Glioblastoma multiforme: State of the art and future therapeutics

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Abstract

Background: Glioblastoma multiforme (GBM) is the most common and lethal primary malignancy of the central nervous system (CNS). Despite the proven benefit of surgical resection and aggressive treatment with chemo- and radiotherapy, the prognosis remains very poor. Recent advances of our understanding of the biology and pathophysiology of GBM have allowed the development of a wide array of novel therapeutic approaches, which have been developed. These novel approaches include molecularly targeted therapies, immunotherapies, and gene therapy.

Methods: We offer a brief review of the current standard of care, and a survey of novel therapeutic approaches for treatment of GBM.

Results: Despite promising results in preclinical trials, many of these therapies have demonstrated limited therapeutic efficacy in human clinical trials. Thus, although survival of patients with GBM continues to slowly improve, treatment of GBM remains extremely challenging.

Conclusion: Continued research and development of targeted therapies, based on a detailed understanding of molecular pathogenesis can reasonably be expected to yield improved outcomes for patients with GBM.

Key Words: Glioblastoma multiforme, gene therapy, immunotherapy, molecularly targeted therapy

BIOLOGY OF GLIOBLASTOMA MULTIFORME

Glioblastoma multiforme (GBM), a World Health Organization (WHO) grade IV glioma, is the most common and lethal primary malignancy of the central nervous system (CNS). More than 10,000 new cases are reported annually in the United States. Despite aggressive treatment including surgical resection, chemo- and radiotherapy, median survival time for patients with GBM is only 14.6 months. GBM is an incurable disease that almost invariably leads to neurological demise and death. Due to its high degree of invasiveness, radical resection of the primary tumor mass is not curative. Infiltrating tumor cells invariably remain within the surrounding brain, leading to disease progression or recurrence, either locally or distant from the primary tumor.

Experimental evidence supports the hypothesis that GBM contains a subpopulation of highly tumorigenic cells, glioblastoma stem cells (GSCs), from which recurrent GBM is thought to derive. CSCs have the capacity to self-renew, differentiate into multiple lineages, and for tumorigenesis.
Unlike the bulk of the rapidly dividing tumor cells, CSCs are thought to be relatively quiescent, rendering them resistant to conventional chemo- and radiotherapy.\[^{32}\] Failure of conventional treatments combined with its poor prognosis highlights the need for novel approaches for GBM that are targeted at residual tumor cells in order to prevent recurrence.

**Primary versus secondary GBM**

GBM can be classified as primary or secondary. Primary GBM occurs de novo; without evidence of a less malignant precursor, whereas secondary GBM develops from an initially low-grade diffuse astrocytoma (WHO grade II) or anaplastic astrocytoma (WHO grade III). The majority of GBMs (~90%) are primary.\[^{140}\] Patients with primary GBM tend to be older (mean age = 55 years) than patients with secondary GBM (mean age = 40 years). Secondary GBM is associated with better prognosis and increased overall survival time compared with primary GBM. Although largely indistinguishable based upon histopathology, primary and secondary GBMs evolve from different genetic precursors and harbor distinct genetic alterations.\[^{160}\] Genetic alterations more typical for primary GBM are epidermal growth factor receptor (EGFR) overexpression, PTEN mutations, and loss of chromosome 10.\[^{75,159,161,258}\] Whereas genetic alterations more commonly seen in secondary GBM include isocitrate dehydrogenase-1 (IDH1) mutations, TP53 mutations, and 19q loss.\[^{7,77,118,155,159,161,269,280}\] Although different gene expression patterns in primary and secondary GBM were identified, characterization of the IDH1 mutation has allowed for reliable molecular differentiation of primary from secondary GBM.\[^{9,158,160,269,280}\]

Prior to the discovery of IDH1 mutation in secondary GBM, primary and secondary GBM were distinguished based upon clinical findings. The diagnosis of primary GBM was made in patients without radiologic or histologic evidence of a less malignant precursor, whereas a diagnosis of secondary GBM was made in patients with preexisting low-grade astrocytoma or evidence of a less malignant precursor either radiographically or histologically.\[^{160}\] In 2008, it was reported that IDH1 mutations occur in a disproportionately large portion of younger patients and in the majority of patients with secondary GBM.\[^{164}\] Additionally, IDH1 mutations appear to be associated with better prognosis and increase in overall survival.\[^{160}\] Interestingly, IDH1 mutations are also found in greater than 80% of diffuse astrocytoma and anaplastic astrocytomas, which are precursors of secondary GBM. In subsequent studies of primary and secondary GBM, IDH1 mutations were found in greater than 80% of secondary GBM and less than 5% of primary GBM.\[^{9,158,269,280}\] Thus, IDH1 mutations are a reliable, objective molecular marker for secondary GBM over clinical and pathological criteria.\[^{158,160}\]

**GBM subtypes**

Targeting the underlying genetic alterations leading to GBM is critical to develop effective treatment strategies. Although GBM is a highly heterogeneous tumor, high dimensional genomic profiling has allowed GBM to be categorized into four subtypes based upon characteristic genetic alterations and distinct molecular profiles. In order to better understand the pathogenesis of GBM, high dimensional genomic profiling has been used to identify genetic abnormalities driving GBM tumorigenesis. Using gene expression-based molecular classification of GBM that integrates multidimensional genomic data to establish patterns of aberrant gene expression and copy number alterations seen in GBM, four subtypes of GBM were identified. These GBM subtypes are classical, mesenchymal, proneural, and neural.\[^{15,20,25,154,169,238,258}\]

Each subtype harbors distinct genetic alterations and expression profiles.\[^{20,258}\] The classical subtype is strongly enriched in the gene expression pattern observed in astrocytes.\[^{23,258}\] It is characterized by aberrant EGFR activity, leading to EGFR overexpression.\[^{25,169,258}\] Additionally, loss of chromosome 10 is frequently observed in the classical subtype. Mutations in TP53, NF1, and IDH1 are uncommonly found in the classical subtype.\[^{258}\] The mesenchymal subtype is also enriched in the gene expression pattern seen in astrocytes, but in addition, expresses mesenchymal markers as well as microglia markers.\[^{23,258}\] It is characterized by alterations in the gene for NF1. PTEN deletions, which lead to alterations in the PI3K/AKT/mTOR intracellular signaling pathways, are also commonly seen in the mesenchymal subtype. EGFR overexpression is less commonly seen in the mesenchymal subtype compared with other GBM subtypes.\[^{258}\] The proneural subtype is enriched in proneural genes as well as the gene expression patterns seen in oligodendrocytes.\[^{23,258}\] It is characterized by alterations in TP53, platelet-derived growth factor receptor (PDGFR), and IDH1.\[^{7,77,118,258,269,280}\] Although overexpression of PDGFR is observed in many GBMs, focal amplification of PDGFR with resultant high levels of PDGFR expression is most frequently seen in the proneural subtype. The proneural subtype is also associated with a younger age at diagnosis.\[^{258}\] Neural subtype gene expression pattern is the most similar to that of normal brain tissue. It is strongly enriched in the gene expression pattern seen in neurons, and expresses both astrocytic and oligodendrocytic markers.\[^{23,258}\]

GBM without IDH1 mutation has been identified as classical, mesenchymal, proneural, and neural. The majority of GBM with IDH1 mutation have the proneural gene expression pattern; however, only 30% of GBM with proneural gene expression patterns have the IDH1 mutation.\[^{160}\] These findings support the hypothesis that secondary GBM are a more homogeneous group characterized by an IDH1 mutation and a proneural
pattern of gene expression, whereas primary GBM are a more heterogeneous group with a diverse array of genetic mutations and aberrant gene expression profiles.\textsuperscript{[168]}

Overall, the proneural subtype is diagnosed at a younger age than other subtypes.\textsuperscript{[238]} This is as expected given that secondary GBM, almost always the proneural subtype, is generally diagnosed in younger patients.\textsuperscript{[160,169]} The proneural subtype is associated with better prognosis and increased survival compared with the other subtypes.\textsuperscript{[160,238]} Interestingly, however, aggressive treatment with chemo-and radiotherapy has been shown to significantly decrease mortality in patients with classical or mesenchymal subtypes, but has not been shown to significantly alter mortality in the proneural subtypes.\textsuperscript{[238]}

The discovery that GBM consists of various subtypes with different genetic alterations and gene expression patterns suggests that no single therapy will be efficacious across all subtypes. Due to the heterogeneity of GBM, it is likely that future therapies will be tailored to target the underlying molecular abnormalities seen in individual patients.

**CURRENT STANDARD OF CARE AND TREATMENT**

The current standard of care for patients with GBM includes maximal safe resection, followed by concurrent radiation therapy (RT) to the resection cavity and chemotherapy (with temozolomide (TMZ), followed by adjuvant TMZ).\textsuperscript{[39,236]} [Table 1]. Surgical resection alone results in a median survival of approximately 6 months. Combined, surgical resection and RT extend median survival to 12.1 months. Addition of TMZ further extends the median survival to 14.6 months.\textsuperscript{[237]}

**Surgical resection**

Surgery remains an important component in the treatment of GBM. It allows for a histologic confirmation of the diagnosis as well as cytoreduction. Surgery may also serve a therapeutic role by reducing the intracranial pressure, and depending on the location of the tumor, occasionally leads to recovery of some neurological function. The principal contraindications to resective surgery are poor performance status (Karnofsky performance scale [KPS] <70), advanced age, eloquent location or extensive bitemporal involvement.

The goal of surgery is to achieve gross total resection of the contrast enhancing component of the tumor, without compromising neurological function.\textsuperscript{[168]} Gross total resection may not be possible based on anatomic structures invaded by the tumor. Advances in surgical imaging techniques, such as intraoperative magnetic resonance imaging (MRI), diffusion tensor imaging, awake craniotomy, cortical mapping, stereotactic guidance, and fluorescent-guided resection, have facilitated delineation of tumor borders and can help optimize maximal safe surgical resection\textsuperscript{[4,97,168]} [Table 2]. Fluorescent-guided resection utilizes a pharmacologic agent that localizes to tumor but not the surrounding normal brain and fluoresces when exposed to light of a specific wavelength. This fluorescence can be used to guide tumor resection by identifying tumor tissue that may otherwise appear normal.\textsuperscript{[4,97,171,232]} Studies comparing fluorescence-guided resection with standard white light resection have shown that patients undergoing fluorescence-guided resection were more likely to have a gross total resection and were more likely to be progression free at 6 months.\textsuperscript{[97,171,232]} Although maximal surgical resection remains important, ultimately GBM does not have a surgical “answer”.

**Chemo-and radiotherapy**

The combination of RT plus TMZ is the most efficacious adjuvant therapy to prolong survival after primary resection. Treatment following surgery usually consists of 6 weeks of RT to the surgical cavity and TMZ, followed by 6 adjuvant cycles of TMZ.\textsuperscript{[234,237]}

The current standard of care for RT in GBM is focal, fractionated external beam radiation therapy (EBRT) to the surgical resection cavity and to a 2 cm margin of surrounding brain tissue.\textsuperscript{[4]} Usually, 60 Gy of RT is delivered in fractions of 2 Gy over 6 weeks.\textsuperscript{[128,222]} Ionizing radiation induces single-strand and double-strand breaks in the DNA of proliferating cells. Other modes of delivering RT have been investigated, including brachytherapy, but none have been proven more effective than the current standard approach.\textsuperscript{[39]}

TMZ, an oral alkylating chemotherapeutic agent, causes DNA damage and triggers a cascade of events leading to tumor cell apoptosis.\textsuperscript{[97]} Recently, TMZ was added to the standard of care for GBM treatment. Previously, chemotherapy had no demonstrable clinical benefit, and RT alone remained the standard of care after surgical resection.\textsuperscript{[4]} In 2005, a clinical trial demonstrated that concurrent RT and TMZ followed by adjuvant TMZ significantly prolonged the median survival more than that of radiation alone (14.6 months versus 12.1 months; \( P < 0.001 \)). At the 5-year analysis of this study, more patients treated with TMZ were alive (9.5% versus 1.9%; \( P < 0.001 \)).\textsuperscript{[237]} These findings established the therapeutic

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**Table 1: Current standard of care**

| Maximal safe surgical resection (Consider microscope, stereotaxy, intraoperative MRI, mapping, fluorescence) |
| Radiation therapy (fractionated external beam with 2 cm margin) |
| Temozolomide |

**Table 2: Surgical aids**

| Intraoperative imaging |
| Stereotactic surgery |
| Fluorescent-guided resection |
| Cortical grids/mapping |
benefit of TMZ in combination with RT, establishing the so-called "Stupp regimen" standard of care for GBM treatment.\cite{264,265} Despite these advances, the median progression-free survival time is only 7 months.\cite{295} When given in combination with RT, patients receive 75 mg/m$^2$/day of TMZ for 6 weeks. For adjuvant therapy following completion of RT, patients receive 150 mg/ day of TMZ for 5 days every 28 days for at least 6 cycles.\cite{227}

As mentioned earlier, TMZ derives its therapeutic benefit from adding a methyl group to purine bases of DNA, which leads to DNA damage and triggers a cascade of events that leads to tumor cell apoptosis.\cite{47,285} The primary cytotoxic target of TMZ is O6-methylguanine. The methyl group added to O6-methylguanine can be removed by O6-methylguanine methyltransferase (MGMT), which is a DNA repair protein that functions to remove methyl groups from the O6 position of guanine. Removal of this methyl group confers resistance of tumor cells to TMZ and other alkylating chemotherapeutic agents by protecting cells from their DNA-damaging effects.\cite{208,299,205}

In some patients, MGMT expression has been decreased or silenced by methylation of the promoter regions of the MGMT gene, preventing it from removing methyl groups from the O6 position of guanine.\cite{259} Thus, the methylation status of the promoter region of the MGMT gene is one of the main mechanisms contributing to TMZ sensitivity or resistance in patients with GBM.\cite{54,54,54,294,293,295,299} Patients with an unmethylated MGMT are much less responsive to TMZ, whereas MGMT methylation confers sensitivity to TMZ in patients with GBM.\cite{60,51,100,283}

Implantation of carmustine wafers into the resection cavity is another Food and Drug Administration (FDA) approved treatment of GBM.\cite{227} Similar to TMZ, carmustine is a DNA alkylating agent.\cite{47} Biodegradable wafers impregnated with carmustine line the tumor resection cavity, allowing for delivery of chemotherapy. Carmustine is released into the surrounding brain tissue immediately after tumor resection and its effects last for several weeks.\cite{4} In clinical trials, carmustine wafers used in combination with radiation and TMZ have been shown to modestly prolong survival in subsets of patients. However, because there are complications associated with the use of wafers, including infection, swelling, need for removal, and impairment of wound healing,\cite{58,54,272} they are not used routinely at most centers.

THERAPIES UNDER INVESTIGATION

MOLECULARLY TARGETED THERAPIES

Genetically, GBM is a highly heterogeneous tumor harboring multiple recurrent and nonrecurrent genetic alterations.\cite{8} Within the same tumor, cytogenetically related and unrelated clones coexist.\cite{8} Due to recent progress in genomics, several aberrantly activated pathways and mutated genes have been implicated in the pathogenesis and malignant progression of GBM.\cite{8,212,255} These findings have inspired the investigation of molecularly targeted therapies designed to target tumor-specific recurrent genetic alterations as a novel approach to treating GBM.\cite{8,255} Many mutations occur in receptor tyrosine kinases (RTKs) or components of their downstream signaling pathways, making them potential targets for drug development and evaluation in clinical trials.\cite{165,212,255,2} The majority of growth factor receptors are transmembrane glycoprotein RTKs with extracellular ligand-binding domains and intracellular kinase domains.\cite{8,212} Activation of these RTKs triggers a cascade of downstream signaling events, and inappropriate activation of these signaling pathways is thought to drive tumor growth, survival, invasion into normal brain, and secretion of angiogenic factors\cite{212} [Figure 1]. Thus, inhibition of these pathways and their downstream intracellular signaling components is the goal of molecularly targeted approaches to treatment of GBM. See Table 3 for a list of these various pathways.

Molecularly targeted therapies can broadly be divided into small molecule inhibitors and monoclonal antibodies. Small molecule inhibitors are nonpolymeric, organic compounds able to cross cell membranes and target specific intracellular constituents.\cite{47} Many small molecule inhibitors are tyrosine kinase inhibitors (TKIs), which function by selectively targeting the intracellular kinase domain of RTKs, blocking receptor activation of
In 2009, Cetuximab binding also leads to internalization and downregulation of EGFR, and it has been suggested that cetuximab may have antibody dependent cytoxic effects on the GBM cells it binds. In preclinical models of GBM, cetuximab decreased growth and prolonged survival. Unfortunately, in clinical trials, cetuximab has shown limited therapeutic benefit and has not improved patient outcomes. Overexpression of PDGFR signaling is another frequent alteration found in GBM. PDGF stimulates tumor growth through autocrine signaling, promoting angiogenesis through paracrine effects on adjacent endothelial cells. Imatinib is a small molecule multikinase inhibitor that binds PDGFR as well as several other RTKs. Similar to EGDF TKIs, imatinib blocks the PDGFR kinase domain to prevent activation of its downstream signaling pathways. Unfortunately, similar to EGFR inhibitors, clinical trials with imatinib demonstrated a lack of therapeutic efficacy and minimal clinical benefit. Initially, these results were attributed to the presence of drug efflux pumps at the blood-brain barrier actively removing imatinib from the brain. Thus, subsequent clinical trials administering hydroxyurea with imatinib were conducted. Results of these trials, however, showed that addition of hydroxyurea did not improve outcomes.

### Inhibition of growth factor pathways

Amplification of EGFR signaling is one of the most common genetic alterations seen in GBM. Over 60% of GBM overexpress EGFR, and in about half of these, overexpression is the result of a mutant form of the receptor, EGFRvIII, which has a constitutively active kinase domain. EGFR overexpression promotes tumor growth, survival, angiogenesis, and invasion. EGFR is also overexpressed in other cancers, and a variety of small molecule TKIs and some monoclonal antibodies have been investigated for their potential to block aberrant EGFR signaling.

Gefitinib and erlotinib are oral small molecule EGFR TKIs that have been extensively studied in preclinical and clinical trials of GBM. Binding to intracellular kinase domain of EGFR, they inhibit the activation of its downstream signaling pathways. In preclinical models of GBM, gefitinib and erlotinib have demonstrated antiproliferative and antiinvasive effects, potent inhibition of EGFR, and prolonged survival. Unfortunately, in clinical trials, they have shown limited therapeutic efficacy. Both gefitinib and erlotinib have been studied as monotherapy in recurrent GBM and in combination with RT and TMZ for newly diagnosed GBM, but neither agent provided any clinical benefit. Several additional clinical trials using erlotinib in combination with radiation and TMZ have shown that erlotinib enhances tumor cell sensitivity to radiation. Other studies, however, have not shown the same results. Overall, small molecule EGFR TKIs have provided minimal to no benefit in clinical outcomes.

Cetuximab is a chimeric murine-human monoclonal antibody that blocks activation of EGFR by its ligands. Cetuximab binding also leads to internalization and downregulation of EGFR, and it has been suggested that cetuximab may have antibody dependent cytoxic effects on the GBM cells it binds. In preclinical models of GBM, cetuximab decreased growth and prolonged survival. Unfortunately, in clinical trials, cetuximab has shown limited therapeutic benefit and has not improved patient outcomes. Overexpression of PDGFR signaling is another frequent alteration found in GBM. PDGF stimulates tumor growth through autocrine signaling, promoting angiogenesis through paracrine effects on adjacent endothelial cells. Imatinib is a small molecule multikinase inhibitor that binds PDGFR as well as several other RTKs. Similar to EGDF TKIs, imatinib blocks the PDGFR kinase domain to prevent activation of its downstream signaling pathways. Unfortunately, similar to EGFR inhibitors, clinical trials with imatinib demonstrated a lack of therapeutic efficacy and minimal clinical benefit. Initially, these results were attributed to the presence of drug efflux pumps at the blood-brain barrier actively removing imatinib from the brain. Thus, subsequent clinical trials administering hydroxyurea with imatinib were conducted. Results of these trials, however, showed that addition of hydroxyurea did not improve outcomes.

### Inhibition of angiogenic pathways

GBM is a highly vascularized tumor characterized by extensive angiogenesis. Vascular endothelial growth factor (VEGF), a critical mediator of angiogenesis, is highly overexpressed in GBM. VEGF has been correlated with clinical outcomes, including time to recurrence and survival. Several small molecule inhibitors and monoclonal antibodies have been developed to block this signaling. Antiangiogenic therapies are currently the most advanced group of targeted therapies for GBM, and there have been multiple clinical trials demonstrating their therapeutic efficacy.

Bevacizumab (trade name Avastin) is a humanized monoclonal antibody that binds and neutralizes the VEGF ligand, preventing activation of its receptors. In clinical trials of recurrent GBM, bevacizumab demonstrated clinical benefit and improved progression-free survival. In 2009, based on the results of two clinical trials, bevacizumab was approved by the FDA for use as monotherapy in recurrent GBM. Bevacizumab is thought to improve...
outcomes by reducing angiogenesis, decreasing growth of tumor cells expressing VEGF, disrupting the microvasculature of the tumor leading to increased tumor hypoxia, and increasing tumor cell sensitivity to RT.[24,67,106,134] It is currently undergoing clinical trials for use in combination with radiation and TMZ for treatment of newly diagnosed GBM. To date, the results of these clinical trials have shown some promising results, but additional trials are also currently underway to confirm clinical benefit.[95,112,168,262,263] Results of a phase III trial recently presented at American Society of Clinical Oncology (ASCO) demonstrated that the addition of bevacizumab to RT and TMZ does not improve overall survival in patients with newly diagnosed GBM over that of RT and TMZ alone. The addition of bevacizumab was shown to slightly improve progression-free survival in patients with newly diagnosed GBM; however, this did not reach statistical significance. Compared with RT and TMZ alone, RT and TMZ in combination with bevacizumab was associated with higher rates of toxicities. Although full interpretation of the long-term results of this study are ongoing, current results suggest that addition of bevacizumab to RT and TMZ for treatment of patient with newly diagnosed GBM does not improve prognosis.[82,164]

Cedatinib, sunitinib, and vatalanib are multikinase TKIs with predominant VEGF inhibition that have been used to block angiogenesis in GBM. Cedarinib and sunitinib are inhibitors of VEGF, PDGFR, and c-kit. Despite encouraging results in preclinical trials, clinical trials with these agents have demonstrated limited therapeutic benefit, and in some trials, were associated with high toxicity rates.[12,110,150,194,198] In clinical trials, similar to cedatinib and sunitinib, vatalanib, an oral inhibitor of VEGF and PDGFR, provided no clinical benefit.[194]

Cilengitide is another novel antiangiogenic therapy being studied in GBM. It is a cyclized pentapeptide small molecule inhibitor that selectively blocks activation of the ανβ3 and ανβ5 integrins. The integrins are a family of cell adhesion molecule transmembrane glycoprotein receptors that mediate cell-matrix and cell-cell interactions. In GBM, the ανβ3 and ανβ5 integrins are widely overexpressed in GBM cells and tumor vasculature, and in addition to VEGF, they are key mediators of angiogenesis, tumor growth, and metastasis.[13,213,224] Several clinical trials have shown that cilengitide is well tolerated with minimal toxicity, and ongoing clinical trials are investigating its therapeutic efficacy and clinical benefit.[196,197,213,235] The results of a phase III trial recently presented at ASCO showed that the addition of cilengitide to RT and TMZ for treatment of newly diagnosed GBM does not improve progression-free survival or overall survival compared with RT and TMZ alone. In this trial, cilengitide was well tolerated and its safety profile was confirmed. To date, results of this trial suggest that the addition of cilengitide to RT and TMZ for treatment of newly diagnosed GBM does not improve prognosis.[233]

**Inhibition of intracellular signaling pathways**

Downstream signaling of many RTKs, including EGFR and PDGFR, lead to activation of the PI3K/AKT/mTOR and RAS/RAF/MAPK secondary messenger systems. Dysregulation of these intracellular signaling pathways often occurs in GBM.[8,39] PTEN and NFI are endogenous inhibitors of PI3K and RAS, and these inhibitors are often lost or mutated in GBM.[97,142] Other signaling molecules in the PI3K and RAS pathways are also commonly mutated in GBM. These alterations further contribute to inappropriate PI3K and RAS activity in GBM. The identification of these alterations, however, allows for the investigation of other approaches besides inhibition of RTK to interfere with aberrant signaling pathway activity.

Pi3K signals through activation of AKT and subsequently mTOR. Perifosine is a small molecule inhibitor of AKT that has been shown in preclinical trials to reduce signaling through the PI3K pathway. Currently, clinical trials with perifosine are being conducted to evaluate its clinical efficacy.[39,172] Rapamycin, temsirolimus, sirolimus, and everolimus are all small molecule inhibitors of mTOR. In clinical trials, these agents have been well tolerated, but they have demonstrated limited therapeutic efficacy.[41,47,78,179,218,265] RAS signals through activation of RAF and MAPK proteins. The rate-limiting step in RAS signaling is farnesylation. Accordingly, inhibitors of farnesyl transferase, including tipifarnib and lonafarnib, have been investigated in GBM.[40,218,225] Preclinical studies have shown reduced signaling through the RAS pathway, but in clinical trials, they have not provided benefit.[40,47,218] Downstream of RAS, RAF signaling occurs through activation of its kinase domains. Sorafenib is a multikinase TKI of RAF, VEGF, PDGFR, and several other kinases. In clinical trials, sorafenib was been well tolerated, but only had limited therapeutic benefit.[165,198]

**Multi-targeted kinase inhibitors and combination therapy**

Clinical trials of small molecule TKIs have demonstrated limited efficacy in patients with GBM. This is likely the result, at least in part, of multiple mutations and pathways driving tumor growth. Due to the intratumor heterogeneity, lack of a dominant single oncogenic “driver” mutation and redundancy of signaling pathways in cancer cells has become increasingly clear that targeting a single receptor or signaling pathway is unlikely to succeed in GBM.[512] Therefore, several strategies have been developed to improve the therapeutic efficacy of targeted molecular therapies. These strategies include using combinations of multiple single-targeted agents and designing small molecule compounds that target multiple kinases simultaneously. These strategies have
the potential for greater efficacy by inhibiting multiple pathways, but they also have an increased risk of toxicity from systemic inhibition. Several multi-target agents have been described in the above sections based on the primary pathway they target. Several clinical trials have evaluated the use of multi-kinase inhibitors and combinations of molecularly targeted agents. Unfortunately, while toxicities often increased substantially, no “combination therapy” has demonstrated superior clinical benefit over single agents. 

**IMMUNOTHERAPY**

Immunotherapy attempts to harness the immune system to selectively destroy tumor cells. It includes both passive and active strategies. Passive immunotherapy uses antibodies, immune cells, or other components of the immune system to target the tumor cells. This approach does not require activation of the patient’s native immune response; rather, immune cells are activated ex vivo and then reinjected. Passive immunotherapy involves use of monoclonal antibodies, cytokine-mediated therapies, and adoptive cell transfer approaches. Monoclonal antibodies were discussed earlier and will be further discussed later in the “Gene Therapy” section that follows. Active immunotherapy attempts to stimulate the patient’s native immune response against the tumor. Active immunotherapy includes peptide-based and cell-based approaches. These therapies are often referred to as cancer vaccines. Currently, most immunologic therapies available are antibody-based therapies, some of which were discussed earlier. There are, however, several cancer vaccines being evaluated as treatment for GBM in clinical trials. See Table 4 for a list of the various active and passive immunotherapeutic approaches.

The immune system can be divided into the innate and the adaptive immune systems. The innate immune system consists of macrophages, monocytes, neutrophils, basophils, eosinophils, natural killer (NK) cells, and complement. The cells of the innate immune response are able to recognize pathogen-associated molecular patterns and defend against the foreign invaders. The adaptive immune system consists of T and B lymphocytes and antigen-presenting cells (APCs), and in contrast to the innate immune system, the cells of the adaptive immune system must be activated by antigens. T lymphocytes can be divided into cytotoxic and helper. Activation of cytotoxic T lymphocytes leads to cell-mediated killing, whereas activation of T helper lymphocytes leads to release of cytokines and recruitment of more immune cells. Activated B lymphocytes are responsible for production of antibodies and antibody-mediated cell cytotoxicity. APCs internalize antigen by phagocytosis or endocytosis, then present a fragment to bind and activate T cells. Dendritic cells (DCs) are the most active APCs, and they are often referred to as professional APCs.

### Passive immunotherapy: Adoptive cell transfer

Adoptive cell transfer uses stimulated immune effector cells to generate cell-based cytotoxic responses to attack GBM cells. In this approach, autologous immune cells are activated ex vivo and readministered. The cells can be given systemically or intratumorally. The two main immune cells used in adoptive cell transfer are lymphocyte-activated killer (LAK) cells and cytotoxic T lymphocytes (CTLs). LAK cells are usually obtained by culture of autologous peripheral lymphocytes in the presence of interleukin-2 (IL-2) to promote maturation and activation, yielding a polyclonal population of both T and NK cells. Although these cells yield a cytotoxic immune reaction, it is nonspecific, and not exclusively directed at tumor cells. CTLs are obtained from peripheral blood mononuclear cells or tumor-infiltrating lymphocytes that are stimulated ex vivo with autologous tumor cells, generating activated CTLs with specificity to the tumor.

Some of the earliest attempts at immunotherapy for treatment of GBM involved LAK cells and/or CTLs. Although results were mixed, CTLs appear to be better tolerated than LAK cells, likely because of their high specificity of targeting tumor cells. Overall, results of clinical trials with LAK cells and CTLs have demonstrated minimal therapeutic efficacy and have not had an impact on survival.

### Active immunotherapy: Cancer vaccines

Active immunotherapy is similar in concept to vaccination. It boosts the patient’s native immune response against the tumor cells by priming it with antigen exposure. Several sources of GBM-related antigen may be used in active immunotherapy, which include intact tumor cells, tumor cell lysate, tumor-derived peptides and mRNA, and synthetic peptides. Active immunotherapy approaches include peptide-based therapies and cell-based therapies. [Figure 2]. In peptide-based therapies, GBM-related antigens are administered to the patient as a vaccine to stimulate an immune response. The antigens used for cancer vaccines are usually small peptides, around nine amino acids in length, which are capable of activating cytotoxic T lymphocytes. Rindopepimut is an injectable peptide vaccine designed to
stimulate an immune response against a specific EGFRvIII antigen.\cite{37,95,208} EGFRvIII variant is a constitutively active, mutant form of EGFR expressed in GBM.\cite{37} A segment of this mutant peptide has been used as a vaccine to generate EGFRvIII-specific antibodies.\cite{37,95,208} In clinical trials of patients with GBM expressing EGFRvIII, rindopepimut has shown some promising results, but clinical trials have not been completed.\cite{208,210}

In cell-based therapies, APCs are activated with GBM-related antigens, rather than the antigen itself, to prime the immune system and then injected into the patients to generate an immune response.\cite{15,16,27,278} As DCs are professional APCs, they are typically used.\cite{27,278} The DCs are typically collected from the patient’s autologous peripheral blood mononuclear cells, cultured in the presence of growth factors and matured, activated ex vivo with tumor-related peptides, and then readministered to generate an antitumor response.\cite{37,95,63,136,276,278} These activated cells can be delivered intradermally, to the lymph nodes or intratumorally.\cite{241} Several clinical trials of cell-based therapies are underway. Overall, DC vaccines appear to be well tolerated, but have not yet resulted in significant therapeutic benefit.\cite{5,6,27,50,115,132,136,181,279,281,282}

**GENE THERAPY**

Gene therapy for the treatment of cancer involves the delivery of genetic material, which includes transgenes, toxins, and viruses, into tumor cells for therapeutic purposes. The genetic material is typically packaged within a vector for delivery into cells. See Table 5 for a list of various gene therapy approaches. There are a variety of different vectors, which include both viral and nonviral vectors. Some gene therapies have shown promising results in preclinical trials, but clinical trials have been unable to demonstrate any significant therapeutic efficacy.\cite{151,242}

Lack of clinical benefit is, at least in part, likely due to the low transfection efficiency of most viral vectors.

**Vectors**

Vectors are classified as viral or nonviral. Viral vectors were the first and most commonly used vectors in gene therapy for GBM. Viral vector-based delivery systems take advantage of the ability of viruses to deliver their genetic information into the host cell.\cite{151} As part of their normal life cycle, viruses bind host cells and, through transduction, introduce their genetic material. For use as vectors in gene therapy, viruses undergo modification; components of their viral genomes are removed and replaced with the therapeutic genetic material to be delivered to the target cell. Depending on which parts of the viral genome are removed, viruses can be engineered into replication-competent or replication-deficient vectors. To generate replication-deficient vectors, the coding regions of the viral genome required for viral replication are removed.\cite{45,151} Thus, replication-deficient vectors can transduce their host cell, but are unable to replicate within their host. Conversely, replication-competent vectors contain coding regions needed for viral replication, enabling them to continue replication and propagate viral infection to surrounding cells.\cite{151}

Vectors ideally transduce tumor cells while sparing normal cells in order to avoid toxicity to the surrounding brain.\cite{45,122,168} Different viruses have been used as vectors, each with their own advantages.

**Table 5: Gene therapy**

| Conditionally cytotoxic (enzyme-prodrug activating therapy; suicide gene therapy) |
| Directly cytotoxic (targeted toxin therapy) |
| Immunostimulatory |
| Oncolytic virotherapy |

Figure 2: In peptide-based therapies, GBM-related antigens are administered to the patient as a vaccine to stimulate an immune response. Once administered, DCs, which are professional APCs, internalize these GBM-related antigens and present them to the immune effector cells to stimulate an immune response. In cell-based therapies, APCs are activated with GBM-related antigens, rather than the antigen itself, to prime the immune system and then injected into the patients to generate an immune response.
and disadvantages. The viral vectors available for gene therapy can be divided into integrating and nonintegrating vectors. Vectors based on retroviruses and lentiviruses integrate their viral genetic material into the chromosomal DNA of the host cell. Vectors based on adenoviruses and herpes simplex viruses type 1 (HSV-1) are able to deliver their genetic material into the nucleus of their host cell, but they are unable to integrate it into the host cell DNA. Retroviruses and adenoviruses are among the earliest and most widely studied viral vectors for use in gene therapy.[20,249,273]

Retroviruses are a large family of enveloped RNA viruses.[119] They contain a reverse transcriptase that, following transduction, allows them to integrate their genetic material in the host DNA.[119] Retroviruses selectively infect actively dividing cells, which allows them to target rapidly dividing GBM cells while sparing normal brain cells.[120] A major drawback is that they may be unable to target the relatively quiescent GSCs that may be responsible for tumor resistance to treatment and recurrence.[45] Another drawback of retroviruses is that they are unstable and difficult to produce at high titers. In order to increase viral titers within the brain, virus-producing cells (VPCs), which continuously produce replication-deficient retrovirus, are often injected into the brain instead of direct viral injection.[187,190] VPCs, however, have a short lifespan and a limited ability to migrate within the brain.[187] Lentiviruses, a subpopulation of retroviruses, are able to infect dividing as well as nondividing cells, allowing them to overcome the drawbacks of traditional retroviruses.[32,119,129]

Adenoviruses, large double-stranded DNA viruses, are unlike retroviruses and lentiviruses. They are unable to integrate their DNA into the host genome following transduction.[45,119,151] They are, however, able to infect dividing and quiescent cells.[64] They express their transgenes at high levels and can be produced at high titers. Herpes simplex virus-1 (HSV1), a member of the herpesvirus family, is also a large, nonintegrating, double-stranded DNA virus. It has neurotropic properties that enable it to efficiently infect both quiescent and proliferative cells of the CNS.[93,64,163] HSV1 has one of the highest vector packing capacities for delivery of genetic material into cells, and this capacity can be utilized for simultaneous delivery of multiple genes with one vector.[64,102,103,126]

In addition to viruses, stem cells have been explored as vectors for delivery of gene therapies in GBM. The three types of stem cells studied as vectors in GBM are neural, embryonic, and mesenchymal.[242] Stem cell vectors appear promising because of inherent tumor trophic properties, which may enhance delivery of genetic material to tumor cells and increase therapeutic efficacy.[2,3,114,122,242,254] Nanoparticles have also been explored as vectors for delivery of gene therapies in GBM. Liposomes are the most widely used nanoparticle vectors. These vectors act by delivering genes to a targeted site based upon their size, charge, and high surface to volume ratio, which provides a powerful source for diffusion.[242]

**Conditionally cytotoxic gene therapy**

Conditionally cytotoxic gene therapy, also referred to as enzyme-prodrug activating therapy or suicide gene therapy, is the most commonly used gene therapy approach in GBM.[242] In conditionally cytotoxic approaches, the transgene for a noncytotoxic enzyme is delivered into tumor cells, and this enzyme remains noncytotoxic until the administration of a noncytotoxic prodrug. Upon prodrug administration, the enzyme converts the noncytotoxic prodrug into a toxic metabolite that induces tumor cell death.[242,255] [Figure 3]. For

![Figure 3](http://www.surgicalneurologyint.com/content/5/1/64)  
**Figure 3:** This figure shows conditionally cytotoxic gene therapy, also referred to as enzyme-prodrug activating therapy or suicide gene therapy, which is the most commonly used gene therapy. In conditionally cytotoxic approaches, as described in the text, the transgene for a noncytotoxic enzyme is delivered into tumor cells using a vector, and this enzyme remains noncytotoxic until the administration of a noncytotoxic prodrug. Upon prodrug administration, the enzyme converts the noncytotoxic prodrug into a toxic metabolite that induces tumor cell death.
enzyme-prodrug activating therapy to be successful, the enzyme transgene must be delivered exclusively into tumor cells, and its enzymatic activity must be high enough to kill tumor cells without causing toxicity to surrounding normal cells or inducing a systemic immune response.\textsuperscript{122} In most enzyme-prodrug therapies, there is a significant bystander effect. This occurs when the cytotoxic metabolite is transmitted, via gap junctions or by diffusing through extracellular space, to cells that were not originally transduced with the enzyme.\textsuperscript{126} Since expression of the enzyme gene will not occur in all tumor cells, this bystander effect is important in amplifying the cytotoxicity of the enzyme-prodrug therapy.\textsuperscript{144}

In GBM, the most commonly studied enzyme/prodrug combination is the herpes simplex virus-1 thymidine kinase (HSV1-TK)/ganciclovir (GCV) system.\textsuperscript{242} HSV1-TK is a thymidine kinase that converts the prodrug GCV into a toxic DNA analog that integrates into replicating DNA triggering tumor cell death.\textsuperscript{14,63,255} In clinical trials of HSV1-TK/GCV for GBM, replication-deficient retroviruses and replication-deficient adenoviruses have been used to successfully deliver the HSV1-TK transgene into the tumor cavity.\textsuperscript{89,122,151,242} As retroviruses can be unstable and difficult to produce at high titers, trials with retroviral vectors typically involve implantation of VPCs producing retroviruses expressing HSV1-TK into the tumor resection cavity to increase titers. Once implanted, these cells produce retroviruses expressing the HSV1-TK transgene, which diffuse into the surrounding brain tissue to infect tumor cells. Adenoviruses, in contrast to retroviruses, can be produced at high titers. Thus, trials with adenoviral vectors usually involve direct injection of virus expressing the HSV1-TK transgene into the surgical resection cavity. Compared with retroviruses, adenoviruses have demonstrated higher gene transfer efficiency into GBM cells.\textsuperscript{214} Overall, numerous clinical trials using either retroviral or adenoviral vectors have shown that HSV1-TK/GCV therapy is well tolerated, but its therapeutic efficacy has been limited.\textsuperscript{70,80,87,98,100,101,134,135,178,185,196,210,231,222,229,242,245}

**Directly cytotoxic gene therapy**

Directly cytotoxic gene therapy, otherwise known as targeted toxin therapy, utilizes surface molecules overexpressed in GBM to target toxins directly into tumor cells to cause tumor cell death. This can be accomplished through viral vector-mediated delivery of transgenes for highly toxic proteins or with recombinant molecules: Immunotoxins. Immunotoxins consist of a tumor-specific monoclonal antibody or ligand conjugated to a toxin.\textsuperscript{63,146,255,256} The antibody or ligand component binds selectively to surface molecules overexpressed in GBM and induces internalization of the immunotoxin. Upon internalization, the toxin triggers a cascade of intracellular events that lead to tumor cell apoptosis. Commonly used toxins are *Pseudomonas* exotoxin (PE) and *Diptheria* exotoxin (DT).\textsuperscript{264} Immunotoxins target surface molecules overexpressed in GBM: EGFR, interleukin-13 (IL-13), urokinase-type plasminogen activator (uPA), and transferrin.\textsuperscript{16,19,46,50,52,63,106,109,137,150,202,277} Despite promising results in preclinical studies, clinical trials of immunotoxins have not shown significant clinical benefit.\textsuperscript{124,125,131,153,162,199,201-207,243,251}

One of the most studied immunotoxins in GBM is IL-13-PE, a recombinant protein of the cytokine IL-13 fused with the PE toxin. In normal cells, IL-13 binds to a heterodimeric receptor complex composed of IL-13 and IL-4 receptors. The majority of GBM cells, however, overexpress a mutant form of the IL-13 receptor, IL-13Rα2.\textsuperscript{124} Unlike the normal receptor for IL-13, the mutant IL-13Rα2 form binds IL-13 in the absence of IL-4, and it binds IL-13 with higher affinity.\textsuperscript{99-103,145,146} As with other immunotoxins, results of clinical trials with IL-13-PE have shown no improvement in patient outcomes.\textsuperscript{124,153,264}

Monoclonal antibodies can be conjugated to radionuclides, small molecule inhibitors, enzymes that require prodrug administration for activation, and chemotherapeutic agents for antibody-mediated targeted delivery into tumor cells.\textsuperscript{220} Radioimmunoconjugates, which consist of tumor-specific monoclonal antibodies conjugated to radioactive iodine, are in essence radiolabeled antibodies that allow targeted destruction of GBM cells through radiation-mediated DNA damage. Radioimmunoconjugates also target surface molecules overexpressed in GBM, including EGFR and integrin ανβ3. Similar to immunotoxins, clinical trials of radioimmunoconjugates have not demonstrated clinical benefits.\textsuperscript{136,227}

**Immunostimulatory gene therapy**

Immunomodulatory gene therapy uses the genetic material of cytokines, lymphocytes, or other immune modulators to enhance the host immune response to the tumor. Immunomodulatory approaches are based on the expectation that antitumor immune responses, activated by tumor-specific antigen expression, would effectively eliminate tumor cells. Unfortunately, often tumors develop mechanisms to evade these immune responses, creating an immune suppressive tumor microenvironment.\textsuperscript{28,102} Thus, the goal of immunostimulatory gene therapy is to improve the immune response against the tumor. Several immunomodulatory approaches have been tested in GBM, including cytokine-mediated gene therapy and immune cell recruitment strategies.

Cytokines are a group of immune effector molecules that play a critical role in initiating, supporting, and inhibiting specific immune pathways. Cytokine-mediated gene therapy aims to use these cytokines to facilitate immune surveillance and stimulate cell-mediated immune responses against tumor cells. It involves specific delivery of transgenes
for various immunostimulatory agents into tumor cells.\textsuperscript{[131]} Tumor cell expression of the gene is used to induce more potent immune responses to the tumor cell.\textsuperscript{[242]}

Immune cell recruitment strategies attempt to recruit DCs or other APCs into the tumor microenvironment in order to prime an immune response against GBM. One strategy to do this combines cytokine-mediated gene therapy with cytotoxic gene therapy. This approach involves delivery of fms-like tyrosine kinase-3 ligand (Flt3L), a cytokine that stimulates recruitment of DCs, as well as HSV1-TK into the surgical resection cavity.\textsuperscript{[122]} Following administration of the GCV, the dying tumor cells release the peptides that will activate the DCs that were recruited to the tumor microenvironment by Flt3L.\textsuperscript{[26,44,242]} This approach has mainly been studied in preclinical trials and has demonstrated an immune response with tumor regression and enhanced survival.\textsuperscript{[26,44]} Clinical trials evaluating this approach in patients with GBM have recently begun.\textsuperscript{[14,122,242]}

Oncolytic virotherapy

In oncolytic virotherapy, unlike in other approaches, viral toxicity is directly responsible for tumor cell death. Oncolytic viral vectors are engineered as conditionally replicating viruses that specifically infect and replicate within tumor cells, while sparing the surrounding normal brain.\textsuperscript{[242]} Oncolytic virotherapy takes advantage of the innate ability of lytic viruses to lyse and kill their host cells following infection.\textsuperscript{[122,131,255]} Upon lysis, virions spread to infect the surrounding tumor cells, propagating their toxicity.\textsuperscript{[45,242]} [Figure 4]. In order to be safe and effective, oncolytic viral vector replication and lytic destruction must be limited to tumor cells only, while being relatively avirulent in normal brain cells.\textsuperscript{[45,131]} This can be accomplished by attenuating the virus to allow restriction of its replication to rapidly dividing cells, permitting them to infect and lyse tumor cells but not normal, nonreplicating brain cells. Additional molecular strategies used to accomplish this include deletion of components of the viral genome necessary for viral replication that are not expressed in normal cells but aberrantly expressed in cancer cells, insertion of promoter sequences into the viral genome that drive the expression of replication genes under the control of unique transcription factors overexpressed in cancer cells, and engineering viral capsid proteins that use unique molecular targeting to selectively transduce cancer cells.\textsuperscript{[131]} Conditionally replicating HSV1 and adenoviruses vectors are the most commonly studied oncolytic viruses in clinical trials of GBM; however, other oncolytic viruses, such as reovirus, measles virus, and Newcastle disease virus, are also being investigated as therapeutic options for GBM.\textsuperscript{[26,71,131,132]}

The first oncolytic virus tested in GBM was conditionally replicating HSV1.\textsuperscript{[134,242]} HSV is a neurotropic virus; hence, it is critical that it is attenuated such that it only infects tumor cells in order to prevent toxicity to surrounding normal brain cells. Since it was first studied, HSV has been modified in different ways to achieve an adequate safety profile. \textsuperscript{[242]} G207 is one of the most widely studied oncolytic HSV vector. It has been genetically engineered to replicate only within rapidly dividing cells.\textsuperscript{[108]} Its HSV1-TK gene has been left intact, which allows oncolytic virotherapy with G207 to be combined with GCV to further increase its cytotoxic effects.\textsuperscript{[122]} In clinical trials, G207 was able to infect and replicate within tumor cells, but it was unable to kill tumor cells efficiently. Oncolytic HSV vectors genetically engineered to encode transgenes for cytotoxic or immunostimulatory proteins are also being explored.\textsuperscript{[16,246]} Overall, clinical trials with G207 and other oncolytic HSV vectors have shown high safety profiles, but they have demonstrated limited therapeutic efficacy.\textsuperscript{[118,119,244]}

Conditionally replicating adenoviruses have also been studied.\textsuperscript{[122]} Adenoviruses have been engineered to specifically replicate and lyse tumor cells in several different ways.\textsuperscript{[122]} An advantage of adenoviruses to HSV oncolytic viruses is that they are naturally nonneurotropic, which enhances their safety profile. ONYX-015 is an adenovirus that has been modified to only replicate in cells with a p53 mutation.\textsuperscript{[36,70,219,255]} Ad5-Delta is another adenovirus that only replicates in cells with defective retinoblastoma function.\textsuperscript{[13,37,34,79,250]} Clinical trials using conditionally replicating adenoviruses have just begun, but early results indicate a favorable safety profile.\textsuperscript{[16]}

A major drawback of oncolytic viruses is the host immune response, which limits their ability to spread
to surrounding cells and reduces their transduction efficiency. Currently, direct delivery of oncolytic virus to the tumor is the only method that has been used in clinical trials. Preclinical trials are investigating novel delivery of these viruses into the bloodstream, which include the use of neural and mesenchymal stem cells as vectors.[115,116,120]

OTHER NOVEL THERAPIES

A variety of other novel approaches for the treatment of GBM are currently being investigated. The NovoTTF-100A is a device that uses alternating electric fields to disrupt cell division, and it is currently being studied as a treatment option for GBM.[117] The use of thermal lasers to denature tumor tissues is another area being investigated.[99] Laser interstitial thermal therapy (LITT) is a minimally invasive thermoablative procedure that uses a laser to create low-powered thermal energy that heats and destroys tumor cells.[90,227] LITT has been used for several decades for the treatment of GBM and other tumors; however, its use has been limited due to several technical limitations, mainly difficulty in regulating the dose of LITT delivered to individual patients.[107,113,215-217,227] In order to overcome these technical limitations, the NeuroBlate System (Monteris Medical, Inc.) was developed with technical advances, such as real-time MRI-guided thermography to facilitate detection of thermal damage to tissue, that allow for safer and more precise delivery of LITT for treatment of GBM. Recently, there have been several Phase I clinical trials done to evaluate the safety of the NeuroBlate System.[91,227] Results of these trials have demonstrated that the NeuroBlate System LITT has a favorable safety profile in patients with GBM; however, additional studies assessing clinical efficacy and outcomes still need to be conducted.[91,227]

CONCLUSION

GBM is the most common and lethal primary malignancy of the CNS. Even with surgical resection and aggressive treatment with chemo-and radiotherapy, the prognosis remains very poor. Due to continued advancements, the understanding of the complex biology of GBM and its pathogenesis, a wide variety of novel therapeutic approaches have been developed and are currently under study as potential treatments for GBM. Despite promising results in preclinical trials, many of these therapies have provided limited or no therapeutic efficacy in human clinical trials. Thus, although survival of patients with GBM continues to slowly improve, treatment of GBM remains extremely challenging. Continued research and development of new molecular targeted and immunotherapeutic approaches, based on a detailed understanding of molecular pathogenesis, can reasonably be expected to lead to increased survival and more favorable prognosis of patients with GBM.

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