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P02.02 SINGLE-CELL RNA SEQUENCING OF NEUROBLASTOMA TUMORS REVEALS IMMUNOREGULATORY INTERACTIONS AS NOVEL TARGETS FOR IMMUNOTHERAPY

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Background Immunotherapy with CAR-T cells, as well as immune checkpoint blockade, show limited clinical efficacy in the pediatric solid cancer neuroblastoma, despite the success in various adult cancers. The lacking efficacy may be due to various immune evasion strategies employed by neuroblastoma tumors, leading to altered functionality of tumor-infiltrating immune cells. We aimed to provide a comprehensive overview of the composition and function of the neuroblastoma immune environment, as well as relevant immunoregulatory interactions (=), to identify novel targets for immunotherapy.

Materials and Methods 25 tumor samples from 20 patients (17 with high-risk disease, 6 with MYCN amplification), were collected during diagnostic biopsy pre-treatment (n=10) or during resection surgery after induction chemotherapy (n=15). Samples were enzymatically digested, single-cell FACS sorted and sequenced by Cel-Seq2 protocol.

Results Lymphoid cells in the TME consisted of $\alpha\beta$ -, $\gamma\delta$ -T cells, NK cells and B cells. Among $\alpha\beta$ -T cells we identified CD8⁺ T cells, two functionally distinct clusters of CD4⁺ T cells, naive-like T cells and FOXP3⁺ regulatory T cells (Tregs). CD8⁺ T cells had reduced cytotoxic capacity compared to blood-derived T cells from a reference group. Tregs expressed high levels of *PRDM1*, *LAYN* and *ICOS*, suggesting an effector Treg profile, which is associated with increased inhibitory capacity. Although NK cells expressed the cytotoxic genes *NKG7*, *KLRF1*, *GNLY*, *GZMB* and *PRF1*, their expression was significantly lower than in blood-derived reference NK cells. Gene set enrichment analysis (GSEA) confirmed a reduced cytotoxic capacity of tumoral NK cells, which correlated with a decreased expression of activating receptors ($r=0.41$, $p<0.001$) and increased TGF β signaling ($r=-0.45$, $p<0.001$). In addition, NK cells highly expressed the heterodimeric receptor *KLRC1:KLRD1*, which can inhibit NK cell function through HLA-E binding. High *HLA-E* expression by endothelial, immune and mesenchymal cells confirmed its inhibitory activity in the TME. Within the myeloid compartment we identified various immunosuppressive populations, comprising a cluster of *IL10* and *VEGFA* expressing macrophages, three clusters of M2 differentiated macrophages expressing *MMP9* and *LGALS3*, and dendritic cells with intact antigen presenting capacity, but high expression of numerous genes encoding immunosuppressive molecules such as *IDO1*, *LGALS1*, *LGALS2*, *CCL22* and *NECTIN2*. In MYCN amplified tumors, specifically, we observed even lower cytotoxic capacity of CD8⁺ T and NK cells. We identified increased TGF β 1 expression and defective antigen presentation by myeloid and tumor cells as potential causes for reduced cytotoxicity in MYCN

amplified tumors. To identify relevant targets for immunotherapy we constructed an unbiased interaction network, which revealed *NECTIN1=CD96* and *MIF=CD74* as active immunoregulatory interactions between tumor and T/NK cells, and *CD80/CD86=CTLA4*, *CLEC2D=KLRB1*, *HLA-E=KLRC1/KLRC2*, *CD99=PILRA*, *LGALS9=HAVCR2*, and *NECTIN2=TIGIT* between myeloid and T/NK cells.

Conclusions Cytotoxic lymphocytes in the neuroblastoma TME show reduced cytotoxic capacity, likely due to highly immunosuppressive myeloid cells, Tregs and numerous immunoregulatory interactions, which may serve as novel targets for immunotherapy in neuroblastoma.

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P02.03 AUTOMATED CELL TYPE SPECIFIC PD-L1 QUANTIFICATION BY ARTIFICIAL INTELLIGENCE USING HIGH THROUGHPUT BLEACH & STAIN 15-MARKER MULTIPLEX FLUORESCENCE IMMUNOHISTOCHEMISTRY IN HUMAN CANCERS

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Background The quantification of PD-L1 (programmed cell death ligand 1) has been used to predict patient's survival, to characterize the tumor immune microenvironment, and to predict response to immune checkpoint therapies. However, a framework to assess the PD-L1 status with a high interobserver reproducibility on tumor cells and different types of immune cells has yet to be established.

Materials and Methods To study the impact of PD-L1 expression on the tumor immune microenvironment and patient outcome, a framework for fully automated PD-L1 quantification on tumor cells and immune cells was established and validated. Automated PD-L1 quantification was facilitated by incorporating three different deep learning steps for the analysis of more than 80 different neoplasms from more than 10'000 tumor specimens using a bleach & stain 15-marker multiplex fluorescence immunohistochemistry panel (i.e., PD-L1, PD-1, CTLA-4, panCK, CD68, CD163, CD11c, iNOS, CD3, CD8, CD4, FOXP3, CD20, Ki67, CD31). Clinicopathological parameter were available for more than 30 tumor entities and overall survival data were available for 1517 breast cancer specimens.

Results Comparing the automated deep-learning based PD-L1 quantification with conventional brightfield PD-L1 data revealed a high concordance in tumor cells ($p<0.0001$) as well as immune cells ($p<0.0001$) and an accuracy of the automated PD-L1 quantification ranging from 90% to 95.2%. Across all tumor entities, the PD-L1 expression level was significantly higher in distinct macrophage/dendritic cell (DC) subsets (identified by CD68, CD163, CD11c, iNOS; $p<0.001$) and in macrophages/DCs located in the Stroma ($p<0.0001$) as compared to intratumoral macrophages/DC subsets. Across all different tumor entities, the PD-L1