LOWER HIV RESERVOIR SIZE IN INDIVIDUALS WHO MAINTAIN HIGHER CD4+ T CELL COUNTS PRIOR TO ANTIRETROVIRAL THERAPY INITIATION: THE STRATEGIC TIMING OF ANTIRETROVIRAL TREATMENT (START) HIV RESERVOIR STUDY. PEBLB13.

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BACKGROUND: Cells latently infected with HIV are the main barrier to HIV cure. Identifying factors that determine the frequency of latently infected cells in people living with HIV (PWH) on antiretroviral therapy (ART) may inform HIV cure strategies. Commencement of ART within 6-12 months of HIV infection is associated with a smaller HIV reservoir[1]. We hypothesised that PLWH who maintain pre-ART CD4+ counts seen in uninfected individuals[2], defined here as ≥800 cells/mm³, would achieve a lower frequency of latently infected CD4+ T-cells on ART than those with 500-599 or 600-799 cells/mm³.

METHODS: Nested study within the Strategic Timing of Antiretroviral Treatment (START) study[3].

Eligibility criteria: randomised to immediate arm of START; received ART for 36-44 months without interruptions >2 weeks; all plasma HIV-RNA levels obtained 8 months after starting ART were <400 copies/mL.

Study participants were enrolled into one of three strata based on CD4+ count at ART initiation: 500-599, 600-799 or ≥800 cells/mm³. Demographic, medical and laboratory analyses were collected at ART initiation. After 36-44 months of ART, peripheral blood mononuclear cells were collected for virological and immunological analyses.

Primary outcome measure: level of total HIV-DNA in peripheral blood CD4+ T-cells after 36-44 months of ART. Secondary outcome measures: (*virological*): 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 ultrasensitive assay; (*immunological*): CD4+ T-cell expression of HLA-DR and PD-1 and 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).

Statistical analyses performed: Kruskal-Wallis rank test; Dunn's multiple pairwise comparison test; generalised negative binomial regression model. Univariate and multivariable analyses.

RESULTS

146 participants were enrolled: median age 39.5 years (IQR 34,48); women n= 87 (59.6%); Black n=124 (84.9%), Hispanic/Latino (13.7%) **(Table 1)**. Median age was significantly different across the CD4+ T-cell strata, oldest in the ≥800 stratum, however other demographic parameters were evenly distributed.

Virological Outcomes

Following 36-44 months of ART, median interquartile range (IQR) levels of total HIV-DNA were 68.4 (13.7-213.1) copies/million cells in the 500-599 stratum, 30.0 (17.1-91.9) copies/million cells in the 600-799 stratum and 16.3 (7.0-117.6) copies/million cells in >800 stratum. Total HIV DNA and plasma HIV RNA but not CA-US RNA or 2LTR DNA were significantly lower in PWH with CD4+ T cell count ≥800 cells/mm³ at ART initiation compared to 600-799 or 500-599 cells/mm³ **(Figure 1A-D)**.

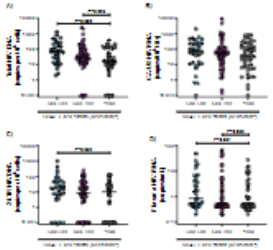

Figure 1. Levels of cell-associated and plasma HIV across strata of CD44 counts at ART initiation

Variable	Overall (N=146)	Strata of CD4+ count (cells/mm³) at ART initiation: 500-599 (N=39)	600-799 (N=60)	≥800 (N=45)	P-value
Age in years, Median (IQR)	39.5 (34, 48)	38.5 (33.5, 41.5)	40 (33.5, 47.5)	44.5 (36.5, 50.5)	0.031
Sex, n (%)					0.452
Male	59 (40.4)	17 (47.2)	25 (41.7)	17 (34.1)	
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)	2.5 (0.5, 7.1)	0.502
Time on ART at time of sampling for HIV reservoir analysis (months), Median (IQR)	38.2 (36.3, 41.7)	39.6 (38.4, 41.5)	39.2 (36.5, 41.5)	37.1 (36.3, 41.3)	0.379
CD4+ count at ART initiation (cells/mm³), Median (IQR)	710.5 (604.5, 854.5)	554.5 (531, 577)	676 (641, 733)	932 (832.5, 1081.5)	N/A
Recorded nadir CD4+ count prior to ART (cells/mm³), Median (IQR)	630 (530, 751)	519 (451.5, 536.5)	612.5 (541, 663.5)	837 (762, 972)	N/A
CD4 CD8 ratio at ART initiation, (Median (IQR)	0.5 (0.4, 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, 4.8)	3.8 (3.0, 4.7)	3.9 (3.0, 4.5)	0.505
Current smoking, n (%)	10 (13.0)	6 (15.7)	3 (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)	0.767
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 (2.6)	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 (%)					0.480
NRTI+PI	9 (6.2)	1 (2.6)	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 (2.6)	0 (0.0)	0 (0.0)	

Table 1. Clinical characteristics at ART initiation

Immunological outcomes

CD4+ T-cell expression of HLA-DR was significantly lower in PWH starting ART with CD4+ counts ≥800 cells/mm³ compared to 500-599 cells/mm³ **(Figure 2A)**, but there were no differences across CD4+ strata in expression of PD-1 or pSTAT5 **(Figure 2 B-D)**.

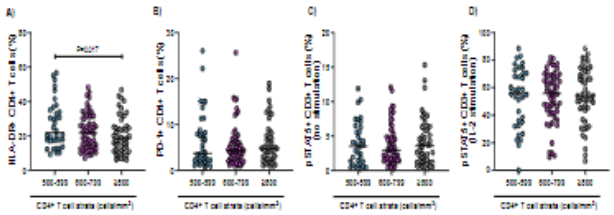

Figure 2. T cell expression of HLA-DR, PD-1 and pSTAT5 across strata of CD44 T-cell count at ART initiation

Correlations between immunological and virological outcome measures

Higher CD4+ T-cell expression of HLA-DR was associated with higher levels of total HIV-DNA and CA-US HIV-RNA in multivariate analyses **(Figure 3)** and higher expression of pSTAT5, was correlated with lower levels of HIV-DNA and CA-US HIV-RNA in both uni- and multivariate analyses.

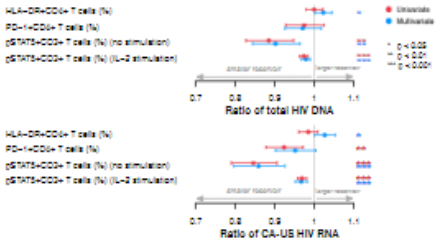

Figure 3. Multivariate and univariate analyses of associations between immune activation /exhaustion parameters and total HIV DNA and CA-US HIV RNA in CD44 T-cells

Associations of clinical characteristics at ART initiation with HIV reservoir size on ART

In multivariate negative binomial regression models, older age and female sex were associated with a lower level of total HIV-DNA. The potential for an association between higher age and preferential survival (Table 1) was addressed in a multivariate analysis: however adjusting for age, sex, enrolment country, plasma viral load at ART initiation and hepatitis B, CD4+ T-cell count at ART initiation remained significantly associated with total HIV-DNA after 36-44 months on ART.

Female sex showed a strong association with a ratio of total HIV-DNA of 0.565 (95% CI 0.350-0.912) compared to males. Virological measures 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 4A)**. To examine whether the lower frequency of total HIV-DNA in the ≥800 cells/mm³ stratum was driven by a higher proportion of females (Table 1), we performed sensitivity analyses stratified by sex. This showed that a lower frequency of HIV-DNA in the ≥800 cells/mm³ stratum could also be found in analyses restricted to females only, whereas for males this difference no longer reached statistical significance **(Figure 4B)**

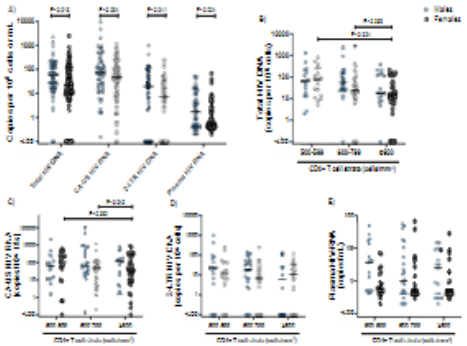

Figure 4. Levels of cell-associated and plasma HIV in males and females across strata of CD44 T-cell count at ART initiation

Conclusions

We report that commencing ART with a CD4+ T cell count ≥800 cells/mm³ compared to 600-799 or 500-599 cells/mm³ 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.

There was a cohort-wide association between total HIV-DNA and CD4+ T-cell count at ART initiation, which remained statistically significant after adjusting for potential confounders in multivariate analyses.

Our study revealed considerable differences between women and men in measures of HIV persistence.

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 our findings indicate that PWH who maintain CD4+ T-cells ≥800 cells/mm³ before ART initiation are endowed with an enhanced capacity to eliminate latently infected cells and may constitute a subgroup that could potentially benefit from interventional cure studies.

References

1. Hocqueloux L, Avettand-Fènoël V, Jacquot S, et al. Long-term antiretroviral therapy initiated during primary HIV-1 infection is key to achieving both low HIV reservoirs and normal T cell counts. J Antimicrob Chemother **2013**; 68(5): 1169-78
2. Rodríguez-Castañón JM, McNaughton A, Cárdenas-Ochoa A, et al. Exceptional T CD4+ recovery post-ART is linked to a lower HIV reservoir with a specific immune differentiation pattern. AIDS Res Hum Retroviruses **2021**.
3. Le T, Wright EJ, Smith DM, et al. Enhanced CD4+ T-cell recovery with earlier HIV-1 antiretroviral therapy. N Engl J Med **2013**; 368(3): 218-30.
4. Lundgren JD, Babiker AG, Gordin F, et al. Initiation of Antiretroviral Therapy in Early Asymptomatic HIV Infection. N Engl J Med **2015**; 373(9): 795-807

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