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REVIEW ARTICLE

Advances in drug development for targeted therapies for glioblastoma

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Abstract

Glioblastoma is the most aggressive primary brain tumor in adults. The prognosis of patients with primary glioblastoma treated with the current standard of care, tumor resection followed by radiation therapy and auxiliary temozolomide, remains poor. Integrative genomic analyses have identified essential core signaling pathways and frequent genetic aberrations, which provide potential drug targets for glioblastoma treatment. Drugs against these therapeutic targets have been developed rapidly in recent years. Although some have shown promising effects on models in preclinical studies, many have shown only modest efficacy in clinical trials. New therapeutic strategies and potent drugs are urgently needed to improve the prognosis of patients with glioblastoma. The goal of this review is to summarize the current advances in drug development for targeted glioblastoma therapies and to reveal the major challenges encountered in clinical trials or treatment. This study will provide new perspectives for future studies of targeted therapeutic drug development and provide insights into the clinical treatment of glioblastoma.

KEYWORDS

drug development, glioblastoma, targeted therapy

Ge Yan and Yunfu Wang contributed equally to this study.

1 | INTRODUCTION

Glioblastoma is the most malignant brain tumor and occurs frequently in the central nervous system.[1] In the USA, the annual incidence of glioblastoma is 3.22/100 000 population, and glioblastoma accounts for 14.6% of all primary brain tumors and 48.3% of malignant brain tumors.[1] The incidence increases with age and is the highest for elderly adults aged 75 to 84 years.[1] Following diagnosis, the 5-year survival rate of patients with glioblastoma (6.8%) is much worse than patients with other malignant brain tumors (35.8%).[1]

According to the World Health Organization (WHO) classification, glioblastoma is the most malignant grade IV glioma.[2] Two types of glioblastoma are defined by the origins of the tumors: primary glioblastoma, which arises de novo as a malignant high-grade glioma (HGG) and accounts for more than 90% of all tumors, and secondary glioblastoma (<10%), which usually develops from a previously established low-grade glioma.[3] Four subtypes of glioblastoma have been identified according to the molecular expression patterns: classical, neural, proneural, and mesenchymal subtypes.[4,5] The specific molecular characteristics of the four subtypes of glioblastoma were revealed in comprehensive genomic studies by The Cancer Genome Atlas (TCGA) program.[6]

Methylation of the O (6)-methylguanine DNA methyltransferase (*MGMT*) gene promoter is one of the most widely studied prognostic biomarkers of glioblastoma[7] and can improve the prognosis of patients with glioblastoma who are treated with temozolomide (TMZ).[8] *MGMT* promoter methylation inhibits the transcription of the *MGMT* gene, which has important functions in the DNA repair process, thus promoting tumor cell death and subsequently improving the beneficial effects of alkylating agents such as TMZ on patients.[8] Mutations in the isocitrate dehydrogenase 1 (*IDH1*) gene, such as *IDH1* (R132H), frequently occur in glioblastoma and are usually considered another prognostic marker for the survival of patients with glioblastoma.[9] *IDH* mutations sensitize tumor cells to radiotherapy and TMZ, thus prolonging the survival of patients with glioblastoma.[9] Mutations in the telomerase reverse transcriptase gene promoter are also a crucial prognostic factor for glioblastoma, but predict poor treatment response in patients.[10,11]

With the recent rapid development of next-generation sequencing technology, many studies have been conducted to explore the genomic landscape of glioblastoma.[6,12] A TCGA Research Network study involved a comprehensive study of more than 500 glioblastoma tumors at the genomic, epigenomic, transcriptomic, and proteomic levels. This multidimensional dataset revealed the most frequent genomic characteristics of glioblastoma tumors.[6] The whole-exome sequencing results in this study identified the most significantly mutated genes or genomic loci, including the phosphatase and tensin homolog (*PTEN*), tumor protein p53 (*TP53*), epidermal growth factor receptor (*EGFR*), phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*), phosphoinositide-3-kinase regulatory subunit 1 (*PIK3R1*), neurofibromin 1 (*NF1*), retinoblastoma 1 (*RB1*), isocitrate dehydrogenase (NADP[+]) 1 (*IDH1*), and platelet-derived growth factor receptor alpha (*PDGFRA*) genes; chromosome 7 (containing the *EGFR*, *MET*, and cyclin-dependent kinase 6 *[CDK6]* genes); chromosome 12 (containing the *CDK4* and *MDM2* genes); and chromosome 4 (containing the *PDGFRA* gene).[6] Moreover, the most frequently occurring gain of function mutations were detected in genes such as SRY (sex-determining region Y)-box 2, the *MYCN* proto-oncogene, cyclin D1, and cyclin E2 (*CCNE2*), and the most frequently occurring loss of function mutations were detected in the cyclin-dependent kinase inhibitor 2A/B (*CDKN2A/B*), chromosome 6q26, parkin RBR E3 ubiquitin protein ligase (*PARK2*), *QKI*, LDL receptor-related protein 1B, neuronal PAS domain protein 3, limbic system associated membrane protein, and SET and MYND domain containing 3 genes.[6]

The current standard of care for patients with newly diagnosed glioblastoma is surgical resection followed by concomitant radiotherapy and TMZ. Although TMZ chemotherapy improves the 2-year survival rate of patients with glioblastoma from 10.4% (radiation only) to 26.5% (radiation + TMZ), the benefit of current treatment is still limited, as the median survival time of patients was only increased by ~2 months.[13] The development of novel

therapeutic strategies and related drugs is urgently needed to substantially improve the prognoses of patients with glioblastoma.

Deep sequencing and comprehensive analysis of the genomic profiles of glioblastoma tumor samples have identified multiple potential therapeutic targets for the development of drugs specific for this malignant disease.[6,12] In this review, we introduce the most extensively studied potential therapeutic targets and related candidate drugs that have been developed for the treatment of glioblastoma (Figure 1). A comprehensive understanding of the current status of targeted therapies for glioblastoma would provide new insights into the future development of drugs to treat patients with glioblastoma.

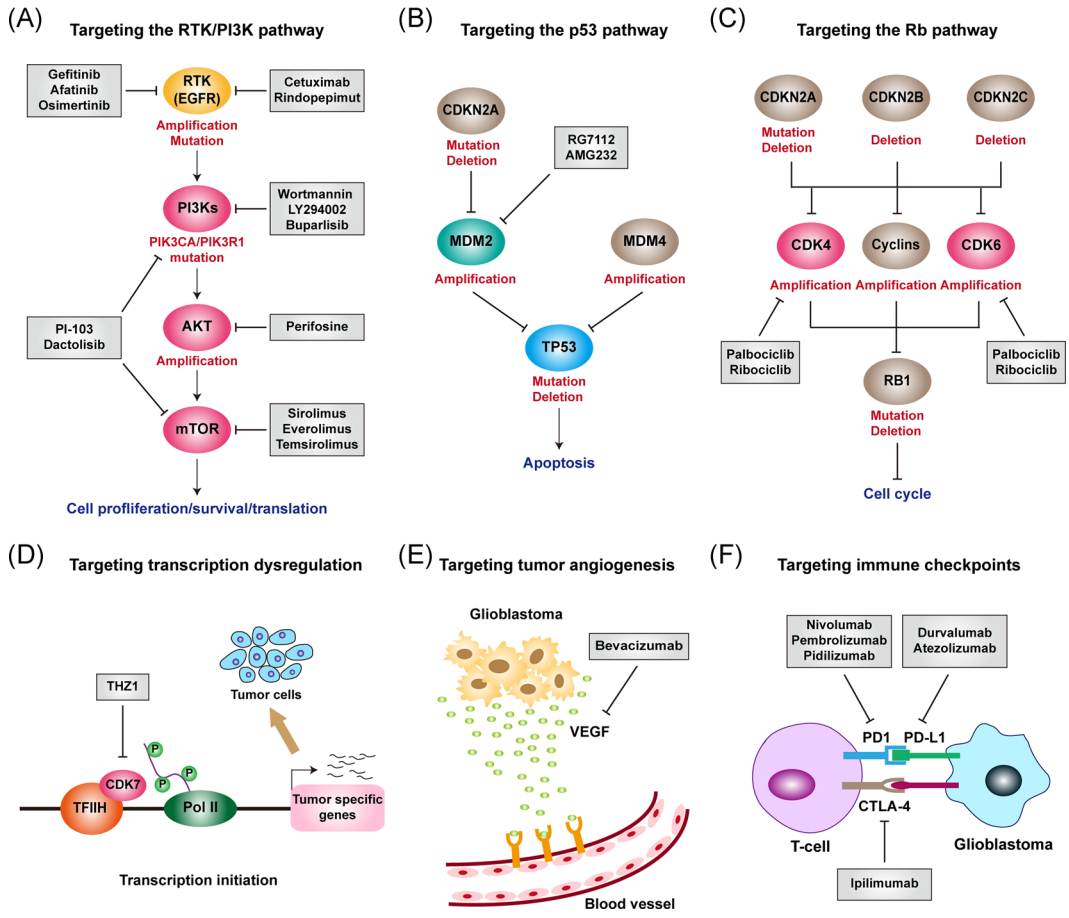

FIGURE 1 Overview of the main strategies for targeted therapies and related drug development for glioblastoma. A, Targeting the RTK/PI3K/AKT/mTOR pathway and cell proliferation/survival/translation processes by EGFR/EGFRvIII inhibitors, PI3K inhibitors, AKT inhibitors, and mTOR inhibitors in glioblastoma. B, Targeting the p53 pathway and apoptosis process by MDM2 inhibitors in glioblastoma. C, Targeting the Rb pathway and cell cycle by CDK4/CDK6 inhibitors in glioblastoma. D, Targeting transcriptional dysregulation by CDK7 inhibitors in tumor cells, including malignant gliomas. E, Targeting tumor angiogenesis by VEGF inhibitors in glioblastoma. F, Targeting immune checkpoints by PD-1 and PD-L1 inhibitors in glioblastoma. CDK, cyclin-dependent kinase; EGFR, epidermal growth factor receptor; PD-1, programmed cell death protein 1; PI3K, phosphatidylinositol 3 kinase; RTK, receptor tyrosine kinase; VEGF, vascular endothelial growth factor [Color figure can be viewed at wileyonlinelibrary.com]

2 | CURRENT STATUS OF DRUG DEVELOPMENT FOR TARGETED THERAPIES FOR GLIOBLASTOMA

2.1 | Targeting core signaling pathways in glioblastoma

Genetic alterations identified in the TCGA study defined three core signaling pathways in glioblastoma: the phosphatidylinositol 3 kinase (PI3K) pathway (with genetic alterations in the *PIK3CA*, *PIK3R1*, *PTEN*, *EGFR*, *PDGFRA*, and *NF1* genes), the p53 pathway (with genetic alterations in the *MDM2*, *MDM4*, and *TP53* genes), and the Rb pathway (with genetic alterations in the *CDK4*, *CDK6*, *CCND2*, *CDKN2A/B*, and *RB1* genes).[6] A rational therapeutic strategy for glioblastoma is to target core signaling pathways involved in glioblastoma.

2.1.1 | Targeting EGFR

EGFR, also known as HER1 or ERBB1, is a transmembrane receptor tyrosine kinase (RTK) that belongs to the ERBB family.[14] By binding to different extracellular ligands, such as epidermal growth factor (EGF), transforming growth factor-α (TGF-α), or heparin-binding EGF-like growth factor (HB-EGF), EGFR forms dimers with either itself or other ERBB family receptors.[15] Dimerized EGFR subsequently causes transphosphorylation of the C-terminal domain and activates downstream signaling pathways (including the RAS/RAF/ERK, PI3K/AKT/mTOR, and Janus kinase/signal transducer and activator of transcription [JAK/STAT] pathways) and numerous physiological processes.[16]

Genetic alterations (gene amplification/mutation/rearrangement/altered splicing) and hyperactivation in *EGFR* were commonly observed in glioblastoma and were present in 57% of invested glioblastoma tumors in the TCGA report.[6] *An EGFR* variant III (*EGFRvIII*) mutation, resulting from the deletion of exons 2 to 7 in the extracellular domain of the EGFR receptor, is the most common oncogenic mutation in glioblastoma.[17] This deletion leads to the expression of an EGFR variant truncated in the extracellular region and the constitutive activation of the EGFR kinase function and downstream pathways.[18,19] In addition to the *EGFRvIII* mutation, other mutations in the extracellular domain of *EGFR*, including R10K, A289, and G598, were observed in 24% of glioblastomas.[6]

Frequent genetic alterations in *EGFR*, such as *EGFR* amplification and the *EGFRvIII* mutation, play critical roles in maintaining the aberrant activation of multiple signaling pathways and important cellular processes in tumor progression, indicating that EGFR/EGFRvIII might be therapeutic targets for glioblastoma drug development.

EGFR/EGFRvIII-targeted drugs

The currently developed EGFR/EGFRvIII-targeted drugs include EGFR tyrosine kinase inhibitors, anti-EGFR antibodies, and anti-EGFRvIII vaccines (summarized in Table 1).

First-generation EGFR inhibitors, such as gefitinib, erlotinib, and lapatinib, were designed to compete with ATP and then bind to and activate the tyrosine kinase domain of EGFR. Although these inhibitors were indicated to exert potent effects on improving cell survival and inhibiting tumor growth in preclinical studies, only limited efficacy was observed in clinical trials.[20-26] Second-generation EGFR inhibitors, including afatinib and dacomitinib, inhibit EGFR activation by irreversibly binding to its tyrosine kinase domain. Afatinib was reported to be safe, but displayed limited activity in treating glioblastoma.[27] Dacomitinib showed potent efficacy in primary cultures and xenografts of EGFR-amplified glioblastoma in preclinical studies,[28] but a phase II study of dacomitinib only revealed limited activity in patients with an EGFR amplification in recurrent glioblastoma.[29] The blood-brain barrier (BBB), which may prevent drugs from penetrating tumors, is a potentially important explanation for the resistance of glioblastoma to EGFR inhibitors.[30] Third-generation EGFR inhibitors, which have irreversible binding activity with EGFR, including rociletinib and osimertinib, were initially designed to target the EGFR (T790M) mutant for the treatment of non–small cell lung cancer (NSCLC).[31-33] A phase I/II clinical trial (NCT01526928) revealed an active

TABLE 1 Clinical trials of PI3K pathway inhibitors in patients with glioblastoma

Target		Drug						
RTK	EGFR/ EGFRvIII	1st gen	Gefitinib	Gefitinib + Temozolomide	Intervention Patients Malignant primary glioma	NCT code NCT00027625	Phase Phase I	Outcome/ publication Prados et al (2008)
				Gefitinib	Recurrent glioblastoma	NCT00250887	Phase II	Hegi et al[22]
				Gefitinib + Radiation	Glioblastoma	NCT00052208	Phase I Phase II	Chakravarti et al[23]
			Erlotinib	Erlotinib + Temozolomide + Radiation	Newly diagnosed glioblastoma	NCT00187486	Phase II	Prados et al[129]
				Erlotinib + Temozolomide + Radiation	Glioblastoma	NCT00039494	Phase II	Brown et al[24]
				Erlotinib	Recurrent malignant glioma	NCT00045110	Phase II Phase II	Raizer et al[130]
				Erlotinib vs Temozolomide/ carmustine	Recurrent glioblastoma	NCT00086879	Phase II	van den Bent et al[25]
			Lapatinib	Lapatinib	Recurrent glioblastoma	…	Phase I Phase II	Thiessen et al[26]
				Lapatinib + Temozolomide + Radiation	Newly diagnosed glioblastoma	NCT01591577	Phase II	Yu et al[131]
		2nd gen	Afatinib	Afatinib/ Afatinib + Temozolomide vs Temozolomide	Recurrent glioblastoma	…	Phase I Phase II	Reardon et al[27]
			Dacomitinib	Dacomitinib	Recurrent glioblastoma with EGFR amplification	…	Phase II	Sepulveda-Sanchez et al[29]

(Continues)

TABLE 1 (Continued)

Target		Drug						
RTK	EGFR/ EGFRvIII	1st gen	Gefitinib	Gefitinib + Temozolomide	Intervention Patients Malignant primary glioma	NCT code NCT00027625	Phase Phase I	Outcome/ publication Prados et al (2008)
		3rd gen	Osimertinib	Osimertinib	EGFR-activated recurrent glioblastoma	NCT03732352	Phase II	Recruiting
		Antibodies	Cetuximab	Cetuximab + Reirradiation	Recurrent glioblastoma	NCT02800486	Phase II	Recruiting
				Cetuximab	Newly diagnosed glioblastoma	NCT02861898	Phase I Phase II	Recruiting
			Nimotuzumab	Nimotuzumab + Temozolomide + Radiation	Newly diagnosed glioblastoma	…	Phase II	Du et al[38]
		Vaccines	Rindopepimut	Rindopepimut + Temozolomide	Newly diagnosed glioblastoma	NCT00458601 (ACT III)	Phase II	Schuster et al[42]
				Rindopepimut + Temozolomide	Newly diagnosed glioblastoma	NCT01480479 (ACT IV)	Phase III	Weller et al[43]
PI3K/Akt/ mTOR pathway	Pan-PI3K		Buparlisib	Buparlisib	Recurrent glioblastoma	NCT01339052	Phase II	Wen et al[52]
	Akt		Perifosine	Perifosine	Recurrent glioblastoma	…	Phase II	Kaley et al[54]
	mTOR		Sirolimus	Sirolimus	Glioblastoma	NCT00047073	Phase I Phase II	Cloughesy et al[55]
				Sirolimus + Erlotinib	Recurrent glioblastoma	NCT00672243	Phase II	Reardon et al[132]
				Sirolimus + Vandetanib	Recurrent glioblastoma	NCT00821080	Phase I	Chheda et al[133]
			Everolimus	Everolimus + Temozolomide	glioblastoma	NCT00387400	Phase I	Mason et al[134]

TABLE 1 (Continued)

Target		Drug						
RTK	EGFR/ EGFRvIII	1st gen	Gefitinib	Gefitinib + Temozolomide	Intervention Patients Malignant primary glioma	NCT code NCT00027625	Phase Phase I	Outcome/ publication Prados et al (2008)
				Everolimus + Temozolomide + Radiation	Newly diagnosed glioblastoma	NCT00553150	Phase I	Sarkaria et al[135]
							Phase II	
				Everolimus + Temozolomide + Radiation	Newly diagnosed glioblastoma	NCT01062399 (RTOG 0913)	Phase I	Chinnaiyan et al[136]
							Phase II	Chinnaiyan et al[137]
				Everolimus + Bevacizumab	Glioblastoma	NCT00805961	Phase II	Hainsworth et al[141]
				Everolimus + Sorafenib	Recurrent high-grade gliomas	NCT01434602	Phase I	Recruiting
							Phase II	
			Temsirolimus	Temsirolimus	Malignant glioma	NCT00022724	Phase I	Chang et al[138]
							Phase II	Chang et al[56]
								Kuhn et al[139]
				Temsirolimus + Erlotinib	Recurrent malignant glioma	NCT00112736	Phase I	Wen et al[140]
							Phase II	
				Perifosine + Temsirolimus	Recurrent/progressive malignant gliomas	NCT02238496	Phase I	Active, not recruiting
	PI3K/ mTOR		Dactolisib	Dactolisib + Everolimus	Advanced cancers, including glioblastoma	NCT01508104	Phase I	Wise-Draper et al[62]

Abbreviations: EGFR, epidermal growth factor receptor; PI3K, phosphatidylinositol 3 kinase; RTK, receptor tyrosine kinase.

efficacy of rociletinib in patients with EGFR-mutated NSCLC.[34] Osimertinib is being evaluated in ongoing clinical trials in patients with NSCLC either as a monotherapy (NCT03790397) or as a combination therapy (NCT02856893 and NCT03133546). According to preclinical studies, osimertinib efficiently inhibits the growth of glioblastoma cell lines both in vitro and in vivo.[35] Although osimertinib shows lower potency than afatinib toward wild-type EGFR in preclinical tumor xenograft models,[31] it is currently being investigated in phase II clinical trial recruiting patients with EGFR-activated recurrent glioblastoma (NCT03732352).

Anti-EGFR antibodies, including cetuximab and nimotuzumab, were designed to bind the extracellular domain of EGFR, inhibit the dimerization of EGFR on the membrane and subsequently trigger the activation of EGFR and downstream signaling pathways.[36] EGFR blockade with cetuximab improves the effectiveness of radiation therapy in EGFR-amplified glioblastoma intracranial mouse models,[37] and two clinical trials (NCT02800486 and NCT02861898) are now recruiting patients with recurrent/newly diagnosed glioblastoma to evaluate the efficacy of cetuximab. The survival of patients with newly diagnosed glioblastoma was increased by nimotuzumab plus temozolomide and radiation therapy in phase II multicenter clinical study.[38]

Anti-EGFRvIII vaccines such as rindopepimut (CDX-110) contain short peptides with EGFRvIII mutation sites and activate the immune system of patients with glioblastoma by specifically targeting EGFRvIII-harboring tumor cells.[39] Although the results of the phase I and phase II trials were promising,[40-42] rindopepimut failed in a currently established phase III trial (NCT01480479) in patients with newly diagnosed glioblastoma.[43]

2.1.2 | Targeting the PI3K/AKT/mTOR signaling pathway

PI3Ks are intracellular lipid kinases that catalyze the phosphorylation of phosphatidylinositol and activate downstream signaling pathways to maintain biological functions such as cell survival, cell cycle, metabolism, and protein translation.[44] According to the preference for different substrates, PI3Ks are divided into three classes: class I-III PI3Ks. Among these classes, class I PI3Ks are involved in the production of phosphatidylinositol-3,4,5-trisphosphate (PIP3) from phosphatidylinositol-4,5-bisphosphate (PIP2). The two subfamilies of class I PI3K proteins, class IA and class IB, are categorized by the membrane receptors that activate them: RTKs and G-protein-coupled receptors (GPCRs), respectively.[45] Class IA PI3Ks are composed of a p85 regulatory subunit and a p110 catalytic subunit. The p85 regulatory subunit has three isoforms, p85α, p85β, and p55γ, which are encoded by the *PIK3R1*, *PIK3R2*, and *PIK3R3* genes, respectively. The p110 catalytic subunit also has three isoforms, p110α, p110β, and p110γ, which are encoded by the *PIK3CA*, *PIK3CB*, and *PIK3CD* genes, respectively. When upstream RTKs are activated by specific ligands, they bind to and cause conformational changes in the p85 subunit of class IA PI3Ks. The p110 catalytic subunit is then activated and catalyzes the phosphorylation of PIP2 to form PIP3, which then activates its downstream effector AKT and initiates the mTORC1 signaling pathway to ultimately induce protein translation.[46]

The comprehensive genomic study of glioblastoma identified the overactivation of the PI3K pathway, which results from alterations in RTK genes, PI3K genes, and *PTEN*, in 89.6% of tumors.[6] In addition, approximately 25.1% of glioblastoma tumors had PI3K mutations, including 18.3% with mutations in the p110α and/or p85α subunits and 6.8% with mutations in other PI3K family members.[6] Inhibition of key effectors of PI3K pathways may result in glioblastoma cell death or tumor suppression. We summarize the development of PI3K-targeted therapeutic drugs in Table 1.

PI3K pathway inhibitors

At the early stage of pan-PI3K inhibitor development, wortmannin and LY294002 showed good effects on tumor inhibition in a glioblastoma cell line in vivo and in vitro models in preclinical studies.[47-49] However, these two drugs did not enter clinical trials because of their toxicity and nonspecific selectivity.[49] Thus, a new type of pan-PI3K inhibitor with higher safety and efficacy, represented by buparlisib, was developed.[50] Buparlisib displays good oral bioavailability and can penetrate the BBB.[50] It showed good potency in preclinical studies and efficiently inhibited tumor growth in intracranial xenograft models of a glioblastoma cell line (U87) with no obvious side effects.[51]

However, buparlisib showed limited efficacy as a single agent in an open-label, multicenter phase II clinical trial (NCT01339052)[52] and is now undergoing widespread testing in several clinical studies in combination with radiotherapy or other chemotherapies.

Perifosine is an extensively studied AKT inhibitor that blocks the activity of the AKT protein and its downstream signaling pathways.[53] Although it showed promising effects to inhibit the PI3K/AKT pathways, a phase II clinical trial did not observe the efficacy of monotherapy with perifosine in improving the survival of patients with recurrent glioblastoma.[54]

Sirolimus (rapamycin), an allosteric mTOR inhibitor, alters the conformation and inhibits the kinase activity of mTOR; however, it is not an effective agent because of its immunosuppressive effects.[55] Thus, the rapamycin analogs everolimus (RAD001) and temsirolimus (CCI-779), with reduced immunosuppressive effects and enhanced pharmacological functions, were designed.[56] Although rapamycin and its analogs showed good efficacy in inhibiting the activity of mTOR in both in vivo and in vitro studies, they inevitably induced overactivation of their upstream regulator AKT via feedback loop.[57]

Dual PI3K/mTOR inhibitors targeting both PI3K and mTOR were produced to avoid feedback or crosstalk between mTOR and its upstream effectors. PI-103, the first-studied dual PI3K/mTOR inhibitor,[58] showed good effects on inhibiting tumor growth in vivo,[59] but did not enter clinical trials because of its unfavorable pharmacological characteristics. Dactolisib (BEZ235) is a newly developed dual PI3K/mTOR inhibitor that significantly improved the survival of glioblastoma cell lines in animal models.[60,61] This drug is a promising candidate for the treatment of malignant tumors but showed limited efficacy in phase I clinical trial combined with everolimus in patients with advanced solid tumors, including glioblastoma (NCT01508104).[62]

2.1.3 | Targeting the p53/ARF/MDM2 signaling pathway

The p53 protein is a transcription factor encoded by the *TP53* gene, which is located on chromosome 17.[63] When the DNA-binding domain of p53 recognizes and interacts with a specific DNA sequence, it triggers the activity of regulatory pathways downstream of p53.[63,64] The p53 protein plays an important role in maintaining cellular homeostasis by controlling multiple cellular processes, such as cell proliferation, cell survival, and genome integrity.[63] In normal cells, the level of the p53 protein is relatively low and the function of p53 is inhibited by interacting with MDM2 and MDM4.[65] DNA damage disrupts the interaction of p53 with MDM2/MDM4, activates the regulatory function of p53, and triggers events such as cell cycle arrest and apoptosis.[66] As a guardian to maintain the genomic integrity of cells, p53 is a tumor suppressor that plays important roles in controlling tumor growth.[67] Deregulated expression of the *TP53* gene and genes encoding other components of the p53/ARF/MDM2 pathway is commonly observed in cancer. According to the TCGA report, 85.3% of glioblastomas exhibited dysregulation of the p53 pathway, including 27.9% with *TP53* gene mutations/deletions, 15.1% with *MDM1/2/4* gene amplifications, and 57.8% with *CDKN2A* gene deletion.[6] The prevalence of dysregulation of the p53/ARF/MDM2 pathway in glioblastoma suggests that targeting this pathway represents a promising therapeutic strategy for this malignant tumor.

MDM2 inhibitors

One of the promising strategies for targeting the p53/ARF/MDM2 signaling pathway is to inhibit the interaction of MDM2 and p53 to reactivate p53 function. Small molecule inhibitors targeting MDM2 have been developed and tested in glioblastoma and other types of cancer. RG7112 is a first-in-class MDM2 inhibitor that exerted good therapeutic effects on patient-derived glioblastoma cells and animal models in preclinical studies.[68] In MDM2-amplified glioblastoma cells, RG7112 exhibited a good ability to restore p53 function and cross the BBB, thus effectively inhibiting tumor growth in xenograft models and prolonging the survival of the animals.[69] Other inhibitors, such as AMG232, were also developed and characterized as potent MDM2 inhibitors in preclinical studies, with good selective inhibitory effects on the tumor initiation of glioma stem cells.[70] AMG232 is being

tested in two phase I studies recruiting patients with advanced solid tumors, including glioblastoma (NCT01723020) and newly diagnosed or recurrent glioblastoma (NCT03107780), for an evaluation of its dose escalation or side effects.

2.1.4 | Targeting the Rb signaling pathway

The Rb signaling pathway plays an important role in controlling the G1-to-S phase cell cycle transition and in regulating DNA replication and cell division.[71,72] CDK4 and CDK6 share highly similar amino acid sequences and functions, and both interact with cyclin D to regulate the phosphorylation of the Rb protein.[73] After activation by upstream mitogenic signaling pathways, such as the PI3K/AKT/mTOR, mitogen-activated protein kinase, Wnt, JAK/STAT, and nuclear factor-κB (NF-κB) signaling pathways, cyclin D interacts with CDK4/6 and activates the function of CDK4/6 to phosphorylate Rb.[74] Phosphorylation of Rb causes the dissociation of the E2 family (E2F) transcription factor from Rb and ultimately activates the target genes of E2F and the G1-to-S cell cycle transition.[75,76]

Dysregulation of the Rb pathway is frequently observed in many types of tumors, including glioblastoma.[12,77] Gene alterations in the Rb pathway are present in 78.9% of glioblastomas, with 7.6% exhibiting *RB1* mutations/deletions, 15.5% exhibiting *CDK4/6* amplification, and 55.8% exhibiting *CDKN2A* deletions.[6] Because of the importance of the Rb pathway in cell cycle control and the prevalence of Rb pathway-related gene alterations in glioblastoma, the major components of this pathway are attractive targets for potential therapies and drug development.[77,78]

CDK4/6 inhibitors

Palbociclib (PD0332991) is a specific CDK4/6 inhibitor that is designed to inhibit the function of CDK4/6, thus reducing the phosphorylation of Rb protein and leading to cell cycle arrest during cancer treatment.[79,80] A phase II clinical trial of palbociclib as a treatment for breast cancer showed that it significantly improves the survival of patients with estrogen receptor-positive and HER2-negative breast cancer.[81] Based on the promising effects observed in clinical trials, palbociclib was approved by the FDA in 2015 as a treatment for breast cancer. In preliminary preclinical studies, palbociclib showed good effects on inhibiting tumor growth in xenograft models of glioblastoma cell lines and primary tumor cells, and thus became a promising candidate for the treatment of glioblastoma.[82,83] However, a phase II study of palbociclib in patients with recurrent Rb-positive glioblastoma (NCT01227434) revealed its lack of effectiveness as a treatment for glioblastoma.[84]

Ribociclib (LEE011) is a specific inhibitor of CDK4/6 with good oral bioavailability.[85] Due to its good therapeutic effects on several types of cancer in both preclinical and clinical studies,[86] ribociclib was approved by the FDA as a treatment for HR-positive and HER2-negative breast cancer in combination with an aromatase inhibitor. Ribociclib showed promising efficacy and good safety at inhibiting neuroblastoma growth in phase I clinical study.[87] As preclinical studies revealed the good ability of ribociclib to penetrate the BBB, several clinical trials were recently developed to explore the possibility of using this drug as a clinical treatment for glioblastoma. A phase 0 clinical trial in patients with recurrent glioblastoma showed that ribociclib exhibited good penetration into tumor regions and effectively inhibited Rb phosphorylation[88]; however, a phase Ib clinical study only identified limited efficacy of ribociclib monotherapy, suggesting that combination therapies with CDK4/6 inhibitors and inhibitors of other signaling pathways might be a new therapeutic strategy for glioblastoma.[89]

2.2 | Targeting transcriptional dysregulation

Precise transcriptional regulation is important for maintaining the specific gene expression patterns of different cell types, and transcriptional dysregulation may be related to many types of diseases, including cancer.[90]

This relationship is partially explained by the discovery that many types of cancer cells rely on aberrant gene expression programs that are regulated by master transcription factors to maintain a specific oncogenic status.[91] Although transcription factors are promising therapeutic targets in cancer cells, the development of direct pharmacological targeted therapies has long been extremely difficult. However, several studies have recently shown that targeting transcriptional cofactors, such as cyclin-dependent kinases (CDKs), represent a new approach to the development of drugs for cancer treatment.[92]

Oncogenic EGFR mutations (such as EGFRvIII) in glioblastoma were considered to promote tumor progression by activating downstream signal transduction. Meanwhile, the efficacies of target therapies for EGFR or components of downstream signaling pathways were not promising. However, a recent study provided an alternative therapeutic strategy for EGFR-mutated glioblastoma, showing that EGFR mutation promoted tumor growth by activating the expression of key transcription factors and remodeling the gene transcriptional program in glioblastoma cells.[93] Based on this finding, targeting transcriptional dysregulation driven by the hyperactivation of oncogenic pathways may be a new strategy for drug development in glioblastoma.

2.2.1 | CDK7 inhibitors

CDK7 is the kinase subunit of the general transcription factor TFIIH and plays an important role in activating transcription initiation by phosphorylating the C-terminal domain of RNA polymerase II (RNA Pol II-CTD).[94] THZ1 is designed to covalently bind to the Cys312 residue outside the kinase domain of CDK7 and shows relatively selective inhibition of CDK7 at lower doses.[92] According to several preclinical studies, THZ1 exhibits potent efficacy in inhibiting tumor cell growth both in vitro and in vivo in several types of cancer, such as T-cell acute lymphoblastic leukemia,[92] small cell lung cancer,[95] triple-negative breast cancer,[96] peripheral T-cell lymphoma,[97] pancreatic cancer,[98] human renal cell carcinoma,[99] and ovarian cancer.[100] Targeting transcriptional dysregulation in diffuse intrinsic pontine glioma (DIPG) by using CDK7 inhibitor THZ1 can effectively reduce the in vitro proliferation of patient-derived DIPG cells and in vivo tumor growth in xenograft models, especially in combination therapy with HDAC inhibition.[101] CDK7 inhibition with THZ1 is also reported to significantly disrupt the survival of patient-derived primary HGG cells, suggesting that CDK7 is a promising therapeutic target for malignant gliomas.[102]

2.3 | Targeting tumor angiogenesis

Angiogenesis is suggested to participate not only in the early stage of tumorigenesis but also in later processes, such as tumor progression and metastasis.[103] Necrosis and microvascular proliferation are the most distinct histopathological characteristics of glioblastoma that distinguish it from low-grade gliomas,[104] suggesting that targeting tumor angiogenesis-related pathways is a rational therapeutic strategy for glioblastoma. Tumor angiogenesis is primarily regulated by proangiogenic factors, including vascular endothelial growth factor (VEGF), PDGF, and basic fibroblast growth factor.[105] Upregulated VEGF expression is usually detected in higher grade glioma and is related to a worse prognosis.[106,107] The VEGF signaling pathway is a widely studied pathway related to tumor angiogenesis, and drugs targeting VEGF signaling have been characterized in clinical studies as good therapeutic candidates for inhibiting cancer including glioblastoma.

2.3.1 | VEGF inhibitors

Bevacizumab is a humanized VEGF-specific monoclonal antibody[108] and is currently the most extensively studied antiangiogenic drug in clinical trials of glioblastoma. It was approved by the FDA for the treatment of multiple types

of cancer, such as colon, lung, kidney, and cervical cancers. Due to its promising effects in phase II trials, bevacizumab received FDA approval for the treatment of recurrent glioblastoma in 2009. Since then, many clinical trials have been conducted in patients with glioblastoma to assess the effects of combination therapy with bevacizumab and standard treatment or other chemotherapies, but promising results have not yet been reported.

2.4 | Targeting immune checkpoint pathways

The immune system is a natural defense mechanism that relies on extensive cooperation among immune cells, tissues, and organs within the body. The immune system functions to recognize and eliminate foreign materials or antigens, and protect the body from invasion by harmful substances, such as transplanted grafts, bacteria, or cancer cells. However, some cancer cells manage to escape immune system surveillance through several mechanisms and maintain their own immunosuppressive microenvironment. Immunotherapy is a promising approach for cancer treatment that has developed rapidly in recent years. Immune checkpoints are stimulatory or inhibitory regulators of the immune response that regulate antigen recognition by T-cell receptors. Inhibitory immune checkpoints, such as cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and programmed cell death protein 1 (PD-1), function to inhibit the overactivation of the immune response and protect normal cells from being erroneously eliminated by the immune system. However, some cancer cells manage to escape the immune response by deregulating the CTLA-4/PD-1 immune checkpoint pathways. Immunotherapy targeting inhibitory immune checkpoints such as CTLA-4/PD-1 reactivate the T-cell-mediated immune response to eliminate tumor cells.

2.4.1 | Immune checkpoint inhibitors

Currently, CTLA-4 and PD-1/PD-L1 are the most extensively studied immunotherapeutic targets in many types of cancers, including glioblastoma (summarized in Table 2).[109] Ipilimumab, a CTLA-4 inhibitor, received FDA approval as a treatment for metastatic melanoma.[110] PD-1/PD-L1 inhibitors, including nivolumab and pembrolizumab, also showed promising effects as treatments for melanoma.[111,112] In addition, clinical trials of nivolumab and pembrolizumab as treatments for brain metastases of other types of cancer showed that these inhibitors effectively cross the BBB and exert activity in the brain.[113,114] The promising results of immune checkpoint inhibitors in other types of cancer have led to extensive preclinical studies and clinical assessments of the activity of these inhibitors in brain tumors, including glioblastoma.[115,116] The results of preclinical studies support the good effectiveness of PD-1 inhibitors in animal models. Clinical trials with combination therapies of ipilimumab and nivolumab are now recruiting patients with newly diagnosed or recurrent glioblastoma (NCT02311920/NCT03233152/NCT03367715). Nivolumab was compared with bevacizumab in a phase III study for recurrent glioblastoma treatment (CheckMate 143, NCT02017717) and was reported to exhibit a failure to meet the primary endpoint of improved overall survival (OS).[117] It is also being assessed in a phase III study (CheckMate 498, NCT02617589) in combination with radiation therapy in patients with newly diagnosed MGMT-unmethylated glioblastoma. The report of CheckMate 498 by Bristol-Myers Squibb (BMS) showed that nivolumab plus radiation therapy failed to meet the primary endpoint of OS in the patients with glioblastoma.[118] In addition, a phase III study (CheckMate 548, NCT02667587) evaluated the efficacy of nivolumab as an adjuvant treatment with TMZ and radiation therapy in patients with MGMT promoter methylation. The BMS report of CheckMate 548 revealed that nivolumab did not meet its primary endpoint, the progression-free survival, of patients with standard therapy in glioblastoma, and the trial is still ongoing as the OS data are not complete.[119] Besides, another two phase II clinical trials with combination therapies of nivolumab are also recruiting patients with newly diagnosed or recurrent glioblastoma (NCT03743662/NCT04195139). Pembrolizumab, another PD-1 inhibitor, is also being assessed either as monotherapy or combined with standard radiation plus TMZ treatment in phase I or phase II studies recruiting patients

with newly diagnosed or recurrent glioblastoma (NCT02530502/NCT03661723/NCT03899857). Pidilizumab, which was also developed as a PD-1 inhibitor, was assessed in an ongoing phase I/II trial in patients with diffuse intrinsic pontine glioma (NCT01952769). The effectiveness of durvalumab, a PD-L1 inhibitor, is currently being evaluated in an ongoing phase II trial in patients with glioblastoma (NCT02336165). A phase I trial (NCT01375842) revealed the good tolerability of atezolizumab, another promising PD-L1 inhibitor, in patients with recurrent glioblastoma,[120] and it is also included in current phase I or phase II studies recruiting patients with glioblastoma (NCT02458638/NCT03174197).

3 | POTENTIAL CHALLENGES AND FUTURE PERSPECTIVES

As mentioned above, the current clinical treatment for glioblastoma includes maximal tumor resection, subsequent radiation therapy, and concurrent TMZ chemotherapy. However, complete surgical resection of tumor tissues in the brain is practically impossible, possibly partially because of the infiltrative characteristics of glioblastoma tumor cells, which rapidly intrude into other parts of the brain. Although the combination therapy with radiation and TMZ showed effects in some clinical studies, the prognosis of patients with glioblastoma remains poor. Chemotherapeutic or biological agents targeting core signaling pathways or key biological processes in tumor progression have been rapidly developed in the last two decades. However, as we previously noted in this review, drug resistance and tumor recurrence are the most common obstacles to drug development in current preclinical or clinical studies of glioblastoma.

Tumor heterogeneity has long been presumed to be an important reason for drug resistance and tumor recurrence in patients with glioblastoma and is a challenge for the drug development in glioblastoma. Intensive genomic exploration has revealed the genetic landscape of glioblastoma and the complexity of genetic alterations in glioblastoma.[6,12] The differences in gene expression profiles among individual tumor samples imply that intertumor heterogeneity is a characteristic of glioblastoma. In addition, the intratumor heterogeneity of glioblastoma has been observed at the single-cell level. Recently, single-cell RNA-seq analysis of five primary glioblastoma tumor samples revealed that diverse transcriptional profiles usually coexist in individual cells from the same tumor.[121] Due to the widespread intertumor or intratumor heterogeneity in glioblastoma, drug resistance is inevitable. Tumor heterogeneity has important implications for the drug development of targeted therapies for glioblastoma. It underscores the importance of developing appropriate prognostic and predictive biomarkers for specific targeted therapies and suggests that combination therapies represent a potentially valid option to avoid the failure of monotherapies.

Most patients with proneural subtype of glioblastoma are young adults and correlated with better clinical outcomes. Proneural tumors are usually characterized with *PDGFRA* amplification/*PDGFRA* mutations/*IDH1* mutations/*TP53* mutations, with high-level expression of oligodendrocytic development genes (eg, *NKX2-2*/*OLIG2*) and proneural development genes (eg, *SOX* genes/*DCX*/*DLL3*/*ASCL1*/*TCF4*).[4] Most classical glioblastoma tumors harbor chromosome 7 amplification and chromosome 10 deletion.[4] High-level expression of *EGFR*/stem cell marker genes (eg, *NES*)/Notch and Sonic hedgehog pathway genes (eg, *NOTCH3*/*JAG1*/*LFNG*), and homozygous deletion of *CDKN2A* are frequently associated with a classical subtype of glioblastoma.[4] Patients with mesenchymal subtype are usually in older age and have worse prognosis than other subtypes. Low-level expression of *NF1*, and high-level expression of mesenchymal marker genes (eg, *CHI3L1*/*MET*) and TNF/NF-κB pathway genes (eg, *TRADD*/*RELB*/*TNFRSF1A*) are correlated with mesenchymal subtype.[4] Neural subtype has a relatively higher level expression of neuron marker genes, such as *NEFL*, *GABRA1*, *SYT1*, and *SLC12A5*.[4] Retrospective studies of clinical trials/cases revealed that the clinical outcomes of a specific treatment verified in patients with different subtypes. A retrospective analysis of phase III clinical trial of bevacizumab plus temozolomide and radiotherapy in patients with newly diagnosed glioblastoma showed that the addition of bevacizumab specifically improved the OS of patients with *IDH1* wide-type proneural subtype.[122] Another retrospective evaluation of the data of bevacizumab treatment

TABLE 2 Clinical trials of immune checkpoint inhibitors in patients with glioblastoma

Target		Drug	Intervention	Patients	NCT Code	Phase	Outcome/ publication
Immune checkpoint pathway	CTLA-4	Ipilimumab	Ipilimumab and/or Nivolumab + Temozolomide	Newly diagnosed glioblastoma or gliosarcoma	NCT02311920	Phase I	Active, not recruiting
			Ipilimumab + Nivolumab	Recurrent glioblastoma	NCT03233152	Phase I	Recruiting
			Ipilimumab + Nivolumab + Radiation	Newly diagnosed MGMT-unmethylated glioblastoma	NCT03367715	Phase II	Recruiting
	PD-1	Nivolumab	Nivolumab vs Bevacizumab	Recurrent glioblastoma	NCT02017717 (CheckMate 143)	Phase III	BMS report[117]
			Nivolumab + Radiation vs Temozolomide + Radiation	Newly diagnosed MGMT-unmethylated glioblastoma	NCT02617589 (CheckMate 498)	Phase III	BMS report[118]
			Nivolumab + Temozolomide + Radiation	Newly diagnosed MGMT-methylated glioblastoma	NCT02667587 (CheckMate 548)	Phase III	BMS report[119]
			Nivolumab + Bevacizumab + Radiation	Recurrent MGMT-methylated glioblastoma	NCT03743662	Phase II	Recruiting
			Nivolumab + Temozolomide	Elderly, newly diagnosed with glioblastoma	NCT04195139	Phase II	Recruiting
		Pembroli-zumab	Pembrolizumab	Newly diagnosed glioblastoma	NCT03899857	Phase II	Not yet recruiting
			Pembrolizumab + + Reirradiation	Naïve and bevacizumab-resistant recurrent glioblastoma	NCT03661723	Phase II	Recruiting
			Pembrolizumab + Temozolomide + Radiation	Newly diagnosed glioblastoma	NCT02530502	Phase I	Active, not recruiting

TABLE 2 (Continued)

Target	Drug	Intervention	Patients	NCT Code	Phase	Outcome/ publication
	Pidilizumab	Pidilizumab	Diffuse intrinsic pontine glioma	NCT01952769	Phase I	Unknown
					Phase II	
PD-L1	Durvalumab	Durvalumab	Glioblastoma	NCT02336165	Phase II	Active, not recruiting
	Atezolizumab	Atezolizumab	Solid tumors, including glioblastoma	NCT01375842	Phase I	Lukas et al[120]
		Atezolizumab	Advanced solid tumors, including glioblastoma	NCT02458638	Phase II	Active, not recruiting
		Atezolizumab + Temozolomide + Radiation	Newly diagnosed glioblastoma	NCT03174197	Phase I	Recruiting
					Phase II	

Abbreviations: CTLA-4, cytotoxic T-lymphocyte-associated antigen 4; PD-1, programmed cell death protein 1.

in patients with recurrent glioblastoma showed that the patients with classical subtype had worse responses to bevacizumab than mesenchymal or proneural subtypes.[123]

The BBB, which is composed of capillary endothelial cells, astrocyte endfeet, and pericytes, is a highly selective and dynamic cellular structure located between the blood compartment and the brain.[124] Under physiological conditions, the normal function of the BBB is to separate the brain from the blood vessels, protecting it from potentially harmful materials, and maintaining the stability of the microenvironment of the brain. Although high-grade gliomas affect the organization of the BBB by altering the normal functions of blood vessels, the function of the tumor BBB resembles the BBB under normal conditions and impedes the penetration of drugs into the tumor environment in the brain.[124] The development of drug delivery techniques to overcome the obstacle of the BBB is another critical challenge in the development of targeted therapies. As mentioned above, some promising therapeutic candidates that showed potent efficacy in preclinical studies showed high rates of failure in subsequent clinical trials. An important cause of these failures was that these drugs were unable to penetrate the BBB and function at the tumor site with effective doses. Novel strategies for drug delivery to overcome the obstacle of the BBB, including using focused ultrasound with microbubbles to mechanically disrupt the BBB [125] or the design of BBB-crossing nanoparticles,[126,127] should be considered in future drug development of targeted therapies for glioblastoma.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

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REFERENCES

1. Ostrom QT, Cioffi G, Gittleman H, et al. CBTRUS statistical report: primary brain and other central nervous system tumors diagnosed in the United States in 2012-2016. *Neuro Oncol*. 2019;21(suppl_5):v1-v100.
2. Wen PY, Huse JT. 2016 World Health Organization classification of central nervous system tumors. *Continuum (Minneap Minn)*. 2017;23(6, neuro-oncology):1531-1547.
3. Ohgaki H, Kleihues P. The definition of primary and secondary glioblastoma. *Clin Cancer Res*. 2013;19(4):764-772.
4. Verhaak RGW, Hoadley KA, Purdom E, et al. Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. *Cancer Cell*. 2010;17(1):98-110.
5. Phillips HS, Kharbanda S, Chen R, et al. Molecular subclasses of high-grade glioma predict prognosis, delineate a pattern of disease progression, and resemble stages in neurogenesis. *Cancer Cell*. 2006;9(3):157-173.
6. Brennan CW, Verhaak RGW, McKenna A, et al. The somatic genomic landscape of glioblastoma. *Cell*. 2013;155(2):462-477.
7. Gerstner ER, Yip S, Wang DL, Louis DN, Iafrate AJ, Batchelor TT. Mgmt methylation is a prognostic biomarker in elderly patients with newly diagnosed glioblastoma. *Neurology*. 2009;73(18):1509-1510.
8. Weller M, Tabatabai G, Kastner B, et al. MGMT promoter methylation is a strong prognostic biomarker for benefit from dose-intensified temozolomide rechallenge in progressive glioblastoma: the DIRECTOR trial. *Clin Cancer Res*. 2015;21(9):2057-2064.
9. Bleeker FE, Atai NA, Lamba S, et al. The prognostic IDH1(R132) mutation is associated with reduced NADP+-dependent IDH activity in glioblastoma. *Acta Neuropathol*. 2010;119(4):487-494.
10. Huse JT. TERT promoter mutation designates biologically aggressive primary glioblastoma. *Neuro Oncol*. 2015;17(1):5-6.

11. Mosrati MA, Malmström A, Lysiak M, et al. TERT promoter mutations and polymorphisms as prognostic factors in primary glioblastoma. *Oncotarget*. 2015;6(18):16663-16673.
12. Cancer Genome Atlas Research Network. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature*. 2008;455(7216):1061-1068.
13. Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med*. 2005;352(10):987-996.
14. Yarden Y, Sliwkowski MX. Untangling the ErbB signalling network. *Nat Rev Mol Cell Biol*. 2001;2(2):127-137.
15. Singh B, Coffey RJ. Trafficking of epidermal growth factor receptor ligands in polarized epithelial cells. *Annu Rev Physiol*. 2014;76:275-300.
16. Mayer BJ. The discovery of modular binding domains: building blocks of cell signalling. *Nat Rev Mol Cell Biol*. 2015; 16(11):691-698.
17. Furnari FB, Cloughesy TF, Cavenee WK, Mischel PS. Heterogeneity of epidermal growth factor receptor signalling networks in glioblastoma. *Nat Rev Cancer*. 2015;15(5):302-310.
18. Huang PH, Xu AM, White FM. Oncogenic EGFR signaling networks in glioma. *Sci Signal*. 2009;2(87):re6.
19. Guo G, Gong K, Wohlfeld B, Hatanpaa KJ, Zhao D, Habib AA. Ligand-Independent EGFR signaling. *Cancer Res*. 2015; 75(17):3436-3441.
20. Halatsch ME, Gehrke EE, Vougioukas VI, et al. Inverse correlation of epidermal growth factor receptor messenger RNA induction and suppression of anchorage-independent growth by OSI-774, an epidermal growth factor receptor tyrosine kinase inhibitor, in glioblastoma multiforme cell lines. *J Neurosurg*. 2004;100(3):523-533.
21. Reardon DA, Groves MD, Wen PY, et al. A phase I/II trial of pazopanib in combination with lapatinib in adult patients with relapsed malignant glioma. *Clin Cancer Res*. 2013;19(4):900-908.
22. Hegi ME, Diserens AC, Bady P, et al. Pathway analysis of glioblastoma tissue after preoperative treatment with the EGFR tyrosine kinase inhibitor gefitinib--a phase II trial. *Mol Cancer Ther*. 2011;10(6):1102-1112.
23. Chakravarti A, Wang M, Robins HI, et al. RTOG 0211: a phase 1/2 study of radiation therapy with concurrent gefitinib for newly diagnosed glioblastoma patients. *Int J Radiat Oncol Biol Phys*. 2013;85(5):1206-1211.
24. Brown PD, Krishnan S, Sarkaria JN, et al. Phase I/II trial of erlotinib and temozolomide with radiation therapy in the treatment of newly diagnosed glioblastoma multiforme: North Central Cancer Treatment Group Study N0177. *J Clin Oncol*. 2008;26(34):5603-5609.
25. van den Bent MJ, Brandes AA, Rampling R, et al. Randomized phase II trial of erlotinib versus temozolomide or carmustine in recurrent glioblastoma: EORTC brain tumor group study 26034. *J Clin Oncol*. 2009;27(8):1268-1274.
26. Thiessen B, Stewart C, Tsao M, et al. A phase I/II trial of GW572016 (lapatinib) in recurrent glioblastoma multiforme: clinical outcomes, pharmacokinetics and molecular correlation. *Cancer Chemother Pharmacol*. 2010;65(2):353-361.
27. Reardon DA, Nabors LB, Mason WP, et al. Phase I/randomized phase II study of afatinib, an irreversible ErbB family blocker, with or without protracted temozolomide in adults with recurrent glioblastoma. *Neuro Oncol*. 2015;17(3): 430-439.
28. Zahonero C, Aguilera P, Ramírez-Castillejo C, et al. Preclinical test of dacomitinib, an irreversible EGFR inhibitor, confirms its effectiveness for glioblastoma. *Mol Cancer Ther*. 2015;14(7):1548-1558.
29. Sepúlveda-Sánchez JM, Vaz MÁ, Balañá C, et al. Phase II trial of dacomitinib, a pan-human EGFR tyrosine kinase inhibitor, in recurrent glioblastoma patients with EGFR amplification. *Neuro Oncol*. 2017;19(11):1522-1531.
30. Roth P, Weller M. Challenges to targeting epidermal growth factor receptor in glioblastoma: escape mechanisms and combinatorial treatment strategies. *Neuro Oncol*. 2014;16(Suppl 8):viii14-viii19.
31. Cross DAE, Ashton SE, Ghiorghiu S, et al. AZD9291, an irreversible EGFR TKI, overcomes T790M-mediated resistance to EGFR inhibitors in lung cancer. *Cancer Discov*. 2014;4(9):1046-1061.
32. Tan CS, Gilligan D, Pacey S. Treatment approaches for EGFR-inhibitor-resistant patients with non-small-cell lung cancer. *Lancet Oncol*. 2015;16(9):e447-e459.
33. Chuang JC, Salahudeen AA, Wakelee HA. Rociletinib, a third generation EGFR tyrosine kinase inhibitor: current data and future directions. *Expert Opin Pharmacother*. 2016;17(7):989-993.
34. Sequist LV, Rolfe L, Allen AR. Rociletinib in EGFR-Mutated non-small-cell lung cancer. *N Engl J Med*. 2015;373(6): 578-579.
35. Liu X, Chen X, Shi L, et al. The third-generation EGFR inhibitor AZD9291 overcomes primary resistance by continuously blocking ERK signaling in glioblastoma. *J Exp Clin Cancer Res*. 2019;38(1):219.
36. Gan HK, Burgess AW, Clayton AH, Scott AM. Targeting of a conformationally exposed, tumor-specific epitope of EGFR as a strategy for cancer therapy. *Cancer Res*. 2012;72(12):2924-2930.
37. Eller JL, Longo SL, Kyle MM, Bassano D, Hicklin DJ, Canute GW. Anti-epidermal growth factor receptor monoclonal antibody cetuximab augments radiation effects in glioblastoma multiforme in vitro and in vivo. *Neurosurgery*. 2005; 56(1):155-162.

38. Du XJ, Li XM, Cai LB, et al. Efficacy and safety of nimotuzumab in addition to radiotherapy and temozolomide for cerebral glioblastoma: a phase II multicenter clinical trial. *J Cancer*. 2019;10(14):3214-3223.
39. Swartz AM, Li QJ, Sampson JH. Rindopepimut: a promising immunotherapeutic for the treatment of glioblastoma multiforme. *Immunotherapy*. 2014;6(6):679-690.
40. Sampson JH, Archer GE, Mitchell DA, et al. An epidermal growth factor receptor variant III-targeted vaccine is safe and immunogenic in patients with glioblastoma multiforme. *Mol Cancer Ther*. 2009;8(10):2773-2779.
41. Del Vecchio CA, Wong AJ. Rindopepimut, a 14-mer injectable peptide vaccine against EGFRvIII for the potential treatment of glioblastoma multiforme. *Curr Opin Mol Ther*. 2010;12(6):741-754.
42. Schuster J, Lai RK, Recht LD, et al. A phase II, multicenter trial of rindopepimut (CDX-110) in newly diagnosed glioblastoma: the ACT III study. *Neuro Oncol*. 2015;17(6):854-861.
43. Weller M, Butowski N, Tran DD, et al. Rindopepimut with temozolomide for patients with newly diagnosed, EGFRvIII-expressing glioblastoma (ACT IV): a randomised, double-blind, international phase 3 trial. *Lancet Oncol*. 2017;18(10):1373-1385.
44. Engelman JA, Luo J, Cantley LC. The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. *Nat Rev Genet*. 2006;7(8):606-619.
45. Zhao H, Wang J, Shao W, et al. Recent advances in the use of PI3K inhibitors for glioblastoma multiforme: current preclinical and clinical development. *Mol Cancer*. 2017;16(1):100.
46. Nojima H, Tokunaga C, Eguchi S, et al. The mammalian target of rapamycin (mTOR) partner, raptor, binds the mTOR substrates p70 S6 kinase and 4E-BP1 through their TOR signaling (TOS) motif. *J Biol Chem*. 2003;278(18):15461-15464.
47. Kubota N, Okada S, Inada T, Ohnishi K, Ohnishi T. Wortmannin sensitizes human glioblastoma cell lines carrying mutant and wild type TP53 gene to radiation. *Cancer Lett*. 2000;161(2):141-147.
48. Tian Q, Cui H, Li Y, Lu H. LY294002 induces differentiation and inhibits invasion of glioblastoma cells by targeting GSK-3beta and MMP. *EXCLI J*. 2012;11:68-77.
49. Rodon J, Dienstmann R, Serra V, Tabernero J. Development of PI3K inhibitors: lessons learned from early clinical trials. *Nat Rev Clin Oncol*. 2013;10(3):143-153.
50. Burger MT, Pecchi S, Wagman A, et al. Identification of NVP-BKM120 as a potent, selective, orally bioavailable class I PI3 kinase inhibitor for treating cancer. *ACS Med Chem Lett*. 2011;2(10):774-779.
51. Koul D, Fu J, Shen R, et al. Antitumor activity of NVP-BKM120--a selective pan class I PI3 kinase inhibitor showed differential forms of cell death based on p53 status of glioma cells. *Clin Cancer Res*. 2012;18(1):184-195.
52. Wen PY, Touat M, Alexander BM, et al. Buparlisib in patients with recurrent glioblastoma harboring phosphatidylinositol 3-kinase pathway activation: an open-label, multicenter, multi-arm, phase II trial. *J Clin Oncol*. 2019;37(9):741-750.
53. Momota H, Nerio E, Holland EC. Perifosine inhibits multiple signaling pathways in glial progenitors and cooperates with temozolomide to arrest cell proliferation in gliomas in vivo. *Cancer Res*. 2005;65(16):7429-7435.
54. Kaley TJ, Panageas KS, Mellinghoff IK, et al. Phase II trial of an AKT inhibitor (perifosine) for recurrent glioblastoma. *J Neurooncol*. 2019;144(2):403-407.
55. Cloughesy TF, Yoshimoto K, Nghiemphu P, et al. Antitumor activity of rapamycin in a phase I trial for patients with recurrent PTEN-deficient glioblastoma. *PLoS Med*. 2008;5(1):e8.
56. Chang SM, Wen P, Cloughesy T, et al. Phase II study of CCI-779 in patients with recurrent glioblastoma multiforme. *Invest New Drugs*. 2005;23(4):357-361.
57. Markman B, Dienstmann R, Tabernero J. Targeting the PI3K/Akt/mTOR pathway--beyond rapalogs. *Oncotarget*. 2010;1(7):530-543.
58. Fan QW, Knight ZA, Goldenberg DD, et al. A dual PI3 kinase/mTOR inhibitor reveals emergent efficacy in glioma. *Cancer Cell*. 2006;9(5):341-349.
59. Bagci-Onder T, Wakimoto H, Anderegg M, Cameron C, Shah K. A dual PI3K/mTOR inhibitor, PI-103, cooperates with stem cell-delivered TRAIL in experimental glioma models. *Cancer Res*. 2011;71(1):154-163.
60. Maira SM, Stauffer F, Brueggen J, et al. Identification and characterization of NVP-BEZ235, a new orally available dual phosphatidylinositol 3-kinase/mammalian target of rapamycin inhibitor with potent in vivo antitumor activity. *Mol Cancer Ther*. 2008;7(7):1851-1863.
61. Liu TJ, Koul D, LaFortune T, et al. NVP-BEZ235, a novel dual phosphatidylinositol 3-kinase/mammalian target of rapamycin inhibitor, elicits multifaceted antitumor activities in human gliomas. *Mol Cancer Ther*. 2009;8(8):2204-2210.
62. Wise-Draper TM, Moorthy G, Salkeni MA, et al. A phase Ib study of the dual PI3K/mTOR inhibitor Dactolisib (BEZ235) combined with everolimus in patients with advanced solid malignancies. *Target Oncol*. 2017;12(3):323-332.
63. Wei CL, Wu Q, Vega VB, et al. A global map of p53 transcription-factor binding sites in the human genome. *Cell*. 2006;124(1):207-219.

64. el-Deiry WS, Kern SE, Pietenpol JA, Kinzler KW, Vogelstein B. Definition of a consensus binding site for p53. *Nat Genet*. 1992;1(1):45-49.
65. Wade M, Li YC, Wahl GM. MDM2, MDMX and p53 in oncogenesis and cancer therapy. *Nat Rev Cancer*. 2013;13(2):83-96.
66. Kastenhuber ER, Lowe SW. Putting p53 in context. *Cell*. 2017;170(6):1062-1078.
67. Lane DP. Cancer. p53, guardian of the genome. *Nature*. 1992;358(6381):15-16.
68. Vu B, Wovkulich P, Pizzolato G, et al. Discovery of RG7112: a small-molecule MDM2 inhibitor in clinical development. *ACS Med Chem Lett*. 2013;4(5):466-469.
69. Verreault M, Schmitt C, Goldwirt L, et al. Preclinical efficacy of the MDM2 inhibitor RG7112 in MDM2-amplified and TP53 wild-type glioblastomas. *Clin Cancer Res*. 2016;22(5):1185-1196.
70. Her NG, Oh JW, Oh YJ, et al. Potent effect of the MDM2 inhibitor AMG232 on suppression of glioblastoma stem cells. *Cell Death Dis*. 2018;9(8):792.
71. Wang H, Zhang X, Teng L, Legerski RJ. DNA damage checkpoint recovery and cancer development. *Exp Cell Res*. 2015;334(2):350-358.
72. Harbour JW, Luo RX, Dei Santi A, Postigo AA, Dean DC. Cdk phosphorylation triggers sequential intramolecular interactions that progressively block Rb functions as cells move through G1. *Cell*. 1999;98(6):859-869.
73. Grossel MJ, Hinds PW. From cell cycle to differentiation: an expanding role for cdk6. *Cell Cycle*. 2006;5(3):266-270.
74. Shapiro GI. Cyclin-dependent kinase pathways as targets for cancer treatment. *J Clin Oncol*. 2006;24(11):1770-1783.
75. Narasimha AM, Kaulich M, Shapiro GS, Choi YJ, Sicinski P, Dowdy SF. Cyclin D activates the Rb tumor suppressor by mono-phosphorylation. *eLife*. 2014;3:e02872.
76. Baker SJ, Reddy EP. CDK4: a key player in the cell cycle, development, and cancer. *Genes Cancer*. 2012;3(11-12):658-669.
77. Roberts PJ, Bisi JE, Strum JC, et al. Multiple roles of cyclin-dependent kinase 4/6 inhibitors in cancer therapy. *J Natl Cancer Inst*. 2012;104(6):476-487.
78. Dickson MA. Molecular pathways: CDK4 inhibitors for cancer therapy. *Clin Cancer Res*. 2014;20(13):3379-3383.
79. Toogood PL, Harvey PJ, Repine JT, et al. Discovery of a potent and selective inhibitor of cyclin-dependent kinase 4/6. *J Med Chem*. 2005;48(7):2388-2406.
80. Fry DW, Harvey PJ, Keller PR, et al. Specific inhibition of cyclin-dependent kinase 4/6 by PD 0332991 and associated antitumor activity in human tumor xenografts. *Mol Cancer Ther*. 2004;3(11):1427-1438.
81. Finn RS, Crown JP, Lang I, et al. The cyclin-dependent kinase 4/6 inhibitor palbociclib in combination with letrozole versus letrozole alone as first-line treatment of oestrogen receptor-positive, HER2-negative, advanced breast cancer (PALOMA-1/TRIO-18): a randomised phase 2 study. *Lancet Oncol*. 2015;16(1):25-35.
82. Michaud K, Solomon DA, Oermann E, et al. Pharmacologic inhibition of cyclin-dependent kinases 4 and 6 arrests the growth of glioblastoma multiforme intracranial xenografts. *Cancer Res*. 2010;70(8):3228-3238.
83. Cen L, Carlson BL, Schroeder MA, et al. p16-Cdk4-Rb axis controls sensitivity to a cyclin-dependent kinase inhibitor PD0332991 in glioblastoma xenograft cells. *Neuro Oncol*. 2012;14(7):870-881.
84. Taylor JW, Parikh M, Phillips JJ, et al. Phase-2 trial of palbociclib in adult patients with recurrent RB1-positive glioblastoma. *J Neurooncol*. 2018;140(2):477-483.
85. Curigliano G, Criscitiello C, Esposito A, Intra M, Minucci S. Pharmacokinetic drug evaluation of ribociclib for the treatment of metastatic, hormone-positive breast cancer. *Expert Opin Drug Metab Toxicol*. 2017;13(5):575-581.
86. Tripathy D, Bardia A, Sellers WR. Ribociclib (LEE011): mechanism of action and clinical impact of this selective cyclin-dependent kinase 4/6 inhibitor in various solid tumors. *Clin Cancer Res*. 2017;23(13):3251-3262.
87. Geoerger B, Bourdeaut F, DuBois SG, et al. A phase I study of the CDK4/6 inhibitor Ribociclib (LEE011) in pediatric patients with malignant rhabdoid tumors, neuroblastoma, and other solid tumors. *Clin Cancer Res*. 2017;23(10):2433-2441.
88. Tien AC, Li J, Bao X, et al. A phase 0 trial of Ribociclib in recurrent glioblastoma patients incorporating a tumor pharmacodynamic- and pharmacokinetic-guided expansion cohort. *Clin Cancer Res*. 2019;25(19):5777-5786.
89. Miller TW, Traphagen NA, Li J, et al. Tumor pharmacokinetics and pharmacodynamics of the CDK4/6 inhibitor ribociclib in patients with recurrent glioblastoma. *J Neurooncol*. 2019;144(3):563-572.
90. Lee TI, Young RA. Transcriptional regulation and its misregulation in disease. *Cell*. 2013;152(6):1237-1251.
91. Bradner JE, Hnisz D, Young RA. Transcriptional addiction in cancer. *Cell*. 2017;168(4):629-643.
92. Kwiatkowski N, Zhang T, Rahl PB, et al. Targeting transcription regulation in cancer with a covalent CDK7 inhibitor. *Nature*. 2014;511(7511):616-620.
93. Liu F, Hon GC, Villa GR, et al. EGFR mutation promotes glioblastoma through epigenome and transcription factor network remodeling. *Mol Cell*. 2015;60(2):307-318.
94. Compe E, Egly JM. TFIIH: when transcription met DNA repair. *Nat Rev Mol Cell Biol*. 2012;13(6):343-354.

95. Christensen CL, Kwiatkowski N, Abraham BJ, et al. Targeting transcriptional addictions in small cell lung cancer with a covalent CDK7 inhibitor. *Cancer Cell*. 2014;26(6):909-922.
96. Wang Y, Zhang T, Kwiatkowski N, et al. CDK7-dependent transcriptional addiction in triple-negative breast cancer. *Cell*. 2015;163(1):174-186.
97. Cayrol F, Praditsuktavorn P, Fernando TM, et al. Corrigendum: THZ1 targeting CDK7 suppresses STAT transcriptional activity and sensitizes T-cell lymphomas to BCL2 inhibitors. *Nat Commun*. 2017;8:14747.
98. Lu P, Geng J, Zhang L, et al. THZ1 reveals CDK7-dependent transcriptional addictions in pancreatic cancer. *Oncogene*. 2019;38(20):3932-3945.
99. Chow PM, Liu SH, Chang YW, Kuo KL, Lin WC, Huang KH. The covalent CDK7 inhibitor THZ1 enhances temsirolimus-induced cytotoxicity via autophagy suppression in human renal cell carcinoma. *Cancer Lett*. 2020;471:27-37.
100. Zhang Z, Peng H, Wang X, et al. Preclinical efficacy and molecular mechanism of targeting CDK7-dependent transcriptional addiction in ovarian cancer. *Mol Cancer Ther*. 2017;16(9):1739-1750.
101. Nagaraja S, Vitanza NA, Woo PJ, et al. Transcriptional dependencies in diffuse intrinsic pontine glioma. *Cancer Cell*. 2017;31(5):635-652.
102. Greenall SA, Lim YC, Mitchell CB, et al. Cyclin-dependent kinase 7 is a therapeutic target in high-grade glioma. *Oncogenesis*. 2017;6(5):e336.
103. Seystahl K, Wick W, Weller M. Therapeutic options in recurrent glioblastoma--an update. *Crit Rev Oncol Hematol*. 2016;99:389-408.
104. Louis DN, Perry A, Reifenberger G, et al. The 2016 World Health Organization classification of tumors of the central nervous system: a summary. *Acta Neuropathol*. 2016;131(6):803-820.
105. Abounader R, Laterra J. Scatter factor/hepatocyte growth factor in brain tumor growth and angiogenesis. *Neuro Oncol*. 2005;7(4):436-451.
106. Zhou YH, Tan F, Hess KR, Yung WK. The expression of PAX6, PTEN, vascular endothelial growth factor, and epidermal growth factor receptor in gliomas: relationship to tumor grade and survival. *Clin Cancer Res*. 2003;9(9):3369-3375.
107. Schmidt NO, Westphal M, Hagel C, et al. Levels of vascular endothelial growth factor, hepatocyte growth factor/scatter factor and basic fibroblast growth factor in human gliomas and their relation to angiogenesis. *Int J Cancer*. 1999;84(1):10-18.
108. Ferrara N, Hillan KJ, Gerber HP, Novotny W. Discovery and development of bevacizumab, an anti-VEGF antibody for treating cancer. *Nat Rev Drug Discov*. 2004;3(5):391-400.
109. Caccese M, Indraccolo S, Zagonel V, Lombardi G. PD-1/PD-L1 immune-checkpoint inhibitors in glioblastoma: a concise review. *Crit Rev Oncol Hematol*. 2019;135:128-134.
110. Hodi FS, O'Day SJ, McDermott DF, et al. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med*. 2010;363(8):711-723.
111. Robert C, Long GV, Brady B, et al. Nivolumab in previously untreated melanoma without BRAF mutation. *N Engl J Med*. 2015;372(4):320-330.
112. Robert C, Ribas A, Wolchok JD, et al. Anti-programmed-death-receptor-1 treatment with pembrolizumab in ipilimumab-refractory advanced melanoma: a randomised dose-comparison cohort of a phase 1 trial. *Lancet*. 2014;384(9948):1109-1117.
113. Margolin K, Ernstoff MS, Hamid O, et al. Ipilimumab in patients with melanoma and brain metastases: an open-label, phase 2 trial. *Lancet Oncol*. 2012;13(5):459-465.
114. Goldberg SB, Gettinger SN, Mahajan A, et al. Pembrolizumab for patients with melanoma or non-small-cell lung cancer and untreated brain metastases: early analysis of a non-randomised, open-label, phase 2 trial. *Lancet Oncol*. 2016;17(7):976-983.
115. Carter T, Shaw H, Cohn-Brown D, Chester K, Mulholland P. Ipilimumab and bevacizumab in glioblastoma. *Clin Oncol*. 2016;28(10):622-626.
116. Preusser M, Lim M, Hafler DA, Reardon DA, Sampson JH. Prospects of immune checkpoint modulators in the treatment of glioblastoma. *Nat Rev Neurol*. 2015;11(9):504-514.
117. Bristol-Myers Squibb. *Bristol-Myers Squibb Announces Results From CheckMate -143, a Phase 3 Study of Opdivo (nivolumab) in Patients With Glioblastoma Multiforme*. 2017. https://news.bms.com/press-release/bmy/bristol-myers-squibb-announces-results-checkmate-143-phase-3-study-opdivo-nivoluma. Accessed 15 April 2020.
118. Bristol-Myers Squibb. *Bristol-Myers Squibb Announces Phase 3 CheckMate -498 Study Did Not Meet Primary Endpoint of Overall Survival with Opdivo (nivolumab) Plus Radiation in Patients with Newly Diagnosed MGMT-Unmethylated Glioblastoma Multiforme*. 2019. https://news.bms.com/press-release/corporatefinancial-news/bristol-myers-squibb-announces-phase-3-checkmate-498-study-did. Accessed 15 April 2020.

119. Bristol-Myers Squibb. *Bristol-Myers Squibb Provides Update on Phase 3 Opdivo (nivolumab) CheckMate-548 Trial in Patients with Newly Diagnosed MGMT-Methylated Glioblastoma Multiforme*. 2019. https://news.bms.com/press-release/corporatefinancial-news/bristol-myers-squibb-provides-update-phase-3-opdivo-nivolumab. Accessed 15 April 2020.
120. Lukas RV, Rodon J, Becker K, et al. Clinical activity and safety of atezolizumab in patients with recurrent glioblastoma. *J Neurooncol*. 2018;140(2):317-328.
121. Patel AP, Tirosh I, Trombetta JJ, et al. Single-cell RNA-seq highlights intratumoral heterogeneity in primary glioblastoma. *Science*. 2014;344(6190):1396-1401.
122. Sandmann T, Bourgon R, Garcia J, et al. Patients with proneural glioblastoma may derive overall survival benefit from the addition of bevacizumab to first-line radiotherapy and temozolomide: retrospective analysis of the AVAglio trial. *J Clin Oncol*. 2015;33(25):2735-2744.
123. Hovinga KE, McCrea HJ, Brennan C, et al. EGFR amplification and classical subtype are associated with a poor response to bevacizumab in recurrent glioblastoma. *J Neurooncol*. 2019;142(2):337-345.
124. Daneman R, Zhou L, Kebede AA, Barres BA. Pericytes are required for blood-brain barrier integrity during embryogenesis. *Nature*. 2010;468(7323):562-566.
125. Deng Z, Sheng Z, Yan F. Ultrasound-induced blood-brain-barrier opening enhances anticancer efficacy in the treatment of glioblastoma: current status and future prospects. *J Oncol*. 2019;2019:2345203.
126. Kim SS, Harford JB, Pirollo KF, Chang EH. Effective treatment of glioblastoma requires crossing the blood-brain barrier and targeting tumors including cancer stem cells: the promise of nanomedicine. *Biochem Biophys Res Commun*. 2015;468(3):485-489.
127. Tang W, Fan W, Lau J, Deng L, Shen Z, Chen X. Emerging blood-brain-barrier-crossing nanotechnology for brain cancer theranostics. *Chem Soc Rev*. 2019;48(11):2967-3014.
128. Prados MD, Yung WKA, Wen PY. Phase-1 trial of gefitinib and temozolomide in patients with malignant glioma: a North American brain tumor consortium study. *Cancer Chemotherapy and Pharmacology*. 2008;61(6):1059–1067. http://doi.org/10.1007/s00280-007-0556-y
129. Prados MD, Chang SM, Butowski N, et al. Phase II Study of Erlotinib Plus Temozolomide During and After Radiation Therapy in Patients With Newly Diagnosed Glioblastoma Multiforme or Gliosarcoma. *Journal of Clinical Oncology*. 2009;27(4):579–584. http://doi.org/10.1200/jco.2008.18.9639
130. Raizer JJ, Abrey LE, Lassman AB, et al. A phase I trial of erlotinib in patients with nonprogressive glioblastoma multiforme postradiation therapy, and recurrent malignant gliomas and meningiomas. *Neuro-Oncology*. 2010;12(1):87–94. http://doi.org/10.1093/neuonc/nop017
131. Yu A, Faiq N, Green S, et al. Report of safety of pulse dosing of lapatinib with temozolomide and radiation therapy for newly-diagnosed glioblastoma in a pilot phase II study. *Journal of Neuro-Oncology*. 2017;134(2):357–362. http://doi.org/10.1007/s11060-017-2533-6
132. Reardon DA, Desjardins A, Vredenburgh JJ, et al. Phase 2 trial of erlotinib plus sirolimus in adults with recurrent glioblastoma. *Journal of Neuro-Oncology*. 2010;96(2):219–230. http://doi.org/10.1007/s11060-009-9950-0
133. Chheda MG, Wen PY, Hochberg FH, et al. Vandetanib plus sirolimus in adults with recurrent glioblastoma: results of a phase I and dose expansion cohort study. *Journal of Neuro-Oncology*. 2015;121(3):627–634. http://doi.org/10.1007/s11060-014-1680-2
134. Mason WP, MacNeil M, Kavan P, et al. A phase I study of temozolomide and everolimus (RAD001) in patients with newly diagnosed and progressive glioblastoma either receiving or not receiving enzyme-inducing anticonvulsants: an NCIC CTG study. *Investigational New Drugs*. 2012;30(6):2344–2351. http://doi.org/10.1007/s10637-011-9775-5
135. Sarkaria JN, Galanis E, Wu W, et al. North Central Cancer Treatment Group Phase I Trial N057K of Everolimus (RAD001) and Temozolomide in Combination With Radiation Therapy in Patients With Newly Diagnosed Glioblastoma Multiforme. *International Journal of Radiation Oncology*Biology*Physics*. 2011;81(2):468–475. http://doi.org/10.1016/j.ijrobp.2010.05.064
136. Chinnaiyan P, Won M, Wen PY, et al. RTOG 0913: A Phase 1 Study of Daily Everolimus (RAD001) in Combination With Radiation Therapy and Temozolomide in Patients With Newly Diagnosed Glioblastoma. *International Journal of Radiation Oncology*Biology*Physics*. 2013;86(5):880–884. http://doi.org/10.1016/j.ijrobp.2013.04.036
137. Chinnaiyan P, Won M, Wen PY, et al. A randomized phase II study of everolimus in combination with chemoradiation in newly diagnosed glioblastoma: results of NRG Oncology RTOG 0913. *Neuro-Oncology*. 2018;20(5):666–673. http://doi.org/10.1093/neuonc/nox209
138. Chang SM, Kuhn J, Wen P, et al. Phase I/pharmacokinetic study of CCI-779 in patients with recurrent malignant glioma on enzyme-inducing antiepileptic drugs. *Investigational New Drugs*. 2004;22(4):427–435. http://doi.org/10.1023/b:drug.0000036685.72140.03
139. Kuhn JG, Chang SM, Wen PY, et al. Pharmacokinetic and Tumor Distribution Characteristics of Temsirolimus in Patients with Recurrent Malignant Glioma. *Clinical Cancer Research*. 2007;13(24):7401–7406. http://doi.org/10.1158/1078-0432.ccr-07-0781

140. Wen PY, Chang SM, Lamborn KR, et al. Phase I/II study of erlotinib and temsirolimus for patients with recurrent malignant gliomas: North American Brain Tumor Consortium trial 04-02. *Neuro-Oncology*. 2014;16(4):567–578. http://doi.org/10.1093/neuonc/not247
141. Hainsworth JD, Shih KC, Shepard GC, et al. Phase II study of concurrent radiation therapy, temozolomide, and bevacizumab followed by bevacizumab/everolimus as first-line treatment for patients with glioblastoma. *Clin Adv Hematol Oncol*. 2012;10(4):240–246.

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