TTV marqueur d'immunosuppression en greffe d'organe ?

S Alain

CNR des Herpèsvirus, CHU de Limoges

Viruses and SOT

CMV

EBV
PTLD

HSV/VZV

HHV-6
HHV-7

HHV-8

BKV/JCV
ADV
Virus
respiratoires

CMV
GCV/VGCV
Prophylaxis

EBV
PTLD

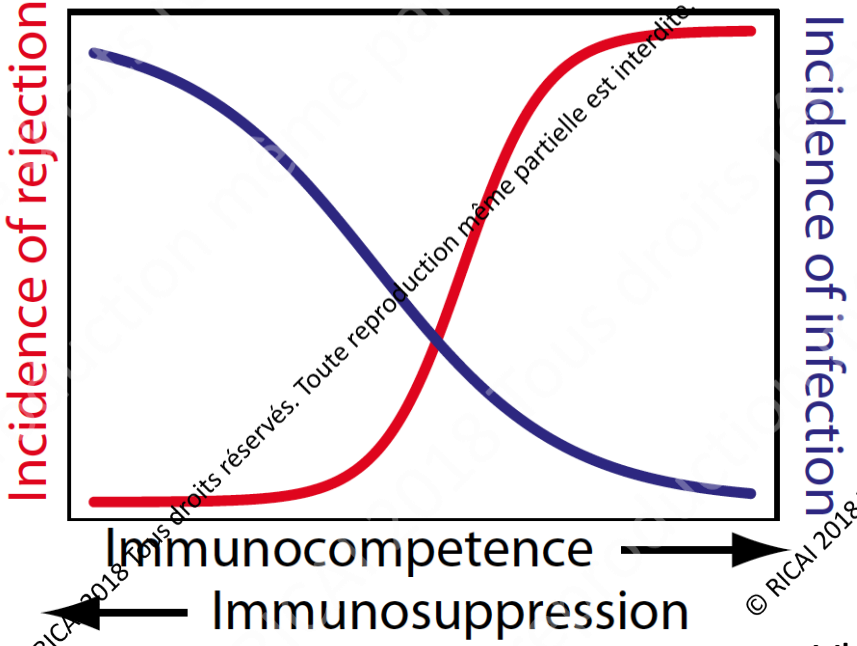
HSV/VZV
prophylaxis ACV

HHV-6
HHV-7

HHV-8

BKV/JCV
ADV
Virus
respiratoires

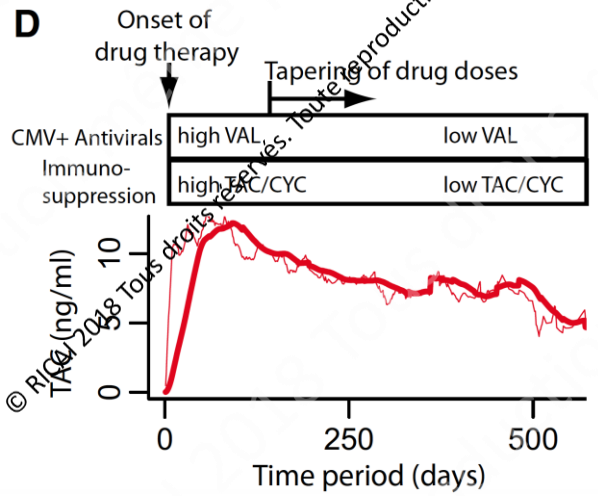
Close relationship between immune status rejection and infection

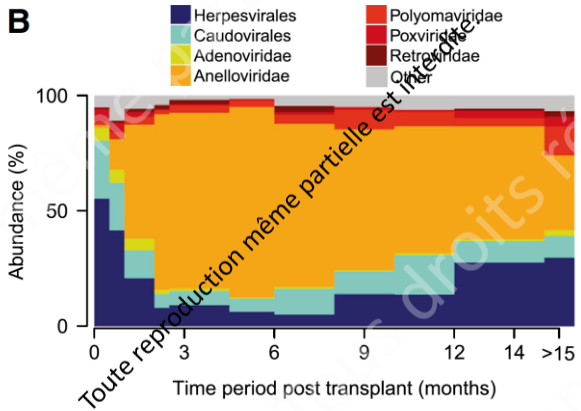


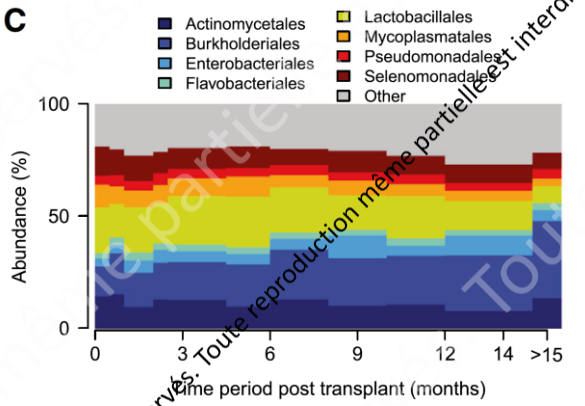
Vlaminck et al., Cell 2013

First insight : virome analysis

96 organ recipients, 41 adult heart, 24 pediatric heart, 31 adult lung, 656 plasma samples

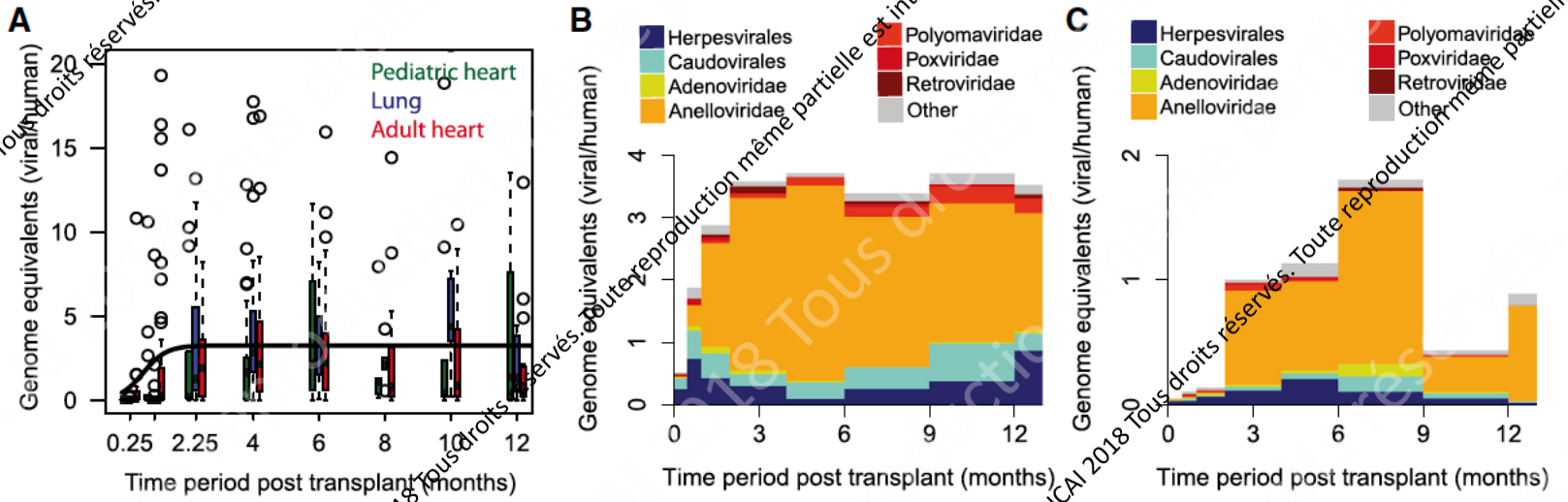




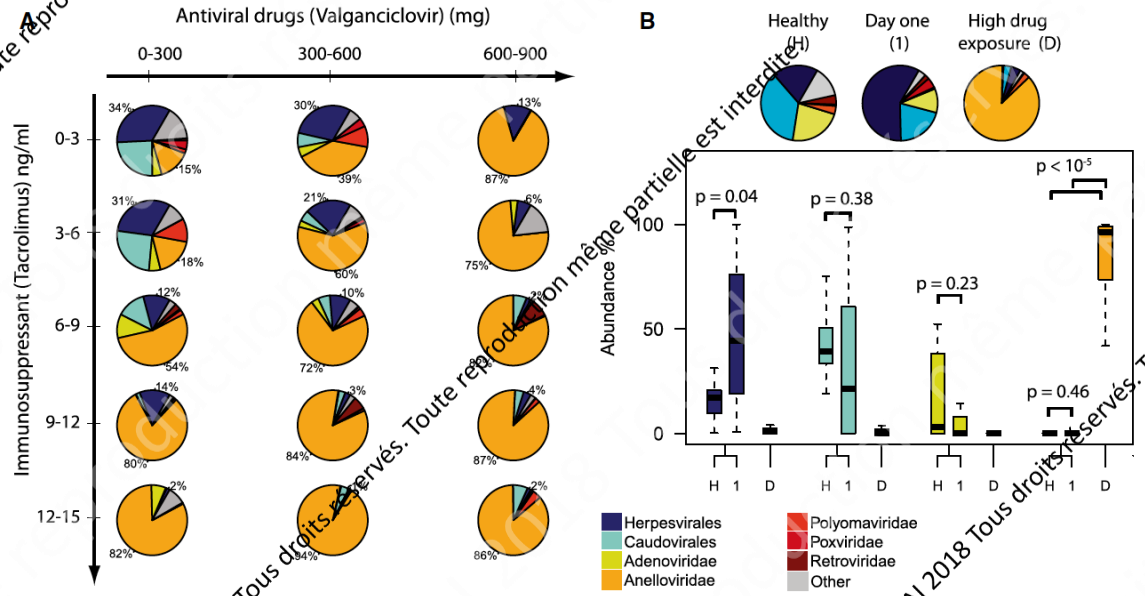


Vlaminck et al., Cell 2013

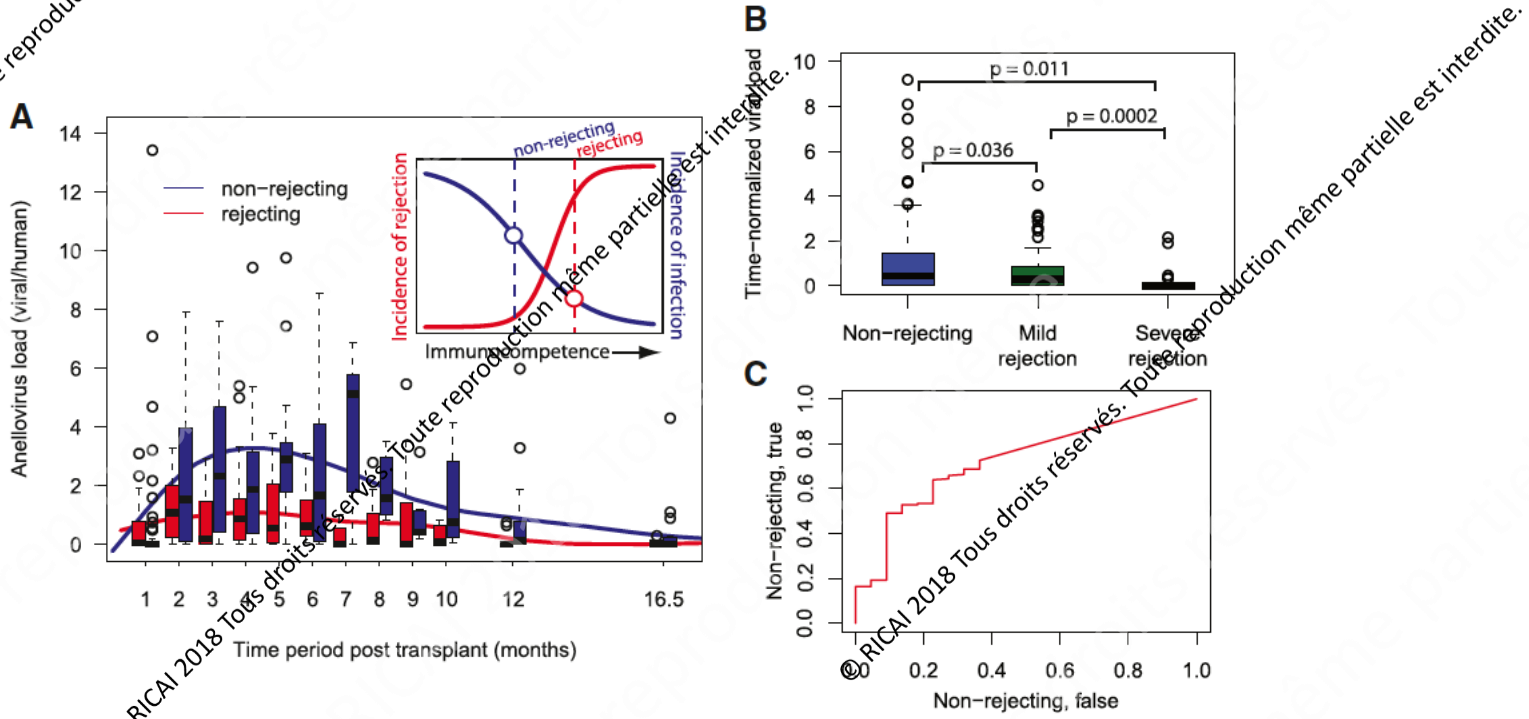
Virome evolution in presence or absence of prophylaxis



virome evolution with antiviral treatment



Anellovirus viral load decreases with graft rejection



How can we quantify TTVs ,

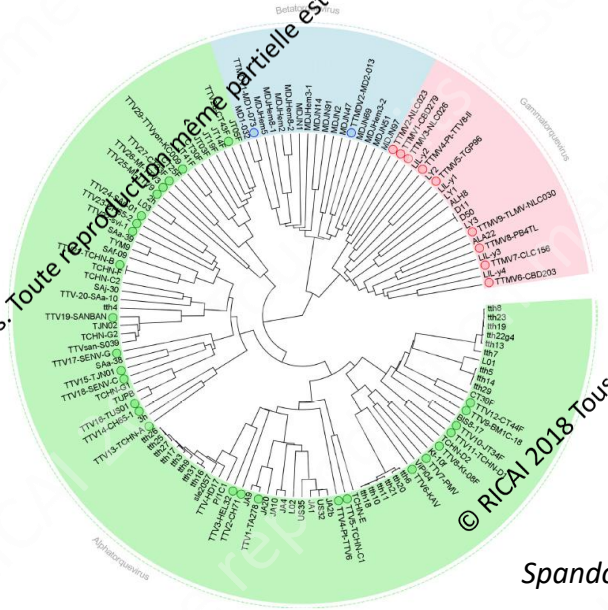
- Not one virus but a family of viruses TTV, TTMV et TTMDV
- Quantify at least the 12 more frequent TTV in human being, not TTMV or TTMDV
- Targeting UTR (better than ORF1)

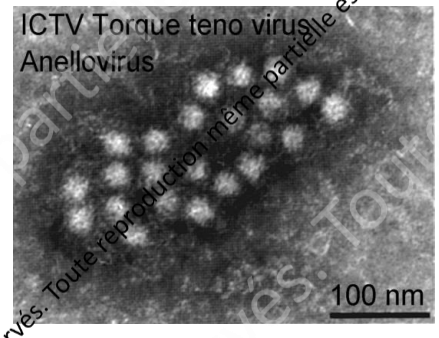
- Several in house PCRs
- 1 standardized assay (TTV Rgene®) (2-10log copies/mL)

Two potential matrices : plasma/whole blood

- Opening the way to standardized evaluation

Possible association with other viral load measurement





TTV (*Ytoh, BBRC, 2001)*

Spandole et al, 2015, Kulifaje et al., JCV 2018)

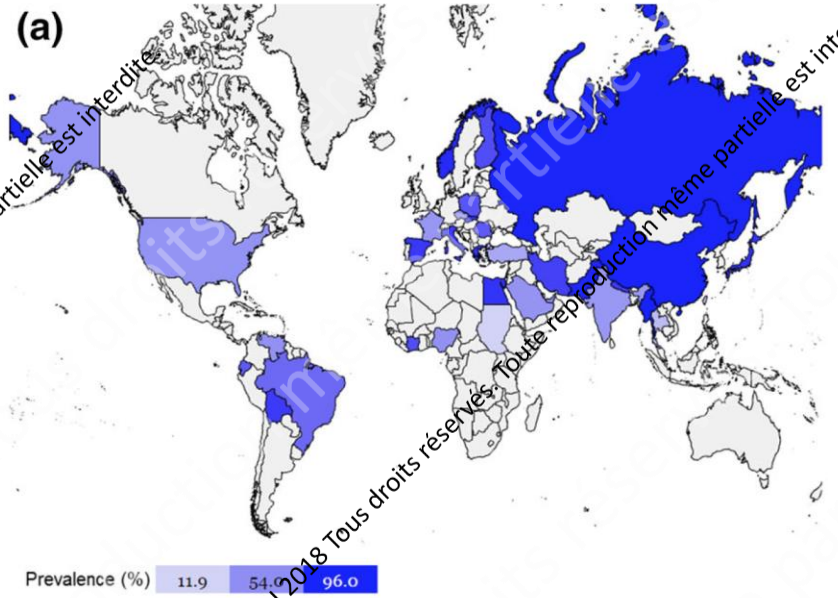
Evaluation of immunosuppression post solid organ allograft (ex CMV) few tests available in routine use

- **Innate immunity**
- NK cells functions
- γδ (gamma-delta) T lymphocytes
- genetic polymorphisms IL28B, Kir genes, TLRs
- **Non specific adaptive immunity**
- Hypogammaglobulinemia (26-70% in some series)
- Lymphopenia
- ImmuKnow (Cylex/Viracor-Eurofins, USA) amount of ATP produced by CD4+ T cells in response to whole blood stimulation by phytohemagglutinin (predictive value not known)
- **Specific adaptive immunity : only 3 tests linked to viremia or disease**
- QuantiFERON –CMV ®, 2 interventional studies (Kumar et al, 2017, kidney and Westall et al., Lung)
- Elispot assay (CD4 + CD8)
- Intracellular cytokines
- consensus recommendations : not clearly recommended but need for interventional studies

Kotton et al. 2017, Transplantation

TTV skills as a biomarker

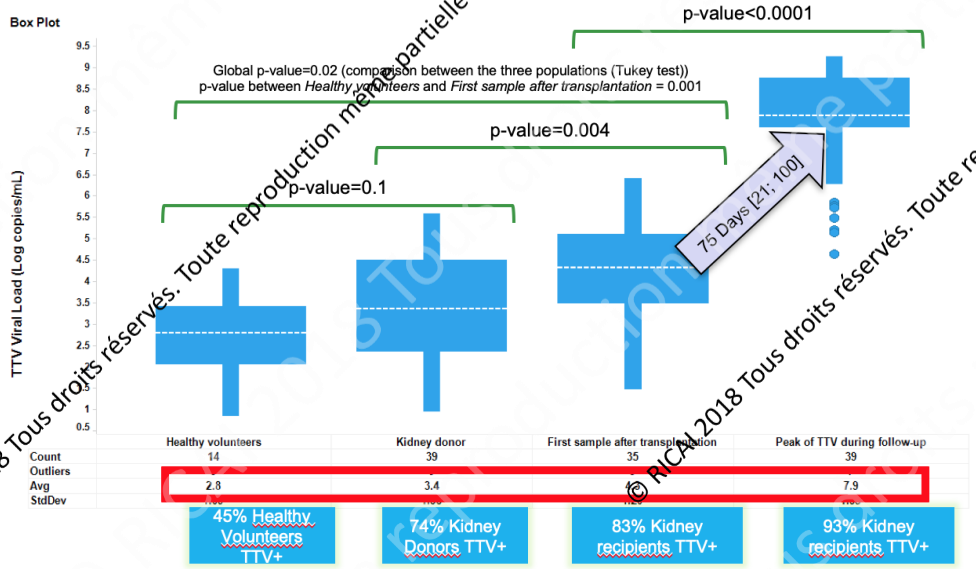
- **Worldwide distribution**
- **Early acquisition**
- **High prevalence of DNA detection** regardless of age, ethnicity, sex, and socio-economic status
- **High frequence of TTV DNA detection in plasma from immunocompromised patients :**
  - 70% Heart-lung (*De Vlaminck et al, 2013*),
  - 84% lung (*Gorzer et al, 2014*)
  - 74% Liver (*Simonetta et al, 2017*)
  - 83% Kidney (*Kulifaje* et al., 2018)
- **Fluctuations associated with the immune status**



Prevalence of DNA detected in UTR region (spandole et al., 2015)

TTV skills as a biomarker for immunosuppression

- High rate of replication : 10^{10} genome units per day, and rapid turn over from plasma
- Plasma viral loads from 10^2 to 10^9 genome copies/mL
- Kinetics associated with CD8+57+ T lymphocytes (CSH)



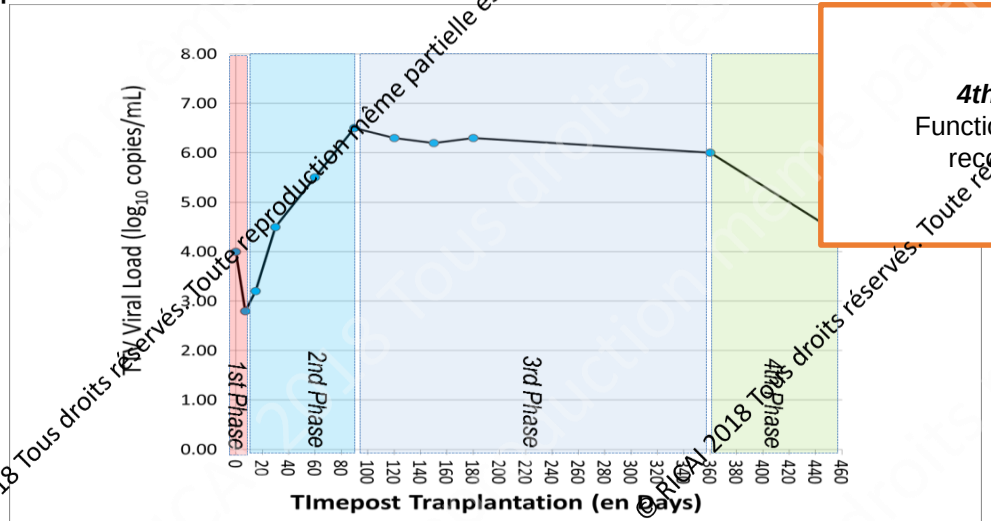
Viral load kinetics in the solid organ transplantation model

- 4 phases (adapted from Maggi et al. ESCV 2017)
  - ≈15 days post-transplantation: viral load diminution
  - ≈15 days-3 months post-transplantation: viral load increase from 3 to 6-7 log_{10} copies / mL (kidney), 9-10 log_{10} copies / mL (lung).
  - ≈3 months- 1 year post-transplantation: viral load stabilization
  - After 1 year post-transplantation: viral decrease and return to basal level

TTV : A Tool to adjust immunosuppression: (IS drug dosages are used for toxicity)

Threshold?

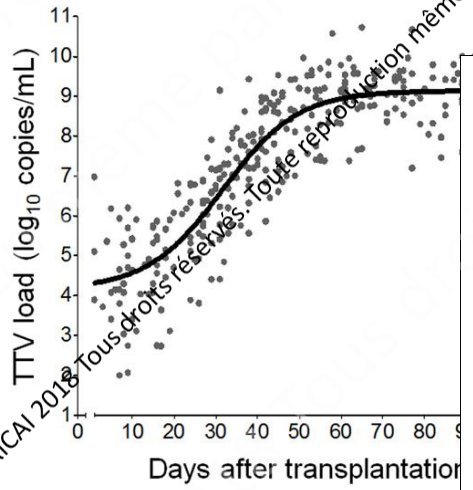
Kinetics?

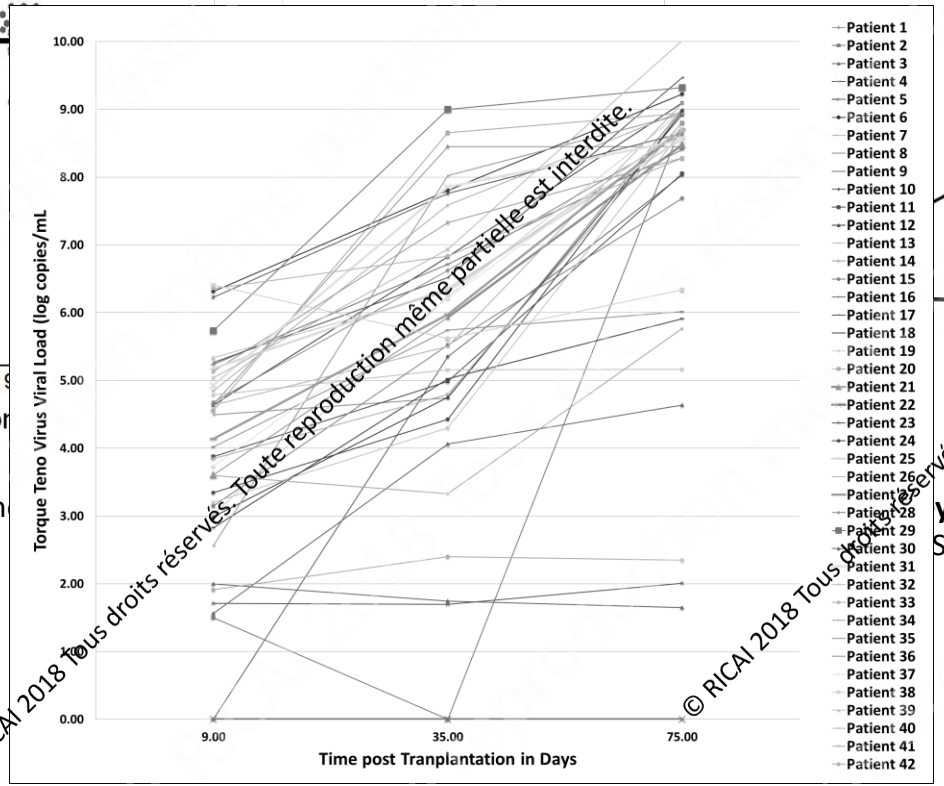


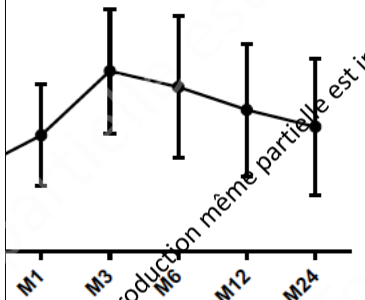
TTV Viral load Kinetic in SOT transplanted patients
(Adapted from Maggi *et al, Oral Communication ESCV 2017*)

4th Phase :
Functional immune reconstitution

Closely similar kinetics whatever the organ but viral load at plateau differ in lung and kidney and early kinetics vary







46 **lung** transplant recipients in h
PCR plasma
(Gorzer et al. Plos one 2015)

y recipients, TTV Rgene®
Solis et al., 2017)

PRÉDICTIVE VALUE OF TTV VIRAL LOAD IN ORGAN RECIPIENTS

kidney and liver

Lung

Kidney / BK virus

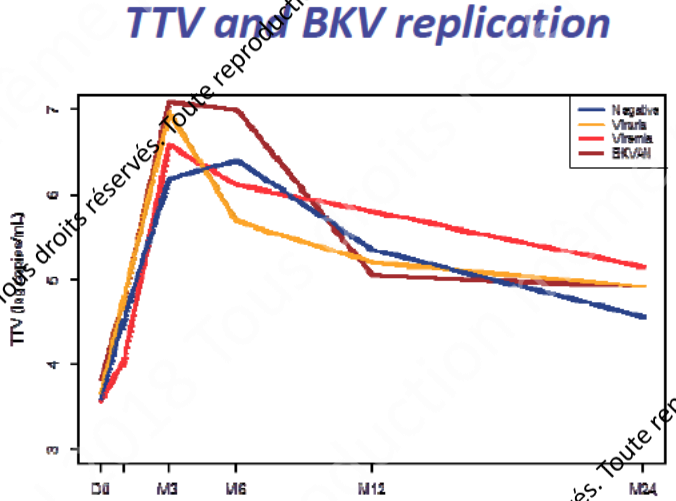
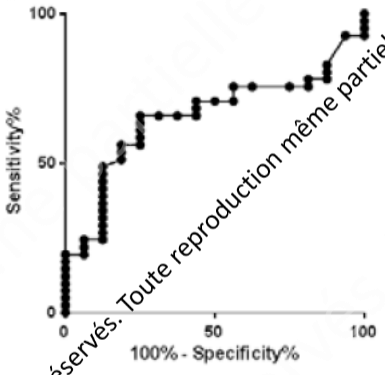


Figure 5. TTV load increase at month 1 and BKV replication.
An increase of TTV viremia by 1 log_{10} copies/mL between D0 and M1 was associated with **BKV viruria development** (p=0.068; sensitivity: 64%; specificity: 75%).

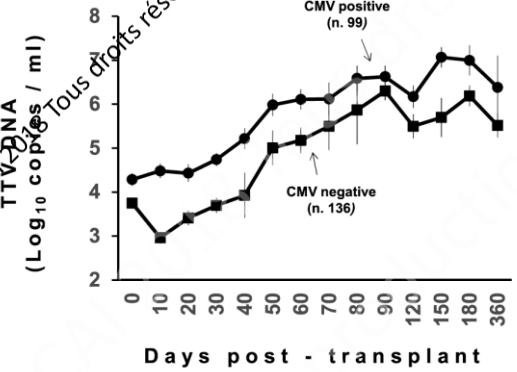


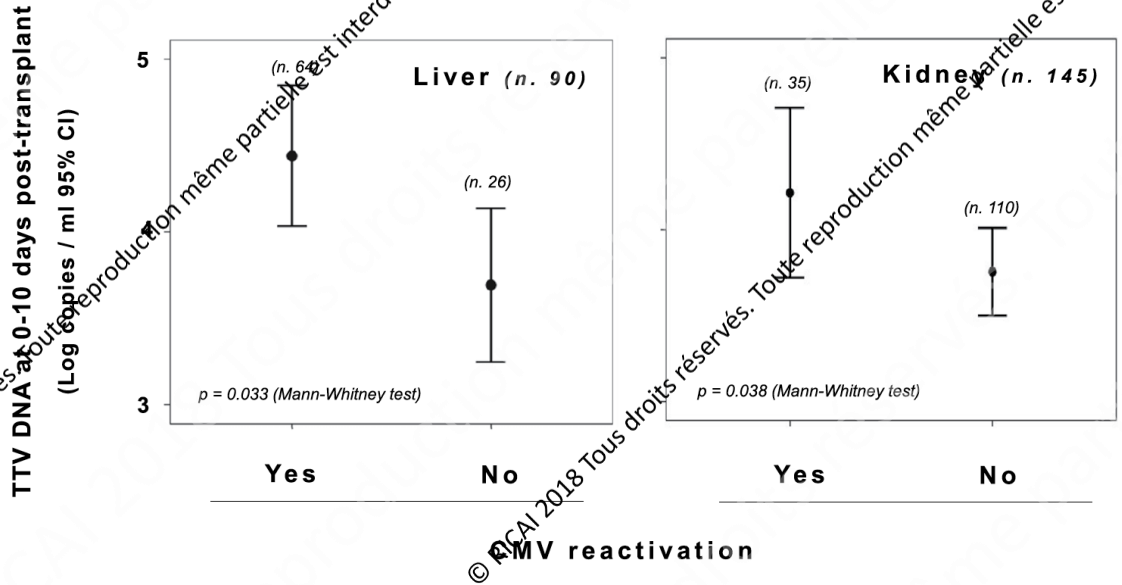
72 kidney recipients , TTV Rgene® on plasma

Solis et al., ESCV 2017

Kidney/Liver CMV

- 280 kidney and liver recipients monitored prospectively for TTV

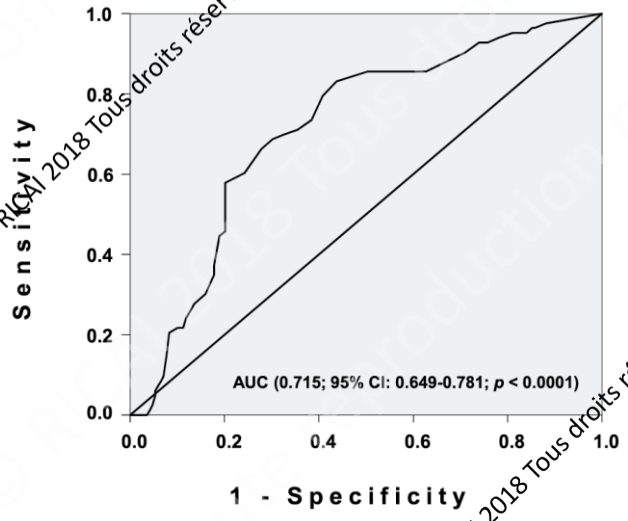




Maggi et al., Sc Reports, 2018

kidney /liver CMV (2)

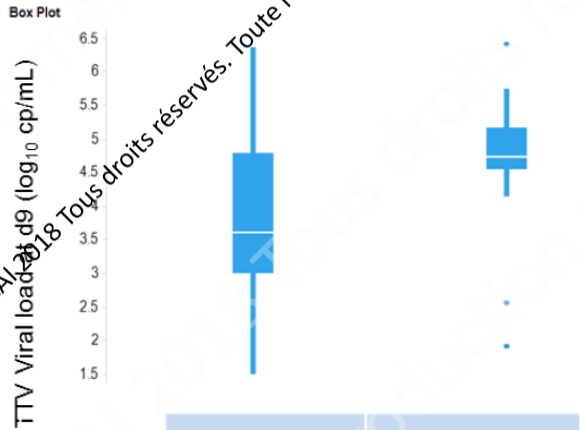
TTV viremia above **3.45** log DNA copies/ml within the first 10 days post-transplant correlates with higher propensity to CMV reactivation within the first four months post transplantation



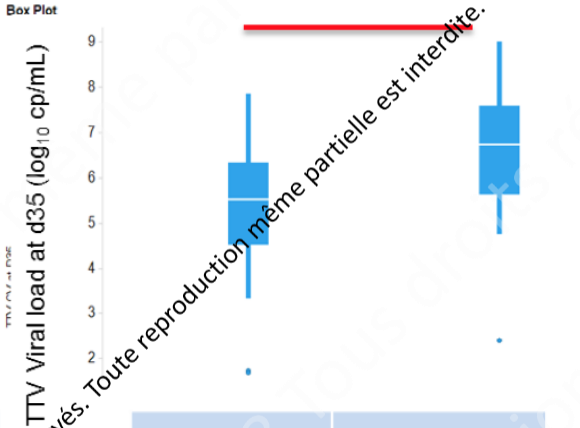
Variable	OR	OR (95% CI)	*p* value
TTV loads at day 0–10 post-tranplant	1.5	1.0–2.3	0.03
Use of anti-CMV prophylaxis	0.4	0.1–1.2	NS
CMV negative serostatus	0.8	0.2–3.1	NS
Mean Tacrolimus levels at month 1 post-transplant	1.0	0.8–1.3	NS

Maggi et al., Sc Reports, 2018

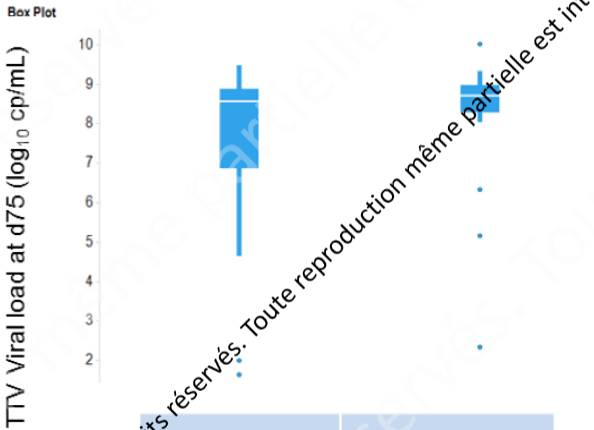
Kidney : All types of viral infection (EBV, BKV or CMV, > 3log copies/mL) by year 1



	No Viral Reactivation	Viral Reactivation
Viral load in mean (log_{10} cp/mL)	3.78	4.63



No Viral Reactivation	Viral Reactivation
5.34	6.57



No Viral Reactivation	Viral Reactivation
7.56	8.08

At d35, TTV VL was significantly different (p=0.0277) between reactivating patient and not reactivating patient

kulifaje et al., unpublished

All infections « related to immunosuppression »

Table 3. Predictive performance of plasma alphatorquevirus DNA loads measured at month 1 (with optimally selected cut-off values) for predicting the occurrence of study outcomes in 1,000 bootstrap samples.

281 Kidney recipients, prospective, Plasma, TTV Rgene® at d7, M1, M3, M6, M12

Cut-off value	Predicted post-transplant event	Sensitivity (95% CI)[a]	Specificity (95% CI)[a]	PPV (95% CI)[a]	NPV (95% CI)[a]
Plasma load >3.15 log_{10} copies/mL	Infection beyond month 1	89.8% (79.6 - 98.0)	30.9% (18.2 - 43.6)	53.8% (42.7 - 64.6)	77.3% (59.1 - 95.5)
Plasma load >4.56 log_{10} copies/mL	iRAE beyond month 1	76.0% (60.0 - 92.0)	65.8% (55.7 - 75.9)	41.3% (28.3 - 54.3)	89.7% (81.0 - 96.6)

CI: confidence interval; iRAE: immunosuppression-related adverse event; NPV: negative predictive value; PPV: positive predictive value.
[a] Mean and 95% bootstrap confidence interval.

AUC at M6 were also significant such as increase between D7 and M1

Ruiz et al., AJT 2018

TTV viral loads correlated with immune response but correlation with immunosuppressive drugs is more controversial

- At M1 and M3 In Ruiz Study
- inverse correlation of TTV viral load with :
- CD3+ cell count, p 0,017 at M3 p<0,0001
- CD4+ p0,015, at M3 p<0,001
- CD8+ p 0,016 (also in Solis 2018)

- No specific association with IS drugs (Maggi, 2018, Solis et al 2018)

Ruiz et al., AJT 2018, Solis et al. RICAI 2018

Relationship between TTV load and immunosuppression

TABLE 4.
TTV load in relation to immunosuppression and kidney function

TTV: median (IQR), copies/mL			
Variables	Yes	No	*P*
Initial immunosuppression			
Induction therapy	3.1×10^5 (2.6×10^4 to 3.7×10^6)	1.4×10^5 (9.8×10^3 to 1.0×10^6)	<0.001
Tacrolimus	2.9×10^5 (2.2×10^4 to 3.1×10^6)	1.4×10^5 (1.3×10^4 to 9.5×10^5)	0.002
Cyclosporine	1.3×10^5 (7.7×10^3 to 6.2×10^5)	3.0×10^5 (2.2×10^4 to 4.4×10^6)	<0.001
mTOR inhibitor	3.9×10^4 (3.6×10^3 to 5.7×10^5)	2.4×10^5 (2.0×10^4 to 1.9×10^6)	0.03
Belatacept	9.5×10^6 (2.1×10^6 to 6.2×10^7)	2.1×10^5 (1.8×10^4 to 1.6×10^6)	<0.001
Mycophenolic acid	2.6×10^5 (1.9×10^4 to 2.5×10^6)	1.6×10^5 (8.2×10^3 to 8.7×10^5)	0.39
Immunosuppression at screening			
Triple immunosuppression	2.8×10^5 (2.2×10^4 to 2.6×10^6)	1.2×10^5 (1.2×10^4 to 7.4×10^5)	0.001
Steroid	2.7×10^5 (2.6×10^4 to 2.4×10^6)	1.3×10^5 (1.4×10^4 to 6.0×10^5)	0.01
Tacrolimus	2.8×10^5 (2.3×10^4 to 2.2×10^6)	1.4×10^5 (1.0×10^4 to 1.2×10^6)	0.01
Tacrolimus through level > median[a]	2.8×10^5 (2.2×10^4 to 1.7×10^6)	2.8×10^5 (2.2×10^4 to 2.2×10^6)	0.20
Cyclosporine	1.0×10^5 (7.0×10^3 to 5.4×10^5)	2.9×10^5 (2.2×10^4 to 2.6×10^6)	<0.001
Cyclosporine through level > median[a]	2.5×10^4 (7.5×10^3 to 8.3×10^5)	1.3×10^5 (6.7×10^3 to 5.2×10^5)	0.46
mTOR inhibitor	6.4×10^4 (6.4×10^3 to 2.8×10^6)	2.4×10^5 (2.1×10^4 to 1.9×10^6)	0.24
Belatacept	1.0×10^7 (3.1×10^6 to 2.6×10^8)	2.1×10^5 (1.9×10^4 to 1.6×10^6)	<0.001
Antimetabolite	2.4×10^5 (2.1×10^4 to 2.2×10^6)	1.0×10^5 (3.9×10^3 to 7.7×10^5)	0.03
Kidney function at screening			
Protein-creatinine ratio > median[a]	2.1×10^5 (1.8×10^4 to 1.7×10^6)	2.5×10^5 (2.3×10^4 to 1.9×10^6)	0.40
eGFR > median[a]	2.7×10^5 (3.7×10^4 to 2.4×10^6)	1.6×10^5 (7.8×10^3 to 1.9×10^6)	0.003

[a] For continuous data the median was selected as a cutoff value to define positivity (tacrolimus level >6 ng/mL, cyclosporine >67 ng/mL, protein-creatinine ratio >154 mg/g, eGFR >42 mL/minute/1.73 m^2).

Ruiz et al., 2018

Lung/ All infections

- 31 lung transplant recipients followed for 2 years retrospectively analyzed
- Prospective study
- PCR in House

- A threshold level of **9.3 log copies of TTV** per ml **at steady state** was predictive for the development of various opportunistic infections in the following timepoint
- not specifically CMV or other..

- the TTV DNA doubling times calculated for 30 or 60 days post-transplantation significantly correlated with the pre-transplant TTV DNA levels (r = 0.61, 0.54, respectively; both P = 0.001).

Gorzer et al., Plos one 2015

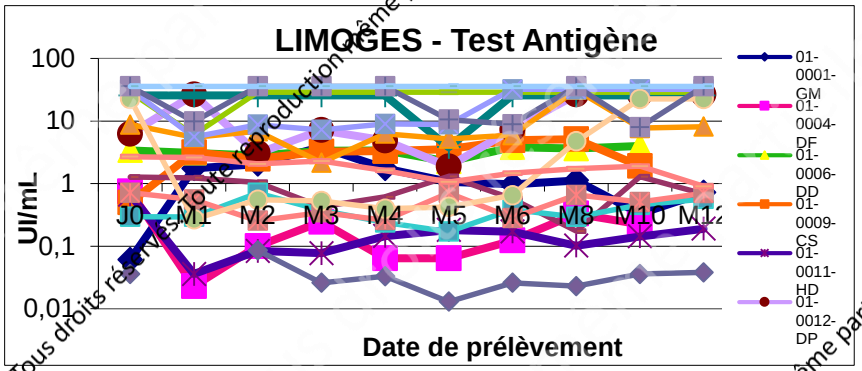
TTV and rejection

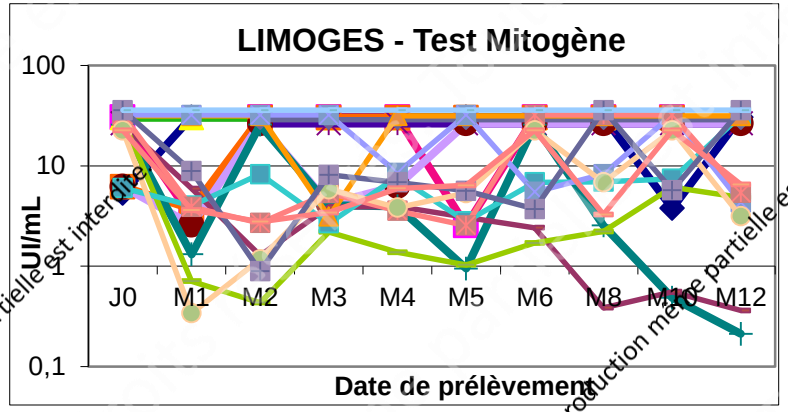
- **Schiemann et al. 2017:** Cross sectional study AMR vs TTV load in 715 kidney recipients
- Risk ratio 0.94 per TTV log level; 95%confidence interval 0.90-0.99; P=0.02)

- **Ruiz et al. 2018**: Prospective follow-up. TTV viral load at baseline is the only marker after multivariate analysis (adjusted HR per 1log10 copies/mL increase 0,69 (0,49-0,97 95% CI),p value 0,030)

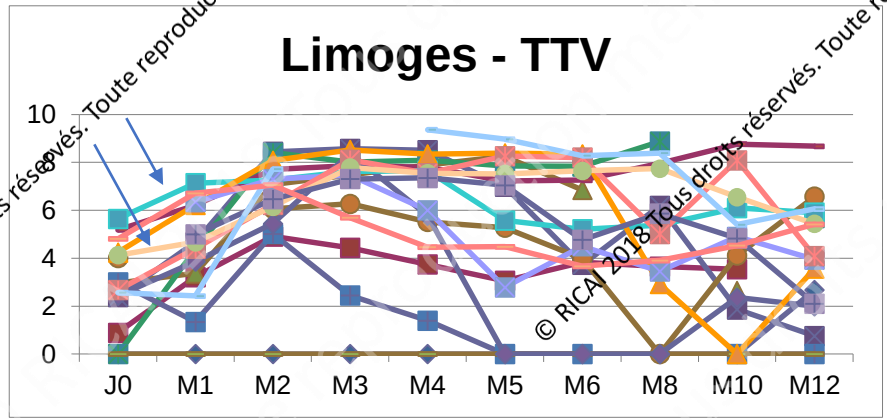
- **Solis et al. 2018:** link between TTV load at baseline threshold 3,4 logs and at M1 threshold 4,2 logscopies/mL plasma

QuantiFERON CMV and TTV viral load :





	Statut CMV	VGCV mois	Infection CMV
D D 06	D+ R+	4	M1 : blip 2,28 Log
C J 27	D+ R+	5	M1 - M2 : blip 4,60 Log M7 infection à CMV
T T 31	D+ R+	3	M9 blips Infection à BKV M5
M A	D+ R+	7	M1 30/12/14 : 2,60 Log M2 26/01/15 : 2,57 Log M 11 2/02/15 : 3,11 Log infection BKV M12



Correlation Antigen/Mitogene/ TTV

CVTTV J0/M1	TTVM1/Ag M1	TTVM1/Mg M1	TTV(M1-J0) -AgM1	TTVM1-Ag M3	TTVM1-Mg M3	TTVM3-Ag M3	TTVM3-Mg M3
0,46	0,08	0,29	0,17	0,099	0,16	0,10	0,04
S	NS	S	NS	NS	NS	NS	NS
p= 0,01		p=0,05	p=0,2		p=0,2		

Spearman test

Conclusion : TTV could be a useful early marker in SOT

- Highly reproducible kinetics in solid organ transplant recipients
- High presomption for a link between TTV viral load in the **early phase** post graft and occurrence of opportunistic viral infections (CMV, BKV…)
- In lung transplant potential link between high viral loads at the plateau with bacterial or fungic infections
- Awaiting interventional studies !

- Standardisation of viral load results is required
  - recent development of the 1st standardized assay
  - 1st QCMD quality control performed in 2018
- Plasma versus whole blood: similar results

- Role of genotypes or TTV lineages in transmission and expansion ?

Acknowledgements

CNR CMV/ UMR Inserm 1092

- Eliza MUNTEANU
- Françoise GARNIER
- Mélissa GOMES
- S Hantz

- D Kulifaje (UMR Inserm/BioMérieux

F Maggi

and D Navarro

CHU Limoges

M Essig

B Durgueil

BioMérieux