Optimization of Lipid Nanoparticle-based CRISPR-activation to Improve HIV Latency Reversal

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Background:
Current latency reversal agents (LRAs) lack specificity and potency. We previously harnessed the inherent sequence specificity of CRISPR activation (CRISPRa) to induce HIV transcription *ex vivo* but observed incomplete proviral reactivation, which may be related to poor expression of the CRISPRa construct in primary CD4+ T cells. CRISPRa may also be limited by the vast sequence diversity of HIV. Here, we optimised CRISPRa in a lipid nanoparticle (LNP)-based delivery system to improve its activity and coverage for diverse HIV subtypes.

Methods:
CRISPRa expression was quantified by intracellular staining (ICS) for dCas9 protein. mRNA expression was optimised by altering its 5' and 3' Untranslated Regions (UTRs). LNPs were modified by conjugating T cells specific targeting antibodies (CD7) to its surface. CRISPRa activity in primary T cells was evaluated using CD25-targeting gRNA and measurement of surface CD25 expression by flow cytometry. Cross-subtype gRNAs were designed by generating variants of HIV-targeting gRNAs matched to non-B sequences from the Los Alamos database. CRISPRa HIV latency reversal was assessed in the J-Lat A2 cell line via GFP expression.

Results:
High expression of dCas9 was observed in JLatA2 (90.6% dCas9+) but not in activated (3.2%) or resting CD4+ T cells (0.9%). Altered dCas9 mRNA enhanced expression in J-Lat A2 (9.7-fold increase in dCas9 expression) but not in resting CD4+ T cells. CD7-targeted LNPs improved CRISPRa activity (6.1-fold increase in CD25 expression). gRNA pools of up to 9 gRNAs improved coverage to greater than 75% for subtype A1, B, C, D, AE sequences. The CRISPRa pan-genotypic LNPs

retained activity in the J-Lat A2 cell line, with slight reduction in potency (70.6% activity Cf. subtype B specific gRNAs).

Conclusion:
CRISPRa activity was improved through alteration in mRNA and use of CD7-targeted LNPs. Sequence coverage was improved by combination of gRNA variants with a minor loss of potency.

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