### EPEN-25. EXCEPTIONAL CLINICAL AND IMAGING RESPONSE TO TRK-INHIBITION IN A PATIENT WITH SUPRATENTORIAL EPENDYMOMA HARBORING NTRK2 GENE FUSION

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BACKGROUND: Patients with metastatic pediatric ependymoma have limited therapeutic options and poor outcomes. Approximately ¾ of supratentorial ependymomas are driven by *C11ORF95-RELA* fusions, and the remaining by a heterogeneous group of fusion events. We present a six year-old male diagnosed with supratentorial ependymoma with leptomeningeal carcinomatosis harboring an *NTRK2*-fusion. Local and distant multifocal, intracranial and intraspinal tumor recurrence occurred seven months following gross total resection of the primary lesion and proton beam craniospinal irradiation. METHODS: DNA and RNA from FFPE tumor were used for targeted sequencing using an 81-gene fusion panel and 124-gene mutation panel. Separately, capture transcriptome sequencing, exome sequencing, and copy number array were performed as part of the Texas KidsCanSeq study, an NHGRI/NCI-funded Clinical Sequencing Evidence-Generating Research (CSER) consortium project. All sequencing was carried out in CLIA-certified laboratories. RESULTS: An in-frame fusion between 5' exons 1–3 of *KANK1* and 3' exons 16–21 of *NTRK2*, predicted to retain the kinase domain, was identified. At tumor recurrence, therapy was initiated with Larotrectinib, an FDA-approved pan-TRK inhibitor. Clinical improvement in cognitive speed, motor strength, and coordination was observed at two weeks with significant tumor response on MRI at two and four months. CONCLUSION: TRK gene fusions have not previously been reported in ependymoma. Further tumor characterization by methylation profiling is underway and will be of diagnostic interest given the apparent discordance between tumor histology and molecular findings. This case highlights the potential impact of clinical genomic analysis for children with CNS tumors.

### EPEN-26. NON-CANONICAL NF-ΚB SIGNALING DRIVES MESENCHYMAL EPENDYMAL CELL SUBPOPULATION IN PFA EPENDYMOMA

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NF-κB signaling is a hallmark of PFA1 ependymoma. Loss of LDOC1, through epigenetic silencing, leads to constitutively active NF-κB signaling and chronic IL-6 secretion. In this study, we investigate the loss of LDOC1 within the PFA tumor clusters. Using our PFA scRNAseq database, in which there are 5 clusters within the tumor cell compartment: mesenchymal (MEC), ciliated (CEC), transportive (TEC), and undifferentiated (UEC). LDOC1 expression was significantly reduced and had an inverse correlation with genes defining the unfavorable MEC subpopulation, predominate in PFA1. This is consistent with our findings that MEC was defined by an NF-κB2 signaling profile. In contrast, LDOC1 expression was higher and positively correlated with genes defining the favorable CEC subpopulation, mostly seen in PFA2. RELA expression, which we studied as a target of LDOC1, was not localized to MEC and was wide-spread throughout the PFA compartment. RELB, part of non-conical NF-κB signaling, was expressed only the MEC subpopulation correlating with IL-6 gene expression found only in this subpopulation. In MAF-811, a PFA cell line with more CEC-like gene phenotype, RELB co-immunoprecipates with the active form of NF-κB2 in both the nucleus and cytoplasm. IL-6 gene expression is almost completely lost when NF-κB2 is knock-down using shRNA. Additionally, loss of LDOC1 leads to over 3 fold increase in NF-κB2 expression. Combined with our previous work, this would suggest that NF-κB2 drives IL-6 expression by binding with RELB in MEC subpopulation and targeting loss of LDOC1 may shift the MEC subpopulation toward the more favorable CEC subpopulation.

### EPEN-27. *CDKN2A* DELETION IN SUPRATENTORIAL EPENDYMOMA WITH *RELA* ALTERATION INDICATES A DISMAL PROGNOSIS – A RETROSPECTIVE ANALYSIS OF THE HIT EPENDYMOMA TRIAL COHORT

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INTRODUCTION: Since supratentorial *RELA*-fusion positive ependymomas are considered a biologically distinct disease, we aimed to identify histological and genetic predictors of outcome in a defined cohort of pediatric patients. MATERIALS AND METHODS: We analyzed 54 *RELA* ependymomas in pediatric patients treated according to HIT2000-E protocols. All cases underwent central neuropathological review. Genome-wide copy number alterations were assessed by molecular inversion probe or SNP array. *RELA* alterations were detected by RT-PCR, sequencing and assessment of nuclear p65-RelA protein. Copy number alteration of the *CDKN2A (cyclin dependent kinase inhibitor 2A)* locus and concordant p16 protein expression were analyzed. RESULTS: Fifty-two tumors were classified as WHO-grade III (96.3%) with high mitotic activity in 39 cases (72.2%), vascular proliferation in 47 (87.0%), necrosis in 43 (79.6%) and clear cell morphology in 19 (35.2%). All tumors harbored *RELA* alterations. Homo- or heterozygous *CDKN2A* deletions were detected in 9 (16.7%) and 14 (25.9%) cases, respectively. p16 protein expression was lost in all cases with homozygous deletion. Median follow-up was 5.4 years with 5-years EFS and OS of 74.1% and 92.6%. In Kaplan-Meier analysis high mitotic activity was related to shorter EFS (p=0.016) and clear cell morphology to longer OS (p=0.039); *CDKN2A* deletion was associated with shorter OS (homozygous deletion, p=0.009; homo-or heterozygous deletion, p=0.034). No correlation between *CDKN2A* deletion and high mitotic activity was found but with higher age at diagnosis (p=0.001). CONCLUSION: Deletion of *CDKN2A* occurred in 42.6% of supratentorial ependymomas with *RELA* alteration and represented a genetic predictor of worse overall outcome in pediatric patients.

### EPEN-28. NOVEL ONCOGENE AMPLIFICATION IN SPINAL EPENDYMOMA INVOLVING THE MYC LOCUS (8Q24)

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BACKGROUND: We report a unique case of spinal ependymoma with classic histology and aggressive clinical behavior which harbored a focal *MYC* (8q24) amplification. CASE REPORT: A-12-year old male presented with a three months history of back pain and acute onset weakness with ataxia. A spine MRI revealed an avidly enhancing intradural, extramedullary mass occupying the dorsal spinal canal from C6 through T2. The tumor demonstrated mild diffusion restriction and was associated with severe cord compression and mild edema. He underwent gross total resection. Pathological diagnosis was classic grade II ependymoma. Eleven months later, he re-presented with acute onset lower extremity paresthesia and left-handed weakness. Spine MRI demonstrated tumor recurrence extending from C2 through T1-T2 with resultant severe cord compression, again demonstrating avid enhancement and restricted diffusion. He underwent subtotal resection of the mass and focal proton beam irradiation. MOLECULAR CHARACTERISTICS: The patient was enrolled on an institutional comprehensive genomic profiling protocol. The tumor's copy number profile was complex, including homozygous loss of 17p and notably, amplification of the *MYC*

oncogene. Using fluorescence *in situ* hybridization, we identified >20 copies of *MYC* in interphase cells, confirming the gene amplification, while two copies of *MYCN* (2p24) were seen. DNA methylation further classified this tumor as clustering near posterior fossa group A (score=0.6073) tumors. CONCLUSION: We report a unique case of an adolescent male with aggressive spinal ependymoma harboring focal *MYC* amplification. Testing for *MYC* amplification may be reasonable in newly-diagnosed spinal ependymomas to aid in characterization.

**EPEN-30. C11ORF95-RELA FUSION PROTEIN ENGAGES NOVEL GENOMIC LOCI TO DRIVE MURINE EPENDYMOMA GROWTH**

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RATIONALE: Over 70% of supratentorial (ST) ependymoma are characterized by an oncogenic fusion between C11ORF95 and RELA. C11ORF95-RELA fusion is frequently the sole genetic driver detected in ST ependymoma, thus ranking this genomic event as a lead target for therapeutic investigation. RELA is a transcription factor (TF) central to mediating NF-kB pathway activation in processes such as inflammation, cellular metabolism, and chemotaxis. HYPOTHESIS: We posited that C11ORF95-RELA acts as an oncogenic TF that aberrantly shapes the tumor epigenome to drive aberrant transcription. Approach: To this end we developed an *in utero* electroporation (IUE) mouse model of ependymoma to express C11ORF95-RELA during embryonic development. Our IUE approach allowed us to develop C11ORF95-RELA driven tumor models and cell lines. We comprehensively characterized the epigenome and transcriptome of C11ORF95-RELA fusion driven mouse cells by H3K27ac ChIP-seq, ATAC-seq, and RNA-seq. RESULTS: This data revealed that: 1) C11ORF95-RELA directly engages 'open' chromatin and is enriched at regions with known RELA TF binding sites as well as novel genomic loci/motifs, 2) C11ORF95-RELA preferentially binds to both H3K27ac (active) enhancers and promoters, and 3) Bound C11ORF95-RELA promoter loci are associated with increased transcription of genes shared with human ependymoma. CONCLUSION: Our findings shed light on the transcriptional mechanisms of C11ORF95-RELA, and reveal downstream targets that may represent cancer dependency genes and molecular targets.

**EPEN-31. SINGLE-CELL RNASEQ OF CHILDHOOD EPENDYMOMA REVEALS DISTINCT NEOPLASTIC CELL SUBPOPULATIONS THAT IMPACT ETIOLOGY, MOLECULAR CLASSIFICATION AND OUTCOME**

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Ependymoma (EPN) is a brain tumor commonly presenting in childhood that remains fatal in the majority of children. Intra-tumoral cellular heterogeneity in bulk-tumor samples significantly confounds our understanding of EPN biology, impeding development of effective therapy. We therefore used single-cell RNA sequencing to catalog cellular heterogeneity of 26 childhood EPN, predominantly from ST-RELA, PFA1 and PFA2 subgroups. ST-RELA and PFA subgroups clustered separately, with ST-RELA clustering largely according to individual sample-of-origin. PFA1 and PFA2 subgroup EPNs cells were intermixed and revealed 4 major subpopulations – 2 with characteristics of ependymal differentiation (transporter and ciliated phenotype subpopulations), an undifferentiated subpopulation and a mesenchymal phenotype. Pseudotime analysis showed the undifferentiated progenitor subpopulation either differentiating into ependymal differentiation subpopulations or transitioning into the mesenchymal subpopulation. Histological analysis revealed that undifferentiated and mesenchymal subpopulations cells colocalized to perinecrotic/perivascular zones, the putative ependymoma stem cell niche. Deconvolution of PFA bulk transcriptome data showed that undifferentiated and mesenchymal subpopulations were associated with a poor prognosis; whereas the ciliated ependymal cell-differentiated subpopulation was associated with a good prognosis. In conflict with current distinct classification paradigms, the ratio of mesenchymal and ciliated subpopulations determined bulk-tumor subgroups assignment to PFA1 and PFA2 respectively. This atlas of EPN cellular heterogeneity provides an important advance in our understanding of EPN biology, identifying high-risk associated subpopulations for therapeutic targeting.

**EPEN-33. PHARMACOGENOMICS REVEALS SYNERGISTIC INHIBITION OF ERBB2 AND PI3K SIGNALING AS A THERAPEUTIC STRATEGY FOR EPENDYMOMA**

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Subgroups of ependymoma, especially RELA fusion-positive and posterior fossa type A tumors, are associated with poor prognosis. Curative therapeutic strategies have not yet been identified. We set up a high-throughput drug screening (HTS) pipeline to evaluate clinically established compounds (n=196) in primary ependymoma cultures (n=12). As culturing ependymoma is challenging, assay miniaturization to 1536-well microplates emerged as a key feature to process HTS despite smallest cell numbers. DNA methylation profiling showed that entity and subgroup affiliation from primary diagnosis was maintained in primary cultures, as assessed through molecular neuropathology 2.0 based classification (MNP 2.0, Capper, D. et al., Nature, 2018). A comparison of HTS data of ependymoma and other pediatric brain tumor models (n=48) revealed a remarkable chemoresistance in vitro. However, we identified Neratinib, an irreversible ERBB2 inhibitor, as the most prominent candidate which was preferentially active in a subset of the investigated ependymoma cultures (n=5). Combinatory treatment with Copanlisib, a PI3K inhibitor, was able to overcome resistance to single agent treatment using Neratinib in established cell lines of ependymoma (n=3) and 2/4 primary cultures for which combinatory treatment could be tested. Finally, we validated efficacy of Neratinib combined with Copanlisib in mice bearing ependymoma xenografts which revealed significantly reduced tumor size compared to vehicle-treated animals. In summary, our study demonstrates that HTS may reveal targeted therapies for pediatric brain tumors. Specifically, we found a synergistic interaction of Neratinib and Copanlisib for treatment of ependymoma, thereby providing a novel therapeutic approach in an otherwise largely chemoresistant entity.

**EPEN-34. THE CRISPR-CAS9 SYSTEM-MEDIATED ENDOGENOUS GENE REARRANGEMENT INDUCED C11ORF95-RELA FUSION IN VITRO AND IN VIVO THAT LED TO THE DEVELOPMENT OF EPENDYMOMA-LIKE TUMOR**

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Recent large-scale genomic studies of ependymal tumors have identified recurrent RELA and YAP1 fusion genes in supratentorial ependymomas. The formation of the C11orf95-RELA fusion gene has been attributed to massive genomic rearrangement involving chromosome 11q termed Chromothripsis in many cases. However, the causal relationship has not been clarified experimentally. In this study, we developed a system to reproduce the oncogenic gene rearrangement using the CRISPR-Cas9 system and examined whether consequent endogenous ependymoma fusion genes are competent to form brain tumors in mice. Initially, to investigate whether C11orf95-RELA fusion can be formed by inducing the relevant gene rearrangement in vitro, we designed multiple guide RNAs on the human and mouse genomic loci and introduced them into cultured cells. RT-PCR and immunoblot analyses detected endogenous C11orf95-RELA fusion transcript and protein in both human and mouse cultured cells. Subsequently, we lentivirally introduced the gRNAs into a mouse brain. Brain tumor formation was observed from around 2 months after the lentivirus injection, thus indicating successful gene rearrangement followed by C11orf95-RELA fusion expression *in vivo*. Analysis of the tumor tissue con-