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Glioblastoma Multiforme: An Overview of Emerging Therapeutic Targets

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Glioblastoma multiforme (GBM) is the most common and aggressive malignant primary brain tumour in humans and has a very poor prognosis. The existing treatments have had limited success in increasing overall survival. Thus, identifying and understanding the key molecule(s) responsible for the malignant phenotype of GBM will yield new potential therapeutic targets. The treatment of brain tumours faces unique challenges, including the presence of the blood brain barrier (BBB), which limits the concentration of drugs that can reach the site of the tumour. Nevertheless, several promising treatments have been shown to cross the BBB and have shown promising pre-clinical results. This review will outline the status of several of these promising targeted therapies.

Keywords: glioblastoma, targeted therapeutics, anti-cancer drugs, brain cancer, immunotherapy

INTRODUCTION

Glioblastoma multiforme (GBM) is the most common and aggressive primary malignant brain tumour and accounts for 60% of brain tumours in adults (1). The global incidence of GBM is <10 per 100,000 persons (2) and has increased over the last decade (3). GBM patients have a poor prognosis with a 1-year survival rate of 37.2%, a 5-year survival rate of 5.1% (4) and a median survival of ∼10 months (5). GBM is divided into three subgroups based on isocitrate dehydrogenase 1 (IDH1) and IDH2 mutation status: IDH-mutant, IDH-wild-type and NOS (not otherwise specified) (6–8). However, despite this classification, the majority of GBM patients receive identical treatments, and few targeted therapies currently exist, contributing to the poor outcomes typically experienced by GBM patients. This review will outline the current treatment options for GBM and discuss some of the more recent developments in targeted therapies being investigated for the treatment of GBM.

CURRENT TREATMENT OPTIONS

The treatment of brain tumours faces unique challenges, most notably the presence of the blood brain barrier (BBB), a highly selective semipermeable barrier that separates blood from the brain. The BBB is comprised of the endothelial cells of capillaries, astrocytes surrounding the capillary, and pericytes embedded in the capillary basal lamina. Physiochemical properties including molecular weight, lipophilicity and charge affect the ability of a molecule to cross the BBB (9). The BBB prevents nearly all large molecules (>400 Da) (10) and ∼98% of small molecule drugs from entering the central nervous system (CNS) (11). The current treatment pipeline begins with surgical resection of the tumour, if applicable and safe to do so, followed by radiotherapy and concomitant chemotherapy (12, 13).

The initial therapeutic approach for GBM is surgery, where maximal resection is associated with longer progression-free survival (PFS) and overall survival (OS) (14). Resection is not a curative approach; hence, patients typically undergo radiotherapy and chemotherapy as an adjunct (15). Radiotherapy, at a total dose of 60 Gy, is administered as either primary treatment or following surgery (16), both resulting in improvements to PFS and OS. Concomitant administration of temozolomide [150–200 mg/m^2/day for 5 days each 28-days cycle (12, 13)], an oral alkylating agent, significantly increases OS in patients with newly diagnosed GBM from 12.1 months with radiotherapy alone to 14.6 months with radiotherapy and temozolomide (13). However, despite this increase in survival with radiotherapy and temozolomide, tumour progression and recurrence typically occur (17, 18), due to the development of resistance to temozolomide (19, 20). Once GBM recurrence occurs, therapeutic options for patients are limited (21, 22). Recently, tumour treating fields (TTFields; Optune), which deliver electric fields to the tumour location to disrupt cancer cell division, have emerged as an FDA-approved treatment for both recurrent and newly diagnosed GBM (23). However, the identification of new targets to facilitate the development of novel targeted therapies is warranted.

EMERGING TARGETED THERAPIES

GBM is an invasive tumour with hallmarks of neoangiogenesis and intratumour heterogeneity, contributing to the poor prognosis observed (24). A variety of genetic and epigenetic alterations have been identified in GBM that influence patient prognosis (**Table 1**). Despite this heterogeneity, a large-scale analysis of genetic aberrations in GBM identified three main signalling pathways that are commonly dysregulated: activation of the receptor tyrosine kinase (RTK)/Ras/phosphoinositide 3-kinase (PI3K) pathway (88%), inhibition of p53 (87%), and retinoblastoma protein (Rb) signalling pathways (78%) (34). Drugs targeting many of these commonly observed alterations have been investigated as potential targeted therapies for GBM.

EphA3 Receptor Inhibitors

The EphA3 receptor is overexpressed in 40–60% of GBM tumours and is commonly overexpressed in recurrent GBM (**Table 1**) (25). EphA3 is highly expressed on the tumour-initiating cell population in glioma, maintains tumour cells in a less differentiated and stem cell-like state, and its expression mediates the tumourigenic potential in GBM cells *in vitro* (26), suggesting that EphA3 may be a potential target for the treatment of GBM (**Table 2**).

A small molecule inhibitor of the EphA3 receptor, GLPG1790, has demonstrated superior tumour reduction in U251MG and U87MG subcutaneous xenograft models when compared to radiotherapy alone, however, GLPG1790 was not as effective as treatment with radiotherapy and concomitant temozolomide (54). Whilst GLPG1790 did not exhibit improved benefit over the current therapies, additional strategies for targeting EphA3 are being examined. An EphA3 monoclonal antibody (IIIA4) that binds the EphA3 globular ephrin-binding domain has been developed, and the humanised version (ifabotuzumab) is the subject of an investigator-sponsored Phase 0/1 clinical trial currently underway in patients with recurrent GBM (55) to identify the optimal dose for tumour penetration. The IIIA4 antibody conjugated to the cytotoxic microtubule-targeting agent maytansine (IIIA4-USAN), induced apoptosis in four primary GBM cell lines *in vitro*, and significantly increased survival in an orthotopic model *in vivo* (55). Providing further evidence for the potential suitability of monoclonal antibodies targeting EphA3, a bispecific antibody against EphA2/A3 reduced clonogenicity *in vitro* and decreased tumour burden *in vivo* (56). Taken together, these studies indicate that EphA3 receptor inhibitors may be promising treatments for EphA3 receptor-amplified GBM, including recurrent disease, however, this remains to be tested in the clinic.

EGFR Inhibitors

Epidermal Growth Factor Receptor (EGFR) amplifications and mutations are detected in 40–60% of GBM cases (28, 96) (**Table 1**) and are generally indicative of poor prognosis (97). EGFR (also referred to as ERBB1 or HER1) is a member of the HER superfamily of RTKs, along with ERBB2, ERBB3, and ERBB4. Binding of a ligand to the ligand-binding site of these receptors induces receptor homo- or heterodimerisation, producing a conformational change that activates the intracellular tyrosine kinase domain. This results in autophosphorylation of the cytoplasmic tail and induces a variety of downstream signalling pathways. The overexpression or mutation of EGFR leads to downstream signalling that impairs apoptosis, enhances proliferation, and angiogenesis. The most common mutant form found in GBM is ΔEGFR (EGFRvIII, or de2-7EGFR), arising through an 801 base pair in-frame deletion from the extracellular domain (98). Due to the high incidence of EGFR amplifications, a variety of EGFR inhibitors have been examined both pre-clinically and clinically (**Table 2**).

Small Molecule Inhibitors

Small molecule tyrosine kinase inhibitors are the most widely studied EGFR inhibitors in GBM, and include erlotinib, gefitinib, and lapatinib. Erlotinib inhibits anchorage-independent growth of GBM cells *in vitro* in an EGFR expression-dependent manner and induces greater levels of apoptosis in more malignant GBM phenotypes (57). The tumour-initiating cell population, which is resistant to radiotherapy (99), is sensitive to erlotinib in a phosphatase and tensin homolog (PTEN) and Akt dependent manner (59), suggesting that erlotinib may eliminate this population *in vivo*. Further, treatment with erlotinib was shown to reduce tumour burden in two GBM patient-derived xenograft (PDX) models (60). However, further studies using additional GBM PDX models demonstrated that tumours overexpressing EGFR were only sensitive to erlotinib if they also expressed PTEN (61). As PTEN expression is downregulated in ∼34% of GBM patients (29), this indicates that erlotinib may not be a suitable treatment for the majority of GBM patients overexpressing EGFR. Indeed, erlotinib was not effective as a monotherapy in recurrent GBM patients and was only marginally beneficial following radiotherapy for non-progressive

TABLE 1 | Commonly identified genetic alterations in GBM.

Name	Function	Expression status	Prevalence	Prognosis	References
EPHA3	Regulation of adhesive and repulsive mechanisms including cell motility and adhesion	Overexpressed	40–60%	Poor; over-expression common in recurrent GBM	(25–27)
EGFR	Regulation of processes involved in cell growth, division and survival	Overexpressed	40–60%	Poor	(28–31)
MGMT	Prevention of mismatch errors	Methylated	40–60%	Favourable	(32, 33)
CDKN2A	Regulation of cell cycle and retinoblastoma activation	Decreased	49–52%	Poor	(34)
PTEN	Regulation of cell signalling. Involved in cell proliferation and survival	Deleted and/or mutated	34%	Poor	(29, 34–37)
PIK3CA	Regulation of processes involved in cell growth, division and survival	Overexpressed and/or mutated	15%	Poor; can predict recurrence	(34, 38, 39)
PDGFRA	Regulation of processes involved in cell growth, division and survival	Overexpressed	13%	Poor	(30, 34, 40, 41)
IDH1	Production of NADPH	Mutated	5–10%	Favourable	(42, 43)
MDM2	Regulation of p53 activity	Overexpressed	8–9%	Unclear	(44, 45)
MET	Regulation of proliferation, survival and motility	Overexpressed and/or mutated	4–6%	Poor	(34, 46–49)
SF/HGF	Activating ligand for HGFR/c-MET. Tumour growth and angiogenesis	Overexpressed	1.6–4%	Poor	(28, 47, 50)
VEGF	Promotion of angiogenesis	Overexpressed and/or mutated		Poor	(51–53)

EPHA3, ephrin type-A receptor 3; EGFR, epidermal growth factor receptor; MGMT, O-6-methylguanine-DNA methyltransferase; CDKN2A, cyclin dependent kinase inhibitor 2A; PTEN, phosphatase and tensin homolog; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; PDGFRA, platelet derived growth factor receptor alpha; IDH1, isocitrate dehydrogenase 1; MDM2, double minute 2 protein; MET, hepatocyte growth factor receptor; SF/HGF, scatter factor/hepatocyte growth factor; VEGF, vascular endothelial growth factor.

GBM patients (62). Despite a limited number of complete and partial responses in a Phase II study in first-relapse GBM, the 6-months PFS and median survival was similar to that previously reported for patients undergoing chemotherapy (64), suggesting that erlotinib may be useful in this setting. However, due to the non-randomised nature of this trial, these results must be interpreted cautiously. By contrast, improved survival (19.3 vs. 14.1 months) was observed when combined with temozolomide and radiotherapy (63), suggesting that erlotinib may be beneficial when combined with other treatments, rather than as a monotherapy.

In contrast to erlotinib, gefitinib exhibits anti-tumour activity independent of the expression level of EGFR (100). Gefitinib inhibits GBM cell migration (65), reduces proliferation of human glioma tumour-initiating cells *in vitro* (59) and enhances survival in an intracranial GBM mouse xenograft model *in vivo* (58). Taken together, these pre-clinical studies indicate that gefitinib may be clinically beneficial. However, despite gefitinib reaching high concentrations in GBM tumour tissue (22-fold higher compared to plasma) and the significant dephosphorylation of EGFR achieved (66), limited clinical effects have been observed in Phase II trials. Several Phase I/II studies have demonstrated that whilst the addition of gefitinib to radiotherapy is well-tolerated, it has no survival benefit (67, 68).

Monoclonal Antibodies

Although tumour immunotherapy has shown some success for the treatment of melanoma and haematological cancers, the applicability to GBM presents more of a challenge. Monoclonal antibodies directed against wild-type EGFR and ΔEGFR have been developed, with the best characterised in GBM being cetuximab. Pre-clinical studies have shown that treatment with cetuximab alone and in combination with radiotherapy increases survival *in vivo* (69) and can also completely eliminate tumours in EGFR-amplified PDX models (70). A phase II trial examining cetuximab treatment in patients with recurrent high-grade glioma showed that cetuximab was well-tolerated, but exhibited limited activity in this patient population (71).

VEGF Inhibitors

With the largely disappointing clinical results for EGFR inhibitors, additional targets are being investigated, including vascular endothelial growth factor (VEGF), which is highly expressed in glioma cells. High VEGF expression is directly associated with the poor prognosis and malignancy of gliomas (51–53, 101). VEGF is a dimeric polypeptide that binds to the VEGF receptors 1 (VEGFR-1) and VEGFR-2, and the co-receptors neuropilin 1 and 2. Following this interaction, VEGF mediates angiogenesis and cell proliferation. Under hypoxic conditions, hypoxia-inducible transcription factors translocate to the nucleus which activate *VEGF* leading to increased angiogenesis in an attempt to counteract hypoxia (102). GBM tumours are commonly hypoxic and have increased VEGF expression that contributes to the irregular vasculature in GBM, prompting the investigation of VEGF as a potential therapeutic target (**Table 2**).

Small Molecule Inhibitors

Several VEGF inhibitors have been examined for the treatment of GBM, including the small molecule inhibitors, tivozanib, and

TABLE 2 | Targeted therapeutic outcomes in GBM.

Target	Drug	Stage	Study design	Response	References
EphA3	GLP1790	*In vitro*	T98G, A172, U251MG, U87MG monolayer, neurospheres and clonogenic assay	Decreased proliferation of cell cultures and cancer stem cells	(54)
		In vivo	U87MG, U251MG, T98G subcutaneous xenografts−30 mg/kg/day orally GLP1790, one dose of 4 Gy radiotherapy, temozolomide 16 mg/kg for 5 consecutive days, or radiotherapy + temozolomide	Increased survival *in vivo* compared to radiotherapy alone, but not temozolomide or radiotherapy + temozolomide	
		In vivo	U87MG intracranial xenografts−30 mg/kg/day orally GLP1790, one dose of 4 Gy radiotherapy, temozolomide 32 mg/kg for 5 consecutive days	Increased survival *in vivo* compared to radiotherapy alone, but not temozolomide or radiotherapy + temozolomide	
	IIIA4-USAN	*In vitro*	Four primary GBM samples	Decreased cell viability	(55)
		In vivo	U251MG, two GBM patient samples intracranial xenografts−(10 mg/kg, twice weekly intravenously)	Increased survival *in vivo* compared to unlabelled IIIA4 and vehicle treatment	
	EPHA2/A3 BsAb	*In vitro*	BT241, BT972 neurosphere and clonogenicity assays	Inhibits clonogenicit	(56)
		In vivo	BT241 intracranial xenografts−intracranial treatment twice a week with 9.4 μl EPHA2/A3 BsAB	Non-significant reduction in tumour burden compared to IgG control	
EGFR	Erlotinib	*In vitro*	Nine GBM cell lines	Inhibits anchorage-independent growth and induces apoptosis	(57)
		In vitro	060919 and 020919 (GBM oncosphere lines)	No inhibition of growth	(58)
		In vitro	Tumour initiating cells isolated from seven GBM patient samples	Time and dose-dependent inhibition of cell proliferation in all cultures (except GBM2)	(59)
		In vivo	Mayo39 and Mayo59 subcutaneous xenografts−40 mg/kg crizotinib daily by gavage, 100 mg/kg erlotinib daily by gavage, or crizotinib + erlotinib	Recues tumour burden	(60)
		In vivo	Primary GBM (GBM12 and GBM14) intracranial xenograft−100 mg/kg or 150 mg/kg daily erlotinib by gavage	Enhanced survival in wild-type PTEN and EGFR amplified tumours; Tumours lacking PTEN exhibit no survival benefit	(61)
		Phase II	Recurrent malignant glioma (*n = 53*) and non-progressive GBM (*n = 43*) following radiation therapy−150 mg/day erlotinib	Single agent activity was minimal for recurrent gliomas and marginally beneficial following radiotherapy for non-progressive GBM	(62)
		Phase II	Newly diagnosed GBM (*n = 65*)−100 mg/day erlotinib with radiotherapy and temozolomide and 150 mg/day after radiotherapy, with erlotinib dose escalated after radiotherapy until patients developed tolerable grade 2 rash or until maximum allowed dose reached	Improved survival times compared to historical controls	(63)
		Phase II	First relapse GBM (*n = 48*)−150 mg of erlotinib	Overall response rate of 6.3%, 6-months progression free survival of 20%, median survival of 9.7 months	(64)
	Gefitinib	*In vitro*	Tumour cell migration in GBM organotypic slice cultures	Decreased tumour cell migration in EGFR-amplified tumours	(65)
		In vitro	020913 and 060919(GBM oncosphere lines)	Slight growth inhibition with gefitinib alone, enhanced with the addition of sunitinib; block of oncosphere regrowth following gefitinib and sunitinib co-treatment	(58)
		In vivo	020913 intracranial xenograft−75 mg/kg gefitinib, 15 mg/kg sunitinib, or gefitinib + sunitinib	Gefitinib alone increased survival compared to vehicle and sunitinib treated; Addition of sunitinib did not further increase survival	
		In vitro	Tumour initiating cells isolated from seven GBM patient samples	Time and dose-dependent inhibition of cell proliferation in all cultures (except GBM2)	(59)
		Phase II	Recurrent glioblastoma (*n = 22*)−500 mg gefitinib for 5 days prior to surgery, followed by post-operative gefitinib until recurrence	Median survival after initiation of gefitinib treatment was 8.8 months; no difference between patients with amplified or normal EGFR.	(66)
		Phase I/II	Newly diagnosed glioblastoma patients (phase I *n = 31*; Phase II *n = 147*)−daily oral gefitinib commenced at the time of conventional cranial radiotherapy and continued post-radiotherapy for 18 months or until progression	No overall survival benefit of the addition of gefitinib when compared to historical cohort of patients treated with radiotherapy alone	(67)
		Phase II	Newly diagnosed GBM (*n = 96*)−500 mg/day gefitinib	Addition of gefitinib produced no survival benefit when compared to historical cohort of patients treated with radiotherapy alone	(68)

(Continued)

TABLE 2 | Continued

Target	Drug	Stage	Study design	Response	References
	Cetuximab	*In vitro*	Primary GBM (Ros57, Jon52, Mor56)	Induced apoptosis as a monotherapy and when combined with radiotherapy	(69)
		In vivo	Ros57 subcutaneous xenograft−0.5 mg cetuximab intraperitoneally twice per week for 5 weeks, or cetuximab + 2 or 4 Gy radiotherapy	Arrest tumour growth (depended on size of tumour at treatment commencement)	
		In vivo	Ros57 or Jon52 intracranial xenografts−0.5 mg cetuximab intraperitoneally twice weekly for duration	Increased survival	
		In vitro	U373MG, U87MG, Ros57, Jon52, Mor56, Bai, Roc	Induced apoptosis in EGFR-amplified lines	(70)
		In vivo	Ros57, Jon52 subcutaneous xenograft−0.5 or 1 mg cetuximab intraperitoneally twice weekly	Decreased tumour burden and increased survival, and eliminated Ros57 tumours	
		Phase II	Recurrent high-grade glioma ($n = 55$)−400 mg/m^2 on week 1 cetuximab intravenously, followed by weekly dose of 250 mg/m^2	Well-tolerated but limited activity	(71)
VEGF	Bevacizumab	*In vivo*	G55 intracranial xenograft−10–100 μg intraperitoneally twice weekly	Decreased tumour growth and vessel density	(72)
		In vivo	U87MG intracranial and intradermal xenograft−98.4 μg intraperitoneally every third day	Reduced vessel permeability and tumour volume	(73)
		In vivo	U87MG subcutaneous xenograft−100 μg intraperitoneally every second day, six doses combined with radiotherapy	Decrease in tumour burden when used as a monotherapy, and at least an additive increase when combined with radiotherapy	(74)
		In vivo	U87MG intracerebral xenograft−1 mg intraperitoneal every third day, three doses	Decreased tumour growth	(75)
		Meta-analysis	Four clinical trials ($n = 607$)	No difference in overall survival, modest increase in progression-free survival when combined with chemotherapy, compared with bevacizumab or chemotherapy alone. Higher incidence of treatment-related adverse events in bevacizumab treated patients.	(76)
	Tivozanib	*Phase II*	Recurrent glioblastoma ($n = 10$)−1.5 mg tivozanib daily, 3 weeks on/1 week off in 28-days cycles	Despite functional changes in tumour vasculature, limited anti-tumour activity was observed	(77)
	Pazopanib	*Phase II*	Recurrent glioblastoma ($n = 35$)−800 mg daily on 4-weeks cycles	Despite demonstrating biological activity (determined by radiographic responses), single-agent pazopanib did not prolong progression free survival	(78)
PDGFR	Imatinib	*In vitro*	U251MG and SF539 cell lines	Gleevec sensitised GBM cells to irradiation	(79)
		In vivo	GL261 intracranial xenograft model−3 mg of Gleevec by gavage on days 5, 7, and 9, 3 Gy radiotherapy on Days 5–9, or Gleevec + radiotherapy	Gleevec monotherapy improved survival at a level similar to radiotherapy, the combination of Gleevec and radiotherapy significantly enhanced survival	(80)
		In vivo	U87MG intracranial xenograft−50 mg/kg intraperitoneally	Increased survival	(81)
		Phase II	Recurrent GBM ($n = 51$)−800 mg/d with dose escalation to 1,000 mg/d	Well-tolerated but has limited anti-tumour activity	(82)
		Phase III	Progressive pre-treated GBM resistant to standard dose temozolomide ($n = 240$)−600 mg/d imatinib in combination with 1,000 mg/d of hydroxyurea, or 1,500 mg/d of hydroxyurea alone	The addition of imatinib did not increase progression free survival	(83)
	Sunitinib	*In vitro*	U87MG, GL15 cells implanted into organotypic brain slices	Sunitinib induced apoptosis and decreased proliferation	(84)
		In vivo	U87MG intracerebral xenograft model−80 mg/kg sunitinib orally, 5 days on, 2 days off	Improved median survival and reduced microvessel density	
		In vivo	PDGF-driven mouse model (PDGF-RES-Cre retrovirus infection of adult glial progenitors in mice carrying conditional deletions of PTEN and p53)−60 mg/kg sunitinib gavaged daily on a 5 day on, 2 days off cycle, 2 or 6 Gy radiotherapy, or a combination of both	Sunitinib or high-dose radiotherapy alone delayed tumour growth and increased survival. The addition of sunitinib to low-dose radiotherapy delayed tumour growth, with no survival benefit. Sunitinib combined with high dose radiotherapy induced a fatal toxicity.	(85)
		Phase II	Recurrent GBM ($n = 6$)−37.5 mg/day sunitinib for 14 days	Overall response rate was 17%, and 6-months progression free survival was not reach. Trial terminated due to insufficient activity.	(86)

(Continued)

TABLE 2 | Continued

Target	Drug	Stage	Study design	Response	References
		Phase II	First-line treatment of patients with GBM ($n = 47$)−2 Gy/day, 75 mg/m^2 oral temozolomide daily, 6 months of maintenance temozolomide therapy with 150 mg/m^2 oral on Days 1–5 every 28 days, and sorafenib (400 mg twice daily orally)	Addition of sorafenib did not improve progression free survival when compared with standard therapy	(87)
PI3K Pathway Inhibitors	Buparlisib	*In vitro*	U87MG	Decreases cell growth	(88)
		In vivo	U87MG subcutaneous xenograft−30 or 60 mg/kg Buparlisib orally daily	Decreased tumour growth	
		In vitro	U373MG, LNZ308, U251MG, SNB19, LN751, LN428, U87-V111, U87-E, U343, LN229, U251-E, D54, U-251-V111, A172, U87-PTEN-V, LN18, U87-PTEN-E, T98G, U87MG, SF767	Dose-dependent growth inhibition, and differential sensitivity pattern with respect to p53 status (wild-type p53 more sensitive than mutant or p53 null)	(89)
		In vivo	U87MG intracranial xenograft−20 or 40 mg/kg buparlisib once per day on a 5 days on, 2 days off schedule for 4 weeks	Increased survival	
		Phase II	Recurrent glioblastoma ($n = 50$)−100 mg daily	Minimal effect on progression free and overall survival	(90)
	Sonolisib	*In vitro*	U251MG, U87MG, LN229, LN18	Whilst sonolisib did not induce apoptosis, it inhibited invasion and angiogenesis	(91)
		In vivo	U87MG intracranial xenografts−2 mg/kg sonolisib orally on a 5 days on, 2 days off schedule for 4 weeks	Increased the median survival time	
		In vitro	U87MG, LNZ308, LN229	Combination with BBR3610 resulted in synergistic killing	(92)
			U87MG intracranial xenograft−0.1 mg/kg BBR3610 intravenously once a week for 3 weeks, 2 mg/kg sonolisib orally three times a week for a total of 12 treatment, or combination of BBR3610 and sonolisib	Enhanced survival time	
		Phase II	Recurrent GBM ($n = 33$)−8 mg of sonolisib daily in 8 weeks cycles	Overall response rate was low	(39)
HGFR/MET inhibitors	SGX532	*In vitro*	U87MG, U373MG, A172, DAOY, GBM10 and glioma stem cells 1228−30 nM−1.5 μM SGX532, 1 h	Inhibition of tumour growth, invasion and migration	(93)
		In vivo	U87MG intracranial xenograft−50 mg/kg SGX532 every 12 h for 3 weeks	Decreased tumour growth	(93)
	Amuvatinib (MP470)	*In vitro*	SF763, SF268, SF295, SF126, SF188, SF767, U87MG and SF210−5–10 μM MP470 1 h prior to irradiation	Enhanced radiosensitivity	(94)
	Crizotinib	*In vivo*	Mayo39 and Mayo59 intracranial xenografts−40 mg/kg crizotinib daily for 7 continuous days by gavage	Inhibition of tumour growth and depletion of sphere-forming cells	(95)
		In vivo	Mayo39 and Mayo59 subcutaneous xenografts−40 mg/kg crizotinib daily by gavage, 100 mg/kg erlotinib daily by gavage, or crizotinib + erlotinib	Reduced tumour burden and vascular density (when given in combination with erlotinib)	(60)

pazopanib. Phase II studies of tivozanib (77) and pazopanib (78) in recurrent glioblastoma showed that these inhibitors exhibited limited anti-tumour activity and did not prolong PFS in this patient population. These trials highlight the limitations of anti-VEGF monotherapy.

Monoclonal Antibodies

Bevacizumab, a humanised monoclonal antibody against VEGF, blocks angiogenesis and thereby reduces tumour growth in a variety of GBM mouse models as a monotherapy and when combined with radiotherapy (72–75). These promising pre-clinical studies led to the clinical investigation and subsequent approval of bevacizumab for the treatment of recurrent GBM (103). However, a meta-analysis of four clinical trials including 607 patients demonstrated that the addition of bevacizumab to standard chemo-radiotherapy in the upfront setting only improves PFS, with no improvement in OS, but with an increase in the number of treatment-related adverse events (76). Of note, a decline in neurocognitive function is more frequently observed following bevacizumab treatment, as bevacizumab impairs hippocampal synaptic plasticity and decreases dendritic spine number and length (104). The modest treatment responses combined with the increased treatment-related adverse events raises concerns about the suitability of the use of bevacizumab as a treatment for GBM.

Inhibition of Multiple RTKs

Multiple RTKs are coactivated in GBM tumours (105), introducing redundancy and limiting the efficacy of therapies targeting single RTKs. EGFR and platelet derived growth factor

receptor A (PDGFRA) protein co-expression occurs in 37% of GBM (106). PDGFRA is the second most frequently amplified (10–13%) receptor tyrosine kinase in GBM (28, 107) (**Table 1**). A variety of multi-RTKs inhibitors have been examined both pre-clinically and clinically (**Table 2**).

Imatinib is a small molecule that inhibits PDGFRA and PDGFRB, as well as the RTKs c-Abl and c-Kit, and is a radiation-sensitising agent for glioma cells *in vitro* (79) and in orthotopic GBM models *in vivo* (80, 81). These promising pre-clinical studies led to the initiation of clinical trials. However, whilst imatinib was well-tolerated in recurrent GBM patients, it exhibited limited anti-tumour activity (82). Subsequent Phase III trials have examined imatinib in combination with hydroxyurea, rather than as a monotherapy (83). This trial showed that there was no PFS benefit to the addition of imatinib to hydroxyurea, or hydroxyurea alone. Further, a recent study has demonstrated that imatinib treatment can increase GBM cell migration and invasion *in vitro* (108), providing further potential insight as to why imatinib has failed in clinical trials for GBM.

Following the failure of imatinib in clinical trials, additional multi-RTK inhibitors have been studied. Sunitinib is an oral, small molecule that inhibits PDFR and VEGFR, and thereby reduces vascularisation and triggers apoptosis to produce tumour reduction. In pre-clinical models, sunitinib treatment induced apoptosis *in vitro*, and improved survival in an intracerebral GBM mouse model (84). Further, sunitinib treatment delayed tumour growth and increased survival in a PDGFF-driven mouse model, both as a monotherapy and in combination with low dose radiotherapy (85). By contrast, a Phase II trial and systematic review of the literature indicated that compared to conventional chemotherapy or bevacizumab, sunitinib has limited clinical activity in recurrent GBM as a monotherapy (86) or when combined with temozolomide or radiotherapy and temozolomide as a first-line treatment of patients with GBM (87).

PI3K Pathway Inhibitors

Genetic aberrations in GBM, including *EGFR*, *PDGFRA*, *PTEN*, *TP53*, and *PIK3CA*, drive the dysfunction of signalling pathways, including PI3K/Akt/mTOR, p53, and Rb1 (34). The PI3K/Akt signalling pathway is activated in most GBMs and plays a critical role in the regulation of signal transduction, and mediates a variety of cellular processes, including proliferation, survival, migration and angiogenesis in GBM. The PI3K/Akt pathway is typically initiated via the activation of RTKs or G protein-coupled receptors, were the conformational changes in the C-terminal kinase domain produced by autophosphorylation provides binding sites for the regulatory subunits of PI3K, which leads to elevated lipid kinase activity of PI3K, and activation of Akt. Despite the limited clinical efficacy of the previously described RTK inhibitors, as activation of each of these receptors leads to downstream activation of the PI3K/Akt pathway, it has therefore been suggested that PI3K pathway inhibitors may be beneficial in GBM. More than 50 PI3K inhibitors have been designed and are under investigation as treatments for a range of cancers. Several PI3K inhibitors have demonstrated pre-clinical efficacy in GBM (**Table 2**) and have entered into clinical trials for GBM treatment.

Buparlisib, a pan PI3K inhibitor, reduces GBM cell growth both *in vitro* and *in vivo* (88, 89). Buparlisib is the most frequently used PI3K inhibitor in clinical trials for GBM treatment, as it is well-tolerated and BBB permeable. However, single agent efficacy in Phase II trials in recurrent glioblastoma has been minimal (90). The lack of clinical efficacy was explained by incomplete blockade of the PI3K pathway in the tumour tissue. Whilst buparlisib showed minimal single-agent efficacy, the study of other PI3K inhibitors that achieve more-complete pathway inhibition may still be warranted.

Sonolisib is an irreversible wortmannin analogue that demonstrates a more persistent inhibitor effect on PI3K than wortmannin. Sonolisib inhibits invasion and angiogenesis in GBM cell lines *in vitro* and extends survival benefit in orthotopic xenograft models *in vivo* (91, 92). Despite these promising pre-clinical results, the response rate to sonolisib in a Phase II study in patients with recurrent GBM was low, and the study did not meet its primary endpoint (109).

HGFR/MET Inhibitors

Brain tumours secrete scatter factor (SF)/hepatocyte growth factor (HGF), the activating ligand for HGFR/MET (50), which has been associated with poor prognosis for GBM patients (**Table 1**) (110, 111). HGF is overexpressed in 1.6–4% of GBM patients, and via activation of MET, enhances tumour growth and angiogenesis (28). The *MET* proto-oncogene encodes for MET, an RTK that is overexpressed in 4–6% of GBM patients. A mutated, constitutively active variant of MET, $MET^{\Delta 7-8}$, has been identified in 6% of patients with high grade gliomas, including GBM, enhancing downstream signalling to promote tumour progression and angiogenesis (46).

SGX-523 is a small molecule inhibitor of HGFR/MET tyrosine kinase activity that inhibits tumour cell growth, migration and invasion in a panel of glioma cells *in vitro* and reduced tumour growth in a murine xenograft model of GBM using the U87MG GBM cell line (**Table 2**) (93). Additionally, amuvatinib (MP470) is a small molecule inhibitor that acts on multiple tyrosine kinases, including MET, has been shown to radiosensitise GBM cell lines both *in vitro* and *in vivo* (**Table 2**) (94).

Another small molecule inhibitor of MET kinase activity, crizotinib, inhibits the growth, sphere-forming capacity and expression of stem cell markers in a subcutaneous xenograft model of GBM using the U87MG cell line (95). However, in a subcutaneous xenograft model using Mayo39 and Mayo59 GBM cell lines, crizotinib was only effective at reducing tumour burden and vascular density when used in combination with the EGFR inhibitor erlotinib (60).

CONCLUDING REMARKS

GBM is an often-fatal disease and the standard treatment options available to patients are only minimally effective. There is a growing body of evidence suggesting that a personalised therapeutic approach for the stratification of GBM patients to novel treatment regimens is necessary if survival rates for GBM patients are to improve. Indeed, genetic profiling of GBM biopsies has revealed aberrant expression of several potential

therapeutic targets, including a number of RTKs (EphA3, EGFR, VEGF, PDGFR, and MET), however, there has been varied and limited clinical success in the use of inhibitors of these targets as anti-cancer therapies. This highlights that a better understanding of the basic biology of GBM is required so that additional targets can be identified. Indeed, promising pre-clinical effects have been observed with EphA3 inhibitors, however, it remains to be seen whether this translates into the clinic. Whilst PI3K inhibitors have exhibited limited effects in clinical trials, they did not completely inhibit the PI3K pathway. Despite the promising outlook for personalised therapeutic approaches to treating GBM patients, the identification of therapeutics that can cross the BBB, whilst maintaining therapeutic concentrations, remains a challenge and is often not reported. Further, although targeted therapies show limited efficacy as single agents, the combination of several targeted therapies may be of benefit to GBM patients. Thus, additional research is urgently required to identify therapeutic targets in GBM and to design novel therapeutic strategies for the treatment of GBM.

AUTHOR CONTRIBUTIONS

OT and JB drafted the manuscript and reviewed the literature. OT, JB, and KS contributed to the preparation and editing of the manuscript. KS formulated the idea.

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