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Targeted Therapies for the Treatment of Glioblastoma in Adults

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Abstract

Purpose of Review Targeted therapies are part of biomarker-driven strategies that exploit actionable molecular targets and have gained traction following survival benefits demonstrated in various systemic malignancies. In glioblastoma, where therapeutic options remain scarce and prognosis poor, targeted therapies offer an attractive treatment alternative and are actively examined in clinical trials. In this review, we summarize the targeted therapies, including traditional small molecule inhibitors and monoclonal antibodies as well as immunotherapeutic approaches that are examined in clinical trials, and discuss the challenges of using them for the treatment of glioblastoma.

Recent Findings Despite initial speculations, phase II/III trials of targeted therapies in adult patients with glioblastoma have largely failed. Recent trials have focused on improving patient stratification, drug-tissue penetration, and target and compensatory pathway inhibition to optimize treatment response. In contrast to traditional small molecule and monoclonal antibody therapies, cancer immunotherapy may target specific molecular or immune checkpoint target(s) to trigger immune responses against glioblastoma. Early phase clinical trials of immunotherapy have shown encouraging results, and larger randomized trials are ongoing.

Summary Targeted therapies are being actively studied in clinical trials. Patients with glioblastoma should be prioritized for clinical trial participation.

Keywords Glioblastoma · Immunotherapy · Molecular targeted therapy · Receptor protein-tyrosine kinase

Introduction

Glioblastomas (GBM) are tumors that arise from glial or precursor cells and are the most common primary malignant brain tumor in adults [1]. Despite standard first-line treatment, almost all tumors recur. Salvage therapeutic options are limited and overall prognosis is poor [1, 2]. In search of better therapeutic strategies, researchers have sought to better understand the pathophysiology underlying the development of GBM. The Cancer Genome Atlas (TCGA), a government-funded initiative to catalog and ascertain genomic alterations in cancer pathogenesis, used GBM as its first study disease. It has since identified three core pathways that lead to the development of GBM: (i) receptor tyrosine kinase (RTK)/Ras/phosphoinositide 3-kinase (PI3K), (ii) p53, and (iii) retinoblastoma (Rb) pathways [2, 3] (Fig. 1).

RTKs are essential for signal transduction events at a cellular level [4]. Activated RTK stimulates the PI3K/AKT/PTEN/mTOR pathway which controls cellular division, proliferation, differentiation, metabolism, and survival. This in turn enhances the growth of GBM and is essential for tumorigenesis [5]. At least one RTK was found altered in 67.3% of GBM [3]. In contrast, *TP53* and *RB* genes are classically known as tumor suppressor genes. p53 is pertinent in the coordination of cell responses to various stressors by regulation of genes involved in apoptosis, DNA repair, and neovascularization [6], while hypophosphorylated Rb protein prevents transactivation of genes required for progression through the cell cycle [7]. Together, the Rb and p53 pathways are important guardians of the G1-to-S phase transition, and inactivating mutations of these pathways render cells to inappropriate cell division and aids in the proliferation of tumor cells. Over the past decade, the elucidation of the RTK, p53, and Rb pathways have facilitated the development of direct inhibitors that bind to these pathway molecular targets to thwart GBM growth and proliferation.

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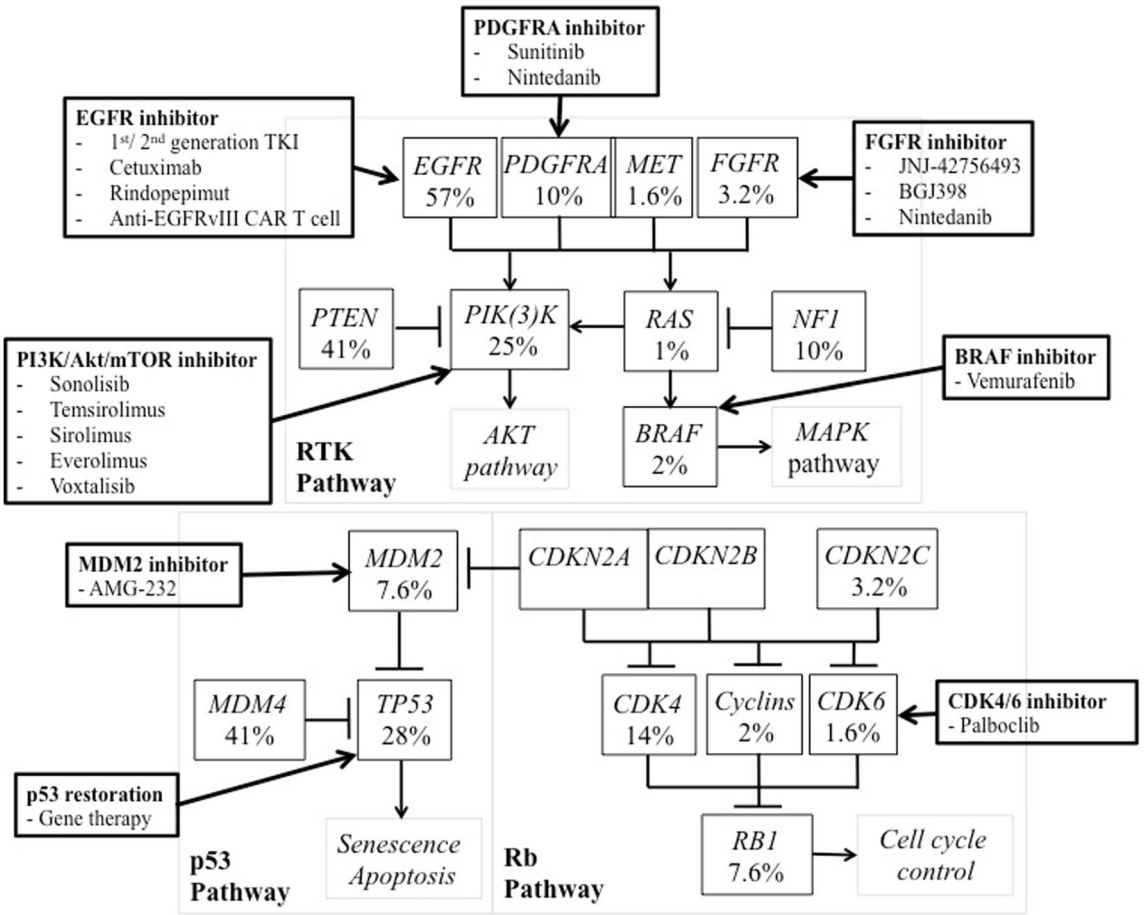

Fig. 1 Targeted therapies against canonical receptor tyrosine kinase (RTK), p53 and retinoblastoma (Rb) pathways in glioblastoma. This simplified diagram depicts the canonical receptor tyrosine kinase (RTK) signaling, and p53 and retinoblastoma (Rb) tumor suppressor pathways and their alteration rates in adult patients with glioblastoma; examples of targeted therapies against these pathway targets are shown. Adapted from Brennan CW, Verhaak RG, McKenna A, Campos B, Noushmehr H, and Salama SR et al. The somatic genomic landscape of glioblastoma. Cell. 2013;155(2):462–77. doi:https://doi.org/10.1016/j.cell.2013.09.034, with permission from Elsevier

More recent efforts have focused on cancer immunotherapy, which has achieved significant survival in patients with systemic malignancies [8–11]. Historically, the central nervous system (CNS) was regarded as an immune-privileged site that is well protected from immune access by the blood-brain barrier (BBB) and an absent lymphatic system. It was widely believed that a disrupted BBB was required for the immune system to mount a response to endogenous tumor antigens in the CNS. Recent studies provided clarifications to this misconception by demonstrating an ability to circulate immune cells to penetrate an intact BBB [12], and a lymphatic drainage system in the CNS [13, 14••]. More importantly, the studies illustrate the existence of adaptive immunity in the CNS where immune responses are actively suppressed by a variety of cellular and molecular mechanisms [15]. In brain malignancies, these immune-suppressive mechanisms are further augmented, thereby allowing tumor cells to escape immune surveillance. Immunotherapy exploits strategies that inhibit and modulate these immunosuppressive pathways to activate immune-mediated anti-tumor effects.

In this review article, we will discuss targeted therapies examined in GBM. We will explore both direct inhibitors and immunotherapeutic approaches and discuss the challenges of using targeted therapies for the treatment of GBM.

Small Molecule and Monoclonal Antibody Therapies

Receptor Tyrosine Kinase Pathways

Epidermal Growth Factor Receptor Inhibitors

In GBM, epidermal growth factor receptor (EGFR) is an important signal in the RTK pathway, where amplification, rearrangement, or point mutations occur in over 40% of GBM [16, 17]. Approximately 50% of EGFR-amplified GBMs also express EGFRvIII, a constitutively active oncogenic variant that results from the deletion of exons 2–7 of EGFR. Given its relatively high prevalence of alteration in GBM, EGFR is an

attractive target for the study and development of targeted therapy.

EGFR kinase inhibitors have been studied and used most extensively in the treatment of EGFR-mutated lung cancer. Despite their success in the treatment of lung cancer, first-generation EGFR kinase inhibitors, such as erlotinib and gefitinib, have not been proven to be effective in patients with GBM [18–20]. Studies of first-generation EGFR inhibitors in patients with GBM showed variable inhibition of EGFR or its downstream effector, which suggest that these RTK inhibitors are unable to inhibit the EGFR signaling network sufficiently [21]. Second-generation EGFR inhibitors, including afatinib, dacomitinib, and neratinib, brought new hope. However, phase I/randomized phase II study of afatinib with and without temozolomide in GBM patients at first or second recurrence showed limited activity in unselected patients [22]; in subgroup analysis, patients whose tumor harbor high levels of EGFRvIII immunoreactivity, EGFR amplification, or PTEN loss showed prolonged progression-free survival (PFS) compared to unselected patients, but these results need prospective validation. A more recently published clinical trial of dacomitinib showed limited single-agent activity in recurrent GBM harboring EGFR amplification, suggesting that other molecular marker(s) may be necessary to identify the patients who may benefit from EGFR inhibition [23].

While the above therapies target the kinase domain of EGFR, monoclonal antibody therapies target EGFR extracellular domain to prevent ligand binding and subsequent EGFR kinase domain activation. Cetuximab is a monoclonal EGFR targeting antibody that is well tolerated but showed limited activity for recurrent GBM in phase II clinical trial [24]. One strategy employed to optimize drug-tissue penetration of these large-size antibodies is the use of intra-arterial approach—a recent phase I trial of superselective intra-arterial cerebral infusion (SIACI) of cetuximab after osmotic disruption of the BBB with mannitol in patients with recurrent malignant glioma showed acceptable safety and tolerability profiles [25]; phase I/II trials in newly diagnosed and recurrent GBM are currently ongoing (NCT02861898, NCT01238237).

Fibroblast Growth Factor Receptor Inhibitors

The identification of chromosomal rearrangement that results in the fusion of 2 genes with oncogenicity has led to the successful development of targeted therapies in various malignancies and marks an important discovery in human cancer genetics [26–28]. Two examples are imatinib for the treatment of chronic myelogenous leukemia [26] and crizotinib for the treatment of anaplastic lymphoma kinase (ALK)-positive non-small cell lung cancer [27, 28]. The discovery of the chromosomal rearrangement and oncogenic fusion of the fibroblast growth factor receptor (FGFR) tyrosine kinases to the transforming acidic coiled-coil (TACC) proteins (FGFR-TACC fusion) in about 3–8.3% of patients with GBM hence raised hopes of finding an effective treatment for this deadly disease [29, 30•, 31].

Notably, a phase I basket trial of JNJ-42756493, an oral pan-FGFR inhibitor, showed encouraging activity with partial response in 2 patients with GBM and stable disease in 16/23 patients with GBM, urothelial, and endometrial cancer; a phase II trial is underway [32]. Currently, there are ongoing phase II trials of other FGFR inhibitors conducted in recurrent GBM expressing activating mutations in FGFR 1, 2, or 3 (NCT01975701).

Vascular Endothelial Growth Factor and VEGF Receptor Inhibitors

Vascular endothelial growth factor receptor (VEGFR) is a key regulator of angiogenesis, which is a pathological hallmark of GBM [33]. Initial studies with monotherapy bevacizumab [34], a VEGF inhibitor, or bevacizumab in combination with irinotecan [35] in patients with recurrent GBM, showed improved outcomes compared to historical controls. However, the evaluation of bevacizumab in phase III clinical trials in newly diagnosed GBM failed to show improvement in overall survival when compared to control [36]. It soon became clear that treatment with bevacizumab, due to its ability to alter angiogenesis and reduce cerebral edema, results in an initial dramatic improvement in clinical and radiographic responses but does not exert sufficient anti-tumor effects to impact disease progression. Consistent with these findings, the BELOB phase II trial showed that bevacizumab alone arm did not meet pre-specified criteria to warrant further studies and has been dropped from the subsequent phase III trial that is examining lomustine alone or in combination with bevacizumab (NCT01290939). Vatalanib [37], tivozanib [38], and cediranib [39] are examples of VEGFR inhibitors that have been unsuccessfully tested for the treatment of GBM. Similarly, vandetanib, a VEGFR-2 and EGFR inhibitor, showed little antitumor effects on recurrent GBM in a phase II trial [40]. Ongoing trials of VEGFR inhibitors include the phase II study of apatinib for treatment of recurrent high-grade glioma (NCT03390062).

Platelet-Derived Growth Factor Receptor and Multi-TKR Inhibitors

Platelet-derived growth factor receptor (PDGFR)-α amplification has been found in almost 15% of gliomas. Dasatinib, a multi-targeted RTK inhibitor, was studied in a phase II trial in patients with recurrent GBM. Patients were selected based on the overexpression of at least 2 dasatinib targets (SRC, c-KIT, EPHA2, and PDGFR) but no clinical benefit was reported, and the trial was closed without proceeding to phase II [41]. Sunitinib, another multi-targeted RTK (PDGFR, VEGF,

CD117, RET, CD114, and CD135) inhibitor that has been FDA approved for the treatment of renal cell carcinoma, also did not show any activity in a phase II trial for recurrent malignant glioma [42]. Similarly, two recent phase II trials of nintedanib, a triple-targeted RTK (PDGFR α/β, FGFR 1–3 and VEGF 1–3) inhibitor, showed limited activity in patients with recurrent GBM [43, 44]. In contrast, dovitinib, multi-RTK (FGFR, VEGFR, PDGFR β, and c-Kit) inhibitor, showed safety and preliminary activity in patients with recurrent GBM unselected for target expression [45]; the phase II study had concluded but results are not yet available.

Phosphatidylinositol-3-Kinase/Akt and the Mammalian Target of Rapamycin Pathway Inhibitors

Phosphatidylinositol-3-kinase (PI3K)/Akt and the mammalian target of rapamycin (mTOR) pathway are activated by RTK, transmembrane integrins, and G protein-coupled receptors, and in turn, inhibits transcription of pro-apoptotic proteins and activates translation of protein required for cell growth, cell cycle progression, and cell metabolism. Alterations in key genes in the PI3K/Akt/mTOR pathway is observed up to 90% of GBM studied [3], and represents one of the most attractive targets for treatment.

A phase II trial showed the poor activity of sonolisib, an irreversible PI3K inhibitor, in recurrent GBM [46]. Similarly, phase II trials of mTOR inhibitors—temsirolimus [47], sirolimus [48], and everolimus [49]—alone or in combination with an RTK inhibitor showed limited clinical efficacy in patients with recurrent GBM. A more recent phase I trial of voxtalisib, a PI3K/mTOR inhibitor showed good safety profile and encouraging activity in newly diagnosed malignant gliomas [50]. AZD2014 is an mTOR inhibitor currently being examined in a clinical trial (NCT02619864).

Notably, WEE1 is an adaptive-resistant gene which has been found to be activated after targeted inhibition of PI3K. One preclinical study showed that the inhibition of WEE1 potentiated the effectiveness of PI3K-targeted inhibition, suggesting a potential role of combination therapy with WEE1 and PI3K inhibition in GBM [51]. A recent phase 0 trial of AZD1775 provided the first evidence of biological activity in human GBM [52].

Mitogen-Activated Protein Kinase/Extracellular Signal-Regulated Kinase Pathway Inhibitors

The MAPK signaling pathway is involved in all GBM and is an obvious target for treatment. The BRAF V600E mutation, a targetable oncogene, can be found in a subset of patients with GBM [53]. Vemurafenib, a BRAF inhibitor, showed promising results in patients with BRAF-mutant (BRAF V600E) high-grade gliomas [54, 55••]. However, limited clinical efficacy was seen when sorafenib (RAF multikinase inhibitor) was studied either alone or in combination with temozolomide [56, 57], temsirolimus [58], bevacizumab [59], or temozolomide and radiation therapy [58].

p53 Pathway

The p53 protein is an essential tumor suppressor protein and its pathway (*MDM2*, *MDM4*, and *TP53*) dysregulation is found in more than 80% of GBM [3]. Given its tumor suppressive abilities, several gene therapy strategies have been developed in an attempt to restore wild-type p53 function in GBM. However, a phase I trial of p53 gene therapy using an adenovirus vector showed limited transduced cells [60], possibly related to low potency and poor BBB penetration. A separate phase II trial of SGT-53, a complex cationic liposome that encapsulates a normal human wild-type p53 DNA sequence, is currently under investigation (NCT02340156).

Mouse double minute (MDM) 2 is a negative regulator of p53, and its amplification inactivates the p53 pathway [61]. Preclinical studies show promising results of MDM2 inhibitors as novel therapies for GBM patients [62–64]. A phase I trial of AMG-232 is ongoing (NCT03107780).

Retinoblastoma Pathway

The Rb pathway (*CDK4*, *CDK6*, *CCND2*, *CDKN2A/B*, and *RB1*) is also found to be affected by almost 80% of GBM [3]. In recent years, several novel agents targeted towards the inhibition of CDK4 and CDK6 has shown promising efficacy against RB1 wild-type GBM models [65]. One example currently being examined in a clinical trial is palbociclib, an inhibitor of CDK4/6 that reverses the inhibition of phosphorylated Rb (NCT02933736).

Other Targets

In low-grade gliomas, monoallelic point mutations of isocitrate dehydrogenase type 1 (IDH1) occur commonly and early in tumorigenesis [66]. Hence, IDH1 is an attractive target for secondary GBM [67]. Preliminary data from phase I clinical trials involving patients with cancers with IDH1 mutation treated with Ag-120 (ivosidenib), an IDH1 mutant enzyme inhibitor, demonstrated promising clinical activity [68]. Clinical trials of Ag-120 and Ag-221 targeting IDH1 and IDH2 respectively are currently ongoing (NCT02074839, NCT02073994, NCT02273739). A more recently identified target is the H3K27M mutation, which arises from the substitution of lysine for methionine at position 27 in histone H3. H3K27M mutation was initially thought to occur exclusively in the pediatric population, but it is now recognized that approximately 30% of adult midline gliomas harbor the mutation [69]. Interestingly, ONC201 affected a partial response in an H3K27M-mutated recurrent glioblastoma [70]. Although

ONC201's specific mechanism of action against the H3K27M mutation has yet been characterized, several phase II clinical trials of ONC201 in adult H3K27M-mutated recurrent high-grade gliomas have been initiated (NCT03295396, NCT03134131, NCT02525692).

A list of recently completed and ongoing phase II trials of targeted therapies in adult patients with glioblastoma is included in Table 1.

Immunotherapy

In contrast to direct inhibitors, immunotherapy inhibits specific GBM target(s) to mount an innate and/or adaptive immune response against GBM. GBM immunotherapy targets tumor cell antigens or immune checkpoint pathways, and treatment strategies can be broadly classified as vaccine therapy, immune modulators, and cellular therapy.

Tumor Cell Antigens/Molecular Targets

As discussed, EGFRvIII and IDH are attractive therapeutic targets given their common occurrence in primary and secondary GBM respectively. Rindopepimut is a synthetic mutated EGFRvIII neoantigen-specific peptide conjugated to the adjuvant keyhole limpet hemocyanin (KLH) and designed to target GBM harboring EGFRvIII. Despite encouraging results from phase 2 trials [71–73], the phase 3 trial of rindopepimut in upfront treatment of EGFRvIII-expressing GBM was terminated at interim analysis for futility, and final analysis showed no significant difference in overall survival in the rindopepimut groups versus control group (HR1.01, 95% CI 0.79–1.30, $p = 0.93$) [74]. One potential contributing factor to the failure of the trial is the administration of KLH in the control arm that could have potentially triggered anti-tumor immune effects. Another strategy developed against EGFRvIII is the chimeric antigen receptor (CAR) T cell therapy. CAR T cells are genetically engineered receptors that couple a single-chain variable fragment domain of a targeting antibody to intracellular signaling and costimulatory domains of CTL receptors, thereby inducing antigen expression in CTLs. CAR T cells engineered from EGFR and EGFRvIII are currently being examined in clinical trials (NCT01454596, NCT02331693, NCT02664363, NCT02209376). A peptide vaccine against IDH R132H-mutated secondary GBM has also been generated and shown to effect major histocompatibility complex (MHC) class II-restricted mutation-specific anti-tumor immune response in mouse models [75]; this peptide vaccine is now being studied in patients with IDH1 R132H-mutated grade III-IV gliomas (NCT02454634).

Another target antigen is the Wilms tumor 1 (WT1) gene located at chromosome 11p13. WT1 is overexpressed in a number of malignancies and is associated with poor prognosis in diffuse astrocytomas [76, 77]. WT1 peptide vaccine was well tolerated and produced clinical activity in patients with WT1/HLA-A*2402-positive recurrent GBM [78], and when combined with standard upfront treatment in patients with newly diagnosed GBM [79]. Additionally, WT1 has been used to generate dendritic cell (DC) vaccine. DCs are antigen-presenting cells with high expression of MHC class II and costimulatory molecules and play crucial roles in both innate and adaptive immune responses [80]. DCs reside as immature cells in almost every organ; when faced with pathogens, they ingest antigens, migrate to secondary lymphoid tissues, and present the antigens to T helper and cytotoxic T lymphocytes (CTL) [81]. The phase I study of WT1-pulsed and/or tumor lysate-pulsed DC vaccine demonstrated safety, immunogenicity, and feasibility in patients with relapsed malignant gliomas [82].

In contrast to these vaccines, others have designed vaccines using multiple tumor-associated antigens. IMA950 is a multipeptide vaccine containing 11 synthetic HLA-A2-restricted tumor-associated peptides found in the majority of GBMs. A two-cohort, phase 1 trial of IMA950, given concurrently with temozolomide and radiation therapy (RT) in patients with newly diagnosed GBM, showed that IMA950 plus granulocyte macrophage colony-stimulating factor (GM-CSF) was well tolerated with primary immunogenicity endpoint exceeded [83]. SL-701, another multivalent vaccine, is being examined in combination with poly-ICLC (inosinic-polycytidylic acid (poly[I:C]) stabilized by lysine and carboxymethyl cellulose) and bevacizumab in patients with recurrent GBM; preliminary results showed that SL-701 is well tolerated with anti-tumor activity and a promising survival tail [84]. A separate phase 1 study was conducted in 21 GBM patients who were HLA-A1 and/or HLA-A2-positive using ICT 107, an autologous pulsed DC vaccine derived from six TAAs, namely AIM-2, MAGE1, TRP-2, gp100, HER2/neu, and IL-13Rα2 that are expressed on gliomas and overexpressed in glioma stem cell population [85]. Preliminary results from the ICT-107 randomized double-blind placebo-controlled phase 2 study demonstrated statistically significant improvement in PFS but not OS in the investigational arm (NCT01280552) [86]; a subsequent phase 3 trial has been suspended due to lack of funding (NCT02546102).

With the shift towards personalized medicine, researchers have also sought to develop personalized vaccines generated from immunogenic antigens unique to an individual patient's tumor. Two such vaccines are the actively personalized vaccine (APVAC) and NeoAntigen Cancer Vaccine (NeoVax) vaccine. While APVAC has been reported to have expected safety profile and high biological activity in newly diagnosed GBM [87], the phase I trial of NeoVax in newly diagnosed MGMT unmethylated GBM is still ongoing (NCT02287428).

Table 1 Examples of recently completed and ongoing phase II/III trials of targeted therapies in adult patients with glioblastoma

ClinicalTrials.gov Identifier	Population	Treatment arms	Treatment target	Status[a]	Sponsor
NCT02800486	Relapsed/refractory GBM, AA and AOA with EGFR overexpression	Single-arm experimental: intra-arterial cetuximab with re-irradiation	EGFR	Recruiting	Northwell Health
NCT02861898	Newly diagnosed GBM with EGFR overexpression	Single-arm experimental: intra-arterial cetuximab after BBB disruption	EGFR	Recruiting	Northwell Health
NCT01257594	Recurrent high-grade glioma with EGFRvIII expression	Experimental arm 1: no cytoreductive surgery planned + erlotinib Experimental arm 2: cytoreductive surgery + erlotinib	EGFR	Completed	Columbia University
NCT00052208	Newly diagnosed GBM	Single-arm experimental: gefitinib and radiation therapy	EGFR	Completed	National Cancer Institute
NCT01975701	Recurrent resectable or unresectable GBM or other glioma subtypes that harbor FGFR1-TACC1, FGFR3-TACC3 fusion and/or activating mutation in FGFR1, 2 or 3	Single-arm experimental: BGJ398	FGFR1-TACC1, FGFR3-TACC-3 fusion, FGFR1, FGFR2, FGFR3	Active	Novartis Pharmaceuticals
NCT01290939	First recurrence of GBM	Experimental arm 1: lomustine + bevacizumab Active comparator arm 2: lomustine	VEGF-A	Active, not recruiting	European Organization for Research and Treatment of Cancer—EORTC
NCT03390062	Recurrent high-grade glioma	Single-arm experimental: apatinib (YN968D1)	VEGFR2	Not yet recruiting	West China Hospital
NCT03291314	Recurrent glioblastoma	Experimental arm 1: combination of axitinib and avelumab Experimental arm 2: axitinib (+ avelumab depending on corticosteroids dose)	VEGFR PDL-1	Completed	Universitair Ziekenhuis Brussel
NCT01498328	Relapsed EGFRvIII-positive glioblastoma	Experimental: group 1a: bevacizumab naïve patients will receive bevacizumab + rindopepimut. Experimental: group 1b: bevacizumab naïve patients will receive bevacizumab + KLH control Experimental: group 2 and 2C: patients refractory to bevacizumab will be administered rindopepimut/GM-CSF while continuing/restarting (if stopped) bevacizumab	EGFR VEGF	Completed	Celldex Therapeutics
NCT01753713	Recurrent or progressive GBM	Experimental Arm 1: Dovitinib in patients who have progressed without anti-angiogenic therapy Experimental arm 2: dovitinib in patients who have progressed on anti-angiogenic therapy	Multi-targeted RTK (FLT3/c-KIT, FGFR1/3, VEGFR1-4)	Completed	National Cancer Institute Novartis
NCT02364206	Newly diagnosed GBM	Single-arm experimental: LY2228820 + temozolomide + radiotherapy	p38 MAPK	Active, not recruiting	Centre Jean Perrin
NCT03561870	Recurrent IDH-mutant glioma	Single-arm experimental: olaparib	PARP	Not yet recruiting	Hospices Civils de Lyon
NCT03557359	Recurrent or progressive IDH mutant gliomas	Single-arm experimental: nivolumab	PD-1	Recruiting	Columbia University
NCT03718767		Single-arm experimental: nivolumab	PD-1		

Table 1 (continued)

ClinicalTrials.gov Identifier	Population	Treatment arms	Treatment target	Status[a]	Sponsor
	Glioma with IDH1 or IDH2 mutation and hypermutator phenotype			Not yet recruiting	National Cancer Institute (NCI)
NCT02968940	Glioblastoma with IDH mutation	Single-arm experimental: avelumab and hypofractionated radiation therapy (NFRT)	PD-L1	Active, not recruiting	New York University School of Medicine
NCT03743662	Recurrent MGMT methylated GBM	Experimental arm 1: patients not planned for surgical debulking will be given re-irradiation, bevacizumab and nivolumab Experimental arm 2: patients planned for surgical debulking will be given re-irradiation, bevacizumab, and nivolumab, followed by re-resection thereafter	PD-1 VEGF	Recruiting	Memorial Sloan Kettering Cancer Center

[a] Source: Clinicaltrials.gov (January 2019)

AA, anaplastic astrocytoma; *AOA*, anaplastic oligoastrocytoma; *EGFR*, endothelial growth factor receptor; *FGFR-TACC*, fibroblast growth factor receptor-transforming acidic coiled-coil; *GBM*, glioblastoma; *IDH*, isocitrate dehydrogenase; *KLH*, keyhole limpet hemocyanin; *MAPK*, mitogen-activated protein kinase; *MGMT*, methylguanine-DNA methyltransferase; *PARP*, poly ADP ribose polymerase; *PD-1*, programmed cell death-1; *PD-L1*, programmed death-ligand 1; *VEGF*, vascular endothelial growth factor

Immune Checkpoint

Blockade of cytotoxic T lymphocyte-associated protein 4 (CTLA-4), programmed cell death protein 1 (PD-1), and programmed cell death protein 1 ligand (PD-L1) represents a paradigm shift in immunotherapy from the usual strategy that directly activates the immune system. Collectively known as immune checkpoint inhibitors, these agents unblock inhibition of CTL by tumor cells, resulting in CTL activation and killing of tumor cells. This therapy has gained traction with successes in the treatment of multiple tumor types [8–11]. In gliomas, immune checkpoint receptors have been shown to be upregulated and associated with tumor grade [88–90], providing the basis for the use of these inhibitors in GBM.

In the safety lead-in phase of a phase 3 trial comparing the combination of nivolumab, a PD-1 inhibitor, plus ipilimumab or nivolumab alone versus bevacizumab in recurrent GBM, only patients in the combination arm of nivolumab and ipilimumab developed grade 3 or more toxicities and required treatment discontinuation from drug-related adverse events [91]. As a result, the combination arm was discontinued, and subsequent patients were randomized to receive nivolumab or bevacizumab. At World Federation of Neuro-Oncology Societies meeting 2017, the trial was announced to be closed prematurely due to the failure of nivolumab to prolong OS in patients with recurrent GBM [92]. A phase II trial of pembrolizumab presented at American Society of Clinical Oncology (ASCO) 2016 also showed limited anti-tumor activity for recurrent GBM [93]. These trial failures may be related to poor BBB penetration of the large molecular nivolumab, and impaired CTL interaction with GBM from systemic lymphopenia and reduced expression or effector function of PD-1. Of note, preliminary data presented at conferences have shown that immune checkpoint inhibitors resulted in durable responses in a subset of patients with GBM, suggesting better patient stratification may improve treatment outcome [92–94]. Clinical trials of other PD-1 and PD-L1 inhibitors, in various combinations, are underway.

Challenges in Using Targeted Therapies for Patients with GBM

Targeted therapies are part of biomarker-driven strategies that exploit actionable molecular or immune marker targets. Despite the extensive number of clinical trials conducted over the past decade, none of the targeted therapies have been shown to improve the outcome for patients with GBM. Initial clinical trial failure could in part be accounted for by the lack of stratification according to individual tumor molecular makeup, but subsequent clinical trials that recruited patients based on the presence of molecular target continued to fail. Several identified contributory factors to the lack of

efficacy of targeted therapies in the treatment of GBM are inadequate drug-tissue penetration, inadequate target inhibition [95], insufficient suppression of downstream signaling [21], GBM heterogeneity [96], and alternative compensatory activation of signaling mediators [97]. It is clear that the current understanding of tumor genetic landscape, heterogeneity, and biological complexity, as well as CNS drug delivery, is not yet sufficient to translate targeted therapies into effective GBM treatment.

Indeed, GBM exhibits extensive intratumoral heterogeneity. In a surgical multisampling study, each of 11 GBMs was shown to harbor subpopulations of tumor cells with distinct genotypes and unique patterns of cancer evolution [96]. These spatial differences and temporal changes challenge the choice of targeted therapies for upfront treatment of GBM and at tumor recurrence. While single-targeted therapies may inhibit one subpopulation, drive the clonal expansion of another, and cause overall treatment failure [98], concurrent use of multiple targeted therapies increases the risk of toxicities, drug-drug interactions, and adverse effects [99]. Additionally, tumor sampling to define new mutational profiles may not be representative of the entire tumor or possible at tumor recurrence. The key is to develop non-invasive methods that can reliably delineate ongoing tumor genetic evolution and to design combination therapies that will target parallel and/or redundant molecular pathways with optimal CNS penetration and safety.

Several innovative molecular-driven, genomics-informed clinical trials are ongoing. One study necessitated multiple biopsies at enhancing and non-enhancing regions of GBM, followed by tumor profiling using genome-wide tumor/normal exome sequencing and tumor RNA sequencing [100•]; the genomic results, in addition to patients' clinical history, would then be made available to a multidisciplinary molecular tumor board within 35 days of surgery for treatment recommendation. This precision medicine approach has been shown to be feasible, though overall cost-effectiveness is unknown. A separate phase II ongoing trial, the molecular analysis for therapy choice (MATCH) trial, is recruiting patients with advanced refractory solid tumors, lymphomas, and multiple myeloma and matching their next-generation sequencing results with one or more of 30 available drugs (NCT02465060). More interestingly, a new international clinical trial, the GBM AGILE trial, using the adaptive trial design is underway for patients with GBM. Unlike traditional randomized controlled trials, GBM AGILE trial adopts a flexible approach that allows for dynamic adjustment for parallel treatment arms of various targeted therapies and hence has the potential to substantially reduce the time for biomarker discovery and drug development. These trials shed new hope to the successful development of targeted therapies for GBM.

Conclusion

Although targeted therapies using small molecular inhibitors, monoclonal antibodies, and immunotherapy have dramatically changed the treatment paradigm for numerous malignancies, clinical trials of targeted therapies in patients with glioblastoma have largely failed. Notably, rare durable responses to targeted therapies have been observed, suggesting the need for identification of more precise biomarkers to help in patient selection. Currently, new treatment strategies with improved BBB penetration, and target and compensatory pathway inhibition are being examined in clinical trials. Where possible, patients with glioblastoma should be recruited in clinical trials.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

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