

A phase I pilot study of safety and feasibility of stem cell therapy for AIDS lymphoma using stem cells treated with a lentivirus vector encoding multiple anti-HIV RNAs

John A. Zaia, M.D.

Project Leader

John J. Rossi, Ph.D.

Scientific Coordinator

Disclosure

- Received restricted stock shares from Nanoviricides as a Scientific Advisor.

Goals of the Study

A phase I pilot study of safety and feasibility of stem cell therapy for AIDS lymphoma using stem cells treated with a lentivirus vector encoding multiple anti-HIV RNAs

Specific Aims--


1. Determine the safety of the strategy in terms of
 - adverse events
 - effects on HIV-1 infection
2. Determine the feasibility of the strategy in terms of
 - quantity, duration and character of vector-marked progeny cells following autologous transplantation
 - integration analysis

Rationale for the Study

Management of HIV-1 infection

- **Problems with conventional anti-retroviral therapy:**
 - **HIV-1 is detectable in tissue and recurs if treatment is stopped.**
 - **Potential for resistant HIV-1 to emerge**
 - **Serious side-effects**
 - **Treatment is expensive**
- **Gene transfer is proposed as a method of ‘adjuvant therapy’ which could modify the need for continued antiviral therapy**
- **Development of a new method of management of HIV-1 infection is the ultimate reason for initiating this clinical trial**

Why do we want to do the study?

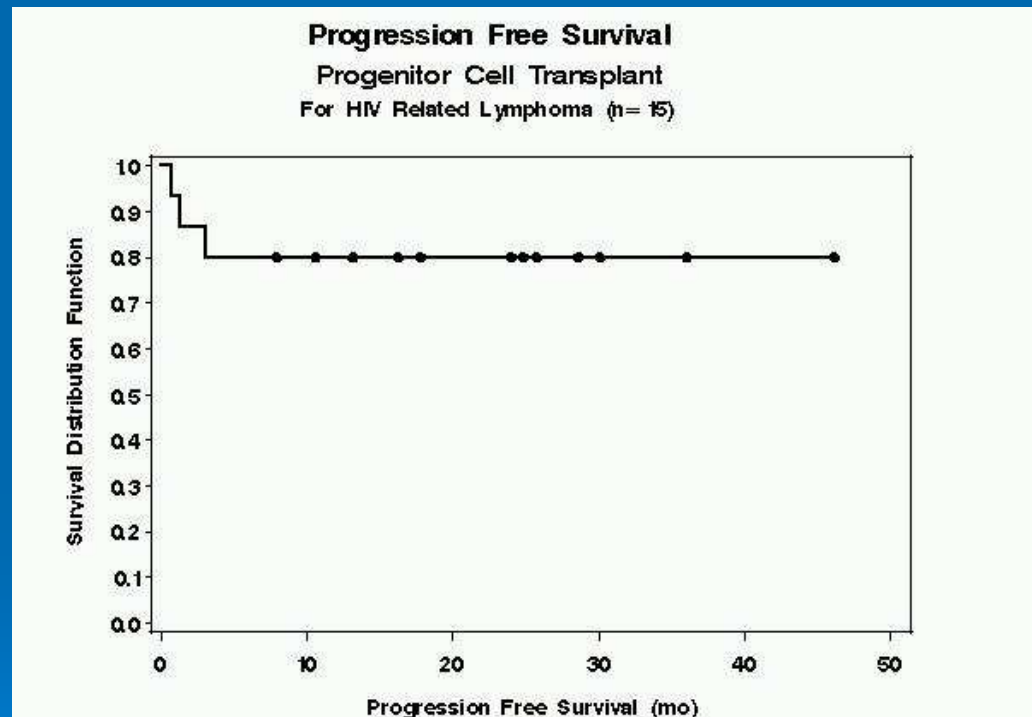
- This is a next step toward the eventual development of a genetic therapy for AIDS
 - This study will provide information needed for determining the safety of this lentivirus vector, and the use of shRNA in a HSC setting.
- 

Why use a lymphoma treatment setting?

- The means of *ex vivo* delivery of anti-HIV-1 genes involves primarily the use of T cells or blood progenitor cells.
- This study proposes to deliver the anti-HIV-1 genes to a patient using blood progenitor cells.
- The assessment of gene delivery using blood progenitor cells is limited by the requirement for myeloablative pre-treatment of the recipient to optimize the engraftment of the cells.
- Thus, the setting of autologous transplantation after dose-intensive therapy for AIDS lymphoma is an ethical and scientifically appropriate clinical setting for evaluating of a new genetic vector.

What is known about the safety of this approach?

- The transplantation procedure itself is therapeutic, and the investigators are very experienced



- The study design has been used before in our study of retrovirus-based delivery of anti-HIV ribozymes in AIDS lymphoma patients (A. Krishnan, P.I.)

Lymphoma Rx

G-CSF (10 ug/kg)

1 2 3 4 5 6 7 8.....
HPC-A Mobilization (days)

Aphereses

#1 #2 #3 #4

Fraction A

CD34+ Selection

Cryopreservation Untransduced

Fraction B

Cryopreservation

Transduction with HIV-shI-TAR-CCR5RZ

Conditioning Regimen:

BCNU

BCNU

BCNU

VP16

Cytosan

-7

-6

-5

-4

-3

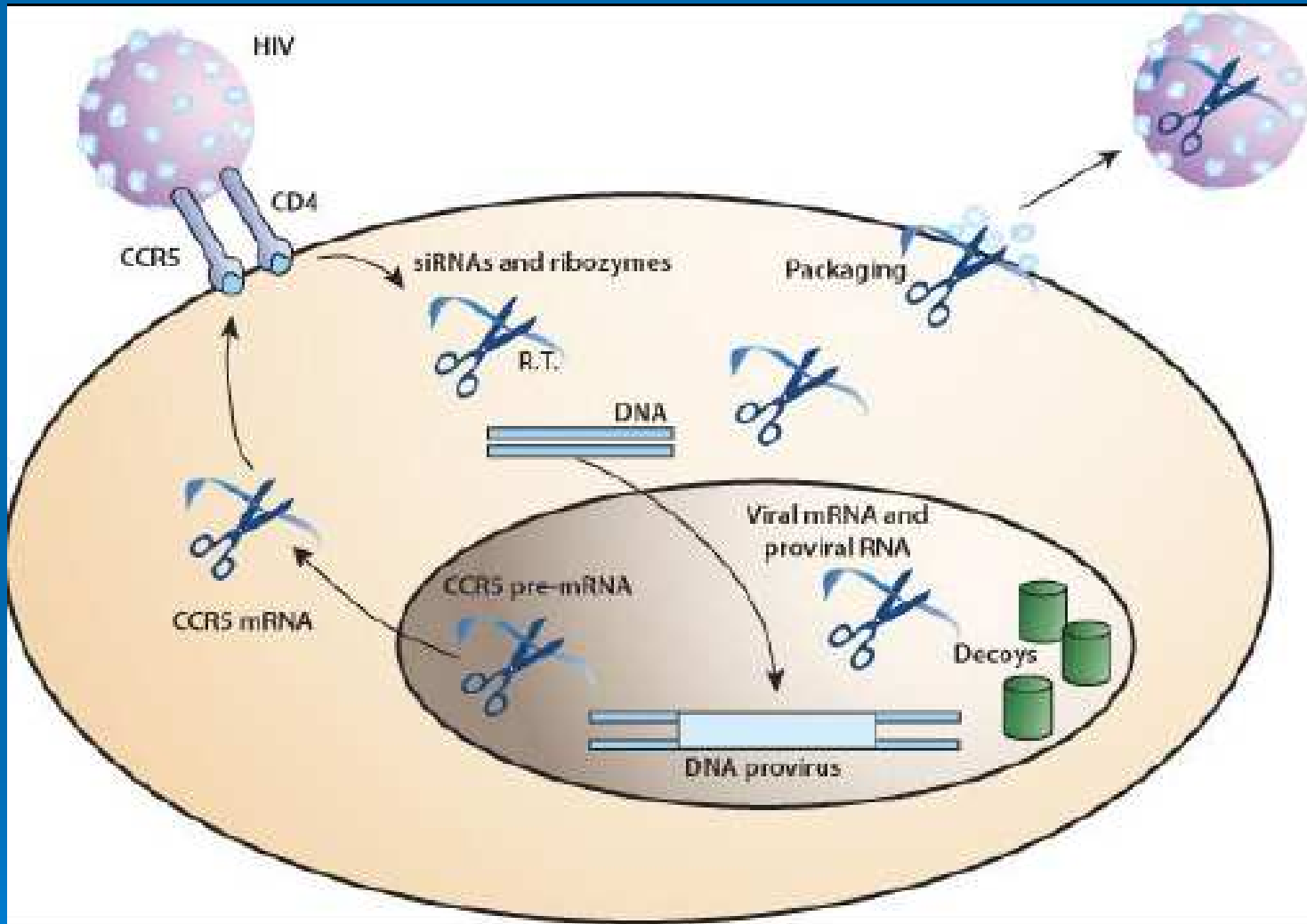
-2

0

+1

Days Pre-and Post-transplant

Multiple small RNA gene therapy approach

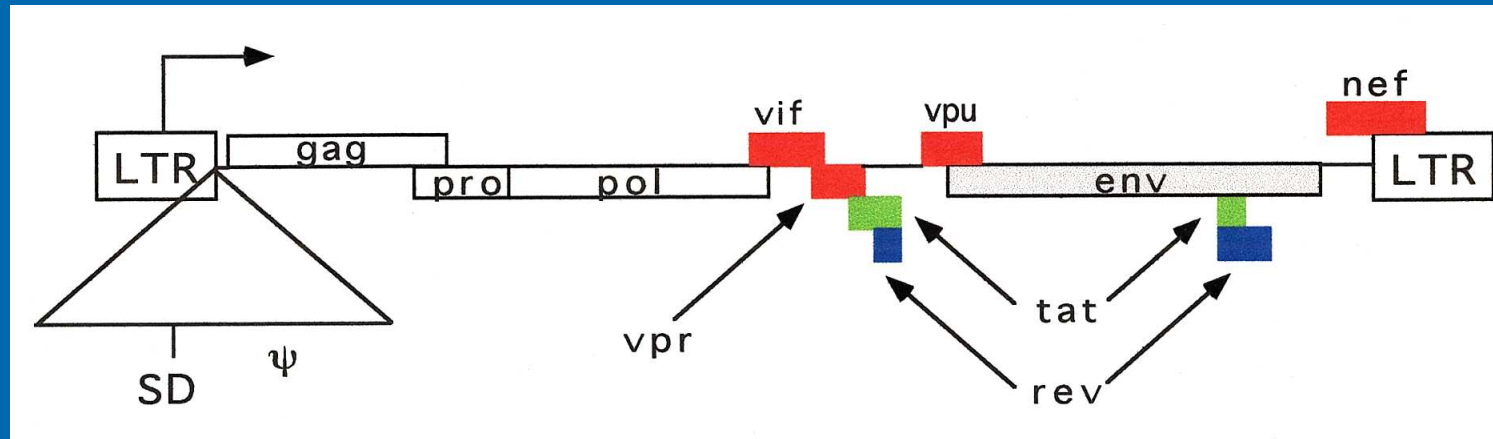


Rationale for the Anti-HIV-1 Design

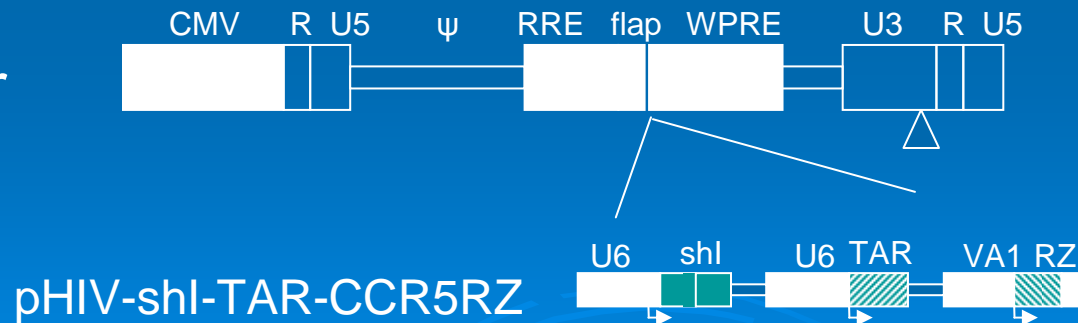
- siRNA is a potent inhibitor of HIV-1 *in vitro*
highly specific molecular target
potency is sufficient to force induction of viral resistance
Lee N. et al. 2002, Nat. Biotechnol.
Li M. et al. 2005, Mol Ther.
- TAR is an RNA element which can efficiently inhibit HIV-1 by serving as a decoy and blocking essential virus interaction with TAT and is expressed with snoRNA for nucleolar localization to achieve optimal effect
Michienzi et al. 2002, PNAS
- CCR5 ribozyme can down-regulate the expression of CCR5, the secondary receptor used for virus entry during new infection
Cagnon & Rossi 2000, Antisense Nucl Acid Drug Dev

HIV-1 vs Lentivirus Vector

HIV-1



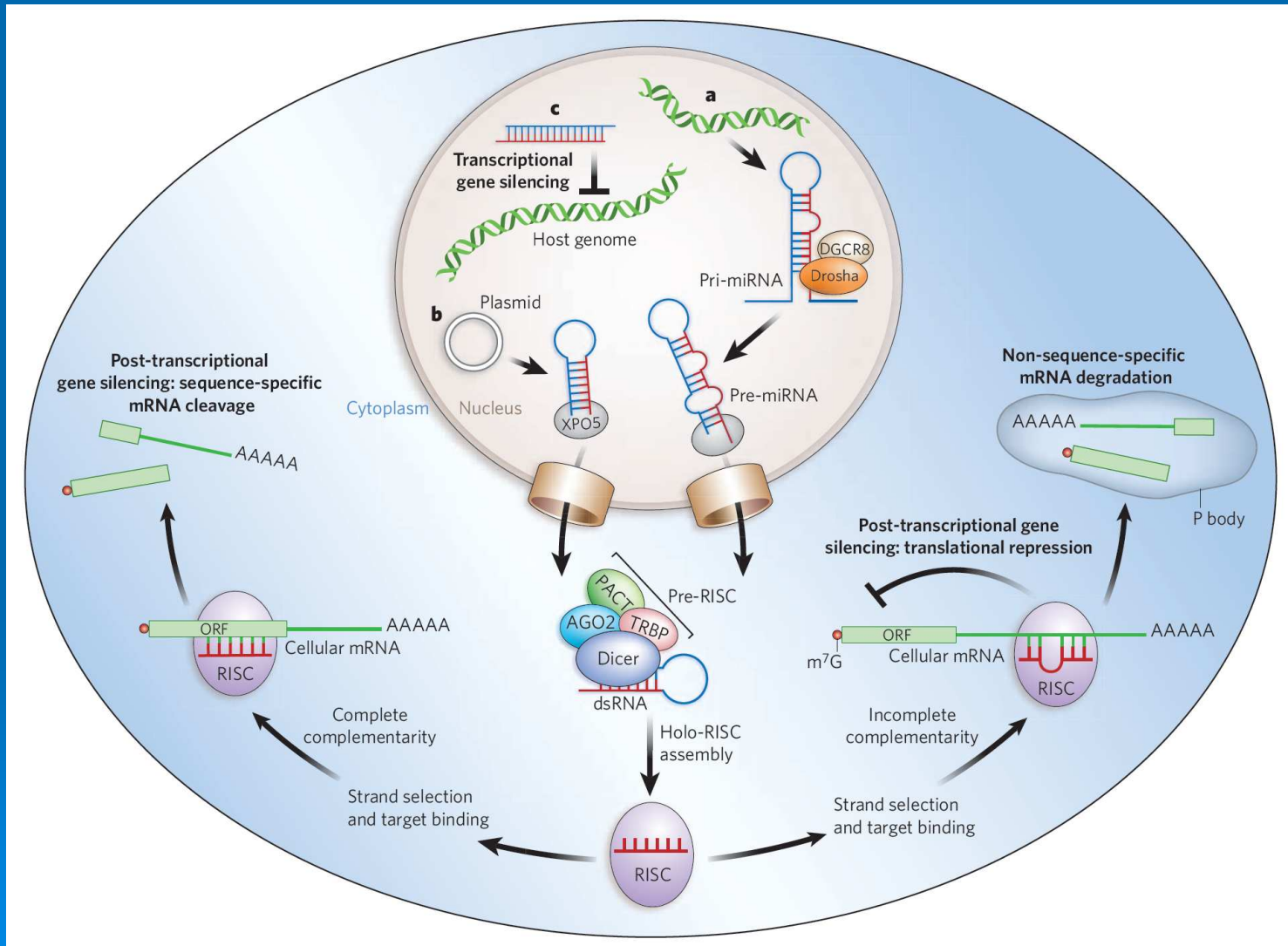
Vector



What is special about this vector?

- The vector is derived from HIV-1 in such a way that the vector is unable to replicate and express those viral genes associated with disease
- The vector is a third-generation or 'self-inactivating' lentivirus vector
- The vector expresses RNAs that can inhibit HIV-1 replication
- This is the first use of gene transfer of RNA interference as a strategy in a clinical trial

RNAi mechanism



Castanotto & Rossi. *Nature* 457: 426-433 (2009)

Is siRNA Safe?

Off-target considerations

- Are there significant alterations of miRNA profiles?
- Are there significant disturbances of cell function as measured by cell, differentiation, or immune activation suggesting a perturbation of non-targeted cellular genes?
- Does the sense strand of shRNA enter RISC thereby adding another level of off-targeting?

Are there significant alterations of miRNA profiles?

Micro RNAs are important regulators of post-transcriptional gene expression in mammalian cells, and they use the same components as the shRNA proposed here.

miRNA array analyses were done using a triple hairpin shRNA construct expressing shRNA to site 1 (and two other anti-rev and tat shRNAs) versus vector backbone in CEM and CD34+ cells. Result:

miRNA 224-up regulated 2 S.D.

miRNA 337-down regulated 2 S.D.

miRNA 338-down regulated 1 S.D.

These differences could not be seen using Northern hybridization analyses for miRNAs 224 and 337.

Are there significant disturbances of cell function suggesting perturbation of non-targeted cellular genes?

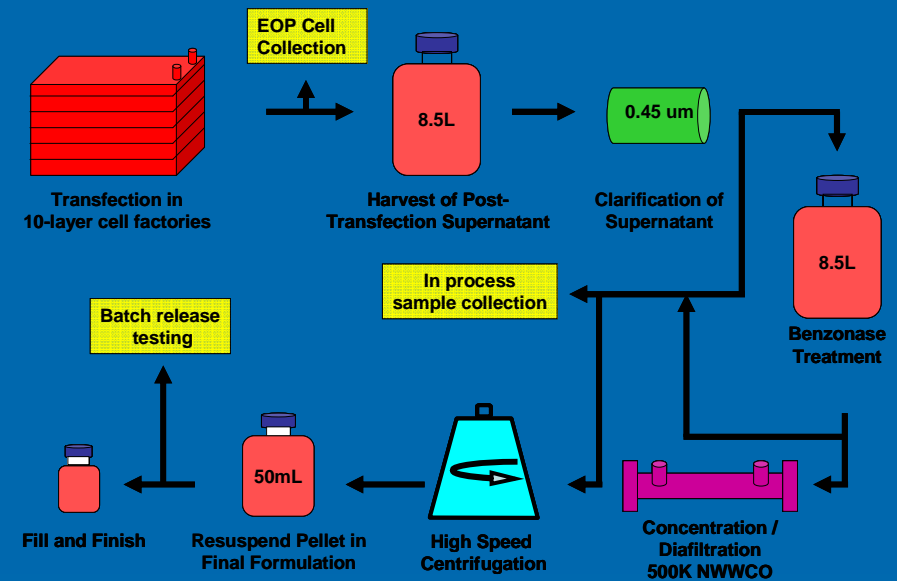
- Danger motifs in RNA: 5' GUCCUCAA 3' and 5' UGUGU 3'
- In siRNA/shRNA, these induce IFN production by plasmacytoid dendritic cells via Toll-like receptor 7 (TLR7) (Hornung et al., Nat. Med., 2005; Judge et al. Nat. Biotech., 2005).
- Pol III shRNA induces IFN alpha (Bridge et al. 2003) and siRNAs activate IFN inducible genes in cultured cell lines (Sledz et al., Nat. Genetics, 2003)
- Can IFN genes be activated in CD34+ derived hematopoietic lineages?

RNA Safety Summary

- All *ex vivo* experiments demonstrated no toxicity of Pol III expressed anti-HIV RNAs in HSC's
- miRNA array analyses showed no dysregulation of endogenous miRNA profiles
- Clinical vector-expressing macrophages have normal function (Li *et al.* Mol. Therapy, 2005)
- *In vivo* analyses in SCID-mice demonstrated that triple vector transduced CD34+ cells differentiated normally into T-lymphocytes and are resistant to HIV challenge (Anderson, et al. Mol. Therapy 2007)
- Fetal monkeys inoculated with siRNA-expressing vectors developed normally (A. Tarantal, UC Davis)

Center for Biomedicine and Genetics

Beckman Research Institute
at City of Hope



Clinical grade triple vector production produced enough vector to treat 5 BMT and 5 autologous T-cell patients.

Requirements:

Pyrogen free, no contaminating cellular DNA above 500 bp, no replication competent recombinants. Pre-clinical transduction data showing no toxicity in HSCs.

Summary

- **RACC presentation 9/21/05, Pre-IND FDA 5/2006. IND filing Nov. 2006. FDA approval for HSC trial May-2007.**
- **Clinical trial for AIDS/lymphoma patients. Autologous tem-cell trials initiated Feb. 2008-four patients under treatment-no adverse events and gene expression and multilineage marking out to two years. No loss of siRNA gene expression during course of trial-therefore no obvious siRNA toxicity (Science Translational Medicine-submitted).**
- **Autologous T- cell trials for mid 2010-FDA approval April 2010 largely based on HSC trial results and use of same vector .**

Marking outcomes to date

