Update on protocol 0107-488: A phase I trial with a single dose of autologous T cells transduced with VRX496 in HIV positive subjects

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HIV-1 vector gene therapy for HIV/AIDS

- **Over 40 million infected world wide**
  - Approximately 1 million in North America
- **68 million people are predicted to die between 2000 and 2020**
- **Combination drug therapy is not a cure**
  - holds disease while patients take a strict multi-drug regimen – compliance?
- **Drug therapy is ultimately toxic**
  - Some drug induced toxicities include GI & liver disfunction, lipid disorders
- **Resistance to drug therapy is increasing**
  - 15% of newly infected individuals are infected with resistant HIV

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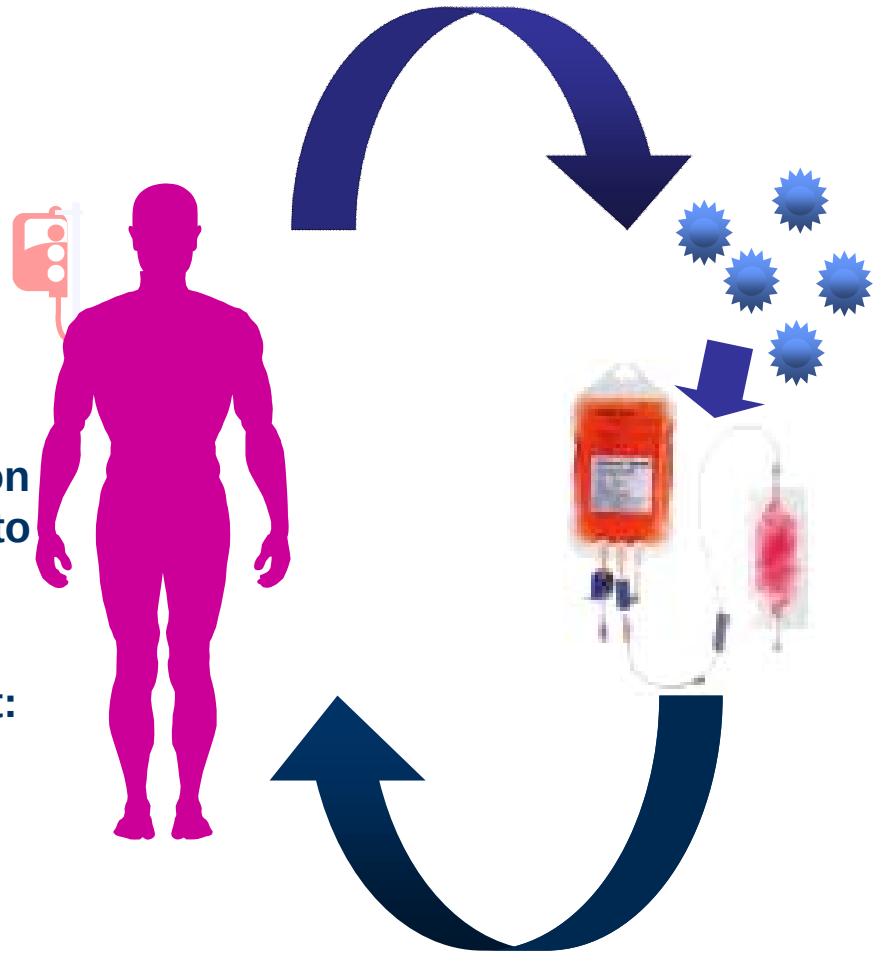
Goal of HIV vector gene therapy

- **Create T cells in the body of HIV infected patients that:**
  - **Inhibit HIV replication by a anti-HIV antisense payload that specifically binds and destroys HIV genetic material in cells**
  - **Permanently decrease HIV replication in the body to levels not conducive to symptomatic AIDS**
  - **It is known from clinical studies that:**
    - **Higher HIV replication → more rapid disease progression**
    - **Lower HIV replication → longer postpone the development of symptomatic AIDS**
    - **Lower HIV replication → lower transmission rates**



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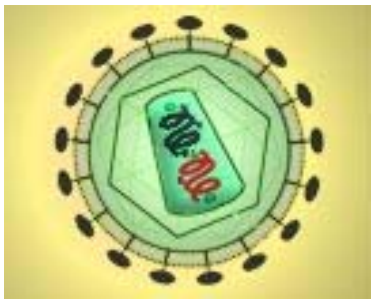
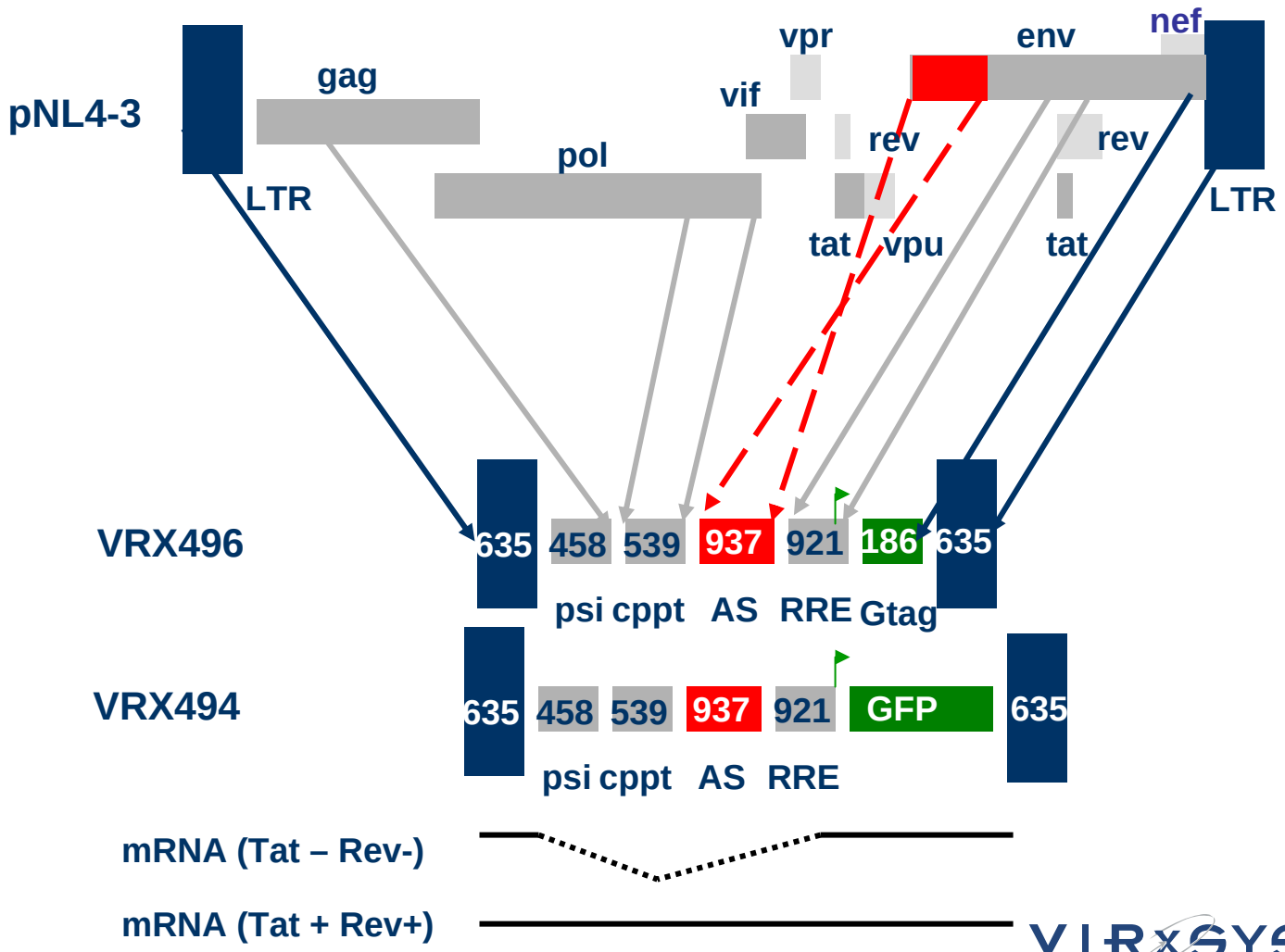
The HIV vector contains a 937 nucleotide anti-HIV antisense sequence





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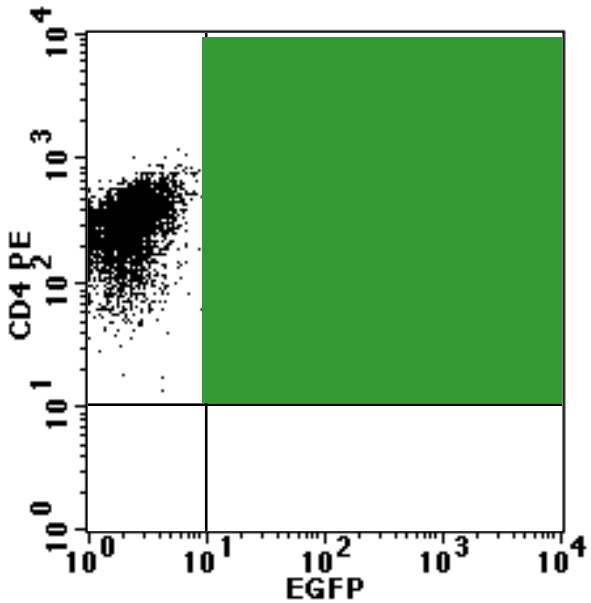
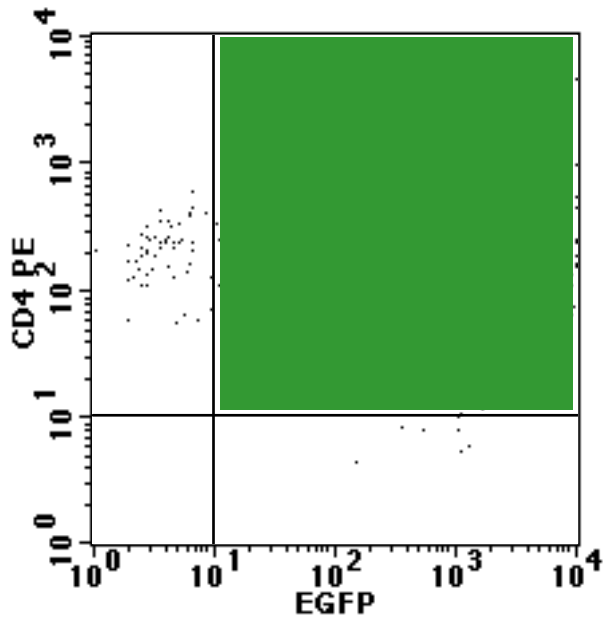
Highly efficient T cell transduction using HIV based vectors



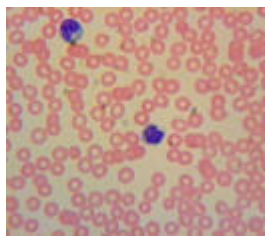
0%

Control



>99%

+ vector- GFP



CD4 T cells

Vector + iCD3 Ab
+ iCD28 Ab

FACS

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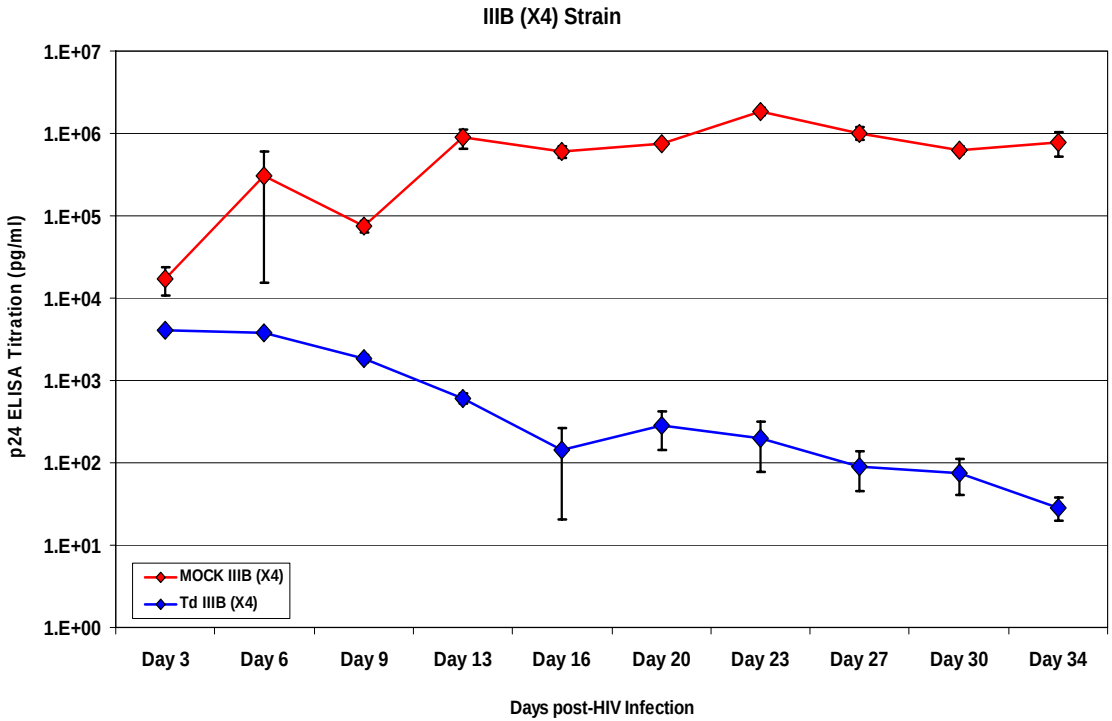
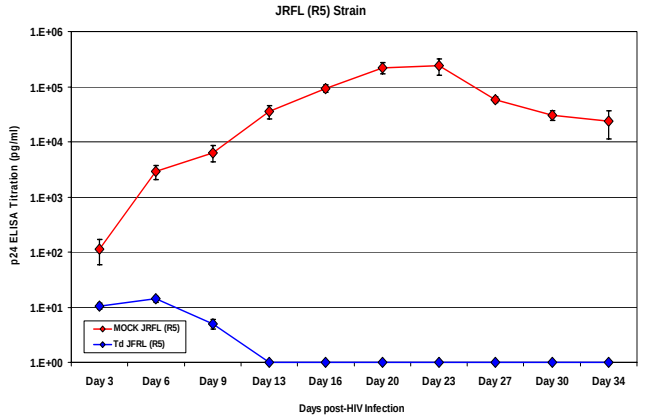
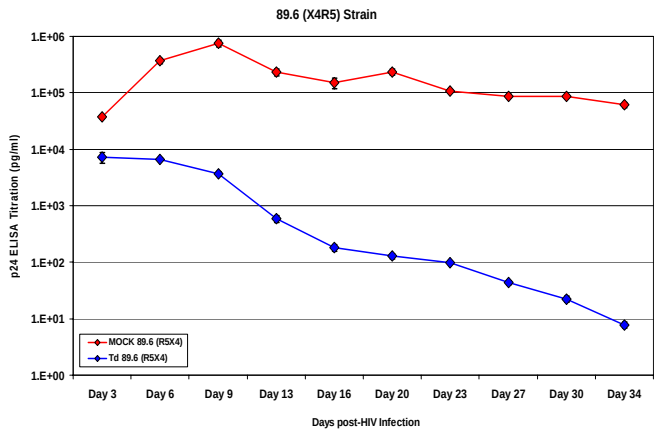
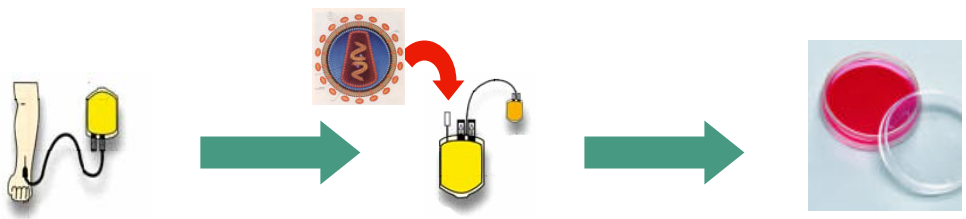
Inhibition of HIV replication in cells after challenge with HIV strains









HIV free blood

Infect with diverse HIV strains

Grow cells in culture

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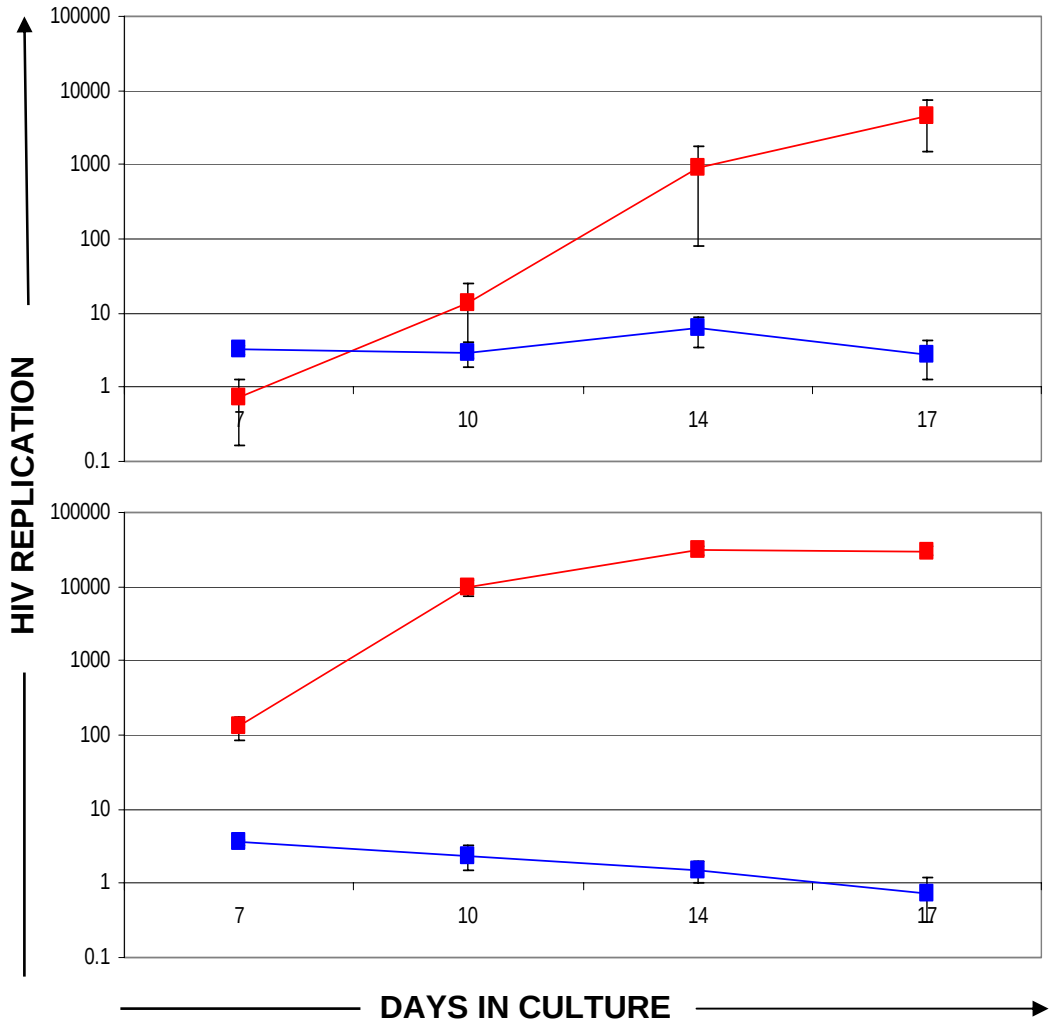
Inhibition of HIV in patient cells >99%



Blood Sample
Viral Load =222,612
CD4 T cell count = 403

Blood Sample
Viral Load =95,591
CD4 T cell count = 288

HIV Positive blood cells

Add vector to cells

Grow cells in culture

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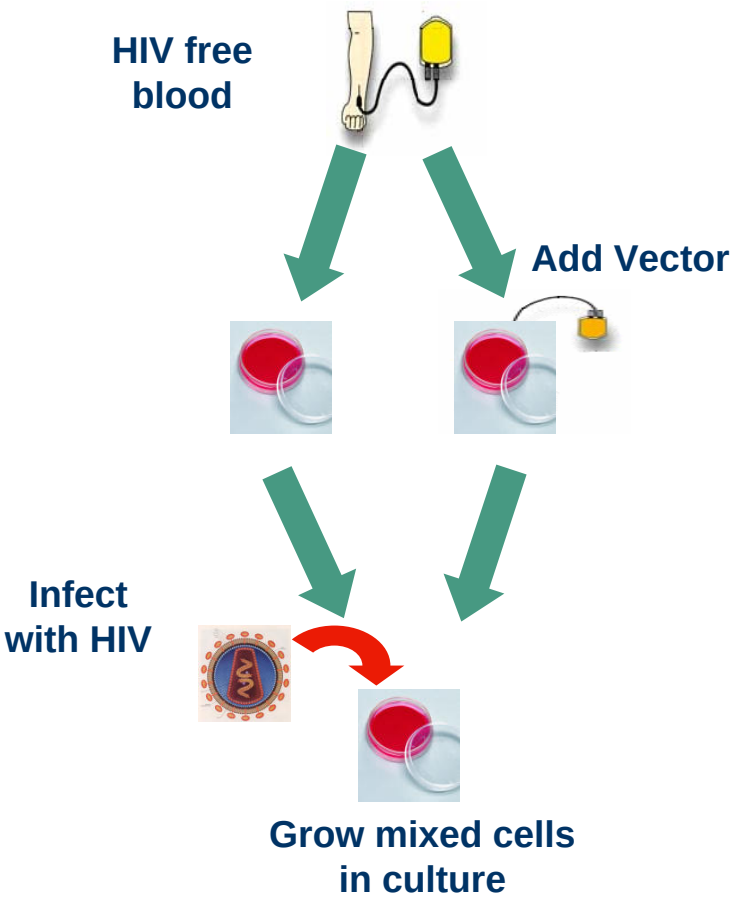
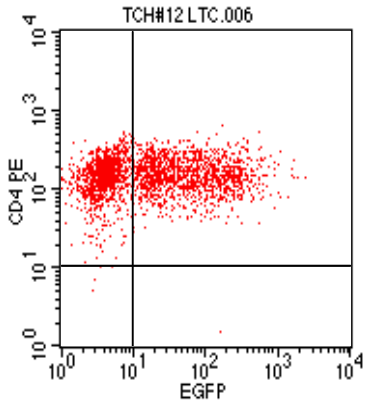
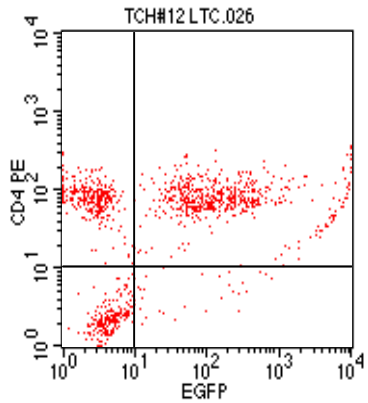
Cells containing vector show selective resistance to productive HIV infection (CD4 downregulation)



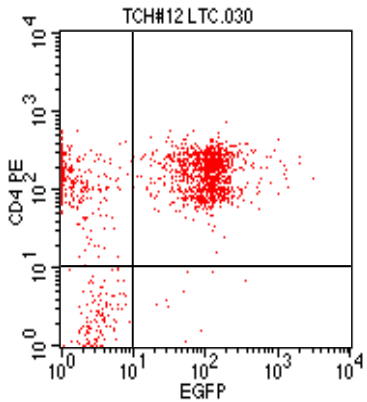
CONTROL: NO HIV



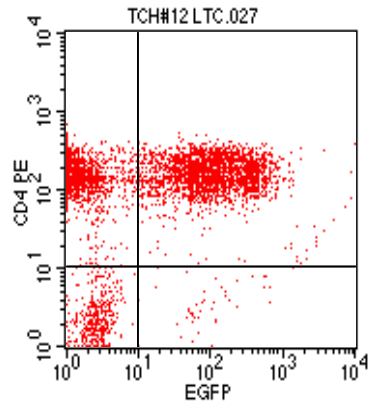
BK132 at MOI 0.001



US1 at MOI 0.05



Ba-L at MOI 0.001



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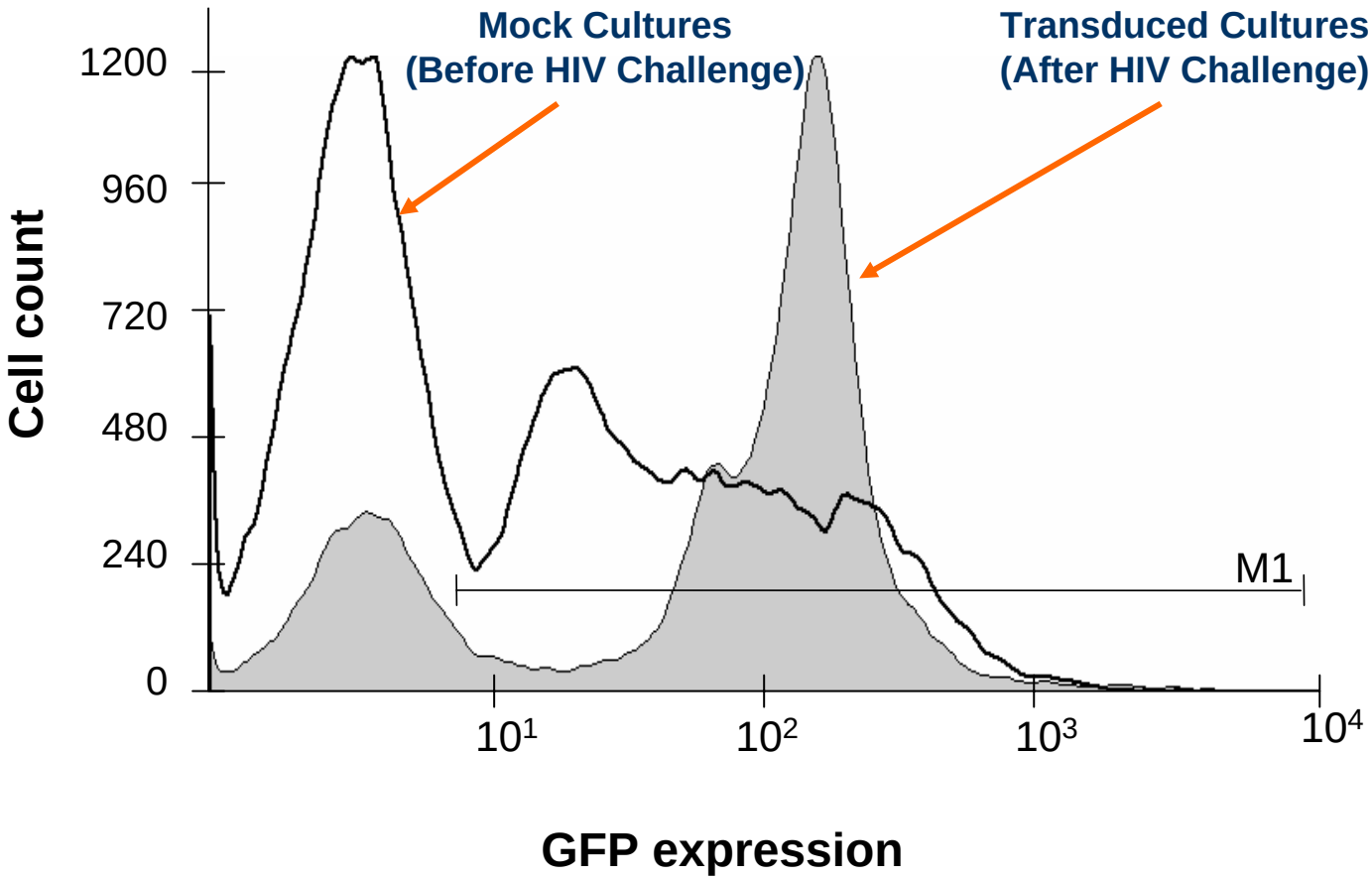
Selection for transduced cells after culture is challenged with HIV



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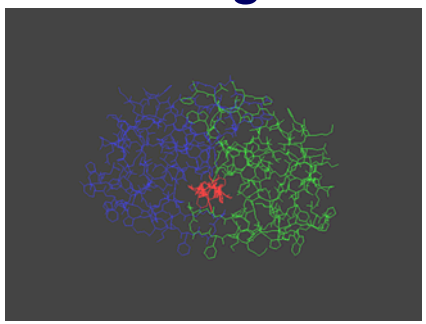
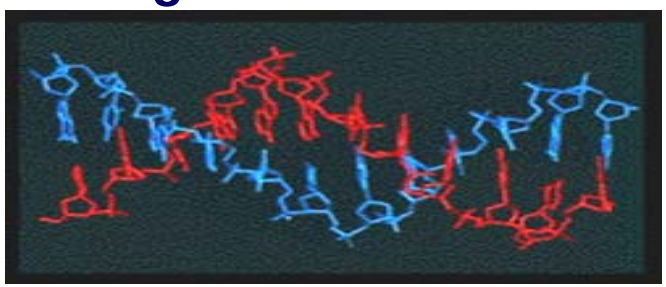
Long anti-HIV antisense may address issue of HIV resistance

- **Anti HIV drugs**
  - **Targets small number of sites on target molecule**



- **Long antisense RNA**
  - **Targets 937 sites along HIV RNA strand**



Number of Mutations needed for Resistance	Consequence of the mutations upon HIV Replication
Small	**Small** ⬇
Large	**Large** ⬇

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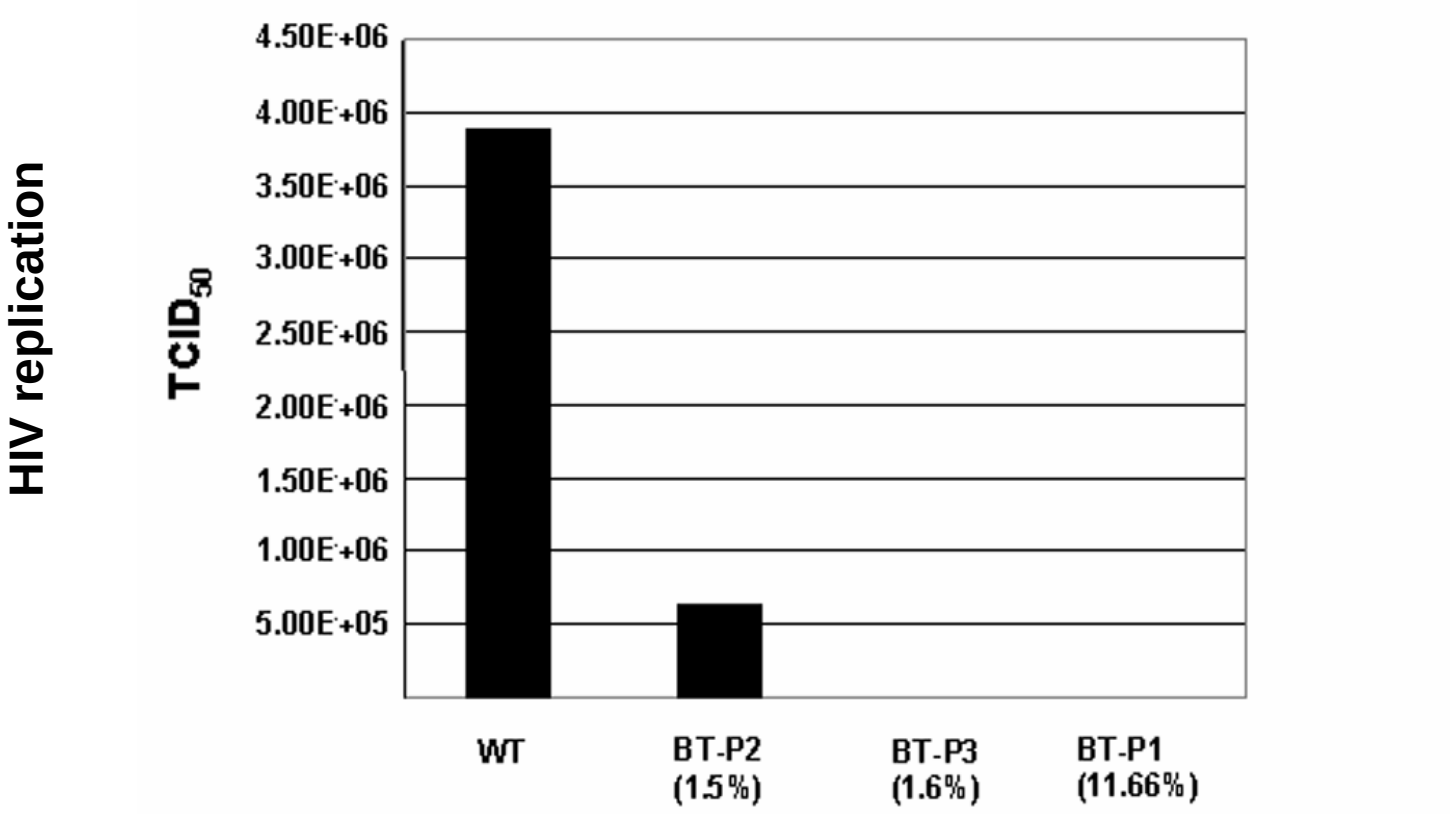
Mutated HIVs are severely attenuated for replication



Number of Mutations	0	15	16	108

Journal of Virology, July 2004

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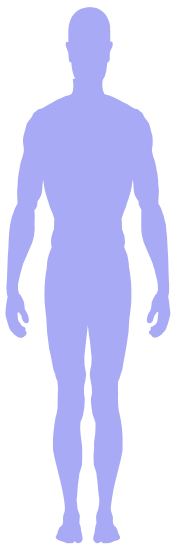
Phase I clinical trial procedure

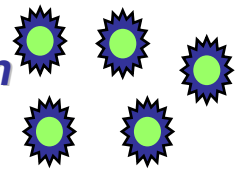
Failed 2 Regimens of HAART
No Opportunistic Infections
CD4 200 - 500
VL >5000



n = 5
serial dosing

T Cell Isolation

Vector Production



QC on vector

Ex-vivo transduction

T CELLS

T Cell Expansion

QC on vector modified cells

Single Dose
10^{10} cells

End-point: Safety

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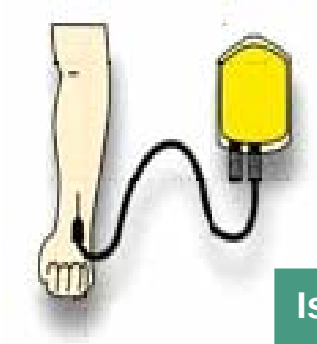
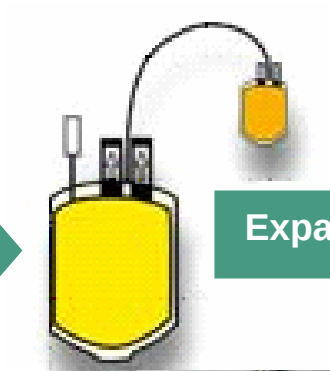
Autologous gene transfer into T cells

Aphaeresis: patient's white cells selectively removed



Isolate T cells

Vector added to cells



Expand cells

Frozen cells are thawed

Modified cells returned to patient

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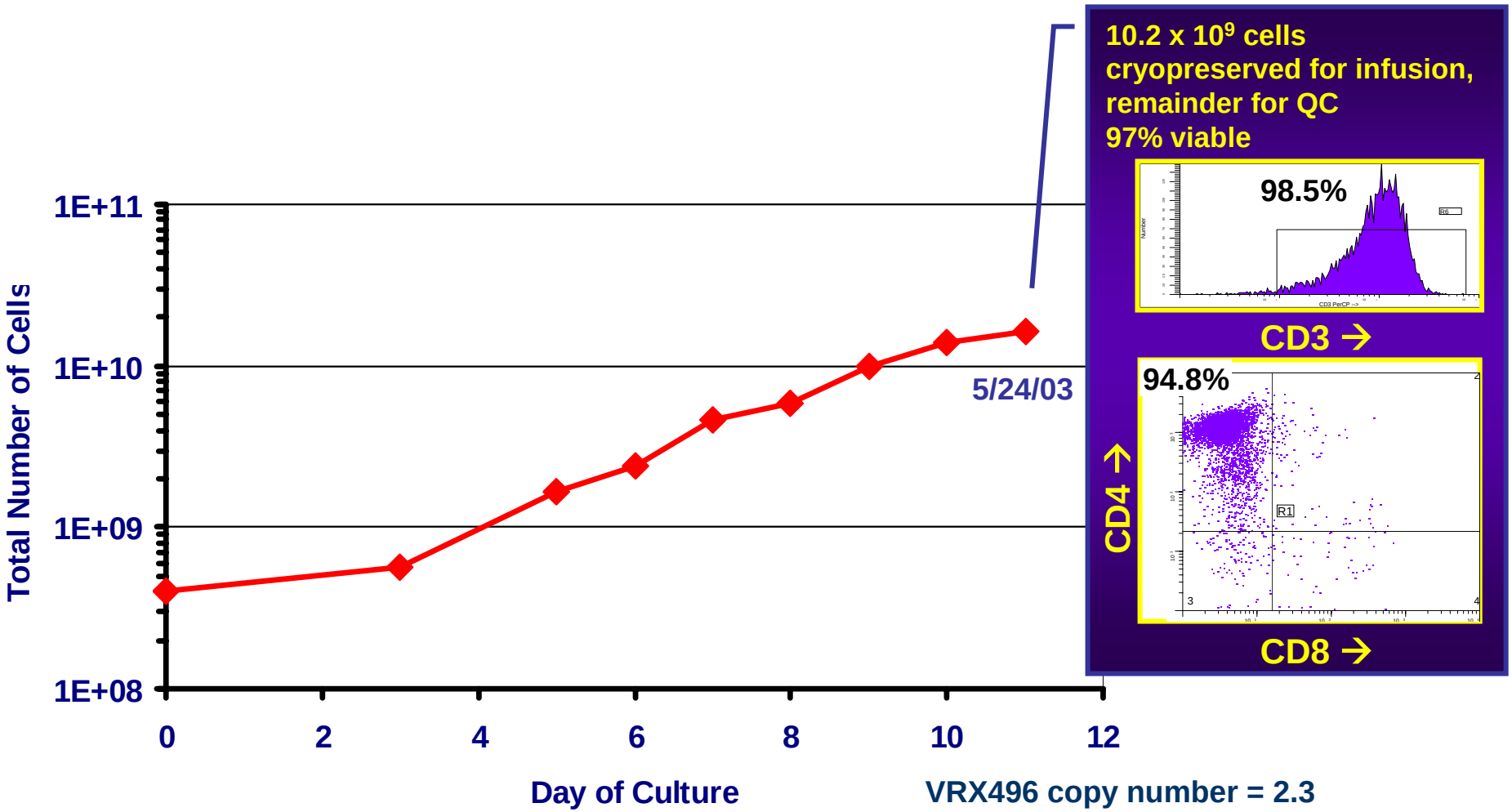
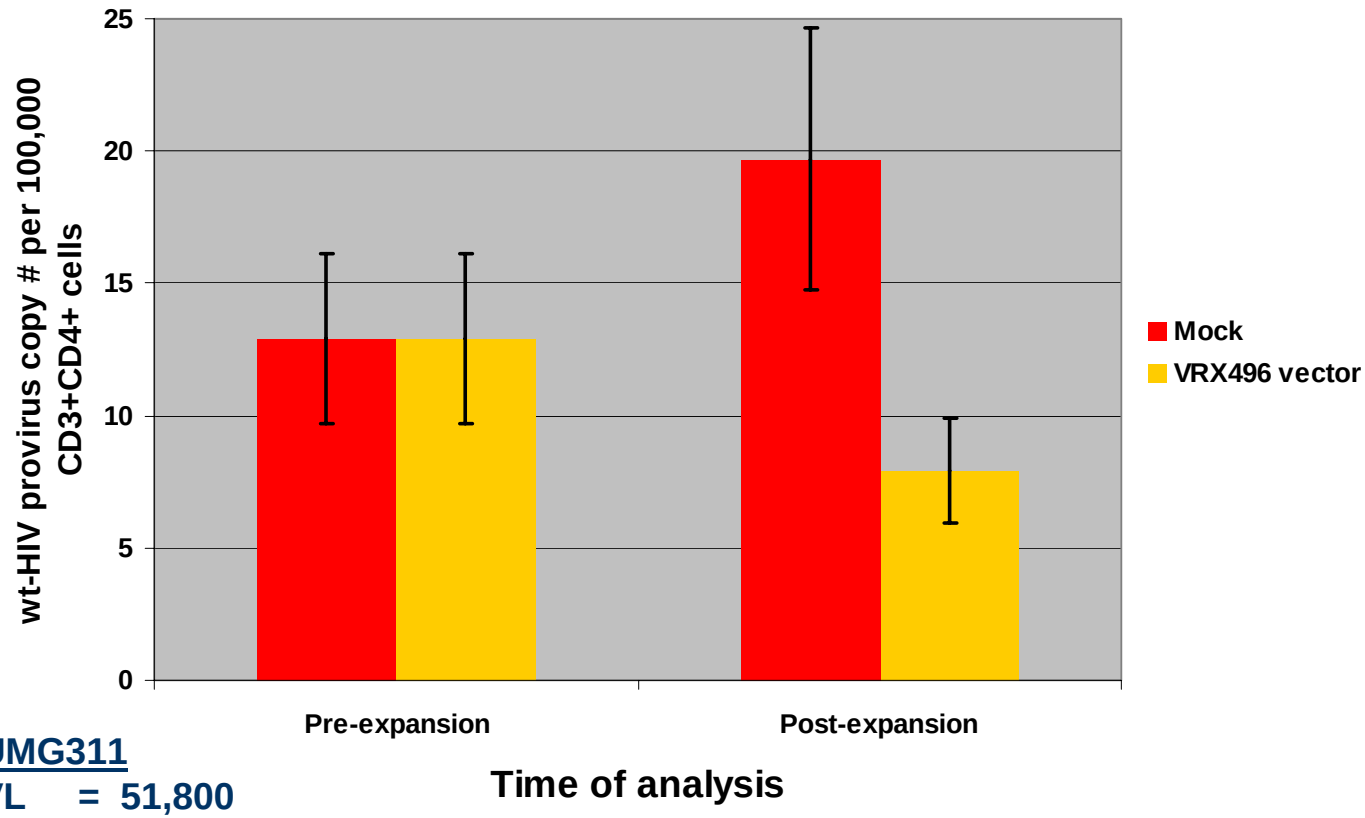
Patient 1 cell growth and phenotype



Ex vivo expansion of HIV infected T cells treated with VRX496 does not increase the number of proviral wt-HIV copies in product



UMG311
VL = 51,800
CD4 = 372

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VRX496 T cell Culture Results

Subject	#1	#2	#3	#4
# cells infused	10^{10}	10^{10}	0.6 x 10^{10}	nd
Viability (%)	71	91	76	nd
Vector Copy #	2.3	1.8	1.0	8.3 (not dosed)
%CD3+	95	100	80	96
%CD3+CD4+	93	76	74	64
P24 (ng/ml)	<50	<50	<50	nd
RCL	Not detected	Not detected	Not detected	Not done

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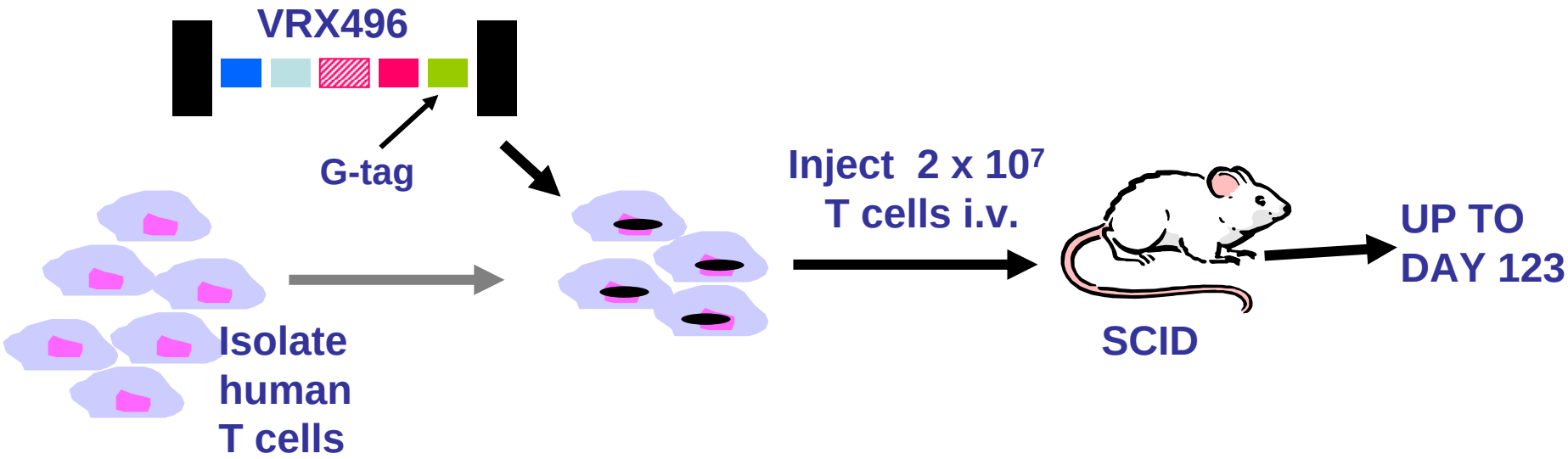
Preclinical animal data



- A total of 50 mice were dosed with VRX496 T cells in this study
- Average copy number in VRX496 T cells = 6 vector copies per cell
- All of the human T cells cleared by day 123
- No pathologic findings related to VRX496 T cells found in mice

- Copy number specification for clinical trial = 0.5 to 5

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Phase I trial: Study overview

Design:	Open label, single center
Sample size:	5 patients
Patient Type:	Failed at least 2 HAART regimens
	Viral Load $\geq$5,000
	CD4 between 150 to 500 counts/mm^3
Dosing:	Autologous VRX496 transduced CD4 T cells Single infusion (10^{10} cells)
	Patients dosed serially after DSMB review
Objectives:	Safety and Tolerability
	Changes in VL & CD4, Immune responses

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Patient visit schedule

Screen	Apheresis	Baseline

Dose	24-48-72 Hours	7-14-21-42 days	3-6-9 Months

Yearly for 15 years

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Patient monitoring

- Viral Load
- CD4 counts
- Vector persistence
- Physical Examination
- Chemistry/Hematology/Urinalysis
- Adverse Experiences
- VSV-G DNA
- VSV-G antibody
- TCR vβ repertoire
- RCL

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Independent Data Safety Monitoring Board (DSMB)

- **Judith Currier, M.D.,** Adjunct Assistant Professor of Infectious Diseases at UCLA Medical Center, Los Angeles
- **Andrew Zolopa, M.D.,** Associate Professor of Medicine, Infectious Diseases at Stanford University Medical Center, Stanford
- **Joel Gallant, M.D.,** Director of the AIDS Service, Johns Hopkins University Hospital, Baltimore

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Patient Characteristics

Characteristic	Patient 1	Patient 2	Patient 3	Patient 4
Age	41	44	40	27
Gender	M	M	M	M
Ethnic Group	Caucasian	Caucasian	African American	African American
Mean Viral Load	188,500	54,100	46,150	54,213
Mean CD4	228	316	241	308
HIV infection (Yrs)	15	13	15	10
Discontinued Therapy	ddC; D4T; Sanquinavir Norvir	AZT; 3TC; Ritonavir Nelfinavir Delavirdine	AZT; Viracept Zerit; Videx Combivir Ziagen Trizivir	D4T; ddI Viramune Viracept Sustiva
Current Therapy	Sustiva Ziagen Kaletra 3TC;Viread	DDI; Amprenavir Lopinavir Tenofovir	None	None

First patient treated with a lentiviral vector

First infusion of VRX496T on July 21, 2003
University of Pennsylvania GCRC

Patient VRX496T- 001

Failed 2 Regimens of HAART

VL = 218,000
CD4 = 202

3 patients dosed

Unanimous approval by the DSMB to dose fourth and fifth patients given cumulative safety data on first 3 patients

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Patient Data Highlights

- Patients experienced no adverse events related to infused product
- ELISA to detect anti-VSV-G antibodies negative
- No detection of VSV-G nucleic acids in patient cells or plasma
- No change in T cell repertoire or anti-HIV immune response
- No persistent adverse effects on viral load or CD4 count
- Viral load of first three patients is lower than baseline at 9, 6 and 3 months post infusion respectively, but significance of this decrease is not established
- DSMB has recommended dosing of final two patients based on the cumulative safety data of the first three patients

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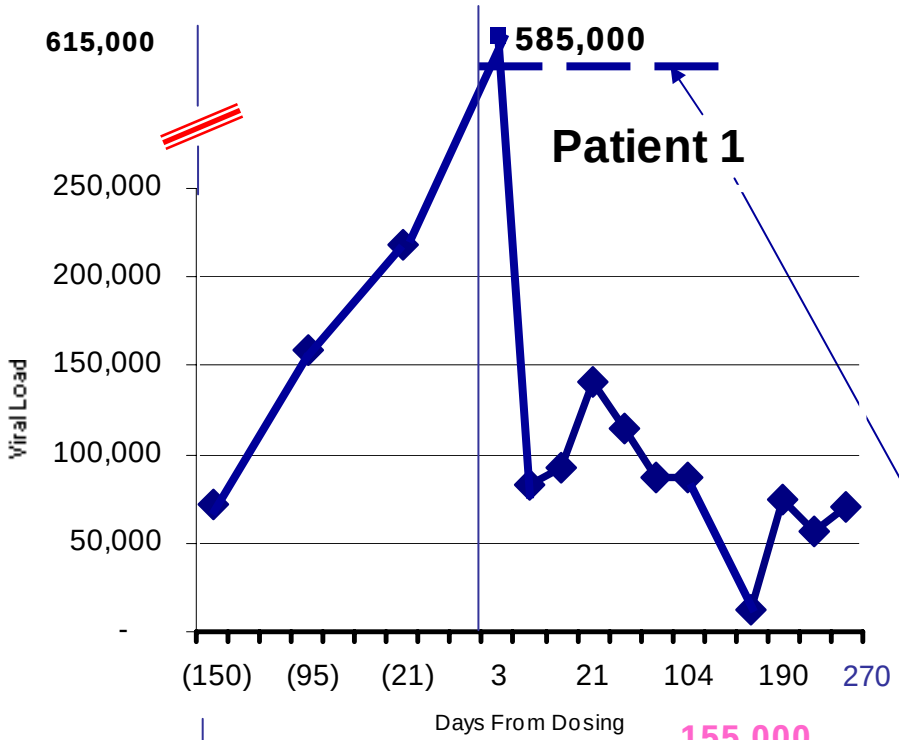
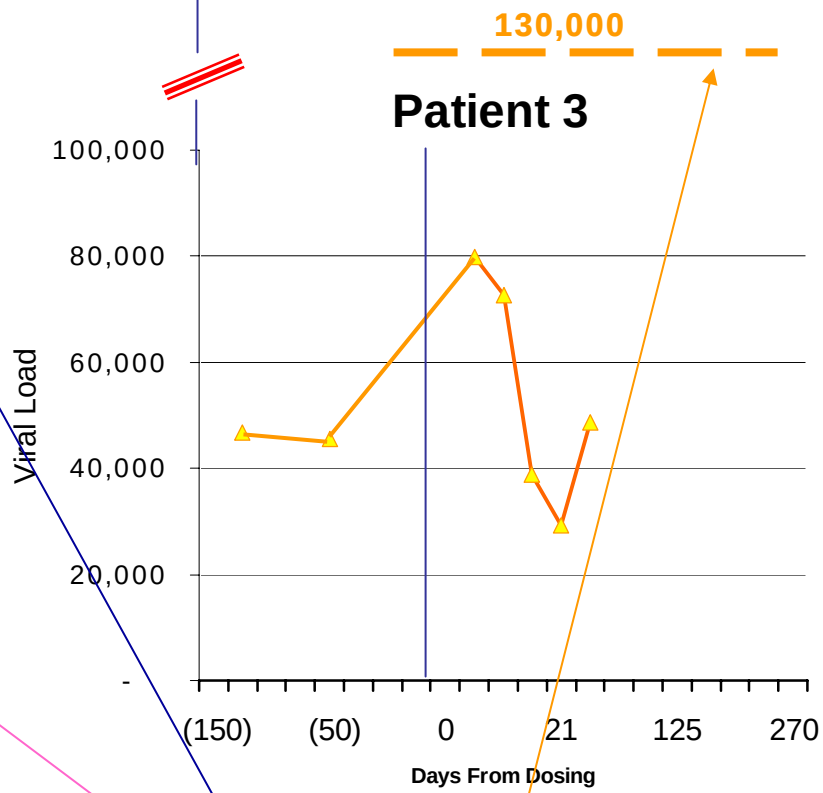
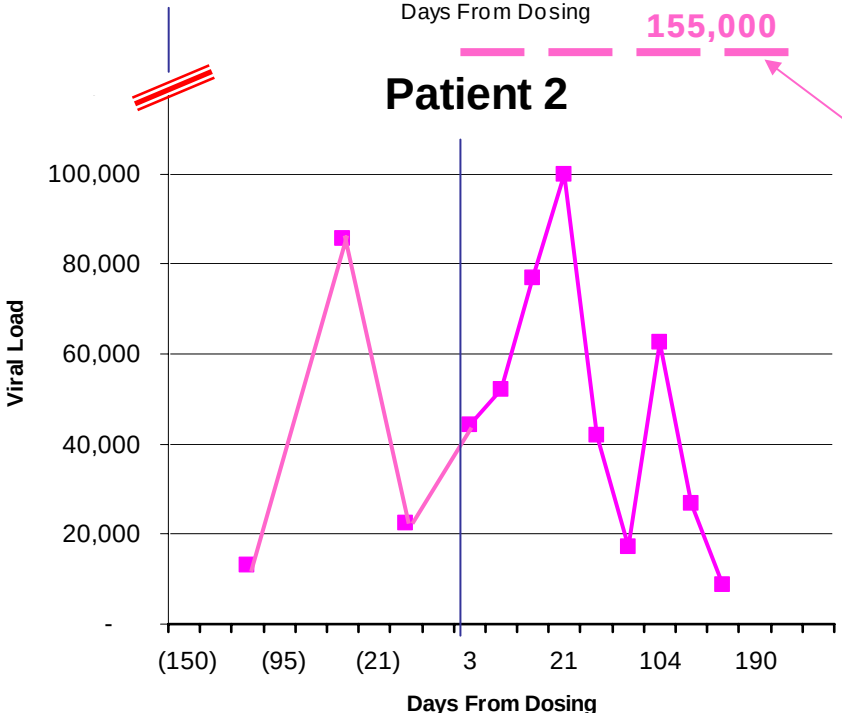
Viral Load







Safety Thresholds

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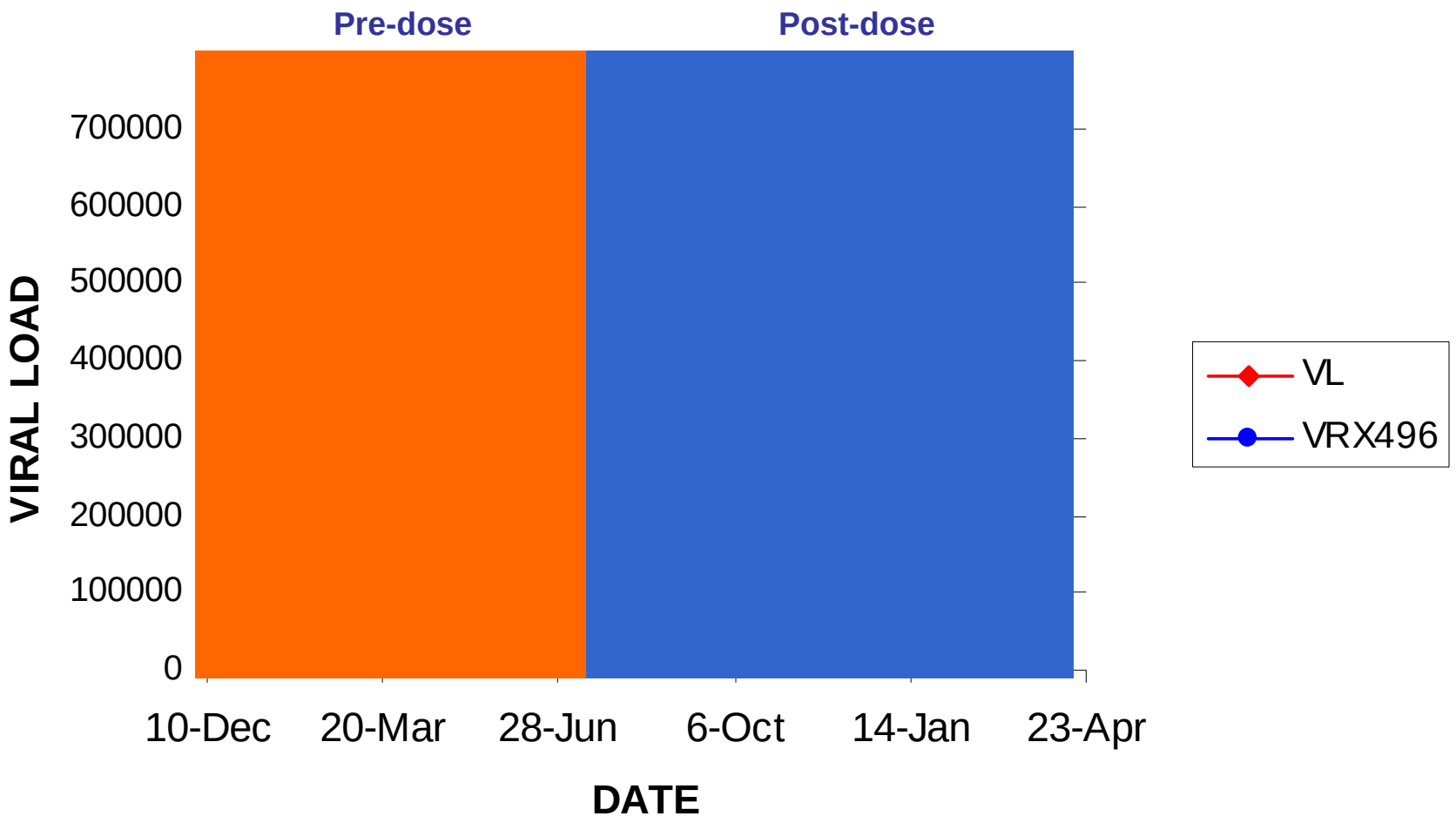
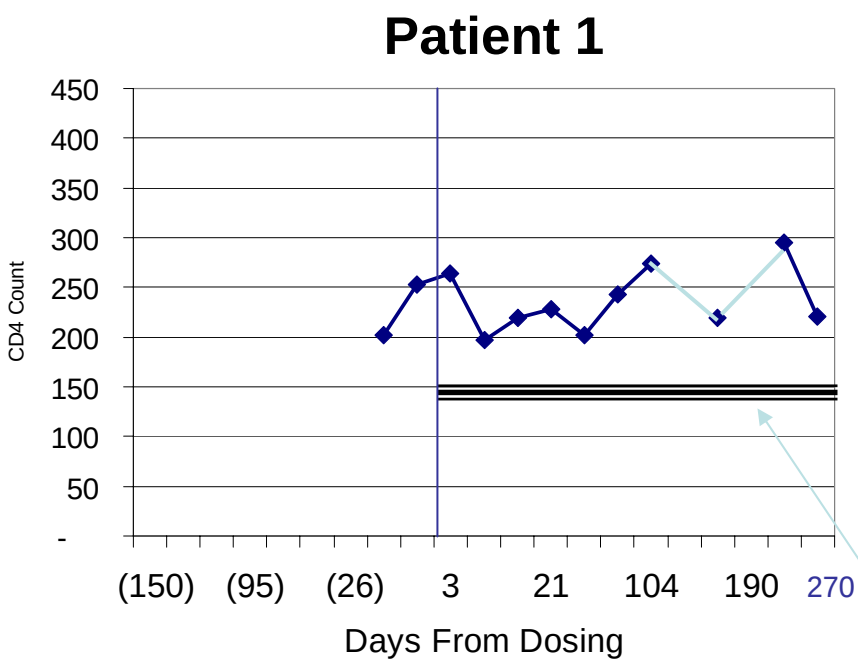
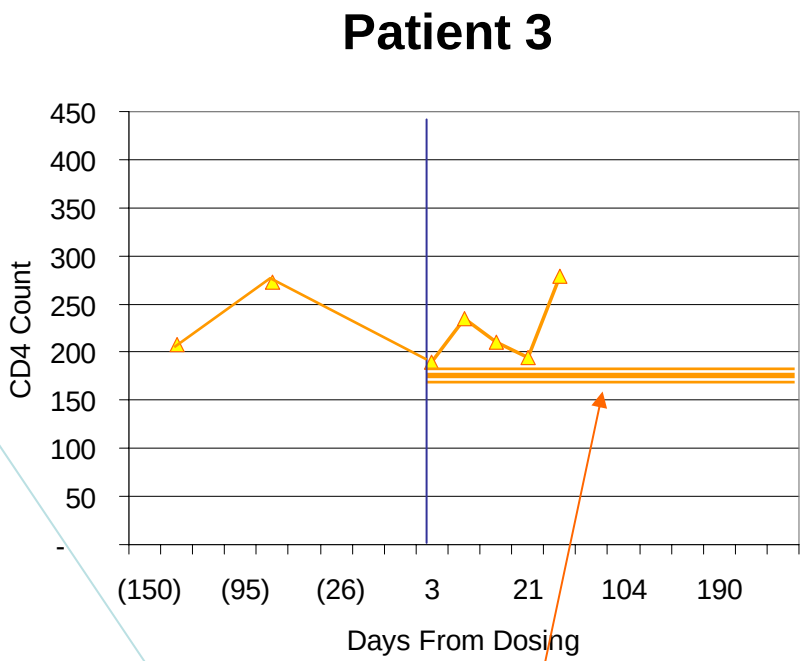
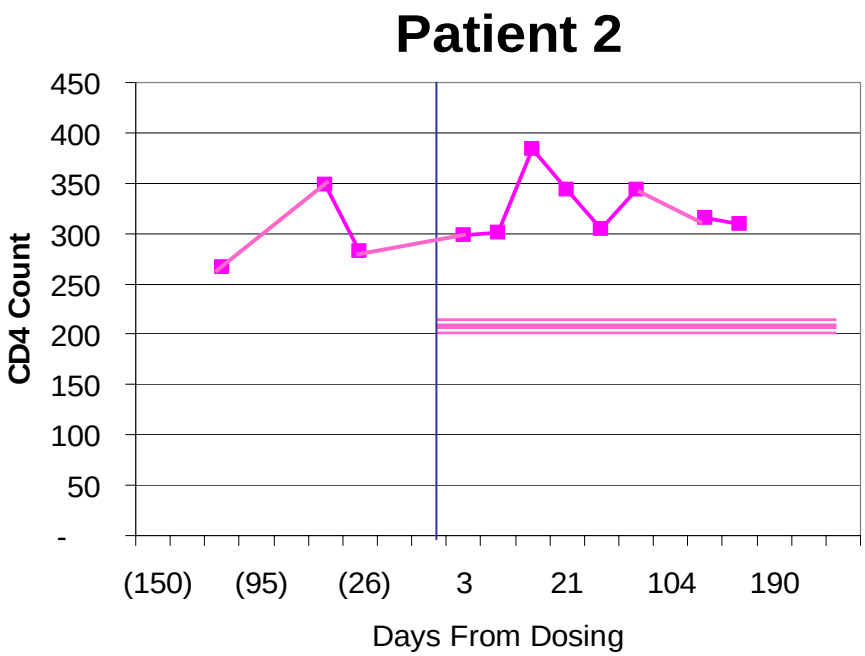
Differential Viral Load



CD4 Counts







Safety Thresholds

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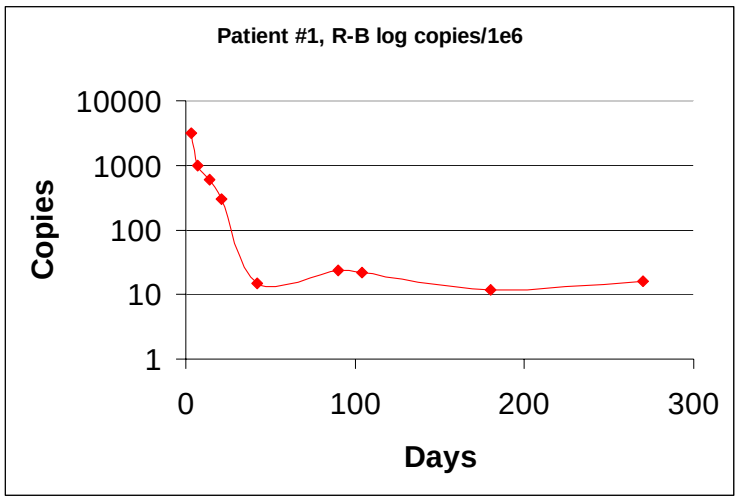
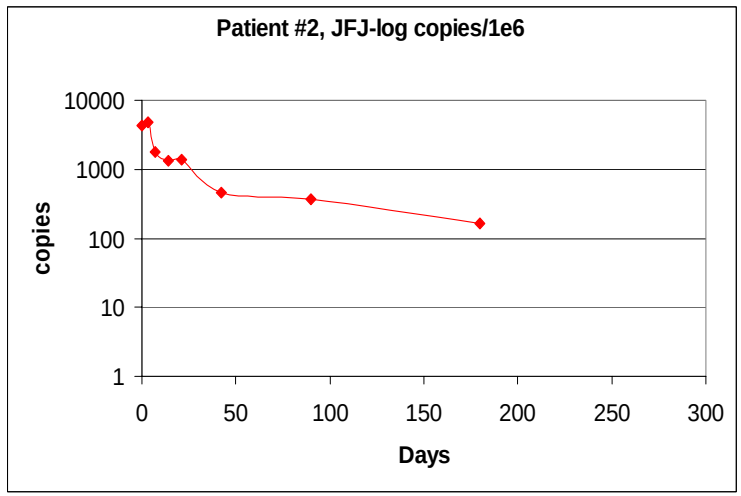
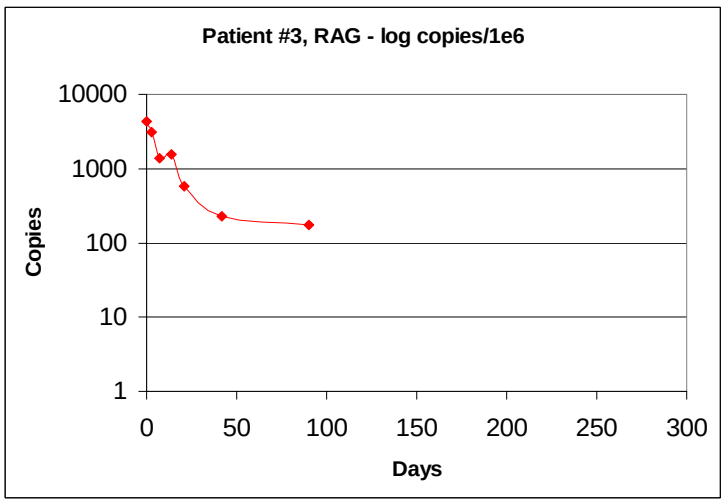
Persistence of VRX496 CD4 T cells in the blood post-infusion







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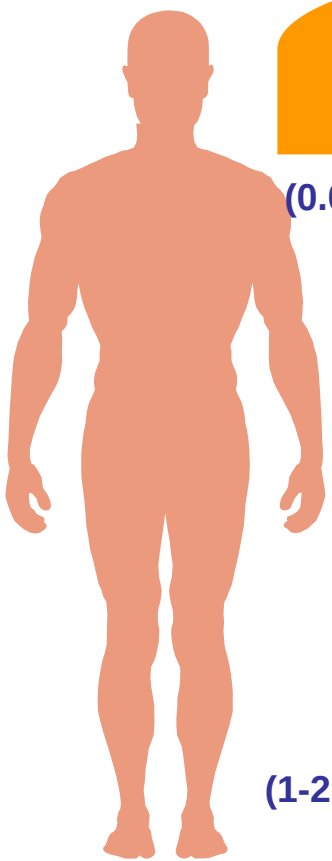
Dosing of VRX496 CD4 T cells in the ongoing phase I clinical trial



200,000,000
CD4 T cells isolated by aphaeresis
(0.04% of total 500,000,000,000 CD4 T cell mass)

T Cells

vector

10,000,000,000
CD4 T cells ex vivo
expanded 50X after
addition of vector

T Cells

10,000,000,000
CD4 T cells injected into patient
(1-2% of total 500,000,000,000 CD4 T cell
mass – normal level)

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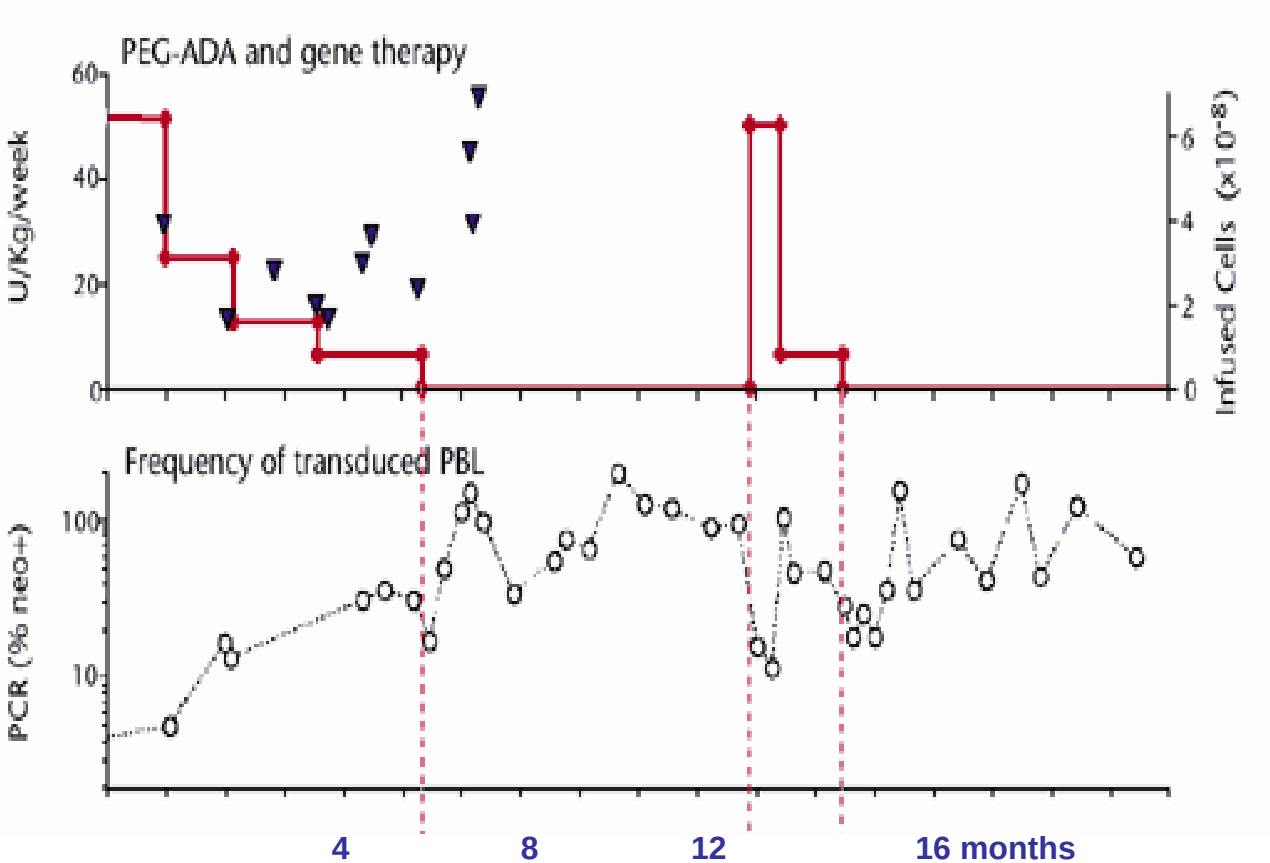
Lymphocytes with a selective advantage can expand up to 100% of body's lymphocytes

- **Persistence and expression of the ADA gene for 12 years…**
  (Muul et al Blood 2003:101:2563)

- **Immune reconstitution of ADA-SCID after PBL gene therapy**
  (Aiuti et al Nature Medicine 2002: 8:423)



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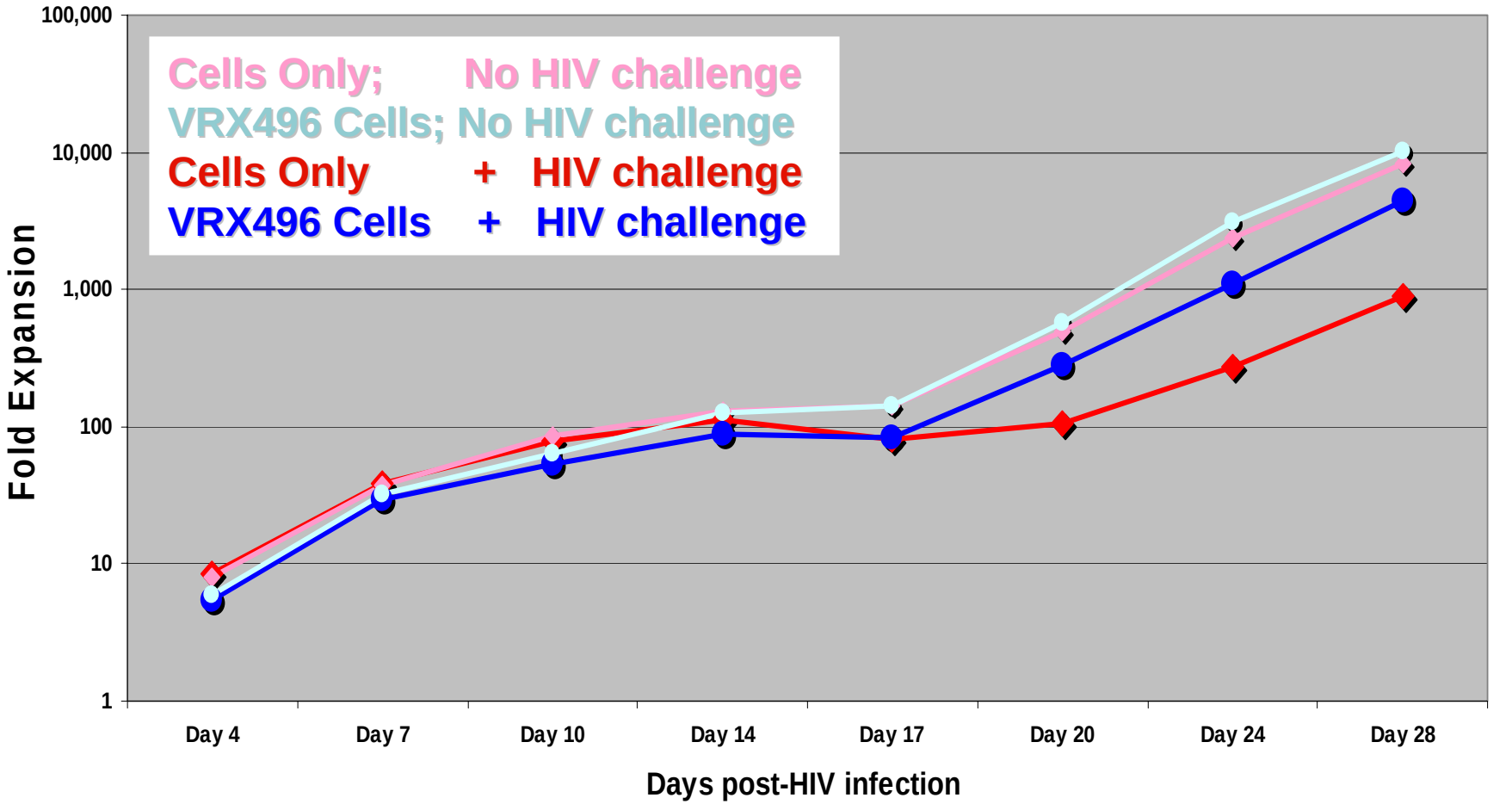
VRX496-containing CD4 T cells show selective survival advantage in vitro



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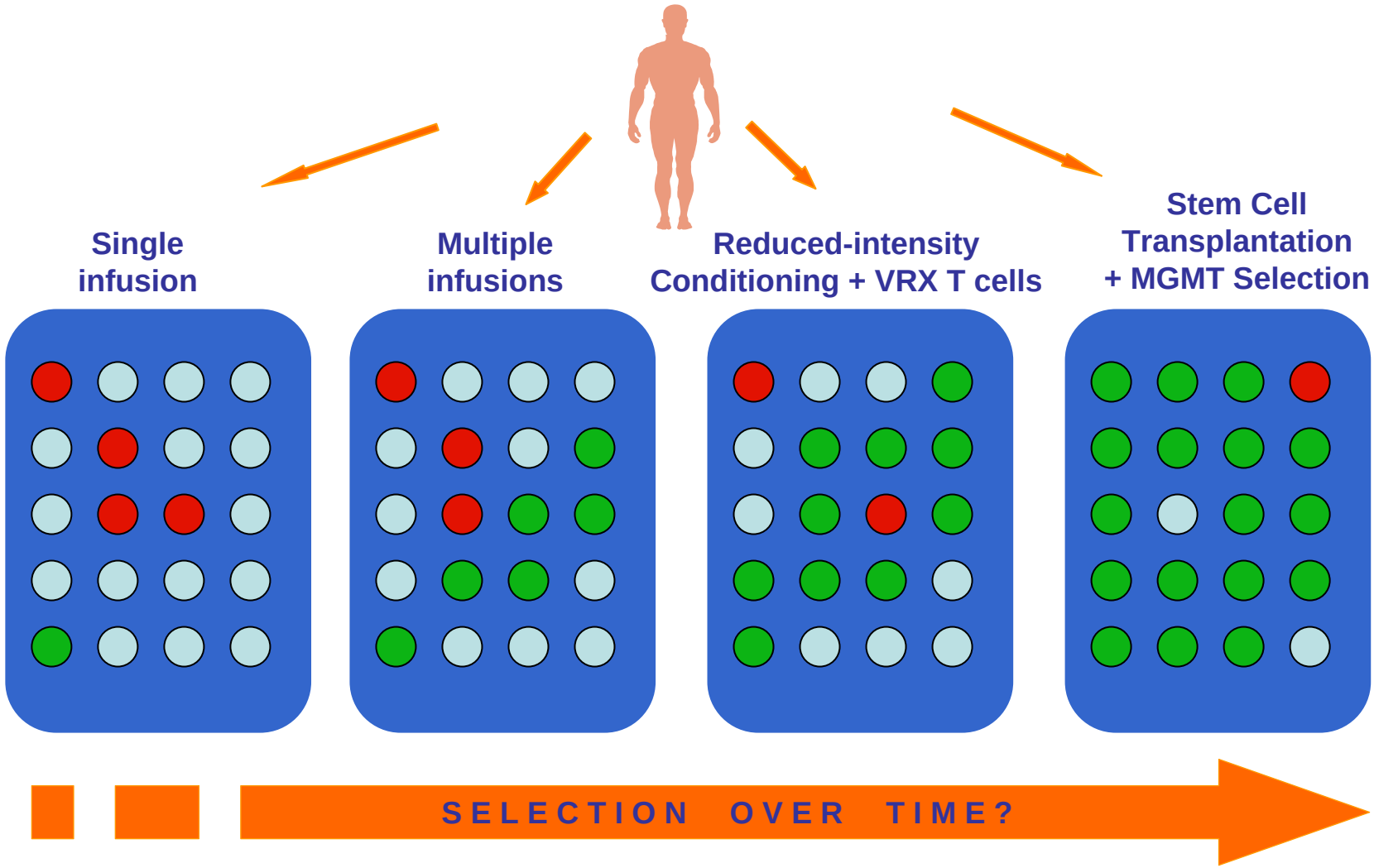
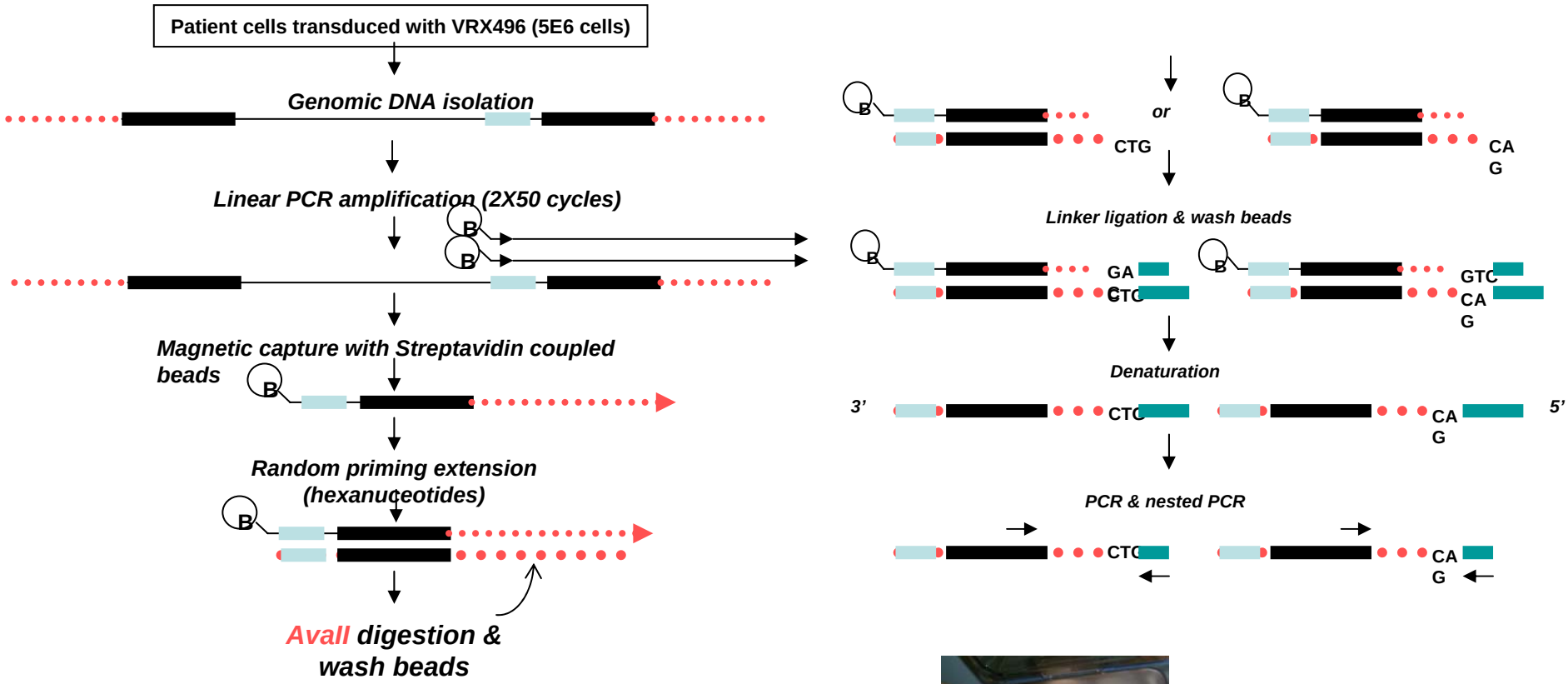
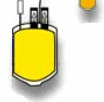
Clinical options for dosing VRX496 cells



LAM-PCR was used to detect the integration sites in infused cell product



HIV patients undergo aphaeresis



Add vector and grow cells to target dose

Final cell product is analyzed by LAM-PCR

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Preliminary insertion site analysis

	% insertions					% insertions			
Chromosome number	Schroeder *et al* HIV vector	Schroeder *et al* wt-HIV	Lu *et al* *S001-010*	Lu *et al* *S001-002*	Chromosome number	Schroeder *et al* HIV vector	Schroeder *et al* wt-HIV	Lu *et al* *S001-010*	Lu *et al* *S001-002*
1	10	2	8	3	*13*	2	2	3	3
2	5	7	5	10	*14*	2	2	3	0
3	5	19	3	3	*15*	5	2	5	7
4	5	0	5	3	*16*	7	5	0	13
5	4	5	0	0	*17*	8	9	3	0
6	8	5	3	0	*18*	0.2	0	0	0
7	5	2	5	7	*19*	6	7	3	26
8	4	0	5	10	*20*	4	0	0	3
9	2	5	8	0	*21*	1	5	0	0
10	2	5	1	0	*22*	2	0	3	0
11	5	7	27	7	*X*	3	7	3	3
12	5	9	5	3					

	number of insertions					number of insertions			
Chromosome number	Schroeder *et al* HIV vector	Schroeder *et al* wt-HIV	Lu *et al* *S001-010*	Lu *et al* *S001-002*	Chromosome number	Schroeder *et al* HIV vector	Schroeder *et al* wt-HIV	Lu *et al* *S001-010*	Lu *et al* *S001-002*
1	49	1	3	1	*13*	8	1	1	1
2	24	3	2	3	*14*	10	1	1	0
3	25	8	1	1	*15*	24	1	2	2
4	22	0	2	1	*16*	34	2	0	4
5	21	2	0	0	*17*	38	4	1	0
6	36	2	1	0	*18*	1	0	0	0
7	24	1	2	2	*19*	31	3	1	8
8	17	0	2	3	*20*	18	0	0	1
9	10	2	3	0	*21*	6	2	0	0
10	7	2	1	0	*22*	13	0	1	0
11	25	3	10	2	*X*	14	1	1	1
12	24	4	2	1	**TOTAL:**	**481**	**43**	**37**	**31**

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Preliminary insertion site analysis of clinically relevant CD4 T cells containing VRX496

Chromosome	Band	Gene		Chromosome	Band	Gene
1	1q22	ASH1		*13*	13q33.3	n.k.
2	2q36.2	CUL3		*14*	14q32.32	Dynein HC
3	3p24.3	n.k.		*15*	15q21.3	n.k.
4	4q12	STXBP1L1		*16*	16p11.2	n.k.
5	n.d.	n.d.		*17*	17p13.1	n.k.
6	6p21.31	n.k.		*18*	n.d.	n.d.
7	7q32.3	n.k.		*19*	19q13.2	MAP4K1
8	8q24.3	PLEC1		*20*	n.d.	n.d.
9	9p22.3	PSIP2		*21*	n.d.	n.d.
10	10p14	n.k.		*22*	22q12.3	n.k.
11	11p15.4	n.k.		*X*	xq28	n.k.
12	12q13.2	RNF41				

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VRX496 integration Site distribution in the infused CD4 T cell product

Gene coding reference sequence databases

Data set	Acembly	GeneScan	RefSeq	Unigene
VRX496 Clinical product (n=133)	108 (81.20%)	105 (78.95%)	77 (57.89%)	90 (67.67%)
Summary HIV data **(Bushman)** (n=2274)	1851 (81.40%)	1754 (77.13%)	1336 (58.75%)	1538 (67.63%)
Random (n=2022)	903 (44.66%)	1303 (64.44%)	524 (25.91%)	675 (33.38%)

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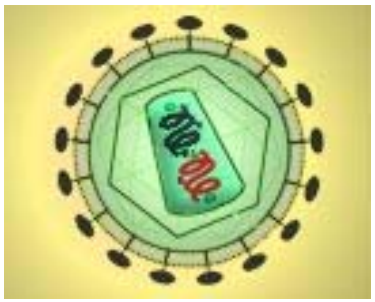
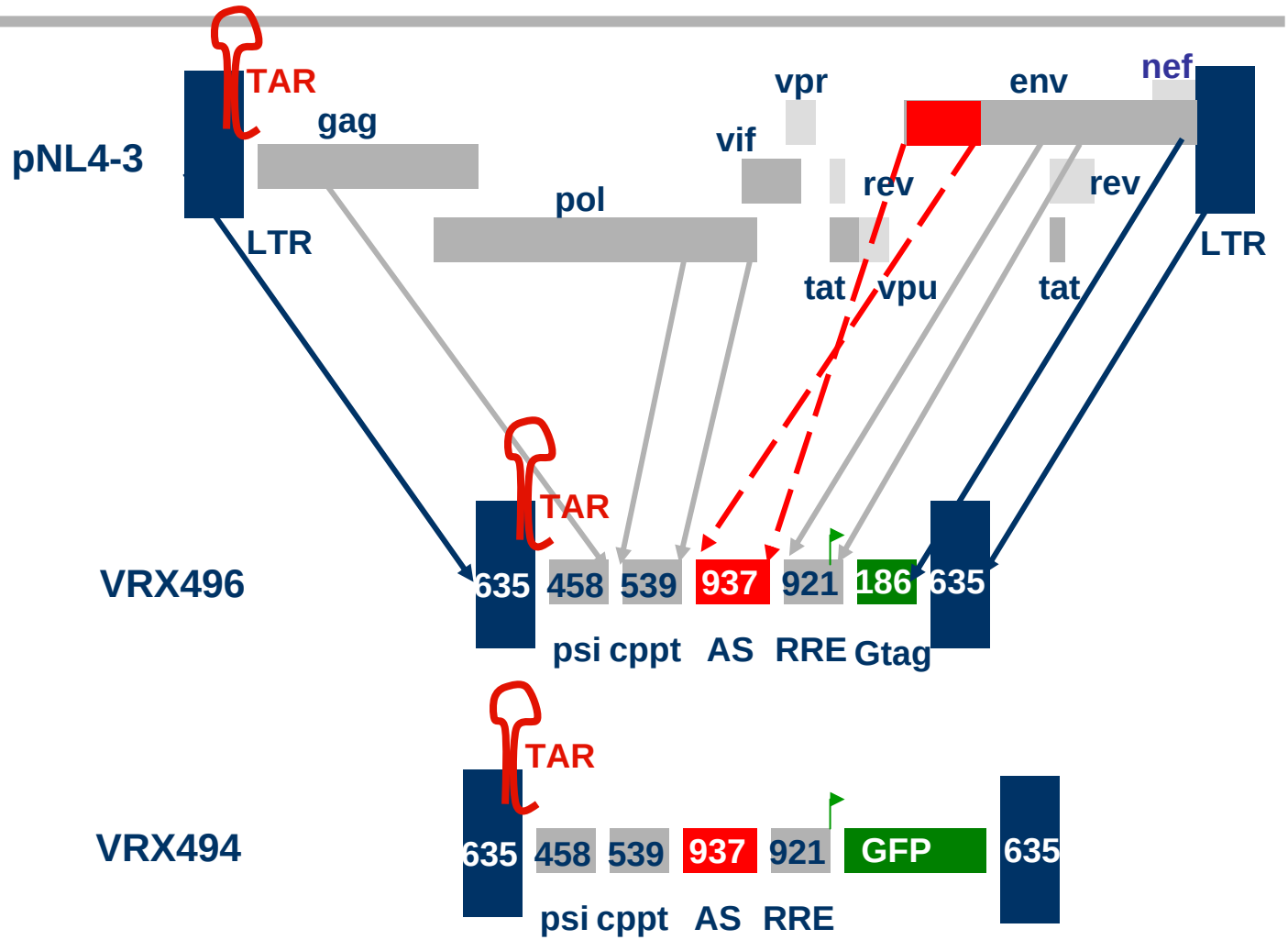
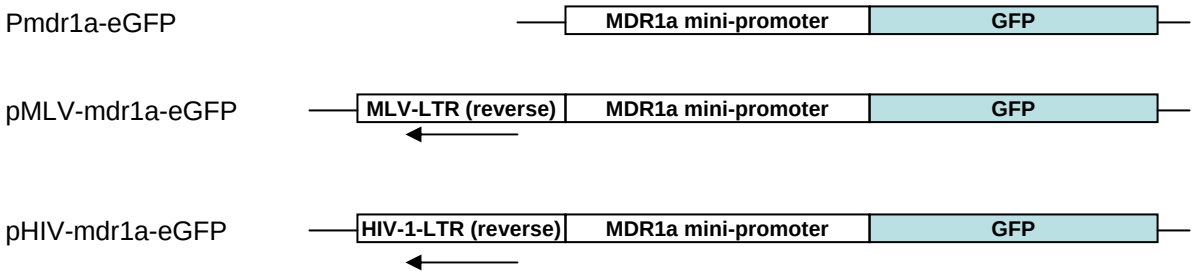
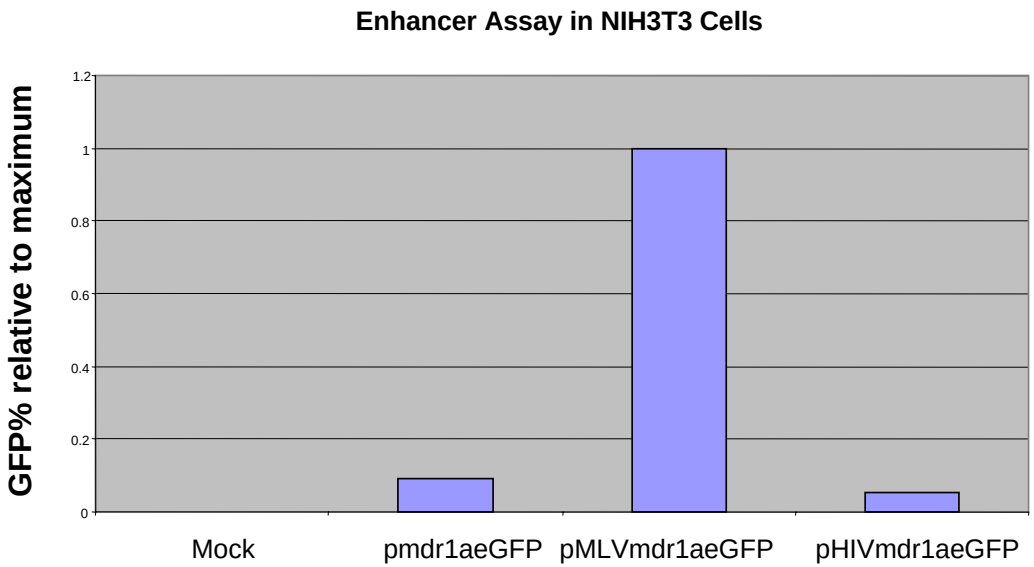
HIV vectors express GFP and antisense from the native HIV LTR

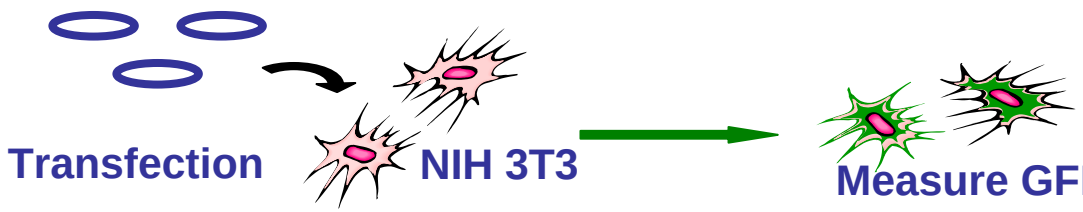




Relative enhancer activity of a MLV and HIV LTR upon a minimal MDR-1 promoter sequence







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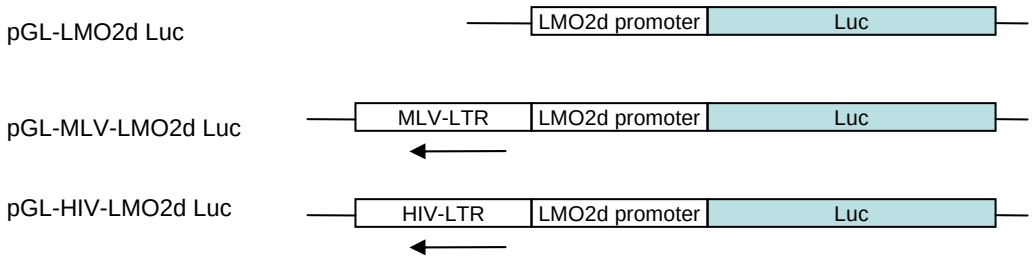
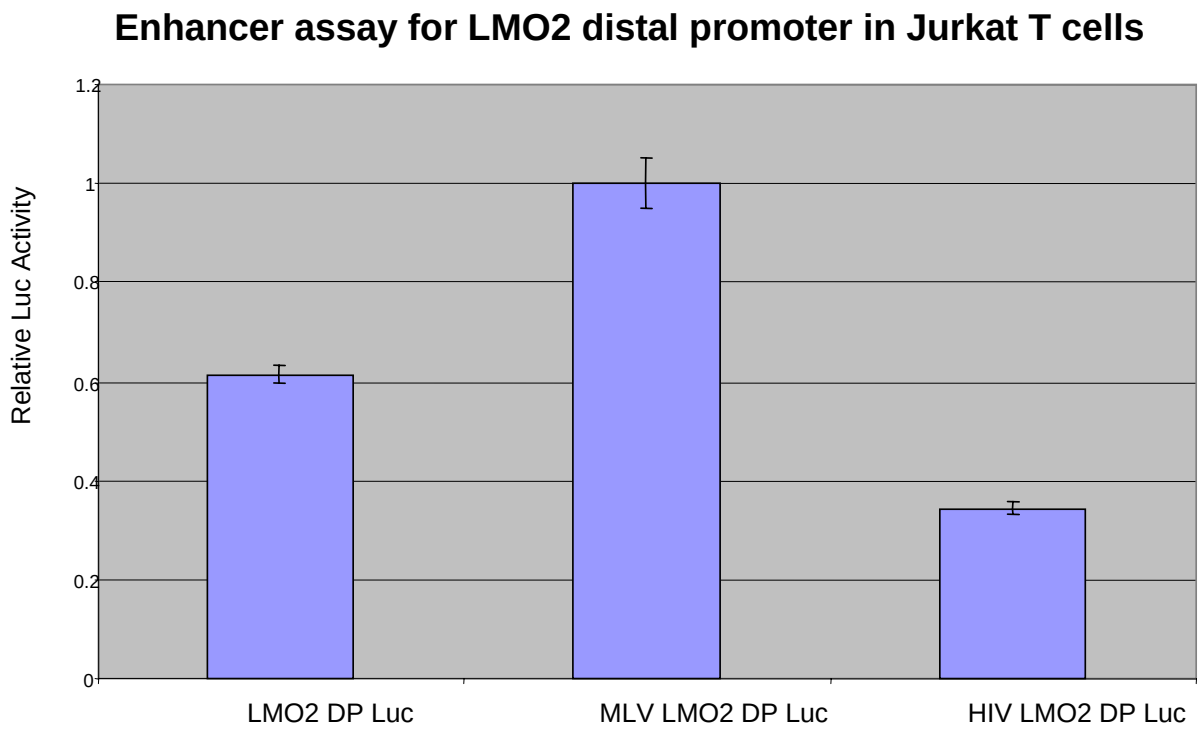
Relative enhancer activity of a MLV and HIV LTR upon the distal LMO2 promoter sequence





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Plan for Phase II trials

DESIGN: Open label, multi-center, multiple infusion

STUDY CENTERS: South Africa; United States

SAMPLE SIZE: up to 30 patients in each country

PATIENT TYPE:

South Africa	United States
Treatment naïve	Failed 1 HAART
Viral Load ≥5,000	Viral Load ≥5,000
CD4 ≥ 350 counts/mm^3	CD4 above 150/mm^3

DOSING: Autologous, multiple infusions (10^9 - 10^{10} VRX496 CD4 T cells)

OBJECTIVES: Safety and Tolerability

Changes in VL & CD4, Immune responses (activity)

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Phase II multidose clinical trial plan

- Each patient will receive up to 8 infusions; 2 cycles of 4 infusions each
- Criteria for proceeding to the next dose
  - ✓ Safety
  - ✓ Viral Load

    - decrease is not greater than 1 log from pre-established baseline

    - if VL decrease > 1 log → monitor

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Summary

- HIV-based lentiviral vector expressing antisense
  - transduction of primary human T cells >90%
  - inhibition of HIV replication by >100 fold
- A phase I clinical trial using VRX496 has been initiated and 3 patients have been dosed
  - No adverse events related to VRX496
- Integration site analysis on VRX496 containing clinical samples in progress
- A phase II clinical trial is planned for the US and South Africa – multiple dosing regimen

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