CUSTOM GENE THERAPY VECTORS FOR RESTING T CELLS

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Background: Gene therapy is considered a potential HIV cure strategy, with its ability to target the latent reservoir. Lentiviral vectors currently represent the gene delivery platform for primary T cells. However, lentiviral vectors are unable to efficiently transduce resting T cells, a major component of the HIV latent reservoir. The reduced efficiency exists due to the enzyme SAMHD1, which antagonizes lentiviral reverse transcription. The accessory protein, Vpx, found in HIV-2 and SIV has been shown to overcome this through inhibition of SAMHD1.

Methods: This project aims to incorporate Vpx into lentiviral vector systems to overcome the restriction in resting $CD4^+$ T cells to gene delivery. Using publicly available Vpx sequences, a diverse panel was selected and incorporated into lentiviral vectors. Gene delivery to primary resting $CD4^+$ T cells was assessed by delivery of green fluorescent protein (GFP). In parallel, the longevity of gene delivery enhancement was observed through Vpx delivery with a viral like particle and later, lentiviral delivery of GFP. The latter ensures that enhancement of HIV infection does not remain after gene delivery. Flow cytometry was used for GFP analysis.

Results: Our Vpx screens revealed not all Vpx proteins enhanced gene delivery equally. A HIV-2 Vpx variant was observed to have the greatest enhancement of gene delivery by a magnitude of 5.53 in 8 independent resting T cell donors. Furthermore, whilst most Vpx variants enhanced gene delivery and HIV infection over 5 days, our lead Vpx variant did so only for 4 days.

Conclusion: The identified HIV-2 Vpx variant enables enhancement of gene delivery in the short-term. This combined with a fusogenic HIV-1 envelope, led to gene delivery of greater than 30% across independent donors, representing significantly greater rates of gene delivery than present platforms. With this gene delivery platform, we have addressed one of the major limitations of HIV gene therapy.

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