to better understand the biology and clinical phenotype. We summarize our institutional experience with spinal MPE including methylation-profiling. METHODS: A retrospective analysis of charts during the period of 2001 to 2019 of histologically proven MPE was done. We performed methylation profiling for 12 patients by Infinium MethylationEPIC Kit. RESULTS: 26 patients with spinal MPE were identified, median age of diagnosis was 34.2 years with a range of 11 to 59.9 years. Ten patients were below 30 years of age, lumbar spine location was commonest and 6 had leptomeningeal spread at diagnosis. All the patients underwent surgery and 11 received radiation following surgery. Eight patients below the age of 30 received radiation due to residual disease or metastases. Methylation profiling revealed 11,752 CpGs differentially methylated between the younger and older patients ($p < 0.05$), however only one CpG cg22496254 associated with gene NCAPG/DCAF16 (role in promoting mitosis) was detectable with FDR < 0.25 that overly methylated in the younger age group. This is a new finding in MPE. CONCLUSIONS: Spinal MPE is a rare spinal tumor. Our study though limited by numbers, showed younger patients had aggressive phenotype, most requiring radiation. Methylation profiling reaffirmed this finding and trend in the younger patients. Prospective studies in a larger cohort of patients with methylation profiling are needed to identify prognostic variables and new targets for treatment.

EPEN-11. ONGOING RESPONSE IN A MULTIPLY RELAPSED METASTATIC POSTERIOR FOSSA EPENDYMOMA A AFTER VORINOSTAT AND CONCOMITANT IRRADIATION

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BACKGROUND: Among the nine molecular subgroups of ependymoma identified, posterior fossa ependymoma A (PF-EPN-A) confers the worst prognosis. These tumors often relapse despite aggressive resection and irradiation, resulting in limited therapeutic options. Although the genomic profile of PF-EPN-A does not typically show any recurrent alterations; they demonstrate distinct epigenetic changes which can be targeted with modulators such as histone deacetylase (HDAC) inhibitors. These inhibitors have shown efficacy in pre-clinical studies in both their anticancer and radiosensitizing properties. CASE: We describe a male diagnosed with a posterior fossa ependymoma at 3 years of age. After initial therapy with resection and focal irradiation, he went on to have a number of recurrences requiring multimodal therapy. Most recently, he developed diffuse intraventricular and leptomeningeal disease not amenable to surgical intervention. Genetic evaluation demonstrated a BCOR mutation and methylation profile was consistent with PF-EPN-A. He received 23.4 Gray craniospinal irradiation with a 30.6 Gray boost to the nodular lesions. Vorinostat was given concomitantly for radio-sensitization in 2 week intervals for a total of 6 weeks. Serial imaging after irradiation revealed decreased tumor burden with almost complete resolution of disease at 15 months. Unfortunately, MRI at 18 months exhibited mild interval growth of 2 lesions. CONCLUSIONS: To our knowledge, this is the first report of a clinical response in a pediatric patient with PF-EPN-A following irradiation administered concomitantly with vorinostat therapy. This response highlights the importance of further studies evaluating this combination therapy and its potential use in this population.

EPEN-13. PRIMARY EXTRADURAL SACROCOCCYGEAL SUBCUTANEOUS MYXOPAPILAR EPENDYMOMA MISDIAGNOSED AS PILONIDAL CYST IN A 7 YEAR-OLD BOY: A CASE REPORT

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BACKGROUND: Ependymomas occur in the brain or spinal cord and rarely as an extraspinal variety at the sacrococcygeal region, separated from the spinal cord. This rare presentation is thought to originate from a group of heterotopic ependymal cells called the coccygeal medullary vestige. There are few reports of this occurrence in children. CLINICAL CASE: A 7-year-old male presented with a history of a soft mass arising in the sacrococcygeal area 3 years earlier, diagnosed as pilonidal cyst at primary level and treated with surgery twice, as this mass recurred the boy was sent to our hospital, a 3rd surgery was performed, all tumoral tissue was removed, no attachment with dural space was founded, pathology revealed myxopapilar ependymoma with positivity for PS100, EMA and Vimentin. After surgery a Follow up MRI of cranium and spine showed absence of disease, no radiotherapy neither chemotherapy was implemented. He has been on surveillance from 3 years now without recurrence. CONCLUSION: This report highlights the fact that pediatric ependymoma can have an extradural presentation and can be confounded with pilonidal cyst, total resection is needed to control the disease. Potential for recurrence or metastatic disease can continue 20 years from the time of primary tumor, so prolonged surveillance is important.

EPEN-14. GENERATION OF A C11ORF95-RELA FUSION TARGETING ANTIBODY AS A DIAGNOSTIC TOOL FOR SUPRATENTORIAL EPENDYMOMA

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Ependymomas account for 10% of paediatric brain tumours and arise in the ventricular walls of the central nervous system. Ependymomas were previously classified as one tumour type and all patients received similar treatment. However, recent genomic studies have identified nine different molecular subgroups of the disease, including one supratentorial subtype characterized by a novel fusion gene C11ORF95-RELA. When introduced into neural stem cells, this fusion is a potent driver of tumorigenesis and its presence in patient samples has previously also been shown to negatively correlate with overall survival. Accurate diagnosis of this subgroup is currently limited to sophisticated approaches such as break-apart FISH or RNA sequencing. Here, we report the generation of a C11ORF95-RELA Fusion-specific antibody that can be used for routine immunohistochemistry (IHC). Candidate antibodies were first selected using phage display and favourable leads were subjected to affinity maturation using ribosome display after a screening process involving immunoblotting and IHC. Further IHC-based screening of affinity-matured candidates using fusion-positive and -negative mouse tissue as well as human fusion-negative ependymoma tumour tissue produced one lead antibody. The antibody detects fusion-specific nuclear staining pattern on fusion-positive tissue and does not react with fusion-negative tissues. This candidate antibody is currently being tested on human fusion-positive ependymoma tissue. This accurate diagnostic tool holds great promise to transform the management of patients with supratentorial ependymoma.

EPEN-16. TRANSCRIPTIONAL REGULATORY CIRCUITRIES AS MOLECULAR TARGETS IN EPENDYMOMA

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Genomic sequencing has driven precision-based oncology therapy; however, genetic drivers remain unknown or non-targetable for many malignancies, demanding alternative approaches to identify therapeutic leads. Ependymomas comprise histologically similar tumor entities driven by distinct molecular mechanisms, such as fusion oncoproteins, genome-wide chromosomal instability, or disruption of DNA methylation patterns. Despite these differences, ependymomas resist chemotherapy and lack available targeted agents for clinical trial development. Based on our previous findings, we hypothesized that mapping chromatin landscapes and super enhancers (SE) could uncover transcriptional dependencies as targets for therapy (Mack, Pajtler, Chavez et al., Nature, 2018). To functionally test the requirement of these SE genes for ependymoma cellular growth, we designed a pooled RNA interference screen against 267 SE associated genes. Our screen was performed in one *C11ORF95-RELA*-fusion model and two PF-EPN-A models as controls in biological triplicates. As an indication that our screen was successful, positive controls scored among lead hits including *KIF11, BUB1B, PHF5A and MYC*. Importantly, we identified many subtype specific dependencies in both *C11ORF95-RELA*-fusion and PF-EPN-A models, thus revealing novel pathways that potentially govern subgroup-specific ependymoma cell growth. Further, several candidates detected across all ependymoma lines were also identified as pan-cancer dependencies or glioma/glioblastoma specific essential genes from the DepMap Cancer Dependency Gene Resource. Our findings reveal novel targets and pathways that are essential for ependymoma cell growth, which may provide new insight into therapeutic strategies against these aggressive brain tumors.

EPEN-17. FAVORABLE OUTCOME TO INTENSIVE CHEMOTHERAPY WITHOUT IRRADIATION IN INFANTILE METASTATIC EPENDYMOMA WITH A NOVEL MOLECULAR PROFILE: A CASE REPORT

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Metastatic disease at initial presentation of intracranial ependymoma is an uncommon occurrence with only rare reports of survival and is reportedly more prevalent in the youngest of children. Clinical and molecular characteristics associated with metastatic presentation, their prognostic implications, as well as optimal treatment options for such patients, have not been identified. CASE REPORT: A seven months old child presented with posterior fossa anaplastic ependymoma; following sub-total resection of primary tumor, a spine MRI revealed leptomeningeal dissemination along the cervical spinal cord and nerve roots of the cauda equina. The patient was successfully treated with five cycles of intensive induction chemotherapy (as per Head Start with high-dose methotrexate) followed by three sequential cycles of marrow-ablative chemotherapy and autologous hematopoietic progenitor cell rescue (AuHPCR) without irradiation; he is currently without evidence of the disease now 60 months following initial diagnosis. MOLECULAR/GENOMIC RESULTS: The patient was enrolled on a patient-centric comprehensive molecular profiling protocol, which included paired tumor-normal whole-exome sequencing, RNA sequencing of the disease-involved tissue, and DNA methylation classification. The genomic profile of the tumor was relatively unremarkable, revealing only a terminal gain of chromosome 3p and a terminal deletion of chromosome 22q, suggestive of an unbalanced translocation. Using RNA sequencing, we identified a novel *SPECC1L-RAF1* gene fusion. The tumor harbors unique transcriptomic and DNA methylation profiles, failing to discretely classify with well-established ependymoma subgroups. CONCLUSION: Use of genomic profiling techniques provides meaningful information for disease characterization allowing for further expansion of the molecular spectrum associated with malignant disease.

EPEN-18. CROSS-SPECIES GENOMICS IDENTIFIES *GLI2* AS AN ONCOGENE OF *C11ORF95* FUSION-POSITIVE SUPRATENTORIAL EPENDYMOMA

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The majority of supratentorial ependymomas (ST-EPN) are driven by fusions between *RELA* and a zinc finger containing gene, *C11orf95*. Apart from fusions to the Hippo effector *YAP1,* which affects a small group of infant patients, the oncogenic mechanism of remaining ST-EPNs is unclear. Aiming at refining the molecular classification of ST-EPNs, we analyzed methylation profiles, RNA and DNA sequencing results as well as clinical data in a cohort of 617 ST-EPNs. Unsupervised clustering analysis of DNA methylation data revealed four distinct clusters that formed in addition to the known molecular groups ST-EPN-RELA and –YAP1. Tumors within these additional clusters were characterized by fusions of *C11orf95* to numerous fusion partners different from *RELA,* e.g. *MAML2*, *MAML3*, *NCOA2* and *SS18*, suggesting a general role of *C11orf95* in tumorigenesis of ST-EPN. Transforming capacity of newly identified fusion genes was validated using an electroporation-based *in vivo* gene transfer technology. All fusion genes were sufficient to drive malignant transformation in the cerebral cortex of mice and resulting tumors faithfully recapitulated molecular characteristics of their human counterparts. We found that both, the partner gene and the zinc finger DNA binding domain of *C11orf95,* were essential to exert tumorigenesis. When exploring genes commonly upregulated in *C11orf95* fusion-expressing tumors of human and murine origin, the Sonic Hedgehog effector gene *Gli2* was identified as a promising downstream target. Subsequent co-expression of *C11orf95:RELA* and a dominant negative form of *Gli2* indeed hampered tumorigenesis. We thus propose *GLI2* as a potential therapeutic downstream target of *C11orf95* fusion-dependent oncogenic signaling in ST-EPN.

EPEN-20. EZHIP/CATACOMB COOPERATES WITH PDGF-A AND P53 LOSS TO GENERATE A GENETICALLY ENGINEERED MOUSE MODEL FOR POSTERIOR FOSSA A EPENDYMOMA

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BACKGROUND: PFA ependymoma is a pediatric brain tumor with only 30% long-term survival. Recently a gene called *CXORF67/EZHIP/CATACOMB* (henceforward: *CATACOMB*) was found to be overexpressed in PFA ependymoma. CATACOMB's mechanism of action has been found to be analogous to that of the H3K27M mutation as its expression reduces H3K27me3 via inhibition of PRC2 catalytic activity. METHODS: We infected NESTIN- or GFAP-expressing neonatal hindbrain progenitors with wild-type *CATACOMB* or a loss of function (LOF) point mutant (M406K), alone, with PDGFA, and with and without p53 deletion. RESULTS: CATACOMB overexpression alone or with p53 loss was insufficient to induce tumorigenesis. CATACOMB overexpression with PDGFA and p53 loss was sufficient to induce tumorigenesis using either the LOF mutant (M406K) or the wild-type CATACOMB in both cells-of-origin. The histology appeared more ependymoma-like when CATACOMB was expressed in GFAP-expressing progenitors. Median survival for the model initiated in NESTIN progenitors was 99.5 days for the CATACOMB mutant (n=26) group and 61 days for the CATACOMB wild-type (n=28; log-rank test p=0.0033). Median survival for the model initiated in GFAP progenitors were 144 days for the CATACOMB mutant (n=19) group and 65 days for the CATACOMB wild-type (n=21; log-rank test is P<0.0013). Immunohistochemistry for H3K27me3 demonstrated that CATACOMB wild-type tumors had reduced H3K27me3 compared to CATACOMB mutant tumors. CONCLUSIONS: Disrupting CATACOMB inhibitory activity toward PRC2 significantly increases survival in mice in both models, suggesting this activity plays a critical role in accelerating tumorigenesis. Ependymoma-like histology was more commonly observed in the model initiated in the GFAP-expressing progenitors.

EPEN-21. IMPAIRED NEURONAL-GLIAL FATE SPECIFICATION IN PEDIATRIC EPENDYMOMA REVEALED BY SINGLE-CELL RNA-SEQ

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