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NEURO-ONCOLOGY ADVANCES

ABSTRACTS

ABSTRACTS FROM THE 2021 SNO-NCI JOINT SYMPOSIUM: TARGETING CNS TUMOR METABOLISM

Submission Categories and Abbreviations:
BIMG - Metabolic Biomarkers and Imaging
DDRE - Metabolic Drug Targets, Resistance
ETMM - Epigenome, Transcriptome, Metabolome and Modeling
FSMP - Metabolic Fluxes and Signaling of Metabolic Pathways
TBMT - Technologies for Studying Brain Metabolism

METABOLIC BIOMARKERS AND IMAGING

BIMG-02. IMAGING IMMORTALITY: TERT EXPRESSION ALTERS GLUCOSE METABOLISM IN LOW-GRADE GLIOMAS IN A MANNER THAT CAN BE LEVERAGED FOR NONINVASIVE METABOLIC IMAGING

Pavithra Viswanath[1], Georgios Batsios[1], Anne Marie Gillespie[1], Hema Artee Luchman[2], Joseph Costello[1], Russell Pieper[1], Sabrina Ronen[1]; [1]University of California San Francisco, San Francisco, CA, USA, [2]University of Calgary, Calgary, AB, Canada

Telomerase reverse transcriptase (TERT) is essential for tumor immortality and uncontrolled proliferation, including in low-grade oligodendrogliomas (LGOGs). Since it is silenced in somatic cells, TERT is also a therapeutic target. Non-invasive imaging of TERT can differentiate tumor from normal brain or lesions such as gliosis and allow assessment of response to therapy. The goal of this study was to identify magnetic resonance spectroscopy (MRS)-detectable metabolic alterations associated with TERT that can be leveraged for noninvasive imaging in LGOGs. We examined patient-derived BT54 neurospheres in which TERT expression was silenced by RNA interference. ^{1}H-MRS showed that steady-state levels of NAD(P)/H, glutathione, aspartate and AXP were elevated in $BT54_{TERT+}$ neurospheres relative to $BT54_{TERT-}$. Glucose flux through the pentose phosphate pathway (PPP) is essential for generating NADPH, which maintains glutathione homeostasis. ^{13}C-MRS confirmed that [2-^{13}C]-glucose flux through the PPP was elevated in $BT54_{TERT+}$ neurospheres relative to $BT54_{TERT-}$, an effect associated with higher activity of the PPP enzyme glucose-6-phosphate dehydrogenase (G6PDH). Hyperpolarized ^{13}C-MRS is a method of increasing the signal to noise ratio of ^{13}C-MRS such that it can monitor metabolic fluxes noninvasively in cells, animals and patients. Consistent with elevated PPP flux and G6PDH activity, hyperpolarized [U-^{13}C]-glucose metabolism via the PPP to 6-phosphogluconate (6-PG) was elevated in $BT54_{TERT+}$ neurospheres relative to $BT54_{TERT-}$. Importantly, examination of an additional patient-derived LGOG model, the SF10417 model which readily forms orthotopic tumor xenografts in rats, showed that 6-PG production from hyperpolarized [U-^{13}C]-glucose demarcated tumor from normal brain. Furthermore, LGOG patient biopsies had elevated NAD(P)/H, glutathione, aspartate, AXP and G6PDH activity relative to gliosis biopsies, confirming the clinical validity of our observations. Collectively, we have identified a metabolic signature of TERT expression that can be leveraged via hyperpolarized [U-^{13}C]-glucose to improve diagnosis and treatment response monitoring for LGOG patients.

BIMG-03. MOLECULAR IMAGING OF GLUCOSE METABOLISM FOR INTRAOPERATIVE FLUORESCENCE GUIDANCE DURING GLIOMA SURGERY

Evgenii Belykh[1,2], Jubran Jubran[1,3], Laeth George[1,3], Liudmila Bardonova[1], Deborah Healey[1], Joseph Georges[4], Chad Quarles[1], Jennifer Eschbacher[1], Shwetal Mehta[1], Adrienne Scheck[1], Peter Nakaji[3], Michael Lawton[1], Mark Preul[1]; [1]Barrow Neurological Institute, Phoenix, Arizona, USA, [2]Rutgers University, New Brunswick, New Jersey, USA, [3]University of Arizona College of Medicine - Phoenix, Phoenix, Arizona, USA, [4]Philadelphia College of Osteopathic Medicine, Philadelphia, PA, USA

PURPOSE: This study evaluated the utility of using molecular imaging of fluorescent glucose analog 2-(*N*-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl)Amino)-2-Deoxyglucose (2-NBDG) as a discriminatory marker for intraoperative tumor border identification in a mouse glioma model. **PROCEDURES:** 2-NBDG and were assessed in GL261 and U251 orthotopic tumor bearing mice. Intraoperative fluorescence of 2-NBDG administered topical and intravenous in normal and tumor regions was assessed with operating microscope, handheld confocal laser scanning endomicroscope (CLE) and benchtop confocal laser scanning microscope (LSM). Additionally, 2-NBDG fluorescence in tumors was compared to 5-aminolevulinic acid-induced protoporphyrin IX fluorescence. **RESULTS:** Intravenously administered 2-NBDG was detectable in brain tumor and absent in contralateral normal brain parenchyma on wide field operating microscopy imaging. Intraoperative and benchtop CLE showed preferential 2-NBDG accumulation in the cytoplasm of glioma cells (tumor-background ratio of 2.76±0.43). Topically administered 2-NBDG did not create a sufficient tumor-background contrast for white field operating microscopy imaging, or under benchtop LSM (tumor-background ratio 1.42 ± 0.72). However, topical 2-NBDG did create sufficient contrast to evaluate cellular tissue architecture and differentiate tumor cells from normal brain parenchyma. PpIX imaging resulted in a more specific delineation of gross tumor margins than IV or topical 2-NBDG, and a significantly higher tumor-normal brain fluorescence intensity ratio. **CONCLUSION:** After intravenous administration, 2-NBDG selectively accumulated in the experimental brain tumors and provided bright contrast under wide field fluorescence imaging with a clinical grade operating microscope. Topical 2-NBDG was able to create a sufficient contrast to differentiate tumor from normal brain cells based on visualization of cellular architecture with CLE. 5-ALA demonstrated superior specificity in outlining tumor margins and significantly higher tumor-background contrast. Given its non-toxicity, using 2-NBDG as a topical molecular marker for noninvasive in vivo intraoperative microscopy is encouraging, and warrants further clinical evaluation.

BIMG-04. MAPPING HETEROGENEITY OF HIGH-GRADE GLIOMA METABOLISM USING HIGH RESOLUTION 7T MRSI

Gilbert Hangel, Cornelius Cadrien, Philipp Lazen, Sukrit Sharma, Julia Furtner, Alexandra Lipka, Eva Heckova, Lukas Hingerl, Stanislav Motyka, Stephan Gruber, Bernhard Strasser, Barbara Kiesel, Mario Mischkulnig, Matthias Preusser, Thomas Roetzer, Adelheid Wöhrer, Georg Widhalm, Karl Rössler, Siegfried Trattnig, Wolfgang Bogner; Medical University of Vienna, Vienna, Austria

OBJECTIVES: Neurosurgical resection in gliomas depends on the precise preoperative definition of the tumor and its margins to realize a safe maximum resection that translates into a better patient outcome. New metabolic imaging techniques could improve this delineation as well as designate targets for biopsies. We validated the performance of our fast high-resolution whole-brain 3D-magnetic resonance spectroscopic imaging (MRSI) method at 7T in high-grade gliomas (HGGs) as first step to this regard. **METHODS:** We measured 23 patients with HGGs at 7T with MRSI covering the whole cerebrum with 3.4mm isotropic resolution in 15 min. Quantification used a basis-set of 17 neurochemical components. They were evaluated for their reliability/quality and compared to neuroradiologically segmented tumor regions-of-interest (necrosis, contrast-enhanced, non-contrast-enhanced+edema, peritumoral) and histopathology (e.g., grade, IDH-status). **RESULTS:** We found 18/23 measurements to be usable and ten neurochemicals quantified with acceptable quality. The most common denominators were increases of glutamine, glycine, and total choline as well as decreases of N-acetyl-aspartate and total creatine over most tumor regions. Other metabolites like taurine and serine showed mixed behavior. We further found that heterogeneity in the metabolic images often continued into the peritumoral region. While 2-hydroxy-glutarate could not be satisfyingly quantified, we found a tendency for a decrease of glutamate in IDH1-mutant HGGs. **DISCUSSION:** Our findings corresponded well to clinical tumor segmentation but were more heterogeneous and often extended into the peritumoral region. Our results corresponded to previous knowledge, but with previously not feasible resolution. Apart from glycine/glutamine and their role in glioma progression, more research on the connection of glutamate and others to specific mutations is necessary. The addition of low-grade gliomas and statistical ROI analysis in a larger cohort will be the next important steps to define the benefits of our 7T MRSI approach for the definition of spatial metabolic tumor profiles.

BIMG-05. TO BE OR NOT TO BE GLYCOLYTIC: DEUTERATED GLUCOSE-BASED ASSESSMENT OF THE WARBURG EFFECT ALLOWS NON-INVASIVE IMAGING OF TUMOR BURDEN AND TREATMENT RESPONSE IN MUTANT IDH GLIOMAS *IN VIVO*

Celine Taglang[1], Georgios Batsios[1], Meryssa Tran[1], Anne Marie Gillepsie[1], Hema Artee Luchman[2], Russell O. Pieper[3], Sabrina M. Ronen[1], Pavithra Viswanath[1]; [1]Department of Radiology and Biomedical Imaging, University of California San Francisco, San Francisco, CA, USA,

[2]Department of Cell Biology and Anatomy and Hotchkiss Brain Institute, University of Calgary, Calgary, AB, Canada, [3]Department of Neurological Surgery, University of California San Francisco, San Francisco, CA, USA

The Warburg effect, characterized by elevated glucose uptake and flux to lactate, is a metabolic hallmark of cancer. Recent studies have identified deuterium ^{2}H-magnetic resonance spectroscopy (MRS) using 6,6'-^{2}H-glucose as a novel method of imaging the Warburg effect in high-grade primary glioblastomas (GBMs). However, its utility for imaging low-grade gliomas has not been tested. The goal of this study was to determine whether 6,6'-^{2}H-glucose can be used for imaging tumor burden and treatment response in mutant isocitrate dehydrogenase (IDHmut) low-grade gliomas *in vivo*. We examined mice bearing orthotopic tumors of the patient-derived BT257 astrocytoma model. ^{1}H-MRS, providing a readout of steady-state metabolite levels, confirmed the presence of 2-hydroxyglutarate, the product of IDHmut, in BT257 tumor tissue but not normal brain. Previous studies comparing IDHmut gliomas with GBMs suggest that IDHmut gliomas undergo lactate dehydrogenase silencing, potentially leading to a non-glycolytic phenotype. Nevertheless, our results indicated that, compared to normal brain, glucose uptake and concomitant flux to lactate were significantly higher in BT257 tumor tissue. Importantly, 6,6'-^{2}H-glucose metabolism to lactate was observed in BT257 tumor-bearing mice, but not tumor-free mice. Furthermore, imaging studies confirmed spatial localization of lactate production to the tumor vs. contralateral normal brain. We then examined the ability of 6,6'-^{2}H-glucose to assess treatment response. Poly-(adenosine 5′-diphosphate-ribose) polymerase inhibitors (PARPi) inhibit IDHmut glioma growth and are in clinical trials for IDHmut glioma patients. Treatment with the PARPi niraparib reduced 6,6'-^{2}H-glucose flux to lactate in BT257 tumor-bearing mice. Importantly, this reduction was observed at early time-points when no difference in tumor volume could be detected using anatomical imaging, pointing to the ability of 6,6'-^{2}H-glucose to assess pseudoprogression. Collectively, our results suggest that IDHmut gliomas display a glycolytic phenotype amenable to non-invasive ^{2}H-MRS-based imaging of tumor burden and treatment response.

BIMG-06. RESPONSE ASSESSMENT OF BEVACIZUMAB THERAPY FOR GLIOBLASTOMA BY USING MULTIPLE PET TRACERS

Keisuke Miyake, Daisuke Ogawa, Tetsuhiro Hatakeyama, Takashi Tamiya; Department of Neurological Surgery, Kagawa University Faculty of Medicine, Miki, Kagawa, Japan

OBJECTIVE: Use of the positron emission tomography (PET), such as 18F-fluorodeoxyglucose (FDG), 11C-Methionine (MET), 18F-Fluorothymidine (FLT), and 18F-Fluoromisonidazole (FMISO), is expected to lead the way for novel applications aimed at achieving efficient malignancy grading and treatment of gliomas. The aim of this study was to assess FDG, MET, FLT, and FMISO PET studies to evaluate the biological effects induced by bevacizumab (BEV) therapy in glioblastoma patients. **METHODS:** Seventy-one patients with glioblastoma were treated biweekly with BEV from July 2013 to November 2020. FDG, MET, FLT, and FMISO PET scans were obtained at baseline and at follow-up (4 weeks after treatment onset). Measures of FDG, MET, FLT, and FMISO avidity were recorded; the measures were SUVmax, metabolic tumor volume (MTV; volume of tumor with SUV>42% of SUVmax), SUVmean (within the MTV), tumor-to-normal ratio (TNR), tumor-to blood ratio (TBR), and total lesion avidity (TLA; calculated as MTV x SUVmean). The prognostic analysis was performed in relation to the response assessment by multiple PET tracers using progression-free survival (PFS) and overall survival (OS). **RESULTS:** Under the assessment of the Cox proportional hazard model, increased changes of FDG SUVmax, MET TLA at follow-up, FLT TLA at follow up, increased changes of FLT TLA, increased changes of FMISO TBR and FMISO MTV were significant prognostic factor of PFS. Increase changes of FDG TLA and FLT TLA and increased changes of FMISO TBR were significant prognostic factor of OS. **CONCLUSION:** Increased changes in FLT TLA and FMISO-PET after BEV therapy may be a useful biomarker for predicting PFS and OS in glioblastoma.

BIMG-07. PHARMACOLOGICAL ASCORBATE ENHANCES RADIATION AND TEMOZOLOMIDE EFFECTIVENESS IN GLIOBLASTOMA BY A MECHANISM MEDIATED BY REDOX ACTIVE IRON

Bryan Allen[1], Michael Petronek[2], Varun Monga[1], Kellie Bodeker[1], Brian Smith[2], Garry Buettner[2], Jeremy Greenlee[1], Mark Smith[1], Douglas Spitz[2], Joseph Cullen[1], Vincent Magnotta[2], John Buatti[1]; [1]University of Iowa Hospitals and Clinics, Iowa City, IA, USA, [2]University of Iowa, Iowa City, IA, USA

Pharmacological ascorbate (P-AscH^{-}; high dose intravenous infusions of vitamin C generating milli-molar plasma concentrations) has re-emerged as an anti-cancer therapy. Phase 1 clinical trials combining P-AscH^{-} with chemotherapy and ionizing radiation demonstrate safety and promising clinical outcomes in a variety of malignancies. In a first-in-human trial, subjects with newly diagnosed glioblastoma (GBM) and undetectable *MGMT* promoter methylation were treated with P-AscH^{-}, ionizing radiation, and temozolomide. Results demonstrate median progression-free survival (PFS) of 10 months and median overall survival (OS) of 23 months, comparing favorably to historical GBM patients expressing MGMT. P-AscH^{-}'s anti-cancer mechanism is dependent upon the presence of redox active labile iron. In the presence of redox active iron, the formation of hydrogen peroxide, which causes oxidative stress and eventual cell death, selectively forms in cancer cells. Treatment with P-AscH^{-} increased cancer cells' labile iron pool, further enhancing sensitivity to P-AscH^{-}. We investigated the capability of MR imaging (T_2* relaxation time) to measure the redox active iron and predict response to P-AscH^{-}. T_2* relaxation time is influenced by in-field inhomogeneities, such as redox active paramagnetic iron. The active phase 2 trial evaluating P-AscH^{-}, radiation, and temozolomide for GBM, obtains T_2* imaging prior to (baseline) and immediately after ascorbate infusion (NCT02344355). A preliminary analysis of the baseline scan for the first 15 subjects suggests those with faster GBM T_2* relaxation times (≤ 58 ms) have more redox active labile iron pools as well as an improved median PFS (11.4 months) compared to those with slower T_2* relaxation times (> 58 ms; median PFS of 8.5 months). Pre-clinical studies evaluating the effectiveness of iron nano-particle supplementation in GBM animal models are on-going. (Supported by P01 CA217797, R01 CA169046, U01 CA140206, T32 CA078586, P30 CA086862, as well as the Gateway for Cancer Research grant G-17–1500.)

BIMG-08. DEUTERIUM MAGNETIC RESONANCE SPECTROSCOPY USING ^{2}H-PYRUVATE ALLOWS NON-INVASIVE *IN VIVO* IMAGING OF TERT EXPRESSION IN BRAIN TUMORS

Georgios Batsios, Celine Taglang, Meryssa Tran, Anne Marie Gillespie, Joseph Costello, Sabrina Ronen, Pavithra Viswanath; UCSF, San Francisco, CA, USA

Telomere shortening constitutes a natural barrier to uncontrolled proliferation and all tumors must find a mechanism of maintaining telomere length. Most human tumors, including high-grade primary glioblastomas (GBMs) and low-grade oligodendrogliomas (LGOGs) achieve telomere maintenance via reactivation of the expression of telomerase reverse transcriptase (TERT), which is silenced in normal somatic cells. TERT expression is, therefore, a driver of tumor proliferation and, due to this essential role, TERT is also a therapeutic target. However, non-invasive methods of imaging TERT are lacking. The goal of this study was to identify magnetic resonance spectroscopy (MRS)-detectable metabolic biomarkers of TERT expression that will enable non-invasive visualization of tumor burden in LGOGs and GBMs. First, we silenced TERT expression by RNA interference in patient-derived LGOG (SF10417, BT88) and GBM (GS2) models. Our results linked TERT silencing to significant reductions in steady-state levels of NADH in all models. NADH is essential for the conversion of pyruvate to lactate, suggesting that measuring pyruvate flux to lactate could be useful for imaging TERT status. Recently, deuterium (^{2}H)-MRS has emerged as a novel, clinically translatable method of monitoring metabolic fluxes *in vivo*. However, to date, studies have solely examined ^{2}H-glucose and the use of [U-^{2}H]pyruvate for non-invasive ^{2}H-MRS has not been tested. Following intravenous injection of a bolus of [U-^{2}H]pyruvate, lactate production was higher in mice bearing orthotopic LGOG (BT88 and SF10417) and GBM (GS2) tumor xenografts relative to tumor-free mice, suggesting that [U-^{2}H] pyruvate has the potential to monitor TERT expression *in vivo*. In summary, our study, for the first time, shows the feasibility and utility of [U-^{2}H]pyruvate for *in vivo* imaging. Importantly, since ^{2}H-MRS can be implemented on clinical scanners, our results provide a novel, non-invasive method of integrating information regarding a fundamental cancer hallmark, i.e. TERT, into glioma patient management.

BIMG-09. GLUTAMINE AND GLYCINE BY MR SPECTROSCOPY IDENTIFY AGGRESSIVE GLIOMAS

Changho Choi, Pegah Askari, Elena Daoud, Kimmo Hatanpaa, Jack Raisanen, Michael Levy, Edward Pan, Toral Patel, Bruce Mickey, Elizabeth Maher; University of Texas Southwestern Medical Center, Dallas, TX, USA

Cancers reprogram their metabolism and the resulting alterations in metabolite abundance can be monitored in patients noninvasively using proton magnetic resonance spectroscopy (MRS). We evaluated glutamine, glycine and 2-hydroxyglutarate (2HG) in 27 adult subjects with gliomas (17 male and 10 female; age 22 - 69, median 39 years) using optimized MRS at 3T (PRESS TE 97ms) and examined their association with post-gadolinium enhancement, cell proliferation rate (MIB-1 labeling index), and overall survival of patients. The tumors included 9 glioblastomas (3 IDH mutated and 6 IDH wildtype), 10 astrocytomas (7 IDH mutated and 3 IDH