PHASE I/II TRIAL OF STEM CELL GENE THERAPY FOR HIV IN AIDS LYMPHOMA PATIENTS

Mehrdad Abedi, Joseph Anderson, Gerhard Bauer,

UC Davis Medical Center, Bone marrow Transplant Unit and Center For Regenerative Cures.

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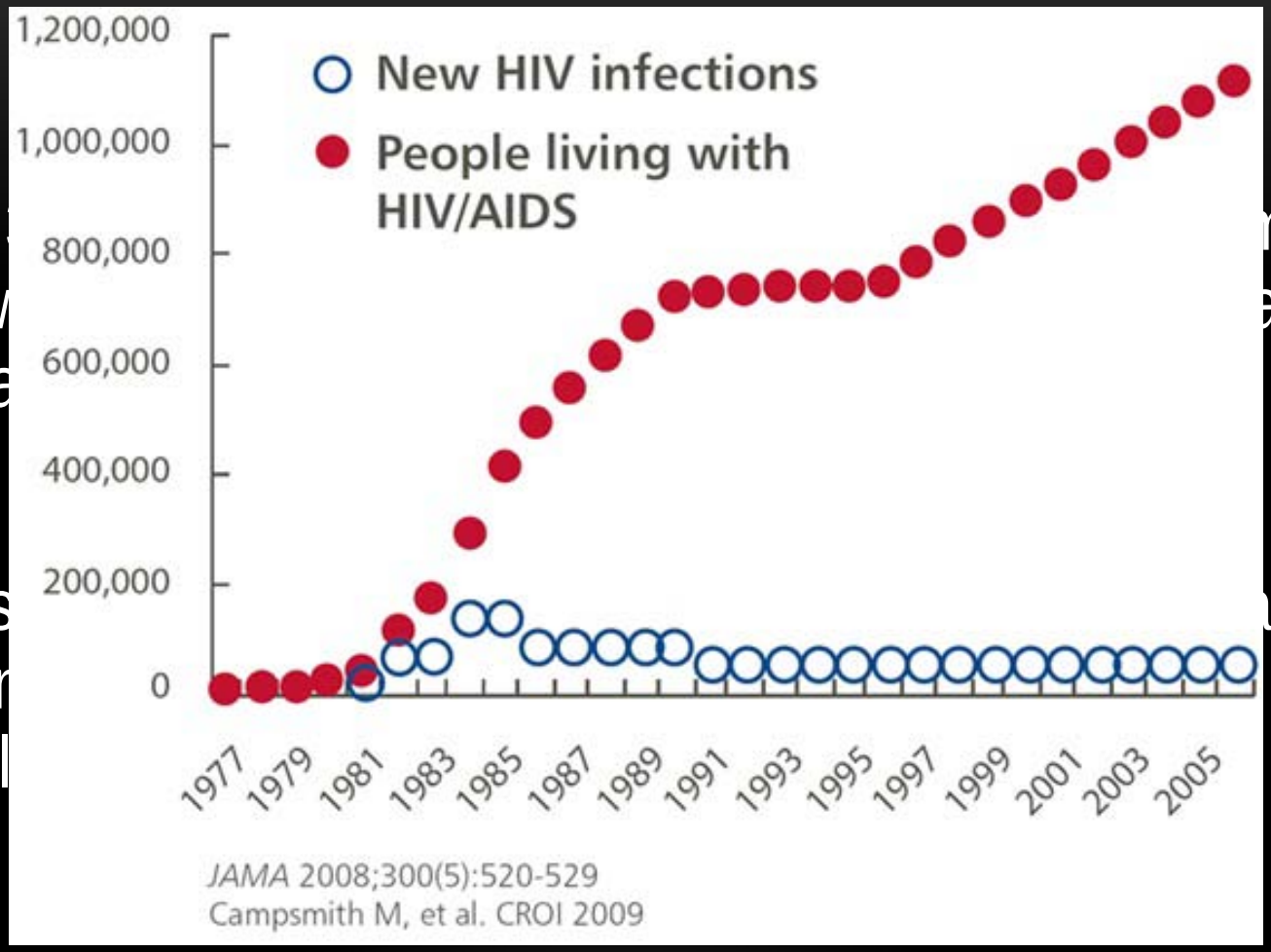
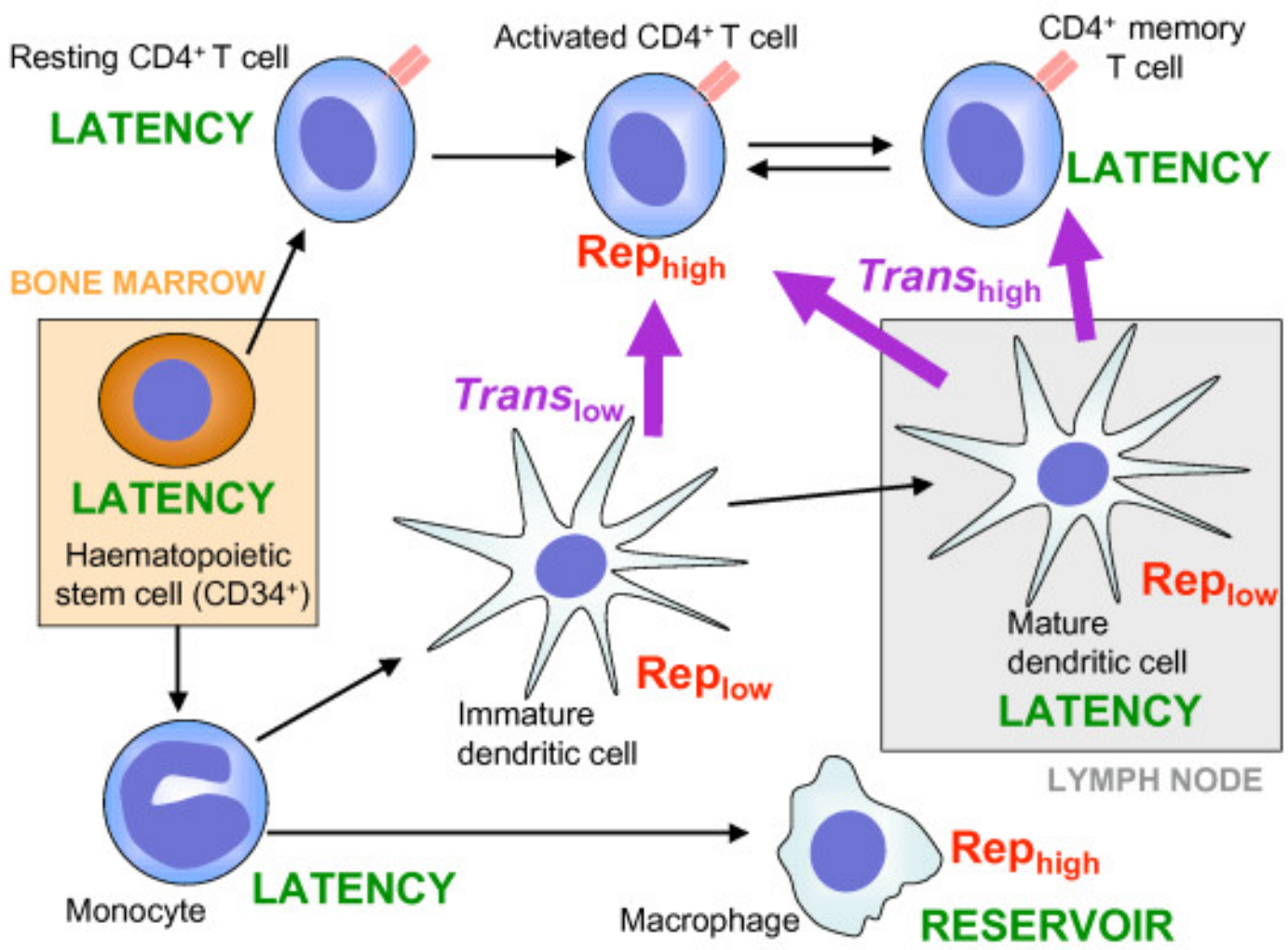


Progress report 2011: Global HIV/AIDS response

Epidemic update and health sector progress towards universal access
WHO, UNICEF, UNAIDS

LATENT RESERVOIRS OF HIV



Microglial and Kupffer cells

CURRENT APPROACHES OF GENE THERAPY IN HIV

- Different gene products
- Peripheral T-cell therapies
- Hematopoietic stem cell therapies

HEMATOPOIETIC STEM CELL THERAPY APPROACH

- Pros:

Effect of the treatment is permanent

Does not require multiple interventions

May eradicate “at least theoretically” all the HIV reservoirs

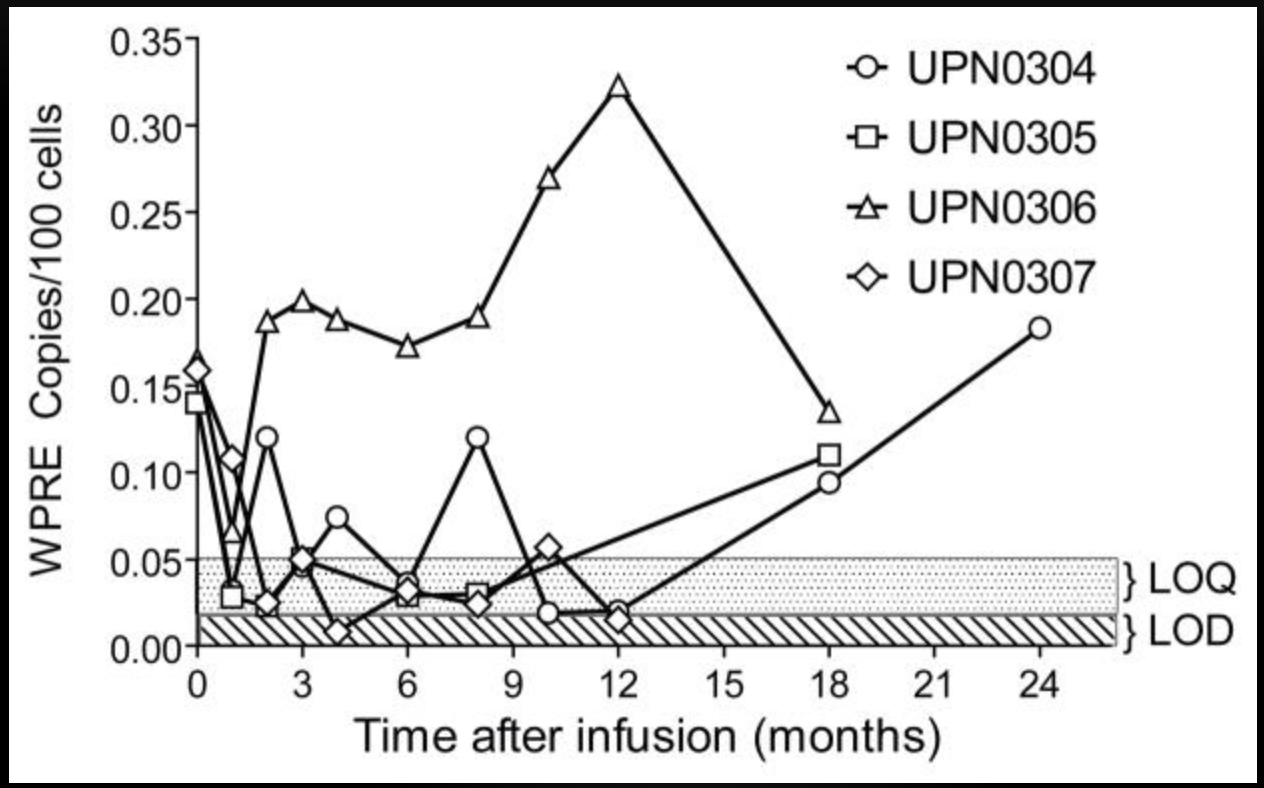
- Cons

Very small transduction efficiency (clinically irrelevant)

Possibility of selection for resistant clones

Higher chance of “again theoretically” abnormal clone development

RECENT ANTI-HIV GENE THERAPY CLINICAL TRIALS



DiGiusto et al. Sci Transl Med. 2010 June 16; 2(36): 36ra43.

HOW CAN WE IMPROVE THE OUTCOME

- Improve the transduction efficiency
- Improve the viability of the product
- Prevent the development of the escape mutant
- Prevent/decrease the risk of insertional mutagenesis
- Augment the expansion of gene modified immune cell clones

HOW CAN WE IMPROVE THE OUTCOME

- Improve the transduction efficiency

  Much better transduction efficiency of the product

- Improve the viability of the product

  Changing the sequence of transduction/freezing/thawing

- Prevent the development of the escape mutants

  Triple anti-HIV gene approach

- Prevent/decrease the risk of insertional mutagenesis

  Self-inactivating vector with a poly-A signal in the 3'UTR; U6 promoters specific transcription termination signals.

- Augment the expansion of gene modified immune cell clones

  Therapeutic ART withdrawal

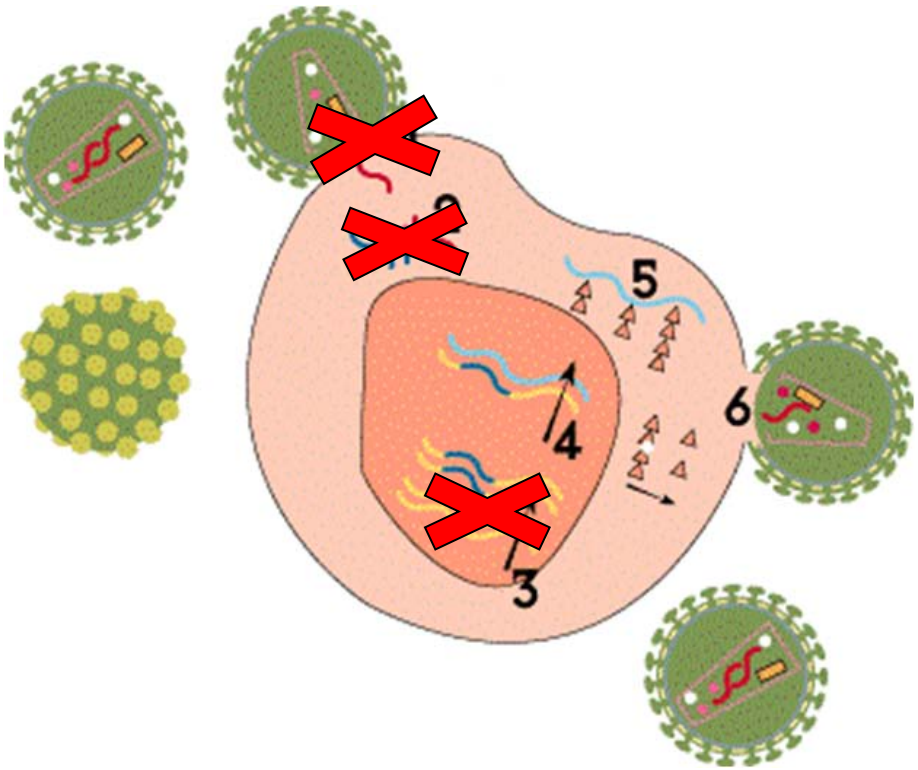
CANDIDATE PRODUCT

HIV resistant gene modified HSC

- an shRNA against CCR5, Cell entry phase
- a chimeric Trim5α: pre-integration stage by disrupting viral capsid uncoating
- a TAR decoy: post-integration/pre-transcription

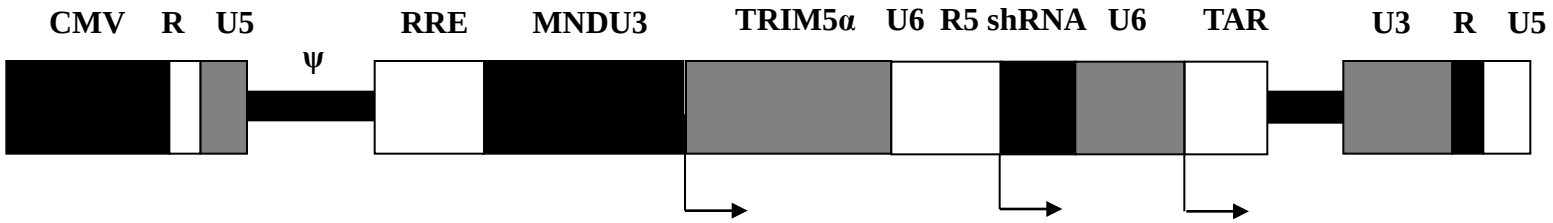
HIV Web Study (www.HIVwebstudy.org)

Combination Anti-HIV Lentiviral Vector



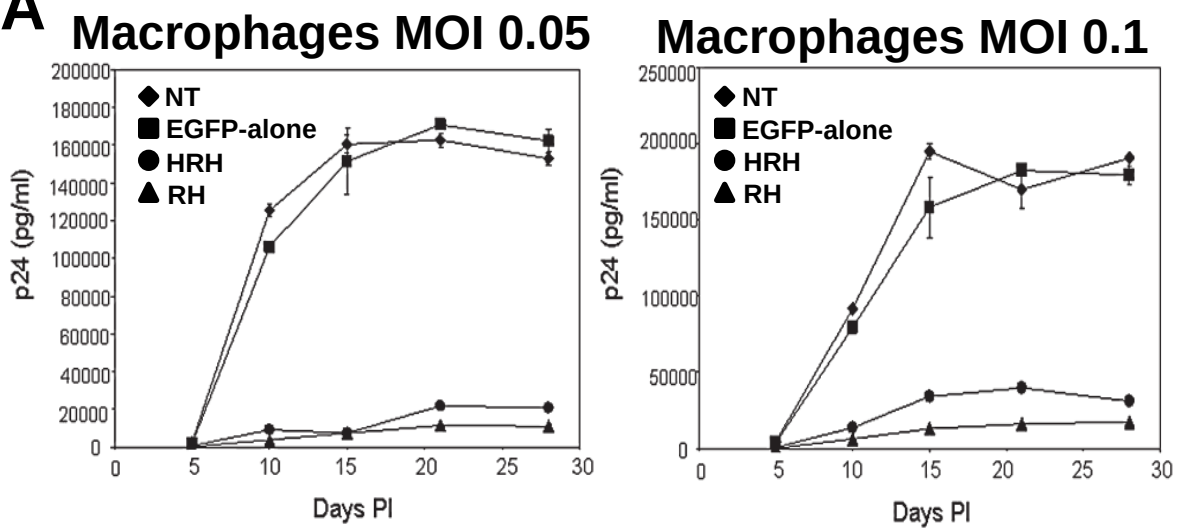
1) CCR5 shRNA (block entry)
2) TRIM5alpha (block integration)
3) TAR decoy (block replication)

CCLc-Combination-anti-HIV



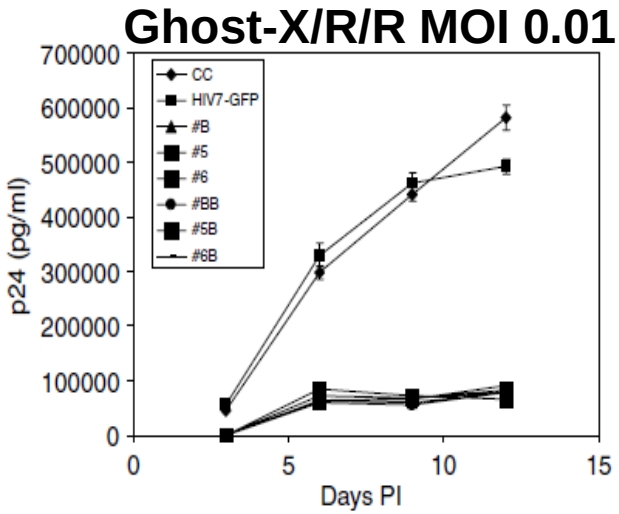
Efficacy of Single Constructs

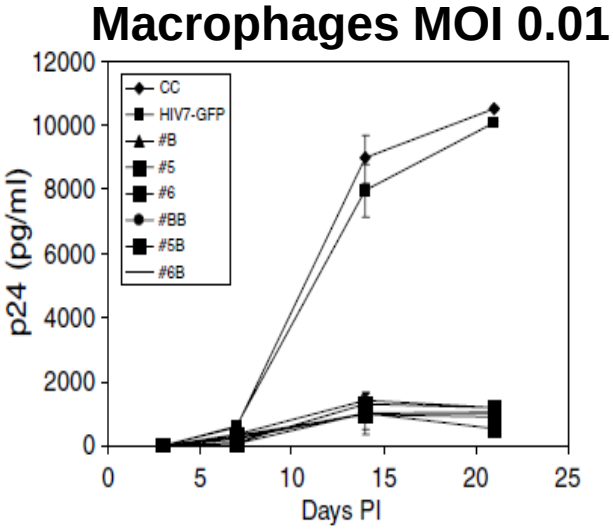
A



TRIM5α-Anderson et al. Human Gene Therapy, 2008

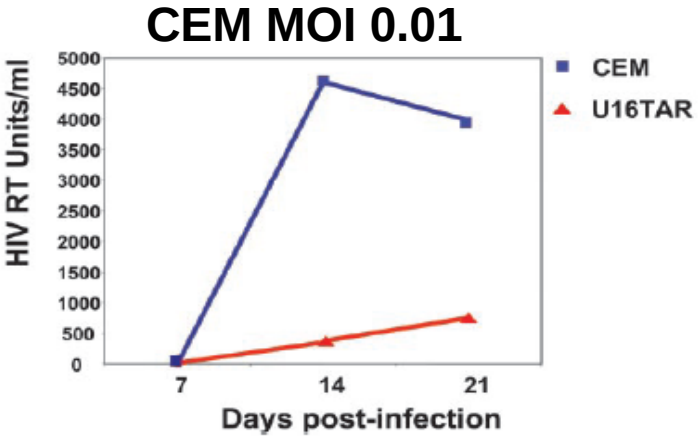
B





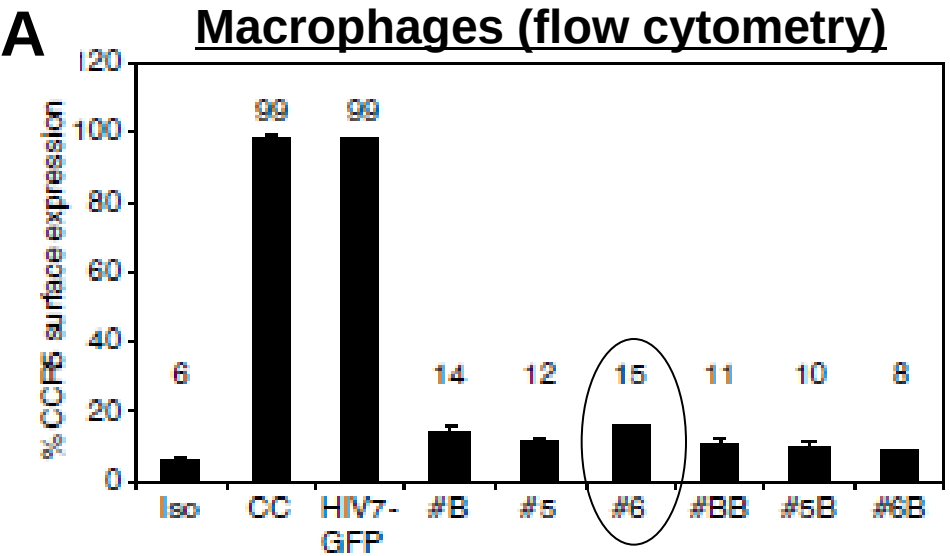
CCR5 shRNA-Anderson et al. Gene Therapy, 2007

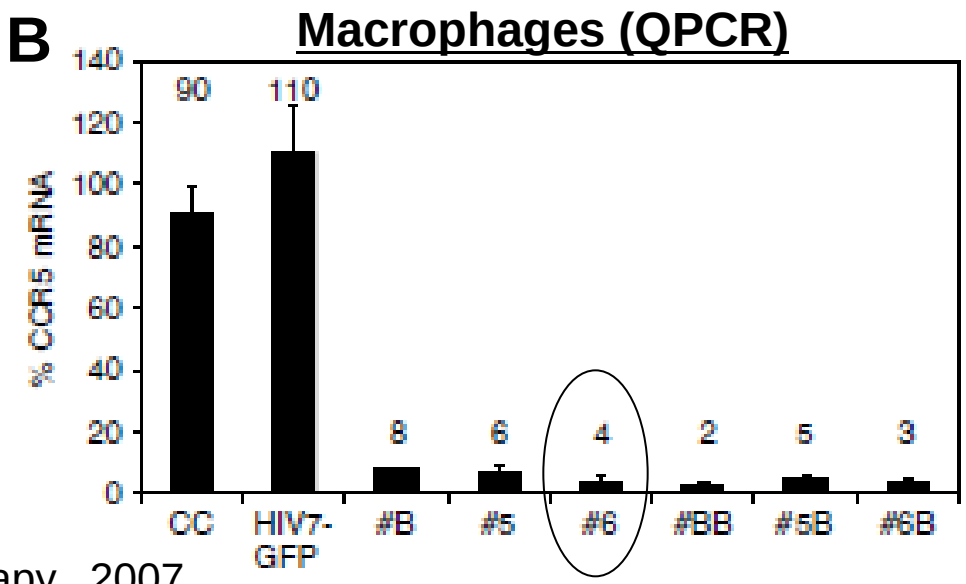
C



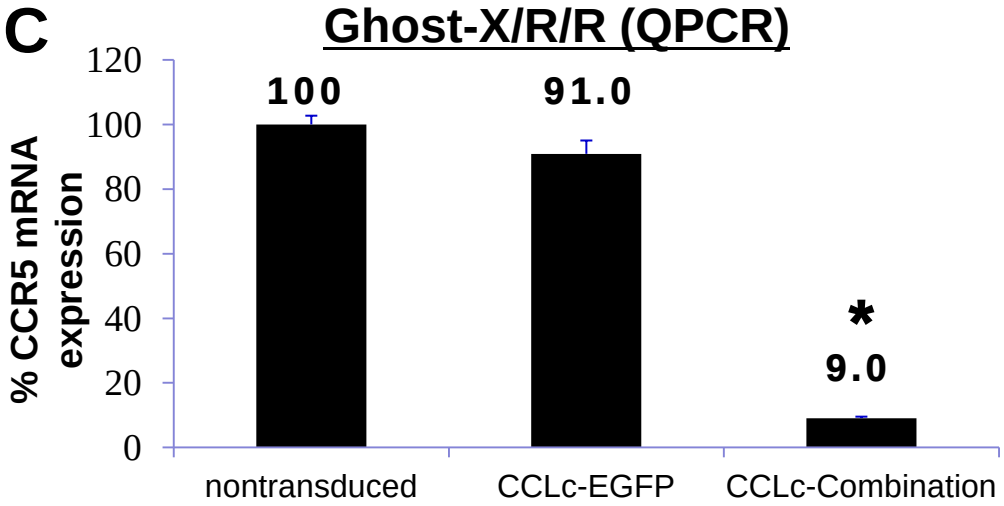
TAR Decoy-Michienzi et al. PNAS, 2002

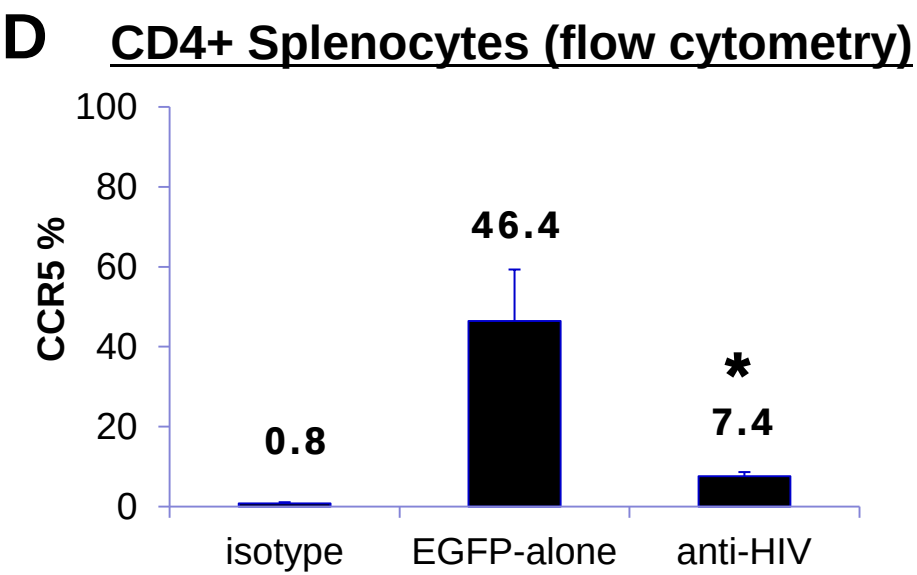
Down Regulation of CCR5





Anderson et al. Gene Therapy, 2007

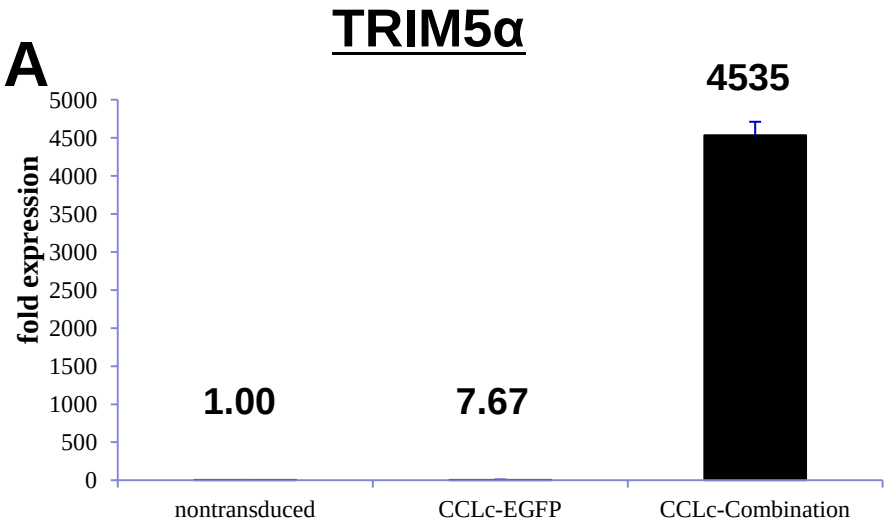


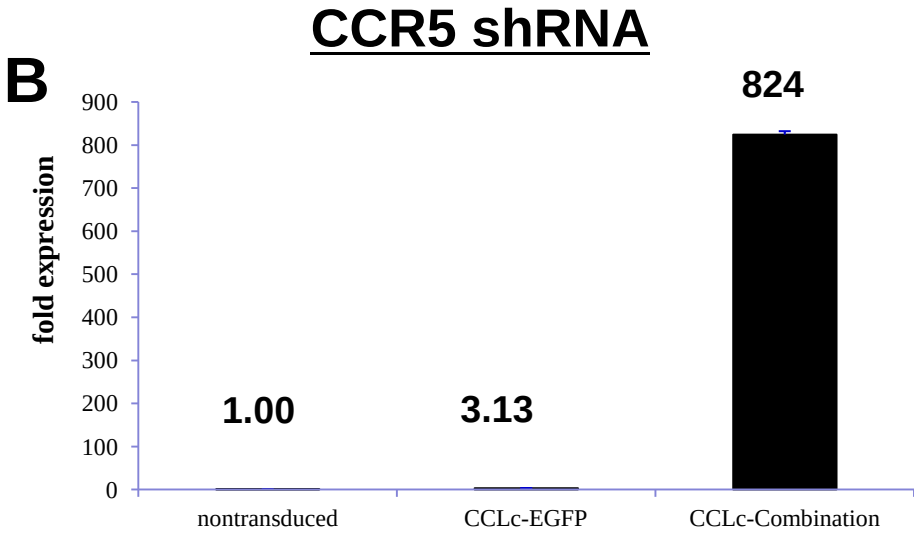


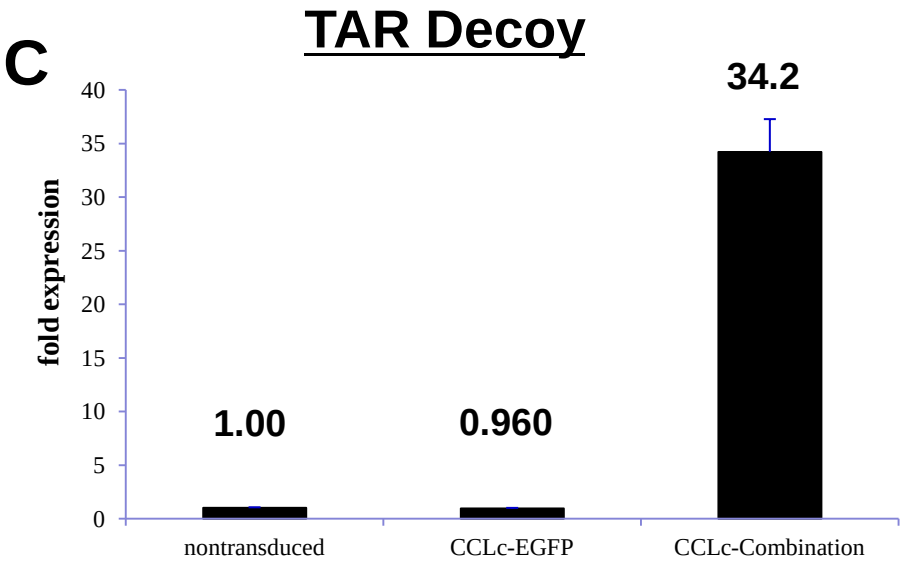
Anderson et al. Molecular Therapy, 2009

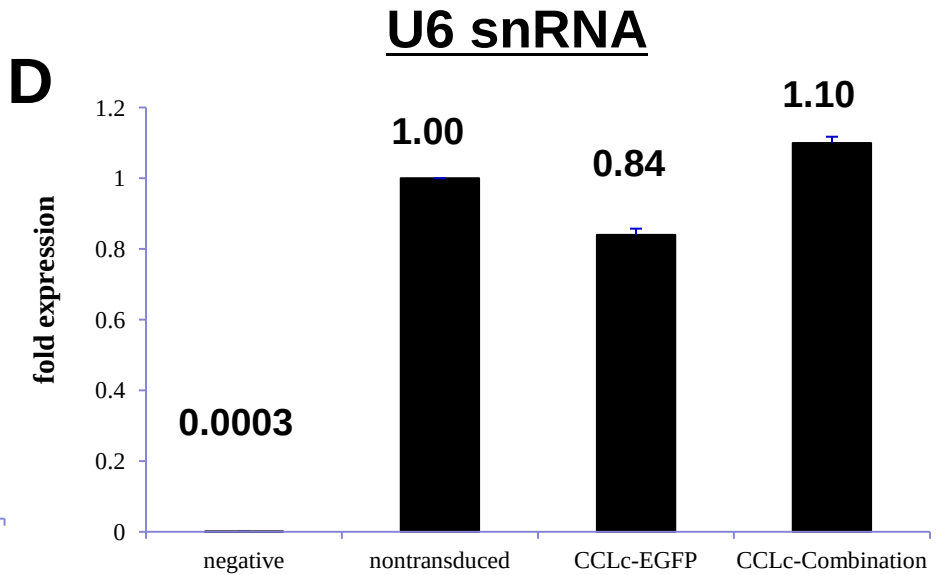
Safety of Combination Anti-HIV Lentiviral Vector

Expression of Anti-HIV Genes



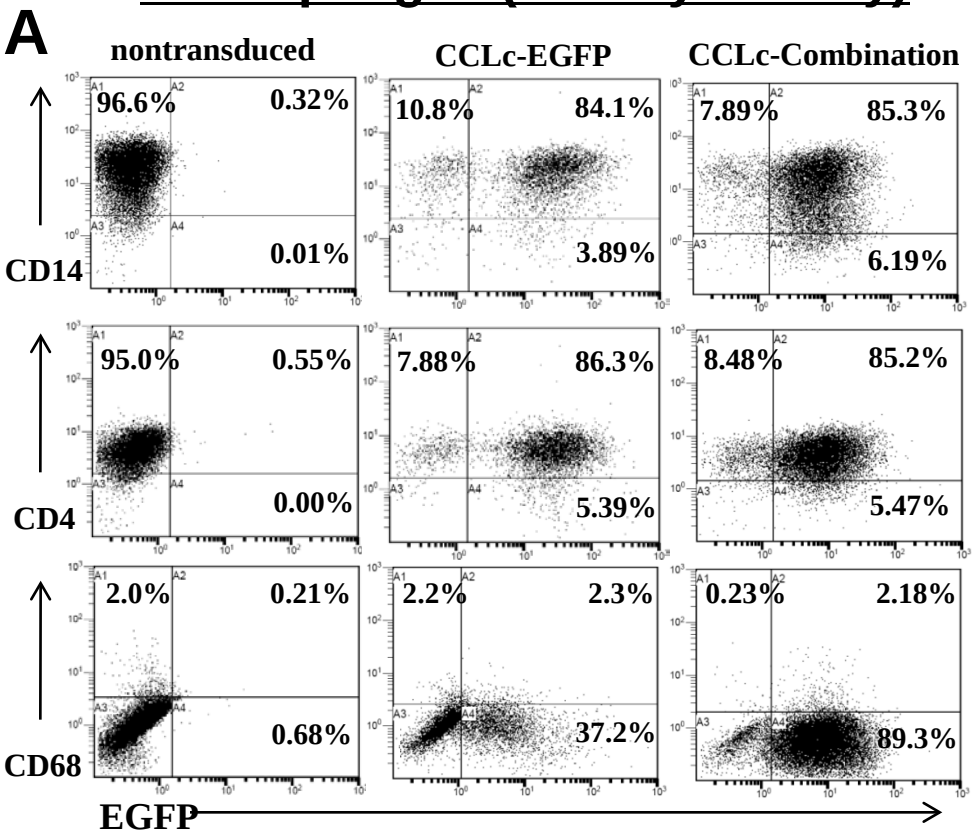




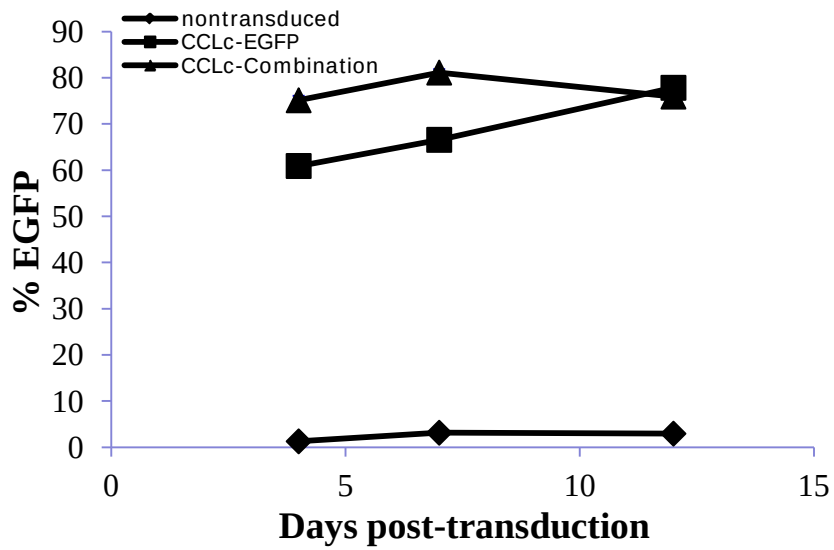


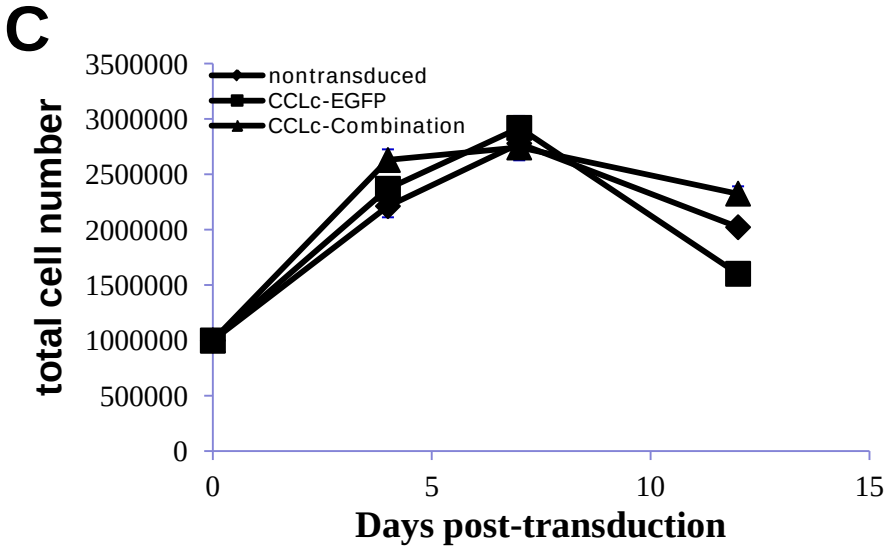
In Vitro Safety Experiments

Macrophages (flow cytometry)



PBMCs (EGFP+/cell counts)



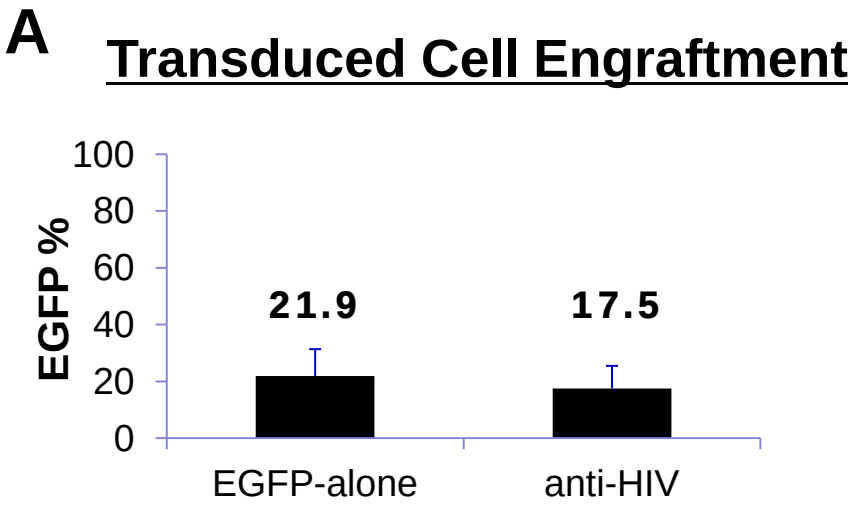


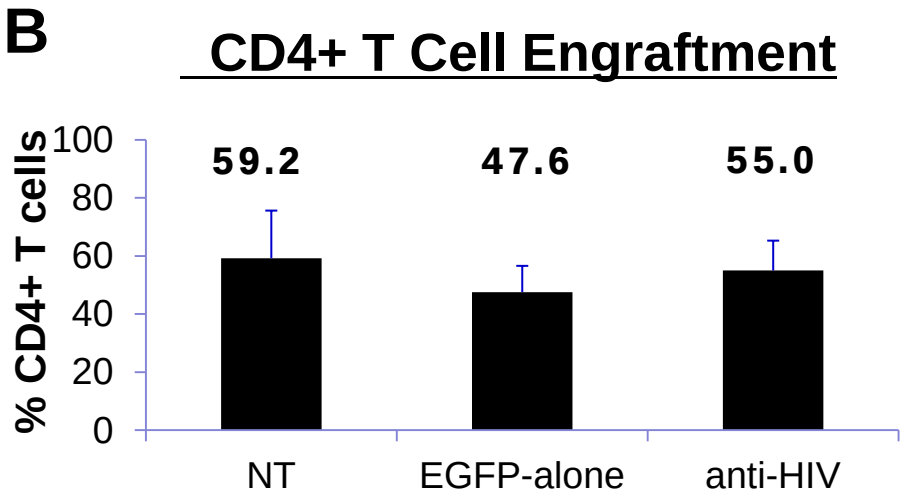
Peripheral Blood Engraftment

TABLE 1 Engraftment of the peripheral blood in transplanted NRG mice

Mouse	Peripheral Blood Engraftment			
	EGFP%		CD4+/CD3+	
NT-1	N/A		59.9	avg. 59.2 % std dev. 16.4
NT-2	N/A		51.9	
NT-3	N/A		56.7	
NT-4	N/A		46.9	
NT-5	N/A		55.2	
NT-6	N/A		31.6	
NT-7	N/A		62.0	
NT-8	N/A		55.0	
EGFP-alone-1	14.8	avg. 21.9 % std. dev. 9.4	58.9	avg. 47.6 % std dev. 9.0
EGFP-alone-2	29.1		44.9	
EGFP-alone-3	36.6		42.7	
EGFP-alone-4	30.7		63.2	
EGFP-alone-5	16.1		41.2	
EGFP-alone-6	13.2		47.7	
EGFP-alone-7	16.1		44.7	
EGFP-alone-8	12.5		45.0	
anti-HIV-1	13.9	avg. 17.5 % std dev. 8.0	52.0	avg. 55.0 % std dev. 10.3
anti-HIV-2	22.0		50.3	
anti-HIV-3	15.9		69.8	
anti-HIV-4	12.6		70.5	
anti-HIV-5	32.0		50.3	
anti-HIV-6	34.3		49.0	
anti-HIV-7	16.8		34.5	
anti-HIV-8	20.7		49.2	

Peripheral Blood Engraftment





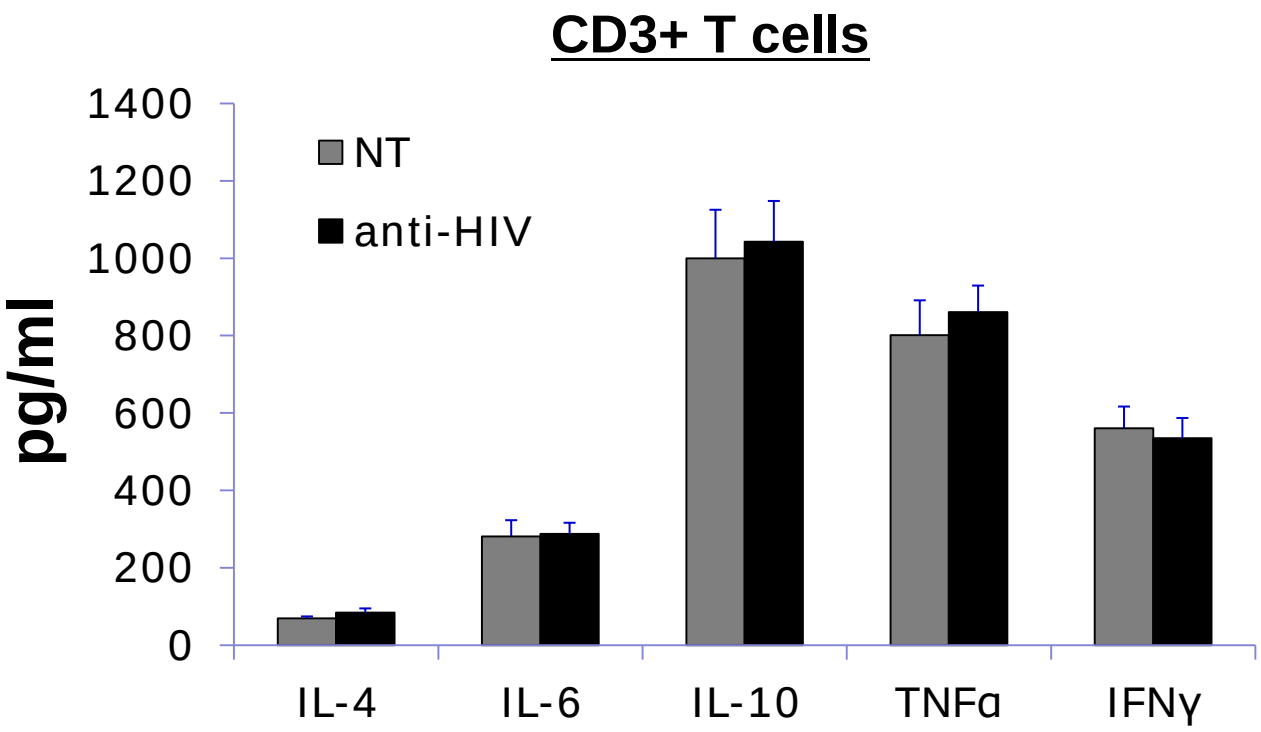
Lymphoid Organ Engraftment

TABLE 2 Engraftment of lymphoid organs in transplanted NRG mice

Mouse	Blood		Thymus			Spleen					Bone Marrow			
	CD4/ CD3	EGFP/ CD45	CD4/ CD8	EGFP/ CD3	EGFP/ CD45	CD3/ CD45	EGFP/ CD3	CD4/ CD3	CD19/ CD45	EGFP/ CD19	CD19/ CD45	EGFP/ CD19	CD14/ CD45[a]	EGFP/ CD14
NT-9	76.9	N/A	89.5	N/A	N/A	89.9	N/A	94.7	6.7	N/A	28.8	N/A	34.3	N/A
NT-10	79.0	N/A	95.4	N/A	N/A	38.3	N/A	85.0	58.8	N/A	56.9	N/A	16.3	N/A
NT-11	60.0	N/A	86.8	N/A	N/A	58.0	N/A	83.8	37.2	N/A	29.9	N/A	33.5	N/A
NT-12	31.8	N/A	89.2	N/A	N/A	28.7	N/A	72.3	57.6	N/A	37.3	N/A	35.2	N/A
NT-13	86.7	N/A	65.5	N/A	N/A	27.0	N/A	45.6	56.2	N/A	67.0	N/A	17.4	N/A
NT-14	75.5	N/A	72.5	N/A	N/A	47.2	N/A	78.7	41.6	N/A	23.8	N/A	38.3	N/A
EGFP-alone-9	63.2	39.1	72.2	45.4	40.7	31.0	35.2	60.0	41.0	33.0	81.3	36.3	13.5	32.5
EGFP-alone-10	40.7	17.2	87.0	26.3	16.3	21.8	24.5	46.2	74.0	15.5	56.7	12.3	2.9	19.2
EGFP-alone-11	37.3	21.5	79.6	12.0	17.0	35.4	17.3	52.9	59.3	20.2	24.2	24.2	4.3	25.2
EGFP-alone-12	41.2	16.1	85.6	23.4	19.9	30.6	18.8	54.1	66.2	23.3	47.0	22.6	4.5	21.7
anti-HIV-9	66.1	10.9	61.7	8.9	16.6	14.4	7.6	64.5	84.1	8.9	83.6	11.3	8.5	10.5
anti-HIV-10	52.0	13.9	83.2	17.4	8.8	19.9	9.0	54.2	62.9	10.3	53.7	9.8	10.7	10.1
anti-HIV-11	50.3	7.7	85.7	9.0	5.6	59.7	3.6	64.8	25.2	7.9	54.6	7.7	16.4	5.9
anti-HIV-12	60.8	12.3	65.1	5.8	6.6	68.1	6.0	33.1	29.6	8.3	9.6	7.8	9.7	8.5
anti-HIV-13	47.9	9.5	81.5	5.7	5.7	20.6	4.6	57.7	66.0	8.0	14.6	10.2	7.8	7.8
anti-HIV-14	66.8	22.2	53.6	21.4	11.4	89.1	9.8	75.8	7.9	10.5	77.9	11.4	18.2	14.0

[a] There was a statistically significant difference ($p<0.05$) between the average levels of CD14/CD45 positive cells in the bone marrow of anti-HIV vector transduced cell engrafted mice compared to nontransduced (NT) cell engrafted mice.

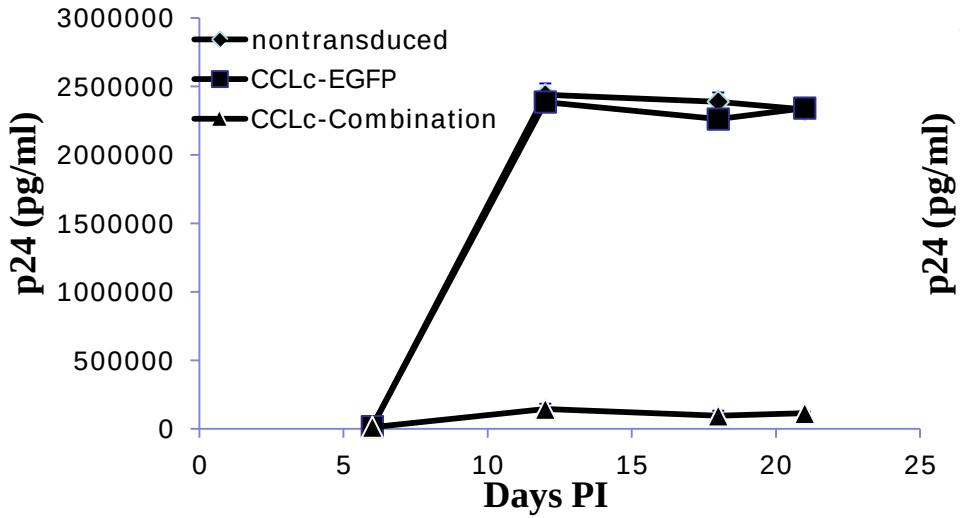
Ex Vivo Cytokine Expression



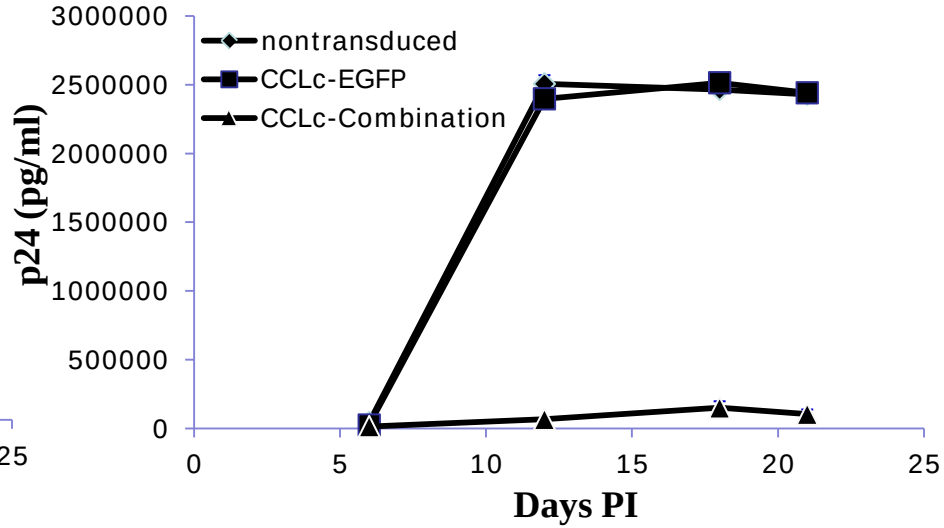
Efficacy of Combination Anti-HIV Lentiviral Vector

In Vitro Challenge Experiments

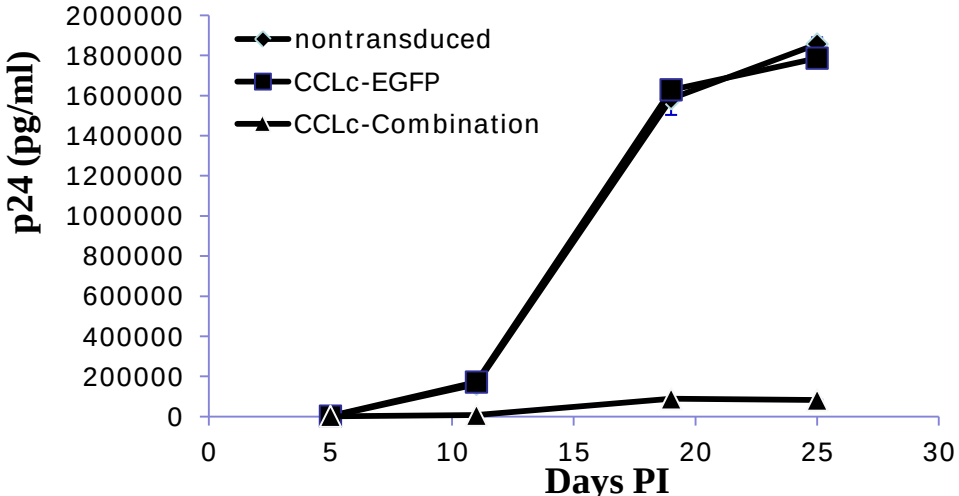
A Ghost-X/R/R BaL-1 MOI 0.05



B Ghost-X/R/R NL43 MOI 0.05

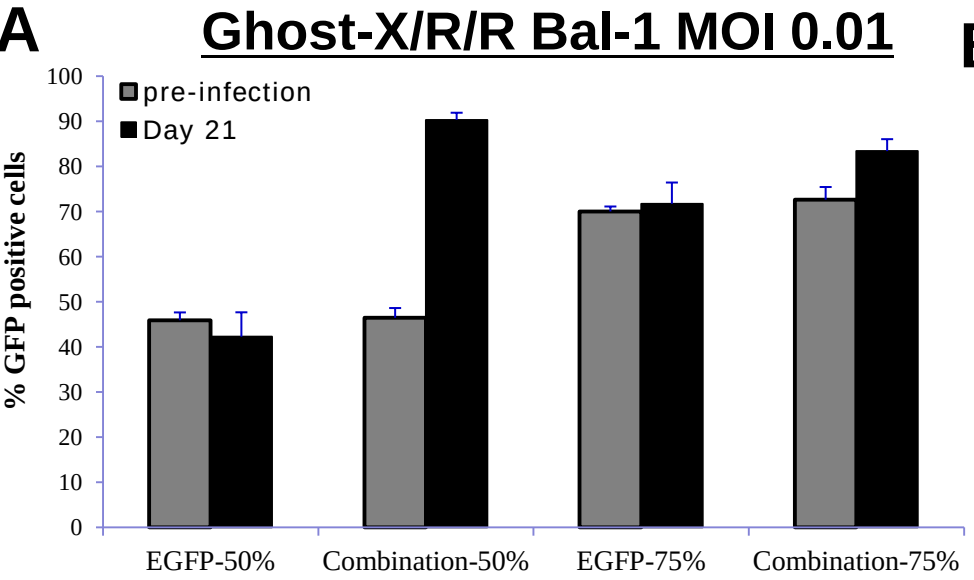


C Macrophages BaL-1 MOI 0.05



In Vitro Selective Survival Advantage

A



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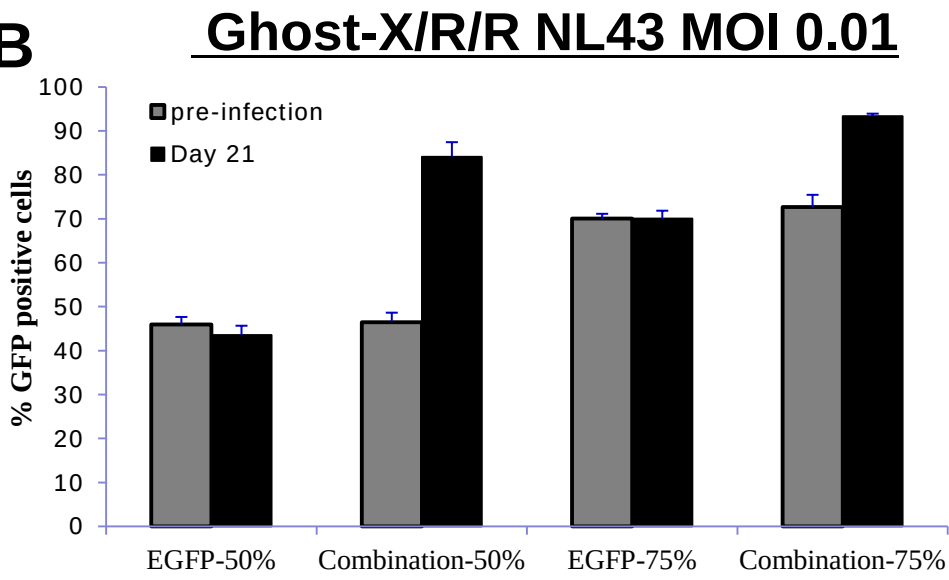
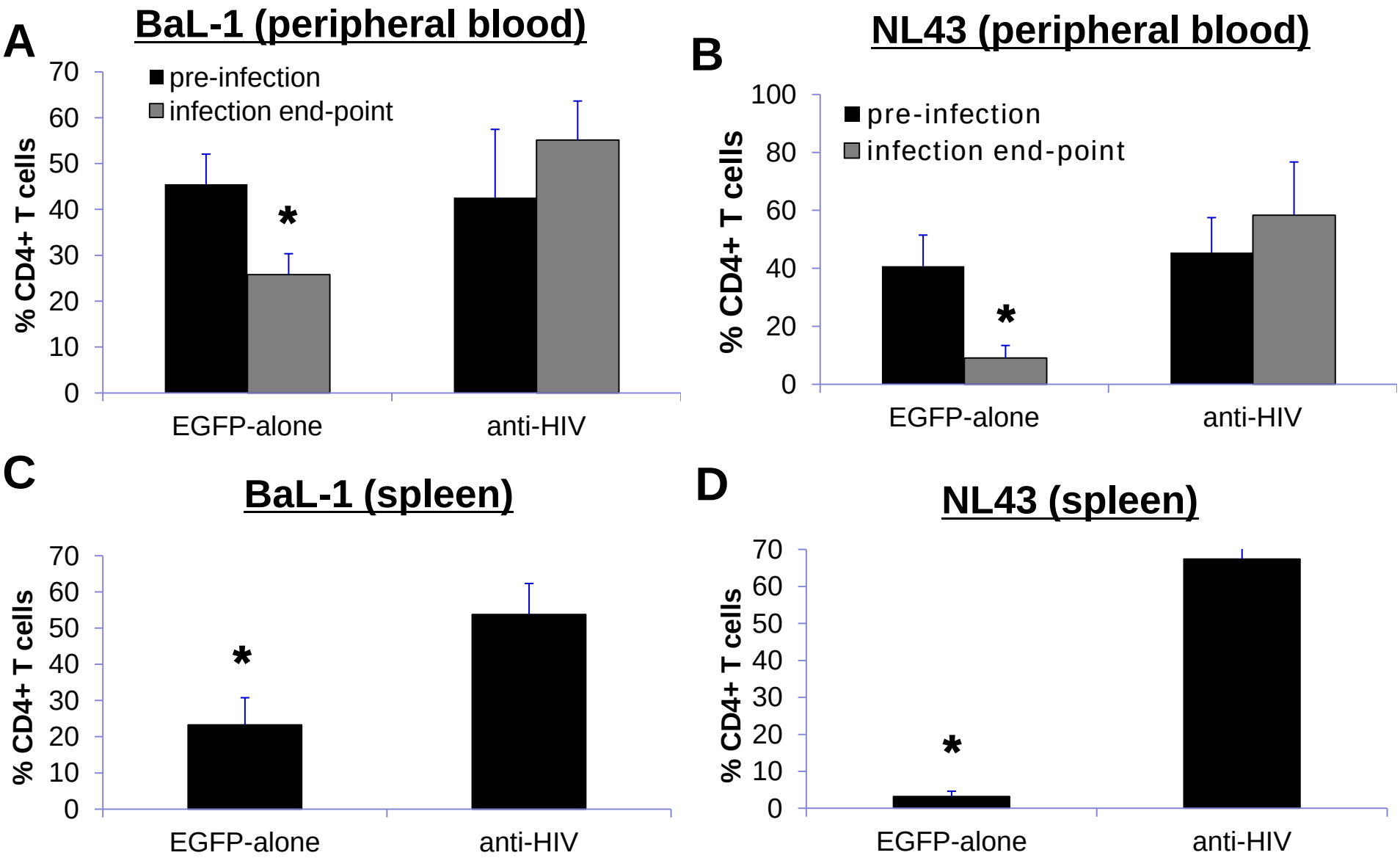


In Vivo Maintenance of CD4+/CD45+ cells



In Vivo Selective Survival Advantage

Table 1. Selective survival advantage CD4+ cell counts from BaL-1 infected RAG1 mice

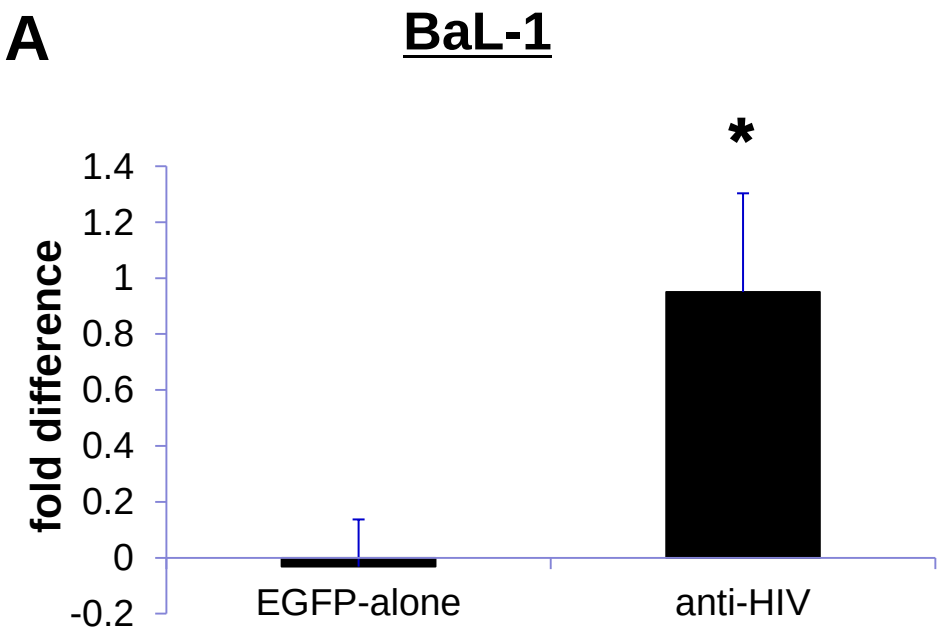
	Day 0		Infection end point	
	EGFP-	EGFP+	EGFP-	EGFP+
EGFP-alone 1	947	225	1373	363
EGFP-alone 2	686	330	970	343
EGFP-alone 3	168	34	619	103
EGFP-alone 4	183	35	178	40
anti-HIV 1	489	59	791	169
anti-HIV 2	710	68	143	26
anti-HIV 3	1160	146	269	101
anti-HIV 4	389	49	278	78

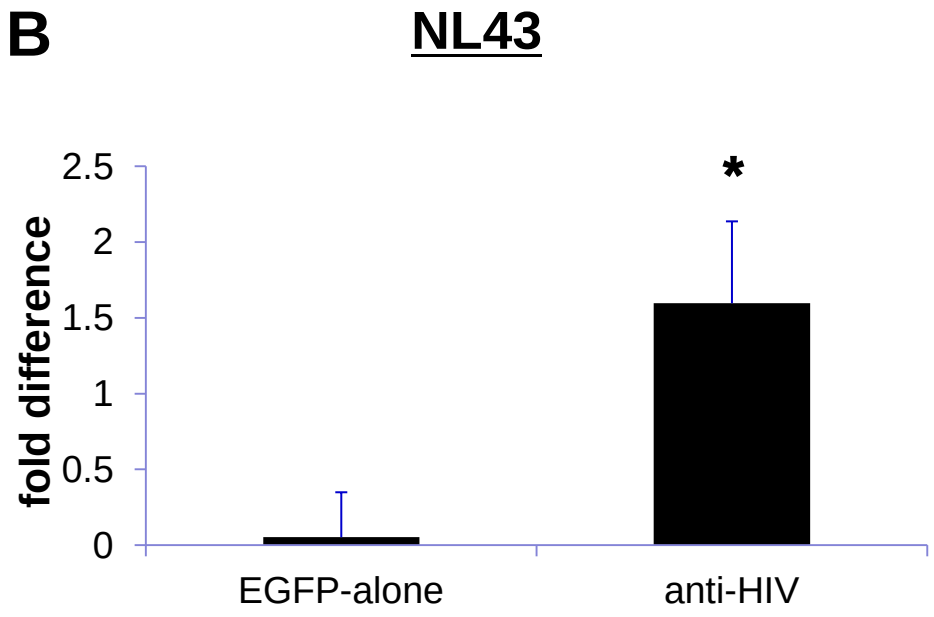
Table 2. Selective survival advantage CD4+ cell counts from NL43 infected RAG1 mice

	Day 0		Infection end point	
	EGFP-	EGFP+	EGFP-	EGFP+
EGFP-alone 1	234	67	524	129
EGFP-alone 2	309	108	498	219
EGFP-alone 3	345	60	345	91
EGFP-alone 4	214	57	262	48
anti-HIV 1	748	45	450	101
anti-HIV 2	240	30	152	44
anti-HIV 3	334	36	445	126
anti-HIV 4	177	16	150	47

In Vivo Selective Survival Advantage

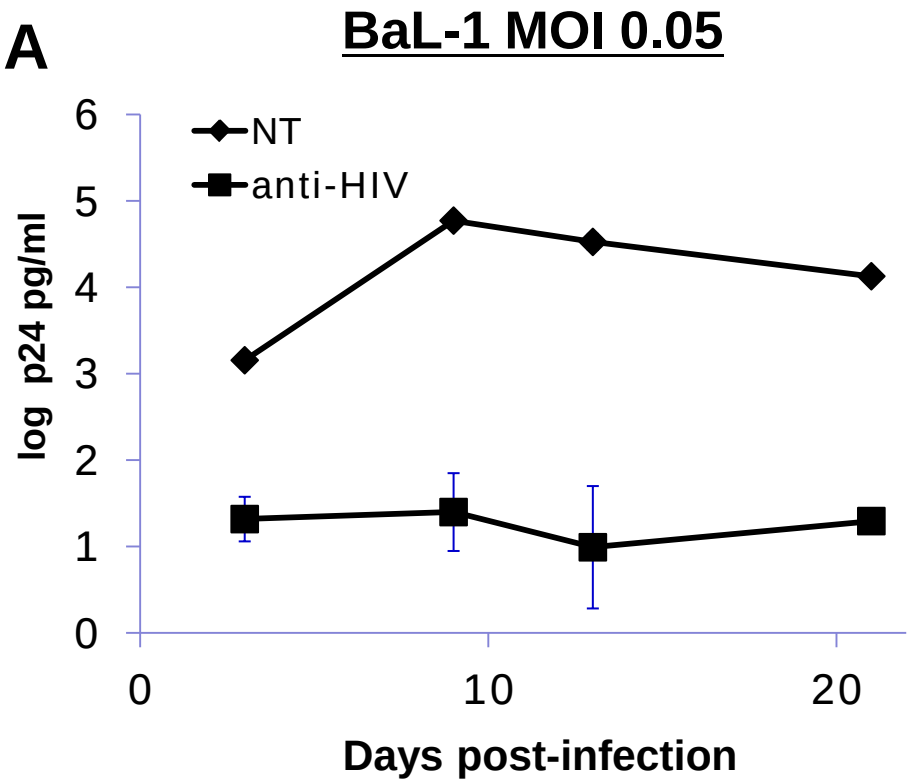
CD4+/EGFP+ cells

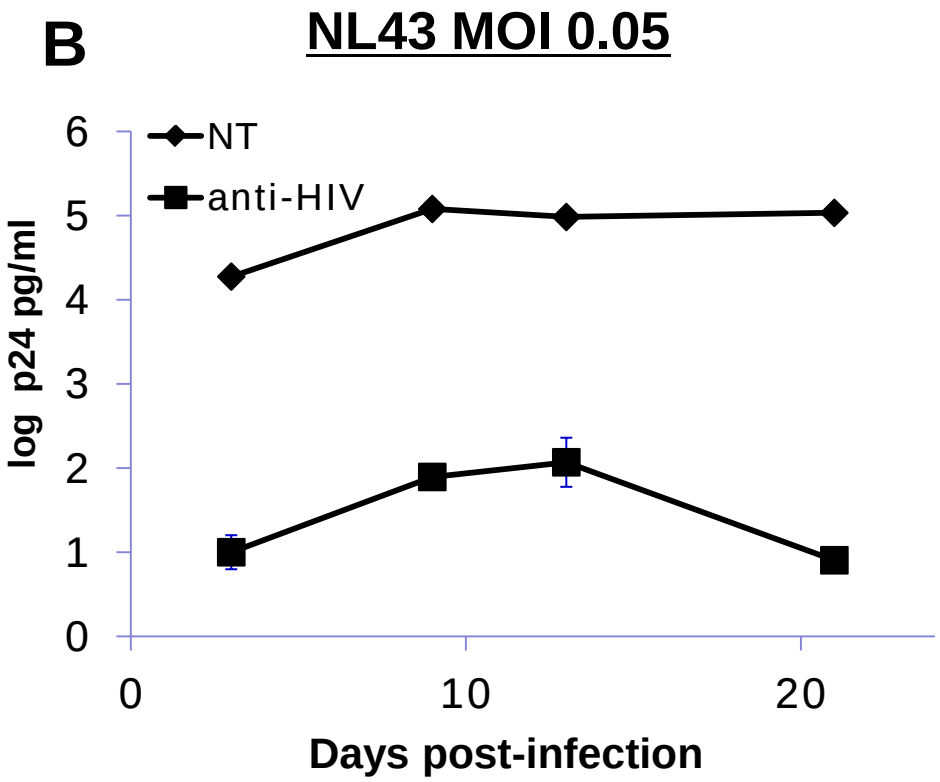




HIV-1 Challenge

CD3+ T cells





Preclinical Results

- **Expression of three anti-HIV genes in transduced cells**
- **Engraftment of anti-HIV vector transduced cells (>8 months)**
- **Potent inhibition of HIV-1 infection, *in vitro* and *ex vivo***
- **Maintenance of CD4+ cell levels in infected mice**
- **Selective survival advantage of anti-HIV vector transduced cells**

PHASE I/II TRIAL OF STEM CELL GENE THERAPY FOR HIV IN AIDS LYMPHOMA PATIENTS

- *Primary Objective: To evaluate the clinical safety, feasibility and efficacy of our approach using our development candidate.*

- *Secondary Objective: To evaluate immune reconstitution with HIV resistant immune cells, viral load and HIV status, integration sites of vector sequences in circulating cells, presence of any in vivo clonal expansion of transduced cells and any immune reaction to transduced cells.*

- *Exploratory Objective: To explore the role of ART interruption on expansion of HIV resistant HIV target cells, including immune cells, both in the peripheral blood and the gut mucosa.*

THE PRIMARY ENDPOINTS

- Safety defined as timely engraftment (the collective establishment of a persistent absolute neutrophil count of at least 500/mm^3 for 3 consecutive days and platelet count of 20,000/mm^3) at one month post transplant, in the absence of any study candidate specific grade III and IV non-hematopoietic organ toxicity or any clonal expansion;

and

- Efficacy of the candidate product defined as establishment of >0.5% mononuclear blood cells expressing anti-HIV genes in the peripheral blood at 3 months post-transplant.

INCLUSION CRITERIA:

Inclusion criteria associated with type and status of lymphoma:

- 1. intermediate or high grade non-Hodgkin's lymphoma,
- 2. Hodgkin's lymphoma,
- 3. Burkitt's lymphoma,
- 4. Plasmoblastic lymphomas or peripheral T cell lymphoma (with the exception of ALK+ type in first or 2nd complete remission).

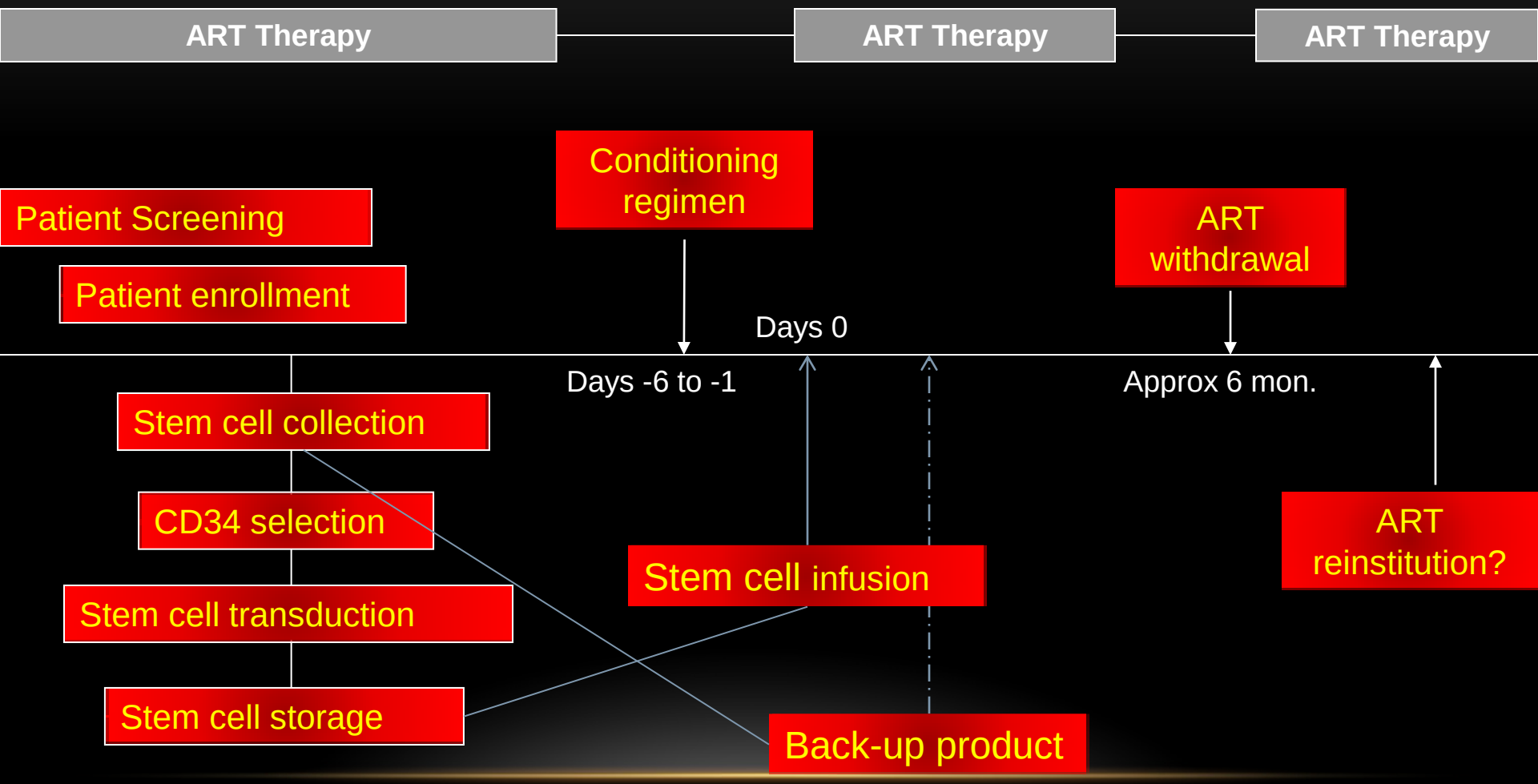
INCLUSION CRITERIA:

Inclusion criteria associated with HIV status:

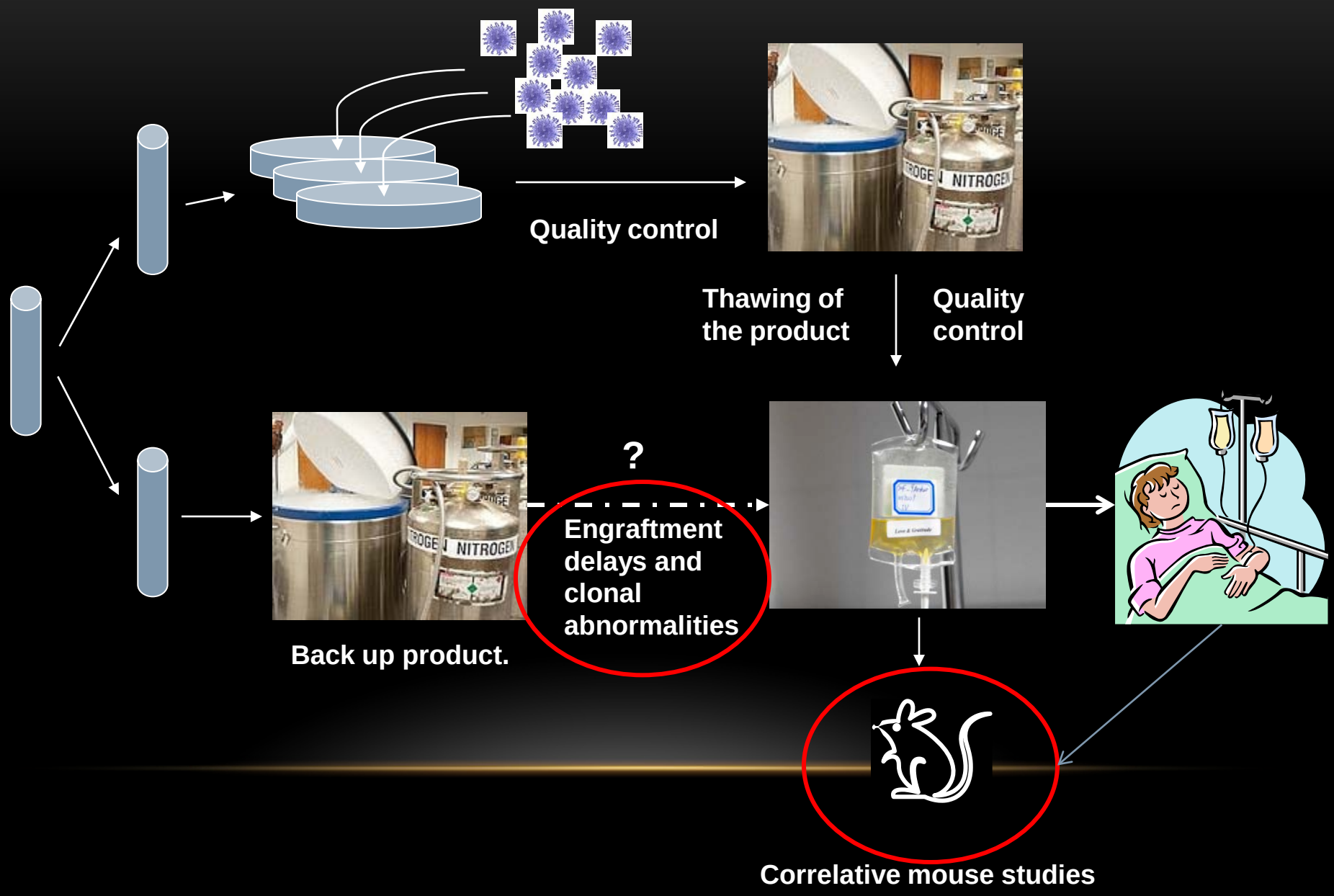
- Must be on a multi-drug anti-HIV regimen (excluding zidovudine [AZT]) and have an HIV viral load < 50 copies/mL by RT-PCR at the time of study enrollment.
- Must agree to have anti-HIV regimen (ART) temporarily stopped at the time of the transplant and about 6 months post transplant once the immune system has shown signs of recovery.

EXCLUSION CRITERIA:

- Patients with > 5% involvement of bone marrow by malignant cells (either by manual count or flow cytometry) prior to stem cell collection.
- Patients with any abnormal cytogenetics in the bone marrow, not related to the lymphoma and not deemed to be constitutional.
- Presence of any active CNS disease at the time of evaluation (parenchymal or leptomeningeal).
- Any history of HIV-associated encephalopathy.
- History of other AIDS-related syndromes that are perceived to cause excessive risk for morbidity post-transplantation, as determined by the principal investigator.
- Symptomatic / active bacterial or fungal infection.
- Active CMV retinitis or other active CMV-related organ dysfunction.
- Relapse of pneumocystis carinii pneumonia within the past year.
- Intractable, severe diarrhea, defined as >1,500 cc diarrheal fluid per day, or diarrhea causing persistent severe electrolyte abnormalities or hypoalbuminemia.
- History of active myocardial ischemia, cardiomyopathy, uncontrolled dysrhythmia or congestive heart failure within the last 6 months before the evaluation.
- Dementia of any kind.
- Seizures within the past 12 months.
- History of grade III hemorrhagic cystitis due to prior cyclophosphamide chemotherapy.
- History of other prior malignancy, except squamous cell carcinoma of the cervix or anus, superficial basal cell or squamous cell skin cancer, or other malignancy curatively treated more than 5 years ago.
- Active psychosocial condition that would hinder study compliance and follow-up.
- Any perceived inability to directly (and without the means of a legal guardian) provide informed consent.
- Any medical or physical contraindication or other inability to undergo HPC collection.


ART Therapy
ART Therapy
ART Therapy
Conditioning regimen
Patient Screening
Patient enrollment
ART withdrawal
Days 0
Days -6 to -1
Approx 6 mon.
Stem cell collection
CD34 selection
Stem cell transduction
Stem cell storage
Stem cell infusion
Back-up product
ART reinstitution?

PHASE I/II


Quality control
Thawing of the product
Quality control
?
Engraftment delays and clonal abnormalities
Back up product.
Correlative mouse studies

PHASE I/II, SAFETY-IN GROUP

- 3 patients → 15 patients

- FDA concerns.
- Feasibility and safety, and "*practicality*".
- Troubleshooting.

Assay/test	Pre-transplant/screening						Post-transplant																	
	Months		Weeks				Weeks						Months											
	-3	-2	-4	-3	-2	-1	1	2	3	4	5	6	2	3	4	5	6	7	8	9	10	12-24	3-5 yr	6-15 yr
Hepatitis, HSV, toxoplasma, VZV, syphilis, HIV, CMV																								
EKG, DLCO, FEVI, FVC, creatine clearance; bone marrow biopsy; central pathology review																								
Karnofsy performance status																								
CXR, quantitative Igs,																								
Staging/restaging																						mos 12, 24	2/yr	1/yr
GI biopsy																								
DNA monitoring																						mos 12, 18, 24	2/yr	1/yr
Immune assessment																						mos 12, 18, 24		
Assess engraftment																								
CMV PCR																							2/yr	1/yr
HIV Viral Load & single copy																						q2m	2/yr	1/yr
H & P (incl. post-transplant toxicity assessment)																						q2m	2/yr	1/yr
CBC																						q2m	2/yr	1/yr

MONITORING/FOLLOW UP STUDIES.

- Engraftment Studies.
- Immunological Studies.
- DNA monitoring/clonality studies.
- Parallel NSG mice studies.
- Viral studies.

IMMUNOLOGY SPECIALTY LABORATORY

- Funded by NIAID
- One of 4 in the US
- Performs immunology on patients in ACTG-sponsored clinical trials
- Focus on opportunistic pathogens and responses to HIV
- Currently focused on VZV and HPV vaccine response
- Soon will do influenza- and TB-specific responses

IMMUNE MONITORING STUDIES.

I. Reconstitution of Immune System in Peripheral Blood:

a. Subsets of T cells including CD4, CD8 and regulatory T Lymphocytes:

b. Determine the memory and naïve CD4 and CD8 T cells as well as the T cell activation

c. Determine NK and NK-T cell subsets

2. Functional Responses Upon Reconstitution of T Cell Immunity

a. Total Th1/Th2/Th17 subsets will be measured after mitogen stimulation

b. Antigen specific T cell responses will be measured after stimulations with CEF and HIV-gag peptides

c. If adequate PBMC are collected, proliferation responses will be tested using CFSE assays

d. Response to neoantigens: vaccination to Tetanus Toxoid.

3. Immunological Reaction to TRIM 5-alpha

ELISA, CTL assays

4. Analysis of the extra-vascular compartment of the immune system

Duodenal biopsies.

DNA MONITORING STUDIES

- Anti-HIV gene integration sites
- Clonality studies
- Parallel engraftment studies in immunodeficient mice using an aliquot of anti-HIV transduced HSCs for each patient.
- Evaluate transduction efficiency, gene marking, and copy number in anti-HIV vector transduced CD34+ cells
- Cell lineage specific differentiation of anti-HIV vector gene modified CD34+ cells

RATIONALE AND SAFETY CONSIDERATIONS FOR TREATMENT INTERRUPTION

- ART interruptions, either for a predetermined time or linked to a CD4 threshold have been used in a numerous research settings[1]

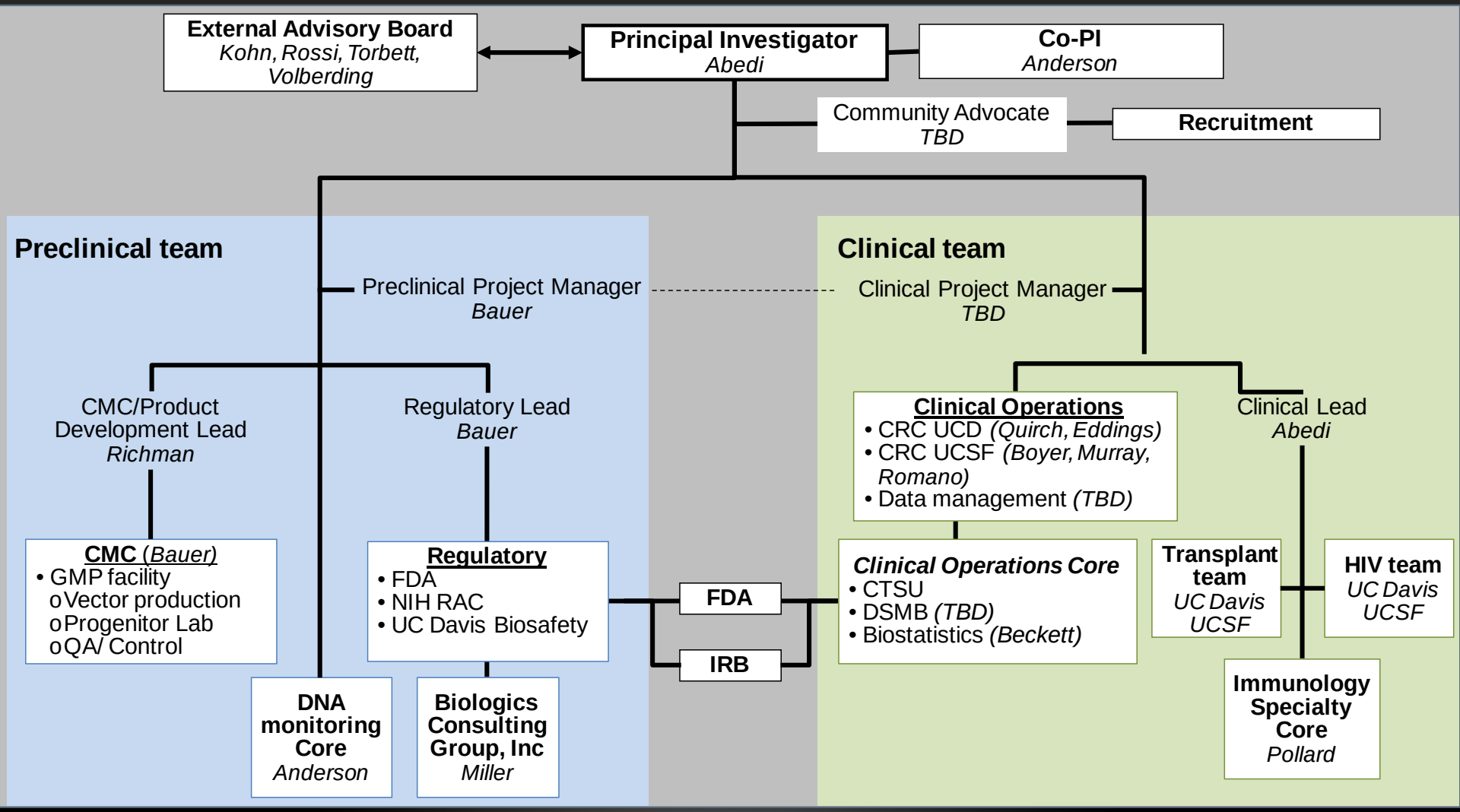
- 'autovaccination': cycled re-exposure to autologous virus to boost immunity to HIV
- 'analytical': to demonstrate the efficacy of immunomodulatory treatment or therapeutic vaccinations administered during virologic suppression where the anticipated virologic/immunologic endpoints would be compared to the control group
- 'strategic': designed to reduce exposure to antiretroviral therapy where the attendant expense, toxicities, and pill burden could be reduced

- Criteria for the restarting therapy for this study have been informed by the safety lessons derived from previous experience to maximize the potential benefits of the intervention while minimizing risk to participants

[1]Pai NP, Tulsky, JP, Lawrence J, et al. Structured treatment interruptions (STI) in chronic suppressed HIV infection in adults (review). Cochrane Library 2008; 1: 1-37.

TREATMENT INTERRUPTION GUIDELINES

- Threshold for restarting ART at 200 CD4+ T-cells (confirmed within 2 weeks) is based on historic knowledge of depressed CD4+ ranges for this population post-transplant. Frequent monitoring of immunologic and virologic parameters will reduce risk to subjects. For the subject with >400 CD4+ T-cells at the time of the treatment interruption, a fall of >50% will also trigger ART restart.
- Threshold for restarting ART if viral load exceeds 300,000 cp/mL (confirmed within 2 weeks [turn around time 4 days at Quest Commercial lab]) will reduce risk to subjects
- Threshold for restarting ART for opportunistic infections per discretion of patient and primary care provider will reduce risk to subjects
- Our infectious disease team have extensive experience with many similar trials including two currently active therapeutic vaccination trials with ART cessation components and has extensive experience achieving successful, documented, study subject comprehension regarding this issue.


External Advisory Board
Kohn, Rossi, Torbett, Volberding
Principal Investigator
Abedi
Co-PI
Anderson
Community Advocate
TBD
Recruitment
Preclinical team
Preclinical Project Manager
Bauer
Clinical team
Clinical Project Manager
TBD
CMC/Product Development Lead
Richman
Regulatory Lead
Bauer
Clinical Operations
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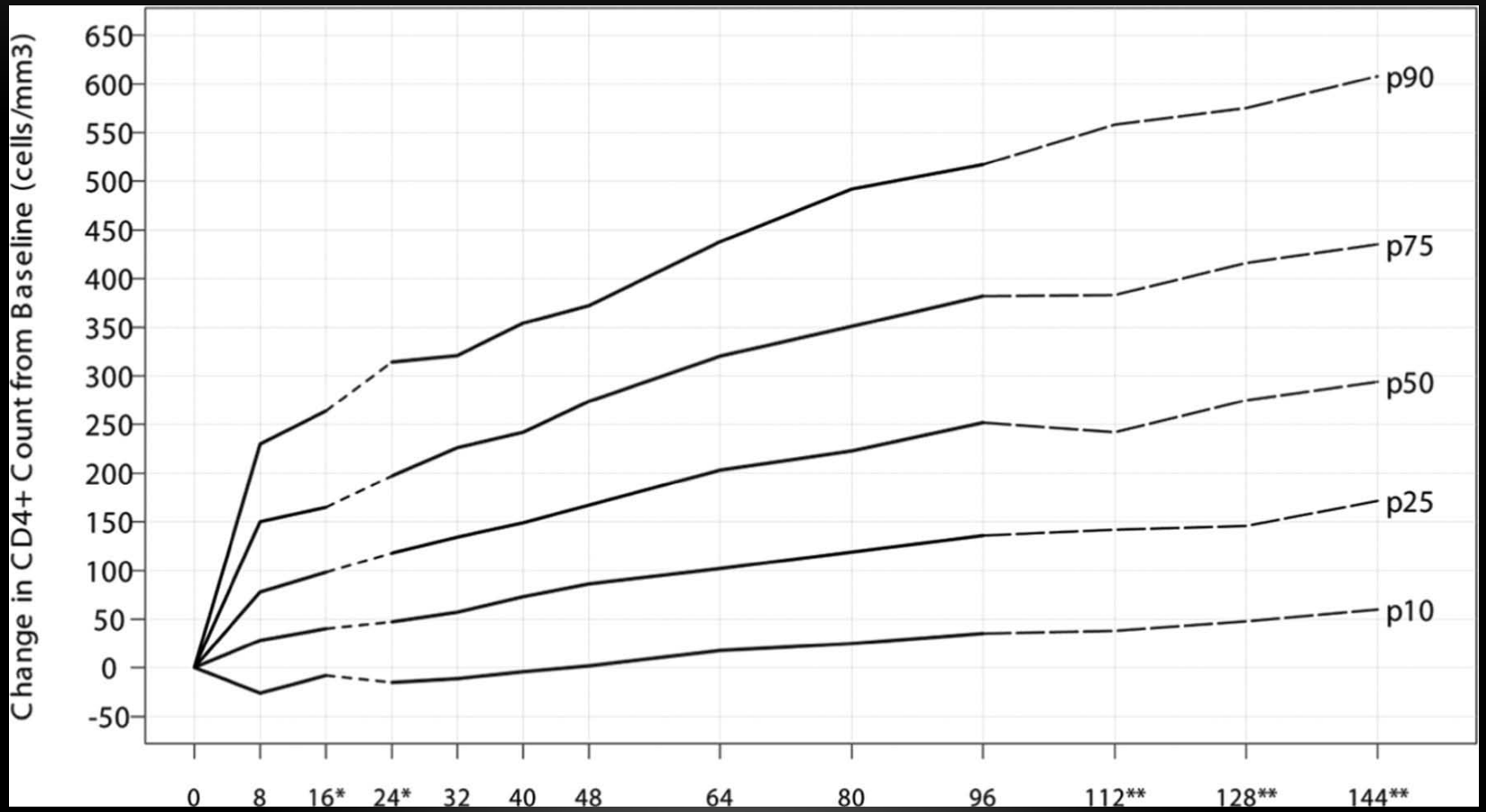
• CRC UCSF (Boyer, Murray, Romano)
• Data management (TBD)
Clinical Lead
Abedi
CMC (Bauer)
• GMP facility
o Vector production
o Progenitor Lab
o QA/ Control
Regulatory
• FDA
• NIH RAC
• UC Davis Biosafety
FDA
IRB
Clinical Operations Core
• CTSU
• DSMB (TBD)
• Biostatistics (Beckett)
Transplant team
UC Davis
UCSF
HIV team
UC Davis
UCSF
DNA monitoring Core
Anderson
Biologics Consulting Group, Inc
Miller
Immunology Specialty Core
Pollard

Change in CD4+ cell count from baseline in percentiles (10th, 25th, 50th, 75th, and 90th) for all AIDS Clinical Trials Group (ACTG) protocol 384 patients (). np978 This plot is based on HIV-positive antiretroviral therapy-naive patients after initiating HAART in ACTG protocol 384 with a median (interquartile range) baseline CD4+ cell count of 279 (98-444) cells/mm3. *At weeks 16 and 24, the ΔCD4+ cell count was positively associated with the baseline CD4+ cell count.



Clinical Infectious Diseases