CRISPR-ACTIVATION FOR HIV LATENCY REVERSAL USING LIPID NANOPARTICLE DELIVERY

Authors:
Moso MA[1,2], Cevaal PM[1], Tan A[1], Kan S[1], Ali A[1], Fisher BM[1], Kim Y[1], Ong J[1], Purcell D[3], Pouton C[4], Carouso F[5], Fareh M[6,7], Quadeer A[3,8], McKay M[3,8], Lewin SR[1,2,9], Roche M[1]

[1]Department of Infectious Diseases, The University of Melbourne at the Peter Doherty Institute for Infection and Immunity; [2]Victorian Infectious Diseases Service, The Royal Melbourne Hospital at the Peter Doherty Institute for Infection and Immunity; [3]Department of Microbiology and Immunology, The University of Melbourne at The Peter Doherty Institute for Infection and Immunity; [4]Monash Institute of Pharmaceutical Sciences, Monash University; [5]Department of Chemical Engineering, The University of Melbourne; [6]Cancer Immunology Program, Peter MacCallum Cancer Centre; [7]Sir Peter MacCallum Department of Oncology, The University of Melbourne; [8]Department of Electrical and Electronic Engineering, The University of Melbourne; [9]Department of Infectious Diseases, Alfred Hospital and Monash University.

Background:
Latency reversing agents for HIV cure have been limited by low potency and off-target effects. CRISPR activation (CRISPRa) is a promising approach to improve potency and specificity for HIV-infected cells, using HIV-specific guide RNAs (gRNAs) and catalytically inactive Cas9 to bind a 20-base sequence on the HIV promoter and stimulate transcription through recruitment of transcriptional activators. We sought to assess potency and specificity of CRISPRa using single or multiplexed gRNAs and lipid nanoparticle (LNP) delivery (CRISPRa-LNPs).

Methods:
LNPs were synthesised by combining CRISPRa mRNAs, HIV-targeting gRNAs (gRNA-L[1], O[1], 1, 2, 3), and an optimised lipid mix. Potency of CRISPRa-LNPs was assessed in the latently infected Jlat-A2 cell line by quantifying GFP expression. Mismatch tolerance of CRISPRa was evaluated using single- or double-base mutations of gRNA-L. Activity of CRISPRa-LNPs in primary CD4+ T-cells was assessed using a gRNA targeting the host gene, CD25. Ex vivo CRISPRa activity was assessed in resting CD4+ T-cells from people with HIV on antiretroviral therapy (n=8) and measured using digital RT-PCR to quantify a range of HIV transcripts.

Results:
HIV-targeting CRISPRa-LNPs led to potent HIV transcription in Jlat-A2 cells, with highest potency observed using multiplexed gRNAs L+O. Single base mutations from position 1-10 and sequential double mismatches up to positions 3,4 retained >50% CRISPRa activity, indicating a degree of mismatch tolerance. There was significant loss of activity with single mutations beyond position 10. CD25-targeting CRISPRa-LNPs led to 3.2-fold increase in CD25 expression in resting CD4+ T-cells, demonstrating successful delivery of CRISPRa to primary cells. CD4+ T-cells treated ex vivo with CRISPRa-LNPs demonstrated median 2.0-fold increase in cell-associated unspliced (Pol) HIV transcripts compared to untreated control cells, indicating increased HIV transcription.

Conclusion:
LNPs can efficiently deliver CRISPRa to a cell line model with great potency and high specificity. Multiplexed gRNA delivery enables greater potency with broader HIV sequence and subtype coverage. Further optimisation of delivery of CRISPRa is being undertaken to improve potency of this approach in resting CD4+ T-cells.

Disclosure of Interest Statement:
No conflicts of interest to disclose.