Combination anti-HIV gene therapy using shRNAs, aptamers and U1i RNAs strongly inhibit HIV-1 replication in T-cells without inducing cellular toxicity.

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Background

Proof of cure

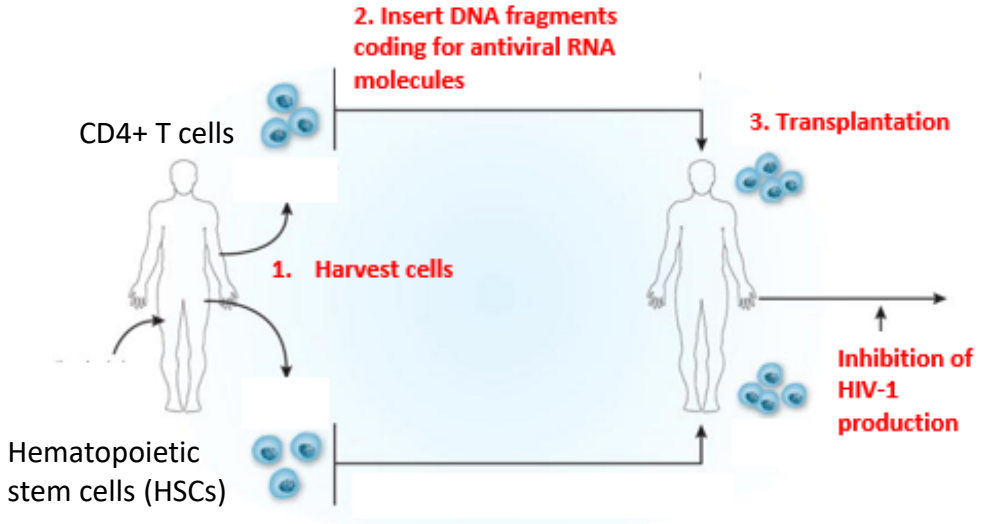
Timothy Brown (the '**Berlin Patient**') and Adam Castillejo (the '**London Patient**') are considered cured from HIV-1 after receiving hematopoietic stem cell (HSC) transplants from resistant donors (CCR5-Δ32). Additionally, a third patient (the '**Düsseldorf patient**') also received a similar transplant and has discontinued cART. Recently, an **HIV-1 positive woman** received a cord blood transplant from a resistant donor (CCR5-Δ32) and has also discontinued cART.

Limitations:

- Very few resistant donors exist
- High risk for recipient (graft versus host disease)

Alternative: Modify patient's cells by gene therapy

Cell transplant protocol[1]



Project objective

Optimize anti-HIV gene therapy candidates by identifying the optimal promoter for expression as well as the best molecular design of antiviral RNAs to maximize inhibitory potency in the absence of cytotoxicity to establish an effective combination gene therapy.

Methods

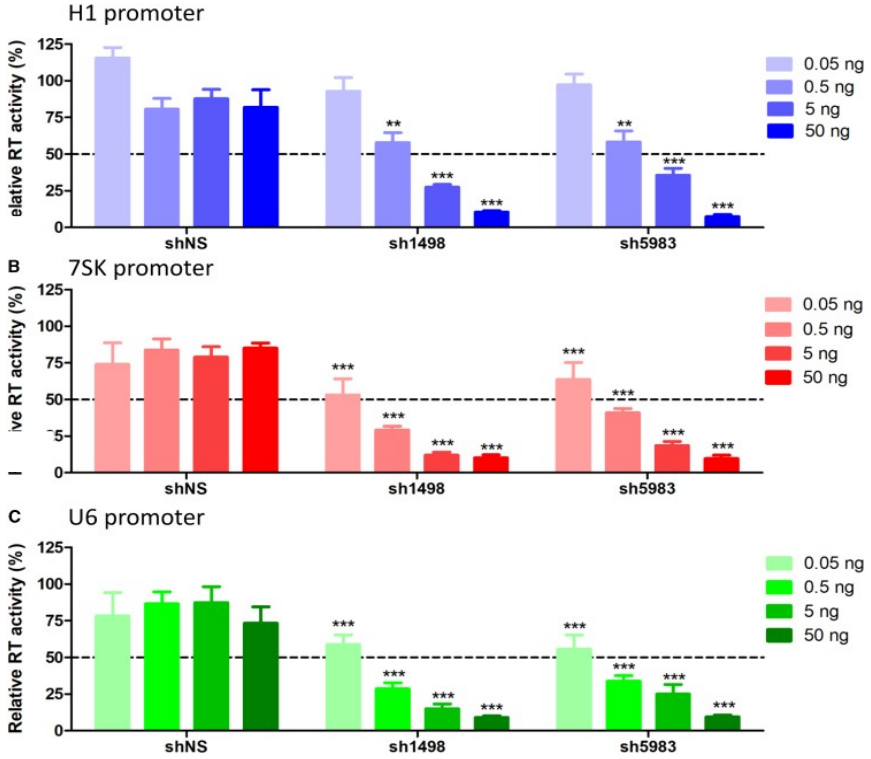
Co-transfection assays: Plasmids were created that express a nonsense shRNA (shNS) as well as HIV specific sh1498[2] and sh5983[3] RNA molecules with either the human H1, 7SK or U6 promoter. Co-transfections in HEK293T cells with HIV-1 (NL4-3 molecular clone) and the constructed plasmids were performed. Reverse transcriptase (RT) activity was measured to estimate the efficiency of the molecules at reducing viral production.

Northern blots: The guide strand of the shRNAs expressed from the different promoters was detected by Northern blot to estimate gene expression levels by quantifying the different band intensities normalized to the 5S rRNA loading control using Fiji software.

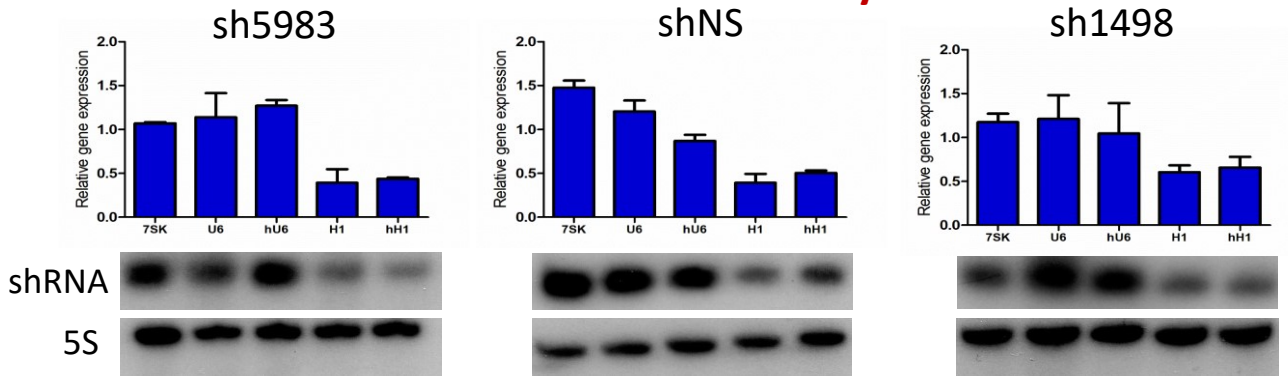
Competitive growth assays and HIV-1 infections: Various genes coding for anti-HIV-1 RNAs were included within lentiviral vectors to be transduced in the lymphocytic cell line SupT1. Transduced SupT1 cells were sorted by GFP expression and subsequently challenged with HIV-1 (NL4-3 molecular clone). Infection kinetics were established by measuring RT activity at various time points. Competitive growth assays were also put into place by mixing 50% GFP negative and 50% GFP positive cells after sorting. The percentage of GFP was measured overtime to detect a growth advantage for GFP negative or GFP positive cells.

Results

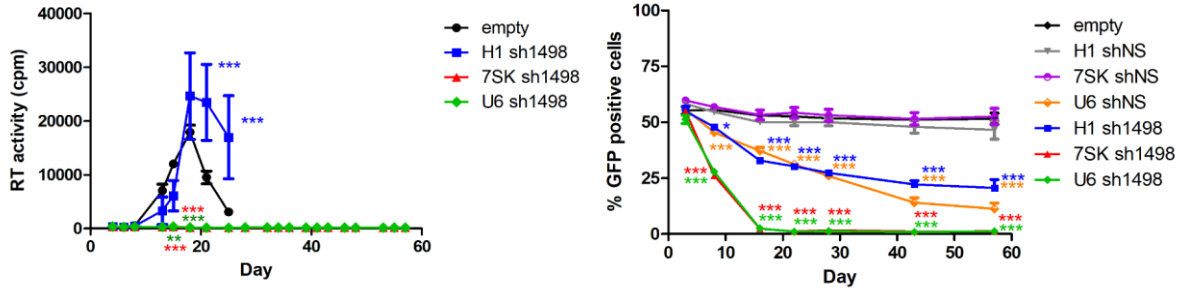
1. Anti-HIV-1 shRNAs are more potent when expressed from the 7SK and U6 promoters in co-transfection experiments[4]



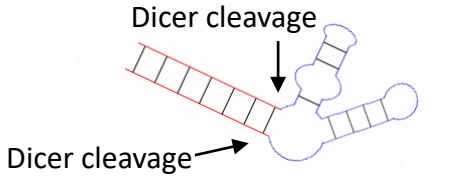
2. Expression levels of shRNAs from each RNA Pol III promoter correlates with their antiviral activity[4]



3. Transduced cells expressing sh1498 from the 7SK and U6 promoter restrict viral replication but have severe growth disadvantages compared to untransduced cells[4]



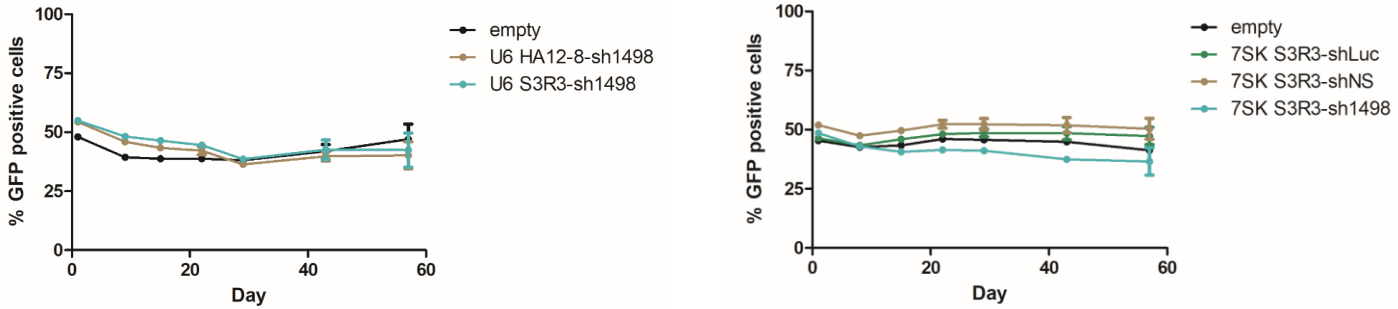
4. Aptamer-shRNA chimeras prevent cytotoxicity of U6 and 7SK promoted anti-HIV shRNAs



Blue: S3R3 Integrase aptamer

Red: shRNA

An anti-HIV (S3R3) and an anti-influenza (HA12-8) aptamer were incorporated within the terminal loop of shRNAs.

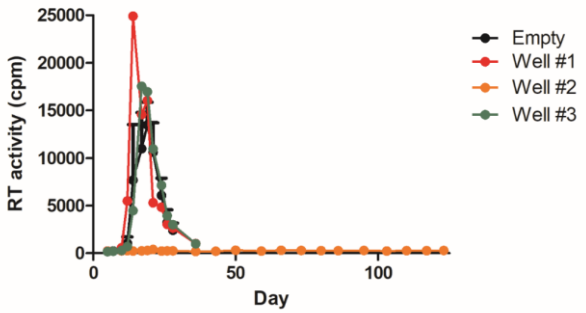


Results

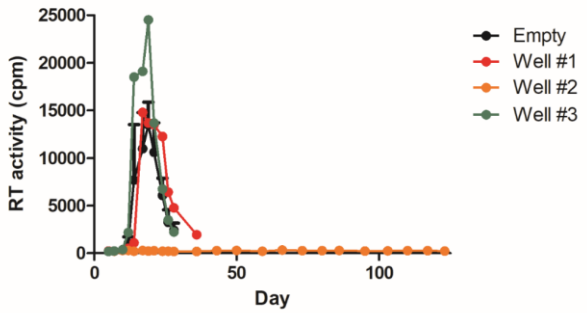
5. Double molecule combination therapy with U1 T6 5’ end length variants[5] and shRNAs can prevent HIV-1 infection

U1i RNAs (U1 T6) are reengineered U1 small nuclear RNAs which can be directed to bind to HIV-1 transcripts to cause recruitment of spliceosomal components which will inhibit viral replication by enhancing splicing or interfering with polyadenylation.

U1 T6 5’3 + H1 sh1498



U1 T6 5’6 + U6 PR10.9-8N-sh1498



Future directions

- Determine why aptamer-shRNA chimeras prevent shRNA mediated cytotoxicity: Dicer cleavage patterns may vary in aptamer-shRNA chimeras, or expression levels of aptamer-shRNA chimeras may differ compared to shRNAs expressed alone.
- Identify a double molecule combination which can completely inhibit viral replication.

References

1. Rossi, J.J., June, C.H. & Kohn, D.B. Genetic therapies against HIV. *Nat Biotechnol* **25**, 1444-1454 (2007).
2. Scarborough, R.J.*, et al.* A Conserved Target Site in HIV-1 Gag RNA is Accessible to Inhibition by Both an HDV Ribozyme and a Short Hairpin RNA. *Mol Ther Nucleic Acids* **3**, e178 (2014).
3. DiGiusto, D.L.*, et al.* RNA-based gene therapy for HIV with lentiviral vector-modified CD34(+) cells in patients undergoing transplantation for AIDS-related lymphoma. *Sci Transl Med* **2**, 36ra43 (2010).
4. Goguen, R.P.*, et al.* Efficacy, accumulation, and transcriptional profile of anti-HIV shRNAs expressed from human U6, 7SK, and H1 promoters. *Mol Ther Nucleic Acids* **23**, 1020-1034 (2021).
5. Del Corpo, O.*, et al.* A U1i RNA that Enhances HIV-1 RNA Splicing with an Elongated Recognition Domain Is an Optimal Candidate for Combination HIV-1 Gene Therapy. *Mol Ther Nucleic Acids* **18**, 815-830 (2019).

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