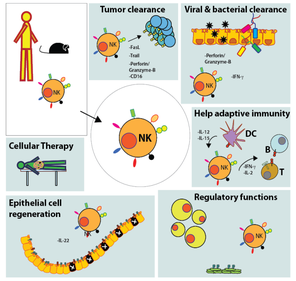
# Transcriptional profiling of Natural Killer Cells Reveals Dysfunction and Immune Suppression in Metastatic HNSCC

**Mohamed Khalil[1,2,3], Elaheh Hashemi[1,2], Abdullah Memon[3], Joseph Zenga[3], Jennifer Bruening[3], Subramaniam Malarkannan[1,2,3]**

[1]Laboratory of Molecular Immunology and Immunotherapy, Blood Research Institute, Versiti, Milwaukee, WI. [2]Department of Microbiology & Immunology, Medical College of Wisconsin, Milwaukee, WI. [3]Department of Otolaryngology, Medical College of Wisconsin, Milwaukee, WI.

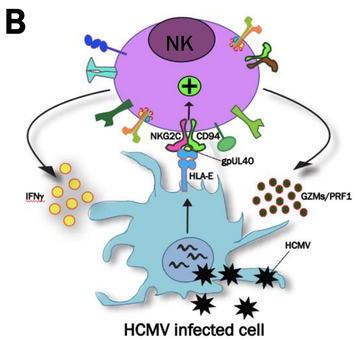
## BACKGROUND

A




- **Natural Killer** (NK) cells serve as the hosts initial defense against **viral infections** and **tumor** clearance
- NK cells have gained significant popularity in various **cellular immunotherapies**

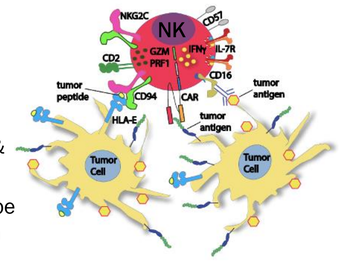
B




- **NKG2C⁺ NK cells** respond to human cytomegalovirus (HCMV) infections in a memory-like response
- Activated NKG2C⁺ NK cells undergo robust clonal expansion, forming a reservoir of **long-lived NKG2C⁺ memory cells**

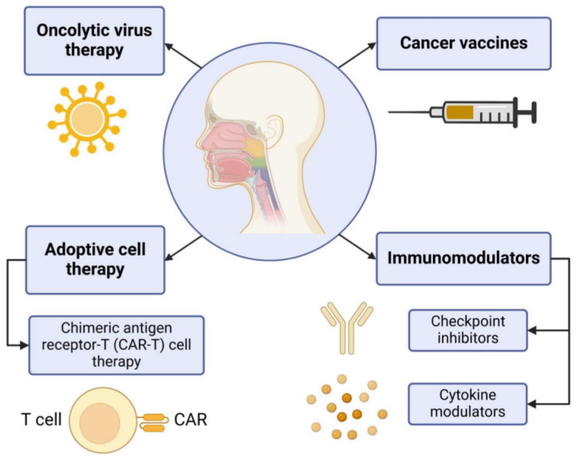
C

- Developing a Memory NK "**super solider**" to target tumor cells via various mechanisms
- **Memory NK cells** can target tumor cells via their activating CD16 & NKG2C receptors
- Memory NK cells can be **safely administered** in the allogenic setting without the risk of graft versus host disease




## KNOWLEDGE GAPS

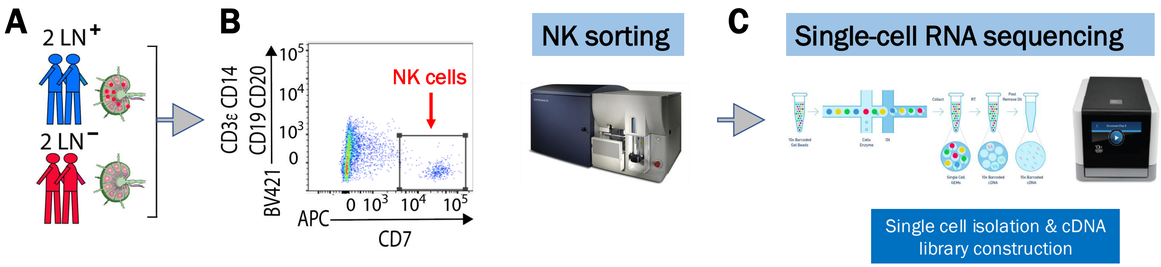
**Novel immunotherapy agents for head and neck cancers**




Khalid et al., Advances and challenges in immunotherapy in head and neck cancer. *Frontiers in Immunology* 2025 Jun 6;16:1596583 PMCID: PMC 12179090

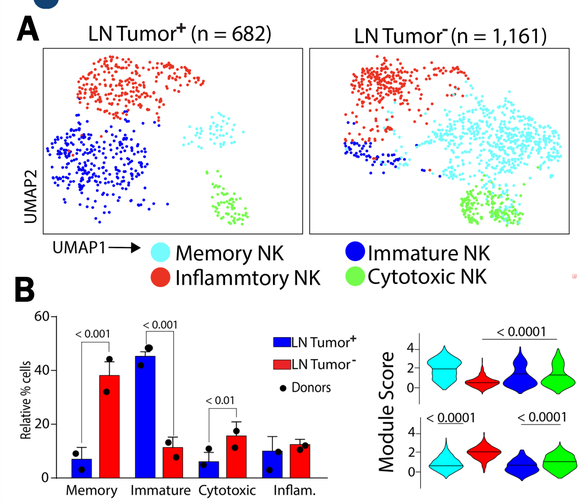
- **Oncolytic viruses** selectively infect and replicate within cancer cells. An FDA-approved example is T-VEC, a modified herpes simplex virus used to treat melanoma. While effective in controlling local lesions, T-VEC has not demonstrated improved overall survival.
- **Cancer vaccines** represent another immunotherapy strategy. They can be administered prophylactically, such as the HPV vaccine, to prevent cancer development. They can also be given therapeutically, training the immune system to recognize and target tumor-specific antigens.
- **Immunomodulators** are agents that alter the activity of the immune system to enhance or suppress immune responses. Recent evidence from KEYNOTE-689 demonstrated an overall survival benefit of pembrolizumab when used in the neoadjuvant setting for locally advanced head and neck cancers.
- **Adoptive cell therapy** such as CAR-T, have been trialed in head and neck cancers. A key limitation of CAR-T therapy is its reliance on the patient's own T cells, which are often depleted or dysfunctional after multiple rounds of chemotherapy and radiation. Memory NK cells can overcome this limitation, as healthy donor NK cells can be administered safely in the allogeneic setting.

## METHODS




- **Figure A.** Lymph node specimens were obtained from four adult patients with primary head and neck squamous cell carcinoma at the Department of Otolaryngology, Medical College of Wisconsin. Two patients had positive lymph nodes (LN⁺) and two had negative lymph nodes (LN⁻).
- **Figure B:** Lymph node specimens were processed into single-cell suspensions, and CD7⁺ NK cells were isolated and sorted using the gating strategy CD3ε/CD14/CD19/CD20⁻ CD7⁺ to capture all NK cells, including early progenitor cells.
- **Figure C:** NK cells (CD3ε/CD14/CD19/CD20⁻ CD7⁺) from lymph node specimens were then utilized to perform single-cell RNA sequencing via the 10x genomic protocol. Sequencing data were aligned with the Cell Ranger pipeline and analyzed using multiple bioinformatic tools to assess transcriptional profiles.

## RESULTS




- **Figure A.** UMAP plot displaying unbiased clustering of lymph node NK cells (n = 4). Each dot represents a cell, and the color represents the transcriptionally unique NK cell subset to which the cell belongs. Number of recovered cells is displayed by the n value. Four unique NK clusters identified.
- **Figure B.** Relative percent of each defined NK cell subset. The input number by each donor is normalized to be equal.
- **Figure C.** Module scores were calculated using both upregulated DEGs and other NK subsets, with 10-20 genes used to calculate each module score.

## Future directions

Our future work will focus on expanding the sample size and validating our scRNA-seq findings at the protein level. In addition, we plan to perform ChIP-seq and ATAC-seq on Memory NK cell population to further elucidate the epigenetic mechanisms regulating their function and anti-tumor response.

## Acknowledgements

This work was supported in part by the National Center for Advancing Translational Sciences, National Institutes of Health, Grant Number TL1 TR001437. NIH grants R01 A1064828, R01 AI102893; NCI grant R01 CA179363 (S.M.), and Nicholas Family Foundation. We thank the MCW Deparment of Otolaryngology for providing travel funds. The content is solely the responsibility of the author(s) and does not necessarily represent the official views of the NIH.