dren. In the last few years, tumor classification through DNA methylation profiling has been demonstrated to be a powerful diagnostic tool which could be especially informative in this setting. METHODS: We reviewed original diagnosis and molecular profile of childhood CNS embryonal tumors other than medulloblastoma or AT/RT from a retrospective single-center cohort. Sixteen FFPE tissue samples from 14 unique patients (diagnosed from 1996 to 2017) were analyzed using DNA methylation arrays and matched with the Heidelberg classifier. Then, cohort characteristics and outcome were re-evaluated according to the results of the array. RESULTS: Median age at diagnosis was 2.7 years; there was no statistically significant difference between ETMRs and CNS embryonal tumors, NOS. Male to female ratio was 4:3. Median OS was 17,5 months (IQR 10.2-103.3 months) and ETMRs presented the worst outcome. Methylation profiling matched with an adequate score in 50% of samples (8/16). DNA methylation profile was consistent with ETMR in two samples but only one showed amplification of C19MC. Seven CNS embryonal tumors, NOS were properly reclassified as supratentorial ependymoma and diffuse pediatric-type HGG (4 and 1) or better defined as CNS neuroblastoma, FOXR2-altered (2). Methylation profiling added a unique diagnostic contribution in 64.3% of all cases (9/14). After the integration of methylation array results, survival markedly differed according to the novel integrated diagnoses; supratentorial ependymomas presented the longest median OS while no patients refined as CNS neuroblastoma or HGG survived. CONCLUSIONS: Our study confirmed that DNA methylation profiling provides relevant information for the classification of rare neoplasms like CNS embryonal tumors. Especially for selected cases with ambiguous histology, implementation of this tool should be considered to improve diagnostic precision and tailor patients' management.

ETMR-08. TREATMENT STRATEGY FOR PINEOBLASTOMA IN INFANT

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Pineoblastoma is a rare malignant brain tumor that occurs in infancy and young adulthood. Although its prognosis has improved in recent years, it remains one of the difficult tumor types to treat. We will retrospectively review the treatment of pineoblastoma at our hospital and propose the possibility of a new treatment for this tumor type. Three cases were studied. All of them presented at less than three years of age and were treated for hydrocephalus simultaneously as the biopsy. Chemotherapy was administered after a possible resection, and local radiotherapy was administered at the age of 3 years. Overall survival ranged from 7 to 91 months, with one case of long-term survival. To date, the prognostic factors for pineoblastoma are the age of onset and the presence of radiation therapy. This is interpreted to mean that the prognosis is worse in infants and young children who cannot be immediately treated with radiation therapy, indicating that radiation therapy is essential for treating this tumor type. On the other hand, radiation therapy for infants can significantly interfere with the development of the central nervous system, and there is much controversy about its potential compatibility with tumor control. We have identified a favorable prognosis group based on the molecular biological background of this tumor type. We propose that early radiotherapy may improve the prognosis.

ETMR-09. *IN VITRO* MODELLING OF EMBRYONAL TUMORS WITH MULTILAYERED ROSETTES (ETMR) AND OTHER NOVEL BRAIN TUMOR TYPES.

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Over the last decade, molecular characterization has resulted in many tumors previously classified as central nervous system primitive neuroectodermal tumors (CNS-PNETs) now being classified into their own distinct tumor types. These novel types are often characterized by very specific genomic aberrations. For instance, embryonal tumors with multilayered rosettes (ETMR) harbor amplifications of miRNA cluster *C19MC* or complex *DICER1* mutations, while in CNS neuroblastoma with *FOXR2* activation structural aberrations result in aberrant *FOXR2* expression. Despite the presence of distinct oncodrivers, our understanding of these tumors is still limited. To elucidate tumor biology and to discover tumor specific treatments, we need to uncover how these oncodrivers contribute to tumorigenesis. However, a bottleneck in basic and translational research of these novel tumor types, is the lack of representative preclinical models, especially in vitro. To overcome this hurdle, we aim to mimic tumor development in genetically modified brain organoids. Human brain organoids derived from pluripotent stem cells are generated to represent either the developing forebrain or cerebellum. To mimic oncodriving events, DNA plasmids are introduced via electroporation into the proposed cell-of-origin populations to knockout tumor suppressor genes or overexpress oncogenes. By detecting fluorescent proteins encoded by the plasmids, electroporated cells are followed over time. Based on our preliminary data, for instance, overexpression of *C19MC* results in ectopic expansion of the electroporated cells. Ongoing histological and molecular characterizations, including (single cell) transcriptomic and epigenomic analyses, will reveal to which extend these organoid models resemble the specific human tumor types. Although further validation is required, these organoid models provide a novel avenue to study especially brain tumor types with distinct oncodriving events for which patient-derived models have not yet been established. They also allow for in-depth analyses of the potential cells of origin and the contribution of different mutations to tumor biology.

ETMR-10. RETROSPECTIVE MOLECULAR RE-EVALUATION OF CNS PNETS; A POPULATION-BASED STUDY

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BACKGROUND: The heterogeneous group of tumors, primitive neuro-ectodermal tumors of the central nervous system (CNS-PNETs), is a group of rare childhood embryonal tumors associated with a poor prognosis. In recent years, molecular analyzes have shown that CNS-PNETs consist of high-grade gliomas (HGG), ependymomas, different embryonal entities like atypical teratoid /rhabdoid tumors (AT/RT), CNS neuroblastoma FOXR2 and embryonal tumor with multi-layered rosettes (ETMR). Each of these tumor types is unusual and long-term clinical follow-up data are sparse. METHODS: We retrospectively re-evaluated all children (0-18 years old) diagnosed with a CNS-PNET in Sweden during 1984-2015. In total, 88 supratentorial CNS-PNETs were identified in the Swedish Childhood Cancer Registry and from these formalin-fixed paraffin-embedded tumor material was available for 69 patients. All tumors were reviewed histopathologically by an experienced neuropathologist and were analyzed using genome-wide DNA methylation profiling and classified by the MNP brain tumor classifier. RESULTS: The largest entities, after re-evaluation, were HGG (30%), CNS NB-FOXR2 (12%), AT/RT (10%) and ETMR (8%). Some tumors were difficult to classify and will be further evaluated molecularly. Some examples: Best treatment results were seen for patients with CNS-NB FOXR2 (5-year PFS: 100%) where all patients had received craniospinal radiotherapy (CSI). Patients with ETMR were all very young and survival data show early progression and poor survival (5-year OS 34%). CONCLUSIONS: Although the patient material is relatively small, it is population-based with long follow-up times. Our findings are in line with other studies and shows that CSI is important for cure for CNS-NB FOXR2 and that intensive multi-modal therapies needs to be evaluated in up-front studies for these rare embryonal tumors.

ETMR-11. TRANSCRIPTIONAL CHANGES UPON KNOCKDOWN OF ALTERED *BCOR/BCORL1* TRANSCRIPTS IN PRECLINICAL MODELS OF CNS EMBRYONAL TUMORS WITH BCOR-RELATED ALTERATIONS

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BCL-6 transcriptional corepressor (*BCOR*) is an epigenetic regulator that silences gene expression mainly via the polycomb repressive complex 1.1 (PRC1.1). *BCOR* genomic alterations are found in a variety of different tumors and recently central nervous system (CNS) tumors with *BCOR* internal tandem duplication (ITD) were classified as a distinct molecular subgroup. We established and characterized two cell models derived from *BCOR* altered CNS tumor patients. One model is characterized by a frameshift mutation in the *BCOR* gene resulting in the expression of a truncated protein lacking the C-terminal PUFD domain required for correct assembly of the PRC1.1. Additionally, this model harbors a translocation of the *BCOR* homologue *BCORL1*. The second model has a characteristic internal tandem duplication (ITD) within the *BCOR* gene. To study the effects of mutated *BCOR/BCORL1* on gene expression, we performed siRNA mediated knockdown of altered *BCOR/BCORL1* transcripts in both models and analyzed transcriptional changes by mRNA expression array. Differentially expressed genes in *BCOR/BCORL1* knockdown versus wild type conditions were enriched for signaling pathways involved in cell cycle progression, cell growth, DNA replication and cancer. This suggests that the alterations in *BCOR/BCORL1* might have pro-oncogenic effects and thereby contribute to the aggressive phenotype of this disease. Especially in the *BCOR* ITD model knockdown of *BCOR* led to transcriptional downregulation of genes associated with the development of brain tumors such as *FGF18*, *PDGFA* and *PDGFRA*. Our results indicate that specific *BCOR/BCORL1* alterations might impair its endogenous function as transcriptional repressor and deregulate the expression of multiple PRC1.1 target genes. An in depth characterization of epigenetic and transcriptional changes in *BCOR/BCORL1* altered CNS tumors could lead to the identification of critical downstream effectors and ultimately reveal new therapeutic vulnerabilities.

ETMR-12. NOVEL CELL MODELS OF CNS TUMORS WITH BCOR FUSION OR INTERNAL TANDEM DUPLICATION SUGGEST FGFR AND PDGFR AS PROMISING THERAPY TARGETS

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Central nervous system (CNS) tumors with *BCOR* internal tandem duplications (CNS-BCOR ITD) are aggressive malignancies recently included in the 2021 WHO Classification of CNS tumors. This entity is characterized by ITDs within the PUFD domain of *BCOR*, potentially interfering with protein-protein interactions and preventing non-canonical polycomb repressive complex 1.1 (ncPRC1.1) complex formation. Additionally, other *BCOR* alterations like frame shift mutations and gene fusions have been described. However, the underlying molecular mechanisms promoting tumor aggressiveness remain unknown. We established cell models from one patient harboring a *BCOR* frameshift mutation and another one with a concomitant *BCORL1*-fusion. Two additional models were derived from a patient with a CNS-BCOR ITD tumor. Multidrug screening uncovered high sensitivity against defined receptor tyrosine kinase (RTK) inhibitors (TKIs). In detail, ponatinib, nintedanib, and dovitinib reduced cell viability at half maximal inhibitory concentrations (IC50) in the low micro-molar range (<2.5 µM). Expression analyses of the respective TKI targets suggested fibroblast growth factor receptor 3 (FGFR3) and platelet derived growth factor receptor A (PDGFRA) as central players in this response. RTK inhibition resulted in strongly impaired downstream MAPK and Pi3K/AKT signaling. Vice versa, exposure to the RTK ligands bFGF and PDGFAA increased S6, Erk and Akt phosphorylation. Next, we treated two patients – one with a *BCOR* frame shift mutation/*BCORL1*-gene fusion and one with an ITD with nintedanib – within a multimodal treatment approach and achieving complete remission and disease stabilization, respectively. Ultimately, we analyzed respective RTK ligands in patient cerebral spinal fluid (CSF) and found FGF18 and PDGFA to correlate with tumor treatment response and progression. Summarizing, we uncover a central role of defined RTK signaling modules in the malignant phenotype of CNS-BCOR-ITD and tumors harboring *BCOR* alterations and elucidate their potential as therapeutic targets. Currently, we aim to dissect the interconnection between *BCOR/BCORL1* alterations and RTK hyperactivation.

ETMR-13. EMBRYONAL TUMOR WITH MULTILAYERED ROSETTES IN AN INFANT: CASE REPORT

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Embryonal tumor with multilayered rosettes (ETMR) is a highly malignant tumor (WHO grade 4) seen predominantly in infants. It includes morphologically distinct embryonal tumors namely, embryonal tumor with abundant neuropil and true rosettes, ependymoblastoma, and medulloepithelioma. The presence of multilayered rosettes and C19MC amplification at chromosome 19q13.42 confirms the diagnosis. The median overall survival is less than a year and the prognosis is generally poor. We report the case of a 1-year-old girl who presented with vomiting, lethargy, and increasing head circumference over a period of six months. On admission, she was drowsy and irritable. Verbal output was limited to moans and motor response was localizing. She was macrocephalic with a head circumference of 51 cm. MRI showed a large 5 x 5 x 6.5cm contrast-enhancing cerebellar vermian tumor with obstructive hydrocephalus. There was no evidence of leptomeningeal disease or spinal metastasis at this time. She underwent a right frontal ventriculoperitoneal shunt insertion, followed by suboccipital craniotomy and subtotal tumor resection one week later. Her shunt was ligated two days after tumor excision, due to development of bilateral subdural hygromas. The patient regained full consciousness, but still had spastic lower extremities and inability to swallow at the time of discharge. Histopathology and immunostains were consistent with an embryonal tumor, possibly ETMR, and the patient was for advised chemotherapy. Before initation of chemotherapy, the patient was admitted in another instution because of alteration in sensorium. Repeat imaging showed progression of the patient's subdural hygromas, requiring insertion of a subduroperitoneal shunt. The patient died seven weeks after tumor resection due to progression of her tumor residual. Management options for ETMR are limited, especially in low- and middle-income countries. International linkages may help facilitate the accurate diagnosis and early treatment of these patients with rare but aggressive brain tumors.

ETMR-14. THE SINGLE-CELL LANDSCAPE OF PINEOBLASTOMA IDENTIFIES DEVELOPMENTAL ORIGINS AND EXPOSES NOVEL THERAPEUTIC VULNERABILITIES.

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Pineoblastoma (PB) is a rare and aggressive childhood brain tumor with highly variable age and treatment-associated outcomes. Our recent bulk tumor analyses of DNA methylation and mutational landscapes uncovered four discrete PB molecular subgroups (PB-miRNA1, PB-miRNA2, PB-MYC/FOXR2, and PB-RB), providing a major advance in our understanding of biological and clinical heterogeneity. However, developmental origins of PB subgroup heterogeneity and mechanisms governing how specific genetic alterations promote malignancy remain unknown. To resolve the cellular origins of PB, we assembled a large single-nucleus RNA-sequencing cohort (n=32) of primary PB tumors, including representatives from each subgroup. Transcriptomic analysis identified subgroup-specific gene expression programs driving intra-tumoral heterogeneity. In addition, we discovered substantial differences in the expression of miRNA biogenesis genes between the PB-miRNA1 and PB-miRNA2 subgroups, providing mechanistic support for their distinct subgroup identities despite overlapping driver events. The MYC/FOXR2 subgroup was characterized by over-expression of the FOXR2 proto-oncogene in bulk RNA-seq, which we validated in single-nuclei and identified co-expressed downstream