Antiretroviral Initiation at ≥800 CD4+ Cells/mm³ Associated With Lower Human Immunodeficiency Virus Reservoir Size

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Background. Identifying factors that determine the frequency of latently infected CD4+ T cells on antiretroviral therapy (ART) may inform strategies for human immunodeficiency virus (HIV) cure. We investigated the role of CD4+ count at ART initiation for HIV persistence on ART.

Methods. Among participants of the Strategic Timing of Antiretroviral Treatment Study, we enrolled people with HIV (PWH) who initiated ART with CD4+ T-cell counts of 500–599, 600–799, or ≥800 cells/mm³. After 36–44 months on ART, the levels of total HIV-DNA, cell-associated unspliced HIV-RNA (CA-US HIV-RNA), and two-long terminal repeat HIV-DNA in CD4+ T cells were quantified and plasma HIV-RNA was measured by single-copy assay. We measured T-cell expression of Human Leucocyte Antigen-DR Isotype (HLA-DR), programmed death-1, and phosphorylated signal transducer and activator of transcription-5 (pSTAT5). Virological and immunological measures were compared across CD4+ strata.

Results. We enrolled 146 PWH, 36 in the 500–599, 60 in the 600–799, and 50 in the ≥800 CD4+ strata. After 36–44 months of ART, total HIV-DNA, plasma HIV-RNA, and HLA-DR expression were significantly lower in PWH with CD4+ T-cell count ≥800 cells/mm³ at ART initiation compared with 600–799 or 500–599 cells/mm³. The median level of HIV-DNA after 36–44 months of ART was lower by 75% in participants initiating ART with ≥800 vs 500–599 cells/mm³ (median [interquartile range]: 16.3 [7.0–117.6] vs 68.4 [13.7–213.1] copies/million cells, respectively). Higher pSTAT5 expression significantly correlated with lower levels of HIV-DNA and CA-US HIV-RNA. Virological measures were significantly lower in females.

Conclusions. Initiating ART with a CD4+ count ≥800 cells/mm³ compared with 600–799 or 500–599 cells/mm³ was associated with achieving a substantially smaller HIV reservoir on ART.

Keywords. HIV; HIV reservoir; antiretroviral therapy; HIV cure.

Despite long-term virological suppression with antiretroviral therapy (ART), human immunodeficiency virus (HIV) persists in long-lived and proliferating CD4+ T cells [1]. Because latently infected cells constitute the main barrier to a cure, identifying factors that determine their frequency may provide insights into HIV cure strategies. Initiating ART early (eg, during seroconversion, within 6 or 12 months of infection) is associated with a lower frequency of latently infected CD4+ T cells (ie, lower HIV reservoir size) [2–11], faster decay of cell-associated HIV-DNA [12], better CD4+ and CD8+ T-cell recovery [13–15], and better preserved B- and T-cell function.

Received 20 June 2021; editorial decision 28 February 2022; published online 9 April 2022.

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Clinical Infectious Diseases® 2022;75(10):1781–91

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https://doi.org/10.1093/cid/ciac249

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However, the capacity to preserve higher levels of CD4+ counts, regardless of duration of untreated infection, may also be an important factor in restricting or reducing the latent HIV reservoir. For example, ART initiation at higher CD4+ T-cell counts has been associated with a lower frequency of CD4+ T cells containing HIV-DNA in people with HIV (PWH), regardless of duration of untreated infection [4, 18, 19]. Also, exceptional CD4+ T-cell recovery on ART, defined as achieving a CD4+ T-cell count of ≥1000 cells/mm³, has been associated with a smaller HIV reservoir [20].

Thus, PWH who have not yet received ART, have the capacity to preserve CD4+ T-cell counts to levels typically observed in otherwise healthy HIV-seronegative persons, may constitute an elite immunological subgroup. Mean CD4+ T-cell counts ranged from 771 to 1109 cells/mm³ in HIV-negative individuals across 25 studies [15]. It is unknown whether maintaining a CD4+ T-cell count within this normal range despite HIV infection, and regardless of duration of untreated infection, is associated with a lower reservoir size on ART.

Biological sex may also affect HIV persistence on ART. Women comprise approximately 50% of the 38 million PWH worldwide [21]. Studies have demonstrated differences between men and women in the dynamics of the HIV reservoir [22]; however, females are greatly underrepresented in HIV persistence studies [23]. Adult females have greater longevity and are more immunocompetent with stronger innate and adaptive immune responses compared with men [24, 25]. Additionally, a recent study demonstrates that across all age ranges, females have a greater capacity to preserve a marker of immunologic resilience that associates with resistance to immunodeficiency syndrome (AIDS) and COVID-19 (ie, higher CD4+ counts and a relatively lower degree of CD8+ T-cell expansion [26]). Hence, this female-biased capacity may associate with a lower HIV reservoir size in females versus males.

The Strategic Timing of Antiretroviral Treatment (START) Study was a randomized clinical trial in which PWH with >500 CD4 cells/mm³ were randomized to initiate ART immediately, or defer ART until CD4+ T-cell counts decreased to <350 cells/mm³ [27]. We enrolled START study participants randomized to the immediate ART arm to test the hypothesis that preservation of CD4+ T-cell counts ≥800 versus 500–599 or 600–799 cells/mm³ before ART initiation associates with a lower frequency of latently infected CD4+ T cells on suppressive ART.

METHODS

Study Design and Participants

Details of the START study are described elsewhere [27]. We enrolled a subset of START study participants randomized to the immediate ART arm into the HIV reservoir study; participants were eligible if they had received ART for 36–44 months without interruption >2 weeks, and if all plasma HIV-RNA levels obtained 8 months after initiating ART were <400 copies/mL (Figure 1). Study participants were categorized according to whether their CD4+ count at ART initiation was 500–599, 600–799, or ≥800 cells/mm³. We selected 800 cells/mm³ as a threshold because it approximated the lower bounds of the interquartile range of median CD4+ counts in healthy HIV-seronegative persons [15]. Virological and immunological analyses were performed in peripheral blood mononuclear cells collected after 36–44 months of ART (Figure 1). Study participants were enrolled at 8 sites in Peru, South Africa, and Uganda; sites were chosen based on their rapid early enrollment into the main START study.

The study was approved by the Alfred Hospital Research and Ethics Committee and the Institutional Review Boards/Ethics Committees at the recruiting sites and was conducted in accordance with the principles of the Declaration of Helsinki (1996). Each participant provided written informed consent before any study procedures.

Outcomes

The primary/secondary measures and associations are outlined in Figure 1. The primary outcome measure was the level of total HIV-DNA in peripheral blood CD4+ T cells after 36–44 months of ART [28]. Secondary virological outcome measures were the level of cell-associated unspliced HIV-RNA (CA-US HIV-RNA) and 2-long terminal repeat (2-LTR) HIV-DNA in CD4+ T cells and plasma HIV-RNA measured by an ultrasensitive assay [29]. Secondary immunological outcome measures were (1) CD4+ T-cell expression of the activation marker Human Leucocyte Antigen-DR Isotype (HLA-DR) and the exhaustion marker programmed death-1 (PD-1) [28] and (2) as a measure of T-cell responsiveness, the proportion of CD3+ T cells expressing phosphorylated signal transducer and activator of transcription-5 (pSTAT5) without or following ex vivo stimulation with interleukin-2 (IL-2). See Supplementary Methods for more details.

START Study Data

Baseline START study data collected were age, sex, race, estimated (self-reported) duration of HIV infection, CD4+ T-cell count, CD4+ T-cell nadir, plasma HIV-RNA, CD4+ T-cell percentage, CD4:CD8 ratio, co-infection with hepatitis B or C, ART regimen, and current smoking, medical history including cardiovascular disease, hypertension, and diabetes. We also accessed START study data on plasma levels of IL-6, high-sensitivity C-reactive protein and D-dimer at ART initiation.

Statistical Analyses

Based on prior work [30], we assumed a standard deviation for the level of total HIV-DNA of 0.65 log₁₀ per million CD4+ T cells. Using this estimate, and after inflating our calculations by 20%, we estimated that 150 participants would provide 80%
statistical power at a 5% significance level to detect a difference in total HIV-DNA of at least 0.4 log₁₀ per million CD4+ T cells between participants commencing ART at CD4+ T-cell count ≥ 800 compared with 500–599 or 600–799 cells/mm³.

We compared virological and immunological measures across the CD4+ strata using Kruskal-Wallis equality of populations rank test and the Dunn multiple pairwise comparison test. To analyze associations of immunological measures or clinical characteristics at ART initiation with HIV reservoir size, we applied a generalized negative binomial regression model with all replicate data used in the analysis as previously described [30]. We assessed covariates for collinearity using the variance inflation factor together with Akaike's Information Criterion. We performed both univariate and multivariable analyses using stepwise regression in the multivariate model.

RESULTS

Study Participants
We enrolled 146 study participants, 36 in the 500–599, 60 in the 600–799, and 50 in the ≥ 800 CD4+ T-cells/mm³ strata. Of these, 59 (40%) were males and 87 (60%) were females (Table 1). The median age was significantly different across the CD4+ strata, oldest in the ≥ 800 followed by the 600–799 cells/mm³ stratum. Congruently, the median CD4:CD8 ratio was significantly different across the 3 CD4+ T-cell strata, highest in the ≥ 800, and then decreasing to the lowest in the 500–599 cells/mm³ stratum. However, other parameters were evenly distributed across the CD4+ strata (Table 1). Plasma levels of IL-6, D-dimer, and high-sensitivity C-reactive protein at ART initiation did not differ across the CD4+ T-cell strata (Supplementary Figure S1). Age
is associated with CD4+ lymphopenia [26]; thus, the older age of individuals within the ≥ 800 cells/mm³ stratum may reflect a length-time bias wherein START study participants with ≥ 800 cells/mm³ stratum had preserved higher CD4+ counts a longer time before HIV diagnosis.

**Primary and Secondary Virological Outcome Measures**

We quantified the level of total HIV-DNA in peripheral blood CD4+ T cells as a proxy for the frequency of infected cells, acknowledging that this measurement includes unintegrated, integrated, defective, and intact virus [31, 32]. The frequency of cells containing HIV-DNA was significantly lower in participants initiating ART with CD4+ ≥ 800 compared with either 600–799 (P = .023) or 500–599 (P = .002) cells/mm³ (Figure 2A). Median (interquartile range) levels of total HIV-DNA in persons initiating ART with 500–599, 600–799, and ≥ 800 cells/mm³ were 68.4 (13.7–213.1), 30.0 (17.1–91.9), and 16.3 (7.0–117.6) copies/million cells, respectively. Hence, the median level of HIV-DNA in the ≥ 800 stratum was less than 25% of that in the 500–599 cells/mm³ stratum.

We quantified CA-US HIV-RNA as a measure of persistent HIV transcription on ART and 2-LTR circles as a measure of recently infected cells. Although these measures were slightly lower in the ≥ 800 stratum, differences were not statistically significant (P = .55 for CA-US HIV-RNA and P = .27 for 2-LTR using Kruskal-Wallis test; Figure 2B and 2C). Analysis of residual viremia on ART (quantified by an ultrasensitive assay with lower limit of detection of 1 copy per milliliter) revealed that plasma HIV-RNA

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**Table 1. Clinical Characteristics at ART Initiation**

| Variable                                                                 | Overall (n = 146) | 500–599 (n = 36) | 600–799 (n = 60) | ≥800 (n = 50) | P-Value |
|--------------------------------------------------------------------------|-------------------|------------------|------------------|--------------|---------|
| Age, median (IQR), y                                                      | 39.5 (34, 48)     | 36.5 (30.0, 41.5) | 40 (35.0, 47.5) | 44.5 (36.0, 50.0) | .021    |
| Sex, n (%)                                                               |                   |                  |                  |              |         |
| Male                                                                     | 59 (40.4)         | 17 (47.2)        | 25 (41.7)        | 17 (34.0)    | .452    |
| Female                                                                   | 87 (59.6)         | 19 (52.8)        | 35 (58.3)        | 33 (66.0)    | .188    |
| Race, n (%)                                                              |                   |                  |                  |              |         |
| Black                                                                    | 124 (84.9)        | 28 (77.8)        | 53 (88.3)        | 43 (86.0)    | .372    |
| Hispanic/Latino                                                          | 20 (13.7)         | 8 (22.2)         | 5 (8.3)          | 7 (14.0)     |         |
| Other                                                                    | 2 (1.37)          | 0                | 2 (3.3)          | 0            |         |
| Country where participant enrolled, n (%)                                |                   |                  |                  |              |         |
| Peru                                                                     | 20 (13.7)         | 8 (22.2)         | 5 (8.3)          | 7 (14.0)     | .379    |
| South Africa                                                             | 57 (39.0)         | 11 (30.6)        | 25 (41.7)        | 21 (42.0)    |         |
| Uganda                                                                   | 69 (47.3)         | 17 (47.2)        | 30 (50.0)        | 22 (44.0)    |         |
| Estimated (self-reported) duration of HIV infection before ART initiation, median (IQR), y | 2.0 (0.4, 5.4) | 1.5 (0.4, 5.5) | 1.7 (0.4, 4.3) | 2.8 (0.5, 7.1) | .502 |
| Time on ART at time of sampling for HIV reservoir analyses, median (IQR), m | 38.2 (36.3, 41.7) | 39.2 (36.5, 41.8) | 38.6 (36.4, 41.8) | 37.1 (36.3, 41.3) | .379 |
| CD4+ count at ART initiation (cells/mm³), median (IQR)                   | 710.3 (604.0, 854.5) | 678 (641, 733) | 564.3 (533, 577) | 932 (852.5, 1081.5) | N/A     |
| Recorded nadir CD4+ count before ART (cells/mm³), median (IQR)           | 630 (530, 781)    | 612.5 (541, 663.5) | 519 (491.5, 536.5) | 837 (762, 972) | N/A     |
| CD4:CD8 T-cell ratio at ART initiation, median (IQR)                      | 0.8 (0.6, 1.0)    | 0.7 (0.6, 0.9)   | 0.6 (0.4, 0.8)   | 0.9 (0.7, 1.2) | .000    |
| Plasma HIV RNA at ART initiation (log10 copies/mL), median (IQR)         | 3.9 (3.1, 4.7)    | 3.9 (3.0, 4.5)   | 4.4 (3.7, 4.9)   | 3.9 (3.0, 4.5) | .505    |
| Current smoking, n (%)                                                    | 19 (13.0)         | 9 (15.0)         | 6 (16.7)         | 4 (8.0)      | .403    |
| Positive cardiovascular disease, n (%)                                   | 0                 | 0                | 0                | 0            | N/A     |
| Positive diabetes, n (%)                                                 | 3 (2.1)           | 2 (3.3)          | 0                | 1 (2.0)      | .787    |
| Positive hypertension, n (%)                                             | 21 (14.4)         | 12 (20.0)        | 2 (5.56)         | 7 (14.0)     | .143    |
| Positive hepatitis B, n (%)                                              | 6 (4.1)           | 3 (5.0)          | 1 (2.8)          | 2 (4.0)      | 1.000   |
| Positive hepatitis C, n (%)                                              | 2 (1.4)           | 1 (1.7)          | 0 (0.0)          | 1 (2.0)      | 1.000   |
| ART regimen prescribed, n (%)                                            |                   |                  |                  |              | .450    |
| NRTI + PI                                                                | 9 (6.2)           | 1 (2.8)          | 5 (8.3)          | 3 (6.0)      |         |
| NRTI + NNRTI                                                             | 136 (93.2)        | 34 (94.4)        | 55 (91.7)        | 47 (94.0)    |         |
| NRTI only (protocol deviation)                                           | 1 (0.70)          | 1 (2.8)          | 0 (0.0)          | 0 (0.0)      |         |

Abbreviations: ART, antiretroviral therapy; IQR, interquartile range; N/A: not applicable; NNRTI, nonnucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

*Acute myocardial infarction, stroke, or coronary revascularization.

*Diabetes mellitus diagnosis or receiving antidiabetic medication (insulin, metformin, sulfonylureas, thiazolidinediones or biguanides, or other) or 8-hour fasting glucose ≥ 126 mg/dL.

*Systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg or receiving blood pressure medication (beta blockers, diuretics, angiotensin converting enzyme inhibitors, angiotensin receptor blockers, calcium channel antagonists, or other).
was significantly lower in participants initiating ART with ≥ 800 versus 500–599 \( (P = .031) \) and trending lower versus the 600–799 cells/mm\(^3\) stratum \( (P = .056) \) (Figure 2D). Collectively, these analyses showed that of those participants randomized to immediate ART, those initiating ART at ≥ 800 cells/mm\(^3\) had a much lower frequency of latently infected CD4+ T cells and a lower level of residual viremia after 36–44 months on ART.

We did not detect an association between participants’ highest CD4+ T-cell counts reported after 36–44 months of ART, and total HIV-DNA and did not find a significant association overall, or across the CD4+ cell strata (Supplementary Table S8).

**Secondary Immunological Outcome Measures**

We found that CD4+ T-cell expression of HLA-DR was significantly lower in PWH initiating ART with CD4+ T-cell counts ≥ 800 compared with 500–599 cells/mm\(^3\) (Figure 3A). However, expression of programmed death-1 or pSTAT5 on T cells did not differ by CD4+ strata (Figure 3B–D).

**Correlations Between Immunological and Virological Outcome Measures**

In multivariate analyses, higher levels of CD4+ T-cell expression of HLA-DR after 36–44 months of ART were associated with higher levels of both total HIV-DNA and CA-US HIV-RNA (Figure 4; Supplementary Tables S1 and S3). At the same timepoint, although expression levels of pSTAT5 were similar across CD4 strata, there was a highly significant association between pSTAT5 levels and measures of HIV persistence. Higher expression of pSTAT5, with or without ex vivo IL-2 stimulation, was correlated with lower levels of HIV-DNA and CA-US HIV-RNA in both univariate and multivariate analyses, with the latter including adjustment for CD4+ count at ART initiation (Figure 4; Supplementary Tables S1–S4). Together, these results suggest that T-cell activation was modestly associated with a larger HIV reservoir and that higher pSTAT5 expression correlated with a lower frequency of infected cells as well as lower HIV transcriptional activity, independent of CD4+ count at ART initiation.

**Associations of Clinical Characteristics at ART Initiation With HIV Reservoir Size on ART**

Older age associated with a lower level of total HIV-DNA (Table 2), which may relate to the length-time bias discussed earlier (Table 1). Female sex showed a strong association with lower total HIV-DNA (ratio 0.565; 95% confidence interval, .350–0.912) compared with male sex (Table 2). Correspondingly,

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**Figure 2.** Levels of cell-associated and plasma HIV across strata of CD4+ T-cell count at ART initiation. The frequency of total HIV-DNA (A), CA-US HIV-RNA (B), and 2-LTR circles (C) in CD4+ T cells, and the level of plasma HIV-RNA measured by single-copy assay (D) within each stratum of CD4+ T-cell count as indicated. 2-LTR: 2-long terminal repeat; CA-US HIV-RNA, cell-associated unspliced HIV-RNA. Each symbol represents a different participant and the horizontal black line the median value.
we found significantly lower levels of total HIV-DNA, CA-US HIV-RNA, 2-LTR HIV-DNA, and plasma HIV-RNA in females compared with males (Figure 5A). To examine whether the lower frequency of total HIV-DNA in the ≥ 800 cells/mm$^3$ stratum related to a higher proportion of females, we performed sensitivity analyses stratified by sex. In the analyses restricted to females, initiating ART with ≥ 800 cells/mm$^3$ associated with lower frequency of HIV-DNA (Figure 5B). In contrast, in males, the slightly lower median levels of total HIV-DNA in the 500–599 and 600–799 CD4 cells/mm$^3$ strata compared with the ≥ 800 cells/mm$^3$ stratum, no longer reached statistical significance, possibly because of loss of statistical power (Figure 5B). For females, there was also a consistent trend toward a lower level of CA-US HIV-RNA in the ≥ 800 cells/mm$^3$ stratum compared with the 500–599 and 600–799 cells/mm$^3$ strata (Figure 5C), whereas no differences across CD4+ strata for either sex was found for 2-LTR HIV-DNA and plasma HIV-RNA (Figure 5D and 5E).

Because of the older age of study participants in strata with higher CD4+ counts (Table 1), we examined whether age confounded the main finding of lower total HIV-DNA in the ≥ 800 CD4+ T cells/mm$^3$ stratum. In a multivariate analysis adjusted for age, sex, enrollment country, plasma viral load at ART initiation and hepatitis B, the CD4+ T-cell count at ART initiation remained significantly associated with total HIV-DNA after 36–44 months on ART (Table 2). The pre-ART CD4:CD8 ratio was significantly associated with total HIV-DNA in univariate analysis (0.37; 95% confidence interval, 0.216–0.639). However, given the higher correlation between CD4+ counts and CD4:CD8 ratio values (collinearity), the association of the ratio with HIV-DNA was no longer significant in the multivariate analysis (Table 2). There was no association with type 2 diabetes or hypertension, whereas enrollment at Ugandan compared with Peruvian sites was associated with higher total HIV-DNA (Table 2; Supplementary Figure S2). Similar observations were observed in univariate analyses (Supplementary Figure S2 and Supplementary Table S5). Hepatitis B surface antigen positivity was significantly associated with a lower total HIV-DNA, but the significance of this association is unclear because only 6 study participants were seropositive.
Exploratory Outcomes

We analyzed the association between CA-US HIV-RNA and clinical characteristics at ART initiation. Being hepatitis B surface antigen positive and longer time on ART at time of sample collection were both significantly associated with a lower level of CA-US HIV-RNA (Supplementary Figure S2).

Table 2. Multivariate Analysis of Associations Between Total HIV-DNA in CD4+ T Cells and Clinical Characteristics at ART Initiation

| Variable                                                                 | Overall (N = 146) | Ratio      | 95% CI     | P-Value |
|--------------------------------------------------------------------------|-------------------|------------|------------|---------|
| CD4+ count at ART initiation (cells/mm^3)                                 | 0.998             | .997       | 1.000      | .009    |
| Age, y                                                                   | 0.973             | .951       | .995       | .015    |
| Sex (referent, male)                                                     |                   |            |            |         |
| Female                                                                   | 0.565             | .350       | .912       | .019    |
| Current smoking                                                          | -                 | -          | -          | .201    |
| Country where participant enrolled (referent, Peru)                       |                   |            |            |         |
| South Africa                                                             | 0.705             | .330       | 1.507      | .367    |
| Uganda                                                                   | 2.491             | 1.151      | 5.392      | .021    |
| Estimated (self-reported) duration of HIV infection before ART initiation, y | -                 | -          | -          | .631    |
| Time on ART at time of sampling for HIV reservoir analyses, m             | -                 | -          | -          | .942    |
| CD4:CD8 ratio at ART initiation                                           | -                 | -          | -          | .420    |
| Plasma HIV RNA at ART initiation (log10 copies/mL)                       | 1.189             | 1.000      | 1.413      | .050    |
| Positive hypertension^a                                                   | -                 | -          | -          | .427    |
| Positive hepatitis B^b                                                    | 0.224             | .068       | .738       | .014    |
| ART regimen (referent, PI/NRTI)                                           |                   |            |            |         |
| NRTI/NNRTI                                                               | -                 | -          | -          | .333    |
| Plasma IL6 (pg/mL) at ART initiation                                      | 0.842             | .676       | 1.047      | .122    |
| Plasma d-dimer (µg/mL) at ART initiation                                  | 0.753             | .568       | .998       | .048    |
| Plasma hs-CRP (µg/mL) at ART initiation                                   | 1.005             | .985       | 1.025      | .645    |

Adjusted for: age, sex, country, viral load, hepatitis B.

^aSystolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg or receiving blood pressure medication (beta blockers, diuretics, angiotensin converting enzyme inhibitors, angiotensin receptor blockers, calcium channel antagonists, other).

^bHepatitis B measured as being hepatitis B surface antigen positive.
and Supplementary Tables S6 and S7), whereas being on a nonnucleoside reverse transcriptase inhibitor-based regimen compared with a protease inhibitor-based regimen was associated with a higher level of CA-US HIV-RNA. Current smoking afforded a nearly 3-fold change in CA-US HIV-RNA. These exploratory analyses suggested that factors associated with the frequency of infected cells were distinct from those associated with transcriptional activity of the reservoir.

DISCUSSION

Among participants randomized to the immediate arm of the START study, we found that levels of total HIV-DNA and plasma HIV-RNA were significantly lower in participants who initiated ART with CD4+ T-cell count ≥ 800 compared with either 600–799 or 500–599 cells/mm³. The median level of HIV-DNA assessed after 36–44 months on ART was lower by 75% in participants initiating ART with ≥ 800 versus 500–599 cells/mm³. In multivariate analyses, the association of total HIV-DNA with CD4+ T-cell count at ART initiation remained statistically significant after controlling for potential confounders including age and sex. Notably, HIV persistence on ART was greater in males than females. Finally, we found that higher CD4+ T-cell expression of HLA-DR was associated with a higher frequency of infected CD4+ T cells, whereas higher pSTAT5 expression correlated with a lower frequency of cells containing HIV-DNA and US HIV-RNA. Collectively, these findings suggest that PWH with the elite capacity to preserve CD4+ T cells ≥ 800/mm³ before commencing ART manifest a substantially lower HIV reservoir on suppressive ART.

Others have found a lower frequency of latently infected cells following early ART initiation [2–11]. However, this is the first study to directly address the role of the CD4+ T-cell count at ART initiation using prespecified CD4 strata regardless of duration of untreated infection. Because the study was conducted exclusively among PWH who initiated ART with CD4+ T-cell counts > 500 cells/mm³, we were uniquely positioned to address nuanced effects of higher CD4+ T-cell counts on the HIV reservoir under current treatment guidelines.

The larger proportion of females in the study facilitated the identification of differences in measures of HIV persistence on ART between sexes. This is aligned with cross-sectional studies in PWH on ART [22, 33–35] and may relate to the stronger...
innate and adaptive immune responses in adult females [24, 25] and the potential role of estrogen in HIV persistence through its effect on the HIV LTR whereupon it inhibits HIV transcription [36].

The transcription factor STAT5 is activated through phosphorylation, responding to drivers of T-cell proliferation, in particular IL-7 and IL-2 [37], and plays a key role in in shaping the CD4+ T-cell immune response [38]. Thus, pSTAT5 levels serve as a proxy for the functionality/responsiveness of T cells. STAT5 activity is involved in driving tumor-specific [39] and cytomegalovirus (CMV)-specific [40] T-cell polyfunctionality and cytokine production, thus emphasizing the potential role of STAT5 in the generation of a potent immune response. Hence, the significant associations between pSTAT5 and HIV reservoir size underscore the importance of reconstituted immunologic health in reducing the frequency of infected cells that is independent of CD4+ T-cell count at ART initiation.

It is plausible that the association we observed between higher levels of pSTAT5 and a lower HIV reservoir size could be explained by greater homeostatic proliferation of CD4+ T cells. However, our finding that levels of pSTAT5 were not significantly different between the 3 CD4+ cell strata and that there was no significant difference either overall, or between the CD4+ cell strata in highest levels of CD4+ cells gained during 36–44 months of ART makes this a less likely explanation for our findings.

Several limitations of our study require consideration. First, factors other than CD4+ T-cell count at ART initiation have the potential to confound our findings. We addressed this by performing stepwise regression in multivariate analyses but recognize there may be additional unrecognized confounders. Second, because samples were not collected for HIV reservoir analyses at the time of ART initiation, we were unable to longitudinally track levels of cell-associated HIV-DNA, RNA, or pSTAT5. Third, we did not analyze the HIV subtypes in study participants, which would have varied between countries. However, adjustment for country of enrollment may mitigate potential confounding because of this factor. Fourth, because of limitations in cell numbers, we were unable to analyze HIV-specific T-cell function, quantify the frequency of cells containing intact HIV provirus, or measure the frequency of cells with inducible replication competent virus. It has been estimated, using near full-genome sequencing of HIV provirus, that only approximately 2.5% of HIV provirus is intact [41]. If enhanced immune-mediated elimination of virus-expressing cells among PWH with CD4+ T-cell counts \( \geq 800 \text{ cells/mm}^3 \) at ART initiation is the main mechanism leading to a lower HIV reservoir in this stratum after 36–44 months of ART, this may primarily be directed against cells containing intact HIV. It would therefore have been of great interest to compare levels of intact HIV DNA across the three strata, but unfortunately this was not feasible in the present study. Fifth, we analyzed pSTAT5 in total CD3+ T cells; hence, it is uncertain whether this association reflected pSTAT5 levels in CD8+ or CD4+ T cells, or both. Our data revealed no difference between pre-ART CD4 strata in pSTAT5 after 36–44 months of ART but do not rule out that such differences might have been present at ART initiation. Finally, CMV antibody tests were not available for study participants. Hence, we were unable to determine potential associations between CMV infection and HIV persistence. CMV antibody positivity rates are very high in PWH [42]; hence, these high rates across CD4+ strata would have likely precluded the power to detect a significant association.

In conclusion, we found that initiating ART with a CD4+ count \( \geq 800 \text{ compared with 600–799 or 500–599 cells/mm}^3 \) was associated with a significantly lower level of total HIV-DNA, plasma HIV-RNA, and T-cell activation after 36–44 months of suppressive ART. Higher pSTAT5 expression correlated with a lower level of HIV-DNA independent of CD4+ T-cell count at ART initiation. Additionally, we observed that reservoir sizes were lower in females, which we suggest is related to the impact of estrogen, which represses HIV reactivation, and because of an enhanced innate immune response in females compared with males, in response to similar levels of HIV RNA. Taken together, these findings suggest that PWH who are able to preserve CD4+ T cells \( \geq 800 \text{ cells/mm}^3 \) before ART initiation, especially females, have a smaller reservoir on ART. Interventional cure studies in this subgroup could potentially have a favorable outcome.

### Supplementary Data

Supplementary materials are available at Clinical Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copiededit and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

### Notes

**Author contributions.** E. J. W. and S. R. L. conceived the study. E. J. W., S. R. L., S. K. A., and J. N. designed the study. E. J. W., L. L., M. J. V., and E. H. oversaw all aspects of the clinical study including study protocol, ethics submission, and management of study participants. C. C. contributed to the design of the laboratory protocol. E. J. W., S. R. L., A. R., J. C., M. J. V., F. H., S. P., R. M., S. B. F., J. K., J. L., P. P., H. M., E. K., P. A. M., C. G., A. L. R., and R. W. coordinated all sample collection, planned sample analyses, and coordinated all data generation. A. R. and J. C. performed polymerase chain reaction analyses of CA-US HIV-RNA, 2-LTR, HIV-DNA, and all flow cytometry analyses. S. P. and K. F. oversaw and performed analyses of plasma HIV-RNA. L. K. and K. P. performed all statistical analysis. E. W., S. R. L., T. A. R., J. N., and S. K. A. performed data analysis and interpretation. T. A. R. drafted the manuscript. All authors reviewed and provided input to the manuscript and approved the final version.

**Acknowledgments.** The authors acknowledge the study participants and all human and animal participants of previous HIV cure studies. The authors also acknowledge the excellent work of other researchers that we have not been able to cite in this publication. This manuscript is dedicated to Dr. Fred Gordin.

**Financial Support.** The study was funded by grants from Gilead Sciences Inc. (grant number CO-US-253-1804), Merck (grant number 51859), The Australian National Health and Medical Research Council (program grant APP119990, National Health Medical Research Council practitioner fellowship APP 1135851, and early career fellowship APP 1092160) and The
Australian Centre for HIV and Hepatitis Virology Research. This work was also supported by the Delaney AIDS Research Enterprise (DARE) to Find a Cure (U19AI096109 and U1UM1AI26661-01). The INSIGHT Washington International Coordinating Center provided in-kind effort and supplemental support to participating sites.

Potential conflicts of interest. T. A. R. has received funding from the Danish Research Council, Region Midt Denmark, The Australian Centre for HIV and Hepatitis Virology Research, Melbourne HIV Cure Consortium, and Gilead outside the submitted work and payment for lectures from Gilead Sciences. T. A. R. also reports a leadership or fiduciary role with the HIV Cure Community Partnership Steering Committee in Australia, the 18th European AIDS Conference Scientific Committee, and the Australasian Society for HIV Medicine (ASHM), Taskforce on Bloodborne Viruses (BBV), Sexual Health and COVID-19. D. P. D. was funded by a grant from the National Cancer Institute (grant number RO1-CA228172). S. P. has received funding from the National Health and Medical Research Council of Australia (NHMRC), National Institutes of Health (NIH), amfAR, the Foundation for AIDS Research, and The Australian Centre for HIV and Hepatitis Virology Research. P. M. declares that her institution received funding from the INSIGHT Network to undertake the START study and received a grant from Alfred Health to conduct sample collection and shipment for the HIV Reservoirs substudy of the START trial. C. C. declares receipt of funding from the NHMRC for an Early Career Fellowship. S. B.-F. declares that her institute received funding from the INSIGHT Network to undertake the START study. K. P. declares that she has received unconditional research grants from Viiv Healthcare and Gilead Sciences. P. P. holds shares in Gilead Sciences and GlaxoSmithKline. S. R. L. has received funding from the Australian NHMRC, the Australian Center for HIV and Hepatitis Virology Research NIH, amfAR (Magnet grant award number 19-02602), Gilead Sciences (clinical research grants), Merck, Viiv, and Leidos outside the submitted work. S. R. L. also reports consulting fees from Abivax, Geovax, Viiv, and Tetralogic, honoraria from Gilead Sciences, Bristol Myers Squibb, and Merck Sharpe & Dohme, an International PCT patent (PCTAU2017050631), and participation on advisory boards for Abivax, Bionor, Viiv, Calimmune, InniVirVax, Aelix Therapeutics, Immuncore, and the French Agency for Research on AIDS and Viral Hepatitis (ANRS) Emerging Infectious Diseases. E. J. W. received research grants from Gilead Sciences, Merck Sharp & Dohme, and The Australian Centre for HIV and Hepatitis Virology Research for this submitted work. E. J. W. reports receipt of research grants from the Victorian, Tasmanian, and South Australian governments. E. J. W. has received free study drug from Gilead Sciences for the VicPrEP study and her institution has received funding from Gilead Sciences, Viiv Healthcare, Abbott, Merck Sharp & Dohme, Boehringer Ingelheim, and Janssen-Cilag. E. J. W. also reports payment for lectures from Gilead Sciences and the Australasian Society of HIV Medicine and participation on an Advisory Board for Gilead Sciences. All other authors report no potential conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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