Slow or fast viral load decay as a predictor of residual viremia level in HIV-infected patients undergoing successful first-line cART

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INTRODUCTION

Current guidelines (AIDSinfo NIH Guidelines 2017; EACS Guidelines 2017) define the long-term suppression of HIV-RNA below 50 copies/ml as the main marker of a successful cART. Although this goal is currently achieved by the majority of patients retained in care (Viswanathan et al. 2015), ultrasensitive assays have demonstrated that residual HIV-RNA remains detectable in the plasma and stable around a median of 3-10 copies/ml in about 80% of effectively treated patients (Palmer et al., 2008; Zheng et al., 2013). The RV may represent either the product of ongoing viral replication from sanctuary sites or occasional virus release from latently infected CD4 lymphocytes; probably both mechanisms coexist (Doyle et al., 2012). Despite evidence of a progressive slow decay over time (Ripamonti et al., 2013; Riddler et al., 2016), RV persists for many years (Palmer et al., 2008; Zheng et al., 2013), even after treatment intensification with integrase inhibitors (Llibre et al., 2012). The clinical meaning of RV and its impact on the long-term management of HIV-infected patients remain controversial. Several studies have demonstrated the association between RV and the risk of virological rebound above 50 copies/ml in successfully treated patients (Doyle et al., 2012; Calcagno et al., 2015), but this association has not been always found (Charpentier et al., 2012). Furthermore, a possible role of RV in the enhancement of chronic immune activation and in the emergence of drug resistance in non-adherent patients has been hypothesized, but deserves further investigation (Sahu 2015; Riddler et al., 2016).

Several factors have been associated with a higher risk of persistence of RV: high HIV-RNA levels pre-cART (Havlir et al., 2005; Palmer et al., 2008; Ripamonti et al., 2013), virological suppression longer than two years (Zheng et al., 2013; Calcagno et al., 2015), high amounts of HIV-DNA in PBMC (Havlir et al., 2005), older age (Zheng et al., 2013), immunological parameters (lower CD4/CD8 ratio, higher CD8 count) (Riddler et al., 2016), lower CD4 nadir (Sahu, 2015) and use of protease inhibitors (PI) instead of non-nucleoside reverse transcriptase inhibitors (NNRTI) (Doyle et al., 2012; Llibre et al., 2012).

This prospective observational study analyzed RV kinetics during one year following cART-induced steady VS to detail if different trends occur at single-patient level. The RV was measured with the ultrasensitive protocol of the Abbott Realtime HIV-1 assay, able to measure up to 5 copies/ml HIV-RNA with high accuracy and precision.
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(Amendola et al., 2011). Further, predictors of achievement and maintenance of ultra-deep RV suppression (URVS), defined as plasma HIV-RNA below 5 cp/ml, were evaluated.

MATERIALS AND METHODS

Participants

This study included HIV-infected patients 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.

Patients met the following criteria:

1) being cART-naive and starting a first-line regimen;
2) not having primary mutations conferring resistance to antiretrovirals at baseline;
3) not interrupting cART or switching to a second-line regimen;
4) achieving VS within 60 weeks from cART start;
5) maintaining stable VS after first HIV-RNA <50 copies/ml for one year;
6) completing the study visits. First-line cART was prescribed by the caring physician.

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

Virological and immunological investigations

Blood samples were collected at initiation of cART (baseline; t0), every 4 weeks after t0 until VS (t1), and thereafter at 6 (t2), 12 (t3), 18 (t4), and 24 (t5) months after t1. Routine viral load measurement was performed with the Abbott RealTime HIV-1 assay (LLOD: 40 copies/ml) according to the manufacturer’s instructions. RV was quantified with the ultrasensitive protocol of the Abbott RealTime HIV-1 assay (LLOD: 5 copies/ml), obtained applying the following modifications to the standard procedure (Amendola et al., 2011): 1) higher sample volume (3.2 ml) concentrated by ultracentrifugation; 2) calibration curve extended towards lower HIV-RNA levels; 3) reduced volume of internal control; 4) “open” software. Each patient underwent multiple quantifications of RV (on average 4.5 tests/patient during the first year of VS, and 2 tests/patient during the second year of VS). To reduce bias and differences, RV was quantified at the end of the study, on samples stored at -80°C, by including all samples of the same patient (batch of 4-5 specimens) in the same working run. HIV-RNA values <5 copies/ml were included in the statistical analysis, although these results fell outside the 95% CI (Amendola et al., 2011).

Total HIV-DNA (HIV-DNA) was quantified by real-time PCR targeting HIV-LTR and hTERT to refer HIV-DNA copies to a million of PBMC (Rozera et al., 2010) or of CD4 (Josefsson et al., 2011). Immunological tests included CD4 and CD8 count according to standard monitoring procedures.

Statistical analyses and study definitions

Descriptive statistics of log$_{10}$-transformed viral results and of immunological values were summarized with the median and the interquartile ranges (IQR) at the following fixed time-points: cART initiation (t0), at VS (t1), 6 (t2), 12 (t3), 18 (t4) and 24 (t5) months after t1. Dates were rounded to the nearest time point (+/-2 weeks). Wilcoxon signed rank sum test was used to test the equality of medians; McNemar and Cochran’s Q tests to analyse proportions of patients reaching URVS; Mann-Whitney test to compare HIV-RNA between two independent groups observed at the same time point. Covariates considered in the analyses were: age, gender; HIV transmission mode (MSM versus heterosexual), HIV-1 subtype (B versus non-B subtype), phase of HIV infection (PHI versus CHI), cART regimen (triple versus quadruple), baseline HIV-RNA (considered as continuous variable in Cox analyses or as categorical variable in the graphs: below versus above the median observed in the entire cohort), CD4 (considered as continuous variable in the Cox analyses or as categorical variable in the graphs: <200 versus ≥200 cells/mm$^3$), CD4/CD8 ratio (<1 versus ≥1), time to achieve VS after cART initiation (<12 versus >12 weeks) and HIV-DNA (as continuous variable). Kaplan-Meier was used to show temporal trends across categorical variables. Cox proportional hazard models, with exact partial likelihood to handle tied failures and time measured in weeks, were carried out to analyse factors associated with:

1. time to URVS (baseline: start of cART);
2. time to first viremia above 5 copies/ml following URVS (baseline: first URVS).

In the multivariable models, all variables associated with the outcome in the univariable analysis with a $p$-value equal or lower than 0.1 (unless otherwise specified) were included.

Ethics statement

The study received the INMI’s ethics committee approval (No. 22/2011 of the INMIs trials register) and patients participating in the study provided written informed consent.

RESULTS

Patient population

Of the 87 patients considered, 60 met the inclusion criteria (reasons for exclusion: virological failure (n=1); two or more viral blips during follow-up (n=8); more than 60 weeks of cART to obtain VS (n=10); withdrawal of consent (n=8)). Patient’s baseline demographic, clinical and viro-immunological characteristics were: 54 (90%) males; HIV acquisition through sexual intercourse [22 (36.7%) heterosexual; 38 (63.3%) MSM]; 11 (18.3%) PHI; non-B HIV-1 subtype in 33.3%; CD4 <200/mm$^3$ in 16 (26.7%); median HIV-RNA 4.89 (IQR, 4.45-5.54) log$_{10}$ copies/ml; median HIV-DNA 3.99 (IQR, 3.63-4.49) log$_{10}$ copies/10$^6$PBMC. First-line cART in 10 out of 11 patients with PHI, was a quadruple regimen based on two nucleoside reverse transcriptase inhibitors (NRTI) plus a boosted protease inhibitor (PI/b) together with raltegravir.

All other patients received a triple regimen based on two NRTIs plus either a non-nucleoside reverse transcriptase inhibitor (NNRTI) or PI/b.
Longitudinal RV assessment towards ultra-deep RV suppression (URVS)

Figure 1A shows the HIV-RNA, CD4/CD8 ratio and HIV-DNA from cART initiation (t0) to last observation. VS (t1) was obtained within 12 weeks from t0 in 26 (43.3%) patients, within 24 weeks in another 26 (43.3%), and thereafter in the remaining 8 (14%) patients. Median log10 HIV-RNA decreased from 4.89 copies/ml (IQR, 4.44-5.54) at baseline to 0.81 copies/ml (IQR, 0.40-1.23) at t1. Median CD4/CD8 ratio rose from 0.42 (IQR, 0.26-0.64) at baseline, to 0.84 (IQR, 0.57-1.22) at t3 and 0.83 (IQR, 0.57-1.13) at t5. Median log10 copies/1x10^6 PBMC HIV-DNA decreased from 3.99 (IQR, 3.63-4.49) at baseline to 3.50 (3.04-3.82) at t1 (p<0.0001), remaining quite stable until week 120.

During the first year of cART-induced VS, a steady decrease of RV occurred [median HIV-RNA 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) log10 copies/ml (or 6.5, 2.5 and 1.5 copies/ml), respectively; t1 versus t2 and versus t3: p<0.0005] (Figure 1B). In all but one of 38 patients who had a two-year VS, RV continued to decrease, with median RV at t1, t3, t4 and t5 of 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) log10 copies/ml HIV-RNA, respectively (t1 versus t4 and versus t5: p<0.0002) (Figure 1B). Cross-sectional analysis showed a significant increase in the number of patients reaching undetectable HIV-RNA: 4 (6.7%) at t1, 19 (31.7%) at t2, 27 (45.0%) at t3 (p<0.001). Among the 38 patients with an additional year of VS follow-up, the proportion of those showing undetectable 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 of VS, each patient achieved URVS (HIV-RNA <5 copies/ml) at least once, after a mean time of 15.3 weeks (SD, +/- 19.9) from t0 (Figure 1C).

Factors predictive of URVS achievement

To identify the factors predictive of the fast achievement of URVS, patients were stratified according to the main viro-immunological characteristics and significant differences were actually observed (Figure 2 A, B, C, D and Supplementary Figure 1). Patients achieving the VS within 12 weeks from the start of cART reached URVS earlier, when compared to those achieving the VS later (differences between groups at t3: p<0.001) (Figure 2A). In these patients with faster URVS, median HIV-RNA values at t1, t2 and t3 were 0.98 (IQR, 0.50-1.50), 0.16 (IQR, 0.20-0.50), and 0.00 log10 copies/ml (IQR: 0.00-0.00), (or 9.5, 1.1 and 0.0 copies/ml), respectively. Conversely, patients who reached the VS slowly showed a lesser decline of viremia, with median HIV-RNA at t1, t2 and t3 of 0.97 (IQR, 0.10-0.90) and 0.48 log10 copies/ml (IQR, 0.10-0.85), (or 5.5, 5.0 and 3.0 copies/ml), respectively. Notably, baseline viral loads were similar in the two patients groups: median log10 copies/ml HIV-RNA 4.73 (IQR, 4.19-5.78) and 4.94 (IQR, 4.65-5.49), respectively (p=0.516).

Patients with pre-cART ≥200/mm³ CD4 or pre-cART HIV-RNA below the median of the study population obtained URVS earlier than those with <200/mm³ CD4 or HIV-RNA above the median at baseline, already 4 weeks after t1, and remained ultra-suppressed in higher proportion (Figure 2B, C).

The proportions of PHI and CHI reaching URVS are depicted in Figure 2D. PHI reached URVS earlier than CHI, having obtained the VS quickly [median weeks: 8

Figure 1 - HIV-RNA, CD4/CD8 cell ratio, HIV-DNA and proportion of patients reaching ultra-deep RV suppression (URVS) from start of cART (t0) to end of follow-up. (A) Median HIV-RNA (green line) and CD4/CD8 ratio (red line) are shown on the left axis; median HIV-DNA in PBMC (orange line) is on the right axis over 135 weeks of observation from the start of cART (t0). (B) HIV-RNA values (and median) measured in each participant (green stained) at the main time points of the study: start of cART (t0), time of VS achievement (t1), 24 weeks after t0 (t2), 48 weeks after t0 (t3) and, in 38 patients (grey stained), 18 months (t4) and 24 months after t0 (t5). The above horizontal dashed line indicates the conventional clinical cut-off for VS (1.70 log10 copies/ml, corresponding to 50 copies/ml HIV-RNA). The lower horizontal dashed line indicates the LLOD of the ultrasensitive assay (0.70 log10 copies/ml, corresponding to 5 copies/ml HIV-RNA). (C) All patients achieved URVS at least once during the VS following cART start.
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**Figure 2** - Achievement of URVS after start of cART (t0) in patients stratified according to the main viro-immunological characteristics. (A) Patients stratified according to the interval of time to VS from start of cART: within 12 weeks (blue line) or after 12 weeks (red line). (B) Patients distinguished on the base of CD4 at start of cART: ≥200/mm³ (blue line) or <200/mm³ (red line). (C) Patients selected above (red line) or below (blue line) pre-cART median viremia (4.89 log₁₀ copies/ml HIV-RNA). (D) Patients enrolled during PHI (blue line) or CHI (red line).

**Table 1** - Favorable factors associated with achievement of ultra-deep RV suppression (URVS) at Cox proportional hazard model.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>First URVS from baseline (t0)</th>
<th>Univariable</th>
<th>p-value</th>
<th>Multivariable</th>
<th>aHR (95%CI)</th>
<th>p-value</th>
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<td>Age (per 10 years increase)</td>
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<tr>
<td>Female</td>
<td>2.33 (0.79-6.86)</td>
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<td>HIV transmission mode</td>
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<td>MSM</td>
<td>1.14 (0.61-2.17)</td>
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<td>HIV-1 Subtype</td>
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<tr>
<td>NB</td>
<td>1.21 (0.62-2.34)</td>
<td>0.578</td>
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<td>Primary HIV Infection (PHI)</td>
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<td>No</td>
<td>Ref.</td>
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<tr>
<td>Yes</td>
<td>3.64 (1.46-9.06)</td>
<td>0.006</td>
<td>5.83 (2.03-16.71)</td>
<td>0.001</td>
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<td>BL CD4 cells/mmc</td>
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<td>≥200</td>
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<td>&lt;200</td>
<td>0.49 (0.24-1.01)</td>
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<td>1.63 (0.77-3.46)</td>
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<td>BL CD4 to CD8 ratio</td>
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<td>&lt;1.0</td>
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<td>≥1.0</td>
<td>1.32 (0.41-4.21)</td>
<td>0.638</td>
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<td>BL HIV-RNA (log₁₀ copies/mL)</td>
<td>0.70 (0.45-1.07)</td>
<td>0.100</td>
<td>0.52 (0.33-0.82)</td>
<td>0.005</td>
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<td>Type of cART regimen</td>
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<td>- NNRTI-based 3 ARVs</td>
<td>Ref.</td>
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<tr>
<td>- IP/β-based 3 ARVs</td>
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<td>0.340</td>
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<tr>
<td>- INI-based 4 ARVs</td>
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<td>0.014</td>
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Abbreviations - HR: Hazard Ratio; aHR: adjusted Hazard Ratio; 95%CI: 95% Confidence Interval; ARVs: antiretroviral therapies.
(IQR, 3-12) vs 24 (IQR, 12-24); p<0.001]: they achieved lower RV level at t1 [median HIV-RNA 0.48 log_{10} copies/ml (IQR, 0.35-1.23)], with a further reduction during follow-up: at t3, 8 out 11 patients (72.7%) showed undetectable HIV-RNA [median HIV-RNA: 0.00 (IQR, 0.00-0.60) log_{10} copies/ml]. On the contrary, CHI showed a lesser decrease of RV [t1 and t3 median HIV-RNA: 0.95 (IQR, 0.48-1.29) and 0.30 (IQR, 0.00-0.74) log_{10} copies/ml, respectively] and, at t3, only 19 out of 49 (38.7%) patients had undetectable HIV-RNA. In parallel, the HIV-DNA, although similar between PHI and CHI at baseline, diminished mainly in PHI [median log_{10} copies/1x10^6 PBMC at t0, t1, and t3: 4.01 (IQR, 3.45-4.79), 3.01 (IQR, 2.67-3.63), and 2.49 (IQR, 2.34-3.27)] and less in CHI [at t0, t1, and t3: 3.93 (IQR, 3.65-4.42), 3.62 (IQR, 3.21-3.96), 3.62 (IQR, 3.33-3.87)], with a significant difference between groups at t3: p=0.002.

At univariable Cox regression analysis (Table 1), factors associated with fast achievement of URVS were cART initiation during PHI, quadruple raltegravir-based cART and pre-treatment >200/mm^3 CD4. In multivariable analysis, cART initiation during PHI and lower HIV-RNA below the median at baseline remained associated to this outcome. HIV-DNA in PBMC and in CD4 was also evaluated, even though this information was available only for 43 patients, but it did not provide significant contribution to both univariable and multivariable Cox regression analysis.

Factors predictive of URVS maintenance

After URVS achievement, 27 (45%) out of 60 participants maintained this condition throughout the follow-up (me-
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Median: 72 weeks, IQR: 36-96) and the proportion of patients reaching undetectable HIV-RNA increased over time: 2/27 (7.4%) at t1, 11/27 (59.2%) at t3 and 15/17 (88.2%) at t5 (t1 versus t3: p<0.001; t3 versus t5: p<0.001) (Figure 3A). On the contrary, the remaining 33 patients (55.0%) showed RV rebound above 5 copies/ml: 13 (21.6%) just 12 weeks after URVS, others (33.3%) within 72 weeks (median 24 weeks, IQR: 12-36). In these patients, RV fluctuated long around a set-point of 5-10 copies/ml HIV-RNA before showing a tendency to decline only at the end of follow-up (Figure 3 B).

The factors associated with the loss of URVS and RV rebound to fluctuating levels were analyzed and the time to VS, pre-cART CD4 and pre-cART HIV-RNA resulted highly influential (Figure 4 A, B, C). At univariable analysis (Table 2), predictors of URVS loss were pre-treatment CD4 <200/mm³, each log increase of pre-cART HIV-RNA and the long time to reach VS (>12 weeks); in the multivariable analysis, all of them remained associated with this outcome.

**DISCUSSION**

Despite intensive investigation, the clinical significance and long-term consequences of RV are still not fully understood. For example, higher levels of RV in cART-treated patients are predictive of virologic rebound (Doyle et al., 2012) or associated with viral evolution (Libbre et al., 2012), development of drug resistance (Santoro et al., 2014), incomplete medication adherence (Li et al., 2014), and greater chronic inflammatory status (Ostrowski et al., 2008). However, some of these findings were not always confirmed (Kieffer et al., 2004; Evering et al., 2012; Teira et al., 2016) and the utilization of low viremia in clinical management remains a matter of debate (Ryscavage et al., 2014; Doyle et al., 2016). In addition, single-patient detailed RV kinetics during the first years of VS is lacking and studying the RV trend in depth could help clarify the characteristics of RV persistence and its role.

This longitudinal analysis conducted on 60 patients starting first-line cART and maintaining VS up to two years

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>First viremia above 5 copies/ml after URVS</th>
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<tr>
<td></td>
<td>Univariable</td>
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<tr>
<td></td>
<td>HR (95%CI)</td>
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<tr>
<td>Age (per 10 years increase)</td>
<td>1.13 (0.81-1.58)</td>
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<td>Gender</td>
<td>Male Ref.</td>
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<td>HIV transmission mode</td>
<td>Heterosexual Ref.</td>
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<td>HIV-1 Subtype</td>
<td>B Ref.</td>
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<td>Primary HIV infection (PHI)</td>
<td>No Ref.</td>
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<td>BL CD4 cells/mmc</td>
<td>&gt;200 Ref.</td>
</tr>
<tr>
<td>BL CD4 to CD8 ratio</td>
<td>&lt;1.0 Ref.</td>
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<tr>
<td>BL HIV-RNA (log₁₀ copies/mL)</td>
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<tr>
<td>Type of cART regimen</td>
<td>NRRTI-based 3 ARVs Ref.</td>
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<td>- INI-based 4 ARVs 0.45 (0.12-1.63)</td>
<td>0.224</td>
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<td>Time to VS</td>
<td>&lt;12 weeks Ref.</td>
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Abbreviations - HR: Hazard Ratio; aHR: adjusted Hazard Ratio; 95%CI: 95% Confidence Interval; ARVs: antiretroviral therapies.
shows that RV steadily decreases when median residual HIV-RNA levels were considered: in fact, RV dropped below 5 copies/ml at least once in each patient. However, after this drop, almost half the patients maintained stable URVS and during the follow-up the proportion of those reaching undetectable HIV-RNA gradually increased. Conversely, the other patients showed a RV rebound that persistently fluctuated around 5-10 copies/ml before a slight decline at the end of the study.

Our findings are consistent with two recently published studies showing a continuous RV decay beyond the first year of suppressive cART (Ripamonti et al., 2013; Riddler et al., 2016), but seem to be in contrast to prior reports describing a biphasic pattern of RV decay with a plateau after the first year of VS (Palmer et al., 2008; Hatano et al., 2010). Our data describe considerable inter-individual differences in RV kinetics during VS following successful cART. Despite the achievement of URVS by the whole population, two different trends of RV in terms of both viral decay rate and URVS maintenance were indeed observed. The slope of RV decay was influenced by pre-cART viro-immunological conditions and stage of infection in which treatment was started: lower pre-cART HIV-RNA and therapy initiation during PHI were significantly associated with the fast achievement of URVS. Instead URVS maintenance was dependent on the short time to achieve VS and favorable pre-cART viro-immunological conditions.

The correlation between RV persistence and both pre-treatment HIV-RNA concentration and immunological status has already been described in several reports (Palmer et al., 2008; Doyle et al., 2012; Ripamonti et al., 2013; Sahu 2015; Riddler et al., 2016). In our study, the faster URVS achievement in patients with lower baseline HIV-RNA could be explained by the direct relationship existing between baseline HIV-RNA and time needed to VS (Rizzardi et al., 2000). The association between pre-treatment higher viremia and loss of URVS may conversely suggest a link between the extent of baseline viral spread and the infection of long-lived reservoirs, as previously postulated (Palmer et al., 2008).

The correlation of RV with the stage of HIV infection at cART initiation has not been fully elucidated. Previous studies failed to find significant differences in RV levels between patients who started cART during PHI and CHI, although a faster viral decrease in patients treated during PHI was observed (Buzon et al., 2014). Our findings confirm the achievement of lower levels of RV and a higher probability of maintaining URVS during PHI: this may be explained by the faster decay of viremia observed in this patient group, the smaller size of the cellular reservoir pool and the better immune competence in patients undergoing early cART, as previously observed (Jain et al., 2013; Hoenigl et al., 2016). Indeed, the reservoir size was similar in our PHI and CHI patients at baseline, but it declined significantly during cART-induced VS only in PHI, in association with fast URVS achievement and maintenance. In CHI, conversely, after initial reduction, HIV-DNA remained stable, accordingly to previous studies showing a gradual decrease of HIV-DNA during the first year of cART and then less noticeable, until the achievement of a plateau (Besson et al., 2014). Evidence of a possible correlation between RV and HIV-DNA during cART remains uncertain and two recent papers demonstrate conflicting results (Besson et al., 2014; Kiselinova et al., 2015), but neither study analyzed the first phase of evolution immediately after cART start. Nevertheless, it is not possible to exclude that the faster decline of viremia in patients treated during PHI is attributable to the preferential use of an integrase inhibitor in the quadruple regimen.

This study has some limitations. Firstly, despite the large number of samples analyzed with an accurate ultrasensitive assay, the size of the study population is limited. Hence our findings need to be confirmed in larger clinical studies, particularly in patients starting cART during PHI. Secondly, this study lacks of information on patient medication adherence.

In conclusion, we describe an association between the time to VS and the probability of maintaining stable URVS over time, after adjustment for confounding factors such as pre-cART viremia and CD4, and stage of HIV infection. This finding strongly supports the recommendations of current guidelines to start cART with an integrase inhibitor-based regimen as soon as possible, independently from CD4 and HIV-RNA.

**Abbreviations**

HIV: HIV-1; cART: combination antiretroviral therapy; BL: baseline; CD4: CD4+ T cells; CD8: CD8+ T cells; PHI: HIV primary infection; CHI: HIV chronic infection; LLOD: low limit of detection; VS: virological suppression (viral load <50 cp/ml HIV-RNA); RV: residual viremia (viral load detectable <50 copies/ml HIV-RNA); URVS: ultra-deep RV suppression (viral load <5 cp/ml HIV-RNA); PBMC: peripheral blood mononuclear cells; MSM: man who have sex with man; HET: heterosexual; GRT: genotype resistance test; PI: protease inhibitor; ARV: antiretroviral therapies.

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**Competing interests**

None declared.

**Ethical approval**

The study received the INMI’s ethics committee approval (No. 22/2011 of the INMI’s trials register) and patients participating to the study provided written informed consent.

**Authorship**

The study was planned and designed by A.A. and E.G.; A.A.2 and A.A.3, C.P. and A.M. enrolled the patients and were responsible for their management; I.A. and G.R. contributed to collect patient’s data; A.A. and G.B. contributed to the execution of experiments, data input and first analysis of the data; A.N. and E.G. contributed to the statistical analyses and interpretation of data; A.A., AA3, M.R.C. and E.G. wrote the article.

All authors have approved the final article and approve its publication.

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Footnotes
Parts of these data, accepted for poster presentation, were presented at the Conference on Retroviruses and Opportunistic Infections (CROI) 2017, held in Seattle, USA.

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