Selection of Safe and Effective Antiviral RNAs for an HIV-1 Functional Cure

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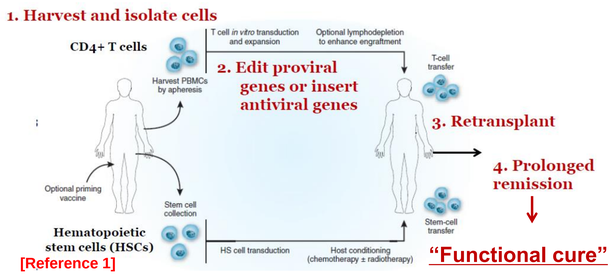
Cell transplant for an HIV cure

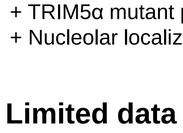
General cell transplant protocol



[Reference 1]

Proof of principle case

Timothy Brown ('the Berlin patient'), cured of HIV-1 with a cell transplant from an HIV-1 resistant donor (CCR5Δ32/Δ32). Off medication since 2007.



- Limited number of known donors.
- Transplant from a donor is risky.
- Potential for selection of CXCR4 using viruses

Other cases of CCR5Δ32Δ32 transplant

Patient/transplant location	Reason for transplant	Age[1]	Outcome
Timothy Brown/Berlin	Acute myeloid leukemia	40	No viremia for >12 years following transplant and discontinuation of cART [2]
NR/Utrecht	Myelodysplastic syndrome	53	Relapse of myelodysplastic syndrome and death 2 months after transplant [7]
NR/Münster	Non-Hodgkin's lymphoma	51	Infection and death 4 months after transplant [7]
NR/Minneapolis	Acute lymphoblastic leukemia	12	GVHD and death 3 months after transplant [7]
NR/Santiago	Non-Hodgkin's lymphoma	46	Pneumonia and death shortly after transplant [7]
NR/Barcelona	Non-Hodgkin's lymphoma	37	Relapse of lymphoma and death 3 months after transplant [6]
NR/Essen	Non-Hodgkin's lymphoma	27	Viral rebound with CXCR4 using virus 27 days after discontinuation of cART (20 days after transplant). Relapse of lymphoma and death 12 months after transplant [8]
NR/Halifax	Chronic myeloid leukemia	58	Decreased viral reservoir 9 months after transplant. Death from myocardial infarction 1.5 years after transplant [L. Barrett, personal communication]
NR/London	Hodgkin's lymphoma	NR	No viremia for 18 months following discontinuation of cART [1]
NR/Düsseldorf	Acute myeloid leukemia	49	No viremia for 4 months following discontinuation of cART [9]

NR: not reported.
[1] Age at time of transplant.

[Reference 2]

Cell transplant clinical trials

CCR5 gene editing

SB-728:
Zn finger nuclease edited CCR5 gene

Sangamo BioSciences

Transduction of genes expressing antiviral peptides or RNAs

CCR5 mRNA targeting shRNA
+ membrane anchored CD4 peptide

CALIMMUNE

CCR5 mRNA targeting ribozyme
+ HIV-1 RNA targeting shRNA (sh5983)
+ Nucleolar localized TAR decoy (U16TAR)

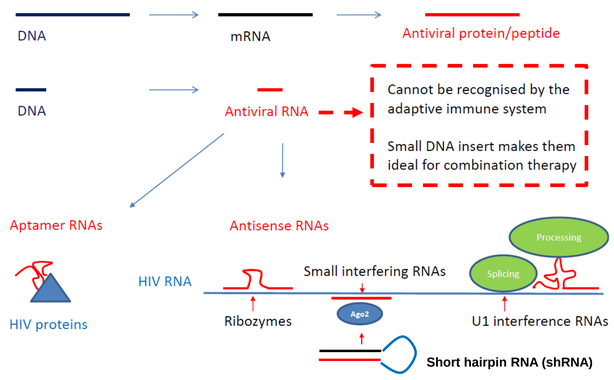
City of Hope

CCR5 mRNA targeting shRNA
+ TRIM5α mutant protein
+ Nucleolar localized TAR decoy (U16TAR)

AMC AIDS Malignancy Consortium

Limited data available on the comparative efficacy and safety of antiviral genes.

Anti-viral genes



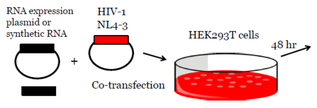
Objectives

Directly compare the efficacy and safety of anti-HIV RNAs from different classes

Compare different promoters for optimal expression of anti-HIV RNAs

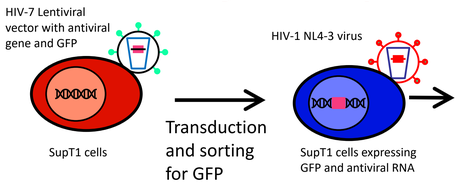
Methods

Efficacy: HIV-1 production

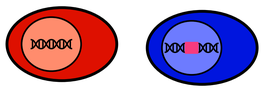


Reduction in HIV-1 Reverse Transcriptase activity (RT) relative to empty RNA expression plasmid

Efficacy: HIV-1 replication

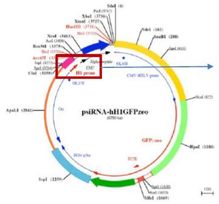


Safety: Competitive Growth Assay



Mix transduced with non-transduced cells and monitor percent GFP positive cells over time

Alternative promoter analysis



Generate RNA expression plasmids with same backbone but with different RNA Polymerase III promoters (H1, hH1, U6, hU6, 7SK)

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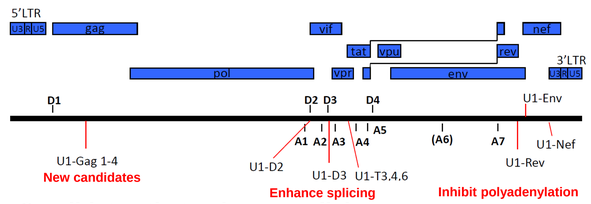
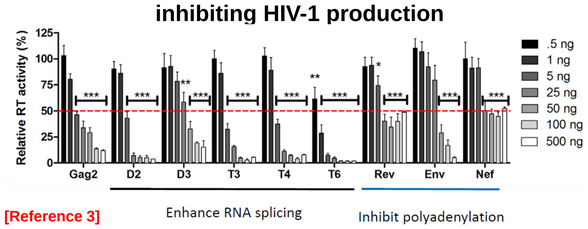
Compare efficacy on HIV-1 production and evaluate transcription profile by RNA-seq

Results: U1 interference RNAs



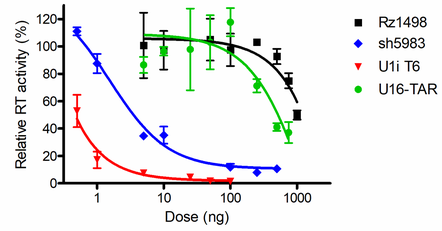
Candidates that enhance splicing are most potent at inhibiting HIV-1 production



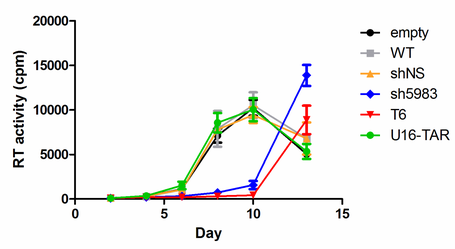
[Reference 3]

Results: An shRNA and a U1i RNA are more potent compared to a ribozyme and a decoy RNA

U1-T6 splicing enhancer and the clinical shRNA candidate (sh5983) are several fold more potent at inhibiting HIV-1 production compared to the clinical decoy candidate (U16-TAR) and a ribozyme (Rz, Ref. 4)

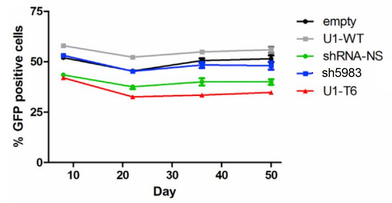


Both U1-T6 and sh5983 inhibit HIV-1 replication compared U1 control (WT) and nonsense shRNA (shNS). At similar transduction levels, the U16-TAR decoy was not effective.



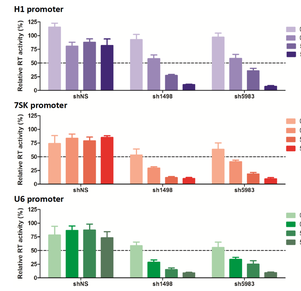
Results: safety

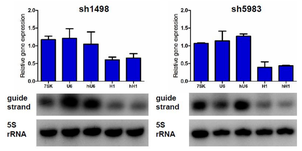
U1-T6 and sh5983 do not affect cell proliferation in transduced cells compared to controls



Results: Promoter analysis

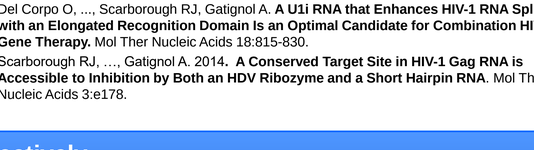
Two anti-HIV-1 shRNAs (sh1498 and sh5983) are more effective inhibitors of viral production when expressed from the U6 and 7SK promoters compared to the H1 promoter





shRNA potency correlates with expression level of the guide strand determined by Northern blot

RNA-seq data suggests that the U6 promoter results in the most accurate transcription initiation at the intended +1 site



The potency of the shRNAs does not seem to be affected by the accuracy of the start site and is determined mainly by the expression level

Conclusions

U1i RNA U1-T6 and shRNA sh5983 are several fold more potent at inhibiting HIV-1 production compared to a ribozyme and a decoy RNA (U16-TAR).

Use of the U6 and 7SK promoters results in more potent inhibition of HIV-1 production by shRNAs compared to the H1 promoter.

The U6 promoter provides the most accurate transcription initiation site, but shRNA potency is mainly determined by the expression level.

Future Directions

Compare the efficacy and safety of additional molecules.

Evaluate potential synergy and antagonism of the top candidates from each class in both efficacy and safety assays.

Compare different promoters for additional shRNAs and other molecules.

References

1. Rossi JJ, June CH, Khon DB. 2007. **Genetic therapies against HIV.** Nat Biotechnol. 25(12):1444-54.
2. Scarborough RJ, Goguen RP, Gatignol A. 2019. **A second patient cured of HIV infeciton: hopes and limitations.** Virologie 23(3):E1-E4.
3. Del Corpo O, …, Scarborough RJ, Gatignol A. **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.
4. Scarborough RJ, …, Gatignol A. 2014. **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.

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