in the clinical laboratory, the performance characteristics of different supervised classification models has not been directly compared. We developed 3 methods using methylation profiles to classify CNS tumors: an exact bootstrap k-nearest neighbor (kNN), a multi-layer perceptron neural net (NN), and a random forest classifier (RF). We trained these methods on the publicly available CNS tumor reference cohort (GSE90496) with 2,801 profiles and 91 classes. We evaluated the performance of these methods by leave-out-25% cross-validation. The relative performance of these methods were evaluated in terms of accuracy, precision, and recall for class or class family. The kNN, RF, and NN classifier had an estimate error rate of 10.74%, 4.01%, and 1.89%, respectively for class prediction and an error rate for family prediction of 5.97%, 0.90%, and 0.6%, respectively. At perfect recall for class assignment, the RF and kNN had a precision of 0.96 and 0.89 while the NN reached 0.98. For family assignment, the precision for the three classifiers was almost 1.0 with recall of nearly 0.8. At the recall rate of 1.0, the precision dropped to 0.94, 0.991 and 0.994 for kNN, RF, and NN, respectively. Overall, the NN showed improved performance metrics compared to the kNN and RF in CNS tumor classification for both class and class family assignment.

PATH-23. ADULT SPINAL CORD ASTROBLASTOMA WITH EWSR1-BEND2 FUSION

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The most recurrent fusion of CNS high-grade neuroepithelial tumor with MN1alteration(HGNET-MN1) is *MN1- BEN Domain Containing 2*(BEND2) fusion. Recently, there was a report of a 3-month-old boy with spinal astroblastoma, classified as CNS HGNET-MN1 by DKFZ methylation classification but positive for EWSR1-BEND2 fusion(Yamasaki, 2019). Here, we report a 36-year old man with a spinal cord astroblastoma with EWSR1 alternation. The patient presented with back pain, gait disorder and dysesthesia in lower extremities and trunk was referred to our hospital. MRI showed intramedullary tumor in Th3-5 level, displaying low-intensity on T1 weighted image, high-intensity on T2 weighted image, and homogeneous gadolinium enhancement. Partial removal was performed with the laminectomy. The tumor extended to extramedullary and its boundary was unclear. Histological examinations showed the epithelium-like tumor cells with eosinophilic cytoplasm with high cellularity palisade, intracellar fibrosis, and mitosis. Immunohistochemical staining showed positive for Olig2, GFAP, EMA, SSTR2, S-100, but negative for p53, PgRAE1/AE3. The tumor was diagnosed as astroblastoma, and was classified as HGNET-MN1 by the DKFZ methylation classifier. However, the MN1 alternation was not detected by fluorescence in situ hybridization, instead EWSR1 and BEND2 alternations which suggested EWSR1-BEND2 fusion were detected. After radiation therapy of 54Gy/30fr with bevacizumab and temozolomide, the residual tumor reduced the size and his symptoms improved. This case provides evidence that *EWSR1-BEND2* fusion is recurrent in HGNET-MN1 and, as previously reported, suggests the importance of BEND2 in this entity. These two cases suggested that it may be the BEND2 alteration that biologically defines the HGNET-MN1 subclass rather than MN1.

PATH-24. MOLECULAR CLASSIFICATION OF HIGH RISK INFANT EMBRYONAL BRAIN TUMORS ENROLLED IN THE ACNS0334 TRIAL: A REPORT FROM THE CHILDREN'S ONCOLOGY GROUP

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Young children with embryonal brain tumors including medulloblastoma (MB), supratentorial primitive neuro-ectodermal tumor, or pineoblastoma have historically been considered high-risk patients with poor outcomes despite the use of intensive radiation-sparing treatment. In the ACNS0334 phase III trial, 91 consented children <36 months old with the above diagnoses were randomized to intensive induction chemotherapy with or without methotrexate followed by consolidation with stem cell rescue. Here we present the results of a centralized integrated molecular analysis including global methylation profiling (65/91), and whole exome sequencing of tumor (46/91) and germline (35/91) DNA. Unsupervised clustering analyses of methylation profiles using multiple orthogonal methods against a reference dataset of 1200 pediatric brain tumors, revealed known and new molecular entities. For tumors diagnosed as MB on central pathology review, 7.3% (3/41) had a non-MB molecular diagnosis (2 embryonal tumor with multiple rosettes/ETMR, 1 group MYC pineoblastoma), with the remainder as MB Group SHH (11/41), Group3 (25/41), and Group4 (2/41). Among histologic non-MBs, 3/24 (12.5%) were molecular entities not intended for trial inclusion (1 each for ATRT, pleomorphic xanthoastrocytoma, and high-grade glioma). ETMR, historically considered a rare entity, was molecularly identified in a significant proportion (14/65; 21.5%) of samples. Among MB-SHH, we detected deleterious PTCH1 mutations in 6/9 tumors but none among 5 germline samples tested; a germline SUFU frameshift mutation with tumor LOH was also observed in MB-SHH. Correlation of these and other molecular features to the parallel clinical analysis will yield important markers of risk stratification and predictors of treatment response.

PATH-25. GENOME-WIDE METHYLATION ANALYSIS CAN SEGREGATE RADIATION-INDUCED GLIOBLASTOMA FROM LATE RECURRENCE OF MEDULLOBLATOMAS

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It could be difficult to diagnose recurrent medulloblastoma with conventional diagnostic tools because other lesions mimic relapse of the tumor from both a morphological and radiological standpoint, particularly when it happens late. We report two medulloblastoma cases, both of which seemed to develop late-recurrence more than 5 years from the initial surgery. Genome-wide methylation analysis revealed that one of the recurrent tumors was in fact a radiation-induced glioblastoma. The first patient was a 6-year-old female patient who developed a posterior fossa tumor. The pathological diagnosis was medulloblastoma with focal desmoplasia. She was in complete remission for 9 years after the treatment but developed an intradural lesion in her thoracic spine. The lesion was biopsied and pathologically confirmed as recurrence of the tumor. The second patient was a female patient who developed non-metastatic medulloblastoma at the age of 10. She suffered local recurrence 5 years after the diagnosis. Biopsy was performed, and the pathological diagnosis was relapse of the tumor. We performed unsupervised hierarchical clustering of the methylation data from our cases and reference data. In contrast to consistency of methylation profiling and copy number abnormalities between primary and recurrent tumors of case 1, the analysis revealed that the recurrent tumor of case 2 was distinct from medulloblastomas and clustered with "IDH-wild type glioblastomas", which suggested that the recurrent tumor was radiation-induced glioblastoma. This report highlights the clinical utility of molecular genetic/epigenetic approach to confirm diagnosis of brain tumor recurrence.

PATH-26. RNA SEQUENCING OF FORMALIN-FIXED PARAFFIN-EMBEDDED SPECIMENS IN DIAGNOSTIC ROUTINE IDENTIFIES CLINICALLY RELEVANT GENE FUSIONS

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Pediatric brain tumor entities harbor a variety of gene fusions. Whilst other molecular parameters like somatic mutations and copy number alterations have become pivotal for brain tumor diagnostics, gene fusions are only less well covered by routinely applied methylation arrays or targeted next-generation sequencing of DNA. In a routine diagnostic setting we established and optimized a workflow for investigation of gene fusions in formalin-fixed paraffin-embedded (FFPE) tumor tissues by using RNA sequencing. Assessing different tools for calling fusions from raw data, we found relevant fusions in 66 out of 101 (65%) analyzed cases in a prospective cohort collected over 26 months. In 43 (43%) cases the fusions were of decisive diagnostic relevance and in 40 (40%) cases the fusion genes rendered a druggable target. Besides the relevance of pathognomonic fusions for diagnostics, especially the detection of druggable gene fusions yields direct benefit to the patients. This approach allows for an unbiased search for fusion events in the tested samples. Besides rare variants of established fusions which were not detected by prior targeted analyses, we identified previously unreported fusion events. Exemplified on KIAA1549:BRAF fusion, we in addition provide an overview of the detection accuracy of different methods, including breakpoint detection in DNA methylation array data and fusion gene detection in DNA panel sequencing data. Our data show that RNA sequencing has great diagnostic as well as therapeutic value by clinically detecting relevant alterations.

PATH-27. MUTATION DETECTION USING PLASMA CELL-FREE DNA IN CHILDREN WITH CENTRAL NERVOUS SYSTEM TUMORS

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BACKGROUND: The role of plasma cell-free DNA (cfDNA) as a cancer biomarker for tracking treatment response and detecting early relapse has been well described for solid tumors outside the central nervous system (CNS). However, the presence of a blood-brain barrier complicates the application of plasma cfDNA analysis for patients with CNS malignancies. METHODS: cfDNA was extracted from plasma of pediatric patients with CNS tumors utilizing a QIAmp® MinElute® kit and quantitated with Qubit 2.0 Fluorometer. Extensive genomic testing, including targeted DNA and RNA solid tumor panels, exome and transcriptome sequencing, as well as copy number array, was performed on matched tumor samples as part of the Texas KidsCanSeq study. An Archer® Reveal ctDNA28 NGS kit was then used for assaying the sensitivity of detecting tumor-specific mutations in the plasma of these patients. RESULTS: A median of 10.7ng cfDNA/mL plasma (Interquartile range: 6.4 – 15.3) was extracted from 78 patients at time of study enrollment. Longitudinal samples from 24 patients exhibited a median yield of 7.7ng cfDNA/mL plasma (IQR: 5.9 – 9.1). An initial cohort of 6 patients was identified with 7 somatic variants covered by the Archer® Reveal kit. Four of seven mutations identified in matched tumor specimens were detected in patient plasma at variant allele frequencies ranging from 0.2–1%. CONCLUSIONS: While challenging, detection of cfDNA in the plasma of pediatric patients with CNS tumors is possible and is being explored in a larger patient cohort along with pilot studies investigating cerebrospinal fluid as an additional source for tumor-specific cfDNA.

PATH-28. MOLECULAR DIAGNOSIS FOR CENTRAL DIAGNOSIS OF BRAIN TUMORS FROM 2016 TO 2019— A REPORT FROM THE JAPAN CHILDREN'S CANCER GROUP (JCCG)

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INTRODUCTION: Since 2016, the Japan Children's Cancer Group (JCCG) has established a nationwide network that prospectively provides pathological review and molecular analysis. METHODS: Patients who were diagnosed with brain tumors between ages 0 and 29 were eligible. The central office at National Center for Child Health and Development served as a hub for the hospitals involved and institutions conducting pathological and molecular analysis, and managed the patients' clinical information and tumor samples. Histopathology of all cases were centrally reviewed. Routine non-NGS based analyses were conducted based on histological diagnosis and included pyrosequencing for glioma-associated hot spot mutations and PFA/PFB classification for ependymoma, RT-PCR for *RELA* fusion and *BRAF* fusion, and nanostring for subgrouping medulloblastoma. In selected cases, methylation analysis, RNA sequencing and exon sequencing of 93 genes were performed in selected cases. RESULTS: In total, 985 cases were registered to this study in four years. Frozen samples were collected from approximately 80% of cases. The number increased from 152 in 2016 to 326 in 2019. They includes glioma (n=268), medulloblastoma (n=161), ependymoma (n=103), germ cell tumor (n=93), ATRT (n=29) and others. In 55 % of the glioma cases, at least one abnormality was detected by the routine analysis. The detailed analysis for atypical cases identified targetable alternations. DISCUSSION: This nationwide central diagnostic system has now been well established. Current issues and future prospective of the system will be discussed.

PATH-29. HIGH FREQUENCY OF CLINICALLY-RELEVANT TUMOR VARIANTS DETECTED BY MOLECULAR TESTING OF HIGH-RISK PEDIATRIC CNS TUMORS – PRELIMINARY FINDINGS FROM THE TEXAS KIDSCANSEQ STUDY

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BACKGROUND: DNA and RNA-based tumor sequencing tests have the potential to guide the clinical management of children with CNS tumors. However, data describing the utility of these tests are limited. METHODS: Children with high-risk or recurrent CNS tumors are included in the diverse cohort of patients enrolling in the KidsCanSeq study from six Texas sites. DNA and RNA from FFPE tumor is subjected to targeted sequencing using a 124-gene mutation panel and an 81-gene fusion panel. Tumor capture transcriptome sequencing, exome sequencing, and copy number array (as well as germline panel and exome testing) are also performed. Tumor variants are classified using AMP/ASCO/CAP consensus guidelines. RESULTS: A total of 74 children with high-risk/recurrent CNS tumors enrolled as of 1/28/20. Targeted tumor DNA and RNA panel testing was completed for 57 patients with varied diagnoses. At least one tumor variant with strong or potential clinical significance was identified in 43 of 57 (75%) tumors, with therapeutic significance in 20 of 57 (35%) tumors. The 38 therapeutically-relevant variants most frequently affected MAPK signaling (BRAF x9, EGFR x3, FGFR2, FGFR3, KRAS, NF1, NTRK2) and the AKT/mTOR pathway (PIK3CA x3, PTEN x2, mTOR, TSC1, PIK3R1). Most had not been detected by prior targeted diagnostic testing (27/38, 71%). CONCLUSION: Integrated DNA and RNA-based panel testing identified variants with potential to impact clinical decision-making in a majority of children with high-risk/recurrent CNS tumors. The comparative yield of panel testing vs. exome/transcriptome/array will be evaluated in the KidsCanSeq study cohort.

PATH-30. EXOSOMES AS A SOURCE OF PLASMA CTDNA TO IDENTIFY POINT MUTATIONS IN PEDIATRIC GLIOMA PATIENTS

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