Glioblastoma precision therapy: From the bench to the clinic

Yuan Zhou, Weijian Wu, Hongye Bi, Dayong Yang, Chunzhi Zhang

PII: S0304-3835(20)30041-0

DOI: https://doi.org/10.1016/j.canlet.2020.01.027

Reference: CAN 114669

To appear in: *Cancer Letters*

Received Date: 15 November 2019

Revised Date: 22 January 2020

Accepted Date: 23 January 2020

Please cite this article as: Y. Zhou, W. Wu, H. Bi, D. Yang, C. Zhang, Glioblastoma precision therapy: From the bench to the clinic, *Cancer Letters*, https://doi.org/10.1016/j.canlet.2020.01.027.

Glioblastoma precision therapy: From the bench to the clinic

Yuan Zhou[1,2*], Weijian Wu[3*], Hongye Bi[4*], Dayong Yang[3#], Chunzhi Zhang[1#]

[1]Department of Radiation Oncology, Tianjin Hospital, Tianjin 300211, China

[2]Tianjin Medical University, Tianjin 300070, China

[3]Frontier Science Center for Synthetic Biology, Key Laboratory of Systems Bioengineering (MOE), School of Chemical Engineering and Technology, Tianjin University, Tianjin 300350, P.R. China

[4]Department of Neurology, Tianjin Union Hospital, Tianjin 300000, China

[*]Yuan Zhou, Weijian Wu and Hongye Bi contributed equally to this work.

[#]Corresponding authors: Chunzhi Zhang (zhchzh_6@hotmail.com) and Dayong Yang (dayong.yang@tju.edu.cn)

Abstract

Glioblastoma (GBM) is the most common malignancy of the central nervous system, and most patients with GBM die of the disease despite standard treatment. By clarifying the molecular abnormalities that drive the malignant phenotype of GBM, various drugs that specifically target tumor cells and the tumor microenvironment have been developed. These drugs, including drugs targeting growth factor receptors and their downstream signaling pathways, angiogenesis, aberrant metabolism, epigenetic deregulation, and aberrant immune microenvironments, have been investigated in preclinical or clinical trials. However, these drugs that significantly inhibited the growth of GBM in the preclinical stage have not produced survival benefits in patients with GBM. One reason for their failure is the lack of a definite driver gene to select patients most likely to benefit. Another reason is the inadequate pharmacokinetic properties of the drugs owing of the blood-brain barrier. In the present review, we discuss progress in the development of target therapeutic strategies. Furthermore, we discuss the development of nanomaterials that act as local drug delivery systems to penetrate the blood-brain barrier for managing GBM.

Keyword: glioblastoma; targeted therapy; antiangiogensis; immunotherapy; nanoparticles; targeting drug delivery

1. Introduction

With the introduction of next-generation sequencing and computable analytical approaches into clinical practice, large-scale genomic profiling of malignant samples from patients to identify specific genomic alterations that guide the selection of optimal therapies for individual patients has become possible [1]. This is termed precision medicine, which is nearly synonymous with targeted therapy. The presence of defined driver genes determines the rational use of individual drugs. After various studies of the molecular mechanism of carcinogenesis, researchers identified some driver genes and produced drugs targeting these alterations. Moreover, these drugs have been successful in the clinical treatment of cancer, including trastuzumab in *HER2*-positive breast cancer [2], erlotinib and gefitinib in lung adenocarcinoma harboring sensitizing *epidermal growth factor receptor*(*EGFR*) mutations [3], and vemurafenib and dabrafenib in $BRAF^{V600E}$ melanoma patients [4-5].

Glioblastoma (GBM), which is the most common primary malignant tumor in the brain, has a poor prognosis despite treatment with surgery, radiotherapy, and chemotherapy. Although the addition of tumor-treating fields has improved survival in patients with GBM, the cost is prohibitive, and overall survival (OS) is extended by only 4.9 months [6]. With progress in understanding the molecular biology underlying GBM, some aberrant molecules in several signaling pathways were found, such as aberrant activation of receptor tyrosine kinase (RTK) genes, activation of the phosphatidylinositol-3-OH kinase (PI3K) pathway, and inactivation of the p53 and retinoblastoma tumor suppressor pathways [7]. According to TCGA data, GBM has been divided into different subtypes based on these aberrant molecules, namely proneural, classical, and mesenchymal GBM [8]. The intertumoral heterogeneity of GBM results in a remarkably variable clinical course. Thus, the 2016 WHO classification of GBM incorporated prevalent molecular alterations as adjuncts to the traditional histopathology [9]. Furthermore, these aberrant molecules also represented attractive therapeutic targets for drug development. However, these drugs did not produce clinical benefits in patients with GBM. Thus, anti-angiogenic treatment and immunotherapy have been investigated in clinical trials for GBM.

In the present review, we summarize some advances of targeted therapies, anti-angiogenic treatments, and immunotherapies for GBM. In addition, we also identified factors responsible for the failure of these therapeutics. Furthermore, we described drug-delivery systems for overcoming

the failure of these treatments. Finally, we forecast expected therapeutic improvements for GBM.

2. Target aberrant molecules in GBM

According to the 2016 WHO classification, GBM was divided into isocitrate dehydrogenase (IDH) 1/2 mutant and IDH wild-type groups based on mutations in IDH1/2. IDH wild-type GBM accounts for more than 90% of all cases of GBM [10]. However, this classification is not predictive of patient survival. Therefore, the identification of molecular markers that predict the clinical course of GBM is welcomed. Via integrative analysis of TCGA data, 74% of patients were found to harbor aberrations in the RTK/RAS/PI3K, p53, and RB pathways [7]. Additionally, various drugs targeting these aberrant molecules have been studied in the clinic (Figure 1).

2.1. ***RTK/RAS/PI3K pathway***

Disruption of the RTK/RAS/PI3K pathway is considered to play an important role in the tumorigenesis and progression of GBM. RTKs are membrane-spanning proteins with extracellular ligand-binding domains and intracellular catalytic domains. By binding to extracellular domains, ligands induce RTK oligomerization, which activates the intracellular catalytic domains. RTK activation initiates a signaling cascade that results in specific cellular responses. In the RTK/RAS/PI3K pathway of GBM, the aberrant activation includes frequent EGFR amplification/mutation, ERBB2 mutation, PDGFRA amplification, MET amplification, PI3K mutation, homozygous PTEN deletion/mutation, AKT amplification, FOXO mutation, homozygous NF1 deletion/mutation, and RAS mutation. These aberrant molecules could emerge as therapeutic targets for GBM [7].

RTK-encoding genes, including EGFR, PDGFRA, ERBB2, and MET, have been identified to play important roles in the development of GBM. Among these genes, EGFR is the most frequently aberrant RTK [7]. Because EGFR-targeting small-molecule tyrosine kinase inhibitors (TKIs) have been successful in the treatment of EGFR-mutated lung cancer [11], mutant EGFR is a rational therapeutic target for GBM. However, neither first-generation nor second-generation TKIs have produced satisfactory clinical results in the treatment of GBM [12]. These are several reasons for these failures. EGFR mutation in GBM occurs in the extracellular domain, whereas EGFR mutation in lung adenocarcinoma occurs in the kinase domain. The particular deletion of exons 2–7 of EGFR in GBM, e.g., EGFRvIII, renders TKIs ineffective against GBM. EGFRvIII alters the extracellular domain of EGFR, making the protein constitutively active. The EGFRvIII

peptide vaccine rindopepimut displayed therapeutic efficacy in preclinical models and early-stage trials [13]. However, the addition of rindopepimut to standard therapy failed to improve OS in patients with newly diagnosed EGFRvIII-positive GBM in a phase 3 study [14]. Another EGFRvIII-targeted therapy is ABT-414, which is an antibody drug conjugate consisting of an EGFR-directed monoclonal antibody conjugated to monomethyl auristatin F, an anti-microtubulin agent [15]. In one study of this therapy, the objective response rate was 6.8%, the 6-month progression-free survival (PFS) rate was 28.8%, and the 6-month OS rate was 72.5%. Furthermore, ABT-414 had an acceptable safety and pharmacokinetic profile in patients with GBM [16]. Another study demonstrated that the objective response, 6-month PFS, and 6-month OS rates were 14.3, 25.2, and 69.1%, respectively, for the combination of ABT-414 and temozolomide (TMZ) in patients with EGFR-amplified recurrent GBM [17]. The encouraging clinical efficacy and manageable adverse events of this combination warrant further clinical development. In addition, several EGFR-targeting agents have been evaluated as monotherapies or in combination with other agents or radiotherapy [18]. However, these treatments failed to produce excellent clinical outcomes for patients with GBM. Thus, further studies are needed to evaluate the value of EGFR inhibition in the treatment of EGFR-amplified GBM.

PDGFRA amplification is found in 13% of cases of GBM [7], and it represents another therapeutic target for GBM. Dasatinib, an oral PDGFRA inhibitor, is a multi-targeted kinase inhibitor that also targets Src family kinases, breakpoint cluster region-Abelson murine leukemia, c-kit, and the ephrin receptor. However, the RTOG 0627 trial failed to reveal significant activity of dasatinib against recurrent GBM [19]. MET amplification also represents a therapeutic target for GBM. However, the clinical efficacy of the oral c-Met inhibitor crizotinib against GBM was dismal [20]. Therefore, combinations of RTK inhibitors have been used in patients with GBM. Alberto et al. reported the use of dasatinib plus crizotinib in patients with recurrent or progressive high-grade glioma. The results illustrated that the combination was poorly tolerated, and its activity was minimal [21]. At present, new c-Met inhibitors, such as a drug-dye conjugate between the anaplastic lymphoma kinase inhibitor crizotinib and heptamethine cyanine dye IR-786 [22], are being studied preclinically. As another potential therapeutic target, HER2-specific chimeric antigen receptor-modified virus-specific T cells were used to treat 17 patients with GBM. The results revealed the safety and clinical benefit of this treatment for patients with progressive GBM

[23]. The clinical efficacy of the agent needs further to be evaluated in larger patient groups. In summary, new RTK inhibitors are needed to improve clinical outcomes for patients with GBM.

Because of the disappointing clinical efficacy of these RTK inhibitors, it is attractive to interfere with downstream molecules. In GBMs, PI3K signaling is highly active. Buparlisib, a pan-PI3K inhibitor, has been tested in patients with recurrent GBM. The results indicated that buparlisib achieved significant brain penetration. However, because of its incomplete blockade of the PI3K pathway, buparlisib had minimal efficacy in patients with PI3K-activated recurrent GBM [24]. Hainsworth et al. examined the combination of bevacizumab with PKM120, another oral pan-class I PI3K inhibitor, in patients with relapsed/refractory GBM. The results demonstrated that the combination has similar efficacy as single-agent bevacizumab [25]. Some young patients with GBM carry the oncogenic $BRAF^{V600E}$ mutation. Woo et al. used vemurafenib, a BRAF inhibitor, together with cobimetinib, a MEK inhibitor, to treat two patients with $BRAF^{V600E}$ mutant GBM. The combination treatment produced dramatic clinical responses. However, the duration of disease control was extremely short [26]. At present, new agents, such as the dual PI3K/mTOR inhibitor XL765 [27], Akt inhibitor MK-2206 [28], and dual PI3K/mTOR inhibitor PI-103 [28], are being investigated preclinically for the treatment of GBM.

*2.2. **p53/ARF/MDM2 pathway***

The p53/ARF/MDM2 pathway is another core deregulated pathway in the pathogenesis of GBM pathogenesis, being aberrant in 84% of patients. *TP53* mutations in GBM are mostly point mutations and gain-of-function oncogenic variants. MDM2 and MDM4, which are the upstream molecules of p53, inactivate p53, leading to the loss of tumor suppression. Thus, targeting different molecules to reactivate or restore p53 function could represent promising precision therapy approaches for GBM.

Because p53 point mutations in GBM result in the overexpression of mutant p53 (mut-p53) and gain of function, effective drugs must change the conformation of the mut-p53 protein to reactivate wild-type p53 (wt-p53). PRIMA-1 (2, 2-bis(hydroxymethyl)-3-quinuclidinone), which is a small-molecular-weight compound, may restores the conformation of mut-p53 in GBM. It alters the mutant protein folding to restore the conformation of mutant p53 proteins [29]. Ksenya et al. reported PRIMA-1 significantly inhibited the growth of GBM by normalization of mut-p53. Furthermore, they found that intermittent dosing regimens of PRIMA-1 are more effective than

traditional chronic dosing in restoring wild-type tumor-suppressor function onto mutant, inactive p53 proteins[30]. PRIMA-1^{MET}, which is a methylated form of PRIMA-1, is more active than PRIMA-1. PRIMA-1^{MET} significantly inhibits the tumor growth of GBM cells [31]. Unfortunately, there have been no clinical trials on PRIMA-1 and PRIMA-1^{MET} for treating GBM patients yet. Other agents, such as CP-31398 [32] and the dietary compound PEITC [33], have been examined in preclinical research.

MDM2 amplification occurs in 14% of patients with GBM [7]. MDM2 is an E3 ubiquitin ligase. By inducing the degradation of p53, MDM2 negatively regulates p53. Therefore, inhibition of the MDM2/p53 interaction could be an effective treatment strategy for GBM. Several MDM2 inhibitors, including RG7112, SAR405838, AMG232, and RG7388, successfully suppressed GBM growth in preclinical studies[34]. However, these results must be verified in patients with GBM.

2.3. ***CDK4/CDK6/RB pathway***

The CDK4/CDK6/RB pathway is another core deregulated pathway in the pathogenesis GBM pathogenesis, being aberrant in 78% of patients. Consequently, some CDK4/CDK6 inhibitors have been developed for treating GBM, but their clinical efficacy has been dismal. Taylor et al. reported that monotherapy with palbociclib, an oral CDK4/CDK6 inhibitor, was ineffective for treating recurrent GBM [35]. Tien et al. revaluated the clinical efficacy of the CDK4/CDK6 inhibitor ribociclib in GBM, finding that this treatment was also ineffective as monotherapy against recurrent GBM [36].

3. Targeting angiogenesis in GBM

Histologically, GBM is characterized by increased angiogenesis and an aberrant microvascular network. Therefore, anti-angiogenic therapies represent highly plausible treatment options. In the angiogenesis pathway of GBM, various molecules, such as vascular endothelial growth factor (VEGF), integrins, PDGF and c-kit receptors, play critical roles, making them pharmaceutical targets (Figure 2) [37]. Among these aberrant molecules, VEGF-A was identified as a crucial angiogenic factor in GBM 25 year ago [38]. Bevacizumab, a recombinant and monoclonal IgG1 antibody targeting VEGF-A, significantly improved PFS in patients with recurrent GBM in uncontrolled phase II clinical trials. In 2009, bevacizumab was awarded FDA approval for the treatment of recurrent GBM based on the results of early clinical trials [38].

However, in the RTOG0825 trial, first-line bevacizumab failed to improve OS in patients with newly diagnosed GBM. Moreover, PFS was only prolonged by 3.4 months [39]. Similar findings were also reported in the AVAglio study [39]. Why did the PFS benefit fail to translate into an OS benefit when bevacizumab was added to standard treatment in GBM? The potential explanation is that the PFS benefit was an imaginary effect, or pseudoresponse [40], and no real gain in PFS was achieved. However, an analysis of AVAglio data according to the IDH mutation status suggested that patients with IDH1–wild-type proneural GBM may experience an OS benefit from first-line bevacizumab therapy [40]. Therefore, it is important to identify and establish predictive biomarkers for bevacizumab treatment. However, no such clinical biomarker relevant to bevacizumab has been established to date. Ratai et al. utilized magnetic resonance spectroscopy (MRS) as an early indicator of response to bevacizumab. The results indicated that decreased Cho/Cr and increased NAA/Cr and NAA/Cho levels in the tumor periphery were associated with both 6-month PFS and 1-year OS. Thus, alterations in NAA and Cho levels as assessed via MRS may be useful for predicting the response to bevacizumab treatment [41]. Another study found that increased bidimensional lesion enhancement (2D-T1) predicted significantly worse OS than post-contrast T1-weighted images. Meanwhile, FLAIR added value beyond 2D-T1 in predicting OS after bevacizumab treatment [42].

Another possible cause of a lack of response is primary or adaptive resistance to bevacizumab treatment in GBM. For example, Voutouri et al. reported that vessel cooption is a strategy for promoting tumor growth without new blood vessel growth. Moreover, cooption is also related to tumor resistance to anti-angiogenic therapy. To control GBM growth, it is imperative to co-inhibit angiogenesis and cooption in a sequential mode [43]. Mastrella et al. found that blocking VEGFA/VEGFR2 signaling in GBM downregulated apelin, which is a cognate ligand of the pro-angiogenic apelin receptor APLNR, and accelerated the invasion of APLNR-expressing GBM cells. Apelin-F13A, which is a mutant APLNR ligand, inhibited angiogenesis and cell invasion in GBM. Therefore, co-inhibiting VEGFR2 and APLNR synergistically improved the survival of mice bearing proneural GBM tumors [44]. Scholz et al. reported that angiopoietin-2 (Ang-2) was upregulated in bevacizumab-treated GBM. As a potential resistance mechanism to bevacizumab, Ang-2 mediated endothelial cell/myeloid cell crosstalk and promoted the resistance of endothelial cells to bevacizumab. Furthermore, they found that combined inhibition of VEGF

and Ang-2 improved survival, decreased vascular permeability, depleted tumor-associated macrophages, enhanced pericyte coverage, and increased the numbers of intratumoral T lymphocytes [45]. In addition, VEGF and integrins are mutual positive regulators of each other. Gerstner et al. examined the combination of cediranib (an oral pan-VEGFR tyrosine kinase inhibitor) with cilengitide (an integrin inhibitor with anti-invasive and anti-angiogenic properties) in patients with recurrent GBM, but the treatment failed to significantly improve survival [46].

For the success of anti-angiogenic therapy against GBM, new anti-angiogenic agents and new therapeutic strategies combining bevacizumab with other agents, such as TMZ [47], the oral PI3K inhibitor BKM120 [25], and dasatinib [48], have been developed. Kebir et al. reported that regorafenib, a potent multi-kinase inhibitor, produced disappointing results in six patients with recurrent high-grade astrocytoma [49]. However, anti-angiogenic therapy, mainly bevacizumab, can lower steroid use and improve quality of life and patient performance. Based on these findings, bevacizumab remains in use in later stages of GBM in the US and Europe even though the drug has not be authorized in Europe for recurrent GBM [50].

4. Targeting aberrant metabolism in GBM

Aberrant cellular metabolism is a hallmark of cancer. GBM cells also exploit altered metabolic pathways to maintain malignant progression, such as increasing glycolysis, lipid metabolism, and glutamine metabolism [51-52]. These aberrant metabolic pathways represent potential therapeutic targets for GBM (Figure 3). Sanzey et al. reported that certain genes, which mainly encode glycolytic enzymes, are upregulated in response to severe hypoxia in GBM cells. Meanwhile, depletion of these genes (*HK2*, *PFKP*, *ALDOA*, *ENO1*, *ENO2*, and *PDK1*) significantly inhibited the growth of GBM. In particular, PFKP and PDK1 were proposed as the most promising therapeutic targets for GBM [53]. Kofuji et al. found that IMP dehydrogenase-2 (IMPDH2), the rate-limiting enzyme for de novo guanine nucleotide biosynthesis, was overexpressed in GBM. IMPDH2 overexpression was linked to increased rRNA and tRNA synthesis, stabilization of the nucleolar GTP-binding protein nucleostemin, and enlarged, malformed nucleoli. IMPDH2 inhibition reversed these effects and inhibited cell proliferation in GBM [54]. More importantly, Bag et al. demonstrated that GBM required synchronization of growth-driving signaling and metabolic pathways. So, they defined cancer-specific signaling-metabolic interconnected networks [55]. Some studies reported that aberrant signaling

pathways play critical roles in rewiring cellular metabolic reprogramming in GBM. For example, 36% of GBM cells feature homozygous PTEN deletion/mutation. Qian et al. demonstrated that PTEN directly interacted with phosphoglycerate kinase 1, which acts as a glycolytic enzyme and protein kinase via intermolecular autophosphorylation, to inhibit its autophosphorylation. This results in the inhibition of glycolysis, ATP production, and GBM cell proliferation [56]. Liu et al. found that high levels of glucose or glutamine blocked mTOR dimerization and promoted mTOR2 protein activity by enhancing the release of mTOR2 from the mTOR complex. Then, mTOR2 upregulated c-myc, which transcriptionally regulated the expression of fructose-6-phosphate aminotransferase 1 (GFAT1). Finally, GFAT1 promoted the growth of GBM cells by increasing glycolysis [57]. Wang et al. uncovered that the metabolic aberrations in GBM stem cells (GSCs) link core genetic mutations in GBM to dependency on de novo pyrimidine synthesis. Targeting the pyrimidine synthetic critical downstream enzyme dihydroorotate dehydrogenase (DHODH) inhibited GSC survival, self-renewal, and tumor initiation in vivo [58]. Echizenya et al. found that 10580, which effectively antagonizes the enzymatic activity of DHODH, induced cell cycle arrest and decreased the expression of stem cell factors in GSCs [59].

In additions, two metabolic enzymes, IDH1 and PKM2, change the phenotype of GBM cells by regulating their epigenetics. Mutation of IDH1 leads to the production of 2-hydroxyglutaric acid (2-HG) from alpha ketoglutarate (α-KG). 2-HG induces a hypermethylator phenotype in glioma by competitively inhibiting multiple α-KG–dependent dioxygenases, including histone demethylases and the ten-eleven translocation family of 5-methylcytosine hydroxylases. It has been identified that AGI-5198, which is a selective R132H-IDH1 inhibitor, inhibited the activity of mutant IDH1 to produce 2-HG and promoted gliomagenic differentiation in vitro and in vivo [60]. Recently, another compound (AG120), which targets mutant IDH1, was approved by the FDA for the treatment of relapsed or refractory IDH1-mutated acute myeloid leukemia [61]. Whether AG120 is adaptive to glioma with IDH1 mutation remains to be clarified.

PKM2 is the last rate-limiting enzyme in glycolysis, and it catalyzes the conversion of phosphoenolpyruvate to pyruvate. PKM2 can form active tetramers in normal cells and inactive dimers in cancer [62]. Usually, inactivated PKM2 promotes tumor growth. EGFR phosphorylates PKM2 and induces its translocation to the nucleus. In the nucleus, PKM2 phosphorylates histone 3 at threonine 11 (H3-T11) to facilitate the dissociation of HDAC3 from the promoters of β-catenin

target genes [62]. In addition, PKM2 directly interacts with JMJD5, a Jumonji C domain-containing dioxygenase, and prolylhydroxylase 3. PKM2 also acts as an attractive target for treating GBM. Dimethylaminomicheliolide (DMAMCL), a small-molecule compound, has been used in clinical trials for recurrent GBM. PKM2 activation by DMAMCL results in the rewiring of aerobic glycolysis and suppression of GBM cell proliferation [63].

5. Targeting aberrant epigenetic deregulation in GBM

Epigenetic modifications also play important roles in the development of GBM. Four interconnected layers mediate epigenetic modifications in GBM, including DNA methylation, histone modification, chromatin remodeling, and non-coding RNA [64]. However, targeting DNA methylation and non-coding RNAs is restricted in research [65-66]. Therefore, the present review will mainly discuss histone modification and chromatin remodeling in regulating GBM, as well as some advances in targeting these aberrant epigenetic regulators (Figure 4).

In GBM, histones are modified by acetylation and methylation to promote transient drug resistance [67]. The addition of acetyl groups to H3 and H4 lysines favors transcription, whereas deacetylation removes these acetyl groups and represses transcription. These processes are regulated by histone acetyltransferases and histone deacetylases (HDACs). HDACs are upregulated in GBM, making them potential therapeutic targets. The FDA has approved several HDAC inhibitors, including vorinostat, romidepsin, belinostat, panobinostat, valproic acid, and entinostat. Unfortunately, the excellent performance of these drugs in preclinical experiments did not translate into survival benefits for patients with GBM [68].

Histone methylation is the other mode of histone modification. Methylating or demethylating certain lysines and arginines of H3 and H4 of histone can activate or repress transcription. In pediatric high-grade glioma, *H3F3A*, encoding the histone variant H3.3, develops mutations involving the histone tail at lysine (K) 27(K27M) and glycine (G) 34 (G34R/V). These two critical single-point mutations are involved in key regulatory post-transcriptional modifications [69]. Moreover, the K27M H3.3 mutation mainly occurs in the brainstem and midline regions in pediatric patients with glioma, and it is a poor prognostic factor [70]. Additionally, PRC2-EZH2 methylases and UTX (KDM6A) and KDM6B demethylases are also involved in regulating the methylation status of H3K27. The small-molecule GSK-J4 suppresses the growth of GBM by inhibiting the activity of KDM6B [71]. Meanwhile, targeting EZH2 also reverses tumor growth by

modulating histone methylation. Wiese et al. attempted to use tazemetostat, an EZH2 inhibitor, to treat pediatric patients with glioma and wild-type or mutated H3, finding that the drug was ineffective against pediatric high-grade glioma [72]. Differing from pediatric high-grade gliomas, adult GBM seldom features mutation of H3.3-*H3FA*, and it commonly overexpresses histone demethylases, such as KDM1. Pharmacological inhibitors of KDM1 upregulate p53 target genes, increase the levels of H3K4-me2 and H3K9-Ac histone modification, and reduce H3K9-me2 modification. These changes promote the apoptosis of glioma cells [73]. However, the relevant studies are in the preclinical stage.

Chromatin remodeling is an extremely complex and difficult target for drugs. The tumor suppressor SWI/SNF complex and PARP-1 polymerase are involved in chromatin remodeling. PARP inhibition has been studied for treating GBM [74].

6. Targeting aberrant immune checkpoints in GBM

Immunotherapies, including vaccines, oncolytic viral therapies, and chimeric antigen receptor T cell therapies, have led to improvements in the survival of patients with GBM [75-76]. The figure 5 showed that some aberrant immune checkpoints are responsible for immunosuppression, excluding the special immune microenvironment of the brain. For example, CTLA-4 and PD-1/programmed death-ligand 1 (PD-L1) are overexpressed in GBM, making them potential therapeutic targets. Moreover, anti-PD-1 and anti-CTLA-4 therapies have produced promising results in SB28 and GL261 mouse glioma models [77]. However, the CheckMate 143 trial failed to demonstrate the superiority of nivolumab over bevacizumab in patients with recurrent GBM [78]. In addition, TIM-3 also acts as an immune checkpoint to promote immune escape by exhausting T cells. High TIM-3 expression is an independent indictor of poor prognosis in patients with GBM. In preclinical experiments, combined anti-TIM-3 and radiotherapy/anti-PD-1 therapy remarkably improved survival in mice with GBM [79]. IDO also suppresses T cell activation and NK cell function. Meanwhile, IDO is highly expressed in GBM and involved in tumor immune escape. Sun et al. found that the IDO inhibitor PCC0208009 significantly enhanced the efficacy of TMZ in GL261 and C6 models [80]. Until today, no breakthrough advancements have been made, and these therapies are not standard treatments. Thus, additional work is needed to elucidate the mechanism of immunosuppression and improve the efficacy of immunotherapy in GBM.

7. Development of targeting drug delivery using nanomaterials

The treatment of GBM remains challenging because the blood-brain barrier (BBB) restricts paracellular diffusion between blood capillaries and the central nervous system (CNS) [81]. The BBB consists of brain capillary endothelial cells (BCECs) surrounded by astrocytic perivascular pseudopodium and pericytes through the basal lamina (Fig. 6A) [82]. The tight junctions between BCECs form a compact barrier that limits the paracellular transport of more than 98% of small molecules [83]. In recent years, nanoparticles (NPs) have become increasingly important for transporting therapeutic agents in patients with GBM. NPs can both improve penetration across the BBB and increase drug uptake by brain tumor cells. The size and surface characteristics of NPs offer them the capacity for passive or active targeting to brain cancer cells or tumor endothelium in a desirable manner, thus minimizing side effects. In addition, nanocarriers can protect drugs in vivo and prolong their circulation half-life.

Size plays a vital role in the accumulation of NPs in tumors [84]. Generally, NPs ranging from 20 to 200 nm in size can facilitate improved tumor accumulation because of the enhanced permeability and retention (EPR) effect, which was considered passive targeting [85]. The basic features of EPR physiology include the highly permeable tumor vasculature, which enhances the permeability of large particles including macromolecules, liposomes, and other soluble particles (Fig. 6B). Regarding delivery to GBM lesions, the optimal diameter of NPs ranges from 30 to 100 nm [84]. Liu et al. studied the effect of size on BBB penetration using AIEgen (fluorogens with aggregation-induced emission characteristics) NPs with sizes of 10, 30, and 60 nm [86]. In a photothrombotic ischemia rat model, 30-nm AIEgen NPs produced the best effect in the BBB damage evaluation. In another example, after focused ultrasound-mediated BBB opening, 60-nm polystyrene–PEG NPs exhibited better diffusion than 110-nm NPs in the normal rat brain [87]. In addition, ultrasmall NPs (<6 nm) may be cleared rapidly by the kidneys [88]. Thus, for brain tumor delivery, NPs ranging in size from 20 to 70 nm are considered ideal [89].

Various targeting moieties can be used for the surface modification of NPs to allow active targeting of the drug carriers to the BBB and tumor tissues. Antibodies, proteins, peptides, and nucleic acids are widely used as targeting elements in NPs (Fig. 6B). The main targets identified for the treatment of glioma include transferrin (Tf) receptors (TfRs), folate, epidermal growth factor receptor (EGFR), VEGF, $\alpha_v\beta_3$ integrins, matrix metalloproteinases (MMPs), lipoprotein receptor-related protein, and vascular cell adhesion molecule 1 [90]. For example, TfR-1 is

overexpressed in both the BBB and many types of brain tumor cells. Several Tf-modified NPs have been studied for delivering drugs to the brain. Voelcker and coworkers described a Tf-based dual-targeting method for serial BBB penetration and glioma targeting [91]. In their work, they developed Tf-modified pSiNPs as targeted nanocarriers to promote small-molecular chemotherapeutic delivery into gliomas. Systematical studies proved that Tf functionalization allowed pSiNPs to target gliomas and increased their internalization efficiency through clathrin-mediated endocytic mechanisms. In monoculture and co-culture BBB models, pSiNPs was more toxic to GBM cells. The EGFP–EGF1 fusion protein can bind to tissue factors overexpressed in glioma cells, and it has been used as a conjugate to target NPs to brain tumors [92]. In vivo studies illustrated that the penetration of EGFP–EGF1 NPsin tumor tissues was 2.38-fold greater than that of NPs without EGFP–EGF1 conjugation. Chlorotoxin (CTX) is one of the most important targeting agents for glioma because of its high affinity for chloride channels and MMP-2 isoforms, which are upregulated in brain cancer tissues. Fang et al. developed CTX-conjugated chitosan (CS) nanocarriers loaded with TMZ (CS–TMZ–CTX) for GBM therapy [93]. The in vivo study found that 2 h after i.v. injection, CS–TMZ–CTX widely spread in the brain, including both parts distal from blood vessels and avascular regions. Aptamers are usually DNA or RNA sequences or peptides that can identify targets on cells and bind to them specifically. A variety of aptamers that target glioma are available. For example, recent studies demonstrated that the AS1411 aptamer forms a stable G-quartet structure to bind with nucleolin in GBM cells. Xie and coworkers used the AS1411 aptamer to functionalize a Se-based NP loaded with ruthenium complexes to achieve tumor imaging and therapy [94]. The results indicated that the cellular uptake and selectivity of this NP were significantly improved. In additions, we have developed a novel magnetic field-controlled DNA nanogel for targeted drug delivery[95]. We synthesized the carrier as a core-shell structure with magnetic Fe_3O_4 nanoparticle core and DNA shell. Under the control of magnetic field, an enhanced targeted drug delivery to U87MG cells was observed. This research provides a new way to realize targeted delivery of drugs to brain.

8. Concluding remarks

Understanding of aberrant molecular pathways that drive the malignant phenotype of GBM will facilitate the development of precise medicine for GBM. However, the table 1, which summarized all clinical results of targeted therapies against GBM mentioned above, showed that

the clinical efficacy is extremely disappointing, although several explanations can be postulated. First, GBM is a complex ecosystem composed of non-tumor cells, such as microglia, astrocytes, and neurons. These non-tumor cells interact with GBM cells to affect tumor growth and sensitivity to treatment. Second, intratumoral heterogeneity, such as distinct phenotypes, genotypes, and epigenetic states, results in the lack of a unified sensitivity to therapy. More importantly, the BBB limits the access of drugs to GBM lesions. Despite their efficacy against a few malignancies, immune checkpoint inhibitors have not produced survival advantages in GBM. The unique immunological microenvironment and lymphocytic infiltration deficiency are two main reasons for this lack of efficacy. Finally, the rapid development of resistant phenotypes is responsible for the failure of various targeted therapies. In sum, it is imperative to explore the mechanism of GBM development systematically to identify new treatment targets. Various molecules can be used to stratify patients with GBM who will experience survival benefits from various treatments. Regarding immunotherapy, it is a priority to understand the immune microenvironment of GBM.

Acknowledgement

We thank Joe Barber Jr., PhD, from Liwen Bianji, Edanz Editing China (www.liwenbianji.cn/ac), for editing the English text of a draft of this manuscript. This work was supported in part by Tianjin Natural Science Foundation (Basic research plan, Grant Nos.: 18JCJQJC47600).

Conflict of interest statement

We declare that we have no conflict of interest

References

[1] W.S. El-Deiry, R.M. Goldberg, H.J. Lenz, A.F. Shields, G.T. Gibney, A.R. Tan, J. Brown, B. Eisenberg, E.I. Heath, S. Phuphanich, E. Kim, A.J. Brenner, J.L. Marshall, The current state of molecular testing in the treatment of patients with solid tumors, 2019, CA Cancer J Clin, 69 (2019) 305-343.

[2] G. von Minckwitz, C.S. Huang, M.S. Mano, S. Loibl, E.P. Mamounas, M. Untch, N. Wolmark, P. Rastogi, A. Schneeweiss, A. Redondo, H.H. Fischer, W. Jacot, A.K. Conlin, C. Arce-Salinas, I.L. Wapnir, C. Jackisch, M.P. DiGiovanna, P.A. Fasching, J.P. Crown, P. Wulfing, Z. Shao, E. Rota Caremoli, H. Wu, L.H. Lam, D. Tesarowski, M. Smitt, H. Douthwaite, S.M. Singel, C.E. Geyer, Jr., K. Investigators, Trastuzumab Emtansine for Residual Invasive HER2-Positive Breast Cancer, N Engl J Med, 380 (2019) 617-628.

[3] R.S. Herbst, D. Morgensztern, C. Boshoff, The biology and management of non-small cell lung cancer, Nature, 553 (2018) 446-454.

[4] S. Corre, N. Tardif, N. Mouchet, H.M. Leclair, L. Boussemart, A. Gautron, L. Bachelot, A. Perrot, A. Soshilov, A. Rogiers, F. Rambow, E. Dumontet, K. Tarte, A. Bessede, G.J. Guillemin, J.C. Marine, M.S. Denison, D. Gilot, M.D. Galibert, Sustained activation of the Aryl hydrocarbon Receptor transcription factor promotes resistance to BRAF-inhibitors in melanoma, Nat Commun, 9 (2018) 4775.

[5] L. Heinzerling, T.K. Eigentler, M. Fluck, J.C. Hassel, D. Heller-Schenck, J. Leipe, M. Pauschinger, A. Vogel, L. Zimmer, R. Gutzmer, Tolerability of BRAF/MEK inhibitor combinations: adverse event evaluation and management, ESMO Open, 4 (2019) e000491.

[6] R. Stupp, S. Taillibert, A. Kanner, W. Read, D. Steinberg, B. Lhermitte, S. Toms, A. Idbaih, M.S. Ahluwalia, K. Fink, F. Di Meco, F. Lieberman, J.J. Zhu, G. Stragliotto, D. Tran, S. Brem, A. Hottinger, E.D. Kirson, G. Lavy-Shahaf, U. Weinberg, C.Y. Kim, S.H. Paek, G. Nicholas, J. Bruna, H. Hirte, M. Weller, Y. Palti, M.E. Hegi, Z. Ram, Effect of Tumor-Treating Fields Plus Maintenance Temozolomide vs Maintenance Temozolomide Alone on Survival in Patients With GBM: A Randomized Clinical Trial, JAMA, 318 (2017) 2306-2316.

[7] N. Cancer Genome Atlas Research, Comprehensive genomic characterization defines human GBM genes and core pathways, Nature, 455 (2008) 1061-1068.

[8] Q. Wang, B. Hu, X. Hu, H. Kim, M. Squatrito, L. Scarpace, A.C. deCarvalho, S. Lyu, P. Li, Y. Li, F. Barthel, H.J. Cho, Y.H. Lin, N. Satani, E. Martinez-Ledesma, S. Zheng, E. Chang, C.G. Sauve, A. Olar, Z.D. Lan, G. Finocchiaro, J.J. Phillips, M.S. Berger, K.R. Gabrusiewicz, G. Wang, E. Eskilsson, J. Hu, T. Mikkelsen, R.A. DePinho, F. Muller, A.B. Heimberger, E.P. Sulman, D.H. Nam, R.G.W. Verhaak, Tumor Evolution of Glioma-Intrinsic Gene Expression Subtypes Associates with Immunological Changes in the Microenvironment, Cancer Cell, 32 (2017) 42-56 e46.

[9] D.N. Louis, A. Perry, G. Reifenberger, A. von Deimling, D. Figarella-Branger, W.K. Cavenee, H. Ohgaki, O.D. Wiestler, P. Kleihues, D.W. Ellison, The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary, Acta Neuropathol, 131 (2016) 803-820.

[10] K. Aoki, H. Nakamura, H. Suzuki, K. Matsuo, K. Kataoka, T. Shimamura, K. Motomura, F. Ohka, S. Shiina, T. Yamamoto, Y. Nagata, T. Yoshizato, M. Mizoguchi, T. Abe, Y. Momii, Y. Muragaki, R. Watanabe, I. Ito, M. Sanada, H. Yajima, N. Morita, I. Takeuchi, S. Miyano, T. Wakabayashi, S. Ogawa, A. Natsume, Prognostic relevance of genetic alterations in diffuse lower-grade gliomas, Neuro Oncol, 20 (2018) 66-77.

[11] K. Nakagawa, E.B. Garon, T. Seto, M. Nishio, S. Ponce Aix, L. Paz-Ares, C.H. Chiu, K. Park, S. Novello, E. Nadal, F. Imamura, K. Yoh, J.Y. Shih, K.H. Au, D. Moro-Sibilot, S. Enatsu, A. Zimmermann, B. Frimodt-Moller, C. Visseren-Grul, M. Reck, R.S. Investigators, Ramucirumab plus erlotinib in patients with untreated, EGFR-mutated, advanced non-small-cell lung cancer (RELAY): a randomised, double-blind, placebo-controlled, phase 3 trial, Lancet Oncol, (2019).

[12] M.M. Kwatra, A Rational Approach to Target the Epidermal Growth Factor Receptor in GBM, Curr Cancer Drug Targets, 17 (2017) 290-296.

[13] D.C. Binder, E. Ladomersky, A. Lenzen, L. Zhai, K.L. Lauing, S.D. Otto-Meyer, R.V. Lukas, D.A. Wainwright, Lessons learned from rindopepimut treatment in patients with EGFRvIII-expressing GBM, Transl Cancer Res, 7 (2018) S510-S513.

[14] M. Weller, N. Butowski, D.D. Tran, L.D. Recht, M. Lim, H. Hirte, L. Ashby, L. Mechtler, S.A. Goldlust, F. Iwamoto, J. Drappatz, D.M. O'Rourke, M. Wong, M.G. Hamilton, G. Finocchiaro, J. Perry, W. Wick, J. Green, Y. He, C.D. Turner, M.J. Yellin, T. Keler, T.A. Davis, R. Stupp, J.H. Sampson, A.I.t. investigators, Rindopepimut with temozolomide for patients with newly diagnosed, EGFRvIII-expressing GBM (ACT IV): a randomised, double-blind, international phase 3 trial, Lancet Oncol, 18 (2017) 1373-1385.

[15] D.A. Reardon, A.B. Lassman, M. van den Bent, P. Kumthekar, R. Merrell, A.M. Scott, L. Fichtel, E.P. Sulman, E. Gomez, J. Fischer, H.J. Lee, W. Munasinghe, H. Xiong, H. Mandich, L. Roberts-Rapp, P. Ansell, K.D. Holen, H.K. Gan, Efficacy and safety results of ABT-414 in combination with radiation and temozolomide in newly diagnosed GBM, Neuro Oncol, 19 (2017) 965-975.

[16] M. van den Bent, H.K. Gan, A.B. Lassman, P. Kumthekar, R. Merrell, N. Butowski, Z. Lwin, T. Mikkelsen, L.B. Nabors, K.P. Papadopoulos, M. Penas-Prado, J. Simes, H. Wheeler, T. Walbert, A.M. Scott, E. Gomez, H.J. Lee, L. Roberts-Rapp, H. Xiong, E. Bain, P.J. Ansell, K.D. Holen, D. Maag, D.A. Reardon, Efficacy of depatuxizumab mafodotin (ABT-414) monotherapy in patients with EGFR-amplified, recurrent GBM: results from a multi-center, international study, Cancer Chemother Pharmacol, 80 (2017) 1209-1217.

[17] A.B. Lassman, M.J. van den Bent, H.K. Gan, D.A. Reardon, P. Kumthekar, N. Butowski, Z. Lwin, T. Mikkelsen, L.B. Nabors, K.P. Papadopoulos, M. Penas-Prado, J. Simes, H. Wheeler, T. Walbert, A.M. Scott, E. Gomez, H.J. Lee, L. Roberts-Rapp, H. Xiong, P.J. Ansell, E. Bain, K.D. Holen, D. Maag, R. Merrell, Safety and efficacy of depatuxizumab mafodotin + temozolomide in patients with EGFR-amplified, recurrent GBM: results from an international phase I multicenter trial, Neuro Oncol, 21 (2019) 106-114.

[18] L. Ma, C. She, Q. Shi, Q. Yin, X. Ji, Y. Wang, Y. Fan, X. Kong, P. Li, Z. Sun, X. Zhang, Z. Zhang, J. Wang, T. Wang, Y. Xu, W. Li, TNFalpha inhibitor C87 sensitizes EGFRvIII transfected GBM cells to gefitinib by a concurrent blockade of TNFalpha signaling, Cancer Biol Med, 16 (2019) 606-617.

[19] A.B. Lassman, S.L. Pugh, M.R. Gilbert, K.D. Aldape, S. Geinoz, J.H. Beumer, S.M. Christner, R. Komaki, L.M. DeAngelis, R. Gaur, E. Youssef, H. Wagner, M. Won, M.P. Mehta, Phase 2 trial of dasatinib in target-selected patients with recurrent GBM (RTOG 0627), Neuro Oncol, 17 (2015) 992-998.

[20] A.S. Chi, T.T. Batchelor, E.L. Kwak, J.W. Clark, D.L. Wang, K.D. Wilner, D.N. Louis, A.J. Iafrate, Rapid radiographic and clinical improvement after treatment of a MET-amplified recurrent GBM with a mesenchymal-epithelial transition inhibitor, J Clin Oncol, 30 (2012) e30-33.

[21] A. Broniscer, S. Jia, B. Mandrell, D. Hamideh, J. Huang, A. Onar-Thomas, A. Gajjar, S.C. Raimondi, R.G. Tatevossian, C.F. Stewart, Phase 1 trial, pharmacokinetics, and pharmacodynamics of

dasatinib combined with crizotinib in children with recurrent or progressive high-grade and diffuse intrinsic pontine glioma, Pediatr Blood Cancer, 65 (2018) e27035.

[22] P.J. Choi, E. Cooper, P. Schweder, E. Mee, R. Faull, W.A. Denny, M. Dragunow, T.I. Park, J. Jose, The synthesis of a novel Crizotinib heptamethine cyanine dye conjugate that potentiates the cytostatic and cytotoxic effects of Crizotinib in patient-derived GBM cell lines, Bioorg Med Chem Lett, 29 (2019) 2617-2621.

[23] N. Ahmed, V. Brawley, M. Hegde, K. Bielamowicz, M. Kalra, D. Landi, C. Robertson, T.L. Gray, O. Diouf, A. Wakefield, A. Ghazi, C. Gerken, Z. Yi, A. Ashoori, M.F. Wu, H. Liu, C. Rooney, G. Dotti, A. Gee, J. Su, Y. Kew, D. Baskin, Y.J. Zhang, P. New, B. Grilley, M. Stojakovic, J. Hicks, S.Z. Powell, M.K. Brenner, H.E. Heslop, R. Grossman, W.S. Wels, S. Gottschalk, HER2-Specific Chimeric Antigen Receptor-Modified Virus-Specific T Cells for Progressive GBM: A Phase 1 Dose-Escalation Trial, JAMA Oncol, 3 (2017) 1094-1101.

[24] P.Y. Wen, M. Touat, B.M. Alexander, I.K. Mellinghoff, S. Ramkissoon, C.S. McCluskey, K. Pelton, S. Haidar, S.S. Basu, S.C. Gaffey, L.E. Brown, J.E. Martinez-Ledesma, S. Wu, J. Kim, W. Wei, M.A. Park, J.T. Huse, J.G. Kuhn, M.L. Rinne, H. Colman, N.Y.R. Agar, A.M. Omuro, L.M. DeAngelis, M.R. Gilbert, J.F. de Groot, T.F. Cloughesy, A.S. Chi, T.M. Roberts, J.J. Zhao, E.Q. Lee, L. Nayak, J.R. Heath, L.L. Horky, T.T. Batchelor, R. Beroukhim, S.M. Chang, A.H. Ligon, I.F. Dunn, D. Koul, G.S. Young, M.D. Prados, D.A. Reardon, W.K.A. Yung, K.L. Ligon, Buparlisib in Patients With Recurrent GBM Harboring Phosphatidylinositol 3-Kinase Pathway Activation: An Open-Label, Multicenter, Multi-Arm, Phase II Trial, J Clin Oncol, 37 (2019) 741-750.

[25] J.D. Hainsworth, K.P. Becker, T. Mekhail, S.A. Chowdhary, J.F. Eakle, D. Wright, R.M. Langdon, K.J. Yost, G.D.A. Padula, K. West-Osterfield, M. Scarberry, C.A. Shaifer, M. Shastry, H.A. Burris, 3rd, K. Shih, Phase I/II study of bevacizumab with BKM120, an oral PI3K inhibitor, in patients with refractory solid tumors (phase I) and relapsed/refractory GBM (phase II), J Neurooncol, 144 (2019) 303-311.

[26] P.Y.M. Woo, T.C. Lam, J.K.S. Pu, L.F. Li, R.C.Y. Leung, J.M.K. Ho, J.T.F. Zhung, B. Wong, T.S.K. Chan, H.H.F. Loong, H.K. Ng, Regression of BRAF (V600E) mutant adult GBM after primary combined BRAF-MEK inhibitor targeted therapy: a report of two cases, Oncotarget, 10 (2019) 3818-3826.

[27] H. Zhao, G. Chen, H. Liang, Dual PI3K/mTOR Inhibitor, XL765, suppresses GBM growth by inducing ER stress-dependent apoptosis, Onco Targets Ther, 12 (2019) 5415-5424.

[28] C.S. Djuzenova, V. Fiedler, S. Memmel, A. Katzer, D. Sisario, P.K. Brosch, A. Gohrung, S. Frister, H. Zimmermann, M. Flentje, V.L. Sukhorukov, Differential effects of the Akt inhibitor MK-2206 on migration and radiation sensitivity of GBM cells, BMC Cancer, 19 (2019) 299.

[29] Y. Zhang, C. Dube, M. Gibert, Jr., N. Cruickshanks, B. Wang, M. Coughlan, Y. Yang, I. Setiady, C. Deveau, K. Saoud, C. Grello, M. Oxford, F. Yuan, R. Abounader, The p53 Pathway in GBM, Cancers (Basel), 10 (2018).

[30] K. Shchors, A.I. Persson, F. Rostker, T. Tihan, N. Lyubynska, N. Li, L.B. Swigart, M.S. Berger, D. Hanahan, W.A. Weiss, G.I. Evan, Using a preclinical mouse model of high-grade astrocytoma to optimize p53 restoration therapy, Proc Natl Acad Sci U S A, 110 (2013) E1480-1489.

[31] M. Patyka, Z. Sharifi, K. Petrecca, J. Mansure, B. Jean-Claude, S. Sabri, Sensitivity to PRIMA-1MET is associated with decreased MGMT in human GBM cells and GBM stem cells irrespective of p53 status, Oncotarget, 7 (2016) 60245-60269.

[32] J. Wischhusen, U. Naumann, H. Ohgaki, F. Rastinejad, M. Weller, CP-31398, a novel

p53-stabilizing agent, induces p53-dependent and p53-independent glioma cell death, Oncogene, 22 (2003) 8233-8245.

[33] Y.C. Chou, M.Y. Chang, H.T. Lee, C.C. Shen, T. Harnod, Y.J. Liang, R.S. Wu, K.C. Lai, F.T. Hsu, J.G. Chung, Phenethyl Isothiocyanate Inhibits In Vivo Growth of Xenograft Tumors of Human GBM Cells, Molecules, 23 (2018).

[34] A. Berberich, T. Kessler, C.M. Thome, S. Pusch, T. Hielscher, F. Sahm, I. Oezen, L.M. Schmitt, S. Ciprut, N. Hucke, P. Ruebmann, M. Fischer, D. Lemke, M.O. Breckwoldt, A. von Deimling, M. Bendszus, M. Platten, W. Wick, Targeting Resistance against the MDM2 Inhibitor RG7388 in GBM Cells by the MEK Inhibitor Trametinib, Clin Cancer Res, 25 (2019) 253-265.

[35] J.W. Taylor, M. Parikh, J.J. Phillips, C.D. James, A.M. Molinaro, N.A. Butowski, J.L. Clarke, N.A. Oberheim-Bush, S.M. Chang, M.S. Berger, M. Prados, Phase-2 trial of palbociclib in adult patients with recurrent RB1-positive GBM, J Neurooncol, 140 (2018) 477-483.

[36] A.C. Tien, J. Li, X. Bao, A. Derogatis, S. Kim, S. Mehta, N. Sanai, A Phase 0 Trial of Ribociclib in Recurrent GBM Patients Incorporating a Tumor Pharmacodynamic- and Pharmacokinetic-Guided Expansion Cohort, Clin Cancer Res, 25 (2019) 5777-5786.

[37] C. Anthony, N. Mladkova-Suchy, D.C. Adamson, The evolving role of antiangiogenic therapies in GBM multiforme: current clinical significance and future potential, Expert Opin Investig Drugs, 28 (2019) 787-797.

[38] F. Winkler, M. Osswald, W. Wick, Anti-Angiogenics: Their Role in the Treatment of GBM, Oncol Res Treat, 41 (2018) 181-186.

[39] O.L. Chinot, W. Wick, W. Mason, R. Henriksson, F. Saran, R. Nishikawa, A.F. Carpentier, K. Hoang-Xuan, P. Kavan, D. Cernea, A.A. Brandes, M. Hilton, L. Abrey, T. Cloughesy, Bevacizumab plus radiotherapy-temozolomide for newly diagnosed GBM, N Engl J Med, 370 (2014) 709-722.

[40] P.Y. Wen, S.M. Chang, M.J. Van den Bent, M.A. Vogelbaum, D.R. Macdonald, E.Q. Lee, Response Assessment in Neuro-Oncology Clinical Trials, J Clin Oncol, 35 (2017) 2439-2449.

[41] E.M. Ratai, Z. Zhang, B.S. Snyder, J.L. Boxerman, Y. Safriel, R.C. McKinstry, F. Bokstein, M.R. Gilbert, A.G. Sorensen, D.P. Barboriak, Magnetic resonance spectroscopy as an early indicator of response to anti-angiogenic therapy in patients with recurrent GBM: RTOG 0625/ACRIN 6677, Neuro Oncol, 15 (2013) 936-944.

[42] J.L. Boxerman, Z. Zhang, Y. Safriel, J.M. Rogg, R.L. Wolf, S. Mohan, H. Marques, A.G. Sorensen, M.R. Gilbert, D.P. Barboriak, Prognostic value of contrast enhancement and FLAIR for survival in newly diagnosed GBM treated with and without bevacizumab: results from ACRIN 6686, Neuro Oncol, 20 (2018) 1400-1410.

[43] C. Voutouri, N.D. Kirkpatrick, E. Chung, F. Mpekris, J.W. Baish, L.L. Munn, D. Fukumura, T. Stylianopoulos, R.K. Jain, Experimental and computational analyses reveal dynamics of tumor vessel cooption and optimal treatment strategies, Proc Natl Acad Sci U S A, 116 (2019) 2662-2671.

[44] G. Mastrella, M. Hou, M. Li, V.M. Stoecklein, N. Zdouc, M.N.M. Volmar, H. Miletic, S. Reinhard, C.C. Herold-Mende, S. Kleber, K. Eisenhut, G. Gargiulo, M. Synowitz, A.L. Vescovi, P.N. Harter, J.M. Penninger, E. Wagner, M. Mittelbronn, R. Bjerkvig, D. Hambardzumyan, U. Schuller, J.C. Tonn, J. Radke, R. Glass, R.E. Kalin, Targeting APLN/APLNR Improves Antiangiogenic Efficiency and Blunts Proinvasive Side Effects of VEGFA/VEGFR2 Blockade in GBM, Cancer Res, 79 (2019) 2298-2313.

[45] A. Scholz, P.N. Harter, S. Cremer, B.H. Yalcin, S. Gurnik, M. Yamaji, M. Di Tacchio, K. Sommer, P. Baumgarten, O. Bahr, J.P. Steinbach, J. Trojan, M. Glas, U. Herrlinger, D. Krex, M. Meinhardt, A. Weyerbrock, M. Timmer, R. Goldbrunner, M. Deckert, C. Braun, J. Schittenhelm, J.T. Frueh, E. Ullrich,

M. Mittelbronn, K.H. Plate, Y. Reiss, Endothelial cell-derived angiopoietin-2 is a therapeutic target in treatment-naive and bevacizumab-resistant GBM, EMBO Mol Med, 8 (2016) 39-57.

[46] E.R. Gerstner, X. Ye, D.G. Duda, M.A. Levine, T. Mikkelsen, T.J. Kaley, J.J. Olson, B.L. Nabors, M.S. Ahluwalia, P.Y. Wen, R.K. Jain, T.T. Batchelor, S. Grossman, A phase I study of cediranib in combination with cilengitide in patients with recurrent GBM, Neuro Oncol, 17 (2015) 1386-1392.

[47] E. Gerstner, K.E. Emblem, K. Chang, B. Vakulenko-Lagun, Y.F. Yen, A.L. Beers, J. Dietrich, S.R. Plotkin, C. Catana, J.M. Hooker, D.G. Duda, B. Rosen, J. Kalpathy-Cramer, R.K. Jain, T. Batchelor, Bevacizumab reduces permeability and concurrent temozolomide delivery in a subset of patients with recurrent GBM, Clin Cancer Res, (2019).

[48] E. Galanis, S.K. Anderson, E.L. Twohy, X.W. Carrero, J.G. Dixon, D.D. Tran, S.A. Jeyapalan, D.M. Anderson, T.J. Kaufmann, R.W. Feathers, C. Giannini, J.C. Buckner, P.Z. Anastasiadis, D. Schiff, A phase 1 and randomized, placebo-controlled phase 2 trial of bevacizumab plus dasatinib in patients with recurrent GBM: Alliance/North Central Cancer Treatment Group N0872, Cancer, 125 (2019) 3790-3800.

[49] S. Kebir, L. Rauschenbach, A. Radbruch, L. Lazaridis, T. Schmidt, A.K. Stoppek, D. Pierscianek, M. Stuschke, M. Forsting, U. Sure, K. Keyvani, C. Kleinschnitz, B. Scheffler, M. Glas, Regorafenib in patients with recurrent high-grade astrocytoma, J Cancer Res Clin Oncol, 145 (2019) 1037-1042.

[50] H.G. Wirsching, G. Tabatabai, U. Roelcke, A.F. Hottinger, F. Jorger, A. Schmid, L. Plasswilm, D. Schrimpf, C. Mancao, D. Capper, K. Conen, T. Hundsberger, F. Caparrotti, R. von Moos, C. Riklin, J. Felsberg, P. Roth, D.T.W. Jones, S. Pfister, E.J. Rushing, L. Abrey, G. Reifenberger, L. Held, A. von Deimling, A. Ochsenbein, M. Weller, Bevacizumab plus hypofractionated radiotherapy versus radiotherapy alone in elderly patients with GBM: the randomized, open-label, phase II ARTE trial, Ann Oncol, 29 (2018) 1423-1430.

[51] Y. Fu, D. Wang, H. Wang, M. Cai, C. Li, X. Zhang, H. Chen, Y. Hu, X. Zhang, M. Ying, W. He, J. Zhang, TSPO deficiency induces mitochondrial dysfunction, leading to hypoxia, angiogenesis and a growth-promoting metabolic shift towards glycolysis in GBM, Neuro Oncol, (2019).

[52] P. Mukherjee, Z.M. Augur, M. Li, C. Hill, B. Greenwood, M.A. Domin, G. Kondakci, N.R. Narain, M.A. Kiebish, R.T. Bronson, G. Arismendi-Morillo, C. Chinopoulos, T.N. Seyfried, Therapeutic benefit of combining calorie-restricted ketogenic diet and glutamine targeting in late-stage experimental GBM, Commun Biol, 2 (2019) 200.

[53] M. Sanzey, S.A. Abdul Rahim, A. Oudin, A. Dirkse, T. Kaoma, L. Vallar, C. Herold-Mende, R. Bjerkvig, A. Golebiewska, S.P. Niclou, Comprehensive analysis of glycolytic enzymes as therapeutic targets in the treatment of GBM, PLoS One, 10 (2015) e0123544.

[54] S. Kofuji, A. Hirayama, A.O. Eberhardt, R. Kawaguchi, Y. Sugiura, O. Sampetrean, Y. Ikeda, M. Warren, N. Sakamoto, S. Kitahara, H. Yoshino, D. Yamashita, K. Sumita, K. Wolfe, L. Lange, S. Ikeda, H. Shimada, N. Minami, A. Malhotra, S. Morioka, Y. Ban, M. Asano, V.L. Flanary, A. Ramkissoon, L.M.L. Chow, J. Kiyokawa, T. Mashimo, G. Lucey, S. Mareninov, T. Ozawa, N. Onishi, K. Okumura, J. Terakawa, T. Daikoku, T. Wise-Draper, N. Majd, K. Kofuji, M. Sasaki, M. Mori, Y. Kanemura, E.P. Smith, D. Anastasiou, H. Wakimoto, E.C. Holland, W.H. Yong, C. Horbinski, I. Nakano, R.J. DeBerardinis, R.M. Bachoo, P.S. Mischel, W. Yasui, M. Suematsu, H. Saya, T. Soga, I. Grummt, H. Bierhoff, A.T. Sasaki, IMP dehydrogenase-2 drives aberrant nucleolar activity and promotes tumorigenesis in GBM, Nat Cell Biol, 21 (2019) 1003-1014.

[55] A.K. Bag, S. Mandloi, S. Jarmalavicius, S. Mondal, K. Kumar, C. Mandal, P. Walden, S. Chakrabarti, C. Mandal, Connecting signaling and metabolic pathways in EGF receptor-mediated

oncogenesis of GBM, PLoS Comput Biol, 15 (2019) e1007090.
[56] X. Qian, X. Li, Z. Shi, Y. Xia, Q. Cai, D. Xu, L. Tan, L. Du, Y. Zheng, D. Zhao, C. Zhang, P.L. Lorenzi, Y. You, B.H. Jiang, T. Jiang, H. Li, Z. Lu, PTEN Suppresses Glycolysis by Dephosphorylating and Inhibiting Autophosphorylated PGK1, Mol Cell, (2019).
[57] B. Liu, Z.B. Huang, X. Chen, Y.X. See, Z.K. Chen, H.K. Yao, Mammalian Target of Rapamycin 2 (MTOR2) and C-MYC Modulate Glucosamine-6-Phosphate Synthesis in GBM (GBM) Cells Through Glutamine: Fructose-6-Phosphate Aminotransferase 1 (GFAT1), Cell Mol Neurobiol, 39 (2019) 415-434.
[58] X. Wang, K. Yang, Q. Wu, L.J.Y. Kim, A.R. Morton, R.C. Gimple, B.C. Prager, Y. Shi, W. Zhou, S. Bhargava, Z. Zhu, L. Jiang, W. Tao, Z. Qiu, L. Zhao, G. Zhang, X. Li, S. Agnihotri, P.S. Mischel, S.C. Mack, S. Bao, J.N. Rich, Targeting pyrimidine synthesis accentuates molecular therapy response in GBM stem cells, Sci Transl Med, 11 (2019).
[59] S. Echizenya, Y. Ishii, S. Kitazawa, T. Tanaka, S. Matsuda, E. Watanabe, M. Umekawa, S. Terasaka, K. Houkin, T. Hatta, T. Natsume, Y. Maeda, S.I. Watanabe, S. Hagiwara, T. Kondo, Discovery of a new pyrimidine synthesis inhibitor eradicating GBM-initiating cells, Neuro Oncol, (2019).
[60] D. Rohle, J. Popovici-Muller, N. Palaskas, S. Turcan, C. Grommes, C. Campos, J. Tsoi, O. Clark, B. Oldrini, E. Komisopoulou, K. Kunii, A. Pedraza, S. Schalm, L. Silverman, A. Miller, F. Wang, H. Yang, Y. Chen, A. Kernytsky, M.K. Rosenblum, W. Liu, S.A. Biller, S.M. Su, C.W. Brennan, T.A. Chan, T.G. Graeber, K.E. Yen, I.K. Mellinghoff, An inhibitor of mutant IDH1 delays growth and promotes differentiation of glioma cells, Science, 340 (2013) 626-630.
[61] K.J. Norsworthy, L. Luo, V. Hsu, R. Gudi, S.E. Dorff, D. Przepiorka, A. Deisseroth, Y.L. Shen, C.M. Sheth, R. Charlab, G.M. Williams, K.B. Goldberg, A.T. Farrell, R. Pazdur, FDA Approval Summary: Ivosidenib for Relapsed or Refractory Acute Myeloid Leukemia with an Isocitrate Dehydrogenase-1 Mutation, Clin Cancer Res, 25 (2019) 3205-3209.
[62] S.T. Sizemore, M. Zhang, J.H. Cho, G.M. Sizemore, B. Hurwitz, B. Kaur, N.L. Lehman, M.C. Ostrowski, P.A. Robe, W. Miao, Y. Wang, A. Chakravarti, F. Xia, Pyruvate kinase M2 regulates homologous recombination-mediated DNA double-strand break repair, Cell Res, 28 (2018) 1090-1102.
[63] J. Guo, Q. Xue, K. Liu, W. Ge, W. Liu, J. Wang, M. Zhang, Q.Y. Li, D. Cai, C. Shan, C. Zhang, X. Liu, J. Li, Dimethylaminomicheliolide (DMAMCL) Suppresses the Proliferation of GBM Cells via Targeting Pyruvate Kinase 2 (PKM2) and Rewiring Aerobic Glycolysis, Front Oncol, 9 (2019) 993.
[64] H.C. Affronti, K.E. Wellen, Epigenetic Control of Fatty-Acid Metabolism Sustains Glioma Stem Cells, Cancer Discov, 9 (2019) 1161-1163.
[65] Q. Xie, T.P. Wu, R.C. Gimple, Z. Li, B.C. Prager, Q. Wu, Y. Yu, P. Wang, Y. Wang, D.U. Gorkin, C. Zhang, A.V. Dowiak, K. Lin, C. Zeng, Y. Sui, L.J.Y. Kim, T.E. Miller, L. Jiang, C.H. Lee, Z. Huang, X. Fang, K. Zhai, S.C. Mack, M. Sander, S. Bao, A.E. Kerstetter-Fogle, A.E. Sloan, A.Z. Xiao, J.N. Rich, N(6)-methyladenine DNA Modification in GBM, Cell, 175 (2018) 1228-1243 e1220.
[66] W. Yu, S. Liang, C. Zhang, Aberrant miRNAs Regulate the Biological Hallmarks of GBM, Neuromolecular Med, 20 (2018) 452-474.
[67] M. Ranjit, M. Hirano, K. Aoki, Y. Okuno, F. Ohka, A. Yamamichi, A. Kato, S. Maeda, K. Motomura, K. Matsuo, A. Enomoto, Y. Ino, T. Todo, M. Takahashi, T. Wakabayashi, T. Kato, A. Natsume, Aberrant Active cis-Regulatory Elements Associated with Downregulation of RET Finger Protein Overcome Chemoresistance in GBM, Cell Rep, 26 (2019) 2274-2281 e2275.
[68] E. Galanis, S.K. Anderson, C.R. Miller, J.N. Sarkaria, K. Jaeckle, J.C. Buckner, K.L. Ligon, K.V.

Ballman, D.F. Moore, Jr., M. Nebozhyn, A. Loboda, D. Schiff, M.S. Ahluwalia, E.Q. Lee, E.R. Gerstner, G.J. Lesser, M. Prados, S.A. Grossman, J. Cerhan, C. Giannini, P.Y. Wen, O. Alliance for Clinical Trials in, Abtc, Phase I/II trial of vorinostat combined with temozolomide and radiation therapy for newly diagnosed GBM: results of Alliance N0874/ABTC 02, Neuro Oncol, 20 (2018) 546-556.

[69] A.S. Harutyunyan, B. Krug, H. Chen, S. Papillon-Cavanagh, M. Zeinieh, N. De Jay, S. Deshmukh, C.C.L. Chen, J. Belle, L.G. Mikael, D.M. Marchione, R. Li, H. Nikbakht, B. Hu, G. Cagnone, W.A. Cheung, A. Mohammadnia, D. Bechet, D. Faury, M.K. McConechy, M. Pathania, S.U. Jain, B. Ellezam, A.G. Weil, A. Montpetit, P. Salomoni, T. Pastinen, C. Lu, P.W. Lewis, B.A. Garcia, C.L. Kleinman, N. Jabado, J. Majewski, H3K27M induces defective chromatin spread of PRC2-mediated repressive H3K27me2/me3 and is essential for glioma tumorigenesis, Nat Commun, 10 (2019) 1262.

[70] A.B. Silveira, L.H. Kasper, Y. Fan, H. Jin, G. Wu, T.I. Shaw, X. Zhu, J.D. Larson, J. Easton, Y. Shao, D.A. Yergeau, C. Rosencrance, K. Boggs, M.C. Rusch, L. Ding, J. Zhang, D. Finkelstein, R.M. Noyes, B.L. Russell, B. Xu, A. Broniscer, C. Wetmore, S.B. Pounds, D.W. Ellison, J. Zhang, S.J. Baker, H3.3 K27M depletion increases differentiation and extends latency of diffuse intrinsic pontine glioma growth in vivo, Acta Neuropathol, 137 (2019) 637-655.

[71] M. Romani, A. Daga, A. Forlani, M.P. Pistillo, B. Banelli, Targeting of Histone Demethylases KDM5A and KDM6B Inhibits the Proliferation of Temozolomide-Resistant GBM Cells, Cancers (Basel), 11 (2019).

[72] M. Wiese, F. Schill, D. Sturm, S. Pfister, E. Hulleman, S.A. Johnsen, C.M. Kramm, No Significant Cytotoxic Effect of the EZH2 Inhibitor Tazemetostat (EPZ-6438) on Pediatric Glioma Cells with Wildtype Histone 3 or Mutated Histone 3.3, Klin Padiatr, 228 (2016) 113-117.

[73] B. Banelli, A. Daga, A. Forlani, G. Allemanni, D. Marubbi, M.P. Pistillo, A. Profumo, M. Romani, Small molecules targeting histone demethylase genes (KDMs) inhibit growth of temozolomide-resistant GBM cells, Oncotarget, 8 (2017) 34896-34910.

[74] S.A. Jannetti, G. Carlucci, B. Carney, S. Kossatz, L. Shenker, L.M. Carter, B. Salinas, C. Brand, A. Sadique, P.L. Donabedian, K.M. Cunanan, M. Gonen, V. Ponomarev, B.M. Zeglis, M.M. Souweidane, J.S. Lewis, W.A. Weber, J.L. Humm, T. Reiner, PARP-1-Targeted Radiotherapy in Mouse Models of GBM, J Nucl Med, 59 (2018) 1225-1233.

[75] E.A. Chiocca, F. Nassiri, J. Wang, P. Peruzzi, G. Zadeh, Viral and other therapies for recurrent GBM: is a 24-month durable response unusual?, Neuro Oncol, 21 (2019) 14-25.

[76] M. Lim, Y. Xia, C. Bettegowda, M. Weller, Current state of immunotherapy for GBM, Nat Rev Clin Oncol, 15 (2018) 422-442.

[77] V. Genoud, E. Marinari, S.I. Nikolaev, J.C. Castle, V. Bukur, P.Y. Dietrich, H. Okada, P.R. Walker, Responsiveness to anti-PD-1 and anti-CTLA-4 immune checkpoint blockade in SB28 and GL261 mouse glioma models, Oncoimmunology, 7 (2018) e1501137.

[78] M. Weller, E. Le Rhun, Immunotherapy for GBM: quo vadis?, Nat Rev Clin Oncol, 16 (2019) 405-406.

[79] J.E. Kim, M.A. Patel, A. Mangraviti, E.S. Kim, D. Theodros, E. Velarde, A. Liu, E.W. Sankey, A. Tam, H. Xu, D. Mathios, C.M. Jackson, S. Harris-Bookman, T. Garzon-Muvdi, M. Sheu, A.M. Martin, B.M. Tyler, P.T. Tran, X. Ye, A. Olivi, J.M. Taube, P.C. Burger, C.G. Drake, H. Brem, D.M. Pardoll, M. Lim, Combination Therapy with Anti-PD-1, Anti-TIM-3, and Focal Radiation Results in Regression of Murine Gliomas, Clin Cancer Res, 23 (2017) 124-136.

[80] S. Sun, G. Du, J. Xue, J. Ma, M. Ge, H. Wang, J. Tian, PCC0208009 enhances the anti-tumor

effects of temozolomide through direct inhibition and transcriptional regulation of indoleamine 2,3-dioxygenase in glioma models, Int J Immunopathol Pharmacol, 32 (2018) 2058738418787991.

[81] R. Alyautdin, I. Khalin, M.I. Nafeeza, M.H. Haron, D. Kuznetsov, Nanoscale drug delivery systems and the blood-brain barrier, Int J Nanomedicine, 9 (2014) 795-811.

[82] N.J. Abbott, L. Rönnbäck, E. Hansson, Astrocyte–endothelial interactions at the blood–brain barrier, Nature Reviews Neuroscience, 7 (2006) 41-53.

[83] W.Y. Liu, Z.B. Wang, L.C. Zhang, X. Wei, L. Li, Tight junction in blood-brain barrier: an overview of structure, regulation, and regulator substances, CNS Neurosci Ther, 18 (2012) 609-615.

[84] W. Tang, W. Fan, J. Lau, L. Deng, Z. Shen, X. Chen, Emerging blood-brain-barrier-crossing nanotechnology for brain cancer theranostics, Chem Soc Rev, 48 (2019) 2967-3014.

[85] H. Chen, W. Zhang, G. Zhu, J. Xie, X. Chen, Rethinking cancer nanotheranostics, Nat Rev Mater, 2 (2017).

[86] X. Cai, A. Bandla, D. Mao, G. Feng, W. Qin, L.D. Liao, N. Thakor, B.Z. Tang, B. Liu, Biocompatible Red Fluorescent Organic Nanoparticles with Tunable Size and Aggregation-Induced Emission for Evaluation of Blood-Brain Barrier Damage, Adv Mater, 28 (2016) 8760-8765.

[87] E. Nance, K. Timbie, G.W. Miller, J. Song, C. Louttit, A.L. Klibanov, T.-Y. Shih, G. Swaminathan, R.J. Tamargo, G.F. Woodworth, J. Hanes, R.J. Price, Non-invasive delivery of stealth, brain-penetrating nanoparticles across the blood − brain barrier using MRI-guided focused ultrasound, Journal of Controlled Release, 189 (2014) 123-132.

[88] M. Longmire, P.L. Choyke, H. Kobayashi, Clearance properties of nano-sized particles and molecules as imaging agents: considerations and caveats, Nanomedicine, 3 (2008) 703-717.

[89] M. Aldea, I.A. Florian, G. Kacso, L. Craciun, S. Boca, O. Soritau, I.S. Florian, Nanoparticles for Targeting Intratumoral Hypoxia: Exploiting a Potential Weakness of GBM, Pharmaceutical Research, 33 (2016) 2059-2077.

[90] F. Danhier, To exploit the tumor microenvironment: Since the EPR effect fails in the clinic, what is the future of nanomedicine?, J Control Release, 244 (2016) 108-121.

[91] M. Luo, G. Lewik, J.C. Ratcliffe, C.H.J. Choi, E. Mäkilä, W.Y. Tong, N.H. Voelcker, Systematic Evaluation of Transferrin-Modified Porous Silicon Nanoparticles for Targeted Delivery of Doxorubicin to GBM, ACS Applied Materials & Interfaces, 11 (2019) 33637-33649.

[92] B. Zhang, H. Wang, Z. Liao, Y. Wang, Y. Hu, J. Yang, S. Shen, J. Chen, H. Mei, W. Shi, Y. Hu, Z. Pang, X. Jiang, EGFP-EGF1-conjugated nanoparticles for targeting both neovascular and glioma cells in therapy of brain glioma, Biomaterials, 35 (2014) 4133-4145.

[93] C. Fang, K. Wang, Z.R. Stephen, Q. Mu, F.M. Kievit, D.T. Chiu, O.W. Press, M. Zhang, Temozolomide Nanoparticles for Targeted GBM Therapy, ACS Applied Materials & Interfaces, 7 (2015) 6674-6682.

[94] X.C. Chen, Y.L. Deng, Y. Lin, D.W. Pang, H. Qing, F. Qu, H.Y. Xie, Quantum dot-labeled aptamer nanoprobes specifically targeting glioma cells, Nanotechnology, 19 (2008) 235105.

[95] C. Yao, Y. Yuan, D. Yang, Magnetic DNA Nanogels for Targeting Delivery and Multistimuli-Triggered Release of Anticancer Drugs, ACS Applied Bio Materials, 1 (2018) 2012-2020.

Figure Legends

Figure 1. Various agents targeting aberrant molecules in three core pathways of GBM pathogenesis, including RTK/RAS/PI3K, p53, and RB pathways, have been investigated in GBM. (A) Some drugs target aberrant molecules in the RTK/RAS/PI3K pathway. (B) Some drugs target aberrant molecules in the p53 and RB pathways.

Figure 2. Various molecules play critical roles in angiogenesis in GBM, such as VEGF, integrins, PDGF, and c-kit receptors. Various drugs inhibit the growth of GBM by targeting these aberrant pharmaceutical targets.

Figure 3. Aberrant metabolic pathways are potential therapeutic targets for patients with GBM. Some agents targeting these aberrant metabolic pathways have been assessed for the treatment of GBM.

Figure 4. Histone modification and chromatin remodeling are involved in regulating GBM. Drugs targeting these aberrant epigenetic regulators have been examined for the treatment of GBM.

Figure 5. Aberrant immune checkpoints are responsible for immunosuppression, including CTLA-4, PD-1/PD-L1, TIM-3, and IDO. Some agents that restore immune activity in GBM by targeting CTLA-4, PD-1/PD-L1, and IDO have been developed.

Figure 6. The use of various nanomaterials to facilitate the local delivery of drugs to GBM lesions. (A) Schematic illustration of the cellular components of the BBB and its restriction of paracellular diffusion. (B) Comparison of passive targeting and active targeting using drug-loaded nanostructures.

Highlight:

- By clarifying the molecular abnormalities that drive the malignant phenotype of GBM, various drugs that specifically target tumor cells and the tumor microenvironment have been developed.
- These drugs, including drugs targeting growth factor receptors and their downstream signaling pathways, angiogenesis, aberrant metabolism, epigenetic deregulation, and aberrant immune microenvironments, have been investigated in preclinical or clinical trials.
- However, these drugs that significantly inhibited the growth of GBM in the preclinical stage have not produced survival benefits in patients with GBM.
- One reason for their failure is the lack of a definite driver gene to select patients most likely to benefit. Another reason is the inadequate pharmacokinetic properties of the drugs owing of the blood-brain barrier.
- In the present review, we discuss progress in the development of target therapeutic strategies. Furthermore, we discuss the development of nanomaterials that act as local drug delivery systems to penetrate the blood-brain barrier for managing GBM.

Table1 summarized all clinical results, which is related to targeted therapies against GBM, mentioned in this paper.

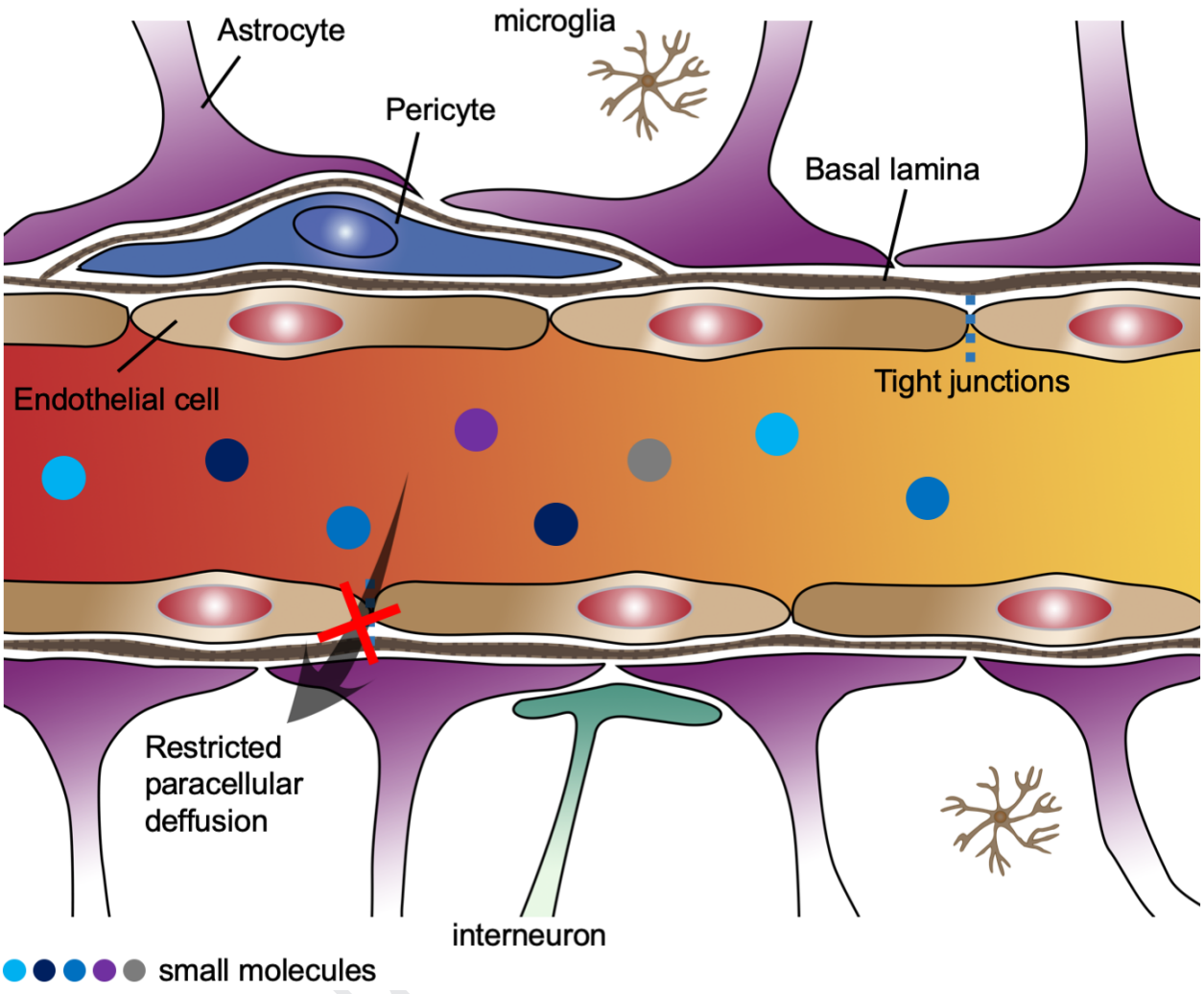
Reference& &selected trails	Intervention	Patient population and	Enrichment	Design	Primary endpoint	PFS	OS	Conclusions
EGFR								
Weller et al. 2017[21]	Rindopepimut	Newly diagnosed, rindopepimut plus TMZ/RT → TMZ (371) versus TMZ/RT → TMZ (374)	EGFRvIII expression	Randomized phase III, placebo controlled	OS	Median PFS (months) Rindopepimut 7.1 Placebo 5.6	Median OS (months) Rindopepimut 20.1 Placebo 20.0	Rindopepimut is inactive in newly diagnosed glioma
van den Bent et al. 2018[23]	Depatuxizumab / ABT-414	Recurrent, ABT-414 plus TMZ (88) versus ABT-414 (86) versus TMZ or CCNU (86)	EGFR amplification	Randomized phase II, open label	OS	Median PFS (months) ABT-414 plus TMZ 3 ABT-414 1.9 TMZ/CCNU 2.0	Median OS (months) ABT-414 plus TMZ 9.6 ABT-414 7.9 TMZ/CCNU 8.2	ABT-414 may be active in combination with TMZ in recurrent glioma
Reardon DA et al. 2017[22]	ABT414	Newly diagnosed(45)PlusTMZ/RT-TMZ	EGFRvIII expression	Singel arm phase □	PFS	Median PFS(months) 6.1	ND	ABT414 may be active in combination with RT Plus TMZ in newly diagnosed glioma

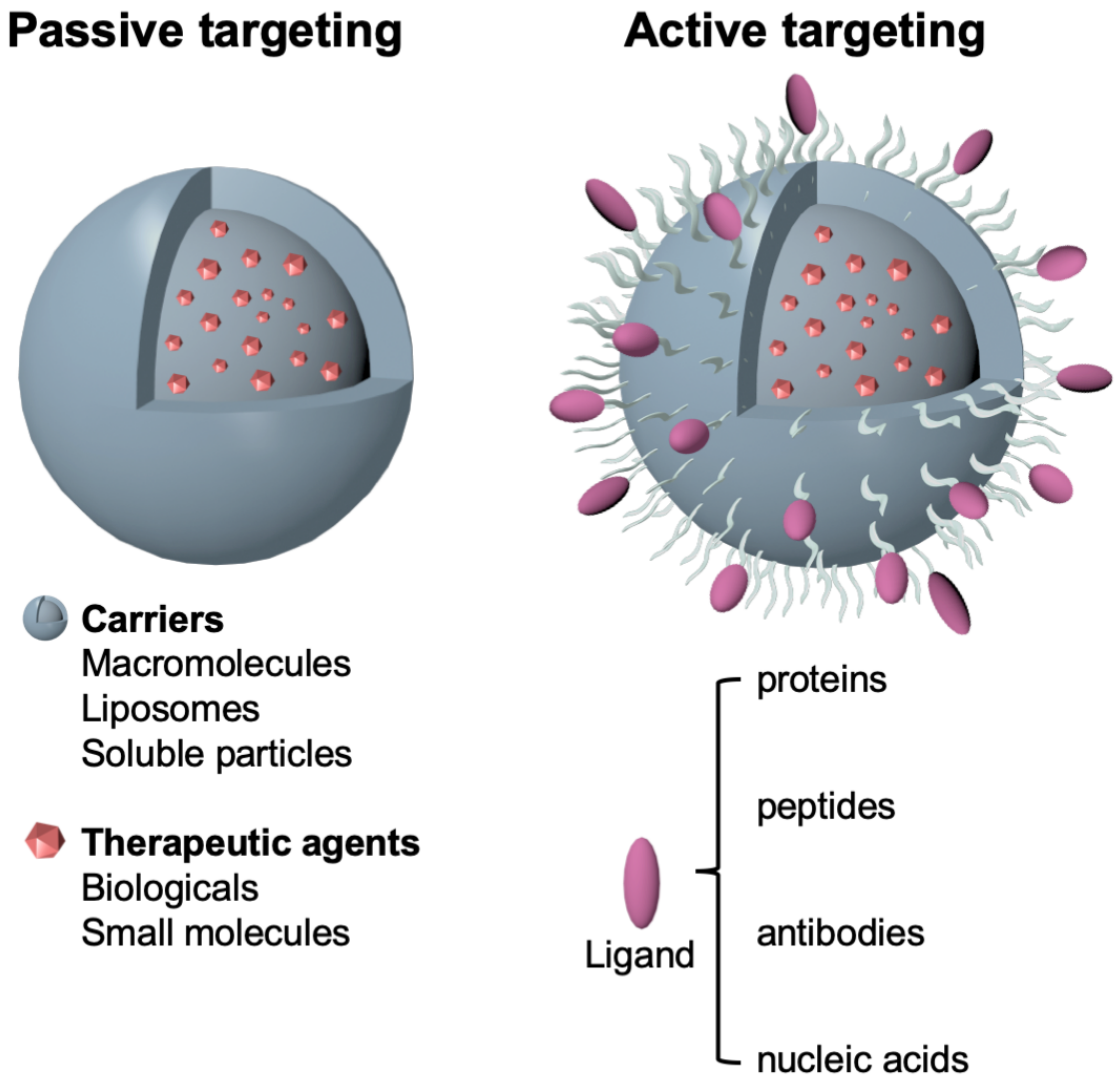
Gan HK et al. 2018[24]	Depatuxizumab/ TMZ	newly diagnosed or recurrent glioblastoma(38)	None	Randomized phase□,open label	OS	PFS-6 30.8%	Median OS 10.7	Depatux-m alone or in combination with temozolomide may be safaty and pharmacokinetic profile in glioblastoma
Lassman AB et al 2019[25]	Depatuxizumab/ TMZ	Recurrent, with prior TMZ therapy(60)	EGFR amplification	Randomized phase□,open label	OS	PFS-6 25.2%	OS-6 69.1%	Depatux-m in combination with temozolomide may be active in glioma
Lassman AB et al. 2015[29]	Dasatinib	Recurrent(17)	None	Randomized phase □	OS	Median PFS 1.7	Median OS 7.9	Dasatinib Is inactive in recurrent glioma
HER-2								
Ahmed N et al. 2017[34]	HER2-CAR VST	HER2-positive glioma(17)	HER2-positive	Singel arm phase □	None	ND	ND	HER2-CAR VSTs is safe and can be associated with clinical benefit for patients with progressive glioblastoma
PI3K								
Wen PY et al. 2019[35]	Buparlisib	Recurrent (65)	None	Randomized phase □,open label	PFS	PFS-6 8% Median PFS(months) 1.7	ND	Buparlisib may be inactive in recurrent glioma

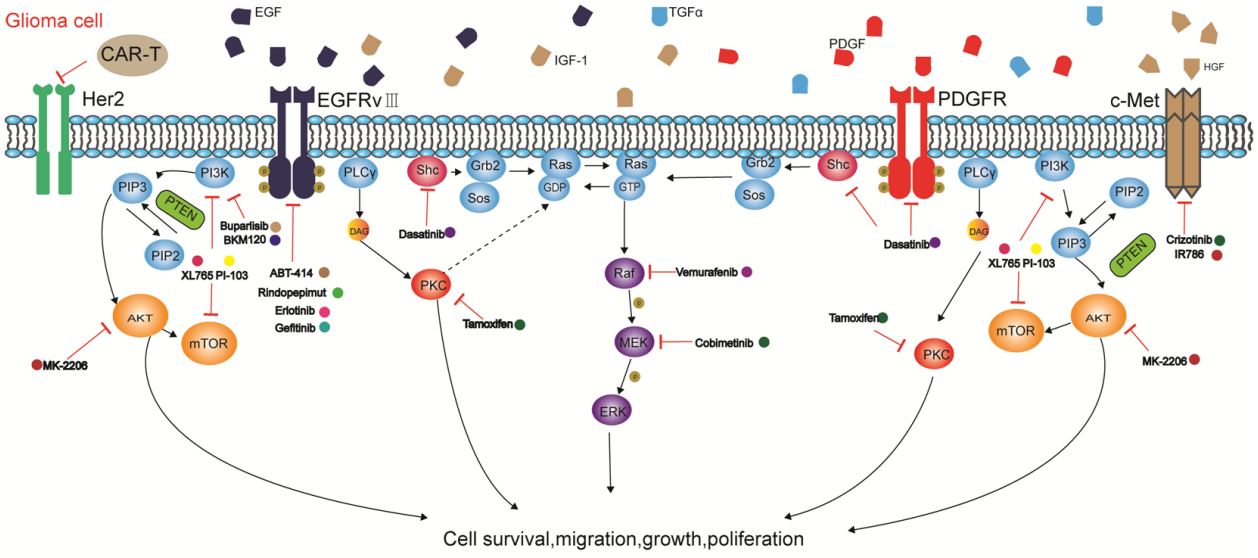
Hainsworth JD et al. 2019[36]	PKM120	Recurrent with prior surgical therapy(88)	None	Single arm phase □/□	ORR	PFS-6 36.5%	ND	PKM120 is inactive in recurrent glioma
CDK4/CDK6								
Taylor JW et al. 2018[49]	palbociclib	Recurrent with RB-positive glioma(22)	None	Randomized phase □	OS	PFS(weeks) 5.14	OS(weeks) 15.4	Palbciclib is inactive in recurrent glioma
VEGFR								
Gilbert MR et al. 2014 [54]	Bevacizumab	Newly diagnosed Bevacizumab(312) versus Placebo(309)	None	Randomized phase III, placebo controlled	OS	PFS (months) control 7.3 bevacizumab. 10.7	OS control 16.1 bevacizumab 15.7 months	Bevacizumab can not prolong OS
Chinot OL et al. 2014[55]	bevacizumab	Newly diagnosed Bevacizumab(458) versus placebo (463)	None	Randomized phase III, placebo controlled	OS	PFS (months) Bevacizumab 10.6 Placebo 6.2	OS(months) Bevacizumab 16.8 Placebo 16.7	Bevacizumab can not prolong OS
Gerstner ER et al. 2015[65]	Cediranib Plus Cilengitide	Recurrent with prior anti-VEGFR therapy(45)	None	Randomized phase □	OS	PFS(months） 1.9	OS(months) 6.5	Cediranib Plus Cilengitide Is inactive in recurrent glioma

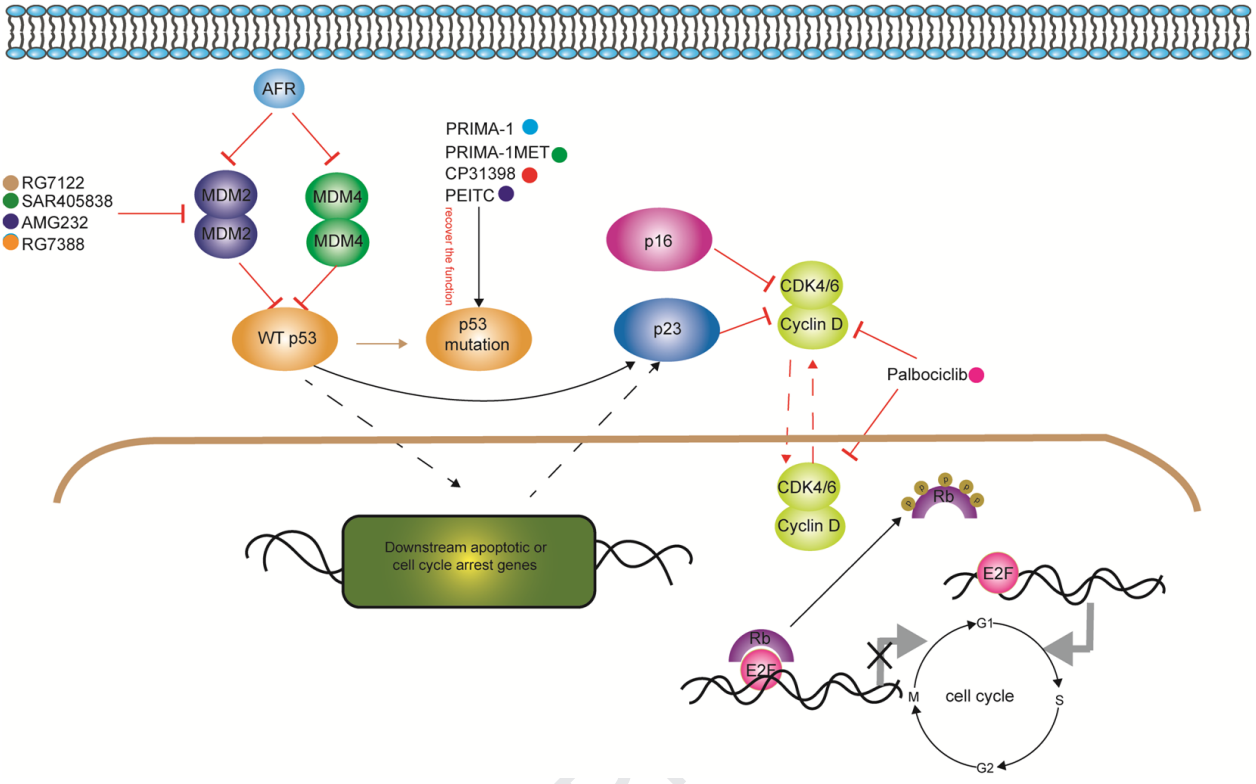
Galanis E et al. 2019[68]	dasatinib	Recurrent Bevacizumab plus dasatinib(128) Bevacizumab(83)	None	Randomized phase □	OS	PFS-6 Bevacizumab plus dasatinib 26.3% Bevacizumab 15.7%	OS(months) Bevacizumab plus dasatinib 7.3 Bevacizumab 7.7	Bevacizumab plus dasatinib does not prolong OS
HDAC								
Iwamoto FM et al. 2011[98]	Romidepsin	Recurrent （34）Plus RT/TMZ-TMZ	None	Singe arm phase□	OS	PFS(weeks) 8	OS(weeks) 34	Romidepsin Is inactive in recurrent glioma
CD155								
Desjardins et al. 2018[108]	PVSRIPO	Recurrent(61) Plus RT/TMZ-TMZ	None	Randomized phase □	OS-24	ND	OS-24 21%	PVSRIPO may be active in recurrent glioma

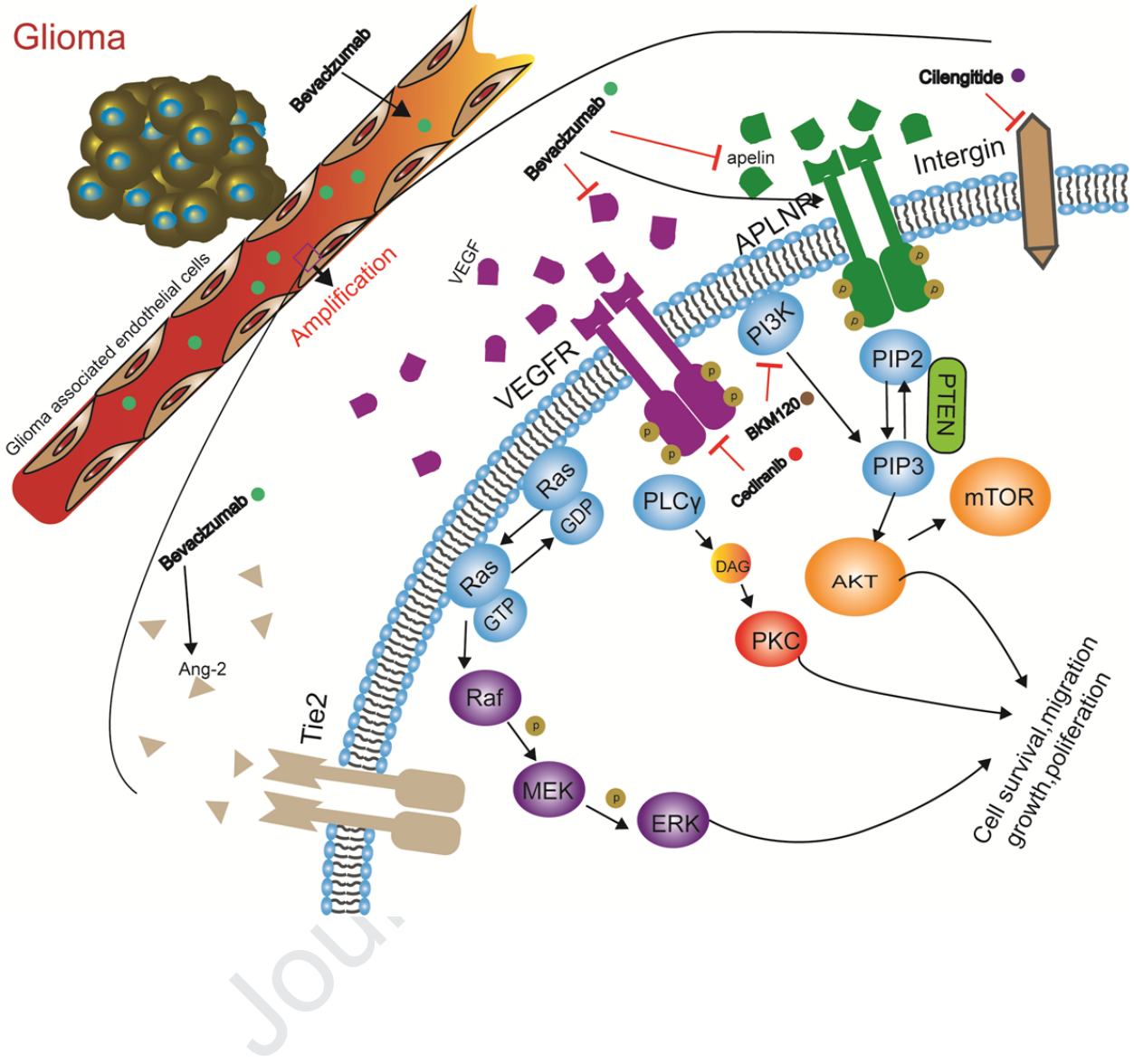
Abbreviation: no data (ND); overall survival (OS); progression-free survival (PFS); objective response rate (ORR); temozolomide (TMZ)

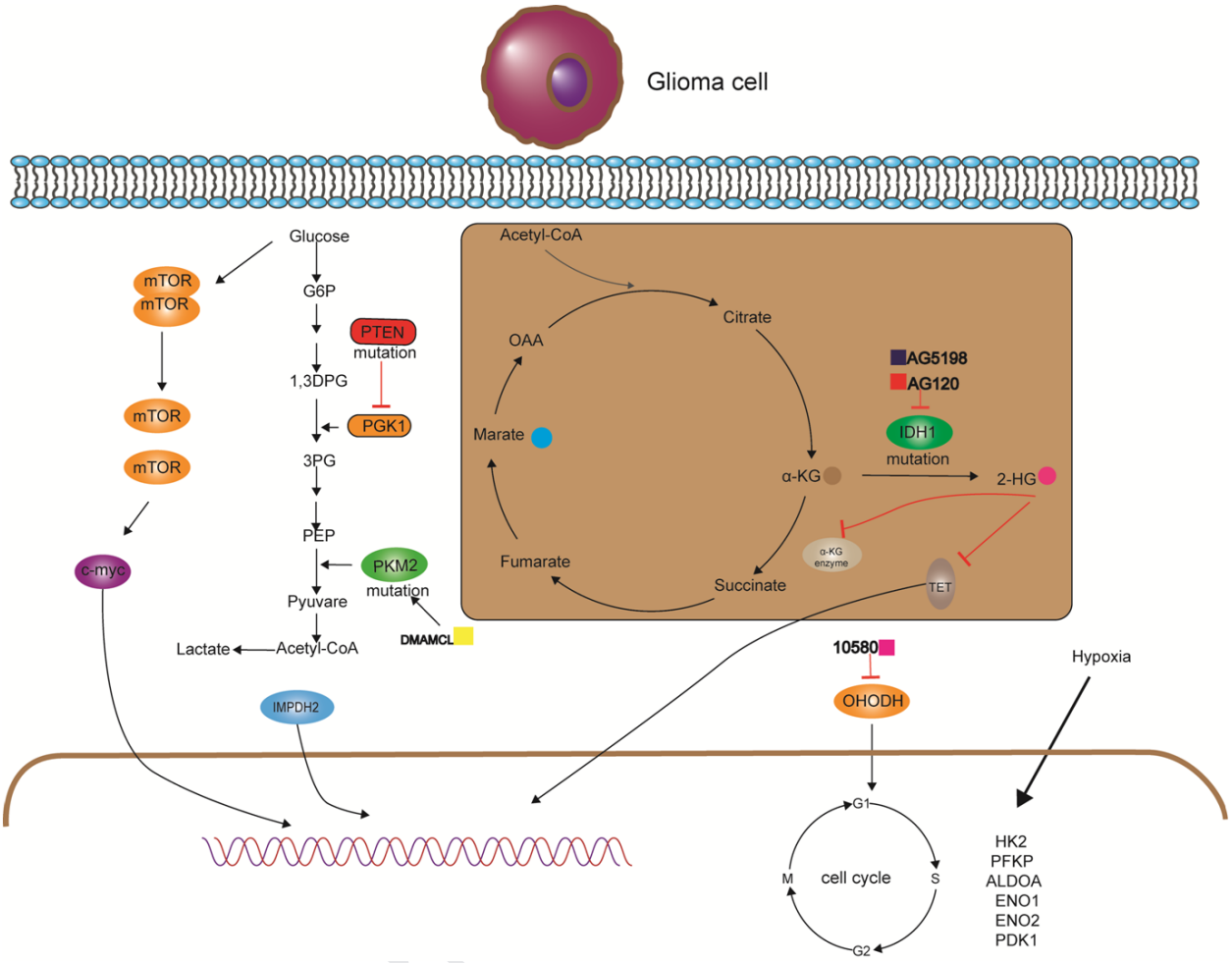

Astrocyte
microglia
Pericyte
Basal lamina
Endothelial cell
Tight junctions
Restricted
paracellular
deffusion
interneuron
small molecules

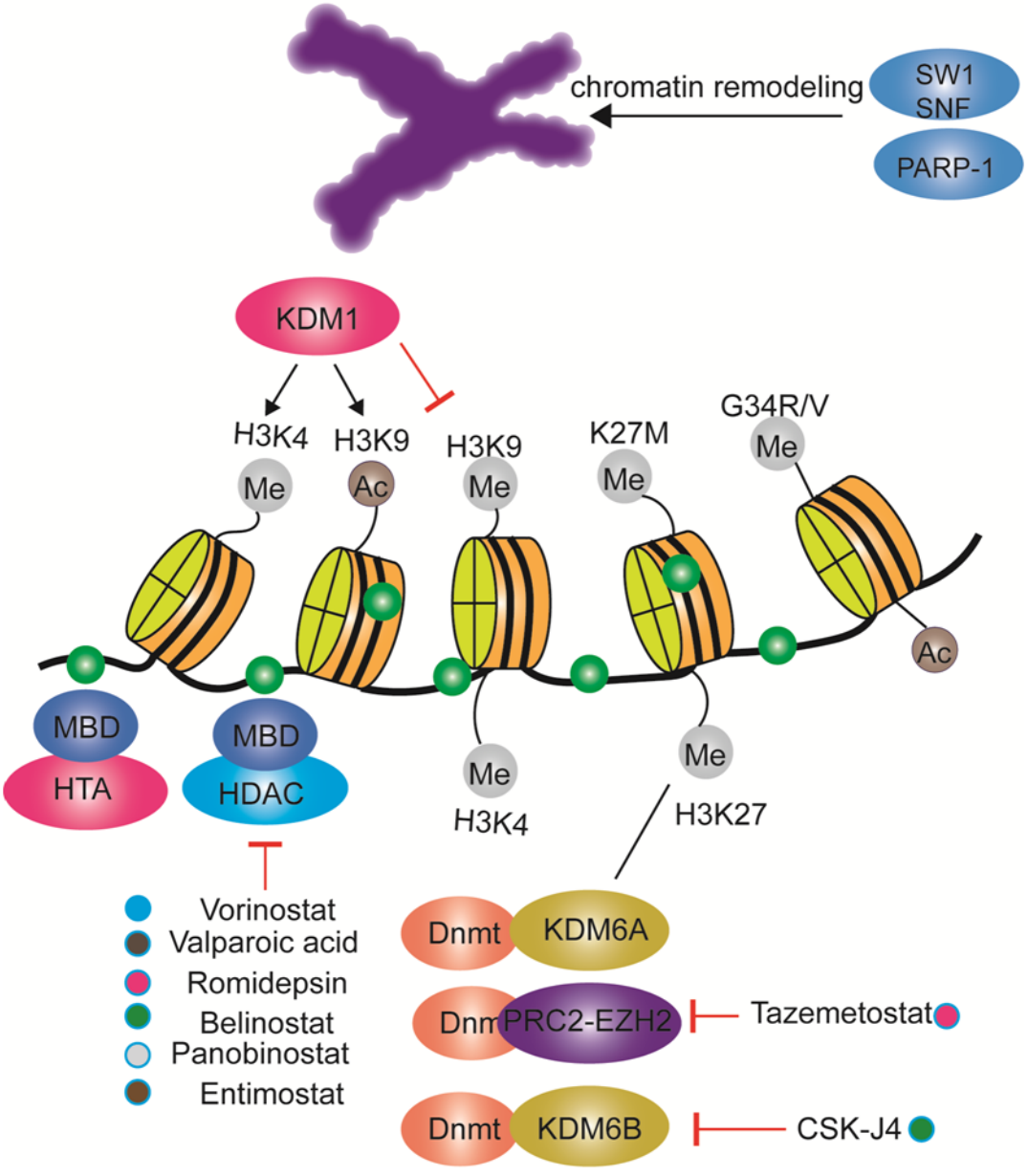

Passive targeting
Active targeting
Carriers
Macromolecules
Liposomes
Soluble particles
Therapeutic agents
Biologicals
Small molecules
Ligand
proteins
peptides
antibodies
nucleic acids






Glioma
Bevacizumab
Glioma associated endothelial cells
Amplification
Bevacizumab
Cilengitide
apelin
Intergin
APLNR
VEGF
VEGFR
PI3K
PIP2
PTEN
PIP3
BKM120
Cediranib
mTOR
Ras
GDP
Ras
GTP
PLCγ
DAG
AKT
PKC
Bevacizumab
Ang-2
Tie2
Raf
MEK
ERK
Cell survival,migration
growth,poliferation


Glioma cell
Glucose
G6P
1,3DPG
3PG
PEP
Pyuvare
Lactate
Acetyl-CoA
mTOR
mTOR
mTOR
mTOR
c-myc
PTEN
mutation
PGK1
PKM2
mutation
DMAMCL
IMPDH2
Acetyl-CoA
Citrate
OAA
Marate
Fumarate
Succinate
α-KG
2-HG
AG5198
AG120
IDH1
mutation
α-KG enzyme
TET
10580
OHODH
Hypoxia
G1
S
G2
M
cell cycle
HK2
PFKP
ALDOA
ENO1
ENO2
PDK1


chromatin remodeling
SW1
SNF
PARP-1
KDM1
H3K4
Me
H3K9
Ac
H3K9
Me
K27M
Me
G34R/V
Me
Ac
MBD
HTA
MBD
HDAC
Me
H3K4
Me
H3K27
Vorinostat
Valparoic acid
Romidepsin
Belinostat
Panobinostat
Entimostat
Dnmt
KDM6A
Dnm
PRC2-EZH2
Tazemetostat
Dnmt
KDM6B
CSK-J4