

Conclusions Our ex vivo and in vivo data demonstrate that healthy donor NK cells can limit metastasis through the directed cytotoxicity against pioneering K14+ invasive cells. However, prolonged exposure to tumors reprogram NK cells from tumor killing to tumor promoting, specifically in promoting the outgrowth of macrometastases. Further, we can neutralize this effect using NK cell specific inhibitory antibodies and epigenetic modifiers. This is the first time inhibitory signaling on NK cells have been linked with a growth promoting phenotype. These data can provide insight into when the use of NK cell directed therapies can be used to treat or prevent clinically relevant metastatic disease.

<http://dx.doi.org/10.1136/jitc-2020-SITC2020.0529>

530

T-CELL IMMUNOGLOBULIN- AND MUCIN DOMAIN-CONTAINING (TIM)-3 DOWNREGULATION IN RESPONSE TO EX VIVO ACTIVATION AND CANCER TARGETS CORRELATES TO NK CELL FUNCTIONALITY

Tram Dao*, Sandro Matosevic, Sagar Utturkar, Nadia Lanman. *Purdue University, West Lafayette, IN, USA*

Background Natural killer (NK) cells are part of the innate immune system, but are capable of participating in both innate and adaptive immune responses due to their wide range of cytolytic activities, from degranulation, secretion of cytokines to antibody-dependent cell-mediated cytotoxicity. These are possible due to the cells' ability to recognize self and non-self-entities via the net signal generated from their activating and inhibitory receptors upon engagement. TIM-3 is a part of the NK receptor repertoire, expressed commonly on different lymphocytes. In T cells, TIM-3 is established as an inhibitory marker. However, in NK cells, the role of TIM-3 could be agonistic or antagonistic to NK cytotoxicity based on the disease type and activation status, though limited information is known about its role in cancer and its correlation to NK cell effector functions.

Methods We measured TIM-3 expression upon activation of human NK cells under various conditions. NK cells were isolated from peripheral blood of healthy donors and expanded either in K562-based feeder media or feeder-free OpTmizer™ media. After expansion, they were co-cultured for 4 hours with patient-derived glioblastoma multiforme cells (GBM43) at effector:target ratios of 2.5:1 and 10:1. To evaluate the effect of TIM-3 expression on NK cells, 7AAD/CFSE killing assays, CD107a degranulation and IFN γ secretion assays were carried out while blocking TIM-3 with neutralizing antibodies. Bioinformatics analysis of GBM patient RNAseq data was carried out to correlate TIM-3 expression with in vivo function, and this analysis is supplemented by phenotyping TIM-3 on NK cells isolated from patient samples in order to infer the role of this receptor in GBM.

Results We found that TIM-3 was downregulated on OpTmizer™ -cultured NK cells once exposed to cancer targets, and this correlated to a decreased in NK killing capacity when compared to feeder media-cultured NK cells, where the downregulation was not observed. Culturing NK cells in different derivatives of both media suggested that a combination of serum and cytokines can induce TIM-3 expression change to cancer targets. Flow cytometric assays revealed that while

degranulation remained the same, the decreased in cytotoxicity corresponded to a decrease in IFN γ secretion. In GBM patient datasets, TIM-3 expression correlates to high IFN γ levels and associates with both pro- and anti-tumorigenic functions. Here, we report a new role of TIM-3 in modulating NK functionality by correlating its loss to a loss in NK cell effector functions, and how its expression can be modified by ex vivo activation.

Conclusions TIM-3 expression on NK cells can be induced by ex vivo expansion, and this change in expression could influence NK cytotoxicity and cytokine secretion. Our data suggested that TIM-3 is not necessarily an inhibitory marker in GBM, and more likely to be a status marker or an activation limiter, working in conjunction with other receptors to modulate NK cell anti-tumor responses.

Ethics Approval This study was approved by Purdue Intuition's Ethics Board, approval number [1804020540].

<http://dx.doi.org/10.1136/jitc-2020-SITC2020.0530>

531

AFM13-TARGETED BLOOD AND CORD-BLOOD-DERIVED MEMORY-LIKE NK CELLS AS THERAPY FOR CD30+ MALIGNANCIES

¹Lucila Kerbauy, ²Nancy Marin*, ¹Mecit Kaplan, ¹Pinaki Banerjee, ²Melissa Berrien-Elliott, ²Michelle Becker-Hapak, ¹Rafet Basar, ²Mark Foster, ³Luciana Garcia Melo, ²Carly Neal, ²Ethan McClain, ¹May Daher, ¹Ana Karen Nunez Cortes, ²Sweta Desai, ¹Francesca Wei Inng Lim, ¹Mayela Carolina Mendt, ²Timothy Schappe, ¹Li Li, ¹Hila Shaim, ¹Mayra Hernandez Sanabria, ²Pamela Wong, ¹Enli Liu, ¹Sonny Ang, ¹Rong Cai, ¹Vandana Nandivada, ¹Vakul Mohanty, ¹Yifei Shen, ¹Natalia Baran, ¹Natalie Fowlkes, ¹Ken Chen, ¹Luis Muniz-Feliciano, ⁴Joachim Koch, ⁵Martin Treder, ⁴Wolfgang Fischer, ⁶Oswaldo Keith Okamoto, ¹Yago Nieto, ¹Richard Champlin, ¹Elizabeth Shpall, ²Todd Fehniger, ¹Katy Rezvani. ¹The University of Texas MDACC, Houston, USA; ²Washington University School of Medicine, St Louis, MO, USA; ³The University of Texas MD, Houston, USA; ⁴Affimed GmbH, Heidelberg, Germany; ⁵Arjuna Therapeutics, Santiago de Compostela, Spain; ⁶Hospital Israelita Albert Einstein, Sao Paulo, Brazil

Background Natural killer (NK) cells are a nascent cellular immunotherapy for hematologic malignancies. Target recognition of NK cell-resistant cancers remains a substantial barrier to broad application of NK cell therapy. One solution are bispecific engagers that trigger NK cells via an NK activating receptor when simultaneously engaging a tumor-specific antigen.

Methods Here, we investigated single NK cell responses stimulated by the tetravalent bispecific innate cell engager (ICE[®]) AFM13 that binds CD30 on leukemia/lymphoma targets and CD16A on several types of NK cells.

Results Multidimensional mass cytometry revealed heterogeneity within AFM13-directed conventional (c)NK cell responses, as well as consistent polyfunctional activation of mature terminally differentiated NK cells across donors. The source of NK cells also impacted the AFM13 response, with cNK cells from healthy donors exhibiting superior responses to those from Hodgkin lymphoma patients. IL-12, IL-15, and IL-18-induced memory-like NK cells from peripheral blood exhibited enhanced killing of CD30+ lymphoma targets directed by AFM13, compared to cNK cells. Cord-blood expanded NK cells that were pre-activated with IL-12, IL-15 and IL-18 also exhibited enhanced killing with AFM13 stimulation, via upregulation of signaling pathways related to NK cell effector function. These cells were stably pre-loaded with AFM13 enhancing responses to CD30+