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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

target genes. To map PB subgroups to their putative developmental beginnings, we created a single-cell transcriptional atlas of the murine pineal gland across 11 developmental stages (E11-P21). Trajectory inference within the developing pineal gland revealed a differentiation continuum of early, mid, and mature alpha-/beta pinealocytes. Cross-species correlation and deconvolution identified significant associations between multiple PB subgroups and specific differentiation states of the pinealocyte lineage, suggestive of developmental origins. Characterization of pinealocyte development informed generation of biologically faithful disease models, including a novel genetically engineered mouse model of the PB-RB subgroup. PB-Rb1 mouse tumors were histologically and molecularly validated for their fidelity to human tumor counterparts, exhibiting up-regulation of key pinealocyte lineage markers that are diagnostic in patients. Finally, high-throughput drug screening identified several promising pharmacological candidates that may attenuate consequences of Rb1 deficiency in affected children.

ETMR-15. CENTRAL NERVOUS SYSTEM EMBRYONAL TUMOR WITH EWSR1 TRANSLOCATION: EVOLVING CHANGES IN HISTOLOGY, SEQUENCING, AND EPIGENETICS AT RELAPSE IN 2 PATIENTS AND POTENTIAL TREATMENT IMPLICATIONS

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INTRODUCTION: Histologically diagnosed embryonal tumors (ET) of the central nervous system (CNS) in the pediatric population represent a group of aggressive malignancies with historically poor prognoses. Immunohistochemistry (IHC) and cytogenetics refined tumor classification and improved treatment strategies. Modern advancements in molecular methods including methylation profiling provide further delineation of distinct ET subclasses. CASE DESCRIPTION: We present two cases with initial diagnosis of a CNS embryonal tumor with EWSR1 rearrangement. Relapsed tissue in both cases displayed acquisition of INI-1 loss consistent with atypical teratoid/rhabdoid tumor (AT/RT). The first case presented at 14 months of age with vomiting and altered mental status. Imaging revealed right frontal lobe hemorrhagic mass with signs of increased intracranial pressure; pathology returned as a CNS embryonal tumor with EWSR1-PLAGL1 translocation. Patient ultimately experienced multiple relapses with tumor sample each with INI-1 loss. The second case presented at 11 months of age with new onset seizures and was found to have right frontal tumor; pathology returned as CNS embryonal tumor with EWSR1-PLAGL1 translocation. Patient experienced recurrence in original tumor bed and pathology was consistent with malignant recurrence with INI-1 loss. Methylation profiling for diagnostic samples in both patients was consistent with ET not otherwise specified, while methylation profiling of relapsed tissue in both cases was consistent with AT/RT. EWSR1 translocation was persistent from diagnostic to relapsed samples. CONCLUSION/DISCUSSION: These cases illustrate the need for ongoing analysis in patients with CNS primitive neuroectodermal tumors and EWSR1 rearrangements, and the unique role of molecular studies in relapsed tissue. The emergence of treatment resistance in a subpopulation of cells more consistent with AT/RT suggests these patients could benefit from upfront and experimental treatment approaches for AT/RT.

ETMR-16. EMBRYONAL TUMORS WITH MULTI-LAYERED ROSETTES: A CASE SERIES IN TREATMENT FOR NEWLY DIAGNOSED CHILDREN

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BACKGROUND: Embryonal tumors with multi-layered rosettes (ETMRs) are rare pediatric brain tumors with poorly defined prognostic features, standard of care treatments or outcome data. Recent data suggest that high-dose chemotherapy and radiotherapy is correlated with improved survival compared to chemotherapy alone. CASE DESCRIPTIONS: Four patients with newly diagnosed ETMRs were treated with 2 cycles of induction chemotherapy per PBTC-026 using isotretinoin, vorinostat, vincristine, cisplatin, etoposide, cyclophosphamide with added intrathecal topotecan. Second look surgery was performed if not in complete remission (CR). Consolidation was with three cycles of marrow-ablative chemotherapy (carboplatin and thiotepa) with autologous hematopoietic cell rescue followed by focal irradiation and 12 cycles of maintenance chemotherapy with intrathecal topotecan, vorinostat and isotretinoin. Patient 1 was a 3-year-old female with right parietal tumor, localized, and achieved gross total resection (GTR). Patient 2 was an 11-month-old male with posterior fossa tumor, localized, and achieved subtotal resection. Patient 3 was a 9-month-old female with posterior fossa tumor with near GTR, and metastasis to T12/L1 and L3/L4. [DTB1] [HNT2] Patient 4 was a 34-month-old male with a right frontal lobe tumor, localized and achieved GTR. Patient 1 is now 18 months from diagnosis and in CR. Patient 2 had second look surgery both after induction and consolidation but suffered neurologic injury to the brainstem which led family to decline further therapy. He is currently 14 months from diagnosis with stable residual disease. Patient 3 had local disease recurrence following radiation therapy at 10 months post diagnosis[DTB3] [HNT4] , prior to maintenance. Patient 4 remains in CR at 13 months from diagnosis, currently in maintenance with vorinostat and isotretinoin but declined intrathecal topotecan. CONCLUSION: This case series adds to current knowledge of intensive multi-modal therapy for newly diagnosed ETMR. Further study to define standard optimal treatments in this high-risk group of patients is warranted.

GERM CELL TUMORS

GCT-01. PEDIATRIC INTRACRANIAL GERM CELL TUMORS AND PRIMARY POLYURIA-POLYDIPSIA SYNDROME: A 13-YEAR SINGLE INSTITUTIONAL EXPERIENCE

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Intracranial germ cell tumors (IGCTs) represent about 4% of all childhood brain tumors. They are common in both the pineal and pituitary regions and sometimes they can be bifocal. Suprasellar and bifocal IGCTs usually present stereotypical symptoms, including primary polyuria-polydipsia syndrome (PPS). Consolidated IGCTs' therapy is based on the International Society of Pediatric Oncologic (SIOP) CNS GCT II protocol consisting of primary pre-radiation chemotherapy combining etoposide, carboplatin and/or cisplatin and ifosfamide. PPS management in these patients requires monitoring of electrolytes and fluids during chemotherapy, especially for cisplatin and/or ifosfamide-based cycles, for which hyperhydratation is required. We report the results of our single-center cohort of patients with IGCTs treated between 2008 and 2021, focusing on the clinical presentation, treatment and long-term follow-up. Thirty-one patients were analyzed (median age=13 years, 87% male). Twelve children (39%) presented a PPS and needed desmopressin treatment, maintained at long-term follow-up data update in all. Over these PPS patients, 6 had bifocal germinomas, 4 suprasellar germinomas, 1 metastatic germinoma and 1 non-germinomatous IGCT. Eleven PPS children (92%) received cisplatin and/or ifosfamide-based chemotherapy: all of them had optimal biochemical urine and blood investigations before, during and after chemotherapy. None of them presented serious complications during treatment. After a median follow-up of 5 years, two patients (6.5%) died (1 IGCT-related, 1 non-cancer related) and one had a second malignancy (parotid gland mucoepidermoid carcinoma, 6 years after IGCT diagnosis). Childhood IGCTs have an excellent prognosis, but present a significant risk of long-lasting severe endocrine sequelae which may be worsened by the primary oncological strategy, requiring careful management of complications related to fluid and electrolytes disturbances. In order to avoid post-treatment pituitary insufficiency, guidelines for diabetes insipidus management when cisplatin and/or ifosfamide-based protocols are used should be established and all patients should receive meticulous endocrine follow-up.

GCT-02. IMAGING RESPONSE ASSESSMENT FOR CENTRAL NERVOUS SYSTEM GERM CELL TUMOURS: CONSENSUS RECOMMENDATIONS FROM THE EUROPEAN SOCIETY FOR PAEDIATRIC ONCOLOGY BRAIN TUMOUR GROUP (SIOPE-BTG) AND NORTH AMERICAN CHILDREN'S ONCOLOGY GROUP (COG)

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