

Benitec - City of Hope human trial update

23rd October 2008, Melbourne, Australia: Leading developer of RNA interference (RNAi)-based therapeutics Benitec Limited (ASX: BLT) announced today that Dr John Zaia, had presented an update on the human HIV trial at the 2nd Annual Cambridge Healthtech Institute's "RNAi for Therapeutics" conference held in Boston, MA USA 22-23rd October 2008.

Dr Zaia is the Chair, Division of Virology, Beckman Research Institute and is a key collaborator for the pilot human HIV study being undertaken at the City of Hope in Duarte, California. The oral presentation was entitled "Delivery of shRNA and other anti-HIV RNAs by autologous transplantation of lentivirus-transduced cells - a feasibility study".

This pilot feasibility study is a collaboration between Benitec and the City of Hope and is Benitec's first human trial. It uses a triple therapy delivered using a lentiviral vector for the treatment of HIV. The rHIV7-shI-TAR-CCR5RZ vector suppresses HIV by expressing three therapeutic nucleic acids that are directed against key steps in HIV replication.

"The final outcomes of the study are still pending however to date we have seen safe engraftment in three patients at 10 days. We have also seen gene markers in the blood months later which is very encouraging" said Dr Zaia.

"We are very encouraged by these initial findings. This is an extremely important trial as it is the first human clinical trial with expressed RNA interference trigger (shRNA) and the first triple gene therapy combination trial for HIV/AIDS. It is the also the first human trial for AIDS using lentiviral vectors transduced with hematopoietic stem cells (HSCs)." said Sue MacLeman, Chief Executive Officer, Benitec Limited.

A copy of the conference presentation is attached. This trial is now fully enrolled. A further update on the trial including further analysis of the gene markers will be presented at the American Society of Hematology in December 2008.

The Study

The study with City of Hope is entitled, "A pilot study of the safety and feasibility of stem cell therapy for AIDS lymphoma using stem cells treated with a lentiviral vector-encoding multiple anti-HIV RNA's."

The pilot study is designed to determine the safety and feasibility of RNA-based anti-HIV therapy with lentivirus-transduced hematopoietic progenitor cells (HPC) in patients undergoing autologous hematopoietic stem cell transplantation (HCT) for intermediate and high grade AIDS lymphoma.

The lentivirus vector encodes three forms of anti-HIV RNA: RNAi in the form of a short hairpin RNA (shRNA) targeted to an exon in HIV-1 tat/rev (shI), a decoy for the HIV TAT-reactive element (TAR), and a ribozyme that targets the host cell CCR5 chemokine receptor (CCR5RZ). The vector, used to transduce autologous CD34-selected HPC, is called rHIV7-shI-TAR-CCR5RZ and was manufactured by the Center for Biomedicine and Genetics at City of Hope.

Following standard mobilization of HPC and collection by apheresis (HPC-A), a portion of the cells were cryo-preserved and left unmanipulated for later use as treatment. The remaining portion of the cells were enriched for CD34+ cells using a Miltenyi CliniMACS™ system, cryo-preserved, and later genetically modified by infection with rHIV7-shI-TAR-CCR5RZ.

The subjects underwent conditioning therapy and at the time of autologous HCT, the rHIV7-shI-TAR-CCR5RZ transduced cells were infused, followed 24-hrs later by the infusion of untransduced autologous HPC-A.

CONTACT:

BENITEC LTD

Sue MacLeman
Chief Executive Officer
+61 437 211 200

Forward-looking Statements

This press release contains forward-looking statements that reflect the Company's current expectations regarding future events. Forward-looking statements involve risks and uncertainties. Actual events could differ materially from those projected herein and depend on a number of factors including the success of the Company's research strategy, the applicability of the discoveries made therein, the successful and timely completion of clinical studies and the uncertainties related to the regulatory process.

About Benitec

Benitec is an Australian biotechnology company focused on licensing its extensive intellectual property portfolio and developing therapeutics to treat serious diseases using its proprietary ddRNAi technology. Its current therapeutic program is focused on infectious diseases and cancer. For additional information, please visit www.benitec.com.

About City of Hope

City of Hope is a leading research and treatment center for cancer, diabetes and other life-threatening diseases. Designated as a Comprehensive Cancer Center, the highest honor bestowed by the National Cancer Institute, and a founding member of the National Comprehensive Cancer Network, City of Hope's research and treatment protocols advance care throughout the nation. City of Hope is located in Duarte, Calif., just northeast of Los Angeles, and is ranked as one of "America's Best Hospitals" in cancer and urology by *U.S. News & World Report*. Founded in 1913, City of Hope is a pioneer in the fields of bone marrow transplantation and genetics. For more information, visit www.cityofhope.org.

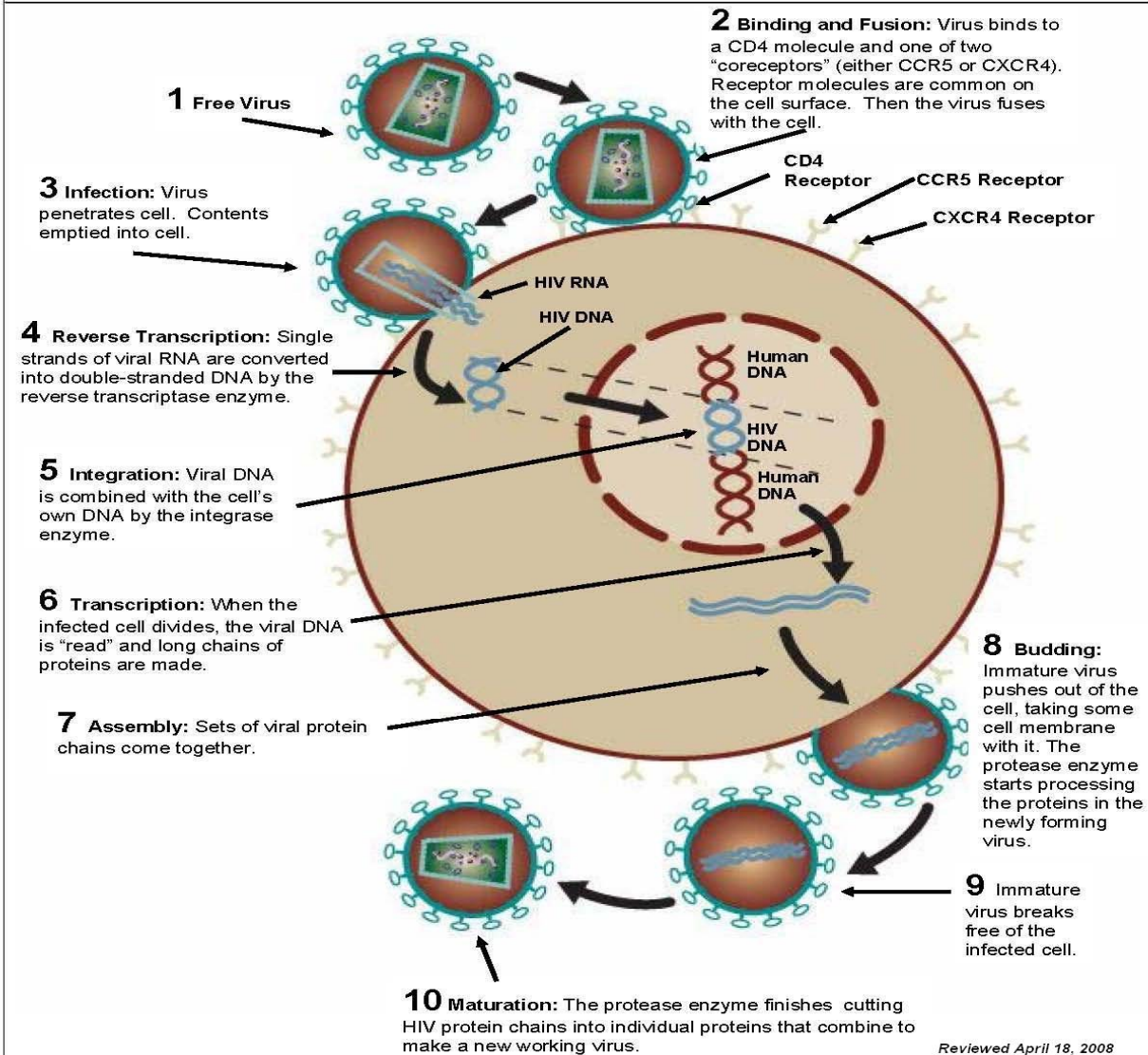
Delivery of shRNA and other anti-HIV RNAs by autologous transplantation of lentivirus-transduced cells: a feasibility study

John A. Zaia, M.D.

October 23, 2008



HIV LIFE CYCLE



Reviewed April 18, 2008

HIV Structure

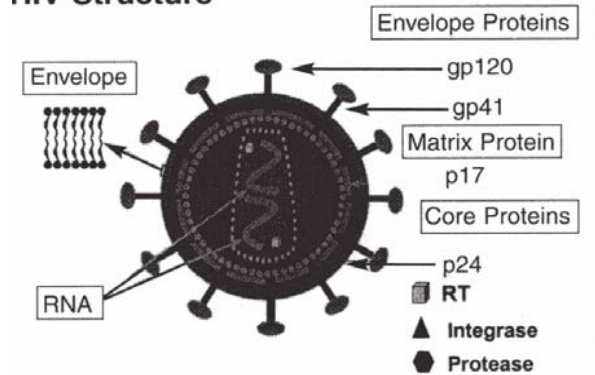
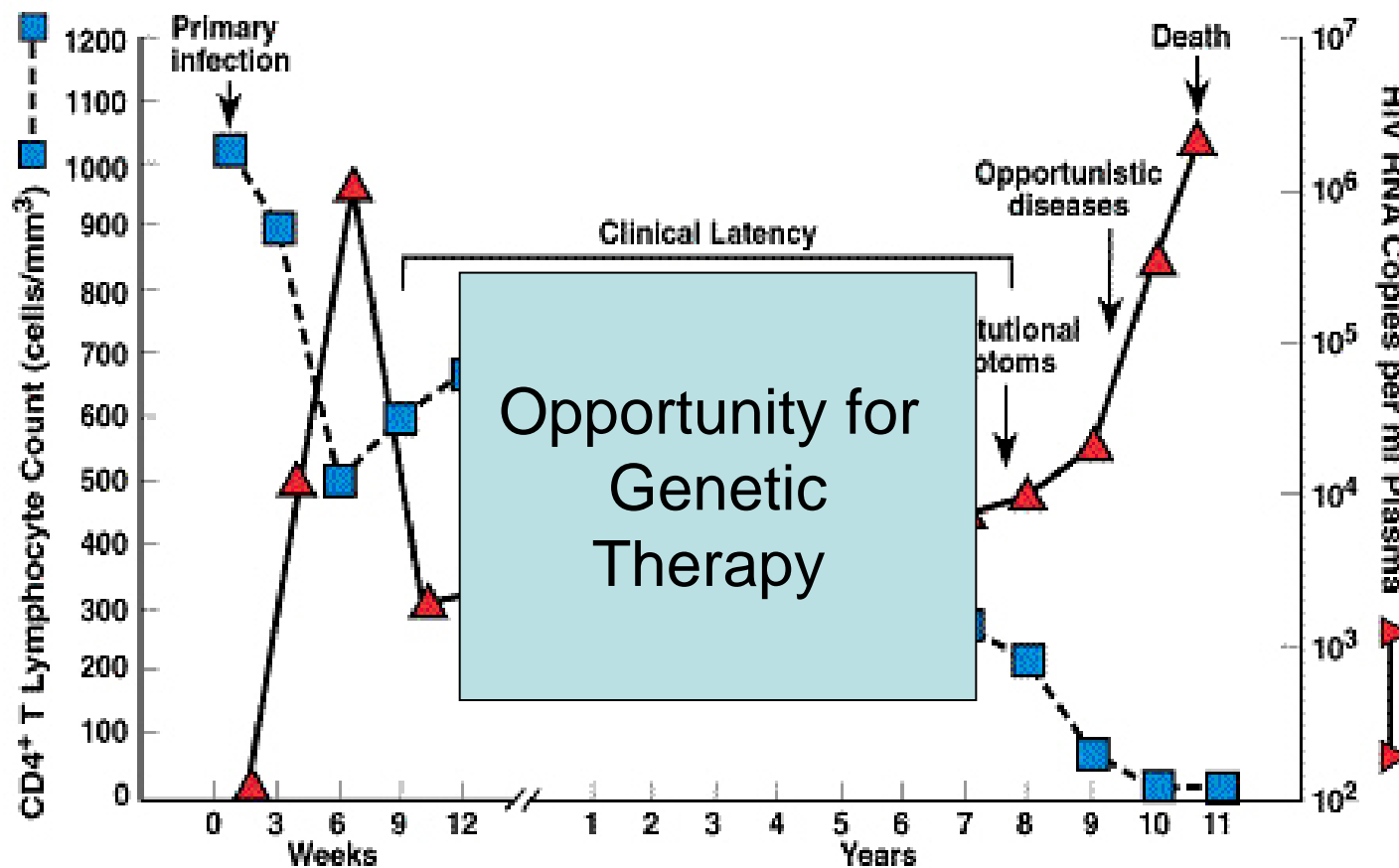


Figure 3. The Human Immunodeficiency Virus

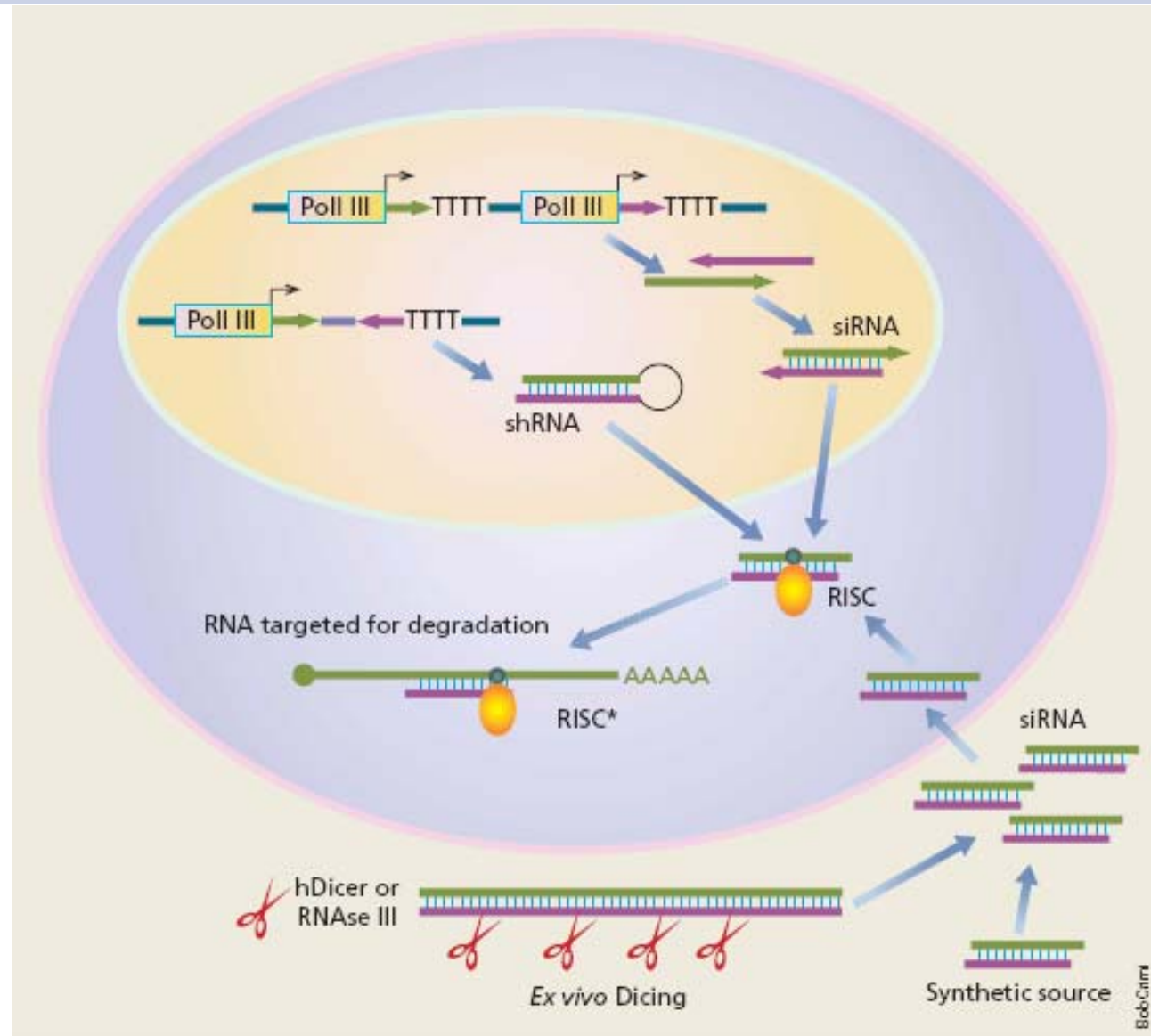
Typical Course of HIV Infection



Modified From: Fauci, A.S., et al, *Ann. Intern. Med.*, 124:654, 1996

Methods for introduction of siRNAs into cells

From Scherer &
Rossi
Nat Biotechnol.
2003;21:1457-65.



Significant issues for discussion

- What is the best strategy for design and delivery of RNAi for control of HIV/AIDS? Optimal target site?
 - Synthetic RNAi vs
 - T cell transduction and expansion
 - Blood stem cell transplantation
- Can RNAi inhibit HIV for long periods of time in vivo and alter the pathogenesis of HIV infection, the progression to AIDS, or the time to initial treatment ?

Gene therapy of HIV using natural mutations: a test of feasibility

- IF there were a natural mutation known to influence the progression of HIV infection, the transfer of such a “surrogate” transgene would provide a test of the concept of gene therapy for HIV
- $\Delta 32$ -CCR5 deletion is naturally present in a fraction of the population. These people cannot be infected with R5 tropic HIV but can be infected with other strains of HIV
- IF use of the natural mutation were to be successful, then this would be important for future RNAi-based system of gene therapy of HIV.

The Hutter Case

- 40 yo male with HIV since 1996 on ART without AIDS developed AML [M4] in 2006
- Transplanted in 2007 using MUD $\Delta 32/\Delta 32$ after moderate-intensity fludarabine, Ara-C, amsacrin (FLAMSA)-reduced intensity conditioning (RIC)
- Relapsed d332 and treated with donor lymphocyte infusion and TBI 200 cGy
- Currently in remission but course complicated by encephalitis leading to virus work-up: negative brain bx, neg HIV PCR, neg for CNS viruses

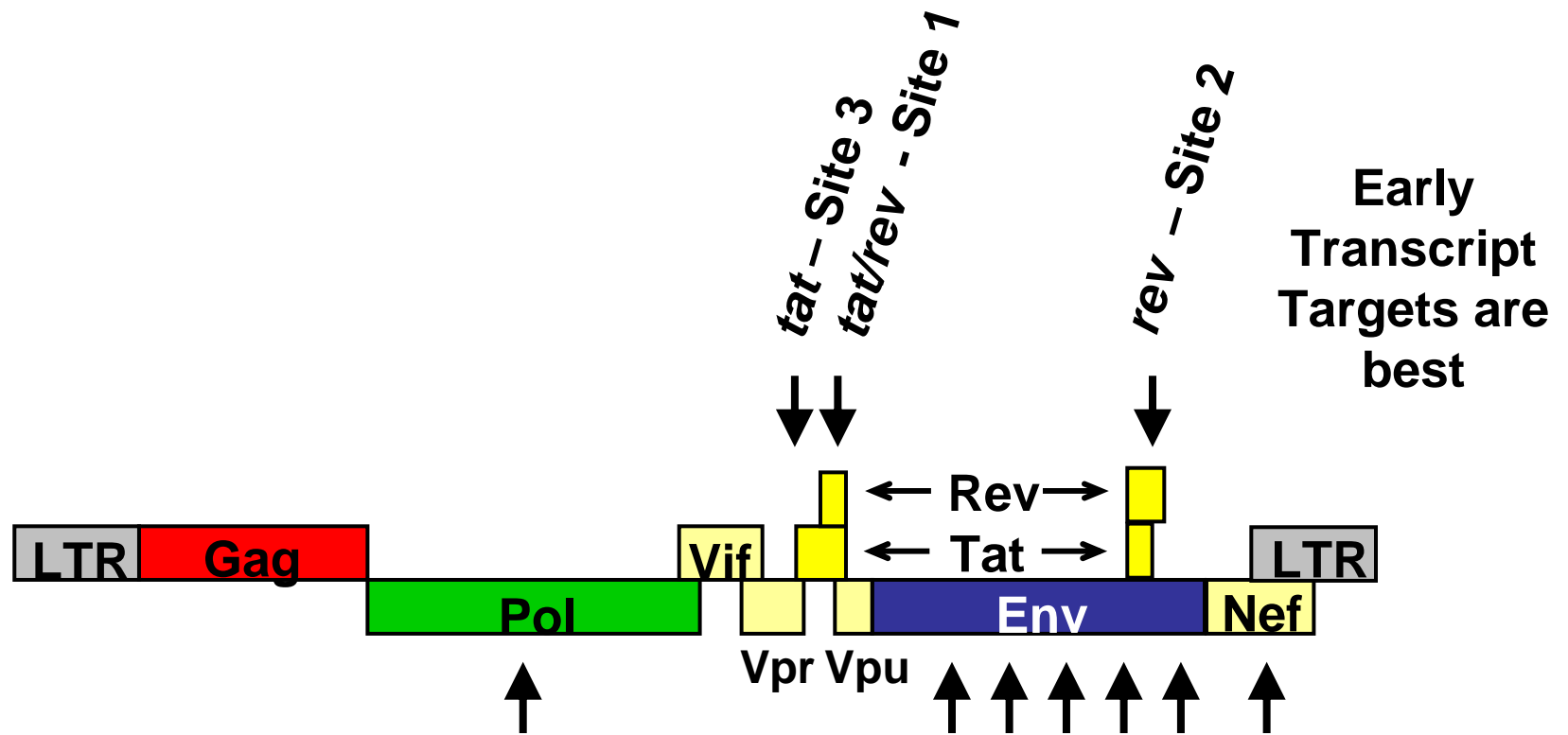
Gero Hutter, CROI abst #719; Boston, Feb 2008

Virology of the Hutter Case

- Pre-HCT HIV load was negative, provirus in PBMC 24 gc/cell while on ART; HIV R5 by V3
- High HIV load immediately post-HCT but became <15 gc/ml on ART
- HIV pro-viral DNA undetectible at d60 and ART discontinued
- Mucosal bx showed C4 cells neg for CCR5, some CCR5 on macrophages, but no HIV
- Brain biopsy negative for HIV
- Remains off ART with no HIV detectible in blood or tissue for >1 year

Gero Hutter*, CROI abstr #719; Boston, Feb 2008

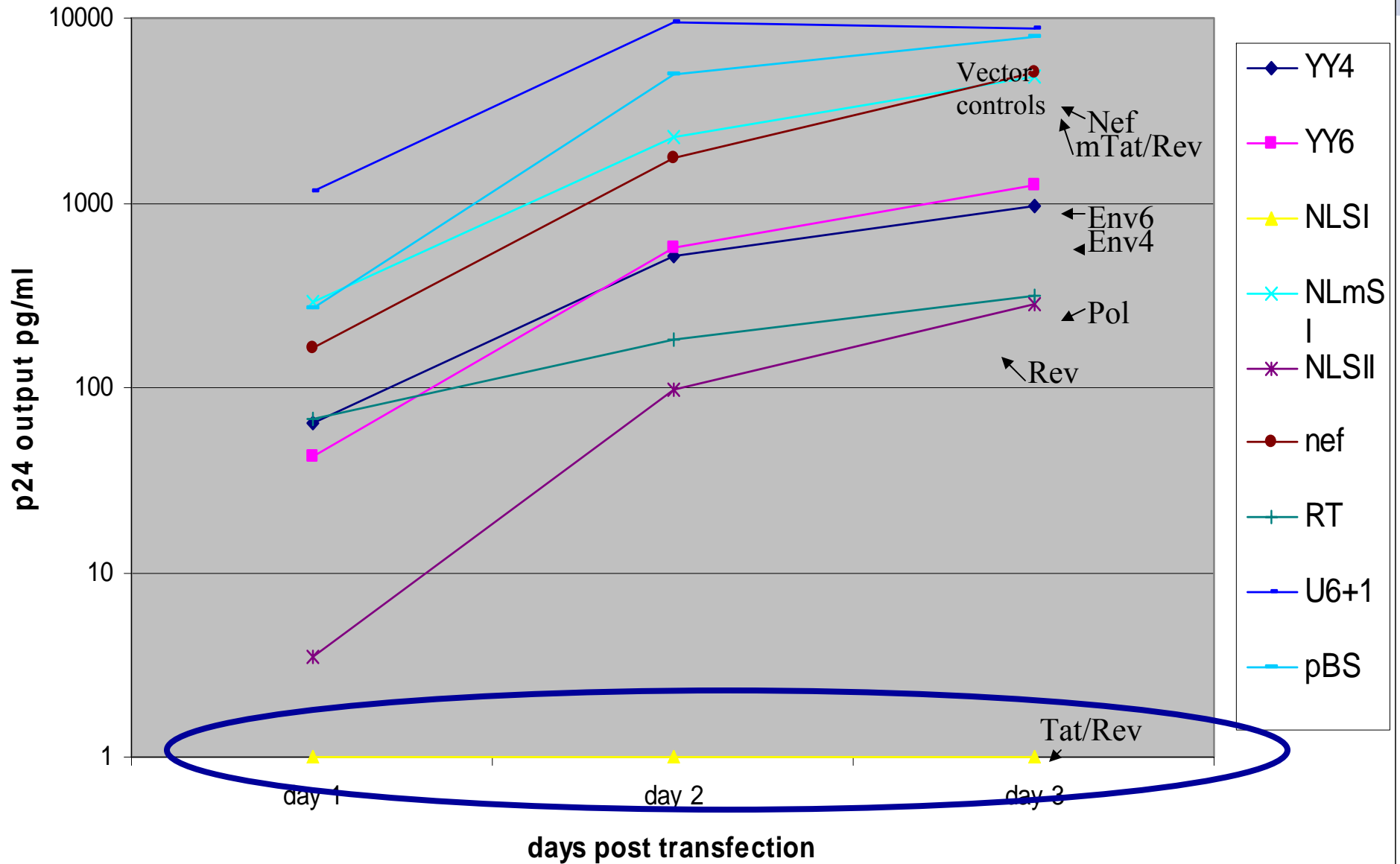
Choosing most potent siRNA/viral target combinations



Anti-HIV Assay: Co-transfect shRNA with HIV proviral DNA, measure p24 antigen.

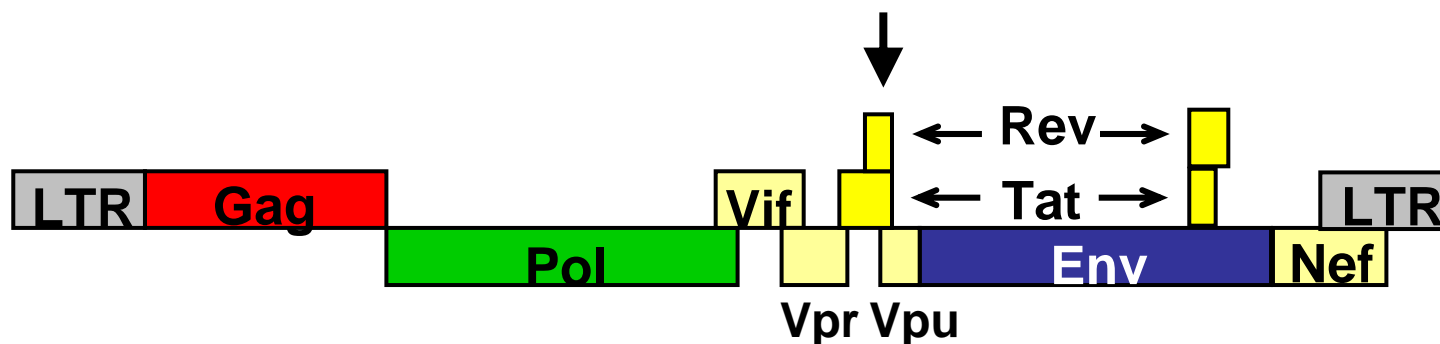
From J. Rossi unpublished

Comparative site-specific anti-HIV effects

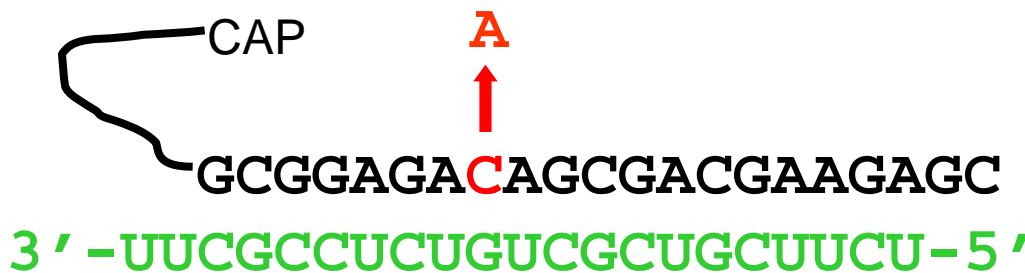
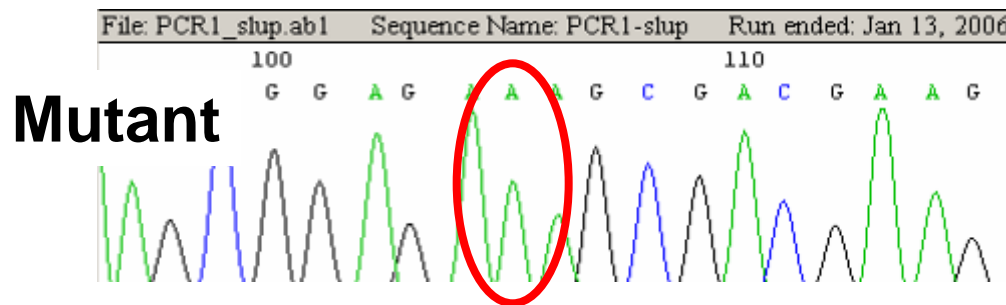
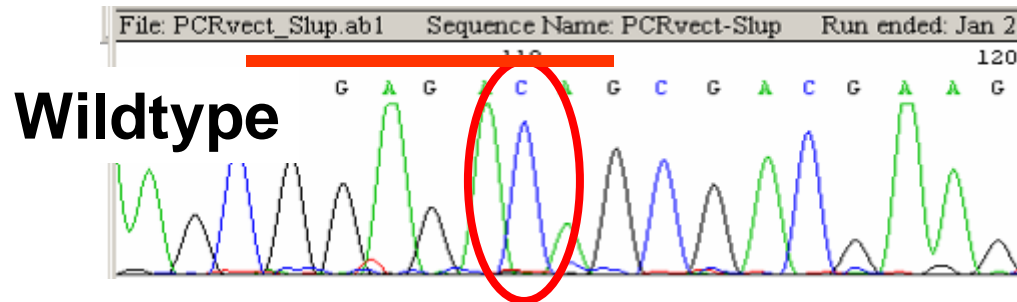


Conclusion: optimal target site in HIV

Targeting Early Transcripts encoding Tat and Rev has been most effective since these are key proteins for subsequent steps in the viral life cycle. In particular the common exon shared by Tat and Rev.



Viral escape mutants emerge with single siRNA targets



Overlapping Reading frames

Tat -Gln to Lys

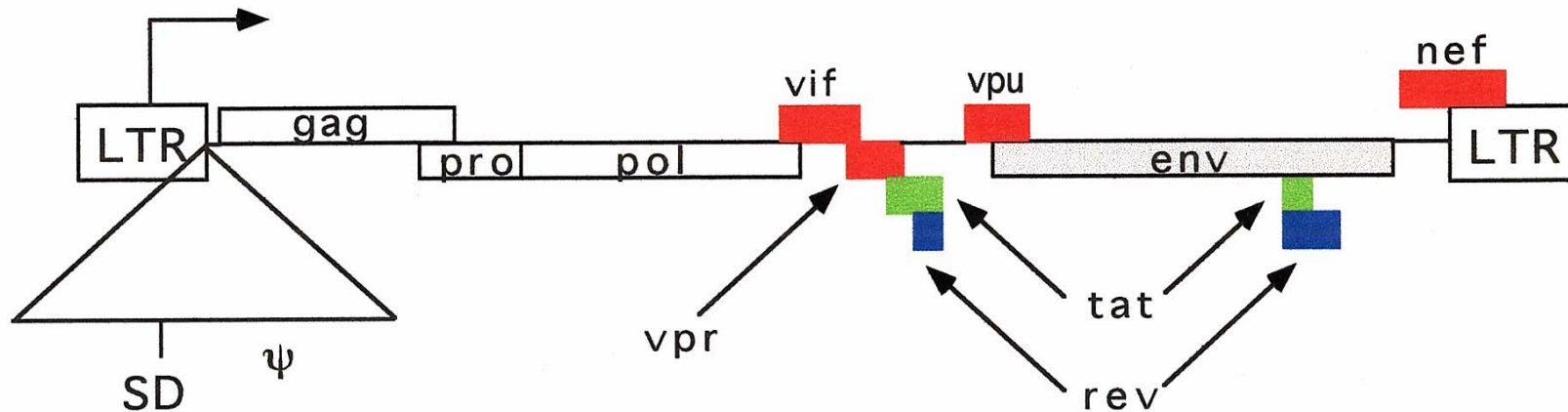
Rev-Asp to Glu

If mutant used to challenge cells expressing shRNA to tat/rev, mutation persists, but if grown on naive T-cells, wild type comes back as predominant species

Combinatorial RNA based therapy for HIV

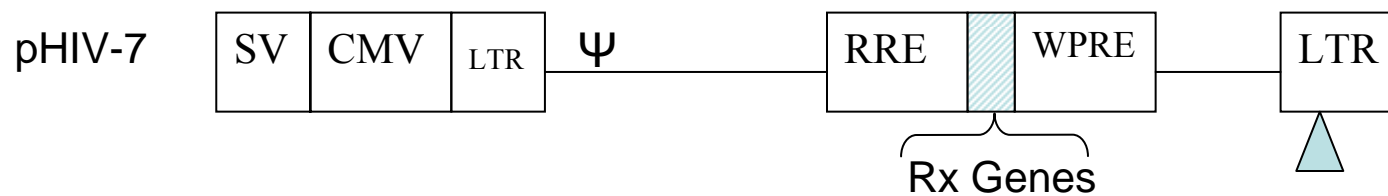
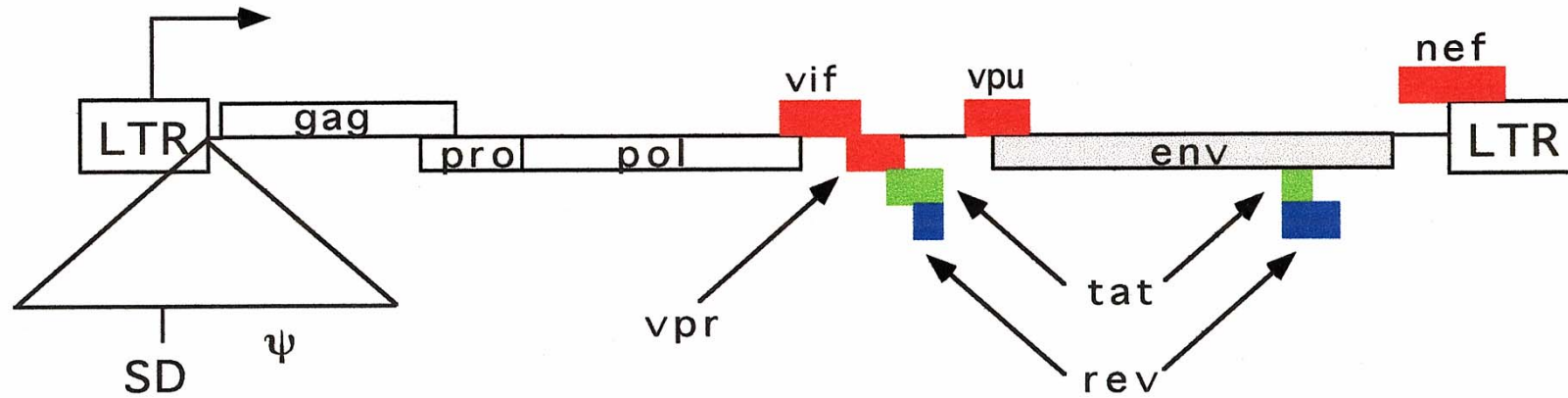
- Most efficacious drug therapy for HIV uses combination of two or three drugs targeting HIV RT and protease. Combinations prolong and sometimes prevent resistant viral variants.
- Can effective combinatorial gene therapy from a single vector be accomplished?

City of Hope Lentivirus Vector



- First generation lentivirus—made by substituting VSVg for env
- Second generation lentivirus—made by removal of accessory genes
- Third generation lentivirus—made by substituting CMVp for LTR-U3 deletion; so-called “self-inactivating vector”

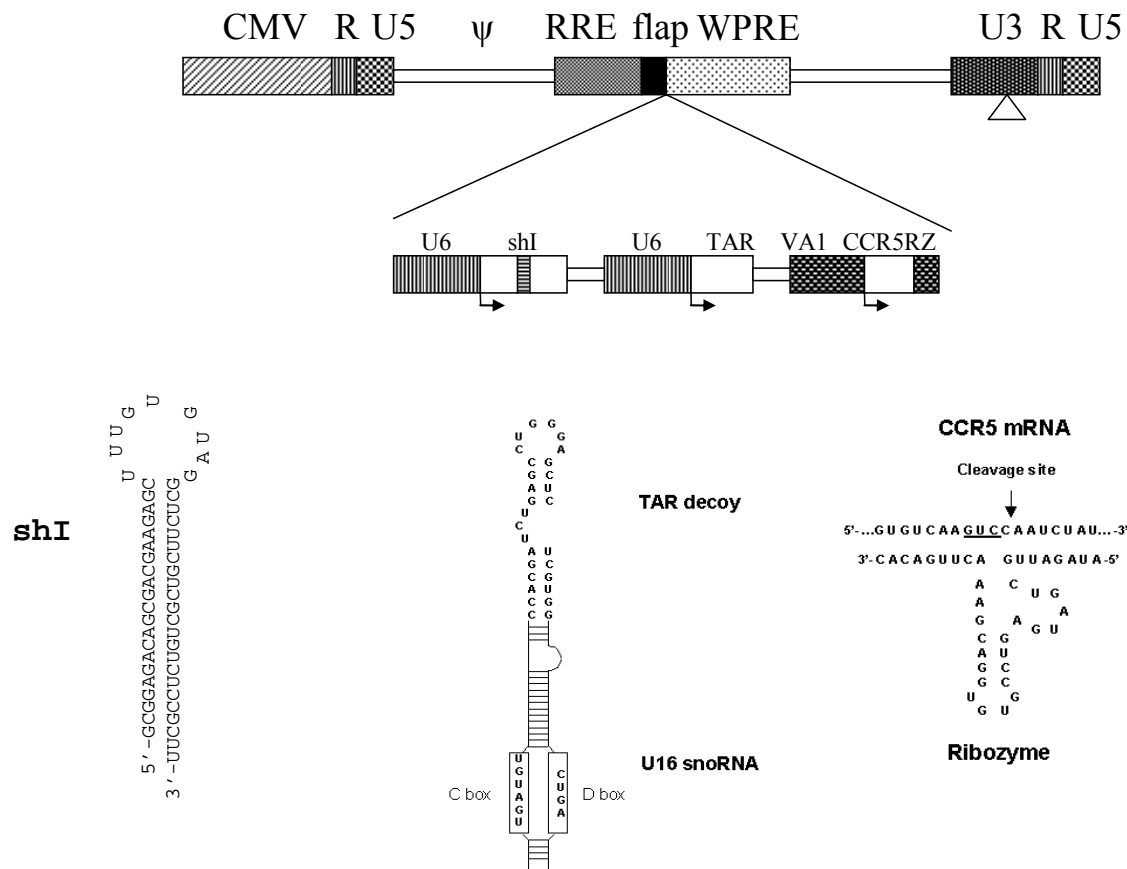
City of Hope Lentivirus Vector



Adapted from Yam et al. Mol Ther 2004

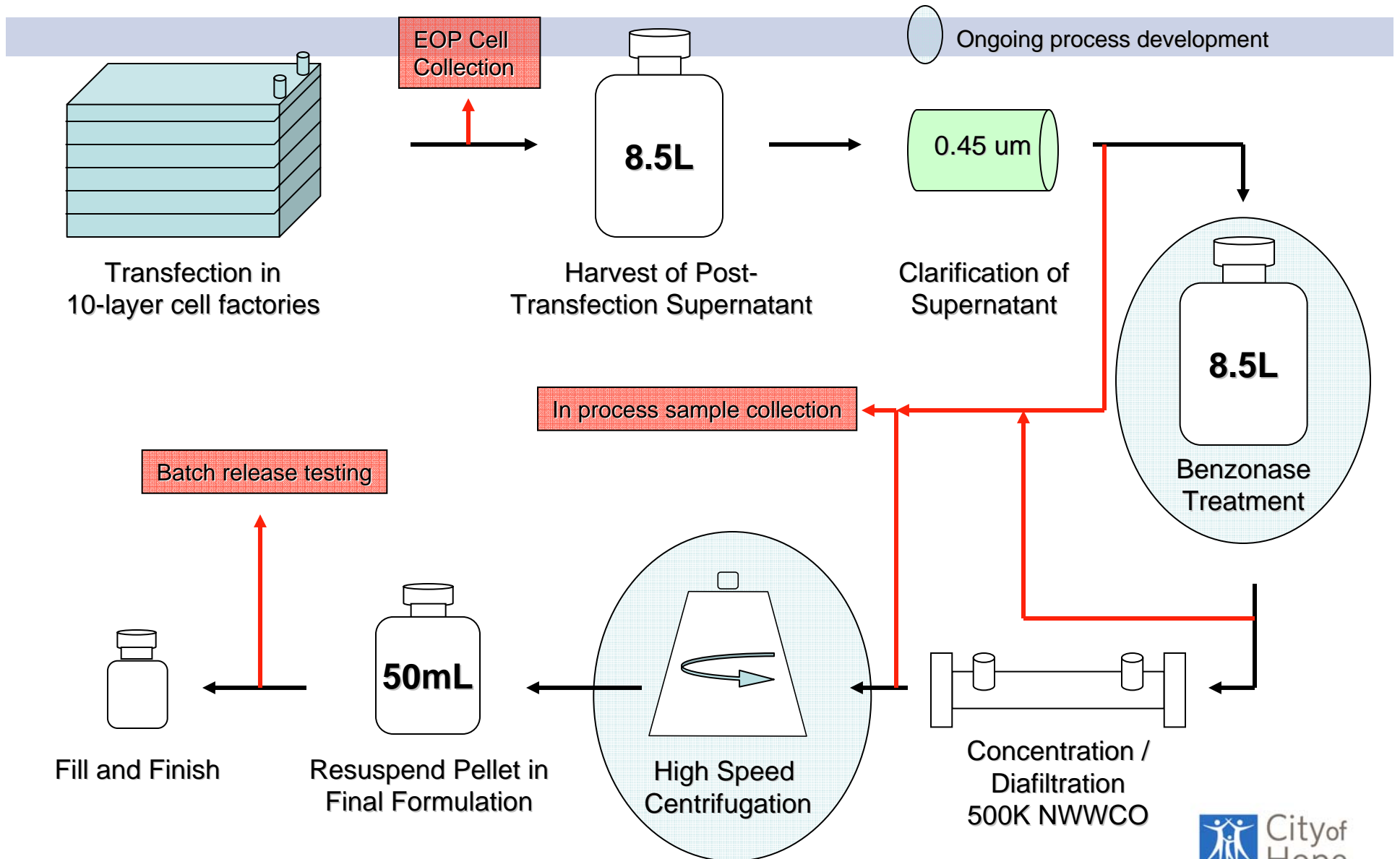
City of Hope Clinical Lentivirus Vector

rHIV7-shI-TAR-CCR5RZ



From M. Li et al Mol Ther 2005

Clinical grade triple vector production at COH

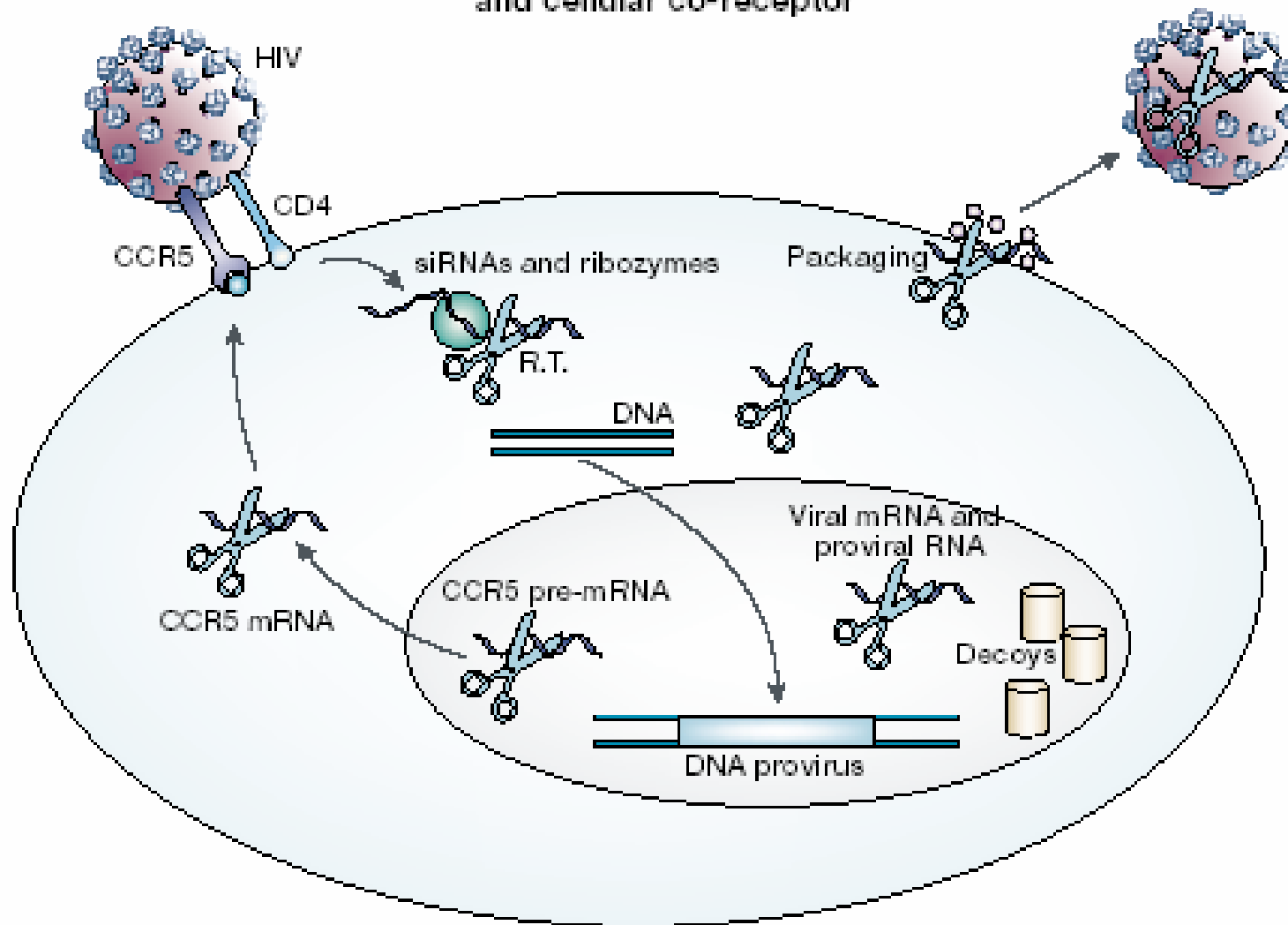


From L. Couture and D. Hsu



Figure 3: 15.04.03

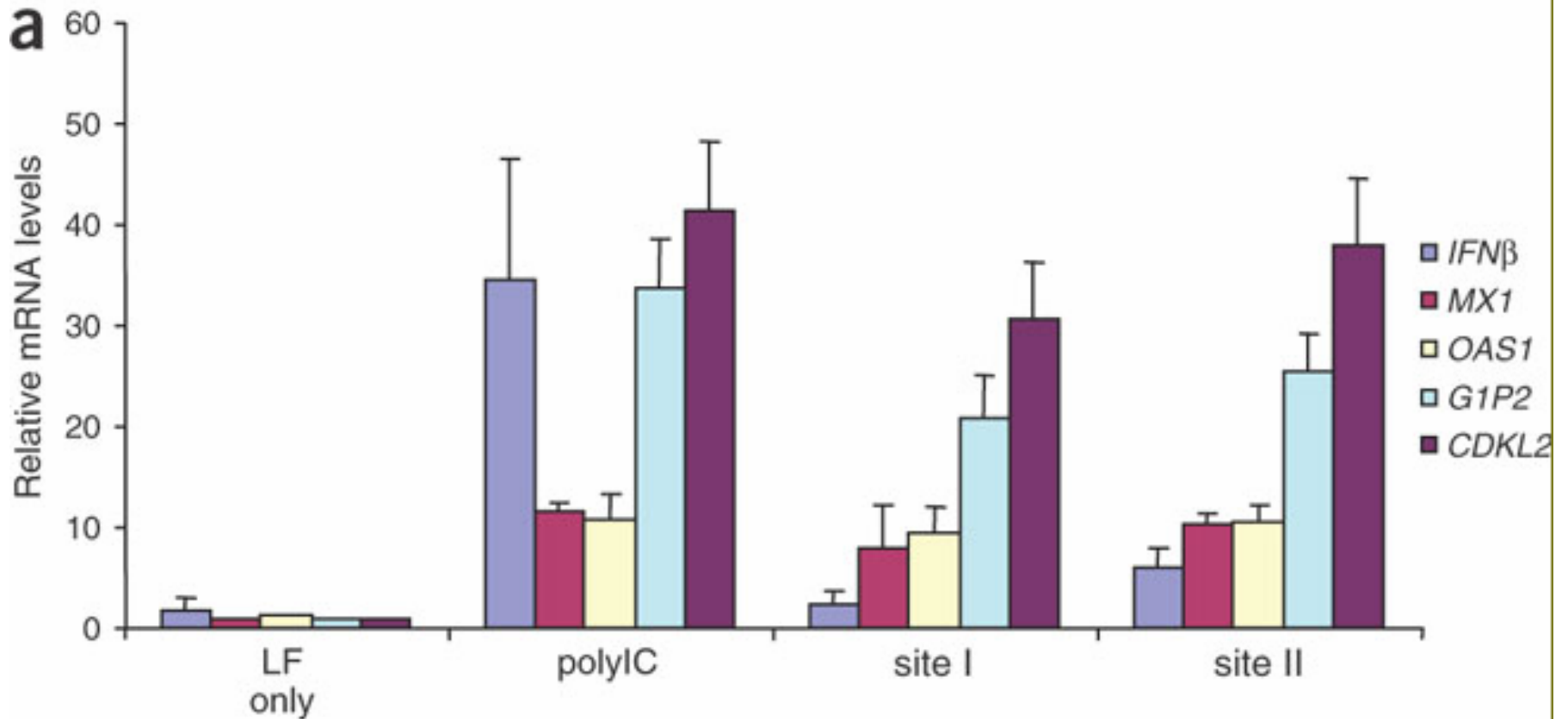
Combinatorial targeting of HIV-1 and cellular co-receptor



Preclinical testing: rHIV7-shI-TAR-CCR5rz

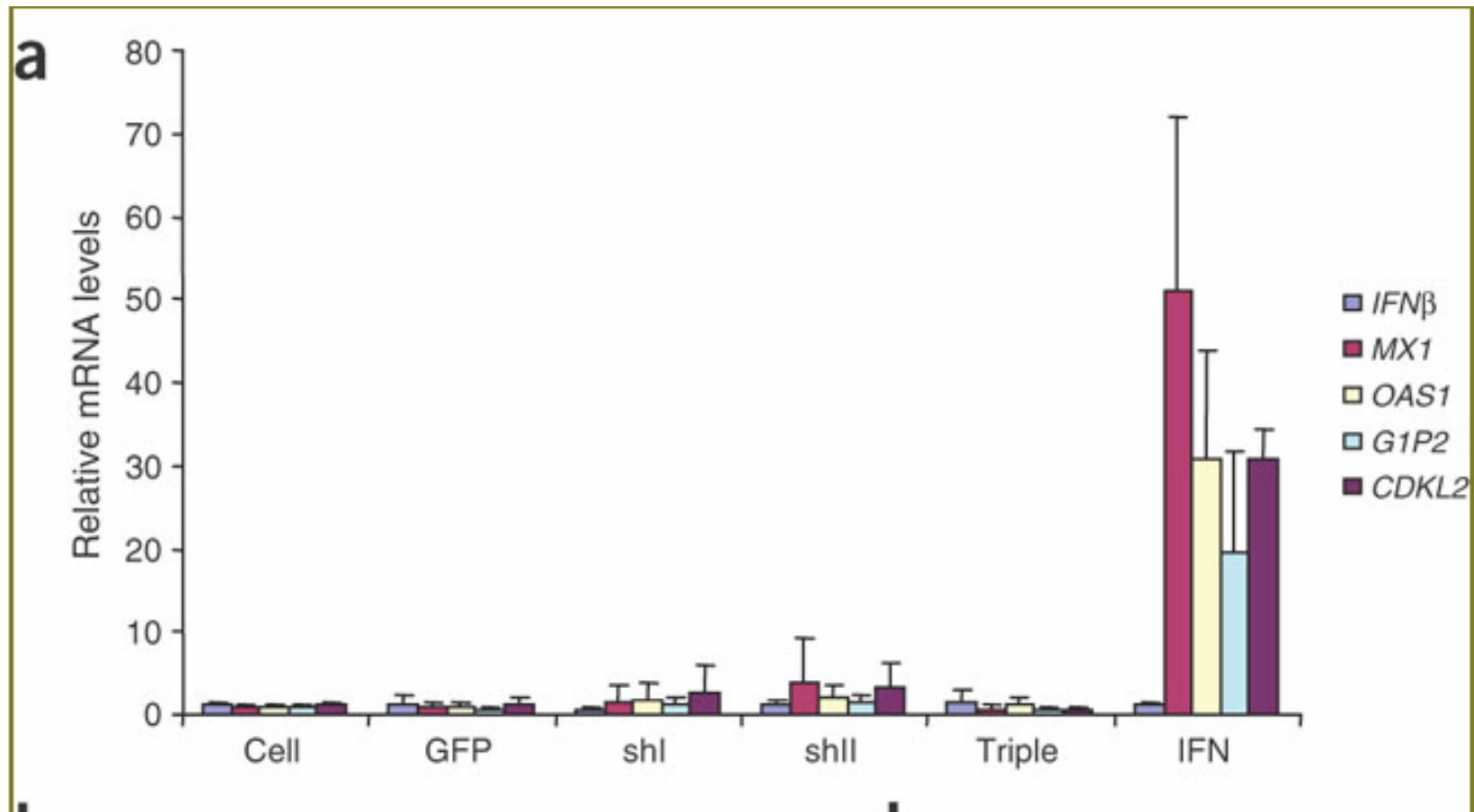
- **RCL free vector**
- **Absence of cell toxicity:**
 - CD34 differentiation in SCIDhu/thy mice
 - Absence of interferon pathway induction
 - Absence of disturbed miRNA array pattern
 - HIV-like integration pattern
- **Antiviral effect after cell differentiation to T cells**
- **Demonstration of intact integration element in transduced cells**

IFN pathway activation by synthetic siRNA in CD34+ cells



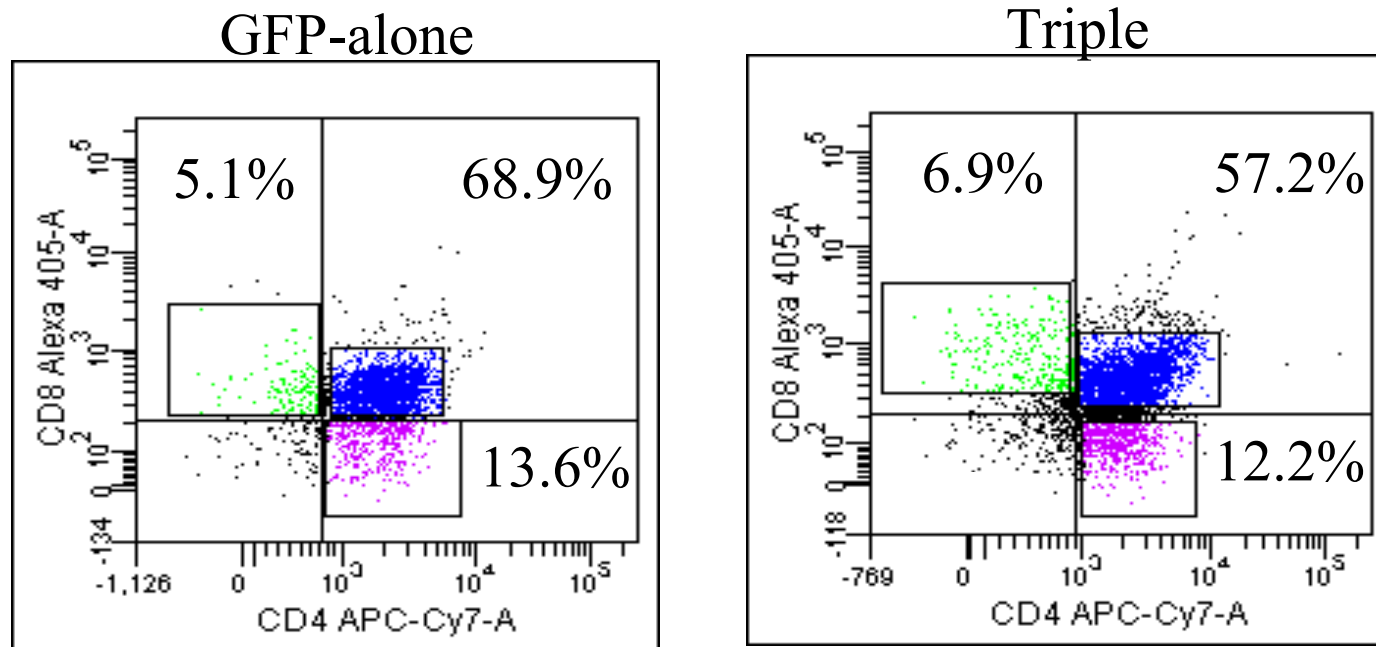
From Robbins et al. Nature Biotech 2006; 24: 566-71

No IFN pathway activation by transcribed siRNA in CD34+ cells



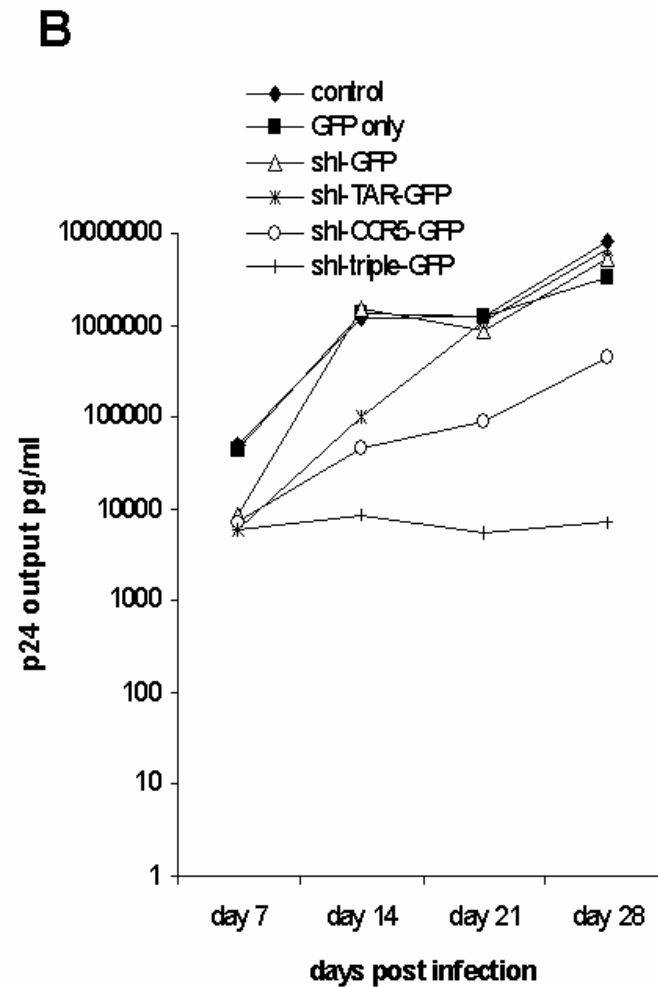
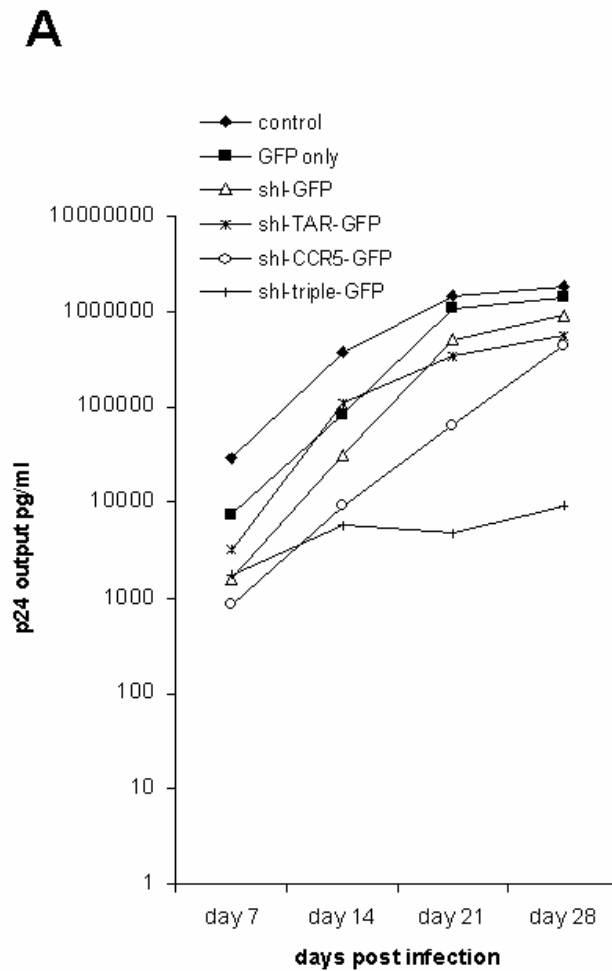
From Robbins et al. Nature Biotech 2006; 24: 566-71

Triple Vector Transgenic Stem Cells Expanded in SCID-hu Thymocytes

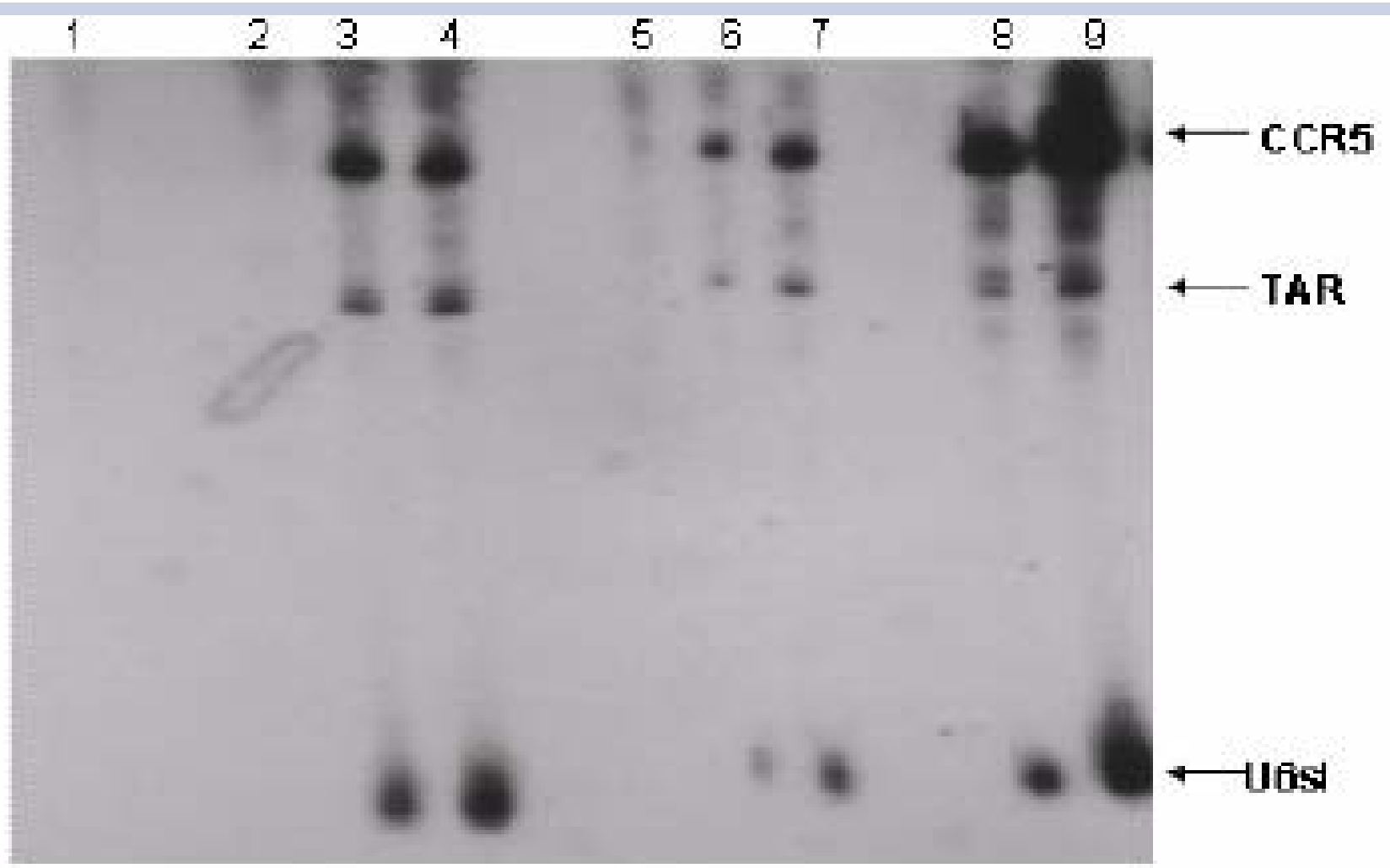


From Anderson et al. Mol Ther 2007; 15: 1182-88

Antiviral effect of triple anti-HIV RNAs



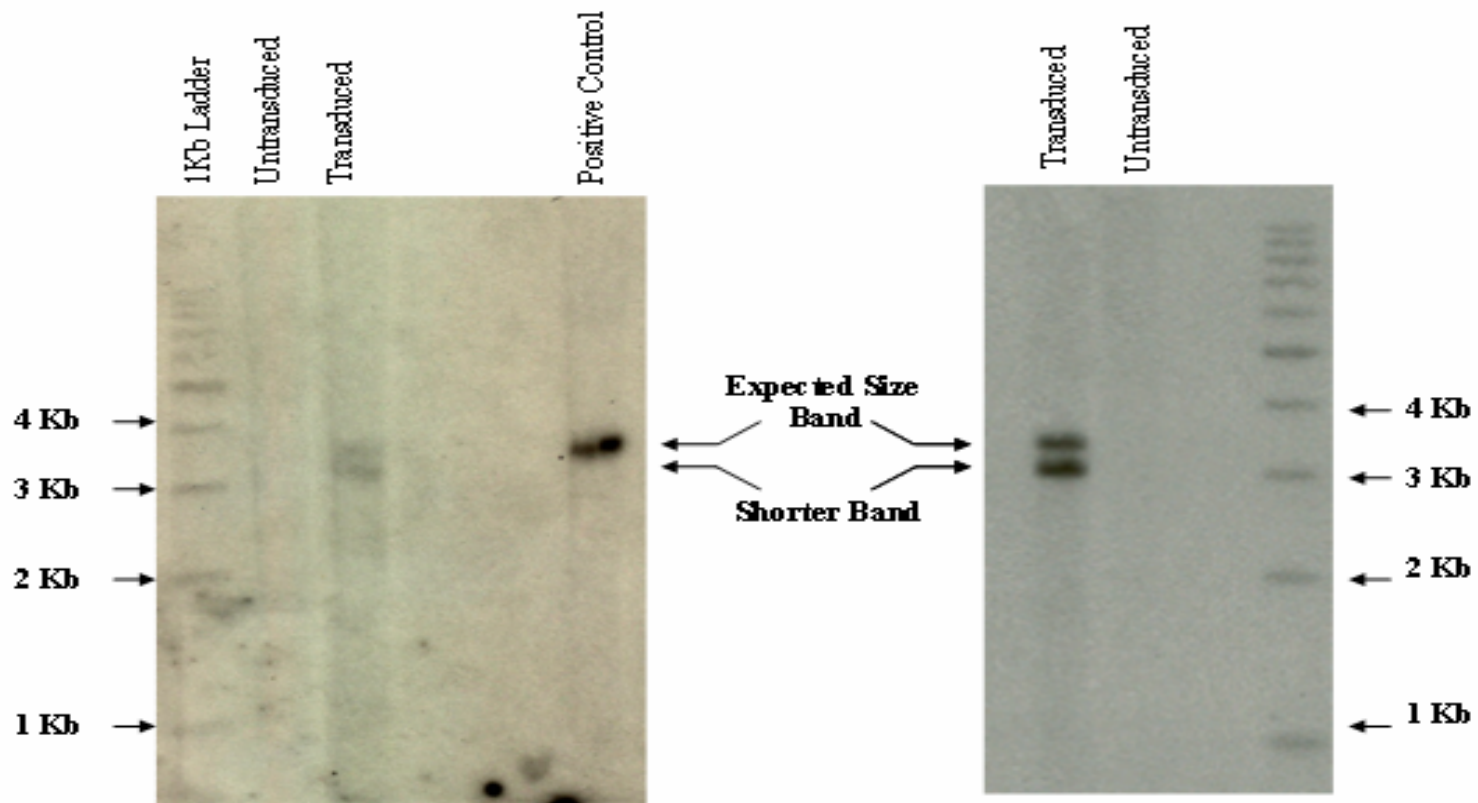
From M. Li et al Mol Ther 2005



From J. Rossi

Proviral integration pattern of triple vector

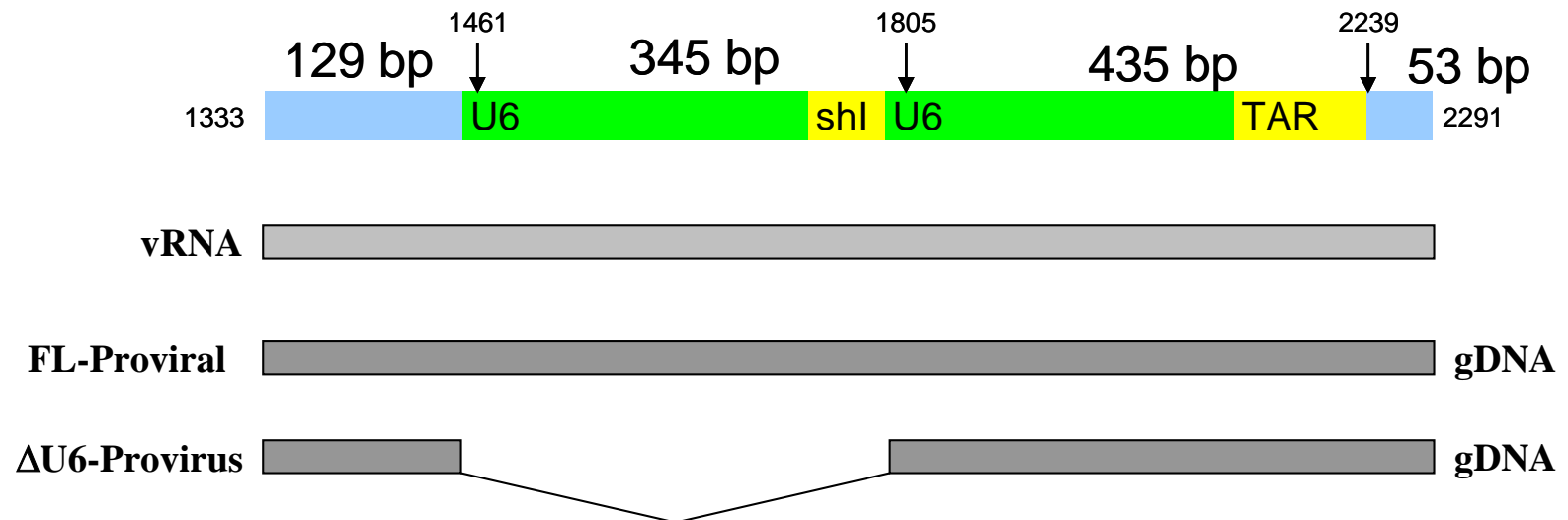
Detection of therapeutic insert in transduced HeLa cells by Southern probe



Integration pattern of triple vector

Schematic representation of full-length and deleted sequences.

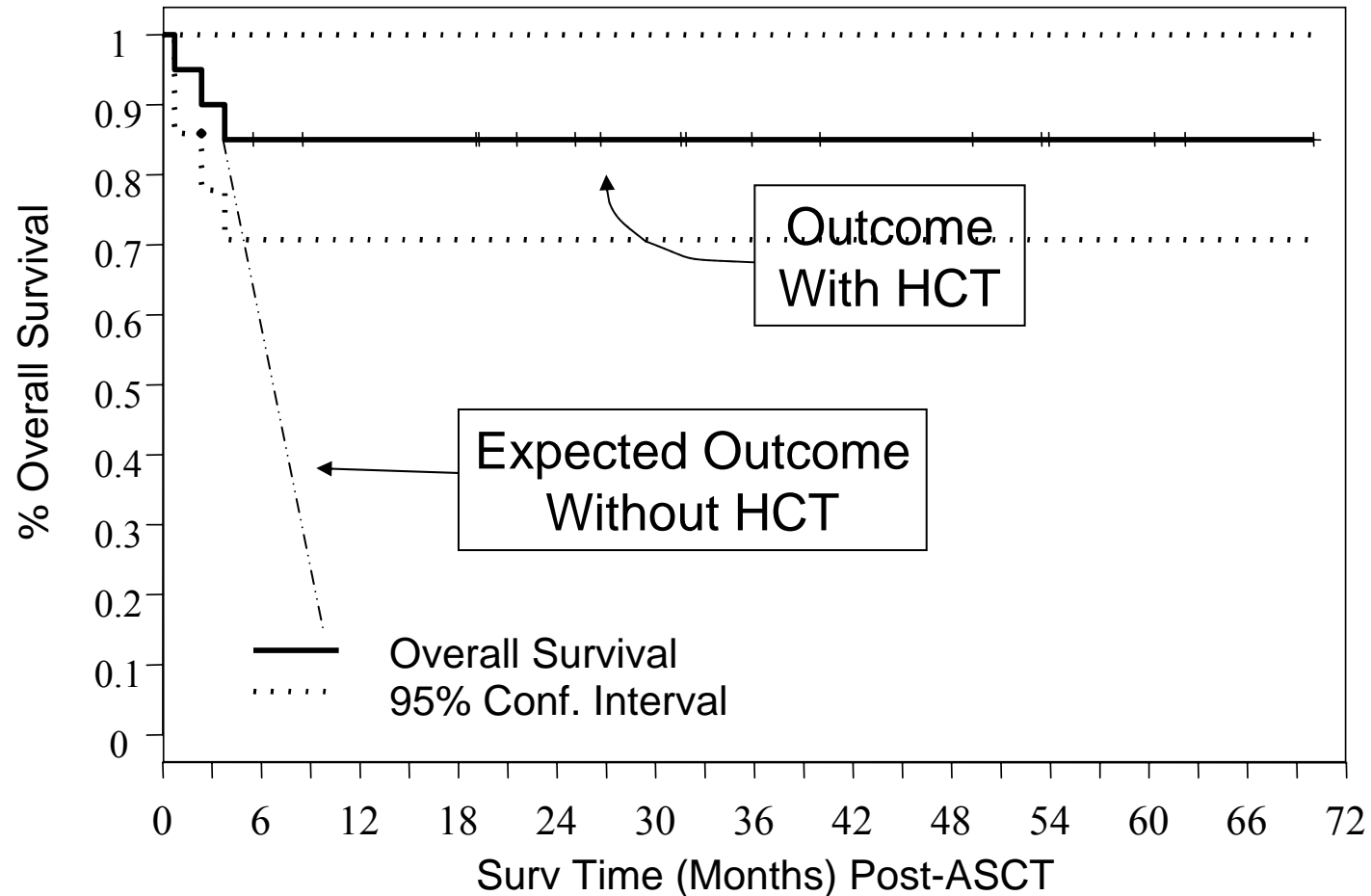
vRNA, viral RNA; FL, full-length; gDNA, genomic DNA; Δ U6, U6 deletion.



Summary of Preclinical Testing

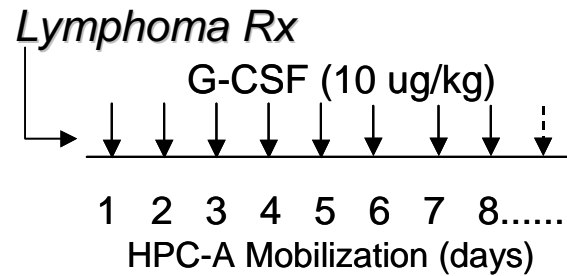
- Long term protection against HIV replication in primary CD34+ derived monocytes and macrophages (Li et al., Mol. Therapy 2005)
- Lack of immunogenicity and normal myeloid differentiation: (Robbins et al., Nature Biotech 2006)
- Normal thymic T-cell development and resistance to T-tropic HIV (Anderson, et al., Molecular Therapy, 2007)

Autologous HCT for High Risk AIDS Lymphoma: COH Experience



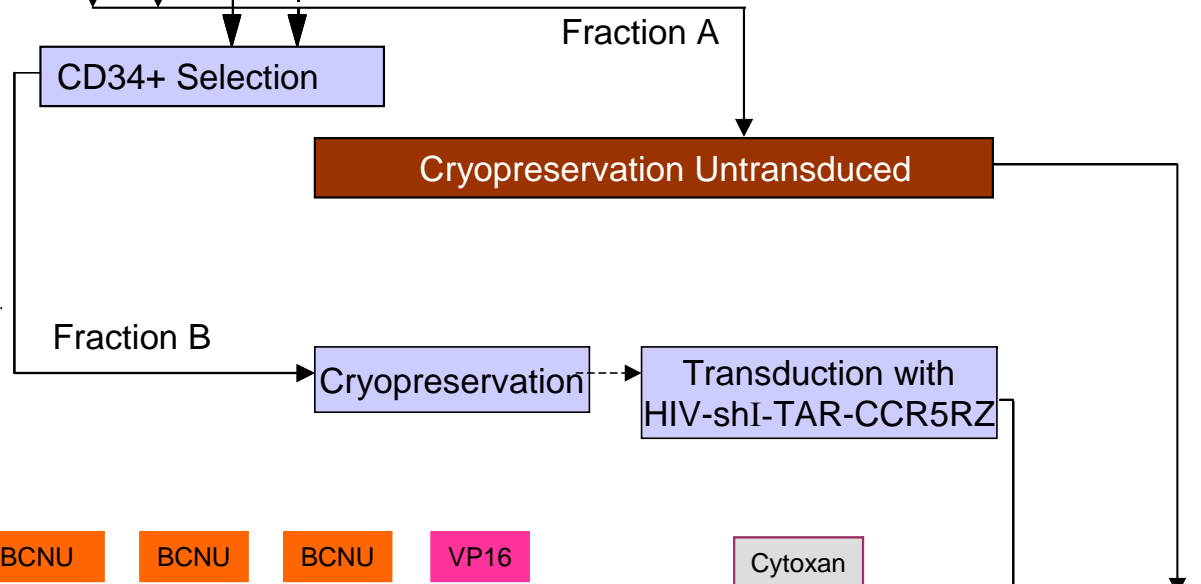
Modified from A. Krishnan et al. Blood 2005; 105:874-8

Study #1: AIDS Lymphoma



Aphereses

#1 #2 #3 #4



-7 -6 -5 -4 -3 -2 0 +1

Days Pre-and Post-transplant

Eligibility Criteria

- Age 18-60 years
- AIDS related lymphoma:
 - Intermediate grade or high grade non-Hodgkin's lymphoma (working formulations D-H and J), and \geq partial response or first relapse after remission with standard chemotherapy
 - Hodgkin's lymphoma any subtype except nodular L&H lymphocyte predominant, and partial response or less, or relapse after standard chemotherapy
- HIV load <50,000 gc/ml on anti-HIV chemotherapy, off AZT
- On appropriate prophylactic antibiotics, if CD4 <200/uL
- Organ functions consistent with routine transplantation screens

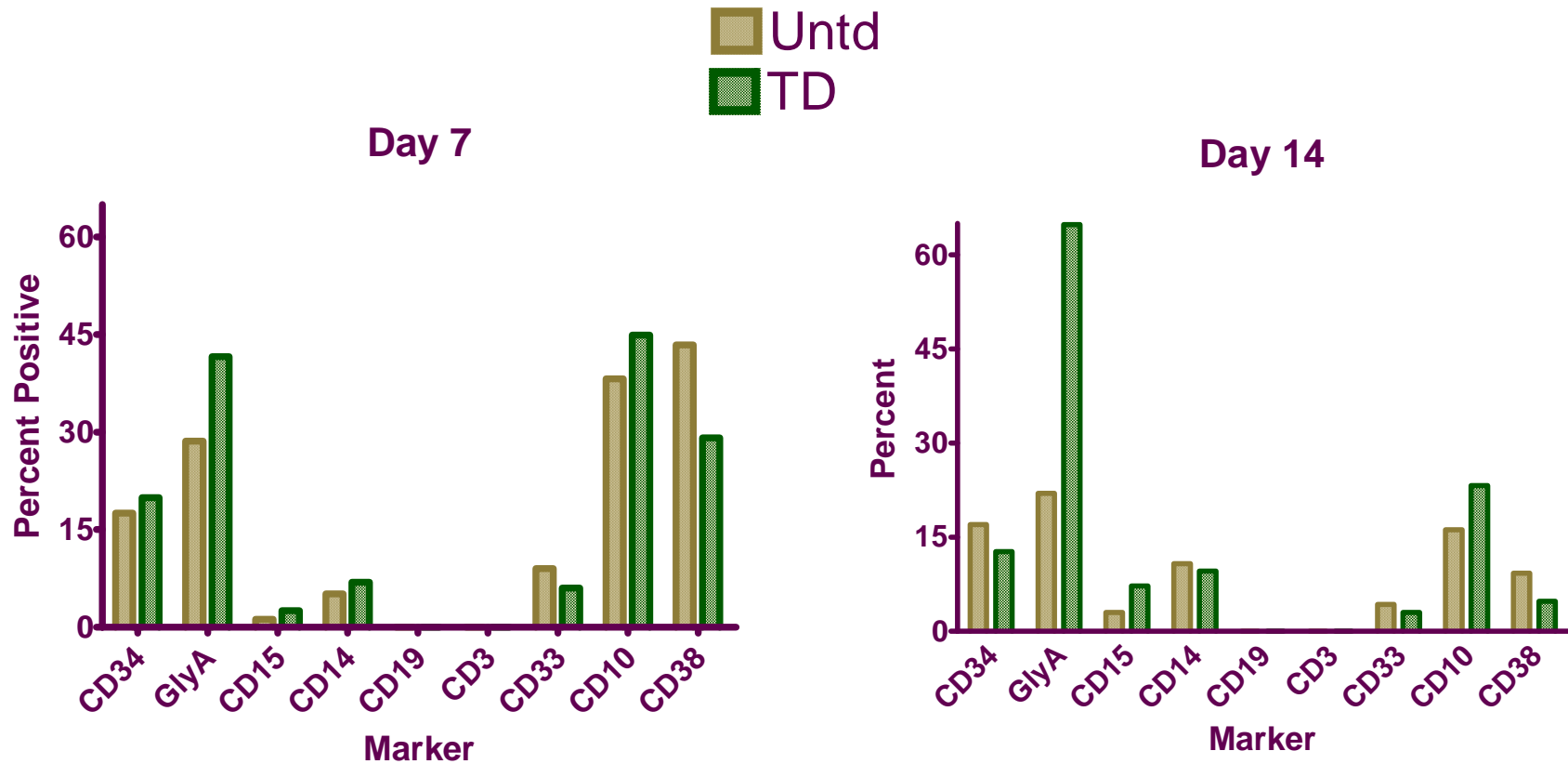
AIDS Lymphoma Study Recruitment

UPN #	Diagnosis	Status
0301	Diffuse Large B cell	Cell Product failed release
0302	Burkitt	Failed eligibility due to infection
0303	Burkitt	Failed eligibility due to low mobilization of PBPC
0304	Diffuse Large B cell	Transplanted Feb 19, 2008
0305	Diffuse Large B cell	Transplanted Mar 13, 2008

AIDS Lymphoma Study Recruitment

UPN #	Diagnosis	Status
0306	Plasmablastic lymphoma	Transplanted Aug 20, 2008
0307	Diffuse Large B cell	Oct 1, 2008

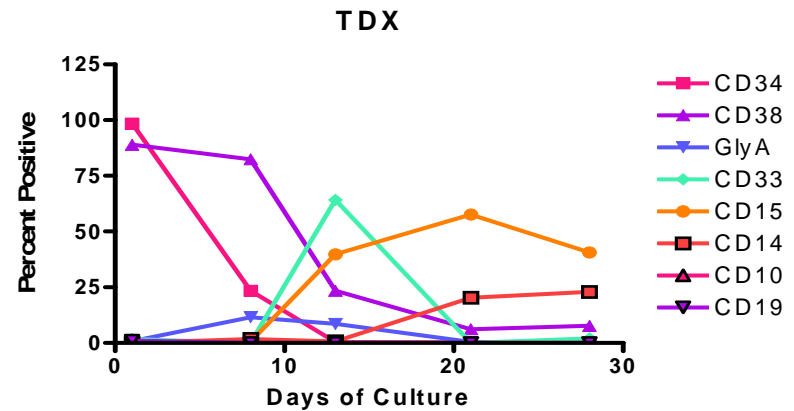
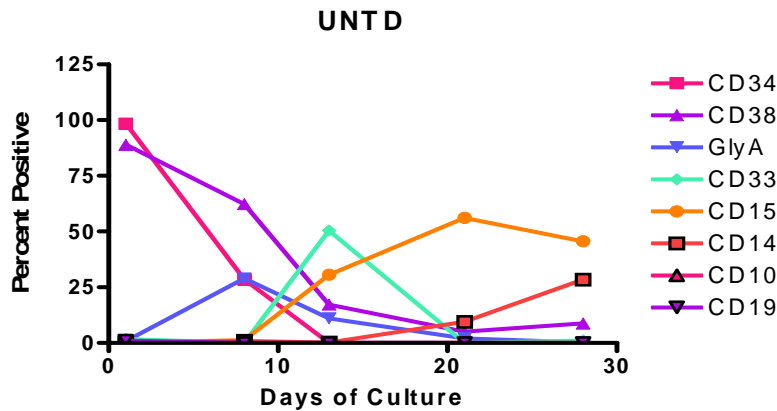
Phenotype of Liquid Culture CD34+ Cells



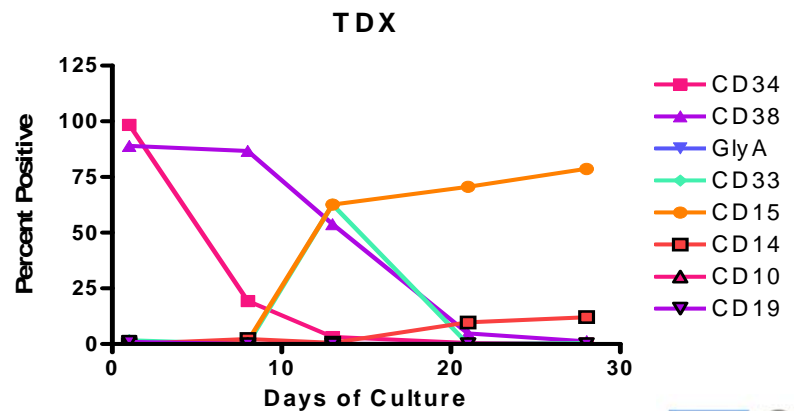
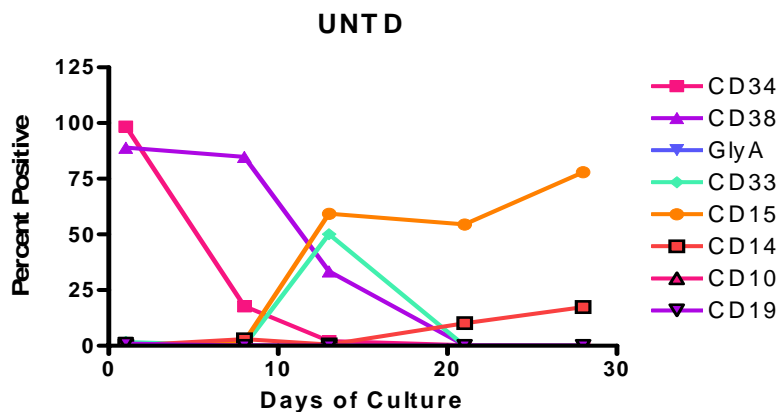
From D. DiGiusto and L. Li

Phenotype Kinetics from 28D Culture of CD34+ Cells

Liquid Culture

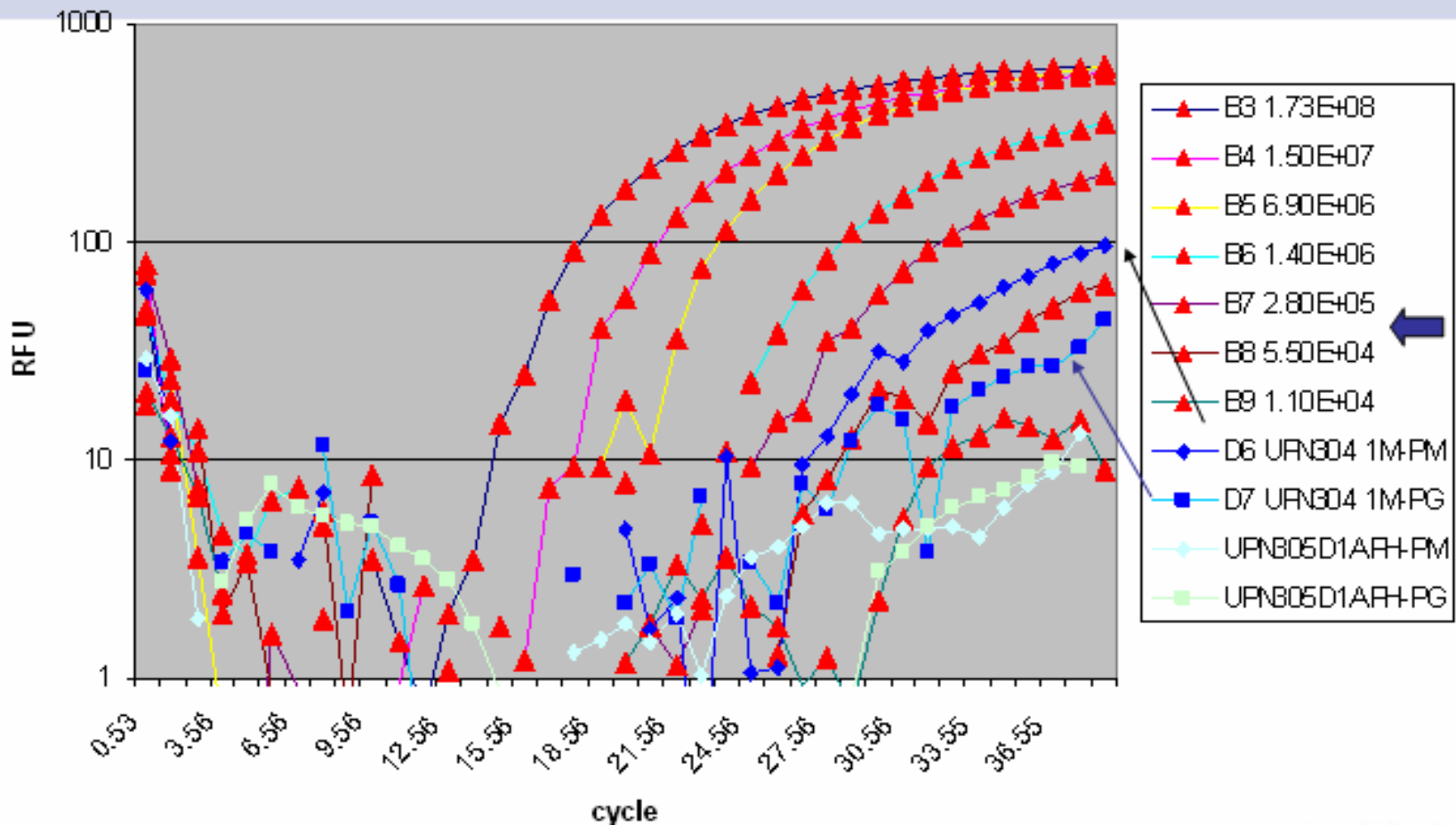


Stromal Culture



From D. DiGiusto and L. Li

qPCR assay for anti-tat/rev siRNA expression in patient cells: UPN0304



From J. Rossi and H. Li

Competition for Engraftment:

transduced: untransduced cells

- **$\geq 2.5 \times 10^6$ CD34/kg frozen unmanipulated**
- **$\sim 2.5 \times 10^6$ CD34/kg frozen & thawed for transduction**
- **Post-Miltenyi column, freeze thaw, transduction process yield = $\sim 50-60\%$**
- **Final CD34 infusion =**
 - 1 x 10^6 /kg transduced cells (1% long term transduction = 10^4 /kg)
 - 2.5 x 10^6 /kg unmanipulated cells
 - Ratio-- 1:250 = 0.4%

Outcome of Study is Pending

- Safe engraftment in 10 days in three patients
- Gene marking in blood: Results to be presented at ASH Annual Meeting, Dec 2008
- Enrollment has ended and patients continue to be followed

Trial highlights

- 1st human clinical trial using lentiviral vector transduction of HSCs.
- 1st human trial with expressed RNA interference trigger (shRNA).
- 1st triple gene therapy combination trial for HIV/AIDS.

Future Directions

- **Clinical approach**
 1. Improve PBPC collection numbers
 2. Use only transduced CD34 in HCT
 3. Consider AMD3100 mobilization
 4. Use maraviroc during and after HCT
- **Technical improvements**
 1. Use fresh CD34 for transduction
 2. Alter transduction conditions
 3. Modify vector: modify U6 promoters; envelope
- **Operational improvements**

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NIAID; NHLBI;
NCI; NCRR

Benitec Ltd

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Li, Lijing
Li, Shirley
Meisenzahl, Rose
Mi, Shu
Potter, Barbara
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Reed, Ken
Rossi, John
Shad, Yasmine
Smith, Eileen
Snyder, David
Stillings-Farris, Amy
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Stan, Rodica
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Wardlow, Michelle
Wang, Sean
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