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Ependymoma represents a heterogeneous disease affecting the entire neuraxis. Extensive molecular profiling efforts have identified molecular ependymoma subgroups based on DNA methylation. However, the intratumoral heterogeneity and developmental origins of these groups are only partially understood, and effective treatments are still lacking for about 50% of patients with high-risk tumors. We interrogated the cellular architecture of ependymoma using single cell/nucleus RNA-sequencing to analyze 24 tumor specimens across major molecular subgroups and anatomic locations. We additionally analyzed ten patient-derived ependymoma cell models and two patient-derived xenografts (PDXs). Interestingly, we identified an analogous cellular hierarchy across all ependymoma groups, originating from undifferentiated neural stem cell-like populations towards different degrees of impaired differentiation states comprising neuronal precursor-like, astroglial-like, and ependymal-like tumor cells. While prognostically favorable ependymoma groups predominantly harbored differentiated cell populations, aggressive groups were enriched for undifferentiated subpopulations. Projection of transcriptomic signatures onto an independent bulk RNA-seq cohort stratified patient survival even within known molecular groups, thus refining the prognostic power of DNA methylation-based profiling. Furthermore, we identified novel potentially druggable targets including IGF- and FGF-signaling within poorly prognostic transcriptional programs. Ependymoma-derived cell models/PDXs widely recapitulated the transcriptional programs identified within fresh tumors and are leveraged to validate identified target genes in functional follow-up analyses. Taken together, our analyses reveal a developmental hierarchy and transcriptomic context underlying the biologically and clinically distinct behavior of ependymoma groups. The newly characterized cellular states and underlying regulatory networks could serve as basis for future therapeutic target identification and reveal biomarkers for clinical trials.

EPEN-22. SINGLE-CELL RNA SEQUENCING IDENTIFIES UPREGULATION OF IKZF1 IN PFA2 MYELOID SUBPOPULATION DRIVING AN ANTI-TUMOR PHENOTYPE

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We have previously shown immune gene phenotype variations between posterior fossa ependymoma subgroups. PFA1 tumors chronically secrete IL-6, which pushes the infiltrating myeloid cells to an immune suppressive function. In contrast, PFA2 tumors have a more immune activated phenotype and have a better prognosis. The objective of this study was to use single-cell(sc) RNAseq to descriptively characterize the infiltrating myeloid cells. We analyzed approximately 8500 cells from 21 PFA patient samples and used advanced machine learning techniques to identify distinct myeloid and lymphoid subpopulations. The myeloid compartment was difficult to interrupt as the data shows a continuum of gene expression profiles exist within PFA1 and PFA2. Through lineage tracing, we were able to tease out that PFA2 myeloid cells expressed more genes associated with an antiviral response (MHC II, TNF- α , interferon-gamma signaling); while PFA1 myeloid cells had genes associated with an immune suppressive phenotype (angiogenesis, wound healing, IL-10). Specifically, we found expression of IKZF1 was upregulated in PFA2 myeloid cells. IKZF1 regulates differentiation of myeloid cells toward M1 or M2 phenotype through upregulation of either IRF5 or IRF4 respectively. IRF5 expression correlated with IKZF1,

being predominately expressed in the PFA2 myeloid cell subset. IKZF1 is also involved in T-cell activation. While we have not completed our characterization of the T-cell subpopulation, we did find significantly more T-cell infiltration in PFA2 than PFA1. Moving forward these studies will provide us with valuable information regarding the molecular switches involved in the tumor-immune microenvironment and to better develop immunotherapy for PFA ependymoma.

EPEN-23. A COMPUTATIONAL ANALYSIS OF THE TUMOUR IMMUNE MICROENVIRONMENT IN PAEDIATRIC EPENDYMOMA

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Ependymoma is the third commonest childhood brain tumour. Relapse is frequent, often fatal and current therapeutic strategies are inadequate. Previous ependymoma research describes an immunosuppressive environment with T-cell exhaustion, indicating a lack of response to T-cell directed immunotherapy. Understanding the immune microenvironment is therefore critical. We present a computational analysis of ependymoma, gene expression derived, immune profiles. Using 465 ependymoma samples from gene expression datasets (GSE64415, GSE50385, GSE100240) and two RNA-seq databases from UK ependymomas, we applied bulk tumour deconvolution methods (CIBERSORT and xCell) to infer immune cell populations. Additionally, we measured checkpoint blockade related mRNAs and used immunohistochemistry to investigate cell populations in ependymoma sections. CIBERSORT indicated high proportions of M2-like macrophages and smaller proportions of activated natural killer (NK) cells, T follicular helper cells, CD4⁺ memory T-cells and B-cells. xCell overlapped with the M2-like macrophage and CD4⁺ memory T-cell signatures seen in CIBERSORT. On immunohistochemistry, T and B cells were scarce, with small numbers of CD8⁺, CD4⁺ and CD20⁺ cells in the parenchyma but greater numbers in surrounding regions. CD68 was more highly expressed in the parenchyma. Analysis of nine checkpoint ligands and receptors demonstrated only the *TIM3/GAL9* combination was reliably detectable. *GAL9* is implicated in tumour interactions with T-cells and macrophages elsewhere, possibly contributing to poorer outcomes. Our study supports the presence of myeloid cells being leading contributors to the ependymoma immune microenvironment. Further work will delineate the extent of myeloid contribution to immunosuppression across molecular subtypes. Modulation of tumour immunity may contribute to better clinical outcomes.

EPEN-24. SIOP EPENDYMOMA II: CENTRAL EPENDYMOMA MANAGEMENT ADVISORY GROUP – THE UK EXPERIENCE

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Paediatric Ependymoma is the second most common malignant brain tumour of childhood with approximately 50% of cases recurring. It has been described as a "surgical" disease since patients who have undergone a gross total surgical resection (GTR) have a better prognosis than those who have a subtotal resection (STR). Analysis of the UKCCSG/SIOP 1992 04 clinical trial has shown that only 49% of cases had a GTR, with 5-year survival rates for STR of 22–47% and GTR of 67–80%. As part of the SIOP II Ependymoma trial the UK established a panel of experts in the treatment of Ependymoma from Neuro-oncology, Neuro-radiology and Neuro-surgery. Meeting weekly, cases are discussed to provide a consensus on radiological review, ensuring central pathological review, trial stratification and whether further surgery should be advocated on any particular case. Evaluation of the first 68 UK patients has shown a GTR in 47/68 (69%) of patients and STR in 21/68 (31%) of patients. Following discussion at EMAG it was felt that 9/21 (43%) STR patients could be offered early second look surgery. Following this 2nd look surgery the number of cases with a GTR increased to 56/68 (82%). There has been a clear increase in the number of patients for whom a GTR has been achieved following discussion at EMAG and prior to them moving forwards with their oncological treatment. This can only have beneficial effects in decreasing their risk of tumour recurrence or CSF dissemination and also in reducing the target volume for radiotherapy.