Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Genome Editing with Zinc Finger Nuclease Modified Autologous CD4 T-cells

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Expanded Methods

STUDY DESIGN

This Phase 1 trial (ClinicalTrials.gov number NCT00842634) was an open label, uncontrolled, nonrandomized study of patients with chronic HIV infection. The study was conducted at the University of Pennsylvania and Jacobi Medical Center, NY between May 2009 and July 2012. The primary objective of the study was to assess the safety and tolerability of a single dose of autologous CD4 enriched T cells modified at the CCR5 gene by ZFNs. Secondary objectives included the assessment of increases in CD4 cell count, persistence of the modified cells, homing to gut mucosa, and effects on viral load. Descriptive statistics were calculated for study variables. The outcomes of cell counts were tested for normality using the Shapiro-Wilk test and analyzed using nonparametric tests (e.g., Mann-Whitney test for comparison of independent samples, Sign test for matched paired data) because the data was not normally distributed. Primary data at specific time points were summarized by providing median with range. Changes of a variable from baseline were summarized by median+SD or ranges when appropriate. The clinical protocol is included as a Supplementary Appendix. The cells were manufactured as described. 1 The dose, percentage of T cells and percentage of cells with CCR5 modification are shown in Table 1. Details of a concurrent control cohort are in Table S3.

The study was conducted at two centers in the United States between May 2009 and July 2012. The primary objective of the study was to assess the safety and tolerability of a single dose of autologous CD4 enriched T cells modified at the CCR5 gene by ZFNs. Secondary objectives included the assessment of increases in CD4 cell count, persistence of the modified cells, homing to gut mucosa, and effects on viral load.
Subjects were eligible for enrollment if they were 18 years of age or older and were infected with HIV, as documented by ELISA and confirmed by Western blot. Subjects must be aviremic (undetectable HIV-RNA by ultrasensitive PCR), receiving stable antiretroviral therapy (HAART), have adequate venous access and no contraindications for leukapheresis. The key exclusion criteria included a SNP at the CCR5 zinc finger nuclease target region, known infection with a CXCR4 tropic or dual tropic virus, current or prior AIDS diagnosis, receiving therapy with maraviroc or immunosuppressives, and hepatitis B or hepatitis C infection. The final protocol, amendments, and consent documents were reviewed and approved by the institutional review board and independent ethics committee at each study center as well as the Recombinant DNA Advisory Committee of the National Institute of Health. All subjects provided written informed consent.

A total of 12 subjects were enrolled into two cohorts in this study, six subjects in each cohort. Cohort 1 enrolled six immune responders (IR) who were defined as subjects with CD4 counts >450 cells/mm$^3$ at screening with a documented CD4 nadir of not lower than 300 cells/mm$^3$. Six subjects with CD4+ T cell counts that were persistently between 200 to 500 cells/mm$^3$ despite a minimum of 2 years of stable HAART were enrolled in Cohort 2, the immune nonresponders (INR). Subjects underwent a 10 liter leukapheresis to collect autologous CD4 T cells for the production of SB-728-T. Following CCR5 modification of the CD4 T-cells, the cells (0.5-1 x 10$^{10}$ cells) were reinfused into the subjects over 15-20 minutes. Subjects in Cohort 1 were followed weekly for the initial 4 weeks and then underwent a 12-week Analytical Treatment Interruption (TI) beginning on week 4. During the TI, subjects were examined every other week for the first two months and then every 4 weeks thereafter until day 252. Subjects in cohort 2 were followed weekly for the first 3 weeks and subsequently on days 42, 56, 84, 168 and 252.
Rectal biopsies were performed at baseline on all subjects and again on days 21 and 112 in cohort 1. Subjects in cohort 2 were re-biopsied on days 42 and 252. All subjects were followed for 36 weeks following study drug administration and were then enrolled in a 10 year follow-up study for monitoring for delayed adverse events potentially associated with the ZFN mediated genomic modification.

**INVESTIGATIONAL AGENT**

SB-728-T refers to autologous CD4+ enriched T cells that have been transduced *ex vivo* with SB-728, a replication deficient recombinant Ad5/35 viral vector encoding the CCR5 specific ZFNs (SBS8196z and SBS8267), resulting in modification of the CCR5 gene. SB-728 supports only transient expression of genes encoded by the vector. The two ZFNs bind to a composite 24-bp sequence found specifically in the region encoding the first transmembrane domain of the CCR5 gene, just upstream from the naturally occurring CCR5-Δ32 mutation. Expression of the CCR5-specific ZFNs induces a double stranded break in the cell’s DNA which is repaired by cellular machinery leading to random sequence insertions or deletions in ~25% of transduced cells. These insertions and deletions disrupt the CCR5 coding sequence leading to frameshift mutation and termination of protein expression. CCR5 modified CD4 T cells could be tracked due to the acquisition of a unique chromosomal 5-nucleotide (pentamer) DNA sequence, CTGAT, in approximately 25% of the modified cells.

The SB-728 T cells were manufactured as described. Briefly, study subjects undergo a 10 liter leukapheresis to collect >10^9 white blood cells. The leukapheresis product is enriched for CD4+ cells by depleting monocytes via counterflow centrifugal elutriation, and by magnetically depleting CD8+ T-cells, both employing a single-use closed-system disposable set. The resulting enriched CD4+ T-cells are activated with anti-CD3/anti-CD28 mAb coated paramagnetic beads.
Cells are then expanded and cultured in a closed system with X-VIVO-15 media. T-cell expansion continues after transfer to a WAVE Bioreactor for additional expansion under perfusion conditions. At the end of the culture period, cells are depleted of magnetic beads, washed, concentrated, and cryopreserved. The mean CCR5 modification in the final product for the 12 subjects was 21.2 ± 5.7% (range: 10.9-27.7%).

**ANALYTICAL METHODS**

**Rectal Biopsy**

Mucosal mononuclear cells were isolated from sigmoid colon biopsies obtained by endoscopy via a combination of collagenase digestion and teasing with 18G needles. Tissues were processed essentially as described in Anton and Shacklett.³,⁴

**CCR5 Modified CD4 T cells by Polymerase Chain Reaction**

ZFN mediated gene modification can generate a wide range of frame-shift mutations to disrupt the CCR5 gene locus. A PCR-based assay was developed to measure a specific five-nucleotide duplication modification (Pentamer) which is found in approximately 25% of cells after repair of the ZFN cleavage site. Genomic DNA (gDNA) is extracted from peripheral blood mononuclear cells (PBMCs) using a commercially available kit (Masterpure DNA Purification kit, Epicenter, Madison, WI). A standard PCR is performed with 5µg of gDNA to amplify a 1.1 kb region that contains the CCR5 modifications. This 1.1 kb amplicon is subsequently evaluated with the two independent qPCRs, one specific for the pentamer duplication-modified CCR allele (by using a primer that contains the pentamer duplication), and a second that amplifies all CCR5 alleles. The ratio of pentamer duplication specific templates multiplied by 4 to the total number of CCR5
alleles yields Pentamer duplications per 1 million PBMCs. The assay has a sensitivity of one modified CCR5 allele per $10^5$ total CCR5 alleles.

**HIV-RNA by Polymerase Chain Reaction**

HIV-RNA was determined by the AMPLICOR HIV-1 MONITOR Test (Roche Molecular Systems, Pleasanton, CA) with a quantitative limit of detection of 50 copies/mL.

**HIV-DNA by Digital Droplet Polymerase Chain Reaction**

Genomic DNA (gDNA) was extracted from PBMCs using a commercially available kit (Masterpure DNA Purification kit, Epicenter, Madison, WI). 1.5 to 2 µg of gDNA is digested with the restriction enzyme DdeI at 37°C for 1 hour. PCR droplets are prepared according to manufacturer's recommendations. Briefly, a 20µl of multiplex PCR mixture is prepared by mixing 250 or 500 ng of the digested gDNA with the ddPCR™ 2x Master Mix and two Taqman primer/probe sets. The Taqman primer/probe sets amplify a conserved region in gag, as described:

- HIVgag forward CATGTTTTTCAGCATTATCAGAAGGA,
- HIVgag reverse TGCTTGATGTCCCCCCACT,
- HIVgag probe, FAM-CCACCCCACAAGATTAAACACCACCATGCTAA-BHQ)
- RPP30 forward GATTTGGACCTGCGAGCG,
- RPP30 reverse GCGGCTGTCTCCACAAGGT, RPP30 probe VIC-CTGACCTGAAGGCTCT-MGB-BHQ).

PCR droplets are generated in a DG8™cartridge using the QX-100 droplet generator, where each 20 µl PCR mixture is partitioned into approximately 15,000 nano-liter size droplets. PCR droplets are transferred into a 96-well PCR plate and sealed with foil. Standard PCR is performed with a Bio-Rad C1000 Thermal Cycler (95°C (60sec), 40 cycles of 94°C (30sec)/60°C (60sec), 98°C (600 sec)). HIV-DNA copy number is evaluated using the QX-100 Digital Droplet PCR system (Bio-Rad, Hercules, CA). The PCR-positive and PCR-negative droplets for...
HIVgag and RPP30 are determined and template concentrations are calculated by Poisson analysis. HIV copy number is determined by normalizing HIVgag concentration to RPP30 concentration. The lower limit of detection of the assay is 10 copies per 10^6 PBMC and the lower limit of quantitative detection is 100 copies per 10^6 PBMC.

**Concurrent Control Cohort**

As a concurrent control group we selected 8 participants in the clinical core cohort of the University of Pennsylvania Center for AIDS Research (CFAR). The characteristics of the subjects and samples tested for HIV DNA are listed in Supplemental Table 3. These patients were male, 4 on protease inhibitor based regimens (2 on NNRTIs, and one of integrase and triple nucleoside regimens). They had a median age of 43 years (range 24-60), undetectable plasma HIV viremia with the ultrasensitive assay (<75 copies/mL) for a median of 22 months (range 8-39 months) before the first PBMC specimen was evaluated for the amount total HIV DNA and provided 5 PBMC specimens for a total follow up of 4.3 years (range 3.2-4.7), during which the plasma HIV viremia remained below the limit of quantification at all time points.

**STATISTICAL ANALYSIS**

Descriptive statistics were calculated for study variables. The outcomes of cell counts were tested for normality using the Shapiro-Wilk test and analyzed using nonparametric tests (e.g., Mann-Whitney test for comparison of independent samples, Sign test for matched paired data) because the data was not normally distributed. Primary data at specific time points were summarized by providing median with range. Changes of a variable from baseline were summarized by median+SD of the change.
For the half-life of CCR5-modified cells, a rate constant associated with the terminal (log-linear) portion of an exponential decay curve for the cell counts versus time was assumed. This rate constant was estimated by maximizing the $R^2$, equivalently, by minimizing the squared errors between the fitted and observed data on the curve. Half-life was calculated by dividing natural log (2) with that of the estimated rate constant.

The rates of decline in CCR5-modified cell count and the non-modified CD4 cells (derived by subtracting CCR5-modified CD4 cells from the total CD4 count) during treatment interruption (week 1 to week 16) were estimated as slopes from a mixed quantile regression analysis where between-subject variability and potential correlations among the repeated cell count measures over time can be controlled using random intercepts. The difference in slopes were tested for significance of an interaction term between the time variable and an indicator of cell type (modified versus not). For all comparisons, a two-sided $P<0.05$ was considered statistically significant. Statistical analysis was performed using STATA 12.0 (Stata Corp, College Station, TX, USA). Graphical presentation was performed using Sigmaplot (Systat Software, San Jose, CA, USA).

**Expanded Results**

Cohort 1 enrolled six immune responders who were defined as subjects with CD4 counts $>450$ cells/mm$^3$ at screening with a documented CD4 nadir of not lower than 300 cells/mm$^3$. Six subjects with CD4+ T cell counts that were persistently between 200 to 500 cells/mm$^3$ despite a minimum of 2 years of stable HAART were enrolled in Cohort 2, the immune non-responders. This cohort did not have a nadir requirement. Cohort 1 patients underwent a planned analytic treatment interruption in order to assess the safety and antiviral effects of SB-728-T in the
presence of viremia; cohort 2 patients did not undergo treatment interruption due to the low nadir CD4 counts. The race and ethnicity of the study subjects was diverse with 5 Caucasians, 4 Blacks, 1 Asian, 1 Hispanic and 1 East Indian. The majority of the subjects were male (10 males, 2 females) and ranged in age from 31 to 60 years (median: 49.0 yrs). As expected, the median CD4 count at baseline was significantly higher in the immune responders (662 cells/µL, range: 554-997 cells/µL) than the immune non-responders (272 cells/µL, range: 193-328 cells/µL, Mann-Whitney test, P=0.003). The median CD4/CD8 ratio of the group was 0.99 (range: 0.50-1.99) and was higher in the immune responders (1.41) than the immune non-responders (0.72, Mann-Whitney test, P=0.05). The subjects were infused with a median of 1.0x10^{10} total cells (range 0.5-1.08x10^{10}), with subjects in cohort 1 (1.0x10^{10} total cells) and cohort 2 (0.85x10^{10} total cells) receiving a similar median dose. All subjects completed the 36 week study and were then enrolled in an ongoing ten year follow-up study to monitor for delayed adverse events resulting from the ZFN-mediated genomic modification.

**Safety and Tolerability**

There were a total of 130 adverse events (AEs) reported by the 12 subjects following study drug infusion; 68 in cohort 1 and 62 in cohort 2. The majority of the AEs (82 AEs, 63%) were mild in severity while the remaining 48 AEs (37%) were moderate in severity. Seventy one of the AEs (55%) were judged unrelated to study drug while only 32 AEs (25%) were related to study drug; the relationship of the remaining 27 AEs was equivocal. Thirty of the AEs related to the study drug occurred within 48 hrs of study drug infusion while the remaining 2 AEs occurred 8 and 30 days after study drug infusion. The most common AEs related to study drug infusion were fever, chills, myalgia, arthralgia, and headache; symptoms that are consistent with a transfusion
reaction. Garlic-like body odor was commonly observed and is related to the metabolism of DMSO used as a cryopreservative.

Expanded Discussion

The magnitude of the increase in CD4 counts above baseline was substantially more when compared with the results of previous adoptive T cell transfer studies in HIV-infected subjects that used the same cell culture technology.\textsuperscript{7-11} The number of gene modified cells was initially greater in immune responders than in immune non-responders despite both cohorts receiving essentially the same number of gene-modified cells. This may be related in part to the large inter-individual variations in CD4 T cell replicative capacity, differences in trafficking kinetics of modified CD4 cells, turnover rates, and telomere lengths seen in HIV infected subjects receiving HAART.\textsuperscript{12-14}

The pharmacokinetics of SB-728-T in peripheral blood most likely consists of an expansion phase for the first 2 to 4 weeks following infusion followed by decay with first order kinetics. It is also possible that differential trafficking and sequestration of CCR5 modified CD4 cells may also contribute to the observed temporal pattern of CD4 counts. The cells appear to be long-lived with a circulation half-life of approximately 1 year. With long term follow-up, gene modified cells have persisted in the circulation of all subjects from this study, the longest of which was >42 months post infusion as of June, 2013. Results from the rectal biopsies performed indicate that some of the infused cells have trafficked to, and are residing within mucosal tissues within 3 weeks of infusion. In a subsequent study, these cells were also detected in the inguinal lymph nodes of three subjects who were biopsied 9 to 18 months following SB-728-T infusion at levels similar to or greater than that in their circulation.\textsuperscript{15} Given that studies have estimated that about
98% of the total lymphocyte mass resides within lymphoid organs, including about 50% in mucosal tissues, it is possible that the total number of CCR5-modified T cells in the patients exceeds the number of cells that were infused.

The safety of CCR5-modified CD4 T-cells was assessed during a 12-week treatment interruption in the immune responders. Unfortunately, the treatment interruption was prematurely terminated in two subjects and thus, data for the entire 12 weeks is available for only 4 subjects. These 4 subjects had a 0.6-, 0.8-, 1.1- and 2.2-log decrease in HIV-RNA from their peak levels before protocol-specified resumption of anti-viral therapy. Despite this decline, the viral load at the end of the treatment interruption in 3 of these subjects was similar in magnitude to that of their historical viral setpoints. However, in one subject, the peak viral load was ~2-log lower than his viral setpoint and was below the limit of detection of detection by the end of the treatment interruption and before resumption of antiviral therapy. The subject’s serum was tested to exclude potential surreptitious use of anti-retrovirals. Interestingly, all participants were geneotyped and this subject was discovered to be the only CCR5Δ32 heterozygote. Therefore, we hypothesize that biallelic modification of the CCR5 CD4 cell compartment may have been greater in this subject. In preliminary analysis, we found a correlation between HIV-RNA and the estimated number of biallelicCCR5-modified CD4 cells in this study.

An interesting observation from this study is the absence of dramatic changes in total HIV-DNA in the PBMC reservoir during the treatment interruption. The development of digital droplet PCR which allows for precise and reproducible measurements of low total HIV-DNA copy numbers enabled us to assay samples with previously undetectable levels using traditional qPCR. The increased sensitivity of the assay can be attributed to the fractionation process which dilutes the background genomic DNA and thereby increases the signal to noise ratio. Despite a 3.1 log
increase in the mean HIV-RNA in the 6 subjects who underwent a treatment interruption, proviral DNA was stable in five of the six subjects. These data are in contrast to that previously published using traditional qPCR technology in which HIV-DNA increased or fluctuated over time in the majority of subjects who discontinued HAART. Therefore, our finding suggests that short-term interruption of HAART is unlikely to substantially modify the size of the latent HIV pool.
Supplementary Figures

Figure S1. Changes in circulating CD4 Lymphocytes.

The CD4 T-lymphocyte values for subjects in the two cohorts are presented in greater detail than in Figure 1 of the main text.
Figure S2. CCR5-modified CD4 T-lymphocytes.

The median percentage of CCR5-modified CD4 T-cells within the PBMC compartment.
Figure S3. HIV-DNA and -RNA during Treatment Interruption.

Panel A. HIV-DNA was measured using digital droplet technology along with HIV viremia for each subject in cohort 1. Despite marked increases in HIV-RNA following HAART interruption, HIV-DNA was stable for 5 of the 6 subjects. For Subject 205, a small change in viral RNA in the absence of a change in proviral DNA can be observed when scale for the VL was expanded (see Figure S3 in the Supplementary Appendix). Note that viral load is plotted on log10 scale and that HIV DNA is plotted on a linear scale. Panel B. The scale for viral load shown in Figure 5 of main text was expanded and plotted for Subject 205 on a linear scale.

A.
B. Subject 205 HIV Proviral Load and HIV Viremia

Subject 205 HIV Proviral Load and HIV Viremia

Treatment Interruption

Viral load (copies/mL)

Proviral DNA

Viral load

Proviral DNA

Days

HIV gag (copies/10^6 CD4 cells)
Figure S4. HIV DNA in PBMC in Cohort 2.

HIV-DNA was measured in PBMC using digital droplet PCR technology for each subject in cohort 2. The patients were aviremic by standard assay (< 75 copies/ml plasma). HIV DNA is plotted on a log scale. The results were analyzed by linear regression and the slope ±SE is indicated for each subject. The lower limit of quantitative detection (LLOQ) and the limit of detection (LOD) are indicated.
Figure S5. HIV DNA in Concurrent Control Cohort, subjects 1 to 4.

HIV-DNA was measured in PBMC using digital droplet PCR technology for each subject in the concurrent control cohort that did not receive SB-728 T cell infusions. Demographics for this cohort are in Table S3. The patients were aviremic by standard assay (< 75 copies/ml plasma). HIV DNA is plotted on a log scale. The results were analyzed by linear regression and the slope±SE is indicated for each subject. The lower limit of quantitative detection (LLOQ) and the limit of detection (LOD) are indicated.
Figure S6. HIV DNA in Concurrent Control Cohort, subjects 5 to 8.

HIV DNA was measured in control subjects #5 to 8 as described above in Figure S5.

- Pt # 110881
  slope -0.000386 ± 6.532e-5

- Pt # 110935
  slope 1.40e-6 ± 0.000112

- Pt # 110951
  slope -4.264e-5 ± 5.064e-5

- Pt # 130104
  slope 0.000620 ± 0.000402
Figure S7. Calculation of estimated biallelic cell modification during treatment interruption.

ZFN mediated gene modification can generate a wide range of frame-shift mutations to disrupt the CCR5 gene locus. A PCR-based assay was developed to measure a specific five-nucleotide duplication at the ZFN cleavage site. This five-nucleotide modification is detected in approximately 25% of the total number of CCR5 modified cells. Therefore, the total number of modified cells is calculated by multiplying the number of pentamers by 4. Clonal analysis of ZFN modified cells indicated that approximately 33% of cells underwent bi-allelic modification, a rate which is doubled if one CCR5 allele was already modified by the natural delta32 mutation. Accordingly, the number of CCR5 modified cells (pentamer x 4) is then multiplied by 1/3 in subjects with wild type CCR5 and by 2/3 in subject 205 who is a delta32 heterozygote.
Supplemental Tables

Table S1. Cohort 1: Tabular listing of CD4 counts and CCR5 modified (SB 728-T) cells in blood.

| Subject | Screen | Pre-Dose Safety Eval | Day 3 | Day 7 | Day 14 | Day 21 | STI Wk 4 | STI Wk 6 | STI Wk 8 | STI Wk 10 | STI Wk 12 | WTI Wk 14 | ART Wk 20 | ART Wk 24 | ART Wk 28 | ART Wk 32 | ART Wk 36 |
|---------|--------|----------------------|-------|-------|--------|--------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| Total CD4 Cells/mm³ |        |                      |       |       |        |        |          |          |          |          |          |          |          |          |          |          |          |          |
| 201     | 546    | 665                  | 800   | 938   | 824    | 627    | 790      | 719      | 644      | 701      | 610      | 861      | 725      | 644      | 614      | 640      | 829      |          |
| 203     | 996    | 659                  | 829   | 2728  | 2523   | 2084   | 1983     | 1584     | 1304     | 1122     | 1088     | 882      | 1224     |          |          |          |          | 1007     |          |
| 204     | 788    | 621                  | 849   | 837   | 748    | 722    | 720      | 746      | 464      | 462      | 577      | 596      | 599      | 805      | 756      |          |          |          |          |
| 205     | 1121   | 955                  | 2025  | 3113  | 2364   | 2082   | 2388     | 2341     | 2049     | 2031     | 2190     | 1789     | 1664     | 1641     |          |          |          |          |          |
| 251     | 951    | 554                  | 1937  | 2014  | 1879   | 1508   | 1714     | 1838     | 1335     | 1068     | 538      | 805      | 958      | 526      | 1196     | 1102     | 1255     |          |
| 253     | 1123   | 997                  | 2750  | 4022  | 2896   | 2148   | 2881     | 1915     | 2485     | 1818     | 1932     | 1616     | 1645     | 1648     | 1754     | 1670     |          |          |
| CCR5 Modified CD4 Cells/mm³ |        |                      |       |       |        |        |          |          |          |          |          |          |          |          |          |          |          |          |          |
| 201     | ND     | 52                   | 63    | 51    | 19     | 26     | 24       | 22       | 21       | 20       | 17       | 17       | 13       | 14       | 13       | 23       | 13       |          |
| 203     | ND     | 210                  | 545   | 765   | 600    | 546    | 402      | 399      | 216      | 125      | 144      | 136      |          |          |          |          |          |          |          |
| 204     | ND     | 54                   | 48    | 70    | 65     | 56     | 60       | 30       | 23       | 36       | 31       | 21       | 29       | 41       |          |          |          |          |          |
| 205     | ND     | 229                  | 414   | 480   | 359    | 403    | 405      | 269      | 301      | 272      | 192      | 187      | 230      |          |          |          |          |          |          |
| 251     | ND     | 185                  | 230   | 169   | 163    | 114    | 173      | 194      | 130      | 73       | 107      | 102      | 97       | 91       | 77       |          |          |          |
| 253     | ND     | 528                  | 694   | 331   | 374    | 433    | 300      | 367      | 228      | 239      | 187      | 198      | 184      | 202      | 153      |          |          |          |
Table S2. Cohort 2: Tabular listing of CD4 counts and CCR5 modified (SB 728-T) cells in blood.

<table>
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<td>305</td>
<td>308</td>
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<td><strong>CCR5 Modified CD4 Cells/mm³</strong></td>
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<td>308</td>
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<td>35</td>
<td>30</td>
<td>30</td>
<td>18</td>
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<td>93</td>
<td>99</td>
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<td>ND</td>
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<td>19</td>
<td>13</td>
<td>15</td>
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</table>
Table S3. Concurrent Control Cohort: Demographics and specimens tested for HIV DNA by digital droplet PCR.

<table>
<thead>
<tr>
<th>CFAR ID</th>
<th>Gender</th>
<th>Regimen (at first visit)</th>
<th>Months undetectable before first PBMC specimen</th>
<th>PBMC specimens tested for HIV DNA</th>
<th>Age(^a)</th>
<th>Duration of PBMC follow up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median</td>
<td>100% M</td>
<td>50% PI based, 25% NNRTI based, 13% triple nucleoside, 13% integrase based</td>
<td>22</td>
<td>43 4.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td></td>
<td></td>
<td>8-39</td>
<td>24-60 3.2-4.7</td>
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</tbody>
</table>

\(^a\) Age at the time of first PBMC sample
**Table S4. Changes in HIV DNA per Million Cells in Blood in Cohort 2 and the Aviremic Control Cohort.**

<table>
<thead>
<tr>
<th>Change in HIV DNA /1x10^6 PBMC per year</th>
<th>Concurrent Control Cohort (N=8)</th>
<th>Cohort 2 (N=6)</th>
<th>Mean difference (p-value)**</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Median</td>
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<td></td>
<td>-0.0049193</td>
<td>0.1080231</td>
<td>-0.0094207</td>
</tr>
</tbody>
</table>

* See Table S3 for demographics of the control cohort.
Supplemental References


17. Imamichi H, Crandall KA, Natarajan V, et al. Human immunodeficiency virus type 1 quasi species that rebound after discontinuation of highly active antiretroviral therapy are similar to the viral quasi species present before initiation of therapy. J Infect Dis 2001;183:36-50.