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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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