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 diagnostic material and profile of children with 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 1962 to 2017) were analyzed using DNA methylation arrays and matched with the corresponding tissue. Then, cohort of neuroectodermals, including (single) genomic aberrations and expression profiles 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, NOH. Male to female ratio was 4:1. Methylation index (MIQ) was 15.3 months and ETMRs presented the worst outcome. Methylated 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, NOH were properly reclassified as supratentorial ependymoma and diffuse pediatric-type HGG (4 and 1) or better defined as CNS neuroblastoma, FOXR2-altered (2). Methylated 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 ependymoma presented the longest median OS while no patients refined as CNS neuroblastoma or HGG survived. CONCLUSION: 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

MARIO SUNIZU1, TUZABURO SHINIMIZU2, OSAKI AKIYAMA1, JUNYA FUJIMURA2, ACHIM HEBEL2, DIEM KIM NHU3, STATHI POLYCHRONOPOULOS1, ZELDA ODE4, JORIS MAAS5, MARCEL KOOL1,2, PRINCESS MAXIMA CENTER FOR PEDIATRIC ONCOLOGY, Utrecht, Netherlands. 2HOPP CHILDREN’S CANCER CENTER (KITZ), HEIDELBERG, GERMANY. 3DEPARTMENT OF NEUROSURGERY, JUNTEDO UNIVERSITY FACULTY OF MEDICINE, TOKYO, JAPAN. 4DEPARTMENT OF PEDIATRICS AND ADOLESCENT MEDICINE, JUNTEDO UNIVERSITY FACULTY OF MEDICINE, TOKYO, JAPAN.

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 3 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. Understanding that radiation therapy is essential for treating this tumor type. On the other hand, radiotherapy 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

JENS BOUT1, MIEKE ROOSSEN1, PHYLCIA STATH1, PANAGIOTIS POLYCHRONOPOULOS1, ZELDA ODE4, JORIS MAAS5, MARCEL KOOL1,2, PRINCESS MAXIMA CENTER FOR PEDIATRIC ONCOLOGY, Utrecht, Netherlands. 2HOPP CHILDREN’S CANCER CENTER (KITZ), HEIDELBERG, GERMANY.

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 tumors 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 oncocdriving events, DNA plasmids are introduced via electroporation into the proposed cell-of-origin populations to knockout tumor suppressor genes or overexpress oncogenic drivers, depending on the tumor type. These tumors are then reprogrammed as organoids and analyzed for their response to a variety of treatments, including (single) genomic aberrations, 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 (0-11 years old) who received craniospinal radiotherapy (CSI) 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-NOX2R 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

MARTIN PIONTEK1,2, DOMINIK KIRCHHOFFER1, LISSA GABLER1,2, DANIELA LOTTSCH-GEJO1, CHRISTINE PIKTER2, WALTER REGEN1, CHRISTIANE MEDIHOFER1,2, DANIELA LOTTSCH-GEJO1,2, DANIELA MACIERECK1,2, WALTER REGEN1, CHRISTIANE MEDIHOFER1,2, DANIELA LOTTSCH-GEJO1,2, DANIELA MACIERECK1,2, WALTER REGEN1, CHRISTIANE MEDIHOFER1,2, DANIELA LOTTSCH-GEJO1.
ETM12. NOVEL CELL MODELS OF CNS TUMORS WITH BCOR FUSION OR INTERNAL TANDEM DUPLICATION SUGGEST FGFR AND PDGFR AS PROMISING THERAPY TARGETS

Pierangelo Kerschbaumer1, Daniela Lützhöft2, Lisa Gabler4, Carola N. Jaenecker1, Martin Ponttek2, Lisa Mayz3, Bernhard Engelger1,4, Christine Pirker1, Thomas Mohr1,2, Anna Lämmerer1,2, Felix Schmitt-Hofner1,2, Bernd Boedt1, Stefan Kubicek2,3, Andreas Pfeiff1, Amadeo A. Azor1, Christian Dorfer1, Christine Haberer1,2, Marcel Kool1, Walter Berger1, Johannes Gogo1
1Center for cancer research (CCR), Comprehensive Cancer Center (CCC), Medical University of Vienna, Vienna, Vienna, Austria. 2Department of Pediatrics and Adolescent Medicine, Comprehensive Cancer Center Vienna, Vienna, Vienna, Austria. 3Department of Oncology, Dana-Farber/Boston Children’s Cancer and Blood Disorders Center, Boston, Massachusetts, USA. 4ScienceCenter, DKTK, Heidelberg, Germany. 5Division of Pediatric Neurooncology, German Cancer Research Center (DKTK), Heidelberg, Baden-Württemberg, Germany. 6Center for Molecular Medicine (CeMM), Austrian Academy of Sciences, Vienna, Vienna, Austria. 7Christian Doppler Laboratory for Chemical Epigenetics and Anti-Infectives, Vienna, Vienna, Austria. 8Institute of Neuroradiology and Neurochemistry, Department of Neurology, Vienna, Vienna, Austria

Central nervous system (CNS) tumors with BCOR internal tandem duplication (ITD) are aggressive malignancies recently included in the 2021 WHO Classification of CNS tumors. This entity is characterized by ITDs within the PUF domain of BCOR, potentially interfering with protein-protein interactions and preventing canonical polycomb repressive complex 1 (ncPRC1) 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 frame shift mutation and another one with a concomitant BCOR/BCORL1-fusion. Two additional models were derived from a patient with a CNS-BCOR ITD tumor. Multigrid 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 (PDGFRA) as central players in this response. RTK inhibition resulted in stiffly impaired downstream MAPK and AKT signaling. Vice versa, exposure to the RTK ligands hFGF and PDGFAA increased S6, Erk and Akt phosphorylation. Next, we treated two patients – one with a BCOR frame shift mutation/BCOR/BCORL1-fusion and one with an ITD with nintedanib – with a multimodal treatment approach and achievement of complete remission and disease stabilization, respectively. Ultimately, we analyzed respective RTK ligands in patient cerebral spinal fluid (CSF) and found FGF18 and PDGFα to correlate with tumor treatment response and progression. Summarizing, we uncover a central role of defined RTK signaling modulation 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.

ETM13. EMBRYONAL TUMOR WITH MULTILAYERED ROSETTES IN AN INFANT: CASE REPORT

Lars Sandulf Xu, Eric Paolo Palabay, Ronnie Baticulan, JRMMCC, Manila, Philippines

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 neaurpil and true rosettes, ependymoblastoma, and medulloepithelioma. The presence of multilayered rosettes and C19MC amplification at chromosome 19q13.42 are markers that are highly correlated with this tumor. 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. She refused oral feeding and motor responses seemed decreased. She was macrocephalic with a head circumference of 51 cm. MRI showed a large 5 x 5 x 6.3cm contrast-enhancing cerebellar vermician tumor with obstructive hydrocephalus. There was no evidence of leptomeningeal disease or spinal metastasis at this time and the patient was referred for suboccipital craniotomy and subtotal 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 subtle lower extremities weakness which slowly improved.

Histopathology and immunostains were consistent with an embryonal tumor, possibly ETMR, and the patient was advised chemotherapy. Before initiation of chemotherapy, the patient was admitted in another institution because of altered sensorium. Repeat imaging showed progression of the patient’s subdural hygromas, requiring insertion of a subduralperitoneal 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.

ETM14. THE SINGLE-CELL LANDSCAPE OF PINEOBLASTOMA IDENTIFIES DEVELOPMENTAL ORIGINS AND EXPOSES NOVEL THERAPEUTIC VULNERABILITIES

Brian Gunderman1, Bernhard Engelger2, Anthony P.Y. Liew3,5, Steven Lammer Alumad2, Elke Pfitzner4, Lea Paul1, Jennifer Hadley1, Melissa Batts1, Paul Klimo Jr.1,6, Frederick A. Boop1,8, Amar Gajjar1, Giles Robinson1, Brent Orr7, Hong Lin1, Sandra Alexandrescu1, David T.W. Jones1,4, Mariella Gajjar, Paul A. Northcott7, St. Jude Children’s Research Hospital, Memphis, TN, USA. 2Center for Cancer Research, National Cancer Institute, Bethesda, MD, USA. 3Department of Neurosurgery, University of Vienna, Vienna, Austria. 4ScienceCenter, DKTK, Heidelberg, Germany. 5Division of Pediatric Neurooncology, German Cancer Research Center (DKTK), Heidelberg, Baden-Württemberg, Germany. 6Center for Molecular Medicine (CeMM), Austrian Academy of Sciences, Vienna, Vienna, Austria. 7St. Jude Children’s Research Hospital, Memphis, TN, USA. 8Department of Neurosurgery, University of Vienna, Vienna, Austria. 9Department of Pediatrics and Adolescent Medicine, Comprehensive Cancer Center Vienna, Vienna, Vienna, Austria.

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 distinct PB molecular subgroups (PB-miRNA1, PB-miRNA2, PB-MYCOFOX2, and PB-RAI), 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 these significant gaps, we generated a single-cell 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. Notably, 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 MYCOFOX2 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...