ENGINEERING A PSEUDOTYPED LENTIVIRAL PLATFORM TO ENABLE LINEAGE-SPECIFIC TRANSDUCTION OF IMMUNE CELLS

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Background Cell-specific transduction remains one of the next frontiers for virally-delivered gene therapy. Efforts to achieve cell-specific transduction have largely been limited to borrowing of preexisting viral glycoproteins and pseudotyping viral surface envelopes to result in altered tropism. VSVG is derived from vesicular stomatitis virus (VSV) and is one of the most commonly used lentiviral (LV) pseudotype glycoproteins as its cognate receptor (LDLR) is present on nearly all dividing and non-dividing cells, enabling broad tropism of VSVG-pseudotyped LVs.

Methods Our lab recently developed a “receptor-blinded” version of VSVG, in which point mutations (K47Q, R354A) of this glycoprotein result in a mutated VSVG with inability to bind and infect LDLR-expressing cells. This mutant viral glycoprotein, which we call “VSVGmut”, thereby loses its broad tropism, but critically retains its fusogen ability, enabling co-display of a new LV pseudotype ligand to drive LV tropism.

Results Initial experiments displaying high-affinity anti-CD19 scFvs alongside VSVGmut on the LV surface demonstrated infection of CD19+ cells, but not CD19- cells. Subsequent work using endogenous ligands (CD80), Fabs (a-CD3e), cytokines (IL-13), viral glycoproteins (SARS-CoV-2 RBD), and peptide MHCs (pMHCs) revealed the modularity of this platform for achieving potent transduction of on-target cells, with minimal infection of bystander cells, across a range of affinities (pM to uM) and at frequencies as low as 1 in 100,000. This technology allowed for library screening of 96 viral pMHC-displaying LVs against a repertoire of >450,000 TCRs in pool, which accurately uncovered EBV- and Flu-specific TCRs through scRNA sequencing.

Conclusions The VSVGmut platform has resulted in our lab’s ability to pair pMHCs with cognate TCRs and viral surface antigens with cognate BCRs, in addition to achieving lineage-specific transduction of T and B cell subsets, setting the stage for cell-specific gene therapy.

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