Engineering Natural Killer Cells for Cancer Therapy: Strategies to Enhance Persistence, Metabolic Fitness, and Cytotoxicity

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Introduction

Natural Killer (NK) cells are a crucial component of the innate immune system and a broader family of cells known as innate lymphoid cells(Mace 2023). They help protect the body and system by targeting and destroying infected or abnormal cells. What makes NK cells unique is that, unlike cytotoxic lymphocytes, they can destroy any potential threats without prior exposure to that specific pathogen (Lanier). This means they do not need to be activated by previous exposure to recognize and kill the infected cells. This is different from other lymphocytes that require previous exposure to recognize specific antigens and become activated so that they can kill the abnormal cells. Understanding how NK cells remain alive and function properly is crucial because it could aid in the development of new treatments utilizing NK cells for cancer and other infectious diseases. Recent advances in single-cell profiling and transcriptomics have revealed significant NK cell heterogeneity, with subsets specialized for anti-viral defense, tumor control, or immune regulation(Rebuffet, Zheng).

Despite their therapeutic promise, NK cell-based therapies face several challenges. One major challenge is the limited lifespan and functional persistence of NK cells in the tumor microenvironment. NK cells can become metabolically exhausted or fail to maintain function, reducing their effectiveness in long-term treatment (Sohn). Therefore, enhancing NK cell fitness, defined as sustained proliferation and metabolic stability, is a major goal in advancing NK cell therapy. In this review, we will explore recent strategies aimed at bettering NK cell function, with a particular focus on genetically engineered approaches such as CAR-NK cell therapy. The purpose of this review is to highlight recent advances and strategies, particularly genetic engineering approaches, that aim to improve NK cell persistence, function, and clinical efficacy

Role of Natural Killer Cells in Immunity

Natural killer (NK) cells are cytotoxic innate lymphoid cells that play an important role in the early immune response against virally infected and transformed cells. Unlike T or B cells, NK cells do not require prior sensitization or antigen specificity to recognize and eliminate target cells. Their function is tightly regulated through a balance between activating and inhibitory receptors, allowing them to rapidly respond to threats while minimizing damage to healthy tissue (Vacca et al., 2023). An important part of NK cell-mediated cytotoxicity is the formation of an immunological synapse with the target cell, followed by the directed release of lytic granules. These granules contain perforin, which forms pores in the target cell membrane, and granzymes, which enter through these pores and induce apoptosis (Vacca et al., 2023).

NK cells also serve important immunomodulatory functions. Upon activation, they secrete pro-inflammatory cytokines such as interferon-gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α), and granulocyte-macrophage colony-stimulating factor (GM-CSF), as well as chemokines like CCL3, CCL4, and CCL5 (Vacca) . These signaling molecules recruit and activate additional immune effectors, increasing the breadth and effectiveness of the overall

immune response (Vacca et al., 2023). Recent studies also demonstrate the capacity of NK cells to exhibit memory-like features, a concept that challenges traditional distinctions between innate and adaptive immunity. For example, cytomegalovirus (CMV) infection can increase the expansion of "adaptive" NK cells with increased functional responses upon re-exposure (Vacca et al., 2023). Understanding NK cells is critical for advancing therapeutic strategies against infections and cancers. Many pathogens and tumor cells have evolved mechanisms to evade NK cell recognition or suppress their activity, limiting the effectiveness of natural immune responses. By shedding light on how NK cells detect targets, regulate cytotoxicity, and adapt to repeated challenges, researchers can develop interventions that enhance their effectiveness while preventing off-target tissue damage. This knowledge is especially valuable for improving NK cell immunotherapies, optimizing their persistence and efficacy in clinical areas.

Cytokine Engineering for NK Cell Persistence

Cytokines are small signaling proteins that play a vital role in regulating immune cell development, differentiation, survival, and activation (Vivier et al., 2011; Abel et al., 2018). In NK cells, cytokines such as interleukin (IL)-2, IL-12, IL-15, IL-18, and IL-21 are particularly important for maintaining effector function, proliferation, and cytotoxicity (Waggoner et al., 2021; Liu et al., 2021). Among these, IL-15 plays a uniquely important role in increasing NK cell development, survival, and homeostasis by supporting metabolic fitness and memory-like properties (Fehniger & Caligiuri, 2001; Romee et al., 2016; Zhou et al., 2023). However, in the context of adoptive NK cell therapy, systemic administration of cytokines like IL-15 can result in nontargeted effects, systemic toxicity, and short-lived signaling because of rapid clearance and nonspecific distribution (Tang et al., 2021; Zhou et al., 2023). To address these challenges, researchers are engineering NK cells to express cytokines such as IL-15 internally, allowing for support that increases NK cell persistence, reduces exhaustion, and supports long-term activity in immunosuppressive environments like tumors (Liu et al., 2021; Zhou et al., 2023).

Chimeric Antigen Receptor (CAR) Engineering of NK Cells

Chimeric antigen receptor (CAR) engineering has been an important part of T cell therapies, and now a similar strategy is being applied to NK cells (Zhang & Liu et al., 2020; Rezvani & Rouce, 2015). CAR-NK cells are genetically modified to express synthetic receptors that specifically recognize tumor-associated antigens, triggering NK cell activation and targeted cytotoxicity (Chu et al., 2022; Liu & Zhang et al., 2020). CAR-NK therapies have several advantages, including a reduced risk of cytokine release syndrome (CRS) and graft-versus-host disease (GvHD), making them safer for clinical application (Liu & Zhang et al., 2020; Shimasaki et al., 2020; Tang et al., 2021). Also, NK cells retain their innate cytotoxicity via natural activating receptors, which allows them to kill tumor cells independently of CAR, providing a broader killing capacity (Rezvani et al., 2017; Miao et al., 2024). However, CAR-NK cells have challenges in the immunosuppressive tumor microenvironment, like metabolic exhaustion and inhibitory signals that limit their persistence and antitumor efficacy (Mace et al., 2016; Zhou et al., 2023). To address these issues, current research is focused on combining CAR engineering with additional genetic modifications aimed at enhancing NK cell survival, metabolic fitness, and functional persistence (Liu & Zhang et al., 2021; Zhang et al., 2022).

Table 1. Recent Advancements in CAR-NK Engineering Strategies

	Gene Knockout for NK Cell Memory	**Cytokine Engineering for Metabolic Fitness**	**CISH deletion for Enhanced Responsiveness**
Engineering strategy	Gene knockout of BCL2 and FLI1 using CRISPR to study survival and memory in NK cells.	CAR-engineering of NK cells and IL-15 cytokine to improve metabolic fitness and persistence.	Knockout of CISH to increase NK cell cytokine responsiveness through CRISPR.
Model/Technique	Mouse model (MCMV infection), adoptive transfer of Ly49H^{+} NK cells, scRNA-seq, ATAC-seq, CRISPR.	In vitro assays and in vivo mouse tumor models; metabolic profiling; mitochondrial analysis.	Cord blood–derived NK cells; in vitro killing assays; in vivo tumor xenografts in mice.
Efficacy Outcomes	BCL2 is important for NK cell survival; FLI1 represses memory NK formation because its deletion improves persistence.	IL-15–expressing CAR-NK cells showed better function of the mitochondria and better tumor control.	CISH-deleted NK cells showed greater expansion, stronger cytotoxicity, and longer persistence.

Safety	No safety concerns were in the study.	Improved safety in the CAR-NK cells vs. CAR-T; no major cytokine release or toxicity in preclinical models.	No toxicity in preclinical models.
Key outcomes	Identified mechanisms in NK cell memory formation, found that BCL2 is important for NK cell survival, and that FLI1 acts as a repressor of NK cell formation and memory.	The IL-15 improved NK survival, metabolism, and antitumor efficacy.	Removing internal gene checkpoints like CISH increases NK cell fitness and tumor-killing capacity.

In Study A, researchers examined the molecular mechanisms crucial for the survival and differentiation of natural killer (NK) cells during viral infection, with a focus on identifying key factors that influence the formation of memory-like NK cells (Riggan et al, 2022). Using single-cell RNA sequencing, ATAC-seq, CRISPR gene editing, and mouse models, the researchers identified that a subset of NK cells, specifically $Ly6C^-$ NK effector cells, had better survival and are the main precursors of memory NK cells. These cells were found to express higher levels of the anti-apoptotic protein BCL2, promoting cell survival. In contrast, the transcription factor FLI1, regulated by STAT5 signaling, was shown to repress NK cell persistence by upregulating the pro-apoptotic protein BIM. The deletion of FLI1 through CRISPR improved NK cell survival and expansion, suggesting it acts as a negative regulator of memory NK cell formation.

While transcription factors play a role in NK cell survival, other research has focused on external factors like cytokines to boost NK cell function. In another study, scientists focused on increasing the metabolic fitness of CAR-engineered NK cells by adding the cytokine IL-15, which is how well the body can produce energy, utilize nutrients, and regulate hormones. CAR-engineered NK cells are natural killer cells that have been genetically modified to express chimeric antigen receptors (CARs) on their surface (Does Reis). These artificial receptors allow NK cells to better recognize and bind to specific proteins on cancer cells, which aids their ability to target and kill tumors more effectively. This technique helps improve NK cell responses against cancers. Cytokines are small proteins that act as signaling molecules in the immune

system; they help immune cells grow, communicate, and respond to any attacks. In this study, researchers engineered NK cells to produce IL-15 themselves, giving them a constant supply of cytokines to properly support them. This helps prevent metabolic exhaustion, which is when cells become weak or inactive because they run out of energy or nutrients, especially when they are in environments like tumors. The IL-15-expressing NK cells had healthier mitochondria (the part of the cell that produces energy) and showed better performance in lab tests and mouse models. This way of increasing NK activity shows that helping NK cells maintain their energy production can make them last longer and be more effective at combating cancer.

Another study took a different approach by focusing on a gene called CISH (Cytokine-Inducible SH2-containing protein), which regulates how NK cells respond to cytokine signals. CISH suppresses or limits the signaling pathways inside NK cells that increase their activation, growth, and survival. By reducing the strength of these signals, CISH helps prevent excessive immune responses, but it can also reduce the ability of NK cells to effectively attack cancer cells (Bernard, Pierre-Louis et al, 2022). When cytokines like IL-15 bind to NK cells, they send signals to activate the cell, but CISH works to turn those signals down. The researchers in this study used CRISPR, a gene editing tool, to remove CISH in cord blood-derived NK cells. This deletion made the cells more responsive to cytokines, activating pathways like mTOR and Akt, which support cell growth and survival. These NK cells expanded more, killed tumor cells more effectively, and survived longer without causing harmful side effects. This method improves NK cell function from the inside by removing limits to their activation.

Discussion and Conclusion

The various strategies explored for enhancing NK cell activity each present unique advantages in the pursuit of more effective immunotherapies. Among these, a synergistic approach combining IL-15 engineering and CISH deletion appears to offer the most promising path to fully maximize NK cell potential. It gives them external support from outside signals like cytokines, while also making the cells more receptive from within by deleting CISH. IL-15 engineering provides critical extrinsic support, bolstering NK cell survival and metabolism, while CISH deletion intrinsically enhances the cells' responsiveness to existing cytokine signals, overcoming inherent inhibitory mechanisms. This dual approach addresses both external and internal limitations on NK cell function. While approaches targeting transcription factors, such as FLI1 deletion, hold promise for inducing long-term, intrinsic changes in NK cell memory and persistence, they may also carry a higher risk of off-target genetic modifications. In contrast, IL-15 engineering and CISH deletion offer a more direct and potentially simpler path to immediate functional enhancement, making them particularly attractive for acute or short-term therapeutic interventions. However, the synergistic combination of these two methods could potentially bridge the gap between short-term efficacy and sustained therapeutic benefit. Combining CISH deletion and IL-15 engineering would provide the best results by creating NK cells that are engineered to express IL-15 and edited to remove CISH. This could lead to NK cells that are very active and resistant to exhaustion, in the future leading to improved immunotherapies.

Citations:

Mace, Emily M. “Human natural killer cells: Form, function, and development.” The Journal of allergy and clinical immunology vol. 151,2 (2023): 371-385. doi:10.1016/j.jaci.2022.09.022

Lanier, Lewis L., et al. "Natural killer cells: definition of a cell type rather than a function." The Journal of Immunology 137.9 (1986): 2735-2739.

Rebuffet, Lucas, et al. "High-dimensional single-cell analysis of human natural killer cell heterogeneity." Nature immunology 25.8 (2024): 1474-1488.

Riggan L, Ma F, Li JH, Fernandez E, Nathanson DA, Pellegrini M, O'Sullivan TE. The transcription factor Fli1 restricts the formation of memory precursor NK cells during viral infection. Nat Immunol. 2022 Apr;23(4):556-567. doi: 10.1038/s41590-022-01150-0. Epub 2022 Mar 14. PMID: 35288713; PMCID: PMC8989647.

Zheng, Liangtao, et al. "Pan-cancer single-cell landscape of tumor-infiltrating T cells." Science 374.6574 (2021): abe6474.

Sohn, Hyogon, and Megan A Cooper. “Metabolic regulation of NK cell function: implications for immunotherapy.” Immunometabolism (Cobham, Surrey) vol. 5,1 e00020. 23 Jan. 2023, doi:10.1097/IN9.0000000000000020

Dos Reis, Filipa D et al. “CAR-NK cells: harnessing the power of natural killers for advanced cancer therapy.” Frontiers in immunology vol. 16 1603757. 30 May. 2025, doi:10.3389/fimmu.2025.1603757

Baughan SL, Folsom T, Johnson M, Kreuger J, Webber B, Moriarity BS. Perspective: IL-15 cytokine-armored NK cells as ready-to-use immunotherapy for diverse malignancies: therapeutic potential and toxicity risks. Front Immunol. 2025 Nov 19;16:1704404. doi: 10.3389/fimmu.2025.1704404. Erratum in: Front Immunol. 2026 Jan 15;17:1779800. doi: 10.3389/fimmu.2026.1779800. PMID: 41346590; PMCID: PMC12672865.

Bernard PL, Delconte R, Pastor S, Laletin V, Costa Da Silva C, Goubard A, Josselin E, Castellano R, Krug A, Vernerey J, Devillier R, Olive D, Verhoeyen E, Vivier E, Huntington ND, Nunes J, Guittard G. Targeting CISH enhances natural cytotoxicity receptor signaling and reduces NK cell exhaustion to improve solid tumor immunity. J Immunother Cancer. 2022 May;10(5):e004244. doi: 10.1136/jitc-2021-004244. PMID: 35589278; PMCID: PMC9121483.

Vacca, Paola, et al. "Origin, phenotype and function of human natural killer cells in pregnancy." Trends in immunology 32.11 (2011): 517-523.

Mace EM. Human natural killer cells: Form, function, and development. J Allergy Clin Immunol. 2023 Feb;151(2):371-385. doi: 10.1016/j.jaci.2022.09.022. Epub 2022 Oct 1. PMID: 36195172; PMCID: PMC9905317.

Chen, S., Zhu, H. & Jounaidi, Y. Comprehensive snapshots of natural killer cells functions, signaling, molecular mechanisms and clinical utilization. Sig Transduct Target Ther 9, 302 (2024). https://doi.org/10.1038/s41392-024-02005-w

Solimando, A., Bilotta, M. T., Desantis, V., Tumino, N., & Vacca, P. (2025). Progressive NK cell dysfunction and ILC imbalance as drivers of immune evasion in multiple myeloma. Journal of Clinical Oncology, 43(16_suppl). https://doi.org/10.1200/jco.2025.43.16_suppl.e19557

Ochayon DE, Waggoner SN. The Effect of Unconventional Cytokine Combinations on NK-Cell Responses to Viral Infection. Front Immunol. 2021 Mar 19;12:645850. doi: 10.3389/fimmu.2021.645850. PMID: 33815404; PMCID: PMC8017335.

Vivier E, Raulet DH, Moretta A, Caligiuri MA, Zitvogel L, Lanier LL, Yokoyama WM, Ugolini S. Innate or adaptive immunity? The example of natural killer cells. Science. 2011 Jan 7;331(6013):44-9. doi: 10.1126/science.1198687. PMID: 21212348; PMCID: PMC3089969.

Abel, A.M., et al. (2018) Natural Killer Cells: Development, Maturation, and Clinical Utilization. Frontiers in Immunology, 9, 1869. https://doi.org/10.3389/fimmu.2018.01869

Liu S, Galat V, Galat Y, Lee YKA, Wainwright D, Wu J. NK cell-based cancer immunotherapy: from basic biology to clinical development. J Hematol Oncol. 2021 Jan 6;14(1):7. doi: 10.1186/s13045-020-01014-w. PMID: 33407739; PMCID: PMC7788999.

Cooper MA, Fehniger TA, Caligiuri MA. The biology of human natural killer-cell subsets. Trends Immunol. 2001 Nov;22(11):633-40. doi: 10.1016/s1471-4906(01)02060-9. PMID: 11698225.

Romee R, Rosario M, Berrien-Elliott MM, Wagner JA, Jewell BA, Schappe T, Leong JW, Abdel-Latif S, Schneider SE, Willey S, Neal CC, Yu L, Oh ST, Lee YS, Mulder A, Claas F, Cooper MA, Fehniger TA. Cytokine-induced memory-like natural killer cells exhibit enhanced responses against myeloid leukemia. Sci Transl Med. 2016 Sep 21;8(357):357ra123. doi: 10.1126/scitranslmed.aaf2341. PMID: 27655849; PMCID: PMC5436500.

Zhou Y, Cheng L, Liu L, Li X. NK cells are never alone: crosstalk and communication in tumour microenvironments. Mol Cancer. 2023 Feb 16;22(1):34. doi: 10.1186/s12943-023-01737-7. PMID: 36797782; PMCID: PMC9933398.

Tang E, Chen JY. Shaping the glioblastoma microenvironment to enhance CAR-NK immunotherapy. Front Immunol. 2025 Dec 1;16:1681966. doi: 10.3389/fimmu.2025.1681966. PMID: 41403931; PMCID: PMC12702947.

Zhang C, Liu Y. Targeting NK Cell Checkpoint Receptors or Molecules for Cancer Immunotherapy. Front Immunol. 2020 Jun 23;11:1295. doi: 10.3389/fimmu.2020.01295. PMID: 32714324; PMCID: PMC7344328.

Rezvani K, Rouce RH. The Application of Natural Killer Cell Immunotherapy for the Treatment of Cancer. Front Immunol. 2015 Nov 17;6:578. doi: 10.3389/fimmu.2015.00578. PMID: 26635792; PMCID: PMC4648067.

Chu J, Gao F, Yan M, Zhao S, Yan Z, Shi B, Liu Y. Natural killer cells: a promising immunotherapy for cancer. J Transl Med. 2022 May 23;20(1):240. Doi: 10.1186/s12967-022-03437-0. PMID: 35606854; PMCID: PMC9125849.

Shimasaki N, Jain A, Campana D. NK cells for cancer immunotherapy. Nat Rev Drug Discov. 2020 Mar;19(3):200-218. doi: 10.1038/s41573-019-0052-1. Epub 2020 Jan 6. PMID: 31907401.

Rezvani K, Rouce R, Liu E, Shpall E. Engineering Natural Killer Cells for Cancer Immunotherapy. Mol Ther. 2017 Aug 2;25(8):1769-1781. doi: 10.1016/j.ymthe.2017.06.012. Epub 2017 Jun 28. PMID: 28668320; PMCID: PMC5542803.

Miao L, Lu C, Zhang B, Li H, Zhao X, Chen H, Liu Y, Cui X. Advances in metabolic reprogramming of NK cells in the tumor microenvironment on the impact of NK therapy. J Transl Med. 2024 Mar 3;22(1):229. doi: 10.1186/s12967-024-05033-w. PMID: 38433193; PMCID: PMC10909296.

Mace, E., Gunesch, J., Dixon, A. et al. Human NK cell development requires CD56-mediated motility and formation of the developmental synapse. Nat Commun 7, 12171 (2016). https://doi.org/10.1038/ncomms12171

Zhang H, Yang L, Wang T, Li Z. NK cell-based tumor immunotherapy. Bioact Mater. 2023 Aug 9;31:63-86. doi: 10.1016/j.bioactmat.2023.08.001. PMID: 37601277; PMCID: PMC10432724.