Use of bevacizumab in recurrent glioblastoma

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Practice points

Indications & usage

- Bevacizumab is US FDA approved for the following indications:
- Metastatic colorectal cancer;
- Recurrent non-small-cell lung cancer;
- Metastatic renal cell carcinoma;
- Recurrent glioblastoma (GBM);
- Metastatic cervical cancer.
- This paper reviews the use of bevacizumab in recurrent GBM and the Phase II clinical trials that involved bevacizumab monotherapy and bevacizumab-containing regimens.

Dosage & administration

- Bevacizumab is administered at 10 mg/kg intravenously every 2 weeks.
- Initial infusion is over 90 ± 10 min.
- Subsequent infusion can range from 30 to 60 min ± 10 min.

Clinical pharmacology

- Bevacizumab is a humanized monoclonal antibody to VEGF.
- Mechanisms of antitumor action include reduction in tumor blood supply, normalization of tumor vasculature and inhibition of tumor vascularization.
- Resistance to bevacizumab can develop and can lead to a pattern of disease progression characterized by nonenhancing disease on MRI imaging.
- Terminal half-life is 13 days and time to reach steady state is 100 days.

Clinical evidence

- Phase II clinical trials utilizing bevacizumab-containing regimens improved 6-month progression-free survival to 42.6–50.3% in comparison to historical Phase II studies in recurrent GBM at usually 9–16%.
- Based on clinical data from the BRAIN study, bevacizumab monotherapy was FDA approved for recurrent GBM.
- Addition of further chemotherapy or signaling agents has not improved survival in comparison to bevacizumab monotherapy.
- Use of concomitant stereotactic radiosurgery or devices such as NovoTTF-100A is possible future considerations for adjuncts with bevacizumab in recurrent GBM.

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Practice points (cont.)

Adverse reactions

- Common adverse events include fatigue, hypertension and proteinuria.
- Of note, development of hypertension during bevacizumab is a potential surrogate marker for a positive response to therapy.
- Serious but rare adverse events can include hemorrhage, epistaxis, venous thromboembolism (deep venous thrombosis or pulmonary embolism), arterial thrombotic event (cardiovascular and cerebrovascular), congestive heart failure, (left ventricular systolic dysfunction), gastrointestinal perforation, perforation, fistula formation, wound dehiscence and reversible posterior leukoencephalopathy.

Drug interactions

- No significant interactions with drug metabolism.
- Concomitant use with prothrombotic agents can lead to an additive risk of venous and arterial thrombosis.

Use in special populations

- Recurrence after bevacizumab initiation is challenging to treat and clinical trials have not seen a significant improvement in survival.
- Discontinuation of bevacizumab after recurrence can lead to a rapid clinical decline and radiographic progression.
- Timing for the initiation and discontinuation of bevacizumab in recurrent GBM remains to be determined.

SUMMARY Glioblastoma (GBM) is the most common adult primary brain neoplasm. Despite advances in treatment, GBM continues to be associated with considerable morbidity and mortality as compared with other malignancies. Standard treatment for GBM results in survival of 12.9 months (95% CI: 12.3–13.7 months) with a median progression-free survival of 7.2 months (95% CI: 6.4–8.2 months) in a modern GBM cohort. These aggressive tumors recur and treatment for recurrent GBM continues to have very poor outcomes. Prior to the use of bevacizumab, monoclonal antibody to VEGF, 6-month progression-free survival in clinical trials for recurrent GBM ranged from 9 to 15%. Trials utilizing bevacizumab and its subsequent US FDA approval have given more hope to recurrent GBM and this concise review discusses bevacizumab in recurrent GBM. This review focuses on time-to-event outcomes (overall survival, progression-free survival and 6-month progression-free survival) in clinical trials utilizing bevacizumab for the treatment of recurrent GBM. For this review, we have chosen to focus primarily on Phase II clinical trials that have been published and available in the literature (PubMed). While we focused primarily on time-to-event variables, toxicity and safety of bevacizumab is very important and this agent can be associated with serious life-threatening toxicities. We have included a general section of toxicities but for a more lengthy review please see the excellent study by Odia and colleagues.

KEYWORDS

- bevacizumab
- glioblastoma • Phase II
- recurrent

Despite advances in the care and treatment of malignant gliomas, median survival remains dismal at less than 20 months [1]. Current standard of care follows the Stupp regimen which includes: chemoradiation with daily temozolomide (75 mg/m² for 6 weeks) followed by six cycles of 5 day temozolomide (dosed at 150–200 mg/m² for 5 days of 28 day cycle) [2]. Malignant gliomas express VEGF on their cell surface [3,4]. It has been suggested that VEGF

is a marker of prognosis with more expression of VEGF portending a worse prognosis [5,6]. In mouse xenograft studies, the growth of malignant gliomas has been inhibited by monoclonal antibodies to VEGF [7]. As a humanized monoclonal IgG1 antibody, bevacizumab acts to bind to human VEGF and inhibits its activity. Bevacizumab's efficacy and tolerability have been studied in a variety of tumors, including glioblastoma (GBM) [8–12].

Indications & usage

Bevacizumab has been used for a variety of cancers as standard therapy or through investigational use on Phase I, II and III clinical trials. In February 2004, bevacizumab received US FDA approval for use in metastatic colorectal cancers for first-line treatment in combination with 5-FU based chemotherapy regimens. The FDA approval was based on results from a randomized Phase III study evaluating patients with metastatic colorectal cancer receiving bevacizumab + irinotecan, fluoruracil and leucovorin (IFL) versus IFL therapy without bevacizumab. Median overall survival (OS) was 20.3 months versus 15.6 months in the bevacizumab + IFL and placebo + IFL groups, respectively [11].

In April 2006, bevacizumab received FDA approval for use in non-small-cell lung cancer following the results from the E4599 randomized Phase III trial evaluating patients with recurrent or advanced non-small-cell lung cancer treated with bevacizumab in combination with paclitaxel and carboplatin versus paclitaxel and carboplatin without bevacizumab [13]. In the chemotherapy + bevacizumab group, the OS was 12.3 months and median progression-free survival (PFS) was 6.2 months. In the chemotherapy alone group, the median OS was 10.3 months and median PFS was 4.5 months [13].

Bevacizumab received further indications in 2009 with the FDA approval for use in recurrent GBM and metastatic renal cell carcinoma. For this manuscript, we will explore in detail the Phase II clinical trials in recurrent GBM and will focus on time-to-event outcomes (6-month PFS, PFS and OS). FDA approval for metastatic renal cell carcinoma followed results from AVOREN randomized Phase III study evaluating previously untreated patients with metastatic renal cell carcinoma receiving IFN- α -2a with or without bevacizumab [14,15]. Results included median OS for bevacizumab + interferon arm and interferon-only group which were 23.3 and 21.3 months, respectively [14,15].

The approval of bevacizumab for breast cancer has been controversial as it has been revoked of its FDA-approved status. In 2008, the FDA gave accelerated approval for the use of bevacizumab in chemotherapy-naïve patients with metastatic HER2-negative breast cancer. Bevacizumab use was approved for combination therapy with paclitaxel following the results of a Phase III E2100 trial which compared paclitaxel

alone versus paclitaxel combined with bevacizumab [16]. Of the 722 patients studied, the median PFS for paclitaxel alone and paclitaxel + bevacizumab were 5.9 and 11.8 months, respectively. The median OS did not show improvement in the combination therapy group [16]. Following this accelerated approval, the AVADO and RIBBON 1 study were both performed to validate bevacizumab's efficacy on PFS. Unfortunately, neither study was able to replicate the PFS found in the E2100 trial, which contributed to the FDA revoking the approval status for bevacizumab in metastatic breast cancer [17,18].

More recently, the FDA approved the use of bevacizumab in metastatic cervical cancer (in 2014) following the results of the GOG 240 randomized Phase III trial evaluating combination chemotherapy (either cisplatin and paclitaxel or topotecan and paclitaxel with or without bevacizumab). The addition of bevacizumab to combination chemotherapy resulted in an additional 3.7 months to median OS [19].

Therefore, bevacizumab has utility in multiple forms of cancer, in particular metastatic/recurrent cancers. Results from the breast cancer studies and subsequent revocation of FDA approval bring into question the best end points for evaluation of bevacizumab's efficacy. There is similar controversy in the primary brain tumor community and warrants further discussion.

Dosage & administration

Bevacizumab is supplied in 5cc (100 mg) and 20cc (400 mg) glass vials, which contain 4 ml or 16 ml bevacizumab, respectively (all at 25 mg/ml). Vials contain no preservative and are suitable for single use only. Opened vials must be used within 8 h. Vials should remain refrigerated at 2–8°C (36–46°F) until just prior to use. Vials should be protected from light and care must be given to prevent freezing or shaking of the vials.

Bevacizumab is administered at 10 mg/kg every 2 weeks. In certain circumstances, bevacizumab can be dosed at 15 mg/kg every 3 weeks. Bevacizumab is diluted in 0.9% sodium chloride injection to a total volume of 100 ml. Bevacizumab is administered as a continuous intravenous (iv.) infusion. Initial dose of bevacizumab is infused over 90 $\pm$ 10 min. The second dose is infused over 60 $\pm$ 10 min. Subsequent infusions are administered over 30 $\pm$ 10 min.

Clinical pharmacology

• Mechanism of action

Bevacizumab is a humanized monoclonal IgG1 antibody with anti-VEGF properties. GBMs are highly vascularized tumors which have over-expression of VEGF. It has been widely presumed that VEGF is the primary growth factor responsible for tumor angiogenesis. Angiogenesis is used by tumor cells; forming new blood vessels from pre-existing vasculature which provides tumor cells access to nutrients and oxygen. VEGF inhibitors are antiangiogenic agents with several proposed mechanisms of action. First, anti-VEGF therapy is believed to decrease tumor blood supply and may act to sensitize tumor endothelial cells to cytotoxins [20–24]. The second potential mechanism of anti-VEGF therapy is normalization of tumor vasculature allowing improved chemotherapy and radiation effects [25]. With this normalization of tumor vasculature, it has been shown that this change in hydrostatic pressure leads to increase transport of large molecules into tumor tissue [26]. Synergistic cell kill and tumor growth inhibition was seen in *in vivo* studies when bevacizumab was combined with doxorubicin, topotecan, paclitaxel and doxetaxel [27–30]. *In vitro* and *in vivo* models have shown that delivery of antiangiogenic agents, including bevacizumab, with radiation promotes endothelial cell apoptosis and a reduction in tumor blood vessel formation which both led to abrogation of tumor growth [31–33]. The latter mechanism is based on the proposed abnormal structure and functioning of tumor blood vessels. A third additional effect of anti-VEGF therapy is continued inhibition of tumor vascularization [34].

Inhibition of VEGF by antiangiogenics such as bevacizumab in GBM has been considered one of the first promising step toward targeted therapy in this disease, but resistance to antiangiogenics can develop in GBM and perhaps leading to very difficult to treat disease [35]. Loss of a dependence on VEGF signaling leading to antiangiogenic resistance may even lead to a more aggressive tumor phenotype [35]. Mouse models have shown that VEGF inhibition can lead to increased tumor invasion [36]. Clinically, this behavior is demonstrated by the development of invasive nonenhancing tumor progression on MRI imaging and this has been shown to occur in 35–57% of progressive cases [37,38]. This nonenhancing progression on MRI

imaging is not the only pattern that can be seen on progression as new enhancing disease and distant disease can also be identified as patterns of progression [37,38]. The specific mechanism by which VEGF inhibition leads to tumor invasiveness is still unclear, but upregulating of receptor tyrosine kinase MET signaling by VEGF inhibition could play a role leading to more aggressive tumor phenotype, in particular a more invasive tumor [39].

In addition to promotion of a more invasive tumor phenotype, antiangiogenic resistance can arise from accessory modes of neovascularization. The growth of tumor vasculature is controlled by angiogenesis and vasculogenesis, but tumor cells that have been exposed to VEGF inhibition can have further growth promotion through the development of alternative vascular channels through the process of vasculogenic mimicry [40]. In this process, glioma stem-like cells thought to promote the development of vasculogenic mimicry such that vessel-like channels that support the recurrent tumor. The mechanics of this mechanism are unclear but glioma stem-like cells (CD133-positive cells) are thought to be responsible for the promotion of behavior [41,42]. These glioma stem-like cells may not only promote vasculogenic mimicry but have the potential to development into endothelial cells leading to tumor progression and growth [43]. *In vitro* and *in vivo* studies have shown that glioma-stem-like cells can develop into endothelial cells dependent and independent of VEGF signaling [44,45]. It is this VEGF-independent signaling that could represent another mechanism for bevacizumab resistance.

Much attention has been paid to concomitant use of bevacizumab with radiation and chemotherapy, but *in vitro* and *in vivo* observations suggest that concomitant use is not necessarily recommended. In work by Arjaans and colleagues, they found that normalization of tumor vasculature by bevacizumab can, in fact, decrease the uptake of monoclonal antibody such as trastuzumab, monoclonal antibody to HER2/Neu, in murine tumor xenograft [46]. Therefore, design of clinical trials should be done carefully to consider bevacizumab's possible effect on tumoral distribution of therapeutic monoclonal antibodies.

• Pharmacodynamics

As an antibody to VEGF, bevacizumab was shown to inhibit tumor angiogenesis in tumor

mice models and this in turn led to an inhibition of tumor growth [47].

• Pharmacokinetics

Terminal half-life of bevacizumab is 13 days [48]. Time to reach steady state is 100 days. Discontinuation results in slow elimination over several months. There is no available antidote for bevacizumab. Clearance of concomitant chemotherapy is not influenced by bevacizumab [49].

Clinical evidence

• Overview of clinical trials

For this manuscript, we focused on Phase II clinical trials utilizing bevacizumab monotherapy or in combination with other therapies (Table 1). In 2007, Vredenburgh *et al.* published data from two Phase II trials, which concluded acceptable toxicity and efficacy in the combination therapy of bevacizumab and irinotecan in recurrent malignant glioma. The first study assessed 32 patients receiving bevacizumab and

Table 1. Phase II studies of bevacizumab monotherapy or combination chemotherapy in recurrent glioblastoma.

Study (year)	Agent	Median progression-free survival, time (95% CI)	6-month progression-free survival, % (95% CI)	Median overall survival, time (95% CI)	Ref.
Bevacizumab-naïve recurrent glioblastoma					
Vredenburgh <i>et al.</i> (2007)	Bevacizumab + irinotecan	20 weeks	30% (16–57%)	40 weeks	[50]
Vredenburgh <i>et al.</i> (2007)	Bevacizumab + irinotecan	24 weeks (18–36 weeks)	46% (32–66%)	42 weeks (35–60 weeks)	[51]
Friedman <i>et al.</i> (2009)	Bevacizumab monotherapy	4.2 months (2.9–5.8 months)	42.6% (29.6–55.5%)	9.2 months (8.2–10.7 months)	[10]
	Bevacizumab + irinotecan	5.6 months (4.4–6.2 months)	50.3 (36.8–63.9%)	8.7 months (7.8–10.9 months)	
Reardon <i>et al.</i> (2009)	Bevacizumab + etoposide	18 weeks (13–40 weeks)	44.4% (26–62%)	46.4 weeks (25–70 weeks)	[54]
Hasselbalch <i>et al.</i> (2010)	Bevacizumab + cetuximab + irinotecan	16 weeks (13–20 weeks)	33% (19–48%)	20 weeks (23–37 weeks)	[55]
Sathornsummette <i>et al.</i> (2010)	Bevacizumab + erlotinib	18 weeks (12.0–23.9 weeks)	29.2% (13.0–47.6%)	44.6 weeks (28.4–68.7 weeks)	[56]
Reardon <i>et al.</i> (2012)	Bevacizumab + carboplatin + irinotecan	Not applicable	46.5% (30.4–61.0%)	8.3 months (5.9–10.7 months)	[57]
Lassen <i>et al.</i> (2013)	Bevacizumab + temsirolimus	8 weeks	Not applicable	15 weeks	[58]
Desjardins <i>et al.</i> (2012)	Bevacizumab + daily temozolomide	15.8 weeks	18.8% (7.6–33.7%)	37 weeks	[59]
Soffietti <i>et al.</i> (2014)	Bevacizumab + fotemustine	5.2 months (3.8–6.6 months)	42.6% (29.3–55.2%)	9.1 months (7.3–10.3 months)	[60]
Taal <i>et al.</i> (2014)	Bevacizumab monotherapy	3 months (3–4 months)	16% (7–27%)	8 months (6–9)	
	Lomustine monotherapy	1 month (1–3 months)	13% (5–24%)	8 months (6–11)	
	Bevacizumab + lomustine (110 mg/m ²)	11 months (1–27 months)	50% (15–77%)	16 months (2–34)	
	Bevacizumab + lomustine (90 mg/m ²)	4 months (3–8 months)	41% (26–55%)	11 months (8–12 months)	
Lee <i>et al.</i> (2015)	Bevacizumab + panobinostat	5 months (3–9 months)	30.4% (12.4–50.7%)	9 months (6–19 months)	[61]
Bevacizumab recurrent glioblastoma					
Kreisl <i>et al.</i> (2009)	Bevacizumab + irinotecan	30 days	N/A	N/A	[52]
Reardon <i>et al.</i> (2011)	Bevacizumab + carboplatin + irinotecan	2.3 months (1.8–3.6 months)	16% (5.0–32.5%)	5.8 months (4.0–7.0 months)	[62]
Reardon <i>et al.</i> (2011)	Bevacizumab + daily temozolomide	4.1 weeks (3.0–7.9 weeks)	0%	12.6 weeks (4.6–23.3 weeks)	[63]
	Bevacizumab + etoposide	8.1 weeks (4.1–12.0 weeks)	7.7% (4.8–29.2%)	19.0 weeks (11.0–25.7 weeks)	

irinotecan (recurrent grade III-IV gliomas with 23 [72%] having GBM) [50]. It is important to note that this trial's primary end point was response rate with a benchmark from previous trials on recurrent glioma had response rates of <10%. For this study, 63% (n = 20) had a radiographic response with one complete response and 19 partial responses recorded. For patients with recurrent GBM, 6-month PFS was 30% (95% CI: 16–57%) with PFS of 20 weeks and OS was 40 weeks [50]. Important toxicities included three thromboembolic events (two pulmonary emboli and two deep venous thromboses) and one cerebrovascular accident [50]. The second study focused on only recurrent WHO grade IV assessed 35 recurrent GBM patients receiving bevacizumab and irinotecan which revealed similar results to the previous mentioned study with PFS at 6 months being 46% (95% CI: 32–66%) and OS being 42 weeks (95% CI: 35–60 weeks) [51]. Similar to previous study, there was a purported increased risk of thromboembolism with four patients experiencing either deep venous thrombosis and/or pulmonary emboli [51]. It is also important to note that one patient experiencing an intracranial bleed associated with this treatment [51]. Monotherapy versus combination therapy was evaluated further in the BRAIN study and by Kreisl and colleagues [10,52]. The BRAIN study concluded that bevacizumab was active and well tolerated, when used either alone or in combination with irinotecan [10]. These conclusions were based on 42.6 and 50.3% 6-month PFS in the bevacizumab alone and the bevacizumab + irinotecan groups, respectively, with no statistically significant difference between bevacizumab monotherapy and combination with irinotecan [10]. Serious grade 3–4 toxicities for both treatment groups included fatigue (n = 10), venous thromboembolism (n = 10), hypertension (n = 8), arterial thromboembolism (n = 4), wound-healing complications (n = 3), gastrointestinal perforation (n = 2) and intracranial hemorrhage (n = 1) all of which have the potential for life-threatening complications [10]. Kreisl and colleagues evaluated addition of irinotecan in recurrent GBM after a first recurrence on bevacizumab monotherapy thereby studying the utility of chemotherapy in combination with bevacizumab [52]. Prior to recurrence on bevacizumab, 6-month PFS was similar to Vredenburgh and colleagues with 29% (95% CI: 18–48%) [52]. Yet, addition of irinotecan after progression did

not improve outcomes with disease progression occurring in 95% of patients by the second cycle of bevacizumab and irinotecan [52]. Therefore, bevacizumab alone rather than in combination with other agents was viewed as a compelling therapy for recurrent GBM. In Kreisl *et al.* clinical trial, venous thromboembolism remained the key toxicity associated with discontinuation of study regimen [52]. Five thromboembolic events were recorded and of note, one bowel perforation occurred in the study with no grade 5 events. These studies led to the FDA granting an accelerated approval to bevacizumab monotherapy for the treatment of recurrent GBM [53].

Following the FDA approval of single agent bevacizumab for recurrent GBM, there have been numerous clinical trials assessing the use of bevacizumab in combination with chemotherapy for recurrent GBM. Of importance, more recent studies evaluating PFS use MRI criteria deemed to be more sensitive than the previous McDonald Criteria. The BRAIN study employed the McDonald Criteria, whereas newer studies use the Response Assessment in Neuro-Oncology (RANO) criteria for assessing disease progression. There are several differences among the two criteria, but the major difference revolves around the RANO criteria using FLAIR imaging to measure nonenhancing tumor [10,64–66]. Early trials such as Vredenburgh and colleagues had utilized objective radiographic response as pivotal outcome, but the observation that bevacizumab can lead to a pseudoresponse on imaging making radiographic response a less desirable end point [50,51,67]. Pseudoresponse can be described as a demonstrable change in enhancement on MRI scan that in fact does not reflect a tumor reduction in tumor growth but rather a change in vascular permeability. Controversy continues to exist in regards to the best end point for bevacizumab-related studies in GBM but time-to-event end points such as OS, PFS and 6-month PFS are generally acceptable [67].

Results from the BELOB study have been recently published and it is a Phase II study randomized trial in recurrent GBM employing use of lomustine, lomustine + bevacizumab or bevacizumab monotherapy [68]. The trial analyzed 50 patients receiving bevacizumab alone, 46 receiving lomustine (CCNU) alone and 44 patient receiving bevacizumab + CCNU. The study assessed 9-month OS which was found to be 38% in the bevacizumab alone group, 43% in the CCNU alone group, 59% in the

bevacizumab + CCNU (90 mg/m²) group and 87% in the bevacizumab + CCNU (110 mg/m²) group. Findings of the BELOB trial, prompted the authors to suggest an EORTC Phase III trial randomizing patients to either CCNU alone or bevacizumab + CCNU will be needed [68]. While the higher-dose of CCNU (110 mg/m²) did have better results over the lower dose of CCNU (90 mg/m²), the presence of increased toxicity (resultant cytopenias) in the higher dose group warranted a reduction to 90 mg/m² by the study investigators. Common grade 3 toxicities seen in this study related to bevacizumab monotherapy included hypertension with 13 patients (26%) experiencing this problem [68]. Another Phase II study has been conducted by the Italian Association of Neuro-Oncology using another nitrosourea, fotemustine, in combination with bevacizumab in recurrent GBM [60]. The combination of fotemustine + bevacizumab had a 6-month PFS of 42.6% (95% CI: 29.3–55.2%) and this was not a statistical improvement in PFS when compared to data from historical controls on bevacizumab alone [60]. Important serious toxicities included two grade 4 pulmonary emboli, one grade 4 cerebrovascular accident, one grade 4 hypertension and one death due to intracranial hemorrhage. This again highlights the common serious toxicities seen with bevacizumab.

Other alkylating agents in combination with bevacizumab have been utilized in Phase II studies and have included temozolomide and carboplatin. Temozolomide has been evaluated in the recurrent GBM setting, especially in patients with prior dose related adverse effects related to dose dense temozolomide. A Phase II study, published in 2012, evaluated 32 patients treated with temozolomide 50 mg/m² daily and bevacizumab 10 mg/kg every 2 weeks [59]. The PFS was 15.8 weeks and median OS was 37 weeks. The study concluded that combination therapy with metronomic temozolomide and bevacizumab had adequate tolerability but the addition of temozolomide did not improve time-to-event outcomes with 6-month PFS being 18.8% (95% CI: 7.6–33.7%) in comparison to bevacizumab monotherapy [59]. Although study results were inferior to historical studies of single agent bevacizumab, it should be noted that the authors suggested one reason for the inferiority may have been due to patient population studied. In the study, patients were accepted without limits on the number of prior

progressions or the type of regimen previously used [59]. Serious toxicities in this study included prolong thrombocytopenia (presumably due to temozolomide) and grade 4 pancreatitis [59]. An interesting study performed by Reardon and colleagues evaluated bevacizumab in combination with carboplatin and irinotecan [57]. The bevacizumab-naïve Phase II study evaluated 40 recurrent GBM patients treated with carboplatin AUC 4 intravenously every four weeks with bevacizumab and irinotecan delivered intravenously every two week. The 6-month PFS was 46.5% (95% CI: 30.4–61.0%) and the median OS was 8.3 months (95% CI: 5.9–10.7 months). However, 11 patients (28%) discontinued the study due to toxicity with another 17 patients (43%) requiring dose modification. Presumably due to carboplatin and irinotecan the most common dose reductions were due to cytopenias. Moreover, one patient died due to gastrointestinal perforation. In conclusion, bevacizumab in combination with carboplatin and irinotecan showed a significant increase in toxicity without improvement in antitumor activity compared with single-agent bevacizumab in the bevacizumab-naïve patients, in the recurrent GBM setting [57].

Etoposide, inhibitor of topoisomerase II, is often used as salvage chemotherapy in patients with recurrent GBM. In 2009, Reardon *et al.* published analysis from a Phase II trial of recurrent malignant glioma patients treated with combination therapy consisting of bevacizumab 10 mg/kg every 2 weeks and etoposide 50 mg/m² daily for 21 days (7 days off) [54]. Among the 27 patients with GBM, the 6-month PFS was 44.4% (95% CI: 13–40%) and median OS was 44.4 weeks (95% CI: 25–70 weeks). Common serious toxicities included thromboembolic disease (grade 4–5, n = 3) with one death attributable to pulmonary embolism. Significant grade 2 toxicities attributable to etoposide were seen and included anemia and neutropenia. Unfortunately, this study had similar conclusions as did other studies of chemotherapy combined with bevacizumab such that the addition of chemotherapy did not improve survival over bevacizumab monotherapy but rather increased toxicity [54].

GBM commonly have overexpression or amplification of EGF receptor (EGFR) and use of inhibitors of EGFR has been studied as a possible provocative agent in recurrent GBM. Erlotinib, EGFR tyrosine kinase inhibitor has been studied

in combination with bevacizumab. A Phase II study evaluated combination therapy with bevacizumab 10 mg/kg every 2 weeks and erlotinib dosed at either 200 mg/day or 500 mg/day in patients with recurrent malignant glioma [56]. Dosing of erlotinib was dependent on concurrent use of enzyme-inducing antiepileptic drugs (EIAEDs) with higher dose used for patients on EIAEDs. Among the 25 patients with GBM, the 6-month PFS was 28% (95% CI: 13–47.6%) and median OS was 42 weeks (95% CI: 28.4–68.7 weeks). The study concluded that the combination therapy was adequately tolerated and provided similar PFS benefit when compared with historical bevacizumab containing therapy regimens [56]. Cetuximab, a monoclonal antibody to EGFR, has been studied in combination with bevacizumab and irinotecan. In a Phase II study by Hasselbalch and colleagues, recurrent GBM patients were treated with a combination of cetuximab, irinotecan and bevacizumab [55]. In this study, the 6-month PFS was 33% (95% CI: 19–48%) and median OS was 30 weeks (95% CI: 23–37 weeks). Therefore, despite the molecular importance of EGFR signaling in GBM, the addition of EGFR inhibitors when combined with bevacizumab did little to change outcomes in recurrent GBM.

Novel agents thought to enhance antiangiogenic action have been evaluated in Phase II clinical trials with bevacizumab. For example, Lassen and colleagues performed a Phase II study evaluating bevacizumab + temsirolimus, mTOR inhibitor, in recurrent GBM patients [58]. Patients were given temsirolimus 25 mg iv. on days 1 and 8 with bevacizumab 10 mg/kg iv. on day 8 every 2 weeks. Unfortunately, the trial was terminated early, after accrual of only 13 patients due to no evidence of responses. The median 6-month PFS was 8 weeks and 6-month OS was 15 weeks [58]. Use of histone deacetylase inhibitors has been evaluated in combination with bevacizumab and this included panobinostat [61]. In this study by Lee and colleagues, panobinostat, a histone deacetylase inhibitor, in combination with bevacizumab did not improve 6-month PFS in comparison to historical controls on bevacizumab alone. In this study, 6-month progression-free survival was 30.4% (95% CI: 12.4–50.7%) at interim analysis and in light of these results this study did not meet criteria for continuation [61].

On the basis of preclinical studies suggesting the radio-sensitizing effects of VEGF inhibition,

Gutin and colleagues assessed the use of bevacizumab with concurrent reradiation in patients with recurrent malignant gliomas [69]. In this study, 20 recurrent GBM patients received bevacizumab in combination with 30 Gy of hypofractionated stereotactic radiotherapy in five fractions. The 6-month PFS was 65% (95% CI: 40–82%) and OS was 12.5 months (95% CI: 6.9–22.8 months). They concluded that the use of bevacizumab with hypofractionated stereotactic radiotherapy is safe and well tolerated in patients with recurrent GBM [69].

Delivery of bevacizumab is usually administered every 2 weeks, but alternate schedules of delivery and modes of delivery have been explored. Raizer and colleagues published a Phase II trial of single agent bevacizumab dosed 15 mg/kg every 3 weeks for patients with recurrent GBM [70]. Of the 50 patients with GBM, the 6-month PFS was 25% and median OS was 25.6 weeks. Raizer and colleagues concluded that single agent bevacizumab dosed 15 mg/kg every 3 weeks showed benefit with tolerable adverse effects for recurrent patients. The study is important since it supports the use of bevacizumab dose at 15 mg/kg every 3 weeks, which is employed by physicians when patients have difficulty tolerating the typical 10 mg/kg every 2 weeks dosing [70]. The mode of delivery of bevacizumab is intravenous but some interesting studies have shown that intra-arterial delivery of bevacizumab could be safely done in patients with recurrent GBM [71]. Burkhardt and colleagues published a study of 14 recurrent GBM patients treated with intra-arterial bevacizumab [72]. In this study, median PFS was 10 months with OS was 8.8 months (four patients passed without progression) and based on these results there are ongoing studies to investigate this interesting mode of bevacizumab delivery [72].

Recent data using the NovoTTF-100A system, a novel antimitotic therapy utilizing alternating tumor treating fields, have shown efficacy in the recurrent GBM population [73]. In an article published by Mrugala and colleagues detailing outcomes from a patient registry dataset (PRiDe), NovoTTF-100A lead to median OS of 9.6 months in a group of community-treated patients in comparison to 6.6 months in the randomized Phase II clinical trial (EF-11) [73]. Interestingly since these patients were not treated under the auspices of a clinical trial, bevacizumab could be given concurrently with

NovoTTF-100A [73]. Therefore in light of these findings with NovoTTF-100A, it will be important to evaluate this device in combination with bevacizumab in future clinical trials.

In conclusion, bevacizumab monotherapy appears to have antitumor activity in Phase II clinical trials in recurrent GBM. Important care should be paid to common serious toxicities including but not limited to venous thromboembolism, cerebrovascular accidents, gastrointestinal perforation and intracerebral hemorrhage. Unfortunately, combination of bevacizumab with traditional chemotherapeutic and novel agents has not improved outcomes over bevacizumab alone. Despite this lack of synergy with chemotherapy, there is room for positive consideration for combination with radiation therapy and even novel therapies such as intra-arterial therapy and devices such as NovoTTF-100A.

Adverse reactions

From the analysis of bevacizumab-related toxicities in three large National Cancer Institute Phase II clinical trials in 210 patients, fatigue, hypertension and proteinuria were the two most common adverse reactions occurring at 26.7, 25 and 25%, respectively [74]. Interestingly, Zhong and colleagues have shown that development of hypertension during bevacizumab therapy for recurrent GBM is associated with a better outcome [75]. In fact, patients that remained normotensive had a median PFS of 2.5 months whereas patients that developed hypertension had a median PFS of 6.7 months [75]. Hematologic adverse events associated with bevacizumab monotherapy included thrombocytopenia at 12.5% but this was associated more frequently with prolonged use of the agent and history of combination with other chemotherapeutic agents. Toxicity associated with bevacizumab appears to be cumulative with increased frequency of adverse events if the agent is continued for greater than 24 months. Other reported common toxicities include hoarse voice, dry skin/nares and joint pain. In general, bevacizumab is well tolerated and can be given concomitantly with other chemotherapeutic agents. Rare but serious adverse events have been reported and can include the following: hemorrhage, epistaxis, venous thromboembolism (deep venous thrombosis or pulmonary embolism), arterial thrombotic event (cardiovascular and cerebrovascular), congestive heart failure (left ventricular systolic

dysfunction), gastrointestinal perforation, perforation, fistula formation, wound dehiscence and reversible posterior leukoencephalopathy. Particular care should be taken in recurrent GBM patients that are on concomitant corticosteroids and bevacizumab as this combination can lead to ulcerated striae distensae and gastrointestinal perforation of peptic ulcer disease or diverticulitis [76–79].

Some patients may experience an infusion-associated adverse event characterized as development of dyspnea or clinically significant hypotension. In addition, patients may experience an allergic reaction/hypersensitivity, adult respiratory distress syndrome or bronchospasm.

During treatment with bevacizumab, blood pressure is monitored routinely with optimal blood pressure control to follow normotensive blood pressure ranges. Patients are monitored by urine dipstick or 24-h urine at least every 4 weeks, to monitor for proteinuria. If patients require elective major surgery, bevacizumab should be held for 6 weeks prior to the surgical procedure. Following major surgery, treatment with bevacizumab should not begin or restart until 4 weeks after the surgical procedure. However, bevacizumab should not begin or restart until 6 weeks after high risk procedures (i.e., neurosurgery, liver resection or thoracotomy). Of course these recommendations in regards to timing should be done on a case-by-case basis and with the close collaboration between treating medical providers.

Drug interactions

Bevacizumab does not have any specific interactions in regards to drug metabolism in patients. However, when bevacizumab is administered concomitantly with agents that are prothrombotic such as epoetin alfa and hormonal agents, there is an additive risk of venous and arterial thrombosis.

It is unknown whether or not bevacizumab crosses the placenta or secreted in human milk. Teratogenic effects have occurred in animals treated with bevacizumab. However, there have been no similar studies reproduced in humans. Current recommendations suggest weighing risks versus benefits before considering treatment of pregnant women and/or breast feeding mothers. Females of reproductive age should be notified of the increased risk of ovarian failure with bevacizumab use. The long-term effects on fertility are not known, but bevacizumab may

damage fertility and recovery may not occur after cessation of use.

There is not enough information to recommend use of bevacizumab in pediatric patients. Safety, efficacy and pharmacokinetics have not been determined. In elderly patients, there have been no reports suggesting decreased efficacy, but there is a trend toward more adverse effects in patients older than 65 years.

Use in specific populations

Most studies have been designed to evaluate recurrent GBM patients that are naive to bevacizumab. Unfortunately for patients that recur on bevacizumab, the disease is often very difficult to treat and can exhibit a very infiltrative pattern of progression (see the section ‘Mechanism of Action’) [35]. In fact, discontinuation of bevacizumab can often lead to dramatic tumor growth and rapid clinical deterioration [80]. Studies by Kreisl *et al.* and Reardon *et al.* have evaluated GBM patients that recurred on bevacizumab. In the Kreisl *et al.* study, recurrent GBM patients were treated with bevacizumab + irinotecan after tumor progression on single-agent bevacizumab [52]. The addition of irinotecan to bevacizumab regimen did not provide antitumor activity such that median PFS was only 30 days and a majority of patients (95%) had disease progression very early after the addition of irinotecan [52]. Reardon *et al.* study evaluated bevacizumab in combination with carboplatin and irinotecan for recurrent GBM patients after progression on bevacizumab therapy [62]. The Phase II study evaluated 25 recurrent GBM patients treated with carboplatin AUC 4 intravenously every four weeks with bevacizumab 10 mg/kg every 2 weeks and irinotecan dosed 340 mg/m² (on EIAEDs) or 125 mg/m² (not on EIAEDs) every 2 weeks in a 4-week cycle. All patients had at least one prior progression on bevacizumab with over half (56%) of patients having their second or third overall progression. The 6-month PFS was 16% and OS was 5.8 months. Nine patients (38%) ultimately required dose modification due to toxicity. The study concluded that the triple regimen therapy provided modest antitumor activity with adequate safety in recurrent GBM patients previously treated with bevacizumab [62]. In a similarly designed Phase II study by Reardon and colleagues, they evaluated use of either metronomic etoposide (50 mg/m² orally daily for 21 days in 28-day cycle) or temozolomide

(50 mg/m² daily for 28 days in 28-day cycle) with bevacizumab 10 mg/kg every 2 weeks [63]. While 52% of patients had a stable radiographic response, the 6-month PFS was poor at 4.4% (95% CI: 3.1–18.2%) [63]. In order to understand whether there is a role for continuation of bevacizumab failure in recurrent GBM, Reardon and colleagues evaluated the pooled outcomes of 99 recurrent GBM patients after failure on Phase II clinical trials utilizing bevacizumab containing regimens [81]. For GBM patients that remained on bevacizumab, median OS was 5.9 months (95% CI: 4.4–7.6 months) versus 4.0 months (95% CI: 2.1–5.4 months) for patients that had discontinued bevacizumab [81]. In this study, the results supported that continued use of bevacizumab after recurrence was an independent predictor of survival (HR = 0.64; *p* = 0.04). It is important to note that this does not represent a Phase II clinical trial but rather a practice-driven model from a single institution. Special care needs to be taken when interpreting these results and consideration of increased risk of toxicity is present as one continues to administer bevacizumab. Recurrent GBM patients that have already received bevacizumab are likely to remain a challenging population to treat and new strategies to improve outcomes are needed.

Conclusion

Because of the aggressive nature and biology of GBM, patients with this diagnosis are highly likely to develop recurrent disease. More randomized controlled trials are needed to evaluate other therapies in comparison to bevacizumab, but this, too is controversial because it remains to be seen if bevacizumab can truly improve OS. Well-designed studies such as BELOB study represent an appropriate and needed avenue to this line of inquiry and are likely to provide needed information on bevacizumab’s continued role in the treatment of recurrent GBM [68]. Furthermore, most clinical trials for recurrent GBM are designed for patients that are naive to bevacizumab. With the increased use of bevacizumab for recurrent disease in GBM with community oncologists, there is a great need for more studies to evaluate agents for treatment after bevacizumab failure and/or consideration of continuation of bevacizumab with other therapies.

Note

For further information of mechanism of action, dosage administration, pharmacodynamics and pharmacokinetics,

please see package insert for bevacizumab (http://www.gene.com/download/pdf/avastin_prescribing.pdf).

Disclosure

In addition to the peer-review process, with the author(s) consent, the manufacturer of the product(s) discussed in this article was given the opportunity to review the manuscript for factual accuracy. Changes were made at the discretion of the author(s) and based on scientific or editorial merit only.

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