DESIGN OF A GENE THERAPY ULTRAPARTICLE TO SUCCESSFULLY MEDIATE A GENE THERAPY APPROACH TO HIV-1 CURE

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Background: A pathway to HIV-1 cure is through gene therapy; a practice of genetically modifying cells to confer protection against HIV-1 infection or spread. Historical inroads into HIV-1 gene therapy cures were undermined by inefficient gene transfer into resting $CD4^+$ T cells; the majority constituent of the long-lived viral reservoir. These cells are recalcitrant to genetic modification due to inefficient cytoplasmic and nuclear transgene delivery. The former, mediated by the plasma membrane, can be overcome with superfusogenic pseudotypes. The latter, mediated by a restriction factor (SAMHD1), can be neutralized by accessory proteins (Vpx). We sought to develop an ultraparticle that synergized superfusogenicity with Vpx to circumvent shortcomings in clinical gene therapy.

Methods: To identify a lead superfusogenic envelope, we assessed over 20 pseudotyping methods. To overcome SAMHD1, we tested over 24 Vpx variants derived from HIV-2 and SIV. Both approaches were performed in primary resting $CD4^+$ T cells. To validate our envelope and Vpx selection, we challenged target cells with an eGFP-encoding vector to ascertain gene modification efficiency. Flow cytometry was used to quantify SAMHD1 degradation and percentage of cells expressing eGFP.

Results: The lead pseudotype mediated fusion with at least 92% of resting $CD4^+$ T cells. The Vpx variants KIRoV-1 (HIV-2) and Mac239 (SIV) degraded 90%+ of SAMHD1 in these cells. 35%+ of SAMHD1-depleted cells were successfully transduced, representing orders of magnitude greater transduction efficiency over control. The window of transduction enhancement by KIRoV-1 was shorter (<1 week) than Mac239 (2+ weeks). This is an important clinical consideration, as Vpx can make cells vulnerable to HIV-1 infection, and therefore risk expanding the viral reservoir.

Conclusion: Genetic delivery into the most challenging cell type was achieved with a “fire-and-forget” ultraparticle realized through an superfusogenic envelope synergized with an effective Vpx. These results encouragingly validate a gene therapy platform that is ready for pre-clinical applications.

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