6th INTERNATIONAL CONGRESS

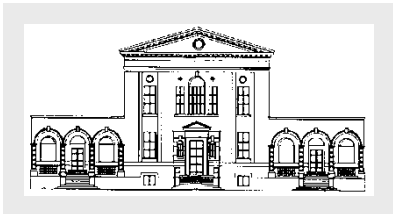
INFECTIONS & TRANSPLANTATION

Varese, 18-20 Maggio 2017

Diagnosis of viral infections: state of the art and perspectives

M.R.Capobianchi

INMI L. Spallanzani, Rome, Italy

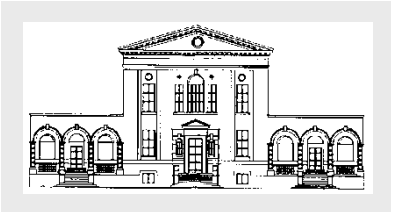


World Health Organization

WHO Collaborating Center for clinical care, diagnosis, response and training on Highly Infectious Diseases

Thanks to:

Isabella Abbate
Chiara Agrati

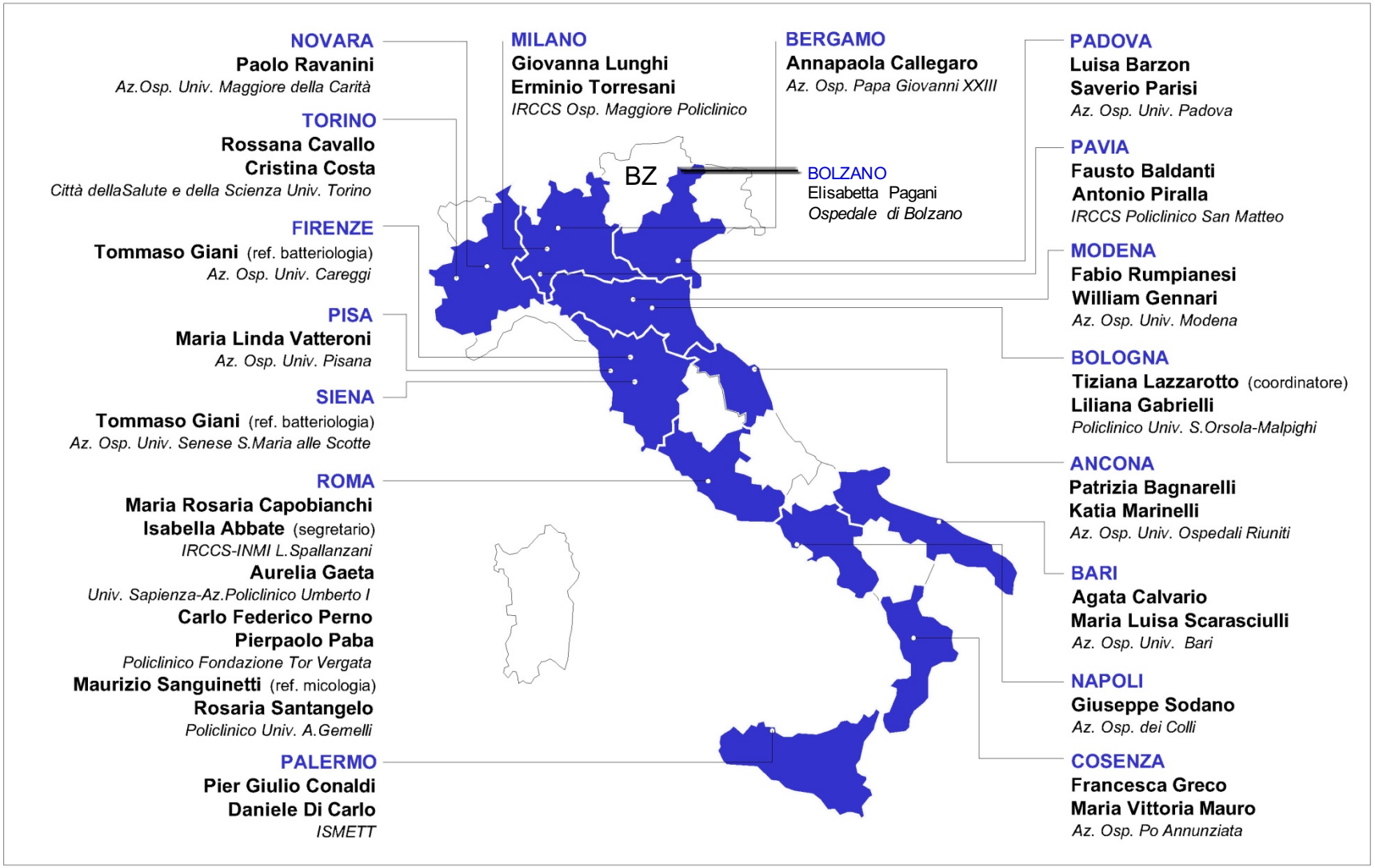


Fausto Baldanti

Tiziana Lazzarotto

GLAIT

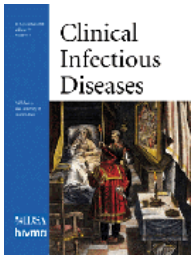
AMCLI Working group on Infections in Transplant GLaIT (since 2008)



21 laboratories of microbiology and virology

Outline

- CMV laboratory monitoring
  - Historical excursus of methods
  - DNAemia
  - Need for standardization
  - Sampling issues
  - Immunological monitoring
  - Diagnostic innovation



The laboratory-transplant connection

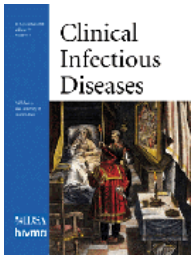
Prophylaxis

- **Rationale:** antiviral therapy for a predefined time to all patients at risk for CMV
- **Laboratory implication:** monitoring not so crucial; driven by symptoms occurrence (clinical surveillance) and called after completion of prophylaxis

Pre-emptive

- **Rationale:** detection of low-level asymptomatic CMV in blood precedes the onset of CMV syndrome and tissue-invasive disease.
- **Laboratory implication:** Regular monitoring of CMV in blood and Tx only when the virus is detected at threshold evels

Legendre C CID 2008



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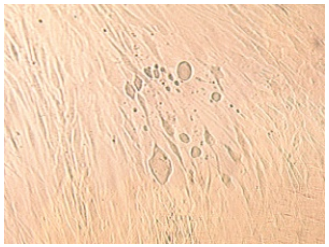
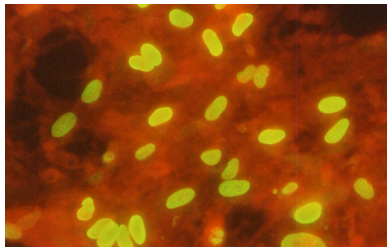
The success of preemptive therapy is dependent upon appropriate diagnostic testing

Legendre C CID 2008

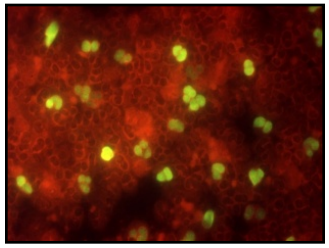
Technological evolution for for CMV monitoring developed since the 80s:

- Virus isolation (standard isolation with virus recovery or rapid isolation by shell-vial)

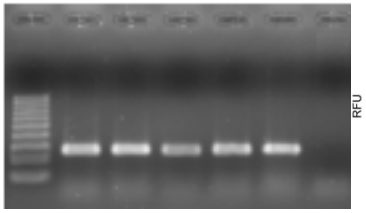


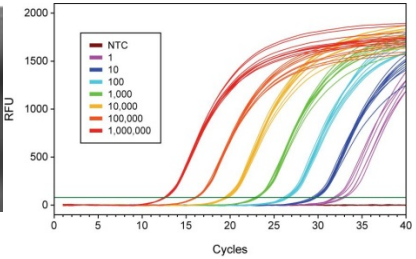


- Detection of pp65 in peripheral blood leukocytes (pp65-antigenemia)



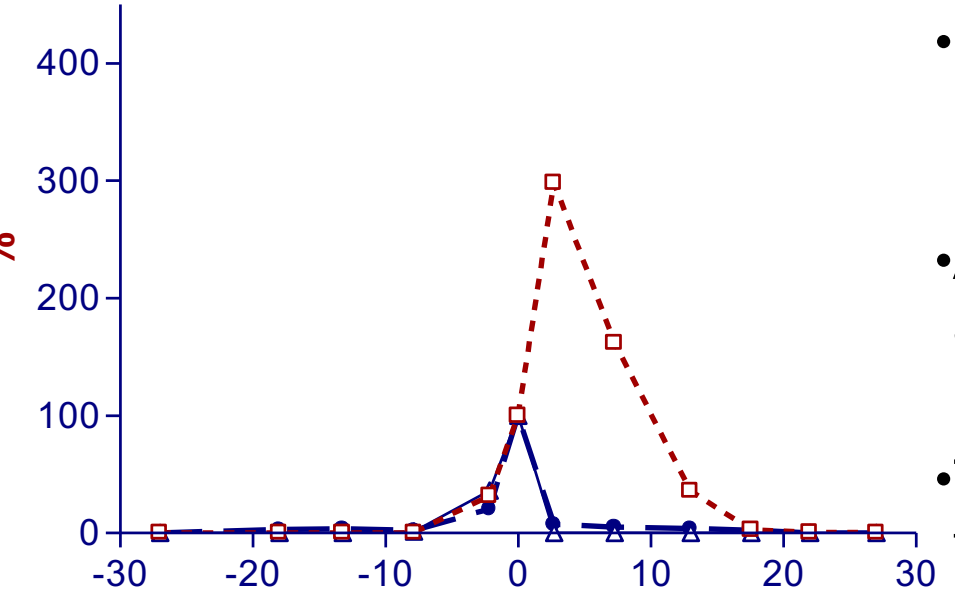
- Detection and quantification of viral DNA in different blood compartments (DNAemia)





- Sequencing (classical and NGS) for detection/quantification of resistance mutations

Rising levels of antigenemia during GCV treatment



- pp65 is passively transferred from infected endothelial cells to leukocytes
- A paradoxical increase in antigenemia levels during treatment in D+/R- is consistently observed.
- This increase is not expression of treatment failure

G. Gerna et al, Transplantation 2003

ARM	Incidence of infection	Incidence of number patients requiring antiviral treatment
99 DNAemia	81/99 81.8%	23/99 23%
101 antigenemia	87/99 86.1%	42/101 41%
	p= ns	p= 0.01

Patients monitored with DNAemia require less Tx, without detriment for successfulmanagement

Gerna et al, Antivir Ther 2007

Table 2. Body fluid specimens that may be used for cytomegalovirus nucleic acid testing

Specimen type	Clinical utility	Advantages	Disadvantages
Serum	Guide preemptive therapy	Indicates active viral replication	Requiring pretest processing (simple)
	Diagnosis of disease	Highly specific	
	Treatment monitoring		
Plasma	Guide preemptive therapy	Indicates active viral replication	Requiring pretest processing (simple)
	Diagnosis of disease	Highly specific	
	Treatment monitoring		
Whole blood	Guide preemptive therapy	Does not require pretest processing	May detect latent virus (low copy numbers)
	Diagnosis of disease	Detects lower level of viral replication, may allow for earlier antiviral therapy (higher sensitivity than plasma/serum)	Low specificity (at low-level viremia)
	Treatment monitoring		
Leukocyte preparation	Guide preemptive therapy	Higher sensitivity than plasma/serum	May detect latent virus
	Diagnosis of disease		Requires pretesting processing
	Treatment monitoring		
Bronchoalveolar fluid	Diagnosis of CMV pneumonia	Higher viral load may correlate with active disease	May indicate viral shedding
			Sample obtained via invasive procedure
Cerebrospinal fluid	Diagnosis of central nervous system CMV disease	High sensitivity and specificity in samples with low leukocyte count	May detect latent virus in samples contaminated with high numbers of leukocytes
	Surrogate marker of treatment response	Higher viral loads may indicate more severe disease	Sample obtained via invasive procedure
Aqueous or vitreal humour	Diagnosis of CMV retinitis (although characteristic funduscopic findings are sufficient for diagnosis)	High sensitivity and specificity	Sample obtained via invasive procedure
Urine	Diagnosis of primary CMV nephritis (not routinely recommended)	Diagnostic in CMV seronegative kidney recipients	Not useful in seropositive patients (up to 50% viral shedding in posttransplant period without clinical significance)

CURRENT OPINION

Clinical utility of cytomegalovirus viral load in solid organ transplant recipients

Curr Opin Infect Dis 2015

Maria V. Dioverti and Raymund R. Razonable

Table 1. Summary of the clinical utility of quantitative cytomegalovirus nucleic acid tests

Clinical utility	
Rapid diagnosis	
	Rapid turn-around time
	High sensitivity and specificity
	Allows early initiation of antiviral therapy
Preemptive therapy	
	Routine monitoring to guide the initiation of antiviral drugs for preemptive treatment of subclinical CMV infection
Prognosis	
	Higher viral loads correlates with symptomatic and more severe disease
Treatment response	
	Viral load decline correlates with clinical response to antiviral therapy
Disease recurrence	
	Failure to achieve an undetectable viral load, and suboptimal or slow decline in viral load correlates with a higher risk for disease relapse
Resistance	
	Lack of viral clearance or an increase in viral load during antiviral therapy should raise concerns for drug-resistant CMV disease

Clinical utility of cytomegalovirus viral load in solid organ transplant recipients

Curr Opin Infect Dis 2015

Maria V. Dioverti and Raymund R. Razonable

Because several platforms were available for CMV QNAT, there was a wide **inter-assay variability** in the viral load reporting, and this limited the generation of widely applicable viral load thresholds that can be used for various clinical applications.

Journal of Clinical Virology 82 (2016) 76–83

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Nation-wide measure of variability in HCMV, EBV and BKV DNA quantification among centers involved in monitoring transplanted patients

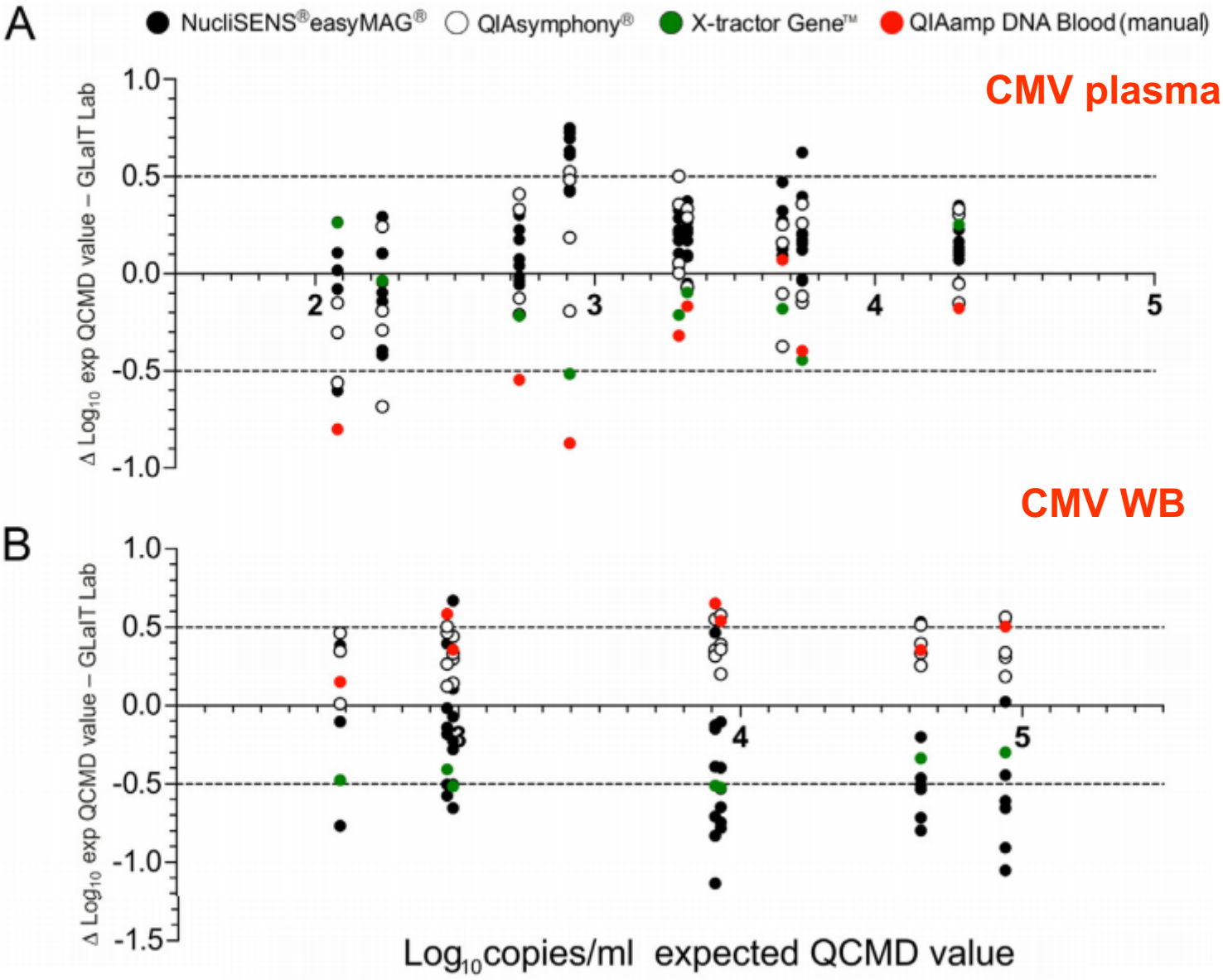
Isabella Abbate[a,1], Antonio Piralla[b,1], Agata Calvario[c], Annapaola Callegaro[d], Cristina Giraldi[e], Giovanna Lunghi[f], William Gennari[g], Giuseppe Sodano[h], Paolo Ravanini[i], Pier Giulio Conaldi[j], Marialinda Vatteroni[k], Aurelia Gaeta[l], Pierpaolo Paba[m], Rossana Cavallo[n], Fausto Baldanti[b,o,*], Tiziana Lazzarotto[p], and the AMCLI – Infections in Transplant Working Group GLaIT[2]

"QCMD 2007 Human CMV DNA EQA Programme"

Methods for viral DNA extraction and quantification

Center #	Nucleic acid extraction		Input volume (µl)	Output volume (µl)	Amplification method	Real-time PCR instrument
	Instrument	Protocol				
1	QIA Symphony	DSP virus/pathogen (modified)	400 (200 for WB)	90 (90 for WB)	CMV Trender Affigene	Stratagene xp3000
2	NucliSENS EasyMag	generic 2.0.1	250 (100 for WB)	25 (25 for WB)	CMV Alert Real-Time, ELITechGroup	ABI Prism 7300
3	QIA Symphony	DSP virus/pathogen (modified)	1000	110	CMV ELITe MGB Kit, ELITechGroup	ABI Prism 7300
4	NucliSENS EasyMag	generic 2.0.1 and specific 2.0 for WB	500 (200 for WB)	55 (55 for WB)	in house PCR (target US8)[19]	ABI Prism 7300
5	NucliSENS EasyMag	generic 2.0.1	100	50	CMV ELITe MGB Kit, ELITechGroup	ABI Prism 7300
6	x-tractor gene UV light	Helix DNA Corbet	400 200 for WB	60 150 for WB	CMV Alert Real-Time, ELITechGroup CMV ELITe MGB Kit, ELITechGroup	ABI Prism 7300
7	NucliSENS EasyMag QIA Symphony	generic 2.0.1 blood 200V6 for WB	500 200 for WB	50 200 for WB	CMV ELITe MGB Kit, ELITechGroup	ABI Prism 7500
8	NucliSENS EasyMag	generic 2.0.1	400 (200 for WB)	60 (85 for WB)	CMV Alert Real-Time, ELITechGroup	ABI Prism 7300
9	NucliSENS EasyMag	generic 2.0.1	1000 (200 for WB)	25 (55 for WB	CMV Alert Real-Time, ELITechGroup	ABI Prism 7300
10	QIA Symphony	DSP virus/pathogen and DSP DNA for WB	500 (200 for WB)	90 (90 for WB)	CMV ELITe MGB Kit, ELITechGroup	ABI Prism 7300
11	QIA Symphony	DSP virus/pathogen and DSP DNA for WB	500 (200 for WB)	140 (90 for WB)	CMV ELITe MGB Kit, ELITechGroup	ABI Prism 7300
12	NucliSENS EasyMag	specific 2.0 (modified for WB)	500 (100 for WB)	100 (50 for WB)	CMV ELITe MGB Kit, ELITechGroup	ABI Prism 7300
13	NucliSENS EasyMag	generic 2.0.1	500 (100 for WB)	55 (55 for WB)	CMV Alert Real-Time, ELITechGroup	ABI Prism 7300
14	NucliSENS EasyMag	generic 2.0.1	500 (200 for WB)	55 (55 for WB)	CMV Alert Real-Time, ELITechGroup	ABI Prism 7300
15	Manual extraction	QIAamp blood mini kit	200	100	CMV r-gene Argene-Biomerieux	ABI Prism 7500

Bland-Altman plots describe the Log difference between the GLaIT laboratory results and QCMD values.



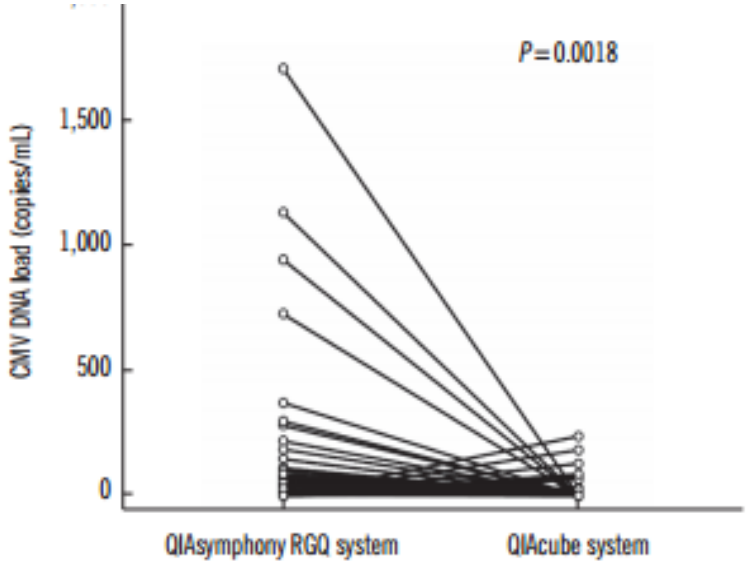
Original Article
Diagnostic Genetics
Ann Lab Med 2017;37:129-136
https://doi.org/10.3343/alm.2017.37.2.129
ISSN 2234-3806 • eISSN 2234-3814
ANNALS OF LABORATORY MEDICINE

Automated Nucleic Acid Extraction Systems for Detecting Cytomegalovirus and Epstein-Barr Virus Using Real-Time PCR: A Comparison Study Between the QIAsymphony RGQ and QIAcube Systems

Hanah Kim, M.D.[1], Mina Hur, M.D.[1], Ji Young Kim, M.T.[1], Hee-Won Moon, M.D.[1], Yeo-Min Yun, M.D.[1], and Hyun Chan Cho, M.D.[2]

Department of Laboratory Medicine[1], Konkuk University School of Medicine, Seoul; Department of Laboratory Medicine[2], Hallym University College of Medicine, Seoul, Korea

Automated nucleic acid extraction systems have different performances and significantly affect the detection of viral pathogens.



Source of variability is more than extraction

Target	Source or description	Variation (%)	*P value*
CMV	Overall total for target	67.4	
	Commercial detection reagent	47.1	<0.0001
	Amplification target gene	9.6	<0.0001
	Interaction of commercial detection reagent and amplification target gene	7.2	<0.0001
	Method for nucleic extraction	3.4	0.0077

165 Labs enrolled in the College of American Pathologists (CAP) 2009 viral load proficiency testing

Hayden RT, J Clin Microbiol 2011

In October 2010, a World Health Organization (WHO) International Reference Standard became available from the National Institute of Biological Standards and Controls (United Kingdom).

The standard was made from a clinical isolate (Merlin) and has a titer of $5x10^6$ IU/ml

All commercial and laboratory developed tests should be recalibrated and show colinearity to the WHO International Standard and results should be reported as IU/mL.

Biologicals 44 (2016) 242–251

Contents lists available at ScienceDirect

Biologicals

journal homepage: www.elsevier.com/locate/biologicals

A collaborative study to establish the 1st WHO International Standard for human cytomegalovirus for nucleic acid amplification technology

Jacqueline F. Fryer [a, *], Alan B. Heath [b], Philip D. Minor [a], the Collaborative Study Group [1]

CURRENT OPINION

Clinical utility of cytomegalovirus viral load in solid organ transplant recipients

Curr Opin Infect Dis 2015

Maria V. Dioverti and Raymund R. Razonable

Because several platforms were available for CMV QNAT, there was a wide **inter-assay variability** in the viral load reporting, and this limited the generation of widely applicable viral load thresholds that can beused for various clinical applications.

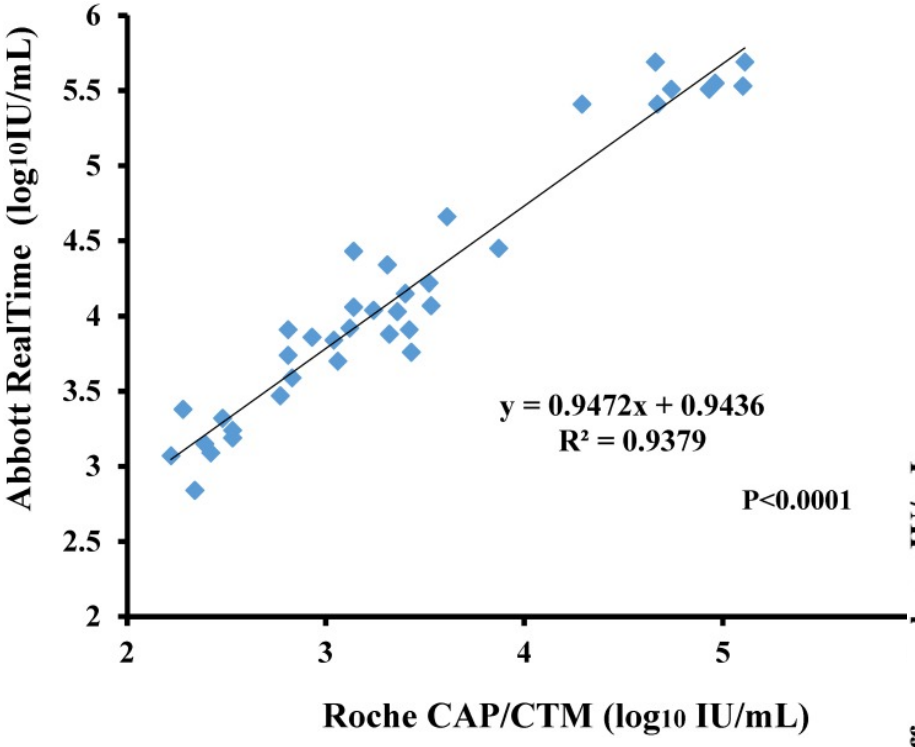
With the recent availability of international standard and certified reference materials, **there is now opportunity to standardize viral load reporting**, with the goal of deriving viral load thresholds for various clinical applications, such as

- rapid diagnosis of CMV infection and disease,
- predicting the risk of disease and assessing the severity of illness
- monitoring efficacy of antiviral therapies
- assessing the risk of viral relapse and drug resistance.

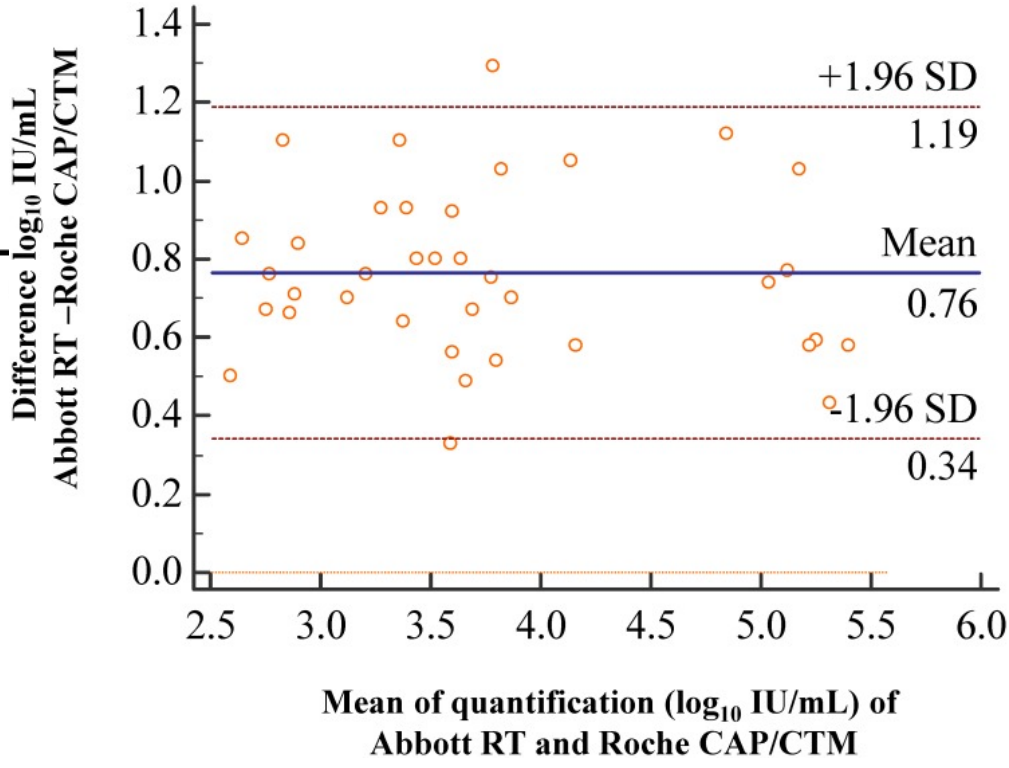
PLOS ONE 2016

Comparison of Two Commercial Automated Nucleic Acid Extraction and Integrated Quantitation Real-Time PCR Platforms for the Detection of Cytomegalovirus in Plasma

Huey-Pin Tsai[1,3], You-Yuan Tsai[1], I-Ting Lin[1], Pin-Hwa Kuo[1], Tsai-Yun Chen[2], Kung-Chao Chang[1], Jen-Ren Wang[1,3,4,5]*



CMV VL measured with Abbott real time on average 0.76 log10 IU/mL higher than Roche CAP/CTM assay (P<0.0001)



Evaluation of COBAS AmpliPrep/COBAS TaqMan CMV Test for use in hematopoietic stem cell transplant recipients

Poornima Ramanan & Raymund R. Razonable

Pages 1-7 | Received 06 Feb 2017, Accepted 28 Apr 2017, Accepted author version posted online: 04 May 2017, Published online: 15 May 2017

While the WHO IS has improved the inter-laboratory result variances, there are still important factors that continue to contribute to assay variability. This lack of harmony among NAT highlights the need for further standardization.

The Clinical Utility of Whole Blood Versus Plasma Cytomegalovirus Viral Load Assays for Monitoring Therapeutic Response

Transplantation 2011

Luiz F. Lisboa,[1] Anders Åsberg,[2] Deepali Kumar,[1] Xiaoli Pang,[3] Anders Hartmann,[4] Jutta K. Preiksaitis,[1] Mark D. Pescovitz,[5] Halvor Rollag,[6] Alan G. Jardine,[7] and Atul Humar[1,8]

The results of a plasma-based PCR assay were compared with a real-time PCR assay of whole blood and assessed for their ability to predict recurrence.

Good correlation but significant difference in absolute value and clearance kinetics.

Positive predictive value of persistent viremia at day 21 for virologic recurrence was 41.9% plasma and 36.3% WB

Enhanced detection of residual viremia using WB does not seem to offer significant clinical advantages nor allows for better prediction of recurrence of CMV viremia

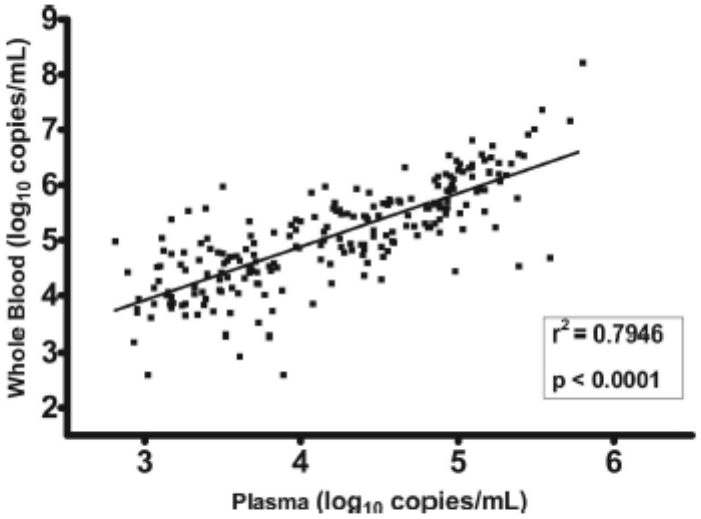


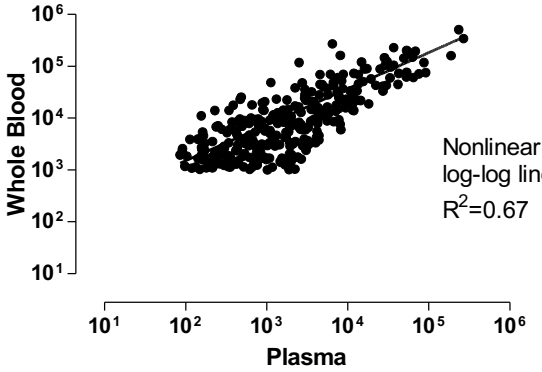
FIGURE 1. Day 0 viral loads in whole blood versus plasma. All viral loads are in log_{10} copies/mL.

Characteristics	Whole blood	Plasma	p
Day 0 VL	118,950 (400-160,000,000)	17,950 (645-635,000)	P<0.001
Log-change in VL (d0-3)	-1.19 (-4.08 to +0.86)	-0.28 (-1.29 to +0.56)	P<0.001
Log-change in VL (d0-7)	-1.30 (-4.28 to +0.19)	-0.37 (-1.88 to +0.49)	P<0.001
Undetectable by end of treatment (d21)	65/219 (29.7%)	114/219 (52.1%)	P<0.001

Kinetics of CMV-DNA load in WB and plasma samples of allogeneic HSCT recipients (1138 samples)

620 samples with quantified CMV-DNA

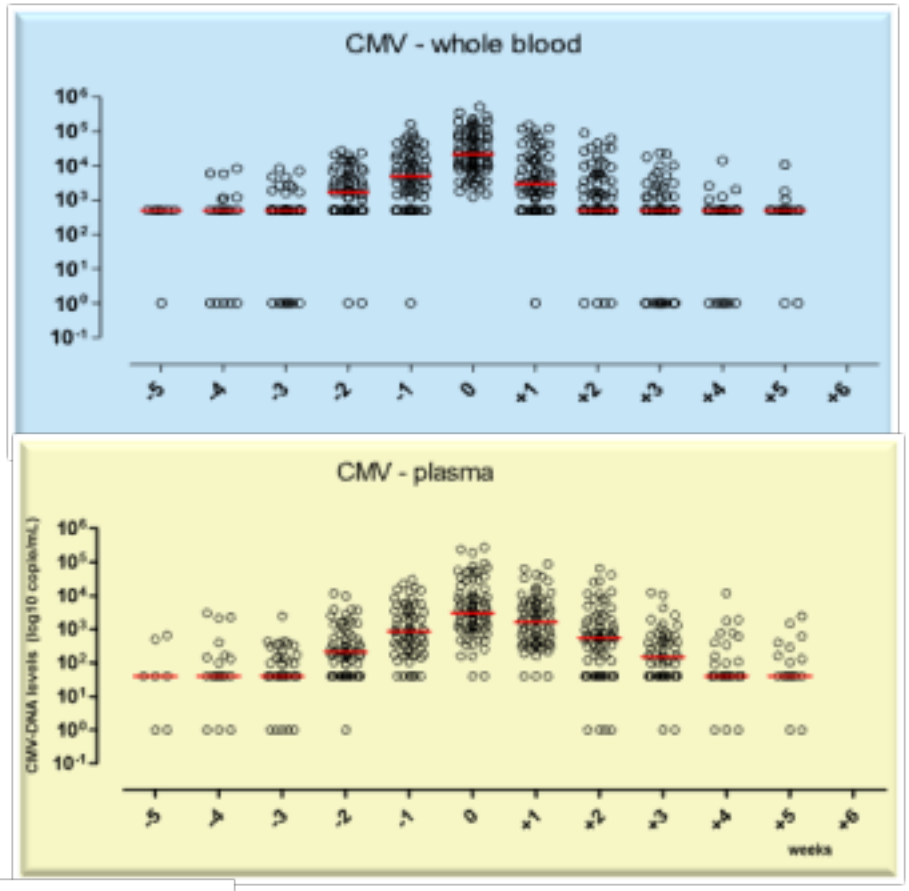
Good correlation plasma vs WB

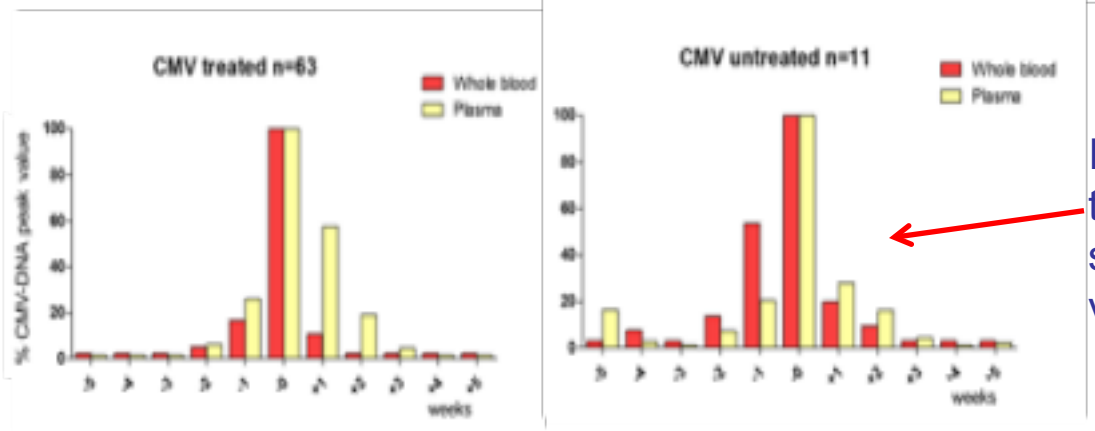


Spearman correlation coefficient r=0.73

Increase and decrease of CMV-DNA level were very similar

Before peak CMV DNA levels in plasma were lower of about 1 Log than WB



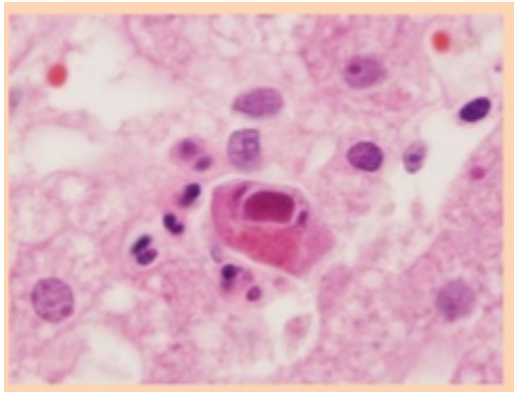


In patients receiving pre-emptive therapy, CMV DNA levels in plasma showed a slower decline after peak value

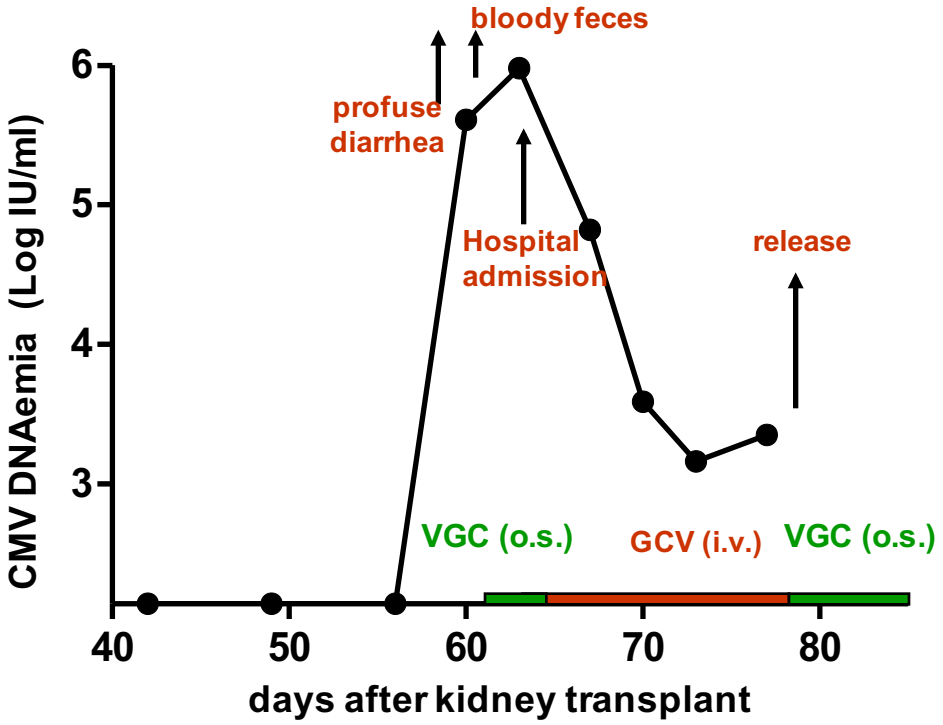
GLaIT

Tissue-invasive CMV disease

The gold standard for definite diagnosis of tissue-invasive CMV disease requires a tissue biopsy and histological demonstration of viral cytopathic effects, or the detection of virus genome/antigens by in-situ hybridization and immunohistochemistry staining.



Obtaining tissue biopsy is not always possible due to the invasive nature of the procedure, and the probable diagnosis of tissue-invasive CMV disease sometimes relies on the exclusion of other potential causes, together with the detection of viremia in a patient with compatible symptoms



For example, a patient with diarrhoea and CMV viremia will have a probable diagnosis of tissue-invasive gastrointestinal CMV disease, as long as there is no other potential cause of the diarrhoea.

BeaM E. Curr Infect Dis Rep 2014

(*Transplantation* 2013;96: 00–00)

Updated International Consensus Guidelines on the Management of Cytomegalovirus in Solid-Organ Transplantation

Camille N. Kotton,[1,8] Deepali Kumar,[2] Angela M. Caliendo,[3] Anders Åsberg,[4] Sunwen Chou,[5] Lara Danziger-Isakov,[6] and Atul Humar,[7] on behalf of The Transplantation Society International CMV Consensus Group

- QNAT is preferred for diagnosis, decisions regarding preemptive therapy, and monitoring response to therapy due to the ability to harmonize and standardize these tests (strong, moderate).
- Either plasma or whole blood is an acceptable specimen for QNAT, with an appreciation of the differences in viral load values and viral kinetics. Specimen type should not be changed when monitoring patients (strong, moderate)
- Commercial and laboratory-developed tests must be calibrated and show colinearity to the WHO international standard; results should be reported as IU/mL (strong, moderate)
- Until harmonization of viral load tests is achieved, it is not possible to establish universal quantitative levels for trigger points of therapy or treatment endpoints
- Histology/immunohistochemistry is the preferred method for diagnosis of tissue-invasive disease
- Culture and QNAT of tissue specimens have a limited role in the diagnosis of invasive disease but may be helpful in gastrointestinal disease, where blood QNAT may not be positive. Positive culture of BAL samples may not always correlate with disease (strong, moderate).

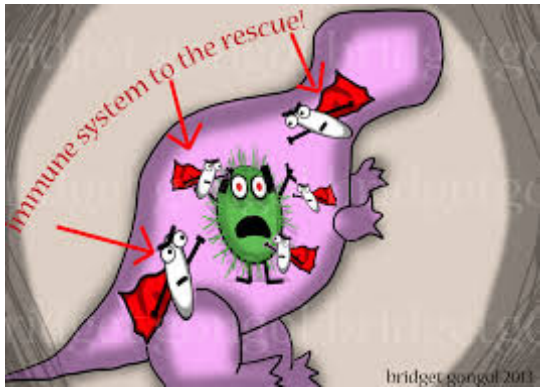
Functional impairment of cell-mediated immunity (CMI) in the course of immunosuppression, such as in solid-organ transplant recipients, is a major cause of uncontrolled CMV replication and related clinical complications

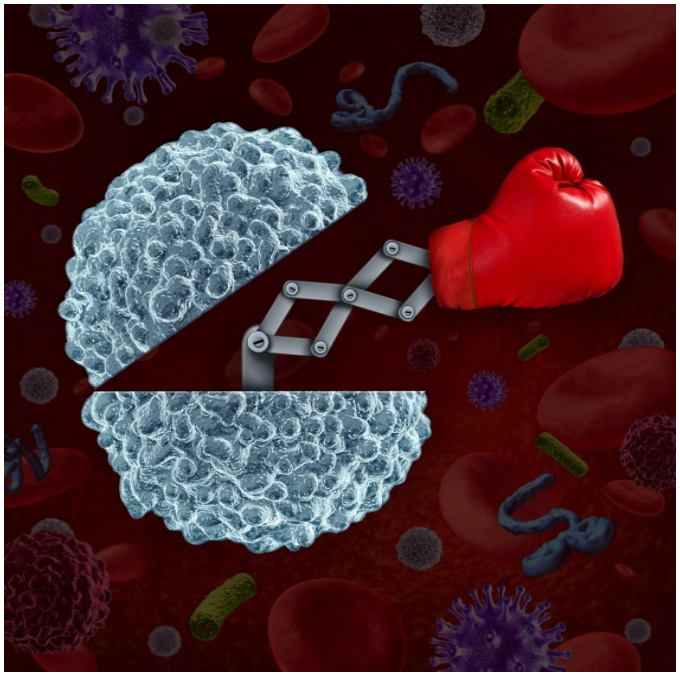
Sund F et al. Clin Transplant. 2010

Fernández-Ruiz M, et al. Clin Transl Immunol. 2014 .

Kotton CN. Nat Rev Nephrol. 2010

Lisboa LF et al. Transplantation. 2012





Monitoring competence of T-cell immunity

- Total lymphocyte counts
- $CD4^+$ and $CD8^+$ T cell counts
- CD4 response to PHA (Cylex)

Non specific
- phenotypic and
- functional parameters

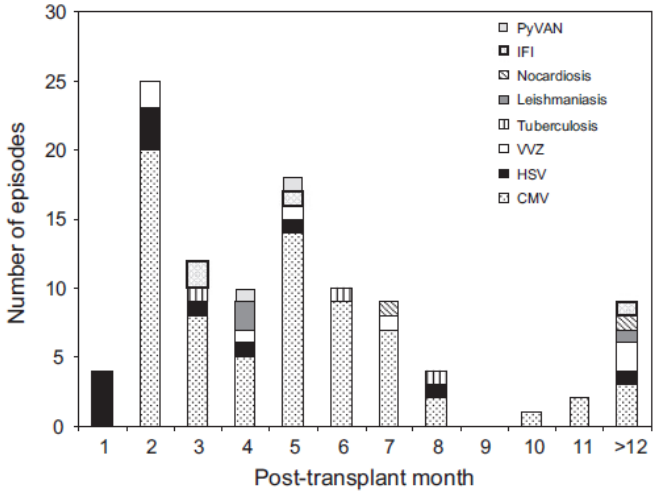
- Tetramer Ag-staining
- Intracellular IFN-γ (flow cytometry, Ag-specific cellular subsets)
- Quantiferon (antigen-specific $CD8^+$ T-cell response)
- Elispot (antigen-specific $CD4^+$ and $CD8^+$ T-cell response)
- Cultured-ELISPOT (central memory antigen-specific $CD4^+$ and $CD8^+$ T-cell response)

CMV-specific
- phenotypic and
- functional parameters

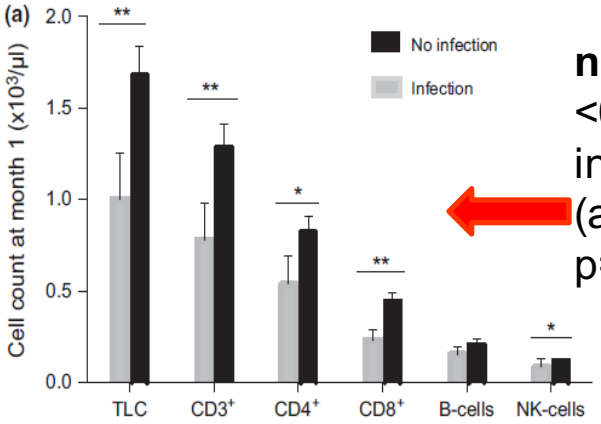
Transplant International 2014

Kinetics of peripheral blood lymphocyte subpopulations predicts the occurrence of opportunistic infection after kidney transplantation

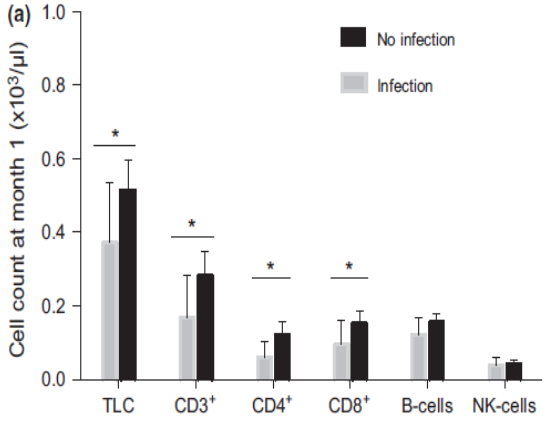
Mario Fernández-Ruiz,[1] Francisco López-Medrano,[1] Luis M. Allende,[2] Amado Andrés,[3] Ana García-Reyne,[1] Carlos Lumbreras,[1] Rafael San-Juan,[1] José M. Morales,[3] Estela Paz-Artal[2] and José M. Aguado[1]

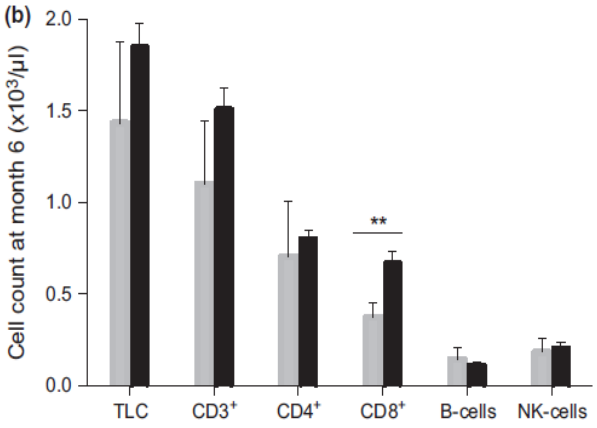


Patients not receiving (164) or receiving (140) antithymocyte globulin (ATG) as induction therapy

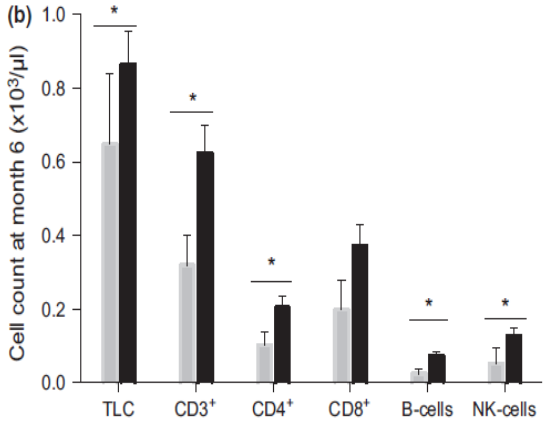


non-ATG: CD8+ T-cell count <0.100 x10^3 cells/ul independent risk factor for OI (adjusted hazard ratio: 3.55; p=0.002)





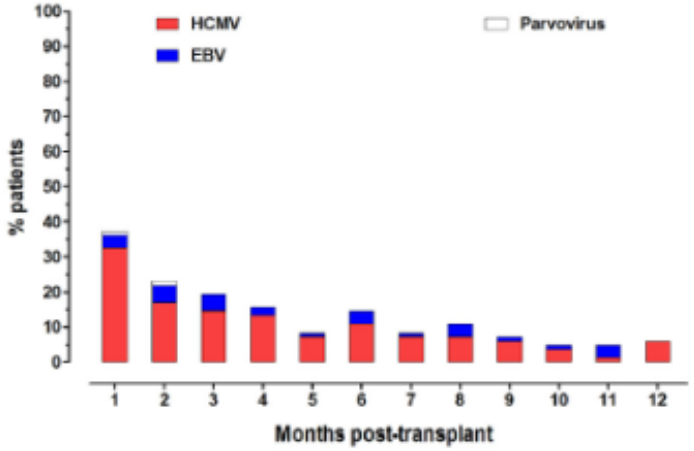
ATG: CD4+ T-cell count at month 1 <0.050 x10^3 cells/ul NPV 0.92 for subsequent OI (overall and CMV)



T-lymphocyte subsets in lung transplant recipients: association between nadir CD4 T-cell count and viral infections after transplantation

Sandra A. Calarota[a], Antonella Chiesa[a], Annalisa De Silvestri[b], Monica Morosini[c], Tiberio Oggionni[c], Piero Marone[a], Federica Meloni[c,d], Fausto Baldanti[a,e,*]

Journal of Clinical Virology 69 (2015) 110–116



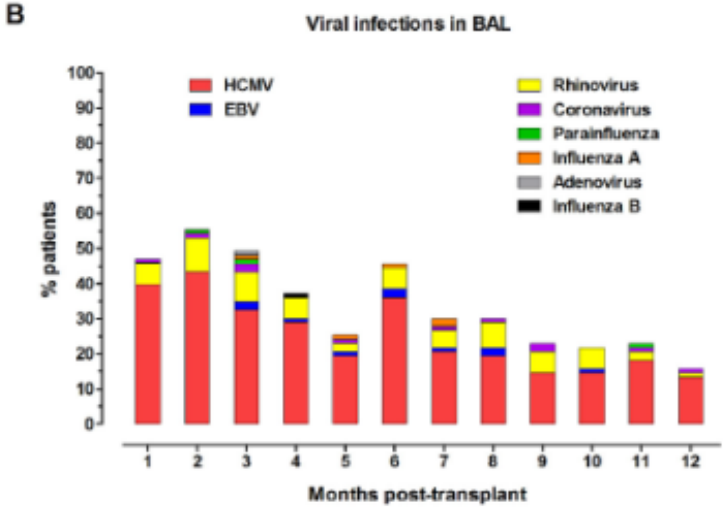
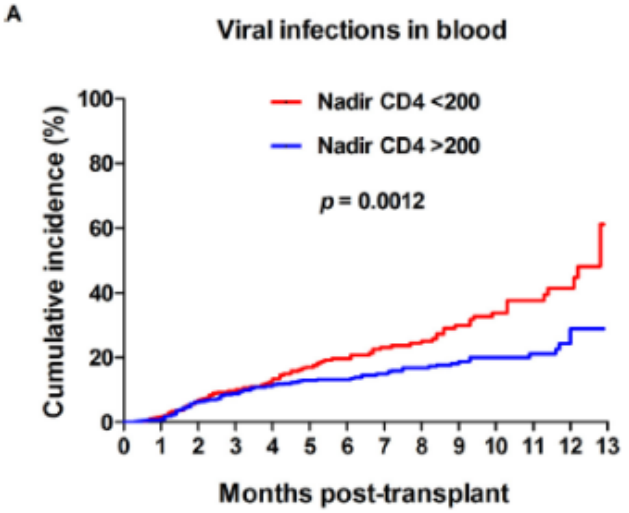


Fig. 2. Occurrence of viral infections in 83 lung transplant recipients within the first 12 months post-transplant. Percentage of patients developing viral infections



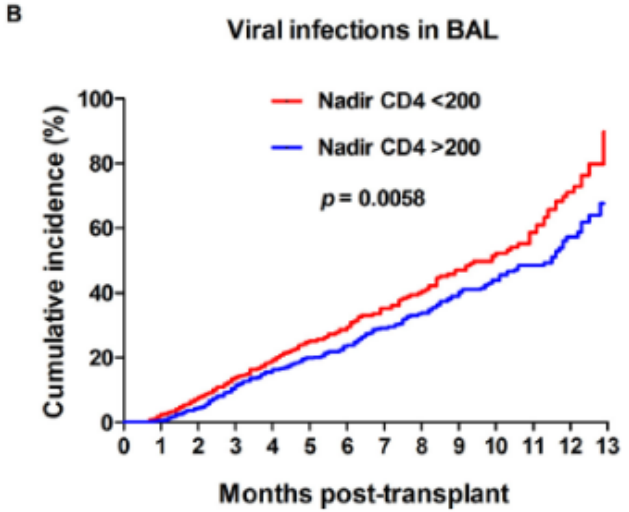


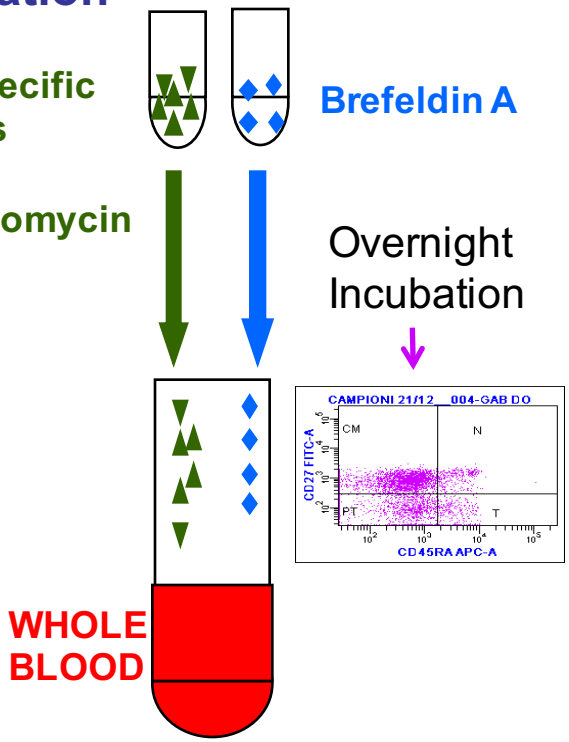
Fig. 3. Cumulative incidence of viral infections in 83 lung transplant recipients in the first 12–13 months post-transplant stratified by nadir CD4 T-cell count (<200

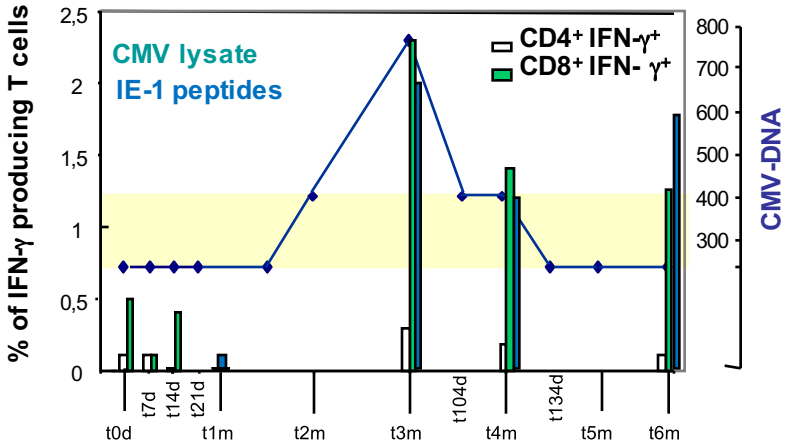
Detection of antigen specific IFN-gamma producing CD4 and CD8 T cells by IFNγ intracellular staining and flow cytometry

Controls
PMA/Ionomycin
Medium

Viral antigens
•CMV AD169 (whole cell lysate, IE-1 peptide pool)
•BKV AS (LT+VP1 peptide pool)

Stimulation





Frequency (median) of CMV-specific IFNγ+ T cells

		Therapy needed	Spontaneous control	P
CMV lysate	CD4	0.15	0.10	
	CD8	0.35	0.20	
IE1-1 pool	CD4	0.00	0.01	0.038
	CD8	0.09	0.32	0.023

Abbate I et al., ESCV meeting 2009, Istanbul

American Journal of Transplantation 2011; 11: 2463–2471
Wiley Periodicals Inc.

Virologic and Immunologic Monitoring of Cytomegalovirus to Guide Preemptive Therapy in Solid-Organ Transplantation

G. Gerna[a,*], D. Lilleri[a], A. Chiesa[b], P. Zelini[b], M. Furione[b], G. Comolli[b,c], C. Pellegrini[d], E. Sarchi[e], C. Migotto[f], M. Regazzi Bonora[g], F. Meloni[h] and E. Arbustini[a]

Immunologic monitoring CMV-specific CD4+ and CD8+ T cells by cytokine flow cytometry, using CMV-infected dendritic cells as stimulus

'protective' immunity: ≥0.4 CD4+ and CD8+ CMV-specific T cells/uL blood

patients reconstituting HCMV-specific CD4+ and CD8+ T-cell immunity at 60 days posttransplant onward were able to control HCMV infection

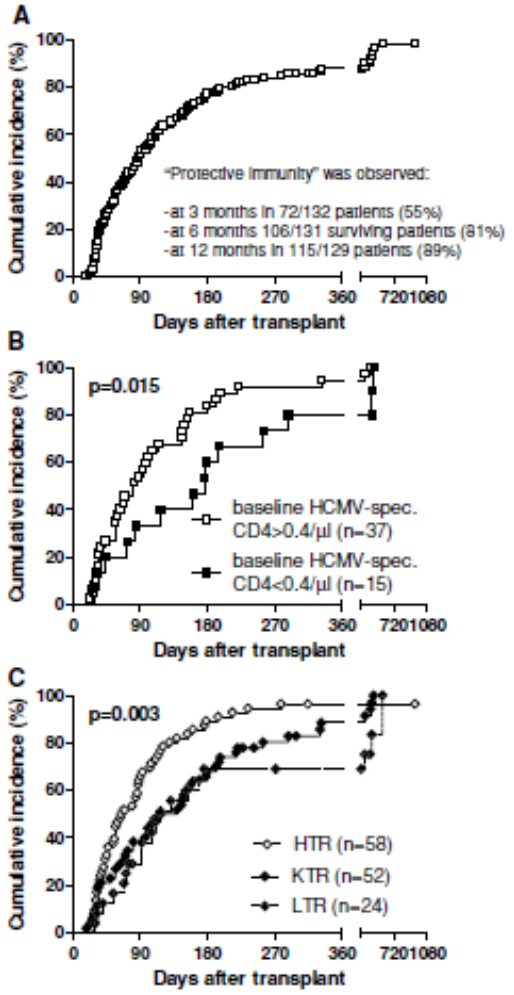


Figure 2: Reconstitution of both HCMV-specific CD4+ and CD8+ T-cell immunity (>0.4 cells/µL blood for both subpopulations [11]). (A) The number of patients reconstituting protective levels of T-cell immunity rose from 72/132 (55%) after 3 months to 115/129 (89%) after 12 months follow-up. (B) Reconstitution according to baseline specific CD4+ levels. (C) Reconstitution according to heart (HTR), kidney (KTR) or lung (LTR) transplantation.

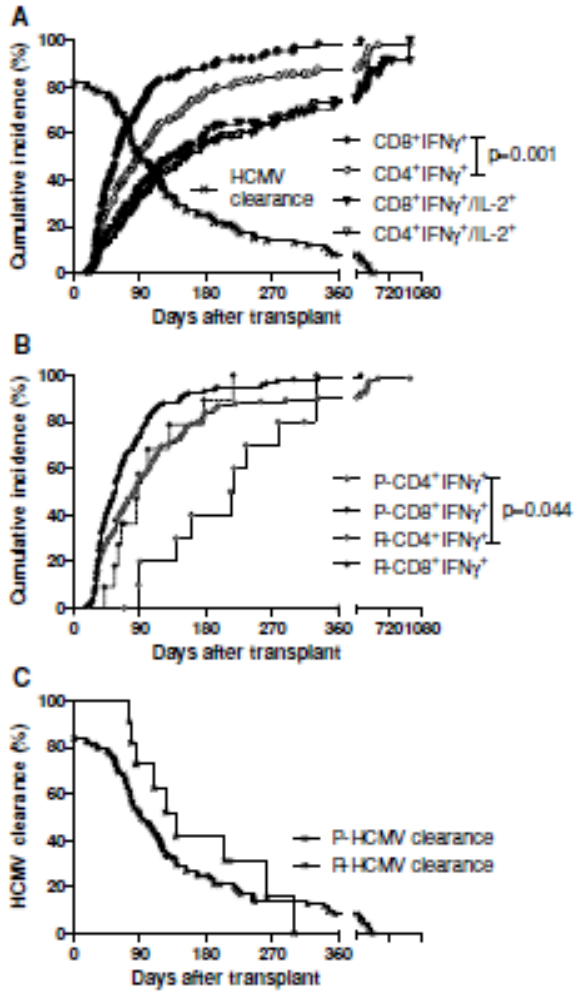


Figure 3: Cumulative incidence of patients with HCMV-specific CD4+ and CD8+ T cells and HCMV clearance from blood. (A) CD8+ T cells preceded CD4+ T cells (median time 57 vs. 87 days, p = 0.001, log-rank test). IFN-γ+/IL-2+CD8+ and CD4+ T-cell appearance was delayed (median time 132 and 155 days, respectively). Virus clearance from blood significantly correlated (p < 0.005) with time to detection of HCMV-specific IFN-γ+ CD4+ T cells and detection of HCMV-specific IFN-γ+/CD8+ T-cells. (B) Cumulative incidence of HCMV-specific CD4+ and CD8+ T-cell appearance in patients with primary (P) or reactivated (R) HCMV infection. (C) HCMV clearance in transplanted patients with primary (P) or reactivated (R) HCMV infection.

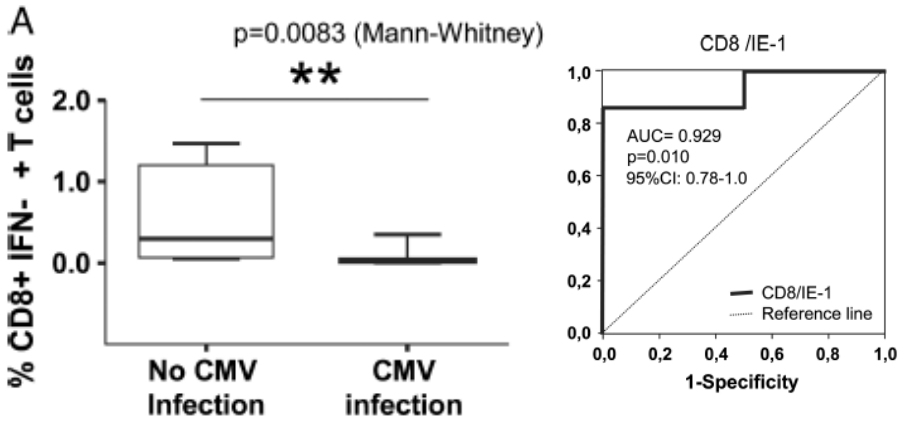
- 15 CMV IgG+ Kidney recipients (FU:1-year)
- Pre-transplant:
  - ✓ ~50% negative cellular response to IE-1
  - ✓ absence of IE-1-responsive T CD8 in pts who developed CMV infection
  - ✓ median CD8 T-cell responses to IE-1 higher in pts who did not develop CMV infection

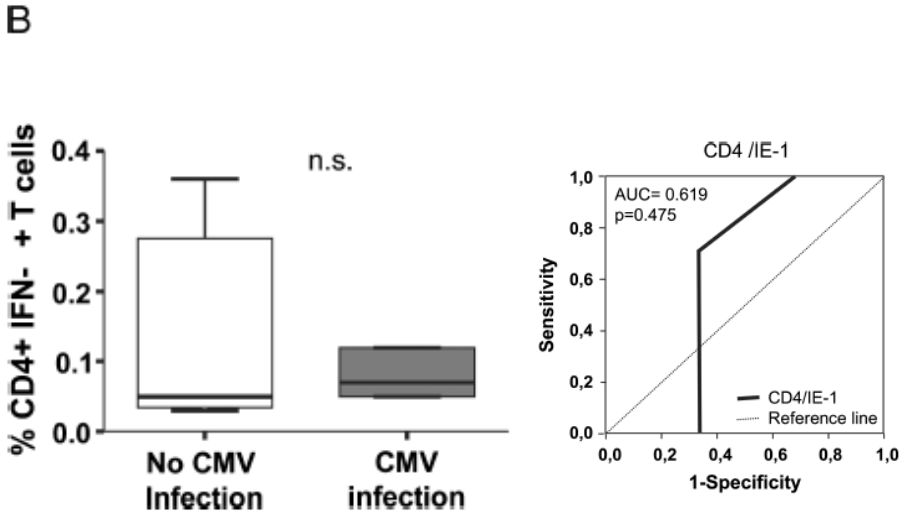
Pretransplant CD8 T-Cell Response to IE-1 Discriminates Seropositive Kidney Recipients at Risk of Developing CMV Infection Posttransplant

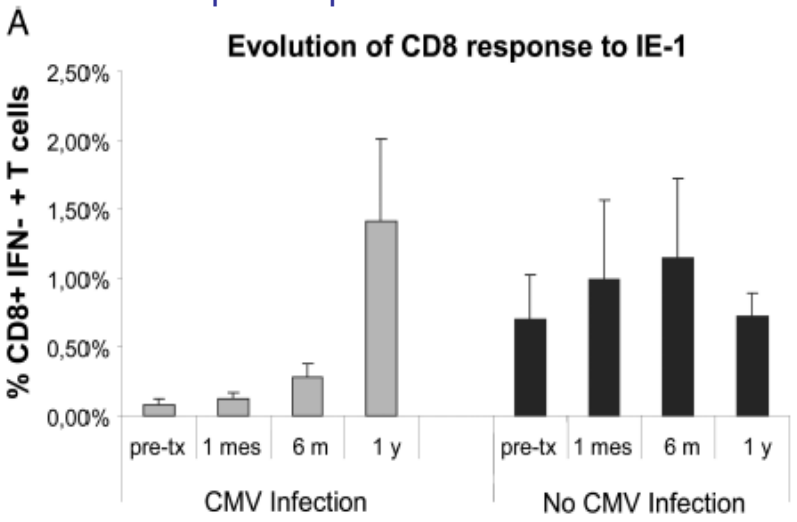
Maria Ovidia López-Oliva,[1] Virginia Martinez,[2] Águeda Buitrago,[2] Carlos Jiménez,[1] Begoña Rivas,[1] Fernando Escuin,[1] María José Santana,[1] Rafael Selgas,[1,2] and Teresa Bellón[2,3]

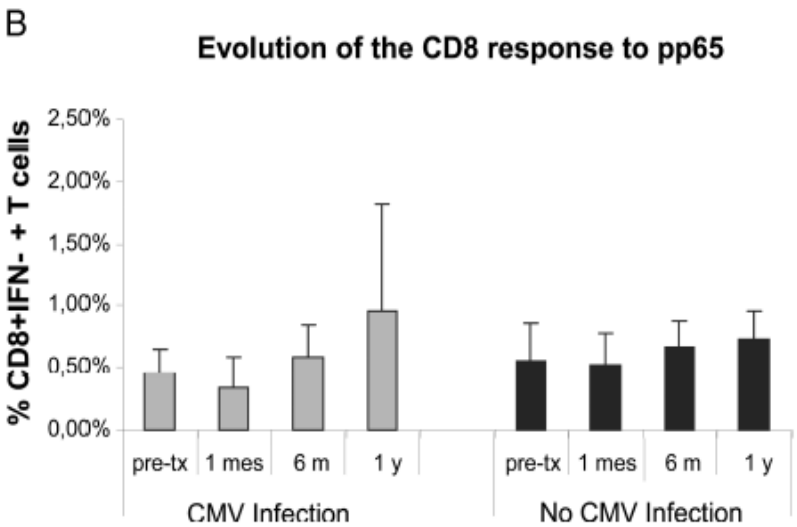
Transplantation • Volume 97, Number 8, April, 2014

Pts who developed CMV infection did not develop IE1-specific CD8 after 6 m









CMV-specific T-cell immunity test using QF-CMV to predict recurrent CMV infections in pediatric allo-HSCT recipients within the first year post-transplantation.

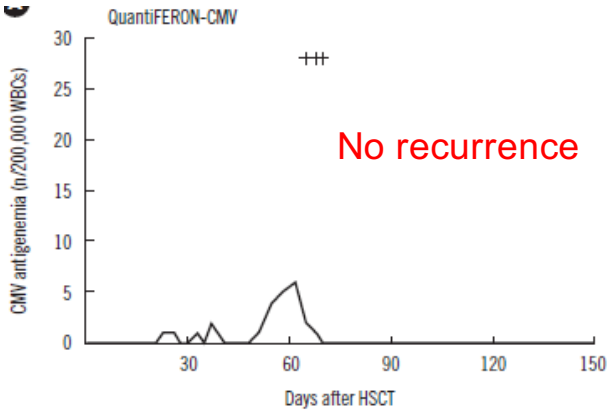
- Patients with positive QF-CMV results following initial CMV infection had no recurrent infections thereafter

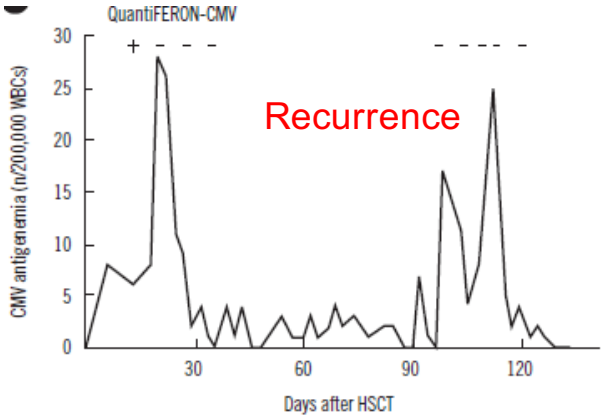
Table 2. Occurrence of recurrent CMV infection in patients with positive, negative, and indeterminate QuantiFERON-CMV results measured at the end of the first CMV infection post allogeneic HSCT

	QuantiFERON-CMV at the end of the first CMV infection			*P*
	Positive (N=4)	Negative (N=8)	Indeterminate (N=3)	
Presence of recurrent CMV infection (N of patients)	0 (0.0%)	5 (62.5%)	3 (100.0%)	0.019
Absence of recurrent CMV infection (N of patients)	4 (100.0%)	3 (37.5%)	0 (0.0%)	

Data are expressed as number (percentage).
Abbreviations: CMV, cytomegalovirus; HSCT, hematopoietic stem cell transplantation.

- Establishment of CMV-specific T-cell immunity following initial CMV infection contributes to the prevention of recurrent CMV infection episodes

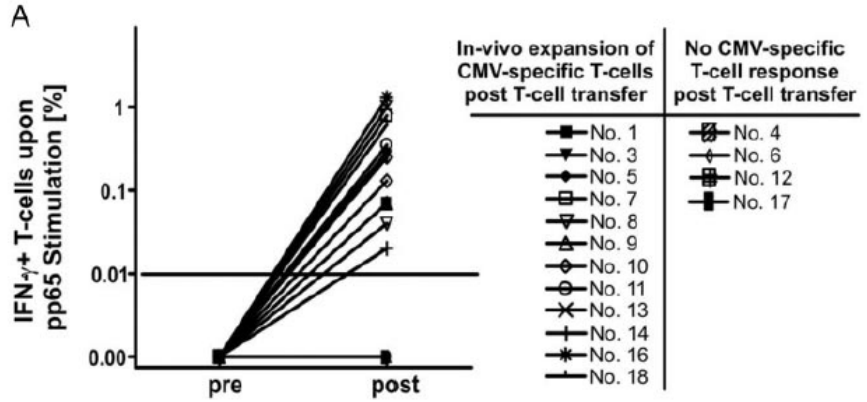




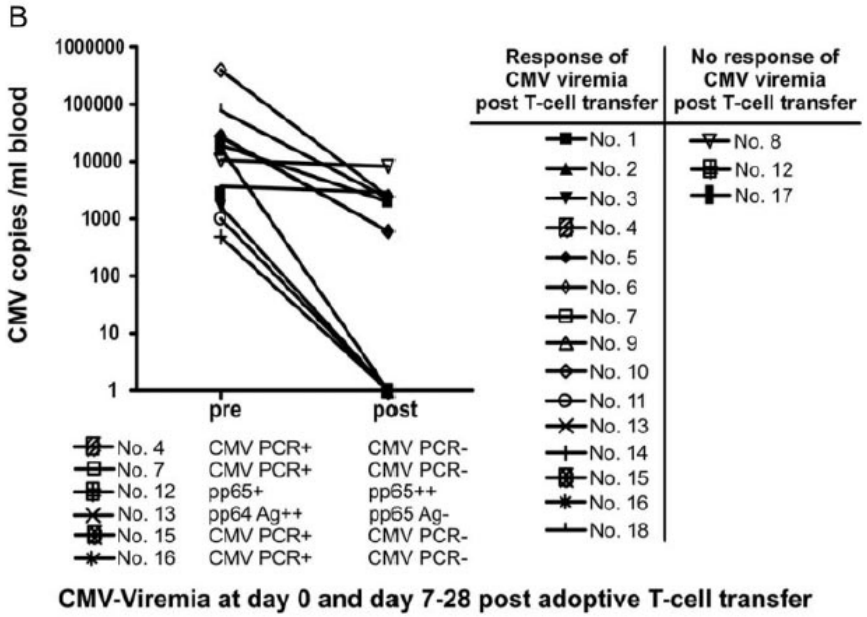
Longitudinal patterns of CMV-specific T-cell immunity in relation to CMV infection

Lee SM, Ann Lab Med 2017

In vivo T-cell response and virologic response after adoptive transfer of pp65-specific T cells in allo-SCT



CMV-specific T-cell response day 0 and day 7-28 post adoptive transfer



CMV-Viremia at day 0 and day 7-28 post adoptive T-cell transfer

The infusion of low numbers of CMV-specific T cells is safe, feasible, and effective as a treatment on demand for refractory CMV infection and CMV disease after allo-SCT.

Feuchtinger T, Blood 2010

- In the transplant setting, emergence of resistance mutations concerns 0.45–2.2% of recipients (i.e. 2.2–15% of patients developing CMV infection) depending on therapeutic strategies, studies definition and analysis criteria (Lurain NS et al. , Clin.Microbiol. Rev. 2010; Hantz S et al, J.Antimicrob. Chemother. 2010).

- Complex virus population dynamics have been reported in immunocompromised patients during drug selective pressure (Baldanti F et al., Transplantation. 1998; Baldanti F et al., Clin Infect Dis. 2002; Baldanti F et al., J Antimicrob Chemother. 2004; Campanini G, J Clin Virol. 2012; Chou S et al., Antimicrob Agents Chemother. 2014)

- The majority of HCMV drug-resistant strains show mutations in the UL97 gene which impair GCV and VGCV intracellular phosphorylation

- Double mutants may also develop

SOT, Spain (Sept 2013-Aug 2015) 9 out of 39 (23%) patients tested showed a CMV resistance mutation

Resistance mutations in the UL97 and UL54 genes.

Patient	Mutation detected UL97	Ratio[a] GCV	Mutation detected UL54	Ratio[a] GCV/POS/CDV
10	A594V	8.3		
19	M460V	8.3		
29	L595S	9.2		
30	C592G	2.9		
34	H520Q	10		
35	M460V	8.3	P522A	3/1/4.1
36	L595S	9.2		
38	M460V	8.3		
42	M460I	5	D413A	6.5/0.8/11

[a] IC_{50} of mutant/IC_{50} of wild type.

Mutations associated with:

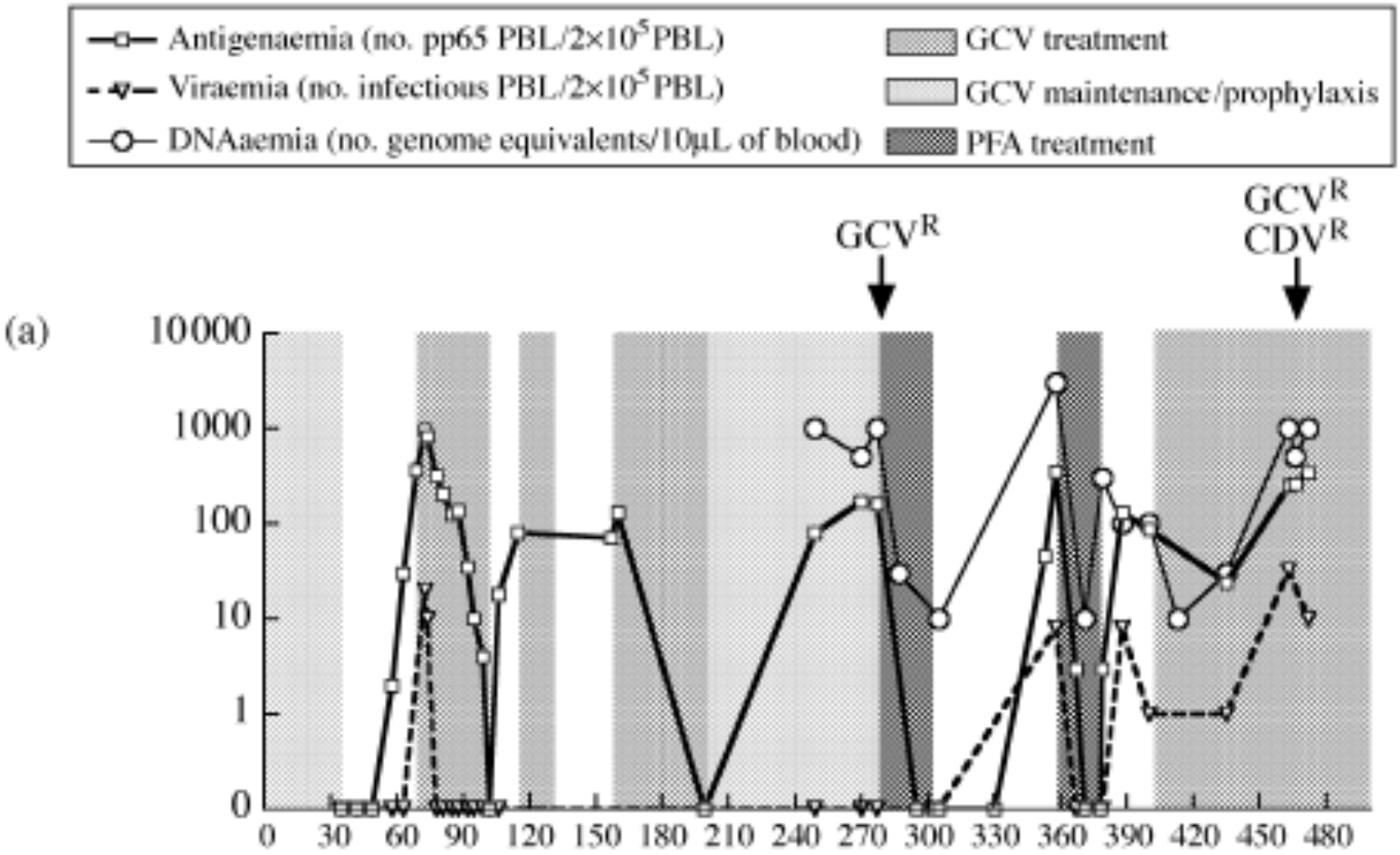
- Lung transplantation,
- Prophylaxis ≥6 months
- ↑ time between transplantation and suspicion of resistance
- Longer previous treatment with GCV or VGCV

López-Aladid R, J Clin Virol 2017

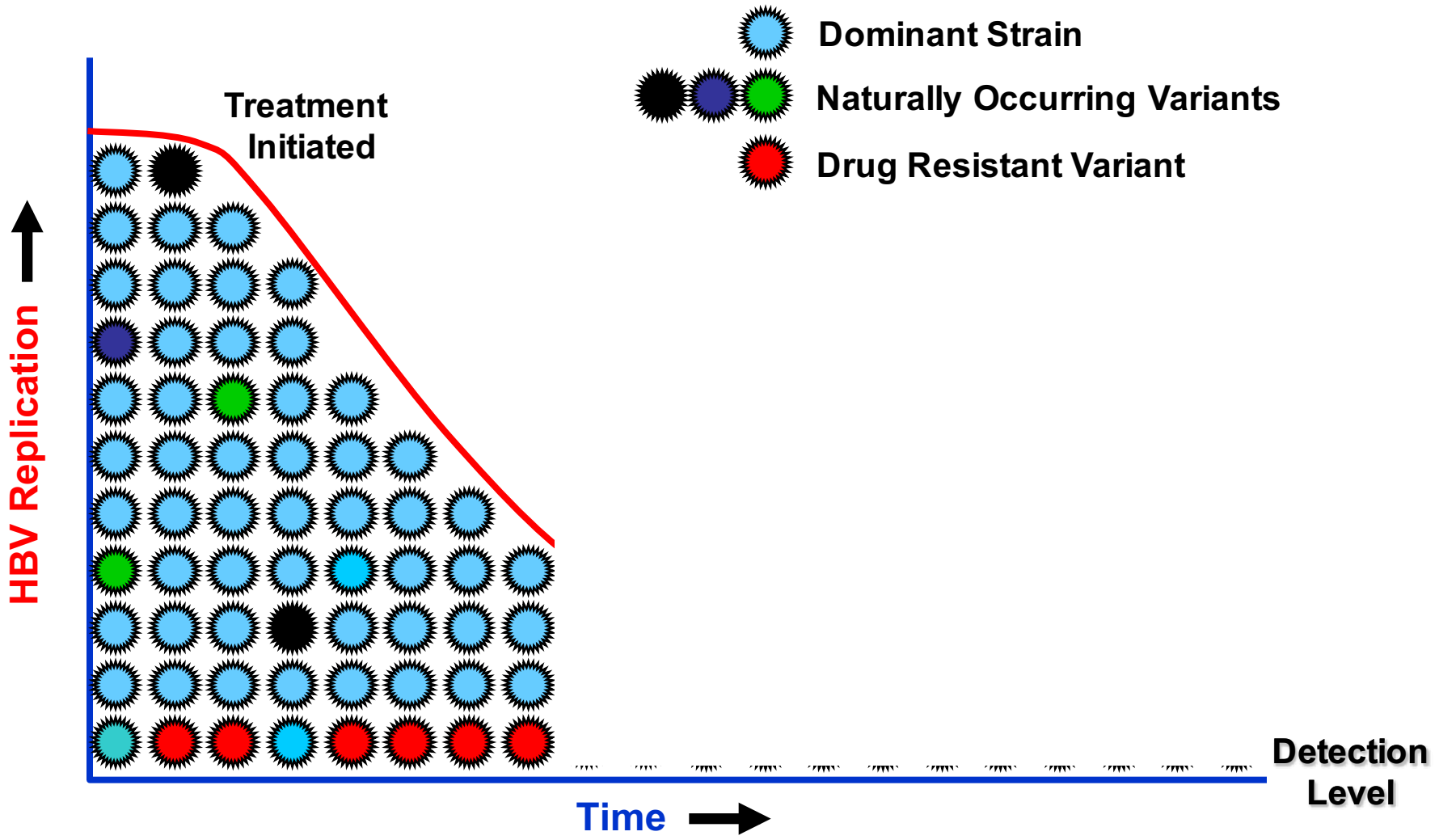
Human cytomegalovirus double resistance in a donor-positive/ recipient-negative lung transplant patient with an impaired CD4-mediated specific immune response

Fausto Baldanti[1,2], Daniele Lilleri[1], Giulia Campanini[1], Giuditta Comolli[1,2], Anna Lisa Ridolfo[3], Stefano Rusconi[3] and Giuseppe Gerna[1]*

Journal of Antimicrobial Chemotherapy (2004) **53**, 536–539

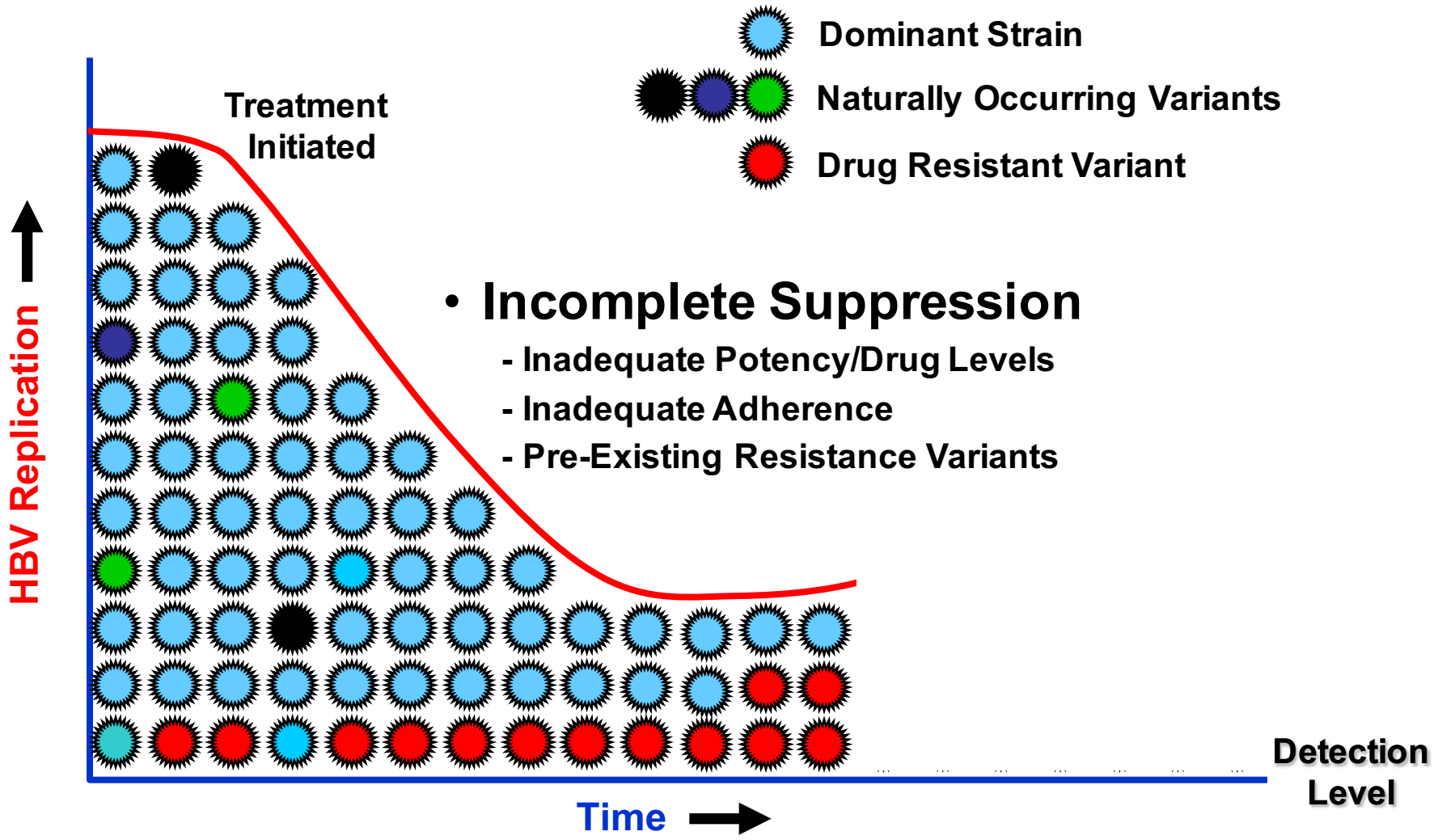


Incomplete suppression of virus replication leads to selection of mutants



Fung SK & Lok ASF. Antivir Ther 2004; 9:1013–1026
Locarnini S, et al. Antivir Ther 2004; 9:679–693

Incomplete suppression of virus replication leads to selection of mutants



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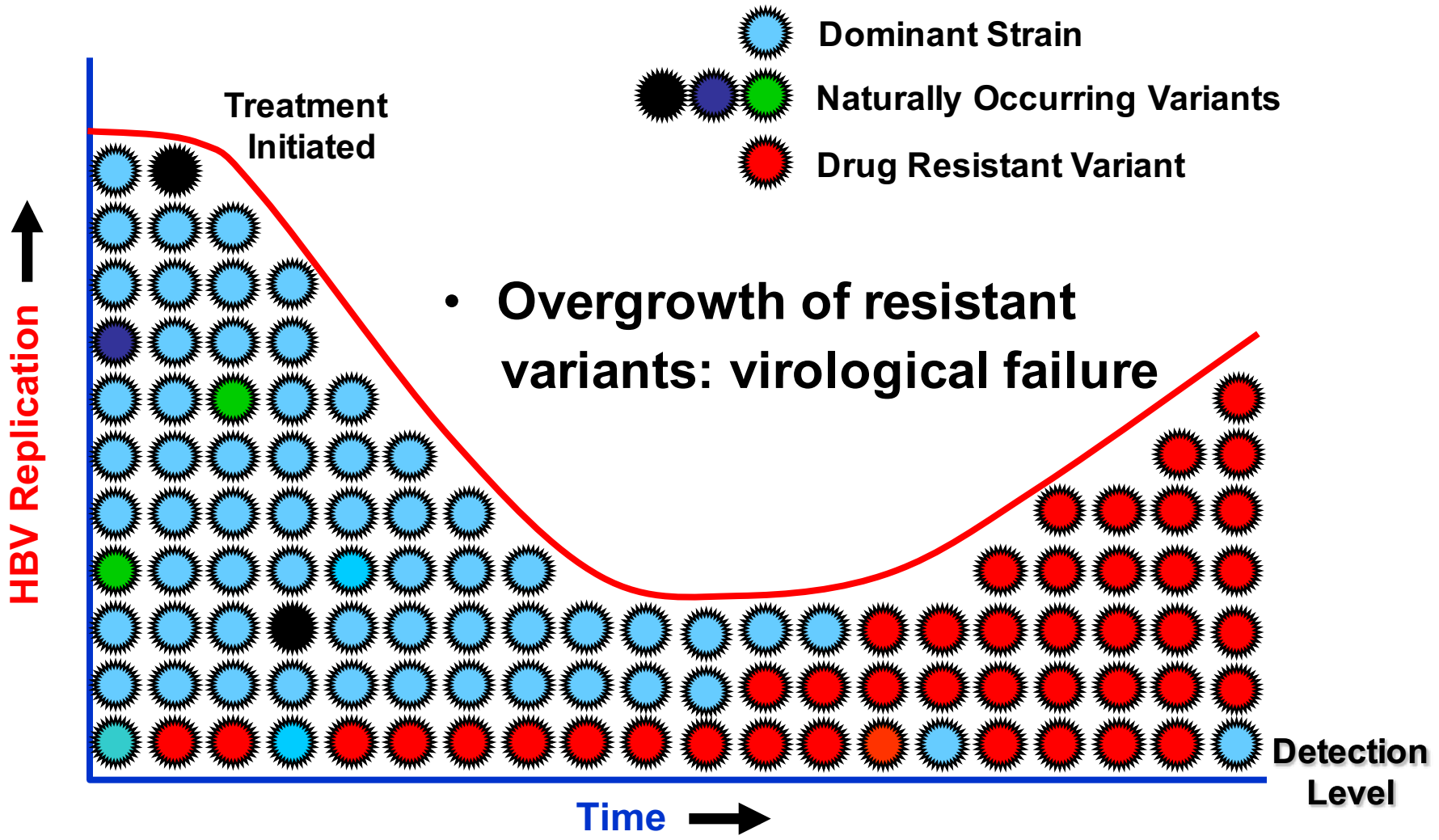
Incomplete suppression of virus replication leads to selection of mutants



Fung SK & Lok ASF. Antivir Ther 2004; 9:1013–1026
Locarnini S, et al. Antivir Ther 2004; 9:679–693

The NEW ENGLAND JOURNAL of MEDICINE

FOCUS ON RESEARCH

February 7, 2008 .

The New Age of Molecular Diagnostics for Microbial Agents

Richard Whitley, M.D.

Published in final edited form as:
J Clin Virol. 2014 September ; 61(1): 9–19. doi:10.1016/j.jcv.2014.06.013.

Deep Sequencing: Becoming a Critical Tool in Clinical Virology

Miguel E. QUIÑONES-MATEU[1,2,*], Santiago AVILA[3,4], Gustavo REYES-TERAN[3,4], and Miguel A. MARTINEZ[5]

...rch 2014
...nb.2014.00025

frontiers in
CELLULAR AND INFECTION MICROBIOLOGY

The diagnosis of infectious diseases by whole genome next generation sequencing: a new era is opening

Marc Lecuit[1,2,3,4] *and Marc Eloit*[5,6]*

Journal of Clinical Virology 58 (2013) 346–350

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Review

Next-generation sequencing technologies in diagnostic virology

Luisa Barzon*, Enrico Lavezzo, Giulia Costanzi, Elisa Franchin, Stefano Toppo, Giorgio Palù

NATURE REVIEWS | MICROBIOLOGY

Application of 'next-generation sequencing technologies to microbial genetics

Daniel MacLean, Jonathan D. G. Jones and David J. St

OPEN ACCESS Freely available online

PLoS GENETICS

Perspective

The Next Generation Becomes the Now Generation

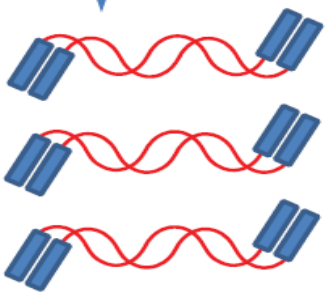
Diego A. Martinez*, Mary Anne Nelson

Department of Biology, University of New Mexico, Albuquerque, New Mexico, United States of America

Amplicon principle:

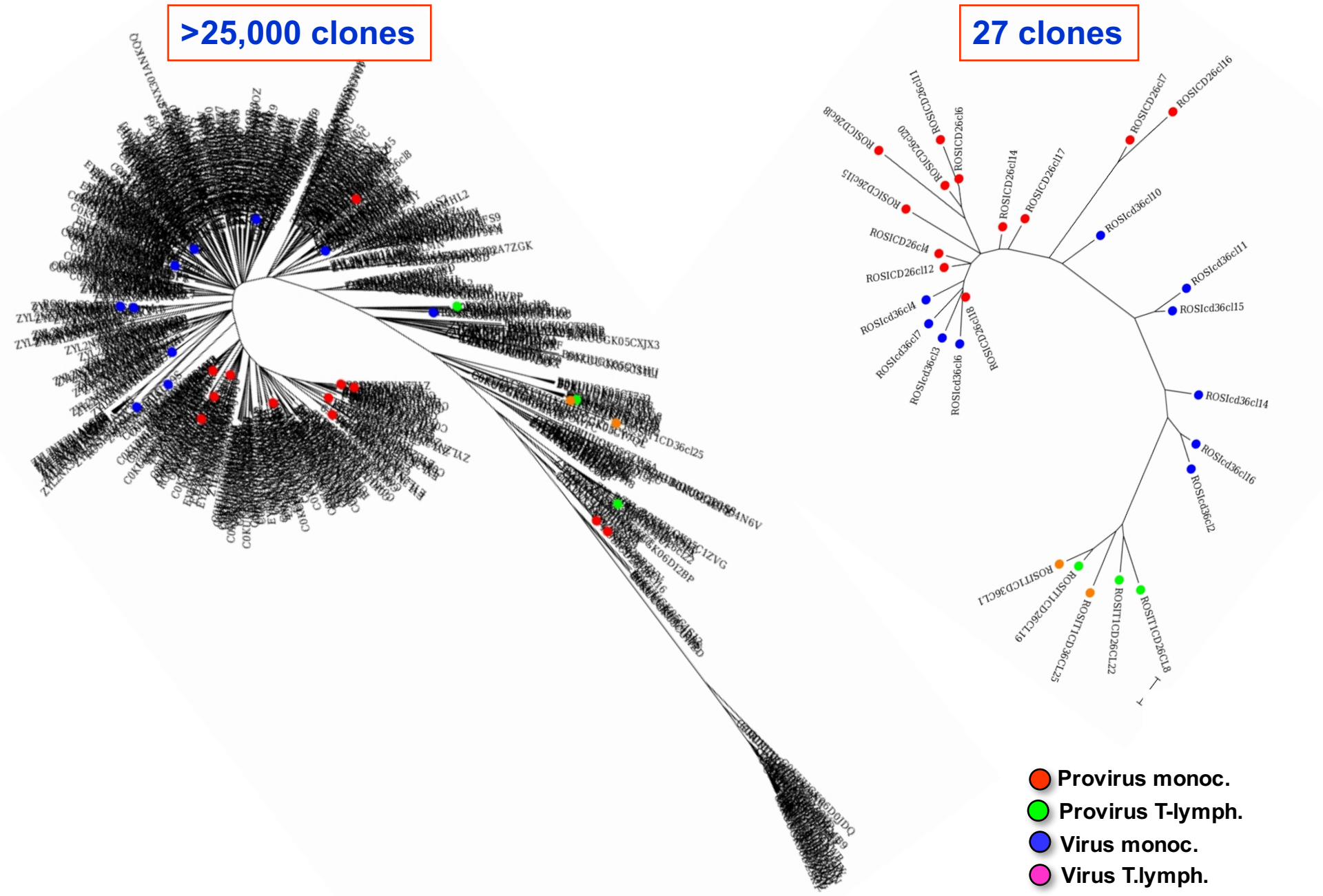
(a) Template DNA

- Generate sequence-targeted amplicons (lenght depending on system)
- Sequence all the amplicons



(b) Random shearing, size selection and adapter ligation

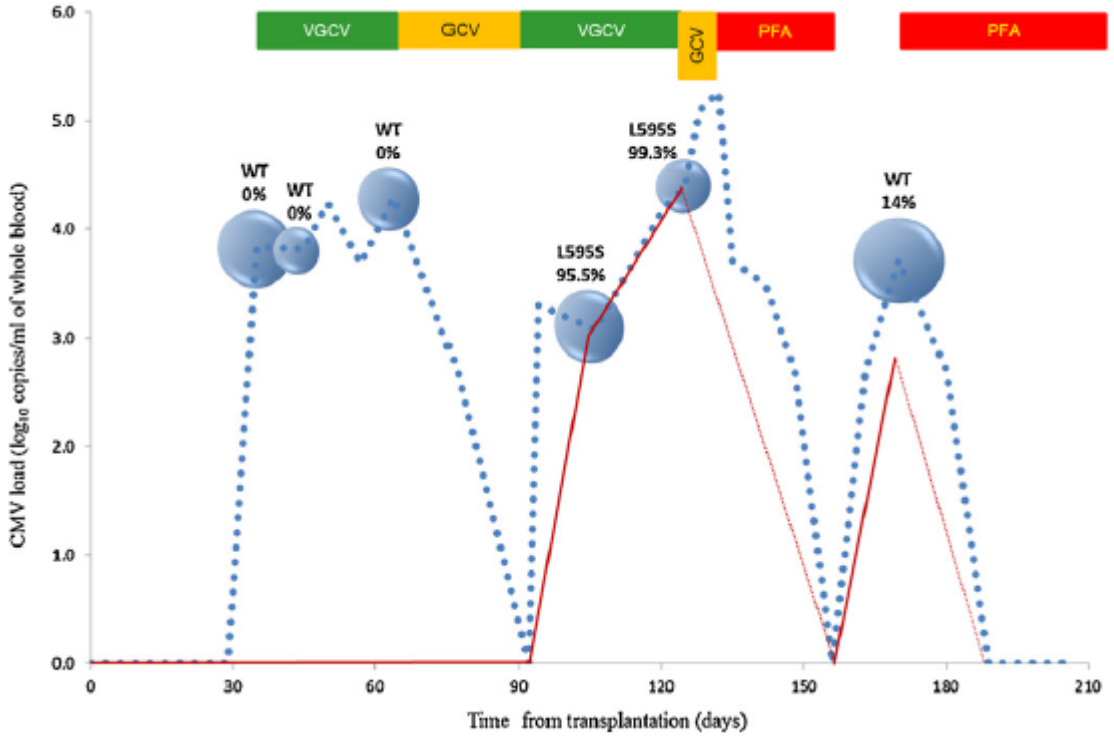
(c) Targeted PCR amplification


>25,000 clones
27 clones
ROSICD26cl11
ROSICD26cl6
ROSICD26cl16
ROSICD26cl8
ROSICD26cl20
ROSICD26cl7
ROSICD26cl15
ROSICD26cl14
ROSICD26cl17
ROSIcd36cl10
ROSICD26cl4
ROSICD26cl12
ROSIcd36cl11
ROSIcd36cl15
ROSIcd36cl4
ROSICD26cl18
ROSIcd36cl7
ROSIcd36cl3
ROSIcd36cl6
ROSIcd36cl14
ROSIcd36cl16
ROSIcd36cl2
ROSITICD36CL1
ROSITICD26CL19
ROSITICD36CL25
ROSITICD26CL22
ROSITICD26CL18
Provirus monoc.
Provirus T-lymph.
Virus monoc.
Virus T.lymph.

Contribution of next generation sequencing to early detection of cytomegalovirus *UL97* emerging mutants and viral subpopulations analysis in kidney transplant recipients

Journal of Clinical Virology 80 (2016) 74–81

Isabelle Garrigue[a,b,c,**], Rémi Moulinas[d,e,f,g], Patricia Recordon-Pinson[b,c], Marie-Laure Delacour[e,f], Marie Essig[h], Hannah Kaminski[i], Jean-Philippe Rerolle[h], Pierre Merville[i], Hervé Fleury[a,b,c], Sophie Alain[d,e,f,g,*]



Emergence of L595S mutation under GCV was followed up.

After PFA rescue therapy, in two patients,L595S mutant re-emerged, but was only detected by NGS technology (14% and 9.6%).

NGS improved sensitivity helps in studying viral abundance, dynamics and diversity, that are difficult with Sanger sequencing.

How far are we from current clinical aplication of NGS?

In the current clinical context, viral resistance is investigated with Sanger sequencing. In case of a negative Sanger result, it could be pertinent to turn to a more sensitive detection with NGS, particularly in fragile patients with high risk of developing antiviralr esistance, giving at the same time an image of the viral global variability.

In a routine perspective, there is a need to determine how early the detection of emergent resistant variants should influence therapeutic adaptation.
When a new viral replication occurs, this also raises the question of sensitive detection of mutation persistence before a new treatment.

This deserves to be studied on a larger scale.

Front Line Genomics

Unzipping genes for the good of humanity

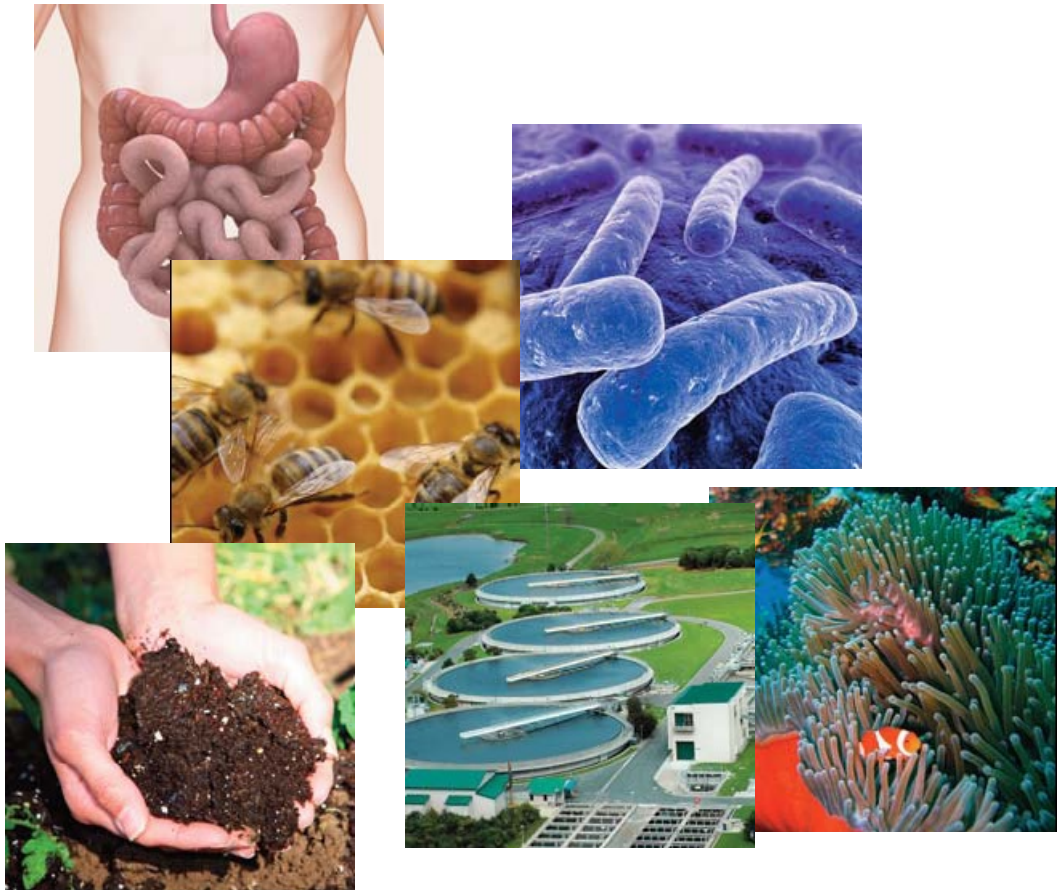
MinION becomes first to sequence DNA in space. 30 August 2016

NASA Astronaut Kate Rubins sequenced DNA in space for the first time ever for the Biomolecule Sequencer investigation, using the MinION sequencing device / NASA

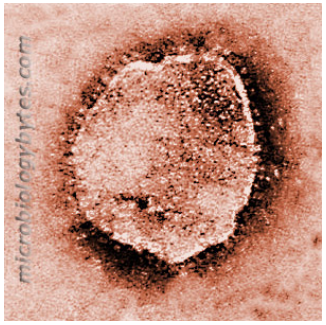
What is Metagenomics?

Contemporary analysis of all genomes present in a given environment:

- **Serching for new species (microbial, viral…)**
- **Quantitative description of microbial communities (viroma, microbioma)**
- **Microbial variability**
- **Diagnostics…..**



- **Human body**
- **Soil samples**
- **Extreme environments**
- **Marine ecosystem**



The NEW ENGLAND JOURNAL of MEDICINE

N Engl J Med 2008;358.

A New Arenavirus in a Cluster of Fatal Transplant-Associated Diseases

Gustavo Palacios, Ph.D., Julian Druce, Ph.D., Lei Du, Ph.D., Thomas Tran, Ph.D., Chris Birch, Ph.D., Thomas Briese, Ph.D., Sean Conlan, Ph.D., Phenix-Lan Quan, Ph.D., Jeffrey Hui, B.Sc., John Marshall, Ph.D., Jan Fredrik Simons, Ph.D., Michael Egholm, Ph.D., Christopher D. Paddock, M.D., M.P.H.T.M., Wun-Ju Shieh, M.D., Ph.D., M.P.H., Cynthia S. Goldsmith, M.G.S., Sherif R. Zaki, M.D., Ph.D.,

454 pyrosequencing of the patients' samples provided, amidst a host background of 103,632 total reads, 14 reads corresponding to a novel, deadly arenavirus

CORRESPONDENCE

Volume 358:2638-2639 June 12, 2008 Number 24

A New Arenavirus in Transplantation

Allander T, de Lamballerie X, Simmonds P

Although the study correctly shows how ultra-highthroughput sequencing is a powerful tool for microbiology, it appears that the current study is an example of its unnecessary use. Sequence diversity within a species is not the same thing as a "new virus."

Temporal Response of the Human Virome to Immunosuppression and Antiviral Therapy

Cell *155*, 1178–1187, November 21, 2013 ©2013

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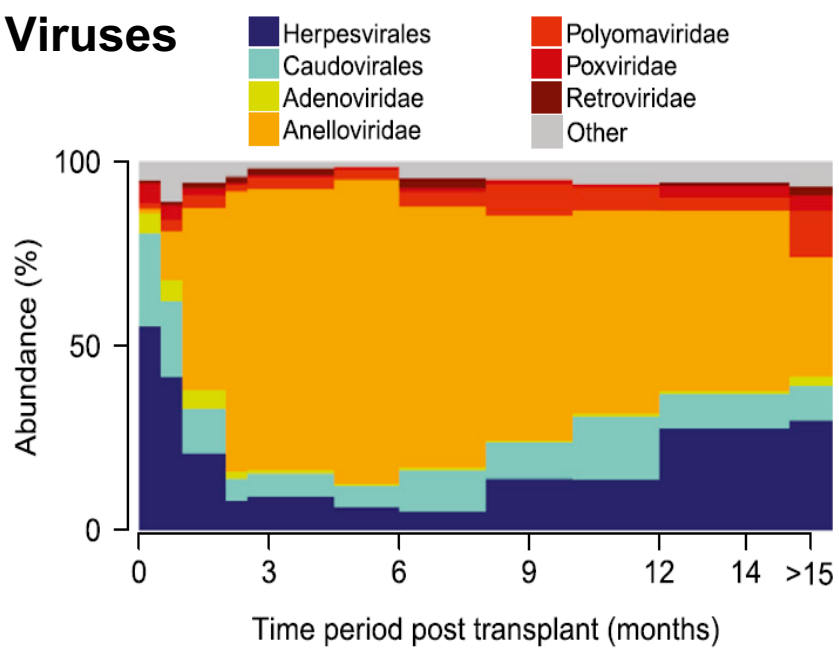
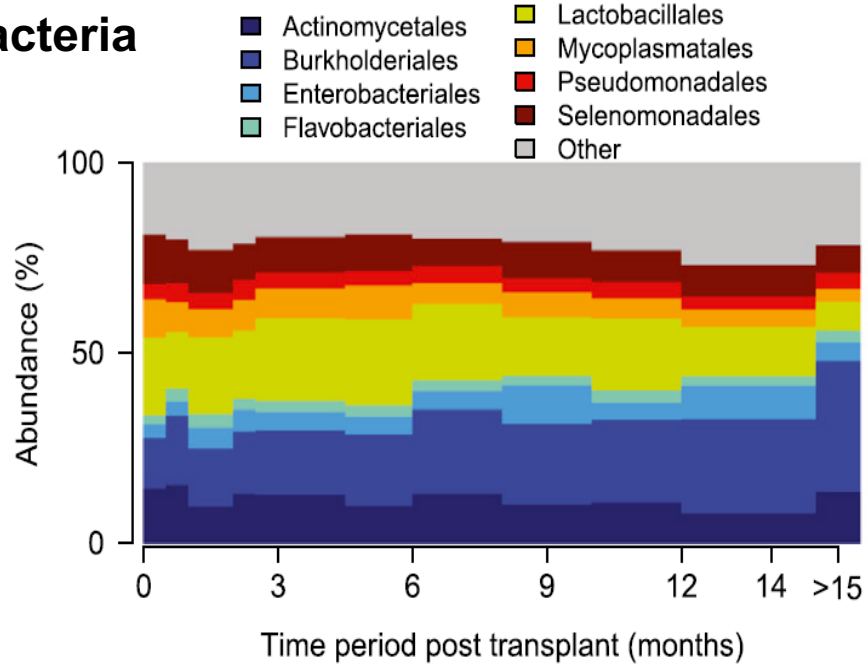
- marked virome composition dynamics at the onset of the therapy
- the bacterial component of the microbiome remains largely unaffected





Take home messages

- Monitoring of viral infections remains mandatory in the post transplant period
- The availability of international standards for quantification of DNA of major opportunistic viruses is a step forward in the identification of cut-off values for preemptive treatment
- Immunologic monitoring is helpful for risk stratification of transplant recipients
- Combining molecular monitoring with immunological monitoring may provide additional information for personalized intervention
- New techniques are rapidly emerging with potential of
  - refining virological monitoring
  - predicting complications
  - tailoring therapeutic intervention
- Still many (trivial?) points need better definition


THANK
YOU!