surrogates for individual patients making them suitable for patient population studies including evaluating the response to IO drug candidates in vitro.

**Results** We co-cultured organoids expressing tumor associated antigen (TAA) of interest with bispecific T cell engagers and CAR-T cells recognizing the TAAs. Our data demonstrated antigen-specific T cell killing of tumor organoids and tumor antigen reactivity of bispecific antibody activated T cells and CAR-T. We engineered tumor organoids to express CD19 and a luciferase reporter gene and measured luciferase activity to monitor the growth and killing of tumor organoids by CD19 CAR-T cells. The luciferase activity in organoids reflected the killing efficiency in a very sensitive, robust and high throughput manner. Immune checkpoint molecules are differentially expressed on individual tumor organoids and we evaluated the potency of immune check blockade using tumor organoids cocultured with allogenic T cells. Killing of tumor organoids and T cell activation was enhanced by PD-1/PD-L1 blockade. We profiled the expression of immune checkpoint molecules on our banked tumor organoids which will provide a valuable resource to choose tumor models and cancer types for preclinical testing of IO drugs.

**Conclusions** In conclusion, we demonstrated the feasibility of in vitro patient-derived model system in the field of IO research using tumor organoid co-culture with immune cells, and their application in IO target and drug discovery.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0527

---

**528 SEXUAL DIMORPHISM IN MYELOID-DERIVED SUPPRESSOR CELLS PROMOTE GBM PROGRESSION IN FEMALES VIA IL-1Β**

1Defne Bayik*, 1Yadi Zhou, 1Chihyun Park, 1Chngjin Hong, 1Danielle Silver, 1Dionysios Watson, 2Alice Lo, 1Tae Hyun Hwang, 1Feixiong Cheng, 1Peter Sims, 1Antonio lavorone, 1Justin Lathia, 1Cleveland Clinic, Cleveland, OH, USA; 2Case Western Reserve University, Cleveland, OH, USA; 3Columbia University, New York, NY, USA

**Background** A potently immunosuppressive tumor microenvironment facilitates progression of glioblastoma (GBM). Immuno-therapies have had variable success in improving the outcome of GBM patients, suggesting that there is a need to gain insight into the mechanisms of immunosuppression. Our findings indicated that proliferating monocyteic MDSCs (mMDSCs) accumulate in tumors of male mice and patients, while female tumor-bearing mice had an increase in circulating granulocytic MDSC (gMDSC) frequency, and a high gMDSC subset accumulation patterns in GBM-bearing mice. The accumulation of gMDSCs were the primary source of IL-1β and that its neutralization provided a female-specific survival advantage by reducing circulating gMDSCs. This was accompanied by declines in tumor infiltration of microglia, microglia activation status and tumor cell proliferation. In vitro, IL-1β inhibition reduced viability and expression of activation markers by primary microglia.

**Conclusions** These findings highlight a novel peripheral gMDSC-microglia IL-1β mediated communication axis in female GBM and indicate expression differences in MDSC subsets can be leveraged for improved immunotherapy efficacy in a sex-specific, precision medicine strategy.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0528

---

**529 CANCER CELLS EDUCATE NATURAL KILLER CELLS TO A METASTASIS PROMOTING CELL STATE**

1Isaac Chan*, 2Hildur Knútsdóttir, 3Gayatri Ramakrishnan, 4Veena Padmanaban, 5Manisha Warnier, 1Juan Carlos Ramirez, 1Joel Bader, 1Elizabeth Jaffe, 2Andrew Ewald

**Background** Metastatic disease drives breast cancer mortality. We recently discovered that leading cells at the invasive edge of mammary tumor organoids retain a conserved basal epithelial program defined by their expression of keratin-14 (K14), establishing K14 as a good marker of invasive breast cancer cells. K14-positive invasive cells also exhibit characteristics that make them targets of immunosurveillance by natural killer (NK) cells. While NK cells are key immune mediators in the control of metastasis, our understanding of the specific mechanisms behind this regulation and its eventual evasion by metastatic cells remains incomplete.

**Methods** We have developed a novel preclinical 3D co-culture assay to discover mechanisms behind interactions between K14 + invasive breast cancer cells and NK cells. Combined with in vivo assays of metastasis, we are able to determine how NK cells limit the early stages of metastasis and also how tumor cells can influence key NK cell properties.

**Results** In ex vivo co-culture assays of NK cells isolated from healthy mouse donors and mammary tumor organoids from MMTV-PyMT and C31T mouse models of breast cancer, we demonstrate that NK cells limit the metastatic invasion of K14+ breast cancer cells. Antibodies to invasive K14+ cells were able to enhance the ability of NK cells to limit colony formation, suggesting antibody-dependent cell mediated cytotoxicity. Surprisingly, when isolated from tumor bearing mice, NK cells did not limit invasion and instead promoted colony formation. The in vivo adoptive transfer of NK cells from healthy donors prevents the progression of early lung metastatic seeds to macrometastases, while the adoptive transfer of cells isolated from tumor bearing donors promotes macrometastatic development. Transcriptomic analysis of reprogrammed NK cells demonstrate they have similar profiles to resting NK cells. This growth promoting phenotype can be reversed with antibodies targeting inhibitory cell surface receptors or the epigenome.
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 Utrurkar, 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 cultured with OpTmizer® media. After expansion, they were co-cultured isolated from peripheral blood of healthy donors and human NK cells under various conditions. NK cells were activated and cultured with OpTmizer® media. After expansion, they were co-cultured with patient-derived glioblastoma multiforme 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 antibody. 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 correlated 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

1Lucia Kerbauy, 1Nancy Marin*, 2Mecit Kaplan, 1Pinaki Banerjee, 3Melissa Berrien-Elliott, 1Marta Hernandez Sanabria, 4Pamela Wong, 4Erin Liu, 1Sonny Ang, 1Rong Cai, 1Vandana Nandivada, 4Vakul Mohanty, 1Yifei Shen, 1Natalia Baran, 1Natalie Fowlkes, 5Keren Chen, 1Luc Muzin-Feliciano, 1Joachim Koch, 1Martin Treder, 1Wolfgang Fischer, 4Oswaldo Keith Okamoto, 1Yago Nieto, 1Richard Champlin, 1Elizabeth Shpall, 1Todd 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. 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 antibody. 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 correlated 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