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Deregulated Signaling Pathways in Glioblastoma Multiforme: Molecular Mechanisms and Therapeutic Targets

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Abstract

Glioblastoma multiforme (GBM) is the most malignant and aggressive type of brain tumor with an average life expectancy of less than 15 months. This is mostly due to the highly mutated genome of GBM, which is characterized by the deregulation of many key signaling pathways involving growth, proliferation, survival, and apoptosis. It is critical to explore novel diagnostic and therapeutic strategies that target these pathways to improve the treatment of malignant glioma in the future. This review summarizes the most common and important pathways that are highly mutated or deregulated in GBM and discusses potential therapeutic strategies targeting these pathways.

Keywords

Signal transduction; Therapy; GBM

INTRODUCTION

Gliomas are the most common type of primary brain tumor, and are histologically differentiated as astrocytomas, oligodendrogliomas, and ependymomas. The World Health Organization (WHO) classifies central nervous system tumors into four different grades: I, II, III and IV (1, 2). Grade IV glioblastoma multiforme (GBM) is the most frequent, devastating, and malignant astrocytic glioma. It is characterized by a high degree of cellularity, vascular proliferation, tumor cell chemoresistance, and necrosis (3, 4). Even after neurosurgical resection, followed by aggressive chemotherapy and radiotherapy, GBM is still considered an incurable malignancy (5). No effective treatment agent against GBM has been identified; thus, the median life expectancy of patients diagnosed with GBMs is about 14 months (3). Of all primary tumors, brain tumors constitute ~2% and the annual global incidence rate of it is about seven per 100,000 individuals (6). In the USA, the overall incidence rate of primary brain tumors is 18.1 per 100,000 individuals. The overall prevalence rate was estimated to be 209.0 per 100,000 in 2004 and 221.8 per 100,000 in 2010 in the USA (7). Although the mechanisms of GBM onset and progression remain largely unknown, recent advances in the understanding of the signaling pathways that underlie GBM pathogenesis have led to the development of new therapeutic approaches

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targeting multiple oncogenic signaling aberrations associated with GBM. This review describes the signaling pathways that are physiologically and pathologically relevant to GBM and discusses some of the most promising therapeutic approaches attempting to target these pathways.

p53 SIGNALING PATHWAY IN GBM

The p53 pathway is the most commonly mutated pathway in tumorigenesis. A well-known tumor suppressor, p53 responds to DNA damage and various genotoxic and cytotoxic stresses by inducing cell cycle arrest and apoptosis. It not only plays a crucial role in tumor suppression, but is also a broad transcription factor. It regulates more than 2,500 genes (6, 8), most of which are involved in tumor development, tumorigenesis, and tumor invasion. GBMs are divided into primary and secondary subtypes. Primary GBMs develop quickly and robustly while secondary GBMs develop progressively from low-grade astrocytoma. p53 mutations play a particularly significant role in the development of secondary GBMs and are often the earliest detectable genetic alteration in primary brain tumors, as they are present in ~65% of precursor low-grade diffuse astrocytomas. Mutations in the p53 pathway are also detected in primary gliomas, although less frequently (1, 9). Mutations in the p53 pathway occur through different mechanisms in primary versus secondary GBM. A majority of p53 mutations in secondary GBM have been shown to be located at two key codons 248 and 273; however, p53 mutations are more widely distributed in primary GBMs. These results suggest that p53 mutations in primary GBM arise as a secondary event due to general genomic instability of the GBM tumor microenvironment (9, 10).

MDM2 is an E3 ubiquitin ligase, which is also an important regulator of the p53 pathway. It negatively regulates p53 in two ways: transcriptional inhibition by direct binding, and degradation through its E3 ligase activity (11–13). Amplification of MDM2 occurred in about 10% of all malignant GBMs, but only in tumors without a p53 mutation, indicating that MDM2 overexpression may provide alternative means for tumors to inactivate p53-regulated growth control without having to alter p53 itself (14). Similarly, MDM4 (MDMX), encoded by 1q32, is another important regulator of p53. Due to its overexpression in malignant gliomas (15, 16), MDM4 has been extensively studied to elucidate its potential therapeutic effects. Whether the amplification of MDM4 causes p53-dependent or independent human malignant glioma remains unclear, but one previous report suggested that overexpression of MDM4 could have possibly caused GBM via a different p53-independent growth control pathway (15), similar to that of MDM2.

The tumor suppressor protein ARF ($p14^{ARF}$) is one of the upstream regulators of the p53 pathway. ARF regulates the transcriptional activities of p53 by directly binding to MDM2 and subsequently inhibiting its E3 ubiquitin ligase activity (17, 18). Inactivation or mutation of ARF was commonly found in both low-grade and high-grade gliomas (19). ARF and INK4a ($p16^{INK4A}$), both encoded by the CDK2A locus, are crucial regulators of the key growth control pathways p53 and pRB, respectively. One of the most frequent mutations found in GBMs is the homozygous deletion of this locus ($p16^{INK4a}/p14^{ARF}/p15^{INK4b}$) (20). Furthermore, in mouse model studies and analyses of human glioma samples, Labuhn *et al.* found that the co-deletion of ARF and INK4a increased accordingly with tumor progression from low- to high-grade gliomas (21). This finding suggests that the locus containing ARF and INK4a is a key contributor to GBM pathogenesis, and that deletion of this locus may be a fundamental event in the development of GBM (20).

Chromosomes 9p, 10q23.3, and 10q 25–26 are the most frequently mutated or deleted regions in human gliomas. These chromosomes encode several critical regulators of cell survival, growth, and apoptosis, including CDK2A, CDK2B, ARF, MDM2, EGFR, and

PTEN. Overall, the p53 pathway is disrupted in approximately 80% of high-grade (WHO Grades III and IV) gliomas. Additionally, EGFR overexpression and PTEN mutations are also commonly found in high-grade gliomas (22, 23). As the most commonly mutated pathway in tumorigenesis, p53 represents a viable therapeutic target for the treatment of GBM. Increasing wild type p53 expression in tumor cells through the use of adenovirus-mediated p53 gene therapy is one possible treatment modality to overcome p53 mutation. In a phase I trial, Lang and colleagues (24) showed that intratumoral injection of a p53-containing adenovirus vector resulted in the transfer of the p53 gene and expression of functional exogenous p53 in all patients. However, transfected cells were only found within a short distance from the injection site, indicating no systemic viral dissemination. Two phase I trials evaluating the transduction efficiency and effectiveness of wild-type Ad5CMV-p53 gene therapy (NCT00004041) or recombinant adenovirus-p53 SCH-58500 (NCT00004080) in combination with surgery have also been completed (February 2009, data not published yet).

pRB SIGNALING PATHWAY IN GBM

The tumor suppressor retinoblastoma (pRB) plays a crucial role in inhibiting cell cycle progression by binding and inhibiting transcription factors of the E2F family. It is encoded by the RB gene, which is regulated by the complex of cyclin-dependent kinases (CDKs). The RB gene is located at 13q14.1-q14.2, which has been shown to be involved in the malignant progression of astrocytomas (25). Mutations in this area are detected in more than 20% of high-grade gliomas. The loss of pRB expression has been detected in GBM (26), and the loss of 13q was also associated with the transition from low- to intermediate-grade gliomas (25, 27). In the G1 phase, pRB is normally inactivated by Cyclin D/CDK4/CDK6-induced phosphorylation, which leads to the release of pRB from E2F and the subsequent stimulation of cell progression into the S phase. CDKN2B, which is a CDK inhibitor and is commonly inactivated in GBM, forms a complex with CDK4 or CDK6, thus preventing the activation of CDKs. The downstream effect of this inhibition is to prevent cell growth and the cell cycle through G1 progression by maintaining the activation state of pRB. In addition to the inactivation of CDKN2B, amplification of CDK4 and CDK6 in glioblastomas is also common, demonstrating that both CDK4 and CDK6 play pivotal roles in astrocytic tumorigenesis and glioma progression (28).

Since the pRB pathway is inactivated by the kinase activity of the CDK4/CDK6/Cyclin D complex, inhibition of CDK4/6 may be a novel chemotherapeutic treatment strategy in GBM patients with aberrantly expressed pRB. A phase II study (NCT01227434) is currently recruiting patients to determine the efficacy of PD 0332991, a novel small molecule inhibitor of CDK4 and CDK6, in patients with recurrent Rb-positive glioblastoma. Overall, the average pathway amplification rates in GBM are: EGFR 35.7%, GLI/CDK 4–13.4%, MDM 2–9.2%, and PIK3C2B/MDM 4–7.7%. The CDKN2A/ CDKN2B locus was deleted in 46.4% of the combined cases (16). Even though other groups have found different frequency rates for these gene mutations, the pRB and p53 pathways consistently appear to be among the most frequently mutated pathways in GBM. Therefore, treatment strategies targeting these signaling pathways may possess broad therapeutic potential in GBM.

PI3K–PTEN-Akt-mTOR Signaling Pathway In GBM

The PI3K-PTEN-Akt-mTOR pathway regulates normal cellular functions that can also be critical in tumorigenesis, including cellular proliferation, apoptosis, cell invasion, and mobility. Activation of phosphatidylinositol 3-kinase (PI3K) complex, the first intracellular member of this signaling pathway, is regulated by many growth factors in conjunction with their receptors, such as epidermal growth factor (EGF) and its receptor (EGFR). Activated

PI3K generates phosphatidylinositol-3,4,5-triphosphate, which in turn activates Akt. Akt then activates mTOR, which integrates several upstream signals into effector actions on multiple downstream targets involved in cell growth and division (29). Tumor suppressors such as PTEN inhibit this pathway.

The PI3K complex links growth factors with cellular proliferation, differentiation, metabolism, and survival. Constitutive activation and mutation of the PI3K signaling pathway play crucial roles in the development of GBM (30–32). The PI3K pathway is altered in about 70% of GBMs, either by deletion of PTEN or amplification of EGFR and/or vascular endothelial growth factor receptor (VEGFR)/platelet-derived growth factor receptor (PDGFR) alpha (33). Overexpression of EGFR is one of the most frequent signaling mutations in GBM, and its amplification leads to increased activation of the PI3K pathway (34, 35). EGFRvIII, an EGF ligand-independent mutant with the deletion of exons 2–7 of the EGFR gene, is another common mutation in GBM (36) and is a significant predictor of poor prognosis (37). The average amplification rate for EGFR in GBM is about 35% (16) and the rate for the EGFRvIII mutant is approximately 40% (6). Additionally, a close correlation exists between amplification of wild-type EGFR and over-expression of EGFRvIII mutant (37). Taken together, these data suggest that EGFR and EGFRvIII are viable targets for therapeutic approaches to GBMs (38).

mTOR (mammalian target of rapamycin) is composed of two parts, mTOR complexes 1 (mTORC1) and 2 (mTORC2), and acts both as a downstream effector and upstream regulator of PI3K, thus playing an important role in GBM tumorigenesis (32). mTORC1 promotes oncogenesis by driving the translation of oncogenes, inhibiting autophagy, upregulating hypoxia-inducible factor 1α to promote angiogenesis, and stimulating lipid uptake via activation of sterol regulatory element binding protein 1c (SREBP1c). Similarly, mTORC2 promotes cancer growth by stimulating glucose uptake via activation of Akt and activating serum/glucocorticoid regulated kinase (SGK), which assists in proliferation and survival (29). Previous reports demonstrated that activation of the mitogenic kinase pathway (MAPK) involves a feedback mechanism between mTOR and PI3K, supporting the argument for combined inhibitors in GBM treatment (39).

PTEN (phosphatase and tensin homologue, located on chromosome TEN) acts as a tumor suppressor gene, and mutations of PTEN lead to the development of many cancers including GBM. PTEN negatively regulates the PI3K/AKT/PKB pathway (40) by blocking Akt signaling via the reduction of intracellular levels of phosphatidylinositol-3,4,5-triphosphate. Thus, PTEN plays a critical role in regulating its downstream target genes including mTOR. Loss of heterozygosity of chromosome 10, which causes deletions or mutations of PTEN, is a common event in GBMs. It is likely that induced expression of functional PTEN may serve as a therapeutic strategy in GBMs with amplified or mutated EGFR-PI3K-Akt-mTOR pathways.

RAS/MAPK SIGNALING PATHWAY IN GBM

Human RAS genes (Rat Sarcoma) are transforming oncogenes, including three highly related genes called H-Ras, N-Ras, and K-Ras. RAS belongs to the G protein family; therefore, the activation and deactivation of RAS is controlled by its binding to guanosine triphosphate (GTP) or guanosine diphosphate (GDP), respectively (41, 42). Activated RAS further activates RAF kinase through direct binding, which then regulates downstream signaling pathways such as the mitogen-activated protein kinase (MAPK) pathway (43, 44). RAS also regulates the activities of other pathways including the PI3K pathway; consequently, RAS plays a pivotal role in regulating cell proliferation, differentiation, signal transduction, apoptosis, and tumorigenesis.

Growth factor receptors, such as EGFRs, PDGFRs, and receptor tyrosine kinases (RTKs) (45) have an additional role in regulating the activities of RAS through direct associations. Upon activation, RAS then activates RAF and phosphorylates a series of kinases including MEK and MAPK, through which RAS can regulate downstream target gene transcription and cell activities. Aberrant expression or defects in the RAS/MAPK pathway causes abnormal cell growth and proliferation, inducing other abnormal cellular activities such as invasion and apoptosis.

Many cancers have exhibited a defective RAS/MAPK pathway. In high-grade astrocytoma and GBM, the over-expression of RAS was detected in multiple cases (46–48). Besides EGFR, other growth factor receptors that regulate RAS such as PDGFR or RTKs are also overexpressed, leading to the implication of a positive correlation between expression of PDGFR and RTKs and glioma pathogenesis. It is likely that the abnormal activation of the RAS/MAPK pathway could be a potential target for glioma treatment therapy.

STAT3 and ZIP4 SIGNALING IN GBM

STAT protein complexes (Signal transducers and activators of transcription) are a family of cytoplasmic proteins with SH2 (Src Homology-2) domains that act as transcription factors. STAT proteins regulate cellular responses to cytokines and growth factors by transducing signals from the plasma membrane to the nucleus (49), thus activating transcription of target genes and regulating cell activities such as proliferation, invasion, and apoptosis. STAT3 is one of the STAT family proteins that can be activated by EGF, and it is upregulated in multiple tumors including GBM (50). Past studies have indicated that STAT3 is a significant player in the development of neural stem cells and astrocytes (51–53). Interestingly, STAT3 was once thought to possess only oncogenic properties, but it was recently found to function in opposing tumor suppressive or oncogenic roles in GBM, depending on the genetic profile of the tumor (54).

Zinc is an essential trace element and catalytic/structural component used by many metalloenzymes and zinc-dependent transcription factors. Recent studies indicate a possible correlation between zinc levels and cancer risk. Our previous studies have indicated that the zinc transporter, ZIP4, is overexpressed in human pancreatic cancer (55). Overexpressed ZIP4 also increases cell proliferation and zinc uptake of pancreatic cancer cells, indicating that aberrant ZIP4 upregulation may contribute to cancer pathogenesis and progression. While investigating the signal transduction pathway through which ZIP4 regulates cancer growth (56), our lab found that overexpression of ZIP4 increases IL-6 transcription through cAMP response element-binding (CREB), which in turn activates STAT3 and leads to increased cyclin D1 expression. This ultimately results in increased cell proliferation and tumor progression. Our recent data suggest that ZIP4 is overexpressed in GBM, and correlates with the overall survival GBM patients. Therefore, ZIP4 may modulate gliomagenesis and malignant gliomas by regulating the activity of STAT3 and its related molecules, making ZIP4 and STAT3 interesting therapeutic targets in controlling glioma malignancy and invasion.

TREATMENT OF GLIOBLASTOMA MULTIFORME

Despite a better understanding of the mechanisms that underlie the development of GBM gained in the last several decades, GBM treatment still lacks efficacy. The current standard of care for GBM includes surgical resection, followed by chemotherapy (mainly temozolomide (TMZ), a DNA alkylating agent) and/or radiation therapy. At present, the average survival rate for GBM is less than 15 months. Improving therapeutic efficacy remains a significant challenge in GBM treatment, and targeting the previously mentioned

signaling pathways may enhance current GBM therapies and positively impact patient outcomes (Table 1).

TMZ

Temozolomide (TMZ), an oral alkylating and chemotherapeutic agent, was first used to treat primary brain tumors in 1993 (57) and became a major agent for treating primary brain tumors following surgical resection and radiotherapy. It alkylates or methylates DNA, causing cancer cells to die. O-6-methylguanine-DNA methyltransferase (MGMT) or O-6-alkylguanine-DNA alkyltransferase can diminish the effect of TMZ by repairing the DNA damage (58).

Previous studies have shown that GBMs are highly resistant to single inhibitor, suggesting that combinational strategies involving standard chemotherapies like TMZ and pathway inhibitors might be a possible future direction for treating GBM. Recently, TMZ has been combined with other inhibitors in many phase I and II clinical trials to try to improve the drug's therapeutic efficacy. Temozolimide together with the EGFR inhibitor, erlotinib, and radiotherapy have recently been reported to improve patient survival time from 14.1 months to 19.3 months (59). The study showed PTEN expression correlates well with survival rates, suggesting that erlotinib regulates the PTEN/mTOR pathway. MGMT promotes methylation, which also correlates well with survival rates, suggesting a synergistic function between TMZ and erlotinib.

EGFR

As mentioned above, EGFRs are overexpressed in multiple GBM cases and play a significant role in regulating other intracellular signaling pathways that contribute to GBM pathogenesis including mTOR/PI3K/Akt and RAS/MAPK. Therefore, inhibiting EGFR may be a possible therapeutic strategy for GBM treatment. Many EGFR inhibitors, such as erlotinib, lapatinib, and gefitinib have been evaluated as single-agent therapies in phase I or phase II trials (60–63), but these drugs had minimal activity in GBM patients and did not improve overall survival or progression-free survival. However, a phase II trial recently showed that vaccinating patients who possessed the EGFRvIII variant, one of the most frequent mutations in GBM, using an EGFRvIII-targeted peptide significantly prolonged progression-free survival (14.2 vs. 6.3 months) and overall survival (26 vs. 15 months) compared to a contemporaneously treated patient cohort matched for EGFRvIII expression and past treatment (64). A randomized, controlled phase III trial to thoroughly evaluate the vaccine is currently in the planning stages.

mTOR-PI3K-Akt-PTEN

Several mTOR-PI3K-Akt-PTEN signaling pathway inhibitors have been developed in the past decade. Among the most widely tested are mTOR inhibitors, including rapamycin and its intravenous and oral derivatives temosirolimus and everolimus, respectively. These drugs inhibit mTOR through the formation of a complex with FK-binding protein 12 (FKBP-12), which then binds to mTOR, preventing its activation and inhibiting tumor cell growth (65). Multiple studies have been conducted using each of these drugs, alone or in combination with other therapies, but results have been limited (66–70). Numerous clinical trials are ongoing to further elucidate the effects of these mTOR inhibitors. Additionally, a phase I study (NCT00704080) is currently recruiting patients to evaluate the preliminary efficacy of XL765, a novel small molecule inhibitor of mTOR and PI3K, in combination with TMZ.

Vascular endothelial growth factor receptors (VEGFRs) and platelet-derived growth factor receptors (PDGFRs) contribute to the growth potential of tumors by activating the PI3K/Akt

pathway (71, 72) and are possible targets for GBM treatment. Bevacizumab (Avastin), a vascular endothelial growth factor (VEGF) monoclonal antibody, was recently found to be effective for recurrent malignant glioma and inhibit angiogenesis by blocking VEGF-A (73). Aberrant vascular proliferation, necrosis, and infiltration of surrounding brain tissues are considered “hallmarks” of GBM. Blocking aberrant vascular proliferation by bevacizumab therapy could block the infiltration of high-grade gliomas. Recently, several groups reported the clinical trial results of bevacizumab alone or combined with different inhibitors such as carboplatin (a DNA alkylating reagent) (74) and erlotinib for treating GBM (75–78). Bevacizumab reduced tumor size and prolonged progression-free survival (PFS) even though the overall survival rates remained unchanged.

Cediranib is another VEGFR inhibitor with anti-cancer potential. In a phase II study (79), cediranib monotherapy in patients with recurrent GBM resulted in a radiographic response proportion, progression-free survival, and overall survival that compared favorably with data from historical controls. A phase III, randomized trial (NCT00777153) is currently ongoing to assess the efficacy of cediranib in patients with recurrent GBM as a single-agent and in combination with lomustine, a DNA alkylating drug primarily used in brain tumor treatment. Other VEGFR/PDGFR inhibitors (80, 81) such as sorafenib (82), imatinib (83, 84), pazopanib (85), and vatalanib (86) have shown no significant clinical benefit due to their poor ability to penetrate the blood-brain barrier, which is an important cause of the high resistance of glioblastoma to chemotherapies. Several phase I and II trials are ongoing to further evaluate the efficacy of bevacizumab, cediranib, and other VEGFR/PDGFR inhibitors in the treatment of GBM.

Akt is another viable molecular target within the mTOR-PI3K-Akt-PTEN pathway, since high activity of Akt in GBM leads to increased cell survival and decreased apoptosis. Enzastaurin is a novel antineoplastic agent that acts on Akt by inhibiting protein kinase C-beta, which regulates Akt activation (87). However, enzastaurin was recently shown to possess no superior efficacy compared with lomustine in a phase III trial involving patients with recurrent GBM (88). Two recently developed Akt inhibitors, nelfinavir and perifosine, interfere with Akt by downregulating its phosphorylation (80, 81) and are being assessed in several ongoing phase I and II trials (NCT01020292, NCT00915694, NCT00590954).

RAS/MAPK

Constitutively activated RAS protein is one of the most common mutations involved in GBM tumorigenesis, and is a prime target for GBM chemotherapy. A recently completed phase I/II trial (NCT00050986) was conducted to evaluate the safety and efficacy of Zarnestra (tipifarnib), a farnesylation inhibitor that interferes with the post-translational processing of RAS, in combination with TMZ. Oncolytic viral vectors also target RAS mutation by selectively replicating in and killing cancer cells with an activated Ras pathway (89). In a phase I trial (90), Forsyth and colleagues showed that intratumoral administration of reovirus, an oncolytic virus, in patients with recurrent malignant gliomas led to a median survival of 21 weeks (range 6–234) and median time-to-progression of 4.3 weeks (range 2.6–39). Another phase I/II study (NCT00528684) evaluating an engineered reovirus (Reolysin, Oncolytics Biotech) in patients with malignant glioma was recently completed, but results have not been published.

CONCLUSION

The combination of inhibitors of multiple pathways and other therapies is considered one of many future directions for GBM treatment. In this review, we have described the major pathways that are highly mutated or deregulated in GBM as well as some newly developed

therapies targeting those pathways. With the knowledge that we have learned from the human tumor biology, we believe that generating GBM mouse models, which mimic human GBM, could provide insight into potentially active drug combinations for future treatment. Inhibitors plus shRNA or small molecules that block/activate major pathways can be tested by using these GBM models, which will help us to elucidate the mechanisms that underlie the tumorigenesis and progression of GBM and eventually discover effective therapies for treating GBM.

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Table 1

Current clinical trials targeting key aberrant signaling pathways in GBM

Pathway	Target	Therapy	Trial specifications	Outcomes	Reference
p53	p53	Ad-53 adenovirus	Phase I (15 patients with recurrent GBM)	Exogenous p53 detected within tumor; no systemic dissemination	Lang *et al.* 2003 (24)
	p53	Ad5CMV-p53	Phase I (patients with recurrent GBM)	Completed; not published (NCT00004041)	
	p53	Ad-p53 SCH-58500	Phase I (patients with recurrent GBM or AA)	Completed; not published (NCT00004080)	
pRB	CDK4/6	PD 0332991	Phase II (patients with recurrent pRB-positive GBM)	Recruiting (NCT01227434)	
PI3K–mTOR-Akt-PTEN	EGFR	Erlotinib + RT + TMZ	Phase II (65 patients with newly diagnosed GBM vs. historical control)	Median OS 19.3 vs. 14.1 months 1 ($p = 0.01$); Median PFS 8.2 vs. 4.9 months ($p = 0.001$)	Prados *et al.* 2009 (59)
	EGFR	Gefitinib	Phase II (98 patients with newly diagnosed GBM vs. historical control)	OS-1 yr 54.2% vs. 48.9% ($p > 0.05$); PFS-1 yr 16.7% vs. 30.3% ($p > 0.05$)	Uhm *et al.* 2011 (63)
	EGFRvIII	EGFRvIII vaccination	Phase II (21 patients with EGFRvIII-expressing newly diagnosed GBM)	PFS-6 67%; Median PFS 14.2 vs. 6.3 months in control group ($p = 0.041$); Median OS 26.0 vs. 15.0 months ($p = 0.001$)	Heimberger et al. 2009 (91)
	mTOR	Temsirolimus	Phase II (65 patients with recurrent GBM)	Median PFS 2.3 months; Median OS 4.4 months	Galanis *et al.* 2005 (68)
	mTOR + PI3K	XL765 + TMZ	Phase I (patients with AA or GBM)	Recruiting (NCT00704080)	
	VEGFR	Bevacizumab	Phase II (50 patients with recurrent GBM)	PFS-6 25%; Median OS 25.6 weeks	Raizer *et al.* 2010(78)
	VEGFR	Cediranib	Phase II (31 patients with recurrent GBM vs. historical control)	PFS-6 25.8% vs. 15%; Median PFS 117 vs. 63 days; Median OS 227 vs. 175 days	Batchelor *et al.* 2010(79)
	VEGFR + PDGFR	Sorafenib + TMZ	Phase II (32 patients with recurrent GBM)	PFS-6 9.4%	Reardon *et al.* 2011 (82)
	VEGFR + PDGFR	Pazopanib	Phase II (35 patients with recurrent GBM)	Median PFS 12 weeks; Median OS 35 weeks	Iwamoto 2010 (92)
	PDGFR	Imatinib + hydroxyurea	Phase II (231 patients with recurrent GBM)	PFS-6 10.6%; Median OS 26.0 weeks	Reardon *et al.* 2009 (84)
	Akt	Enzastaurin	Phase III (266 patients with recurrent GBM)	PFS 1.5 vs. 1.6 months in lomustine control; OS 6.6 vs. 7.1 months)	Wick *et al.* 2010(88)
	Akt	Nelfinavir + RT + TMZ	Phase I	Recruiting (NCT01020292)	
	Akt	Perifosine	Phase II	Ongoing (NCT00590954)	

Pathway	Target	Therapy	Trial specifications	Outcomes	Reference
RAS	RAS	Reovirus	Phase I (12 patients with recurrent GBM)	Median PFS 4.3 weeks; Median OS 21 weeks	Forsyth *et al.* 2008 (90)
	RAS	Reolysin	Phase I/II	Completed; not published (NCT00528684)	
	RAS	Zarnestra + TMZ	Phase I/II	Completed; not published (NCT00050986)	

The majority of clinical trials involving therapies targeting commonly deregulated signaling pathways in GBM have shown little to no clinical efficacy.

Abbreviations: AA, WHO Grade III anaplastic astrocytoma; GBM, WHO Grade IV glioblastoma multiforme; OS, Overall survival; OS-1 yr, Overall survival one year after initiation of treatment; PFS, Progression-free survival; PFS-6, Progression-free survival six months after initiation of treatment; RT, Radiation therapy; TMZ, Temozolomide.