Hamlyn, E; Ewings, FM; Porter, K; Cooper, DA; Tambussi, G; Schechter, M; Pedersen, C; Okulicz, JF; McClure, M; Babiker, A; Weber, J; Fidler, S; INSIGHT SMART and SPARTAC Investigators, (2012) Plasma HIV viral rebound following protocol-indicated cessation of ART commenced in primary and chronic HIV infection. PLoS One, 7 (8). e43754. ISSN 1932-6203 DOI: https://doi.org/10.1371/journal.pone.0043754

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Introduction

Long-term use of antiretroviral therapy (ART) in HIV-positive persons may be challenged by the need for high-level adherence, development of drug resistance, toxicities, and cost. Treatment strategies conferring durable virological control, whilst minimising ART exposure are highly desirable. With this goal in mind, strategic interruption of ART was the focus of several studies [1–3].

However, interruption of ART is no longer a recommended strategy [2] and the level of HIV plasma viral load (pVL) following ART stop has been shown to reach levels comparable to pre-treatment values [2–4], increasing onward transmission risk [5]. Inaccessible reservoirs of latently-infected resting memory CD4 T-cells are hypothesised to be the major source contributing to viraemia rebound after stopping ART [6,7].

Recent research has shown the dramatic effect of ART to prevent onward viral transmission [8], and mathematical models predict that it may potentially be possible to eliminate HIV infection at a population level with universal treatment coverage for all HIV-positive individuals, irrespective of CD4 count [9]. However, although not recommended, consideration of the...
potential impact of individuals choosing to stop ART could be considerable, and data are needed on subsequent viral rebound to better inform future transmission models. Furthermore, final results from SPARTAC suggested that ART initiated in primary HIV infection (PHI) was associated with a change in pVL set-point out to 60 weeks after stopping therapy [10] whilst the SMART trial reported that interruption of ART in its chronic infection (CHI) was associated with an increased risk of all-cause mortality.

The level of viral rebound following interruption of ART commenced at different stages of HIV infection is, therefore, highly relevant from both a clinical and public health perspective and warrants further investigation.

We, therefore, wanted to compare the pVL changes observed after cessation of ART initiated in chronic HIV infection with those in PHI by comparing viral rebound between individuals enrolled in two protocol-indicated ART interruption studies; SPARTAC and SMART.

Methods

Ethics statement

The SPARTAC trial was approved by the following authorities: Medicines and Healthcare products Regulatory Agency (UK), Ministry of Health (Brazil), Irish Medicines Board (Ireland), Medicines Control Council (South Africa), and the Uganda National Council for Science and Technology (Uganda). It was also approved by the following ethics committees in the participating countries: Central London Research Ethics Committee (UK), Hospital Universitario Clementino Fraga Filho Ethics in Research Committee (Brazil), Clinical Research and Ethics Committee of Hospital Clinic in the province of Barcelona (Spain), The Adelaide and Meath Hospital Research Ethics Committee (Ireland), University of Witwatersrand Human Research Ethics Committee, University of KwaZulu-Natal Research Ethics Committee and University of Cape Town Research Ethics Committee (South Africa), Uganda Virus Research Institute Science and ethics committee (Uganda), The Prince Charles Hospital Human Research Ethics Committee and St Vincent’s Hospital Human Research Ethics Committee (Australia), and the National Institute for Infectious Diseases Lazzaro Spallanzani, Institute Hospital and the Medical Research Ethics Committee, and the ethical committee Of the Central Foundation of San Raffaele, Monte’ Tabor (Italy). The INSIGHT SMART trial was approved by the University of Minnesota institutional review board. All participants signed a written informed consent.

Study populations

Viral dynamics following treatment interruption were compared using data from SPARTAC and SMART participants. SPARTAC is an international RCT comparing no therapy, 12-week ART, or 48-week ART initiated within a maximum of 6 months from the last documented HIV negative test date. The primary outcome measure was time to confirmed CD4 cell count <350 cells/mm³, or the initiation of long-term therapy. PHI was identified according to the trial protocol. The trial recently reported a significant difference in time to the primary endpoint for the 48-week, but not the 12-week, ART arm compared to no therapy, although not significantly longer than the time already spent on therapy [10]. SMART is an international RCT which compared a 414-guided strategy of planned treatment interruptions versus continuous ART in chronically HIV-infected individuals. Eligible participants with CD4>350 cells/mm³ were randomised to either a Drug Conservation (DC) or Viral Suppression (VS) arm. Enrolment was stopped on 11th January 2006 and participants in the DC arm were recommended to re-initiate ART as interim results clearly indicated superiority of the VS arm [2].

Individuals were included in this analysis if they underwent protocol-induced ART cessation, i.e. on ART at time of randomisation to the DC arm in SMART (hereafter, the chronically-infected population) or randomised to one of the two treatment arms in SPARTAC (hereafter, the PHI population), and had a CD4 cell count and pVL available at the time of ART stop. Subsequent pVL measurements were scheduled at 4, 12, 24, 36, 48 and 4, 8, 16, 32, 40, 48 weeks after ART stop in SPARTAC and SMART respectively. pVLs were determined locally; for included participants, 40, 59, <1 and <1% from SPARTAC and 20, 74, 4 and 2% from SMART were measured using bDNA, PCR, NASBA and other assays, respectively.

Statistical methods

We examined the time following ART stop for pVL to reach pre-ART levels in the PHI population, using survival methods. We then compared pVL levels at 4 weeks after stopping ART in the PHI participants, and up to 48 weeks afterwards, with those in chronically-infected individuals. Using linear and ordered logistic regression, respectively, we examined differences in absolute levels and in the proportions with pVL<400, 400–3999, 3500–9999, 10,000–49,999 or ≥50,000 copies/ml at 4 weeks after ART stop [5]. Using linear mixed models, we examined differences in pVL levels and slopes over 4–48 weeks after ART stop, and estimated predicted pVLs at 4 and 36 weeks after ART stop for representative PHI and chronically-infected participants (male infected through sex with men, aged 40 years and with CD4 600 cells/mm³ at ART stop). We estimated the complete viral burden, through a time-averaged area-under-pVL curve, over the whole period after stopping ART. We then categorised the chronically-infected participants according to their nadir CD4 count at ART stop in order to assess whether any differences over 48 weeks between the populations could be explained solely by nadir CD4.

Follow-up began from the date of first stopping all drugs in the ART regimen and was censored at the last pVL measurement, the 48 week visit, when ART was re-initiated, or 11th January 2006 for SMART participants, whichever was earliest.

We restricted analyses to participants who had suppressed pVL to <400 copies/ml at the time of ART stop, as this was the limit of the least sensitive assay used across both trials. pVL data were log_{10}-transformed and values <400 copies/ml were treated as = 400 copies/ml for all participants to avoid confounding by trial, as a greater proportion of pVLs were measured using this detection limit in the PHI compared to chronically-infected participants (13 versus 6% <400 copies/ml and 9 vs. 16%, respectively, <50 copies/ml). CD4 and pVL at ART stop were defined by those closest to ART stop (up to 24 and 12 weeks before, respectively, and no more than 2 weeks after). Subsequent pVLs were defined by those closest to the scheduled visits (allowing a +/-2 week window around the week 4 and 6 visits and a +/-4 week window around subsequent visits). Pre-ART pVLs in PHI participants were estimated as the mean of all available pVLs before ART initiation (9%, 85%, 5%, 1% participants had 1, 2, 3 and 4 pre-ART pVLs available, respectively). We also restricted analyses to include sexually-infected individuals only as few were from other risk groups in the PHI population.

Models were adjusted for the effect of sex/risk group (sex between men (MSM), heterosexual men or heterosexual women), age and CD4 at ART stop. No adjustments were made for time on ART, as this is confounded by duration of infection and, therefore, by trial, nor for ART class (also confounded by trial). Using only
data from the chronic population, however, we investigated the possible effect of ART class on viral rebound. We also investigated whether there were differences in the effects of participant characteristics at ART stop for PHI compared to chronically-infected participants, and CD4 nadir up to ART stop, where appropriate, using interactions.

As SMART participants were enrolled into the trial with prevalent HIV infection, duration of HIV infection and time since first initiation of ART may not have been known and so reported values should be regarded as best estimates. In particular, the duration of infection is based on first known HIV positive result, therefore, the intervals are likely to be underestimates.

Results

Description of the population

Of the 243 SPARTAC participants randomised to one of the two treatment arms, 16 were excluded because they did not initiate ART (n = 5), did not stop ART (n = 6), were on ART for <15 days (n = 4) or did not have a pVL at ART stop (n = 1). Of the 2290 SMART participants on ART at the time of randomisation to the DC arm, 256 were excluded because they did not stop ART (n = 42, 25 of whom were randomised in the month prior to 11th January 2006), stopped ART after 11th January 2006 (n = 20), did not have a pVL at ART stop (n = 1) or did not have any subsequent pVLs after stopping ART (n = 193, 153 of whom only stopped in the month prior to 11th January 2006). Additional exclusions were as follows: 43 SPARTAC and 384 SMART participants with pVL ≥400 copies/ml at ART stop, 125 SMART participants with reported risk group IDU, and 2 SPARTAC and 75 SMART participants with other/unknown route of HIV transmission. Therefore, 182 PHI and 1450 chronically-infected participants were included in our analyses.

Participant demographics, ART exposure and CD4 at time of treatment discontinuation are shown in Table 1. Compared to those chronically-infected, PHI participants were younger (median age 28 versus 35 years), more likely to be female (33% versus 24%), and had considerably less ART exposure (6% versus 44% ever exposed to ≥3 drug classes) and were more likely to be on a protease inhibitor regimen at the time of ART stop (94% versus 36%). At ART stop, median CD4 was 200 cells/mm3, compared to PHI participants. Lower CD4 nadir was higher in chronically-infected participants with nadir CD4

500 cells/mm3, compared to PHI participants. Lower CD4 nadir was associated with faster rebound (Figures 2a and 2b). For every 8 copies/ml, the pVL in PHI participants increased, on average, by 0.17 (95% CI 0.14, 0.20) log10 copies/ml compared to 0.07 (0.01, 0.29), 0.33 (0.21, 0.44), 0.46 (0.36, 0.57) and 0.71 (0.60, 0.81) log10 copies/ml in chronically-infected participants with nadir CD4 <200 cells/mm3 and PHI participants (p = 0.001).

The associations between longer-term pVL rebound and sex/risk group, CD4 cell count and age at ART stop were qualitatively similar as those for the week 4 pVL rebound (results not shown).

pVL rebound after ART stop, categorising the chronically-infected participants by nadir CD4 count

Over 4–48 weeks after ART stop, pVL remained significantly higher in chronically-infected participants with nadir CD4 <500 cells/mm³, compared to PHI participants. Lower CD4 nadir was associated with faster rebound (Figures 2a and 2b). For every 8 weeks the pVL in PHI participants increased, on average, by 0.17 (95% CI 0.14, 0.20) log10 copies/ml compared to 0.17 (0.01, 0.29), 0.33 (0.21, 0.44), 0.46 (0.36, 0.57) and 0.71 (0.60, 0.81) log10 copies/ml in chronically-infected participants with nadir CD4 <200, 200–349 and <350 cells/mm³, respectively.

We observed a similar relationship for viral burden: chronically-infected participants with a lower nadir CD4 had higher viral burden, compared with PHI participants (0.39 [95% CI 0.27, 0.51], 0.29 [0.18, 0.41] and 0.20 [0.06, 0.33] log10 copies/ml higher viral burden for nadir CD4 < 200, 200–349 and 350–499 cells/mm³, respectively), but there was no difference in viral burden between chronically-infected participants with CD4 nadir ≥500 cells/mm³ and PHI participants (0.04 [−0.12 to 0.20] log10 copies/ml).

pVL rebound after ART stop by PHI versus chronically-infected participants

At 4 weeks after ART stop, the proportions with pVL <400 copies/ml were similar in the two groups (Table 2), but median pVL levels were significantly lower among PHI compared to chronically-infected participants (unadjusted median 3.7 versus 4.4 log10 copies/ml, respectively; adjusted pVL 0.45 [95% CI 0.26, 0.64] log10 copies/ml lower, p < 0.001; Table 3). Higher CD4 cell count at ART stop was weakly associated with lower week 4 pVL, and persons infected through heterosexual contact had lower week 4 pVL compared to MSM, but with no evidence that this effect differed between the PHI and chronically-infected groups (p = 0.4).

There was no association between age at ART stop and week 4 pVL (p = 0.6).

Considering only the chronically-infected participants, pVL was significantly higher at 4 weeks after ART stop for those on PI-based or triple NRTI regimens, compared to those on NNRTI-based regimens (0.69 [0.56, 0.81] and 0.58 [0.30, 0.79] log10 copies/ml, respectively). There was no evidence of a difference for other highly-active or suboptimal regimens, compared to NNRTI-based regimens (0.36 [−0.01, 0.74] and 0.17 [−0.07, 0.42] log10 copies/ml, respectively).

Over 48 weeks after ART stop, median pVL remained lower in individuals with PHI compared to chronically-infected participants (Figure 1a), with evidence to suggest that chronically-infected participants rebounded more rapidly than PHI participants (i.e. had steeper slope; adjusted p < 0.0001; Figure 1b). Predicted pVLs for representative participants are given in Figure 1b.

The median (IQR) viral burden was 1.12 (0.56, 1.69) and 1.55 (1.03, 1.99) log10 copies/ml amongst PHI and chronically-infected participants, respectively. After adjustment, viral burden was, on average, 0.28 (95% CI 0.17, 0.39) log10 copies/ml lower for PHI versus chronically-infected participants (p < 0.001).

The associations between longer-term pVL rebound and sex/risk group, CD4 cell count and age at ART stop were qualitatively similar as those for the week 4 pVL rebound (results not shown).

Time to pVL reaching pre-ART levels in PHI participants

Among the PHI participants, the median (IQR) pre-ART pVL was 4.5 (3.9, 5.1) log10 copies/ml. Eleven participants had pre-ART pVL < 400 copies/ml and were, therefore, omitted from the analyses of estimating time to reaching pre-ART pVL. Nine participants, who had higher median pre-ART pVL (5.3 log10 copies/ml), were censored before reaching pre-ART levels due to ART re-initiation (one at 5 weeks and the remainder ≥25 weeks after ART stop). A total of 71 (42% of 171) participants were observed to rebound to pre-ART pVL levels, at a median of 50 (95% CI 48, 51) weeks. A quarter of participants had rebounded to pre-ART levels by 15 (95% CI 12, 26) weeks.

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The effects of sex/risk group and age at ART stop were similar to those for week 4 pVL rebound (results not shown). However, we found evidence of an interaction between nadir CD4 and CD4 cell count at stop ($p = 0.04$ and $0.0006$ in the linear mixed model for pVL up to 48 weeks and the linear regression for viral burden, respectively). CD4 count 100 cells/mm$^3$ higher at ART stop was associated with a higher viral burden of $0.05$ (95% CI $0.03$, $0.08$) log$_{10}$ copies/ml lower per 100 cells/mm$^3$ higher CD4 at ART stop, respectively. Among chronically-infected participants with nadir CD4 $200–349$ cells/mm$^3$, there was no evidence of such an association (viral burden $0.002$ [−0.03, 0.04] and $−0.01$ [−0.05, 0.03] log$_{10}$ copies/ml higher per 100 cells/mm$^3$ higher CD4 at ART stop, respectively). Among PHI participants, there was evidence to suggest that higher CD4 at ART stop was associated with lower viral burden ($0.04$ [95% CI $0.003$, $0.08$] log$_{10}$ copies/ml lower per 100 cells/mm$^3$ higher CD4 at ART stop). The effect on viral burden of PHI versus chronic infection/nadir CD4 remained robust with or without adjustment for CD4 count at ART stop.

**Discussion**

This is the first study to compare HIV pVL dynamics between PHI and chronically-infected individuals undergoing a protocol-induced ART interruption. We observed that pVL rebound after stopping ART initiated in PHI was lower than that observed in chronic infection, at 4 weeks after treatment interruption, and this...
difference was sustained over 48 weeks of follow-up. In addition, the overall viral burden, as estimated by the area under the pVL by time curve, was significantly lower in PHI compared to chronically-infected participants.

Our findings support those from a smaller study which observed significantly shorter time to viral rebound following treatment interruption in participants who initiated treatment in PHI compared to chronic infection [11]. However, they did not consider the stage of infection prior to commencing therapy. We found that, as anticipated, when participants with chronic infection were stratified by nadir CD4 at time of stopping ART, lower nadir CD4 was associated with higher pVL after stopping therapy. Compared to PHI, viral rebound was higher in chronically-infected participants with nadir CD4 < 500 cells/mm³, but similar to levels experienced by those with nadir CD4 ≥ 500 cells/mm³. Interestingly, in chronically-infected participants with CD4 nadir < 200 cells/mm³, higher CD4 count at ART stop was associated with subsequent higher viral burden. Thus, it could be hypothesised that the degree of viral rebound may be related to the degree of immune reconstitution occurring during ART, or to the number of CD4 target cells available for viral infection at ART stop [12].

The observed difference in virological impact of stopping ART in PHI versus chronic infection may reflect differences in viral reservoir size although no data were available from either trial on HIV reservoir size and we were, therefore, unable to directly examine this. A study of ART initiated during PHI found that 36

<table>
<thead>
<tr>
<th>Table 2. pVL levels at 4 weeks after ART stop by PHI versus chronically-infected participants.</th>
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<tbody>
<tr>
<td>pVL, copies/ml</td>
</tr>
<tr>
<td>&lt;400</td>
</tr>
<tr>
<td>400–3499</td>
</tr>
<tr>
<td>3500–9999</td>
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<tr>
<td>10,000–49,999</td>
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<tr>
<td>≥50,000</td>
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</tbody>
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Values are n (%). pVL = plasma viral load. Adjusted p-value from ordered logistic regression <0.001. Of participants with a week 4 pVL available. doi:10.1371/journal.pone.0043754.t002

Table 3. Factors associated with pVL level (log₁₀ copies/ml) at 4 weeks after ART stop (from adjusted linear regression model*).

<table>
<thead>
<tr>
<th></th>
<th>Coefficient (95% CI)</th>
<th>P</th>
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<tbody>
<tr>
<td>PHI, versus chronically-infected</td>
<td>-0.45 (-0.64, -0.26)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age at ART stop, per 10 years</td>
<td>0.01 (-0.04, 0.07)</td>
<td>0.6</td>
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<tr>
<td>Sex/risk group, vs men infected through sex with men</td>
<td></td>
<td></td>
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<tr>
<td>Male, infected through sex with women</td>
<td>-0.14 (-0.30, 0.03)</td>
<td></td>
</tr>
<tr>
<td>Female, infected through sex with men</td>
<td>-0.33 (-0.47, -0.20)</td>
<td></td>
</tr>
<tr>
<td>CD4 count at ART stop, per 100 cells/mm³</td>
<td>-0.02 (-0.04, 0.002)</td>
<td>0.08</td>
</tr>
<tr>
<td>Constant**</td>
<td>4.26 (4.18, 4.34)</td>
<td>-</td>
</tr>
</tbody>
</table>

CI = confidence interval. pVL = plasma viral load. Coefficients are interpreted as the value of log₁₀ copies/ml lower pVL for a negative sign, and higher for a positive sign. *Adjusted for factors in the table. **Mean week 4 pVL for a chronically-infected male infected through sex with men, aged 40 years and with CD4 600 cells/mm³ at ART stop. doi:10.1371/journal.pone.0043754.t003
therapy may be related to prognosis. In our analysis, the protocol-indicated ART cessation in both trial populations minimised the effect of this potential source of bias, although this study has some limitations. It was not possible to adjust for ART duration, which was longer for the chronically-infected compared to PHI participants, or for ART class. It is also possible that some SMART participants may have initiated ART in primary infection, although this information is not captured. Our analyses, restricted to the chronically-infected participants only, however, indicated that those previously on NNRTI-based regimens had lower week 4 pVL rebound compared to those on other regimens. Since a greater proportion of chronically-infected, compared to PHI participants, were previously on NNRTI-based regimens, adjustment for ART class would have only served to augment the differences reported here between the groups. Longitudinal analysis of both populations is also subject to bias due to informative censoring, in particular due to exclusion of data for individuals who re-initiated ART. However, since a higher proportion of chronically-infected compared to PHI participants reintiated therapy (32% versus 9% before week 48), the results presented here are likely to be an underestimate of the difference between the two populations. Although pVL assays varied according to location, it is unlikely, given the pVL ranges in these analyses, that use of different commercial pVL assays would significantly affect the results.

In both trials, pVL was not measured until 4 weeks following treatment interruption. Although PHI participants were not observed to rebound to pre-ART pVL levels until a median of 50 weeks, the pVL levels may have been greater before week 4. Earlier and more frequent testing would give a better indication of immediate viral dynamics following ART cessation. The move in the HIV prevention field to explore a universal “test and treat” strategy [9,17–18] is currently receiving much scientific and advocacy interest. Although mathematical models are encouraging, the effectiveness of such an approach will depend on sustained adherence to therapy. Transmission risk has been shown to be higher in those with pVL>1500 copies/ml [5,19]. The data presented in our analysis show that, irrespective of disease stage and nadir CD4 count, the level of rebound viraemia on stopping ART in the vast majority of individuals reaches a level above which transmission can occur. Targeting individuals with ART during PHI could have a marked impact on HIV transmission [20], but it is crucial that strategies investigating the use of ART as transmission prevention examine the consequences of ART discontinuation and viral rebound on onward transmission. In addition to the impact of pVL on transmission, the sexual behaviour of those individuals critically impacts their transmission risk at a population level [21]. In the SMART trial, individuals did not reduce high-risk sexual behaviour despite treatment interruption, and detectable pVL [22]. This was not investigated in SPARTAC. However, in a recent study looking at onward HIV transmission amongst 47 individuals treated in PHI who stopped ART, there were at least five new primary infection events originating from these persons within 16–61 weeks after stopping early ART [23]. This analysis provides estimates for the viral rebound following cessation of ART initiated in PHI or chronic infection, and may inform mathematical models evaluating the potential population effect of universal treatment on HIV incidence for individuals stopping ART. The demonstrated differences in viral load dynamics following ART cessation between PHI and chronic infection indicate that the consequences of treatment interruption may differ, potentially reflecting differences in immunological status, HIV activation and reservoir size. This analysis supports

Figure 2. pVL after ART stop in primary (PHI) and chronic HIV infection (CHI), with CHI participants categorised by nadir CD4 count. a. median (IQR) pVL up to 48 weeks after ART stop. b. predicted pVL over 4–48 weeks after ART stop, based on a representative participant (male infected through sex between men, aged 40 years and CD4 count 600 cells/mm³ at ART stop; values in brackets are the 95% CI). CI = confidence intervals, IQR = interquartile range, pVL = plasma viral load.

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11. Imperial College Trial Secretariat: S Keeling, A Becker. Imperial College DMSM Secretariat: C Boocock.

* Left the study team before the trial ended

SMART investigators


