Molecular Therapy
Nucleic Acids
Commentary

Engineering NK cells with LNP-enabled IL-2 mRNA transfection: Steps toward affordable precision immuno-therapeutics

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The natural killer (NK) cells of the immune system have emerged as potent warriors, capable of targeting and eliminating malignant cells without prior sensitization.[1] Work carried out by Pichon and colleagues presents a significant advancement in the field of immunotherapy and in particular in cancer treatment.[2] This study encompasses engineering of the innate immune cells, NK cells, toward intrinsically secreting interleukin-2 (IL-2), a critical cytokine that amplifies immune responses by employing a lipid nanoparticle (LNP)-enabled IL-2 mRNA delivery. This innovative approach not only enhances the efficacy of NK cells but also opens new avenues for improved immune therapy, potentially revolutionizing the treatment of various cancers. The present study addresses the limitations of current NK cell therapies and introduces a novel strategy that could be applicable across a range of malignancies. However, the safety and efficacy of this approach in humans remain to be demonstrated.

This study has been woven around the understanding that NK cells play a crucial role in the immune system's surveillance against cancer. Historically, the use of NK cells in cancer therapy has been hampered by their short persistence in the tumor microenvironment and their over-reliance on external cytokine support for activation and expansion. The development of mRNA-based technologies has opened new avenues for enhancing NK cell function by allowing for the transient expression of therapeutic proteins without risking genomic integration.[3] More importantly, IL-2-secreting NK cells circumvent these challenges by providing a continuous, localized supply of IL-2, which not only sustains the NK cells but also enhances their anti-tumor activity.

Toward developing a safer and efficient platform to deliver the IL-2 mRNA to NK cells, the authors performed a range of optimizations in developing imidazole lipid-based nanoparticles (iLNPs) including the method of complexation with mRNAs for selectivity of co-lipids. iLNPs outperformed prevailing methods including viral vectors and electroporation, offering a safer and more cost-effective alternative. Superior transfections of these iLNPs are attributed to the presence of distinctive mRNA-rich bleb structures in their LNPs, in turn enhancing intracellular delivery.[4] Intriguingly, screening of base modifications on mRNA demonstrated that unmodified mRNAs had superior translation in NK cells compared to the base modified mRNAs. The recent studies have shown N^1-methyl-pseudouridine modification (N^1MeψU) has superior translations in hematopoietic cells such as dendritic cells.[5] The present study throws light on the importance of screening base modifications on mRNA in a cell-specific manner for enhanced translation as other hematopoietic, NK cells do not require base modifications. Optimizations on both iLNPs and mRNA fronts led to efficient IL2 mRNA transfections into NK cells at a therapeutically relevant fidelity.

IL-2 mRNA-transfected NK cells demonstrated sustained secretion of IL-2 cytokine, leading to enhanced proliferation, persistence, and cytotoxicity against tumor cells *in vitro*. Intrinsic secretion of IL-2 creates a favorable immunological milieu that not only bolsters the NK cells' own functions but also recruits and activates other immune cells, which helps to elicit a robust anti-tumor immune response. This is a notable achievement since IL-2 has been a key cytokine for NK cell activation and expansion.[6] mRNA-enabled IL-2 protein replacement may simplify the process of NK cell therapy, and it reduces adverse effects associated with systemic recombinant IL-2 protein administration (Figure 1).

The present finding is biomedically relevant as it addresses the immediate enhancement of NK cell therapy and shifts the current paradigms of cancer immunotherapy.[7] This study suggests that autonomous cytokine production by immune cells could be a powerful strategy to circumvent the immunosuppressive tumor microenvironment. Furthermore, it may potentially improve patient outcomes by augmenting the anti-tumor activity of NK cells and minimizing the toxicities associated with current treatments.

mRNA-loaded LNPs could facilitate the co-delivery of multiple therapeutic mRNAs, hence expanding the therapeutic potential of NK cell-based treatments. Also, the loading of mRNAs encoding cytokines or chimeric antigen receptors in the LNP formulation could augment the therapeutic potential of NK cells. Interestingly, recent findings have demonstrated that the depletion of endonuclease Regnase-1 mRNA enhances NK cell anti-tumor activity.[8] The use of iLNP systems, along with CRISPR or

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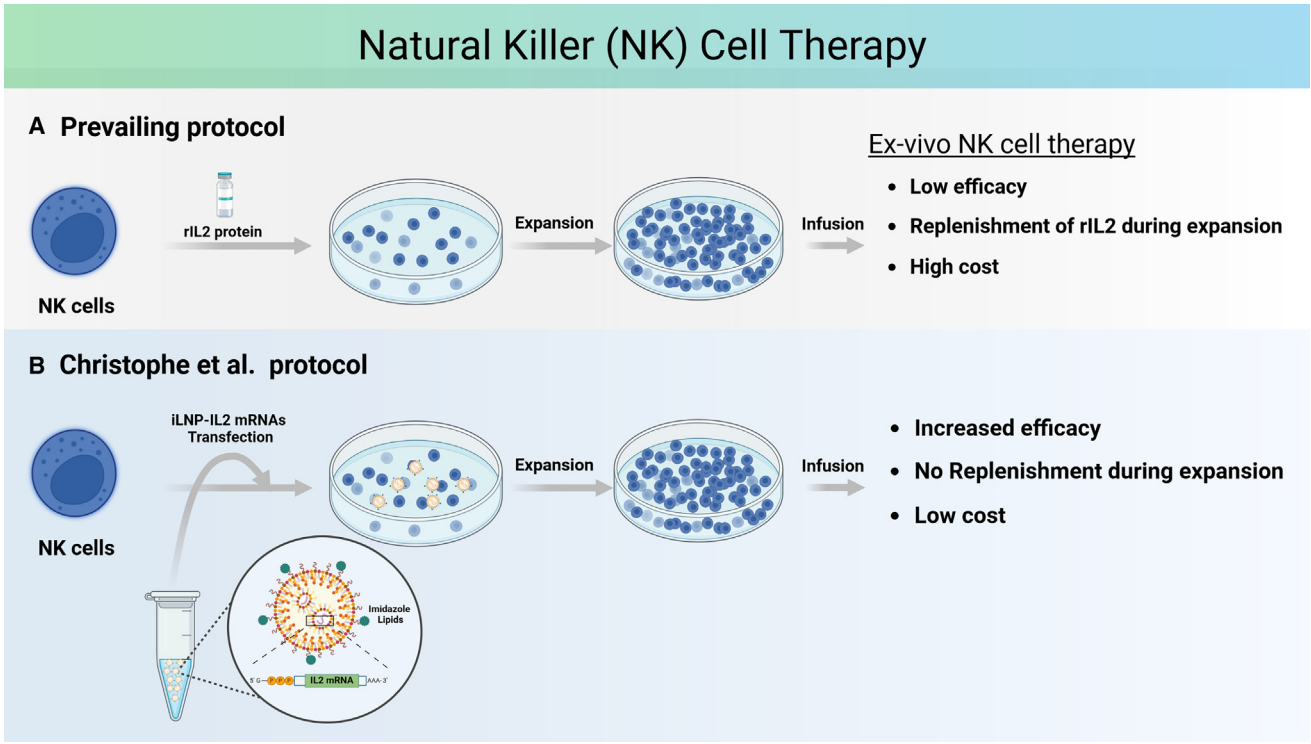

Figure 1. IL-2 supplementation in *ex vivo* NK cell expansion

(A) Recombinant IL-2 supplementation in *ex vivo* expansion of NK cells. (B) Lipid nanoparticle-enabled IL-2 mRNA delivery for intrinsic secretion of IL-2 in *ex vivo* expansion of NK cells.

RNA interference tools targeting Regnase-1 mRNA, may be a valuable consideration for future NK cell therapy. This approach can be explored for other diseases including infectious and autoimmune disorders wherein the immune modulation is both beneficial and required.

However, the long-term effects of continuous IL-2 secretion on the immune system and the potential for inducing harmful inflammatory responses need to be carefully evaluated. The *in vivo* efficacy and safety of the imidazole lipid-based mRNA platforms need to be thoroughly investigated. Additionally, these findings must be attested in larger animal models and ultimately in a clinical setting to determine the safety and efficacy of this approach in humans. As we gaze into the crystal ball, this work is of utmost importance during the molecular and cellular therapies. The ability to endow NK cells with the power of intrinsic IL-2 secretion could lead to the development of off-the-shelf, ready-to-use NK cell products that are more effective and easier to administer. This could democratize access to advanced immune therapies, making them available to a wider patient population. The long-term effects of mRNA transfection on NK cell function and the potential for off-target effects are also important considerations. The authors acknowledge these points and suggest that further work is needed to investigate the targeting efficiency of their formulations *in vivo* and to explore the possibility of using their technology for CRISPR-Cas9 gene editing.[9]

In conclusion, the study on instigating NK cells with intrinsically secreting IL-2 using an imidazole lipid-based mRNA platform offers a novel approach to enhance the therapeutic potential of these cells. It not only offers a promising new approach to enhance the efficacy of NK cell-based treatments but also paves the way for innovative strategies that leverage the immune system's own arsenal against disease. The potential to improve the efficacy and safety of NK cell therapies, and the broader implications for the field of gene editing, make this study a significant contribution to the advancement of cancer immunotherapy.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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