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## INTRODUCTION

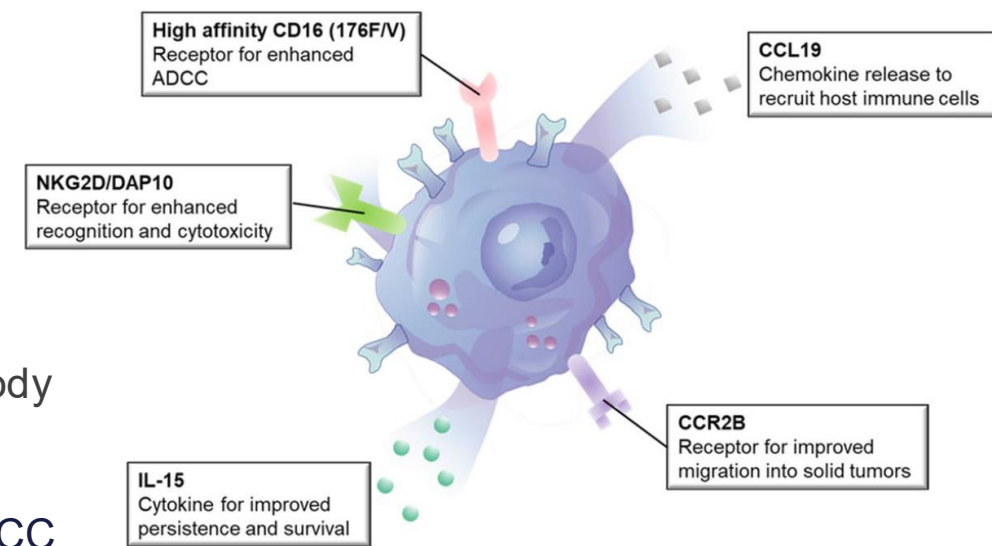
Uveal melanoma (UM) preferentially metastasizes to the liver, leading to a poor prognosis. The immunosuppressive microenvironment of the liver, the low mutation burden and specific driver mutations of metastatic UM (MUM) contribute to the limited efficacy of immunotherapies, including immune checkpoint inhibitors and adoptive T-cell transfers. Existing evidences highlight the crucial role of Natural Killer (NK) cells in monitoring and targeting circulating MUM cells, suggesting that therapeutic strategies that enhance NK cell activity may offer clinical benefits. Donor variability and limited expansion capacity restricted clinical application of primary NK cells. NK cells derived from induced pluripotent stem cells (iPSCs) present distinct benefits, especially in their ability to be genetically modified to produce cells with improved antitumor characteristics. Therefore, we investigated the antitumor activity of iPSC-NK cells against MUM.

## METHODS

eNK is being developed as AKT-01/HLCN061 through a collaboration between Akatsuki Therapeutics Inc. and Healios K.K.<sup>1,2</sup>. The functions of iPSC-NK cells were enhanced by gene editing, resulting in the production of engineered iPSC-NK cells (eNK):

### In vitro activities of eNK cells:

- Intracellular cytokine staining
- CD107a degranulation assay
- Transwell migration assay
- Cytotoxic assay
- ADCC assay with anti-GD2 antibody



### Cell lines used:

- NK92 cell line purchased from ATCC
- UM001 and UM004- MUM cell line established at TJU
- K562 – erythroleukemic cells that are highly sensitive to NK cell-mediated lysis purchased from ATCC

## RESULTS

Figure 1. Expression of transgene proteins in eNK cells

Expression of the transgene receptors as determined by FACS\*

	NKG2D	CD16	CCR2B
NK92	96.1	0.94	1.26
eNK	29.2	85.98	94.98

\*% positive cells

Secretion of soluble mediators as determined by ELISA\*

	IL15	CCL19
NK92	0	0
eNK	315	20

\* pg/ml

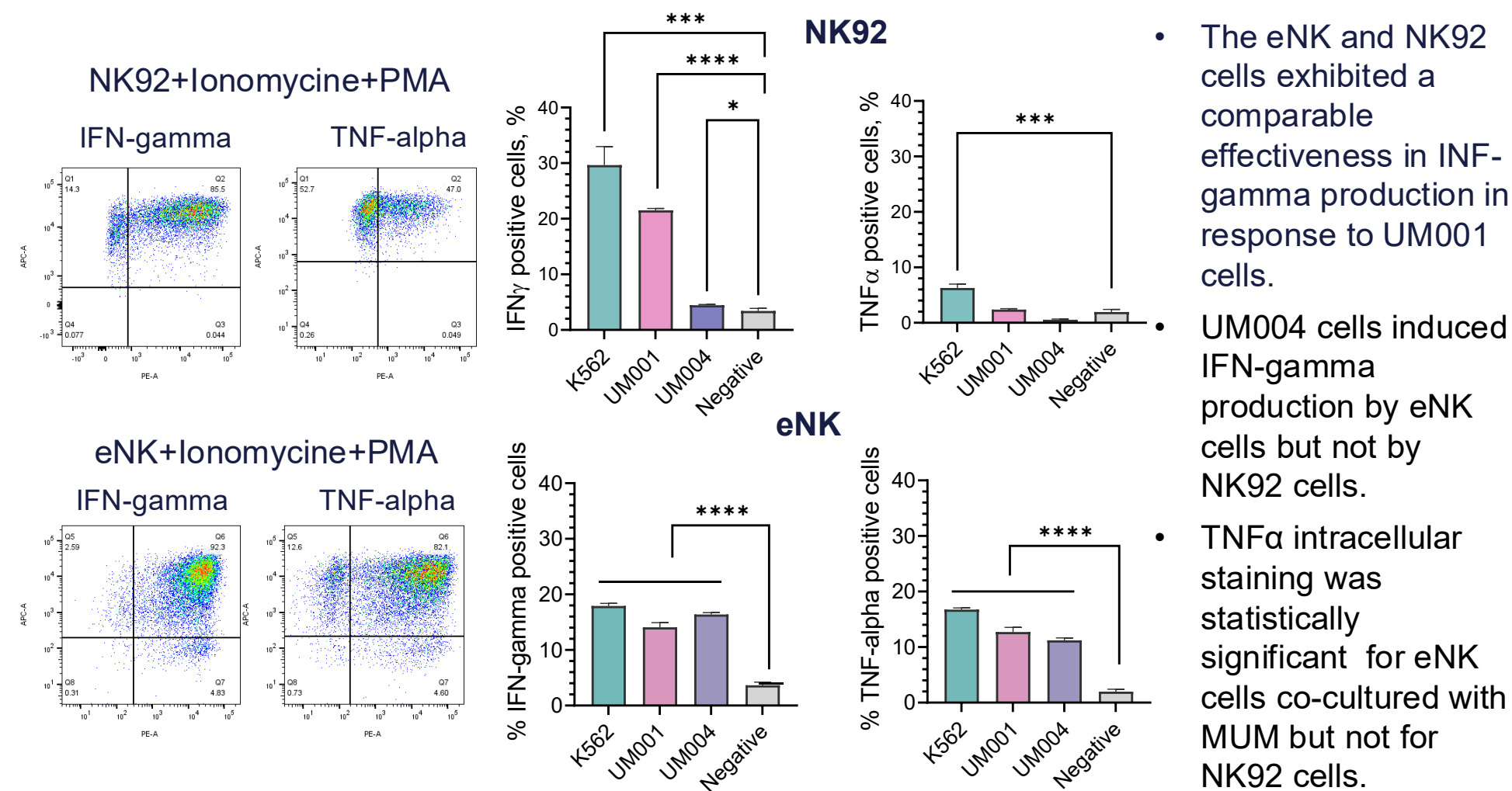
- eNK cells produced all transgene proteins introduced at iPSC stage

## ACKNOWLEDGMENTS

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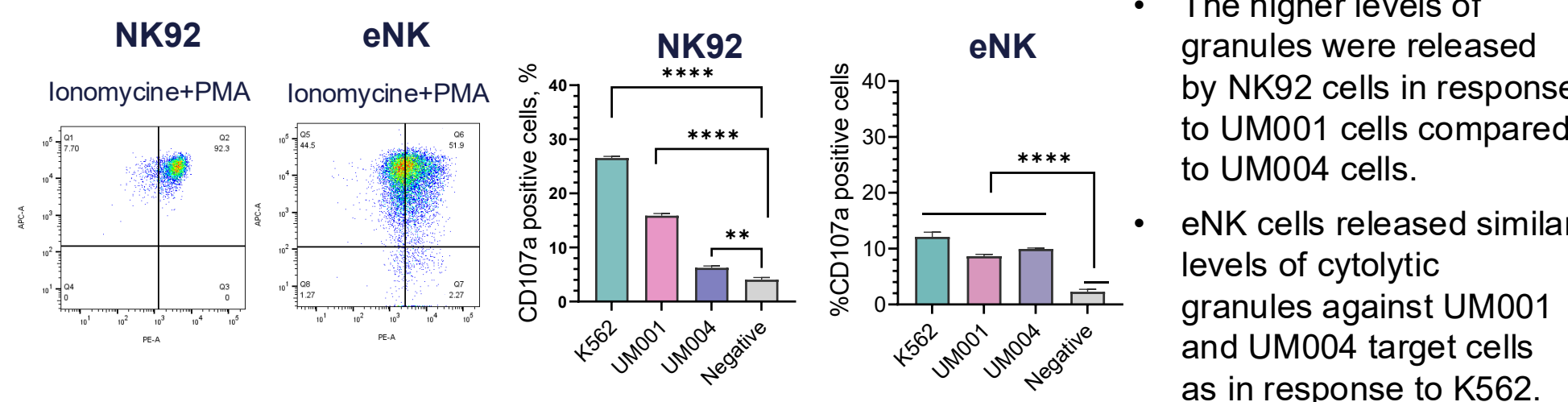
## RESULTS

Figure 2. eNK cell cytokine production



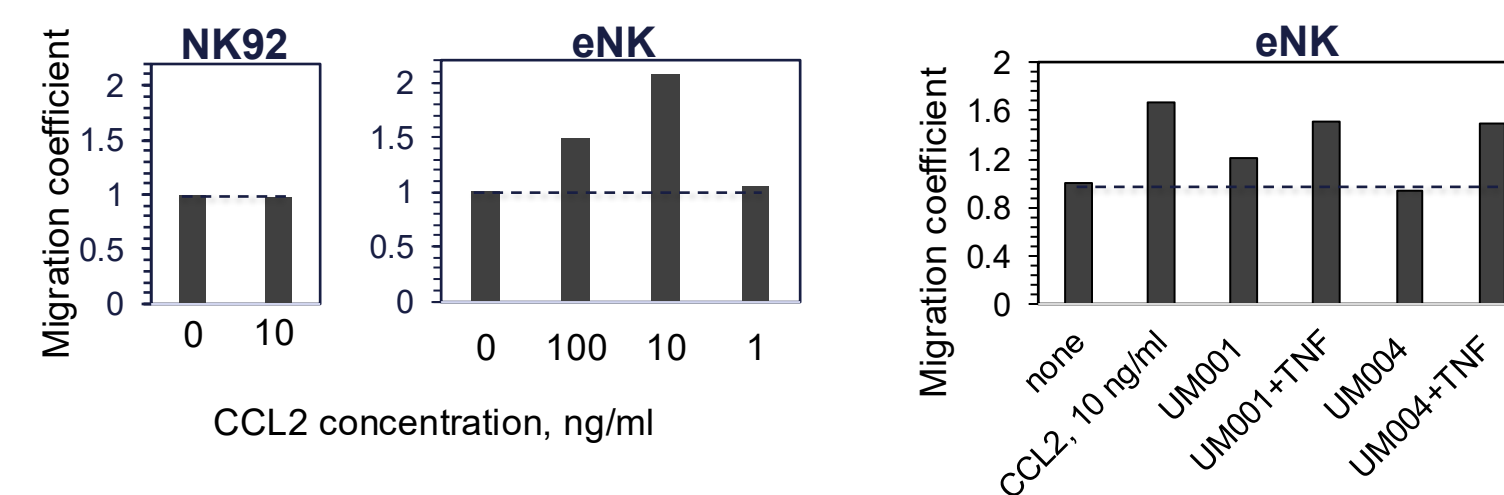
- The eNK and NK92 cells exhibited a comparable effectiveness in INF-gamma production in response to UM001 cells.
- UM004 cells induced IFN-gamma production by eNK cells but not by NK92 cells.
- TNF $\alpha$  intracellular staining was statistically significant for eNK cells co-cultured with MUM but not for NK92 cells.

Figure 3. eNK cell cytolytic granule release



- The higher levels of granules were released by NK92 cells in response to UM001 cells compared to UM004 cells.
- eNK cells released similar levels of cytolytic granules against UM001 and UM004 target cells as in response to K562.

Figure 4. NK cell migration assay

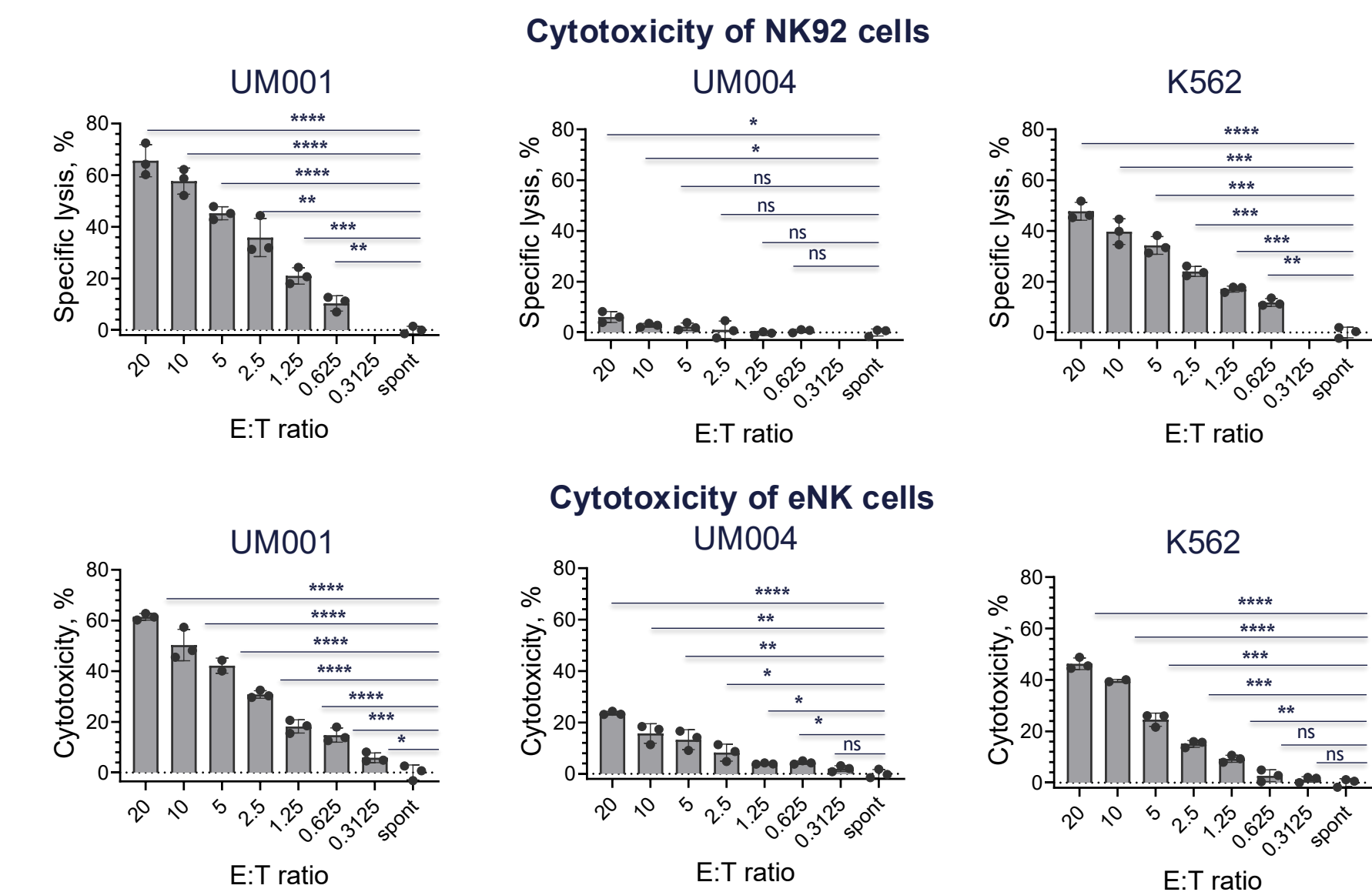


- eNK cells but not NK92 migrated toward CCL2
- eNK cells exhibited enhanced migration into chambers containing supernatants from TNF $\alpha$ -stimulated UM001 or UM004 cells

## REFERENCES

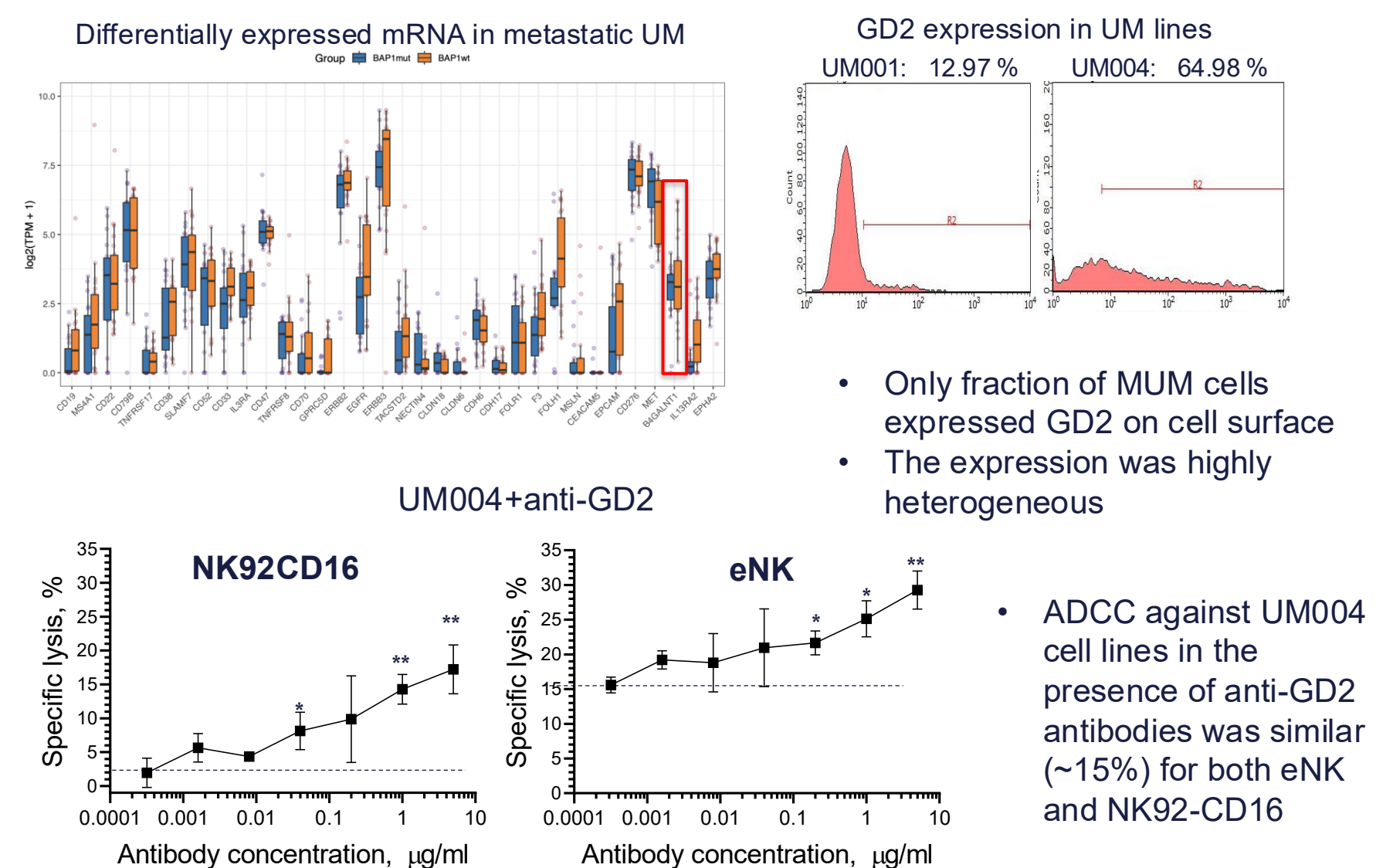
- Nakamura et al. Antitumor effects of natural killer cells derived from gene-engineered human-induced pluripotent stem cells on hepatocellular carcinoma. *Cancer Immunology, Immunotherapy* (2025) 74:99.
- Fukutani et al. Human iPSC-derived NK cells armed with CCL19, CCR2B, high-affinity CD16, IL-15, and NKG2D complex enhance anti-solid tumor activity. *Stem Cell Research & Therapy* (2025) 16:373.

Figure 5. NK cell cytotoxic assay



- The eNK and NK92 cells exhibited a comparable effectiveness in targeting UM001 cells.
- NK92 cells showed negligible cytolytic activity against UM004 cells.
- In contrast, eNK cells induced approximately 25% tumor cells killing, indicating greater cytotoxic efficacy.

Figure 6. NK cell ADCC



- Only fraction of MUM cells expressed GD2 on cell surface
- The expression was highly heterogeneous

- ADCC against UM004 cell lines in the presence of anti-GD2 antibodies was similar (~15%) for both eNK and NK92-CD16

## SUMMARY

**eNK cells have enhanced activity against UM cells, showing potential to improve the adoptive NK cell therapy of metastatic UM.**