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Review Article

Metformin and glioma: Targeting metabolic dysregulation for enhanced therapeutic outcomes

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ABSTRACT

Glioma, a highly aggressive form of brain cancer, continues to pose significant therapeutic challenges in the field of medicine. Its invasive nature and resistance to traditional treatments make it particularly difficult to combat. This review examines the potential of metformin, a commonly prescribed antidiabetic medication, as a promising new treatment option for glioma. The potential of metformin to target crucial metabolic pathways in cancer cells presents an encouraging approach to improve therapeutic outcomes. The review explores the complexities of metabolic reprogramming in glioma and metformin's role in inhibiting these metabolic pathways. Preclinical studies demonstrate metformin's efficacy in reducing tumor growth and enhancing the sensitivity of glioma cells to chemotherapy and radiotherapy. Furthermore, clinical studies highlight metformin's potential in improving progression-free survival and overall survival rates in glioma patients. The review also addresses the synergistic effects of combining metformin with other therapeutic agents, such as temozolomide and radiotherapy, to overcome drug resistance and improve treatment efficacy.

Despite the promising findings, the review acknowledges the need for further clinical trials to establish optimal dosing regimens, understand the molecular mechanisms underlying metformin's antitumor effects, and identify patient populations that would benefit the most from metformin-based therapies. Additionally, the potential side effects and the long-term impact of metformin on Glioma patients require careful evaluation.

In conclusion, this review underscores the potential of metformin as a repurposed drug in glioma treatment, emphasizing its multifaceted role in targeting metabolic dysregulation. Metformin holds promise as part of a combination therapy approach to improve the therapeutic landscape of glioma and offers hope for better patient outcomes.

Introduction

Gliomas are a group of rare but poorly treated cancers of neuroglia, more commonly referred to as glia or glial cells. The term neuroglia, which literally means "nerve glue" was first used by Rudolf Virchow to identify cells surrounding the neurons in the brain [1]. It is now known that glial cells play an important role in supporting neuronal function [2]. The relative ratio of glia/neurons in the brain has been controversial, but it is generally believed there are at least the same number of glial cells in the brain as neurons [3]. Glial cells are divided into different types depending on their function and histology. For example, the four common glial cells in the brain are astrocytes, oligodendrocytes, ependymal cells, and microglial cells [4]. Gliomas can be subdivided according to cell types they originate from; for example, astrocytomas are tumors of astrocytes. However, the majority of the literature uses the umbrella term glioma and we will do the same in this review.

Causes of glioma are uncertain but both genetic [5] and environmental factors [6] may be responsible. Generally speaking, glioma falls into definition of rare cancers (average global incidence below 6 per 100,000 persons per year). However, all recent analyses of national and international cancer registries have shown an increase in incidence of glioma over the past decades [7–9]. A recent analysis of data from 204 countries and territories has shown that both incidence and deaths from brain cancer have steadily increased since 1990 reaching 347,992 new

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cases and 246,253 deaths in 2019 [10]. Over this period, there has also been significant innovations in surgery, radiotherapy as well as clinical introduction of new medicines [11–13] for glioma patients. However, despite many welcomed improvements in individual treatment centers worldwide [14,15], survival rates in glioma have remained stubbornly poor and have not significantly improved overall. In glioblastoma multiforme (GBM, stage 4 glioma) which is the most advanced form of the disease, median survival is below 15 months and 5 year survival rates are around 5 %, regardless of treatment modality [16].

In view of this poor prognosis for gliomas, the search for new treatments is an urgent priority. In this context, repurposing of existing approved drugs is a particularly important endeavor as it significantly shortens the time and cost of bringing new therapies to patients [17–19].

One of the hallmarks of cancer is the ability for metabolic reprogramming which can then promote tumor growth, metastasis, and chemoresistance [20]. Rapid proliferation of cancer cells requires fast access to energy and biosynthetic precursors. In order to meet these requirements, cancer cells exhibit an increased rate of glycolysis and glutaminolysis [21]. Hence, the use of drugs that modulates multiple metabolic pathways in combination with the mainstay protocol treatments, can be a more successful approach. Here, we review the role of metabolic reprogramming in glioma, as well as preclinical and clinical evidence for the use of metformin (MET), a well-tolerated antidiabetic drug for treatment of gliomas.

Metabolic reprogramming in glioma

In normal cells, metabolism of glucose and production of ATP is conducted in three main stages of glycolysis, tricarboxylic acid (TCA) cycle and oxidative phosphorylation (OXPHOS). During glycolysis, one molecule of glucose is converted to two molecules of pyruvate over nine enzyme-catalyzed steps with a net gain of 2 ATP and 2 NADH molecules. Each pyruvate is then converted to acetyl-CoA which enters the TCA cycle during which 3 NADH, 2 FADH2 and one GTP molecules are produced. Finally, in OXPHOS, each FADH2 and NADH molecule is converted to two and three ATP molecules respectively with a concomitant consumption of molecular oxygen, making this stage the major source of ATP production in normal cells. In cancer cells, including glioma cells, metabolic reprogramming promotes glycolysis as the main provider of ATP, whilst concurrently stalling TCA cycle and OXPHOS. Whilst this makes the yield of ATP per molecule of glucose less efficient, this is compensated for by faster production of ATP. Mechanisms for this reprogramming are discussed below.

Upregulation of glycolysis

One of the most dysregulated signaling pathways in all cancers, including glioblastoma, is the PI3K/akt pathway [22] This pathway has a wide ranging influence in cancer through promotion of cell survival, proliferation, angiogenesis, and invasion. In particular, it is shown in glioma that PI3K/akt pathway regulates key glycolytic proteins such as glucose transporters (GLUTs) [23] and hexokinase-2 (HK2) [24]. Activation of the PI3K/akt pathway can occur through various routes including activation of growth factor receptors, such as the Epidermal Growth Factor Receptor (EGFR) [25]. EGFR is overexpressed in nearly 60 % of glioblastomas and can be activated through mechanisms such as increased autocrine expression of EGFR ligands, amplification, and mutation leading to a constitutively active form known as EGFRvIII [26].

The PI3K/akt pathway is also activated by the deletion or mutation of Phosphatase and Tensin Homolog (PTEN), a crucial enzyme that inhibits the PI3K/akt pathway. In GBM, PTEN is either deleted or mutated in approximately 40 % of tumors [25].

Activation of this pathway also stabilizes Hypoxia-Inducible Factor-1α (HIF-1α), an important transcription factor that becomes active and stable in hypoxic conditions [27]. HIF-1α plays a role in cellular metabolic adaptation to survive under hypoxic conditions by shifting main pathway for ATP production from oxidative phosphorylation (OXPHOS) to glycolysis, thus ensuring continued energy supply [27]. HIF-1α promotes glycolysis by regulating multiple proteins including increasing expression of Hexokinase 2 (HK2) and pyruvate dehydrogenase kinase 1 (PDK1).

HK2 phosphorylates glucose, in a step which is the rate-limiting step for glycolysis [28]. Elevated HK2 expression has been associated with higher-grade gliomas, potentially contributing to their aggressive nature [24]. Additionally, increased HK2 expression has been linked to reduced sensitivity of GBM to temozolomide (TMZ), the standard agent used in GBM treatment [29]. HIF-1α also increases the expression of PDK1 [34] which in turn, inhibits pyruvate dehydrogenase activity. This impedes pyruvate oxidation, which reduces acetylCoA production, leading to slowing down of the TCA cycle [27]. HIF-1α further promotes the conversion of pyruvate to lactate by activating lactate dehydrogenase and accumulating lactate within the cell. This accumulated lactate can either be converted back to glucose *via* glucogenesis or be exported outside the cell via lactate transporter MCT4, the upregulation of which is also mediated by HIF-1α [27,30].

Another dysregulated pathway in GBM is the RAS/RAF/MEK/ERK pathway [25], which influences metabolism, proliferation, differentiation, and apoptosis by phosphorylating various transcription factors, including c-Myc [31]. c-Myc is a transcription factor downstream of both the PI3K/akt [32] and RAS/RAF/MEK/ERK pathways [31], contributing to the Warburg effect in GBM [33].

This transcription factor regulates the expression of several proteins and enzymes involved in various roles within glycolytic pathways, including glucose uptake (GLUT1), glycolysis (HK2, phosphofructokinase, enolase, pyruvate kinase M-2), lactate production (lactate dehydrogenase A), and can also promote lactate export through the plasma membrane (MCT1) [34].

TP53 is a tumor suppressor gene encoding for the p53 protein, often referred to as "The Guardian of the Genome" [35]. *TP53* is activated mainly following DNA damage either following treatment or radiation, even resulting from errors during cell replication. p53 Plays a central role in preventing the transformation of normal cells into malignant ones by regulating DNA repair, the cell cycle, metabolism, and apoptosis. One of the myriad functions of wild type p53 is to favor oxidative phosphorylation (OXPHOS) over glycolysis, by suppressing the expression of glucose transporters (GLUT1, GLUT4) and downregulating the pyruvate dehydrogenase inhibitor (PDK2) [36]. Additionally, p53 can repress MYC expression by binding to the MCY gene promoter [37]. However, *TP53* is also one the most mutated gene in cancers and particularly in glioma where 49 % of patient samples from grades II and III and 84 % of patient samples from grade 4 showing this mutation [38,39]. Mutated p53 will no longer be able to promote oxidative phosphorylation (OXPHOS) over glycolysis and also loses tumor suppressor function. Furthermore, mutated p53 has been shown to upregulate genes such as *MYC* and *EGFR*, which promote cancer growth, and downregulate genes involved in inhibiting cancer growth, such as *PTEN* [40].

Modifications to TCA cycle and oxidative phosphorylation

As mentioned earlier, most studies have demonstrated high dependence of glioma on glycolysis as the main metabolic pathway. Nonetheless, the GBM microenvironment may lead cells to adapt to a different metabolic pathway rather than glycolysis. It was observed that GBM cells in the interior region of the tumor have an increased expression of HIF-1α leading to lactate formation which is secreted through MCT4 to the extracellular space [25]. In contrast, GMB cells in the periphery have shown an increased expression of MCT1 and c-MYC, thus promoting lactate uptake (lactate shuttling) and utilization through Cori Cycle.

Another manifestation of metabolic reprogramming in gliomas is modifications to TCA cycle [41], and the increased reliance of tumor

cells on glutamine metabolism or so called "glutamine addiction"(see below).

During a normal turn of the TCA cycle, citrate is converted to isocitrate by aconitase which in turn is converted by isocitrate dehydrogenase (IDH) to α-ketoglutarate (α-KG) with a loss of CO_2 and gain of one NADPH. Aconitase is particularly susceptible to deactivation in the presence of reactive oxygen species (ROS) generated during hypoxia [42] which is common in many cancers including glioma. Furthermore, the *IDH* gene, encoding for isocitrate dehydrogenase, is often mutated in gliomas [43–45]. Either or both of these result in a buildup of citrate and a concurrent depletion of α-KG, which in turn impedes the turn of the TCA cycle. In cancer cells, this excess citrate is exported back to the cytoplasm where it is converted to acetyl-CoA and then to fatty acids. In addition, cancer cells can circumvent the need to produce citrate by directly converting oxaloacetate to α-KG via glutamate-linked aminotransferase. Finally, tumor cells can replenish levels of α-KG by metabolism of glutamine (anaplerosis).

We should note that even though gliomas rely heavily on glycolysis to gain energy, recent evidence suggests that a subset of glioma cells with stem cell–like properties can switch back to OXPHOS [26]. Of course, gliomas are characterized by both heterogeneity and plasticity between different phenotypes, which is a major reason for difficulty in treating them.

Glutamine metabolism

Cancer cells take up glutamine (gln) through several transporters, including SLC1A5 (ASCT2), SLC38A1, and SLC6A14, to meet their high metabolic demands [46]. It has been shown that the higher expression of the transporter ASCT2 that mediates influx of glutamine into cells correlates with malignancy in higher grade glioma compared to lower grade glioma and normal brain [47].

Once inside the cell, glutamine is hydrolyzed in the mitochondria by glutaminase enzymes (GLS1 and GLS2) to produce ammonia and glutamate [48]. The ammonia produced is crucial for the biosynthesis of nucleic acids, which are essential for the rapid proliferation of cancer cells. Glutamate, the other product of glutamine hydrolysis, is converted to α-ketoglutarate (α-KG) by glutamate dehydrogenase (GDH). This conversion process produces a molecule of NADPH, which is vital for maintaining the redox balance within the cell. Additionally, glutamate can assist in the transformation of oxaloacetate to α-KG via glutamate-linked aminotransferase, further fueling the tricarboxylic acid (TCA) cycle and supporting the energetic and biosynthetic needs of the rapidly proliferating cancer cells. Furthermore, gln is excreted by LAT1 in exchange for the extracellular amino acid, leucine that is crucial for several reasons [49]. Leucine is a potent activator of the mTORC1 pathway, which regulates cell growth and proliferation. This mechanism also allows cancer cells to adapt their metabolism based on nutrient availability.

In gliomas, both GLS1 and GLS2 play crucial roles in supporting tumor metabolism and survival. GLS1 is the primary isoform expressed in many cancers and is extensively studied for its role in converting glutamine to glutamate, supplying α-ketoglutarate (α-KG) for the tricarboxylic acid (TCA) cycle, essential for energy production and biosynthesis. This overexpression of GLS1, driven by oncogenes like MYC, supports the high proliferative and metabolic demands of glioma cells. Inhibitors targeting GLS1, such as BPTES, have shown potential in reducing tumor growth by disrupting glutamine metabolism [50]. Although GLS2 is less studied and exhibits different roles in various cancers, in gliomas, it holds the same importance as GLS1 in gliomas [51]. GLS2 also catalyzes the conversion of glutamine to glutamate but is crucial for maintaining redox balance and providing metabolic flexibility under stress conditions. Its regulation by cellular signals ensures a steady supply of metabolic intermediates necessary for glioma cell survival and adaptation. Targeting both GLS1 and GLS2 may offer a comprehensive therapeutic strategy by impairing the metabolic and redox homeostasis of glioma cells.

An often overlooked pathway in both normal and some cancer cells involves the conversion of glutamine to α-KG by glutamine transaminases (GTK and GTL), coupled with ω-amidase. This alternative pathway becomes significant when GLS1 is inhibited, as shown in pancreatic cancer cells treated with GLS1 inhibitors, where increased GTK activity helps maintain glutamate production and adapt to treatment [50]. Genetic inhibition of GTK has shown significant tumor volume reduction in xenograft models, highlighting its potential as a therapeutic target [52].

Glutathione synthesis

Glutathione (GSH) synthesis occurs in two steps following glutaminase-mediated conversion of glutamine to glutamate. The glutamate-cysteine ligase (GCL) catalyses the reaction between glutamate and cysteine to produce γ-l-Glutamyl-l-cysteine. Then glutathione synthetase(GS) catalyses the joining of γ-l-Glutamyl-l-cysteine with glycine to produce GSH [53].

GSH acts as a ROS scavenger, helping to neutralize harmful oxidative molecules within cells. It is converted to its oxidized form, GSSG, during this process. Additionally, GSH plays a significant role in detoxifying xenobiotics, including chemotherapeutic agents like temozolomide (TMZ). This detoxification is mediated by glutathione S-transferase (GST), which conjugates GSH with xenobiotics, reducing their cytotoxic effects and contributing to chemoresistance [54].

The transcription factor NRF2 is crucial in regulating the synthesis and utilization of GSH, especially under oxidative stress conditions. NRF2 enhances GSH production and upregulates the expression of GST, further promoting the detoxification process and aiding in the development of resistance to chemotherapy [55].

EGF-MEC-ERC-ELK1-GDH1

As previously mentioned, the overactivity of EGFR is observed in a large number of GBM patients and significantly impacts glycolysis. A newly discovered pathway, EGF-MEK-ERK-ELK1-GDH1, highlights its role in glutamine metabolism in GBM cells. There is a significant correlation between EGFR activation and increased glutamine metabolism. Specifically, EGF-stimulated EGFR activation leads to the phosphorylation of MEK1, ERK1/2, and ELK1. As a transcription factor, ELK1 enhances the gene expression of the GDH1 enzyme, which accelerates the conversion of glutamate into α-ketoglutarate (α-KG) within the TCA cycle, fueling ATP production. This pathway underscores the importance of glutamine metabolism in glioma progression by demonstrating that EGFR activation promotes glutaminolysis in a GDH1-dependent manner [56].

mTOR/MYC-C/GFAT1

Glucose and glutamine are crucial nutrient for glioblastoma [57], with each of them being involved in pathways in cancer cell. At the same time there is a cross pathway between them involving mTOR2/MYC-C/GFAT1 [58]. GBM cells have been known to accumulate glucose-glutamine which is found to induce mTOR2. mTOR2 directly activates MYC-C and as its role transcriptional factor, gene expression of glutamine fructose-6-phosphate aminotransferase (GFAT) increases [59]. GFAT acts on its substrate fructose-6-phosphate and glutamine to produce glucosamine 6 phosphate and glutamate [50]. Glioma cells are then able to use them as building block for lipid glycosylation and protein synthesis.

Lipid metabolism

Lipids play different roles within cells which includes structural component in membrane, signaling molecules, and as alternative source

of energy. The latter can be utilized by cancer cells to support their proliferation, survival, migration and invasion in nutrient deprived conditions [60]. Several proteins involved in lipid metabolism are upregulated in glioma. These proteins include ATP citrate lyase, which converts cytosolic citrate to acetyl-CoA, the LDL receptor which is responsible for the uptake of LDL, and Acetyl-CoA carboxylase which synthesizes malonyl-CoA from acetyl-CoA for fatty acid synthesis [61]. Glioma cells also show higher amount of triglyceride and lipid droplets compared to their non-malignant counterparts in the brain [62]. This can be related to upregulation of Sterol O-acyltransferase 1 in glioma cells. This protein esterifies cholesterol for storage in lipid droplets [63] and serves to impede the negative feedback mechanism of cholesterol on the transcription factor SREBP, which is responsible for initiating de novo lipid synthesis within the cellular environment (see below). This not only offers an additional energy source for the glioma cell to utilize as needed, but also serves to prevent the buildup of cholesterol from impeding lipid production within the cell.

It may seem logical to think of fatty acids as an alternative energy source for glioma cells in nutrient deficient conditions [64]. However, in nutrient favorable environment fatty acid metabolism seems to support mesenchymal subtype proliferation by providing acetate for synthesis of β-hydroxybutyrate (β-HB). This metabolite which is normally produced by the liver to provide energy for tissue in starvation state activates a G-protein coupled receptor GPR109A. This provides an autocrine signal to the mesenchymal subtype cell that decreases the level of cAMP and subsequently inhibits the expression of the cyclin dependent kinase inhibitor p27 and promotes continuation of the cell cycle.

The SREBP proteins are transcription factors that play a crucial role in the regulation of fatty acid and cholesterol production [64]. The proteins in question have been demonstrated to function as a downstream target within the PI3K/akt pathway. Furthermore, the stabilization of SREBPs can be achieved by the inhibition of GSK3 by akt and mTORC2. This inhibition prevents GSK3 from phosphorylating SREBPs, which in turn prevents their ubiquitination and subsequent destruction. The upregulation of SREBP-1, a specific subtype observed in GB [61], has been demonstrated to have a protective effect against oxidative stress [65]. The observed phenomenon can be attributed to the heightened ratio of saturated and mono-unsaturated fatty acids, which are generated by cancer cells, in contrast to polyunsaturated fatty acids in the cell membrane. This disparity in fatty acid composition may contribute to a reduced susceptibility to peroxidation. This observation highlights the significance of lipogenesis in glioblastoma (GBM), as glioma stem cells (GSC) have elevated levels of fatty acid synthase (FASN) expression. FASN plays a crucial role in the synthesis of palmitate from malonyl-CoA and acetyl-CoA, and its expression is shown to be higher in GSC compared to differentiated GBM cells [66]. The enzyme inhibition exerted an impact on glioblastoma stem cells (GSCs) by diminishing their viability, invasiveness, and expression of stemness markers, while showing no notable influence on the viability of differentiated glioblastoma multiforme (GBM) cells.

Clinical use of metformin

Metformin (MET) is a member of the biguanide class of drugs which also include phenformin and buformin. It was first synthesized in 1922 following the isolation of a naturally occurring bioactive biguanide, galegine from *Galega officinalis* (Goat's rue plant) in 1914 [67]. Although galegine itself is mildly toxic and inadvertent ingestion by grazing cattle can occasionally cause death [68], safety of metformin has been confirmed through numerous clinical studies [69–71]. Indeed, metformin is superior to other biguanides due to its better tolerability by most patients. Since the discovery of its glucose lowering effects in the late 50′s, metformin has become the first line medication for diabetes mellitus type 2 (DMII) worldwide [72] and often features in the list of most commonly prescribed drugs [73]. It is not surprising that because of its safety profile and tolerability, metformin has also been investigated as a treatment in a number of other indications, and particularly in cancer.

However, despite its wide use and long clinical history, the various modes of action of metformin as a glucose-lowering agent (and also for use in other treatments), are not yet fully worked out. The most likely reason is that metformin plays a complex and multifaceted role in modifying biological pathways [74]. In other words, the observed physiological effects and clinical outcomes from administration of metformin is likely to be a culmination of multiple modifications, some complementary and some contradictory, in biological/cellular pathways. We will first look at evidence linking metformin administration and cancer, followed by analysis of the data supporting potential mode of action of metformin in these cases.

Many studies have demonstrated that DM patients are at an increased risk of cancer, including colorectal [75], gastric [76], esophageal cancer [77] and lung [78]. Additionally, many epidemiological studies and meta analyses have associated the use of metformin with lower rate of cancer incidence [79]. We should note however that in contrast, Lee et al. have shown an increased risk of prostate cancer with the use of metformin [80]. Nonetheless, metformin has been evaluated as a potential cancer treatment in different cancer types including breast [81], colon [82], esophageal [83], and cervical cancers [84]. It has been demonstrated that metformin can reduce cell proliferation, promote apoptosis, and cause cell cycle arrest. These metformin anti-cancer effects are mediated particularly through its role in disrupting the metabolic cycle in caner [85]. Furthermore, the use of metformin in combination with other anti-cancer treatments exhibited many favorable effects [81,82]. It has been demonstrated that metformin can enhance the chemotherapeutic and/or radiotherapy responses [86]. Additionally, it has been suggested that metformin may be crucial in inhibiting the multidrug resistance 1 (MDR1) gene, which is linked to chemotherapy resistance [87]. In regard to glioma, metformin activity has been evaluated in different *in vitro*, and *in vivo* studies which demonstrated the anti-proliferative and anti-migratory effects of the drug [88–90]. These anticancer effects were also seen against the glioblastoma stem cells [91]. Altogether, these data have shown a promising role of metformin as a potential anticancer treatment in glioblastoma. In addition, researchers have evaluated metformin as a adjunct treatment to achieve a more effective anticancer properties. A study carried on glioblastoma cell line U87 treated with metformin showed an increase in exosome secretion [92] which is known as a factor which spreads resistance to the neighboring cell [93]. This observation may point to a role for metformin in increasing chemotherapy resistance. In the following section we will explore the mechanisms of action of metformin on its own or as a combination treatment in glioblastoma.

Molecular targets of metformin in cancer

The anti-cancer mechanism of action of metformin is not completely understood [94]. However, it is believed that metformin can affect cancer cells through either indirect or direct routes. The indirect effect involves the reduction of circulating glucose [95] mainly by improving insulin-mediated suppression of glycogen breakdown in the liver, and by enhancing insulin-stimulated glucose disposal in skeletal muscle.

These effects are particularly noticeable in cancer types such as endometrial carcinoma, where there is overexpression of the insulin receptor (IRα), leading to enhanced proliferative signaling. Hyperinsulinemia resulting from insulin resistance is identified as a risk factor for several cancers, including endometrial cancer [96].

Additionally, elevated insulin levels are associated with increased plasma concentrations of IGF-1, which correlates with upregulation of its receptor in breast cancer. Binding of IGF-1 to its receptor activates pathways such as PI3K/Akt/mTOR and Ras/Raf/MEK, promoting cancer cell proliferation [97]. Metformin exerts its effects indirectly by reducing blood insulin levels, subsequently lowering IGF-1 concentrations.

The direct effect of metformin in cancer is exerted through modulation of several molecular targets and pathways [96,98–100]including: AMP-activated protein kinase (AMPK), which plays an essential role in cancer metabolism under energy stress, PI3K/akt/mTOR and Ras/-Raf/MEK pathways which control growth of cancer cells, reducing the synthesis of proinflammatory TNF, IL-6, and NF-κB, which collectively induce the production of VEGF; and finally, inhibition of the multidrug resistance 1 (MDR1) gene [101]. We will now discuss how metformin modulates these molecular targets in gliomas specifically.

AMPK pathway

AMPK is an energy sensor in the cell to which AMP will bind when the AMP:ATP ratio increase [102,103]. After AMP binding, this protein can be phosphorylated and activated by several other proteins including LKB1 (liver kinase B1). When activated, AMPK inhibits many anabolic pathways that consume ATP including fatty acid and cholesterol synthesis and activates catabolic pathways to produce ATP including glucose uptake and fatty acid metabolism. The overall result is inhibition of cell growth and cell cycle arrest at G1 phase. Another mechanism by which activated AMPK can induce cell cycle arrest is through activation of tumor suppressor proteins p27, p53 [104]and retinoblastoma (Rb) [105,106]. Activated AMPK can also inhibit the function of multiprotein complex mTORC1 [102,107] reducing cellular growth and proliferation. Overall, AMPK's tumor-suppressing and tumor-promoting capacities, determining its role in carcinogenesis, is clearly context-dependent.

In gliomas, AMPK was found to be overexpressed in glioblastoma and constitutively activated [108,109]. The expression was higher in highly proliferating cells in vitro and in vivo, elevated levels of activated AMPK was present in areas of high proliferation surrounding the blood vessels [108]. Furthermore, there is a correlation between the levels of phosphorylated (activated) AMPK and phosphorylated Rb *in vitro* and *in vivo*. [82] However whilst Rios et al. report that both knockdown and small molecule inhibition of AMPK reduced cell proliferation in vitro and tumor growth in vivo [108], Chippa et al. report this only in glioma stem cell lines [83].

Metformin reduces ATP production via inhibition of mitochondrial electron transport chain complex I (ETC1) (see later) and thus activates AMPK [109]. It has been shown in A172 glioblastoma cell line, that metformin produces an increase in AMPK expression and phosphorylation (activation), and a decrease in mTOR protein expression, reducing tumor cell growth and promoting tumor cell death [101].

AMPK activation is also implicated in cell autophagy. Autophagy has been proven to be one of the anti-tumor mechanisms of metformin in treatment for leukemia [110], melanoma [111] and GBM [112]. However, autophagy is also a rescue mechanism for tumors under stress conditions [113,114]

However, as previous results may show benefit of activating AMPK in glioma, other research showed that. The degree and/or mechanism of AMPK activity, the specific expression of AMPK isoforms, AMPK subcellular localization, the activity of other signaling networks in the cell, and extracellular environmental conditions could all influence whether AMPK plays a positive or negative role in tumor growth [115]. When resources are sufficient, loss of LKB1–AMPK signaling can obviously induce a proliferative and metabolic phenotype beneficial for tumor cells.

AMPK and glioma stem cells (GSC)

Metformin's influence on stem cell-like glioma cells, commonly known as Glioma Stem Cells (GSC), is another possible mode of action [94]. This is a type of tumor cell that has a strong ability for self-renewal, migration, and cytostatic resistance [116]. They are also blamed for tumor recurrence and chemotherapy resistance [117]. Metformin stimulated FOXO3 through AMP-activated protein kinase (AMPK), causing stem-like glioma-initiating cells to differentiate in vitro and successfully prevent tumor growth in vivo. Sato et al. used a mouse model to show that metformin has a role in FOXO3 activation through altering AMPK [90]. This results in a non-cancerous alteration in the direction of GSC transformation. The tumor stem cell population responsible for self-renewal is depleted as a result of this shift, resulting in a considerable increase in the survival of mice with glioma. However, the key mechanism involved in metformin's impact on GSC viability may not be AMPK activation [91].

PI3K/akt

Akt is a serine/threonine kinase that plays a vital part in the PI3K/akt signaling system which regulates cell metabolism, proliferation, protein synthesis, angiogenesis and apoptosis [118]. It is known that the PI3K/akt/mTOR signaling pathway is hyperactived in a variety of cancers, and that its activation can promote cancer cell proliferation and migration, while inhibiting apoptosis [119]. An in vitro study found that metformin significantly reduced PIK3CA, p-akt, and phospho-p70S6K expression levels in cervical cancer [84]. In GBM cell lines metformin also showed anti-invasive and anti-migratory actions by inactivating akt pathway [88].

Metformin reduce ATP production

As described before, metformin's capacity to reduce mitochondrial ATP synthesis is a feature of its anti-cancer action [94]. Metformin can interfere with electron transport chain activity through a direct inhibition of electron transport chain complex I (ETC1) [120], an enzyme that catalyzes the first step of the electron transport chain by triggering the oxidation of NADH to NAD+ [121]. Through this mode of action, metformin reduces total ATP production and oxygen consumption by attenuating but not completely inhibiting ETC1 [120]. GBM adapts to these effects by boosting ATP synthesis through glycolysis.

Metformin mediate cell cycle arrest

Metformin's action as an antiproliferative drug in GBM could be explained by its ability to arrest the cell cycle in the G1 phase [122] during mitosis. This arrest causes the cell cycle to halt in the G1 phase, leading to cell death and apoptosis as common outcomes [120].

Chloride intracellular channel-1 (CLIC1) is a direct target of metformin in human glioblastoma cells

CLIC1 is a member of chloride channel protein family that can move from cytoplasm to plasma membrane due to oxidative stress during cell cycle transition, where it functions as a chloride conductive pore, allowing cells to shift from G1 to S phase [89]. CLIC1 has been shown to contribute in proliferation, self-renewal and poor prognosis in glioma [123]. A bioinformatics analysis comparing normal brain cells with various glioma grades [124] revealed overexpression of CLIC1 in gliomas compared to normal cells, with GBM showing higher CLIC1 expression than other gliomas, particularly in the mesenchymal subtype of GBM.

Gritti et al. investigated whether metformin affects CLIC1 and is involved in inhibiting cell growth in the U87 human GBM cell line. Metformin interacts with CLIC1 on the outer side of the membrane, likely targeting the side chain of Arg29, which is crucial for destabilizing the channel in its closed state. Metformin may displace the arginine side chain from this polar region, maintaining the closed state of the channel and potentially blocking the pore, thereby exerting antiproliferative effects by blocking G1/S transition and arresting the cell cycle in the G1 phase [89]. However, metformin does not inhibit the activity of CLIC1 with a mutated Arg29.

Metformin increase expression of REDD1/DDIT4

The Regulated in Development and DNA Damage Response 1 (REDD1), also known as DNA-damage Inducible Transcript 4 (DDIT4) is a protein upregulated by stress condition such as hypoxia [125], DNA damage, or ATP depletion [126] and act as tumor suppressant. In LNCaP prostate cancer [127], and LN18, SF767, U87 and U251 glioma cell lines [120], treated with metformin due to its ability to increase REDD1 expression,REDD1 offer an alternative pathway that metformin can inhibit mTOR activity in independently on AMPK activation, once REDD1 is highly express its directly phosphorylation of TSC1 which negatively control mTOR activity and contribute in metformin anti-proliferative effect on GBM [120].

Preclinical data on metformin in glioblastoma

Numerous preclinical studies have investigated the use of metformin in combination with various chemotherapy drugs across different cancer types. For example, studies have shown that combining metformin with chemotherapy agents such as gemcitabine in pancreatic cancer [154], paclitaxel in melanoma [155], and cisplatin in lung cancer [156], may enhance anticancer effects compared to chemotherapy alone. However, some studies have reported no significant improvement with the combination [157]. Similarly, metformin combined with hormone therapy drugs, like tamoxifen, has demonstrated a greater anticancer effect than hormone therapy alone [158]. Furthermore, combining metformin with targeted therapy drugs, such as trastuzumab, has shown enhanced anticancer effects in breast cancer compared to targeted therapy alone [159].

For glioblastoma, preclinical research also provides strong evidence of how metformin can be repurposed to target cancer-specific metabolic pathways and improve treatment outcomes. Below, we summarize the preclinical findings on metformin in combination with various agents. These studies illustrate the synergistic effects, potential mechanisms of action, and the overall impact on glioblastoma cells, particularly glioma stem cells (GSCs). This compilation of data provides a foundational understanding of metformin's multifaceted role in glioblastoma treatment and sets the stage for subsequent clinical investigations (Table 1).

Metformin in combination with chemotherapeutics in glioma

Several preclinical studies have examined the use of metformin in combination with chemotherapeutics such as Temozolomide [120,132,134,137,138,160–166], Sorafenib [164], crizotinib [145], and Vismodegib [167], as potential treatments for glioma. Most of these studies suggest that the combination of metformin and chemotherapy has a greater anticancer effect compared to chemotherapy alone. Additionally, some investigations have demonstrated that metformin can sensitize cells previously resistant to single-agent chemotherapy [120,134,162,165,167] while others have confirmed the combination's activity against glioma stem cells (GSC) [132,164].

Dual targeting of metabolic pathways through the administration of metformin in conjunction with another metabolic modulator is a promising strategy due to the heightened metabolic demand and vulnerability of cancer cells. Numerous preclinical studies have explored the combination therapy of metformin with other metabolic agents, such as 2-deoxyglucose [137,138] and dichloroacetate [139,140,168–170], demonstrating promising results. Both combinations have shown anti-proliferative and antimigratory effects, although 2-deoxyglucose did not consistently exhibit cytotoxic effects across all treated cell lines.

Below, we highlight the potential of some of these combinations in more detail

Temozolomide

Temozolomide (TMZ) is an alkylating agent used in combination with radiotherapy and/or resection and is known as the gold standard for treatment of glioblastoma. However, the introduction of TMZ to the treatment modalities has only increased the median overall survival from 12.1 to 14.6 months [171], whilst resistance [172], or development of resistance mediated by TMZ itself [173], is responsible for treatment failure.

The combination of metformin and TMZ has a synergistic effect against glioblastoma and increased effects on downstream targets compared to the effect of each one alone [160]. Both TMZ [174] and metformin [100], increase ROS, AMPK activation, and p53 phosphorylation. However, there are caveats. As indicated earlier, the effect of increasing ROS by metformin is not seen in all glioma subtypes and in some is reported to decrease ROS [134]. Also, TMZ increases the p-akt levels [161], which could impair its AMPK activating activity effect when used alone [175]. Regardless, the combined increase in p53 phosphorylation is shown to sensitize the cell for TMZ-induced DNA damage.

Interestingly, the effect of metformin alone on glioma stem cells (GSC) shows it has a unique inhibitory effect on akt phosphorylation [132], but not on differentiated cells [91], and it selectively decreases the survival of GSC. Although, the role of metformin in overcoming TMZ resistance is not exclusive to GSC glioblastoma cells [120,162].

Dichloroacetate

Dichloroacetate (DCA) is a pyruvate analog, which inhibits pyruvate dehydrogenase kinase and its isoforms (PDK1-4). PDK1-4 are inhibitors of pyruvate dehydrogenase complex (PDC), the enzyme that converts pyruvate to acetyl-CoA. Therefore, by inhibiting PDKs, dichloroacetate indirectly activates PDC [176]. These perturbations of the PDC/PDK axis induce a "glycolytic shift" whereby affected cells favor ATP production by glycolysis over OXPHOS and cellular proliferation over cellular quiescence [177]. DCA as monotherapy tends to block the glycolytic pathway but did not have a statistically significant effect on glioblastoma cells. However, DCA and metformin synergistically induce apoptosis or lethal effects in vitro and in vivo by inhibiting the metabolic pathway [139], and increasing ROS production [140] in glioblastoma cancer cells. A consequence of the glycolytic shift induced by dichloroacetate is an increase in the production of lactate.

Lactate plays a unique role in the tumor microenvironment, encouraging tumor cell proliferation and development as well as providing peripheral cancer cells with an alternative means of energy production (see lactate shuttling above) [178]. Lactate is also an agonist for the G-protein-coupled receptor GPR81, the activation of which leads to an increase in angiogenesis, immunological evasion, and chemoresistance [179]. Finally, lactate acts as a positive feedback mechanism for HIF-1α [177]. Unrelated to glycolysis, lactate can also be produced by the breakdown of glutamine [180] in tumors, in a process which is coordinated by c-Myc [179]. Consequently, a combination of DCA and metformin produces more lactate than DCA alone [139]. Glioblastomas are shown to have elevated lactate concentrations compared to normal brain tissue [181]. This suggests that a triple combination of DCA, metformin, and a glutaminase inhibitor, such as CB839, can lead to suppression and lowering the production of lactate.

2-Deoxyglucose

2-Deoxyglucose (2-DG), a non-metabolizable glucose analogue, inhibits both protein glycosylation and prevents glycolysis. 2-DG suppresses glycolysis at the start stage by inhibiting glucose-6-phosphate isomerase, the enzyme that transforms glucose-6-phosphate to fructose-6-phosphate. As a result, 2-DG can deplete ATP and biosynthesis of glycans necessary for protein glycosylation [182]. By itself or in combination with other anticancer therapeutics, 2-DG can kill human breast cancer cells [183], suppress hepatocellular carcinoma [184], and other carcinoma cells [184]. This effect was accompanied by a drop in the

Table 1
Preclinical data on metformin in glioblastoma.

Combination treatment	Result		Model	Reference
	In vitro	*In vivo*		
Diclofenac	The combination showed anti-migratory effect more than metformin alone in GSC and GBM cells. The higher antimigratory effect was not significant in the combination compared to diclofenac alone in some cell lines.	—	BTIC-8, −11, −13, and −18 U87- HTZ349 cell lines	[128]
Stattic (STAT3 inhibitor)	GSC and GBM cells treated with static alone inhibited proliferation, migration and invasion. While the combination treatment have additive antiproliferation effect on GSC they have no additive effect on migration or invasion in GSC and didn't show any additive effect in GBM cells.	—	GSCs isolated from surgically resected tumor from patients and a subset was treated to induce differentiation into GBM.	[129]
Temozolomide	Combination in high doses is more cytotoxic, induce apoptosis than each one alone.	Marked reduction in tumor growth rate and increase survival	U87- U251, and A172 GBM orthotopic mice models	[130]
Temozolomide	MET and TMZ inhibited GSC and GBM cells proliferation synergistically and increased apoptosis in GSCs. AMPK activation is not solely responsible for the synergism.	Combination treatment prolonged mice survival.	U87 and U251 GSCs and GBM cells in vivo Human tumor xenografts in SCID mice injected with U87 cells	[131]
Temozolomide	TMZ plus MET synergistically inhibiting proliferation and induced apoptosis in GSC, also significant reduction in the size and number of gliospheres of GSC	—	U87 and C6 cell lines	[132]
Temozolomide	The combination reduced cell viability in both cell lines in hypoxia compared to normoxic cells. Only the combination and MET produced this effect in TZM resistant cells where the combination produced it earlier than MET alone.	—	T98 cells resistant to TZM and U251 cells	[133]
Temozolomide	the combination improved treatment response and act additivily in TZM non resistant cells and increased sensitivity and act synergistcaly to TZM resistant cells.	In U251 cells model show decrease tumor volume to the combination that is similar to TZM only in T98G cell model TMZ monotherapy had no effect on tumor proliferation in contrast, combination therapy has synergistic effect in decrease in tumor growth rate	U251 and T98G cell lines U251 and T98G model mice	[134]
Temozolomide - radiotherapy	metformin combination with TZM and radiation was more effective than each one alone. MET sensitize TZM resistant cells to TZM	—	U87, LN18, U251 and SF767 cell lines	[112]
Temozolomide - radiotherapy	metformin radio-sensitized cells when combined with TZM. When metformin was combined with TZM, it resulted in significantly higher pAMPK levels in MGMT methylated cells, but not in non-methylated MGMT cells.	—	LN18 and LN229 cell lines	[135]
Sorafenib or temozolomide	MET and sorafenib decreased GSC proliferation, increased ROS for longer time, produced highest apoptotic rate more than each one alone or in combination of MET with TZM. In GBM cells only MET and sorafenib combination showed a significant reduction of cell proliferation. Sorafenib and TZM combination with MET or sorafenib alone inhibited drug efflux from GSCs.	—	GSCs isolated from surgically resected tumors of patients	[136]
2-Deoxyglucose	The combination significantly inhibits proliferation, sphere formation, and invasion.	Combination treatment also have survival benefit and anti-invasion in xenograft model	TS13–20, TS15–88, TS09–03, GSC11, and U87 cell linesGSC11 mice	[137]
ABT-263 and 2-Deoxyglucose	In pediatric GBM cells, triple combination of ABT-263 and metformin and 2DG was significantly highly effective in inducing apoptosis than either metformin/ABT-263 or 2DG/ABT-26 alone.		SF188, KNS42 cell lines	[138]
Dichloroacetate	Combination show synergistic cytotoxicity reduce cells number to 40 % of control, induce apoptosis, especially late apoptosis increased in percentage by 13.6 %.in vivo, after prolong combination result in significant tumor size reduction	after prolong treatment combination result in significant tumor size reduction	U87 cell line C57BL/6 mice	[139]
Dichloroacetate	The addition of metformin to DCA significantly potentiate cytotoxicity by		VM-M3	[140]

(*continued on next page*)

Table 1 (*continued*)

Combination treatment	Result		Model	Reference
	In vitro	*In vivo*		
	increase ROS production in AMPK independent action.			
Dichloroacetate	——	DCA didn't increase life span in rates. The combination of MET with DCA increased life span of rates more than metformin alone.	C6 cell line	[141]
Dichloroacetate -radiotherapy	MET and DCA combination reduced DIPG proliferation synergistically, decreased ATP more than each one alone, increased ROS production in mitochondria and cytoplasm which lead to more double strand break in DNA compared to monotherapy and didn't affect lung fibroblast non-cancerous cells. The triple combination (radiation, DCA and metformin) led to a more potent therapeutic effect.	MET didn't prolong survival. Combination of radiotherapy with MET prolonged survival but the longest survival was obtained with triple combination with greatest DNA damage.	Patient-derived diffuse intrinsic pontine gliomas (DIPG). xenograft orthotopic mouse model with patient derived DIPG	[142]
Dichloroacetate	MTF significantly enhanced the cytotoxic activity of DCA, also increased apoptosis and cell cycle arrest against C6 cell.	MET alone has more survival benefits than DCA alone. Combination has synergistic effect in inducing apoptosis and reduce tumor invasion	C6 cell line	[143]
9-*cis* Retinoic acid	The combination inhibited proliferation and synergistically induce apoptosis the GSC significantly more than each drug alone.	——	C6 cell line	[144]
Crizotinib	Combining crizotinib with metformin result in synergistic decrease in proliferation of tumor cells	——	U87 cell line	[145]
Vismodegib	Metformin treatment in combination with Vismodegib synergistically reduced viability and increased apoptosis in the two Vismodegib resistant cell lines.	The combination decreased growth of the tumor significantly and showed increased caspase 3 compared to monotherapy with each.	MB11 and DAOY both generated to form resistance to Vismodegib by culturing with sublethal concentrations of Vismodegib. Two MB11 and other two DAOY smoothened mutated receptor. Nude mice injected subcutaneously with MB11 cells.	[146]
Disulfiram –Cu-Radiosensitization	The combination has synergistic effect in induce apoptosis and sensitizing U87 cell to radiation		U87	[147]
cold atmospheric plasma	the combination is synergistic decrease cell number and strongly inducing apoptosis in H_2O_2 dependent.	——	U251 and U87	[148]
Akt1/akt2 inhibitor	The combination treatment potentiate reduction in CSC sphere size, cell number and also decrease living cell by 95 % percentage of control group	synergistic effect in reduction tumor volume and tumor weight	U87	[149]
H_2O_2 - n-C60	Combination of metformin with either H_2O_2 or n-C60 has a synergistic cytotoxic by increase ROS level.		C6 cells	[150]
epigallocatechin gallate (EGCG) and TMZ	——-	The median survival rate of the triple-drug combination TDC-treated rats (24 weeks) was significantly higher than that of the tumor-control group (7.5 weeks) and dual treatment groups TMZ-MET (18.5) week and TMZ-EGCG (15) week. Additionally, 50 % of the rats administered with the TDC TME had a significantly improved tumor inhibition and a prolonged period of survival (>25 weeks).	C6 rat glioma cell line Wistar rats	[151]
Simvastatin	Treatment of both cell lines in combination significantly lowered the migration of GBM cells, decreased the release of vascular endothelial growth factor, and markedly elevated apoptosis in comparison to the control, MET, or simvastatin-treated groups.	Compared to control tumors and tumors treated with metformin or simvastatin, the combined treatment significantly lowered the size and mass of the tumors and upregulated important genes linked to apoptosis.	U-87 MG and U-118 MG	[152]
Artesunate (ART)	results demonstrated that MET combined with ART can significantly suppress the invasion and migration capacity of GBM cells and significantly increase the apoptosis rate in GBM cells compared to that in the control, ART, and MET groups.	————	U251 and U-118 cells	[153]

amount of ATP in the cell.

In five different glioblastoma-derived spheroid models, 2-DG had a moderate antiproliferative effect. This modest reduction could be explained by cancer cells' ability to maintain ATP generation, which would result in a compensatory increase in mitochondrial metabolism. Combined therapy with 2-DG and metformin showed a strong synergistic, anti-stemness effect, almost completely impairing sphere formation of glioblastoma-cell spheroids [137]. Presumably, this was because cells became more quiescent due to lack of energy as the two important ATP production pathways were inhibited.

Sorafenib

The epidermal growth factor receptor (EGFR) is a key regulator of growth and progression in several cancers. In particular, EGFR is commonly amplified, overexpressed, or constitutively active in primary glioblastoma [185]. Sorafenib is an inhibitor of the RAS/RAF/MAPK pathway, which alongside the PI3K/akt/mTOR pathway, is the downstream signaling conduit for EGFR. Clearly, an inhibition of both axes could provide a more complete suppression of EGFR signaling for cancer cell proliferation, antiapoptotic effect, metastasis, and angiogenesis [185]. It is shown by Holland et al. that combined activation of akt and RAS was needed to induce glioblastoma formation [186]. Furthermore, sorafenib also inhibits other growth factor receptors including platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF) both of which are overexpressed in glioma and contribute to the tumor growth and are activators of the RAS/RAF/MAPK pathway [187,188].

Moreover, the oncogenic transcription factor MYC, which is regulated by both RAS and akt, is also involved in modulation of tumor metabolism and growth [37]. The combined effects of metformin and inhibition of the RAS pathway can complement each other by acting on MYC through multiple mechanisms. These include p53, which is activated by AMPK, and can downregulate MYC transcription by binding to the MYC promoter [189]. In addition, AMPK can activate FOXO3a, which antagonizes MYC in a variety of ways, including increased expression of endogenous MAX inhibitors which binds to MYC and prevents MYC/MAX heterodimerization, as well as promoting MYC breakdown [34].

Indeed, a study on the effects of sorafenib and metformin on glioma stem cells found that this combination had a higher antiproliferative action than metformin plus TMZ as TMZ had no significant effect on GSC when it is used alone and no significant difference from metfromin alone was seen in the metformin plus TMZ combination [164]. The response was not limited to GSCs, and it was seen in non-stem cells as well. The combination also suppressed drug efflux pumps and maintained oxidative stress better than either one alone.

Vismodegib

Vismodegib is a drug that binds to and is an antagonist of the smoothened receptor, leading to inhibition of abnormal activation of the hedgehog (Hh) pathway. The Hh pathway is a signaling system that plays a role in a variety of developmental events, including proliferation, survival, and differentiation. While this route is quiescent in most normal tissues, it has been found to be abnormally activated in a number of cancers [190]. Sonic Hh (SHH) signaling controls the oncogenesis of medulloblastoma (MB). GLI protein, a measure of Hh pathway activity, is present in MB tissues [191]. The SHH/GLI1 pathway is controlled by the AMPK signaling cascade, according to recent research [192]. As an energy and metabolic sensor, AMPK suppresses SHH/GLI1 signaling by phosphorylating GLI1 at three different locations, causing GLI1 degradation and inactivation [192]. Also, GLI1 inhibition may be mediated by AMPK, with S6K (downstream of mTOR) [193]. The combination therapy of metformin as an AMPK activator with vismodegib has been tested on vismodegib-resistant MB cell lines [167]. The combination resulted in considerable synergism in lowering cell viability, indicating that the combo treatment was able to defeat the vismodegib-resistant cell line. This result was due to another pathway through the AMPK–PI3K–mTOR pathway since PI3K signaling was identified as another possible resistance mechanism of SMO antagonists [194]. The combined treatment greatly slowed the growth of the MB11 tumor in vivo. The combined treatment rats had much less proliferative and higher expression of the apoptosis caspase-dependent gene [154].

Although the studies on vismodegib focus predominantly on medulloblastoma, its mechanism of action through metabolic reprogramming pathways, particularly AMPK and mTOR signaling, has significant implications for glioblastoma research. Glioblastoma shares several dysregulated pathways with medulloblastoma, including PI3K and mTOR signaling, both of which are targeted by metformin. These parallels suggest that combining metformin with smoothened receptor antagonists like vismodegib warrants exploration in glioblastoma models, especially given the role of AMPK in both cancers.

Crizotinib

Crizotinib is a tyrosine kinase inhibitor that targets ALK, ROS1, and c-MET, and is approved for treatment of ALK-rearranged non–small-cell lung cancer (NSCLC). Since c-MET fusions are associated with many cancers including glioblastoma, and since ALK (Anaplastic Lymphoma Kinase) is overexpressed in more than a third of gliomas [195], crizotinib appears to be a potentially important chemotherapeutic for some patient sub-population in this disease [196]. For example, a study of *ALK, ROS1*, and *MET* status and their corresponding proteins in nine glioblastoma stem cell lines revealed slight overexpression of ALK and c-MET proteins, suggesting a role for crizotinib in eradicating glioma stem cells [197].

To date however, there has been only one investigation of crizotinib in combination with metformin for glioma. It is known that c-MET inhibition drives metabolic reprogramming including higher oxidative metabolism [145]. In view of metformin's ability to inhibit respiratory chain, it is shown that combining crizotinib with metformin leads to synergistic reduction in proliferation of U87 tumor cells. [145] However, combining metformin with crizotinib and other agents may be also potentially interesting. For instance, metformin has been shown to restore crizotinib sensitivity in crizotinib-resistant human lung cancer cells through its inhibition of IGF1-R signaling pathway [198]. In view of the importance of IGF1-R signaling pathway in glioma [199], combination of metformin and crizotinib may also have a role in glioblastoma chemotherapy. Furthermore, combination of crizotinib with temozolomide (TMZ) in FIG-ROS1-positive U118MG cell line and patient-derived tissue (which highly express phosphorylated ALK, c-Met, and ROS), showed a significant increase in cell death compared to TMZ alone. However, a similar effect was absent in U87 (FIG-ROS1--negative) cells and tissue [200]. To date however, combination of the three agents together is not yet explored.

Artesunate

Artesunate (ART) is one of the most commonly and efficient drug used for the treatment of malaria [201]. On its mechanism of anticancer, It has been demonstrated that ART activates the AMPK pathway, directly triggers the AMPK-mTOR axis by generating ROS, which in turn triggers autophagy in tumor cells, and non-specific capture of cytosolic components damages tumor cells irreversibly, finally resulting in tumor cell death [202] and as MET also activate AMPK pathway as mentioned earlier it is not surprising for producing a synergistic effect toward GBM cells. Together, they impair the mitochondrial metabolic behavior of tumor cells, significantly reducing their invasion and migration capacities, as demonstrated by Ding et al. [153].

Clinical studies on metformin in glioblastoma

The promising preclinical findings highlight the potential of metformin as a valuable adjunct in glioblastoma treatment, supporting its progression to clinical trials. The subsequent clinical studies aim to validate these preclinical results and assess the efficacy and safety of metformin in combination with standard and novel therapies in human patients. Below is a summary of clinical trials involving metformin in glioblastoma (Table 2).

Table 2
Clinical studies involving metformin.

Combination of treatment	Phase	Patient number	Result	Ethnicity	Dosage	Reference
MET+TMZ+Radiotherapy	Phase I	33	Thirty-three patients, 19 females and 14 males were included. Median age was 54 years, median ECOG score was 1 At a median follow up of 20.3 months (range 2.0–55.8) for patients at risk, the median survival was 38.2 months (95 % CI, 12–59), with 20 out of 33 patients alive at the date of this report. At 2 years the overall survival was 65.5 %, and 50.5 % at 3 years. Median PFS was 15.4 months (95 % CI, 10–36). For 27 patients with gross total resection, median survival was 38.84 months compared to 16.8 months for 6 patients with partial resection (p Z 0.02). The median survival of patients aged 〈 60 years was 38.8 months and 17.1 for patients 〉 60 years (p Z 0.16). The median survival for 12 patients with methylated MGMT was not reached compared to 38.2 months for 21 patients with unmethylated MGMT (p Z 0.12). Adjuvant TMZ/ Metformin was discontinued in 2 patients, one with disease progression, and another one following prolonged grade 4 thrombocytopenia. There were no unexpected severe adverse events. Thirteen patients were re-operated for findings suggestive of disease progression. Six patients of the 13 re-operated patients had treatment-related necrosis with no viable residual GBM. Two of these 6 patients later developed disease progression. Eighty-one percent and 70.9 % of the patients were free from treatment-related necrosis at 2 and 3 years, respectively	Not Specified	Metformin: 850 mg twice daily. Temozolomide (TMZ): 75 mg/m^2 daily.	[203]
TMZ+MET+Memantine+Mefloquine	Phase I-II	85	The maximum tolerated doses (MTDs) for doublet therapy were memantine 20 mg twice daily, mefloquine 250 mg 3 times weekly, and metformin 850 mg twice daily. For triplet therapy, the MTDs were memantine 10 mg twice daily, mefloquine 250 mg 3 times weekly, and metformin 850 mg twice daily. For quadruplet therapy, the MTDs were memantine 10 mg twice daily, mefloquine 250 mg 3 times weekly, and metformin 500 mg twice daily. Dose-limiting toxicities included dizziness (memantine) and gastrointestinal effects (metformin). Lymphopenia was the most common adverse event (66 %). From study entry, the median survival was 21 months, and the 2-year survival rate was 43 %.	Not Specified	Metformin: Starting dose 1000 mg twice daily (reduced to 850 mg or 500 mg BID in combination arms due to toxicity). Temozolomide (TMZ): 75 mg/m^2 daily during radiotherapy; 150–200 mg/m^2 daily on days 1–5 of a 28-day cycle as adjuvant. Memantine (10–30 mg BID) and Mefloquine (250 mg three times weekly).	[204]
Metformin + chloroquine	Phase Ib	15	the combination treatment of metformin and chloroquine toxicity profile is well tolerated, but the combination did not induce a clinical response in this patient group	Not Specified	Metformin: Dose escalation up to 1500 mg twice daily (3000 mg total daily dose). Chloroquine: Fixed dose of 200 mg once daily.	[205]
Vincristine+ irinotecan+MET+TMZ	Phase I	26	The children treated with vincristine 1.5 mg/m^2 intravenous Days 1 and 8, irinotecan 50 mg/m^2 intravenous Days 1–5, temozolomide 50 mg/m^2 oral Days 1–5 in a 21-day cycle in combination with metformin. It was found that the combination was tolerable with phase II recommended dose being 166mg/m^2/ day.	Caucasian (81 %) and African-American (15 %)	Metformin: Dose levels ranged from 666 mg/m^2/day to 2000 mg/m^2/day, with a recommended Phase II dose at 1666 mg/m^2/day. Chemotherapy Regimen Vincristine: 1.5 mg/m^2 intravenous on Days 1 and 8. Irinotecan: 50 mg/m^2 intravenous on Days 1–5. Temozolomide: 100 mg/m^2 oral	[206]

(*continued on next page*)

Table 2 (*continued*)

Combination of treatment	Phase	Patient number	Result	Ethnicity	Dosage	Reference
					on Days 1–5 (reduced to 50 mg/m^2/day after an amendment).	
MET +TMZ	Phase I/II	22	The phase I part of the study included 7 patients treated with TMZ 150 & 200 mg/m^2/day in combination with MET showed tolerability of the with metformin dose up to 2250 mg/day (the highest dose tested) with no dose limiting toxicities.	primarily Japanese	Metformin: from 1500 mg/day to 2250 mg/day, with 2250 mg/day recommended as the Phase II dose. Temozolomide (TMZ): 75 mg/m^2 daily with radiotherapy, followed by 150–200 mg/m^2 for 5 days in 28-day cycles during maintenance.	[207]
MET + low carbohydrate diet + radiotherapy	Phase I	13	The study concluded that 850 mg three times daily was not tolerable and recommended twice daily regimen for phase II trials.	Not specified	Metformin: Dose escalation up to 850 mg three times daily (2550 mg total daily dose) in combination with a Modified Atkins Diet (ModAD). Radiotherapy: For newly diagnosed high-grade gliomas (HGG), 60 Gy over six weeks; for recurrent gliomas, 30–35 Gy over two weeks.	[208]
MET+niacinamide+ lapatinib+Plerixafor +Radiotherapy+TMZ	case report	1	30 months after the start of his adjunctive therapy, his clinical condition has steadily stabilized with no sign of his tumor returning. He is in clinical and radiologic remission.	Not specified	Temozolomide: 150 mg/m^2 daily for five days, then escalated to 200 mg/m^2 monthly for five days. Plerixafor: 0.24 mg/kg once weekly. Lapatinib: Initially 1000 mg daily, later increased to 2500 mg twice a day, two days per week. Metformin: Started at 500 mg daily, escalated to 1000 mg twice daily. Niacinamide: 60 mg/kg daily.	[209]

Conclusion

In conclusion, incorporating metformin into treatment regimens for glioblastoma has the potential to improve patient outcomes by targeting metabolic pathways that are relevant to cancer. The preclinical experiments discussed in this review show that metformin can effectively hinder the growth of tumors, trigger programmed cell death, and make glioblastoma cells, including glioma stem cells, more responsive to standard chemotherapeutic drugs and other targeted therapies. These data have strongly encouraged the progression of metformin into clinical trials in order to confirm its effectiveness and safety profile in patients with glioblastoma.

Of course, there are a number of issues surrounding the clinical use of metformin and translation of preclinical potency into clinical efficacy for this drug. Generally speaking, metformin is not metabolized and has good tissue distribution and cell penetration which is mediated by active transported into cytoplasm via organic cation transporters (OCTs). [210]. However, there are substantial variations in glycemic control following metformin administration [211], mainly due to polymorphisms in the genes that control metformin cellular uptake and therefore pharmacokinetics and pharmacodynamics of the drug [210, 211]. There are no reasons that similar factors may not influence clinical efficacy of metformin for cancer patients.

Nevertheless, the clinical studies discussed in this review demonstrate the potential clinical advantages of combining metformin with different treatments, such as temozolomide, radiation, and targeted therapies. While the findings of these trials are varied, with some demonstrating notable enhancement in patient outcomes and others suggesting no meaningful impact, the collective data indicate that metformin may serve as a beneficial supplementary element in the comprehensive strategy to treating glioblastoma.

Metformin belongs to the biguanide chemical class and we should note that whilst a number of other biguanides have been studied in preclinical investigations for their potency against glioma [212–215], none has shown sufficient promise to progress further so it seems that for the time being, metformin is the only drug in this class with clinical potential.

Future research should prioritize the investigation of the exact molecular pathways responsible for metformin's anticancer effects, the discovery of biomarkers that may be used to stratify patients who would most benefit from it, and the enhancement of combination treatment plans and dosage techniques. Moreover, it is imperative to conduct additional clinical trials with bigger groups of patients and longer observation periods in order to ascertain the enduring advantages and potential hazards of integrating metformin into conventional treatment procedures for glioblastoma.

By further investigating and confirming the efficacy of metformin in glioblastoma treatment, we have the potential to enhance patient prognosis and quality of life, providing a newfound sense of optimism for those fighting this formidable and complex brain tumor.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT and Quill Bot to improve language and readability. After using these technologies, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

CRediT authorship contribution statement

Haneen A. Basheer: Writing – review & editing, Writing – original draft, Supervision, Conceptualization. **Nadeem M. Salman:** Writing – review & editing, Writing – original draft. **Rami M. Abdullah:** Writing – review & editing, Writing – original draft. **Lina Elsalem:** Writing – review & editing. **Kamyar Afarinkia:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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