Review and Public RAC Discussion of Protocol #0508-725

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

Additional Key Personnel

Amrita Krishnan, M.D. Principal Investigator
Clinical Investigator
Hematopoietic Cell Transplantation
AIDS Lymphoma

Larry Couture, Ph.D. Manufacturing/Regulatory Affairs
Senior VP for Applied Technology Development
Beckman Research Inst of City of Hope

Linda Couto, Ph.D. Research Liaison
Director, Pre-clinical Development
Benitec Inc

- Rationale for the Study—
  - Why do we want to do this study?
  - What will it teach the field of gene therapy?
  - Why is this so important?

- Rationale for the Vector Design—
  - What is special about this vector?

- Safety of the procedure—
  - What is known about the safety of this approach?

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

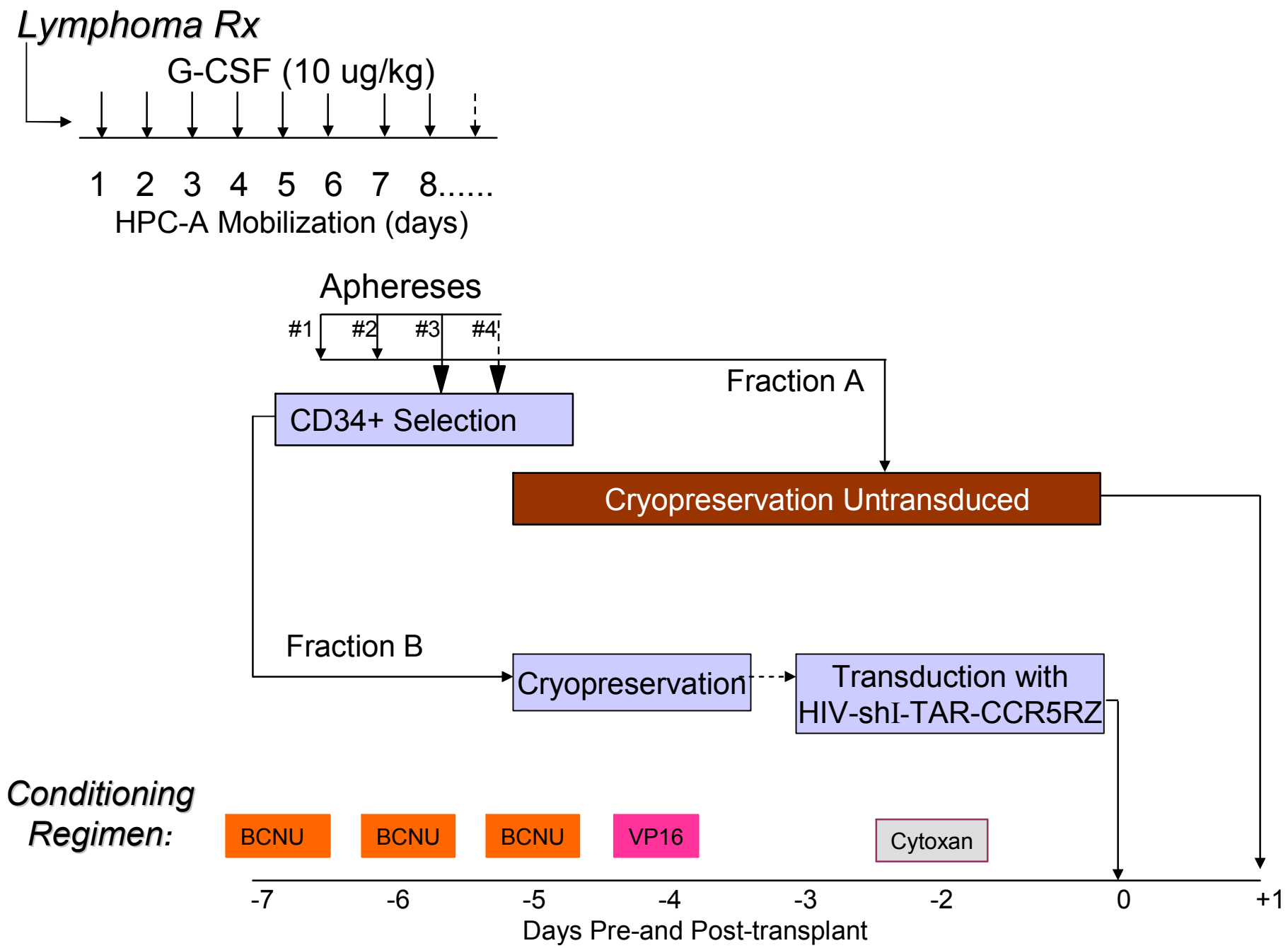
Why do we want to do the study?

- 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, a vector that has potential in other areas of gene transfer research

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 evaluation of a new genetic vector.


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
Cytoxan
-7 -6 -5 -4 -3 -2 0 +1
Days Pre-and Post-transplant

Why is this study important to the field?

- The study will provide some indication of whether the use of a lentivirus vector is feasible in the setting of blood progenitor transplantation-based gene delivery.

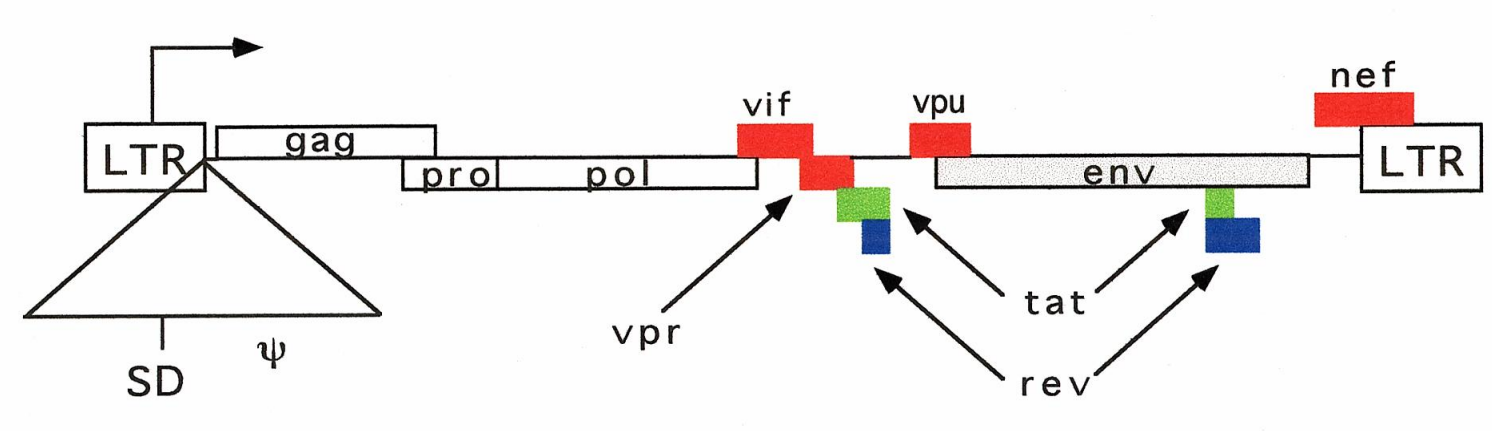
- If this lentivirus vector is effective in this setting, it will have application in other settings appropriate for hematopoietic progenitor cell gene therapy.

What is special about this vector?

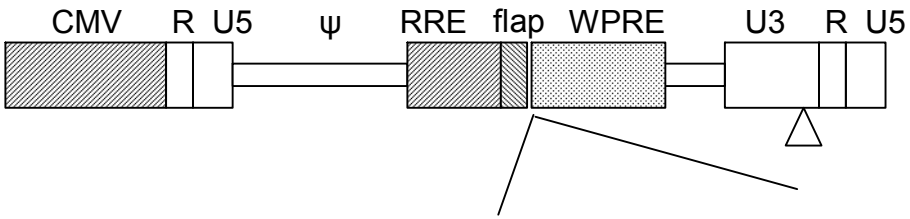
- The vector is derived from HIV-1 in such a way that the vector is unable to replicate and express those viral genes associated with disease
- The vector is a third-generation or ‘self-inactivating’ lentivirus vector
- The vector expresses RNAs that can inhibit HIV-1 replication
- This is the first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

HIV-1 vs Lentivirus Vector

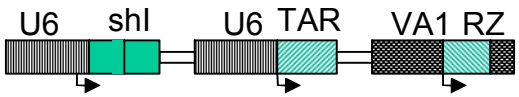
HIV-1



Vector

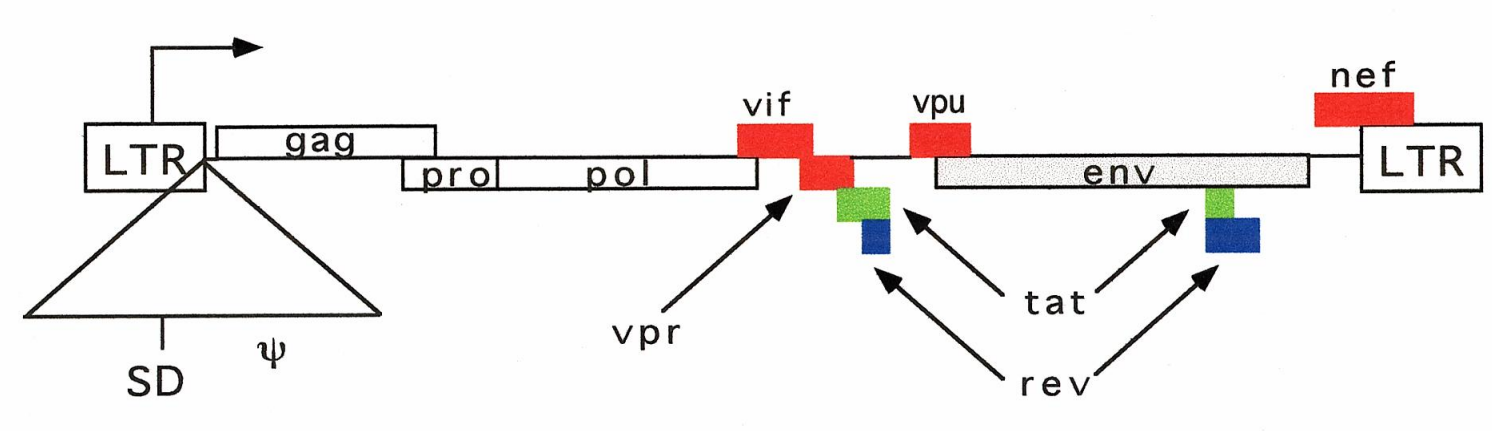


pHIV-shI-TAR-CCR5RZ

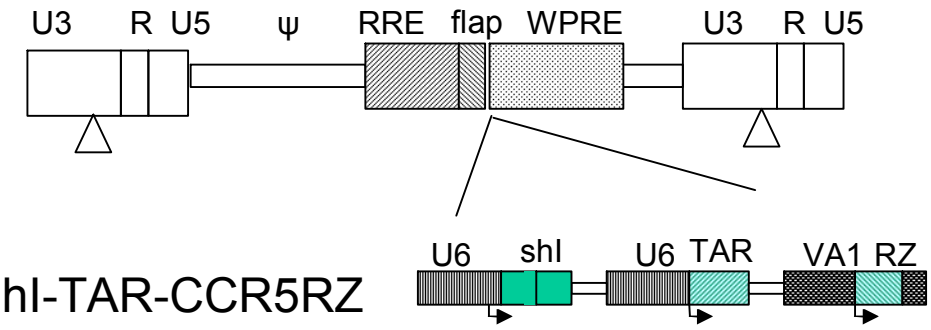


HIV-1 vs Lentivirus Vector

HIV-1



Integrated Vector



pHIV-shI-TAR-CCR5RZ

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. 20:500-505,
  Li M. et al. 2005, Mol Ther, in press
- 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 99: 14047-14052
- 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 8:251-61

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.)

Is siRNA Safe?

Off-target considerations

- Are there significant alterations of miRNA profiles?
- Are there significant disturbances of cell function as measured by cell replication, 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: in an array of 250 miRNAs--

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’ GUCCUUCAA 3’ and 5’ UGUGU 3’
- In siRNA/shRNA, these induce IFN production by plasmacytoid dendritic cells via Toll-like receptor 7

  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?

Method of Experimentation

CD34+ cord blood cells

↓

Transduction with vector

↓ 7 days

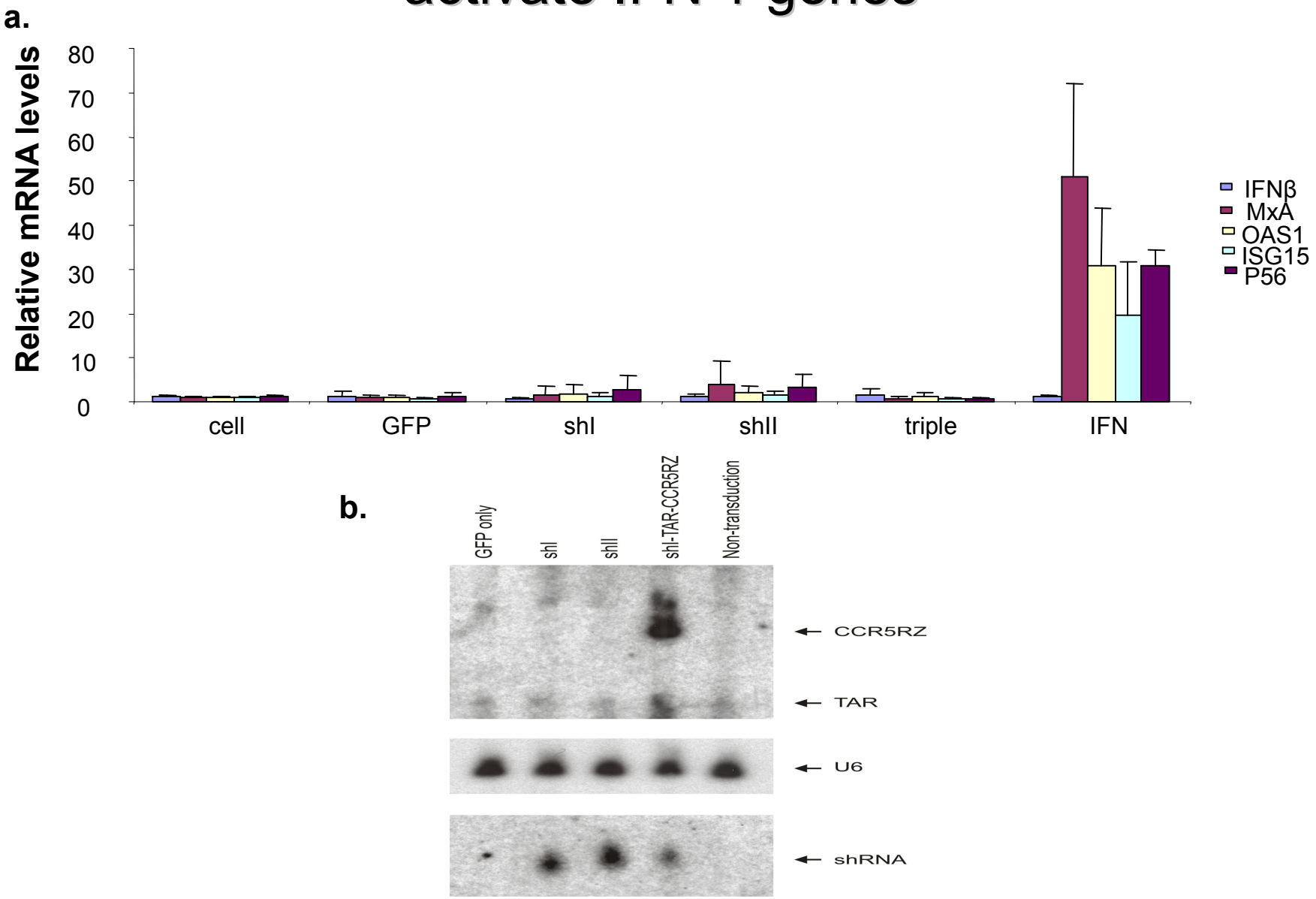
Sorting by FACS

↓ GM-CSF and M-CSF
8 days

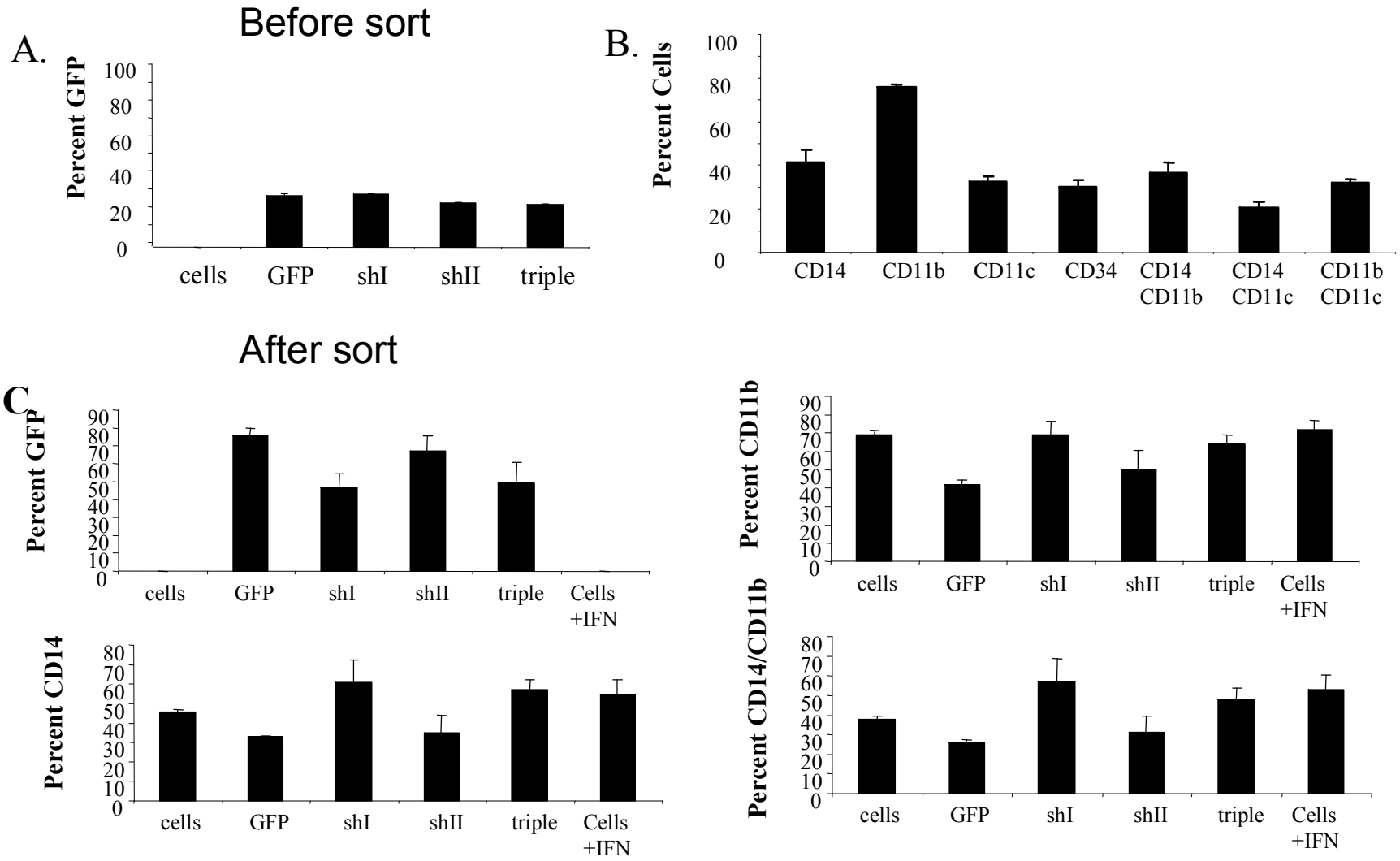
Mature monocyte/macrophages

- FACS Characterization
- IFN induction
- IFN inducible proteins
- Cytokine release
- Macrophage Function

Expressed hairpin siRNAs with UGU motifs do not activate IFN 1 genes

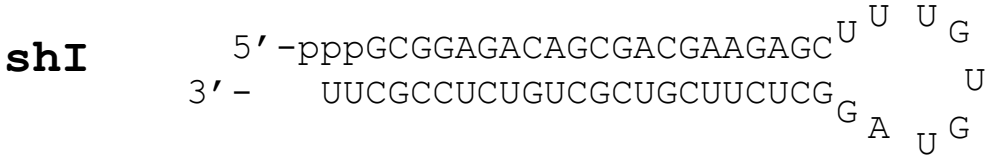


Lentiviral vector-transduced cells expressing shRNAs show normal *in vitro* marker differentiation



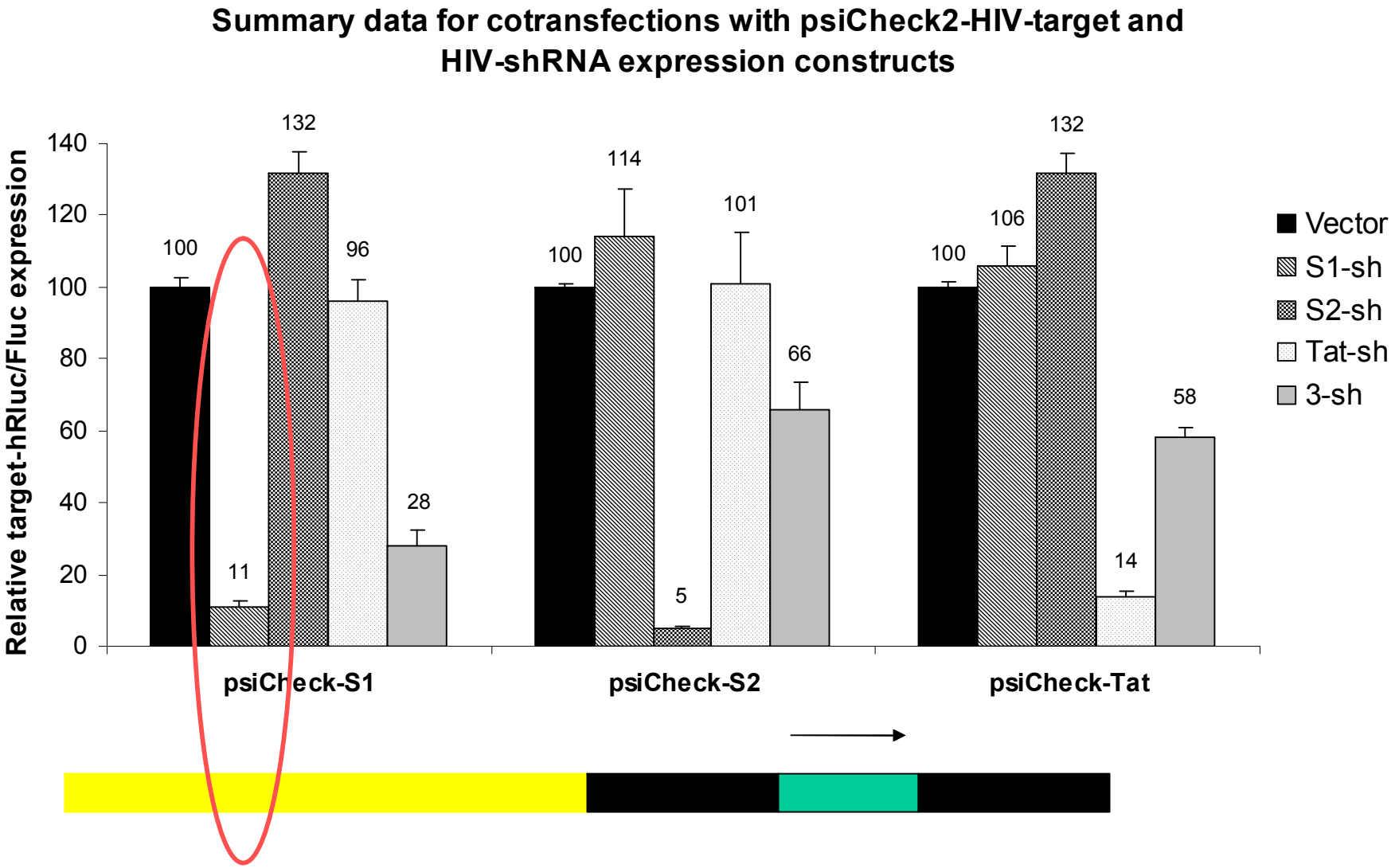
Does the sense strand of shRNA enter RISC thereby adding another level of off-targeting?

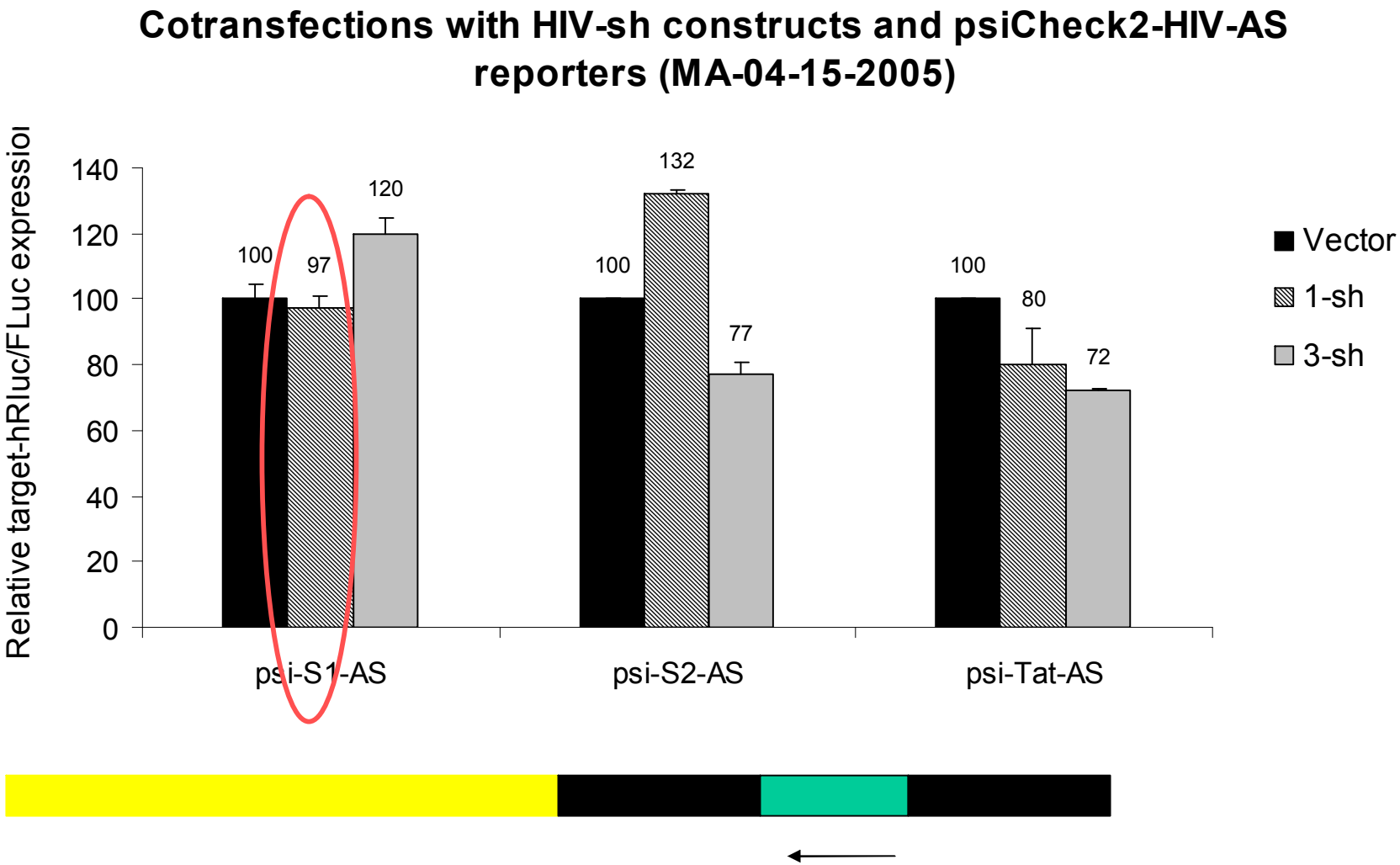
Strand selection into RISC



Off-targeting can occur from either strand-is the sense strand effectively entering RISC?

Renilla Luciferase with HIV target in 3’ UTR


Summary data for cotransfections with psiCheck2-HIV-target and HIV-shRNA expression constructs
Relative target-hRluc/Fluc expression
140
120
100
80
60
40
20
0
100
11
132
96
28
100
5
114
101
66
100
14
106
132
58
psiCheck-S1
psiCheck-S2
psiCheck-Tat
Vector
S1-sh
S2-sh
Tat-sh
3-sh


Cotransfections with HIV-sh constructs and psiCheck2-HIV-AS reporters (MA-04-15-2005)
Relative target-hRluc/FLuc expressio
140
120
100
80
60
40
20
0
100
97
120
100
132
77
100
80
72
psi-S1-AS
psi-S2-AS
psi-Tat-AS
Vector
1-sh
3-sh

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 (R. Akkina CSU)
- Fetal monkeys inoculated with siRNA-expressing vectors developed normally (A. Tarantal, UC Davis)

Conclusions

- This proposal will evaluate a new lentivirus vector that expresses anti-HIV-1 RNAs; the goal is to advance the treatment methods for AIDS by means of gene therapy

- The study will evaluate the safety of a potent new form of gene therapy--RNA interference– and will have application to other gene transfer studies in the future

- The clinical setting of transplantation for AIDS lymphoma has been selected as particularly appropriate and will also inform future trials of gene-modified blood stem cells