1996-2013: The evolution of HIV viral load testing

Anna Maria Geretti

Institute of Infection & Global Health

UNIVERSITY OF LIVERPOOL

QIAGEN would like to thank our speaker, Prof. Anna Maria Geretti, for her presentation.

The HIV Virology Timeline

HIV-1 isolated

HIV-1 genome sequenced

HIV replicates at high levels throughout the infection

HIV replication drives immune compromise

HAART

Plasma HIV RNA "viral load" suppression as goal of therapy

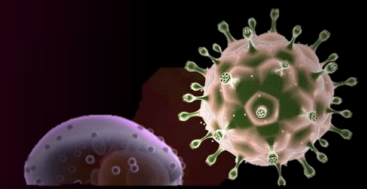
HIV replication causes disease through immune activation & inflammation

HIV eradication strategies

1982 1985 1991 1995 1996 2009 2010 →

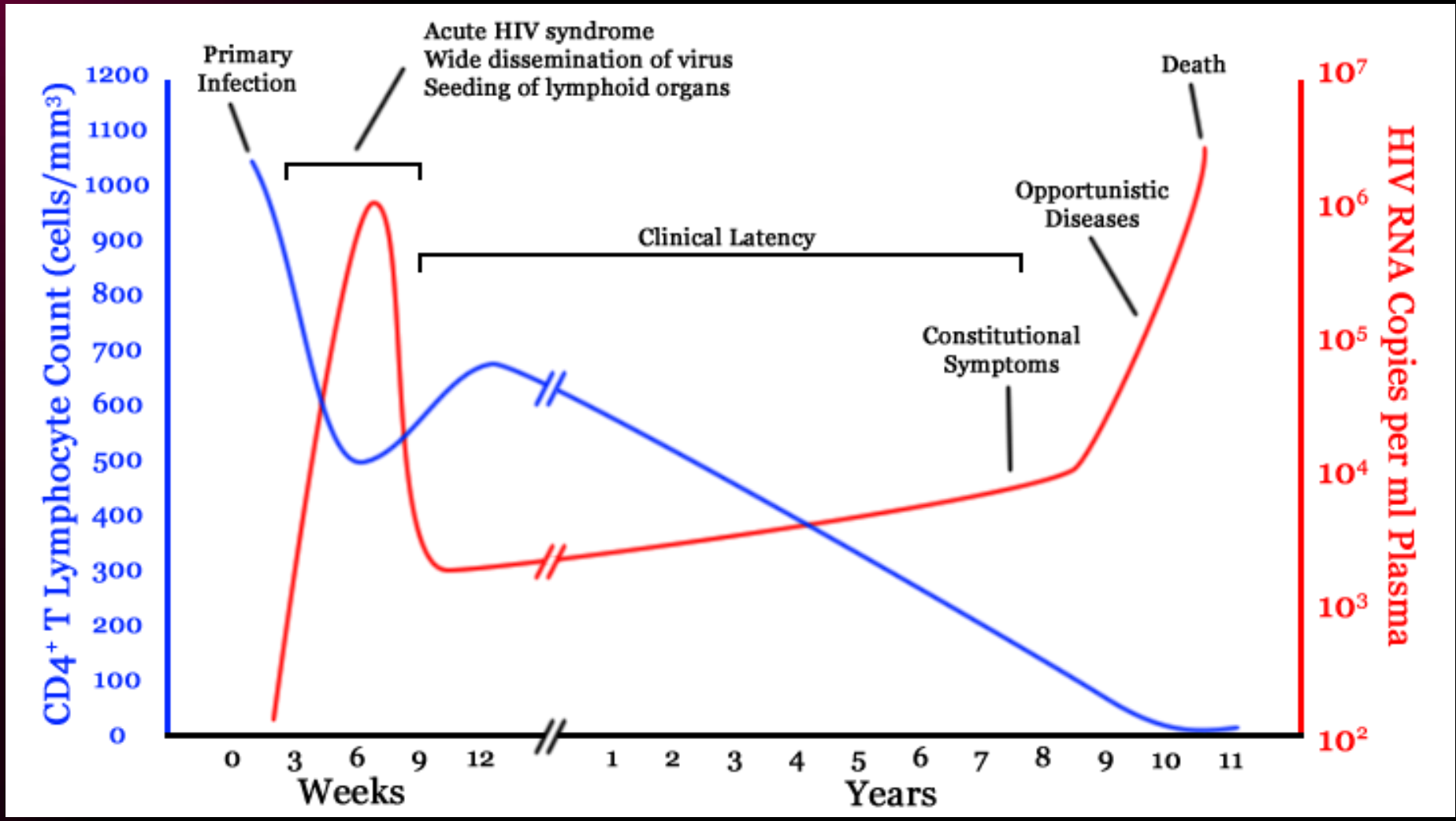
Key virological characteristics of HIV infection

- **High virus replication rate**
  10^9-10^{10} virus particles produced each day
- **Rapid virus clearance**
  $T_{½}$ of virus producing cells: <1 day
  $T_{½}$ of free virus in plasma: a few hours
- **Virus latency – integration into host DNA**
- **Continuous genetic evolution**

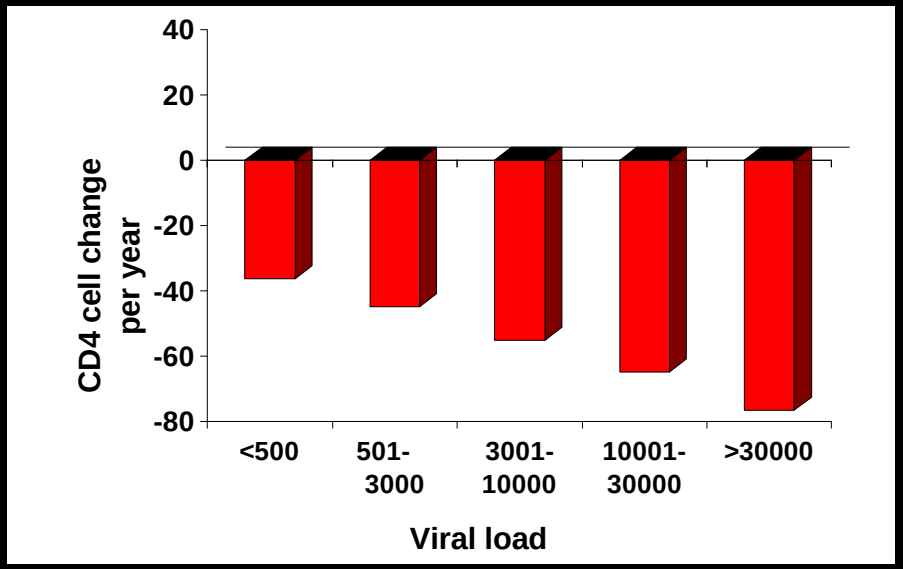


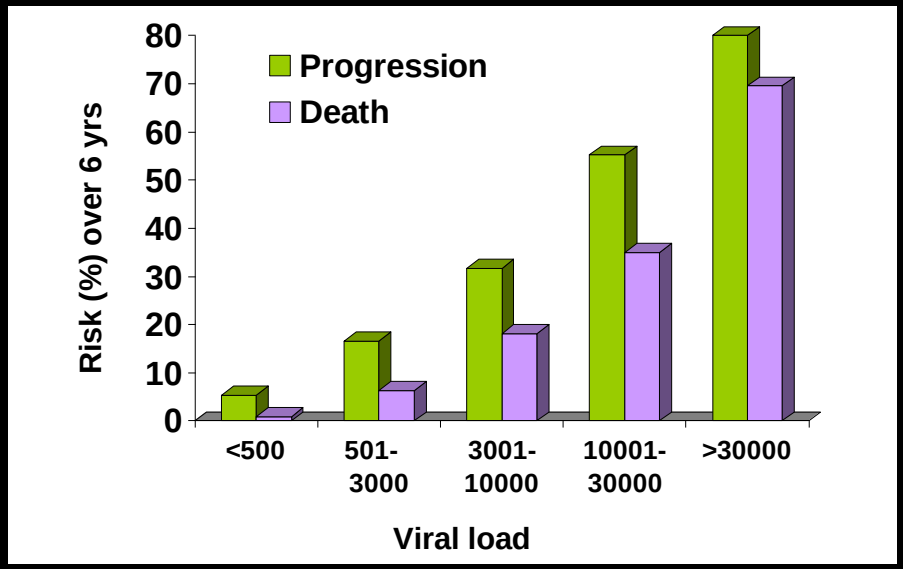
Wong et al. PNAS 1997; Wong et al. Science 1997; Chun et al. Nature 1997; Chun et al. PNAS 1997; Siliciano et al. Nat Med 2003; Strain et al. PNAS 2003

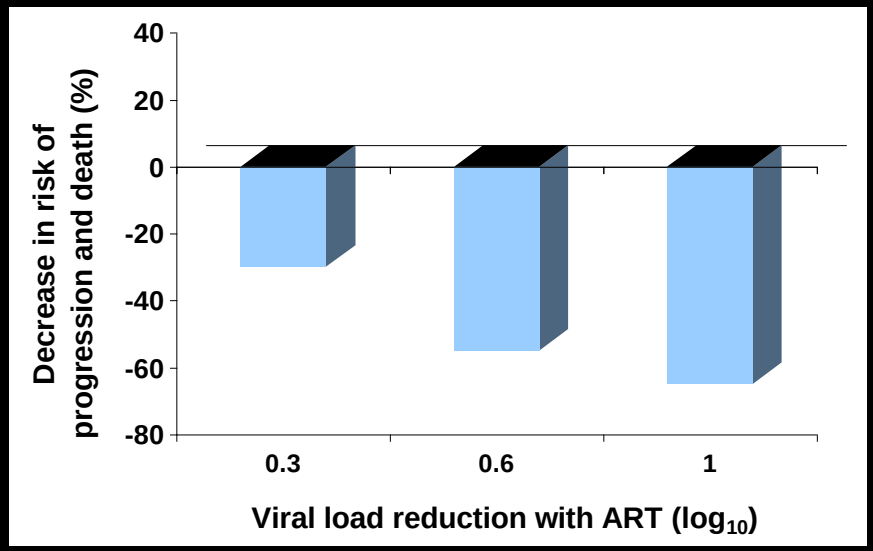
Natural history of HIV infection



Viral load predicts disease progression and mortality



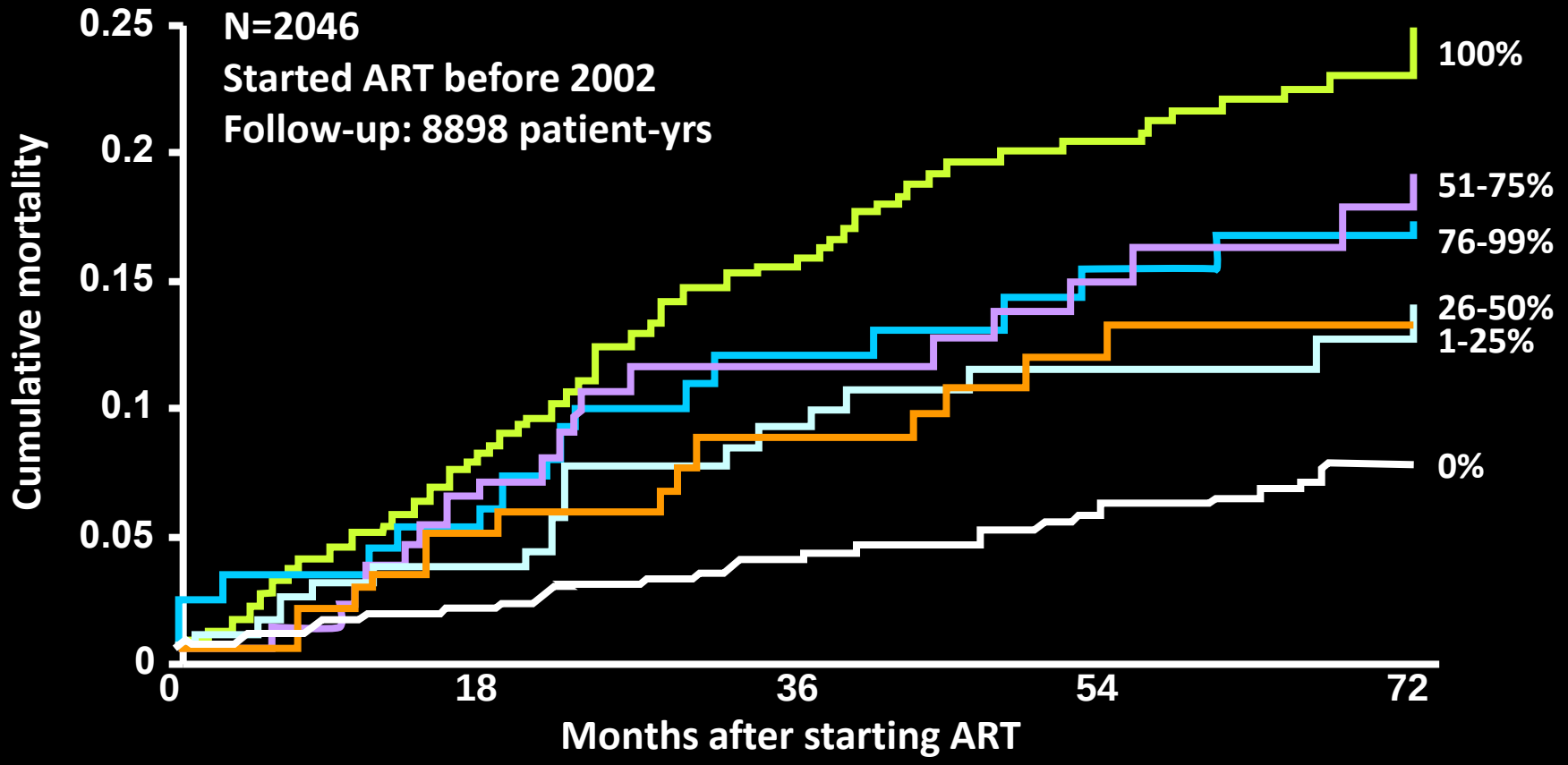




Mellors et al. Science 1996

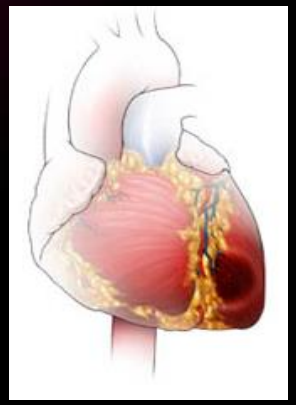
Mortality according to frequency of viral load measurements >400 cps during first-line ART

Cumulative mortality stratified by % of VL measurements ≥400 over 18 months after ART initiation



Lohse et al. Clin Infect Dis 2006

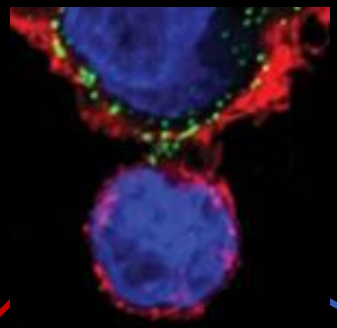
SMART Study: Stopping ART is associated with a risk of disease and mortality



No.												
DC	2495	1839	1441	1112	915	733	569	449	375	297	178	34
VS	2512	1848	1434	1122	931	754	601	490	398	310	184	41

El-Sadr et al. NEJM 2006

Pathogenesis of HIV infection



Immune activation
Inflammation

Immune compromise

Cardiovascular, bone, kidney disease; neuro-cognitive disorders; cancer; etc

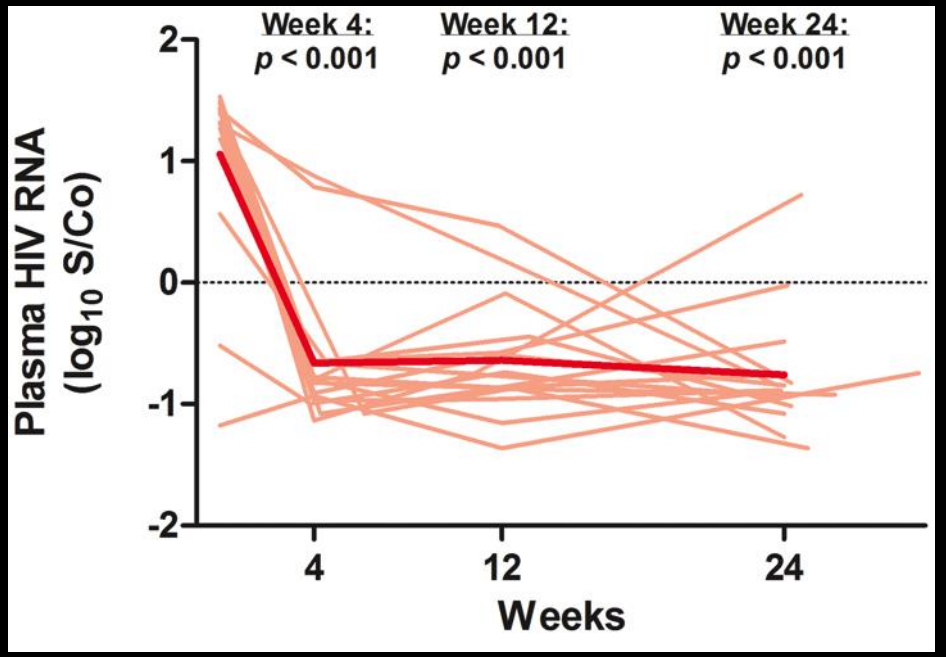
Liver disease due to hepatitis B or C

Accelerated aging

AIDS
Cancer

Viral load in “Elite Controllers”

- ART-naïve patients (n=16)
- HIV+ for 10 yrs (4.5, 24)
- Viral load 77 cps (40, 324)
- CD4 count 615 cells (476, 801)

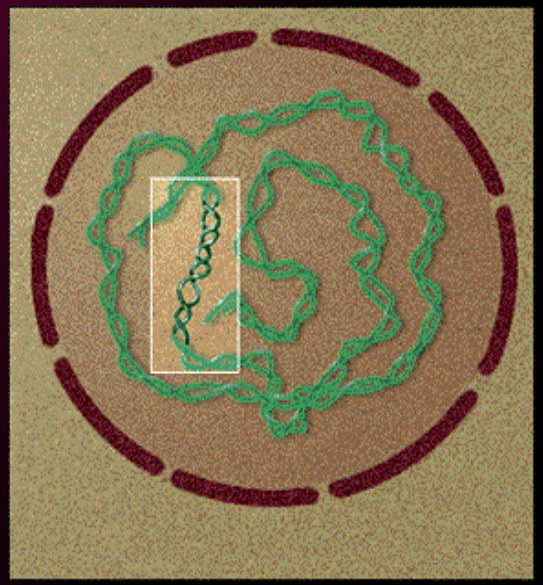


- <u>TDF/FTC + RAL for 24 wks</u>

- Decline in viral load and immune activation
- No significant change in CD4 counts

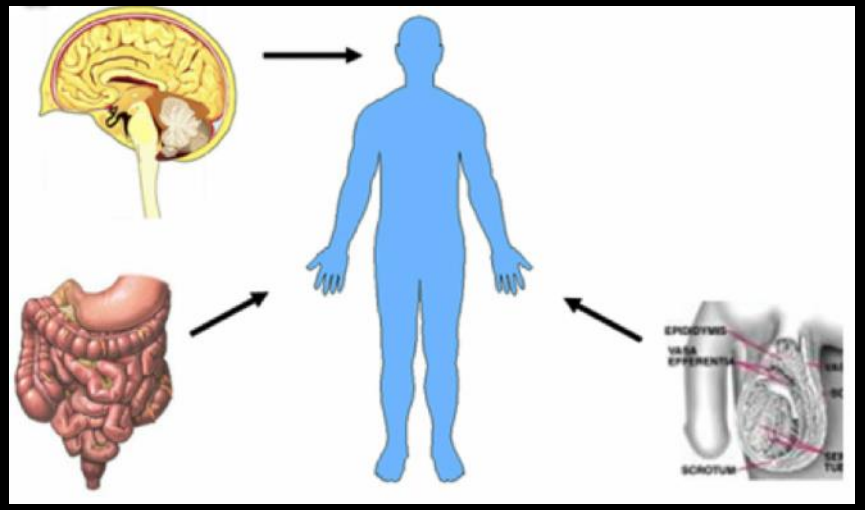
Hatano et al. Plos Pathog 2013

Obstacles to HIV eradication with ART

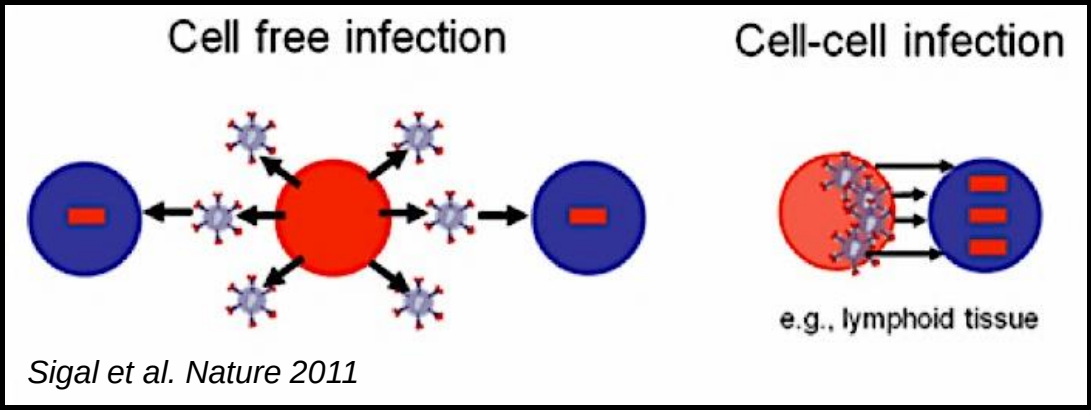


Integration and latency

Sanctuary sites



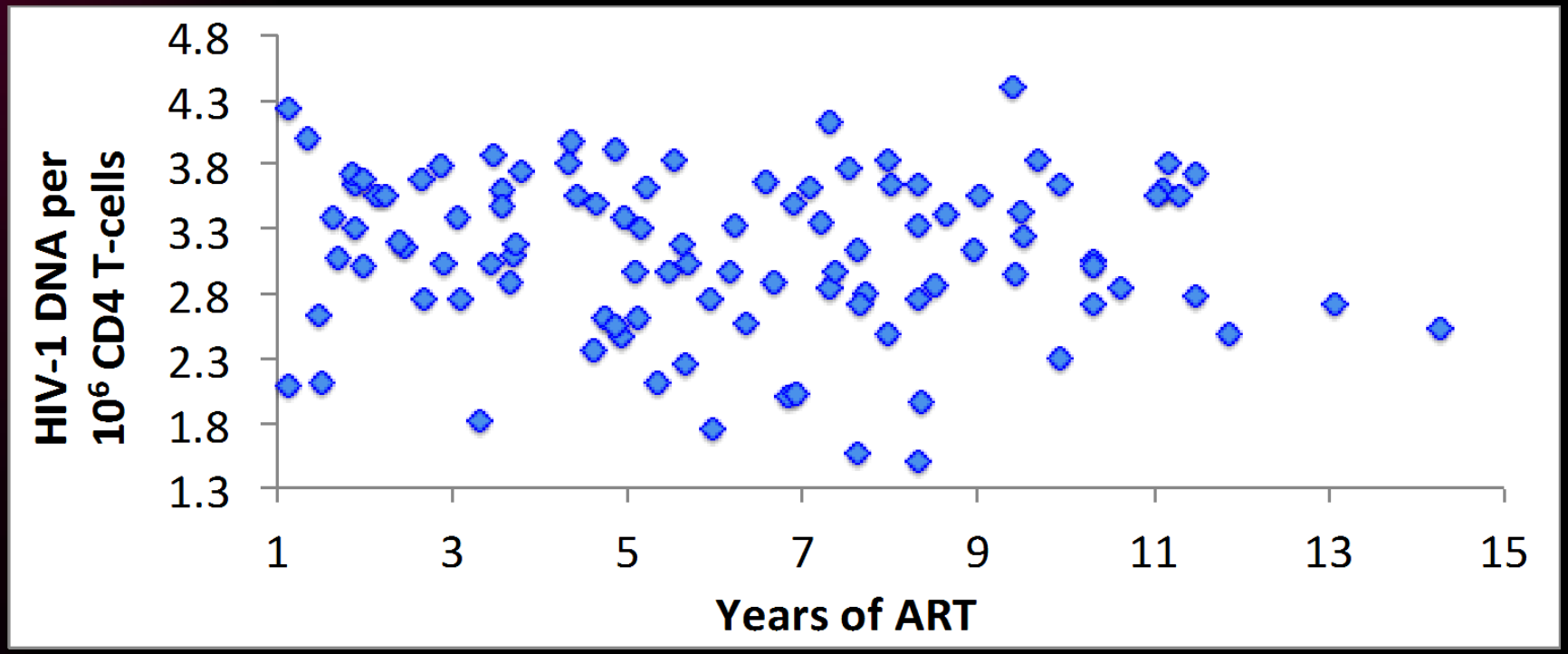
Cell-to-cell virus spread



Sigal et al. Nature 2011

HIV-1 DNA detection during suppressive ART

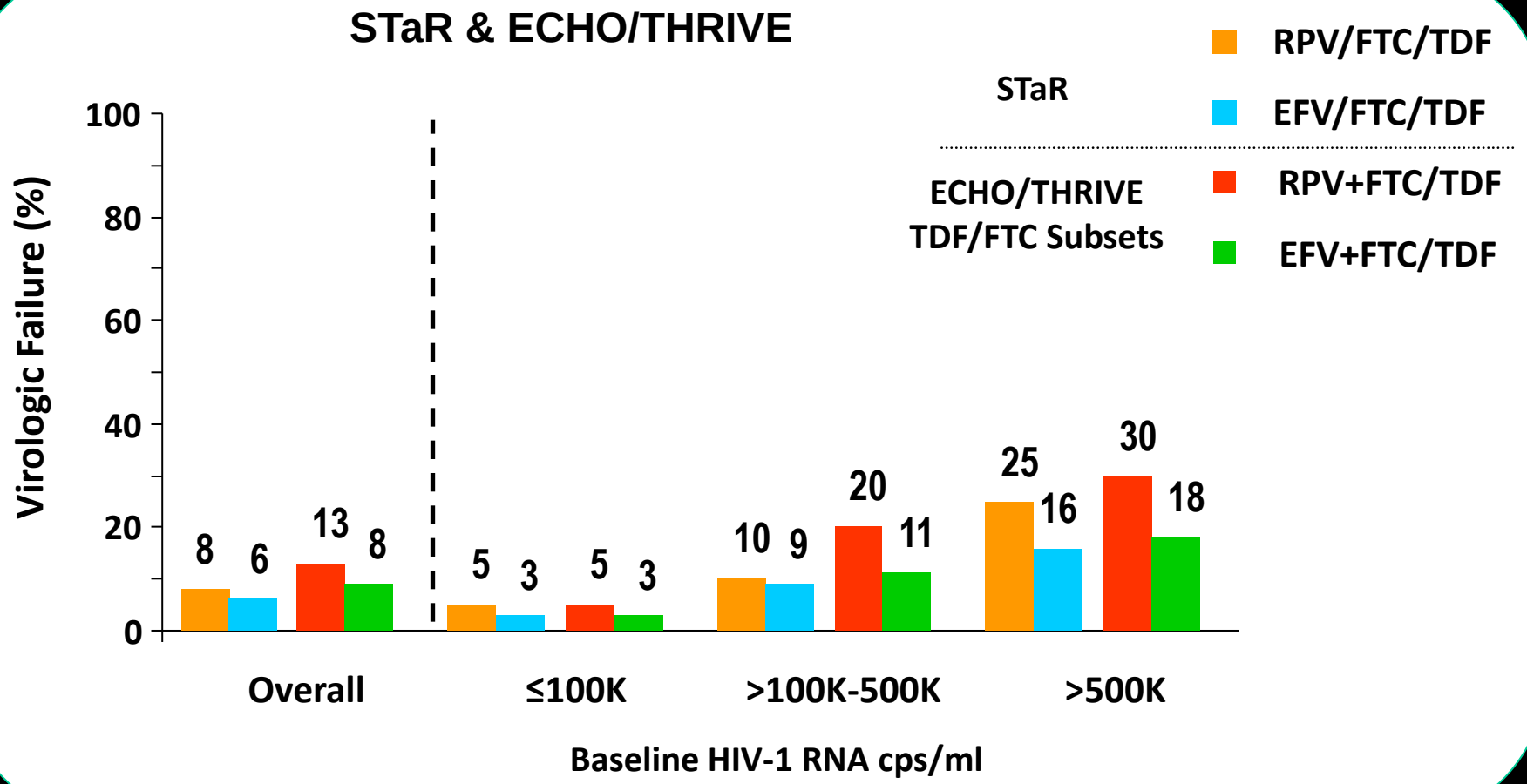
- HIV-1 DNA quantified in PBMC from 104 patients receiving consistently suppressive ART (<50 cps) for 1 to 15 years



PBMC = Peripheral blood mononuclear cells

Geretti et al. International Workshop on HIV & Hepatitis Viruses Drug Resistance 2013

Rilpivirine for first-line ART: Virological failure at wk 48 by baseline viral load



Cohen et al. ICDTHI 2012

Rilpivirine for first-line ART: Resistance analysis

	EFV (n=392)	RPV (n=394)	RPV (n=550)	EFV (n=546)
Any resistance	1%	4%	7%	2%
NNRTI resistance	1%	4%	6%	2%
NRTI resistance	0.3%	4%	7%	1%
By baseline HIV-1 RNA				
≤100,000	1%	2%	2%	1%
100,001–500,000	0	5%	9%	2%
>500,000	4%	19%	21%	7%

Cohen et al. ICDTHI 2012

Viral load suppression as the goal of ART

- Viral load *"undetectability"*
- Defined by the technical properties of the assay
  - ***First-generation assays 400 cps***
  - ***Second-generation assays 50 cps***

    (e.g. Roche Amplicor v1.5; Bayer bDNA v3)
  - ***Third-generation assays 20 cps, 40 cps, 45 cps + qualitative RNA detection below these cut-offs***

    (e.g. Roche TaqMan v1/v2; Abbott RealTime; Qiagen Artus$_{HIV}$)

Defining viral load cut-offs

- Patients who achieve and maintain viral load suppression <50 cps have a small risk of rebound during follow-up, and the risk declines further the longer the viral load stays <50 cps

Virological failure according to current guidelines

DHHS 2013: Inability to achieve or maintain <200 cps

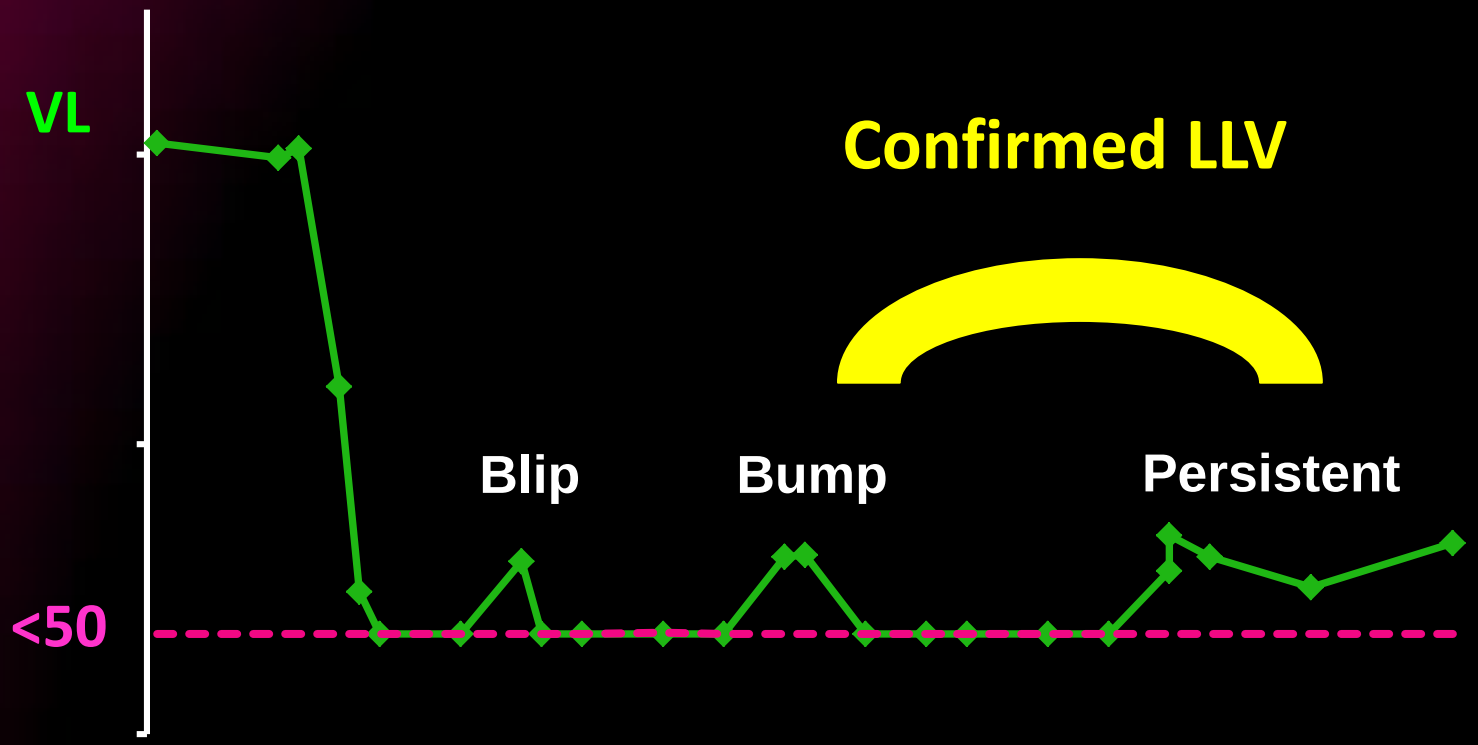
IAS-USA 2012: Sustained elevation 50- 200 cps should prompt evaluation of factors leading to failure and consideration of changing ART

BHIVA 2012: Failure to achieve <50 cps 6 months after commencing ART, or confirmed rebound >400 cps following suppression <50 cps

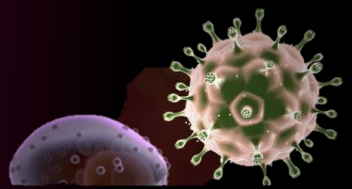
EACS 2013: Confirmed >50 cps 6 months after initiation or modification of ART


Welcome to the grey zone

Low-level viraemia (LLV) during ART: Definitions



Magnitude: 50-200; 50-400; <1000 cps/ml



What causes low-level HIV-1 RNA detection during ART?

Technique-related

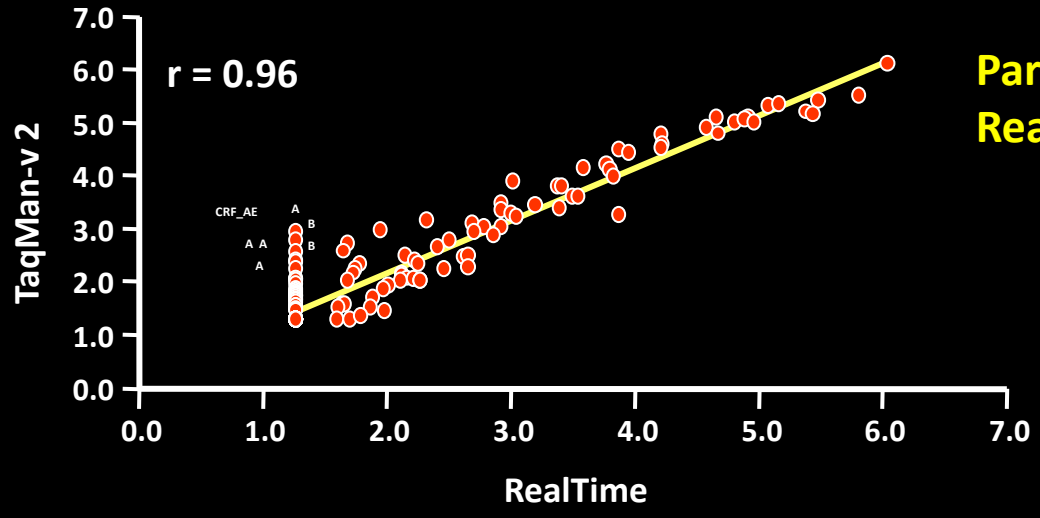
Assay concordance at the 50 cps cut-off

		>50 cps			
		Amplicor 1.5	RealTime	TaqMan v2	$Artus_{HIV}$
<50 cps	Amplicor 1.5	-	NA	6%-23%	-
	RealTime	NA	-	13%	5%
	TaqMan v2	5%	7%	-	-
	$Artus_{HIV}$	-	5%	-	-

- Assays that capture both RNA and DNA during the extraction step are vulnerable to certain conditions of specimen collection and handling (e.g., PTT vs. EDTA collection tubes, delays in plasma separation)[4]

1. The International Viral Load Assay Collaboration. JCM (in press);
2. Garcia et al. JCV 2013; 3. Taylor et al. PLOS One 2013; 4. Adachi et al. IAS 2013

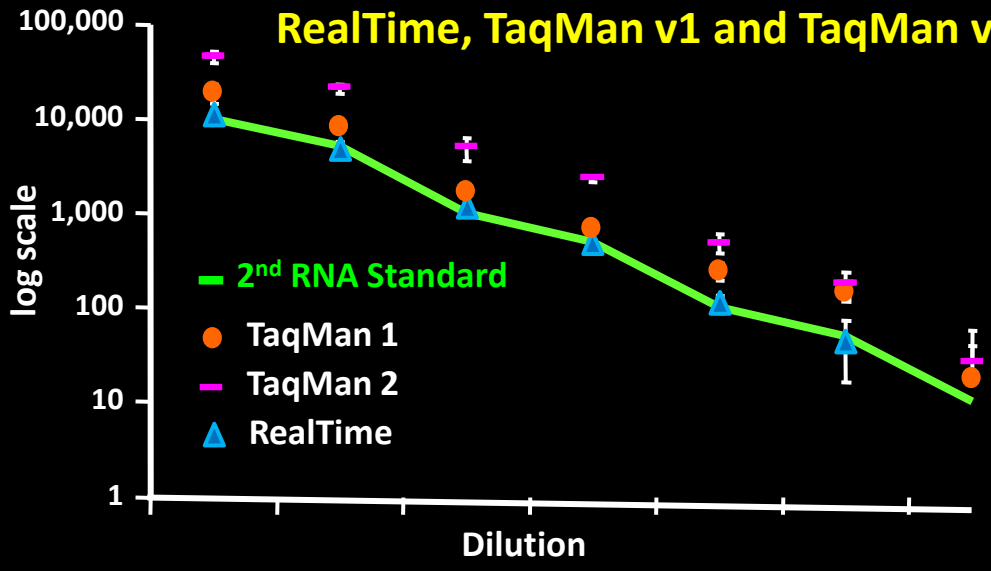
RealTime vs. TaqMan assays



Parallel testing of clinical samples
RealTime vs. Taqman v2

Lower limit of quantification
Abbott RealTime = 40 cps/ml
Roche TaqMan-v1 = 40 cps/ml
Roche TaqMan-v2 = 20 cps/ml

Parallel testing of WHO 2nd IS
RealTime, TaqMan v1 and TaqMan v2

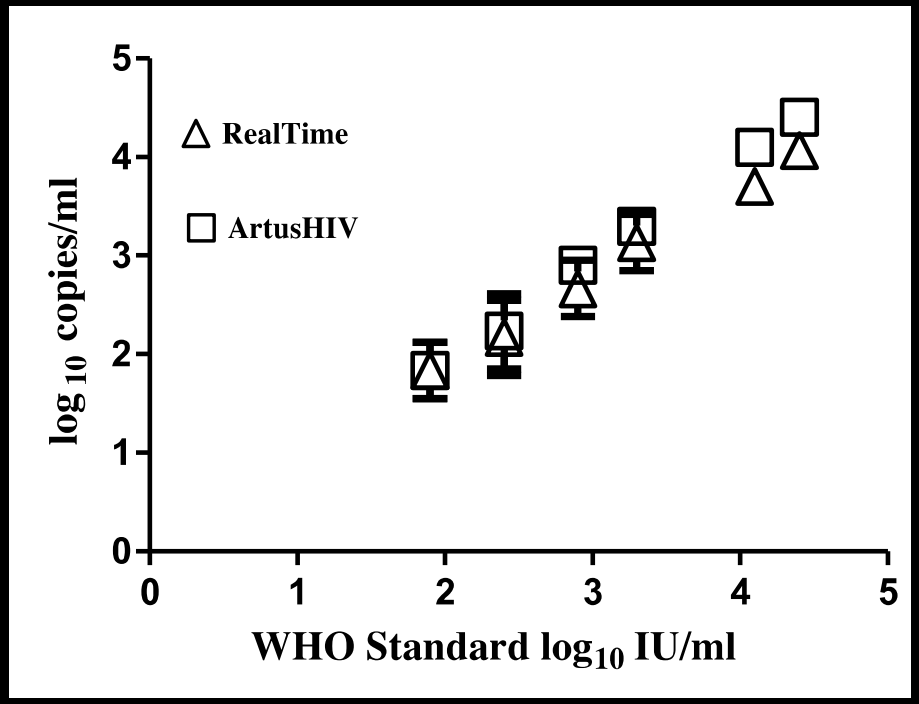


Median difference (log_{10} cps/ml):
RealTime 0.0 (-0.1, 0.1)
TaqMan-v1 0.2 (0.1, 0.5)
TaqMan-v2 0.7 (0.4, 0.7)

Geretti et al, 12th European AIDS Conference, Cologne, Germany, 2009

Artus HIV-1 QS-RGQ assay

- Qiagen QIASymphony SP for sample preparation, QIAsymphony AS for set-up, Rotor-Gene Q for real-time PCR
- Targets a 93-nucleotide region in the 5'LTR of HIV-1 group M
- IC introduced into each specimen during sample preparation
- Dynamic range 100 to 10^8 IU/ml (45 to 4.5 x 10^7 cps/ml)

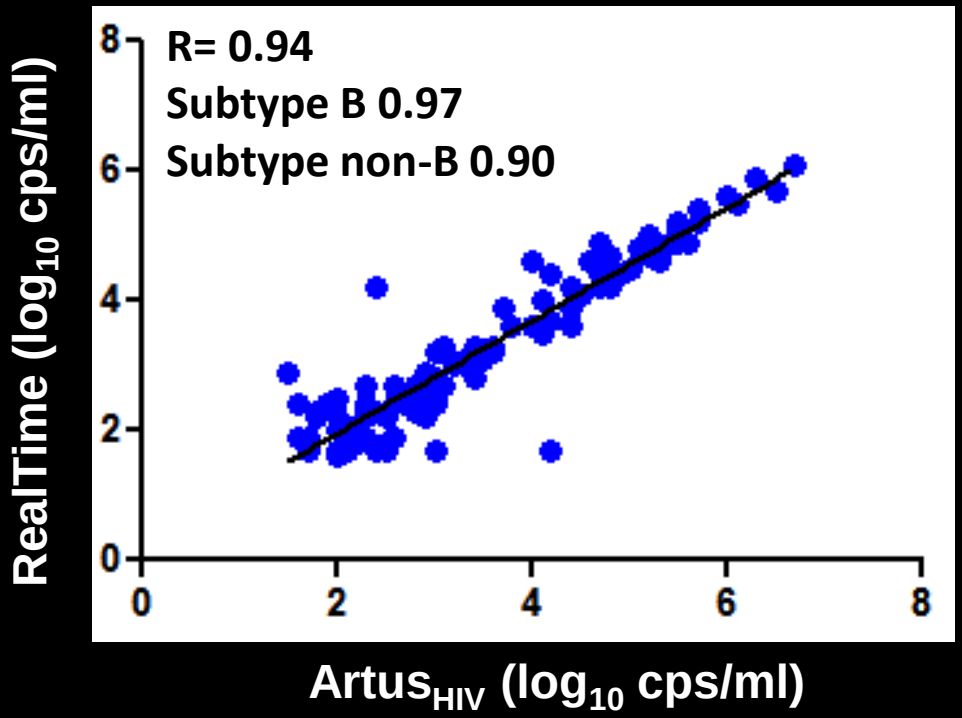


Assay performance with 2nd WHO International Standard for HIV-1 RNA

Garcia et al. JCV 2013

$Artus_{HIV}$ vs. RealTime: Correlation analysis

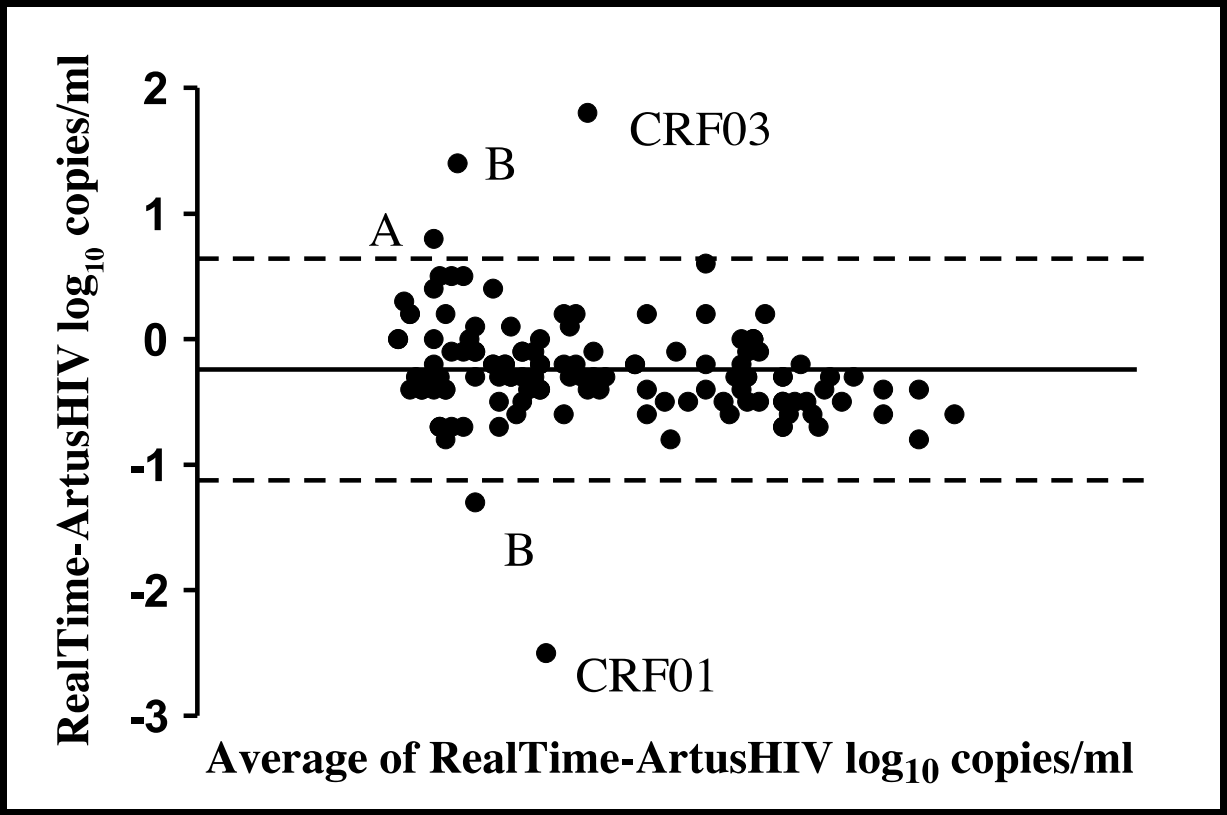
61/211 (29%) samples from treated patients <LLQ of both assays
125/211 (59%) samples quantified by both assays



HIV-1 Subtype	No. of samples	Mean log_{10} copies/ml (SD)	
		$Artus_{HIV}$	RealTime
A	12	3.5 (1.3)	2.4 (0.7)
B	71	4.1 (1.0)	4.6 (1.3)
C	19	3.6 (1.3)	2.6 (1.2)
D	4	3.4 (1.5)	3.2 (1.3)
F	1	2.8	2.4
G	5	4.8 (1.8)	4.2 (1.7)
CRF01	4	4.1 (1.4)	3.6 (1.2)
CRF02	4	2.7 (0.8)	2.6 (0.6)
CRF012	1	2.0	1.9
CRF14	1	3.7	3.3
Complex	3	4.8 (1.3)	4.4 (1.2)

Garcia et al. JCV 2013

$Artus_{HIV}$ vs. RealTime: Agreement analysis



5/125 (4%) results outside the limit of agreement:

3 higher by RealTime (by 0.8 to 1.8 log_{10})

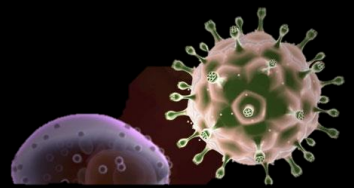
3 higher by $Artus_{HIV}$ (by 1.3 to 2.5 log_{10})

4/125 (3%) samples differed by >1 log_{10}
22/125 (18%) samples differed by >0.5 log_{10}
Values generally higher with $Artus_{HIV}$

Garcia et al. JCV 2013

$Artus_{HIV}$ vs. RealTime: Discordance rates

- 13/211 (6%) samples quantified by RealTime alone
  median VL 54 cps/ml (range 40 -824)
- 11/211 (5%) samples quantified by $Artus_{HIV}$ alone
  median VL 78 cps/ml (range 42-2,193)
- All samples from patients on ART
- At the 50 cps threshold, 15/147 (10%) results discordant:
  - 8 quantified by RealTime alone
    median VL 57 (range 57-824) cps
  - 7 quantified by $Artus_{HIV}$ alone
    median VL 78 (range 67-2193) cps



Garcia et al. JCV 2013

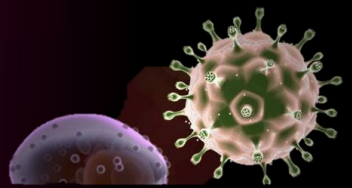
What causes low-level HIV-1 RNA detection during ART?

Technique-related

Patient-related

Low-level HIV-1 RNA detection during ART varies according to patient characteristics

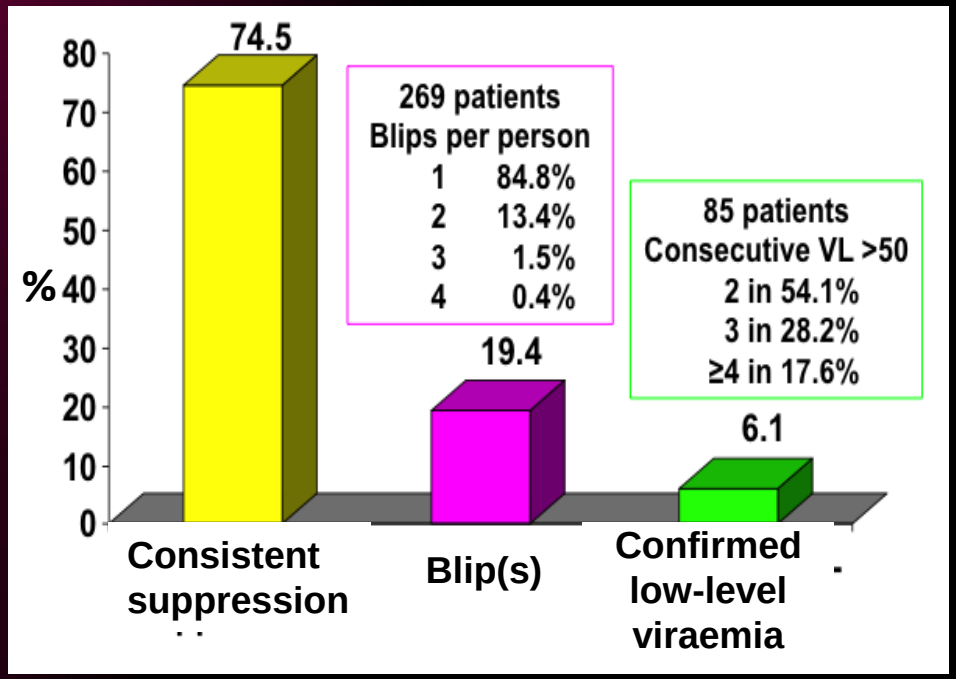
- **Patients less likely to show low-level HIV-1 RNA detection**
  - **Older** — *p <.0001*
  - **White MSM** — *p =0.006*
  - **Lower pre-ART viral load** — *p =0.04*
  - **On ART for longer** — *p <.0001*
  - **Viral load <50 cps for longer** — *p <.0001*
  - **Higher CD4 count** — *p <.0001*
  - **On NNRTI-based ART** — *p <.0001*
  - **Greater adherence** — *p <.0001*



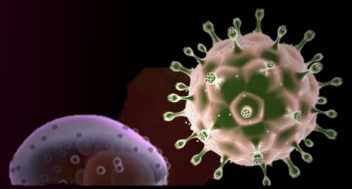
Univariate analysis

Doyle et al. Clin Infect Dis 2012

Low-level HIV-1 RNA detection during ART predicts viral load >400 cps



- In the first year after achieving <50 cps, confirmed VL 50-400 cps predicts rebound >400 cps during median 2 yrs of follow-up[1]
- *Adjusted RR of rebound 2.18 [95% CI 1.15-4.10]*



Geretti et al. Antiv Ther 2008

Low-level HIV-1 RNA detection during ART predicts viral load >1000 cps

- Patients with ≥12 months of ART who achieved <1000 cps
- Persistent viraemia for ≥6 months (blips excluded)
- Stratified in 50-199, 200-499, and 500-999 cps
- Versant bDNA v3 (1999-2010), Abbott Real-Time (from 2010)

Cox modeling of univariate and multivariate analyses of the association between persistent low-level viraemia and viral load rebound >1000 cps after 1 year

Persistence Duration, HIV Load	Univariate		Multivariate[a]	
	HR (95% CI)	*P* Value	Adjusted HR (95% CI)	*P* Value
≥6 mo				
<50 RNA copies/mL	1.00 (reference)		1.00 (reference)	
50–199 RNA copies/mL	2.61 (1.88–3.63)	<.001	2.22 (1.60–3.09)	<.001
200–499 RNA copies/mL	2.92 (1.99–4.28)	<.001	2.15 (1.46–3.17)	<.001
500–999 RNA copies/mL	5.57 (3.67–8.46)	<.001	4.85 (3.16–7.45)	<.001

Adjusted for age, sex, race, sexual orientation, IDU, monthly income, type of employment, date of HIV diagnosis, baseline CD4 count, IDU, ART use

Laprise et al. Clin Infect Dis 2013

Detection of resistance-associated mutations at low viral load levels

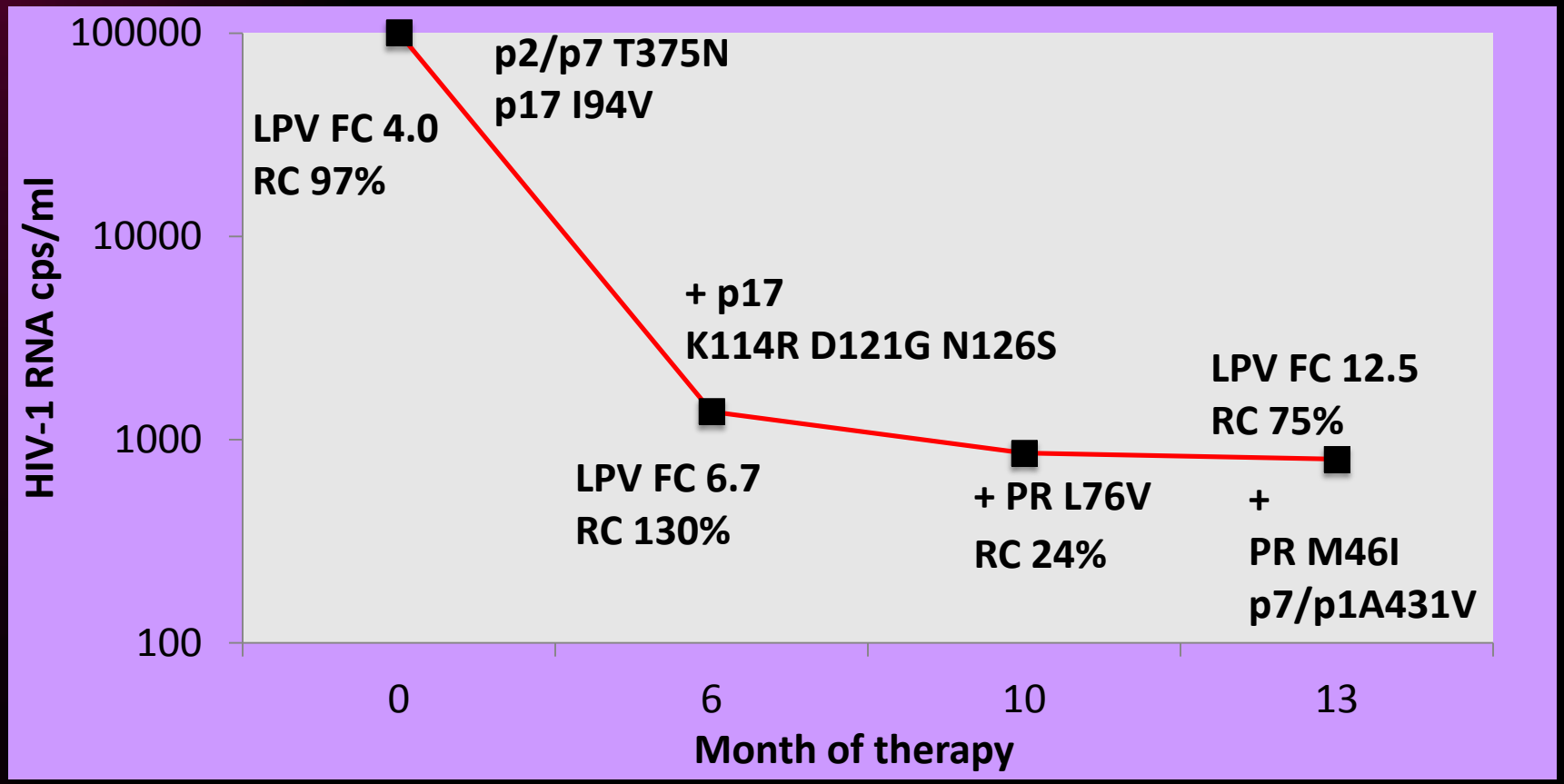
VL cps	n	% RAMs	RR (95% CI)
<300	449	60	0.94 (0.87-1.01)
300-1000	552	72	0.99 (0.94-1.04)
1000-3000	1120	76	1
3000-10000	1312	77	1.01 (0.97-1.05)
10000-30000	1326	67	0.91 (0.87-0.95)
30000-100000	1438	60	0.84 (0.80-0.88)
≥100000	1682	49	0.70 (0.66-0.74)

RAMs: Resistance-associated mutations
RR: Relative risk of RAM detection

RAM	VL <1000	VL >1000	
M41L	20.7	27.0	NRTIs
D67N	21.5	23.3	
K70R	17.1	16.1	
L210W	12.2	16.0	
T215Y/F	19.1	25.6	
T215F	6.2	7.6	
K219Q	7.0	7.7	
K219E	6.2	5.7	
K65R	5.34	4.18	
L74V	3.27	6.50	
M184V	38.8	39.3	
K103N	38.0	35.6	NNRTIs
Y181C	15.7	19.7	
G190A	12.2	15.2	
D30N	5.4	6.1	PIs
M46I	12.3	10.5	
V82A	10.7	11.7	
I84V	5.4	11.2	
L90M	14.0	21.2	

Mackie et al. J Infect Dis 2010

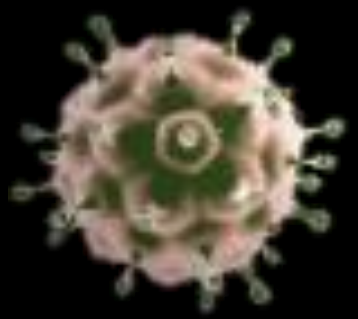
HIV protease and gag evolution during low-level viraemia on TDF 3TC LPV/r



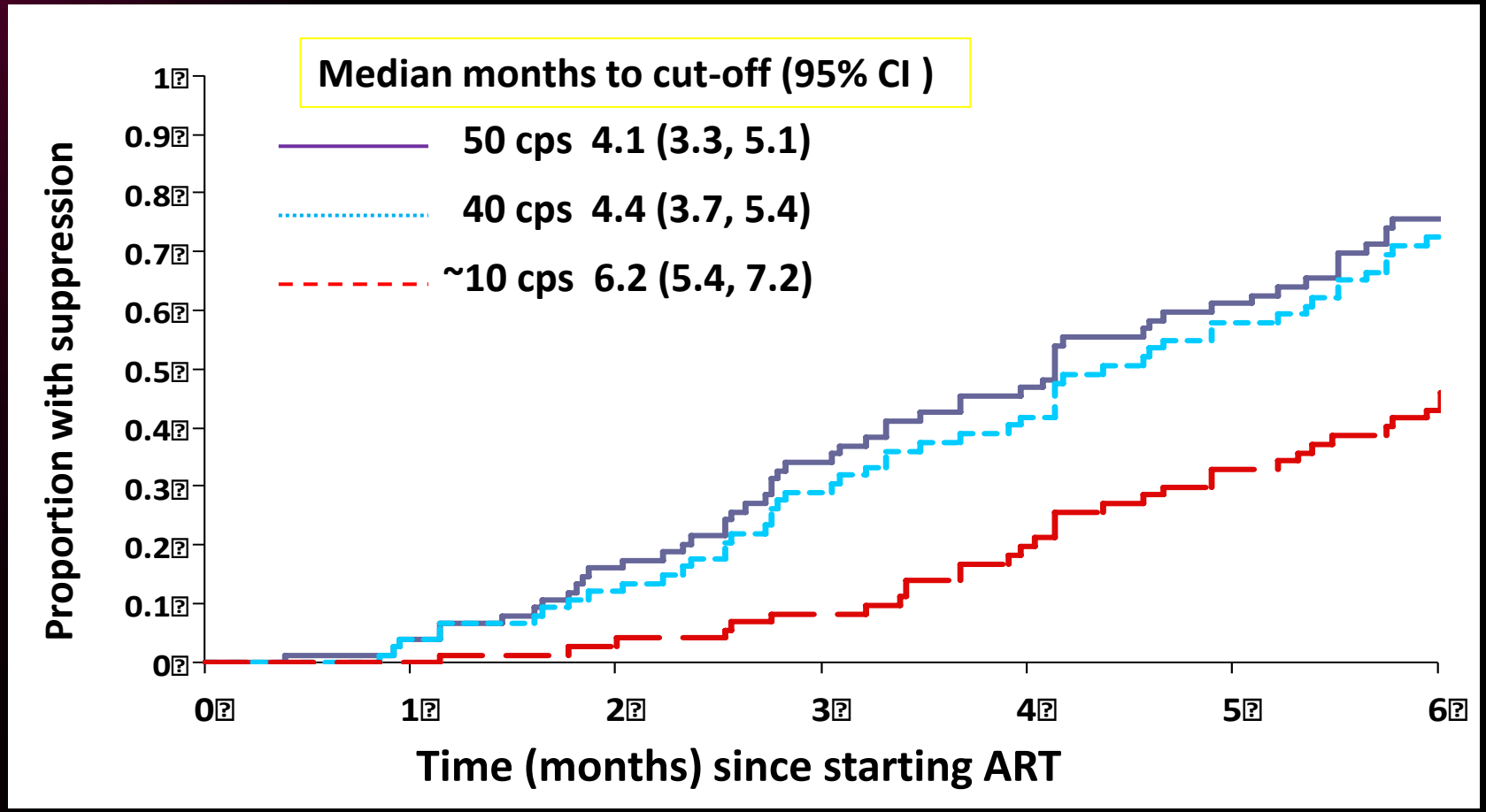
Geretti et al. (unpublished)

1. *Is there any level of HIV replication that can be regarded as “safe”?*

2. *Does HIV RNA detection always indicate ongoing virus replication?*



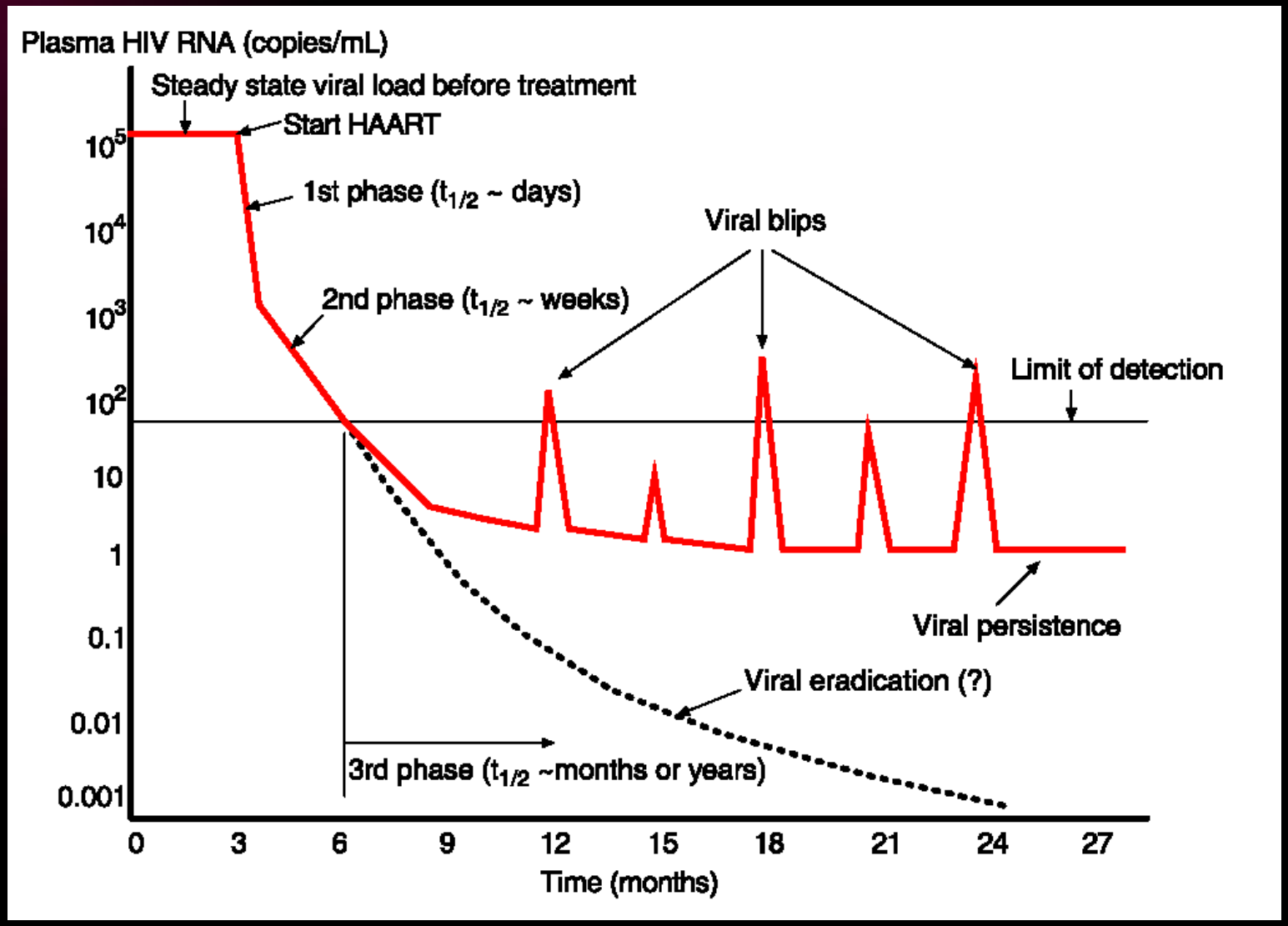
HIV-1 RNA kinetics after starting first-line ART with TDF/FTC + EFV or LPV/r

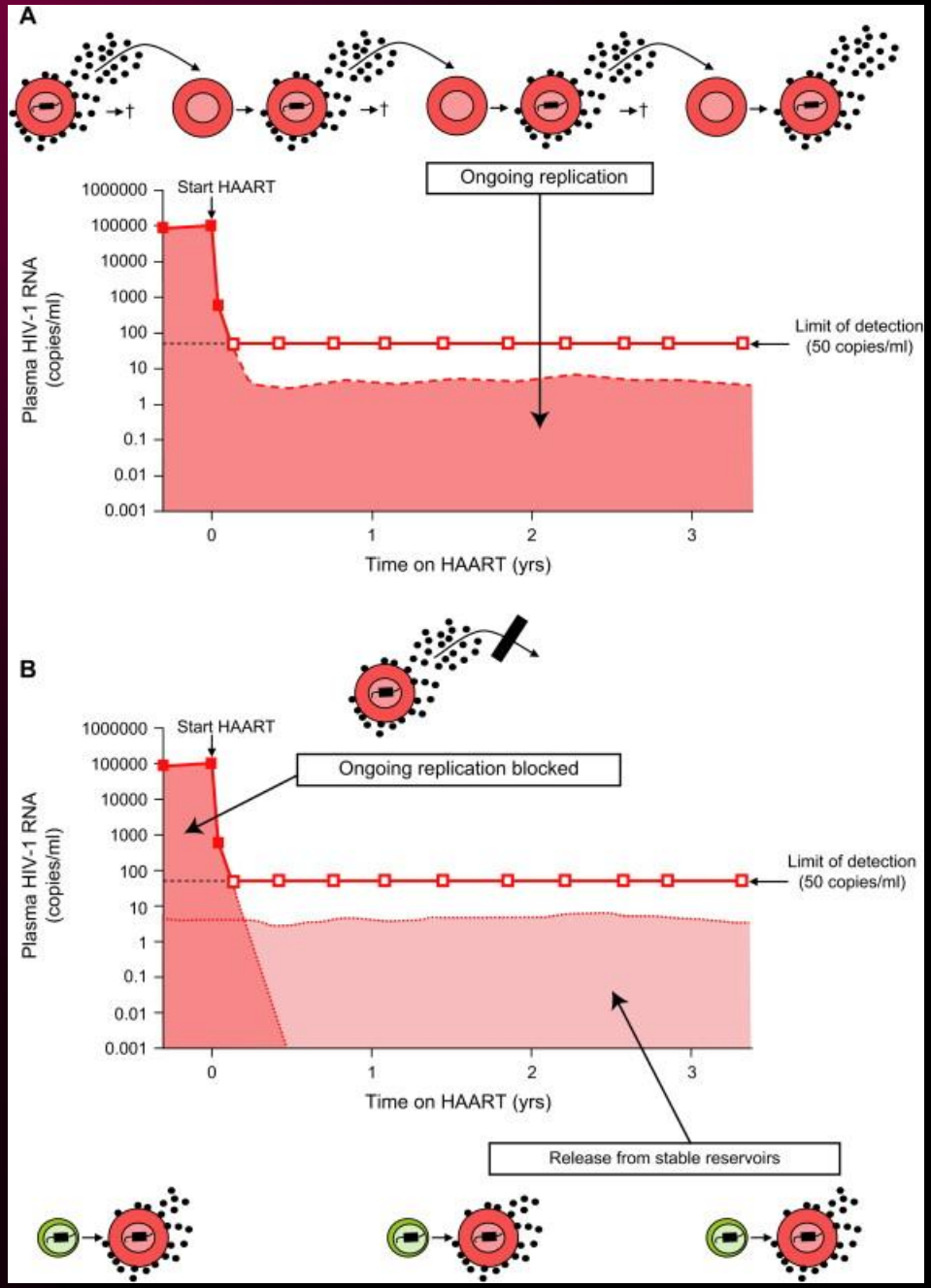


Abbott RealTime HIV-1 assay

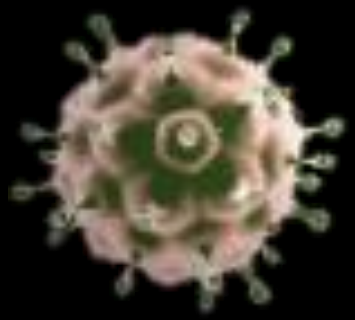
Doyle et al. Clin Infect Dis 2012

Plasma HIV-1 RNA kinetics during ART



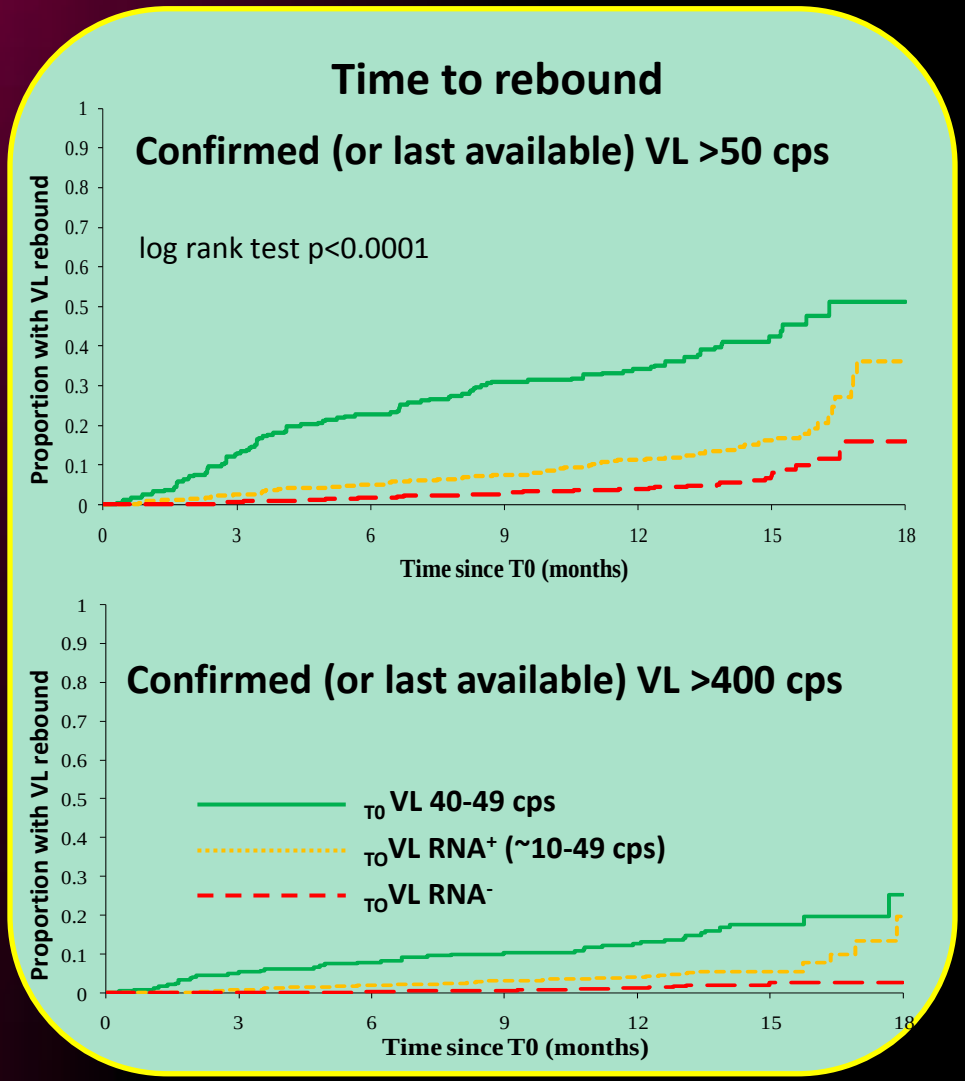


Ongoing virus replication

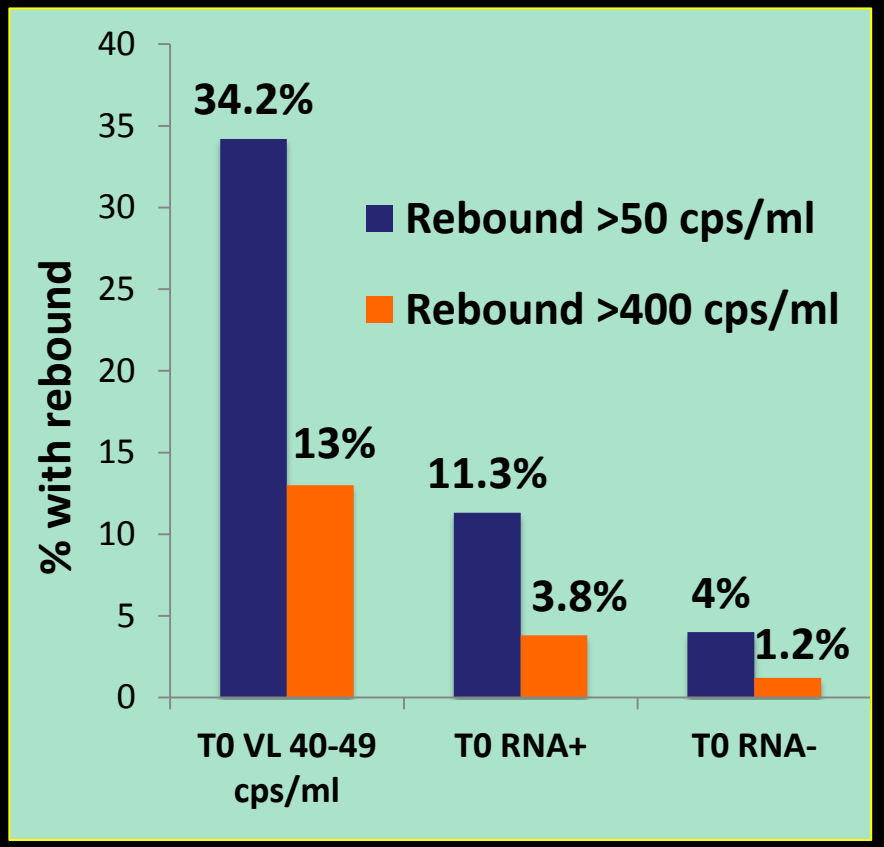


Bursts of virus replication

HIV-1 RNA detection below 50 cps predicts rebound >50 cps and >400 cps



- 1247 patients with viral load <50 cps at an arbitrarily selected time point during ART (=T0)



Abbott RealTime assay

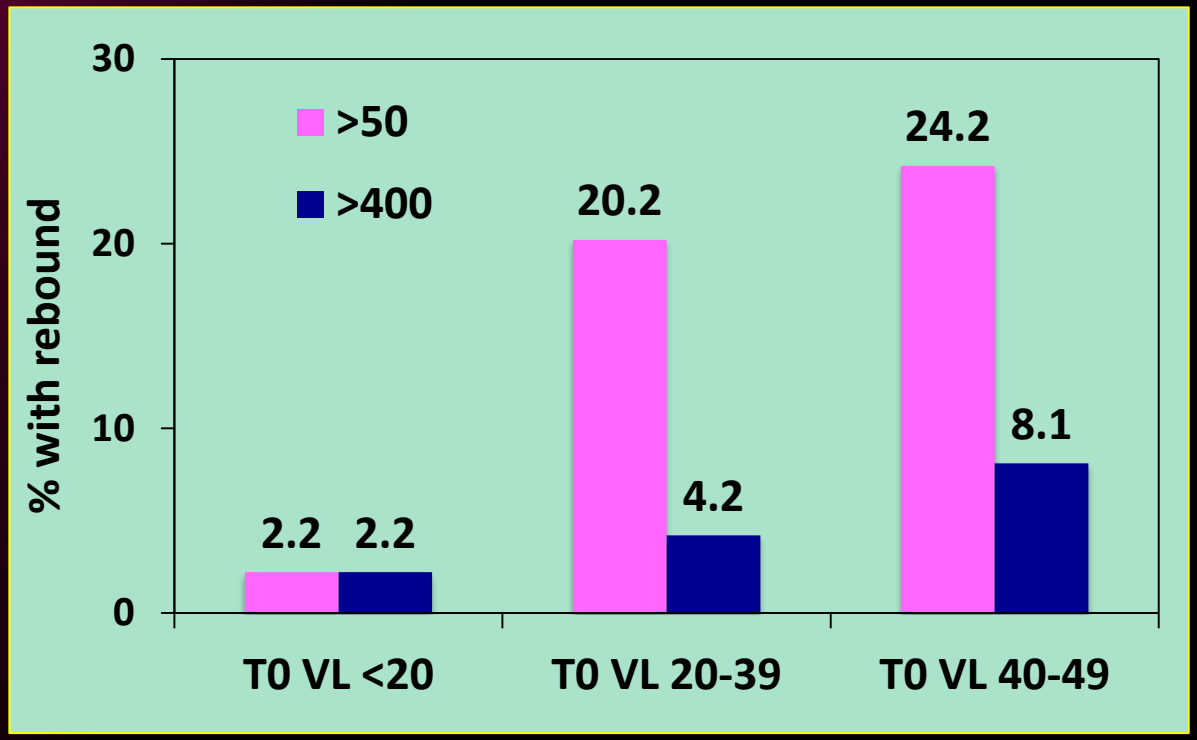
Doyle et al. Clin Infect Dis 2012

Factors associated with viral load rebound

Multivariate model		VL >50 cps/ml			VL >400 cps/ml		
T_0 VL	**40-49 cps/ml**	**4.68**	**2.40, 9.12**	**<0.0001**	**10.71**	**3.30, 34.81**	**<0.0001**
	RNA detected	**2.33**	**1.26, 4.31**		**3.78**	**1.23, 11.59**	
	RNA not detected	**1.00**	**-**		**1.00**	**-**	
Length VL<50 cps/ml	Per yr longer	0.79	0.69, 0.91	0.0005	0.88	0.72, 1.06	0.15
ART duration	Per yr longer	1.06	0.99, 1.15	0.10	1.14	1.02, 1.27	0.03
Gender	Male	0.81	0.45, 1.45	0.47	1.49	0.65, 3.38	0.35
	Female	1.00	-		1.00	-	
Age	Per 10 yrs older	0.80	0.61, 1.04	0.09	1.07	0.71, 1.62	0.74
Ethnicity	White	1.00	-	0.11	1.00	-	0.17
	Black	1.91	1.00, 3.63		2.40	0.91, 6.36	
	Other	1.50	0.75, 2.98		1.85	0.59, 5.83	
Risk group	Homosexual	1.00	-	0.36	1.00	-	0.63
	Heterosexual	0.83	0.41, 1.70		1.36	0.48, 3.85	
	Other	1.70	0.63, 4.64		2.07	0.50, 8.60	
ART regimen	NNRTI based	0.40	0.21, 0.77	0.002	0.46	0.17, 1.23	0.23
	Other/Unknown	1.40	0.79, 2.48		0.99	0.40, 2.46	
	PI based	1.00	-		1.00	-	
Adherence	Not available	0.59	0.32, 1.10	0.23	0.99	0.39, 2.47	0.99
	<95%	1.00	-		1.00	-	
	>95%	0.87	0.54, 1.39		0.96	0.45, 2.07	
CD4 count	Per 100 cells higher	0.92	0.84, 1.00	0.06	1.00	0.87, 1.15	0.97
Pre-ART VL	Per 1 $\log_{10}$ cps higher	1.04	0.80, 1.33	0.79	0.74	0.52, 1.05	0.10

Doyle et al. Clin Infect Dis 2012

HIV-1 RNA detection below 50 cps predicts rebound >50 cps and >400 cps



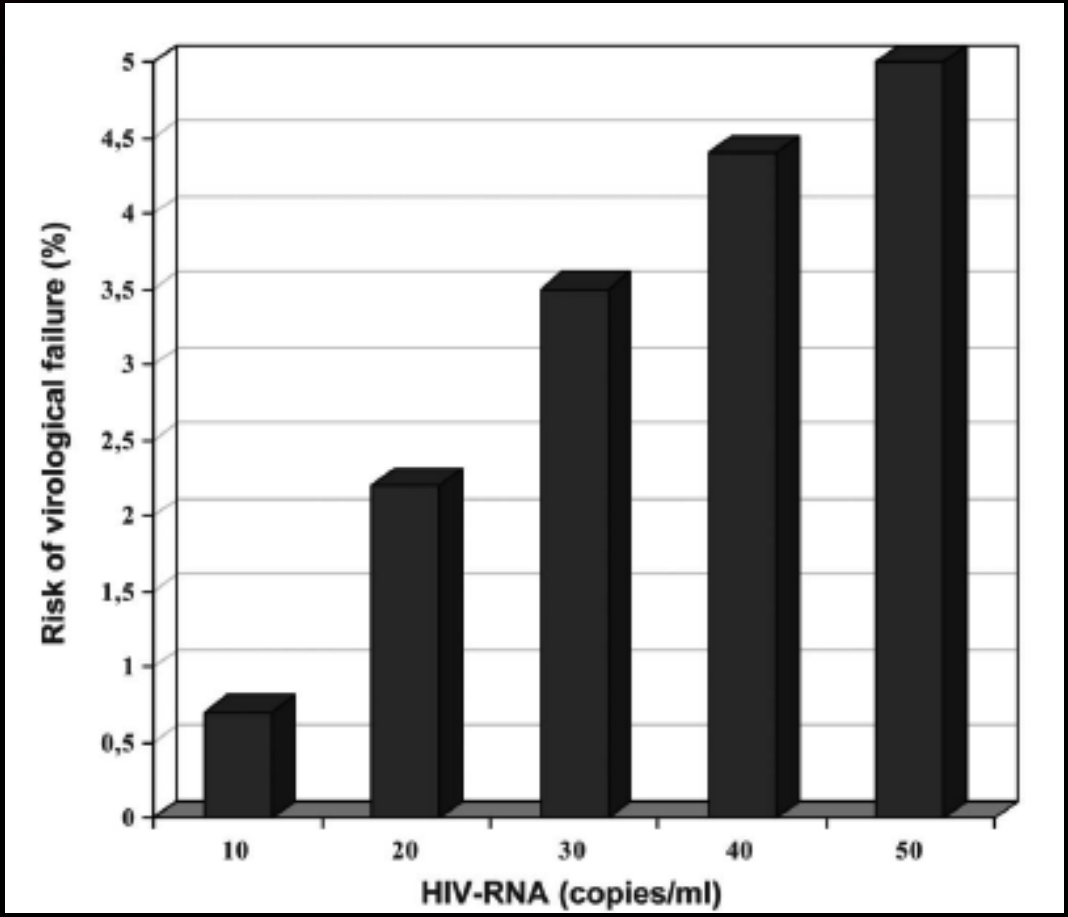
	VL rebound (cps)	
T0 VL	>50	>400
<20	3/134	3/134
20-39	19/94	4/94
40-49	15/62	5/62
P	*<0.001*	*0.118*

TaqMan v2 assay

Álvarez et al. J Clin Microbiol 2013

Risk of confirmed rebound >50 cps according to previous (4 months) HIV-1 RNA level

- 1214 patients followed for mean 378 days

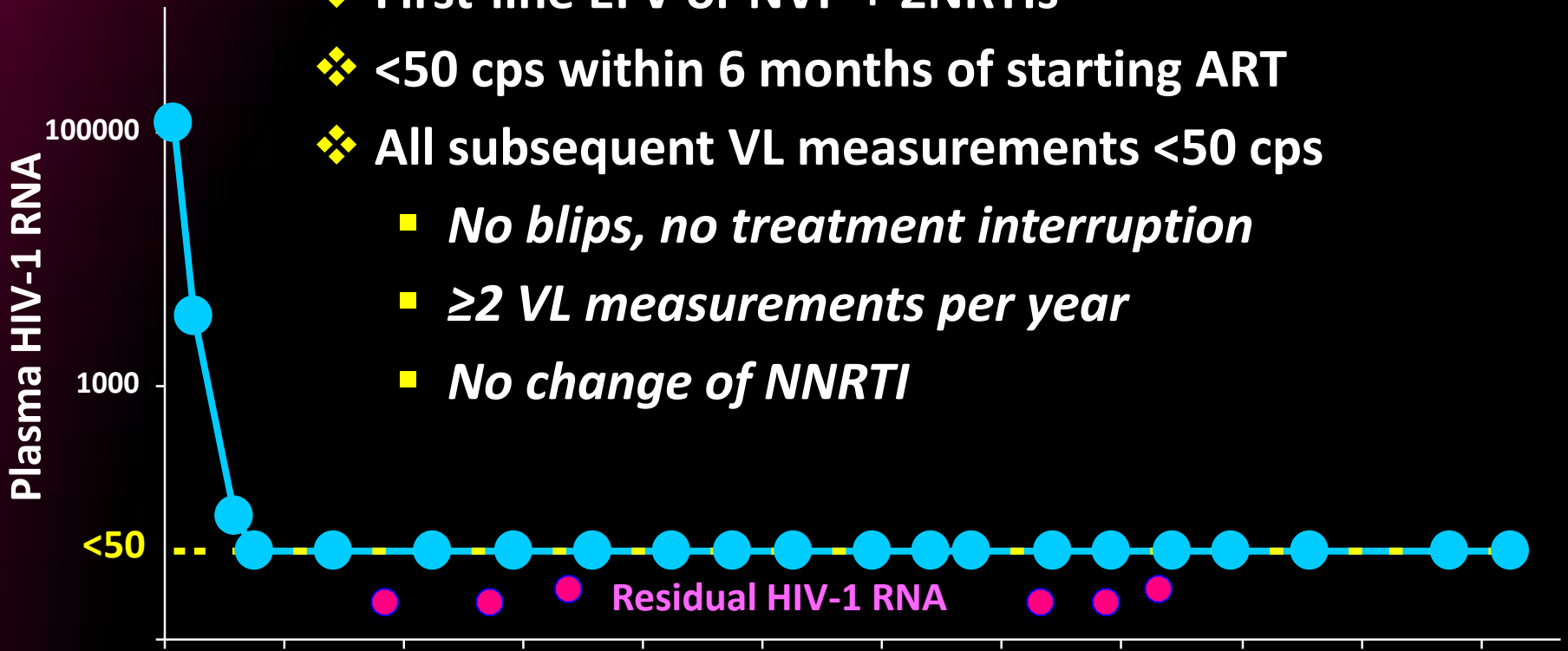


Siemens Ultrasensitive Assay

Maggiolo et al. J Acquir Immune Defic Syndr 2012

HIV-1 RNA detection in patients with consistent suppression <50 cps for ≤15 years

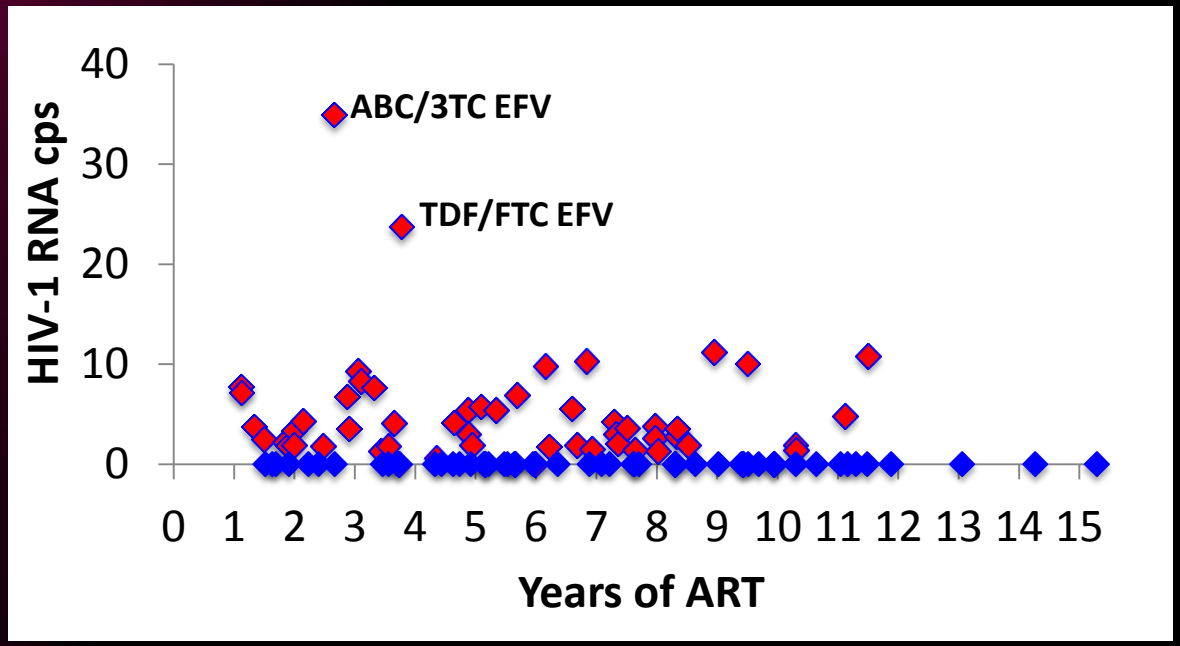
- First-line EFV or NVP + 2NRTIs
- <50 cps within 6 months of starting ART
- All subsequent VL measurements <50 cps
  - *No blips, no treatment interruption*
  - *≥2 VL measurements per year*
  - *No change of NNRTI*



Geretti et al. International Workshop on HIV & Hepatitis Viruses Drug Resistance 2013

HIV-1 RNA detection in plasma

- Plasma HIV-1 RNA detected in 52/104 (50%) patients



- No association with age, sex, race, risk group, duration of HIV diagnosis, nadir & current CD4 count, pre-ART viral load, NNRTI used, NNRTI concentration

HIV-1 RNA cps/ml	Years VL <50 cps/ml			Total (n=104)	*P*
	0-4 (n=31)	5-7 (n=33)	8-15 (n=40)		
Median (range)	3 (1, 35)	3 (1, 10)	3 (1, 11)	3 (1, 35)	*0.451*
Mean $\log_{10}$ (SD)	0.6 (0.3)	0.5 (0.2)	0.5 (0.2)	0.5 (0.2)	*0.451*

Geretti et al. International Workshop on HIV & Hepatitis Viruses Drug Resistance 2013

What causes low-level HIV-1 RNA detection during ART?

Technique-related

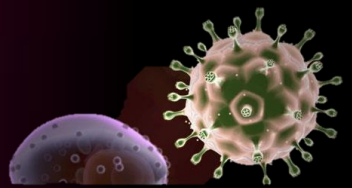
Patient-related

Drug-related

Virus-related

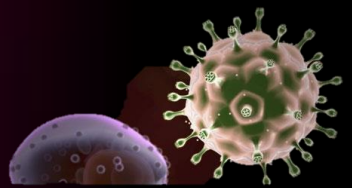
Take away points: Viral load testing

- Importance of lab-clinic dialogue
  - *Assay selection, sample handling, result interpretation*



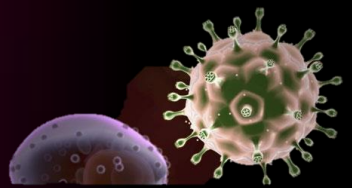
Take away points: the 50 copies cut-off

- Well validated as a target of ART
- Importance of regular monitoring in the first year after achieving <50 cps
  - *Persistent viral load <50 cps during this period predicts a very low risk (5%) of subsequent rebound*
- Importance of confirming viral load rebound >50 cps in a subsequent sample
  - *Confirmed low-level viraemia predicts increased risk of rebound >400 and >1000 cps*
- Most (but not all) new assays read “50” as older assays did



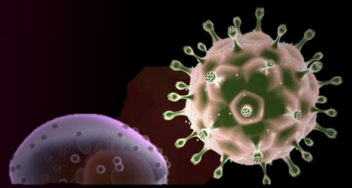
Take away points: New cut-offs

- Detection of HIV-1 RNA levels of 10-50 cps predicts rebound >50 and >400 cps
  - *Suggestive of ongoing virus replication in at least a subset of patients with LLV detection below the 50 cps threshold*
- During long-term, seemingly suppressive ART, HIV-1 RNA remains detectable in plasma at levels ~ 3 cps using research assays
  - *Different population from that with levels ~10-49 cps*
  - *Not associated with a risk of rebound*
  - *Viral load not usually detectable by current commercial assays*

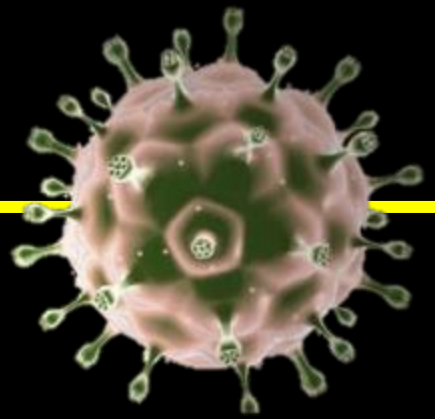


Looking ahead

- Studies required to define the optimal management of patients with low-level HIV-1 RNA detection both above and below the 50 cps cut-off
- At present, readings below 50 cps obtained with available commercial assays can be used to define need for patient review and optimal frequency of monitoring



- Andrew Phillips, Alessandro Cozzi-Lepri, UCL, London
- Ana Garcia, Royal Free NHS Foundation Trust, London
- Stacey King, Andrew Owen, University of Liverpool
- Nicola Mackie, Imperial College Healthcare NHS Trust, London
- Jonathan Ainsworth, Anele Waters, N. Middlesex University Hospital, London
- Frank Post, King's College Hospital, London
- Simon Edwards, Central & Northwest London Community NHS Foundation Trust, London
- Julie Fox, St Thomas' Hospital, London



Thank you