Antibody-targeted Tat-LNP X enables selective Tat mRNA delivery to CD4$^+$ T cells for enhanced latency reversal *ex vivo*

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Background:
One strategy to reduce the size of the HIV reservoir is to reactivate proviral transcription using latency reversing agents. Lipid nanoparticles (LNPs) encapsulating mRNA encoding the HIV Tat protein (Tat-LNP X) potently reverse latency in CD4+ T cells from people with HIV (PWH) on suppressive antiretroviral therapy (ART). However, we hypothesise that the *in vivo* efficacy of Tat-LNP X may be limited by nonspecific uptake by bystander cells, necessitating a more targeted delivery approach. Here, we engineered a T cell-targeted Tat-LNP X and assessed its ability to selectively deliver Tat mRNA to CD4+ T cells for potent latency reversal in the presence of bystander cells.

Methods:
Tat-LNP X was formulated through microfluidic mixing. Targeted Tat-LNP X was generated by nanobody-based surface conjugation of antibodies specific for internalised T cell receptors CD7 or CD2. Transfection efficiency was assessed *in vitro* using isolated CD4+ T cells and peripheral blood mononuclear cells (PBMCs) by flow cytometry. HIV latency reversal was assessed *ex vivo* in PBMCs from PWH on ART, using digital PCR.

Results:
Untargeted Tat-LNP X potently transfected 80.5±4.1% (mean±SEM) of isolated CD4+ T cells, but only 29.3±6.7% of CD4+ T cells in the presence of bystander PBMCs. Surface functionalisation with anti-CD7 or anti-CD2 antibodies enhanced transfection of CD4+ T cells in the presence of PBMCs to 59.3±9.1% and 56.4±9.1%, respectively. Consistent with improved delivery, CD7- and CD2-targeted Tat-LNP X potently reversed latency in PBMCs from PWH on ART by upregulating elongated HIV transcripts 20.2- and 23.1-fold compared to untreated control, even at a low dose of 100ng per 10^6 cells.

Conclusion:
Targeted Tat-LNP X amplifies the delivery of Tat mRNA to CD4+ T cells for potent latency reversal in PBMCs treated *ex vivo*. Future studies will determine whether this approach can drive latency reversal *in vivo* in humanised mice.

Disclosure of Interest Statement:
Authors declare no conflicts of interest