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EXTH-85. MAGMAS INHIBITION AS A POTENTIAL TREATMENT STRATEGY IN MALIGNANT GLIOMA

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peutic agents/regimen. In this study, we tested a series of patient-derived primary glioma stem cells (GSCs) to 3 GSIs (RO4929097, BMS-708163, and LY3039478) by sphere formation assay. GSI sensitivity was defined by statistically significant inhibition of sphere forming ability. 46% of the GSC lines showed significant impairment by both GSIs ($p<0.05$), which were defined as the GSI-sensitive GSCs. To identify a predictive signature, we performed enrichment and correlation analyses using RNA sequencing, RPPA, and methylation data of the GSI-sensitive and resistant GSCs. Analysis of integrated data identified GSI-sensitive GSCs harbored significantly elevated NOTCH expression (high NOTCH1 expression and low NOTCH1 methylation). In addition, GSI-sensitive cells showed increased P53 activity (increased expression of P53 targets-P21, BAX and TIGAR), high wild-type P53 frequencies, and decreased PI3K/AKT activity (high PTEN expression and low p-AKT-Thr308 expression) ($p<0.05$). As GSIs alone showed only modest efficacy, we turned to identifying a combination strategy to strengthen GSI effect on cell growth. We showed that concomitant inhibition of NOTCH signaling with GSI together with doxorubicin (induces DNA damage and thus restores P53 activity) had a synergistic growth inhibitory effect in P53 wild-type GSI-sensitive cells, confirming doxorubicin enhanced GSI efficacy in a subset of GBMs. In summary, we elucidated the genetic characteristics (high NOTCH, wild-type p53, and low PI3K/AKT) of GSI-sensitive GSCs and developed a combination strategy based on the genetic markers. High NOTCH1 expression and wild-type P53 predicted the synergistic effect of GSI and doxorubicin, providing a potential new therapeutic regimen for a molecularly selected subset of GBM patients.

EXTH-83. TARGETING DNA REPAIR AND SURVIVAL PATHWAYS THROUGH HEAT SHOCK PROTEIN INHIBITION USING AT13387 TO SENSITIZE GLIOMA TO CHEMORADIATION THERAPY

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BACKGROUND: Glioblastoma (GBM) is the most lethal of adult primary brain tumors. Resistance to the standard chemoradiation therapy with temozolomide (TMZ) emerges due to increased expression of DNA-alkylating proteins and potent DNA repair. This is in part through activation of the heat-shock response, an evolutionally conserved defense mechanism mediated by heat-shock proteins (HSP). We recently reported that AT13387, an orally-bioavailable second generation HSP90 inhibitor, efficiently crosses the blood brain barrier (BBB) and is effective preclinically against gliomas as a single agent and in combination with TMZ. **METHODS AND RESULTS:** To test whether its potent effects on DNA-repair pathways could sensitize GBM to chemoRT, we examined the effects of AT13387 in glioma cell lines and patient-derived glioma stem-like cells. AT13387 potently induced apoptosis, inhibited key oncogenic pathways and inhibited proliferation and migration of glioma cells. RPPA and RNAseq analysis revealed reduced expression of several key DNA-repair proteins and genes. AT13387-treated glioma cells exposed to radiation showed impaired ability to repair DNA in NHEJ/HR DNA-repair assays and increased DNA damage in a Comet assay. The combination of AT13387 with TMZ and RT showed *in vivo* efficacy with significant improvement in survival in intracranial GBM-xenotransplanted zebrafish and in an intracranial glioma PDX nude mouse model compared with radiotherapy alone or chemoRT. Lastly, AT13387-treated *ex vivo* human glioma slice cultures showed reduced levels of DNA repair and survival pathway proteins and increased g-H2AX levels indicating DNA damage compared with untreated controls. **CONCLUSIONS:** The oral bioavailability, ability to cross the BBB and multi-target pathway inhibition by AT13387 makes it a promising agent for therapeutic targeting of GBM. Our results also strongly support the potential for Hsp90 inhibition with AT13387 as a therapeutic strategy against gliomas in combination with chemoradiation therapy. A phase I/II trial of the combination in newly-diagnosed GBM patients is currently under planning.

EXTH-84. TARGETING THE SALVAGE PATHWAY OF NAD+ GENERATION IN GLIOMAS BY KPT-9274, A NOVEL DUAL INHIBITOR OF PAK4 AND NAMPT

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BACKGROUND: Current treatment of glioblastoma (GBM) with surgery and chemoRT yields an average survival of ~1.5 years, highlighting the need for more effective therapies. We have previously reported that PAK4 is a key modulator of radioresistance in GBM. Additionally, pathways that regulate differential energy metabolism in gliomas and circumvent tumor heterogeneity have recently emerged as promising therapeutic targets. We hypothesized that inhibition of NAMPT, the rate-limiting enzyme of the NAD+ salvage pathway, a key pathway preferentially used in glioma energy metabolism, would be effective against GBM. **METHODS:** We tested the efficacy of KPT9274, a potent first-in-class dual inhibitor of PAK4 and NAMPT, currently in human trials against solid tumors and lymphomas (NCT02702492), on NAD levels and cell viability in glioma cell lines and patient-derived glioma stem-like cells (GSC). We also examined enzyme-kinetics of various components of the NAD salvage pathway and effects of NADH rescue by nicotinamide, NMN and NAD. Lastly, we examined the biological relevance of NAMPT inhibition in glioma cells and GSC using a combination of cell adhesion, wound-healing, transwell-migration and microtubule formation assays. **RESULTS:** Treatment with KPT9274 resulted in potent depletion of NAD in glioma cells which was rescued by nicotinamide, NMN and NAD demonstrating its specificity against NAMPT. KPT-9274 treatment resulted in potent inhibition of cell adhesion, migration, cell invasion, endothelial tube formation and proliferation in glioma cells and GSC. In vivo studies in mouse bioluminescent intracranial glioma PDX model as well as PK studies are ongoing to confirm the pre-clinical significance of targeting NAMPT in gliomas and will be presented. **CONCLUSIONS:** Our study demonstrates a key role for NAMPT in NAD generation in gliomas as seen by the potent biological effects of KPT-9274 against these tumors and supports the potential for targeting the NAD salvage pathway as a novel therapeutic strategy for treatment of GBM.

EXTH-85. MAGMAS INHIBITION AS A POTENTIAL TREATMENT STRATEGY IN MALIGNANT GLIOMA

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OBJECTIVES: Magmas (mitochondria-associated protein involved in granulocyte-macrophage colony-stimulating factor signal transduction) is a nuclear gene that encodes for the mitochondrial import inner membrane translocase subunit Tim16. Magmas is highly conserved and ubiquitously expressed in all mammalian cells, and is essential for cell viability. Its expression levels are increased in a significant proportion of human prostate cancers, independently of mitochondria content. In addition, Magmas mRNA is over expressed in two ACTH-secreting pituitary adenoma cell lines as compared to normal pituitary in mouse, as well as in 47 out of 64 pituitary adenomas compared to normal pituitary in human. Moreover, Magmas silencing sensitizes to pro-apoptotic stimuli and induces a G0/G1 accumulation. Based on the above findings, we believed that inhibition of Magmas by small molecule inhibitors could be beneficial for glioma treatment. **METHODS:** In this study, we tested the capability of a Magmas inhibitor-BT#9 to cross the blood brain barrier in mice. The anti-tumor effect of BT#9 was investigated using glioma cell lines. **RESULTS:** Our in vivo results showed that while the plasma level of BT#9 reaches a Cmax within 5 minutes and is obviously eliminated by 720 minutes, brain levels of BT#9 increase over the first 240 minutes after IV exposure and then slowly decrease, indicating that BT#9 may cross the blood brain barrier. In vitro study using glioma cell lines revealed that Magmas inhibition by BT#9 significantly decreased cell proliferation, induced apoptosis along with vacuole

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formation, blocked migration and invasion. Since Magmas is a ROS regulator, BT#9 treatment resulted in a decrease in respiratory function of glioma cells. **DISCUSSION:** This is the first study about the role of Magmas in glioma. Our findings suggest that Magmas plays a key role in glioma cell survival and targeting Magmas by Magmas inhibitor has the potential to become an therapeutic strategy in gliomas.

GENETICS AND EPIGENETICS

GENE-01. STABILITY OF ACTIONABLE MUTATIONS IN PRIMARY AND RECURRENT GLIOBLASTOMAS

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INTRODUCTION: Efforts to improve patient survival in recurrent glioblastomas (GBMs) are often based on targeting the tumors acquired genetic changes. However, resections on recurrent GBMs are infrequently performed and molecular data is extrapolated from the initial tumor. We therefore initiated a large study to assess whether genetic changes of initial GBMs are still present at recurrence. **METHODS:** DNA was isolated from pairs of initial and recurrent GBM (FFPE) tumor samples and sequenced on a panel of 419 cancer associated genes. We also determined *EGFRvIII* expression status and *MGMT* promoter methylation status. All patients were treated according to the Stupp protocol. **RESULTS:** Tumor pairs from 226 patients from 10 medical centers in six countries were included in the analysis. Median survival was 21.1 months and median time to second surgery 11.1 months. Higher age, greater lesion size at second surgery and steroid treatment were associated with poorer survival in the recurrent tumor. ~4% of tumors were *IDH1* mutated (median OS 33.7 months). Of the 114 tumor pairs sequenced to date, 73 patients (64%) gained or lost a variant in a recurrent driver oncogene while the median retention rate of driver variants was 80%. Common genes with gained mutations include *TP53* (13 patients) and *NF1* (8 patients), common losses of mutations were found in *EGFR* (12 out of 23). *EGFRvIII* was expressed in 25.5% of initial tumors and was lost at recurrence in 50% of cases. Two patients developed a hypermutated tumor at recurrence (276 and 431 coding variants after 12 and 17 cycles of temozolomide respectively). **CONCLUSION:** Preliminary analysis indicates that there are distinct gains and losses in each tumor, including in some driver mutations at tumor recurrence. This has important consequences on the need for re-biopsies in targeted treatment studies in recurrent glioblastoma.

GENE-02. PERIPHERAL BLOOD DNA METHYLATION PROFILES IDENTIFY IDH1/2 MUTATION STATUS IN ADULTS WITH DIFFUSE GLIOMA

Andreas Kloetgen[1], Jonathan Serrano[1], Seema Patel[1], Christopher Bowman[2], Guomiao Shen[1], David Zagzag[1], Matthias Karajannis[3], John Golfinos[1], Dimitris Placantonakis[1], Aristotelis Tsirigos[1], Andrew S. Chi[1] and Matija Snuderl[1]; [1]Laura and Isaac Perlmutter Cancer Center, NYU Langone Medical Center, New York, NY, USA, [2]Department of Pathology University of California San Francisco, San Francisco, CA, USA, [3]Memorial Sloan Kettering Cancer Center, New York, NY, USA

INTRODUCTION: Identification of IDH1/2 mutations and association with better outcome revolutionized diagnosis and management of patients with gliomas. Liquid biopsy allows identification of driver mutations from blood; however detection of circulating tumor DNA or circulating tumor cells from patients with brain tumors is difficult due to the low amount of tumor material in the peripheral blood. We hypothesized that brain tumors alter the epigenetic profiles of circulating leukocytes and sought to identify DNA methylation patterns in peripheral blood that would identify molecular subtypes of glioma. **METHODS:** We analyzed brain tumors and matched whole peripheral blood collected in EDTA tubes from 63 patients with newly diagnosed diffuse glioma and whole peripheral blood from 40 individuals with no known history of brain tumor. Tumor IDH1/2 mutation status was analyzed using next-generation sequencing and circulating leukocyte genomic DNA methylation was analyzed using Illumina 850k EPIC array. Methylation data were analyzed with the R Bioconductor package minfi, including quality control, data normalization and differentially methylated CpG site analysis. Subsequent filtering was performed using a p-value cutoff = 0.01 and a minimal mean difference of the Beta-value of = 0.1. **RESULTS:** Peripheral blood methylation profiles of patients with brain tumors were distinctly different from controls. DNA methylation profiles from peripheral leukocytes identified a total of 222 differentially methylated CpG sites in males and 515 CpG sites in females between IDH mutated versus wild-type diffuse glioma patients. Blood DNA methylation distinguished patients with IDH mutant and wildtype diffuse gliomas with 98% accuracy, nearly perfectly separating groups by clustering. **CONCLUSIONS:** Diffuse gliomas induce epigenetic changes in the methylome of the circulating leukocytes. IDH wildtype and mutant gliomas can be distinguished with high accuracy by profiling the epigenome of circulating leukocytes. Our study demonstrates the potential of peripheral blood DNA methylation assessment for non-invasive diagnosis of brain tumors.

GENE-03. SERUM LONG NONCODING RNA HOTAIR AS NOVEL DIAGNOSTIC AND PROGNOSTIC BIOMARKER IN GLIOBLASTOMA MULTIFORME

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Glioblastoma multiforme (GBM) is the most common malignant adult brain tumor. However, detecting tumor progression via MRI is often inconclusive which can lead to delayed treatment. Therefore, there is an urgent need to develop a peripheral biomarker for GBM. Utilizing single molecule sequencing, we have previously demonstrated that hundreds of long noncoding RNAs, including HOTAIR, are strongly dysregulated in GBM and HOTAIR is critical in GBM cells proliferation. The purpose of this study was to investigate the prognostic and diagnostic values of serum HOTAIR in GBM. The HOTAIR expressions were measured in 32 GBM serum samples and 40 healthy controls using qRT-PCR. The PCR products were subsequently subcloned into pCRTM4-TOPO®TA vectors for DNA sequencing. A ROC curve was generated to examine HOTAIR's prognostic value and correlations of serum HOTAIR levels with clinicopathological features were analyzed. Serum exosomes were also isolated and validated by Western blot and NanoSight analysis. We further detected serum HOTAIR levels in mice with GBM PDX-39 tumors implanted intracranially. GBM patients had a significantly higher serum HOTAIR expression than those of healthy controls (P<0.0001, Mann-Whitney test). At the cut-off value of 5297 HOTAIR level in serum, the area under the ROC curve distinguishing GBM patients from normal controls was 0.776 (95% CI: 0.664–0.888, P<0.0001), with 81.3% sensitivity and 72.2% specificity. In addition, Pearson correlation analysis indicated a medium correlation of serum HOTAIR levels and the corresponding tumor HOTAIR levels (r=0.734, P<0.01). Clinical data also indicated that HOTAIR was correlated with higher WHO grades of glioma. HOTAIR was found to be enriched in exosome fraction of GBM serum. Moreover, the presence of GBM tumor in mice lead to significant increase in serum HOTAIR expression. Our results, for the first time, demonstrated that the serum HOTAIR could be used as a novel biomarker for diagnosis and prognosis in GBM.

GENE-04. COMPREHENSIVE GENOMIC CHARACTERIZATION OF AGGRESSIVE MENINGIOMAS IDENTIFIES MOLECULAR SIGNATURES THAT PREDICT CLINICAL OUTCOMES

Harish Vasudevan[1], Steve Braunstein[1], Joanna J. Phillips[2], Melike Pekmezci[1], Ashley Wu[1], Gerald Reis[1], Stephen Magill[1], Susan Chang[1], Penny Sneed[1], Michael McDermott[1], Arie Perry[1] and David Raleigh[1]; [1]UCSF, San Francisco, CA, USA, [2]Department of Neurological Surgery and Pathology, University of California, San Francisco (UCSF), San Francisco, CA, USA

BACKGROUND: Current pathological grading schemes do not fully predict meningioma behavior, and the molecular basis for meningioma is incompletely understood. Here, we perform whole exome sequencing (WES), DNA methylation arrays, RNA-seq, NanoString (NS), and immu-

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