Advances in the Treatment of Malignant Gliomas

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Abstract Local control with surgery, radiation, and temozolomide chemotherapy remain the pillars of treatment for high-grade gliomas. Novel therapeutic strategies, including a variety of antiangiogenic agents, are under investigation. One of these agents, bevacizumab, was recently given accelerated approval by the US Food and Drug Administration as a single agent for recurrent glioblastoma. Recent trial results are generating important clinical questions regarding which patients to treat and when, and how best to monitor response. Encouraging results of recent studies are driving willingness to undertake aggressive treatment and to improve outcomes in this population. In this era, better understanding of biology, molecular aspects of cancer, and clinical trial methodology are crucial for clinicians. This review focuses on recent advances in the treatment of malignant gliomas, especially antiangiogenic therapy.

Keywords Glioblastoma · Bevacizumab · Antiangiogenic therapy · Temozolomide

Introduction

Approximately 20,000 patients are diagnosed with gliomas each year in the United States [1]. The majority of these are malignant, comprising glioblastomas (GBMs; World Health Organization [WHO] grade IV tumors) and anaplastic astrocytomas (AAs; WHO grade III), as well as other less common variants. GBM is the most common and most aggressive subtype. Low-grade (WHO grade II) gliomas also have the potential to become highly malignant neoplasms.

This review focuses on recent advances in the treatment of malignant gliomas, particularly GBMs, with an emphasis on antiangiogenic therapy.

Standard of Care for Newly Diagnosed Glioblastoma

For decades, there was debate regarding the benefit of chemotherapy for patients with newly diagnosed GBM. The debate was settled in 2005 when results of a phase 3 trial conducted by the European Organization for Research and Treatment of Cancer (EORTC) and the National Cancer Institute of Canada (NCIC) were reported. This study demonstrated that temozolomide (TMZ), a DNA alkylating agent that is administered orally and well tolerated, improved median survival and increased the likelihood of long-term survival when given concurrently with radiotherapy (RT) and then following RT, instead of RT alone following maximal surgical resection [2].

In the initial report, median overall survival (OS) was 14.6 months, and the 2-year overall survival rate (2yOS) was 27% following RT/TMZ versus a median OS of 12.1 months and 2yOS of 11% following RT alone [2]. Longer follow-up is now available and demonstrated that the benefit of administering TMZ at diagnosis is sustained. For example, the 5-year overall survival rate (5yOS) was 10% following RT/TMZ versus 2% following RT alone [3••].

Patients in the EORTC-NCIC phase 3 study without disease progression discontinued adjuvant TMZ after six monthly cycles in part because of concerns regarding
long-term toxicity such as myelodysplasia. Although no data are yet available demonstrating improvement in survival with prolonged adjuvant therapy, reports of serious side effects from prolonged TMZ use are rare [4], and most neuro-oncologists in the United States advocate at least 12 post-RT adjuvant TMZ cycles for patients without disease progression. Ongoing clinical trials also typically incorporate 12 adjuvant TMZ cycles.

Treatment of Newly Diagnosed Anaplastic Gliomas

After the initial report in 2005 [2], the EORTC-NCIC regimen of maximal surgical resection followed by concurrent RT and TMZ followed by at least 6 monthly adjuvant TMZ cycles became the standard of care for newly diagnosed GBM. The treatment of newly diagnosed anaplastic gliomas is less straightforward. Patients with AA are often treated analogously as those with GBM, and treatment strategies for anaplastic oligodendroglioma vary widely depending on 1p19q deletion status [5].

A recent phase 3 German trial suggested equivalence of RT before or following chemotherapy (with TMZ or procarbazine, lomustine, and vincristine [PCV]) for patients with anaplastic tumors of various histologies [6]. However, despite the relatively large size of the trial, each cohort was relatively small when divided by histology and other clinically important prognostic factors, and the complex trial design adds difficulty to incorporating results into practice [7•]. Recently opened trials, such as CATNON (Concurrent and/or Adjuvant Temozolomide for 1p19q NON deleted tumors) and CODEL (for patients with 1p19q CO-DELeted tumors), will evaluate various strategies for treatment of anaplastic gliomas, including RT alone, RT with concurrent and/or adjuvant TMZ, or TMZ alone (the latter in CODEL only).

MGMT Expression and Promoter Methylation

Among the strongest predictors for survival in the EORTC-NCIC study for newly diagnosed GBM was epigenetic silencing of the MGMT (O6-methylguanine-DNA-methyltransferase) gene by promoter methylation [3••]. Patients with tumors harboring MGMT promoter methylation clearly benefit most from combined RT/TMZ. For example, median survival was 23 months following RT/TMZ versus 15 months for RT alone, and 5yOS rate was 14% versus 5%.

Patients with unmethylated tumors also appear to benefit, but to a lesser degree, with a median OS of 12.6 months for combined RT/TMZ versus 11.8 months for RT alone, 2yOS of 14.8 versus 1.8%, and approximately 10% of patients living after 3, 4, and 5 years versus none [3••]. Conventional wisdom suggests that epigenetic silencing of the MGMT promoter reduces MGMT protein expression, reducing the resistance to TMZ-induced DNA methylation. However, patients with MGMT methylated tumors treated with RT alone survived longer than patients with unmethylated tumors also treated with RT alone [3••]. As such, MGMT promoter methylation appears to represent both a predictive factor for TMZ sensitivity and a prognostic factor for survival, independently of the specific therapy administered. Therefore, the mechanism of MGMT-mediated TMZ resistance is more complex than currently understood, further supported by the lack of correlation between MGMT protein expression (by immunohistochemistry) and survival [8]. Methylation-specific polymerase chain reaction, best performed on fresh frozen biopsies, is increasingly available and is the standard at this time to assess MGMT status rather than tests for protein expression [9].

As TMZ is generally well tolerated, patients with newly diagnosed GBM outside of a clinical trial are currently treated with RT and TMZ regardless of MGMT methylation status. Several trials are underway to determine whether intensifying the TMZ dosing schedule can increase benefit in patients with MGMT unmethylated tumors.

Pseudoprogression

Radiographic worsening within several months after the completion of RT is increasingly recognized as a possible effect of RT/TMZ that is transient, rather than true tumor progression. When these abnormalities stabilize or improve without altering treatment, the phenomenon is called pseudoprogression. This occurs in up to two thirds of patients with GBM treated with RT and concurrent TMZ, and may be more common in MGMT methylated cases [10].

Whether pseudoprogression occurs more commonly following RT and TMZ than RT alone is debated [11], but undisputed is the increased surveillance and awareness of the issue in the past several years. Patients with asymptomatic increases in contrast enhancement on brain imaging that could represent pseudoprogression are often continued on adjuvant TMZ. In this setting, the post-RT MRI should be considered as a new baseline, rather than an assessment of response to chemoradiotherapy. This is essential to avoid overestimating the benefits of second-line treatment. In symptomatic patients or those with a marked increase in contrast-enhanced abnormality, surgery should be considered, as no imaging modality adequately distinguishes pseudoprogression from tumor growth. If mainly necrosis is noted during surgery, continuation of adjuvant TMZ is an option [12•].

Treatment for true progression of malignant gliomas, either proven histologically or presumed from continued radio-
graphic progression, constitutes a major challenge. Nitrosoureas such as lomustine and carmustine were often considered the standard approach, but they have limited effectiveness in this setting, with response and 6-month progression-free survival (6mPFS) rates not more than 20% for recurrent GBM [13]. Nitrosoureas are also associated with significant bone marrow and pulmonary toxicities. TMZ is often reintroduced using either a metronomic or dose-dense schedules aimed at depleting MGMT and reintroducing TMZ sensitivity [14]. Most single-arm phase 2 trials of targeted agents yield disappointing results, partly because of inadequate drug delivery [15] and partly because of molecular intra- and intertumoral heterogeneity. Enrichment by molecular testing of pretreatment tissue may improve results [16].

Perhaps the most exciting development in the treatment of recurrent (and possibly newly diagnosed) malignant gliomas has been the use of antiangiogenic therapies such as bevacizumab.

**Antiangiogenic Therapy for Recurrent Gliomas**

The dependence of tumor growth on the development of new blood vessels is now a well-established aspect of cancer biology. Angiogenesis is essential for the supply of oxygen, nutrients, growth factors, and growth of tumor and spread of tumor cells. Angiogenesis is regulated by several proteins that promote or prevent the process. During tumor progression, growth is sustained by nutrients and oxygen through passive diffusion. Once new blood vessels form, the tumor starts to grow and spreads faster [17]. Molecular changes or alterations in the local environment, including the release of growth factors or hypoxia, may induce the change from a less to a more aggressive tumor. This is also called the *angiogenic switch* [18]. In malignant gliomas, angiogenesis is typically associated with an increase in vascular endothelial growth factor (VEGF), a protein that stimulates new blood vessel formation. Activation of the VEGF receptor starts many processes that promote endothelial cell growth, migration, and survival from preexisting blood vessels. In addition, VEGF mediates permeability (leakiness) of blood vessels and is involved in the mobilization of the so-called endothelial progenitor cells from the bone marrow to distant sites of new vessel formation [19].

Bevacizumab is a humanized monoclonal anti-VEGF antibody studied in several phase 2 trials for malignant gliomas. Data first emerged on the activity of bevacizumab in 2005 in a cohort of 21 patients with malignant glioma [20]. There was an unprecedented radiographic response rate of 43% following treatment with bevacizumab and irinotecan (adapted from the regimen used for colon cancer). Subsequent studies reported responses in up to 63% of patients [21–23••].

The largest study of bevacizumab for recurrent GBM was a noncomparative phase 2 trial (now called the “BRAIN” trial), which was not designed to compare the two arms against each other. In this study, 167 patients were randomly assigned to treatment with bevacizumab alone or in combination with irinotecan [23••]. The 6 m-PFS rate was 43% in the bevacizumab monotherapy cohort and 50% in the bevacizumab–irinotecan group. Median OS was 9.2 months and 8.7 months, respectively. Typical toxicities from bevacizumab were observed, including hypertension, myelosuppression. Diarrhea was seen in the bevacizumab–irinotecan group. Asymptomatic intracranial hemorrhage was reported in approximately 3% of patients.

Both arms were associated with superior radiographic response rates, median PFS, and 6mPFS rates relative to historic controls. Controversy surrounds whether bevacizumab alone is sufficient for benefit, as suggested by the BRAIN study. Irinotecan is used mainly in the treatment of metastatic colorectal cancer in which there is undisputed efficacy as a single agent. The combination of bevacizumab and irinotecan has also shown clinical benefit in metastatic colorectal cancer, whereas bevacizumab alone was associated with inferior survival in the same population. The exact efficacy of irinotecan in recurrent malignant gliomas is debated, but the impact is likely modest at best [24•].

Therefore, debate surrounding the contribution of irinotecan led to efforts to enhance the efficacy of bevacizumab through combination with other agents such as etoposide, carboplatin, and TMZ [25, 26]. Re-irradiation, previously relegated to a treatment of last resort, was demonstrated to have surprisingly minimal toxicity and a very high response rate when combined with bevacizumab. In a pilot study combining bevacizumab and hypofractionated re-irradiation (30 Gy divided in 5 fractions over 15 days) for patients with recurrences less than 3.5 cm in size, there was no radionecrosis in 25 patients, suggesting a protective effect from concurrent bevacizumab. In addition, 50% of patients with GBM responded and the 6mPFS rate was 65%, suggesting synergistic efficacy of the combined modalities [27]. Higher RT fractions are under exploration for patients with recurrent disease, and the shortened RT course with concurrent bevacizumab (and TMZ) is under investigation for newly diagnosed disease as well (below).

**Bevacizumab for Newly Diagnosed Glioblastoma**

The encouraging results with bevacizumab for recurrent disease led to investigation of potential benefit in the newly diagnosed setting. However, there are serious toxicity concerns, including breakdown of recently closed surgical wounds. Therefore, an assessment of risk versus benefit is
essential, as almost 50% of GBM patients with MGMT methylated tumors survive 2 years following RT and TMZ without bevacizumab as initial therapy [3••]. Therefore, it is entirely possible that the addition of bevacizumab to RT and TMZ may shorten survival in some patients because of the development of a fatal toxicity. Moreover, the concern has been raised that bevacizumab promotes a more invasive aggressive tumor biology that could also shorten survival in some cohorts compared with RT/TMZ alone.

In one phase 2 study of 51 patients, 20 were treated with standard RT/TMZ with the addition of bevacizumab 29 days following surgery, followed by up to six cycles of adjuvant TMZ with bevacizumab. In addition, 31 patients received similar treatment without bevacizumab. The 6mPFS rate was 77.5% in the bevacizumab group and 51.6% in the standard treatment arm. Median PFS in the bevacizumab arm was 17 months compared with 7 months in the other arm [28]. However, this small study was underpowered to make definitive comparisons, tempering overinterpretation of benefit.

The recursive partitioning (RPA) technique can be employed to refine the stratification and design of malignant glioma trials [29]. The available phase 2 data on bevacizumab suggests that patients with a less favorable RPA class may benefit most [30]. For example, a pilot study of 10 patients followed by a 60-patient expansion phase was conducted at the University of California, Los Angeles and Kaiser-Permanente [31]. After completion of standard radiotherapy and TMZ combined with bevacizumab, patients were then placed on a maintenance phase of bevacizumab every 2 weeks and standard adjuvant TMZ until progression or 24 months, after which they were maintained on bevacizumab only. Serious but uncommon adverse events included ischemic stroke, pulmonary embolus, wound breakdown, gastrointestinal bleeding/perforation, and renal dysfunction. Preliminary analysis demonstrates a nonsignificant trend toward longer survival (compared with historic controls treated with RT/TMZ without bevacizumab) for patients with a lower RPA class (ie, older/poorer performance status) but with no advantage for patients with a high RPA class. Longer PFS was seen regardless of RPA class.

Radiation Therapy Oncology Group (RTOG) trial 0825 and a European study are randomized, placebo-controlled, phase 3 trials that will evaluate the potential benefit of bevacizumab for newly diagnosed GBM.

**Pseudoprogression**

In addition to the inability of contrast-enhanced MRI to distinguish genuine progression from “pseudoprogression,” contrast MRI is unable to identify “pseudoprogression” seen during bevacizumab or other antiangiogenic therapy for recurrent malignant gliomas [32]. A nonenhancing tumor pattern of progression is common in this setting (Fig. 1), and a retrospective study suggested this pattern predicts shorter survival [33]. Despite efforts to utilize modalities such as MR spectroscopy and MRI perfusion, no imaging modality is widely accepted to distinguish true response from pseudoprogression (or true progression from pseudo-progression). Research positron emission tomography (PET) tracers [34] and restricted diffusion [35] may be helpful in the future.

**Salvage Treatment After Progression on Bevacizumab**

Although bevacizumab is now an established treatment option for patients with recurrent GBM, treatments after bevacizumab failure provide only transient tumor control. Chemotherapy in recurrent malignant glioma in this setting is an area of considerable challenge. Patients who progress and discontinue bevacizumab typically show minimal benefit from further treatment [33]. Continuing bevacizumab but adding irinotecan [21] or changing to a different concurrent chemotherapeutic agent, such as carboplatin, also appears to be ineffective [36].

Translational insight into the mechanism of resistance to antiangiogenic therapy has shown that several growth factors, including fibroblast growth factor, stroma cell-derived factor-1α, and viable circulating endothelial cells, increased when tumors escaped treatment; circulating progenitor cells increased when tumors progressed after drug interruption [37•]. Efforts to combine bevacizumab with agents that inhibit these processes are ongoing. However, given the lack of effective post-bevacizumab (salvage) therapies, consideration should be given to deferring treatment with bevacizumab until a second or later recurrence in patients with high performance status and small tumors that are not rapidly enlarging.

**Other Antiangiogenic Therapies**

Various small molecule agents that directly or indirectly interfere with angiogenesis have been investigated in a number of early phase 1 trials. Some of these agents have undergone further development such as vandetanib, cediranib (AZD2171), aflibercept (formerly VEGF-Trap), and sunitinib. The oral tyrosine kinase inhibitors (TKIs) of VEGF receptors, cediranib for instance, showed benefit of alleviating edema and reducing contrast-enhancing tumor [37•]. Serial MRIs and PET scans in a phase 2 study showed changes interpreted as “vascular normalization,” which is proposed to
offer a therapeutic normalization window to deliver cytotoxic chemotherapy \[37\]. There are several randomized trials adding small molecule anti-VEGF TKIs, including a randomized phase 2 study with vandetanib for newly diagnosed GBM and a phase 3 study with cediranib for recurrent GBM. The latter has completed accrual, and the results are expected soon.

Cilengitide

Integrins are transmembrane receptors that bind multiple extracellular ligands. This binding activates integrins to regulate tumor cell invasion, migration, proliferation, survival, and angiogenesis. Integrins are widely expressed by both GBM cells and tumor vasculature. VEGF can trigger activation of endothelial integrins alpha (v) beta3 (\(\alpha v\beta3\)) \[38\]; tumor cell integrin \(\alpha v\beta3\) activation promotes angiogenesis \[39\]. Primary endothelial cells undergo apoptosis when deprived from integrin-mediated attachment in vitro \[40\].

Cilengitide, an inhibitor of \(\alpha v\beta3\) and alpha (v) beta5 integrin receptors, has been investigated in recurrent GBM in two phase 2 trials with some benefit as a single agent \[41, 42\]. In a subsequent study, cilengitide was added to standard RT/TMZ in a randomized phase 2 trial with promising efficacy, particularly in patients with tumors with \(MGMT\) promotor methylation \[43\]. Currently, an international phase 3 trial (CENTRIC) is enrolling patients with GBM harboring \(MGMT\) promoter methylation, adding cilengitide to standard RT/TMZ compared with RT/TMZ without cilengitide. There is also at least one phase 2 cilengitide trial actively accruing for patients with \(MGMT\) unmethylated tumors.

Talampanel

The glutamatergic system is important in the proliferation and migration of GBM. In a single-arm phase 2 study, talampanel, a glutamate receptor blocker, was added to standard RT/TMZ in 72 patients, yielding a median survival time of 18.3 months, a statistically significant improvement compared with historic controls \[44\]. Talampanel was well tolerated and will be investigated in a phase 3 trial.

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**Fig. 1** Pseudoresponse to bevacizumab. Pre-bevacizumab MRI shows marked contrast enhancement in the right posterior parietal/temporal area (a) with associated edema and non-enhancing tumor on the fluid-attenuated inversion recovery (FLAIR) sequence (b) in a recurrent glioblastoma (GBM). Following 3 months of treatment with bevacizumab, the contrast enhancement disappeared (c), but the FLAIR abnormality significantly worsened (d), demonstrating, at least in part, progression of nonenhancing tumor. Moreover, there was a new lesion on FLAIR imaging in the contralateral hemisphere (d, arrow) also consistent with progressive disease.
Although this appears promising in comparison to the median survival from the EORTC-NCIC study of RT/ TMZ [2], the apparent positive clinical trial results may also reflect a more aggressive approach toward patients with GBM, as enthusiasm and interest in the field has risen with a change in the historic controls required (see below).

Vaccination and Immunomodulation for Malignant Gliomas

Polyinosinic-polycytidylic acid stabilized with polylysine and carboxymethylcellulose (poly-ICLC) may stimulate the release of cytokines and, by inducing interferon-γ production, may increase the tumoricidal activities of various immunohematopoietic cells. Two phase 2 studies with poly ICLC suggested survival advantage awaiting further confirmation [45, 46].

Another early phase study with dendritic cell vaccines including 32 GBM patients reported that vaccine responders exhibited significantly longer time to progression relative to nonresponders. However, further research confirmation is needed [47].

The CDX-110 vaccine, which targets an epidermal growth factor variant III (EGFRvIII)-specific peptide, has been studied in a phase 2 trial in newly diagnosed EGFRvIII expressing GBM. Eligibility mandated the absence of any enhancing postradiotherapy abnormality on MRI, which is a favorable prognostic feature. Although the preliminary results are encouraging, the patient selection criteria makes the results difficult to interpret in view of the lack of appropriate historic controls [48].

Conclusions

Despite all available treatment modalities, prognosis remains poor for patients with malignant gliomas generally and GBM in particular, especially compared with improvements in treatment for other cancers such as lymphoma or breast cancer. Strategies including a wide variety of biological response modifiers are underway. Recently, bevacizumab was approved by the US Food and Drug Administration as a single agent for recurrent GBM. However, with antiangiogenic therapies including bevacizumab, the issue of pseudoresponse is emerging as a new challenge both for clinical practice, trial design, and interpretation [49].

In addition to bevacizumab, several single-arm phase 2 studies for newly diagnosed GBM report encouraging results with talampanel, poly ICLC, and cilengitide [50•]. This either reflects activity of each agent, or alternatively, activity of none if the historic control data from EORTC-NCIC no longer reflect current outcomes of patients treated with radiation and temozolomide. This prolonged survival may reflect improvement in the overall care and attention to this disease. Therefore, adequately powered randomized trials, rather than single-arm studies, will be required to differentiate these possibilities. For example, RTOG 0525, a phase 3 trial comparing RT/TMZ with standard versus intensified adjuvant TMZ dosing, may shed light on this issue if the “control” arm demonstrates significantly longer survival than the same treatment in the EORTC-NCIC study.

Future clinical trials must take anatomic, biologic, and translational aspects into consideration. Inter- and intratumoral heterogeneity in malignant gliomas requires tailored therapies based on individual molecular signatures. This will allow maximal benefit and avoidance of toxicities in those who will not respond to a particular treatment but only develop side effects. Understanding molecular aspects of cancer may be essential to reach improved outcomes in this disease.

Disclosure Dr. Andrew B. Lassman has served on scientific advisory boards and/or consulted for Schering-Plough Corporation, Sigma-Tau Pharmaceuticals, Bristol-Myers Squibb, ImClone Systems, Genentech, Eisai, Cephalon, and Enzon Pharmaceuticals. He also serves on the editorial board of the Journal of Neuro-Oncology and the speakers’ bureau for Schering-Plough Corporation.

No other potential conflicts of interest relevant to this article were reported.

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


23. Friedman HS, Prados MD, Wen PY, et al.: Bevacizumab alone and in combination with irinotecan in recurrent glioblastoma. J Clin Oncol 2009, 27:4733–4740. This is the study that led to accelerated approval by the US Food and Drug Administration of bevacizumab for recurrent GBM.


