



SAFETY in the HIV/AIDS Program

Lentiviral Vectors: Former and recent data strongly support a low theoretical risk of cancer associated with lentiviral vector-mediated gene therapy, but not gene therapy mediated by previously available retroviral vectors. This is one of the significant technological advances of VIRxSYS' HIV-derived lentiviral gene therapy vector.

Despite several retroviral gene therapy trials without a serious adverse event, the theoretical risk of insertional oncogenesis¹ was notoriously realized in the X-linked SCID trial where two of the nine children cured developed leukemia within three years after treatment with a retroviral vector distinct from the lentiviral² class; the leukemia was subsequently linked to vector-associated insertional oncogenesis. Accordingly, the risk of insertional oncogenesis and clonal outgrowth of modified cells has become a pressing question in the field of gene therapy (Hacein-Bey-Abina et al, 2002, Int. J. Hemat.).

Lentivirus gene therapy vector may be safer than the murine oncoretroviral-based (MLV) vectors recently used the successful treatment of disease in three gene therapy trials to date including the X-linked SCID trial mentioned above (Hacein-Bey-Abina et al, 2002, Int. J. Hemat.; Aiuti, 2002, Curr. Opin. Mol. Ther.; Blaese et al, 1995, Science). Contrary to the MLV used in these trials, *lentiviruses are not associated with oncogenesis and therefore may represent a safety advantage over oncoretroviral gene therapy vectors.*

Importantly, leukemia is not a recognized side effect of HIV patients even though memory T cells are known to harbor integrated virus for years (Baum et al, 2003, Blood; Siliciano et al, 2003, Nat. Med.). Thus, there is ample long-term evidence demonstrating that an HIV-derived lentiviral vector is unlikely to have a pro-cancer effect in patients.

In particular, lentivirus vectors may be safer than onco-retroviral vectors regarding regional gene activation³ since the lentivirus LTR⁴ does not contain the same enhancer³ activity as the oncoretrovirus LTRs. We performed a study directly comparing the ability of the HIV LTR and onco-retroviral LTR to activate regional genes; *we found that the HIV LTR does not have the ability to distally activate promoters at all in stark contrast to onco-retroviral LTRs.* This data has been independently confirmed by Christoff von Kalle's group⁵ and was presented at the recent American Society of Gene Therapy in June this year. Finally, lentiviruses have less poly-A read through⁶ and hence a lower risk of unintentional transcriptional activation of neighboring genes (Zaiss et al, 2002, J. Virol.).

Cellular Therapy for Gene Delivery: Autologous⁷ cellular therapy for treatment of HIV has evaluated in clinical trials since the mid 1990's. Safety is well established, but efficacy has been difficult to achieve due to low gene transfer efficiency. VIRxSYS' proprietary gene therapy vector is significantly more efficient in delivering genetic material to cells than any other gene therapy vector developed to date (Humeau et al, 2004, Mol. Ther.), and therefore is expected to overcome this roadblock.

For HIV: Each of these trials were similar in that the gene delivery vector was a retroviral vector (MLV) not of the lentiviral class², and cells were modified and expanded *ex vivo*, and so treatment was given as a cellular therapeutic. These trials laid the groundwork for cellular-based therapeutic approaches to HIV/AIDS treatment, which remains the primary approach in clinical trials today.

- 1) Morgan and Walker, (1996, Hum. Gene Ther.), established for the first time that cellular therapy appeared to be a feasible and safe therapeutic approach.
- 2) Woffendin *et al.* (PNAS, 1996) represents the first safety trial examining autologous T lymphocyte transfer for gene therapy.
- 3) Wong-Staal, Poeschla, and Looney (Hum. Gene Ther., 1998) involved the use of HIV-specific hairpin ribozymes in an autologous T lymphocyte setting. Although details from this study are scarce, the safety of this approach was established.



SAFETY in the HIV/AIDS Program

For Multiple Dosing

- 1) The most recent T lymphocyte-based approach for HIV/AIDS gene therapy engineered an enhanced anti-HIV immune response by modifying CD4 and CD8 T lymphocytes to express a CD4 receptor coupled to the CD3 ζ signaling chain (Walker *et al.*, Blood, 2000; Mitsuyasu *et al.*, Blood, 2000; Deeks *et al.*, Mol. Ther., 2002). In one of the Phase I trials, trafficking of modified lymphocytes to the mucosal tissue in the rectum was observed, in combination with a greater than 0.5 log decrease in viral load at the site (Mitsuyasu *et al.*, 2002). *This is the first example of gene-modified T lymphocytes that were modified and expanded ex vivo, trafficking to a site of infection in vivo and effecting a response.* Multiple doses of vector modified cells were given with no subsequent adverse events, *demonstrating the safety of a multiple dosing approach to treatment.*

For Conditioning: A potential future approach for dosing of VRX-modified T cells to HIV patients is the pre-conditioning of these patients with a drug that kills off their existing lymphocytes. This is anticipated to improve treatment by 1) making “room” for the vector-modified T cells to repopulate the body, and 2) killing off latent-HIV infected T cells that could contribute to future disease.

This approach has recently been demonstrated to be safe in a Phase I clinical trial recently conducted at the NIH by the world-renowned cancer investigator Dr. Steven Rosenberg (Dudley et al, J. Immunother., 2002). He used this treatment to improve transplantation of anticancer lymphocytes into his patients. VIRxSYS proposes a similar approach, only instead anti-cancer lymphocytes, we would be infusing HIV resistant lymphocytes.

Summary: VIRxSYS is taking a safe step-wise approach to reaching efficacy in its HIV/AIDS program. The safety of lentiviral vectors over pre-existing retroviral vectors has been well recognized in the scientific and FDA community. Gene therapy delivery is achieved using a very well established platform of autologous T cell therapy. The “next steps” to improving the therapeutic index will also use the methods of 1) multiple dosing, and 2) conditioning, both of which have also been previously shown to be safe in Phase I clinical trials.

¹Insertional oncogenesis is the development of cancer resulting from the stable insertion of retrovirus DNA into cellular DNA. This insertion may cause disruption of normal function of genes that control cell growth. Dysregulation of such genes can cause uncontrolled cell growth, or cancer.

²The lentiviral class of retroviral vectors, is a subclass of the retroviral group. Lentiviruses are known for being slow-growing viruses, hence the name “lenti” for slow. They have several features that set them apart from onco-retroviruses which are known for their ability to cause cancer. Among these features are the ability to infect non-dividing cells, and no known connection to cancer. Onco-retroviruses were previously the retrovirus of choice used to make gene therapy vectors.

³Regional gene activation caused by an “enhancer” element in a retrovirus or retroviral vector is a manner by which insertion of the viral DNA into the cellular DNA may dysregulate genes by causing unnatural over-expression of these genes.

⁴The LTR is the name of the element in a retrovirus or retroviral vector that enables 1) insertion of the viral DNA in the cellular DNA and 2) expression of the viral DNA once inserted. The LTR is the viral genetic element that contains the “enhancer”.

⁵Christoff von Kalle is the investigator in the group that has been studying the underlying mechanisms of development of leukemia in the children in the X-linked SCID trial mentioned at the start of this white-paper. He is highly respected in the field.

⁶Poly-A is a stop signal inside the vector DNA that helps to prevent unnatural over-expression of closely located cellular genes. High levels of “read through” this stop can contribute to unnatural over-expression of cellular genes.

⁷Autologous means “self”. Transplantation works best when using your own tissue. Use of tissue from other persons often leads to rejection.