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## Integrin $\alpha_4\beta_7$ expression on peripheral blood CD4<sup>+</sup> T cells predicts HIV acquisition and disease progression outcomes

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## Abstract

The gastrointestinal (GI) mucosa is central to HIV pathogenesis, and the integrin  $\alpha_4\beta_7$  promotes the homing of immune cells to this site. Data from SIV animal models suggest that  $\alpha_4\beta_7$  blockade provides prophylactic and therapeutic benefits. Here we show that pre-HIV infection levels of  $\alpha_4\beta_7^+$  peripheral blood CD4<sup>+</sup> T cells, independent of other T cell phenotypes and genital inflammation, were associated with increased rates of HIV acquisition in South African women. This association was stronger when infection was caused by HIV strains containing V2 Env motifs with a preference for  $\alpha_4\beta_7$  binding. A similar acquisition effect was observed in a Kenyan cohort, and in non-human primates (NHPs) following intravaginal SIV challenge. In addition, pre-HIV  $\alpha_4\beta_7^+$  CD4<sup>+</sup> T cells predicted higher set point viral load and a >2-fold increased rate of CD4 decline. These results were confirmed in SIV-infected NHPs. Increased frequencies of pre-HIV  $\alpha_4\beta_7^+$  CD4<sup>+</sup> T cells were also associated with higher post-infection expression of LPS binding protein, a microbial translocation marker, suggestive of more extensive gut damage. CD4<sup>+</sup> T cells expressing  $\alpha_4\beta_7$  were rapidly depleted very early in HIV infection, particularly from the GI mucosa, and were not restored by early antiretroviral therapy (ART). This study provides a link between  $\alpha_4\beta_7$  expression and HIV clinical outcomes in humans, in line with observations made in NHPs. Given the availability of a clinically approved anti- $\alpha_4\beta_7$  monoclonal antibody for treatment of inflammatory bowel disease, these data support further evaluation of targeting  $\alpha_4\beta_7$  integrin as a clinical intervention during HIV infection.

### One sentence summary:

The role of  $\alpha_4\beta_7^+$  CD4<sup>+</sup> T cells in HIV pathogenesis

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## Introduction

Several lines of evidence suggest that the gastrointestinal (GI) mucosa and the associated lymphoid tissue play a critical role in HIV pathogenesis. Infections by HIV and SIV rapidly deplete CD4<sup>+</sup> T cells from this site during the first weeks of infection(1-3), and the resulting, extensive damage to the homeostasis of gut tissue persists into chronic HIV infection. One consequence of the extensive gut damage is the translocation of gut-resident bacterial products into the blood, which has been proposed to be a major source of chronic immune activation that drives HIV pathogenesis(4).

The homing of immune cells to the inductive and effector sites of the large and small intestine is facilitated by the expression of the integrin  $\alpha_4\beta_7$  on these cells and its preferred ligand MAdCAM-1, which is constitutively expressed on the high endothelial venules of all GI tissues(5). Several lines of evidence suggest that  $\alpha_4\beta_7$ -expressing CD4<sup>+</sup> T cells are important in HIV pathogenesis. These include direct binding of  $\alpha_4\beta_7$  to some HIV strains(6-9), not as an HIV entry co-receptor *per se* (10-12), but rather as a molecule that may facilitate attachment of the virus to its optimal target cells. This role for  $\alpha_4\beta_7$  in facilitating localization and attachment of virus to cells may be particularly important during HIV transmission, when availability of target cells is a rate-limiting step for the virus. Many laboratories have shown that CD4<sup>+</sup> T cells expressing  $\alpha_4\beta_7$  are preferentially infected both *in vitro* and *ex vivo* (6, 8, 12-14), including during acute SIV infection and in experiments

using HIV clade C viruses(15), the predominant clade in South Africa. We have previously shown that  $\alpha_4\beta_7$  expression on HIV target cells in the female reproductive tract (FRT) was associated with other markers of optimal HIV target cells, including CCR5 expression(16).

The *in vivo* implications of modulating  $\alpha_4\beta_7$  have been highlighted by studies that have utilized a primatized anti- $\alpha_4\beta_7$  monoclonal antibody (mAb) (derived from the Act1 clone). When administered just prior to and during acute intravenous SIVmac239 infection, Act1 mediated moderate reductions in plasma viral load but substantial reductions in gut pro-viral DNA(17). More dramatic results were obtained when Act1 was administered prior to low-dose repeat vaginal challenge with SIVmac251; a significant delay in SIV acquisition was observed(18). Animals that eventually acquired SIV showed markedly reduced damage to gut-associated lymphoid tissue (GALT), in addition to other lymphoid and mucosal tissues. When given in combination with antiretroviral therapy (ART), anti- $\alpha_4\beta_7$  promoted potent therapeutic effects, leading to post-treatment virological control in 8/8 treated animals compared to 0/7 controls(19). These findings suggest that  $\alpha_4\beta_7$  expressing cells play a central role in SIV transmission and pathogenesis.

One important gap in the literature is the role of  $\alpha_4\beta_7$  at the time of HIV exposure in humans. Here we report evidence that frequencies of  $\alpha_4\beta_7$  expressing CD4<sup>+</sup> T cells predict both increased risk of HIV acquisition and more rapid disease progression in a cohort of high-risk women from KwaZulu-Natal, South Africa. With a number of anti- $\alpha_4\beta_7$  blockers in various stages of clinical development, these findings inform the potential translation of these drugs in the treatment of HIV disease.

## Results

### Participants

We compared levels of  $\alpha_4\beta_7$  integrin expression on CD4<sup>+</sup> T cells in blood samples from individuals who later acquired HIV to controls that remained HIV uninfected for the duration of the CAPRISA 004 study. Cases were sampled at the last available pre-HIV infection visit, with a sampling median of 110 days (IQR 65, 182) pre-HIV infection. Controls (n = 106) were matched to cases (n = 59) at a 2:1 ratio on the basis of study arm, age (5-year window), and month of enrolment. No major differences between cases and controls were observed for a number of demographic, clinical, and behavioral variables (Table 1). Additional analyses were conducted in study cohorts from Kenya, Uganda, the RV254/SEARCH 010 cohort in Thailand (Table S1-3), and in non-human primates (NHPs).

### Pre-HIV frequencies of integrin $\beta_7^{\text{Hi}}$ CD4<sup>+</sup> T cells are associated with HIV acquisition

Previous studies have demonstrated that  $\beta_7^{\text{Hi}}$  cells in the blood are >99%  $\alpha_4\beta_7^+$  (6, 20); therefore  $\beta_7^{\text{Hi}}$  CD45RA<sup>-</sup> gating was used to quantify  $\alpha_4\beta_7$  expression on CD4<sup>+</sup> T cells (herein referred to as  $\beta_7^{\text{Hi}}$  cells). We identified three populations of CD4<sup>+</sup> T cells based on the relative density of the integrin  $\beta_7$  and CD45RA expression that were consistently measured across the study population:  $\beta_7^{\text{Hi}}$  CD45RA<sup>-</sup>,  $\beta_7^{\text{Int}}$  CD45RA<sup>+</sup> and  $\beta_7^{\text{Neg}}$  CD45RA<sup>-</sup> (Fig. 1A). Because it is difficult to sample cases immediately prior to HIV infection, we investigated the stability of  $\beta_7^{\text{Hi}}$  frequencies in blood samples over multiple HIV uninfected

visits in a subset of participants (range 2-5 visits, Fig. 1B). The median co-efficient of variation (CV) was 15% (IQR 11-36), indicating relatively stable expression in most individuals. We then determined whether any association between HIV infection and higher frequencies of  $\beta_7^{\text{Hi}}$  CD4<sup>+</sup> T cells might be explained by sampling carried out closer to the estimated time of HIV infection (Fig. 1C). Our analysis indicates that this was not the case; the frequencies of  $\beta_7^{\text{Hi}}$  CD4<sup>+</sup> T cells were consistent among cases and controls regardless of sample timing, congruent with the stability data presented in Figure 1B.

In our primary endpoint analysis, the frequency of  $\beta_7^{\text{Hi}}$  cells was higher in samples from cases (median 9.7%, IQR 8.1-12.3%) than controls (median 8.7%, IQR 6.5-10.7%). In conditional logistic regression analyses, each percent of pre-HIV infection  $\beta_7^{\text{Hi}}$  CD4<sup>+</sup> T cells correlated with 17% increased risk of HIV acquisition (OR 1.17, 95% 1.05 –1.32,  $p=0.007$ , Fig. 1D). In contrast, no significant associations were observed for either  $\beta_7^{\text{Int}}$  or  $\beta_7^{\text{Neg}}$  populations and HIV acquisition ( $p=0.242$  and  $0.882$  respectively, Fig. S1).

We next carried out multivariable modeling to adjust for variables that may confound the HIV acquisition analysis. Integrin  $\beta_7^{\text{Hi}}$  cell frequency remained associated with HIV acquisition with a similar effect estimate after adjusting for study site, HSV-2 sero-status, abnormal vaginal discharge, number of sexual partners and sex acts/month, condom and depomedroxyprogesterone acetate (DMPA) usage (aOR 1.16, 95% 1.03-1.32,  $p=0.016$ , Table 2). We also adjusted the analysis for genital inflammation (defined as 5/9 pro-inflammatory cytokines in the upper quartile(21)), and found that frequencies of  $\beta_7^{\text{Hi}}$  cells remained a predictor of HIV outcome in a model that included all of the other covariates listed above (aOR 1.15, 95% 1.02-1.30,  $p=0.028$ ). Additionally, frequencies of  $\beta_7^{\text{Hi}}$  cells remained a predictor of HIV outcome in a model that included both CD4<sup>+</sup> T cell activation (aOR 1.18, 95% 1.05-1.33,  $p=0.005$ ) and CD8<sup>+</sup> T cell activation (aOR 1.18, 95% 1.05-1.32,  $p=0.007$ ) defined by HLA-DR and CD38 co-expression. We carried out further phenotypic profiling to compare levels of CCR5, Ki67, CD38, and HLA-DR expression between the three main  $\beta_7$  subsets (Fig. S2).  $\beta_7^{\text{Hi}}$  cells were comparable to at least one of the other  $\beta_7$ -associated subsets with respect to phenotypic markers that have been associated with HIV pathogenesis, further indicating that the observed effect is specific to  $\beta_7^{\text{Hi}}$  expression.

The HIV acquisition results were validated in additional 41 participants (11 cases and 30 controls) from an independent cohort of female sex workers from Nairobi (Table S3), with a very similar odds ratio for HIV acquisition risk observed as in CAPRISA 004 (Table S4, OR 1.19, 95% CI 0.94-1.52,  $p=0.148$ ). Combining the data from the two cohorts ( $n=206$ ), we found that each percent increase in  $\beta_7^{\text{Hi}}$  expression correlated with an 18% increase in HIV risk (OR 1.18, 95% CI 1.06-1.31,  $p=0.002$ ). These data demonstrate that  $\alpha_4\beta_7$  is a consistent predictor of HIV acquisition risk in two independent human cohorts.

We further analyzed pre-infection  $\alpha_4\beta_7$  expression on blood CD4<sup>+</sup> T cells as a predictor of SIV acquisition in NHPs exposed to weekly intra-vaginal challenges with SIVmac251. These animals were rhesus macaques (RMs) that were in the control arm (irrelevant IgG) of a published study(18). After adjusting for age, parity, and menses, RMs with higher  $\alpha_4\beta_7$  levels acquired SIV more rapidly than RMs with lower  $\alpha_4\beta_7$  frequencies (aHR 1.20/%  $\alpha_4\beta_7$ , 95% CI 0.99-1.44,  $p=0.057$ , Fig. S3). These results are congruent with the previous NHP

observations (14, 22) and our human cohort data, confirming that  $\alpha_4\beta_7$  expression on CD4<sup>+</sup> T cells is associated with both HIV and SIV infection risk.

### Levels of $\alpha_4\beta_7$ integrin expression by CD4<sup>+</sup> T cells correlated in corresponding samples from blood and cervix

We next explored whether levels of  $\alpha_4\beta_7^+$  CD4<sup>+</sup> T cells in the blood reflected levels in the female reproductive tract (FRT), the main site of HIV exposure during heterosexual transmission. While cervical specimens were not available in CAPRISA 004, we evaluated whether  $\alpha_4^+\beta_7^{\text{Hi}}$  cells in the blood correlated with  $\alpha_4^+\beta_7^{\text{Hi}}$  cells from endocervical cytobrushes in women from Uganda and Kenya (Fig. 1E). Positive correlations were observed consistently between cohorts (combined  $r=0.65$ ,  $p<0.0001$ ,  $n=57$ ). These data suggest that assessing systemic  $\beta_7^{\text{Hi}}$  cells is likely reflective of  $\alpha_4^+\beta_7^{\text{Hi}}$  levels in the FRT.

### Pre-HIV levels of $\alpha_4\beta_7$ are associated with early HIV env sequences

Several reports have suggested that  $\alpha_4\beta_7$  can bind to the gp120 second variable loop (V2) of some strains of HIV Env directly(7, 9, 23, 24). Specifically, the P/SDI/V V2 motif has been associated with increased  $\alpha_4\beta_7$ -dependent *in vitro* replication and is over-represented in the South African epidemic, particularly in KwaZulu-Natal(7, 15). With these observations in mind, we hypothesized that the frequency of  $\beta_7^{\text{Hi}}$  cells pre-HIV infection would correlate with the V2 sequences of early-transmitting viruses encoding P/SDI/V motif. Sequences of acute/early HIV envelopes were available for 32 CAPRISA 004 participants at a median of 5 weeks post-HIV infection (IQR 3, 7). Indeed, participants infected by viruses with V2 loops containing the P/SDI/V motif had higher levels of pre-HIV  $\beta_7^{\text{Hi}}$  cells than those infected by viruses with LDI/V motifs (median 11.5, IQR 8.9, 13.8; vs. median 9.1, IQR 6.0-10.9,  $p=0.0366$ , Fig. 1F and 1G). These data suggest that the risk of HIV acquisition mediated by  $\alpha_4\beta_7$  might be particularly pronounced when exposure involves viruses containing certain V2 motifs associated with enhanced  $\alpha_4\beta_7$  binding.

### Pre-HIV infection $\beta_7^{\text{Hi}}$ frequencies are associated with HIV disease progression

To determine if pre-HIV infection frequencies of  $\beta_7^{\text{Hi}}$  CD4<sup>+</sup> T cells predicted the rate of HIV disease progression, we correlated the frequency of these cells with both peak and set-point viral load (VL), the rate of CD4<sup>+</sup> T cell decline prior to ART initiation, and the median CD4:CD8 ratio post-HIV infection (Fig. 2). Modest correlations between  $\beta_7^{\text{Hi}}$  cell frequency and both peak (<180 days post-HIV) and set point VL (average of >180 day measurements) were observed ( $r=0.232$ ,  $p=0.112$  for peak;  $r=0.345$ ,  $p=0.016$  for set point, Fig. 2A and 2B); in contrast, no correlations were observed for  $\beta_7^{\text{Int}}$  or  $\beta_7^{\text{Neg}}$  cells (Fig. S4A and B).

The frequency of  $\beta_7^{\text{Hi}}$  cells was a strong predictor of CD4<sup>+</sup> T cell decline below 500 cells/ $\mu\text{l}$ ; individuals with  $\beta_7^{\text{Hi}}$  cells above the median of  $\beta_7^{\text{Hi}}$  expression progressed to CD4<500 at more than twice the rate of those below the  $\beta_7^{\text{Hi}}$  median (HR 2.38, 95% CI 1.25-4.51,  $p=0.008$ , Fig. 2C). At day 500, approximately 80% of those above the  $\beta_7^{\text{Hi}}$  median had progressed, compared to approximately 50% of those below the  $\beta_7^{\text{Hi}}$  median. In multivariable Cox regression models (Table 3), inclusion of plasma VL as a covariate had an impact on the strength of association, suggesting that the  $\beta_7^{\text{Hi}}$ -associated rate of disease

progression might be mediated in part by higher levels of HIV replication. However, in the full model, including VL and other important covariates (age, study site, study arm, DMPA use and HSV-2 status at baseline), pre-HIV levels of  $\beta_7^{\text{Hi}}$  cells remained a significant predictor of the rate of CD4<sup>+</sup> T cell decline (aHR 2.14, 95% CI 1.04-4.39,  $p=0.039$ , Fig. 2D).

Furthermore, we observed an inverse association between pre-HIV  $\beta_7^{\text{Hi}}$  cells and post-HIV CD4:CD8 ratio, another marker of disease progression(25) ( $r=-0.322$ ,  $p=0.026$  for measurements <180 days post-HIV, and  $r=-0.347$  and  $p=0.016$  for measurements >180 days post-HIV, Fig. 2E and 2F). Again, neither  $\beta_7^{\text{Int}}$  or  $\beta_7^{\text{Neg}}$  cell frequencies were associated with CD4<sup>+</sup> T cell decline < 500 cells/ $\mu\text{l}$  or CD4:CD8 ratio (Fig. S4C-E). We did not find any associations between activation markers expressed on bulk pre-infection CD4<sup>+</sup> T cells and disease progression (Fig. S5, Table S5).

In order to establish a link between human and NHP data we characterized  $\alpha_4\beta_7^+$  CD4<sup>+</sup> T cell frequencies in 14 NHPs prior to IV injection with SIVmac239. Nine animals had low levels (<30%) while the remaining 5 animals had higher levels >30%) of  $\alpha_4\beta_7^+$  on CD4<sup>+</sup> T cells; these patterns of expression were similar in both the blood and the gut tissue. RMs with higher  $\alpha_4\beta_7$  expression experienced higher set point VL than animals with lower  $\alpha_4\beta_7$  expression (median from week 3-16, 331,680 versus 82,230 copies/ml), which was statistically significant in a linear mixed model analysis ( $p=0.033$ , Fig S6A). As observed in humans, RMs with higher  $\alpha_4\beta_7$  expression had a faster rate of CD4 decline ( $p<0.001$ , Fig S6B) demonstrating consistency between different primate species.

We hypothesized that the association between  $\beta_7^{\text{Hi}}$  cells and the rate of HIV disease progression might be mediated by more efficient transit of virus-infected cells into the GALT, leading to more extensive gut damage and its associated pathogenic effects. To test this hypothesis, we measured plasma levels of microbial translocation markers prospectively following acute HIV until ART initiation. We found that levels of LPS binding protein (LBP) were elevated in CAPRISA 004 participants with  $\beta_7^{\text{Hi}}$  cell frequencies above the median at all visits post-HIV infection, whether compared as median values (Fig. 2G) or at 6-month intervals (beta 0.54, 95% CI: 0.06-1.03,  $p=0.028$ , Fig. 2H). To determine if this was simply a reflection of more rapid progression, we utilized linear mixed models adjusting for viral load and CD4:CD8 ratio, measured at the same time points as LBP. LBP remained associated with higher  $\beta_7^{\text{Hi}}$  levels in the adjusted models (beta 0.59, 95% CI: 0.01-1.16,  $p=0.045$ ). Additionally, we quantified plasma levels of another two commonly used markers: intestinal fatty-acid binding protein (I-FABP) and sCD14. While we did not observe a statistically significant difference in I-FABP and sCD14 levels (Table S6) in relation to  $\beta_7^{\text{Hi}}$  levels, this could be due to recent reports that have suggested these markers may not be specific to microbial translocation and are influenced by other causes of GI disease(26-28) and monocyte activation(29), respectively.

### Depletion of $\beta_7^{\text{Hi}}$ CD4<sup>+</sup> T cells in the blood and gastrointestinal mucosa during acute HIV infection

To determine the impact of acute HIV infection on the  $\beta_7^{\text{Hi}}$  CD4<sup>+</sup> T cell population early in HIV infection in both the blood and the GI tract we compared frequencies of both CCR5<sup>+</sup>

and  $\beta_7^{\text{Hi}}$  cells at Fiebig (F) stages I, II, and III in participants enrolled in the RV254 early infection cohort to chronic HIV infected and uninfected participants (Fig. 3)(30). In the blood,  $\beta_7^{\text{Hi}}$  cells increased transiently in FI followed by a modest drop in those recruited from FII onwards (Fig. 3A). Similar kinetics were observed for blood CCR5 depletion, where levels of CCR5<sup>+</sup> cells remained comparable to HIV uninfected levels in FI and significantly decreased during FII and FIII (Fig. 3B). In contrast in the GI tract,  $\beta_7^{\text{Hi}}$  depletion was evident in all Fiebig stages, with major depletion occurring from FI (Fig. 3C). This occurred more rapidly than CCR5 depletion, where the major loss of CCR5<sup>+</sup> cells was only evident during the transition from FII to FIII (Fig. 3D). These data confirm that  $\alpha_4\beta_7^+$  CD4<sup>+</sup> T cells are depleted very early in HIV infection, particularly in the gut, providing a potential explanation as to why these cells predicted higher rates of HIV acquisition and disease progression in CAPRISA 004 study.

Since ART was initiated upon diagnosis in RV254, we were not able to assess disease progression in this cohort. However, we determined the impact of ART on  $\beta_7^{\text{Hi}}$  and CCR5<sup>+</sup> CD4<sup>+</sup> T cell frequencies in both colon biopsies and blood over 2 years following very early HIV treatment at the time of acute diagnosis. In participants who initiated ART in either FI or FIII, two years of ART failed to restore blood  $\beta_7^{\text{Hi}}$  CD4<sup>+</sup> T cells (Fig. 3E). Interestingly, although not statistically significant, a continuous loss of  $\beta_7^{\text{Hi}}$  CD4<sup>+</sup> T cells in peripheral blood could be observed despite the fact that these participants initiated treatment during FI, suggesting that if anything these frequencies may continue to decline during treatment. Similarly, ART initiation failed to restore blood CCR5<sup>+</sup> CD4<sup>+</sup> T cells (Fig. 3F). However, the impact of ART on  $\beta_7^{\text{Hi}}$  and CCR5<sup>+</sup> CD4<sup>+</sup> T cells in the colon was strikingly different. The frequency of colonic  $\beta_7^{\text{Hi}}$  CD4<sup>+</sup> T cells in patients who initiated ART in either FI or FIII were already reduced at initial baseline measurement and these levels showed no sign of recovery after 24 months of treatment (Fig 3G). In contrast, in participants who initiated ART in FI, colonic CCR5<sup>+</sup> CD4<sup>+</sup> T cells were not depleted and their frequency at 24 months of therapy was maintained at a level similar to healthy controls. Participants who initiated therapy in FIII showed an initial loss and a gradual, “near” recovery by month 24 (Fig. 3H). These data suggest that  $\beta_7^{\text{Hi}}$  CD4<sup>+</sup> T cell depletion occurs very early during acute HIV infection, including in the GI tract compared to blood. It is also clear that ART is unable to restore the frequency of those CD4<sup>+</sup> T cell frequencies in the GI tract, even when provided at the earliest time point, when gut damage is relatively minimal compared with chronic HIV.

## Discussion

The integrin  $\alpha_4\beta_7$  plays an important role in promoting immune cell trafficking to the inductive and effector sites of the gastrointestinal tract, both of which are irreversibly damaged during acute HIV infection. The main aim of the present study was to evaluate the role of  $\alpha_4\beta_7$  integrin in humans at risk of HIV infection, an important step towards the translation of these promising pre-clinical studies. In line with our *a priori* hypotheses, expression of  $\alpha_4\beta_7$  on blood memory CD4<sup>+</sup> T cells measured prior to HIV infection in South African women predicted both higher risk of HIV acquisition and a more rapid rate of HIV disease progression.

Although the association of  $\alpha_4\beta_7$  expression and HIV acquisition was relatively modest, results were consistent in independent cohorts in two different countries and in NHPs. Higher pre-infection  $\alpha_4\beta_7$  levels were previously associated with susceptibility to rectal SIV infection (14, 22). Additionally in a low-dose vaginal challenge, SIV infection was significantly delayed by blocking  $\alpha_4\beta_7$  using Act1(18). Consistencies in odds ratios for HIV acquisition risk between CAPRISA 004 and Kenyan FSW cohort demonstrate that  $\alpha_4\beta_7$  associates with HIV acquisition risk in women from different geographic locations. Reported findings demonstrate consistency in humans and show that results can be translated between human and non-human primates.

Interactions between  $\alpha_4\beta_7$  and HIV *env* may assist the virus in locating its ideal target cells(31). The findings herein show that higher frequency of  $\alpha_4\beta_7$  CD4<sup>+</sup> T cells was associated with preferential infection by HIV-1 containing gp120 V2 motifs (P/SDV/I) that have been associated with higher  $\alpha_4\beta_7$  binding and are over-represented in clade C sequences from KwaZulu-Natal, South Africa, the region where the CAPRISA 004 study was conducted (7, 15). The  $\alpha_4\beta_7$ -binding motif has been implicated as an important epitope for HIV antibody responses, including both bNAbs (32, 33) and those that correlated with protection against HIV infection in the RV144 vaccine study(34). The role of pre -infection  $\alpha_4\beta_7$  expression on HIV pathogenesis likely depends on the nature of the transmitting virus.

Levels of  $\alpha_4\beta_7$  expression had a strong impact on the rate of HIV disease progression; in particular, as measured by the rate of CD4<sup>+</sup> T cell decline. As observed with HIV acquisition, the progression effect was highly similar between RMs and humans. In addition, the data from RV254 suggest that  $\alpha_4\beta_7$ <sup>+</sup> CD4<sup>+</sup> T cells are targeted very early in the blood and gut. In humans, a high proportion of initial HIV-target cells are likely  $\alpha_4\beta_7$ <sup>+</sup>, and the rapid gut depletion of CD4<sup>+</sup> T cells may be driven by the preferential depletion of cells expressing  $\alpha_4\beta_7$  (even earlier than CCR5). Therefore, in individuals with higher frequency of  $\alpha_4\beta_7$ <sup>+</sup> cells one may expect increased viral replication and associated destruction of many CD4 compartments including the GI mucosa, the latter of which has a major effect on disrupting immune homeostasis. While we were not able to link rapid gut depletion with pre-HIV  $\alpha_4\beta_7$ , due to lack of pre-HIV samples in RV254 and lack of gut sampling in CAPRISA004, our finding of raised LBP levels at all stages of untreated HIV infection in individuals with higher  $\alpha_4\beta_7$  expression supports this concept, linking  $\alpha_4\beta_7$  expression with subsequent microbial translocation and gut damage.

Initiation of antiretroviral therapy (ART), particularly at early stages of HIV infection(35), restores some immune cell populations, but rarely to their pre-infection frequency and/or function(36). The majority of CD4 depletion occurs in the GALT during primary infection(3, 37) and ART administration at first detection of VL failed to prevent depletion or facilitate reconstitution of gut  $\alpha_4\beta_7$ <sup>+</sup> CD4<sup>+</sup> T cells in RV254. The fact that ART alone does not lead to immune-restoration, but ART in combination with anti-  $\alpha_4\beta_7$  did so in NHPs(19), suggests that interventions in addition to ART may be needed to achieve functional CD4<sup>+</sup> T cell restoration.

One of the limitations of our study is the lack of paired cervical and blood sampling, which is not available in CAPRISA 004. Nevertheless, data from two independent East African



cohorts demonstrate that the frequency of  $\alpha_4\beta_7^+$  cells correlated strongly between blood and cervical CD4<sup>+</sup> T cells, suggesting that the correlation between increased frequencies of blood  $\alpha_4\beta_7^+$  CD4<sup>+</sup> T cells and HIV acquisition may be explained, at least in part, by a higher concentration of target cells at the site of HIV exposure(38). While the results presented here are specific to heterosexual transmission of HIV, previous NHP studies support a similar role for  $\alpha_4\beta_7$  during other modes of exposure.

This study defines the importance of  $\alpha_4\beta_7$  in HIV acquisition and disease progression in a prospective natural history study of high-risk women. One model to explain these data is that preferential infection of  $\alpha_4\beta_7^+$  CD4<sup>+</sup> T cells at the time of HIV exposure leads to more pronounced local infection of these cells, which then migrate rapidly to the gut mucosal and lymphoid tissue, where rapid viral replication contributes to establishment of the latent HIV reservoir. Recent work by our group has suggested that the administration of a primatized analogue of the anti- $\alpha_4\beta_7$  mAb in SIV-infected macaques receiving early ART led to sustained spontaneous control of SIV and repopulation of GI tract with CD4<sup>+</sup> T cells in the absence of further treatment(19). Combined with the findings of the current study, these data suggest that  $\alpha_4\beta_7$  integrin might be a useful target for HIV prevention and/or treatment in humans. Further evaluation of this concept is clinically feasible, given that a humanized version of the Act1 monoclonal antibody clone (called Vedolizumab) has been proven safe and effective and is FDA-approved for the treatment of adults with moderate to severe ulcerative colitis and Crohn's disease(39, 40).

## Materials and Methods

### Study design

We carried out a nested retrospective case-control analysis to correlate the expression of integrin  $\beta_7^{\text{Hi}}$  on CD4<sup>+</sup> T cells to rates of HIV acquisition. Cases were then followed prospectively to compare disease progression outcomes stratified by  $\beta_7^{\text{Hi}}$  CD4<sup>+</sup> T cells at pre-HIV infection time points. The main outcomes for disease progression were set point and peak HIV viral loads measured post-infection prior to the initiation of ART. Survival analyses were used to compare the time to CD4 decline below 500 cells/ $\mu\text{l}$ . In RV254 study different acute HIV stages were compared cross-sectionally at diagnosis, and followed longitudinally following early ART initiation. All experiments were performed in a blinded fashion. The detailed study cohorts, sample collection and processing information is provided in Supplementary Material and Methods. The sample size for each experiment is included in the figures and/or figure legends.

### Flow cytometry analysis

PBMC were thawed, washed to remove the cryopreserving fluid and then rested for 3 hours (RPMI 1640 supplemented with 10% fetal bovine serum) at 37°C, 5% CO<sub>2</sub>, and stained with a panel of antibodies designed to profile  $\beta_7$  expression on different CD4<sup>+</sup> T cell subsets in terms of their memory, activation, and target cell properties. Detailed methods can be found in Supplementary Material and Methods.

### Soluble biomarker analysis

Plasma LBP levels were measured using Human LBP DuoSet ELISA, DY870-05 (R&D Systems Inc., Minneapolis, USA). All assays were performed following manufacturer's instructions. Samples with values below the lower detection limit were assigned the value half the lower limit of quantification, LLOQ/2.

### Viral sequencing

The sequence of transmitted/founder *envelopes* were inferred as the consensus of acute/early sequences obtained from a median of 5 weeks post-HIV infection (IQR: 3 – 7.25, range: 2 – 13 weeks). Viral *envelope* sequences were generated by Sanger sequencing of amplicons generated by single genome amplification of viral RNA, performed as previously described(45, 46). Additional methods can be found in Supplementary Material and Methods.

### Statistical approaches

To compare HIV acquisition risk, we carried out conditional logistic regression with strata defined by matching criteria that was used to select cases and controls. The main explanatory variables in all models included 3 integrin  $\beta_7$ -defined subsets, each modeled separately in bivariate and multivariable models adjusting for a number of potential confounding variables. Disease progression rates were evaluated using several readouts, including correlation analyses (Spearman rank correlation) with HIV VL and CD4:CD8 ratio, measured both as peak (highest value in the first 180 days of infection) and set point (average value in measurements made after 180 days infection until ART initiation). Rates of CD4 decline were compared in bivariate and multivariable Cox regression models with the endpoint defined as any two CD4 counts below 500/ $\mu$ l prior to ART initiation. Survival analyses excluded CD4 counts measured during the first 180 days of follow up; these were censored to exclude transient CD4 drops during acute HIV infection, as previously described(48). D'Agostino and Pearson omnibus normality test was utilized for Gaussian distribution of the data. For data that did not follow normal distribution, non-parametric tests including Kruskal-Wallis test and Spearman correlation were performed. All statistics are two-tailed. Linear mixed models were used to compare LBP levels prospectively, with  $\beta_7^{\text{Hi}}$  above and below the median as the primary predictor, and adjustments made for progression variables including VL and CD4:CD8 ratio.

### Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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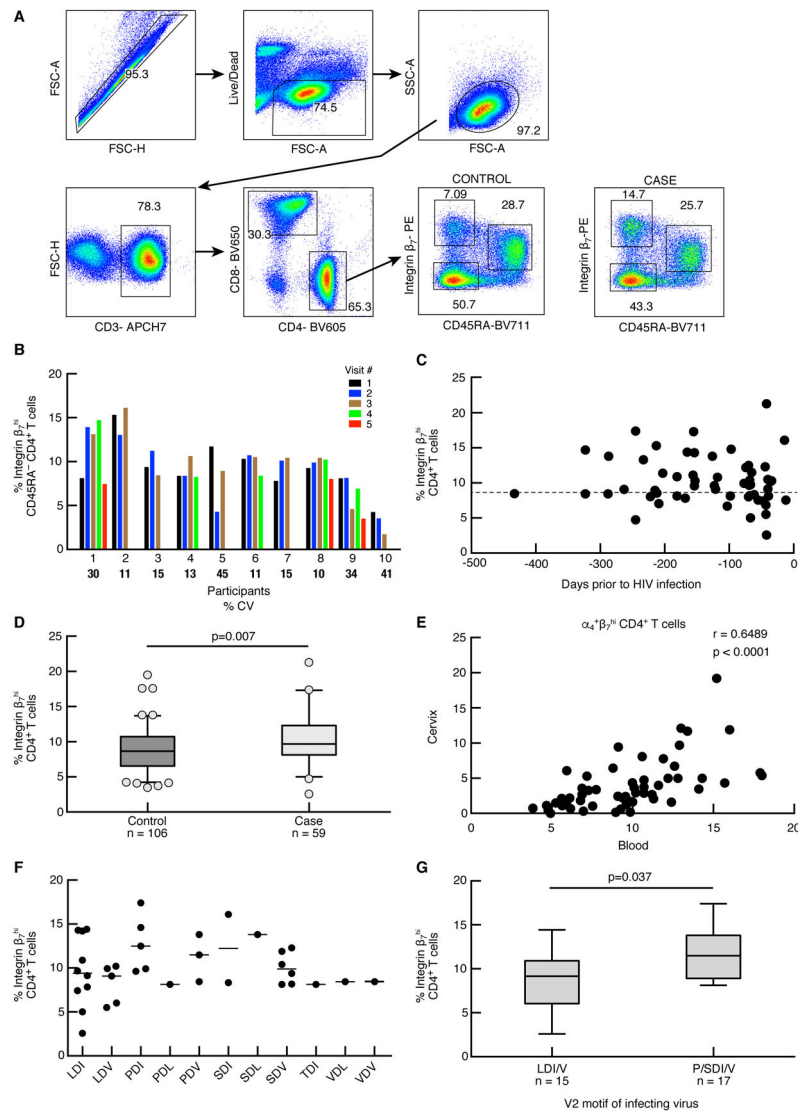
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**Figure 1. Effect of pre-infection  $\beta_7^{\text{Hi}}$  CD45RA<sup>-</sup> CD4<sup>+</sup> T cell frequency on HIV acquisition risk in CAPRISA 004 study.**

**A)** Parent gating strategy for the analysis of frozen PBMCs from the CAPRISA 004 study. The staining profile of PBMC from a representative HIV-uninfected participant is shown.  $\beta_7$  gating is shown for one representative control and one case sample obtained at a HIV-uninfected time point. **B)** Stability of  $\beta_7^{\text{Hi}}$  CD4<sup>+</sup> T cells overtime. Samples from 10 patients (x axis) were assayed from 3-5 HIV uninfected visits (colored bars) depending on sample availability. Median coefficient of variation (CV) between HIV uninfected time points for every individual was calculated. Individual CVs are indicated in the graph. **C)** Sampling time points for the pre-HIV  $\beta_7^{\text{Hi}}$  measurements. The number of days prior to HIV infection that the sample was obtained (x-axis) is plotted against  $\beta_7^{\text{Hi}}$  frequency (y-axis). The dashed line represents the median Integrin  $\beta_7^{\text{Hi}}$  expression by CD4<sup>+</sup> T cells **D)** The frequency of  $\beta_7^{\text{Hi}}$  CD45RA<sup>-</sup> CD4<sup>+</sup> T cells in cases (n=59) and controls (n=106). Conditional logistic regression analysis was used to measure the effect of pre-infection  $\beta_7^{\text{Hi}}$  levels on HIV acquisition. **E)** Spearman correlation between  $\alpha_4\beta_7^{\text{Hi}}$  CD4<sup>+</sup> T cell frequency between cervix

and blood in the Nairobi/Uganda study (n=54) **F**) Infecting viral V2 motifs and pre-HIV frequencies of  $\beta_7^{\text{Hi}}$  CD4+ T cells **G**) Pre-HIV frequencies of  $\beta_7^{\text{Hi}}$  CD4+ T cells in cases infected by viruses with V2 loops containing the P/SDI/V and LDI/V motifs (n=32). Differences between groups were analyzed using unpaired t-test.

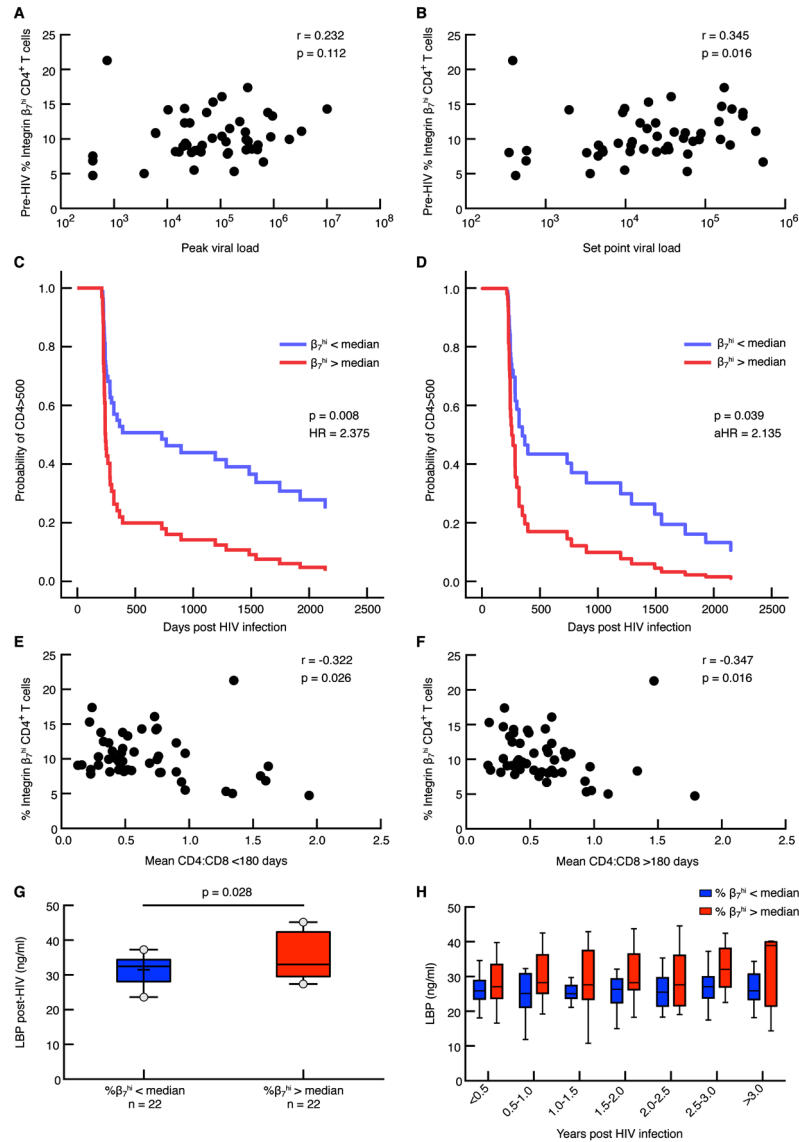
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**Figure 2. Effect of pre-infection  $\beta_7^{\text{Hi}}$  CD45RA<sup>-</sup> CD4<sup>+</sup> T cell frequency on disease progression in patients that became infected in CAPRISA 004/002 study.**

**A)** Correlation between pre-infection  $\beta_7^{\text{Hi}}$  frequency and peak VL (n= 49) **B)** Correlation between pre-infection  $\beta_7^{\text{Hi}}$  frequency and set point viral load (n=49) **C).** The frequency of pre-infection  $\beta_7^{\text{Hi}}$  cells as a predictor of CD4<sup>+</sup> T cells decline <500 cells/ $\mu\text{l}$  (n=48) analyzed using Cox regression models **D)** Pre-infection  $\beta_7^{\text{Hi}}$  cells as a predictor of CD4<sup>+</sup> T cells decline <500 cells/ $\mu\text{l}$  in a multivariable Cox regression model correcting for age, study site, study arm, set point viral load, DMPA use and HSV-2 status at baseline **E)** Correlation between pre-infection  $\beta_7^{\text{Hi}}$  frequency and mean CD4:CD8 ratio < 180 days post infection (n=48) **F)** Correlation between pre-infection  $\beta_7^{\text{Hi}}$  frequency and mean CD4:CD8 ratio > 180 days post infection (n=48) **G)** Median post-infection plasma levels of LBP expression in cases with pre-infection  $\beta_7^{\text{Hi}}$  CD4<sup>+</sup> T cell expression above (n=22) and below (n=22) median. **H)** Longitudinal plasma LBP levels at 6 month intervals in CAPRISA 002 cases.

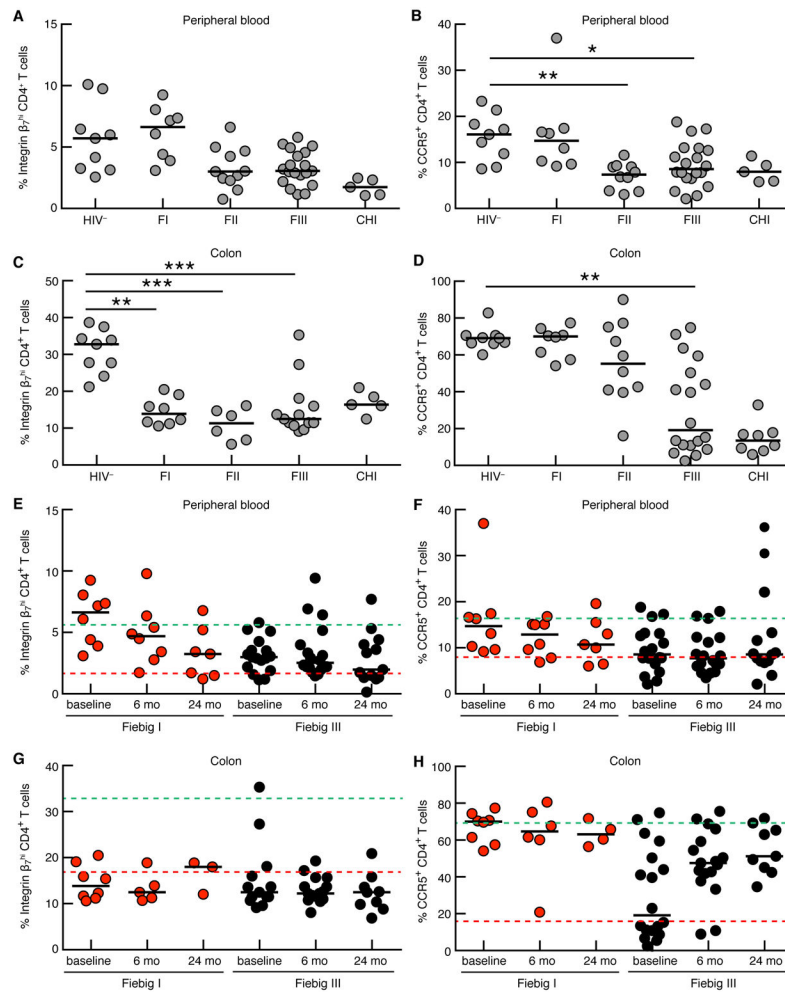
Linear mixed models were used to compare LBP levels over time. Spearman correlation was used to analyze associations between the two variables.

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**Figure 3. Depletion and post cART recovery of  $\beta 7^{\text{Hi}}$  and  $\text{CCR5}^+ \text{CD4}^+$  T cells in peripheral blood and cells isolated from sigmoid colon during acute infection in RV254.** Frequencies of (A)  $\beta 7^{\text{Hi}}$  and (B)  $\text{CCR5}^+ \text{CD4}^+$  T cells in the peripheral blood at different Fiebig (F) stage [HIV- (n=9), FI (n=8), FII (n=11), FIII(n=20) and chronic HIV infected (CHI, n=5)] (C) Frequencies of  $\beta 7^{\text{Hi}} \text{CD4}^+$  T cells in the colon [HIV- (n=9), FI (n=8), FII (n=6), FIII(n=13) and CHI (n=5)] (D) Frequencies of  $\text{CCR5}^+ \text{CD4}^+$  T cells in the colon [HIV- (n=9), FI (n=8), FII (n=10), FIII(n=18) and CHI (n=8)]. Frequencies of (E)  $\beta 7^{\text{Hi}}$  and (F)  $\text{CCR5}^+ \text{CD4}^+$  T cells in the peripheral blood following ART initiation in either FI [red data points, at baseline (n=8), 6m(n=8), 24m(n=7)] or FIII [black data points, at baseline (n=18), 6m(n=18) and 24m(n=14)]. (G) Frequencies of  $\beta 7^{\text{Hi}} \text{CD4}^+$  T cells in the colon following ART initiation in either FI [red data points, baseline (n=8), 6m(n=5), 24m(n=3)] or FIII [black data points, baseline (n=13), 6m(n=15) and 24m(n=9)] (H)  $\text{CCR5}^+ \text{CD4}^+$  T cells in the colon following ART initiation in either FI [red data points, at baseline (n=8), 6m(n=6), 24m(n=4)] or FIII [black data points, at baseline (n=18), 6m(n=17) and 24m(n=9)]. The dashed lines represent the median of cells in healthy control participants (green, n=9) and in CHI patients (red, n=5). For A to D, Kruskal Wallis test was used followed by a Dunn's multiple comparisons test to look for differences between HIV uninfected (HIV-) group and groups at different Fiebig stages. For E to H Friedman test

was used to test for significant differences within each ART initiation group (FI and FIII).  
\*P<0.05, \*\*P< 0.01 and \*\*\*P<0.001.

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**Table 1.**

Characteristics of the CAPRISA 004 study population

Variable	Cases (n=59) Median (IQR)	Controls (n=106) Median (IQR)	P value
Age *	23 (20, 25)	22 (20, 28)	.864
Urban site	19/59 (32.2)	40/106 (37.7)	.503
TFV arm *	23/59 (39.0)	43/106 (40.6)	.87
DMPA use	50/59 (84.7)	85/106 (80.2)	.532
Vaginal discharge	25/59 (42.4)	35/106 (33.0)	.242
Ulcers	1/59 (1.7)	6/106 (5.7)	.423
HSV-2 sero-status (at trial entry)	32/59 (54.2)	53/106 (50.0)	.629
Sex acts, past 30 days	5 (2.5, 6.6)	5 (3, 8)	.281
Parity	1 (1, 2)	1 (1, 1)	.878

\* Part of the matching criteria

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**Table 2.**Multivariable analysis of HIV acquisition and integrin  $\beta_7^{\text{Hi}}$  CD4<sup>+</sup> T cells using conditional logistic regression

Variable	P value	aOR	95.0% CI	
			Lower	Upper
Integrin $\beta_7^{\text{Hi}}$ CD45RA <sup>-</sup> CD4 <sup>+</sup> T cells	<b>.016</b>	<b>1.163</b>	<b>1.029</b>	<b>1.316</b>
Urban study site	.091	.349	.103	1.181
HSV-2 seropositive at trial entry	.223	1.606	.750	3.438
Abnormal vaginal discharge	.248	1.646	.707	3.831
Number of new casual partners in the last 30 days	.669	.916	.614	1.367
Median # of sex acts / month	.100	1.110	.980	1.258
Condom use	Never (ref)	.486	1	-
	Always	.134	.399	.120
	Occasionally	.371	.623	.221
	Most times	.740	.824	.262
DMPA use	.294	1.880	.578	6.120

**Table 3.**Multivariable analysis of CD4 decline and integrin  $\beta_7^{\text{Hi}}$  CD4<sup>+</sup> T cells using Cox regression

Variable	P value	aHR	95.0% CI	
			Lower	Lower
Integrin $\beta_7^{\text{Hi}}$ CD45RA <sup>-</sup> CD4 <sup>+</sup> T cells	<b>.039</b>	<b>2.135</b>	<b>1.039</b>	<b>4.389</b>
Age	<b>.041</b>	<b>1.106</b>	<b>1.004</b>	<b>1.219</b>
Urban study site	.520	.747	.308	1.814
Study arm	.159	.598	.293	1.223
Log10 pVL (set point)	<b>&lt;.001</b>	<b>2.538</b>	<b>1.649</b>	<b>3.905</b>
DMPA use	.829	1.126	.384	3.304
HSV-2 seropositive at baseline	.952	.975	.429	2.217

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