firmed the expression of the endogenous C11orf95-RELA fusion gene. These results suggested that a gene rearrangement is a primary mechanism to form the C11orf95-RELA fusion which is the direct driver of tumorigenesis. Our system to simulate a genomic event will provide significant insights into the understanding of the tumorigenic mechanism in ependymomas.

EPEN-35. PERITONEAL CARCINOMATOSIS OF ANAPLASTIC EPENDYMOMA: FIRST REPORTED CASE

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Peritoneal Carcinomatosis of anaplastic ependymoma is not a previously reported entity. The authors report on a child with multiple successfully treated brain and spine disease occurrences who subsequently develops carcinomatosis of the abdomen and no evidence of CNS recurrence. Ependymoma accounts for up to 10% of childhood CNS tumors diagnosed in the United States with a median age of 51–71 months. Typical locations are based on age. Disease is typically treated with surgical resection followed by radiation. The role of chemotherapy has not been proven but currently being examined with open clinical trials. We will describe patient's presentations, clinical treatment and recurrence with subsequent treatment and outcome at time of meeting.

EPEN-36. THE TREATMENT OUTCOME OF PAEDIATRIC SUPRATENTORIAL C11ORF95-RELA FUSED EPENDYMOMA: A COMBINED REPORT FROM E-HIT SERIES AND AUSTRALIAN NEW ZEALAND CHILDREN'S HAEMATOLOGY/ONCOLOGY GROUP

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AIM: Advances in molecular classification of paediatric ependymoma have been pivotal in improving risk stratification and understanding of this disease. *C11orf95-RELA* fused supratentorial ependymoma (ST-EPN) have been reported to have a poor outcome, with 10-year overall survival (OS) of 49% and progression free survival (PFS) of 19%. A cohort of patients from multiple international institutions with molecularly confirmed *C11orf95-RELA* fused ST-EPN were reviewed to assess their disease behaviour. METHOD: We reviewed patients with molecularly determined *C11orf95-RELA* supratentorial ependymoma diagnosed between 1999 – 2019. Demographic information, extent of surgical resection, use of radiotherapy and/or chemotherapy, disease recurrence, treatment at recurrence and clinical outcome data was collected. PFS and OS of all patients were estimated using Kaplan-Meier method. RESULTS: A total of 76 ST-EPN patients with *C11orf95-RELA fusion* were identified (median age: 7 years3 months, range: 5 months – 18 years7 months). 58 patients (76.3%) had complete surgical resection. 70 patients(92.1%) received radiotherapy. 55 patients(72.3%) received chemotherapy. The 10-year OS of *C11orf95-RELA* fused ST-EPN was 72.4% and PFS was 63.8%. In contrast, ST-EPN at a single institution with unconfirmed molecular status had an OS of 61.1% and PFS of 34.9%. CONCLUSION: Detailed molecular analysis identified distinct subgroups of patients with ST-EPN. Patients from this cohort with *C11orf95-RELA* methylation profiles had a significantly higher OS compared to previous reports and those with unconfirmed fusion status, emphasising the critical importance of complete molecular profiling to assist in treatment decision making. Complete molecular analysis in future prospective cohorts is essential for accurate risk stratification and treatment selection.

EPEN-37. TREATMENT OUTCOME OF RECURRENT EPENDYMOMA IN CHILDREN IN NORTHERN EGYPT

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INTRODUCTION: 1/3 of Ependymoma patients will develop recurrence with only 25% are long term survivors. Treatment is usually between surgery, radiotherapy or combinations. PATIENTS AND METHODS: Retrospective review of children with recurrent Ependymoma in northwest of Egypt between 2005 and 2019 in Alexandria School of medicine records. RESULTS: 27 patients were identified 19 of them after 2010. The median age is 9.7 years (1.5–19), with 16 males and 11 females. Pathology were 11 grade II Ependymoma and 16 anaplastic Ependymoma. 16 had gross residual disease after 1st surgery and 22 received radiotherapy initially at median dose of 53.5 Gy, 4 patients received suboptimal radiotherapy. The initial site was14 supratentorial tumors and 13 infratentorial. Median time to recurrence is 27.6 months(3–84), and recurrences were 17 local and 9 CSF disseminated, and one patient had recurrence at the scar with lung metastasis. At a median follow up of 56.6 months 14(51.8%) are still alive. Treatment was surgery only in 6(4 alive) radiotherapy alone in 2(1alive), combined in 15(9 alive) and 4 patients received neither. The best outcome were in patients with late local relapse treated with complete resection and CSI after 2010. Radiotherapy dose was between 54 to 57.3 Gy and one patient developed reirradiation injury at brain stem. 5 of the 14 living patients is having toxicity in form of hearing aids (4) and low TSH(1). CONCLUSION: Aggressive treatment of recurrent Ependymoma with surgery and radiotherapy is feasible and about half of the patients are salvageable.

EPEN-38. EZH2 INHIBITORY PROTEIN (EZHIP/CXORF67) EXPRESSION IS HIGHLY CONCORDANT WITH H3K27ME3 LOSS AND IS A PROMISING SURROGATE MARKER FOR POSTERIOR FOSSA TYPE A EPENDYMOMAS

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BACKGROUND: Gene expression and DNA methylation have identified 2 distinct clinicopathological subgroups among the WHO Grade II/III posterior fossa (PF) ependymomas (EPN), of which the PF-A molecular subgroup associates with poor outcome. OBJECTIVE: To analyse the utility of immunohistochemistry for H3K27me3, Tenascin C, EZHIP (Cxorf67), EZH2 and fluorescence-in-situ-hybridisation for chromosome 1q21 locus gain in the prognostic stratification of PF-EPNs. METHODS: All PF Grade II/III tumors were retrieved (2009–2019). Immunohistochemistry for H3K27me3, H3K27M-mutation-specific antibody, EZH2, EZHIP, Tenascin-C and fluorescence in-situ hybridisation for 1q21 locus was performed and compared with outcome. RESULTS: 71 PF-EPNs were included. H3K27me3 loss (PF-A) was seen in 65% (46/71) of cases, of which majority were positive for EZHIP (73%, 24/33) and Tenascin C (65%, 28/43). Minority showed chromosome 1q gain (19%, 8/42). An EZHIP negative PF-A tumor was immunopositive for H3K27M-mutant staining, while all others were negative. PF-A EPNs occurred at a median age of 4.5 years (range 1–53), were predominantly grade III (Grade III:II – 1.6:1), and 50% (10/20) of patients on follow-up experienced tumor progression. EPNs with retained H3K27me3 (PF-B) did not show EZHIP expression (0/20) or 1q gain; however, tenascin C expression was seen in 47% (8/25) of them. They occurred predominantly in adults, showed Grade II preponderance and only 2/11 patients on follow-up experienced progression. EZH2 expression did not correlate with H3K27me3 loss but positively correlated with EZHIP expression (p=0.015). CONCLUSION: H3K27me3 is a reliable surrogate for prognostic classification of PF-EPNs. EZHIP expression is highly concordant with H3K27me3 loss and is a valuable adjunct.

EPEN-39. CLINICAL STRATIFIED TREATMENT OF LOCALIZED PEDIATRIC INTRACRANIAL EPENDYMOMA WITH COMBINED LOCAL IRRADIATION AND CHEMOTHERAPY WITHIN THE PROSPECTIVE, MULTICENTER E-HIT TRIAL – THE MOLECULAR SUBGROUP MATTERS

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BACKGROUND: Pediatric ependymoma is a heterogenous disease. Subgroup-specific clinical information on prospectively treated patients will help to improve treatment stratification. METHODS: Within the population based, prospective, multicenter E-HIT-trial (2001–2011) patients with localized ependymoma confirmed by neuropathological central-review, received hyperfractionated local radiotherapy (68Gy, 2x1Gy/day) followed by chemotherapy (stratum-A), or chemotherapy followed by local radiotherapy (54Gy, 1.8Gy/day) (children < 4years, stratum-B), or age-adapted radiotherapy with pre-/post-irradiation chemotherapy (residual tumor, diagnosis after 2005, stratum-C). Retrospective classification of DNA-methylation was available for n=164 E-HIT-trial participants, and n=80 patients with comparable treatment and prospective registration in the subsequent HIT-interim-registry (2012–2014). FINDINGS: For 291 E-HIT-trial patients, 5-year progression-free (PFS) and overall survival (OS) were 61±3%, and 81±2%. Five-year PFS/OS after complete resection were 71±4% and 87±3% in stratum-A (n=127), and 64±5% and 86±4% in stratum-B (n=86). Outcome was poor after incomplete resection, irrespective of treatment-stratum (n=78, 5-year PFS/OS: 43±6%, 68±5%). In the pooled trial- and registry-cohort, there were 152 patients with PF-EPN-A (5-year PFS/OS: 44±4%, 77±4%), 40 of them with 1q-gain (5-year PFS/OS: 28±7%, 66±8%), 21 with PF-EPN-B (5-year PFS/OS: 90±7%, 100%), 59 with ST-EPN-RELA (5-year PFS/OS: 63±7%, 87±5%), and 4 with ST-EPN-YAP1 (2 progression/relapse, no death). CONCLUSION: Outcome differed between molecular subgroups and insufficient survival rates were achieved for patients with PF-EPN-A with 1q-gain, despite combined radio- and chemotherapy treatment. Treatment reduction in the context of a clinical trial may be considered for PF-EPN-B.

EPEN-41. C11ORF95-RELA FUSION REGULATES ABERRANT GENE EXPRESSION THROUGH THE UNIQUE GENOMIC BINDING SITES FOR EPENDYMOMA FORMATION

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A majority of supratentorial ependymoma is associated with recurrent C11orf95-RELA fusion ($RELA^{FUS}$). The presence of RELA as one component of the $RELA^{FUS}$ leads to the suggestion that NF-kB activity is involved in the ependymoma formation, thus being a viable therapeutic target in these tumors. However, the oncogenic role of another C11orf95 component in the tumorigenesis is not still determined. In this study, to clarify the molecular mechanism underlying tumorigenesis of $RELA^{FUS}$, we performed $RELA^{FUS}$-ChIP-Seq analysis in cultured cells expressing the $RELA^{FUS}$ protein. Genomic profiling of $RELA^{FUS}$ binding sites pinpointed the transcriptional target genes directly regulated by $RELA^{FUS}$. We then identified a unique DNA binding motif of the $RELA^{FUS}$ different from the canonical NF-kB motif in de novo motif discovery analysis. Significant responsiveness of $RELA^{FUS}$ but not RELA to the motif was confirmed in the reporter assay. An N-terminal portion of C11orf95 was sufficient to localize in the nucleus and recognizes the unique motif. Interestingly, the $RELA^{FUS}$ peaks concomitant with the unique motif were identified around the transcription start site in the $RELA^{FUS}$ target genes as previously reported. These observations suggested that C11orf95 might have served as a key determinant for the DNA binding sites of $RELA^{FUS}$, thereby induced aberrant gene expression necessary for ependymoma formation. Our results will give insights into the development of new ependymoma therapy.

EPEN-42. MOLECULAR PROFILING REVEALS DISTINCT SUBGROUPS OF PEDIATRIC SPINAL EPENDYMOMA

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Paediatric spinal ependymomas are important, albeit uncommon, malignant central nervous system tumours. Unlike adults, children with these tumours are likely to experience a more aggressive disease course, with a higher rate of local failure and a higher rate of metastases. The clinical and molecular factors underlying these differences remain poorly characterized. We analyzed spinal ependymoma (SEPN) tumour samples from 27 paediatric patients (female: 11, male: 15; age range: 4–18 years) using genome-wide DNA methylation profiling, copy-number analysis, as well as transcriptome profiling. Using DNA methylation profiles, two distinct unsupervised consensus-clustering approaches, hierarchical clustering and non-negative matrix factorization reliably identified two subgroups. These subgroups were designated as Myxopapillary ependymomas (SP-MPE) and spinal ependymomas (SP-EPN) based on the online Classifier tool (MNP2.0). The genome-wide copy-number analysis showed differences in numbers and pattern of copy-number alterations between these groups. The gain of chromosome 20 (39%) followed by loss of chromosomes 6 (28%), 10 (28%), and 13 (28%) were detected in the SP-MPE group, whereas loss of chromosome 22 was frequent (60%) in the SP-EPN group. Transcriptomics analysis showed that genes associated with oxidative phosphorylation, TCA cycle components, electron transport, and Interferon-gamma production characterize the SP-MPE group whereas potassium ion import and regulation of astrocyte differentiation characterize the SP-EPN group. Western blot analysis validated the increased protein expression of oxidative phosphorylation complexes in SP-MPE. With this study, we provide a foundation for further molecular characterization of pediatric SEPN subgroups. Our results suggest that mitochondrial oxidative phosphorylation may drive the regulation of energy metabolism of SP-MPE tumours.

EPEN-43. TARGETING INTRA-TUMOUR HETEROGENEITY IN PAEDIATRIC EPENDYMOMA: AN INTEGRATED OMICS STUDY TOWARDS PATIENT-TAILORED THERAPY

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Ependymoma (EPN) is the second most common malignant paediatric brain tumour, which despite extensive genomic sequencing, no novel therapeutic options have been discovered. Multi-omics are anticipated to reveal dysregulated pathways that may be predictive of patient-specific biomarkers. Given the close association between gene expression, active biochemical signaling and metabolism, there is an unmet scientific challenge to determine whether EPN gene expression correlates with aberrant metabolic pathways, thus presenting therapeutic vulnerabilities. We first compared two distinct subgroups of EPN, PF-A and ST-RELA, identifying 115 metabolites and 1580 upregulated genes between the two subgroups, therefore validating previously reported genetic clustering of these two subtypes. We next integrated transcriptomics and metabolomics, comparing 28 intra-tumour tissue regions from eight primary PF-A EPN patients. Polar metabolites and RNA were simultaneously extracted from the same population of cells. RNAseq identified dysregulated genes and liquid chromatography-mass spectrometry (LC-MS) detected 98 significantly altered metabolites between 18 multi-regions, the majority mapping onto the arginine and proline pathways. Integration of genes and metabolites using pathway-based network analysis revealed 124 aberrant gene-metabolite interactions between intra-tumour regions, with large numbers occurring in the glucogenesis and glycine metabolic pathways in 6/8 patients. These may represent ubiquitous and therapeutically relevant metabolic pathways critical for EPN survival. Additionally, patients presented at least one unique intra-tumour genomic-metabolomic interaction, applicable for patient-tailored therapy. This is the first exploration of EPN multi-omic integration and intra-tumour heterogeneity. Selected drug targets predicated on aberrant gene-metabolite networks will be validated in multi-region patient-derived cell lines and orthotopic models using repurposed therapeutics.