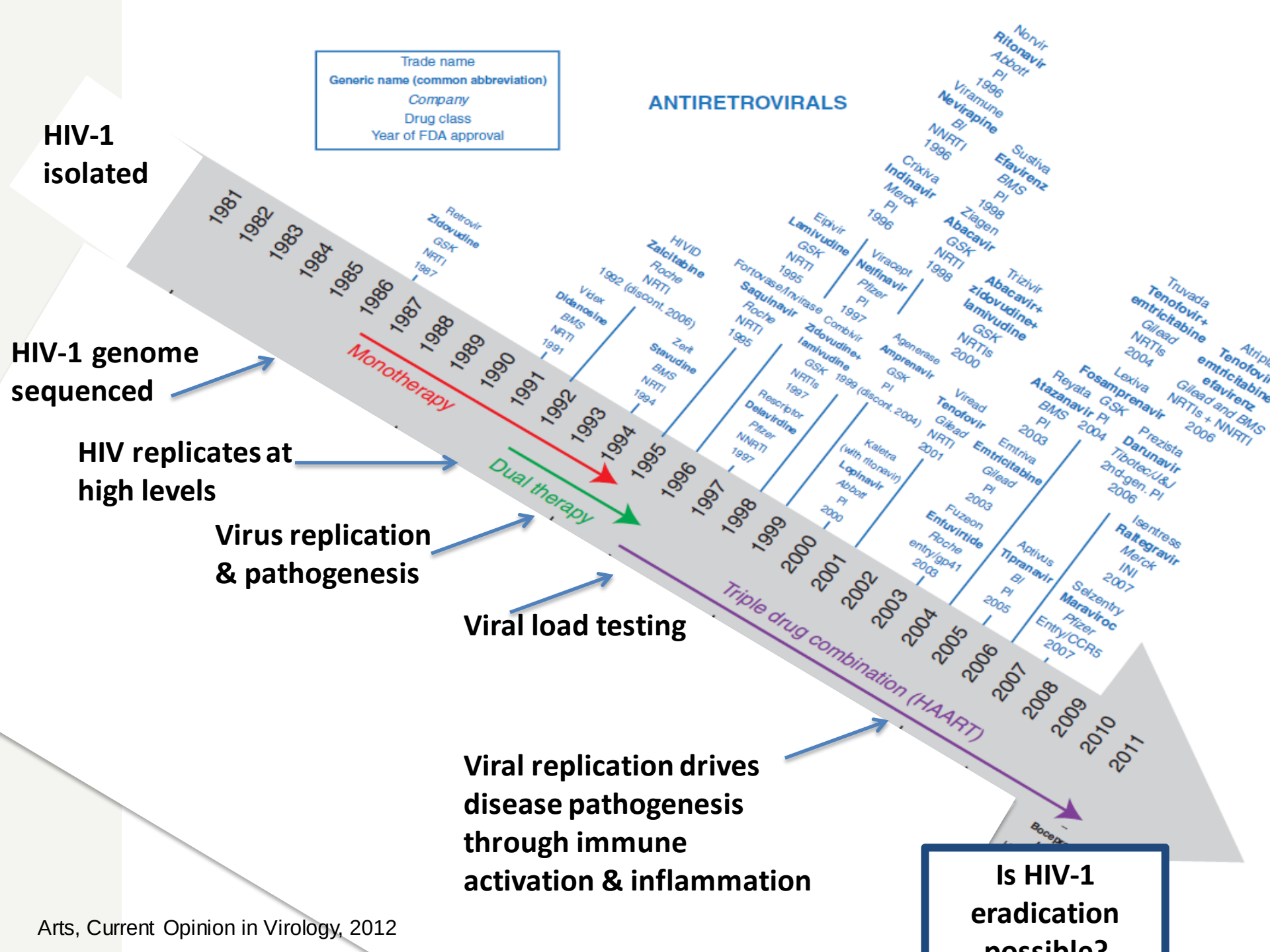
The need for ultrasensitive determinations in HIV diagnostics

Filippo Canducci

University of Insubria-Varese

San Raffaele Hospital-Milano

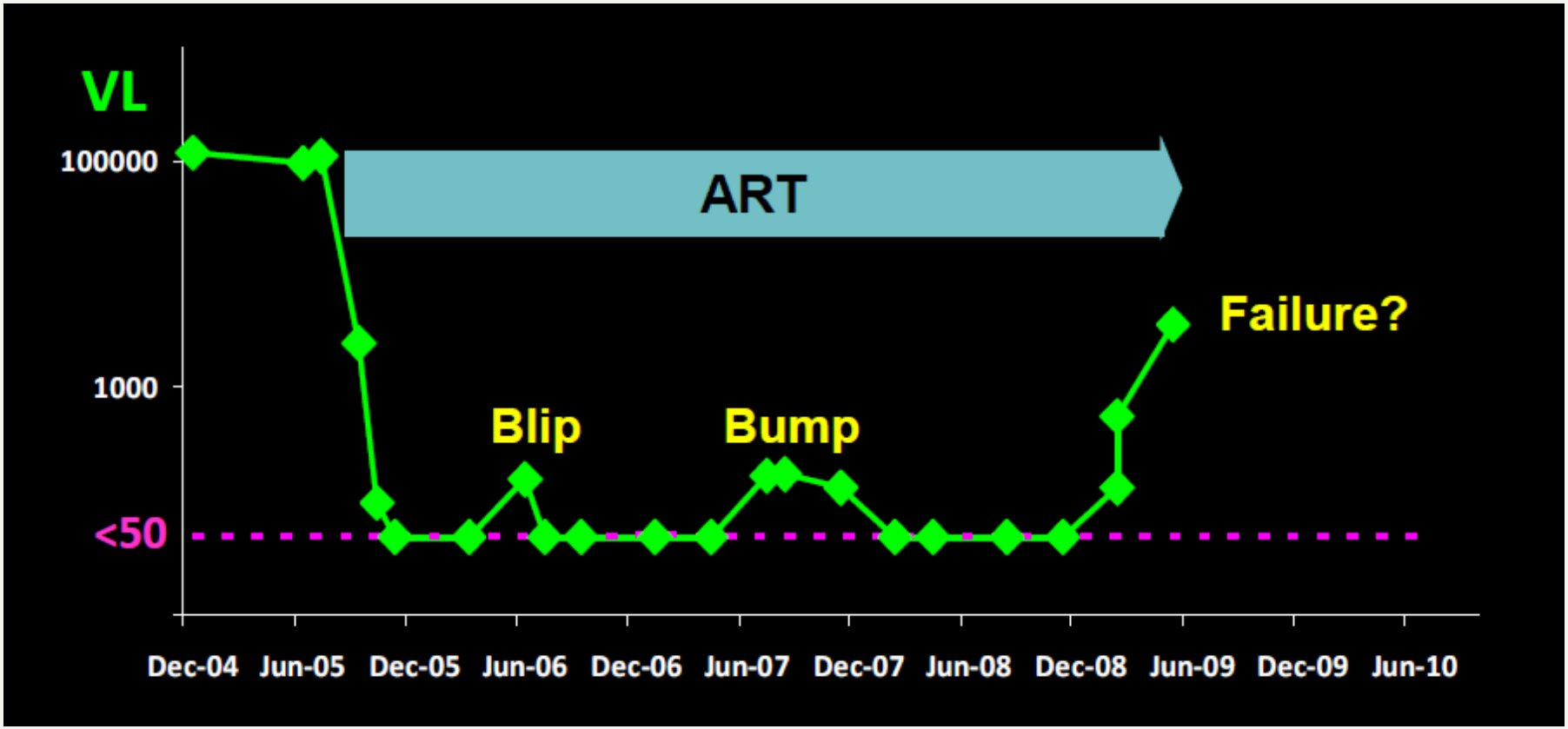


Arts, Current Opinion in Virology, 2012

Linee Guida Italiane sull'utilizzo dei farmaci antiretrovirali e sulla gestione diagnostico-clinica delle persone con infezione da HIV-1

PAZIENTE	IMPIEGO	RACCOMANDAZIONE (FORZA/EVIDENZA)
Tutti i pazienti dal momento in cui entrano in cura.	La viremia è l'indicatore più importante di risposta terapeutica; pertanto, essa va misurata ad intervalli regolari, soprattutto nei pazienti che iniziano una cART.	[AI]
Naïve alla cART.	La viremia andrebbe determinata con regolarità ogni 3/4 mesi fino al momento dell'eventuale inizio della terapia.	[AII]
All'inizio del percorso terapeutico o di un cambio terapeutico per fallimento virologico.	La viremia andrebbe misurata immediatamente prima e non oltre 4 settimane dall'inizio della terapia, per verificare l'efficacia iniziale del trattamento.	[AII]
	Ripetere la determinazione della viremia ogni 4-8 settimane dall'inizio della terapia, fino al raggiungimento di viremia non rilevabile (< 50 copie/mL).	[BII]
In regime terapeutico con soppressione virologica stabile.	Determinare la viremia ogni 3/4 mesi.	[AII]
	Se il paziente è in soppressione virologica stabile da almeno 2-3 anni, è sicuramente aderente, ed è in buono stato clinico e immunologico, è possibile, in casi particolari e a giudizio del medico curante, estendere l'intervallo fra le determinazioni della viremia fino a 6 mesi.	[BIII]
In cambio di terapia per semplificazione o tossicità.	La viremia andrebbe misurata al momento del cambio della terapia, quindi entro 2-4 settimane dal cambio, e poi a distanza regolare di 3-4 mesi fino ad un anno dal cambio, al fine di confermare l'efficacia del nuovo regime.	[BIII]
In caso di mancato raggiungimento della soppressione virologica a 6 mesi dall'inizio della cART, oppure in caso di incremento della viremia dopo iniziale soppressione.	Un attento monitoraggio della viremia (anche molto ravvicinato nel tempo) andrebbe effettuato al fine di distinguere un fallimento precoce (rialzo viremico) da una lenta e graduale riduzione della viremia stessa, oppure da un blip. In tali circostanze, la definizione di fallimento virologico andrà commisurata anche alla viremia basale.	[BII]

Defining viral load rebound during ART



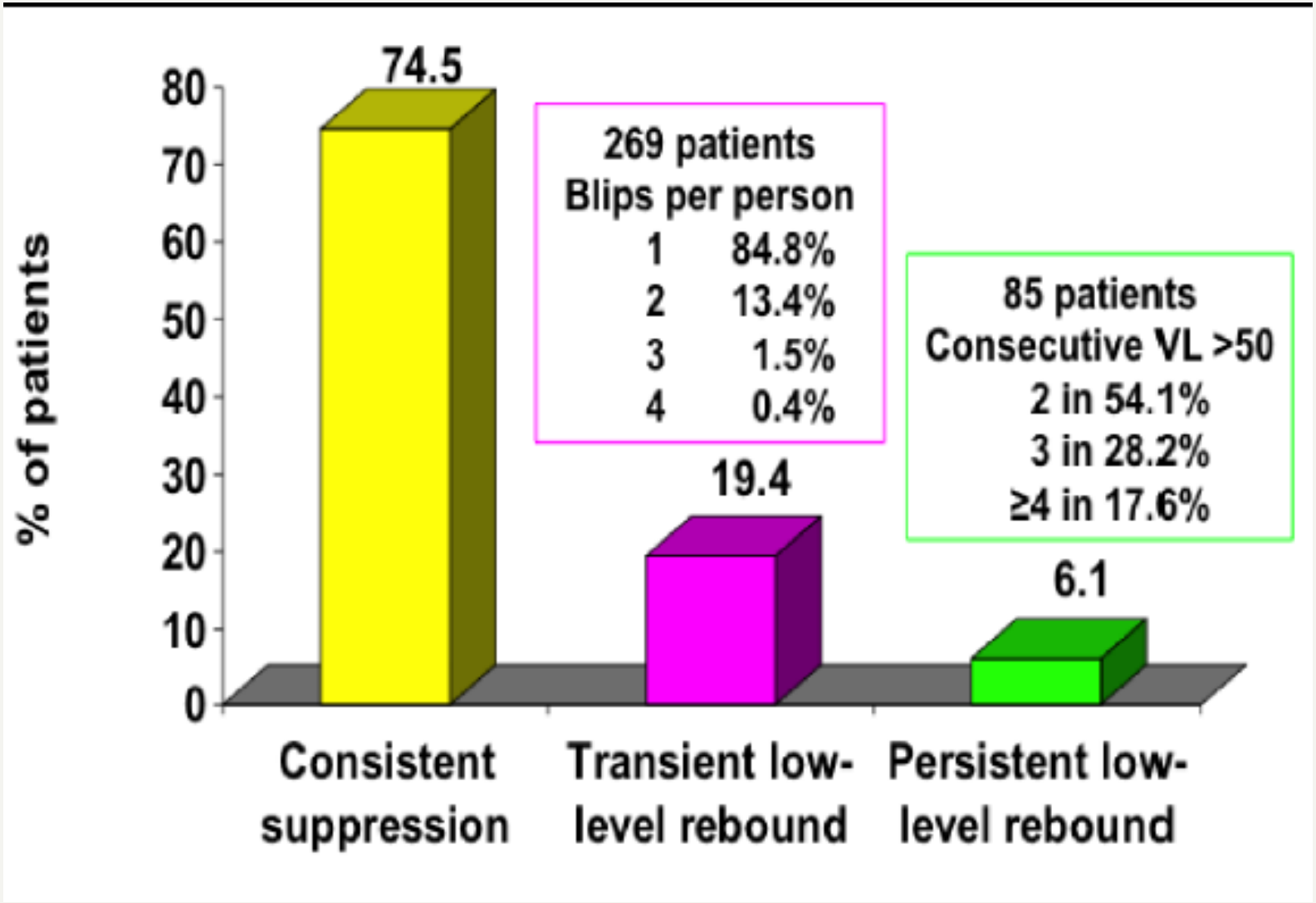
Defining virological failure

DHHS 2011
The inability to achieve or maintain a VL <200 cps/ml

BHIVA 2012
Failure to achieve a VL <50 cps/ml 6 months after commencing ART or following suppression to <50 cps/ml a VL rebound to >400 cps/ml on two consecutive occasions

IAS-USA 2012
Sustained VL elevation between 50 and 200 cps/ml should prompt evaluation of factors leading to failure and consideration of switching ART

Viral loads in the HAART era



Geretti, Antiv Ther 2008

The need for ultrasensitive determinations in HIV-1 diagnostics

- ✓ *Virological rebound in human immunodeficiency virus-infected patients with or without residual viraemia: results from an extended follow-up*

- ✓ *Clinical evaluation and comparizion of QIAsymphony RGQ Automated Sample Preparation assay with the Versant HIV-1 RNA kPCR 1.0 (Siemens Diagnostics) assay for quantitative detection of human immunodeficiency virus type 1 in plasma samples*

Clinical evaluation of the *artus* HI Virus-1 QS-RGQ kit for quantitative detection of HIV-1 in plasma samples using the QIAsymphony RGQ Automated Sample Preparation assay

	artus® HI Virus-1 QS-RGQ CE-IVD kit	VERSANT HIV-1 RNA 1.0 Assay (kPCR)
detection limits	45 copies/ml	1 cp/ml
dynamic range	45–4.5 × 10^7 copies/ml	37-10^8 cp/ml
Linear range	45-112 copies/ml	37-10^8 cp/ml

Study design

From January 2013 to July 2013, a total of 192 plasma specimens from 192 patients with HIV-1 infection were collected.

Each of the routine clinical samples was analyzed for the quantification of HIV-RNA using artus® HI Virus-1 QS-RGQ CE-IVD kit and results were compared with those obtained by the Siemens VERSANT HIV-1 RNA 1.0 assay (kPCR).

Sample processing

VERSANT HIV-1 RNA 1.0 assay (kPCR)

850 micro liters of EDTA plasma were processed by this system (1050 micro liters l dead volume required).

Nucleic acid was extracted by the COBAS AmpliPrep system prior to being automatically transferred to the COBAS TaqMan system for amplification and detection.

Results were given in c/ml and were transferred directly to the Laboratory Information Management System.

Sample processing

QIAsymphony SP/AS system

1000 l of plasma were processed prior to transfer to the AS module.

QIAGEN artus HIV-1 QS-RGQ test PCR set-up PCR set-up was performed with the Rotor-Gene Q stated in the artus HIV-1 QS-RGQ test handbook.

The data was analysed at a threshold of 0.04 for HIV-1 and 0.03 for the internal control. HIV-1 viral loads were calculated as IU/l of eluate and converted to IU/ml of original sample.

In order to compare results with those obtained with the Roche HIV-1 v2.0 test, a conversion factor of 0.45 copies/IU was used to convert the artus HIV-1 QS-RGQ test results to c/ml.

Both assays allow using primary tubes and bar-code reading

Results

3 samples (1.56%) were eliminated from the study due to amplification inhibition

157/189 (81.8%) had positive results with mean log difference of 0.14 and

17/189 (8.84%) were negative for HIV-RNA, in both assays

3/189 samples (1.56%) were below the Siemens linear range, and negative in the artus® assay

12 samples (6.24%) showed discordant results , (mean log difference of 1.74)

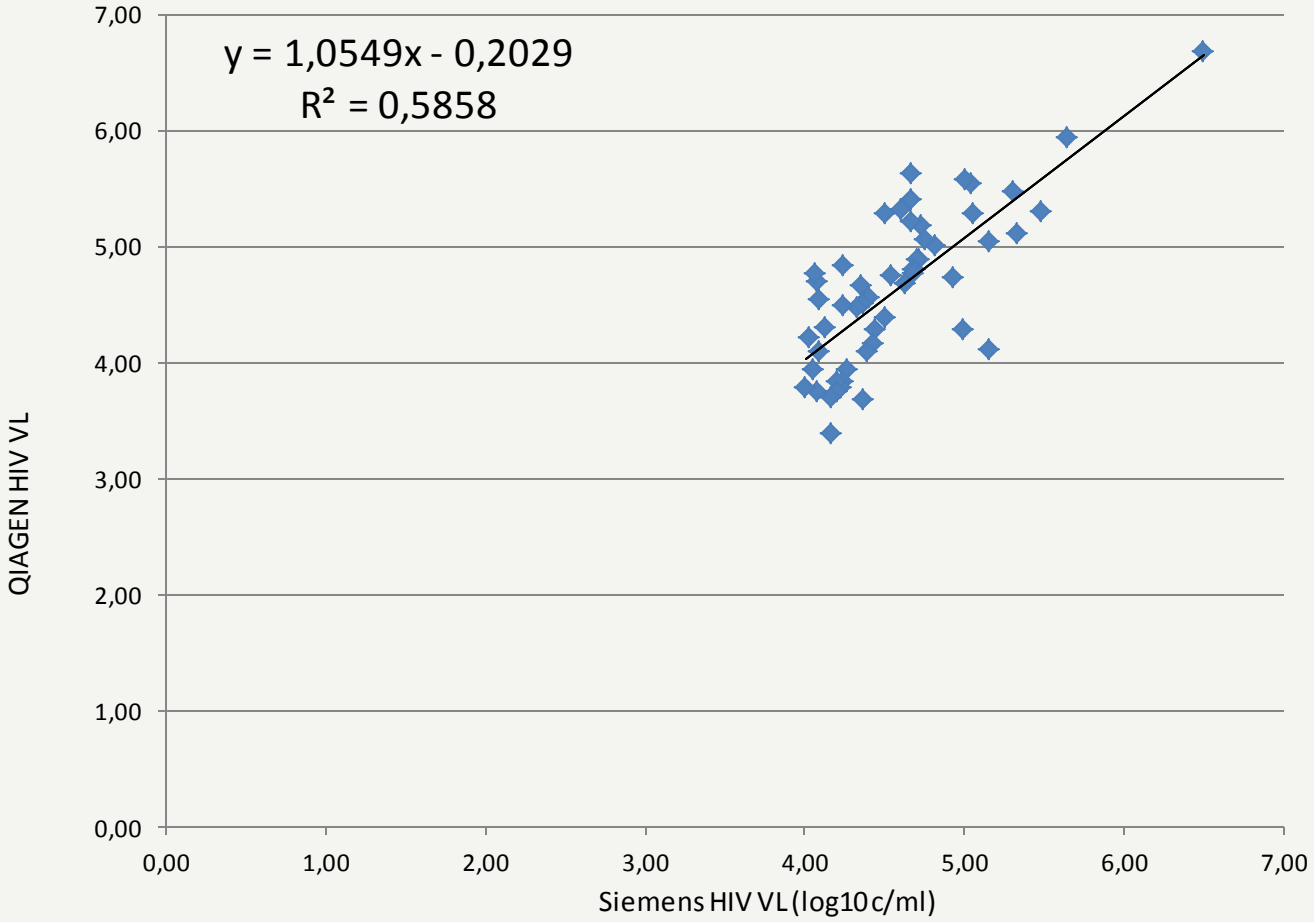
Results

Siemens	
Mean	3.26
Standard Error	0.10
Median	3.23
Mode	1.82
Standard Deviation	1.18
Sample Variance	1.40
Kurtosis	-1.13
Skewness	0.22
Range	4.87
Minimum	1.63
Maximum	6.50
Sum	504.60
Count	155.00
Largest(1)	6.50
Smallest(1)	0.48
Confidence Level(95.0%)	0.19

QIAGEN	
Mean	3.13
Standard Error	0.11
Median	3.21
Mode	0.85
Standard Deviation	1.43
Sample Variance	2.04
Kurtosis	-0.93
Skewness	0.02
Range	6.37
Minimum	0.30
Maximum	6.67
Sum	485.49
Count	155.00
Largest(1)	6.67
Smallest(1)	0.30
Confidence Level(95.0%)	0.23

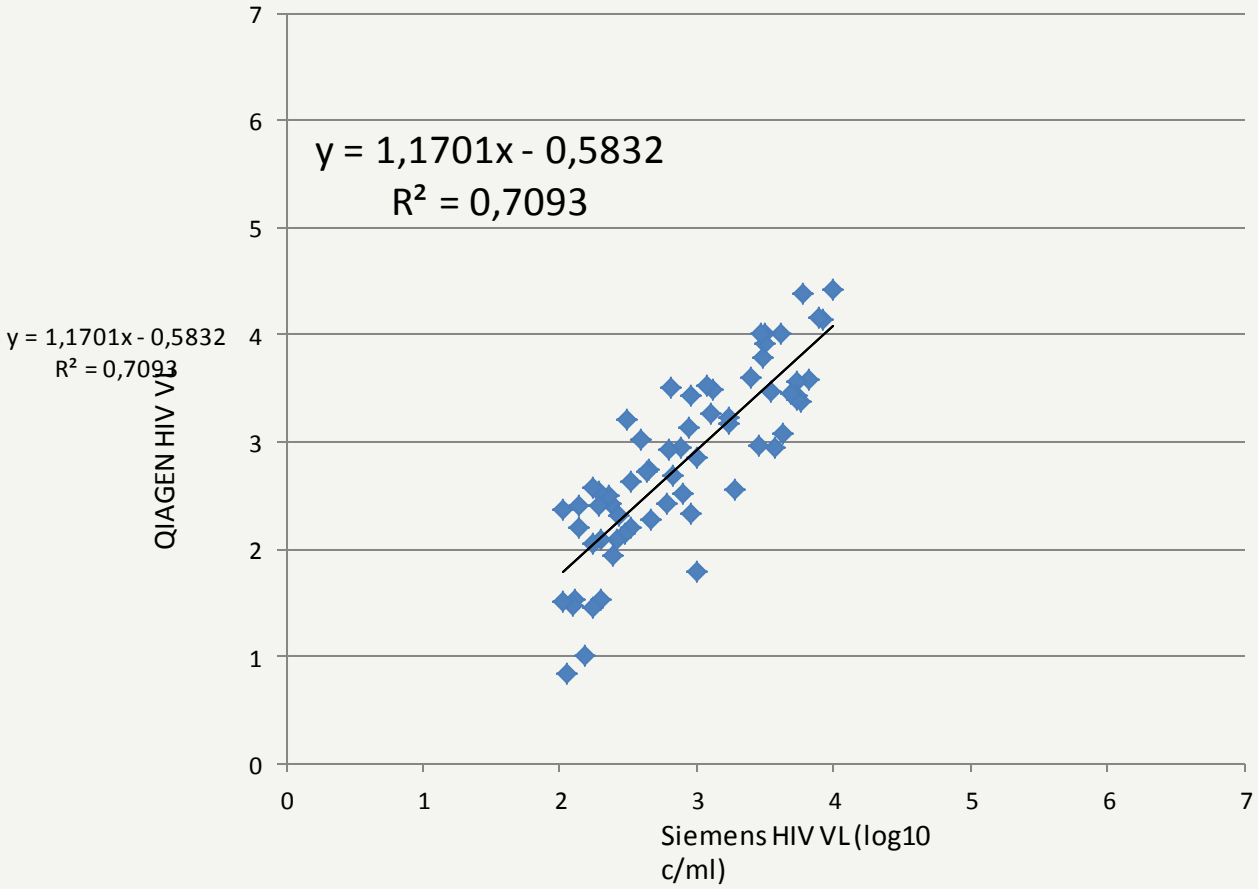
Results

Linear correlation co-efficient: 4-6.5 log range



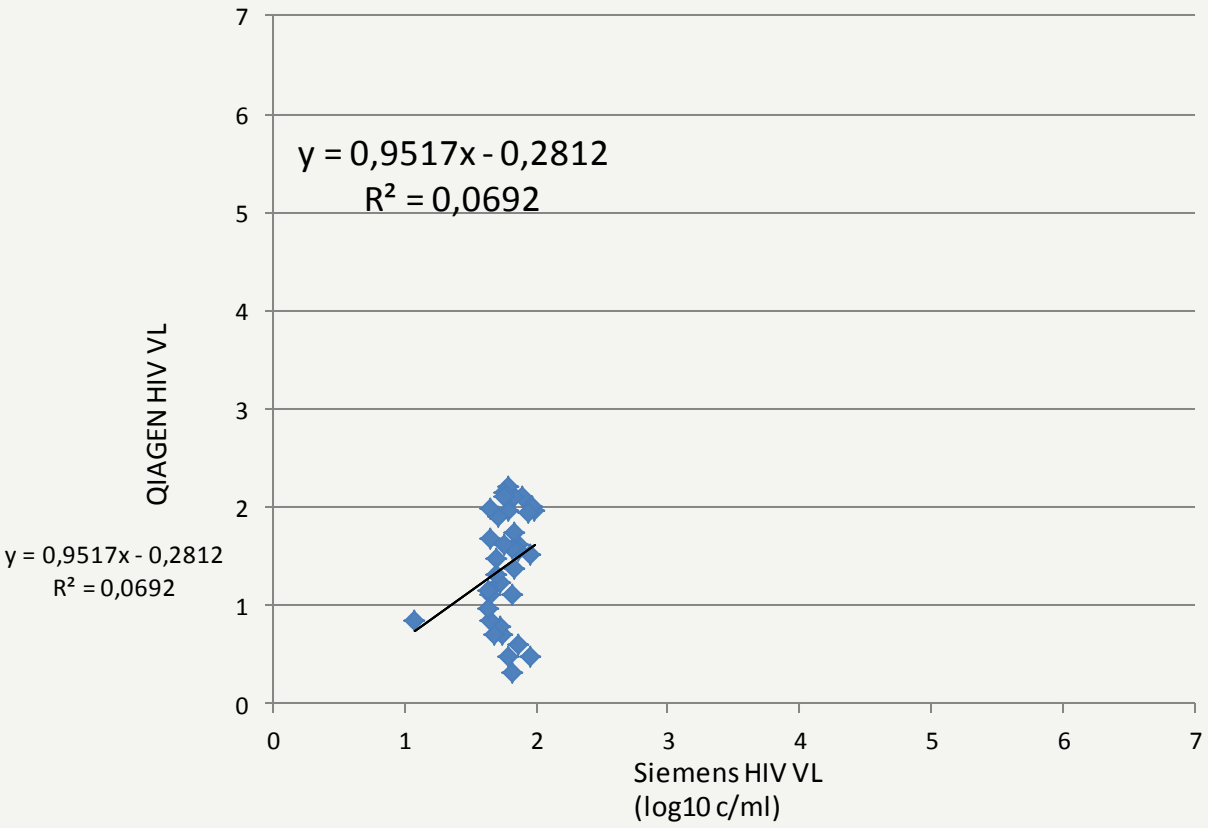
Results

Linear correlation coefficient 2-4 log range



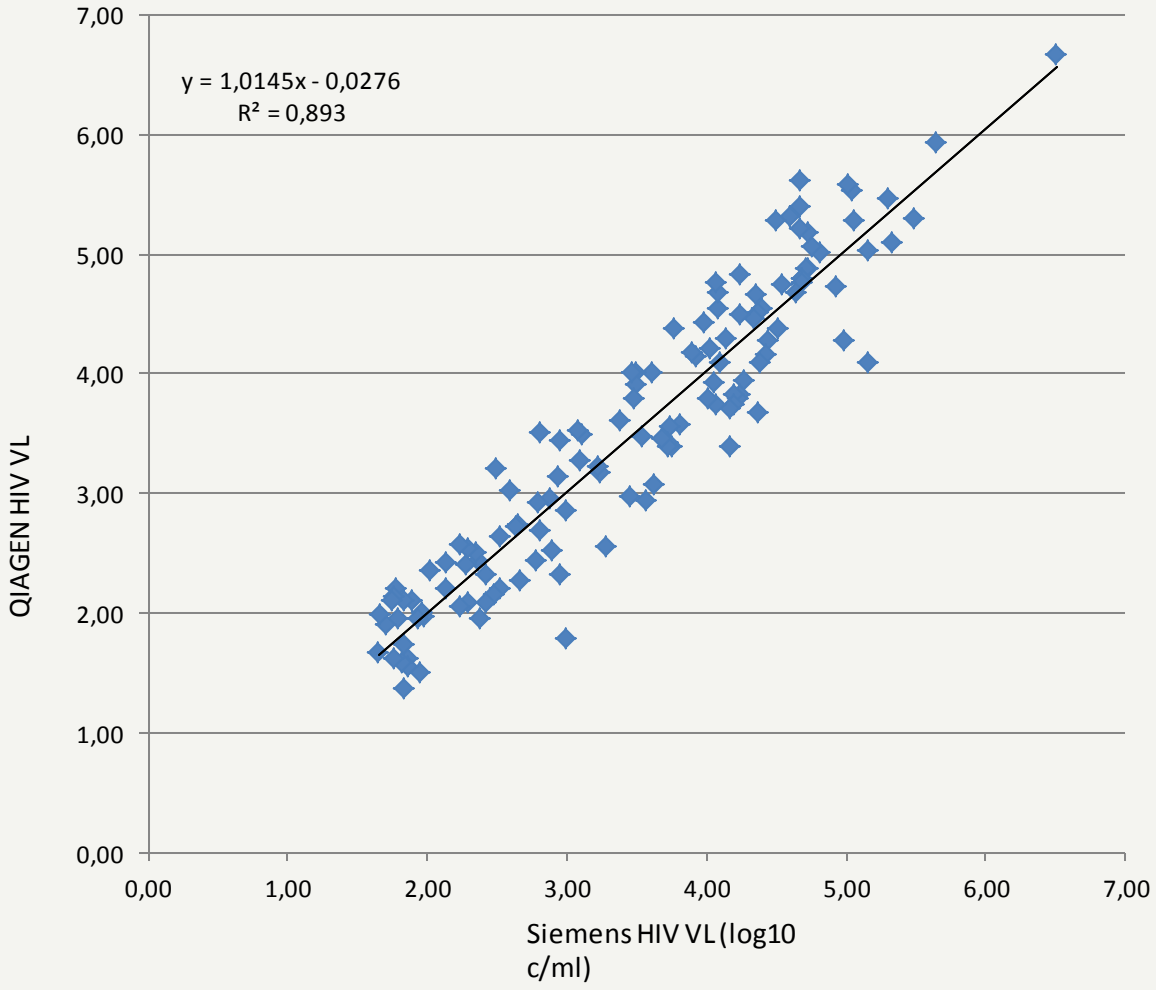
Results

Linear correlation coefficient: 1-2 log range



Results

Linear correlation co-efficient



Samples with viral loads within the linear range of both assays in an XY scatter plot.

Results

HIVRNA12A QCMD Panel	Siemens (c/ml)	QIAGEN (c/ml)
3 (C.)	610	117
6 (A/G)	3028	27403
8 (C.)	6824	1992

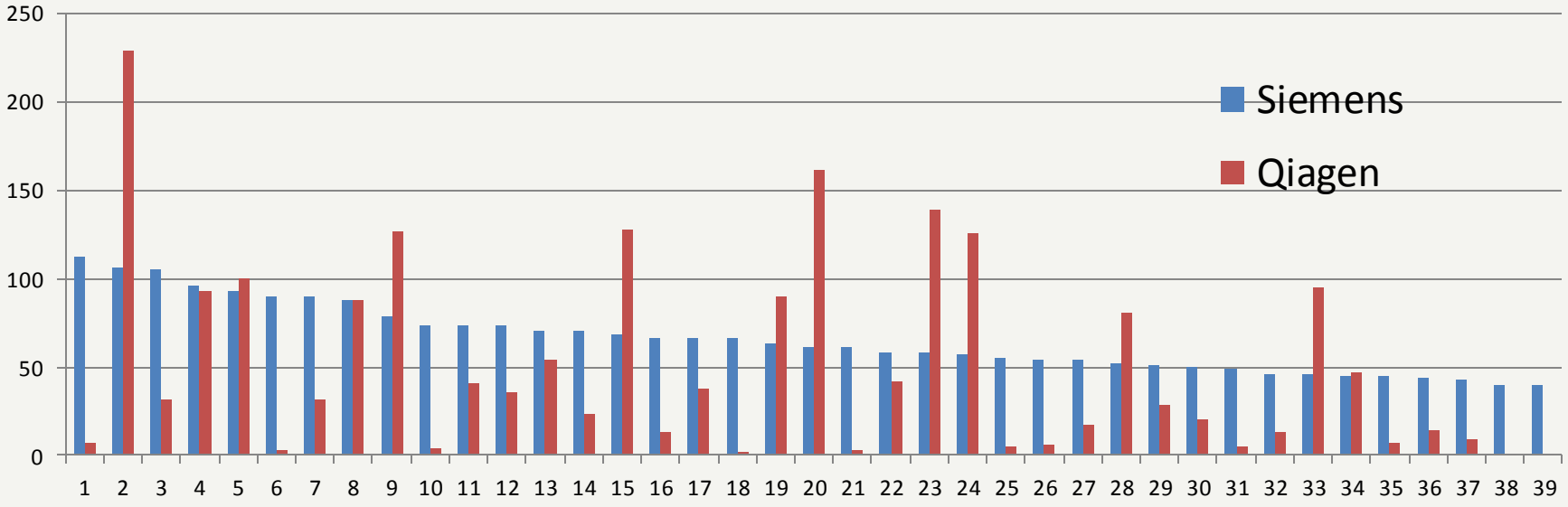
Results

Negative samples with both assays

Sample ID	Siemens (c/ml)	Siemens (log c/ml)	QIAGEN (c/ml)	QIAGEN (log c/ml)
1	0	0.00	0	0.00
2	0	0.00	0	0.00
3	0	0.00	0	0.00
4	0	0.00	0	0.00
5	0	0.00	0	0.00
6	0	0.00	0	0.00
7	0	0.00	0	0.00
8	0	0.00	0	0.00
9	0	0.00	0	0.00
10	0	0.00	0	0.00
11	0	0.00	0	0.00
12	0	0.00	0	0.00
13	0	0.00	0	0.00
14	0	0.00	0	0.00
15	0	0.00	0	0.00
16	0	0.00	0	0.00
17	0	0.00	0	0.00
18	0	0.00 inib		0.00

Results

39 samples with values between 135 and 40 cp/ml with Siemens



Results

Discordant samples below Qiagen linear Range, within analytical sensitivity

HIVRNA12A	Siemens (c/ml)	Siemens (log c/ml)	QIAGEN (c/ml)	QIAGEN (log c/ml)	
1	42	1.62	0		0.00
2	44	1.64	0		0.00
3	55	1.74	2		0.30
4	59	1.77	0		0.00
5	67	1.83	0		0.00
6	74	1.87	0		0.00
7	47	1.67	0		0.00
8	65	1.81	0		0.00
9	50	1.70	0		0.00
10	50	1.70	0		0.00
11	135	2.13	1		0.00
12	48	1.68	1		0.00
	61.3				

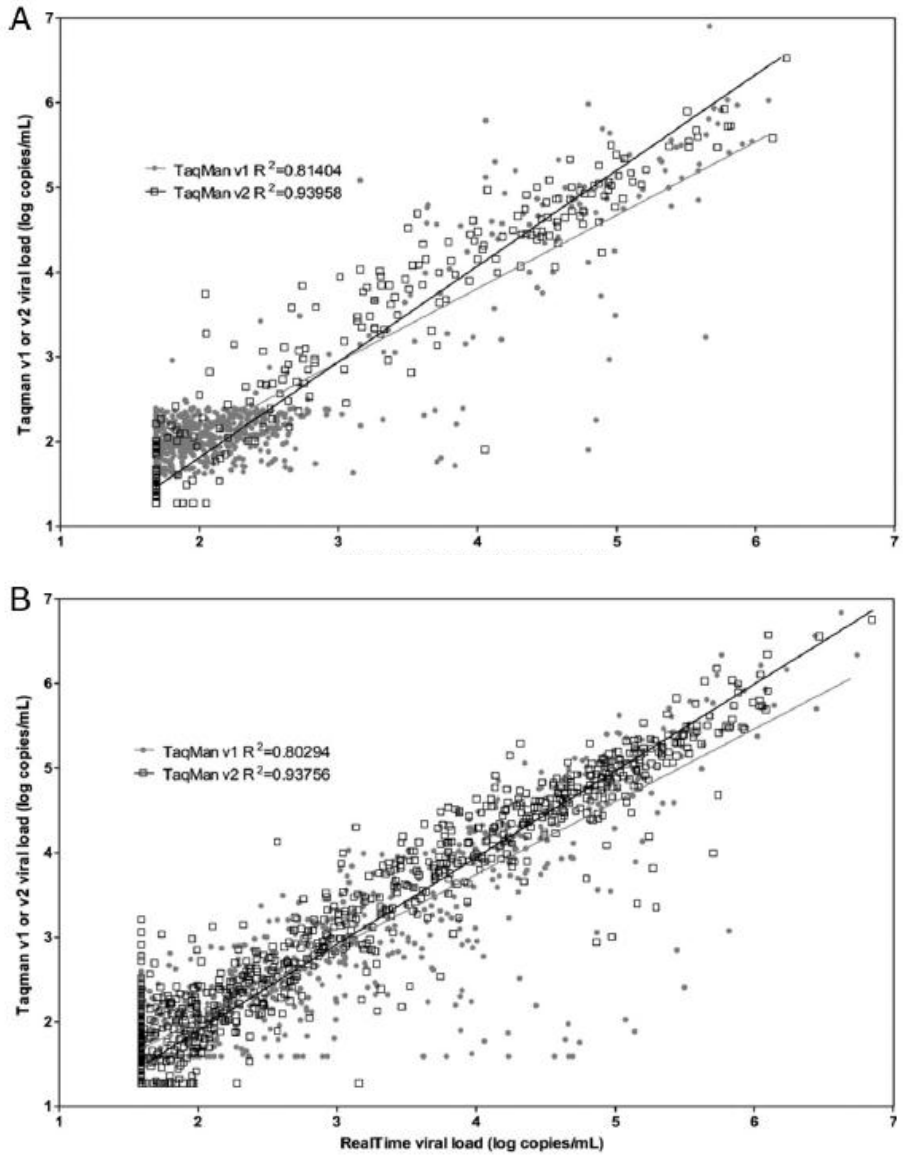
Comparative Performances of HIV-1 RNA Load Assays at Low Viral Load Levels

TABLE 1 Correlation coefficients for interassay comparisons at viral loads of <1,000 copies/ml[a]

Assay detection (at <1,000 copies/ml)	Correlation coefficients of discordant results of ≥1,000 copies/ml			
	Amplicor	RealTime	TaqMan v1	TaqMan v2
Amplicor		NA[b]	0.52	0.85
RealTime	NA		0.74	0.82
TaqMan v1	0.45	0.54		0.69
TaqMan v2	0.80	0.78	0.68	

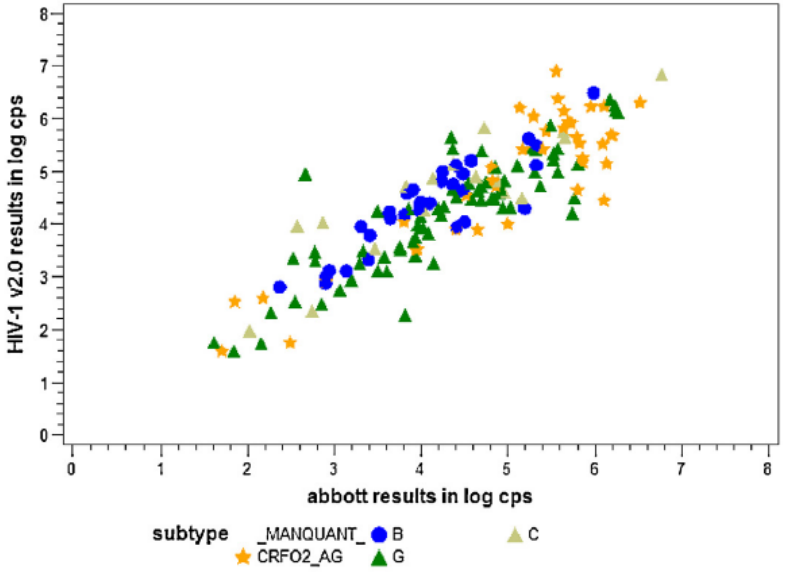
[a] Correlation coefficients of the interassay comparisons were calculated using the results with a restriction of at least one viral load assay giving a result of <1,000 copies/ml. The assay restricted to <1,000 copies/ml is shown in the left-hand column, and its comparator assay is indicated in the columns. For example, when TaqMan v1 was restricted to values of <1,000 copies/ml, the matching Amplicor test results had an *R* value of 0.45. Conversely, when the Amplicor test was restricted to <1,000 copies/ml, the matching TaqMan v1 results had an *R* value of 0.52.

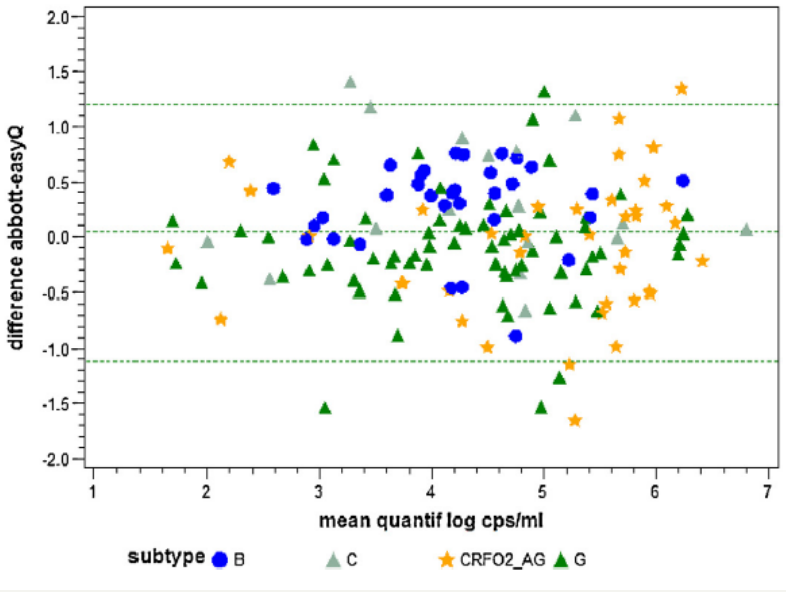
[b] NA, not available.



Swenson, JCM 2013

Comparison of the NucliSENS EasyQ HIV-1 v2.0 with Abbott m2000rt RealTimeHIV-1 assay for plasma RNA quantitation in different HIV-1 subtypes





Attention when switching platforms during longitudinal viral load monitoring

Gomes JVM 2013

Conclusions

- Both assays have the advantages of **CE marking, automation, standardised reagents** and having broad dynamic ranges over several log10 values.
- Comparing the results the XY scatter plot showed a good correlation between the two systems.
- Bland–Altman analysis also demonstrated that the difference between the two assays was low, and that overall the artus HIV-1 QS-RGQ test did not differ significantly from the Siemes kPCR assay.
- 3 samples exhibited inhibition with the artus HIV-1 QS-RGQ test
- The new docked QIAsymphony SP/AS platform increases the automation of QIAGEN artus real-time PCR assays.
- An advantage of the QIAsymphony SP/AS system is that as an open platform, in-house real time PCR assays can also be set up and a variety of different extraction protocols and sample types can be performed

Implications for clinical care

Caution is required when extrapolating findings obtained with older VL assay to populations monitored with current assays

A novel sample may be more informative than the repetition of the same sample from the same patient

What are the causes of low-level HIV-1 RNA detection in plasma during ART?

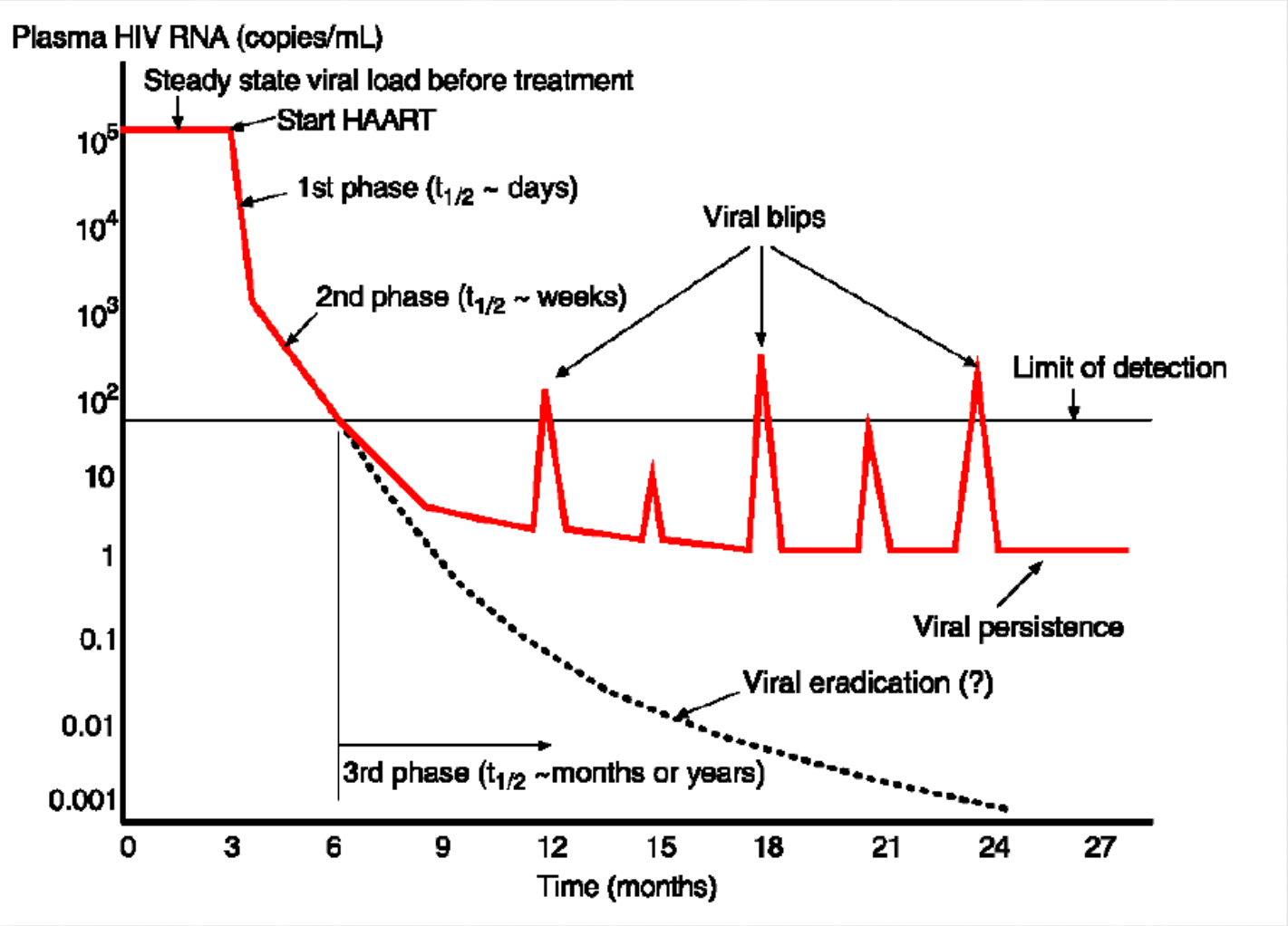
Virus-related?

Drug-related?

Patient-related?

Technique-related?

What are the causes of low-level HIV-1 RNA detection in plasma during ART?

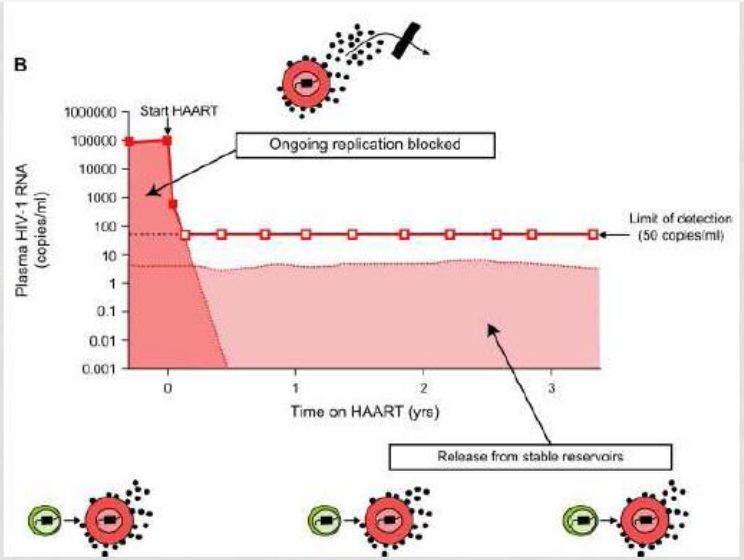


ART intensification* does not affect residual viraemia
***with EFV, ATV/r, LPVr, RAL (x4), MVC (x2), or T20**

Rong, PLoS Comp Biol 2009

What are the causes of low-level HIV-1 RNA detection in plasma during ART?

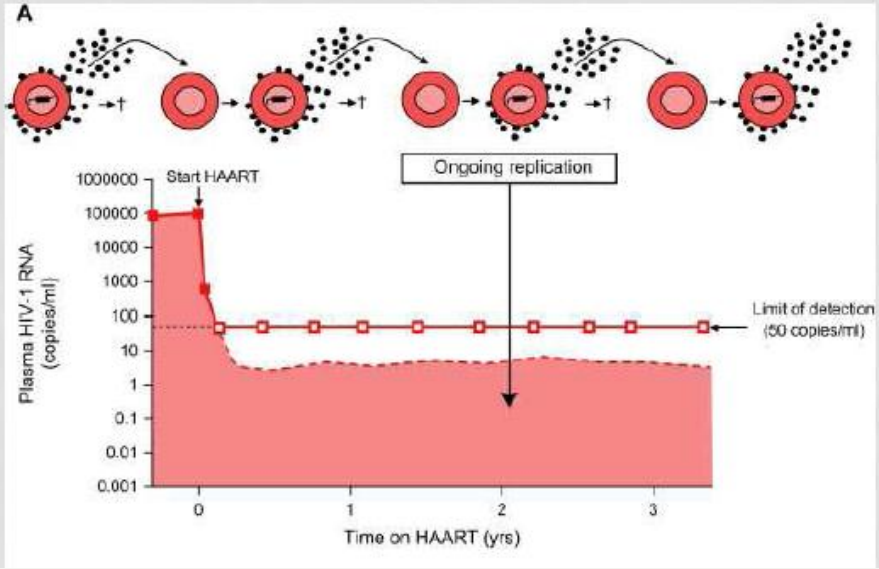
Virus reactivation in latently infected cells in response to antigenic stimulation, with presence of ART ensuring that new cells cannot be productively infected



Shen, J Allergy Clin Immunol 2008

What are the causes of low-level HIV-1 RNA detection in plasma during ART?

Ongoing virus replication in sanctuary cellular or body compartments due to poor drug penetration or activity



The other view: Residual viraemia during ART reflects ongoing virus replication

Adding ABC to EFV+IDV in patients with a VL <50 but >2.5 cps/ml lowers the VL <2.5 cps/ml

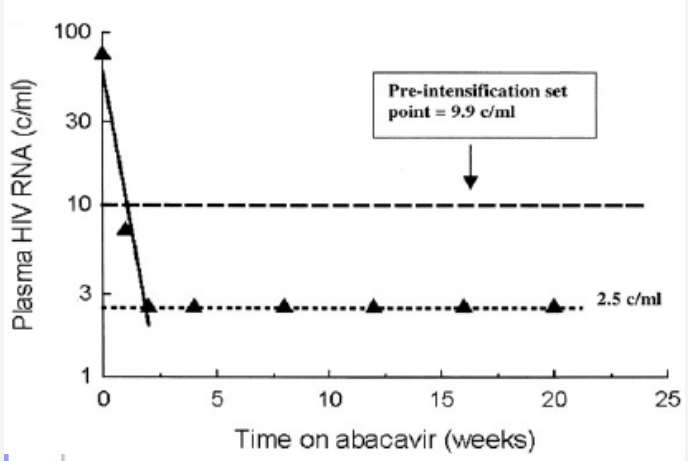
RNA levels increase gradually before rebounding >50 cps/ml after simplifying triple ART to ATV/r Monotherapy

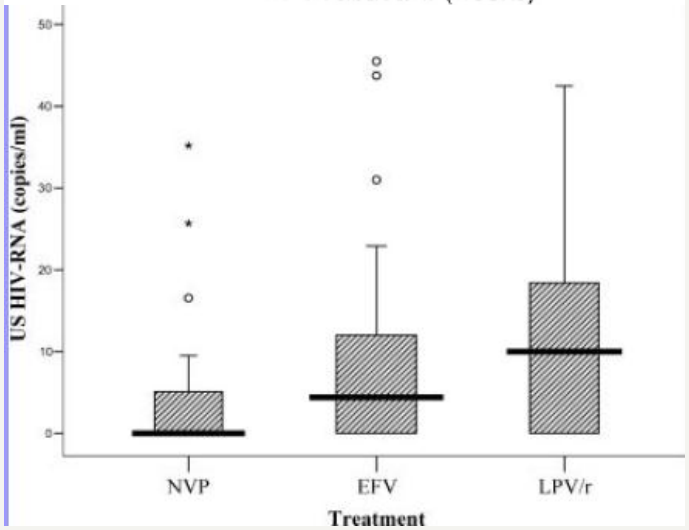
HIV-1 genetic evolution occurs at RNA levels >6.5 cps/ml

The 3rd drug in triple ART is associated with residual RNA levels in patients with VL <50 cps/ml

IVIG transiently decrease residual viraemia

Some papers but not alla show viral evolution despite suppression





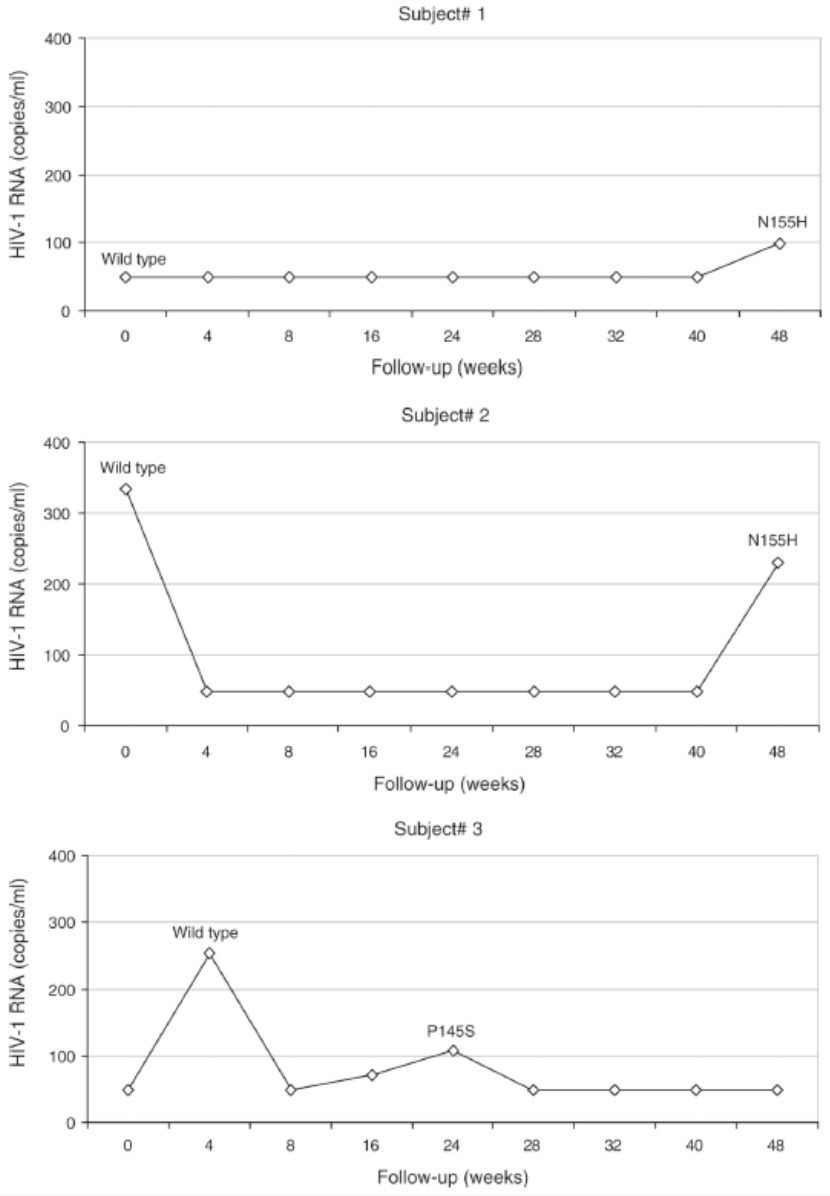
Doyle 2012

Exploring the clinical significance of plasma RNA detection <50 cps/ml

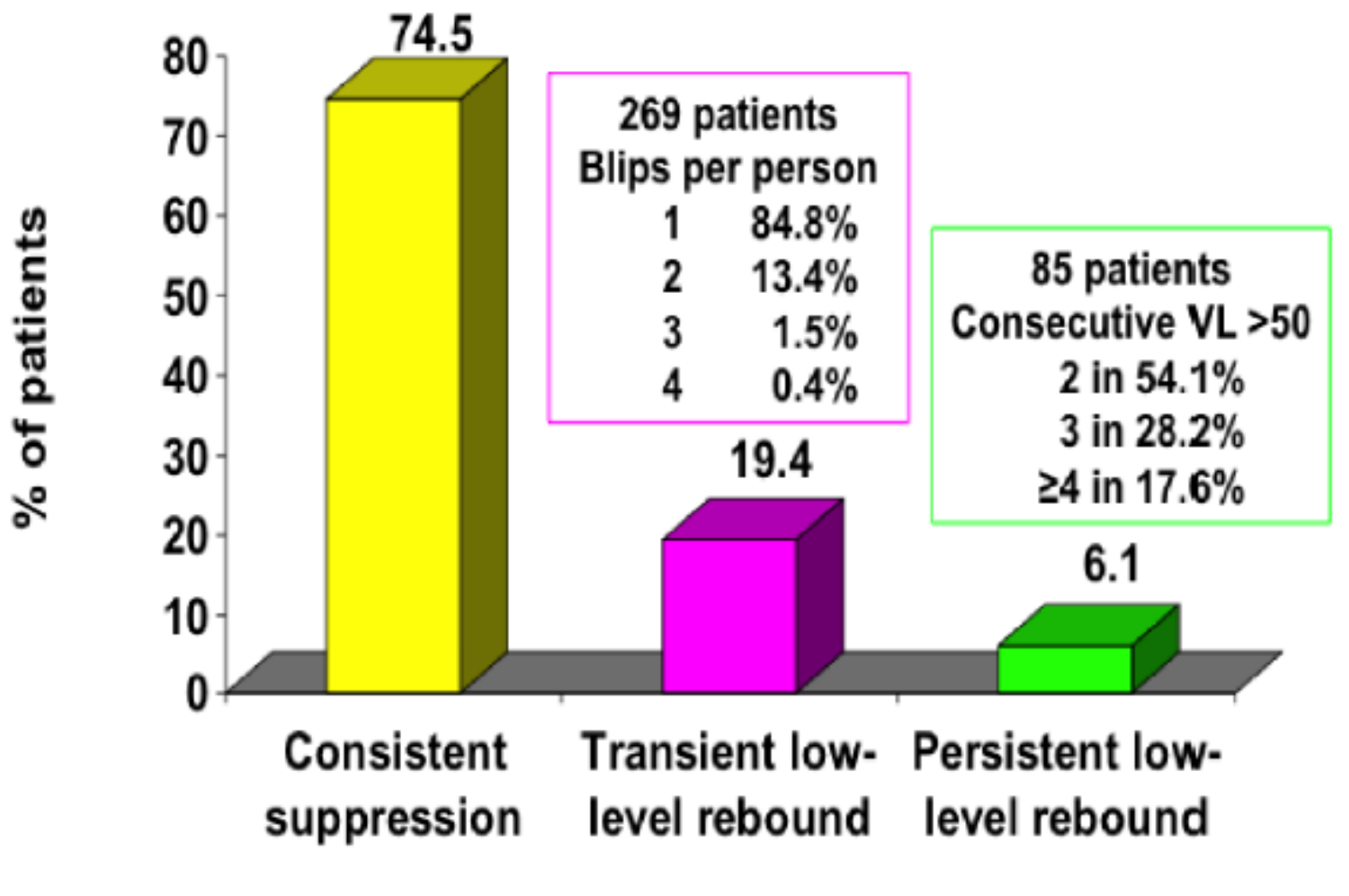
AIDS. 2011 March 13; 25(5): 665–669. doi:10.1097/QAD.0b013e3283445834.

Emerging integrase inhibitor resistance mutations in raltegravir-treated HIV-1-infected patients with low-level viremia

Sébastien Gallien[a], **Constance Delaugerre**[b], **Isabelle Charreau**[c], **Joséphine Braun**[c], **Thomas Boulet**[c], **Aurélie Barrail-tran**[e], **Nathalie de Castro**[d], **Jean-Michel Molina**[d], and **Daniel R. Kuritzkes**[a]



Viral loads in the HAART era



Geretti, Antiv Ther 2008

HIV-1 DNA persistence during ART

A high HIV-DNA load was associated with **faster progression** in patients with primary HIV infection and the **risk of clinical progression** was predicted with more accuracy by HIV-DNA quantification than by other makers (including CD4+ cell counts)

Pre-ART cellular **HIV-1 DNA levels** predict the detection of residual viraemia during suppressive ART also in naïve patients

In some studies, Pre-ART cellular **HIV-1 DNA load and residual HIV-1 RNA** levels also predict episodes of HIV-1 RNA elevation >50 cps/ml during ART or in patients that simplifyed therapy

Goujard,C. 2006
Kostrikis,L.G. 2002
Rouzioux,C. 2005
Hatzakis,A.E. 2004

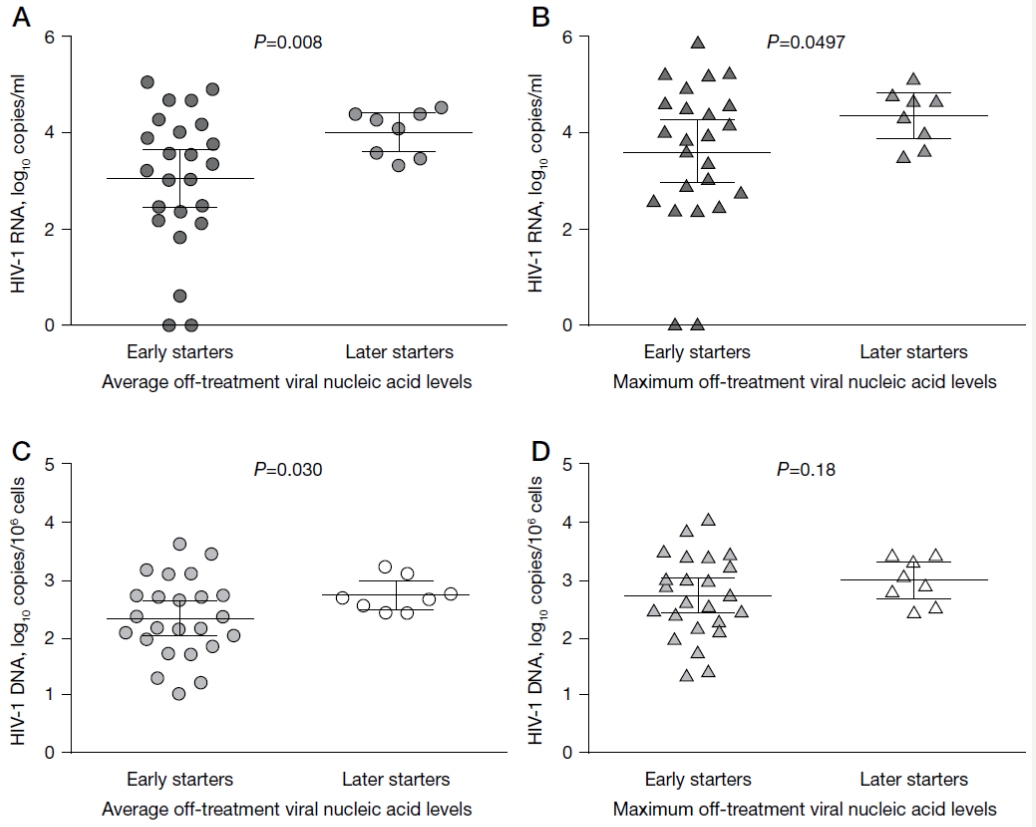
TW Chun, JID 2011
Parisi SG, J Clin Microbiol 2011
Sarmati L, J Med Virol 2007

Original article

Effect of early antiretroviral therapy during primary HIV-1 infection on cell-associated HIV-1 DNA and plasma HIV-1 RNA

Sara Gianella[1,2†], Viktor von Wyl[1†], Marek Fischer[1†], Barbara Niederoest[1], Manuel Battegay[3], Enos Bernasconi[4], Matthias Cavassini[5], Andri Rauch[6], Bernard Hirschel[7], Pietro Vernazza[8], Rainer Weber[1], Beda Joos[1], Huldrych F Günthard[1], the Swiss HIV Cohort Study*

Figure 3. Post-treatment cessation HIV-1 nucleic acid levels from patients stratified according to the time of ART initiation



(A&B) Plasma viraemia (log_{10} HIV-1 RNA copies/ml). Early starters (antiretroviral therapy [ART] initiation ≤60 days after infection) are displayed by dark grey symbols and later starters (start >60 days after infection) by light grey symbols. **(C&D)** Cell-associated HIV-1 DNA (log_{10} HIV-1 DNA copies/10^6 cells). Early starters are displaye by filled grey symbols and later starters by open symbols. Circles show mean setpoints and triangles show maximum off-treatment measurements. *P*-values indicate t results of the Student's *t*-test using Welch's correction for unequal variance. Error bars show means and their 95% CIs.

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

Lack of correlation between the size of HIV proviral DNA reservoir and the level of immune activation in HIV-infected patients with a sustained undetectable HIV viral load for 10 years

Isabelle Poizot-Martin[a,*], Olivia Faucher[a], Véronique Obry-Roguet[a], Corinne Nicolino-Brunet[b], Sylvie Ronot-Bregigeon[a], Françoise Dignat-George[b], Catherine Tamalet[c]

Retrospective cross sectional study included 62 patients with a median duration of undetectable pVL of 10.3 years.

The patients were separated into two groups: 27 non-blippers (sustained pVL < threshold value during all the visits) and 35 blippers (≥1 episodes of pVL > threshold but <1000 copies/ml).

DNA reservoir & immune activation

Table 1
Baseline characteristics and history of antiretroviral treatment for all 62 HIV1-infected patients and according to HIV RNA level during the study period.

Characteristics	All *N*/median (*n* = 62)	Non-blippers (*n* = 27)	Blippers (*n* = 35)	*p*
Male/female, *n*/*n*	40/22	16/11	24/11	0.447
Age, y [IQR]	46 [42–52]	45 [42–51]	47[42–54]	0.624
HIV transmission risk group, *n* (%)				0.997
MSM	15 (24.2)	6 (22.2)	9 (25.7)	
Heterosexual contact	18 (29)	8 (29.6)	10 (28.6)	
Injection drug use	19 (33.3)	8 (29.6)	11 (31.4)	
Other/unknowm	10 (16.1)	5 (18.5)	5 (14.3)	
Hepatitis C co-infection (PCR+), *n* (%)	23 (33.8)	9 (33.3)	7 (20.0)	0.234
Duration of follow up of HIV infection, y [IQR]	15.1 [11.8–15.9]	13.7 [10.7–21.3]	16.8 [12.7–20.9]	0.287
AIDS diagnosis before LTIT, *n* (%)	15 (24.2)	4 (14.8%)	11 (31.4%)	0.130
HAART				
ART exposure prior LTIT, y [IQR]	2.8 [1.0–4.5]	3.2 [1.8–4.6]	2.6 [0.8–4.7]	0.386
Number of treatments [IQR]	1 [0–3]	1 [0–2]	2 [1–3]	0.117
First-line therapy, *n* (%)	16 (25.8)	12 (44.6)	6 (17.1)	0.019
Time to viral suppression, mo [IQR]	3 [1.8–4.8]	2.8 [1.6–3.7]	3.8 [1.9–5.5]	0.231
Duration of viral suppression, y [IQR]	10.3[9.5–10.9]	9.9 [9.3–10.6]	10.7 [9.7–11.1]	0.023
LTIT, *n* (%)				
PI-based cART	42 (67.7)	14 (51.9)	28 (80.0)	0.093
NNRTI-based cART	9 (14.5)	5 (18.5)	4 (11.4)	
3 NRTI	5 (8.1)	4 (14.8)	1 (2.9)	
2 NRTI	6 (9.7)	4 (14.8)	2 (5.7)	
Others[a]				
Nadir $CD4^+$ T, cells/μl [IQR]	202 [52–287]	233 [149–342]	178 [55–275]	0.277
At initiation of LTIT				
$CD4^+$ T, cells/μl [IQR]	260 [150–446]	348 [163–472]	227 [141–336]	0.110
CD4 > 500, cells/μl (%)	4 (6.5)	4 (18.2)	0	–
$CD8^+$ T, cells/μl [IQR]	884 [499–1350]	839 [531–1286]	1050 [463–1489]	0.518
CD4: CD8 ratio [IQR]	0.3 [0.20–0.48]	0.42 [0.21–0.60]	0.27 [0.19–0.42]	0.075
CD4: CD8 ratio > 1 (%)	1 (1.6)	1 (4.5)	0	0.431
HIV RNA level, log/ml [IQR]	4.13 [2.09–4.57]	3.87 [2.79–4.75]	4.17 [3.20–4.52]	0.884

Data are median (range) or number (%); LTIT, long term inhibition treatment; patients were stratified according to HIV RNA level during the study period: the non-blippers patients had pVL under the threshold value at all time points. The blipper-patients had ≥1 episode of viremia over threshold value but less than 1000 copies/ml.

[a] Others: 1NRTI+1PIr: *n* = 1; 1NRTI+1PI+1NNRTI: *n* = 2; patient groups were compared by use Chi-square and Mann–Whitney *U* tests *p* values > 0.05 were NS.

DNA reservoir & immune activation

Table 3
Analysis of the proportion of functional markers among $CD4^+$ T cells and activation markers among $CD8^+$ T cells: (a) blippers versus non-blippers; (b) according to IC HIV DNA level.

(a)

Markers	Non-blippers ($n=27$) % [IQR]	Blippers ($n=35$) % [IQR]	p
$CD4^+45RA^+62L^+/CD4^+$	41.8 [29.3–51.1]	39 [32.8–50.1]	0.723
$CD4+CD28^+/CD4^+$	95.96 [88.45–99.93]	96.61 [93.60–98.89]	0.584
$CD4^+DR^+/CD4^+$	6.42 [5.06–8.77]	6.42 [5.06–8.77]	0.265
$CD8^+DR^+/CD8^+$	15.1 [9.31–21.27]	21.08 [14.42–30.44]	0.036
$CD8^+CD38^+/CD8^+$	15.72 [11.06–27.42]	21.53 [16.11–27.34]	0.095

(b)

Markers	IC HIV DNA < 20 copies/10^6 PBMCs ($n=18$)	IC HIV DNA ≥20 copies/10^6 PBMCs ($n=44$)	p
$CD4^+45RA^+62L^+/CD4^+$	45.9 [38.6–51.3]	37.6 [29.3–49.4]	0.068
$CD4^+CD28^+/CD4^+$	98.04 [94.12–99.99]	95.71 [91.31–98.49]	0.128
$CD4^+DR^+/CD4^+$	6.47 [5.80–8.09]	7.06 [5.60–12.22]	0.299
$CD8^+DR^+/CD8^+$	19.78 [14.79–31.92]	18.45 [13.32–27.19]	0.816
$CD8^+CD38^+/CD8^+$	17.58 [12.80–25.58]	17.16 [11.06–28.52]	0.889

Results: The median IC HIV DNA rate was 34 copies/106 PBMCs (71% ≥20 copies/106 PBMCs) with no significant difference between the groups.

The proportion of CD8+CD38+ and CD8+DR+ T cells was higher in blipper patients.

No correlation was found between markers of immune activation on CD4+ and CD8+ T cells and the IC HIV-DNA level.

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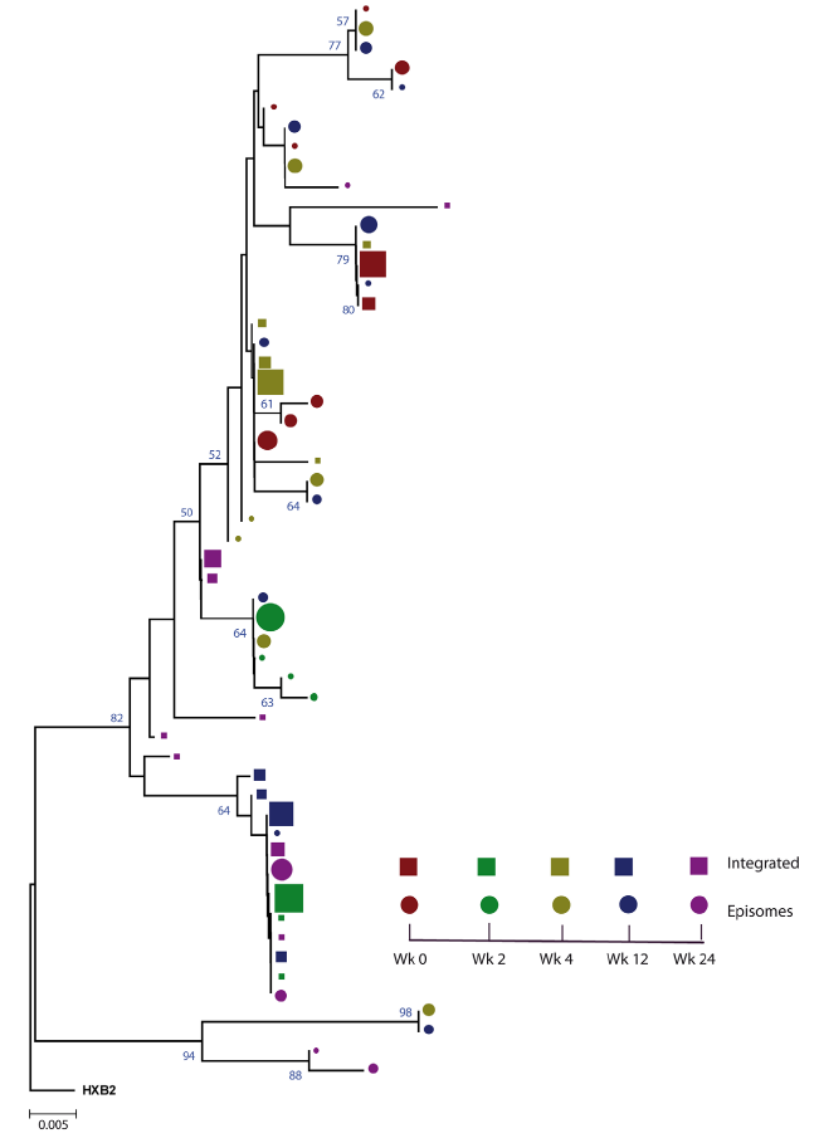
Deep Molecular Characterization of HIV-1 Dynamics under Suppressive HAART

Maria J. Buzón[1☯a], Francisco M. Codoñer[1☯b], Simon D. W. Frost[2], Christian Pou[1], Maria C. Puertas[1], Marta Massanella[1], Judith Dalmau[1], Josep M. Llibre[3], Mario Stevenson[4], Julià Blanco[1], Bonaventura Clotet[1,3], Roger Paredes[1,3], Javier Martinez-Picado[1,5*]

Although phylogenies showed that both DNA forms were intermingled within the phylogenetic tree, compartmentalization between episomal and proviral DNA samples exist and suggest that they belong to different viral populations.



The need for ultrasensitive determinations in HIV-1 diagnostics

- ✓ *Virological rebound in human immunodeficiency virus-infected patients with or without residual viraemia: results from an extended follow-up*

Methods

- At HSR, bDNA (limit of quantification 50 HIV RNA copies/mL) used up to February 2009
- Since March 2009, all patients routinely tested by kPCR
- kPCR assay gives three possible outputs:
  - A quantitative result for HIV RNA >37 copies/mL
  - A semi-quantitative result for HIV RNA between 1 and 37 copies/mL
  - A qualitative result ('undetectable') if HIV RNA is below the limit of quantification (1 copy/mL).

Patients selection

- Patients included in this analysis if last 4 consecutive HIV RNA values were below 50 copies/mL, that is
  - two consecutive HIV RNA viral loads (VLs) of <50 copies/mL as tested by bDNA, *followed by*
  - two consecutive HIV RNA VLs of <50 copies/mL by kPCR.
- Two patient groups were identified on the basis of the kPCR results:
  - patients with an HIV RNA load of <1 copy/mL confirmed in two consecutive samples (group A)
  - patients with residual viraemia, defined as an HIV RNA load of <1 copy/mL in one sample and not in the other or two HIV RNA values of between 1 and 49 copies/mL (group B).

Gianotti N, et al. J Antimicrob Chemother. 2012 Jan;67(1):213-7.

Statistical analysis

- Primary analysis: time to virological rebound (Kaplan–Meier method)
  - curves of groups A and B compared by the log-rank test.
- Patients who changed any of the antiretroviral drugs in their regimen during follow-up while their HIV RNA load was <50 copies/mL were censored at the time of the switch.

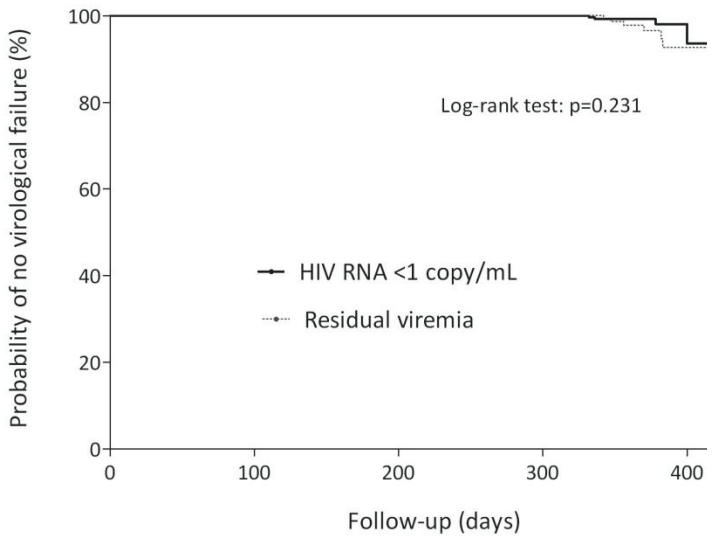
Baseline characteristics

Gianotti N, et al. J Antimicrob Chemother. 2012 Jan;67(1):213-7.

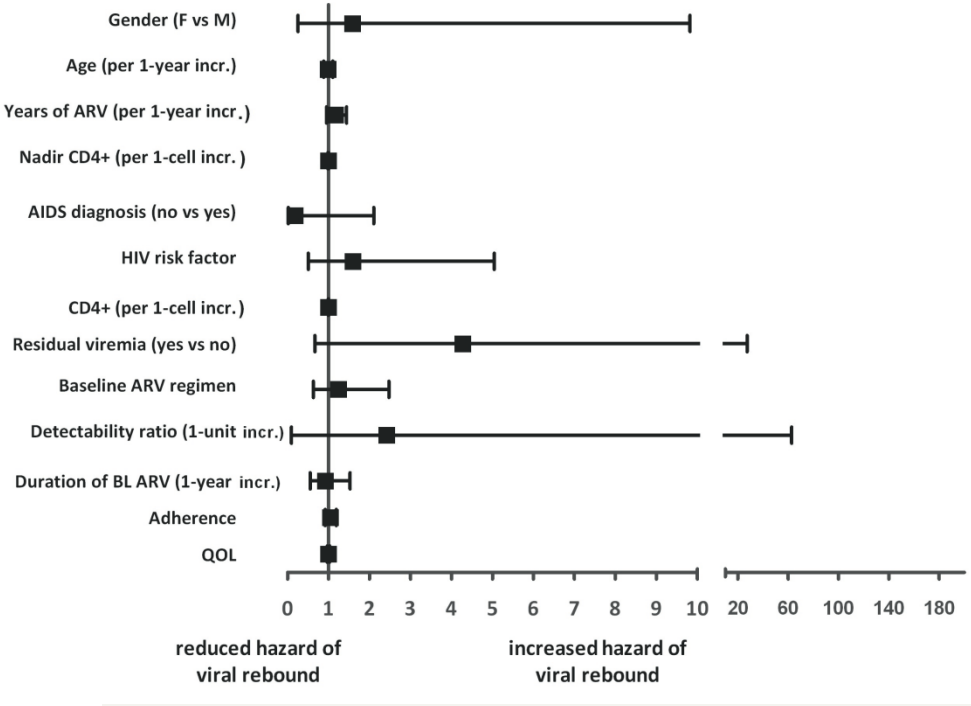
Table 1. Demographic and clinical characteristics of the study subjects at baseline

	Overall ($n=739$)	HIV RNA <1 copy/mL ($n=446$)	Residual viraemia ($n=293$)	P value
Gender, *n* (%)				0.012[a]
male	569 (77.0)	329 (73.8)	240 (81.9)	
female	170 (23.0)	117 (26.2)	53 (18.1)	
Median age, years (Q1, Q3)	46.6 (42.2, 51.5)	46.5 (42.1, 51.5)	46.7 (42.6, 51.4)	0.728[b]
AIDS diagnosed before baseline, *n* (%)	168 (22.7)	99 (22.1)	69 (23.5)	0.720[a]
Hepatitis C virus infection, *n* (%)				0.692[a]
yes	218 (29.5)	133 (29.8)	85 (29.0)	
no	440 (59.5)	261 (58.5)	179 (61.1)	
unknown	81 (11.0)	52 (11.7)	29 (9.9)	
Median nadir CD4+ count, cells/μL (Q1, Q3)	213 (107, 297)	213 (108, 300)	214 (107, 290)	0.772[b]
Median CD4+ count at first kPCR, cells/μL (Q1, Q3)	528 (373, 716)	515 (372, 701)	552 (374, 734)	0.340[b]
Median DR (Q1, Q3)	0.42 (0.20, 0.65)	0.37 (0.17, 0.58)	0.50 (0.24, 0.71)	<0.0001[b]
Median duration of antiretroviral treatment, years (Q1, Q3)	11.1 (5.3, 13.9)	11.0 (5.7, 13.8)	11.3 (4.7, 14.0)	0.699[b]
Median duration of baseline antiretroviral therapy, years (Q1, Q3)	1.41 (0.61, 2.9)	1.38 (0.59, 3.03)	1.44 (0.67, 2.66)	0.896[b]

One-year results



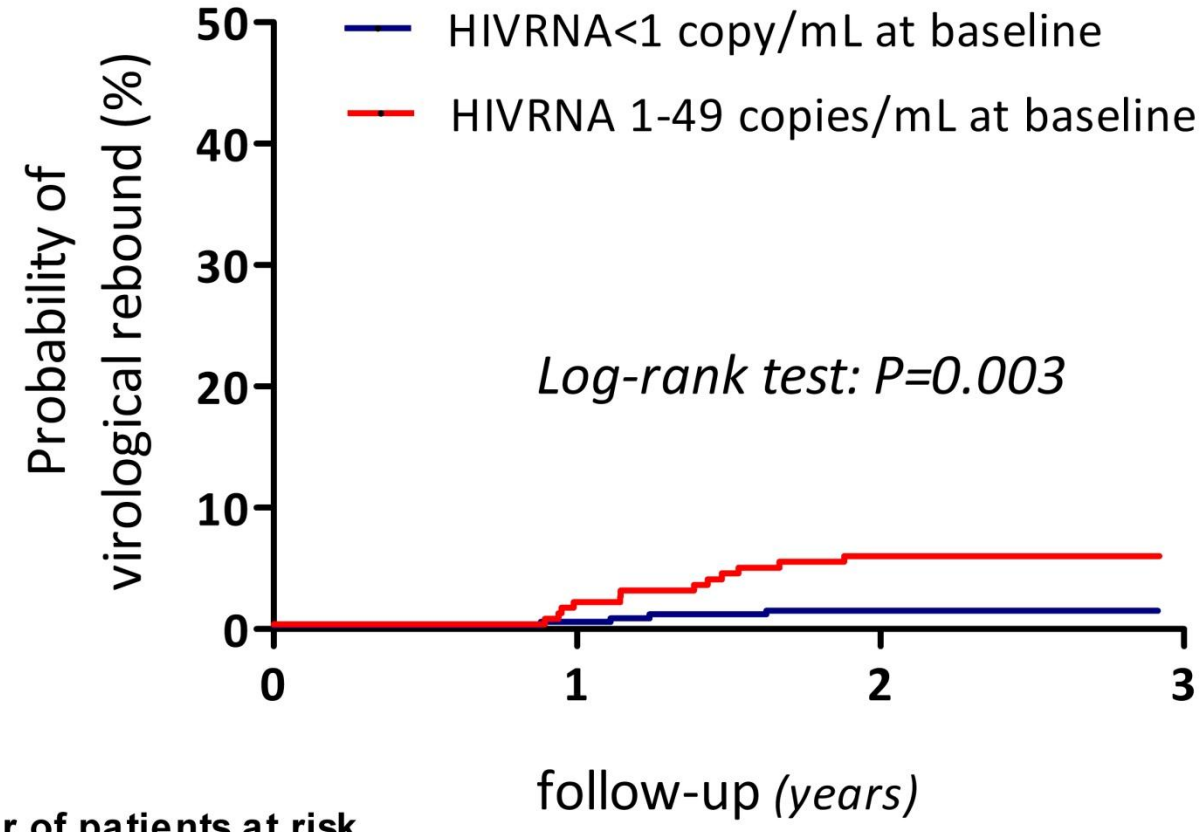
Number of patients at risk					
HIV RNA <1 copy/mL	446	437	430	335	22
Residual viremia	293	290	284	229	15



The adjusted CD4+ change [median (Q1, Q3)] in group A was +21.2 (22.5, 53.2) cells/µL/year and +14.3 (27.7, 43.9) cells/µL/year in group B (p=0.036)

Gianotti N, et al. J Antimicrob Chemother. 2012 Jan;67(1):213-7.

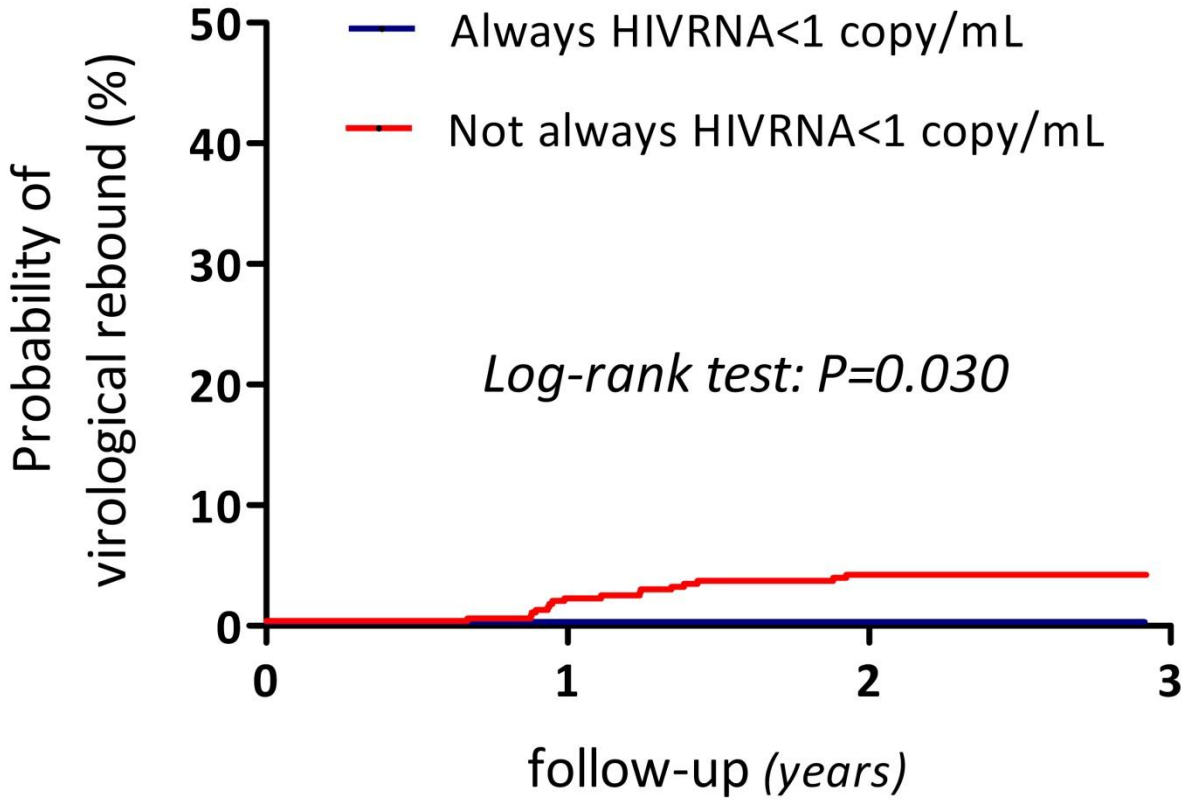
Results (extended follow-up)



Number of patients at risk

<1 copy/mL	***446***	***325***	***306***	***260***
1-49 copies/mL	***293***	***210***	***190***	***163***

Results (extended follow-up)



Number of patients at risk

Always <1 copy/mL	***168***	***122***	***116***	***99***
Not always <1 copy/mL	***571***	***419***	***392***	***335***

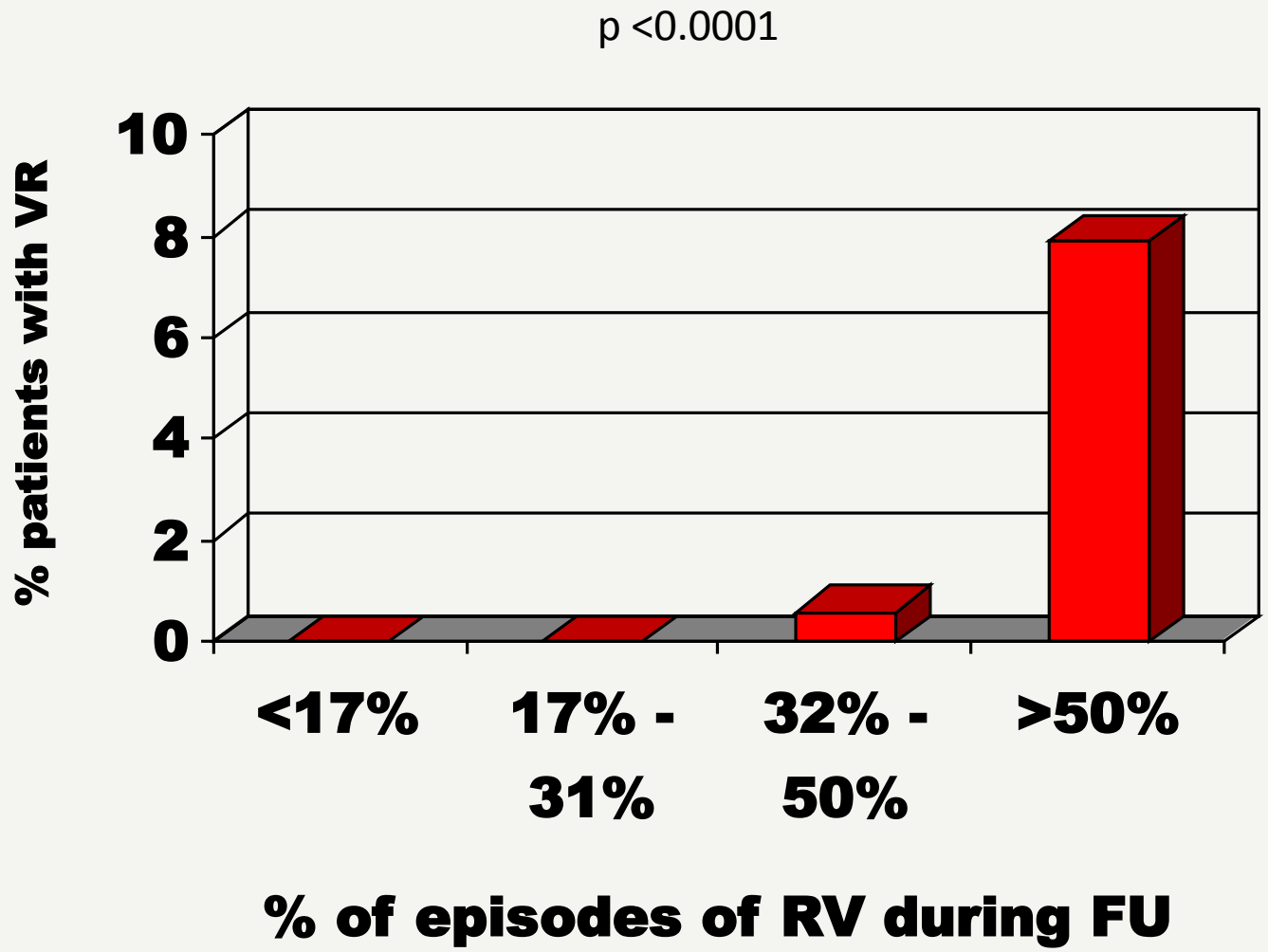
Proportional hazard Cox regression model: dependent variable = virological rebound through133 weeks

Gianotti et al, CMI, 2013

Independent variable	HR	95% CI	p
RV vs <1 copy/mL at BL	**4.829**	**1.355-17.216**	**0.015**
Gender (M vs F)	0.338	0.079-1.455	0.145
Age (<50 yrs vs ≥ 50 yrs)	5.117	0.908-28.842	0.064
Years of ARV therapy (per 5-years increments)	**1.137**	**1.007-1.283**	**0.038**
Duration of last ARV regimen (per 1-year increments)	0.960	0.645-1.430	0.841
Detectability ratio up to BL	**182.358**	**6.871-4839.491**	**0.002**
CD4+ nadir (≤200 vs >200 cells/μL)	**5.193**	**1.364-19.770**	**0.015**
HIV stage C3 (no vs yes)	3.023	0.594-15.374	0.183
IVDU vs UKN/other	1.149	0.187-7.047	0.880
Heterosexual vs UKN/other	1.706	0.287-10.132	0.557
MSM vs UKN/other	5.419	0.915-32.086	0.063
BL CD4+ (per 100 cells/μL increments)	1.000	0.988-1.002	0.963
NNRTI-based vs PI/r-based regimen at BL	1.803	0.289-11.234	0.528
Unboosted-PI vs PI/r-based regimen at BL	2.838	0.748-10.769	0.125
New drugs-based* vs PI/r-based regimen at BL	**0.080**	**0.008-0.834**	**0.035**
** Mostly RAL+ETR+MRV or DRV/r*			

VR according to the proportion of episodes of RV

Gianotti et al, CMI, 2013



The need for ultrasensitive determinations in HIV-1 diagnostics

- VR rate still very low, even among patients with RV
- RV favors VR at almost 3 years of FU and RV confers roughly 5-fold risk of VR
- The risk of VR increases with **increasing episodes of RV** (>50% = highest risk)
- The **detectability ratio** had the highest impact is on the risk of VR suggesting the hypothesis that patients with RV do not have relevant ongoing replication in most cases. (budget/guidelines?)
- The meaning of very low plasma copies is still controversial: can it be **DNA shedding** from reservoir?

HIV-DNA loads, plasma residual viraemia and risk of virological rebound in heavily treated, virologically suppressed, HIV-infected patients.

Nicola Gianotti[1], Filippo Canducci[2,3], Laura Galli[1], Francesca Cossarini[1], Stefania Salpietro[1], Andrea Poli[1], Silvia Nozza[1], Vincenzo Spagnuolo[1], Massimo Clementi[2,4], Michela Sampaolo[2,4], Elisa Rita Ceresola[2,4], Sara Racca[2], Adriano Lazzarin[1,4] and Antonella Castagna[1]

1) Malattie Infettive, IRCCS San Raffaele, Milano
2) Microbiologia e Virologia, IRCCS San Raffaele, Milano
3) Dipartimento di Medicina Clinica e Sperimentale, Università dell'Insubria, Varese
4) Università Vita-Salute San Raffaele, Milano

In press……

Is HIV-DNA more informative than ultrasensitive assays to measure residual viremia (RV) with regard to the risk of VR ?

Aims

- to investigate whether HIV-DNA is a useful tool to predict virological rebound (VR) in treatment –experienced patients who attained <50 HIV-RNA copies/mL under cART

- to investigate whether RV is associated with higher loads of HIV-DNA also in patients with history of virological failure

Methods

- Single-center, retrospective study
- Patients on antiretroviral therapy (cART), with
  - HIV-RNA<50 copies/mL
  - history of virological failure
  - clade B HIV
  - samples for HIV-DNA and plasma viral load measurement collected on the same day

Methods

- **HIV-RNA** was quantified by Versant kPCR (Siemens Diagnostics; limit of detection: 1 copy HIV RNA/mL)
- RV was defined by HIV-RNA values of 1-49 copies/mL

- **HIV-DNA** was extracted from $1x10^6$ PBMCs and quantified by Real Time PCR with wo amplification protocols targeting two distinct regions of the viral genome were amplified:
  - ***HIV-1 pol*** (*method 1 = SIVIM consensus, De Rossi et al. 2010*)
  - ***HIV-1 LTR*** *(method 2, Chun et al. 2011)*
  - ***Unintegrated 2LTR HIV-DNA*** *(unintegrated, Sharkey 2011)*

(Main) Demographic and clinical characteristics at the time of first paired determination of HIV-RNA and HIV-DNA among study subjects

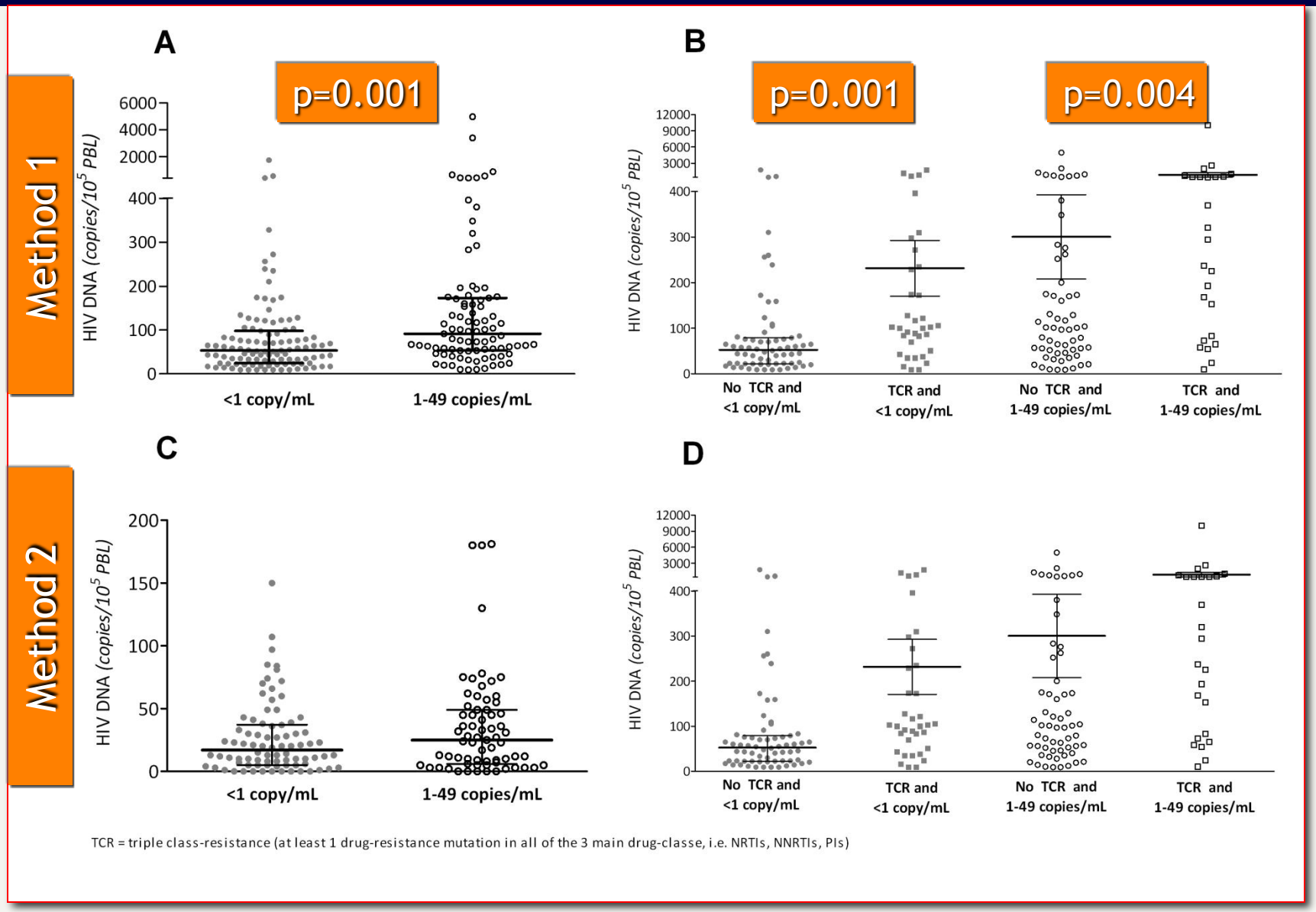
	OVERALL (n=194)	*HIV RNA < 1copy/mL* (n=104)	*HIV RNA 1-49 copies/mL* (n=90)	*P value*
Previous AIDS diagnosis, *n(%)*	46 (24%)	23 (22%)	23 (26%)	0.614
Median nadir CD4+ count, *cells/mm^3 (Q1, Q3)*	164 (57-264)	149 (50-277)	166 (71-260)	0.581
Median CD4+ count, *cells/mm^3 (Q1, Q3)*	489 (357-640)	502 (362-742)	479 (356-624)	0.461
Median detectability ratio *(Q1, Q3)*	0.63 (0.45-0.79)	0.61 (0.43-0.76)	0.67 (0.50-0.82)	0.036
Median duration of ART, *years (Q1, Q3)*	13.5 (6.7-16.3)	13.7 (8.6-16.3)	13.5 (6.1-16.8)	0.596
Type of antiretroviral treatment, *n (%)*				0.478
NRTI-based regimen	6 (3%)	4 (4%)	2 (2%)	
NNRTI-based regimen	12 (6%)	9 (9%)	3 (3%)	
PI-based regimen	81 (42%)	43 (41%)	38 (43%)	
New-drugs-based regimen	95 (49%)	49 (47%)	46 (52%)	
At least 1 drug-resistance mutation for, *n(%)*				
NRTIs	135 (73%)	75 (74%)	60 (71%)	0.740
NNRTIs	90 (49%)	52 (52%)	38 (45%)	0.461
PIs	98 (53%)	54 (53%)	44 (52%)	0.999
NRTIs and NNRTIs and PIs	60 (32%)	35 (35%)	25 (30%)	0.530

HIV-DNA load at baseline

	OVERALL	*HIV RNA < 1 copy/mL*	*HIV RNA 1-49 copies/mL*	*P value*
Median HIV-DNA, *copies/10^5 PBL (Method 1)* (n=194)	78 (38-193)	64 (28-120)	109 (53-320)	0.001
Median HIV-DNA, *copies/10^5 PBL (Method 2)* (n = 149)	21 (5-469	17 (5-37)	25 (6-49)	0.225

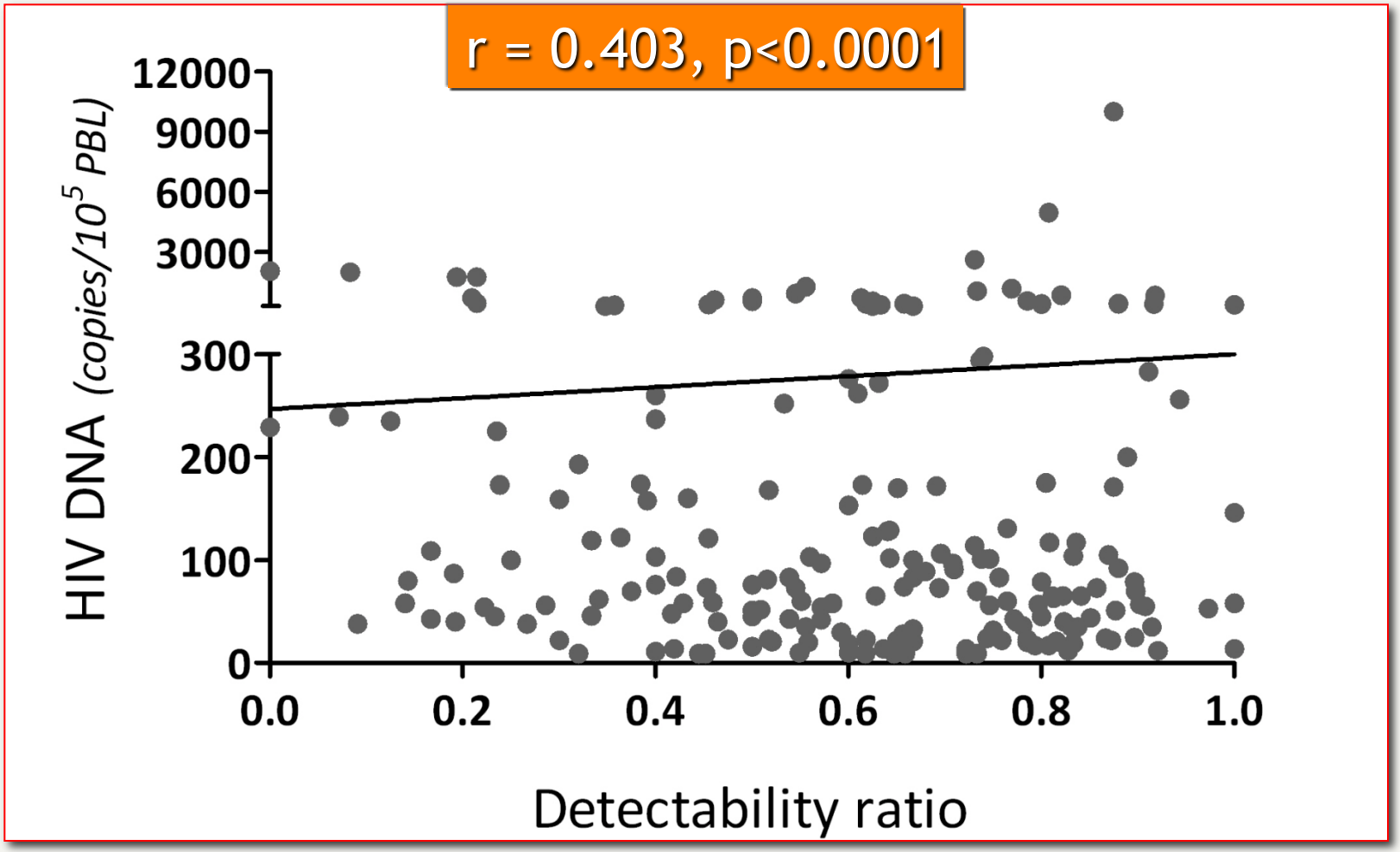
Unintegrated 2LTR DNA was detected only in 3 patients. One experienced VR

Associations between HIV-DNA loads and RV/TCR

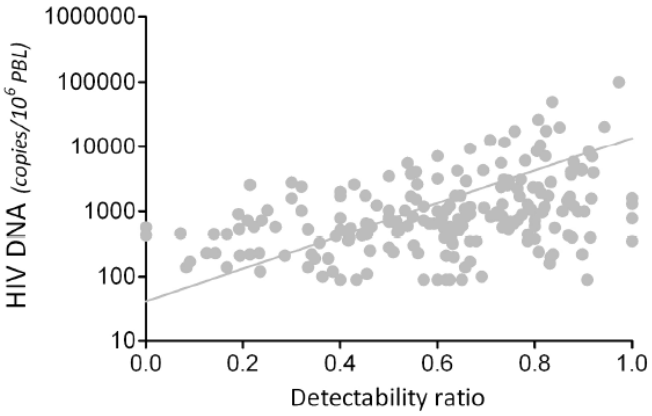


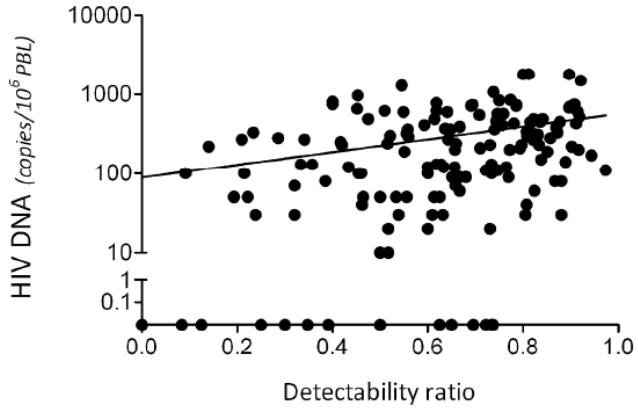
TCR = triple class-resistance (at least 1 drug-resistance mutation in all of the 3 main drug-classe, i.e. NRTIs, NNRTIs, PIs)

Associations between HIV-DNA loads and DR



Associations between HIV-DNA loads and DR

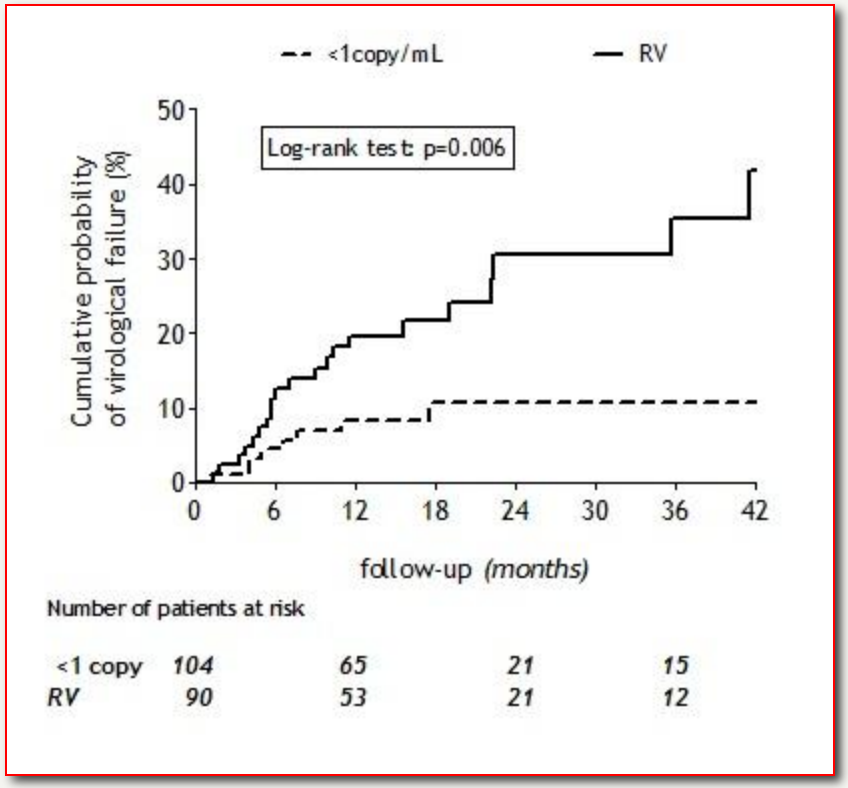


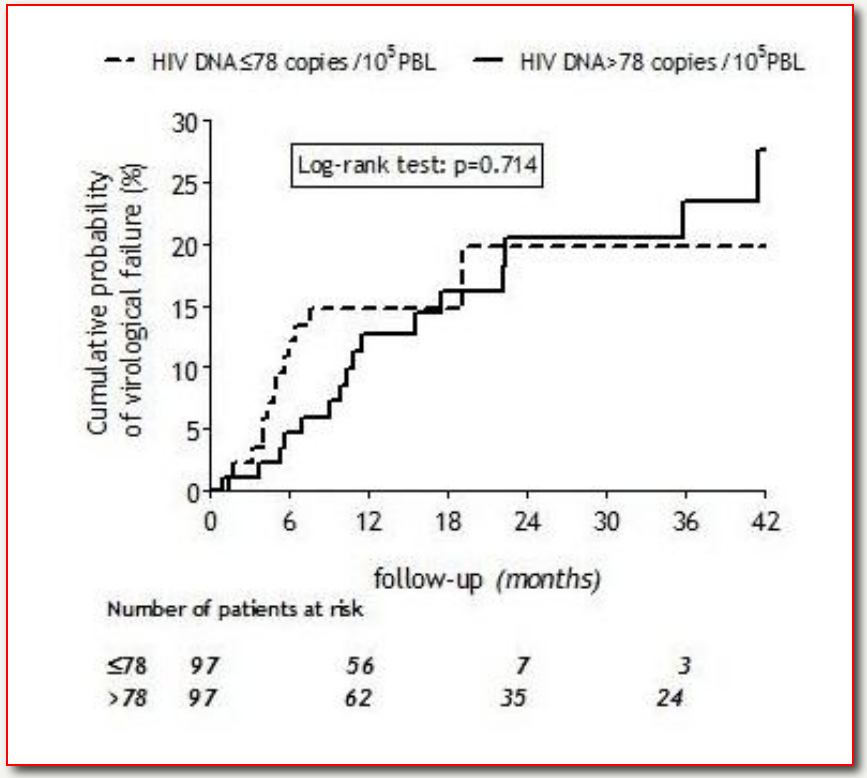


Correlation between HIV-DNA loads and the detectability ratio calculated up to the first paired determination of HIV-RNA and HIV-DNA loads. Panel A: HIV-DNA load measured by the HIV-1 pol method (method 1); panel B: HIV-DNA load measured by the HIV-1 LTR method (method 2).
189x250mm (300 x 300 DPI)

Probability of VR during follow-up according to RV and HIV-DNA loads

Median follow-up = 17.5 (13.5-31.5) months
VR occurred in 29/194 (15%) subjects during follow-up
viral load at failure was 167 (81-1932) HIV-RNA copies/mL





Multivariate Cox proportional hazard model (stepwise regression): influence of demographic and HIV-related characteristics on the hazard of virological rebound

Characteristics	*Model 1 (n=194)*			*Model 2 (n=149)*		
	AHR	**95% CI**	***P value***	**AHR**	**95% CI**	***P value***
Age *(per 5-years older)*			0.890			0.097
Years since first HIV positive test *(per 5-years longer)*	**1.528**	**1.035 - 2.256**	***0.031***			0.099
HCV Infection *(Yes vs No vs Unknown)*			0.534			0.168
Type of antiretroviral treatment			0.254			0.102
Nadir CD4+ cell count *(per 100-cells/µL higher)*	**0.605**	**0.391 - 0.937**	***0.010***	**0.521**	**0.289-0.939**	***0.030***
Previous AIDS diagnosis *(Yes vs No)*			0.081			0.057
Detectability ratio *(per 10% higher)*			0.313			0.289
Current CD4+ cell count *(per 100-cells/µL higher)*			0.666			0.307
Current HIV-RNA *(<1 copy/mL vs 1-49 copies/mL)*	**0.203**	**0.073 - 0.469**	***0.002***			0.090
HIV-DNA Method 1 *(per 20-copies/10^5PBL higher)*			0.139		*Not included in the model*	
HIV-DNA Method 2 *(per 20-copies/10^5PBL higher)*		*Not included in the model*				0.170
Triple-class resistance *(No vs Yes)*			0.462			0.529

Conclusions

- In treatment-experienced patients with undetectable viral load in plasma, **HIV-DNA load is independently and directly associated with RV**

- Nevertheless, in this setting **the risk of viral rebound was not independently associated with HIV-DNA loads**

- **Lack of internationally validated methods** may still limit clinical usage of HIV-1 DNA testing

Limits

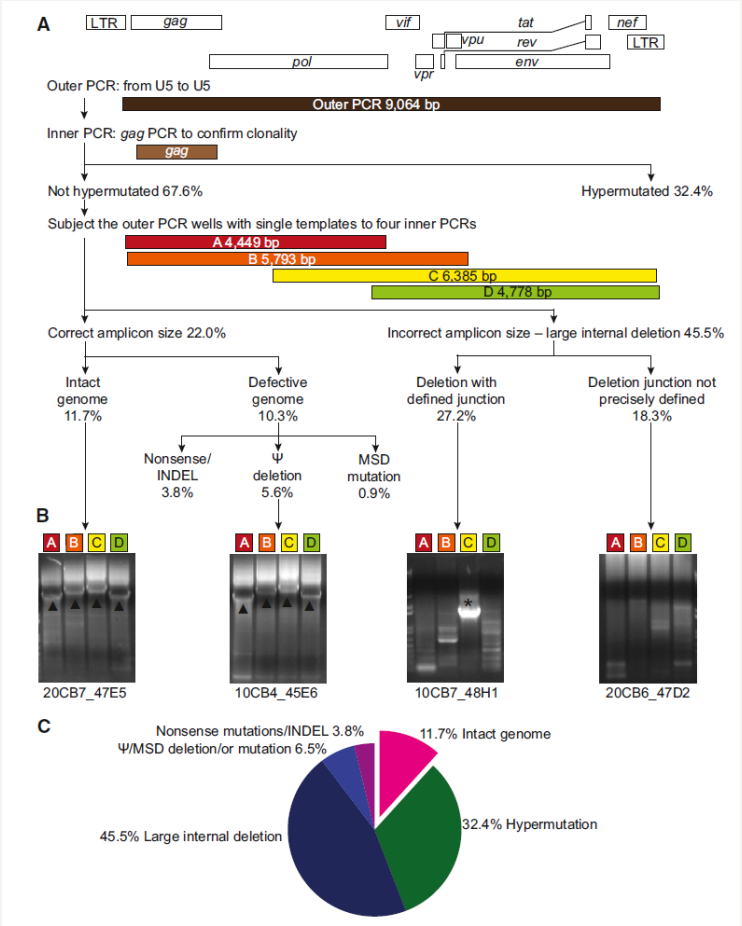
- Retrospective design
  - the similar results observed with two different methods to quantify HIV-DNA loads provide a high consistency to our findings
- Relatively small sample size
  - this sample size was large enough to detect and independent association between RV and VR
  - we are confident about the pre-eminent effect of RV, with respect to HIV-DNA load, on VR in these patients.
- The design of our **study does not enable us to define if RV is the cause or the effect of increased levels of HIV-DNA** or if the two phenomena are simply two "parallel" **consequences of a higher exposure to uncontrolled HIV replication in the previous years** (association with DR

Cell

Replication-Competent Noninduced Proviruses in the Latent Reservoir Increase Barrier to HIV-1 Cure

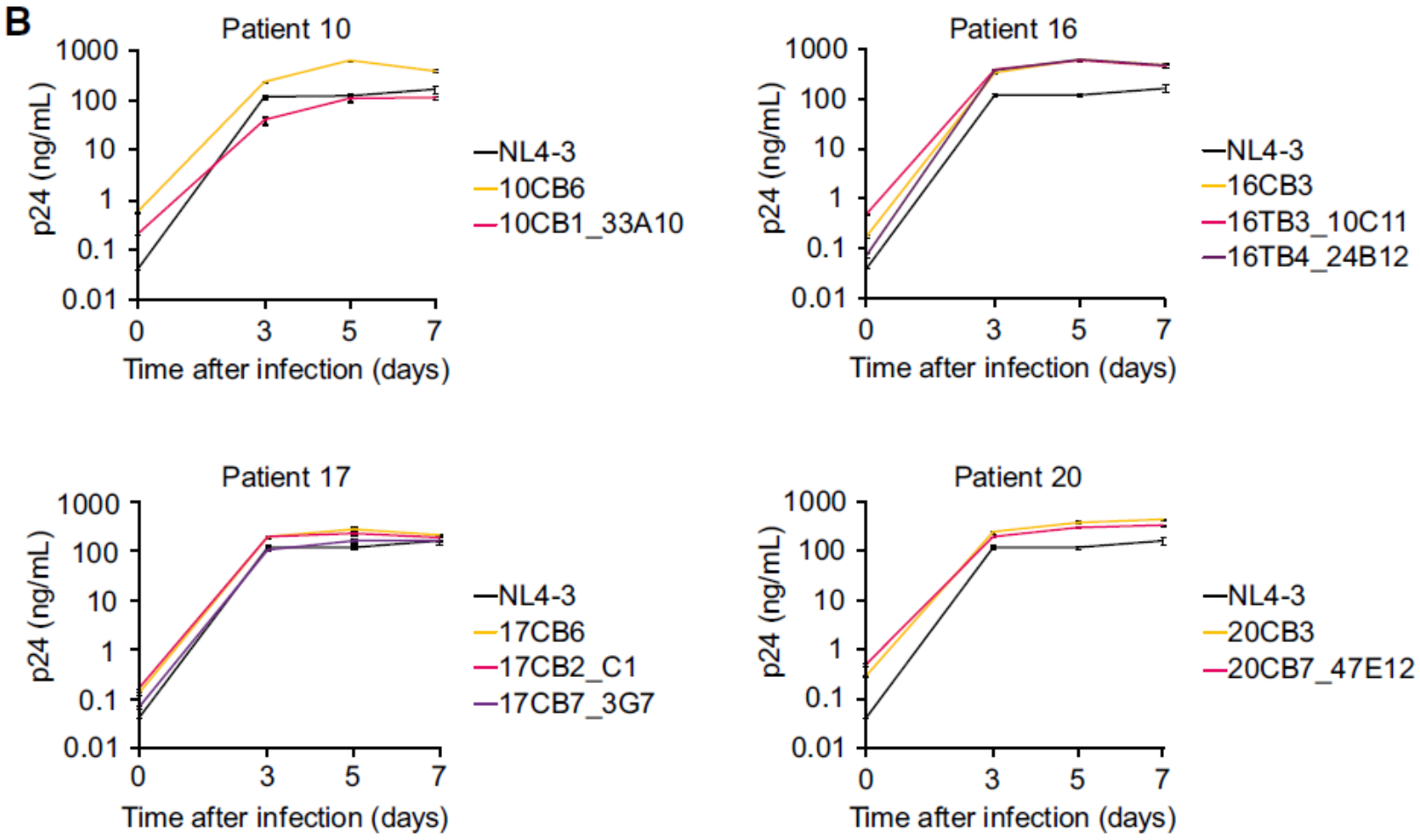
Ya-Chi Ho,[1] Liang Shan,[1,5] Nina N. Hosmane,[1] Jeffrey Wang,[2] Sarah B. Laskey,[1] Daniel I.S. Rosenbloom,[3] Jun Lai,[1] Joel N. Blankson,[1] Janet D. Siliciano,[1] and Robert F. Siliciano[1,4,*]

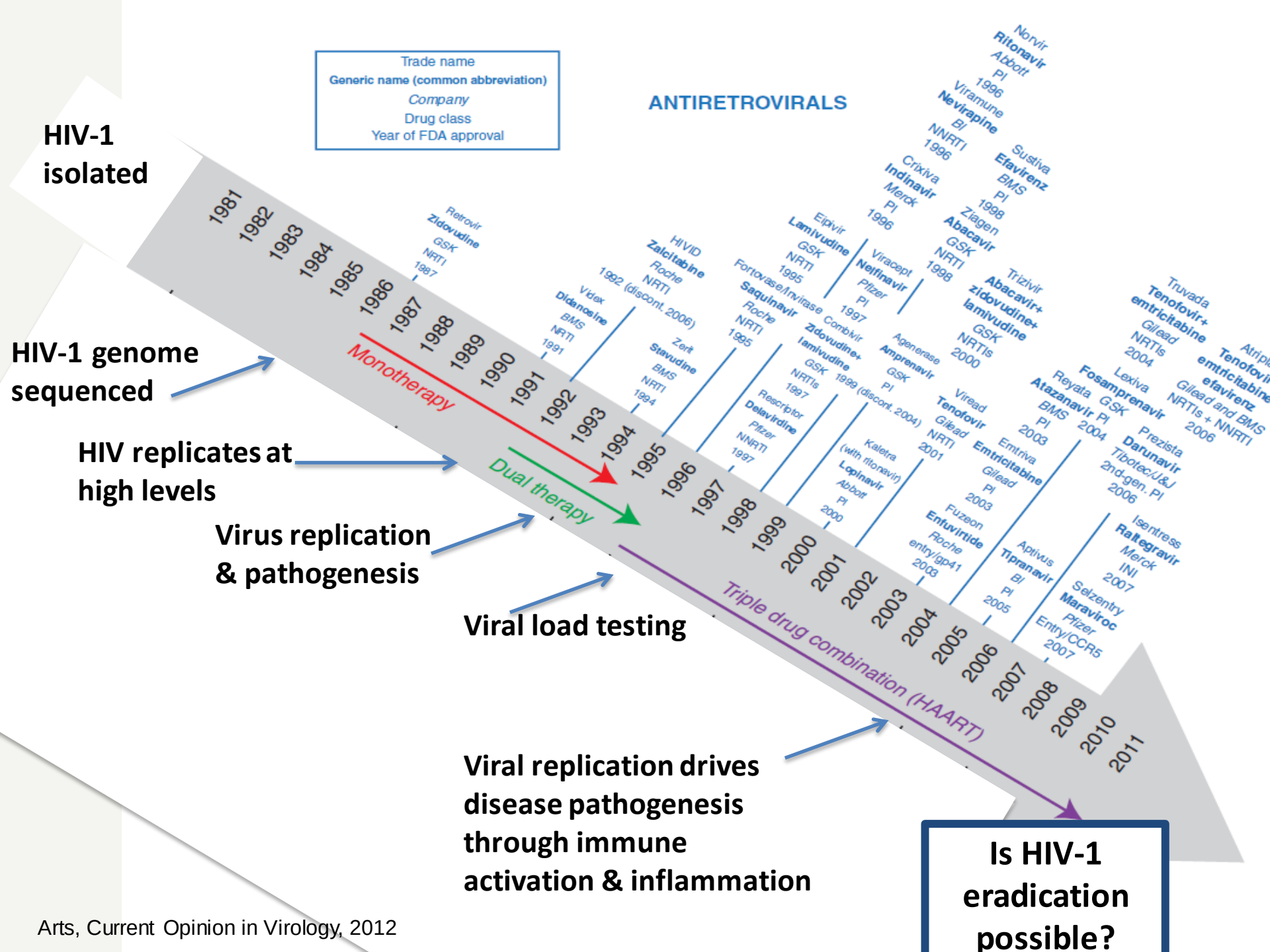
Analysis of 213 noninduced proviral clones from treated patients showed 88.3%with identifiable defects but 11.7% with intact genomes and normal long terminal repeat (LTR) function.



Cell

Replication-Competent Noninduced Proviruses in the Latent Reservoir Increase Barrier to HIV-1 Cure





Arts, Current Opinion in Virology, 2012

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