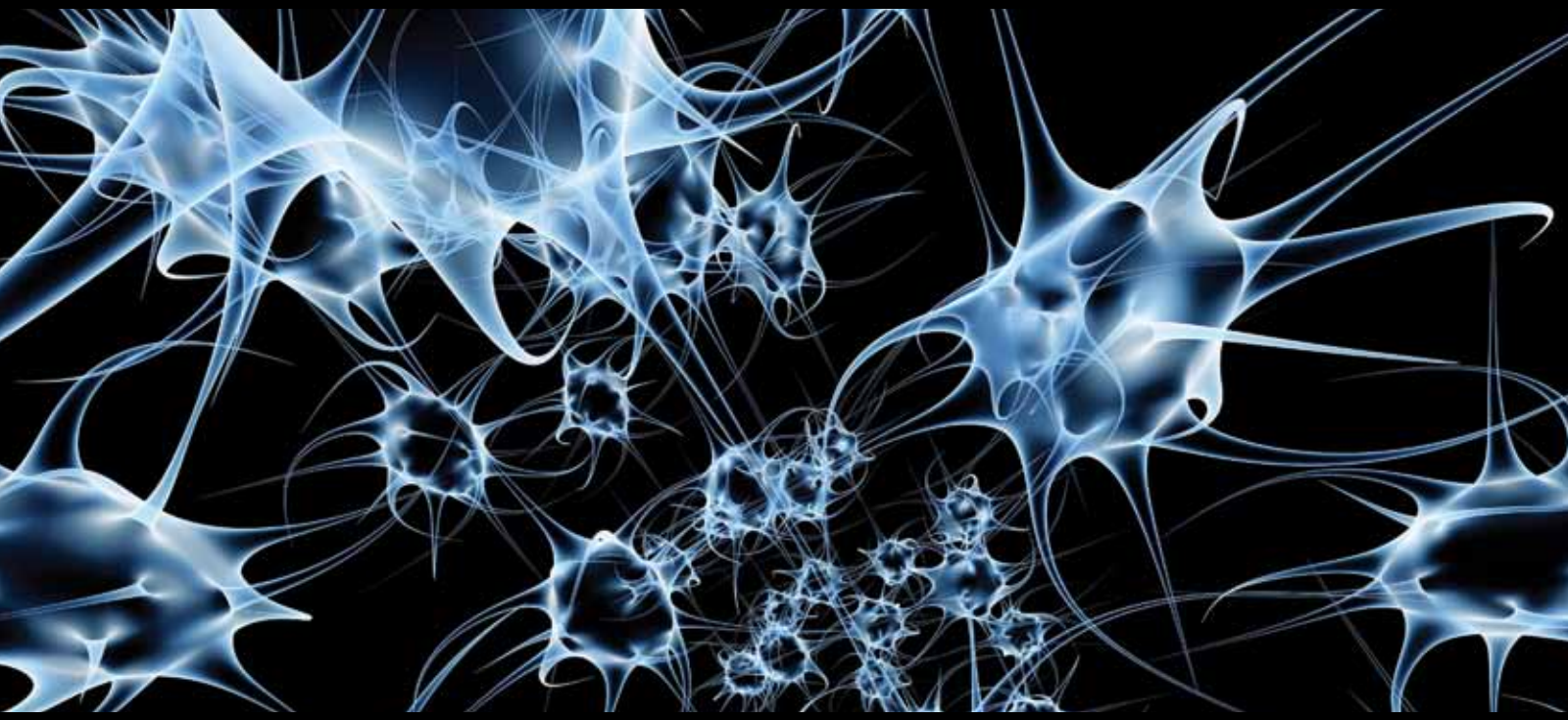
Neurology Research International

Glioblastoma Multiforme

Guest Editors: Jonas M. Sheehan, Robert Cavaliere, Elena Farace, Markus Bredel, and Stuart H. Burri



Glioblastoma Multiforme

Neurology Research International

Glioblastoma Multiforme

Guest Editors: Jonas M. Sheehan, Robert Caveliere, Elana Farace, Markus Bredel, and Stuart Burri

Editorial Board

Contents

Hindawi Publishing Corporation
Neurology Research International
Volume 2012, Article ID 819304, 1 page
doi:10.1155/2012/819304

Editorial

Glioblastoma Multiforme

Jonas M. Sheehan,[1] Robert Cavaliere,[2] Elena Farace,[3] Markus Bredel,[4] and Stuart H. Burri[5]

[1] *Penn State Institute of the Neurosciences and Penn State Cancer Institute, Hershey, PA 17033, USA*
[2] *The Ohio State University College of Medicine, Columbus, OH 43210, USA*
[3] *Departments of Health Evaluation Sciences and Neurosurgery, Penn State College of Medicine/Penn State Milton S. Hershey Medical Center, Hershey, PA 17033, USA*
[4] *Radiation Oncology Brain Tumor Laboratory, University of Alabama at Birmingham, Birmingham, AL 35233, USA*
[5] *Division of Radiation Oncology, Levine Cancer Institute, Carolinas HealthCare System, Charlotte, NC 28203, USA*

Correspondence should be addressed to Jonas M. Sheehan, jsheehan@hmc.psu.edu

Received 13 February 2012; Accepted 13 February 2012

Despite recent advances in medicine, including the molecular and genetic advances in the field of oncology, glioblastoma remains one of the deadliest cancers in humans. Advances over the past 30 years have led to measurable but modest improvement in survival. New approaches to treatment, including vaccine therapy, based on sound laboratory and preclinical data, are encouraging, but the key to a substantial impact in patients with glioblastoma has remained elusive.

This special issue was undertaken in an effort to demonstrate some of the novel approaches to basic science, diagnosis, and treatment for patients with glioblastoma. The first manuscript discuses the use of a leading edge MR imaging technique, texture analysis, to differentiate glioblastoma from malignant glioneuronal tumors, highlighting advanced imaging techniques that should be further explored across brain tumors.

Basic science advances are also discussed. One article discusses the cause/effect role of leptin in glioblastoma, with a discussion regarding a potentially central role in tumor progression. In addition, E. A. El Habr et al. elucidate the clinical and prognostic role of the AKT-mTOR pathway in astrocytomas. These basic science developments are important as clinically relevant therapies are developed by logical extension from basic and translational knowledge.

Two other manuscripts focus on current and future trends in the management of glioblastoma. While the outcomes for patients with this deadly disease remain poor, it is our sincere hope that the work and effort demonstrated by our authors, and scores of researchers around the world, will lead to substantial advances in our understanding and management of glioblastoma.

Jonas M. Sheehan
Robert Cavaliere
Elena Farace
Markus Bredel
Stuart H. Burri

Hindawi Publishing Corporation
Neurology Research International
Volume 2012, Article ID 878425, 13 pages
doi:10.1155/2012/878425

Review Article

Current Trends in Targeted Therapies for Glioblastoma Multiforme

Fumiharu Ohka, Atsushi Natsume, and Toshihiko Wakabayashi

Department of Neurosurgery, Nagoya University School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan

Correspondence should be addressed to Atsushi Natsume, anatsume@med.nagoya-u.ac.jp

Received 1 August 2011; Revised 21 October 2011; Accepted 7 December 2011

Academic Editor: Jonas Sheehan

Glioblastoma multiforme (GBM) is one of the most frequently occurring tumors in the central nervous system and the most malignant tumor among gliomas. Despite aggressive treatment including surgery, adjuvant TMZ-based chemotherapy, and radiotherapy, GBM still has a dismal prognosis: the median survival is 14.6 months from diagnosis. To date, many studies report several determinants of resistance to this aggressive therapy: (1) O^6-methylguanine-DNA methyltransferase (MGMT), (2) the complexity of several altered signaling pathways in GBM, (3) the existence of glioma stem-like cells (GSCs), and (4) the blood-brain barrier. Many studies aim to overcome these determinants of resistance to conventional therapy by using various approaches to improve the dismal prognosis of GBM such as modifying TMZ administration and combining TMZ with other agents, developing novel molecular-targeting agents, and novel strategies targeting GSCs. In this paper, we review up-to-date clinical trials of GBM treatments in order to overcome these 4 hurdles and to aim at more therapeutical effect than conventional therapies that are ongoing or are about to launch in clinical settings and discuss future perspectives.

1. Introduction

Glioblastoma multiforme (GBM) is one of the most frequently occurring tumors in the central nervous system and the most malignant tumor among gliomas. A subanalysis in an international randomized trial by the European Organization for Research and Treatment of Cancer/National Cancer Institute of Canada (EORTC/NCIC) compared the results of radiotherapy (RT) alone with those of concomitant RT and temozolomide (TMZ) and found that the addition of TMZ to radiotherapy for newly diagnosed GBM resulted significant survival benefit [1], additionally the subgroup analysis of the 5-year survival data of the EORTC/NCIC trial also revealed its benefit [2]. Since then, TMZ has been the current first-line chemotherapeutic agent for GBM. However, despite aggressive treatment including surgery, adjuvant TMZ-based chemotherapy, and RT, GBM still has a dismal prognosis: the median survival is 14.6 months from diagnosis. Many studies aim to overcome several determinants of resistance to conventional therapy by using various approaches to improve the dismal prognosis of GBM such as modifying TMZ administration and combining TMZ with other agents, developing novel molecular-targeting agents, and novel strategies targeting GSCs. In this paper, we review up-to-date clinical trials of GBM treatments in order to overcome determinants and to aim at more therapeutical effect than conventional therapy that are ongoing or are about to launch in clinical settings and discuss future perspectives.

2. Overcoming Alkylating Agent Resistance due to MGMT

MGMT is capable of counteracting the cytotoxicity induced by O^6-alkylating agents. Furthermore, increased MGMT expression is well correlated with *in vitro* and *in vivo* glioma resistance to TMZ [3–6]. However, in this process, MGMT is rapidly degraded via the ubiquitin-proteasome pathway after receiving alkyl groups from DNA; the repletion of cellular MGMT pools also depends on the resynthesis of the molecule [7]. This makes MGMT a suitable target for intervention to improve the therapeutic efficacy of TMZ.

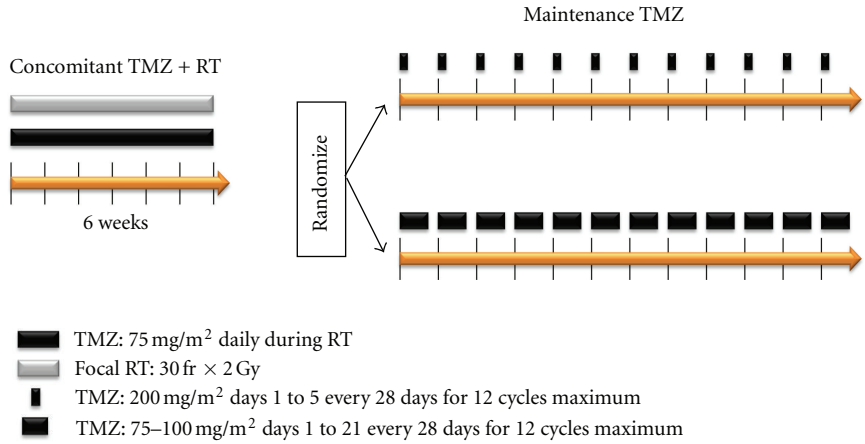


Figure 1: RTOG 0525/EORTC 26052-22053 (dose-dense) study. The study aimed to determine if intensified TMZ (75–100 mg/m^2 × 21 days, q4weeks) improves overall survival or progression-free survival compared to the standard arm (150–200 mg/m^2 × 5 days, q4weeks).

Additional treatment options are limited in cases of relapse after a standard-dose TMZ treatment (150–200 mg/m^2× 5 days, q4weeks). Supported by the assumption that continuous treatment with alkylating agents induces the depletion and exhaustion of MGMT activity, many researchers have investigated the effects of different dose- and time-modified TMZ schedules.

2.1. RTOG 0525/EORTC 26052-22053 (Dose-Dense) Study. This is a randomized phase III trial comparing standard adjuvant TMZ with a dose-dense schedule in newly diagnosed GBM [8]. This trial was based on a report indicating that dose-dense TMZ prolongs MGMT depletion in blood mononuclear cells and possibly tumors; the study aimed to determine if intensified TMZ (75–100 mg/m^2× 21 days, q4weeks) improves overall survival (OS) or progression-free survival (PFS) compared to the standard arm (150–200 mg/m^2× 5 days, 4weeks) after the standard concomitant RT+TMZ (Figure 1). No significant difference was observed between the standard and experimental arms with respect to median OS (16.6 versus 14.9 months, $P = 0.63$), median PFS (5.5 versus 6.7 months, $P = 0.06$), or *MGMT* methylation status. In addition, the experimental arm significantly increased grade ≥3 toxicity including lymphopenia and fatigue. This study did not demonstrate improved efficacy of dose-dense TMZ for newly diagnosed GBM regardless of *MGMT* methylation.

2.2. Continuous Dose-Intense TMZ in Recurrent Malignant Glioma: The RESCUE Study. There is no consensus on the optimal approach for patients with recurrent GBM, in which recurrence occurs after TMZ is initially used followed by 12 or more cycles of adjuvant therapy. Protracted drug exposure may reduce MGMT activity as described above. In addition, protracted TMZ dosing may inhibit endothelial cell recovery in the tumor and the activity of circulating endothelial precursors as well as upregulate thrombospondin-1, leading to an antiangiogenic effect [9–12]. Ninety-one patients with GBM were prospectively divided into 3 groups according to the timing of progression during adjuvant therapy: early, extended, and rechallenge [13] (Figure 2). All patients received 50 mg/m^2/day TMZ on a continuous (28/28) basis for a maximum of 12 months or until progression occurred. The primary endpoint of this study was 6 months PFS (PFS6). PFS6 was 27.3%, 7.4%, and 35.7% in the early, extended, and rechallenge groups, respectively; 1-year survival from time of study entry was 27.3%, 14.8%, and 28.6% for the 3 groups, respectively. The results of the RESCUE study suggest that patients who progress early compared with those who progress late or after a treatment-free interval may respond differently to the continuous dose-intense TMZ re-treatment. However, given that no consensus treatment option exists for patients with recurrent GBM, it would be of note that continuous dose-intense TMZ serves as a useful platform for combination strategies.

2.3. One-Week-on/One-Week-off TMZ in Elderly Patients with Newly Diagnosed Malignant Gliomas: The NOA-08 Study. Both surgery and radiation therapy are less tolerated in elderly patients than in younger ones. To reevaluate the widespread therapeutic nihilism with malignant glioma in the elderly (age >65), the Neurooncology Working Group (NOA) of the German Cancer Society conducted a randomized phase III trial to compare a 1-week-on/1-week-off TMZ schedule at 100 mg/m^2 with dose modification in 25 mg steps in both directions and involved field RT (54–60 Gy) in elderly patients with newly diagnosed anaplastic astrocytoma or GBM (NOA-08) [14]. The primary endpoint was the median OS during follow-up in the 12 months after the operation. Patient characteristics were balanced between arms in the intention-to-treat population ($n = 373$) except for more resections and more anaplastic astrocytomas in the RT arm. TMZ was not demonstrated to be superior. However, patients in the TMZ arm had an increased risk of death (HR = 1.24 [95% CI: 0.94–1.63]) compared with those in the RT arm. The rates of adverse and serious adverse events were also higher in the TMZ arm. This trial failed

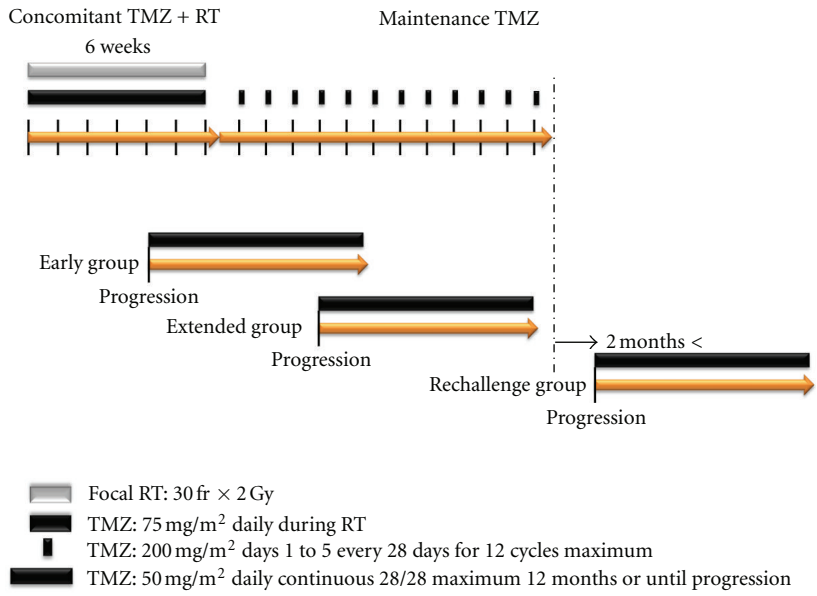


Figure 2: Continuous dose-intense TMZ in recurrent malignant glioma: the RESCUE study. Ninety-one patients with GBM were prospectively divided into 3 groups according to the timing of progression during adjuvant therapy: early, extended, and rechallenge.

to demonstrate the noninferiority of dose-intensified TMZ alone compared with RT alone in the primary treatment of older patients with malignant gliomas. Whether RT plus TMZ is superior to RT alone, is being addressed in an ongoing companion trial conducted by the NCIC, EORTC, and RTOG.

2.4. Combination with 1,3-Bis (2-Chloroethyl)-1-Nitrosourea: The TEMOBIC Study. Although TMZ replaced nitrosoureas such as 1,3-*bis* (2-chloroethyl)-1-nitrosourea (BCNU) as the standard initial chemotherapy in the treatment of GBM, the DNA damage induced by nitrosoureas and TMZ is partially repaired by MGMT. Thus, combined administration of nitrosoureas and TMZ might overcome MGMT-mediated resistance via MGMT depletion, yielding superior treatment results compared with the administration of TMZ alone. A phase II study was conducted in newly diagnosed anaplastic oligodendrogliomas [15]. This study assessed the efficacy and safety of BCNU (150 mg/m^2, day 1) and TMZ (110 mg/m^2, days 1–5) every 6 weeks for up to 6 cycles before conventional RT (60 Gy/30 fr) in 54 patients. Grade 3-4 toxicities included thrombopenia ($n = 20$), neutropenia ($n = 13$), and elevated transaminases ($n = 5$). Treatment was discontinued in 4 patients, and possible treatment-related deaths occurred in 3 patients. The combination should be carefully considered due to its significant toxicity.

2.5. Combination with O^6-Benzylguanine. O^6-benzylguanine (O^6-BG) is a potent inhibitor that irreversibly inactivates MGMT by covalently transferring its benzyl group to the cysteine residues of MGMT's active site [16]. As a result, O^6-BG enhances TMZ cytotoxicity in MGMT-proficient glioma cells both *in vitro* and *in vivo* but not in MGMT-deficient cells [17]. Since patients with MGMT overexpression in tumors respond more poorly to alkylating agents, co-administration of O^6-BG to deplete the tumor pools of MGMT to enhance drug cytotoxicity has been previously attempted in a clinical setting [18, 19]. However, systemic delivery of O^6-BG increased the myelotoxicity caused by MGMT depletion in bone marrow cells. Therefore, the dose of alkylating agents was reduced to a subtherapeutic level. Consequently, none of the patients responded to this drug combination. Therefore, the therapeutic potential of adding O^6-BG to enhance TMZ cytotoxicity in tumor cells has been discouraging thus far.

2.6. Combination with Cilengitide: The CORE Study. Cilengitide, a selective $\alpha v\beta 3/5$ integrin inhibitor, exhibits dose-dependent antitumor activity in patients with recurrent GBM [20]. A randomized controlled phase II trial (CORE) was designed as stepwise cilengitide intensification in subjects with newly diagnosed GBM and unmethylated *MGMT* promoter status [21]. The toxicity from this treatment was minimal. A further phase trial testing the use of intensified cilengitide (2000 mg, 5 days/week) in combination with concomitant RT with TMZ is now recruiting patients with newly diagnosed GBM with unmethylated *MGMT* status.

2.7. Combination with Interferon-β: The JCOG0911 (INTEGRA) Study. Interferon (IFN)-β exerts pleiotropic biological effects and is widely used either individually or in combination with other antitumor agents to treat malignant gliomas and melanomas [22]. In the treatment of malignant gliomas, IFN-β can act as a drug sensitizer by enhancing the toxicity of chemotherapeutic agents against various neoplasms when administered in combination with nitrosoureas. Combination therapy with IFN-β and nitrosoureas is primarily used

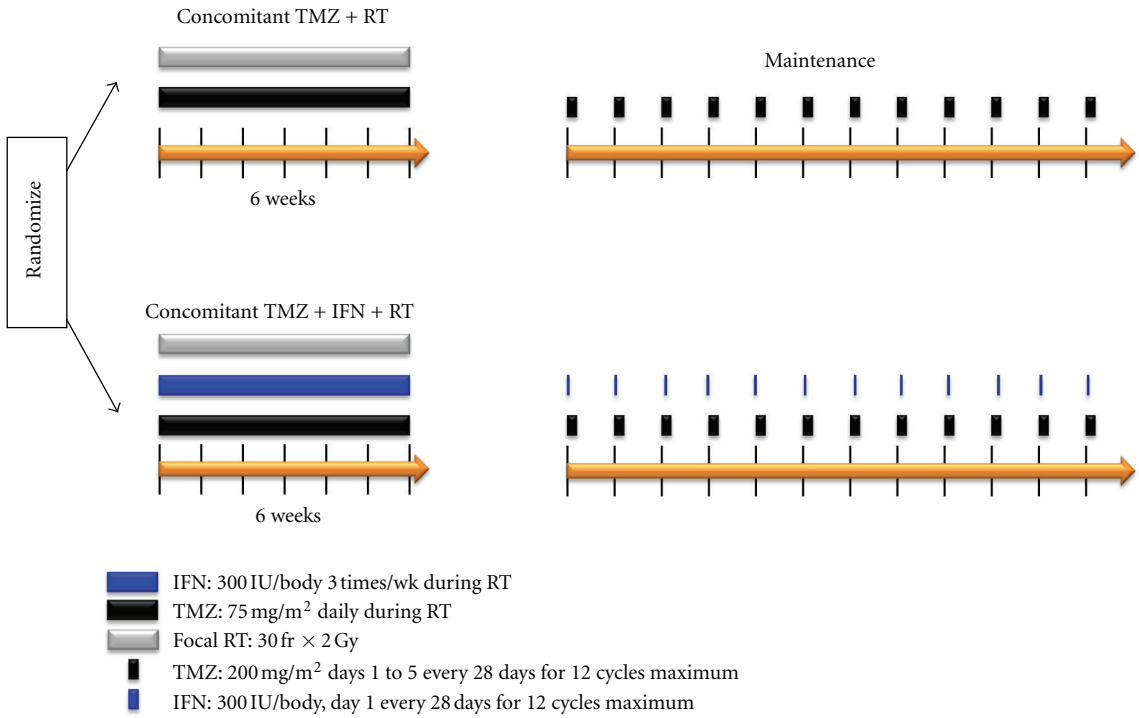


Figure 3: Combination with interferon-β: the JCOG0911 (INTEGRA) study. A randomized phase II clinical trial in patients with newly diagnosed GBM is under way to compare the standard-of-care regimen with the addition of IFN-β in the upfront settings.

for the treatment of gliomas in Japan [23]. In a previous *in vitro* study in human glioma cells, we found that IFN-β markedly enhances chemosensitivity to TMZ [24]; this finding suggests that one of the major mechanisms by which IFN-β enhances chemosensitivity is the downregulation of MGMT transcription via p53 induction. This effect was also observed in an experimental animal model [25]. The results of these 2 studies suggest that compared with chemotherapy with TMZ alone and concomitant RT, chemotherapy with IFN-β and TMZ with concomitant RT might further improve the clinical outcomes of malignant gliomas. To evaluate the safety, feasibility, and clinical effectiveness of combination therapy with IFN-β and TMZ, a phase I clinical study, the Integrated Japanese Multicenter Clinical Trial: a Phase I Study of Interferon-β and TMZ for Glioma in Combination with RT (INTEGRA study), was conducted. The primary endpoint was the incidence of adverse events. The exploratory endpoints were PFS and OS. The study population comprised 16 patients with newly diagnosed gliomas and 7 with recurrent high-grade gliomas. Grade 3-4 leukocytopenia and neutropenia were observed in 6.7% and 13.3% of the patients, respectively. Overall, 40% of the patients exhibited an objective response to therapy. In patients with newly diagnosed GBM, the median OS time was 17.1 months and the rate of 1-year PFS was 50%.

This regimen is safe and well tolerated by the patients, and may prolong the survival of patients with GBM. A randomized phase II clinical trial in patients with newly diagnosed GBM is under way to compare the standard-of-care regimen with the addition of IFN-β in the upfront settings (Figure 3).

3. Strategies Targeting the Altered Signaling Pathways (Figure 4)

3.1. VEGF Signaling. GBM is characterized by sustained angiogenesis—the key regulator of which is vascular endothelial growth factor (VEGF). Bevacizumab (Avastin, BEV) is a humanized monoclonal antibody that binds to and inhibits the activity of VEGF [26–29]. In preclinical models, BEV has been shown to exhibit activity against GBM both alone and in combination with RT and TMZ. The benefit and safety profile of BEV was confirmed in a randomized, noncomparative phase II trial (BRAIN study; AVF3708g) in GBM patients who experienced first or second recurrence following standard-of-care with TMZ [27]. In both the BEV ($n = 85$) and BEV plus irinotecan ($n = 82$) cohorts, objective response rate and PFS6 were significantly higher than those of the external historical controls. It might be important to state that the addition of irinotecan to bevacizumab did not improve outcome, and PFS6 as a primary endpoint is a controversial particularly in patients treated with antiangiogenic therapies (such as bevacizumab) that can lead to improved imaging findings without actual tumor response (a so-called pseudoresponse). In any case, in light of these results, studies were initiated to evaluate BEV in combination with RT plus TMZ as the upfront treatment for newly diagnosed GBM. In a noncomparative study in 70 newly diagnosed GBM patients, BEV in combination with RT plus TMZ resulted in median OS and PFS times of 19.6 and 13.6 months, respectively. In another nonrandomized phase II study [30], the effects of RT plus TMZ were compared with ($n = 25$) and without ($n = 31$) BEV;

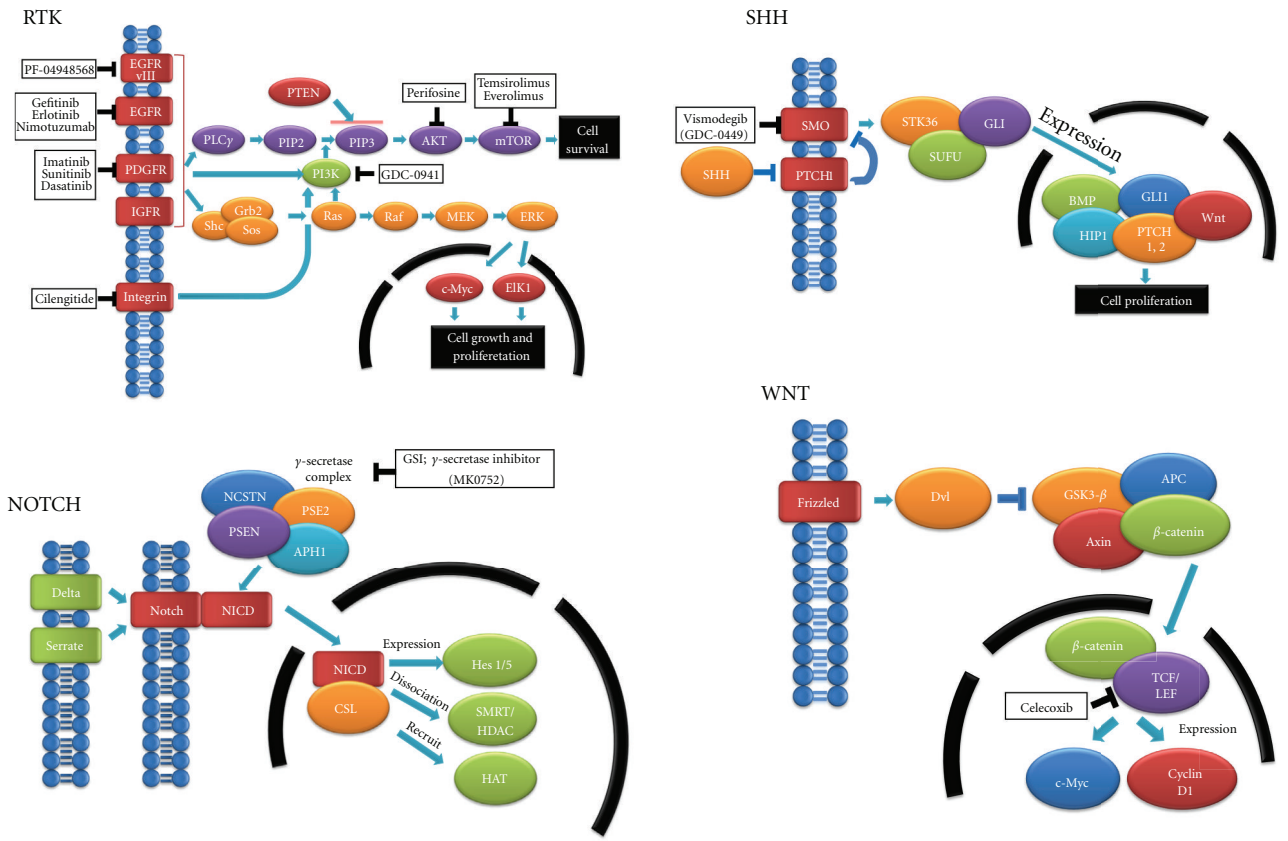


Figure 4: Altered signaling pathways and inhibitors. Receptor tyrosine kinase (RTK), *EGFR* and *platelet-derived growth factor receptor (PDGFR)* are receptor tyrosine kinases (RTKs). In GBM, 40–60% of cases exhibit *EGFR* amplification and high EGFR protein expression levels. *PDGFR* is overexpressed at the transcriptional level. *EGFR* activation initiates signal transduction such as the PI3K/Akt pathway. *EGFR variant III (EGFRvIII)* is a constitutively activated mutation of *EGFR*, that is, expressed in approximately 25% of GBM cases but not in normal tissues; Sonic Hedgehog (SHH), SHH is a secreted protein critical for pattern formation in the central nervous system. SHH ligand binding to its receptors, patched homolog (PTCH) and smoothened homolog (SMO), leads to the activation of gliotactin (GLI) transcription factors that are translocated into the nuclease to regulate various cellular activities, including the maintenance of cell stemness, survival, and proliferation; NOTCH, The Notch pathway is initiated by the binding of transmembrane ligands on one cell to the notch receptors on an adjacent cell. This binding causes the γ-secretase-mediated proteolytic release of the Notch intracellular domain (NICD). Released NICD translocates into the nucleus and then turns CSL (a transcriptional factor) from a repressor to an activator, causing various effects; WNT, Wnt signals are divided into 2 different pathways: the canonical or WNT/β-catenin pathway is involved in cell fate determination and the noncanonical pathway is involved in the control of cell movement and tissue polarity [35]. Following the binding of Wnt protein to a receptor complex comprising Frizzleds/low-density lipoprotein receptor-related protein (Fz/LRP), cytoplasmic disheveled (Dvl) is phosphorylated. The phosphorylation of Dvl inhibits the activity of glycogen synthase kinase-3β (GSK-3β), elevating nonphosphorylated β-catenin levels in the cytoplasm. β-Catenin translocates into the nucleus and forms a complex with members of the T-cell transcription factor (TCF)/lymphoid enhancer-binding factor (LEF) family of transcription factors.

RT plus TMZ with BEV resulted in increased PFS6 (87% versus 52%), median PFS (12 versus 7 months, $P = 0.0001$), 2-year OS rate (50% versus 22%), and median OS (24.0 versus 17.5 months, $P = 0.09$). A large randomized double-blind placebo-controlled phase III trial (AVAglio, BO21990, NCT00943826) is currently recruiting approximately 920 newly diagnosed GBM patients from 140 centers worldwide [31]. However, there is evidence suggesting that anti-VEGF treatment increases tumor cell invasion in GBM [32]. While BEV strongly decreases contrast enhancement, this is accompanied by a 68% increase in infiltrating tumor cells in the brain parenchyma. These data strongly suggest that vascular remodeling induced by anti-VEGF treatment leads to a more hypoxic tumor microenvironment. This favors a metabolic change in the tumor cells toward glycolysis, which leads to tumor cell invasion into normal brain tissue.

Cediranib is another potent oral VEGF signaling inhibitor that exhibits activity against all 3 VEGF receptors [33]. REGAL (NCT00777153) is a randomized phase III study comparing cediranib and lomustine (CCNU) in patients with recurrent GBM. Between October 2008 and September 2009, 325 patients from 10 countries were enrolled. The first clinical results of the REGAL trial were negative [34]. Other clinical trials are under way to assess cediranib either as a monotherapy or in combination with other agents.

3.2. EGFR and PDGF Signaling. To date, various genetic alterations are reported in GBMs such as e*pidermal growth factor receptor* (*EGFR*) amplification, *CDKN2A* loss, *phosphatase and tensin homolog* (*PTEN*) loss, and so forth. Among these various alterations, several alterations deregulate pathways involving the RTK/PI3K/Akt/mTOR pathway [36, 37], which is regarded as the most amenable pathway to pharmacologic intervention [38]. This pathway contains a number of key kinase intermediates as shown below. *EGFR* and *platelet-derived growth factor receptor (PDGFR)* are receptor tyrosine kinases (RTKs). In GBM, 40–60% of cases exhibit *EGFR* amplification and high EGFR protein expression levels [39, 40]. *PDGFR* is overexpressed at the transcriptional level. This *EGFR* activation initiates signal transduction such as the PI3K/Akt pathway. The phosphatidylinositol-3′ kinases (PI3Ks) are lipid kinases that are activated downstream of growth factor receptor signaling. Activated PI3Ks phosphorylate the lipid phosphatidylinositol (4, 5)-bisphosphate (PIP2) to generate phosphatidylinositol (3, 4, 5)-triphosphate (PIP3). PIP3 recruits Akt, which is the major effector of this pathway, to the cell membrane. Activated Akt enhances cell growth, survival, and proliferation and indirectly enhances the activity of mammalian target of rapamycin (mTOR), which controls cell growth by regulating various cellular processes. *PTEN* suppresses Akt phosphorylation by reversing PI3K-driven phosphorylation, resulting in the inhibition of the PIP3 signal thus suppressing cell proliferation [36, 41, 42]. Considering that these various alterations affect cell survival and proliferation, many studies suggest a novel strategy targeting these small molecules to improve the dismal prognosis of GBM [43].

To date, the small-molecule inhibitors of *EGFR* that were introduced in clinical trial include gefitinib, erlotinib, and nimotuzumab [44–46]. Gefitinib is an oral low-molecular-weight adenosine triphosphate mimetic of the anilinoquinazoline family. Gefitinib is an efficient therapeutic agent for a subset of patients with nonsmall-cell lung cancers (NSCLC), particularly the ones who harbor an activating *EGFR* mutation. However, several clinical trials evaluating the efficacy of gefitinib with or without TMZ in GBM report disappointing efficacy. Uhm et al. report that patients who exhibit adverse reactions to gefitinib (e.g., rash and diarrhea) have prolonged overall survival [47] Erlotinib is also an *EGFR* tyrosine kinase inhibitor (EGFR-TKI). This EGFR-TKI exhibits clinical activity particularly in tumors that have mutations in the adenosine triphosphate binding pocket of the tyrosine kinase domain of the *EGFR* gene. Although several clinical trials have evaluated the efficiency of erlotinib, almost all failed to demonstrate its efficiency or any additional benefit [45, 48]. Nimotuzumab was tested in an open-label randomized phase III trial in 150 patients with newly diagnosed GBM, but the interim analysis has failed to demonstrate efficacy [49]. *EGFR variant III (EGFRvIII)* is a constitutively activated mutation of *EGFR* that is expressed in approximately 25% of GBM cases but not in normal tissues. PF-04948568 is a vaccine containing a 13-amino acid sequence unique to EGFRvIII. A randomized phase IIb/III ACTIII study was initiated; the primary objective was to reject the null hypothesis that less than 53% of patients will be progression-free at 5.5 months from first vaccination [50]. Since this vaccination was well tolerated and the null hypothesis was rejected, further study is warranted. Afatinib, an irreversible erbB family blocker, exhibits high *in vitro* activity in tumor cell lines resistant to reversible EGFR inhibitors. This study compared the afatinib alone and afatinib with TMZ therapies with TMZ alone therapy [51]. The results show that afatinib alone is less effective than TMZ alone. Furthermore, afatinib with TMZ is comparable to TMZ alone.

Another TKI activating the PI3K/Akt/mTOR pathway is *PDGFR*. Imatinib blocks the ATP-binding site of tyrosine kinase proteins including *PDGFR* and inhibits the activity of *PDGFR*. The clinical efficiency of imatinib against other cancers such as chronic myeloid leukemia (CML) and gastrointestinal stromal tumors (GISTs) has been demonstrated [52–54]. In addition, since imatinib is active in GBM cell lines and mouse models, several clinical trials have evaluated its efficiency in GBM patients [43, 55, 56]. However, most of these clinical trials were not able to demonstrate any advantage of imatinib. It should be noted that most of these clinical trials to date enrolled unselected patients in whom the relative importance of each dysregulated molecule affecting tumor growth was largely unknown, which may be why these clinical trials demonstrate no advantage. Sunitinib is an orally available multitarget TKI of FDR, PDGFR, and c-kit. It exhibits broad-spectrum antiangiogenic activity. A phase II trial with stratification of prior use of BEV was designed for recurrent GBM to assess the safety and efficacy of 37.5 mg sunitinib administered on a continuous daily schedule. Twenty-eight and twenty-one patients have been enrolled in the BEV-resistant and BEV-naïve arms, respectively [57]. However, no patient has reached PFS6 at all. The efficacy of dasatinib, a PDGF and Src inhibitor, was evaluated retrospectively in recurrent malignant gliomas [58]. Twenty patients were treated with dasatinib alone, and in combinations with BEV and other anticancer drugs, or BEV-naïve. Dasatinib alone or in combination with BEV did not exhibit activity because low central nervous system penetration may limit its activity.

3.3. Targeting the PI3K/Akt/mTOR Pathway. Following the activation of RTK, the activated PI3K/Akt/mTOR pathway induces cell growth and proliferation. In addition to the RTK inhibitors described above, several studies suggest potential therapeutic targets of PI3K, Akt, and mTOR [38, 59, 60]. A preclinical study demonstrated that LY294002 and wortmannin inhibit PI3K. Because of toxic effects, poor pharmaceutical properties, and a lack of selectivity, the use of these agents was restricted in the preclinical study. Recently, the thienopyrimidine drug GDC-0941 was found to exhibit excellent oral anticancer activity in a preclinical study and is now undergoing a phase I clinical trial in cancer patients [59]. In addition, imidazopyridines, pyridopyrimidines, quinazolyne derivatives, thiazoles, azolepyrimidine derivatives, and other chemotypes are reported to inhibit the PI3K. Akt is also heavily targeted for therapy. The phospholipid perifosine is suggested to interfere with the association of

the Akt PH domain with PIP3, thus blocking the membrane localization of the protein. Perifosine is currently undergoing phase II clinical trials for prostate, head and neck, breast, and pancreatic cancers, melanomas, and sarcomas [61]. The mTOR kinase is intimately linked to PI3K/Akt signaling as well as the regulation of protein synthesis, cell growth, and survival. The dysregulation of mTOR is regarded as a therapeutic target. In addition, rapamycin and its analogs inhibit mTOR kinase via a rather indirect fashion. At present, 2 rapamycin analogs, temsirolimus and everolimus, are approved for the treatment of metastatic renal cell cancer [62]. Several clinical trials evaluating the efficiency of mTOR inhibitors such as temsirolimus in gliomas have been performed. The results of these trials suggest that monotherapy with temsirolimus does not prolong survival but combination therapy enhances its efficiency [43].

3.4. Glutamate Pathway. Glutamate is a major excitatory neurotransmitter in CNS. It is stored in synaptic vesicles and released as neurotransmitter. After it serves as neurotransmitter, it is rapidly took up at the plasma membrane of neurons, glial cells, and terminated. Glioma cells released glutamate in concentration, and glutamate reuptake is reduced due to reduction of glutamate transporter. This increased glutamate influences the surrounding cells and the glutamatergic system is associated with the proliferation, survival and migration of gliomas. Talampanel is an antagonist of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) glutamate receptor. The phase 2 trial of Talampanel combined with conventional TMZ and radiation for 72 newly diagnosed GBM patients showed median survival time of 18.3 months. This trial suggested the efficiency of Talampanel [63]. The other phase 2 trial evaluated the efficiency of Talampanel as single agent for recurrent malignant glioma patients. This trial shows no significant activity as single agent (median OS; 13 weeks) [64].

3.5. Histone Deacetylase. Vorinostat is an oral histone deacetylase (HDAC) inhibitor. HDAC acts on nucleosomal histones, leading to the tight coiling of chromatin and silencing of the expression of various genes. HDAC regulates cell survival, proliferation, tumor cell differentiation, cell cycle arrest, and apoptosis. There is preclinical evidence that vorinostat has antitumor activity against malignant glioma cell lines *in vitro* and orthotopic xenografts *in vivo*. Animal experiments also support the conclusion that vorinostat crosses the blood-brain barrier [65]. On the basis of these results, a phase I study of vorinostat in combination with TMZ was performed and revealed that this treatment is well tolerated; a phase II trial is under way [66].

4. Strategy Targeting Glioma-Initiating Cells

Several studies revealed that gliomas harbor a small population of cells termed glioma stem-like cells (GSCs) [67, 68]. GSCs have the ability to undergo self-renewal and initiate tumorigenesis. GBM forms extensively heterogeneous bulk tumors; this heterogeneity may be crucial for treating this disease. The presence of GSCs may be an important clue in clarifying the details of this heterogeneity. In addition, GSCs are resistant to a wide variety of chemotherapeutic agents and possess a remarkable ability to recover from cytotoxic therapy [69]. Furthermore, GSCs play a crucial role in RT failure, as tumors surviving RT are enriched in GSCs. Therefore, an alternative strategy involving selective targeting of this functionally distinct chemo- and radiation-resistant small group of GSCs rather than the bulk of the tumor may be more successful in treating this deadly disease [70]. GSCs exhibit various alterations to signaling pathway activity including PTEN, sonic hedgehog (SHH), notch, wingless-type MMTV integration site family member (WNT), maternal embryonic leucine-zipper kinase (MELK), and B lymphoma Mo-MLV insertion region 1 (BMI1), which are associated with self-renewal and neoplastic proliferation. These altered pathways may represent possible targets for GSCs.

4.1. PI3K/Akt/mTOR Pathway Including the PTEN Pathway. PTEN suppresses Akt phosphorylation by reversing PI3K-driven phosphorylation, resulting in the inhibition of PIP3 signaling and the suppression of cell proliferation. The *PTEN* gene is mutated in 15–40% of primary GBM cases. PTEN deletions with retinoblastoma-associated protein (pRb) inactivation or ABCG2 transporter activation accelerate the formation of aggressive high-grade tumors and GSC-like neurosphere formation capacity in a transgenic mouse model of glioma [71–75]. Although PTEN is one of the most remarkable targets involved in GSC activity, its status in GSCs has yet to be elucidated. The dysfunction of PTEN leads to the activation of the PI3K/Akt/mTOR pathway. Therefore, therapy targeting the PI3K/Akt/mTOR pathway described above may be also effective against GSCs exhibiting PTEN dysfunction. Thus, these results suggest the potential of efficiency of such therapies for GSCs.

4.2. SHH Pathway. SHH is a secreted protein critical for pattern formation in the central nervous system. SHH ligand binding to its receptors, patched homolog (PTCH) and smoothened homolog (SMO), leads to the activation of glio-tactin (GLI) transcription factors that are translocated into the nuclease to regulate various cellular activities, including the maintenance of cell stemness, survival, and proliferation. Altaba et al. demonstrate that SHH signaling regulates the expression of stemness genes such as *Nanog homeobox* (*NANOG*), *SRY-box containing gene 2* (*SOX2*), and *octamer-binding protein 4* (*OCT4*). In addition, they demonstrate that SHH-GLI signaling is required not only for sustained glioma growth and survival but also for GSC survival and proliferation [76]. Considering these reports, the inhibition of the SHH signaling pathway may be a target of therapy. The novel SMO inhibitor, vismodegib (GDC-0449), exhibits antitumor activity in a mouse model of medulloblastoma and primary human tumor cell xenograft models including colorectal cancer and pancreatic carcinoma. A phase I clinical trial was initiated on the basis of these preclinical tests; the results demonstrate that vismodegib is generally well tolerated with an acceptable safety profile in refractory

locally advanced metastatic solid tumors, including basal cell carcinoma and medulloblastomas [77]. Therefore, the SHH signaling pathway may be a potential target for therapy against GSCs.

4.3. Notch Pathway. The Notch pathway is initiated by the binding of transmembrane ligands on one cell to the notch receptors on an adjacent cell. This binding causes the γ-secretase-mediated proteolytic release of the Notch intracellular domain (NICD). Released NICD translocates into the nucleus and then turns CSL (a transcriptional factor) from a repressor to an activator, causing various effects [78]. Notch controls the specification, proliferation, and survival of nonneoplastic neural precursors and is aberrantly activated in embryonal brain tumors, suggesting a molecular link between neural stem cells and medulloblastomas. Previously, Sullenger et al. demonstrated that GSCs promote radioresistance compared with GBM tumor bulk because GSCs preferentially activate the DNA damage-response pathway. Notch signaling plays an important role in this DNA damage response pathway via the activation of the PI3K/Akt pathway and the pro-survival protein, Mcl-1. Notch pathway inhibition using γ-secretase inhibitors (GSIs; MK0752) impairs cell growth, clonogenic survival, and tumor formation ability and sensitizes GSCs to radiation at clinically relevant doses [79, 80].

4.4. Wingless-Type MMTV Integration Site Family Member (Wnt) Pathway. Wnt signals are divided into 2 different pathways: the canonical, or WNT/β-catenin pathway is involved in cell fate determination and the noncanonical pathway is involved in the control of cell movement and tissue polarity [35]. Following the binding of Wnt protein to a receptor complex comprising Frizzleds/low-density lipoprotein receptor-related protein (Fz/LRP), cytoplasmic disheveled (Dvl) is phosphorylated. The phosphorylation of Dvl inhibits the activity of glycogen synthase kinase-3β (GSK-3β), elevating nonphosphorylated β-catenin levels in the cytoplasm. β-Catenin translocates into the nucleus and forms a complex with members of the T-cell transcription factor (TCF)/lymphoid enhancer-binding factor (LEF) family of transcription factors [81]. Epigenetic silencing and loss-of-function mutations of negative regulators of WNT signaling are observed in a variety of human cancers. It is suggested that Wnt signaling is also involved in the regulation of cancer stem cells because of the many similarities in the pathways that regulate normal and cancer stem cells. Therefore, the inhibition of Wnt signaling may disrupt the maintenance of the stemness of GSCs. Although they include preclinical agents, several agents inhibiting the Wnt pathway are suggested for potential clinical use in a review by Takahashi-Yanaga and Kahn [82]. Of these agents, those that are used clinically are NSAIDs such as aspirin, sulindac, and celecoxib; celecoxib is the only NSAID approved by the Food and Drug Administration (FDA) for the treatment of familial adenomatous polyposis. The inhibition of the Wnt pathway by celecoxib has been shown by its ability to induce the degradation of TCF.

These various pathways altered in GBM or in the presence of GSCs with altered signaling pathways may induce resistance to conventional therapy. In addition, in other cancers, several studies suggest the efficiency of various small-molecule inhibitors. Although several clinical trials of these inhibitors in GBM have been performed, almost all failed to demonstrate the efficiency of these inhibitors compared with conventional therapy. We expect that combinations of these agents may overcome resistance to treatment or change the definition of patients who should be treated by each agent to induce a more favorable response.

5. Bypassing the Blood-Brain Barrier

The blood-brain barrier blocks most molecules that are larger than ~500 Da. Many drugs are denied access to the very regions where they would be effective, thus limiting the clinical application of most anticancer drugs for treating brain tumors. Each anticancer agent showed various permeability for BBB, although the relationship of its permeability to therapeutic efficacy is not clear [83]. Although several local therapies are attempted to overcome this BBB or "blood-tumor barrier," local therapies should be more developed to deliver therapeutic agents in more distant locations due to highly infiltrative nature of high-grade gliomas.

5.1. Gliadel (Carmustine, BCNU) Wafers. A meta-analysis combining the results of the randomized phase III trial published by Westphal et al. [84] and an earlier randomized phase III study by Valtonen et al. [85] demonstrates that the subgroup of GBM treatments with BCNU wafers increases mean survival to 13.1 months compared with 10.9 months for placebo patients ($P = 0.03$). The results of the 2 trials led the FDA to approve Gliadel for the treatment of newly diagnosed GBM in 2003. A combination of local BCNU wafer treatment and concomitant radiochemotherapy with TMZ is attractive not only because it may significantly reduce the toxicity of a systemic combination of BCNU and TMZ as described above but also because it may take advantage of the sensitizing effect of TMZ and BCNU on their respective resistance by MGMT [86]. However, several complications are associated with the implantation of BCNU wafers, including cerebral edema, healing abnormalities, cerebral spinal fluid leaks, intracranial infections, seizures, hydrocephalus, and cyst formation. The rates for these adverse events were well established in 2 randomized phase III trials that compared BCNU wafers with placebo ones. Gliadel wafer implantation may add to the toxicity of concomitant radiochemotherapy with systemic TMZ. Therefore, the combined approach requires special attention [87].

5.2. Convection-Enhanced Delivery. A direct intracerebral approach called convection-enhanced delivery (CED) may be used as a strategy to address these issues [88–90]. CED employs positive pressure that generates a local pressure gradient to distribute agents in the extracellular space. Unlike diffusion delivery, CED is not significantly affected by the concentration, molecular weight, or particle size of the agent. Furthermore, CED ensures high concentrations and the

homogenous distribution of a drug throughout a given target tissue.

5.3. CED of IL13-PE38QQR (Cintredekin Besudotox) for Recurrent GBM: The PRECISE Study. Interleukin (IL)-13 is a cytokine derived from type 2 T-helper cells and can bind to 2 receptor chains: IL-13Rα1 and IL-13Rα2; IL-13 has low affinity for the IL-13Rα1 chain and high affinity for the IL-4Rα chain. It forms a receptor complex with the IL-4Rα chain, which is involved in IL-13-induced signal transduction via either Janus kinase/signal transducer and activator of transcription (JAK-STAT) or PI3K [91]. The IL-13Rα2 chain binds to IL-13 with high affinity and internalizes it after ligand binding without the involvement of other chains.

IL-13R is overexpressed in a majority of glioma cell lines and resected GBM specimens [92]. A chimeric fusion protein composed of human IL-13 and mutated forms of *Pseudomonas aeruginosa* exotoxin A (PE38QQR) has been developed and shown to affect the specific cytotoxicity of glioma cell lines [92, 93].

IL-13-PE is reported to be more active against glioma cell lines than IL-4-targeted toxins *in vitro* [93]. In a phase I trial, 51 patients with GBM were administered IL-13-PE38QQR via CED [94]. A phase III study was conducted to compare the efficacy of IL-13-PE to that of Gliadel wafers in patients with malignant gliomas [93]. PFS was longer (17.7 versus 11.4 weeks) in patients treated with IL-13-PE than in patients treated with Gliadel wafers; however, there was no significant difference in the median survival time between the 2 groups.

Overall, IL-13-based toxins can be potentially used in adjuvant therapy for malignant gliomas, but their use requires further clinical studies.

5.4. CED of TGF-β Antisense: The SAPPHIRE Study. Transforming growth factor-*beta* (TGF-β) is a multifunctional regulatory polypeptide belonging to a ligand superfamily that includes the TGF-βs, activins, and bone morphogenetic proteins (BMPs). TGF-β controls many aspects of cellular function including proliferation, differentiation, migration, apoptosis, adhesion, angiogenesis, immune surveillance, and survival. Three TGF-β isoforms have been found: TGF-β1, TGF-β2, and TGF-β3. The TGF-β2 isoform is specifically overexpressed in malignant gliomas. Increased TGF-β2 levels are associated with advanced disease stage and cause immunodeficiencies in patients with gliomas [95]. TGF-β2 not only inhibits lymphocyte proliferation but also has multiple effects on the immune system. These effects include inhibition of immune cell activation, blockage of antitumor activity, shift in cytokine balance toward immunosuppression, and inhibition of antigen presentation. Thus, the targeted inhibition of TGF-β2 should have an antitumor effect and allow an immune-mediated response. Several approaches to block TGF-β function are currently being studied including the use of monoclonal antibodies against TGF-β, recombinant fusion proteins containing the ectodomains of TGF-β receptor (TβR)II and TβRIII to prevent the binding of TGF-β ligands, ATP competitive inhibitors at the ATP-binding site of TβRI kinase, and antisense oligonucleotides specific for TGF-β2 [96–101].

Trabedersen (AP-12009) is a synthetic antisense oligodeoxynucleotide designed to block TGF-β2 production [102]. In a randomized controlled phase IIb trial involving patients with brain tumors, the survival rates of patients for whom trabedersen was intratumorally administered were higher than those of patients receiving standard chemotherapy [103]. An international clinical phase III trial is currently recruiting patients with recurrent or refractory anaplastic astrocytoma. A randomized controlled dose-finding phase IIb study evaluated the efficacy and safety in 145 patients with recurrent or refractory high-grade gliomas [104]. The patients were randomly assigned to receive 10 or 80 μM trabedersen or standard chemotherapy. The primary end point was 6-month tumor control rate. Although this study failed to meet the primary end point, it could be due to the pseudoprogression that occurs with immune therapies. A prescribed anaplastic astrocytoma subgroup analysis found a significant benefit for 10 μM trabedersen with respect to the 14-month tumor control rate. The 2-year survival rate for 10 μM trabedersen tended to be superior to those of the other treatments. An international clinical phase III trial is currently recruiting patients with recurrent or refractory anaplastic astrocytomas with end points of 14-month progression rate and 2-year survival rate.

6. Future Perspectives

The Cancer Genome Atlas (TCGA) is a project that catalogs genomic abnormalities that are involved in the development of cancer [105, 106]. TCGA published the results of its first study in a large GBM cohort consisting of 206 patient samples. Techniques that are currently used for detection of abnormalities include gene-expression profiling, copy-number variation profiling, single-nucleotide polymorphism (SNP) genotyping, genome-wide methylation profiling [107], microRNA profiling [108], and exon sequencing. Since the publication of the first paper, several analysis groups within the TCGA Network have presented the results of highly detailed analyses of GBM. Verhaak et al. [109] recently subclassified GBM into proneural, neural, classical, and mesenchymal subtypes by integrating multidimensional data on gene expression, somatic mutations, and DNA copy number. The major features of the Proneural class are focal amplification of *PDFRA*, *IDH1* mutation, and *TP53* mutation and/or loss of heterozygosity. Moreover, the group showed high expression of genes associated with oligodendrocyte development, such as *PDGFRA, NKX2-2*, and *OLIG2*. The Neural subtype is characterized by the expression of neuron markers such as *NEFL*, *GABRA1*, *SYT1*, and *SLC12A5*. The Classical subtype features high *EGFR* expression associated with chromosome 7 amplification and low expression of *p16INK4A* and *p14ARF*, resulting from focal 9p21.3 homozygous deletion. Neural stem cell markers such as nestin, as well as components of the Notch and Sonic hedgehog signaling pathways, are highly expressed in the Classical type. The Mesenchymal subtype is characterized by focal hemizygous deletions at 17q11.2 that contains *NF1*

and high expression of *YKL-40 (CHI3L1)*, *MET*, *CD44*, and *MERTK*. Genes in the tumor necrosis factor superfamily pathway and NF-kappaB pathway, such as *TRADD*, *RELB*, and *TNFRSF1A*, are highly expressed in this subtype. The classification of GBM may lead to establishment of personalized therapies for groups of patients with GBM. However, the results of clinical studies of EGFR and PDGFR inhibitors as monotherapy are disappointing thus far. While research and development of more promising molecular-targeted agents are needed in the laboratory, molecular-targeted agents are likely to have synergistic antitumor effects in combination. On the other hand, strategies of how to evaluate better ways to design early phase clinical trials, to choose better endopoints should avoid trials that will not provide helpful answers. The discrepancy between PFS and OS as endpoints are still controversial; also the question when and how to integrate new therapies into the backbone of standard therapy still remains.

Authors' Contribution

Equally contributed to preparation of this manuscript.

References

[1] R. Stupp, W. P. Mason, M. J. van den Bent et al., "Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma," *The New England Journal of Medicine*, vol. 352, no. 10, pp. 987–996, 2005.

[2] R. Stupp, M. E. Hegi, W. P. Mason et al., "Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase IIII study: 5-year analysis of the EORTC-NCIC trial," *The Lancet Oncology*, vol. 10, no. 5, pp. 459–466, 2009.

[3] J. P. Fruehauf, H. Brem, S. Brem et al., "In vitro drug response and molecular markers associated with drug resistance in malignant gliomas," *Clinical Cancer Research*, vol. 12, no. 15, pp. 4523–4532, 2006.

[4] J. Ma, M. Murphy, P. J. O'Dwyer, E. Berman, K. Reed, and J. M. Gallo, "Biochemical changes associated with a multidrug-resistant phenotype of a human glioma cell line with temozolomide-acquired resistance," *Biochemical Pharmacology*, vol. 63, no. 7, pp. 1219–1228, 2002.

[5] M. Hermisson, A. Klumpp, W. Wick et al., "O6-methylguanine DNA methyltransferase and p53 status predict temozolomide sensitivity in human malignant gli-oma cells," *Journal of Neurochemistry*, vol. 96, no. 3, pp. 766–776, 2006.

[6] D. M. Kokkinakis, D. B. Bocangel, S. C. Schold, R. C. Moschel, and A. E. Pegg, "Thresholds of O6-alkylguanine-DNA alkyltransferase which significant resistance of human glial tumor xenografts to treatment with 1,3-bis(2-chloroethyl)-1-nitrosourea or temozolomide," *Clinical Cancer Research*, vol. 7, no. 2, pp. 421–428, 2001.

[7] K. S. Srivenugopal, X. H. Yuan, H. S. Friedman, and F. Ali-Osman, "Ubiquitination-dependent proteolysis of O6-methylguanine-DNA methyltransferase in human and murine tumor cells following inactivation with O6-benzylguanine or 1,3-bis(2-chloroethyl)-1-nitrosourea," *Biochemistry*, vol. 35, no. 4, pp. 1328–1334, 1996.

[8] M. Gilbert, "RTOG 0525: a randomized phase IIII trial comparing standard adjuvant temozolomide (TMZ) with a dose-dense (dd) schedule in newly diagnosed glioblastoma (GBM)," *Journal of Clinical Oncology*, vol. 29, supplement, 2011, Abstract no. 2006.

[9] R. S. Kerbel and B. A. Kamen, "The anti-angiogenic basis of metronomic chemotherapy," *Nature Reviews Cancer*, vol. 4, no. 6, pp. 423–436, 2004.

[10] F. Bertolini, S. Paul, P. Mancuso et al., "Maximum tolerable dose and low-dose metronomic chemotherapy have opposite effects on the mobilization and viability of circulating endothelial progenitor cells," *Cancer Research*, vol. 63, no. 15, pp. 4342–4346, 2003.

[11] U. Emmenegger, S. Man, Y. Shaked et al., "A comparative analysis of low-dose metronomic cyclophosphamide reveals absent or low-grade toxicity on tissues highly sensitive to the toxic effects of maximum tolerated dose regimens," *Cancer Research*, vol. 64, no. 11, pp. 3994–4000, 2004.

[12] G. Bocci, G. Francia, S. Man, J. Lawler, and R. S. Kerbel, "Thrombospondin 1, a mediator of the antiangiogenic effects of low-dose metronomic chemotherapy," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 22, pp. 12917–12922, 2003.

[13] J. R. Perry, K. Bélanger, W. P. Mason et al., "Phase II trial of continuous dose-intense temozolomide in recurrent malignant glioma: RESCUE study," *Journal of Clinical Oncology*, vol. 28, no. 12, pp. 2051–2057, 2010.

[14] W. Wick, "NOA-08 randomized phase IIII trial of 1-week-on/1-week-off temozolomide versus involved-field radiotherapy in elderly (older than age 65) patients with newly diagnosed anaplastic astrocytoma or glioblastoma (Methusalem)," *Journal of Clinical Oncology*, vol. 28, supplement, 2010, Abstract no. LBA2001.

[15] O. Chinot, " TEMOBIC: a ANOCEF phase II study of BCNU and temozolomide (TMZ) combination prior to radiotherapy (RT) in anaplasic oligodendroglial gliomas (AOG)," *Journal of Clinical Oncology*, vol. 29, supplement, 2011, Abstract no. 2034.

[16] L. Liu and S. L. Gerson, "Targeted modulation of MGMT: clinical implications," *Clinical Cancer Research*, vol. 12, no. 2, pp. 328–331, 2006.

[17] T. Kanzawa, J. Bedwell, Y. Kondo, S. Kondo, and I. M. Germano, "Inhibition of DNA repair for sensitizing resistant glioma cells to temozolomide," *Journal of Neurosurgery*, vol. 99, no. 6, pp. 1047–1052, 2003.

[18] J. A. Quinn, J. Pluda, M. E. Dolan et al., "Phase II trial of carmustine plus O6-benzylguanine for patients with nitrosourea-resistant recurrent or progressive malignant glioma," *Journal of Clinical Oncology*, vol. 20, no. 9, pp. 2277–2283, 2002.

[19] J. A. Quinn, A. Desjardins, J. Weingart et al., "Phase I trial of temozolomide plus O6-benzylguanine for patients with recurrent or progressive malignant glioma," *Journal of Clinical Oncology*, vol. 23, no. 28, pp. 7178–7187, 2005.

[20] D. A. Reardon, K. L. Fink, T. Mikkelsen et al., "Randomized phase II study of cilengitide, an integrin-targeting arginine-glycine-aspartic acid peptide, in recurrent glioblastoma multiforme," *Journal of Clinical Oncology*, vol. 26, no. 34, pp. 5610–5617, 2008.

[21] B. Nabors, "Cilengitide in patients with newly diagnosed patients with glioblastoma multiforme and unmethylated MGMG gene promoter: safety run-in results from a randomized controlled phase II study (CORE)," *Journal of Neuro-Oncology*, vol. 12, p. iv75, 2010, Abstract no. OT-26.

[22] M. Chawla-Sarkar, D. J. Lindner, Y. F. Liu et al., "Apoptosis and interferons: role of interferon-stimulated genes as mediators of apoptosis," *Apoptosis*, vol. 8, no. 3, pp. 237–249, 2003.

[23] T. Wakabayashi, N. Hatano, Y. Kajita et al., "Initial and maintenance combination treatment with interferon-β, MCNU (Ranimustine), and radiotherapy for patients with previously untreated malignant glioma," *Journal of Neuro-Oncology*, vol. 49, no. 1, pp. 57–62, 2000.

[24] A. Natsume, D. IshII, T. Wakabayashi et al., "IFN-β downregulates the expression of DNA repair gene MGMT and sensitizes resistant glioma cells to temozolomide," *Cancer Research*, vol. 65, no. 17, pp. 7573–7579, 2005.

[25] A. Natsume, T. Wakabayashi, D. IshII et al., "A combination of IFN-β and temozolomide in human glioma xenograft models: implication of p53-mediated MGMT downregulation," *Cancer Chemotherapy and Pharmacology*, vol. 61, no. 4, pp. 653–659, 2008.

[26] J. J. Vredenburgh, A. Desjardins, J. E. Herndon et al., "Bevacizumab plus irinotecan in recurrent glioblastoma multiforme," *Journal of Clinical Oncology*, vol. 25, no. 30, pp. 4722–4729, 2007.

[27] H. S. Friedman, M. D. Prados, P. Y. Wen et al., "Bevacizumab alone and in combination with irinotecan in recurrent glioblastoma," *Journal of Clinical Oncology*, vol. 27, no. 28, pp. 4733–4740, 2009.

[28] M. C. Chamberlain, "Emerging clinical principles on the use of bevacizumab for the treatment of malignant gliomas," *Cancer*, vol. 116, no. 17, pp. 3988–3999, 2010.

[29] A. D. Norden, J. Drappatz, and P. Y. Wen, "Novel antiangiogenic therapies for malignant gliomas," *The Lancet Neurology*, vol. 7, no. 12, pp. 1152–1160, 2008.

[30] M. Gruber, "Bevacizumab in combination with radiotherapy plus concomitant and adjuvant temozolomide for newly diagnosed glioblastoma: update progression-free survival, overall survival, and toxicity," *Journal of Clinical Oncology*, vol. 27, supplement, p. 15s, 2009, abstract no. 2017.

[31] O. L. Chinot, T. de La Motte Rouge, N. Moore et al., "AVAglio: phase 3 trial of bevacizumab plus temozolomide and radiotherapy in newly diagnosed glioblastoma multiforme," *Advances in Therapy*, vol. 28, no. 4, pp. 334–340, 2011.

[32] O. Keunen, M. Johansson, A. Oudin et al., "Anti-VEGF treatment reduces blood supply and increases tumor cell invasion in glioblastoma," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 108, no. 9, pp. 3749–3754, 2011.

[33] T. T. Batchelor, D. G. Duda, E. di Tomaso et al., "Phase II study of cediranib, an oral pan-vascular endothelial growth factor receptor tyrosine kinase inhibitor, in patients with recurrent glioblastoma," *Journal of Clinical Oncology*, vol. 28, no. 17, pp. 2817–2823, 2010.

[34] T. T. Batchelor, "The efficacy of cediranib as monotherapy and in combination with lomustine compared to lomustine alone in patients with recurrent glioblastoma: a phase IIII randomized study," *Neuro-Oncology*, vol. 12, p. iv75, 2010, Abstract no. OT-25.

[35] M. Katoh and M. Katoh, "WNT signaling pathway and stem cell signaling network," *Clinical Cancer Research*, vol. 13, no. 14, pp. 4042–4045, 2007.

[36] C. K. Cheng, Q. W. Fan, and W. A. Weiss, "PI3K signaling in glioma—animal models and therapeutic challenges," *Brain Pathology*, vol. 19, no. 1, pp. 112–120, 2009.

[37] H. Ohgaki and P. Kleihues, "Genetic alterations and signaling pathways in the evolution of gliomas," *Cancer Science*, vol. 100, no. 12, pp. 2235–2241, 2009.

[38] Q. W. Fan and W. A. Weiss, "Targeting the RTK-PI3K-mTOR axis in malignant glioma: overcoming resistance," *Current Topics in Microbiology and Immunology*, vol. 347, pp. 279–296, 2010.

[39] H. Ohgaki, P. Dessen, B. Jourde et al., "Genetic pathways to glioblastoma: a population-based study," *Cancer Research*, vol. 64, no. 19, pp. 6892–6899, 2004.

[40] L. Frederick, X. Y. Wang, G. Eley, and C. D. James, "Diversity and frequency of epidermal growth factor receptor mutations in human glioblastomas," *Cancer Research*, vol. 60, no. 5, pp. 1383–1387, 2000.

[41] A. M. Martelli, C. Evangelisti, F. Chiarini, and J. A. McCubrey, "The phosphatidylinositol 3-kinase/Akt/mTOR signaling network as a therapeutic target in acute myelogenous leukemia patients," *Oncotarget*, vol. 1, no. 2, pp. 89–103, 2010.

[42] T. L. Yuan and L. C. Cantley, "PI3K pathway alterations in cancer: variations on a theme," *Oncogene*, vol. 27, no. 41, pp. 5497–5510, 2008.

[43] P. C. De Witt Hamer, "Small molecule kinase inhibitors in glioblastoma: a systematic review of clinical studies," *Neuro-Oncology*, vol. 12, no. 3, pp. 304–316, 2010.

[44] J. N. Rich, D. A. Reardon, T. Peery et al., "Phase II trial of gefitinib in recurrent glioblastoma," *Journal of Clinical Oncology*, vol. 22, no. 1, pp. 133–142, 2004.

[45] M. J. van den Bent, A. A. Brandes, R. Rampling et al., "Randomized phase II trial of erlotinib versus temozolomide or carmustine in recurrent glioblastoma: EORTC brain tumor group study 26034," *Journal of Clinical Oncology*, vol. 27, no. 8, pp. 1268–1274, 2009.

[46] D. Strumberg, B. Schultheis, M. E. Scheulen et al., "Phase II study of nimotuzumab, a humanized monoclonal anti-epidermal growth factor receptor (EGFR) antibody, in patients with locally advanced or metastatic pancreatic cancer," *Investigational New Drugs*. In press.

[47] J. H. Uhm, K. V. Ballman, W. Wu et al., "Phase II evaluation of gefitinib in patients with newly diagnosed Grade 4 astrocytoma: Mayo/North Central Cancer Treatment Group Study N0074," *International Journal of Radiation Oncology, Biology, Physics*, vol. 80, pp. 347–353, 2010.

[48] D. A. Reardon, A. Desjardins, J. J. Vredenburgh et al., "Phase 2 trial of erlotinib plus sirolimus in adults with recurrent glioblastoma," *Journal of Neuro-Oncology*, vol. 96, no. 2, pp. 219–230, 2010.

[49] F. Bach, "Current status of a phase III trial of nimotuzumab (ti-EGF-R) in newly diagnosed glioblastoma," *Journal of Clinical Oncology*, vol. 29, supplement, 2011, Abstract no. 2059.

[50] R. Lai, "Final analysis of act IIII: a phase II trial of PF04948568 (CDX-110) in combination with temozolomide (TMZ) in patients (PTS) with newly diagnosed glioblastoma (GBM)," *Neuro-Oncology*, vol. 12, p. iv76, 2010, Abstract no. OT-31.

[51] D. D. Eisenstat, "A phase II study of daily afatinib (BIBW 2992) with or without temozolomide (21/28 days) in the treatment of patients with recurrent glioblastoma," *Journal of Clinical Oncology*, vol. 29, supplement, 2011, Abstract no. 2010.

[52] B. J. Druker, M. Talpaz, D. J. Resta et al., "Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase

in chronic myeloid leukemia," *The New England Journal of Medicine*, vol. 344, no. 14, pp. 1031–1037, 2001.

[53] J. Verweij, A. Van Oosterom, J. Y. Blay et al., "Imatinib mesylate (STI-571 Glivec, Gleevec) is an active agent for gastrointestinal stromal tumours, but does not yield responses in other soft-tissue sarcomas that are unselected for a molecular target: results from an EORTC Soft Tissue and Bone Sarcoma Group phase II study," *European Journal of Cancer*, vol. 39, no. 14, pp. 2006–2011, 2003.

[54] G. D. Demetri, M. Von Mehren, C. D. Blanke et al., "Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors," *The New England Journal of Medicine*, vol. 347, no. 7, pp. 472–480, 2002.

[55] P. Y. Wen, W. K. A. Yung, K. R. Lamborn et al., "Phase I/II study of imatinib mesylate for recurrent malignant gliomas: North American Brain Tumor Consortium Study 99-08," *Clinical Cancer Research*, vol. 12, no. 16, pp. 4899–4907, 2006.

[56] E. Razis, P. Selviaridis, S. Labropoulos et al., "Phase II study of neoadjuvant imatinib in glioblastoma: evaluation of clinical and molecular effects of the treatment," *Clinical Cancer Research*, vol. 15, no. 19, pp. 6258–6266, 2009.

[57] T. Kreisl, "A phase II trial of sunitinib in the treatment of recurrent glioblastoma (GBM)," *Neuro-Oncology*, vol. 12, p. iv71, 2010, Abstract no. OT-12.

[58] C. Lu-Emerson, A. D. Norden, J. Drappatz et al., "Retrospective study of dasatinib for recurrent glioblastoma after bevacizumab failure," *Journal of Neuro-Oncology*, vol. 104, no. 1, pp. 287–291, 2011.

[59] P. Workman, P. A. Clarke, F. I. Raynaud, and R. L. M. Van Montfort, "Drugging the PI3 kinome: from chemical tools to drugs in the clinic," *Cancer Research*, vol. 70, no. 6, pp. 2146–2157, 2010.

[60] B. M. Slomovitz, K. H. Lu, T. Johnston et al., "A phase 2 study of the oral mammalian target of rapamycin inhibitor, everolimus, in patients with recurrent endometrial carcinoma," *Cancer*, vol. 116, no. 23, pp. 5415–5419, 2010.

[61] E. Calvo, M. V. Bolós, and E. Grande, "Multiple roles and therapeutic implications of Akt signaling in cancer," *OncoTargets and Therapy*, vol. 2, pp. 135–150, 2009.

[62] H. Azim, H. A. Azim, and B. Escudier, "Targeting mTOR in cancer: renal cell is just a beginning," *Targeted Oncology*, vol. 5, pp. 269–280, 2010.

[63] S. A. Grossman, X. Ye, M. Chamberlain et al., "Talampanel with standard radiation and temozolomide in patients with newly diagnosed glioblastoma: a multicenter phase II trial," *Journal of Clinical Oncology*, vol. 27, no. 25, pp. 4155–4161, 2009.

[64] F. M. Iwamoto, T. N. Kreisl, L. Kim et al., "Phase 2 trial of talampanel, a glutamate receptor inhibitor, for adults with recurrent malignant gliomas," *Cancer*, vol. 116, no. 7, pp. 1776–1782, 2010.

[65] E. Galanis, K. A. Jaeckle, M. J. Maurer et al., "Phase II trial of Vorinostat in recurrent glioblastoma multiforme: a north central cancer treatment group study," *Journal of Clinical Oncology*, vol. 27, no. 12, pp. 2052–2058, 2009.

[66] P. Y. Wen, "Phase I study of vorinostat in combination with temozolomide in patients with malignant gliomas," *Journal of Clinical Oncology*, vol. 29, supplement, 2011, Abstract no. 2032.

[67] S. K. Singh, C. Hawkins, I. D. Clarke et al., "Identification of human brain tumour initiating cells," *Nature*, vol. 432, no. 7015, pp. 396–401, 2004.

[68] J. Lee, M. J. Son, K. Woolard et al., "Epigenetic-mediated dysfunction of the bone morphogenetic protein pathway inhibits differentiation of glioblastoma-initiating cells," *Cancer Cell*, vol. 13, no. 1, pp. 69–80, 2008.

[69] A. Eramo, L. Ricci-Vitiani, A. Zeuner et al., "Chemotherapy resistance of glioblastoma stem cells," *Cell Death and Differentiation*, vol. 13, no. 7, pp. 1238–1241, 2006.

[70] A. Natsume, S. Kinjo, K. Yuki et al., "Glioma-initiating cells and molecular pathology: implications for therapy," *Brain Tumor Pathology*, vol. 28, no. 1, pp. 1–12, 2011.

[71] M. Groszer, R. Erickson, D. D. Scripture-Adams et al., "Negative regulation of neural stem/progenitor cell proliferation by the Pten tumor suppressor gene in vivo," *Science*, vol. 294, no. 5549, pp. 2186–2189, 2001.

[72] A. M. Bleau, D. Hambardzumyan, T. Ozawa et al., "PTEN/PI3K/Akt pathway regulates the side population phenotype and ABCG2 activity in glioma tumor stem-like cells," *Cell Stem Cell*, vol. 4, no. 3, pp. 226–235, 2009.

[73] C. Gregorian, J. Nakashima, J. L. Belle et al., "Pten deletion in adult neural stem/progenitor cells enhances constitutive neurogenesis," *Journal of Neuroscience*, vol. 29, no. 6, pp. 1874–1886, 2009.

[74] C. H. Kwon, D. Zhao, J. Chen et al., "Pten haploinsufficiency accelerates formation of high-grade astrocytomas," *Cancer Research*, vol. 68, no. 9, pp. 3286–3294, 2008.

[75] A. Xiao, H. Wu, P. P. Pandolfi, D. N. Louis, and T. Van Dyke, "Astrocyte inactivation of the pRb pathway predisposes mice to malignant astrocytoma development that is accelerated by PTEN mutation," *Cancer Cell*, vol. 1, no. 2, pp. 157–168, 2002.

[76] V. Clement, P. Sanchez, N. de Tribolet, I. Radovanovic, and A. Ruiz i Altaba, "HEDGEHOG-GLI1 signaling regulates human glioma growth, cancer stem cell self-renewal, and tumorigenicity," *Current Biology*, vol. 17, no. 2, pp. 165–172, 2007.

[77] P. M. LoRusso, C. M. Rudin, J. C. Reddy et al., "Phase I trial of hedgehog pathway inhibitor vismodegib (GDC-0449) in patients with refractory, locally advanced or metastatic solid tumors," *Clinical Cancer Research*, vol. 17, no. 8, pp. 2502–2511, 2011.

[78] X. Fan, L. Khaki, T. S. Zhu et al., "NOTCH pathway blockade depletes CD133-positive glioblastoma cells and inhibits growth of tumor neurospheres and xenografts," *Stem Cells*, vol. 28, no. 1, pp. 5–16, 2010.

[79] M. M. Lino, A. Merlo, and J. L. Boulay, "Notch signaling in glioblastoma: a developmental drug target?" *BMC Medicine*, vol. 8, article 72, 2010.

[80] A. Pannuti, K. Foreman, P. Rizzo et al., "Targeting Notch to target cancer stem cells," *Clinical Cancer Research*, vol. 16, no. 12, pp. 3141–3152, 2010.

[81] W. J. Nelson and R. Nusse, "Convergence of Wnt, β-Catenin, and Cadherin pathways," *Science*, vol. 303, no. 5663, pp. 1483–1487, 2004.

[82] F. Takahashi-Yanaga and M. Kahn, "Targeting Wnt signaling: can we safely eradicate cancer stem cells?" *Clinical Cancer Research*, vol. 16, no. 12, pp. 3153–3162, 2010.

[83] M. W. Pitz, A. Desai, S. A. Grossman, and J. O. Blakeley, "Tissue concentration of systemically administered antineoplastic agents in human brain tumors," *Journal of Neuro-Oncology*, vol. 104, no. 3, pp. 629–638, 2011.

[84] M. Westphal, Z. Ram, V. Riddle, D. Hilt, and E. Bortey, "Gliadel wafer in initial surgery for malignant glioma:

long-term follow-up of a multicenter controlled trial," *Acta Neurochirurgica*, vol. 148, no. 3, pp. 269–275, 2006.

[85] S. Valtonen, U. Timonen, P. Toivanen et al., "Interstitial chemotherapy with carmustine-loaded polymers for high-grade gliomas: a randomized double-blind study," *Neurosurgery*, vol. 41, no. 1, pp. 44–49, 1997.

[86] J. Plowman, W. R. Waud, A. D. Koutsoukos, L. V. Rubinstein, T. D. Moore, and M. R. Grever, "Preclinical antitumor activity of temozolomide in mice: efficacy against human brain tumor xenografts and synergism with 1,3-bis(2-chloroethyl)-1- nitrosourea," *Cancer Research*, vol. 54, no. 14, pp. 3793–3799, 1994.

[87] H. C. Bock, M. J. A. Puchner, F. Lohmann et al., "First-line treatment of malignant glioma with carmustine implants followed by concomitant radiochemotherapy: a multicenter experience," *Neurosurgical Review*, vol. 33, no. 4, pp. 441–449, 2010.

[88] K. S. Bankiewicz, J. L. Eberling, M. Kohutnicka et al., "Convection-enhanced delivery of AAV vector in Parkinsonian monkeys; in vivo detection of gene expression and restoration of dopaminergic function using pro-drug approach," *Experimental Neurology*, vol. 164, no. 1, pp. 2–14, 2000.

[89] P. Hadaczek, M. Kohutnicka, M. T. Krauze et al., "Convection-enhanced delivery of adeno-associated virus type 2 (AAV2) into the striatum and transport of AAV2 within monkey brain," *Human Gene Therapy*, vol. 17, no. 3, pp. 291–302, 2006.

[90] M. T. Krauze, S. R. Vandenberg, Y. Yamashita et al., "Safety of real-time convection-enhanced delivery of liposomes to primate brain: a long-term retrospective," *Experimental Neurology*, vol. 210, no. 2, pp. 638–644, 2008.

[91] T. A. Wynn, "IL-13 effector functions," *Annual Review of Immunology*, vol. 21, pp. 425–456, 2003.

[92] W. Debinski, N. I. Obiri, S. K. Powers, I. Pastan, and R. K. Puri, "Human glioma cells overexpress receptors for interleukin 13 and are extremely sensitive to a novel chimeric protein composed of interleukin 13 and Pseudomonas exotoxin," *Clinical Cancer Research*, vol. 1, no. 11, pp. 1253–1258, 1995.

[93] M. Mut, J. H. Sherman, M. E. Shaffrey, and D. Schiff, "Cintredekin besudotox in treatment of malignant glioma," *Expert Opinion on Biological Therapy*, vol. 8, no. 6, pp. 805–812, 2008.

[94] S. Kunwar, S. M. Chang, M. D. Prados et al., "Safety of intraparenchymal convection-enhanced delivery of cintredekin besudotox in early-phase studies," *Neurosurgical Focus*, vol. 20, no. 4, p. E15, 2006.

[95] C. Kjellman, S. P. Olofsson, O. Hansson et al., "Expression of TGF-β isoforms, TGF-β receptors, and Smad molecules at different stages of human glioma," *International Journal of Cancer*, vol. 89, no. 3, pp. 251–258, 2000.

[96] P. Hau, P. Jachimczak, R. Schlingensiepen et al., "Inhibition of TGF-β2 with ap 12009 in recurrent malignant gliomas: from preclinical to phase I/II studies," *Oligonucleotides*, vol. 17, no. 2, pp. 201–212, 2007.

[97] M. D. Hjelmeland, A. B. Hjelmeland, S. Sathornsumetee et al., "SB-431542, a small molecule transforming growth factor-β-receptor antagonist, inhibits human glioma cell line proliferation and motility," *Molecular Cancer Therapeutics*, vol. 3, no. 6, pp. 737–745, 2004.

[98] U. Naumann, P. Maass, A. K. Gleske, S. Aulwurm, M. Weller, and G. Eisele, "Glioma gene therapy with soluble transforming growth factor-β receptors II and IIII," *International Journal of Oncology*, vol. 33, no. 4, pp. 759–765, 2008.

[99] T. Schneider, A. Becker, K. Ringe, A. Reinhold, R. Firsching, and B. A. Sabel, "Brain tumor therapy by combined vaccination and antisense oligonucleotide delivery with nanoparticles," *Journal of Neuroimmunology*, vol. 195, no. 1-2, pp. 21–27, 2008.

[100] T. T. Tran, M. Uhl, Y. M. Jing et al., "Inhibiting TGF-β signaling restores immune surveillance in the SMA-560 glioma model," *Neuro-Oncology*, vol. 9, no. 3, pp. 259–270, 2007.

[101] M. Uhl, S. Aulwurm, J. Wischhusen et al., "SD-208, a novel transforming growth factor β receptor I kinase inhibitor, inhibits growth and invasiveness and enhances immunogenicity of murine and human glioma cells in vitro and in vivo," *Cancer Research*, vol. 64, no. 21, pp. 7954–7961, 2004.

[102] K. H. Schlingensiepen, R. Schlingensiepen, A. Steinbrecher et al., "Targeted tumor therapy with the TGF-β2 antisense compound AP 12009," *Cytokine and Growth Factor Reviews*, vol. 17, no. 1-2, pp. 129–139, 2006.

[103] L. Valliéres, "Trabedersen, a TGFβ2-specific antisense oligonucleotide for the treatment of malignant gliomas and other tumors overexpressing TGFβ2," *IDrugs*, vol. 12, no. 7, pp. 445–453, 2009.

[104] U. Bogdahn, P. Hau, G. Stockhammer et al., "Targeted therapy for high-grade glioma with the TGF-β2 inhibitor trabedersen: results of a randomized and controlled phase IIb study," *Neuro-Oncology*, vol. 13, no. 1, pp. 132–142, 2011.

[105] R. McLendon, A. Friedman, D. Bigner et al., "Comprehensive genomic characterization defines human glioblastoma genes and core pathways," *Nature*, vol. 455, no. 7216, pp. 1061–1068, 2008.

[106] D. W. Parsons, S. Jones, X. Zhang et al., "An integrated genomic analysis of human glioblastoma multiforme," *Science*, vol. 321, no. 5897, pp. 1807–1812, 2008.

[107] H. Noushmehr, D. J. Weisenberger, K. Diefes et al., "Identification of a CpG Island Methylator Phenotype that Defines a Distinct Subgroup of Glioma," *Cancer Cell*, vol. 17, no. 5, pp. 510–522, 2010.

[108] T. M. Kim, W. Huang, R. Park, P. J. Park, and M. D. Johnson, "A developmental taxonomy of glioblastoma defined and maintained by microRNAs," *Cancer Research*, vol. 71, no. 9, pp. 3387–3399, 2011.

[109] R. G. W. Verhaak, K. A. Hoadley, E. Purdom et al., "Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1," *Cancer Cell*, vol. 17, no. 1, pp. 98–110, 2010.

Hindawi Publishing Corporation
Neurology Research International
Volume 2012, Article ID 428565, 14 pages
doi:10.1155/2012/428565

Review Article

Novel Therapies in Glioblastoma

James Perry, Masahiko Okamoto, Michael Guiou, Katsuyuki Shirai, Allison Errett, and Arnab Chakravarti

Department of Radiation Oncology, Arthur G. James Comprehensive Cancer Center and Richard L. Solove Research Institute, The Ohio State University, Columbus, OH 43210, USA

Correspondence should be addressed to James Perry, james.perry@osumc.edu

Received 16 August 2011; Accepted 9 December 2011

Academic Editor: Stuart Burri

Conventional treatment of glioblastoma has advanced only incrementally in the last 30 years and still yields poor outcomes. The current strategy of surgery, radiation, and chemotherapy has increased median survival to approximately 15 months. With the advent of molecular biology and consequent improved understanding of basic tumor biology, targeted therapies have become cornerstones for cancer treatment. Many pathways (RTKs, PI3K/AKT/mTOR, angiogenesis, etc.) have been identified in GBM as playing major roles in tumorigenesis, treatment resistance, or natural history of disease. Despite the growing understanding of the complex networks regulating GBM tumors, many targeted therapies have fallen short of expectations. In this paper, we will discuss novel therapies and the successes and failures that have occurred. One clear message is that monotherapies yield minor results, likely due to functionally redundant pathways. A better understanding of underlying tumor biology may yield insights into optimal targeting strategies which could improve the overall therapeutic ratio of conventional treatments.

1. Introduction

Glioblastoma (GBM) is a grade IV glioma and accounts for approximately 75% of all high-grade gliomas with approximately 9,000 new cases per year diagnosed in the United States alone, making it the most common adult brain tumor. GBM is the most aggressive glial neoplasm, and despite advances in medical management, the outcomes remain quite poor. The current standard of care for high-grade glioma patients is maximum surgical resection combined with radiation and concomitant and adjuvant temozolomide (TMZ) therapy. The addition of radiotherapy for the treatment of GBMs led to the first significant improvement in patient survival starting in the late 1970s. More recently, Stupp et al. have shown that the addition of the chemotherapeutic agent TMZ can increase survival further to approximately 15 months.

2. Development of Standard of Care (RT + TMZ)

Surgery is a critical component of standard of care, allowing histological diagnosis, but more critically, tumor debulking. This greatly reduces the number of cells to be killed by radiation or chemotherapy. It also decreases intracranial pressure which, depending on the location of the tumor, may result in recovery of CNS function or decrease in usage of corticosteroid. Recently, the effectiveness of aggressive surgical resection on survival was suggested by some prospective analyses [1–3].

Unfortunately, most glioblastomas recur following surgery. The efficacy of radiation therapy (RT) was reported in the 1970s [4, 5], and RT has become a standard adjuvant therapy in patients with malignant glioma. In 2005, the efficiency of concomitant and adjuvant TMZ was suggested by a phase III study that was conducted by the European Organization for Research and Treatment of Cancer (EORTC) and the National Cancer Institute of Canada (NCIC) [6]. In the EORTC/NCIC study, a total of 573 patients with newly diagnosed glioblastoma were enrolled. The authors reported the combined therapy of TMZ and RT increased median survival time (MST) when compared with RT-alone (14.6 months versus 12.1 months, $P < .001$). At the 5-year analysis of this study, the 5-year overall survival rate was 9.8% for the combination therapy group versus 1.9%

for the RT alone group ($P < .001$), with a median follow-up of 61 months [7]. With this strong evidence, combination therapy with TMZ and RT is widely prescribed and currently considered the standard treatment for patients with newly diagnosed glioblastoma.

Some investigators had suggested that the epigenetic silencing of a DNA repair enzyme named *O*-6-methylguanine-DNA methyltransferase (MGMT) by promoter methylation was associated with good prognosis for patients with glioblastoma treated with alkylating agents such as TMZ [8, 9]. In agreement with previous studies, patients with a methylated MGMT promoter had significantly improved MST when compared with patients with an unmethylated MGMT promoter (21.7 months versus 15.3 months, $P < .001$) in the EORTC/NCIC trial [10]. Furthermore, this study indicated that patients with an unmethylated MGMT promoter received less benefit from the combined therapy. MGMT promoter methylation status is widely used to predict the efficacy for combination therapy of RT and TMZ for newly diagnosed glioblastoma.

Although the combination therapy of RT and TMZ has become standard, most patients will still eventually recur. Thus the development of a new treatment strategy is needed in order to overcome the resistance of glioblastoma to current therapy. One strategy is increasing the intensity of radiation dose. However, neither radiosurgery boost [11] nor brachytherapy boost [12] shows improvement in survival. Another strategy is the optimization of TMZ usage by approaches such as dose-dense regimens. RTOG 0525/EORTC 26052-22053, a prospective randomized trial, was conducted by the Radiation Therapy Oncology Group International (RTOG) and EORTC. It aimed to determine whether a dose-dense TMZ regimen is more effective than the standard TMZ regimen in the adjuvant setting, and the results of this study showed no significant difference between the two arms [13]. Moreover, the adverse effects, especially in the field of lymphopenia and fatigue, were significantly increased in the dose-dense arm.

3. Technological Advancements in GBM Therapy

Current research efforts in both the basic and clinical sciences are improving clinicians' ability to more accurately target and treat GBM. In addition to targeted therapies, which will be discussed later in this paper, there have been advancements in the technological arena that are improving patient care.

3.1. Target Delineation. MRI remains the gold standard for delineating tumor in both the pre- and postoperative setting. Gross tumor volume is felt to be best represented by areas of contrast-enhanced T1 signal while areas of T2/FLAIR enhancement reflect regions of infiltrative tumor. These volumes form the basis for radiotherapy target delineation. A limitation of contrast-enhanced MRI is that it relies on surrogate markers of tumor presence (tumor-associated breakdown of the blood-brain barrier and cerebral edema) versus a direct measure of actual gross tumor mass and spread. Functional imaging techniques such as positron emission tomography (PET) play a pivotal role in the staging and planning of cancers in other parts of the body. Unfortunately, the most widely used radiotracer [^{18}F]-fluorodeoxyglucose is relatively insensitive at delineating malignancy in the brain due to the high basal metabolic rate of normal brain tissue [14]. Other clinically available radiotracers such as L-methyl-^{11}C-methionine (MET) and *O*-(2-[^{18}F]fluoroethyl)-L-tyrosine(FET) have shown promise in localizing gliomas. Recent studies have shown MET PET can more accurately identify areas of active tumor versus traditional MRI [15, 16]. MET is actively taken up by gliomas but shows only low uptake in normal brain. In a study of 14 patients with high-grade glioma, MET PET was highly correlated with areas of endothelial proliferation and mitotic activity [17], a more direct marker of tumor activity that can be visualized using MRI. In a study of 26 patients with GBM, Lee et al. [15] showed that 5 of 26 patients had areas of MET PET positivity outside radiotherapy volumes defined by MRI. All of these patients had noncentral failures. In 14 patients where MET PET-positive areas were covered in the high-dose radiotherapy volume, none had noncentral failures. FET PET has also been shown to correlate with areas of active tumor. In a study of 31 patients with GBM, FET PET was highly correlated with areas of active tumor on biopsy [18]. FET shows minimal uptake in macrophages or inflammatory tissues, indicating that it may be superior to MET in identifying areas of active tumor, especially in the postoperative setting. In a study comparing FET PET to MRI, Piroth et al. showed that areas active on FET PET not covered by MRI-based radiotherapy target volumes predict a shorter disease-free survival (5.1 versus 9.6 months) and overall survival (6.9 versus 20 months) further supporting the integration of metabolically based treatment planning methods into radiation planning for GBM [19]. An emerging technique that may also play an important role in target delineation for GBM is diffusion tensor imaging (DTI). DTI uses specialized MRI sequences to measure the diffusion of water in the brain, the greatest values of which lie along white matter tracts. Postmortem studies in GBM patients show that glioma cells tend to migrate the greatest distance from the primary site along these tracts. In a study of 14 patients with recurrent GBM, Krishnan et al. were able to show a strong relationship between the sites of recurrence and the DTI maps emanating from the primary site [20]. While none of the aforementioned imaging techniques have been tested in a phase III study, they provide promising tools by which clinicians cannot only more accurately identify areas of active tumor but also potentially predict their most likely route of spread to help refine radiotherapy treatment fields.

3.2. Disease Monitoring. Treatment-related effects such as postoperative scarring and hemorrhage, peritumoral edema, inflammation, and microvascular changes make radiographic assessment problematic as these changes can mimic progressive disease. This has been termed pseudoprogression. Additionally, given the high doses and often large volumes required to treat GBM, up to 30% of patients develop radionecrosis which can also mimic disease recurrence on

MRI [21]. The front-line treatment for radionecrosis is steroids and time. Some patients require surgical resection for more advanced cases resistant to steroid treatment. The ability to diagnose true progression and initiate second-line therapy is paramount in a patient population with such poor disease-free and overall survival. Both PET imaging and magnetic resonance spectroscopy (MRS) have been shown to be superior to MRI in helping distinguish active disease from pseudoprogression or radionecrosis. The specificity of FET PET and MET PET to detect recurrent disease is approximately 90% and 70%, respectively [19]. Areas active on FET PET show a high correlation with biopsy-proven areas of active disease [18], and regions of enhancing tissue on MRI are often negative on FET PET. Numerous studies of magnetic resonance spectroscopy (MRS), which measures the differential concentrations of metabolites in the brain, also show improved accuracy in distinguishing recurrent disease versus MRI (71% versus 55%) [22]. Classic MRS is hampered by poor spatial resolution and nonvolumetric data; however, recent advances in technology now permit volumetric MRS which may improve its diagnostic value. Integration of these metabolically based imaging techniques may help to improve our ability to detect disease progression early and optimize 2nd-line therapy for patients.

3.3. Treatment in the Recurrent Setting. The vast majority of patients with GBM recur within 8–12 months following completion of therapy highlighting the importance of developing efficacious treatments for patients with recurrent disease. Studies examining reirradiation with or without chemotherapy have shown the most promising results. Given the fact that the majority of patients fail within 2 cm of the primary site, numerous studies examining the efficacy of stereotactic radiosurgery (SRS), as delivered by a variety of different methods, have been completed [23–25]. SRS provides a modest improvement in outcome but with significant risk of radionecrosis requiring surgical resection. Patients undergoing fractionated stereotactic radiotherapy (FSRT) at the time of recurrence have median survival ranging from approximately 7 to 14 months for all patients but with reduced rates of morbidity [23, 26–28]. Dosing regimens with a cumulative dose exceeding 40 Gy appear to be associated with a greater degree of radiation damage [29]. Response on postprocedure MRI seems to be an important prognostic factor for either SRS or FSRT. In a study of 36 patients (26 SRS, 10 FSRT) patients showing response on posttreatment MRI had median survival of 15.8 months versus 7.3 months for nonresponders [23].

Combination chemoradiotherapy has shown some efficacy in the recurrent setting. In a study of 25 patients with recurrent high-grade glioma (20 GBM, 4 anaplastic astrocytoma), treatment with FSRT (30 Gy in 5 fractions) plus concurrent bevacizumab resulted in posttreatment median survival of 12.5 months [30] with comparable toxicity rates as reported by other studies of GBM patients treated with bevacizumab. Three of 25 patients had to discontinue treatment due to CNS toxicity, wound complications, and bowel perforation. A recent phase I dose-escalation trial of gefitinib, an epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI), plus FSRT (18 to 36 Gy in 3 fractions) in 15 patients (11 GBM, 4 anaplastic astrocytoma) showed this combination was well tolerated at all dose levels. Median progression-free survival was 7 months with a 6-month progression-free survival of 63% and a 1-year overall survival of 40%. Of course, given the mixture of grade III and grade IV histologies, it is difficult to compare outcomes with studies of GBM patients alone, but these initial results show promise. Studies combining cytotoxic chemotherapy with FSRT agents have shown similar response rates with acceptable toxicity profiles [31–33].

Recurrent malignant gliomas can also be managed with chemotherapy. Modifications in the dosing regimen of TMZ show modest improvements in progression-free survival. Standard dosing regimens of TMZ (200 mg/m^2) produce a 6 month progression-free survival of 21% [34]. TMZ delivered in a low-dose, protracted schedule (75 mg/m^2 for 21 days of a 28-day cycle) in an attempt to deplete MGMT produced PFS-6 of 30% [35].

4. Targeted Therapy

Considering glioblastoma treatment is the most expensive cancer treatment per capita in the United States, and outcomes are still so universally poor, there is a great need for more effective therapeutic options. Targeted therapy and personalized medicine are currently two of the more aggressively pursued ideas in cancer treatment. While targeted therapies aim to affect a specific alteration, most chemotherapies are generic, broad-based DNA-damaging agents that affect all cells in a similar manner. Targeted therapies offer the possibility of selectively killing cancer cells and sparing normal tissue. An example of a commonly targeted pathway in glioblastoma is the EGFR receptor tyrosine kinase.

5. Receptor Tyrosine Kinase (RTK) Inhibitors

5.1. EGFR. As mentioned above, EGFR is known to be an important player in gliomagenesis and in the aggressive and therapeutic-resistant phenotype demonstrated by this tumor. In addition to its critical role in several survival signaling pathways, alterations in EGFR are some of the most common mutations found in GBM. EGFR is overexpressed in approximately 50% of tumors, and of those nearly half express the constitutively active EGFRvIII mutant [36]. These combined facts have made this growth factor receptor a very popular target for molecular therapies. Many clinical trials have examined the efficacy of EGFR inhibitors, and to date there is little evidence to support their use in a monotherapy setting. While gefitinib, a selective inhibitor of EGFRs tyrosine kinase domain (Figure 1), is approved for use in non-small-cell lung cancer, a recent phase II clinical trial of gefitinib in newly diagnosed GBM patients by the Mayo/North Central Cancer Treatment Group showed no significant improvement in either overall survival or progression-free survival [37]. Another recent phase II trial studying the efficacy of erlotinib, which also inhibits the tyrosine kinase domain of EGFR (Figure 1), and TMZ with RT for newly diagnosed GBM had to be stopped

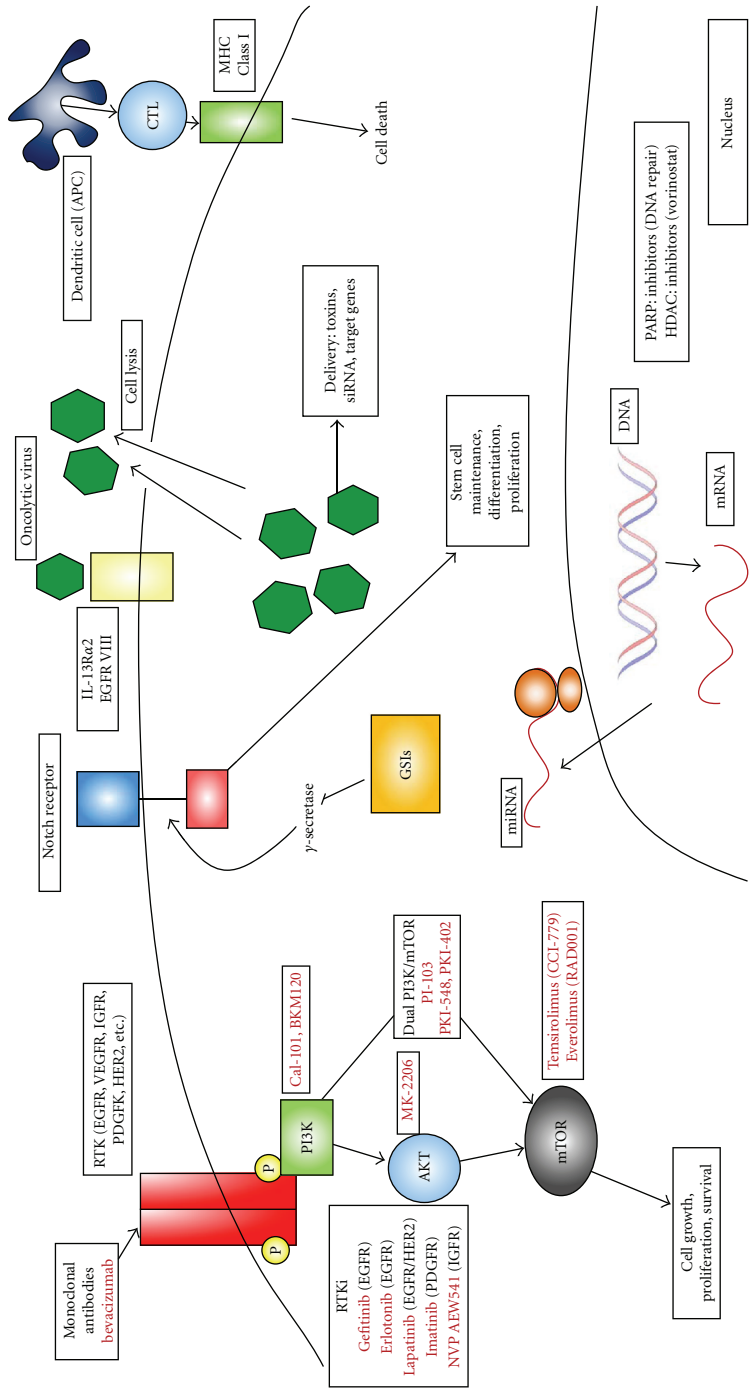


Figure 1: Novel therapies in GBM. RTKs and survival signaling pathways are major drug targets in GBM. Receptors have been targeted extracellularly by monoclonal antibodies or intracellularly at the tyrosine kinase domain. Major nodes in survival signaling pathways (P13K, AKT, mTOR) have been the focus of intense study and drug development. More recent approaches include stem-cell targeting (GSIs), inhibition of DNA rapair (PARP inhibitors), and targeting a host of cellular pathways through microRNA manipulation. Novel tumor cell killing approaches are also being studied. Oncolytic virus therapy, either alone or in combination with targeted agent delivery and immunotherapy, are being employed to efficiently kill tumor cells while sparing normal tissue. RTK, receptor tyrosine kinase; EGFR, epidermal growth factor receptor; VEGFR, vascular endothelial growth factor receptor; IGFR, insulin-like growth factor receptor 1; PDGFR, platelet-derived growth factor receptor; mTor, mammalian target of rapamycin; GSI, gamma-secretase inhibitor; APC, antigen-presenting cell; CTL, cytotoxic T lymphocyte, MHC class I, major histocompatibility complex I; PARP, poly(ADP-ribose) polymerase; HDAC, histone deacetylase inhibitor.

short of full accrual due to the lack of benefit and the unreasonable toxicity, including at least three treatment-related deaths [38]. A different phase II trial of erlotinib in the same setting showed less toxicity and even demonstrated improved median survival (19.3 months versus 14.1 months) compared to historical controls [39]. Interestingly, the first study only escalated erlotinib dose to 150 mg/day while the second study, which showed improved toxicities as compared to historical controls, escalated to a maximum dose, in some patients, of 300 mg/day. Overall results from multiple clinical trials show monotherapy EGFR inhibition, or addition of these inhibitors to standard-of-care treatments has shown little benefit to patients and a dramatically increased toxicity profile.

In terms of current multitherapy strategies, combining several RTK inhibitors, or attacking several important glioma survival strategies, is likely to improve outcomes over monotherapies, while also possibly reducing toxicity. A recent study showed that EGFR expression in a GBM xenograft model increased the efficacy of an anti-vascular endothelial growth factor (VEGF) (vandetanib or cediranib) therapy in combination with irradiation when compared to xenografts lacking EGFR expression [40]. A preclinical study of monoclonal antibody inhibition of both EGFR and VEGFR-2 has demonstrated improved efficacy in an orthotopic xenograft model [41]. This research was not performed in the presence of IR and therefore may have less relevance to glioblastoma therapy. However, it does demonstrate the importance, as well as the complexity, of designing combination therapies. In a similar vein, it was recently shown that HER2 inhibition might help overcome EGFR resistance and increase radiosensitivity in a GBM cell model [42]. A different combination of EGFRvIII inhibition with C-met inhibition showed synergy against PTEN null/EGFRvIII positive tumors, a very aggressive tumor population [43]. With the promise shown, even novel inhibitors are being tested in combination. A recent study reported that inhibition of autophagy was able to enhance the cell-death-inducing capabilities of erlotinib in a glioma cell model [44]. Lovastatin, a member of the statin family (normally used to reduce cholesterol), was also shown to increase the efficacy of EGFR inhibitor therapies [45]. However, in a clinical phase I/II trial of lapatinib, a dual EGFR/HER2 inhibitor, showed poor results [46]. Even rational combinations targeting this pathway have not led to expected results, as shown by the underwhelming results in a recent phase II trial examining erlotinib therapy in combination with an mTOR inhibitor (sirolimus) [47]. While EGFR inhibitor combination treatments have produced improved results compared to monotherapy, we are still a long way from being able to determine what combinations will provide benefit for which patients.

5.2. Insulin-Like Growth Factor Receptor I (IGFR). While EGFR has been the major focus of targeted receptor tyrosine kinase therapies, there has been work into other known survival signaling activating receptors such as IGFR. It has previously been established that there is significant cross-talk between IGFR and EGFR receptors, and the similar cellular responses to signaling through these receptors could play a large role in mediating resistance to anti-EGFR therapies [48]. In 2002, Chakravarti et al. showed upregulation of the IGFR gene in a GBM cell line resistant to AG1478, an EGFR TKI. In this work, they demonstrated that upregulation of IGFR in this resistant cell line correlated with sustained activation of the PI3K pathway. Cotargeting of the IGFR and the EGFR receptors led to increased apoptosis, as well as a reduction in invasive potential [49]. A more recent study demonstrated that combination of the IGFR inhibitor NVP-AEW541 with dasatinib led to increased apoptosis in GBM cell lines, but not in nonneoplastic human astrocytes, and synergistically inhibited clonogenic survival [50]. These studies highlight the possible efficacy of cotargeting IGF and EGF receptors to overcome therapeutic resistance and enhance therapeutic gain.

5.3. Platelet-Derived Growth Factor Receptor (PDGFR). PDGF signaling is another commonly altered signaling pathway in glioblastoma. A recent study in GBM cell lines showed that varying concentration of imatinib, a PDGFR inhibitor, had either cytostatic effects, at low concentrations, and possibly cytotoxic events at high concentrations [51]. Comparatively, another report in GBM cell lines showed treatment with imatinib actually led to the activation and sustained signaling through the ERK1/2, PI3K, and other important cell survival signaling pathways [52]. Some reports have even identified that PDGFR status was not predictive of imatinib efficacy even though it was shown to be a prognostic marker [53]. These results indicate that while PDGFR inhibition might be an interesting target, much more study is needed.

6. PI3K/AKT/mTOR Inhibitors

In addition to EGFR and other RTK therapies, there has been a major focus on inhibiting downstream survival signaling pathways stimulated by these receptors. The two most prominent and most studied pathways are the MAPK signaling cascade and the PI3K/AKT/mTOR pathway. Initially it was believed PI3K signaling was responsible for cell survival, while the MAPK pathway was involved in cell proliferation. Now, these two pathways are thought to share a significant amount of overlap and to both be involved in cell growth, proliferation, and survival. As targeted therapies have become a more important piece of the cancer treatment arsenal, these pathways have been the focus of a significant amount of research effort.

One of the initial works identifying PI3K inhibitors as viable for the treatment of GBM was a paper by Kubota et al. which showed the early PI3K inhibitor wortmannin sensitized GBM cells to radiation regardless of p53 status [54]. Wortmannin was then later shown to reverse the growth advantage seen in GBM cells which both lacked PTEN expression and overexpressed the EGFRvIII "always-on" variant growth receptor [55]. However wortmannin, while potent, has significant levels of nonspecific kinase inhibition and is soluble in organic solvents, which has limited its applicability for human clinical trials [56]. Following the proof of principle of this novel targeting therapy, many

new PI3K inhibitors were developed and used in clinical trials. These include perifosine, cal101, px-866, pi-103, and others with some PI3K inhibitors even being specifically assessed in glioblastoma (XL765, XL147, and BKM 120) (Figure 1). Results of many of these trials have been poor; however, therapies using these drugs in combination with other inhibitors have recently become a focus.

In addition to PI3K inhibition, small-molecule inhibitors have been developed both upstream (i.e., AKT inhibitors) and downstream (i.e., mTOR inhibitors) in this pathway. AKT has been targeted because this kinase is the central node in the RTK/PI3K/AKT/mTOR signaling cascade. Direct inhibition of this molecule would prevent downstream signaling similar to RTKi or PI3K inhibition. The importance of developing these novel inhibitors at different stages of the signaling cascade has become even more obvious with the development of RTKi refractory tumors. Only one AKT inhibitor, mk-2206, has currently made it into phase II clinical trials. Recently GlaxoSmithKline has begun phase I trials with two different AKT inhibitors, with mixed results. The initial phase I study of drug GSK690693 was withdrawn, and trials of drug GSK2141795 are currently not recruiting patients. Also, a phase II trial of MK-2206 had been planned in recurrent glioma; however, that trial has since been withdrawn. However promising it has been preclinically, AKT inhibition has proven difficult to translate into clinical efficacy.

Probably the most targeted member of this pathway is the mammalian target of rapamycin (mTOR). In gliomas, it has been observed that the mTORC2 complex promotes growth and cell motility [57]. An early study demonstrating efficacy of targeting this pathway showed increased radiosensitization of a U87 xenograft [58]. Based on available data, it seems likely that in order to effectively block mTOR activity in cells, both mTORC1 and mTORC2 complexes will need to be targeted [59]. These preclinical results have been critical to planning the numerous clinical trials that have been performed with mTOR inhibitors in glioblastoma. A phase I trial of rapamycin in PTEN-deficient glioblastoma patients, while showing some promising results, also demonstrated the inherent difficulties of targeting this protein. In this trial, multiple patients were observed who showed elevated levels of pAKT following mTOR inhibition, which was correlated with shorter time to progression [60]. The AKT activation observed was likely due to alteration of signaling feedback loops, again highlighting the complexity of targeted therapy. Combination therapy to block these feedback loops may also improve efficacy [61]. Despite this complexity, promising results have pushed mTOR inhibitors to further trials. Several of these mTOR inhibitors have been or are currently being tested in the clinical trials setting specifically in gliomas, including temsirolimus, everolimus (RAD001), and sirolimus. Temsirolimus (CCI-779) has been the most extensively studied drug in clinical trials. A phase I study determined the clinically effective dose to be 250 mg IV weekly [57]. Phase II trials with CCI-779 as a monotherapy in recurrent GBM showed no effectiveness despite low toxicity and initial disease stabilization [62]; however, a North Central Cancer Treatment Group study showed a statistically significant time to progression increase in temsirolimus responders (5.4 months versus 1.9 months, 2.3 months overall) [63].

Because of the promise of combination therapy, currently there is a significant emphasis on dual PI3K/mTOR inhibitors. Several novel small-molecule inhibitors have been developed that have dual specificity for these targets. XL765 has recently been shown to reduce cell viability in vitro and in limited animal study showed a possible effectiveness when combined with TMZ therapy [64]. Similarly PKI-587 and PKI-402 were shown to have a strong in vitro antitumorigenic effect across multiple cell types including glioma cells, while also slowing tumor growth in xenograft models [65, 66]. Another dual PI3K/mTOR inhibitor, PI-103, which is known to have monotherapy efficacy in glioma [67] was recently shown to specifically reduce tumor volumes in combination with NSC-delivered s-trail in an orthotopic intracranial xenograft model [68]. PI-103 combination therapy has also proven effective in sensitizing cells to both chemotherapy [69] and radiation [70] through reducing DNA damage repair. RAD001 is currently being used in multiple combination treatment studies, including studies employing an oncolytic virus [71], Raf inhibitors [72], and VEGFR-2 [73]. In a GBM orthotopic xenograft model, it was shown however that PTEN does not serve as a predictive marker for RAD001 effectiveness, despite the importance of PI3K/AKT signaling activation in mTOR upregulation [74]. There has also been evidence for targeting this signaling pathway from both ends, with a report showing rapamycin promotes a response to EGFR inhibitors in either PTEN-sufficient or PTEN-deficient GBM cells by reducing tumor cell growth. This treatment combination also results in tumor cell death in PTEN-deficient cells [75]. While there is hope clinical trials with these novel dual-targeting agents will demonstrate better efficacy than current monotherapies, there are still significant difficulties in trying to determine the best role for these targeted therapies in cancer treatment.

7. Antiangiogenic Therapies

Antiangiogenic therapy, which has been well studied in many types of cancer, has also emerged as a novel therapy for glioblastoma. Glioblastoma is characterized by vascular proliferation or angiogenesis [76], and advances in molecular biology have allowed us to target angiogenesis of glioblastoma. VEGF, a critical mediator of angiogenesis, is highly expressed in glioblastoma and regulates tumor angiogenesis [77, 78]. Preclinical studies have shown that VEGF inhibitors inhibit the growth of glioma cells [79, 80]. Antiangiogenic therapies for glioblastoma are currently the most advanced of any targeted therapy, and many clinical trials have demonstrated their efficacy. In fact, bevacizumab has been approved by the US food and Drug Administration in the setting of recurrent glioblastoma.

Bevacizumab is a humanized monoclonal antibody against VEGF and prevents the activation of VEGF receptor tyrosine kinases (Figure 1) [81]. This drug is considered a well-established antiangiogenic therapy in several angiogenic tumors [82]. In glioblastoma, a phase II study of the addition of bevacizumab to the standard treatment of TMZ and

radiotherapy was conducted for 70 newly diagnosed patients [83]. The median overall survival and PFS were 19.6 and 13.6 months, respectively. Another phase II study of additional bevacizumab to standard therapy showed median PFS was 13.8 months in 125 newly diagnosed glioblastoma patients [84]. Recently two clinical trials of bevacizumab have been reported in 2011 ASCO annual meeting for newly diagnosed glioblastoma. Vredenburgh et al. performed a phase II study of bevacizumab, TMZ, and radiotherapy followed by bevacizumab, TMZ, and oral topotecan [85]. The 6-month event-free survival was 90%, and median overall survival has not been reached. Although the regimen was tolerable, there were 2 treatment-related deaths, including CNS hemorrhage and pneumonitis. Omuro et al. conducted a phase II trial of bevacizumab, TMZ, and hypofractionated stereotactic radiotherapy for newly diagnosed glioblastoma patients with tumor volume under 60 cc [86]. The median PFS was 11 months, and objective response rate was 90%. Furthermore, 1-year overall survival was 90% with median follow-up of 13 months. Despite a more aggressive radiotherapy schedule, the regimen was well tolerated and had promising results. Additional bevacizumab seems to have favorable effects; however, it is still unclear whether this regimen can improve overall survival. Two randomized phase III trials, RTOG 0825 and AVAGLIO, are ongoing to demonstrate the efficacy and safety of combined therapy of bevacizumab, TMZ, and radiotherapy for newly diagnosed glioblastoma [87, 88]. The addition of bevacizumab to TMZ and radiotherapy is expected to become frontline treatment for glioblastoma, and is already thought of by some as nearly a part of standard of care.

While bevacizumab has been thoroughly investigated in clinical and preclinical studies, other drugs have also been studied as antiangiogenic therapy for glioblastoma patients [89]. Cilengitide, which is selective for $\alpha v\beta 3$ and $\alpha v\beta 5$ integrin receptors, is considered a novel antiangiogenic therapy for glioblastoma. A phase II study of recurrent glioblastoma treated by cilengitide showed that 6 month PFS was 15%, and treatment was well tolerated [90]. Furthermore a phase I/IIa study of cilengitide combined with TMZ and radiotherapy was performed for 52 newly diagnosed glioblastoma patients [91]. This regimen was well tolerated and showed promising results with median overall survival of 16.1 months. Currently, two randomized trials, CENTRIC and CORE, are ongoing and are expected to show the benefits of additional cilengitide to standard therapy for newly diagnosed glioblastoma patients [88, 92]. Other antiangiogenic therapies (e.g., VEGF receptor tyrosine kinase inhibitors) have also been performed in clinical and preclinical studies [89], although there have not been any drugs to show strong antiglioma effects compared with bevacizumab. Further investigation is warranted to establish the efficacy and safety of novel antiangiogenic therapy for glioblastoma.

8. Novel Targeted Therapies

8.1. Notch Inhibitors. One of the most controversial topics in cancer biology is the theory of cancer stem cells or tumor-initiating cells. Despite the split opinions regarding the existence of this cell population, mounting evidence has spurred development of novel therapeutics to target this proposed group of cells. While proper definition and identification of this stem cell population is still ongoing, researchers have used clues about pathways critical to known stem cell populations to design novel therapies. One such pathway is the notch signaling pathway, which is important in both normal and neoplastic development in the central nervous system by controlling proliferation, apoptosis, stem cell maintenance, differentiation, and homeostasis [93]. Specifically in gliomas, notch has been linked to overexpression of EGFR [94]; however, it is more likely notch's role in the maintenance of stem cell populations that make it an interesting therapeutic target [95, 96]. Gamma secretase inhibitors (GSIs), which are known to inhibit notch, have been shown to inhibit glioma stem cell growth [97], while overexpression of notch induces tumor growth and can be blocked by treatment with GSIs (Figure 1). While notch inhibition is still very novel, it has shown efficacy in preclinical models and shows a strong possibility for combination therapy targeting both tumor cell bulk with conventional therapies as well as the tumor-initiating cell population.

8.2. Virotherapy/Gene Therapy for GBMs. Cancer therapy using viruses comes in a variety of approaches including direct viral cytotoxicity and targeted toxin delivery. The use of viruses to deliver tumor suppressors, or siRNAs, to knockdown oncogene expression, immune modulating compounds, or antiangiogenic compounds, is also underway, yet these attempts are often designed in combination, or as a method to enhance oncolytic viruses (Figure 1).

The general goal behind direct viral cytotoxicity or oncolytic virotherapy is to design a virus that specifically and faithfully infects and replicates only in tumor cells. This is usually accomplished by attenuating the virus to restrict their replication to actively dividing cells (i.e., tumor cells) while sparing normal, nonreplicating tissue. Viruses such as herpes simplex virus 1 (HSV), adenovirus, and reovirus have been attenuated so as to conditionally replicate within cancer cells [98–100]. Oncolytic viral therapy has undergone major changes, using the virus not only as a cytotoxic therapy, but also as a delivery mechanism. Researchers have used oncolytic HSV-1 to deliver vasculostatin, an antiangiogenic compound [101], as well as chondroitinase ABC I [102]. This method has demonstrated significantly enhanced therapeutic efficacy over virus alone. Similar approaches with adenoassociated virus (aav) particles [103] have shown codelivery of oncolytic virus with antitumor molecules has enhanced efficacy and improved survival in xenograft models. Each of these oncolytic viruses has also been examined in phase I clinical trials in glioma and have been shown to be well tolerated [104–106].

Targeted toxin delivery is similar to oncolytic virus therapy in that it is a virus-mediated cytotoxic therapy. While oncolytic viruses are directly responsible for tumor cell death, the strategy for this therapy is to use nonreplicating virus particles, such as aav, to deliver a powerful toxin,

that is, *Pseudomonas* exotoxin (PE), specifically to cancer cells by targeting preferentially expressed receptors. Some of the receptors include the IL-13R variant $\alpha 2$, which varies from the receptor expressed on normal brain cells [107], as well as EGFR [108] or the EGFRvIII variant [109]. Clinical trials have been performed with an IL-13R$\alpha 2$ targeting virus delivering cintredekin besudotox (CB). A phase III study of this therapy compared to Gliadel Wafer administration at first recurrence showed no survival benefit [110]. In general, despite promising preclinical results with viral therapy for gliomas, clinical trials resulting from this work have yet to show any significant survival benefits. This pattern is not uncommon in cancer therapy, yet it does point out the need for more research. Despite the poor clinical trial results to date, the positive data coming from viral therapy research indicate that this form of therapy has significant future potential.

8.3. Immunotherapy. One alternative to target gliomas without affecting normal cells is by utilizing the body's natural defenses to kill tumor cells. There are currently two main immunotherapy strategies being tested against gliomas: adoptive immunotherapy and active immunotherapy.

Adoptive immunotherapy is the process of stimulating immune cells *ex vivo* and then readministering them to the patient in hope of therapeutic benefit. This can be done either intravenously or directly into the tumor. The two major cell types used are lymphocyte activated killer (LAK) cells or cytotoxic T lymphocytes (CTLs). Recent reports have identified a synergistic response between cytokine-induced killer cells (CIKs) and TMZ [111]. Specific targeting of HER-2 by T cells was shown to induce regression in HER-2-positive autologous tumor xenografts as well as to target the tumor-initiating cell (TIC) population, demonstrating a targeted approach may prove to be more effective [111, 112]. A significant number of phase I or phase I/II trials have been performed using LAK cells or CTLs. These trials were largely in the recurrent setting and demonstrated limited efficacy combined with significant rates of toxicity for LAK cells [113], and better tolerance and improved survival for CTLs [113]. While responses to the adoptive therapies have shown only limited efficacy to date, it is possible that combination therapy or more targeted immunotherapeutic approaches may be of use.

Active immunotherapy is similar to vaccination, the idea being to stimulate the patient's immune system by using tumor-related sources of antigen (whole tumor cells, tumor protein lysates, mRNA, synthetic peptides). These sources of antigen can either be injected alone or coupled to dendritic cells [113]. Dendritic cells are powerful antigen-presenting cells, and dendritic cell therapy is designed to increase antigen presentation by incubating tumor antigens with these cells before injecting them back into the body (Figure 1). This method of active tumor immunotherapy has been the most extensively studied with widely varying results. Some of the trials performed using this method have shown promising results, while others demonstrated no benefit [113]. A major problem in interpreting results from these trials is the wide variation in protocols for everything from acquiring cells, type of tumor antigen chosen, and number of cells used. However, targeting this type of therapy may lead to enhanced clinical benefit. One major trial using patient tumor cell cultures infected with Newcastle Disease virus followed by gamma irradiation demonstrated significant increases in progression-free survival, 40 weeks versus 26 weeks, as well as overall survival, 100 weeks versus 49 weeks [114]. This trial also reported increased 1-year (91% versus 45%), 2-year (39% versus 11%), and long-term survivors (4% versus 0%). These results are very promising; however, the trial was nonrandomized and studied a limited number of subjects (23 patients receiving immunotherapy with 87 controls). Controls were also not treated using current standard of care as this trial was performed in 2004 before the results of the Stupp trial were published. Human cytomegalovirus (CMV) has been identified to be associated with tumors in a significant proportion of glioblastoma patients (50–90%) [115, 116]. Currently two clinical trials are ongoing at the Duke Brain Tumor Immunotherapy Program attempting to utilize this knowledge. The Vaccine Therapy in Treating Patients with Newly Diagnosed Glioblastoma Multiforme (ATTAC) [117] and the Evaluation of Recovery From Drug-Induced Lymphopenia Using Cytomegalovirus-Specific T-Cell Adoptive Transfer (ERaDICATe) [118] trials are either recruiting or in a data analysis phase, with results likely to be reported soon.

8.4. DNA Damage. DNA damage repair is a double-edged-sword in the cancer world. Lack of proper DNA repair can lead to genomic instability and the generation of cancer. However, once cancer is established, DNA repair genes undermine many effective cancer therapeutics. Both radiotherapy and chemotherapies are designed around a DNA-damaging strategy designed to induce cell death and tumor regression. In the presence of DNA repair proteins, these therapies have reduced efficacy. By targeting DNA repair proteins in cancer cells, we can again render them sensitive to radiation and chemotherapies. PARP plays a role in single stranded DNA, repair and inhibitors are currently being tested in a number of cancer sites. If PARP is inhibited, single-stranded nicks will not be repaired and will lead to DNA strand breaks during replication. Double-stranded DNA breaks are extremely toxic lesions to cells, and thereby PARP inhibition should increase cell death in proliferating cells (Figure 1). PARP inhibitors E7016 and AZD2281 have been shown to radiosensitize GBM cells both *in vitro* and *in vivo* [119, 120], with an enhanced effect when combined with heat shock protein 90 (HSP90) inhibition [121]. PARP inhibitors have also been shown to increase the efficacy of chemotherapies such as DNA topoisomerase I poisons, TMZ, iriniotecan, or cilengitide [122–124]. It has also been observed that PTEN loss can negatively affect homologous recombination, thereby increasing the efficacy of PARP inhibition as well as other DNA-damaging modalities [125]. In addition to PARP inhibition, other targets have been identified to utilize DNA repair as a therapeutic strategy. Inhibition of PP2A has been shown to augment DNA-damaging agents by inducing Plk-1 and AKT activation and decreasing p53 expression which has led to complete

remission or significant tumor regression, when combined with TMZ or doxorubicin, in a large number of tumors in a xenograft model [126].

8.5. Autophagy. Autophagy is an evolutionarily conserved process through which the cell is able to degrade damaged organelles and other cell components [127]. Reports identify autophagy to serve a dual role in cancer. Limited and controlled autophagy can be a survival method for cancer cells which allows them to overcome current therapies such as chemotherapy and radiation [128–130]. However, autophagy has also been identified as a possible therapeutic target because sustained and uncontrolled autophagy can lead to cell death [131, 132]. This process is interlinked with several crucial cancer survival pathways including p53 signaling and the PI3K pathway, as well as apoptotic signaling molecules like Bif-1 [127]. Currently, attempts are being made to control this switch and tip cells into a pro-death state. This work is similar to the rationale behind apoptotic research. Resistance to apoptosis is one of the major hallmarks of many cancer types, and the ability to regulate this cellular process would allow for not only increased efficacy of current treatments but also a novel target for future therapy. As with most therapies discussed so far, combination therapy will likely play a key role in future treatment plans. Recent studies have demonstrated autophagy can be induced in glioma cells by current standard-of-care therapy [130, 133] and AKT signaling plays a major role in the prosurvival effects of this process [129, 134]. This data indicates that cotargeting AKT will be important in regulating and controlling the prodeath role of autophagy in glioma.

8.6. HDAC Inhibitors. HDAC inhibitors have been studied in the setting of GBM and shown positive indications in both preclinical and clinical testing. In preclinical models, HDACs have been shown to sensitize cells to chemotherapy [135], to have antiproliferative activity by increasing PTEN and AKT expression while reducing phosphorylation of the proteins to their active forms [136], to increase apoptosis in GBM cells through activation of the JNK pathway and reduction in telomerase activity [137], and to sensitize cells to radiation [138]. In addition, in a phase II clinical trial, the potent HDAC inhibitor, vorinostat, was shown to have modest single-agent efficacy and to be well tolerated [139].

8.7. MicroRNA. As microRNAs have become better studied, the possible roles in therapeutic scenarios have increased. Issues still remain in targeted delivery of these microRNA constructs to tumor cells, but many possible targets have been identified for almost all cancer types. In glioblastoma, some of these targets include miR-124 and miR-137, which could target both tumor-initiating cells, by inducing differentiation, and normal glioblastoma cells by arresting cell growth [140]. The miR 302–367 cluster has also been shown to induce TIC differentiation as well as to reduce the infiltrative properties of these cells [141]. A miR-21 inhibitor has been shown to sensitize the glioblastoma cell line U251 to ionizing radiation [142]. miR-34-a has been shown to inhibit glioblastoma growth by targeting the c-Met and notch pathways, two well-known signaling pathways linked to glioma pathogenesis [143]. miR-10b has been linked to glioblastoma cell growth [144], and miR-124a has been linked to migration and invasion [145]. This sampling of microRNA targets linked to glioblastoma growth, survival, TIC maintenance, and migration/invasion underscores the possible therapeutic application of microRNAs to regulate major pathways linked to disease severity.

9. Discussion

In this paper we have discussed RTK inhibition, angiogenesis inhibitors, and the PI3K/Akt/mTOR inhibition in detail due to the substantial amount of research conducted in these areas and have also briefly discussed several very novel areas of research including Notch inhibition, viral and immunotherapies, and DNA repair pathways. Within the heavily researched pathways, the overarching theme in glioblastoma therapy is that monotherapies demonstrate limited efficacy. Because single-agent therapies have shown no significant benefit, it is critical to begin designing rational combinations. Different RTK inhibitors combined with PI3K/mTOR dual inhibitors or antiangiogenic agents combined with Akt inhibition are already being examined. It is likely that many of the novel therapies discussed in this work will demonstrate greater efficacy when paired with the more studied targeted therapies. Because many of these targets are within the same signaling cascade, inhibiting pathways horizontally rather than vertically should remove some of the compensatory mechanisms glioblastomas use to overcome treatment. It is also important to note that many of the molecular biology advancements will be augmented by advancements in current treatments. Improved tumor border delineation or detection of microscopic disease will enhance the efficacy of upfront surgical and radiotherapy interventions while better methods for posttreatment image surveillance will improve treatments in the recurrent setting. Critically, it should be acknowledged that these therapies will need to work in conjunction with the current standard of care, highlighting treatments that can serve as radio-or chemosensitizers. Glioblastoma carries a very poor prognosis, but with improved technology and novel, personalized, rational, targeted therapies patient survival and quality of life will be greatly improved.

References

[1] E. R. Laws, I. F. Parney, W. Huang et al., "Survival following surgery and prognostic factors for recently diagnosed malignant glioma: data from the glioma outcomes project," *Journal of Neurosurgery*, vol. 99, no. 3, pp. 467–473, 2003.

[2] W. Stummer, U. Pichlmeier, T. Meinel, O. D. Wiestler, F. Zanella, and H. J. Reulen, "Fluorescence-guided surgery with 5-aminolevulinic acid for resection of malignant glioma: a randomised controlled multicentre phase III trial," *Lancet Oncology*, vol. 7, no. 5, pp. 392–401, 2006.

[3] V. Vuorinen, S. Hinkka, M. Farkkila, and J. Jaaskelainen, "Debulking or biopsy of malignant glioma in elderly

people—a randomised study," *Acta Neurochirurgica*, vol. 145, no. 1, pp. 5–10, 2003.

[4] K. Kristiansen, S. Hagen, T. Kollevold, A. Torvik, I. Holme, and R. Nesbakken, "Combined modality therapy of operated astrocytomas grade III and IV. Confirmation of the value of postoperative irradiation and lack of potentiation of bleomycin on survival time: a prospective multicenter trial of the scandinavian glioblastoma study group," *Cancer*, vol. 47, no. 4, pp. 649–652, 1981.

[5] M. D. Walker, E. Alexander Jr, W. E. Hunt, C. S. MacCarty, M. S. Mahaley Jr, and J. Mealey Jr, "Evaluation of BCNU and/or radiotherapy in the treatment of anaplastic gliomas. A cooperative clinical trial," *Journal of Neurosurgery*, vol. 49, no. 3, pp. 333–343, 1978.

[6] R. Stupp, W. P. Mason, M. J. van den Bent et al., "Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma," *New England Journal of Medicine*, vol. 352, no. 10, pp. 987–996, 2005.

[7] R. Stupp, M. E. Hegi, W. P. Mason et al., "Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial," *The Lancet Oncology*, vol. 10, no. 5, pp. 459–466, 2009.

[8] M. Esteller, J. Garcia-Foncillas, E. Andion et al., "Inactivation of the DNA-repair gene MGMT and the clinical response of gliomas to alkylating agents," *New England Journal of Medicine*, vol. 343, no. 19, pp. 1350–1354, 2000.

[9] M. E. Hegi, A. C. Diserens, S. Godard et al., "Clinical Trial Substantiates the Predictive Value of O-6-Methylguanine-DNA Methyltransferase Promoter Methylation in Glioblastoma Patients Treated with Temozolomide," *Clinical Cancer Research*, vol. 10, no. 6, pp. 1871–1874, 2004.

[10] M. E. Hegi, A. C. Diserens, T. Gorlia et al., "MGMT gene silencing and benefit from temozolomide in glioblastoma," *New England Journal of Medicine*, vol. 352, no. 10, pp. 997–1003, 2005.

[11] L. Souhami, W. Seiferheld, D. Brachman et al., "Randomized comparison of stereotactic radiosurgery followed by conventional radiotherapy with carmustine to conventional radiotherapy with carmustine for patients with glioblastoma multiforme: report of radiation therapy oncology group 93-05 protocol," *International Journal of Radiation Oncology Biology Physics*, vol. 60, no. 3, pp. 853–860, 2004.

[12] N. J. Laperriere, P. M. K. Leung, S. McKenzie et al., "Randomized study of brachytherapy in the initial management of patients with malignant astrocytoma," *International Journal of Radiation Oncology Biology Physics*, vol. 41, no. 5, pp. 1005–1011, 1998.

[13] M. R. Gilbert, M. Wang, K. D. Aldape et al., "RTOG 0525: a randomized phase III trial comparing standard adjuvant temozolomide (TMZ) with a dose-dense (dd) schedule in newly diagnosed glioblastoma (GBM)," *Journal of Clinical Oncology*, vol. 29, no. 15, 2011.

[14] D. Pauleit, G. Stoffels, A. Bachofner et al., "Comparison of (18)F-FET and (18)F-FDG PET in brain tumors," *Nuclear Medicine and Biology*, vol. 36, no. 7, pp. 779–787, 2009.

[15] I. H. Lee, M. Piert, D. Gomez-Hassan et al., "Association of 11C-methionine PET uptake with site of failure after concurrent temozolomide and radiation for primary glioblastoma multiforme," *International Journal of Radiation Oncology Biology Physics*, vol. 73, no. 2, pp. 479–485, 2009.

[16] N. Kawai, Y. Maeda, N. Kudomi et al., "Correlation of biological aggressiveness assessed by 11C-methionine PET and hypoxic burden assessed by 18F-fluoromisonidazole PET in newly diagnosed glioblastoma," *European Journal of Nuclear Medicine and Molecular Imaging*, pp. 1–10, 2010.

[17] N. Sadeghi, I. Salmon, C. Decaestecker et al., "Stereotactic comparison among cerebral blood volume, methionine uptake, and histopathology in brain glioma," *American Journal of Neuroradiology*, vol. 28, no. 3, pp. 455–461, 2007.

[18] D. Pauleit, F. Floeth, K. Hamacher et al., "O-(2-[18F]fluoroethyl)-L-tyrosine PET combined with MRI improves the diagnostic assessment of cerebral gliomas," *Brain*, vol. 128, no. 3, pp. 678–687, 2005.

[19] M. D. Piroth, M. Pinkawa, R. Holy et al., "Prognostic Value of Early [18F]Fluoroethyltyrosine Positron Emission Tomography After Radiochemotherapy in Glioblastoma Multiforme," *International Journal of Radiation Oncology, Biology, Physics*, 2010.

[20] A. P. Krishnan, I. M. Asher, D. Davis, P. Okunieff, and W. G. O'Dell, "Evidence That MR Diffusion Tensor Imaging (Tractography) Predicts the Natural History of Regional Progression in Patients Irradiated Conformally for Primary Brain Tumors," *International Journal of Radiation Oncology Biology Physics*, vol. 71, no. 5, pp. 1553–1562, 2008.

[21] I. Yang and M. K. Aghi, "New advances that enable identification of glioblastoma recurrence," *Nature Reviews Clinical Oncology*, vol. 6, no. 11, pp. 648–657, 2009.

[22] W. Hollingworth, L. S. Medina, R. E. Lenkinski et al., "A systematic literature review of magnetic resonance spectroscopy for the characterization of brain tumors," *American Journal of Neuroradiology*, vol. 27, no. 7, pp. 1404–1411, 2006.

[23] M. Patel, F. Siddiqui, J. Y. Jin et al., "Salvage reirradiation for recurrent glioblastoma with radiosurgery: radiographic response and improved survival," *Journal of Neuro-Oncology*, vol. 92, no. 2, pp. 185–191, 2009.

[24] P. C. Hsieh, J. P. Chandler, S. Bhangoo et al., "Adjuvant gamma knife stereotactic radiosurgery at the time of tumor progression potentially improves survival for patients with glioblastoma multiforme," *Neurosurgery.*, vol. 57, no. 4, pp. 684–692, 2005.

[25] T. Biswas, P. Okunieff, M. C. Schell et al., "Stereotactic radiosurgery for glioblastoma: retrospective analysis," *Radiation Oncology*, vol. 4, p. 11, 2009.

[26] S. E. Fogh, D. W. Andrews, J. Glass et al., "Hypofractionated stereotactic radiation therapy: an effective therapy for recurrent high-grade gliomas," *Journal of Clinical Oncology*, vol. 28, no. 18, pp. 3048–3053, 2010.

[27] E. Fokas, U. Wacker, M. W. Gross, M. Henzel, E. Encheva, and R. Engenhart-Cabillic, "Hypofractionated stereotactic reirradiation of recurrent glioblastomas: a beneficial treatment option after high-dose radiotherapy?" *Strahlentherapie und Onkologie*, vol. 185, no. 4, pp. 235–240, 2009.

[28] S. E. Combs, C. Thilmann, L. Edler, J. Debus, and D. Schulz-Ertner, "Efficacy of fractionated stereotactic reirradiation in recurrent gliomas: long-term results in 172 patients treated in a single institution," *Journal of Clinical Oncology*, vol. 23, no. 34, pp. 8863–8869, 2005.

[29] S. F. Shepherd, R. W. Laing, V. P. Cosgrove et al., "Hypofractionated stereotactic radiotherapy in the management of recurrent glioma," *International Journal of Radiation Oncology Biology Physics*, vol. 37, no. 2, pp. 393–398, 1997.

[30] P. H. Gutin, F. M. Iwamoto, K. Beal et al., "Safety and Efficacy of Bevacizumab With Hypofractionated Stereotactic Irradiation for Recurrent Malignant Gliomas," *International*

Journal of Radiation Oncology Biology Physics, vol. 75, no. 1, pp. 156–163, 2009.

[31] J. Glass, C. L. Silverman, R. Axelrod, B. W. Corn, and D. W. Andrews, "Fractionated stereotactic radiotherapy with cisplatinum radiosensitization in the treatment of recurrent, progressive, or persistent malignant astrocytoma," *American Journal of Clinical Oncology*, vol. 20, no. 3, pp. 226–229, 1997.

[32] G. Lederman, M. Wronski, E. Arbit et al., "Treatment of recurrent glioblastoma multiforme using fractionated stereotactic radiosurgery and concurrent paclitaxel," *American Journal of Clinical Oncology*, vol. 23, no. 2, pp. 155–159, 2000.

[33] R. E. Wurm, D. A. Kuczer, L. Schlenger et al., "Hypofractionated stereotactic radiotherapy combined with topotecan in recurrent malignant glioma," *International Journal of Radiation Oncology Biology Physics*, vol. 66, no. 4, pp. S26–S32, 2006.

[34] W. K. A. Yung, R. E. Albright, J. Olson et al., "A phase II study of temozolemide vs. procarbazine in patients with glioblastoma multiforme at first relapse," *British Journal of Cancer*, vol. 83, no. 5, pp. 588–593, 2000.

[35] A. A. Brandes, A. Tosoni, G. Cavallo et al., "Temozolomide 3 weeks on and 1 week off as first-line therapy for recurrent glioblastoma: phase II study from Gruppo Italiano Cooperativo di Neuro-Oncologia (GICNO)," *British Journal of Cancer*, vol. 95, no. 9, pp. 1155–1160, 2006.

[36] M. Grzmil and B. A. Hemmings, "Deregulated signalling networks in human brain tumours," *Biochimica et Biophysica Acta—Proteins and Proteomics*, vol. 1804, no. 3, pp. 476–483, 2010.

[37] J. H. Uhm, K. V. Ballman, W. Wu et al., "Phase II evaluation of gefitinib in patients with newly diagnosed grade 4 astrocytoma: Mayo/North central cancer treatment group study N0074," *International Journal of Radiation Oncology, Biology, Physics*, 2010.

[38] D. M. Peereboom, D. R. Shepard, M. S. Ahluwalia et al., "Phase II trial of erlotinib with temozolomide and radiation in patients with newly diagnosed glioblastoma multiforme," *Journal of Neuro-Oncology*, vol. 98, no. 1, pp. 93–99, 2010.

[39] M. D. Prados, S. M. Chang, N. Butowski et al., "Phase II study of erlotinib plus temozolomide during and after radiation therapy in patients with newly diagnosed glioblastoma multiforme or gliosarcoma," *Journal of Clinical Oncology*, vol. 27, no. 4, pp. 579–584, 2009.

[40] P. R. Wachsberger, Y. R. Lawrence, Y. Liu, B. Daroczi, X. Xu, and A. P. Dicker, "Epidermal growth factor receptor expression modulates antitumor efficacy of vandetanib or cediranib combined with radiotherapy in human glioblastoma xenografts," *International Journal of Radiation Oncology Biology Physics*, vol. 82, no. 1, pp. 483–491, 2012.

[41] Y. Diao, X. H. Tian, Y. Huang, L. K. Chen, X. N. Lin, and Z. W. Zhuang, "Enhanced cancer therapy with the combination of EGFR and VEGFR-2 targeting in an orthotopic glioblastoma model," *Journal of Chemotherapy*, vol. 22, no. 6, pp. 407–412, 2010.

[42] S. Berezowska, S. Diermeier-Daucher, G. Brockhoff et al., "Effect of additional inhibition of human epidermal growth factor receptor 2 with the bispecific tyrosine kinase inhibitor AEE788 on the resistance to specific EGFR inhibition in glioma cells," *International Journal of Molecular Medicine*, vol. 26, no. 5, pp. 713–721, 2010.

[43] B. Lal, C. R. Goodwin, Y. Sang et al., "EGFRvIII and c-Met pathway inhibitors synergize against PTEN-null/EGFRvIII+ glioblastoma xenografts," *Molecular Cancer Therapeutics*, vol. 8, no. 7, pp. 1751–1760, 2009.

[44] B. Lal, C. R. Goodwin, Y. Sang et al., "EGFRvIII and c-Met pathway inhibitors synergize against PTEN-null/EGFRvIII+ glioblastoma xenografts," *Molecular Cancer Therapeutics*, vol. 8, no. 7, pp. 1751–1760, 2009.

[45] C. Cemeus, T. T. Zhao, G. M. Barrett, I. A. Lorimer, and J. Dimitroulakos, "Lovastatin enhances gefitinib activity in glioblastoma cells irrespective of EGFRvIII and PTEN status," *Journal of Neuro-Oncology*, vol. 90, no. 1, pp. 9–17, 2008.

[46] B. Thiessen, C. Stewart, M. Tsao et al., "A phase I/II trial of GW572016 (lapatinib) in recurrent glioblastoma multiforme: clinical outcomes, pharmacokinetics and molecular correlation," *Cancer Chemotherapy and Pharmacology*, vol. 65, no. 2, pp. 353–361, 2010.

[47] D. A. Reardon, A. Desjardins, J. J. Vredenburgh et al., "Phase 2 trial of erlotinib plus sirolimus in adults with recurrent glioblastoma," *Journal of Neuro-Oncology*, vol. 96, no. 2, pp. 219–230, 2010.

[48] J. van der Veeken, S. Oliveira, R. M. Schiffelers, G. Storm, P. M. P. Van Bergen En Henegouwen, and R. C. Roovers, "Crosstalk between epidermal growth factor receptor- and insulin-like growth factor-1 receptor signaling: implications for cancer therapy," *Current Cancer Drug Targets*, vol. 9, no. 6, pp. 748–760, 2009.

[49] A. Chakravarti, J. S. Loeffler, and N. J. Dyson, "Insulin-like growth factor receptor I mediates resistance to anti-epidermal growth factor receptor therapy in primary human glioblastoma cells through continued activation of phosphoinositide 3-kinase signaling," *Cancer Research*, vol. 62, no. 1, pp. 200–207, 2002.

[50] D. R. Premkumar, E. P. Jane, and I. F. Pollack, "Co-administration of NVP-AEW541 and dasatinib induces mitochondrial- mediated apoptosis through Bax activation in malignant human glioma cell lines," *International Journal of Oncology*, vol. 37, no. 3, pp. 633–643, 2010.

[51] E. Ranza, G. Mazzini, A. Facoetti, and R. Nano, "In-vitro effects of the tyrosine kinase inhibitor imatinib on glioblastoma cell proliferation," *Journal of Neuro-Oncology*, vol. 96, no. 3, pp. 349–357, 2010.

[52] Y. Dong, L. Jia, X. Wang et al., "Selective inhibition of PDGFR by imatinib elicits the sustained activation of ERK and downstream receptor signaling in malignant glioma cells," *International Journal of Oncology*, vol. 38, no. 2, pp. 555–569, 2011.

[53] J. Paulsson, M. B. Lindh, M. Jarvius et al., "Prognostic but not predictive role of platelet-derived growth factor receptors in patients with recurrent glioblastoma," *International Journal of Cancer*, vol. 128, no. 8, pp. 1981–1988, 2011.

[54] N. Kubota, S. Okada, T. Inada, K. Ohnishi, and T. Ohnishi, "Wortmannin sensitizes human glioblastoma cell lines carrying mutant and wild type TP53 gene to radiation," *Cancer Letters*, vol. 161, no. 2, pp. 141–147, 2000.

[55] M. Klingler-Hoffmann, P. Bukczynska, and T. Tiganis, "Inhibition of phosphatidylinositol 3-kinase signaling negates the growth advantage imparted by a mutant epidermal growth factor receptor on human glioblastoma cells," *International Journal of Cancer*, vol. 105, no. 3, pp. 331–339, 2003.

[56] J. LoPiccolo, G. M. Blumenthal, W. B. Bernstein, and P. A. Dennis, "Targeting the PI3K/Akt/mTOR pathway: effective combinations and clinical considerations," *Drug Resistance Updates*, vol. 11, no. 1-2, pp. 32–50, 2008.

[57] S. M. Chang, J. Kuhn, P. Wen et al., "Phase I/pharmacokinetic study of CCI-779 in patients with recurrent malignant glioma on enzyme-inducing antiepileptic drugs," *Investigational New Drugs*, vol. 22, no. 4, pp. 427–435, 2004.

[58] J. S. Eshleman, B. L. Carlson, A. C. Mladek, B. D. Kastner, K. L. Shide, and J. N. Sarkaria, "Inhibition of the mammalian target of rapamycin sensitizes U87 xenografts to fractionated radiation therapy," *Cancer Research*, vol. 62, no. 24, pp. 7291–7297, 2002.

[59] N. Gulati, M. Karsy, L. Albert, R. Murali, and M. Jhanwar-Uniyal, "Involvement of mTORC1 and mTORC2 in regulation of glioblastoma multiforme growth and motility," *International Journal of Oncology*, vol. 35, no. 4, pp. 731–740, 2009.

[60] T. F. Cloughesy, K. Yoshimoto, P. Nghiemphu et al., "Antitumor activity of rapamycin in a phase I trial for patients with recurrent PTEN-deficient glioblastoma," *PLoS Medicine*, vol. 5, no. 1, pp. 0139–0151, 2008.

[61] L. Albert, M. Karsy, R. Murali, and M. Jhanwar-Uniyal, "Inhibition of mTOR activates the MAPK pathway in glioblastoma multiforme," *Cancer Genomics and Proteomics*, vol. 6, no. 5, pp. 255–261, 2009.

[62] S. M. Chang, P. Wen, T. Cloughesy et al., "Phase II study of CCI-779 in patients with recurrent glioblastoma multiforme," *Investigational New Drugs*, vol. 23, no. 4, pp. 357–361, 2005.

[63] E. Galanis, J. C. Buckner, M. J. Maurer et al., "Phase II trial of temsirolimus (CCI-779) in recurrent glioblastoma multiforme: a north central cancer treatment group study," *Journal of Clinical Oncology*, vol. 23, no. 23, pp. 5294–5304, 2005.

[64] G. Prasad, T. Sottero, X. Yang et al., "Inhibition of PI3K/mTOR pathways in glioblastoma and implications for combination therapy with temozolomide," *Neuro-Oncology*, vol. 13, no. 4, pp. 384–392, 2011.

[65] R. Mallon, I. Hollander, L. Feldberg et al., "Antitumor efficacy profile of PKI-402, a dual phosphatidylinositol 3-kinase/Mammalian target of rapamycin inhibitor," *Molecular Cancer Therapeutics*, vol. 9, no. 4, pp. 976–984, 2010.

[66] Q. W. Fan, Z. A. Knight, D. D. Goldenberg et al., "A dual PI3 kinase/mTOR inhibitor reveals emergent efficacy in glioma," *Cancer Cell*, vol. 9, no. 5, pp. 341–349, 2006.

[67] Q. W. Fan, Z. A. Knight, D. D. Goldenberg et al., "A dual PI3 kinase/mTOR inhibitor reveals emergent efficacy in glioma," *Cancer Cell*, vol. 9, no. 5, pp. 341–349, 2006.

[68] T. Bagci-Onder, H. Wakimoto, M. Anderegg, C. Cameron, and K. Shah, "A dual PI3K/mTOR inhibitor, PI-103, cooperates with stem cell-delivered TRAIL in experimental glioma models," *Cancer Research*, vol. 71, no. 1, pp. 154–163, 2011.

[69] M. A. Westhoff, J. A. Kandenwein, S. Karl et al., "The pyridinylfuranopyrimidine inhibitor, PI-103, chemosensitizes glioblastoma cells for apoptosis by inhibiting DNA repair," *Oncogene*, vol. 28, no. 40, pp. 3586–3596, 2009.

[70] R. Prevo, E. Deutsch, O. Sampson et al., "Class I PI3 kinase inhibition by the pyridinylfuranopyrimidine inhibitor PI-103 enhances tumor radiosensitivity," *Cancer Research*, vol. 68, no. 14, pp. 5915–5923, 2008.

[71] M. M. Alonso, H. Jiang, T. Yokoyama et al., "Delta-24-RGD in combination with RAD001 induces enhanced anti-glioma effect via autophagic cell death," *Molecular Therapy*, vol. 16, no. 3, pp. 487–493, 2008.

[72] A. B. Hjelmeland, K. P. Lattimore, B. E. Fee et al., "The combination of novel low molecular weight inhibitors of RAF (LBT613) and target of rapamycin (RAD001) decreases glioma proliferation and invasion," *Molecular Cancer Therapeutics*, vol. 6, no. 9, pp. 2449–2457, 2007.

[73] R. K. Goudar, Q. Shi, M. D. Hjelmeland et al., "Combination therapy of inhibitors of epidermal growth factor receptor/vascular endothelial growth factor receptor 2 (AEE788) and the mammalian target of rapamycin (RAD001) offers improved glioblastoma tumor growth inhibition," *Molecular Cancer Therapeutics*, vol. 4, no. 1, pp. 101–112, 2005.

[74] L. Yang, M. J. Clarke, B. L. Carlson et al., "PTEN loss does not predict for response to RAD001 (everolimus) in a glioblastoma orthotopic xenograft test panel," *Clinical Cancer Research*, vol. 14, no. 12, pp. 3993–4001, 2008.

[75] M. Y. Wang, K. V. Lu, S. Zhu et al., "Mammalian target of rapamycin inhibition promotes response to epidermal growth factor receptor kinase inhibitors in PTEN-deficient and PTEN-intact glioblastoma cells," *Cancer Research*, vol. 66, no. 16, pp. 7864–7869, 2006.

[76] J. C. Reijneveld, E. E. Voest, and M. J. B. Taphoorn, "Angiogenesis in malignant primary and metastatic brain tumors," *Journal of Neurology*, vol. 247, no. 8, pp. 597–608, 2000.

[77] N. Ferrara, H. P. Gerber, and J. LeCouter, "The biology of VEGF and its receptors," *Nature Medicine*, vol. 9, no. 6, pp. 669–676, 2003.

[78] I. H. Chaudhry, D. G. O'Donovan, P. E. C. Brenchley, H. Reid, and I. S. D. Roberts, "Vascular endothelial growth factor expression correlates with tumour grade and vascularity in gliomas," *Histopathology*, vol. 39, no. 4, pp. 409–415, 2001.

[79] D. F. Stefanik, W. K. Fellows, L. R. Rizkalla et al., "Monoclonal antibodies to vascular endothelial growth factor (VEGF) and the VEGF receptor, FLT-1, inhibit the growth of C6 glioma in a mouse xenograt," *Journal of Neuro-Oncology*, vol. 55, no. 2, pp. 91–100, 2001.

[80] S. Bao, Q. Wu, S. Sathornsumetee et al., "Stem cell-like glioma cells promote tumor angiogenesis through vascular endothelial growth factor," *Cancer Research*, vol. 66, no. 16, pp. 7843–7848, 2006.

[81] L. G. Presta, H. Chen, S. J. O'Connor et al., "Humanization of an anti-vascular endothelial growth factor monoclonal antibody for the therapy of solid tumors and other disorders," *Cancer Research*, vol. 57, no. 20, pp. 4593–4599, 1997.

[82] F. Kazazi-Hyseni, J. H. Beijnen, and J. H. M. Schellens, "Bevacizumab," *Oncologist*, vol. 15, no. 8, pp. 819–825, 2010.

[83] J. J. Vredenburgh, A. Desjardins, J. P. Kirkpatrick et al., "Addition of Bevacizumab to Standard Radiation Therapy and Daily Temozolomide Is Associated with Minimal Toxicity in Newly Diagnosed Glioblastoma Multiforme," *International Journal of Radiation Oncology, Biology, Physics*, 2010.

[84] J. J. Vredenburgh, A. Desjardins, J. P. Kirkpatrick et al., "Addition of bevacizumab to standard radiation therapy and daily temozolomide is associated with minimal toxicity in newly diagnosed glioblastoma multiforme," *International Journal of Radiation Oncology Biology Physics*, vol. 82, no. 1, pp. 58–66, 2012.

[85] J. J. Vredenburgh, A. Desjardins, D. A. Reardon et al., "Bevacizumab, temozolomide, and radiation therapy followed by bevacizumab, temozolomide, and oral topotecan for newly-diagnosed glioblastoma multiforme (GBM)," *Journal of Clinical Oncology*, vol. 13, supplement 3, pp. iii858–iii91, 2011.

[86] A. M. P. Omuro, K. Beal, and S. Karimi, "Phase II study of bevacizumab (BEV), temozolomide (TMZ), and hypofractionated stereotactic radiotherapy (HFSRT) for newly diagnosed glioblastoma (GBM)," *Journal of Clinical Oncology*, vol. 13, supplement 3, pp. iii41–iii68, 2011.

[87] A study of avastin (bevacizumab) in combination with temozolomide and radiotherapy in patients with newly

diagnosed glioblastoma, 2010, http://clinicaltrials.gov/ct2/show/NCT00943826.

[88] Temozolomide and radiation therapy with or without bevacizumab in treating patients with newly diagnosed glioblastoma, 2011, http://clinicaltrials.gov/ct2/show/NCT00884741?term=rtog+0825&rank=1.

[89] K. Beal, L. E. Abrey, and P. H. Gutin, "Antiangiogenic agents in the treatment of recurrent or newly diagnosed glioblastoma: analysis of single-agent and combined modality approaches," *Radiation Oncology*, vol. 6, no. 1, 2011.

[90] D. A. Reardon, K. L. Fink, T. Mikkelsen et al., "Randomized phase II study of cilengitide, an integrin-targeting arginine-glycine-aspartic acid peptide, in recurrent glioblastoma multiforme," *Journal of Clinical Oncology*, vol. 26, no. 34, pp. 5610–5617, 2008.

[91] R. Stupp, M. E. Hegi, B. Neyns et al., "Phase I/IIa study of cilengitide and temozolomide with concomitant radiotherapy followed by cilengitide and temozolomide maintenance therapy in patients with newly diagnosed glioblastoma," *Journal of Clinical Oncology*, vol. 28, no. 16, pp. 2712–2718, 2010.

[92] Cilengitide, temozolomide, and radiation therapy in treating patients with newly diagnosed glioblastoma and methylated gene promoter status (CENTRIC), 2011, http://clinicaltrials.gov/ct2/show/NCT00689221?term=centric&rank=1.

[93] M. M. Lino, A. Merlo, and J. Boulay, "Notch signaling in glioblastoma: a developmental drug target?" *BMC Medicine*, vol. 8, 2010.

[94] C. Brennan, H. Momota, D. Hambardzumyan et al., "Glioblastoma subclasses can be defined by activity among signal transduction pathways and associated genomic alterations," *PLoS ONE*, vol. 4, no. 11, Article ID e7752, 2009.

[95] X. Fan, L. Khaki, T. S. Zhu et al., "NOTCH pathway blockade depletes CD133-positive glioblastoma cells and inhibits growth of tumor neurospheres and xenografts," *Stem Cells*, vol. 28, no. 1, pp. 5–16, 2010.

[96] M. Ying, S. Wang, Y. Sang et al., "Regulation of glioblastoma stem cells by retinoic acid: role for Notch pathway inhibition," *Oncogene*, vol. 30, no. 31, pp. 3454–3467, 2011.

[97] J. Chen, S. Kesari, C. Rooney et al., "Inhibition of Notch signaling blocks growth of glioblastoma cell lines and tumor neurospheres," *Genes and Cancer*, vol. 1, no. 8, pp. 822–835, 2010.

[98] I. V. Ulasov, M. A. Tyler, A. A. Rivera, D. M. Nettlebeck, J. T. Douglas, and M. S. Lesniak, "Evaluation of E1A double mutant oncolytic adenovectors in anti-glioma gene therapy," *Journal of Medical Virology*, vol. 80, no. 9, pp. 1595–1603, 2008.

[99] R. Chambers, G. Y. Gillespie, L. Soroceanu et al., "Comparison of genetically engineered herpes simplex viruses for the treatment of brain tumors in a scid mouse model of human malignant glioma," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 92, no. 5, pp. 1411–1415, 1995.

[100] M. E. Wilcox, W. Yang, D. Senger et al., "Reovirus as an oncolytic agent against experimental human malignant gliomas," *Journal of the National Cancer Institute*, vol. 93, no. 12, pp. 903–912, 2001.

[101] J. Hardcastle, K. Kurozumi, N. Dmitrieva et al., "Enhanced antitumor efficacy of vasculostatin (Vstat120) expressing oncolytic HSV-1," *Molecular Therapy*, vol. 18, no. 2, pp. 285–294, 2010.

[102] N. Dmitrieva, L. Yu, M. Viapiano et al., "Chondroitinase ABC I-mediated enhancement of oncolytic virus spread and antitumor efficacy," *Clinical Cancer Research*, vol. 17, no. 6, pp. 1362–1372, 2011.

[103] D. H. Meijer, C. A. Maguire, S. G. Leroy, and M. Sena-Esteves, "Controlling brain tumor growth by intraventricular administration of an AAV vector encoding IFN-β," *Cancer Gene Therapy*, vol. 16, no. 8, pp. 664–671, 2009.

[104] J. M. Markert, M. D. Medlock, S. D. Rabkin et al., "Conditionally replicating herpes simplex virus mutant G207 for the treatment of malignant glioma: results of a phase I trial," *Gene Therapy*, vol. 7, no. 10, pp. 867–874, 2000.

[105] E. A. Chiocca, K. M. Abbed, S. Tatter et al., "A phase I open-label, dose-escalation, multi-institutional trial of injection with an E1B-attenuated adenovirus, ONYX-015, into the peritumoral region of recurrent malignant gliomas, in the adjuvant setting," *Molecular Therapy*, vol. 10, no. 5, pp. 958–966, 2004.

[106] P. Forsyth, G. Roldán, D. George et al., "A phase I trial of intratumoral administration of reovirus in patients with histologically confirmed recurrent malignant gliomas," *Molecular Therapy*, vol. 16, no. 3, pp. 627–632, 2008.

[107] J. Wykosky, D. M. Gibo, C. Stanton, and W. Debinski, "Interleukin-13 receptor α2, EphA2, and Fos-related antigen 1 as molecular denominators of high-grade astrocytomas and specific targets for combinatorial therapy," *Clinical Cancer Research*, vol. 14, no. 1, pp. 199–208, 2008.

[108] H. Horita, J. Thorburn, A. E. Frankel, and A. Thorburn, "EGFR-targeted diphtheria toxin stimulates TRAIL killing of glioblastoma cells by depleting anti-apoptotic proteins," *Journal of Neuro-Oncology*, vol. 95, no. 2, pp. 175–184, 2009.

[109] P. Grandi, J. Fernandez, O. Szentirmai et al., "Targeting HSV-1 virions for specific binding to epidermal growth factor receptor-vIII-bearing tumor cells," *Cancer Gene Therapy*, vol. 17, no. 9, pp. 655–663, 2010.

[110] S. Kunwar, S. Chang, M. Westphal et al., "Phase III randomized trial of CED of IL13-PE38QQR vs Gliadel wafers for recurrent glioblastoma," *Neuro-Oncology*, vol. 12, no. 8, pp. 871–881, 2010.

[111] J. Jin, K. M. Joo, S. J. Lee et al., "Synergistic therapeutic effects of cytokine-induced killer cells and temozolomide against glioblastoma," *Oncology Reports*, vol. 25, no. 1, pp. 33–39, 2011.

[112] N. Ahmed, V. S. Salsman, Y. Kew et al., "HER2-specific T cells target primary glioblastoma stem cells and induce regression of autologous experimental tumors," *Clinical Cancer Research*, vol. 16, no. 2, pp. 474–485, 2010.

[113] E. Vauleon, T. Avril, B. Collet, J. Mosser, and V. Quillien, "Overview of cellular immunotherapy for patients with glioblastoma," *Clinical and Developmental Immunology*, vol. 2010, Article ID 689171, 2010.

[114] H. H. Steiner, M. M. Bonsanto, P. Beckhove et al., "Antitumor vaccination of patients with glioblastoma multiforme: a pilot study to assess feasibility, safety, and clinical benefits," *Journal of Clinical Oncology*, vol. 22, no. 21, pp. 4272–4281, 2004.

[115] K. G. Lucas, L. Bao, R. Bruggeman, K. Dunham, and C. Specht, "The detection of CMV pp65 and IE1 in glioblastoma multiforme," *Journal of Neuro-Oncology*, vol. 103, no. 2, pp. 231–238, 2011.

[116] D. A. Mitchell, W. Xie, R. Schmittling et al., "Sensitive detection of human cytomegalovirus in tumors and peripheral blood of patients diagnosed with glioblastoma," *Neuro-Oncology*, vol. 10, no. 1, pp. 10–18, 2008.

[117] Vaccine therapy in treating patients with newly diagnosed glioblastoma multiforme (ATTAC), 2011, http://www.clinicaltrials.gov/ct2/show/NCT00639639?term=ATTAC&rank=1.

[118] Evaluation of recovery from drug-induced lymphopenia using cytomegalovirus-specific T-cell adoptive transfer (ERaDICATe), 2011, http://www.clinicaltrials.gov/ct2/show/NCT00693095?term=ERaDICATe&rank=1.

[119] A. L. Russo, H. C. Kwon, W. E. Burgan et al., "In vitro and in vivo radiosensitization of glioblastoma cells by the poly (ADP-ribose) polymerase inhibitor E7016," *Clinical Cancer Research*, vol. 15, no. 2, pp. 607–612, 2009.

[120] F. A. Dungey, D. A. Loser, and A. J. Chalmers, "Replication-dependent radiosensitization of human glioma cells by inhibition of poly(ADP-Ribose) polymerase: mechanisms and therapeutic potential," *International Journal of Radiation Oncology Biology Physics*, vol. 72, no. 4, pp. 1188–1197, 2008.

[121] F. A. Dungey, K. W. Caldecott, and A. J. Chalmers, "Enhanced radiosensitization of human glioma cells by combining inhibition of poly(ADP-ribose) polymerase with inhibition of heat shock protein 90," *Molecular Cancer Therapeutics*, vol. 8, no. 8, pp. 2243–2254, 2009.

[122] G. Cimmino, S. Pepe, G. Laus et al., "Poly(ADPR)polymerase-1 signalling of the DNA damage induced by DNA topoisomerase I poison in D54p53wt and U251p53mut glioblastoma cell lines," *Pharmacological Research*, vol. 55, no. 1, pp. 49–56, 2007.

[123] L. Tentori, C. Leonetti, M. Scarsella et al., "Systemic Administration of GPI 15427, a Novel Poly(ADP-Ribose) Polymerase-1 Inhibitor, Increases the Antitumor Activity of Temozolomide against Intracranial Melanoma, Glioma, Lymphoma," *Clinical Cancer Research*, vol. 9, no. 14, pp. 5370–5379, 2003.

[124] S. J. Miknyoczki, S. Jones-Bolin, S. Pritchard et al., "Chemopotentiation of temozolomide, irinotecan, and cisplatin activity by CEP-6800, a poly(ADP-ribose) polymerase inhibitor," *Molecular cancer therapeutics*, vol. 2, no. 4, pp. 371–382, 2003.

[125] B. McEllin, C. V. Camacho, B. Mukherjee et al., "PTEN loss compromises homologous recombination repair in astrocytes: implications for glioblastoma therapy with temozolomide or poly(ADP-Ribose) polymerase inhibitors," *Cancer Research*, vol. 70, no. 13, pp. 5457–5464, 2010.

[126] J. Lu, J. S. Kovach, F. Johnson et al., "Inhibition of serine/threonine phosphatase PP2A enhances cancer chemotherapy by blocking DNA damage induced defense mechanisms," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 28, pp. 11697–11702, 2009.

[127] C. H. Eng and R. T. Abraham, "The autophagy conundrum in cancer: influence of tumorigenic metabolic reprogramming," *Oncogene*, vol. 30, no. 47, pp. 4687–4696, 2011.

[128] W. Zhuang, B. Li, L. Long, L. Chen, Q. Huang, and Z. Q. Liang, "Knockdown of the DNA-dependent protein kinase catalytic subunit radiosensitizes glioma-initiating cells by inducing autophagy," *Brain Research*, 2010.

[129] Q. W. Fan, C. Cheng, C. Hackett et al., "Akt and autophagy cooperate to promote survival of drug-resistant glioma," *Science Signaling*, vol. 3, no. 147, Article ID ra81, 2010.

[130] S. L. Lomonaco, S. Finniss, C. Xiang et al., "The induction of autophagy by γ-radiation contributes to the radioresistance of glioma stem cells," *International Journal of Cancer*, vol. 125, no. 3, pp. 717–722, 2009.

[131] E. C. Filippi-Chiela, E. S. Villodre, L. L. Zamin, and G. Lenz, "Autophagy interplay with apoptosis and cell cycle regulation in the growth inhibiting effect of resveratrol in glioma cells," *PLoS ONE*, vol. 6, no. 6, Article ID e20849, 2011.

[132] W. T. Liu, C. H. Lin, M. Hsiao, and P. W. Gean, "Minocycline inhibits the growth of glioma by inducing autophagy," *Autophagy*, vol. 7, no. 2, pp. 166–175, 2011.

[133] M. Natsumeda, H. Aoki, H. Miyahara et al., "Induction of autophagy in temozolomide treated malignant gliomas," *Neuropathology*, vol. 31, no. 5, pp. 486–493, 2011.

[134] Q. W. Fan and W. A. Weiss, "Autophagy and Akt promote survival in glioma," *Autophagy*, vol. 7, no. 5, pp. 536–538, 2011.

[135] A. Bangert, S. Hacker, S. Cristofanon, K. M. Debatin, and S. Fulda, "Chemosensitization of glioblastoma cells by the histone deacetylase inhibitor MS275," *Anti-Cancer Drugs*, vol. 22, no. 6, pp. 494–499, 2011.

[136] W. J. Huang, C. W. Lin, C. Y. Lee et al., "NBM-HD-3, a novel histone deacetylase inhibitor with anticancer activity through modulation of PTEN and AKT in brain cancer cells," *Journal of Ethnopharmacology*, vol. 136, no. 1, pp. 156–167, 2011.

[137] V. Sharma, N. Koul, C. Joseph, D. Dixit, S. Ghosh, and E. Sen, "HDAC inhibitor, scriptaid, induces glioma cell apoptosis through JNK activation and inhibits telomerase activity," *Journal of Cellular and Molecular Medicine*, vol. 14, no. 8, pp. 2151–2161, 2010.

[138] J. H. Kim, J. H. Shin, and I. H. Kim, "Susceptibility and radiosensitization of human glioblastoma cells to trichostatin A, a histone deacetylase inhibitor," *International Journal of Radiation Oncology Biology Physics*, vol. 59, no. 4, pp. 1174–1180, 2004.

[139] E. Galanis, K. A. Jaeckle, M. J. Maurer et al., "Phase II trial of vorinostat in recurrent glioblastoma multiforme: a north central cancer treatment group study," *Journal of Clinical Oncology*, vol. 27, no. 12, pp. 2052–2058, 2009.

[140] J. Silber, D. A. Lim, C. Petritsch et al., "miR-124 and miR-137 inhibit proliferation of glioblastoma multiforme cells and induce differentiation of brain tumor stem cells," *BMC Medicine*, vol. 6, p. 14, 2008.

[141] M. Fareh, L. Turchi, V. Virolle et al., "The miR 302-367 cluster drastically affects self-renewal and infiltration properties of glioma-initiating cells through CXCR4 repression and consequent disruption of the SHH-GLI-NANOG network," *Cell Death & Differentiation*, vol. 2, no. 19, pp. 232–244, 2012.

[142] Y. Li, S. Zhao, Y. Zhen et al., "A miR-21 inhibitor enhances apoptosis and reduces G2-M accumulation induced by ionizing radiation in human glioblastoma U251 cells," *Brain Tumor Pathology*, vol. 28, no. 3, pp. 209–214, 2011.

[143] Y. Li, F. Guessous, Z. Ying et al., "MicroRNA-34a inhibits glioblastoma growth by targeting multiple oncogenes," *Cancer Research*, vol. 69, no. 19, pp. 7569–7576, 2009.

[144] G. Gabriely, M. Yi, R. S. Narayan et al., "Human glioma growth is controlled by microRNA-10b," *Cancer Research*, vol. 71, no. 10, pp. 3563–3572, 2011.

[145] A. Fowler, D. Thomson, K. Giles et al., "miR-124a is frequently down-regulated in glioblastoma and is involved in migration and invasion," *European Journal of Cancer*, 2011.

Hindawi Publishing Corporation
Neurology Research International
Volume 2012, Article ID 454957, 13 pages
doi:10.1155/2012/454957

Review Article

The Clinical and Prognostic Significance of Activated AKT-mTOR Pathway in Human Astrocytomas

Elias A. El Habr,[1] Christos Adamopoulos,[2] Georgia Levidou,[1] Aggeliki A. Saetta,[1] Penelope Korkolopoulou,[1] and Christina Piperi[2]

[1] *First Department of Pathology, Laiko Hospital, National and Kapodistrian University of Athens Medical School, 11527 Athens, Greece*
[2] *Department of Biological Chemistry, National and Kapodistrian University of Athens Medical School, 11527 Athens, Greece*

Correspondence should be addressed to Elias A. El Habr, elhabere@yahoo.com

Received 30 July 2011; Revised 5 December 2011; Accepted 9 December 2011

Academic Editor: Stuart Burri

Astrocytomas, the most common type of gliomas, and especially grade IV glioblastomas are "endowed" with strong proliferation and invasion potentials, high recurrence rate, and poor patients' prognosis. Aberrant signaling of AKT-mTOR (mammalian target of rapamycin) has been implicated in carcinogenesis. This paper is focused on the impact of deregulated AKT-mTOR signaling components in the clinical outcome and prognosis of human astrocytomas. Current therapeutic targeting of astrocytomas with AKT-mTOR inhibitors in preclinical and clinical stage is also discussed, including future perspectives regarding the management of these devastating tumors.

1. Introduction

Gliomas present the commonest type of tumor of the central nervous system. Based on the World Health Organization (WHO) criteria, diffuse astrocytomas, the most aggressive type of gliomas, are further classified into varying degrees of malignancy ranging from grade II to IV [1]. The life expectancy of patients diagnosed with a grade IV astrocytoma is on average 14 months after diagnosis despite differential treatment strategies including surgery, radiation, and/or chemotherapy.

The development of phosphorylation state-specific antibodies (PSSAs) has enabled a static determination of protein phosphorylation in the spatially complex structures of cells and tissues [2]. What is expected from PSSAs is the ability to confer a "screenshot" of intracellular signal transduction pathways, so as to provide important information to the clinician regarding prognosis, prediction, and/or therapy [2].

It is well established that the deregulation of AKT-mTOR (mammalian target of rapamycin) signaling is involved in carcinogenesis and plays a major role in the development of an aggressive phenotype influencing prognosis and determining response to therapies. The aim of this paper is to critically discuss and compose the results of several reports dealing with the role of activated AKT-mTOR pathway in human diffuse astrocytomas. We will focus on reports that used PSSAs for the activated form of AKT, mTOR, p70S6K, S6, and 4E-BP1 and highlight the role of these molecules in gliomagenesis.

2. The AKT-mTOR Pathway

2.1. A Quick Overview (Figure 1). AKT is one of the most important downstream targets of PI3K (phosphoinositide 3′-kinase). The AKT-mTOR pathway is initially activated at the level of cell membrane, and propagation of the activation signal occurs through PI3K class IA. A variety of signaling molecules including tyrosine kinase growth factor receptors (e.g., epidermal growth factor receptor (EGFR), insulin-like growth factor-1 receptor (IGF-1R)) as well as G-protein-coupled receptors, cell adhesion molecules, and oncogenes may lead to PI3K activation [3]. Phosphatidylinositol-3,4,5-triphosphate (PIP3), PI3K product, binds to 3′-phosphoinositide-dependent kinase 1 (PDK-1) and AKT through the pleckstrin homology domains (PH). This allows

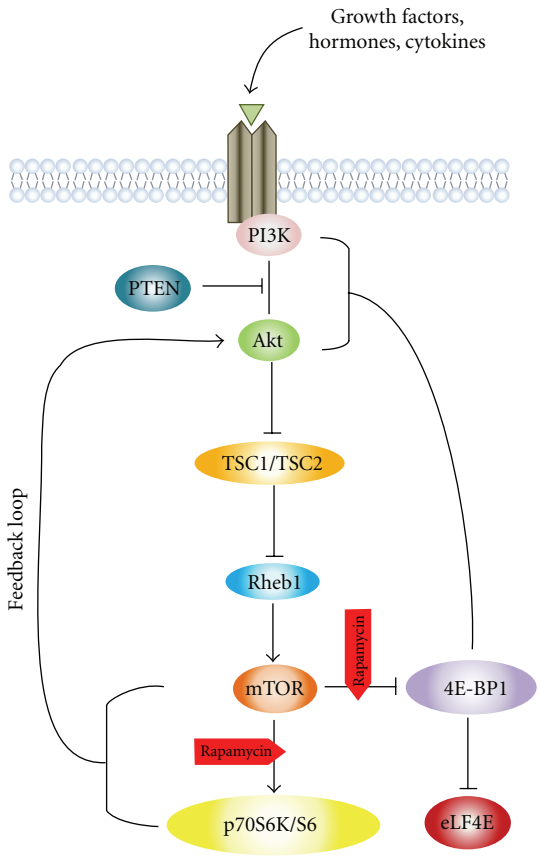


Figure 1: Schematic representation of AKT-mTOR signaling pathway showing rapamycin inhibition sites in astrocytomas.

the translocation of both proteins to the cell membrane, followed by their activation [4]. Colocalization of AKT with PDK1 results in phosphorylation of AKT at Thr308 and its partial activation [5]. In order for AKT to be fully activated, an additional Ser473 phosphorylation by the putative kinase PDK2 is required, which is believed to be mTORC2 (mTOR complex 2) [6]. PI3K is antagonized by PTEN (phosphatase and tensin homolog deleted on chromosome 10) through dephosphorylation of PIP3, thereby preventing AKT translocation and subsequent activation of both AKT and PDK-1. Once activated, AKT moves to the cytoplasm and nucleus, where it phosphorylates, activates, or inhibits many downstream targets to regulate various cellular functions including cell metabolism, protein synthesis, cell survival/inhibition of apoptosis, and cell cycle progression.

mTOR, a serine/threonine kinase, presents AKT's most well-studied downstream substrate. mTOR can be either directly phosphorylated and activated by AKT or indirectly activated through phosphorylation and subsequent inactivation of TSC2 (tuberous sclerosis complex 2, known as tuberin). TSC2 usually inhibits mTOR via Rheb (Ras homolog enriched in brain), a GTP-binding protein. Phosphorylation of TSC2 leading to its inactivation allows Rheb kept in the GTP-bound state, further inducing increased mTOR activation [3]. Two complexes of mTOR exist including the complex of mTORC1, with mTOR binding to Raptor, and the complex mTORC2, with mTOR binding to Rictor. Among several functions of mTOR, the regulation of translation is the best studied in relation to oncogenesis [7]. Two downstream pathways of mTORC1 involved in the translation machinery are responsible for ribosome recruitment to mRNA: phosphorylation and inactivation of 4E-BP1 (eukaryotic translation initiation factor 4E-binding protein 1), the repressor of mRNA translation, and activation of S6K1 (ribosomal S6 kinase 1), the promoter of mRNA translation [8]. 4E-BP1 phosphorylation (p-4E-BP1) prevents eIF4E interaction, liberating it to interact with eIF4G to form the eIF4F complex that enhances the cell's overall translational machinery [9]. Because eIF4E is the least abundant among the initiation factors, its dissociation from 4E-BPs represents the rate-limiting event for cap-dependent initiation of translation. Many growth- and proliferation-related proteins encoded by "weak" mRNAs show great reliance on the availability of eIF4E [10]. S6K1, which is phosphorylated and activated by mTORC1 on a hydrophobic motif, further phosphorylates S6, the 40S ribosomal protein, enhancing mRNAs translation with a 5′-TOP (5′-terminal oligopolypyrimidine). All ribosomal proteins and elongation factors are encoded by 5′-TOP [11].

2.2. AKT Protein: A Basic "AKTor" in Human Astrocytomas. AKT (also known as protein kinase B, PKB) is a serine/threonine kinase with three isoforms being identified in mammalian cells: AKT1 (PKBα), AKT2 (PKBβ), and AKT3 (PKBγ). They are all encoded by different genes with ubiquitous expression in normal cells and variable levels of expression among tissues [27]. AKT is a central node of PI3K signaling pathway, with crosstalk and feedback loops influencing its regulation. Altered expression of AKT has been associated with carcinogenesis [28].

Several studies [12–26] have investigated the clinical and prognostic significance of AKT and its activated form (phosphorylated AKT, p-AKT) in human astrocytomas (Table 1), without discrimination between the nuclear and cytoplasmic localization of this molecule apart of a few reports. The subcellular localization of p-AKT is, however, very important since current data indicate differentially shaped signaling in a spatially restricted way [13]. Interestingly, increased levels of nuclear p-AKT expression were correlated with higher levels of cytoplasmic immunostaining [13, 15]. These correlations between nuclear and cytoplasmic immunoexpression, along with the concurrent nuclear and cytoplasmic localization of p-AKT, are compatible with the dual regulatory role of gene expression targeting proteins in both cytoplasmic and nuclear level. It is worthy of note the findings of the study of Annovazzi et al. [18] showing that the immunostaining of p-AKT was nuclear in low-grade gliomas and cytoplasmic in high-grade gliomas. The reason for this disparity is unclear despite the explanation given by the latter group supporting that the prevailing nuclear localization in low-grade gliomas, where AKT expression is infrequent, could mean that the regulatory mechanism is different in comparison with tumors with cytoplasmic localization.

All reports dealing with different grades of human astrocytomas, except one, showed that increased total, nuclear,

Table 1: Summary of reports investigating the role of AKT and p-AKT in astrocytomas.

Report (number of cases and grades)	Antibody used	Immunostaining percentage	Correlations with clinicopathological features	Other correlations	Survival analysis
Yang et al. [12] (96 patients: 16 grade II, 35 III, and 45 IV)	Rabbit monoclonal anti-p-AKT (Ser473) ab (CST), at a concentration of 1.5 Ig/mL (IHC)	92.7% (89/96) showed nuclear and cytoplasmic staining	p-AKT with (i) higher grade (ii) lower KPS score	—	(i) p-AKT associated with a worse prognosis (ii) Multivariate analysis: p-AKT as an independent prognostic factor
Saetta et al. [13] (82 patients: 20 grade II, 14 III, and 48 IV)	Rabbit polyclonal anti-p-AKT1/2/3 ab (SCB), diluted 1 : 250 (IHC)	p-AKT: (i) nuclear 93.05% (67/72) (ii) cytoplasmic 59.72% (43/72)	(i) Nuclear and cytoplasmic p-AKT with tumor grade (ii) Cytoplasmic p-AKT with patients' age	(i) Nuclear p-AKT with cytoplasmic p-AKT (ii) Nuclear and cytoplasmic p-AKT with nuclear and cytoplasmic p-ERK (iii) Nuclear p-AKT with VEGF and MVD	Multivariate analysis: cytoplasmic p-AKT as independent predictor of survival (higher survival probability)
Li et al. [14] (87 patients: 27 grade I-II, 24 III, and 36 IV)	Rabbit monoclonal anti-p-AKT ab (EP), diluted 1 : 200 (IHC)	(i) 72.4% (63/87) showed nuclear and/or cytoplasmic staining (ii) 36.1% of grade IV showed strong expression	High p-AKT levels with tumor grade	—	—
El-Habr et al. [15] (71 patients: 7 grade II, 5 III, and 59 IV)	Rabbit polyclonal anti-p-AKT1/2/3 ab (SCB), diluted 1 : 250 (IHC)	p-AKT: (i) nuclear 97% (22/24) (i) cytoplasmic 100%	Cytoplasmic p-AKT with tumor grade	Nuclear p-AKT with cytoplasmic p-AKT	—
Wang and Kang [16] (48 patients: 16 grade II, 23 III, and 9 IV)	(i) Mouse monoclonal anti-AKT2 ab SCB, diluted 1 : 100 for IHC and 1 : 500 for WB (ii) p-AKT (information not provided) diluted 1 : 500 for WB	64.6% (31/48) showed cytoplasmic AKT2 staining	AKT2 and p-AKT with tumor grade	AKT2 with Ki-67	—
Suzuki et al. [17] (64 patients grade IV)	Rabbit polyclonal anti-p-AKT (Ser473) ab (CST), diluted 1 : 200 (IHC)	(i) 68.8% (44/64) (ii) 29.7% (19/64) had greater than 50% p-AKT positivity	—	—	(i) p-AKT positive, lower survival rate than p-AKT negative (ii) Multivariate analysis: higher expression of p-AKT with poor prognosis
Annovazzi et al. [18] (54 patients: 10 grade II, 10 III, and 34 IV)	(i) Mouse monoclonal anti-p-AKT (Ser473) ab (CST), diluted 1 : 100 (IHC) (ii) Rabbit monoclonal anti-p-AKT (Ser473) ab (CST), diluted 1 : 1000 (WB)	(i) 0%, 50%, and 56.6% in grade II, III, and IV, respectively (ii) Nuclear in grade II and III but mainly cytoplasmic in grade IV	p-AKT with tumor grade	p-AKT with (i) EGFR amplification (ii) p-mTOR	No significant correlation

Table 1: Continued.

Report (number of cases and grades)	Antibody used	Immunostaining percentage	Correlations with clinicopathological features	Other correlations	Survival analysis
Matsutani et al. [19] (24 patients)	Mouse monoclonal anti-AKT1 (B-1) ab (SCB) (IHC)	58.3% (14/24), cytoplasmic staining	—	(i)Positive AKT with tumor recurrences (ii) Overexpressed AKT with invasive recurrence into surrounding brain	AKT overexpression with: (i) shorter OS (ii) PFS Multivariate analysis: AKT overexpression as a significant prognostic factor for shorter PFS
Hlobilkova et al. [20] (89 patients: 42 grade I-II and 47 grade III-IV)	Mouse monoclonal anti-p-AKT (Ser473) ab (IHC)	86% of low grade and in 79% of high grade	No correlation with grade	p-AKT with EGFR activation	—
Mizoguchi et al. [21] (82 patients: 27 grade III and 55 IV)	Rabbit polyclonal anti-p-AKT (Ser473) ab (CST), diluted 1 : 100 (IHC)	(i) 78.2% of glioblastomas (43/55) positive nuclear and/or cytoplasmic (ii) 18.5% of anaplastic astrocytomas (5/27) positive nuclear and/or cytoplasmic	p-AKT with tumor grade	p-AKT with (i) EGFRvIII (ii) EGFRwt (iii) p-STAT3	p-AKT marginally predictive of worse prognosis
Pelloski et al. [22] (268 grade IV)	Rabbit polyclonal anti-p-AKT (Ser473) ab (CST), diluted 1 : 300 (IHC)	Not provided	—	p-AKT with (i) p-ERK (ii) p-p70S6K (iii) p-mTOR (iv) YKL-40	No significant correlation
Riemenschneider et al. [23] (29 grade IV)	Rabbit monoclonal anti-p-AKT (Ser473) ab (CST), diluted 1 : 50 (IHC)	Not provided	—	p-AKT with (i) p-TSC2 (ii) p-S6K (iii) p-S6	—
Wang et al. [24] (128 patients: 9 grade II, 49 III, and 70 IV)	Rabbit polyclonal anti-p-AKT (Ser473) ab (CST), diluted 1 : 50 (IHC)	p-AKT in: (i) 84% (59/70) grade IV (ii) 44% (20/46) grade III (iii) 22% (2/9) grade II	p-AKT with tumor grade	p-AKT with activated NFκB	—
Chakravarti et al. [25] (11 grade II, 13 III, and 56 IV)	p-AKT (Thr308) ab (CST) (WB)	66% (50/92) of grade IV	p-AKT with tumor grade	p-AKT with (i) p-PI3K (ii) p-p70S6K (iii) inversely with cCas3	p-AKT with (i) adverse outcome (ii) reduced time to death
Choe et al. [26] (45 grade IV)	Rabbit polyclonal anti-p-AKT (Ser473) ab (CST), diluted 1 : 50 (IHC)	Not provided	—	p-AKT with (i) PTEN protein loss (ii) p-FKHR (iii) p-S6 (iv) p-mTOR	—

ab: antibody, cCas3: cleaved caspase 3, CST: Cell Signaling Technology (Beverly, MA), EP: Epitomics (CA, USA), IHC: immunohistochemistry, KPS: Karnofsky Performance Status, MVD: microvessel density, OS: overall survival, p-ERK: phosphorylated extracellular-signal-regulated kinase, PFS: progression-free survival, SCB: Santa Cruz Biotechnology, VEGF: vascular endothelial growth factor, WB: Western blot.

and/or cytoplasmic AKT and p-AKT correlated with tumor histological grade (Table 1) indicating that activation of AKT participates in astrocytic tumor progression. On the contrary, Hlobilkova et al. [20] found the same proportions of p-AKT expression between low- and high-grade astrocytomas, suggesting that its activation is a starting event in gliomagenesis. Yang et al. [12] showed a significant correlation of p-AKT expression with KPS (Karnofsky Performance Status) score, whereas a significant association between cytoplasmic p-AKT expression and patients' age

was denoted by Saetta et al. [13]. Also, from the same study [13], correlations of nuclear p-AKT expression with VEGF (vascular endothelial growth factor) and microvessel density were observed, which implicate p-AKT as major component of angiogenesis in astrocytic gliomas. Matsutani et al. [19] found that patients positive for AKT with residual tumors postoperatively exhibited tumor recurrences, and invasive recurrence into surrounding brain was present only in tumors overexpressing AKT.

Two independent studies [13, 22], unlike Mizoguchi et al. [21] and Choe et al. [26], observed that p-AKT expression correlates to expression of p-ERK in gliomas. This correlation can be explained by the simultaneous activation of these parallel pathways by common receptor tyrosine kinases (e.g., EGFR) acting upstream. This can be further supported by the correlation of p-AKT activation with EGFR gene amplification and with progression from anaplastic astrocytomas to glioblastoma [18, 21]. More specifically, the immunostaining of wild type-EGFR (EGFRwt) [18, 21] and mutant EGFR (EGFRvIII) [21] correlated with activation of AKT. Also, from the latter report [21], a strong association between p-AKT and expression of p-STAT3 was denoted, whereas the statistical analysis of two independent studies [23, 24] failed to confirm such a relation. On the other hand, the immunohistochemical data from the microarray study of Wang et al. [24] showed an important correlation between p-AKT and activated NF-κB, suggesting that AKT activation may lead to NF-κB activation in diffuse astrocytomas. The finding of Chakravarti et al. [25] that shows an inverse association of p-AKT levels with cleaved caspase 3 (an apoptotic marker) levels indicates that PI3K members may act towards suppression of apoptosis, suggesting a possible explanatory mechanism by which the PI3K pathway may enhance resistance to radiation therapy in grade IV astrocytomas. Such a finding did not emerge from the study of Annovazzi et al. [18] probably due to technical reasons.

Since the expression status of mutant *IDH1* (isocitrate dehydrogenase 1) has been recently proposed as a prognostic factor in astrocytic tumors [29], Saetta et al. [13] included the IDH1-R132H antibody in their immunohistochemical analysis, which is a surrogate marker of the *IDH1* mutant, in a subset of cases in order to evaluate any potential relationship with p-AKT, but the expression of the former was not related neither to nuclear nor to cytoplasmic p-AKT. Also, the same group in a latter study [30] (see below) examined any possible correlation of IDH1-R132H expression with the activated forms of mTOR, p70S6K, and 4E-BP1. All three proteins were found to be unrelated to IDH1-R132H expression.

The relationships of p-AKT with p-mTOR and its downstream molecules will be discussed in next sections. From some reports, several correlations of p-AKT and its upstream regulators have emerged. *In vitro* experiments have shown that addition of YKL-40 protein to cells of connective tissue resulted in increased cell proliferation through AKT activation and MAPK pathways, nominating *YKL-40* as a potential regulator of these signaling pathways, since its secreted form is the product of one of the most expressed genes in glioblastoma with prognostic potential for these tumors [22]. YKL-40 expression was marginally correlated with expression of p-AKT in the study of Pelloski et al. [22], but, from the same study, no correlation was observed among expression of p-AKT and PTEN. On the contrary, Choe et al. [26] found a strong inverse correlation between AKT activation and PTEN loss, which remained significant during multivariate analysis. Also, from the same report [26], a statistical correlation between p-AKT and p-FKHR emerged and was maintained as an independent association during multivariate analysis. Since AKT is an important mediator of PI3K, a significant association among the activation states of these two proteins was expected [25].

The prognostic importance of AKT or p-AKT in astrocytic tumors is controversial based on current literature. The study of Matsutani et al. [19] found overexpression of AKT to be significantly associated with progression-free survival (PFS) and shorter overall survival (OS). In other reports, p-AKT expression was predictive of worse prognosis [12, 17, 21, 25]. Chakravarti et al. [25] showed elevated expression levels of p-AKT to be significantly associated with reduced time to death in the entire cohort, but such a relation did not remain significant during multivariate survival analysis. They then examined the prognostic significance of p-AKT only in patients diagnosed with a grade IV astrocytoma treated by surgery combined with postoperative radiation and showed a correlation of p-AKT expression with adverse outcome, suggesting an important role of this pathway in radiation resistance. Yang et al. [12] found also that p-AKT expression was statistically associated with worse prognosis, but, unlike Chakravarti et al. [25], in multivariate survival analysis, p-AKT emerged as an independent factor of prognosis. In the same context, the report of Suzuki et al. [17] showed that rate of survival for p-AKT positive tumor patients was lower than that of p-AKT negative tumors, whereas multivariate survival analysis nominated p-AKT as a strong independent prognostic factor. During univariate survival analysis of Saetta et al. [13], p-AKT expression did not correlate with survival, whereas multivariate analysis revealed cytoplasmic p-AKT expression as an independent prognostic factor, implying a higher survival probability. The latter finding is not one of a kind, since it is in accordance with current observations in lung and ovarian tumors [31, 32]. Finally, in two independent reports [18, 22], p-AKT expression failed to attain prognostic significance in gliomas.

2.3. The Role of mTOR Protein in Astrocytomas. The mTOR proteins belong to the PIKK (phosphoinositide 3-kinase-related kinase) family, transmitting signals associated with cellular growth, proliferation, and stress responses [33]. mTOR is a large protein of ~300 kDa Mwt with a COOH-terminal catalytic domain homologous to PI3K, functioning exclusively as a protein serine/threonine kinase [34].

Some reports [14, 18, 22, 23, 26, 30] have investigated the clinical and prognostic significance of activated mTOR (phosphorylated mTOR, p-mTOR) in human astrocytomas. Table 2 summarizes the important findings of these reports. In the report of Korkolopoulou et al. [30], mTOR was seen predominantly in the cytoplasm/cell membrane but

Table 2: Summary of reports investigating the role of mTOR activation in astrocytomas.

Report (number of cases and grades)	Antibody used	Immunostaining percentage	Correlations with clinicopathological features	Other correlations	Survival analysis
Korkolopoulou et al. [30] (111 patients: 25 grade II, 15 III, and 71 IV)	Rabbit monoclonal anti-p-mTOR (Ser2448) ab (CST), diluted 1 : 50 for IHC and 1 : 1.000 (WB)	(i) 84.7% (94/111) cytoplasmic/membranous (ii) 2.7% (3/111) nuclear	Gade III and IV marginally higher p-mTOR expression than grade II	p-mTOR with (i) nuclear and cytoplasmic p-AKT (ii) p-p70S6K (iii) p-4E-BP1 (iv) VEGF	p-mTOR with worse DFS
Li et al. [14] (87 patients: 27 grade I-II, 24 III, and 36 IV)	Rabbit monoclonal anti-p-mTOR (Ser2448) ab (EP), diluted 1 : 100 (IHC)	(i) 74.7% (65/87) nuclear and/or cytoplasmic (ii) 44.4% of grade IV strong expression	p-mTOR with tumor grade	—	—
Annovazzi et al. [18] (54 patients: 10 grade II, 10 III, and 34 IV)	Rabbit polyclonal anti-p-mTOR (Ser2448) ab (CST), diluted 1 : 75 (IHC)	0%, 70%, and 81.8% nuclear in grade II, III, and IV, respectively	p-mTOR with tumor grade	p-mTOR with (i) p-AKT (ii) inversely Beclin 1	No significant correlation
Pelloski et al. [22] (268 grade IV)	Anti-p-mTOR (Ser2448) ab (CST), diluted 1 : 100 (IHC)	Not provided	—	p-mTOR with (i) p-ERK (ii) p-p70S6K (iii) p-AKT (iv) PTEN (v) YKL-40	(i) p-mTOR with shorter OS (ii) Multivariate analysis: not retained as an independent prognostic factor
Riemenschneider et al. [23] (29 grade IV)	Rabbit polyclonal anti-p-mTOR (Ser2448) ab (CST), diluted 1 : 75 (IHC)	Not provided	—	No significant correlation	—
Choe et al. [26] (45 grade IV)	p-mTOR (Ser2481) ab (CST), diluted 1 : 50 (IHC)	Not provided	—	p-mTOR with (i) p-AKT (ii) p-S6 (iii) EGFRvIII	—

ab: antibody, CST: Cell Signaling Technology (Beverly, MA), DFS: disease-free survival, EP: Epitomics (CA, USA), IHC: immunohistochemistry, OS: overall survival, p-ERK: phosphorylated extracellular-signal-regulated kinase, VEGF: vascular endothelial growth factor, WB: Western blot.

also occasionally in the nucleus, suggesting that this nuclear import of mTOR may have an important role in activating its cytoplasmic signaling. On the other hand, Li et al. [14] found that the immunostaining of p-mTOR was both nuclear and cytoplasmic, whereas Annovazzi et al. [18] observed a nuclear immunostaining. The reason for the disparity in mTOR immunostaining may be due to the difference in the antibodies used and requires further investigation.

It is worthy of note that 3 independent reports [14, 18, 30] dealing with different grade of astrocytomas denoted a correlation of p-mTOR expression with tumor grade. These findings imply that activation of mTOR may be a late event during gliomagenesis, facilitating the acquisition of a malignant phenotype.

An association between VEGF and cytoplasmic p-AKT levels in astrocytic tumors was discussed previously in Section 2.2 [13]. The same group [30] denoted that this relationship also applies to p-mTOR and its target proteins p-4E-BP1 and p-p70S6K, which was illustrated in their similar distribution in perinecrotic areas of glioblastomas (Tables 3 and 4). Their findings are supported by recent data clearly placing HIF-1a the driving force of VEGF upregulation and angiogenesis under hypoxic conditions downstream of mTOR [37], as well as from *in vitro* experiments where decreased VEGF levels with suppression of angiogenic phenotype followed mTOR inhibition or enhanced translation of VEGF mRNA followed hypoxic activation of 4E-BP1 and eIF4E [30]; Beclin 1, expression of which decreases with malignancy, was inversely correlated with p-mTOR [18].

Since AKT and mTOR proteins interact with and activate each other as discussed in Section 2.1, it is expected the activated forms of these two proteins to be correlated together. Indeed, several reports have denoted a correlation of these two proteins [18, 22, 26, 30]. Choe et al. [26] found a strong correlation of p-AKT and p-mTOR in univariate analysis, but, in multivariate, this association did not reach significance, concluding that additional inputs (e.g., nutrients, amino acids, cellular ATP, and phosphatidic acid) to mTOR activation are involved, some of which may be associated with other parts of the signaling pathway. Only one report [23] did not observe association between p-AKT and p-mTOR suggesting that multiple AKT-independent

Table 3: Summary of reports investigating the role of p70S6K and S6 activation in astrocytomas.

Report (number of cases and grades)	Antibody used	Immunostaining percentage	Correlations with clinicopathological features	Other correlations	Survival analysis
Korkolopoulou et al. [30] (111 patients: 25 grade II, 15 III, and 71 IV)	Rabbit polyclonal anti-p-p70S6K (Thr421/Ser424) ab (specific for p70 subunit) (SCB), diluted 1 : 250 for IHC and 1 : 200 for WB	99.1% (99/111) showed nuclear staining	No significant correlation	p-p70S6K with (i) cytoplasmic p-AKT (ii) p-mTOR (marginal) (iii) VEGF	No significant correlation
Yang et al. [12] (96 patients: 16 grade II, 35 III, and 45 IV)	Rabbit polyclonal anti-p-S6 (Ser235/236) ab (CST), at a concentration of 0.125 Ig/mL (IHC)	82.3% (79/96) cytoplasmic	p-S6 with (i) tumor grade (ii) lower KPS score	—	(i) p-S6 with worse prognosis (ii) Multivariate analysis: p-S6 as an independent prognostic factor
Li et al. [14] (87 patients: 27 grade I-II, 24 III, and 36 IV)	Rabbit monoclonal anti-p-p70S6K (Thr389) ab (EP), diluted 1 : 50 (IHC)	(i) 72.4% (63/87) nuclear and/or cytoplasmic (ii) 41.7% of grade IV showed strong expression	p-p70S6K with tumor grade	—	—
McBride et al. [35] (45 patients: 22 astrocytomas grade II)	(i) Rabbit polyclonal anti-p-S6 (Ser235/236) ab (SCT), diluted 1 : 200 (IHC) (ii) Rabbit polyclonal anti-p-S6 (Ser240/244) ab (CST), diluted 1 : 200 (IHC)	(i) p-S6 (Ser235/236): 76% (29/38) (ii) p-S6 (Ser240/244): 76% (29/38)	—	p-S6 (Ser235/236) with (i) p-PRAS40 (ii) p-S6 (Ser240/244) p-S6 (Ser240/244) with (iii) PTEN methylation (iv) inversely PTEN expression	(i) p-S6 (Ser235/236) with OS (ii) p-S6 (Ser240/244) with OS
Annovazzi et al. [18] (54 patients: 10 grade II, 10 III, and 34 IV)	Rabbit polyclonal anti-p-S6 (Ser240/244) ab (CST), diluted 1 : 100 for IHC. Not provided for WB	(i) 0%, 30%, and 82.3% in grade II, III, and IV, respectively (ii) Nuclear in grade II and III but mainly cytoplasmic in grade IV	p-S6 with tumor grade	p-S6 IHC with (i) p-S6 WB (ii) Ki-67 (iii) inversely with Beclin 1	No significant correlation
Ermoian et al. [36] (71 patients: 28 grade II, 17 III, and 26 IV	Anti-p-S6 ab (CST)	Not provided	p-S6 unrelated to tumor grade	No significant correlation	No significant correlation
Pelloski et al. [22] (268 grade IV)	Anti p-p70S6K ab (CST), diluted 1 : 1000 (WB)	Not provided	—	p-p70S6K with (i) p-ERK (ii) p-mTOR (iii) p-AKT (iv) PTEN (v) YKL-40	(i) p-p70S6K with shorter OS (ii) Multivariate analysis: not retained as an independent prognostic factor
Riemenschneider et al. [23] (29 grade IV)	(i) Mouse monoclonal anti-p-p70S6K (Thr389) ab (CST), diluted 1 : 200 (IHC) (ii)Rabbit monoclonal anti-p-S6 (Ser235/236) ab (CST), diluted 1 : 200 (IHC)	Not provided	—	(i) p-p70S6K with p-AKT (ii) p-S6 with p-AKT	—

Table 3: Continued.

Report (number of cases and grades)	Antibody used	Immunostaining percentage	Correlations with clinicopathological features	Other correlations	Survival analysis
Ckakravarti et al. [25] (11 grade II, 13 III, and 56 IV)	Anti-p-p70S6K (Thr389) ab (CST), diluted 1 : 50 (WB)	39.1% (36/92) of grade IV	p-p70S6K with tumor grade	p-p70S6K with (i) p-PI3K (ii) p-AKT (iii) inversely cCas3	p-p70S6K with (i) adverse outcome (ii) reduced time to death Multivariate analysis: p-p70S6K as an independent prognostic factor
Choe et al. [26] (45 grade IV)	Anti p-S6 (Ser235/236) ab (CST), diluted 1 : 50 (IHC)	Not provided	—	p- S6 with (i) EGFRwt (ii) EGFRvIII (iii) p-mTOR (iv) p-ERK	—

ab: antibody, cCas3: cleaved caspase 3, CST: Cell Signaling Technology (Beverly, MA), EP: Epitomics (CA, USA), IHC: immunohistochemistry, KPS: Karnofsky Performance Status, OS: overall survival, p-ERK: phosphorylated extracellular-signal-regulated kinase, SCB: Santa Cruz Biotechnology, VEGF: vascular endothelial growth factor, WB: Western blot.

signaling pathways on mTOR regulation could be involved. Pelloski et al. [22], unlike Korkolopoulou et al. [30], showed a strong p-mTOR correlation with p-ERK, which could be explained by the simultaneous stimulation of these parallel pathways by EGFR, the expression of which was correlated with both proteins in the analysis of Choe et al. [26]. Pelloski et al. [22], also, found a correlation of p-mTOR with YKL-40 (see Section 2.2) and PTEN expression. The latter finding is in accordance with previous data of a relationship between high PTEN expression and AKT activation [22]. The contradictory findings in the literature suggest a tumor specific relationship between PTEN expression and activation of the AKT pathway and this should be better assessed by alternative methodology than immunohistochemical analysis.

As mentioned above, the most important effectors of mTOR kinase in the regulation of translation are 4E-BP1 and p70S6K, S6 ribosomal protein, being the major effector of the latter. In this context, Choe et al. [26] found an association between p-mTOR and p-S6, whereas Pelloski et al. [22] denoted a strong association of the former protein with p-p70S6K. Korkolopoulou et al. [30] found also correlations of p-mTOR with p-4E-BP1 and p-p70S6K, the former of marginal significance, but, despite these interrelationships, the three proteins were topographically distinct, since only p-mTOR was found in pseudopalisading perinecrotic cells in glioblastomas. This finding is one of a kind and could attribute a functional link with tumor hypoxia, confirmed by *in vitro* data indicating upregulation of mTOR by hypoxia inducible factor-1a (HIF-1a) [37].

Regarding the prognostic significance of activated mTOR, few reports [18, 22, 30] have investigated the influence of p-mTOR on patients' survival. From the survival analysis of Annovazzi et al. [18], no significant correlations of p-mTOR with patients' survival emerged. On the contrary, Pelloski et al. [22] and Korkolopoulou et al. [30] found that p-mTOR expression was correlated with short OS and free of disease survival, respectively, but such a relation did not remain significant during multivariate survival analysis.

2.4. Implication of p70S6K and Its Major Effector S6 Protein in Astrocytomas. There are two isoforms of S6K1 (cytoplasmic p70S6K1 and nuclear p85S6K1) present in mammals, produced by alternative splicing from the same transcript [38]. The large isoform, p85S6K1, is composed of an N-terminal 23-aa long, which directs p85S6K1 to the nucleus. On the contrary, the short form, p70S6K1, is mainly located in the cytoplasm. Current data indicate a similar regulation of p85S6K1 and p70S6K1. Initial mapping of the main residues for p70S6K1 activation was to T229 (of activation loop) and T389 (of the hydrophobic motif) and subsequently to S371 of the linker domain [39].

Table 3 shows the reports dealing with the significance of p70S6K in human astrocytomas. p70S6K is a nuclear cytoplasmic shuttling protein, activated in the nucleus by mTOR and relocalized in the cytoplasm after mRNA translation initiation [40]. Thus, it is not surprising that p-p70S6K has been reportedly seen in the cytoplasm and/or in the nucleus [14, 30]. Two independent reports [14, 25] denoted a correlation of p-p70S6K with tumor grade suggesting that the activation of this protein takes place later during gliomagenesis. On the contrary, Korkolopoulou et al. [30] denoted that p70S6K activation may take place at an earlier stage of the neoplastic process, since no difference existed between low- and high-grade cases.

The latter group [30] observed a correlation of cytoplasmic p-AKT with p-p70S6K. Interestingly, in multidimensional analysis in glioblastomas, p-p70S6K, but not with p-mTOR, clustered together with p-AKT implying that the downstream effect of p-AKT is primarily conveyed by S6K signaling through TSC2 [23]. The same correlation between p-AKT and p-p70S6K was denoted by 3 different groups [22, 23, 25]. Since p70S6K is an important downstream mediator

Table 4: Summary of reports investigating the role of phosphorylated 4E-BP1 in astrocytomas.

Report (number of cases and grades)	Antibody used	Immunostaining percentage	Correlations with clinicopathological features	Other correlations	Survival analysis
Korkolopoulou et al. [30] (111 patients: 25 grade II, 15 III, and 71 IV)	Rabbit polyclonal anti-p-4E-BP1/2/3 (Ser36) ab (SCB), diluted 1 : 50 for IHC and 1 : 200 for WB	82.4% (61/74) nuclear	p-4E-BP1 with tumor grade	p-4E-BP1 with (i) nuclear p-AKT (ii) cytoplasmic p-ERK (iii) p-mTOR (iv) VEGF	(i) p-4E-BP1 adversely affected survival in the entire cohort and marginally in glioblastomas (ii) Multivariate analysis: p-4E-BP1 as an independent predictor of survival in the entire cohort as well as in glioblastomas
Ermoian et al. [36] (71 patients: 28 grade II, 17 III, and 26 IV	Anti-p-4E-BP1 ab (CST) (WB)	Not provided	p-4E-BP1 unrelated to tumor grade	p-4E-BP1 with p-AKT	No significant correlation
Riemenschneider et al. [23] (29 grade IV)	Rabbit polyclonal anti-p-4E-BP1 (Ser65) ab (CST), diluted 1 : 50 (IHC)	Not provided	—	No significant correlation	—

ab: antibody, CST: Cell Signaling Technology (Beverly, MA), IHC: immunohistochemistry, p-ERK: phosphorylated extracellular-signal-regulated kinase, SCB: Santa Cruz Biotechnology, VEGF: vascular endothelial growth factor, WB: Western blot.

of PI3K, a significant correlation among the activation states of both proteins is expected and further predicted by preclinical models. In accordance, p-PI3K levels and p-p70S6K were correlated in the study by Chakravarti et al. [25]. Regarding YKL-40 as discussed in Section 2.2 and 2.3, its expression correlated significantly with p-p70S6K [22]. The same report denoted a strong correlation of the latter with p-ERK; since the activation of AKT was inversely associated with cleaved caspase 3, it was expected that the same correlation will occur between p-p70S6K and the latter, which was found by Chakravarti et al. [25], suggesting an implication of activated p70S6K in the suppression of apoptosis.

Pelloski et al. [22] found that p-p70S6K was associated with reduced overall survival time on univariate but not in multivariate analysis. A previous study [25] showed this marker to be significantly correlated to survival on both analysis types. This can be explained statistically since the percentage of p-p70S6K-negative cases in the former study (6%) was lower than that in the latter study (44%) or because Pelloski et al. [22] included, in their study, additional markers (namely, YKL-40 and p-ERK) with more dominant molecular effects within tumor cells. Finally, the survival analysis of Korkolopoulou et al. [30] did not reveal any association of p-p70S6K with patients' survival.

The 40S ribosomal subunit protein S6 is the first identified and the most well-studied substrate of S6K1. Its phosphorylation has been found to correlate with increased protein synthesis. After stimulation, S6K1 phosphorylates S6 on five serine residues (S235, S236, S240, S244, and S247) within the C-terminus [38].

Table 3 summarizes the findings of several reports discussing the role of activated S6 (phosphorylated S6, p-S6) in human astrocytomas. Yang et al. [12] denoted a cytoplasmic immunostaining for p-S6, whereas Annovazzi et al. [18] found that grade II had different immunostaining for p-S6 than grade III and IV (nuclear for grade II and III, and cytoplasmic for grade IV). Similar finding emerged for p-AKT in the same report and was discussed in Section 2.2. Also, noteworthy is the findings of McBride et al. [35] who used two different antibodies for the activated form of S6 (Table 3). Both antibodies showed the same percentage of immunostaining, and their expressions were strongly correlated, which served as a good internal validation for their IHC (immunohistochemistry) protocol. Also, Annovazzi et al. [18] validated their IHC by WB (Western blot) analysis and found a strong correlation between the findings of both techniques; a significant correlation of p-S6 expression with tumor grade was denoted by 2 different groups [12, 18], whereas Ermoian et al. [36] did not find such a relation; lower KPS was correlated with p-S6 [12].

A protein-positioned downstream of PTEN, namely, PRAS40, does not inhibit the mTOR pathway in its phosphorylated form (discussed in Section 2.1), and it can therefore be assumed that upon activation of the mTOR pathway, these proteins are linked and correlate in terms of phosphorylation status. In accordance, McBride et al. [35] showed association of S6 and PRAS40 phosphorylation along with *PTEN* methylation. In addition, S6 phosphorylation and PTEN expression were negatively correlated. In the same context, Riemenschneider et al. [23] found a strong correlation between p-AKT and p-S6. Finally, Annovazzi et al. [18]

found a strong positive correlation of p-S6 with Ki67 and negative with Beclin 1 (see Section 2.3).

McBride et al. [35] performed two different survival analyses for their cohort, one with the anti-p-S6 antibody for the 235/236 epitope and one for the epitope 240/244. In both cases, an inverse correlation between OS and phosphorylation of S6 was denoted. In the same context, Yang et al. [12] found a strong correlation between p-S6 expression and worse prognosis, which in multivariate survival analysis emerged as an independent prognostic factor. On the contrary, the survival analysis of Annovazzi et al. [18] and Ermoian et al. [36] did not reveal any correlation of p-S6 with patients' survival.

2.5. 4E-BP1 Protein: The Less Studied in the Pathogenesis of Astrocytomas. 4E-BP1 (also known as PHAS-1) is a translation repressor protein and one of the main effectors of mTOR in the PI3K/AKT signaling pathway that integrates signals from extracellular stimuli, amino acid availability, and oxygen and energy status of the cells. 4E-BPs contain a TOS (TOR signaling) motif that binds the mTORC1, which controls the activity of 4E-BP1 [41]. mTORC1 activation leads to 4E-BP1 phosphorylation at Thr36, Thr45, Ser64, and Thr69 sites and 4E-BP1 release from eIF4E [41].

Table 4 summarizes the important findings of the few reports dealing with the role of activated 4E-BP1 (p-4E-BP1) in human astrocytomas. In the report of Korkolopoulou et al. [30], the immunostaining of p-4E-BP1 was nuclear (nuclear cytoplasmic shuttling protein). Although the mechanisms of the nuclear localization of p-4E-BP1 have not been clarified, there is current evidence that eIF4E functions as a nuclear regulator of several RNAs exportation implicated in proliferation and cell growth [42]. Also, Korkolopoulou et al. [30] showed that p-4E-BP1 expression levels increased with grade, whereas, in the report of Ermoian et al. [36], no correlation was elicited between p-4E-BP1 mRNA or protein levels (as measured using immunoblotting) and glioma grade.

p-4E-BP1 expression correlated with cytoplasmic p-ERK expression in the report of Korkolopoulou et al. [30], consistent with *in vitro* data indicating the emerging role of ERK signaling in regulating 4E-BP1 [43]. Also, from the latter report [30], a significant correlation of p-4E-BP1 expression with nuclear p-AKT was denoted, which was in accordance with the data of Ermoian et al. [36] that observed a similar association; an important finding emerging from these two reports is the absence of correlation between p-4E-BP1 and p-S6 [36], from on one hand, and among p-4E-BP1 and p-p70S6K, on the other hand [30]. This finding shows that, in the regulation and activation of these two molecules, other pathways may be implicated; VEGF, which is a marker of angiogenesis (see previous sections), was also found to be correlated with p-4E-BP1 [30].

One of the most important findings emerging from the investigation of Korkolopoulou et al. [30] is the independent adverse prognostic significance of p-4E-BP1 expression, in the entire cohort and glioblastomas, which has not been documented thus far. *In vitro* experiments have documented sensitization of U87 glioblastoma xenografts to irradiation following 4E-BP1 targeting and decreased hypoxia tolerance, indicating that glioblastomas, which are known to be hypoxic, are expected to rely more on translation regulation pathways for critical functions than their lower grade counterparts [44] thus corroborating the finding of Korkolopoulou et al. [30] that the prognostic effect of 4E-BP1 was established in glioblastomas.

2.6. Therapeutic Targeting of AKT-mTOR Pathway in Astrocytomas. The development of small kinase inhibitors has improved clinical practice for several solid tumors and presented a reasonable strategy for astrocytomas treatment. Primary glioma cell cultures as well as U87 and U251 cell lines where a strong activation of AKT/mTOR pathway was observed exhibited increased sensitivity to rapamycin, the archetypic mTOR inhibitor which did not, however, caused a compensatory AKT activation resulting from mTORC1 negative feedback via insulin receptor substrate (IRS1, Figure 1) [30, 45]. Additionally, rapamycin was effective in inhibiting the phosphorylation of both p70S6K and 4E-BP1 even at 48 hours [30] being a clinical promise in recurrent glioblastoma patients displaying higher levels of p-p70S6K in baseline tumor samples [46].

Unfortunately, single agent mTOR inhibitors such as rapamycin (sirolimus, CCL-779) that specifically complexes with FK506-binding protein 12 (FKBP12), thereby interacting allosterically and inhibiting mTOR (Figure 1), has produced minimal clinical activity and improvement in neuroimaging of recurrent glioblastoma patients [47, 48]. The PFS6 (progression-free survival at 6 months) was rather low at 7.8%, whereas the median OS was 4.4 months. Additional, the rapalogs, temsirolimus (CCI-779), deforolimus (AP23573), and everolimus (RAD001) have also been generated without major clinical benefit, and, although they are all well tolerated, some toxicities have been reported including lymphopenia, hyperlipidemia, stomatitis, and increased risk of opportunistic infections [46, 48, 49]. Furthermore, a rapamycin phase I trial was conducted in recurrent glioma patients deficient in PTEN based on preclinical data where PTEN mutant/null tumors exhibited an enhanced susceptibility to mTOR inhibition [50–52]. Interestingly, a complete inhibition of proliferation was observed in half of the patients which correlated to mTORC1 inhibition.

Taking into account the complexity of intracellular signal transduction pathways and the ability of tumor cells to compensate for acute changes, it is not surprising that single-agent therapies have a limited efficacy against most solid tumors, including gliomas [45]. The possibility of an upstream positive feedback loop at the PI3K level has driven therapeutic targeting towards multiple signaling molecules of the EGFR-PI3K-mTOR axis and identification of agents showing additive or synergistic antitumor effects [28]. Indeed, preclinical studies of combined EGFR and mTOR inhibitors have shown antiproliferative and proapoptotic effects against gliomas [45, 53]. In accordance, several pilot studies with drug combinations in recurrent gliomas showed

either a promising PFS6 at 25% for glioblastoma patients [54] or disease stabilization [55].

Initial phase I studies indicated that combination treatment in recurrent glioblastoma patients was well tolerated resulting either in stabilization of the disease for some patients or partial response rates [54, 56]. However, in a more recent phase I and II study of recurrent glioblastoma, combined treatment use of erlotinib and temsirolimus caused increased toxicity without major clinical advantages. Temsirolimus was maximally tolerated at 15 mg per week (lower dose than used in monotherapy) and erlotinib at 150 mg per day. Most common grade 3 toxicity was rash followed by severe mucositis, thrombocytopenia, and diarrhea. On phase II trial, PFS6 of 40 glioblastoma patients was 12.5% with the median PFS being 8 weeks and only 30% of patients exhibiting stable disease. Trials involving combinations of cytotoxic drugs with mTOR inhibitors have been initiated recently aiming in reducing the immunosuppressive consequences.

Several factors may contribute to this minimal antitumor response to combination therapy of EGFR and mTOR inhibitors in glioblastomas, including mainly therapy resistance or use of inadequate drug doses [57]. It is evident that glioblastomas exhibit molecular heterogeneity involving EGFRvIII and PTEN mutations, increased AKT activation, or MGMT promoter methylation that may differentiate the efficiency of EGFR-AKT-mTOR cascade targeting among patients. Although several studies have been performed aiming to establish molecular profiles that would predict predictive clinical responses towards EGFR and/or mTOR inhibition, a convincing molecular signature for glioblastoma is still elusive. Additional to identification of driver mutations as therapeutic targets, efficient target inhibition is also important. The use of rapamycin and its derivatives as potent mTOR inhibitors at the current clinical doses has been challenged recently, especially regarding inhibition of 4E-BP1 and proliferation in some patients [58]. A better activity towards this pathway is expected from next generation mTOR inhibitors [58, 59]. It has been argued that modulation of 4E-BP1 function may serve to determine the sensitivity of tumor cells to PI3K/AKT pathway inhibitors. It is thus conceivable that 4E-BP1 modulation may have a place in the treatment options for glioblastoma as an adjunct to the existing or upcoming cytotoxic therapies.

3. Conclusion

It is evident that AKT-mTOR pathway activation is highly implicated in glioma biology contributing to tumor progression and angiogenesis. Many reports, however, analyzing the same molecule in gliomas had often controversial or discrepant findings, preventing us to generate reliable conclusions regarding the clinical and prognostic importance of these molecules in astrocytomas. This discrepancy could be attributed to differences in techniques used, antibodies, cohorts, and so forth. What was missing from most reports cited in this paper was the validation of their findings. For example, in the study of Korkolopoulou et al. [30], the IHC was validated by WB and by Rapamycin treatment, whereas McBride et al. [35] used two different antibodies for the same protein (p-S6), which served as an internal validation for their IHC. On the other hand, Pelloski et al. [22] validated the findings of their IHC by using a validation cohort different from their main cohort enrolled in the study.

Despite these remarks, the implication of activated AKT-mTOR pathway in the pathogenesis of human astrocytomas could not be ignored. Some findings of the cited reports were similar and allowed us to generate a safe conclusion. For example, activated AKT was seen to correlate with tumor grade in all cited reports except one. More studies are needed in order to validate the findings of the cited reports, especially for mTOR and the effectors 4E-BP1 and p70S6K.

Furthermore, the AKT-mTOR pathway remains a promising and potential target for glioblastoma therapy. Due to the molecular heterogeneity of glioblastomas, it is evident that strategies targeting this pathway require considerable advances in genetics and pharmacology. Genomic approaches of large scale are required for identification of main driver mutations and establishment of molecular signatures leading to successful application of mTOR-targeted therapy. Screening tumor samples for compensatory receptor tyrosine kinases will identify those patients that require combinations of multiple kinase inhibitors for successful downstream signaling suppression [60]. The recent categorization of glioblastoma subtypes is an important tool in identifying tumors that are more susceptible to mTOR targeting [61].

Radiotherapy in combination with mTOR inhibition (temsirolimus) is an alternative schedule that has proved efficient in preclinical studies [62] and is currently under evaluation in a clinical study of newly diagnosed glioblastomas in comparison to chemoirradiation and lack of MGMT methylation [63].

Finally, advances in pharmacological approaches have resulted in production of novel mTOR inhibitors with an expected superior activity than rapalogs [59, 64]. Interesting clinical data are expected from a current phase I trial where combination of mTORC1 and 2 inhibitors as well as combination of mTORC1 and 2 and PI3K inhibitors has been applied to patients with glioblastomas that have undergone radiation therapy.

Authors' Contribution

E. A. El Habr and C. Adamopoulos have equally contributed to the paper.

References

[1] D. N. Louis, H. Ohgaki, O. D. Wiestler et al., "The 2007 WHO classification of tumours of the central nervous system," *Acta Neuropathologica*, vol. 114, no. 2, pp. 97–109, 2007.

[2] J. W. Mandell, "Immunohistochemical assessment of protein phosphorylation state: the dream and the reality," *Histochemistry and Cell Biology*, vol. 130, no. 3, pp. 465–471, 2008.

[3] J. LoPiccolo, G. M. Blumenthal, W. B. Bernstein, and P. A. Dennis, "Targeting the PI3K/Akt/mTOR pathway: effective

combinations and clinical considerations," *Drug Resistance Updates*, vol. 11, no. 1-2, pp. 32–50, 2008.

[4] J. A. Engelman, J. Luo, and L. C. Cantley, "The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism," *Nature Reviews Genetics*, vol. 7, no. 8, pp. 606–619, 2006.

[5] K. E. Anderson, J. Coadwell, L. R. Stephens, and P. T. Hawkins, "Translocation of PDK-1 to the plasma membrane is important in allowing PDK-1 to activate protein kinase B," *Current Biology*, vol. 8, no. 12, pp. 684–691, 1998.

[6] E. M. Sale and G. J. Sale, "Protein kinase B: signalling roles and therapeutic targeting," *Cellular and Molecular Life Sciences*, vol. 65, no. 1, pp. 113–127, 2008.

[7] G. V. Thomas, "mTOR and cancer: reason for dancing at the crossroads?" *Current Opinion in Genetics and Development*, vol. 16, no. 1, pp. 78–84, 2006.

[8] N. Hay and N. Sonenberg, "Upstream and downstream of mTOR," *Genes and Development*, vol. 18, no. 16, pp. 1926–1945, 2004.

[9] A. C. Gingras, S. P. Gygi, B. Raught et al., "Regulation of 4E-BP1 phosphorylation: a novel two step mechanism," *Genes and Development*, vol. 13, no. 11, pp. 1422–1437, 1999.

[10] A. de Benedetti and J. R. Graff, "eIF-4E expression and its role in malignancies and metastases," *Oncogene*, vol. 23, no. 18, pp. 3189–3199, 2004.

[11] M. A. Bjornsti and P. J. Houghton, "The TOR pathway: a target for cancer therapy," *Nature Reviews Cancer*, vol. 4, no. 5, pp. 335–348, 2004.

[12] J. Yang, D. Liao, Z. Wang, F. Liu, and G. Wu, "Mammalian target of rapamycin signaling pathway contributes to glioma progression and patients' prognosis," *Journal of Surgical Research*, 2009.

[13] A. A. Saetta, G. Levidou, E. A. El-Habr et al., "Expression of pERK and pAKT in human astrocytomas: correlation with IDH1-R132H presence, vascular endothelial growth factor, microvascular characteristics and clinical outcome," *Virchows Archiv*, vol. 458, no. 6, pp. 749–759, 2011.

[14] X. Y. Li, L. Q. Zhang, X. G. Zhang et al., "Association between AKT/mTOR signalling pathway and malignancy grade of human gliomas," *Journal of Neuro-Oncology*, pp. 1–6, 2010.

[15] E. A. El-Habr, P. Tsiorva, M. Theodorou et al., "Analysis of PIK3CA and B-RAF gene mutations in human astrocytomas: association with activation of ERK and AKT," *Clinical Neuropathology*, vol. 29, no. 4, pp. 239–245, 2010.

[16] G. Wang, C. Kang, and P. Pu, "Increased expression of Akt2 and activity of PI3K and cell proliferation with the ascending of tumor grade of human gliomas," *Clinical Neurology and Neurosurgery*, vol. 112, no. 4, pp. 324–327, 2010.

[17] Y. Suzuki, K. Shirai, K. Oka et al., "Higher pakt expression predicts a significant worse prognosis in glioblastomas," *Journal of Radiation Research*, vol. 51, no. 3, pp. 343–348, 2010.

[18] L. Annovazzi, M. Mellai, V. Caldera, G. Valente, L. Tessitore, and D. Schiffer, "mTOR, S6 and AKT expression in relation to proliferation and apoptosis/autophagy in glioma," *Anticancer Research*, vol. 29, no. 8, pp. 3087–3094, 2009.

[19] T. Matsutani, Y. Nagai, S. Mine, H. Murai, N. Saeki, and Y. Iwadate, "Akt/protein kinase B overexpression as an accurate prognostic marker in adult diffuse astrocytoma," *Acta Neurochirurgica*, vol. 151, no. 3, pp. 263–268, 2009.

[20] A. Hlobilkova, J. Ehrmann, E. Sedlakova et al., "Could changes in the regulation of the PI3K/PKB/Akt signaling pathway and cell cycle be involved in astrocytic tumor pathogenesis and progression?" *Neoplasma*, vol. 54, no. 4, pp. 334–341, 2007.

[21] M. Mizoguchi, R. A. Betensky, T. T. Batchelor, D. C. Bernay, D. N. Louis, and C. L. Nutt, "Activation of STAT3, MAPK, and AKT in malignant astrocytic gliomas: correlation with EGFR status, tumor grade, and survival," *Journal of Neuropathology and Experimental Neurology*, vol. 65, no. 12, pp. 1181–1188, 2006.

[22] C. E. Pelloski, E. Lin, L. Zhang et al., "Prognostic associations of activated mitogen-activated protein kinase and Akt pathways in glioblastoma," *Clinical Cancer Research*, vol. 12, no. 13, pp. 3935–3941, 2006.

[23] M. J. Riemenschneider, R. A. Betensky, S. M. Pasedag, and D. N. Louis, "AKT activation in human glioblastomas enhances proliferation via TSC2 and S6 kinase signaling," *Cancer Research*, vol. 66, no. 11, pp. 5618–5623, 2006.

[24] H. Wang, H. Wang, W. Zhang, H. J. Huang, W. S. L. Liao, and G. N. Fuller, "Analysis of the activation status of Akt, NFκB, and Stat3 in human diffuse gliomas," *Laboratory Investigation*, vol. 84, no. 8, pp. 941–951, 2004.

[25] A. Chakravarti, G. Zhai, Y. Suzuki et al., "The prognostic significance of phosphatidylinositol 3-kinase pathway activation in human gliomas," *Journal of Clinical Oncology*, vol. 22, no. 10, pp. 1926–1933, 2004.

[26] G. Choe, S. Horvath, T. F. Cloughesy et al., "Analysis of the phosphatidylinositol 3′-kinase signaling pathway in glioblastoma patients in vivo," *Cancer Research*, vol. 63, no. 11, pp. 2742–2746, 2003.

[27] T. F. Franke, "PI3K/Akt: getting it right matters," *Oncogene*, vol. 27, no. 50, pp. 6473–6488, 2008.

[28] Q. W. Fan and W. A. Weiss, "Targeting the RTK-PI3K-mTOR axis in malignant glioma: overcoming resistance," *Current Topics in Microbiology and Immunology*, vol. 347, pp. 279–296, 2010.

[29] C. Hartmann, B. Hentschel, W. Wick et al., "Patients with IDH1 wild type anaplastic astrocytomas exhibit worse prognosis than IDH1-mutated glioblastomas, and IDH1 mutation status accounts for the unfavorable prognostic effect of higher age: implications for classification of gliomas," *Acta Neuropathologica*, pp. 1–12, 2010.

[30] P. Korkolopoulou, G. Levidou, E. A. El-Habr et al., "Phosphorylated 4E-Binding Protein (p-4E-BP1): anovel prognostic marker in human astrocytomas," *Histopathology*. In press.

[31] J. Woenckhaus, K. Steger, K. Sturm, K. Münstedt, F. E. Franke, and I. Fenic, "Prognostic value of PIK3CA and phosphorylated AKT expression in ovarian cancer," *Virchows Archiv*, vol. 450, no. 4, pp. 387–395, 2007.

[32] A. Shah, W. A. Swain, D. Richardson et al., "Phospho-Akt expression is associated with a favorable outcome in non-small cell lung cancer," *Clinical Cancer Research*, vol. 11, no. 8, pp. 2930–2936, 2005.

[33] R. T. Abraham, "PI 3-kinase related kinases: 'big' players in stress-induced signaling pathways," *DNA Repair*, vol. 3, no. 8-9, pp. 883–887, 2004.

[34] R. T. Abraham and J. J. Gibbons, "The mammalian target of rapamycin signaling pathway: twists and turns in the road to cancer therapy," *Clinical Cancer Research*, vol. 13, no. 11, pp. 3109–3114, 2007.

[35] S. M. McBride, D. A. Perez, M. Y. Polley et al., "Activation of PI3K/mTOR pathway occurs in most adult low-grade gliomas and predicts patient survival," *Journal of Neuro-Oncology*, vol. 97, no. 1, pp. 33–40, 2010.

[36] R. P. Ermoian, T. Kaprealian, K. R. Lamborn et al., "Signal transduction molecules in gliomas of all grades," *Journal of Neuro-Oncology*, vol. 91, no. 1, pp. 19–26, 2009.

[37] C. C. Hudson, M. Liu, G. G. Chiang et al., "Regulation of hypoxia-inducible factor 1α expression and function by the mammalian target of rapamycin," *Molecular and Cellular Biology*, vol. 22, no. 20, pp. 7004–7014, 2002.

[38] O. Meyuhas, "Chapter 1 physiological roles of ribosomal Protein S6: one of its kind," *International Review of Cell and Molecular Biology*, vol. 268, pp. 1–37, 2008.

[39] S. H. Um, D. D'Alessio, and G. Thomas, "Nutrient overload, insulin resistance, and ribosomal protein S6 kinase 1, S6K1," *Cell Metabolism*, vol. 3, no. 6, pp. 393–402, 2006.

[40] F. Vega, L. J. Medeiros, V. Leventaki et al., "Activation of mammalian target of rapamycin signaling pathway contributes to tumor cell survival in anaplastic lymphoma kinase-positive anaplastic large cell lymphoma," *Cancer Research*, vol. 66, no. 13, pp. 6589–6597, 2006.

[41] C. G. Proud, "Amino acids and mTOR signalling in anabolic function," *Biochemical Society Transactions*, vol. 35, no. 5, pp. 1187–1190, 2007.

[42] S. Darb-Esfahani, A. Faggad, A. Noske et al., "Phospho-mTOR and phospho-4EBP1 in endometrial adenocarcinoma: association with stage and grade in vivo and link with response to rapamycin treatment in vitro," *Journal of Cancer Research and Clinical Oncology*, vol. 135, no. 7, pp. 933–941, 2009.

[43] Q. B. She, E. Halilovic, Q. Ye et al., "4E-BP1 Is a Key Effector of the Oncogenic Activation of the AKT and ERK Signaling Pathways that Integrates Their Function in Tumors," *Cancer Cell*, vol. 18, no. 1, pp. 39–51, 2010.

[44] L. Dubois, M. G. Magagnin, A. H. G. Cleven et al., "Inhibition of 4E-BP1 sensitizes U87 glioblastoma xenograft tumors to irradiation by decreasing hypoxia tolerance," *International Journal of Radiation Oncology Biology Physics*, vol. 73, no. 4, pp. 1219–1227, 2009.

[45] R. D. Rao, A. C. Mladek, J. D. Lamont et al., "Disruption of parallel and converging signaling pathways contributes to the synergistic antitumor effects of simultaneous mTOR and EGFR inhibition in GBM cells," *Neoplasia*, vol. 7, no. 10, pp. 921–929, 2005.

[46] E. Galanis, J. C. Buckner, M. J. Maurer et al., "Phase II trial of temsirolimus (CCI-779) in recurrent glioblastoma multiforme: a north central cancer treatment group study," *Journal of Clinical Oncology*, vol. 23, no. 23, pp. 5294–5304, 2005.

[47] T. Schmelzle and M. N. Hall, "TOR, a central controller of cell growth," *Cell*, vol. 103, no. 2, pp. 253–262, 2000.

[48] S. M. Chang, P. Wen, T. Cloughesy et al., "Phase II study of CCI-779 in patients with recurrent glioblastoma multiforme," *Investigational New Drugs*, vol. 23, no. 4, pp. 357–361, 2005.

[49] J. N. Sarkaria, E. Galanis, W. Wu et al., "NCCTG phase I trial of temsirolimus (CCI-779) and temozolomide (TMZ) in combination with radiation therapy (RT) in newly diagnosed glioblastoma multiforme (GBM) patients," *Journal of Clinical Oncology*, vol. 27, supplement 15, p. 2019, 2009.

[50] T. F. Cloughesy, K. Yoshimoto, P. Nghiemphu et al., "Antitumor activity of rapamycin in a phase I trial for patients with recurrent PTEN-deficient glioblastoma," *PLoS Medicine*, vol. 5, no. 1, pp. 0139–0151, 2008.

[51] M. S. Neshat, I. K. Mellinghoff, C. Tran et al., "Enhanced sensitivity of PTEN-deficient tumors to inhibition of FRAP/mTOR," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 98, no. 18, pp. 10314–10319, 2001.

[52] K. Podsypanina, R. T. Lee, C. Politis et al., "An inhibitor of mTOR reduces neoplasia and normalizes p70/s6 kinase activity in Pten+/- mice," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 98, no. 18, pp. 10320–10325, 2001.

[53] M. Y. Wang, K. V. Lu, S. Zhu et al., "Mammalian target of rapamycin inhibition promotes response to epidermal growth factor receptor kinase inhibitors in PTEN-deficient and PTEN-intact glioblastoma cells," *Cancer Research*, vol. 66, no. 16, pp. 7864–7869, 2006.

[54] L. Doherty, D. C. Gigas, S. Kesari et al., "Pilot study of the combination of EGFR and mTOR inhibitors in recurrent malignant gliomas," *Neurology*, vol. 67, no. 1, pp. 156–158, 2006.

[55] P. Therasse, S. G. Arbuck, E. A. Eisenhauer et al., "New guidelines to evaluate the response to treatment in solid tumors. European organization for research and treatment of cancer, national cancer institute of the United States, national cancer institute of Canada," *Journal of the National Cancer Institute*, vol. 92, no. 3, pp. 205–216, 2000.

[56] D. A. Reardon, J. A. Quinn, J. J. Vredenburgh et al., "Phase 1 trial of gefitinib plus sirolimus in adults with recurrent malignant glioma," *Clinical Cancer Research*, vol. 12, no. 3, pp. 860–868, 2006.

[57] S. M. Chang, J. Kuhn, K. Lamborn et al., "Phase I/II study of erlotinib and temsirolimus for patients with recurrent malignant gliomas (MG) (NABTC 04-02)," *Journal of Clinical Oncology*, vol. 27, supplement 15, p. A2004, 2009.

[58] D. A. Guertin and D. M. Sabatini, "The pharmacology of mTOR inhibition," *Science Signaling*, vol. 2, no. 67, p. pe24, 2009.

[59] C. C. Thoreen, S. A. Kang, J. W. Chang et al., "An ATP-competitive mammalian target of rapamycin inhibitor reveals rapamycin-resistant functions of mTORC1," *Journal of Biological Chemistry*, vol. 284, no. 12, pp. 8023–8032, 2009.

[60] J. M. Stommel, A. C. Kimmelman, H. Ying et al., "Coactivation of receptor tyrosine kinases affects the response of tumor cells to targeted therapies," *Science*, vol. 318, no. 5848, pp. 287–290, 2007.

[61] R. G. W. Verhaak, K. A. Hoadley, E. Purdom et al., "Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1," *Cancer Cell*, vol. 17, no. 1, pp. 98–110, 2010.

[62] J. S. Eshleman, B. L. Carlson, A. C. Mladek, B. D. Kastner, K. L. Shide, and J. N. Sarkaria, "Inhibition of the mammalian target of rapamycin sensitizes U87 xenografts to fractionated radiation therapy," *Cancer Research*, vol. 62, no. 24, pp. 7291–7297, 2002.

[63] M. W. Ronellenfitsch, J. P. Steinbach, and W. Wick, "Epidermal growth factor receptor and mammalian target of rapamycin as therapeutic targets in malignant glioma: current clinical status and perspectives," *Targeted Oncology*, vol. 5, no. 3, pp. 183–191, 2010.

[64] D. A. Guertin and D. M. Sabatini, "The pharmacology of mTOR inhibition," *Science Signaling*, vol. 2, no. 67, p. pe24, 2009.

Hindawi Publishing Corporation
Neurology Research International
Volume 2012, Article ID 870807, 6 pages
doi:10.1155/2012/870807

Review Article

Leptin Promotes Glioblastoma

Johnathan E. Lawrence,[1,2] Nicholas J. Cook,[1,2] Richard A. Rovin,[2,3] and Robert J. Winn[1,2]

[1] *Biology Department, Northern Michigan University (NMU), Marquette, MI 49855, USA*
[2] *Upper Michigan Brain Tumor Center, NMU and Marquette General Hospital, Marquette, MI 49855, USA*
[3] *Neurosurgery, Marquette General Hospital, Marquette, MI 49855, USA*

Correspondence should be addressed to Robert J. Winn, rwinn@nmu.edu

Received 15 July 2011; Accepted 20 October 2011

Academic Editor: Stuart H. Burri

The hormone leptin has a variety of functions. Originally known for its role in satiety and weight loss, leptin more recently has been shown to augment tumor growth in a variety of cancers. Within gliomas, there is a correlation between tumor grade and tumor expression of leptin and its receptor. This suggests that autocrine signaling within the tumor microenvironment may promote the growth of high-grade gliomas. Leptin does this through stimulation of cellular pathways that are also advantageous for tumor growth and recurrence: antiapoptosis, proliferation, angiogenesis, and migration. Conversely, a loss of leptin expression attenuates tumor growth. In animal models of colon cancer and melanoma, a decline in the expression and secretion of leptin resulted in a reduction of tumor growth. In these models, positive mental stimulation through environmental enrichment decreased leptin secretion and improved tumor outcome. This review explores the link between leptin and glioblastoma.

1. Introduction

Leptin is the product of the *obese* gene, located on chromosome 7 in humans. Mice with mutation in the *obese* gene are obese and insatiable [1]. When exogenous leptin is injected into leptin-deficient obese mice (ob/ob mice), the protein promotes satiety and weight loss [2–5]. The effects of leptin on these obese mice sparked a leptin intense focus in obesity research over the past 15 years. Unlike the ob/ob mice, obese humans are not leptin deficient. Obese humans have high circulating leptin levels which are directly correlated to the total amount of adipose tissue [6]. Leptin helps regulate bodyweight in humans by negative feedback promoting satiety when energy stores are elevated [7]. The current model suggests that obesity in humans is due to a desensitization to leptin. Obese subjects have a diminished response to leptin, and in some subjects the diminished response is due to a mutation in the leptin receptor gene [8]. The high prevalence of obesity in the USA is strongly correlated with the risk of multiple diseases, including cancer [9]. The association between cancer and obesity may, in part, be explained by elevated circulating leptin.

2. Leptin in Cancer

Leptin has been classified as a growth factor because it stimulates three key pathways well known for their roles in cell growth: proliferation, survival, and motility and migration (Figure 1). It is well documented that the binding of leptin to the leptin receptor (ObR) activates the Janus kinase-signal transducer and activator of transcription (JAK-STAT), the mitogen-activated protein kinase (MAPK), and the phosphatidylinositol 3-kinase (PI3K) pathways in both normal [10–22] and malignant cells [20, 23–41]. Supporting a role of leptin in cancer pathogenesis are reports that DNA polymorphisms in the leptin and ObR genes are associated with increased risk and progression of breast [42], prostate [43], and oral cancer [44].

Evidence generally supports leptin as a growth factor, promoting cell division and evasion of cell death [45]. Numerous reports indicate that leptin has both antiapoptotic [28, 33, 34, 36, 46–54] and proliferative effects [24, 25, 27, 29–31, 33, 34, 36, 41, 47, 49, 50, 52, 53, 55–59] (Table 1). It appears that leptin-mediated proliferation of these cancers occurs through the activation of the JAK-STAT [25, 27, 29–31, 34, 41], PI3K [24, 31, 33, 36], and MAPK [24, 31]

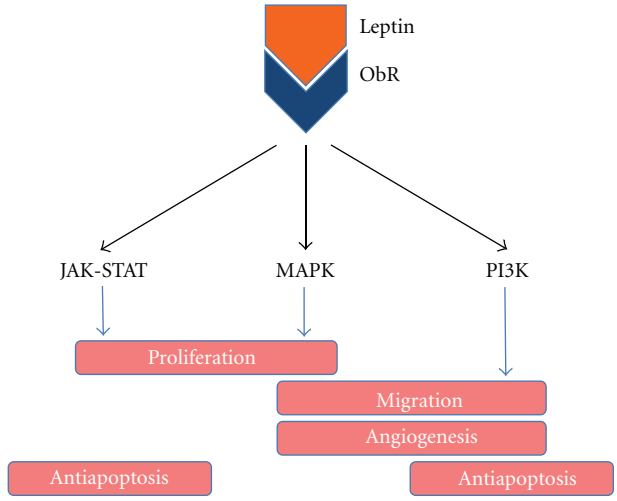


Figure 1: Cellular pathways activated through leptin receptor (ObR) stimulation.

Table 1: Summary of the Literature: leptin's role in cancer promotion*.

Cancer type	Antiapoptosis	Proliferation	Migration	Angiogenesis
Bone		24		
Breast	28, 46	27, 56	23, 65	38, 69
Cartilage			32	
Colon	48	57, 58	20, 62, 64	58
Endometrial	34	30, 31, 34		
Esophageal	51			
Gallbladder		53	53	
Gastric		25, 59		
Glioma	77		74	
Kidney		29		
Large B-cell lymphoma	33			
Leukemia	47	47		71
Liver	52	41, 52	26	70
Lung	53			
Neuroblastoma	49	49		
Ovarian		55		
Prostate	50	50	37, 39, 40	
Thyroid	36	36	63	37
Uterine				68

* Numbers correspond to works cited.

pathways, whereas apoptosis avoidance is promoted by leptin via the JAK-STAT [28, 34] and PI3K [33, 36] pathways (Figure 1).

Migration is enhanced by leptin in several normal [10, 20–22, 60, 61] and cancerous tissues [20, 23, 26, 32, 35, 37, 39, 40, 62–64] (Table 1). Leptin treatment increases the growth and migration of cholangiocarcinoma cells *in vitro* and cholangiocarcinoma is inducible in obese fa/fa Zucker (faulty ObR) rats [53]. In metastatic colon cancer cells, leptin provokes the formation of lamellipodia and augments invasion through the MAPK and PI3K pathways [62]. It has since been confirmed that leptin increases migration through the MAPK and PI3K pathways in prostate [37, 39, 40], liver [26], cartilage [32], and breast [23, 40, 64] cancers, as well as the JAK-STAT pathway in colon [35], prostate [39], liver [26], and breast [23] cancers. Compounding the complexity of leptin's role in carcinogenesis is that leptin may have differential responses in closely related cells; leptin induces migration in papillary thyroid cancer cells but not in anaplastic and follicular thyroid cancer cells [63].

In addition to its role in cellular proliferation, apoptosis avoidance, and migration, leptin is a potent angiogenic factor. Using an *in vitro* angiogenesis assay, leptin enhances the formation of capillary-like tubes by human umbilical venous

endothelial cells [65]. In 5- to 6-week-old C57BL/6J mice, leptin induces fenestrated blood vessel growth [66]. This response is synergistic with vascular endothelial growth factor (VEGF) and fibroblast growth factor-2 [66]. Myometrial cells and the blood-vessel walls of uterine myomas contain leptin, though the surrounding normal tissue does not. This suggests that leptin may be involved in angiogenesis and the development of uterine cancer [67]. VEGF levels are augmented by leptin in various cancers [37, 38, 58, 68]. It has been reported that the leptin-induced upregulation of VEGF may be due to activation of the IL-1 system [38]. This leptin-mediated IL-1 up-regulation appears to be accomplished by activation of the MAPK and PI3K pathways, among others [37, 38]. Leptin and ObR expression are correlated with the grade of the tumor, differentiation, and microvessel density [58, 69]. VEGF expression is also correlated to these variables [58]. It is noteworthy to mention that Per Ole Iverson and coworkers blocked the ObR which suppressed rat leukemia cell growth by inhibiting angiogenesis [70]. Interestingly, hypoxia can induce VEGF production in cells, and it has been demonstrated that leptin expression is also augmented under similar conditions [71].

3. The Leptin GBM Connection

It was once thought that adipocytes were the sole producers of leptin. However, leptin expression and secretion has since been demonstrated in several tissues of the body (cancerous and noncancerous) including the pituitary gland and hypothalamus [72]. Barbara Morash and colleagues provided the first report of leptin expression in glioma following detection of leptin expression in the rat C6 glioma cell line [72]. It was later shown that C6 cells express more leptin and ObR than normal glial tissue [73]. Leptin and ObR expression subsequently has been confirmed in human primary GBM tissue as well as established human GBM cells lines [74]. Leptin and ObR are overexpressed in human primary brain tumors when compared to normal glial tissue [74]. Furthermore, the expression of the leptin-ObR system correlates with histological grade: GBM has the greatest levels of leptin and ObR while low-grade gliomas have the least [74]. This suggests that leptin/ObR autocrine/paracrine signaling increases the malignant characteristics of gliomas.

Leptin/ObR overexpression in glioma [74], coupled with recent evidence that the release of leptin from adipose tissue promotes melanoma and colon cancer [75], provides strong evidence that leptin plays a role in cancer pathogenesis. In the rat C6 cell line, leptin knockdown using RNA interference produced a reduction of both leptin mRNA and leptin protein. This knockdown caused a twofold increase in cell death suggesting that endogenous leptin promotes cell survival [76]. Furthermore, exogenous leptin enhances migration and invasion of the rat C6 cells through increased levels of matrix metalloproteinase-13 (MMP-13) [73]. The leptin-mediated up-regulation of MMP-13 occurs through the MAPK pathway [73].

While there is increasing evidence of leptin's role in angiogenesis [37, 38, 58, 68], no studies (to our knowledge) have indicated how leptin might affect angiogenesis in GBM. However, hypoxia, which is a characteristic of solid tumors, is more pronounced with higher grades of glioma [77] and may explain the increased expression of leptin and ObR in GBM compared to lower-grade glioma [74].

4. Environmental Enrichment Modulates Leptin Levels

It is increasingly evident that the enhanced mental stimulation from environmental enrichment (EE) delays the advancement of neurodegenerative disorders such as Huntington's, Parkinson's, and Alzheimer's [78], slows the progression of cancer [75, 79–81], and increases the activity of natural killer cells [82]. Environmental enrichment refers to the living conditions of the subject. In the context of the rodent, EE is achieved through conditions that allow the rodent to roam more freely, engage with the surroundings, be housed with other rodents, and have better access to exercise equipment. For humans, increased social and physical activity leads to EE. Interestingly, EE can reduce peripheral leptin expression and release [75].

The response to EE is related to the type of stress the subject experiences: EE increases eustress and decreases distress. Eustress is the result of positive stressors like exercise and social interaction whereas distress is the result of negative stressors like mental stress and social isolation. The augmentation of eustress and the reduction of distress are associated with longer survival and slower tumor growth [75, 79, 81]. Probably the most significant human data to date are those reported by Barbara Andersen and her colleagues who showed that distress reduction through psychological intervention resulted in a 45% decrease in the risk of breast cancer recurrence [79] and a 59% reduction in the risk of dying following breast cancer recurrence [81]. The physiological basis for this finding is an active area of investigation. Using mouse models for melanoma and colon cancer, Cao et al. demonstrated that EE enhances brain-derived neurotrophic factor (BDNF) expression [75]. BDNF in turn activates sympathetic nerve fibers innervating white adipose tissue. This beta-adrenergic stimulation suppresses leptin secretion resulting in cancer inhibition and remission [75].

5. Environmental Enrichment and GBM

A study has yet to be designed that blocks ObR or alters leptin levels in GBM subjects or animal models. One viable option for GBM treatment may be through EE. Recall that EE-induced activation of the brain-adipocyte BDNF/leptin axis causes cancer remission and inhibition in mice [75], and distress reduction lowers the rate of recurrence in breast cancer patients [79]. Environmental enrichment and psychological treatment increase BDNF and thereby reduce systemic leptin via sympathetic activation of beta-adrenergic receptors in adipose tissue. This hypothalamic-sympathoneuronal-adipocyte axis does not address the potential leptin-ObR autocrine signaling loop of GBM. Factors that influence the transcriptional regulation of the leptin gene in the rat C6 cells are different than those in adipose tissue [83, 84], and

therefore successful treatments may need to be more specific to GBM. Therapies that are successful at crossing the blood-brain barrier and reducing the leptin-ObR signaling loop in GBM are needed and should be a focus of future research.

6. Summary

Leptin, which may be controlled by specific stimulation of the brain via EE or psychological intervention, has significant influence on tumor growth. In GBM and other cancer cells, leptin promotes cancer by stimulating cellular pathways that are advantageous for proliferation, angiogenesis, and evasion of death. Unfortunately, most of what is known about leptin and glioma stems from the rat C6 cell line. Future studies should focus on established human GBM cell lines and primary GBM neurosphere cultures both *in vitro* and *in vivo*.

References

[1] Y. Zhang, R. Proenca, M. Maffei, M. Barone, L. Leopold, and J. M. Friedman, "Positional cloning of the mouse obese gene and its human homologue," *Nature*, vol. 372, no. 6505, pp. 425–432, 1994.

[2] L. A. Campfield, F. J. Smith, Y. Guisez, R. Devos, and P. Burn, "Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks," *Science*, vol. 269, no. 5223, pp. 546–549, 1995.

[3] J. L. Halaas, K. S. Gajiwala, M. Maffei et al., "Weight-reducing effects of the plasma protein encoded by the obese gene," *Science*, vol. 269, no. 5223, pp. 543–546, 1995.

[4] M. A. Pelleymounter, M. J. Cullen, M. B. Baker et al., "Effects of the obese gene product on body weight regulation in ob/ob mice," *Science*, vol. 269, no. 5223, pp. 540–543, 1995.

[5] J. Rentsch, N. Levens, and M. Chiesi, "Recombinant ob-gene product reduces food intake in fasted mice," *Biochemical and Biophysical Research Communications*, vol. 214, no. 1, pp. 131–136, 1995.

[6] M. Maffei, J. Halaas, E. Ravussin et al., "Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects," *Nature Medicine*, vol. 1, no. 11, pp. 1155–1161, 1995.

[7] J. M. Friedman, "Leptin, leptin receptors, and the control of body weight," *Nutrition Reviews*, vol. 56, no. 2, pp. S38–S46, 1998.

[8] K. Clément, C. Vaisse, N. Lahlou et al., "A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction," *Nature*, vol. 392, no. 6674, pp. 398–401, 1998.

[9] X. Pi-Sunyer, "The medical risks of obesity," *Postgraduate Medicine*, vol. 121, no. 6, pp. 21–33, 2009.

[10] A. K. Nath, R. M. Brown, M. Michaud, M. R. Sierra-Honigmann, M. Snyder, and J. A. Madri, "Leptin affects endocardial cushion formation by modulating EMT and migration via Akt signaling cascades," *Journal of Cell Biology*, vol. 181, no. 2, pp. 367–380, 2008.

[11] C. Martín-Romero and V. Sánchez-Margalet, "Human leptin activates PI3K and MAPK pathways in human peripheral blood mononuclear cells: possible role of Sam68," *Cellular Immunology*, vol. 212, no. 2, pp. 83–91, 2001.

[12] V. Sanchez-Margalet and C. Martin-Romero, "Human leptin signaling in human peripheral blood mononuclear cells: activation of the JAK-STAT pathway," *Cellular Immunology*, vol. 211, no. 1, pp. 30–36, 2001.

[13] F. Machinal-Quélin, M. N. Dieudonné, M. C. Leneveu, R. Pecquery, and Y. Giudicelli, "Proadipogenic effect of leptin on rat preadipocytes in vitro: activation of MAPK and STAT3 signaling pathways," *American Journal of Physiology*, vol. 282, no. 4, pp. C853–C863, 2002.

[14] S. Najib and V. Sánchez-Margalet, "Human leptin promotes survival of human circulating blood monocytes prone to apoptosis by activation of p42/44 MAPK pathway," *Cellular Immunology*, vol. 220, no. 2, pp. 143–149, 2002.

[15] A. Pérez-Pérez, J. Maymó, J. L. Dueñas et al., "Leptin prevents apoptosis of trophoblastic cells by activation of MAPK pathway," *Archives of Biochemistry and Biophysics*, vol. 477, no. 2, pp. 390–395, 2008.

[16] B. Mattioli, L. Giordani, M. G. Quaranta, and M. Viora, "Leptin exerts an anti-apoptotic effect on human dendritic cells via the PI3K-Akt signaling pathway," *FEBS Letters*, vol. 583, no. 7, pp. 1102–1106, 2009.

[17] M. Arias-Álvarez, R. M. García-García, L. Torres-Rovira, A. González-Bulnes, P. G. Rebollar, and P. L. Lorenzo, "Influence of leptin on in vitro maturation and steroidogenic secretion of cumulus-oocyte complexes through JAK2/STAT3 and MEK 1/2 pathways in the rabbit model," *Reproduction*, vol. 139, no. 3, pp. 523–532, 2010.

[18] Y. C. Chen, C. H. Chen, Y. H. Hsu et al., "Leptin reduces gentamicin-induced apoptosis in rat renal tubular cells via the PI3K-Akt signaling pathway," *European Journal of Pharmacology*, vol. 658, no. 2-3, pp. 213–218, 2011.

[19] Z. Guo, H. Jiang, X. Xu, W. Duan, and M. P. Mattson, "Leptin-mediated cell survival signaling in hippocampal neurons mediated by JAK STAT3 and mitochondrial stabilization," *Journal of Biological Chemistry*, vol. 283, no. 3, pp. 1754–1763, 2008.

[20] S. Attoub, V. Noe, L. Pirola et al., "Leptin promotes invasiveness of kidney and colonic epithelial cells via phosphoinositide 3-kinase-, Rho-, and Rac-dependent signaling pathways," *The FASEB Journal*, vol. 14, no. 14, pp. 2329–2338, 2000.

[21] A. Oda, T. Taniguchi, and M. Yokoyama, "Leptin stimulates rat aortic smooth muscle cell proliferation and migration," *Kobe Journal of Medical Sciences*, vol. 47, no. 3, pp. 141–150, 2001.

[22] S. Goetze, A. Bungenstock, C. Czupalla et al., "Leptin induces endothelial cell migration through Akt, which is inhibited by PPARγ-ligands," *Hypertension*, vol. 40, no. 5, pp. 748–754, 2002.

[23] S. Kim, A. Nagalingam, N. K. Saxena, S. V. Singh, and D. Sharma, "Benzyl isothiocyanate inhibits oncogenic actions of leptin in human breast cancer cells by suppressing activation of signal transducer and activator of transcription 3," *Carcinogenesis*, vol. 32, no. 3, pp. 359–367, 2011.

[24] B. Burguera, A. Brunetto, A. Garcia-Ocana et al., "Leptin increases proliferation of human steosarcoma cells through activation of PI(3)-K and MAPK pathways," *Medical Science Monitor*, vol. 12, no. 11, pp. BR341–BR349, 2006.

[25] L. M. Judd, K. Bredin, A. Kalantzis, B. J. Jenkins, M. Ernst, and A. S. Giraud, "STAT3 activation regulates growth, inflammation, and vascularization in a mouse model of gastric tumorigenesis," *Gastroenterology*, vol. 131, no. 4, pp. 1073–1085, 2006.

[26] N. K. Saxena, D. Sharma, X. Ding et al., "Concomitant activation of the JAK/STAT, PI3K/AKT, and ERK signaling is involved in leptin-mediated promotion of invasion and migration of hepatocellular carcinoma cells," *Cancer Research*, vol. 67, no. 6, pp. 2497–2507, 2007.

[27] N. K. Saxena, P. M. Vertino, F. A. Anania, and D. Sharma, "Leptin-induced growth stimulation of breast cancer cells involves recruitment of histone acetyltransferases and mediator complex to CYCLIN D1 promoter via activation of Stat3," *Journal of Biological Chemistry*, vol. 282, no. 18, pp. 13316–13325, 2007.

[28] H. Jiang, J. Yu, H. Guo, H. Song, and S. Chen, "Upregulation of survivin by leptin/STAT3 signaling in MCF-7 cells," *Biochemical and Biophysical Research Communications*, vol. 368, no. 1, pp. 1–5, 2008.

[29] L. Li, Y. Gao, L. L. Zhang, and D. L. He, "Concomitant activation of the JAK/STAT3 and ERK1/2 signaling is involved in leptin-mediated proliferation of renal cell carcinoma Caki-2 cells," *Cancer Biology and Therapy*, vol. 7, no. 11, pp. 1787–1792, 2008.

[30] S. Catalano, C. Giordano, P. Rizza et al., "Evidence that leptin through STAT and CREB signaling enhances cyclin D1 expression and promotes human endometrial cancer proliferation," *Journal of Cellular Physiology*, vol. 218, no. 3, pp. 490–500, 2009.

[31] J. Gao, J. Tian, Y. Lv et al., "Leptin induces functional activation of cyclooxygenase-2 through JAK2/ STAT3, MAPK/ERK, and PI3K/AKT pathways in human endometrial cancer cells," *Cancer Science*, vol. 100, no. 3, pp. 389–395, 2009.

[32] S. N. Yang, H. T. Chen, H. K. Tsou et al., "Leptin enhances cell migration in human chondrosarcoma cells through OBRl leptin receptor," *Carcinogenesis*, vol. 30, no. 4, pp. 566–574, 2009.

[33] S. Uddin, R. Bu, M. Ahmed et al., "Leptin receptor expression and its association with PI3K/AKT signaling pathway in diffuse large B-cell lymphoma," *Leukemia and Lymphoma*, vol. 51, no. 7, pp. 1305–1314, 2010.

[34] Y. Liu et al., "Leptin activates STAT3 and ERK1/2 pathways and induces endometrial cancer cell proliferation," *Journal of Huazhong University of Science and Technology Medical*, vol. 31, no. 3, pp. 365–370, 2011.

[35] J. Ratke, F. Entschladen, B. Niggemann, K. S. Zänker, and K. Lang, "Leptin stimulates the migration of colon carcinoma cells by multiple signaling pathways," *Endocrine-Related Cancer*, vol. 17, no. 1, pp. 179–189, 2010.

[36] S. Uddin, P. Bavi, A. K. Siraj et al., "Leptin-R and its association with PI3K/AKT signaling pathway in papillary thyroid carcinoma," *Endocrine-Related Cancer*, vol. 17, no. 1, pp. 191–202, 2010.

[37] K. A. Frankenberry, P. Somasundar, D. W. McFadden, and L. C. Vona-Davis, "Leptin induces cell migration and the expression of growth factors in human prostate cancer cells," *American Journal of Surgery*, vol. 188, no. 5, pp. 560–565, 2004.

[38] W. Zhou, S. Guo, and R. R. Gonzalez-Perez, "Leptin pro-angiogenic signature in breast cancer is linked to IL-1 signalling," *British Journal of Cancer*, vol. 104, no. 1, pp. 128–137, 2011.

[39] D. D. Deo, A. P. Rao, S. S. Bose et al., "Differential effects of leptin on the invasive potential of androgen-dependent and -independent prostate carcinoma cells," *Journal of Biomedicine and Biotechnology*, vol. 2008, no. 1, Article ID 163902, 2008.

[40] C. Y. Huang, H. S. Yu, T. Y. Lai et al., "Leptin increases motility and integrin up-regulation in human prostate cancer cells," *Journal of Cellular Physiology*, vol. 226, no. 5, pp. 1274–1282, 2011.

[41] D. Sharma, J. Wang, P. P. Fu et al., "Adiponectin antagonizes the oncogenic actions of leptin in hepatocellular carcinogenesis," *Hepatology*, vol. 52, no. 5, pp. 1713–1722, 2010.

[42] K. Snoussi, A. D. Strosberg, N. Bouaouina, S. B. Ahmed, A. N. Helal, and L. Chouchane, "Leptin and leptin receptor polymorphisms are associated with increased risk and poor prognosis of breast carcinoma," *BMC Cancer*, vol. 6, article 38, 2006.

[43] R. Ribeiro, A. Vasconcelos, S. Costa et al., "Overexpressing leptin genetic polymorphism (-2548 G/A) is associated with susceptibility to prostate cancer and risk of advanced disease," *Prostate*, vol. 59, no. 3, pp. 268–274, 2004.

[44] C. Yapijakis, M. Kechagiadakis, E. Nkenke et al., "Association of leptin -2548G/A and leptin receptor Q223R polymorphisms with increased risk for oral cancer," *Journal of Cancer Research and Clinical Oncology*, vol. 135, no. 4, pp. 603–612, 2009.

[45] P. Somasundar, D. W. McFadden, S. M. Hileman, and L. Vona-Davis, "Leptin is a growth factor in cancer," *Journal of Surgical Research*, vol. 116, no. 2, pp. 337–349, 2004.

[46] S. De Assis, G. Khan, and L. Hilakivi-Clarke, "High birth weight increases mammary tumorigenesis in rats," *International Journal of Cancer*, vol. 119, no. 7, pp. 1537–1546, 2006.

[47] M. Hino, T. Nakao, T. Yamane, K. Ohta, T. Takubo, and N. Tatsumi, "Leptin receptor and leukemia," *Leukemia and Lymphoma*, vol. 36, no. 5-6, pp. 457–461, 2000.

[48] P. Rouet-Benzineb, T. Aparicio, S. Guilmeau et al., "Leptin counteracts sodium butyrate-induced apoptosis in human colon cancer HT-29 cells via NF-κB signaling," *Journal of Biological Chemistry*, vol. 279, no. 16, pp. 16495–16502, 2004.

[49] V. C. Russo, S. Metaxas, K. Kobayashi, M. Harris, and G. A. Werther, "Antiapoptotic effects of leptin in human neuroblastoma cells," *Endocrinology*, vol. 145, no. 9, pp. 4103–4112, 2004.

[50] P. Somasundar, K. A. Frankenberry, H. Skinner et al., "Prostate cancer cell proliferation is influenced by leptin," *Journal of Surgical Research*, vol. 118, no. 1, pp. 71–82, 2004.

[51] O. Ogunwobi, G. Mutungi, and I. L. P. Beales, "Leptin stimulates proliferation and inhibits apoptosis in Barrett's esophageal adenocarcinoma cells by cyclooxygenase-2-dependent, prostaglandin-E2-mediated transactivation of the epidermal growth factor receptor and c-Jun NH2-terminal kinase activation," *Endocrinology*, vol. 147, no. 9, pp. 4505–4516, 2006.

[52] C. Chen, Y. C. Chang, C. L. Liu, T. P. Liu, K. J. Chang, and I. C. Guo, "Leptin induces proliferation and anti-apoptosis in human hepatocarcinoma cells by up-regulating cyclin D1 and down-regulating Bax via a Janus kinase 2-linked pathway," *Endocrine-Related Cancer*, vol. 14, no. 2, pp. 513–529, 2007.

[53] G. Fava, G. Alpini, C. Rychlicki et al., "Leptin enhances cholangiocarcinoma cell growth," *Cancer Research*, vol. 68, no. 16, pp. 6752–6761, 2008.

[54] Y. Shen, Q. Wang, Q. Zhao, and J. Zhou, "Leptin promotes the immune escape of lung cancer by inducing proinflammatory cytokines and resistance to apoptosis," *Molecular Medicine Reports*, vol. 2, no. 2, pp. 295–299, 2009.

[55] J. H. Choi, S. H. Park, P. C. K. Leung, and K. C. Choi, "Expression of leptin receptors and potential effects of leptin on the cell growth and activation of mitogen-activated protein kinases in ovarian cancer cells," *Journal of Clinical Endocrinology and Metabolism*, vol. 90, no. 1, pp. 207–210, 2005.

[56] M. N. Dieudonne, F. Machinal-Quelin, V. Serazin-Leroy, M. C. Leneveu, R. Pecquery, and Y. Giudicelli, "Leptin mediates a proliferative response in human MCF7 breast cancer cells," *Biochemical and Biophysical Research Communications*, vol. 293, no. 1, pp. 622–628, 2002.

[57] J. C. H. Hardwick, G. R. Van Den Brink, G. J. Offerhaus, S. J. H. Van Deventer, and M. P. Peppelenbosch, "Leptin is a growth

factor for colonic epithelial cells," *Gastroenterology*, vol. 121, no. 1, pp. 79–90, 2001.

[58] L. Hui, W. Desen, P. Zhizhong et al., "Expression and biological significance of leptin, leptin receptor, VEGF, and CD34 in colorectal carcinoma," *Cell Biochemistry and Biophysics*, vol. 60, no. 3, pp. 241–244, 2011.

[59] R. Pai, C. Lin, T. Tran, and A. Tarnawski, "Leptin activates STAT and ERK2 pathways and induces gastric cancer cell proliferation," *Biochemical and Biophysical Research Communications*, vol. 331, no. 4, pp. 984–992, 2005.

[60] M. Suzukawa, H. Nagase, I. Ogahara et al., "Leptin enhances survival and induces migration, degranulation, and cytokine synthesis of human basophils," *Journal of Immunology*, vol. 186, no. 9, pp. 5254–5260, 2011.

[61] K. Schram, R. Ganguly, E. K. No, X. Fang, F. S. Thong, and G. Sweeney, "Regulation of MT1-MMP and MMP-2 by leptin in cardiac fibroblasts involves Rho/ROCK-dependent actin cytoskeletal reorganization and leads to enhanced cell migration," *Endocrinology*, vol. 152, no. 5, pp. 2037–2047, 2011.

[62] T. Jaffe and B. Schwartz, "Leptin promotes motility and invasiveness in human colon cancer cells by activating multiple signal-transduction pathways," *International Journal of Cancer*, vol. 123, no. 11, pp. 2543–2556, 2008.

[63] S. P. Cheng, P. H. Yin, Y. C. Chang, C. H. Lee, S. Y. Huang, and C. W. Chi, "Differential roles of leptin in regulating cell migration in thyroid cancer cells," *Oncology Reports*, vol. 23, no. 6, pp. 1721–1727, 2010.

[64] N. K. Saxena, L. Taliaferro-Smith, B. B. Knight et al., "Bidirectional crosstalk between leptin and insulin-like growth factor-I signaling promotes invasion and migration of breast cancer cells via transactivation of epidermal growth factor receptor," *Cancer Research*, vol. 68, no. 23, pp. 9712–9722, 2008.

[65] A. Bouloumié, H. C. A. Drexler, M. Lafontan, and R. Busse, "Leptin, the product of Ob gene, promotes angiogenesis," *Circulation Research*, vol. 83, no. 10, pp. 1059–1066, 1998.

[66] R. Cao, E. Brakenhielm, C. Wahlestedt, J. Thyberg, and Y. Cao, "Leptin induces vascular permeability and synergistically stimulates angiogenesis with FGF-2 and VEGF," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 98, no. 11, pp. 6390–6395, 2001.

[67] A. Markowska, A. S. Belloni, M. Rucinski et al., "Leptin and leptin receptor expression in the myometrium and uterine myomas: is leptin involved in tumor development?" *International Journal of Oncology*, vol. 27, no. 6, pp. 1505–1509, 2005.

[68] B. B. Knight, G. M. Oprea-Ilies, A. Nagalingam et al., "Survivin upregulation, dependent on leptin-EGFR-Notch1 axis, is essential for leptin-induced migration of breast carcinoma cells," *Endocrine-Related Cancer*, vol. 18, no. 4, pp. 413–428, 2011.

[69] D. Ribatti, A. S. Belloni, B. Nico, M. Di Comite, E. Crivellato, and A. Vacca, "Leptin-leptin receptor are involved in angiogenesis in human hepatocellular carcinoma," *Peptides*, vol. 29, no. 9, pp. 1596–1602, 2008.

[70] P. O. Iversen, C. A. Drevon, and J. E. Reseland, "Prevention of leptin binding to its receptor suppresses rat leukemic cell growth by inhibiting angiogenesis," *Blood*, vol. 100, no. 12, pp. 4123–4128, 2002.

[71] G. Ambrosini, A. K. Nath, M. Rocío Sierra-Honigmann, and J. Flores-Riveros, "Transcriptional activation of the human leptin gene in response to hypoxia. Involvement of hypoxia-inducible factor 1," *Journal of Biological Chemistry*, vol. 277, no. 37, pp. 34601–34609, 2002.

[72] B. Morash, A. Li, P. R. Murphy, M. Wilkinson, and E. Ur, "Leptin gene expression in the brain and pituitary gland," *Endocrinology*, vol. 140, no. 12, pp. 5995–5998, 1999.

[73] W. L. Yeh, D. Y. Lu, M. J. Lee, and W. M. Fu, "Leptin induces migration and invasion of glioma cells through MMP-13 production," *GLIA*, vol. 57, no. 4, pp. 454–464, 2009.

[74] M. Riolfi, R. Ferla, L. D. Valle et al., "Leptin and its receptor are overexpressed in brain tumors and correlate with the degree of malignancy," *Brain Pathology*, vol. 20, no. 2, pp. 481–489, 2010.

[75] L. Cao, X. Liu, E. J. D. Lin et al., "Environmental and genetic activation of a brain-adipocyte BDNF/leptin axis causes cancer remission and inhibition," *Cell*, vol. 142, no. 1, pp. 52–64, 2010.

[76] R. Brown, B. Morash, E. Ur, and M. Wilkinson, "RNAi-mediated silencing of leptin gene expression increases cell death in C6 glioblastoma cells," *Molecular Brain Research*, vol. 139, no. 2, pp. 357–360, 2005.

[77] S. M. Evans, K. D. Judy, I. Dunphy et al., "Hypoxia is important in the biology and aggression of human glial brain tumors," *Clinical Cancer Research*, vol. 10, no. 24, pp. 8177–8184, 2004.

[78] G. Laviola, A. J. Hannan, S. Macrì, M. Solinas, and M. Jaber, "Effects of enriched environment on animal models of neurodegenerative diseases and psychiatric disorders," *Neurobiology of Disease*, vol. 31, no. 2, pp. 159–168, 2008.

[79] B. L. Andersen, H. C. Yang, W. B. Farrar et al., "Psychologic intervention improves survival for breast cancer patients: a randomized clinical trial," *Cancer*, vol. 113, no. 12, pp. 3450–3458, 2008.

[80] K. A. Esser, C. E. Harpole, G. S. Prins, and A. M. Diamond, "Physical activity reduces prostate carcinogenesis in a transgenic model," *Prostate*, vol. 69, no. 13, pp. 1372–1377, 2009.

[81] B. L. Andersen, L. M. Thornton, C. L. Shapiro et al., "Biobehavioral, immune, and health benefits following recurrence for psychological intervention participants," *Clinical Cancer Research*, vol. 16, no. 12, pp. 3270–3278, 2010.

[82] N. Benaroya-Milshtein, N. Hollander, A. Apter et al., "Environmental enrichment in mice decreases anxiety, attenuates stress responses and enhances natural killer cell activity," *European Journal of Neuroscience*, vol. 20, no. 5, pp. 1341–1347, 2004.

[83] B. Morash, J. Johnstone, C. Leopold et al., "The regulation of leptin gene expression in the C6 glioblastoma cell line," *Molecular and Cellular Endocrinology*, vol. 165, no. 1-2, pp. 97–105, 2000.

[84] A. W. Li, B. Morash, A. N. Hollenberg, E. Ur, M. Wilkinson, and P. R. Murphy, "Transcriptional regulation of the leptin gene promoter in rat GH3 pituitary and C6 glioma cells," *Molecular and Cellular Endocrinology*, vol. 176, no. 1-2, pp. 57–65, 2001.

Hindawi Publishing Corporation
Neurology Research International
Volume 2012, Article ID 195176, 7 pages
doi:10.1155/2012/195176

Research Article

Can Dynamic Contrast-Enhanced Magnetic Resonance Imaging Combined with Texture Analysis Differentiate Malignant Glioneuronal Tumors from Other Glioblastoma?

Pierre-Antoine Eliat,[1] Damien Olivié,[2,3] Stephan Saïkali,[4] Béatrice Carsin,[3] Hervé Saint-Jalmes,[1,2,5] and Jacques D. de Certaines[1,5]

[1] *PRISM, IFR 140, Biogenouest, Université de Rennes 1, Campus de Villejean, 35043 Rennes, France*
[2] *LTSI, INSERM U642, Université de Rennes 1, 35000 Rennes, France*
[3] *Department of Radiology, CHU Rennes, 35000 Rennes, France*
[4] *Department of Neuropathology, CHU Rennes, 35000 Rennes, France*
[5] *Cancer Institute Eugène Marquis, 35000 Rennes, France*

Correspondence should be addressed to Pierre-Antoine Eliat, pierre-antoine.eliat@univ-rennes1.fr

Received 2 July 2011; Accepted 29 August 2011

Academic Editor: Jonas Sheehan

An interesting approach has been proposed to differentiate malignant glioneuronal tumors (MGNTs) as a subclass of the WHO grade III and IV malignant gliomas. MGNT histologically resemble any WHO grade III or IV glioma but have a different biological behavior, presenting a survival twice longer as WHO glioblastomas and a lower occurrence of metastases. However, neurofilament protein immunostaining was required for identification of MGNT. Using two complementary methods, dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) and texture analysis (MRI-TA) from the same acquisition process, the challenge is to *in vivo* identify MGNT and demonstrate that MRI postprocessing could contribute to a better typing and grading of glioblastoma. Results are obtained on a preliminary group of 19 patients a posteriori selected for a blind investigation of DCE T1-weighted and TA at 1.5 T. The optimal classification (0/11 misclassified MGNT) is obtained by combining the two methods, DCE-MRI and MRI-TA.

1. Introduction

Glioblastomas represent the majority of the glial tumors, but their phenotypic and genotypic heterogeneities are large, as attested by the appellation "glioblastoma multiforme". Their World Health Organization (WHO) classification is mainly based on histological criteria. As the result of a consensus, the classification associates several concepts that are discussed and debated. New approaches of malignant gliomas grading are requested for treatment individualization as well as for the development of new drugs and treatment strategies.

An interesting approach has been proposed by Saint-Anne Hospital (Paris) to differentiate malignant glioneuronal tumors (MGNTs) from WHO grade III and IV malignant gliomas: in a previous study concerning 49 patients classified as WHO grade IV, 10 have been identified as MGNT with a survival twice longer as glioblastomas and a lower occurrence of metastases. Gross total surgical resection may be curative in some cases [1–3]. Though MGNT histologically resemble any WHO grade III or IV glioma, they have a different biological behavior. Neurofilament protein (NFP) immunostaining is strictly required for identification of MGNT by pathologist [3] and has been considered as the gold standard for this study even if it is not the only one discriminant parameter taken into account by pathologists.

Compared to histology or molecular biology, magnetic resonance imaging: (i) associates a large range of complementary acquisition and postprocessing modalities as, for instance, diffusion weighted imaging (DWI) or susceptibility-weighted imaging (SWI), dynamic contrast-enhanced (DCE) MRI or texture analysis (MRI-TA) [4–8], (ii) is performed *in vivo* and then could contribute to

early diagnosis and treatment followup, and (iii) allows the mapping of intra or peritumoral heterogeneity which is a highly difficult challenge when analysing biopsies or surgical pieces. Even if genomics and proteomics will probably be in a near future the gold standard for tumor subtyping, our challenge, on the basis of the example of MGNT, was to illustrate a potential important contribution from presurgical functional MRI data.

Using two complementary methods on the same set of MR images, a static one (MRI-TA) and a dynamic one (DCE-MRI), the challenge was to *in vivo* characterize the subclass of MGNT and to demonstrate that MRI could usefully contribute to revisit the malignant glioma typing even if an early typing of these tumors do not modify the surgery strategy.

2. Patients and Methods

2.1. Patients. Nineteen patients (9 female and 10 male patients; median age, 57 years; range 40–71 years) with grade IV gliomas according to the WHO classification were selected a posteriori for this study: 8 Glioblastoma (GBM) and 11 MGNT according to the Saint-Anne classification.

2.2. Pathology. All patients underwent a subtotal or a gross total resection, and a single experienced neuropathologist reviewed histological specimens. The evaluated histological parameters included necrosis, vascular proliferation, mitosis, presence of giant cells and cell, density. Tumor necrosis was recorded as present when observed in at least one area in the total histological samples available. Percentage of necrosis was estimated by analysis of the totality of the paraffin-embedded tissue. Vascular proliferation was noted as low rate when endothelial cells begin confluence and as high rate when glomeruloid aspects were noted. Mitosis was analyzed in the most mitotically active tumoral area by counting number of mitosis per 10 contiguous high-power magnification fields. Cellularity was defined as low, moderate or high, according to the presence or absence of neuropil between tumor cells. For each case, perivascular lymphocytes, multinucleated giant cells, and dense reticulin network were noted when present.

Systematic immunohistochemistry study was performed for all cases on representative paraffin-embedded tumoral tissue with glial fibrillary acidic protein (GFAP) (clone 6F2) and neurofilament (clone 2F11). When at least one tumoral cell exhibited a positive labelling for neurofilament, case was considered as a MGNT, in agreement with the Saint-Anne protocol.

As neurofilament is rather heterogeneous inducing potential diagnosis error related to sampling for pathology examination, we have considered as MGNT all tumors exhibiting at least one cell with positive labeling for neurofilament. MGNT diagnosis also includes other information but, according to [3], we have considered that this criteria could be at this time used as the gold standard for this study.

2.3. Dynamic MRI Acquisition and Longitudinal Relaxation Rate Measurements. 2D MRI serial acquisitions were performed with a head coil on a 1.5 T imager (GE Signa, Milwaukee, USA). The quantitative MR Imaging protocol has been adapted from previously published studies [9, 10]. 2D fast multiplanar spoiled gradient echo (FMPSPGR) sequences (TR/TE = 150/5.6 ms and variable flip angle, $\theta_1 = 10^\circ$, $\theta_2 = 90^\circ$) were performed. Thirteen sagittal slices were acquired with a field of view of 180 mm × 240 mm, a slice thickness of 5 mm, and a 192 × 256 matrix leading to spatial resolution of 0.94 mm × 0.94 mm. Five longitudinal relaxation time (T1) calibration vials (T1 ranging from 120 ms to 2174 ms at 1.5 T and 19°C) were positioned in the coil and simultaneously imaged with the patient. For parameter extraction, regions of interest (ROI) including about 1500 pixels were manually positioned on the tumor and on the calibration vials on postcontrast images and on the corresponding part of the precontrast image. Before contrast injection, the initial longitudinal relaxation rate R_{10} value of each ROI was obtained with the two images acquired with the 2 different flip angles ($\theta_1 = 10^\circ$, $\theta_2 = 90^\circ$). After a bolus injection of a 0.1 mmol·Kg^{-1} dose of Gd-DOTA (DOTAREM, Guerbet, France), the dynamic relaxometry curves were recorded during 15 minutes. In order to increase temporal resolution, only one T_1 weighted acquisition with a $\theta_2 = 90^\circ$ flip angle was performed for each measurement. Indeed, for small flip angles the signal is independent on R_1 and does not change after gadolinium injection. For each set of images, the temporal resolution was 28 seconds.

2.4. Physiological Parameters Extraction from DCE-MRI. Postprocessing of DCE-MRI was done with tools developed for ImageJ (ImageJ, Rasband, W.S., US National Institutes of Health, Bethesda, MD, USA http://rsb.info.nih.gov/ij/, 1997–2011) and IDL 5.2 (Research System Incorporation, Boulder, CO, USA). After injection of a bolus D (mmol·kg^{-1}) of Gd-DOTA, which distributes in the extracellular space, the relaxation rate of the tissue at time t after the injection is

$$R_1(t) = R_{10} + r v_e C_{\mathrm{EES}}(t), \tag{1}$$

where r (mmol·s^{-1}) is the relaxivity of the extracellular fluid, C_{EES} the contrast agent concentration in the extravascular extracellular space (EES) at time t, R_{10} (s^{-1}) the relaxation rate before contrast injection, and $R_1(t)$ (s^{-1}) the relaxation rate after contrast injection.

At time t after contrast injection, the plasma concentration is given by the exponential relation(6)

$$C_p(t) = D \cdot \sum\nolimits_{i=1,2} a_i \cdot e^{-m_i t}, \tag{2}$$

a_i and m_i are plasmatic constants describing the early mixing phase ($i = 1$) and the later extraction phase ($i = 2$). On the Basis of Weinmann et al. data [11], Tofts and Kermode established that for Gd-DTPA $a_1 = 3,99\,\mathrm{kg{\cdot}l^{-1}}$, $a_2 = 4.78\,\mathrm{kg{\cdot}l^{-1}}$, $m_1 = 0.144\,\mathrm{min^{-1}}$, and $m_2 = 0.011\,\mathrm{min^{-1}}$. By

Table 1: MRI and pathology data (pathology).

	Patient n°	Neurofilament immunostaining	a	N of mitosis (in 10 areas)	Vascular proliferation	Cell density	Giant cells
Other WHO Glioblastomas	P02	−	50	12	+	+	−
	P09	−	35	6	+	+	+
	P26	−	50	12	+	+	−
	P29	−	70	5	+	+	+
	P31	−	30	36	+	+	−
	P41	−	80	65	+	+	−
	P42	−	60	31	+	±	+
	P53	−	20	12	+	+	−
	GBM	**8/8 ⊖**	**49, 38 ± 20, 43**	**22, 38 ± 20, 60**	**8/8 ⊕**	**7/8 ⊕ 1/8 ⊜**	**3/8 ⊕ 5/8 ⊖**
MGNT	P01	+	20	30	+	±	+
	P18	+	60	40	+	+	+
	P20	+	35	48	+	+	+
	P22	+	20	36	+	+	−
	P23	+	40	11	+	+	−
	P36	+	20	5	+	±	+
	P43	+	50	3	+	±	−
	P47	+	50	4	+	±	−
	P48	+	50	10	+	±	−
	P51	+	40	17	+	+	+
	P52	+	35	21	+	+	−
	MGNT	**11/11⊕**	**38, 18 ± 13, 83**	**20, 45 ± 15, 81**	**11/11 ⊕**	**6/11⊕ 5/11⊜**	**5/11⊕ 6/11 ⊖**

using the model described by Tofts and Kermode [12], the contrast agent concentration in EES is

$$C_{\mathrm{EES}}(t) = \frac{K^{\mathrm{trans}}}{v_e} \cdot \sum_{i=1,2} \frac{a_i \cdot \left(e^{-(K^{\mathrm{trans}}/v_e)t} - e^{-m_i t}\right)}{m_i - (K^{\mathrm{trans}}/v_e)}, \quad (3)$$

K^{trans} (min^{-1}) is the transfer constant which characterizes the blood-tissue exchange processes. From (1) and (3), we obtain

$$\Delta R_1(t) = R_1(t) - R_{10} = K^{\mathrm{trans}} \cdot r \cdot \sum_{i=1,2} \frac{a_i \cdot \left(e^{-(K^{\mathrm{trans}}/v_e)t} - e^{-m_i t}\right)}{m_i - (K^{\mathrm{trans}}/v_e)}, \quad (4)$$

K^{trans} and v_e are calculated by fitting the experimental data on (4) using the Levenberg-Marquadt nonlinear regression method. In addition to transfer constant ($K^{\mathrm{trans}}\,\mathrm{min}^{-1}$) and EES fraction v_e, the following parameters were computed from the fitted $\Delta R_1(t)$ curves: maximum relaxation rate ($\Delta R_1 \mathrm{max}\ \mathrm{s}^{-1}$), time to peak (TTP s), and slopes at 30 s ($s30\ \mathrm{s}^{-2}$).

2.5. MRI Texture Analysis. On each tumor, one Region Of Interest (ROI) of around 1500 pixels was selected by the radiologist. Mazda imaging analysis software (Institute of Electronics, Technical University of Lodz, Poland, version 3.20 http://www.eletel.p.lodz.pl/programy/mazda/) was used for texture parameters calculation in ROIs. Three statistical methods of TA were applied giving different texture parameters: (i) gray level histogram (GLH) parameters (mean, variance, skewness, kurtosis, and percentiles) that provide statistics on signal intensity distribution, (ii) co-occurrence matrix (COM) parameters (e.g., entropy, energy, correlation, contrast, inverse difference moment, etc.); this matrix provides statistics on the joint gray-level relationships by evaluating the probability that a gray level i occurs, at a distance d and angle θ, from another gray level j in the image, thus evaluating image homogeneity, directionality and internal arrangement, and (iii) the run-length distribution matrix (RLM) parameters (gray level distribution, run-length distribution, run percentage, etc.); these parameters describe mainly the coarseness of the image. A "run" is defined as a sequence of identical gray level values in a certain

Table 2: MRI and pathology data (magnetic resonance imaging).

					DCE					Texture analysis					
	Patient n°	Ring	Meningo tropism	Ratio: diffuse infiltrative area/tumor	K^{trans} (min^{-1})	v_e%	s30 (s^{-2})	TTP (s)	$\Delta R_1 max$ (s^{-1})	hRLN	hGLN	ASM $\times 10^{-3}$	SumEntr	Entr	HAC DCE-MRI + TA
Other WHO Glioblastomas	P02	−	−	0,81	0,03	0,08	0,05	457	0,12	130	2,78	7,58	1,79	2,14	+
	P09	+	−	0,53	0,18	0,32	0,26	341	0,53	157	2,84	5,99	1,80	2,23	+
	P26	+	−	0,74	0,07	0,25	0,11	568	0,37	151	2,34	7,36	1,78	2,15	−
	P29	+	±	0,45	0,12	0,19	0,17	311	0,32	119	1,96	9,23	1,67	2,04	−
	P31	+	±	0,54	0,18	0,19	0,21	237	0,34	126	2,69	8,61	1,77	2,07	−
	P41	+	±	0,48	0,27	0,17	0,24	162	0,31	175	2,67	5,58	1,87	2,26	−
	P42	+	±	0,32	0,13	0,42	0,22	520	0,65	118	3,06	8,40	1,69	2,09	−
	P53	+	±	0,41	0,07	0,38	0,12	771	0,53	90	2,14	1,19	1,56	1,93	+
	GBM	**7/8⊕ 1/8 ⊖**	**1/8 ⊕ 4/8 ⊜ 3/8 ⊖**	**0,54 ± 0,16**	**0,13 ± 0,08**	**0,25 ± 0,12**	**0,17 ± 0,07**	**421 ± 198**	**0,40 ± 0,17**	**133 ± 27**	**2,56 ± 0,38**	**8,08 ± 1,98**	**1,74 ± 0,10**	**2,12 ± 0,11**	**5/8 ⊖ 3/8 ⊕**
MGNT	P01	−	−	0,49	0,04	0,22	0,07	747	0,32	178	3,56	7,88	1,75	2,12	+
	P18	−	±	0,44	0,41	0,41	0,48	222	0,72	172	3,17	6,47	1,84	2,21	+
	P20	−	+	0,34	0,40	0,44	0,48	241	0,77	125	2,53	1,03	1,68	2,01	+
	P22	−	+	0,79	0,26	0,30	0,31	250	0,51	161	2,75	7,73	1,74	2,13	+
	P23	±	+	0,28	0,22	0,41	0,32	350	0,67	156	4	7,07	1,79	2,18	+
	P36	±	±	0,40	0,39	0,21	0,29	140	0,38	221	3,79	5,10	1,92	2,31	+
	P43	±	±	0,54	0,21	0,26	0,27	256	0,44	215	3,03	6,68	1,80	2,19	+
	P47	±	+	0,65	0,15	0,25	0,22	318	0,42	197	2,52	5,09	1,91	2,30	+
	P48	±	+	0,28	0,11	0,14	0,14	266	0,24	150	3,05	8,57	1,73	2,08	+
	P51	±	+	0,44	0,07	0,14	0,10	396	0,23	183	3,21	5,61	1,90	2,26	+
	P52	±	+	0,70	0,20	0,16	0,21	188	0,29	256	4,24	4,58	1,91	2,37	+
	MGNT	**0/11 ⊕ 7/11⊜ 4/11 ⊖**	**7/11⊕ 3/11 1/11 ⊖**	**0,49 ± 0,17**	**0,22 ± 0,13**	**0,27 ± 0,11**	**0,26 ± 0,14**	**307 ± 163**	**0,45 ± 0,19**	**83 ± 37**	**3,26 ± 0,58**	**6,83 ± 1,72**	**1,82 ± 0,09**	**2,20 ± 0,11**	**11/11 ⊕ 0//11 ⊖**

Table 3: Positive predictive value, negative predictive value, sensitivity and specificity (in %) obtained from hierarchical ascendant classification of both MRI-texture analysis run length matrix parameters (RLM) and co-occurrence matrix parameters (COM) and dynamic contrast enhancement MRI parameters for *in vivo* discrimination of MGNT from glioblastoma type.

	Positive predictive value	Negative predictive value	Sensitivity	Specificity
MRI-TA RLM	58	43	64	37
MRI-TA COM	75	71	82	62
DCE-MRI	71	80	64	50
MRI-TA COM + DCE-MRI	79	100	100	62

direction. This method calculates the probability function $p(i, d)$ in which each gray level i has a run of length d. Further details of these methods can be found in [13].

2.6. Statistical Analysis. The Mann-Whitney test was used for the estimation of statistical significance of imaging parameters. A P value less than 0.05 was considered as statistically significant. Principal component analysis (PCA) and hierarchical ascendant classification (HAC) were performed for each class of TA parameters and for parameters from DCE-MRI. Analysis were performed with Xlstat (Addinsoft, Paris, France). Positive predictive value (PPV), negative predictive value (NPV), sensitivity, and specificity have been calculated from HAC.

3. Results

3.1. Pathology. All tumors exhibited GFAP expressing cells with great variation in tumor areas. Neurofilament positive tumor cells were present in 11 cases. These tumoral cells were cytologically indistinct from other tumor cells with great variation in their number between the different cases. All tumors exhibited marked nuclear atypia and high rate of vascular proliferation. Mitosis were present in all cases with a mean of 25 mitosis/10 HPF for GBM group and 21 mitosis/10 HPF for MGNT group. Tumoral necrosis was noted in all cases with a mean of 50% for GBM group and 38% for MGNT group. Only one GBM displayed moderate cellular density (12%), other cases presenting a high cellular density. In MGNT group, 5 cases were moderately cellular (45%). Numerous multinucleated giant cells were observed in 2 GBM (25%) and in 5 MGNT (45%). Accentuation of the reticulin network was always noted and lymphocytic perivascular cuffing was seen in 2 GBM (25%) and 7 MGNT (63%) (Tables 1 and 2).

3.2. MRI Visual Examination. Visual examination of MR images by the neuroradiologist before and after contrast agent does not discriminate MGNT. The two expected MRI morphological signs for MGNT characterization, meningotropism in MGNT and ring structure in other WHO glioblastomas, are not significant (Tables 1 and 2). However, Figure 1 shows typical well delimited ring pattern of GBM and a more complex "grape-like" appearance of the presented MGNT.

3.3. DCE-MRI. Parameters describing enhancement curve and physiological parameters have been calculated for each patient (Tables 1 and 2). These parameters can be presented as physiological maps (Figure 1).

3.4. Texture Analysis. Parameters from the GLH, COM, and RLM were extracted from postinjection T1 weighted images (Tables 1 and 2).

3.5. Statistical Analysis. Using independently the two methods, MRI-TA and DCE-MRI, a rather poor discrimination between GBM and MGNT is obtained. Although higher values of K^{trans} and s30 are observed for MGNT, there are no statistical significant differences for DCE-MRI related parameters. From the best selected TA features horizontal run length nonuniformity (hRLN) and horizontal grey level nonuniformity (hGLN) from the run length matrix method present a statistical significant difference between MGNT and GBM with P values, respectively, $P = 0.008$ and $P = 0.017$. When associating both MRI-TA and DCE-MRI, 45% of the information is given by the 3 first factorial axis which are mainly weighted by hRLN, hGLN and by angular second moment (ASM), sum entropy and entropy from the cooccurrence matrix method. 11/11 correct classification of MGNT is obtained (Table 3) with a positive predictive value of 79%, a negative predictive value of 100%, a sensitivity of 100%, and a specificity of 62%.

4. Discussion

Though DCE-MRI has already been used in neuro-oncology, previous results have mainly concerned low versus high WHO grades [14–16] and have not revisited the WHO grading of malignant gliomas. Also, MRI texture analysis has opened exciting perspectives in the evaluation of intra-, peri-, and extratumoral heterogeneity but has not been already oriented to an attempt of malignant gliomas subgrading [4, 5].

This study associating DCE and TA is, at our knowledge, the first attempt to *in vivo* indentify the subclass of MGNT from glioblastoma. Associating DCE-MRI and MRI-TA, a sensitivity of 100% and a specificity of 62% have been obtained in the limited population already studied. The rather low specificity may be improved by adding texture parameters from T2 and/or diffusion weighted images as well as by 3D TA approaches. This preliminary result suggests that *in vivo* MRI associated with appropriate postprocessing methods could characterize MGNT up to now identified by *ex vivo* immunostaining histology. One important parameter in pathology typing of MGNT is the angiogenic status, well expressed by the K^{trans} parameter in DCE MRI. It has

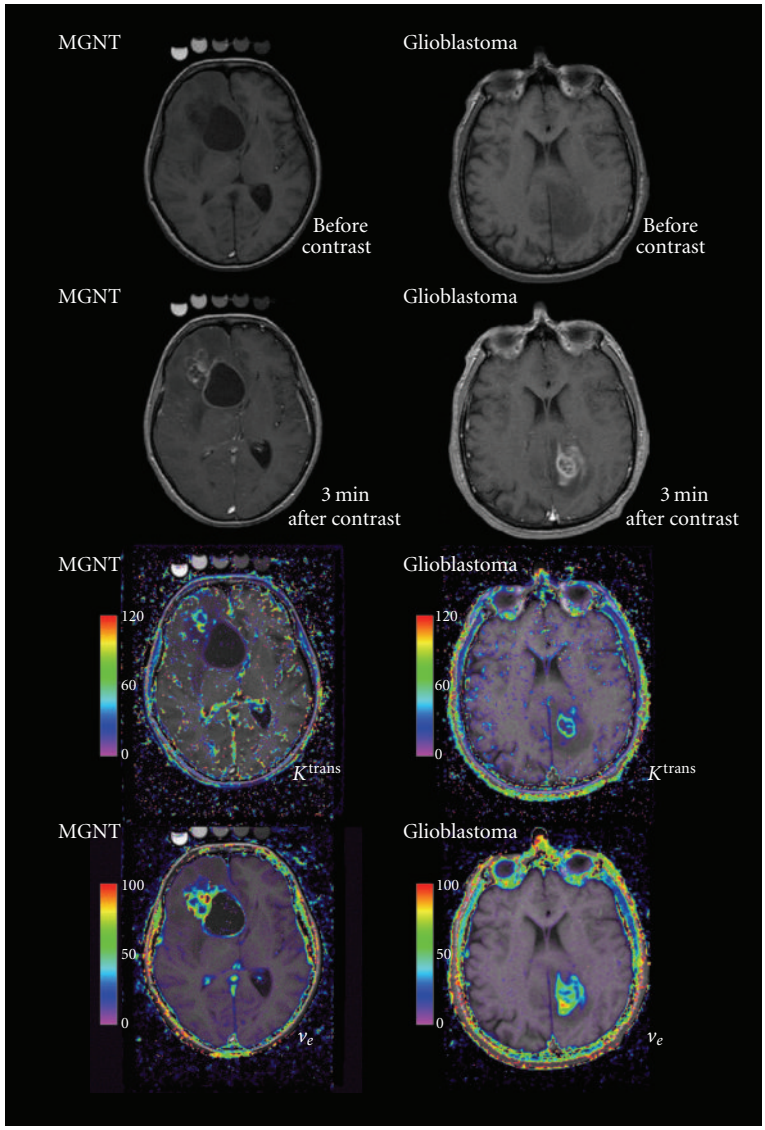


Figure 1: From top to bottom T1-weighted MR image before and 3 min after contrast agent injection, calculated map of transfer constant (K^{trans} in min^{-1}) and of extracellular extravascular space fraction (v_e in %). An example of a malignant glioneuronal tumor is on the left and of a glioblastoma is on the right. The color code increases from purple to red. On the postcontrast T1 images the MGNT shows a complex structure, whereas the GBM sows a typical enhanced ring.

also to be noticed that the relevant MRI-TA parameters are second order parameters, then not detected by the visual observation of the radiologist who only can detect first order texture. Concerning other WHO high-grade gliomas, it has to be noticed a large heterogeneity concerning these DCE-MRI and MRI-TA parameters, suggesting again the clinical interest of tumor individualization.

5. Conclusion

These preliminary results show the potential interest of DCE-MRI and MRI-TA association for *in vivo* tumor characterization, an exciting challenge for subtyping of glioblastoma. Furthermore, if associated with molecular tumor characterization by genomics or proteomics, functional imaging has the potential to provide additional information on tumor heterogeneity, a highly relevant parameter for tumor grading.

References

[1] C. Daumas-Duport, F. Beuvon, P. Varlet, and C. Fallet-Bianco, "Gliomas : WHO and Sainte-Anne Hospital classifications," *Annales de Pathologie*, vol. 20, no. 5, pp. 413–428, 2000.

[2] P. Varlet, A. Jouvet, C. Miquel, G. Saint-Pierre, F. Beuvon, and C. Daumas-Duport, "Criteria of diagnosis and grading of oligodendrogliomas or oligo-astrocytomas according to the WHO and Sainte-Anne classifications," *Neurochirurgie*, vol. 51, no. 3-4, pp. 239–246, 2005.

[3] P. Varlet, D. Soni, C. Miquel et al., "New variants of malignant glioneuronal tumors: a clinicopathological study of 40 cases," *Neurosurgery*, vol. 55, no. 6, pp. 1377–1391, 2004.

[4] S. Herlidou-Même, J. M. Constans, B. Carsin et al., "MRI texture analysis on texture test objects, normal brain and intracranial tumors," *Magnetic Resonance Imaging*, vol. 21, no. 9, pp. 989–993, 2003.

[5] D. Mahmoud-Ghoneim, G. Toussaint, J. M. Constans, and J. D. de Certaines, "Three dimensional texture analysis in MRI: a preliminary evaluation in gliomas," *Magnetic Resonance Imaging*, vol. 21, no. 9, pp. 983–987, 2003.

[6] J. M. Provenzale, S. Mukundan, and D. P. Barboriak, "Diffusion-weighted and perfusion MR imaging for brain tumor characterization and assessment of treatment response," *Radiology*, vol. 239, no. 3, pp. 632–649, 2006.

[7] A. Rauscher, J. Sedlacik, M. Barth, E. M. Haacke, and J. R. Reichenbach, "Nonnvasive assessment of vascular architecture and function during modulated blood oxygenation using susceptibility weighted magnetic resonance imaging," *Magnetic Resonance in Medicine*, vol. 54, no. 1, pp. 87–95, 2005.

[8] V. Sehgal, Z. Delproposto, E. M. Haacke et al., "Clinical applications of neuroimaging with susceptibility-weighted imaging," *Journal of Magnetic Resonance Imaging*, vol. 22, no. 4, pp. 439–450, 2005.

[9] P. A. Eliat, V. Dedieu, C. Bertino et al., "Magnetic resonance imaging contrast-enhanced relaxometry of breast tumors: an MRI multicenter investigation concerning 100 patients," *Magnetic Resonance Imaging*, vol. 22, no. 4, pp. 475–481, 2004.

[10] D. Vincensini, V. Dedieu, J. P. Renou, P. Otal, and F. Joffre, "Measurements of extracellular volume fraction and capillary permeability in tissues using dynamic spin-lattice relaxometry: studies in rabbit muscles," *Magnetic Resonance Imaging*, vol. 21, no. 2, pp. 85–93, 2003.

[11] H. J. Weinmann, M. Laniado, and W. Mützel, "Pharmacokinetics of GdDTPA/dimeglumine after intravenous injection into healthy volunteers," *Physiological Chemistry and Physics and Medical NMR*, vol. 16, no. 2, pp. 167–172, 1984.

[12] P. S. Tofts and A. G. Kermode, "Measurement of the blood-brain barrier permeability and leakage space using dynamic MR imaging. 1. Fundamental concepts," *Magnetic Resonance in Medicine*, vol. 17, no. 2, pp. 357–367, 1991.

[13] M. Petrou and P. García Sevilla, *Image Processing: Dealing With Texture*, John Wiley & Sons, New York, NY, USA, 2006.

[14] L. Lüdemann, W. Grieger, R. Wurm, M. Budzisch, B. Hamm, and C. Zimmer, "Comparison of dynamic contrast-enhanced MRI with WHO tumor grading for gliomas," *European Radiology*, vol. 11, no. 7, pp. 1231–1241, 2001.

[15] L. Lüdemann, B. Hamm, and C. Zimmer, "Pharmacokinetic analysis of glioma compartments with dynamic Gd-DTPA-enhanced magnetic resonance imaging," *Magnetic Resonance Imaging*, vol. 18, no. 10, pp. 1201–1214, 2000.

[16] H. C. Roberts, T. P. Roberts, A. W. Bollen, S. Ley, R. C. Brasch, and W. P. Dillon, "Correlation of microvascular permeability derived from dynamic contrast-enhanced MR imaging with histologic grade and tumor labeling index: a study in human brain tumors," *Academic Radiology*, vol. 8, no. 5, pp. 384–391, 2001.