Dynamics of Ultra-Deep Suppressed HIV Viremia (<5 copies/ml HIV-RNA) in cART-treated Patients

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Background: HIV residual viremia (RV), persists in 70-90% of patients under successful cART over extended periods[1,2] and clear association with virological failure risk has been demonstrated[3]; however RV dynamics, particularly during prolonged viral suppression (VS), is not completely defined[4-6].

Objectives: Our aim was to monitor RV (HIV RNA detected between 5 and 50 copies/ml) kinetics during successful cART with an ultrasensitive assay in effectively first-line cART-treated patients. For this purpose, patients were aligned at the time of achievement of first viral suppression (VS; HIV-RNA <50 copies/ml) and followed-up for almost 1 year.

Predictors of reaching and maintaining ultra-deep viral suppression (UVS; HIV-RNA <5 copies/ml)) were also studied.

Methods: This study included 60 HIV-infected patients (Table 1) attending the outpatient facility of the "Lazzaro Spallanzani" Hospital in Rome, enrolled in prospective, observational studies on early response to cART, in chronic (CHI) or primary (PHI) HIV infection, *naïve* for antiretrovirals. First-line cART was not dictated by the study protocol, but prescribed by the caring physician.

The kinetics of RV (HIV-RNA detected between 5 and 50 copies/ml) was assessed in patients reaching VS (HIV-RNA below 50 copies/ml) and maintaining it continuously for one year (n=60 patients) and two year (n=38 out of 60 patients).

Probability to achieve and maintain UVS (HIV-RNA <5 copies/ml) was also established in respect of demographics, primary HIV infection (PHI) status, pre-therapy HIV-RNA and CD4, time to first VS and cART regimen by Cox proportional hazard models.

Patients included in this analysis met the following criteria: i) being cART-naive and starting first-line regimen; ii) not having primary mutations conferring resistance to antiretrovirals at GRT; iii) not interrupting cART or switching to a second-line regimen; iv) achieving first VS within 60 weeks from start of cART; v) maintaining stable VS after first HIV-RNA <50 copies/ml for one year; vi) completing the study visits. First-line cART was not dictated by the study protocol, but prescribed by the caring physician.

Overall, the entire follow-up lasted on average 103 weeks (range, 48-132) from start of cART. For patients with follow-up of 12 months after first VS, the study lasted a mean of 68 weeks (range, 48-108); for those with 24 months follow-up after, the observation period lasted a mean of 116 weeks (range, 96-132). Demographics, clinical and cART-related characteristics were abstracted from clinical charts and collected anonymously.

Blood samples were collected at initiation of cART (baseline), then every 4 weeks. Descriptive statistics of $\log_{10}$-transformed viral results and of immunological values were summarized with the median and the interquartile ranges (IQR). Comparisons of viral loads were carried out at the following fixed time-points: cART initiation (t0), first VS (t1), 6 (t2), 12 (t3), 18 (t4) and 24 (t5) months after t1. Dates were rounded to the nearest time point (+/-2 weeks). Routine viral loads and RV were measured with standard and ultrasensitive (US) protocols[7] of the Abbott Real-Time HIV-1 assay (LLOD: 40 and 5 copies/ml HIV-RNA, respectively).

All patients participating to the study provided written informed consent for analysis of stored samples and anonymous data collection from clinical charts.

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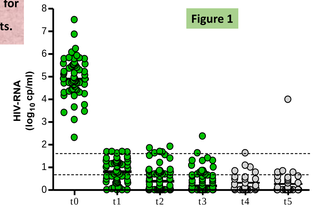
Table 1: Demographic, clinical and viro-immunological characteristics.

	N	%
Gender		
Male	54	90.0
Female	6	10.0
HIV transmission mode		
Heterosexual	22	36.7
MSM	38	63.3
HIV-1 Subtype		
B	40	66.7
Non B	20	33.3
Primary HIV Infection (PHI)		
Yes	11	18.3
No	49	81.7
Baseline CD4 (cells/mmc)		
<200	16	26.7
≥200	44	73.3
Type of cART regimen		
3 ARVs	50	81.2
4 ARVs including raltegravir	10	18.3
Time to first VS (HIV-RNA <50 copies/ml)		
≤12 weeks	26	43.3
>12 weeks	34	56.7
Baseline HIV-RNA ($\log_{10}$ copies/ml)		
Median value (IQR)		4.89 (4.44-5.54)
Baseline total HIV-DNA ($\log_{10}$ copies/10^6PBMC)		
Median value (IQR)		3.99 (3.63-4.49)

Abbreviation: ARVs: antiretroviral therapies.

Figure 1: HIV-RNA values and median measured in 60 patients during first year of steady VS (green circles) at t1, t2 and t3: 0.81 (IQR, 0.40-1.23), 0.39 (IQR, 0.00-0.78), and 0.18 (IQR, 0.00- 0.68) $\log_{10}$copies/ml HIV-RNA (or 6.5, 2.5 and 1.5 copies/ml HIV-RNA), respectively; t1 *vs* t2 and *vs* t3: *p≤0.0005*.
In all but one of 38 patients (grey circles) who had 2-year of VS, RV continued to decrease [median RV at t1, t3, t4 and t5: 0.97 (IQR, 0.54-1.28), 0.18 (IQR, 0.00-0.78), 0.10 (IQR, 0.00-0.48), and 0.00 (IQR, 0.00-0.40) $\log_{10}$ copies/ml HIV-RNA, respectively (t1 *vs* t4 and *vs* t5: *p≤0.0002*).
The above horizontal dashed line indicates the conventional clinical cut-off for VS (1.70 $\log_{10}$ copies/ml or 50 copies/ml HIV-RNA); the lower horizontal dashed line indicates the LLOD of US (0.70 $\log_{10}$ copies/ml, corresponding to 5 copies/ml HIV-RNA).

Figure 1



Results 1: First VS (t1) was obtained within 12 weeks from start of cART (t0) in 26 (43.3%) patients, within 24 weeks in 26 (43.3%), and thereafter in the remaining 8 (14 %) patients. During the first year of continuous VS, **steady decrease of RV occurred** (Figure 1).

Cross-sectional analysis showed a significant **increase in the number of patients reaching not-detectable HIV-RNA during VS follow-up**: 4 (**6.7%**) at first VS (t1), 19 (**31.7%**) at t2, 27 (**45.0%**) at t3 (*p<0.001*). In the 38 patients with an additional year of VS follow-up, the proportion of those showing not-detectable HIV-RNA increased from **5.3%** at t1, to **42.1%** and **63.2%** at t3 and t5, respectively (t1 *versus* t3 and *versus* t5: *p≤0.001*).

During the first year VS, **all patients achieved UVS at least once**, after a mean time of 15.3 weeks (SD, +/- 19.9) from t0. After that, **27 (45%) maintained USV throughout the study; the remaining 33 patients showed persistent RV levels fluctuating around 5-10 copies/ml HIV-RNA** (Figure 2).

Among patients **maintaining UVS** throughout the study, the proportion of those reaching **not-detectable HIV-RNA** increased over time: 2/27 (**7.4%**) at t1, 16/27 (**59.2%**) at t3 and 15/17 (**88.2%**) at t5 (t1 *versus* t3: *p<0.001*; t3 *versus* t5: *p<0.001*).

Results 2: Significant differences in the proportion of subjects **achieving UVS** were observed when time to first VS, pre-cART $CD4^+$ T cell count, pre-cART HIV-RNA and PHI/CHI status were considered (Figure 3). At multivariable Cox regression analysis, **factors associated with UVS achievement** were cART initiation during PHI (aHR 5.83; 95%CI 2.03-16.71; *p=0.001*) and lower HIV-RNA (aHR 0.52; 95%CI 0.33-0.82; *p=0.005*), as expected.

Among patients **maintaining UVS** throughout the follow-up, significant differences were observed when stratifying the study population according to time to first VS, pre-cART $CD4^+$ T cell count and pre-cART HIV-RNA (Figure 4). **Predictors associated with loss of UVS** were >12 weeks to reach first VS, <200/mmc $CD4^+$ T cells counts pre-cART and lower pre-cART HIV-RNA concentration (Table 2).

Figure 3: Proportion of patients achieving UVS after start of cART (t0), stratified according to main viro-immunological characteristics (as shown in the panels).

Figure 2: Kinetics of viremia (median) from start of cART (t0) in patients reaching and maintaining UVS (dashed black line) and in those showing fluctuating RV (solid red line).
The above horizontal dashed line indicates the conventional clinical cut-off for VS (1.70 $\log_{10}$ copies/ml, corresponding to 50 copies/ml HIV-RNA); the lower horizontal dashed line indicates the LLOD of ultrasensitive assay used to measure RV (0.70 $\log_{10}$ copies/ml, corresponding to 5 copies/ml HIV-RNA).

Figure 2

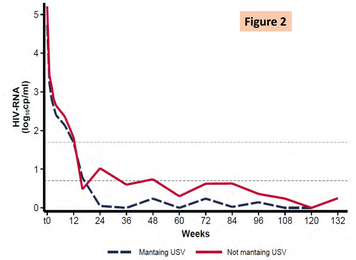


Figure 3

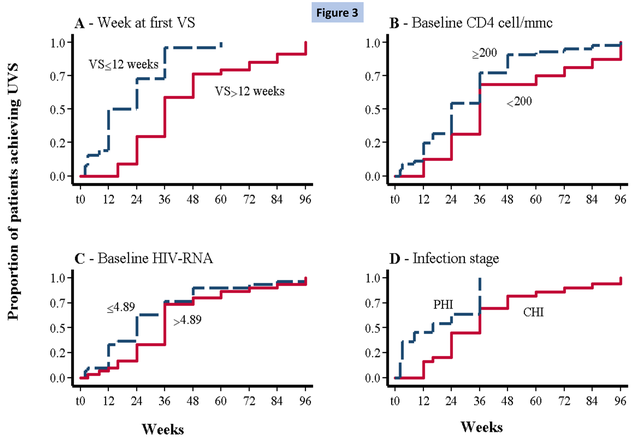


Figure 4

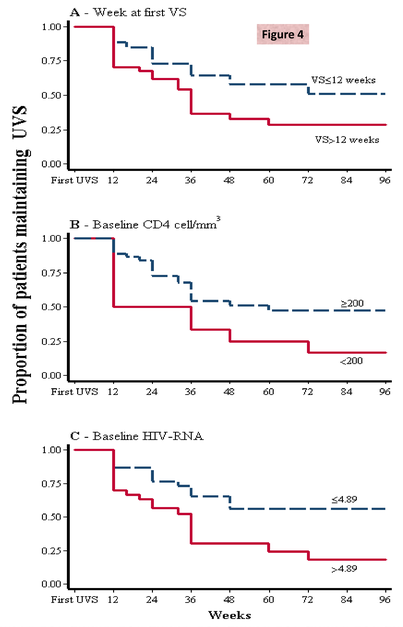


Figure 4: Proportion of patients maintaining UVS, stratified according to main viro-immunological characteristics.

Table 2: Variables associated with loss of UVS at Cox proportional hazard model.

Characteristics	Time to first viremia above 5 copies/ml after first UVS Univariable HR (95%CI)	p-value	Multivariable aHR (95%CI)	*p-value*
Age (per 10 years increase)	1.13 (0.81-1.58)	0.471		
Gender				
Male	Ref.			
Female	1.59 (0.50-5.07)	0.437		
HIV transmission mode				
Heterosexual	Ref.			
MSM	0.64 (0.30-1.36)	0.247		
HIV-1 Subtype				
B	Ref.			
NB	1.72 (0.79-3.71)	0.170		
Primary HIV infection (PHI)				
No	Ref.			
Yes	0.40 (0.11-1.38)	0.147		
BL CD4 T cells (cells/mmc)				
≥200	Ref.			
<200	2.42 (1.09-5.35)	0.029	3.97 (1.61-9.77)	***0.003***
BL CD4 to CD8 ratio				
<1.0	Ref.			
≥1.0	0.66 (0.15-3.03)	0.598		
BL HIV-RNA ($\log_{10}$ copies/mL)	1.88 (1.16-3.04)	0.011	2.14 (1.23-3.72)	***0.007***
Type of cART regimen				
- NNRTI-based 3 ARVs	Ref.			
- IP/b-based 3 ARVs	1.40 (0.63-3.13)	0.410		
- INI-based 4 ARVs	0.45 (0.12-1.63)	0.224		
Time to first VS				
≤12 weeks	Ref.		Ref.	
>12 weeks	2.02 (0.93-4.40)	0.075	2.51 (1.07-5.89)	***0.034***

Abbreviations - HR: Hazard Ratio; aHR: adjusted Hazard Ratio; 95%CI: 95% Confidence Interval; ARVs: antiretroviral therapies.

Conclusions: In this longitudinal analysis, conducted on 60 patients starting their first cART and maintaining VS up to 2 years, a progressive decline in RV towards UVS (<5 copies/ml HIV-RNA) was observed.

In all patients, HIV-RNA decreased below 5 copies/ml at least once during the first year of stable VS and the proportion of those reaching not-detectable HIV-RNA gradually increased over the first year after VS. This trend was confirmed during the second year of stable suppression in the subgroup of patients with a longer follow-up.

UVS was maintained in almost half of patients during follow-up, while the remaining patients showed fluctuating RV levels around a median of 5-10 copies/ml HIV-RNA.

The predictors of UVS achievement and maintenance, particularly shorter time to reach VS (together with pre-cART lower viremia and higher $CD4^+$ cells number), sustain current guideline recommendations to initiate cART early with a potent regimen. In addition, the study suggests a cART-driven progressive purging of viral reservoirs supporting the possibility of functional cure.