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Antiretroviral initiation at ≥800 CD4+ cells/ mm^3 associated with lower HIV reservoir size

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Summary:

In people with HIV, initiating ART with CD4+ counts ≥800 cells/mm^3 versus 600-799 or 500-599 cells/mm^3 was associated with achieving a substantially smaller HIV reservoir on ART. Higher pSTAT5 expression correlated with a smaller HIV reservoir independent of CD4+ count.

Abstract

Background

Identifying factors that determine the frequency of latently infected CD4+ T-cells on antiretroviral therapy (ART) may inform strategies for 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 (START) Study, we enrolled people with HIV (PWH) who initiated ART with CD4+ T-cell counts of 500-599, 600-799 or ≥800 cells/mm^3. After 36-44 months on ART, we quantified levels of total HIV-DNA, cell-associated unspliced HIV-RNA (CA-US HIV-RNA) and 2-long terminal repeat HIV-DNA in CD4+ T-cells and measured plasma HIV-RNA by single-copy assay. We measured T-cell expression of HLA-DR, programmed death-1, 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/mm^3 at ART initiation compared to 600-799 or 500-599 cells/mm^3. 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/mm^3 [median (IQR): 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..

Conclusion

Initiating ART with a CD4+ count ≥800 cells/mm^3 compared to 600-799 or 500-599 cells/mm^3 was associated with achieving a substantially smaller HIV reservoir on ART.

Keywords:

HIV, HIV reservoir, antiretroviral therapy, HIV cure

Introduction

Despite long-term virological suppression with antiretroiral therapy (ART), HIV persists in long-lived and proliferating CD4+ T-cells [1]. As 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 (e.g., during seroconversion, or within 6 or 12 months of infection) is associated with a lower frequency of latently infected CD4+ T-cells (i.e., 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 [2, 9, 16, 17]. 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 ≥1,000 cells/mm^3, has been associated with a smaller HIV reservoir [20].

Thus PWH who prior to 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 771-1109 cells/mm^3 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 to 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 AIDS and COVID-19, i.e., 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 vs males.

The Strategic Timing of Antiretroviral Treatment (START) Study was a randomised clinical trial in which PWH with >500 CD4 cells/mm^3 were randomised to initiate ART immediately, or defer ART until CD4+ T-cell counts decreased to <350 cells/mm^3[27]. We enrolled START study participants randomised to the immediate ART arm to test the hypothesis that preservation of CD4+ T-cell counts above ≥800 vs. 500-599 or 600-799 cells/mm^3 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/mm^3. We selected 800 cells/mm^3 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 eight sites in Peru, South Africa, and Uganda; sites were chosen based on their rapid early enrolment 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 prior to any study procedures.

Outcomes

The primary/secondary measures and associations are outlined (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 (i) CD4+ T-cell expression of the activation marker HLA-DR and the exhaustion marker PD-1 [28] and (ii) 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, 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 (hs-CRP) 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 $\log_{10}$ 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 $\log_{10}$ per million CD4+ T-cells between participants commencing ART at CD4+ T-cell count ≥800 as compared to 500-599 or 600-799 cells/mm^3.

We compared virological and immunological measures across the CD4+ strata using Kruskal-Wallis equality of populations rank test and Dunn's multiple pairwise comparison test. To analyse associations of immunological measures or clinical characteristics at ART initiation with HIV reservoir size, we applied a generalised negative binomial regression model with all replicate data employed in the analysis as previously described [31]. 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/mm^3 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/mm^3 stratum. Congruently, the median CD4:CD8 ratio was significantly different across the three CD4+ T-cell strata, highest in the ≥800 then decreasing to the lowest in the 500-599 cells/mm^3 stratum. However, other parameters were evenly distributed across the CD4+ strata (Table 1). Plasma levels of IL-6, d-dimer and hs-CRP 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/mm^3 stratum may reflect a length-time bias wherein START study participants with ≥800 cells/mm^3 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 [32, 33]. The frequency of cells containing HIV-DNA was significantly lower in participants initiating ART with CD4+ ≥800 compared to either 600-799 (P=0.023) or 500-599 (P=0.002) cells/mm^3 (Figure 2A). Median (IQR) levels of total HIV-DNA in persons initiating ART with 500-599, 600-799 and ≥800 cells/mm^3 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/mm^3 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=0.55 for CA-US HIV-RNA and P=0.27 for 2-LTR using Kruskal-Wallis test; Figure 2B, 2C). Analysis of residual viremia on ART (quantified by an ultrasensitive assay with lower limit of detection of

one copy per mL) revealed that plasma HIV-RNA was significantly lower in participants initiating ART with ≥800 vs. 500-599 (P=0.031) and trending lower vs. the 600-799 cells/mm^3 stratum (P=0.056) (Figure 2D). Collectively, these analyses showed that of those participants randomised 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 (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 to 500-599 cells/mm^3 (Figure 3A). However, expression of PD-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, while 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 uni- 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% CI 0.350-0.912) compared to male sex (Table 2). Correspondingly, we found significantly lower levels of total HIV-DNA, CA-US HIV-RNA, 2-LTR HIV-DNA and plasma HIV-RNA in females compared to 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 as compared to the ≥800 cells/mm^3 stratum, no longer reached statistical significance, possibly due to loss of statistical power (Figure 5B). For females there was also a consistent trend towards a lower level of CA-US HIV-RNA in the ≥800 cells/mm^3 stratum compared to 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, 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, enrolment 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% CI 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 enrolment at Ugandan compared to Peruvian sites was associated with higher total HIV-DNA (Table 2; Figure S2). Similar observations were observed in univariate analyses (Figure S2; Table S5). Hepatitis B surface antigen positivity was significantly associated with a lower total HIV-DNA, but the significance of this association is unclear as only six study participants were seropositive.

Exploratory outcomes

We analysed 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 (Figure S2 and Supplementary Tables S6 and S7), whereas being on a non-nucleoside reverse transcriptase inhibitor -based regimen as compared to 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 randomised 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 to either 600-799 or 500-599 cells/mm^3. The median level of HIV-DNA assessed after 36-44 months on ART was lower by 75% in participants initiating ART with ≥800 vs. 500-599 cells/mm^3. 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 vs. 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/mm^3 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 pre-specified CD4-strata regardless of duration of untreated infection. As the study was conducted exclusively among PWH who initiated ART with CD4+ T-cell counts >500 cells/mm^3, 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, 34-36] and may relate to the stronger innate and adaptive immune responses in adult females [24, 25] and the potential role of oestrogen in HIV persistence through its effect on the HIV LTR whereupon it inhibits HIV transcription [37].

The transcription factor STAT5 is activated through phosphorylation, responding to drivers of T-cell proliferation, in particular IL-7 and IL-2 [38], and plays a key role in in shaping the CD4+ T-cell immune response [39]. Thus, pSTAT5 levels serve as a proxy for the functionality/responsiveness of T-cells. STAT5 activity is involved in driving tumour-specific [40] and CMV-specific [41] T-cell polyfunctionality and cytokine production, thus emphasising 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 three 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 recognise there may be additional unrecognised confounders. Second, as 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 analyse the HIV subtypes in study participants, which would have varied between countries. However, adjustment for country of enrolment may mitigate potential confounding due to this factor. Fourth, due to limitations in cell numbers, we were unable to analyse 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, utilising near full genome sequencing of HIV provirus, that only approximately 2.5% of HIV provirus is intact [42]. If enhanced immune-mediated elimination of virus-expressing cells among PWH with CD4+ T-

cell counts ≥ 800/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 analysed 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 does not rule out that such differences might have been present at ART initiation. Finally, cytomegalovirus (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 [43], 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 ≥800 compared to 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 oestrogen, which represses HIV reactivation, and due to an enhanced innate immune response in females compared to males, in response to similar levels of HIV RNA. Taken together, these findings suggest that PWH who are able to preserve CD4+ T-cells ≥800 cells/mm^3 before ART initiation, especially females, have a smaller reservoir on ART. Interventional cure studies in this subgroup could potentially have a favourable outcome.

NOTES

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Author contributions

EJW and SRL conceived the study. EJW, SRL, SKA and JN designed the study. EJW, LL, MJV, FH oversaw all aspects of the clinical study including study protocol, ethics submission and management of study participants. CC contributed to the design of the laboratory protocol. EW, SRL, AR, JC, MJV, FH, SP, RM, SBF, JK, JL, PP, HM, EK, PAM, CG, ALR, RW coordinated all sample collection, planned sample analyses, and coordinated all data generation. AR and JC performed PCR analyses of CA-US HIV-RNA, 2-LTR, HIV-DNA and all flow cytometry analyses. SP and KF oversaw and performed analyses of plasma HIV-RNA. LK and KP performed all statistical analysis. EW, SRL, TAR, JN and SKA performed data analysis and interpretation. TAR drafted the manuscript. All authors reviewed and provided input to the manuscript and approved the final version.

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Conflicts of interest

TAR has received funding from the Danish Research Council, Region Midt Denmark, The Australian Centre for HIV and Hepatitis Research, Melbourne HIV Cure Consortium and Gilead outside the submitted work and payment for lectures from Gilead Sciences. TAR 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 Blood-borne Viruses (BBV), Sexual Health and COVID-19. DPD was funded by a grant from NCI (grant number RO1-CA228172). SP 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 Research (ACH^2). PM declares that her institution received funding from the INSIGHT Network to undertake the START study and also received a grant from Alfred Health to conduct sample collection and shipment for the HIV Reservoirs substudy of the START trial. CC declares receipt of funding from the NHMRC for an Early Career Fellowship. S B-F declares that her institution received funding from the INSIGHT Network to undertake the START study. KP declares that she has received unconditional research grants from ViiV Healthcare and Gilead Sciences. PP holds shares in Gilead Sciences and GlaxoSmithKline. CC has received funding from the Australian National Health and Medical Research Council for an Early Career Fellowship. SRL has received funding from the Australian NHMRC, the Australian Center for HIV and Hepatitis Virology ResearchNIH, amfAR (Magnet grant award number 19-02602), Gilead Sciences (clinical research grant), Merck, ViiV and Leidos outside the submitted work. SRL also reports consulting fees from Abivax, Geovax, ViiV, and Tetralogic, honoraria from Gilead Sciences, Bristol Myers Sqibb, and Merck Sharpe & Dohme, an International PCT patent (PCTAU2017050631), and participation on advisory boards for

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Figure legends and captions

Figure 1. Consort diagram for study enrolment.

ART: antiretroviral therapy; 2-LTR: 2-long terminal repeat; PD-1: programmed death-1; pSTAT5: phosphorylated signal transducer and activator of transcription-5

Figure 2. Levels of cell-associated and plasma HIV across strata of CD4+ T-cell count at ART initiation.

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Figure 3. T-cell expression of HLA-DR, PD-1 and pSTAT5 across strata of CD4+ T-cell count at ART initiation.

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Figure 4. Multivariate and univariate analyses of associations between immune activation/exhaustion parameters and total HIV-DNA and CA-US HIV-RNA in CD4+ T-cells.

Fold-change in total HIV-DNA and CA-US HIV-RNA in CD4+ T-cells for each unit increase in the indicated parameters for all participants. pSTAT5: phosphorylated signal transducer and activator of transcription-5; PD-1: Programmed death-1; IL: interleukin

Figure 5. Levels of cell-associated and plasma HIV in males versus females across strata of CD4+ T-cell count at ART initiation.

Comparison of virological measures between males and females for the entire cohort (A) including the frequency of total HIV-DNA (B), CA-US HIV-RNA (C), 2-LTR circles (D) and plasma HIV-RNA measured by single-copy assay (E) for males and females within each stratum of CD4+ T-cell counts. Statistical comparisons were done for males versus females across the entire cohort for each virological measure (A) and for males and females separately to compare each measure across the CD4 strata (B-E). Only statistically significant results are shown. CA-US HIV-RNA: Cell-associated unspliced HIV-RNA; 2-LTR: 2-long terminal repeat.

Table 1. Baseline demographics of study participants

IQR: Interquartile range; ART: antiretroviral therapy; N/A: non-applicable; cART: combination antiretroviral therapy; PI: protease inhibitor; NRTI: nucleoside reverse transcriptase inhibitor; NNRTI: non-nucleoside reverse transcriptase inhibitor. 1) Acute myocardial infarction, stroke or coronary revascularisation; 2) Diabetes mellitus diagnosis or receiving anti-diabetic medication (insulin, metformin, sulfonylureas, thiazolidinediones or biguanides or other) or 8 hour fasting glucose ≥126 mg/dL; 3) Systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg or receiving blood pressure medication (beta blockers, diuretics, angiotensin converting enzyme (ACE) inhibitors, angiotensin receptor blockers, calcium channel antagonists, other)

Table 2. Multivariate analysis of associations between total HIV-DNA in CD4+ T-cells and clinical characteristics at ART initiation.

Adjusted for: age, sex, country, viral load, hepatitis B. Hyphen (-) indicates that variables with p- values >0.20 was removed from model by stepwise regression. CI: confidence interval; cART: combination antiretroviral therapy; PI: protease inhibitor; NRTI: nucleoside reverse transcriptase inhibitor; NNRTI: non-nucleoside reverse transcriptase inhibitor; hs-CRP: high-sensitivity C-reactive protein; IL: interleukin. 1) Systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg or receiving blood pressure medication (beta blockers, diuretics, angiotensin converting enzyme (ACE) inhibitors, angiotensin receptor blockers, calcium channel antagonists, other); 2) Hepatitis B measured as being hepatitis B surface antigen positive

Variable	Overall (N=146)	Strata of CD4+ count (cells/mm^3) at ART initiation			P-value
		500-599 (N=36)	600-799 (N=60)	≥800 (N=50)	
Age in years, Median (IQR)	39.5 (34, 48)	36.5 (30.0, 41.5)	40 (35.0, 47.5)	44.5 (36.0, 50.0)	0.021
Sex, n (%)					0.452
Male	59 (40.4)	17 (47.2)	25 (41.7)	17 (34.0)	
Female	87 (59.6)	19 (52.8)	35 (58.3)	33 (66.0)	
Race, n (%)					0.188
Black	124 (84.9)	28 (77.8)	53 (88.3)	43 (86.0)	
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 (%)					0.372
Peru	20 (13.7)	8 (22.2)	5 (8.3)	7 (14.0)	
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 prior to ART initiation (years), Median (IQR)	2.0 (0.4, 5.4)	1.7 (0.4, 4.3)	1.5 (0.4, 5.5)	2.8 (0.5, 7.1)	0.502
Time on ART at time of sampling for HIV reservoir analyses (months), Median (IQR)	38.2 (36.3, 41.7)	38.6 (36.4, 41.8)	39.2 (36.5, 41.8)	37.1 (36.3, 41.3)	0.379

CD4+ count at ART initiation (cells/mm^3), Median (IQR)	710.3 (604.0, 854.5)	564.3 (533, 577)	678 (641, 733)	932 (852.5, 1081.5)	N/A
Recorded nadir CD4+ count prior to ART (cells/mm^3), Median (IQR)	630 (530, 781)	519 (491.5, 536.5)	612.5 (541, 663.5)	837 (762, 972)	N/A
CD4:CD8 ratio at ART initiation, (Median (IQR)	0.8 (0.6, 1.0)	0.6 (0.4, 0.8)	0.7 (0.6, 0.9)	0.9 (0.7, 1.2)	0.000
Plasma HIV RNA at ART initiation (log10 copies/ml), Median (IQR)	3.9 (3.1, 4.7)	4.4 (3.7, 4.9)	3.8 (3.0, 4.7)	3.9 (3.0, 4.5)	0.505
Current smoking, n (%)	19 (13.0)	6 (16.7)	9 (15.0)	4 (8.0)	0.403
Positive cardiovascular disease (CVD), n (%)[1]	0	0	0	0	N/A
Positive diabetes, n (%) [2]	3 (2.1)	0	2 (3.3)	1 (2.0)	0.787
Positive hypertension, n (%) [3]	21 (14.4)	2 (5.56)	12 (20.0)	7 (14.0)	0.143
Positive hepatitis B, n (%)	6 (4.1)	1 (2.8)	3 (5.0)	2 (4.0)	1.000
Positive hepatitis C, n (%)	2 (1.4)	0 (0.0)	1 (1.7)	1 (2.0)	1.000
ART regimen prescribed, n (%)					0.450
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)	

Table 1. Clinical characteristics at ART initiation

IQR: Interquartile range; ART: antiretroviral therapy; N/A: non-applicable; cART: combination antiretroviral therapy; PI: protease inhibitor; NRTI: nucleoside reverse transcriptase inhibitor; NNRTI: non-nucleoside reverse transcriptase inhibitor

1) Acute myocardial infarction, stroke or coronary revascularisation

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

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

Variable	Overall (N=146)			
	Ratio	95% CI		P-value
CD4+ count at ART initiation (cells/mm^3)	0.998	0.997	1.000	0.009
Age in years	0.973	0.951	0.995	0.015
Sex (ref:Male)				
Female	0.565	0.350	0.912	0.019
Current smoking	-	-	-	0.201
Country where participant enrolled (ref:Peru)				
South Africa	0.705	0.330	1.507	0.367
Uganda	2.491	1.151	5.392	0.021
Estimated (self-reported) duration of HIV infection prior to ART initiation (years)	-	-	-	0.631
Time on ART at time of sampling for HIV reservoir analyses (months)	-	-	-	0.942
CD4:CD8 ratio at ART initiation	-	-	-	0.420
Plasma HIV RNA at ART initiation (log10 copies/ml)	1.189	1.000	1.413	0.050
Positive hypertension[1]	-	-	-	0.427
Positive hepatitis B[2]	0.224	0.068	0.738	0.014
ART regimen (ref:PI/NRTI)				
NRTI/NNRTI	-	-	-	0.333
Plasma IL-6 (pg/mL) at ART initiation	0.842	0.676	1.047	0.122
Plasma d-dimer (□g/mL) at ART initiation	0.753	0.568	0.998	0.048
Plasma hs-CRP (□g/m) at ART initiation	1.005	0.985	1.025	0.645

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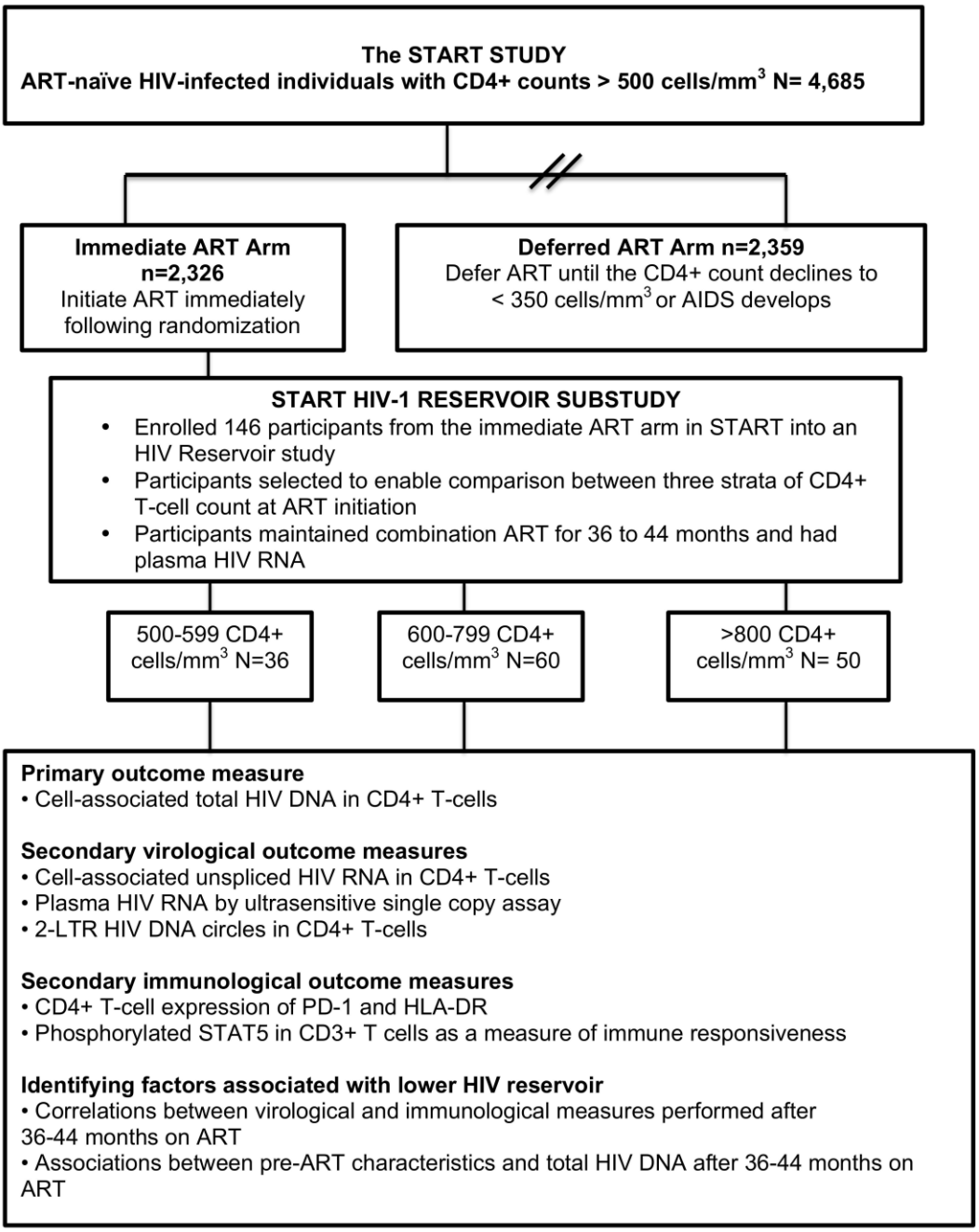
2) Hepatitis B measured as being hepatitis B surface antigen positive



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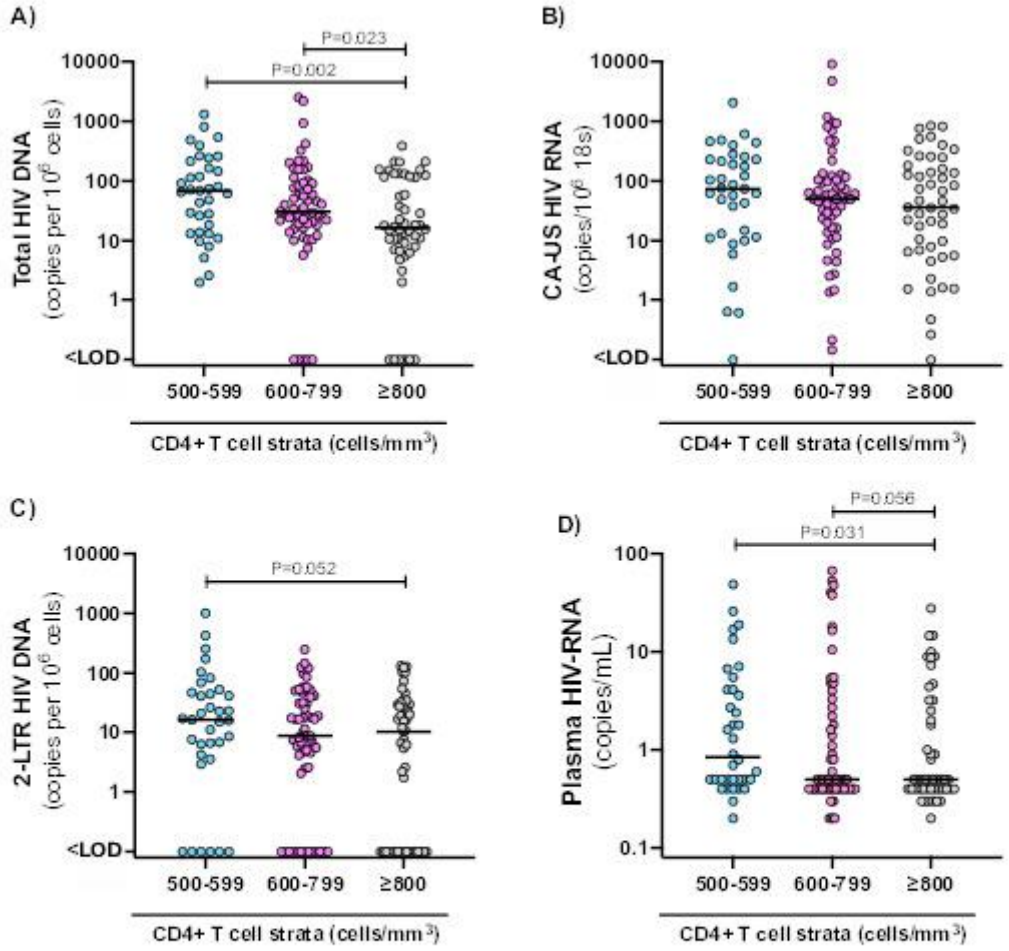

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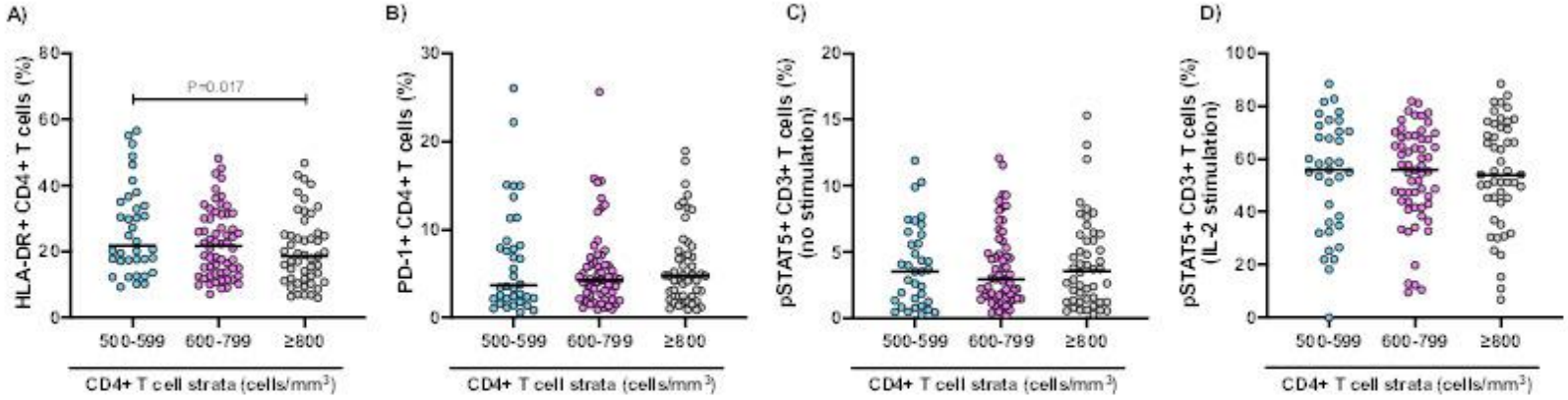

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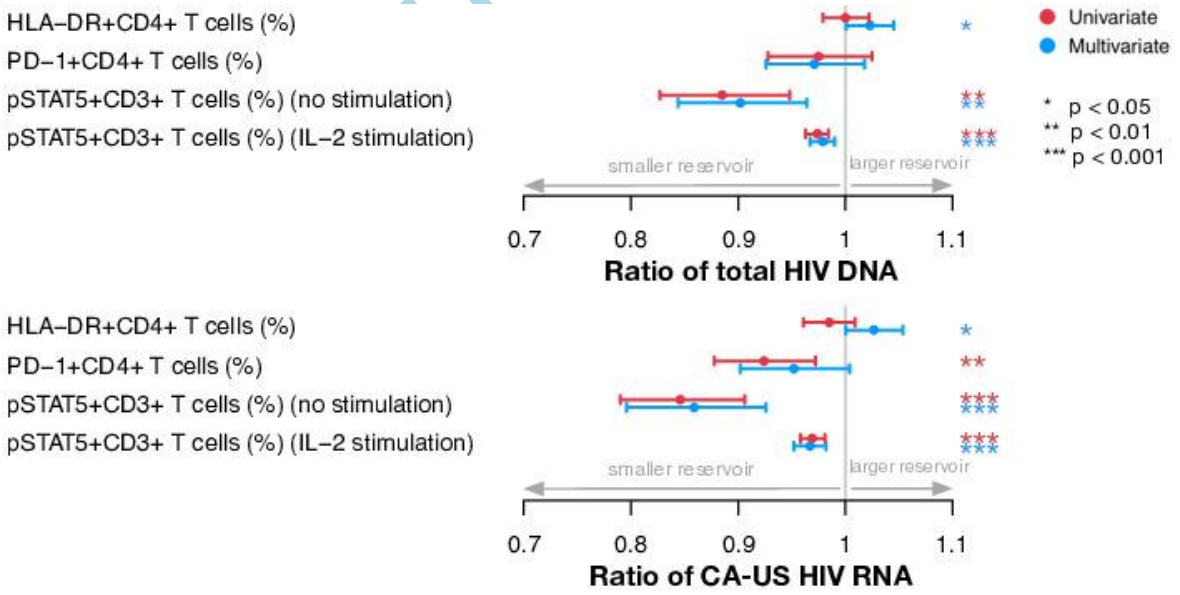

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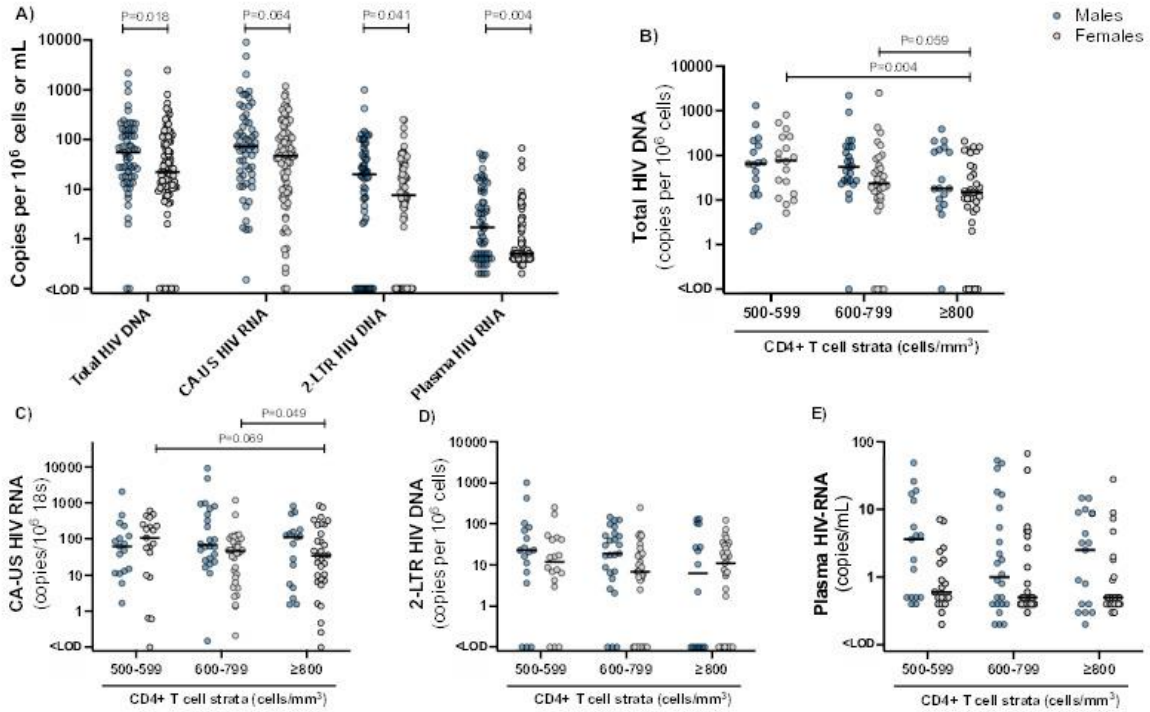

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