

ASX/Media Release

Dr Zaia presents Update on HIV lymphoma study at ASGT Conference

6 June 2008, Melbourne, Australia: Dr John A Zaia presented, over the weekend, an update on the human pilot HIV lymphoma stem cell study at the 11th Annual Meeting of the American Society of Gene Therapy held May 28-June 1, 2008 in Boston, Massachusetts.

His talk was entitled "Gene Therapy Approaches for Treatment of HIV/AIDS: Current Status".

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. This study includes the use of Benitec's technology as a clinical method to fight HIV-AIDS infection. In this ground-breaking pilot-clinical study patients with AIDS-related lymphoma are being treated using vector expressed RNAi aimed at rendering the cells resistant to the HIV-1 virus infection.

This study entitled "A 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 RNA's" commenced in Q3 2007.

In his update Dr Zaia presented 60 day data available on the first two patients in the current study. This is still early data however patients are doing well after transplant and pleasingly the gene markers are detectable in these patients. Safe engraftment was seen at 10 days.

This is early data. However if it can be confirmed, then treatment with gene therapy early after HIV infection may be justified, and, if such treatment delayed the need for antiviral chemotherapy, gene transfer would likely become an important strategy for treatment of HIV infection.

A copy of Dr Zaia's presentation is attached.

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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. For additional information, please visit www.benitec.com.

Gene Therapy Approaches for Treatment of HIV/AIDS: Current Status

John A. Zaia

May 31, 2008

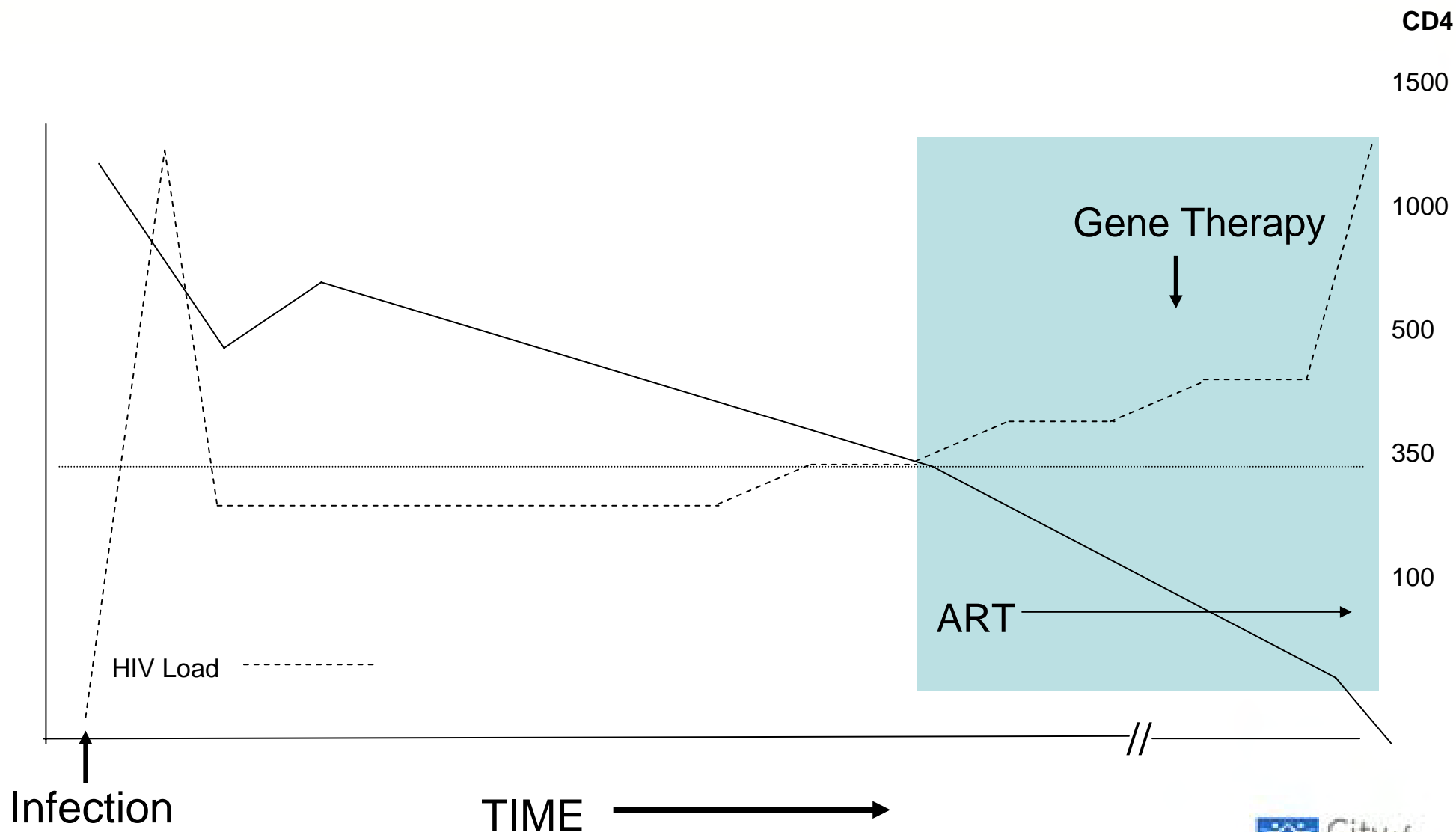
Significant questions

- What is the best strategy for applying gene transfer for control of HIV/AIDS?
- Is there an antiviral gene (or genes) that can 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 ?
- How promising are lentivirus vectors for gene delivery?
- What is the best method of delivery of this gene?
 - T cell transduction and expansion
 - Blood stem cell transduction and transplantation

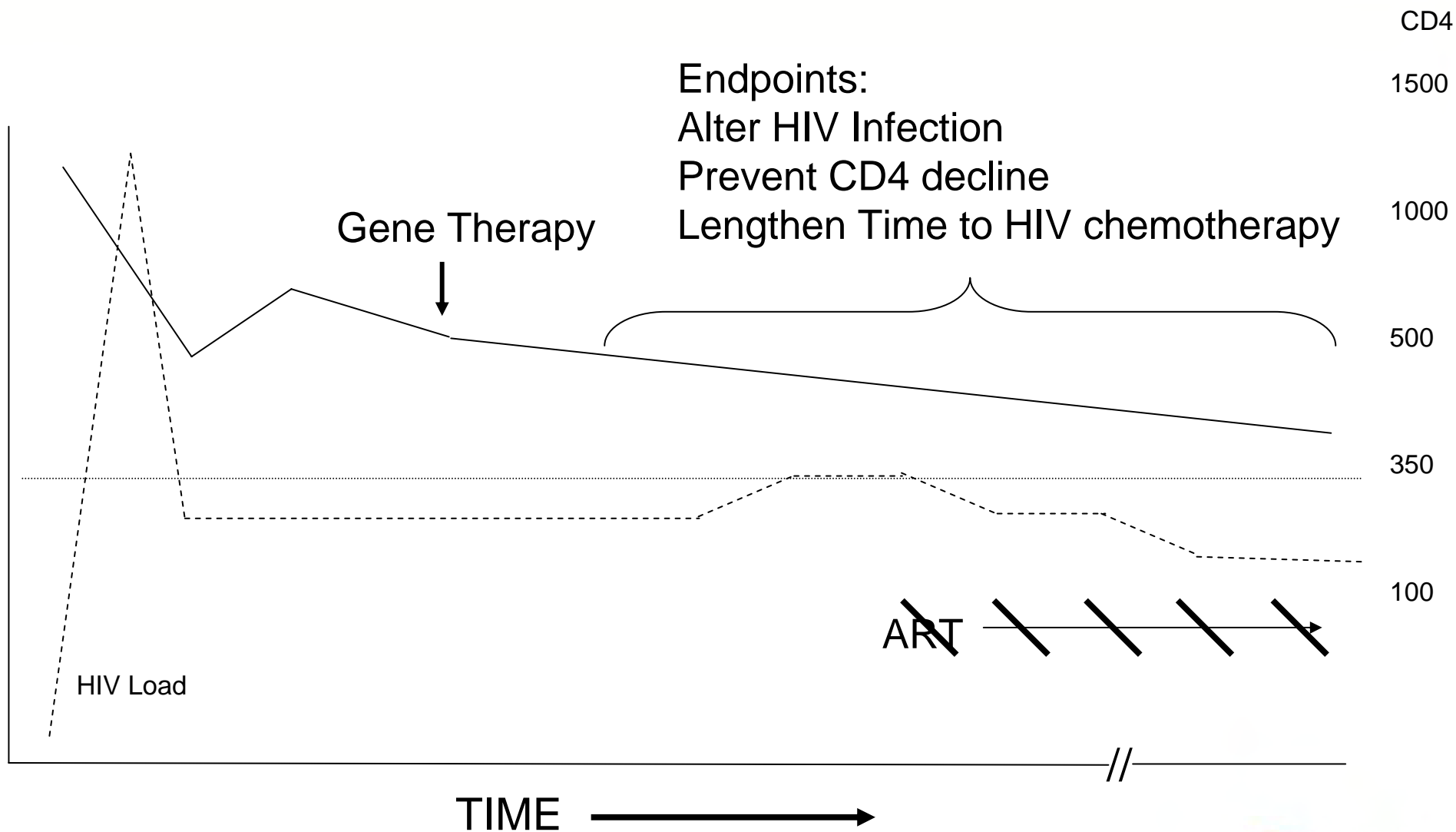
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Natural History of AIDS: Rationale for gene rx



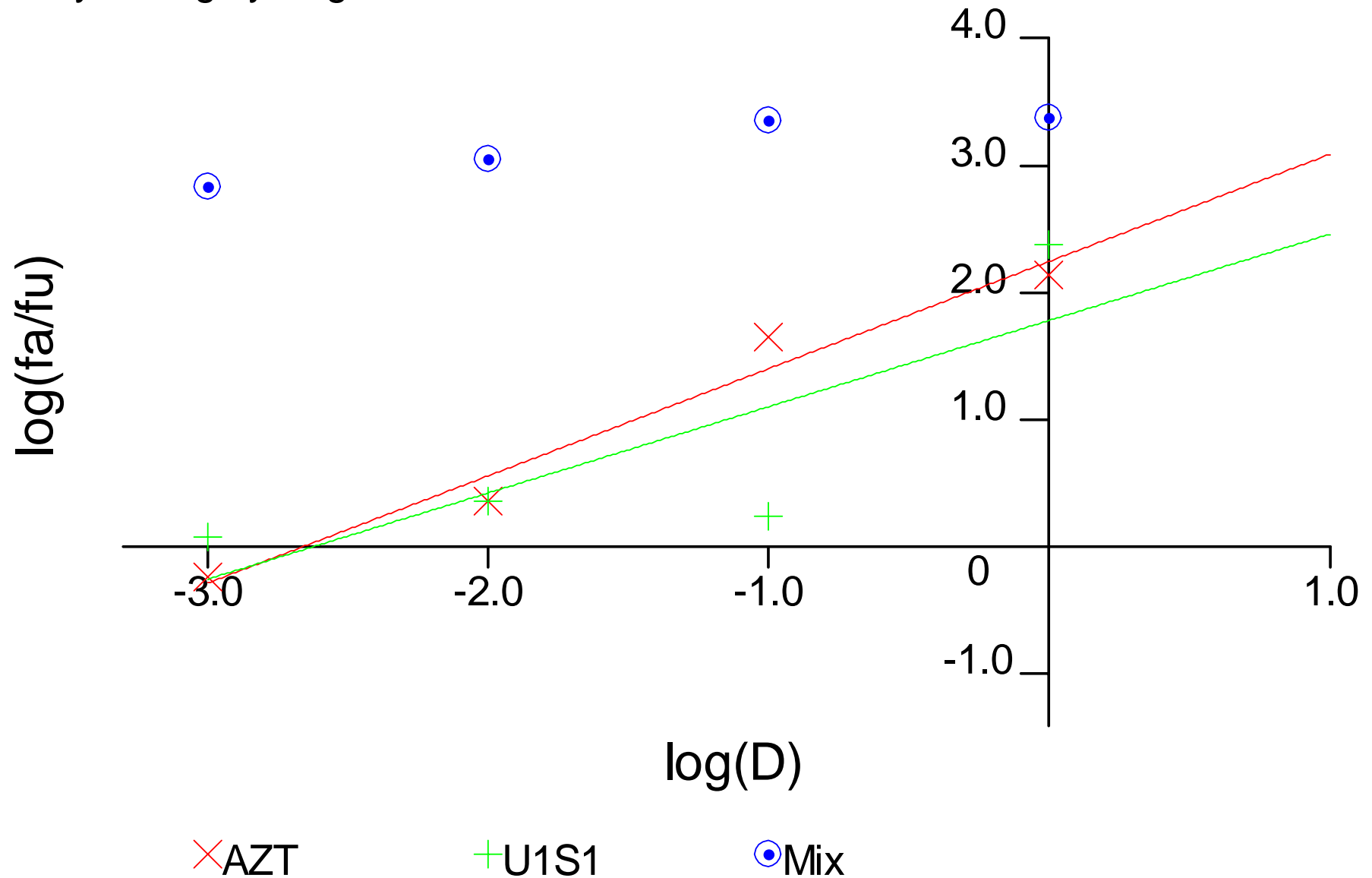
Clinical Strategy



AZT / U1S1

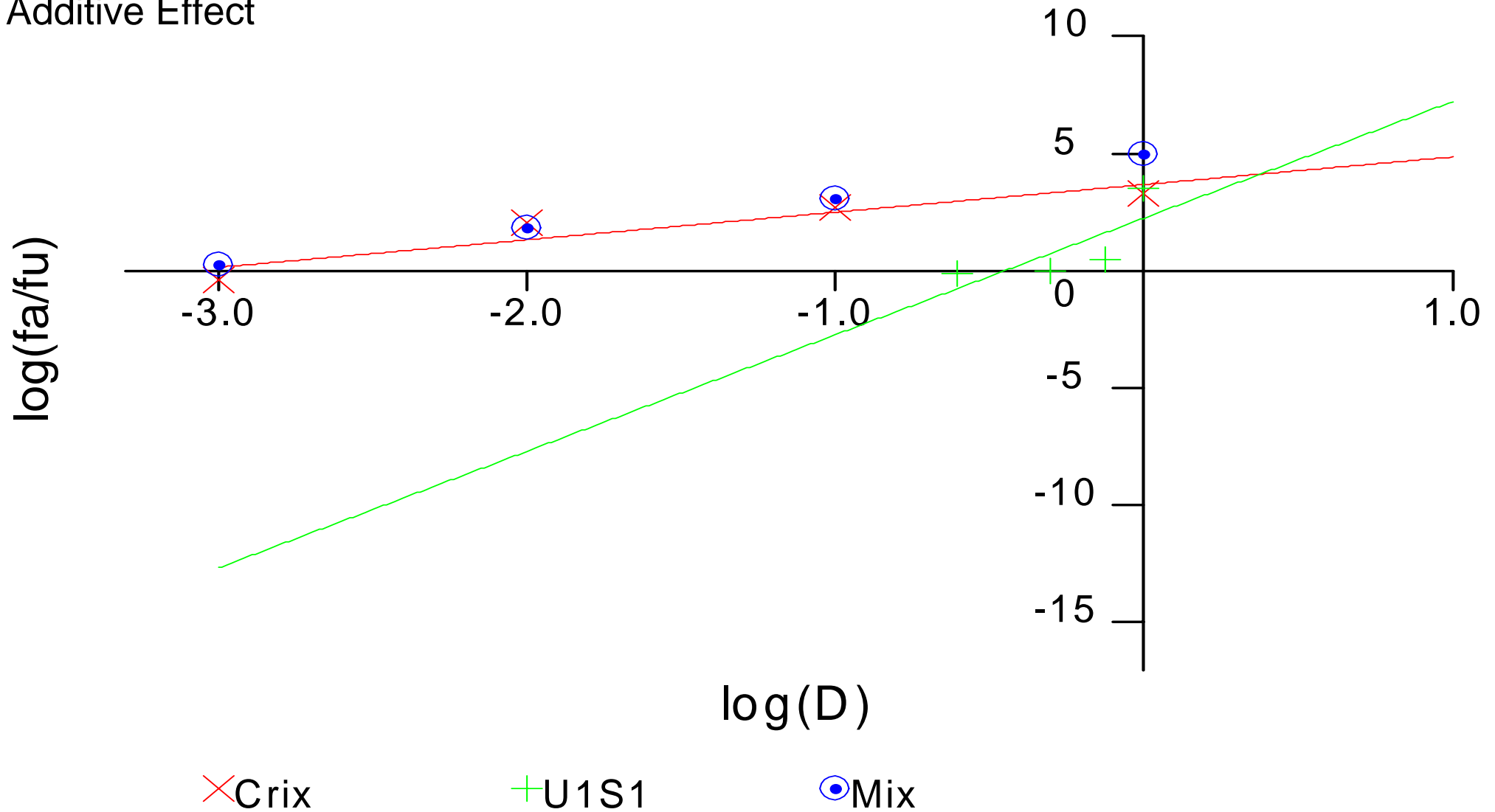
Very strong synergism

Median-effect plot



Median-effect plot

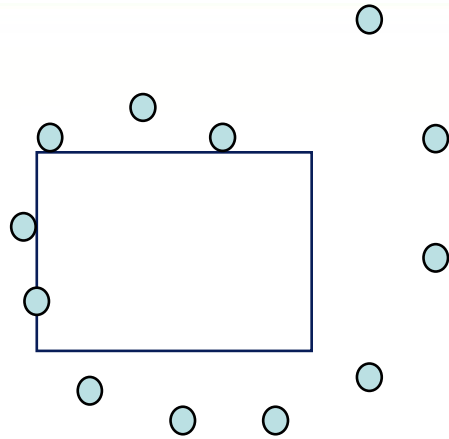
Crix / U1S1
Additive Effect



Significant issues

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HIV Targets for Gene Therapy



Protein Based:

Transdominant Mutants:

Rev; Tat; Gag; Env

Ig Monobodies to Tat, Env, Rev,

Gag, IN, RT

KDEL-signal retention in ER

Toxins: bacterial, viral, IFN

CD8 TCR zeta-Ig hybrid

RNA Based:

Antisense to LTR, TAR, tat, env,
rev, gag

Decoy RNA using TAR, RRE

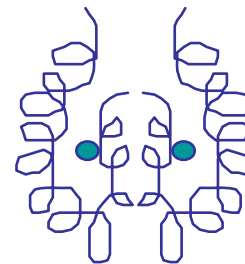
Ribozymes

hammerhead

hairpin

RNAi

miRNA

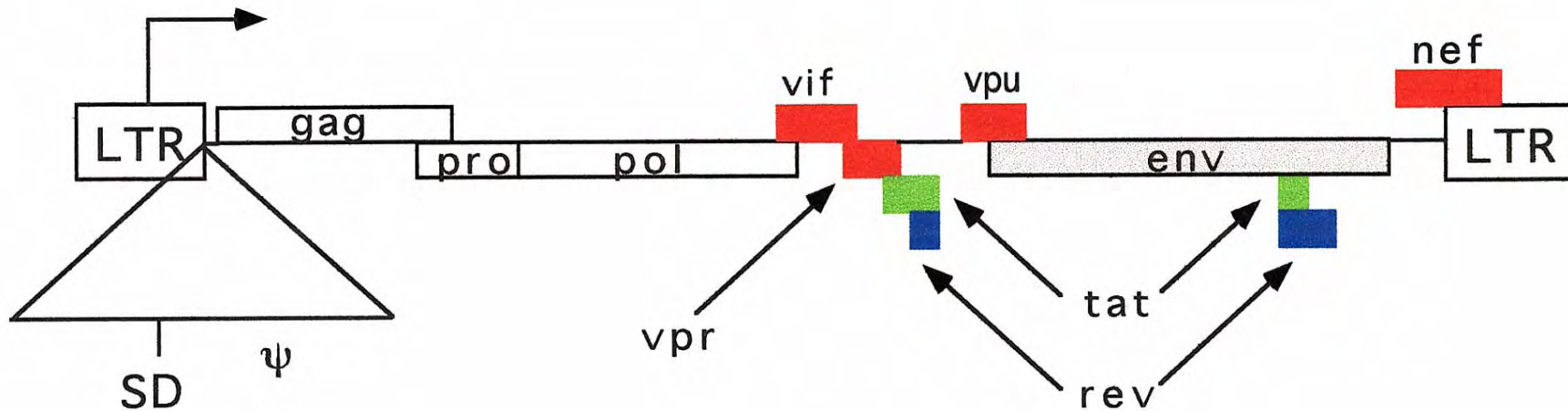


From Zaia, Cairns, Rossi, Chapter 100 in Blume, Forman, Applebaum, Thomas' Hematopoietic Cell Transplantation, Third Edition, Blackwell Scientific Publications, Boston, 2003

Significant issues

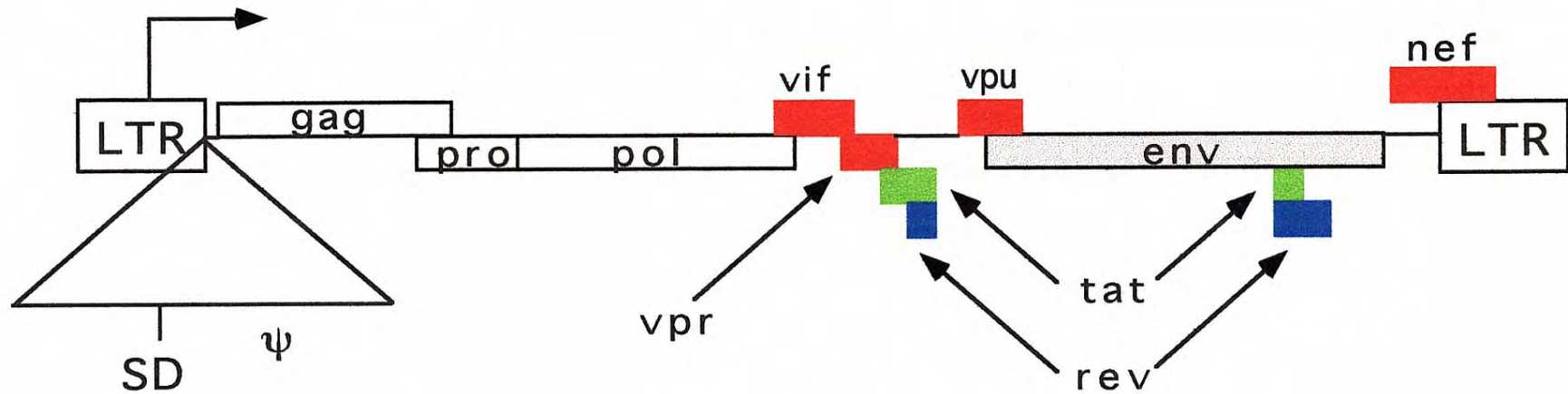
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From HIV-1 to Lentivirus Vector



- 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”

VirXsys & City of Hope Lentivirus Vectors



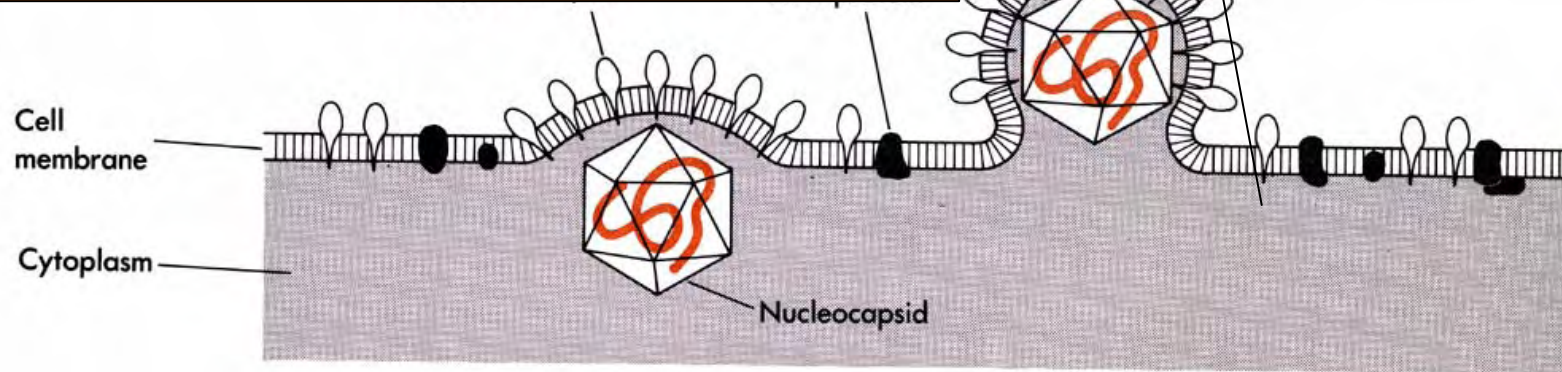
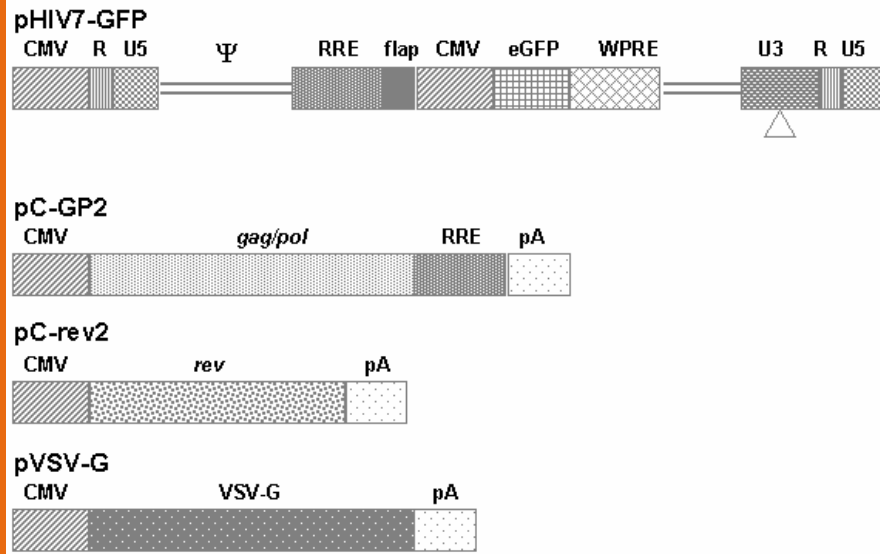
Adapted from Lu et al. J Gene Med 2004



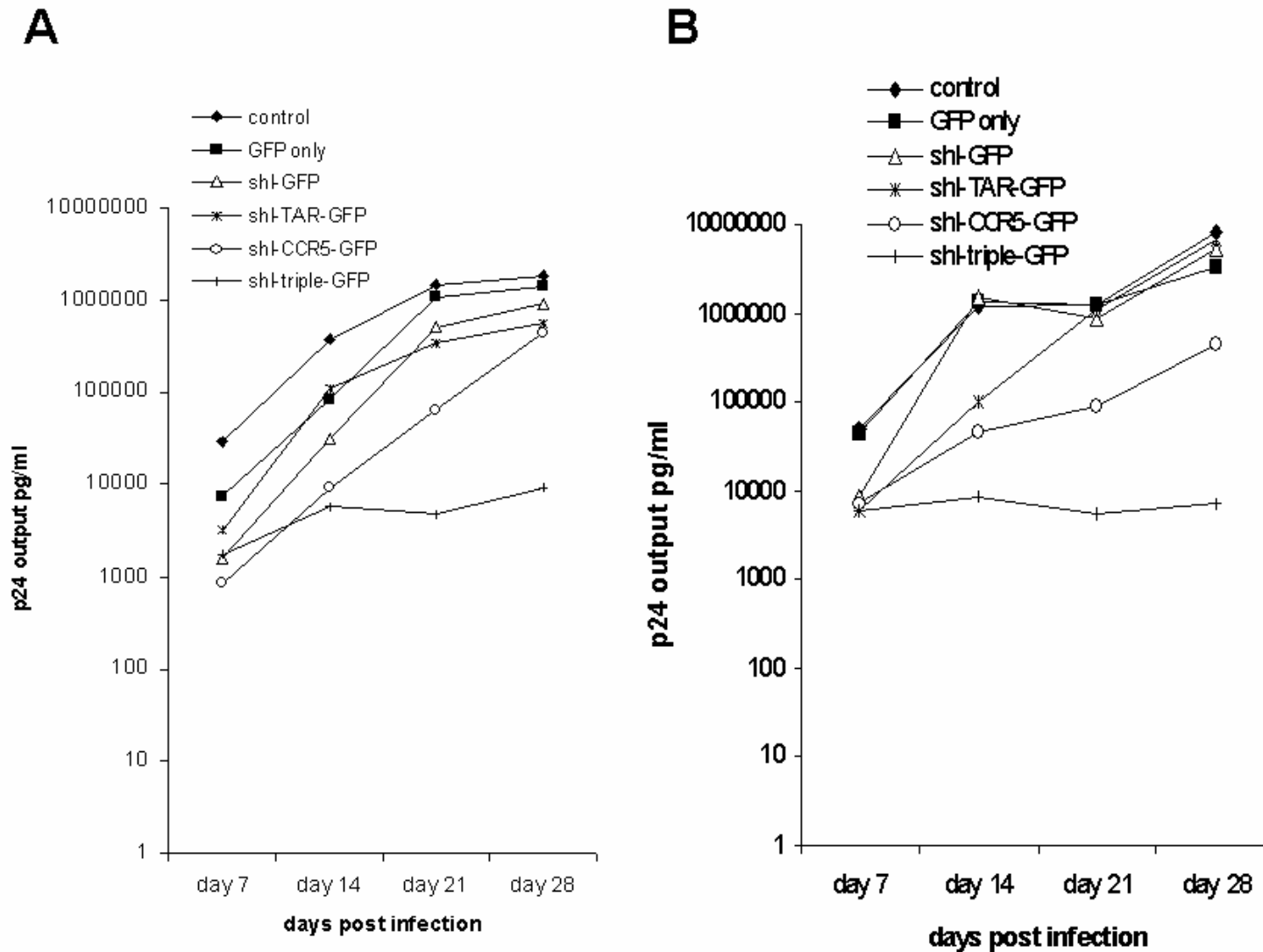
Adapted from Yam et al. Mol Ther 2004

4-plasmid system of lentivirus vector production

DNA Plasmids

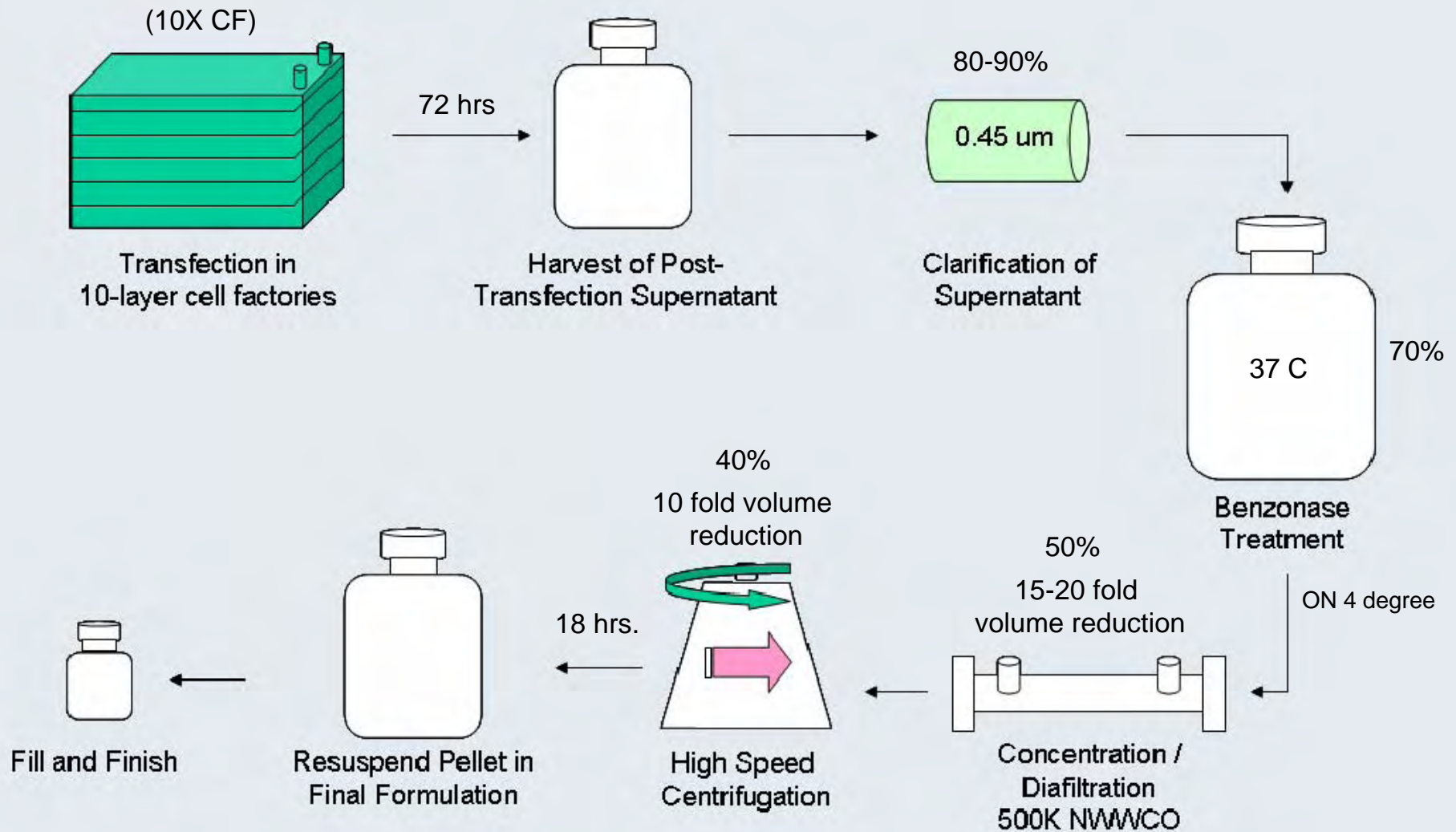


Antiviral effect of triple anti-HIV RNAs



From M. Li et al Mol Ther 2005

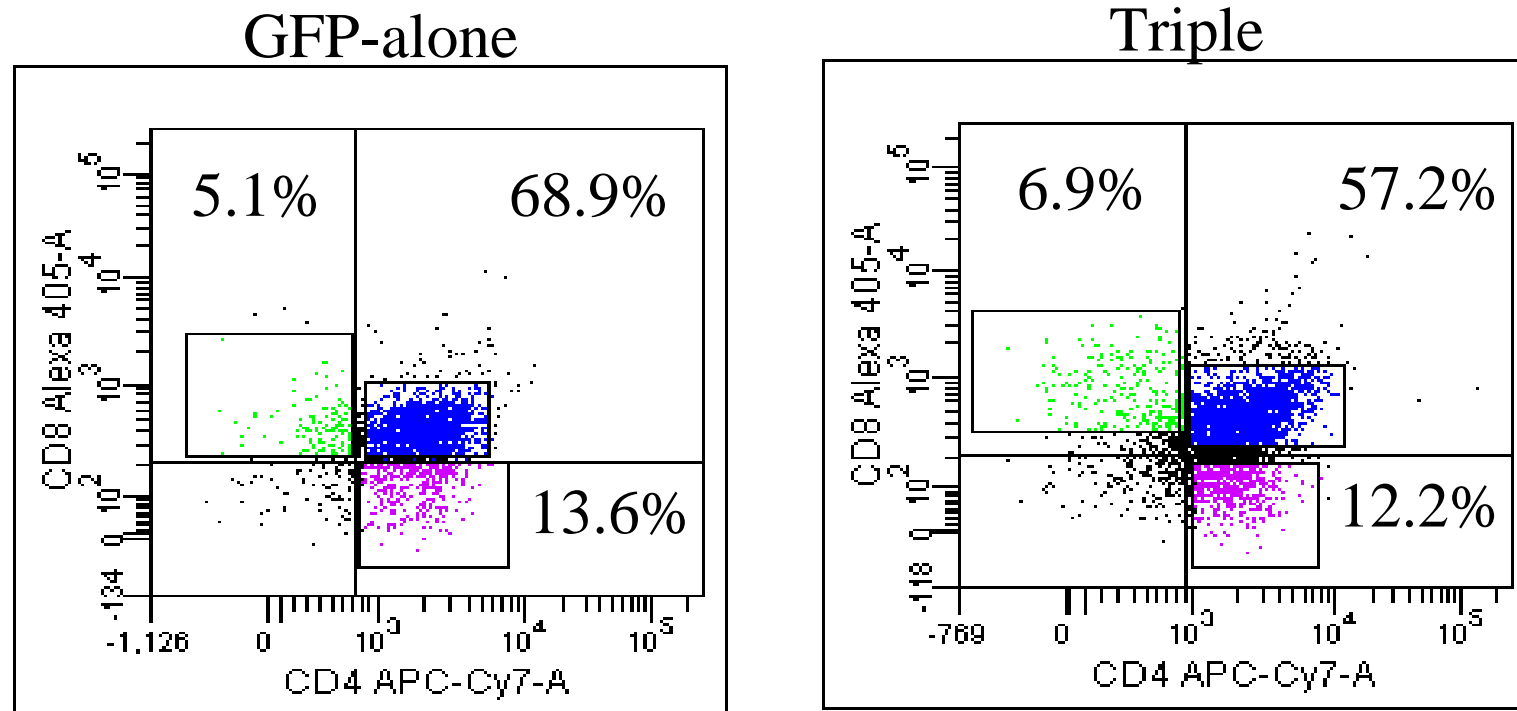
Downstream sub-batch Lentivirus processing



Preclinical testing: rHIV7-shI-TAR-CCR5rz

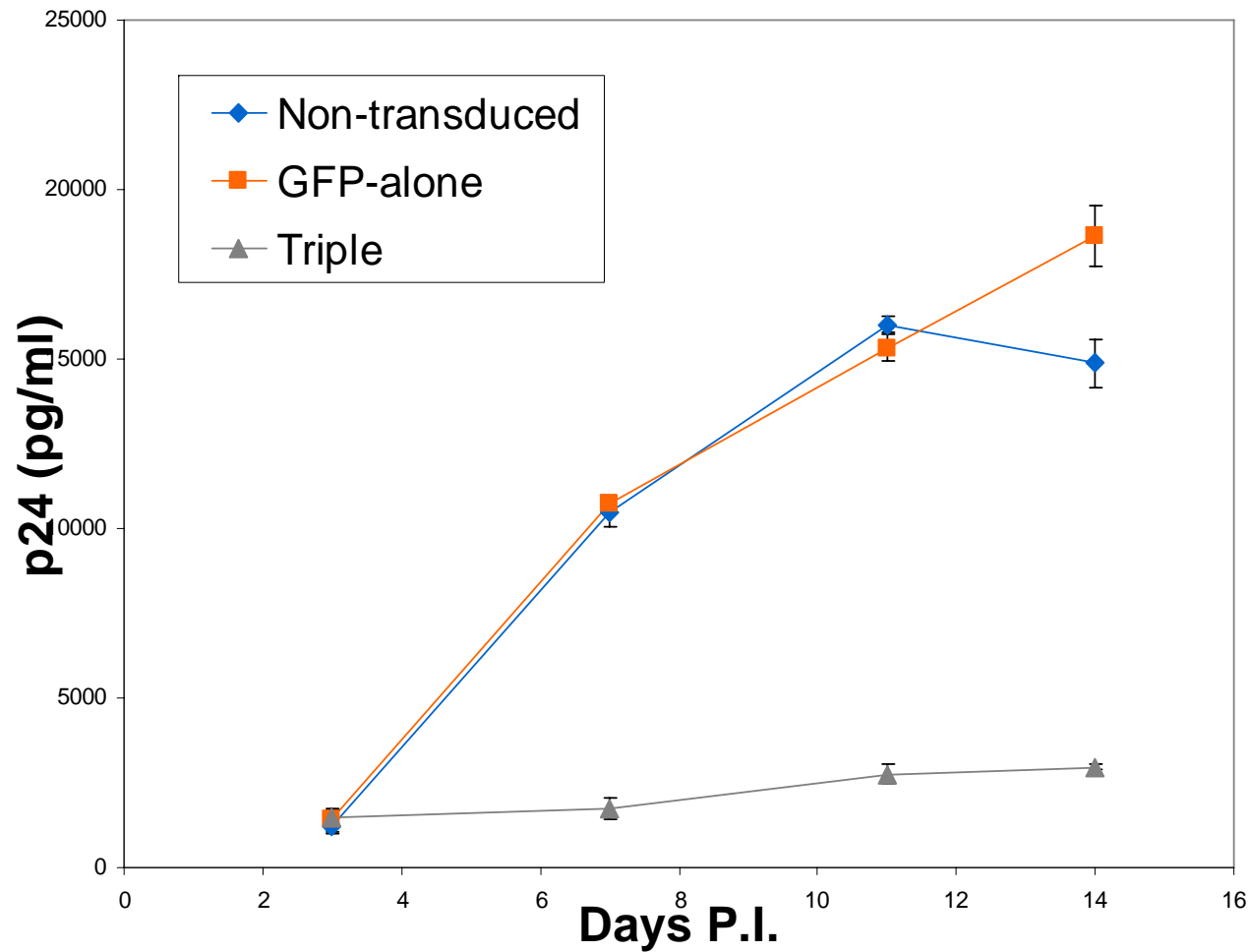
- RCL free vector
- Absence of cell toxicity:
 - CD34 differentiation in SCIDhu/thy mice
 - Absence of interferon pathway induction
 - Absence of disturbed miRNA array pattern
 - HIV-like integration pattern
- Antiviral effect after cell differentiation to T cells
- Demonstration of intact integration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

Triple Vector Transgenic Stem Cells Expanded in SCID-hu Thymocytes



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

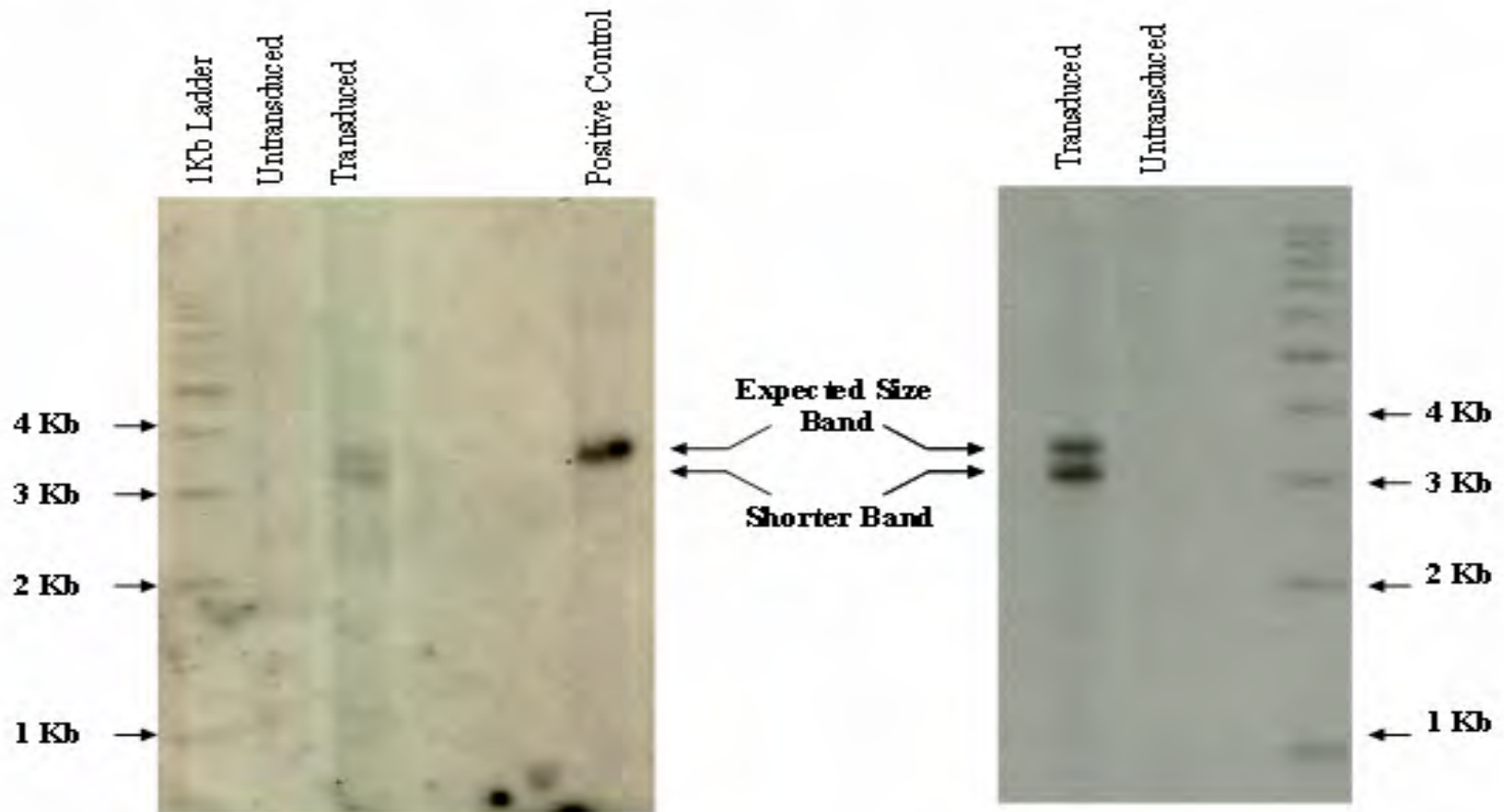
HIV-1 Challenge of Triple Transgenic Thymocytes



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

Proviral integration pattern of triple vector

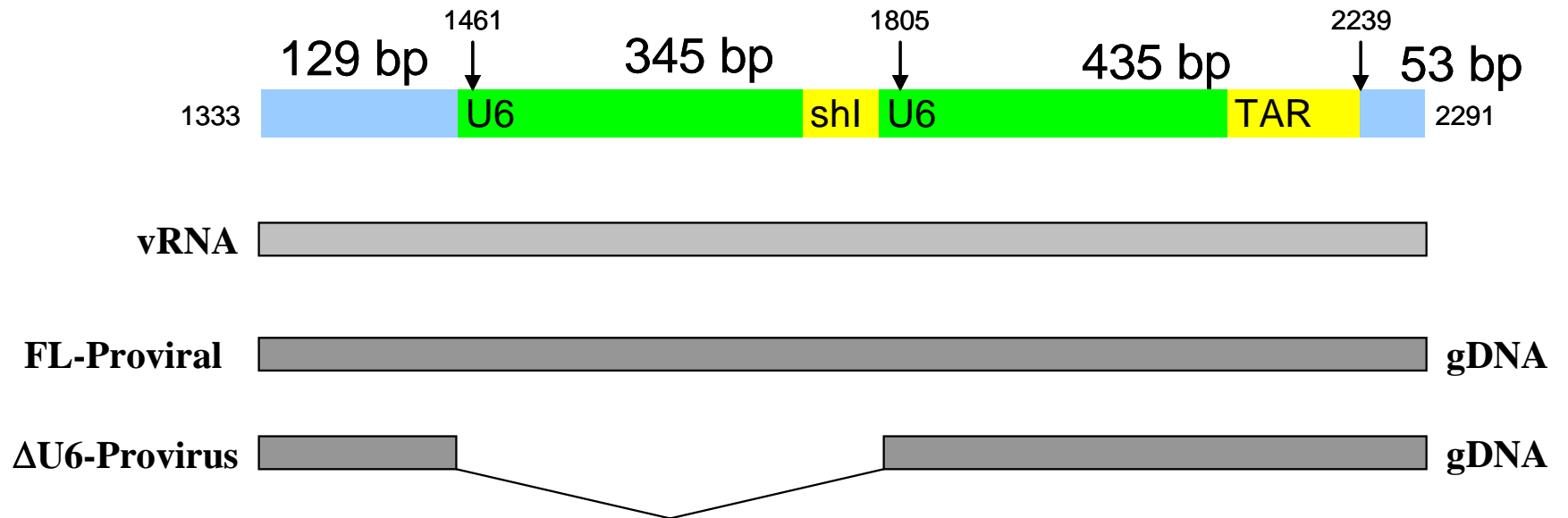
Detection of therapeutic insert in transduced HeLa cells by Southern probe



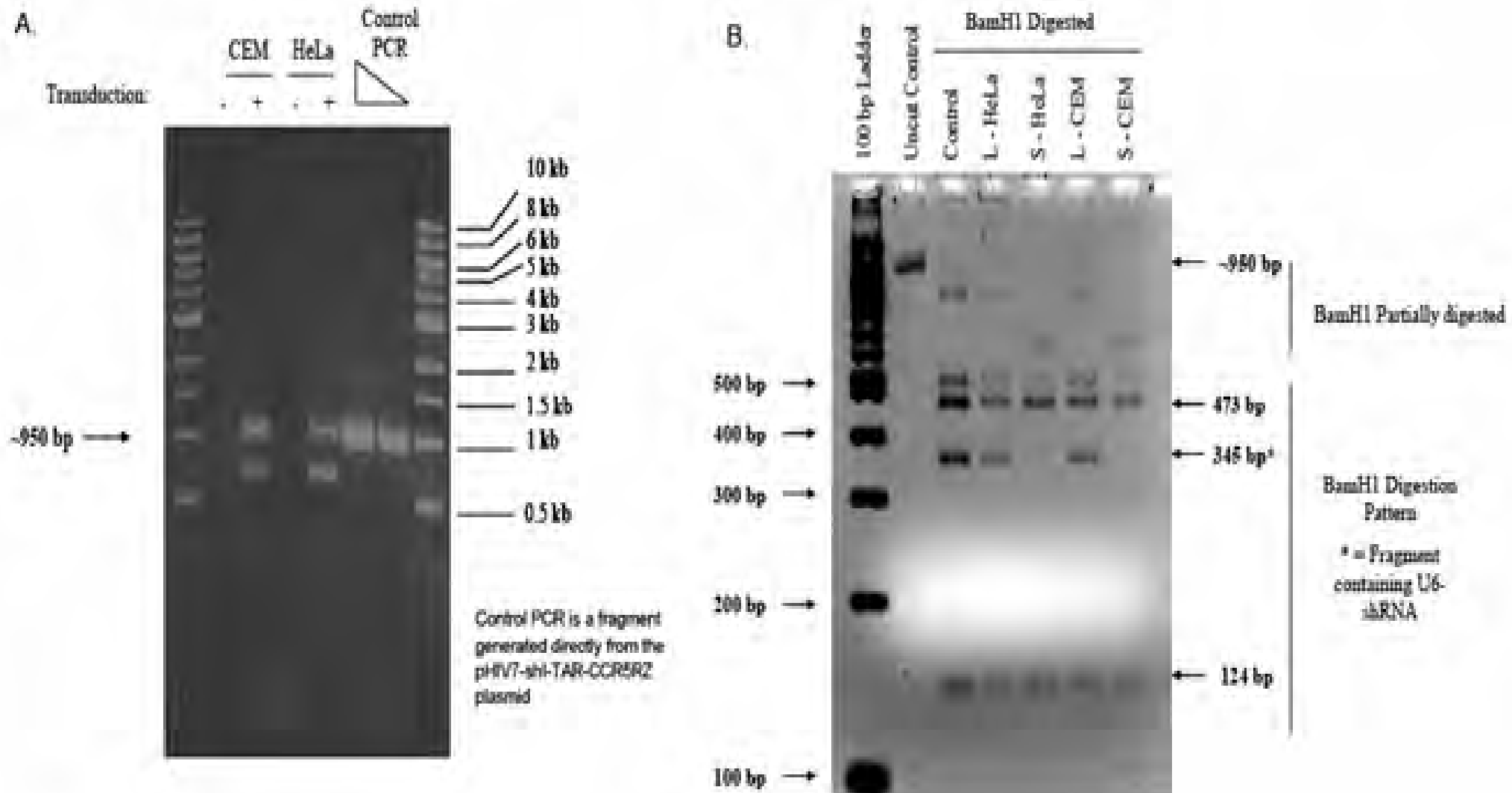
Integration pattern of triple vector

Schematic representation of full-length and deleted sequences.

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



Integration pattern of triple vector



Genomic DNA

BamH1 digestion of bands

Significant issues

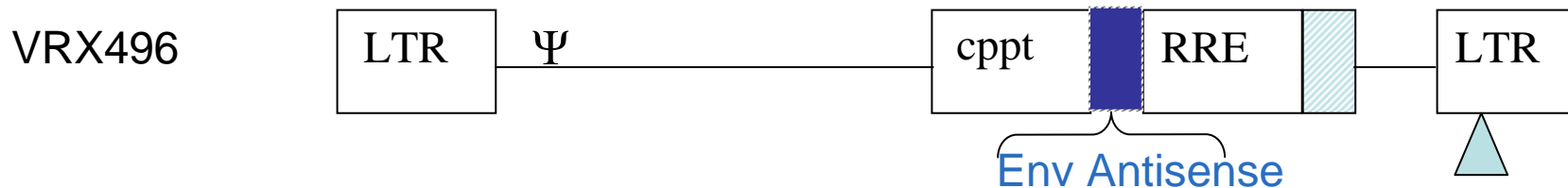
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Retroviral T Cell-based Gene Therapy in HIV/AIDS

- Initial clinical trial of gene rx used T cells transduced with RevM10
C Woffendin et al. PNAS 1996; 93: 2889-2894
U Ranga et al. PNAS 1998; 95: 1201-1206.
- Gene modified T cells can be targeted by transgene-specific immunity
S. Riddell et al. Nat Med 1996; 2: 216-223
- HIV-specific gene modified CD8 cells can traffic to lymph nodes at sites of HIV replication and retain ag-specific potential
S. Brodie et al. J Clin Invest 2000; 105: 1407-1417
- Using a genetically modified TCR, CD4zeta, T cells survived at 1-3% of blood T cells at 8 wks and 0.1% at 1 year
R. Mitsuyasu et al. Blood 2000; 96: 785-793
- Genetically modified syngeneic CD4 and CD8 cells persisted for >1yr
R. Walker et al Blood 2000; 96:467-474

Delivery System: T Lymphocytes for Gene Transfer

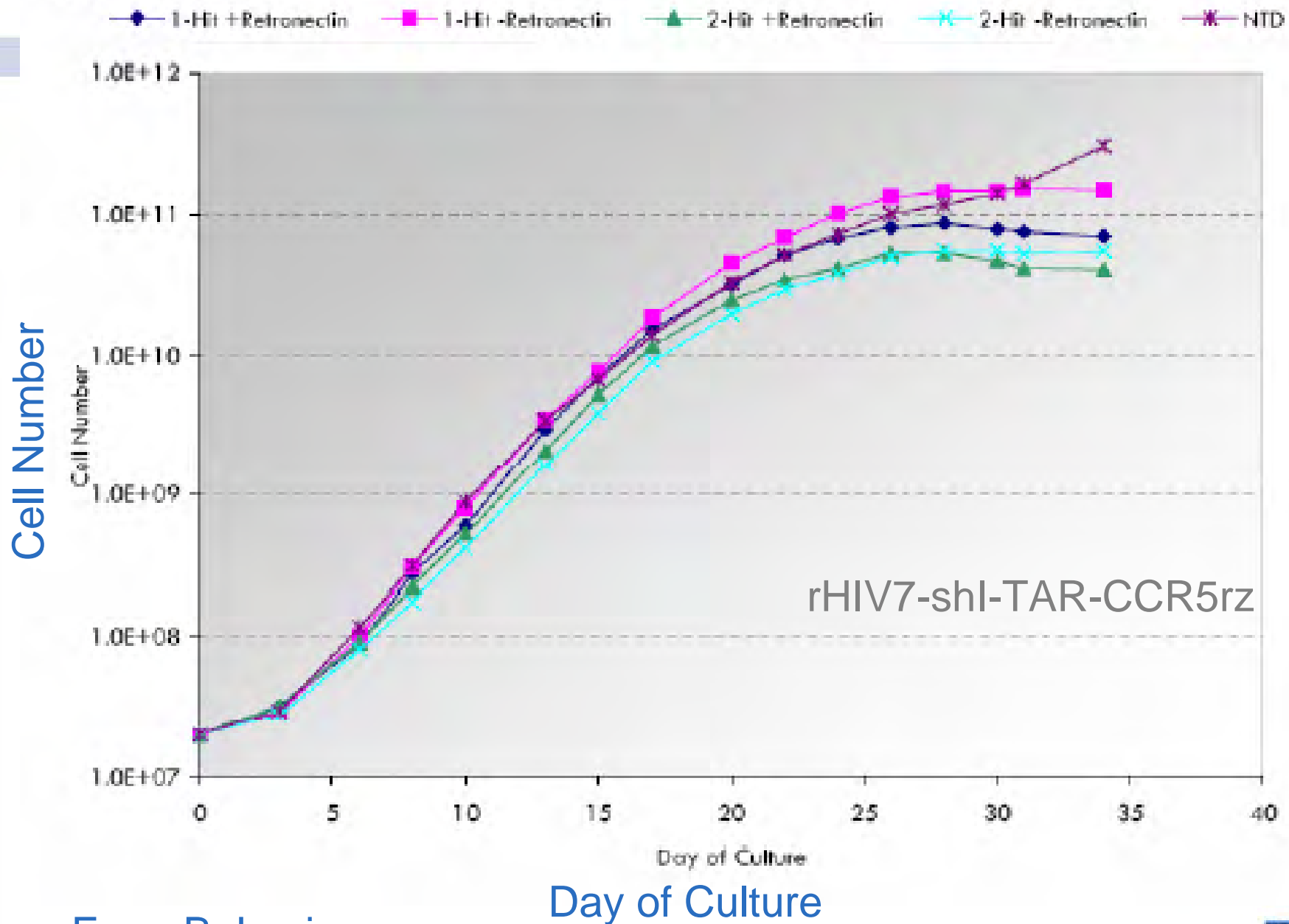
First lentivirus trial in humans completed at UPENN using a vector developed by VirXsys Inc.



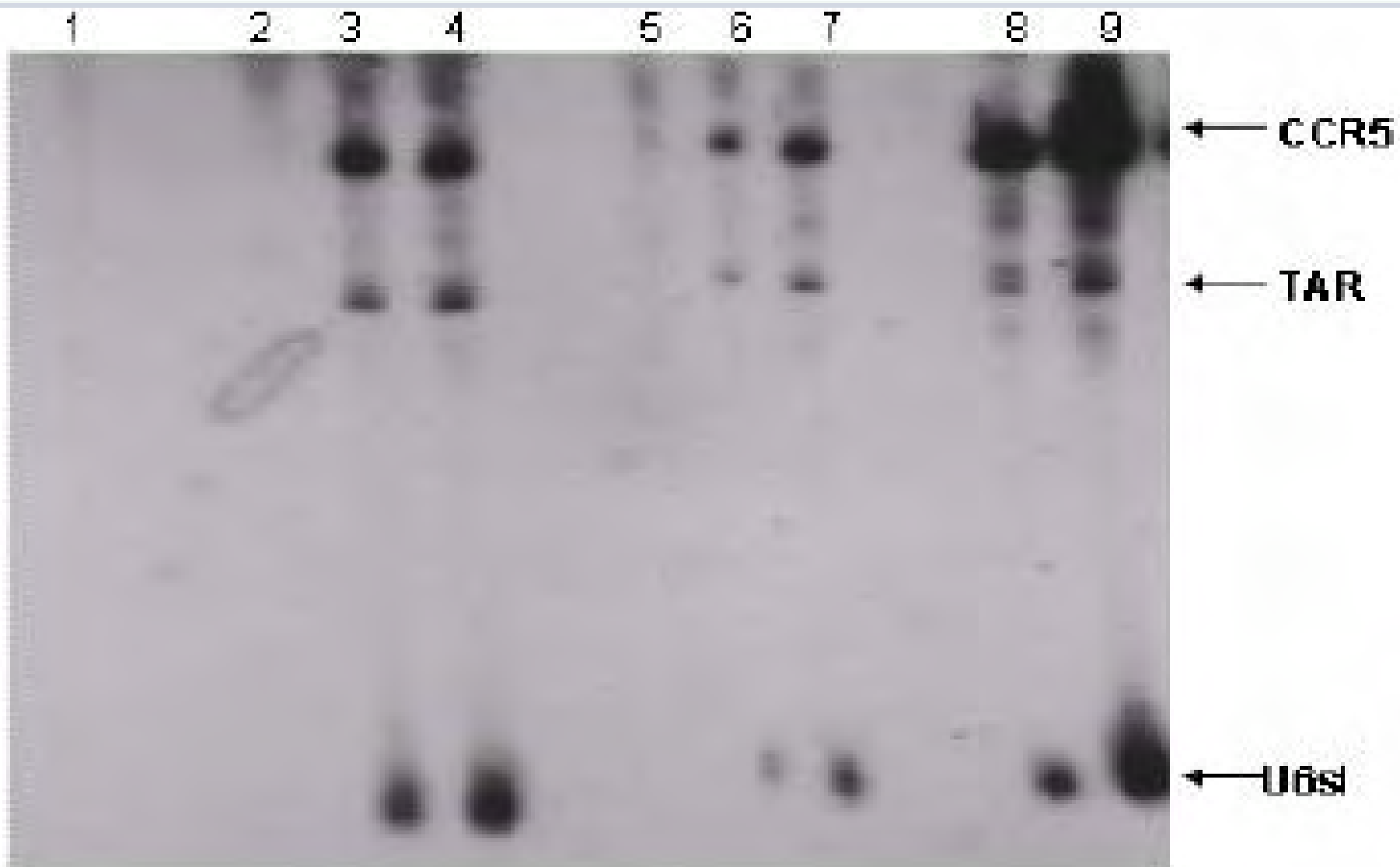
CD4 T cells present rapid in vivo assessment of any relative efficacy of gene therapy, i.e. protection of cells from HIV infection

T-cell based lentivirus gene therapy

- 5 AIDS patients with ART-failure were treated with a single dose of T cells transduced with VRX496 [Levine et al. J Exp Med: 2006]
 1. Safety was demonstrated
 2. Marking of PBMC ~1:1000-10000 cells lasted up to one yr
 3. Mobilization of vector by wild-type HIV
 4. Unexplained drop in HIV load in some patients
- VirXsys Phase 2 Study (40 patients)
- UPENN STI Study (on-going)

A**Total Cell Number**

From B. Levine

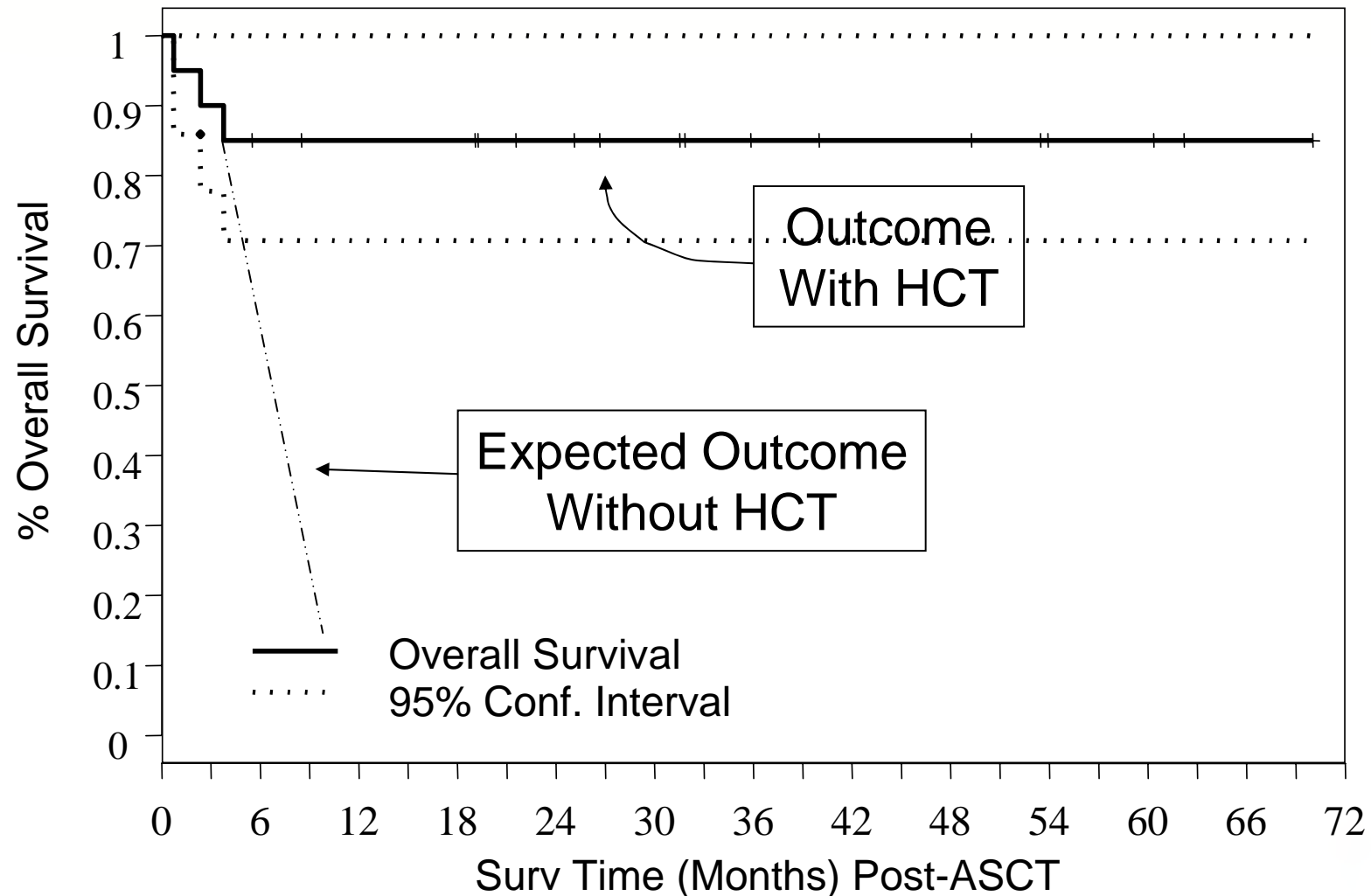


From J. Rossi

Stem Cell Gene Therapy for HIV/AIDS: Ethical considerations

- Partial or complete myeloablation of bone marrow is considered essential for successful engraftment
- Toxicity of stem cell transplantation must be justifiable
- Autologous setting is safer than allogeneic setting
- Use of AIDS patients undergoing autologous HCT is an justifiable population in which to evaluate a new vector system

Autologous HCT for High Risk AIDS Lymphoma: COH Experience



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

Treatment of High Risk AIDS Lymphoma

Dose-intense chemotherapy followed by autologous stem cell “rescue” is the treatment of choice for non-HIV positive patients with relapsed lymphoma.

This approach has been extended to high-risk ARL, as follows:

- Standard therapy cycles are completed (e.g CHOP)
- After the last cycle, the patient’s peripheral blood stem cells are collected using G-CSF
- After carmustine (BCNU), etoposide (VP16), and cyclophosphamide dose-intense chemotherapy, the cells are infused as part of autologous HCT

Treatment of AIDS Lymphoma with Autologous HCT

- 35 patients with high-risk ARL
- Patients had a median of 2 chemo regimens prior to SCT
- Median CD4 = 174/uL; median HIV load = 26,120 gc/ml at Dx
median HIV load at HCT = <500 gc/ml
- Most were on PI-based regimens and some on NRTI/NNRTI-based regimens
- 10.6×10^6 CD34+ cells/kg collected by apheresis
- Median time to engraftment = 11 days [day 23 in one pt on AZT]

Treatment of AIDS Lymphoma with Autologous HCT

- 2 died of early relapse disease; 1 died of cardiomyopathy and renal failure at day +22 post-HCT (age 68)
- HAART continued thru the 3-week HCT hospitalization but <50% patients maintained compliance; HAART resumed in all patients on day 21
- CD4 counts recovered to pre-transplant levels at 1 year and median = 450/uL at 2 years
- HIV load increased during first 2 months in association with non-compliance but was undetectable in 76% at 1 year

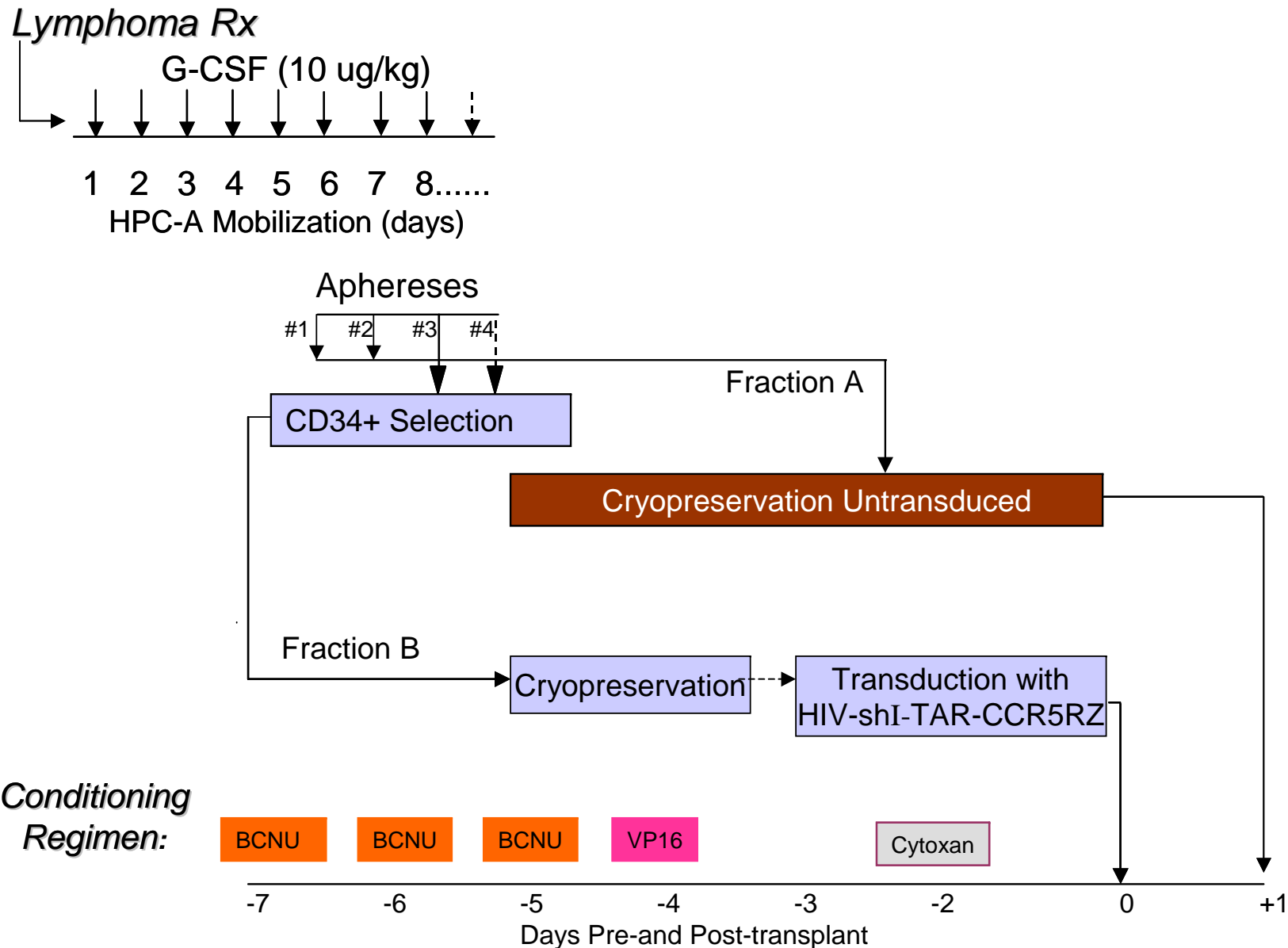
A Pilot Study of Safety and Feasibility of Stem Cell Therapy for AIDS Lymphoma Using Stem Cells Treated with a Lentivirus Vector Encoding Multiple anti-HIV RNAs

Specific Aims:

The primary objective is to determine **safety and feasibility** of lentivirus-transduced hematopoietic stem cells in the setting of autologous HCT for the treatment of AIDS lymphoma

The secondary objective is to determine the **quantity and duration of vector-marked** peripheral blood cells after marrow engraftment

Study #1: AIDS Lymphoma



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

Exclusion Criteria

- History of grade III cystitis due to cyclophosphamide
- CNS lymphoma
- Prior other malignancy except treated basal cell ca, cervical ca, or squamous cell ca
- HIV-associated encephalopathy; dementia; seizures in past year
- No active bacterial, fungal, CMV infection; no OI in past year except treatment-responsive MAI, candida, HSV, VZV, CMV
- Other AIDS-related syndromes, infectious or otherwise, perceived to cause excessive risk for morbidity post-HCT
- Inability to undergo blood stem cell mobilization or any contra-indication for undergoing HCT

Transduction Method

- CD34+ G-CSF mobilized peripheral blood progenitor collected off ART and cells selected on a CliniMax® column (Miltenyi)
- Cells thawed, centrifuged thru HSA to remove DMSO, and cultured overnight in X-Vivo 15 media supplemented with--
 - SCF 100 ng/ml
 - TPO 10 ng/ml
 - Flt-3L 100 ng/ml
- Cells are transduced at MOI = 5 in a Retronectin® (Takara)-coated T-75 flask for 16 hr
- Final suspension in PBS with Ca/Mg, 0.5% EDTA, 0.5% HSA

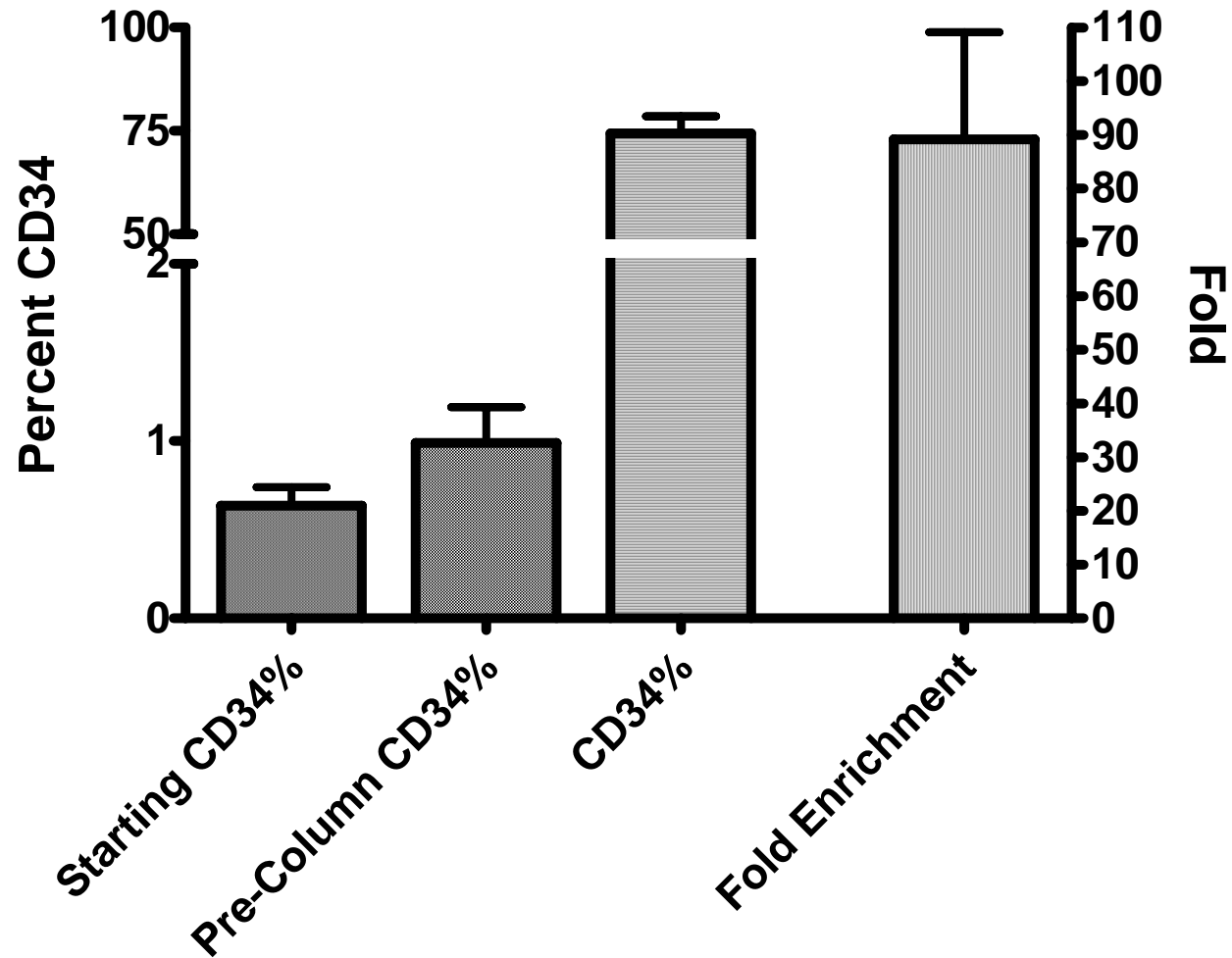
Cell Product Release Tests

- Endotoxin assay
- Gram stain
- ~~Viability >70%~~
 $\geq 5 \times 10^6/\text{kg}$ viable CD34+ cells

AIDS Lymphoma Study Recruitment

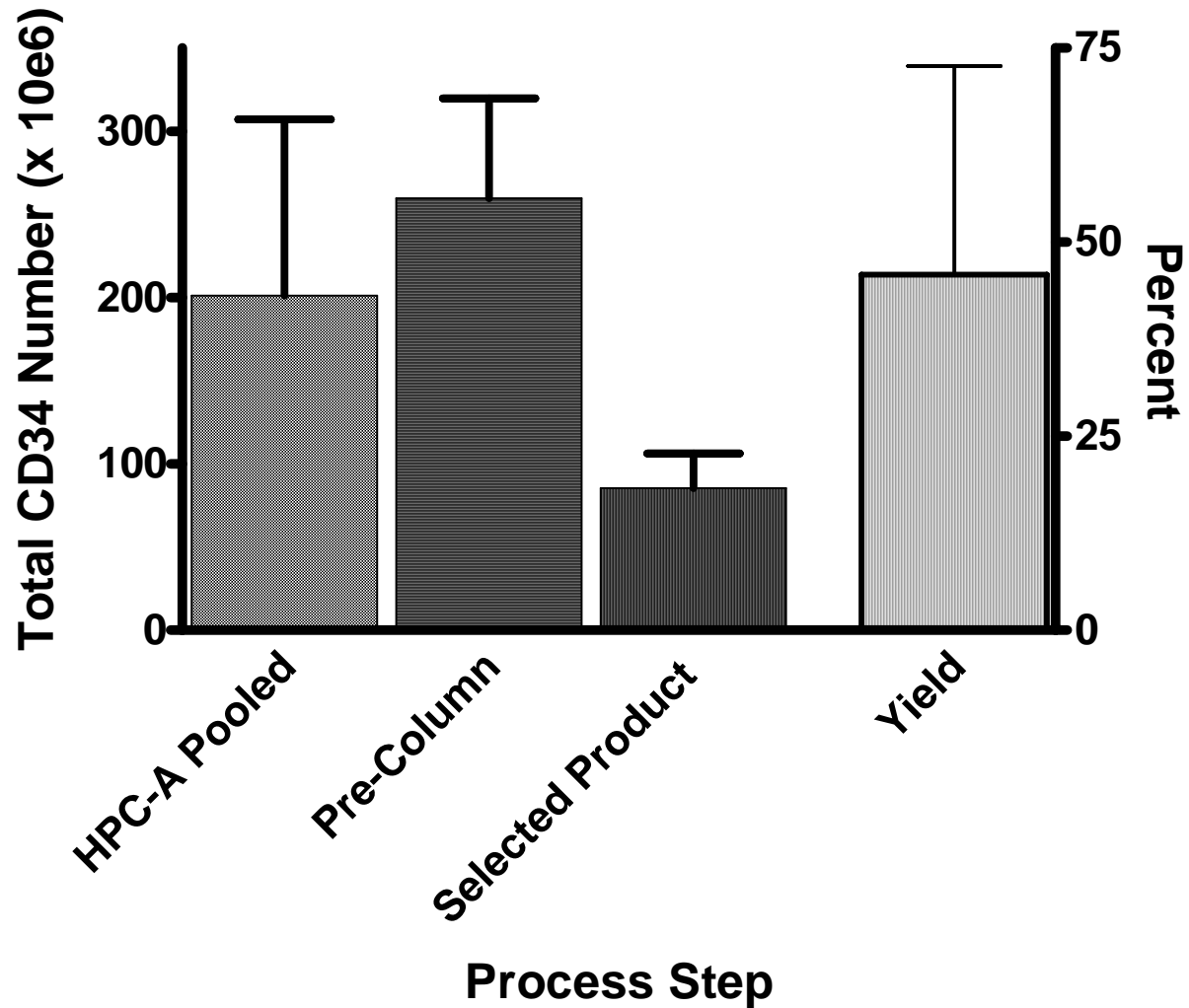
UPN #	Diagnosis	Status
0301	Diffuse Large B cell	Cell Product failed release
0302	Burkitt	Failed eligibility due to infection
0303	Burkitt	Failed eligibility due to low mobilization of PBPC
0304	Diffuse Large B cell	Transplanted Feb 19, 2008
0305	Diffuse Large B cell	Transplanted Mar 13, 2008

CD34 Frequency



From D. DiGiusto and L. Li

Summary CD34 Recovery



From D. DiGiusto and L. Li

Cell Yield

Total Cell Number

% Viable Cells

Date Processed		Thaw	Total Cell Number		Thaw	% Viable Cells	
			After Pre-stimulation	At Harvest		After Pre-stimulation	At Harvest
9/2/2007	UPN0301	9.84E+07	1.23E+08	6.23E+07	76.6	33.3	62
2/2/2008	UPN0304	1.16E+08	1.83E+08	1.36E+08	95.9	72.4	62.8
2/21/2008	UPN0305	1.52E+08	1.42E+08	1.31E+08	95	57.9	52.4

Table 1. Total Cell Number and Viability of CD34 transduction cultures over time.

Viable Cell Number

Date Processed		Thaw	Viable Cell Number	
			After Pre-stimulation	At Harvest
9/2/2007	UPN0301	7.54E+07	4.08E+07	3.86E+07
2/2/2008	UPN0304	1.11E+08	1.32E+08	8.54E+07
2/21/2008	UPN0305	1.44E+08	8.20E+07	6.90E+07

51%
77%
48%
yield

Table 2. Viable cell number in CD34 transduction cultures over time.

Competition for Engraftment: transduced: untransduced cells

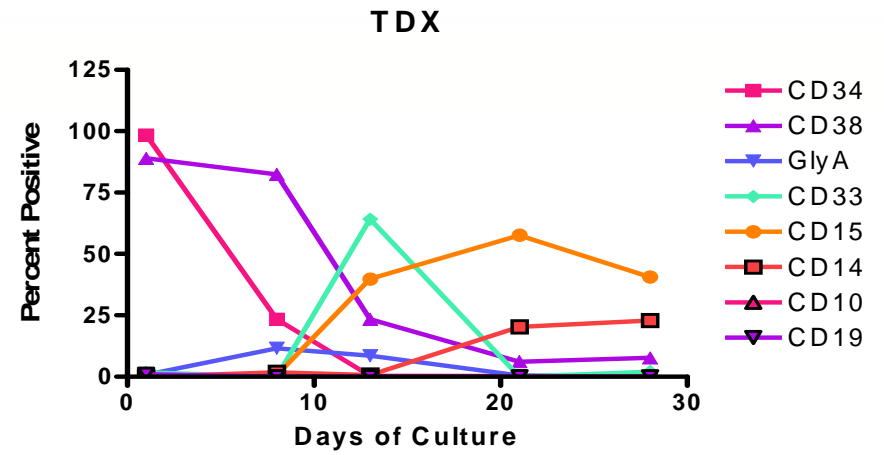
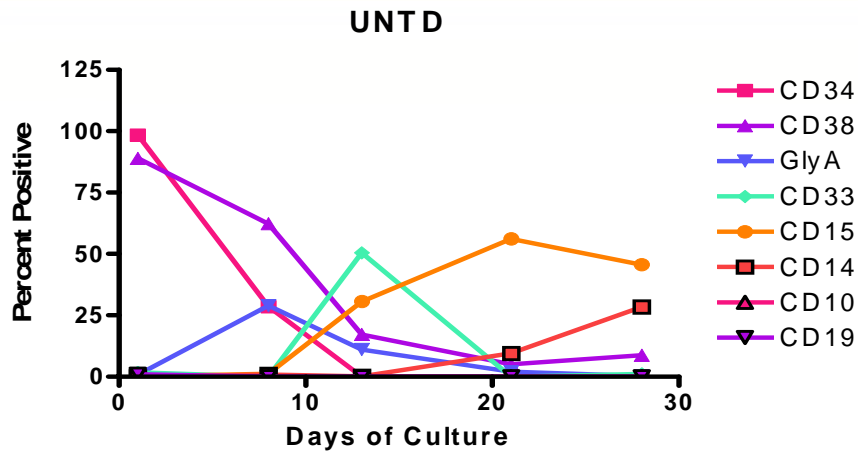
- $\geq 2.5 \times 10^6$ CD34/kg frozen unmanipulated
- $\sim 2.5 \times 10^6$ CD34/kg frozen & thawed for transduction
- Post-Miltenyi column, freeze thaw, transduction
process yield = $\sim 50-60\%$
- Final CD34 infusion =
 - 1×10^6 /kg transduced cells
 - 2.5×10^6 /kg unmanipulated cells
 - Ratio--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

Total Cell Infusion

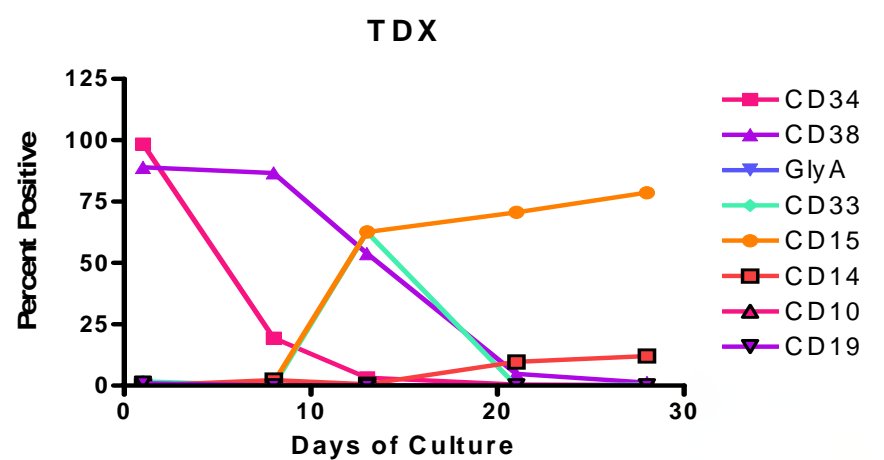
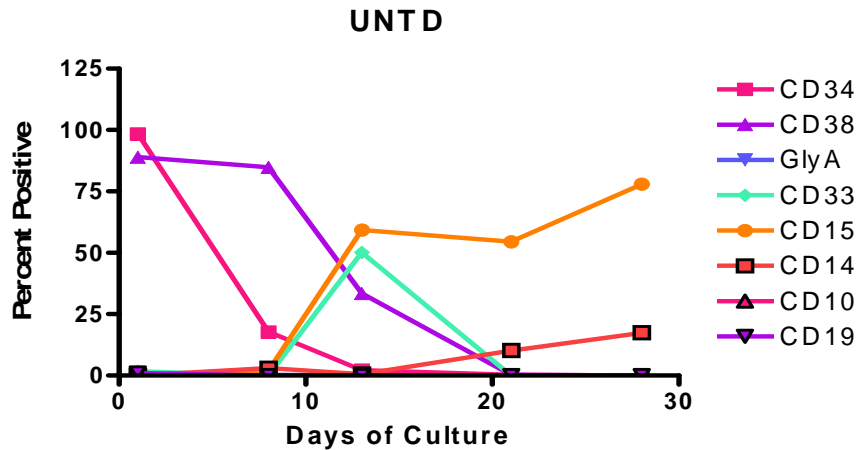
UPN	Cell Product	% Viable Cells in Cell Product		Viable cells/kg
		Non-Transduced	Transduced	
0301	3.86×10^7	62	62	0.29×10^6
0304	8.54×10^7	67	64.5	1.02×10^6
0305	6.9×10^7	53.7	52.4	0.78×10^6

Phenotype Kinetics from 28D Culture of CD34+ Cells

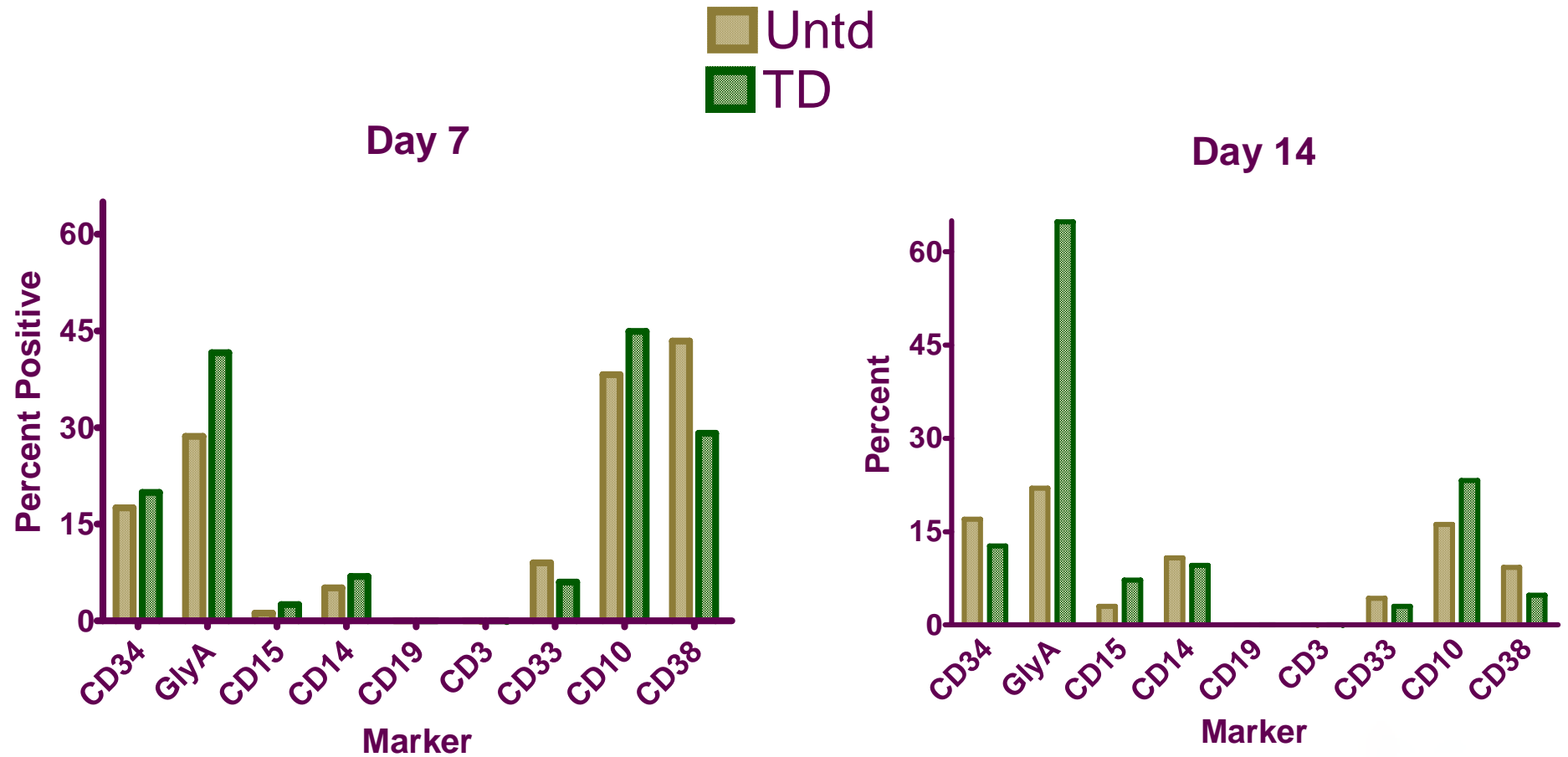
Liquid Culture



Stromal Culture

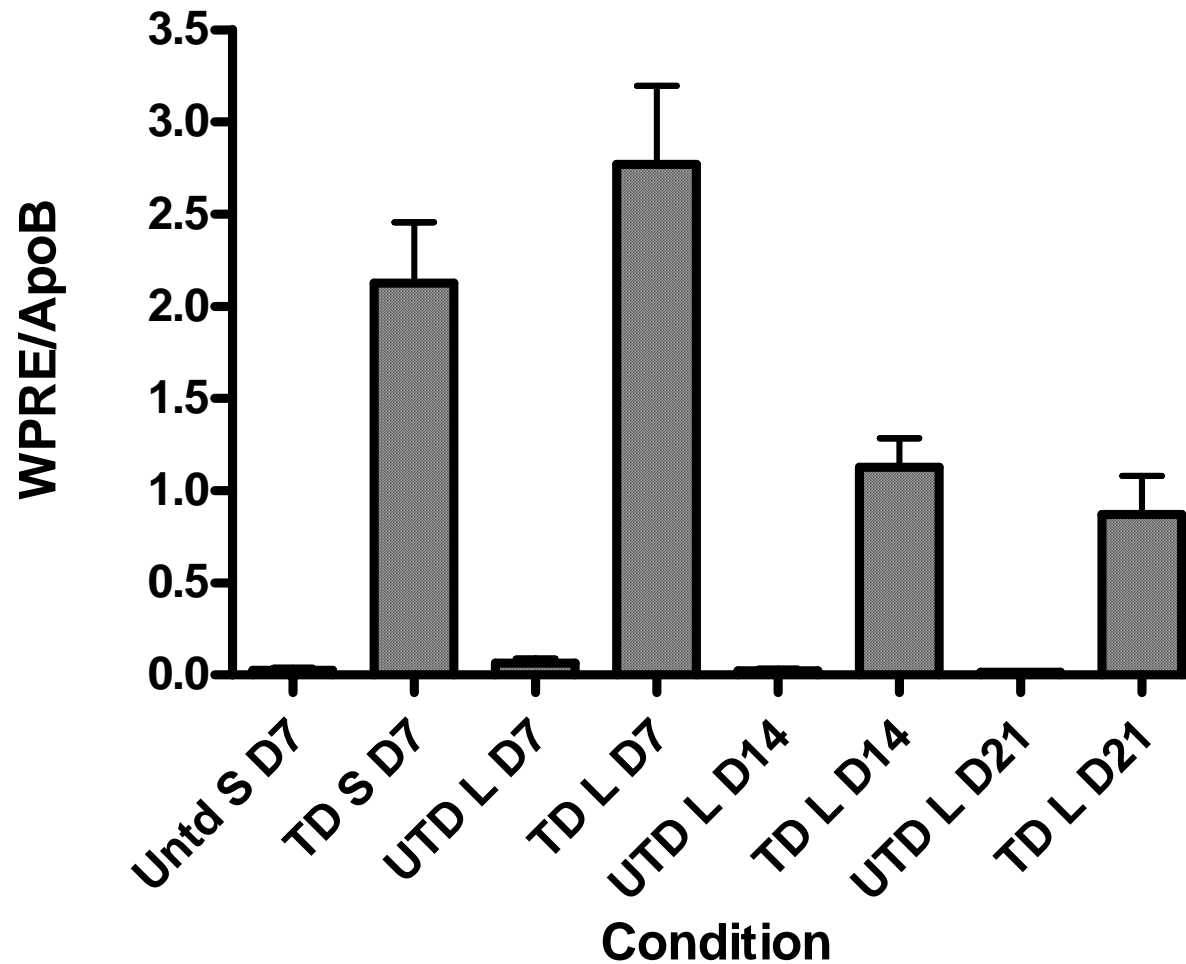


Phenotype of Liquid Culture CD34+ Cells

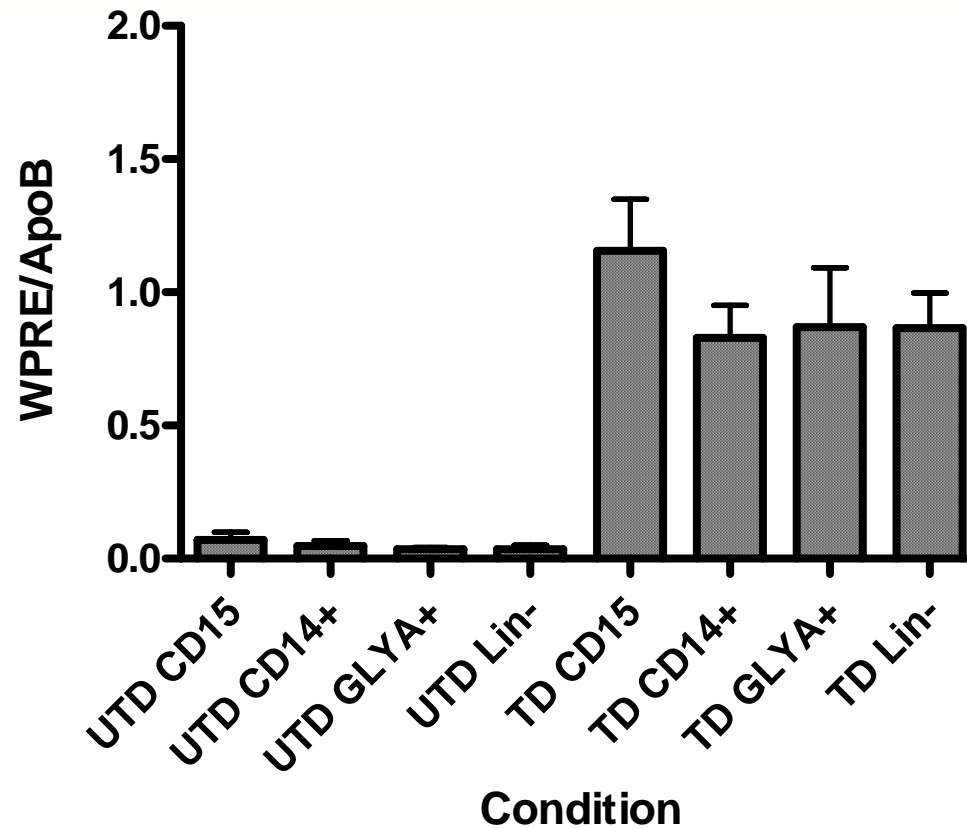


From D. DiGiusto and L. Li

Marking of Bulk Cultures



Marking of Lineage Specific Cells



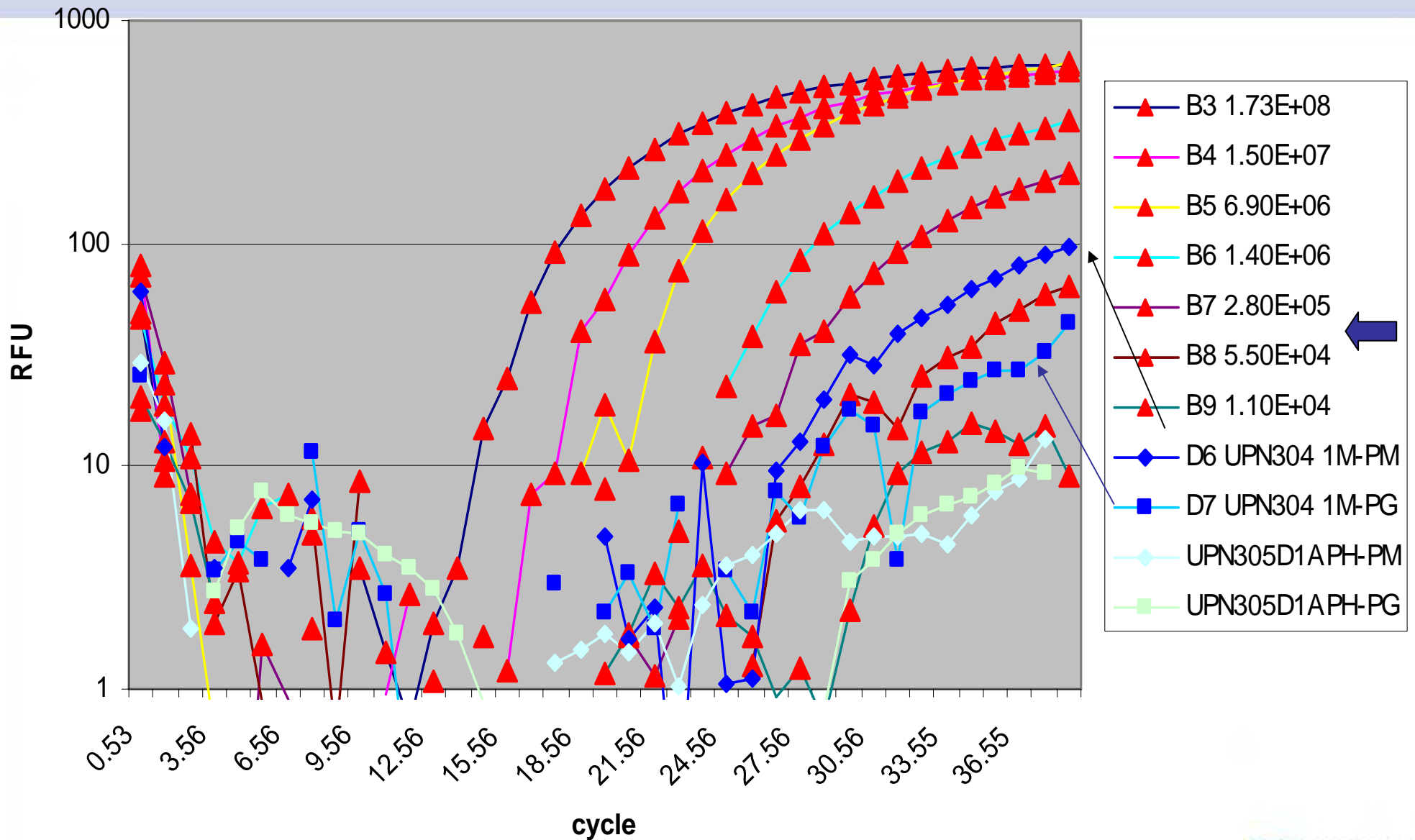
Transduction Analysis of Cultured CD34+ Cells

Liquid culture, stroma culture, and lineage PCR for UPN0305						
	D8	D13	D22	D29		
Liquid Untd	0%	0%	0%	0%		
Liquid TD	23%	3%	1%	1%		
Stroma Untd	ND	0%	0%	0%		
Stroma TD	ND	2%	3%	1%		
	Bulk culture				Stroma	
	CD15	CD14	GlyA	Lin-	CD10+	CD10-
Liquid Untd D13	0%	0%	0%	0%	ND	ND
Liquid TD D13	1%	1%	8%	11%	ND	ND
Stroma Untd D29	0%	0%	ND	0%	ND	ND
Stroma TD D29	1%	1%	ND	1%	ND	ND

UPN0305

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

patient 304 1M



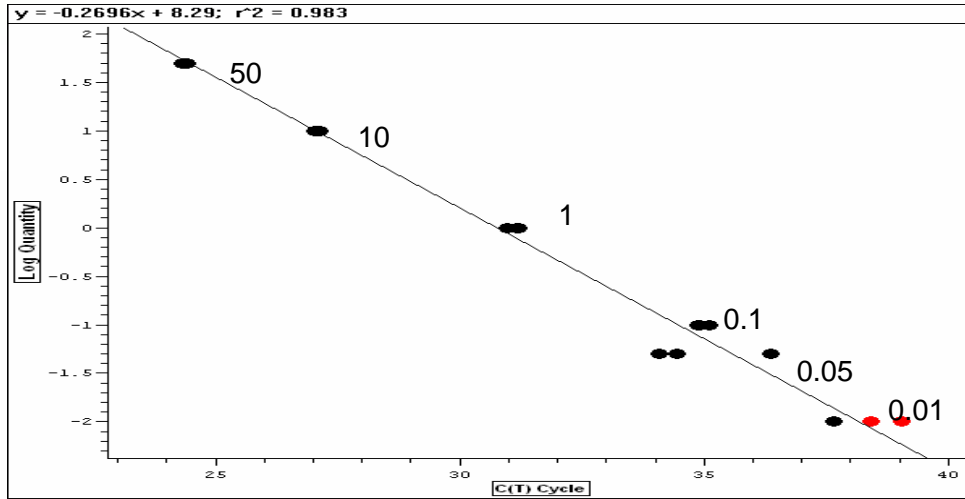
From J. Rossi



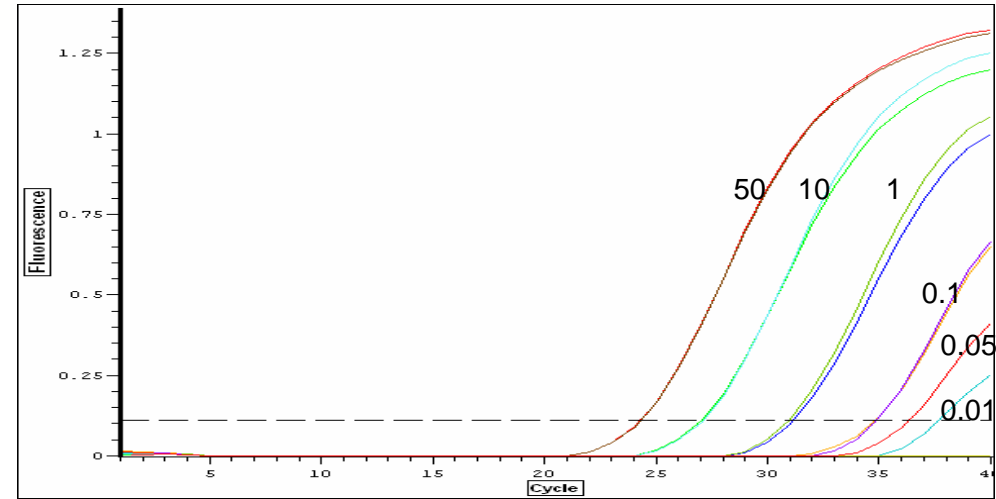
Gene Marking in PBMC: Q-PCR primary data

Primer: WPRE, Standard Curve:H9c1/PBMC (100%-0.002% H9c1-positive)

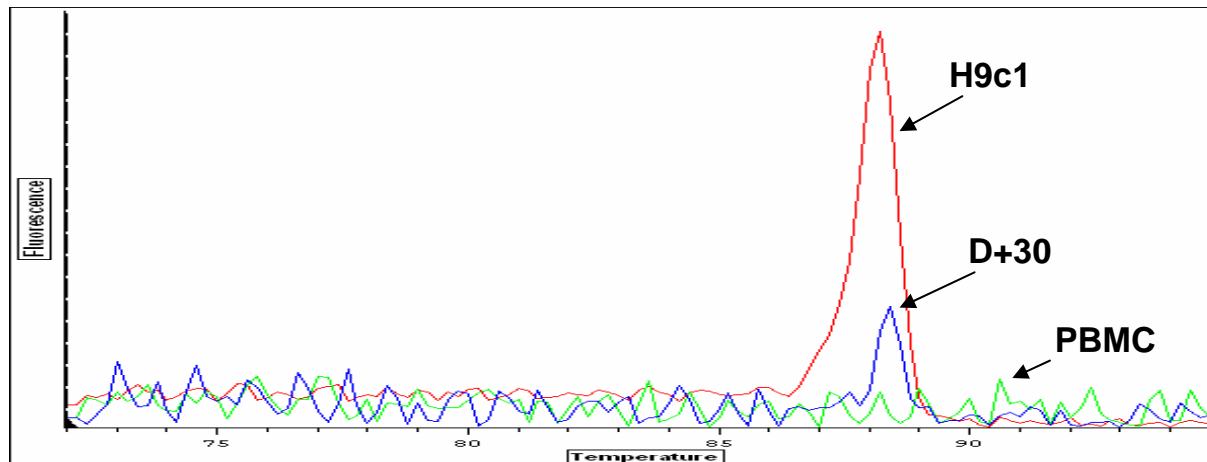
A. Standard Curve



B. Ct curves



C. Melting Curve analysis



Outcome Pending

- Safe engraftment in 10 days in two patients
- Gene marking in blood:

Months:	1	2	3	6	12	18	24
Patient #1	+	+				
Patient #2	+	+				

- Patients continue to be enrolled and followed

Conclusions

- Strategic gene therapy design would treat early after HIV infection
- Multiplex lentivirus vector can be used in HIV-related projects
- shRNA-containing vector can be used in clinical studies
- Further follow-up continues for on-going studies
- Development of T-cell based and stem cell based methods deserve further evaluation

Acknowledgements

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Friedman, Michael
Gonzalez, Nancy
Han, Yongyi
Hsu, David
Kalos, Michael
King, Valerie
Krishnan, Amrita
Krupka, Emily
Land, Colleen Jill
Lee, Mary
Levine, Alexandra
Li, Haitang

UPENN:

C. June
B. Levine

NIAID; NHLBI;
NCI; NCRR

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Reed, Ken
Rossi, John
Shad, Yasmine
Smith, Eileen
Snyder, David
Stillings-Farris, Amy
Stinson, Sherri
Stan, Rodica
Stinson, Sherri
Wardlow, Michelle
Wang, Sean
Williams, Brenda
Yam, Priscilla
Yee, Jiing-Kuan
Zaia, John A.