An HIV-specific TALE mRNA mediated strategy for potent and specific HIV-1 latency reversal

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While suppressive ART has improved the lifespan of people with HIV, the ability of HIV to persist in a latent state within long-lived memory CD4+ T cells, necessitates the development of effective latency reversal therapies. “Shock and kill” strategies are of particular interest, in which latency reversal agents (LRA) can induce HIV transcription to reveal and sensitize infected cells for clearance. Restricting an LRA’s selectivity/specificity to HIV+ cells is critical in reducing off-target effects on uninfected cells. However, the only common signature to all HIV-infected cells (including those in latency) is the integrated viral DNA (i.e. provirus). This presents an opportunity for DNA recognition platforms such as transcription activator-like effectors (TALEs) and CRISPR-Cas9. As TALEs have shown greater targeting efficiency in heterochromatin compared to Cas9, we hypothesized that HIV DNA-specific TALEs fused with transactivation domains (TALEAs) delivered as mRNA would provide transient, potent, translatable, and specific HIV reactivation. We designed mRNA constructs encoding HIV-specific TALEAs containing a chimeric p65-HSF1 transactivation domain targeting various regions within the HIV 5’ LTR. After HIV-TALEA mRNA electroporation of J-Lat 10.6, an in vitro cell line model of HIV latency, we observed high levels of HIV reactivation with greater potency compared to those observed with the current gold standard of viral Tat mRNA. However, in more deeply latent cell lines (J-Lat 6.3, 8.4, and 15.4), we met a stronger barrier to reactivation across all TALEA or Tat conditions tested. To address this, we added the Tat domain to our HIV-specific TALEA and measured greater, though still incomplete, reactivation compared to that of single components, indicating additional reactivation barriers remained unresolved. We therefore tested whether proviral DNA methylation in J-Lats limits TALEA-mediated reactivation. Pre-treatment of J-Lat 6.3, 8.4, and 15.4 cell lines with the DNA methyltransferase inhibitor 5-aza-2′-deoxycytidine for 24 hours followed by mRNA electroporation, potently reactivated all latent HIV cell lines tested, particularly with the TALEA-Tat fusion construct. Altogether, these data demonstrate HIV specific TALEAs can potently reactivate latent HIV models *in vitro* which may contribute to the development of a curative therapy.