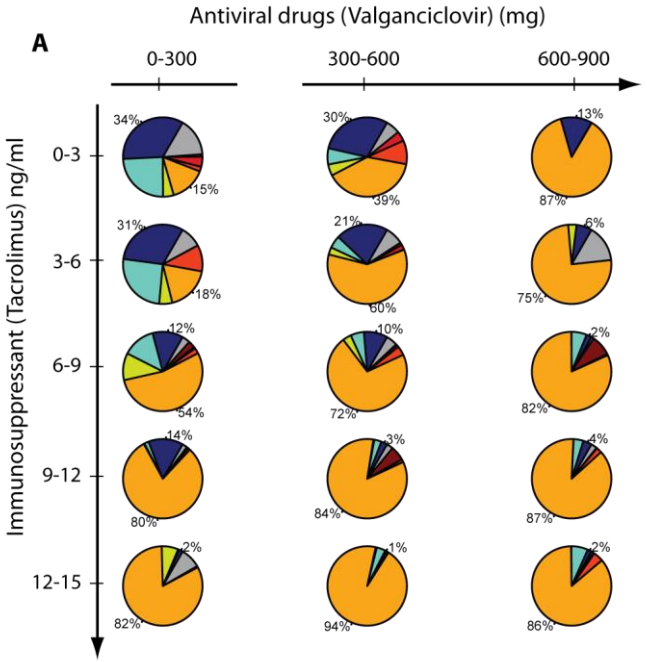
Virome temporal dynamics



De Vlaminck, Cell, 2013

96 patients; 656 samples

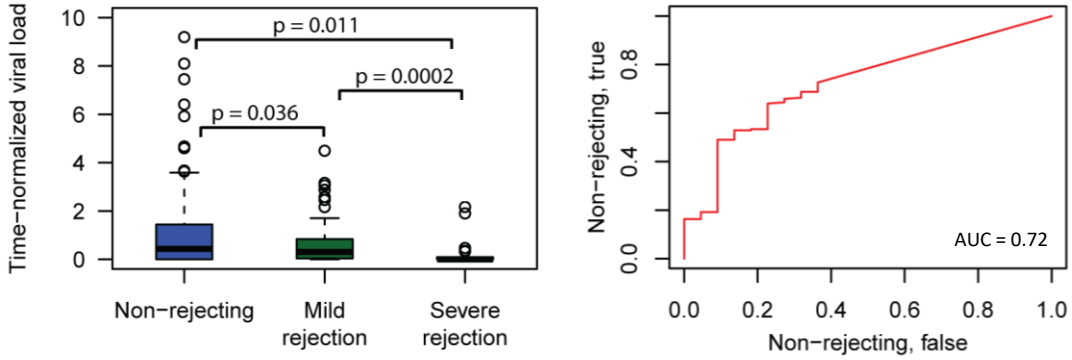
Immunosuppressants and antivirals alter structure of the virome



47 patients, 380 samples

De Vlaminck, Cell, 2013

Anellovirus load for rejecting vs non-rejecting recipients



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

De Vlaminck, Cell, 2013

Conclusions

- Need wide variety of components to test for
- Most too complex for clinical setting
- Should be noninvasive, rapid turn around time, accurate, precise, cost effective, standardizable
- Most studies are single center
- Very few commercial kits