function. This study aimed at a comparative analysis of CTLA-4⁺ cells between different tumor entities.

Materials and Methods To quantify CTLA-4⁺ cells, 4,582 tumor samples from 90 different tumor entities as well as 608 samples of 76 different normal tissue types were analyzed by immunohistochemistry in a tissue microarray format. Two different antibody clones (MSVA-152R and CAL49) were validated and quantified using a deep learning framework for automated exclusion of unspecific immunostaining.

Results Comparing both CTLA-4 antibodies revealed a clone dependent unspecific staining pattern in adrenal cortical adenoma (63%) for MSVA-152R and in pheochromocytoma (67%) as well as hepatocellular carcinoma (36%) for CAL49. After automated exclusion of non-specific staining reaction (3.6%), a strong correlation was observed for the densities of CTLA-4⁺ lymphocytes obtained by both antibodies ($r=0.87$; $p<0.0001$). The mean density of CTLA-4⁺ cells was 674 ± 1482 cells/mm² and ranged from 71 ± 175 cells/mm² in leiomyoma to 5916 ± 3826 cells/mm² in Hodgkin's lymphoma. Within epithelial tumors, the density of CTLA-4⁺ lymphocytes were higher in squamous cell (421 ± 467 cells/mm²) and urothelial carcinomas (419 ± 347 cells/mm²) than in adenocarcinomas (269 ± 375 cells/mm²) and renal cell neoplasms (256 ± 269 cells/mm²). A high CTLA-4⁺ cell density was linked to low pT category ($p<0.0001$), absent lymph node metastases ($p=0.0354$), and PD-L1 expression in tumor cells or inflammatory cells ($p<0.0001$ each). A high CTLA-4/CD3-ratio was linked to absent lymph node metastases ($p=0.0295$) and to PD-L1 positivity on immune cells ($p<0.0026$).

Conclusions Marked differences exist in the number of CTLA-4⁺ lymphocytes between tumors. Analyzing two independent antibodies by a deep learning framework can facilitate automated quantification of immunohistochemically analyzed target proteins such as CTLA-4.

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P02.07 CHARACTERIZATION OF THE TUMOR IMMUNE MICROENVIRONMENT OF PEDIATRIC POSTERIOR FOSSA A EPENDYMOMAS

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Background Ependymoma is the third most common brain tumor in children. At the moment, surgery and radiotherapy are the only effective treatments that can be offered, and despite this, a significant part of the patients relapse with no therapeutic salvage options. Therefore, new treatment modalities are needed. To develop immunotherapies for these children, knowledge of the tumor microenvironment is crucial. The current study aims to unravel the tumor immune microenvironment (TIME) of pediatric posterior fossa A (PFA) ependymomas.

Materials and Methods We used bulk RNA sequencing data of 22 pediatric ependymomas. We defined two groups, hereafter called PFA immune+ (PFAI+) and PFAI-, based on the RNA expression levels of the NanoString panel of Human PanCancer Immune Profiling genes. We performed gene set enrichment analysis and deconvoluted the bulk RNA samples with ependymoma-specific single-cell RNA sequencing datasets. To validate our findings on a protein level, we applied immunohistochemistry with antibodies recognizing tumor-infiltrating lymphocytes, tumor-associated macrophages and microglia.

Results Unsupervised hierarchical clustering of RNA expression of immune-related genes revealed two distinct PFA groups. Differential gene expression analysis showed that PFAI+ have a significantly higher expression of genes associated with immune functions, such as CD3E, CCR2, GZMA, CXCL9 and TRBC2. Accordingly, gene set enrichment analysis demonstrated that several immune pathways, including T-cell signalling, interferon-gamma response and TNF α signalling are enriched in PFAI+ ependymomas. RNA expression of immune checkpoints was also higher in PFAI+ tumors, indicating that these tumors might be more responsive to combinational therapies including immune checkpoint inhibitors. While immunohistochemistry showed low amounts of infiltrating CD3+, CD8+ and CD20+ cells, high numbers of CD163+ and HLA-DRA+ cells were detected. These cells were mainly located in regions of tumor necrosis. Increased amounts of CD4+ and CD8+ lymphocytes were present in PFAI+ tumors compared to PFAI- tumors. Deconvolution of the bulk RNA samples based on single-cell RNA sequencing data revealed an enrichment of myeloid cell populations, especially microglia and macrophages. Furthermore, PFAI+ tumors were found to contain significantly higher relative proportions of T-cells compared to PFAI- tumors (median of 3.76% for PFAI+ compared to 0.03% for PFAI-).

Conclusions We suggest that pediatric posterior fossa A ependymomas can be divided into two groups based on the expression of immune-related genes, in which PFAI+ ependymomas are characterized by higher RNA expression levels of these genes and greater amounts of tumor-infiltrating immune cells. Several techniques showed an enrichment of T-lymphocytes in the PFAI+ ependymomas relative to the PFAI- ependymomas.

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P02.08 THE ROLE OF FOXP3+ REGULATORY T CELLS AND IDO+ IMMUNE AND TUMOR CELLS IN MALIGNANT MELANOMA – AN IMMUNOHISTOCHEMICAL STUDY

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Background Although Malignant Cutaneous Melanoma (CM) is a highly immunogenic cancer, it can evade the immune