Kirby Institute

Block and Lock: A pathway to remission

Tony Kelleher | September, 2019

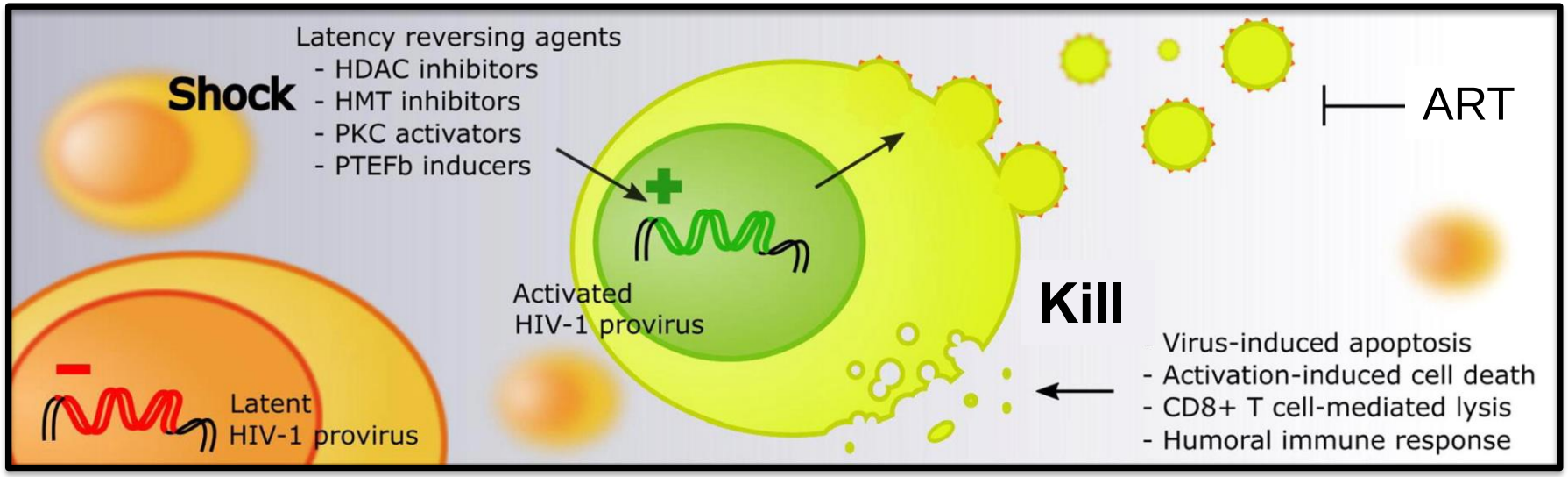
Conflicts of interest

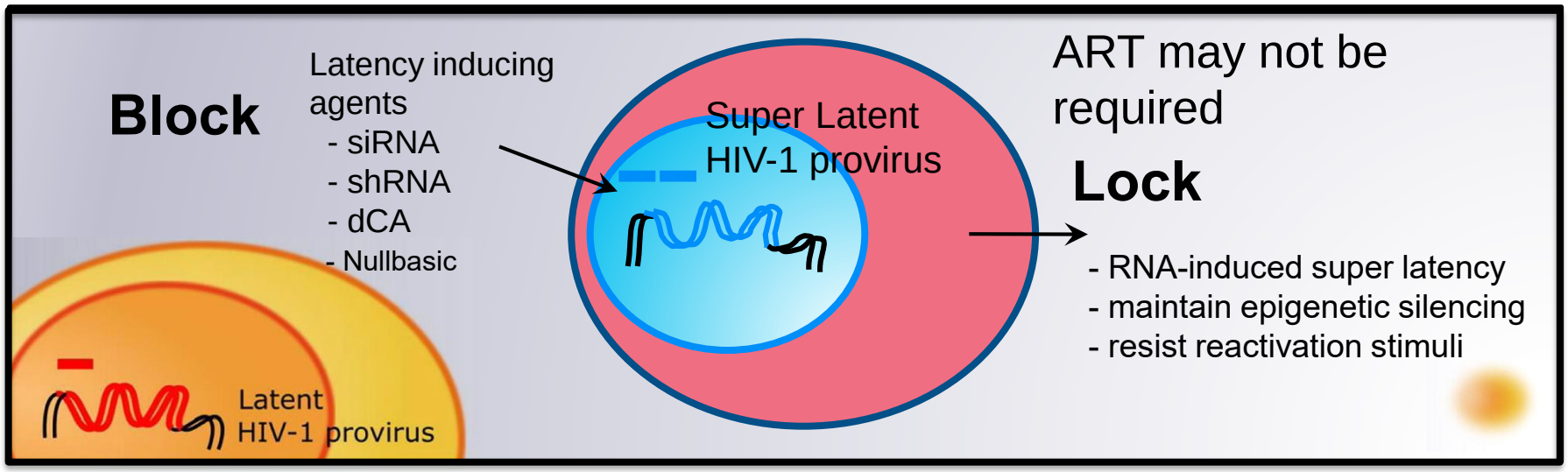
- Inventor on patents regarding Promoter targeted siRNAs
- Calimmune /CSL has provided
  - Anti CCR5 shRNAs
  - retroviral backbones for delivery and expression of sh/siRNAs

  used in the SCID Hu Mouse experiments

HIV cure strategies

- Eradication vs Functional: “Shock & Kill” or “Block & Lock” ???





The virus in the reservoir is in a Latent state

Post-integration Latency

DNA form of the Virus, integrated BUT not transcribing:

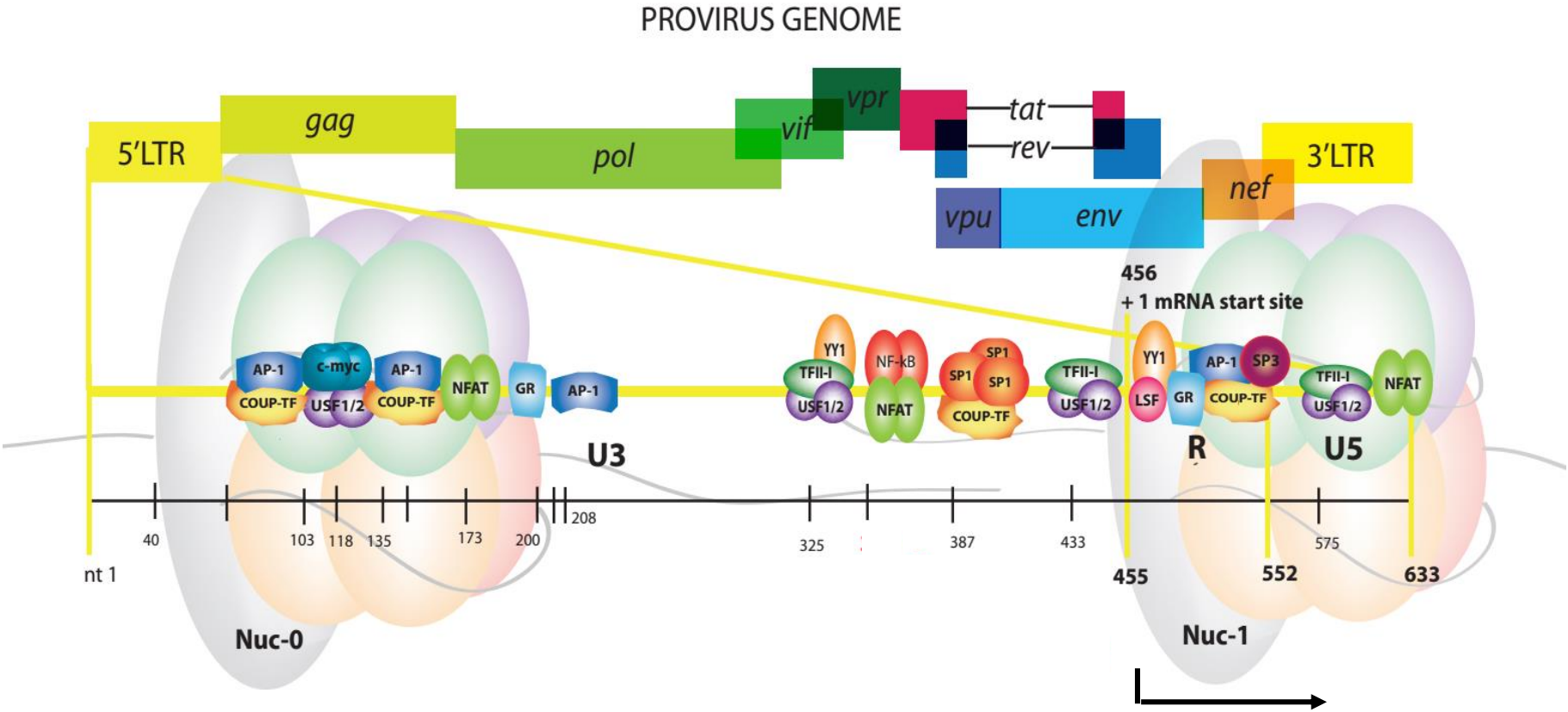
- not making RNA
- gene expression is driven by promoters: in **HIV** this is the **5'LTR**

Mechanisms of latency:

- **epigenetic silencing**
  - change in histone architecture of the 5'LTR: deacetylation and methylation of histones
  - sequestration of host (Transcription Factors) and/or viral factors (Tat) that regulate viral transcription
- **integration into silent genes**
  - HIV tends to integrate into active genes
  - active genes may become silent as cell differentiates or becomes resting
- **defective virus**
  - may not be replication competent, unable to transcribe

Control of Proviral Transcription

Importance of 5'LTR: Major determinant of post Integration latency



5' LTR : binding sites for Tat and host transcription factors

histone methylation: closed/compacted chromatin

histone acetylation: open/accessible chromatin

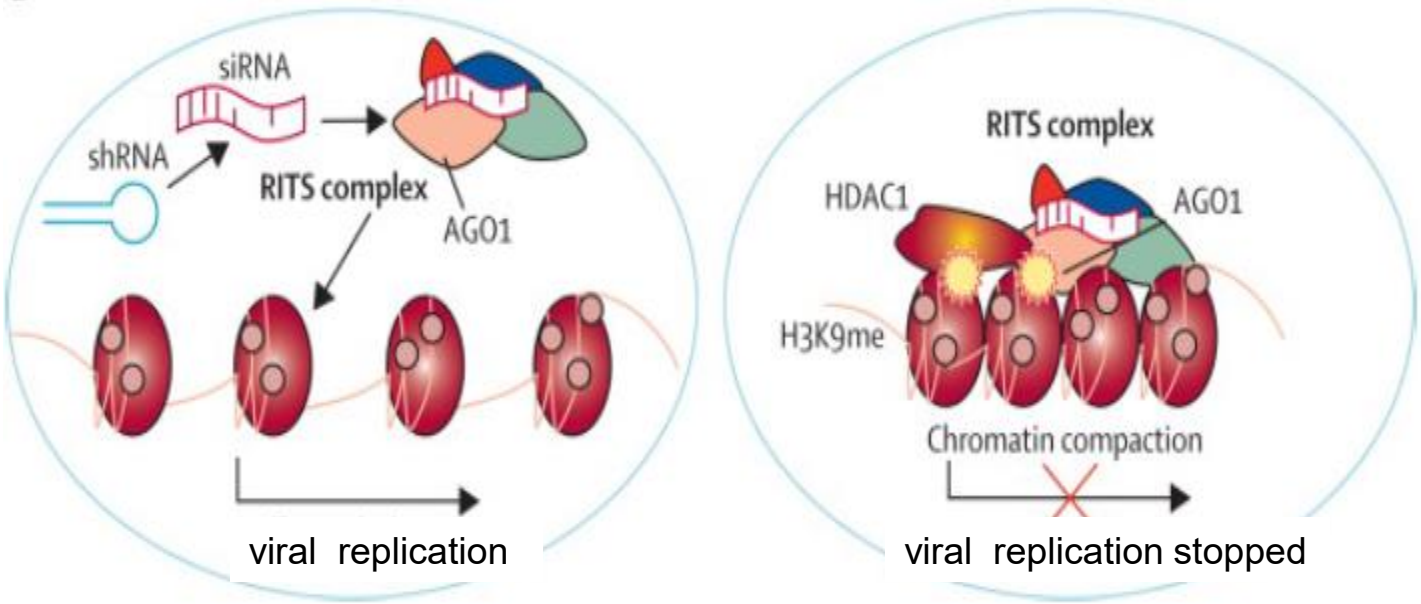
Mendez et al, 2015

Gene therapy for drug free remission of HIV

HIV Functional cure: enforcing viral latency

"BIND and GAG" or "BLOCK and LOCK"

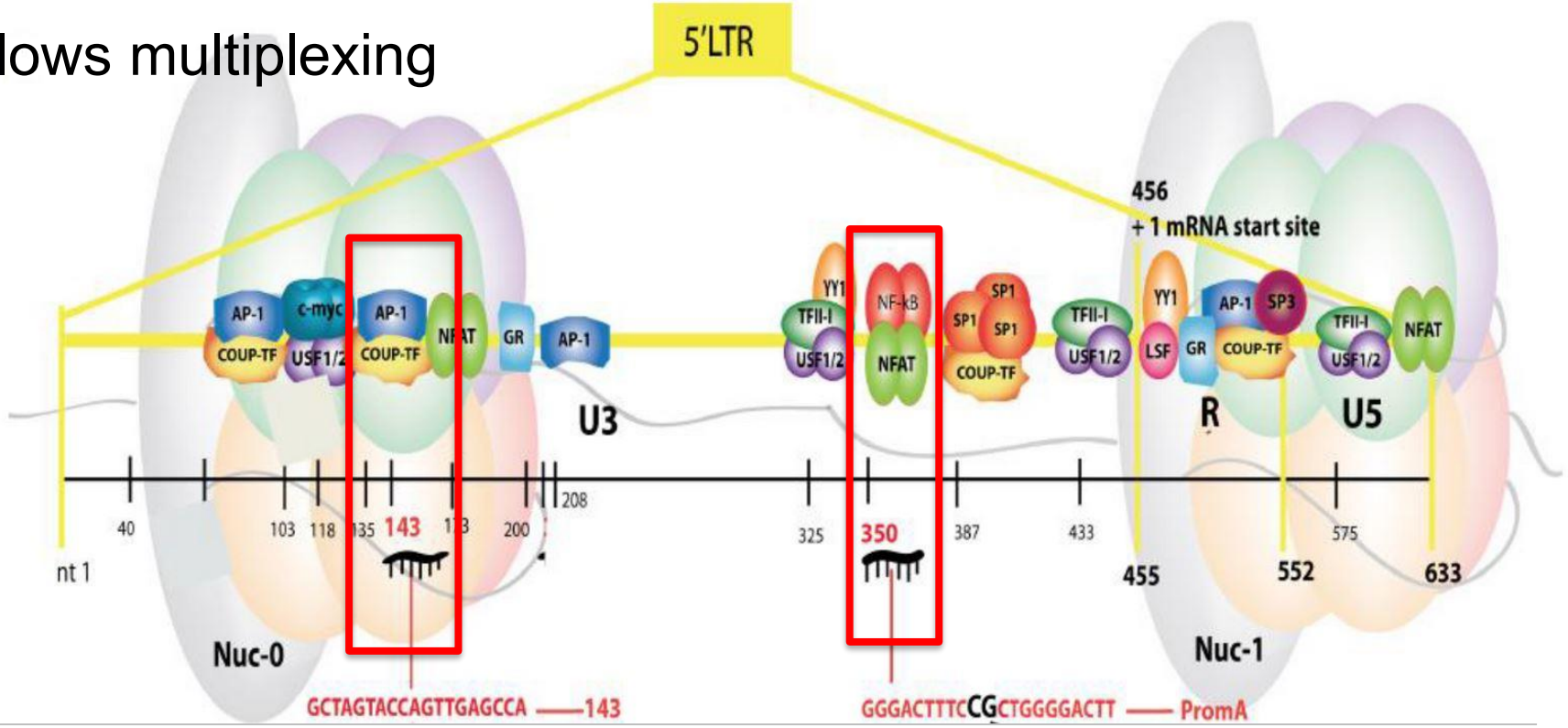
- Gene therapy: si/shRNA which target sequences within HIV promoter
- Induces **long term lock down of virus in latent form,** by inducing changes in histone tails and nucleosome architecture, resistant to a range of reactivation stimuli



Ahlenstiel et al, 2016

HIV-1 Promoter siRNA transcriptional silencing targets

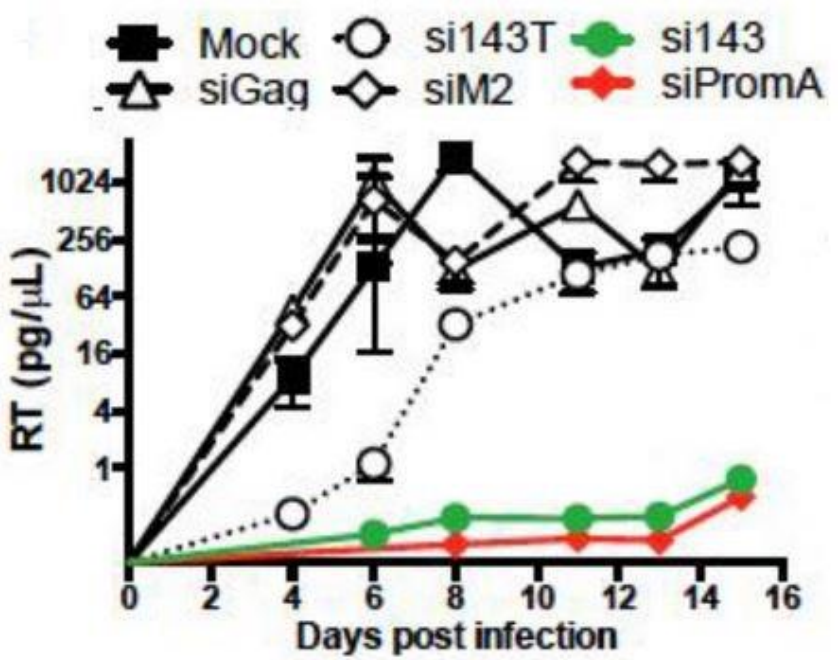
- **siPromA:** targets the unique, tandem NF-κB binding motif
- **si143:** targets region adjacent to AP-1/COUP-TF motif
- both have sequences that differ from those in the human genome, but conserved in HIV-1
- allows multiplexing



Mendez et al, 2015

siPromA/143 are highly specific & potent HIV-1 suppressors

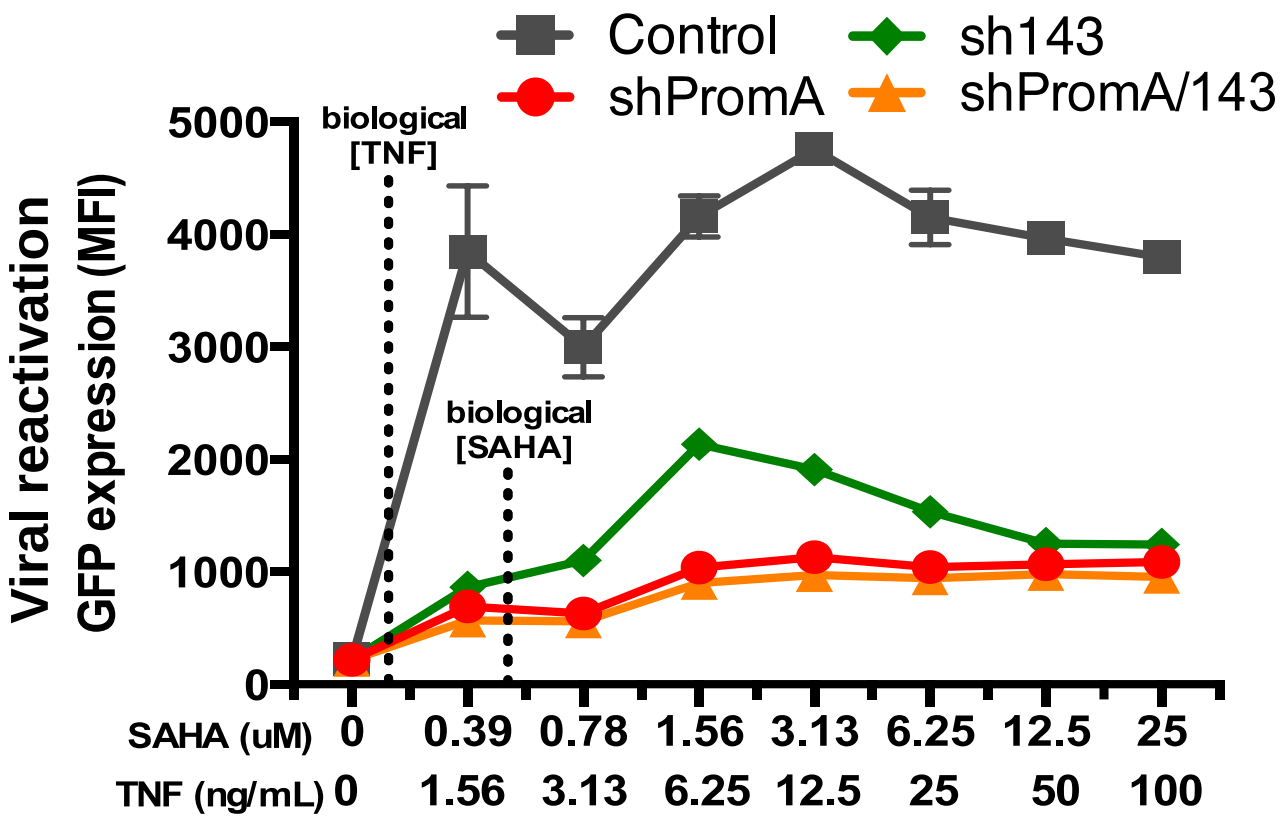
siRNA	Target HIV-1 $_{NL4-3}$ strain sequence
siPromA	GGGACTTTCCGCTGGGGACTT
siM2	GGGACTTTAAGCTGGGGACTT
siScrambled	AAGCTGGGACGTGTGCCTGTT
si143	GCTAGTACCAGTTGCGCCA
si143T	GCTAGTACCAGTTGCTCCA



- siPromA- & si143-transfected cells suppress HIV-1 transcription
- suppression is **profound** (3-4 $\log_{10}$ of viral RNA) and **prolonged** (21+ days
- mutated si- & siScrambled-transfected cells do not suppress virus
- effective in a range of cells: multiple T cell lines, Monocyte derived Macrophages, Astrocytes
- no clear off-target effects: highly specific

Suzuki et al., *RNA Biology* 2011; *MTNA* 2013; Ahlenstiel et al., *NAR* 2012; *MTNA 2015;* Klemm et al., *Genes* 2016

shPromA & sh143 protect cells from reactivation

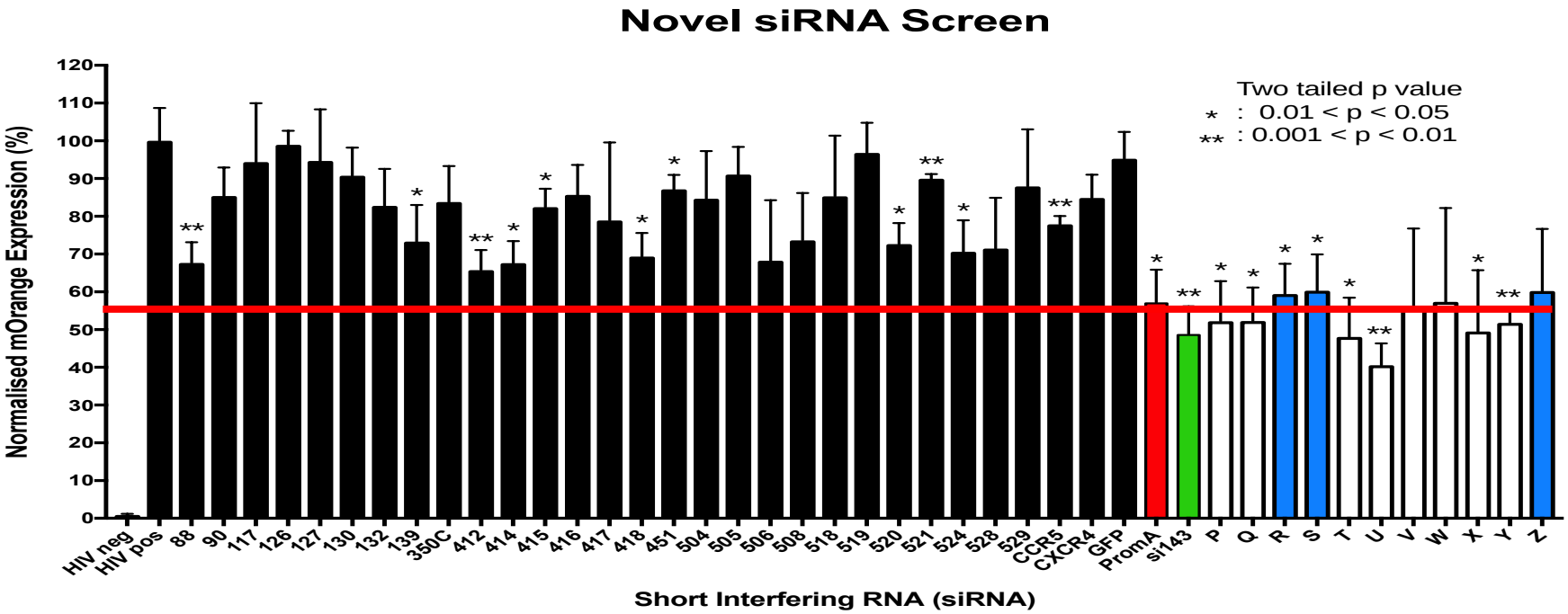


- Latency models show robust resistance to reactivation stimuli when stably transduced with shPromA and/or sh143

Ahlenstiel *et al*., MTNA 2015; Mendez *et al*., Retrovirology, 2018.

Multiplexing siRNAs to overcome HIV-1 diversity

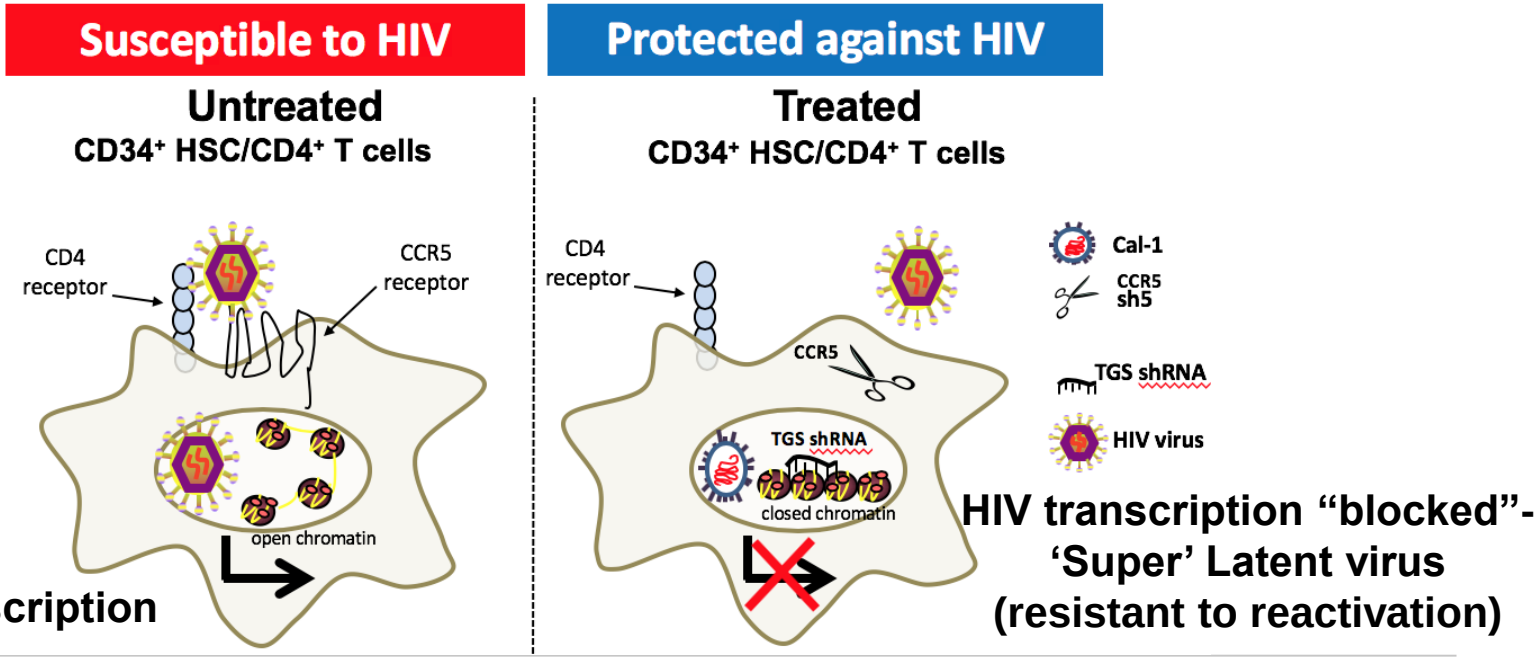
- Screening of 40 custom designed siRNAs targeting 5'LTR
- 11 new targets (P-Z) superior to PromA/143 in pseudovirus suppression



- Multiplexing siPromA/si143 covers: 72.9% all subtypes
- Multiplexing si71/si136/siT/siY covers: 99.89% all subtypes
- Informative for design of nanoparticles & lentiviral constructs

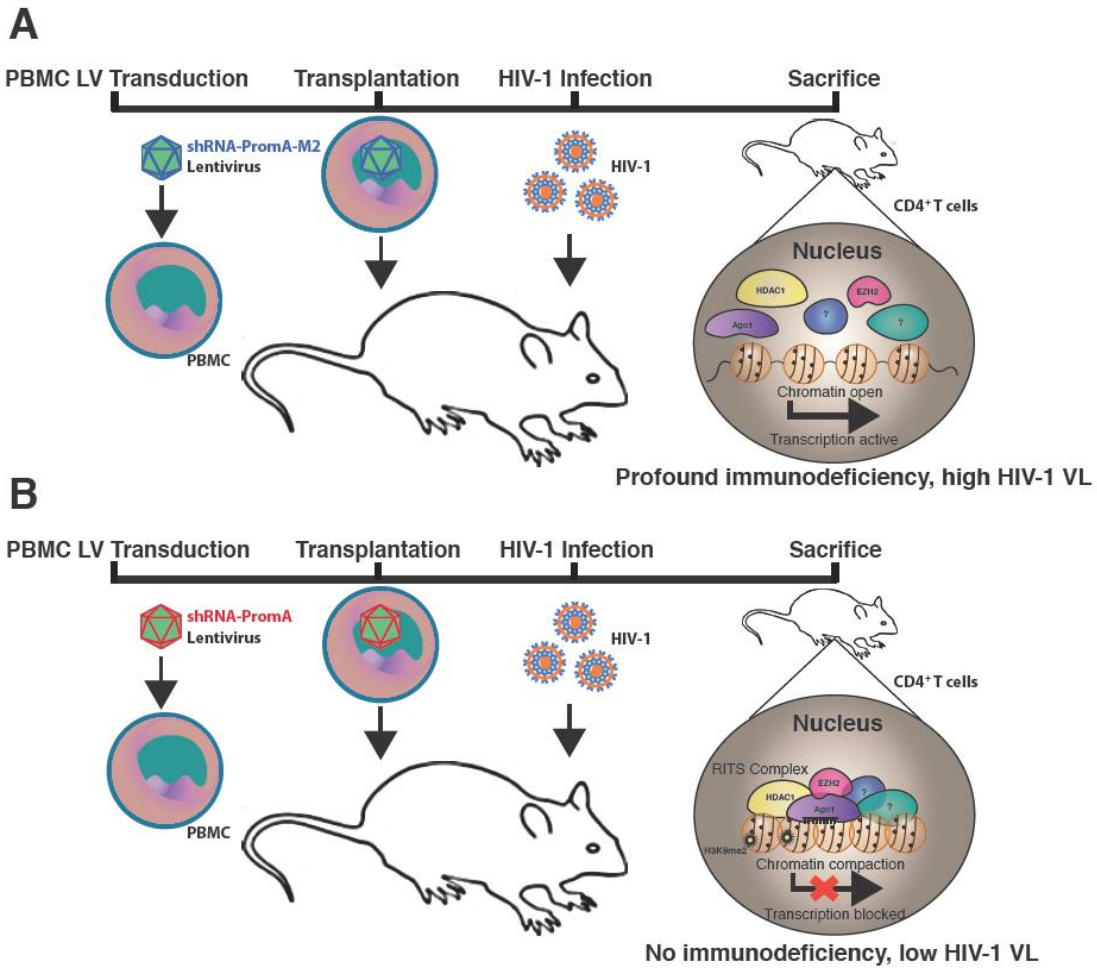
Functional cure: Combined RNA therapeutic targets

- Host factor: **shCCR5 Cal-1** (PTGS)
  - knocks down HIV co-receptor CCR5, prevents virus entry
- Viral factor: HIV-1 5`LTR tandem NF-κB sites (TGS)
  - **shPromA** induces epigenetic silencing of 5`LTR
  - "**block & lock**" approach



Suzuki *et al.*, *J RNAi and Silencing* 2005; Yamagishi *et al.*, *JBC* 2009; Suzuki *et al.*, *RNA Biology* 2011; *MTNA* 2013; Ahlenstiel *et al.*, *NAR* 2012; *MTNA 2015;* Tsukamoto *et al., AIDS 2018,* Mendez *et al., Retrovirology 2018.*

Preclinical mouse data in humanised acute HIV mouse



Susceptible to HIV

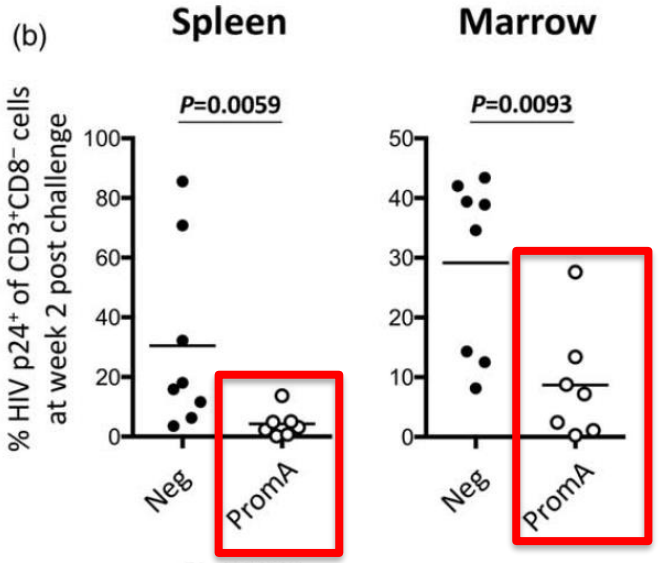
Protected against HIV

Reconstitution of SCID-humanised mice with **shPromA-transduced PBMC or CD34 stem cells** suppresses acute HIV-1 infection

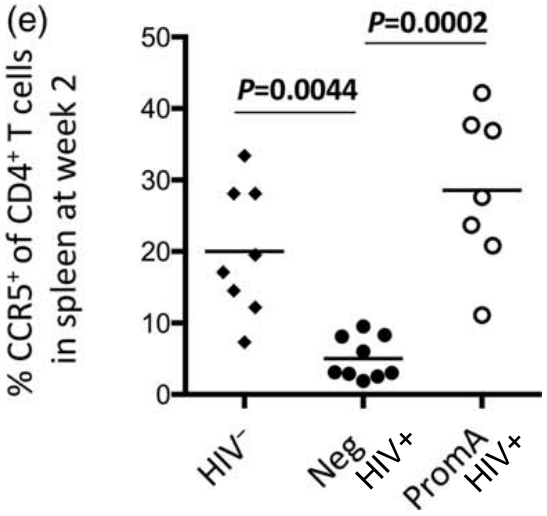
Suzuki et al., MTNA 2013; Ahlenstiel *et al*., Frontiers in Immunology Review 2015

shPromA reduces p24 / RNA transcripts & protects CD4+ T cells

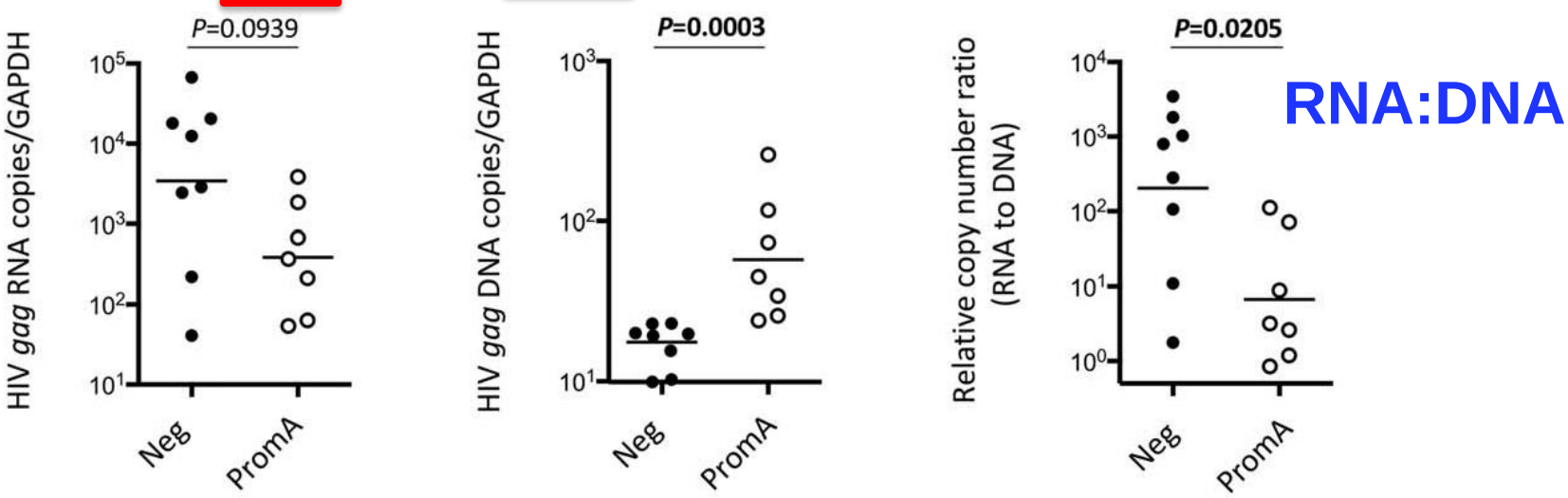
p24



%CD4+



Spleen



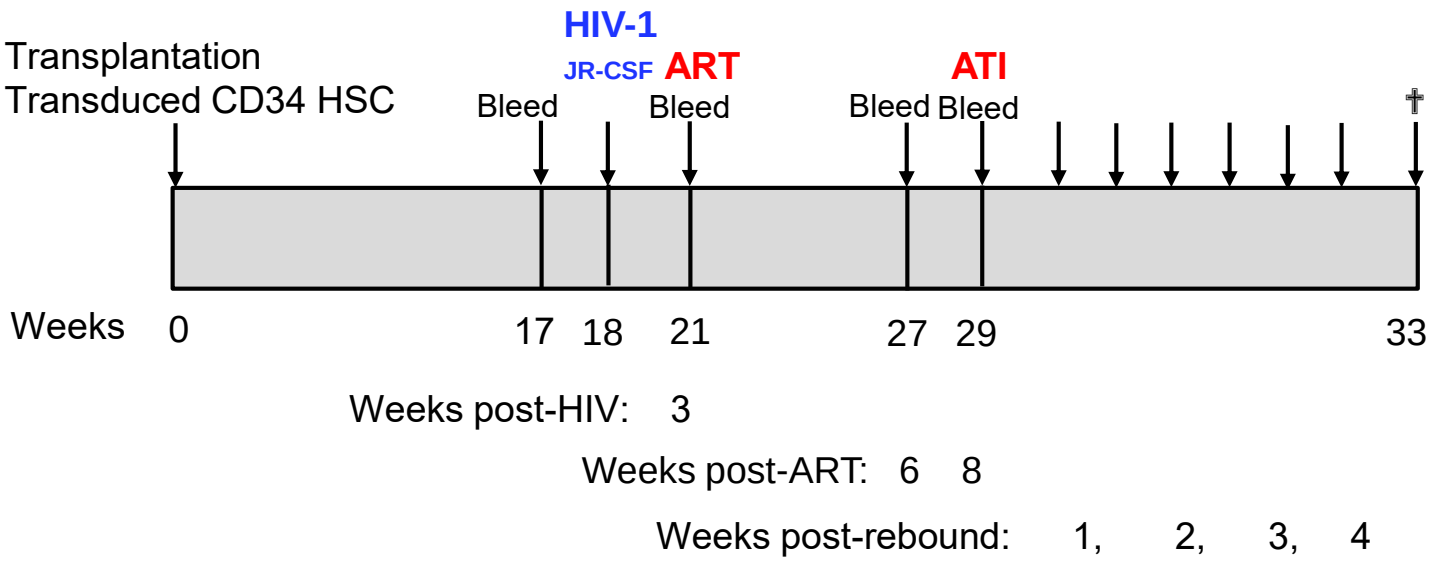
RNA:DNA

RNA

DNA

Tsukamoto *et al*., 2018 AIDS

Schema for humanised mouse expts:
ART treatment plus analytical treatment interruption



- **Mock untransduced CD34+ HSC**
- **LV-GFP shEmpty-transduced CD34+ HSC**
- **LV-GFP shPromA-transduced CD34+ HSC**
- **LV-GFP shCCR5/shPromA-transduced CD34+ HSC** (CSL Cal-1 vector)

Lentiviral MOI 5: 40-70% transduction efficiency

Limitations

- **Despite excellent *in vitro* characteristics**
  - Prolonged and sustained silencing
- ***In vivo* effects limited**
  - Cells protected represent a minority of cells in periphery
  - Extent of protect correlates with degree of shRNA expression

Understand limitations

- Modelling virus rebound following ART interruption

  (collaborating with Prof. Miles Davenport, Kirby Institute)
- Lymph node immunostaining & RNAscope

  (collaborating with Dr. Constantinos Petrovas, VRC, NIH)
- Improve delivery systems

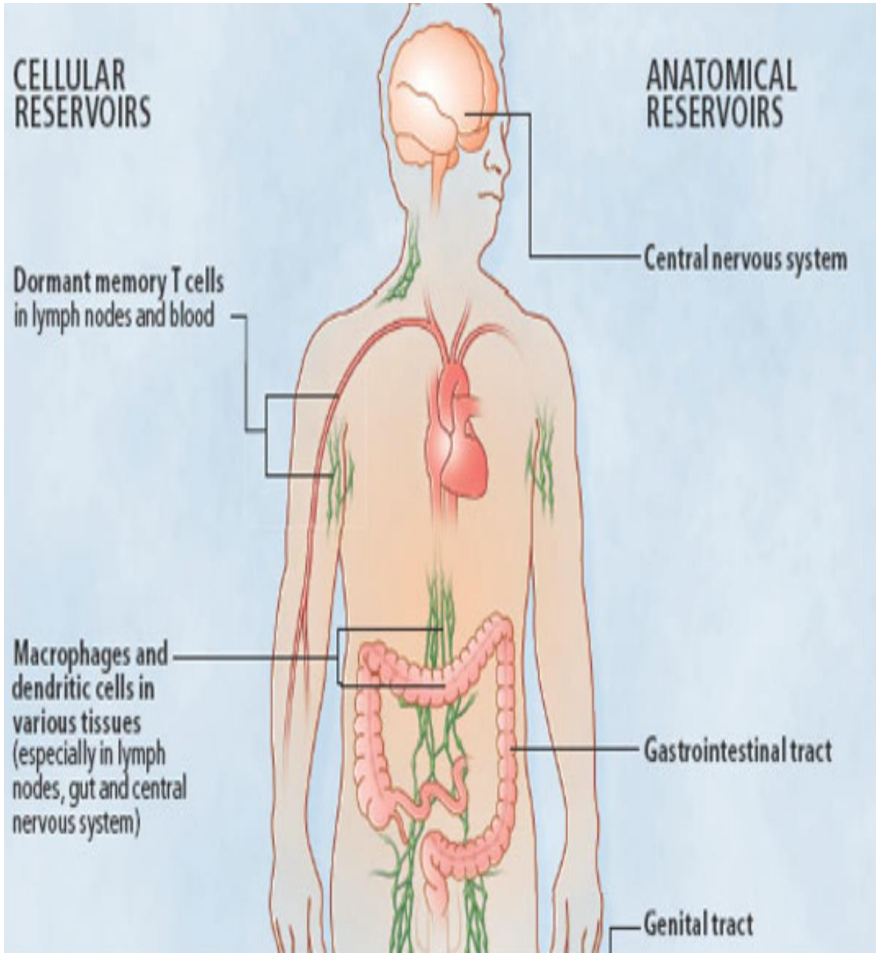
Where is the Reservoir?

Cellular reservoirs

- T cells: all CD4+ T cells
  - varying contribution of subsets
  - Memory: Central > Transitional
    - Effector Memory cells
    - Stem Memory cells, small reservoir, but persist
    - Follicular T helper cells (Tfh) in lymph nodes
  - Naïve: smaller contribution
- these cells are **resting** not activated
- Monocytes/macrophages & DC

Tissue reservoirs

- Brain: Microglia, Astrocytes
- Gut/Genitourinary tracts



Stevenson, 2008

Gene therapy in HIV infection requires efficient delivery to the reservoir

- Manipulation of the reservoir requires delivery of genes into resting T cells
- Standard gene therapy delivery system uses **lentiviruses**
  - Use artificial envelope protein VSVg
  - VSVg useful for many cell types, but not for resting T cells
- Resting T cells have low endocytosis and do not carry VSV-g receptor
- Highly challenging:
  - VSV-g pseudotyped retroviral delivery is limited to CD4+ T cells,
  - needs activation of cells or ways of avoiding viral restriction factors
  - must be done *ex vivo,* complex expensive GMP procedures
  - needs to deliver gene therapy to cells without clear marker of their being latently infected
  - need to access small, difficult to access reservoir

Gene therapy in HIV infection requires efficient delivery to the reservoir

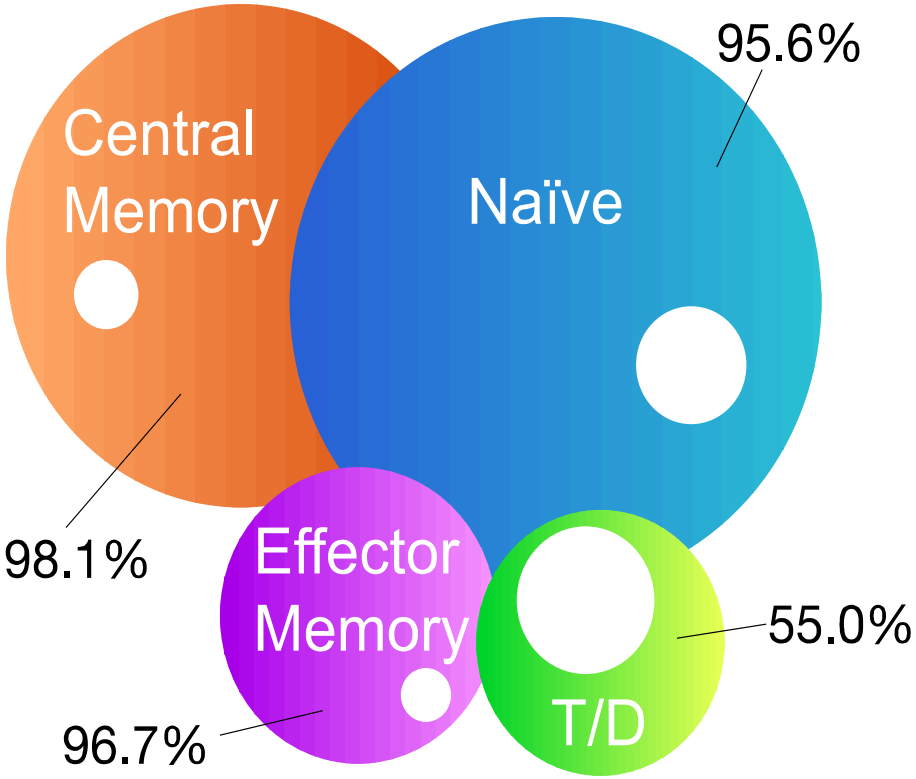
Potential solutions:

- **Retroviral delivery:**
  - build new retrovirus expressing
    - broadly tropic HIV-1 gp120 instead of VSV-g and
    - HIV-2/SIV Vpx
- **Use nanoparticles**
  - allows multiplexing
  - may allow *in vivo* rather than *ex vivo* treatment, with bulk synthesis
  - potentially better safety profile

Lentivirus targeting Resting CD4+ T Cells

Lead Envelope

- Lentiviral (HIV-1/B)
- Dual-Tropic
- Low-CD4 Dependence
- Very efficient vector delivery to a range of Resting T cells



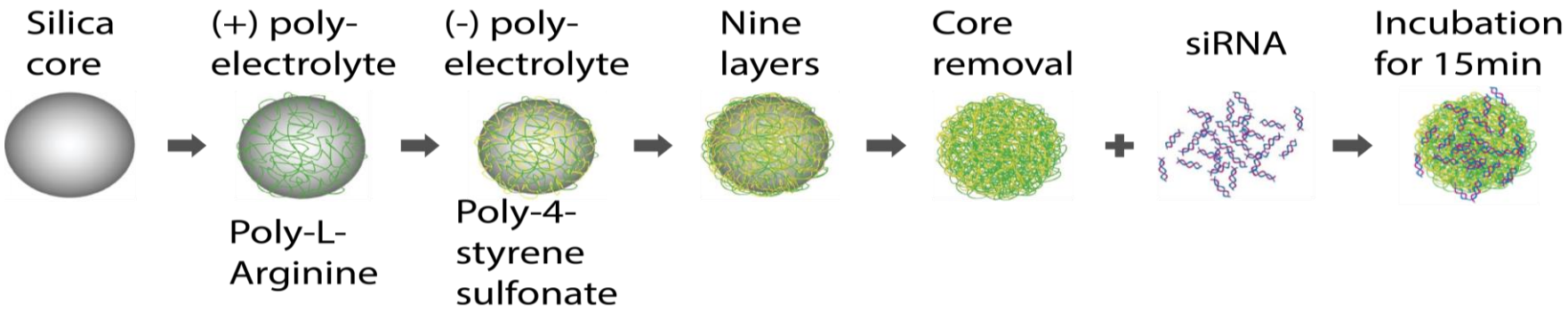
n=8

Wong and Turville, IAS Amsterdam, 2018

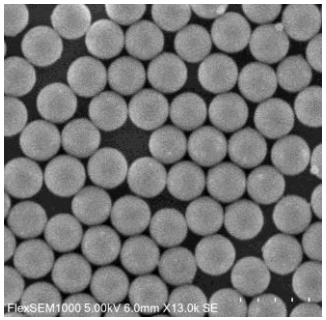
Nanoparticle preparation- Layer by layer

ARC Centre of Excellence in CONVERGENT BIO-NANO SCIENCE & TECHNOLOGY

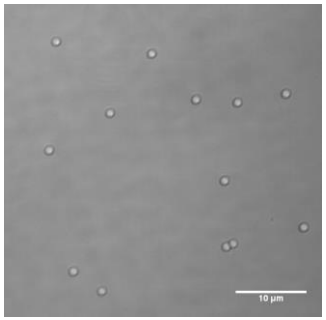
- Layer by Layer (LbL) built around a 900 nm silica particle
  through electrostatic interactions
- Dissolving Silica template to prepare hollow Capsules



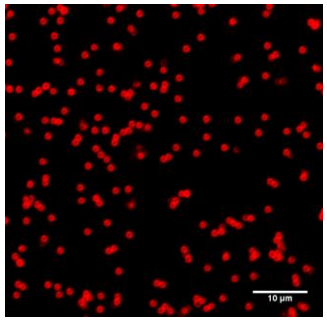
850nm Silica core template



Capsule (after core removal)



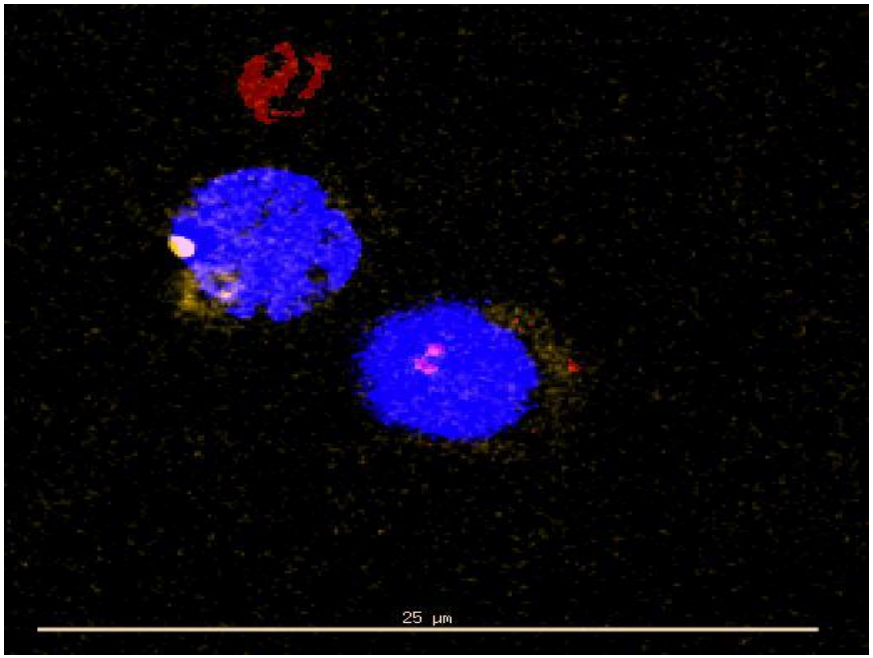
After siRNA adsorption

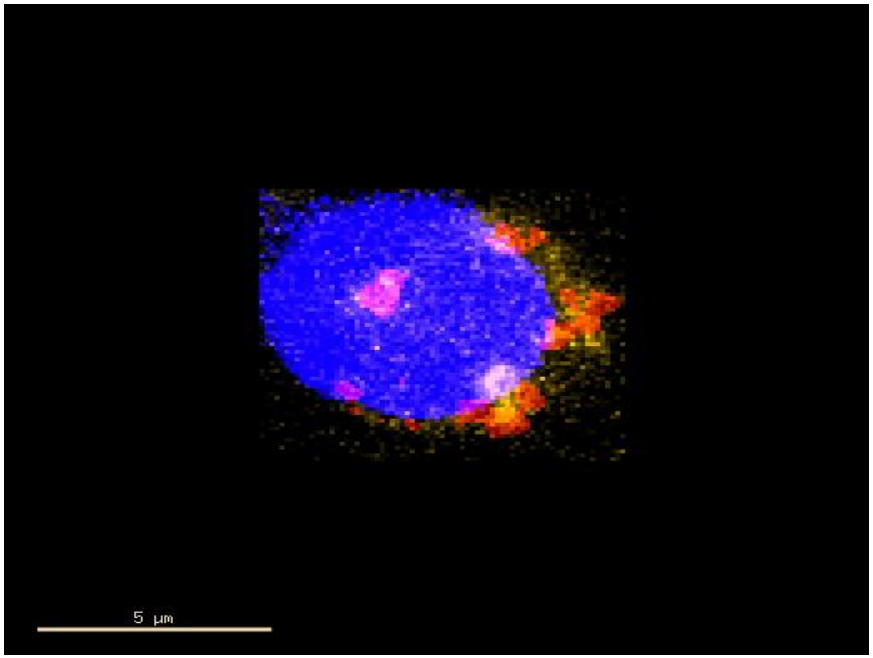


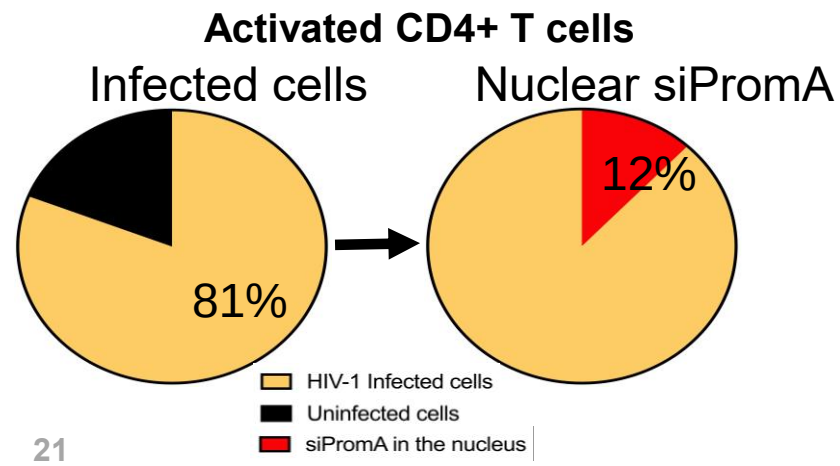
Björnmalm et al. *Nanoengineering Particles through Template Assembly (2016)*

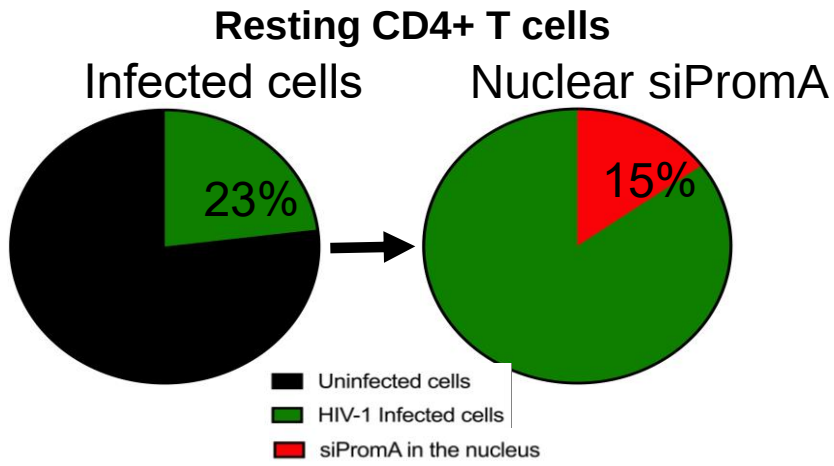
Nanoparticle Transduction of CD4+ T cells

siRNA: Nuclei: Virus

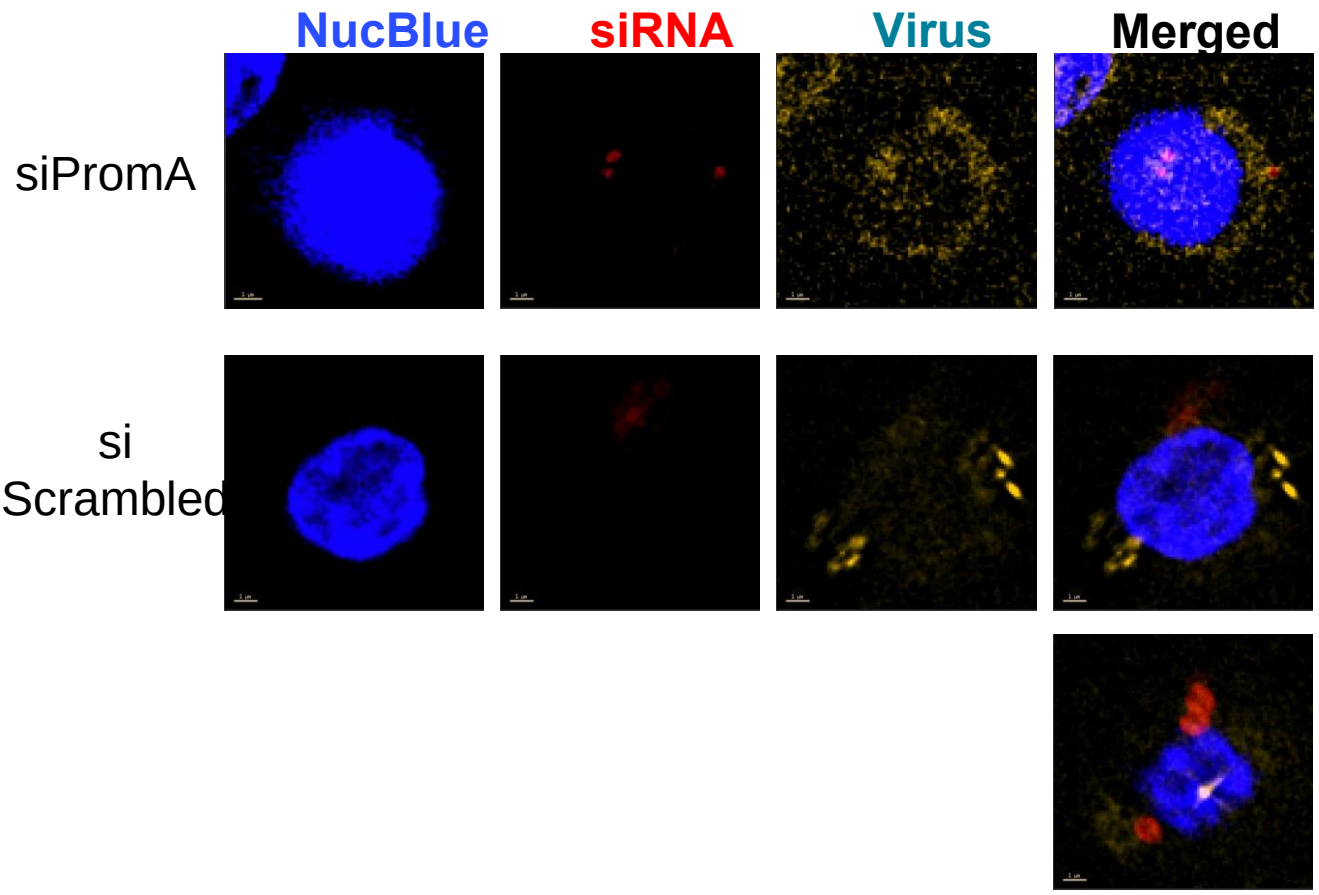


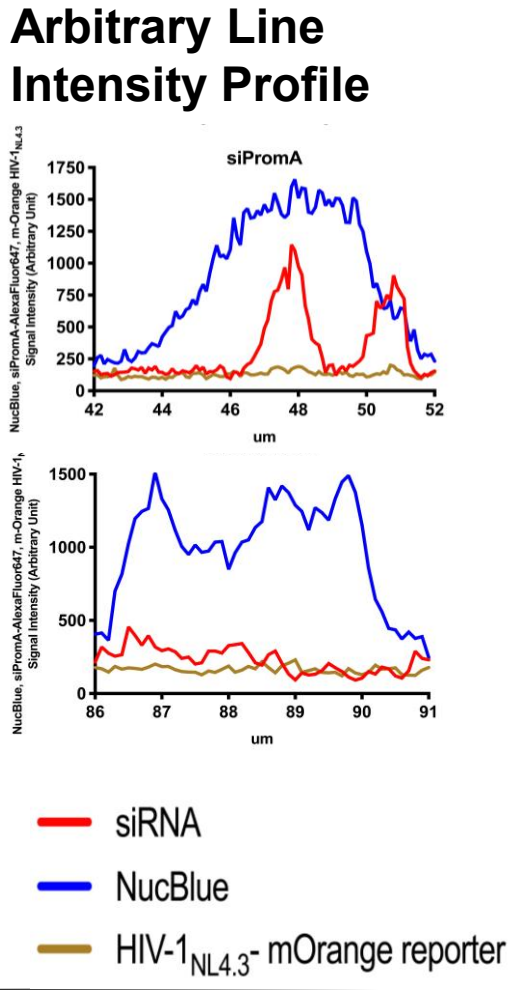






Nuclear delivery in Activated Primary CD4+ T cells is Specific



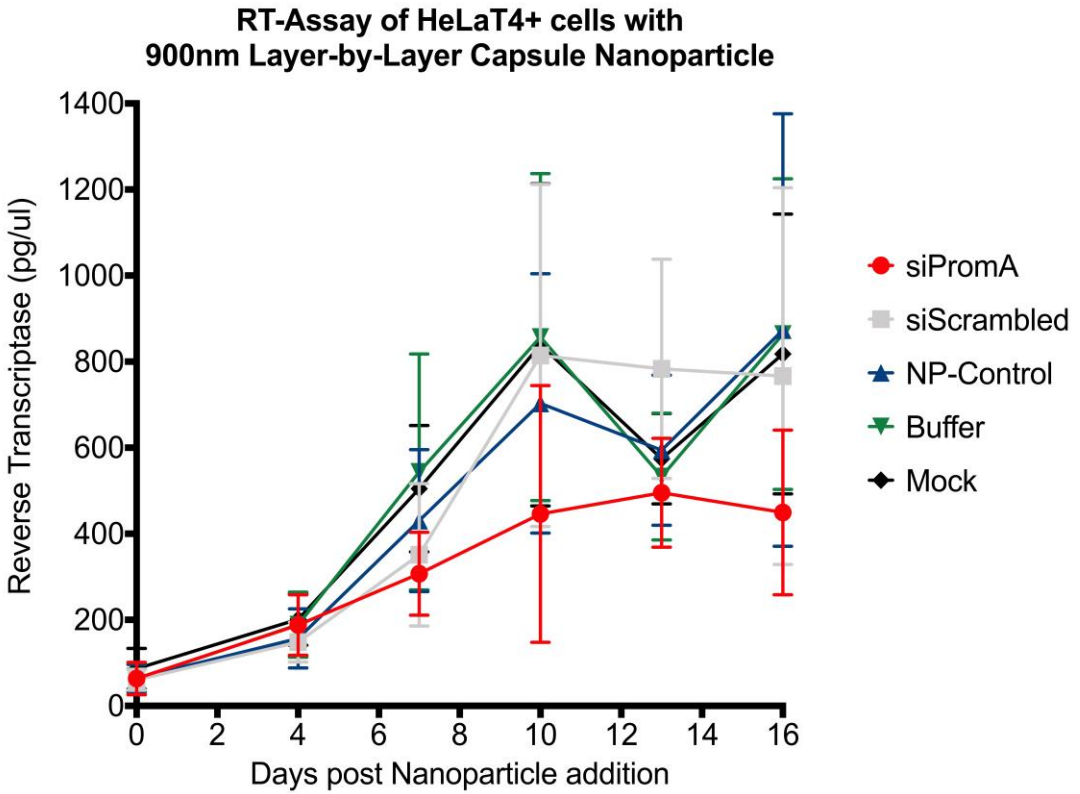


NB: Cell in different z-stack showing **siScrambled** nanoparticles are present

NP delivered siRNA suppresses HIV-1 replication

- Measuring active HIV-1 in the supernatant of infected cells over a time course
- HeLa T4+ cells were infected for 4 days prior to siRNA-NP delivery

➢ **Decreased RT-Activity in cultures with NP delivered siPromA compared to controls**



Conclusions

- **si/shRNAs targeting conserved regions of the HIV-1 promoter induce prolonged, profound & specific silencing**
  - in multiple cell types
    - T cells Macrophages Astrocytes
  - induced latency is resistant to T cell activation: Superlatency
- **Encouraging *in vivo* effects in 2 separate murine models**
    - Effect related to extent of transduction, need more efficient delivery
- **Any gene cargo needs efficient delivery to the reservoir especially resting T cells**
  - existing approaches are inefficient: approach, **Modify The Vector, Not The Cell**
  - Promising results from
    - **engineered retrovirus** carrying specific *env* (>80% of resting $CD4^+$ T cells) and Vpx, 40%+ transduction efficiencies at low MOI for transduction (MOI=0.04, important for upscaling)
    - **LBL Nanoparticles**: may have advantages for siRNA multiplexing and use for direct *in vivo* delivery

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