Background Mesothelioma is a rare but deadly malignancy of cells lining internal organs. It is most commonly found in the pleura (cells lining the lung), but also in the peritoneum. Between 2015 and 2019, half of all mesotheliomas diagnosed were at a distant site, where five-year survival was as low as 9%. Immunotherapy is emerging as a viable systemic therapy both as a first-line and second-line treatment, but there is still room for improvement. We hypothesized that natural killer (NK) cells could be mobilized to contribute to immune control. We set out to test our Tri-specific Killer Engager (TriKE®) platform in the context of mesothelioma. TriKEs consist of a single chain variable fragment (scFv) or camelid nanobody targeting a tumor antigen and camelid nanobody targeting Fc receptor CD16 on NK cells, linked by an IL-15 moiety. We tested two different TriKEs, one targeting mesothelin (cam1615SS1), commonly found on epithelioid mesothelioma, and a second TriKE targeting B7H3 (GTB-5550), a common tumor antigen.

Methods Three pleural mesothelioma lines, H2373, H2596 (both sarcomatoid) and H2461 (epithelioid), and four peritoneal mesothelioma lines, ROB, YOU, HAY and ORT (all epithelioid), were assessed for mesothelin and B7H3 surface expression by flow cytometry. NK cells were isolated from human blood and challenged with these lines in the presence of TriKE or IL-15 as a control. NK cell degranulation and cytokine production were assessed by flow cytometry and tumor cell control was assessed by live cell imaging in three dimensional spheroid cultures.

Results The peritoneal mesothelioma lines had detectable mesothelin and cam1615SS1 induced NK cell degranulation and cytokine responses against these cells. The pleural mesothelioma lines had low levels of surface mesothelin, so although cam1615SS1 triggered NK cell degranulation, it failed to control the tumor in three dimension cultures. B7H3 was expressed on all mesothelioma lines, regardless of subtype, and GTB-5550 induced robust degranulation and cytokine responses in NK cells. Levels of B7H3 and mesothelin did not change with overnight interferon gamma treatment to mimic an inflammatory tumor environment. In long-term spheroid assays, GTB-5550 induced NK cell destruction of tumor spheroids more rapidly than IL-15 treatment (figure 1).

Conclusions GTB-5550 drove NK cell responses towards all mesothelioma subtypes, while cam1615SS1 successfully targeted epithelial peritoneal mesothelioma. In future studies we aim to combine TriKE with immune checkpoint inhibitors to test their potential to drive innate immune responses in the context of currently approved therapies.

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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.