Relationship of HIV Reservoir Characteristics with Immune Status and Viral Rebound Kinetics in an HIV Therapeutic Vaccine Study

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Abstract

Objectives—To evaluate the impact of therapeutic HIV vaccination on the HIV reservoir, and assess the relationship of the viral reservoir with HIV-specific immune status and viral rebound kinetics.

Design—Retrospective analysis of ACTG A5197, a randomized, placebo-controlled trial of a therapeutic rAd5 HIV-1 gag vaccine.

Methods—Participants received vaccine/placebo at weeks 0, 4, and 26 prior to a 16-week analytic treatment interruption (ATI) at week 38. Cell-associated HIV-1 RNA and DNA (CA-RNA and CA-DNA) and HIV-1 residual viremia (RV) were quantified at weeks 0, 8, and 38. HIV-specific CD4+/CD8+ activity were assessed by an intracellular cytokine staining assay.

Results—At study entry, CA-RNA and CA-DNA levels were correlated inversely with the numbers of HIV-specific CD4+ interferon-γ-producing cells (CA-RNA: $r = -0.23$, P=0.03 and CA-DNA: $r = -0.28$, P<0.01, N=93). Therapeutic HIV vaccination induced HIV-specific CD4+ activity, but did not significantly affect levels of CA-RNA or CA-DNA. Vaccine recipients with

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undetectable RV at week 8 had higher frequencies of HIV-specific CD4+ and CD8+ interferon-γ-producing cells (undetectable versus detectable RV: 277 versus 161 CD4+ cells/10^6 lymphocytes, P=0.03 and 1326 versus 669 CD8+ cells/10^6 lymphocytes, P=0.04). Pre-ATI CA-RNA and CA-DNA were associated with post-ATI plasma HIV set point (CA-RNA: $r = 0.51$, P<0.01 and CA-DNA: $r = 0.47$, P<0.01).

Conclusions—Vaccine-induced T-cell responses were associated with a modest transient effect on RV, but more potent immune responses and/or combination treatment with latency-reversing agents are needed to reduce the HIV reservoir. HIV reservoir measures may act as biomarkers of post-ATI viral rebound kinetics.

Introduction

The persistence of HIV within long-lived reservoirs results in the need for life-long antiretroviral therapy (ART). While ART has saved millions of lives, its use is associated with a number of drawbacks that include side-effects, drug-drug interactions, pill fatigue, drug resistance, and high cost. Therefore, developing novel strategies that can reverse HIV latency and boost HIV-specific immune responses to achieve sustained ART-free HIV remission is a priority for HIV research [1]. Spontaneous control of HIV in the absence of ART has been described in HIV controllers. Such control may be mediated, at least in part, by strong HIV-specific CD8+ T cell effector activity [2–5]. One strategy that seeks to induce such responses is therapeutic HIV vaccination, which aims to further boost HIV-specific immune activity [6].

Despite the hope that therapeutic HIV vaccination could play a central role in HIV curative strategies, little is known about the effect of vaccination and vaccine-induced T cell responses on the HIV reservoir. While it is proposed that activating HIV-specific T cells during suppressive ART may reduce the latent reservoir [5], this approach could increase viremia and expand the HIV reservoir due to higher levels of HIV production and target cell availability [7]. ACTG A5197 was a randomized, placebo-controlled trial of a therapeutic rAd5 HIV-1 *gag* vaccine in participants on suppressive antiretroviral therapy. The vaccine induced HIV-specific CD4+ and CD8+ interferon-γ-producing T cells [8]. Factors associated with lower viral rebound set point during the analytic treatment interruption (ATI) included randomization to the vaccine arm, lower pre-ART viral load, and a greater number of HIV-specific CD4+ T cells [8, 9]. In the current study, we explored the impact of therapeutic vaccination and immune status on the HIV reservoir among participants of A5197. In addition, we evaluated whether quantitative measures of the HIV reservoir can serve as useful biomarkers to predict the kinetics of HIV rebound during an ATI.

Patients and methods

Study population

Study design and patient inclusion criteria for ACTG A5197 have been described in detail [8]. Eligible subjects were on ART with CD4+ cell counts 500/mm^3, plasma HIV-1 RNA levels of 50 copies/mL with a history of HIV-1 RNA 500 copies/mL for 24 months prior to enrollment, and screening serum Ad5 antibody titers 200 units/mL. Participants were

randomized 2:1 to receipt of either a replication-defective rAd5 vaccine containing an HIV-1 *gag* insert or placebo at weeks 0, 4, and 26. Starting at week 38, participants underwent a 16-week ATI. Plasma and peripheral blood mononuclear cells (PBMCs) were collected at weeks 0, 8, and 38. Plasma HIV-1 RNA levels were measured at weeks 1, 2, 3, 4, 6, 8, 10, 12, and 16 of the ATI.

HLA typing

HLA class I typing was performed following the PCR-SSOP (sequence-specific oligonucleotide probing) and the PCR-SBT (sequence based typing) protocols recommended by the 13th International Histocompatibility Workshop (http://www.ihwg.org). Participants were grouped into protective, neutral, or unfavorable HLA groups based on race-specific definitions. Protective HLA alleles were defined a priori as HLA B*27, B*57 for both white and black participants, and B*81 for black participants only. Unfavorable HLA alleles were defined as the HLA-B*35-Px variants (B*3502, 3503, 3504, or 5301) for both white and black participants; B*0702 and B*0801 for white participants only; and B*4501 and B*5802 for black participants only. Participants with one protective and one unfavorable allele or those with neither protective nor unfavorable HLA alleles were categorized in the neutral HLA group.

HIV-1 reservoir assays

Cell-associated HIV-1 RNA and DNA (CA-RNA and CA-DNA) and residual viremia (RV) by a single-copy assay (SCA) were quantified at weeks 0, 8, and 38. Cellular RNA and DNA were isolated from cryopreserved PBMCs using the AllPrep DNA/RNA Mini Kit (Qiagen). CA-RNA and CA-DNA were quantified using a real-time PCR approach with primers/probes targeting conserved regions of the HIV LTR/gag as previously described [10].

Low-level HIV-1 viral loads were measured using the SCA as previously described [11]. The efficiency of HIV amplification by the SCA primers and probes was evaluated by sequencing of the HIV-1 *gag* region to assess potential sequence mismatches between the SCA primer/probe and patient sequence. For those samples with any sequence mismatches, SCA was performed on plasma samples with detectable viremia by a commercial assay to confirm concordance of values by the two assays. The limit of detection of the SCA was determined by the amount of available plasma for the assay and standardized to the highest limit for any individual (0.7 copies/mL). The limit of detection was used in the analysis for undetectable SCA results.

HIV-specific CD4+ and CD8+ activity by intracellular cytokine staining assay (ICS) for interferon-gamma

Blood samples obtained at protocol-defined intervals were sent to Merck Research Laboratories by overnight courier. HIV-specific CD4+ and CD8+ responses were evaluated by an intracellular cytokine staining assay as previously described [8, 12, 13]. Lymphocytes were exposed to HIV-1 Gag, Nef, Pol or mock peptide pools for 18 hours. Interferon-gamma (IFN-γ) producing CD4+ and CD8+ T cells were detected by multiparameter flow cytometry.

Neutralizing antibody (NAb) assay

HIV-1 neutralizing antibody levels were measured at pre-ATI (week 38) for a subset of participants using a recombinant-virus assay (Monogram Biosciences) [14]. The neutralizing capacity of pre-ATI plasma was assessed using pseudotyped virus containing participant *env* amplified from plasma HIV-1 RNA obtained at the first post-ATI time point when the viral load was <1,000 HIV-1 RNA copies/mL. The neutralizing antibody titer was calculated as the reciprocal of the plasma dilution conferring 50% inhibition (IC_{50}). The association between NAb titer and viral rebound endpoints were adjusted for baseline CD4+ count, nadir CD4+ count, age, treatment arm assignment, and HIV-specific CD4+ IFN-γ-production.

Statistical analysis

Associations between HIV reservoir levels and either immune status or viral rebound kinetics were assessed by Spearman correlation, Wilcoxon rank sum test and Fisher's exact test for two group comparisons. No adjustments were made for multiple comparisons. For HIV rebound kinetics, the two coprimary endpoints of the study (ATI viral load set point and viral load time-averaged area-under-the-curve [TA-AUC]) and time to viral rebound 200 HIV RNA copies/mL were assessed. The ATI viral load set point was defined as the mean of the week 12 and 16 plasma HIV-1 RNA levels. For the viral load TA-AUC endpoint, viral rebound kinetics for each study participant were estimated by the linear trapezoidal method, with the use of the first, last, and intervening plasma $\log_{10}$ HIV-1 RNA levels observed during the ATI to calculate the AUC, and by dividing the AUC by the number of days between the first and the last observation.

Results

HIV reservoir levels and HIV-specific T cell activity at study entry

At study entry, there were no significant differences between treatment arms in levels of CA-RNA (Vaccine [N=64] versus Placebo [N=32] arms: median 152 copies/10^6 PBMCs versus 179 copies/10^6 PBMCs, P=0.78), CA-DNA (76 copies/10^6 PBMCs versus 93 copies/10^6 PBMCs, P=0.41], or RV (Vaccine [N=64] versus Placebo [N=30]: median 0.7 versus 0.7 copies/mL, P=0.63). Levels of CA-RNA were correlated with levels of CA-DNA (Spearman *r* = 0.55, P<0.01, N=92) and RV (Spearman *r* = 0.23, P=0.04, N=77).

Baseline (week 0) CA-RNA and CA-DNA levels in PBMCs were correlated negatively with the frequency of HIV-specific CD4+ IFN-γ-producing cells (Fig. 1, CA-RNA: Spearman *r* = −0.23, P=0.03, N=93 and CA-DNA: *r* = −0.28, P<0.01, N=93), but not with the frequency of CD8+ IFN-γ-producing cells. The level of RV at study entry did not correlate with either HIV-specific CD4+ or CD8+ activity.

Effect of vaccination on CA-RNA, CA-DNA, and residual plasma viremia

Despite increasing HIV-specific CD4+ IFN-γ activity [8], vaccination did not result in significant changes in CA-RNA or CA-DNA prior to the ATI (Fig. 2). By week 8, after two doses of the vaccine/placebo, modest differences between study arms were noted in the levels of RV (vaccine [N=65] versus placebo [N=30]: median 0.7 versus 1.1 copies/mL,

P=0.08) and proportions of individuals with detectable RV (37% versus 57%, P=0.08). The within-arm comparison of RV changes between baseline and week 8 did not show a significant change either in the vaccine or placebo arm (Wilcoxon signed rank test P=0.25 [vaccine] and P=0.43 [placebo]). Vaccine recipients with undetectable RV at week 8 had significantly higher frequencies of HIV-specific CD4+ IFN-γ-producing cells (undetectable RV [N=39] versus detectable RV [N=24]: 277 versus 161 cells/10^6 lymphocytes, P=0.03) and CD8+ IFN-γ-producing cells (undetectable RV [N=39] versus detectable RV [N=24]: 1326 versus 669 cells/10^6 lymphocytes, P=0.04). At week 38, however, the associations between RV and HIV-specific CD4+ and CD8+ activity had weakened and were no longer statistically significant (undetectable RV [N=35] versus detectable RV [N=25]: 249 versus 153 CD4+ IFN-γ-producing cells/10^6 lymphocytes, P=0.08; and 1007 versus 838 CD8+ IFN-γ-producing cells/10^6 lymphocytes, P=0.51).

Association of HIV reservoir levels with T cell activation and HLA haplotype

At baseline, CA-RNA and CA-DNA levels were significantly associated with the frequency of activated CD8+CD38+ cells (CA-RNA: Spearman $r = -0.26$, P=0.01, N=90; CA-DNA: $r = -0.22$, P=0.04, N=90), but not with frequency of activated CD4+CD38+ T cells. The frequencies of activated CD4+ and CD8+ cells at baseline were not associated with CA-RNA:DNA ratio, RV levels, post-ATI set point, or TA-AUC.

Individuals with unfavorable HLA alleles were found to have significantly higher levels of CA-RNA and RV when compared to participants with neutral HLA alleles (Fig. 3). Those with unfavorable HLA alleles were also found to have the highest levels of CA-DNA, but the difference between HLA types was not statistically significant.

Relationship of NAb titers with HIV reservoir levels and post-ATI rebound kinetics

The median (Q1, Q3) NAb titer on the $\log_{10}$ scale were 1.86 (1.79, 2.04). At the pre-ATI (week 38) time point, NAb titers were positively correlated with RV levels (Supplemental Fig. 1, Spearman $r = 0.42$, P<0.01, N=37) and less so with levels of CA-RNA ($r = 0.31$, P=0.06), CA-DNA (r = 0.22, P=0.18), and CA-RNA:DNA ratio ($r = 0.21$, P=0.21). NAb titer was significantly associated with post-ATI TA-AUC endpoint (P=0.03) after adjusting for potential confounders (HIV-specific CD4+ IFN-γ-producing cells, nadir CD4+ T cell count, and treatment arm), but was not significantly associated with the post-ATI viral load set point.

Association of pre-ATI CA-RNA and CA-DNA levels with HIV rebound kinetics

The plasma HIV-1 RNA set point reached during the ATI was positively correlated with pre-ATI (week 38) CA-RNA and CA-DNA (Fig. 4, CA-RNA: Spearman $r = 0.51$, P<0.01, N=90; CA-DNA: $r = 0.47$, P<0.01, N=93). Pre-ATI CA-RNA and CA-DNA levels were also significantly associated with ATI TA-AUC (CA-RNA: $r = 0.38$, P<0.01; CA-DNA: $r = 0.36$, P<0.01). Pre-ATI CA-RNA:DNA ratio and levels of RV were not significantly associated with either the ATI viral load set point or TA-AUC.

The associations between these HIV reservoir measures and confirmed time to viral rebound 200 HIV RNA copies/mL were weaker and not statistically significant (CA-RNA: *r =*

−0.17, P=0.16, N=72; CA-DNA: $r = −0.15$, P=0.2, N=75; RV: $r = −0.15$, P=0.17, N=88). The results were largely unchanged when the analysis was stratified by treatment arm (Table S1).

Discussion

ACTG study A5197 represents one of the largest therapeutic HIV vaccine studies performed during the modern cART era and offers a valuable opportunity to assess vaccine-associated changes in the HIV reservoir, the relationship of the reservoir with immune status, and whether HIV reservoir measures predict viral rebound kinetics. Early in the vaccination course, higher frequencies of HIV-specific CD4+ and CD8+ cells were associated with lower levels of RV, but this effect waned over time. Despite a higher level of vaccine-induced HIV-specific CD4+ activity, CA-RNA and CA-DNA levels were not significantly altered by vaccination. In addition, we found significant associations between HIV reservoir levels and both T cell activation and HLA type. Finally, higher pre-ATI CA-RNA and CA-DNA levels were associated with higher post-ATI viral load set point.

Vaccination with rAd5-HIV *gag* induced at least two-fold increase in HIV-specific CD4+ IFN-γ activity in approximately 40% of recipients [8]. Levels of HIV-specific CD4+ activity peaked at week 8 (after 2 doses of vaccination) and were predictive of post-ATI viral load set point. Only the week 8 HIV-specific CD4+ activity was associated with lower residual viremia. This finding is consistent with the concept of a stronger initial vaccine-induced immune response that waned over time. In aggregate, these results suggest that the longer vaccination schedule may not have increased vaccine response and that an earlier treatment interruption might have demonstrated a more robust vaccine effect.

Although the use of therapeutic HIV vaccines is being explored as part of a strategy to induce post-treatment HIV control, little is known about how the stimulation of HIV-specific T cells may affect the latent HIV reservoir. While it is hoped that a vaccine-induced immune response may help clear the HIV reservoir [5], a recent study of an autologous dendritic cell-based therapeutic HIV vaccine demonstrated an increased T cell activation as well as residual viremia and cell-associated RNA after vaccination despite continuous ART [7]. In contrast, the vaccines used in A5197 increased HIV-specific CD4+ and CD8+ T cell activity without inducing higher levels of cell-associated HIV RNA or plasma residual viremia. Several factors could explain the differences seen in the level of post-vaccination HIV expression in these studies. These include the vaccine strategy (adenoviral vector versus autologous dendritic cells), differences in the length of time on ART, and participant characteristics. In the dendritic cell study, participants had a median CD4+ count of 486 cells/mm^3 and were only treated with approximately 12 weeks of ART prior to vaccine administration [7]. In contrast, A5197 participants had higher CD4+ counts at baseline (853 cells/mm^3 in the vaccine arm) and all participants were on suppressive ART for at least 2 years prior to study entry. In addition, a small study of a DNA prime, rAd5 boost strategy in 12 vaccine recipients demonstrated an increase in HIV-specific T cell response without significant changes in residual viremia or size of the latent reservoir by viral outgrowth assay [15]. It is also interesting to note that a study of a modified Vaccinia Ankara and Fowlpox-based therapeutic HIV vaccine in young adults showed a transient decrease in the

size of the HIV reservoir by viral outgrowth assay and that the latent reservoir size was correlated with post-vaccination HIV-specific CD4+ IFN-γ+ cells [16]. Evaluation of the HIV reservoir effects of other therapeutic HIV vaccine strategies are needed to further explore these findings.

Certain HLA alleles (e.g., B*27 and B*57) are associated with strong HIV-specific immune responses and are overrepresented in HIV controllers [3]. Such protective HLA alleles have also been associated with lower plasma viral loads and HIV CA-DNA levels [17, 18]. In contrast, unfavorable HLA alleles have been identified (e.g., B*35-Px) that are associated with more rapid HIV disease progression [19]. In this study, we found an increased baseline level of residual viremia and CA-RNA in participants with unfavorable HLA alleles. This association occurred in the setting of suppressive ART and could signify a larger transcriptionally-active HIV reservoir in those with unfavorable HLA alleles. It is possible that a greater distinction in HIV reservoir size may have been detected within certain CD4+ cell subsets, as a previous report showed lower HIV DNA levels within the central memory T cell compartment of long-term nonprogressors harboring either HLA B*27 or B*57 [18]. No significant differences in HIV reservoir were observed between individuals with protective HLA alleles and those harboring either unfavorable or neutral HLA alleles. However, there were a limited number of participants with protective HLA alleles and studies in larger cohorts are needed to explore the finding that those with protective HLA alleles had the lowest levels of CA-DNA.

The induction of NAbs may be an important strategy for delaying the timing of viral rebound after treatment interruption [20]. In an analysis of the ACTG study A5170, McLinden and colleagues found that higher NAb titers were significantly associated with lower viral load during treatment interruption [21]. In contrast, our results showed that higher pre-ATI NAb titers were associated with both higher levels of pre-ATI residual viremia and post-ATI viral load. These results suggest that the extent of pre-ATI NAb titers detected in this study was primarily a reflection of circulating antigen levels and not apparently protective against post-ATI viral rebound. One potential explanation for the disparate results may be assay differences as the A5170 analysis evaluated the extent of heterologous NAb titers against four subtype B isolates whereas the current study measured levels of autologous NAb titers. It is possible that the measurement of heterologous NAbs may be more indicative of broadly neutralizing antibodies specific to more conserved aspects of the viral envelope. In addition, the relationship between NAb titers and extent or timing of viral rebound may be nonlinear [21]. Other studies evaluating the protective ability of either heterologous and/or autologous NAbs during treatment interruption have also shown mixed results [22–27]. More comprehensive studies are needed to fully characterize the impact of both heterologous and autologous NAbs, as well as antibody diversity and effector functions (e.g., Ab-dependent cellular cytotoxicity) on viral rebound kinetics during treatment interruption.

Clinical trials of therapeutic vaccines and interventions targeting the HIV reservoir would ideally include an analytic treatment interruption (ATI) component, as was performed in A5197, to demonstrate an effect on viral rebound kinetics. However, such studies are time consuming, costly, and not without clinical risks [28]. Therefore, many recent studies have

focused their outcome measurements on surrogate endpoints, such as the effects on the viral reservoir. Residual plasma viremia and cell-associated HIV-1 RNA and DNA are commonly used measures of the HIV reservoir size [11, 29–32]. However, few studies have evaluated the ability of these measures to predict the timing and extent of viral rebound, which are the ultimate markers of success. In this study, we show that pre-ATI cell-associated HIV RNA and DNA measured in PBMCs are significantly correlated with plasma viral load set point and TA-AUC after the treatment interruption. These results are supported by several published reports. One study found a correlation between pre-ATI proviral DNA levels and viral set point, but study participants underwent 4 short (2 week) ATI cycles prior to a longer ATI, a study design that significantly complicates interpretation of the results [33]. A second study also found an association between pre-ATI proviral DNA levels and viral control during the ATI, but study participants were on suboptimal regimens (including NRTI-only regimens) and many had multiple short ATI cycles [34]. Overall, these results suggest that HIV reservoir measurements while on suppressive ART could be used to predict some aspects of post-ATI viral rebound dynamics and could have potential as biomarker endpoints of future interventional HIV reservoir studies.

This study has a few notable limitations. Our analyses of the association between pre-ATI CA-RNA and CA-DNA with post-ATI viral kinetics were complicated by the fact that two-thirds of participants received the therapeutic vaccination. A sensitivity analysis showed that the results were largely unchanged when the analysis was restricted to placebo recipients only. While measuring HIV reservoir levels in total PBMCs is more cost-effective and surveys a broader range of cells, it is possible that the association between HIV reservoir measures and viral rebound dynamics may be even stronger if only CD4+ T cells were included or if viral outgrowth assays were performed. The viral outgrowth assay avoids the measurement of replication-defective HIV proviruses, but has other limitations. Viral outgrowth assays may underestimate the presence of replication-competent provirus [35, 36] and the use of viral outgrowth assays has limited scalability for larger studies as it is costly, and time- and labor-intensive [37].

In this study, therapeutic HIV vaccination-induced T cell response were associated with a modest, transient effect on RV, but no significant changes in CA-RNA or CA-DNA levels. More potent immune responses and/or combination treatment with latency-reversing agents are needed to significantly reduce the latent HIV reservoir. While HIV reservoir measures were found to predict post-ATI viral load set point, additional studies are needed to confirm this association and to determine which HIV reservoir characteristic may best predict HIV rebound kinetics. Finding early markers of efficacy for viral eradication strategies could be a key step in streamlining the development of next-generation therapeutics with a goal of sustained ART-free HIV remission.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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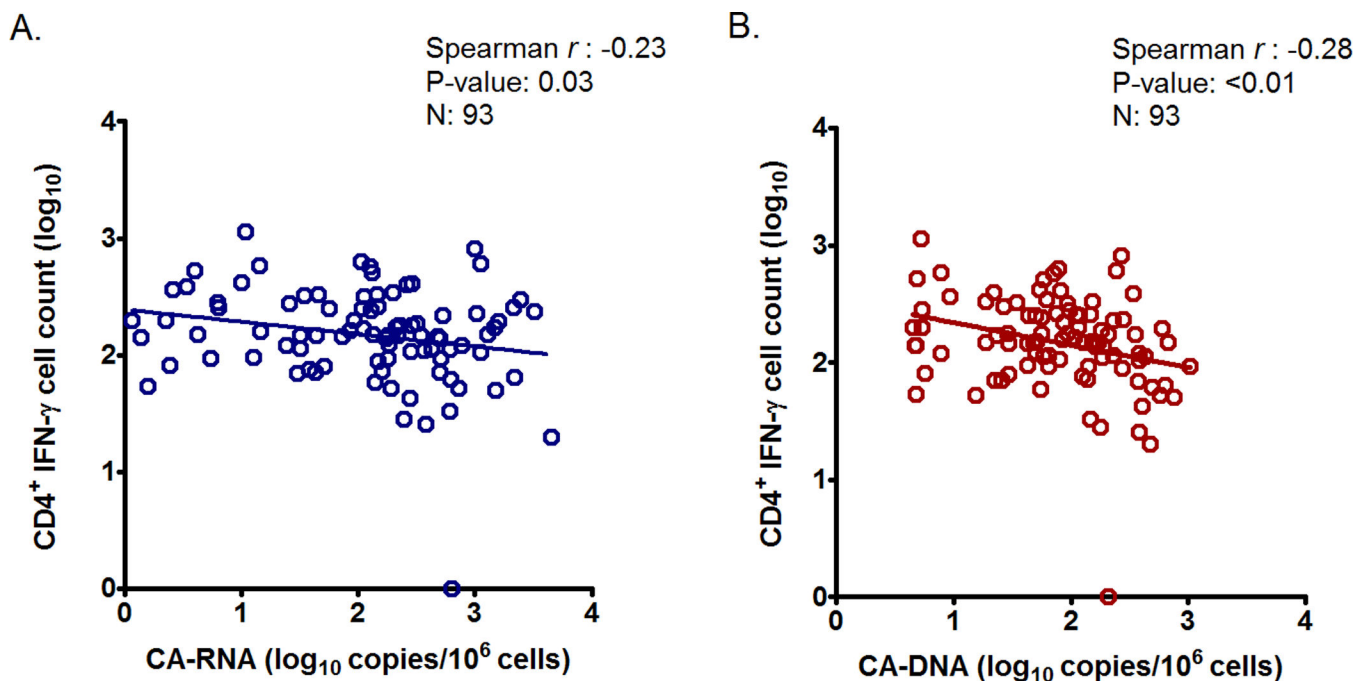
Figure 1. Association between HIV-specific CD4+ activity and HIV reservoir levels at baseline
(A) CA-RNA and (B) CA-DNA at baseline are associated with the number of HIV-specific CD4+ IFN-γ-producing cells. CA-RNA, cell-associated HIV RNA; CA-DNA, cell-associated HIV DNA.
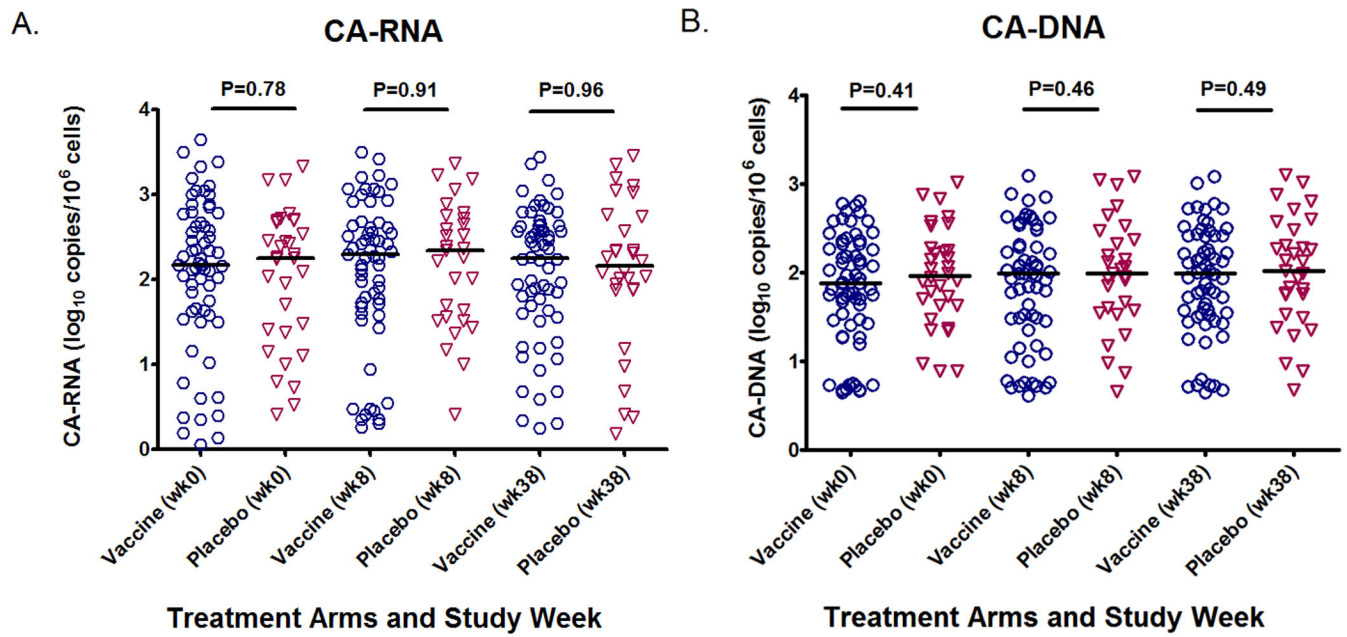
Figure 2. Therapeutic HIV vaccination was not associated with changes in CA-RNA or CA-DNA levels

(A) CA-RNA and (B) CA-DNA levels in participants of A5197 pre- (wk 0) and post-vaccination (wk 8 and 38). Bars represent median values. CA-RNA, cell-associated HIV RNA; CA-DNA, cell-associated HIV DNA.
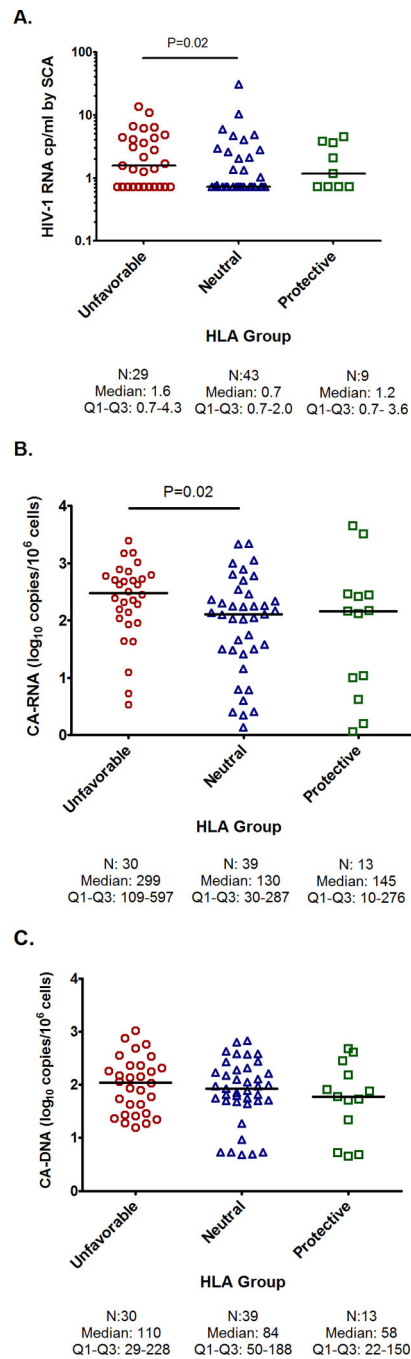
Figure 3. Baseline HIV reservoir levels differ by HLA group
(A) Residual viremia by single-copy assay, (B) CA-RNA, and (C) CA-DNA levels in PBMC in participants of A5197 stratified by the presence of unfavorable, neutral, or protective HLA alleles. SCA, single-copy assay; CA-RNA, cell-associated HIV RNA; CA-DNA, cell-associated HIV DNA; HLA, human leukocyte antigen; PBMC, peripheral blood mononuclear cell.
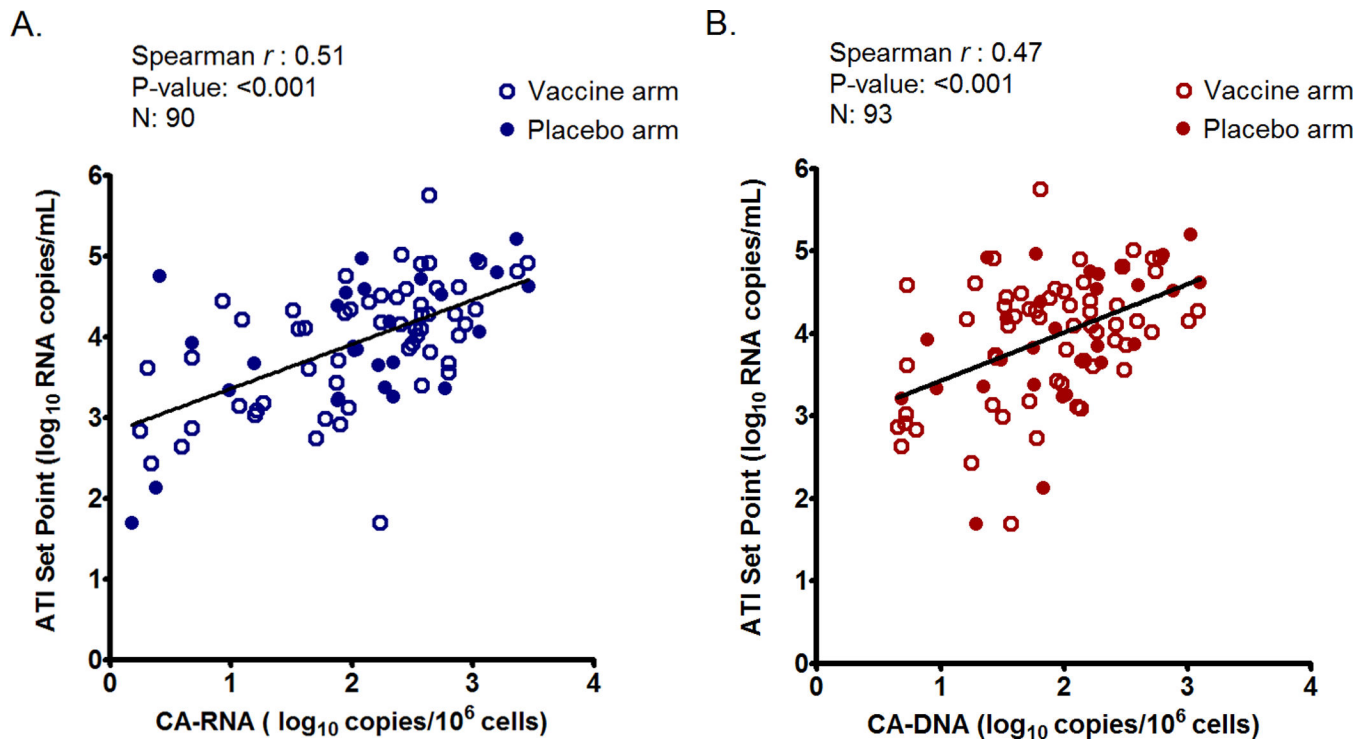
Figure 4. Pre-ATI CA-RNA and CA-DNA predict viral load set point

Pre-ATI week 38 (A) CA-RNA and (B) CA-DNA levels were both significantly correlated with post-ATI viral load set point. ATI, analytic treatment interruption; CA-RNA, cell-associated HIV RNA; CA-DNA, cell-associated HIV DNA.