Metformin as an Adjunct Treatment to Temozolomide for High-Grade Gliomas: A Systematic Review and Meta-Analysis

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■ OBJECTIVE: **High-grade gliomas (HGGs) are aggressive tumors known for their poor prognosis. Despite research into its molecular and clinical aspects, current management minimally impacts survival. It is unclear whether combining temozolomide (TMZ) with metformin (MET) could enhance survival in this population.**

■ METHODS: **A systematic search on PubMed, Embase, and Cochrane Library databases was conducted for studies comparing TMZ + MET versus TMZ alone for HGG. The outcomes of interest were overall survival, progression-free survival, and subgroup analysis with O6-methylguanine-DNA methyltransferase and patients with diabetes. The analysis comprised outcomes reported as hazard ratios (HRs) and odds ratios with corresponding 95% confidence intervals (CIs) as all the outcomes are continuous. A significance level of $P < 0.05$ was considered statistically significant. Heterogeneity was assessed using the I^2 statistic with P values inferior to 0.10, and $I^2 > 25\%$ were considered significant for heterogeneity. The random effects model was employed for all outcomes.**

■ RESULTS: **Ten studies were included, comprising 3623 patients, of which 346 (9.5%) were assigned for TMZ + MET. The TMZ + MET group was associated with a significant reduction in mortality rates when compared to the TMZ alone group (HR 0.74; 95% CI 0.59, 0.93; $P < 0.01$; I^2 29%). There was no significant difference between groups for progression-free survival (HR 0.87; 95% CI 0.68–1.12; $P = 0.29$). In a subgroup analysis restricted to patients who received TMZ + MET, the diabetic subgroup had a significantly higher mortality rate than the normoglycemic subgroup (odds ratio 1.25; 95% CI 1.10, 1.41; $P < 0.01$; I^2 79%).**

■ CONCLUSIONS: **Our results showed that patients who received TMZ + MET had a significantly higher overall survival than patients who received TMZ alone. These findings support the use of MET along with TMZ for the treatment of HGGs.**

Key words
- Astrocytoma
- Glioblastoma
- High-grade gliomas
- Metformin
- Temozolomide

Abbreviations and Acronyms
AMP: Adenosine monophosphate
AMPK: AMP-activated protein kinase
ATP: Adenosine triphosphate
CI: Confidence interval
GBM: Glioblastoma
HGG: High-grade glioma
HR: Hazard ratio
IDH: Isocitrate dehydrogenase
MET: Metformin
MGMT: O6-methylguanine-DNA methyltransferase
mTOR: Mammalian target of the rapamycin
NADP: Nicotinamide adenine dinucleotide phosphate
OS: Overall survival
PFS: Progression-free survival
ROBINS I: Risk Of Bias in Non-Randomized Studies of Interventions
T2DM: Type 2 diabetes mellitus
TMZ: Temozolomide
WHO: World Health Organization

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INTRODUCTION

High-grade gliomas (HGGs) are a group of aggressive, malignant, and diffuse brain tumors with rapid progression. These encompass a variety of entities such as glioblastoma, isocitrate dehydrogenase (IDH)-wildtype (grade 4); astrocytoma, IDH-mutant (grades 3 and 4); oligodendroglioma, IDH-mutant, 1p/19q-codeleted (grade 3). Glioblastoma, the most common malignant primary brain tumor in adults, typically affects individuals between the ages of 55 and 60 years old.[1] The incidence of HGG is estimated to be around 3 to 4 cases per 100,000 people annually in the US.[1]

The prognosis of patients with HGG is influenced by factors such as age and Karnofsky's Performance Status, extent of resection, and subsequent treatments. The initial step in management is maximal safe resection, followed by radiation therapy and chemotherapy with alkylating agents, such as temozolomide (TMZ).[2-6]

Unfortunately, there has been little improvement in survival rates over recent years, with the prognosis remaining dismal. The median overall survival (OS) for patients with glioblastoma, based on population-based studies, is approximately 10 to 17 months.[2] In this context, metformin (MET) has been considered as a potential novel therapy to improve survival in HGG.[7] Lowering glucose levels has been associated with apoptosis of cancer cells and a reduction in tumor progression; also, MET activates adenosine monophosphate (AMP)-kinase and inhibits the mammalian target of the rapamycin (mTOR) pathway, inhibiting protein biosynthesis and tumor cell growth.[8-10] It may directly inhibit tumor cells and indirectly impede cancer cell growth by reducing circulating glucose and insulin levels, consequently affecting insulin-like growth-factor signaling. Experimental studies have demonstrated that the combination of TMZ+MET is superior to monotherapy in terms of cell viability and survival.[11,12] However, the clinical impact on patient prognosis is still unknown.[13] Therefore, our study aimed to conduct an updated systematic review and meta-analysis comparing the effectiveness of TMZ+MET versus TMZ alone for patients with HGG.

METHODS

This systematic review adhered to the recommendations of the Cochrane Handbook for Systematic Reviews of Interventions.[14] The findings were reported following the guidelines set forth by the Preferred Reporting of Systematic Reviews and Meta-Analyses guideline.[15] The study was prospectively registered in the "International Prospective Register of Systematic Reviews" in 2023 under the identification CRD42023432149.

Search Strategy

A search was performed on PubMed, Embase, and Cochrane Library databases using the following search strategy: (GBM OR glioblastoma OR gliomas OR astrocytomas OR HGG) AND (metformin OR antidiabetic* OR Axpinet OR Diagemet OR Glucient OR Glucophage OR Metabet) AND ("standard treatment" OR "gold-standard treatment" OR TMZ OR temozolomide OR "alquilating agent" OR "alkylating agent"). The search was carried out in May 2023 and was updated in December 2023. A search on the reference lists of all included studies was done, as well as prior systematic reviews.

Selection Procedure and Data Extraction

The triage of studies was done manually by two authors (E.B.T.A. and M.Y.F.) with any disagreement resolved through discussion with the senior author (A.D.P.).

Study Selection and Eligibility Criteria

Only the studies that satisfied all the following specified eligibility criteria were included: 1) studies in patients diagnosed with HGG; 2) that compared TMZ+MET versus TMZ alone; and 3) reported at least 1 outcome of interest. We included both observational and randomized studies. Our endpoints of interest were OS and progression-free survival (PFS). We also performed a subgroup analysis based on O6-methylguanine-DNA methyltransferase (MGMT) status and patients with type 2 diabetes mellitus (T2DM). Our exclusion criteria comprised preclinical investigations (with animals or in vitro), studies that included low-grade gliomas, and studies not published in English. There were no restrictions in terms of year of publication.

Statistical Analysis

To preserve time-to-event data, we computed pooled hazard ratios (HRs) with 95% confidence intervals (CIs) in the comparison of OS and PFS between TMZ+MET versus TMZ alone. Subgroup analysis was conducted for studies that evaluated MGMT and patients with T2DM. When appropriate, comparative analyses were executed, with the chosen statistic as the odds ratio for the event in consideration. Outcomes were pooled with a random-effects model. Review Manager version 5.4.1 (Cochrane Center, The Cochrane Collaboration) was used for statistical analysis. Statistical significance was defined as P values of <0.05.

Heterogeneity

Heterogeneity was examined with I^2 statistics and P values inferior to 0.10 or $I^2 > 25\%$ were considered statistically significant for heterogeneity, based on Cochrane's Handbook for Systematic Reviews of Interventions thresholds.

Risk of Bias Assessment

Risk of bias was evaluated using the Cochrane Risk Of Bias In Non-randomized Studies of Interventions (ROBINS-I) tool. The ROBINS-I categorizes studies as having a low, moderate, serious, critical risk of bias, or no information.[16] Randomized studies were analyzed using the Risk of Bias 2 tool and classified in high, low, or some concerns of bias in 5 domains: selection, performance, detection, attrition, and reporting biases.[14]

RESULTS

Study Selection and Baseline Characteristics

As detailed in Figure 1, our search yielded 1516 studies, of which 203 were excluded as duplicates, and an additional 1279 were excluded during the screening based on title and abstract. A total of 34 studies were fully reviewed. After this final step, a total of 10 studies were included, of which 1 was a randomized controlled trial.[17-26] A total of 3623 patients were included, as reported in Table 1, of whom 3277 (90.5%) received only the standard treatment with TMZ, whereas 346 (9.5%) were treated with a combination of TMZ with MET. All patients received the optimal treatment in terms of tumor resection and radiotherapy. The TMZ dose varied between 25 and 50 mg/day, while the MET dose ranged from 400 mg/day to 2550 mg/day.

Systematic Review

In total, ten included studies analyzed OS in the patient population receiving TMZ+MET versus TMZ alone (Table 2). Among these studies, in four of them, a statistically significant difference was observed between the groups, with the results of three studies being associated with an improvement in the MET-treated group, and 1 study reporting overall better outcomes for the TMZ-alone group. Additionally, another four articles reported no statistical difference compared to the control

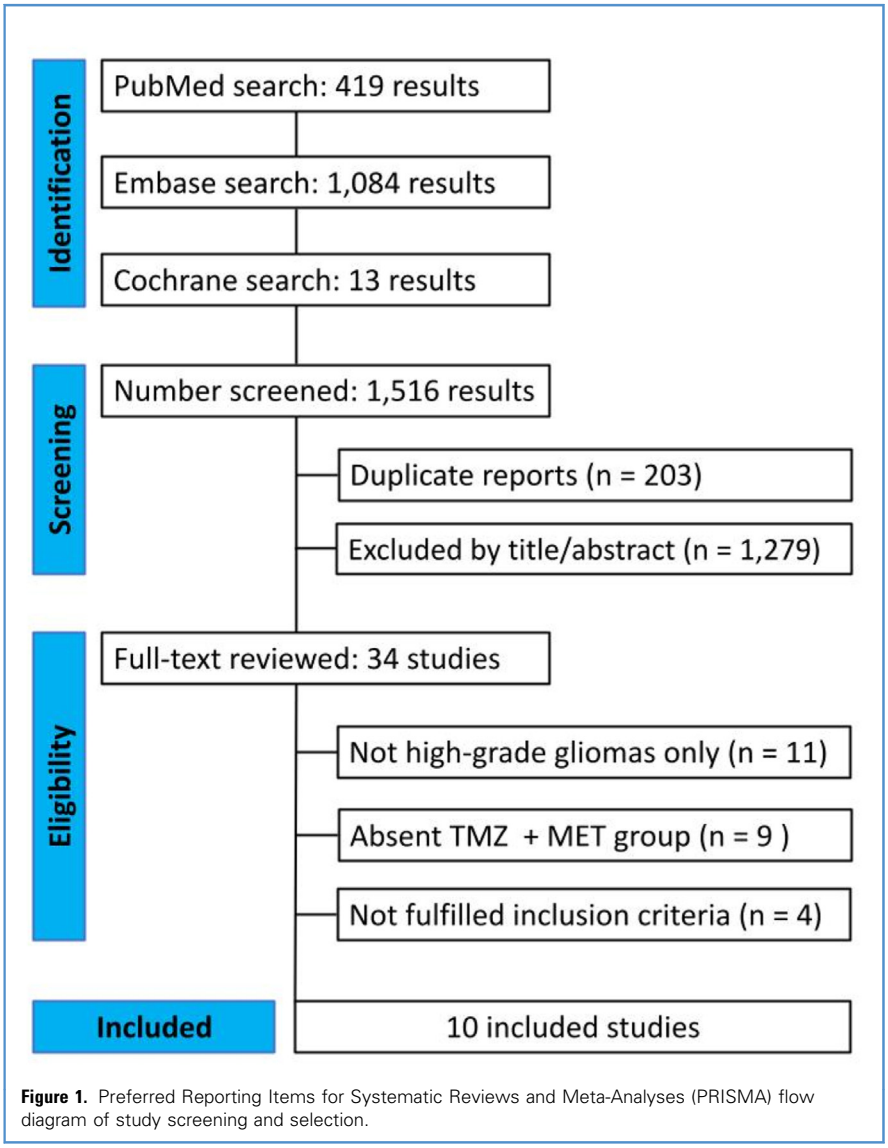


Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram of study screening and selection.

Table 1. Baseline Characteristics of Included Studies

Study	Study Design	Intervention	Control	Age†, y	Follow-Up, mo	Location	Period
Adeberg 2015	Retrospective study	20	20	63	N/A	Germany	2006–2013
Fuentes-Fayos 2023	Retrospective study	25	60	N/A	N/A	Spain	2016–2022
Henderson 2015	Retrospective study	6	220	N/A	N/A	Scotland	2010–2012
Mohammad 2023*	Retrospective study	40	66	N/A	N/A	Canada	N/A
Porper 2021	Retrospective study	10	3	61	18	Israel	2014–2020
Razak 2019	Retrospective study	7	115	60.8	N/A	England	2013–2014
Seliger 2019	Retrospective study	55	1038	59	88	Germany	1998–2013
Seliger 2020	Retrospective study	122	1609	60	24	Germany	N/A
Welch 2013	Retrospective study	18	108	66	N/A	USA	1998–2010
Yoon 2023	RCT	43	38	57.5	N/A	Korea	2006–2020

N/A, not available; RCT, randomized controlled trial.
*Conference abstracts.
†Mean or median.

Table 2. Individual Conclusion of the Studies Included in the Systematic Review

Study	Glioma Type	Outcome (TMZ vs. TMZ+MET)
Adeberg 2015	Glioblastoma (n = 276)	MET use was not associated with improvement in OS ($P = 0.326$).
Fuentes-Fayos 2023	Glioblastoma (n = 85)	The use of MET was not associated with significant differences in OS compared to not using MET (13 vs. 12.2 months; $P = 0.30$).
Henderson 2015	Glioblastoma (n = 226)	The use of MET was associated with increased OS relative to patients who were not treated with MET (19.4 vs. 15.1 months, the P value was not reported). The use of MET was also associated with better OS outcomes in nondiabetic patients with MGMT+ (19.5 vs. 14.1 months; $P = 0.01$).
Mohammad 2023	Glioblastoma (n = 288)	MET use was not associated with a statistically significant improvement in OS patients with MGMT+ (22.0 vs. 14.7 months; $P = 0.13$).
Porper 2021	Glioblastoma (n = 10) Anaplastic astrocytoma (n = 3)	MET was associated with a decrease in OS relative to patients without MET, but without statistical proof (7.9 vs. 11.4 months)
Razak 2019	Glioblastoma (n = 122)	The use of MET was associated with increased OS relative to patients who were treated with TMZ only in relation to months of survival, but without statistical significance (21.1 vs. 11.9 months; $P = 0.12$).
Seliger 2019	Glioblastoma (n = 862) High grade glioma WHO III (n = 231)	The addition of MET was associated with significantly better OS compared to patients without MET (HR = 0.62)
Seliger 2020	Newly diagnosed glioblastoma (n=1731)	MET treatment was associated with a decrease in OS relative to patients without MET (14.5 vs. 18.5 months, HR = 0.90).
Welch 2013	Glioblastoma (n = 123)	The use of MET was not associated with significantly better OS compared to patients without MET (10 vs. 6 months, $P = 0.09$).
Yoon 2023	Recurrent or refractory glioblastoma (n = 81)	The use of MET was associated with increased OS relative to patients who were not treated with MET in relation to months of survival, but without statistical significance for the treatment of recurrent GBM (17.2 vs. 7.7 months; $P = 0.47$).

The types of high-grade glioma from each study were analyzed in addition to the available outcomes. Each study reported on a specific outcome, with only OS time being a common data among them.
MET, metformin; OS, overall survival; GBM, glioblastoma; HR, hazard ratio; WHO, World Health Organization; MGMT, O6-methylguanine-DNA methyltransferase; TMZ, temozolomide.

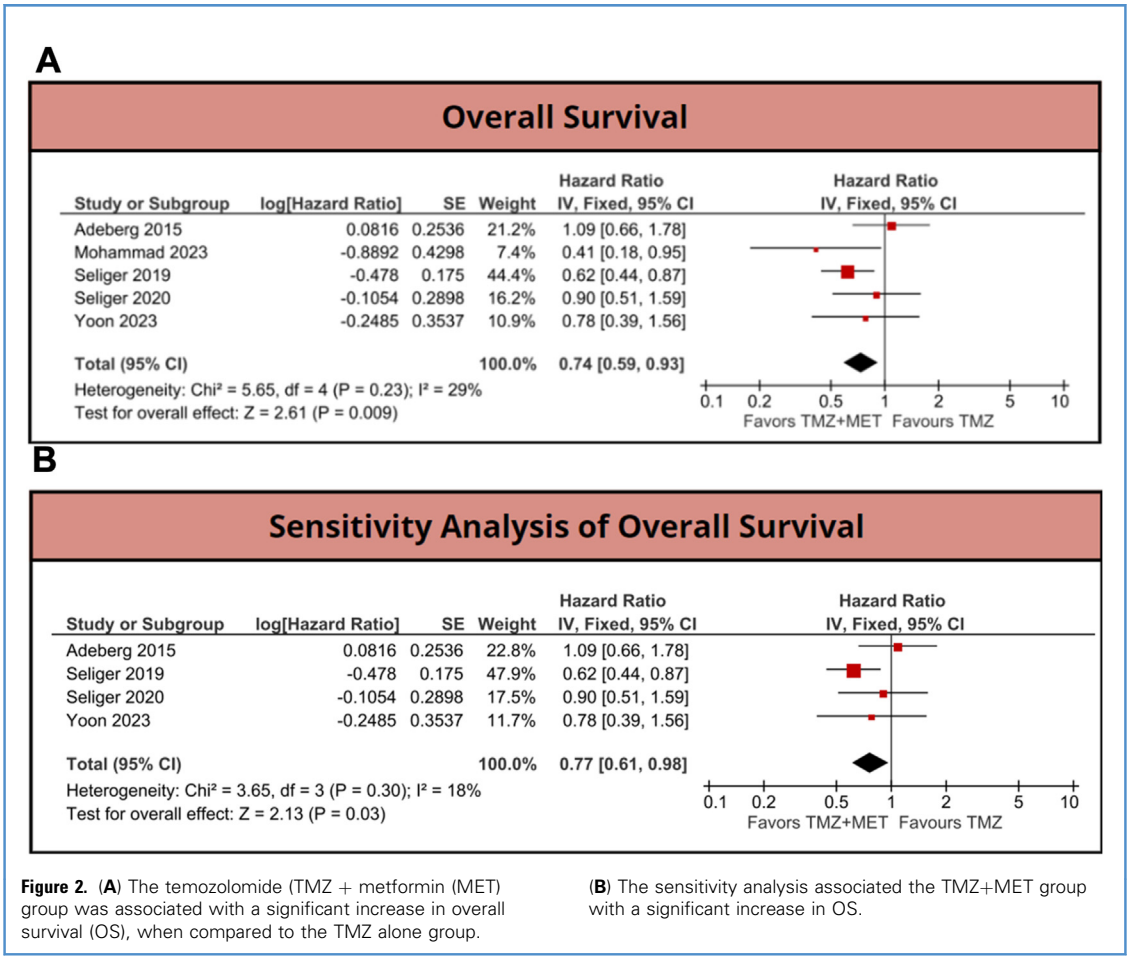


Figure 2. (**A**) The temozolomide (TMZ + metformin (MET) group was associated with a significant increase in overall survival (OS), when compared to the TMZ alone group. (**B**) The sensitivity analysis associated the TMZ+MET group with a significant increase in OS.

and intervention groups for OS outcome. Two studies only reported qualitative analysis, without measuring statistical significance.

Meta-Analysis

The meta-analysis included five out of the ten studies. The outcomes analyzed were OS and PFS for patients who received TMZ+MET treatment versus TMZ alone. Our analysis also explored subgroups, including patients with and without T2DM, as well as those with and without MGMT+ genetic markers.

OS was significantly improved in patients treated with TMZ+MET relative to TMZ alone (HR 0.74; 95% CI 0.59, 0.93; $P < 0.01$; $I^2 = 29\%$; Figure 2A). MET, commonly prescribed for the treatment of T2DM, has shown potential therapeutic benefits for cancer. Currently, HGG has a low survival rate even following the gold standard treatment. The result of the analysis demonstrated an increase in OS for patients in the TMZ+MET group, which suggests that the addition of MET to TMZ treatment may be an effective strategy to improve OS in patients with HGG.

A sensitivity analysis was conducted to ensure the reliability of the OS analysis. In this context, the study of Mohammad et al. was excluded due to a scarcity of data, as it was published only as an abstract.[20] After excluding this study, the P value remains significant and favorable for the TMZ+MET combination. The heterogeneity analysis I^2 was reduced from 29% to 18% (HR 0.77; 95% CI 0.61, 0.98; $P = 0.003$; $I^2 = 18\%$; Figure 2B).

The comparative study between patients with HGG treated with TMZ alone and those who received the combination of TMZ+MET revealed different results. While OS showed a significant improvement in those treated with the TMZ+MET combination compared to the TMZ-alone group, PFS showed no statistically significant differences between the groups (HR 0.87; 95% CI 0.68, 1.12; $P = 0.29$; $I^2 = 0\%$; Figure 3). These results

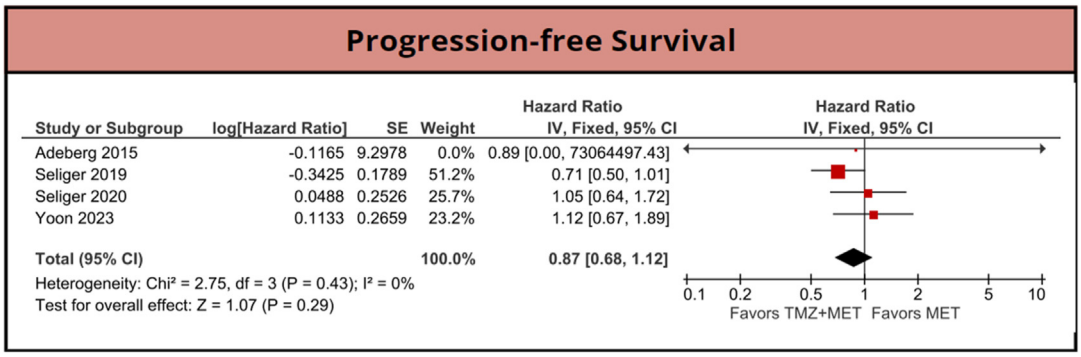


Figure 3. Progression-free survival did not show significantly different results between patients with high-grade gliomas treated with temozolomide (TMZ) and metformin (MET) as compared with TMZ alone.

indicate that although the TMZ+MET combination may improve overall patient survival, it does not appear to have as substantial an impact on disease progression as measured by PFS. This discrepancy highlights the importance of evaluating multiple outcomes in clinical trials to obtain a comprehensive understanding of the effect of a given therapeutic intervention in patients with HGGs.

Subgroup analysis for OS, including only patients with T2DM at baseline compared with those without this diagnosis, revealed distinct results. OS was significantly increased in patients without a diagnosis of T2DM at baseline compared to those who had T2DM (HR 1.25; 95% CI 1.10, 1.41; $P < 0.01$; $I^2 = 79\%$; Figure 4). These findings suggest that not all antidiabetics may be associated with a positive impact on the survival of patients with HGG. The analysis highlights the uniqueness of MET for the treatment of gliomas. The high heterogeneity ($I^2 = 79\%$) observed may result from differences in study design, variations in sample size, and differences in patient baseline characteristics. The studies analyzed employed diverse methodologies, including retrospective data collection and prospectively collected clinical trial data, leading to variations in treatment administration, follow-up durations, and data completeness. Additionally, differences in sample sizes across studies contributed to statistical variability, as some studies had only a few hundred patients while others included over a thousand, affecting effect size estimates and statistical power. Standardizing patient selection criteria, harmonizing data collection methods, and performing stratified analyses based on prognostic factors in future research may help clarify these sources of heterogeneity.

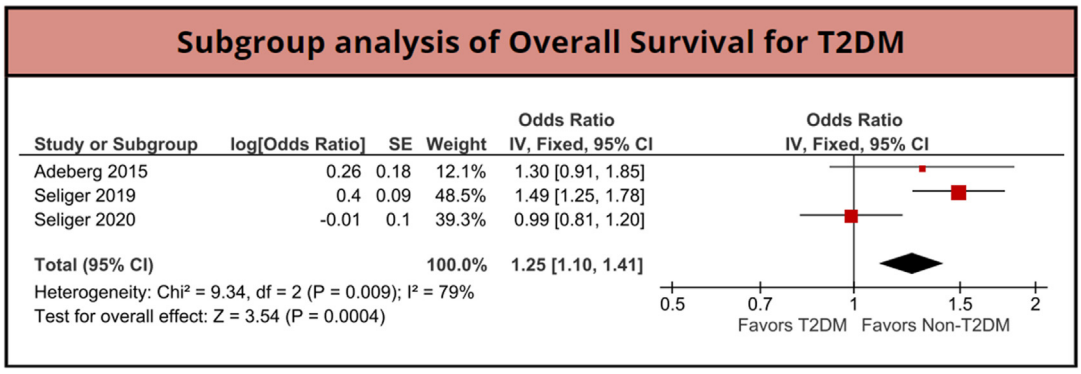


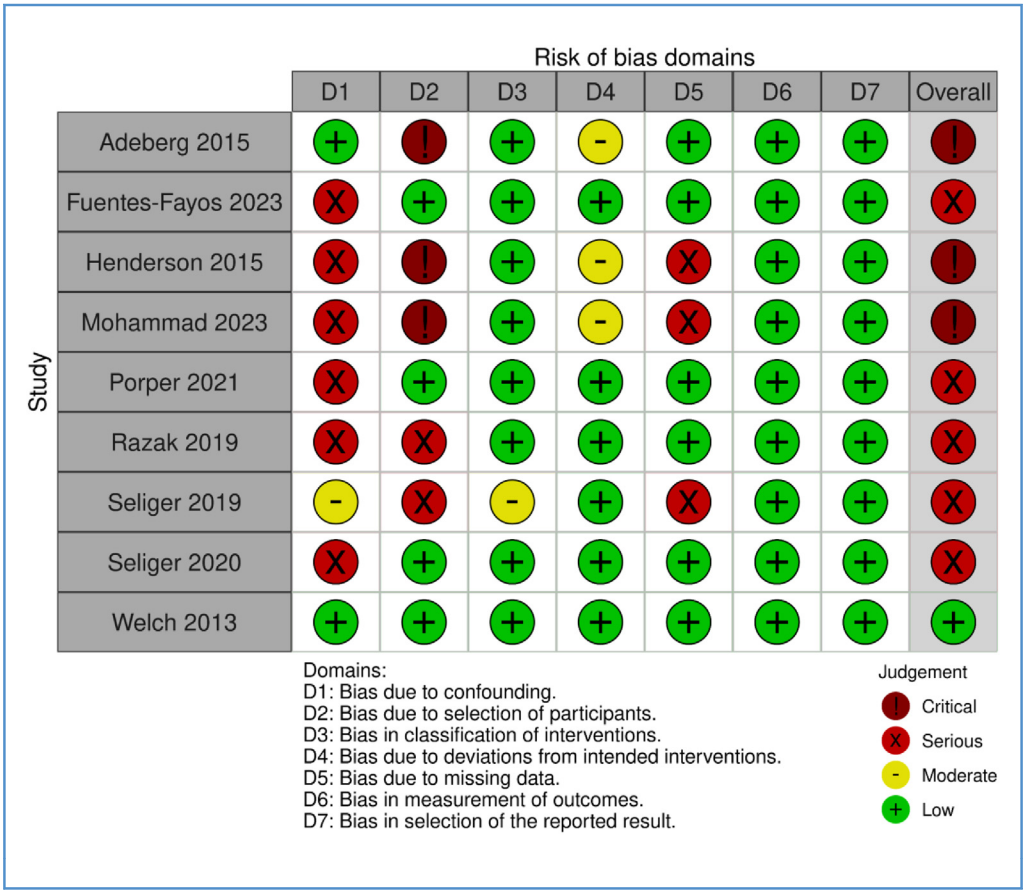
Figure 4. Overall survival (OS) was significantly superior in patients without T2DM prior to initiating combined chemotherapy.

Subgroup analysis of patients with a positive versus negative MGMT+ genetic marker revealed a significant difference in OS between patients treated with MET compared with those who did not receive this treatment, particularly among individuals with the genetic marker positive. Patients with positive MGMT+ who received MET showed a significant increase in OS compared to those who did not receive treatment (HR 0.44; 95% CI 0.37, 0.51; $P < 0.01$; $I^2 = 74\%$, **Figure 5**). These results indicate that the addition of MET to the therapeutic regimen may have a particularly positive impact on the survival of patients with HGGs expressing the MGMT+ genetic marker. This finding is clinically relevant as it suggests that MET may play a crucial role in improving outcomes in a specific subpopulation of glioma patients, highlighting the importance of personalizing treatment based on specific genetic markers. However, the high heterogeneity observed ($I^2 = 74\%$) suggests the need for further investigation to fully understand the underlying mechanisms and confirm the robustness of these results.

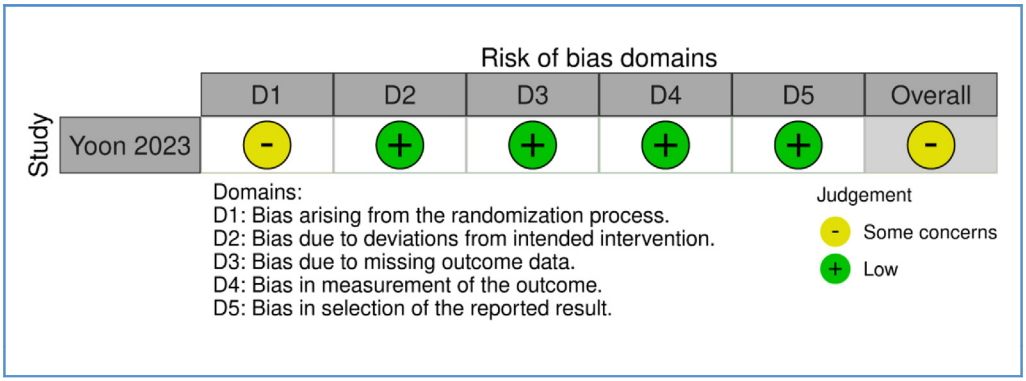
Quality Assessment

When we evaluated the risk of bias for nonrandomized studies using the ROBINS-I tool, two studies were classified as critical, six as serious, and 1 as low risk of bias overall. Thus, the analysis of the results revealed a diversity of levels of bias in the examined studies. The inclusion of the "Critical" and "Serious" categories demonstrates a potentially significant influence of biases on the observed results. These results may be due to factors such as nonrandom selection of treatment groups, confounding of uncontrolled variables, and methodological approaches susceptible to systematic bias. In the only randomized study, the RoB-II tool was applied, obtaining an overall result of "some concerns" for risk of bias. These results show an important limitation for our analysis due to the overall risk of bias of the included studies.

Risk of bias summary for non-randomized studies (ROBINS-I)[16]



Risk of bias summary for randomized studies (RoB-II)[27]



DISCUSSION

In this systematic review and meta-analysis, a total of ten studies were analyzed to assess the advantages of combining MET with the standard treatment of TMZ versus the TMZ-only group. The key findings of our study were: 1) patients who received the TMZ+MET treatment presented a statistically significant increase for OS, when compared to those who received TMZ only, 2) the sensitivity analysis, after removing an abstract, continued to demonstrate the relevance of the better results for OS for the TMZ+MET group compared to the TMZ-only group, 3) there was no significant difference for PFS rates between the TMZ+MET versus the TMZ alone group, 4) OS was significantly superior in patients without T2DM prior to initiating combined chemotherapy, and 5) in patients with the positive MGMT marker (associated with better prognosis), OS was significantly improved with TMZ+MET treatment compared to those who did not have the marker. These findings serve to further support the evidence of the beneficial effects of MET addition in the TMZ treatment of HGG.

A common T2DM medication, MET, regulates glucose levels in the brain by regulating insulin levels. Since glucose serves as the primary energy source for the central nervous system, the influence of MET on glucose modulation is highly significant. This regulation is closely connected to the interplay between two important signaling pathways within cells: the AMP-activated protein kinase (AMPK) pathway and the mTOR pathway. These pathways play a crucial role in controlling cell growth and metabolism.[28] Understanding the interplay between MET, glucose levels, and the AMPK/mTOR pathways provides valuable insights into the mechanisms underlying cell growth regulation.[29] The AMPK regulates cellular metabolism by conserving adenosine triphosphate (ATP) through the inhibition of various anabolic pathways, including the mTOR pathway, which controls cell growth.[30] This mechanism is closely associated with MET since it inhibits mitochondrial complex I by reducing glucose levels, thereby preventing mitochondrial ATP production and increasing the ratios of adenosine diphosphate : ATP and AMP : ATP. The mTOR pathway, known to regulate protein synthesis, cell survival, lipid synthesis,

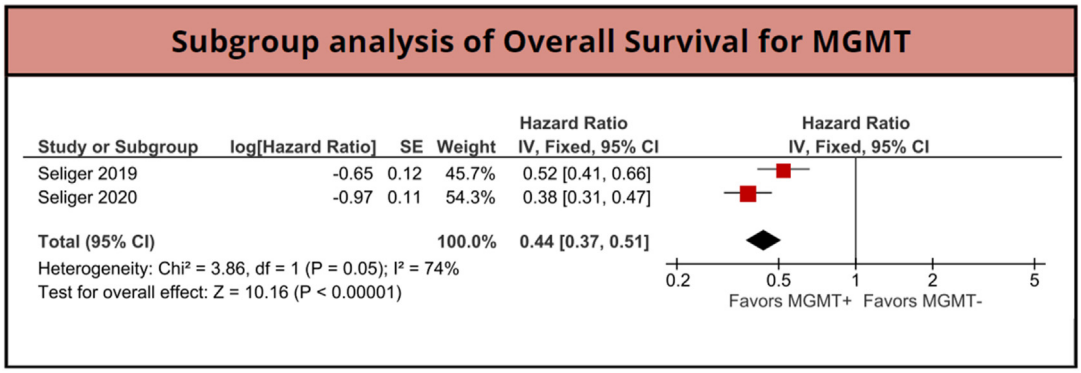


Figure 5. Patients with positive O6-methylguanine-DNA methyltransferase (MGMT) mutation presented a statistically significant increase in overall survival (OS), compared to those MGMT negative.

motility, autophagy, and transcription, influences cell growth and proliferation. Its inactivation is closely linked to the induction of apoptosis in tumor cells.[31,32]

Beyond its role in metabolic regulation, MET exhibits multiple antitumor effects by targeting tumor metabolism, hypoxia, and angiogenesis, which are crucial factors in glioma progression. By modifying cancer cell energy dynamics, MET decreases oxygen consumption and alters mitochondrial function, ultimately limiting the energy supply available for tumor growth.[33,34] This metabolic shift may not only affect cancer cell proliferation but also could influence the tumor microenvironment. In addition to its metabolic effects, MET impacts tumor hypoxia, an important factor of GBM aggressiveness and therapy resistance. By enhancing blood perfusion and reducing oxygen demand, MET alleviates tumor hypoxia, leading to downregulation of HIF-1α, a transcription factor known to promote tumor survival and angiogenesis.[33] Moreover, MET has been shown to partially reverse hypoxia-induced gene signatures in GBM cells, which are associated with poor prognosis.[35] MET also plays a pivotal role in angiogenesis, a process essential for tumor growth and survival. By suppressing angiogenesis-related factors, MET helps inhibit excessive tumor vascularization, ultimately limiting the oxygen and nutrient supply necessary for tumor expansion.[33] Additionally, MET has been found to preserve blood-brain barrier integrity, maintaining endothelial tight junctions and reducing vascular permeability, which may contribute to the mitigation of cerebral edema in glioma patients.[36]

Although this meta-analysis showed the effectiveness of MET for the treatment of HGG, this drug can influence high-grade tumors in different ways depending on their molecular subtype. GBM is the most prevalent form of HGG among the population worldwide.[37,38] Unfortunately, GBM also carries the poorest prognosis within the glioma group, with an average survival ranging from 12 to 24 months.[39] Despite the overall challenging outlook, GBM exhibits specific molecular markers that can significantly impact disease progression and the efficacy of chemotherapy drugs. According to the World Health Organization's (WHO) new classification of central nervous systemtumors, only the most aggressive form of GBM is classified as glioma grade IV, referring to diffuse adult wild-type IDH astrocytoma, where the promoter methylation status of MGMT holds prognostic value.[40]

Patients with MGMT promoter methylation exhibit increased sensitivity to combination therapy with TMZ and MET due to specific biological mechanisms. MGMT promoter methylation silences the gene, reducing DNA repair capacity and increasing tumor sensitivity to alkylating agents.[41] As a result, patients with methylated MGMT promoters show improved survival when treated with alkylating agents like TMZ, while those without methylation benefit less.[41,42] Additionally, the antidiabetic drug MET has been shown to enhance radiosensitivity in GBM cells, particularly in MGMT nonmethylated tumors, by inducing G2/M cell cycle arrest and elevating AMPK levels.[43] This dual role of MET—enhancing the effects of TMZ in MGMT-methylated tumors while also increasing radiosensitivity in MGMT nonmethylated tumors—suggests its potential as a valuable adjunct therapy. Understanding these mechanisms reinforces the importance of personalized therapeutic approaches based on MGMT status to optimize treatment outcomes for glioma patients.

Genetic biomarkers like IDH mutations play a crucial role in shaping the response to MET treatment, particularly in oncology. IDH mutations alter cellular metabolism, leading to the accumulation of oncometabolites that can enhance MET's antitumor effects by disrupting energy homeostasis.[44] The molecular marker IDH1 can be classified as mutant (milder form) or wild-type (more aggressive form).[45] The IDH mutation is associated with various additional molecular alterations in HGG, including loss of the short arm of chromosome 1 and loss of the long arm of chromosome 19q. This genetic modification causes a functional alteration in the enzyme, allowing it to convert isocitrate to D-2-hydroxyglutarate instead of following the normal tricarboxylic acid metabolism. This abnormal conversion results in an excessive accumulation of 2-HG in glioma cells. During the conversion process from isocitrate to D-2-hydroxyglutarate, the IDH mutation also causes a reduction in nicotinamide adenine dinucleotide phosphate (NADP+) to NADPH, and NADPH plays a crucial role in cellular protection against oxidative stress by participating in glutathione regeneration, a cellular antioxidant.[46-49] Mutations in the IDH gene result in the accumulation of D-2HG, an oncogenic metabolite that can affect cell differentiation, reflecting tumor characteristics such as cellularity, growth pattern, and vascularization.[50-52] A study conducted by Cuyàs et al. demonstrated in cell lines that the "metabolic phenotype" of IDH1 mutant cells reveals unexpected metabolic vulnerabilities that can be exploited as therapeutic targets.[44] This happens because the R132H mutation causes significant changes in the bioenergetic and biosynthetic demands of IDH1 mutant cells, making them highly sensitive to the antitumor effects of MET. These findings suggest that MET selectively reduces growth, survival, and self-renewal in cells with this mutation. Studies conducted on patients have supported the hypothesis that the mutant IDH subtype favors treatment with MET compared to patients with wild-type IDH.[24,53]

Another important molecular biomarker that influences the prognosis of GBM is MGMT.[54] The function of MGMT is to repair DNA damage caused by alkylation. MGMT transfers the methyl group from the alkylated site to a different molecule, thereby restoring the original DNA structure. This mechanism is intrinsically opposed to the action of TMZ as chemotherapy, as it is an alkylating agent.[55] Therefore, high expression of MGMT in tumor cells can confer resistance to alkylating therapy, such as TMZ. This is because MGMT is capable of removing the methyl groups added to DNA by the chemotherapy agent, thereby preventing its effectiveness in cancer treatment.[56] Weller et al. demonstrated the effects of MGMT through a study involving 199 patients, where patients with MGMT promoter methylation showed longer OS and median PFS in patients who received TMZ treatment.[57] Furthermore, the research group led by Shao-Wei Feng, through a study using cell lines, demonstrated that MET can help overcome resistance to TMZ in GBM treatment by targeting the induction of MGMT protein expression. According to the study, MET alone or in combination with TMZ can significantly suppress the induction of MGMT protein

expression in a dose-dependent manner, thereby enhancing the effectiveness of TMZ in combating tumor cells.[58] Following, in the context of preclinical studies, a recent meta-analytic study evaluating OS in GBM murine models treated with a combination of TMZ + biguanide compared to those treated with TMZ alone found a significant improvement in OS in the TMZ + biguanide group. Additionally, a significant improvement in OS was reported in a subanalysis specific to TMZ+MET.[59]

Further research is needed to determine the optimal dosage of both MET and TMZ for combination treatment. In a study conducted by Lee et al. and Yu et al., using both in vivo and in vitro models, it was demonstrated that high doses of MET (400 mg/kg/day) in conjunction with reduced doses of TMZ (25 mg/kg/day) significantly reduced tumor growth rates and prolonged OS.[14,53] However, the study emphasized that MET doses exceeding 2000 mg per day may result in unacceptable toxic effects. Furthermore, a randomized study conducted by Yon et al. examined patients with recurrent or refractory GBM who initially received a MET dosage of 1000 mg/day and gradually escalated to a maximum limit of 2550 mg/day while concurrently undergoing TMZ treatment at a dosage of 50 mg/m^2.[26] Although the treatment was well tolerated, it did not show significant benefits for patients with recurrent or refractory glioblastoma. Despite variations in dosage, all three studies confirmed that the combination of MET+TMZ is superior to TMZ monotherapy.

Among the ten studies presented, the majority did not report specific glucose levels, limiting a comprehensive analysis of the role of glucose in glioblastoma outcomes. Specifically, Fuentes-Fayos (2023), Henderson (2015), Mohammad (2023), Porper (2021), and Razak (2019) did not mention glucose levels at all, while Yoon (2023) also lacked relevant data. Among the studies that did provide glucose measurements, Adeberg (2015) classified hyperglycemia into mild (180–299 mg/dl) and excessive (≥300 mg/dl) categories, while Seliger (2018) reported an average serum glucose level of 187.5 (±73) mg/dl. Seliger (2019) mentioned glucose values exceeding 11.1 mmol/L (approximately 200 mg/dl) but did not provide a detailed distribution. Welch (2013) documented a median glucose level of 196 mg/dl, ranging from 98.5 to 321.5 mg/dl. Given prior evidence linking elevated glucose levels to poorer outcomes in glioblastoma, the lack of glucose data in most studies represents a missed opportunity for deeper insights. Future research should prioritize standardized glucose measurements to better understand their impact on glioblastoma prognosis and treatment response.

Heterogeneity was often observed among the studies, which reflected on our risk of bias. Subanalyses on OS upon combination therapy of MGMT+ versus MGMT-patients and diabetic and nondiabetic patients were conducted, yielding substantial heterogeneity, which must be taken into account. Physiologically, it is evident that MET acts differently depending on the molecular subtypes of HGG and is also intrinsically linked to chemotherapy and radiotherapy treatments and their dosages. Our subgroup analysis evidences the probable direct antitumor mechanism of MET. The benefits of increased survival are probably independent of better glycemic control or management of T2DM since patients previously normoglycemic had a better survival performance with combination chemotherapy than diabetic patients. Furthermore, randomized controlled trials should be conducted comparing the effectiveness of joint treatment of TMZ with MET versus TMZ alone to more clearly demonstrate how MET operates in each molecular variation, with a focus on determining which biomarkers truly benefit the most from the utilization of MET, and it is also necessary to define the optimal dosage of MET plus TMZ to ensure an effective treatment in combating these tumors.

Our study has certain limitations. Firstly, the inclusion of nonrandomized studies clearly increases the risk of bias, as the results presented may be influenced by the study's design characteristics. Secondly, the utilization of data extracted from conference abstracts restricts our ability to perform a comprehensive risk of bias, as a few points could not be clearly discussed and were assumed to be the worst-case scenario in our risk of bias analysis. Thirdly, there is a significant imbalance in sample sizes between the MET and non-MET groups in some included studies, which may introduce bias and affect the reliability of the meta-analysis results. Fourthly, more specific investigations are required to examine subgroups, such as comparing patients who were already undergoing treatment with MET versus those who initiated MET treatment after being diagnosed with HGG. Fifthly, it was not possible to explore MET's effects on other molecular markers, such as TERT and ATRX mutations, due to the lack of data on these markers in the included studies. This limits the understanding of MET's impact across different molecular subtypes. Furthermore, additional studies focusing on specific genetic biomarkers such as IDH and MGMT are necessary to establish a more precise understanding of the relationship between these molecular mutations and their impact on MET treatment.

A notable limitation of this meta-analysis is the inclusion of studies using the outdated WHO brain tumor classifications. The new WHO framework integrates key molecular insights for glioma stratification, which these older studies lack. Despite traditional histopathological data's relevance, especially where molecular analysis is inaccessible, the inconsistent tumor classification can introduce outcome variability, complicating therapeutic and prognostic interpretations. Future research should align with the current molecular-based nosology to enhance diagnostic accuracy and treatment approaches for HGGs.

CONCLUSION

Our study concluded that the OS rate of patients who received the MET + TMZ treatment was significantly increased compared to patients who only received the standard treatment with TMZ. These findings suggest that MET should be considered for treatment of HGG along with TMZ.

CRediT AUTHORSHIP CONTRIBUTION STATEMENT

Eloísa Bittencurt Thomaz de Assis: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Marcio Yuri Ferreira:** Writing – review & editing, Writing – original draft, Investigation. **Jéssica Sales de Oliveira:** Writing – review & editing, Writing – original draft,

Investigation. **Lucas Pari Mitre:** Investigation, Formal analysis. **Eduardo Mendes Correa da Silva:** Writing — review & editing, Writing — original draft, Investigation. **Luciano Lobão Salim Coelho:** Writing — original draft, Investigation, Conceptualization. **Daniel Antunes Moreno:** Writing — review & editing. **Allan Dias Polverini:** Writing — review & editing, Writing — original draft, Conceptualization.

REFERENCES

1. Ostrom QT, Gittleman H, Farah P, et al. CBTRUS statistical report: primary brain and central nervous system tumors diagnosed in the United States in 2006-2010. *Neuro Oncol*. 2013;15(Suppl 2): ii1-ii56.
2. Berger K, Turowski B, Felsberg J, et al. Age-stratified clinical performance and survival of patients with IDH-wildtype glioblastoma homogeneously treated by radiotherapy with concomitant and maintenance temozolomide. *J Cancer Res Clin Oncol*. 2021;147:253-262.
3. Gittleman H, Lim D, Kattan MW, et al. An independently validated nomogram for individualized estimation of survival among patients with newly diagnosed glioblastoma: NRG Oncology RTOG 0525 and 0825. *Neuro Oncol*. 2017;19:669-677.
4. Horbinski C, Nabors LB, Portnow J, et al. NCCN Guidelines® insights: central nervous system cancers, version 2.2022: featured updates to the NCCN guidelines. *J Natl Compr Canc Netw*. 2023;21: 12-20.
5. Trifiletti DM, Alonso C, Grover S, Fadul CE, Sheehan JP, Showalter TN. Prognostic implications of extent of resection in glioblastoma: analysis from a large database. *World Neurosurg*. 2017;103:330-340.
6. Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med*. 2005;352:987-996.
7. Krinsley JS. Effect of an intensive glucose management protocol on the mortality of critically ill adult patients. *Mayo Clin Proc*. 2004;79:992-1000.
8. Dowling RJO, Zakikhani M, Fantus IG, Pollak M, Sonenberg N. Metformin inhibits mammalian target of rapamycin–dependent translation initiation in breast cancer cells. *Cancer Res*. 2007;67: 10804-10812.
9. Ben Sahra I, Laurent K, Loubat A, et al. The antidiabetic drug metformin exerts an antitumoral effect in vitro and in vivo through a decrease of cyclin D1 level. *Oncogene*. 2008;27:3576-3586.
10. Vazquez-Martin A, Oliveras-Ferraros C, Menendez JA. The antidiabetic drug metformin suppresses HER2 (erbB-2) oncoprotein overexpression via inhibition of the mTOR effector p70S6K1 in human breast carcinoma cells. *Cell Cycle*. 2009;8:88-96.
11. Kalender A, Selvaraj A, Kim SY, et al. Metformin, independent of AMPK, inhibits mTORC1 in a rag GTPase-dependent manner. *Cell Metab*. 2010;11: 390-401.
12. Rozengurt E. Mechanistic target of rapamycin (mTOR): a point of convergence in the action of insulin/IGF-1 and G protein-coupled receptor agonists in pancreatic cancer cells. *Front Physiol*. 2014;5:357.
13. Lee JE, Lim JH, Hong YK, Yang SH. High-dose metformin plus temozolomide shows increased anti-tumor effects in glioblastoma in vitro and in vivo compared with monotherapy. *Cancer Res Treat*. 2018;50:1331-1342.
14. Higgins JPT, Altman DG, Gøtzsche PC, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *BMJ*. 2011;343:d5928.
15. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *BMJ*. 2009;339:b2535.
16. Sterne JA, Hernán MA, Reeves BC, et al. ROBINS-I: a tool for assessing risk of bias in non-randomised studies of interventions. *The BMJ*. 2016;355:i4919.
17. Adeberg S, Bernhardt D, Ben Harrabi S, et al. Metformin influences progression in diabetic glioblastoma patients. *Strahlenther Onkol*. 2015;191: 928-935.
18. Fuentes-Fayos AC, G-García ME, Pérez-Gómez JM, et al. Metformin and simvastatin exert additive antitumour effects in glioblastoma via senescence-state: clinical and translational evidence. *EBioMedicine*. 2023;90:104484.
19. Henderson D, Chalmers A, White M, Clarke B. PO14FACTORS effecting survival in patients with glioblastoma multiforme. *Neuro Oncol*. 2015; 17(Suppl 8):viii3.
20. Mohammad A, Jatana S, Al-Saadi T, Shenouda G, Diaz R. 867 postoperative metformin is associated with improved survival of glioblastoma patients. *Neurosurgery*. 2023;69(Supplement_1):46.
21. Porper K, Shpatz Y, Plotkin L, et al. A Phase I clinical trial of dose-escalated metabolic therapy combined with concomitant radiation therapy in high-grade glioma. *J Neuro Oncol*. 2021;153: 487-496.
22. Proceedings of the spring 2019 joint meeting of the society of British neurological surgeons and the European association of neuroscience nurses. *Br J Neurosurg*. 2019;33:444-477.
23. Seliger C, Luber C, Gerken M, et al. Use of metformin and survival of patients with high-grade glioma. *Int J Cancer*. 2019;144:273-280.
24. Seliger C, Genbrugge E, Gorlia T, et al. Use of metformin and outcome of patients with newly diagnosed glioblastoma: pooled analysis. *Int J Cancer*. 2020;146:803-809.
25. Welch MR, Grommes C. Retrospective analysis of the effects of steroid therapy and antidiabetic medication on survival in diabetic glioblastoma patients. *CNS Oncol*. 2013;2:237-246.
26. Yoon WS, Chang JH, Kim JH, et al. Efficacy and safety of metformin plus low-dose temozolomide in patients with recurrent or refractory glioblastoma: a randomized, prospective, multicenter, double-blind, controlled, phase 2 trial (KNOG-1501 study). *Discov Oncol*. 2023;14:90.
27. Sterne JAC, Savović J, Page MJ, et al. RoB 2: a revised tool for assessing risk of bias in randomised trials. *BMJ*. 2019;366:l4898.
28. Xiong ZS, Gong SF, Si W, et al. Effect of metformin on cell proliferation, apoptosis, migration and invasion in A172 glioma cells and its mechanisms. *Mol Med Rep*. 2019;20:887-894.
29. Garza-Lombó C, Schroder A, Reyes-Reyes EM, Franco R. mTOR/AMPK signaling in the brain: cell metabolism, proteostasis and survival. *Curr Opin Toxicol*. 2018;8:102-110.
30. Hardie DG, Ross FA, Hawley SA. Ampk - a nutrient and energy sensor that maintains energy homeostasis. *Nat Rev Mol Cell Biol*. 2012;13:251-262.
31. Mazurek M, Litak J, Kamieniak P, et al. Metformin as potential therapy for high-grade glioma. *Cancers*. 2020;12:210.
32. Hay N, Sonenberg N. Upstream and downstream of mTOR. *Genes Dev*. 2004;18:1926-1945.
33. Wang JC, Li GY, Li PP, et al. Suppression of hypoxia-induced excessive angiogenesis by metformin via elevating tumor blood perfusion. *Oncotarget*. 2017;8:73892-73904.
34. Scharping NE, Menk AV, Moreci RS, et al. The tumor microenvironment represses T cell mitochondrial biogenesis to drive intratumoral T cell metabolic insufficiency and dysfunction. *Immunity*. 2016;45:374-388.
35. Calvo Tardón M, Marinari E, Migliorini D, et al. An experimentally defined hypoxia gene signature in glioblastoma and its modulation by metformin. *Biology*. 2020;9:264.
36. Zhao B, Wang X, Zheng J, Wang H, Liu J. Effects of metformin treatment on glioma-induced brain edema. *Am J Transl Res*. 2016;8:3351-3363.
37. Wirsching HG, Galanis E, Weller M. Glioblastoma. *Handb Clin Neurol*. 2016;134:381-397.
38. Wen PY, Kesari S. Malignant gliomas in adults. *N Engl J Med*. 2008;359:492-507.
39. Tran B, Rosenthal MA. Survival comparison between glioblastoma multiforme and other incurable cancers. *J Clin Neurosci Off J Neurosurg Soc Australas*. 2010;17:417-421.

40. Louis DN, Perry A, Wesseling P, et al. The 2021 WHO classification of tumors of the central nervous system: a summary. *Neuro Oncol*. 2021;23:1231-1251.

41. Hegi ME, Liu L, Herman JG, et al. Correlation of O6-methylguanine methyltransferase (MGMT) promoter methylation with clinical outcomes in glioblastoma and clinical strategies to modulate MGMT activity. *J Clin Oncol Off J Am Soc Clin Oncol*. 2008;26:4189-4199.

42. Esteller M, Garcia-Foncillas J, Andion E, et al. Inactivation of the DNA-repair gene MGMT and the clinical response of gliomas to alkylating agents. *N Engl J Med*. 2000;343:1350-1354.

43. Adeberg S, Bernhardt D, Harrabi SB, et al. Metformin enhanced in vitro radiosensitivity associates with G2/M cell cycle arrest and elevated adenosine-5'-monophosphate-activated protein kinase levels in glioblastoma. *Radiol Oncol*. 2017;51:431-437.

44. Cuyàs E, Fernández-Arroyo S, Corominas-Faja B, et al. Oncometabolic mutation IDH1 R132H confers a metformin-hypersensitive phenotype. *Oncotarget*. 2015;6:12279-12296.

45. Hartmann C, Hentschel B, Wick W, et al. Patients with IDH1 wild type anaplastic astrocytomas exhibit worse prognosis than IDH1-mutated glioblastomas, and IDH1 mutation status accounts for the unfavorable prognostic effect of higher age: implications for classification of gliomas. *Acta Neuropathol*. 2010;120:707-718.

46. Capper D, Weißert S, Balss J, et al. Characterization of R132H mutation-specific IDH1 antibody binding in brain tumors. *Brain Pathol*. 2009;20:245-254.

47. Lu C, Ward PS, Kapoor GS, et al. IDH mutation impairs histone demethylation and results in a block to cell differentiation. *Nature*. 2012;483:474-478.

48. Dang L, White DW, Gross S, et al. Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. *Nature*. 2009;462:739.

49. Yan H, Parsons DW, Jin G, et al. IDH1 and IDH2 mutations in gliomas. *N Engl J Med*. 2009;360:765-773.

50. Pasquini L, Napolitano A, Tagliente E, et al. Deep learning can differentiate IDH-mutant from IDH-wild GBM. *J Pers Med*. 2021;11:290.

51. Wu F, Chai RC, Wang Z, et al. Molecular classification of IDH-mutant glioblastomas based on gene expression profiles. *Carcinogenesis*. 2019;40:853-860.

52. Melhem JM, Detsky J, Lim-Fat MJ, Perry JR. Updates in IDH-wildtype glioblastoma. *Neurotherapeutics*. 2022;19:1705-1723.

53. Maraka S, Groves MD, Mammoser AG, et al. Phase 1 lead-in to a phase 2 factorial study of temozolomide plus memantine, mefloquine, and metformin as postradiation adjuvant therapy for newly diagnosed glioblastoma. *Cancer*. 2019;125:424-433.

54. Butler M, Pongor L, Su YT, et al. MGMT status as a clinical biomarker in glioblastoma. *Trends Cancer*. 2020;6:380-391.

55. Mansouri A, Hachem LD, Mansouri S, et al. MGMT promoter methylation status testing to guide therapy for glioblastoma: refining the approach based on emerging evidence and current challenges. *Neuro Oncol*. 2019;21:167-178.

56. Weller M, Stupp R, Reifenberger G, et al. MGMT promoter methylation in malignant gliomas: ready for personalized medicine? *Nat Rev Neurol*. 2010;6:39-51.

57. Weller M, Felsberg J, Hartmann C, et al. Molecular predictors of progression-free and overall survival in patients with newly diagnosed glioblastoma: a prospective translational study of the German glioma network. *J Clin Oncol*. 2009;27:5743-5750.

58. Feng SW, Chang PC, Chen HY, Hueng DY, Li YF, Huang SM. Exploring the mechanism of adjuvant treatment of glioblastoma using temozolomide and metformin. *Int J Mol Sci*. 2022;23:8171.

59. Ferreira MY, Thomaz de Assis EB, Batista S, et al. Survival impact of combined biguanide and temozolomide in glioblastoma preclinical models: a systematic review and meta-analysis. *World Neurosurg*. 2024;183:239-245.e2.

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