Reprogramming cytotoxic natural killer cells via IL-15-conjuguated mRNA-LNP

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Cytotoxic natural killer (NK) cells play a significant role in the defense against viral infections like HIV-1 by releasing cytotoxic molecules such as perforin and granzyme. However, their ability to mount a robust and durable antiviral response against HIV-1 depends not only on their capacity to produce these cytotoxic molecules but also on their ability to access key sites of HIV-1 replication. Secondary lymphoid tissues (SLTs) are sites of productive HIV-1 infection, and it has been shown that cytotoxic NK cells with fail to migrate and accumulate in SLTs due to the lack of expression of key SLT homing receptors such as CD62L and CCR7. These challenges underscore the importance of targeting cytotoxic NK cells to deliver and express SLT-homing mRNAs to promote homing into SLT for effective HIV-1 clearance.

Lipid nanoparticles (LNP) have emerged as a promising platform for the delivery of mRNA-based therapies. Recently our lab has shown that using ligand-based conjugation of mRNA-LNP enable precise targeting of specific immune cell populations. Given that IL-15 is a vital cytokine for NK cell survival, we hypothesize that conjugating IL-15 onto LNP would enable us to target cytotoxic NK cells and modulate their immune function. To do this, we leveraged the use of two IL-15 receptor superagonist, N803 and IL15/IL15Ra, to selectively target IL2Rßγ-expressing NK cells. Specifically, we functionalized N803 and IL15/IL15Ra and conjugated it to the surface of LNP containing mRNA encoding GFP. We found that treating human PBMCs *in vitro* with N803-GFP-LNP and IL15/IL15Ra-GFP-LNP leads to delivery and expression of GFP in NK cells with high specificity and efficiency compared to controls. Additionally, we found that GFP expression in NK cells increased in a dose-dependent manner, with higher mRNA dose resulting in greater GFP expression. Next, we tested whether murine NK cells could be similarly targeted *in vivo*, and we found that murine NK cells in spleen and blood resulted in robust expression of GFP relative to PBS or unconjugated GFP-LNP controls. Taken together these data demonstrate IL-15-conjugated-LNP as a promising platform to target NK cells for immunomodulation to promote their homing to SLTs for effective HIV-1 clearance.