Dana-Farber Cancer Institute | Robert and Renée Belfer Center for Applied Cancer Science

#5235

BRIGHAM AND WOMEN'S HOSPITAL | HARVARD MEDICAL SCHOOL

Combination STING agonist and NK cellular therapy in patient-derived organotypic tumor spheroids

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Background: Increasing the immunogenicity of solid tumors may potentiate durable anti-tumor immunity. Targeting innate pattern recognition receptor signaling is a promising strategy to repolarizing the suppressive tumor immune microenvironment. A potential synergistic approach is the addition of cellular therapy. Tumor models expressing high levels of endogenous STING, but where the pathway is not active, were considered for evaluating STING agonism as a potential intervention. Additionally, prior work has demonstrated that STING induction is toxic to T cells; however, NK cells are unaffected, due to their differential regulation of autophagy. Here we investigate the immunomodulatory properties of STING agonist dazostinag (TAK-676) alone and in combination with donor-derived NK cells using short-term microfluidic culture of fresh patient-derived organotypic tumor spheroids (PDOTS).

Methods: Surgical cases from Brigham and Women's Hospital under an IRB-approved protocol were studied. PDOTS were generated as previously described. *Ex vivo* response was assessed by multiplexed cytokine array and single cell RNA sequencing (scRNAseq). Baseline immune phenotypes were analyzed by FACS from single cells isolated during tumor sample preparation.

Results: Ten explants were studied. We observed significant induction of CXCL10 in all samples treated with STING agonist. IFN-β, IFN-γ, TNF-α, and MIP1-α were also consistently induced by STING agonist treatment. More detailed analysis of two samples by scRNAseq revealed a significant modulation of the tumor immune microenvironment by treatment with dazostinag. We observed profound changes in tumor phenotype, indicating changes in immunogenicity such as upregulation of RAET1E and NECTIN2. Donor-derived NK cell therapy added to PDOTS cultures displayed elevated expression of natural cytotoxicity receptors, pro-inflammatory cytokines such as TNF-a and IL-16, effector lymphocyte chemoattractants such as CCL5, and activating receptors including CD16, 2B4, and DNAM-1 that was not observed in endogenous NKs. Expression of the following pro-inflammatory effector molecules by both endogenous NK and donor-derived NK cells was significantly enhanced by treatment with dazostinag: IFN-γ, TRAIL, CXCL9, CXCL10, and CXCL11. We did not observe concomitant upregulation of immune checkpoints by NK cells.

Figure 1. Characterization of human mesothelioma samples and donor-derived NK cells. (**A**) MPM tumors have high STING expression, but the pathway is silent; high effector lymphocyte infiltration, but tumors considered "cold." (**B**) Organotypic tumor spheroids from 12 patients (10 evaluable for assay) were disaggregated and seeded into 3D microfluidic devices: treatments and endpoint assays shown below. (**C**) Expression of stress ligands by tumor cells, and co-receptor expression on endogenous NK and donor-derived NK cells were evaluated by flow cytometry at baseline prior to treatment. (**D**) Baseline profile of tumors 30293 and 30298 used for 10x scRNAseq.

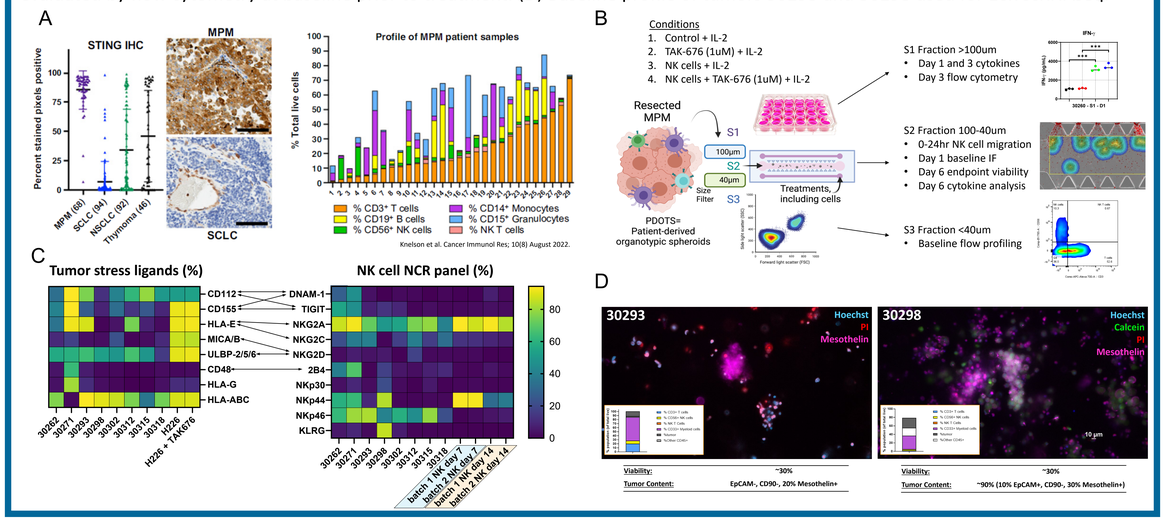


Figure 2. Significant STING agonist-induced secretion of pro-inflammatory chemokines and cytokines in MPM samples. Treatment of pDOTS with dazostinag modulates the tumor microenvironment, including enhancing secretion of CXCR3 chemoattractant, potentially recruiting effector lymphocytes.

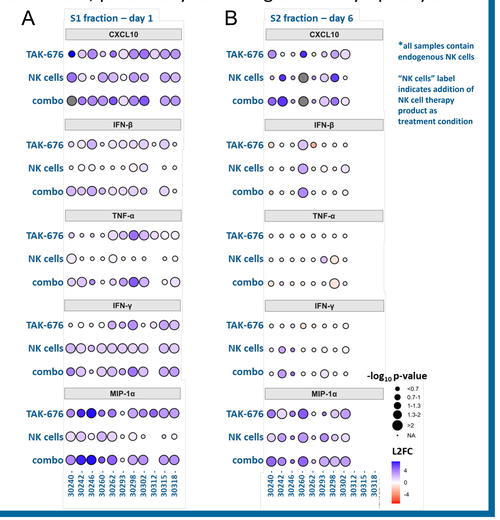


Figure 3. Profound differences in STING agonist-treated vs. untreated. UMAP projections indicating two profiled patients, treatment-based clustering, and cell type clustering. NK cells were further analyzed by donor-derived vs. endogenous NKs.

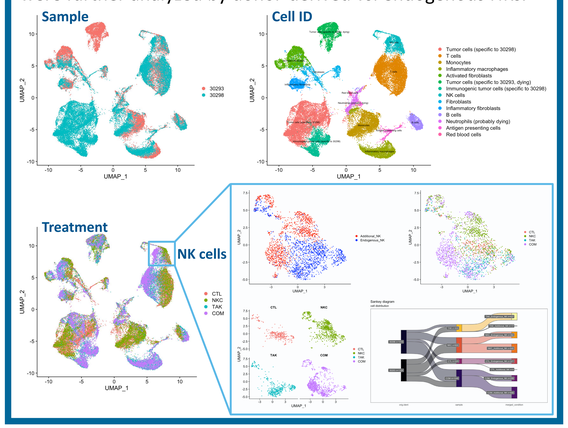
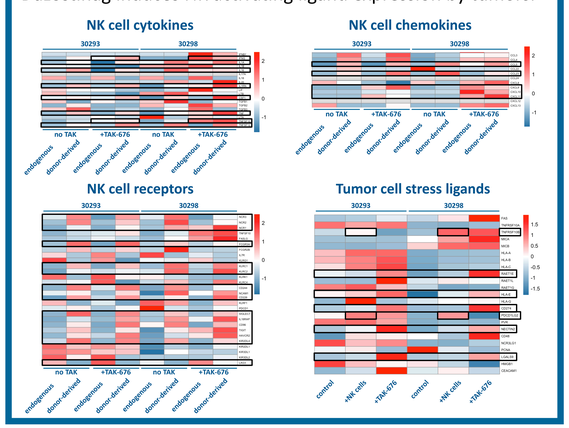


Figure 4. Gene expression profiling endogenous vs. donor-derived NK cells and tumor cells post-dazostinag treatment. Donor-derived NK cells display robust effector phenotype. Dazostinag induces NK activating ligand expression by tumors.



Conclusions: Using tumor explants and a variety of orthogonal techniques, we investigated the response to STING agonism and donor-derived NK cells *ex vivo*. Our results indicate that STING agonism remodels the TME, creating a more immune-permissive environment. STING agonism induces secretion of CXCR3 ligands—which would be expected to recruit effector lymphocytes to the tumor—and enhances local secretion of anti-tumor cytokines. Given these preliminary pharmacodynamic data, future studies will evaluate the extent to which STING agonism may bolster NK-based cell therapy.

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Tuesday April 9, 2024
Location: Section 1

1:30PM-5:00PM
Poster Board Number: 10