



ABSTRACT:

Background: Low levels of free plasma virus are common in patients successfully treated with HAART. The effect of repeated treatment interruptions on this residual viremia has not been reported. We here describe changes in HIV RNA below 50 copies and in HIV DNA in patients enrolled in a substudy of the ISS PART clinical trial.

Methods: The ISS PART was a randomized, controlled trial comparing 24 months of continuous (arm A) with 24 months of intermittent HAART (arm B, 5 Structured Treatment Interruptions of 1 to 3 months duration, each followed by 3 months therapy). Thirty-three arm A and 25 arm B patients participated in this substudy where residual viremia (assay cut-off: 2.5 copies HIV RNA/ml) and HIV DNA (assay cut-off: 10 copies/106 PBMC) were studied at baseline and at month 24 (3 months after the end of the last treatment interruption in arm B). Non-parametric tests were used to compare frequency distributions.

Results: At baseline, arm A and B patients had similar demographic, clinical and immunovirologic characteristics. All of them were on first line HAART and had HIV RNA < 50 copies/ml. Median CD4 cell count/mm³ was 727 (range, 339-1459), with no difference between arms. The proportion of subjects with HIV RNA \leq 2.5 copies was 51.5% in A and 60% in B (p=0.52). HIV DNA values \leq 10 copies/106 PBMC were found in 45.5% of arm A and in 56% of arm B subjects (p=0.42). In arm B median time off-therapy was 274 days (range, 237-298). After 24 months, plasma HIV RNA values were < 50 copies/ml in all patients, with 54.5% subjects in arm A and 32% in arm B having \leq 2.5 copies/ml (difference arm A – Arm B= 22.5; 95% CI -2.4; 47.1, χ^2 test: p=0.09). The analysis of percentile distributions confirmed a different trend in the two arms, with an increase of \geq 14.4 copies in 25% arm B subjects and a reduction of \geq 10.2 copies in 25% arm A subjects (Mann-Whitney test: p=0.024). No significant differences from baseline were seen in the proportion of subjects with \leq 10 copies HIV DNA/106 PBMC. No correlation was found between HIV RNA and HIV DNA values.

Conclusions: In our population, repeated interruptions of HAART over 24 months induced a moderate increase in residual viremia, without affecting HIV-1 proviral DNA load in PBMC. This may reflect increased viral release from reservoirs during off treatment periods and/or ongoing replication. The implications of this finding in terms of viral evolution and long term virologic control deserve further investigation.

BACKGROUND: HAART AND RESIDUAL VIREMIA

- > Residual viremia can be demonstrated in nearly all subjects with HIV RNA persistently < 50 copies/ml and can be reduced by treatment intensification ¹
- > Virus released from latent reservoirs mainly contributes to residual viremia ²
- > No viral evolution and resistance seem to occur when HIV viremia is persistently < 50 copies/ml ³
- > Baseline level of proviral HIV DNA is the major predictor of the magnitude of residual viremia ⁴
- > Lower values of residual viremia are not associated with better immunological or virologic outcome ⁴
- > Rising values of residual viremia may precede virologic failure after treatment simplification ⁵

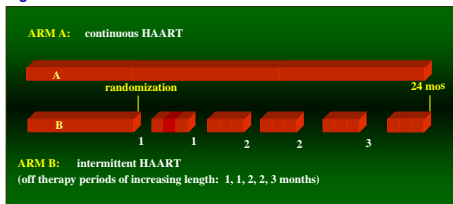
AIM OF THE STUDY

Treatment interruptions have been proposed as a strategy to simplify therapy and reduce drug costs and toxicity. Their feasibility and safety are currently under discussion. Until now, no data have been reported about their impact on residual viremia, once treatment is reinitiated. We therefore investigated the evolution of residual viremia and HIV DNA load in subjects undergoing 5 time-fixed treatment interruptions over a 24 month period, compared with patients continuing their HAART regimen.

METHODS:

PATIENTS AND SAMPLES

This was a substudy of the ISS PART, a randomized, controlled study comparing 24 months of continuous (arm A) versus intermittent HAART (arm B) (Figure 1) in 273 patients with chronic HIV infection ⁶. Fifty-eight patients from 36 clinical sites were enrolled in this substudy. Thirty-three of them had been randomized to arm A and 25 to arm B. Main clinical and demographic characteristics are summarized in Table 1. No differences in any baseline characteristics were found between substudy population and the principal study one. HIV RNA and HIV DNA were measured in stored blood samples drawn at time 0 and after 24 months. The latter time point corresponded to the end of a 3-month therapy period in arm B.



QUANTIFICATION OF RESIDUAL VIREMIA

Residual viremia was quantified in EDTA plasma by an ultrasensitive method based on a modified Roche Amplicor HIV-1 Monitor test, version 1.5, with a limit of detection of 2.5 copies. Modification included pelleting virus from 2 ml of plasma at 23.600 g at 4°C for 2 hours, adding half of the normal volume of quantitation standard (QS) and resuspending the RNA pellet in 50 μ l of diluent. The entire volume of resuspended RNA was assayed by reverse transcription and PCR amplification. These and subsequent detection steps followed the manufacturer's protocol exactly.

Table 1. Patient characteristics

	ARM A	ARM B
No. Patients	33	25
Age (y), median (range)	41 \pm 10.44 (24-62)	38 \pm 9.3 (24-59)
Females: no (%)	5 (15.2)	8 (32)
CD4+ count, cells/mm ³ , median (range)	749 (347-1459)	621 (339-1189)
Pre-HAART CD4+ count, cells/mm ³ , median (range)	387 (148-828)	412 (123-733)
CDC Stage: no (%)		
-CDC A	32 (97)	23 (92)
-CDC B	3 (5.2)	2 (8)
Time from HIV diagnosis (y), median (range)	3 (1-16)	2 (1-10)
Time on HAART (mos), median (range)	29 (13-46)	25 (11-46)
Patients with previous changes of regimen: no (%)	13 (39.4)	9 (36)
HAART regimen: no of patients (%)		
-PI-based	8 (24.2)	3 (12)
-NNRTI-based	19 (57.6)	21 (84)
-All NRTI-based	6 (18.2)	1 (4.0)
Patients with plasma HIV RNA < 50 copies/ml: no (%)	33 (100)	25 (100)

QUANTIFICATION OF HIV-1 DNA IN PBMCs

Total cell DNA was extracted from PBMCs pellets using the QIAamp DNA Blood Mini kit (Qiagen, Italy).

The HIV-DNA load was measured by use of multiplex Real-time polymerase chain reaction (PCR) technology using the ABI Prism 7500 Real Time PCR System (Applied Biosystem, Foster City, CA).

The amount of HIV proviral DNA was determined using HIV-1 specific primers and FAM-MGB probe designed to amplify a conserved gag region (Applied Biosystem). To normalize the gag copies respect the DNA load input RNase-P DNA copies were quantified by RNase-P primer and VIC-TAMRA probe (Applied Biosystem). The 8E5 cell line was used to generate standard curves for HIV-1 DNA and RNase P quantification. HIV-1 and RNase P standard curves had slopes between -3.28 and -3.42 and the coefficients of correlation were >0.98. All samples and controls were run in triplicate and the normalized value of HIV-1 DNA load was expressed like number of HIV copies/10⁶ cells. The limit of detection was 10 copies/10⁶ cells.

STATISTICAL ANALYSIS

Sample size for the original ISS PART study was calculated on the basis of the primary trial endpoint (immunological efficacy). Additional enrolment in this virological substudy was free and no predetermined sample size was calculated for it, representing a secondary analysis of exploratory nature.

The analyses were performed according to an "intention-to-treat" (ITT) approach.

Baseline characteristics were compared between treatment groups using parametric and non parametric tests as appropriate.

Changes over time in the proportion of patients with HIV RNA >2.5 copies/ml and with HIV DNA >10 copies /10⁶ in both arms were assessed by the McNemar test. Proportions of patients with HIV RNA > 2.5 copies and with HIV DNA > 10 copies /106 PBMC at 24 months in the two arms were analyzed by the χ^2 test. HIV RNA and HIV DNA change in the two arms was compared by the Mann-Whitney test

The Pearson's correlation coefficient was used to assess the relationship between HIV RNA and HIV DNA relative change.

in Subjects Undergoing Repeated Treatment Interruptions. PART Clinical Trial.

Maria F Pirillo, Liliana E Weimer, Maria G Mancini, Roberta Amici, Stefano Vella
di Sanità, Rome, Italy

Lucia Palmisano, MD
Istituto Superiore di Sanità
Viale Regina Elena 299
00161 Rome ITALY
Tel. : 39-06-49384021
Fax : 39-06-49387199
E-mail: l.palmisano@iss.it

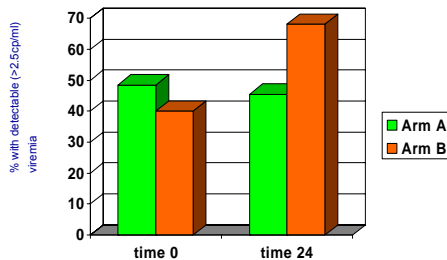
RESULTS:

RESIDUAL VIREMIA

At time 0 detectable levels of HIV RNA (> 2.5 copies/ml) were found in 16/33 (48.5%) patients in arm A and 10/25 (40%) ($P = 0.520$). After 24 months, detectable levels of HIV RNA were observed in 15/33 (45%) subjects in arm A and 17/25 (68%) in arm B (difference Arm B-Arm A = 22.5; 95% CI (-2.4;47.1), $P = 0.0873$). Using percentile analysis, HIV RNA values distributions in A and B were statistically different ($P = 0.012$) (Table 2).

The intragroup comparison showed an increase in the proportion of patients with detectable RNA between time 0 and time 24 in arm B ($P = 0.065$), while no modifications were observed for arm A (Figure 2).

Figure 2. Evolution of residual viremia over 24 months of continuous or intermittent HAART



Difference Arm B-Arm A = 22.5; 95% CI (-2.4;47.1)

Chi square test: $p = 0.0873$

McNemar change test at baseline and 24 mos: Arm A: $p = 1.000$, Arm B: $p = 0.065$

Table 2.

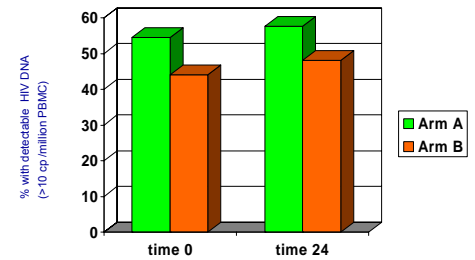
	Arm A (cont)	Arm B (ST)
HIV RNA change (mean±SD)	-0.3±33.8	3.8±26.7
Percentile 25	-10.2	0
50	0	2.1
75	1.3	14.4

Mann-Whitney test: $p = 0.012$

HIV DNA

At baseline, detectable levels of HIV DNA (10 copies/ 10^6 cells) were found in 18/33 (54.5%) arm A and 11/25 (44%) arm B subjects ($P = 0.426$). After 24 months 19/33 patients (57.6%) in arm A and 12/25 (48%) in arm B had detectable HIV DNA (difference B-A = -9.6; 95% CI (-34.9; 16), $P = 0.47$). Intragroup analysis showed no changes for HIV DNA either in A or B (Figure 3).

Figure 3. Evolution of HIV DNA load over 24-months of continuous or intermittent HAART



Difference Arm B-Arm A = -9.6; 95% CI (-34.9;16)

Chi square test: $p = 0.47$

McNemar change test at baseline and 24 mos: Arm A: $p = 1.000$, Arm B: $p = 1.000$

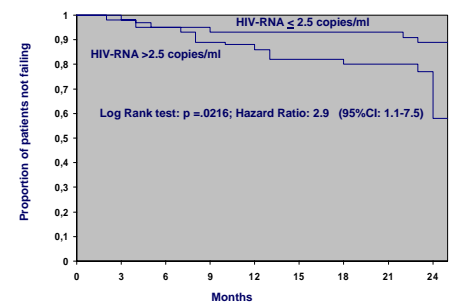
CONCLUSIONS:

This substudy of the ISS PART demonstrates that 2 years of intermittent therapy tend to increase the level of persistent HIV viremia and the proportion of patients with detectable plasma HIV RNA, whereas no significant changes occur in subjects who continue HAART. Conversely, neither in arm A nor in B the HIV DNA load in PBMCs and the proportion of patients with undetectable (< 10 copies/ 10^6 PBMC) HIV DNA changed over time.

This finding may partly explain why in the original ISS PART population we found an association between basal HIV RNA values and rate of virological failure over the whole study period⁶ (Figure 4).

Although the long term consequences of increasing residual viremia are unknown, our observations suggest that repeated HAART interruptions tend to produce a perturbation of viral dynamics that deserves careful clinical monitoring

Figure 4. Time to Virologic Failure in STI arm of the ISS PART study. ITT analysis



REFERENCES:

- Havilr DV, Strain MC, Clerici M, et al. Productive infection maintains a dynamic steady state of residual viremia in human immunodeficiency virus type-1 infected persons treated with suppressive antiretroviral therapy for five years. *J Virol* 2003; 77: 11212-11219
- Kieffer TL, Finucane MM, Nettles RE et al. Genotypic analysis of HIV-1 drug resistance at the limit of detection: virus production without evolution in treated adults with undetectable HIV loads. *J Infect Dis* 2004; 189: 1452-1465
- Bailey JR, Sedaghat AR, Kieffer T et al. Residual Human Immunodeficiency Virus type I viremia in some patients on antiretroviral therapy is dominated by a small number of invariant clones rarely found in circulating CD4+ T cells. *J Virol* 2006; 80: 6441-6457
- Havilr D, Koelsch KK, Strain MC et al. Predictors of residual viremia in HIV-infected patients successfully treated with efavirenz and lamivudine plus either tenofovir or stavudine. *J Infect Dis* 2005; 191: 1154-1158
- Palmisano L, Giuliano M, Nicastrì M et al. Residual viremia in subjects with chronic HIV infection and viral load < 50 copies/ml: the impact of highly active antiretroviral therapy. *AIDS* 2005; 19:1843-1842
- McKinnon JE, Arribas JR, Pulido F et al. The level of persistent HIV viremia does not increase after successful simplification of maintenance therapy to lopinavir/ritonavir alone. *AIDS* 2006; 20: 2331-2335

36 Italian Clinical Sites

Università degli Studi di Milano, Istituto Malattie Infettive, Milano; IRCCS Lazzaro Spallanzani, III Divisione Malattie Infettive, Roma; IRCCS Lazzaro Spallanzani, IV Divisione Malattie Infettive, Roma; Ospedale L. Sacco, I Divisione Malattie Infettive e Servizio di Allergologia, Milano; Policlinico S. Orsola - Matigghi, Sezione di Malattie Infettive, Bologna; Arcispedale S. Anna, Divisione Malattie Infettive, Ferrara; Ospedale Careggi, Divisione Malattie Infettive, Firenze; Ospedale Amedeo di Savoia, U.O. Malattie Infettive B, Torino; Presidio Ospedaliero Villa Igea, Divisione Malattie Infettive, Trento; Università degli Studi di Brescia - Spedali Civili di Brescia, Clinica di Malattie Infettive e Tropicali, Brescia; Università degli Studi di Modena, Clinica Malattie Infettive e Tropicali, Modena; Azienda Ospedaliera di Padova, Divisione Malattie Infettive e Tropicali, Padova; Ospedali Infermi di Rimini, Divisione Malattie Infettive, Rimini; ASMN Reggio Emilia, Divisione Malattie Infettive, Reggio Emilia; Ospedale S. Bortolo, Divisione Malattie Infettive, Vicenza; Azienda Ospedaliera di Parma, Divisione Malattie Infettive, Parma; Ospedale San Raffaele, Centro Ricerca e Cura per le Patologie HIV Correlate, Milano; Ospedale S.M. Annunziata, U.O. Malattie Infettive, Firenze; Ospedale di Prato Misericordia e Dolce, U.O. Malattie Infettive, Prato; Ospedale D. Cotugno, IV Divisione Malattie Infettive, Napoli; Ospedale D. Cotugno, III Divisione Malattie Infettive, Napoli; Policlinico Umberto I - Università degli Studi di Roma La Sapienza, III Divisione Malattie Infettive, Roma; Ospedale Maggiore, U.O. Malattie Infettive, Bologna; Azienda Ospedaliera D. Cotugno, VIII Divisione Malattie Infettive, Napoli; Ospedale Civile, Divisione Malattie Infettive, Pescara; Università degli Studi di Sassari, Istituto di Malattie Infettive, Sassari; Ospedale Civile di Pesaro, Divisione di Malattie Infettive, Pesaro; E.O. Ospedali Galliera, U.O. Malattie Infettive, Genova; Ospedale Civile, Divisione Malattie Infettive, Busto Arsizio; Università degli Studi di Catania, Istituto Malattie Infettive, Catania; Policlinico Umberto I, Istituto Malattie Infettive e Tropicali, Roma; Azienda Usl n.5 Spezzino, Regione Liguria, Ospedale Felettino, Malattie Infettive, La Spezia; Policlinico Le Scotte, U.O. Malattie Infettive 1, Siena.