197 NOVEL FCYR RECOMBINANT FUSION FACILITATES ANTIBODY ARMING OF ENGINEERED IPSC-DERIVED NK CELLS TO ENHANCE TARGETING AND KILLING OF OVARIAN CANCER CELLS

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Background Ovarian cancer is a leading cause of cancer-related deaths among women due to the development of therapeutic resistance. Natural killer (NK) cells are cytotoxic lymphocytes that can kill neoplastic cells without prior sensitization. A key anti-tumor function of human NK cells is antibody-dependent cell-mediated cytotoxicity (ADCC), mediated exclusively by the IgG Fcy (FcyR) receptor CD16A. The mechanism of action of several clinically successful antitumor therapeutic monoclonal antibodies (mAbs) involves ADCC; however, their therapeutic efficacy is reduced due to regulatory checkpoints of CD16A, which include its low IgG binding affinity and rapid downregulation upon NK cell activation by the membrane metalloprotease ADAM17.[1–3] CD64, the highest affinity FcyR, is expressed on myeloid-derived cells and not lymphocytes and is also not cleaved by ADAM17. To enhance ADCC, we generated CD64/16A, a novel recombinant FcyR consisting of extracellular CD64 and intracellular and transmembrane CD16A to mediate high affinity IgG binding and potent cell signaling.[4 5]

Methods Engineered NK cells expressing CD64/16A were derived from human induced pluripotent stem-cells (iPSCs), referred to here as iNKs, which are clonal and clinically scalable NK cells. ADCC and natural cytotoxicity of three ovarian cancer cell lines were measured in vitro using Delfia EuTDA and IncuCyte cytotoxicity assays, and production of anti-tumor cytokines was determined via flow cytometry. Finally, tumor cell killing was assessed in vivo using a human xenograft mouse model of peritoneal metastatic ovarian cancer.

Results Our data show that iNK-CD64/16A cells uniquely facilitate mAb absorption, robustly produce IFN-y and TNF-alpha, and kill several ovarian cancer cell lines in the presence of the therapeutic mAbs trastuzumab or cetuximab, which target HER2 or EGFR, respectively. We found that iNK-CD64/16A cells can capture soluble mAbs and retain antibody arming and ADCC efficacy after cryopreservation. Additionally, iNK-CD64/16A cells robustly mediate ADCC and reduce overall tumor burden of HER2+ tumor cells in vivo in the described metastatic ovarian cancer xenograft model.

Conclusions Our findings provide new insights into using high affinity Fc receptor-based adoptive NK cell therapies and lay the preclinical foundation necessary for developing an 'off-the-shelf' cellular therapy that can be combined with therapeutic tumor-targeting mAbs for the treatment of ovarian cancer. Importantly, iNK-CD64/16A cells can serve as a docking platform for therapeutic antibodies that can be switched and mixed for universal tumor antigen targeting to treat multiple malignancies.

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Ethics Approval This study was approved by the University of Minnesota's Institutional Animal Care and Use Committee, protocol number 1902–36768A.

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