Tri-specific killer engagers target natural killer cells towards mesothelioma

Philippa R. Kennedy[1], Quinlan M. Kile[1], Blake A. Jacobson1, Brianna Ettestad[1], Nicholas A. Zorko[1], Caroline Hallstrom[1], Behiye Kodal[1], Deborah A. Todhunter[2], Daniel A. Vallera[2], Gregory Berk[3], Jeffrey S. Miller[1], Manish R. Patel[1], Martin Felices[1]

[1]Department of Medicine and [2]Department of Radiation Oncology, University of Minnesota, Minneapolis, MN, [3]GT Biopharma, Brisbane, CA, USA.

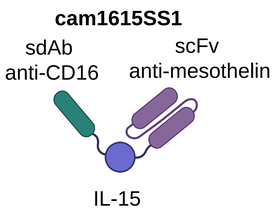
Masonic Cancer Center, University of Minnesota — Comprehensive Cancer Center designated by the National Cancer Institute

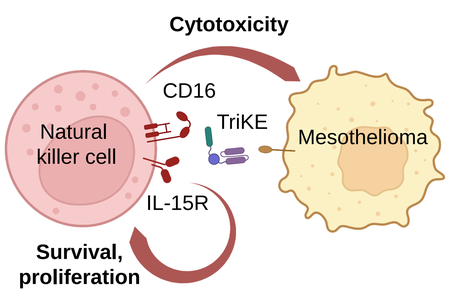
Introduction:

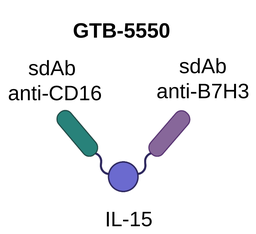
Mesothelioma is a rare but deadly malignancy of cells lining internal organs. It is most commonly found in the pleura (cells lining the lung), but also in the peritoneum. Between 2015 and 2019, half of all mesotheliomas diagnosed were at a distant site, where five-year survival was as low as 9%. Immunotherapy 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.

Tri-specific killer engagers:

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. Cross-linking of CD16 by the tumor antigen triggers NK cell cytotoxicity of the tumor cells bearing the antigen. The IL-15 moiety primes the NK cell, enhancing survival, proliferation and motility. 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.

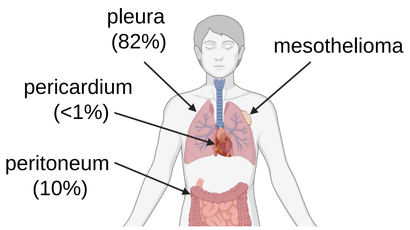






Mesothelioma:

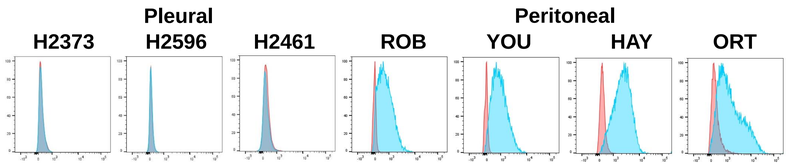
Is a rare cancer with only 62,550 cases reported in the USA between 1999-2018. The majority of these were pleural mesothelioma (cells lining the lung) driven by exposure to asbestos. To assess the suitability of our TriKEs for treating mesothelioma patients we assessed the presence of tumor antigens on various cell lines, either long established, in the case of the pleural lines, or only a few passages away from patients, in the case of the peritoneal lines (PMID: 15274292).



Mesothelioma cell lines		
Pleural		Peritoneal
Sarcomatoid	Epithelioid	Epithelioid
H2373	H2461	ROB
H2596		YOU
		HAY
		ORT

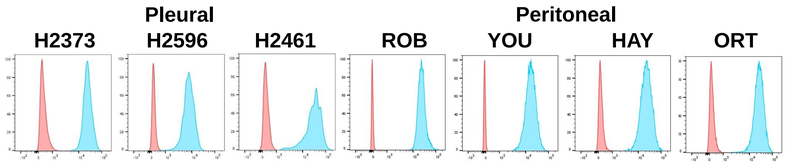
Tumor antigen mesothelin is present on epithelial peritoneal mesothelioma lines

Pleural and peritoneal mesothelioma lines were stained for mesothelin (mAb clone K1) or an isotype control. The staining of live single cells was assessed by flow cytometry.



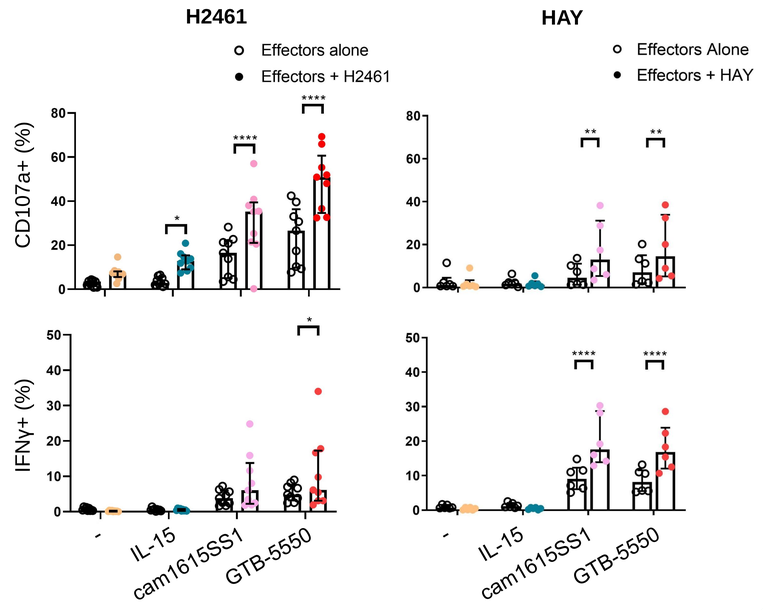
Tumor antigen B7H3 is present on all mesothelioma lines

Pleural and peritoneal mesothelioma lines were stained for B7H3 (mAb clone MIH42) or an isotype control. The staining of live single cells was assessed by flow cytometry.



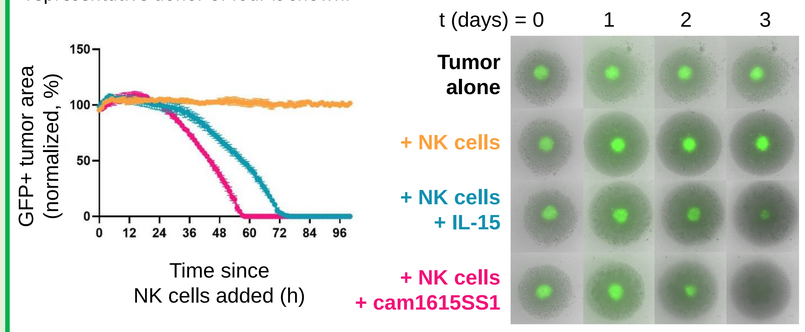
TriKEs targeting mesothelin or B7H3 induce NK cell degranulation and cytokine production

Peripheral blood mononuclear cells from healthy donors were challenged with pleural mesothelioma cells (H2461 is shown as an example) or peritoneal mesothelioma cells (HAY is shown as an example) alone, in the presence of equimolar IL-15, cam1615SS1 or GTB-5550. The degranulation, indicated by CD107a detection, and the cytokine response, indicated by intracellular IFNγ build up, was assessed for live NK cells (CD56+ CD3- live/dead dye-) by flow cytometry. Each dot represents a different donor.



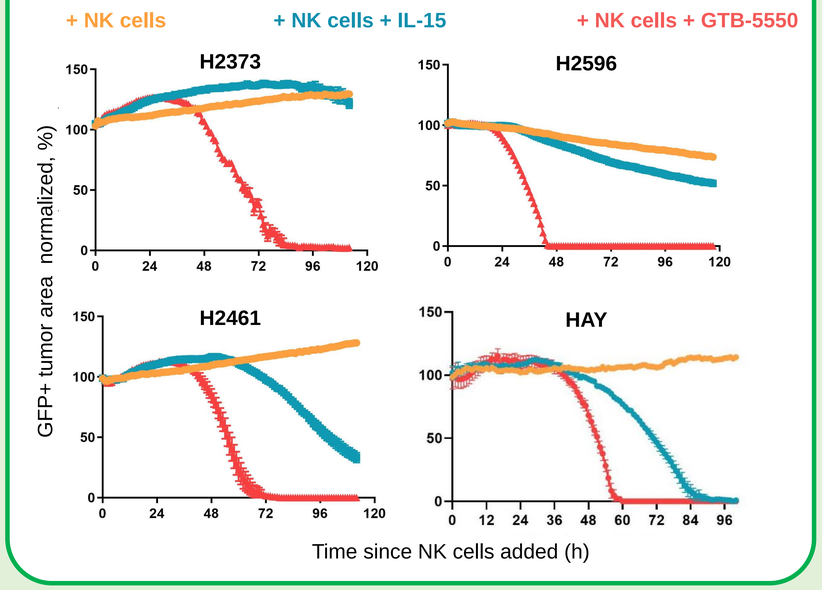
NK cells control peritoneal mesothelioma in three-dimensional spheroid cultures in the presence of cam1615SS1

GFP+ HAY cells (epithelioid peritoneal mesothelioma) were allowed to form spheroids for three days. NK cells alone, with equifunctional IL-15 or 30nM cam1615SS1 were added at t=0 and the survival of tumor cells was tracked through live imaging of the GFP signal. One representative donor of four is shown.



NK cells control mesothelioma in three-dimensional spheroid cultures in the presence of GTB-5550

GFP+ H3273, H2596, H2461 and HAY cells were allowed to form spheroids for one to three days. NK cells alone, with equifunctional IL-15 or 3nM GTB-5550 were added at t=0 and the survival of tumor cells was tracked through live imaging of the GFP signal. One representative donor of four is shown.



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.

Acknowledgments: Ira Pastan (NIH) kindly provided the sequence for the anti-mesothelin scFv, SS1. Raffit Hassan (NIH) kindly provided the peritoneal mesothelioma lines. This project was supported in large part by the Meso Foundation and also by the Department of Defense (CA200922) and National Institutes of Health (R35 CA197292, P01 CA111412). We also thank the Flow Cytometry Resource, University of Minnesota. Schematics in this poster were created in Biorender.com.