epidermoidoma, Posterior Fossa A (PFA), is the most prevalent, occurring in the hindbrain of infants and young children. Lacking highly recurrent somatic mutations, PFAs are thought to be a largely epigenetically driven entity, defined by hypermethylation at the histone 3 lysine 27 residue. Previous transcriptional analysis of PFAs revealed an enrichment of hypoxia signaling genes. Thus, we hypothesized that hypoxic signaling, in combination with a unique metabolic milieu, drive PFA oncogenesis through epigenetic regulation. In this study, we identified that PFA cells control the availability of specific metabolites under hypoxic conditions, resulting in diminished H3K27 trimethylation and increased H3K27 acetylation in vitro and in vivo. Unique to PFA cells, transient exposure to ambient oxygen results in a reversible cellular toxicity. Furthermore, perturbation of anaerobic glycolysis pathways is sufficient to inhibit growth of PFA primary cultures in vitro. PFA cells sequester s-adenosylmethionine while upregulating EZH2, a polycomb repressive complex 2 (PRC2) inhibitor, resulting in decreased H3K27 trimethylation. Furthermore, hypoxia fine-tunes the abundance of alpha-ketoglutarate and acetyl-CoA to fuel demethylase and acetyltransferase activity. Paradoxically, a genome-wide CRISPR knockout screen identified the core components of PRC2 as uniquely essential in PFAs. Our findings suggest that PFAs thrive in a narrow “Goldilocks” zone, whereby they must maintain a unique epigenome and deviation to increased or decreased H3K27 trimethylation results in diminished cellular fitness. Previously, we showed that PFAs have a putative cell of origin arising in the first trimester of development. Using single-cell RNAseq and metabolomics, we demonstrate that PFAs resemble the natural metabolic-hypoxic milieu of normal development. Therefore, targeting metabolism and/or the epigenome presents a unique opportunity for rational therapy for infants with PFA epedymoma.

ETMM-09 TARGETING GliOBLASTOMA MULTIFORME METABOLISM AT THE INVASIVE TUMOR FRONT Joseph H. Garcia1, Saket Jain2, Erin A. Akins3, Angad S. Beniwal1, Keyara J. Wolfe1, Longyou Cha1, Sanyu Kung1, Manish K. Aghi1,2
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Glioblastoma (GBM) is a primary malignant brain tumor with a median survival under two years. The poor prognosis GBM carries is largely due to cellular invasion, which enables escape from resection and drives inevitable recurrence. Numerous factors have been proposed as the primary driving forces behind GBM’s ability to invade adjacent tissues rapidly, including alterations in its cellular metabolism. Though studies have investigated links between GBM’s metabolic profile and its invasive capabilities, these studies have had two notable limitations. First, while infiltrating GBM cells utilize adaptive cellular machinery to overcome stressors in their microenvironment, the cells at the invasive tumor front have rarely been sampled in previous studies, which have primarily used banked tissue taken from the readily accessible tumor core; Second, studies of invasion have primarily used two-dimensional (2D) culture systems, which fail to capture the dimensionality, mechanics, and heterogeneity of GBM invasion. To address these limitations, our team developed two complementary approaches: acquisition of site-directed biopsies from patient GBMs to define regional heterogeneity in invasiveness, and engineering of three-dimensional (3D) platforms to study invasion in culture. Through utilization of these platforms, and by taking advantage of the system-wide, unbiased screens of metabolite profile and gene expression available, our team looked to accomplish the goal of identifying targetable metabolic factors which drive cellular invasion in GBM. Pilot RNA-sequencing data revealed 87 of the top 250 (35%) genes preferentially expressed in the tumor invasive edge, and 30 of the top 250 (12%) genes preferentially expressed in the tumor core were involved in cellular metabolism. KEGG pathways analysis demonstrated enrichment of glycolytic, pentose phosphate, and response to amino acid starvation pathways. To address this, we investigated whether PFA cells can lead to developing effective combination therapies coupling chemotherapeutic strategies with targeting of metabolic programs used by cancer initiating cells.

FSMP-02. CHANGES IN GLUTAMINE METABOLISM INDUCED BY OXALOACETATE IN GLIOBLASTOMA Omkar Ijare1, David Conway2, Alan Cash3, David Baskin1, Kumar Pichumani1, 2Houston Methodist Hospital and Research Institute, Houston, TX, USA, 3MetVital Inc, San Diego, CA, USA.

Anhydrous Enol-Oxaloacetate (AEO) has been shown to significantly increase survival and decrease tumor growth rates in animal models of glioblastoma and hepatocellular carcinoma. In the body, AEO is metabolized to “oxaloacetate” (OAA). Earlier, we demonstrated that AEO drastically reduced Warburg glycolysis in glioblastoma cells which was determined by the increase in pyruvate to lactate ratio and a 48.8% decrease in lactate production in 13C-labeled glucose metabolism studies. We have expanded this previous work to examine 13C-labeled glutamine metabolism. Cultured solid tumor cancer cells strongly rely on both biomass and glutamine to synthesize carbon intermediates for anaplerotic reactions. With treatment of OAA, we hypothesize that glutamine-derived OAA may be reduced which can be tracked through the use GC-MS based 13C-isotopomer labeling experiments. Patient-derived glioblastoma cells were grown in 15 mM glucose and 2 mM glutamine containing DMEM medium supplemented with 2 mM OAA for 10 days. 24 hours prior to harvesting the cells, 4 mM of [U-13C]glutamine was introduced to the medium. OAA treated cells showed significant decrease in the protein levels of lactate dehydrogenase A and C which indicates the switching off of glycolysis to support the utilization of elevated OAA levels during glutamine metabolism in the TCA cycle. 13C mass distribution analysis showed significant decrease in malate, aspartate and citrate pools (malate: 8.8%, p = 0.0098; aspartate: 9.2%, p = 0.0064; citrate: 9.5%, p = 0.0036) in their M+4 isotopomer labeling in OAA treated group compared to the control group. Decrease in lactate generation may rely on cancer proliferation, migration and invasion. Together, these data provide alternative way to modulate energy metabolism in glioblastoma using AEO treatment. Similarly, AEO treatment in other solid tumors (e.g. pancreatic ductal adenocarcinoma) may produce altered glutamine metabolism which may be of therapeutic value.

FSMP-03. INVESTIGATING CO-OPTED METABOLIC PATHWAY IN MELANOMA BRAIN METASTASIS Julie Farrant, Joshua Jackson, Edward Hartsough, Drexel University, Philadelphia, PA, USA.

Melanoma, an aggressive form of skin cancer, frequently metastasizes to the brain. While peripheral melanoma is largely treatable, MBM fail to respond to current therapeutics and is a clear unmet clinical need. Initial
clinical symptoms of Melanoma Brain Metastases (MBM) typically include headaches, seizures and other neurological deficits, suggesting that MBM disrupt normal brain functions. One of the major cell types that melanoma encounters and interacts with during brain metastasis are astrocytes. Astrocytes, the most abundant cell in the brain, interact with neurons and the vasculature, provide trophic and energetic support to neurons, and regulate local blood flow. Metabolic pathways in astrocytes, particularly the glutamine cycle, are essential for the recycling and formation of neurotransmitters needed to maintain the excitation/inhibition balance. We propose that MBM co-opt astrocytic metabolism, fueling MBM growth, and deplete metabolic intermediates crucial for neuronal activity leading to altered neurologic function. We begin to unravel the metabolic interactions between astrocytes and MBM using novel modeling platforms with genetic and pharmacological tools to manipulate the tumor microenvironment. This project investigates the contribution of astrocytic metabolism to MBM growth. We intend on dissecting the distinct metabolic needs of metastatic brain melanoma in the CNS microenvironment and the subsequent neurologic consequences. Completion of this project will provide a platform to study MBM and interaction with the local brain microenvironment. Inhibiting metabolic interactions between melanoma and gial cells may provide new avenue for therapeutic targeting of MBM.

FSMP-05. KETOGENIC DIETS FOR HIGH-GRADE GLIOMA
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BACKGROUND: Given therapeutic challenges posed by high-grade glioma (HGG), multiple concomitant therapies, including metabolic add-ons to standard of care, are warranted. Targeting astrocytes are also energy-dependent on glucose. Preclinical data supports the use of ketogenic diets (KD) in this population to deplete the tumor microenvironment of glucose, thereby exerting anti-tumor effects while the surrounding parenchymal tissue utilizes ketones. OBJECTIVE: The aim of this study was to conduct an up-to-date systematic review of the clinical use of ketogenic diets (KD) in the setting of high-grade glioma treatment and compare study design, outcomes, and challenges in the translation of these methods from bench to bedside. METHODS: We conducted comprehensive searches of the clinicaltrials.gov and pubmed.gov. Trials were included in our review if they were conducted in a patient population with high-grade glioma (either early or refractory) and at least one study arm included the use of a KD. RESULTS: The clinicaltrials.gov search yielded 12 studies of which 11 met inclusion criteria. Five of these trials reported results. The PubMed search yielded 2 additional studies. Seven clinical trials with reported results on a total of 69 patients were considered. CONCLUSIONS: The use of KD has proven to be safe and tolerable in early trials, however, further studies are warranted to examine efficacy. Challenges to feasibility include low patient enrollment and compliance, as dietary changes were reported to negatively affect quality of life. Additionally, variability between administered and planned KDs, duration of KD regimen, carbohydrate: fat ratio, underlying genetic factors that affect the induction of ketosis, and use of steroid therapy in this patient population may all contribute to inconsistent clinical data when compared to preclinical studies. Future larger scale clinical trials and prospective studies are needed to clarify the role of KDs in the treatment of HGG.

FSMP-06. IN VIVO MONITORING OF LDHA EXPRESSION IN GLIOBLASTOMA USING QUANTITATIVE EXCHANGED-LABEL TURNOVER 1H MRS TECHNIQUE
Puneet Bagga1, Laurie Rich2, Mohammad Harris3, Neil Wilson4, Mitch Schnall5, John Dette6, Zoltan Patay7, Amar Gajjar1, Walter Akers4, Jeffrey Steinberg1, Gedre Krenciute8, Suzanne Baker1, Ravinder Reddy8,9
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Most cancers, including glioblastomas (GBMs), rely extensively on glycolysis to support growth, proliferation, and survival. A hallmark of this elevated glycolysis is overexpression of Lactate dehydrogenase-A (LDHA) protein leading to increased uptake of glucose and overproduction of lactate. Various clinical trials using LDHA as a target for diagnosis and treatment have yielded encouraging results. However, in vivo monitoring of LDHA expression has been challenging due to either requirement of administration of radioactive substrates or specialized hardware. In this presentation, we will demonstrate a new method-quantitative exchanged-label turnover MRS (QELT, or simply qMRS)-that increases the sensitivity of magnetic resonance-based metabolic mapping without the requirement for specialized hardware. qMRS relies on the administration of deuterated (2H-labeled) substrates to track the production of downstream metabolites. Since 2H is invisible on 1H MRS, replacement of 2H with 1H due to metabolic turnover leads to an overall reduction in 1H MRS signal for the corresponding metabolite. We applied our qMRS technique to monitor the rate of lactate production in a preclinical GBM model. Infusion of [6,6-2H]glucose led to downstream deuterium labeling of lactate, thereby resulting in a reduction in the 1.33 ppm lactate-CH peak on 1H MRS over time. The subtraction of post-administration 1H MR spectra from the pre-infusion spectra aided in the determination of the kinetics of the lactate turnover. We believe that the detection and quantification of lactate production kinetics may provide crucial information regarding tumor LDHA expression non-invasively in GBMs without requiring biopsy. Hence, qMRS is expected to open up new opportunities to probe LDHA expression differences in a variety of gliomas, including GBMs and astrocytomas. This method takes advantage of the universal availability and ease of implementation of 1H MRS on all clinical and preclinical magnetic resonance scanners.

FSMP-07. CYSTATHIONINE-β-LYASE DRIVES ANTI-OXIDANT DEFENSE IN CYSTEINE-RESTRICTED IDH1 MUTANT ASTROCYTOMAS
Andrés Cano-Gaitan1, Anas Oudin2, Fred Fack2, Maria- Francesca Allega1, David Sumpton1, Elena Martinez-Garcia2, Gunnar Dittmar2, Ann-Christin Hau1, Christel Herold-Mende2, Roland Herwig3,4, Johannes Merkert5,6,7, P. Niclou1,8; Luxembourg Institute of Health, Luxembourg, Luxembourg, Luxembourg, 2Luxembourg Institute of Health, Luxembourg, Luxembourg, Luxembourg, 3Cancer Research UK Beatson Institute, Glasgow, United Kingdom, 4University of Heidelberg, Heidelberg, Germany, 5University of Bergen, Bergen, Norway

Mutations in isocitrate dehydrogenase 1 or 2 (IDH1/ID2) define glioma subtypes and are considered primary events in gliomagenesis, impacting tumor epigenetics and metabolism. IDH enzymes are crucial for the generation of reducing potential, yet the impact of the mutation on the cellular antioxidant system is not understood. Here, we investigate how glutathione (GSH) levels are maintained in IDH1 mutant gliomas, despite an altered NADPH/NADP balance. We find that IDH1 mutant astrocytomas specifically upregulate the cystathionine-β-lyase (CSE), the enzyme responsible for cysteine production upstream of GSH biosynthesis. Genetic and chemical interference with CSE in patient-derived glioma cells carrying the endogenous IDH1 mutation, sensitized tumor cells to cysteine depletion, an effect not observed in IDH1 wild-type gliomas. This correlated with reduced GSH synthesis as shown by in vitro and in vivo serine tracing and led to delayed tumor growth in mice. Thus we show that IDH1 mutant astrocytic gliomas critically rely on NADPH-independent de novo GSH synthesis to maintain the antioxidant defense, which uncovers a novel metabolic vulnerability in this dismal disease.

FSMP-08. TARGETING PYRIMIDINE SYNTHESIS ACCENTUATES MOLECULAR THERAPY RESPONSE IN GLIOBLASTOMA STEM CELLS
Kailin Yang1, Xiuxing Wang1, Qiulian Wu1, Leo Kim1, Andrew Morton1, Ryan Gimple1, Briana Prager1, Weiwai Tao1, Zhiyun Qu1, Linjie Zhao1, Sameer Agnihotri1, Paul Mischel1, Stephen Mack2, Shideng Bao1, Jeremy Rich1; 1Cleveland Clinic, Cleveland, OH, USA, 2University of California, San Diego, La Jolla, CA, USA, 3Case Western Reserve University, Cleveland, OH, USA, 4University of Pittsburgh, Pittsburgh, PA, USA, 5Baylor College of Medicine, Houston, TX, USA

Glioblastoma stem cells (GSCs) reprogram glucose metabolism by hijacking high-affinity glucose uptake to survive in a nutritionally dynamic microenvironment. Here, we trace metabolic aberrations in GSCs to link core genetic mutations in glioblastoma to dependency on de novo pyrimidine synthesis. Targeting the pyrimidine synthetic rate-limiting enzyme carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, dihydroorotase (CAD) or the critical downstream enzyme dihydroorotate dehydrogenase (DHODH) inhibited GSC survival, self-renewal, and in vivo tumor initiation through the depletion of the pyrimidine nucleotide supply in rodent models. Mutations in EGFR or PTEN generated distinct CAD phosphorylation patterns to activate carbon influx through pyrimidine synthesis. Simultaneously, loss of function abrogation of tumor-specific metabolic fluxes and DHODH activity with clinically approved inhibitors demonstrated sustained inhibition of metabolic activity of pyrimidine synthesis and GSC tumorigenic capacity in vitro. Higher expression of pyrimidine synthesis genes portends poor prognosis of patients with glioblastoma. Collectively, our results demonstrate a therapeutic approach of precision medicine through targeting the nexus between driver mutations and metabolic reprogramming in cancer stem cells.