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

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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-intense 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**

CD34+ Selection

**Fraction A**

Cryopreservation Untransduced

**Fraction B**

Cryopreservation

Transduction with HIV-shI-TAR-CCR5RZ

**Conditioning Regimen:**

BCNU  BCNU  BCNU  VP16  Cytoxan

-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

• 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**

- LTR
- gag
- pro
- pol
- vif
- vpu
- env
- rev
- nef

**Vector**

- CMV
- R
- U5
- ψ
- RRE
- flap
- WPRE
- U3
- R
- U5

**pHIV-shl-TAR-CCR5RZ**

- U6
- shl
- U6
- TAR
- VA1
- RZ
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

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.

There 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’ GUCCUUCAA 3’ and 5’ UGUGU 3’
- 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 disregulation 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)
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


- 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

[Graph showing WPRE copies/100 cells over time for different individuals (UPN0304, UPN0305, UPN0306, UPN0307) with markers for LOD and LOQ.]