

Pre-Human Immunodeficiency Virus (HIV) $\alpha 4\beta 7^{\text{hi}}$ CD4⁺ T Cells and HIV Risk Among Heterosexual Individuals in Africa

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Background. CD4⁺ T cells expressing $\alpha 4\beta 7$ are optimal targets for human immunodeficiency virus (HIV) infections, with higher pre-HIV $\alpha 4\beta 7^{\text{hi}}$ expression linked to increased HIV acquisition and progression in South African women. However, similar associations were not observed in men who have sex with men or people who inject drugs in the Americas, indicating need for further research.

Methods. This retrospective case-control study enrolled heterosexual men and women from South Africa (HIV Vaccine Trials Network [HVTN] 503) and East Africa (Partners Preexposure Prophylaxis/Couples' Observational Study [PP/COS]), quantifying $\alpha 4\beta 7$ expression on CD4⁺ T cells as a predictor of subsequent HIV risk using flow cytometry analyses.

Results. Associations between $\alpha 4\beta 7^{\text{hi}}$ expression and HIV acquisition varied across cohorts. In HVTN 503, women had a higher risk estimate compared to men, but this was not significant. In PP/COS, $\alpha 4\beta 7^{\text{hi}}$ expression was generally protective, particularly in Ugandans. Additionally, $\alpha 4\beta 7^{\text{hi}}$ expression inversely correlated with peak viral load in PP/COS but not in HVTN 503; in the latter cohort, $\alpha 4\beta 7^{\text{hi}}$ expression was inversely correlated with the CD4/CD8 ratio and predicted rapid CD4⁺ T-cell decline, similar to what was observed previously in South Africa.

Conclusions. These findings suggest that $\alpha 4\beta 7^{\text{hi}}$ expression on CD4⁺ T cells may not predict HIV acquisition and progression in all contexts, which may be due to cohort effects, modes of transmission, viral clade, or other factors.

Keywords. $\alpha 4\beta 7$; HIV acquisition; HIV progression; Africa; CD4⁺ T cells.

The integrin $\alpha 4\beta 7$ is expressed on the surface of immune cells including CD4⁺ T cells where it mediates homing to gut-associated

lymphoid tissues [1, 2]. Several studies have suggested that $\alpha 4\beta 7$ is involved in human immunodeficiency virus (HIV) pathogenesis. During acute infection, $\alpha 4\beta 7$ -expressing cells are highly susceptible to HIV/simian immunodeficiency virus (SIV) and preferentially depleted; these cells highly coexpress other HIV-susceptible markers such as the main HIV coreceptor, CCR5, and $\alpha 4\beta 7$ is preferentially expressed on Th17 cells [3–5]. Some data suggest that $\alpha 4\beta 7$ may act as a nonessential receptor that aids viral attachment via interaction with gp120 of some HIV type 1 (HIV-1) strains [1, 6, 7]. As a potential cure strategy, 1 study combined blocking $\alpha 4\beta 7$ using a monoclonal antibody with antiretroviral therapy (ART) and reported significant virological control in rhesus macaques infected with SIVmac239-nef-stop virus [8]. Two subsequent studies that tested this $\alpha 4\beta 7$ -blockade strategy in a similar infection model did not replicate this effect [9, 10]. While targeting $\alpha 4\beta 7$ may not be a viable cure approach, CD4⁺ T cells expressing this molecule are nonetheless important targets of lentiviral infection.

Cohort studies examining the role of integrin $\alpha 4\beta 7$ as a predictor of HIV acquisition or progression have also generated

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mixed results. In heterosexual South African women, we showed that women with higher pre-HIV $\alpha 4\beta 7^{\text{hi}}$ CD4⁺ T cells had higher risk of HIV infection and progressed faster compared to women with lower $\alpha 4\beta 7^{\text{hi}}$ CD4⁺ T cells [11]. This was not replicated in a United States–based study among men who have sex with men (MSM) and people who inject drugs (PWID) [12]. Despite similar study design and methods, the characteristics of the populations differed, which may contribute to the discrepancies between the studies. Therefore, gaps still exist regarding understanding the role of $\alpha 4\beta 7$ and HIV outcomes across populations, clades, and geographies.

MATERIALS AND METHODS

Study Design and Population

We carried out a nested, retrospective, matched case-control study to evaluate the association between pre-HIV $\alpha 4\beta 7^{\text{hi}}$ CD4⁺ T cells and HIV outcomes in HVTN 503 and Partners PrEP (preexposure prophylaxis) combined with Couples' Observational Study (COS) cohorts (hereafter PP/COS) [13–17]. All participants in the parent cohorts provided informed written consent to have specimens stored for future immunological research, and the substudies reported here were approved by the institutional review board (IRB) at the University of Manitoba and at local IRBs where the studies were conducted. We analyzed samples from participants who were later diagnosed with HIV (PLDH) and compared these to those who remained HIV uninfected (nonseroconverters [NSCs]). PLDH were sampled at the last available pre-HIV infection visit and matched to controls within the same cohort based on prespecified criteria (Supplementary Methods).

Flow Cytometry Analyses

The expression of $\alpha 4\beta 7$ was quantified on CD4⁺ T cells using flow cytometry of peripheral blood mononuclear cells (PBMCs). In brief, cryopreserved PBMCs were thawed and rested for 3 hours in media (RPMI 1640 + 10% fetal bovine serum + 1% penicillin-streptomycin) at 37°C and 5% carbon dioxide and stained with panels of fluorescently conjugated antibodies (Supplementary Methods).

Statistical Methods

Medians with interquartile range (IQR) and percentages were used to summarize continuous and categorical variables, respectively. Mann-Whitney test was used to compare $\alpha 4\beta 7^{\text{hi}}$ CD4⁺ T cells between groups. Conditional logistic regression (CLR) was used to measure HIV acquisition associations, with 3 $\alpha 4\beta 7^{\text{hi}}$ subsets as primary continuous explanatory variables. Each integrin subset was modeled separately in unadjusted and multivariable analyses, which adjusted for potential confounders. Stratified CLR was used to compare the $\alpha 4\beta 7$ –HIV associations based on sex, country, and treatment arms within each cohort.

Spearman rank correlation test was used to measure associations between $\alpha 4\beta 7$ and HIV peak and set point viral load (VL), and CD4/CD8 ratio post-HIV infection. Cox regression was used to predict time to CD4⁺ T-cell decline to <500 cells/ μL prior to ART initiation. CD4⁺ T-cell counts measured during the first 180 days of follow-up were censored to exclude the short-lived CD4 drops during the acute infection [11]. In Cox regression analyses, $\alpha 4\beta 7$ expression was dichotomized as above/below the median. CLR was performed using SAS version 9.4. Other statistical analyses and data visualizations were computed in RStudio 4.2.2. All statistics are 2-tailed and *P* values $\leq .05$ were considered statistically significant. *P* values were adjusted for multiple testing using the Benjamini-Hochberg method with *p.adjust.method* in R software.

RESULTS

Study Participants

Our study included males and females at risk of HIV infection. The median age was 32 years (IQR, 27–40 years) for PP/COS and 22 years (IQR, 20–26 years) for HVTN 503. Of the 180 samples processed for PP/COS, we excluded 6 (3.3%) due to <50% cell viability. For HVTN 503, we excluded 19 (7.9%) samples due to low viability. In PP/COS, 75% of participants had a baseline risk score ≥ 5 , reflecting seronegative partners with greater exposure to HIV acquisition. Due to the early close of HVTN 503, 52 (24%) and 157 (71%) of participants received only 1 or 2 doses of the vaccine regimen, respectively, while 12 (~5%) completed 3 vaccine doses as per protocol (Table 1).

Effect of Pre-HIV Frequencies of Integrin $\beta 7^{\text{hi}}$ CD4⁺ T-Cell Subsets on HIV Acquisition

We characterized subsets of CD4⁺ T cells based on their expression of integrin $\beta 7$ and CD45RA: $\beta 7^{\text{hi}}$ CD45RA[−], $\beta 7^{\text{int}}$ CD45RA⁺, and $\beta 7^{\text{lo}}$ CD45RA[−] (Figure 1A). Previous reports showed that most blood $\beta 7^{\text{hi}}$ CD4⁺ T cells coexpress integrin $\alpha 4$ [3, 4, 11, 18] and are mainly memory cells [4, 11, 18]. We considered $\beta 7^{\text{hi}}$ CD45RA[−] CD4⁺ T cells to be $\alpha 4\beta 7^{\text{hi}}$. Relatively consistent measurements of $\beta 7^{\text{hi}}$ expression on CD4⁺ T cells from the same healthy volunteer PBMCs were obtained across multiple experimental batches (Supplementary Figure 1). We compared proportions of $\beta 7^{\text{hi}}$ CD4⁺ T-cell subsets by treatment arms, HIV acquisition status, sex, and country (Figure 1B and 1C). The frequency of $\beta 7^{\text{hi}}$ CD4⁺ T cells was only significantly different with treatment arms, with higher expression in the vaccine arm in HVTN 503 (*P* = .014; Figure 1B) and in participants who received oral PrEP in PP/COS (*P* = .026; Figure 1C) compared to the placebo groups. Similarly, the expression levels of $\beta 7^{\text{int}}$ and $\beta 7^{\text{lo}}$ cells were significantly different between PLDH and NSC, and between the PrEP and placebo arms, but only in PP/COS (Supplementary Figure 2).

Table 1. Baseline Characteristics of Participants

Characteristics	HVTN 503		Partners PrEP/COS	
	PLDH (n = 47)	NSC (n = 174)	PLDH (n = 35)	NSC (n = 139)
Age ^a , y	23 (21–27)	22 (20–26)	30.3 (24.6–39.7)	32.2 (28.1–40.1)
CD4 count ^a , cells/ μ L	415.5 (290–496.8)	NA	491 (422.2–632.5)	NA
Time from sampling to infection ^a , d	177 (141–218.5)	NA	234 (147–321.5)	NA
Sex				
Female	26 (55.3)	72 (41.4)	19 (54.3)	61 (43.9)
Male	21 (44.7)	102 (58.6)	16 (45.7)	78 (56.1)
Treatment arm				
Placebo	24 (51.1)	86 (49.4)	15 (53.6)	60 (53.6)
Vaccine	23 (48.9)	88 (50.6)	NA	NA
PrEP	NA	NA	13 (46.4)	52 (46.4)
Cohort				
COS	NA	NA	7 (20.0)	27 (19.4)
Partners PrEP	NA	NA	28 (80.0)	112 (80.6)
HSV-2 positive	22 (46.8)	50 (28.7)	18 (72.0)	66 (63.5)
Treatment status				
Completed	3 (6.4)	9 (5.2)	NA	NA
Off treatment early	44 (93.6)	165 (94.8)	NA	NA
Ad5 titer				
≤ 200	19 (40.4)	69 (39.7)	NA	NA
> 200	28 (59.6)	105 (60.3)	NA	NA
Baseline risk score ^b				
< 5	NA	NA	9 (25.7)	34 (24.5)
≥ 5	NA	NA	26 (74.3)	105 (75.5)
Sites: South Africa				
Cape Town	10 (21.3)	40 (23.0)	NA	NA
eThekweni	11 (23.4)	14 (8.0)	NA	NA
Klerksdorp	8 (17.0)	32 (18.4)	NA	NA
Medunsa	5 (10.6)	22 (12.6)	NA	NA
Soweto	13 (27.7)	66 (37.9)	NA	NA
PHRU	NA	NA	2 (5.7)	4 (2.9)
Sites: Uganda				
Jinja	NA	NA	7 (20.0)	29 (20.9)
Kampala	NA	NA	14 (40.0)	55 (39.6)
Sites: Kenya				
Kisumu	NA	NA	4 (11.4)	32 (23.0)
Thika	NA	NA	2 (5.7)	8 (5.8)
Nairobi	NA	NA	6 (17.1)	11 (7.9)

All data are presented as sample sizes (No.) and corresponding column percentages (%) unless otherwise stated.

^aData are presented as median (interquartile range).

^bPart of the matching criteria.

Abbreviations: Ad5, adenovirus type 5; COS, Couples' Observational Study; HSV-2, herpes simplex virus 2; HVTN, HIV Vaccine Trials Network; NA, not applicable; NSC, human immunodeficiency virus nonseroconverters; PHRU, Perinatal HIV Research Unit (Soweto, South Africa); PLDH, people later diagnosed with human immunodeficiency virus.

Pre-HIV $\beta 7^{\text{hi}}$ CD4⁺ T-cell expression was not significantly associated with HIV acquisition in either cohort. In HVTN 503, the pre-HIV $\beta 7^{\text{hi}}$ odds ratio (OR) was > 1 (OR, 1.06 [95% confidence interval {CI}, .96–1.18]; adjusted OR [aOR], 1.06 [95% CI, .93–1.19]), while PP/COS trended toward a protective effect (OR, 0.92 [95% CI, .83–1.01]; aOR, 0.93 [95% CI, .84–1.03]) (Table 2). In sex-stratified CLR analyses, the odds of HIV acquisition among female participants (aOR, 1.15 [95% CI, .93–1.43]) was higher compared to male participants (aOR, 1.01 [95% CI, .78–1.29]) in HVTN 503 (Figure 2A and 2B). In PP/COS,

preinfection $\beta 7^{\text{hi}}$ CD4⁺ T-cell frequency was significantly associated with reduced HIV acquisition among participants enrolled in Uganda (Figure 2A), but the association was diminished in adjusted analyses (aOR, 0.86 [95% CI, .69–1.06]; Figure 2B).

All results were confirmed with a second flow cytometry panel containing integrin $\beta 7$ and CD45RA, which revealed consistent effect estimates, suggesting the reproducibility of these results (Supplementary Figure 3). Likewise, sensitivity analyses revealed consistent results (Supplementary Table 1 and 2). A summary of pre-HIV $\beta 7^{\text{hi}}$ CD4⁺ T-cell-HIV acquisition associations,

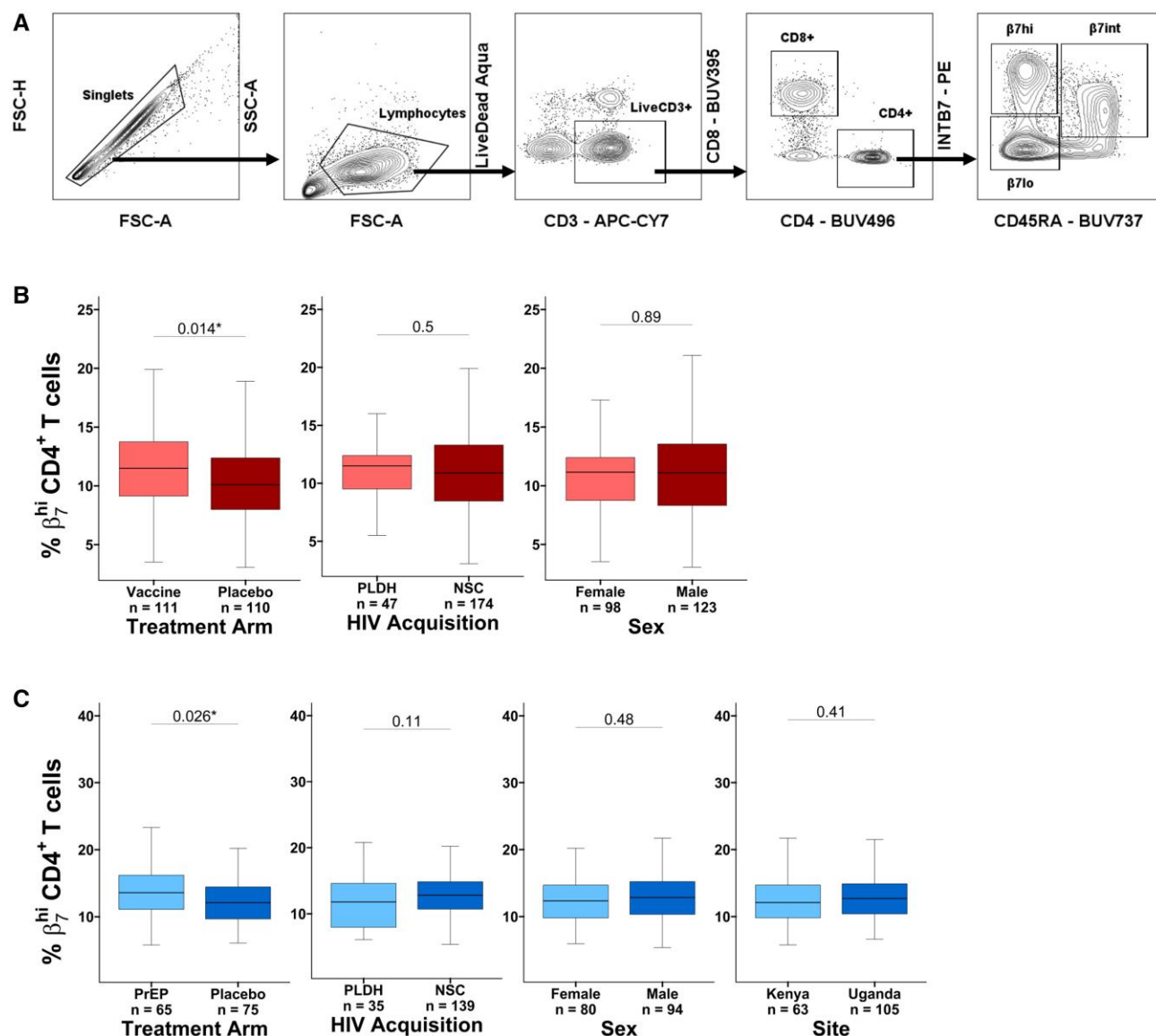


Figure 1. Pre-HIV integrin $\beta 7^{hi}$ CD4⁺ T-cell frequencies across subgroups. *A*, Representative gating strategy for flow cytometry analysis of integrin $\beta 7$ subsets from frozen peripheral blood mononuclear cells. *B*, Frequencies of $\beta 7^{hi}$ CD4⁺ T cells in HIV Vaccine Trials Network 503 cohort. and *C*, Frequencies of $\beta 7^{hi}$ CD4⁺ T cells in Partners PrEP/Couples' Observational Study cohort. Subgroups include treatment arm, HIV acquisition status, sex, and study site. Comparisons between 2 groups were analyzed using the Mann-Whitney test. Statistical significance is denoted as * $P < .05$. Abbreviations: FSC, forward scatter; HIV, human immunodeficiency virus; NSC, human immunodeficiency virus nonseroconverters; PLDH, people later diagnosed with human immunodeficiency virus; SSC, side scatter.

highlighting varying trends across studies, is provided in [Supplementary Table 3](#).

Effect of Pre-HIV Frequencies of Integrin $\beta 7$ CD4⁺ T-Cell Subsets on HIV Progression

We next investigated pre-HIV frequencies of $\beta 7^{hi}$ CD4⁺ T cells as predictors of HIV disease progression, with viral loads (peak and set point VL; [Figure 3A](#) and [3B](#)), CD4/CD8 ratio, and time to CD4⁺ T-cell decline as the main outcome variables. In PP/COS, pre-HIV $\beta 7^{hi}$ cells was inversely correlated with peak VL ($P = .024$; [Figure 3B](#)), while in HVTN 503, $\beta 7^{hi}$ cells correlated

inversely with mean CD4/CD8 ratio >180 days postinfection ($P = .042$; [Figure 3C](#)); a similar correlation was observed with CD4/CD8 ratio at last visit ([Supplementary Figure 4](#)). In unadjusted Cox regression, pre-HIV $\beta 7^{hi}$ CD4⁺ T cells predicted faster CD4⁺ T-cell decline <500 cells/ μ L in HVTN 503 but not in PP/COS ([Figure 3D](#)). Specifically, participants with $\beta 7^{hi}$ cells above the median experienced CD4⁺ T-cell count <500 cells/ μ L at more than twice the rate compared to those with $\beta 7^{hi}$ cells below the median (HVTN: hazard ratio [HR], 2.30 [95% CI, 1.10–5.00]; $P = 0.03$). Pre-HIV $\beta 7^{hi}$ CD4⁺ T cells remained a strong predictor of faster CD4⁺ T-cell decline <500 cells/ μ L in HVTN 503

Table 2. Association of Pre-Human Immunodeficiency Virus (HIV) $\beta 7$ CD4⁺ T Cells With HIV Acquisition

Integrin $\beta 7$ Subsets	Cohorts	Unadjusted Models OR (95% CI)	P Value	Adjusted Models aOR (95% CI)	P Value
Hi	HVTN 503	1.06 (.96–1.18)	.254	1.06 (.93–1.19)	.388
	PP/COS	0.92 (.83–1.01)	.082	0.93 (.84–1.03)	.182
Int	HVTN 503	0.99 (.96–1.01)	.379	1.01 (.98–1.05)	.377
	PP/COS	1.05 (1.01–1.08)	.011*	1.05 (1.01–1.10)	.027*
Lo	HVTN 503	1.01 (.98–1.04)	.508	0.98 (.95–1.01)	.187
	PP/COS	0.96 (.93–.99)	.026*	0.96 (.91–1.00)	.061

The multivariable model for HVTN 503 was adjusted for age, sex, study site, adenovirus type 5 titer, and herpes simplex virus type 2 (HSV-2) status at baseline while PP/COS was adjusted for age, sex, study site, and HSV-2 status at baseline.

Odds ratios were estimated using conditional logistic regression models. Statistical significance is denoted as * $P < .05$.

Abbreviations: aOR, adjusted odds ratio; CI, confidence interval; HVTN, HIV Vaccine Trials Network; OR, odds ratio; PP/COS, Partners PrEP/Couples' Observational Study.

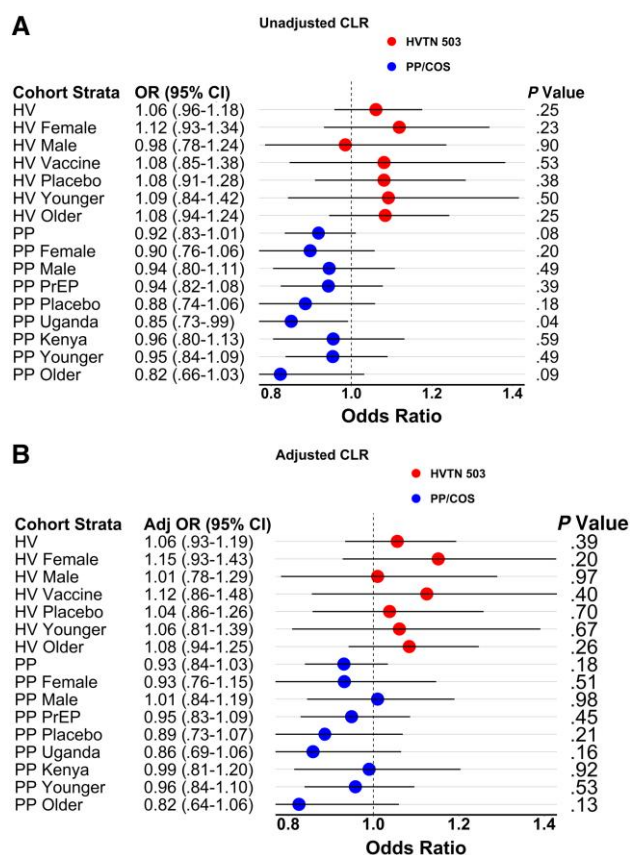


Figure 2. Odds ratios (ORs) for the association between pre-HIV integrin $\beta 7^{\text{hi}}$ CD4⁺ T cells and HIV acquisition. *A*, Unadjusted ORs. *B*, Adjusted ORs. Odds ratios were estimated using conditional logistic regression, with the analysis stratified by sex, age, treatment arm, and HIV prevention intervention where applicable. The multivariable model for HIV Vaccine Trials Network (HVTN) 503 was adjusted for age, adenovirus type 5 titer, and herpes simplex virus type 2 status at baseline, while the model for Partners PrEP/Couples' Observational Study (PP/COS) was adjusted for age and HSV-2 status at baseline. Abbreviations: CI, confidence interval; CLR, conditional logistic regression; COS, Couples' Observational Study; HV, HIV Vaccine Trials Network study 503; OR, odds ratio; PP, Partners PrEP; PrEP, preexposure prophylaxis.

after adjusting for VL, age, sex, study site, adenovirus type 5 (Ad5) titer, study arm, and herpes simplex virus type 2 (HSV-2) status at baseline (HVTN: adjusted HR [aHR], 2.72 [95% CI, 1.13–6.58]; $P = .03$; Figure 3D).

Associations Between Pre-HIV Frequencies of Naive and Central Memory CD4⁺ T Cells Mirror the Associations of Integrin $\beta 7^{\text{int}}$ and $\beta 7^{\text{hi}}$ CD4⁺ T-Cell Subsets on HIV Acquisition

We characterized CD4⁺ T-cell memory phenotypes based on the expression of CCR7, CD45RA, and CD27 (Figure 4A, Supplementary Figure 5). Similar to the findings with $\beta 7^{\text{hi}}$ CD4⁺ T cells, central memory (T_{CM}) cells did not predict HIV acquisition in HVTN 503 but were associated with lower odds of HIV acquisition in PP/COS (PP: OR, 0.94 [95% CI, .90–.98]; $P = .005$; Figure 4B). As observed with $\beta 7^{\text{int}}$ CD4⁺ T cells, naive T cells (T_{N}) were associated with higher rates of HIV acquisition in PP/COS (PP: OR, 1.04 [95% CI, 1.01–1.07]; $P = .006$; Figure 4B). In HIV progression analyses, pre-HIV T_{CM} were associated with lower CD4/CD8 ratio post-infection (Figure 4C, Supplementary Figure 6). In contrast, T_{N} , like $\beta 7^{\text{int}}$ CD4⁺ T cells, positively correlated with CD4/CD8 ratio (Figure 4D, Supplementary Figure 6). Additionally, pre-HIV frequency of T_{CM} inversely correlated with peak VL ($P = .05$; Supplementary Figure 6) in PP/COS, in line with what was observed for $\beta 7^{\text{hi}}$ CD4⁺ T cells. Considering both T_{CM} and $\beta 7^{\text{hi}}$ CD4⁺ T cells are CD45RA⁺ while T_{N} and $\beta 7^{\text{int}}$ CD4⁺ T cells are CD45RA⁺, we estimated correlations between these subsets, observing positive correlations between T_{CM} and $\beta 7^{\text{hi}}$ CD4⁺ T cells ($P = <.001$), effector memory (T_{EM}) and $\beta 7^{\text{lo}}$ CD4⁺ T cells ($P = <.001$), and T_{N} and $\beta 7^{\text{int}}$ CD4⁺ T cells ($P = <.001$) (Supplementary Figure 6). In summary, the associations between pre-HIV frequencies of naive and central memory CD4⁺ T-cell subsets mirror the patterns observed with integrin $\beta 7^{\text{int}}$ and $\beta 7^{\text{hi}}$ subsets, both in HIV acquisition and disease progression, emphasizing their interconnectedness.

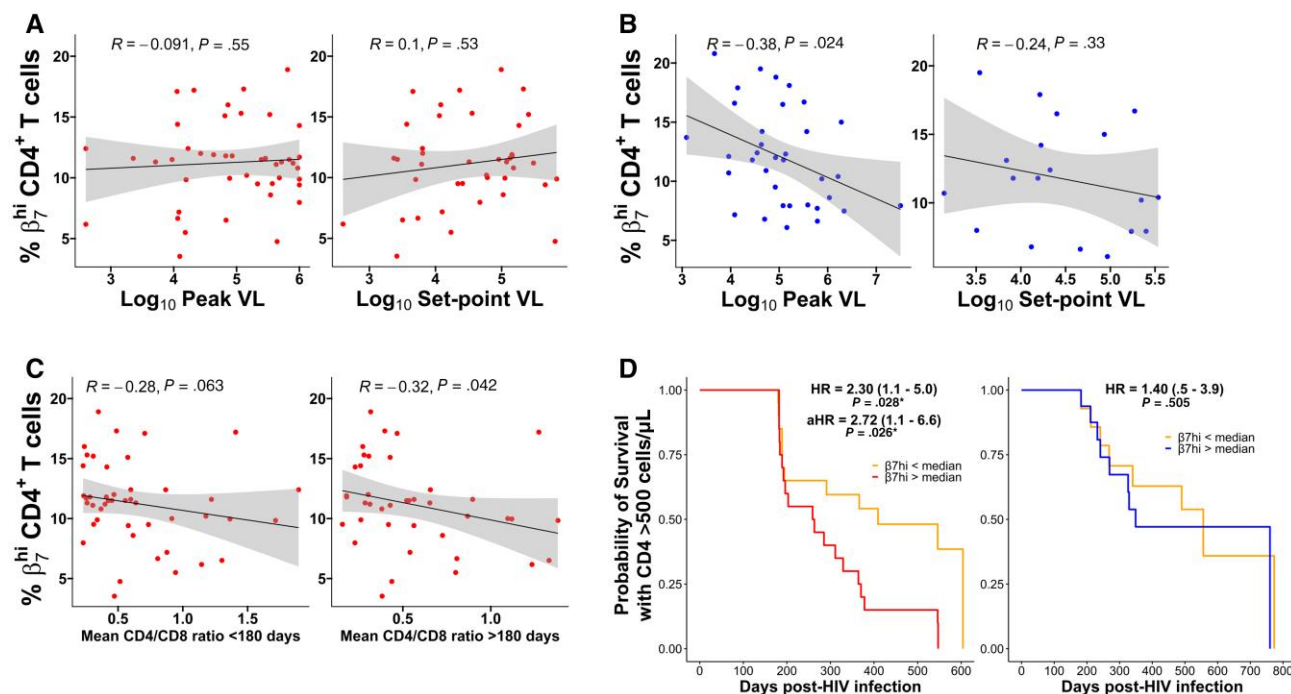


Figure 3. Association between pre-HIV integrin $\beta 7^{\text{hi}}$ CD4^+ T cells and HIV progression. *A* and *B*, Correlation between integrin $\beta 7^{\text{hi}}$ CD4^+ T cells and peak and set point viral load (VL) in HIV Vaccine Trials Network (HVTN) 503 ($n = 45$ and $n = 40$, respectively) and Partners PrEP/Couples' Observational Study (PP/COS) ($n = 35$ and $n = 19$, respectively). *C*, Correlation between integrin $\beta 7^{\text{hi}}$ CD4^+ T cells and mean $\text{CD4}/\text{CD8}$ ratio at <180 d ($n = 45$) and >180 d ($n = 40$) postinfection in HVTN 503. *D*, Unadjusted hazard ratios (HRs) and adjusted HRs (aHRs) for CD4^+ T-cell decline <500 cells/ μL in HVTN 503 ($n = 40$) and PP/COS ($n = 30$). Correlations were analyzed using Spearman rank correlation test and HRs were estimated using Cox regression.

Differential Phenotypic Characteristics of Integrin $\beta 7$ Subsets in HVTN 503 Versus PP/COS Cohorts

Given the divergent point estimates for HIV acquisition risk observed between cohorts, we further investigated the phenotypic characteristics of each integrin $\beta 7$ subset in both cohorts to elucidate the underlying factors that might contribute to these differences. Because of the overlap between $\alpha 4\beta 7$ and Th17 cells, another HIV-susceptible immune subset, we examined the correlation between integrin $\beta 7$ subsets with Th17 cells expressing interleukin 17 (IL-17), interleukin 22, interferon- γ , tumor necrosis factor- α , and granulocyte macrophage-colony-stimulating factor (gating strategy shown in [Supplementary Figure 7](#)). We observed a correlation between $\beta 7^{\text{hi}}$ cells and IL-17-expressing CD4^+ T cells in HVTN 503 ($P < .001$; [Figure 5A](#)) while $\beta 7^{\text{hi}}$ cells were not associated with IL-17 $^+$ CD4^+ T cells in the PP/COS cohort ($P = .334$; [Figure 5B](#)). We next correlated integrin $\beta 7$ subsets with demographic parameters, including age, baseline risk score, and HSV-2 status. We observed that age was the only demographic parameter that was associated with integrin $\beta 7$ subsets. Specifically, in HVTN 503, integrin $\beta 7^{\text{hi}}$ was correlated with age (HVTN: $r = 0.14$, $P = .042$; [Figure 5C](#)) while in PP/COS, integrin $\beta 7^{\text{int}}$ was inversely correlated with age (PP: $r = -0.23$,

$P = .002$; [Figure 5D](#)). We observed similar levels of $\beta 7^{\text{hi}}$ expression between the 2 cohorts; however, the frequency of $\beta 7^{\text{int}}$ cells was significantly lower and $\beta 7^{\text{lo}}$ cells were higher in PP/COS compared to HVTN 503 ([Supplementary Figure 8A](#)). CCR5^+ cells were not significantly different on the $\beta 7^{\text{hi}}$ subsets between the cohorts (gating strategy shown in [Supplementary Figure 9](#)). However, the frequency of activated ($\text{CD38}^+\text{HLA-DR}^+$), proliferating (KI67^+), and $\text{T}_{\text{CM}} \text{CD4}^+$ T cells were higher in PP/COS $\beta 7^{\text{hi}}$ CD4^+ T cells compared to HVTN 503, while $\beta 7^{\text{hi}}$ cells in HVTN 503 were higher in their frequency of T_{EM} and transitional memory (T_{TM}) phenotypes ([Supplementary Figure 8B](#) and [8C](#)). The integrin $\beta 7^{\text{int}}$ in PP/COS samples had significantly higher expression of naive cells than that in HVTN 503 ([Supplementary Figure 8D](#)). Detailed proportions of memory and activated cells within integrin $\beta 7$ subsets are further characterized in [Supplementary Figure 10](#).

DISCUSSION

Studies of $\alpha 4\beta 7$ in HIV have generated conflicting findings. Here we further explored associations of $\alpha 4\beta 7^{\text{hi}}$ expression with HIV outcomes focusing on men and women exposed to HIV through heterosexual transmission. Through analyses of

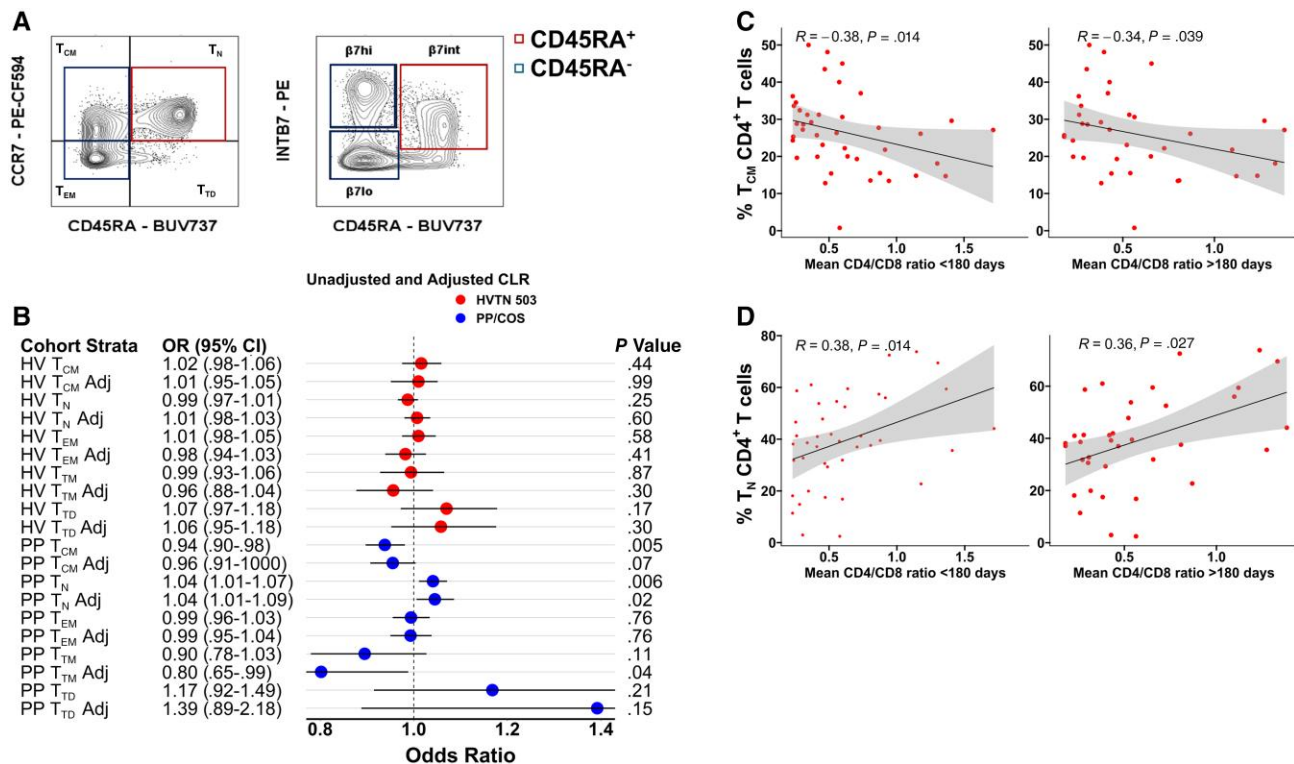


Figure 4. Association between pre-HIV memory CD4⁺ T-cell subsets and HIV outcomes. **A**, Representative plots illustrating the gating strategy overlap between T_{CM} and $\beta 7^{\text{hi}}$ cells, as well as T_N and $\beta 7^{\text{int}}$ cells. **B**, Unadjusted and adjusted odds ratios (ORs) for the association of memory CD4⁺ T-cell subsets with HIV acquisition. **C**, Correlation between T_{CM} and mean CD4/CD8 ratio in HIV Vaccine Trials Network (HVTN) study 503 at <180 d (n = 45) and >180 d (n = 40). **D**, Correlation between T_N and mean CD4/CD8 ratio in HVTN 503 at <180 d (n = 45) and >180 d (n = 40). ORs were estimated using conditional logistic regression. Multivariable models for HVTN 503 were adjusted for age, adenovirus type 5 titer, and herpes simplex virus type 2 (HSV-2) status at baseline, while models for the PP/COS cohort were adjusted for age and HSV-2 status at baseline. Correlations were assessed using Spearman rank correlation test. Abbreviations: CI, confidence interval; CLR, conditional logistic regression; COS, Couples' Observational Study; HV, HIV Vaccine Trials Network; HVTN, HIV Vaccine Trials Network; OR, odds ratio; PP, Partners PrEP; T_{CM}, central memory T cells; T_{EM}, effector memory T cells; T_N, naive T cells; T_{TD}, terminally differentiated T cells; T_{TM}, transitional memory T cells.

2 large cohorts from South and Eastern Africa, we did not observe statistically significant associations between integrin $\alpha 4\beta 7^{\text{hi}}$ expression and HIV susceptibility in either cohort. However, we noted divergent trends between cohorts, with varying estimates observed in subgroup analyses. Of note, we did replicate the association between $\alpha 4\beta 7^{\text{hi}}$ expression and disease progression in South Africans, confirming that $\alpha 4\beta 7^{\text{hi}}$ CD4⁺ T cells were associated with faster disease progression, with a similar effect size as was observed in CAPRISA 004.

It is noteworthy that these cohorts exhibit important differences in the association of $\alpha 4\beta 7$ with HIV acquisition relative to what has previously been reported. We have undertaken an exploration of potential reasons for these disparities, including factors such as age, sex, HIV clade, and the correlation of $\alpha 4\beta 7$ with Th17 and memory phenotypes. Our previous study showed a modest association between systemic $\alpha 4\beta 7^{\text{hi}}$ expression and increased HIV acquisition in heterosexual South African females enrolled in CAPRISA 004 [11]. The present analyses are in general agreement with the acquisition finding

in South Africa women in terms of observing a similar point estimate, for example, a 15% increase in HIV risk per 1% increase in $\alpha 4\beta 7^{\text{hi}}$ expression (albeit with 95% CIs that overlapped with 1). Since the sex-stratified sample sizes were not powered to find HIV acquisition associations in either cohort, these findings require further confirmation. Similar validation studies showed no association between $\alpha 4\beta 7^{\text{hi}}$ and HIV risk in MSM [12]. Males and females exhibit major differences in their sexual route of HIV transmission [19, 20], which may impact the interaction of target cells with HIV. Taken together, 1 interpretation of our data is that the association between $\alpha 4\beta 7^{\text{hi}}$ expression on CD4⁺ T cells and HIV acquisition may be consistent in females from South Africa.

In addition to sex and route of exposure, the stronger associations observed between integrin $\alpha 4\beta 7^{\text{hi}}$ and HIV outcomes in South Africa may be partly due to differences in HIV clades. HIV clades exhibit varying geographic distribution patterns [21, 22]. While clade C is the predominant HIV clade in South Africa, clades A and D are mostly predominant in

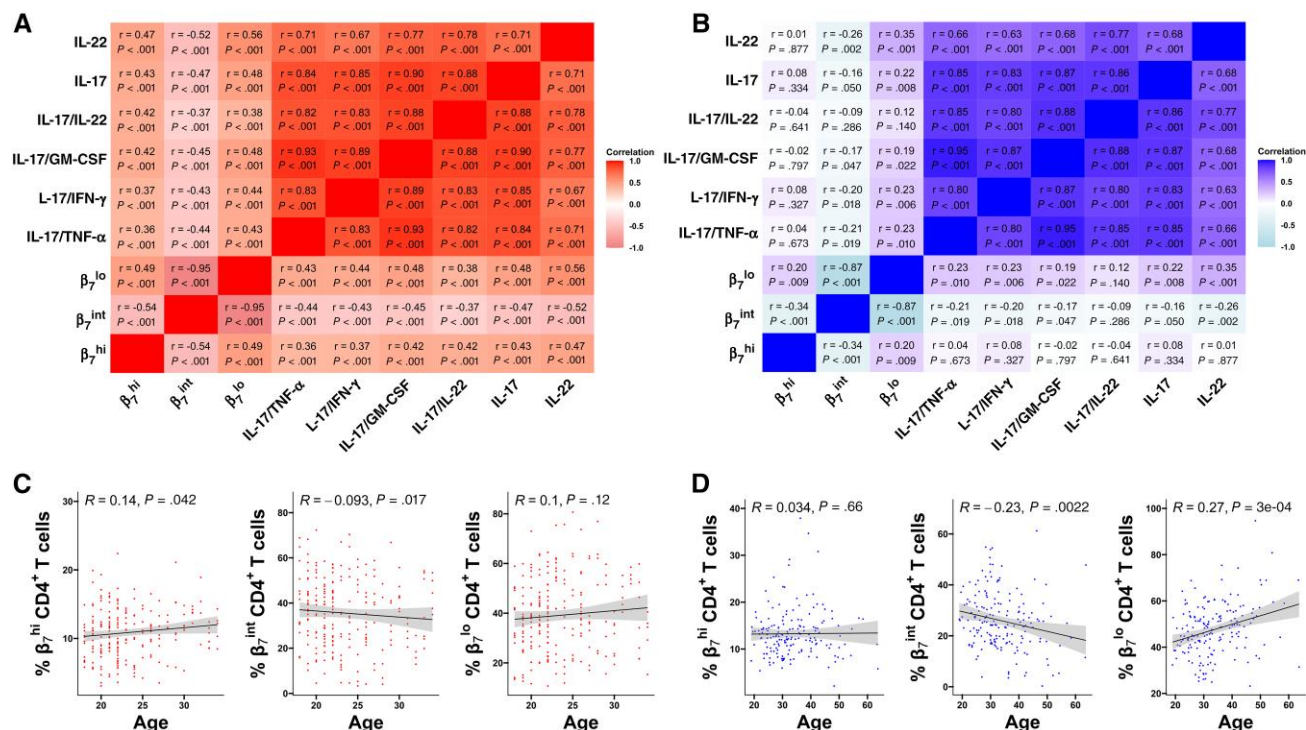


Figure 5. Correlation of integrin β_7 CD4⁺ T-cell subsets with Th17 subsets and age. *A* and *B*, Correlation matrices between integrin β_7 subsets and Th17 CD4⁺ T-cell subsets in HIV Vaccine Trials Network (HVTN) 503 ($n = 130$ – 221) and Partners PrEP/Couples' Observational Study (PP/COS) ($n = 119$ – 174). *C* and *D*, Correlation of integrin β_7 subsets and participant age in HVTN 503 ($n = 221$) and PP/COS ($n = 174$). Sample sizes for the correlation matrices (*A* and *B*) ranged from 119 to 221, based on data availability for each parameter. Correlations were analyzed using Spearman rank correlation test. Abbreviations: GM-CSF, granulocyte macrophage–colony-stimulating factor; IFN, interferon; IL, interleukin; TNF, tumor necrosis factor.

Kenya and Uganda, while subtype B is mostly found in the Americas [21, 23]. Some data suggest that HIV clades may interact differently with $\alpha 4\beta 7$ [7, 24]. For example, $\alpha 4\beta 7$ cells may bind directly to HIV gp120 second variable loop (V2) of some strains of HIV and act as a nonessential receptor for the virus [6, 7]. However, the strongest binding affinity of $\alpha 4\beta 7$ with HIV was found with strains having P/SDI/V binding motifs, which is a motif most prevalent among HIV clade C from South Africa compared to other HIV clade C strains and other clades [7]. We have also previously reported that in South Africa, being infected by HIV strains with a V2 loop containing the P/SDI/V motif was associated with a higher frequency of pre-HIV $\alpha 4\beta 7$ [11]. The type of infecting virus may also influence the rate of HIV progression after infection [22, 25]. Interestingly, our data support the hypothesis that the association between $\alpha 4\beta 7^{hi}$ and HIV acquisition may be influenced by how $\alpha 4\beta 7$ interacts with different HIV clades, with stronger $\alpha 4\beta 7^{hi}$ effects in women from South Africa where clade C predominates.

We showed that individuals in the active arm in HVTN 503 upregulated $\alpha 4\beta 7^{hi}$ frequencies on their blood CD4⁺ T cells compared to individuals who received placebo. The vaccine used in HVTN 503 was an Ad5-vectored trivalent vaccine

designed to induce T-cell immunity [26, 27]. Unfortunately, the vaccines failed to offer protection against HIV infection and may have increased HIV susceptibility of vaccinated individuals seropositive for Ad5 [13, 26], possibly due to the expansion of activated Ad5-specific T cells, including those expressing $\alpha 4\beta 7^{hi}$, thereby increasing the number of HIV target cells [13, 28, 29]. Similarly, in PP/COS, there was a higher expression of $\alpha 4\beta 7^{hi}$ CD4⁺ T cells among participants in the PrEP intervention compared to the placebo arm of that trial. The impact of PrEP on immune cell frequency and distribution has been examined, particularly in mucosal sites where HIV transmission commonly occurs [30]. While some studies have shown that PrEP does not significantly alter the number or distribution of immune cells [31], others have shown transient changes in the frequency of immune cells after initiating PrEP [30, 32]. Overall, our data showed that HIV prevention interventions may influence the expression levels of $\alpha 4\beta 7^{hi}$ on CD4⁺ T cells; however, in neither case did these appear to impact associations between $\alpha 4\beta 7^{hi}$ and HIV risk, which in both cohorts were similar between treatment arms.

Examination of phenotypic differences between integrin β_7 subsets in HVTN 503 and PP/COS cohorts provides valuable insights into the factors contributing to variations in HIV

acquisition risk. These include correlations with IL-17-expressing CD4⁺ T cells, demographic parameters, and distinctive memory T-cell phenotypes. T_{CM} were more strongly correlated with $\alpha 4\beta 7^{\text{hi}}$ cells in HVTN 503 compared to PP/COS. Interestingly, our data suggest that $\alpha 4\beta 7^{\text{hi}}$ cells are predominantly T_{CM}, while $\alpha 4\beta 7^{\text{lo}}$ cells tend to be enriched for T_{EM} [4, 18]. This observation prompts the intriguing possibility that $\alpha 4\beta 7^{\text{hi}}$ cells may exhibit a preference for migration to gut lymphoid tissue. Likewise, integrin $\beta 7^{\text{int}}$ and naive CD4⁺ T cells were associated with increased HIV acquisition in PP/COS but not in HVTN 503, despite strong correlations between these 2 subsets in both cohorts. Additionally, we observed an inverse correlation between integrin $\beta 7^{\text{int}}$ and naive T-cell subsets with increasing age in PP/COS. Notably, participants in the PP/COS group were approximately 10 years older on average compared to HVTN 503 and included several individuals >40 years of age. Age could serve as a significant confounding factor due to decreases in naive cells and increases in memory cells as individuals age. Since the $\alpha 4\beta 7^{\text{hi}}$ CD4⁺ T-cell variable is a proportion, its values may increase as the proportion of naive $\alpha 4\beta 7^{\text{int}}$ CD4⁺ T cells decreases with age. This could potentially influence the associations between integrin $\beta 7$ and HIV. Notably, $\alpha 4\beta 7^{\text{hi}}$ cells correlated with Th17 cell subsets in HVTN 503 but not in PP/COS. Th17, like $\alpha 4\beta 7$ cells, are also highly susceptible to HIV and have been implicated in both HIV acquisition and disease progression [4, 18, 33, 34]. Taken together, the phenotypic differences in $\alpha 4\beta 7^{\text{hi}}$ cells between cohorts may contribute to the associations between $\alpha 4\beta 7$ and HIV outcomes that have been observed.

A strength of our study is that we adopted similar designs and analyses as previous studies [11, 12]. In addition, we adopted multiple quality control procedures to ensure consistency of experiments over time and reproduced our main flow cytometry results using a second panel. One limitation of our study is that we did not have access to corresponding mucosal samples to measure $\alpha 4\beta 7^{\text{hi}}$ expression in the mucosa. However, frequencies of $\alpha 4\beta 7^{\text{hi}}$ CD4⁺ T cells in the blood correlate with those in the genital tract [11, 35]. If feasible, future studies should include mucosal sampling to more accurately capture immune events at the mucosal level. A second limitation was our inability to match cases and controls on exposure risk levels for HVTN 503 as we did in PP/COS [36]. Exposure to HIV may be relatively low among some serodiscordant couples due to low viremia in the HIV-positive partner [37–39] and the risk levels tend to be highest at the early stages of the relationship, waning as the relationship progresses [40]. While matching on baseline risk score in PP/COS ensured that HIV exposure level is comparable between cases and controls, this may have dampened associations in PP/COS compared to HVTN 503. We were unable to examine the effect of our immune phenotypes on CD4/CD8 ratio in PP/COS due to lack of data on the absolute CD8⁺ T-cell count in this cohort. However, we

were able to test HIV progression associations within this cohort by using CD4 and VL as HIV progression parameters. We acknowledge that our sample size limitations may have constrained our ability to detect significant effects in our HIV acquisition analyses. Additionally, due to our limited sample size, we were unable to conduct subgroup analyses in our progression analyses as we did in the acquisition analyses.

In conclusion, our study suggests that associations between pre-HIV $\alpha 4\beta 7^{\text{hi}}$ expression on CD4⁺ T cells and HIV outcomes may vary between different populations. Our data suggest that $\alpha 4\beta 7^{\text{hi}}$ may more consistently predict disease progression compared to acquisition, where the interplay of exposure, susceptibility, and various cofactors makes prediction of acquisition more complex. While $\alpha 4\beta 7^{\text{hi}}$ cells are reliable targets for HIV, their abundance alone may not consistently predict HIV acquisition, which in turn depends on external factors and the specific population being studied. Our research emphasizes the need to recognize the population-specific nature of $\alpha 4\beta 7$ associations with HIV.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (<http://jid.oxfordjournals.org/>). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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