

# Glioblastoma pharmacotherapy: A multifaceted perspective of conventional and emerging treatments (Review)

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**Abstract.** Due to its localisation, rapid onset, high relapse rate and resistance to most currently available treatment methods, glioblastoma multiforme (GBM) is considered to be the deadliest type of all gliomas. Although surgical resection, chemotherapy and radiotherapy are among the therapeutic strategies used for the treatment of GBM, the survival rates achieved are not satisfactory, and there is an urgent need for novel effective therapeutic options. In addition to single-target therapy, multi-target therapies are currently under development. Furthermore, drugs are being optimised to improve their ability to cross the blood-brain barrier. In the present review, the main strategies applied for GBM treatment in terms of the most recent therapeutic agents and approaches that are currently under pre-clinical and clinical testing were discussed. In addition, the most recently reported experimental data following the testing of novel therapies, including stem cell therapy, immunotherapy, gene therapy, genomic correction and precision medicine, were reviewed, and their advantages and drawbacks were also summarised.

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## 1. Introduction

Although various cancer therapies have achieved promising outcomes in clinical trials, resistance to treatment remains a major obstacle (1). By unravelling the underlying mechanism in this process and developing new drugs, it is hoped that superior therapeutic strategies that can improve clinical outcomes will emerge in the near future (2-5).

Brain malignancies are associated with high rates of morbidity and mortality, which require extensive resource allocation (6). Glioma is the most common type of cancer in the primary central nervous system (CNS), comprising >80% of all brain cancer cases, of which 46.3% are glioblastomas, according to the Central Brain Tumor Registry of the United States Statistical Report regarding primary brain and central nervous system tumours diagnosed in the United States between 2008 and 2012 (6). Low-grade gliomas include grade I tumours (such as pilocytic astrocytoma and diffuse glioma) and grade II tumours (such as astrocytoma and oligodendroglioma), whereas high-grade gliomas (HGGs) include grade III tumours (such as anaplastic astrocytomas and anaplastic oligodendrogliomas) and grade IV tumours (such as glioblastoma) (7). Glioblastoma or glioblastoma multiforme (GBM) is considered to be the deadliest and the most common primary malignancy of the CNS, comprising 16% of all primary brain tumours and up to 54% of all gliomas (6). Similar to other types of brain cancer, GBM requires sophisticated medical technology for accurate diagnosis and therapy (6). Despite the application of various multimodal therapy approaches

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*Abbreviations:* PDGFR, platelet-derived growth factor receptor; RTK, receptor tyrosine kinase; Rb1, retinoblastoma protein 1

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combining surgery, chemotherapy and radiotherapy, GBM is typically fatal within 3-4 months of its occurrence without treatment, with a life expectancy of ~14 months with surgery and adjuvant radiation therapy (6). Therefore, there is continuous demand for the development of novel strategies to improve the survival rate of patients with GBM.

GBM can be primary (*de novo*) or secondary, i.e., it can also develop from grade II astrocytoma or grade III anaplastic astrocytoma (8). In addition, by using datasets generated by The Cancer Genome Atlas, a previous study identified the following four subtypes of GBM: Pro-neural; neural; classical; and mesenchymal (8). Each subtype exhibits its own unique distinctive phenotypic and gene expression profiles (8). Inefficiency of drug delivery across the blood-brain barrier (BBB) and drug resistance are the main obstacles to GBM treatment (9). Despite the availability of various treatment strategies against GBM, including chemotherapy, radiotherapy and surgical resection, the median survival time remains unsatisfactory, at only 14.6 months (10).

GBM is a highly aggressive malignant brain tumour that mainly originates from glial cells, and its current treatment options include chemotherapy, radiotherapy and neurosurgery (10-12). Rapid recurrence after therapy significantly worsens the prognosis due to the invasive properties of glioma cells (13). Temozolomide (TMZ) is currently the main chemotherapeutic agent used for the treatment of GBM (14). However, long-term TMZ administration typically results in resistance, limiting its efficacy (15). The stem-like characteristics of GBM cells are primarily responsible for its resistance to chemotherapy, immunotherapy and radiation (16). Therefore, adjuvant therapy, such as chemotherapy or immunotherapy, may be applied to overcome drug resistance and to improve the survival rates of patients with GBM (17). In the era of precision medicine, a combination of molecular techniques can also be applied to target the unique tumour characteristics of individual patients (18-20). To improve the outcome, a comprehensive list of parameters must be understood, including the GBM microenvironment, the pharmacokinetic and pharmacodynamic profiles of the drug of interest, in addition to the delivery and safety profiles (21). Although some drugs (TMZ, lomustine, carmustine and bevacizumab) have obtained Food and Drug Administration (FDA) approval for GBM treatment, others are currently at different stages of clinical trial testing (22). In addition, despite extensive scientific research, a clear standard-of-care (SOC) guideline for recurrent GBM is lacking. Although drug resistance poses numerous challenges, there is hope that future treatments may prove more effective for patients with GBM.

Consistent with other types of cancer, the survival rate of GBM can be improved through early diagnosis (6); however, this is rare in this disease (6). Due to the presence of highly invasive cells, surgery cannot be applied in a large number of cases (23). In addition, due to the high degree of redundancy in the signalling pathways that are dysregulated in GBM (8), a high rate of efficacy cannot be achieved by only applying single-target therapy. Monotherapy is considered to be inadequate due to resistance triggered by the various compensatory feedback mechanisms and the low number of specific predictive biomarkers available (9). Therefore, designing a multi-targeted therapeutic approach for patients with GBM currently remains

a challenge. Furthermore, the targeted site [receptor tyrosine kinases (RTK), PI3K signalling, retinoblastoma protein (Rb) or p53 signalling pathways] requires a complex formulation to obtain the correct molecular structure and highly precise doses with the ability to readily cross the BBB (24).

The DNA alkylating chemotherapeutic agent TMZ is the first-line and SOC treatment method for GBM, both as monotherapy or in combination with radiotherapy (10). The currently used SOC guideline for GBM consists of maximal safe resection (the safest surgical approach, such as biopsy, different degrees of subtotal resection, or gross total resection), radiotherapy and chemotherapy using TMZ (10-12). After surgery, the treatment regimen consists of 6 weeks of radiotherapy in the surgical cavity with TMZ treatment, followed by adjuvant therapy, consisting of six cycles in total of TMZ treatment at a dose of 150-200 mg/m<sup>2</sup> for 5 days during each 28-day cycle (10). TMZ is an alkylating chemotherapeutic agent that can be orally administered and acts by causing DNA damage, which triggers a signalling cascade that leads to the apoptosis of the malignant cells (9). O<sup>6</sup>-methylguanine methyltransferase (MGMT), a DNA repair enzyme, is responsible for transferring the methyl group from a guanine to a cysteine residue, counteracting chemotherapy-induced DNA alkylation and, therefore, leading to cancer drug resistance (9). Furthermore, another FDA-approved treatment method for GBM is the implantation of biodegradable wafers made of polifeprosan 20 with carmustine into the resection cavity (11). These wafers are implants containing carmustine and a biodegradable copolymer (polifeprosan-a polyanhydride copolymer consisting of poly [bis (p-carboxyphenoxy)] propane and sebacic acid at a 20:80 molar ratio), to control the release rate of carmustine. These wafers are white to pale yellow, ~1.45-cm in diameter and with a thickness of 1-mm (25). Carmustine causes cross-links in DNA and RNA, inhibiting DNA synthesis, RNA production and translation. In addition, carmustine binds to glutathione reductase, leading to cell death. However, the risk of infection and impaired wound healing are complications that limit its use (11).

Recent studies have been focusing on developing novel biomaterials that can efficiently deliver TMZ to the active site. Implantable microspheres loaded with TMZ that can release this drug in a sustained manner have been previously studied (26). In addition, functionalised TMZ nanoparticles have been successfully developed, including liposomes functionalised with transferrin (27), chitosan nanoparticle functionalised with biotin (28), poly-lactide-co-glycolide nanoparticle functionalised with a monoclonal antibody against transferrin receptor (29), mesopore silica nanoparticles (30), gold-coated nanofibers (31) and magnetite nanoparticles (32). To improve the bioavailability of active drugs by preventing rapid degradation or drug resistance, other strategies have been used in addition to nanotechnology, such as synergistic substances or tumour-targeting peptides. TMZ may be either administered alone, or in combination with radiotherapy or with other active substances, including acridone derivatives (33), chlorotoxin (28), the bromodomain inhibitor JQ1 (27) or doxorubicin (18), with the aim of improving the treatment outcomes.

When tumour-bearing mice were treated with transferrin nanoparticles loaded with TMZ and the bromodomain

inhibitor JQ1, the DNA damage and cell apoptosis increased, which was associated with an increase in survival compared with equivalent free drug treatment (27).

A synergistic approach has been studied using acridone derivatives combined with TMZ. This combination was found to exert major tumour cytotoxic effects, effectively suppressing malignant cell proliferation in both sensitive and resistant tumour cell subpopulations (33).

TMZ bound to nanoparticles exhibits higher stability at physiological pH, with a half-life 7-fold longer compared with free TMZ. TMZ nanoparticles with chlorotoxin (NP-TMZ-CTX) was able to target GBM cells and achieved 2-6-fold higher uptake and 50-90% reduction of IC<sub>50</sub> at 72 h post-treatment as compared with non-targeted NP-TMZ. NP-TMZ-CTX has shown great promise in its ability to deliver a high therapeutic dose of TMZ to GBM cells, and may serve as a template for targeted delivery of other therapeutics (28).

Molecular targeting is another therapeutic strategy used for GBM, with different approaches, namely to target tubulin and EGFR to modify DNA function or to deactivate NF- $\kappa$ B (34,35). The majority of the targeted molecular therapies against GBM were developed to specifically inhibit tumour angiogenesis (36-38). A large number of drugs proposed for GBM therapy are anti-angiogenic agents that function by inhibiting VEGFR or platelet-derived growth factor (PDGF) receptor (PDGFR) function (36). A previous meta-analysis, which included 11 studies and 3,743 participants, did not reveal an improvement in the overall survival (OS) of patients with HGG who received anti-angiogenic therapy (36). However, an improvement in progression-free survival (PFS) was observed (36). Although the pathophysiological mechanism underlying GBM involves a large number of signalling pathways, the PI3K/AKT/mTOR pathway has been attracting particular attention, since it can regulate protein synthesis, cell proliferation, migration, angiogenesis and apoptosis (39). Previous studies have demonstrated that a dual targeting PI3K/mTOR signalling pathway or a combined therapy with PDGFR and VEGFR inhibitors were more efficient in inducing GBM cell death compared with single treatment (40,41).

Immunotherapy is also becoming an important field of research in GBM. Peptide vaccines, such as Rindopepimut and SurVaxM, in addition to autologous vaccines, including Gliovac and Prophage, are potential candidates for immunotherapy against GBM (42,43). In addition, oncolytic viral therapy (VT) using the parvovirus ParvOryx is an emerging strategy used for the treatment of GBM (44). Data from a previous study showed that the effects of VT were comparable to those of dendritic cell (DC)-based vaccines, with similar outcomes in comparison with standard therapy, including previous GBM single resection, subsequent radiotherapy and first-line therapy with concomitant TMZ/bevacizumab and irinotecan (44). DC-based vaccination is an immunotherapy approach used to boost the potential of the cancer patient's own immune system. Autologous DCs are obtained from monocytes in peripheral blood mononuclear cells, and then loaded with stem-like cell-associated antigens. Generally, 3-5 weeks are required for vaccine production. However, to induce a lasting and potent immune response, several conditions are required, such as the minimum number of injected DCs, a specific vaccination

schedule and route of administration, details which have not yet been clearly outlined. To assess the clinical impact of immunotherapy compared with that following standard HGG treatment (surgical resection with adjuvant hyperfractionated radiotherapy and concomitant TMZ), a comprehensive meta-analysis of previous clinical trials that applied DC therapy and VT was conducted by measuring OS and PFS as the outcome parameters (1). It was found that the OS was greatly improved by DC therapy in patients with both new and recurrent HGG (1). However, VT did not confer statistically significant improvements in either OS or PFS in patients with newly diagnosed HGG. Due to the insufficient number of studies, a meta-analysis on PFS for patients with recurrent HGG who received DC vaccination and a meta-analysis of OS and PFS for patients with recurrent HGG who received VT could not be carried out (1,45).

GBM stem cells (GSCs) represent another cell type in GBM that appear to be important for tumour growth, since they have been reported to be implicated in drug resistance and recurrence after therapy (46). This suggests that GSCs may be suitable targets for the development of novel therapeutic strategies (47).

In terms of drug delivery, different types of biomaterials, including nanoshells, liposomes and nanoparticles, have been developed over the past decade (3,4). The ultimate aims were to accurately deliver antitumour agents to their respective targets and to improve bioavailability whilst minimising side effects (3,4).

It has been widely reported that genetic and epigenetic alterations are major causes of carcinogenesis (8,48). These alterations tend to overlap with the signalling pathways involved in controlling cell proliferation, cell division, apoptosis and cell motility (8). However, they may also cause alterations in other signalling networks that can indirectly promote cancer progression, including inflammation, modulation of the tumour microenvironment and angiogenesis (8). To date, three core signalling pathways have been frequently associated with GBM pathogenesis (32,49,50): The p53, Rb and RTK/Ras/PI3K pathways (Fig. 1). However, other signalling pathways and effectors may be involved in this type of malignant disease and their discovery would uncover the possibility of novel therapies in the future. The currently available therapy options for GBM (51-64) are summarised in Table I.

In the present review, the main strategies applied for GBM treatment are presented by discussing the most recent pharmacological and other medical approaches that are currently under pre-clinical and clinical examination, followed by outlining the future perspectives for optimising GBM management.

## 2. Pharmaceutical products tested in clinical trials

Although GBM is characterised by heterogeneity, analysis of genetic aberrations has identified the following three commonly dysregulated signalling pathways: Activation of the RTK/Ras/PI3K pathway; inhibition of p53; and Rb signalling (48). These are the focus of current research efforts in the search for molecular targets of GBM. Of the numerous strategies employed for GBM treatment, only a small number have successfully reached pharmaceutical marketing. Among

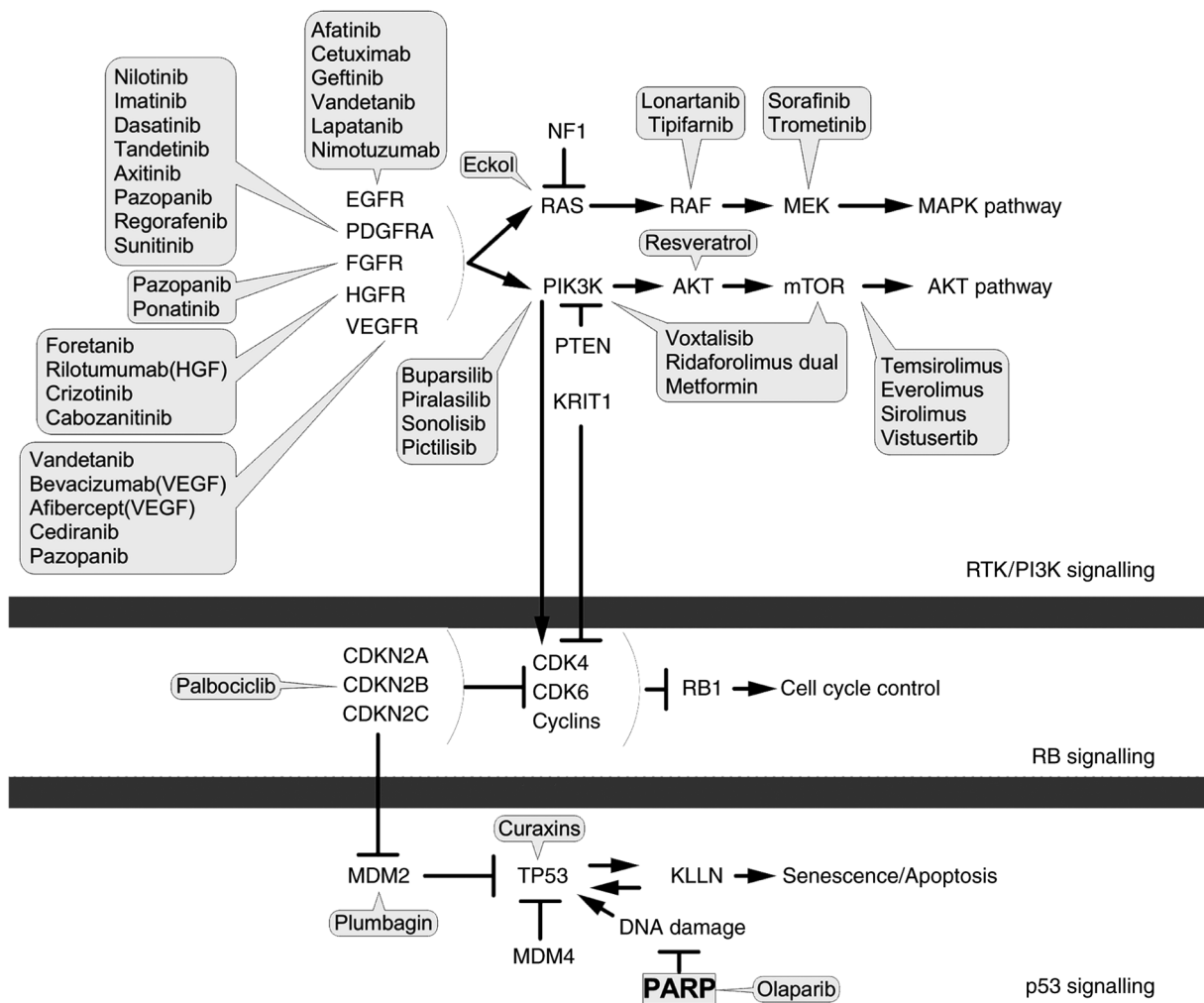


Figure 1. Glioblastoma multiforme signalling pathways. PDGFRA, platelet-derived growth factor receptor A; FGFR, fibroblast growth factor receptor; INSR, insulin receptor; NF1, neurofibromin 1; MAPK, mitogen-activated protein kinase; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; KRIT1, Krev interaction trapped; RTK, receptor tyrosin kinase; CDKN2A/B/C, cyclin-dependent kinase inhibitor 2A/B/C; CDK4/6, cyclin-dependent kinase 4/6; Rb1, retinoblastoma protein 1; MDM2/4, mouse double minute 2/4 homolog; KLLN, killin. Block arrow, inhibition; point arrow, pathway flow.

these, the following are of interest: i) Molecularly targeted therapy; ii) anti-angiogenic drugs; iii) GSC targeting; iv) microRNAs; v) immunotherapy; vi) nanotherapy; vii) gene therapy; and viii) oncolytic viruses (59). As of 2021, a number of compounds have successfully gone through all phases of drug development and have been approved for clinical testing on human subjects (21,37,38,42,44,54,60,61,65-82). They are summarized in Table II.

At present, drugs targeting the RTK/Ras/PI3K, p53, Rb and TGF- $\beta$  signalling pathways are undergoing clinical trial testing, whilst those that target the Wnt (83) and unfolded protein response (84) signalling pathways remain under experimental investigation.

**Chemotherapeutics.** TMZ (trade names, Temodar or Temodal; Schering-Plough) (85) functions via a mechanism that is not fully understood. TMZ is an imidazotetrazine pro-drug of dacarbazine, which acts as an alkylating agent by causing double-stranded DNA breaks, in addition to inhibiting the activity of DNA repair enzymes (9). TMZ primarily exerts cytostatic and pro-senescence effects by preventing cancer cell cycle progression from the G<sub>2</sub> to M phase, but it is

generally not cytotoxic or apoptosis-inducing (86). Previous studies found that, compared with other alkylating agents, prolonged TMZ treatment after radiotherapy appears to be well-tolerated among patients with GBM (10,66,87). However, its therapeutic efficacy is limited. Currently available clinical data suggest that significant benefits were only observed in a small percentage of patients, with a median survival extension of only 2.5 months (10). Therefore, TMZ is typically applied as a part of a complex regimen of combinatorial therapy, such as RIST (rapamycin, irinotecan, sunitinib and TMZ) (88) or CUSP9 (a therapeutic protocol that includes nine repurposed active substances: Aprepitant, minocycline, disulfiram, celecoxib, sertraline, captopril, itraconazole, ritonavir and auranofin, along with low-dose TMZ) (89).

**Carmustine.** Carmustine [or 1,3-bis(2-chloroethyl)-1-nitrosourea] is another DNA and RNA alkylating agent that has been used for GBM therapy (11,67). Gliadel<sup>®</sup> (Guilford Pharmaceuticals, Inc.) is the commercial name of biodegradable polymer wafers containing 3.85% carmustine in a polifeprosan 20 implant (11). It is designed to be inserted into the resection cavity, which then releases the active agent

Table I. Chemical and immunological therapy options in glioblastoma multiforme.

Class	Active ingredient	Mechanism	(Refs.)
Anti-angiogenics	Bevacizumab, panobinostat, altiratinib	Target VEGF	(19,51,52)
	Trebananib	Targets tumour-associated endothelial cells	(53)
	Crenolanib, tandutinib	Inhibit PDGFR	(22)
	Enzastaurin	Inhibits protein kinases	(54)
Kinase inhibitors	Paxalisib	Inhibits PI3K and mTOR	(55)
Molecularly targeted agents	Temozolomide, mibefradil, carmustine	Alter DNA functions	(56)
	Afatinib	Inhibits EGFR	(57)
	Curaxins	p53 activator and NF- $\kappa$ B deactivator	(58)
Vaccine-based immunotherapy	Rindopepimut, survivin	Peptide vaccines	(59)
	Sitoiganap, Prophage	Autologous cancer vaccines	(60)
Antibody-based immunotherapy	Asunercept, depatuxizumab	EGFR-targeting antibodies	(61,62)
Checkpoint inhibitors	Indoximod	Inhibits indoleamine 2,3-dioxygenase pathway	(63)
Oncolytic viruses	ParvOryx	Autonomous protoparvovirus H-1	(64)

directly into the target site (11). The survival benefit of carmustine has demonstrated an increased median survival time of 89.5 weeks in patients with GBM treated with carmustine wafers in combination with radiotherapy compared with radiotherapy alone (90).

Among the 51 clinical studies that tested carmustine for GBM treatment, only one is ongoing (participants are being examined or are receiving an intervention), but not recruiting (potential participants are not currently being enrolled or recruited), and is currently at phase I/II. That study was undertaken to investigate the possible dosage and side effects of TMZ co-administration with carmustine, O<sup>6</sup>-benzylguanine (O<sup>6</sup>BG), radiotherapy and autologous stem cell transplantation in patients with newly diagnosed GBM (91). The results indicate that the blood and bone marrow can be chemoprotected from combined O<sup>6</sup>BG and TMZ chemotherapy-associated myelosuppression using gene modification with the O<sup>6</sup>BG-resistant P140K mutant MGMT. Compared with TMZ alone, the addition of O<sup>6</sup>BG to TMZ treatment appears to provide significant treatment gains for mg of TMZ dose (91).

**Lomustine.** Lomustine, marketed as Gleostine (Next Source Technology, LLC), is a type of nitrosourea that is orally administered during chemotherapy (92). It also functions through an alkylating mechanism (92). Despite modest PFS prolongation, the combination of lomustine and bevacizumab (a monoclonal antibody developed against VEGF) was not found to confer survival advantages compared with lomustine alone in patients with progressive GBM (68). However, recent results indicate that the combination of lomustine and TMZ may improve the survival rate compared with standard TMZ therapy in patients with newly diagnosed GBM positive for MGMT promoter methylation (20).

**Targeted therapy.** In GBM, several pathways have been frequently found to be altered, including the PI3K/AKT/mTOR,

p53 and Rb pathways (39,49), making them potential targets for GBM treatment. Although there is increasing interest in targeting the tumour microenvironment, such as blood vessels, the monocyte-macrophage-microglia compartment and T cells (93), improved clinical trial design by including pharmacodynamic endpoints in patient populations is required to optimise this type of treatment.

Afatinib is an orally administered, third-generation EGFR inhibitor that has been extensively studied for its potential application in GBM therapy (57,70,94,95). Gefitinib, erlotinib, dacomitinib and osimertinib are also examples of EGFR inhibitors (49,96). Although different strategies are used for targeting EGFR in recurrent GBM, it appears that, although the efficacy of afatinib is limited, its safety profile is manageable (94). A recent study demonstrated that afatinib in combination with TMZ inhibited the tumourigenesis of GBM cells by targeting the EGFRvIII/cMet signalling pathway (95). In addition, compared with afatinib monotherapy, its combination with TMZ was revealed to synergistically inhibit GBM cell motility, invasion, proliferation and clonogenic survival whilst inducing senescence (97).

Dasatinib is an orally administered, synthetic small-molecule inhibitor of the Src family of tyrosine kinases (98). A previous clinical trial investigating dasatinib treatment in patients with recurrent GBM found that it was not effective (98).

Regorafenib is an orally administered inhibitor of stromal, oncogenic and angiogenic RTKs (99). REGOMA is a multi-centre, open-label, randomised phase II clinical trial that was performed in Italy, which found that regorafenib treatment conferred a surprisingly good OS rate in patients with recurrent GBM, suggesting that it should be investigated in a subsequent phase III study (99).

Dianhydrogalactitol (Val-083) is a bifunctional hexitol derivative that was first introduced in 1979 for the treatment of gynaecological malignancies (100). However, it was recently

Table II. Clinically tested drugs used in GBM treatment.

Drug (trade name)-manufacturer	Mechanism	Side effects	(Refs.)
<b>Chemotherapeutic drugs</b>			
Temozolomide (Temodar)-Merck KGaA	Alkylating agent	Nausea, vomiting, mouth sores, taste changes, constipation, coughing, headache and tiredness	(66)
Carmustine (BiCNU, Gliadel waffer)-Bristol Mayers Squibb, Arbor Pharmaceuticals	Alkylating agent as injectable solution (BiCNU) or biodegradable polymer implant (Gliadel)	Cerebral oedema, intracranial hypertension and infections, seizures, thromboembolic events	(67)
Lomustine (CeeNU, Gleostine)-Bristol-Myers-Squibb NextSource Biotechnology	Alkylating agent	Hematological toxic events	(68)
<b>Targeted drugs</b>			
Mibefradil-Cavion/Jazz Pharmaceuticals	Selective blocker of T-type channels	Decreased platelet count, increased aspartate and alanine aminotransferase	(69)
Afatinib (Giotrif)-Boehringer Ingelheim	Irreversible inhibitor of EGFR, tyrosine kinase activity and tumour cell proliferation	Grade I adverse events: Nausea, vomiting, weight loss, and fatigue. Grade II adverse events: Rash. Grade III adverse events: Maculopapular rash and paronychia	(70)
Dianhydrogalactitol (Val-083)-DelMar Pharmaceuticals	Binds to GBM cell DNA, leading to cell death	No results reported	(71)
Bevacizumab (BV, Avastin, Mvasi)-Genentech, Amgen	Human monoclonal antibody that inhibits VEGF	Fatigue, headache, hypertension and thromboembolism	(72)
Tandutinib (MLN518)-Millennium Pharmaceuticals	Inhibitor of type III receptor tyrosine kinase (PDGF receptor-b, c-Kit, Fms-like tyrosine kinase 3)	Common grade 3 adverse events include hypertension, muscle weakness, lymphopenia and hypophosphatemia	(73)
Enzastaurin-Eli Lilly	Specifically targets and inhibits protein kinase C	Aortic thrombosis, erysipelas, cerebral hemorrhage and seizures	(54)
Cediranib (AZD2171)-AstraZeneca	Inhibitor of tyrosine kinase with activity on PDGF receptors and c-Kit	Nausea, fatigue, diarrhea, headache, hoarseness and hypertension	(37)
<b>Immunotherapy drugs</b>			
<b>Active immunotherapy</b>			
Rindopepimut (Rintega, CDX-110)-Celldex	Peptide vaccine	All rindopepimut vaccines administered in preclinical and clinical studies were generally well accepted and toxicity never exceeded grade 2	(74)
SurVaxM-MimiVax	Peptide vaccine that targets survivin	Well-tolerated, mostly with grade 1 adverse events and no serious adverse events	(75)
Prophage (G-100, G-200, Vitespen)-Agenus	Heat shock protein peptide complex-96	Adverse events (fatigue, flu-like illness, erythema, diarrhea) in 74% of the patients, with no grade 3 or 4 events related to vaccination	(60)
Gliovac (ERC 1671)-Epitopoietic Research Corporation	Autologous antigens	Mild and transient toxicity: Grade 2 headaches and local erythema at injection site	(42)
IMA950-Immatics Biotechnologies	Immunotherapeutic multiple-peptide vaccine	Minor reactions at injection site, pruritus, rash, fatigue, allergic reactions, neutropenia, anaemia and anaphylaxis	(76)
ICT-107-ImmunoCellular Therapeutics	Autologous dendritic cell vaccine pulsed with class I peptide from tumour-associated antigens	Well-tolerated, with no difference between the treatment and control groups regarding adverse events	(77)
DCVax-L-Northwest Biotherapeutics	Dendritic cell vaccine	Mild side effects: Skin reactions, redness, pain and swelling at the site injection	(78)

Table II. Continued.

Drug (trade name)-manufacturer	Mechanism	Side effects	(Refs.)
<b>Passive immunotherapy</b>			
Depatux-M (ABT-414)-AbbVie	Antibody-drug conjugate that binds to EGFR	Blurred vision, photophobia and eye pain were reported by all patients recruited	(21)
Asunercept (APG101, CAN-008)-Apogenix	Blocker of CD95 pathway	No serious adverse events	(61)
MEDI-3617, MEDI-575-MedImmune	Novel anti-PDGFR antibodies	Prolonged grade 3 edema	(38)
Sirolimus (Rapamune)-PF PRISM CV	mTOR inhibitor	No severe toxicity in any of the patients	(79)
<b>Nanotherapy-based drugs</b>			
Nanocell Doxorubicin-EnGeneIC	Minicell with doxorubicin conjugated against EGFR	Well-tolerated, no dose limiting toxicity	(80)
<b>Viruses</b>			
ParvOryx (H-1PV)-Oryx GmbH	Oncolytic virus	No dose-dependent side effects and no dose-limiting toxicity	(44)
<b>Gene therapy</b>			
TOCA511 combined with TOCAFC-Tocagen	Retroviral replicating vector, permanent integration into the cancer cell genome	Bone marrow depression	(81)
Veledimex-Ziopharm Oncology	Control system that can switch on human IL-12 gene therapy on demand	Well-tolerated, decreased peripheral lymphocytes and platelets, elevated liver transaminases	(82)

GBM, glioblastoma multiforme; PDGFR, platelet-derived growth factor receptor.

rediscovered as a potential treatment option for TMZ-resistant GBM (101). It is currently undergoing phase II examination in patients with bevacizumab-naïve, MGMT-unmethylated recurrent GBM (101).

Mibefradil selectively blocks T-type calcium channels and slows cancer cell proliferation, but does not induce apoptosis (102). Therefore, this drug is typically used in combination with other chemotherapeutic agents, such as TMZ, or with other therapeutic methods, such as radiotherapy (102). A previous phase I study that examined the use of mibefradil dihydrochloride, which was administered together with hypo-fractionated radiation, for patients with recurrent GBM, suggested that it could be safely co-administered, with pharmacologically effective concentrations detected in the resected area of the brain (17 days/200 mg/day mibefradil) (103). In 2017, mibefradil completed phase I clinical trial testing for GBM treatment in combination with TMZ or radiotherapy (104) and is ready to enter phase II trials.

**Anti-angiogenic agents.** GBM is characterised by extensive microvascular proliferation, the presence of pro-angiogenic factors and high levels of VEGF activation (8). Therefore, a number of different agents have been investigated to exploit these characteristics and to expand upon the currently limited treatment options. As such, numerous inhibitors of VEGF and pro-angiogenic signalling have been tested in previous clinical trials. However, with the exception of enzastaurin and cediranib (105), none were able to progress further than phase II

of clinical testing. Due to the development of drug resistance, the responses to anti-angiogenic agents are rarely durable (36). Alternative angiogenic mechanisms are frequently activated to trigger the generation of new blood vessels (36). In addition, local hypoxia has been reported to activate alternative pro-angiogenic pathways involving angiopoietin-1, EGF, fibroblast growth factor, granulocyte colony-stimulating factor, insulin-like growth factor, PDGF, stromal cell-derived factor-1 and TGF (106). Therefore, targeting multiple signalling pathways or concomitant anti-angiogenic agent administration may prevent the development of resistance and prolong patient survival (36).

Bevacizumab (Avastin) is a human monoclonal antibody that functions as an inhibitor of VEGF (38). It was approved by the FDA as a single agent for the second-line treatment of advanced GBM (107). However, clinical trial data showed that the survival rate following bevacizumab treatment was not prolonged for >14.5 months (51). Different combinations of bevacizumab and other drugs are currently under investigation to improve therapeutic efficacy. A study performed in 2019 revealed that patients with recurrent GBM may benefit from a triple-drug therapy regimen consisting of bevacizumab, irinotecan and TMZ plus tumour-treating fields (TTFields; cancer treatment strategy that uses alternating electric fields of intermediate frequency and low intensity to disrupt cell division) (108). By contrast, recent results from another randomised phase I trial and placebo-controlled phase II trial, which investigated the administration of bevacizumab and

dasatinib to patients with recurrent GBM, demonstrated that, despite Src signalling up-regulation, bevacizumab in combination with dasatinib did not significantly improve patient outcomes compared with bevacizumab alone (109).

Ranibizumab, marketed as Lucentis, is the Fab fragment derived from the parent molecule of bevacizumab (110). It was approved by FDA for the treatment of age-related macular degeneration (110) and may represent a viable option for future GBM trials.

Tandutinib (MLN518) is piperazinyl quinazoline that was designed to inhibit type III RTKs (111). Data from a previous phase II study revealed that, after oral administration, tandutinib was distributed to the brain, where its concentrations were higher compared with those in the plasma (111). Unfortunately, this particular study was closed due to lack of efficacy (111).

Cediranib (AZD2171) is a tyrosine kinase inhibitor that mainly targets c-Kit and PDGFR (37). The results of recent clinical trials showed no improvements in the survival rate when cediranib was added to the lomustine alone treatment plan, compared with that in the group treated with lomustine alone (37,112). By contrast, data from another study showed that combined treatment with cediranib and radiotherapy improved the PFS and OS by improving tumour perfusion, compared with chemotherapy alone (113).

Enzastaurin specifically targets and inhibits protein kinase C, which in turn suppresses cell proliferation and tumour growth (114). A clinical trial commenced in 2019 to examine the effects of the combination of enzastaurin and TMZ during and following radiotherapy on patients with newly diagnosed GBM, with or without the novel genomic biomarker *de novo* genomic marker 1, but no results have yet been reported (115). The results from recent clinical studies using enzastaurin at different combinations did not appear to be promising (114,115). Compared with other therapeutic agents, including bevacizumab, TMZ and lomustine, the survival rate did not improve following enzastaurin therapy (115).

*Immunotherapy.* Although immunotherapy achieved some notable successes in the treatment of other types of cancer such as skin, lung or breast cancer (116-118), it remains an emerging area of research in the GBM field. Immunotherapy in GBM includes antibody-mediated treatment, immune stimulation, vaccines and adoptive cell therapy (59). To date, none of the phase III immunotherapy-based clinical trials (119,120) have demonstrated efficacy and, therefore, no FDA-approved immunotherapy against GBM currently exists. Given the challenges faced, a combinatorial approach is required. In addition, due to the high risk of recurrence, biomarker identification for patient selection and disease monitoring is essential (59). A search for 'glioblastoma' and 'immunotherapy' in the ClinicalTrials.gov database (April 2021) (120) yielded a list of 123 clinical trials that involved the following: DC vaccines; synthetic peptide vaccines; autologous T-cell transfer; gene therapy; T-cell transfer combined with tumour cell lysate vaccine; autologous natural killer (NK) or NKT cell transfer; allogeneic T-cell transfer; and immunosuppressive checkpoints (115). Furthermore, there are currently 20 ongoing (not recruiting) clinical trials worldwide that are examining the potential application of immunotherapeutic approaches for GBM treatment (115). Despite the limitations of this search,

the relatively high number reflects the extensive interest in anti-GBM immunotherapy.

*Active immunotherapy.* Extensive research into the mechanism underlying tumour-induced immune suppression led to the discovery of the benefits of targeting immunosuppressive checkpoints, some of which are currently being tested in clinical trials on GBM (64,75). Results from pre-clinical studies demonstrated that several immunotherapeutic strategies can be effectively applied to animal models of GBM, including gene therapy, passive immunotherapy, adoptive T-cell transfer targeting tumour antigens or modified to express chimeric antigen receptors, inhibition of immune checkpoints, active immunotherapy, and using peptide or DC vaccines (74,121).

Rindopepimut (CDX-110) is a peptide vaccine that was designed for patients expressing EGFRvIII, a mutant EGFR that is absent in healthy cells (122) and is present in 20-30% of patients with GBM (123). Findings from previous phase I and II clinical trials demonstrated longer PFS and OS among patients who were EGFRvIII-positive compared with those in patients who were negative for this EGFR mutant (123). However, these results were not consistent with those found in the subsequent phase III clinical trial, where the OS rate was similar between the Rindopepimut + TMZ and the TMZ alone groups (122).

Prophage (G-100, G-200 and Vitespen) contains a heat shock protein peptide complex-96 (43). This is a vaccine that was created using tumour tissue collected from the patient, making it a personalised, patient-specific vaccine (43). In a previous phase II clinical trial, patients with GBM who underwent G-100 treatment and standard therapy (TMZ) exhibited increased PFS and OS, suggesting clinical efficacy (124). However, a larger cohort is required to verify this potential efficacy (43).

SurVaxM is another peptide vaccine, which targets survivin, a cell-survival protein present in 95% of GBMs (75). In 2016, a clinical study on patients with recurrent GBM demonstrated its safety and efficacy, which was shown by the increased PFS and OS observed compared with those in patients treated with chemotherapy alone (TMZ) (75). In March 2020, a phase II study was initiated, which tested the effects of SurVaxM and pembrolizumab on patients with GBM at the first recurrence (125). The aim was to assess its clinical activity, safety profile and tolerability (125). No results have yet been reported for this clinical trial.

Gliovac (ERC-1671) contains autologous antigens obtained from surgically removed tumour tissues from the patient and is administered in combination with allogeneic antigens obtained from other patients with GBM (43). In a previous phase I study, all patients treated with Gliovac survived for 6 months after treatment initiation compared with the control group, in which only 33% of the patients remained alive at 6 months (42). As of March 2021, Gliovac is being investigated in a phase II clinical trial.

IMA950 is a multi-peptide vaccine that has been specifically developed for GBM immunotherapy (76). It consists of tumour-associated peptides from human leukocyte antigen (HLA) surface receptors obtained from the primary human GBM tissue (126). A phase I clinical trial involving patients



positive for HLA-A\*02 reported an antitumour immune response, but no effects on the survival rate were observed (76).

DC immunotherapy is an emerging strategy for the treatment of GBM (77). Recently, phase I and II clinical trials testing DC immunotherapy were conducted on patients with newly diagnosed and recurrent GBM, prior to observing its prognostic effects. The results demonstrated that DC immunotherapy caused tumour shrinkage and elevated numbers of tumour-infiltrating CD8<sup>+</sup> lymphocytes. The study concluded that patients with malignant glioma may benefit from DC-tumour immunotherapy, but this strategy may cause transient, albeit reversible, high levels of serum aspartate aminotransferase and alanine aminotransferase (127).

ICT-107 is an autologous DC vaccine pulsed with class I peptides from tumour-associated antigens (TAAs) designed to target six different TAAs (77). A clinical trial involving ICT-107 administration in patients with GBM revealed promising results regarding safety, reporting low-grade (grade 1) adverse events such as fatigue, flushing, pruritus, skin rash and erythema (128).

Northwest Biotherapeutics recently developed the DCVax-L vaccine, which is currently undergoing phase III trial testing in patients with newly diagnosed GBM (129). This vaccine contains a combination of autologous tumour antigens and antigens from the patient (130). Longer OS was found according to data from a previous phase I/II trial, with mild vaccination-related side effects also observed (130).

*Passive immunotherapy.* Adoptive immunotherapy and serotherapy are examples of passive immunotherapy strategies (130,131). Since VEGF and EGF are highly expressed in GBM (35), recombinant humanised monoclonal VEGFR antibodies and anti-EGFRvIII antibodies represent an area of investigation in this field.

Asunercept (APG101 and CAN-008) inhibits the Fas (CD95) pathway (61). A previous phase II clinical trial revealed that asunercept combined with radiotherapy increased the PFS and the PFS at 6 months compared with those in the group treated with radiotherapy alone (132). Furthermore, the combination of asunercept and radiation also significantly prolonged the time to deterioration and maintained a higher quality of life compared with the radiotherapy alone group (133). However, these findings require further verification in a subsequent phase III clinical trial including a larger number of patients.

Depatux-M (depatuxizumab mafodotin, ABT-414) is an EGFR antibody-drug conjugate (62). Once internalised, it releases the anti-microtubule agent monomethyl-auristatin F, which triggers tumour cell death (62). In a phase I clinical trial, the adverse effects of Depatux-M and TMZ combined treatment were similar to those following TMZ treatment alone (134). In March 2021, a recent phase III clinical trial undertaken to evaluate Depatux-M for the treatment of newly diagnosed GBM was discontinued due to futility, having failed to demonstrate a survival benefit. Of the patients included in that study, 85% had discontinued Depatux-M, mainly due to disease progression (135).

MEDI-3617 and MEDI-575 are two anti-platelet-derived growth factor receptor  $\alpha$  (PDGFR $\alpha$ ) antibodies that were developed for GBM therapy (38,136). However, a phase II clinical

study showed that although MEDI-575 was well-tolerated, it did not exhibit any significant clinical activity (136).

*Nanocarriers.* Nanomedicine is a rapidly evolving field, which entails the combination of nanotechnology, biomedicine and pharmacology (3,4). Several attempts have been made to apply nanomedicine to the treatment of GBM. In 2012, Opaxio, which is paclitaxel conjugated with poliglumex, an innovative macromolecular taxane created to increase the therapeutic index of paclitaxel, received approval and orphan drug status from the FDA for the treatment of GBM (137). SGT-53 (developed by SynerGene Therapeutics, Inc.) is an anti-transferrin antibody fragment and an example of a targeted nano-drug, which is currently undergoing phase I and II trial testing for GBM treatment (115). Nanotherm uses aminosilane-coated superparamagnetic iron oxide nanoparticles for the induction of local hyperthermia for the treatment of GBM (137). Nanocell is a minicell conjugated with doxorubicin (80). However, its toxicity profile has not been reported and its efficacy has yet to be assessed (80).

*Gene therapy.* GBM occurs due to the sequential accumulation of genetic alterations (8), making gene therapy a promising alternative to address the limitations of conventional therapy. Therefore, gene or nucleic acid carriers that are able to successfully penetrate into the tumour tissue have been extensively studied. Although several clinical trials have evaluated the use of viral vectors for GBM gene therapy, none achieved FDA approval, mainly due to inefficient viral delivery, lack of tumour penetration and insufficient efficacy (138,139). However, a number of non-viral vectors have shown promising results in pre-clinical trials and have entered clinical trial testing (140). Several gene therapy agents carried by different vectors have been evaluated in clinical trials for GBM treatment.

Herpes simplex virus-thymidine kinase (tk) converts the antiviral drug ganciclovir into ganciclovir triphosphate (141). Its combination with vectors such as retro- or adenoviruses has been investigated in clinical studies, but the results were not promising, due to tumour progression leading to low OS within 10 months of treatment (77%) (142).

Adenovirus-tk is an adenoviral vector that contains the herpes simplex virus tk gene (143). Its combination with valacyclovir was previously evaluated in a phase I clinical study (12). Although not particularly potent in terms of efficacy, the survival rate was encouraging, prompting the commencement of phase II trial testing, which is currently ongoing (12,144).

Toca 511, a retrovirus vector, is under phase II/III clinical trial examination, and the results were found to be favourable regarding the safety profile and OS of Toca 511 compared with lomustine alone treatment (145). The retroviral replicating vector can permanently integrate the yeast cytosine deaminase gene into the tumour cell genome, thereby converting 5-fluorocytosine into the toxic 5-fluorouracil (146).

Veledimex is an oral activator of human IL-12 that has been shown to increase the survival rate of patients with recurrent GBM in a previous phase I clinical trial (147). A phase I clinical study of Ad-RTS-hIL-12 + Veledimex combined with cemiplimab in patients with recurrent/progressive GBM is currently ongoing (148).

SGT-53 is a p53 gene transfectant that is enclosed in liposomes (149). This active nanoparticle recently underwent phase II clinical evaluation in combination with TMZ for the treatment of recurrent GBM, but the results remain unavailable at present (150).

NU-0129 can transfect small interfering RNAs targeting the *Bcl2l12* oncogene enclosed in spherical nucleic acid gold nanoparticles (151). It recently completed its first human phase 0 clinical trial for GBM treatment (151). In addition to the evidence of crossing the BBB, macrodosing was well-tolerated with no unexpected side effects observed (151).

*Viruses.* ParvOryx (H-1PV) is an oncolytic virus that specifically targets and destroys tumour cells (152). A previous phase I/II clinical trial revealed that H-1PV is well-tolerated, and can cross the BBB and enter the tumour to trigger an immune response (44).

*Old but new.* Currently commercialised for other diseases, several active agents, such as chlorpromazine, chloroquine, metformin or disulfiram, are being investigated for their effects on GBM physiology. Novel chemotherapeutic approaches to GBM treatment have been proposed. However, these processes require additional financial resources and time before marketing (drug development and registration). In this respect, drug re-purposing represents a new option for the pharmaceutical industry. Research efforts to find new uses for drugs originally developed for treating other diseases have been intensified, which is summarised in a number of recent reviews (24,153-157).

Among the most studied repurposing candidates, the following may be mentioned: CNS drugs (chlorpromazine, valproate, fananserin, pimozide, trifluoperazine, imipramine, thioridazine and propentofylline); antimalarial drugs (chloroquine and mefloquine); antidiabetics (biguanides-metformin), lonidamine, disulfiram, rapamycin, everolimus, temsirolimus and ridaforolimus (158-160). Due to the heterogeneity that exists within this tumour, this re-purposing strategy may hold great promise for the treatment of GBM.

Instead of monotherapy alone, superior outcomes may also be achieved by combining different types of therapies. Various combinations of therapeutic agents have been studied in previous clinical trials for GBM treatment (22,161-165) and they are summarized in Table III.

### 3. Drug therapies under development

As conventional treatment methods generally cannot prevent recurrence (2), research focus has been shifted onto developing novel strategies to target residual tumour cells, which is essential in GBM therapy (59). Although treatment methods have improved over the last 20 years (24,96,153-157), the OS of patients with GBM has not reached the levels achieved in other types of solid tumours (6). Novel therapy designs are currently developed for clinical trial testing, which are designed to be applied in combination with the current SOC to improve treatment outcomes (59). The novel therapeutic strategies currently under investigation include the following: Molecularly targeted therapies (including the inhibition of growth factor and angiogenic signalling pathways, multi-kinase inhibitors

and combinatorial therapy); passive/active immunotherapy (including monoclonal antibodies, cytokine-mediated therapies, adoptive cell transfer, and peptide- or cell-based approaches); stem cell therapy; gene therapy; and precision therapy (Fig. 2). Targeted therapies typically use drugs that inhibit specific signalling pathways (22). A wide variety of drugs that function in this manner are currently under investigation. However, the results have not been satisfactory. In one previous clinical study, the first-generation EGFR inhibitor erlotinib did not confer benefits to GBM treatment due to its unsatisfactory effects, with tumour progression and a median overall survival of 5.7 months (49,166). In addition, negative data were reported by clinical studies that tested rapamycin, an mTOR inhibitor, at different phases of GBM treatment (79). A possible reason for this was that, although the concentrations of rapamycin were sufficient for the *in vitro* inhibition of mTOR in the tumour, the magnitude of this inhibition inside the tumour cells varied substantially (79). Another difficulty encountered during GBM treatment is the common occurrence of side effects, such as vomiting and nausea, hair loss, headache, fever and weakness (59).

*Monoclonal antibodies.* One of the leading classes of therapeutics is monoclonal antibodies designed to recognise receptors and ligands expressed on the cell surface (167). They mainly prevent downstream receptor signalling by disrupting receptor-ligand interactions (167).

Bevacizumab is an antibody that targets VEGF and has been approved by the FDA (72). A previous study reported that bevacizumab combined with SOC did not increase the OS compared with SOC alone (168). However, due to limited toxicity compared with SOC, bevacizumab represents one of the leading new treatment methods for GBM (38). AMG595 is another antibody that was recently tested in phase I clinical trials, which specifically targets EGFRvIII (169). AMG595 is an immunoconjugate that cannot be cleaved and consists of a human monoclonal antibody targeting EGFRvIII and mertansine, which is a cytotoxic agent (169). In these trials, AMG595 demonstrated good pharmacokinetic profiles in patients with EGFRvIII-positive GBM (169-171). However, the use of AMG595 remains limited due to increased EGFRvIII mutagenicity (169).

Nimotuzumab is a humanised monoclonal antibody that alters cell division by binding to EGFR (172). A previous phase II trial conducted on patients with high-grade glioma found that, compared with radiation therapy (RT) alone (12.6 months), nimotuzumab and RT improved the median survival time (17.8 months) (173). However, data from another phase III clinical trial on patients with GBM showed no significant changes in the survival time when standard TMZ and RT with or without nimotuzumab was used (174). Additional studies are required to determine whether nimotuzumab can be successfully applied as a front-line therapeutic agent for GBM.

Depatuzumab mafodotin (ABT-414) is a monoclonal antibody-drug conjugate that binds to EGFR, leading to its inhibition. In addition, ABT-414 is conjugated with the tubulin inhibitor monomethyl auristatin F (175). A recent study on patients with recurrent GBM revealed that depatuzumab mafodotin treatment resulted in a median survival time of

Table III. Combined drugs targeting RTKs/mTOR/PI3K signalling in clinical trials of GBM.

Drug combination	Clinical trial	Efficiency and drawbacks	(Refs.)
Bevacizumab + erlotinib	Phase II NCT00671970	Although bevacizumab + erlotinib was adequately tolerated, there was no progression-free survival benefit or improved radiographic response when compared with historical bevacizumab-salvage therapy	(161)
Bevacizumab + trebananib	Phase I/II NCT01290263	The study reported serious adverse events, including blood and lymphatic system disorders, such as decreased platelet and neutrophil counts (2.7%), limb edema and fatigue (2.7%). There was no significant improvement in outcome compared with bevacizumab alone	(22)
Temsirolimus + erlotinib	Phase I/II NCT00112736	The maximum tolerated dosage of temsirolimus + erlotinib was lower than expected and increased toxicity was observed. Minimal antitumour activity was noted due to insufficient tumour drug concentrations	(162)
Temsirolimus + perifosine	Phase I/II NCT01051557	Serious adverse events, including lung infection, hyperglycaemia and thromboembolic event were reported, with a higher risk than temsirolimus alone	(163)
Sirolimus + erlotinib	Phase II NCT00672243	Limited efficacy among patients with recurrent GBM. The most frequent toxicities were rash, mucositis, diarrhoea, fatigue and hyperlipidaemia	(164)
Sirolimus + vandetanib	Phase I NCT00821080	The combination appeared feasible and safe. Hypophosphataemia, lymphopenia, rash and fatigue were the most commonly reported toxicities	(165)
Pembrolizumab + dactolisib	Phase IIB NCT02430363	No results reported	

GBM, glioblastoma multiforme; RTK, receptor tyrosin kinase.

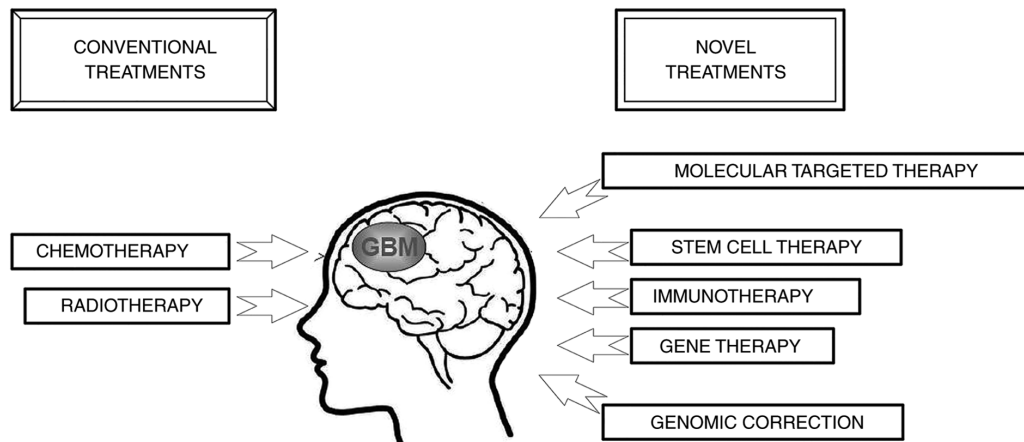


Figure 2. GBM treatment options. GBM, glioblastoma multiforme.

10.7 months (176). At present, only one phase I study has reported the safety and efficacy of ABT-414 when combined with TMZ for recurrent GBM (134).

**Targeting stem-like cells in GBM.** Stem-like cells were first detected in GBM in 2004 by Singh *et al* (177), and they were described as cells that are able to initiate tumour growth *in vivo*. Since malignancy recurrence may be a consequence of the gain of stem cell-like features during disease progression (47),

the cancer stem cell phenotype may prove to be an important therapeutic target. An attractive strategy to regulate the GBM stem cell-like properties and progenitor cell phenotype is to target the epigenetic polycomb repressor complex 1/2 and its key drivers enhancer of zeste 2 polycomb repressive complex 2 subunit and BMI1 (178).

Tumouricidal neural stem cell therapy is a promising new strategy which recently entered clinical trial testing in human patients with GBM (46). The therapy consists in tumouricidal

neural stem cells, which track chemokines to migrate toward solid GBM sites and malignant cells that invade into the healthy areas of the brain (46).

The methods used to identify and target GSCs have not been completely successfully, as the exact mechanisms and functionalities of the putative GSCs markers have not yet been fully elucidated. RNA aptamers that selectively bind to GSCs were previously created on human primary GSCs using cell-systematic evolution of ligand by exponential enrichment methodology (SELEX) (179). Using a cell-SELEX approach, RNA aptamers that selectively bind GSCs were generated. The results proved that they were able to inhibit cell proliferation, migration and stemness, and were able to strongly reduce tumour growth *in vivo*, proving that this approach is a promising innovative diagnostic and therapeutic tool for GBM (179). Due to the crucial role of GSCs in the recurrence and therapy resistance of GBM, these aptamers represent an innovative drug delivery method and are valuable candidates for the treatment of GBM.

*MicroRNAs (miRNAs/miRs).* TargoMiR are miR-16-filled micelles that target EGFR and are especially designed to preserve the expression of the miR-15/16 miRNA family, which have been shown to act as tumour suppressors in cancer (180). However, no clinical findings have been reported to date in terms of GBM treatment.

*Genomic correction.* High-resolution structural genomics has the potential to reveal novel therapeutic options for GBM treatment by integrating structural genomics datasets to identify therapeutic paths in self-renewing cells (181). At <5 kb resolution, contact maps allow the identification of individual DNA loops and large-scale genomic compartmentalisation (182). Differences in looping architectures of genes were previously observed among GSCs collected from different patients, suggesting that the 3D genome architecture may represent a new approach to targeting inter-patient heterogeneity (181).

*Other strategies.* Other novel investigated strategies for GBM treatment involve medical devices (183). NovoTTF-100A is a medical device that can disrupt cell division by using alternating electric fields (184). In addition, thermal lasers can be used to denature tumour tissues (185). Laser interstitial thermal therapy (NeuroBlate System; LAANTERN) can also destroy tumour cells through heating by using a thermoablative procedure and real-time MRI-guided thermography can be applied (186). In 2018, the FDA approved the treatment of recurrent and newly diagnosed GBM by using medical devices that deliver electric fields directly into the tumour (TTFields; Optune®; Novocure GmbH) (187).

Despite advances in the genomic characterisation of GBM, developed targeted drugs have thus far demonstrated insufficient efficacy in clinical trials, with poor patient survival (22,80,98). A large proportion of patients with primary brain tumours typically relapse after standard therapy (20), at which time alternative effective strategies are lacking. To address this issue, research efforts have been directed towards profiling mutations and molecular alterations in the tumours to devise drug treatment regimens that are patient-specific (8). It is hoped that data generated through this

profiling can provide the basis for the development of novel and effective therapies.

Over the past decade, studies have been performed in specific cancers to progressively individualise the therapeutic regimens (6). Treatment with custom-designed tyrosine kinase inhibitors or immunotherapeutics have already been used in certain types of cancer, such as haematological malignancies and melanoma (188).

The  $\beta$ -arrestin family of proteins has been reported to serve an important role in the development of numerous types of cancer (189). However, information regarding its role in gliomas remains poorly understood. Proliferation and response to SOC treatment could be influenced by  $\beta$ -1 arrestin overexpression (190). As a result,  $\beta$ -1 arrestin was overexpressed in a HGG cell line through transfection with a  $\beta$ -1 arrestin plasmid in a previous study (191). Although cells overexpressing  $\beta$ -1 arrestin exhibited higher susceptibility to TMZ after 24 h, this difference became statistically insignificant compared with that in untransfected cells after 48 and 72 h (191). This variation in the HGG cell response to identical treatments highlights the importance of individualised therapy (192). Precision medicine is an innovative approach that can be tailored according to the genetic profile of both disease and patient.

A trial that used molecular profiling for guiding individualised treatment plans in patients with recurrent/progressive GBM was initiated in 2014 (trial no. NCT02060890) (193). The aim was to obtain biopsies from patients for extensive genome-wide profiling and to select drugs that may modulate actionable targets such as EGFR, PDGFRA and v-raf murine sarcoma viral oncogene homolog B1 genes (193).

At present, a number of trials on isocitrate dehydrogenase-wild-type GBM are ongoing, in which integrated treatment strategies incorporating single-cell technologies, multi-omics and computational approaches are being studied (194-196).

In terms of drug development, nanomedicine is a relatively new research field. The main objective is to deliver therapeutic agents to the specific targeted sites, preferably in a controlled manner. In this regard, several types of nanoparticles have been studied as potential drug delivery systems carrying bioactive compounds for GBM treatment (197).

However, the specific characteristics of GBM pose major challenges, regardless of the strategy applied. These challenges include cellular heterogeneity, meaning that some drugs cannot penetrate into the tumour tissue efficiently, posing difficulties in biomarker assessment, since repeat on-treatment biopsies are not feasible (198).

#### 4. Conclusions

GBM is the most aggressive among all types of primary brain tumours. Currently available SOC therapeutic methods have failed to significantly improve the survival rate, emphasising the need to develop novel therapeutic approaches. Novel biomarker-driven strategies have been developed over the last decades, but they have performed poorly during clinical trial testing. This is mainly due to low BBB permeability and increased resistance/tolerance. Most, if not all, clinical trials tend to test a targeted treatment method using a potential 'one size fits all' approach, but there are several biological differences among individual patients. In recent years, although

molecular profiling data of tumour tissues are becoming increasingly available, proof-of-concept studies in GBM are still lacking. The biological complexity, lack of data for precision medicine and low efficiency of drug delivery systems are examples of the numerous challenges encountered in the management of GBM that must be addressed.

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### Authors' contributions

ASS, AC and AD were involved in conceptualisation. CB, SAA, OA and SD were involved in formal analysis. SAA, CB, OA, OS, SD, LGT, VS and ASS prepared the original draft. ASS, LGT, AC and AD reviewed and edited the manuscript. All the authors have read and approved the final manuscript. Data sharing is not applicable.

### Ethics approval and consent to participate

Not applicable.

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### Competing interests

The authors declare that they have no competing interests.

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