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Reduction of HIV Persistence Following Transplantation in HIV Infected Kidney Transplant Recipients

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Abstract

Chronic inflammation may contribute to HIV persistence through a number of potential pathways. We explored the impact of immunosuppressant therapy on peripheral blood measures of HIV persistence following kidney transplantation. Stored plasma and peripheral blood mononuclear cells prior to transplantation and at weeks 12, 26, 52 and 104 post-transplant were obtained from 91 transplant recipients. In a multivariate model, higher pre-transplant plasma HIV RNA level (p<.0001) and a longer duration of follow-up post-transplant (p=0.09) were associated with higher post-transplant plasma HIV RNA levels. A higher baseline HIV DNA (p<.0001) was significantly associated with higher HIV DNA levels post-transplant, while higher CD4+ T cell count (p=0.001), sirolimus use (p=0.04) and a longer duration of follow-up (p=0.06) were associated with lower post-transplant HIV DNA levels. The association between sirolimus exposure and lower frequency of cells containing HIV DNA levels post-transplant suggest that the immune-modifying drugs may affect the level of HIV persistence during effect therapy. Future studies of sirolimus as a reservoir-modifying agent are warranted.

Keywords

Human Immunodeficiency Virus; kidney transplantation; sirolimus

INTRODUCTION

Modern antiretroviral regimens are able to suppress HIV replication and consequent immune system damage. However, these drugs must be taken daily for life, are expensive and often have side effects that make required adherence challenging. Many patients are unable to access or adhere to these drugs indefinitely. Novel curative strategies are now being actively pursued to address these limitations (1). The fact that a single person has apparently been

cured with a hematopoietic stem cell transplant suggests that such an outcome is at least possible (2).

HIV persists at low levels indefinitely despite complete or near complete suppression of HIV replication with therapy (3). Many mechanisms likely account for persistence. Although many groups have focused on the molecular biology of HIV DNA transcription (4, 5), our group has taken a broader approach by focusing on the complex roles that inflammation, immune dysfunction and/or T cell homeostasis might have on the viral reservoir. HIV-associated T cell activation and generalized inflammation often persist during long-term antiretroviral therapy (6, 7). The size and perhaps distribution of the reservoir is associated with frequency of cells expressing markers of activation and dysfunction (e.g., CD38, HLA-DR, Ki67, CCR5 and PD-1) (8, 9). Theoretically, this persistent inflammatory environment might contribute to HIV persistence through at least four independent mechanisms: (a) activating viral replication in latently-infected T cells, (b) increasing the availability of susceptible target cells (making *de novo* infection more likely), (c) inhibiting the function of the adaptive immune system, and/or (d) stimulating the proliferation and expansion of memory T cells harboring replication competent HIV (10). The potential role of immune-based therapeutics that interrupt one or more of these mechanisms as a component of a curative strategy is now recognized as a key research question in the field (1).

Sirolimus (rapamycin) is a naturally occurring macrolide antibiotic that inhibits the mammalian target of rapamycin (mTOR), a key regulatory kinase which controls cell-cycle progression (11). Sirolimus has complex effects on T cell activation and function (12) and blocks progression from the G1 to S phase in activated T cells. In contrast to cyclosporine, the inhibitory effect of sirolimus on cell-cycle progression appears to be limited to T cells that are activated by cytokines and does not occur in T cells activated directly by antigen-TCR engagement (13, 14). Also, in contrast to cyclosporine, MHC class I or class II restricted antigen presentation by dendritic cells is not inhibited by sirolimus, at least *in vitro* (15). In the murine and non-human primate models, sirolimus enhances the formation of T cell memory and antibodies in response to vaccination (16, 17). Moreover, sirolimus appears to enhance T regulatory cell function in humans, which may have both beneficial and harmful effects on immune function (18, 19).

Some have argued that sirolimus may have potential utility in the management of HIV infection (20). Sirolimus reduces CCR5 expression on T cells, which may make them less susceptible to HIV infection (21–23). Dendritic cells exposed to sirolimus *in vitro* failed to make interferon-alpha when exposed to deactivated HIV (24). In a recent pilot study involving seven HIV-infected adults with Kaposi's sarcoma, sirolimus was well-tolerated and resulted in partial remissions of Kaposi's sarcoma in three individuals (25). Sirolimus also blocks the negative effect HIV has on autophagy, which might result in a less favorable environment for HIV replication (26).

Our group has established a prospective cohort assessing the safety and efficacy of liver and kidney transplantation in HIV-infected persons (27). The majority of study participants entered the cohort on a stable fully suppressive antiretroviral drug regimen and remained on

an effective regimen during the transplant and post-transplant periods. Although no standard immunosuppressive regimen was used during the transplantation, most patients received a combination regimen that included cyclosporine, tacrolimus or sirolimus, mycophenolate, and/or prednisone. We performed a retrospective analysis to determine if immunosuppressive therapies result in a reduction in the size of the HIV reservoir, as defined by the level of plasma HIV RNA, cell-associated RNA, and cell-associated DNA ("proviral DNA"). Given the possible beneficial effects that sirolimus may have on the reservoir, we were particularly interested in the effects of this drug on the reservoir relative to other immunosuppressive drugs.

MATERIALS AND METHODS

Cohort

Subjects were identified retrospectively from the "Solid Organ Transplantation in HIV: Multi-Site Study (AI052748)", which is an NIH-funded study of HIV-infected individuals receiving either a kidney or liver transplant. Over 250 HIV-infected adults were enrolled in the parent study. Plasma and PBMCs were collected prior to transplantation ("baseline") and at weeks 12, 26, 52 and 104 post-transplant. Eligible subjects had CD4+ T cell counts 200 cells/mm^3 and an undetectable (<50 copies/mL with the Amplicor Monitor Ultrasensitive PCR assay, or <75 copies/mL with the bDNA Versant version 3.0 assay) plasma HIV RNA level pre-transplant. Post-transplant immunosuppression was required of all subjects to prevent rejection. The decision as to which regimen was used was a clinical decision that was made at the local center, and not standardized across subjects. For this analysis, we identified from this cohort those study participants who were on a stable antiretroviral regimen, and who did not interrupt antiretroviral therapy for more than 3 days in the post-transplant period.

Measurements

Stored plasma and peripheral blood mononuclear cells (PBMCs) at baseline and weeks 12, 26, 52, and 104 were analyzed for plasma HIV RNA levels, cell-associated RNA levels and proviral DNA levels using previously described methods (28–32). We used the isothermal Transcription Mediated Amplification (TMA) (Aptima, Gen-Probe) assay to measure longitudinal plasma HIV RNA levels. This is a nucleic acid-amplification test that has been FDA-approved for the early detection of HIV infection in blood donors and validated for clinical use (33, 34). It is a highly specific and sensitive assay, with a 50% detection limit of 3.6–14 copies RNA/mL when performed in singlicate (35, 36). The sensitivity of the TMA assay is <3 RNA copies/mL when 4 replicates (each testing 0.5 mL of plasma) are performed. The output for the TMA assay is a signal/cutoff (S/Co) ratio (range 0–30), with S/Co <1.0 considered HIV RNA "negative" and S/Co 1.0 considered "positive." In previous studies by our group using this assay, the proportion of subjects with low-level detectable viremia after one year of viral load suppression was 76%–87% and did not change over seven years of viral suppression (31).

We have also recently modified the TMA assay to measure cell-associated RNA (32, 37). As previously described, stored PBMCs were thawed at 37°C and rinsed twice with PBS.

An aliquot of PBMC was then resuspended in 0.5 mL of PBS before the addition of 0.4 mL of lysis buffer containing detergent and denaturing agents as well as target-capture and amplification reagents used for TMA assay (GenProbe, Inc., San Diego, CA). In our recent study of "elite" controllers and long-term antiretroviral treated patients, this assay proved to be very sensitive in detecting residual cell-associated HIV RNA in PBMCs, and demonstrated consistent correlations with host immune response parameters (e.g., T cell activation and HIV-specific immunity) (32, 38). The output for the TMA assay is a signal/cutoff (S/Co) ratio (range 0–30), with S/Co <1.0 considered HIV RNA "negative" and S/Co ≥1.0 considered "positive." All S/Co ratios were normalized to per million CD4+ T cells (derived from the quantitation of human genomic DNA from a parallel real-time PCR amplification targeting a highly conserved region of the DQ-alpha locus, multiplied by the proportion of white blood cells that were CD4+ T cells at each time point).

Proviral DNA was measured using modifications of previously-described methods. HIV gag DNA was amplified and detected using real-time PCR. This assay has an overall sensitivity of 1 copy of HIV DNA/3 μg of input DNA, equivalent to approximately 1 HIV provirus per 450,000 PBMCs. All proviral DNA levels were normalized to per million CD4+ T cells, as above.

Statistical Analysis

Predictors of HIV persistence (which included CD4+ T cell count and exposure to various immunosuppressive drugs as time-dependent covariates) over the two-year period post-transplant were examined using univariate and multivariate linear repeated measures models. Subjects were censored at the time of any virologic failure or graft loss. All variables with $p < 0.1$ from the univariate model were included in an initial multivariate model. For normality of the response variable, $\log_{10}$ (for plasma HIV RNA) and reciprocal (for cell-associated RNA and proviral DNA) transformations were used. Statistical analyses were performed with SAS software, version 9.2 (SAS Institute, Cary, NC, USA); $p < 0.05$ was considered statistically significant.

RESULTS

In the parent study, a total of 150 kidney recipients were enrolled and followed for a median of 3.2 years (interquartile range [IQR] 2.5–4.5) post-transplant. From this group, we selected 91 kidney transplant recipients who did not interrupt antiretroviral therapy for more than 3 days in the post-transplant period, and who had stored plasma and PBMC samples available for analysis. The median age at baseline was 45 years (IQR 40 to 50); 76% were men. The median pre-transplant CD4+ T cell count was 510 cells/mm^3 (IQR 376 to 664). At week 12 post-transplant, 48% of subjects were taking a non-nucleoside reverse transcriptase inhibitor-based regimen (38% on efavirenz and 10% on nevirapine) and 50% were taking a protease inhibitor-based regimen. Immunosuppressive drugs used at week 12 included cyclosporine (21%), tacrolimus (74%), sirolimus (9%), mycophenolate mofetil (73%) and prednisone (76%). Of note, tacrolimus was discontinued in 7 subjects due to toxicity, and ? was replaced with sirolimus. Of 91 subjects, 25 (27%) had anti-thymocyte/lymphocyte globulin (ATG/ALG) induction (all administrations were within 1 week post-transplant).

Excluding any data after a graft loss or virologic failure, 45 subjects had samples available for analysis at all 4 post-transplant time points; 20, 16 and 10 subjects had samples at 3, 2 and 1 time point(s), respectively. Median [IQR] plasma HIV RNA level was 1.0 [0.1–6.3] S/Co at baseline and 2.8 [0.2–7.4] copies S/Co at week 104 post-transplant. Baseline and week 104 proviral DNA levels were 1.8 [1.5–2.9] and 1.8 [1.5–2.6] copies per million CD4+ T cells, respectively. For cell-associated RNA, median [IQR] was 3.2 [2.3–5.8] S/Co per million CD4+ T cells at baseline and 4.4 [2.8–16.6] S/Co per million CD4+ T cells at week 104.

In univariate models, higher baseline plasma HIV RNA levels (per $\log_{10}$ S/Co; p<0.0001), white race (p=0.01), and HCV co-infection (p=0.02) and longer duration of follow-up (per 13 weeks; p=0.09) were associated with increased plasma HIV RNA levels post-transplant. Compared to other drugs, exposure to tacrolimus was associated with decreased plasma HIV RNA levels post-transplant (p=0.03). In the multivariate model, higher baseline plasma HIV RNA level (p<0.0001) and white race (p=0.01) remained significant, while a longer duration of follow-up post-transplant (p=0.09) was marginally significant (Table 1). HCV co-infection and tacrolimus exposure lost their significance in the multivariate model.

In univariate models, higher baseline HIV DNA levels (p<0.0001), lower post-transplant CD4+ T cell count (p=0.03), and cyclosporine use (p=0.09) were associated with increased HIV DNA levels post-transplant, while sirolimus use (p=0.02) and longer duration of follow-up (p=0.09) were associated with decreased levels. In the multivariate model, higher baseline proviral HIV DNA (p0<0.0001) and lower CD4+ T cell count (p=0.001) were associated with increased proviral HIV DNA levels post-transplant, while sirolimus use (p=0.04) and longer duration of follow-up post-transplant (p=0.06) were associated with decreased proviral DNA levels (Table 2).

No significant predictors of cell-associated RNA post-transplant were identified in the multivariate model (data not shown).

DISCUSSION

Although ART reduces HIV replication and HIV-associated inflammation and reverses HIV-associated immunodeficiency, these effects are often incomplete. T cell activation decreases during ART but rarely returns to normal (39). Higher levels of T cell activation (as defined by CD38 and HLA-DR) and dysfunction (as defined by PD-1 expression) have been associated with higher levels of persistent cell-associated HIV DNA and/or RNA (but not necessarily plasma HIV RNA) in long-term treated adults (40). Based in part on these observations, we predicted that the use of immunosuppressive therapy might affect HIV persistence in treated individuals. In order to justify future studies of potentially harmful interventions, we explored the impact of solid-organ transplantation—where potent immunomodulatory drugs are required—on HIV persistence in a large well-characterized cohort. We found that in general, a longer duration of observation post-transplant was associated with small increases in plasma HIV RNA and decreases in HIV DNA in PBMCs. These data suggest that transplant procedure or (more likely) the immune-modifying drugs

required to prevent transplant rejection might have altered T cell biology, leading to activation of the latent reservoir and production of virus.

We were primarily interested in the relative effect of sirolimus (rapamycin) on reservoir size as this drug inhibits T cell proliferation, reduces CCR5 expression and enhances antigen-specific memory T cell formation. Although only a small proportion of the cohort was exposed to this drug, we found that the use of sirolimus was independently associated with lower HIV DNA levels. Theoretically, sirolimus may be reversing HIV latency (causing HIV production and perhaps leading to a decline in HIV DNA), or may be having potentially beneficial effects on CD8+ T cell function (as predicted by non-human primate studies (16)) or reducing T cell homeostasis.

There is no standard method for quantifying the size of the reservoir during long-term antiretroviral therapy. The virus outgrowth assay provides a quantitative assessment of the frequency of cells containing replication-competent virus, but such assay are cumbersome, require large amounts of blood, cannot be easily performed retrospectively, and only detect a small and variable proportion of the total replication-competent virus population (41). Given the lack of a gold-standard, we applied three approaches now being widely used in studies of the reservoir: plasma HIV RNA, cell-associated HIV RNA, and cell-associated HIV DNA. Our group has extensively validated the TMA approach for quantification of HIV RNA in plasma and cells, although we recognized these assays are semi-quantitative, have limited range and have not been widely used in the field. The largely negative results with our RNA-based assays should be considered in the context of these limitations. Given our interest in the impact of sirolimus on T cell proliferation and activation, quantitative measures of cell-associated HIV DNA might be considered optimal, at least for studies such as the one we performed. These assays are sensitive, highly reproducible and provide an overall assessment of the total body burden of infected cells. Immune-based therapeutics that might affect cell proliferation and/or result in clearance of the infected cell population would be expected to reduce the frequency of circulating cells which harbor HIV DNA, as we observed in this exploratory study.

This study has several important limitations. The cohort was not designed to study the impact of immune-based therapeutics on HIV persistence and hence, biologic specimens were not optimally collected. Future controlled studies should look at other measures of the reservoir, including levels of replication-competent HIV and rates of infection in resting memory CD4+ T cells. In addition to the potent immunosuppressive therapies, all subjects received a kidney transplant, which might have influenced outcomes. Finally, the use of the immunosuppressive regimens was not controlled. There may be unmeasured confounding issues that lead to preferential use of sirolimus—which was the focus of our analysis— which contributed to the observed changes in reservoir size. Despite these limitations, we believe our data, as well as recent clinical experience (25) support a controlled clinical trial assessing the impact of sirolimus on immune function and HIV persistence during effective antiretroviral therapy.

Acknowledgments

Abbreviations

HIV	human immunodeficiency virus
mTOR	mammalian target of rapamycin
HCV	hepatitis C
ART	antiretroviral therapy
TMA	Transcription Mediated Amplification

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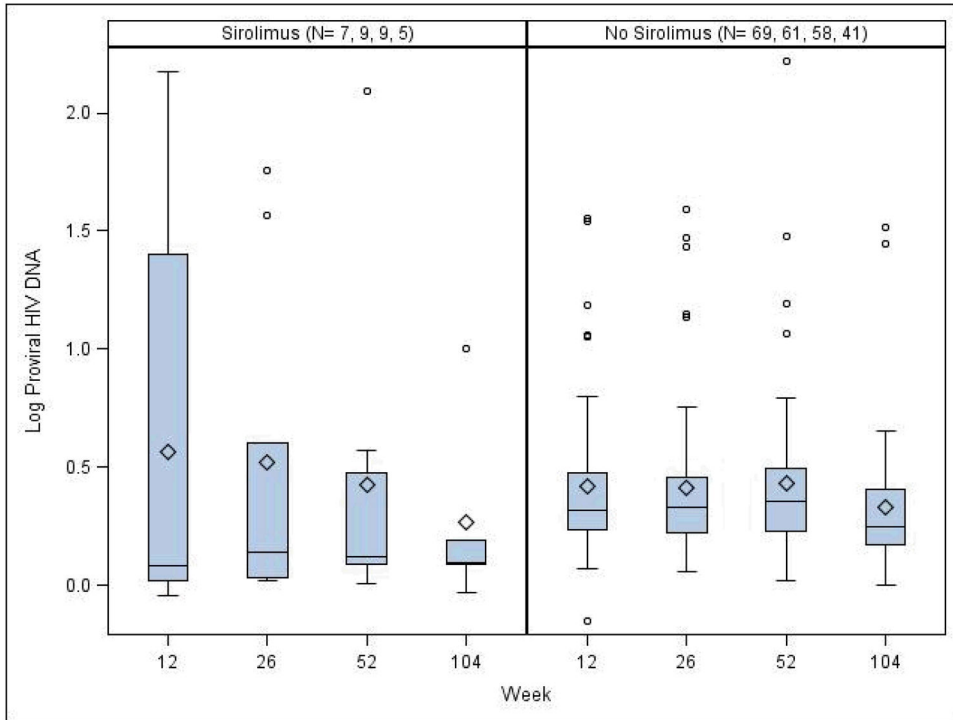
Figure 1. Proviral HIV DNA Levels over Time by Sirolimus Exposure

The level of HIV DNA in PBMCs was measured. The frequency of infection was normalized to the predicted frequency of CD4+ T cells within the PBMCs. Exposure to sirolimus was associated with lower cell-associated HIV DNA levels post-transplant compared to other immunosuppressive drugs after adjustment for baseline HIV DNA level, time post-transplant and CD4+ T cell count. Note that 15 subjects with sirolimus use at 30 time points are reflected in this figure: 7 subjects contributed a single time point, 3 subjects contributed 2 time points, 3 subjects contributed 3 time points and 2 subjects contributed 4 time points.
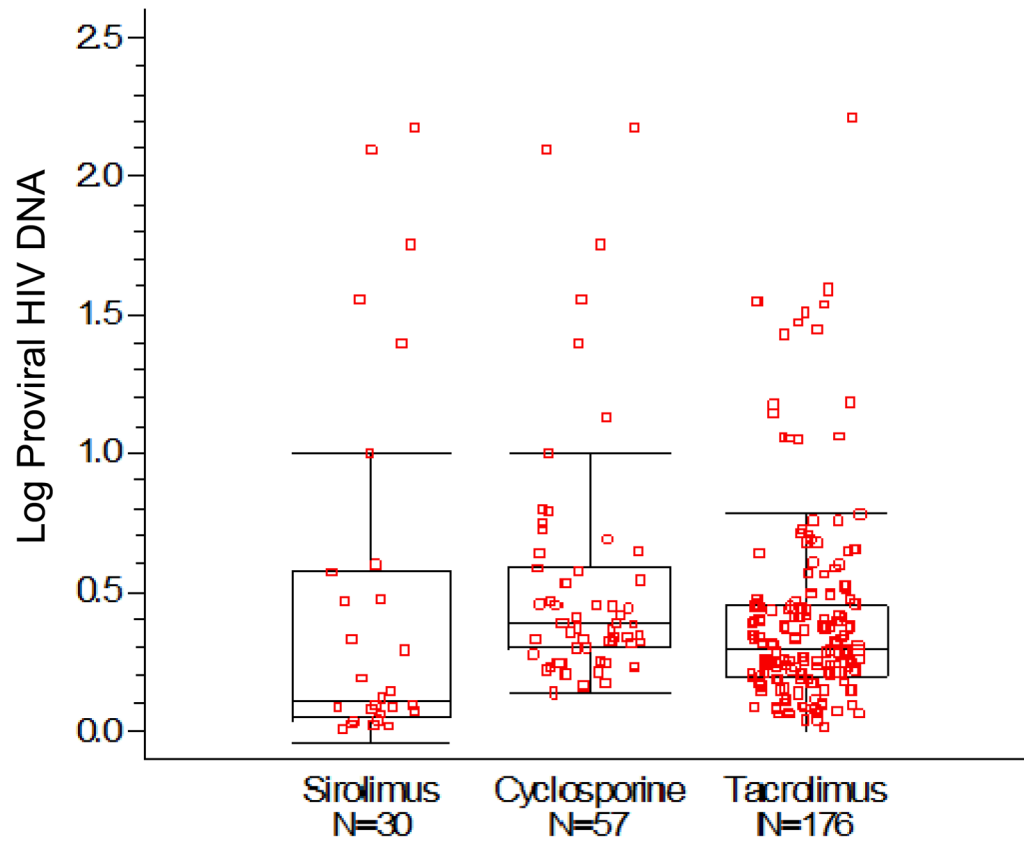
Figure 2. Differential impact of immunosuppressive therapy on Proviral HIV DNA levels post-transplant
Log Proviral HIV DNA Levels by Sirolimus Use Post-Transplant. The level of cell-associated DNA at any time point post-transplant was lower in patients on sirolimus than in those receiving cyclosporine or tacrolimus.

Table 1

Linear Repeated Measures Models for Log_{10} Plasma HIV RNA Levels

Univariate Predictor	Estimate	P Value
Age (per year)	0.0021	0.73
Gender (Male)	0.0974	0.52
Race (White)	0.3645	0.01
Nadir CD4+ T cell Count (per 50 cells/μL)	−0.0178	0.36
Baseline CD4+ T cell Count (per 50 cells/μL)	−0.0053	0.68
HCV Infection Status (Antibody Positive)	−0.3336	0.02
Baseline Plasma HIV RNA (log_{10} S/Co)	0.4277	<.0001
ATG/ALG Induction	0.04710	0.74
CD4+ T cell Count (per 50 cells/μL)[*]	0.0030	0.76
Tacrolimus[*]	−0.2833	0.03
Cyclosporine[*]	0.2146	0.24
Mycophenoalate use[*]	−0.0993	0.43
Sirolimus use[*]	0.0830	0.64
Prednisone use[*]	−0.0568	0.70
Time (per 13 weeks)[*]	0.0367	0.09

Multivariate Predictors	Estimate	P Value
Baseline Plasma HIV RNA (log_{10} S/Co)	0.4024	<.0001
Race (White)	0.3079	0.01
Time (per 13 weeks)[*]	0.0324	0.09

[*] Time-varying covariate

Table 2

Linear Repeated Measures Models for Reciprocal Proviral HIV DNA Levels

Univariate Predictor	Estimate	P Value
Age (per year)	−0.0027	0.23
Gender (Male)	−0.0228	0.67
Race (White)	−0.0662	0.17
Nadir CD4+ T cell Count (per 50 cells/μL)	0.0014	0.81
Baseline CD4+ T cell Count (per 50 cells/μL)	0.0024	0.59
HCV Infection Status (Antibody Positive)	−0.0251	0.63
Baseline Proviral HIV DNA (reciprocal, copies per million CD4+ T cells)	0.5044	<.0001
ATG/ALG Induction	−0.0156	0.76
CD4+ T cell Count (per 50 cells/μL)[*]	0.0058	0.03
Tacrolimus[*]	−0.0214	0.53
Cyclosporine[*]	−0.1182	0.09
Mycophenoalate use[*]	0.0569	0.12
Sirolimus use[*]	0.1222	0.02
Prednisone use[*]	0.0290	0.50
Time (per 13 weeks)[*]	0.0099	0.09
Multivariate Predictors	**Estimate**	**P Value**
Baseline Proviral HIV DNA (reciprocal, copies per million CD4+ T cells)	0.4974	<.0001
CD4+ T cell Count (per 50 cells/μL)[*]	0.0077	0.001
Sirolimus use[*]	0.0869	0.04
Time (per 13 weeks)[*]	0.0093	0.06

[*] Time-varying covariate