Title

PATH-29. CLINICAL SIGNIFICANCE OF TEMOZOLOMIDE-INDUCED SOMATIC HYPERMUTATION IN INITIALLY LOW-GRADE IDH-MUTANT DIFFUSE GLIOMAS

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are not explained by the current genomic classification. We aim to identify clinically and biologically relevant subgroups within IDH-mut low grade gliomas to gain a deeper insight and improve classification. We used 412 IDH-mut gliomas profiled by The Cancer Genome Atlas (TCGA) Network, utilising methylation, mRNA and mutation datasets to identify unique molecular signatures. We found that IDH-mut gliomas further subdivide into 2 groups based on mutational rate. High mutation load predicts poor survival in IDH-mut glioma. Analysis of differentially expressed genes in high versus low-mutational rate showed significant enrichment of HOX genes, 24/40 HOX genes were up regulated in this group. Interestingly, both overexpression and hyper-methylation of specific HOX genes were associated with worse survival. We further show that 7 of these HOX genes (HOXA4, HOXA7, HOXA10, HOXA13, HOXD3, HOXD9, and HOXD10) are the most significant in determining survival. Signed average of 7 Hox genes significantly improved survival and hazard ratio (HR) based on high versus low methylation (HR=4.3, p<0.0001) and high versus low mRNA expression (HR=2.8, p=0.00095). Similarly, effect on survival based on high expression and hyper-methylation of HOX genes was not only observed in IDH-mut 1p/19q-codeleted and non-codeleted groups independently, but also in IDH-wild-type low grade glioma. Multivariate analysis adjusted for confounding factors (grade, age and codeletion status) showed prognostic factors associated with survival in high versus low methylated group (HR=3.2, p=0.0036). Interestingly, only the same direction (high-high and low-low groups) of both mRNA and methylation showed significance and increased HR, which challenges the current understanding of methylation of genes and gene expression. We show that IDH-mut gliomas can further be stratified into clinically relevant categories based on high mRNA expression and hyper-methylation of Hox genes.

PATH-26. NEURO-ONCOLOGY NEXT-GENERATION SEQUENCING 219-GENE PANEL FOR COMPREHENSIVE CLINICAL TESTING

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Molecular parameters were incorporated in the WHO classification of central nervous system (CNS) tumors. Despite advances in understanding the molecular biology of CNS tumors, targetable genomic abnormalities are lacking. To identify diagnostically, prognostically and potential therapeutically relevant abnormalities, we developed a next generation sequencing (NGS) assay for formalin-fixed paraffin-embedded (FFPE) tissue that evaluates 219 genes associated with adult and pediatric CNS tumors. This test consists of a DNA and an RNA subpanel for the detection of sequence alterations and gene rearrangements (known gene fusions and abnormal transcript variants, and novel fusion transcripts that contain any of the interrogated genes as a partner). The assay utilizes an amplicon-based approach with molecular barcode chemistry (to allow traceability of PCR artifacts/duplicates), Illumina sequencing and custom bioinformatics pipelines. Analytical validation included 175 samples. Overall concordance with alternative methods were 99% and 96% and success rates were 97% and 95% for the DNA and RNA subpanels, respectively. Inter and intra-assay reproducibility was 100% for both subpanels. The limit of detection (analytical sensitivity) for nucleic acid input and tumor content were 8.5 ng and 30%, and 10 ng and 10% for the DNA and RNA subpanels, respectively. The analytical specificity was high, with per base DNA sequencing false positive rate <0.4% and absent fusion transcript detection in non-neoplastic samples. We developed a robust 219-gene neuro-oncology NGS assay suitable for clinical testing of FFPE specimens, including small biopsies with low tumor content. This test, combined with chromosomal microarray analysis, detects nearly all single nucleotide variants, fusion rearrangements and copy number changes associated with CNS tumors. These tests are intended to assist in the diagnosis, prognosis and therapeutic management of adult and pediatric patients with CNS tumors, and have the potential to unravel novel genomic abnormalities and expand the understanding of the molecular biology of such tumors.

PATH-27. IDENTIFYING THE GENETIC SIGNATURE OF RESPONSE IN A PHASE II STUDY OF TUMOR TREATING FIELDS IN RECURRENT GLIOBLASTOMA

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Prognosis of relapsed glioblastoma (GBM) is dismal and current treatment fails to provide prolonged survival. Small subsets of patients respond well to some novel therapeutics probably due to the genetic variations of tumors and patients. In the Phase 3 EF-11 recurrent GBM trial, a small subset of patients derived significant benefit from TTFields alone. This proof-of-concept trial [NCT01954576] will study adult patients with relapsed GBM treated with TTFields by genetic analysis of primary and recurrent tumors. Post-hoc correlations will be used between clinical response, mutational analyses and quantitative gene expression to define genomic signatures of response. Whole exome and RNA sequencing will be used to identify genomic signature of responders to TTFields. Fifteen patients with bevacizumab-naïve recurrent GBM and 15 patients with bevacizumab-refractory GBM will be treated with TTFields for 6 and 4 months respectively. Patients will undergo standard brain MRI scans without and with gadolinium contrast and perfusion imaging every 8 weeks. Tissue from the primary tumor at recurrence will be genetically analyzed. Genomic DNA (gDNA) will be extracted from patients tumor and blood samples. Purified gDNA fragments will be used for Illumina sequencing library construction. Certain germ-line variants may contribute to gliomagenesis and be associated with somatic mutations within the tumor and subtypes of GBM more or less sensitive to TTFields. Analyses will be conducted on all patients: bevacizumab-naïve and bevacizumab-refractory GBM separately, and patients with objective radiographic response (complete response + partial response (CR + PR) and stable disease (SD) separately. With 50% bevacizumab-refractory GBM patients, response rate will be significantly higher than the baseline rate of 14%. Using an Exact test with type I error of 0.05 and 80% power, the estimated sample sizes will detect a statistical difference on response rate in the TTFields group compared to historical controls. To-date 4 patients have been enrolled.

PATH-28. THE NATURAL HISTORY OF BRAF V600E-MUTATED GLIOBLASTOMAS IN ADULTS

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BACKGROUND: BRAF V600E-mutations are rare but noteworthy in primary brain tumors given their potential as a targetable mutation and the lack of efficacious therapies for glioblastoma. BRAF V600E mutations may serve as a prognostic marker in pediatric and low-grade gliomas, and are associated with improved survival in young adults with glioblastoma; however, its prognostic significance in adults >35 years is uncertain given the very small number of patients evaluated to date. METHODS: Patients aged >18 with WHO III-IV glioma and a BRAF V600E mutation were identified from the National Institutes of Health, the Johns Hopkins Hospital, and a previous publication (PMID:27503138). Paired control cases were identified at each institution based on age, sex, degree of resection, performance status at diagnosis/first encounter, MGMT and IDH status, and first-line treatment. Log-rank test was used to compare survival curves. RESULTS: The present cohort consisted of 23 patients (6 from each institution and 11 from a published cohort) with median age of 39 (range 20–70 years), 78% female, and 87% with a glioblastoma diagnosis. No tumors had an IDH mutation. 39% of patients were aged >50 years. All but one were treated with radiation and temozolomide at diagnosis (exception went into hospice and died shortly thereafter). The median overall survival was 33.4 ± 8.4 months in all patients. For 13 patients aged 35 or older, median survival was 34.5 ± 12.1 months compared to 18.0 ± 3.0 months in case-matched controls (p=0.03). Two patients were treated with dabrafenib and trametinib; one is still on therapy (26 months), the other progressed after 8 months. CONCLUSIONS: Adults aged >35 with BRAF V600E mutation may have improved survival compared with matched controls, similar to results in young adults. BRAF V600E mutations occur in patients with glioblastoma aged >50 years and testing in this population should be considered as well.

PATH-29. CLINICAL SIGNIFICANCE OF TEMOZOLOMIDE-INDUCED SOMATIC HYPERMUTATION IN INITIALLY LOW-GRADE IDH-MUTANT DIFFUSE GLIOMAS

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INTRODUCTION: Diffuse low-grade gliomas (DLGG) treated with temozolomide (TMZ) can develop somatic hypermutation. We present data on the incidence and prognostic importance of somatic hypermutation in IDH-mutant DLGGs. METHODS: We analyzed 120 patients treated on a phase II clinical trial of TMZ for sub-totally resected DLGGs to estimate the risk of recurrence and transformation after TMZ. To understand the prognostic significance of somatic hypermutation, we determined hypermutation status by exome or targeted sequencing on tumors from 81 patients with recurrent IDH-mutant DLGGs. 63/81 patients received TMZ before recurrence, including 28 patients treated on-trial. RESULTS: With median follow-up of 8.7 years, 89 patients from the phase II trial progressed, 60 underwent 1 re-operation, and 36 had histologically confirmed transformation. The 8-year freedom from transformation was 48.2% and 59.9% for IDH-mutant astrocytomas and oligodendrogliomas, respectively; risk of transformation increased with pre-TMZ tumor volume (HR 2.5 per 100cc, p<0.001). In the recurrent glioma cohort, 65/81 patients transformed to grade III or IV; hypermutation was identified at transformation in 30/53 (57%) treated with TMZ. Hypermutation occurred in 31 patients all had received TMZ and 30/31 had developed transformed tumors. Analyzing by specimen, hypermutation was associated with transformation (bootstrapped logistic regression p < 0.001). After transformation to grade III disease (n=47), hypermutation was associated with diminished survival (HR 5.6, p=0.007), controlling for molecular subtype and age at diagnosis. Patients with transformation to glioblastoma (n=18) had poor prognosis regardless of hypermutation (p=0.53). Four cases of spinal dissemination were identified, all of whom had hypermutated gliomas. CONCLUSIONS: Somatic hypermutation is common in transformed, initially low grade IDH-mutant diffuse gliomas treated with TMZ. After anaplastic transformation, somatic hypermutation is associated with reduced survival, independent of molecular subtype. These data have implications for the management of newly diagnosed and recurrent DLGG, and indicate a potential role for immunotherapy.

PATH-30. RECONSIDERATION FOR POOR PROGNOSIS OLIGODENDROGLIAL TUMOR CASES BASED ON WHO2007 AND WHO 2016

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Mounting evidence suggests that oligodendroglial tumor is associated with a more favorable prognosis comparing with astrocytic tumor. Treatment strategy of our department is as follows 1) grade 2 oligodedroglioma with less than 90% resection are treat with post operative radiation following with RTOG 9802. 2)grade 3 anaplastic oligodendroglioma are treated with ACNU and radiation therapy. Instead of better prognosis of oligodendroglial tumor, there are several patients with unfavorable clinical course of malignant transformation. In this study, we retrospectively analyse clinical data of unfavorable oligodendroglioma cases diagnosed by WHO2007 and WHO2016. Overall 237 cases of newly diagnosed by WHO2007 in 2001 to 2014 were further analysed. Fetal cases were diagnosed as Oligodendroglioma(OL) 5, Oligoastrocytoma(OA) 3, Anaplastic oligodendroglioma(AO) 13 and Anaplastic oligoastrocytoma(AOA) 3 in WHO2007. These tumor were re-evaluated with WHO2016 (OL 120, AO 65, NOS 22). Fetal cases of oligodedroglial tumor were OD-mt 5, AO-mt 4, NOS 1 and the lest were all astrocytic tumor, Diffuse astrocytoma(DA)-mt 3, DA-wt 1, Anaplastic astrocytoma(AA)-mt 2 and AA-wt 7 cases. OS of all oligodendroglial tumor was not reached, PFS was 8.5 months in OD and not reached in AO. Median OS and median PFS were 57,1 and 40,1 months in OD fetal cases and 31,3 and 18,6 months in AO fetal cases. Five OD fetal cases were all partial removal and 3 were treated by post-operative therapy. One out of four AO fetal cases was total removal and all cases were treated with post-operative therapy. There were several poor prognosis oligodendroglial tumor instead of their genomic alteration such as 1p19q LOH and IDH-mt. Aggressive tumor resection may also alter the natural history of unfavorable oligodendroglial tumor.

PATH-31. GIANT CELL GLIOBLASTOMAS: ANALYSIS OF MISMATCH-REPAIR (MMR) PROTEINS EXPRESSION, POLIMERASE ε (POLE) MUTATIONS AND THEIR ROLE IN TUMOR IMMUNORESPONSE

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Giant cell glioblastoma (gcGBM) is a rare (<1%) variant glioblastoma (GBM), in younger patients. Unlike the IDH-wild type GBM, they have a better prognosis. Mutations in the POLE and in MMR genes accelerate tumorigenesis, generating in some tumours an ultra-mutated phenotype. The lack of proofreading activity generates production of neoantigens, recalling tumour infiltrating lymphocytes, and immune-checkpoint ligands exposition. Aim of our study was to investigate MMR proteins expression, POLE mutations, related checkpoint molecules and the tumor immune-microenvironment in a group of gcGBMs compared to IDH-wild type GBMs. We performed a molecular and immunohistochemical analysis on 60 primary gcGBMs. All tumours where characterized for EGFR, PTEN, p53, IDH1, MGMT status by immunohistochemistry and/or molecular analysis. We investigated MMR protein (MSH6, MSH2, PML2 and MLH1), PD-L1, CTLA-4 and CD28 expression by immunohistochemistry in gcGBMs and in a group of standard GBMs. POLE mutations have been studied by direct sequencing of exons encoding its exonuclease activity. Then we assess the immunological status investigating the presence of lymphocytic infiltrates, microglia and macrophages, by CD3, CD4, CD8, CD68, CD163, MHC class II and IBA1. All the results obtained have been related to clinical data. The median survival time was 21 months, with 4 patients long survivors (>5 years), higher than in the standard group. The main findings where partial loss of expression of MMR proteins (overall MSH2 and MSH6) on 30% of cases, mostly related to presence of inflammatory infiltrates, also showing CD28 immunostaining. Microglia IBA1+ was significantly present in patients with longer survival. Correlation with PD-L1 and CTLA4 was found only in 2 cases. POLE sequencing displays mutation F367S on 20% of cases. Our results show that gcGBMs are an histological variant with increased tendency to ultra-mutated phenotype with a better prognosis and suggesting these patients as candidates for immunotherapy.

PATH-32. BRAIN TUMOR CLASSIFICATION UPDATES FROM cIMPACT-NOW, THE CONSORTIUM TO INFORM MOLECULAR AND PRACTICAL APPROACHES TO CNS TUMOR CLASSIFICATION

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cIMPACT-NOW (the Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy) was created to provide a forum to evaluate and recommend proposed changes to future CNS tumor classifications. While it is understood that the major impact on international brain tumor classification comes about through the WHO classification update process, it is anticipated that this additional process will see impact in selected tumor types and in time periods between the WHO classification updates. Over the past year, cIMPACT-NOW has convened three working committees (WC), each of which has focused on different classification issues. WC1 has debated the grading of diffuse gliomas relative to IDH status, and has formulated criteria for IDH-wildtype grade II and grade III diffuse astrocytic tumors that are likely to behave as glioblastomas, such as EGFR amplification and +7/-10 copy number changes. WC2 is developing a molecular classification of pediatric low-grade gliomas, focusing on the diffuse gliomas. WC3 has addressed miscellaneous issues, including clarifications of Not Otherwise Specific (NOS) and Not Elsewhere Classified (NEC) diagnoses and refined criteria for diffuse astrocytoma (e.g., the use of ATRX and p53 immunohistochemistry relative to 1p/19q testing) and diffuse midline glioma, H3 K27M-mutant. To date, two publications have resulted from WC3 and guidelines are expected soon from WC1 and WC2. The combined recommendations of the current cIMPACT-NOW WCs will be discussed in light of the WHO classification.

PATH-33. HEXOKINASE 2 KNOCKOUT VIA CRISPR REDUCES DOWNSTREAM GENE EXPRESSION, IMPLICATING A REDUCTION IN CELL PROLIFERATION AND DRUG RESISTANCE

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HK2 has a prominent role in aerobic glycolysis and has been implicated in many cancer types including GBM, with overexpression associated with drug resistant phenotypes. Previously, we have demonstrated HK2 expression was upregulated between 6 to >1000 fold in GBM biopsy tissue (n=100) and patient derived cell cultures (n=13), compared to normal brain tissue. In the