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## Clinical targeting of altered metabolism in high grade glioma

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### Abstract

High grade gliomas are among the deadliest of all cancers despite standard treatments, and new therapeutic strategies are needed to improve patient outcome. Targeting the altered metabolic state of tumors with traditional chemotherapeutic agents has a history of success, and our increased understanding of cellular metabolism in the past two decades has reinvigorated the concept of novel metabolic therapies in brain tumors. Here we highlight metabolic alterations in advanced gliomas and their translation into clinical trials using both novel agents and already established drugs repurposed for cancer treatment in an effort to improve outcome for these deadly diseases.

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High grade gliomas (HGGs) are the predominant advanced malignancies of the central nervous system and among the deadliest of all cancer types. Historically, HGG referred to astrocytomas or oligodendrogliomas that were grade 3 (anaplastic) or grade 4 (glioblastoma) according to conventional histopathology<sup>1,2</sup>. As our molecular understanding of these tumors has improved, the term HGG has persisted, but changed with respect to the tumor entities it encompasses<sup>3</sup>. For the purposes of this review, we will use HGG to describe IDH mutant astrocytomas (grade 3 and 4), IDH mutant oligodendrogliomas (grade 3) and IDH wild type glioblastomas (grade 4).

HGGs are estimated to have afflicted 25,000 Americans in 2020 with near uniform lethality and an average patient survival time of only six months<sup>4,5</sup>. Standard therapies

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#### Conflict of Interest

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for gliomas include surgical resection, radiation, and alkylating chemotherapy, yet HGGs still have particularly poor prognoses due to near-universal recurrence following treatment<sup>6-9</sup>. Despite our growing understanding of tumor biology and a host of new technologies and pharmaceutical approaches, HGGs remain exceedingly difficult to eradicate even with current therapies<sup>10,11</sup>.

Large-scale genomic analyses have identified numerous genetic aberrations in HGGs that disrupt normal functioning of growth signaling<sup>12-20</sup>, cell cycle<sup>21-24</sup>, autophagy<sup>25</sup>, and cell death<sup>26,27</sup>. These findings have significant potential for molecularly targeted cancer-specific therapeutics, although attempts to target precise molecular alterations in HGGs have not yet yielded clinical benefit. This lack of success is likely to be due to the extensive intratumoral heterogeneity characteristic of HGGs<sup>28</sup>. Indeed, the only therapies that improve survival in HGGs do not require specific oncogenic mutations for efficacy<sup>8,29-31</sup>. Standard therapies (temozolomide, TMZ; and radiation therapy, RT) exert their beneficial effects through the induction of DNA damage, and additional pro-tumorigenic processes are under intense investigation as candidate targets for the treatment of HGG<sup>32,33</sup>.

Altered cellular metabolism is a hallmark of cancer and a promising therapeutic vulnerability in the clinic<sup>34</sup>. Since distinct genetic alterations can lead to common metabolic adaptations within a tumor<sup>35</sup>, targeting metabolism in genomically heterogeneous tumors such as HGGs may be more successful than targeting specific genomic alterations. Metabolism influences virtually all cellular phenotypes, and targeting metabolism has a long history of success in a variety of diseases. Notable examples include methotrexate, gemcitabine and asparaginase for the treatment of cancer<sup>36-38</sup>, statins for hypercholesterolemia<sup>39</sup>, and metformin for diabetes<sup>40</sup>. Moreover, antimetabolites against nearly every known pathway are already known and characterized, many of which are currently used for other purposes in patients and hold potential for use in cancer.

A general map of central carbon metabolism and metabolic targets relevant to current and emerging therapies for HGGs is shown in Figure 1. Most common metabolic processes can be traced to the central carbon backbone pathway known as glycolysis. Glycolysis converts glucose to pyruvate through a series of consecutive enzymatic reactions generating ATP and NADH. Glycolytic intermediates can also be used for biosynthetic processes via the pentose phosphate pathway. Pyruvate can enter the tricarboxylic acid (TCA) cycle (alternatively known as the citric acid cycle or Krebs cycle), which is a central metabolic hub in the mitochondria for a variety of pathways including breakdown and synthesis of amino acids and lipids, as well as the production of the redox cofactors NADH and FADH<sub>2</sub>. These cofactors are often used to fuel electron transport (ETC) and oxidative phosphorylation (OXPHOS) for mitochondrial ATP production. In the context of cancer, cells require both ATP and macromolecule biosynthesis to survive and fuel growth and proliferation. The biologically delicate act of balancing these fundamentally opposing processes makes cancer cells particularly sensitive to metabolic perturbations.

The profoundly altered metabolic state of cancer cells was first posited by Otto Warburg in the 1920s<sup>41</sup>, who observed dramatically increased glycolytic activity in cancer cells despite abundant oxygen<sup>42</sup>. This aerobic glycolysis is now thought to sustain the biosynthesis

required for continued proliferation<sup>43</sup>. The intense dependence of cancer cells upon increased metabolic activities for growth and invasion makes targeting metabolism an appealing therapeutic strategy.

Increased sensitivity of cancer cells to metabolic targeting is highlighted by the successes of metabolic agents used as anti-cancer therapeutics as early as the 1940s. Antagonists of folate metabolism (required for cell proliferation and nucleotide synthesis) were among the first antimetabolites used in patients, with methotrexate causing remission in children with leukemia in 1948<sup>44</sup>. In the 1950s, the purine analogs 6-mercaptopurine and 6-thioguanine, and the pyrimidine analog 5-fluorouracil, were first used to treat cancer patients<sup>45–48</sup>. Targeting these pathways has expanded to include other antimetabolites such as gemcitabine and pemetrexed<sup>49</sup>. In addition to their efficacy as single agents, these anti-metabolites can potentiate the efficacy of other treatments in a variety of cancer types<sup>50</sup>.

As our understanding of metabolism in normal physiology and disease has expanded over the decades, so too has our treatment arsenal against diseases characterized by aberrant metabolism. In this review, we highlight the metabolically targeted therapeutic strategies that are under clinical investigation for patients with HGG.

## Glycolysis as a diagnostic tool and metabolic target

Warburg's observation of increased glucose uptake in cancer cells is routinely observed clinically when tumors incorporate higher levels of the radioactive glucose analog 2-deoxy-2-[<sup>18</sup>F]-fluoro-glucose (FDG) than normal tissues in patients. FDG contains an <sup>18</sup>F atom attached to the 2-carbon of glucose, whereas normal glucose contains an oxygen atom at the 2-position (2-OH). Hexokinase phosphorylates FDG to generate [<sup>18</sup>F]FDG-6-phosphate, which is trapped intracellularly but cannot undergo further glycolytic metabolism due to the absence of the hydroxyl group on the 2-position. Therefore, the absence of 2-OH in FDG allows [<sup>18</sup>F]FDG-6-phosphate to accumulate to high, detectable levels in many highly glycolytic cancerous tissues. FDG-PET imaging has therefore emerged as an important imaging tool for numerous cancers. In HGG, the utility of FDG-PET is somewhat limited by the glucose-avidity of normal cortex, however this modality can be useful for some applications such as distinguishing progressive HGG from treatment-related necrosis<sup>51</sup>.

Removing the 2-OH from glucose also has therapeutic potential. 2-Deoxyglucose (2DG) has the 2-OH group replaced with a hydrogen instead of [<sup>18</sup>F]. Like FDG, 2DG is phosphorylated by hexokinase but cannot be further catabolized through glycolysis. 2DG-6-phosphate exerts product inhibition on hexokinase and thus can inhibit glycolysis. Treatment with 2DG can enhance RT in preclinical models of HGG<sup>52</sup> and has been tested with RT in patients. Initial clinical trials using 2DG with RT in GBM patients suggested that 2DG was safe, reasonably well tolerated and could be efficacious<sup>53–55</sup>. These findings prompted a randomized trial of RT alone in comparison to RT with 2DG for patients with GBM that was performed in India. While the results of this trial have never been published, an abstract from 2014 indicated that 2DG treatment did not confer a survival benefit<sup>56</sup>. These disappointing results could in part be due to the poor drug-like properties of 2DG, which

require millimolar concentrations for efficacy<sup>55</sup>. While 2DG analogs could have some therapeutic promise in the future, as of 2021 there are no listed trials on [ClinicalTrials.gov](https://ClinicalTrials.gov) utilizing 2DG as a therapeutic intervention.

Nitrosoureas are a class of alkylating agents used in GBM patients as an alternative to TMZ<sup>57</sup> but some have also been reported to inhibit glycolysis (Figure 1). The brominated derivative of pyruvate, 3-bromopyruvate (3-BrPA) is an alkylating agent that also inhibits glycolysis and depletes ATP<sup>58</sup>. Using a single drug to induce DNA damage and simultaneously inhibit glycolysis is an attractive therapeutic strategy, and while 3-BrPA has been used in patients, it has not been studied in rigorous clinical trials and has serious safety concerns<sup>57</sup>. A potential alternative and more stable 3-BrPA derivative known as 3-bromo-2-oxopropionate-1-propyl ester (3-BrOP) has been shown to sensitize TMZ- and carmustine-resistant GBM stem cells (GSCs) to both agents<sup>59</sup>. Moreover, 3-BrPA analogs can reverse hypoxia-induced nitrosourea resistance<sup>60</sup> and further suggest potential opportunities to inhibit glycolysis for improved therapeutic responses.

Recent data have shown that oxaloacetate, a naturally occurring metabolite in the TCA cycle, suppresses the Warburg effect in GBM<sup>61</sup>. This may be due to the ability of oxaloacetate to inhibit the glycolytic enzyme lactate dehydrogenase (LDHA, Figure 1)<sup>62</sup>. Increasing oxaloacetate levels reduces tumor growth and improves survival in GBM animal models<sup>63,64</sup>, and a phase 2 clinical trial with a proprietary oxaloacetate pro-drug called Anhydrous Enol-Oxaloacetate (AEO) in combination with standard therapy for GBM patients is now underway ([NCT04450160](https://clinicaltrials.gov/ct2/show/study/NCT04450160)).

## TCA Cycle and IDH mutations

The TCA cycle utilizes pyruvate (derived from glycolysis) as an electron source for the mitochondrial respiratory chain and is a central metabolic hub for a variety of synthetic pathways. The isocitrate dehydrogenase (IDH) enzymes, which function in the TCA cycle and other metabolic processes, hold particular relevance to gliomas. In normal tissues, IDH1 catalyzes the NADP-dependent reversible conversion of isocitrate to  $\alpha$ -ketoglutarate ( $\alpha$ -KG) in the cytosol, while IDH2 catalyzes the same reaction in the mitochondria. IDH3 is a mitochondrial enzyme that is linked to NAD<sup>+</sup> instead of NADP<sup>+</sup> and thus is an important producer of NADH in the TCA cycle. Subsequently, IDH-produced  $\alpha$ -ketoglutarate be used to produce the amino acids glutamate and glutamine (Figure 1). IDH1 and IDH2 can also run in the “reverse” direction to produce isocitrate, which can be used for lipid synthesis.

With the dawn of advanced genomics in the 2000s, exome sequencing studies identified a mutation in IDH1 and IDH2 in many cancers including about 10% of GBM patients<sup>65</sup>. The majority of IDH mutations occur at a residue essential for isocitrate binding (R132 in IDH1; R172 and R140 in IDH2) and are most prevalent in grade II-III gliomas and secondary GBMs<sup>65-67</sup>. IDH mutant (IDHmt) gliomas contain one wildtype IDH allele, catalyzing the conversion of isocitrate to  $\alpha$ -KG, while the mutant allele loses normal catalytic activity and undergoes a gain of function to catalyze the production of 2-hydroxyglutarate (2HG) from  $\alpha$ -KG<sup>68</sup>. Notably, 2HG is considered an oncometabolite due to its ability to directly regulate tumorigenesis. Due to its structural resemblance to

$\alpha$ -KG, 2HG can act as a substrate inhibitor of  $\alpha$ -KG-dependent epigenetic regulators, in particular TET enzymes and histone demethylases<sup>69,70</sup>. IDHmt-mediated inhibition of these proteins blocks differentiation of non-transformed cells, which is thought to promote gliomagenesis<sup>71</sup>.

Inhibitors of mutant IDH have been developed, are FDA-approved for patients with IDHmt leukemias, and are under clinical investigation for patients with IDHmt gliomas. The IDHmt inhibitor AGI-5198 delays growth and promotes differentiation of IDHmt but not wtIDH glioma cells<sup>72</sup>, and two additional IDHmt inhibitors, AGI-120 (ivosidenib) and AGI-881 (vorasidenib) have shown acceptable safety and potential efficacy in cancer patients. Both agents inhibit 2HG production in human gliomas<sup>73</sup>. Ivosidenib is an inhibitor of the mutant IDH1 isoform (IDH1mt) and approved by the FDA as a first-line treatment for acute myeloid leukemia (AML), an additional tumorigenic context in which IDH is frequently mutated<sup>74–76</sup>. Ivosidenib has shown safety and potential efficacy in patients with IDH1mt advanced gliomas<sup>77</sup>. Vorasidenib is an inhibitor of both mutant IDH1 and mutant IDH2 that penetrates the brain in multiple species and blocks 2HG production in glioma tissue by >90%<sup>78</sup>. A randomized phase 3 trial (NCT04164901) to determine single agent efficacy of vorasidenib vs. placebo in patients with IDH1/2 mutant gliomas is ongoing<sup>79</sup>. Some studies suggest that 2HG may also promote DNA repair and RT resistance, especially when the IDH mutation occurs alongside common mutations present in IDHmt astrocytomas<sup>80</sup>. These findings suggest that vorasidenib may have utility in combination with genotoxic agents such as RT and temozolomide

## Targeting vulnerabilities conferred by IDH mutations

The most efficacious strategy to exploit the IDH mutation may not be through its catalytic inhibition. IDH mutations in glioma are associated with improved survival compared to IDHwt tumors<sup>65</sup>, but whether this is due to the mutation itself or the tumor's natural history is an open question. IDHmt and 2HG appear particularly important for tumor growth early in tumorigenesis<sup>67,81,82</sup>. This is matched by clinical data showing that inhibition of mutant IDH may slow the growth of less aggressive non-enhancing IDHmt low grade gliomas but be less effective for higher grade contrast-enhancing IDHmt gliomas<sup>77</sup>. These findings suggest that inhibition of mutant IDH may be initially beneficial but become less effective once the tumor is established<sup>83</sup>.

## IDH mutation sensitizes tumors to PARP inhibitors

Rather than directly targeting the IDH mutation itself, several groups have instead defined and targeted the downstream vulnerabilities conferred by 2HG. A handful of novel IDH-mediated therapeutic vulnerabilities have been recently identified and targeted in clinical trials. In a variety of genetic contexts, the 2HG produced by mutant IDH inhibits DNA repair due to defective homologous recombination (HR)<sup>84,85</sup>. When HR is defective, for example when BRCA is inactive, cells become reliant on alternative mechanisms of DNA repair such as non-homologous end joining or single strand base repair. Inhibitors of poly-ADP ribose polymerase (PARP) have increased efficacy in cells lacking HR, including both BRCA and IDH mutant cells<sup>86,87</sup>. This concept is now being tested in IDHmt brain

tumor patients with the PARP inhibitor BGB-290, which is being given in combination with temozolomide for patients with IDHmt brain tumors ([NCT03914742](#)). Preliminary trials treating IDHmt mesenchymal sarcoma patients with monotherapy olaparib, another PARP inhibitor, have suggested therapeutic benefit in individuals with IDHmt chondrosarcoma and pulmonary epithelioid hemangioendothelioma <sup>88</sup>. Early results from a separate trial combining olaparib with TMZ, also an inducer of DNA damage, in recurrent GBM indicates that olaparib reliably penetrates many HGGs <sup>89</sup>, and this agent could be useful in IDHmt brain tumors as well.

### **2HG inhibits metabolic enzymes BCAT1 and BCAT2 and sensitizes tumors to glutaminase inhibition**

Increased levels of 2HG produced by IDHmt can also directly inhibit metabolic enzymes that require the structurally similar molecule  $\alpha$ -KG as a cofactor. This biology raises the possibility of IDHmt-dependent metabolic vulnerabilities. This is particularly true of glutamate production in IDHmt tumors. Glutamate is a precursor for the antioxidant molecule glutathione, which protects cells against oxidative stress (Figure 1). Glutamate can be produced from one of several mechanisms. Among these are the deamidation of glutamine via glutaminase (GLS) or branched chain amino acid transamination via branched chain amino acid transaminase 1 (BCAT1) and 2 (BCAT2). BCAT1 and BCAT2 are  $\alpha$ -KG-dependent and are competitively inhibited by 2HG <sup>90</sup>. Glutaminase inhibition thus selectively depletes glutamate, and consequently the glutamate contaminating tripeptide antioxidant glutathione, levels in IDHmt cells and sensitizes IDHmt gliomas to RT in laboratory models <sup>91</sup>. In light of these therapeutically significant findings, a clinical trial combining the glutaminase inhibitor CB-839 with RT in IDHmt anaplastic astrocytoma and IDHmt diffuse astrocytoma patients is underway ([NCT03528642](#)).

### **Modulators of mitochondrial activity and oxidative stress**

In healthy tissues, redox cofactors such as NADH generated in the TCA cycle are used to fuel the mitochondrial electron transport chain (ETC). The ETC is a series of protein complexes that builds an electrochemical gradient across the inner mitochondrial membrane. The stored free energy across this gradient is then used to power ATP synthesis during the process of oxidative phosphorylation. Electron leak from the ETC makes mitochondria the main source of cellular reactive oxygen species (ROS, Figure 1), which are potentially toxic at high levels. At low levels, ROS stimulate pro-survival and proliferation signaling pathways, while high levels cause cell death. ROS are normally kept in check by antioxidant molecules such as glutathione and thioredoxin, but this delicate balance is dysregulated in tumors. In the context of cancer, tumor cells often exploit mitochondrial activity for ROS signaling. However, this also makes cancer cells more vulnerable to mitochondrial targeting and inducers of oxidative stress <sup>92</sup>.

The biguanide metformin has been used clinically for over 60 years and is the first line treatment for type 2 diabetes. Metformin inhibits glucose production in the liver and improves insulin signaling to increase glucose uptake in skeletal myocytes. Both mechanisms reduce hyperglycemia and associated clinical symptoms. Metformin has

been extensively studied and reviewed elsewhere <sup>93,94</sup>, but new mechanistic insights and therapeutic uses continue to be discovered. Recent epidemiological data has suggested that it also holds the potential for use in a variety of cancers. Metformin inhibits complex I of the ETC, which depletes ATP and NAD<sup>+</sup> and causes activation of AMP-activated protein kinase (AMPK) <sup>93</sup>. Metformin therapy is associated with prolonged progression free survival in diabetic GBM patients <sup>95</sup>, suggesting its anti-tumorigenic metabolic activity may be useful clinically. Metformin is currently being studied in a 33-patient phase 2 trial in combination with TMZ and RT in GBM patients ([NCT02780024](#)). Metformin treatment appears to be safe and feasible and promising survival results, including a 3-year overall survival over 50% have been presented in abstract form <sup>96</sup>. Whether these encouraging results are due to systemic changes in glucose and insulin or direct action of metformin on glioma cells is not certain.

Recent studies of ascorbate (vitamin C) in glioma cells have found that pharmacological levels of ascorbate dramatically elevate ROS, inhibit glycolysis, and increase labile iron <sup>97-99</sup>. Ascorbate also induces double strand DNA breaks and increases sensitivity of GBM cells to RT due to excessive ROS and oxidative stress <sup>100,101</sup>. A phase 1 trial ([NCT01752491](#)) with high-dose ascorbic acid infusions in GBM patients receiving standard chemoradiation has shown minimal toxicity and favorable progression-free and overall survival <sup>97,102,103</sup> compared to historical controls <sup>30</sup>. A phase 2 trial ([NCT02344355](#)) assessing efficacy of ascorbate with chemoradiation in GBM patients is ongoing. The anticancer mechanisms and therapeutic potential of high-dose ascorbate are reviewed in detail in reference <sup>104</sup>.

## Metabolic Nutritional Therapies for HGGs

The links between metabolic inputs, or nutrition, and wellbeing have been long recognized. However, understanding the complex relationships among the numerous genetic and environmental factors of diet and metabolism and their potential for intervention in diseases such as cancer has been challenging <sup>105</sup>. Altering tumor biology using “precision nutrition,” or the restriction of specific nutrients in food <sup>106</sup>, may be a feasible dietary intervention strategy when based on metabolic activities of both the tumor and systemic environment within individual patients.

The ketogenic diet (KD) is a potential precision nutrition therapy that has recently received substantial interest in oncology. The KD is based on restricted carbohydrate intake to rewire systemic metabolism through the decreased level of circulating glucose and insulin, and consequently the production of ketones as an alternative fuel source. This may produce an unfavorable metabolic environment for tumors that rely on insulin as a growth factor and glucose as a fuel <sup>107,108</sup>. While the preclinical rationale for the KD is strong, especially in combination with other anti-cancer agents <sup>109</sup>, results from clinical trials investigating feasibility and efficacy of KD in cancer patients are mixed or limited to case studies and numerous clinical trials are ongoing <sup>107</sup>. Initial studies of the ketogenic diet in patients with GBM have shown that the diet is reasonably well-tolerated though logistically difficult to administer <sup>110</sup>. Indeed, a recent phase 1 study of the KD ([NCT02046187](#)) attempted to determine if KD could enhance standard chemoradiation in newly diagnosed GBM patients;

however, this was terminated due to excessive protocol deviations due to the strict nature of dietary requirements. More recently, a modified dietary intervention termed the GLAD diet (Glioma modified Atkins Diet) was developed as a less restrictive diet that was still ketogenic, but also involved periods of intermittent fasting<sup>111</sup>. HGG patients (both IDHwt and IDHmt astrocytoma) adhered to the GLAD diet reasonably well and most achieved ketosis. However, GLAD was initiated in patients with stable brain tumors after completion of most RT and chemotherapy, which likely improved patient compliance. Whether these dietary interventions will be feasible in patients with newly diagnosed HGG undergoing chemoradiation, and whether they provide therapeutic benefit, remain unanswered questions.

## Purine synthesis is a targetable vulnerability that promotes therapy resistance in IDHwt GBM

Nucleotide metabolism has been successfully targeted in numerous cancers and recent data suggest its inhibition could provide therapeutic benefit in HGG. Nucleotides are a class of biomolecules that are the building blocks of nucleic acids (DNA, RNA, ribosomes) but also play a wide variety of cellular functions including roles as signaling molecules and comprising chemically accessible energy (e.g., ATP, GTP). Structurally, nucleotides contain a nitrogenous base attached to a sugar unit (ribose or deoxyribose) that contains a mono-, di-, or triphosphate group. Nucleotide species can be classified as either purines (containing a double ring nitrogenous base structure) or pyrimidines (containing a single ring), which are synthesized through distinct pathways.

Glioma cells synthesize nucleotides using different pathways than non-malignant cortical cells, which provides a potential therapeutic window for targeting these metabolic pathways (Figure 2). In non-malignant cells, nucleotides are typically generated through salvage pathways in which pre-formed nitrogenous bases from the diet or breakdown of nucleic acids are directly conjugated to activated ribose (phosphoribosyl pyrophosphate, which is generated from glucose in the pentose phosphate pathway, Figure 1). By contrast, proliferating cells typically generate nucleotides from scratch using the *de novo* synthetic pathways. In *de novo* pyrimidine synthesis, the nitrogenous base is constructed from carbamoyl phosphate and aspartate, and then conjugated to PRPP. In contrast, *de novo* purine synthesis occurs through the building of the nitrogenous base directly on the PRPP using several different amino acids, one-carbon carbon units, and a substantial quantity of free energy from ATP. Both *de novo* purine and pyrimidine synthesis are activated by numerous oncogenic abnormalities including AKT/mTOR activation, receptor tyrosine kinase signaling and MYC activation<sup>112–117</sup>.

While nucleotide salvage does occur in HGG<sup>118</sup>, glioma cells, especially glioma stem-like cells, appear to primarily rely on *de novo* pyrimidine and purine synthesis<sup>112,119,120</sup>. Both *de novo* purine and pyrimidine synthesis in HGG regulate tumor stemness<sup>112,119</sup>. *De novo* purine synthesis also mediates other key oncogenic features of HGG including tumor growth and ribosomal biogenesis<sup>121</sup>. Purine metabolism also regulates double strand DNA break repair, and thus mediates resistance to both radiation and temozolomide, the primary treatments used for HGG<sup>122,123</sup>. Despite these key links between purine metabolism and

HGG growth and treatment resistance, anti-folate therapy, which inhibits purine metabolism, has largely been ineffective for the treatment of HGG. These findings may be due to relatively high concentrations of hypoxanthine in the brain, which could allow HGG to refill purine pools by generating inosine monophosphate through salvage pathways when the proximal steps of *de novo* purine synthesis are inhibited (Figure 2) <sup>124</sup>.

Inhibiting purine synthesis downstream of the hypoxanthine salvage step may be more efficacious for the treatment of HGG. Mycophenolate mofetil (MMF) is an FDA-approved agent that is metabolized by the liver into mycophenolic acid (MPA) which directly inhibits the purine synthetic enzyme inosine monophosphate dehydrogenase <sup>125</sup> (Figure 2). MMF is routinely used as an immunosuppressant for organ transplant patients, is well tolerated and appears to cross the blood-brain barrier <sup>126,127</sup>. While MMF is an immunosuppressant, which may raise concerns for use in cancer patients, preclinical studies in orthotopic patient-derived GBM models demonstrate that short-term MMF treatment can overcome both RT and temozolomide resistance without compromising animal health <sup>122,123</sup>. Given these promising findings, our research group is now performing a phase I clinical trial combining MMF with RT for patients with GBM (NCT04477200).

Other inhibitors of nucleotide metabolism may prove clinically beneficial for HGG as well. Gemcitabine is a cytidine analog used in other cancers that can be incorporated into DNA or impair deoxynucleotide production via inhibition of ribonucleotide reductase. Gemcitabine can cross the blood brain barrier and accumulate into tumors such as GBM at active concentrations <sup>128,129</sup>. A clinical trial combining gemcitabine and radiation for patients with HGGs demonstrated acceptable safety and promising clinical outcomes, though further investigation was halted due to the success of the cytotoxic chemotherapy temozolomide <sup>130</sup>. Nucleotide biosynthesis is an established therapeutic target in multiple disease states, and we are optimistic that increasing our understanding of altered nucleotide metabolism in GBM will lead to therapeutic advances.

## Conclusions and Future of Metabolic Therapy in HGG

Advances in therapy for HGG have stalled due, in part, to the plasticity of glioma cells and the genomic and epigenomic heterogeneity of these tumors. Targeting metabolism, which is the level of biology closest to phenotype, may be a promising strategy to improve HGG outcomes despite this heterogeneity. Over the past decade we have developed a tremendous understanding of how many of the oncogenic molecular drivers of HGG activate common metabolic pathways, which in turn promote key oncogenic phenotypes. New methods of measuring metabolism including *in vivo* stable isotope tracing in HGG patients <sup>131,132</sup>, spatial mass spectrometry <sup>133</sup> and single cell RNA sequencing <sup>134</sup> will enable additional discoveries regarding cell-type specific metabolic alterations and cross-talk between malignant and non-malignant cells in HGG. As we work to translate our understanding of these metabolic alterations into clinical implementation through metabolic inhibitors and dietary modulation, we are optimistic that the proximity of metabolism to phenotype may allow metabolically targeted therapies to move the needle and further improve outcomes for these deadly diseases.

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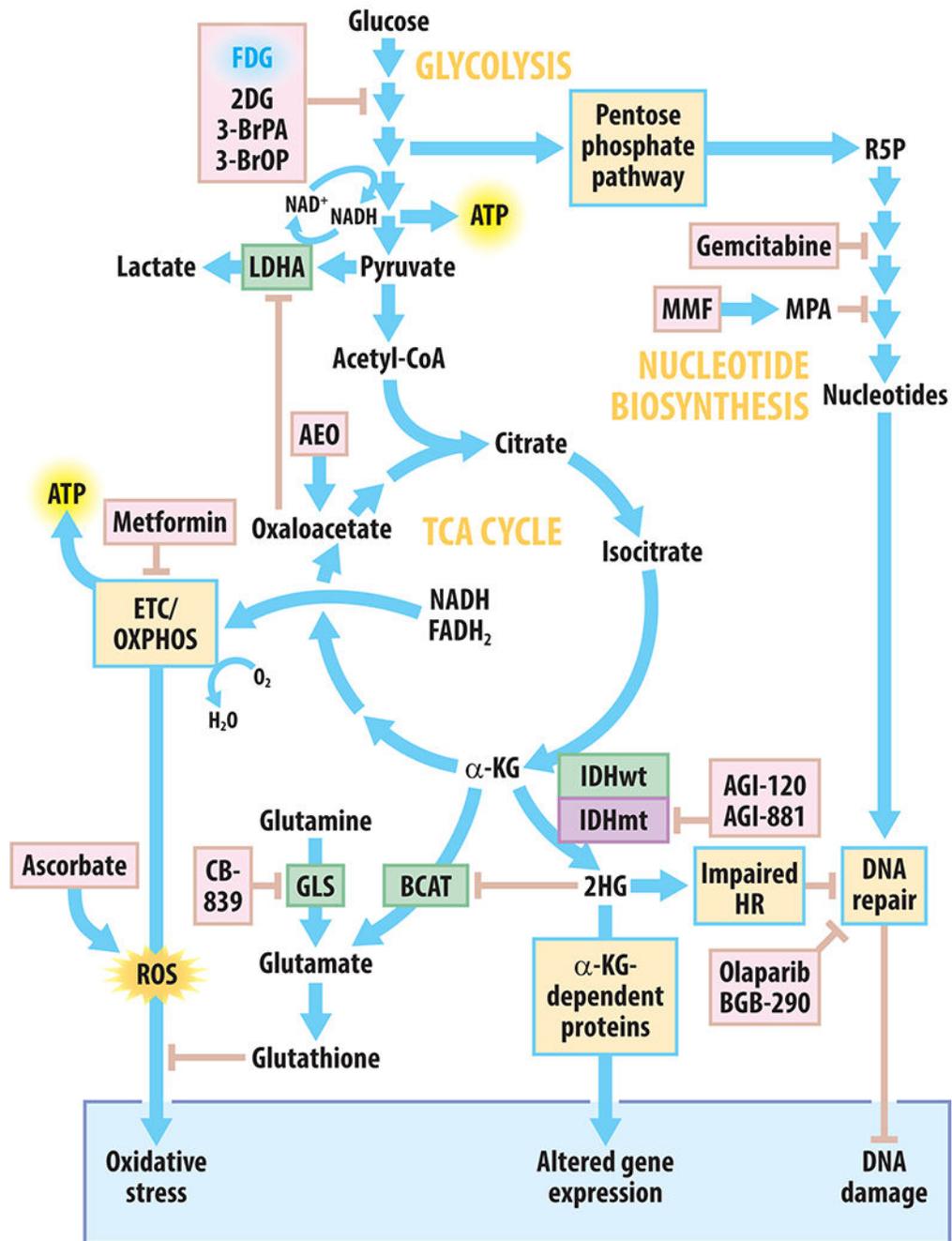
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**Figure 1. Overview of tumor metabolism and therapeutic strategies to target metabolic activity in HGGs.**

Blue arrows indicate biochemical conversions, metabolic pathway steps, or positive regulation; peach arrows with flat heads indicate inhibition or negative regulation; green boxes indicate metabolic enzymes; violet boxes indicate mutant enzymes; yellow boxes indicate general processes; pink boxes indicate chemical agents that can disrupt metabolism and are used clinically or undergoing preclinical studies. Abbreviations used: 2DG, 2-deoxyglucose; 3-BrOP, 3-bromo-2-oxopropionate-1-propyl ester; 3-BrPA, 3-bromopyruvate; AEO, anhydrous enol-oxaloacetate; ATP, adenosine triphosphate; BCAT, branched-chain

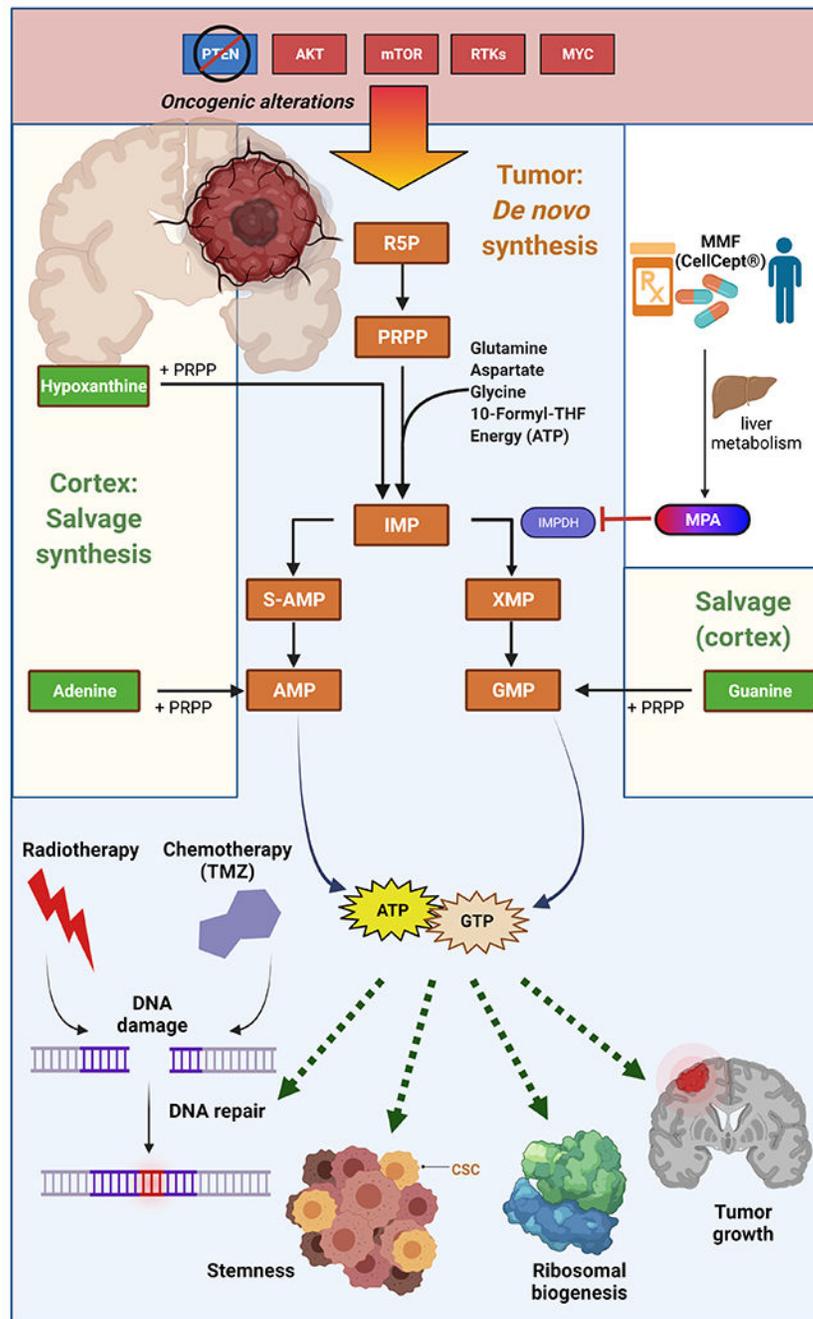
amino acid aminotransferase; ETC, electron transport chain; FDG, 2-deoxy-2-[<sup>18</sup>F]-fluoro-glucose; GLS, glutaminase; HR, homologous recombination; IDHwt/mt, isocitrate dehydrogenase wildtype/mutant; LDHA, lactate dehydrogenase A; MMF, mycophenolate mofetil; MPA, mycophenolic acid; NAD, nicotinamide adenine dinucleotide; OXPHOS, oxidative phosphorylation; ROS, reactive oxygen species;  $\alpha$ -KG,  $\alpha$ -ketoglutarate.

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**Figure 2. *De novo* purine synthesis is increased by oncogene activation, promotes pro-tumor cellular activities, and represents a therapeutic liability in HGG.**

*De novo* purine synthesis is activated by oncogenic alterations including PTEN deletions and upregulation or mutations in AKT, mTOR, receptor tyrosine kinases (RTKs), and MYC. In the *de novo* purine synthetic pathway, ribose 5-phosphate (R5P) is activated to phosphoribosyl pyrophosphate (PRPP), allowing the biochemical construction of a purine ring upon the ribose unit to form inosine monophosphate (IMP). IMP can be converted to adenylosuccinate (S-AMP) and then adenosine monophosphate (AMP) and adenosine triphosphate (ATP). IMP can also be converted to xanthosine monophosphate (XMP) via

IMP dehydrogenase (IMPDH) and then guanosine monophosphate (GMP) and guanosine triphosphate (GTP). In non-malignant brain tissue, purines are produced through salvage synthesis, in which free nitrogenous bases are conjugated to PRPP. Increased purine levels in HGGs promote stemness, tumor growth, ribosomal biogenesis, and DNA repair and subsequent therapeutic resistance. Purine synthesis can be pharmacologically targeted using mycophenolate mofetil (MMF/CellCept®), which is converted to mycophenolic acid (MPA) in the liver. MPA inhibits IMPDH to suppress purine synthesis and gliomagenesis in preclinical models. Figure created with BioRender.

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