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IL VIROMA COME STRUMENTO DIAGNOSTICO

*Lo studio del
microbiota: il ruolo
dell'AMCLI*

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A new concept: the human virome

Cell

Leading Edge
Review

Redefining Chronic Viral Infection

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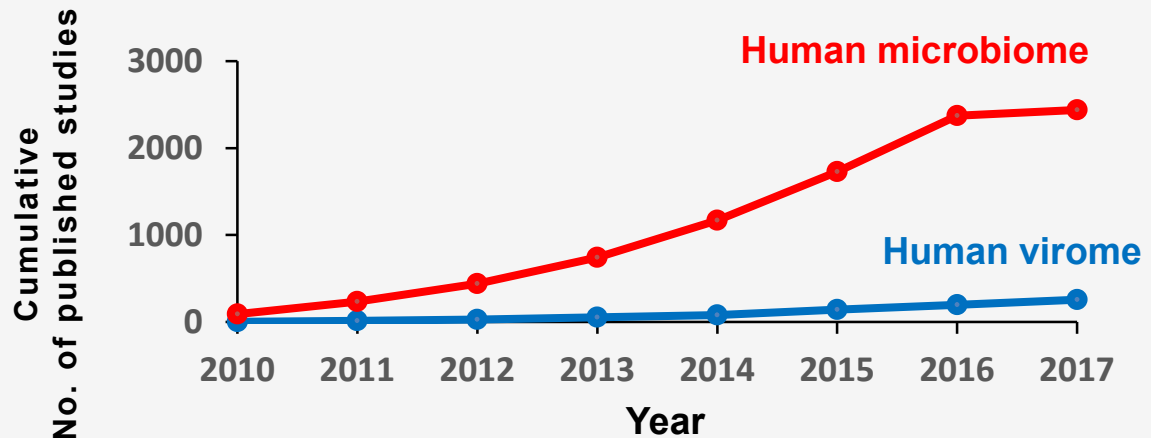
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Viruses that cause chronic infection constitute a stable but little-recognized part of our metagenome: our virome. Ongoing immune responses hold these chronic viruses at bay while avoiding immunopathologic damage to persistently infected tissues. The immunologic imprint generated by these responses to our virome defines the normal immune system. The resulting dynamic but metastable equilibrium between the virome and the host can be dangerous, benign, or even symbiotic. These concepts require that we reformulate how we assign etiologies for diseases, especially those with a chronic inflammatory component, as well as how we design and interpret genome-wide association studies, and how we vaccinate to limit or control our virome.



Limitations of human virome studying

Feature	Bacteria	Viruses	Challenges
Genome	dsDNA	dsDNA, ssDNA, partially dsDNA, ssRNA or dsRNA	Different extraction protocols for different viruses. RNA viruses require cDNA synthesis and ssDNA second strand synthesis
Gene conservation	Highly conserved, essential genes (for example, 16s rRNA) enabling broad microbiome studies and surveys of taxa	No homologous genes between viruses of different phyla	Lack of conserved homology between viral phyla prevents universal primer-based surveys of viromes
Culture	Often straightforward to culture and obtain pure, highly enriched bacterial DNA and RNA	Challenging to culture, and require a host cell for replication	Cultured viruses are heavily contaminated with host cell nucleic acids, which decreases viral sequencing output
Clinical specimens	Hardy bacterial cells with cell walls can often be separated from human cells in clinical specimens using differential lysis methods or flow cytometry ¹⁴⁴ prior to extraction	Viruses are intracellular pathogens, and although separation from the host is possible (for example, by filtration or antibody pull-down), viruses cannot easily be separated from clinical samples prior to extraction	Clinical specimens are heavily contaminated with host nucleic acids, which decreases viral sequencing output
Methylation patterns	Bacteria use different methylation patterns from eukaryotes; host DNA can be depleted post-extraction using restriction endonucleases that are directed against CpG methylation ¹⁴⁵	DNA viruses are often methylated by the host intracellular machinery, and may have similar methylation patterns	DNA digestion according to methylation patterns is less effective as a means of host depletion for viral sequencing

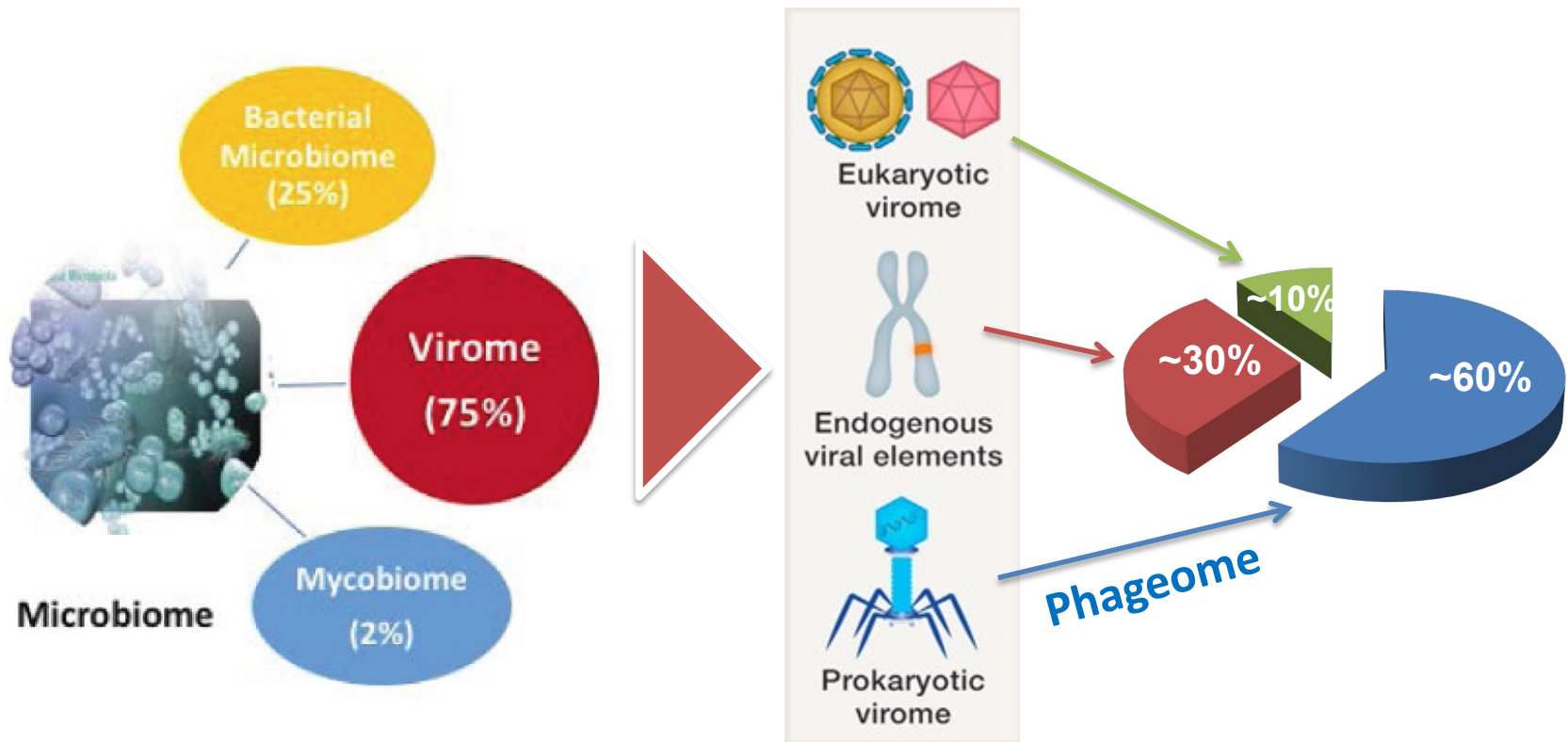


- **inadequate bioinformatic tools for virome analysis**
- **lack of robust virome database**
- **of the US\$ 920 million invested in microbiome research from 2012 to 2014, only 3% of research was dedicated to studies of virome**

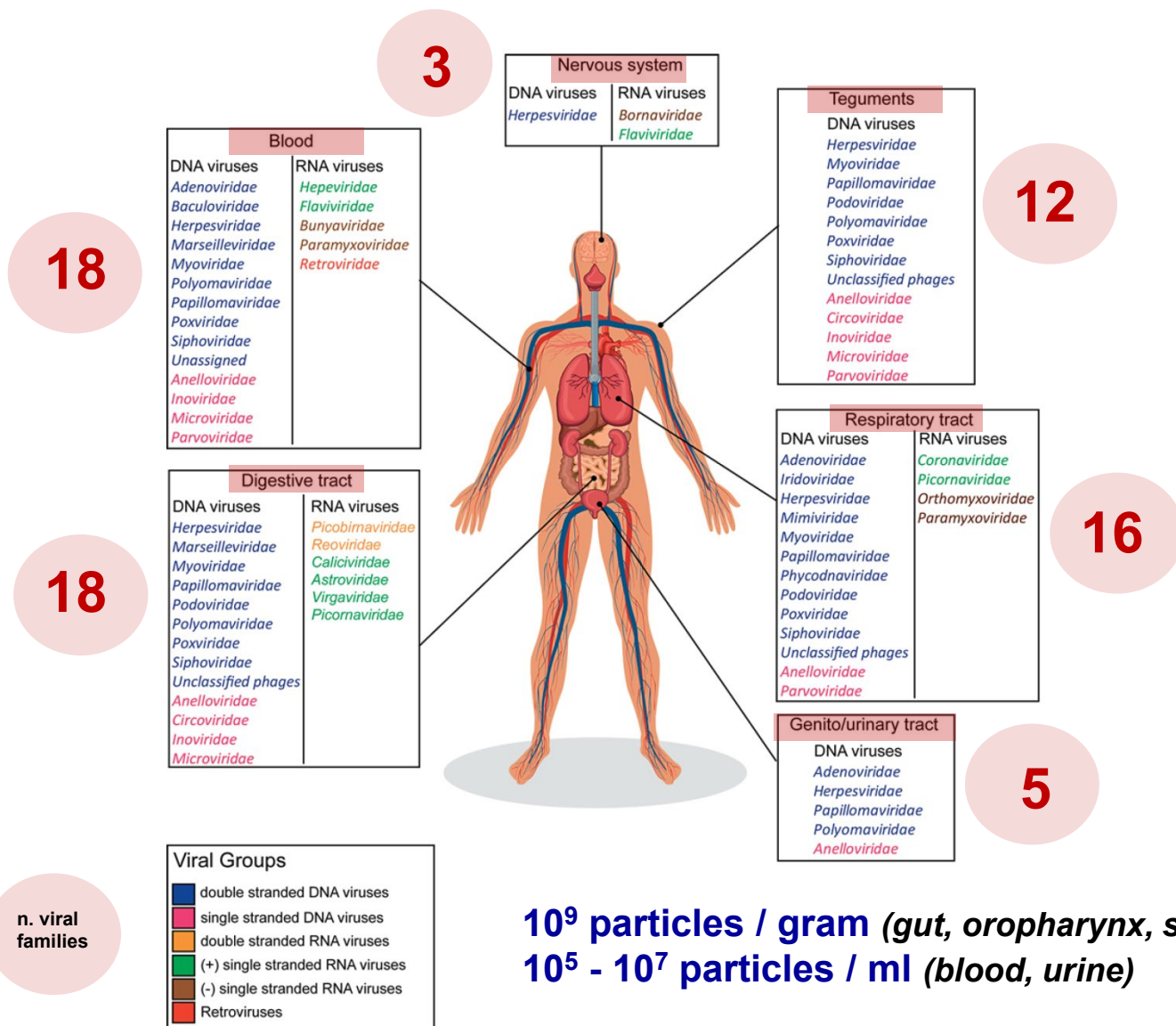


Definition

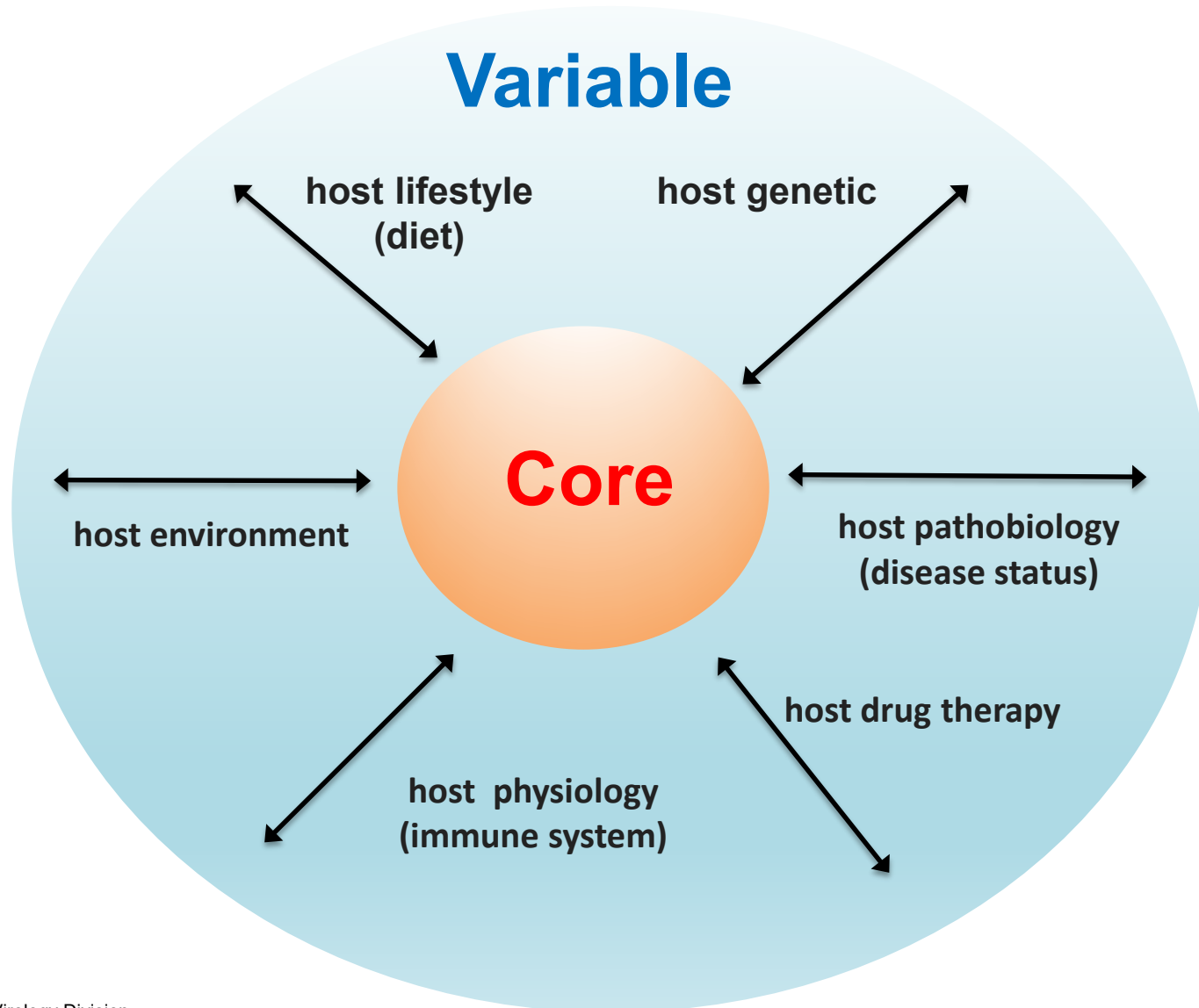
- all the nucleic acids (ssDNA, dsDNA, ssRNA, dsRNA) belonging to the virus-like particles associated with a particular habitat, defined over a range of scales from the entire body to a specific organ or tissue



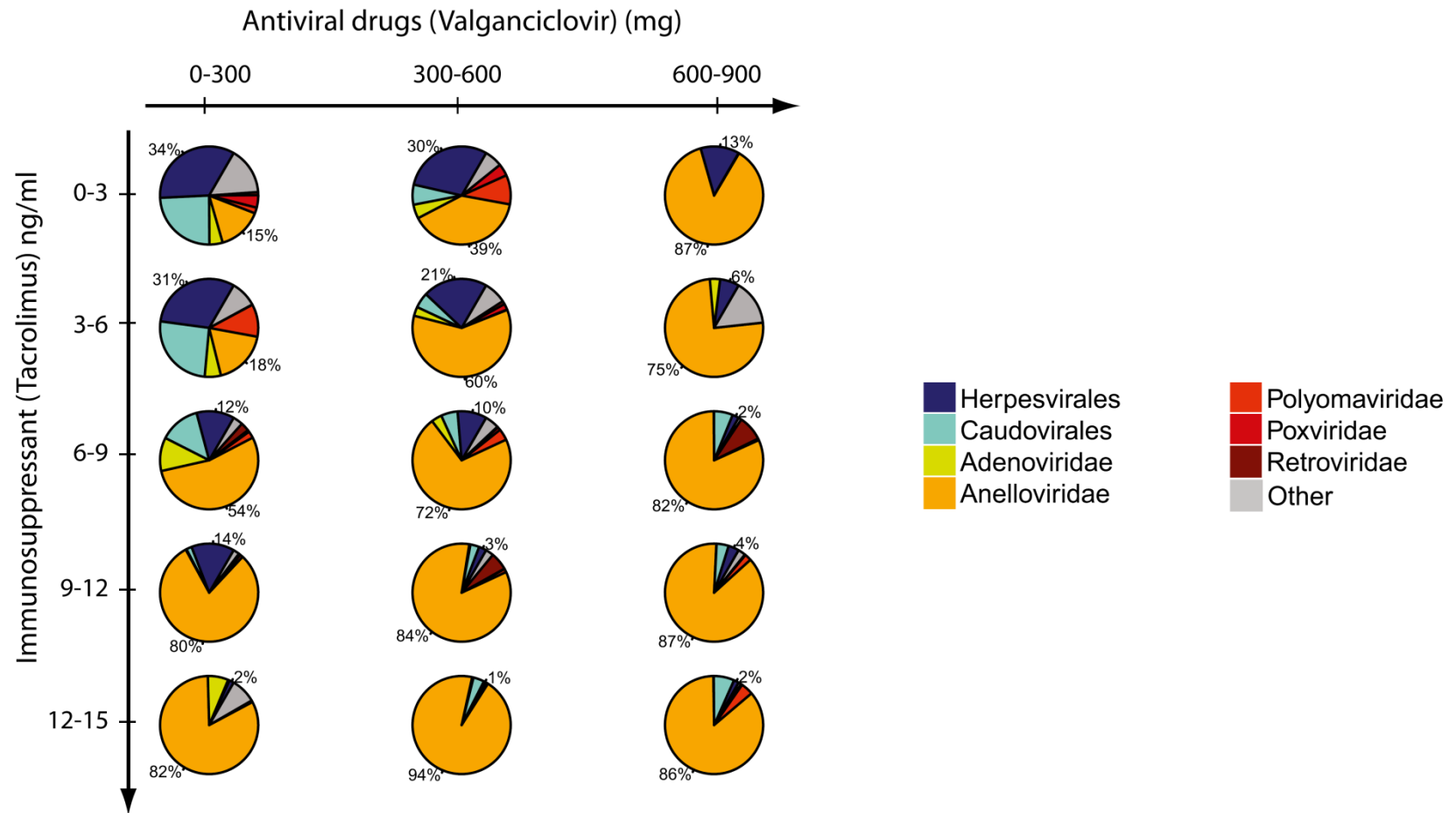
Composition



Structure



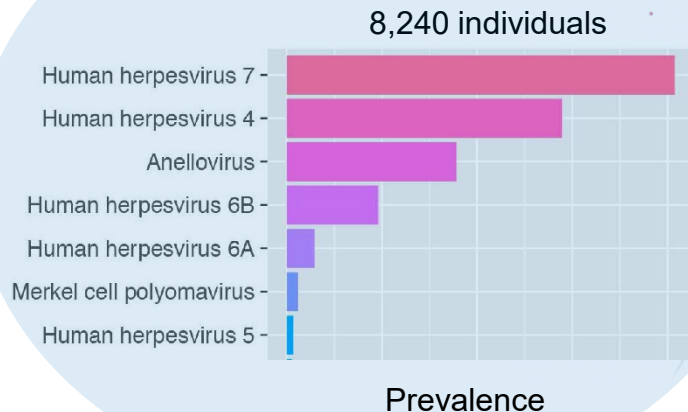
Virome structure is affected by immune modulation and antiviral therapy



The healthy virome



BLOOD



GUT

Bacteriophages

in ~ 50% of individuals:

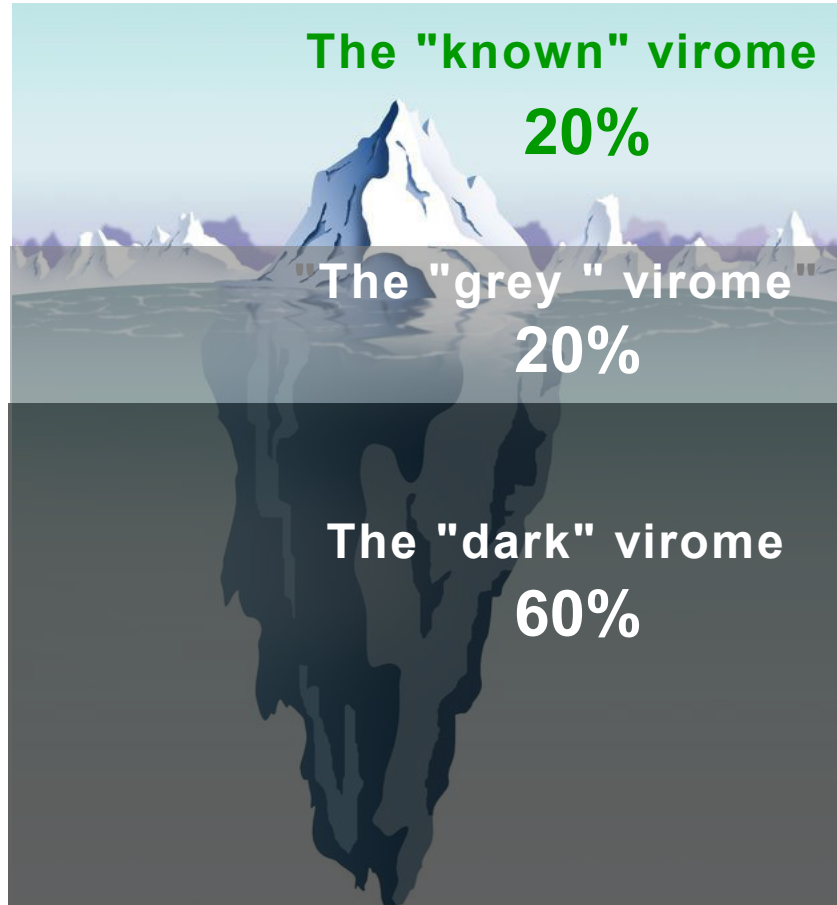
- *Caudovirales*
- *Microviridae*

Eukaryotic viruses

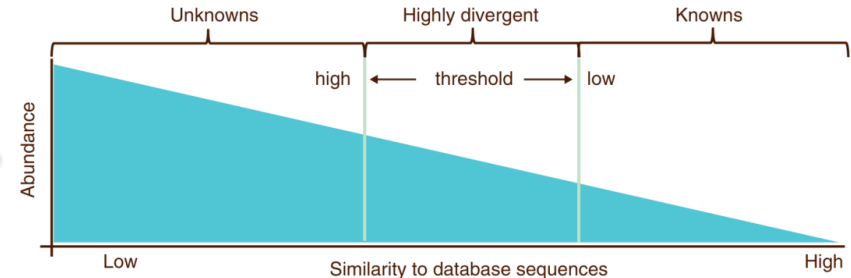
- *Anelloviridae*
- *Herpesviridae*
- *Papillomaviridae*
- *Adenoviridae*



The complete virome



- ✓ "Mega" virome
- ✓ "Low-level" virome



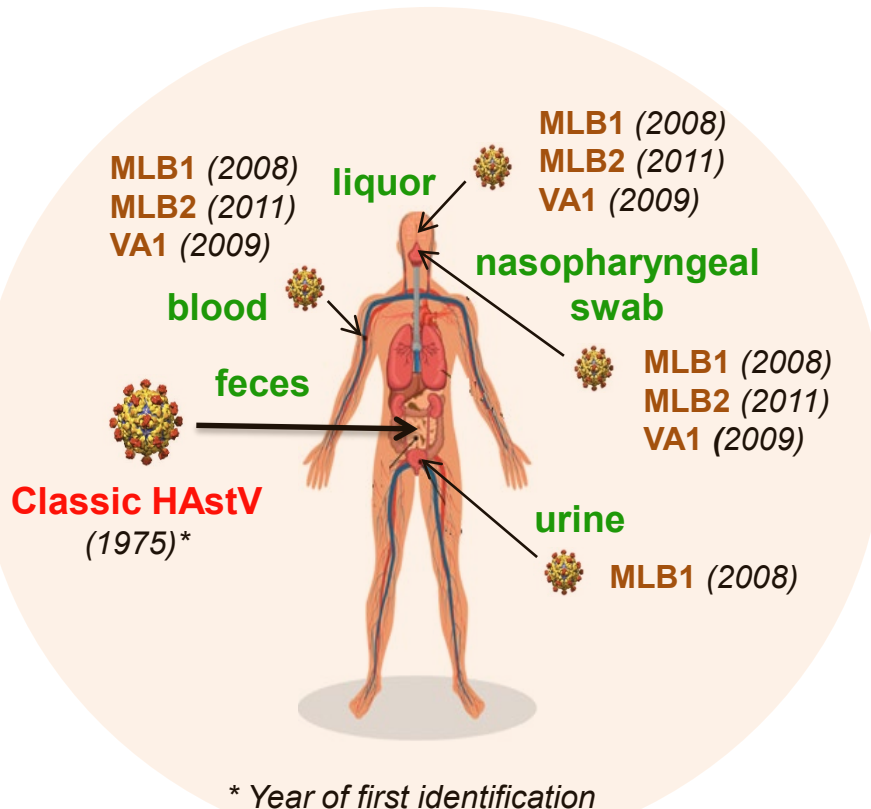
The most sequence reads by metagenomic analyses has features of viral genetic material but no taxonomic classification



The implications for clinical practice: the "expanding" viruses

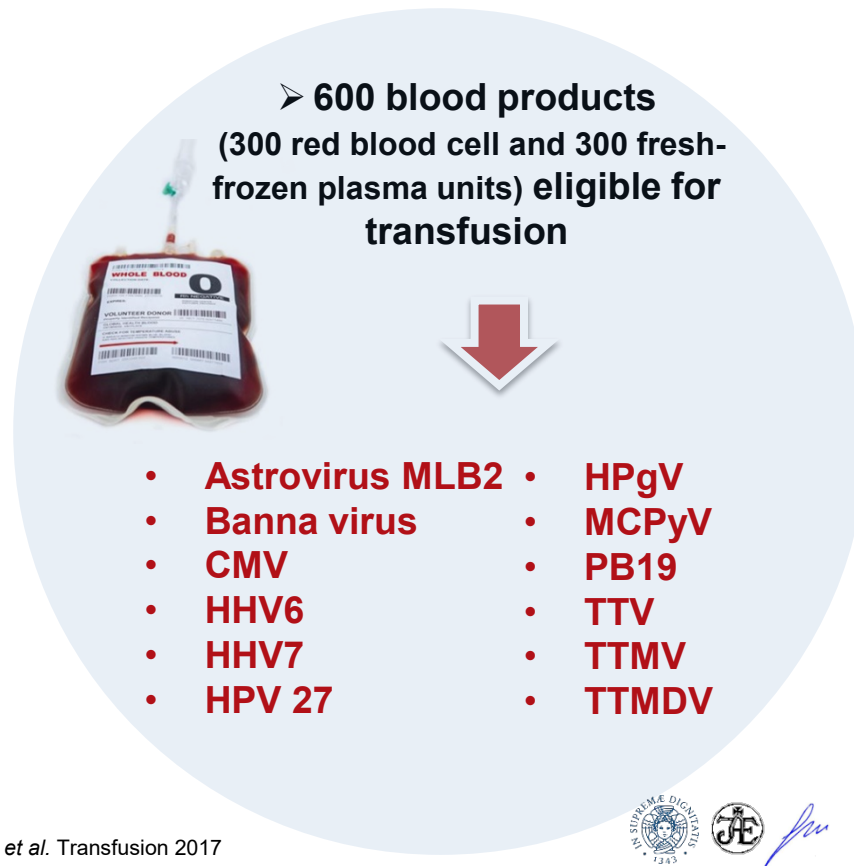
➤ The expanding tissue tropism of known viruses

The "Astrovirus story"



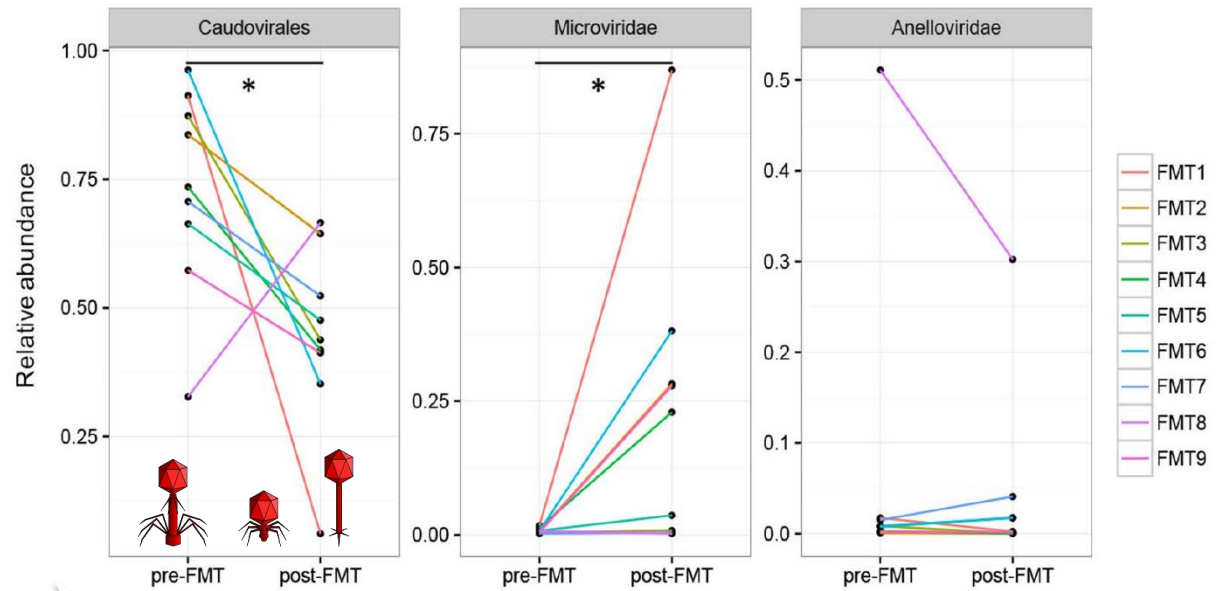
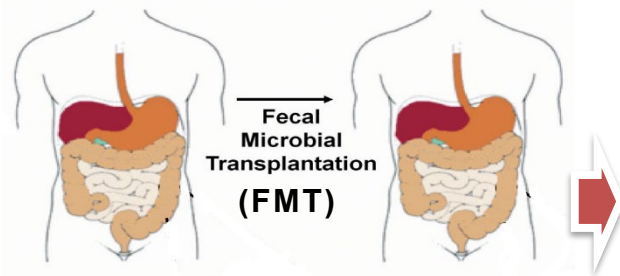
➤ The expanding viral landscape in clinical samples

The " Blood products story"



Phages and fecal microbial transplantation

Pts. with *Clostridium difficile* infection (CDI)



CDI recipients with Caudovirales:

- *higher richness in donor*
- *higher colonisation level of donor-derived taxa*

FMT responders

- ✓ **The restoration of virome community is as important as that of bacterial microbiome in FMT**
- ✓ **Donor selection based on virome characteristics should be considered in FMT practice**

Virome in transplant recipients: novel virus-disease associations

Virus	Specimen Type	Transplant	Clinical Presentation
Astrovirus VA1/ HMO-C-UK1	Brain, CSF, plasma, and stool	HCT	42 year old man presenting with encephalitis and bilateral hearing loss; 18 month old boy presenting with encephalopathy
Tick-borne Encephalitis Virus	Brain, CSF from the recipients	Kidney, Liver	Donor-derived virus resulting in fatal febrile illness and encephalopathy among 3 organ transplant recipients from a deceased organ donor.
Dandenong virus	Transplanted organ tissue (kidney, liver), blood, and CSF	Kidney, Liver	Donor-derived virus resulting in fatal febrile illness and encephalopathy among 3 organ transplant recipients from a deceased organ donor.
New Jersey Polyomavirus	Muscle, skin	Pancreas	33 year old woman presenting with weakness, retinal blindness, and skin plaques
Novel Poxvirus	Skin	Kidney	45 year old man with ulcerative skin lesions across flank.
Human Mastoadenovirus type 70	Stool	HCT	62 year old man with presenting with diarrhea.

The Perils of Pathogen Discovery

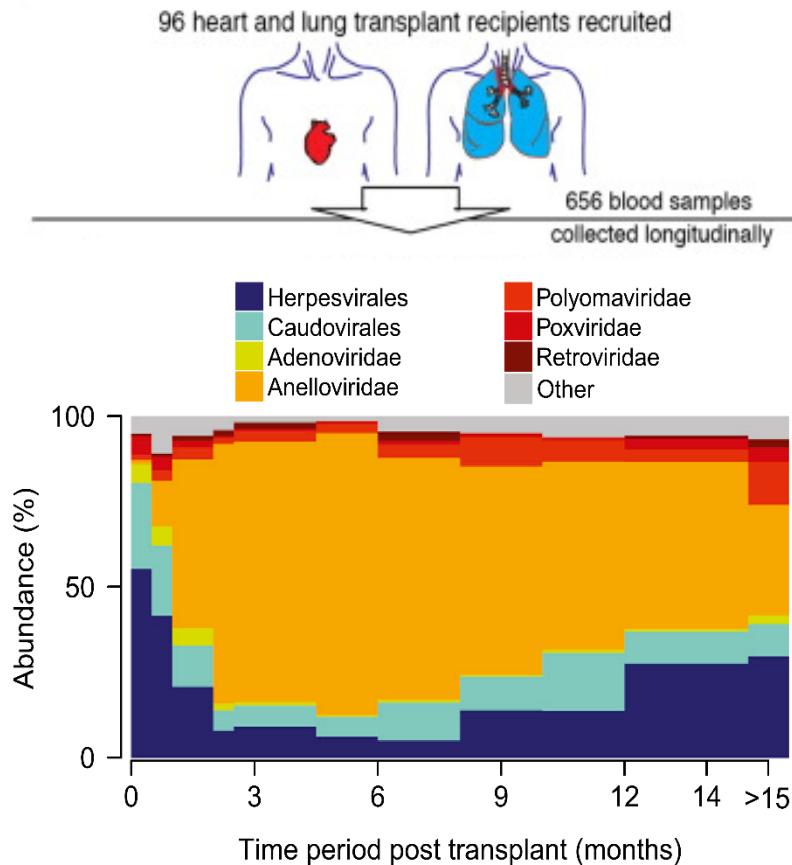


- **Xenotropic murine leukemia virus-related virus (XMRV)**
- **Parvovirus-like hybrid virus (PHV)**

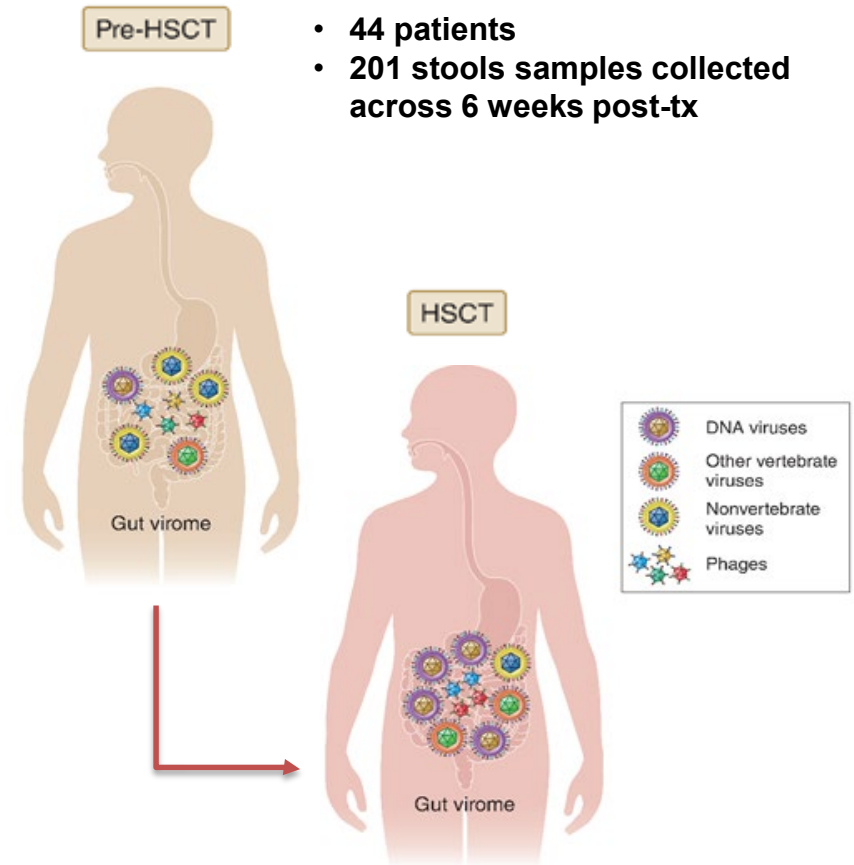


Virome changes in post-transplantation

Blood Virome



Gut Virome



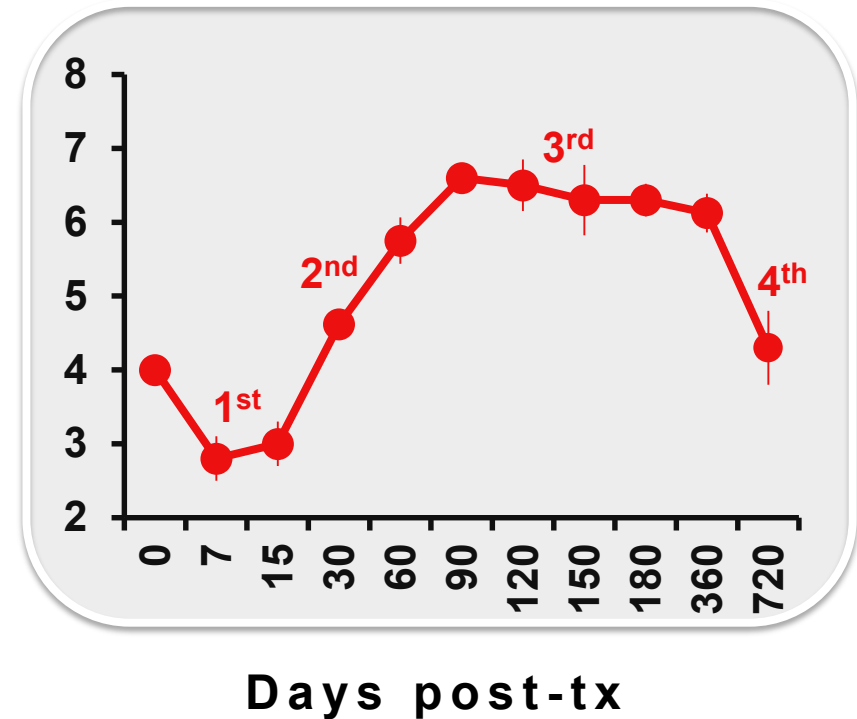
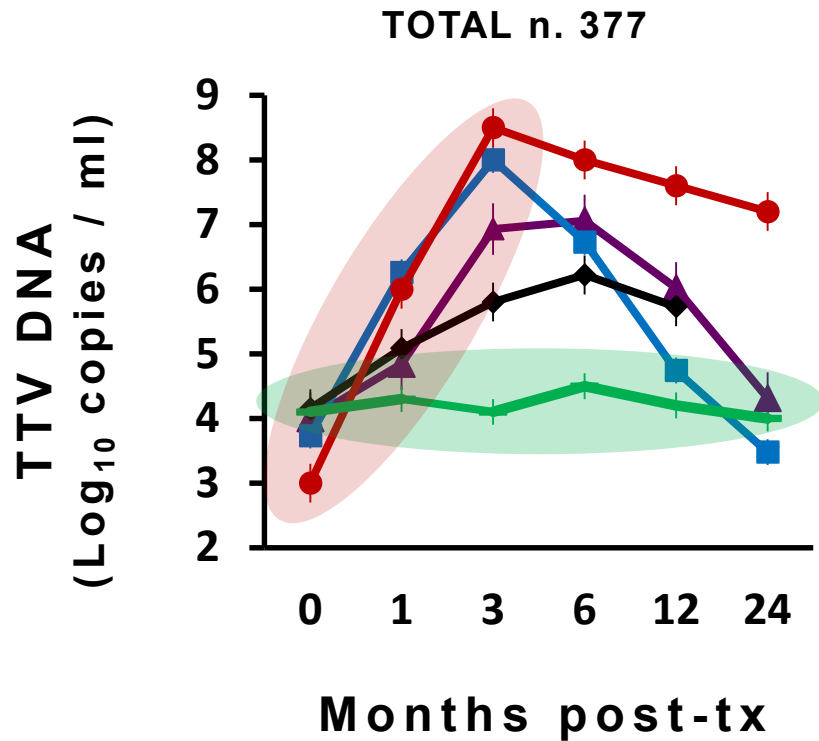
70%

← Anellovirus TTV →

40%



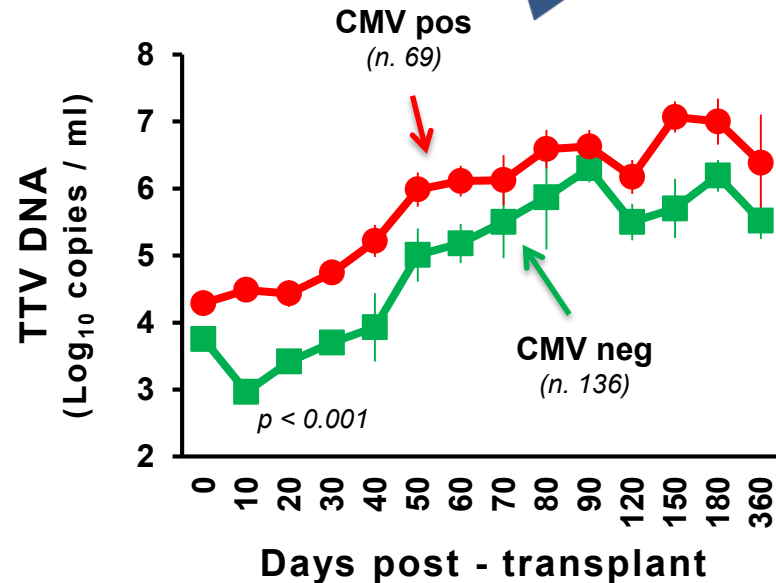
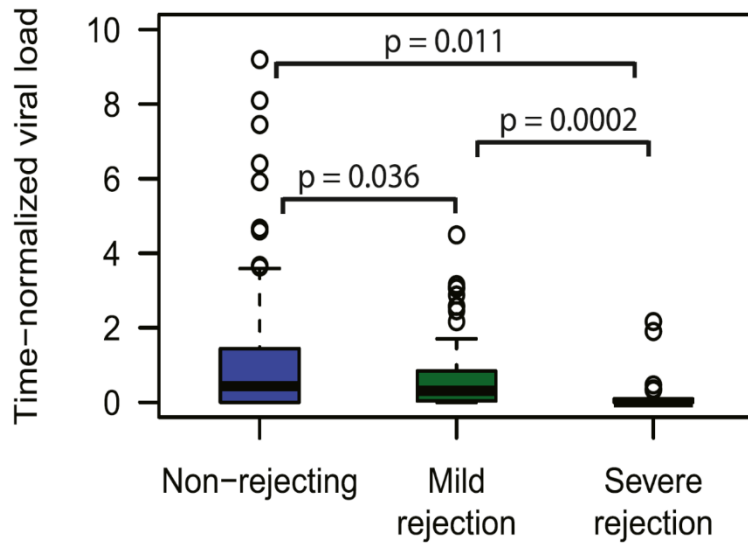
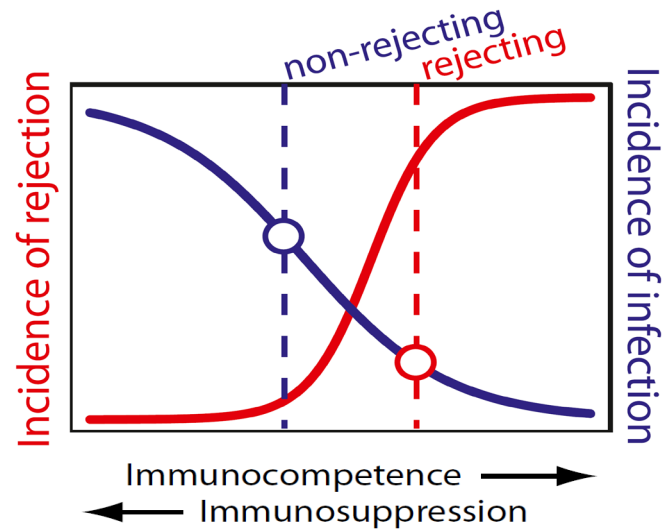
TTV kinetics in transplantation



Lung (n.31), Pancreas (n.26), Kidney (n.146)
Liver (n.144), Healthy donors (n.30)



TTV viremia and post-transplant complications

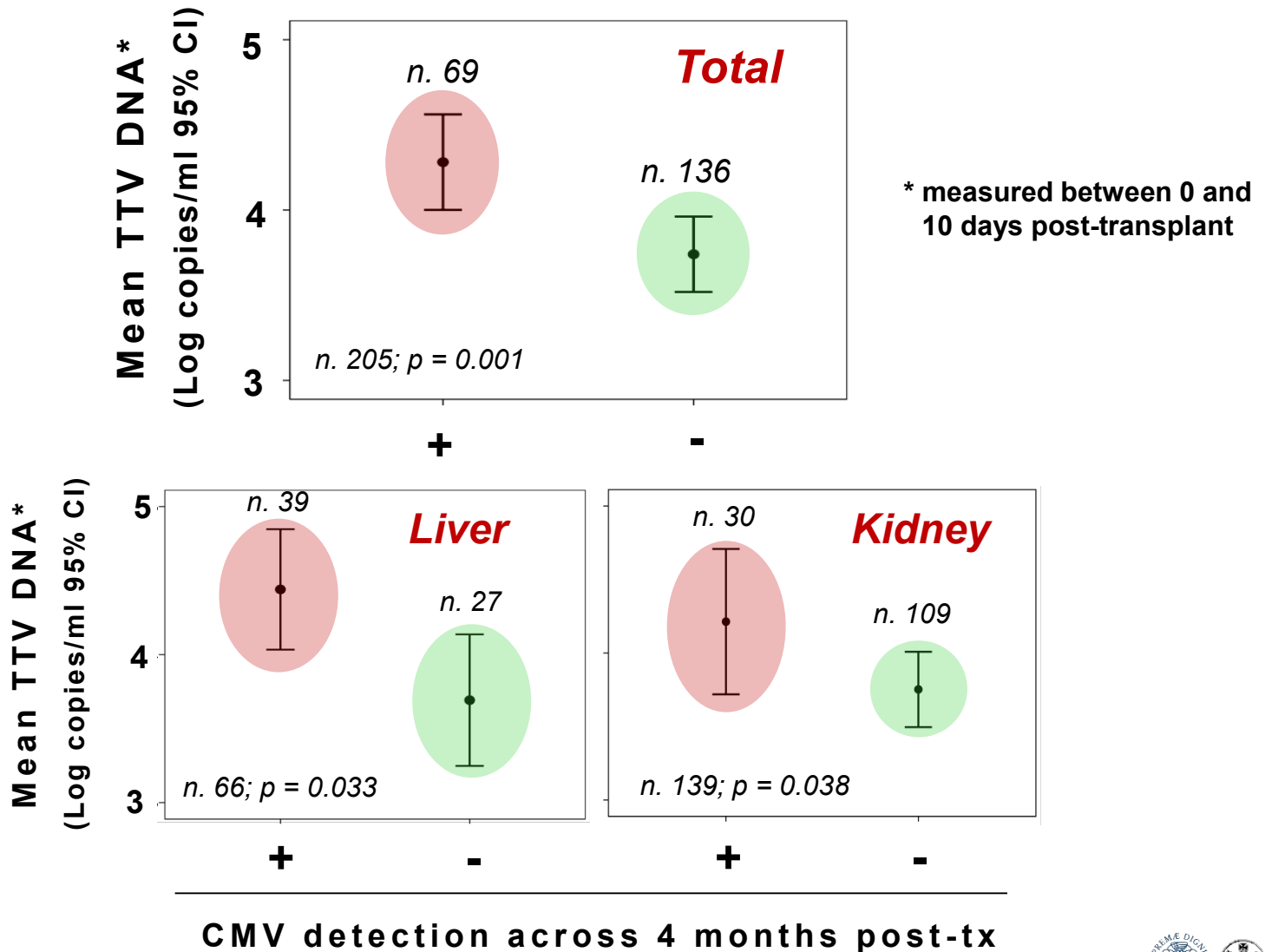


Predictive levels of TTV viremia

Parameter (higher risk)	Transplant type (no. pts.)	Plasma TTV viremia		Reference
		Phase	Predictive level (log DNA copies/ml)	
Viral reactivation				
	HSCT (23)	2 nd (at day 30)	> 8.4	Gilles et al. 2017
	Lung (31)	3 rd	> 9.3	Gorzer et al. 2014
Graft rejection				
	Liver (39)	1 st (at day 0)	< 1.3	Simonetta et al. 2017
	Lung (57)	1 st (at day 14)	< 4.0	Blatter et al. 2017
	Lung (20)	3 rd	< 7.0	Gorzer et al. 2017
	Heart / Lung (96)	All	< 4.0	De Vlaminck et al. 2014
	Kidney (715)	All	< 4.8	Schiemann et al. 2017



TTV viremia predicts CMV reactivation



TTV index in liver / kidney transplant recipients

$\leq 3.45 \log_{10}$ copies/ml
lower probability of CMV reactivations



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

TAKE-HOME MESSAGE :

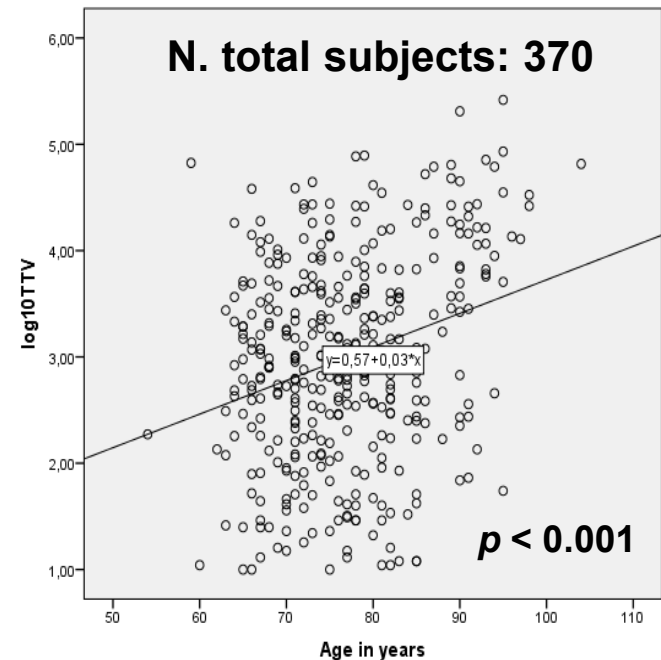
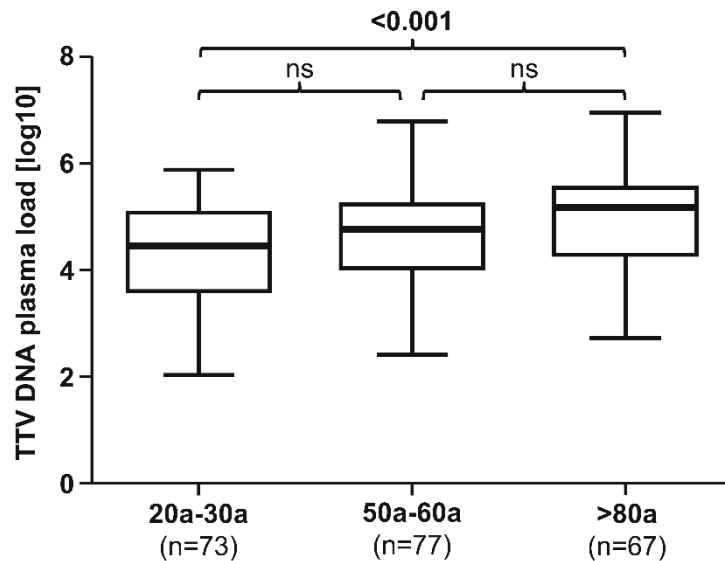
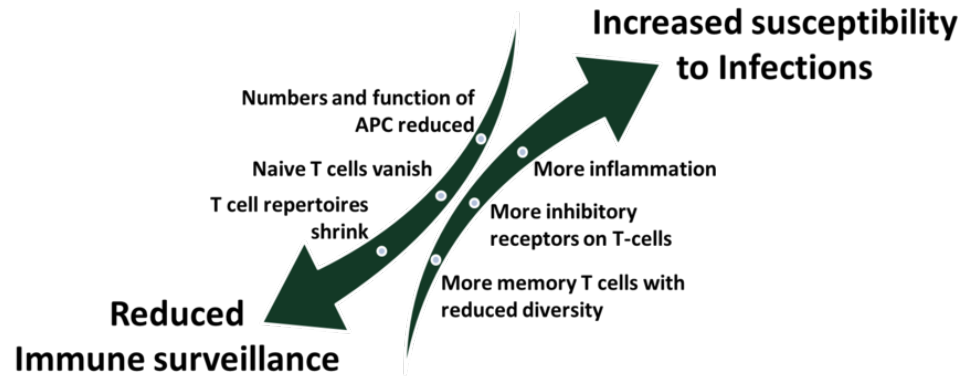
TTV viremia above 3.45 log DNA copies/ml within the first 10 days post-transplant correlates with higher propensity to CMV reactivation following liver/kidney transplantation

Biomarker assays useful in predicting post-transplant complications

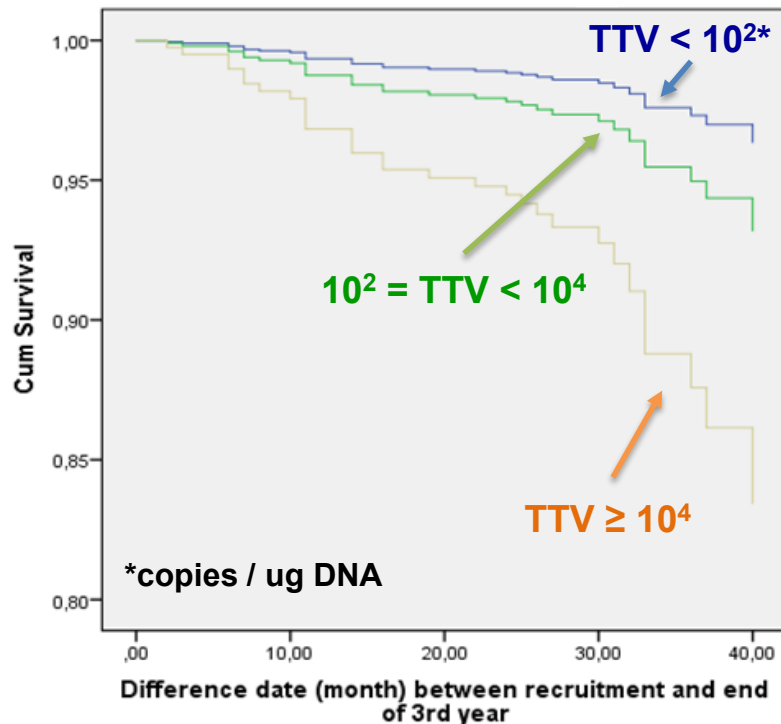
Assay	Test Specifications	Availability
Torque teno virus	Measures level of viral DNA in body fluid	Research setting only
Epstein-Barr virus DNAemia	Measures level of viral DNA in body fluid	Commercially available, multiple labs
Cylex Immunoknow	Nonspecific cell-mediated immunity assay measuring adenosine 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



Virome and immunosenescence



TTV and mortality in elderly subjects



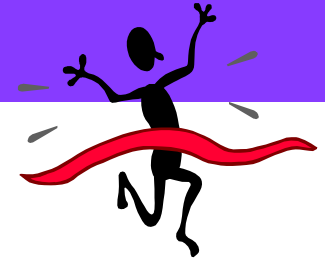
The proportion of pts died after 3 yrs of follow-up:

- 22% for pts with TTV copies ≥ 4.0
- 5% for pts with TTV copies < 4.0

Independent variable	Dependent variable "Death"	
	β	<i>p</i>
Age	- 0.095	0.004
Triglycerides	0.001	0.789
HDL	-0.003	0.848
Cu/Zn ratio	-0.749	0.058
Gender	0.409	0.466
TTV load	1.386	0.009



Conclusions



- ✓ Our understanding of the human virome is still fragmented and standardized methods are required for providing a more accurate study of the virome
- ✓ Assessing the human virome as a whole will be of interest in medical diagnosis and give highly valuable information to monitor the patient management
- ✓ By now, novel associations and routinely unemployed kinetics of viruses within the virome have important clinical implications for the relationships between transplantation, graft rejection and viral reactivation