RNA interference (RNAi) based therapeutics for the treatment of HIV-1

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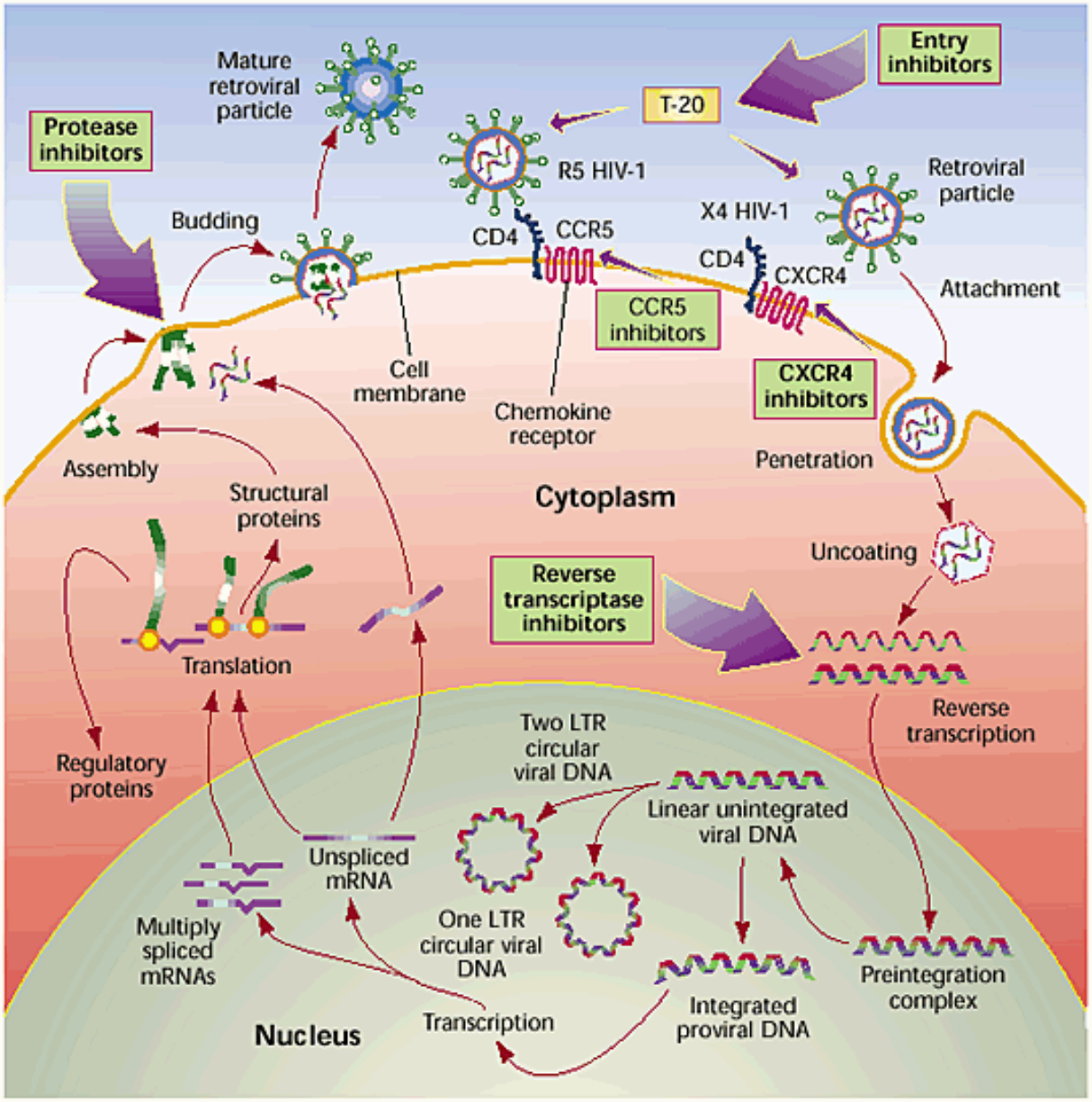
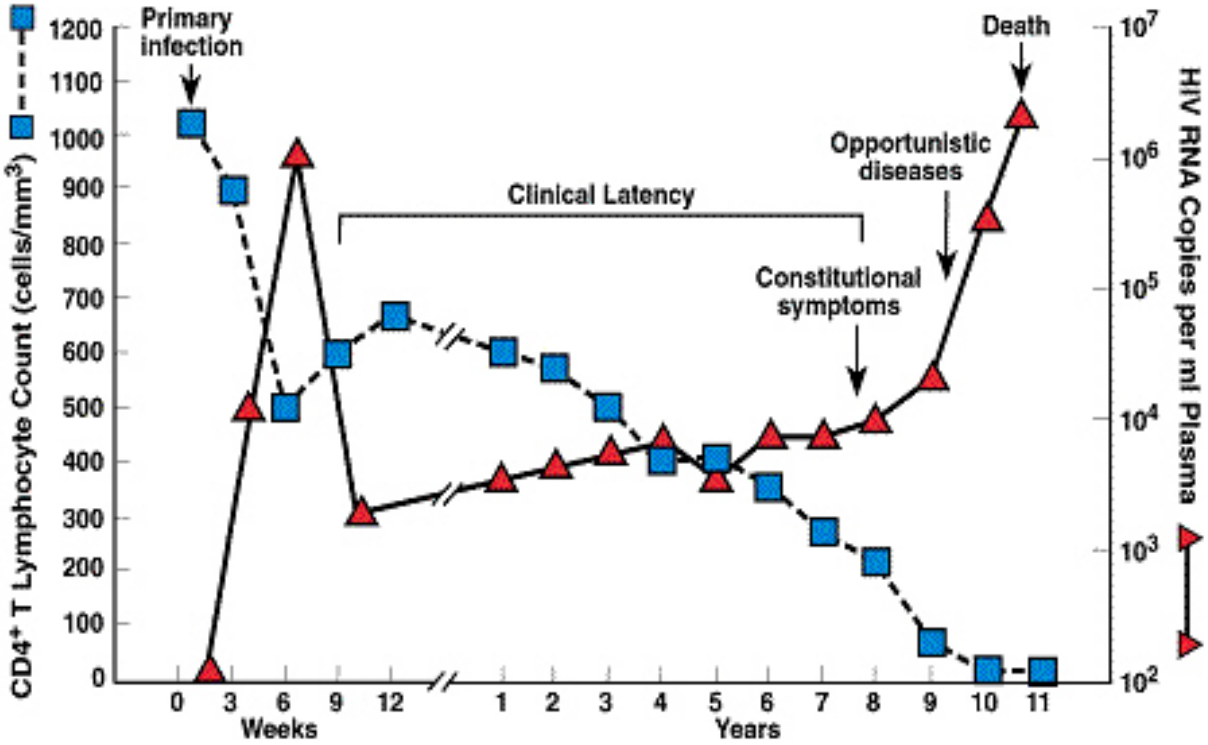
City of Hope


Mature retroviral particle
Protease inhibitors
Budding
T-20
Entry inhibitors
R5 HIV-1
CD4
CCR5
X4 HIV-1
CD4
CXCR4
Retroviral particle
Attachment
CCR5 inhibitors
CXCR4 inhibitors
Cell membrane
Chemokine receptor
Penetration
Assembly
Structural proteins
Cytoplasm
Uncoating
Reverse transcriptase inhibitors
Translation
Reverse transcription
Regulatory proteins
Two LTR circular viral DNA
Linear unintegrated viral DNA
Unspliced mRNA
Multiply spliced mRNAs
One LTR circular viral DNA
Integrated proviral DNA
Preintegration complex
Transcription
Nucleus
Bob Crimi

Typical Course of HIV Infection



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

Why Do We Need New HIV drugs?

- HIV infection is chronic and lifelong treatment required.
- Increasing number of patients with drug resistant virus-few if any options.
- Toxicities of current chemotherapy agents (in Highly Active Anti-Retroviral Therapy –cocktail).
- Difficult dosing regimens for patients, and drugs must be taken daily
- Gene and/or Systemic small RNA therapies could replace or be used as adjuvants for chemotherapy

Major Goals of anti-HIV therapies

- Reduce viral load
- Use combination therapies to mitigate emergence of drug resistant viral mutants
- Prevent CD4 T-cell decline
- If possible, eradicate latent viral reservoirs

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.
- We hypothesized that it should be possible to develop effective small RNA based combinatorial therapies.
- Gene therapy versus systemic RNA therapy-or both?

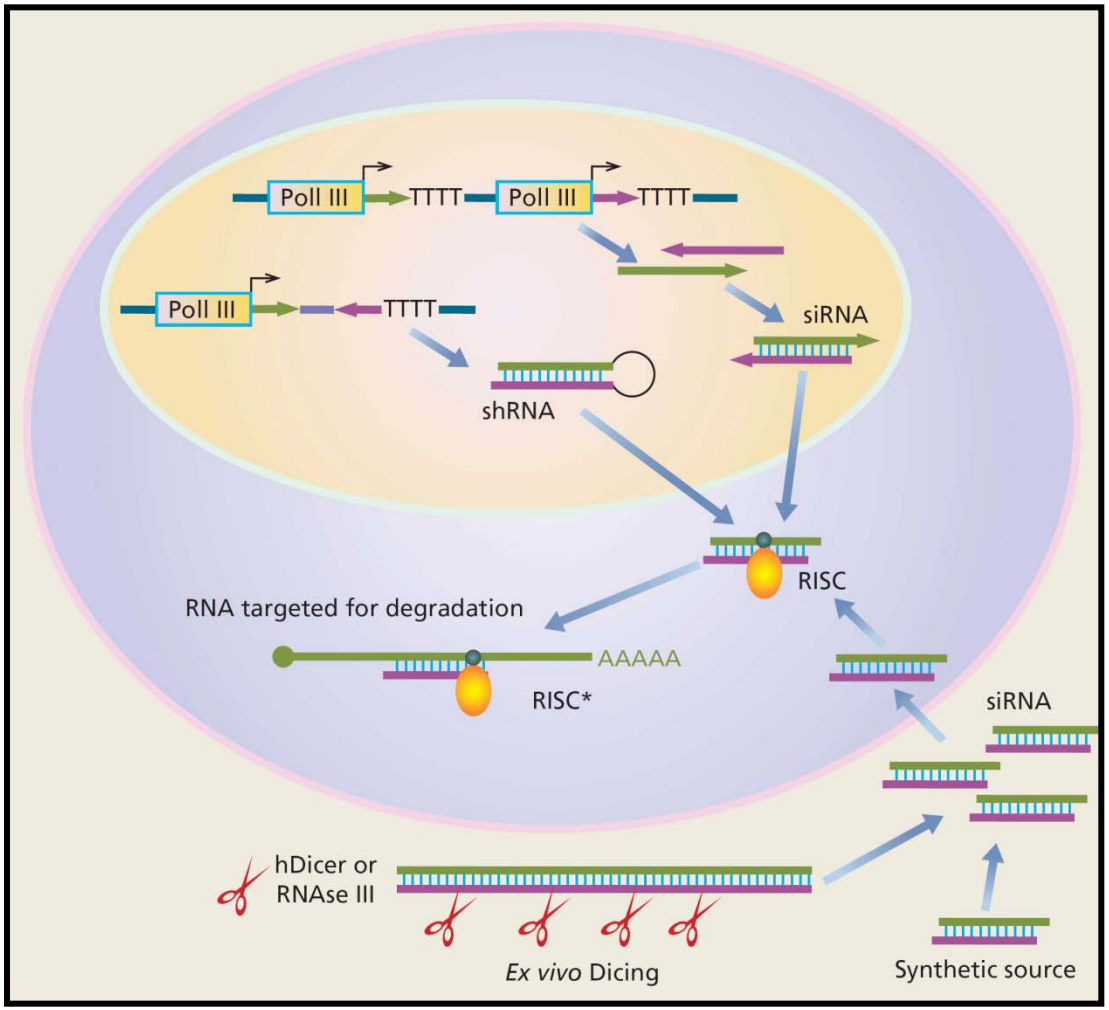
Applying RNAi for the treatment of human diseases

Powerful mechanism for targeted inhibition of gene expression.

Only a two dimensional design (Watson-Crick base pairing) is required for drug design.

Amenable to systemic delivery or a gene therapy approach.

Triggering RNAi in Mammalian Cells

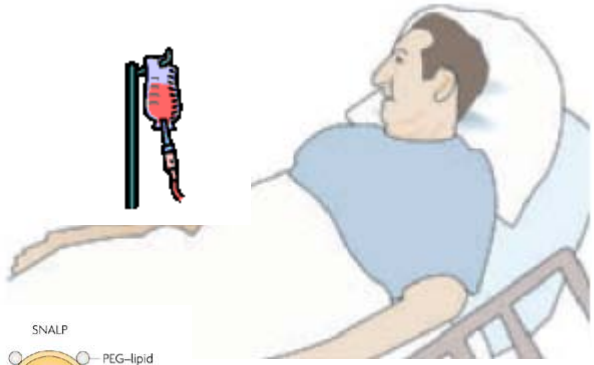


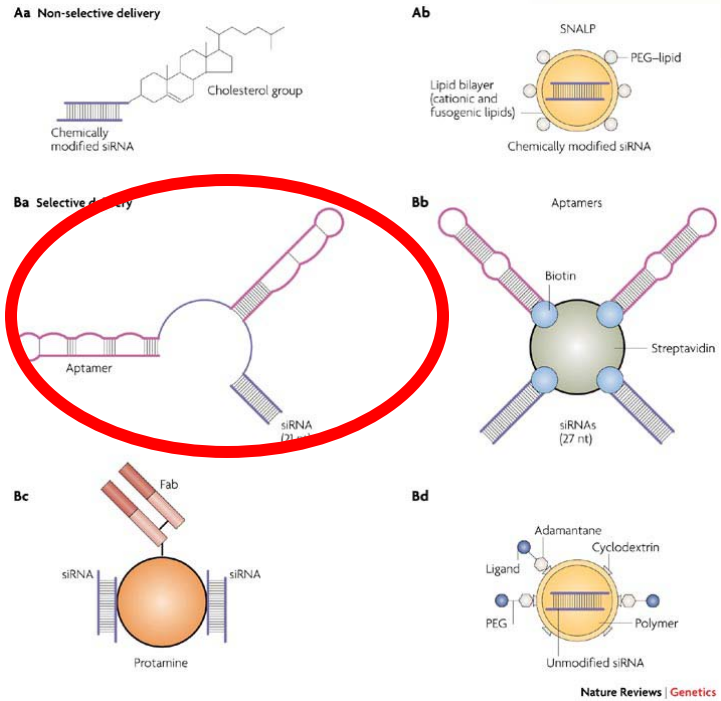
shRNA = short hairpin RNA; siRNA = short interfering RNA; RISC = RNA-induced silencing complex

Scherer LJ, Rossi JJ. *Nat Biotechnol*. 2003;21:1457-65.

Challenge-Treat HIV infection by systemic delivery off small RNAs

siRNA

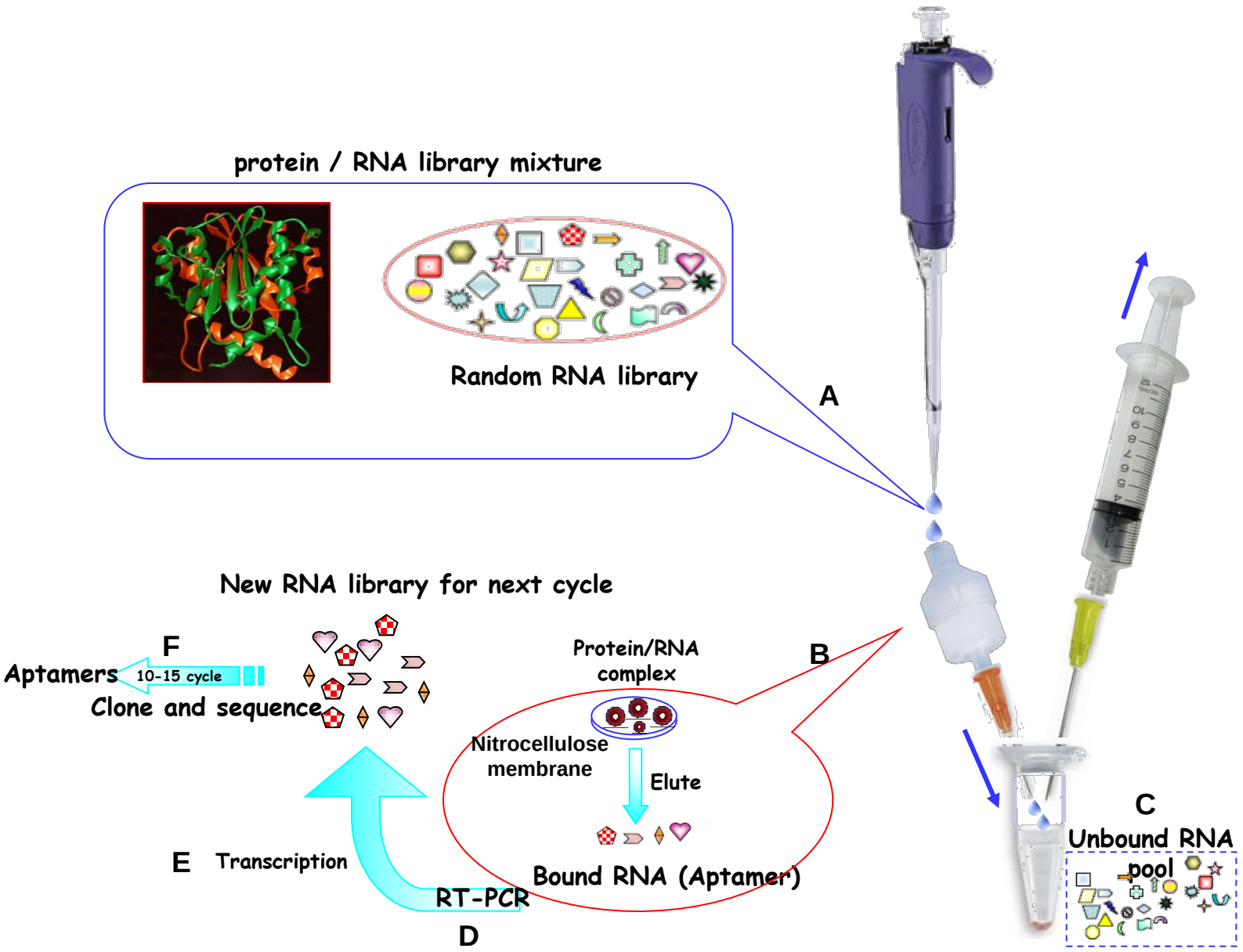




Aptamers

- In vitro evolved nucleic acids that bind to selected ligands (proteins,carbohydrates, other nucleic acids) with high affinities, similar to antibodies.
- Selection process involves use of libraries of random sequences to select tight binders out of as many as 10^{14} molecules.

T7 PGGGAGGACGAUGCGG- N20 -40 CAGACGACUCGCCCGA-



Nitrocellulose-based *in vitro* SELEX

Targeted delivery using dual Function Aptamer- siRNA treatment of HIV infection

- We evolved RNA aptamers that selectively bind to the HIV envelope protein gp 120. The binding neutralizes a broad range of viral isolates (Zhou et al, NAR 2009).
- The gp120 aptamer has also been developed as a delivery vehicle of anti-HIV siRNAs to HIV infected cells expressing envelope at the cell surface(Zhou et al., Molecular Therapy 2008, Nuc.Acids Res. 2009) making dual function inhibitors.

Gp 120 binding affinitiesof individual RNA aptamer

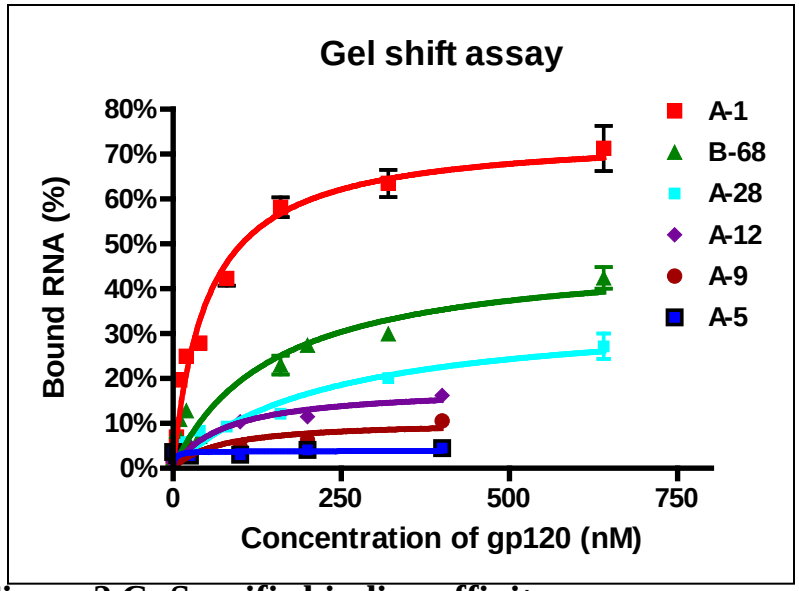
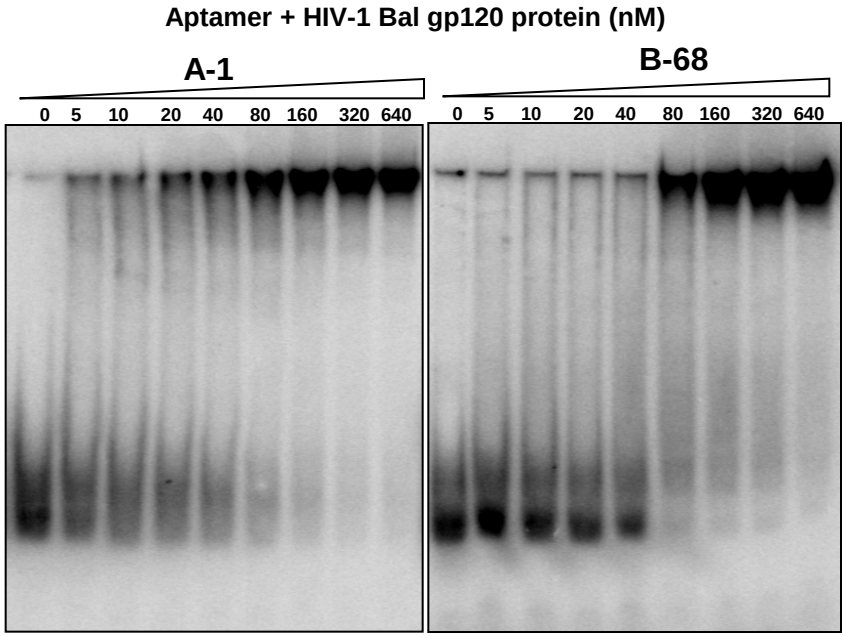
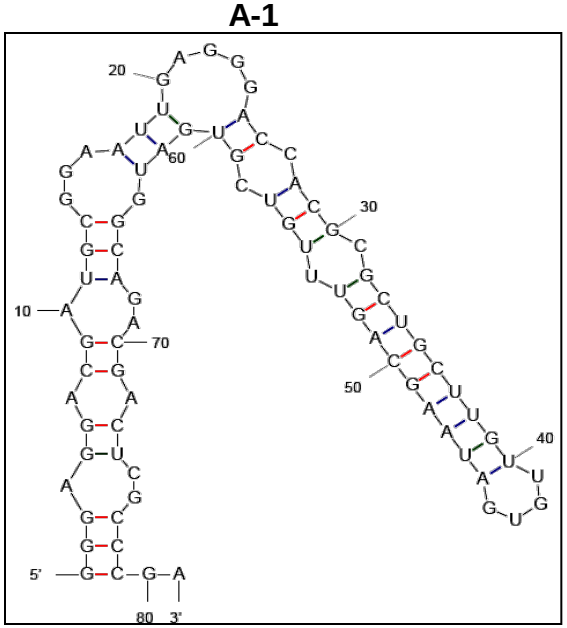


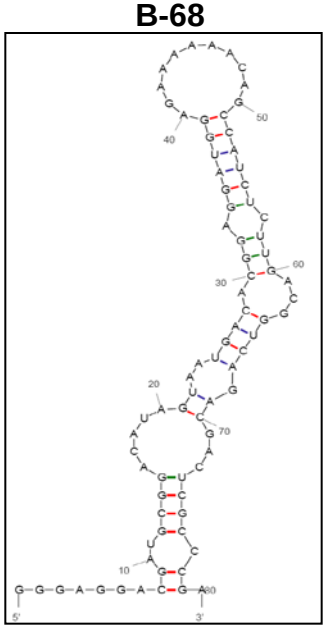
Figure 2 C: Specific binding affinity



Predicted secondary structures



K_D (nM) 52.83 ± 7.02 nM



K_D (nM) 97.76 ± 18.77 nM

Real-time live cells confocal microscopy

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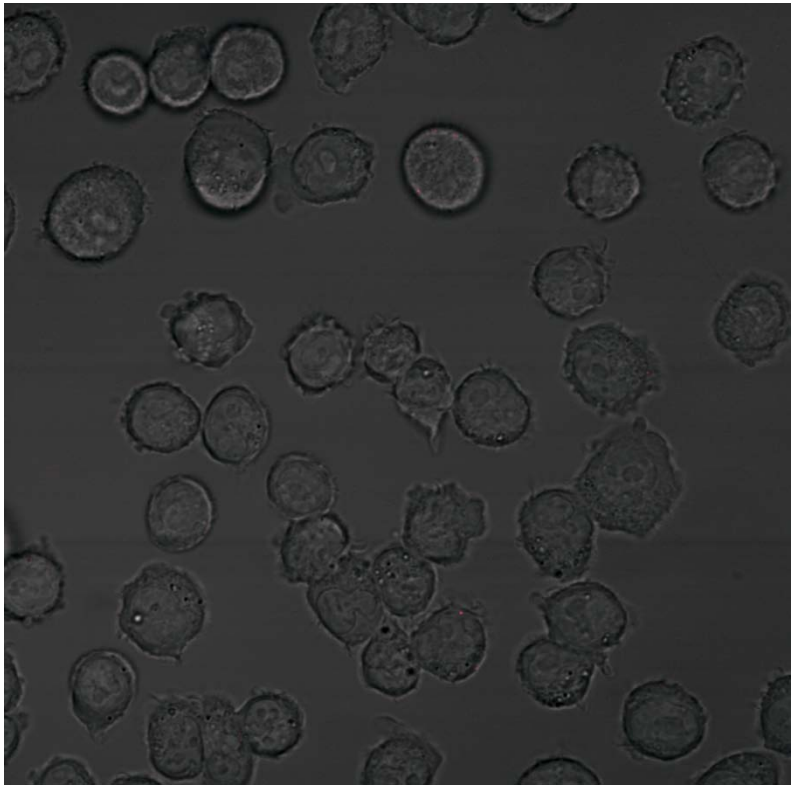
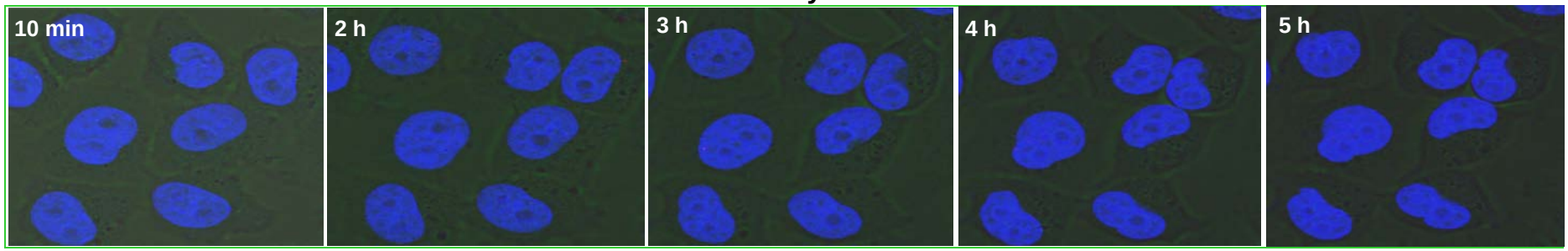
Internalization in CHO-gp160 cells of Cy3-gp120 aptamer

Movie (10 min to 6 h)

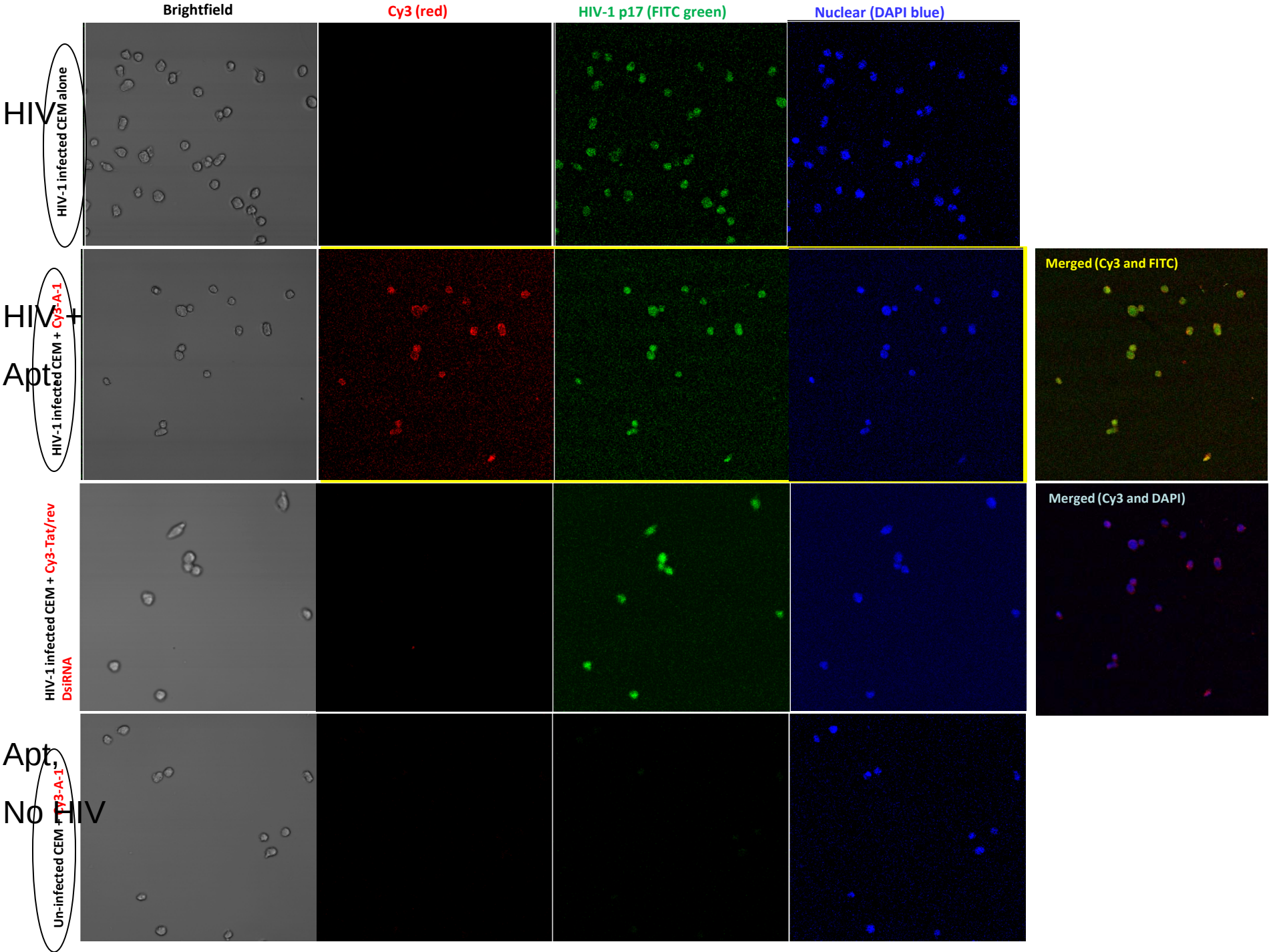


CHO-EE control + Cy3-labled Chimeras A-1



Antigp120 Aptamer- only taken up by HIV infected cells

- Infect CEM cells with NL4-3-
- Treat with Aptamer-labeled with CY3
- Treat cells with anti-p17 antibody-FITC labeled secondary Ab
- Controls: infected plus tat/rev siRNA-CY3;uninfected plus aptamer-siRNA-CY3


Brightfield
Cy3 (red)
HIV-1 p17 (FITC green)
Nuclear (DAPI blue)
HIV
HIV-1 infected CEM alone
HIV+
Apt
HIV-1 infected CEM + Cy3-A-1
Merged (Cy3 and FITC)
HIV-1 infected CEM + Cy3-Tat/rev DsiRNA
Merged (Cy3 and DAPI)
Apt
No HIV
Un-infected CEM + Cy3-A-1

Aptamer-targeted RNA interference for HIV-1 therapy

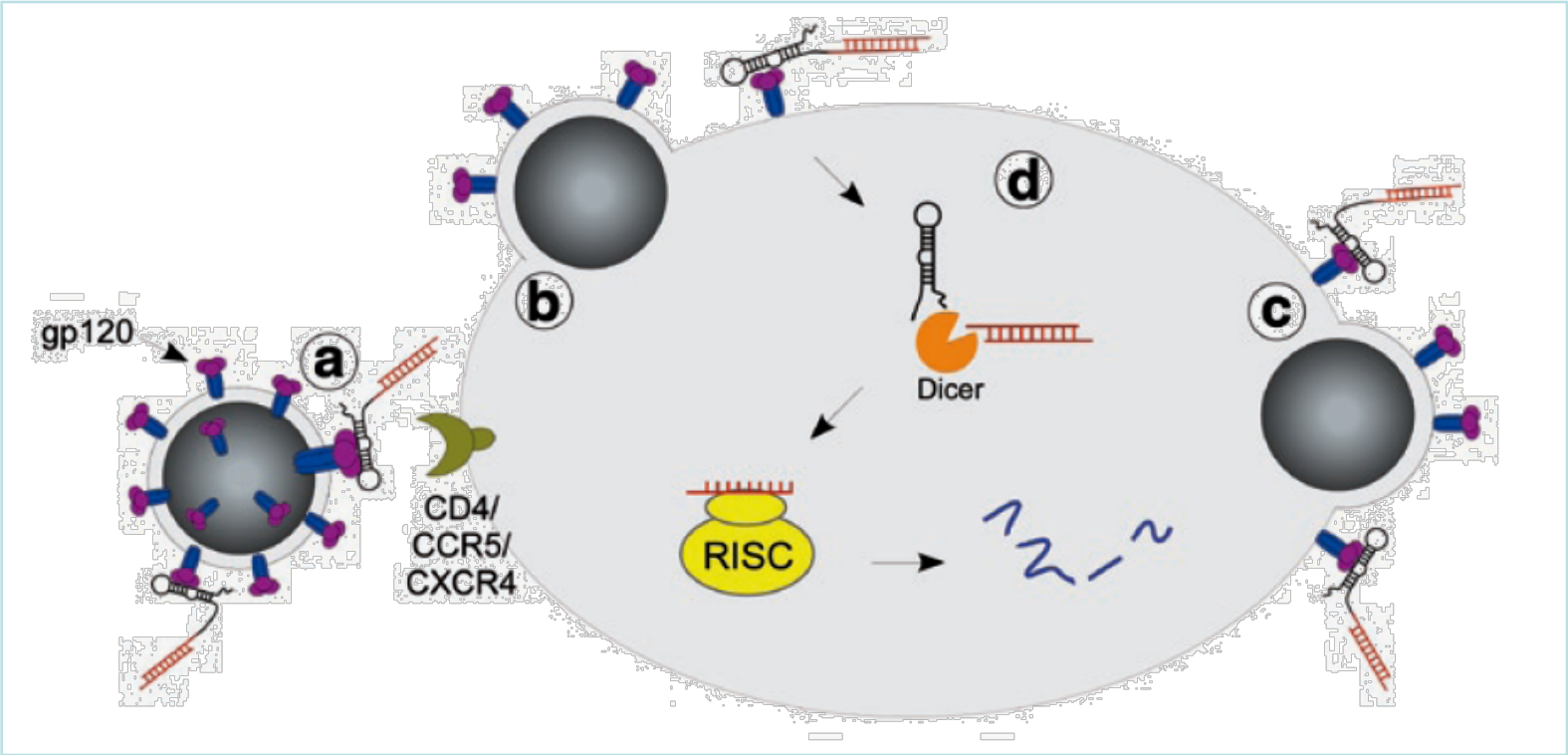
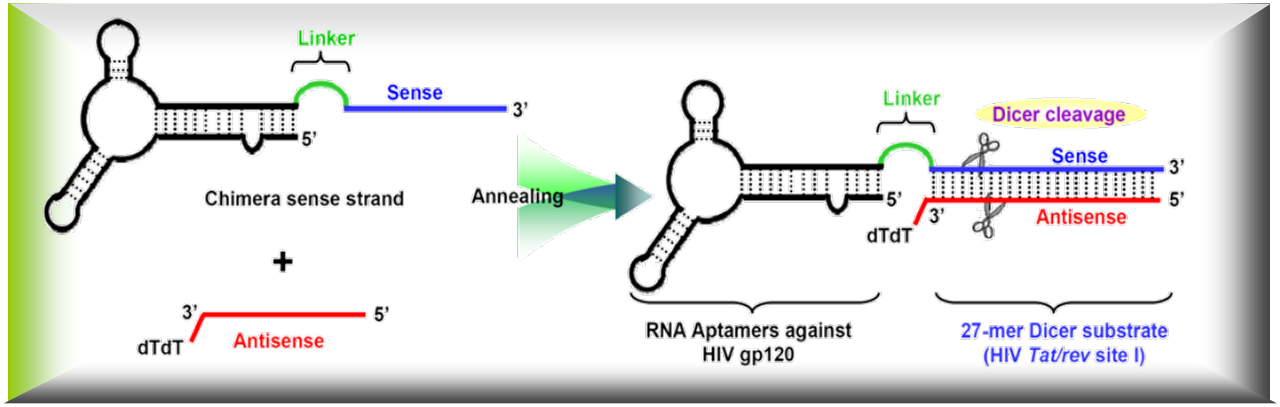


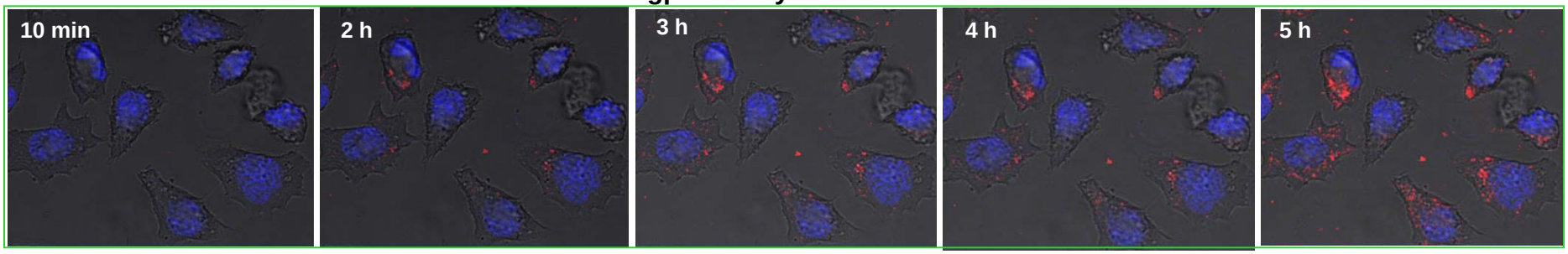
Figure 1 Hitchhiking constructs for inhibiting HIV. Aptamer (black) and siRNA (red) chimeras can bind to gp120 to block infection of cells (**a**). As virions fuse (**b**) or assemble to bud (**c**), gp120 at the cell surface can be targeted by the chimeras. Chimeras co-internalized with gp120 are processed by Dicer (**d**) and fed to the RNA-induced silencing complex to degrade target viral RNA (blue).

Amy Yan and Andrew Ellington, "A Hitchhiker's Guide to HIV", vol. 16, 1356-1358. (2008)
J. H. Zhou, H. T. Li, S. Li, J. Zaia, J. Rossi, Molecular Ther. (2008)
J. H. Zhou, C. P. Neff, P. Swiderski, H. T. Li, J. Zhang, R. Akkina, J. J. Rossi, Nucleic Acid Res. (2009)

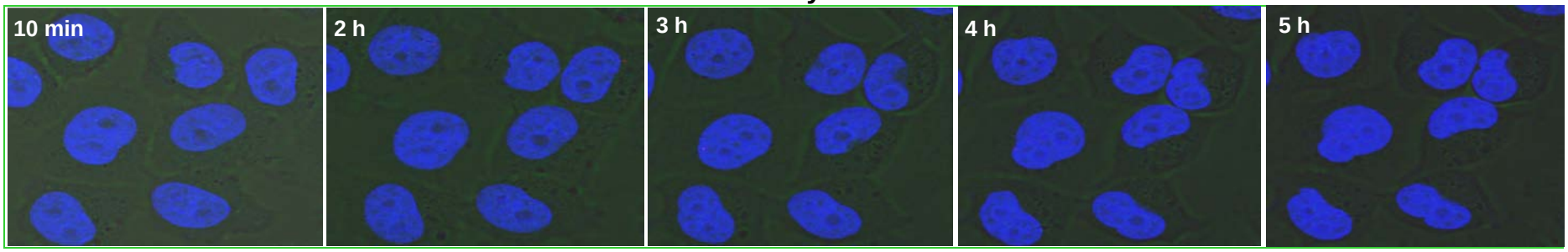
Chimeric-siRNA Aptamer Uptake Into gp120 Expressing Cells



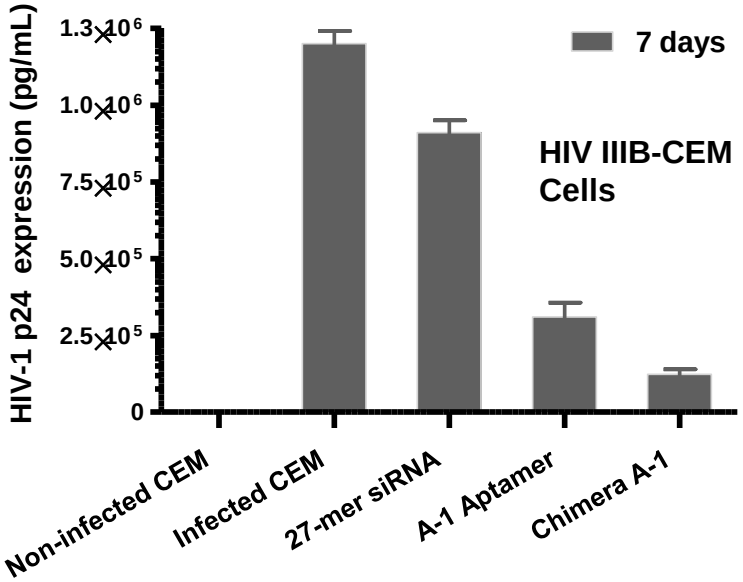
CHO-WT gp160 + Cy3-labled Chimeras A-1

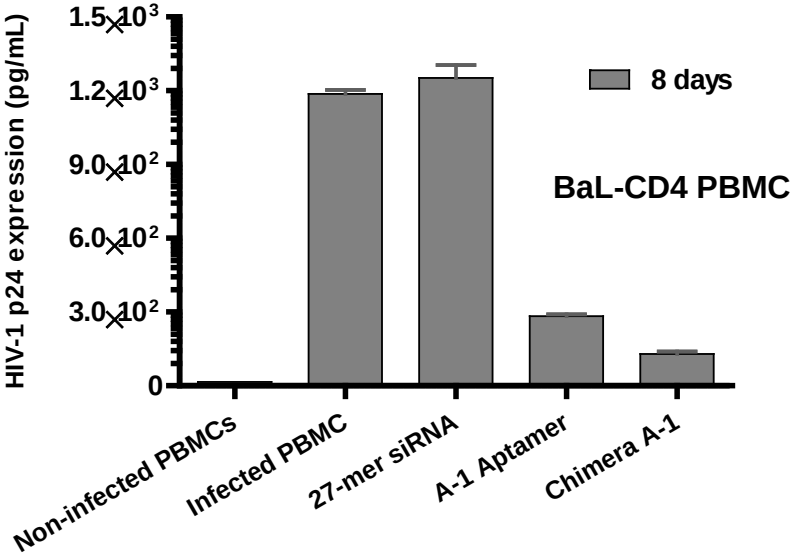


CHO-EE control + Cy3-labled Chimeras A-1



Aptamer and Aptamer chimera mediated inhibition of HIV-1 in PBMCs and Cultured T-lymphocytes





Small animal model for studying anti-HIV RNAi based therapies

Rag2−/−gc−/− (RAG-hu) mice with a capacity for multilineage human hematopoietic cell engraftment. P. W. Denton, J. V. Garcia, Novel humanized murine models for HIV research. Curr. HIV/AIDS Rep. 6, 13–19 (2009).

HIV-1–infected RAG-hu mice can sustain chronic viremia lasting for more than a year.

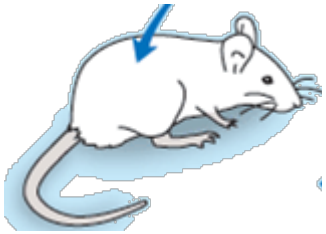
HIV -1 infected animals display a continuous declining trend of CD4+ T cell levels, mirroring the main features of human HIV-1 infection.

Thus, the RAG-hu mouse model of HIV-1 infection is an excellent model for in vivo pathogenesis studies and the evaluation of new anti-HIV therapeutic strategies (Neff et al, Science Translational Medicine 2011).

In vivo testing of aptamer-siRNA chimera in humanized RAG-hu mice

Rag2-/-gamma c-/- mouse

HIV NL4-3 infection



1th week infection

3 -5 weeks infection

3 to 5 weekly injections of aptamer, siRNA

Chimeras A-1

Control RNAs

5th week

6th week

7th week

8th week

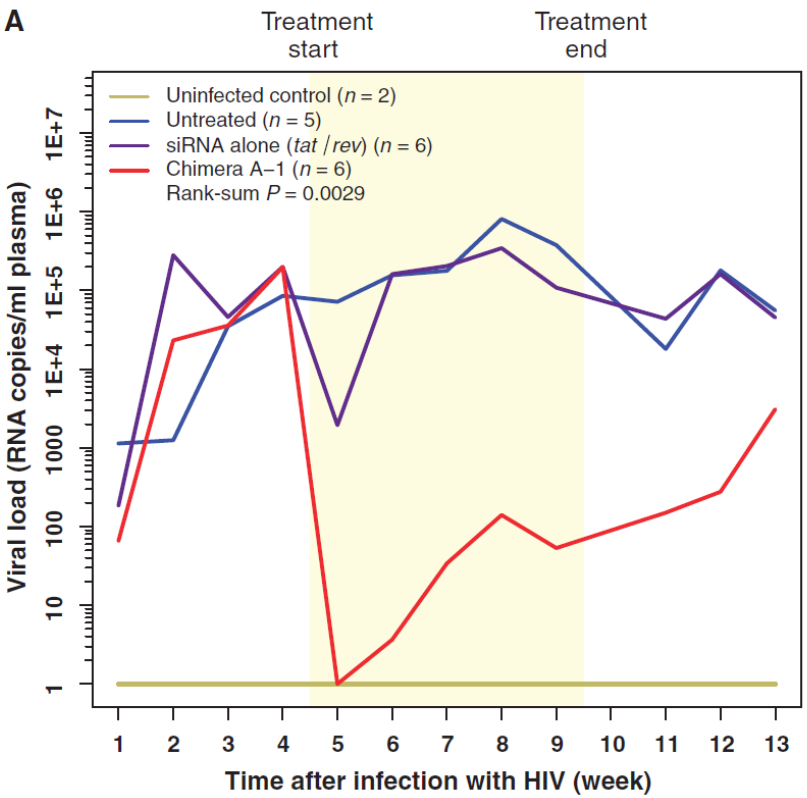
injection

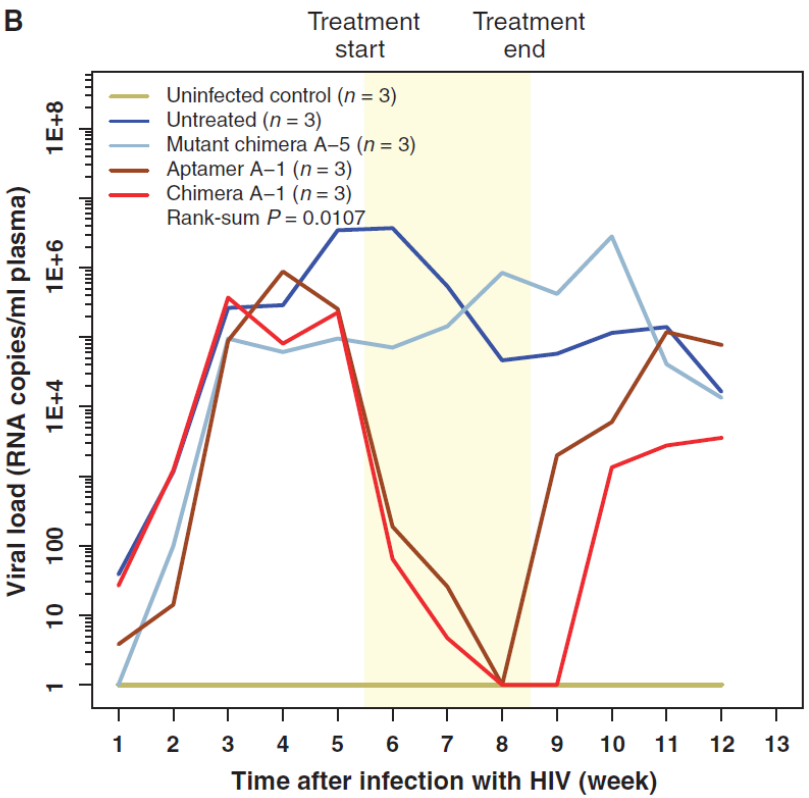
Untreated

- The humanized Rag2-/-gc-/- mice (RAG-hu) engrafted with CD34 hematopoietic progenitor cells were infected with HIV-1 NL4.3 virus intraperitonially.
- One injection of 10 g experimental RNA per week.
- 5th week sample for siRNA and Tat/rev detection
- 1-9 weeks samples for viral loading test monitored by real-time PCR.

Neff et al, STM 2011

Both aptamer and aptamer chimera inhibit HIV infection

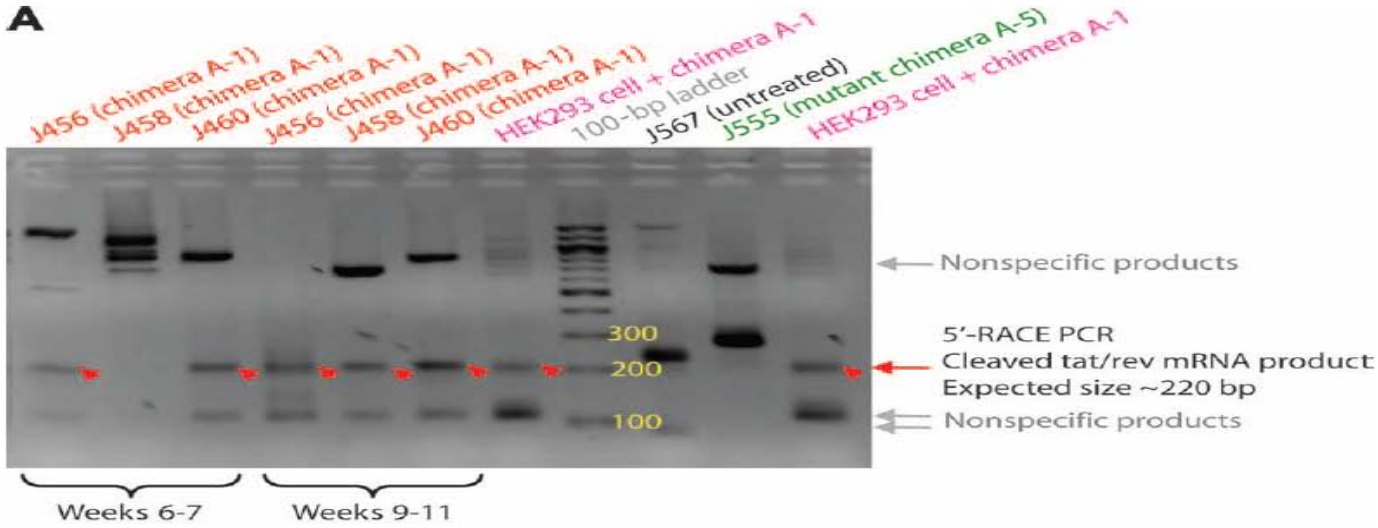


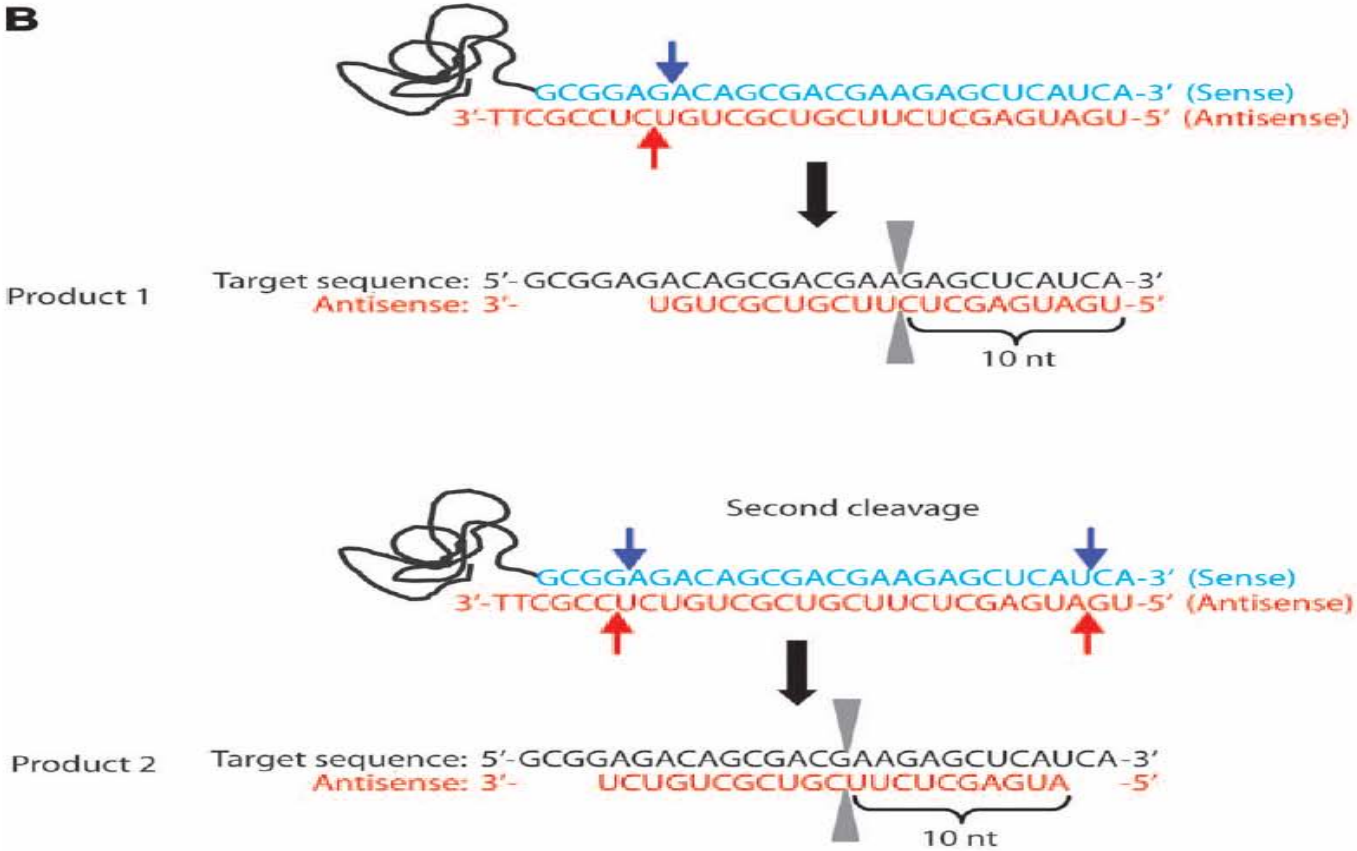


Neff et al, STM 2011

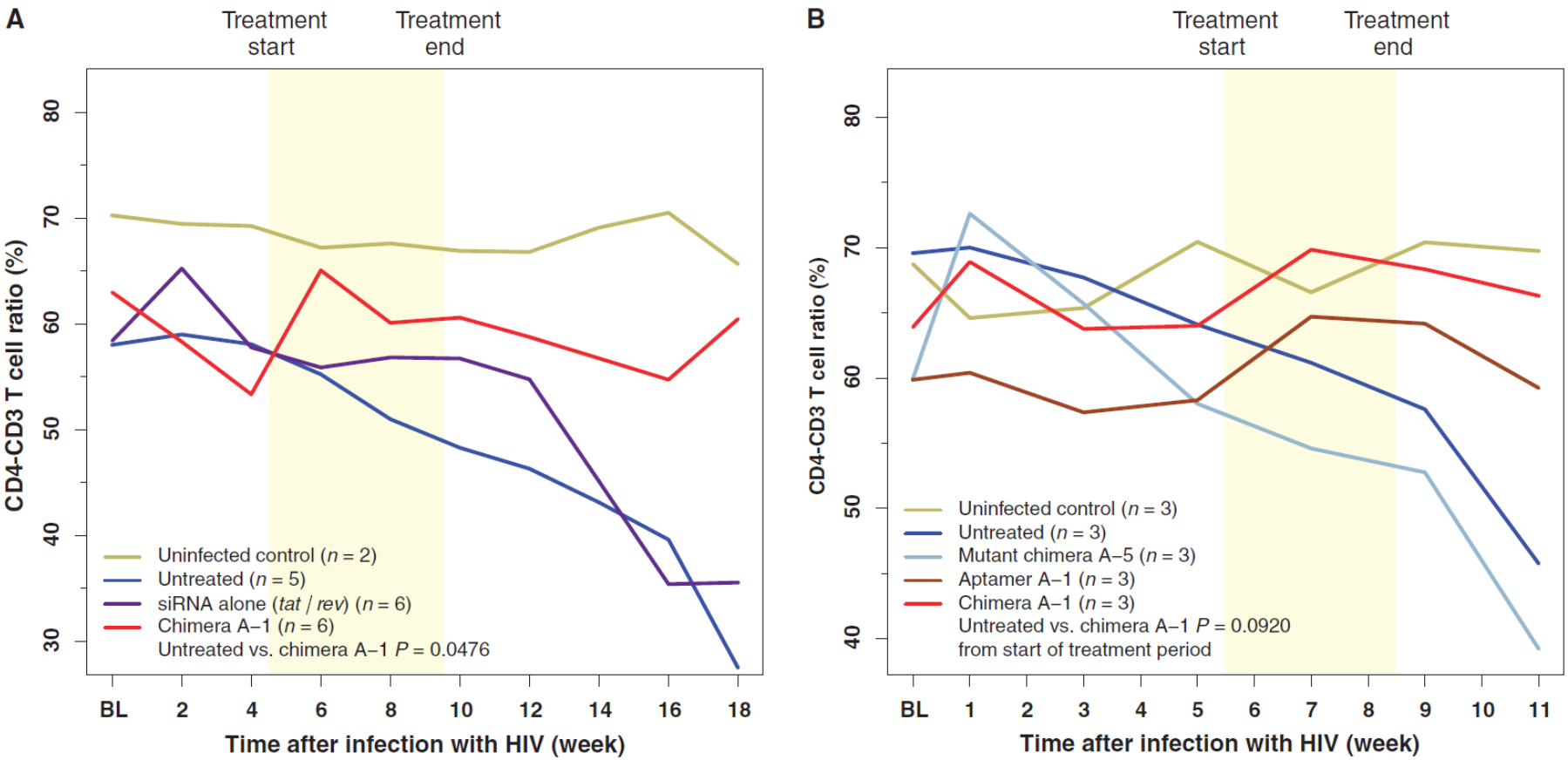
siRNA enhances duration of anti-HIV activity over aptamer alone

		Week1	Week2	Week3	Week4	Week5	Week6	Week7	Week8	Week9	Week10	Week11	Week12
Uninfected Control	J570	1	1	1	1	1	1	1	1	1	1	1	1
	J571	1	1	1	1	1	1	1	1	1	1	1	1
	J572	1	1	1	1	1	1	1	1	1	1	1	1
	Average	1	1	1	1	1	1	1	1	1	1	1	1
Non-treated	J565	15	46	70653	40173	1611916	2655213	978487	60589	13505	107329	81927	13975
	J566	64	16469	526913	167200	3062118	8307855	270938	291214	335899	1731311	988012	46677
	J567	60	2016	491653	3579558	8307830	2302355	593930	5652	42427	8190	33857	6770
	Average	46	6177	363073	1262310	4327288	4421808	614452	119152	130610	615610	367932	22474
Mutant Chimera A-5	J554	1	1146	23277	56175	39446	24263	320608	716431	841330	2035506	49469	24459
	J555	1	849	70653	24425	20593	14871	59073	1257084	39690	6266084	14665	7238
	J556	1	1	526913	166751	1070324	987725	155435	661132	2205710	1733448	93791	13743
	Average	1	665	206948	82450	376788	342286	178372	878216	1028910	3345013	52641	15147
Aptamer A-1	J547	1	3	93229	599425	911450	**83**	**176**	**1**	**709**	**26559**	**93791**	**113621**
	J548	1	12	302753	494757	494491	**188**	**1**	**1**	**2961**	**4489**	**29650**	**38287**
	J549	56	79	24952	2296677	36377	**429**	**98**	**1**	**3791**	**1820**	**599471**	**106580**
	Average	19	31	140312	1130286	480773	233	92	1	2487	10956	240971	86162
Chimera A-1	J456	1	1921	308042	137626	562806	**628**	**104**	**1**	**1**	**1102**	**2435**	**3346**
	J458	37	1921	125161	40783	479243	**429**	**1**	**1**	**1**	**1304**	**6800**	**8774**
	J460	510	495	1319090	93745	42772	**1**	**1**	**1**	**1**	**1675**	**1278**	**1539**
	Average	183	1445	584097	90718	361607	353	35	1	1	1360	3504	4553


A
J456 (chimera A-1)
J458 (chimera A-1)
J460 (chimera A-1)
J456 (chimera A-1)
J458 (chimera A-1)
J460 (chimera A-1)
HEK293 cell + chimera A-1
100-bp ladder
J567 (untreated)
J555 (mutant chimera A-5)
HEK293 cell + chimera A-1
Nonspecific products
300
200
100
5'-RACE PCR
Cleaved tat/rev mRNA product
Expected size ~220 bp
Nonspecific products
Weeks 6-7
Weeks 9-11

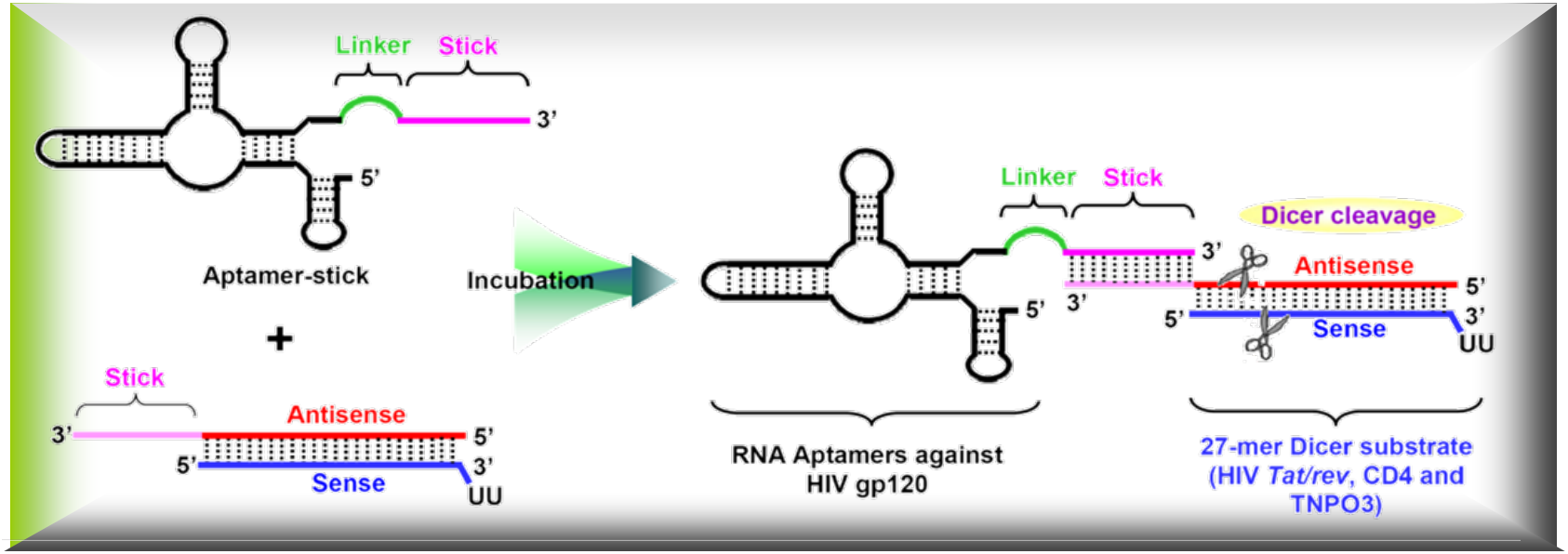

B
GCGGAGACAGCGACGAAGAGCUCAUCA-3' (Sense)
3'-TTCGCCUCUGUCGCUGCUUCUCGAGUAGU-5' (Antisense)
Product 1
Target sequence: 5'-GCGGAGACAGCGACGAAGAGCUCAUCA-3'
Antisense: 3'- UGUCGCUGCUUCUCGAGUAGU-5'
10 nt
Second cleavage
GCGGAGACAGCGACGAAGAGCUCAUCA-3' (Sense)
3'-TTCGCCUCUGUCGCUGCUUCUCGAGUAGU-5' (Antisense)
Product 2
Target sequence: 5'-GCGGAGACAGCGACGAAGAGCUCAUCA-3'
Antisense: 3'- UCUGUCGCUGCUUCUCGAGUA -5'
10 nt

Aptamer and chimeric aptamer-siRNA prevent CD4+ cell decline



Neff et al, STM 2011

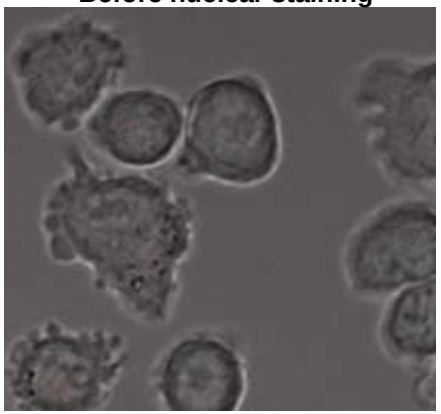
Modular aptamer-that allows separate synthesis of aptamer and dicer substrate siRNAs. Can be used for multiplexing siRNAs



Internalization in CHO-gp160 cells of aptamer-stick-siRNA

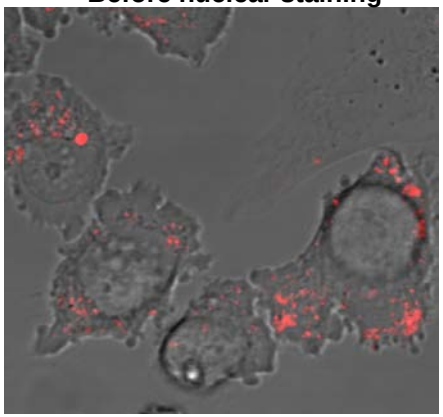
10 min incubation

Before nuclear staining

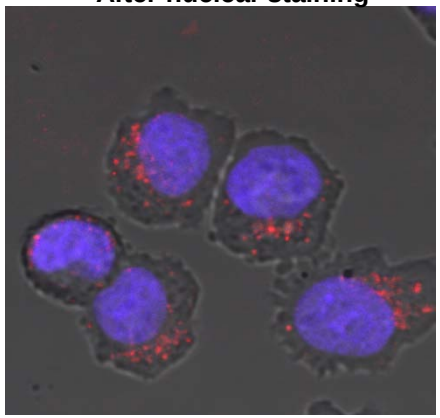


Overnight incubation

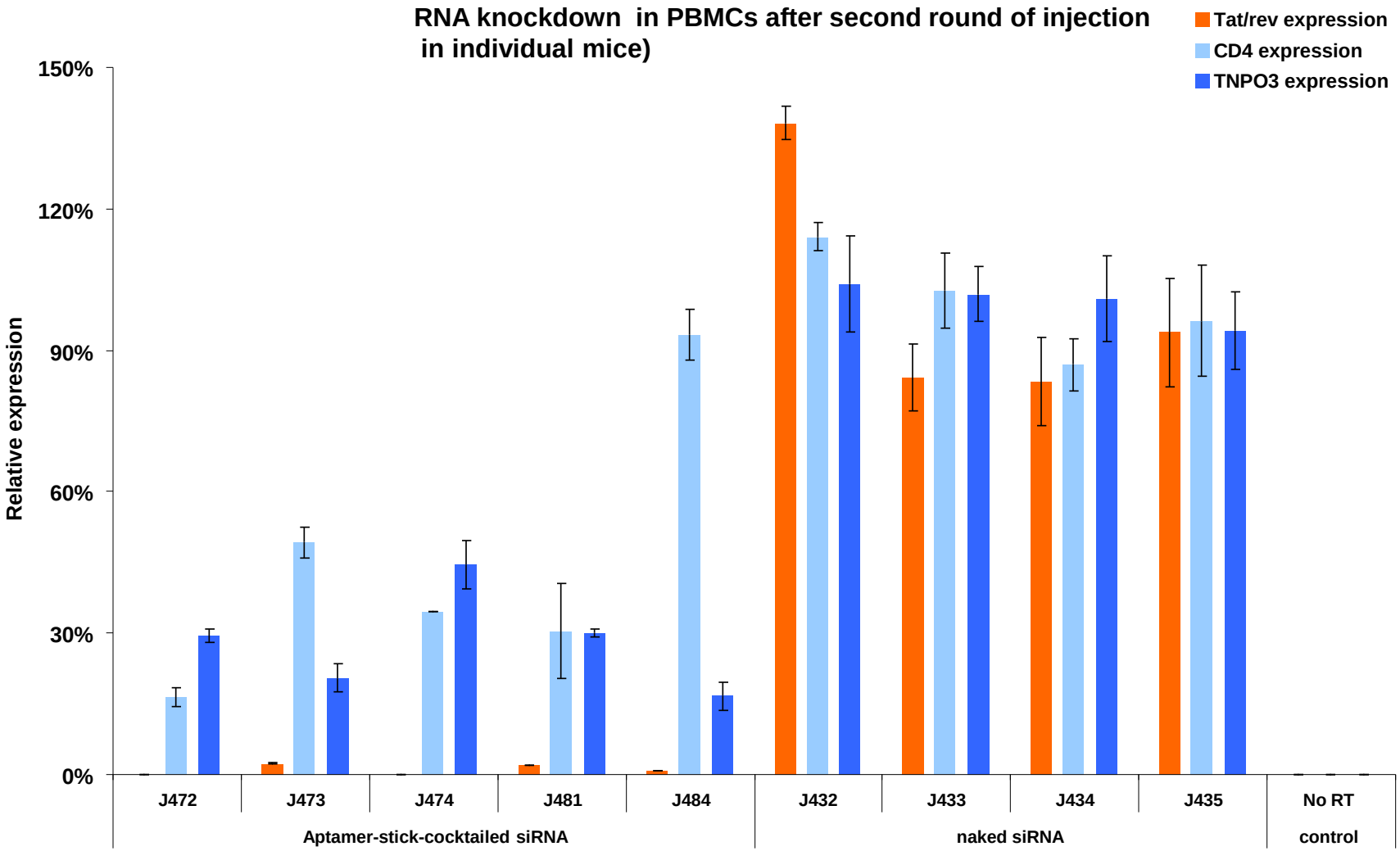
Before nuclear staining



After nuclear staining



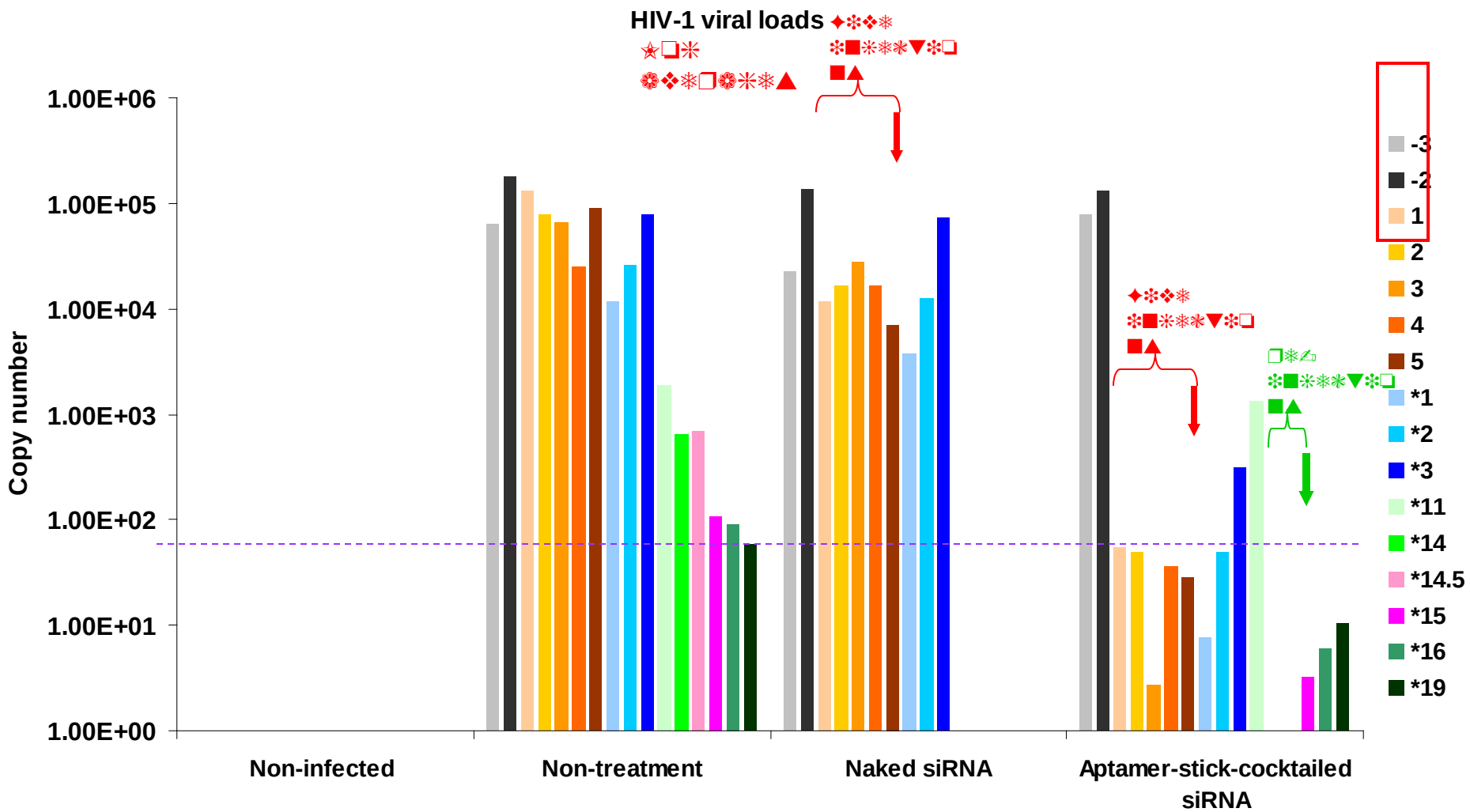
Multiplexed aptamer-dsiRNAS down regulate multiplexed targets in humanized mouse model.



❖Five injections were performed from 1 week to 5 week.

❖Two more injections were performed at *12.5 week and *13.5 week. (re-treatment: aptamer-cocktail siRNAs)

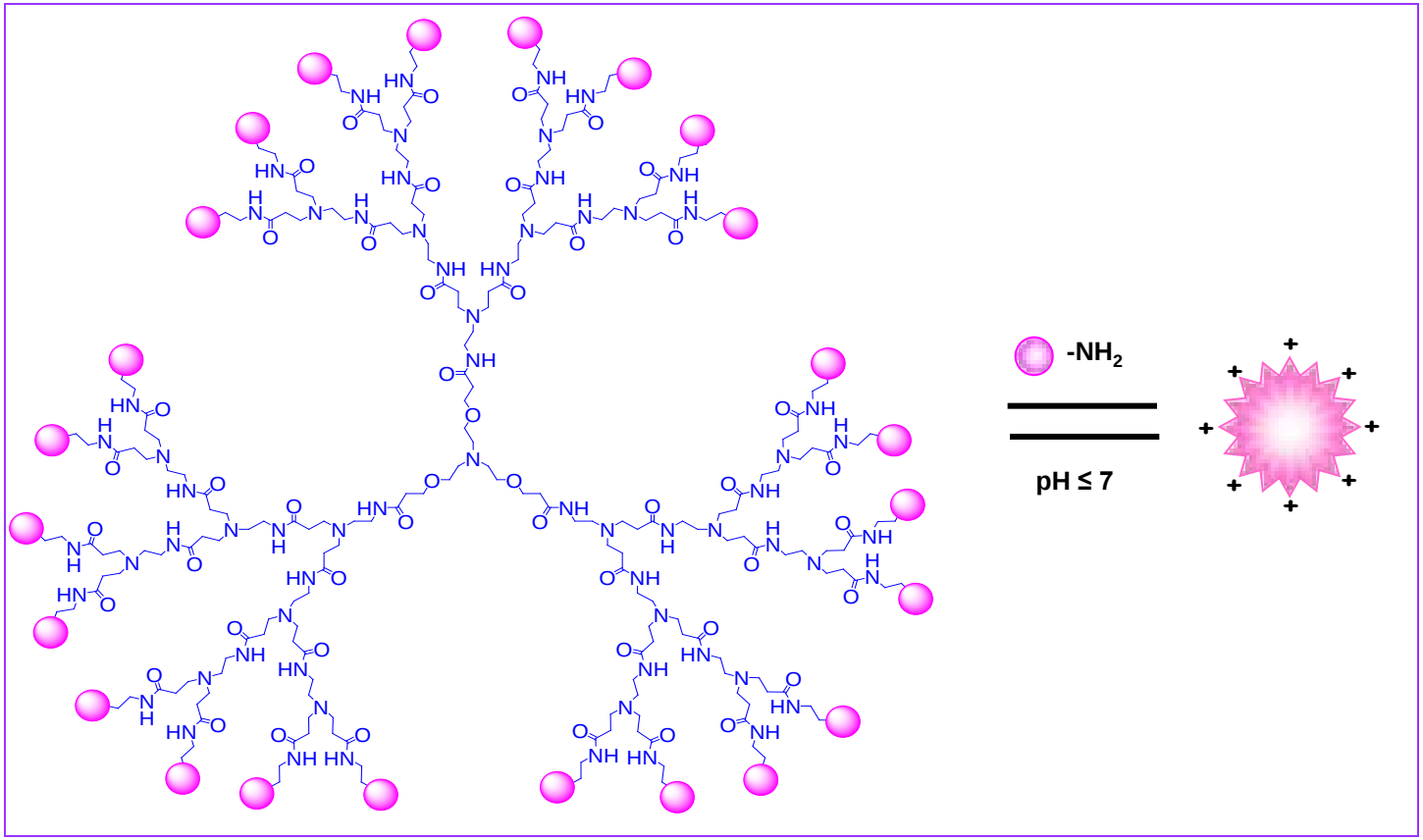
❖One injection of 0.25 nmol (10 g) experimental RNA per week.



Summary of Aptamer studies

- **Once a week intravenous injections of 10 ugms/animal of Anti-gp120 aptamer-siRNA chimeras provided long term inhibition of HIV-1 replication in a humanized mouse model of HIV infection.**
- **Aptamer-siRNA chimeras provide dual function inhibition.**
- **A synthetic version of the anti-gp120 aptamer with a sticky-bridge sequence allowed mixing of a cocktail of siRNAs for effective target knockdown and inhibition of HIV in the humanized mice.**
- **The aptamer approach presents the possibility of purging HIV infected cells via selective delivery of cyto-toxic siRNAs to HIV infected cells.**

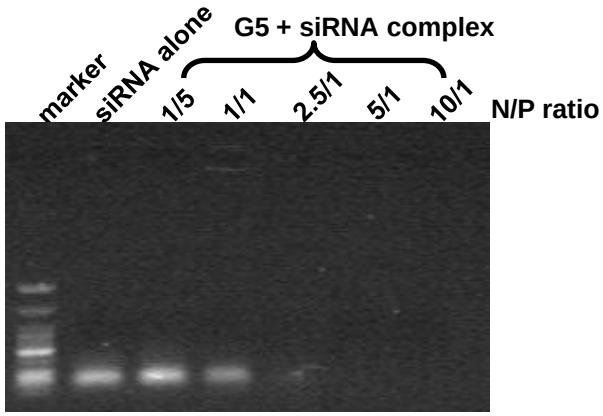
Flexible PAMAM dendrimers with a triethanolamine core (for clarity, G3 dendrimer as an example)



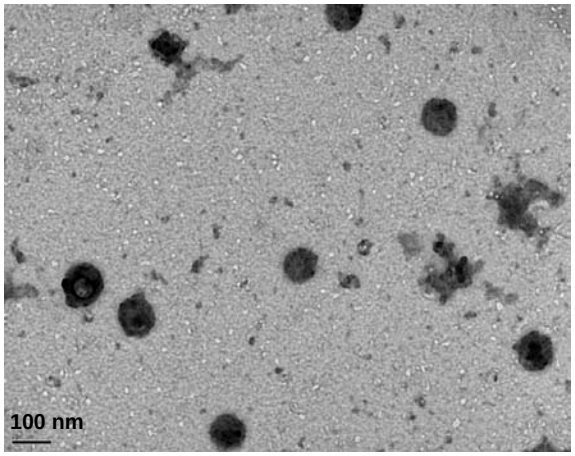
Dendrimers –plus siRNAs form nanoparticles and protect siRNA from degradation

Formation of G5-siRNA complexes

gel shift assay

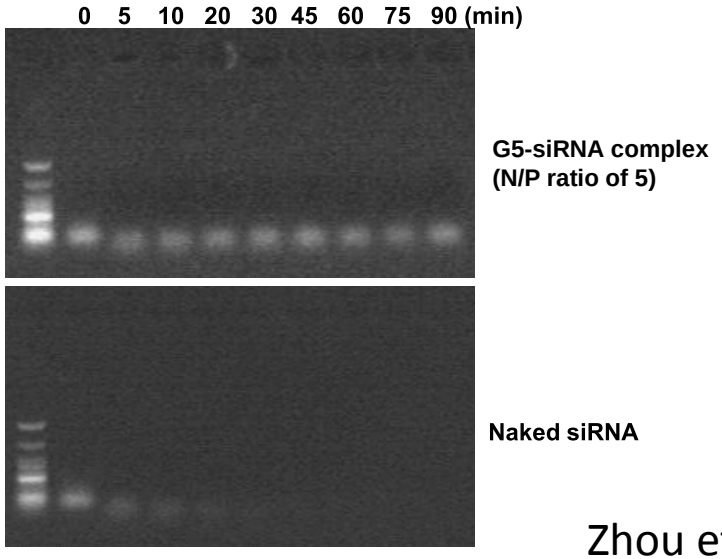


TEM image of G5-siRNA nanoparticles

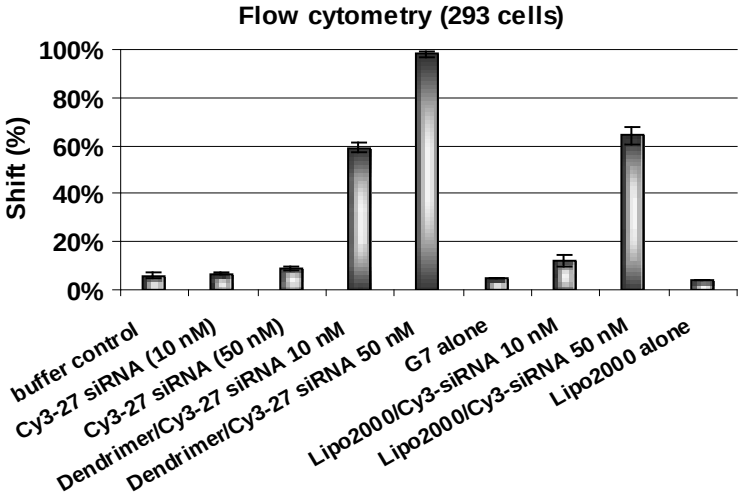


RNase resistance of G5-siRNA complexes

siRNA stability



Cellular uptake of G5-siRNA complex in HEK293 cells



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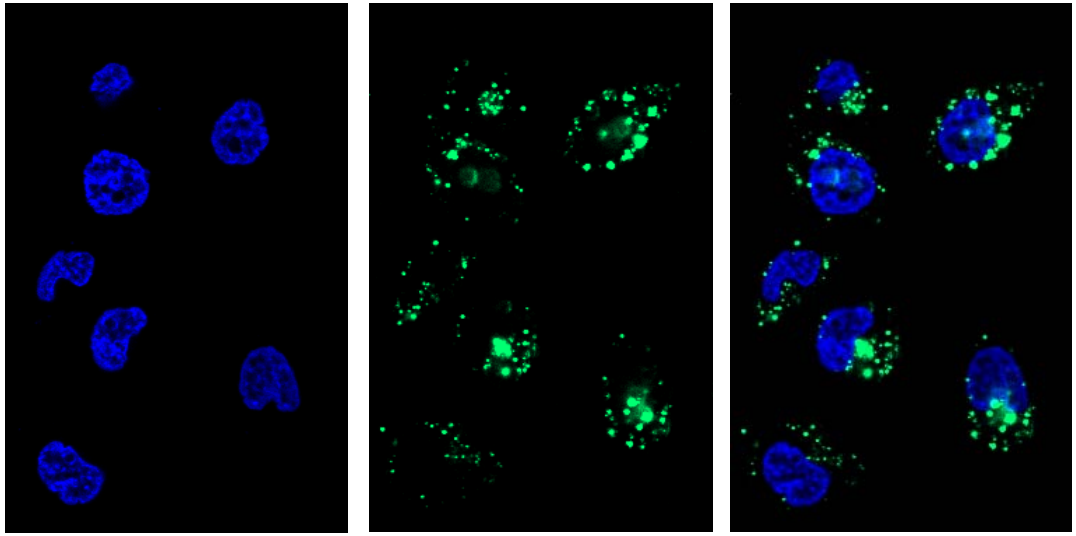
Cellular uptake study of dendrimer-siRNA complexes-mediated by macropinocytosis

G_5-Alexa488 dsiRNA (4 h incubation)

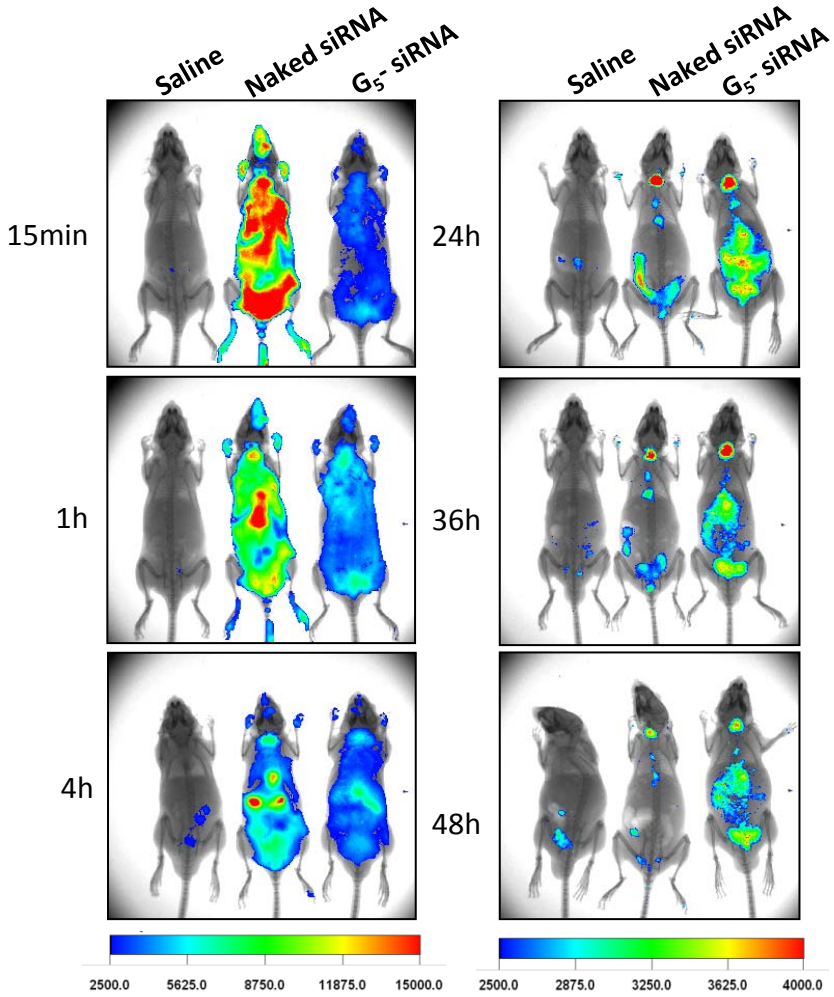
Blue
Nucleus
of Hela cells

Green
G_5-dsiRNA
in HeLa cell

Merged



In vivo distribution and cryosection observation of G_5 dendrimer-Cy5 siRNA complexes



Yuanyu Huang, Quan Du, and Zicai Liang
Institute of Molecular Medicine, Peking University
Zhou et al., Molecular Therapy 2011

Multiplex siRNAs for HIV-1 treatment

Anti-HIV tat/rev siRNA

The Tat and Rev regulatory proteins encoded by HIV-1 are both expressed early in the viral life cycle and are both essential for viral replication.

Anti-CD4 siRNA

Primary receptor required for HIV entry.

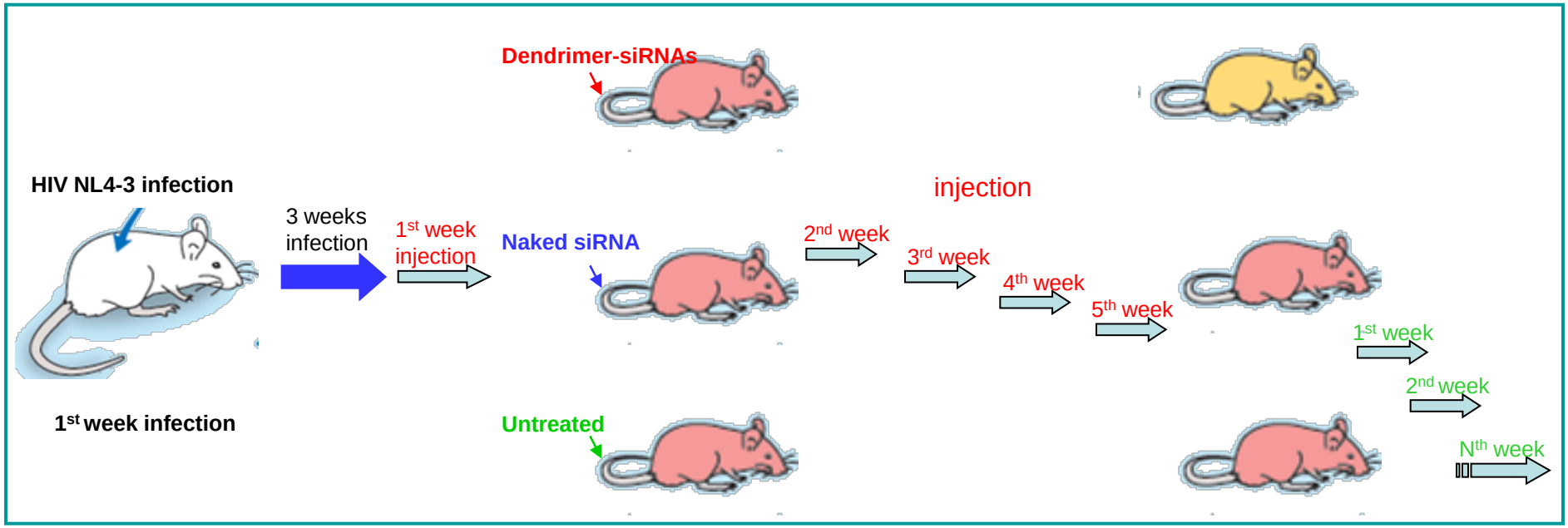
Anti-TNPO3 siRNA

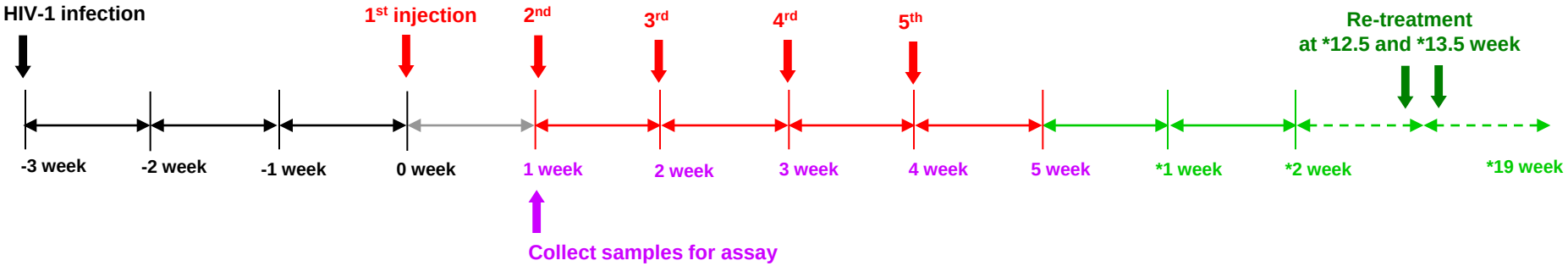
TNPO3 is required for nuclear import of the HIV-1 preintegration complex (PIC).

In vivo analyses of G5 Dendrimer Delivered siRNAs

- Cocktail of three (TNPO3, tat/rev, CD4)
- Dendrimer-Tat/rev alone
- Dendrimer-mutant non-functional tat/rev siRNA
- Uncomplexed combination of siRNAs
- Dendrimer alone

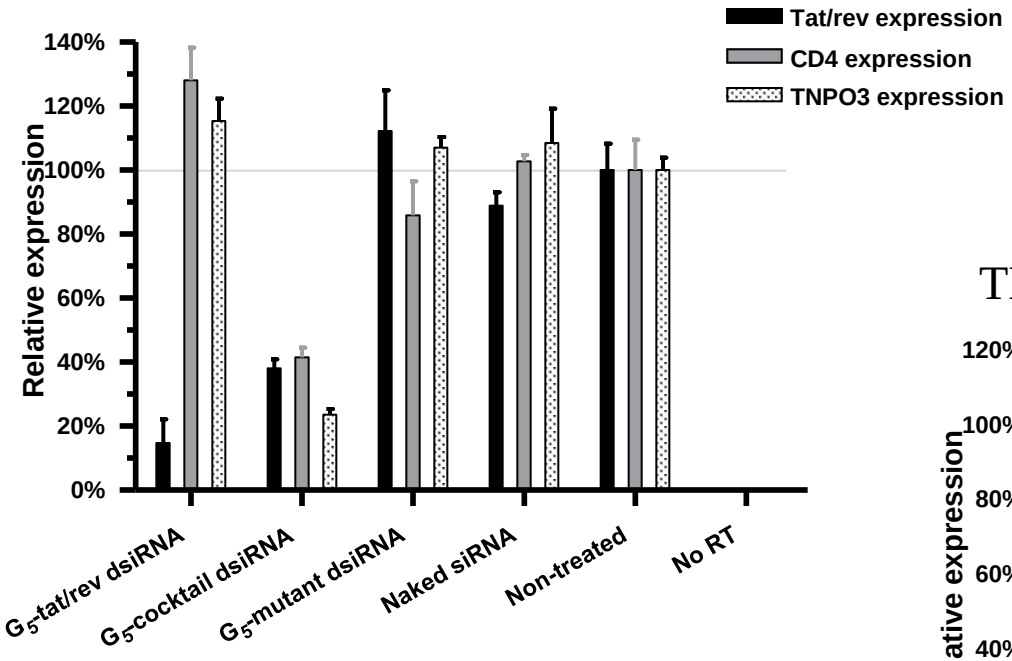
humanized Rag2-/-γc-/- mice (RAG-hu), injected with human CD34+ hematopoietic progenitor cells-differentiate in T-cells, macrophages,monocytes, dendritic cells



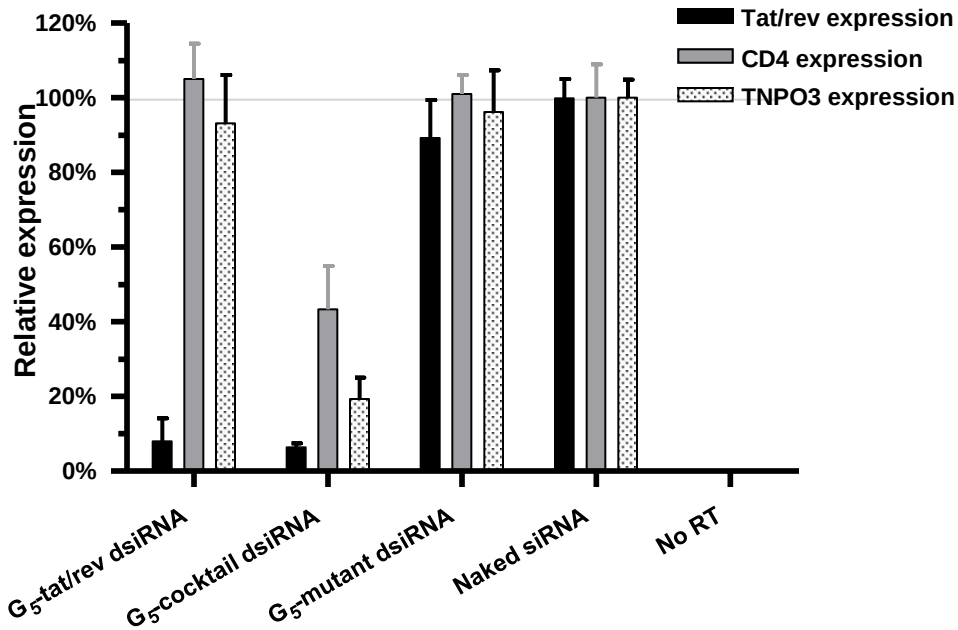


Targeted mRNA reductions in Dendrimer/siRNA treated animals following second and third injections.

SECOND WEEK



THIRD WEEK



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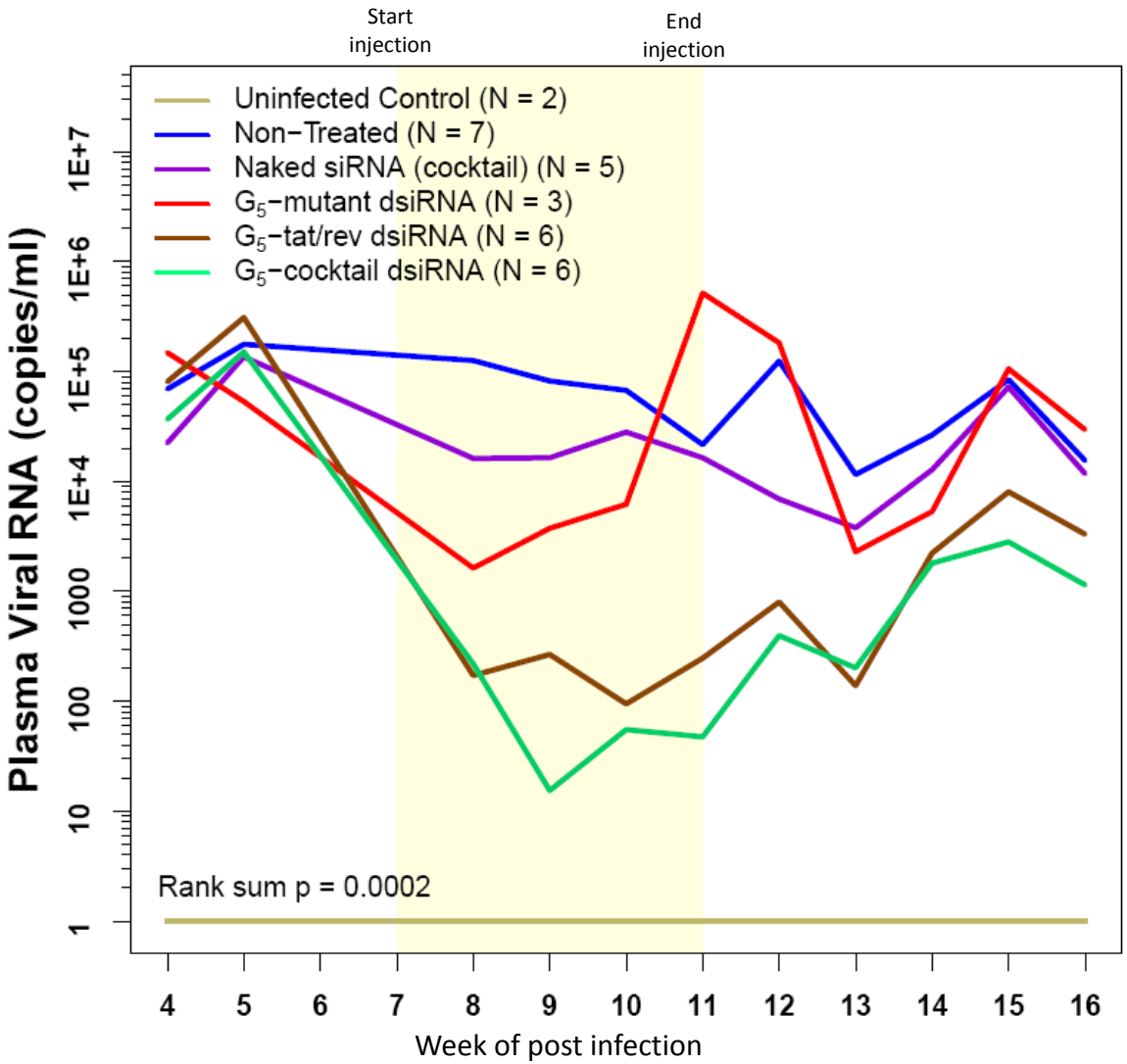
REDUCTIONS IN HIV LOADS FOLLOWING DENDRIMER SiRNA INJECTIONS



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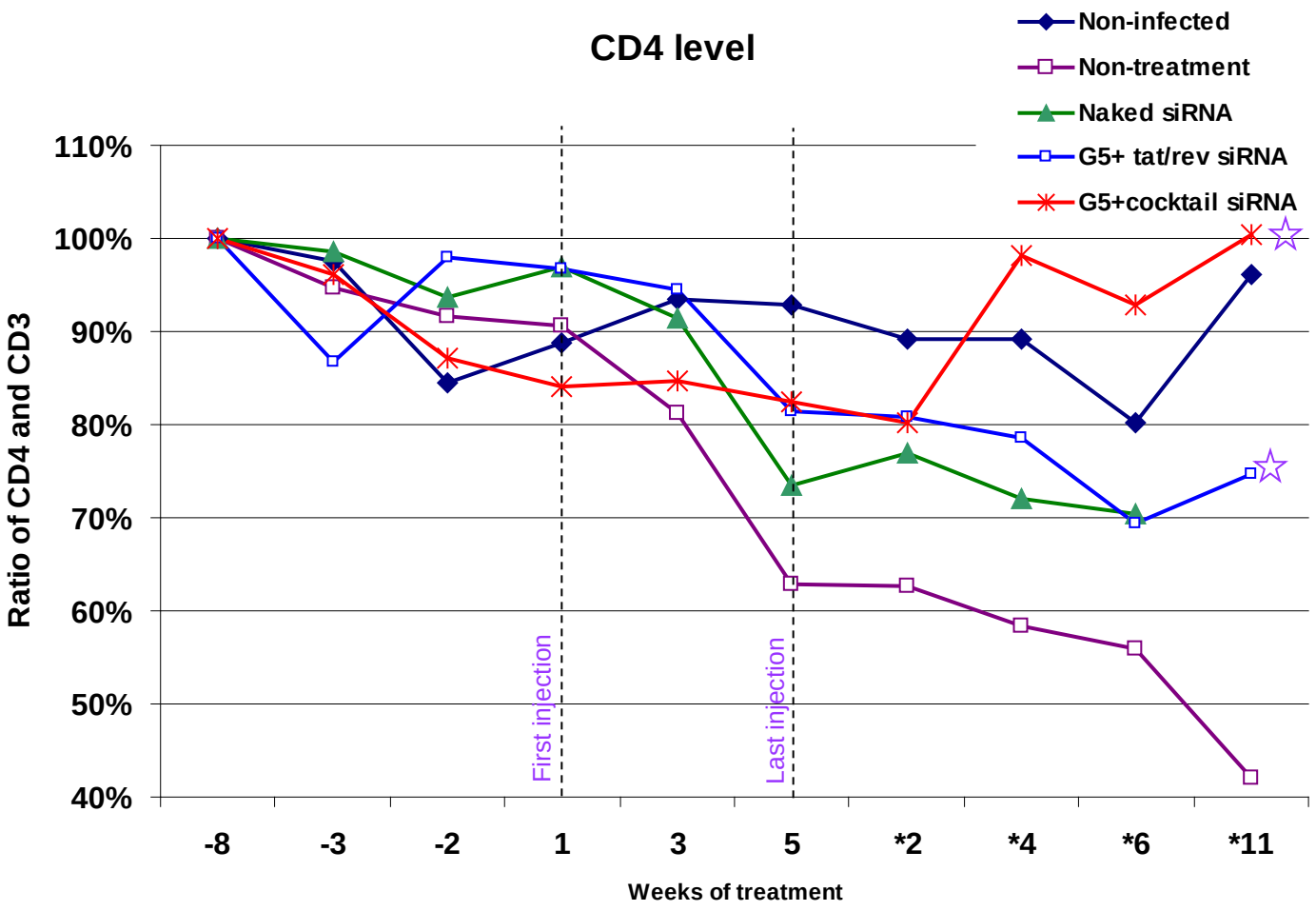
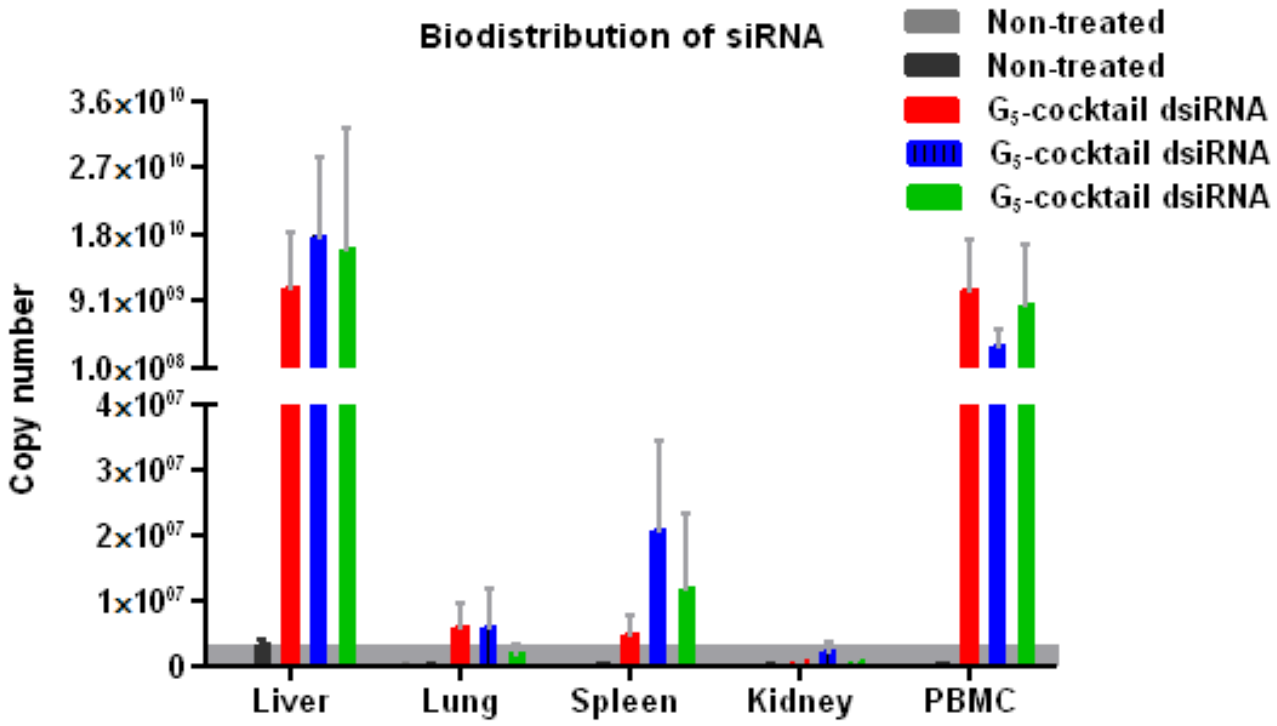
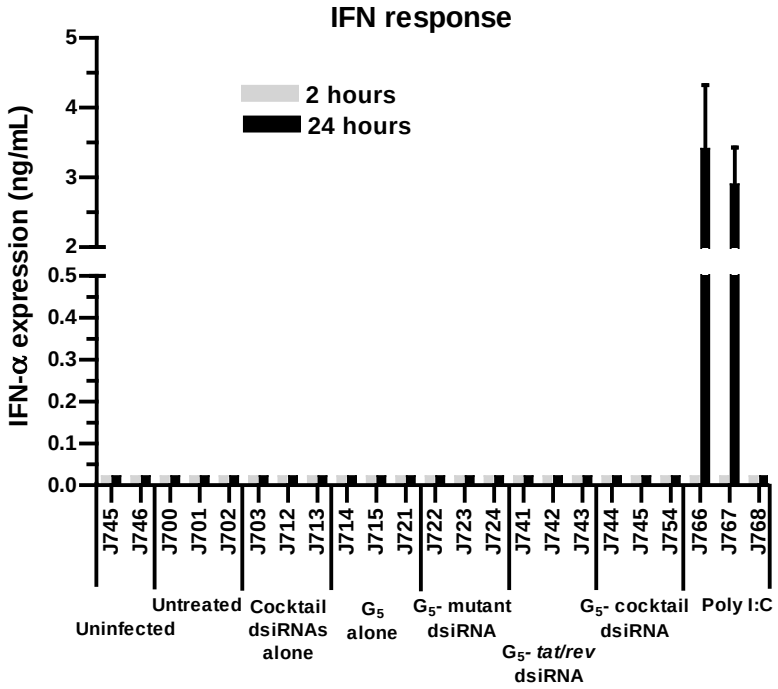
Dendrimer-siRNA complexes protect HIV infected RAG-hu mice from CD4 T cell loss.

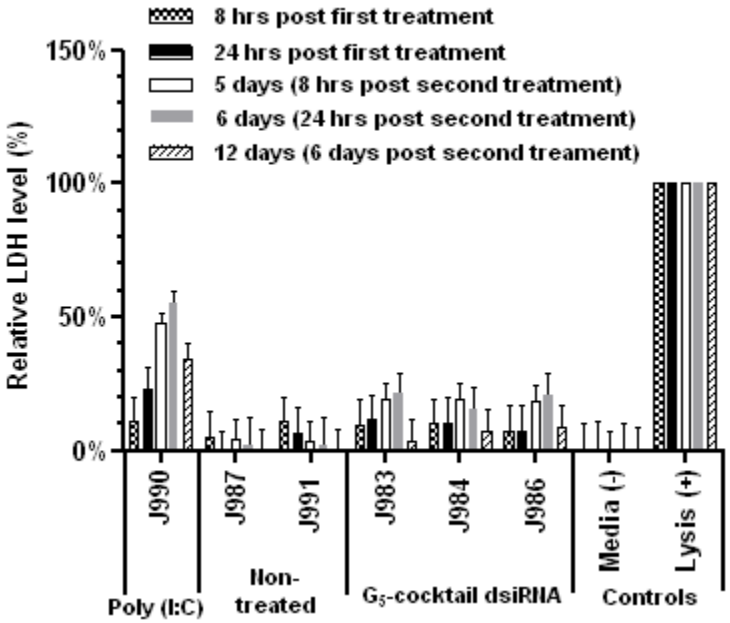


Bio distribution of G5 dendrimer siRNAs



No toxicities following dendrimer siRNA treatments





Summary of Dendrimer studies

- PAMAM dendrimers are safe and effective delivery vehicles of for dsiRNAs/siRNAs
- Effective in vivo delivery to lymphocytes which are normally refractory to most delivery vehicles.
- These dendrimers are candidates for delivery of cocktails of therapeutic dsiRNAs/siRNAs in HIV infected patients who have HAART resistant virus.

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RAG-hu mice:

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Colorado State University

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