Title

Virologic and immunologic effects of adding maraviroc to suppressive antiretroviral therapy in individuals with suboptimal CD4+ T-cell recovery

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Virologic and immunologic effects of adding maraviroc to suppressive ART in subjects with suboptimal CD4+ T-cell recovery

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Abstract

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Background—Combination antiretroviral therapy (ART) suppresses HIV-1 replication, but does not restore CD4+ T-cell counts in all subjects. To investigate the effects of maraviroc on HIV-1 persistence and the relations between virologic and immunologic parameters in subjects with incomplete CD4+ T-cell recovery, we performed a prospective, open-label pilot trial in which maraviroc was added to a suppressive ART regimen for 24 weeks.

Design—A5256 was a single-arm trial in which subjects on suppressive ART with incomplete CD4+ T-cell recovery added maraviroc for 24 weeks.

Methods—We quantified low-level, residual viremia in plasma and total HIV-1 DNA and 2-LTR circles in peripheral blood mononuclear cells before and after maraviroc intensification. We also evaluated markers of CD4+ and CD8+ T-cell immune activation (%CD38+HLA-DR+) and apoptosis (%caspase3+/Bcl-2−).

Results—No effect of maraviroc was found on the probability of detectable plasma viremia (≥ 1 copy/mL; n=31, exact McNemar p=1.0) or detectable 2-LTR circles (n=28, p=0.25) or on total HIV-1 DNA (n=28, 90% confidence interval: −0.1, +0.3 $\log_{10}$ copies/10^6 CD4+ T-cells). Pre-maraviroc HIV-1 DNA levels were inversely related to pre-maraviroc %CD38+HLA-DR+ CD4+ T-cells (Spearman=−0.52, p=0.004), and lower pre-maraviroc HIV-1 DNA levels were associated with larger decreases in %CD38+HLA-DR+ CD4+ T-cells during maraviroc intensification (Spearman=0.44, p=0.018).

Conclusions—In subjects on suppressive ART with incomplete CD4+ T-cell recovery, maraviroc intensification did not affect measures of HIV-1 persistence but did decrease persistent CD4+ T-cell immune activation especially in subjects with low pre-intensification levels of HIV-1 DNA.

Introduction

Antiretroviral therapy (ART) for HIV-1 infection rapidly suppresses levels of plasma viremia to levels undetectable by clinical assays (HIV-1 RNA <50 copies per milliliter) [1,2]. In the majority of subjects, suppressing viral replication with ART correspondingly increases CD4+ T-cell counts [3–5]. However, in a subset of subjects known as immunological non-responders, CD4+ T-cell levels do not recover, leaving these subjects at greater risk of opportunistic infections and mortality [6–11]. A meta-analysis of 46 treatment groups from 17 phase 2–3 clinical studies showed that CCR5 antagonists increased CD4+ T-cell counts more than comparator regimens independently of viral suppression [12], suggesting that maraviroc may improve CD4+ T-cell counts in immunologic non-responders to ART.

We previously reported, however, that maraviroc intensification for 24 weeks in AIDS Clinical Trials Group (ACTG) study A5256 did not increase in CD4+ T-cell counts in the periphery, but did lower levels of immune activation and apoptosis in both the CD4+ and

CD8+ T-cell compartments [13]. Other studies with maraviroc have observed trends towards decreased immune activation in CD4+ T-cells [14], CD8+ T-cells [15] or CD4+ and CD8+ T-cells [16]. In HIV-1 uninfected patients receiving hematopoietic stem cell transplantations for cancer, maraviroc limited visceral graft versus host disease, suggesting that blockade of CCR5 can inhibit lymphocyte trafficking [17]. However, a placebo controlled study by Hunt et al. found increased levels of HLA-DR+ CD8+ T-cells in the periphery, and increased levels of HLA-DR+ CD4+ and CD8+ T-cells in rectal tissue during maraviroc intensification [18]. Interestingly, changes in low-level plasma viremia were not observed despite the changes in immune activation. In addition, a study by Gutierrez et al. also found a transient increase in 2-LTR circles despite a decrease in immune activation [16], although this effect was not observed in a different study [15].

These divergent findings indicate that the impact of maraviroc intensification on immune activation and residual plasma viremia, and the relationships between measures of viral persistence and immune activation and apoptosis in the context of maraviroc intensification remain incompletely understood. To more thoroughly evaluate the impact of maraviroc intensification on virologic and immunologic parameters in subjects with stable but suboptimal CD4+ T-cell recoveries on suppressive ART, we longitudinally measured residual plasma viremia, total and 2-LTR HIV-1 DNA in PBMC and levels of immune activation and apoptosis in CD4+ and CD8+ T-cells in patients receiving maraviroc in ACTG study A5256. We also evaluated relationships between these parameters to gain further insight into the impact of maraviroc intensification.

Methods

Study Design and Patient Enrollment

The study design and patient enrollment for ACTG A5256 have been previously reported [13]. Briefly, A5256 was a prospective multicenter 48-week single-arm open-label trial in which HIV-1 infected subjects with plasma viremia (HIV-1 RNA) suppressed on ART and suboptimal CD4+ T-cell recovery (defined as <250 cells/μL and a slope of annual change in the CD4+ T-cell count from −20 to +20 cells/μL during the past year) intensified their ART regimen by adding maraviroc (Pfizer) for 24 weeks; maraviroc was then stopped and pre-intensification ART was continued for an additional 24 weeks. Subjects had 2 assessments prior to maraviroc intensification (pre-entry and week 0) and then provided samples at 4, 8, 12, 16, 22 and 24 weeks after maraviroc intensification, followed by sampling at 12, 22 and 24 weeks after maraviroc discontinuation (study weeks 36, 46, and 48). Plasma HIV-1 RNA was quantified by an FDA-approved assay at each visit to confirm viral suppression. The primary endpoint in A5256 was the change in CD4+ T-cell counts from the average of two pre-maraviroc measurements to the average of CD4+ T-cell counts from week 22 and week 24 during maraviroc plus ART.

Quantification of Residual Plasma Viremia

Stored plasma samples from the two pre-maraviroc timepoints and study weeks 12, 22, 24 and 36 were used to assess residual plasma viremia using a previously described assay with single copy sensitivity (SCA) [19]. Briefly, plasma was thawed and an internal standard

(replication competent avian sarcoma virus, RCAS) was spiked into the plasma. Virions were then pelleted by centrifugation at $170,000 \times g$ for 30 minutes and nucleic acid was isolated and resuspended in 55 μL of 5 mM Tris-HCl supplemented with 1 μM DTT and 1000 units of recombinant RNasin. A two-step quantitative real-time PCR (qRT-PCR) assay was then used to quantify levels of HIV-1 RNA and the internal standard RCAS from the isolated nucleic acid, as described previously [19]. A control assay without reverse transcriptase was included with each extraction to confirm the absence of HIV-1 DNA. The limit of detection for the single copy assay (SCA) is based on the input volume of plasma. With a typical volume of 3.0 milliliters of plasma, the limit of detection is 0.6 copies per milliliter of plasma.

Quantification of Total and 2-LTR Circle HIV-1 DNA

To quantify total and 2-LTR HIV-1 DNA, nucleic acid was isolated from cryopreserved peripheral blood mononuclear cells (PBMC) and qRT-PCR assays were performed as described previously [20]. Briefly, PBMC were thawed and nucleic acid was isolated using the Qiagen FlexiGen kit for total HIV-1 DNA and the Q1Aprep Spin Miniprep Kit for 2-LTR circles [21,22]. Separate qPCR assays using previously established primers and probes for total HIV-1 DNA [23] and 2-LTR circles [20] were used to evaluate cellular levels of HIV-1 DNA. A total of 12.5 μL of nucleic acid extract (equivalent to 6.25×10^5 PBMC, as determined by Quant-iT PicoGreen dsDNA kit [24]) was added to 37.5 μL of TaqMan Platinum Quantitative PCR Super Mix-UDG with Rox qRT-PCR cocktail for each assay [20]. Levels of total and 2-LTR HIV-1 DNA in this study were evaluated at 2 pre-maraviroc time points, and week 12 and week 24 post-maraviroc. All qRT-PCR was performed using the Applied Biosystems 7900 Real-time PCR platform with appropriate positive and negative controls. Total HIV-1 DNA values below the limit of quantification were imputed to a value of 10 copies/10^6 PBMC. Samples with HIV-1 DNA below the limit of detection were infrequent (4 samples at study pre-entry, 1 at entry, 2 at week 12, and 1 at week 24, from a total of 5 unique donors). To determine the amount of HIV-1 DNA or 2-LTR circles per 10^6 CD4+ T-cells, the levels of HIV-1 DNA per 10^6 PBMC were normalized using the percent CD4+ T-cells from that timepoint.

Immunological Assessments

Levels of immune activation and apoptosis within the T-cell compartment were assessed at the 2 pre-maraviroc timepoints and weeks 12, 22, 24, 36, 46 and 48 as previously reported [13]. Peripheral blood samples were collected in heparinized vacutainer tubes and shipped overnight to a central processing lab where PBMC were isolated by density gradient centrifugation and immunologic measurements were performed. CD4+ and CD8+ T-cell activation was evaluated by measuring the frequency of either CD3+CD4+ or CD3+CD8+ cells that were CD38+HLA-DR+. Cells were fixed in 2% formaldehyde and evaluated within 24 hours. The frequency of CD4+ and CD8+ T-cells that were negative for the intracellular marker Bcl-2 or positive for caspase-3 were quantified by performing surface staining and then fixing and permeabilizing the cells to evaluate the extent of apoptosis within the T-cell compartment. All cells were analyzed on an LSR II Flow Cytometer (BD) using FACS Diva software, v.6.1.1. Analysis of flow data was performed using FlowJo software, v.8.8.6 (Tree Star).

Statistical Analysis

Change in residual viremia ($</$ 1 copy/mL) and 2-LTR circles (positive/negative) over 24 weeks of maraviroc intensification were assessed with an exact McNemar's test. In the event of repeated measures at a given timepoint (e.g., two pre- and two post- maraviroc timepoints for residual viremia), detectability was defined based on detection at either timepoint. The median change in total HIV-1 DNA ($\log_{10}$ copies/10^6 CD4+ T-cells) and accompanying exact Hahn and Meeker 90% confidence interval (CI) were estimated, where the 2 pre-maraviroc values were averaged. Only subjects with results available for both pre-maraviroc and the week 24 timepoint on maraviroc were included. Associations between total HIV-1 DNA and markers of CD4+ and CD8+ T-cell immune activation and apoptosis were evaluated using Spearman's rank correlations. Relationships between pre-maraviroc covariates or change after maraviroc intensification and pre-maraviroc 2-LTR circles and SCA results were evaluated with exact Wilcoxon rank-sum test or Kruskal-Wallis test where appropriate.

Results

Study Population

A total of 34 subjects were enrolled in A5256. Baseline characteristics of this study population have been described previously [13]. Of the 34 subjects, 32 were male (94%). Study participants had a median age of 50 (interquartile range: 47 to 55) years and a median duration of HIV-1 RNA suppression of 3 years (Q1–Q3: 1.8–4.5). The median CD4+ T-cell count at baseline was 153 (Q1–Q3: 119–203) cells/μL, and the median CD8+ T-cell count at baseline was 559 (Q1–Q3: 416–872) cells/μL. Longitudinal plasma HIV-1 RNA testing with an FDA-approved assay showed that 2 subjects (6%) experienced virologic failure (2 consecutive measurements of HIV-1 RNA levels at or above the limit of detection of the assay used) during maraviroc intensification and discontinued maraviroc. These 2 subjects were excluded from further analysis, as well as one subject with a viral rebound at week 24.

Effect of Maraviroc on Residual Plasma Viremia

Table 1 and Figure 1 show residual viremia as measured by SCA during maraviroc intensification through 12 weeks post maraviroc. Overall, 48% of subjects had detectable residual viremia (HIV-1 RNA 1 copy/mL) at either of the pre-maraviroc timepoints and likewise at week 22/24; 7 of 31 subjects never had detectable viremia. There was no apparent difference in the probability of detectable residual viremia between pre-maraviroc timepoints and week 22/24 after maraviroc intensification (p=1.0); 74% of subjects had concordant outcomes (i.e., had detectable residual viremia at both pre-maraviroc and week 22/24 or had no detectable virus at both timepoints). Among subjects with detectable residual viremia, median levels of residual viremia were 2.9 and 2.4 copies per milliliter of plasma pre-maraviroc versus 4.9 and 3.9 copies per milliliter after 22 and 24 weeks of maraviroc dosing. Twelve weeks after stopping maraviroc, 43% of subjects had detectable viremia, with a median level of 4.3 copies per milliliter of plasma. When comparing week 22/24 versus week 36, no difference in the probability of detectable residual viremia was found (67% of subjects with concordant outcomes, p=0.75).

Effect of Maraviroc on Total and 2-LTR Circle HIV-1 DNA

The median HIV-1 DNA level at pre-maraviroc was 4.3 (Q1–Q3: 4.0–4.6) $\log_{10}$ copies per 10^6 CD4 cells, compared with 4.3 (Q1–Q3: 4.1, 4.7) and 4.4 (Q1–Q3: 3.8–4.8) $\log_{10}$ copies per 10^6 CD4 cells after 12 and 24 weeks of maraviroc intensification, respectively (Table 2). The median change in total HIV-1 DNA from pre-maraviroc to week 24 was +0.2 $\log_{10}$ copies of HIV-1 DNA per 10^6 CD4 cells (90% CI: −0.1, +0.3).

2-LTR circles were not detected in 82% of subjects during 24 weeks of maraviroc intensification; 14% of subjects had detectable 2-LTR circles at either of the pre-maraviroc timepoints and 4% at week 24 (Table 3). Comparing pre-maraviroc to week 24, there was no evidence of a difference in the probability of detectable 2-LTR circles (p=0.25); 89% of subjects had concordant outcomes (i.e., detectable 2-LTR circles at both pre-maraviroc and week 24 or had no detectable 2-LTR circles at both timepoints).

Relationships between Virologic and Immunologic Parameters

A strong inverse correlation was found between pre-maraviroc levels of total HIV-1 DNA and pre-maraviroc levels of cellular immune activation in CD4+ T-cells as measured by percentage of CD38+HLA-DR+ (Figure 2A; rho= −0.52, p=0.004). The relationship between total HIV-1 DNA and immune activation in CD4+ T-cells was qualitatively similar but not statistically significant at week 24 following maraviroc treatment (rho= −0.34, p=0.07). Conversely, there was no relationship found between levels of cellular activation on CD8+ T-cells and total HIV-1 DNA levels (Figure 2B; rho= −0.14, p=0.482). There was evidence of an inverse correlation between levels of apoptotic CD4+ T-cells (percentage of Bcl-2−) and the level of HIV-1 DNA (Figure 2C, rho= −0.36, p=0.057); however, this association was not seen for percentage of caspase3+ CD4+ T-cells (data not shown), or between pre-maraviroc levels of apoptotic CD8+ T-cells and HIV-1 DNA levels.

Following the addition of maraviroc for 24 weeks, the levels of cellular activation, as measured by CD38+HLA-DR+, decreased 1.3% (90% CI: −1.8% to −0.3%) on CD4+ T-cells and decreased 1.4% (90% CI: −3.0% to −0.3%) on CD8+ T-cells [13]. The change in %CD38+HLA-DR+ CD4+ cells was positively correlated with pre-maraviroc levels of total HIV-1 DNA (Supplemental figure 1A; rho=0.44, p=0.018), reflecting that subjects with the lowest pre-maraviroc HIV-1 DNA levels had the highest pre-maraviroc CD4+ immune activation and largest decreases following maraviroc intensification. An association between changes in %CD38+HLA-DR+ CD8+ cells and pre-maraviroc levels of total HIV-1 DNA was not apparent (Supplemental figure 1B; rho=0.23, p=0.240). Additionally, no association was seen between pre-maraviroc covariates (i.e., age, CD4+, CD8+, immune activation) or change after maraviroc intensification and pre-maraviroc detectability of residual viremia or detectability of 2-LTR circles (data not shown).

Discussion

In this study, we evaluated the impact of maraviroc ART intensification in patients with suboptimal CD4+ T-cell recovery despite suppressive ART. We did not observe an effect of maraviroc intensification on the levels of residual viremia in our small cohort of patients,

which is consistent with the results of previous ART intensification studies with antiretrovirals from other drug classes including protease inhibitors, non-nucleoside RT inhibitors and integrase inhibitors [18, 25–28]. A report by Madrid-Elena et al. also found no change in residual viremia following maraviroc administration, but did show that maraviroc upregulated NF-κB signaling *in vivo* and increased levels of cellular HIV-1 RNA [29], suggesting that maraviroc may have at least partial agonist activity despite being regarded as a CCR5 antagonist [30]. However, these findings are in contrast to a recent report demonstrating that maraviroc intensification does not affect gene expression in CD4+ T-cells [31].

There was no observed change in total or 2-LTR HIV-1 DNA in PBMC following maraviroc intensification. Unchanged levels of HIV-1 DNA are consistent with stability of HIV-1 DNA in subjects on long-term ART [32]. With regard to 2-LTR circles, Guiterrez et al. reported a statistically significant increase in 2-LTR circle detection after 12 and 24 weeks of maraviroc intensification in 9 subjects on suppressive ART [16]. The authors proposed that the increase in 2-LTR circle detection may be the result of a partial CCR5 agonist activity of maraviroc or from increased availability of cytokines following CCR5 blockade, leading to greater virus production and replication. In our study, 2-LTR detection was rare and did not appear related to maraviroc intensification, arguing against increased virus production. Consistent with our findings, Lafeuillade et al. rarely detected 2-LTRs and did not find an increase in 2-LTR circles during maraviroc intensification [15].

When evaluating relationships between virologic and immunologic measurements prior to maraviroc intensification, we found a strong inverse correlation between percentage of HLA-DR+CD38+ CD4+ T-cells and levels of total HIV-1 DNA. This inverse correlation contrasts with the modest positive correlation between total HIV-1 DNA and immune activation reported by Hatano et al. (rho = 0.16, p = 0.057) [33]. The reason for this discrepancy is unknown, but differences in the patient groups studied (immunologic non-responders in the current study versus mostly immunologic responders in the other study [33], see explanation below) and lack of standardized immunophenotyping assays between laboratories may have contributed. The overall higher level of immune activation in subjects with suboptimal CD4+ T-cell recovery [34,35] enrolled in A5256 may have also contributed to the observed differences. Future studies of maraviroc should carefully consider standardization of immunophenotyping and selection of patients by time on ART and CD4+ T-cell count to more clearly elucidate the effects of maraviroc intensification on virologic persistence and levels of immune activation.

In untreated HIV-1 infection, the levels of immune activation are correlated with apoptosis in CD4+ T-cells [36], and both immune activation and apoptosis are reduced following suppression of viral replication on ART [37]. In the current study, we found evidence to suggest greater pre-maraviroc CD4+ T-cell apoptosis (%Bcl-2−) in subjects with lower pre-maraviroc levels of HIV-1 DNA (and correspondingly higher levels of CD4+ T-cell activation). The increased apoptosis observed may be driven by persistently higher levels of immune activation in immunologic non-responders [35], leading to greater cell death and lower HIV-1 DNA levels. The accelerated death of HIV-1 infected cells is likely to be accentuated by the characteristically high levels of T-cell turnover and failure of

homeostatic proliferation present in immunological non-responders [34,38]. The blockade of ongoing viral replication with stable, suppressive ART would prevent the generation of new HIV-1 infected cells, resulting in the lower HIV-1 DNA levels observed among individuals with higher T-cell activation and turnover.

We also found that lower pre-maraviroc levels of HIV-1 DNA were associated with higher levels of pre-maraviroc CD4+ T-cell immune activation and greater reductions in CD4+ T-cell immune activation following maraviroc intensification. As noted previously [13], we did not observe increases in CD38+HLA-DR+ CD8+ T-cells following maraviroc intensification as reported by Hunt et al. [18]. The explanation for these conflicting results remains elusive, but may have been caused by differences in sample processing between the studies, as discussed previously [13]. Other studies investigating the impact of maraviroc on immune activation have generally observed trends towards reduced CD4+CD38+ T-cells [14, 39–40] or no change in T-cell activation phenotype [30]. A decrease in CD4+ T-cell activation following maraviroc intensification in those with higher levels of baseline activation could result from blockade of CCR5-mediated trafficking to effector sites (e.g., lymph nodes, gut mucosa), where T-cells would encounter cognate antigens and become activated. Preventing the interaction of CD4 cells with antigen-presenting cells could lead to lower levels of CD4+ T-cell activation in the periphery, though this hypothesis requires further study.

In conclusion, our results are consistent with full suppression of viral replication with standard ART in that maraviroc did not have an effect on low-level viremia, 2-LTR HIV-1 DNA, or the number of HIV-1 infected cells. Although maraviroc has an established role in the prevention of graft versus host disease by preventing the trafficking of T-cells to visceral sites [17], its effects on T-cell activation in the context of HIV-1 infection are still unclear. Overall, the effects of maraviroc on T-cell activation in well-controlled HIV-1 infection appear to be modest and variable across studies [13–15, 18, 31, 39–40], indicating limited potential for reducing chronic immune activation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
The impact of maraviroc intensification on low level plasma viremia. Levels of plasma viremia were quantified by the single copy assay (SCA) at pre-entry, at maraviroc intensification (week 0) and 12, 22 and 24 weeks of maraviroc intensification and 12 weeks after maraviroc discontinuation. The limit of detection was 1 copy/mL (gray dashed line).

Figure 2.
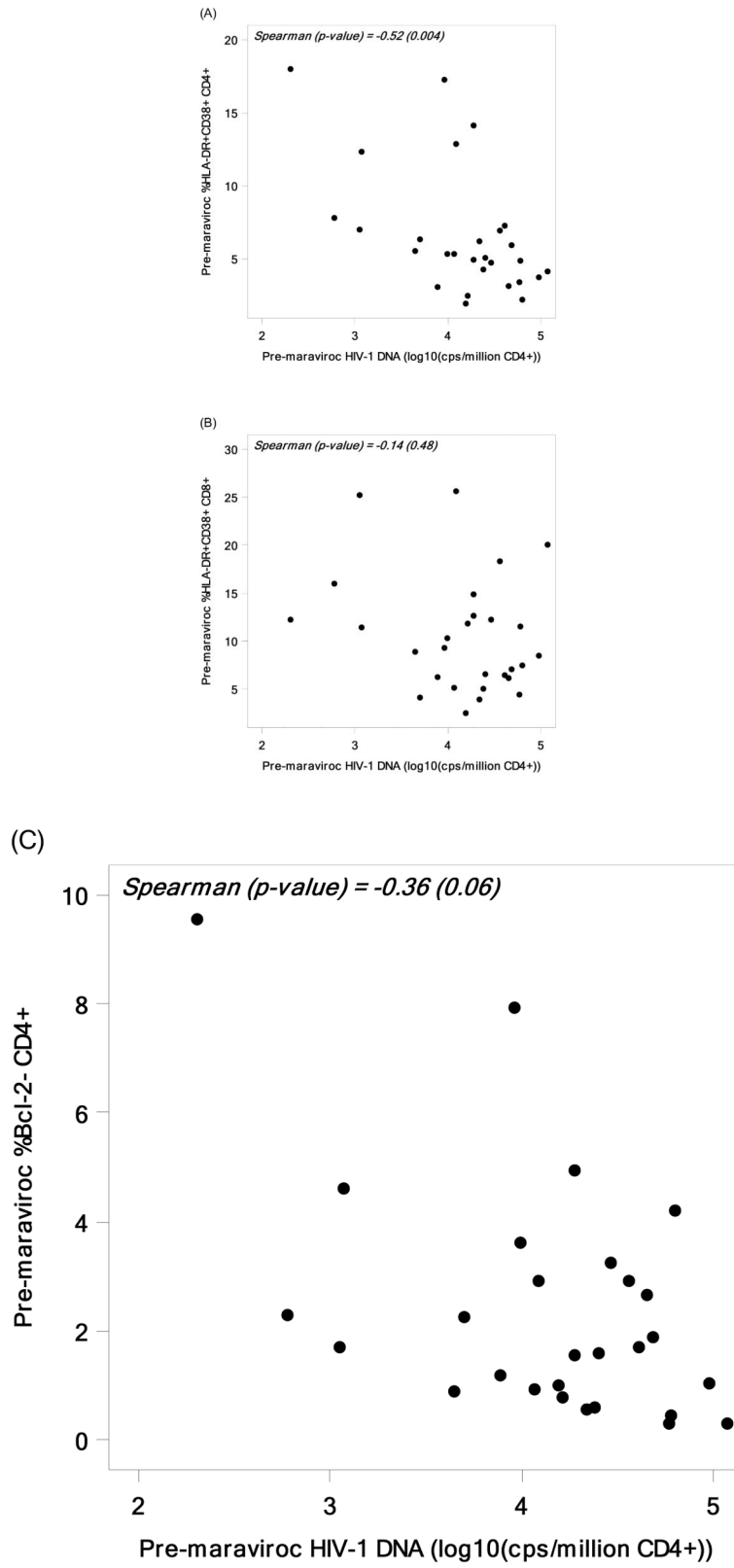
Relationships between total HIV-1 DNA levels and immune activation and apoptosis in CD4+ and CD8+ T-cells prior to maraviroc intensification. Correlations between activated (%CD38+HLA-DR+) CD4+ T-cells (A) and activated CD8+ T-cells (B) with pre-maraviroc HIV-1 DNA ($\log_{10}$ copies/10^6 CD4+ T-cells). C) Relationship between apoptotic (Bcl-2−) CD4+ T-cells and pre-maraviroc HIV-1 DNA.

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Table 1

Residual Plasma Viremia by Study Week

		Study Week						
		Pre-maraviroc		Maraviroc				Post-maraviroc
		pre-entry	0	12	22	24		36
		N=31	N=31	N=30	N=31	N=29		N=30
SCA < 1 copy/mL		20 (65%)	21 (68%)	17 (57%)	22 (71%)	15 (52%)		17 (57%)
SCA ≥ 1 copy/mL		11 (35%)	10 (32%)	13 (43%)	9 (29%)	14 (48%)		13 (43%)
Levels of Virus in Plasma (≥ 1 copy/mL)	Median	2.9	2.4	1.8	4.9	3.9		4.3
	Q1, Q3	2.4, 7.3	1.6, 5.9	1.2, 4.9	1.8, 13	1.8, 6.7		2.2, 6.1
	Min, Max	1.2, 11	1.2, 7.9	1.2, 13	1.2, 24	1.2, 12		1.1, 37
SCA ever ≥ 1 copy/mL		15 (48%)			15 (48%)			

Table 2

Total HIV-1 DNA Levels by Study Week

		Study Week		
		0[*]	12	24
		N=29	N=27	N=28
Log10 (HIV-1 DNA Copies per 10^6 CD4+ T cells)	Median	4.3	4.3	4.4
	Q1, Q3	4.0, 4,6	4.1, 4.7	3.8, 4.8
	Min, Max	2.3, 5.1	1.9, 5.2	2.2, 5.1

[*]Calculated as the average of the two pre-maraviroc results

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Table 3

2-LTR Circles by Study Week

		Study Week				
		Pre-maraviroc		Maraviroc		
		pre-entry	0	12	24	
		N=27	N=29	N=27	N=28	
Negative 2-LTR circles		24 (89%)	28 (97%)	25 (93%)	27 (96%)	
Positive 2-LTR circles		3 (11%)	1 (3%)	2 (7%)	1 (4%)	
Positive 2-LTR copies/10⁶ PBMC	Min, Max	5, 26	59, 59	2, 3	11, 11	
Positive 2-LTR copies/10⁶ CD4+ cells	Min, Max	24, 200	738, 738	13,33	153, 153	
Ever positive 2-LTR circles		4 (14%)		1 (4%)		