selectivity and so we are interested in identifying which transporters are particularly important in the metabolic adaptation to hypoxia. Using CRISPR and siRNA technologies we have identified transporters that are functionally required to maintain cell proliferation of glioma cell lines and patient tumour cells. Furthermore, using stable isotope-enriched nutrients, we have identified novel means by which glioma cell metabolism can be perturbed by inhibition of these transporters. Characterising which SLC25A transporters are important for hypoxic tumour metabolism could therefore expose a way to exploit these hypoxic areas subsequently making them more vulnerable to treatment and thus impacting patient survival.

DDRE-26. THE IMMUNO-METABOLIC ENZYME FASN PREVENTS CANCER-CELL INTRINSIC TYPE I INTERFERON RESPONSES IN GLIOBLASTOMA

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Glioblastoma (GBM) is a devastating primary brain cancer with a median survival of 11–15 months. Radiation therapy (RT), the standard of care for GBM, can generate type I interferon responses (IFN-I) to jumpstart antitumor immunity. However, these effects are sometimes mitigated by inhibitory mechanisms that are exacerbated by RT. RT can modify GBM metabolism to promote *de novo* lipogenesis via the fatty acid synthase (FASN). Because FASN was found to impair IFN-I in antiviral immunity, we hypothesize that FASN is preventing RT-induced IFN-I responses to promote GBM survival and evade immune recognition. We first defined RT-induced metabolic changes in the GL261 murine GBM model. We observed an increase in mitochondrial respiration, glycolysis and in lipid metabolism-related pathways in 10 gray (Gy) irradiated GL261 cells. Additionally, we found upregulation of FASN by western blot and lipid accumulation by BODIPY staining in 10 Gy-GL261 cells. RT-induced lipid accumulation was reverted when GL261 cells were incubated with a FASN inhibitor. Next, to ask whether FASN was impairing IFN-I, GL261 cells were engineered to express an inducible shRNA silencing FASN (GL261shFASN) or its non-silencing control (GL261shNS). As expected, irradiation of GL261shNS cells enhanced the secretion of IFN-beta and CXCL10. This effect was more pronounced when FASN was abrogated in GL261 independently from the presence of RT. Finally, GL261shNS and GL261shFASN cells were orthotopically implanted in mice and IFN-I signaling was blocked by anti-IFN-I receptor antibody (a-IFNAR). Mice bearing GL261shFASN tumors presented a median survival of 51 days vs. 35 days for GL261shNS tumors, a significant prolongation of mice survival that was completely abrogated with a-IFNAR treatment. Overall, our findings suggest that FASN-mediated lipogenesis prevents RT-induced cancer cell intrinsic IFN-I to promote GBM survival. Consequently, it is possible that FASN acts as an immuno-metabolic checkpoint capable to regulate the immune system upon metabolic cues generated by RT.

DDRE-27. IDH MUTATED GLIOMAS PROMOTE EPILEPTOGENESIS VIA D-2-HYDROXYGLUTARATE DEPENDENT MTOR HYPERACTIVATION

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INTRODUCTION: Epileptic seizures in patients with low-grade, isocitrate dehydrogenase (IDH) mutated gliomas reach 90%, a major source of morbidity for these patients. Albeit there are multiple features that contribute to tumor related epileptogenesis, IDH mutations are determined to be an independent factor, although the pathogenesis remains poorly understood. We demonstrate IDH-mutated tumors promote epileptogenesis through D-2-hydroxyglutarate (D-2-HG) dependent mTOR hyperactivation and metabolic reprogramming. **METHODS:** Human epileptic and nonepileptic cortex were identified via subdural electrodes in patients with IDH-mutated gliomas (n=5). An *in vitro* rat cortical neuronal model on microelectrode arrays were utilized to investigate the role of D-2-HG on neuronal excitability. mTOR and lysine demethylase (KDM) modulators were applied to elucidate the epileptogenic mechanism. Tetrodotoxin was utilized to evaluate the contribution of neuronal activity to mTOR signaling and metabolism. mTOR signaling was evaluated through western blot analysis and multiplex immunofluorescence. Metabolic function were analyzed via Seahorse assays and metabolomic analysis. **RESULTS:** D-2-HG increased normalized bursting rate in the neuronal cultures ($p<0.0001$). Inhibition of mTOR with rapamycin corrected bursting levels to control levels. Furthermore, D-2-HG induced mTOR hyperactivation, independent of bursting activity, which correlated with upregulation of mTOR signaling in human epileptic tissue. KDM inhibition resulted in mTOR hyperactivation and neuronal hyperexcitability, which we demonstrated with D-2-HG, succinate, and PFI-90, a small molecule KDM inhibitor. Epileptic cortex and D-2-HG-treated neurons, have distinct metabolisms independent of neuronal activity compared to peritumoral nonepileptic cortex and control, respectively. **CONCLUSION:** We demonstrate IDH-mutated gliomas promote epileptogenesis through a D-2-HG dependent mTOR hyperactivation via KDM inhibition, a putative mechanism and potential therapeutic targets. Furthermore, we argue mTOR hyperactivation results in metabolic reprogramming, independent of neuronal firing, which may contribute to epileptogenesis, a heretofore unrecognized aspect of pathologic mTOR signaling in neurological diseases.

DDRE-28. MECHANISTIC AND THERAPEUTIC LINKS BETWEEN PURINE BIOSYNTHESIS AND DNA DAMAGE IN GLIOBLASTOMA

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Glioblastoma (GBM) is the most common and aggressive adult brain cancer. Radiation therapy (RT) is a critical treatment modality, and development of RT resistance is the predominant cause of recurrence and mortality in GBM patients. Using cell line models as well as patient-derived xenografts and neurospheres in orthotopic brain tumor models, we have identified increased rates and dependence upon *de novo* purine biosynthesis as a hallmark of GBM RT resistance. More recently, we have discovered that radiation treatment acutely stimulates flux through *de novo* purine synthesis in cell line and neurosphere models of GBM. This RT-induced increase in *de novo* purine synthesis is dependent on signaling through the DNA damage response and thus appears to be an adaptive mechanism to supply purines to repair radiation-induced DNA damage. To determine whether this regulatory mechanism also exists *in vivo*, we have used advanced metabolomic and metabolic tracing techniques with ^{13}C-labeled glucose and ^{15}N-labeled glutamine in mice bearing RT-resistant GBM patient-derived orthotopic brain tumors. We found that that orthotopic GBM PDXs had elevated activity of *de novo* purine synthesis that increased further after RT, while normal cortex had little activity even after RT. These observations have therapeutic relevance, as targeting this metabolic pathway with the FDA-approved purine biosynthesis inhibitor mycophenolate mofetil (MMF) overcomes GBM radiation resistance in mouse models *in vivo*. The lack of *de novo* purine synthesis in normal cortex suggests that targeting this pathway may be tumor specific. Collectively our data suggest that *de novo* synthesis of purines mediates RT resistance in GBM and that treatment of brain tumors with MMF in combination with RT may be a promising therapeutic strategy in patients.

DDRE-29. DE NOVO PYRIMIDINE SYNTHESIS IS A TARGETABLE VULNERABILITY IN IDH-MUTANT GLIOMA

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70–90% of lower-grade gliomas and secondary glioblastomas harbor gain-of-function mutations in isocitrate dehydrogenase 1 (*IDH1*), causing overproduction of the oncometabolite (*R*)-2-hydroxyglutarate [(*R*)-2HG]. Although inhibitors of mutant IDH enzymes are effective in other cancers, including leukemia, they have shown guarded efficacy in preclinical and clinical brain tumor studies, thus underscoring the need to identify additional therapeutic targets in *IDH* mutant glioma. We sought to identify tumor-specific metabolic vulnerabilities induced by *IDH1* mutations that could be exploited therapeutically. To un-

cover such vulnerabilities, we conducted a chemical synthetic lethality screen using isogenic *IDH1* mutant and *IDH1* wild-type (WT) glioma cell lines and a novel metabolic inhibitor screening platform. We discovered that *IDH1* mutant cells are hypersensitive to drugs targeting enzymes in the de novo pyrimidine nucleotide synthesis pathway, including dihydroorotate dehydrogenase (DHODH). This vulnerability is specific because inhibitors of purine nucleotide metabolism did not score in our screen. We validated that the cytotoxicity of pyrimidine synthesis inhibitors is on-target and showed that *IDH1* mutant patient-derived glioma stem-like cell lines are also hyperdependent on de novo pyrimidine nucleotide synthesis compared to *IDH1* WT lines. To test pyrimidine synthesis dependence of *IDH1* mutant gliomas in vivo, we used a brain-penetrent DHODH inhibitor currently undergoing evaluation in leukemia patients, BAY 2402234. We found that BAY 2402234 displays monotherapy activity against gliomas in an orthotopic xenograft model of *IDH1* mutant glioma, with an effect size that compared favorably with radiotherapy. We also developed novel genetically engineered and allograft mouse models of mutant IDH1-driven anaplastic astrocytoma and showed that BAY 2402234 blocked growth of orthotopic astrocytoma allografts. Our findings bolster rationale to target DHODH in glioma, highlight BAY 2402234 as a clinical-stage drug that can be used to inhibit DHODH in brain tumors, and establish *IDH1* mutations as predictive biomarkers of DHODH inhibitor efficacy.

DDRE-30. THERAPEUTIC TARGETING OF DISRUPTED METABOLIC STATE IN DIFFUSE INTRINSIC PONTINE GLIOMA

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BACKGROUND: Diffuse Intrinsic Pontine Glioma (DIPG) is a uniformly fatal pediatric brainstem tumor and the leading cause of brain-tumor related deaths in children. It is therefore imperative to identify novel treatment strategies for this aggressive and devastating disease. Metabolic reprogramming in tumors and in the tumor microenvironment contribute to evasion of therapy and tumor recurrence. The goal of this study was to identify and therapeutically target metabolic vulnerabilities in DIPG that mediate aggressiveness and treatment resistance. **METHODS:** DIPG tumors are marked by cellular heterogeneity and are driven by a population of cells with stem cell properties. We took a comprehensive metabolomics and transcriptomic screening approach to determine the operative pathways in the tumor driving stem cell compartment. To demonstrate efficacy and potential therapeutic window of activity, we treated DIPG tumors with clinically available and brain-penetrant inhibitors of the identified dysregulated metabolic pathways. **RESULTS:** Our multi-omics analyses revealed that tumorigenic patient-derived DIPG cells significantly upregulate metabolic programs including cholesterol biosynthesis and mitochondrial oxidative phosphorylation (OXPHOS) compared to DIPG cells that were induced to undergo differentiation (events associated with a loss of tumorigenic capabilities). The therapeutic targeting of DIPG tumors with clinically available and brain penetrant inhibitors of OXPHOS and cholesterol biosynthesis resulted in tumor cell killing and growth inhibition both in vitro and in vivo. Moreover, there was a therapeutic window of activity in tumorigenic DIPG cells compared with differentiated gliomas and non-malignant cells. **CONCLUSION:** Our findings demonstrate that DIPG harbor perturbations in metabolic programs that can be exploited for therapeutic benefits. The results from this study defined the metabolic pathways operative in the tumor-driving population in DIPG and demonstrated efficacy of targeting these pathways.

DDRE-31. FEASIBILITY AND BIOLOGIC ACTIVITY OF A KETOGENIC / INTERMITTENT FASTING DIET IN GLIOMA PATIENTS

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BACKGROUND: There has been increasing interest in exploring ketogenic diet therapies (KDT) in patients with glioma given the poor prognosis. The purpose of this single-arm, open label phase 2 study was to rigorously examine the feasibility, safety, systemic biological activity, and cerebral activity of a KDT in patients with glioma. **METHODS:** 25 patients with biopsy-confirmed WHO Grade 2–4 astrocytoma with stable disease following adjuvant chemotherapy were enrolled in an 8-week GLioma Atkins-based Diet (GLAD). GLAD consisted of 2 fasting days (calories<20% calculated estimated needs) interleaved between 5 modified Atkins diet days (net carbohydrates≤20 gm/day) each week. The primary outcome was dietary adherence by food records. Markers of systemic and cerebral activity included weekly urine ketones, serum insulin, glucose, hemoglobin A1c, IGF-1, and MR spectroscopy at baseline and week 8. **RESULTS:** 21 patients completed the study. 80% of patients reached ≥40 mg/dL urine acetoacetate during the study. 48% of patients were adherent by food record. The diet was well-tolerated with two grade 3 adverse events (neutropenia, seizure). Measures of systemic activity including hemoglobin A1c, insulin, and fat body mass decreased significantly, while lean body mass increased. MR spectroscopy demonstrated increased ketone concentrations (β-hydroxybutyrate (bHB) and acetone (Ace)) in both lesional and contralateral brain, compared to baseline. Higher total choline (tCho) and glutamine (Gln) levels were observed in lesional as compared to contralateral brain at baseline, and both decreased following intervention. Average ketonuria correlated with cerebral ketones in lesional (tumor) and contralateral brain (bHB Rs0.52, p=0.05). There were no differences in cerebral metabolites in IDH-mutant glioma after controlling for ketonuria. **CONCLUSIONS:** The GLAD dietary intervention, while demanding, produced meaningful ketonuria, and significant systemic and cerebral metabolic changes in participants. Participant ketonuria correlated with cerebral ketone concentration and appears to be a better indicator of systemic activity than patient-reported food records.

DDRE-32. THERAPEUTIC TARGETING OF A NOVEL METABOLIC ADDICTION IN DIFFUSE MIDLINE GLIOMA

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Diffuse midline glioma (DMG) is a uniformly fatal pediatric cancer that is in need of urgent "outside the box" therapeutic approaches. Recent studies show that tumor cells adapt to stresses created by oncogenic mutations and these oncogene-induced adaptations create vulnerabilities that can be exploited to therapeutic ends. To uncover these oncogene-induced vulnerabilities in DMGs we conducted a genome-wide CRIPSR knockout screen in three DMG lines. The top common DMG dependency pathway that we discovered is *de novo* pyrimidine biosynthesis. Under normal conditions pyrimidine nucleotide needs are met through the salvage pathway. However, in DMG tumorigenesis, pyrimidine nucleotide synthesis is rewired such that the cells become dependent on the *de novo* biosynthesis pathway. *De novo* pyrimidine synthesis is catalyzed by CAD, DHODH and UMPS; all three genes are identified as dependencies in our screen and have been validated using shRNA mediated gene knockdown. Interestingly, DMG cells did not exhibit a dependency on the *de novo* purine biosynthesis pathway. Using a small molecule inhibitor of DHODH, BAY2402234 [currently studied in phase I trial for myeloid malignancies (NCT03404726)], we have demonstrated and validated, (i) efficacy and specificity of *de novo* pyrimidine synthesis inhibition *in vitro* in DMG cells; (ii) *de novo* pyrimidine addiction is not attributable to cell proliferation; (iii) DHODH inhibition induces apoptosis by hindering replication and inciting DNA damage; (iv) DHODH and ATR inhibition act synergistically to induce DMG cell death; and (v) critical *in vivo* efficacy. The *in vivo* experiment documents that BAY2402234 crosses the blood-brain barrier, is present in the brain at therapeutically relevant concentrations, suppresses *de novo* pyrimidine biosynthesis in intracranial DMG tumors in mice, and prolongs survival of orthotopic DMG tumor bearing mice. Taken together, our studies have identified a novel metabolic vulnerability that can be translated for the treatment of DMG patients.

DDRE-33. MELATONIN AS A MASTER METABOLIC SWITCH FOR GLIOBLASTOMA

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Glioblastoma (GBM) is the most common form of malignant primary brain cancer in adults with a median survival of only 15 months. Therefore, new therapies to suppress malignant brain cancer are needed. Brain Tumor Initiating Cells (BTICs) are a GBM subpopulation of cells with a highly