



**FDA BIOLOGICAL RESPONSE MODIFIERS
ADVISORY COMMITTEE MEETING
BRIEFING PACKAGE**

**AUTOLOGOUS T CELLS TRANSDUCED WITH
VRX496,
AN HIV-1 BASED LENTIVIRAL VECTOR FOR THE TREATMENT OF
PATIENT-SUBJECTS INFECTED WITH HIV-1**

SUBMITTED BY

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OCTOBER 26TH, 2001

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1.0 Introduction

VIRxSYS Corporation of Gaithersburg, Maryland, is planning to submit an Investigational New Drug Application to United States (U.S.) Food and Drug Administration (FDA) in 2001. The VIRxSYS drug product is autologous human T cells transduced with the HIV-1 based lentiviral vector, VRX496. VIRxSYS is proposing to develop T cells transduced with VRX496 as a gene transfer product for the treatment of human immunodeficiency virus (HIV) infection. VIRxSYS is submitting this briefing package in anticipation of the FDA's October 25th-26th Biological Response Modifiers Advisory Committee (BRMAC) meeting.

1.1 Sponsor Background

VIRxSYS Corporation, located in Gaithersburg, Maryland, was founded in April 1998, as a biotechnology company to develop HIV vector gene transfer platforms for the treatment of individuals infected with HIV and other diseases. The company currently employs 40 employees and occupies 20,000ft² of office & laboratory space, including a class 10,000 vector manufacturing suite. The company's vector gene transfer product is based on research that was conducted at Dr. Boro Dropulic's laboratory at The Johns Hopkins University (JHU), in Baltimore. VIRxSYS was established to optimize the vector system developed at JHU for clinical trials and for eventual treatment of HIV infection. Dr. Dropulic is the Founder and Chief Scientific Officer of VIRxSYS Corporation and is also an Adjunct Assistant Professor in Oncology at the Johns Hopkins University School of Medicine.

1.2 Product Background

VIRxSYS Corporation (VIRxSYS) of Gaithersburg, Maryland is developing a proprietary gene transfer product for the treatment of HIV infection. VIRxSYS' goal is to improve the management of HIV infection through the use of a novel gene transfer approach that utilizes an HIV-based lentiviral vector containing an anti-HIV antisense sequence targeted to the HIV -1 envelope gene. The vector is produced via co-transfection of a 293 cell line with two distinct plasmid DNA vectors. The first

plasmid DNA is the VRX496 vector. A second or helper vector, called VIRPAC or VRX170, supplies the genes necessary *in trans* for production of the antisense viral vector.

VRX496 is intended for *ex vivo* transduction of autologous T cells from HIV-infected patients that are subsequently re-introduced to the patient intravenously (i.v.). The HIV vector containing the anti-HIV env antisense would target wt-HIV RNA and destroy it, and hence, decrease productive HIV replication from T cells with the ultimate goal of decreasing viral loads *in vivo*. The vector may also increase the survival of VRX496 modified T cells by interfering with productive HIV replication.

Decreasing viral loads and increasing T cell survival in HIV-infected individuals could provide enormous benefit to HIV infected individuals. It could prolong or postpone progression to acquired immune deficiency syndrome (AIDS) and provide the immune system with a better chance to control the infection.

1.3 Scientific Background and Rationale

1.3.1 Human Immunodeficiency Virus (HIV)

HIV infects 36.1 million people worldwide (UNAIDS, 2000). In the United States (U.S.), it is estimated that over 425,000 people are infected with HIV, and over 45,000 new cases are reported each year (CDC, 2000). AIDS is defined by the Centers for Disease Control (CDC) as occurring in all HIV-infected individuals that have a CD4+ T cell count of less than 200/mm³. This definition also includes 26 conditions affecting people with HIV, including *P. carinii* pneumonia, HIV wasting syndrome, CMV disease, tuberculosis, Kaposi's sarcoma, disseminated *M. avium*, chronic Herpes simplex, recurrent bacterial pneumonia, HIV-associated dementia, and toxoplasmosis (Bartlett, 2001). The majority of these conditions are opportunistic infections that are often severe and sometimes fatal, because the ravaged immune system can no longer fight off infection. The mortality due to HIV/AIDS is estimated to be approximately 3 million deaths annually worldwide, and 15,000 in the U.S. (UNAIDS, 2000; CDC, 2000).

HIV belongs to a family of retroviruses known as lentiviruses. These ribonucleic acid (RNA) viruses are characterized by an enzyme known as reverse transcriptase that transcribes the viral RNA into provirus deoxyribonucleic acid (DNA) that becomes integrated into the host cell genome. The primary target cells for HIV-1 infection are human CD4 T-lymphocytes (T cells) and macrophages. Infection by HIV-1 is initiated by sequential binding of the viral envelope glycoprotein gp120 to the CD4 receptor and to one other co-receptor (CCR5 or CXCR4) and subsequent gp41-mediated fusion of the viral envelope with the cell membrane. After uncoating, the viral core and its components (viral RNA, gag and pol gene products) are released into the cytoplasm. The viral enzyme reverse transcriptase converts single-stranded viral RNA into a first strand DNA copy and subsequently into a viral DNA duplex of two strands. The viral DNA duplex is transported to the cell nucleus where it is integrated by the HIV integrase enzyme into the host cell genome. Once integrated into the host cell genome as provirus DNA, HIV can establish a productive infection or a latent infection depending upon critical host factors, particularly whether the infected cell is in an activated or resting state.

As of April 1999 there were 16 approved drug products for the treatment of HIV infection (FDA, 2001). The current standard of treatment for HIV/AIDS is the highly active antiretroviral therapy (HAART). This therapy typically consists of a triple "cocktail" of a nucleoside reverse transcriptase inhibitor (NRTI), a non-nucleoside reverse transcriptase inhibitor (NNRTI) and a protease inhibitor (PI). Although these cocktails have been successful in reducing viral loads and restoring immune function, they do not represent a cure, and there are concerns regarding adverse effects associated with long-term usage of HAART. Specifically, a variety of metabolic disorders including HIV-associated lipodystrophy, central adiposity, dyslipidaemia, hyperlipidaemia, hyperglycemia and insulin resistance have been reported as resulting from HAART (Vigouroux et al., 1999; Behrens et al., 2000). These reactions, combined with complex and cumbersome dosing regimens, can have an adverse impact on patient adherence to therapy (Lucas et al., 1999; Max et al., 2000).

1.3.2 Genetic Therapy for HIV infection

Over the past several years, several gene transfer strategies have been in clinical trials for HIV -1 infection. All of these studies have used Moloney Murine Leukemia Virus (MoMLV)-based vectors to deliver a variety of anti-HIV payload sequences. These sequences include transdominant negative proteins, RNA decoys, antisense RNA and ribozymes (Dropulic and Jeang 1994; Veres et al., 1998). Antisense RNA gene transfer offers a significant advantage over several other genetic antiviral approaches since it is not a protein and thus not immunogenic. Expression of an immunogenic non-native protein (e.g. a transdominant mutant protein or a single chain antibody targeted to a HIV protein) in transduced CD4 T cells could lead to rejection of the transduced cells by the host's immune system and thus, make the gene transfer ineffective. In all the patient groups that have been tested so far, none of MoMLV-vector based anti-HIV therapies have been demonstrated to be effective in humans (Kohn, 2001).

The advantages of HIV vectors over other vectors for HIV gene therapy are: 1) HIV vectors are very efficient in transducing human CD4 T cells; 2) inserting the anti-HIV payload in the context of HIV promotes its subcellular co-localization to the target wt-HIV RNA; and 3) inserting the payload upstream of the vector splice acceptor site in the RRE makes its expression Tat and Rev dependent, thus restricting its expression only to HIV infected cells. Previous studies have shown that anti-HIV payloads placed in the context of its target sequence can efficiently inhibit HIV replication (Dropulic et al. 1996). It is important to note that the anti-HIV effects of HIV vectors are not solely restricted to the anti-HIV antisense payload, but competition between many cis-acting sequences (e.g. TAR, RRE) present in the vector and wt-HIV RNA occurs for factors that promote their replication. The above synergistic effects provide several important advantages for the use of HIV based vectors over the MoMLV-based vector system.

1.3.3 VRX496: an HIV-based lentiviral vector

VRX496 is an HIV -based lentiviral vector harboring an anti-HIV antisense sequence targeted to the HIV env coding sequence. Importantly, VRX496 is a fully-gutted vector and does not encode for any viral proteins (figure 1). The vector is a replication defective vector. It is derived from the pNL4-3 molecular clone and contains a 937bp antisense sequence targeted to the env gene sequence in the wt-HIV genome. Briefly, fragments from the HIV -1 LTR, Gag, Pol and RRE were sub-cloned from the pNL4-3 molecular clone to form the basic vector. The anti-HIV antisense envelope payload was also sub-cloned from the same molecular clone and placed in the reverse orientation upstream of the rre fragment. The RRE fragment also contains a spliced acceptor site that renders anti-HIV antisense expression to be both Tat and Rev dependent. This restricts expression of the antisense payload only to CD4 T cells that become infected with wt-HIV. In addition, a stop codon was engineered in the Gag region to prevent translation of the Gag protein from genomic vector RNA if recombination between the vector and helper Gag-Pol RNA should occur in this region.

VRX496 is produced by transient transfection of 293 cells with distinct plasmid DNA vectors containing the anti-HIV sequences and helper sequences required for viral vector production. The helper plasmid, designated VRX170 or VIRPAC, is unique. The helper plasmid was constructed to contain both the Gag-Pol open reading frames and the VSV-G envelope open reading frames on the same plasmid, but driven off separate promoter systems. The Gag-Pol genes are expressed off the CMV promoter and are Rev-dependent, while the Tat and Rev genes are expressed from a spliced mRNA that is Rev independent. The Rev and Tat genes were codon degenerated to increase their expression during vector production. The Rev and Tat genes are expressed as a spliced mRNA with Tat, expressed downstream of an internal ribosome entry site. The bovine growth hormone poly A sequence is used to terminate the Gag-Pol-Rev-IRES-Tat transcriptional unit. To increase the safety of the helper construct, the first 42 nucleotides of the Gag gene sequence was codon

degenerated to decrease the chance for recombination with the Gag sequence present in the vector. To further increase the safety of the helper construct, synthetic poly A signals containing strong transcriptional pause sequences were inserted proximally to the EF-1 α /HTLV and CMV promoters to prevent transcriptional read-through between the Gag-Pol and VSV-G envelope open reading frames (figure 2).

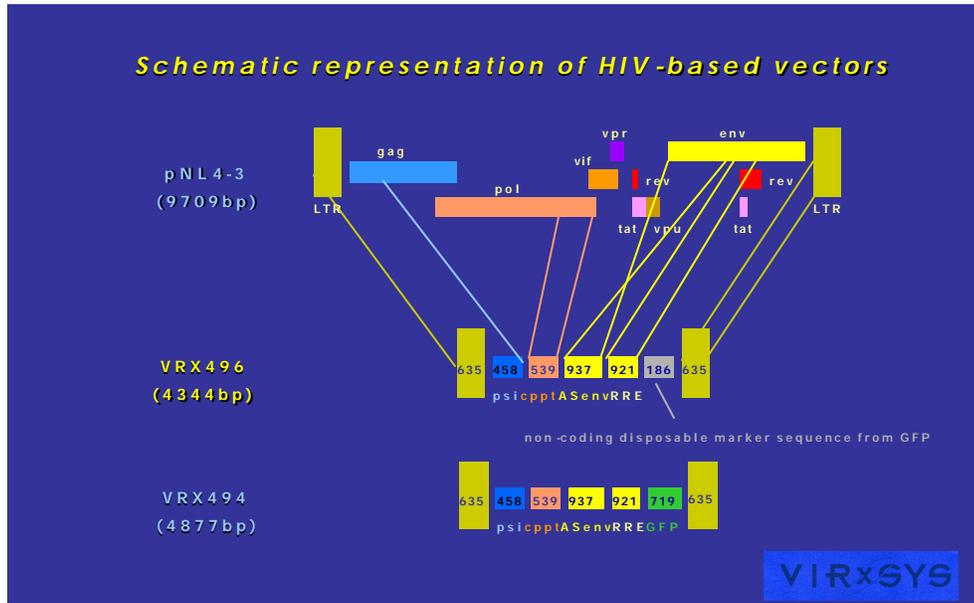


Figure 1. Schematic Representation of the VRX496 showing all the elements of the vector and the regions of wt-HIV from which they were derived. The vector elements are all cis-acting elements. No proteins are expressed from VRX496. VRX494 is an analog of VRX496 that expresses GFP.

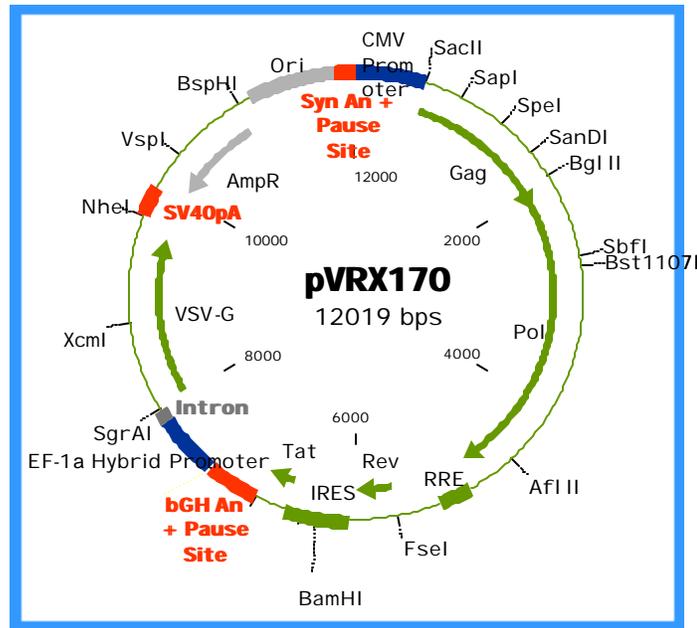


Figure 2. Schematic Representation of the VIRPAC (VRX170) helper plasmid showing transcriptional partition of structural (gag-pol) and envelope (VSV-G) genes (green) by the transcriptional pause sites (red) and polyadenylation sites (red). The CMV and EF-1alpha hybrid promoters (blue) drive expression of the structural and envelope genes respectively. Plasmid backbone sequences are shown in grey.

VRX496 is intended for *ex vivo* transduction of autologous CD4 cells from HIV infected patients that are subsequently autologously re-introduced back to the patient intravenously (figure 3). VRX496 expressing anti-HIV antisense env would target wt-HIV RNA and destroy it, and hence, decrease productive HIV replication from CD4 T cells with the goal of decreasing viral loads *in vivo*. Furthermore, expression of the antisense in VRX496 containing T cells may prevent productive HIV infection of CD4 T cells, and hence, promote their survival. Decreasing viral loads and increasing CD4 T cell survival in HIV-infected individuals would provide enormous benefit to the patient. It could prolong or postpone progression to AIDS and provide the immune system with a better chance to control the infection.

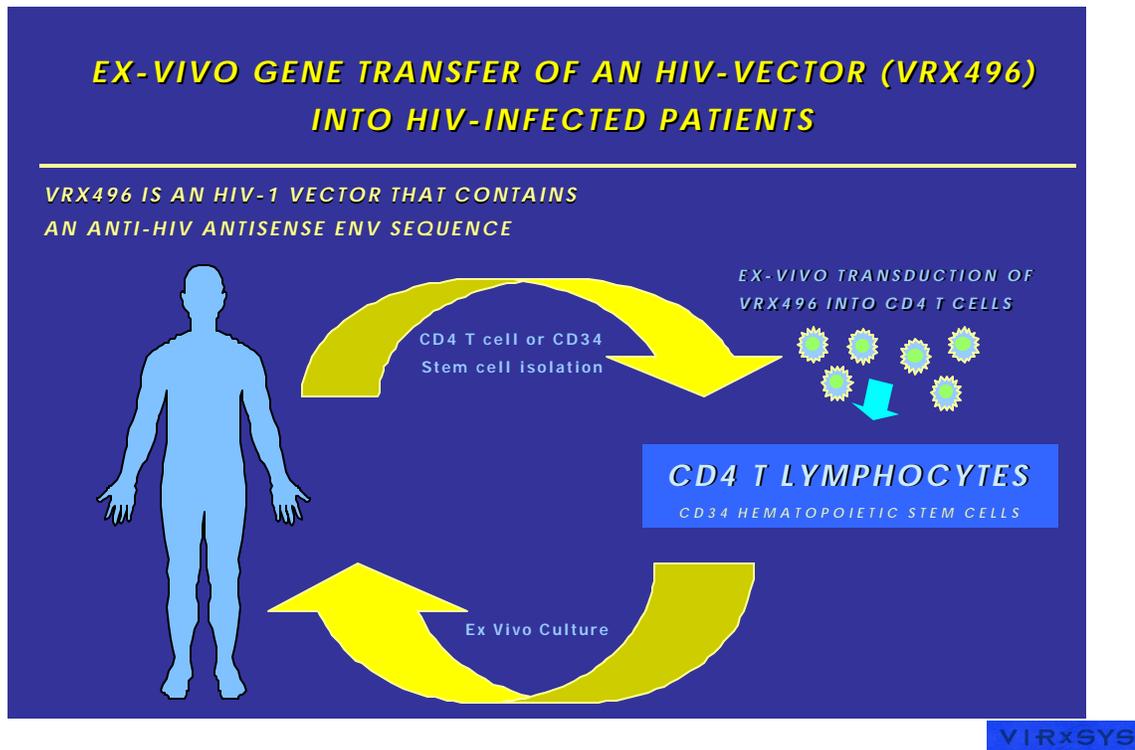


Figure 3. The proposed scheme for ex vivo gene transfer of VRX496

It is important to note that although that the proposed clinical trial scheme targets T cells for transduction and autologous infusion into HIV-infected patient-subjects, VRX496 could be used to target other cell types, such as CD34+ hematopoietic stem cells (HSC). VRX496 modified HSC could be introduced into HIV-infected patients by bone marrow transplantation (BMT). Modified HSC would mature and give rise to VRX496 modified T cells. The advantage of the BMT approach is that a significant portion of the transduced cells can be modified with vector, and patient-subject conditioning regimens during BMT would allow for significant numbers of modified T cells to be present relative to unmodified cells. Nevertheless, the T cell approach holds great promise for HIV gene therapy since these cells can survive long term *in vivo* after *ex vivo* expansion. Recently, June and colleagues (Mitsuyasu et al, 2000) have shown persistence of CD4 T cells transduced with a MoMuLV vector expressing the CD4 ζ gene in HIV infected patient-subjects (Figure 4). CD4 T cells transduced with CD4 ζ -MoMuLV could be detected for almost a year *in vivo*,

without significant loss of vector containing cells during the course of the study. The data demonstrates that *ex vivo* expanded T cells can survive long-term in HIV-infected individuals *in vivo*.

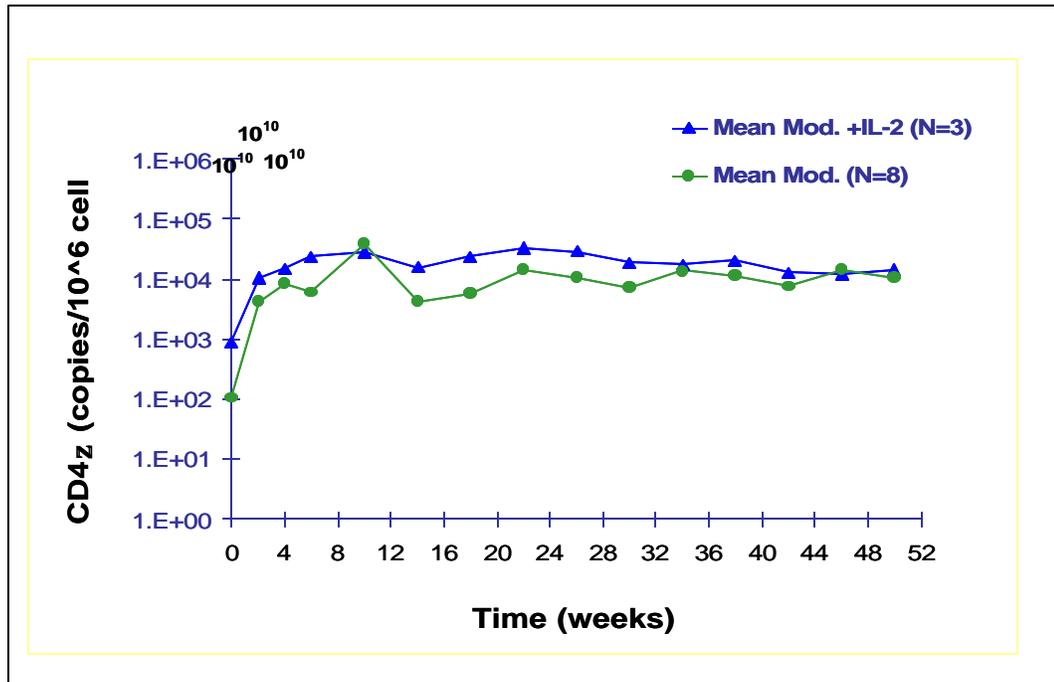


Figure 4. Long term persistence of CD4 ζ modified T cells in HIV-infected patient-subjects. Patient subjects were infused with 3 times with 10^{10} cells (0, 2 & 4 weeks; upper left of graph) that were previously transduced with a CD4 ζ expressing MoMuLV vector. Patients were monitored for the persistence of CD4 ζ marked cells. The genetically marked cells persisted for the duration of the study (50 weeks). The blue triangles are the mean data for CD4 ζ modified cells expanded in *i*CD3/28 + IL-2, while the green circles are the mean data for the CD4 ζ modified cells expanded only in *i*CD3/28.

In summary, it is recognized that a successful T cell therapeutic approach would require sufficient numbers of VRX496 modified T cells to survive *in vivo* so as to decrease viral loads and help promote the anti-HIV immune response *in vivo*. This could be achieved by selective survival of VRX496 modified cells, due to their

resistance to productive wt-HIV infection, or other enrichment procedures could be used once safety of the vector is demonstrated during the phase I clinical trial.

1.3.4 Safety features of VRX496

VRX496 gene transfer of autologous T cells was selected as most appropriate for initial gene transfer of lentiviral vectors into HIV-infected patient-subjects based on the following safety features:

- 1) The components of VRX496 are wholly derived from wt-HIV, even the anti-HIV payload sequence is entirely derived from wt-HIV. Therefore, any recombination event that could possibly occur between VRX496 and wt-HIV cannot produce a novel pathogenic virus;
- 2) The backbone of the vector is derived from pNL4-3, the prototypic HIV molecular clone that was created from HIV strains common to North America;
- 3) VRX496 is a fully-gutted vector. No genes encoding proteins are present in VRX496 that could potentially increase the pathogenicity of wt-HIV (i.e. if the gene would be incorporated into the resident wt-HIV genome by recombination with the vector);
- 4) VRX496 does not contain heterologous sequences such as internal viral promoters that could potentially increase the pathogenicity of wt-HIV (i.e. if the heterologous sequence would be incorporated into the resident wt-HIV genome by recombination with the vector);
- 5) Expression of the anti-HIV env antisense payload is targeted only to cells that are infected with wt-HIV. The anti-env antisense is located upstream of a major splice acceptor site and therefore is expressed only after both Tat and Rev are provided by the infecting wt-HIV;
- 6) Expression of the anti-HIV antisense payload within the context of HIV RNA permits effective colocalization of the payload to target sequences, and hence mediates their effective destruction (i.e. the antisense is flanked by HIV vector sequences that are analogous to wt-HIV, and thus permits effective targeting of antisense-containing vector RNA to wt-HIV RNA);

- 7) VRX496 has weak mobilizing capabilities that was purposely selected to enhance safety of the first vector of this class to be introduced into humans; and
- 8) The target cells, T cells, are the most appropriate for initial safety studies in humans because they are the cells that are directly infected by wt-HIV.

VRX496 has been designed for maximum safety, while maintaining a high transduction efficiency of human CD4 T cells and a high degree of anti-HIV activity. VRX496 has been maximally gutted so that no viral proteins are expressed from the vector. To increase the safety of the vector, a stop codon sequence was engineered into the vector so that if a recombination event would occur with the helper, a non-functional gag sequence would result. Therefore a single recombination event between the vector and helper will not yield a functional gag-pol open reading frame. A second frame shift mutation would also be required to restore the gag-pol open reading frame sequence.

The VRX496 is a lentiviral vector, containing two long terminal repeats (LTR), a packaging signal (ψ) overlapping with the beginning of the gag gene, a central polypurine tract (cPPT), a rev responsive element (RRE) and a 937 base antisense sequence against the HIV-1 envelope gene. To prevent expression of a short polypeptide from the gag sequence, a frameshift mutation was inserted 42 nucleotides downstream (Cla I site) of the first gag ATG. This frameshift mutation results in a stop signal 84 nucleotides from the ATG. The stop signal also serves to prevent expression of a functional gag-pol open reading frame if the vector should recombine with either wt-HIV or helper downstream of the frameshift mutation site.

1.4 Clinical Rationale and Indication

VRX496 has been developed as a gene transfer product for the treatment of HIV infection, via autologous T cell transduction *ex vivo* and subsequent reintroduction to the patient.

HIV-based vectors could potentially provide several important advantages over current HIV combination therapies. First, the vector, once introduced, would permanently have its anti-HIV effect for the life of the cell, in contrast to drug therapy that requires constant supply of drug. Since studies to date have indicated no apparent toxicity of VRX496 to primary T cells, it is anticipated that the anti-HIV effect would occur without any biological toxicity, in contrast to present drug therapies whose toxicities are cumulative and leads to treatment failure.

Preliminary *in vitro* testing has repeatedly showed a reduction in HIV replication by as much as 99% in human T cells (from normal and HIV-infected donors) that were *ex vivo* modified with anti-HIV vectors similar to VRX496 and then, in the case of the normal donors, infected with wt-HIV. Also, productive infection of wt-HIV in VRX496 modified CD4 T cells was significantly reduced, indicating a survival advantage for VRX496 modified cells over cells not modified with the vector. These data suggest that HIV vectors such as VRX496 have the potential to reduce viral replication and promote T cell survival *in vivo*. Decreasing viral loads and increasing T cell survival in HIV-infected individuals without the toxic effects of HAART therapy could provide enormous benefit to HIV infected individuals. It could prolong or postpone progression to acquired immune deficiency syndrome (AIDS) and provide the immune system with a better chance to control the infection.

2.0 Product Manufacturing and Characterization

2.1 Overview

The VIRxSYS drug product consists of human T cells transduced with the HIV vector, VRX496 (VRX496 modified T cell product). VRX496 is produced from 293 cells transfected with two plasmids, a lentiviral vector plasmid and a helper

packaging vector plasmid encoding structural and envelope genes. Following co-transfection, the vector is concentrated and purified from the supernatant using ultrafiltration, diafiltration and chromatographic steps. The vector product is supplied in a liquid formulation in bags. Production of VRX496 vector product will be conducted at VIRxSYS' facilities located in Gaithersburg, Maryland using current Good Manufacturing Procedures (cGMP) conditions. This vector product is then used to transduce patient-subject T cells *ex vivo*, which are subsequently re-introduced to the patient (final drug product). Transduction of patient-subject T cells *ex vivo* will be performed at the University of Pennsylvania Hospital's Clinical Cell Production Facility using cGMP conditions.

2.2 Viral Vector Description and Characterization

VRX496 is a HIV-1 based lentiviral vector carrying an anti-HIV antisense sequence targeted to the HIV env gene. VRX496 is a fully-gutted vector that does not encode for any protein gene sequence. The vector contains a 937 base antisense env sequence that inhibits wild-type HIV replication. VRX496 is used for *ex vivo* transduction of autologous T cells for subsequent reintroduction into the patient.

2.3 Components and Manufacturing of Viral Vector

2.3.1 Plasmid VRX496 (pN1cla(cPT2)ASenvGtag)

The VRX496 lentiviral vector plasmid is derived from a wt-HIV viral clone, pNL4-3, that has been gutted to remove any protein coding sequences and is thus replication defective. Briefly, fragments from the HIV-1 LTR, Gag, Pol and RRE were sub-cloned from the pNL4-3 molecular clone to form the basic vector. The anti-HIV payload consists of a 937bp antisense sequence (also sub-cloned from the pNL4-3 molecular clone) targeted to the env gene sequence in the wt-HIV genome. The antisense sequence was inserted in a region of the vector that is upstream of the RRE splice acceptor so that it is expressed only in the presence of Tat and Rev, which are supplied by the wt-HIV upon infection of vector-transduced cells. A map of the VRX496 plasmid is presented in figure 5 below:

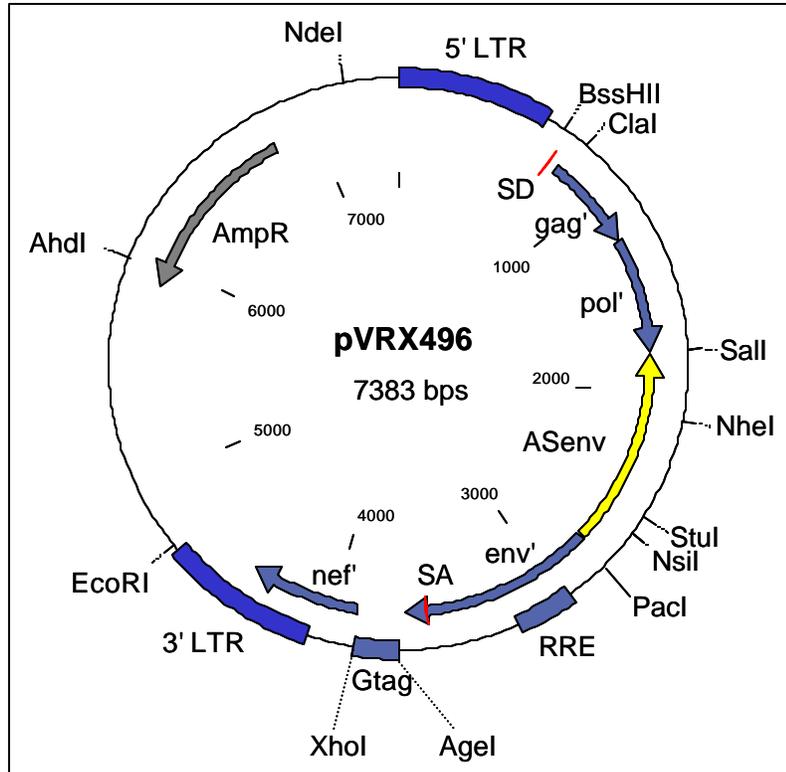


Figure 5: The VRX496 Plasmid Construct showing the 186 G-tag marker sequence that will be used to detect vector containing cells and the anti-env antisense payload in yellow. gag', pol', env' and nef' designate non-coding regions containing the packaging, cPPT, RRE and 3'LTR sequences respectively.

The VRX496 plasmid was incorporated into *Escherichia coli* strain STBL2™ competent cells and a master cell bank generated after selection of 1 of 10 clones based on the testing in table 1 below. VRX496 plasmid DNA produced from the master cell bank was tested as described in table 2 on page 18.

Table 1 Bacterial Master Cell Banks		VRX496 & VRX170
Parameter	Assay	Specification
Purity	Ampicillin Resistance Test for Plasmid Maintenance	≥ 95% Ampicillin Resistant colonies
Identity	Characterization of Plasmid DNA by Restriction Digest	Corresponds to reference standard for correct size and restriction digest pattern
	DNA Sequence Analysis	Corresponds to reference standard sequence
Potency	Bacterial Cell Plasmid Copy Number Measurement by TaqMan DNA PCR	1 - 2000 copies

2.3.2 Helper Plasmid VIRPAC-1.2 (VRX170)

The VIRPAC-1.2 (VRX170) helper plasmid used for production of VRX496 has been designed with maximum safety considerations in mind. For example, the first 41 nucleotides of the gag sequence were degenerated to decrease the region of homology with the vector. Similarly, RRE2 has been used instead of RRE1 to decrease the homology between these regions in the vector and helper. A map of the VIRPAC-1.2 (VRX170) plasmid is presented in figure 2 above on page 9.

The VIRPAC-1.2 plasmid was incorporated into *Escherichia coli* strain DH10B™ competent cells and a master cell bank generated after selection of 1 of 10 clones based on the tests shown in table 1 above. VRX170 plasmid DNA produced from the master cell bank was tested as described in table 2 below.

Table 2		Release Tests for Plasmid DNA VRX496 & VRX170	
Parameter	Criteria	Test Method	Specification
Identity	Restriction Analysis	Characterization of Plasmid DNA by Restriction Analysis	Plasmid DNA present is of the correct size and yields correct restriction digest pattern
	pH and Conductivity	pH and Conductivity of Plasmid DNA	0.5-3 mS
Purity	Agarose Gel	Agarose Gel Test for Purity of the Final Purified DNA Product	≥ 60% Supercoiled form

2.3.3 293 Cell Line

The Master Cell Bank of 293 cells is being manufactured by VIRxSYS Corporation, Gaithersburg, MD. The tests performed on the MCB are listed below in Table 3 below. We have previously used these specifications to certify a 293T cell line that will now not be used for vector production due to the inability to accurately determine the passage history of the 293T cell line (personal communication, Dr Michelle Calos, UCSF, California, USA). The 293 cell line is presently being certified has a well characterized origin and passage history.

Table 3: Release Testing Assays for 293 Master Cell Bank		
Test	Assay	Specification
Identity	Cell Culture Identification and Characterization	Isoenzyme migration most consistent with cells of human origin
Safety	Mycoplasma	Negative
	Sterility (Direct Inoculation Method)	No growth observed
	Test for Presence of Inapparent Viruses	Negative
	Porcine Parvovirus Detection in Cell Lysates	Not detected
	PCR for HIV-1/2 DNA	Negative
	PCR for HBV DNA	Negative
	RT-PCR for Hepatitis C Virus	Negative
	PCR for HHV-7 DNA	Negative
	PCR for EBV DNA	Negative
	PCR for CMV DNA	Negative
	PCR for HHV-6 DNA	Negative
	PERT Assay for the Detection of Retrovirus in Biological Samples	Negative
	In Vitro assay for the Presence of Bovine Viruses	Not detected
	In Vitro assay for the Presence of Viral Contaminants	Not detected
	PCR for HTLV-I/II DNA	Negative
	PCR for Human Parvovirus B19 DNA	Negative
	PCR for AAV DNA	Negative
Transmission Electron Microscopic Evaluation of Cultured Cells	No identifiable virus-like particles nor any microbial agents	

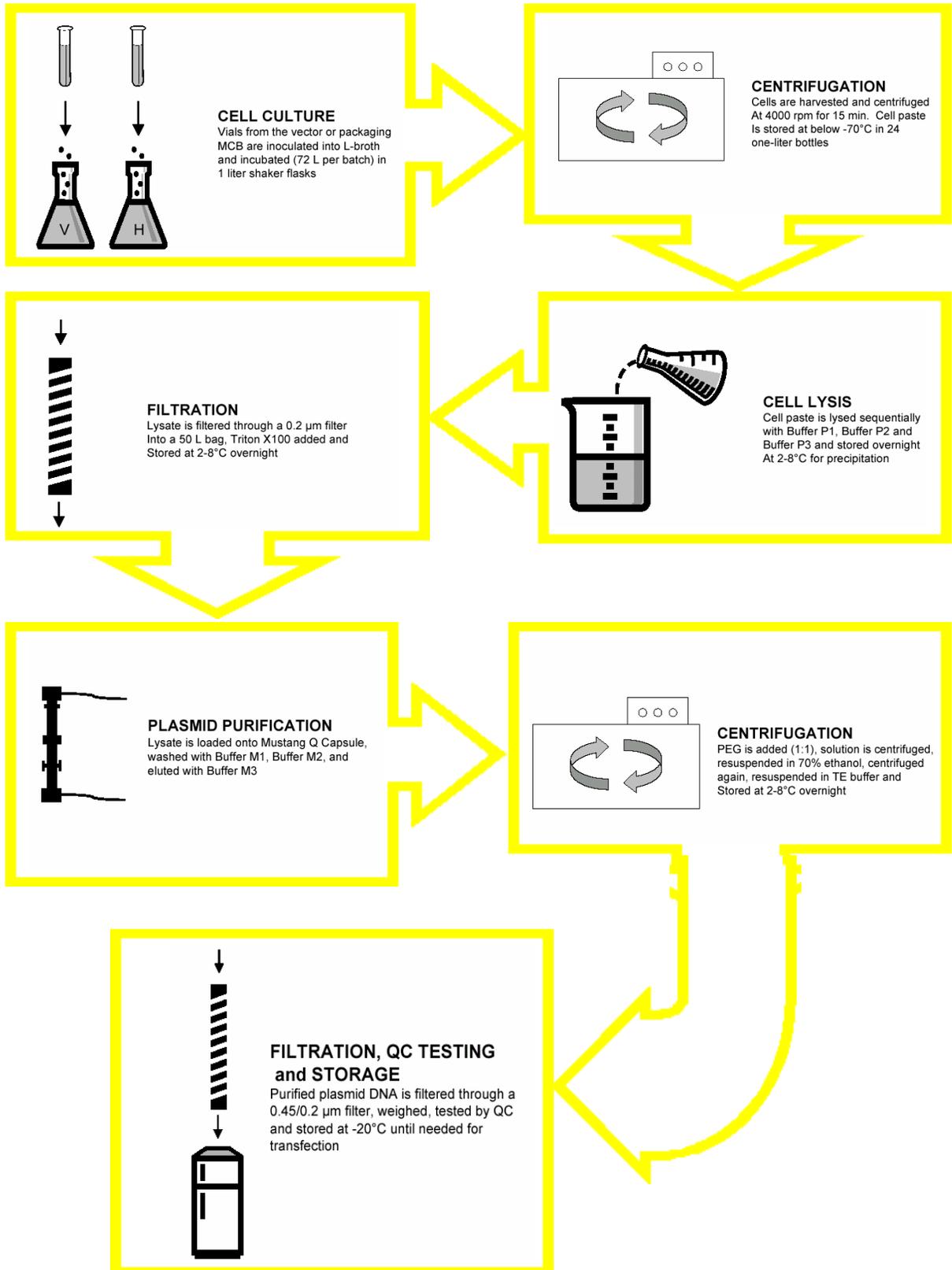
2.4 The Manufacturing Process

Vector manufacturing operations are performed in dedicated suites (plasmid vector preparation, host cell transfection, and vector purification) using critical raw materials of the highest grade available (USP or ACS, if applicable). Manufacturing

operations are conducted according to formal manufacturing procedures. Whenever possible, manipulations are performed using closed systems; all open operations are performed under class 100 biological safety cabinets (BSCs) dedicated to specific operations. Vector and helper plasmids will be prepared in an area isolated from both mammalian cells and virus. Upstream processes, including host cell expansion, transfection, and harvest will take place in a clean room (class 10,000) bio-safety level (BSL) 2. Downstream viral vector purification and storage will be performed under BSL 2 conditions in the clean room. Current and future batches of VRX496 for non-clinical and clinical studies are being produced at the VIRxSYS vector manufacturing facility in Gaithersburg, Maryland using cGMP conditions.

Briefly, plasmids pN1cla(cPT2)ASenvGtag (VRX496) and helper plasmid VIRPAC-1.2 (VRX170) are prepared from bacterial cell banks and seeded into standard bacterial growth medium. Cells are centrifuged and lysed followed by plasmid DNA purification (via chromatography) and storage at minus 20°C. 293 cells are thawed and expanded from stock and co-transfected with both vector and helper plasmid via calcium phosphate precipitation. Supernatant containing recombinant vector virus is collected over the next 48 hours and stored at 4°C. Viral harvests are pooled and purified by ultrafiltration, diafiltration and column chromatography. Bulk viral vector is stored at 4°C pending Quality Control assessment. Bulk vector is filled into bags and stored at minus 20°C. Flow diagrams of the manufacturing processes for plasmid DNA purification and viral vector production are presented in figures 6 & 7 below.

Figure 6: Plasmid Manufacturing Process



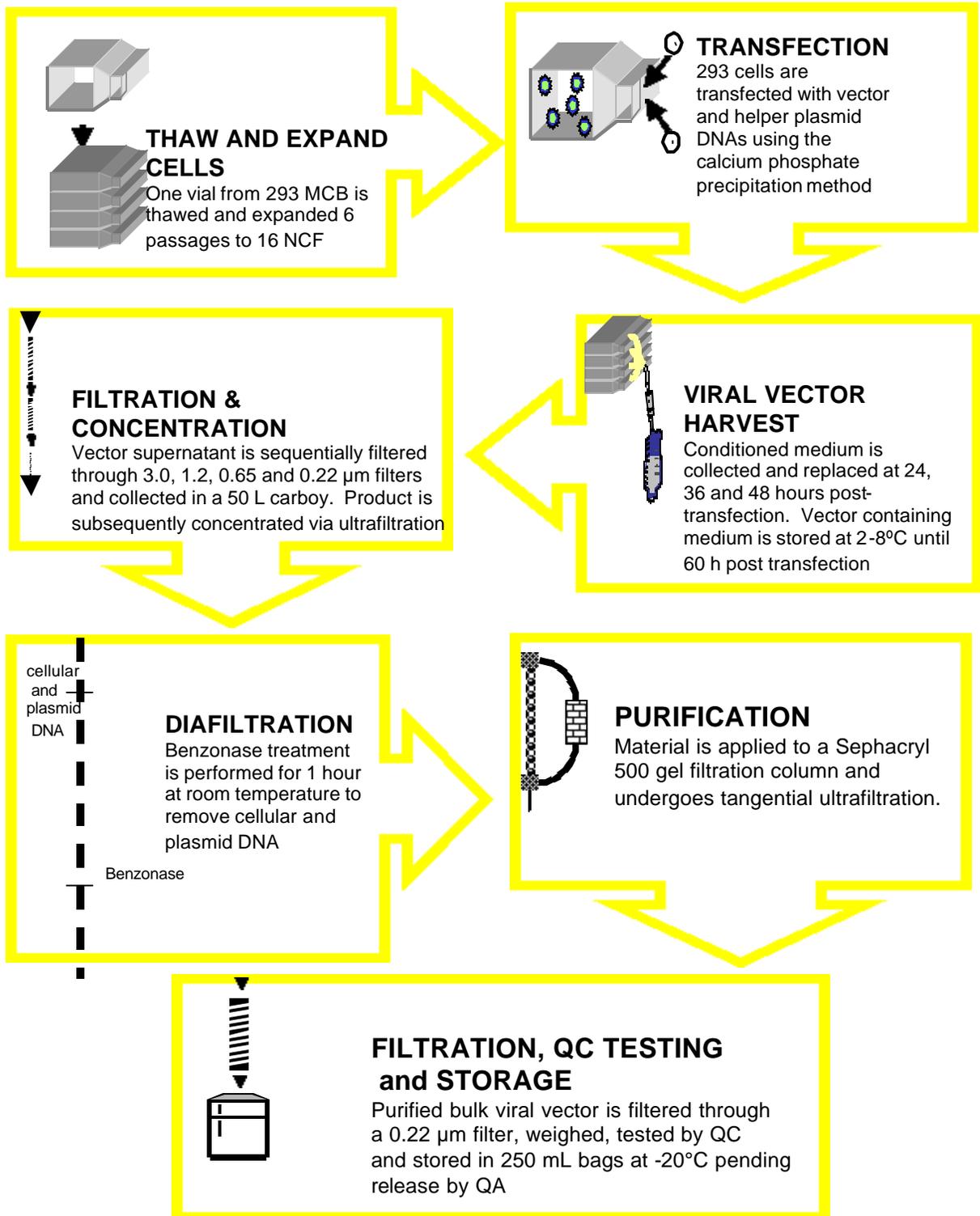


Figure 7: Vector Product Manufacturing Process

2.4.1 Plasmid DNA Production

Production of vector and helper plasmid DNA is performed in a dedicated room. All open manipulations involving bacterial (*E. coli*) cultures are performed in a class II biological safety cabinet. The fermentation process for plasmid DNA production is carried out in batches of 72 liters. Each MCB vial is thawed, aseptically transferred into a sterile shake flask and incubated at $30\pm 1^{\circ}\text{C}$ for 20-24 h. The fermentation broth containing *E. coli* is then transferred into one-liter centrifuge bottles. Centrifugation is performed and the supernatant is decanted. The plasmid DNA is isolated from bacteria by a standard alkaline lysis procedure. It is then purified using anion exchange module, precipitated with isopropanol, and formulated in 10mM Tris-HCl, 1mM EDTA buffer, pH 8.0. Plasmid DNA is tested by QC, released by QA and is then stored at minus 20°C until needed for transfection.

2.4.2 Transfection of Plasmid DNA into 293 Cells

Transfection of plasmid DNA into 293 cells is accomplished using the calcium phosphate precipitation method (CaPi). All upstream (cell culture) and downstream (purification) work is done in the class 10,000 clean room. All open manipulations with cells and virus vector is performed in a class II biological safety cabinet (class 100 air). 293 cells from the MCB will be expanded into 16 10-layer Nunc Cell Factories (NCF). Vector and helper plasmid DNA in HEPES-phosphate buffer will be co-precipitated by addition of CaCl_2 . After CaPi/DNA complexes are formed they will be aseptically transferred into plastic bags, mixed, and equally distributed into NCF. After transfection, the DNA-containing medium will be replaced with regular DMEM/10% FBS medium.

2.4.3 VRX496 (Viral Vector) Product Harvest

Twenty-four hours after transfection, conditioned medium in NCF containing vector product is aseptically removed into bags and replaced with fresh DMEM/10% FBS medium. This process is repeated at 36 and 48 h after transfection. Vector-

containing medium is stored at 2-8°C until 60 h post transfection. At this time all medium from bags is pooled aseptically into a 50L sterile flexible carboy.

2.4.4 VRX496 (Viral Vector) Purification and Formulation

Downstream purification begins with clarification of the vector-containing medium by filtration through cartridge filters with decreasing pore diameter. Clarified medium is collected in a sterile 50L carboy. Vector is subsequently concentrated by ultrafiltration and introduced into the buffer optimal for benzonase treatment by diafiltration. Benzonase treatment is performed for the removal of cellular and plasmid DNA. The material is applied to a gel-filtration column with Sephacryl-500, equilibrated with buffer. The product is formulated to the appropriate concentration by tangential flow ultrafiltration. The eluted material is sterile filtered and is identified as Bulk Vector Product. The purified bulk viral vector is filtered through a 0.22 µm filter, weighed, tested by QC and stored in 250 ml bags at -20°C pending release by QA (figure 7 & table 4). A scheme for RCR release testing is shown in figure 8.

Table 4: Viral Vector Product (DRAFT)			
Parameter	Criteria	Test Method	Specification
Identity	RT-PCR for G tag	Qualitative and RT-PCR	Positive
Potency	Wt-HIV Inhibition	HIV-1 Challenge Assay in Sup T1 Cells	≥ 1 log inhibition
Purity	Residual Benzonase	Benzonase ELISA	≤ 100 ng/ml
	Host Cell Protein	Host Cell Protein Characterization by SDS-PAGE	For information only
	Host Cell DNA	Qualitative DNA-PCR (Human marker specific)	≤ 100 pg of cellular DNA/dose

Table 4: Viral Vector Product (DRAFT)			
Parameter	Criteria	Test Method	Specification
Safety	Sterility <ul style="list-style-type: none"> • Bulk Harvest • Bulk Fill • Final Fill 	21 CFR 610.12	Negative Negative Negative
	Mycoplasma	21 CFR 610.30 PTC, 1993	Negative
	Endotoxin	USP <85>	< 100 EU/ml
	Adventitious Viruses	In Vitro Assay for the Presence of Viral Contaminants (PTC, 1993)	Negative
	RCR Assay	Passage supernatant six times on H9 cells and then perform RT PCR for VSV-G and HIV-gag on supernatant	No Gag or VSV-G sequence detected
	RT-PCR for wt-HIV	Qualitative RT-PCR Assays on Virus Vector Supernatant	No wt-HIV sequence detected
	TaqMan RT-PCR for VSV-G RNA	Quantitative RT-PCR Assay for VSV-G RNA Detection	For information only
	TaqMan DNA PCR for VSV-G DNA	Quantitative DNA PCR Assay for VSV-G DNA Detection	For information only
Other	E1a & E1b	Quantitative DNA-PCR assay for E1a and E1b specific sequences	For information only *If E1a and E1b sequences detected, final transduced cells will be tested
	Titer	Virus Titer Measurement on Primary T cells by Quantitative (TaqMan) DNA-PCR	1-10 copies

Table 4: Viral Vector Product (DRAFT)			
Parameter	Criteria	Test Method	Specification
	Concentration	HIV-1 p24 Antigen Capture Assay	≥ 10 ug/ml
	Total Protein	Total Protein by Lowry Assay	0.3 to 3.0 mg DNA /L of culture
	Fill Volume	Fill Volume, Appearance, pH and Conductivity of Final Viral Vector Supernatant	> stated volume recovered
	pH		7.0 – 7.4
	Conductivity		4-7 mS
	Appearance		colorless, clear to slightly turbid

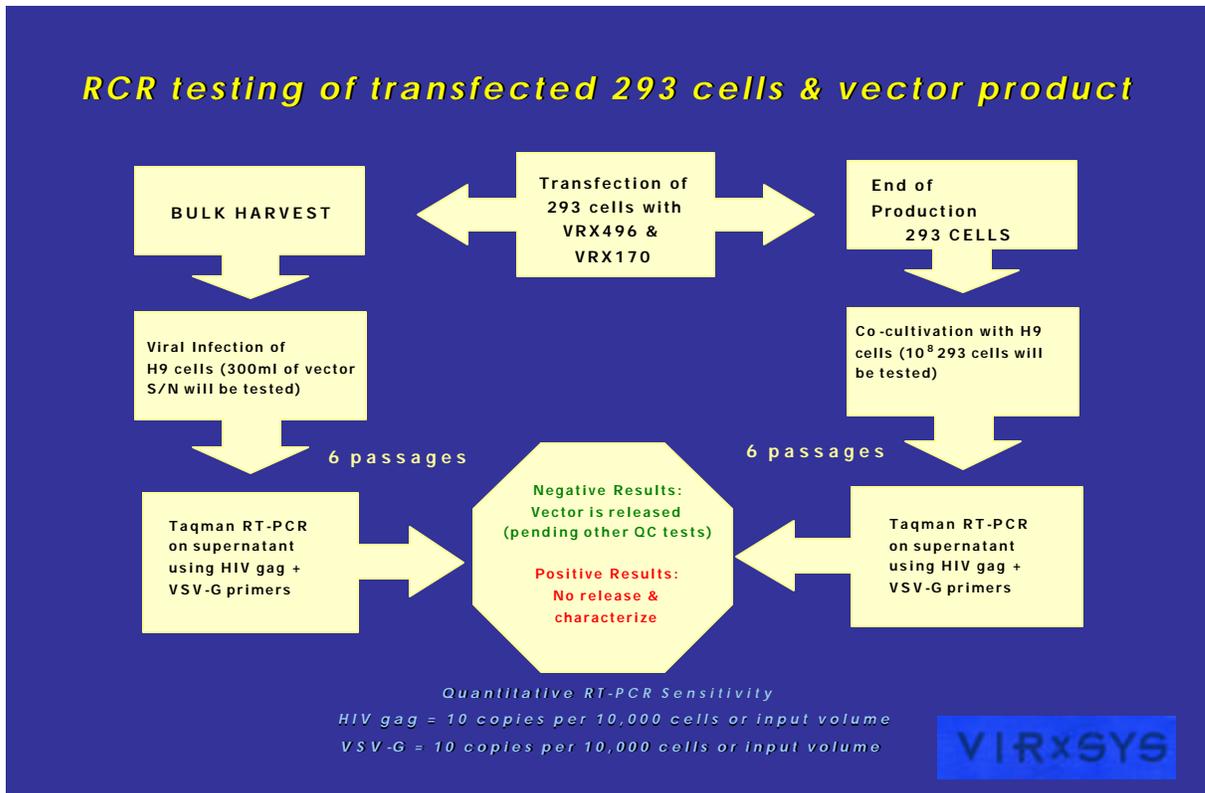


Figure 8 (above) RCR testing of vector product and end of production cells. Samples are infected or co-cultivated on H9 cells, passaged six times prior to assay for HIV Gag and VSV-G

RNA. The vector product will only be released for transduction if all assays are negative, pending testing of other QC assays.

2.5 Final Drug Product: Autologous T Cells Transduced with VRX496

2.5.1 Manufacturing Facility

Patients will undergo aphaeresis to obtain the starting T cell population in the Hospital of the University of Pennsylvania (HUP) Blood Bank. Isolation and *ex vivo* transduction of T-cells will be performed at the Clinical Cell and Vaccine Production Facility (CVPF) at the University of Pennsylvania Cancer Center.

The CVPF is a core facility within the Leonard and Madlyn Abramson Family Cancer Research Institute that can perform processing of a variety of cell types in support of adoptive cell therapy clinical trials. A variety of cell-based therapies involving bone marrow derived cells such as lymphocytes, dendritic cells, stem cells are currently in development there. In addition, non-marrow derived cells such as neural stem cells, islet cells and myoblasts can be processed in the CVPF. Processing includes isolation/enrichment of particular cell lineages, cryopreservation and storage of cells and tissues, RNA isolation from tumor, growth and expansion of cells, and transduction (insertion) of experimental therapeutic genes into cells.

The CVPF is developing procedures and instituting policies to become compliant with the standards of the Foundation for the Accreditation of Hematopoietic Cell Therapy. The International Society for Hematotherapy and Graft Engineering and the American Association of Blood Banks have jointly developed these standards in response to guidance offered by the Food and Drug Administration.

2.5.2 Cell Processing Procedures

The final drug product, autologous T cells transduced with VRX496, is produced according to the flow diagram depicted in figure 9.

2.5.3 Aphaeresis and T Cell Isolation

Patient-subjects enrolling in the clinical trial for VRX496 at the University of Pennsylvania Cancer Center undergo aphaeresis at the Hospital of the University of Pennsylvania (HUP) Blood Bank. Aphaeresis is a procedure by which the white blood cells are removed from the blood and red cells and other components are returned to the subject. The procedure usually takes about 3 hours and is a common procedure performed in blood banks. The aphaeresis product can then be transported to the HUP blood bank cell-processing laboratory for cryopreservation in liquid nitrogen. Fresh cells or cryopreserved cells then proceed to the CVPF. The CVPF may also freeze cells obtained from aphaeresis. The cells are washed in a COBE 2991 or equivalent device and depleted of monocytes.

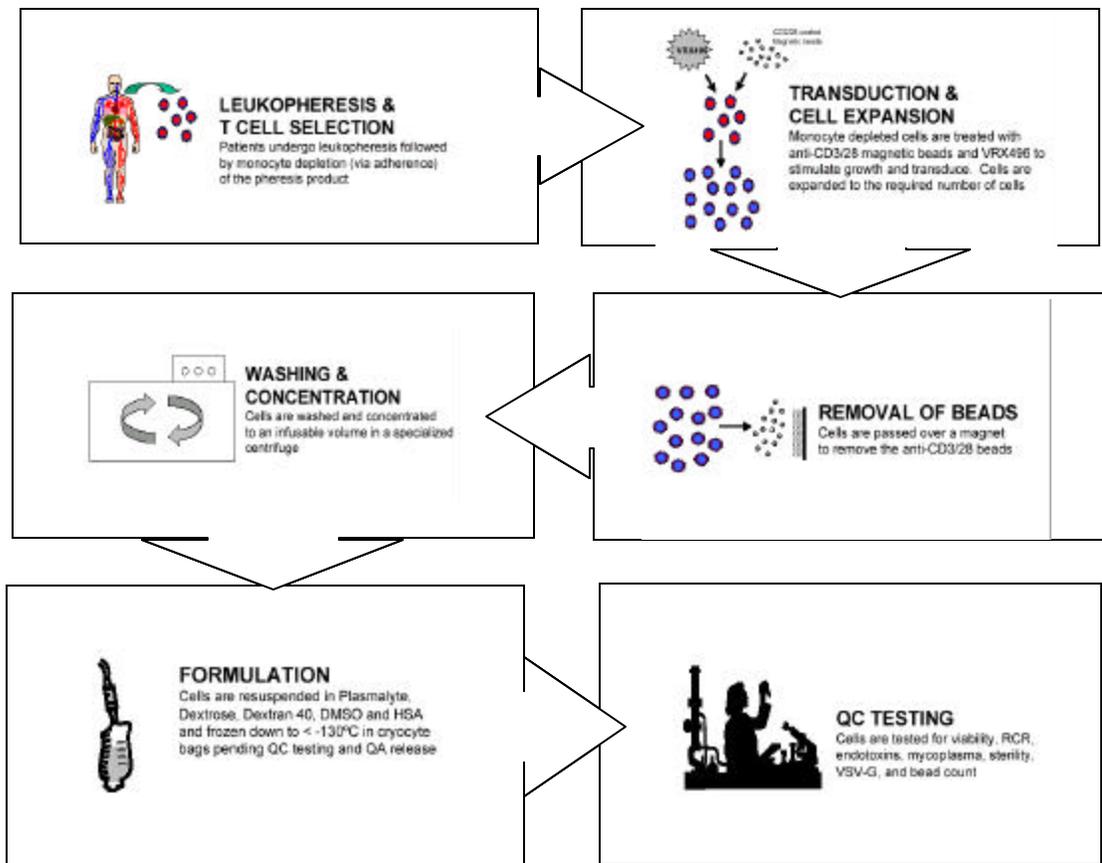


Figure 9. The T cell isolation procedure and transduction process

2.5.4 Cell Transduction and Expansion

Subject T cells are cultured in a nutrient media, and stimulated to divide and grow via the addition of magnetic beads coated with anti-CD3/anti-CD28 monoclonal antibodies (iCD3/28) directed against cell surface receptors. The VRX496 viral vector product is added simultaneously. The whole mixture of cells, growth media, VRX496 and monoclonal antibodies immobilized on beads is added to a gas permeable plastic bag and the cells are placed in a humidified 37°C 5% CO₂ incubator. Tubing leads on the bags and a variety of connecting devices allow the cells to be grown in a closed system with minimal risk of contamination. The cultures will be maintained for up to 11 days prior to harvesting and preparation for reinfusion. The cells will be counted daily or every other day and fresh medium will be added to maintain the cells at an appropriate density.

2.5.5 Cell Harvesting and Formulation

The anti-CD3/anti-CD28 beads must be removed prior to reinfusing the cells to the subject. To do this, the cell culture is passed over the Baxter MaxSep™ magnet. The beads are retained on the magnets and the cells flow through. The next step prior to infusion is to wash the cells out of the nutrient media and into an infusible solution. At times, the volume of the cell culture can be as much as 10 liters (2.5 gallons) or more. Washing and concentration is performed in a Baxter Fenwal Harvester COBE 2991 cell processor, or equivalent device maintaining a closed system.

After washing, the cells are resuspended and frozen down in an infusion solution (cryomedia) consisting of 31.25% PlasmaLyte A, 31.25% Dextrose 5%, 0.45% NaCl, 5% Human Serum Albumin (HSA), 1% Dextran 40, 7.5% DMSO. Cryopreservation is performed by allowing the product to cool at one degree (1°C) per minute until the product reaches the point of phase transition; then the freezing rate is increased until

the temperature reaches minus 90°C. Containers of cryopreserved cells are stored at minus 130°C in an electric freezer with liquid nitrogen back-up pending the results of quality control release testing. The Cell Manufacturing Protocol is described in detail in IND #6675. It is cross-referenced here because it contains confidential information not accessible to VIRxSYS Corporation.

Table 5: Transduced T Cell product QC Release Testing (Draft)			
Test	Test Item (Timing)	Method	Specification
Residual Bead Count	Final product (after harvest and before addition of cryopreservative)	Calculated based on visual count	< 100 beads per 3×10^6 cells
Viability	Sentinel vial post thaw (concurrent with product administration)	Trypan blue dye exclusion	> 60%
RCR	Culture supernatant and cells after 6 passages of the day of harvest on 293T cells	Co-cultivation assay followed by TaqMan RT-PCR detection for VSV-G and HIV-gag	Negative
Mycoplasma	1. Pre-harvest/ washing (day of harvest) 2. Final product (after harvest and before addition of cryopreservative)	PCR	Negative
Endotoxin	1. Pre-harvest/ washing (day of harvest) 2. Final product (after harvest and before addition of cryopreservative)	Limulus Amoebocyte Lysate (LAL)	< 1 EU/ml
Bacterial or fungal contamination	Final product (after harvest and addition of cryopreservative)	Culture per 21 CFR 610.12	No growth
VSV-G DNA analysis	Cells (day of harvest) 1% or 10^8 cells will be tested	TaqMan DNA-PCR	Not detectable for VSV-G DNA sequences
VSV-G RNA analysis	Cell supernatant (day of harvest) 3 x 200ul of cell supernatant will be tested	TaqMan RT-PCR	Not detectable for VSV-G RNA sequences

2.5.6 Transduced T Cell Release Testing and Quality Control Methods

QC testing is performed prior to administration of cells to the patient. QC testing includes a bead count, viability, RCR, PCR test for VSV-G sequences, mycoplasma, endotoxin, bacterial & fungal contamination. Specifications are listed in table 5 above.

2.5.7 RCR Assays

Biological Replication Competent Retrovirus (RCR) assays will be used to detect for a putative HIV-based RCR that could occur from recombination of vector or wt-HIV genome with VSV-G RNA or DNA. Both the viral vector product and the transduced cell product will undergo biological RCR testing. Briefly, viral vector product and cell product will be incubated on H9 cell and 293 cells respectively. Since the viral product does not contain wt-HIV, there should be no amplification and detection of Gag or VSV-G sequences on the H9 cell line, which is permissive to wt-HIV replication. The cells infected with the viral vector product will be passaged 6 times (twice weekly) for a total of 3 weeks and the supernatant will then be assayed by TaqMan RT-PCR for VSV-G or HIV-gag RNA. Similarly, VRX496 transduced cell product will be co-cultivated with 293T cells (a wt-HIV non-permissive cell line) and then the cells will be passaged 6 times as above and assayed by TaqMan RT-PCR for VSV-G & HIV-gag RNA. The vector will only be released if the all biological RCR assays are negative, pending release of other QC assays. A scheme showing the RCR release testing criteria is shown in figure 10.

2.5.8 DNA and RNA PCR (Detection of VSV-G)

The vector transduced cell release assay will use DNA and RNA PCR to detect VSV-G helper sequences in cells transduced with vector and cell supernatant, respectively. Our release testing criteria require that no functional VSV-G DNA be detected in vector-transduced cells. Also, our release testing criteria require that no functional VSV-G RNA be detected in the supernatant of vector-transduced cells. When both VSV-G DNA and RNA sequences are below the detection limit of highly

sensitive PCR assays the cells will be released. Although the VSV-G DNA and RNA PCR assays are very stringent methods to determine for the presence of VSV-G DNA or RNA, it is important to note that no assay can guarantee that absolutely no VSV-G nucleic acid will be present in the final VRX496 modified T cell product. Therefore, it is an intrinsic risk of the proposed clinical study to use the broadly tropic VSV-G as the envelope protein for HIV-1 based vectors in HIV-infected individuals since there is the theoretical possibility that the VSV-G DNA could recombine with vector or wt-HIV to produce a RCR. A non-detectable VSV-G sequence in the final product can significantly reduce the risk of recombination by reducing the number of molecules available for a recombination event. In addition, all biological RCR assays to date indicate that no such virus is produced *in vitro*, as detected by the above described biological RCR assays. A scheme showing the RCR release testing criteria for the transduced cell product is shown in figure 10.

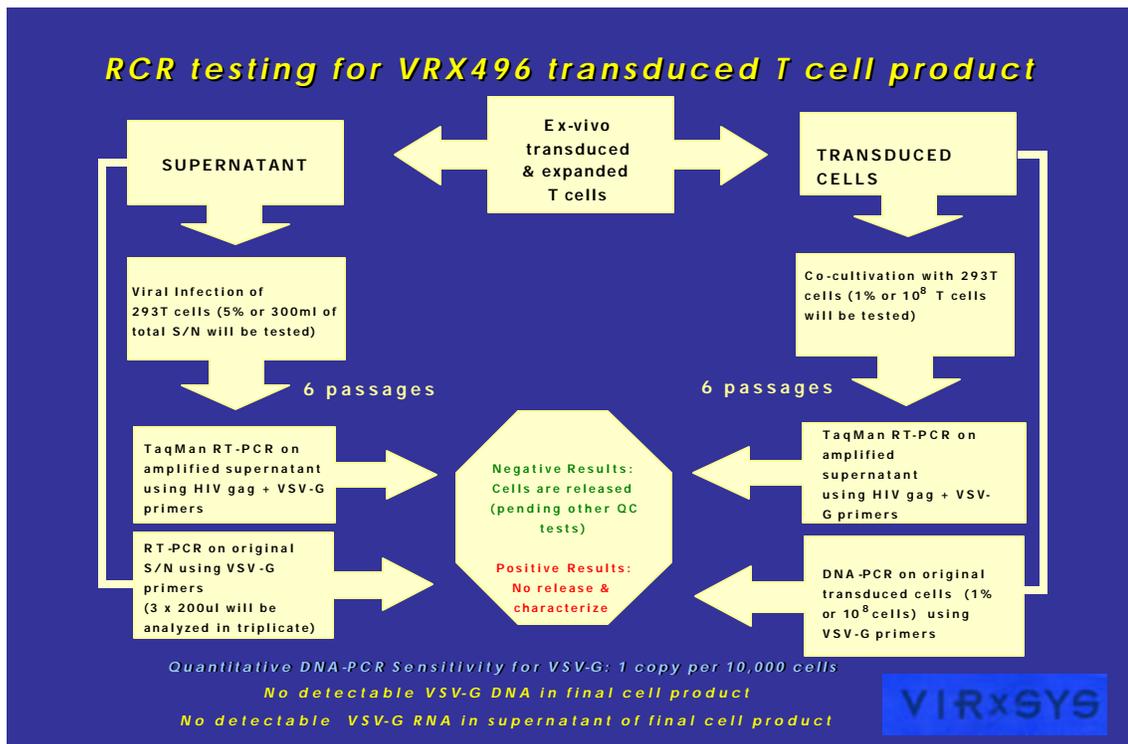


Figure 10. RCR testing for VRX496 modified vector product. Supernatant and transduced cells will be assayed by biological RCR testing by infection of 293T and co-cultivation with 293T cells for six passages before sensitive TaqMan PCR is used to detect Gag or VSV-G sequences. In addition the

cell supernatant and transduced cells will be tested directly for VSV-G RNA and DNA respectively by quantitative PCR. Only if all assays are negative will the transduced product be released, pending the release of other QC assays.

3.0 Experimental preclinical Studies

3.1 Transduction of Primary Human CD4 T cells

Primary human CD4 T cells were isolated from normal human donors and transduced with VRX494 vector (a GFP expressing analog of VRX496, the candidate vector for phase I clinical trials) at an MOI of 20 in the presence of immobilized CD3 and CD28 (iCD3/28) antibodies. After seven days in culture the cells were examined for EGFP expression by FACS analysis (figure 11). The cells demonstrated greater than 99% transduction efficiency.

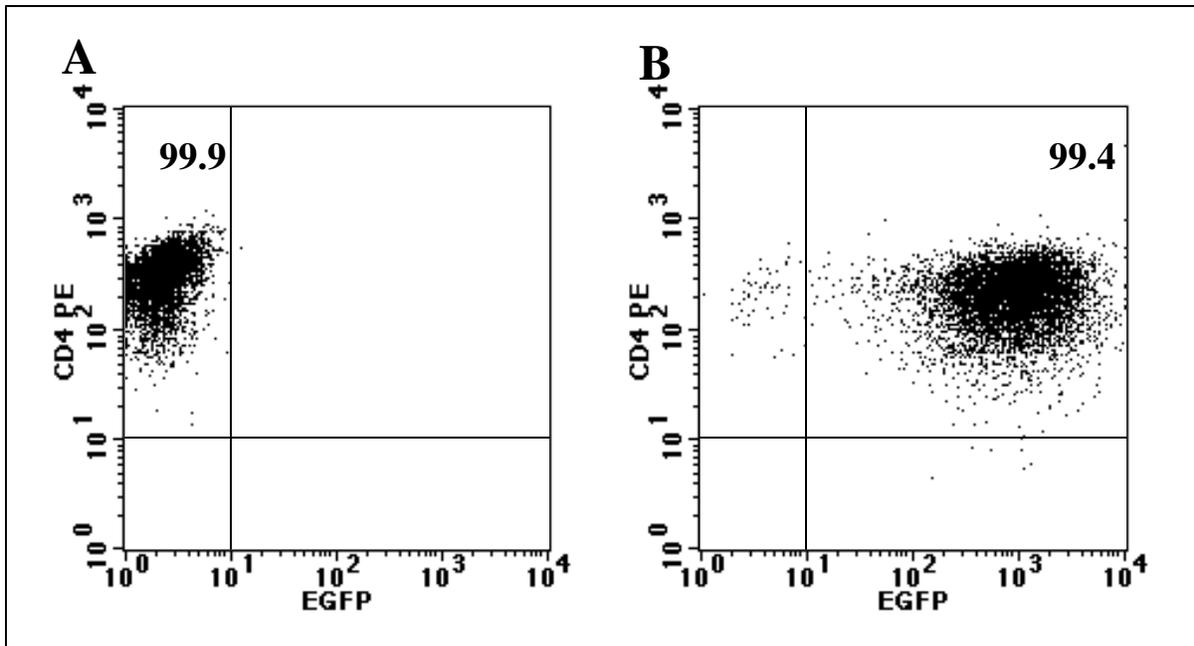


Figure 11. High level of transduction of primary human CD4 T cells with VRX494, a GFP positive version of the VRX496 anti-HIV vector. CD4 T cells were isolated from normal human donors and transduced with VRX494 in the presence of iCD3/28 antibodies. The cells were then cultured for one week in iCD3/28 at which time the culture was analyzed by FACS. The Y-axis shows the percentage of CD4 cells obtained from the isolation procedure, while the X-axis shows the percentage of vector containing cells as shown by GFP expression. The control cells are shown in panel A while the test cells are shown in panel B.

In a separate experiment, cells containing the vector were expanded ex vivo over a 29 day period and examined for the percentage of GFP expressing cells and the vector copy number by TaqMan PCR. The average transduction efficiency of CD4 T cells was found to be greater than 95%, as determined by FACS analysis for GFP expression. Taqman PCR showed that the vector copy number was stable at about 9 copies per cell during the course of the experiment (figure 12). Furthermore, transduced cells demonstrated no toxic effects from the vector, as untransduced and transduced cells expanded similarly. The transduced CD4 T cells showed remarkable stability of vector copy number and GFP expression considering the greater than 1000 fold expansion that occurred during the ex vivo culture period (figure 12).

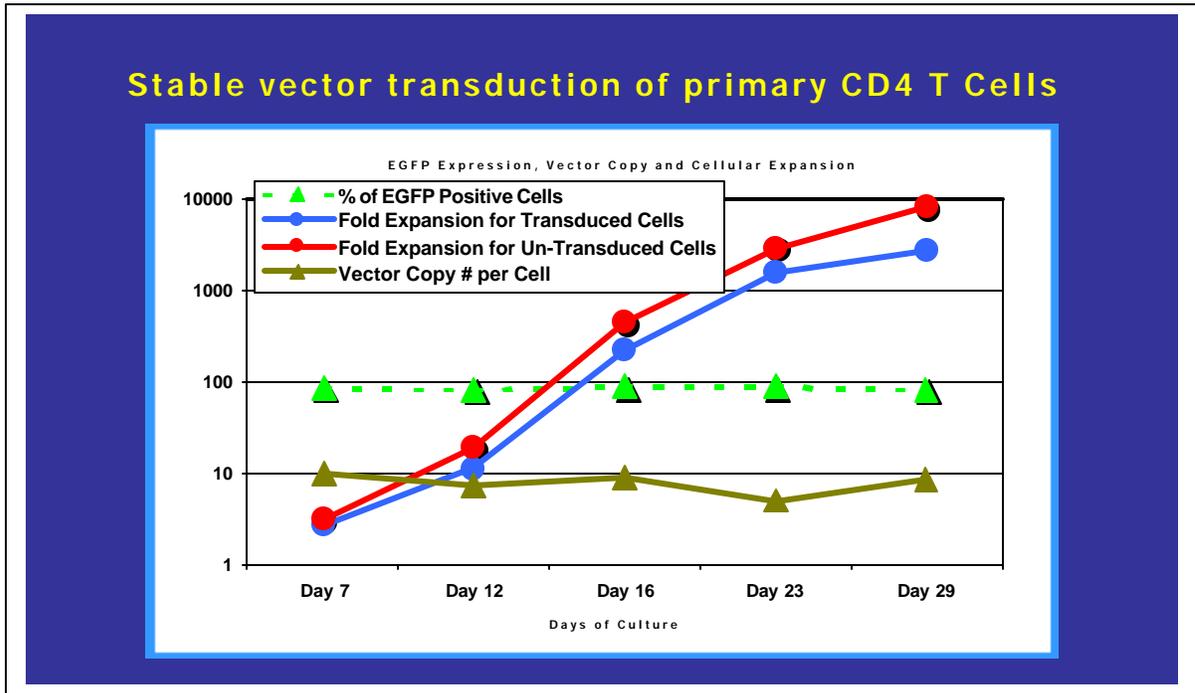


Figure 12. Normal primary human CD4 T cells isolated from a normal human donor were transduced with vector (VRX494 – an GFP positive version of VRX496) with an MOI of 20 and cultured for 29 days ex vivo. During this time, the number of GFP positive cells remained stable at >95% GFP positive (green triangles). Furthermore, the vector copy number, as determined by TaqMan PCR was also stable at about 9 copies per cell (brown triangles). The stability of vector copy number and marker gene expression was seen during an over 1000 fold expansion of the cells in culture. No

significant difference in expandability was seen between cells that did not contain the vector (red circles) and cells that contained the vector (blue circles). In toto, these results indicate that the vector is extremely stable in primary CD4 T cells and that there does not appear to be any toxic effects due to the vector that would significantly affect CD4 T cell expansion ex vivo.

3.2 Inhibition of wt-HIV replication by VRX496 analog (VRX494) modified cells

CD4 T cells transduced with an analog VRX496 were then challenged with NL4-3 strain of HIV -1. Since VRX494 can attain such high transduction efficiencies, no selection for transduced cells was required. While control cells devoid of VRX494 replicated wt-HIV to predictable levels (figure 13; red circles), cells transduced with VRX494 inhibited NL4-3 HIV-1 replication by over 3 logarithmic units of p24 during the peak of infection (figure 4; blue circles). Furthermore, no breakthrough virus was seen during the extended culture period. The data demonstrates that an analog VRX496 can potentially inhibit wt-HIV replication in primary human CD4 T cells.

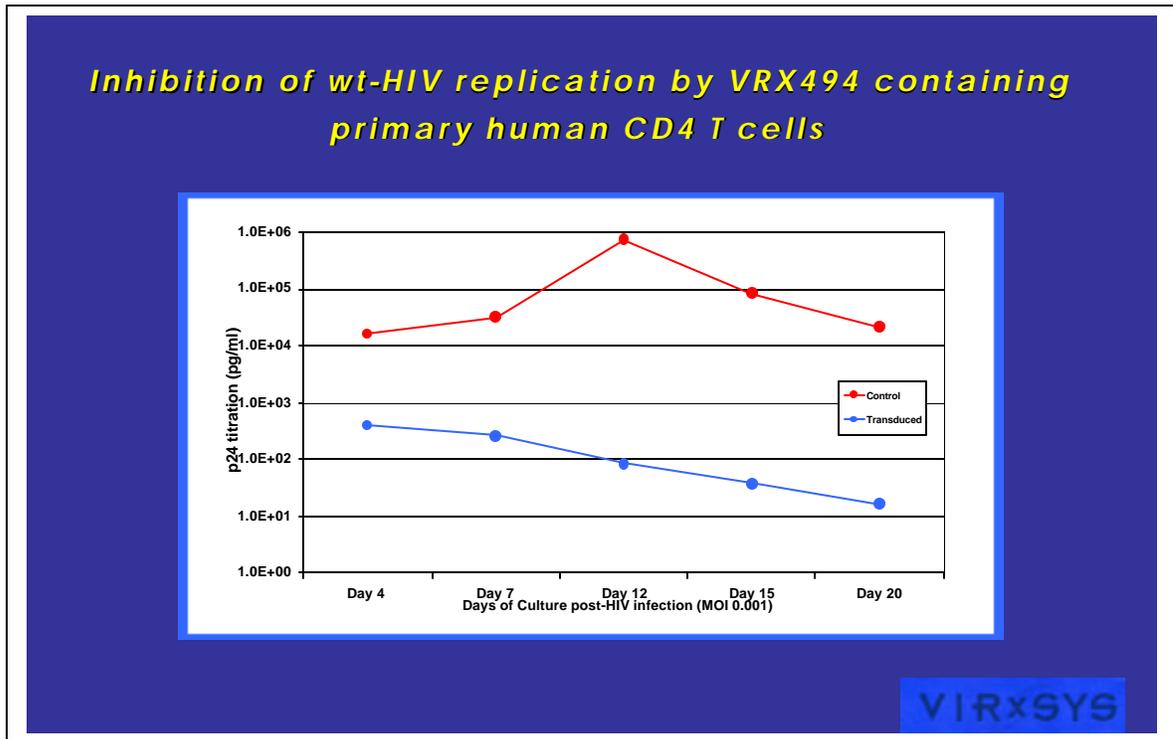


Figure 13. Inhibition of wt-HIV replication in cells transduced with vector. Cells transduced with VRX494 (blue) and control untransduced cells (red) were challenged with HIV NL4-3 virus at a MOI of 0.001 five days after transduction. The cells were kept in culture for 20 days post-HIV infection (x-

axis) during which time p24 was assayed for wt-HIV replication (y-axis: scale is logarithmic units of p24; $1.0E+01=10$ pg/ml, $1.0E+02=100$ pg/ml, $1.0E+03=1000$ pg/ml etc.) The above graph is a representative of three independent experiments.

3.3 Resistance to productive HIV infection by VRX496 analog (VRX494) modified cells

The above cultures were examined for down-regulation of surface CD4 expression by FACS analysis. Down-regulation of surface CD4 expression would indicate productive HIV infection that results from expression of HIV nef, gp120 or Vpu proteins (Benson et al., 1993). While the above HIV -1 infected cultures demonstrated down-regulation of surface CD4 in control untransduced primary CD4 T cell cultures, no significant surface CD4 down-regulation was seen in VRX496 modified cell cultures (figure 5). The data demonstrates that VRX496 confers resistance to productive HIV replication in CD4 T cells.

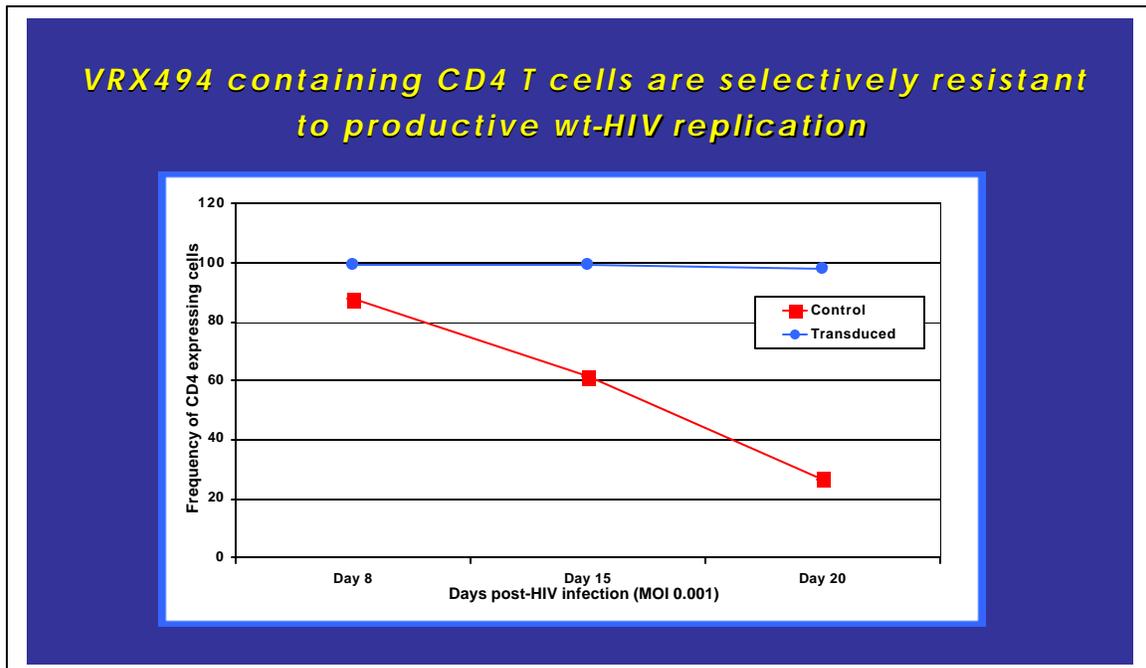


Figure 14. Resistance to productive HIV infection by analog VRX496 transduced (blue circles) but not untransduced control cells (red squares). The percentage of CD4 positive cells was determined by FACS analysis.

3.4 Inhibition of the replication of wt-HIV primary strains with analog VRX496 (VRX494) modified cells.

To test for the ability of HIV vectors to inhibit X4 and R5 strains of HIV -1, CD4 T cells from normal human donors were isolated and challenged with wt-HIV strains and then assayed for wt-HIV replication by measuring for p24 activity. The data shows that that the analog VRX496 (VRX494) can effectively control HIV -1 replication of all strains (both X4 and R5 strains) of HIV -1 analyzed (figure 15). However, VRX494 was more effective in initially controlling the replication of X4 strains of HIV (average of 3.5 logarithmic units of inhibition of p24) than R5 strains of HIV (average of 2.5 logarithmic units of inhibition of p24), indicating that the antisense payload is more effective in controlling X4 strains of HIV. This is consistent with the preferential sequence specificity of the anti-HIV antisense payload sequence, which would preferentially inhibit X4 strains of HIV -1 since it is derived from the X4 NL4-3 strain. At later time-points, the vector was able to effectively control R5 HIV strains by over 3 logarithmic units of inhibition of p24 (figure 15). The data demonstrates that VRX496 can broadly inhibit a wide variety of X4 and R5 strains of HIV -1 .

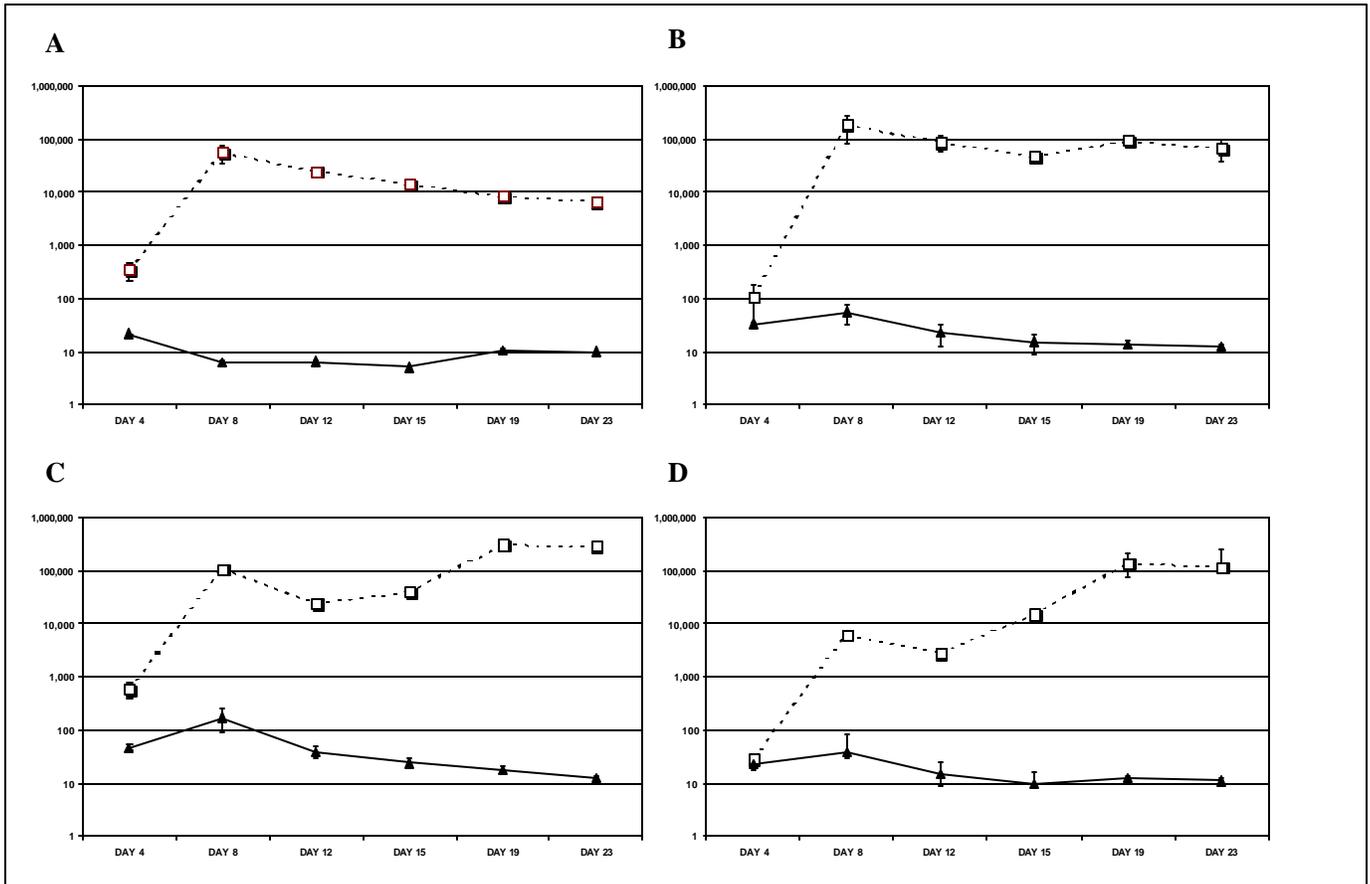


Figure 15. Inhibition of primary HIV strains by an analog VRX496 (VRX494) vector in primary human CD4 T cells. Cells from normal human donors were obtained and CD4 T cells isolated by MACS positive CD4 selection. The purity of the recovered CD3+CD4+ fraction was estimated to be 95.4%. The CD4 T cells were then transduced with VRX494 at a MOI of 20. The transduction frequency was determined to be 98.51% EGFP positive CD4 T cells. Five days after transduction, the cells were challenged with the following HIV strains at a MOI of 0.001. Primary HIV Isolate Challenge: Results displayed are Average +/- Standard Deviation (n=3). June and colleagues have shown that cells stimulated with iCD3/28 are capable of supporting the replication of both X4 and R5 strains of HIV-1. After transduction, iCD3/28 bead stimulation was added again on day 4 to facilitate cell expansion. Open squares-black dashed lines: HIV infection into untransduced (MOCK) CD4+ T cells. Closed triangles- black plain lines: VRX494 Exp#65 MOI 20-1 round Transduced CD4+ T cells – 98.5% EGFP+CD4+ - Average vector copy number: 13 (day7) A: CXCR4 laboratory strain NL4-3 at MOI 0.001; B: CXCR4 primary isolate BK-132 at MOI 0.001 C: CCR5 primary isolate Ba-L at MOI 0.001; D: CCR5 primary isolate US1 at MOI 0.001 The HIV-1 NL4-3 is the cognate virus from which

the vector was constructed. It is a X4 strain of HIV. Primary isolate HIV-1 BK132 was isolated from an HIV-1 infected individual and grown up in PBMCs for a single passage. It has been determined to be an X4 strain of HIV-1. HIV-1 Ba-L strain is the prototypic R5 strain of HIV. HIV-1 US1 is a primary isolate that has an R5 phenotype.

3.5 Selective resistance of VRX496 analog (VRX494) modified cells to productive HIV infection

To determine whether VRX496 could provide selective resistance to CD4 T cells, the following experiment was performed. CD4 T cells isolated from normal human donors were transduced with vector so that the transduced and untransduced populations were approximately 50% each. The 50% mixture of cells was then challenged with different strains of HIV -1. As shown in figure 16, while control cells that did not contain the vector down-regulated surface CD4 expression (lower left panel), cells transduced with the vector did not display such significant down-regulation (upper right panel). The data demonstrates that VRX496 provides CD4 T cells with a selective resistance to productive HIV infection over cells that do not contain the vector

(figure 16).

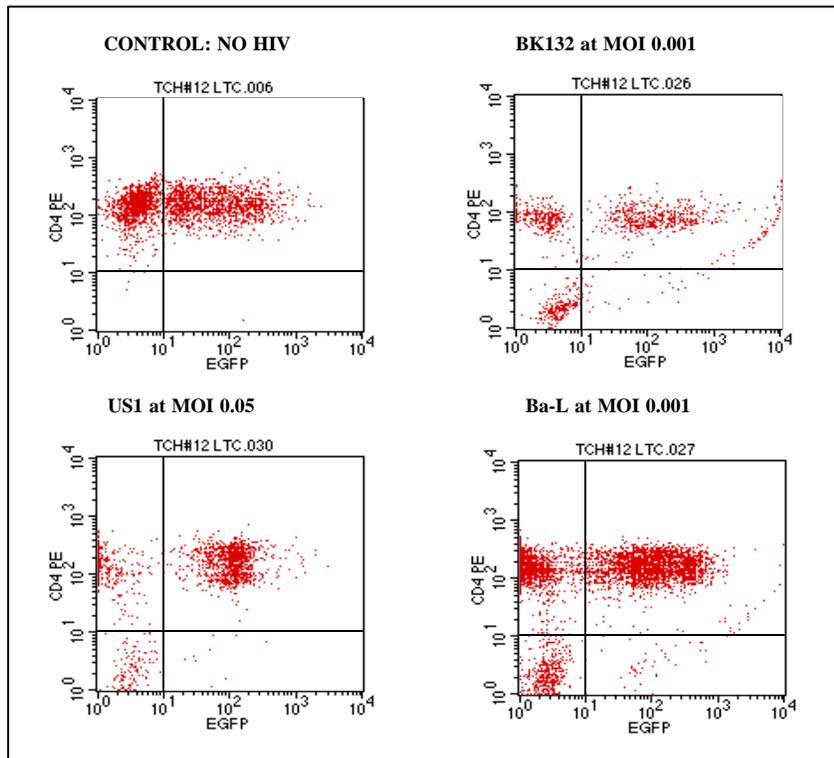


Figure 16 (above). Selective resistance to CD4 downregulation in VRX494 modified cells. Transduced with vector to achieve 50% transduction and then challenged with wt-HIV prior to being analyzed by FACS 36 days after challenge. The strain of virus and the MOI used for infection is shown above each panel. Cells without vector (not expressing GFP) show selective down-regulation of CD4 over cells containing vector (GFP positive).

3.6 Lack of Toxicity of HIV vectors on transduced CD4 T cells

VRX496 is a VSV-G pseudotyped lentiviral vector and thus may be toxic to cells. We examined whether VRX496 transduction of normal primary T cells affected their doubling time, cell size, viability or surface antigen expression. The data demonstrates that VRX496 is not toxic to primary T cells. There was no significant difference in the population doubling time, cell size, viability or surface antigen expression between untransduced control cells (red) and VRX496 transduced cells (blue) (figure 17).

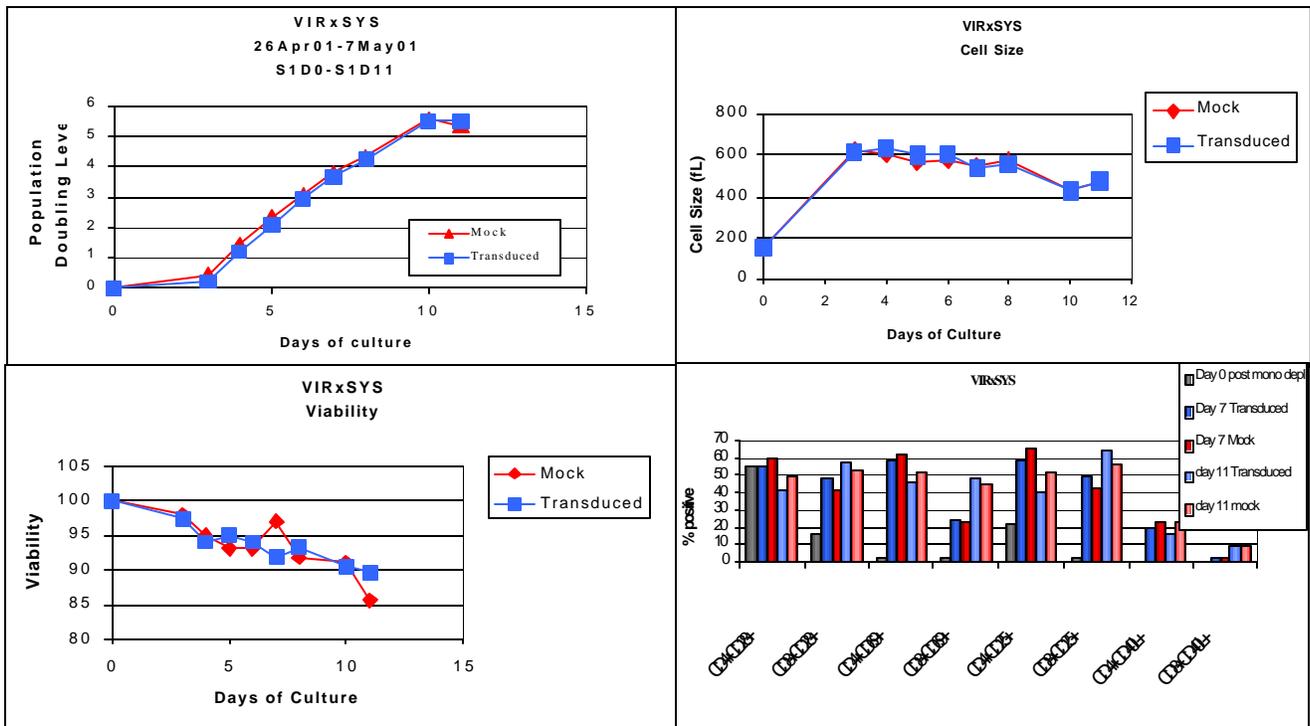


Figure 17. *Biological analysis of cells transduced with VRX496 (blue) as compared to untransduced control cells (red). T cells isolated from normal human donors were transduced with VRX496 and analyzed for their doubling time (upper left), cell size (upper right), viability (lower left) and surface antigen expression (lower right). No significant differences were seen between transduced and untransduced populations. In the lower right panel, surface antigen expression was examined by FACS on day 7 and day 11 post-transduction. (Day 0 pretransduction – grey; day 7 transduced – dark blue; day 7 mock – dark red; day 11 transduced – light blue; day 11 mock – light red).*

3.7 Inhibition of HIV replication in CD4 T cells from an HIV infected individual

Evidence indicating that VRX496 modified cells may be able to inhibit HIV replication in CD4 T cells from HIV-1 infected individuals has recently been obtained. Blood from a single HIV-1 infected donor was obtained from The Gary Lambert Research Center at The Johns Hopkins University School of Medicine. The patient had the following characteristics: CD4 counts of 685 per ul, no opportunistic infections, discontinued HAART therapy and a viral load of 92,000 copies per ml. CD4 T cells were isolated from the blood of the donor (donor code: PG01) and then transduced with VRX496. The cells were then monitored for viral replication by p24 assay. The data shows that vector transduced cells could inhibit endogenous wt-HIV replication by over 2 logarithmic units of p24 from cells derived from the HIV infected donor (figure 18).

3.8 Preferential resistance of VRX496 transduced cells to productive HIV replication

CD4 T cells from donor PG01 that were challenged above were monitored for surface CD4+ expression to determine if there was any preferential resistance of transduced cells to productive wt-HIV replication. The data shows that the number of productively HIV infected cells that were down-regulated for CD4 expression was considerably less in the cells treated with VRX496 than the untreated mock control cell cultures (figure 19). The data suggests that VRX496 can provide CD4 cells with

a preferential resistance to productive HIV infection, and hence, a survival advantage over cells not modified with the vector.

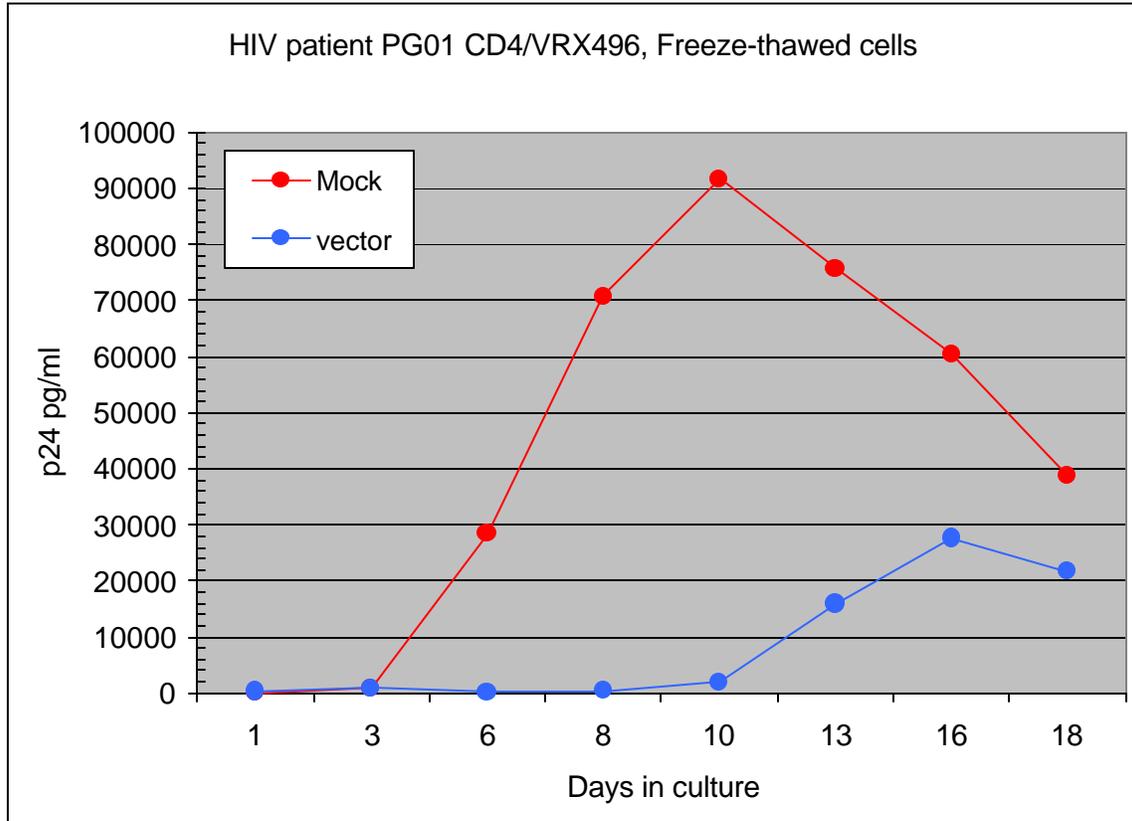


Figure 18. Inhibition of HIV replication from a HIV-1 infected donor by VRX496 transduced cells. Peripheral blood CD4 cells from an HIV infected donor (donor code: PG01) were purified by negative selection transduced with VRX496 in the presence of anti-CD3 and anti-CD28 antibody coated beads and IL-2 and cultured for 9 days before the cells were frozen down (during a clinical trial this would be the stage at which QC would be performed). The cells were then thawed and grown in anti-CD3/28 and IL-2 for 18 days during which time virus replication was assayed by p24 antigen capture assay. The data demonstrates strong inhibition (over 2 logarithmic units of p24) of wt-HIV by the transduced cells.

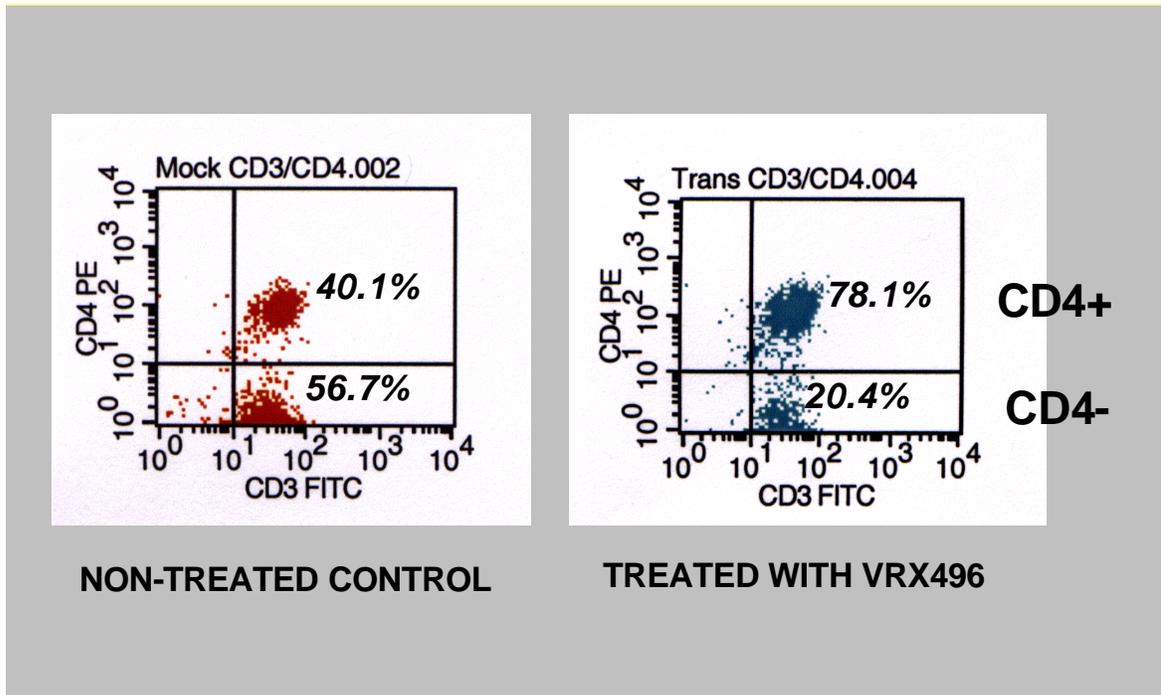


Figure 19. Preferential resistance of VRX496 treated CD4 T cells to productive HIV replication. While non-treated control cells displayed only 40.1% of the cells being CD4+, almost twice as many cells (78.1%) of the cells were CD4+ after treatment with VRX496. The data suggests that VRX496 treated cells are selectively resistant to productive HIV infection over non-treated cells in an HIV infected environment.

3.9 Determination of the nature of the breakthrough virus

The nature of the breakthrough virus seen in the transduced culture (blue) in figure 18 was studied. RT-PCR was performed on the supernatant of all the cultures using wt-HIV and vector specific primers. As shown in figure 20, while in control mock cultures only the wt-HIV band is seen during the course of the experiment, a significant vector band and a lighter wt-HIV band is seen in transduced cultures during the time of viral breakthrough. The data shows that the viral breakthrough

consists of predominantly packaged vector genomes that were selectively packaged into progeny viral particles at the expense of wt-HIV.

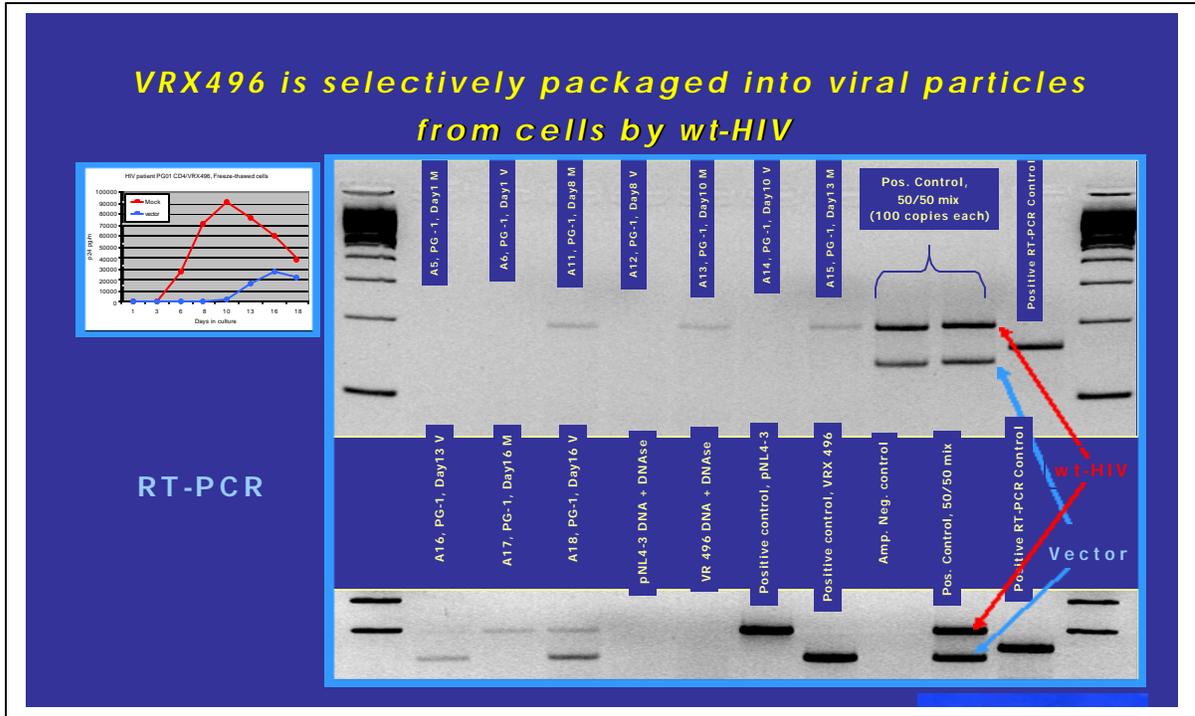


Figure 20 RT-PCR of viral supernatants from cells obtained from a HIV-infected donor (PG01) that was transduced with VRX496 and later expanded in vitro using iCD3/28 antibodies. Lanes are marked as mock (M) or vector (V) transduced cell supernatant. The controls are clearly marked in the figure.

3.10 Analysis of the packaged vector genomes

The mobilization potential of VRX496 was examined by passaging the supernatants that contained packaged vector genomes onto naïve T cells. Formal mobilization of the packaged vector genomes into the genome of naïve T cells could not be demonstrated using conventional DNA PCR of cellular DNA. However, when the cellular DNA was analyzed by TaqMan PCR, a low level of vector mobilization was detected (30 copies per 10,000

cells analyzed). The data shows that VRX496 weakly mobilizes into primary CD4 T cells after infection of vector containing cells with wt-HIV.

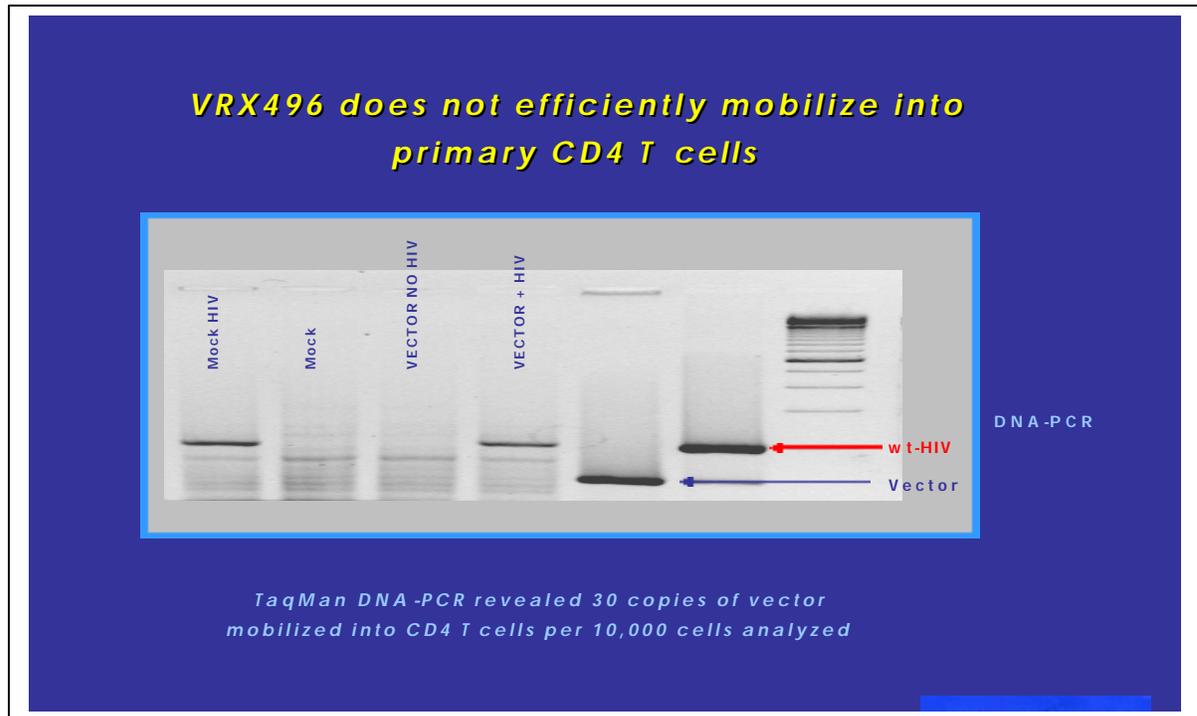


Figure 21. Weak mobilization of VRX496 into primary CD4 T cells. Supernatants from cells transduced with vector that were infected with wt-HIV were incubated on primary uninfected CD4T cells. The DNA was then isolated and PCR performed to detect wt-HIV and vector genomic DNA. Although wt-HIV could be seen in mock and vector + wt-HIV cultures, no vector band was seen in the infected cultures. To determine if any vector mobilization had occurred, TaqMan PCR was performed on the cells. This assay revealed that only 30 copies of vector had mobilized into the genome of primary CD4T cells per 10,000 cells analyzed.

3.11 Mobilization properties of the VRX496 analog, VRX494 (GFP+)

The mobilization properties of VRX496 were examined using an analog vector (VRX494) that expresses the green fluorescent protein (GFP). Primary CD4 T cells and a T cell line (Sup T1 cells) were transduced with VRX494 and then challenged with wt-HIV at a MOI of 0.2. The supernatants of the challenged cells were then

infected onto naive MT4 cells, a CD4 T cell line that contains the HTLV-1 Tax gene and is highly permissive for HIV replication. Untransduced, VRX430 transduced and VRX494 transduced cells were challenged with wt-HIV. VRX430 is a payload deficient version of VRX494, where the anti-HIV env antisense sequence is absent in this vector. In figure 22, the data shows that both VRX430 and VRX494 show low mobilization properties from Sup T 1 cells and particularly from primary CD4 T cells. Furthermore, it appears that the antisense payload inhibits vector mobilization since in both primary CD4 T cells and Sup T1 cells, the antisense containing vector (VRX494) mobilized less efficiently than the payload-deficient vector (VRX430).

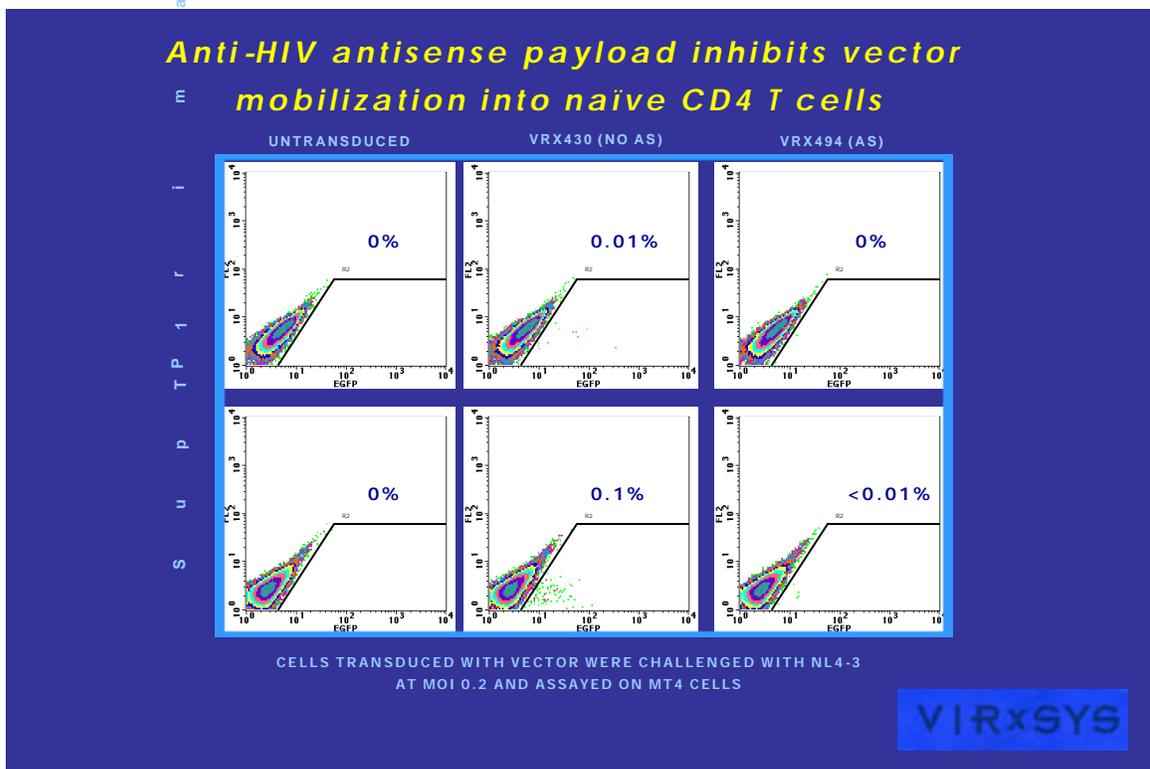


Figure 22. Low levels of vector mobilization from primary CD4 T cells and CD4 T cell lines. CD4 T cells (untransduced, VRX430 transduced & VRX494 transduced) were challenged with wt-HIV and 3 days later the supernatants were infected onto MT4 cells. FACS analysis was performed on MT4 cells 3 days after infection. Mobilization events are scored as positive for GFP expression. Cells containing VRX494 had lower levels of mobilization as compared to cells that contained the payload-deficient VRX430 vector.

3.12 In Vivo mobilization properties of the vectors

An *in vivo* mobilization study was performed using the SCID/SCID mouse xenotransplantation model to determine if the vector would adversely mobilized *in vivo* upon challenge with vector containing cells with wt-HIV. CD4 cells were isolated from a normal human donor and separated into two lots for transduction with VRX496 analog vectors expressing either EGFP (VRX494) or EYFP (VRX530). The cells were challenged with wt-HIV (MOI = 0.2) and then injected intraperitoneally into SCID/SCID mice that had previously been injected intraperitoneally with peripheral blood human mononuclear cells. Control CD4+ T cells consisted of cells that were not infected with wt-HIV. Mice were sacrificed at 3 and 7 days post injection. Preliminary studies have shown that days 3 and 7 post wt-HIV challenge are the days most likely to produce mobilization events *in vivo*. After harvest of the cells from the peritoneal cavity the cells were examined by FACS for vector mobilization. The experimental design for this study is shown below in table 6 and figure 23.

Table 6: In Vivo Mouse Mobilization Study---Experimental Design						
Group	Mice/group (Males)	Transducing Vector(s)	HIV Challenge ¹	MOI	Mice Sacrificed	
					Day 3	Day 7
1	10	VRX494 (EGFP) VRX530 (EYFP)	No	0.2	5	5
2	10	VRX494 (EGFP) VRX530 (EYFP)	Yes	0.2	5	5

¹ Cells infected ex vivo with HIV NL4

Each mouse was injected intraperitoneally with 4×10^6 VRX494 transduced CD4+ T cells and 4×10^6 VRX530 transduced CD4+ T Cells. Group 1 mice were injected with uninfected CD4+ cells and Group 2 mice were injected with NL4-3 HIV infected CD4+ cells.

An authentic or non-adverse mobilization event would consist of mobilization of the vector genome from CD4 cell to CD4 cells resulting from wt-HIV envelope mediated spread, provided by the infecting wt-HIV. This type of mobilization would be visualized by double positive EGFP/EYFP expressing cells since double positive cells would be the result of mobilization of the vector from either GFP or YFP

expressing cells to YFP or GFP expressing cells, respectively. An adverse mobilization event would be spread of the vector to a cell type not normally infected by wt-HIV. An example of such spread would be incorporation of the VSV-G envelope into the packaged vector genome and confer it with broad tropism to infect a wide variety of cell types. Such adverse mobilization would be visualized by detection of GFP or YFP expression in a CD4 negative cell line. For the purposes of this study, B cells (CD19 cells) were chosen as the marker cell to detect for an adverse mobilization event. It is known that B cells are not infected by wt-HIV and thus this cell type is a definite cell type for which to look for adverse mobilization. The scheme for the experiment is shown in figure 23.

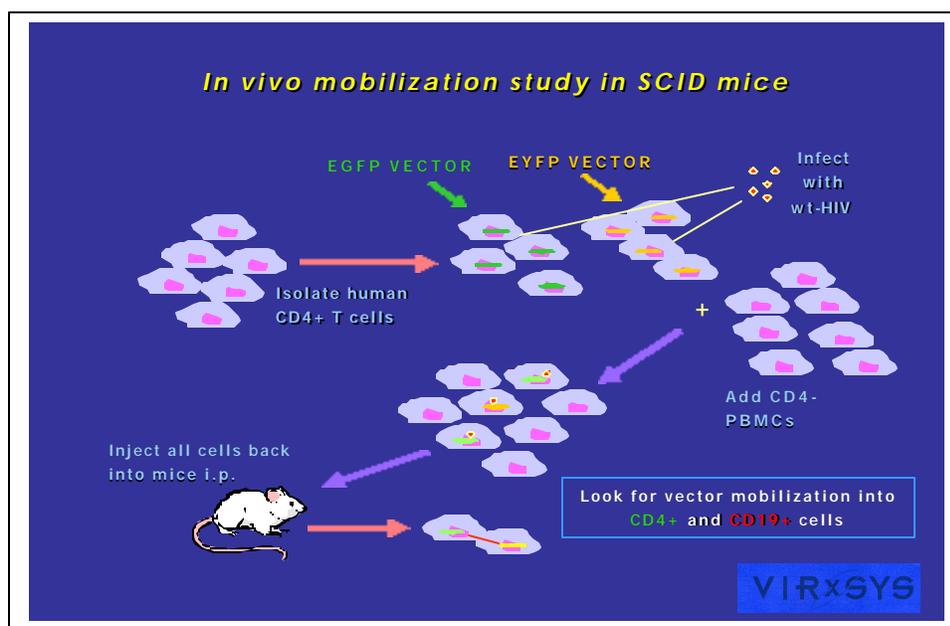


Figure 23. Scheme for the in vivo mobilization study in SCID mice.

In this study, two groups of ten male mice were injected intraperitoneally with either VRX494/VRX530 transduced CD4+ T Cells or HIV NL4-3 infected VRX494/VRX530 transduced cells. After three days, no mobilization was evident in either the control mice or those injected with HIV NL4-3 infected cells, and after 7 days, three of five mice injected with HIV infected EGFP/EYFP cells exhibited between 1 and 2% EGFP+/EYFP+ cells as determined by FACS analysis (figure 24). At no time was

the percentage of dually EGFP/EYFP labeled cells greater than 2%, suggesting that a low level of mobilization was occurring between CD4+T cells. Mice injected with uninfected cells each exhibited less than 1% of cells expressing both EGFP and EYFP. Furthermore, no mobilization was observed from CD4+T cells to the surrogate CD19 B cells (figure 25). This data shows that no adverse mobilization event occurred after wt-HIV challenge of VRX496 analog vector containing cells.

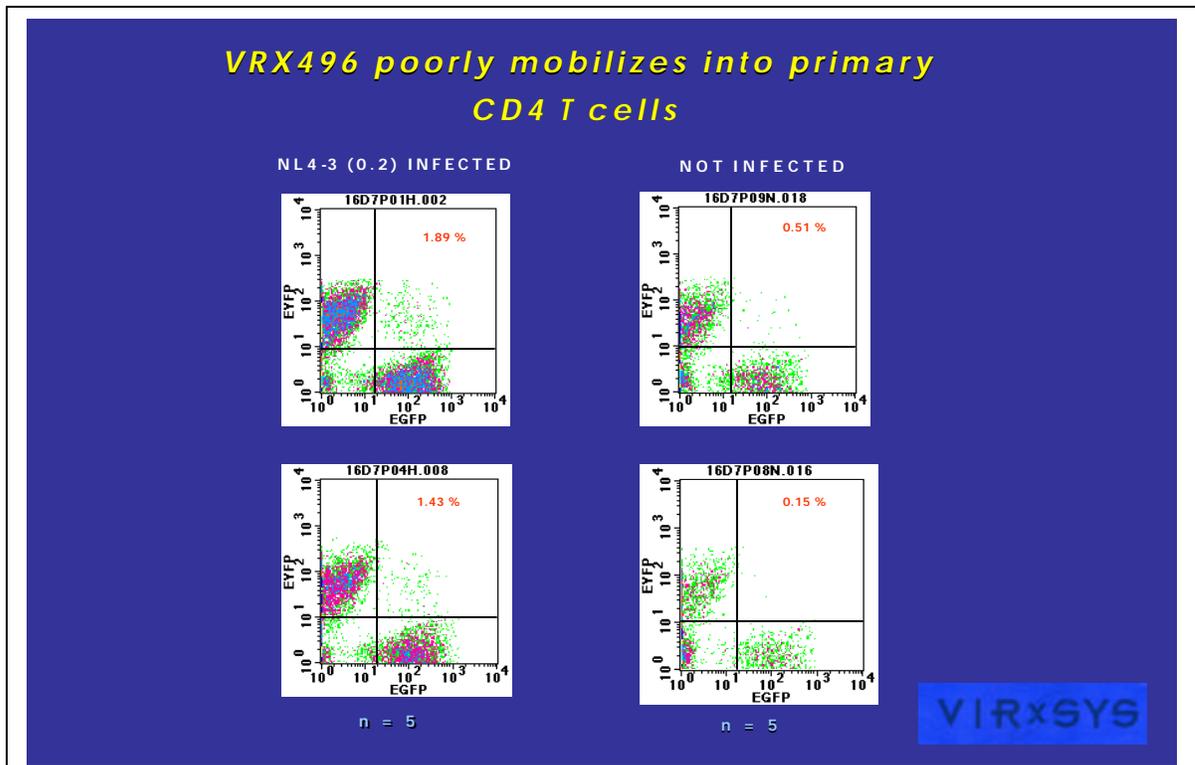


Figure 24. Weak mobilization of VRX496 analogs into primary CD4 T cells. The left two panels show between 1-2% EGFP/EYFP double positive cells, indicating weak mobilization of the VRX496 analog vectors between CD4 T cells. The right two panels show cells that were mock infected with wt-HIV, showing the level of background double positive staining (<1%).

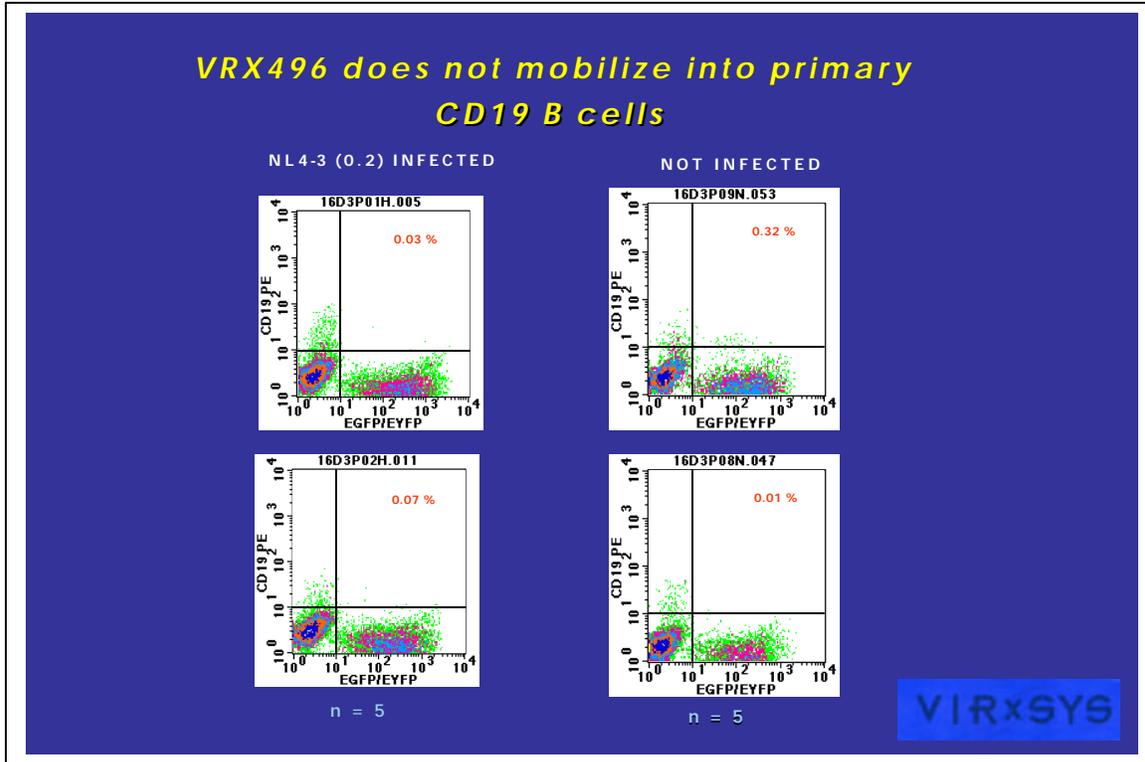


Figure 25. Authentic mobilization of VRX496 analog vectors. Cells present in the peritoneum of mice were isolated and underwent FACS analysis for the presence of EGFP or EYFP in CD19 B cells. The left panels show no double positive events in cells challenged with wt-HIV. The right panels show the level of background double positive events. The data shows that no adverse mobilization events were detected in surrogate CD19 B cells.

3.13 Summary of the experimental preclinical data

The above described experimental results have demonstrated that:

- Transduction of primary CD4 cells resulted in a >90% transduction efficiency with no apparent toxicity of the transduced cells.
- Vector containing cells challenged with NL4-3 and primary strains demonstrated potent inhibition of wt-HIV replication
- Vector containing cells challenged with HIV show resistance to productive HIV infection over cells that do not contain the vector
- Vector demonstrated a weak level of authentic mobilization. No adverse mobilization events were detected.

4.0 Biodistribution and Toxicity Studies in animals

To evaluate the biodistribution and toxicity of VRX496 transduced human T cells in an animal model, the SCID/SCID murine model was chosen because:

- 1) Human T cells carrying the VRX496 test article could be used during the course of the animal study;
- 2) The SCID/SCID murine model permits medium-term survival of human T cells to permit sufficient time for RCR amplification in human cells in vivo to maximize the chance for detection of an adverse event; and
- 3) The SCID/SCID model has residual NK activity that eventually destroys all human cells to permit for discriminatory detection of an adverse event in murine tissue without the lingering background of residual human cells.

The scheme for detection of RCR or pre-RCR mobilization into murine tissue is depicted in figure 26 below.

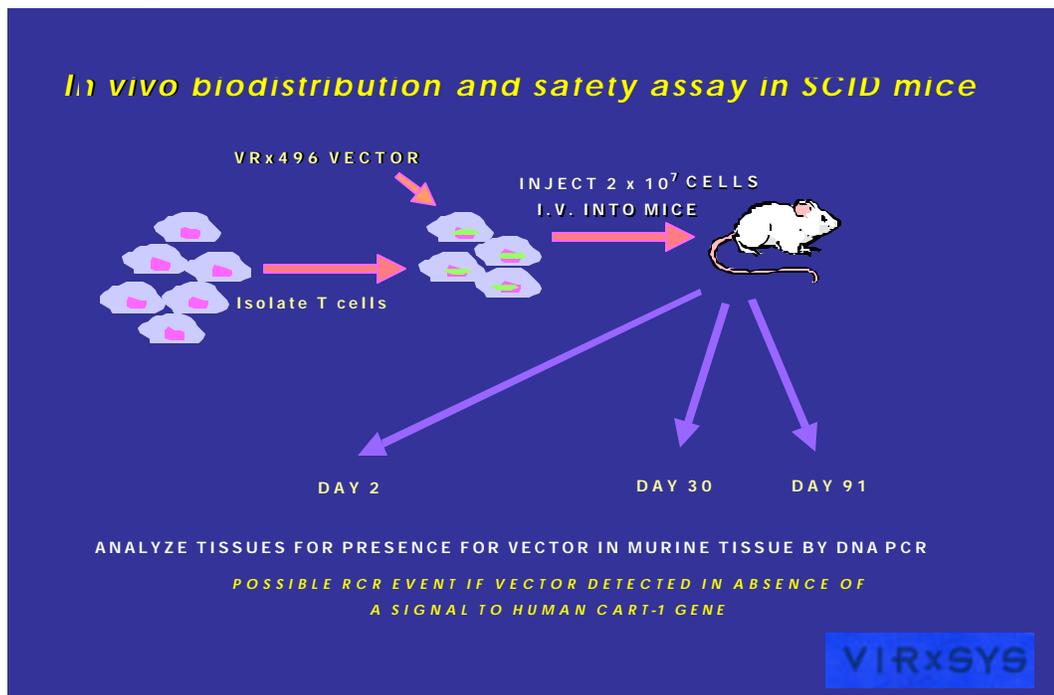


Figure 26 (above) The scheme for the *in vivo* biodistribution study. Patient-subject scale T cells were isolated from a whole leukapheresis preparation and entirely transduced with pilot large scale

lot of VRX496 (cells in figure 17). The VRX496 modified cells were then injected i.v. into SCID/SCID mice and the mice were then sacrificed at days 2, 30 and 91 post injection. Various organs were harvested and DNA extracted from the tissues. The DNA was then analyzed for the presence of vector and human DNA using vector (G-tag) and human (CART-1) specific primers. The CART-1 gene was selected to detect human cells because there are regions of relatively low homology between human and murine CART-1 to permit their discrimination. An adverse event would be if a PCR product for vector (G-tag) was detected in the absence of a PCR product for human CART-1 gene. This would indicate that the vector autonomously mobilized from the human cell and infected mouse tissue. Such an event may indicate the presence of a RCR that gains entry into murine tissue by using the VSV-G envelope protein, warranting further characterization. No such events were detected during the course of this study.

4.1 Biodistribution Study in Mice

A definitive GLP biodistribution study has been conducted in SCID/SCID mice. A total of 192 mice were divided into four groups of 24 mice/sex/group and given a 0.3 mL intravenous injection of either infusion media control, mock transduced human T cells, 3×10^5 vector transduced T cells or 20×10^6 vector transduced T cells as outlined in Table. Mock transduced human T cells (cells transduced by a vector storage buffer) and an infusion media of plasma light and 5% dextrose in a 1:1 ratio served as the T cell control and media control, respectively.

Table 6 Study 1173-100		Bio-distribution of VRX496 T-Cells in SCID Mice				
Group	Treatment (Dose)	Time of Sacrifice				Total Mice/ Group
		Day 2	Day 15	Day 30	Day 91	
		Number of Mice/Sex				
1	Media Only	6 (5) ¹	6 (5)	6 (5)	6 (5)	24 (20)
2	T-Cell Control (20×10^6 /mouse)	6 (5)	6 (5)	6 (5)	6 (5)	24 (20)
3	Vector Transduced T-Cells (low dose, 3×10^5 /mouse)	6 (5)	6 (5)	6 (5)	6 (5)	24 (20)
4	Vector Transduced T-Cells (high dose, 20×10^6 /mouse)	6 (5)	6 (5)	6 (5)	6 (5)	24 (20)

¹ *One extra mouse will be included per sex per group for each time-point. Samples will be taken from only five mice/group.*

Parameters evaluated included mortality, clinical observations, physical examinations and body weights. Clinical observations included examination of skin and fur, eye and mucus membranes, respiratory, circulatory, autonomic and central nervous systems, and somatomotor and behavioral patterns. In addition, mice were observed for potential signs of toxicity, including tremors, convulsions, salivation, diarrhea, lethargy, coma, or other atypical behavior. Individual body weights and physical examinations were performed at randomization, prior to dosing on Day 1, and weekly thereafter. Blood samples for PCR analysis were obtained on Study Day 2, 30 and 91 from 5 animals/sex/group/interval. Following weighing and blood collection on Study Day 2, 30, or 91, the designated animals were euthanized by carbon dioxide asphyxiation. Tissue samples were collected for PCR analysis from the injection site, inguinal, mesenteric and submaxillary lymph nodes, testes or ovaries, heart, bone marrow, skeletal muscle, adrenals, spleen, kidney, liver, lung, brain, pancreas and vertebrae. All tissue vials were then frozen in liquid nitrogen and stored at approximately minus 80°C until analysis. Samples analyzed by PCR included the injection site (tail), inguinal lymph nodes, lung, heart, bone marrow, liver, blood, spleen and testes or ovaries.

There were no changes or differences in body weight between groups and there were no remarkable clinical observations at any dose of vehicle or VRX496. Tissues from control-treated mice tested negative for VRX496 and Human DNA sequences at 2, 30 and 91 days post-dose. For both the low and high-dose VRX496 treated mice, all tissues examined by PCR analysis revealed the presence of VRX496 and Human DNA at both 2 and 30 and 91 days post-dosing. At day 91, only 4 tissues among 4 independent animals were positive. The results from the 120-day sacrifice will be included in the IND submission.

4.1.1 Sensitivity of the DNA-PCR Assay

FDA regulations stipulate that the level of detection of DNA-PCR based assays be less than 100 copies per microgram of DNA per tissue sampled. A total of 3ug of DNA is extracted from each tissue to be analyzed: 1ug is spiked with a positive control DNA and 2ug of DNA from animal tissue without spiked positive control DNA. The HOTSHOT nucleic acid extraction method was used (Truett et al., 2000). Both DNA and RNA are extracted by the HOTSHOT method. Since each tissue has a variable level of RNA expression, a numerical correction factor (called here the Z factor) taking into account the level of RNA in the extracted nucleic acid preparation must be established for each tissue type. To validate the Z factor for each tissue type, the nucleic acid from each tissue was divided into two samples where one was treated with RNAase to digest the RNA in the sample. The samples were then run on an agarose gel in triplicate and the signal intensity determined by phosphoimage analysis. The Z factor was determined by dividing the signal intensity of the undigested sample (DNA + RNA) by the signal intensity of the RNAse digested sample (DNA only) to obtain the correction factor for that type of tissue. The Z factor has been validated to a 95% confidence level for all tissues that have been analyzed.

After correction for the correct DNA concentration of the mouse tissue, the sensitivity of the DNA-PCR assay is 50 copies of the vector or human specific sequence per microgram of DNA analyzed. This is well below the 100 copy detection sensitivity recommended by the FDA. The vector specific sequence detected by DNA-PCR is a fragment of GFP coding sequence, G-tag. The size of the G-tag PCR product is 142 base pair. The human specific sequence detected by DNA-PCR is the 3' untranslated region of human CART-1 gene. The size of CART-1 PCR product is 156 base pair.

4.1.2 Murine tissue can be infected with HIV-1 vector

In order to determine if a vector derivative (such as an RCR or pre-RCR) could autonomously mobilize and infect murine tissues, we determined whether murine tissues could be transduced with a VRX496 analog vector. If the vector could not transduce murine tissues then the biodistribution assay would not be able to detect for adverse events in mouse tissues. Murine hematopoietic cells were found to be efficiently transduced with the vector, even at relatively low MOIs (figure 27).

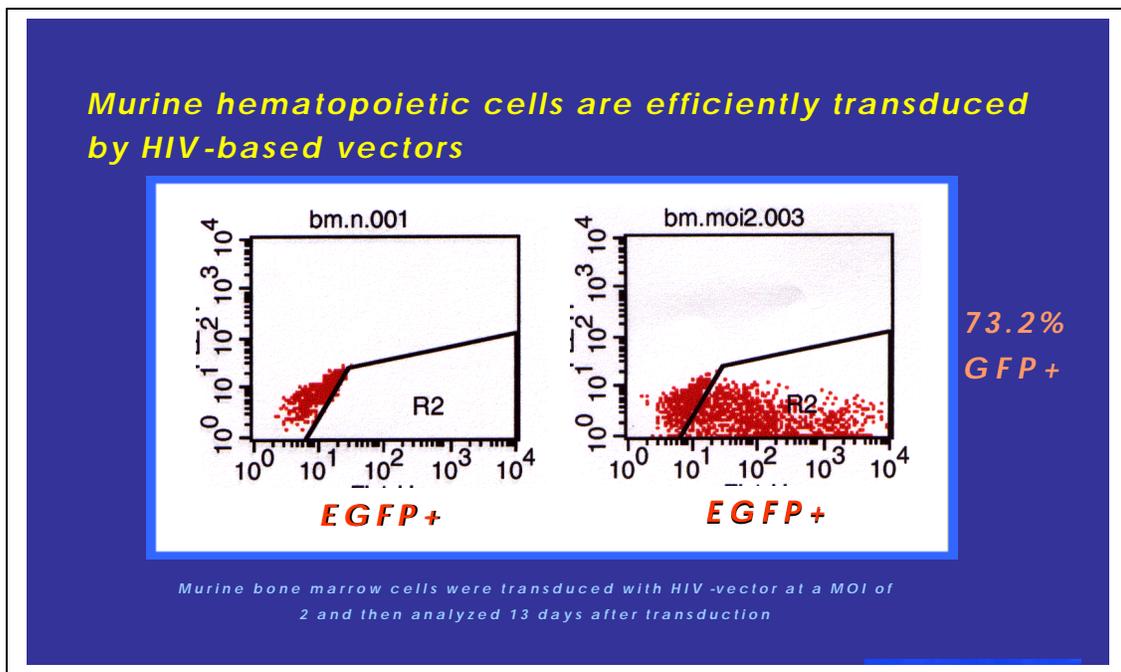


Figure 27. Showing efficient transduction of murine hematopoietic cells by HIV-based vectors

4.1.3 Representative data from the DNA-PCR analysis

DAY 2 POST-INJECTION: DNA from murine tissues were isolated and analyzed for the presence of vector DNA (Gtag). Analysis of the control group of animal samples that were injected with unmodified cells revealed, as expected, lack of vector sequence detection (figure 28). The spiked control in each group displayed a band

of the correct size, demonstrating the sensitivity of the assay (50 copies per ug DNA). All the animals receiving a high dose of vector-modified cells were expectedly positive for the vector (G-tag) sequence (figure 29). When G-tag positive tissue was analyzed for Hu-CART DNA, a positive PCR product of the correct size was detected (figure 30). The data demonstrates that detection of G-tag in the tissues was due to vector-containing cells. This result is expected since at day 2 post-injection there are many human cells that are circulating in the mouse that produce the positive vector and human DNA PCR signals (Table 7).

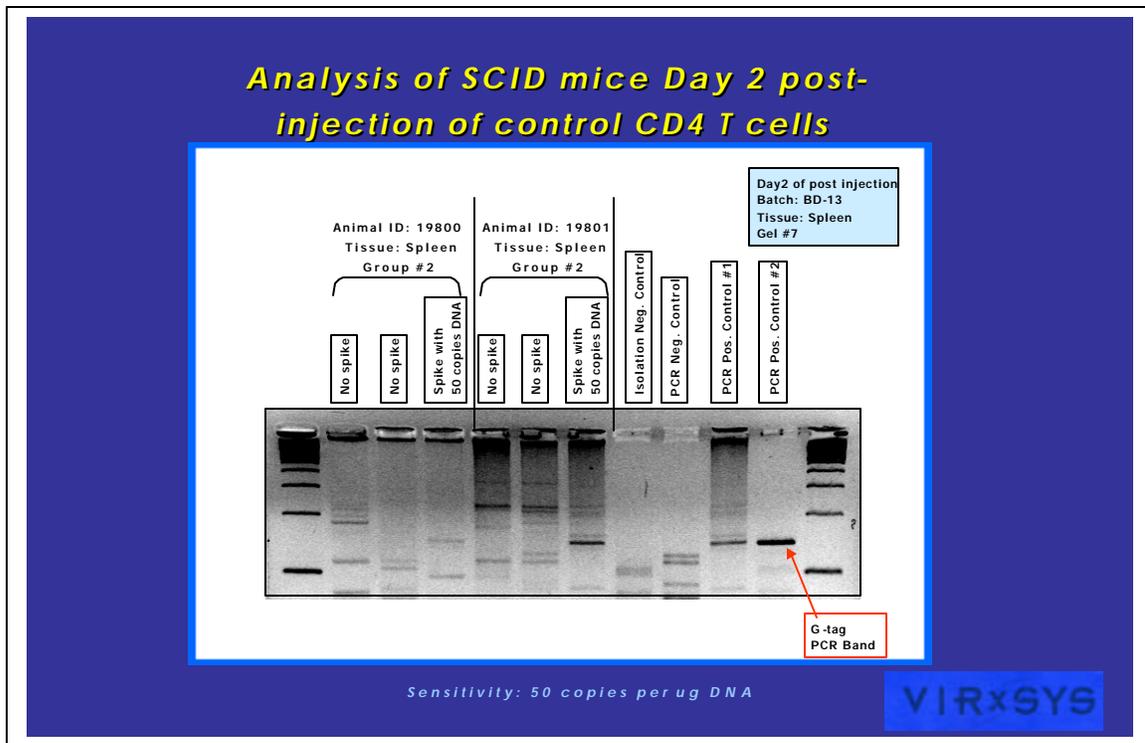


Figure 28 Day 2 data of the control CD4 T cell group (no vector) showing no vector positive signal in animal tissues. The spike controls show the sensitivity of the assay (50 copies).

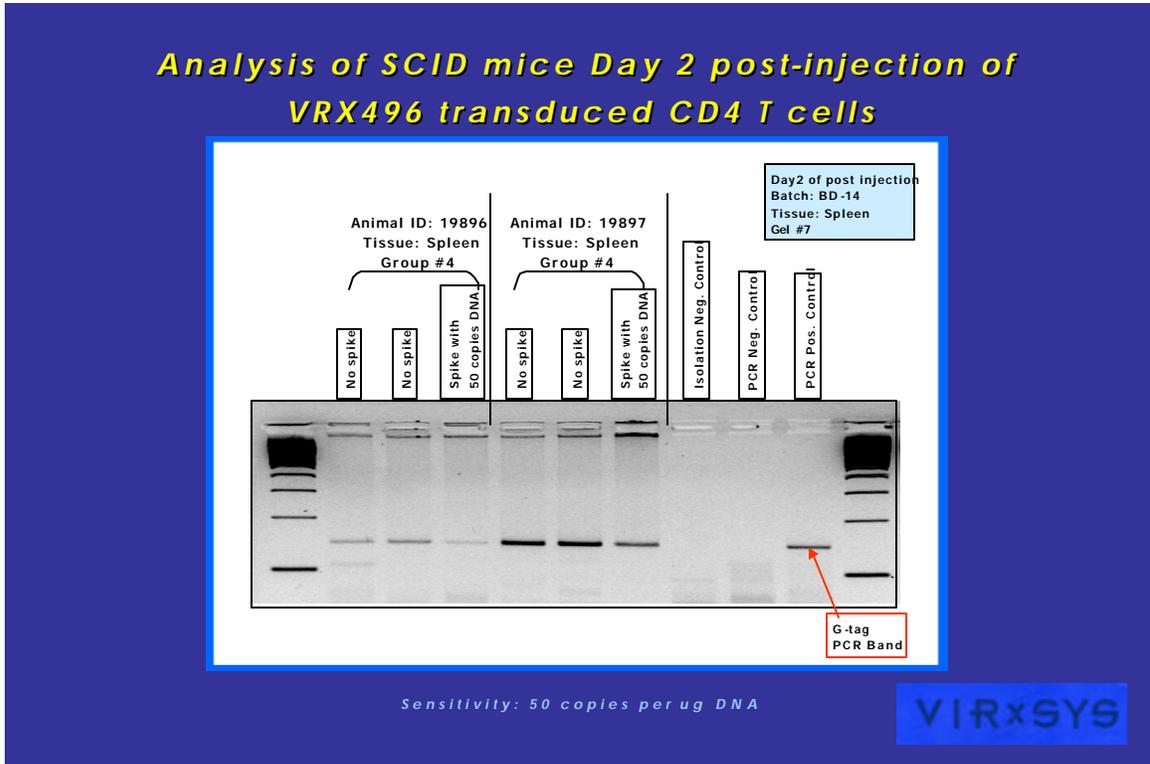


Figure 29 Day 2 data of the VRX496 containing CD4 T cell group showing detection of the vector G-tag signal in essentially all tissues examined.

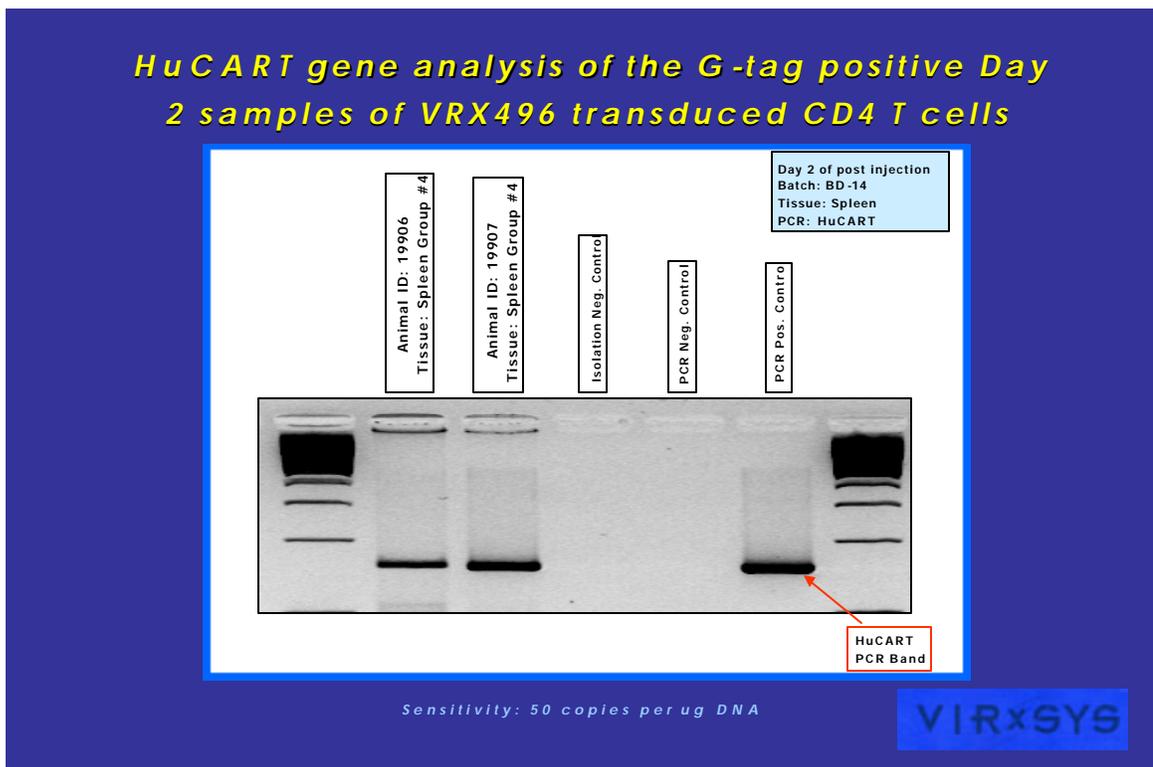


Figure 30 (above) Day 2 data of the vector positive samples showing detection of the HuCART-1 gene in all tissues containing the vector.

Summary of the Day 2 DNA-PCR data

	Heart Vector/Human	Testes Vector/Human	Ovary Vector/Human	Liver Vector/Human	Ing-LN Vector/Human
Group #1	Neg. / ND	Neg. / ND	Neg. / ND	Neg. / ND	Neg. / ND
Group #2	Neg. / ND	Neg. / ND	Neg. / ND	Neg. / ND	Neg. / ND
Group #3 (low dose)	1 (-) / ND 9 + / 9 +	4 (-) / ND 1 + / 1 +	5 (-) / ND	10 (+) / 10 (+)	6 (-) / ND 4 (+) / 4 (+)
Group #4 high dose)	10 (+) / 10 (+)	5 (+) / 5 (+)	3 (+) / 3 (+)	10 (+) / 10 (+)	1 (-) / ND 9 (+) / 9 (+)

	Bone Marrow Vector/Human	Lung Vector/Human	Spleen Vector/Human	Tail Vector/Human	Blood Vector/Human
Group #1	Neg. / ND	Neg. / ND	Neg. / ND	Neg. / ND	Neg. / ND
Group #2	Neg. / ND	Neg. / ND	Neg. / ND	Neg. / ND	7 (-) / ND 3 False Pos.*
Group #3 (low dose)	10 (+) / 10 (+)	10 (+) / 10 (+)	10 (+) / 10 (+)	4 (-) / ND 6 (+) / 6 (+)	2 (-) / ND 8 (+) / 8 (+)
Group #4 high dose)	10 (+) / 10 (+)	10 (+) / 10 (+)	10 (+) / 10 (+)	10 (+) / 10 (+)	1, Fail** 9 (+) / 9 (+)

Group 1=medium only; Group 2=no vector cells;
Group 3=low dose VRX496 cells;
Group 4=high dose VRX496 cells

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Table 7 Summary of the day 2 DNA PCR data showing that a great majority of the tissues tested contained vector-containing cells for those groups of animals injected with VRX496 cells. It was determined that all tissues that were positive for vector were also positive for the human CART gene. The day 2 data serves as a positive control for the study, showing that most organs are perfused with VRX496 T cells after tail-vein injection.

DAY 30 POST-INJECTION: Analysis of tissues from day 30 animals for the vector (G-tag) sequence revealed that many of the human cells had died during the interim period since many samples lacked detection of both G-tag and Hu-CART (figure 31 & table 8). This provides an opportunity to determine if a RCR or a pre-RCR has infected mouse tissue. Such an event would be detected by the presence of G-tag and the absence of a Hu-CART signal. This would then be considered an adverse event and would then be characterized. However, all the animals that were positive

for G-tag (vector) were also positive for Hu-CART, indicating that the signal is due to the presence of remnant vector containing cells (figure 32 & table 8).

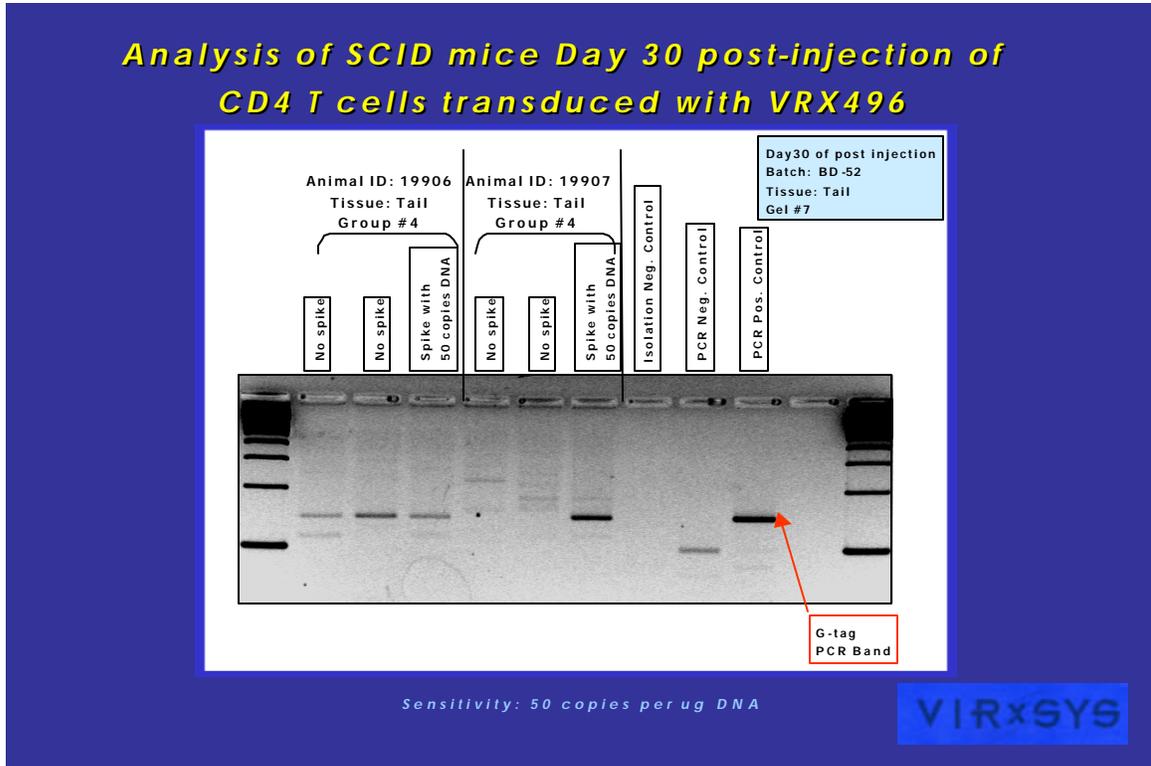


Figure 31 Day 30 data showing occasional detection of the vector (G-tag) in some of the tissues analyzed.

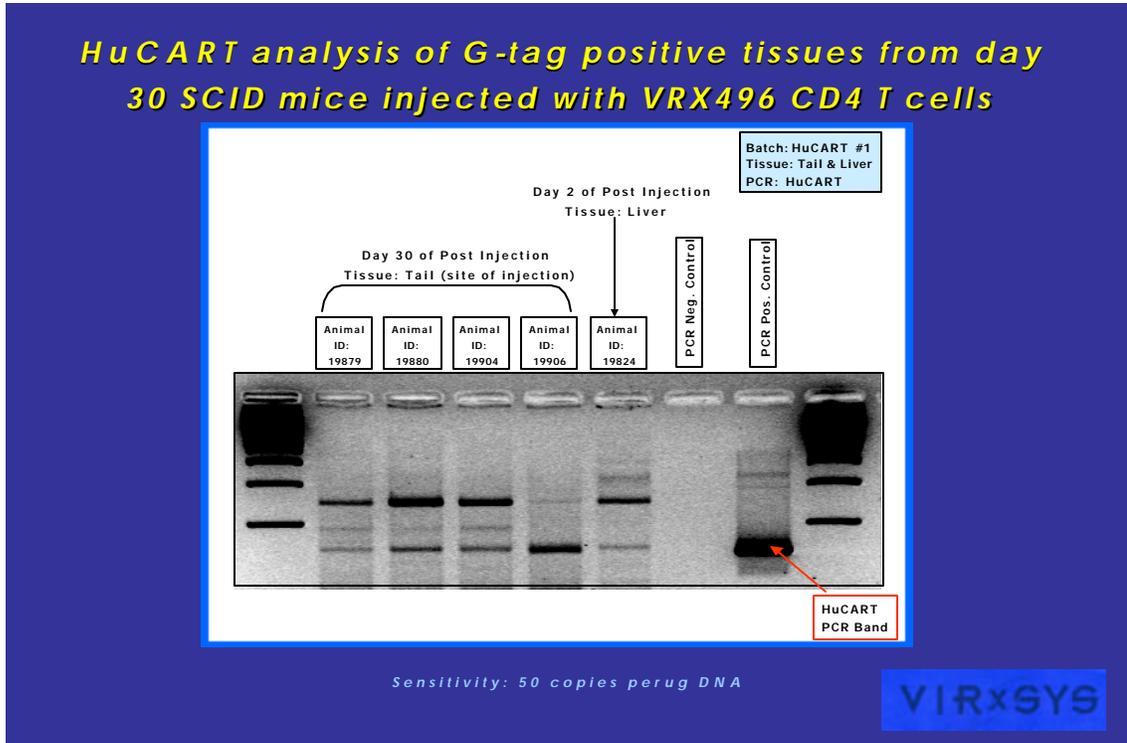


Figure 32 Day 30 data of the VRX496 containing CD4 T cell group demonstrating the presence of HuCART DNA when the vector is detected in the tissue.

Summary of the Day 30 SCID data

	Heart <i>Vector/Human</i>	Testes <i>Vector/Human</i>	Ovary <i>Vector/Human</i>	Liver <i>Vector/Human</i>	Ing-LN <i>Vector/Human</i>
Group #1	Neg. / ND	Neg. / ND	Neg. / ND	Neg. / ND	Neg. / ND
Group #2	Neg. / ND	Neg. / ND	Neg. / ND	Neg. / ND	Neg. / ND
Group #3 low dose)	5 (-) / ND 5 (+) / 5 (+)	4 (-) / ND 1 (+) / 1 (+)	4 (-) / ND 1 (+) / 1 (+)	7 (-) / ND 3 (+) / 3 (+)	6 (-) / ND 4 (+) / 4 (+)
Group #4 high dose)	7 (-) / ND 3 (+) / 3 (+)	3 (-) / ND 2 (+) / 2 (+)	2 (-) / ND 3 (+) / 3 (+)	9 (-) / ND 1 (+) / 1 (+)	8 (-) / ND 2 (+) / 2 (+)
	Bone Marrow <i>Vector/Human</i>	Lung <i>Vector/Human</i>	Spleen <i>Vector/Human</i>	Tail <i>Vector/Human</i>	Blood <i>Vector/Human</i>
Group #1	Neg. / ND	Neg. / ND	Neg. / ND	Neg. / ND	Neg. / ND * 5 Failed
Group #2	Neg. / ND	Neg. / ND	Neg. / ND	Neg. / ND	Neg. / ND ** 1 Failed
Group #3 low dose)	8 (-) / ND 2 (+) / 2 (+)	9 (-) / ND 1 (+) / 1 (+)	10 (-) / ND	8 (-) / ND 2 (+) / 2 (+)	5 (-) / ND 3 (+) / 3 (+) *** 2 Failed
Group #4 high dose)	10 (-) / ND	6 (-) / ND 4 (+) / 4 (+)	9 (-) / ND 1 (+) / 1 (+)	6 (-) / ND 4 (+) / 4 (+)	6 (-) / ND 4 (+) / 4 (+)

Group 1=medium only; Group 2=no vector cells;
Group 3=low dose VRX496 cells;
Group 4=high dose VRX496 cells

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Table 8 (above) Summary of the day 30 data showing gradual disappearance of the vector-containing cells in murine tissues. In those tissues that still show a vector (G-tag) signal, a concomitant Hu-CART signal was seen.

DAY 91 POST-INJECTION: At day 91, essentially all of the human cells had died, as shown by a negative vector signal for all but 4 independent tissues in 4 independent animals. These 4 tissue samples were analyzed for Hu-CART and shown to be positive, demonstrating that the G-tag signal is due to residual vector-containing cells and not some type of adverse event (figure 33 & table 9).

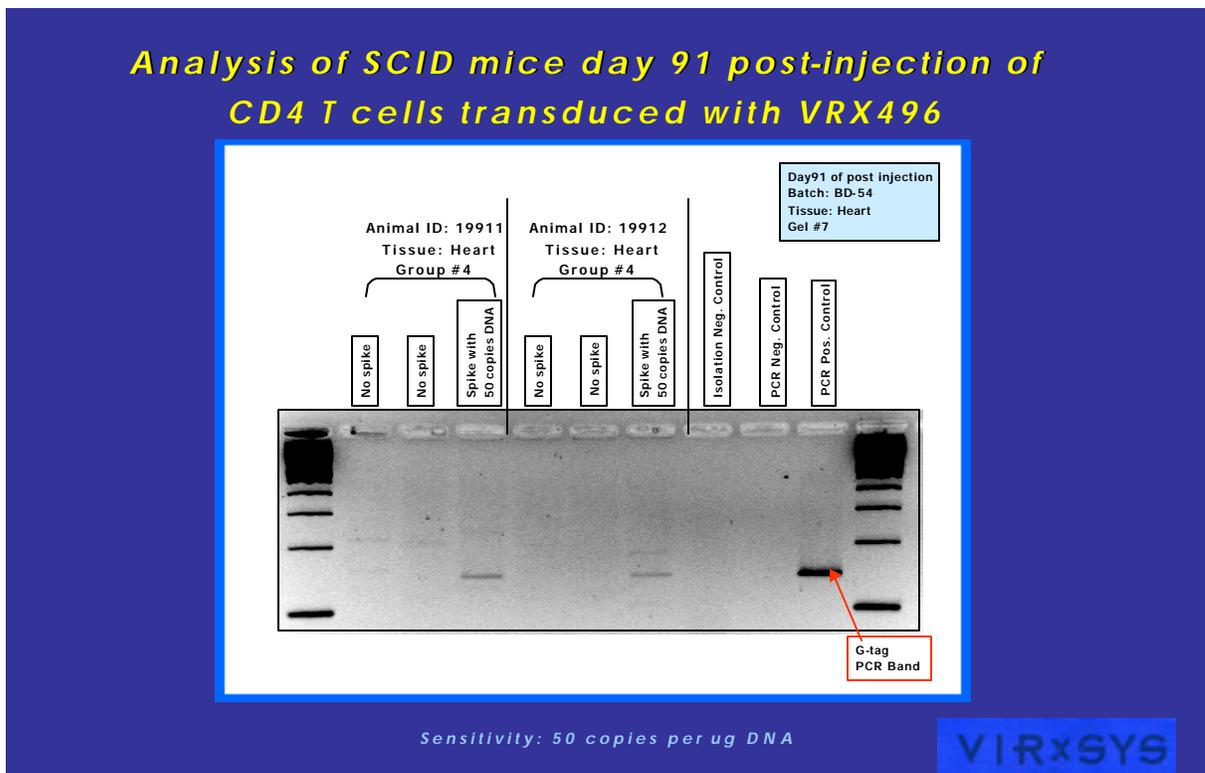


Figure 33 Day 91 data of the VRX496 containing CD4 T cell group demonstrating lack of vector signal in essentially all of the tissue samples examined.

Summary of the Day 91 SCID data

	Heart Vector/Human	Testes Vector/Human	Ovary Vector/Human	Liver Vector/Human	Ing-LN Vector/Human
Group #1	Neg. / ND	Neg. / ND	Neg. / ND	Neg. / ND	Neg. / ND
Group #2	Neg. / ND	Neg. / ND	Neg. / ND	Neg. / ND	Neg. / ND
Group #3 (low dose)	10 (-) / ND	5 (-) / ND	5 (-) / ND	10 (-) / ND	10 (-) / ND
Group #4 (high dose)	10 (-) / ND	5 (-) / ND	5 (-) / ND	9 (-) / ND (+) / 1 (+)	10 (-) / ND

	Bone Marrow Vector/Human	Lung Vector/Human	Spleen Vector/Human	Tail Vector/Human	Blood Vector/Human
Group #1	Neg. / ND	Neg. / ND	Neg. / ND	Neg. / ND	Neg. / ND
Group #2	Neg. / ND	Neg. / ND	Neg. / ND	Neg. / ND	Neg. / ND
Group #3 (low dose)	10 (-) / ND	9 (-) / ND (+) / 1 (+)	10 (-) / ND	10 (-) / ND	7 (-) / ND *3 failed
Group #4 (high dose)	10 (-) / ND	10 (-) / ND	9 (-) / ND (+) / 1 (+)	9 (-) / ND (+) / 1 (+)	6 (-) / ND *4 failed

*Group 1=medium only; Group 2=no vector cells;
 Group 3=low dose VRX496 cells;
 Group 4=high dose VRX496 cells*

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Table 9 Summary of the day 91 data showing essentially complete disappearance of the vector-containing cells in murine tissues. In four independent tissue samples a vector signal was detected and a concomitant Hu-CART signal was seen.

CONCLUSIONS

At day 2 and day 30 post-injection, all tissues analyzed from all the animals in control groups (group #1 and #2) are negative for VIRxSYS vector specific sequence by DNA-PCR analysis.

At day 2 post-injection, essentially all the tissues analyzed were positive for VIRxSYS vector specific sequence by DNA-PCR analysis.

At day 30 post-injection, some of the tissues analyzed were positive for VRX496 specific sequence by DNA-PCR analysis

At day 91 post-injection, essentially all of the tissues analyzed were negative for VRX496 specific sequence by DNA-PCR analysis.

All the samples positive for vector specific sequence were found to be positive for human specific sequence as detected by DNA-PCR.

No adverse event was seen in all the samples tested so far. The final report will include day 120 data.

4.2 Toxicology

The toxicity of the VRX496 vector was assessed in SCID/SCID mice injected intravenously with VRX496 transduced human CD4 cells (from healthy donors). Human CD4+ T-cells were to be transduced with VRX496 ex-vivo, and injected intravenously into SCID/SCID mice at 2 dose levels. Mice were to be sacrificed at Days 2, 30 or 91 days post-exposure. Six mice per sex per group will be treated, however only five mice in each group will be necropsied.

Table 7: VRX496 Cell Product Mouse Safety/Toxicity Study					
Group	Treatment (Dose)	Time of Sacrifice			Total Mice/Group
		Day 2	Day 30	Day 91	
		Number of Mice/Sex			
1	Media Only	6 (5) ¹	6 (5)	6 (5)	24 (20)
2	T-Cell Control (20×10 ⁶ /mouse)	6 (5)	6 (5)	6 (5)	24 (20)
3	Vector Transduced T-Cells (low dose, 3×10 ⁵ /mouse)	6 (5)	6 (5)	6 (5)	24 (20)
4	Vector Transduced T-Cells (high dose, 20×10 ⁶ /mouse)	6 (5)	6 (5)	6 (5)	24 (20)

¹ One extra mouse will be included per sex per group for each time-point. Samples will be taken from only five mice/group

Selected tissues were to be collected and fixed from five animals per group at the time of necropsy. Histopathological analyses were to be performed on tissues from the control and high dose groups (Groups 1 and 4, respectively). If any target organ toxicities are identified in the high dose animals, selected tissues from the low dose and T-cell control dose animals were to be examined histopathologically. Clinical

chemistry and hematological analysis were to be performed on all animals upon necropsy.

Preliminary results from animals sacrificed at Days 2, 30 and 91 showed no signs of toxicity and had no effect of mortality, body weight, physical examination findings, hematology, serum chemistry, gross pathology findings, organ weights, and histopathology results through 90-days post dosing.

Through the Day 30 interim sacrifice, three animals have died on study. A group 2 male was found dead on Day 15, a group 3 female was sacrificed moribund on Day 15, and a group 4 female was found dead on day 28. These deaths were not considered attributable to test article treatment. In the animals found dead or sacrificed moribund, gross pathology findings were noted only in the group 3 female and included widespread red discharge from the ventral and abdominal areas, legs and tail. These findings were not considered related to test article treatment.

Gross pathology findings in the animals sacrificed on day 2 were limited to enlarged uterus in one female from each group and a discolored spleen in one group 4 female. There were no histopathological findings in the males on day 2.

In the animals sacrificed on day 30, gross pathology findings were limited to epicardial mineralization in animals of both sexes in all groups. No other findings were noted in the males. Other finding in the females included a group 1 female with enlarged mandibular lymph nodes and distended uterus, a group 3 female with fluid in the uterus, and two group 4 females with distended uterus. The epicardial mineralization does not appear to be treatment related, and the various uterine observations reflect normal estrous cycle activity.

There were no treatment-related differences between control and treated groups with respect to body weights or clinical pathology or organ weights. No significant histopathological effects were noted. The epicardial mineralization was apparent in all control and test article treated groups, and these changes are considered incidental or secondary to the injection procedure. Thus, through 30 days post-

dosing, VRX496 appears safe when administered at doses up to 20×10^6 cells per mouse. This dose represents 2.5 times the proposed maximal clinical dose and 75 times the starting clinical dose of 10^9 transformed T cells per patient. The day 90 and 120 data is presently being analyzed and will be forthcoming in the IND submission.

5.0 Proposed Clinical Development

5.1 Summary of Previous Human Experience

Autologous T cells transduced with VRX496 have not been previously investigated in human subjects in the U.S. or other countries.

5.2 Overview of Clinical Rationale

As previously discussed, the proposed clinical approach involves administration of autologous CD4 T cells that have been transduced with the lentiviral vector VRX496. VRX496 is a lentiviral vector that carries an anti-HIV antisense sequence targeted to the HIV env gene. Data from preliminary studies suggest that HIV vectors such as VRX496 could potentially reduce viral loads in HIV-infected individuals and thus could delay the onset to AIDS while promoting CD4 T cell survival and providing the immune system with a better chance to control the infection.

VRX496 was selected as a candidate for therapy because, in addition to the preliminary efficacy data noted above, the proposed patient population VRX496 will be used to transduce CD4 T cells is the most appropriate for safety studies. Additionally, as this patient population is already laden with wt-HIV the vector will not be introducing any novel genetic sequences. Furthermore, the components of VRX496 are wholly derived from wt-HIV (the anti-HIV gene is an anti-env antisense), therefore any recombination event between VRX496 and wt-HIV cannot produce a novel pathogenic virus. Finally, VRX496 is a fully-gutted vector: no proteins are expressed from VRX496, and the anti-HIV env antisense payload is expressed only after wt-HIV infects vector containing cells.

HAART, the current standard of treatment for HIV/AIDS typically consists of a NRTI, NNRTI and PI triple “cocktail.” Although these cocktails have been successful in reducing viral loads and restoring immune function, they do not represent a cure, and there are concerns regarding adverse effects associated with long-term usage of HAART. The goal of VRX496 cellular therapy is to attenuate wt-HIV replication in vivo so as to decrease the overall viral load set point, thereby indefinitely delaying the onset of disease. Preliminary result from experiments in SCID mice (mice with transplanted human immune cells) indicate that the human cells transduced with VRX496 and implanted into the SCID mice do not elicit any overt adverse effects.

5.3 Proposed Phase 1 Protocol Synopsis

Study Drug:	Autologous T Cells Transduced with VRX496
Protocol Number:	VRX496-01-01
Protocol Title:	A Phase 1 Open-Label Clinical Trial of the Safety and Tolerability of Single Escalating Doses of Autologous T Cells Transduced with VRX496 in HIV Positive Patient-subjects
Study Phase:	1
Study Design:	A single center, open-label, dose escalation study
Sample Size:	N = up to 24 evaluable patient-subjects
Study Population:	Male and female HIV positive patient-subjects, 18 to 60 years of age who have discontinued, or who are failing a treatment regimen of HAART.
Treatment Groups:	Individual cohorts of 3 patient-subjects or up to 6 patient-subjects will be dosed with autologous T Cells transduced with VRX496 at one of the following dose levels: 1.0×10^9 ; 3.0×10^9 ; 1.0×10^{10} ; and 3.0×10^{10} transduced T cells. Dosing will begin in the initial 3 patient-subjects at the 1.0×10^9 dose level. After safety has been adequately demonstrated at the lower dose level, subsequent cohorts of 3 patient-subjects will be dosed with the next higher dose level.
Treatment Duration:	Autologous T Cells transduced with VRX496 will be infused intravenously over approximately 30 minutes. The dose volume administered will be approximately 10 to 200 mL, depending on dose.

- Evaluation Schedule:** Patient-subjects will be evaluated during pre-treatment screening, at aphaeresis, during the infusion of Autologous T Cells transduced with VRX496, through 6 hours post infusion, at 24, 48, 72 hours, 7 days, 14 days, 28 days, 3, 6 months and yearly for the life of the patient-subject.
- Objectives:** The primary objective is to assess the safety and tolerability of Autologous T Cells transduced with VRX496 in HIV positive patient-subjects.
- The secondary objectives are:
- To determine the number of VRX496 modified T-lymphocytes in the blood
 - To determine the levels of wt-HIV and VRX496 in the plasma.
- Safety Criteria:** Adverse experiences through 28 days post-dosing, Chemistry, Hematology, Urinalysis, precipitous and sustained decrease in CD4 T-cell counts, precipitous and sustained increase in wt-HIV viral load, sustained VSV-G RNA detection in plasma followed by biological detection of a VSV-G containing replication competent retrovirus (RCR).
- # of VRX496 modified cells:** The number of VRX496 containing cells in the blood at 72 hours, 7, 28 days, 3, 6 months and yearly for life post dosing.
- Secondary Criteria:** Changes in the anti-HIV immune response, CD4 T-cell counts, differential viral load, VSV-G antibody responses, TCR V β diversity analysis.

5.4 Description of Trial Design

Protocol VRX496-01-01 is a single-center, open-label, single-dose, dose- escalation study of the safety and tolerability of intravenous administration of VRX496 modified T-cells in HIV positive patient-subjects.

Up to 24 HIV positive patient-subjects meeting the study inclusion criteria will be enrolled into the study with up to 6 patient-subjects treated at each of the following 4 dose levels:

- Dose Level 1 – approximately 1.0×10^9 VRX496 modified T-cells,
- Dose Level 2 – approximately 3.0×10^9 VRX496 modified T-cells,
- Dose Level 3 – approximately 1.0×10^{10} VRX496 modified T-cells, and
- Dose Level 4 – approximately 3.0×10^{10} VRX496 modified T-cells.

The initial patient-subjects enrolled will be assigned to treatment with the first dose level (1.0×10^9 VRX496 modified T-cells). After the 28-day patient-subject clinical and laboratory safety has been established at the current dose level, dosing will proceed to the next higher dose level according to the dose escalation scheme outlined below.

The maximum tolerated dose (MTD) will be defined as the dose level immediately below the level at which greater than or equal to two patient-subjects develop DLT.

5.5 Dose Escalation Scheme

Following dosing with VRX496 modified T-cells, patient-subjects will be evaluated for dose limiting toxicity through 28-days post-dosing. Severity of observed toxicities will be graded using the AIDS Clinical Trials Group (ACTG) toxicity criteria. Dose limiting laboratory toxicity (DLT) will be defined as any of the following :

- hematologic toxicity greater than or equal to ACTG Grade 3 (*Note: If a patient-subject demonstrates Grade 3 or greater Laboratory Toxicity the investigator will immediately repeat the test to confirm the result. For the purpose of dose escalation, if the repeat test does not confirm the toxicity, the patient-subject will not be considered to have experienced a dose limiting laboratory toxicity*), or
- non – hematologic toxicity of ACTG Grade 4, or
- sustained (i.e., persistent over 7 days in duration) increase from baseline in wt-HIV viral load of a magnitude of 0.5 log or greater, or

- sustained (i.e., persistent over 7 days in duration) decrease from baseline in CD4+ T-cell count of 50% or greater, or
- sustained (i.e., persistent over 7 days in duration) presence of VSV-G RNA in the plasma followed by a positive biological RCR test.

Dose escalation will proceed as follows:

- Three patient-subjects will be enrolled at each dose level and followed for dose limiting toxicity through 28-days post dosing. Dose escalation will proceed upon approval of the Data Safety Monitoring Board (DSMB). *Note: for Dose Level 1, the initial patient-subject treated must be followed through 28-days prior to the treatment of the remaining patient-subjects in the Dose Level. For Dose Levels 2, 3 and 4 the initial patient-subjects may be treated and followed concurrently.*
- If no dose limiting toxicity is observed, dosing may proceed to the next higher dose level.
- If one of the initial three patient-subjects demonstrates a dose limiting toxicity, an additional three patient-subjects will be studied at the current dose level. If no additional dose limiting toxicity is observed dosing may proceed to the next higher dose level.
- The Data and Safety Monitoring Board will review data from each dose level and approve escalation to the next higher dose level.
- Patient-subject treatment and dose escalation will cease if two (2) patient-subjects at a dose level exhibit a dose limiting toxicity.
- Patient-subject treatment and dose escalation will cease if a single (1) patient-subject demonstrates the presence of a Replication Competent Retrovirus (RCR), as defined by the biological RCR test.

5.6 Primary Safety Endpoints

The primary safety endpoints are:

- The incidence of adverse events at each dose level studied from dosing through 28-days post-dosing,
- The incidence of serious adverse events and dose limiting toxicity at each dose level studied from dosing through 28-days post-dosing, and
- The changes in clinical chemistry, hematology and urinalysis test results at each time point from dosing through 28 days post-dosing.
- The changes in viral load and CD4 T cell count from dosing through 28-days post-dosing.

5.7 Secondary Endpoints

The secondary endpoints of this study will focus on long term safety, changes in indices of HIV infection and cell survival of VRX496 containing T-lymphocytes as follows:

- The incidence of serious adverse events through 6 months post-dosing,
- The change in differential viral load from pre-dose levels through 6-months post dosing, and
- The change in CD4+ T-cell counts from pre-dose levels through 6-months post dosing.
- Immune function (by HIV virus specific CD4 cell proliferative responses, Tetanus Toxoid specific CD4 proliferative responses, ELISPOT measurement of IFN-gamma producing CD8 T cells, & TCR V β diversity analysis)
- VSV-G antibody responses to the product vector

5.8 Survival of VRX496 modified T-cells

The survival of VRX496-modified T-cells will be determined by measuring the average vector copy number in the blood by TaqMan PCR through 6 months post dosing and then yearly for the life of the patient.

5.9 Prior and Concomitant Therapy

Patient-subjects are prohibited from taking the following medications during the course of the study (i.e., from screening through 6-month post treatment follow-up):

- Immunomodulating agents (IL-2, IFN-Gamma, Granulocyte colony stimulating factors, Megace)
- Any experimental therapy for HIV or other indications
- Corticosteroids
- Hydroxyurea
- Additional antiretroviral medication regimes. If a patient-subject is currently receiving an antiretroviral regimen, that regimen must be continued for the 6-month duration of the trial.

5.10 Inclusion Criteria

- Male and female patient-subjects 18 – 60 years of age who are HIV positive.
- Karnofsky Performance Score of 80 or higher.
- Patient-subjects who have received HAART therapy for at least 6 months and have either discontinued, or are failing treatment.
- Patient-subjects with a documented CD4 T-cell count greater than $200/\text{mm}^3$ but less than $600/\text{mm}^3$ within 30 days prior to screening.
- Patient-subjects with a documented viral load of greater than 5000 copies within 30 days prior to screening.
- Patient-subjects who understand and agree to be compliant with the requirements for the 6-month duration of the study and the necessity for annual follow up for life. At the time of death an autopsy will be performed.
- Patient-subjects who have provided written informed consent after the nature of the study has been explained.

5.11 Exclusion Criteria

- Patient-subjects who have not been treated with a previous regimen of HAART.
- Patient-subjects that demonstrate only CCR5 utilizing strains of HIV
- Patient-subjects who are pregnant or breast-feeding.
- Patient-subjects who have a recent (within 1 year) history of drug abuse and or a positive urine drug/alcohol test at time of screening.
- Patient-subjects who are currently taking corticosteroids or who have taken corticosteroids within the past 30 days.
- Patient-subjects who have received hydroxyurea within the prior 30 days.
Note: Patient-subjects currently taking hydroxyurea may be enrolled if the use of hydroxyurea is discontinued at least 30 days prior to apheresis.
- Patient-subjects taking immunomodulating agents (e.g., IL-2, IFN-Gamma, Granulocyte Colony stimulating factors, Megace)
- Previous treatment with HIV experimental vaccine(s).
- Patient-subjects taking antibiotics within one week prior to receiving study medication.
- Patient-subjects currently in the treatment or recovery phase of an acute infection (e.g., sinusitis).
- Patient-subjects who have received acute treatment of a serious Opportunistic Infection (OI) within the past 30 days.
- Patient-subjects who have undergone leukopheresis or lymphopheresis within 90 days of entry into the study.
- Patient-subjects who have active CNS disease or seizures within 1 year.
- Patient-subjects who have active MAI Infection or CMV Disease.

- Patient-subjects who have a history of cerebral toxoplasmosis or cryptococcal meningitis.
- Patient-subjects with a history of cancer.
- Patient-subjects with a history of Class III or IV congestive heart failure
- Patient-subjects with the following laboratory abnormalities: Hemoglobin of less than 10 for males and less than 9.5 for females; absolute neutrophil count less than 1000/iL; platelet count of less than 100,000/mm³; serum creatinine greater than 1.5 mg/dL; AST or ALT greater than 2.5 times the upper limit of normal; total serum bilirubin greater than 1.5 times the upper limit of normal; amylase & lipase outside normal range; and proteinuria of 2+ or greater.
- Patient-subjects who have participated in a prior gene therapy trial.
- Patient-subjects who have previously received treatment under this protocol or are currently being treated under another research protocol.
- Patient-subjects with a significant medical history who in the opinion of the investigator would be placed at risk by being enrolled in this study.

5.12 Discontinuation Criteria

A patient-subject may be withdrawn from the study prior to drug treatment at the investigator's discretion if any of the following occurs:

- Clinically significant deterioration that impairs the global status/condition of the patient-subject.
- Investigator decides discontinuation is in the best interest of the patient-subject.
- Patient-subject requests withdrawal from the study.

- Patient-subject is enrolled and subsequently becomes ineligible for study participation due to a change in patient-subject status that meets exclusionary criteria. (e.g., addition of prohibited medication, pregnancy).

Patient-subjects withdrawing from the study for any of the above reasons will be considered terminated and will be replaced. The reason for study termination will be documented in the patient-subject's medical record and recorded on the appropriate page of the case report form.

All patient-subjects receiving VRX496-modified T-cells must be followed for the entire 6 months. In the event a patient-subject fails to complete the follow up requirements through Day 28, the patient-subject will be replaced to permit the determination of the maximum tolerated dose. If a patient-subject fails to keep follow-up appointments, the site must document all attempts to contact the patient-subject. Minimum follow-up contact requirements will include at least 3 telephone contacts (on different days and at different times of the day) and a certified letter.

VIRxSYS may at their discretion terminate the study as a whole if conditions warrant that this action be taken. If the study is stopped, treated patient-subjects must be followed for the entire 6-month follow up period. The study site investigator maintains responsibility for orderly discontinuation of the study at his/her study site.

5.13 Potential Phase 2 Studies

Subsequent to this initial Phase 1 study, additional Phase 2 studies may be conducted based upon data obtained in Phase 1. Phase 2 studies would be designed to further examine the safety and efficacy of cells transduced with VRX496 in patients with HIV-infection. Potentially, additional Phase 1 and 2 studies would be designed to examine additional antisense constructs or combinations of constructs, including some designed to have increased mobilization.

6.0 Overall Summary

VIRxSYS is developing a novel antisense vector that has exhibited the ability to efficiently transduce into primary human CD4 T cells. Furthermore, VRX496 transduced T cells have shown marked inhibition of wt-HIV replication and enhanced resistance to productive HIV infection. The VRX496 HIV vector has weak mobilizing capabilities that were purposely selected to enhance safety of the first vector of this class to be introduced into humans. Preliminary studies have shown the safety of VRX496 in SCID mice injected with human T cells transduced with VRX496. The first-in-humans study with VRX496 is proposed in HIV-infected patients where T cells transduced with VRX496 will be re-introduced into patients. The primary objective of this Phase 1 study is to examine the safety of autologous T cells transduced with VRX496 in HIV patients.

Beyond phase I clinical trials, several strategies could be employed to increase the efficacy of this class of vectors: 1) multiple dosing regimens could be used to increase the number of vector-containing cells in vivo; 2) light conditioning regimens could be used to enhance engraftment of VRX496 modified cells; 3) selection of VRX496 modified cells with a selection gene; or 4) modification of the vector to increase mobilization and thus potentially extend the range of anti-HIV vector action and therapeutic benefit of autologous *ex vivo* cell transduction.

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