Title: Cytotoxic Potential of Ascites-Derived NK Cells in Ovarian Cancer: Implications for Immunotherapy

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Ovarian cancer (OC) is the deadliest gynecologic malignancy worldwide, with a 5-year survival rate of only 37%. As infiltration of certain immune subtypes, including Natural Killer (NK) cells, is associated with improved survival of OC patients, immunotherapy emerges as a promising therapeutic approach. To better understand the potential of NK cells in this context, fluid accumulation in the peritoneal cavity (ascites) of OC patients provides a valuable resource for studying tumor-associated immune cells. Within the ascites, immune populations are known to be nearly identical to those in the primary tumor, allowing for assessment of endogenous, antitumor NK cells without tumor biopsies. We compared the cytotoxic capabilities of ascites-derived NK cells to NK cells derived from the blood of matched patients and from healthy donor controls. Contrary to existing literature describing ascites-derived NK cells as exhausted and inactive, we found their cytotoxic activity against OVCAR8 cells to significantly surpass (by 2-fold) that of NK cells from blood of matched patient and healthy donor controls. To investigate potential drivers of this enhanced cytotoxicity, we performed mass cytometry to characterize differences in protein expression. This analysis revealed significant alterations in several receptors, including decreased PD1 and increased expression of NKG2D and CD2 on ascites-derived NK cells. Correspondingly, OVCAR8 cells exhibit high RNA expression of PDL1, MICA/MICB, and CD58—the respective ligands for these receptors—suggesting that these interactions may contribute to the heightened cytotoxic activity of ascites-derived NK cells. Based on these findings, we hypothesize that targeting these receptor-ligand interactions represents a viable strategy to enhance NK cell-mediated antitumor responses in OC patients. Current efforts are focused on developing novel engager molecules designed to activate NK cells within the ascites and primary tumor by leveraging receptors identified as contributing to enhanced cytotoxicity, with future studies planned to evaluate their therapeutic efficacy preclinically.