into PPIX. Our data suggest that different microenvironments within the tumour alter the activity of the heme biosynthetic pathway, resulting in differential fluorescence in glioblastoma. It paves the way for work to alter the glioblastoma microenvironment in order to further improve the use of FGS in guiding surgery across these devastating tumours.

BIMG-18. ELEVATED CYSTATHIONINE IN MEDULLOBLASTOMA DEMONSTRATES TUMOR-SPECIFIC METHIONINE METABOLISM

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We investigated tumor-specific metabolism medulloblastoma using a non-biased MS-imaging screen and identified a pattern of methionine flux that may present a therapeutic opportunity. We studied brain tumors that form in mice genetically engineered to develop Sonic Hedgehog (SHH)-driven medulloblastoma. We subjected sagittal sections including brain and medulloblastoma to MS-imaging, generating concentration maps for hundreds of metabolites MW 100–400. We then confirmed results by analyzing tumor, brain and blood by LC-MS/MS, high-resolution NMR and 2D NMR-TOCSY, and used immunohistochemistry to determine the cellular localization of implicated enzymes. MS imaging, accomplished by matrix-assisted laser desorption electrospray ionization (MALDESI), detected cystathionine at an order of magnitude higher concentration in medulloblastomas compared to adjacent brain. No other metabolite showed such a strong, tumor-specific localization. LC-MS/MS and NMR methods confirmed cystathionine elevation. As cystathionine is the product of homocysteine and serine, catalyzed by cystathionine beta-synthase (CBS), we investigated CBS expression by IHC. Consistent with prior studies, we found that only astrocytes expressed CBS, both in the normal brain and within the tumors. ScRNA-seq confirmed *Cbs* only in astrocytes, and showed tumor cells express methionine-metabolizing enzymes *Mat2a*, *Dnmt1*, *Ancy* and *Mtr*. Together, these findings show that tumor cells generate and export homocysteine, which astrocytes convert to cystathionine. Tumor cystathionine generation responded to changes in methionine- cycle metabolites. *In vivo*, systemic administration of homocysteine increased tumor cystathionine which decreased in response to systemic folate, the methyl donor for homocysteine methyltransferase. Cystathionine itself was inert in tumors as tumor cells cultured in up to 8 mM cystathionine showed no change in cell cycle progression. Our studies show that medulloblastomas utilize methionine and generate homocysteine, but avoid folate-dependent homocysteine-methionine recycling by exporting homocysteine for detoxification by local astrocytes. This model suggests that treatments that impose methionine scarcity, folate scarcity or CBS inhibition may produce anti-tumor effects in medulloblastoma.

BIMG-19. ^{18}F-FLUCICLOVINE PET/CT TO DISTINGUISH RADIATION NECROSIS FROM TUMOR PROGRESSION IN BRAIN METASTASES TREATED WITH STEREOTACTIC RADIOSURGERY: A PROSPECTIVE PILOT STUDY

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PURPOSE: To estimate the accuracy of ^{18}F-Fluciclovine PET/CT in distinguishing radiation necrosis (RN) from tumor progression (TP) among patients with brain metastases (BM) having undergone prior stereotactic radiosurgery (SRS) who presented with a follow-up MRI brain (with DSC-MR perfusion) which was equivocal for RN versus TP. **METHODS:** Within 30 days of equivocal MRI brain, subjects underwent ^{18}F-Fluciclovine PET/CT on a Siemens Biograph mCT scanner with a 10 mCi bolus dose immediately prior to PET. Data were collected in list mode for 25 minutes post-injection and were reconstructed as a static image of data 10–25 minutes post-injection, and as a dynamic series of four 5-minute frames between 5–25 minutes post-injection. Quantitative metrics for each lesion were documented including SUVmax, SUVmean, SUVpeak, and normal brain SUVmean. Lesion to normal brain ratios were calculated. The reference standard was clinical follow-up with MRI brain (with DSC-MR perfusion) every 2–4 months until multidisciplinary consensus (or tissue confirmation) for diagnosis of RN versus TP. **RESULTS:** From 7/2019-11/2020, 16 of 16 planned subjects enrolled and underwent ^{18}F-Fluciclovine PET/CT for evaluation of 21 brain lesions. Primary histology included NSCLC (n=7), breast (n=5), melanoma (n=3), and endometrial (n=1). Ranges of quantitative metrics were: SUVmax, 2.18–12.1; SUVmean, 1.16–7.37; SUVpeak, 1.06–5.14; normal brain SUVmean, 0.19–0.50; SUVmax/normal ratio, 7.5–45.4; SUVmean/normal ratio, 4.2–26.3; and SUVpeak/normal ratio, 3.9–26.4. Among the patients 10 patients with 12 lesions who completed follow up, estimates of the area under the receiver operating characteristic curve for SUVmax, SUVmean, and SUVpeak were: 0.93, 0.93, and 0.82, respectively. **CONCLUSION:** In this population, ^{18}F-Fluciclovine PET/CT favorably produces a wide range of lesion quantitative metric values, low uptake in the normal brain, and promising accuracy to distinguish RN from TP. Completion of follow-up for all patients is required. Phase II and III studies are under development.

BIMG-20. METABOLIC BIOMARKERS IN MICRODIALYSATE OF IDH-1 MUTANT TUMORS

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Glioblastoma (GBM) is a common deadly malignant brain cancer of the central nervous system, with a median survival of 12–15 months. Scientific advancements are lacking in developing effective therapies for both primary GBM, as well as secondary GBMs, that typically originate as malignant transformation of lower-grade isocitrate dehydrogenase (IDH) 1-mutant tumors. The unique metabolomic profile of IDH1-mutant tumors presents opportunities to develop biomarker signatures of therapeutic efficacy. Microdialysis is an extracellular fluid sampling collection technique utilizing a perfused semipermeable catheter to permit diffusion of molecules between brain interstitium and the perfusate. We hypothesized that microdialysis may identify a metabolomics-based biomarker response to therapy in IDH1-mutant tumors. To test this hypothesis, orthotopic xenografts were generated from patient-derived xenografts (PDX) harboring mutant IDH-1 (R132H). Perfusates were collected from intra-cranial tumors in athymic nude mice sampled at baseline and 72h post treatment with temozolomide (TMZ), an oral alkylating agent used to treat IDH1-mutant gliomas, compared with vehicle treatment. Perfusates were analyzed via untargeted metabolomic profiling using liquid chromatography-mass spectrometry. Tumor specific metabolites such as (D)-2 hydroxyglutarate, were detected in microdialysate from IDH-1 mutant tumor bearing mice compared to non-tumor bearing mice. We also found high levels of metabolites such as 5-methylthioadenosine, and dimethylarginine and wide range of amino acids in microdialysate from IDH-1 mutant tumor bearing mice. TMZ treatment induced changes to metabolites in creatine and histidine metabolism. Our results indicate that microdialysis is a feasible technology to identify metabolomics-based biomarkers in IDH1-mutant gliomas and their response to therapy. We suggest that *in vivo* intratumoral microdialysis over several days could yield metabolic pharmacodynamic biomarkers of value to therapeutic translation for IDH-mutant gliomas.

BIMG-21. DEUTERIUM METABOLIC IMAGING (DMI), A NEW, MRI-BASED TECHNIQUE FOR MAPPING BRAIN TUMOR METABOLISM IN VIVO

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Deuterium Metabolic Imaging (DMI) combines 3D deuterium (^{2}H) magnetic resonance spectroscopic imaging (MRSI) with administration of a ^{2}H-labeled substrate to map uptake and metabolism of the substrate. DMI has been implemented on a 4 Tesla clinical research MRI scanner, and on an 11.7 Tesla preclinical MRI scanner, and has been used with ^{2}H-labeled glucose, acetate and choline. DMI data are presented as color maps of concentration of the ^{2}H-labeled substrate and its metabolites, overlaid on anatomical MR images. In rat and mouse models of glioblastoma, DMI data acquired at 5 to 8 uL resolution following intravenous ^{2}H-glucose infusion clearly showed the Warburg effect in the tumor lesions. The Warburg effect is indicated by the ratio of ^{2}H-labeled lactate/glutamate+glutamine (Glx). High levels of ^{2}H-labeled lactate and low levels of ^{2}H-labeled Glx are the result of a high rate of glycolysis and low rate of oxidative glucose metabolism. Because DMI detects both glucose and its downstream metabolism, the technique does not suffer from low image contrast with normal brain, as is the case with FDG-PET that detects glucose uptake only. For clinical research studies patients orally consumed 0.75g/kg of ^{2}H-glucose dissolved in water. The observations made in the animal models were confirmed in several patients with recurrent GBM, showing hotspots in the lac/Glx maps (8 mL resolution), coinciding with the area of the tumor lesion. In patients with meningioma, no Warburg effect was detected using DMI. Furthermore, DMI data acquired in a patient with GBM one week after finishing 30 days of radiation therapy, also showed no high levels of ^{2}H-labeled lactate in the lesion. These data indicate that the presence of the Warburg effect could correlate with tumor grade and/or aggressiveness, and that DMI of glucose metabolism could potentially be a biomarker of therapy effect.

BIMG-22. DEEP LEARNING SUPER-RESOLUTION MR SPECTROSCOPIC IMAGING TO MAP TUMOR METABOLISM IN MUTANT IDH GLIOMA PATIENTS

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Metabolic imaging can map spatially abnormal molecular pathways with higher specificity for cancer compared to anatomical imaging. However, ac-

quiring high resolution metabolic maps similar to anatomical MRI is challenging due to low metabolite concentrations, and alternative approaches that increase resolution by post-acquisition image processing can mitigate this limitation. We developed deep learning super-resolution MR spectroscopic imaging (MRSI) to map tumor metabolism in patients with mutant IDH glioma. We used a generative adversarial network (GAN) architecture comprised of a UNet neural network as the generator network and a discriminator network for adversarial training. For training we simulated a large data set of 9600 images with realistic quality for acquired MRSI to effectively train the deep learning model to upsample by a factor of four. Two types of training were performed: 1) using only the MRSI data, and 2) using MRSI and prior information from anatomical MRI to further enhance structural details. The performance of super-resolution methods was evaluated by peak SNR (PSNR), structure similarity index (SSIM), and feature similarity index (FSIM). After training on simulations, GAN was evaluated on measured MRSI metabolic maps acquired with resolution 5.2×5.2 mm^2 and upsampled to 1.3×1.3 mm^2. The GAN trained only on MRSI achieved PSNR = 27.94, SSIM = 0.88, FSIM = 0.89. Using prior anatomical MRI improved GAN performance to PSNR = 30.75, SSIM = 0.90, FSIM = 0.92. In the patient measured data, GAN super-resolution metabolic images provided clearer tumor margins and made apparent the tumor metabolic heterogeneity. Compared to conventional image interpolation such as bicubic or total variation, deep learning methods provided sharper edges and less blurring of structural details. Our results indicate that the proposed deep learning method is effective in enhancing the spatial resolution of metabolite maps which may better guide treatment in mutant IDH glioma patients.

BIMG-23. SINGLE-VOXEL VERSUS MULTI-SLICE MRSI IN PATIENTS WITH GLIOMA ON A KETOGENIC DIET INTERVENTION

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BACKGROUND: Ketogenic diet therapies (KDTs) may be beneficial by exploiting glioma metabolic vulnerabilities. The GLioma modified Atkins-based Diet study (GLAD; NCT02286167) evaluated systemic and cerebral (MR spectroscopy) biomarkers to determine the feasibility and biological effects of a KDT in glioma patients. While we observed metabolic changes in tumor and normal brain after KDT using single-voxel MRS (SV-MRS), optimal voxel placement was not always achieved. AIMS: We performed an exploratory analysis comparing cerebral metabolite changes using multi-slice MRSI (MS-MRSI) versus SV-MRS acquisition. **METHODS:** We evaluated four patients from the GLAD study (mean age 39years; 2 female, 3 AA IDH-mutant, 1 GBM IDH-wildtype) who underwent MRS at baseline and following eight weeks of KDT. SV-MRS (sLASER, TR/TE 2.2s/34ms) was acquired from a 2x2x2cm voxel placed in the residual tumor and the contralateral homologous brain. MS-MRSI was acquired with a multi-slice spin echo sequence (TR/TE 3.6/144ms, 4 slices, nominal resolution 13x7x7mm, SENSE factor 3) and maps of total choline (tCho), total N-acetyl-aspartate (tNAA), and lactate (Lac) were reconstructed and normalized relative to creatine. Metabolite levels were measured on the MS-MRSI maps using a region of interest placed in the same areas studied with the SV-MRS. **RESULTS:** Lesional tCho and tNAA levels showed strong correlation between SV-MRS and MS-MRSI both at baseline (Pearson's r=0.92 and 0.97, respectively) and after 8 weeks of KDT (r=0.96 and 0.84, respectively). tCho and tNAA correlated less robustly between SV-MRS and MS-MRSI in the contralesional region (r=0.56–0.96). Lesional Lac was significantly lower after KDT (1.01±0.48 versus 0.59±0.24, paired t-test p=0.02). **CONCLUSIONS:** While SV and MS-MRSI provided generally concordant lesional results, MS-MRSI offers added potential to map regional variations not captured by SV-MRS and thus may better define the control regions. MS-MRSI detected a decrease in tumoral lactate levels following study intervention, suggesting KDT-related changes in tumoral energy metabolism.

METABOLIC DRUG TARGETS, RESISTANCE

DDRE-01. METABOLIC PLASTICITY AND HETEROGENEITY IN IDH1MUT CELL LINES PRODUCES RESISTANCE TO GLUTAMINASE INHIBITION BY CB839

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BACKGROUND: Mutant IDH1 ($IDH1^{mut}$) gliomas have characteristic genetic and metabolic profiles and exhibit phenotype that is distinct from their wild-type counterparts. The glutamine/glutamate pathway has been hypothesized as a selective therapeutic target in $IDH1^{mut}$ gliomas. However, little information exists on the contribution of this pathway to the formation of D-2-hydroxyglutarate (D-2HG), a hallmark of IDH^{mut} cells, and the metabolic consequences of inhibiting this pathway. **METHODS:** We employed an untargeted metabolic profiling approach in order to detect metabolic changes arising from glutaminase (GLS) inhibition treatment. Subsequently, ^{13}C metabolic tracing analysis through a combined Nuclear Magnetic Resonance and Liquid Chromatography-Mass Spectrometry approach, we explored the fate of glutamine and glucose under treatment with CB839 a glutaminase-GLS-inhibitor and their respective contributions to D-2HG formation. **RESULTS AND CONCLUSIONS:** The effects of CB839 on cellular proliferation differed among the cell lines tested, leading to designations of GLS-inhibition *super-sensitive*, *-sensitive* or *-resistant*. Our data indicates a decrease in the production of downstream metabolites of glutamate, including those involved in the TCA cycle, when treating the sensitive cells with CB839 (glutaminase -GLS- inhibitor). Notably, CB839-*sensitive* $IDH1^{mut}$cells respond to GLS inhibition by upregulating glycolysis and lactate production. In contrast, CB839-*resistant*$IDH1^{mut}$ cell lines do not rely only on glutamine for the sustenance of TCA cycle. In these cells, glucose contribution to TCA is enough to compensate the downregulation of glutamine-derived TCA metabolites. This investigation reveals that the glutamine/glutamate pathway contributes differentially to D-2HG in a cell-line dependent fashion on a panel of IDH^{mut} cell lines. Further, these results demonstrate that there is a heterogeneous landscape of $IDH1^{mut}$ metabolic phenotypes. This underscores the importance of detailed metabolic profiling of $IDH1^{mut}$ patients prior to the decision to target glutamine/glutamate pathway clinically.

DDRE-02. SMOOTHENED-ACTIVATING LIPIDS DRIVE RESISTANCE TO CDK4/6 INHIBITION IN HEDGEHOG-ASSOCIATED MEDULLOBLASTOMA

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BACKGROUND: Medulloblastoma is an aggressive pediatric brain tumor that is associated with misactivation of the Hedgehog (HH) pathway. Our lab has shown that *CDK6*, a critical activator of the cell cycle, is a direct transcriptional target of oncogenic HH signaling, and that inhibiting CDK6 blocks the growth of HH-associated medulloblastoma in mice. A clinical trial exploring the efficacy of CDK6 inhibition in medulloblastoma patients is underway, but prior attempts to target the HH pathway in medulloblastoma have been encumbered by resistance to molecular monotherapy. Thus, we sought to identify mechanisms of resistance to CDK6 inhibition in HH-associated medulloblastoma. **METHODS:** We performed orthogonal CRISPR and CRISPR interference screens in HH-associated medulloblastoma cells treated with pharmacologic inhibitors of CDK6 *in vitro*, and RNA-sequencing of HH-associated medulloblastomas with genetic deletion of CDK6 *in vivo*. Mechanistic and functional validation of resistance pathways was performed using CRISPR interference, immunoblotting, immunofluorescence, genetics, and pharmacology. Lipid quantification was carried out by ultra-high performance liquid chromatography-tandem mass spectrometry. **RESULTS:** Our results reveal that decreased ribosomal protein expression underlies resistance to CDK6 inhibition in HH-associated medulloblastoma, leading to endoplasmic reticular (ER) stress and activation of the unfolded protein response (UPR). We show that ER stress and the UPR increase the activity of enzymes producing Smoothened-activating sterol lipids that sustain oncogenic HH signaling in medulloblastoma despite CDK6 inhibition. These discoveries suggest that combination molecular therapy against CDK6 and HSD11ß2, an enzyme producing Smoothened-activating lipids, may be an effective treatment for HH-associated medulloblastoma. In support of this hypothesis, we demonstrate that concurrent genetic deletion or pharmacological inhibition of CDK6 and HSD11ß2 additively blocks the growth of multiple models of HH-associated medulloblastoma in mice. **CONCLUSIONS:** Smoothened-activating lipid biosynthesis underlies resistance to CDK6 inhibition in HH-associated medulloblastoma, revealing a novel combination therapy to treat the most common malignant brain tumor in children.

DDRE-03. IDH1-MUTANT GBM CELLS ARE HIGHLY SENSITIVE TO COMBINATION OF KDM6A/B AND HDAC INHIBITORS

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