genes such as PDCD1 (PD-1), ENTPD1 (CD39), HAVCR2 (TIM3), LAG3, GZMB, and GZMK. This gene expression profile suggests chronic activation due to exposure to tumor antigens. Melan-A tetramer-positive cells predominantly localized to Cluster C0, and the largest clonotype was associated with this cluster. We also assessed systemic recirculation by sequencing TCRs in PBMCs, revealing varying recirculation levels among different T-cell clusters. While chronically activated (C0) T cells displayed limited recirculation, T cells with a memory phenotype (C2), cytotoxic functions (C4), and effector T-cell responses (C6) were more present in the peripheral blood.

Conclusions: These findings challenge the notion of the eye as an immune-privileged site, as we consistently observed immune responses within UM tumors. The mechanism by which tumor antigens prime naïve T cells in this site remains mysterious.

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188P Expression of the co-stimulatory checkpoint protein OX40L (TNFSF4) in the melanoma micro-environment

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Background: Immune checkpoint inhibitors revolutionized treatment of both locally advanced and metastatic melanoma patients, yet some patients fail to achieve long-lasting benefit or progress on treatment, highlighting the need for new immune-modulatory agents. The co-stimulatory ligand OX40L and its receptor OX40 (also named TNFSF4/ TNFRSF4) were previously shown to elicit anti-tumor immune responses in pre-clinical cancer models. This led to several clinical trials of OX40-agonism as therapeutic interventions in cancer, which showed limited efficacy thus far. We previously analyzed RNA-sequencing data of OX40L expression in melanoma and found that low mRNA expression was associated with worse prognosis and with worse outcome following anti-PD1 treatment. These findings encouraged further studies to substantiate the role of OX40L in the melanoma microenvironment.

Methods: Multiplex immunofluorescent microscopy was used to evaluate the expression of OX40L/OX40 in FFPE surgical specimens of primary/metastatic melanoma tumors. Previously established cell-specific markers were used in parallel to identify OX40L- expressing cell phenotypes. Quantification was performed with GEN5 PRIME software.

Results: OX40L+ cells were detected in 15/16 tumors tested, in variable abundances, which correlated with the abundance of OX40+ cells and with co-localization events of OX40L+/OX40+ cells, suggesting functional OX40 ligand-receptor interactions. OX40L expression was frequently detected on Macrophages/Dentritic-cells, less on CD4+ or CD8+ T cells and rarely on melanoma cells. Unexpectedly, we now identified high prevalence of OX40L expression on Foxp3+ regulatory T cells (Treg), in addition to OX40, which was previously shown to antagonize their suppressive function.

Conclusions: Here we show that OX40L is abundantly expressed, together with OX40, on Treg within the melanoma micro-environment. These novel findings may suggest a role for OX40L in modulating Treg suppressive activity. If further proven, our observations may be important in prognostication and prediction of response in melanoma. Furthermore, they may point to new IO combinations for further research and have therapeutic implications towards 'heating' up tumors.

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189P The impact of immune microenvironment subpopulations on soft tissue sarcomas

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Background: Malignant tumors are sophisticated, organized ecosystems in addition to collections of cancer cells. It has long been established that the formation, recurrence, and metastasis of cancers are all tightly correlated with their immune components. However, the impact of the tumor microenvironment in soft tissue sarcoma (STS) remains mainly unclear. Studying how the immunological microenvironment affects the clinical presentation and prognosis of soft tissue sarcomas is the objective.

Methods: Retrospective research was conducted on 168 soft tissue sarcoma patients who received care at the Republican specialized Scientific and practical Medical Center of Oncology and Radiology of Uzbekistan. Through immunohistochemistry analyses of CD3, CD4, CD8, CD20, and CD68, lymphocyte subpopulations that infiltrate tumours and their subpopulations were investigated.

Results: In the immunological microenvironment, CD 68+ macrophages and CD 3+ T cells were the most prevalent cell types. There was a substantial positive correlation between CD 68 expression and the probability of local recurrence ($p = 0.014$) in a multivariate analysis employing the Fine & Gray risk regression model with death as a competing event, independent of age, resection margins, and the presence of B cells. Furthermore, B cell abundance was significantly lower ($p = 0.013$) and macrophage abundance was much higher in patients older than middle age. Regarding histological subtypes, undifferentiated pleomorphic sarcoma and myxofibrosarcoma were more frequently found to have these cells, which had a high total number of tumor-infiltrating lymphocytes in general and macrophages in particular.

Conclusions: The significant number of CD 68 macrophages in soft tissue sarcomas and their detrimental effect on the prognosis for local recurrence are both confirmed by this investigation.

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190P Immune-related roles of B7H3 in glioblastoma

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Background: Glioblastoma is the most frequent and aggressive primitive brain cancer, associated with dreadful prognosis. This project focuses on a protein called B7H3, member of the B7 « immune checkpoint » protein family. Two isoforms of B7H3 protein (i.e. 2Ig and 4Ig) are produced by alternative splicing and expressed at the cell membrane. Of note, the ectodomain of B7H3 exists as a soluble protein. B7H3 expression in several cancers is associated with poorer prognosis and increased aggressiveness, however its precise role in immune cell modulation in the context of glioblastoma remains elusive.

Methods: Using a murine glioblastoma cell line (CT2A), we allografted immunocompetent mice with CT2A glioma cells overexpressing (OE) the 2Ig isoform of B7H3 (vs cells with control vector). We also engineered a human cell line (U87 OE 2Ig B7H3 vs control vector) to decipher the role of the B7H3 protein on immune cell modulation. Finally patient-derived glioblastoma stem-like cells (GSCs) were used for additional in vitro experiments.

Results: We observed a slight increase in survival in CT2A OE-B7H3 mice, which surprisingly indicated a tumor suppressive effect. We are currently reproducing the experiment using another murine cell line (GL261). Conversely, in vitro coculture experiments revealed no impact of B7H3 on T cell activation or apoptosis, but preliminary results suggest that B7H3 lowers T cell proliferation in vitro. We showed that patient-derived GSCs release soluble B7H3 in the extracellular space and is especially found on extracellular vesicles (EVs).

Conclusions: These results suggest that B7H3 may exert diverse functions in different aspects of glioblastoma immunogenicity. We are exploring the roles of B7H3 isoforms as well as the role it could play on glioblastoma cell-derived EVs, to understand its impact on the immune system.

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