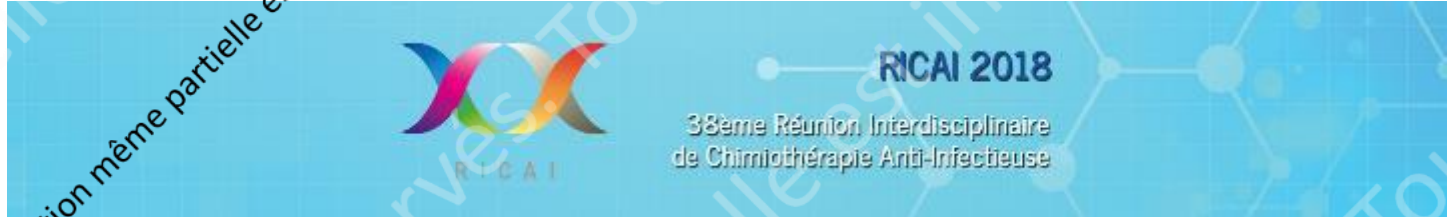


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Speaker's name :

Fabrizio Maggi,

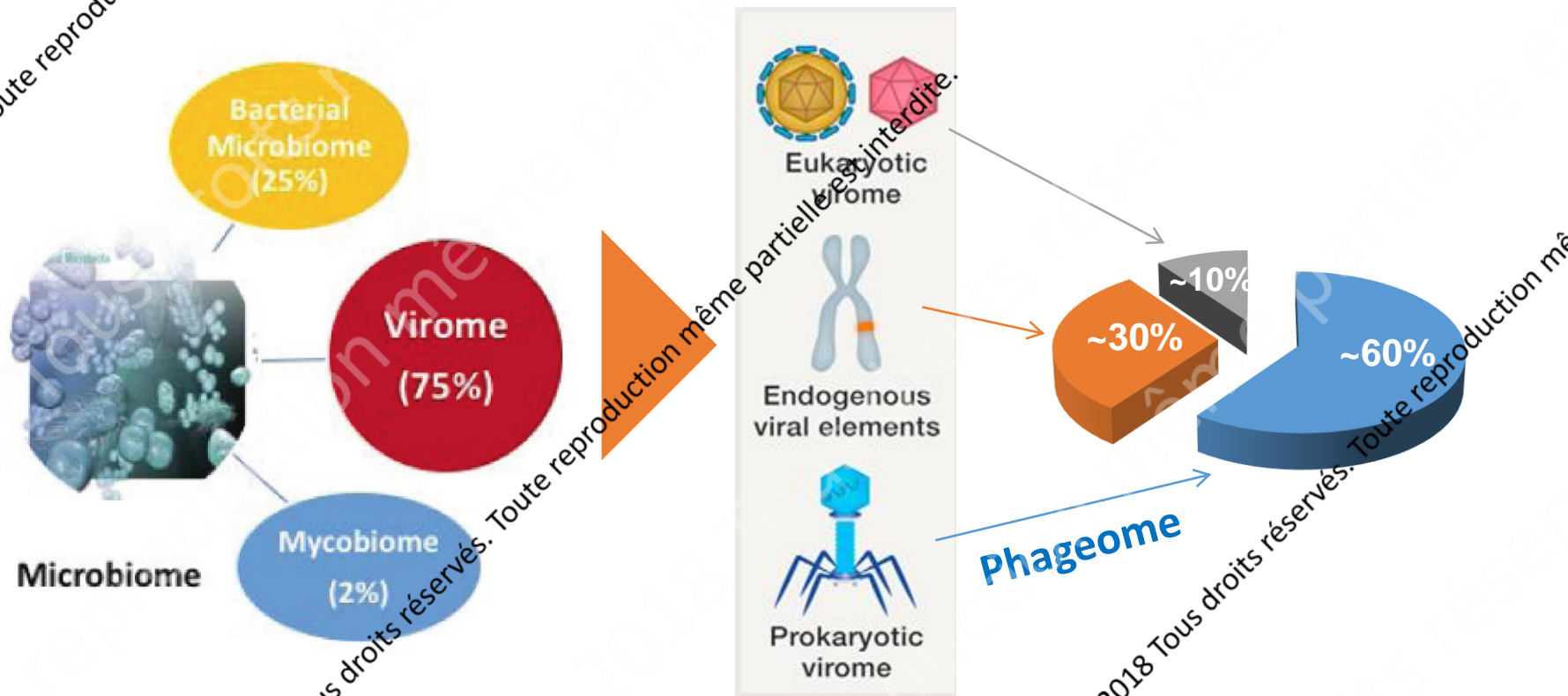
**Department of Translational Research, University of Pisa
Virology Division, Pisa University Hospital**

TTV agent pathogène? ou marqueur de pollution anthropique?

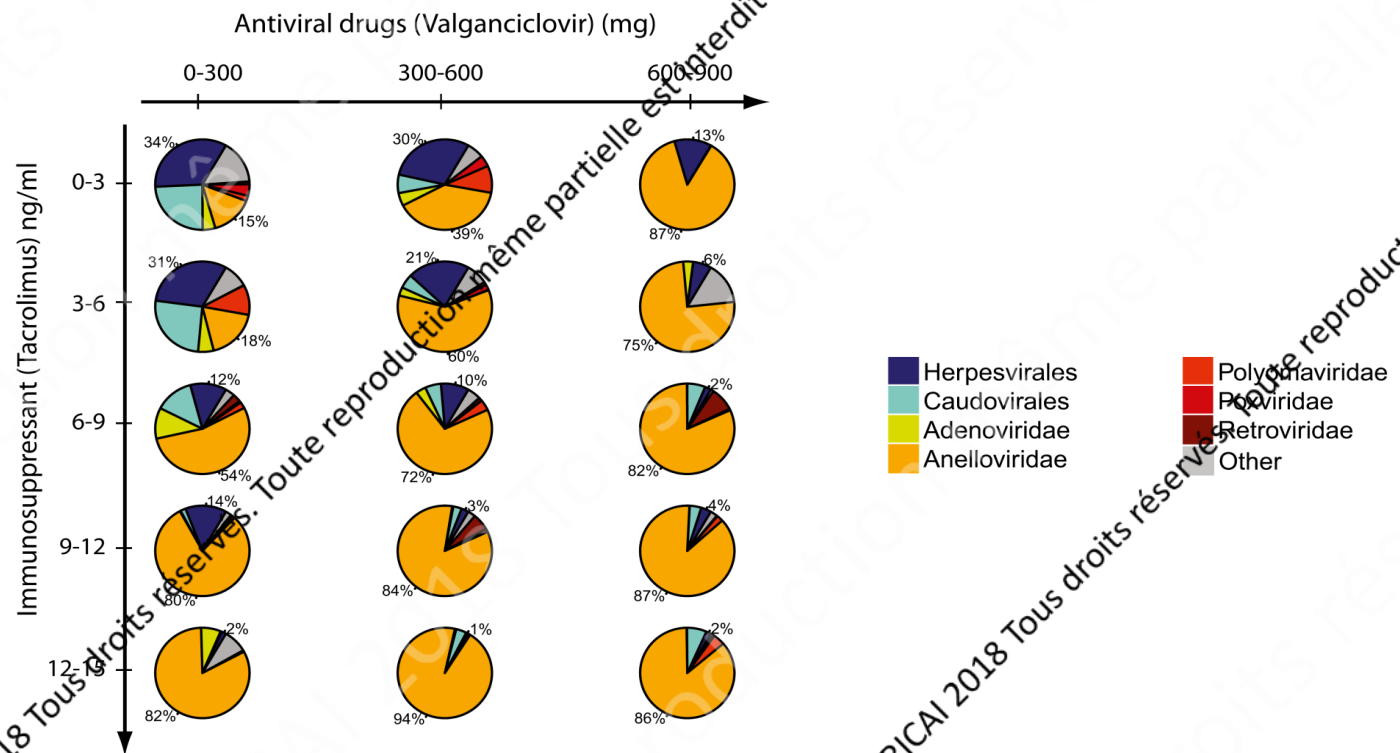
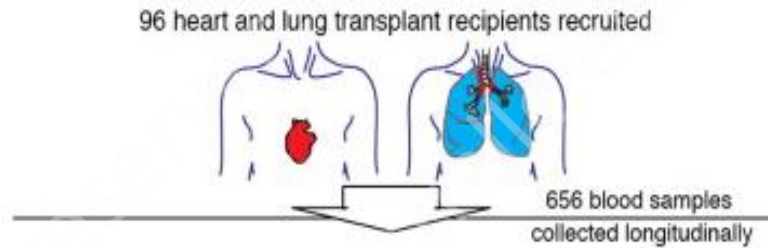
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Virome

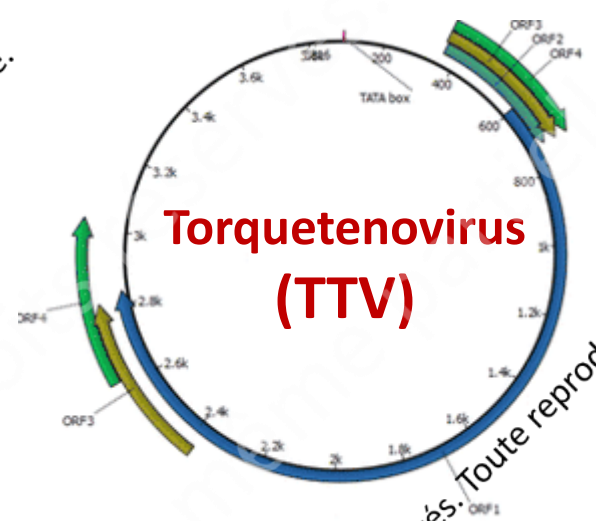
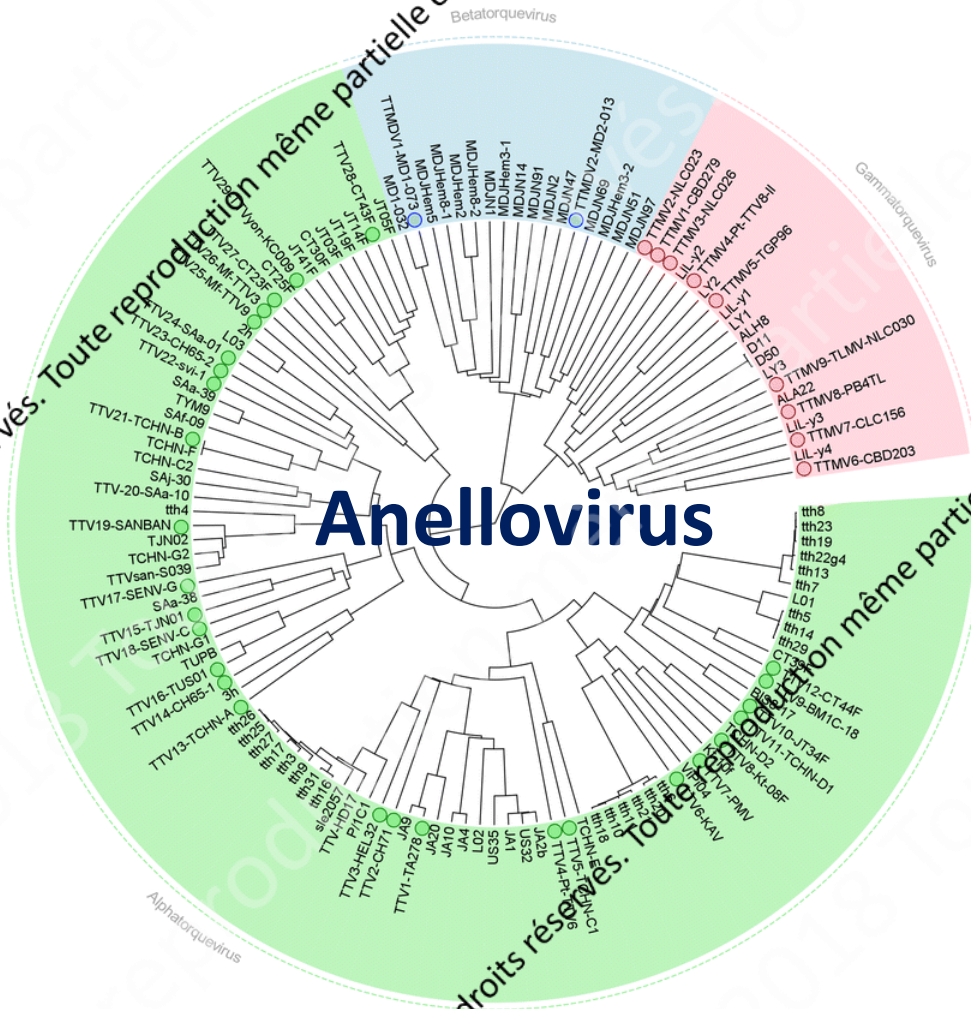
- **the full compendium of viruses from a particular habitat, including not only pathogenic viruses but also viruses essentially devoid of pathogenic potential**



Anelloviruses: the most prevalent component of blood virome



TTV: the prototype of anelloviruses



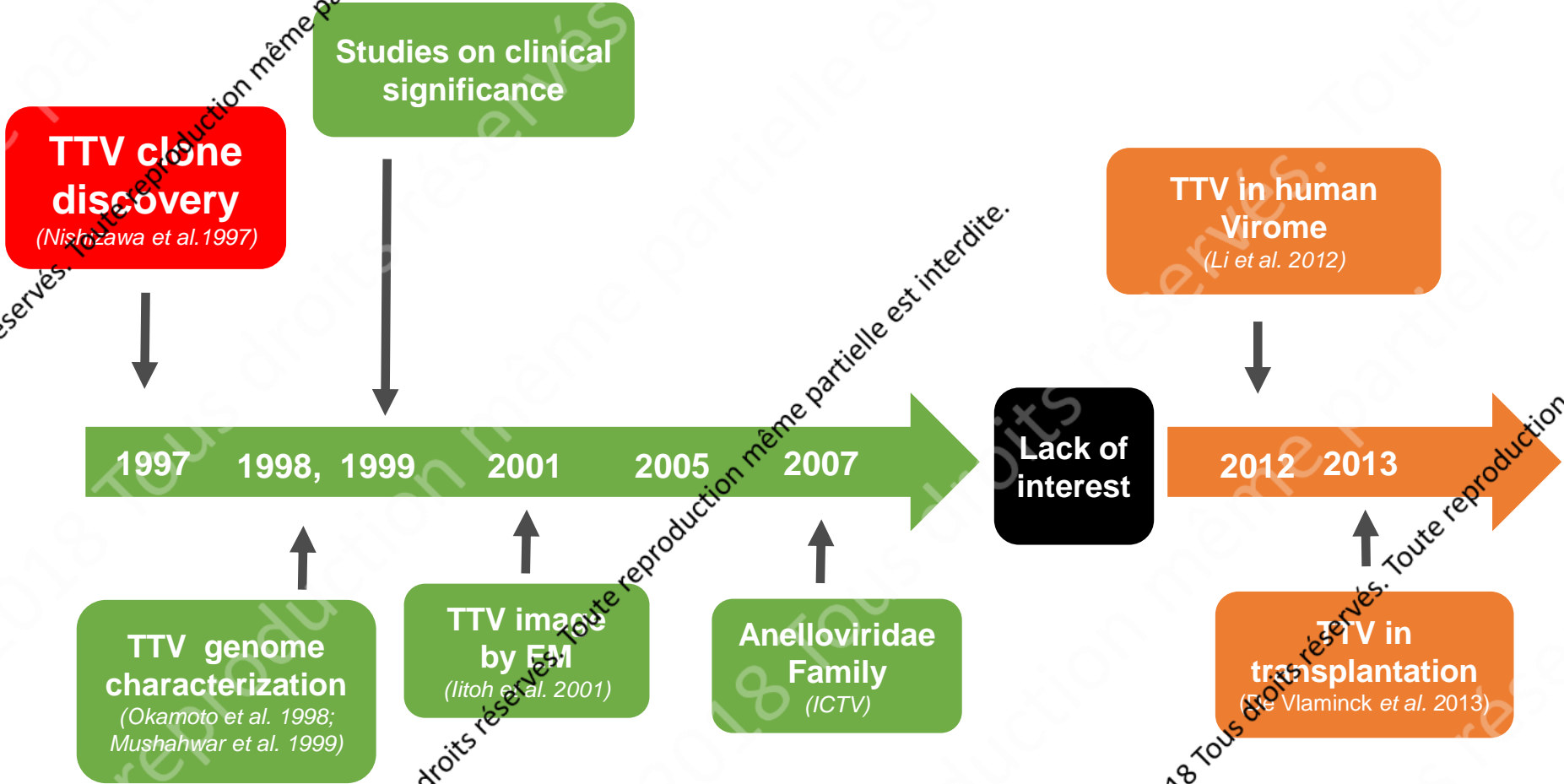
Derivation of name

Torque: from Latin torques, "necklace"
Teno: from Latin tenuis, "thin"

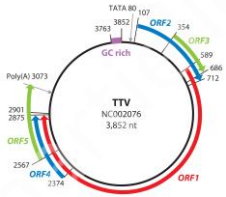
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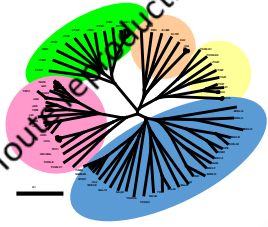
Human TTV story



TTV properties



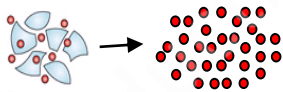
✓ **GENOME:** small circular ssDNA (2.2 to 3.7 kb)
(the genetically simplest of all known replication-competent viruses hitherto detected in humans)



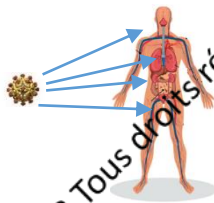
✓ **GENETIC VARIABILITY:** extremely high
(at least 29 human TTV species, each consisting of numerous types)



✓ **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



Dynamics of chronic TTV viremia, relative to other viruses

Virus	Mean virion half-life in plasma (hrs.)	New virions entering plasma per day	Reference
HBV :	28.8	$> 1.7 \times 10^{13}$	Whalley et al. 2001
HCV :	2.7	$> 1.3 \times 10^{12}$	Neumann et al. 1998
TTV :	4.3	$> 2.0 \times 10^{10}$	Maggi et al. 2010
HIV :	< 1	$> 9.8 \times 10^9$	Ramratnam et al. 1999



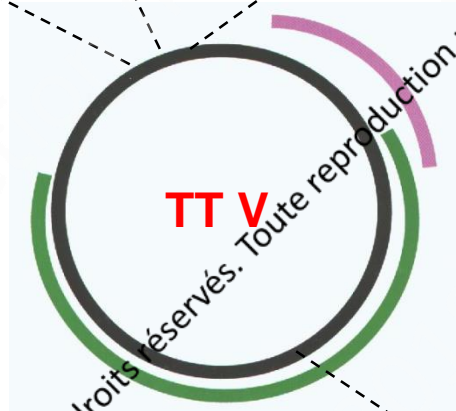
TTV diagnosis

“Universal” real-time UTR PCR

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



UTR



Genogroup-specific PCRs

Genogroups 4 and 5

G4-1	CCATTTTGTGCAGCCCG
G4-2	CGGCCGACTCCACGGCAT
G4-3	AGCCCGCAATTTCTGTT
G4-4	ACGGCATGAYTTTGTGTCTCTG
G5-1	CCAACCTGCAAAGAAAAAACACCT
G5-2	CGCCTCCTTACTCTTCGTCGTC
G5-3	CGAGCACCTCTGGTACGAGTC
G5-4	GTCTGCGAAGTCTGCCACGGG

Genogroups 1, 2 and 3

G1-1	AACGARGACCTAGACCTTTCAGATA
TTV2	CAGTTAGTGGTGAGCCGAGATA
G1-3	TTYAGACACCCARAIGTAGACTT
G1-4	GTGAGCCGAACGGATGATG
G2-1	AATATGACMCCTTGGAGGIGG
G2-2	TGAGCAGAACCGTAACCGCAAG
G2-3	CTGGAGIAGATGAACRTAGA
PMV4	CTGTAAATAAGTGGGGGG
G3-1a	AAYGACCGCTAGACCTTGCCAGATA
G3-1b	AAAYACAGCTAGACCTTIGCYAGAT
G3-2	TTWCGTGTGRGCIGAACCG
G3-4	TGKCTGTACCAITTRTCTWCAA



TTV pathogenicity: an extraordinary difficult issue to untangle

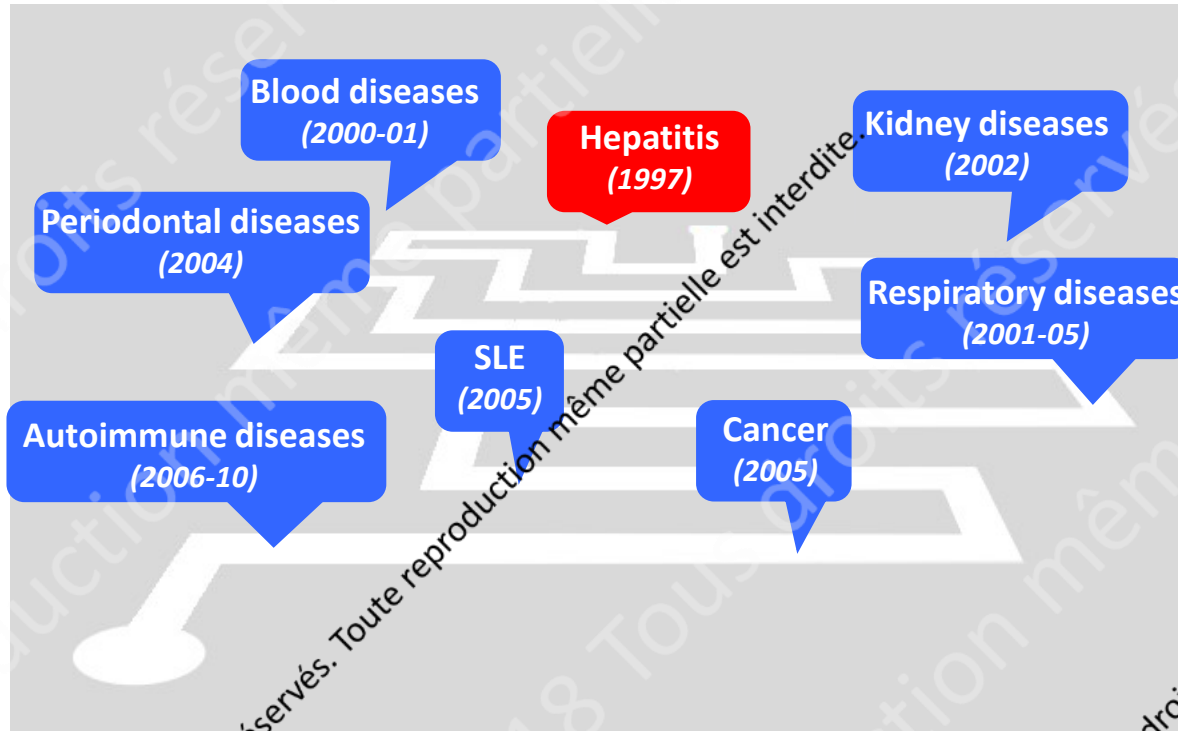


Reasons:

- ✓ extremely high prevalence of active TTV infections among apparently healthy individuals
- ✓ TTV presence in many different tissues
- ✓ existence of numerous TTV species
- ✓ frequent occurrence of co-infections by two or more species of TTV
- ✓ wide range of TTV loads in different individuals

Investigations on the pathogenic potential of TTV

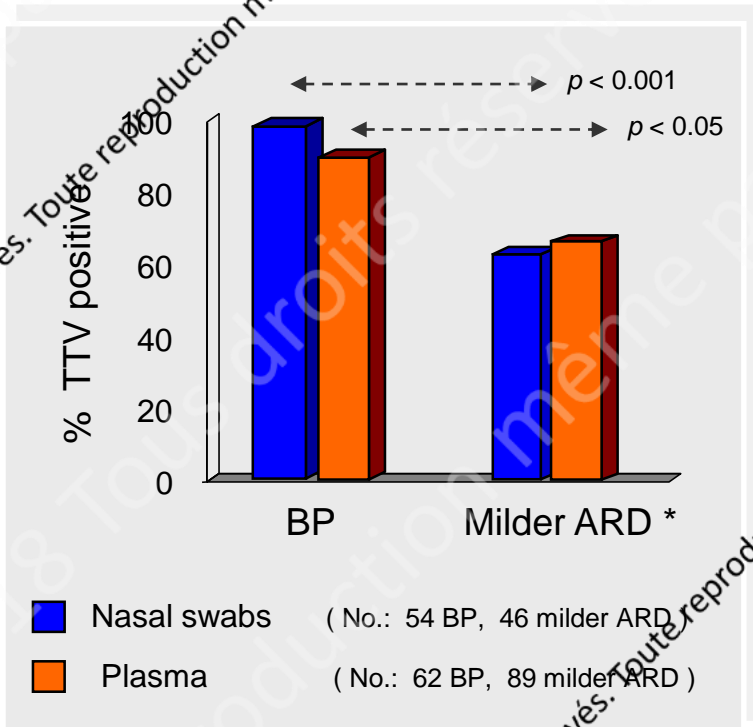
Etiological agent of disease or **cofactor of other diseases** ?



In spite of all efforts, to date 20 years after its discovery, no conclusive evidence has been reached

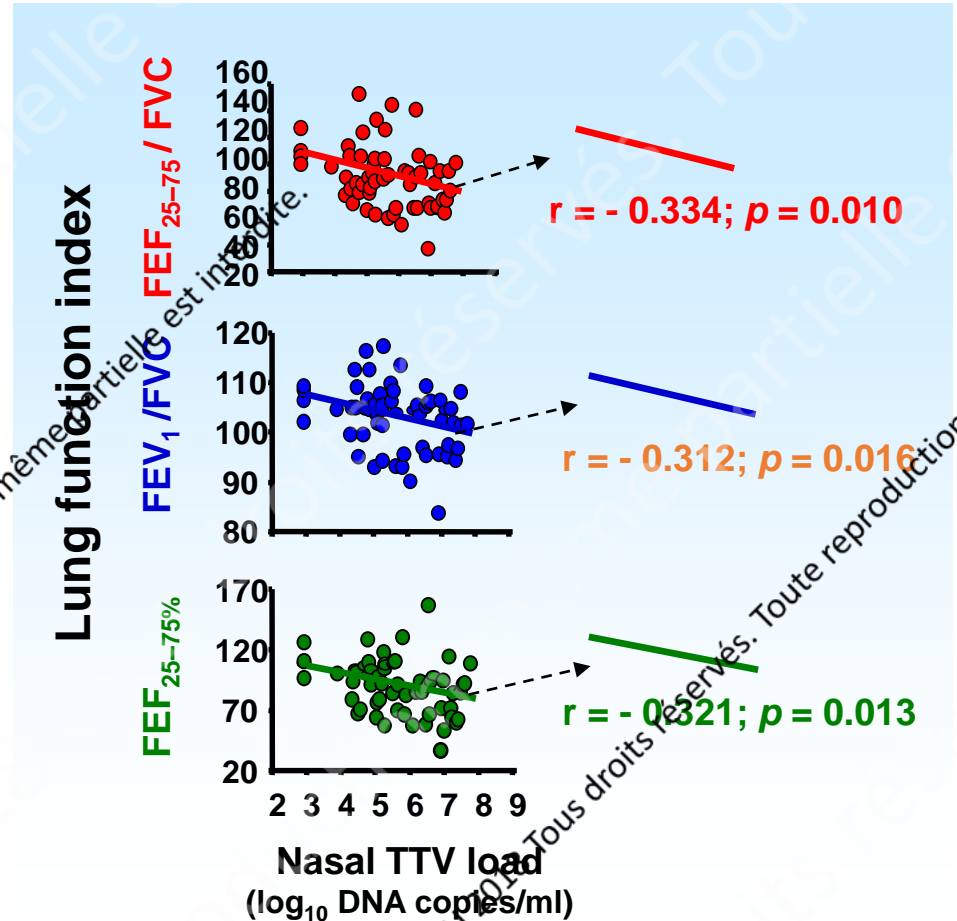
TTV and respiratory diseases

Children with acute respiratory diseases (ARD)



* Including laryngitis, bronchitis, and bronchiolitis

Asthmatic children



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?***
- ***TTV species diverse in their ability to induce pathogenicity?***



TTV as a potential bio-marker

- Prevalence
- Ubiquity

- Fast and cheap quantification by rtPCR
- Insensitivity to antiviral drugs



TTV has proved useful in 2 main fields as marker:

- ✓ to detect anthropic pollution
- ✓ to follow up kinetics of functional immune competence

TTV as a marker of anthropic pollution

✓ **Because of its extremely high resistance**

➤ **TTV is frequently found in:**

- **wastewater (38–100%)**
- **drinking water (5-12%)**
- **river water**
- **surface samples (15%) collected in hospital settings**
- **air samples (16%) collected in hospital settings**



➤ **May be particularly useful for evaluating the efficiency of treatment or cleaning procedures in microbe removal (i.e. in wastewater or in blood products)**

Charest *et al.* J Water Health 2015; Vecchia *et al.* Food Environ Virol 2013; Diniz-Mendes *et al.* J Appl Microbiol 2008; Verani *et al.* Water Sci Technol 2006; Carducci *et al.* J Hosp Infect 2011

TTV as a marker for evaluating the nanofiltration efficacy

Transfusion Medicine, 2009, **19**, 213–217

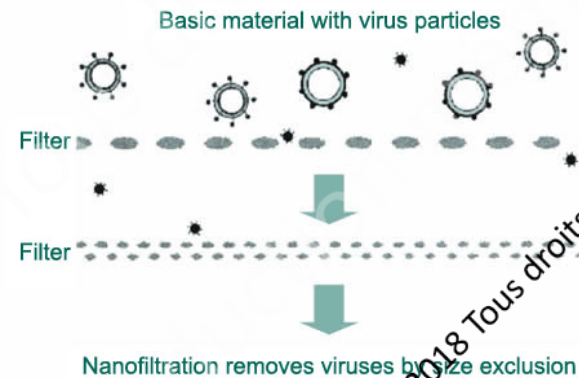
doi: 10.1111/j.1365-3148.2009.00931.x

SHORT COMMUNICATION

Effectiveness of nanofiltration in removing small non-enveloped viruses from three different plasma-derived products

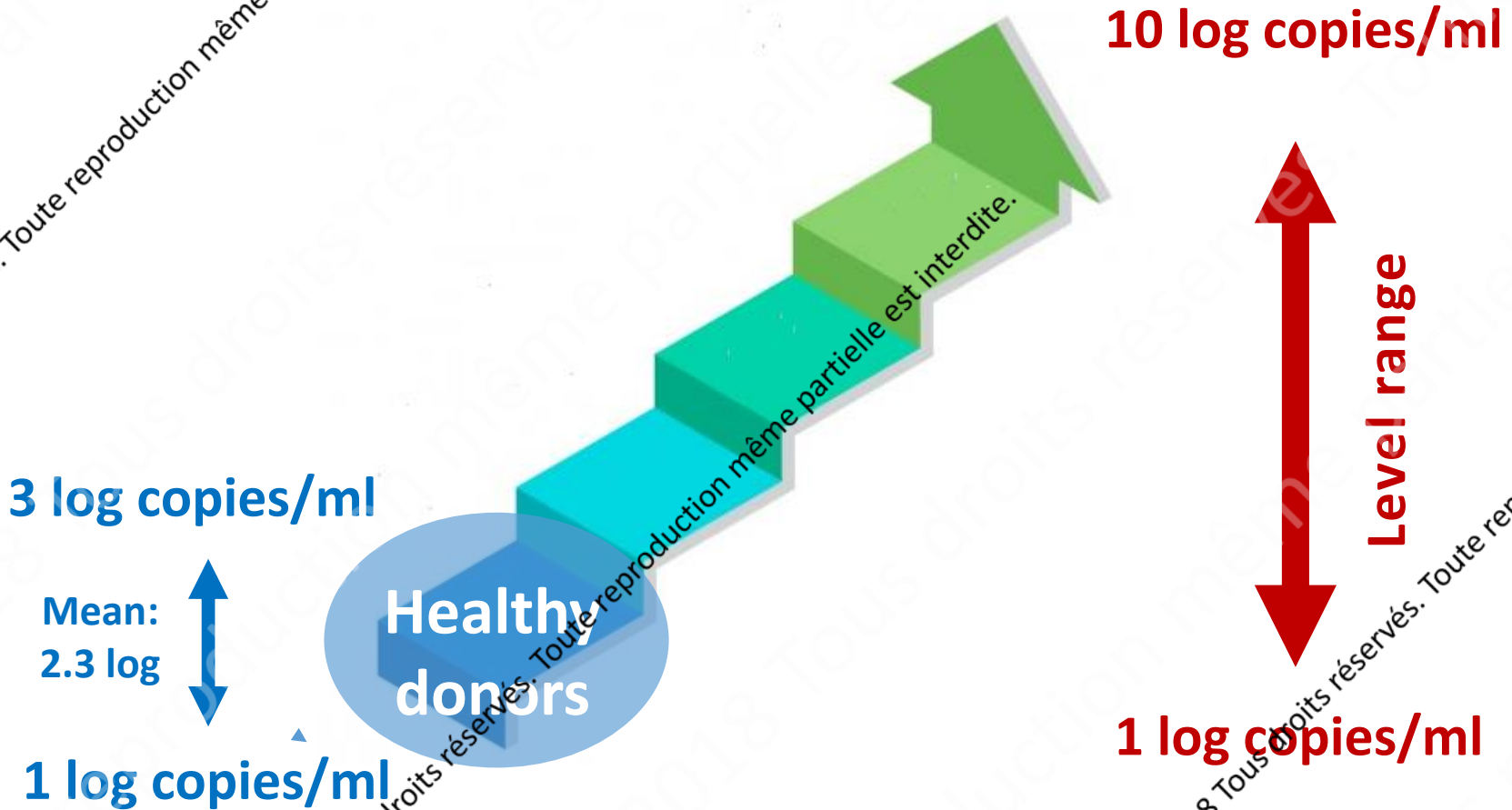
M. C. Menconi,* F. Maggi,† K. Zakrzewska,‡ V. Salotti,§ P. Giovacchini,* C. Farina,*
E. Andreoli,† F. Corcioli,‡ M. Bendinelli,† & A. AZZI‡ * *Keison S.p.A. Research Centre, Castelvechio
Pascoli (LU)*; † *Virology Section and Retrovirus Center, Department of Experimental Pathology, University of Pisa, Pisa*; ‡ *Department of
Public Health, University of Florence, Florence*, and § *Genedia Srl, R&D, Napoli, Italy*

Received 03 December 2008; accepted for publication 06 February 2009

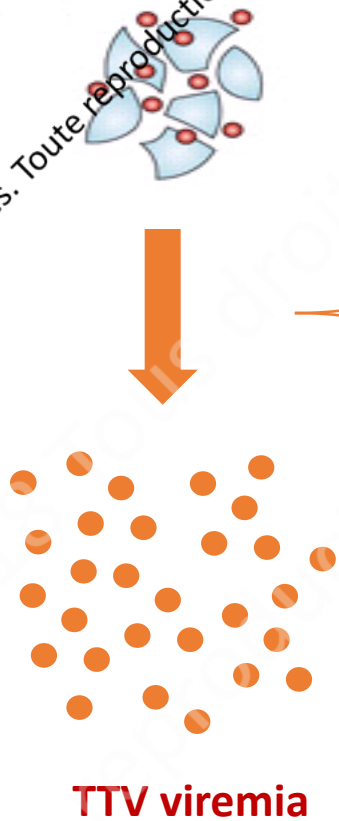


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Levels of TTV viremia



Factors that may impact on the size of TTV viremia



TTV viremia

No./spectrum/turnover of cells replicating TTV(s)	H*
No. of virions produced per cell and daily	H
Rate of virions release/clearance into/from the circulation	H
Proportion of immunocomplexed virions	H
No. of different TTVs harbored	EA
Synergy or interference between the TTVs carried	EA
Acute intercurrent superinfection by a different TTV	EA

Depressed immune responses	P
Changes in the functional integrity and relative proportion of cells participating in such responses	H
Counts of circulating lymphocytes	EA
Immune activation by superimposed exogenous immunogens	EA

Concomitant infections by other pathogens	H
Presence of concomitant noninfectious pathologies (tumours, etc.)	EA

Immunosuppressive therapies	P
Local accumulation of proliferating lymphoid cells	H
Regeneration rate of susceptible cells	H
Age	P

* P: proved; EA: at least some direct evidence available; H: hypothetical

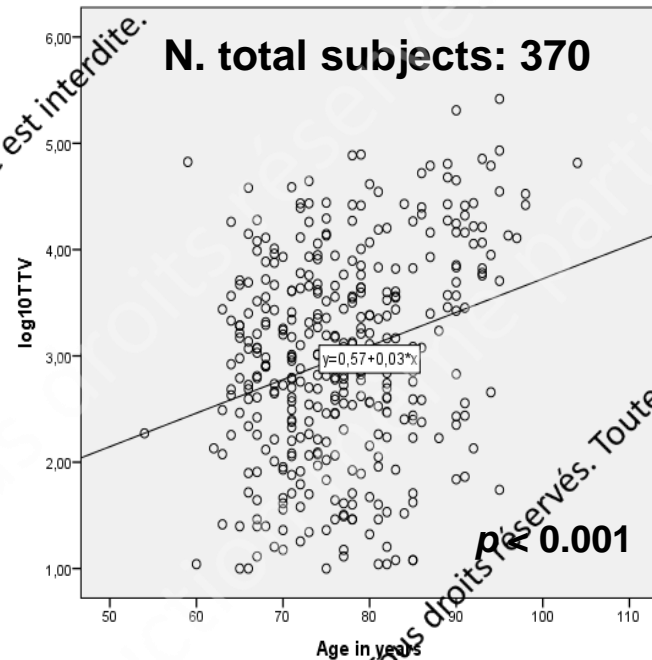
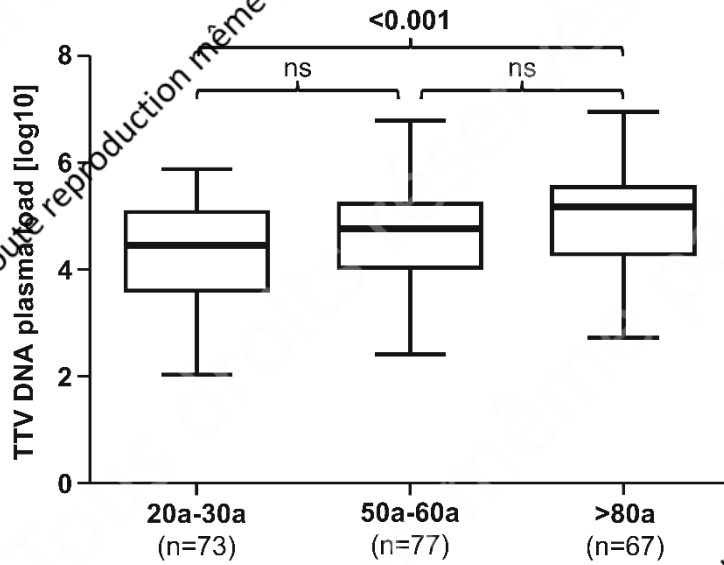
Virus – related

Immunological

Host – related

Factors

TTV viremia and age



TTV and mortality in elderly subjects

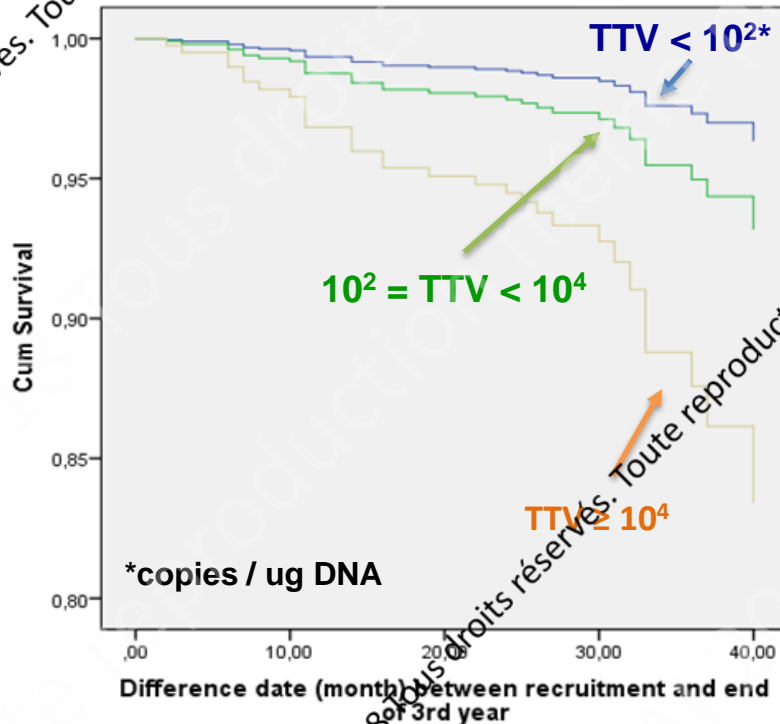


✓ 379 elderly subjects who were followed up for 3 yrs (age range 60–105 yrs)

✓ 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

✓ TTV load is a strong parameter for predicting the mortality (Cox regression analysis)



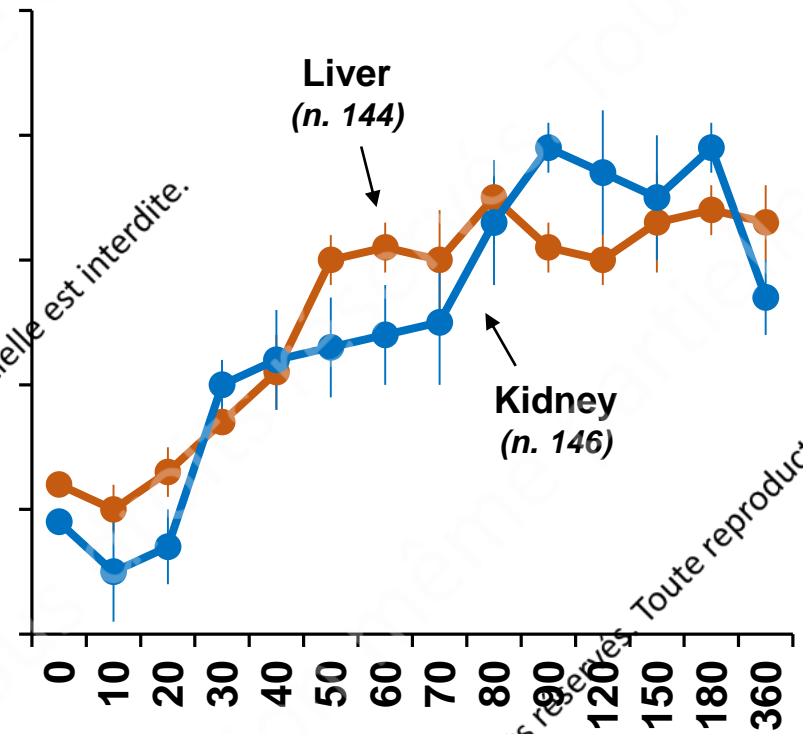
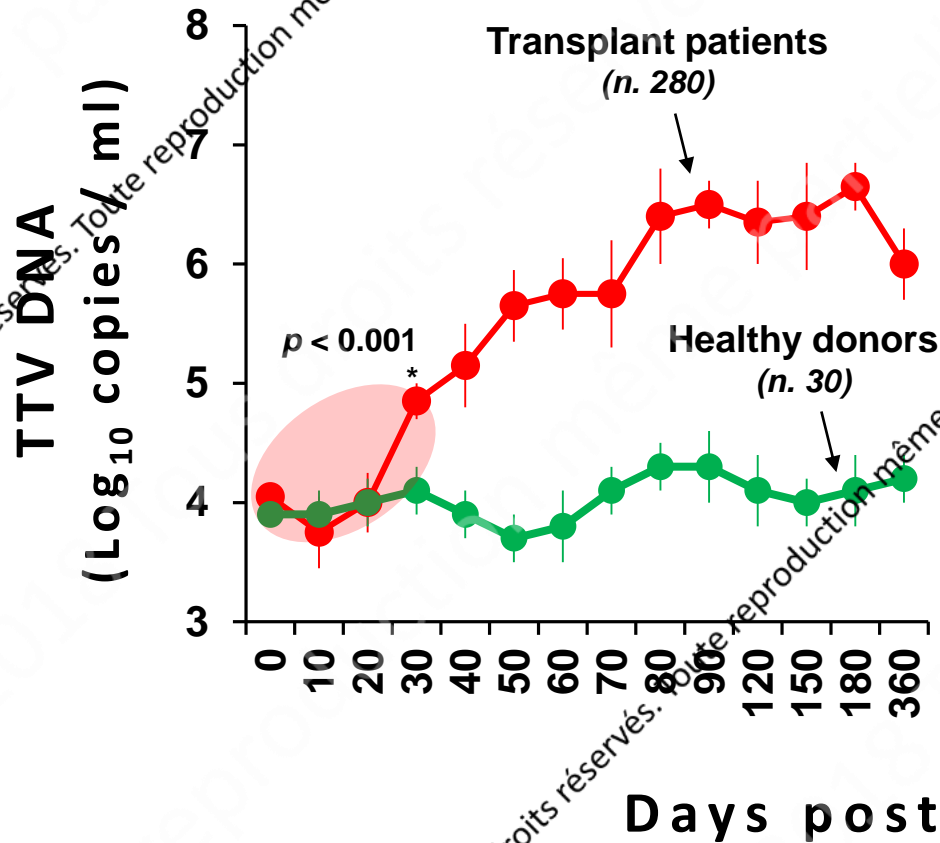
Independent variable	Dependent variable "Death"	
	β	<i>p</i>
Age	- 0.095	0.004
Triglycerides	0.001	0.789
HDL	-0.003	0.848
Cu/Zn ratio	-0.749	0.058
Gender	0.409	0.466
TTV load	1.386	0.009

Biomarker assays useful in predicting post-transplant complications

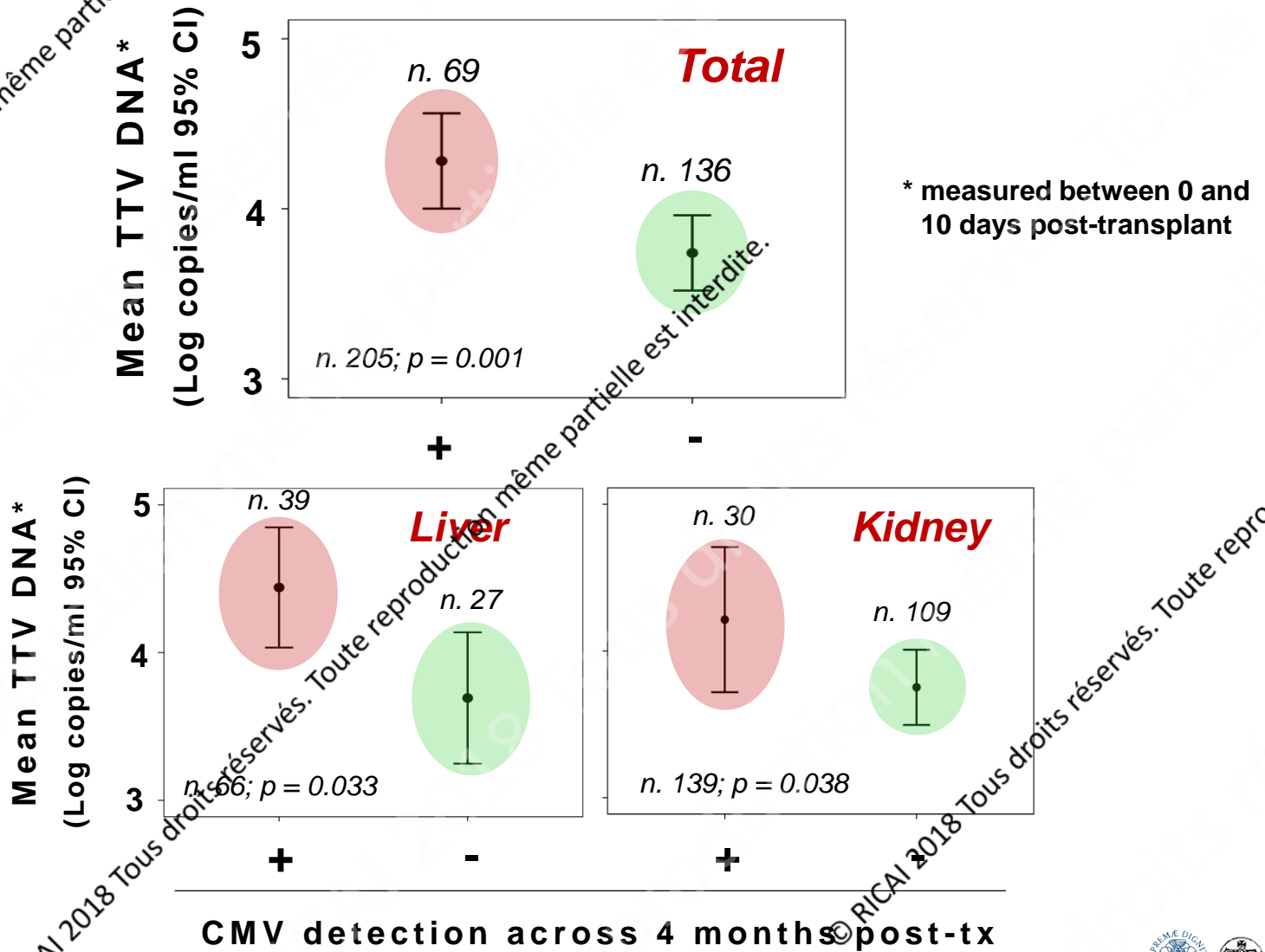
Assay	Test Specifications	Availability
Torque teno virus	Measures level of viral DNA in body fluid	Research setting only
Epstein-Barr virus DNAemia	Measures level of viral DNA in body fluid	Commercially available, multiple labs
Cylex Immunoknow	Nonspecific cell-mediated immunity assay measuring adenosine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



TTV viremia in kidney/liver transplant patients



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 $3.45 \log_{10}$ 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 can be useful**

✓ **In addition to its use as potential viral indicator of anthropic pollution, TTV may serve as a cheap and easy-to-measure surrogate of functional immune competence, and could prove especially useful in the management of transplant patients**

