Design and Validation of Membrane-Tethered IL-21 mRNAs for NK Immunomodulation

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Background: Cytokine immunotherapy is being explored as a potential strategy to enhance immune responses for HIV cure strategies. Recent work has demonstrated that systemic IL-21 administration during SIVmac infection can enhance cytolytic NK cell function and reduce reservoir seeding in lymphoid tissue. However, systemic cytokine administration can be associated with dose-limiting side effects and off-target signaling. Here, we developed an mRNA-encoded IL-21-IL-21 receptor (IL21-IL21R) fusion protein designed to transiently induce autocrine signaling after targeted delivery to NK cells.

Methods: Alpha-fold was applied to IL-21-IL-21R fusion proteins with various linkers. mRNAs encoding two variants with different linkers were synthesized. 293F cells or PBMCs were transfected with each mRNA. 2-24 hours later, cell surface protein expression or intracellular STAT3 phosphorylation (downstream of IL-21R signaling) was measured via flow cytometry.

Results: Regardless of linker inclusion, all IL-21-IL21R constructs demonstrated a predicted fold that matched unliganded IL-21R (PDB: 8ENT; RMSD 0.5-0.7Å). 24 hours following transfection of 293F cells, 10-15x higher expression of the IL-21 fusion protein was detected on the cell surface using an IL-21-specific staining antibody compared to untransduced controls (MFI: 7360, 9124 ; Negative control MFI: 672). 2 hours after electroporation of IL-21-IL-21R mRNA into primary human PBMC, we observed robust phosphoSTAT3 signaling, indicating bioactivity of the IL-21-IL-21R fusion protein. PhosphoSTAT3 staining returned to baseline by 24 hours. While the IL-21 and IL-21R linker variants had minimal differential effect in protein expression in 293F cells, the variant including a linker between IL-21 and IL-21R demonstrated slightly higher phosoSTAT3 levels in primary T cells (MFI: 1845 vs. 1085) and NK cells (MFI: 1763 vs. 1185).

Conclusions: mRNA-encoded IL-21-IL21R shows cell surface expression and retains transient signaling activity. Future directions will explore whether this protein engineering yields autocrine signaling to mitigate systemic off-target effects. This construct will ultimately be tested in murine and primate models using an NK cell-specific delivery method to assess its impact on cytolytic NK activity and the lentiviral reservoir. More broadly, this approach can be applied to other cytokines for targeted immune cell modulation.