Journal of Medical Microbiology (2009), **58**, 121–124 DOI 10.1099/jmm.0.004630-0

Modifications of residual viraemia in human immunodeficiency virus-1-infected subjects undergoing repeated highly active antiretroviral therapy interruptions

Lucia Palmisano, Marina Giuliano, Raffaella Bucciardini, Mauro Andreotti, Vincenzo Fragola, Maria F. Pirillo, Liliana E. Weimer, Maria G. Mancini and Stefano Vella

Department of Therapeutic Research and Medicines Evaluation, Istituto Superiore di Sanità, Rome, Italy

Correspondence
Lucia Palmisano
lucia.palmisano@iss.it

Received 2 July 2008
Accepted 30 September 2008

Residual viraemia is detectable in the majority of human immunodeficiency virus (HIV)-infected subjects with plasma HIV-1 RNA <50 copies ml^{-1}. In the present study, the impact of repeated treatment interruptions on residual HIV-1 viraemia was investigated in 58 subjects enrolled in the ISS-PART, a multicentre, randomized clinical trial comparing 24 months of continuous (arm A) and intermittent (arm B) highly active antiretroviral therapy (HAART). Residual viraemia was measured by a modified Roche Amplicor HIV-1 RNA assay (limit of detection 2.5 copies ml^{-1}). At baseline, the median value of residual viraemia was 2.5 copies ml^{-1} in both arms; after 24 months, the median value was 2.5 in arm A and 8.3 in arm B. The median change from baseline to month 24 was significantly different between patients under continuous or intermittent HAART: 0 copies ml^{-1} (range −125.2 to +82.7) of HIV-1 RNA in arm A versus 2.1 copies ml^{-1} (range −80 to +46.8) in arm B (P=0.024). These results suggest that intermittent HAART tends to modify HIV-1 viraemia set point even if a virological response is achieved after HAART reinstitution.

INTRODUCTION

Residual viraemia is detectable in the majority of highly active antiretroviral therapy (HAART)-treated human immunodeficiency virus (HIV)-infected subjects with plasma viral load <50 copies ml^{-1} (Finzi *et al.*, 1999; Dornadula *et al.*, 1999; Havlir *et al.*, 2003) and persists for at least 7 years of successful HAART (Palmer *et al.*, 2008). Long-lived cells infected before HAART initiation have been proposed as a major source of this low-level viraemia (Maldarelli *et al.*, 2007), the implications of which have not been fully explored. Studies suggest that residual viraemia does not increase after successful HAART simplification to a regimen based on lopinavir/ritonavir alone (McKinnon *et al.*, 2006), and that in the mid term its magnitude is not associated with virological failure in subjects on continuous HAART (Havlir *et al.*, 2005). No data have been reported on the impact that Structured Treatment Interruptions (STIs), a controversial strategy proposed to simplify therapy and reduce drug exposure (Ananworanich & Hirschel, 2007), may have on residual viraemia once HAART is reinstituted and patients regain a full virological response. In the present study, low-level HIV-1 RNA was quantified in subjects undergoing 24 months of intermittent HAART, and the results were compared with those found in patients continuing HAART.

Abbreviations: HAART, highly active antiretroviral therapy; STIs, Structured Treatment Interruptions.

METHODS

The present study is an exploratory, unplanned analysis involving 58 subjects enrolled in the ISS-PART, a randomized, controlled trial comparing 24 months of continuous (arm A) versus intermittent (arm B) HAART in 273 subjects with chronic HIV infection and persistent suppression of viral replication (Palmisano *et al.*, 2007). Intermittent HAART consisted of five STIs of 1, 1, 2, 2 and 3 months duration, each followed by 3 months of therapy. Any regimens in the Treatment Guidelines in force at the time (year 2001) could be used, including those based on an unboosted protease inhibitor.

Residual HIV-1 plasma viraemia was measured using an assay based on a modified Roche Amplicor HIV-1 Monitor test, version 1.5, as previously described (Palmisano *et al.*, 2005). The limit of detection was 2.5 copies of HIV-1 RNA ml^{-1}. Plasma samples collected at time 0 and after 24 months were analysed. The latter time point corresponded to the end of the final 3-month therapy period in arm B. The Wilcoxon test for dependent samples was used to compare HIV-1 RNA values at baseline and after 24 months within the groups. The Mann–Whitney test was used to compare HIV-1 RNA levels and change over time in the two arms. Proportions of patients with detectable (>2.5 copies ml^{-1}) HIV-1 RNA at baseline and 24 months in the two arms were compared by the χ^2 test. In arm

B, Pearson's correlation coefficient was used to assess the relationship between HIV RNA values measured during the last HAART interruption and at month 24.

RESULTS AND DISCUSSION

Thirty-three arm A and 25 arm B subjects were included in this study. Table 1 summarizes their main clinical and demographic characteristics, which were not statistically different from those of the original ISS-PART population.

No statistically significant differences were found between arm A and B for all the considered variables. All patients completed the 24 month follow-up according to the protocol and all of them had less than 50 copies ml^{-1} of plasma HIV-1 RNA at the end of it by the standard Roche Amplicor HIV-1 Monitor test. The total time spent off therapy for subjects in arm B was 274 days (median, range 237–298).

At baseline, median (range) plasma HIV-1 RNA values were 2.5 (2.5–137) copies ml^{-1} in arm A and 2.5 (2.5–82.5) copies ml^{-1} in arm B ($P=0.34$). After 24 months, HIV-1 RNA median values were 2.5 (2.5–97.7) copies ml^{-1} in arm A ($P=0.74$ with respect to baseline) and 8.3 (2.5–49.3) copies ml^{-1} in arm B ($P=0.08$ with respect to baseline) (Fig. 1). Comparing the median change in HIV-1 RNA values in the two arms yielded a significant difference: 0 (range −125 to +82.7) copies ml^{-1} in arm A and 2.1 (range −80 to +46.8) copies ml^{-1} in arm B ($P=0.024$). Detectable (>2.5 copies ml^{-1}) levels of HIV-1 RNA were found in 16/33 (48.5 %) subjects in arm A and 10/25 (40 %) in arm B ($P=0.52$) at baseline and in 15/33 (45 %) in arm A and 17/25 (68 %) in arm B after 24 months, with a trend to an increase in arm B only ($P=0.06$) (Fig. 1) In group B, no correlation was found between the magnitude of HIV-1 RNA rebound during the last STI and the values of residual viraemia at month 24.

This study was conducted to assess whether repeated HAART interruptions may influence the levels of residual HIV-1 viraemia in subjects who regain virological suppression after restarting HAART. For this purpose, an ultrasensitive assay with a limit of detection of 2.5 copies of HIV-1 RNA ml^{-1} was used to measure residual viraemia before and after 24 months of continuous or intermittent HAART in the context of the ISS-PART clinical trial. In agreement with literature data (Havlir *et al.*, 2003), at baseline less than 50 % of patients in the whole study population had detectable (>2.5 copies HIV-1 RNA ml^{-1}) residual viraemia, with a median value of 2.5 copies ml^{-1}. After 2 years, these values remained unchanged in subjects on continuous HAART; this finding is consistent with the hypothesis that a stable set point of low-level HIV-1 RNA is usually achieved after a few months of HAART (Maldarelli *et al.*, 2007) and persists for several years thereafter (Palmer *et al.*, 2008). Conversely, in subjects undergoing five cycles of 'on/off' therapy, the number of HIV-1 RNA copies ml^{-1} tended to increase, as did the proportion of individuals with detectable viraemia. A significant difference was found when the variations in residual viraemia occurring in the two arms after 24 months were compared. These small changes would have gone unnoticed using conventional assays for HIV-1 RNA quantification; in fact, all patients in the two arms had plasma viraemia values <50 copies ml^{-1} after 24 months by using the standard Amplicor assay.

Table 1. Baseline patient characteristics

	Arm A	Arm B
No. of patients	33	25
Age (years), median (range)	37 (24–62)	36 (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)
Years from HIV diagnosis, median (range)	3 (1–16)	2 (1–10)
Time on HAART (months), 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^{-1}: no. (%)	33 (100)	25 (100)

*According to protocol, eligible patients could have changed their HAART regimen once for toxicity or poor compliance (not for virological failure).

†PI, protease inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor.

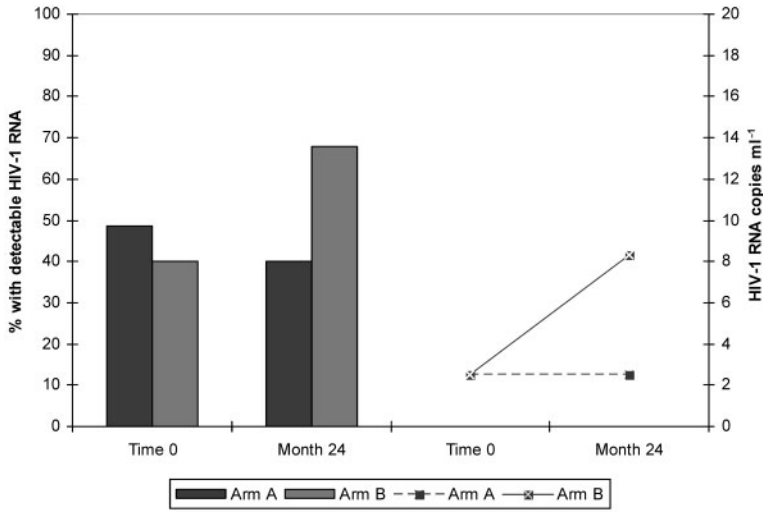

Fig. 1. Changes in HIV-1 RNA during 24 months of continuous (arm A) or intermittent (arm B) HAART. Left panel, proportion of patients with detectable (>2.5 copies ml^{-1}) HIV-1 RNA; right panel, median HIV-1 RNA values.

The mechanisms for this apparent increase in residual viraemia in subjects receiving intermittent HAART in the ISS-PART study are not clear. One explanation could be that 3 months of HAART were insufficient to regain pre-interruption viraemia values; however, the absence of correlation between the magnitude of viral rebound during the last interruption and the values of HIV-1 RNA measured at month 24 makes this hypothesis unlikely, although it cannot be ruled out. On the other hand, a gradual reseeding of viral reservoirs during five successive STIs seems to be a plausible explanation (Frost *et al.*, 2002).

This study has some limitations. The first of these is the relatively small number of patients, which does not allow the capture of possible differences related to the type of regimen. Secondly, two time points only could be analysed for each patient, baseline and month 24, allowing the description of the overall effect of intermittent therapy on residual viraemia but not its dynamics. The strength of the reported observations is represented by the availability of a control group, the high level of patient compliance to study protocol and the homogeneity of the tested population to that of the original ISS-PART study.

In conclusion, the results obtained using a more sensitive method for HIV-1 RNA quantification suggest that 24 months of continuous or intermittent HAART have a different impact on residual viraemia. Further studies with longer follow-up are recommended to confirm these findings and to assess whether they can influence the long-term risk of virological failure and viral evolution.

ACKNOWLEDGEMENTS

The authors wish to thank the participating 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 – Malpighi, 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 Arstizio; 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) and technical staff at Istituto Superiore di Sanità (F. Innocenti, A. Mattei, P. Cocco, D. Diamanti and F. Costa). The study was supported by grants from the Istituto Superiore di Sanità, National AIDS Clinical Research Program, years 2001–2004.

REFERENCES

Ananworanich, J. & Hirschel, B. (2007). Intermittent therapy for the treatment of chronic HIV infection. *AIDS* **21**, 123–124.

Dornadula, G., Zhang, H., VanUitert, B., Stern, J., Livornese, L., Jr, Ingerman, L. J., Witek, J., Kedanis, R. J., Natkin, J. & other authors (1999). Residual HIV-1 RNA in blood plasma of patients taking suppressive highly active antiretroviral therapy. *JAMA* **282**, 1627–1632.

Finzi, D., Blanckson, J., Siliciano, J. D., Margolick, J. B., Chadwick, K., Pierson, T., Smith, K., Liszewicz, J., Lori, F. & other authors (1999). Latent infection of CD4+ T cells provides a mechanism for lifelong persistence of HIV-1, even in patients on effective combination therapy. *Nat Med* **5**, 512–517.

Frost, S. D. W., Martinez-Picado, J., Ruiz, L., Clotet, B. & Brown, A. J. (2002). Viral dynamics during structured treatment interruptions of chronic human immunodeficiency virus type 1 infection. *J Virol* **76**, 968–979.

Havlir, D. V., Strain, M. C., Clerici, M., Ignacio, C., Trabattoni, D., Ferrante, P. & Wong, J. K. (2003). 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* **77**, 11212–11219.

Havlir, D. V., Koelsch, K. K., Strain, M. C., Margot, N., Biao, L., Ignacio, C. C., Miller, M. D. & Wong, J. K. (2005). Predictors of residual viremia in HIV-infected patients successfully treated with efavirenz and lamivudine plus either tenofovir or stavudine. *J Infect Dis* **191**, 1164–1168.

Maldarelli, F., Palmer, S., King, M. S., Wiegand, A., Polis, M. A., Mican, J., Kovacs, J. A., Davey, R. T., Rock-Kress, D. & other authors (2007). ART suppresses plasma HIV-1 RNA to a stable set point predicted by pretherapy viremia. *PLoS Pathog* **3**, e46.

McKinnon, J. E., Arribas, J. R., Pulido, F., Delgado, R. & Mellors, J. W. (2006). The level of persistent HIV viremia does not increase after successful simplification of maintenance therapy to lopinavir/ritonavir alone. *AIDS* **20**, 2331–2335.

Palmer, S., Maldarelli, F., Wiegand, A., Bernstein, B., Hanna, G. J., Brun, S. C., Kempf, D. J., Mellors, J. W., Coffin, J. M. & King, M. S. (2008). Low-level viremia persists for at least 7 years in patients on suppressive antiretroviral therapy. *Proc Natl Acad Sci U S A* **105**, 3879–3884.

Palmisano, L., Giuliano, M., Nicastri, E., Pirillo, M. F., Andreotti, M., Galluzzo, C. M., Bucciardini, R., Fragola, V., Andreoni, M. & Vella, S. (2005). Residual viremia in subjects with chronic HIV infection and viral load < 50 copies/ml: the impact of highly active antiretroviral therapy. *AIDS* **19**, 1843–1847.

Palmisano, L., Giuliano, M., Bucciardini, R., Fragola, V., Andreotti, M., Galluzzo, C. M., Pirillo, M. F., Weimer, L. E., Arcieri, R. & other authors (2007). Determinants of virologic and immunologic outcomes in chronically HIV-infected subjects undergoing repeated treatment interruptions: the Istituto Superiore di Sanita-Pulsed Antiretroviral Therapy (ISS-PART) study. *J Acquir Immune Defic Syndr* **46**, 39–47.