Supplementary Appendix

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

Supplement to: Tebas P, Stein D, Tang WW, et al. Gene editing of *CCR5* in autologous CD4 T cells of persons infected with HIV. N Engl J Med 2014;370:901-10. DOI: 10.1056/NEJMoa1300662

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$\pm$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,[2] 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.[2] 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.[2]

The SB-728 T cells were manufactured as described.[1] 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

and transduced with SB-728 vector.[2] 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 $\pm$ 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[5]: HIVgag forward CATGTTTTCAGCATTATCAGAAGGA, HIVgag reverse TGCTTGATGTCCCCCCACT, HIVgag probe, FAM-CCACCCCACAAGATTTAAACACCATGCTAA-BHQ) and the human Ribonuclease P protein subunit p30 (RPP30 forward GATTTGGACCTGCGAGCG, RPP30 reverse GCGGCTGTCTCCACAAGT, RPP30 probe VIC-CTGACCTGAAGGCTCT-MGB-BHQ).[5,6] 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.[6] 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.[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.[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.[15] Given that studies have estimated that about

98% of the total lymphocyte mass resides within lymphoid organs, including about 50% in mucosal tissues,[3] 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.[6] 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.[16] Therefore, our finding suggests that short-term interruption of HAART is unlikely to substantially modify the size of the latent HIV pool.[17]

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.

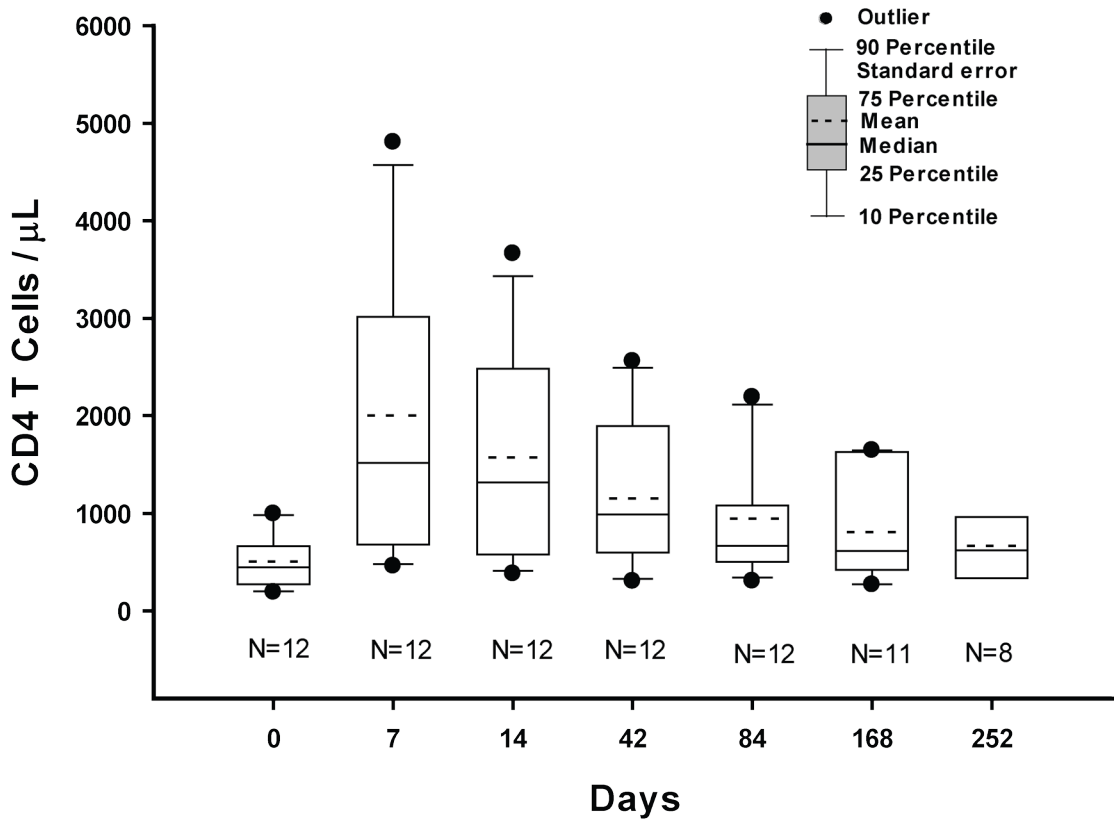


Figure S2. CCR5-modified CD4 T-lymphocytes.

The median percentage of CCR5-modified CD4 T-cells within the PBMC compartment.

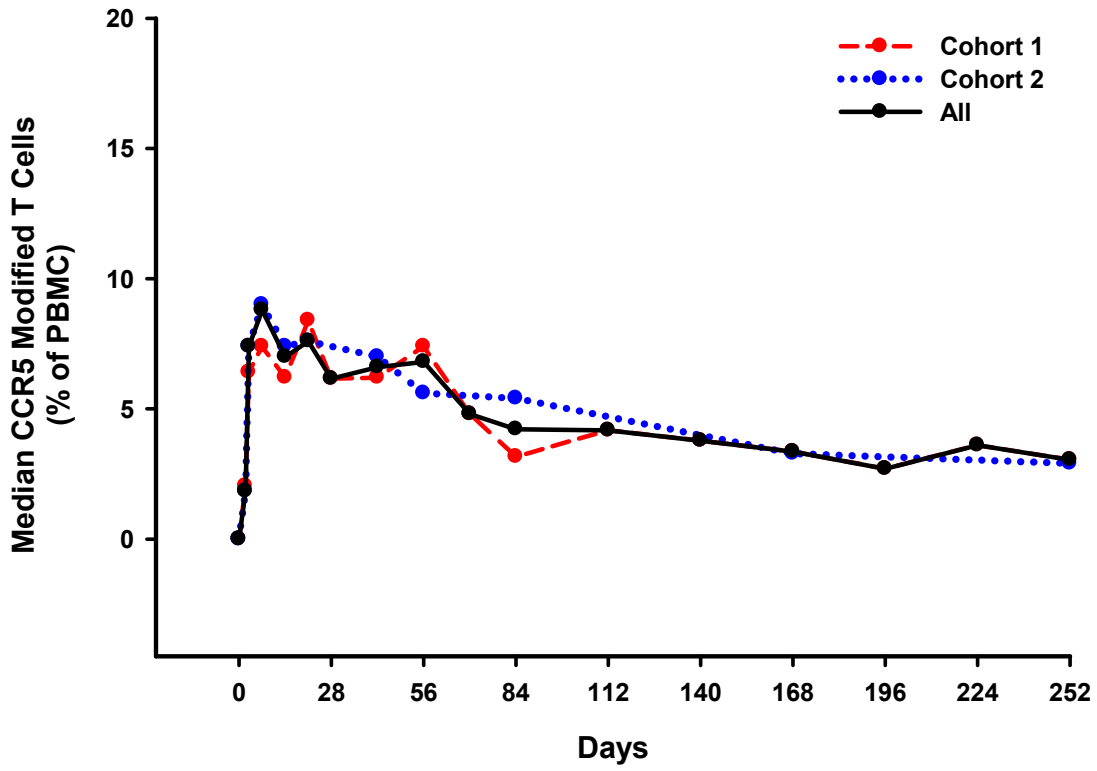
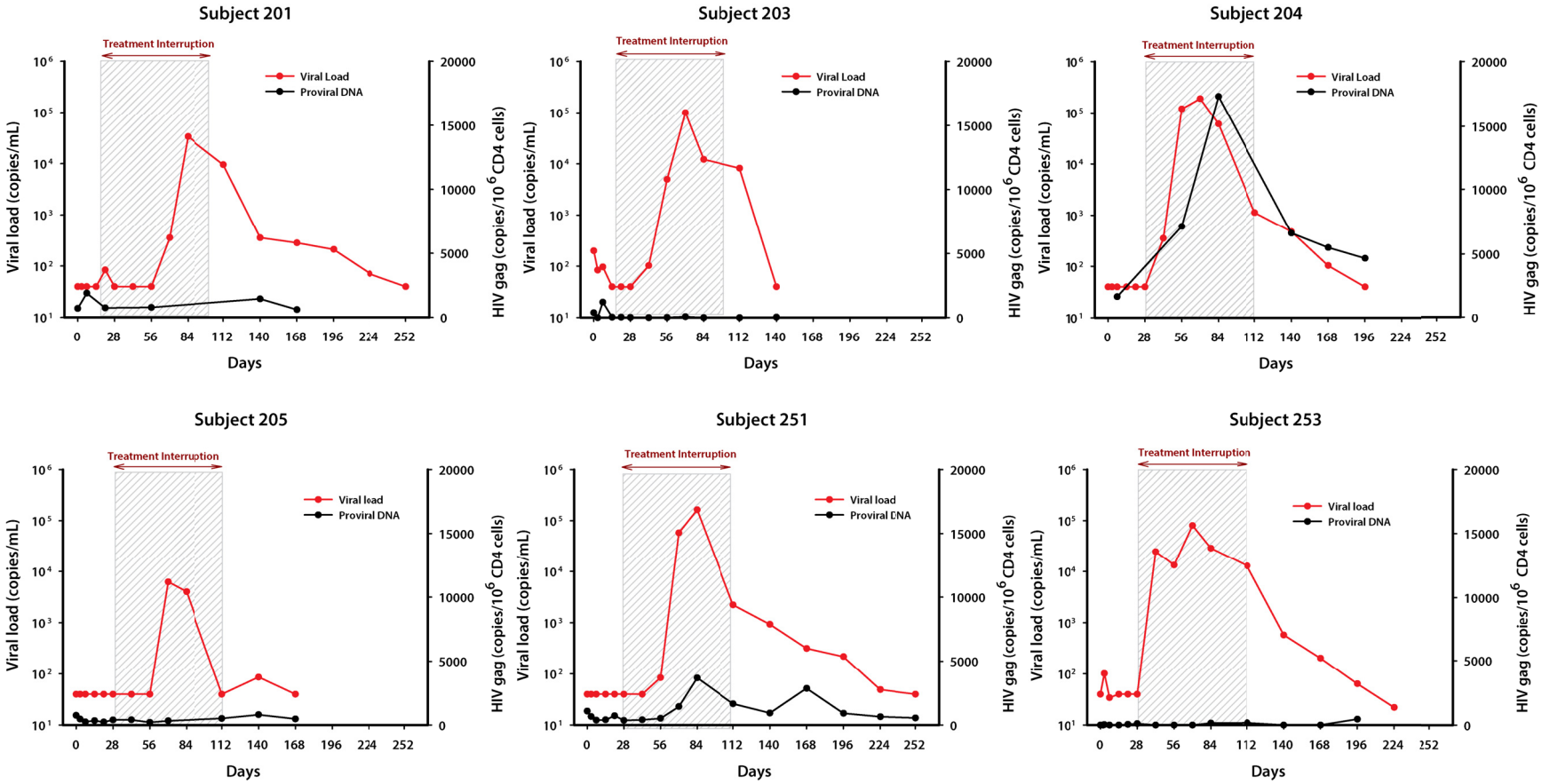


Figure S3. HIV-DNA and -RNA during Treatment Interruption.

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.

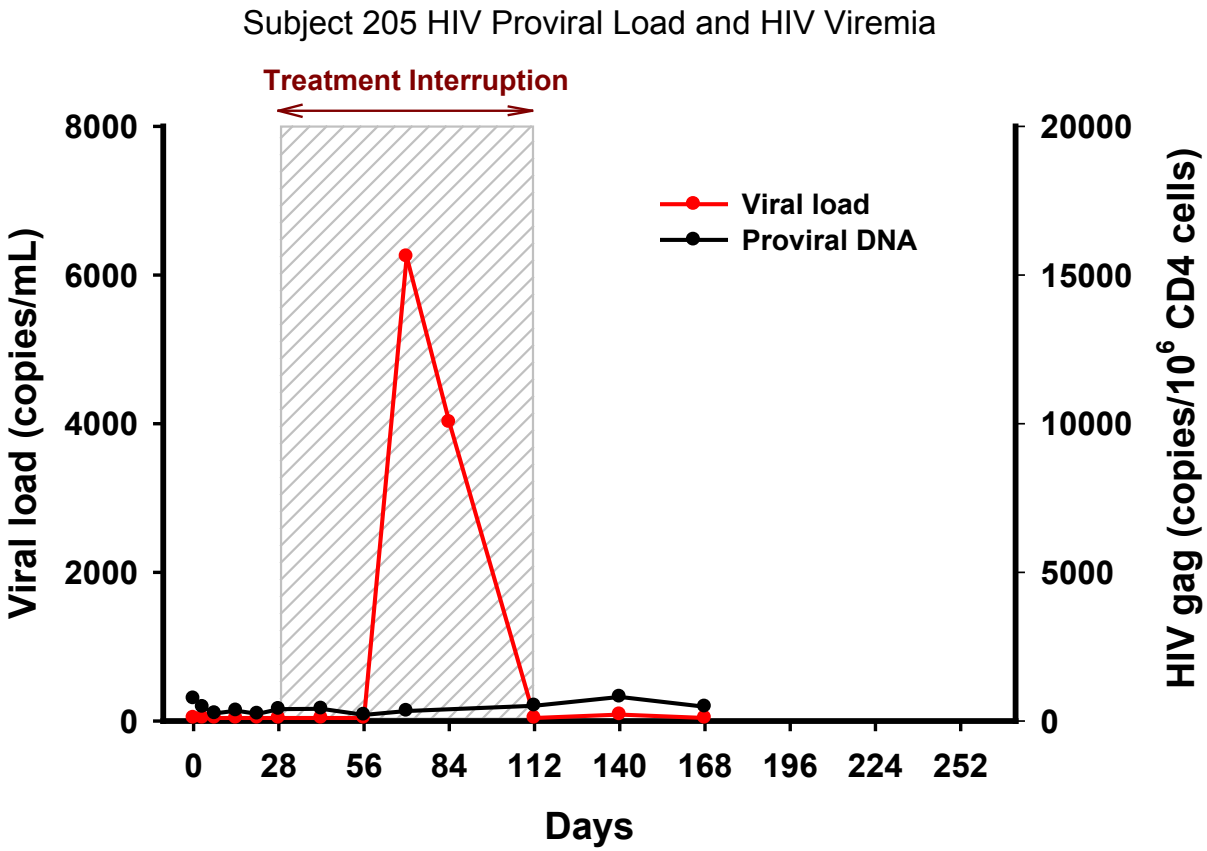

Subject 205 HIV Proviral Load and HIV Viremia
Treatment Interruption
Viral load
Proviral DNA
Viral load (copies/mL)
HIV gag (copies/10^6 CD4 cells)
8000
6000
4000
2000
0
20000
15000
10000
5000
0
0
28
56
84
112
140
168
196
224
252
Days

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.

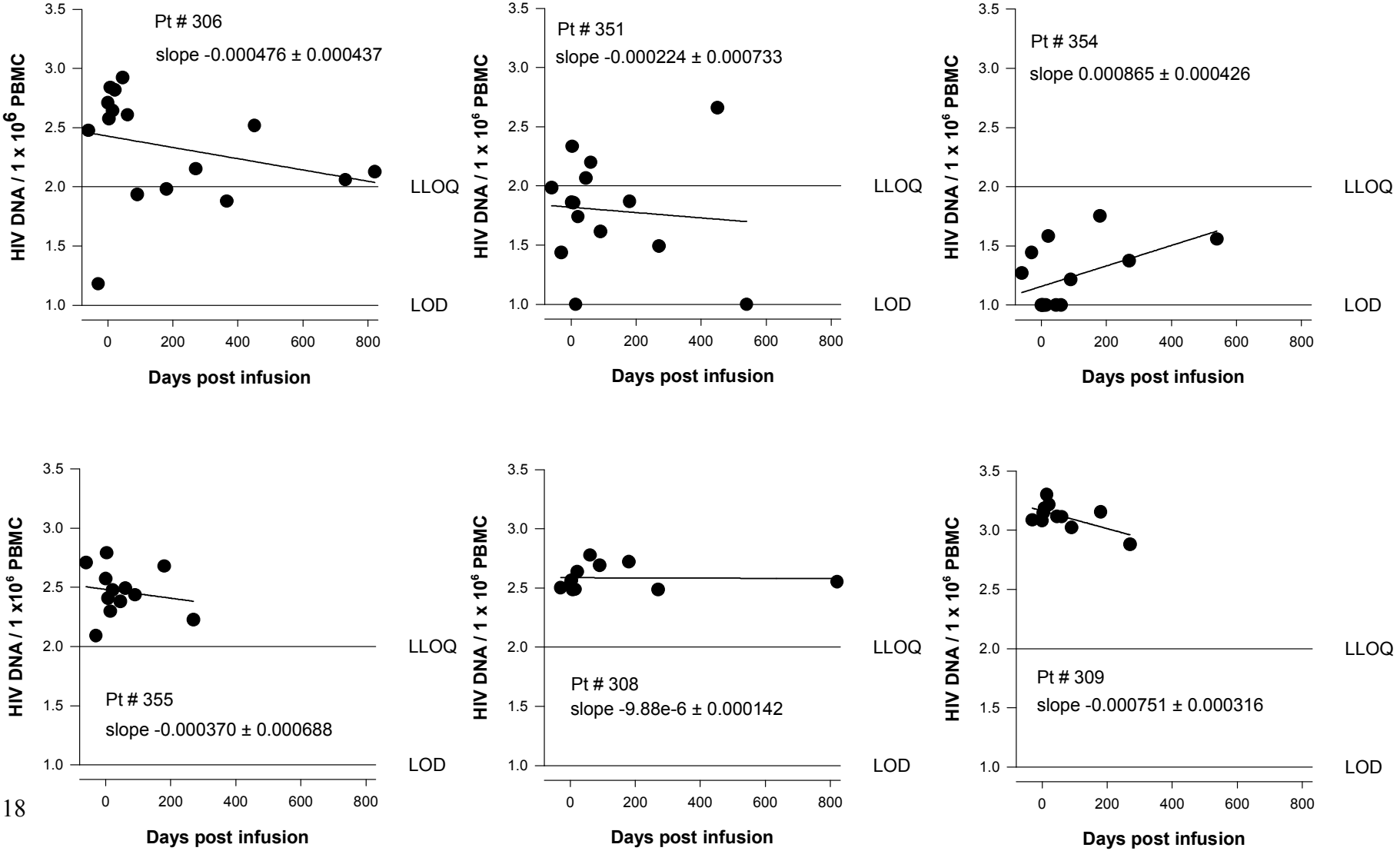


Figure S5. HIV DNA in Concurrent Control Cohort, subjects 1 to 4.

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.

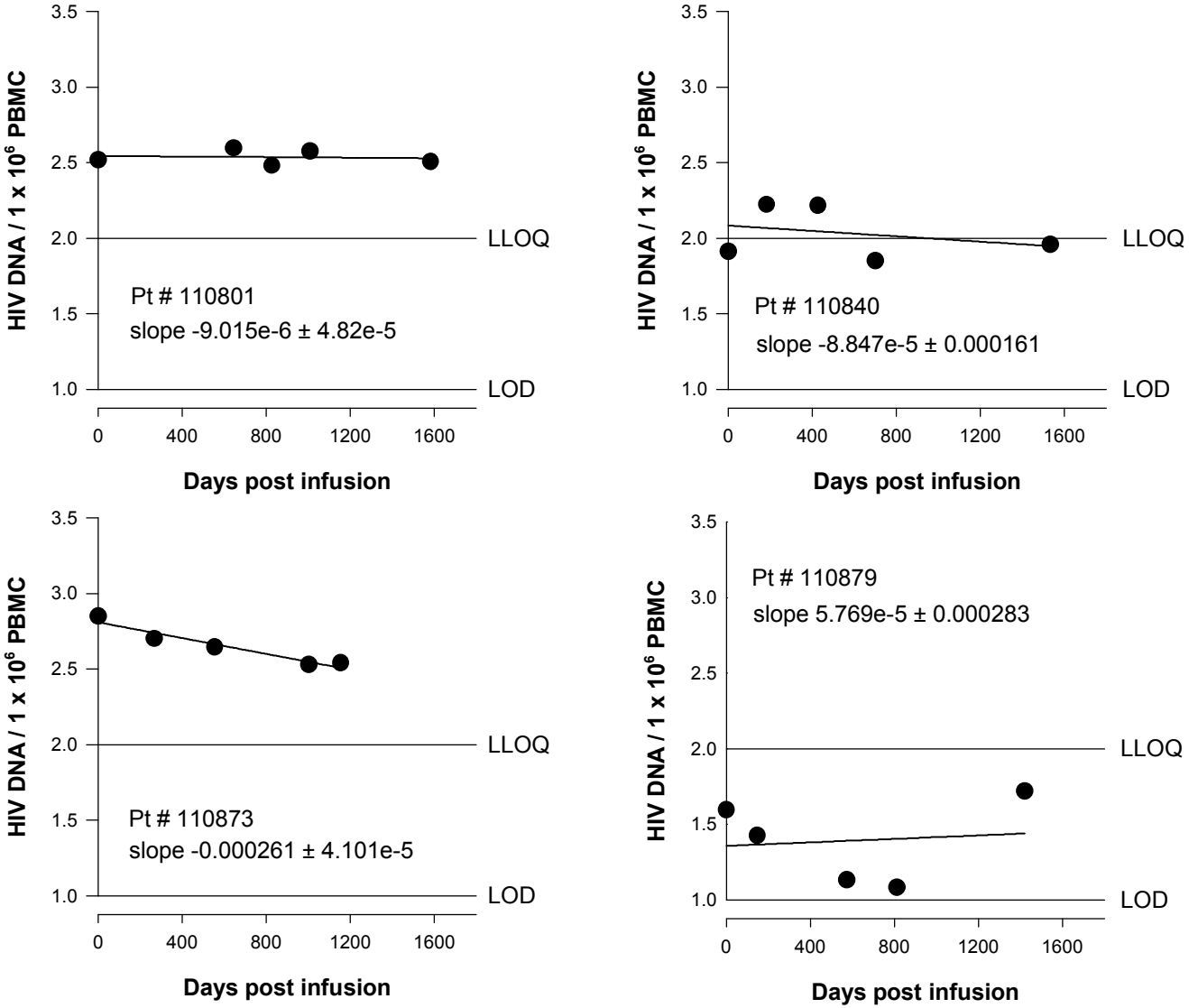


Figure S6. HIV DNA in Concurrent Control Cohort, subjects 5 to 8.

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

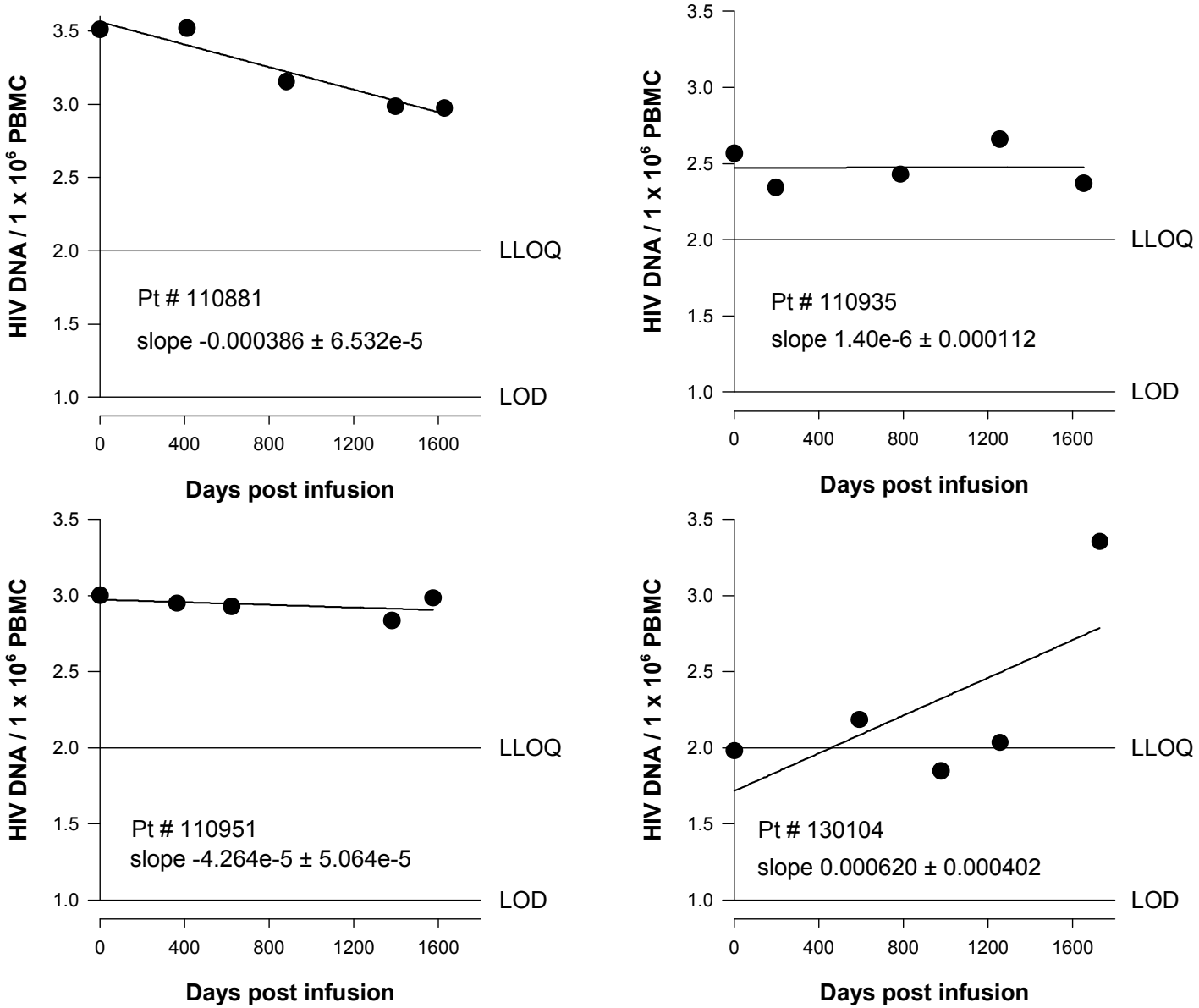
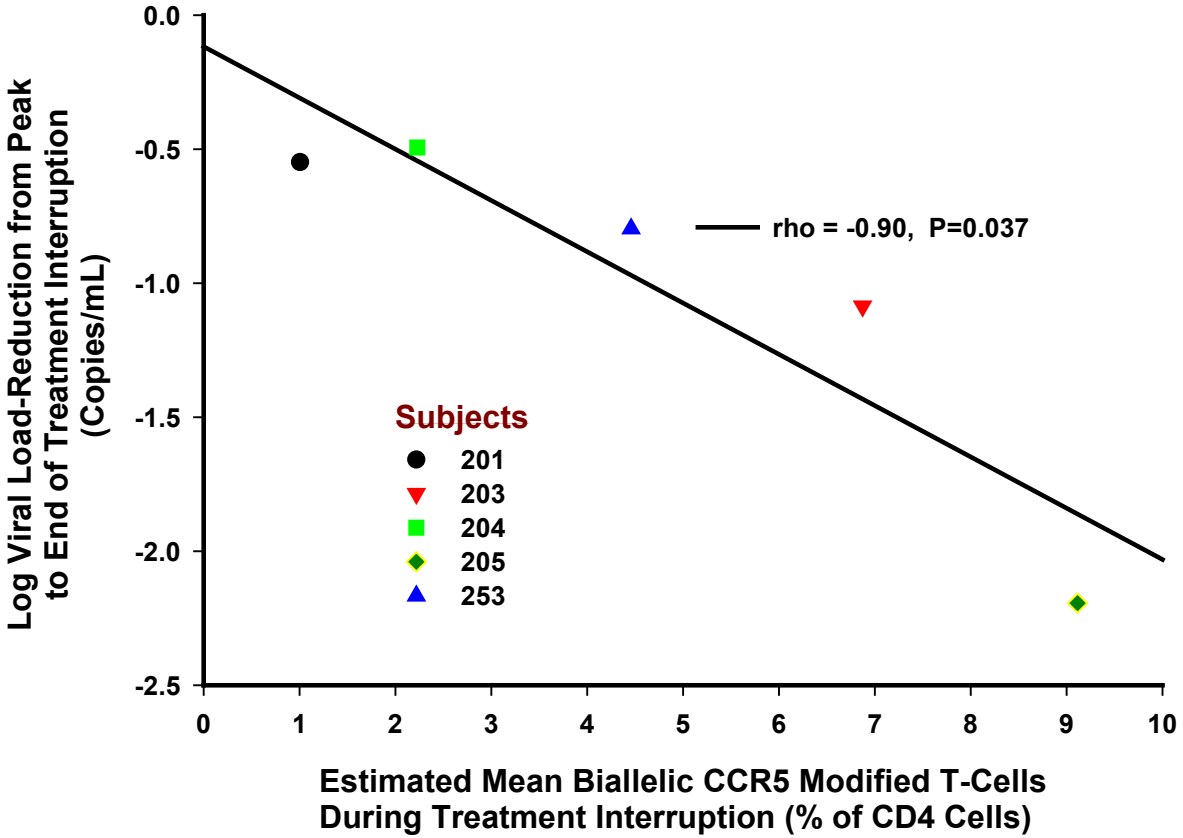


Figure S7. Calculation of estimated biallelic cell modification during treatment interruption.

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 multipled 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/mm^3																	
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/mm^3																	
201	ND	0	52	63	51	19	26	24	22	21	20	17	17	13	14	23	13
203	ND	0	210	545	765	600	546	402	399	216	125	144	136				156
204	ND	0	54	48	70	65	56	60	30	23	36	31	21	29	41		
205	ND	0	229	414	480	359	403	405	269	301	272	192	187	230			
251	ND	0	185	230	169	163	114	173	194	130	73	107	102	101	97	91	77
253	ND	0	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.

Subject	Screen	Pre-Dose Safety Eval	Day 3	Day 7	Day 14	Day 21	Wk 6	Wk 8	Wk 12	Wk 24	Wk 36
Total CD4 Cells/mm^3											
306	243	271	920	1873	1521	1309	1126	787	727	573	651
308	357	328	434	627	473	399	557	476	493	421	382
309	476	341	1614	4808	3664	3154	2560	2263	1068	1631	
351	221	193	560	525	523	410	381	462	418	270	318
354	229	220	739	1162	1118	1119	854	902	732	616	589
355	236	272	363	462	383	343	306	305	308	284	319
CCR5 Modified CD4 Cells/mm^3											
306	ND	0	145	271	181	199	222	117	82	77	62
308	ND	0	35	30	30		18	17	18	16	
309	ND	0	459	1106	921	753	741	457	288	426	
351	ND	0	110	112	94	74	58	58	53	22	26
354	ND	0	155	286	249	232	164	150	143	93	99
355	ND	0	62	57	41	29	19	13	15	12	

Table S3. Concurrent Control Cohort: Demographics and specimens tested for HIV DNA by digital droplet PCR.

CFAR ID	Gender	Regimen (at first visit)	Months undetectable before first PBMC specimen	PBMC specimens tested for HIV DNA					Age[a]	Duration of PBMC follow up
110801	M	PI based	11	06/Jun/2007	11/Mar/2009	09/Sep/2009	10/Mar/2010	05/Oct/2011	46	4.3
110840	M	PI based	20	17/Apr/2007	16/Oct/2007	17/Jun/2008	17/Mar/2009	28/Jun/2011	42	4.2
110873	M	PI based	8	30/Aug/2006	24/May/2007	06/Mar/2008	28/May/2009	27/Oct/2009	42	3.2
110879	M	NNRTI based	10	05/Nov/2007	31/Mar/2008	01/Jun/2009	25/Jan/2010	26/Sep/2011	52	3.9
110881	M	NNRTI based	29	09/Mar/2007	23/Apr/2008	06/Aug/2009	04/Jan/2011	23/Aug/2011	29	4.5
110935	M	Integrase based	31	30/Jul/2007	11/Feb/2008	23/Sep/2009	06/Jan/2011	07/Feb/2012	60	4.5
110951	M	PI based	39	17/Oct/2007	15/Oct/2008	30/Jun/2009	28/Jul/2011	09/Feb/2012	24	4.3
130104	M	NRTI based	24	30/Nov/2006	14/Jul/2008	04/Aug/2009	10/May/2010	25/Aug/2011	43	4.7
Median	**100% M**	**50% PI based, 25% NNRTI based, 13% triple nucleoside, 13% integrase based**	**22**						**43**	**4.3**
Range			**8-39**						**24-60**	**3.2-4.7**

[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.

	Concurrent Control Cohort (N=8)			Cohort 2 (N=6)			Mean difference (p-value)**
Change in HIV DNA /1x10^6 PBMC per year	Mean	SD	Median	Mean	SD	Median	
	-0.0049193	0.1080231	-0.0094207	-0.0585144	0.2044398	-0.1078575	0.0198823 (0.646)

* See Table S3 for demographics of the control cohort.

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