Title

BIOM-38. PI3K/AKT/mTOR SIGNALING PATHWAY ACTIVITY IN IDH-MUTANT DIFFUSE GLIOMA

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small subset of patients appears to benefit, warranting evaluation of predictive markers of improved survival. METHODS: We evaluated 108 patients managed at Dana-Farber Cancer Institute for IDH-wildtype glioblastoma, who received anti-PD-(L)1 therapy and had complete clinical, pathologic, and radiographic data. OS was measured from anti-PD-(L)1 initiation to death or censored at last follow-up. Predictors of OS were evaluated by multivariable Cox regression, adjusted for age at diagnosis, sex, disease setting (newly-diagnosed vs. recurrent), *MGMT* status, chemotherapy and bevacizumab before/during anti-PD-(L)1, extent of resection prior to anti-PD-(L)1, and, at anti-PD-(L)1 initiation: KPS, dexamethasone use, absolute lymphocyte count (ALC), ADC mean value, FLAIR volume, T1 post-contrast volume. RESULTS: Of 108 patients, 28 (25.9%) were newly-diagnosed, 80 (74.1%) were treated in the recurrent setting, and 100 died (92.3%). In multivariable analysis, unmethylated *MGMT* (HR 2.31, 95%CI: 1.38–3.88, p=0.002), and, at the time of anti-PD-(L)1 initiation, baseline dexamethasone use (HR 1.79, 95%CI: 1.07–3.00, p=0.03), lower ADC mean values (reference 2nd quartile [1,130-1,262 10^{-6}mm^2/s]; 3rd quartile [1,268–1,388] HR 0.32, 95%CI: 0.15–0.67, p=0.002; 4th quartile [1,402–11,966] HR 0.30, 95%CI: 0.14–0.66, p=0.003), and higher T1 post-contrast volumes (reference 2nd quartile [4,121–8,157mm^3]; 3rd quartile [8,701–14,663mm^3] HR 2.08, 95%CI: 1.03–4.19, p=0.04; 4th quartile [15,566-64,362mm^3] HR 3.15, 95%CI: 1.49–6.69, p=0.003) independently predicted worse OS. Older age (HR 1.02/year, 95%CI: 1.00–1.05, p=0.07) and lower ALC (HR 0.66/unit, 0.40–1.10, p=0.11) trended towards worse OS. CONCLUSION: Unmethylated *MGMT*, and, at the time of starting anti-PD-(L)1, baseline dexamethasone, low ADC mean value, and high T1 post-contrast volume may be predictive of worse OS in IDH-wildtype glioblastoma patients treated with anti-PD-(L)1. These findings may help better select patients who could benefit from PD-(L)1 inhibitors.

BIOM-35. MULTI ANALYTE ASSAY FOR NON-INVASIVE DIAGNOSIS OF BRAIN TUMORS

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The diagnosis of Central Nervous System (CNS) malignancies such as Gliomas in individuals presenting with Intracranial Space Occupying Lesions (ICSOL) is based on histopathological examination (HPE) of tumor tissue obtained by an invasive brain biopsy. However, brain biopsies are resource intensive and are associated with procedural risks such as haemorrhage, morbidity and mortality. The present study evaluated a non-invasive approach for diagnosis of CNS-M in symptomatic individuals based on evaluation of circulating tumor analytes in peripheral blood. The non-invasive multi-platform approach for diagnosis of CNS-M included Immunocytochemistry (ICC) profiling and Fluorescence *in situ* Hybridization (FISH) of Circulating Tumor Cells (CTCs) and Digital Droplet PCR (ddPCR) of cell-free tumor DNA (ctDNA) and exosomal mRNA. Performance characteristics of each platform were evaluated using blood and tissue samples from 445 individuals including 227 known cases of CNS-M, 47 known cases of benign CNS conditions (CNS-B), 141 known cases of other cancers with brain metastases (OTH-M) and 30 asymptomatic individuals (ASYM). In a set of 37 samples from individuals with radiological ICSOL, suspected of malignancy (CNS-S) complete diagnostic work-up was performed with ICC, FISH and ddPCR. Glial CTCs were detected in 88.8% of 227 CNS-M and undetectable in 89.4% of 47 CNS-B or 100% of 141 OTH-M, indicating high sensitivity and specificity, respectively. The multi-analyte approach discerned CNS-M from CNS-B as well as OTH-M with 91.7% accuracy and accurately inferred lineage in 84.6% of cases. This non-invasive multi-analyte approach can diagnose CNS-M with an accuracy not inferior to standard HPE, can substitute invasive biopsies in most cases and is particularly helpful in cases where a biopsy is not viable.

BIOM-36. THE UNIQUE METABOLOMICS BASED BIOMARKERS OF RESPONSE TO IMMUNOTHERAPY FOR GLIOBLASTOMA

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INTRODUCTION: The adaptive immune response requires robust T cell proliferation and activation. These T cell changes are dependent on metabolic program shifts that can be measured using metabolomic analysis. The objective of this project was to identify a metabolomics profile to serve as a biomarker of response to immunotherapy for the treatment of brain tumors. METHODS: GL261-gp100 tumor-bearing mice were received anti-PD1 or bone marrow-derived dendritic cell (DC) vaccine that was generated ex vivo. Urine samples were collected for Nuclear Magnetic Resonance (NMR) analysis. A more in-depth Sparse Partial Least Squares Discriminant Analysis (sPLS-DA) revealed global metabolic changes induced with immunotherapy. RESULTS: The metabolic changes were most dramatic at 24 hours post DC vaccination and slowly returned to baseline at 7 days post DC vaccination. The main drivers of the differences included creatine, n-dimethyl glycine, alanine, lactate, glucose, glutamine, leucine, citrate and formate. Anti-PD1 therapy-related metabolic changes were largest around day 20 post therapy and the main drivers of the differences included dimethyl sulfone, succinate, lactate and isobutyrate. CONCLUSION: Immunotherapy results in systemic metabolic changes that serve as a biomarker treatment effect. These findings have translational relevance in predicting patients who will develop an immune response to immunotherapy and ultimately have better outcomes with treatment.

BIOM-37. INITIAL RADIOGRAPHIC ASSESSMENT OF ADC VALUES IN PEDIATRIC PATIENTS TREATED WITH DAY101 FOR RECURRENT OR PROGRESSIVE LOW-GRADE GLIOMAS (LGGS) HARBORING MEK/ERK PATHWAY ALTERATIONS

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BACKGROUND: Calculated from a diffusion-weight image (DWI), the apparent diffusion coefficient (ADC) is a quantitative measure that reflects observed net movement of water and correlates to tumor cellularity. We examine the changes in ADC values in patients with LGG treated with dimeric, pan-RAF inhibitor DAY101 (formerly TAK-580/MLN2480). METHODS: Focusing on ADC change with treatment, we reviewed historical, baseline, and on-treatment brain MRIs for 9 patients enrolled on our institutional, IRB-approved phase 1 trial of DAY101 in children and young adults with radiographically recurrent or progressive LGG harboring MEK/ERK pathway alterations. De-identified DICOM MRI files were independently reviewed. Time points selected included baseline, first follow-up and best response. The pmod molecular image software package was utilized for data processing of ADC estimates. ADC changes were displayed as a histogram with mean values. Results were based upon a single read paradigm. RESULTS: A shift to lower ADC values for solid components of these tumors was observed, reflecting cellularity and organization; while necrosis correlated with a shift toward higher ADC values. DWI results show reduced ADCs in responding patients, with greater percent change in ADC from baseline associated with deeper responses among treated patients. DWI for treated patients with resultant stable disease showed no significant change in ADC values from baseline while a shift toward higher ADC values was evident in treated, progressing tumors. CONCLUSION: Preliminary DWI analysis reveals that a reduction in ADC values may correlate with treatment response and a shift toward more normal cellularity in tumors treated with DAY101. This method will be applied to a larger cohort of patients in an ongoing phase 1 trial (NCT03429803) and a planned phase 2 trial.

BIOM-38. PI3K/AKT/MTOR SIGNALING PATHWAY ACTIVITY IN IDH-MUTANT DIFFUSE GLIOMA

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PI3K/AKT/mTOR signaling pathway activation is a common mechanism of tumor progression in diffuse lower grade glioma. Robust and accurate biomarkers are needed to stratify patients for therapies targeting this pathway. To investigate the potential of phosphoprotein quantification to provide a direct and functional pathway readout, we analyzed 90 tumors from 83 patients with IDH-mutant diffuse glioma. The cohort comprised 50 IDH-mutant astrocytomas, 40 IDH-mutant and 1p/19q-codeleted oligodendrogliomas, 7 of whom had paired samples from initial diagnosis and recurrence. We developed and validated a pipeline using multiplex immunofluorescence to quantify tumor cell-specific phospho-protein expression of 3 pathway nodes, ribosomal protein S6 (RPS6), PRAS40, and 4E-BP1. In oligodendroglioma the fraction of tumor cells expressing each of the three phosphorylated proteins increased with tumor grade ($p< 0.05$). Comparing tumors at initial diagnosis (n=48) and at recurrence (n=42), p-RPS6 and p-PRAS40 increased in tumor cells ($p< 0.05$) and there was an overall increase in intertumoral heterogeneity of signaling activity at recurrence ($p< 0.04$). Analysis of paired samples demonstrated increased signaling pathway activity in a subset at recurrence. Robust signaling activity, defined as a phospho-positive tumor cell fraction ≥ median for all three phosphoproteins, was identified in 71.4% of grade 3 IDH-mutant astrocytoma(5/7) and

45.4% of grade 3 IDH-mutant, 1p/19q-codeleted oligodendroglioma(5/11). In a subset of cases analyzed by targeted NGS, robust signaling pathway activity was identified in 38%(11/29) at the protein level while genetic alterations predicted to activate the pathway were present in only 17.2% (5/29). Our results demonstrate robust PI3K/AKT/mTOR signaling activity in a significant fraction of IDH-mutant diffuse glioma, an association with increasing tumor grade in oligodendroglioma, and an increase at recurrence in both oligodendroglioma and astrocytoma. Overall, our data suggest that quantitative evaluation of phosphoproteins may be a sensitive method to detect PI3K/AKT/mTOR pathway activity and may be useful for patient stratification.

BIOM-39. ESTABLISHMENT OF A CONNECTIVITY SIGNATURE FOR GLIOMAS

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Recent studies have demonstrated extensive cell-to-cell connectivity between tumor cells of gliomas with considerable relevance for tumor progression and therapy resistance. Tumor microtubes (TMs) are neurite-like tumor cell extensions that build these tumor cell networks. Measuring the extent of connectivity in individual tumors has been challenging and depended on anatomical parameters that are difficult to evaluate in patient samples. We performed bulk and single-cell (sc)RNA sequencing of connected vs. unconnected tumor cells from patient-derived xenograft tumors using a newly developed technology that exploits SR101 dye transfer within tumor cell networks. scRNA sequencing was performed with 17 human glioblastoma tumor samples. Three diffuse glioma cohorts from The Cancer Genome Atlas (n = 648), the Chinese Glioma Genome Atlas (n = 668) and the NCT Neuro Master Match (n = 38, IDH-wildtype only) were used to assess clinical properties. A connectivity signature both from bulk and scRNA sequencing data of xenografted primary glioblastoma tumor cells was established. Comparative analysis showed better performance and higher biological relevance of the single-cell derived signature that involves 71 genes. Most of the genes are related to neurogenesis and neural tube development, including several previously recognized TM-relevant genes. Highest connectivity was observed in astrocytic-like and mesenchymal-like tumor cells. Induction of connectivity *in vitro* was accompanied with increase of the connectivity signature. The connectivity signature was higher in astrocytic as compared to oligodendrocytic gliomas, and highest in IDH-wildtype gliomas. In accordance, connectivity correlated strongly with dismal survival in all three glioma cohorts. The connectivity signature established here is biologically plausible and associates with prognostically relevant glioma subtypes. It provides the first proof-of-principle that tumor cell connectivity is relevant for the clinical course of patients with gliomas, and at the same time serves as a robust biomarker that can be used for future studies, including prospective clinical trials.

BIOM-40. ANALYSIS OF SERUM MIRNA IN GLIOBLASTOMA PATIENTS: TARGETED ENRICHMENT OF EXTRACELLULAR VESICLES ENHANCES SPECIFICITY FOR PROGNOSTIC SIGNATURE

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Glioblastoma is a devastating disease, for which biomarkers allowing a prediction of prognosis are urgently needed. microRNAs have been described as potentially valuable biomarkers in cancer. Here, we studied a panel of microRNAs in extracellular vesicles (EV) from the serum of glioblastoma patients and also in total serum without prior EV separation, and evaluated their correlation with the survival of these patients. Our study included 55 patients in total, 26 (47.3%) of which were treated within the multicenter Phase III CeTeG/NOA-09 trial and 29 (52.7%) in the Division of Clinical Neurooncology of the University Hospital of Bonn, as well as 10 healthy volunteers (HV). Blood was drawn from patients during the adjuvant chemotherapeutic treatment. A panel of 15 microRNAs was studied by quantitative real-time PCR in EV that were separated by size-exclusion chromatography, followed by CDxx* immunoprecipitation (SEC+CDxx*), and compared with those from total serum of glioblastoma patients and HV. Comparing SEC+CDxx* to total serum, we found evidence for enrichment of miR-21-3p and miR-106a-5p and, conversely, lower levels of miR-15b-3p in SEC+CDxx* EV. miR-15b-3p and miR-21-3p were upregulated in serum of glioblastoma patients compared to healthy subjects. Significant correlation with survival of the patients was found for levels of miR-15b-3p in total serum and miR-15b-3p, miR-21-3p, miR-106a-5p and miR-328-3p in SEC+CDxx* EV. Combining miR-15b-3p in serum or miR-106a-5p in SEC+CDxx* EV with any one of the other three microRNAs in SEC+CDxx* EV allowed for a prognostic stratification of glioblastoma patients. We have thus identified four microRNAs whose levels, in combination, can predict the prognosis for these patients. *=Cluster of Differentiation xx (CDxx); Molecule cannot be specifically mentioned due to pending patent.

BIOM-41. GENETIC MARKERS CORRELATED WITH PROGRESSION-FREE SURVIVAL TIMES IN GLIOBLASTOMA PATIENTS UNDERGOING TREATMENT WITH TUMOR TREATING FIELDS

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INTRODUCTION: Despite advances in surgical approaches, followed by chemo-radiotherapy protocols, the overall prognosis for patients with glioblastoma remains poor. Clinical trials have demonstrated that the use of low intensity alternating electric fields, known as Tumor Treating Fields (TTFields), via the Optune™ device extends overall survival times when combined with standard chemotherapy. However, the response to TTFields varies across patients, and it is currently unclear why some patients show increased time to tumor progression with TTFields treatment while others do not. One possible answer lies in the biological diversity of the tumors themselves. Genetic alterations are known to impact survival times and chemotherapy sensitivity in glioblastoma, suggesting that certain markers may also predict responsiveness to TTFields. Here, we compare the genetic profile of primary glioblastoma tumors with progression times in patients receiving TTFields treatment. METHODS: Patients with primary glioblastoma who chose treatment with the Optune™ device were prospectively enrolled and a sample from their primary tumor resection was sent for FoundationONE CDx™ testing. Genetic alteration results, including mutation burden and copy number alterations, were then compared with clinical data and tumor progression times. RESULTS: Mutations and/or copy number changes in genes that regulate cell growth/proliferation, apoptosis, and interactions with DNA were among the most common alterations observed in our cohort. For patients that recurred within 12 months, we found a common pattern of alterations that includes CDKN2A/2B co-deletion, MTAP deletion, and PIK3 mutations. This pattern was not observed in patients that recurred after 12 months. CONCLUSION: The identification of genetic markers that predict treatment responsiveness may help direct patients toward optimal treatment options. Ongoing work is aimed at expanding our sample size, correlating these genetic markers with overall patient survival, and determining if this pattern of expression is specifically related to TTFields treatment response.

BIOM-42. ASSOCIATION OF NEUTROPHIL-LYMPHOCYTE RATIO WITH GLIOMA GRADING AND SURVIVAL

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INTRODUCTION: Gliomas are the most common primary central nervous system tumor. Inflammatory responses are thought to play an important role in cancer progression. Neutrophil-lymphocyte ratio (NLR) is