Gene transfer in humans using a conditionally replicating lentiviral vector

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We report findings from a clinical evaluation of lentiviral vectors in a phase I open-label nonrandomized clinical trial for HIV. This trial evaluated the safety of a conditionally replicating HIV-1-derived vector expressing an antisense gene against the HIV envelope. Five subjects with chronic HIV infection who had failed to respond to at least two antiviral regimens were enrolled. A single i.v. infusion of gene-modified autologous CD4 T cells was well tolerated in all patients. Viral loads were stable, and one subject exhibited a sustained decrease in viral load. CD4 counts remained steady or increased in four subjects, and sustained gene transfer was observed. Self-limiting mobilization of the vector was observed in four of five patients. There is no evidence for insertional mutagenesis after 21–36 months of observation. Immune function improved in four subjects. Lentiviral vectors appear promising for gene transfer to humans.

clinical trials | HIV | immunotherapy | gene therapy

In preclinical studies, a highly efficient T cell culture system and a lentiviral vector containing a long antisense sequence to HIV envelope were developed (1–3). In a prior phase I trial, autologous CD4 cells from HIV-infected subjects were expanded *ex vivo* by using anti-CD3/CD28 beads as artificial antigen presenting cells, and adoptive transfer of the activated $CD4^+$ T cells was shown to result in dose-dependent increases in the steady-state $CD4^+$ T cell counts by induction of resident $CD4^+$ cell proliferation and a sustained decrease in CCR5 expression *in vivo* (4). Concurrently, an HIV-1-based lentiviral vector expressing a 937-base antisense gene against the HIV envelope, termed VRX496, was developed (Fig. 1*A*). This vector retains the full LTRs of HIV, and, therefore, expression of the antisense is up-regulated upon wild type HIV infection of the cell. This LTR-dependent transcriptional up-regulation is in contrast to self-inactivating vectors, where the LTRs are modified by deletion of the U3 region, and transgene expression is driven from a heterologous internal promoter. An advantage of using a long antisense sequence is that it targets multiple sites of HIV genomic RNA, constraining the pathogenic virus' ability to form resistance mutants without adversely affecting viral fitness (2). In preclinical studies bringing these two technologies together, the rationale for the present clinical study was supported by the potent antiviral effects that were demonstrated *in vitro*, regardless of patient status or the tropism of the infecting virus (5).

After extensive preclinical safety tests (6, 7) approval by institutional review boards, the Food and Drug Administration (FDA), the FDA's Biological Response Modifiers Advisory Committee (now called the Cellular, Tissue, and Gene Therapies Advisory Committee), and the National Institutes of Health Office of Biotechnology Activities, a phase I open label nonrandomized clinical trial, was initiated to investigate the safety and tolerability of autologous T cells modified with the VRX496 vector. In this study, 5 subjects with HIV infection that was resistant to at least two antiviral regimens and who had a viral load >5000 copies per ml and $CD4^+$ T cells counts between 200–500 cells per mm^3 were serially enrolled. Each subject was given a single infusion of $\approx 1 \times 10^{10}$ gene modified autologous $CD4^+$ T cells in a single dose. The primary endpoints for safety were incidence of adverse events, viral load, $CD4^+$ count, and emergence of a replication-competent lentivirus (RCL) derived from the vector (i.e., distinct from wild-type (wt)-HIV). The primary feasibility endpoint was the ability to manufacture autologous lentiviral engineered $CD4^+$ T cells from subjects with drug-resistant HIV infection. Secondary endpoints included measurements of vector persistence and immunological functional assessments.

Results

Patient Characteristics. Baseline characteristics for the five subjects with chronic HIV infection who participated in the study are shown in Table 1. The first subject was infused in July 2003, and the fifth subject was infused in September 2004. All subjects had drug-resistant HIV infection, having received therapy with a mean 7.6 (range 4–10) different antiretroviral agents. Enrolled patients must have been on their present therapy for at least 3 months and agree to continue their therapy for at least 6 months. Four subjects elected to remain on their present failing therapy, and one subject was intolerant of antiretroviral therapy. Fig. 5, which is published as supporting information on the PNAS web site, provides the date of infusion for each patient and the dates of the initiation of their current anti retroviral regimens. Peripheral blood mononuclear cells were obtained by apheresis, the $CD4^+$ T cells were enriched by negative selection, and the cells were transduced with the VRX496 lentiviral vector at a multiplicity of infection (MOI) of 5 as described in *Supporting Methods*, which is published as supporting information on the PNAS web site. All subjects were infused with the target dose of ≈10 billion cells, and it is noteworthy that the $CD4^+$ cells were efficiently transduced, as the average vector copy number per cell for the 5 infused products was 2.1 (range 1.0 to 4.1). This large-scale cell manufacturing process was performed under GMP-compliant conditions, confirming our preclinical data (3, 5), and substantiating the promise of lentiviral vectors as generally more efficient in transduction than other viral vectors in a number

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Conflict of interest statement: L.M.H., T.R., X.L., G.K.B., V.S., F.L., and B.D. were employees of VIRxSYS Corporation at the time this study was performed.

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Abbreviations: PBMC, peripheral blood mononuclear cell; RCL, replication-competent lentivirus; wt, wild type.

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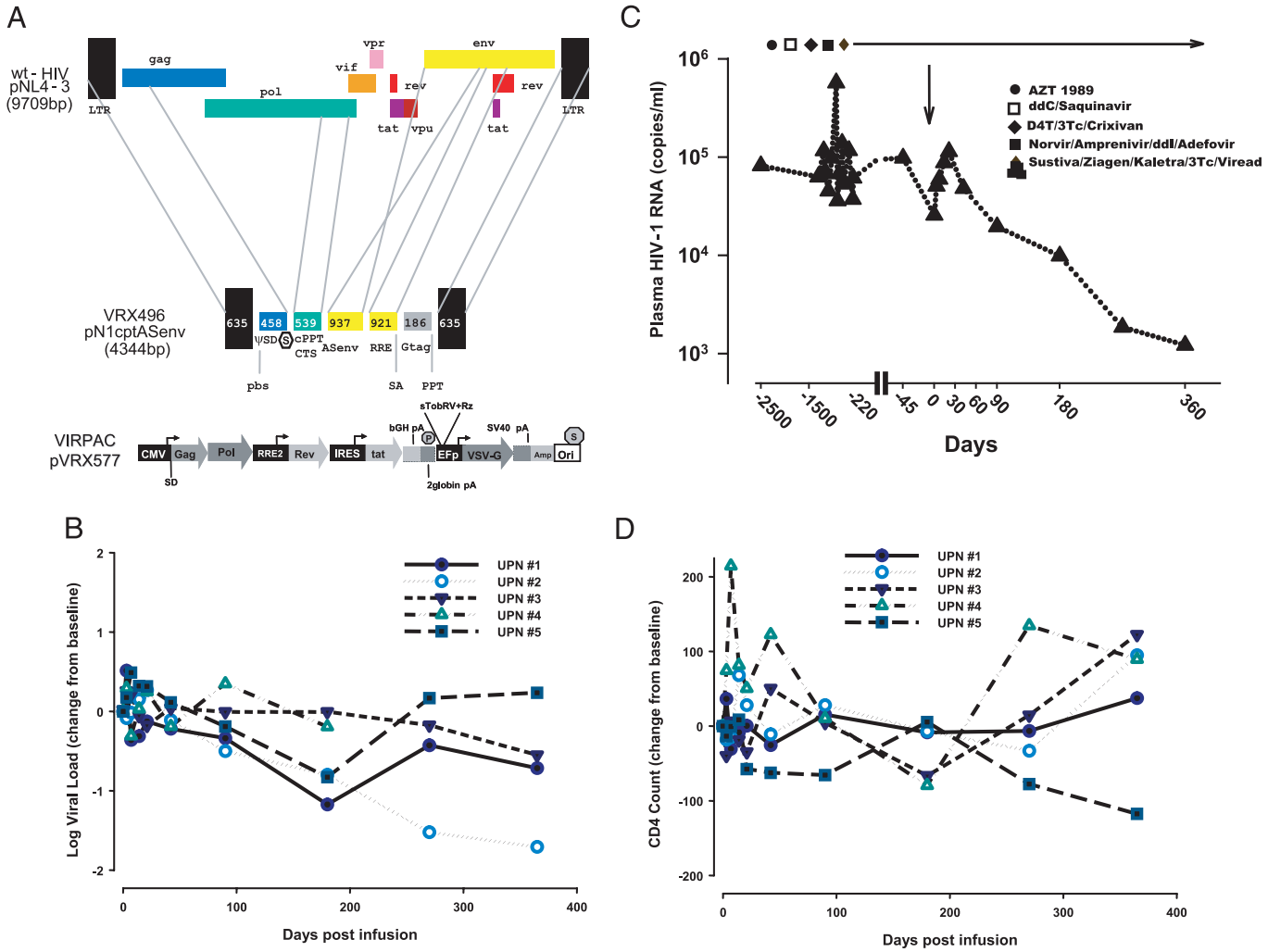

Fig. 1. Schematic representation of the gene-transfer vector, VRX496, and viral loads and CD4 counts after treatment. (*A*) Vector design. (*Upper*) Schematic representation of pN1cptASenv (VRX496), depicting elements of the vector and the regions of wt-HIV$_{NL4-3}$ from which they were derived. The numbers in the vector refer to the size of the genetic elements. VRX496 is derived from the NL4-3 clone of wild-type HIV. The vector expresses a 937-bp antisense segment targeted against HIV envelope gene (ASenv). The antisense payload is Tat- and Rev-dependent, and, thus, basal expression is increased after HIV infects vector-containing cells. HIV-derived elements include the 5′ and 3′ long terminal repeat (LTR), a packaging signal (Ψ), tRNA primer-binding site (pbs), central polypurine tract and central termination sequence (cPPT and CTS), splice acceptor and donor sites (SA and SD), Tat-dependent HIV promoter (P), *Gag* gene, rev response element (RRE), and 3′ polypurine tract (PPT). Engineered elements include a stop codon in gag (3). Gtag is a noncoding marker sequence from GFP. (*Lower*) Schema of pVRX577 (VIRPAC), the helper packaging construct. VRX496 is pseudotyped with a vesicular stomatitis virus protein G (VSV-G) envelope. Gag and pol are expressed under the control of the CMV promoter, Rev under the control of the rev response element derived from HIV-2 (RRE-2), which is used to reduce homology between VRX496 and VIRPAC, tat under the control of an internal ribosomal entry site (IRES), and VSV-G expressed by an elongation factor 1α (EF-1α)/human T cell lymphotrophic virus (HTLV) chimeric promoter. VSV-G is separated from the other packaging genes for safety by several pause signals and a *cis*-acting ribozyme derived from the tobacco mosaic ringspot virus (sTobRV+Rz) that will cleave any read-through RNA. Sequences of *rev* and *tat* genes were partially degenerated to reduce homology with the vector. (*B*) Primary endpoints. The log plasma HIV viral load is depicted as change from baseline. The baseline values are shown in Table 1. Changes >0.5 log were outside the variation of the assay and were considered meaningful. The VRX496 cell infusion was given on day 0. Subject 4 began an antiretroviral therapy regimen 7 months after infusion, and his viral load became undetectable at that point. (*C*) Subject 2 course and detailed viral load. The detailed course for subject 2 is shown, with all available viral loads and a summary of antiretroviral therapy plotted. Note that the *x*-axis scale changes on day 0 to display the available baseline values. The vertical arrow depicts the time of the VRX 496 modified CD4$^+$ cell infusion. The date of infusion was October 13, 2003; the most recent viral load is 1,930 copies per ml (January 2006). (*D*) CD4 cell counts. CD4 cell counts are plotted as a change from baseline after the VRX496 infusion. Baseline values are shown in Table 1. Subject 4 began a new antiretroviral therapy regimen 7 months after infusion, and his viral load became undetectable at that point.

of *in vitro* cell culture models (8). There was no evidence of ongoing HIV replication as measured by before and after cell-expansion proviral copy numbers or the generation of a vector-derived RCL determined by biological RCL assay (*Methods*) in any of the cell products (Table 1), nor in any of the clinical vector lots to date (7). The infusions were well tolerated, and no subjects experienced serious adverse events that were judged as possibly, likely, or definitely related to the VRX496 cells.

Primary Trial Endpoints. Subjects were monitored at 1, 2, 3, 7, 14, and 21 days, 6 weeks and at 3, 6, and 9 months for viral load, CD4 count, emergence of any potential RCL, and for immunological parameters. Importantly, no subject has clinical or laboratory evidence of vector-derived RCL *in vivo*, which was evaluated by monitoring for VSV-G DNA in peripheral blood mononuclear cells (PBMCs), VSV-G RNA in patient plasma, generation of antibody against the VSV-G protein by ELISA (Table 2, which is published as supporting information on the PNAS web site), and a biological RCL assay performed on patient PBMCs 6 months after dosing. Mean viral load at screening ranged from 19,970 to 188,500 copies per ml, with a median viral load of 68,224. The change from baseline in viral load log values in all

Table 1. Baseline subject characteristics

Characteristic	Subject 1	2	3	4	5
Age	41	44	40	27	45
Gender	M	M	M	M	M
Ethnic group	Caucasian	Caucasian	African American	African American	Caucasian
Baseline viral load	188,500	54,100	46,150	32,400	19,970
Baseline CD4 count per mm^3	253	316	273	308	220
HIV infection, y	15	15	15	10	9
Previous ARV experience	4 NRTI, 2 NNRTI, 4 PI	5 NRTI, 4 PI	6 NRTI, 1 PI	2 NRTI, 1 NNRTI, 1 PI	5 NRTI, 1 NNRTI, 1 PI
Therapy at enrollment	1 NRTI, 2 PI	3 NTRI, 1 NNRTI, 1 PI	None	2 NRTI, 1 NNRTI	2 NNRTI, 1 PI
No. of VRX496 CD4 cells infused	1×10^{10}	1×10^{10}	0.6×10^{10}	1×10^{10}	0.9×10^{10}
$CD3^+$ cells*, %	95	100	80	98	94
Average vector copy no. per cell*	2.3	1.8	1.0	4.1	1.2
p24 pg/ml*	<50	<50	<50	<50	<50
RCL	Negative	Negative	Negative	Negative	Negative

NRTI, nucleoside reverse-transcriptase inhibitor; NNRTI, nonnucleoside reverse-transcriptase inhibitor; PI, protease inhibitor.
*Value determined at the time of cell harvest.

patients is shown in Fig. 1*B*. Viral load changes greater than 0.5 log were noted as meaningful because they are outside of the variation of the viral load assay. Transient increases in viral load was observed in patients shortly after dosing presumably because of cytokine release; this increase has previously been observed (9). Subject 1 entered the study with a mean baseline viral load of 188,500 (5.27 logs) copies per ml, and, at the 6 and 12 month points, his viral load declined to 12,700 copies per ml and 36,300 copies per ml, which correlates to a drop of 1.17 and 0.71 logs, respectively. At the 6-month time point, subject 5 had a 0.75 log drop in viral load, and his viral load returned to baseline levels at the 9-month and 1-year time points.

The case history of subject 2 exemplifies our patients, for whom chronic HIV-1 infection was resistant to available antiretroviral agents (Fig. 1*C*). He is a 41 year old who has been HIV positive for at least 15 years. Medical history is significant for hyperlipidemia (related to antiretrovirals), peripheral neuropathy bilaterally in hands and feet (related to antiretrovirals), lymphadenopathy, and buccal wasting related to HIV lipodystrophy. Prior HIV therapies include zidovudine (1989), zalcitabine (1994–1995), sanquinavir (1994–1997), stavudine (1996–1999), lamivudine (1996–1999), indinavir (1996), ritonavir (1997–1999), amprenavir (1997–1999), and didanosine (1998–1999). His regimen at the time of enrollment included efavirenz (April 1998–), abacavir (November 2001–), ritonavir/lopinavir (November 2001–), lamivudine (March 2003–), and tenofovir (March 2003).

Subject 2 was given a single infusion of VRX496 $CD4^+$ T cells on October 13, 2003, when he had a mean baseline viral load of 54,100 copies per ml (4.64 logs). At 6 and 12 months, his viral load declined to 8,627 and 1,063, a drop of 0.8 and 1.7 logs, respectively (Fig. 1*C*). With the exception of subjects 3 and 4, no study participants changed antiretroviral therapy during the 1 year after infusion of VRX496 $CD4^+$ cells. Subjects 1 and 2 changed regimens between years 1 and 2. Fig. 5 provides treatment histories for all patients.

At study entry mean $CD4^+$ T cell counts ranged from 220 to 316 cells per μl (mean, 274). At the 1 year time point, $CD4^+$ T cell counts were elevated relative to baseline in four of five study participants (Fig. 1*D*).

Persistence of the lentiviral engineered cells was assessed by quantitative PCR of peripheral blood mononuclear cells (Fig. 2*A*). There was prolonged engraftment as measured by detection of the unique sequence tag in most, with 2 of 5 subjects having detectable VRX496 $CD4^+$ T cells at one year and later, with frequencies of 0.04% and 0.023% of total PBMCs. The mean half-life of modified cells in circulation was 23.5 ($\pm$7.7) days, with a range from 19 to 37 days during the first 6 months after infusion. However, the apparent decay kinetics are complex and, presumably, represent the death of infused cells, migration to tissues and secondary lymphoid organs, and the accumulation of clonal progeny consequent to cellular division (10, 11). Evidence to support the latter is suggested by the observation that subjects 4 and 2 had detectable frequencies at 1 and 2 years respectively, after being below the limit of assay quantification at 9 months (0.02%). One may speculate that the 6 month drops in viral load observed in subjects 1 and 5 may have been sustained had the modified cells persisted, thus providing rationale for multiple infusions in follow-on trials.

A major concern for the use of a VSV-G pseudotyped HIV vector is the formation of a vector-derived RCL, particularly with VSV-G envelope sequences, because such RCLs would have a broader tropism than HIV. To address this safety issue, we used sensitive molecular assays for detection of VSV-G DNA and RNA in the final T cell product and in peripheral blood, respectively, at 3, 7, 21, and 90 days after infusion. VSV is an RNA virus and natural infection is possible although rare and typically limited to persons who work with livestock. Detection of VSV-G DNA would be a strong indicator that a recombination event has occurred, which may represent an RCL. Because the vector genome does not contain VSV-G sequences, persistent expression of VSV-G nucleic acid, in particular DNA, would indicate a recombination event between the vector and helper during vector production, indicating the possibility of a vector-derived RCL. Had this situation occurred, it would have triggered a full biological RCL assay as confirmatory testing. In addition to testing for VSV-G sequences, a biological amplification assay was used to test for vector-derived RCL in both the final cellular product and in patient PBMCs isolated by apheresis 6 months after infusion. No VSV-G envelope sequences were detected in any final product or in any subject (Table 2). In addition, no subject developed antibodies to VSV-G that could be detected in plasma as measured by ELISA.

Another potential safety concern associated with the use of lentiviral vectors is vector mobilization, also commonly referred to as conditional replication (12). Mobilization is possible for vectors that retain their full LTRs when packaging proteins are provided in trans such as during HIV infection. Although vector mobilization *in vivo* to nontarget tissues may have adverse safety consequences, mobilization of the vector payload into uninfected $CD4^+$ cells could amplify the antiviral effects (12, 13). VRX496

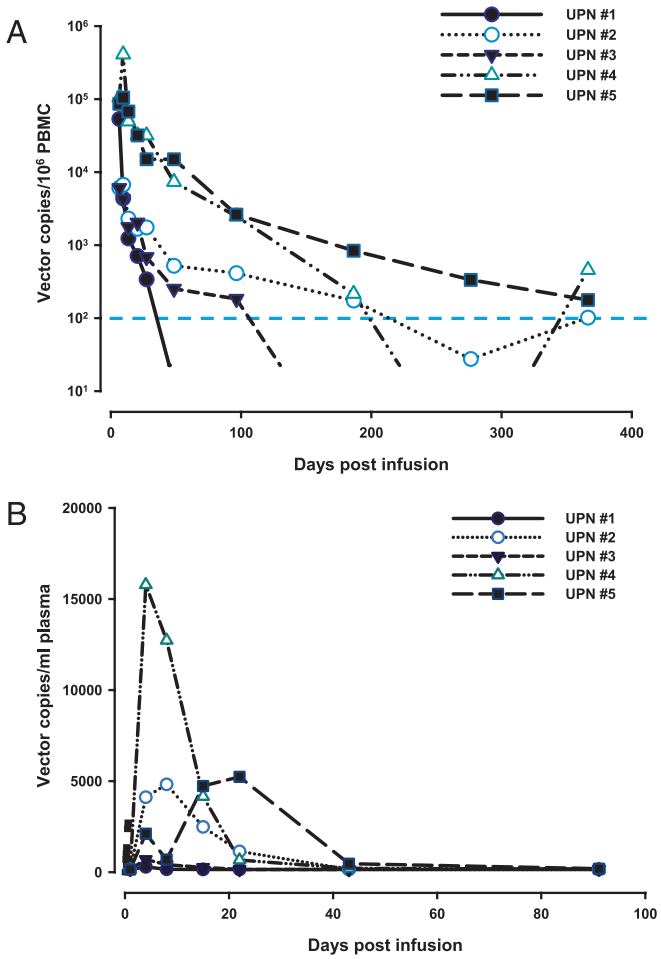

Fig. 2. Engraftment of vector-modified cells and detection of mobilization of vector *in vivo*. (*A*) Prolonged engraftment of lentiviral transduced CD4$^+$ cells. Vector persistence was assessed beginning 20 min after infusion of VRX496-modified CD4$^+$ cells and then at 72 h; 1, 2, 3, and 6 weeks; and 3, 6, 9, and 12 months. PBMC were collected at the indicated time points, and DNA analysis was performed for detection of VRX496 vector sequences by using real-time PCR. The limit of quantitative detection (LOD) is 200 vector copies per 10^6 PBMC. At the 1-year time point, subject 4 has a frequency of engraftment of 0.04% (400 copies) after being undetectable at the 9-month time point, and subject 2 has 0.023% engraftment (233 copies) at 2 years. Subjects 3–5 have not yet completed their 2-year follow up. (*B*) Vector mobilization. To assess for vector mobilization, RT-PCR analysis for vector genomic RNA was done by using primers specific for truncated GFP (Gtag) sequence, which is a component of the vector and allowed it to be distinguished from wt-HIV in the patients (see *Methods*). The presence of genomic vector transcripts in circulation was assessed by isolating RNA from plasma at the indicated time points after infusion on day 0.

is an example of a conditionally replicating HIV vector that replicates only in the presence of wild type HIV, and it was selected for the initial clinical evaluation because it displayed less efficient mobilization *in vitro* than other vectors [(3) and Biological Response Modifiers Advisory Committee Meeting, October 26, 2001, www.fda.gov/ohrms/dockets/ac/01/briefing/3794b3.htm], thereby maximizing safety as much as possible while retaining the wt-LTR for HIV-specific gene expression. To determine vector mobilization *in vivo*, we tested for the presence of VRX496 genomic RNA in the plasma by quantitative RT-PCR assaying for the specific primer to a non-protein-encoding gene fragment of the GFP (Gtag), which is used to distinguish VRX496 from wt-HIV in the patient (Fig. 1*A*). Gtag RNA was detected in all subjects after infusion (Fig. 2*B*). Vector mobilization in plasma was transient as it was not evident after day 60. Subject 4 had the highest magnitude of mobilization, which may be related to the observation that he had the highest average vector copy per cell (4.0) in his infused CD4$^+$ T cell product (Table 1).

To monitor for clonal outgrowth as a consequence of potential insertional mutagenesis, the T cell receptor repertoire was assessed in all subjects by using a PCR-based spectratyping assay. There were no changes toward increased skewing or oligoclonality and, importantly, no evidence of clonal outgrowth at 21–36 months after infusion. An example of the distribution of the T cell receptor V-β gene usage is shown in Fig. 6, which is published as supporting information on the PNAS web site. Insertion-site analysis was also extensively evaluated as described next.

Integration-Site Analysis. Detailed analysis of vector integration sites was performed because of concerns for insertional mutagenesis that have been raised by trials using oncoretroviral vectors (14). The sites of integration of the antisense *env* vector were examined in transduced T cells harvested before infusion into patients. Cells from all five of the subjects were analyzed, yielding a total of 192 unique integration site sequences. Integration sites were mapped onto the human genome sequence (Fig. 3*A*) and the distribution compared with human genomic features and previously determined HIV integration sites (15–18). Studies have shown that integration by HIV or HIV-based vectors was favored in gene-rich regions, and this trend was also seen for the antisense *env* vector (Fig. 3*A*; $P = 2 \times 10^{-10}$ for comparison with random placement). Similarly, integration by the antisense *env* vector was favored in features associated with gene dense regions such as G/C-rich sequences and Alu elements ($P = 6.5 \times 10^{-8}$ and $P < 0.0001$, respectively, for comparison with random). Integration by VRX496 was strongly favored in transcription units, with 74% of sites within these sequences ($P < 1.2 \times 10^{-14}$ for comparison with random), as seen in previous HIV data sets. A similar trend was seen for integration sites from each patient analyzed individually. Comparison with transcriptional profiling data revealed that genes hosting integration events by VRX496 were biased toward relatively high-level expression ($P = 0.004$, Mann–Whitney test). A detailed genomic and statistical analysis of the VRX496 integration events is included in *Appendix*, which is published as supporting information on the PNAS web site.

Of note, integration in gene-dense regions was more strongly favored in the antisense *env* vector data than in previously studied HIV vector or virus data sets (Fig. 3*B*; $P = 2.9 \times 10^{-5}$ for comparison with pooled HIV integration data). The reason for the stronger favoring of gene-rich regions by VRX496 is uncertain; one possibility is vector independent: that the optimally stimulated primary T cells used in this study contain higher levels of cellular factors promoting integration in gene-rich regions. Taken together the data so far demonstrate the vector displays integration similar to wild type HIV.

Immune Assessments. To characterize immune function, we analyzed the response of T cells to HIV antigens by ELISpot. The frequency of T cells secreting IFN-γ was assessed at baseline, 3 and 6 months after infusion by stimulation with a panel of overlapping 15-mer peptides that corresponds to the entire coding region of HIV-1 *env* and *gag*. The frequency of T cells responding to env was elevated in three of five subjects at 3 and 6 months after infusion when compared with baseline (Fig. 4). In addition, the responses to env after infusion were higher in magnitude in three patients at 3 months ($P < 0.05$) and 6 months ($P < 0.001$) than a series of matched control patients with chronic HIV-1. In contrast, the T cells from the subjects generally did not have augmented responses at 3–6 months after therapy after gag stimulation compared with matched controls

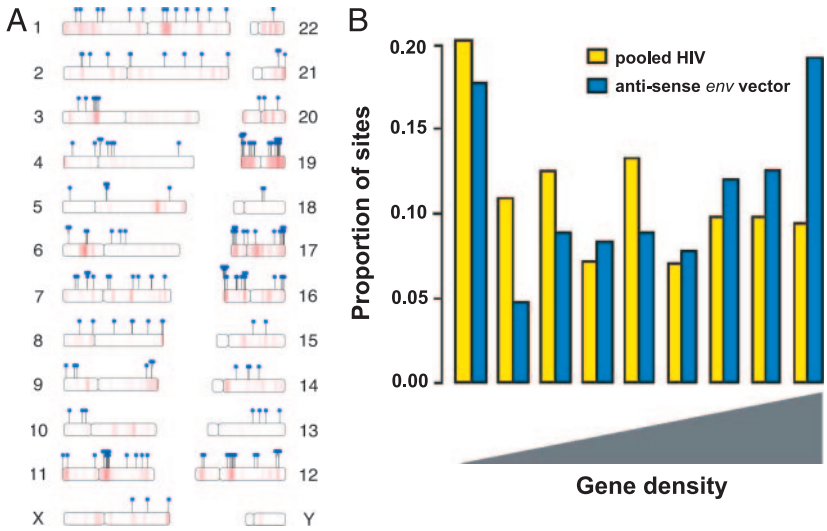

Fig. 3. Analysis of the sites of VRX496 integration in patient cell product prior to infusion. (*A*) Integration of the VRX496 antisense *env* vector in the human genome. Integration sites are mapped on the human chromosomes, with sites of vector integration shown by the blue ''lollipops.'' Gene density is indicated by the red shading on the human chromosomes, with more intense red indicating higher gene density. Vector integration differs from random ($P = 2 \times 10^{-10}$ for comparison with random placement). (*B*) Preferential integration of the VRX496 antisense *env* vector in gene-rich regions. The association of integration sites with genes was assessed by sliding a 250-kb window along the human genome. Values were determined separately for antisense *env* vector sites and summed previously studied HIV sites. The results were pooled, divided into nine intervals, and the proportion of each type of site in each interval assessed. Vector integration in gene-dense regions was more strongly favored than in previously studied HIV vector or virus data sets ($P = 2.9 \times 10^{-5}$ for comparison with pooled HIV integration data). The *P* value is the result of fitting a cubic polynomial to the gene-density values. See the online statistical supplement (*Appendix*) for more details.

($P > 0.5$; Fig. 7*A*, which is published as supporting information on the PNAS web site).

To characterize CD4 memory responses during the protocol, the response to diphtheria toxin was measured. Subjects had absent or low responses at baseline, and three of five subjects had increased responses at 3 and 6 months after infusion (Fig. 7*B*). The after-treatment responses were elevated in comparison with samples from HIV controls ($P < 0.03$). No subject was vaccinated during the protocol, and the immune response changes were documented before any subject changed antiretroviral therapy. Finally, serum

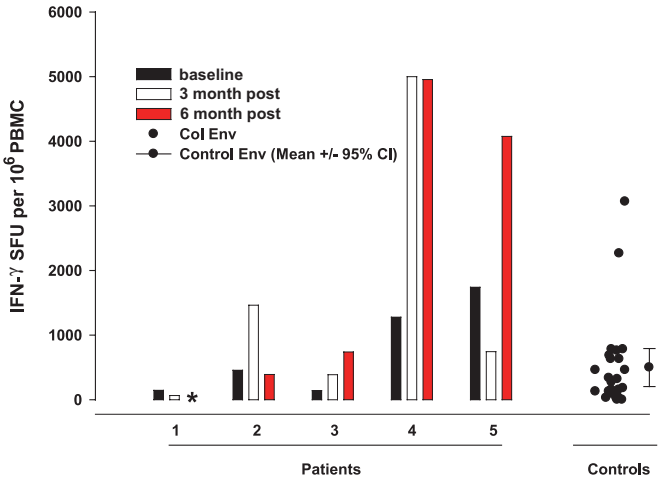

Fig. 4. Immunologic assessment of HIV-1 *env*-specific effector cells secreting IFN- γ. Blood samples were obtained at baseline and at 3 and 6 months after gene-transfer therapy. PBMCs were isolated from study subjects and HIV-1-positive control subjects ($n = 25$) by a standard Ficoll separation technique. IFN-γ production after HIV-1 *env in vitro* stimulation of PBMCs was assessed for an *env* antigen-specific response by a standard ELISPOT. The mean ± 95% confidence interval for the control subjects is plotted. *, subject 1 did not have a 6-month sample available for analysis.

from patients was analyzed for neutralizing antibodies to laboratory HIV strains and to autologous viral isolates (19). No subjects developed neutralizing antibodies to env.

By ELISpot analysis, there was little improvement of the immune response of patient 2 to the HIV gag or env that could possibly explain the mechanism for the drop in viral load. Therefore, additional immune analysis was performed by using intracellular cytokine staining (ICS) (see *Supporting Methods*) after stimulation of patients' PBMCs with overlapping peptides from Gag, Pol, Env, and Nef corresponding to the HIV-1 clade B consensus sequence. The CEF (CMV, EBV, and Flu) peptide was used to measure the cytokine response to other, non-HIV, pathogens. There was a measurable improvement in the anti-Pol and Nef responses as early as 1 year after dosing (Fig. 7*C*) and significant improvement in general immune function at year 2 after dosing. However, it cannot be known whether this improvement is a result of the study treatment alone, or results from the change in medication the patient was taking between years 1 and 2. During this time, while continuing on kaletra and ziagen, the patient went off sustiva and switched from lamivudine and tenofovir to Truvada, which is a combination of tenofovir and tricitabine. However, it should be noted that, to our knowledge, antiretroviral drugs, if efficient in controlling HIV replication, are not able to restore the immune response to HIV or other pathogens in patients who are failing highly active antiretroviral therapy (HAART).

Discussion

Our results demonstrate the expected high efficiency of gene transfer *in vitro* at the clinical scale, and the observed long term persistence of gene-modified T cells *in vivo* in these subjects with late stage HIV infection is encouraging and indicates that the VSV-G pseudotyped vector may not have substantial intrinsic immunogenicity when given in a single cellular dose, as has been observed with most forms of gene transfer therapy. To date our studies have not uncovered evidence of insertional mutagenesis, with observation of 21–36 months. However, given that the

latency period was 3 years for adverse events to become clinically evident in the case of stem cell gene transfer of common γ-chain by using an oncoretroviral vector (14), safety will not be fully established until longer follow up is completed, and more subjects have been treated. In accordance with the long term follow-up guidelines provided by the FDA, the patients enrolled in this trial will be followed annually for 15 years after infusion.

An increase in the cellular responses to HIV was observed in four of five patients, and three of those experienced a concomitant improvement in their T cell memory responses as well. The magnitude of the responses to env were higher than we have observed in studies of patients with chronic HIV-1 infection who have been vaccinated with HIV genes (20). Finally, we observed a robust antiviral effect in one subject who had been refractory to conventional antiviral therapy. The mechanism of the delayed antiviral effect remains unclear and may be related to immune enhancement and/or to vector mobilization (12), consistent with the transient vector mobilization that we have observed. The antiviral response observed in patient 2 cannot yet be definitively linked to vector expression in the T cells instead of an effect from the T cell infusion alone, however, several studies involving T cell infusions in HIV patients have been performed, and sustained control in viral load has not been observed. One can speculate that vector mobilization resulting in greater antisense pressure against HIV leads to the generation of HIV variants in patients with reduced pathogenicity, which, in combination with the immune reconstituting effects of adoptive cellular therapy, could lead to enhanced control of viral replication. This situation would be reminiscent of the subset of individuals with advanced multidrug-resistant HIV that develop an immunologic profile comparable to that of long-term nonprogressors, where it has been suggested that functional immunity can be reconstituted as a result of a de facto attenuated vaccine process that occurs consequent to the generation of drug resistant HIV with reduced fitness (21).

The retention of the LTRs in VRX496 provides the potential for the vector to be packaged by using wt-HIV proteins and, thus, mobilized to CD4-bearing cells. For most applications, SIN vectors are thought to be safer because they cannot mobilize because of a deletion in the U3 region of their LTRs. However, in the setting of HIV, mobilization may be beneficial (12), resulting from production of immunizing virus like particles, spreading of vector to additional T cells, or a combination of both mechanisms. Mathematical modeling evaluating the hypothesis of vector spread to T cells indicates that the antiviral effects of conditionally replicating vectors are complex and depend critically on the efficiency of mobilization (13). Although VRX496 contains an anti-HIV gene and, thus, should inhibit production of HIV proteins capable of packaging the vector, at lower copy numbers, silencing or variegation may result in productive infection and mobilization (22).

This trial provides intriguing data in a pilot study evaluating a gene therapy vector and its application in the setting of HIV. In particular, conditionally replicating lentiviral vectors may have promise in a number of chronic viral infections. Two follow-on studies are presently underway to further evaluate the potential of this vector when given in multiple doses (www.clinicaltrials.gov). Taken together the results support the clinical promise of this efficient T cell culture system and gene transfer using lentiviral vector technology.

Methods

Clinical Protocol. Details of the protocol have been published (23). The final protocol was later modified to remove dose escalation. To be eligible for the study, subjects must have failed at least two HAART regimens as a result of drug resistance or be intolerant to antiretrovirals, with viral load of >5,000 copies per ml and CD4 counts between 150 and 500. In addition, subjects must have had a Karnofsky performance score of at least 80 and no signs of opportunistic infections. Subjects were monitored after infusion at 1, 2, 3, 7, 14, and 21 days, 6 weeks, and at 3, 6, and 9 months for viral load (Amplicor assay; Roche, Indianapolis, IN), CD4 count, emergence of potential RCLs, and for immunological parameters. Adverse events were defined in part by observation of a sustained 0.5-log increase in viral load, or 30% decrease in CD4 count within 3 weeks after dosing. Additional information is provided in *Supporting Methods*, which is published as supporting information on the PNAS web site.

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