504 USING MULTIPLEXED IMMUNOFLUORESCENCE TO QUANTITATIVELY ANALYZE MYELOID DERIVED SUPPRESSOR CELLS (MDSCS) IN RELATION TO TERTIARY LYMPHOID STRUCTURES (TLS) IN BLADDER CANCER

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Background An intriguing phenomenon in many solid tumors is the de novo lymphoid neogenesis of tertiary lymphoid structure (TLS). However, it remains unclear whether spontaneous TLS restricts or promotes tumor progression. MDSCs are major contributors to tumor evasion of adaptive immune responses. We hypothesize that MDSCs repel TLS as a histological pattern in bladder cancers.

Methods To perform a spatial analysis of MDSDs and other immune cell phenotypes in the bladder tumor microenvironment we used MultiOmyx™, an immunofluorescence (IF) assay utilizing a pair of directly conjugated Cyanine dye-labeled (Cy3, Cy5) antibodies per round of staining. Using a 14-marker panel and proprietary cell segmentation and classification algorithms developed at NeoGenomics we have analyzed the presence of TLS (positive for CD20, CD3, and PNAd), followed by a spatial analysis of MDSCs, T cell subtypes, and M1/M2-type tumor-associated macrophages (TAMs) in relation to the TLS in 25 FFPE samples from patients with bladder cancer.

Results To test our initial hypothesis that MDSCs repel TLS in bladder cancer, we quantitated the presence of immune cell phenotypes inside the TLS, and in tissue regions near and far from the TLS. As expected we found a significantly higher density of B cells and T cells present inside the TLS compared to either near or far from the TLS. However, when quantitating MDSCs we found a linear decrease related to their proximity to the TLS, with a 25% lower density in the region near the TLS, and a 46% decrease in the regions far from the TLS. This pattern was not observed for TAMs that were found at the same density inside, near, or far from the TLS, confirming that this pattern is MDSC specific. We are currently performing further analysis of MDSCs co-expressed with either CXCR2 or iNOS for additional answers to the possible mechanisms behind this finding.

Conclusions The finding on the co-abundance of MDSCs and T/B cells following the distance from TLS suggests that MDSCs may repel TLS-derived T and B cells through cell-cell interaction via physical proximity. This study has important implications for bladder cancer immunotherapy.

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505 ACTIVATION OF NK T CELLS PROMOTE THE INFLAMMATORY TUMOR MICROENVIRONMENT AND CONTROL THE GROWTH OF SOLID TUMOR

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Background Type I NK T cells, also known as iNKT cells, can recognize self or microbial lipids presented through CD1d molecules on antigen-presenting cells. Activation of NKT cells induces inflammatory cytokines and help in mounting anti-tumor immunity. How does stimulation of iNKT cells in vivo alter the tumor microenvironment is not clearly understood.

Methods C57BL/6 mice were given a subcutaneous injection of B16F10 melanoma cell line (1 X 10^6 cells). Mice were given intraperitoneal injection of alpha-galactosylceramide (a-GalCer, a ligand for iNKT cells; 2 microgram/injection) on day +1, +5, +10, +15 and +20. NK cells, $Gr1^+$ cells and $F4/80^+$ macrophages in mice were depleted using cell-specific antibodies. The growth of tumors was monitored, and immune cells were characterized using flow cytometry and immunofluorescence staining. Student's t-test and one-way ANOVA were used for statistical analysis.

Results Our results showed that intratumoral NK T cells had significantly low expression of CD25, CD69, CD122, and IFN-gamma receptor molecules and produced lower inflammatory cytokines (IFN-gamma, TNF-alpha, and GM-CSF) as compared to splenic NK T cells. The soluble factor produced by B16F10 cells reduces the expression of these cytokines and cytokine receptors in vitro on the NK T cells purified from the spleen. Treatment of tumor-bearing mice with a-GalCer significantly increased the IFN-gamma-producing NK T cells, $CD8^+$ T cells, and effector Th1 cells in secondary lymphoid organs, and tumors, also significantly reduced the tumor growth. Furthermore, a-GalCer treatment significantly increased the $iNOS^+CD206^-$ M1-macrophages and reduced the $iNOS^-CD206^+$ M2-macrophages in the spleen and tumor. The depletion of $F4/80^+$ macrophages prevented the a-GalCer-induced reduction of tumor growth.

Conclusions Our results showed that tumor produced soluble factors alter the phenotype of NK T cells. Activation of NKT cells with a-GalCer promotes the M1-macrophages, and effector $CD8^+$ T cells, Th1 cells in the secondary lymphoid organs and tumor microenvironment. This finding suggests that activation of NKT cells may provide an effective anti-tumor response.

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Ethics Approval All the procedures performed in the experiments involving mice were in accordance with the ethical standards of (NCCS) Institutional Ethics Committee of Animals Usage (Approval ID: EAF/B-166/2011 and EAF/B-256/2016).

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506 THE TUMOR IMMUNE MICROENVIRONMENT OF METASTATIC OSTEOSARCOMA IS MARKED BY LYMPHOCYTE EXCLUSION AND IMPACTS PATIENT PROGRESSION-FREE SURVIVAL

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Background Patients with relapsed metastatic osteosarcoma have no effective treatments available to them,[1] and immunotherapy thus far has not succeeded in improving outcomes.[2–5] We aim to understand the immune architecture of the tumor microenvironment (TME) of osteosarcoma, with the goal of