SYNERGISTIC APPROACH TO OVERCOME THE SOLID TUMOR MICROENVIRONMENT OF MESOTHELIOMA WITH NATURAL KILLER CELL-FOCUSED IMMUNOTHERAPY

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**Background** Mesothelioma is a rare, but aggressive cancer that occurs in cells that surround internal organs. Immune checkpoint inhibitors (ICI) have been approved for the treatment of mesothelioma (nivolumab, ipilimumab and pembrolizumab), but currently approved strategies do not make use of natural killer (NK) cell mediated antibody-dependent cellular cytotoxicity (ADCC) of mesothelioma cells. We hypothesized that combining IL-15 treatment with an anti-PDL1 ICI that drives ADCC will enhance NK cell control of mesothelioma and lead to more robust immune control of the disease.

**Methods** In vitro assays challenged NK cells with three pleural mesothelioma lines, H2373, H2461 and H2596 and four peritoneal mesothelioma lines, ROB, YOU, HAY and ORT. Flow cytometry was used to assess degranulation and cytokine production by NK cells co-cultured with mesothelioma cells in short-term assays (5 hours). The tumor cells were treated overnight with IFNγ to mimic the inflammatory tumor micro-environment. Natural cytotoxicity was compared with ICI that do not drive ADCC (pembrolizumab), ICI that drive ADCC (avelumab) and IL-15, alone or in combination. Live cell imaging was used to track mesothelioma survival in three dimensional spheroids over 5 days when treated with NK cells and these drugs.

**Results** Mesothelioma lines showed a range of sensitivity to NK cell natural cytotoxicity, with H2373 cells being very resistant and H2596 being more sensitive. More resistant cells tended to have more HLA class I at the cell surface, which can inhibit NK cell function. IFNγ treatment increased the abundance of PDL1 and HLA class I on mesothelioma lines. Lines that lacked PDL1 and were resistant to avelumab treatment (ROB and YOU), were rendered sensitive by increased surface PDL1 following IFNγ treatment. However, lines that were already sensitive to avelumab treatment showed reduced NK cell responses with IFNγ (H2373 and ORT). In spheroid assays, H2596 and H2461, but not H2373, showed synergy between avelumab and IL-15 (figure 1).

**Conclusions** This study demonstrates a novel synergistic approach to treatment of mesothelioma, but variation in mesothelioma sensitivity to the combination of IL-15 and avelumab suggests other factors beyond PDL1 abundance are contributing to NK cell responses. Greater abundance of HLA class I decreased NK cell responses in our system, but would be beneficial for endogenous T cell responses. Optimizing for both the adaptive and innate arms of the immune response has the potential to limit tumor escape and enhance mesothelioma immunotherapy.

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**Ethics Approval** This study was deemed not to constitute human research as determined by the University of Minnesota Institutional Review Board.

**Abstract 465 Figure 1** NK cells with anti-PDL1 and IL-15 decrease the survival time of H2596 spheroids. Live imaging tracks the survival of three-dimensional spheroids made from GFP+ mesothelioma cells over 5 days. The relative two-dimension area observed in widefield microscopy images is plotted against time for one donor, representative of at least five donors.