REVIEW

Therapeutic strategies of glioblastoma (GBM): The current advances in the molecular targets and bioactive small molecule compounds

Hui Liu[a], Weimin Qiu[a], Tianyu Sun[a], Lei Wang[a], Chenxi Du[a], Yanyu Hu[d], Wenyuan Liu[a,e], Feng Feng[c,d], Yao Chen[b,*], Haopeng Sun[a,c,*]

[a]School of Pharmacy, China Pharmaceutical University, Nanjing 211198, China
[b]School of Pharmacy, Nanjing University of Chinese Medicine, Nanjing 210023, China
[c]Jiangsu Drug Development Engineering Research Center for Central Degenerative Disease, Jiangsu Food and Pharmaceuticals Science College, Huaian 223005, China
[d]Department of Natural Medicinal Chemistry, China Pharmaceutical University, Nanjing 211198, China
[e]Department of Pharmaceutical Analysis, Key Laboratory of Drug Quality Control and Pharmacovigilance, China Pharmaceutical University, Nanjing 211198, China

Abstract   Glioblastoma (GBM) is the most common aggressive malignant tumor in brain neuroepithelial tumors and remains incurable. A variety of treatment options are currently being explored to improve patient survival, including small molecule inhibitors, viral therapies, cancer vaccines, and monoclonal antibodies. Among them, the unique advantages of small molecule inhibitors have made them a focus of attention in the drug discovery of glioblastoma. Currently, the most used chemotherapeutic agents are small molecule inhibitors that target key dysregulated signaling pathways in glioblastoma, including receptor tyrosine kinase, PI3K/AKT/mTOR pathway, DNA damage response, TP53 and cell cycle inhibitors. This review analyzes the therapeutic benefit and clinical development of novel small molecule inhibitors discovered as promising anti-glioblastoma agents by the related targets of these major pathways. Meanwhile, the recent advances in temozolomide resistance and drug combination are also reviewed. In the last part, due to the constant clinical failure of targeted therapies, this paper reviewed the research progress of other therapeutic methods for glioblastoma, to provide patients and readers with a more comprehensive understanding of the treatment landscape of glioblastoma.

*Corresponding authors.
  E-mail addresses: 300630@njucm.edu.cn (Yao Chen), sunhaopeng@163.com (Haopeng Sun).

1. Introduction

Glioblastoma (GBM) is the most frequent tumor with poor prognosis in the central nervous system (CNS)[1]. Normally, the median age of clinically diagnosed GBM is 59 years (42−73 years), and the ratio of male to female patients diagnosed with GBM is 1.6[2]. Standard care for GBM includes safe maximal surgical resection, radiotherapy (RT), and chemotherapy with the alkylating agent temozolomide (TMZ)[3]. Among them, both RT and TMZ activate the death of apoptotic cells by inducing DNA double strand breaks (DSBs) to kill GBM cells. Fig. 1 summarizes the standard of care for patients with newly diagnosed GBM. In carefully selected clinical trials, GBM patients still have a median overall survival (OS) of about 15−18 months and a median time to relapse of approximately 7 months, with a 5-year survival of less than 10% and even shorter survival in elderly patients[4]. Moreover, GBM with IDH-wt usually has a worse prognosis and inevitable

relapse[5]. Consistently, there are scarce treatment options for the inexorable recurrency, showing only modest anti-tumoral activity in second-line chemotherapy[6]. Once GBM recurred, the median OS was estimated 24−44 weeks[7]. Standard of care treatment paradigm for recurrent GBM is shown in Fig. 2. DNA alkylating drugs lomustine (CCNU) and carmustine (BCNU) and the anti-angiogenic agent Avastin (bevacizumab) have been approved by the US Food and Drug Administration (FDA) for the treatment of relapsing GBM, but existing treatment options are limited and treatment results in patients are also unsatisfactory[3]. At the same time, the uncontrolled growth of primary and recurrent tumors is mainly due to redundant activation or dysregulation of a large number of signaling pathways in GBM[8]. In order to better understand the clinical needs of GBM patients and the current status of drug development, we will mainly discuss the therapeutic benefit and clinical development of new small molecule inhibitors discovered as promising anti-GBM agents by the related targets of
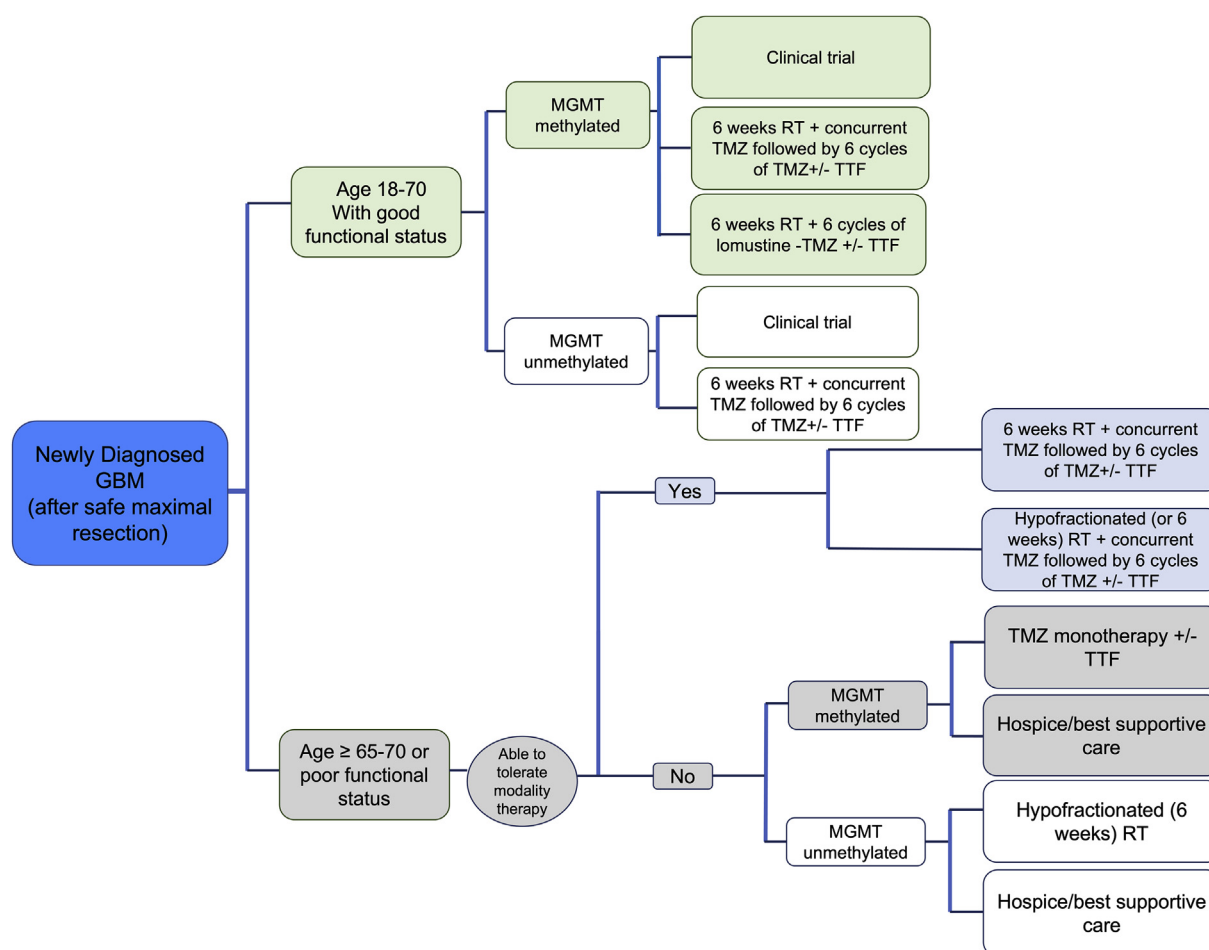
Figure 1   Standard of care for newly diagnosed patients with GBM. In general, 6 weeks of standard RT plus TMZ can be considered for elderly patients with good functional status. Since the MGMT methylation status can predict the efficacy of TMZ, it is considered not to choose TMZ for treatment of patients with non-MGMT methylated tumors, especially when the risks brought by TMZ outweigh the benefits. GBM, glioblastoma; MGMT, O^6-methylguanine-DNA methyltransferase; RT, radiotherapy; TTF, tumor-treating fields; TMZ, temozolomide.

Figure 2 Standard of care therapies for patients with recurrent GBM. Bevacizumab for anti-edema benefit in symptomatic patients may be considered as the common treatment options. Chemotherapy with bevacizumab, including TMZ or nitrosourea (class IIb recommended), may be considered.

these major pathways in details in this review. We will also review the recent advances in TMZ resistance and drug combination. In the final part, the research progress of other therapeutic methods for GBM will be briefly introduced.
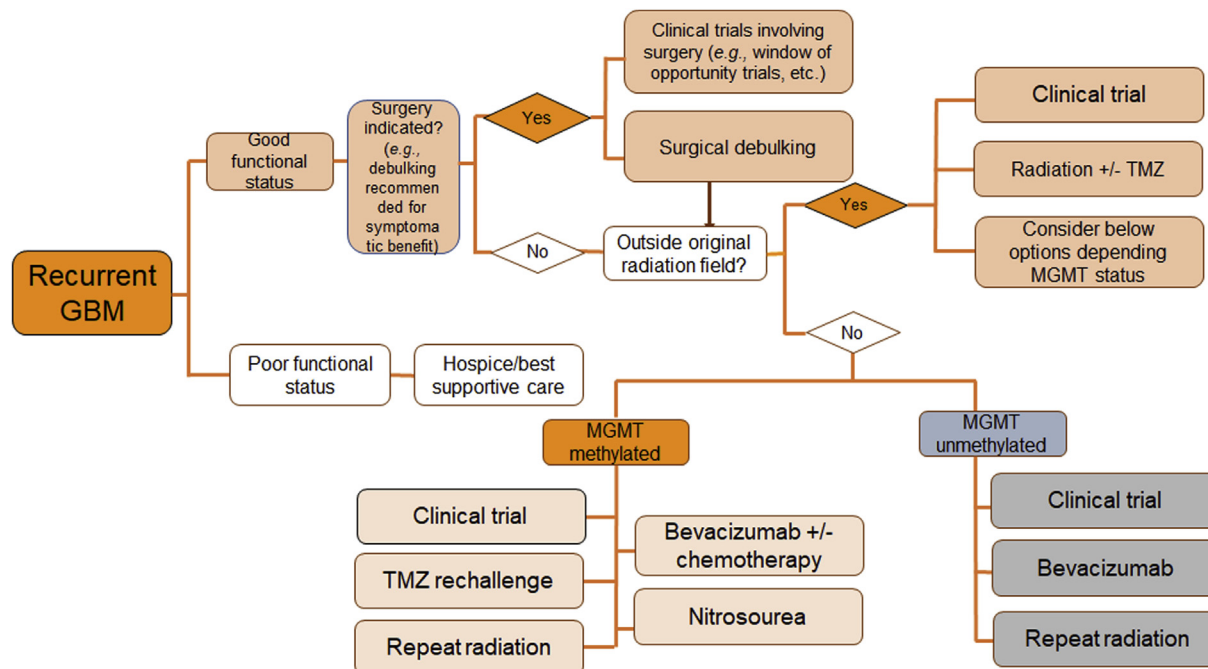
2. Targets of GBM and relevant small-molecule novel therapeutics

There are three main dysregulated pathways in GBM: the receptor tyrosine kinases (RTKs)/Ras/phosphatidylinositol 3-kinse (PI3K) pathway altered in 88% of patients, p53 pathway altered in 87% of patients, and the RB pathway altered in 78% of patients[8]. Fig. 3 shows candidate dysregulated molecular pathways suitable for GBM targeted interventions. In the analysis of more sample data, the most common amplification events on the GBM chromosomes were chromosome 7 (EGFR/MET/CDK6), chromosome 12 (CDK4 and MDM2) and chromosome 4 (PDGFRA)[9]. The candidate amplified gene targets and deleted gene targets in GBM are showed in Table 1. The alternations in these pathways are used to differentiate between molecular and epigenetic subtypes of GBM, which contributed to the development of targeted therapies to influence clinical outcomes and the sensitivity of individual tumors to treatment[10,11].

2.1. Receptor tyrosine kinase (RTK) inhibitors

The RTKs are mutated or amplified in 67.3% of GBM overall: epidermal growth factor receptor (EGFR) (57.4%), PDGFRA (13.1%), mesenchymal−epithelial transition (MET) (1.6%), and fibroblast growth factor receptor 2/3 (FGFR2/3) (3.2%). The alterations of the RTKs affect a wide range of downstream cellular pathways and processes, including apoptosis, growth, survival, and translation[12]. As noted in Fig. 4, a multitude of downstream targets including protein kinase B (AKT), the PI3K, and

mammalian target of rapamycin (mTOR) and their respective pathways are driven by activation of RTKs and G-protein-coupled receptors (GPCRs).

2.1.1. PDGFR and VEGFR inhibitors
A large number of studies have shown that while abnormal angiogenesis supports the growth of GBM stem-like cells (GSCs), these tumor cells may also regulate and promote the tumor vascular system by directly transdifferentiating into endothelial cells or by secreting regulatory growth factors, such as vascular endothelial growth factor (VEGF) and hepatocellular carcinoma derived growth factor (HDGF)[13]. Meanwhile, the platelet-derived growth factor (PDGF) and its receptor (PDGFR) are overexpressed in approximately 16% of GBM[14]. Therefore, concomitant administration of small molecule inhibitors of the VEGF pathway and the PDGF family will likely have a positive impact on chemoradiation treatment outcome[15]. There are several VEGFR/PDGFR inhibitors on the market for cancer treatment. Table 2 shows FDA-approved VEGFR inhibitors for clinical use. Several inhibitors with activity against VEGF and PDGF have been investigated for the treatment of GBM, like sorafenib (**1**, Nexavar, Bayer), and sunitinib (**2**, Sutent, Pfizer). Unfortunately, clinical evaluations have showed that neither **1** nor **2** appeared to provide any significant benefit in GBM patients.

Sorafenib (**1**, Nexavar, Bayer, Fig. 5) is a multi-targeted kinase inhibitor that is active against both types of VEGF (VEGFR-2 and -3) and PDGF (PDGFβ and Kit) families. **1** monotherapy showed a significant survival benefit in preclinical evaluation of U87 cells compared with vehicle treatment ($P < 0.05$)[22]. Regrettably, the clinical **1** treatment provided no survival benefit over historical controls to GBM patients[16]. Vandetanib (**4**, ZD6474, Fig. 5), a small molecule inhibitor of VEGFR, EGFR and RET tyrosine kinases[23], has been approved by FDA for the treatment of advanced medullary thyroid cancer (MTC) in 2011. **4** was well
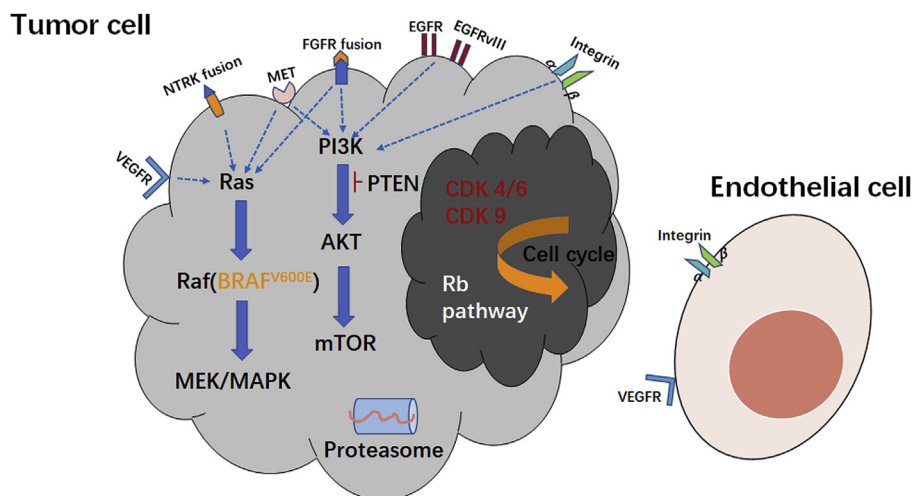
Figure 3   Candidate molecular pathways amenable to targeted interventions in GBM.

tolerated in combination with other conventional chemotherapy agents in clinical trials in patients with GBM. However, results from a phase II study of more than 100 patients with newly diagnosed GBM showed that it was well tolerated but did not significantly extend OS. This led to the premature termination of further treatment studies in GBM[24]. The unsatisfactory therapeutic effect of **4** in GBM may be related to its failure to exert effective effect in brain because of the active efflux at blood–brain barrier (BBB) mediated by P-glycoprotein (P-gp) and breast cancer resistance protein (Bcrp1). Studies have also shown that mTOR inhibitors can increase the effective concentration of **4** in the brain. This could also serve as a future strategy to improve VEGFR inhibitors in GBM[25]. Axitinib (**5**, AG013736, Fig. 5), approved for the treatment of renal cell carcinomas by FDA in 2012, is a second-generation orally effective PDGFR and VEGFR1/2/3 antagonist[26]. In addition to these FDA-approved VEGFR inhibitors, several small molecule compounds with biological activity are also under development.

Table 1   The candidate amplified gene targets and deleted gene targets in GBM. Effective small molecule inhibitors targeting GBM amplification genes are also listed in the table.

Small molecule inhibitor that targets amplified gene	The amplified gene in GBM	The deleted gene in GBM
Abemaciclib, palbociclib, ribociclib	CDK4	CDKN2A/B
Gefitinib, erlotinib, afatinib, lapatinib, osimertinib	EGFR	PTEN
Palbociclib, abemaciclib, ribociclib	CDK6	RB1
Ninedanib	PDGFRA	TP53
AMG232, idasanutlin	MDM2/4	QKI
AZD4547, infrgratinib, erdafitinib	FGFR3/2	CDKN2C
Perifosine, PBI-05204	AKT3/7	LSAMP

Cediranib (**6**, AZD2171, AstraZeneca Pharmaceuticals, UK, Fig. 5) is an oral pan-VEGFR tyrosine kinase inhibitor (TKI) with activity against PDGF receptors and c-Kit, which can normalize tumor vasculature and alleviate edema in GBM to some extent[27]. Unfortunately, the success of **6** has been limited by its low permeability across the BBB. **6** is not clinically feasible due to its short half-life, high toxicity and multiple intracranial procedures required for local administration[28]. Regorafenib (**7**, BAY73-4506, Fig. 5) is an oral multi-kinase inhibitor that inhibits angiogenesis, interstitial, and oncogenic RTKs. The structure of **7** is the result of optimization of **1** structure. A phase II clinical trial (NCT02926222) was completed in GBM multiforme. The results showed a significant improvement in OS after treatment with **7** compared with the lomustine group, with a median OS of 7.4 months[29]. In addition, two clinical trials of **7** are being recruited for the treatment of GBM (NCT04051606, NCT04810182).

Tivozanib (**8**, AV-951, Fig. 5) is a very potent pan-VEGFR inhibitor with nanomolar activity against PDGFR. Clinical trials have shown that **8** can significantly improve the treatment of advanced RCC. However, **8** has shown limited antitumor efficacy in phase II GBM trials, which may indicate that despite GBM tumor vascular abnormalities, anti-VEGFR therapy alone has very limited efficacy[30]. Dovitinib (**9**, TKI-258, Fig. 5) is a multi-target RTK inhibitor that had several clinical trials in patients with brain tumors due to its safety and ability to cross the BBB. **9** showed efficacy in a small subset of patients with recurrent GBM in a phase I trial but showed no therapeutic advantage in patients with recurrent GBM in phase II[31,32]. Nintedanib (**10**, BIBF1120, Fig. 5) is a potent triple angiokinase inhibitor targeting VEGFR, PDGFR, and FGFR. But considering the limited CNS penetration of **10**, the inhibitory effects are difficult to obtain in GBM. Anlotinib (**11**, AL3818, Fig. 5) is an oral, highly selective VEGFR2 inhibitor with similar tolerability to other VEGFR inhibitors in patients. Moreover, **11** has significantly fewer side effects than **2**[33]. In 2018, **11** was approved in China for the treatment of patients with non-small cell lung cancer (NSCLC)[34]. In a case of recurrent GBM, magnetic resonance imaging (MRI) scan showed reduction of tumor size 26 days after **11** treatment. However, the tumor continued to progress 2 months after treatment with **11**[35]. So far, a 50-participant clinical trial of **11** in the treatment of recurrent GBM is being recruited (NCT04004975). Given the limited treatment options available for recurrent GBM, further clinical
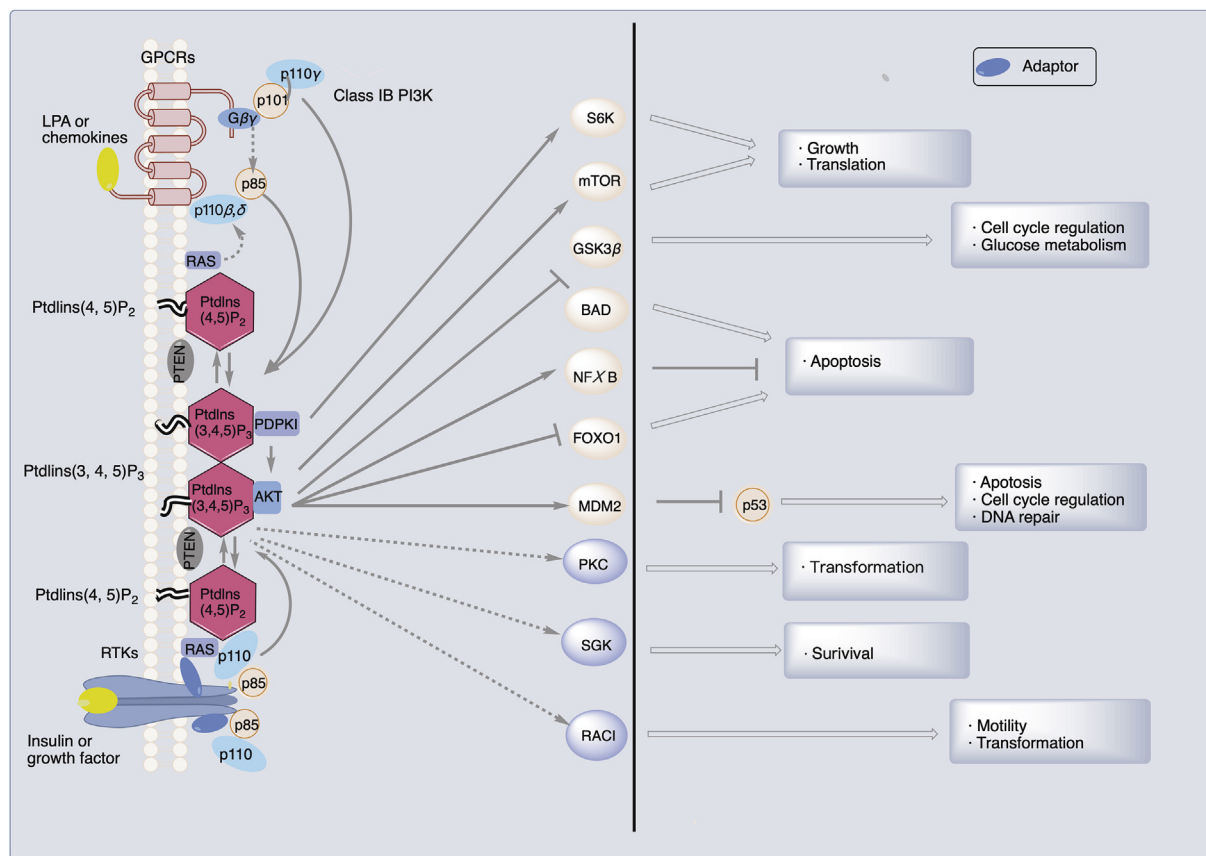
Figure 4 Alterations in RTKs are related to a wide range of downstream cellular pathways and processes.

trials of **11** are very meaningful. Tandutinib (**12**, MLN518, Fig. 5) is a highly active oral PDGFR inhibitor that has been evaluated in several clinical trials for GBM. Phase II results showed that **12** combined with bevacizumab was equally effective but more toxic than bevacizumab monotherapy for patients with recurrent GBM[36]. Another phase II study was discontinued during interim analysis due to lack of efficacy of **12** in patients with recurrent GBM, although GBM with altered PDGF pathway was not extensively studied in this study[37].

Vatalanib (**13**, PTK787, Fig. 5) is a small molecule inhibitor of VEGFR, PDGFR, and c-Kit. To date, three clinical studies using **13** for GBM treatment have been completed, showing that **13** is safe and well tolerated in patients. Cabozantinib (**14**, XL184, BMS-907351, Fig. 5) is a multikinase inhibitor targeting VEGFR-2, MET, and RET with IC_{50} values in the low nanomolar range in enzymatic analysis. It received orphan drug approval from FDA in 2011, which supports its use in the United States as a treatment for a variety of cancers, particularly NSCLC, GBM and MTC[38]. In addition, preclinical studies have shown that **14** can effectively inhibit multiple RTKs in multiple cancer cell lines and animal xenograft models and has significant oral bioavailability and BBB penetration. However, the efficacy of **14** in a phase II clinical trial of GBM was not significant[39]. In addition to these VEGFR/PDGFR inhibitors routinely used in other tumors, a number of small molecules have been developed to treat GBM, which are still at the laboratory level and require additional data to support further research but are worth waiting for. For example, DW10075 (**15**, Fig. 5) is a potent small-molecule inhibitor of VEGFR, the IC_{50} values of VEGFR-1, VEGFR-2 and VEGFR-3 were 6.4, 0.69

and 5.5 nmol/L, respectively[40]. Even at 10 μmol/L, **15** had no inhibitory effect on 18 other kinases including FGFR and PDGFR. Oral administration of **15** could significantly suppress the U87-MG xenograft tumors in nude mice and reduce the expression of CD31 and Ki67 in tumor tissues[40]. The chemical structures of the related VEGFR/PDGFR inhibitors are presented in Fig. 5.

Bevacizumab is the humanized VEGF antibody for GBM. Although bevacizumab has become a standard part of salvage treatment for recurrent GBM, multiple studies have shown that it does not improve survival[41,42]. There is growing evidence that the root cause of angiogenesis is hypoxia, which makes bevacizumab resistant[43]. In addition to being limited by the BBB, another disadvantage of PDGFR/VEGFR inhibitors is that, like other RTK inhibitors, their off-target activities may lead to drug toxicity. Further study of other components of RTK receptor may provide new possibilities for GBM treatment.

2.1.2. EGFRvIII inhibitors and EGFR−STAT3 signaling

As studies shown, the amplification and mutation of EGFR are the most prevailing genetic alterations occurring in more than 50% of GBM (164/291)[9]. Especially, the overexpression of epidermal growth factor receptor variant III (EGFRvIII) in GBM typically ranges from 25% to 81%. Consistently, EGFRvIII is rare in low-grade gliomas. Therefore, its high occurrence in high-grade gliomas supports its important role in the progression of GBM. The EGFRvIII mutant is an in-frame deletion of 267 amino acids from the extracellular domain (ECD) in GBM, which is different from the kinase domain (KD) mutations found in lung cancer. EGFR-vIII targeted vaccines in clinical trials have also demonstrated

Table 2 Approved VEGFR/multitargeted inhibitors and the relevant clinical use.

Drug targeting VEGFR	Preclinical assessment of brain penetration	Clinical trial in GBM	The relevant clinical use
Sorafenib (**1**)	P-gp and Bcrp substrate. Limited brain penetration in mice	NCT00544817 (phase 2; completed) NCT00597493 (phase 2; completed)[16] NCT00445588 (phase 2; completed)[17]	In 2005 sorafenib was approved by FDA for the treatment of advanced renal cell carcinoma (RCC) and in 2007 for the treatment of hepatocellular carcinoma (HCC)[18]
Sunitinib (**2**)	P-gp and Bcrp substrate. Limited brain penetration in mice	NCT00535379 (phase 2; active, not recruiting) NCT02928575 (phase 2; recruiting)	In 2006 sunitinib was approved by FDA for the treatment of RCC and of gastrointestinal stromal tumor (GIST)
Pazopanib (**3**)	P-gp and Bcrp substrate. Limited brain penetration in mice	NCT00459381 (phase 2; completed)[19]	In 2009 pazopanib was approved by FDA for RCC and in 2012 for soft tissue sarcoma[20]
Vandetanib (**4**)	P-gp substrate	NCT00995007 (phase 2; completed) NCT00441142 (phase 1/2; completed)	In 2011 vandetanib was approved by FDA for treatment of late-stage (metastatic) medullary thyroid cancer in adult patients who are ineligible for surgery[21]
Axitinib (**5**)	P-gp and Bcrp substrate. Limited brain penetration in mice	NCT03291314 (phase 2; completed)[4]	In 2012 axitinib was approved by FDA for use in patients with RCC that had failed to respond to a previous treatment

safety and efficacy in patients with GBM[44]. This also suggests that EGFRvIII is an effective therapeutic target for GBM, and the development of EGFRvIII targeted inhibitors is a potential therapeutic approach for the treatment of GBM.

Many EGFR inhibitors (Fig. 5), such as erlotinib (**16**), gefitinib (**17**) and lapatinib (**22**), have been widely evaluated in the clinic for the treatment of GBM[45]. **16** and **17**, the first generation of EGFR-tyrosine kinase inhibitors (TKIs), significantly improved survival in lung patients but had no significant effect in GBM clinical trials. This failure may be related to their inhibition of catalytic activity by competing with ATP binding sites in KD rather than EC[46]. The second-generation EGFR inhibitors, afatinib (**18**) is an FDA-approved irreversible inhibitor targeting EGFR, HER2, HER4 and EGFRvIII. In preclinical evaluation, **18** combined with TMZ significantly prevented tumor progression in nude mice cultured by intracranial injection of U87 EGFRvIII luciferase-transfected cells[47]. Clinical trial results showed limited single-agent activity of **18** in unselected patients with recurrent GBM[48]. However, further studies of **18** in patients with GBM are still meaningful and should be based on appropriate biomarker-based preselection[49].

JCN037 (**19**) is an EGFR inhibitor with anilinoquinazoline scaffold, which reduces the number of rotatable bonds and polar surface area and improves efficiency and BBB penetration by introducing *ortho*-fluorine and *meta*-bromine into aniline ring and fusing 6,7-dialkoxy groups. It showed obvious inhibitory activity in orthotopic GBM xenograft mice[50]. AZD3759 (**20**), a selective EGFR inhibitor, effectively penetrates the BBB and has the same free concentration in the blood, cerebrospinal fluid, and brain tissue[51]. Its penetrant properties and antitumor activities have been demonstrated in an early clinical study in patients with brain metastasis (BM) and leptomeningeal metastasis (LM)[52]. The good permeability of **20** to the BBB and its promising clinical activity support further evaluation of this compound in studies. NT113 (**21**) is a pan-ERBB inhibitor with high brain penetrance. Compared with **16** and **22**, **21** showed the most significant

improvement in survival and the most significant inhibition of tumor growth in intracranial GBM xenograft mice[53]. **21** deserves more clinical investigations in patients with GBM. Dacomitinib (**23**, Fig. 5) is a second-generation irreversible EGFR TKI that penetrates the BBB in a multicenter phase II trial (NCT01520870)[54]. The clinical trials showed that although **23** was not effective in most GBM patients with amplified EGFR, a subset experienced a durable, clinically meaningful benefit.

Rociletinib (**24**, CO-1686, Fig. 5), one of the third generation EGFR TKIs, is active against the EGFR T790M mutation while preserving the wild-type EGFR[55]. Preclinical results showed **24** did not achieve tumor regression in an EGFR-TKI-sensitizing mutations (EGFRm) PC9 mouse brain metastases model[56]. Osimertinib (**25**, AZD09291, Fig. 5) is an oral, third-generation irreversible EGFR inhibitor with significant brain permeability. In preclinical evaluation, **25** showed significant growth inhibition activity against all six GBM cell lines. Among them, the inhibitory effect of **25** on GBM cell proliferation was 10 times that of the first-generation EGFR inhibitors. This anti-GBM cell proliferation mechanism may be different from first-generation EGFR inhibitors, which effectively inhibit the EGFR/ERK pathway[57]. Meanwhile, in the *in-situ* GBM model, **25** can significantly inhibit the growth of GBM tumors in mice and prolong the survival time of animals. Furthermore, the activity result of **25** in a progressive, EGFR mutant GBM patient who was treated with the drug as an off-label salvage therapy indicated that the patient eventually progressed to a separate tumor site, highlighting the heterogeneity of the tumor, which is a major challenge for EGFR-targeted GBM therapy. This case highlights the need for further clinical evaluation of **25** for GBM, including identifying which EGFR alterations are sensitive to this drug[58]. The chemical structures of the related EGFR inhibitors are presented in Fig. 5.

Overall, the main challenges of current EGFR-targeting strategies for GBM are the lack of CNS permeability in most TKIs, the molecular heterogeneity of GBM, and the need to enhance the

specificity of small-molecule inhibitors of GBM-related EGFR mutations. For example, **17**, **18**, and **25** have been reported to act as substrates for P-GP and Bcrp, so brain penetration of these EGFR inhibitors is expected to be limited. Dose-limiting toxicity (DLT) is another reason for the failure of TKIs in clinical application of GBM[58]. Other EGFR-targeting therapies are currently represented by antibody−drug conjugates (ADCs) and human antibodies. For example, depatuxizumab mafodotin (ABT-414) and nimotuzumab have demonstrated anticancer activity in preclinical and clinical evaluations for the treatment of GBM.

2.2. PI3K/AKT/mTOR pathway inhibitors in GBM

Many downstream targets and pathways of RTKs are thought to be important drivers of glioma development, apoptotic escape, and cell growth[9]. Among them, the PI3K/AKT/mTOR pathway is one of the most targeted and dysregulated downstream pathways[59]. In clinical analysis, the PI3K pathway affected by phosphatase and tension homolog (PTEN) mutation, was abnormally activated in GBM patients, resulting in poor prognosis[60].

2.2.1. PI3K inhibitors and their preliminary clinical results
A growing body of preclinical and clinical evidence suggests that PI3K pathway inhibitors will offer promising options for the treatment of cancer patients, including GBM. To better exploit the efficacy of PI3K inhibitors in patients with GBM, future challenges may include identifying which patient populations will

benefit from these inhibitors (PTEN loss *vs* PIK3CA mutations) and optimizing the combination of PI3K pathway inhibitors with other pathway therapeutics.

BKM120 (**26**, buparlisib, Novartis, Fig. 6) is a pan-PI3K inhibitor with significant brain penetration. Indeed, **26** showed anti-tumor activity in GBM cell lines and intracranial U87 tumor mice regardless of PTEN or EGFR status[93]. However, the clinical results showed insufficient overall pathway inhibition by tolerable doses in patients with recurrent GBM. In a recent phase II trial (NCT01339052) of 65 patients with PI3K pathway-activated recurrent GBM, the result of the trial showed only 8% patients reached 6-month PFS, the median PFS is 1.7 months. The reason for the lack of clinical efficacy is that the PI3K pathway cannot be completely blocked in tumor tissues. Recent studies have shown that **26** in combination with the PARP inhibitor rucaparib (**79**, Fig. 12) can complement each other in terms of DNA damage responses and drug accumulation *in vitro* and *in vivo*, improving anti-tumor efficacy compared to each monotherapy[61]. PQR309 (**27**, bimiralisib, Fig. 6) is an effective PI3K/mTOR inhibitor with good BBB penetration and oral bioavailability in mammals. It has a higher inhibitory effect on PI3K than mTOR kinase[62]. **27** has been confirmed to have antitumor activity against GBM *in vitro* and *in vivo*. Moreover, the combination **27** with AKT inhibitor showed stronger anti-GBM activity in nude mouse orthotopic LN-229 glioma xenograft model[63].

GNE-493 (**28**, Fig. 6) is a potent inhibitor of PI3K and mTOR with good pharmacokinetic (PK) parameters[64]. However, its poor
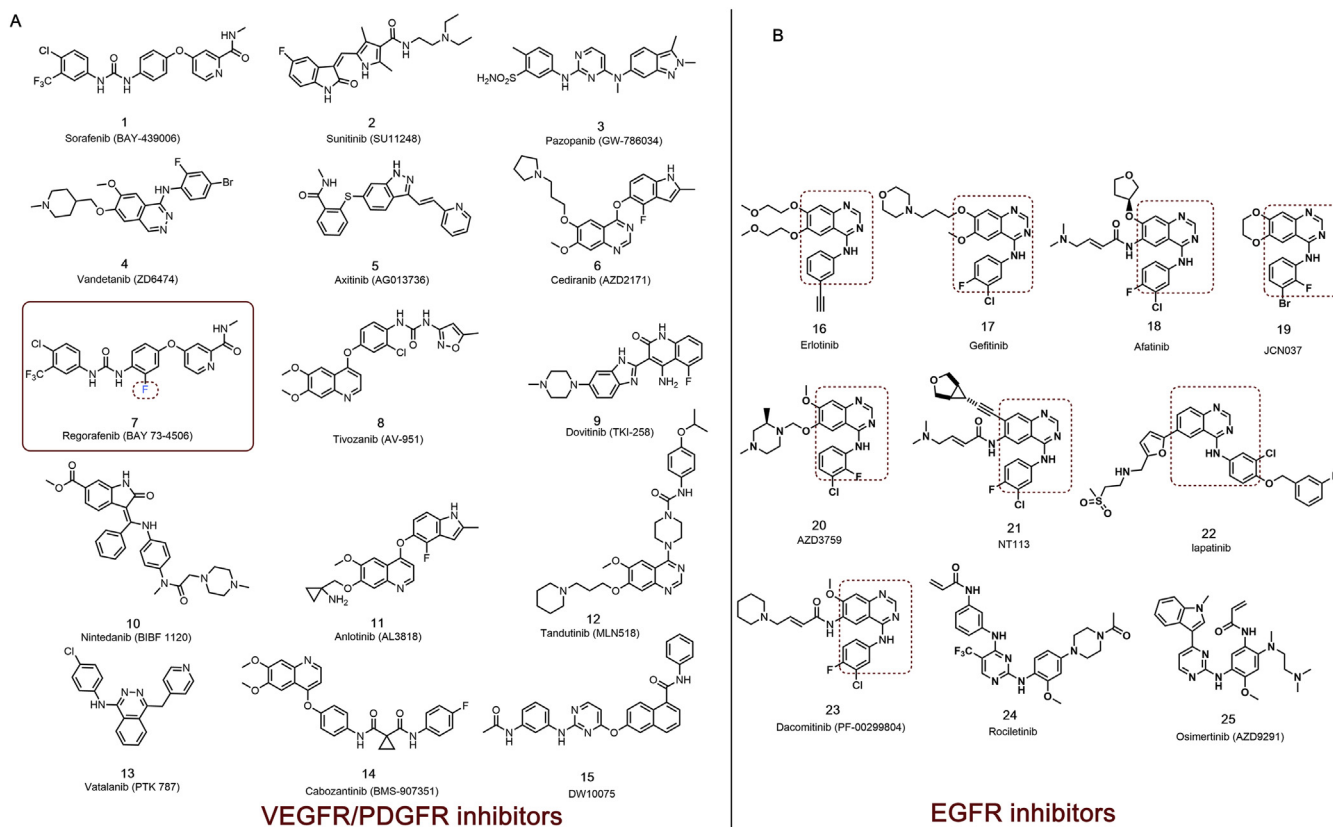
Figure 5 (A) The chemical structures of the related VEGFR/PDGFR inhibitors advanced in clinical study. (B) The chemical structures of the related EGFR inhibitors advanced in clinical study. Both **1** and **7** are oral diphenylurea multi-kinase inhibitors approved by the FDA, **7** is the fluorinated form of **1**. Regorafenib (**7**) is a very promising small molecule inhibitor for the treatment of GBM (its structure is highlighted in box). The chemical structures of **16**–**23** all contain anilinoquinazoline pharmacophore, marked by red boxes in the figure.

Figure 6 Structures and modification of PI3K inhibitors advanced to clinical study for GBM. The PI3K/mTOR reference IC$_{50}$ values for **26** and **27** are given in this figure.[62] **30**, a PI3K inhibitor with BBB permeability and metabolic stability, was found by structural modification of PI3K/mTOR inhibitors **28** and **29**. HBD, hydrogen bond donor.

brain penetration limits its use as a treatment for GBM. **28** was used as the initial lead compound to obtain analogues with better brain permeability by reducing the number of hydrogen bond donors. GNE-317 (**29**, Fig. 6) was designed out of these efforts and is an effective brain penetrant PI3K inhibitor. It markedly inhibits the PI3K pathway in the brains of mice with intact BBB[65]. **29** was uniformly distributed throughout the brain in the U87 and GS2 orthotopic mice of GBM, with tumor growth inhibition rates of 90% and 50%, respectively, showing a survival benefit[65,66]. However, **29** was found to have unacceptable projected human clearance, so it was further optimized to paxalisib (**30**, GDC-0084, Fig. 6). **30** is a PI3K inhibitor with comparable potency and similar BBB permeability to **24**, but with more desirable human PK properties[67]. The effective results of **30** in mice with U87 and GS2 intracranial tumors promoted its clinical trials in GBM[67]. The phase I study of **30** in GBM has shown that **30** has typical PI3K/mTOR inhibitor related toxicity. Fluorodeoxyglucose positron emission tomography (FDG-PET) and concentration data from brain tumor tissue indicated that **30** crossed the BBB[68].

Pilaralisib (**31**, XL147, SAR245408, Fig. 6) is a pan-PI3K inhibitor developed by Sanofi/Exelixis. The clinical results of **31** showed favorable safety in combination with other chemotherapeutic agents in the treatment of solid tumors[69]. The PI3K/mTOR inhibitor voxtalisib (**32**, XL765, SAR245409, Fig. 6) showed an

attractive activity in a range of genetically diverse GBM xenografts, either alone or in combination with conventional therapeutics. The study showed that **32** had superior tumor suppression effects to **31** in cancer cell models[70]. Phase I study showed that **32** combined with TMZ with or without RT had good safety and moderate PI3K/mTOR pathway inhibition in high-grade glioma patients[71,72].

Idelalisib (**33**, Fig. 6), an oral bioavailable small molecule inhibitor, is the first PI3K inhibitor approved by FDA in July 2014 for the treatment of relapsed lymphocytic leukemia (CLL)[73]. However, **33** is difficult to benefit GBM patients due to its poor permeability to the BBB[74]. Sonolisib (**34**, PX-866, Fig. 6) is a kind of oral PI3K inhibitor. In a phase II study of patients with recurrent GBM, the results showed although **34** was relatively well tolerated, the overall response rate was low[75]. The chemical structures of the related PI3K inhibitors are presented in Fig. 6.

2.2.2. AKT/mTOR inhibitors

Protein kinase B (AKT) pathway is associated with most human malignancies, including GBM. Meanwhile, the RTK/PTEN/PI3K pathway leads to elevated levels of activated and phosphorylated AKT in most GBM tumor samples and cell lines, which have been shown to contribute to the uncontrolled growth of glioma cells, avoid apoptosis, and enhance tumor invasion[76]. The mammalian target of rapamycin (mTOR), a component of both the mTOR

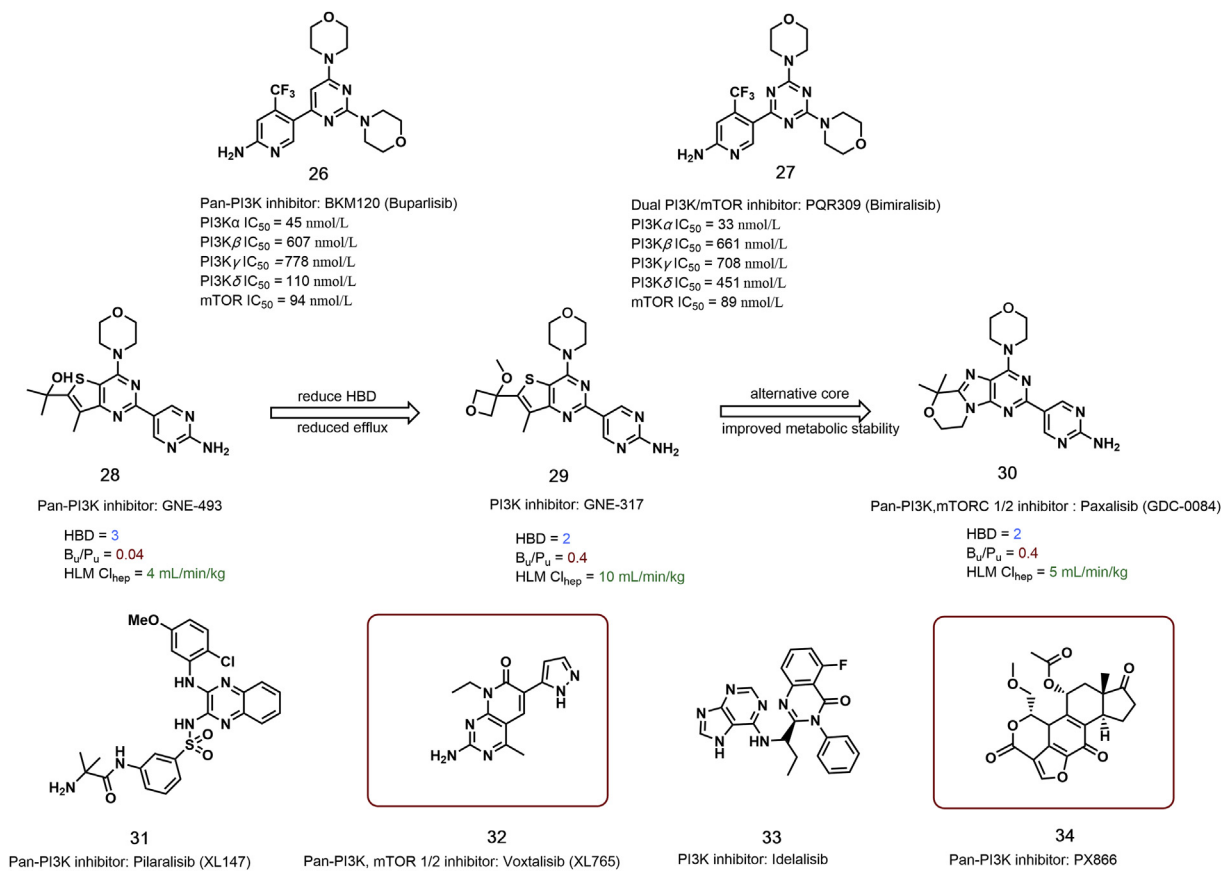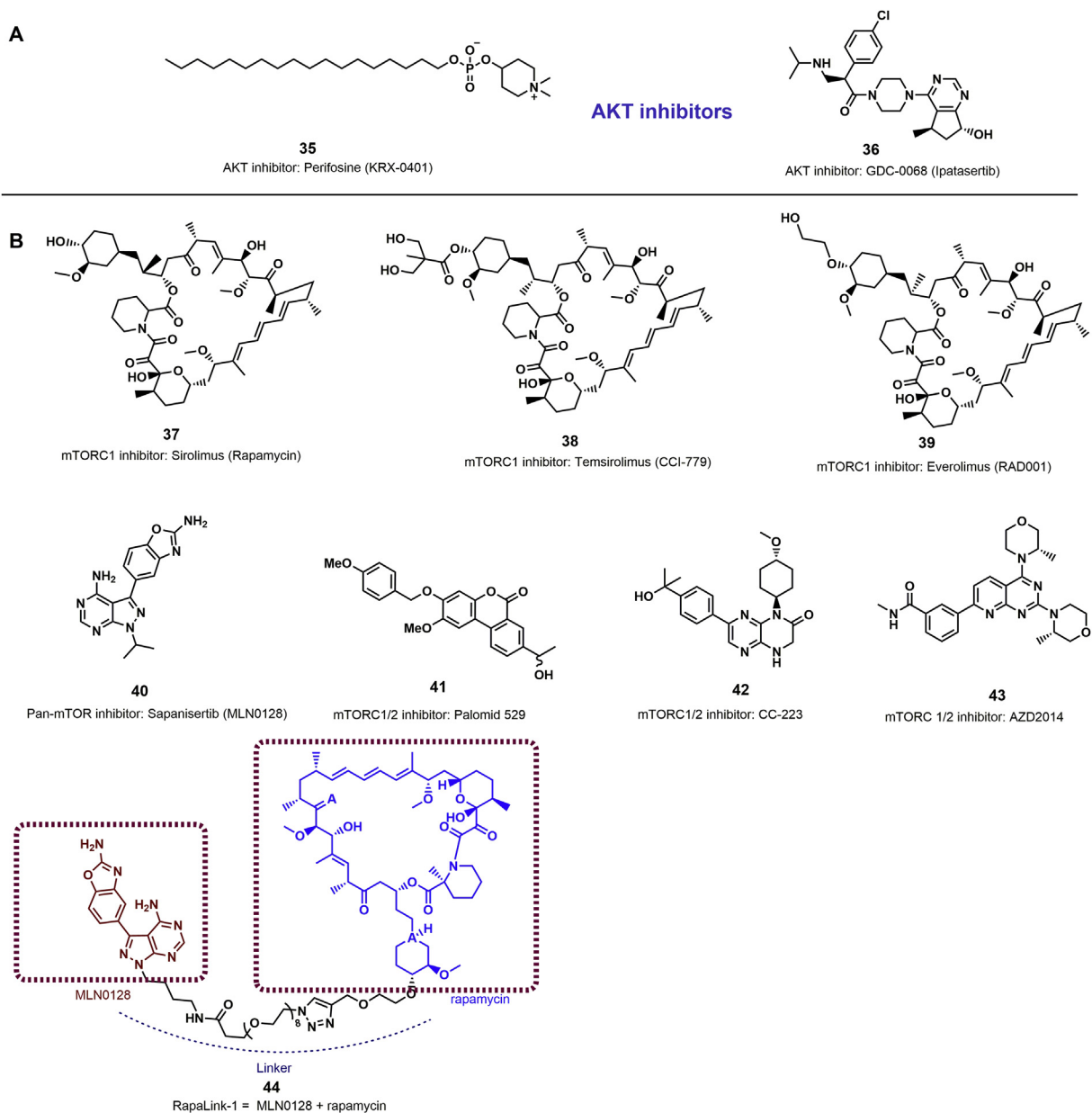**Figure 7** (A) Chemical structures of AKT inhibitors advanced to clinical study for GBM. B: Chemical structures of mTOR inhibitors advanced to clinical study for GBM. **35**−**37** have molecular weights (MW) greater than 500 (MW of **35**, **36** and **37** are 914, 1030 and 958). The chemical structure of RapaLink-1 and the mTOR cocrystallized with rapalink-1. (a) The structure of **42**, a polyethylene glycol-based linker combines rapamycin with **38**. Studies showed a long linker was necessary for improving potency[97]. (b) mTOR catalytic-domain-bearing TORKi PP242 (PDB ID: 4JT5) and mTOR FRB-domain-rapamycin-FKB12 (PDB ID: 1FAP) are two valid co-crystal structures that can be used to construct the molecular binding model.

complex 1 (mTORC1) and the mTOR complex 2 (mTORC2), is a serine/threonine kinase with broad activity in cell proliferation, growth, and survival[77]. Hyperactivation of mTOR is common in GBM and its high expression is associated with poor prognosis and a decrease in survival[78,79].

Perifosine (**35**, KRX-0401, Fig. 7) is the first allosteric AKT inhibitor with brain penetration to enter clinical phase. It attenuates AKT activation by blocking pleckstrin homology (PH) domain-dependent localization of AKT on the cell membrane. Although the result of a phase II clinical trial of **35** for the treatment of recurrent GBM showed that it was well tolerated but

ineffective. Its synergistic effects with other approaches still have potential in the treatment of GBM[80]. GDC-0068 (**36**, ipatasertib, Fig. 7), is a highly selective ATP-competitive pan-AKT inhibitor that preferentially binds to AKT in an activated conformation, resulting in increased anti-proliferative activity in cell lines with the activation of the PI3K/AKT pathway[81]. Preclinical data also showed that **36** can enhance the antitumor activity of classical chemotherapy drugs. Meanwhile, **36** can significantly inhibit the growth of PIK3CA mutated tumor in the BM model of breast cancer, suggesting to some extent that **36** can adequately penetrate the BBB[82]. **36** has also been reported as part of effective AKT

kinase degraders, and future research progress in the treatment of GBM still deserves attention.

Among mTOR inhibitors, sirolimus (**37**, Fig. 7), temsirolimus (**38**, Fig. 7) and everolimus (**39**, Fig. 7) are FDA-approved agents[83]. Sirolimus (**37**, rapamycin, Pfizer), a well-studied natural product with antifungal, immunosuppressive, and antineoplastic activities, is a macrolide antibiotic. **37** is widely recognized for its ability to inhibit the mTOR signaling pathway and has been widely studied for its therapeutic potential. It's reported that **37** and TMZ have a synergistic effect in inhibiting GBM activity, increasing reactive oxygen species (ROS) production and depolarizing mitochondrial membrane potential (MMP)[84]. A phase I study result demonstrated that the combination of **37** with metronomic chemotherapy was well tolerated in children with recurrent or solid and intractable brain tumors[85]. However, **37** works primarily by inhibiting mTORC1, while the uninhibited mTORC2 can activate the AKT signaling pathway to counteract reduced mTOR levels. This may be the main reason why **37** fails in clinical treatment of GBM.

38 and **39**, analogues of **37**, have been submitted for clinical trials to treat a variety of cancers. Temsirolimus (**38**, CCI-779), a water-soluble mTOR inhibitor with molecular weight of a thousand, has been demonstrated that **38** is well tolerated in monotherapy but has limited efficacy in the phase trial II of 65 patients with recurrent GBM[86]. Subsequently, a further phase II study of bevacizumab in combination with **38** in the treatment of recurrent GBM showed that the combination was safe but did not show an advantage over bevacizumab alone[60]. Notably, the patients with newly diagnosed unmethylated GBM may benefit from **38** according to mTOR-Ser2448 phosphorylation in a randomized phase II trial associated with 111 patients. The phase II trial data of RT + TMZ ± **39** (RAD001) in 171 patients with newly diagnosed GBM showed there were no difference in PFS, while the OS for **39** was inferior to that for control patients[87]. Palomide 529 (**41**, P529, Fig. 7) is a dual mTORC1/2 inhibitor that has the potential to enhance the efficacy of RT by delaying the DNA repair mechanism[88]. Meanwhile, **41** has been confirmed to have good brain penetration, which provides support for further evaluation of **41** treatment for GBM[89]. CC-223 (**42**, Fig. 7) is a potent orally bioavailable inhibitor of mTOR kinase and may have theoretical advantages over the first-generation drugs by inhibiting mTORC1 (pS6RP and p4EBP1) and mTORC2 [pAKT(S473)]. A phase II study on **42** described that it resulted in highly durable tumor regression and control of neuroendocrine tumors (NET) carcinoid symptoms. This makes **42** a promising agent for further development[90]. AZD2014 (**43**, vistusertib, Fig. 7) is also a dual mTORC1/2 inhibitor that enhances the radiosensitivity of GSCs both *in vitro* and under orthotopic *in vivo* conditions[91]. Studies results of **41** and **43** suggest that the dual mTORC1/2 inhibitor may be suitable radiosensitizers for GBM treatment. Furthermore, the results of a phase I trial showed that **43** combined with TMZ showed good safety at the tested dose levels in patients with first recurrent GBM[92].

Rapalink-1 (**44**, Fig. 7) is a third generation mTOR inhibitor that links **37** to **40** with better activity than first-generation and second-generation mTOR inhibitors. It showed good inhibitory activity in mice with U87MG intracranial xenografts. It was well tolerated by mice and significantly improved survival compared with the control group[93]. Studies also showed that **44** can

effectively impair the motility and clonogenicity of GBM stem cells and reduce the expression of stem cell molecules[94]. However, **44** has an effect on the aggravation of BBB disruption, which may lead to serious side effect[95]. The chemical structure of **44** and the mTOR co-crystallized with **44** are presented in Fig. 7.

Studies have shown that PTEN tumor suppressor positively regulates autophagy by inhibiting the PI3K/AKT pathway[96]. Common mutations of PTEN and neurofibromin 1 (NF1) occur frequently in gliomas, leading to constitutive activation of PI3K/AKT/mTOR signaling, suggesting that synergistic action of autophagy inhibitors by using inhibitors of the PI3K pathway could contribute to the treatment of GBM.
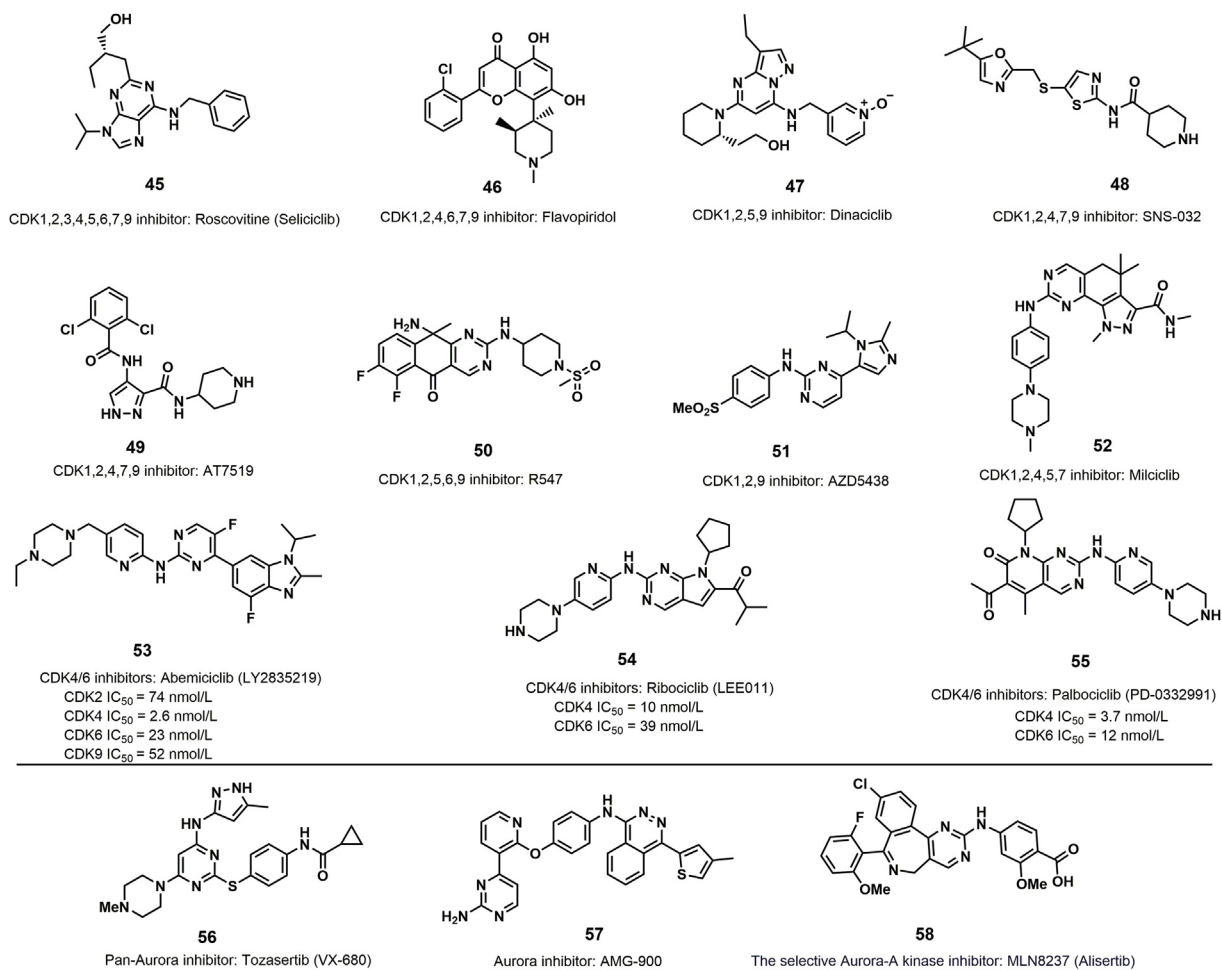
2.3. RB pathway and related small-molecule inhibitors

Studies have demonstrated that cell-intrinsic sex differences in RB regulation and stem-like cell function may be one of the reasons for the large number of male patients with GBM[98]. The CDK-RB-Early 2 factor (E2F) pathway integrates external and internal signals to control the process of G1/S transition in mammalian cell cycle. Alterations in this pathway have been found in GBM.

2.3.1. CDK4/6 and pan-CDK inhibitors in GBM

GBM genomic instability markers were associated with S-phase perturbations and G2/M transitions controlled by CDKs. This provides a theoretical basis for the development of small molecule inhibitors targeting CDKs in the treatment of GBM[99]. There are many compounds in the existing cyclin-dependent kinase inhibitors (CKIs) that have entered clinical trials for GBM treatment, including pan-CDK inhibitors that target CDK1 [seliciclib (**45**), flavopiridol (**46**), dinaciclib (**47**), SNS-032 (**48**), AT7519 (**49**), R547(**50**), AZD5438(**51**), and milciclib (**52**)], the CDK4/6 inhibitors [LY2835219 (**53**), LEE011 (**54**), palbociclib (**55**)]. Clinical studies of cyclin-dependent kinase 9 (CDK9) inhibitors **45**−**48** all had their additional targets, which led to their cancer treatment failure and involved many adverse reactions[100]. Understanding which CDKs are activated in GBM and whether these complexes are uniquely altered to cover cell cycle protection checkpoints could help design more selective inhibitors for GBM therapy. GBM shows overexpression of a protein called cyclin D1, which induces the level of cyclin-dependent kinase 4 (CDK4) and cyclin-dependent kinase 6 (CDK6). This outcome leads to the growth of tumors. GBM is characterized by high-frequent dysregulation of the cyclin-dependent kinase inhibitor 2A(CDKN2A)/cyclin D2 (CCND2)/CDK4/CDK6 pathway. Consequently, dual inhibition of CDK4 and CDK6 may be an attractive approach for the treatment of GBM[101].

Abemaciclib (**53**, LY2835219, Eli Lilly, Fig. 8) is the first selective inhibitor of CDK 4 and CDK6 with safety features that allow continuous administration to achieve sustained targeted inhibition[102]. In laboratory studies, **53** was able to enter the brain, stop CDK4/6 from making cells, and slow growth of mice with GBM. As a CDK4/6 inhibitor, **53** has an ongoing phase II trial (NCT02981940) in recurrent GBM patients with activation of CDK4/6 pathway[103]. A pilot study (NCT04074785) of **53** with bevacizumab in recurrent GBM patients with loss of CDKN2A/B or amplification of CDK4/6 is recruiting by university of Texas Southwestern Medical Center. Ribociclib (**54**, LEE011, Novartis, Fig. 8) is another inhibitor of CDK4/6[104]. A phase Ib study

45
CDK1,2,3,4,5,6,7,9 inhibitor: Roscovitine (Seliciclib)

46
CDK1,2,4,6,7,9 inhibitor: Flavopiridol

47
CDK1,2,5,9 inhibitor: Dinaciclib

48
CDK1,2,4,7,9 inhibitor: SNS-032

49
CDK1,2,4,7,9 inhibitor: AT7519

50
CDK1,2,5,6,9 inhibitor: R547

51
CDK1,2,9 inhibitor: AZD5438

52
CDK1,2,4,5,7 inhibitor: Milciclib

53
CDK4/6 inhibitors: Abemiciclib (LY2835219)
CDK2 IC_{50} = 74 nmol/L
CDK4 IC_{50} = 2.6 nmol/L
CDK6 IC_{50} = 23 nmol/L
CDK9 IC_{50} = 52 nmol/L

54
CDK4/6 inhibitors: Ribociclib (LEE011)
CDK4 IC_{50} = 10 nmol/L
CDK6 IC_{50} = 39 nmol/L

55
CDK4/6 inhibitors: Palbociclib (PD-0332991)
CDK4 IC_{50} = 3.7 nmol/L
CDK6 IC_{50} = 12 nmol/L

56
Pan-Aurora inhibitor: Tozasertib (VX-680)

57
Aurora inhibitor: AMG-900

58
The selective Aurora-A kinase inhibitor: MLN8237 (Alisertib)

Aurora inhibitors with available BBB penetration

Figure 8 The structures for CDK inhibitors and aurora inhibitors with available BBB penetration in clinical trials. **53**−**55** have been approved by FDA for breast cancer therapy. Both **53** and **55** could cross the BBB.

(NCT02345824) has been conducted to determine whether this inhibitor can penetrate tumor tissue and modulate downstream signaling pathways including RB in patients with recurrent GBM. But the results showed that **54** alone seemed ineffective for the treatment of recurrent GBM[105]. Palbociclib (**55**, PD0332991, Pfizer, Fig. 8), a selective inhibitor of both CDK4 and CDK6[106], has been approved for use in breast cancers in 2015[107]. In addition, some preclinical studies have shown **55** is effective for treating GBM[108]. Annexin V *in vitro* assay showed that **55** significantly increased the percentage of cells apoptotic during the G1 cell cycle. Preclinical results support the possibility of **55** as an adjunct therapy to RT and merit further clinical investigation[108].

53–**55** have been approved for the treatment of CDK4/6 inhibitors of HR$^+$/human epidermal growth factor receptor 2 (HER2)-breast cancer, but due to poor BBB permeability, these drugs may not be ideal for the treatment of brain tumors[101]. In terms of adverse reactions, **54** and **55** were mainly toxic to bone marrow, but had no significant effect on gastrointestinal tract. While **53** can cause many gastrointestinal adverse events[109].

Overall, the off-targets and inadequate BBB penetration are major limitations of CDK4/6 inhibitors for GBM treatment.

2.3.2. PLK1/aurora kinase inhibitors

The polo-like kinase 1 (PLK1) and the aurora kinase A (AurA) pathways play key roles in GBM cell growth/migration and in the self-renewal of the GSCs component and have been studied as an innovative strategy to overcome GBM resistance and recurrence[110].

The pan-aurora kinase inhibitor tozasertib (**56**, VX-680, Fig. 8) has been shown to reduce stem cell differentiation by inhibiting AurA kinase in GBM cells[111]. In mice GBMs originating from subventricular zone neural stem cells (NSCs), **56** can enhance the effect of RT, reduce tumor growth, and increase survival. Furthermore, the effect of **56** after RT is better than before RT in the treatment of GBM[112]. AMG900 (**57**, Fig. 8) is also a pan-aurora inhibitor and induces cell cycle arrest and senescence to inhibit the proliferation of A172, U87MG and U118MG GBM cells by upregulating p53 and p21[113]. MLN8237 (**58**, alisertib, Fig. 8) is a selective aurora A kinase inhibitor for the treatment of

hematologic malignancies, ovarian cancer, and other solid tumors in clinical trials. The phase I trial result indicated that **58** tablets with irinotecan and TMZ showed significant antitumor activity in patients with neuroblastoma[114]. Encouragingly, the combination of **58** and TMZ has shown promising results in Phase II clinical trials in patients with v-myc avian myelocytomatosis viral onco-gene neuroblastoma derived homolog (MYCN) non-amplified tumors[115]. However, the number of patients involved in the study was limited and focused on children, the efficacy of **58** in GBM needs to be further studied. The chemical structures of the related CDK inhibitors and aurora inhibitors are presented in Fig. 8.

2.4. p53 pathway and related small-molecule inhibitors

2.4.1. p53 inhibitors in GBM
In GBM patients, more than half were p35 positive, and DNA sequencing analysis showed that p53-positive microvascular proliferating cells were identical to TP53 mutations observed in primary tumors. The p53 protein is encoded by the *TP53* gene, which is thought to be the most mutated gene in human tumors[116]. The components of the deregulated p53 pathway are related to the invasion, migration, proliferation, avoidance of apoptosis and stem cell properties of GBM cells.

2.4.2. Murine double minute-2 (MDM2) inhibitors in GBM
MDM2 inhibitors and proteolysis targeting chimera (PROTAC) degraders have been recognized as attractive cancers treatment strategies for blocking the MDM2/p53 protein−protein interaction (PPI)[117]. At the same time, integrins $\alpha5\beta1$ and p53 are part of the convergence pathway that controls glioma apoptosis, prompting researchers to search for effective molecules that can regulate both target families simultaneously. Compound **59** (Fig. 9) is a potent MDM2/4 and $\alpha5\beta1/\alpha v\beta3$ blocker among these molecules. And in p53-wild glioma cells, **59** arrested cell cycle and proliferation while strongly reducing cell invasive-ness. Further biological studies are needed to deepen the anti-cancer activity of such novel class of compounds[118]. Compound **60** (Fig. 9) is a promising cytotoxic agent targeting TSPO and MDM2, which reactivates p53 function, inhibits the activity of both human GBM cells and glioma tumor stem cells (CSCs). Meanwhile, it sensitizes GBM cells and CSCs to the activity of TMZ[119].

AMG-232 (**61**, KRT-232, Fig. 9) is an effective and selective oral piperidinone inhibitor of MDM2−p53 PPI with favorable toxicological properties *in vitro* and *in vivo*[120]. In addition, the molecule is projected to have a low clearance rate and a long

half-life in humans[120]. It is currently recruiting patients with GBM in phase I with the trial number of NCT03107780[117]. Idasanutlin (**62**, RG7388, Fig. 9) is an MDM2 inhibitor with better efficacy and selectivity than before. Current research data support the process of clinical development of **62**. At the same time, **62** is recruiting phase I/II GBM patients. It has been recognized as an attractive cancer therapeutic strategy to reac-tivation of p53 through inhibiting MDM2 and MDMX, the negative suppressors of p53. However, the acquired resistance and toxicity of p53 activation continue to limit the development of MDM2 inhibitors as clinical anticancer agents. The chemical structures of the related MDM2−p53 inhibitors are presented in Fig. 9.

2.5. Miscellaneous

2.5.1. Integrin $\alpha v\beta3$ inhibitors in GBM
Integrins $\alpha v\beta3$ and $\alpha v\beta5$ regulate tumor angiogenesis and aggressiveness, possibly by regulating the activation of trans-forming growth factor β (TGFβ) pathway[121]. In the brain of pa-tients with GBM, the expression of $\alpha v\beta3$ and its ligand vitronectin are associated with tumor progression and invasive behavior at tumor margins. In preclinical models, integrins $\alpha v\beta3$ and $\alpha v\beta5$ have been identified as therapeutic targets for GBM[122].

Cilengitide (**63**, Fig. 10), the $\alpha v\beta3$ and $\alpha v\beta5$ integrin inhibitor, is a pentapeptide that has undergone several clinical trials in GBM[123]. In the phase II study, data showed compound **63** had antitumor activity in patients with GBM either as a single agent or in combination with TMZ. However, **63** has not been further developed as an anticancer drug because in a phase III trial involving 3417 patients with newly diagnosed GBM with meth-ylated MGMT promoter, its combination with TMZ failed to improve treatment outcomes[124]. Results from clinical trials in GBM using **63** revealed treatment benefits in only a subset of patients in a phase III trial. In the current study, the researchers provided an explanation for the clinical failure of **63** by finding that sensitivity to integrin blockade is not dependent solely on the expression of integrin $\alpha v\beta3$, but rather on the integrin $\alpha v\beta3$−Glut3 axis[125]. Studies showed that integrin $\alpha v\beta3$ activated a PAK4-YAP/TAZ signaling (Fig. 10) axis to enhance Glut3 expression[126]. The researchers also found that the growth and survival of mesenchymal-subtype GBM may be dependent on another glycolytic gene product, further suggesting that these tumors may be also sensitive to p12-activated kinase 4 (PAK4) or Yes-associated protein (YAP)/WW domain-containing transcription regulator 1(WWTR1, also known as TAZ) inhibitors[126]. For example, PF-03758309 (**64**, Fig. 10) is a classic inhibitor of PAK4,

59
IC$_{50}$ $\alpha5\beta1$ = 22 nmol/L
IC$_{50}$ $\alpha v\beta1$ = 1.4 nmol/L
IC$_{50}$ MDM2 = 72 nmol/L
IC$_{50}$ MDM4 = 77 nmol/L

60
IC$_{50}$ MDM2 = 4.3 nmol/L

61
AMG 232
Cellular IC$_{50}$ = 9.1 nmol/L
K_D (SPR) = 0.045 nmol/L
IC$_{50}$ MDM2 = 0.6 nmol/L

62
RG7388
Cellular MTT IC$_{50}$ = 30 nmol/L
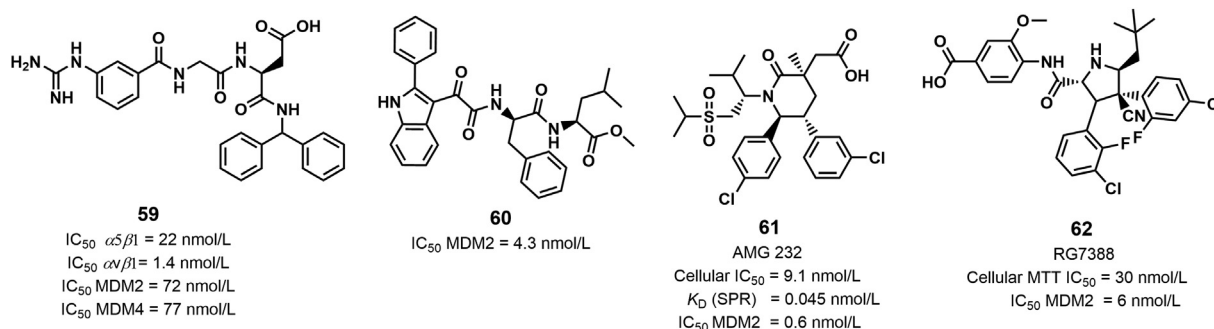IC$_{50}$ MDM2 = 6 nmol/L

Figure 9   The chemical structure of potent small-molecule inhibitors of the MDM2−p53 inhibitors.
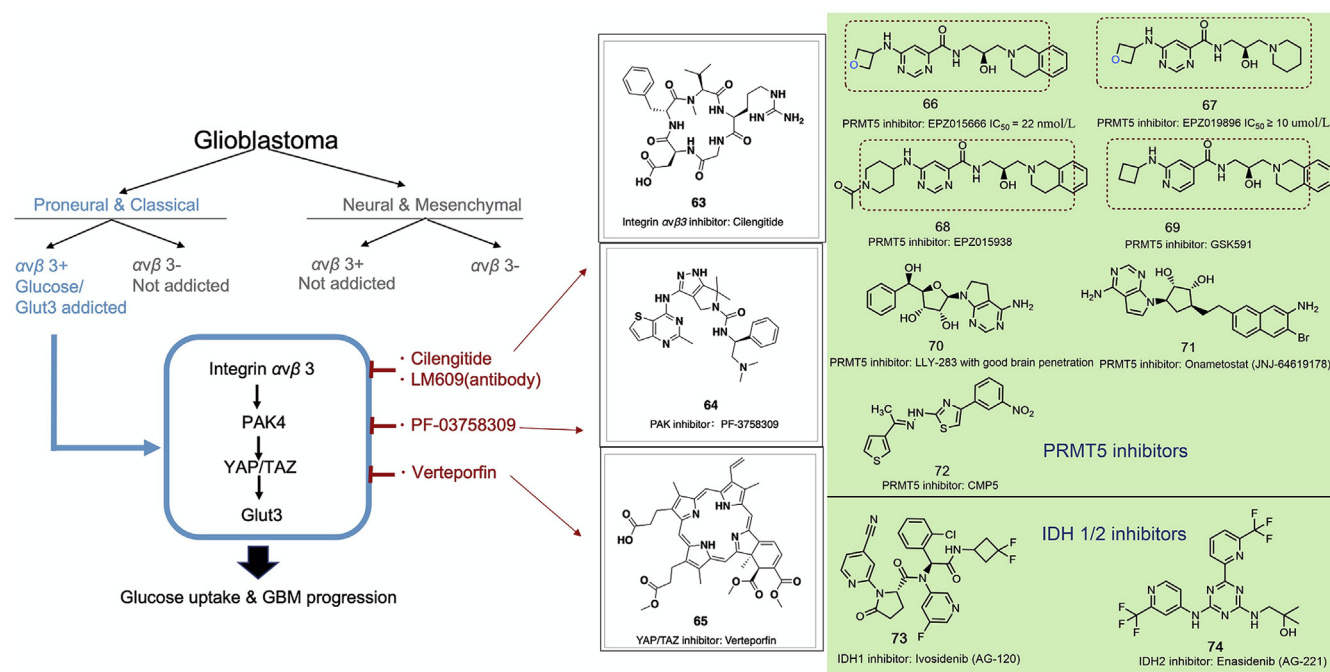
Figure 10   Chemical structure of a variety of other small molecule inhibitors in the treatment of GBM. These mainly include inhibitors targeting the PAK4-YAP/TAZ signaling, PRTM5 inhibitors and IDH1/2 inhibitors in GBM.

verteporfin (**65**, Fig. 10) is a YAP/TAZ inhibitor. They deserve to be tested for their potential in GBM treatment in the future.

2.5.2.   *Protein arginine methyltransferase 5 (PRMT5) inhibitors in GBM*

The expression of protein arginine methyltransferase 5 (PRMT5) is increased in GBM and its expression is negatively correlated with GBM patient survival. It has been recently emerged as a promising target in GBM. PRMT5 inhibition can effectively attenuate the growth and clonogenic capacity of most patient derived GSCs lines[127]. EPZ015666 (**66**, GSK3235025, Fig. 10) is an orally available inhibitor of PRMT5 enzymatic activity with IC_{50} of 22 nmol/L in biochemical assays and broad selectivity against a panel of other histone methyltransferases. **66** showed synergistic anti-GBM effects in combination with mTOR inhibitor *in vitro* and xenograft mouse models[128]. Both EPZ019896 (**67**, Fig. 10) and EPZ2015938 (**68**, Fig. 10) are two effective inhibitors of PRMT5 by further optimizing the structure of **66**[129]. **68** has been licensed to GSK (GSK3326595) and has already been evaluated its safety, PK, and pharmacodynamic (PD) in phase 1 clinical trial (NCT02783300). **69** (GSK591, Fig. 10) is a substrate-competitive inhibitor of the PRMT5-methylosome protein 50 (MEP50) complex. Studies have shown that **69** can significantly increase resveratrol induced cell apoptosis and enhance chemosensitivity of tumors by inhibiting PRMT5[130]. **69** has also shown the ability to suppress the growth of a range of 46 patient-derived GSCs cultures[127]. LLY-283 (**70**, Fig. 10), a potent and selective inhibitor of PRMT5 enzyme activity with good brain penetration, significantly prolonged survival in mice with orthotopic GBM patient-derived xenografts (PDXs) in preclinical data[127]. Onametostat (**71**, JNJ-64619178, Fig. 10) is one of the few inhibitors of PRMT5 currently in clinical trials[131]. CMP5 (**72**, Fig. 10) is a class of agents that can effectively block the activity of PRMT5. *In vivo* treatment of intracranial tumors in zebrafish shows that **72** can effectively inhibit the growth of GBM tumors and improve the survival rate[132].

Future research will focus on drug development of **72** to improve its PD and PK properties for the treatment of GBM.

2.5.3.   *Isocitrate dehydrogenase (IDH) inhibitors in GBM*

Cancer genetics studies have shown that hotspot mutations in isocitrate dehydrogenase 1 (IDH1) and isocitrate dehydrogenase 2 (IDH2) occur frequently in various human cancers, including malignant gliomas, acute myeloid leukemia (AML), intrahepatic cholangiocarcinoma, chondrosarcoma, and thyroid carcinomas[133]. Diffuse gliomas are malignant brain tumors that includes low-grade gliomas (LGGs) and GBM. IDH1 gene mutations are mostly concentrated in LGGs (70%)[134]. IDH mutated gliomas have been reported to be more suitable for surgical resection, and maximum surgical resection may provide a significant survival advantage for patients with IDH mutated gliomas.

Ivosidenib (**73**, AG-120, Fig. 10) is an inhibitor of mutant IDH1 that is being evaluated clinically in patients with solid tumors. The results of a current clinical trial involving 66 patients show that **73** can slow the growth of gliomas that have not yet progressed, thus preventing LGGs from turning into advanced GBM[134]. Enasidenib (**74**, AG-221 Fig. 10) is an orally allosteric inhibitor of mutant IDH2 under clinical development[133]. **74** has a significant clinical survival benefit for patients with AML. There are also ongoing clinical trials of **74** in other IDH2 mutated solid tumors, including gliomas. However, clinical data are not available yet[135].

In general, there are not many small molecule inhibitors that specifically target IDH mutations for high-grade gliomas known as GBM. On the one hand, most of GBM is IDH wild type, but IDH inhibitors can be used to prevent further deterioration of low-grade glioma; On the other hand, for GBM with IDH mutation, it can be seen from current clinical trials that most chemotherapy regimens are formulated by inhibiting other targets, such as using PARP inhibitors and anti-PD-L1/anti-PD-1 antibody combined with TMZ.
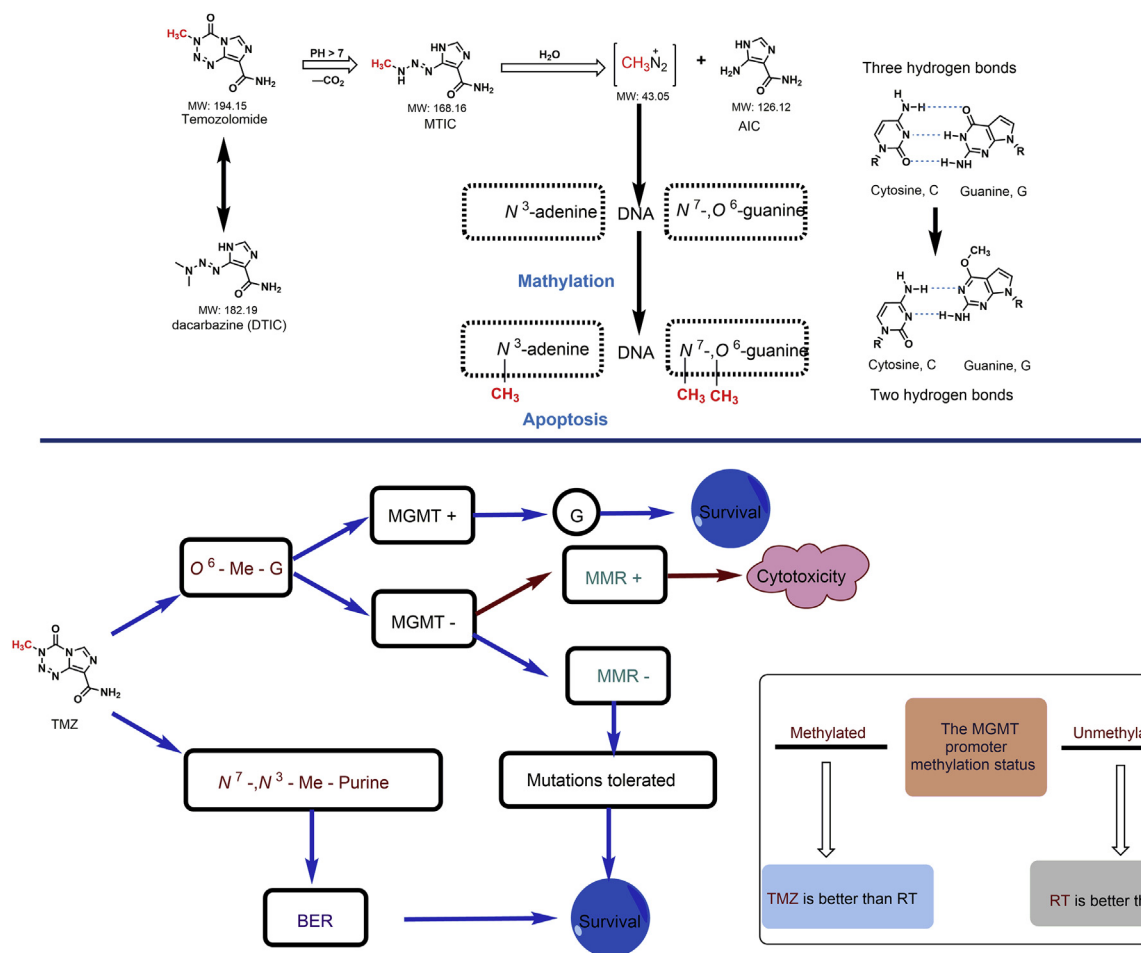
Figure 11 The structure and activation route of prodrug TMZ and repair of O^6-methylguanine-DNA methyltransferase. TMZ hydrolyzed spontaneously to active fragment MTIC and TMZ metabolite at physiological pH. MTIC is further hydrolyzed to 5-amino-imidazole-4-amide (AIC) and methylhydrazine, the former being an intermediate in the biosynthesis of purines and nucleic acids, and the latter being considered as the active fragment of alkylation. Key DNA repair mechanisms that affect cellular response to TMZ. MGMT methylation status is predictive of the efficacy TMZ.

3. TMZ-resistant GBM and recent advances

TMZ is an imidazotetrazine derivative like dacarbazine (DTIC), as an alkylation agent with good ability to cross the BBB. It has been evaluated largely in the therapy of malignant brain tumors and as the first line option treatment, especially in methylated MGMT-carrying patients with GBM[136]. The cytotoxicity of TMZ is based on its methylation capacity of O^6-guanine to result in O^6-methylguanine (O^6-MeG). It is noteworthy that the methyl of O^6-MeG can be removed by MGMT (direct repair, Fig. 11). Additionally, O^6-MeG is tolerated in mismatch repair (MMR)-deficient tumors. Therefore, MGMT or MMR deficiency confers resistance to TMZ[137]. Fig. 11 shows that MGMT methylation status may be a biomarker for predicting the TMZ treatment effect in GBM. The key DNA repair mechanisms that affect the cellular response to TMZ are also shown in Fig. 11.

3.1. Combination therapy to boost temozolomide effects

TMZ combined with other targeted small molecule inhibitors is a significant therapeutic strategy for GBM. In this section, we discuss the potential strategies that might overcome TMZ resistance such as combinations with other chemotherapies, targeting the acquired vulnerabilities associated with resistance to TMZ or suppressing genomic instability. At the same time, Table 3 summarizes the existing completed clinical trials of effective drugs in combination with TMZ.

3.1.1. Combination cannabidiol (CBD) with TMZ therapy

Cannabidiol (75, CBD, Fig. 12), a naturally occurring small molecule compound that can cross the BBB to reach the site of brain tumor, was approved by FDA in 2018 to treat refractory childhood epilepsy. 75 has also been shown to have potential antitumor effects. Previous studies in 2015 showed that 75 could induce autophagy through a transient receptor potential vanilloid-2 (TRPV2)-dependent manner and inhibit the differentiation of GSCs[144]. In 2021, it was further found that 75 can activate transient receptor potential vanilloid-4 (TRPV4) to induce mitophagy and ultimately kill glioma cells[145]. Preclinical studies have shown that the combination of 75 and TMZ therapy for GBM has a more selective inhibitory effect on cancer cells in the brain. Clinical trials have also shown that 75 combined with TMZ therapy can bring significant survival differences in patients, which supports further research on 75 in the treatment of GBM[146]. These

Table 3 Selected completed clinical trials of the combination potent agents with TMZ.

Combined agent	Study design	Study population	Median OS	Median PFS	DLT	Trial (NCT number)
Carboxyamidotriazole orotate (CTO)[138]	Non-randomized phase I study of CTO in combination therapy with TMZ for patients with GBM or recurrent GBM	100 participants (older than 18 years of age)	Newly diagnosed GBM, combination: 2-year OS of 62%. TMZ: 16−19 months	Combination: 15 months; TMZ: 6−7 months	No dose-limiting toxicities were observed	NCT 01107522
BKM120[139]	Non-randomized phase I study of BKM120 in combination with adjuvant TMZ in patients with newly diagnosed GBM	38 participants with newly diagnosed GBM	Novartis has decided not to proceed with the development of BKM120 in newly diagnosed GBM due to the failure to meet the primary objective of the estimated MTD, as well as the challenging safety observed		The MTD of buparlisib in combination with TMZ at stage I: 80 mg/day; the AEs: nausea, fatigue (>50%)	NCT 01473901
Lomustine[140,141]	Randomized phase III trial of CCNU/TMZ combination therapy *vs* standard TMZ therapy for newly diagnosed MGMT-methylated GBM patients	141 participants with MGMT-methylated GBM (18−70 years)	Combination: 41.8 months (32.6 months −not assessable) TMZ: 31.4 months (95% CI 27.7−47.1)	Combination: 16.7 months (95% CI 11.4 −24.2); TMZ: 16.7 months (12.0−32.0)	The AEs of grade 3 (>50%) in combination with CCNU	NCT 01149109
Cilengitide[124]	Randomized phase III study of cilengitide in combination with TMZ/RT *vs* standard treatment alone	545 participants with MGMT-methylated GBM (>18 years)	Combination: 26.3 months (95% CI 23.8 −28.8); TMZ: 26.3 months (23.9−34.7); (HR 1.02, 95% CI 0.81 −1.29, $P = 0.86$)	Combination: 13.5 months (95% CI 10.8 −15.9); TMZ: 10.7 months (8.1 −13.3); HR 0.93, (95% CI; 0.76−1.13, $P = 0.46$)	No overall additional toxic effects with cilengitide, the AEs of grade 3 (<20%)	NCT 00689221
Disulfiram[142,143]	Randomized controlled trial of disulfiram in recurrent GBM	88 participants with previous diagnosis of GBM/23 recurrent TMZ-resistant GBM patients	7.1 months (95% CI: 5.8 −8.5) in recurrent GBM	1.7 months (95% CI: 1.4 −1.9) in recurrent GBM	Only one patient (4%) had DLT, MTD of disulfiram: 500 mg/day	NCT 02678975, NCT 03034135

AEs, adverse events; CI, combination index; CCNU, lomustine; DLT, dose-limiting toxicity; HR, hazard ratio; OS, overall survival; PFS, progression-free survival; RT, radiotherapy; TMZ, temozolomide.

encouraging results, on the one hand, support that **75** can enhance the therapeutic effect of TMZ on GBM, on the other hand, also show that induction of autophagy can be an effective strategy to improve chemotherapy resistance.

3.1.2. Combination autophagy inhibitors with TMZ therapy
At present, many studies have proved that autophagy is crucial to the occurrence and growth of GBM, and the development of GBM can be significantly reduced by inhibiting autophagy[147]. At the same time, studies have shown that autophagy endows glioma with the characteristics of resistance to RT, chemotherapy, and high recurrence. Therefore, the combination of autophagy inhibitors and TMZ is a promising treatment for GBM. Chloroquine (**76**, CQ, Fig. 12) can accumulate in lysosomes, thereby increasing

the pH value in lysosomes by preventing autophagosome-lysosomal fusion to inhibit mitochondrial autophagy[148]. Preclinical evaluation showed that **76** could effectively block autophagy of brain glioma cells in mice U87MG tumor subcutaneous transplantation model and improve their chemosensitivity to TMZ[149]. Early evidence that **76** can reach the CNS was demonstrated in a small randomized clinical trial in which it was used in combination with conventional therapy to improve OS in patients with GBM. Meanwhile, the results of a recent clinical trial also showed that **76** combined with TMZ had good toxicity and extended OS. This supports further clinical studies of **76**[150]. Hychroxychloroquine (**77**, HCQ, Fig. 12) is a derivative of **76** and an autophagy inhibitor. The cumulative retinal toxicity of **77** is smaller than **76**[151]. However, results from a phase I/II clinical trial

showed that **77** combined with TMZ presented significant toxicity and side effects in newly diagnosed GBM patients, with no significant improvement in OS[152]. Further development of compounds with more autophagy inhibitory activity than **77** and low toxicity is worth studying.

3.1.3. ALDH1A3 inhibitors potentiate TMZ efficacy to GSCs

Studies have shown that aldehyde dehydrogenase 1A3 (ALDH1A3) is regulated by autophagy during chemotherapy and can directly participate in the resistance against TMZ treatment and lead to poor prognosis of GBM patients. The researchers found that ALDH1A3 knockout glioma cells were more sensitive to TMZ treatment than ALDH1A3 wild-type glioma cells. They also found that the use of higher concentration of TMZ induces autophagy. These results suggest that autophagy may be induced by chemotherapy, playing a vital role in chemoresistance[153]. Meanwhile, emerging evidence suggests that GSCs can lead to TMZ resistance and tumor recurrence due to their ability to self-renew and invade adjacent tissues[154]. Recent studies have shown that imidazole[1,2-α]pyridine derivatives, **78** (Fig. 12), have a nanomolar to picomolar efficacy against GBM stem cells as ALDH1A3 inhibitors[155]. Therefore, inhibition of ALDH1A3 may be a new and promising anti-GBM method to improve TMZ resistance. Disulfiram (**79**, DSF, Fig. 12) has been widely reported as an ALDH inhibitor, but its inhibitory activity against ALDH is not apparent when used alone. Only copper support can show significant ALDH inhibitory activity in U251MG, U87MG, and U373MG GBM cell lines. As an old drug that has been in clinical use for over 70 years (approved by FDA in 1951), **79** has well-established PK properties and the ability to penetrate BBB. The results of the first small phase I clinical trial of **79** and TMZ in the treatment of GBM showed that the combination is safe and can prolong PFS to some extent, which supports further clinical trials of **79**, especially **79** with concurrent Cu administration, in GBM[156].

3.1.4. Combination PARP inhibitors with TMZ therapy

Inhibition of poly(ADP-Ribose) polymerases 1 and -2 (PARP-1 and PARP-2) activated by DNA single- and double-strand breaks and promotion of their repair through chromatin relaxation and the supplementation of other repair proteins is another strategy to enhance the activity of TMZ. The cytotoxicity of TMZ could potentially enhanced by PARP inhibition through blocking the base excision repair (BER) pathway[157]. So far, olaparib (**80**), rucaparib (**81**) and niraparib (**82**, Fig. 12) have been approved by FDA to treat certain ovarian cancers[158]. While veliparib (**83**) is in the late stage of clinical development[159]. Developed by Pfizer, talazoparib (**84**) was approved in the USA for the treatment of adult patients with harmful or suspected deleterious germline BRCA-mutated, negative HER2, locally advanced or metastatic breast cancer[160]. Olaparib (**80**, AZD2281, Fig. 12) potentiated radiation and TMZ in GBM models but with limited brain penetration. Clinically, **80** aggravates TMZ-associated hematological toxicity and requires intermittent administration. The PK, safety, and tolerability of **80** and TMZ against recurrent GBM were evaluated in the latest OPARATIC trial. The results showed that **80** could reach central GBM tumors at effective concentrations. The clinical results support its further clinical development and highlight the need for better preclinical models[161]. Rucaparib (**81**, AG014699, Fig. 12) is the first PARP inhibitor to be evaluated in clinical trials. In several preclinical studies, **81** significantly improved the efficacy of TMZ in various solid tumor models. However, preclinical studies in the treatment of GBM showed that **81** was excluded from the CNS by the BBB, which limited the efficacy of **81** in the *in-situ* GBM xenotransplantation model[162]. Veliparib (**83**, Fig. 12) combined with TMZ had a stronger inhibitory effect on MSH6-inactivated orthodontic xenograft tumors, compared with TMZ monotherapy[159]. Talazoparib (**84**, BMN 673, Fig. 12) is currently the most potent clinically used PARP inhibitor (PARP-1 IC$_{50}$ = 1.3 nmol/L). Meanwhile, studies have shown that EGFR-amplified GSCs have significant
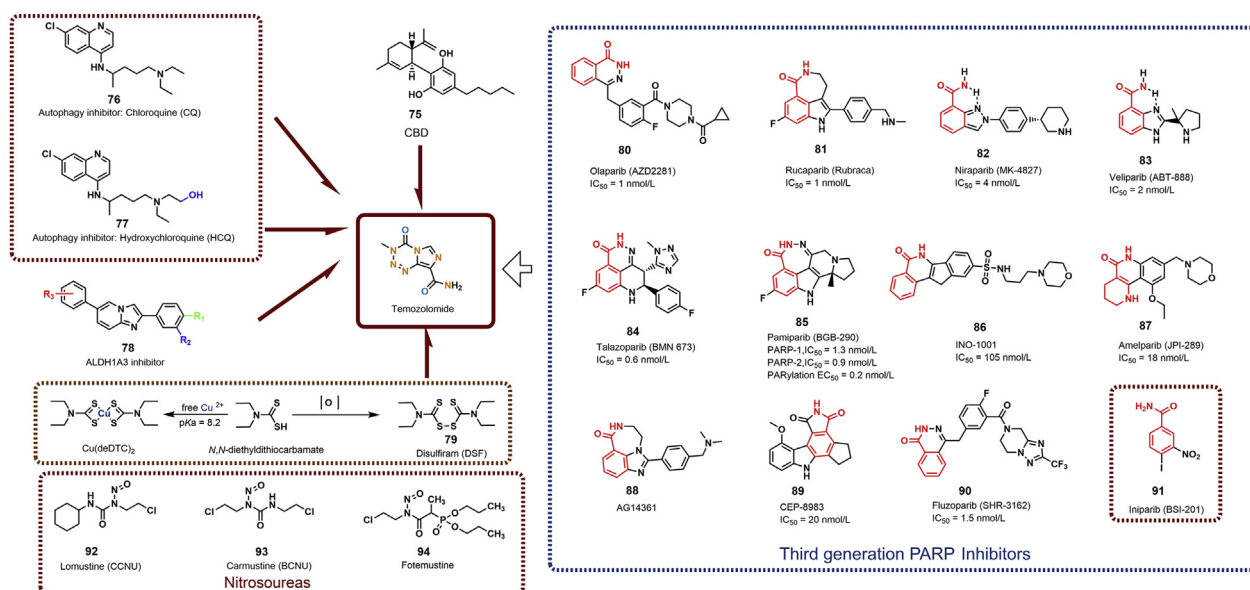

Figure 12   The combination therapy of effective molecules and TMZ (**77**, R$_1$ = H, R$_2$ = H, R$_3$ = 4-F, the IC$_{50}$ values of this ALDH1A3 inhibitor against the PN-157, MES-267, and MES-374 cell lines were 25.2, 63.4 nmol/L, and 2.58 pmol/L, respectively). The chemical mechanism of copper accumulation involved in the neurotoxicity of disulfiram (DSF) is briefly demonstrated. Red coloration of the bond in PARP inhibitors highlights the conserved nicotinamide pharmacophore. The main nitrosoureas with good BBB penetration.

sensitivity to **84** treatment[163]. A Phase II trial of **84** for the treatment of recurrent high-grade gliomas is currently being recruited by the university of Hong Kong (NCT04740190).

Among other PARP inhibitors, BGB-290 (**85**, pamiparib, Fig. 12), a potent and selective third-generation PARP inhibitor (PARP-1 IC_{50} = 1.3 nmol/L, PARP-2 IC_{50} = 0.9 nmol/L) showed the ability to cross the BBB in C57BL/6 mice with brain-to-plasma ratios in the range of 17%−19%[164]. The combination effect of **85** and TMZ was evaluated in cellular assays and in animal models and the results indicated that combination of **85** and TMZ significantly delayed resistance without additional toxicity[157]. In clinical studies, **85** is currently being studied as a maintenance treatment for platinum-sensitive ovarian and gastric cancers in phase III clinical trials[164]. A phase I trial of **85** and TMZ in treating IDH-1/2-mutant gliomas is recruiting to study the side effects and best dose of **85** and TMZ (NCT03749187). INO-1001 (**86**, Fig. 12) is a PARP-1 inhibitor that blocks the repair process of *N*-methylpurines generated by TMZ. The water-soluble PARP-1 inhibitor amelparib, JPI-289 (**87**, Fig. 12) is a potential neuroprotective agent for the treatment of acute ischemic stroke[165]. The neuroprotective effect of **87** may be due to its ability to attenuate the permeability of the BBB and decrease the brain edema. In preclinical evaluation, PARP inhibitor AG14361 (**88**, Fig. 12) combined with TMZ resulted in complete tumor regression in mice for more than 60 days[166]. The study also showed that **88** increased TMZ activity in all MMR-proficient or MMR-deficient cells, overcoming TMZ resistance[167]. CEP-8983 (**89**, Fig. 12) is the selective PARP inhibitor. Studies have shown that **89** and its prodrug CEP-9722 are effective chemo-sensitizing agents against chemotherapy-resistant tumors when used in combination with TMZ. Fluzoparib (**90**, SHR-3162, Fig. 12) is a novel PARP inhibitor undergoing clinical trials[168]. Iniparib (**91**, BSI-201, Fig. 12) was thought as a PARP1 inhibitor that has clinical activity in cancers mediated by mismatch repair defects, such as triple-negative breast cancer and pancreatic cancer with BRCA2 mutations[169]. However, later clinical studies found that **91** did not have the typical characteristics of PARP inhibitors and had no significant inhibitory effect on PARP. Even so, as a prodrug with a simple chemical structure, **91** can cause cell death through intracellular conversion to nitro ions. And a phase II study of **91** has shown that **91** is well tolerated with RT and TMZ in newly diagnosed GBM patients and shows potential antitumor activity. Doses need to be optimized (frequency and sequence) before other efficacy studies can be conducted[170]. In general, PARP inhibitors could improve the efficacy of TMZ in the clinical treatment of GBM, but the brain penetration is a major limitation of the use of PARP inhibitors.

3.1.5. Nitrosoureas-TMZ combination therapy (MGMT methylated)

Nitrosoureas are a kind of the most classical agents in the treatment of GBM. In clinical use of nitrosoureas, lomustine (**92**, Fig. 12), carmustine (**93**, Fig. 12) and fotemustine (**94**, Fig. 12) have good BBB permeability[171]. Data from many clinical trials also indicated that nitrosoureas had better benefits for patients with MGMT methylation than those without MGMT methylation. Lomustine (**92**) a nitrosourea compound, is one of the most effective drugs to treat GBM. **92**−**94** are the most used compounds clinically. Studies have indicated that **92** is generally better than **93** in oral formulation and has better safety profile.

In the setting of a unrandomized phase II trial and a randomized phase III trial (the CeTeG/NOA-09 trial), data suggested that **92**-TMZ plus RT might improve survival compared with TMZ chemoradiotherapy in newly diagnosed GBM with MGMT methylation. However, due to the limited number of patients in clinical trials, the results still should be interpreted with caution[140].

3.2. Adjuvant Tumor-Treating Fields (TTFields) with TMZ in GBM

Tumor-Treating-Fields (TTFields) is a low intensity electric field transformed by medium frequency (200 kHz), which is an anti-mitotic treatment. TTFields can deliver a low intensity alternating electric field to the tumor, which interferes with GBM cell division and organelle assembly. In a 2012 phase III trial of NovoTTF-100A, the chemotherapy-free treatment device appeared to be comparable in efficacy and activity to chemotherapy for recurrent GBM. Although the results showed that treatment with NovoTTF-100A alone did not improve OS, TTFields had some advantages in toxicity and quality of life. Results from a 2015 Phase III clinical trial showed that adding TTFields to the standard treatment of TMZ resulted in significant improvement in patients' PFS and OS[172].

4. Other therapies

4.1. Immunotherapies

A variety of cancers now benefit from these immunotherapies. More and more researchers are trying to use immunotherapy to solve the treatment bottleneck of GBM.

Disialoganglioside (GD2) is highly expressed in most pediatric patients with osteosarcoma, neuroblastoma, and GBM[173]. Dinutuximab (an anti-GD2 monoclonal antibody) was approved by FDA in 2015 and is currently used as a combination immunotherapy regimen for high-risk neuroblastoma in children[174]. Studies data showed that the anti-tumor effect of dinutuximab on GBM cells provided theoretical support for GD2 targeted immunotherapy of GBM[175].

Pembrolizumab is an IgG4 monoclonal antibody targeting PD-1. In a phase II study of pembrolizumab alone or with bevacizumab showed that pembrolizumab was well tolerated but of limited benefit in patients with recurrent GBM[176]. Immune analyses showed that in most GBM patients, pembrolizumab treatment failed to induce an efficient immune response, possibly due to the lack of T cells in the tumor microenvironment and the preponderance of $CD68^+$ macrophage[177].

At present, there are synergistic immunotherapies for GBM to improve the efficacy of immunotherapy in GBM treatment. For example, anti-interleukin-6 (IL-6) treatment was found to reduce CD40 expression in GBM-associated macrophages. It was also found that antibody cocktail immunotherapy combined with checkpoint blockade and dual targets of IL-6 and CD40 may provide a good therapeutic prospect for GBM and other solid tumors.

4.2. Viral therapies and vaccines

Chimeric antigen receptor T (CAR-T) cell therapy is one of the most promising immunotherapies in the last decade. Currently, there are many CAR-T products in clinical stage for GBM treatment. Oncolytic viruses (OVs) are a new class of anticancer drugs that promote tumor regression by preferring replication in tumor

Table 4   Key properties of effective small molecule inhibitors in preclinical and clinical evaluation of GBM.

Compd.	HBD	MW	cLogP	Animal models/ Evaluation index	Preclinical assessment of BBB	Associated with autophagy
VEGFR inhibitor: regorafenib (**7**)	3	483	4.8	BALB/c nude mice or NOD/SCID mice with U87 or U138 cell orthotopic brain tumor/ tumor volume	P-gp substrate limited brain penetration in mice	Inducing lethal autophagy arrest by stabilizing PSAT1 in GBM[184]
EGFRvIII inhibitor: afatinib (**18**)	2	486	4.1	Athymic mice with U373 cell orthotopic brain tumor/Tumor volume[47]	P-gp and Bcrp substrate	EGFRvIII expressing xenografts depend on autophagy for accelerated regrowth after therapy[185]
EGFR inhibitor: AZD3759 (**20**)	1	460	4.4	Triple-knockout mice/ brain-to-plasma ratios	High rat $K_{puu, brain}$ and $K_{puu, CSF}$ in rat; in cyno by PET	No data reported
EGFRvIII inhibitor: NT113 (**21**)	2	506	4.8	Athymic mice with transplanted subcutaneously human GBM tissues/percent survival	High mouse B/P	No data reported
Pan-PI3K inhibitor: BKM120 (**26**)	2	410	2.3	Nude rats carrying orthotopic U87 MG xenografts/Percent survival	Effective inhibition of pAKT in rat/human brain tissue	**26** can induce autophagy. The use of **26** with autophagy inhibitors is a good strategy[186]
Pan-PI3K inhibitor: GDC-0084 (**30**)	2	382	0.3	Mice bearing U87 or GS2 intracranial tumors/ Tumor volume[67]	High mice [Brain]$_u$/ [Plasma]$_u$	No data reported
Aurora A inhibitor: MLN8237 (**58**)	2	519	6.2	Intracerebral-4687 GBM tumor mice/percent survival	Effectively crosses the BBB in PDOX models of pGBM	58 may induce autophagy by decreasing TPR expression[187]

HBD, hydrogen bond donor; MW, molecular weight; PSAT1, phosphoserine aminotransferase 1; TPR, translocated promoter region.

cells, inducing immunogenic cell death, and stimulating host anti-tumor immunity[178]. Daiichi Sankyo recently announced that OV therapy Delytact (teserpaturev/G47Δ) has been granted conditional and time-limited approval by the Ministry of Health, Labor and Welfare (MHLW) in Japan for the treatment of malignant glioma. It's the first oncolytic virus ever approved for treatment of malignant glioma or any primary brain cancer.

Oncolytic adenovirus DNX-2401 is a highly reproducible genetically modified virus capable of infecting and killing glioma cells and stimulating anti-tumor immune responses. The results of clinical trials show that DNX-2401 can prolong the survival of patients with recurrent GBM to a certain extent and has good safety[179]. Collectively, DNX-2401 can induce an immune response to the tumor, which may help some patients to fully respond to significantly improve survival. Further studies could be conducted to determine which patients would benefit the most.

The personalized vaccination trial, based on the analysis of mutations and transcriptomes and immunopeptidomes of newly diagnosed GBM, also shows some feasibility and therapeutic potential[180]. Rindopepimut (CDX-110) is a peptide vaccine targeting EGFRvIII, which had been approved for an international phase III trial (NCT01480479) in 745 patients with EGFRvIII-positive GBM. However, the trial result was negative showing no difference in outcome with addition of rindopepimut to standard chemotherapy[181].

DCVax®-L, an autologous dendritic cell vaccine, has completed its phase III trial in 331 patients with newly diagnosed GBM. Preliminary clinical results suggest that adding DCVax®-L to the standard treatment of patients with GBM is feasible, safe, and may extend survival[182]. ICT-107, also an autologous dendritic cell vaccine, has completed a phase II trial in 124 newly diagnosed GBM patients. The results showed that ICT-107 can significantly improve PFS, which has good clinical significance[183].

So far, trials data from autologous dendritic cell vaccines have shown superior therapeutic efficacy to peptide vaccines, and this approach to GBM treatment warrants further study and analyses.

5.   Future perspectives

In conclusion, we discuss the current therapeutic potential of bioactive small molecule inhibitors of different targets for GBM as well as their current clinical progress and future development potential. We screened 7 small molecules (**7**, **18**, **20**, **21**, **26**, **30**, and **58**) with good activity and high therapeutic potential from the mentioned small molecules (selection criteria: good target inhibitory activity; adequate BBB permeability) summarizes their key properties, models and assessment criteria for preclinical use and current advances in autophagy in Table 4.

Currently, most of small molecule inhibitors have failed at the clinical level of GBM treatment. On the one hand, there is a lack of specific targets. Most of the existing targets, such as PI3K and mTOR, are targets of peripheral tumors, which leads to the existence of relatively high toxicity and side effects. On the other hand, due to the restrictions of BBB, it is difficult for drugs to enter the CNS and reach sufficient effective concentration. Most small molecule inhibitors used for GBM treatment are kinase

inhibitors. However, relatively few kinase inhibitors are currently available specifically for GBM treatment. Only several molecules, **20** (EGFR inhibitor), **21** (EGFR inhibitor), **30** (PI3K inhibitor) were designed to achieve the brain penetration. CNS drug candidates not only require high potency and selectivity against the intended therapeutic target, but also optimal brain penetration to achieve the desired pharmacological exposure. For example, off-target and inadequate BBB penetration are major limitations of CDK4/6 inhibitors for GBM treatment. While according to the existing inhibitors and small molecules with satisfyingly biological activity, EGFR inhibitors with adequate BBB permeability, such as **20** and **21**, deserve more research in the treatment of GBM. Furthermore, **30** and **26** of PI3K inhibitors have significant brain penetration.

Chemoresistance is a hallmark of GBM recurrence, and there is strong experimental evidence that chemoresistance and tumor recurrence are associated with stem cell characteristics in GBM. GBM chemical resistance to TMZ can be improved by targeting the highly active ALDH1 associated with GSCs or directly targeting GSCs. Meanwhile, targeted inhibition of autophagy and adaptive unfolded protein response (UPR) may be a strategy to improve the efficacy of chemotherapeutic agents to overcome drug resistance caused by autophagy and UPR. The high genomic heterogeneity of GBM is the main reason for the lack of efficacy of targeted therapy in patients. Inhibition of purine synthesis may be a promising strategy for overcoming therapeutic resistance in GBM, a genome-heterogeneous disease. Meanwhile, to avoid immune escape and tolerance, the dosage design and timing of administration in combination should also be well studied.

Also, elderly patients have become one of the reasons for poor prognostic factors in GBM with the increasing longevity of people in world population and the higher incidence of GBM in elderly patients. The physical condition of elderly patients and whether the damage of drugs to the aging brain is irreversible are also factors that need to be considered in the process of drug development and optimization.

From the perspective of innovative GBM treatment strategies, NSC-CEAd-S-Pk7, an oncolytic adenovirus delivered to across the BBB via NSCs, is feasible and safe in patients with newly diagnosed high-grade glioma. This demonstrates that drug delivery by NSCs may be an innovative and effective central targeting strategy. Other innovations include combining the capacity of natural proteins and viral particulates to cross the BBB with inhibitors that target specific proteins to achieve GBM treatment *via* intravenous injection. Finally, peptide vaccines are an invaluable tool for the application of immunotherapy in the treatment of GBM.

In the treatment of cancer, though, the efficacy of biomacromolecules is increasingly recognized as a priority. However, in the treatment of GBM, small molecule inhibitors have a greater advantage in entering the CNS to exert efficacy due to their relatively simple structure. Meanwhile, the cost of the compound itself is much lower than that of biological macromolecules, which makes it more acceptable to patients. As the molecular mechanism and biomarkers of GBM are more clearly studied in the future, more targeted small molecule compounds with excellent BBB permeability will still be the main research and development direction of GBM treatment drugs. How to combine the advantages of biological macromolecules and small molecule inhibitors to complement each other and bring benefits to GBM patients is also worthy of researchers' consideration and efforts.

Author contributions

Hui Liu and Haopeng Sun are responsible for writing the whole passage. Weimin Qiu, Lei Wang, Chenxi Du, Yanyu Hu, and Yao Chen are in charge of checking and revision. Others are made by Wenyuan Liu and Feng Feng.

Conflicts of interest

The authors declare no competing financial interest.

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