

IDH-mutant lower-grade astrocytomas have only rarely been investigated. In this study, we recruited 161 IDH-mutant lower-grade astrocytomas, and examined PDGFRA amplification, CDKN2A deletion, and CDK4 amplification by FISH analysis, TERT promoter mutation by Sanger sequencing, and ATRX loss and p53 expression by immunohistochemistry. We identified PDGFRA amplification, CDKN2A deletion, and CDK4 amplification in 18.6%, 14.9%, and 18.0% of our cohort respectively, and these alterations occurred in a mutually exclusive fashion. PDGFRA amplification was associated with shorter PFS ($p < 0.0001$) and OS ($p < 0.0001$). In tumors without PDGFRA amplification, CDKN2A deletion was associated with a shorter PFS ($p = 0.0332$). Tumors were then divided into three risk groups based on the presence of molecular alterations: high-risk (PDGFRA amplification), intermediate-risk (CDKN2A deletion or CDK4 amplification) and low-risk (neither CDKN2A deletion, CDK4 amplification nor PDGFRA amplification). These three risk groups were significantly different in overall survival with mean survivals of 40.2, 62.9, and 71.8 months. The high-risk group also demonstrated a shorter PFS compared to intermediate- ($p = 0.036$) and low-risk ($p < 0.0001$) groups. Our data illustrate that IDH-mutant lower-grade astrocytomas is not a homogeneous group and should be molecularly stratified for risk.

PATH-43. DIAGNOSIS OF GLIOMAS USING CIRCULATING GLIAL CELLS

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Invasive procedures for diagnosis of CNS malignancies carry inherent risks of high morbidity and mortality. Although circulating biomarkers such as cell free DNA (cfDNA) and microvesicle (MV) borne nucleic acids have been proposed as potential diagnostic aids, their stand-alone utility has inherent limitations. However, Circulating Glial Cells (CGCs) combined with cfDNA could offer a viable alternative to invasive biopsies for diagnosis of CNS malignancies; yet the technological challenge in the detection of CGCs in glioma patients presents a formidable challenge. In this study, we evaluated the feasibility of harvesting CGCs from suspected cases of Glioma. From a cohort of 23 suspected cases of CNS malignancies, we used 15ml of peripheral blood and used the CellWizard™ process and related protocol for isolation of CGCs. CellWizard™ is an epigenetically active media with paradoxical chemo-toxicity that selectively induces lethality in normal cells which have a functionally responsive cell death (apoptosis) mechanism, while simultaneously conferring survival privilege on apoptosis resistant cells typically released from a malignant tumor. This paradoxical cytotoxicity of the medium leads to selective elimination of most leukocytes thus facilitating a label free negative enrichment of CGCs, which can be harvested and further characterized. Patients included 11 Glioblastoma, 3 Anaplastic astrocytoma, 2 Medulloblastoma, 5 Oligodendroglioma, 1 Gliosarcoma and 1 meningioma patient. Characterization of CGCs was performed using GFAP, S100 and CD45 markers. CGCs were detected in 16 out of 23 (69.6 %) patients and could be stained positively for both GFAP and S100 and negatively for CD45. Detection of viable CGCs in cases of CNS malignancies can be used for characterization of markers related to the diagnosis.

PATH-44. MULTIPLE BIOMARKER ALGORITHM BASED ON CXCL13, IL-10, IL-2 RECEPTOR, AND β 2-MICROGLOBULIN IN CEREBROSPINAL FLUID TO DIAGNOSE CENTRAL NERVOUS SYSTEM LYMPHOMA

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BACKGROUND: Brain biopsy is the gold standard for the diagnosis of primary central nervous system lymphoma (PCNSL). However, the biopsy procedure has a risk of complications, such as hemorrhage and seizure. We have reported that cerebrospinal fluid (CSF) interleukin-10 (IL-10), soluble IL-2 receptor (sIL-2R), and β 2-microglobulin (β 2-MG) are the useful diagnostic biomarkers for PCNSL. Recently, the C-X-C motif chemokine ligand 13 (CXCL13) has been reported to be another useful biomarker for the PCNSL. The aim of this study was to validate the diagnostic performance of the CSF CXCL13 and diagnostic algorithm based on the combination of these biomarkers. **METHODS:** We retrospectively examined the CSF

CXCL13 concentration, as well as IL-10, sIL-2R, and β 2-MG, in a case-control study ($n=230$). We used logistic regression analyses to create diagnostic algorithms based on these CSF biomarkers. To examine the utility of the multivariate diagnostic algorithm, we conducted the prospective study and evaluated the accuracy of this algorithm. **RESULTS:** In case-control study, we have demonstrated that CSF CXCL13 levels were significantly higher in the patients with PCNSL (area under the curve (AUC)=0.981). A total of 84 patients were enrolled in the prospective study. The multivariate diagnostic algorithm using CSF levels of CXCL13, IL-10, sIL-2R, and β 2-MG demonstrated excellent diagnostic performance: positive predictive value was 89% and negative predictive value was 100%. Four markers' combination (CXCL13 + IL-10 + β 2-MG + sIL-2R) had the highest AUC (AUC=0.998). The misdiagnosis cases of the algorithm were only 3 cases; CNS Sjögren's syndrome, histiocytic sarcoma, and glioblastoma. In addition, CSF CXCL13 levels were prognostic biomarkers in PCNSL patients. **CONCLUSIONS:** Our study suggests that the algorithm based on 4 CSF biomarkers had excellent diagnostic performance in CNS lymphoma. However, this algorithm should be further validated in prospective cohort studies with larger numbers of patients.

PATH-45. DIFFERENT TYPES OF HYPERMUTATOR PHENOTYPE OF GLIOBLASTOMA ACCORDING TO MUTATION PATTERNS OF MISMATCH REPAIR GENES

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BACKGROUND: Although precision medicine has been widely applied to routine care, only few molecular targeted drugs have been developed for glioma. Immune checkpoint inhibitors hold a potential promise to improve outcome of malignant glioma especially with the hypermutator phenotype. Mismatch repair deficiency could induce hypermutation during temozolomide (TMZ) treatment, but the mechanism of mutation acquisition is not well understood. **METHODS:** We present the results of target sequencing of 57 longitudinal specimens from 27 individuals with glioblastoma (GBM). We used Ion AmpliSeq Cancer Hotspot Panel v2 (CHP) and/or Ion AmpliSeq Comprehensive Cancer Panel (CCP) for target sequencing. **RESULTS:** Acquired mutations with G:C >A:T transition at non-CpG sites were found in 70% of recurrent MGMT methylated samples, whereas only 8% in MGMT unmethylated cases ($p < 0.001$). Two cases of hypermutator phenotype were identified in MGMT methylated, IDH wild type recurrent GBMs after TMZ chemotherapy. One case gained mutations in mismatch repair genes of *MLH1*, *MSH2*, *MSH6*, and *PMS1*. Most of the acquired mutations were G:C >A:T mutations typical to TMZ-induced hypermutation. The recurrent tumor was highly aggressive with overall survival after recurrence of 3.7 months. The other case gained mutations in mismatch repair genes of *MLH1* and *PMS1* only. Most of the acquired mutations were insertion mutations. The prognosis after recurrence was much longer. **CONCLUSIONS:** We identified two types of hypermutator phenotype according to mutation pattern of mismatch repair genes. It has been suggested that MSH2-MSH6 complex deficient tumor cannot repair TMZ-induced mismatch mutation, thereby causing hypermutation. Conversely, MLH1-PMS1 complex deficient tumor with intact MSH2-MSH6 complex can repair mismatch mutation but is susceptible to insertion mutation. Taken together, *MSH6* mutation plays a key role in TMZ-induced hypermutation, while *MLH1* and *PMS1* mutations might cause insertion-based hypermutation. Larger and prospective studies are warranted to clarify the mechanism, outcome, and effectiveness of checkpoint inhibitors.

PATH-46. DETECTING MISDIAGNOSED ATYPICAL TERATOID/RHABDOID TUMOR (ATRT) BY DNA METHYLATION-BASED TUMOR CLASSIFICATION

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Brain tumor diagnostics is achieved by combining morphology assessment and biomarker identification. However, the inter-observer variability of the histopathological diagnosis and lack of distinctive biomarkers makes these diagnoses particularly challenging. Recently, the potential for methylation-based characterization has been developed for brain tumors. To provide accurate and efficient brain tumor diagnostics, The Jackson Laboratory developed a DNA methylation array (Illumina Infinium HD Methylation EPIC), combining the "Classifier" established by the German Cancer Research Center (DKFZ), which can identify 82 distinct central nervous system tumors. Here we present a pediatric patient referred to us for medulloblastoma subtype classification by the methylation profile. Surprisingly, the array classified the tumor type as atypical teratoid/rhabdoid tumor (ATRT), subclass SHH. Also, the CNV profile indicated the *SMARCB1/INI1*