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# Long-term Antiretroviral Therapy Mitigates Mortality and Morbidity Independent of HIV Tropism: 18 Years Follow-up in a Women's Cohort

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*KA and CR contributed equally to this article.

**Authors' Contributions:** BW conceived and designed the study, analyzed and interpreted the data, supervised the HIV tropism analyses, wrote the paper, and was responsible for the overall coordination of the study. CR and KA shared senior authorship. CR performed the statistical analyses and prepared the figures, designed the study, interpreted the data, and contributed to writing the paper. KA and HM were the founding and long-term principal investigators of the two WIHS sites and contributed to the study design, interpretation of data, and writing. HB conceived and designed the study, interpreted the data, supervised the tropism analyses, and contributed to writing the paper. BS, KK, TS, and AG developed, validated, and performed the HIV tropism assay. QS contributed to the statistical analysis and Wei Gao performed data management. ER and SH recruited, retained, and followed the WIHS participants.

Conflicts of Interest and Source of Funding

BW, HB and CR are co-inventors of seven patented technologies for the genotypic determination of HIV-1 coreceptor usage. The patents are owned by Health Research, Incorporated, the research foundation for the New York State Department of Health (NYSDOH), and are licensed to Quest Diagnostics. In this study, tropism determinations were performed at the Wadsworth Center of the NYSDOH by coauthors of this paper.

The remaining authors have declared no conflicts of interest and none of the authors has financial relationships with commercial entities relevant to this manuscript.

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## Abstract

**Objective:** CXCR4(X4)-tropic HIV-1 was found previously to herald CD4+ cell depletion and disease progression in individuals who were antiretroviral-naive or took combination antiretroviral therapy (cART) for <5 years. We updated this finding by investigating whether the deleterious effect of X4-tropic strains is mitigated by long-term cART.

**Design:** We examined morbidity and mortality in relation to HIV-1 tropism and cART in 529 participants followed up to 18 years in the Women's Interagency HIV Study; 91% were women of color.

**Methods:** Plasma-derived HIV-1 tropism was determined genotypically.

**Results:** We categorized participants according to number of visits reported on cART after initiation: Group 1) ≤3 visits, 74% of these participants reporting no cART; Group 2) ≥4 visits and <70% of visits on cART; Group 3) ≥70% of visits on cART. AIDS mortality rates for participants in each group with X4-virus compared to those with R5-virus exclusively were, respectively: 1) 62% vs 40% (P=0.0088); 2) 23% vs 22% [Nonsignificant (NS)]; 3) 7% vs 14% (NS). Kaplan-Meier curves showed accelerated progression to AIDS death or AIDS-defining illness in participants with ≤3 cART visits and X4-viruses (P=0.0028), but no difference in progression rates stratified by tropism in other groups. Logistic Regression found that HIV-1 suppression for ≥10 semiannual visits (≥5 years total) mitigated X4-tropism's deleterious effect on mortality, controlling for maximal viral load and CD4 nadir.

**Conclusions:** Long-term cART markedly mitigated the deleterious effect of X4-viruses on AIDS morbidity and mortality. Mitigation was correlated with duration of viral suppression, supporting HIV-1 suppression as a crucial goal.

### Keywords

HIV-1; HIV-1 tropism; HIV-1 coreceptor usage; CXCR4; CCR5; combination antiretroviral therapy; cART; HAART; immunologic nonresponders; HIV infection in women

## INTRODUCTION

HIV-1 coreceptor usage plays a critical role in viral pathogenesis and disease progression [1-7]. HIV-1 strains that initiate infection use the CCR5-coreceptor (R5-viruses) [1-7]. Strains using the CXCR4-coreceptor (X4-viruses) emerge in ~50-80% of persons during chronic infection, with X4- and R5-viruses generally coexisting within the viral swarm [3-6,8-11]. Emergence of X4-viruses usually heralds CD4 cell depletion and disease progression [3-6,8-10,12-15]. Deleterious effects of X4-viruses were primarily demonstrated in combination antiretroviral therapy (cART)-naïve individuals or those who took cART for <5 years. [14,16,18-21]. Initial studies of coreceptor usage, also called tropism, and cART focused on pretreatment tropism, with few years of follow-up on therapy; most showed that detection of X4-viruses before cART was a strong predictor of disease progression on treatment [16-17,19-21].

Sustained cART has led to dramatic reductions in HIV-1 disease [22], yet the question of whether the deleterious effect of X4-tropism persists despite long-term cART has not been studied in detail. Furthermore, most studies of tropism in vivo focused predominantly on men [10,14,18-21]. We therefore examined the relationship of HIV-1 tropism and long-term cART to morbidity and mortality in 529 women followed up to 18 years in the Woman's Interagency HIV Study (WIHS), an investigation of inner city women in the US.

## METHODS

We studied participants in the Bronx and Brooklyn, NY sites of WIHS, a longitudinal study of women with HIV-1. Interviews, examinations, and laboratory tests were performed semiannually; follow-up extended from enrollment in 1994-1995 until 2012 [23]. Each Institutional Review Board approved the investigation and each woman signed informed consent. cART was defined as any three-drug antiretroviral combination [24].

Plasma-derived HIV-1 tropism was determined using a DNA heteroduplex tracking assay. This assay, performed by Wadsworth Center coauthors, was validated by comparison with phenotypic assay results; both assay and validation were published previously [19]. The assay was licensed to Quest Diagnostics, where it was approved by the Food and Drug Administration (FDA) and Clinical Laboratory Improvement Amendments (CLIA) for clinical coreceptor quantification. Tropism was determined at 1--3 time points for each subject; time points were: 1) WIHS Visit 1; 2) WIHS visit preceding first cART visit; 3) WIHS visit following ≥18 months of continuous cART and virologic failure. X4 strains at ≥1 time point counted as detection of X4 tropism.

### Statistical Analyses.

Longitudinal CD4 counts were modeled using mixed effects models. We also fit regression splines to individual CD4 trajectories and overlaid a linear mixed effects model stratified by coreceptor type to look at overall trends. These models were performed using SAS version 9.3. counts over time.

Kaplan-Meier curves stratified by coreceptor usage were employed to model time to AIDS death or a new AIDS-defining illness (ADI). Participants were also categorized by the number of visits on cART and groups were compared using the Log-rank Test. Categorical data were assessed using Fisher's Exact Test. The Kruskal-Wallis Test was used for continuous data.

Logistic regression analysis and Cox proportional hazards models were used for binary outcomes and time to event data respectively. Regression models controlled for maximal viral load and CD4 nadir and were performed using mortality due to AIDS as the outcome.

Primary outcomes for survival analyses were participants' time from date of the first WIHS visit to AIDS death or incident ADI. Ascertainment and classification of deaths in participants have been published [25], with deaths due to ADI and infection classified as AIDS deaths in this analysis. ADI were self-reported and classified as incident as previously described [26].

## RESULTS

We studied 529 women with HIV-1; (See Table, Supplemental Digital Content 1, describing participants' demographic, virologic, and immunologic characteristics.) The cohort's racial and ethnic profile (61% African American, 30% Hispanic, 6% White, and 3% Other) is representative of WIHS and women diagnosed with HIV-1 infection in the US in 2016 [23,27,28].

We categorized participants into three groups according to the reported number of semiannual visits on cART after initiation: Group 1: ≤3 visits on cART, 74% of these participants reporting no cART, called "little or no cART," Group 2: ≥4 visits and <70% of visits on cART, called "intermittent cART," and Group 3) ≥70% of visits on cART, "consistent cART." To determine virologic efficacy, we ascertained the number and percentage of visits with viral suppression (HIV-1 RNA load <80 copies/mL) for each subject over time.

Table 1 presents participants' characteristics in relation to tropism and cART. Thirty percent of participants took little cART; 18% took intermittent cART; and 52%, consistent cART. Notably, the consistent cART participants had the highest prevalence of X4-strains (47%) (P=0.01). The little cART group had higher HIV-1 loads (P=0.0028) and lower CD4 counts (P=0.002) at baseline than the other groups.

Viral suppression was strongly correlated with the number of visits on cART. Only 12% of women with little cART ever had complete viral suppression, compared to 92% with consistent therapy and 74% with intermittent cART (P<0.0001).

X4-viruses were detected, exclusively or in a mixture with R5-variants, in 39% of participants. We compared the CD4 trajectories of participants with X4-variants to those with R5-viruses exclusively, controlling for viral load. Although CD4 counts rose in both groups, participants with X4-strains displayed significantly diminished CD4 counts throughout follow-up (P=0.026). (See Figure, Supplemental Digital Content 2, displaying trajectories of participants' CD4 cell counts over time, stratified by tropism).

Because of the long-term difference in CD4 trajectories, we asked whether X4-tropism may predispose to immunologic nonresponder status [29-31], defined here as CD4 counts remaining <500 cells/$mm^3$ despite viral load <80 copies/mL for ≥5 consecutive years on cART. Fourteen immunologic nonresponders were identified, and eleven (79%) had X4-viruses, suggesting an association of X4-tropism and incomplete CD4 recovery (P=0.018 by Fisher's Exact Test). CD4 cell nadirs in the nonresponders did not differ significantly by tropism.

Next, we analyzed the relationship of tropism to clinical outcomes during cART. Kaplan-Meier curves stratified by tropism were employed to model time to AIDS death or a new AIDS-defining illness (ADI). For participants with little cART, there was a markedly accelerated mortality rate for participants with X4-viruses (P=0.0002) (Fig 1B). If ADI or death was the outcome, the result was similar (P=0.0028) (Fig 1C). By contrast, curves depicting time to mortality in intermittent and consistent cART groups showed no

difference based upon tropism (NS) (Fig 1D, F), nor was there a difference if the outcome encompassed ADI or mortality rates. (NS)(Fig 1E,G). These results support the idea that cART mitigates or eliminates the deleterious effects of X4-viruses.

We also analyzed the relationship of tropism to clinical outcomes using Fisher's Exact Test, (full results in Table 1A&B). Mortality rates for women with X4- versus R5-variants, respectively, were: little cART: 62% vs 40% (P=0.0088); intermittent cART: 23% vs 22% (NS); and consistent cART: 7% vs 14% (NS); these results also support the idea of mitigation by cART.

Logistic regression analysis of all participants found that women who had <10 visits with complete HIV-1 suppression on cART were >3 times [OR 3.342 (1.952-5.72)] more likely to experience AIDS mortality than those who had ≥10 visits with viral suppression (See Table, Supplemental Digital Content 3, showing Logistic Regression analysis of relation of AIDS mortality to cART). Higher CD4 nadir was associated with lower likelihood of mortality due to AIDS [22,31]. Notably, for women achieving HIV-1 suppression for ≥10 continuous or noncontinuous semi-annual visits, the difference in mortality between participants who ever had detectable X4-strains and those with R5-virus exclusively was no longer significant after controlling for maximum viral load and CD4 nadir.

These findings show that complete HIV-1 suppression for ≥10 visits (5 years) mitigated the deleterious effect of X4-strains on mortality.

## DISCUSSION

This study demonstrates that long-term cART greatly mitigates the deleterious effect of CXCR4(X4)-tropic HIV-1 on morbidity and mortality. A body of literature documents that X4-tropism heralds CD4 depletion and disease progression, even in those receiving cART; most individuals studied, however, were antiretroviral-naïve or received cART for only a few years [3-6,8-10,12-14,16-21]. We updated these findings by studying women followed up to 18 years. Multiple analyses point to a role for both long-term cART and viral suppression in mitigating X4-tropism's effects (Table 1B, Fig 1, Supplementary Table 2.)

Logistic regression found that participants who had <10 visits with complete HIV-1 suppression on cART were >3 times [OR 3.342 (1.952-5.72)] more likely to experience AIDS death than those who had ≥10 visits with suppression (Supplemental Digital Content 3). Furthermore, logistic regression revealed that HIV-1 suppression for ≥10 semiannual visits (≥5 years total) mitigated the effect of X4-tropic virus on mortality, controlling for maximal viral load and CD4 nadir.

In this study, participants taking consistent cART had the highest prevalence of X4-variants among cART groups; they also achieved the most visits with complete viral suppression and the lowest morbidity and mortality. Data presented here suggest that mitigation of X4-tropism's effects provides a partial explanation for the success of cART, even conferring mitigation on those taking intermittent cART. Morbidity and mortality rates, however, were lowest in participants who achieved the most visits with viral suppression, underscoring the importance of HIV-1 suppression.

Mechanisms that mitigate X4-viruses' effect are likely to be complex because CD4 count, tropism, and cART are intertwined [5,8-10,13-16,18,19,32]. cART inhibits replication of all viral strains, generally leading to increased CD4 counts and reversal of HIV-1 disease [19,20,33]. In addition, multiple previous studies revealed that during treatment, cART preferentially suppresses X4-tropic viruses in peripheral blood and X4-proviral variants [34-38]. Mechanisms for preferential X4-virus suppression are likely to stem from differences in cellular targets for HIV-1 [35,39-41].

A small group of immunologic nonresponders was identified. They displayed a significant association between X4-tropism and nonresponder status, suggesting that X4-tropism may play a role in incomplete CD4 cell recovery. These data suggest that a larger study of this question would be worthwhile.

Worldwide, 52% of individuals with HIV-1 are women, as are 23% of persons with HIV-1 in the US [28,42]. Most studies of HIV-1 pathogenesis and disease progression, however, have focused predominantly on men [10,14,18-21]. There are numerous ethical and scientific reasons to include women in HIV-1 research (27,42-63). Biological differences between the sexes influence HIV-1 disease progression [27,52-62], as do socioeconomic, demographic, and cultural disparities [28,42,63]. Women with HIV-1 often experience gender inequality and sexual violence [42,63]. In the US, 61% of woman with HIV-1 are Black, and the rate of new infections is 15-fold higher in Black compared to white women [28]. Genetic diversity is relevant to HIV-1 infection. The CCR5 Δ32 heterozygous state, which confers partial resistance to HIV-1 infection in women, is found primarily in whites [2,27,64,65]. A WIHS study found that Black women taking cART experienced more adverse HIV-1 outcomes than whites, adjusting for confounders; these data suggest a possible role for genetic variability in clinical outcomes [23]. To optimize cART, it will be important to perform more studies examining the interplay between HIV-1 pathogenesis and treatment in diverse gender and racial groups.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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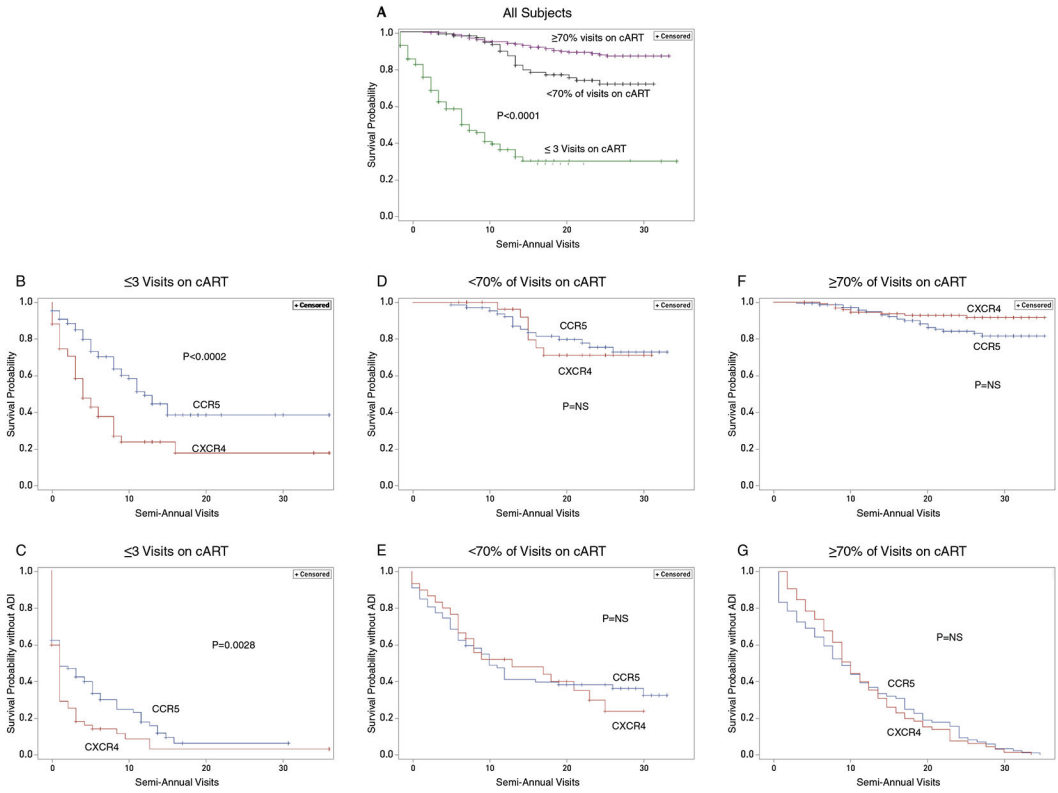


**Figure 1. Kaplan-Meier curves of the time until death due to AIDS or a new AIDS defining illness (ADI) and the relationship to HIV-1 tropism.**

A. Survival time until death due to AIDS for all 529 participants, stratified by number of reported visits on cART. These Kaplan-Meier curves show a significant difference in survival among the three groups defined by number of visits of cART. (P<0.0001 by the log-rank test).

B. Survival time until death due to AIDS in participants with little or no cART (≤3 visits on cART) stratified by coreceptor type (CCR5 or CXCR4). The Kaplan-Meier curves show decreased survival in subjects with X4 strains as compared to participants harboring R5 strains exclusively (P=0.0002 by the log-rank test).

C. Time until progression to a new AIDS-defining illness or death due to AIDS among participants with little or no cART (≤3 visits on cART) stratified by coreceptor type (CCR5 or CXCR4). The Kaplan-Meier curves showed more rapid disease progression or death due to AIDS for those with X4 strains as compared to participants harboring R5 strains exclusively (P=0.0028 by the log-rank test).

D. Survival time until death due to AIDS in participants with intermittent cART (<70% of visits on cART after initiation of therapy) stratified by coreceptor type (CCR5 or CXCR4). The Kaplan-Meier curves were not significantly different between the two coreceptor groups and hence did not demonstrate a difference in mortality rates associated with HIV-1 tropism. [Nonsignificant (NS) by the log-rank test].

E. Time until progression to a new AIDS-defining illness or death due to AIDS in participants taking intermittent cART (<70% of visits on cART after initiation of therapy) stratified by coreceptor type (CCR5 or CXCR4). The Kaplan-Meier curves were not significantly different between the two coreceptor groups and hence did not demonstrate a

difference in the time to clinical disease progression or AIDS death associated with tropism (NS).

F. Survival time after initiating cART until death due to AIDS in participants with consistent cART (≥70% of visits on cART after initiation) stratified by coreceptor type (CCR5 or CXCR4). The Kaplan Meier curves were not significantly different between the two coreceptor groups and hence did not demonstrate a difference in mortality rates associated with HIV-1 tropism (NS by the log-rank test).

G. Time after initiating cART until progression to a new AIDS-defining illness or death due to AIDS in participants with consistent ART (<70% of visits on cART after initiation of therapy) stratified by coreceptor type (CCR5 or CXCR4). The Kaplan-Meier curves were not significantly different between the two coreceptor groups and hence did not demonstrate a difference in the time to clinical disease progression or AIDS death associated with tropism (NS by the log-rank test).

**Table 1.**

Participants' characteristics, morbidity, and mortality in relation to tropism and cART

| **A. Virologic, immunologic, and cART-related profiles** | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Characteristics** | **Participants with Little or No cART ≤3 visits on cART** | | **Participants with Intermittent cART ≥4 and <70% of visits on cART** | | **Participants with Consistent cART ≥70% of visits on cART** | | **P Value** |
| **Number and percentage (%) of participants in each cART category** | 160 (30%) | | 97 (18%) | | 272 (52%) | | |
| **Number and percentage of participants with X4 strains** [a] | 58 (36%) | | 30 (31%) | | 127 (47%) | | 0.01 [b] |
| **Median HIV-1 load at WIHS entry, (log10 copies/mL) [Interquartile Range (IQR)]** | 4.63 [1.21] | | 4.28 [0.97 IQR] | | 4.30 [1.16 IQR] | | 0.0028 [c] |
| **Median CD4 cell count at WIHS entry, cells/mm,3 [IQR]** | 215 [311 IQR] | | 387 [252 IQR] | | 311 [242 IQR] | | 0.002 [d] |
| **Median CD4+/CD8 ratio at WIHS entry, [IQR]** | 0.30 [0.39 IQR] | | 0.47 [0.33 IQR] | | 0.40 [0.32 IQR] | | <0.0001 [e] |
| **Participants who ever achieved complete viral suppression on cART, [Viral load (VL) <80 copies/ mL], Number (%)** | 19 (12%) | | 72 (74%) | | 251 (92%) | | <0.0001 [f] |
| **Mean number of visits on cART with complete viral suppression, (VL<80) [Standard Deviation (SD)]** | 1.2 [0.8 SD] | | 5.6 [5.7 SD] | | 11.9 [8.9 SD] | | <0.0019 [g] |
| **Mortality due to AIDS stratified by cART group, Number (%)** | 77 (48%) | | 22 (23%) | | 30 (11%) | | <0. 001 [h] |
| **B. Morbidity and mortality in relation to tropism and cART** | | | | | | | |
| | **Participants with Little or no cART** | | **Participants with Intermittent cART** | | **Participants with Consistent cART** | | **P value** |
| | **X4** [a] | **R5** [i] | **X4** | **R5** | **X4** | **R5** | |
| **Mortality due to AIDS, stratified by cART group and tropism, Number (%)** | 36(62%) | 41(40%) | 7(23%) | 15(22%) | 9(7%) | 21(14%) | 0.0088 [j], NS [k], NS |
| **Participants who progressed to a new AIDS- defining illness or AIDS death, stratified by cART group and tropism, Number (%)** | 49(85%) | 70(69%) | 20(67%) | 43(64%) | 74(58%) | 87(60%) | 0.037 [l], NS, NS |

cART, combination antiretroviral therapy; X4, HIV-1 strains using the CXCR4 coreceptor; R5, HIV-1 strains using the CCR5 coreceptor; WIHS, Women's Interagency HIV Study.

[a] Participants with X4 strains detected at one or more time point.

[b] Fisher's exact test. The percentage of women with detectable X4 strains in the group with consistent use of cART was higher than expected by chance.

[c] Kruskal-Wallis test. The median HIV-1 RNA load at WIHS entry of participants reporting little or no cART significantly exceeded those of the participants in the two other cART groups.

[d] Kruskal-Wallis test. The median CD4 cell count at WIHS entry differed among the three cART groups, with the lowest counts in the subjects with little or no cART, and the highest in those reporting intermittent cART.

[e] Kruskal-Wallis test. The median CD4/CD8 cell ratio at WIHS entry differed among the three cART groups, with the lowest median ratio in the subjects with little or no cART and the highest in subjects reporting intermittent cART.

[f] Fisher's exact test. The percentage of subjects who achieved complete viral suppression (VL<80) at one or more visits differed among the three cART groups, with the smallest percentage occurring in the participants with little or no cART and the largest in those with consistent cART.

[g] Wilcoxon rank sum and Fisher's exact test. The mean number of visits on cART with complete viral suppression differed among the three cART groups, with the smallest number of visits with VL <80 in the participants with little or no cART and the largest in those with consistent cART.

[h] Fisher's exact test. Mortality rates due to AIDS in the three cART groups differed, with the highest rate in the group with little or no cART and the lowest in those with consistent cART. Outcomes for survival analyses were participants' time from date of the first WIHS visit to AIDS death.

[i] Participants with R5 strains detected exclusively.

[j] Fisher's exact test. P values refer to comparisons of rates of death due to AIDS in participants with X4 strains as compared to those with R5 strains exclusively. In those with little or no cART, participants with X4 strains had a higher mortality rate than those with R5 strains exclusively. The mortality rates in the two other cART groups did not differ when stratified by tropism.

[k] NS, not significant.

[l] Fisher's exact test. P values refer to comparisons of rates of death due to AIDS and new AIDS-defining illnesses, including designated cancers, in participants with X4 strains as compared to those with R5 strains exclusively. In those with little or no cART, participants with X4 strains had significantly higher rates of AIDS death or new AIDS-defining illnesses than those with R5 strains exclusively. The rates of AIDS deaths and new ADI in the two other cART groups did not differ when stratified by tropism.

Primary outcomes for survival analyses were participants' time from date of the first WIHS visit to AIDS death or incident ADI.