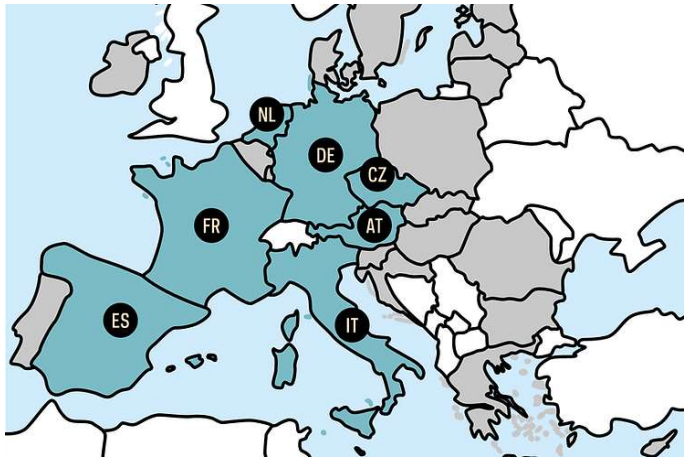
La personalizzazione della terapia immunosoppressiva è possibile?

Fabrizio Maggi
University of Insubria

Varese

Personalisation of immunosuppression by monitoring viral load post kidney transplantation – a randomized controlled phase II trial

TTV GUIDE
Torque Teno Virus
Based Immune Monitoring



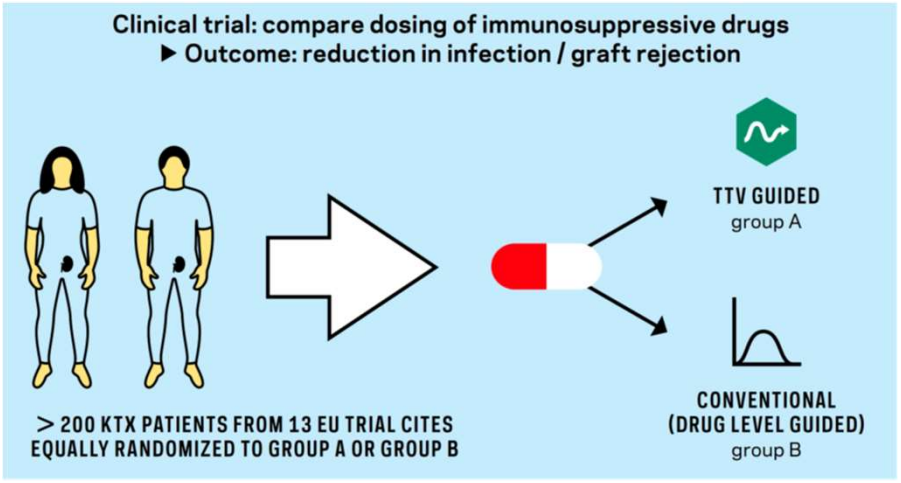
Project Information

TTV GUIDE TX

Grant agreement ID: 896932

Start date	**1 May 2021**
End date	**30 April 2026**
Total cost	**€ 6 099 8319 831**

Members: 19 partners from 7 EU countries
Coordinated by Medizinische Universitaet, Wien, Austria



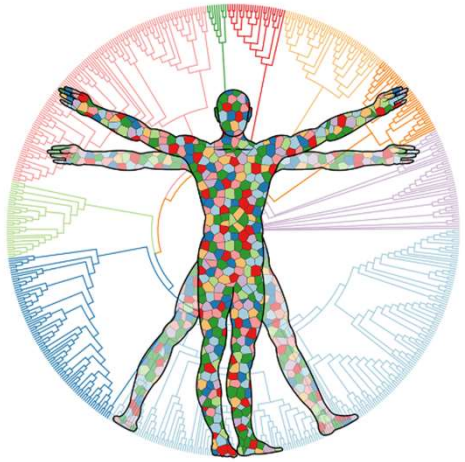
Biomarker assays useful in predicting post-transplant complications

Assay	Test Specifications	Availability
Torque teno virus	Measures level of viral DNA in body fluid	Research setting only
Epstein-Barr virus DNAemia	Measures level of viral DNA in body fluid	Commercially available, multiple labs
Cylex Immunoknow	Nonspecific cell-mediated immunity assay measuring adenosine triphosphate release from $CD4^+$ T cells	Commercially available, Viracor-IBT Laboratories
T-SPOT.PRT	Global cell-mediated immunity assay using common donor antigens	Oxford Immunotec
QuantiFERON MONITOR	Global cell-mediated immunity assay involving proprietary antigens that stimulate both innate and adaptive immunity	Qiagen
T-Track ImmunoScan	Cell-mediated immunity assay involving a mixture of antigens derived from different viruses and bacteria	Lophius
Immunobiogram	Bioassay of cellular immune response to panel of immunosuppressant drugs	Research setting only, BIOHOPE Scientific

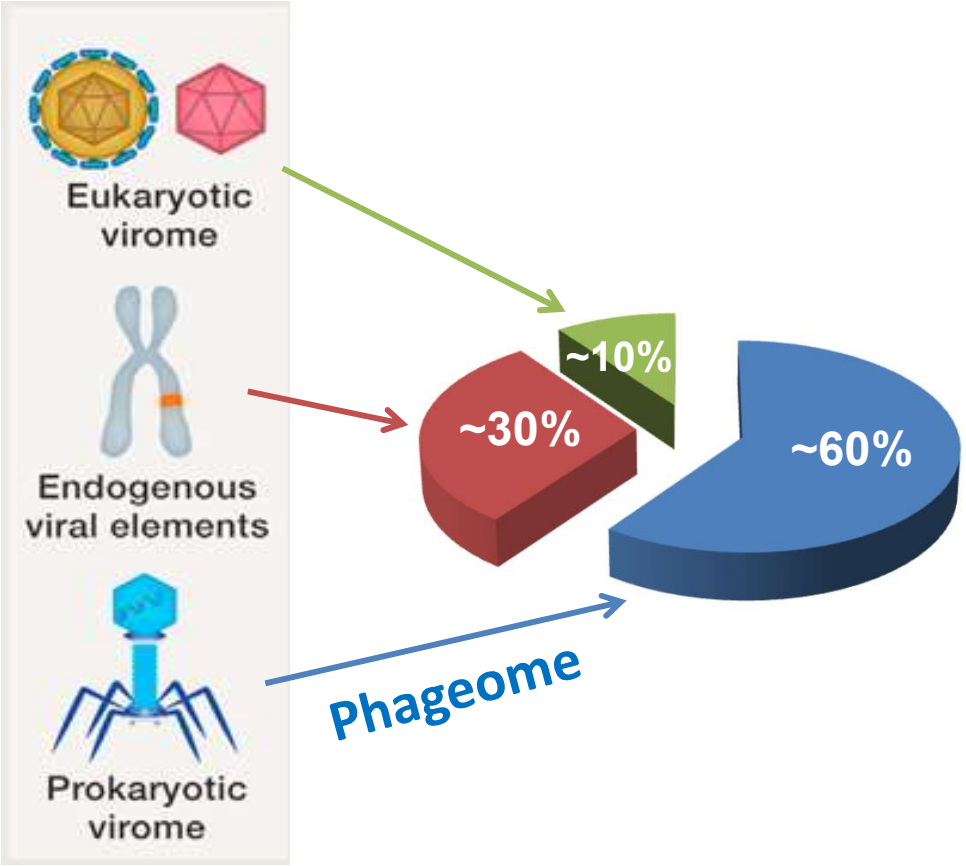
Cotton, J Infect Dis 2018

The human virome

- ***the full compendium of viruses from a particular habitat, including not only pathogenic viruses but also viruses essentially devoid of pathogenic potential***

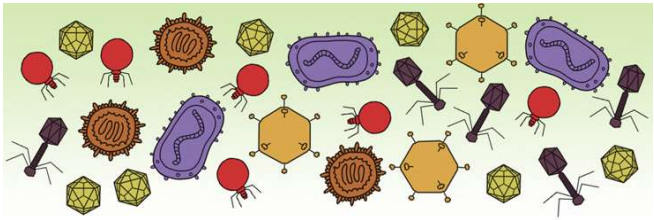


Virome

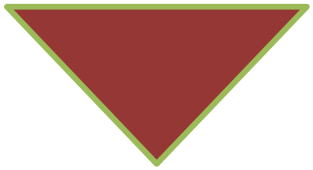


Wylie et al. Transl Res 2012; Virgin, Cell 2014; Zou et al. Microbiome 2016; Freer et al. Curr Med Chem 2018

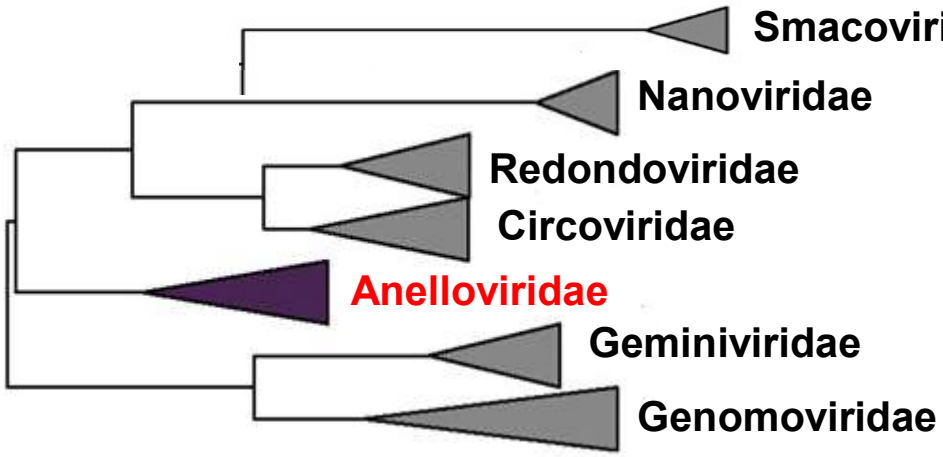
The "core" of virome



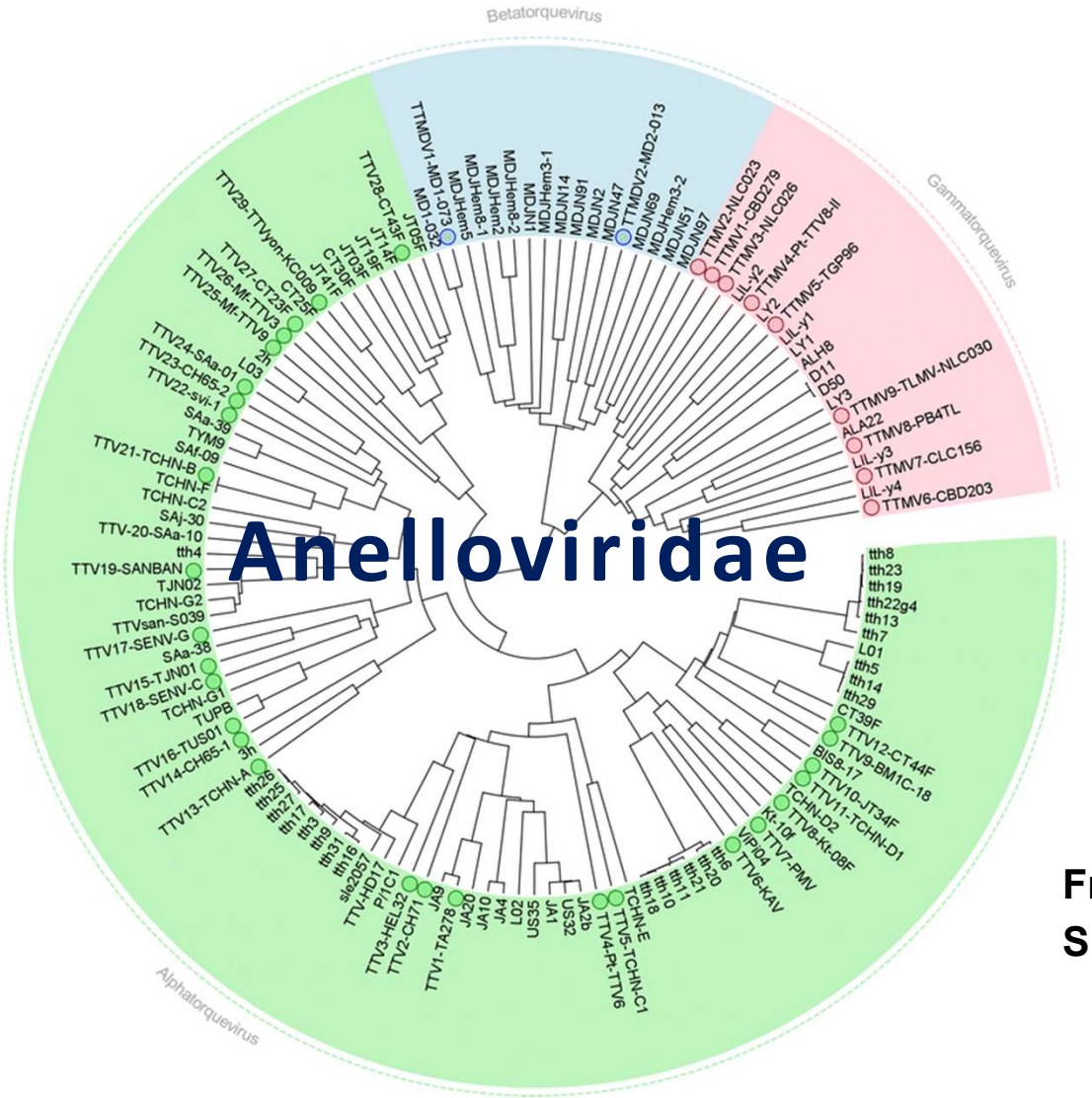
Virome

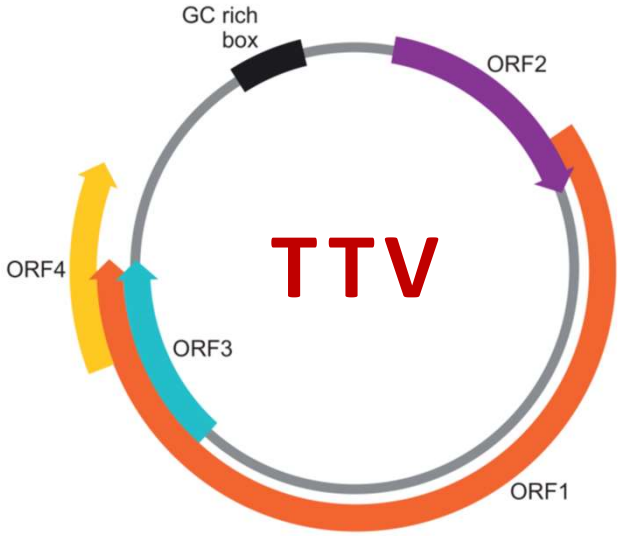


Small circular replication-associated protein (Rep) encoding single-stranded (CRESS) DNA viruses



Torquetenovirus (TTV): the prototype of anelloviruses





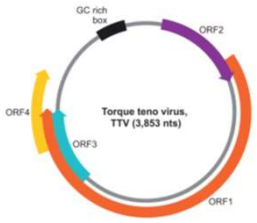
Torque teno virus

From latin: ***torques*** ***tenuis***

Significance: ***necklace*** ***thin***

Spandole et al. Arch Virol 2015

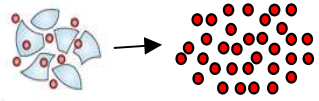
TTV properties



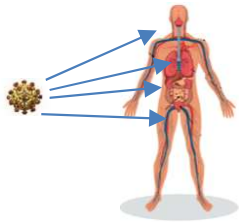
✓ **GENOME:** **small circular ssDNA (~3.8 kb)**
(the genetically simplest of all known replication-competent viruses hitherto detected in humans)

✓ **GENETIC VARIABILITY:** **extremely high**
(at least 22 human TTV species, each consisting of numerous types)

✓ **PREVALENCE:** **very high in the general population (~90%), acquired very early in life through many routes of transmission**



✓ **PERSISTENCE:** **chronic and productive infections (> 80% of infected people)**



✓ **UBIQUITY:** **detected in all the tissues and organs, T lymphocytes probably the main site of virus replication**

Miyata et al. 1999; Takahashi et al. 2000; Okamoto et al. 2001; Bendinelli et al. 2001; Jones et al. 2005; Bendinelli and Maggi, 2005; Ninomiya et al. 2007; 2008; Maggi et al. 2006; 2010

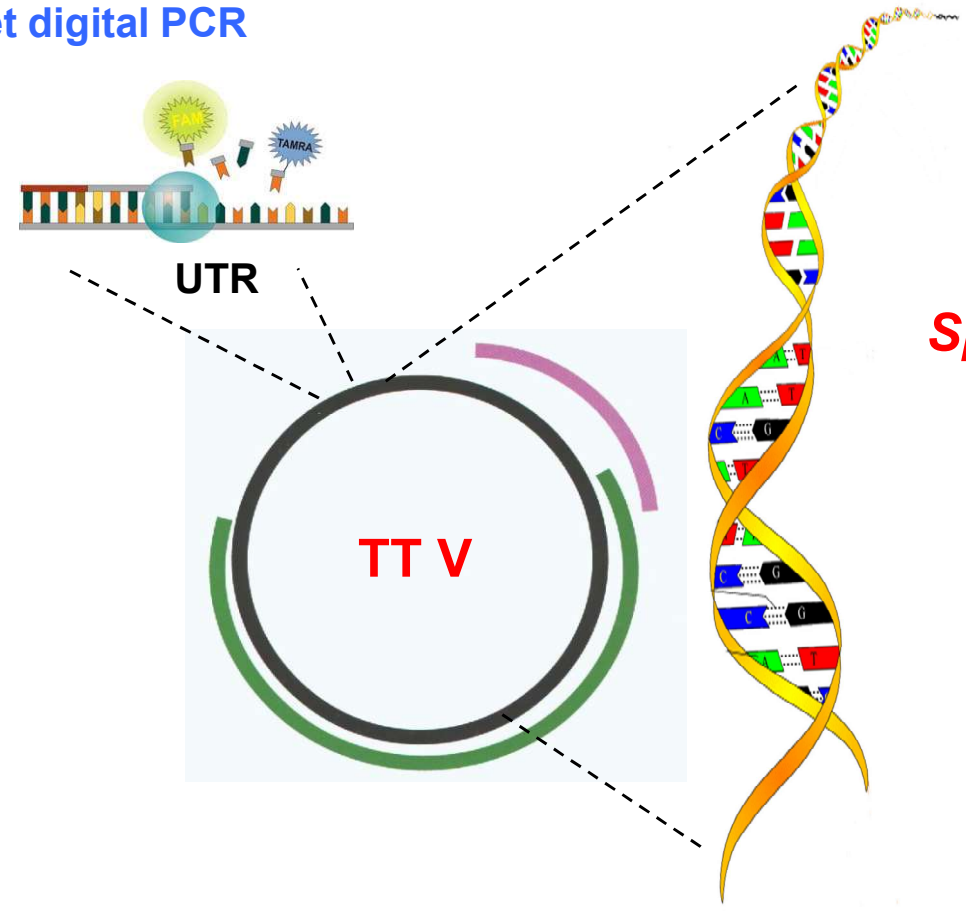
TTV and human diseases

- ✓ **A component of the normal human microflora, essentially devoid of pathogenic potential**
- ✓ **An "orphan" virus waiting to be linked to disease(s):**
  - ***only occasional infections aggressive to become the cause of significant clinical disease?***
  - ***TTV cofactor in some human diseases having a multifactorial origin?*** *(asthma? multiple sclerosis? autoimmune diseases?)*
  - ***TTV species diverse in their ability to induce pathogeneicity?*** *(TTV 7 species in Kawasaki disease?)*

Maggi and Bendinelli, Rev Med Virol 2010; Thissen et al. PLoS One 2018

"Universal" real-time UTR PCR:

1. In house TaqMan rtPCR
2. Commercial R-gene TTV assay
3. Droplet digital PCR

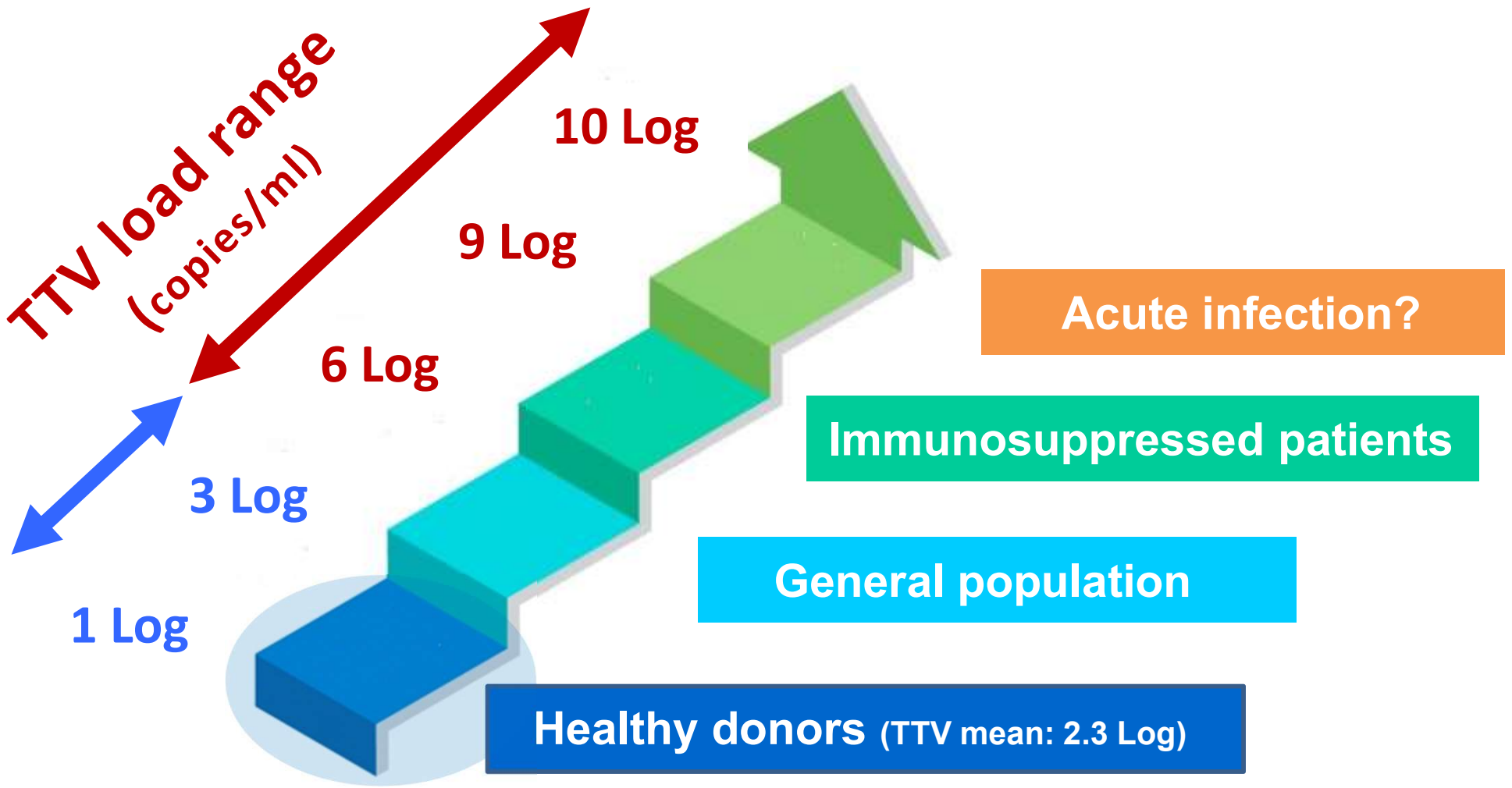


Species-specific rtPCRs

NGS

Maggi *et al.* J Clin Microbiol 2005; Macera *et al.* J Med Virol 2019

Levels of TTV viremia



Focosi et al. Clin Microbiol Infect 2020

Factors impacting on TTV viremia

- *No. of virions produced per cell and daily*
- *No./spectrum/turnover of cells replicating TTV*
- *Rate of virions release/clearance into/from the circulation*
- *Proportion of immunocomplexed virions*
- *Changes in functional integrity/proportion of immune cells*
- *Concomitant infections by other pathogens*
- *Local accumulation of proliferating lymphoid cells*
- *Regeneration rate of susceptible cells*

hypothetical

- *No. of different TTVs harbored*
- *Synergy/interference between the TTVs carried*
- *Acute superinfection by a different TTV*
- *Changes in functional integrity/proportion of immune cells*
- *Counts of circulating lymphocytes*
- *Immune activation by superimposed exogenous immunogens*
- *Presence of concomitant noninfectious pathologies*

at least some direct evidence available

- ***Age***
- ***Depressed immune responses***
- ***Immunosuppressive therapies***

proved

Maggi and Bendinelli, 2010

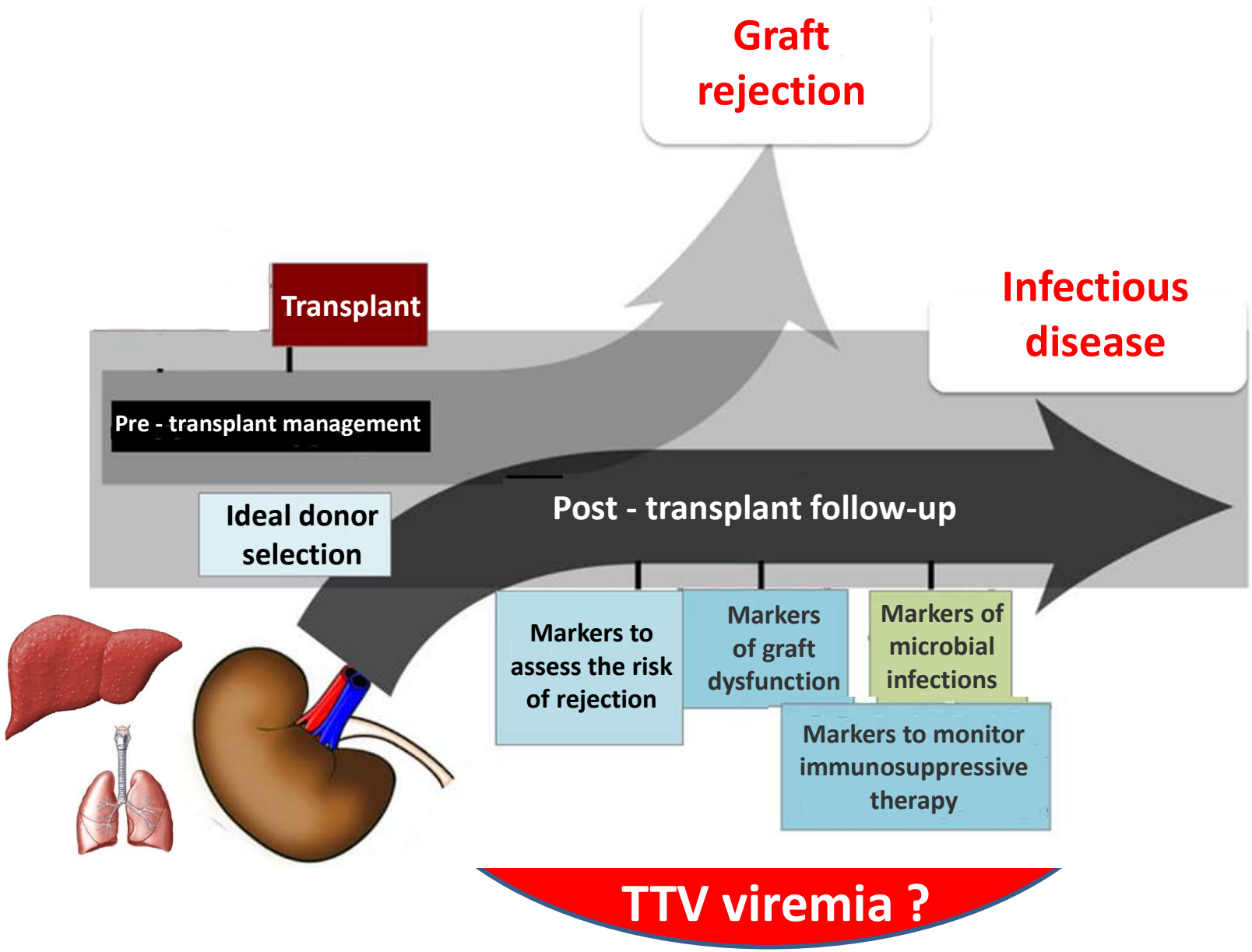
Markers of immune function

Alterations With Age	Non-IRP	IRP
Markers and cells		
CD4:CD8 ratio	> 1	< 1
T cell proliferation	Normal	Reduced
CD28	Increased	Reduced
CD57	Reduced	Increased
CD45RA	Increased	Reduced
CD45RO	Reduced	Normal
KLRG1	Reduced	Increased
Cytokines and growth factors		
Interleukin-2	Increased	Reduced
Interleukin-10	Stable	Stable
Interferon-γ	Increased	Reduced
CMV/EBV		
CMV+ cells	Lower frequencies, mostly KLRG1+	Higher frequencies, mostly KLRG1+
EBV+ cells	Lower frequencies	Higher frequencies

Abbreviations: IRP, immune-risk phenotype; KLRG1, killer cell lectin-like receptor subfamily G, member 1; CMV, cytomegalovirus; EBV, Epstein-Barr virus.

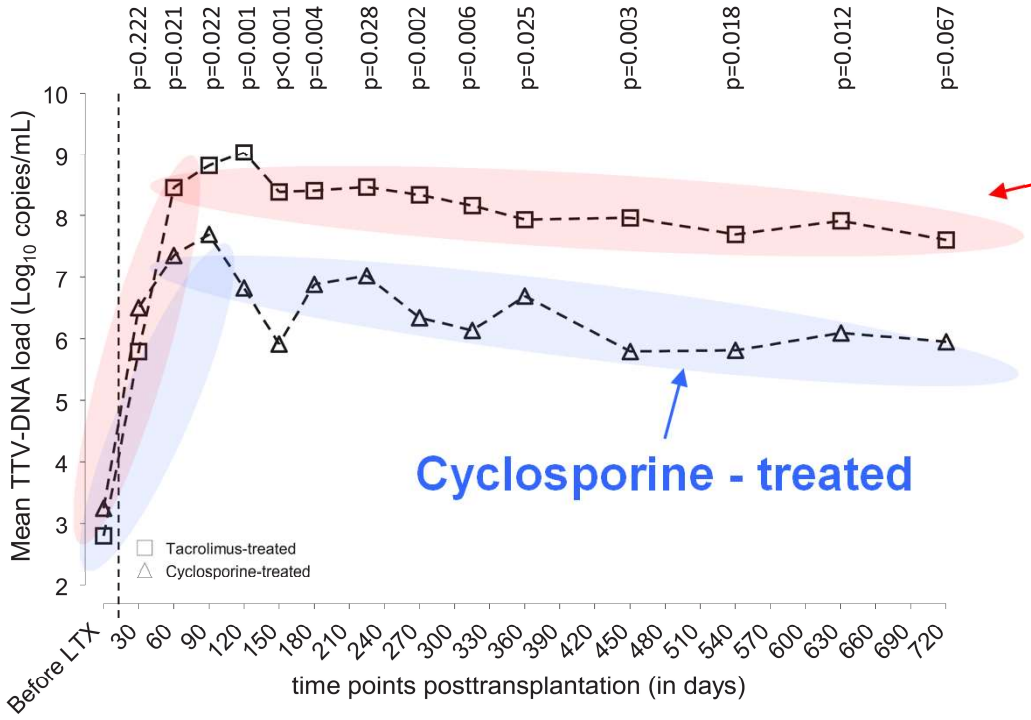
Pawelec *et al.*, Mech Ageing Dev 2001; Focosi *et al.,* J Clin Oncol 2007

Biomarkers in solid organ transplantation



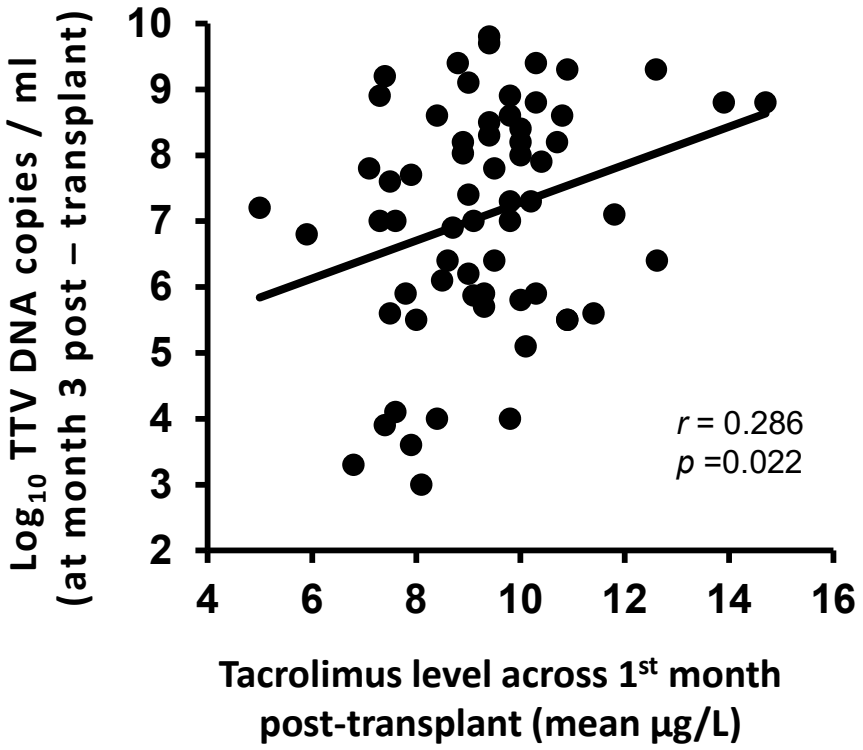
Brunet et al. Ther Drug Monitoring 2016

TTV viremia and immunosuppressants



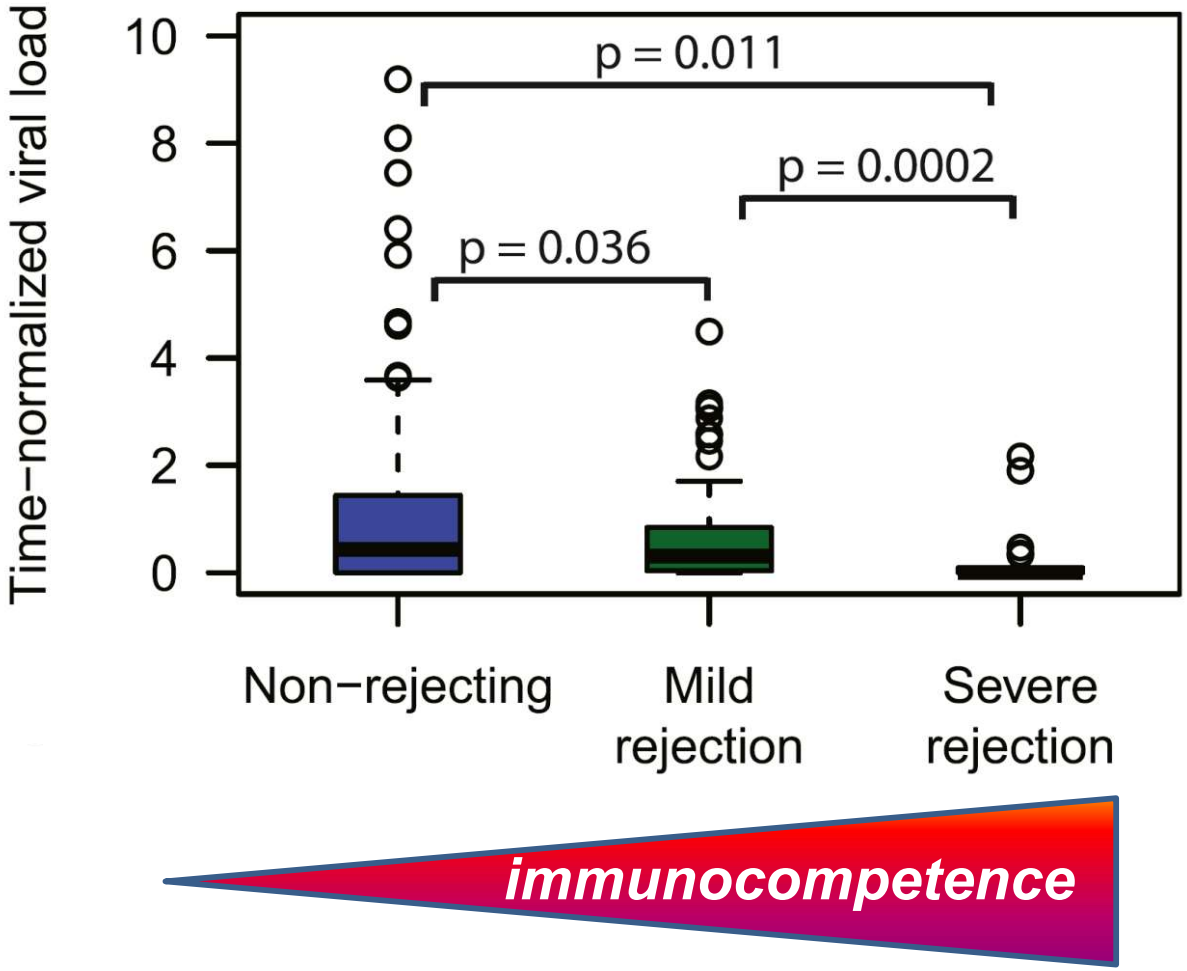
Tacrolimus - treated

Cyclosporine - treated



Gorzer et al. J Heart Lung Transpl 2014; Norden et al. Open Forum Infect Dis 2018

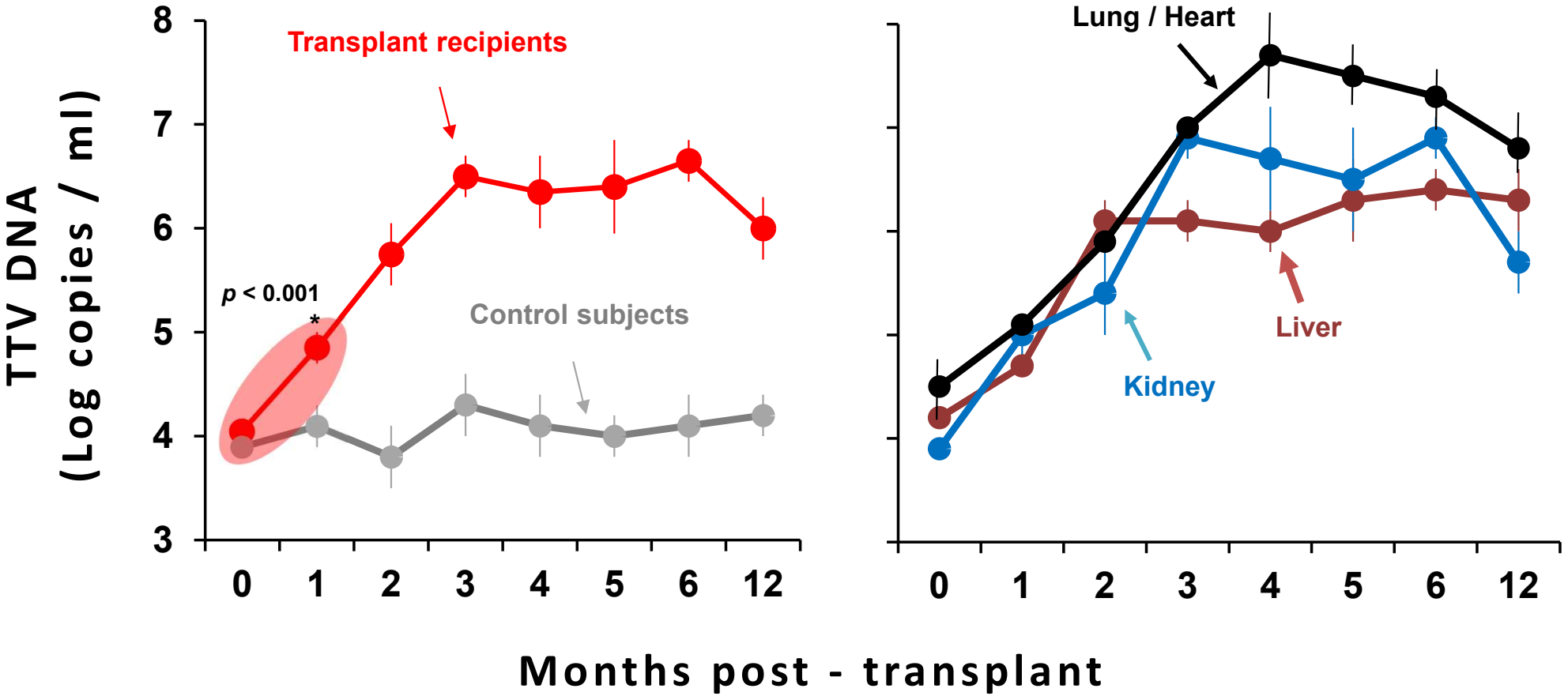
TTV viremia and risk of graft rejection



➢ **Lower levels of TTV viremia correlate with more severe risk of graft rejection**

De Vlaminck et al. Cell 2013

TTV viremia in SOT recipients



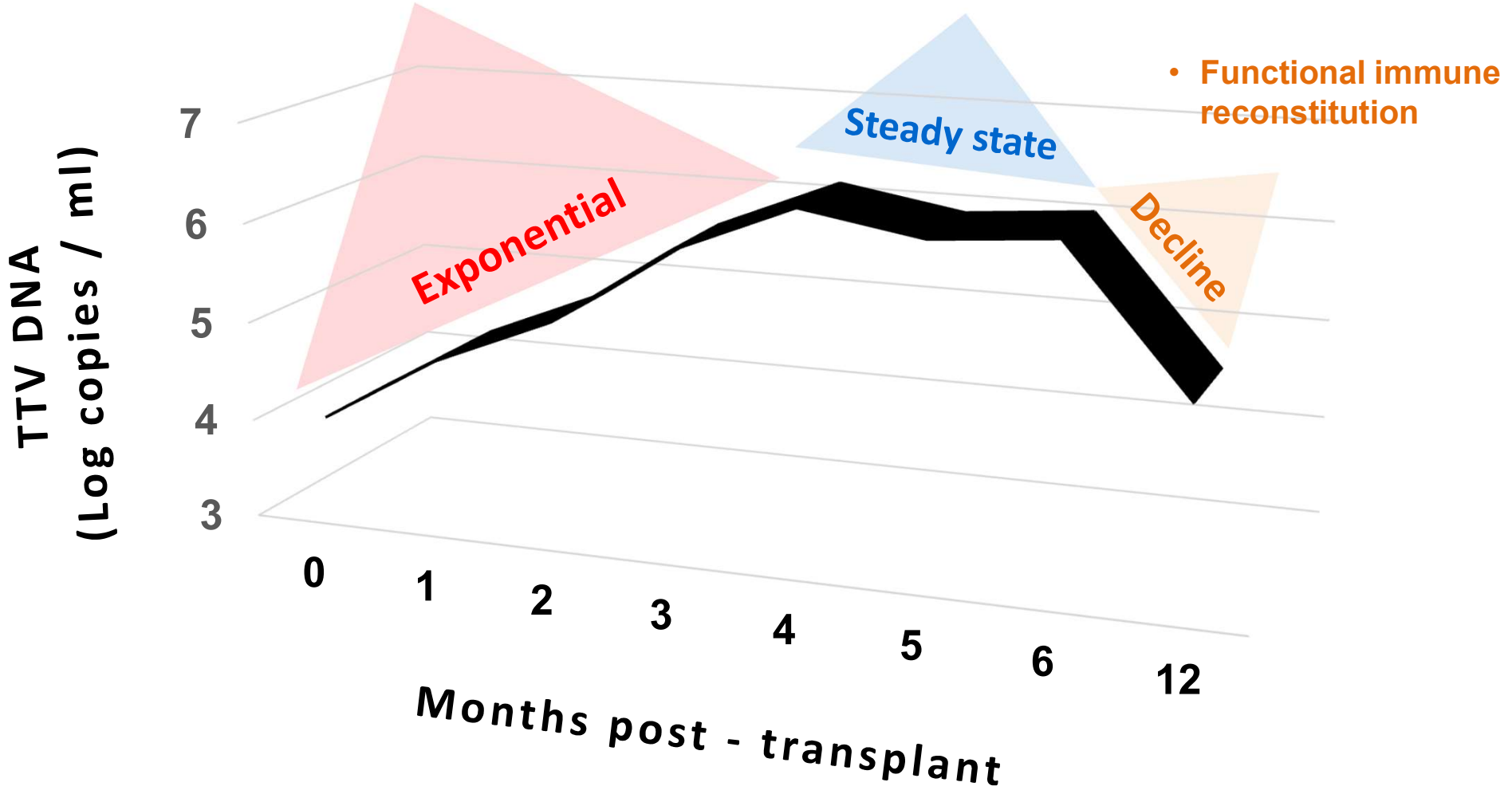
Maggi et al. Sci Rep 2018; Gorzer *et al.* J Heart Lung Transplant 2014; 2017; De Vlaminck *et al.* Cell 2013

Phases of TTV viremia

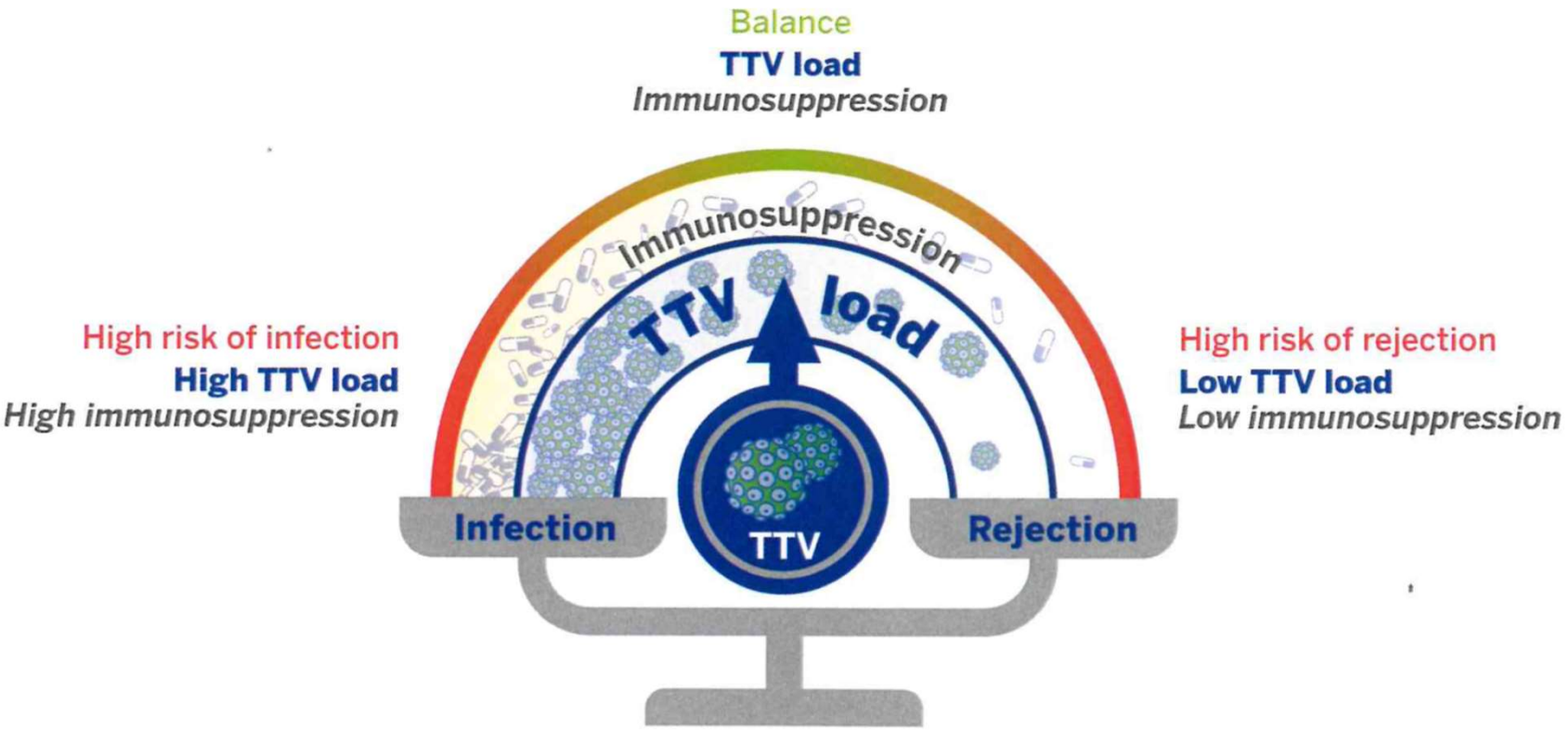
- Induction (type / level)
- Immunosuppressants (type / level)

- Immunosuppressants (level)

- Functional immune reconstitution



Cut-off of TTV viremia predict the risk of complications in kidney transplant patients

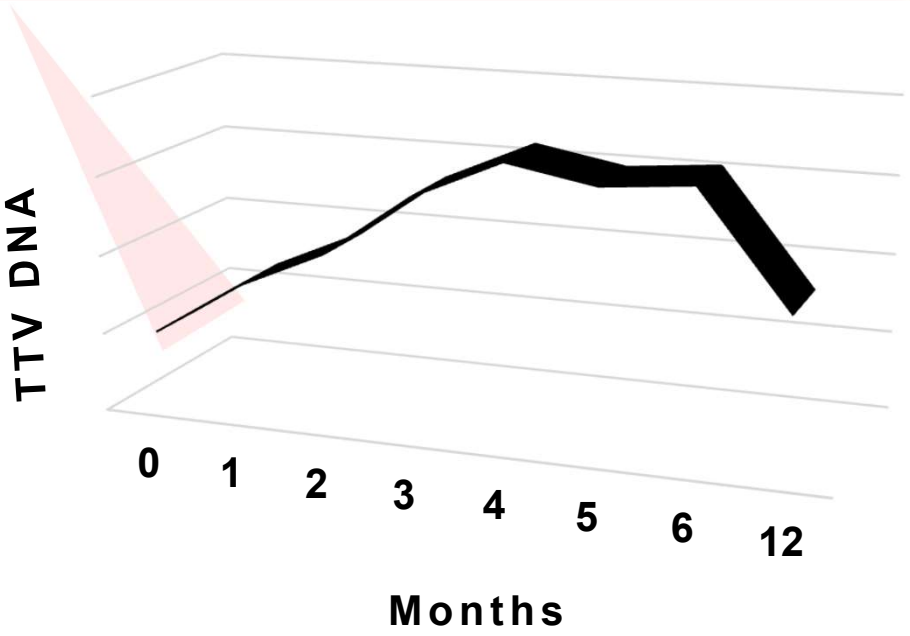


TTV load: 4.6 - 6.2 Log

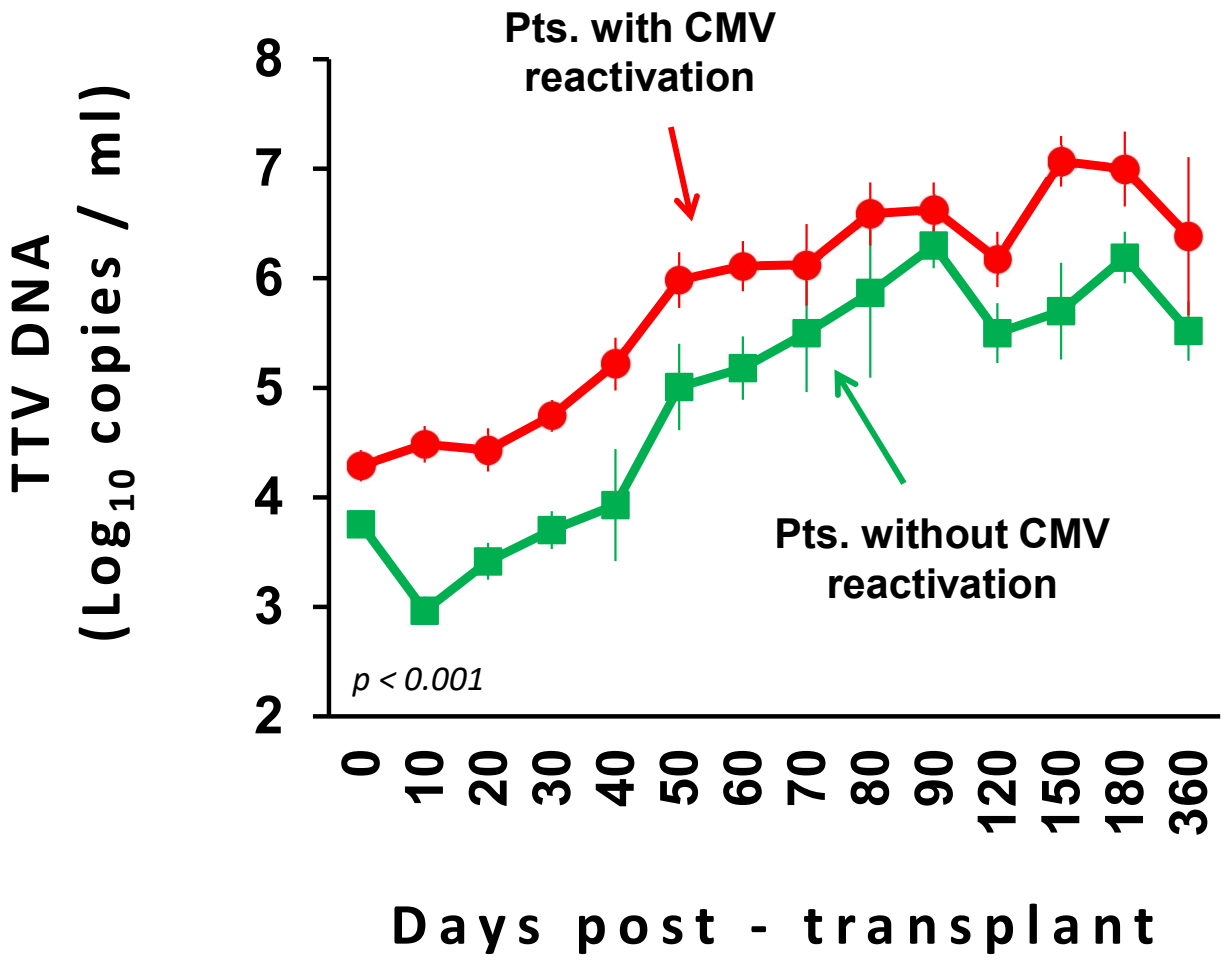
Bond et al. In preparation

Cut-off of TTV viremia as predictive marker of post-transplant complications

Organ	Liver	Kidney	Kidney	Kidney/Liver
Complication	Graft rejection	infections	infections	infections
TTV viremia cut-off (Log copies/ml)	< 1.4	> 3.1	> 3.3	> 3.4
	Simonetta et al. Transpl 2017	*Ruiz et al. AJT 2019*	*Maggi et al. Sci Rep 2018*	*Gore et al. In press*

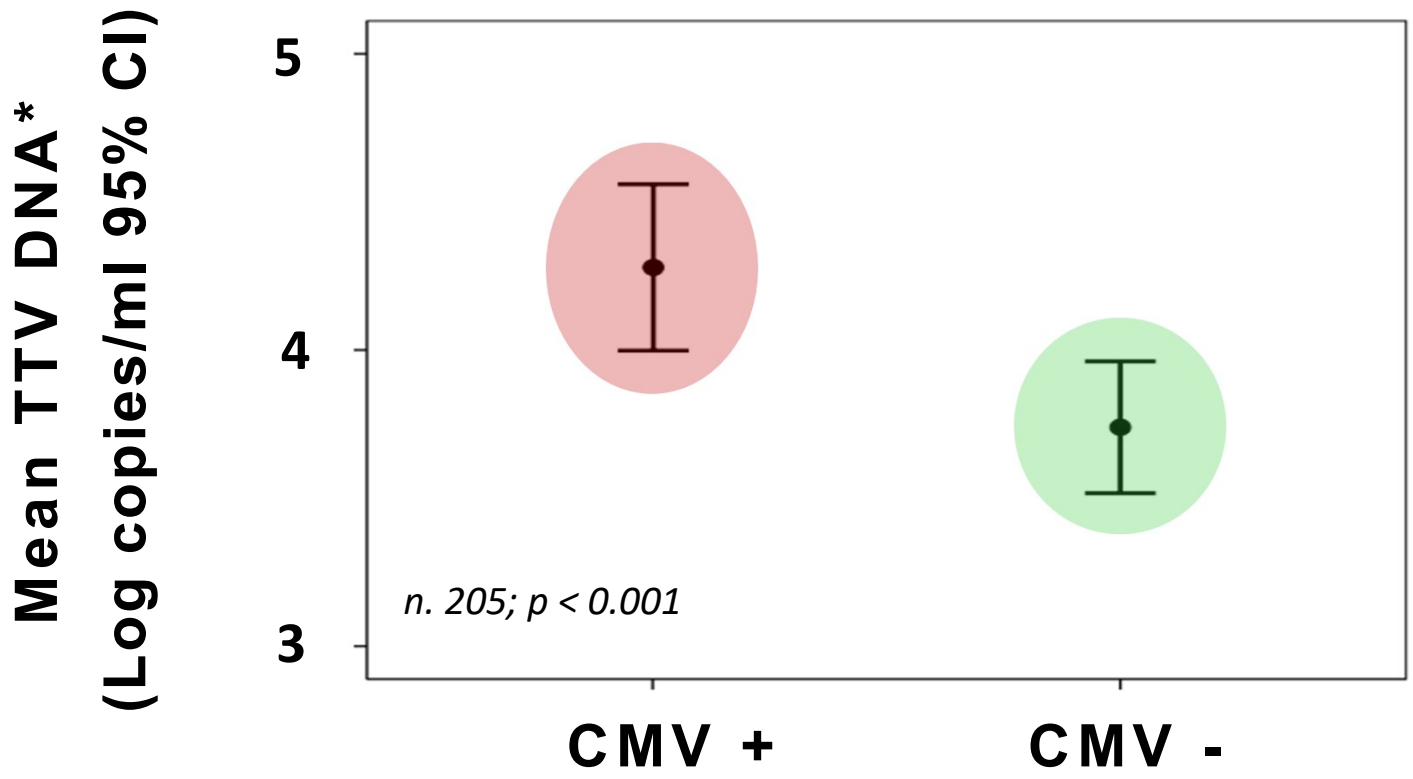


TTV viremia in kidney transplant patients, grouped by CMV status



Maggi et al. Sci Rep 2018

TTV viremia measured within 10 days post-kidney transplant predicts CMV reactivation



*** measured between 0 and 10 days post-transplant**

Maggi et al. Sci Rep 2018

TTV index in kidney transplant recipients

≤ 3.45 log_{10} copies/ml
lower probability of CMV reactivations

> 3.45 log_{10} copies/ml
higher probability of CMV reactivations

TAKE-HOME MESSAGE :

TTV viremia above 3.45 log DNA copies/ml within the first 10 days post-transplant correlates with higher propensity to CMV reactivation following liver/kidney transplantation

Conclusions

- ✓ **TTV plays a substantial role in the human virome, and it has a considerable impact on the host immune system**
- ✓ **Although TTV has not yet been firmly associated with any clinical manifestation, performing the quantification of TTV viremia is useful**
- ✓ **TTV may serve as a cheap and easy-to-measure surrogate of functional immune competence, and could prove especially useful in the management of SOT patients**