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

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**Background** Type 1 NK T cells, also known as iNKT cells, can recognize self or microbial lipids presented through CD1d molecules on antigen-presenting cells. Activation of NK T 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 NK T 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 NK T 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-236/2016).

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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, and immunotherapy thus far has not succeeded in improving outcomes. We aim to understand the immune architecture of the tumor microenvironment (TME) of osteosarcoma, with the goal of...