Developing triplex forming oligonucleotides as a 'block and lock' strategy for an HIV cure

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A joint venture between The University of Melbourne and The Royal Melbourne Hospital

Background:

HIV cure is hindered by latently infected T cells that persist during ART. The virus integrates into the host genome, becoming transcriptionally silenced. It can then reactivate[1].

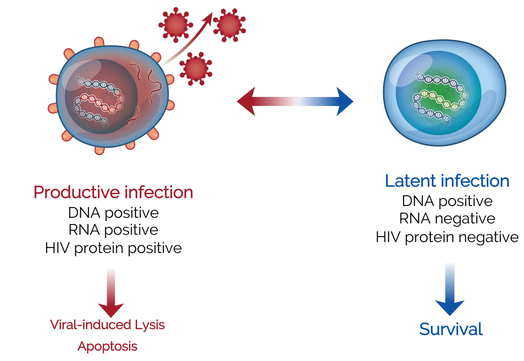


Figure 1. HIV latency hinders a cure to HIV.

Strategy to HIV functional cure: Block and Lock

During long term ART, a shift towards more latent provirus is observed[2].
Similarly, Block and Lock aims to permanently silence HIV provirus transcription and prevent virus from reactivating.

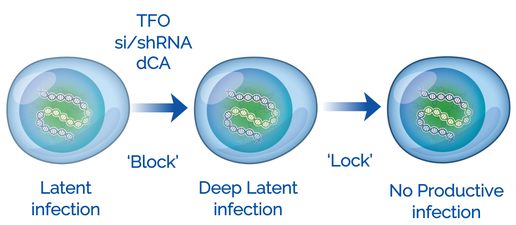


Figure 2. The block and lock strategy and some potential silencing approaches including dCA[3], siRNA and shRNA[4].

Triplex forming oligonucleotides (TFO) are a single strand of DNA that binds to specific duplex DNA sequences in a stable and sequence-specific manner, thereby enabling targeted silencing of specific genes such as HIV by terminating transcription elongation[8]. We hypothesise that TFO binding to the integrated provirus will inhibit constitutive and inducible viral transcription. This creates a 'block and lock' strategy for HIV cure.

Hypothesis:

Binding of HIV-specific TFOs will lock the integrated HIV provirus in a deep latent state, leading to inhibition of virion production in the presence of cellular activation.

Methods:

We designed multiple TFOs targeting HIV gag (n=3), env (n=4) and to GFP (n=4). TFOs were delivered by lipofectamine or a novel nanoparticle to HEK 293T transfected with plasmids expressing gag, env, green-fluorescent-protein (GFP) and full-length HIV NL-4.3.

TFO locations in the HIV sequence

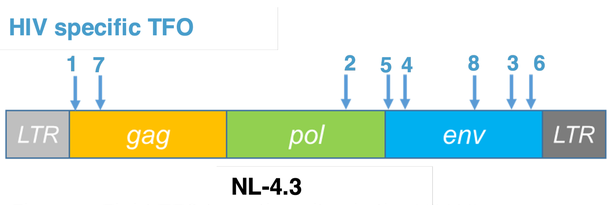


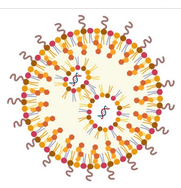
Figure 3. Eight TFO targeting sites in the HIV NL-4.3 sequence were selected on the basis of compatible target sequence

TFO inhibition of full length proviral plasmid expression was determined by measuring release of viral particles in supernatant. This was quantified by co-culture of infected supernatant with TZM-bl cells.

HIV transcription was determined by quantification of cell associated unspliced HIV RNA.

TFO activity on integrated provirus was assessed by transfection of LNP-TFOs and subsequent stimulation of J-Lat A2 and quantification of changes in GFP level(n=4).

TFO delivered by Lipid nanoparticles



Efficient receptor mediated endocytosis[3] and improve TFO endosome escape[5]

Results:

HIV Gag targeting TFO inhibits HIV gag expression when delivered as an unintegrated plasmid

HIV-specific TFOs inhibited HIV gag expression (reduction with 4 uM HIV-TFO7, 67.26±9.19%, p=0.03)

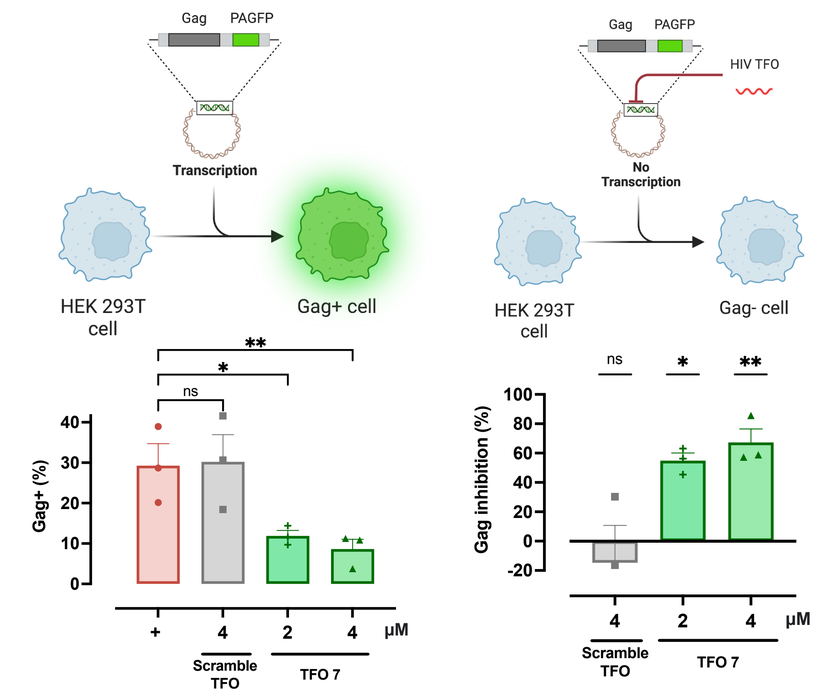


Figure 5. HIV TFO 7 inhibits Gag expression in HEK 293T cells.
Expression of green fluorescent protein was quantified in the presence of different concentrations of HIV TFO 7 and a scrambled TFO (grey) and percentage of positive cells is shown (left). Reduction of expression relative to DMSO (right). The columns represent the mean and error bars the SEM. Statistical significance determined with one-Way ANOVA test, *p<0.05 **p<0.01. (N=3)

HIV Env targeting TFO inhibits HIV env expression

HIV-specific TFOs inhibited HIV env expression (reduction of env expression ranging from 75 to 88%).

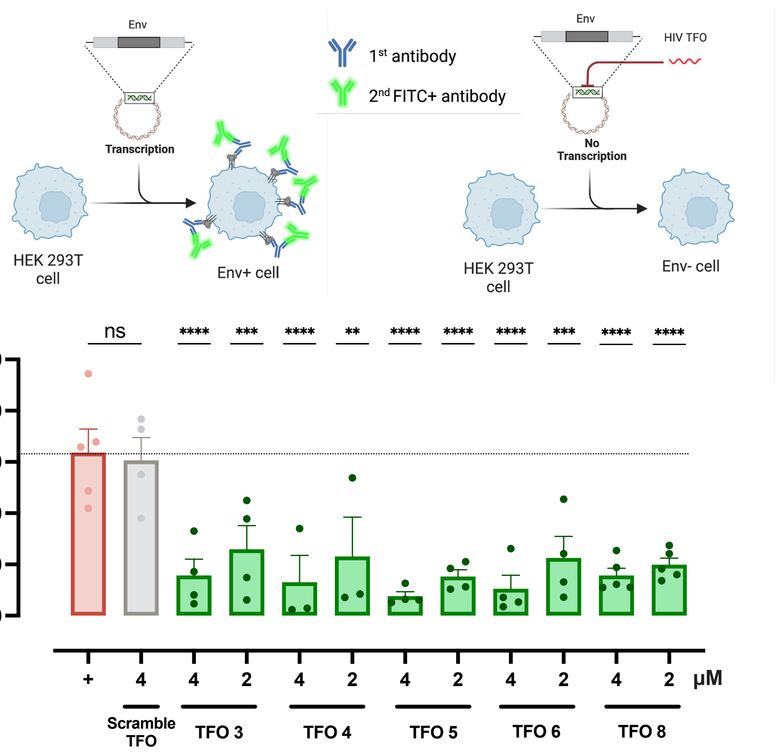


Figure 6. Multiple HIV TFOs reduce HIV envelope expression.
Bar graph depicting Env expression under various conditions. HIV TFOs 3, 4, 5, 6, and 8 more effectively inhibited envelope expression at 4 uM compared to 2 uM. The columns represent the mean and error bars the SEM. with statistical significance tested via one-way ANOVA, **p<0.01, ***p<0.001, ****p<0.0001. (N=4)

HIV targeting TFO inhibits *in vitro* viral production

In HIV NL-4.3 transfected cells, 2uM HIV TFO 4 potently reduced the).59%

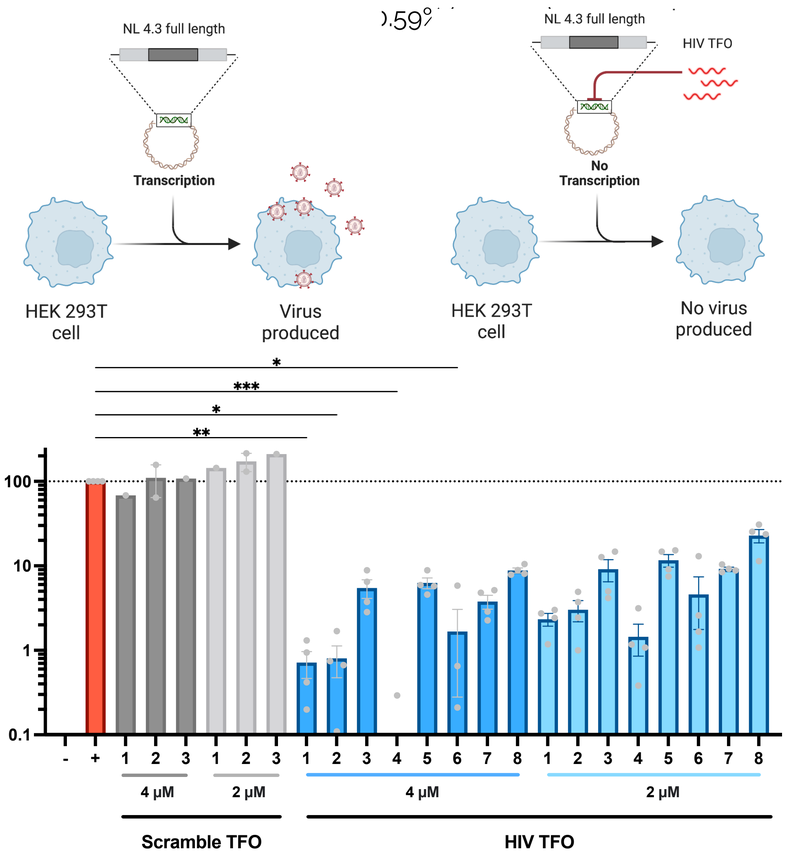


Figure 7. Reduced viral production in full-length NL4.3 transfected HEK 293T cells, as measured by the TZM-bl assay in the presence of multiple TFOs.
HEK293 T cells were transfected with full length NL4.3 and production of virus in supernatant quantified following incubation with the TZMBL reporter cell line where luciferase expression was quantified. The scrambled TFOs (grey) showed similar levels of infectivity to the positive control. All eight HIV TFOs demonstrated significant inhibition of HIV infectivity at both 2 μM and 4 μM concentrations. Statistical significance determined with Kruskal-Wallis test. (n=4). *p<0.05, **p<0.01, ***p<0.001

Results:

Reductions in viral expression occur at the level of HIV transcription

TFOs reduced the level of cell associated unspliced HIV RNA, consistent with interference with HIV transcription.

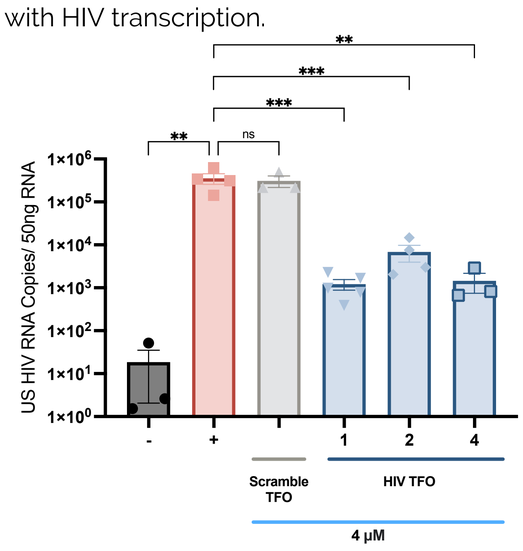


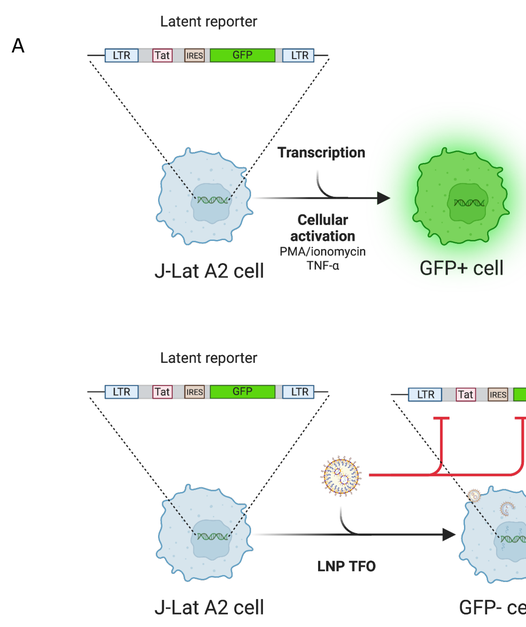
Figure 8. TFOs include a reduction of cell associated unspliced HIV RNA consistent with inhibition of transcription.
HEK293 T cells were transfected with full length NL4.3 and RNA extracted from cells in the presence and absence of TFOs. Cell associated unspliced HIV RNA copies was quantified by RT-PCR. There was a significant decrease in US HIV RNA levels in HIV TFO treated samples compared to the positive control and scramble TFO-treated samples. The columns represent the mean and error bars the SEM. Statistical significance determined with one-Way ANOVA test **p<0.01, ***p<0.001. (N=3)

TFO delivered by lipid nanoparticles (LNP) can inhibit viral reactivation in a latently infected cell line

- J-Lat A2 Cells were incubated with LNP-TFO for 24 h, then stimulated with PMA/ionomycin or TNF-alpha for 24 h

LNP + GFP TFO 2 led to

- **7.4-11.1%** GFP reduction in PMA/ionomycin stimulated cells
- **28.0-32.0%** GFP reduction in TNF-alpha stimulated cells



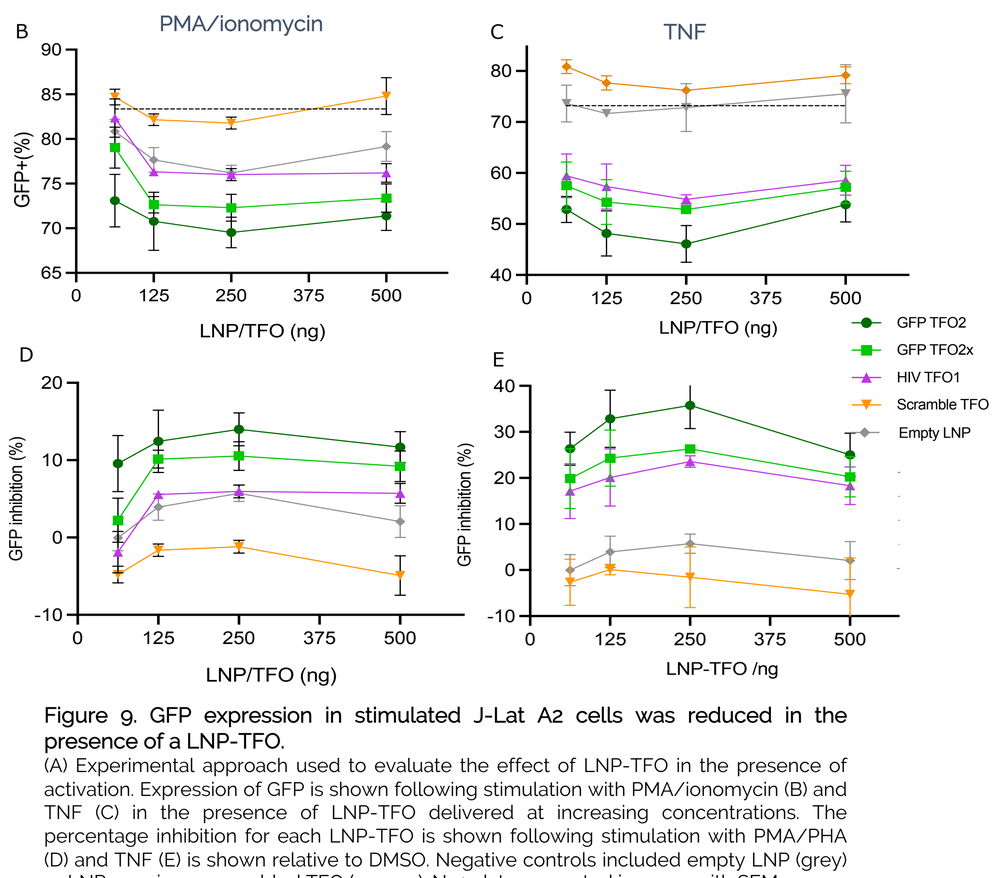


Figure 9. GFP expression in stimulated J-Lat A2 cells was reduced in the presence of a LNP-TFO.
(A) Experimental approach used to evaluate the effect of LNP-TFO in the presence of activation. Expression of GFP is shown following stimulation with PMA/ionomycin (B) and TNF (C) in the presence of LNP-TFO delivered at increasing concentrations. The percentage inhibition for each LNP-TFO is shown following stimulation with PMA/PHA (D) and TNF (E) is shown relative to DMSO. Negative controls included empty LNP (grey) or LNP carrying a scrambled TFO (orange). N=4, data presented in mean with SEM.

Conclusion:

- TFOs can potently inhibit HIV gene expression
- Lipid nanoparticle encapsulated TFOs could moderately reduce HIV reactivation in a non-infectious latently infected T cell line.
- Further improvements to TFOs could involve the addition nuclear targeting peptides[9] and modified 2'-O-methyl oligonucleotides to increase stability[10].
- TFOs potentially present a novel HIV 'block and lock' option to limit HIV transcription.

References:

1. Deeks, Lewin *et. al.* Nature Medicine, 2021
2. Einkauf, K. B et al. Cell, 2022.
3. V. A. Green et al. Virol J, 2012.
4. C. Ahlenstiel et al. Mol Ther, 2015.
5. Cevaal, P.M., et al. ACS Nano, 2021
6. Huo, S., et al., ACS Nano, 2014
7. Maugeri, M. Nat Commun, 2019
8. A. Arcella et al., J Am Chem Soc, 2012.
9. Tarvirdipour et al. Biomater. Sci., 2022
10. Schneider, P.N., et al., Genesis, 2011.

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