Our incomplete understanding of the key players in Medulloblastoma (MB) development and progression, and their roles in modulating highly immune desolate-like microenvironment in MBs present major hurdles in successfully applying existing therapies and developing new therapies for MBs. Here, we demonstrate that Yap1 acts as a critical modulator of SHH-MB (SmoM2; GfiaCre (SG) and Pchps53 SHH-MB mouse models) progression and immune evasion. Yap1 genetic deletion in SG mice significantly extends survival and normalizes brain development by increasing neuronal differentiation. Both bulk and single-cell RNA sequencing analyses show that Yap1 deleted tumors contain cells with more differentiated molecular signatures similar to late CGNPs and differentiating neurons, and less stem-like cells, compared to SG tumors. Additionally, integrated analyses of ChIPseq, RNAseq, and scRNAq data suggest that Yap1 directly binds to the Super enhancer region containing Sox2 and promotes Sox2 expression in SHH MB cells. We postulate that Yap1 expression is maintained or re-activated in SHH MB cells to generate long-term self-renewing tumor cells. Consistently, Yap1-deleted SHH MB or Verteportin (a small molecule inhibitor of Yap1) -treated Pchps53 MB cells lose self-renewal ability in vitro. Furthermore, we hypothesize that a molecular mechanism underlying this stemness promoting function is mediated through Sox2 expression. Intriguingly, Yap1 deletion in SG MBs is accompanied by a significant change in the immune microenvironment, when compared to age-matched SG MBs: a decrease in the number of bone marrow-derived immune cells (including cytotoxic T-cells, neutrophils, and macrophages). RNAseq analysis of rescued tumors shows marked enrichment of interferon-gamma response genes and pro-inflammatory cytokines. This study highlights Yap1 as a crucial mediator of MB progression and a potential therapeutic target in inflammatory immune cell infiltration into SHH MBs. Consequently, our work paves the way for improving immunotherapy treatments in brain malignancies.

**EPENDYMOMA**

**EPEN-01. C11ORF95-RELA DICTATES ONCOGENIC TRANSCRIPTIONAL PROGRAMS TO DRIVE AGGRESSIVE SUPRATENTORIAL EPENDYMOMA**

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Over 60% of supratentorial (ST) ependymomas harbor a gene fusion between C11orf95, an uncharacterized gene, and RELA (also known as p65), a main component of the NF-κB family of transcription factors. While its sufficiency to drive tumor has been established, the mechanism of tumorigenesis remains elusive. To tackle this question, we developed a natively forming mouse tumor model using in utero electroporation of the embryonic mouse brain and performed integrative epigenomic and transcriptomic mapping. Our findings indicate that in addition to direct canonical NF-κB pathway activation, C11orf95-RELA (CR60) dictates a neoplastic transcriptional program and leads to unique sites across the genome enriched with inflammatory immune cell infiltration into SHH MBs. Consequently, our work paves the way for improving immunotherapy treatments in brain malignancies.

**EPEN-02. FUNCTION AND DEPENDENCY OF NF-KB ACTIVITY IN C11ORF95-RELA FUSION EPENDYMOMA**

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Introduction: Ependymoma is an aggressive type of pediatric brain tumor resistant to chemotherapy, with treatment to date limited to surgical resection and radiation. Thus, identification and validation of molecular targets that can translate into clinical trials in ependymoma is desperately needed to improve patient outcomes. Over 70% of supratentorial ependymomas are driven by an oncogenic fusion between C11orf95 and Rela (denoted CR60). CR60 expression initiates ependymoma development in mice by potentially acting as an oncogenic transcription factor, disrupting gene expression programs. We hypothesized that specific CR60 interacting proteins are required for tumor formation and could represent lead therapeutic targets. Methods: To study CR60 ependymoma, a natively forming tumor model of CR60 was generated by in utero electroporation of the developing mouse brain. Tumors were then subjected to nuclear Rap Immunoprecipitation and Mass Spectrometry Analysis of Endogenous Proteins (RIME) of HA-tagged CR60 protein. Immunoprecipitation and Western Blot (IP-WB) were utilized to probe for novel protein interactions. Results: We identified several protein interactions consistent with canonical Rela mediated transcription (NFKB1 and NFKB2) as well as novel protein interactions that converged on RNA splicing and translational regulation. In addition, we identified a large series of novel chromatin-binding proteins. Conclusions: Further study is ongoing to validate the key CR60 protein interaction dependency on tumor development. ChIP-Seq (chromatin immunoprecipitation with massively parallel DNA sequencing) and CUT&RUN (cleavage under target and release using nuclease) assays are being employed to further characterize the transcriptional landscape of canonical Rela pathway members. By interrogating these mechanisms, novel therapeutic targets and pathways may be identified in parallel with dissecting the molecular basis of CR60 driven ependymoma.

**EPEN-03. ZFTA/C11ORF95 FUSIONS DRIVE SUPRATENTORIAL EPENDYMOMA VIA SHARED ONCOGENIC MECHANISMS**

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The majority of supratentorial ependymomas (ST-EPN) are driven by fusion genes between RELA and zinc finger transcription associated, ZFTA, previously named C11orf95. Apart from fusions with a portion of the histone effector YAP1, which affects a small group of infant patients, the oncogenic mechanism of remaining ST-EPNs remains unclear. Aiming at refining the oncogenic repertoire of ST-EPNs, we performed comprehensive analysis of endogenous proteins containing alternative translocations that shared ZFTA-RELAX3C-NFκB pathway dependency on tumor development. ChIP-Seq (chromatin immunoprecipitation with massively parallel DNA sequencing) and CUT&RUN (cleavage under target and release using nuclease) assays were employed to identify the transcriptional landscape of canonical Rela pathway members. By interrogating these mechanisms, novel therapeutic targets and pathways may be identified in parallel with dissecting the molecular basis of ZFTA-RELAX3C-NFκB pathway dependency on tumor development.
Abstracts

EPEN-04. SIOP EPENDYMOIMA I: FINAL RESULTS, LONG TERM FOLLOW-UP AND DEVELOPMENT OF A MOLECULAR AND SINGLE-CELL RNA-SEQ PROFILE OF A MOUSE MODEL OF EPENDYMOIMA

Introduction: Surgery and radiotherapy are established childhood ependymoma treatments. The efficacy of chemotherapy has been debated. We report final results of the SIOP Ependymoma I trial, with 12-year follow-up, in the context of a post-hoc analysis of more recently described biomarkers. Aims and Methods: The trial assessed event free (EFS) and overall survival (OS) of patients aged three to 21 years with non-metastatic intracranial ependymoma, treated with a staged management strategy targeting maximum local control. The study also assessed: the response rate (RR) of subtotally resected (STR) disease to vincristine, etoposide and cyclophosphamide, and surgical outcomes. Children with non-metastatic intracranial recurrence (GTR) received radiotherapy of 54 Gy in 30 daily fractions over six weeks, whilst those with STR received VEC before radiotherapy. We retrospectively assessed methylation and 1q status alongside GTR rates in 1999 and 2007, 89 participants were enrolled, 15 were excluded with metastatic (n=4) or non-ependymoma tumours (n=11) leaving a final cohort of 74. Five- and ten-year EFS was 49.3% and 46.7%, OS was 69.3% and 60.5%. 1q gain was associated with improved EFS (p=0.002, HR=3.00, 95% CI 1.49–6.10), bTERT expression was associated with worse five-year EFS (20.0% vs 83.3%, p=0.014, HR=5.8). GTR was achieved in 33/74 (44.6%) and associated with improved EFS (p=0.006, HR=2.81, 95% confidence interval 1.35–5.84). There was an improvement in GTR rates in the latter half of the trial (1999/2002 52.8%, versus 2003-2007 56.8%). Despite the protocol, 12 participants with STR did not receive chemotherapy. However, chemotherapy RR was 65.3% (19/29, 95% CI 45.7–82.1). Conclusions: VEC exceeded the RR was 65.5% (19/29, 95%CI 45.7–82.1). Conclusions: VEC exceeded the RR was 65.5% (19/29, 95%CI 45.7–82.1). Conclusions: VEC exceeded the RR was 65.5% (19/29, 95%CI 45.7–82.1). Conclusions: VEC exceeded the RR was 65.5% (19/29, 95%CI 45.7–82.1). Conclusions: VEC exceeded the