Identifying Safe and Effective Type 3 RNA Polymerase III Promoted shRNAs on Lentiviral Vectors for Use Against HIV

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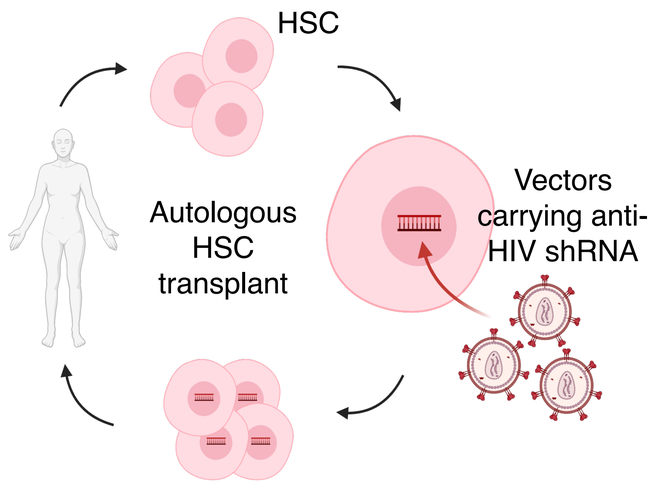
BACKGROUND

- HIV targets and depletes CD4+ T cells, leading to a weakened immune system[1]
- Antiretroviral medication requires daily pills, risking side effects[2-4]
- Autologous HSC transplants involving cells modified with antiviral shRNAs may serve as one-time therapy
- Unknown how the choice of promoter affects the efficacy and toxicity of the most potent shRNAs



- This project aims to investigate how well different promoter-shRNA combinations inhibit HIV replication and whether they exhibit cytotoxic effects.

METHODOLOGY

Cloning shRNAs under 7SK, U6, or H1 promoters

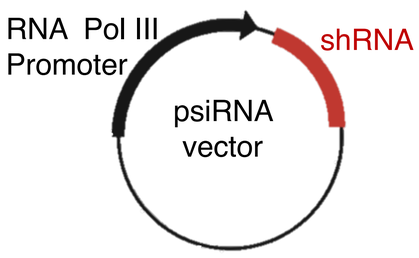
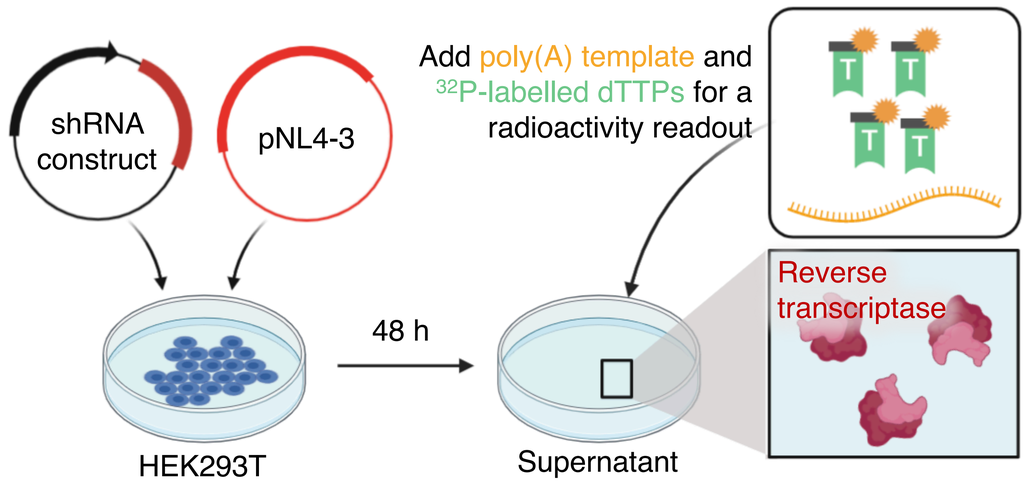


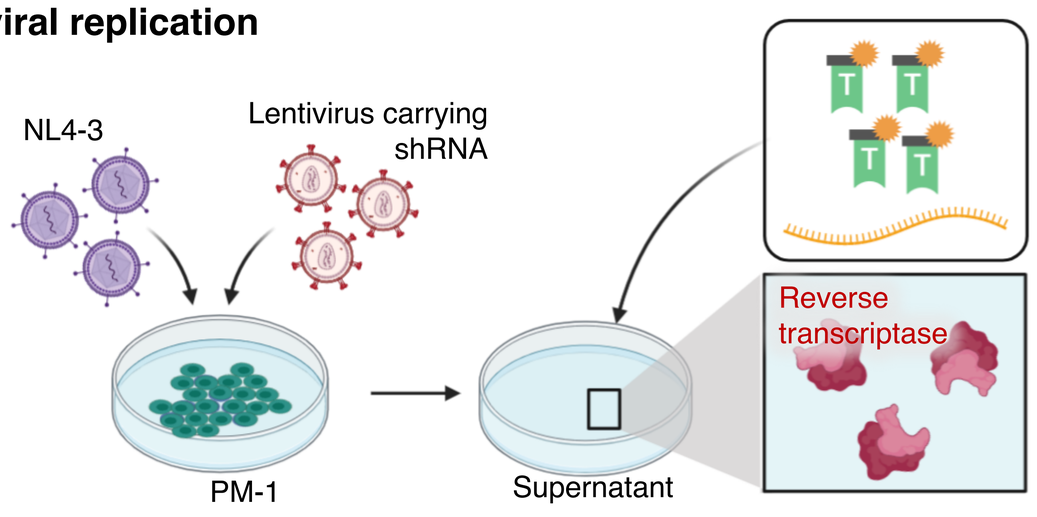
Table 1. shRNA target sites

shRNA	Target
shLdr4	Leader sequence in 5' UTR
shPol247	Pol gene
shShape7	Pol gene

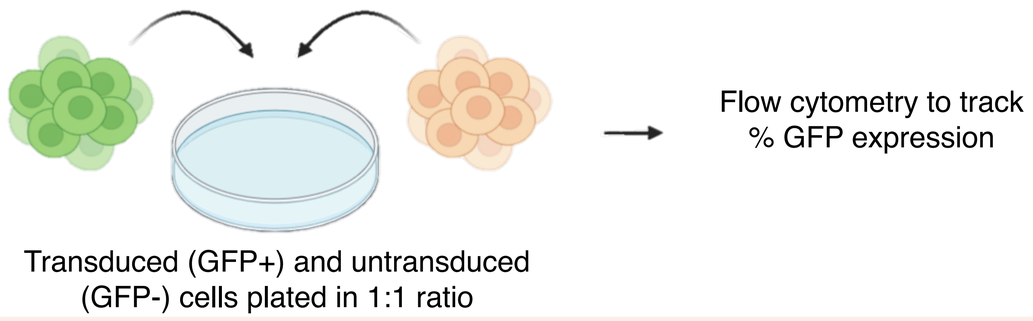
Efficacy of shRNA constructs for inhibiting viral production



Efficacy of shRNA constructs for inhibiting long-term viral replication

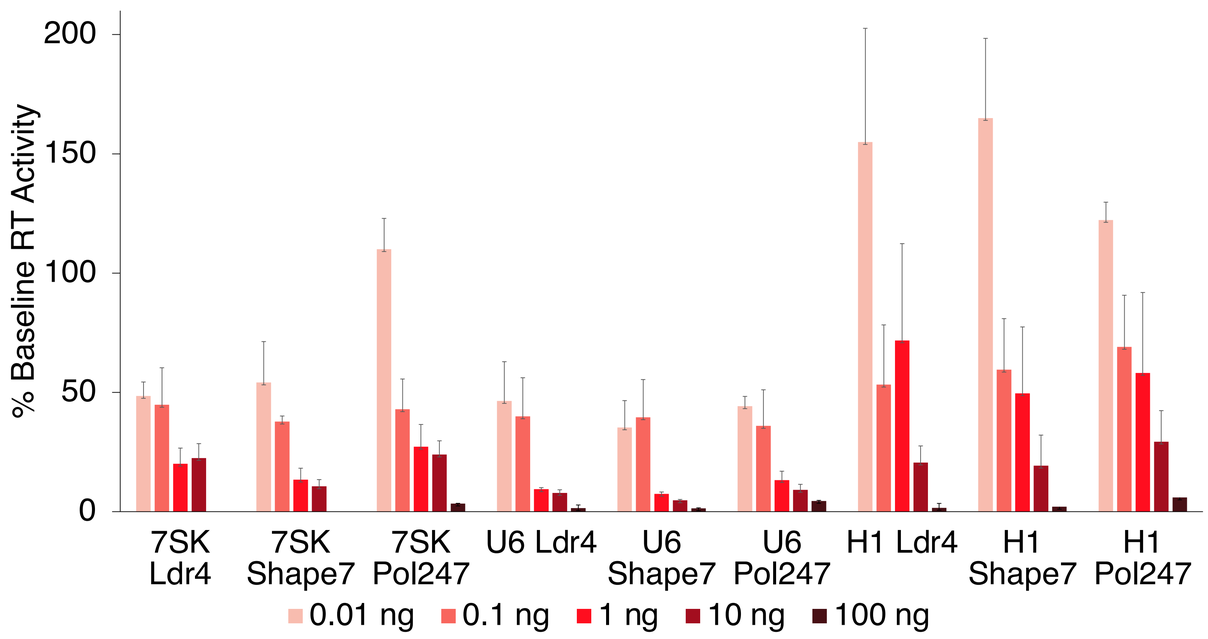


Evaluating toxicity of shRNA constructs

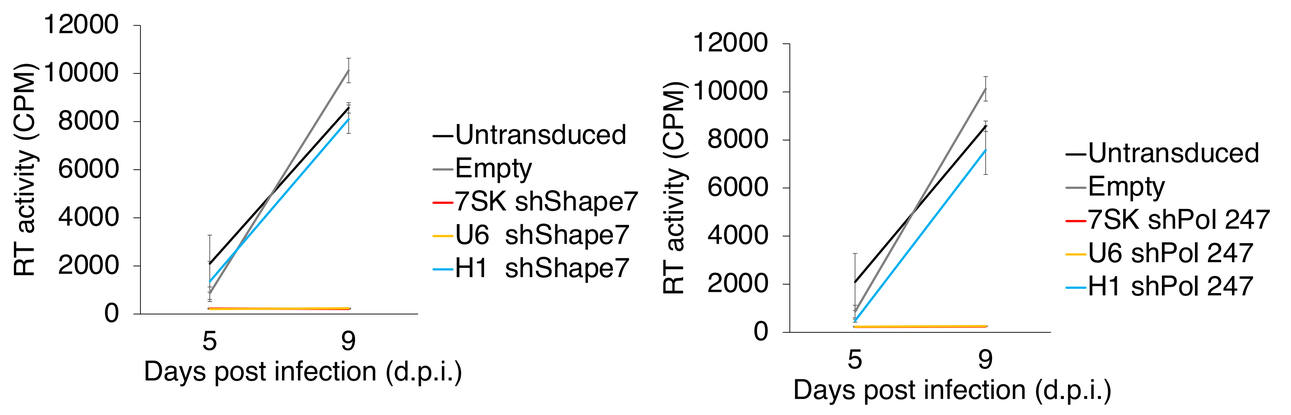


RESULTS

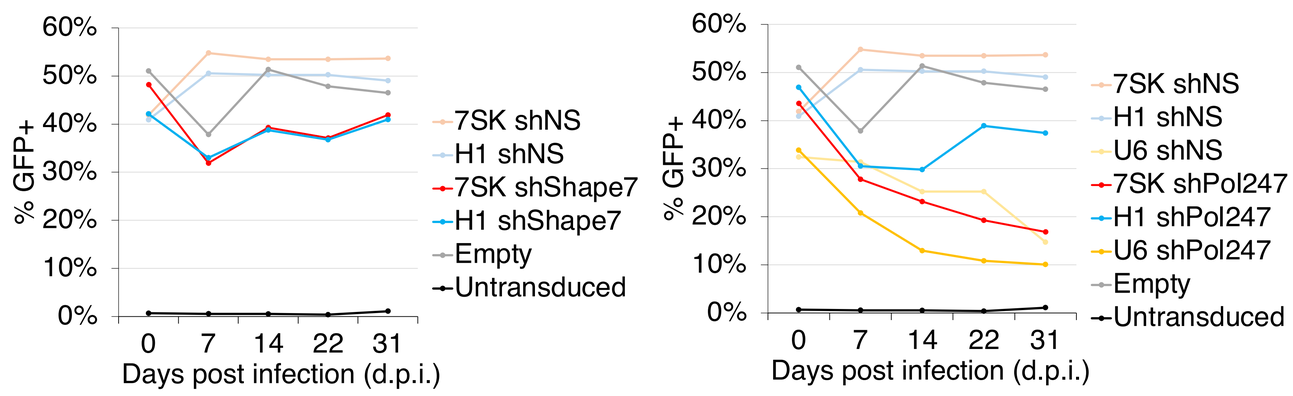
shRNAs expressed from 7SK and U6 promoters exhibit greater activity against viral production



shRNAs expressed from 7SK and U6 promoters delay viral production



7SK- and U6-promoted shRNAs exhibit evidence of toxicity



CONCLUSIONS

1. Pol247 may be toxic when expressed from U6 and 7SK
2. Toxicity may be due to sequence-specificity (7SK Pol247 appeared toxic, but not 7SK Shape7)
3. Toxicity from U6 constructs may be due to off-target effects

ACKNOWLEDGEMENTS

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REFERENCES

1. Babiker, A. G., et al. 2013. START study. *Clin Trials.*
2. Bavinger, C., et al. 2013. Risk of cardiovascular disease from cART. *PLoS One.*
3. Tshikuka, J. G., et al. 2018. Relationship between cART and diabetes. *BMC Public Health.*
4. Carr, A., and D. A. Cooper. 2000. Adverse effects of antiretroviral therapy. *The Lancet.*