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Rendon, L.F.; Tewarie, I.A.; Cote, D.J.; Gabriel, A.; Smith, T.R.; Broekman, M.L.D.; Mekary, R.A.

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SYSTEMATIC REVIEW

Statins and Gliomas: A Systematic Review of the Preclinical Studies and Meta-Analysis of the Clinical Literature

Luis F. Rendon[1,2] · Ishaan A. Tewarie[1,3,4] · David J. Cote[1,5] · Aaron Gabriel[6] · Timothy R. Smith[1] · Marike L. D. Broekman[1,3,4] · Rania A. Mekary[1,6]

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Abstract

Background Gliomas represent most common primary brain tumors. Glioblastoma (GBM) is the most common subtype and carries a poor prognosis. There is growing interest in the anti-glioma properties of statins. The aim of this study was to conduct a systematic review of the preclinical literature and to meta-analyze existing clinical studies to determine what benefit, if any, statins may confer in the context of glioma.

Methods The PubMed, Embase, Cochrane, and Web of Science libraries were queried in May 2021. Preclinical studies were included if they investigated the anti-cancer effects of statins in glioma in vitro and in vivo. Clinical studies were included if they reported incidence rates of glioma by statin use, or mortality outcomes among GBM patients by statin use. Pooled point estimates were calculated using a random-effects model.

Results In total, 64 publications, 51 preclinical and 13 clinical, were included. Preclinical studies indicated that statins inhibited glioma cell proliferation, migration, and invasion. These effects were time- and concentration-dependent. Synergistic anti-glioma effects were observed when statins were combined with other anti-cancer therapies. Clinical observational studies showed an inverse, albeit non-statistically significant, association between statin use and incidence rate of glioma (HR = 0.84, 95% CI 0.62–1.13, I^2 = 72%, p-heterogeneity = 0.003, 6 studies). Statin use was not associated with better overall survival following GBM surgery (HR = 1.05, 95% CI 0.85–1.30, I^2 = 30%, *p*-heterogeneity = 0.23, 4 studies).

Conclusion Statins were potent anti-cancer drugs that suppressed glioma growth through various mechanisms in vitro; these effects have translated into the clinical realm, clinically but not statistically, in terms of glioma incidence but not GBM survival.

Key Points

The preclinical studies indicate that statins possess potent anti-cancer effects.

The clinical studies showed a clinically but not statistically significant benefit from regular statin use and glioma incidence but not GBM survival.

1 Introduction

Glioma is the most common primary brain tumor in adults [1]. Glioblastoma (GBM) accounts for 45% of all gliomas and carries a poor prognosis of 15–18 months' survival

✉ Luis F. Rendon
lrendon@bu.edu

1 Department of Neurosurgery, Computational Neurosciences Outcomes Center, Brigham and Women's Hospital, Harvard Medical School, 75 Francis Street, Boston, MA 02115, USA

2 Department of Neurosurgery, Boston University School of Medicine, Boston, MA 02118, USA

3 Department of Neurosurgery, Haaglanden Medical Center, The Hague, The Netherlands

4 Department of Neurosurgery, Leiden Medical Center, Leiden, The Netherlands

5 Department of Neurosurgery, University of Southern California, Los Angeles, CA 90033, USA

6 Department of Pharmaceutical business and administrative sciences, School of Pharmacy, MCPHS University, Boston, MA 02120, USA

[1–3]. Despite efforts to improve therapeutics, the median survival for GBMs has remained unchanged for nearly two decades [3, 4]. Therefore, it is imperative to innovate new treatments that reduce incidence or improve survival. However, developing new chemotherapeutic agents requires demonstrated efficacy and safety in clinical trials, which is a time-intensive and costly endeavor [5]. Drug repurposing, a process by which an already-approved drug is used for an indication other than that initially intended, has gained increasing popularity in oncology [6, 7]. Patients may immediately benefit from approved pharmacotherapies if these treatments are found to reduce incidence or improve morbidity or mortality for their specific disease [7].

Statins block cholesterol synthesis by inhibiting the hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase enzyme. They were initially approved to treat hypercholesteremia and to prevent coronary artery disease [8]. Recently, statins have also been demonstrated to possess anti-inflammatory and anti-cancer properties [9–11]. Investigators are now aiming to validate the use of statins as a treatment in different primary malignancies, with promising results in prostate, kidney, and lung cancer [12–19]. Malignant glioma cells may be especially sensitive to statin treatment considering that the mevalonate cascade, the pathway that results in cholesterol synthesis, is often dysregulated in this cell type [19–21]. This distinct feature of glioma cells has inspired both preclinical and clinical studies to explore the use of statins as anti-glioma agents. Our study aimed to systematically review and meta-analyze the existing literature to determine the association between statin use and glioma incidence and GBM survival.

2 Methods

2.1 Search Strategy

The PubMed, Embase, Cochrane, and Web of Science libraries were searched for relevant studies following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [22]. The query was conducted on May 20, 2021. A professional librarian was consulted to verify that the search syntax contained all synonyms for glioma, glioma cell lines, and statins. The search syntax can be found in the Online Appendix. Studies were eligible only if they were published in English. No restrictions were placed by the date of publication. Titles and abstracts of relevant studies were independently screened by two authors (LFR and AG). Full-text articles were screened by two independent authors (LFR and AG). Any discrepancies were resolved by a third author (IRT).

2.2 Inclusion Criteria and Exclusion Criteria

Preclinical studies were included if they investigated the anti-tumor effects of statins on malignant glioma cells, glial tumors, or glioma animal models. Clinical studies were included if they examined the possible effects statins have on glioma incidence, morbidity, or mortality. Studies focusing on other mevalonate pathway inhibitors, nonglial brain tumors, or nonglial cell lines were excluded. Case reports, studies performed in a pediatric population, and reviews were excluded.

2.3 Data Extraction

The following variables were collected from the preclinical studies: year of publication, type(s) of statin used, the cell line used, use of adjuvant therapy, effect on proliferation, migration, invasion, autophagy, change in tumor size, method of tumor size assessment, location of tumor placement, route of statin administration, overall survival (OS), and associated mechanisms. For clinical studies, the following variables were collected: year of publication, study design, type(s) of statin used, sample size, duration and/or intensity of statin therapy, effect sizes for glioma incidence rate (IR) or OS.

2.4 Data Analysis

Reported hazard ratios (HR) or odds ratios (OR) comparing participants with the longest statin exposure to the statin unexposed groups were extracted. Odds ratios derived from risk set sampling (incidence density sampling) studies were considered unbiased estimators of HRs [23]. Odds ratios derived from prevalent case control studies (cumulative incidence sampling) were considered unbiased estimates of the relative risk (RR) under the rare disease assumption (< 10%) [23]. Moreover, the RR was assumed to approximate the HR when the outcome is rare. Therefore, the final point estimates in the meta-analysis were all converted to HR. Two analyses were then conducted: (1) a meta-analysis for the incidence rate of glioma by statin use, and (2) a meta-analysis for overall survival in GBM by statin use. A random-effects model with a DerSimonian-Laird (DL) estimator was applied in order to obtain the pooled HRs and their estimated 95% confidence intervals (CIs). Forest plots were generated to depict and summarize the results. Heterogeneity was assessed using the I^2 and Cochran's Q statistic (p-value < 0.10). Low heterogeneity was defined as I^2 values between 0 and 25%, moderate heterogeneity between 25% and 50%, and substantial heterogeneity > 50%. Statistical analysis was conducted in R (Version 3.6.3) [24].

A two-tailed p-value < 0.05 was considered statistically significant unless otherwise indicated.

2.5 Risk of Bias Assessment

Study quality was assessed using the Newcastle-Ottawa Quality Assessment Scale for cohort and case control studies [25]. Points were awarded based on the following criteria: selection (4 maximum points), comparability (2 maximum points), and exposure/outcome (3 maximum points) [25]. The scores were evaluated in accordance with the Agency for Healthcare Research and Quality (AHRQ) standards [26]. Studies with scores > 6, 4–6, and <4 were deemed as being good quality, fair, and poor quality, respectively [26]. Assessment of publication bias was not feasible due to the limited number of studies (< 10) per outcome [27].

3 Results

A total of 7079 unique publications were identified (Fig. 1). After screening studies based on title and abstract, 180 studies were selected for full-text screening. Sixty-four studies, 51 preclinical and 13 clinical, met the inclusion criteria (Supplementary Table 1 and Table 3).

3.1 Proliferation

Forty preclinical studies examined the role of statins as monotherapy on the proliferation of glioma, all of which concluded that statins reduce glioma cell proliferation (Table 1) [10, 12, 13, 28, 30–34, 38–41, 43–45, 47–49, 52, 54–59, 61–67, 69–75]. The observed reduction of glioma cell was dose- and time-dependent proliferation [12, 13, 30–34, 38–41, 43–45, 47–49, 52, 54–59, 62, 64–66, 68, 69, 71–73]. The postulated mechanism was a G0/G1 cell cycle arrest due to the upregulation of P21, a cell cycle regulator [6–30, 30–39, 39–44, 44, 45, 45–52, 52–54, 54–65, 68, 73, 74]. The inhibition of proliferative activity was reversed by the administration of mevalonate pathway intermediates, namely mevalonate or geranylgeranyl pyrophosphate (GGPP), indicating that the blockage of HMG-CoA reductase was specifically responsible for these observations [34, 39, 54–56, 58, 63, 66, 72] (Fig. 2).

3.2 Migration and Invasion

The effects of statin monotherapy on migration or invasion were reported in 11 preclinical studies (Table 1) [10, 28, 31, 35, 37, 40, 42, 48, 51, 73, 75]. All studies observed an inhibition of migration [10, 28, 31, 35, 37, 40, 42, 48, 75]. Reduced glioma cell invasion was reported in seven [28, 37, 42, 48, 51, 73, 75] of eight studies [35]. Several mechanisms were postulated as explanations. First, statins disrupt actin bundles and reduce expression of vimentin, leading to a compromise in the integrity of the cytoskeleton [51]. Second, decreases in matrix metalloproteases, specifically MMP-2, MMP-9, and microglial MT1-MMP affected the capacity for cell migration [38, 42]. Finally, reduced transforming growth factor beta (TGF-β) contributed to changes in cellular morphologies that inhibited invasiveness [42, 75]. Similar to the effects on proliferation, these results occurred in a dose- and time-dependent manner and were reversed by GGPP and mevalonate [40, 48, 51, 73, 75].

3.3 Induction of Apoptosis and Autophagy

Statin monotherapy was found to induce apoptosis in 32 preclinical studies (Table 1) [10, 12, 13, 29–32, 34, 36–38, 40, 41, 44–48, 50–53, 62–65, 68, 71, 73–75]. The driving mechanism was activation of caspases 3, 7, 8, and 9 [13, 29–32, 36, 40, 41, 50, 51, 53, 62, 68, 75], inhibition of the anti-apoptotic bcl-2 protein, increased levels of the proapoptotic Bim protein [30, 31, 52], and activation of the c-Jun N-terminal kinases (JNK) [38, 47, 68]. These pathways culminated in detectable morphological changes reflecting cell death [12, 13, 29, 30, 34, 36, 44, 45, 50, 73]. Apoptosis was induced in a dose- and time-dependent manner [12, 13, 29–32, 34, 36–38, 40, 44, 45, 48, 50, 51, 53, 62, 64, 68, 73, 75], and was reversed by administration of mevalonate or GGPP in 12 studies [29, 34, 36, 37, 41, 47, 50, 55, 63, 65, 72, 75]. Additionally, statin monotherapy resulted in an upregulated, but incomplete, autophagic process. Higher levels of P62 and LC3B levels reflected increased autophagosome formation [10, 36, 51, 53, 59, 62, 63, 65, 72, 75]. Notably, fusion of autophagosomes with lysosomes was inhibited, leading to accumulation of partially degraded cargo inside cells [53, 62]. The altered autophagic process combined with apoptosis induction were the two main mechanisms by which statins induced cell death.

3.4 In Vivo Studies

Twelve studies examined the effect of statin monotherapy on overall tumor growth or survival in vivo (Table 2) [64–75]. No tumor models were pretreated with statins prior to implantation. Tumor size was decreased in 75% of studies [64–75]. Notably, the reduction of tumor size was more robust when statins were combined with other pharmacotherapies [64–75]. Five studies reported the use of orthotopic tumor models, with four administering statins orally and one via intraperitoneal injections [67, 70, 71, 74, 75]. Two studies using orthotopic models and oral statin administration found a decrease in tumor size [67, 75]. Two studies reported an increase in mouse survival with statin monotherapy, one in a cortical glioma model

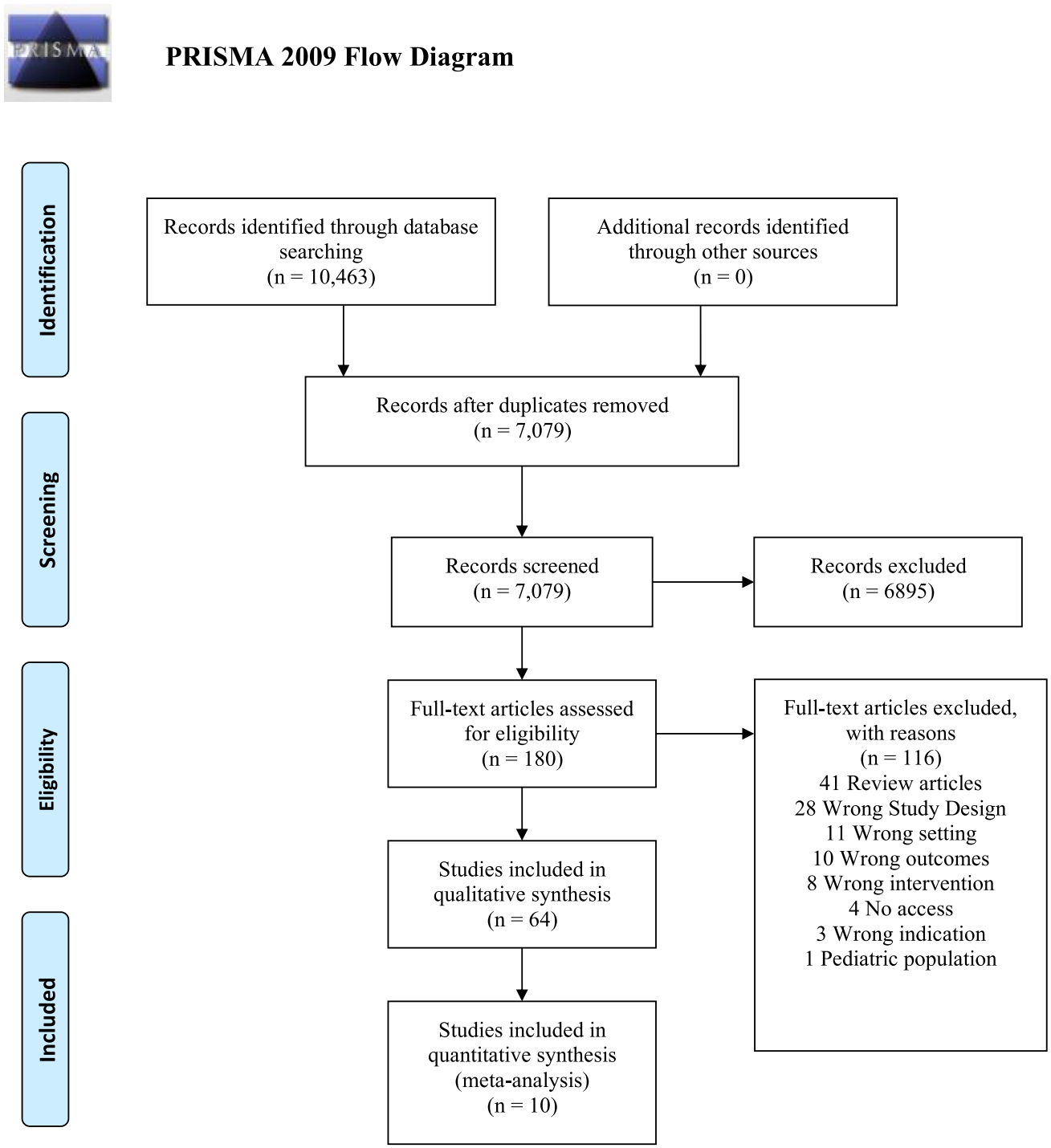

Fig. 1 PRISMA flowchart depicting the study selection process

with intraperitoneal statin injections and one in a subcutaneous glioma model with oral statin treatment [74, 75]. However, two studies, both using cortical glioma models with oral statin administration, failed to detect a difference in survival [70, 71].

3.5 Synergistic Effects

The antitumor effects of statins in combination with other therapies were examined in 29 studies. The other pharmacotherapies included gefitinib, irinotecan, temozolomide

Table 1 Statin monotherapy effects *in vitro*

First author and year	Statin used	Growth conditions	Proliferation	Migration	Invasion	Apoptosis	Autophagy
Soma (1991) [56]	Simvastatin	DMEM, fetal calf serum	↓	x	x	x	x
Soma (1992) [55]	Simvastatin	RPMI 1640, fetal calf serum	↓	x	x	x	x
Jones (1994) [12]	Lovastatin	DMEM, fetal bovine serum	↓	x	x	↑	x
Soma (1994) [39]	Simvastatin	Ham's F10 medium, fetal calf serum, horse serum	↓	x	x	x	x
Soma (1995) [54]	Simvastatin	Ham's F10 medium, fetal calf serum, horse serum	↓	x	x	x	x
Prasanna (1996) [49]	Lovastatin	RPMI 1640, fetal calf serum	↓	x	x	x	x
Kikuchi (1997) [66]	Simvastatin	DMEM, fetal calf serum	↓	x	x	x	x
Choi (1999) [34]	Lovastatin	DMEM, fetal bovine serum	↓	x	x	↑	x
Murakami (2001) [73]	Simvastatin	Agar, fetal calf serum	↓	x	↓	↑	x
Schmidt (2001) [52]	Lovastatin	DMEM, fetal calf serum	↓	x	x	↑	x
Obara (2002) [37]	Cerivastatin	DMEM, fetal bovine serum	x	↓	↓	↑	x
Gliemroth (2003) [35]	Simvastatin	DMEM, fetal bovine serum	↓	↓	–	x	x
Jiang (2004) [47]	Lovastatin	MEM, fetal bovine serum	↓	x	x	↑	↑
Koyuturk (2004) [13]	Simvastatin	DMEM, fetal bovine serum	↓	x	x	↑	x
Rattan (2006) [50]	Lovastatin	DMEM, fetal bovine serum	x	x	x	↑	x
Yao (2006) [61]	Lovastatin	RPMI 1640, fetal bovine serum	↓	x	x	x	x
Cemeus (2008) [43]	Lovastatin	DMEM, fetal bovine serum	↓	x	x	↑	x
Chan (2008) [44]	Lovastatin	DMEM, fetal bovine serum	↓	x	x	↑	x
Gabrys (2008) [64]	Lovastatin	MEM with Earle's Balanced Salts	↓	x	x	↑	x
Bababeygy (2009) [71]	Simvastatin	DMEM, fetal calf serum	↓	x	x	↑	x
Tsuboi (2009) [59]	Pitavastatin	MEM, fetal bovine serum	↓	x	x	–	↑
Wu (2009) [40]	Simvastatin	DMEM, fetal bovine serum	↓	↓	x	↑	x
Tapia-Perez (2011) [58]	Various	MEM with Earle's Balanced Salts, RPMI, fetal calf serum	↓	x	x	x	x
Yanae (2011) [41]	Various	DMEM, fetal calf serum	↓	x	x	↑	x
Misirkic (2012) [36]	Simvastatin	RPMI 1640, fetal bovine serum	x	x	x	↑	↑
Yongjun (2013) [42]	Atorvastatin	DMEM, fetal bovine Serum	x	↓	↓	x	x
Jiang (2014) [65]	Pitavastatin, cerivastatin	Human recombinant EGF, bFGF, heparin	↓	x	x	↑	↑
Jiang (2014) [72]	Various	DMEM, fetal bovine serum	↓	x	x	x	↑
Kula (2014) [67]	Simvastatin	DMEM, fetal calf Serum	↓	x	x	x	x
Slawinska-Brych (2014) [38]	Fluvastatin	DMEM, fetal bovine serum	↓	x	x	↑	x
Afshordel (2015) [28]	Lovastatin	DMEM, fetal calf serum	↓	↓	↓	x	x
Bayat (2016) [31]	Atorvastatin	DMEM, fetal bovine serum	↓	↓	x	↑	x
Fan (2016) [46]	Pitavastatin	DMEM, fetal bovine serum	x	x	x	↑	x
Tapia-Perez (2016) [70]	Atorvastatin, lovastatin	DMEM, fetal calf serum	↓	x	x	x	x
Alizadeh (2017) [29]	Simvastatin	DMEM, fetal bovine serum	x	x	x	↑	x
Chang (2017) [45]	Fluvastatin, lovastatin	DMEM, fetal bovine serum	↓	x	x	↑	x
Liu (2017) [68]	Lovastatin	DMEM, fetal bovine serum	↓	x	x	↑	x
Wang (2017) [74]	Simvastatin	Neurobasal medium, bFGF, EGF	↓	x	x	↑	x

Table 1 (continued)

First author and year	Statin used	Growth conditions	Proliferation	Migration	Invasion	Apoptosis	Autophagy
Bayat (2018) [31]	Atorvastatin	DEMEM-F12, fetal bovine serum	↓	x	x	↑	x
Bayat (2018) [32]	Atorvastatin	DMEM, fetal bovine serum	↓	x	x	↑	x
Beckwitt (2018) [33]	Various	RPMI 1640, GlutaMax supplement, fetal bovine serum	↓	x	x	x	x
Oliveira (2018) [10]	Atorvastatin	DMEM-F12, fetal bovine serum	↓	↓	x	↑	↑
Xiao (2019) [75]	Simvastatin	MEM, fetal bovine serum	↓	↓	↓	↑	↑
Zhu (2019) [62]	Lovastatin	MEM, fetal bovine serum	↓	x	x	↑	↑
Lüetbow (2020) [48]	Atorvastatin	DMEM, fetal bovine serum	↓	↓	↓	↑	x
Samiei (2020) [51]	Simvastatin	DMEM, fetal bovine serum	x	x	↓	↑	↑
Shojaei (2020) [53]	Simvastatin	DMEM, fetal bovine serum	x	x	x	↑	x
Dastghaib (2020) [63]	Simvastatin	DMEM, fetal bovine serum	↓	x	x	↑	↑

DMEM Dulbecco's modified Eagle's medium, *MEM* minimal essential media, *bFGF* basic fibroblast growth factor, *EGF* epidermal growth factor

x = study did not investigate effect, ↓ = dcreased effect with statin therapy, – = no effect observed with statin therapy, ↑ = increased effect with statin therapy

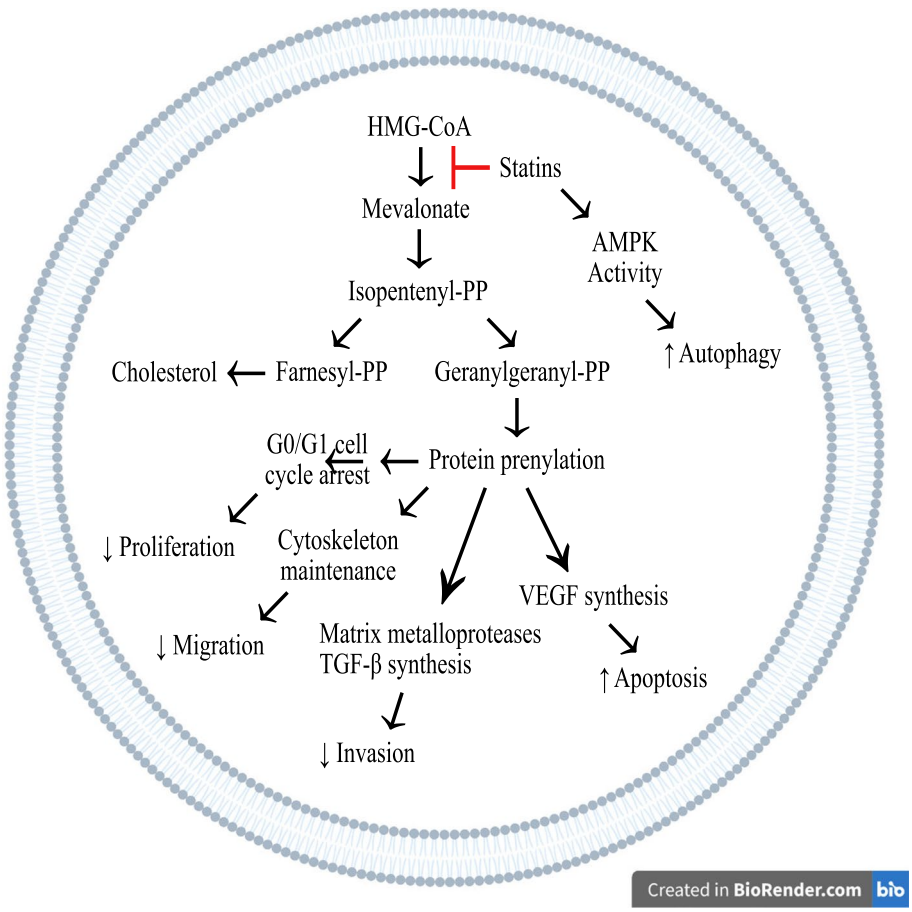

Fig. 2 Diagram summarizing the signaling pathways impacted by statin administration. The ⊥ symbol indicates an inhibitory action

Table 2 Statin monotherapy effects in vivo

First author	Statin used	Tumor placement	Route of statin administration	Method for tumor size assessment	Tumor size	Mouse survival
Kikuchi (1997) [66]	Simvastatin	Subcutaneous	Intratumor injection	Tumor size = (length × width)$^{1/2}$	–	x
Murakami (2001) [73]	Simvastatin	Subcutaneous	Spray, injection	Tumor volume = length × width^2 × 0.5	↓	x
Gabrys (2008) [64]	Lovastatin	Subcutaneous	Intraperitoneal	Tumor volume = length × width^2 × π/6	↓	x
Bababeygy (2009) [71]	Simvastatin	Orthotopic	Oral	Tumor size was determined using Advanced PCI software with sections of brain stained with crystal violet	–	–
Jiang (2014) [65]	Pitavastatin, cerivastatin	Subcutaneous	Intraperitoneal	Tumor volume = length × width^2 × 0.5. Tumors dissected and weighed after sacrifice	–	x
Jiang (2014) [72]	Various	Subcutaneous	Oral, intraperitoneal	Tumor size measured every 2 days, extracted and weighed after sacrifice.	↓	x
Kula (2014) [67]	Simvastatin	Orthotopic	Oral	Tumors dissected and weighed on day 18	↓	x
Tapia-Perez (2016) [70]	Atorvastatin, Lovastatin	Orthotopic	Oral	Tumor volume = length × width^2 × π/6	–	–
Liu (2017) [68]	Lovastatin	Subcutaneous	Intraperitoneal or peritumoral injection	Tumor volume = length × width^2 × 0.5	↓	x
Peng (2017) [69]	Atorvastatin	Subcutaneous	Oral	Tumor volume = length × width^2 × 0.5236	↓	x
Wang (2017) [74]	Simvastatin	Orthotopic	Intraperitoneal	Histologically	↓	↑
Xiao (2019) [75]	Simvastatin	Orthotopic, subcutaneous	Oral	Tumors extracted and weighed after sacrifice	↓	↑

x = study did not investigate effect, ↓ = decreased effect with statin therapy, – = no effect observed with statin therapy, ↑ = increased effect with statin therapy

(TMZ), tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL), and thiazolidinediones, among others [10, 34, 43–70, 72]. Overall, combination therapies were more effective compared to statin monotherapies [10, 34, 43–70, 72]. A notable synergistic effect on cell proliferation, migration, and apoptosis was observed when statins were used in combination with TMZ [10, 51, 53, 60, 62, 63, 69]. Three studies examined statins in combination with radiation, two of which found no added benefit from concurrent treatment [64–72]. The other study reported an increase in radiation-induced cell death and relative survival [59] (see Table 3).

3.6 Meta-Analysis: Incidence Rate of Glioma

Six studies including 6912 cases of glioma and 729,335 controls investigated the association between statin use and the incidence rate of glioma (Table 4) [18, 76–80]. Three studies reported ORs that were approximated to HRs due to risk set sampling methodology [18, 76, 77], one reported an OR, which was approximated to RR under the rare disease

Table 3 Characteristics of the clinical studies

First author (year)	Study design	Statins used	Study population	Study duration (MM/YY)	Statin exposure ascertainment method	Primary outcome(s)	% Male	Average age (years)
Ferris (2012) [80]	Retrospective cumulative case–control	Simvastatin, lovastatin, atorvastatin, rosuvastatin	917	07/2007 – 01/2010	Patient questionnaire	IR	51%	56 (mean)
Gaist (2013) [18]	Retrospective matched case–control (risk-set sampling)	Simvastatin, lovastatin, fluvastatin, atorvastatin, cerivastatin, pravastatin, rosuvastatin	21,136	01/2000 – 12/2009	National registry	IR	62%	< 60: 11,634 > 60: 9502
Gaist (2014) [82]	Retrospective cohort	Atorvastatin, cerivastatin, fluvastatin, lovastatin, pravastatin, simvastatin, rosuvastatin	339	01/2000–12/2009	National Registry	OS	53%	67 (mean)
Mohebbi (2014) [87]	RCT	Atorvastatin	42	03/2011–12/2012	Double-blind administration	Plasma MMP-9	60%	54 (mean)
Bhavsar (2016) [81]	Retrospective cohort	Atorvastatin (43.6%), simvastatin (29.5%), rosuvastatin (14.1%'), pravastatin (10.3%), lovastatin (2.5%)	284	1/2006–7/2015	Electronic Medical Records	OS, PFS	40%	56 (mean)
Chen (2016) [76]	Retrospective matched case–control (risk-set sampling)	Lovastatin, pravastatin, rosuvastatin, fluvastatin, simvastatin, atorvastatin	1,065	01/1996–12/2011	National registry	IR	51%	66 (mean)
Seliger (2016) [77]	Retrospective matched case–control (risk-set sampling)	Simvastatin, atorvastatin, pravastatin, fluvastatin, rosuvastatin	27,159	01/1995–12/2015	National registry	IR	55%	55
Happold (2018) [83]	Retrospective cohort	NA	810	CENTRIC: 10/2008–05/2011 CORE: 02/2009 –8/2013	Electronic Medical Records	OS, PFS	55%	57 (median)
Seliger (2018) [84]	Retrospective cohort	Simvastatin, atorvastatin, cerivastatin, fluvastatin, lovastatin, pravastatin	1093	01/1998–12/2013	Electronic Medical Records	OS, PFS	57%	59 (mean)
Cote (2019) [79]	Prospective cohort[a]	Atorvastatin, lovastatin, pravastatin, rosuvastatin, simvastatin	NHS: 114,419 NHSII: 115,813 HPFS: 50,223	NHS: 01/1994–12/2013 NHSII: 01/1999–12/2013 HPFS: 01/1990–12/2016	Patient questionnaire	IR	NHS: 0% NHSII: 0% HPFS: 100%	NHS: 61 (mean) NHSII: 45 (mean) HPFS: 58 (mean)

Table 3 (continued)

First author (year)	Study design	Statins used	Study population	Study duration (MM/YY)	Statin exposure ascertainment method	Primary outcome(s)	% Male	Average age (years)
Henker (2019) [85]	Retrospective cohort	Simvastatin (85%), pravastatin (10%), atorvastatin (5%)	224	01/2009–12/2016	NA	Clinical and radiological features of epileptogenic tumors	55%	66 (mean)
Altwairgi (2020) [86]	Prospective cohort	Atorvastatin	36	01/2014–01/2017	Prospective administration	OS, PFS	62%	52 (median)
Cote (2021) [78]	Prospective cohort[b]	NA	405,515	01/2006-12/2016	Patient questionnaire	IR	62%	61 (median)

IR incidence rate, *OS* overall survival, *PFS* progression-free survival, *NA* not available

[a]Study combined three previously defined cohorts: Nurses' Health Study, Nurses' Health Study II, Health Professionals Follow-Up Study.

[b]Study used the UK Biobank population-based cohort

assumption [80], and two reported HRs [78, 79]. The RR was approximated to HR under the rare disease assumption as well. Upon contact, the authors of one paper [79] recalculated a multivariable-adjusted HR after exclusion of patients with a history of cancer diagnoses at baseline to be in homogeneity with the original studies included in the meta-analysis and was hence included in our meta-analysis. Pooled results showed that statin users had a non-statistically significant lower incidence rate of glioma (HR = 0.84, 95% CI 0.62–1.13, I^2 = 72%, p-heterogeneity = 0.003, 6 studies) compared to non-users (Fig. 3) [18, 76–80]. A sensitivity analysis where only studies with a duration of statin therapy exceeding ≥10 life-time prescriptions or ≥2 years also revealed a similar non-statistically significant inverse association with glioma incidence rate (HR = 0.76, 95% CI 0.51–1.15, I^2 = 75%, p-heterogeneity = 0.003; 5 studies) [18, 76, 77, 79, 80].

With regard to GBM, one study reported no association between ≥ 2-year statin use and incidence rate of GBM (no point estimate provided) [80] compared to non-users, while another reported an increased incidence rate of GBM in ever statin users (HR = 1.57, 95% CI 1.10–2.07) [79] compared to never users. Three of the four case control studies were scored as being of good quality [18, 76, 77], with the Ferris et al. [80] being assessed as fair quality. Both Cote et al. [78, 79] cohort papers were determined to be of good quality (Supplementary Table 1 and Table 2).

3.7 Meta-analysis: Statins and Survival in GBM

Overall survival with statin therapy in GBM was examined in four studies (Table 5) [81–86]. A total of 539 deaths occurred in 714 patients [81–83]. One study did not provide the number of deaths [84]. Compared to non-users, statin users had a non-statistically significant worse overall survival (HR = 1.05, 95% CI 0.85–1.30, I^2 = 44%, p-heterogeneity = 0.14, four studies, Fig. 4) [81–84]. Low heterogeneity was present. The four studies were determined to be of good quality (Supplementary Table 2) [81–84].

Notably, of the studies that were not included in this meta-analysis, one reported for OS an HR of 1.20 (95% CI 0.70–2.06) in statin users [85]. Those results were limited to patients with small postoperative residual tumor volumes who did not receive adjuvant therapy [85]. Another paper reported no association between statin use and median OS in GBM (19.9 months for incident users vs 19.6 months for nonusers, $p = 0.98$), but used controls that were independent of their study and was, therefore, excluded from the meta-analysis [86]. Two studies additionally found no difference in progression-free survival between experimental and control groups (7.6 months in incident users vs 7.8 months in nonusers, $p = 0.35$, and HR = 1.08, 95% CI 0.83–1.41, $p = 0.75$) [83, 86].

Table 4 List of clinical studies included in the incidence rate (hazard) of glioma by statin use meta-analysis.

First author and year	Study design	Statins used	No. glioma cases	No. controls	Statin duration or intensity in meta-analysis	Effect size (OR/HR) (95% CI)	Variables matched or adjusted for	Conclusions
Ferris (2012) [80]	Retrospective cumulative case–control[a]	Simvastatin, lovastatin, atorvastatin, rosuvastatin	517	400	>120 months	0.44 (0.15–0.97)	Age, gender, ethnicity, educational level, NSAID use, study center	Long-term statin use is inversely associated with glioma
Gaist (2013) [18]	Retrospective matched case–control (risk-set sampling)[b]	Simvastatin, lovastatin, fluvastatin, atorvastatin, cerivastatin, pravastatin, rosuvastatin	2656	18,480	5+ years	0.76 (0.59–0.98)	Birth year, sex, year of index, years of schooling, diabetes, stroke, aspirin, NSAID use	Long-term statin use is inversely associated with risk of glioma
Chen (2016) [76]	Retrospective matched case–control (risk-set sampling)[b]	Lovastatin, pravastatin, rosuvastatin, fluvastatin, simvastatin, atorvastatin	213	852	>11 Prescriptions	0.43 (0.21-0.88)	Age, sex, index date, number of hospitalizations, continuing statin users, diabetes, stroke, NSAID use, use of lipid lowering drugs	Statin use is inversely associated with risk of brain cancer
Seliger (2016) [77]	Retrospective matched case–control (risk-set sampling)[b]	Simvastatin, atorvastatin, pavastatin, fluvastatin, rosuvastatin	2469	24,690	>90 Prescriptions (~10-20 years use)	0.75 (0.48–1.17)	Index date, age, sex, general practice, years in database prior to index date, ethnicity, BMI, smoking, diabetes, congestive heart failure	Statin use is not associated with risk of glioma
Cote (2019) [79]	Prospective cohort	Atorvastatin, lovastatin, pravastatin, rosuvastatin, simvastatin	483	279,972	> 8 years	1.44 (1.03–1.99)[c]	Age, hypertension, diabetes, BMI, smoking status	Long-term statin use may be associated with increased risk of glioma.
Cote (2021) [78]	Prospective cohort	NA	574	404,941	Current use	1.04 (0.81–1.33)	Age, sex, diabetes, hypertension, BMI, smoking status, statin use	No significant association between current statin use and glioma risk

Effect sizes and corresponding 95% confidence Intervals are those reported directly in the study

OR odds ratio, *HR* hazard ratio, *CI* confidence interval, *BMI* body mass index, *NA* not available, *No.* number

[a]In the cumulative case control study design, the odds ratio is a valid estimate of the relative risk when the disease is rare (< 10%)

[b]In the risk set sampling and matched case–control studies, the odds ratio is an unbiased estimate of the hazard ratio

[c]HR has been updated to exclude baseline cancers to improve homogeneity of included studies and, therefore, does not match published HR exactly

3.8 Statins and Morbidity

Data support the notion that statins may provide a morbidity benefit following GBM surgery. A randomized clinical trial (RCT) found that administration of atorvastatin led to improved functioning compared to controls as determined by Karnofsky performance scale (KPS) improvement two months following GBM surgery (11.43 increase in statin users vs 4.00 in the placebo group, $p = 0.03$) [87]. The same paper found that MMP-9 concentrations were not different 24 h postoperatively, although significant reductions in the treated group were detected two weeks after surgery (no point estimate provided, $p = 0.048$) [87]. Lastly, statin intake reduced the occurrence of seizures prior to GBM treatment (OR for non-occurrence = 4.94, 95% CI 1.56–15.7, $p = 0.007$) [86].

4 Discussion

Statins are gaining increasing attention as anti-cancer agents given their newly identified anti-inflammatory and anti-cancer effects. This systematic review and meta-analysis aimed to summarize the current literature regarding the association between statins and pre-clinical and clinical outcomes of interest. The preclinical studies supported the notion that statins inhibited proliferation, migration, and invasion of glioma cells in vitro. Statins also induced apoptosis and autophagy. The clinical studies, on the other hand, did not demonstrate statistically significant associations between long-term statin use and glioma incidence or survival.

The included preclinical studies provide important insights into the specific effects of modulating cholesterol synthesis in glioma. The consequences of inhibiting the HMG-CoA reductase enzyme extend beyond the blockage of cholesterol synthesis. Downstream signaling pathways dependent on precursors from the mevalonate cascade (e.g. GPPP) regulate cell cycle arrest [6–30, 30–39, 39–44, 44, 45, 45–52, 52–54, 54–65, 68, 73, 74], TGF-β activity [42, 75], synthesis of matrix metalloproteases [38, 42], and activation of caspases [13, 29–32, 36, 40, 41, 50, 51, 53, 62, 68, 75]. The reversal of statin's anti-proliferative, anti-migratory, and anti-apoptotic effects by administration of isoprenoids (i.e., GPPP) supports the idea that blockage of HMG-CoA reductase leads to a reduction of isoprenoids. These isoprenoids serve critical functions in the regulation of cell cycle in malignant glioma cells. Although serum-rich growth conditions were used by most studies, three were conducted in the absence of serum, and their results were not different with respect to changes in proliferation and apoptosis [64, 65, 74]. The anticancer effect of statins are therefore not limited to the selection of differentiated glioma cells and are just as efficacious on glioma stem-like cells. Likewise, four studies used tumor cells taken from patient samples instead of established cell lines and those studies observed similar anticancer effects of statins [49, 55, 56, 74].

Through a variety of mechanisms, it appears that statins possess potent anti-cancer properties. The dose- and time-dependent nature of these effects pose an interesting dilemma. For clinically relevant observations, statins must first achieve therapeutic concentrations in the brain microenvironment for prolonged periods. Potency and lipophilicity are, therefore, the two most important characteristics when selecting statins for glioma treatment. According to the DrugBank [88], the statins with the highest capacity to penetrate the blood-brain barrier as per their ADMET scores were simvastatin (ADMET = 0.9422), fluvastatin (ADMET = 0.9382), cerivastatin (ADMET = 0.9381), pitavastatin (ADMET = 0.9296), and lovastatin (ADMET = 0.9287). Importantly, atorvastatin

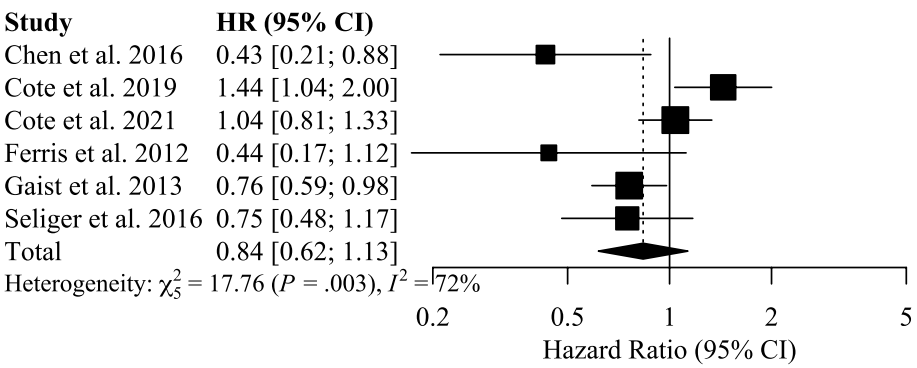

Fig. 3 Forest plot showing the association between statin use and incidence rate of glioma. The solid squares represent the point estimate of each study with the horizontal lines denoting the 95% CI. The size of the square is proportional to the relative weight of each respective study. The center of the diamond is the pooled estimate using a random effects model and its width reflects the 95% CI. *CI* confidence interval, *HR* hazard ratio

Table 5 List of clinical studies included in the overall survival for GBM by statin use meta-analysis.

First author and year	Study design	Statins used	No. deaths	No. patients	Adjuvant therapies	Duration of statin therapy	Effect size (HR) (95% CI)	Variables matched or adjusted for	Conclusions
Gaist (2014) [82]	Retrospective cohort	Atorvastatin, cerivastatin, fluvastatin, lovastatin, pravastatin, simvastatin, rosuvastatin	28	29	Surgery (78.8%) Radiation (76.1%) Chemotherapy (52.2%)	≥ 5 years	0.75 (0.47–1.20)	Year of diagnosis, age at diagnosis, gender, Charlson Comorbidity Index score, diabetes, hospital contact allergy or asthma, years of schooling, anti-asthma drugs, antihistamines, hormone replacement therapy, low dose aspirin use, non-aspirin NSAIDs	Long-term prediagnostic statin use is not associated with improved OS in GBM
Bhavsar (2016) [81]	Retrospective cohort	Atorvastatin (43.6%), simvastatin (29.5%), rosuvastatin (14.1%), pravastatin (10.3%), lovastatin (2.5%)	124	134	Surgery (100%) Chemotherapy + radiation (96.1%)	Preoperative use	1.14 (0.79–1.64)	Age, BMI, sex, American Society of Anesthesiology (ASA) physical status, preoperative dexamethasone use, adjusted Charlson comorbidity index	Preoperative statin use is not associated with improved OS in GBM
Happold (2018) [83]	Retrospective cohort	Atorvastatin, fluvastatin, lovastatin, pitavastatin, pravastatin, rosuvastatin, simvastatin	387	551	Surgery (99.6%)[a] Chemotherapy + radiation (100%)	Ever use	1.31 (1.00–1.70)	Protocol treatment, recursive partitioning analysis, age, extent of surgery, MMSE score, steroid use, MGMT methylation status	Ever use of statin is not associated with improved OS in GBM
Seliger (2018) [84]	Retrospective cohort	Simvastatin, atorvastatin, cerivastatin, fluvastatin, lovastatin, pravastatin	NA	862	Surgery + radiation + chemotherapy (50.8%)	Use at time of GBM diagnosis	0.96 (0.76–1.20)	Age at diagnosis, sex, year of diagnosis, BMI, Karnofsky Performance Score, Extent of resection, MGMT methylation status, IDH mutation status, primary therapy	Statin use at time of diagnosis not associated with improved OS in GBM

Effect sizes and corresponding 95% confidence Intervals are those reported directly in the study

HR hazard ratio, *GBM* glioblastoma Multiforme, *OS* overall survival, *CI* confidence interval, *BMI* body mass index, *MMSE* Mini-Mental State Exam, *NA* not reported, *No.* number

[a]Extent of surgery includes gross total and subtotal resections.

has an ADMET permeability index of 0.7825 [88], while pravastatin is generally considered to have poor permeability through the blood-brain barrier [88, 89]. One study on human subjects found that the lovastatin concentration peaked at 1.3 ng/mL in the cerebrospinal fluid (CSF) 2.5 h after a 40-mg dose of lovastatin [90]. Other experiments found that the maximum statin concentrations in rodent brains 1 h after oral administration was 600 pmol/g brain for simvastatin, 300 pmol/g brain for lovastatin, and 100 pmol/g brain for pravastatin, with large reductions in concentrations observed after 6 and 24 h [91]. The exact mechanism by which these reductions occur has not been described but is likely to be due to metabolism or active transport of statins out of the central nervous system [89, 91]. The concentrations used in the in vitro studies were appreciably higher, as they commonly ranged from 0.1–20 uM. Therefore, permeability across the blood-brain barrier poses a major issue when attempting to translate these promising preclinical findings into clinical practice as currently approved doses of statins may be inadequate for clinically relevant effects.

Although in vivo studies demonstrated benefits from statin therapy, specifically in reducing tumor volume, there was no consensus regarding overall survival benefit in animal model studies [64–75]. The different models (cortical vs subcutaneous) and means of statin administration (oral vs intraperitoneal) used makes any interpretation of the results difficult. Ultimately, the unclear survival benefit highlights the inability of statins to function as a stand-alone therapy in glioma. Multiple preclinical studies reported synergistic anti-cancer effects of statins when combined with other chemotherapies [10, 34, 43–70, 72]. One paper reported that statins sensitize glioma cells to the cytotoxic effects of chemotherapy [63]. Of the approved treatments for GBM, the combination of temozolomide and statins resulted in significant cytotoxic effects [10, 51, 53, 60, 62, 63, 69]. Further in vivo work using cortical glioma models may provide valuable insights into the relationship between chemotherapies, statin administration, and survival.

The meta-analysis demonstrated a non-statistically significant inverse association between statin use and incidence rate of glioma [18, 76–80]. An important source of limitations in the studies included was the lack of distinction between prevalent users, incident users, or mixed users. Restricting the analysis to prevalent statin users might exclude patients who died early (depletion of susceptible) and would artificially inflate the observed point estimate [92], as was reported in three studies [18, 76, 80]. Additionally, one matched case control study included in the meta-analysis failed to adjust for the matching variables, which resulted in selection bias and hence most likely biased the point estimate [76]. Another problem inherent to these observational studies is the possibility of unaccounted time-varying exposure and time-varying confounders [93]. Alterations to individual statin regimens in response to changing HDL, LDL, and total cholesterol levels may mask the true effect size. Moreover, participants who deviate from their assigned "treatment" strategy (statin use vs no use) in the absence of a contraindication should theoretically be censored, although the effect on the overall point estimate may be small if few participants change their treatment regimen significantly. None of the studies accounted for variances in treatment regimen, contributing to heterogeneity in derived HRs [18, 76–80]. Time-varying inverse probability weights may be of use for adjustment after censoring time-varying confounding in future studies [94]. In the absence of RCTs, the HR reported here reflects level 3A evidence as defined as a systematic review of case-control studies [95], thereby supporting the need for further well-designed studies.

This meta-analysis found a worse survival in statin users compared to non-users in GBM [81–84]. However, as in a previous report [96], this result was non-statistically significant. These studies defined statin users as those "prevalent users" who were taking statins prior to their GBM diagnosis [81–84, 92]. This could have led to confounding by indication, as death due to comorbidities in long-term statin users compared to non-users during the study period could skew the calculated HRs in favor of non-users. The lack of information on duration and dosage of statin therapy prior to GBM diagnosis adds heterogeneity to the analysis. Additionally, the lack of standardized treatment regimens contributes to the variance in calculated HRs, as different numbers of patients in each study received surgery, radiotherapy, and/or chemotherapy [81–84, 92]. People on high-intensity statins also suffer from significant other comorbidities, which could have influenced the choice of treatment they received. Only two studies controlled for the Charlson Comorbidity Index score; however, neither controlled for IDH or MGMT status, two critical prognostic factors in GBM [81, 82]. Only one study controlled for both MGMT and IDH status but did not account for comorbid conditions between their study populations [84]. Given the heterogeneity across these studies, the HR presented here reflects level 3A evidence, in terms of hierarchy. A higher level, such as 2A, would be a misclassification as such a determination would suggest homogeneity across the cohort studies included [95]. In order to improve the level of evidence and make actionable recommendations for the use of statins in GBM, future studies must homogenize adjuvant therapies across study populations and control for known prognostic variables in GBM.

The discrepancy between the preclinical studies and the overall survival evidence in GBM patients poses an interesting dilemma. One explanation may be that the overall survival studies did not distinguish results by specific statins. Including statins with poor blood-brain penetration and less

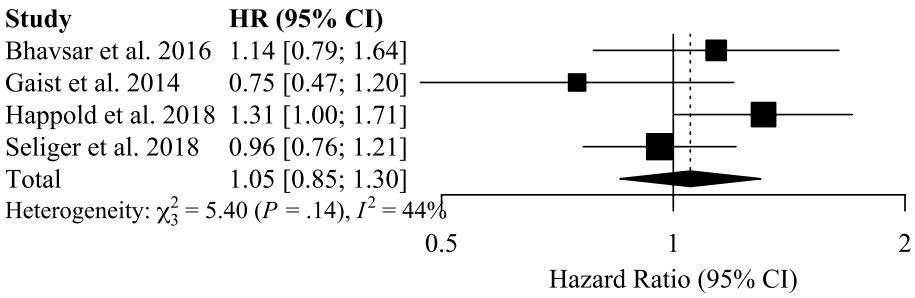

Fig. 4 Forest plot showing the association between statin use and overall survival in GBM. The solid squares represent the point estimate of each study with the horizontal lines denoting the 95% CI. The size of the square is proportional to the relative weight of each respective study. The center of the diamond is the pooled estimate using a random effects model and its width reflects the 95% CI. *CI* confidence interval, *GBM* glioblastoma, *HR* hazard ratio

potent anti-cancer effects could dilute a true survival benefit. As previously mentioned, simvastatin is the most promising candidate given its pharmacodynamic and pharmacokinetic properties, and focusing future clinical studies around this drug, and other lipophilic statin with improved blood-brain barrier penetration, may provide promising results. Another explanation could be attributed to the retrospective nature of the studies, as statin dosages could not be maximized. Subtherapeutic concentrations could also mask any potential clinical benefit. Finally, future studies subgrouping populations based on glioma grade can also provide much needed clarity. Nonetheless, clinical evidence does indicate that statin use may be associated with improved morbidity in GBM. Lower rates of pretreatment seizure, higher KPS scores, and lower MMP-9 plasma concentrations were observed in statin-treated GBM cohorts [86, 87]. Continued assessments of how statins improve quality of life may also help carve out a role for statin therapy in this disease. Consequently, there is a discrepancy between the preclinical and clinical literature. Although in vitro and in vivo preclinical studies overwhelmingly indicate a favorable effect of statin therapy, the results seem to be limited to well-controlled environments and reflect the efficacy of statin, as opposed to the effectiveness of statin, in an uncontrolled environment captured in the observational studies.

There are several limitations our study. First, the preclinical studies reported various magnitudes of anti-cancer effects from statin exposure depending on the cell model used. While we did not examine the specific cell models and instead reported overall results, this may be an important variable to consider when attempting to translate the preclinical effects into the clinical realm. Second, to determine the effect on incidence rate of glioma, we relied on ORs/HRs from the longest statin-exposed groups, which may have increased the heterogeneity of observed effects. Third, clinical heterogeneity was inevitable across the observational studies. For example, statin dosages were not widely reported. Despite these limitations, our study is the first to our knowledge that comprehensively describes the anti-glioma effects of statins in the preclinical and clinical literature and provides valuable insight into the mechanisms that could underlie these interactions.

5 Conclusion

In preclinical studies, statins were shown to exert potent anti-glioma effects through different mechanisms stemming from the inhibition of the mevalonate cascade, resulting in the inhibition of proliferation, migration, invasion, and in the induction of apoptosis and autophagy as the main anti-cancer effects. Synergism was observed between statins and other cancer therapeutics. A meta-analysis of clinical studies showed a non-statistically significant inverse association between statin use and incidence rate of glioma and a non-statistically significant worse overall survival following GBM surgery. These discrepancies support the need for conducting future well-designed comparative studies comprising incident-statin users with standardized treatment dose and duration along with appropriate control for possible time-varying exposure and confounding.

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Declarations

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Ethics approval Not applicable.

Code availability The metafor statistical packages for R are publicly available.

Availability of data and material The datasets generated during the current study are publicly available from the individual studies, and the composite dataset used in this paper is available through the corresponding author on reasonable request.

Consent for publication The authors, jointly and severally, give the publisher the permission to publish the work.

Consent to participate Not applicable.

Authors' contributions LFR: conceptualization, data curation, formal analysis, investigation, methodology, project administration, software, validation, visualization, writing—original draft, writing—review and editing. IAT: conceptualization, methodology, project administration, supervision, writing—original draft, writing—review and editing. DJC: formal analysis, methodology, validation, writing—original draft, writing—review and editing. AG: data curation, formal analysis, investigation, methodology, writing—original draft. TRS: conceptualization, project administration, resources, supervision. MLDB: conceptualization, project administration, resources, supervision, writing – review and editing. RAM: conceptualization, methodology, project administration, resources, supervision, validation, writing—review and editing.

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