Review

A Review of Dose-dense Temozolomide Alone and in Combination with Bevacizumab in Patients with First Relapse of Glioblastoma

FREDRIK JOHANSSON[1], SIMON EKMAN[1], ERIK BLOMQUIST[1], ROGER HENRIKSSON[2], STEFAN BERGSTRÖM[3] and MICHAEL BERGQVIST[1]

[1]Section of Oncology, Department of Radiology, Oncology and Radiation Sciences, Uppsala University, Uppsala, Sweden;
[2]Department of Radiation Sciences and Oncology, Umeå University Hospital, Umeå, Sweden;
[3]Department of Oncology, Karolinska University Hospital, Stockholm, Sweden;
[4]Department of Oncology, Gävle Hospital, Gävle, Sweden

Abstract. *Treatment of patients with glioblastoma improved dramatically when concomitant and adjuvant temozolomide was added to external radiation therapy. The addition of this new treatment schedule as well as the improvements in individually-tailored radiation treatment, has resulted in a larger proportion of patients being fit for further treatment after first relapse. One of the most interesting combinations that have started to become part of the therapeutic arsenal in the daily clinic is dose-dense temozolomide in combination with bevacizumab. We reviewed and compiled the literature concerning the present topic based on a search of the PubMed database (http://www.ncbi.nlm.nih.gov/pubmed/) for the years between 1995 and 2011. The clinical studies that have been performed are small and divergent, making it difficult to grade the scientific evidence for the combinatorial treatment of dose-dense temozolomide and bevacizumab. However, the available studies and the experience we have at our departments suggest that this combination is of interest for glioblastoma patients experiencing first relapse. More randomized clinical trials are needed in order to establish the standard of treatment at first relapse in patients with glioblastoma.*

Correspondence to: Associate Professor Michael Bergqvist, MD, PhD, Department of Oncology, Entrance 78, Uppsala University Hospital, S-751 85 Uppsala, Sweden. Tel: +46 186115534, Fax: +46 186115528, e-mail: Michael.Bergqvist@onkologi.uu.se

In Sweden, approximately 400 new cases of glioblastoma [high-grade (IV) malignant glioma] are diagnosed each year. The majority of patients are diagnosed at around 60 years of age and the median survival is 12 to 14 months (1, 2). Current standard treatment consists of debulking surgery followed by radiotherapy combined with chemotherapy with temozolomide. Novel therapies at relapse have been studied intensely, but most often without any significant clinical improvement. In a study including eight phase II trials on chemotherapy in recurrent high-grade glioma, an overall response rate of 9% was shown. Progression-free survival (PFS) at 6 months was reported to be 21% and the median overall survival (OS) was 30 weeks (3). Recently, a combination of bevacizumab and irinotecan led to an impressive response rate (approximately 30%) (4).

Current Standard Treatment

The recommended standard treatment has long included surgery and radiation therapy. Traditionally, chemotherapy has been saved for second-line treatment. Surgery is usually performed soon after initial radiological diagnosis, while its aim is to obtain material for a conclusive diagnosis, as well as ro remove the tumor mass. If extensive surgery is not possible, at least a biopsy can most often be obtained to establish a histological diagnosis. Surgery is then usually followed by external radiation therapy. Despite intensive research in the field, further advancement in prolonging survival was not successful until it was shown that the oral chemotherapeutic agent temozolomide, used concomitantly with radiotherapy and in the adjuvant setting, significantly prolonged median survival from 12.1 to 14.6 months for

patients with newly diagnosed glioblastoma (5). In the 5-year analysis, 9.8% of patients in the temozolomide arm were still alive, while in the control arm this figure was only 1.9% (6).

Treatments at Relapse

Previously, chemotherapy was used only at relapse, which invariably occurs. The most studied and recommended treatments were historically based on nitrosourea, often lomustine, given in combination with procarbazine and vincristine (PCV) (7). Several other chemotherapeutic combinations have been investigated, resulting in an objective response rate of less than 10% and a PFS of 2-3 months independently of which drug was being tested (3). Until 2009, no significant advances were seen in the relapse setting, but then a combination of bevacizumab and irinotecan suggested a significant clinical improvement (4). In a recent phase III study (8), 447 patients with high-grade glioma at relapse were randomly assigned to six cycles of PCV or to either of the temozolomide arms (200 mg/m^2 for 5 out of 28 days or 100 mg/m^2 for 21 out of 28 days). Treatment in all arms continued for up to 9 months, or until progression. When comparing the combined temozolomide arms with PCV-treated patients, no difference in survival was seen between the treatments. Notably, this study commenced before the results of the study with concomitant and adjuvant temozolomide were published. Therefore, since the patients in this study were chemotherapy-naïve at relapse and most patients are now treated with chemotherapy in the adjuvant setting, the results might not be fully applicable to the current population with relapsed glioblastoma.

Rationale for Current Dosing of Temozolomide

Temozolomide is an alkylating agent given in primary treatment at a standard dose of 150-200 mg/m^2 for 5 consecutive days in a 28-day cycle. In the initial study, this dose was found to be tolerable with an acceptable toxicity, but myelosuppression was dose-limiting (10). Another study concluded that a daily dose of 75 mg/m^2 temozolomide is also generally well-tolerated and that treatment can last for up to 7 weeks without increased toxicity. Use of this dose increases the cumulative dose two-fold compared to use of the standard dose. At higher daily doses (100 mg/m^2) grade IV leucopenia and thrombocytopenia were seen in 25% of patients (11). These doses are now used in the standard treatment, i.e. 75 mg/m^2 daily concomitant with radiation therapy and 150-200 mg/m^2 for 5 out of 28 days in six cycles in the adjuvant setting after radiation therapy.

Efficacy of Temozolomide

The cytotoxic effect of temozolomide and other alkylating agents consists primarily of transferring a methyl group to the O-6 position of guanine in the DNA molecule, causing defective DNA replication and tumour cells to enter apoptosis. However, this type of lesion is normally repaired by the DNA repair enzyme methylguanine-DNA methyltransferase (MGMT). Based on the repair mechanism, it can be estimated that the main cytotoxic effect of alkylating agents is dependent on the MGMT enzyme. A cell containing more MGMT (expression is up-regulated) is more resistant to the effect of the alkylator, while a lower expression renders the cell sensitive. Expression of *MGMT*, like many other genes, is dependent on the methylation status of the promoter, to which transcription factors bind and commence transcription and synthesis of proteins. Methylation of the promoter interferes with the transcription factors and inhibits transcription of the gene associated with that promoter. Promoter methylation effectively silences the gene and the protein levels are consequently low. There are numerous reports of a correlation between *MGMT* methylation status and response to temozolomide treatment and also survival, where the silenced gene correlates to better prognosis (12, 13). Patients with low *MGMT* expression receiving temozolomide treatment had a significantly longer survival than those with a high expression of *MGMT* (13). In fact, the strongest predictor of outcome and benefit of temozolomide treatment was *MGMT* methylation status (6). It should be emphasized that when comparing patients with methylated and those with unmethylated promoters receiving the same treatment, i.e. radiotherapy alone or its combination with temozolomide, OS was lower in the patients with unmethylated promoter in both treatment groups, possibly reflecting differences in tumour biology. This might also be an effect of alkylating agents as salvage treatment, which will possibly further increase the gap between methylated and unmethylated groups as patients with an elevated expression of MGMT are not likely to respond to such treatment in the salvage setting either (13).

MGMT Depletion

MGMT repairs DNA by transferring the alkyl group added by an alkylating agent from the O-6 position of guanine in the DNA molecule to an internal cysteine residue in the active site of MGMT. Thereby, the DNA is repaired and replication can proceed. When this happens, the MGMT enzyme is irreversibly inactivated and the enzyme is marked for destruction in the ubiquitin proteolytic pathway. To restore its function, *de novo* protein synthesis of MGMT is required. This has led to the hypothesis that MGMT can be consumed faster than it is synthesized. Thus, the depletion

of MGMT will reduce the ability of the cells to recover from treatment with alkylating agents and this might also enable patients with high expression of *MGMT* to benefit from temozolomide treatment. Depletion of MGMT can hypothetically be achieved by introducing a false substrate for MGMT, such as *O*-6-benzylguanine, which will consume MGMT at a high rate. The drawback of this method is that it also has the risk of increasing toxicity as MGMT in normal cells is also inactivated. Another option is to add more alkylating agents in order to introduce more alkyl groups, thereby saturating the MGMT system.

MGMT depletion has been studied intensely, mostly in peripheral blood monocytic cells (PBMCs). These are used as a surrogate marker for tumour MGMT depletion but it should be emphasized that further evidence is needed regarding the correlation between MGMT levels in PBMCs and tumour tissue. Tolcher *et al*. measured the levels of MGMT in PBMCs from patients under different regimens of temozolomide treatment and found temozolomide to be effective in depleting MGMT, even at relatively low doses. One arm, receiving temozolomide for 7 out of 14 days at a dose of 75-175 mg/m^2, showed a marked decrease in MGMT activity after 7 days of treatment, which was partly restored after 7 days off-treatment. The resulting decrease was approximately 55% of baseline levels. The other arm, receiving 85-125 mg/m^2 temozolomide for 21 out of 28 days, had a slower decline but the decrease was similar to that of the first arm (14). This implies that a dose-dense regimen of temozolomide might be efficient in depleting MGMT.

Dose-dense Temozolomide

A few clinical trials have been performed on a limited number of patients, where an altered dosing regimen of temozolomide has been evaluated to clarify whether it is possible to increase the cumulative dose and effect without increasing toxicity by depleting the MGMT levels. So far, the concept has only been evaluated in small phase II studies, where the results must be interpreted with precaution as the level of evidence is relatively weak. Two regimens have been investigated more thoroughly; one with temozolomide at 100-150 mg/m^2 for 21 out of 28 days and one with 150 mg/m^2 for 7 out of 14 days (7 days on/7 days off).

The 7 days on/7 days off regimen was evaluated in 21 patients with recurrent glioblastoma and objective response was seen in 10% of the patients whereas PFS at 6 months was 48%. The treatment was well-tolerated (15) and further analyzed in a non-randomized phase II study of 90 patients with recurrent glioblastoma, where PFS at 6 months was found to be 44%. The *MGMT* promoter methylation status of the tumours was analyzed and no differences were seen in survival depending on methylation status. The overall response rate was 10% and the authors concluded that a

possible depletion of *MGMT* was accomplished with the used regimen (16). The 21/28 days schedule, evaluated in 18 patients, was also shown to be feasible with a response rate of 22%. MGMT promoter methylation status was assessed only in seven cases, which was insufficient for any firm conclusions (17). In another study, where the concept was to evaluate the efficacy of temozolomide compared to the previously established recurrent therapy with PCV in glioblastoma, two different temozolomide treatment arms were used. The treatment was given in either the current standard scheme (TMZ-5) or a dose-dense scheme of treatment with 100 mg/m^2 for 21 out of 28 days (TMZ-21). The results of this study indicated an inferiority of the dose-dense regimen, and the authors speculated that the cumulative dose may be less important than the peak doses accomplished with the higher daily dose in the TMZ-5 arm. In this respect, the other option of dose-dense temozolomide with 7 days on, 7 days off seems more appealing (8). In a recent phase II study by Abacioglu *et al*., the efficacy and tolerability of protracted, dose-dense temozolomide therapy (100 mg/m2 for 21 consecutive days of a 28-day cycle) was evaluated in patients with recurrent glioblastoma or grade III glioma who had previously received standard therapy. Out of 25 patients included, two patients had partial responses and 10 had stable disease (60% overall clinical benefit). The median PFS was 3 months (95% confidence interval, CI=1.8-4.2) and the median overall survival was 7 months (95% CI=5.1-8.9 months). The regimen was well-tolerated and the authors concluded that protracted, dose-dense temozolomide had modest activity with manageable toxicity in these patients (9). In a compelling study, the dose-dense scheme (7 days on, 7 days off) was compared with a metronomic regimen of 50 mg/m^2 daily continuously in the adjuvant setting for newly-diagnosed glioblastoma (18). To facilitate benchmarking of the results with the EORTC/NCIC phase III study (5), treatment was given in the same way, but adjuvant temozolomide was given in either a dose-dense or a metronomic scheme. Adjuvant treatment was given in six cycles in both arms. Each arm consisted of approximately 40 patients. The results showed no benefit of administering temozolomide in the metronomic regimen, as no sign of supremacy against historical data of the EORTC/NCIC-study was seen. Moreover, the metronomic regimen was also considered more costly. However, the dose-dense scheme possibly led to a median survival time (17.1 months) superior to the compared results and the authors proposed further studies in a phase III setting. In particular, patients with an unmethylated *MGMT* promoter (which results in high MGMT levels) seemed to benefit from the treatment when compared with historical data (overall survival of 15.4 months, compared to 12.7 months reported from the EORTC/NCIC study) (13). This possibly suggests that the dose-dense scheme of temozolomide was able to deplete MGMT in

tumour tissue (18); however, as can be judged from the results achieved so far, depletion of MGMT seems to add minor improvement to the treatment of high-grade glioma.

Bevacizumab

Rationale. A growing tumour is dependent on oxygen and nutrients. When the tumour is small, sufficient supplies are maintained with simple diffusion of nutrients from surrounding capillaries. To sufficiently support a growing tumour, a crucial step is when the cell acquires the ability to attract blood vessels, the 'angiogenic switch'. When a cell is hypoxic, *i.e.* insufficient oxygen supply, the expression of angiogenic vascular endothelial growth factor (VEGF) is induced to attract surrounding vessels. Endothelial cells and pericytes in the vessel walls respond to the signal by growing in the direction of the signal. Tumour cells, which are often hypoxic as they grow uncontrolled beyond the limits of simple diffusion, also secrete VEGF. A high level of VEGF secretion correlated to dense vascularization and also a high grade of malignancy in glioblastomas (19). However, a high expression of VEGF does not induce the formation of reliable vessels. The tumour-induced vessels vary in size, with calibre shifts and excessive branching, contain shunts and are also much more permeable than is normal. These properties affect the transportation of nutrients in the vessels, which is impaired as the blood flow is not optimal. The possibility of delivering blood-borne drugs effectively is consequently also reduced.

Such malformed vessels leak fluids into surrounding tissues as a consequence of the increased vascular permeability, giving rise to oedema in the surrounding brain tissue. The oedema raises the intracranial pressure, causing severe symptoms in the patients such as headache, nausea and neurological deficits. Oedema can often be relieved by treatment with corticosteroids. As corticosteroids have multiple unwanted side-effects, it must be emphasized that it is of utmost importance to keep the dose to a minimum.

Efficacy. Upon treatment with the anti-angiogenic anti-VEGF antibody bevacizumab, a rapid change in the configuration of the blood vessels has been observed, leading to a decrease in permeability, with a consequent lowering of interstitial fluid pressure and normalized blood flow. As oxygen is supplied to the tumour tissue, sensitivity to radiation therapy may increase when more free oxygen radicals are formed. The delivery of chemotherapeutic agents to the tumour tissue may also be facilitated with normalized blood vessels and reduced interstitial fluid pressure. Anti-angiogenic therapy can therefore be considered to create a treatment opportunity window, where tumour cells are more sensitive to treatment. It is reasonable to believe that anti-angiogenic treatment is most effective when combined with cytotoxic treatment.

If the anti-angiogenic treatment is too intense, the vessels are further diminished increasing the risk of even worse hypoxia and consequent tumour necrosis (20). Furthermore, the hypoxic environment created by an intense anti-angiogenic therapy may also lead to selection of tumour cells insensitive to hypoxia, which are often more malignant than their oxygen-consuming counterparts. Bevacizumab has been studied as a single treatment, and in combination with irinotecan for recurrent glioblastoma. One hundred and sixty-seven patients were enrolled in a randomized phase II study, half of them received bevacizumab at 10 mg/kg and the other half the same dose of bevacizumab, but in combination with irinotecan at 340 mg/m^2 or 125 mg/m^2 (with or without enzyme-inducing antiepileptic drugs, respectively). Bevacizumab was given every two weeks. The regimen was concluded as being tolerable and effective, as objective response rates were seen in 28% and 38% in the bevacizumab and in the combination arm, respectively. PFS at six months was estimated to be 42.6% and 50.3%, respectively, which are impressive numbers compared to historical data. A trend for decreasing corticosteroid use was also seen. The median OS was 8.7 months for the combination arm and 9.2 months for the bevacizumab-alone arm (4).

Combination of Bevacizumab and Temozolomide

Both *in vitro* and *in vivo* studies of the combination of bevacizumab and temozolomide give encouraging results with a superior survival in animal xenograft studies, when compared to the agents used as single treatment (21). Recently, a phase II study was published where 70 patients with newly diagnosed glioblastoma were treated according to the current standard treatment with concomitant and adjuvant temozolomide, but with the addition of bevacizumab (10 mg/kg) every 14 days (22). The study was a single-arm study, but a reference population of patients treated at the same site according to the current standard protocol served as a comparison. The results were also benchmarked with the results of the EORTC-NCIC (5) study regarding PFS and OS. OS was 19.6 months in the study group, and 21.1 months in the control group (p=0.06), while PFS was 13.6 months and 7.6 months (p=0.005), respectively. Regarding OS, both groups were superior to the EORTC-NCIC study, possibly suggesting that new treatments in the salvage setting are of value. In the reference group, 51% received bevacizumab at recurrence. When comparing PFS, the study group was superior to both the EORTC-NCIC study and the reference group. A possible interpretation of these results might be that bevacizumab prolongs survival, either given as first-line therapy or at recurrence. If given adjuvantly, the time-to- recurrence is increased, and when given at recurrence, an equal effect is seen as OS does not differ. The toxicity observed was

acceptable and anticipated for bevacizumab. Currently, a randomized phase III study is evaluating the addition of bevacizumab to the first-line standard treatment (23).

In the relapse setting, there are ongoing studies with bevacizumab at 10 mg/kg every second week in combination with 7 days on/7 days off temozolomide at a daily dose of 100 mg/m^2 . Results published so far consist of five accrued patients out of 30 planned and conclude that it is a well-tolerated and effective regimen (24). In another study, daily temozolomide (50 mg/m^2) in combination with bevacizumab (10 mg/kg) was evaluated in 32 patients with recurrent glioblastoma. All patients had undergone standard radiotherapy and 63% had progressed on a 5-day temozolomide regime. The authors found acceptable toxicity and a response rate of 37.5% (25).

Conclusion

The introduction of the combination of temozolomide and irradiation in 2003 changed the whole treatment panorama of patients with high grade tumours. The 10-year update of this study shows that the addition of temozolomide to irradiation results in nearly a 10% survival rate. Additionally, because radiation treatment has further improved since the study was published, less severe side-effects are seen and altogether, this has given patients with glioblastoma a better chance of having a performance status, which renders them amenable to treatment at first relapse. However, as the current review implies, there is no 'golden standard' in the setting of first relapse. Regarding temozolomide at recurrence, available data are limited. As it is used in the first-line setting, standard dosing of temozolomide is seldom an option at recurrence, making it important to study alternative dosing, such as dose-dense regimens. The proposed value of bevacizumab treatment in prolonging survival is an issue to be addressed in further randomized trials, however, from a clinical point of view, treatment with bevacizumab is of strong value for some patients, since both tumour response, as well as improvement in neurological symptoms, can be seen. Furthermore, the effect of reduced corticosteroid dose and a prolonged time to progression is often of considerable value and may motivate the start of bevacizumab treatment.

As yet, the scientific evidence for the combinatorial treatment of dose-dense temozolomide and bevacizumab is limited, and randomized clinical trials in the first relapse setting are warranted. However, available data suggest that this combination is of interest for patients with a first relapse of glioblastoma.

References

1 Ohgaki H and Kleihues P: Population-based studies on incidence, survival rates, and genetic alterations in astrocytic and oligodendroglial gliomas. J of Neuropathol & Exp Neurol *64(6)*: 479-489, 2005.

2 Filippini G, Falcone C, Boiardi A, Broggi G, Bruzzone MG, Caldiroli D, Farina R, Farinotti M, Fariselli L, Finocchiaro G, Giombini S, Pollo B, Savoiardo M, Solero CL and Valsecchi MG: Prognostic factors for survival in 676 consecutive patients with newly diagnosed primary glioblastoma. Neuro Oncol *10(1)*: 79-87, 2008.

3 Wong ET, Hess KR, Gleason MJ, Jaeckle K, Kyritsis P, Prados MD, Levin V and Yung WK: Outcomes and prognostic factors in recurrent glioma patients enrolled onto phase II clinical trials. J Clin Oncol *17(8)*: 2572-2578, 1999.

4 Friedman HS, Prados MD, Wen PY, Mikkelsen T, Schiff D, Abrey LE, Yung WKA, Paleologos N, Nicholas MK, Jensen R, Vredenburgh J, Huang J, Zheng M and Cloughesy T: Bevacizumab alone and in combination with irinotecan in recurrent glioblastoma. J Clin Oncol *27(28)*: 4733-4740, 2009.

5 Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJB, Belanger K, Brandes A a, Marosi C, Bogdahn U, Curschmann J, Janzer RC, Ludwin SK, Gorlia T, Allgeier A, Lacombe D, Cairncross JG, Eisenhauer E and Mirimanoff RO: Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. New Engl J Med *352(10)*: 987-996, 2005.

6 Stupp R, Hegi ME, Mason WP, van den Bent MJ, Taphoorn MJ, Janzer RC, Ludwin SK, Allgeier A, Fisher B and Belanger K: 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. Lancet Oncol *10(5)*: 459-466, 2009.

7 Huncharek M and Muscat J: Treatment of recurrent high grade astrocytoma; results of a systematic review of 1,415 patients. Anticancer Res *18(2B)*: 1303-1311, 1998.

8 Brada M, Stenning S, Gabe R, Thompson LC, Levy D, Rampling R, Erridge S, Saran F, Gattamaneni R and Hopkins K: Temozolomide *versus* procarbazine, lomustine, and vincristine in recurrent high-grade glioma. J Clin Oncol *28(30)*: 4601-4608, 2010.

9 Abacioglu U, Caglar HB, Yumuk PF, Akgun Z, Atasoy BM and Sengoz M: Efficacy of protracted dose-dense temozolomide in patients with recurrent high-grade glioma. J Neuro-oncol *103(3)*: 585-93, 2011.

10 Newlands ES, Blackledge GR, Slack J a, Rustin GJ, Smith DB, Stuart NS, Quarterman CP, Hoffman R, Stevens MF and Brampton MH: Phase I trial of temozolomide (CCRG 81045: M&B 39831: NSC 362856). Br J Cancer 1992 *65(2)*: 287-291, 1992.

11 Brock CS, Newlands ES, Wedge SR, Bower M, Evans H, Colquhoun I, Roddie M, Glaser M, Brampton MH and Rustin GJ: Phase I trial of temozolomide using an extended continuous oral schedule. Cancer Res *58(19)*: 4363-4367, 1998.

12 Esteller M, Garcia-Foncillas J, Andion E, Goodman SN, Hidalgo OF, Vanaclocha V, Baylin SB and Herman JG: Inactivation of the DNA-repair gene MGMT and the clinical response of gliomas to alkylating agents. New Engl J Med *343(19)*: 1350-1354, 2000.

13 Hegi ME, Diserens A-C, Gorlia T, Hamou M-F, de Tribolet N, Weller M, Kros JM, Hainfellner J a, Mason W, Mariani L, Bromberg JEC, Hau P, Mirimanoff RO, Cairncross JG, Janzer RC and Stupp R: MGMT gene silencing and benefit from temozolomide in glioblastoma. New Engl J Med *352(10)*: 997-1003, 2005.

14 Tolcher A, Gerson S, Denis L, Geyer C, Hammond L, Patnaik A, Goetz A, Schwartz G, Edwards T and Reyderman L: Marked inactivation of *O*-6-alkylguanine-DNA alkyltransferase activity with protracted temozolomide schedules. Br J Cancer *88(7)*: 1004-1011, 2003.

15 Wick W, Steinbach JP, Küker WM, Dichgans J, Bamberg M and Weller M: One week on/one week off: a novel active regimen of temozolomide for recurrent glioblastoma. Neurology *62(11)*: 2113-2115, 2004.

16 Wick A, Felsberg J, Steinbach JP, Herrlinger U, Platten M, Blaschke B, Meyermann R, Reifenberger G, Weller M and Wick W: Efficacy and tolerability of temozolomide in an alternating weekly regimen in patients with recurrent glioma. J Clin Oncol 2007 *25(22)*: 3357-61, 2007.

17 Strik HM, Buhk JH, Bock C, Hoffmann AL, Wrede A, Marosi C, Kaiser U, Christmann M, Kaina B and Bahr M: Tegwondo: Development of a novel near-continuous dose-dense temozolomide regimen for the treatment of recurrent brain tumors. ASCO Meeting Abstracts *26(15 suppl)*: 13016, 2008.

18 Clarke JL, Iwamoto FM, Sul J, Panageas K, Lassman AB, DeAngelis LM, Hormigo A, Nolan CP, Gavrilovic I, Karimi S and Abrey LE: Randomized phase II trial of chemoradiotherapy followed by either dose-dense or metronomic temozolomide for newly diagnosed glioblastoma. J Clin Oncol *27(23)*: 3861-3867, 2009.

19 Lamszus K, Ulbricht U, Matschke J, Brockmann MA, Fillbrandt R and Westphal M: Levels of soluble vascular endothelial growth factor (VEGF) receptor 1 in astrocytic tumors and its relation to malignancy, vascularity, and VEGF-A. Clin Cancer Res *9(4)*: 1399-1405, 2003.

20 Winkler F, Kozin SV, Tong RT, Chae S-S, Booth MF, Garkavtsev I, Xu L, Hicklin DJ, Fukumura D, di Tomaso E, Munn LL and Jain RK: Kinetics of vascular normalization by VEGFR2 blockade governs brain tumor response to radiation: role of oxygenation, angiopoietin-1, and matrix metalloproteinases. Cancer Cell *6(6)*: 553-563, 2004.

21 Mathieu V, De Nève N, Le Mercier M, Dewelle J, Gaussin JF, Dehoux M, Kiss R and Lefranc F: Combining bevacizumab with temozolomide increases the antitumor efficacy of temozolomide in a human glioblastoma orthotopic xenograft model. Neoplasia *10(12)*: 1383, 2008.

22 Lai A, Tran A and Nghiemphu P: Phase II study of bevacizumab plus temozolomide during and after radiation therapy for patients with newly diagnosed glioblastoma multiforme. J Clin Oncol *29(2)*: 142-148, 2011.

23 Chinot OL, de La Motte Rouge T, Moore N, Zeaiter A, Das A, Phillips H, Modrusan Z and Cloughesy T: AVAglio: Phase 3 trial of bevacizumab plus temozolomide and radiotherapy in newly diagnosed glioblastoma multiforme. Adv Ther *28(4)*: 334-340, 2011.

24 Badruddoja M a, Pazzi M, Stea B, Kuzma KM, Bishop MC, Carmody R, Schroeder K, Seeger J, Marsella M and Sanan A: Phase II study of biweekly temozolomide plus bevacizumab for adult patients with recurrent glioblastoma multiforme. ASCO Meeting Abstracts *28(15 suppl)*: e12554, 2010.

25 Maron R, Vredenburgh JJ, Desjardins A, Reardon DA, Quinn JA, Rich JN, Gururangan S, Wagner SA, Salacz ME and Friedman HS: Bevacizumab and daily temozolomide for recurrent glioblastoma multiforme (GBM) ASCO Meeting Abstracts *26(15 suppl)*: 2074, 2008.