

# Bevacizumab and dose-intense temozolomide in recurrent high-grade glioma

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**Background:** Angiogenesis inhibition is a rational treatment strategy for high-grade glioma (HGG). Combined antiangiogenic therapy and chemotherapy could be beneficial, taking advantage of different mechanisms of antitumour activity of both therapies. We carried out a phase I–II clinical trial with the combination of bevacizumab and continuous dose-intense temozolomide (TMZ) for patients with a recurrent HGG after first- or second-line treatment.

**Patients and methods:** Twenty-three HGG patients were treated with bevacizumab (10 mg/kg i.v. every 3 weeks) and TMZ (daily 50 mg/m<sup>2</sup>), until clinical or radiological progression. Conventional and dynamic magnetic resonance imaging (MRI) were carried out on days –4, 3 and 21 and until clinical or radiological progression.

**Results:** Overall response rate (20%), 6-month progression-free survival (PFS6) (17.4%), median progression-free survival (13.9 weeks) and median overall survival (OS) (17.1 weeks) were considerably lower compared with most other studies with bevacizumab-containing regimens. The dynamic MRI parameters contrast transfer coefficient and relative cerebral blood volume decreased rapidly during the early phases of treatment, reflecting changes in vascularisation and vessel permeability but not in tumour activity. In addition, >50% of patients showed oedema reduction and a reduced shift on T1 images.

**Conclusion:** Treatment with bevacizumab and TMZ is feasible and well tolerated but did not improve PFS6 and median OS.

**Key words:** angiogenesis, bevacizumab, MRI, recurrent glioblastoma multiforme, temozolomide

## introduction

Treatment outcome for patients with a high-grade glioma (HGG), i.e. anaplastic astrocytoma (AA, grade III) and glioblastoma multiforme (GBM, grade IV) remains poor, mainly because radical tumour resections are hardly ever achieved and local recurrences are unavoidable [1]. Current standard treatment of primary GBM is neurosurgery followed by 30 times 2-Gy irradiation combined with daily temozolomide (TMZ) chemotherapy (75 mg/m<sup>2</sup>), with six subsequent monthly adjuvant cycles of TMZ chemotherapy (150–200 mg/m<sup>2</sup> daily for 5 days). With this regimen, the median overall survival (OS) is 14.6 months [2].

There is growing interest in alternative TMZ schedules, other than 150–200 mg/m<sup>2</sup> on first 5 days of each 28-day cycle, especially regimens with continuous low-dose TMZ administration. Protracted TMZ regimens may deplete O<sup>6</sup>-methylguanine DNA methyltransferase, an important factor in TMZ resistance [3], and offer a higher dose intensity per month

of delivery. In addition, several preclinical studies demonstrated that continuous daily administration of cytotoxic drugs at low dose, below the maximum tolerated dose (continuous dose-intense or metronomic chemotherapy), has potential antiangiogenic activity [4–9]. Several clinical studies with daily low-dose TMZ regimens in second line demonstrated responses in patients pretreated with TMZ [10].

Given the characteristic high degree of endothelial proliferation, high vascular permeability and increased pro-angiogenic growth factors expression, such as the vascular endothelial growth factor (VEGF), angiogenesis inhibition is a rational treatment strategy for HGG [11]. Single-agent bevacizumab treatment showed an improved median OS of 9.7 months since latest recurrence [12]. The combination of antiangiogenic therapy with chemotherapy is promising, as has been demonstrated by several phase II studies. Studies by Stark-Vance [13], Pope et al. [14], Vredenburgh et al. [15, 16], Norden et al. [17], Guiu et al. [18], Cloughesy et al. [12], Kreisl et al. [19], Poulsen et al. [20] and Nghiemphu et al. [21] demonstrated that treatment with anti-VEGF mAb bevacizumab in combination with the topoisomerase-1 inhibitor irinotecan in patients with relapsed HGG resulted in remarkably high response rates (RRs) according to Macdonald

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criteria [22]—RR: 43%–63%; 6-month progression-free survival (PFS6): 38%–46% and 6-month overall survival: 72%–77%. These data are considerably better than historical controls treated with chemotherapy alone [23]. The underlying mechanisms of angiogenesis inhibition were explored in a study by Batchelor et al. [24], where AZD2171 (a tyrosine kinase inhibitor of the VEGF receptor) was administered as monotherapy in patients with relapsed GBM. AZD2171 induced an immediate effect on tumour vasculature, as indicated by a significant decrease in tumour gadolinium uptake, decreased tumour vessel permeability and significantly alleviated oedema.

In the present study, we explored the combination of these two antiangiogenic strategies, bevacizumab with continuous dose-intense TMZ, in patients with relapsed HGG. Primary end point of the study was PFS6. To quantify effects on tumour vasculature, patients were assessed with dynamic contrast-enhanced magnetic resonance imaging for vascular permeability of the tumour and with dynamic susceptibility contrast magnetic resonance imaging for tumour perfusion, at study inclusion and at 3 and 21 days after starting treatment. In addition, these parameters were evaluated until clinical or radiological relapse. The predicting value for clinical outcome of the different imaging techniques was calculated.

## patients and methods

The trial was carried out at the Academic Medical Center of the University of Amsterdam. Eligible patients included adults with a life expectancy of >8 weeks, a histologically confirmed intracranial HGG (World Health Organization grade III or IV), evidence of tumour recurrence at baseline magnetic resonance imaging (MRI), Karnofsky performance score >70% and adequate recovery from prior treatment. All patients provided written informed consent, and the trial was conducted in accordance with the Declaration of Helsinki and was approved by the Academic Medical Center Institutional Review Board and the Dutch Central Committee on Research investigating Human Subjects (ISRCTN23008679). See supplemental data section (available at *Annals of Oncology* online) for details.

### study treatment

The experimental treatment consisted of continuous dose-intense TMZ [Temodal® (Schering-Plough, Houten, The Netherlands), daily 50 mg/m<sup>2</sup>, orally, continuously], bevacizumab (Avastin®, 10 mg/kg i.v., every 21 days, defined as one cycle) and dexamethasone if needed at inclusion. For these patients, the dexamethasone dose was fixed to 12 mg daily during the first cycle. Bevacizumab was supplied by Roche (Woerden, The Netherlands). TMZ and dexamethasone (Oradexon®, Organon, Oss, The Netherlands) were commercially obtained.

### study evaluations

Clinical study evaluations during treatment included medical interim history, physical examination (e.g. blood pressure, Karnofsky performance status and neurological status), complete blood count and chemistry and urine analysis (protein). Toxicity was evaluated according to the National Cancer Institute—Common Terminology Criteria for Adverse Events Version 3.0. See supplemental data section (available at *Annals of Oncology* online) for detailed toxicity and MRI evaluations.

### statistical considerations

With a sample size of 25 HGG patients, the study was designed to conclude with a nominal 0.05 one-sided significance level and a power of 80% that

for combined treatment with bevacizumab and TMZ, a PFS6 of 30% is higher than historical PFS6. Historical controls were derived from Ballmann et al. [23], with PFS6 of 9% for grade IV patients.

Statistical analysis of progression-free survival (PFS) and OS was carried out according to Kaplan–Meier. Analysis of possible prognostic factors was done with a log-rank test. Variables that were statistically significant were further analysed with multivariate analyses by using the Cox proportional hazards model. Hazard risk ratios and 95% confidence intervals (CIs) are reported with two-tailed probability values. The reported probability values in the Cox model are on the basis of the Wald test, and a probability value <0.05 was considered significant.

## results

### patient characteristics

From April 2007 to December 2007, 23 patients with histologically confirmed HGG were enrolled, 8 AA and 15 GBM (Table 1). Of these, 18 patients were already on corticosteroid treatment (13 GBM and 5 AA). In two patients, bevacizumab was added after the first cycle to explore early (at days 3 and 21)

**Table 1.** Patient response characteristics

Characteristic	Value		
	Grade III	Grade IV	Overall
Total number of patients	8	15	23
Age (years)			
Mean	36.0	50.9	45.7
Median	36.0	55.0	44.6
Range	17.5–54.9	24.2–68.7	17.5–68.7
Sex			
Male	5	10	15
Female	3	5	8
% Male	62.5	66.7	65.2
% Dexamethasone at start	62.5	86.7	78.3
Time from first diagnosis (months)			
Median	36	13	17
Range	17.6–56.5	8.8–55.1	8.8–56.5
CE-T1 ORR (%)			
Day 3	37	7.7	19
Day 21	25	16.7	20
3D-NEV ORR (%)			
Day 3	50	46	47
Day 21	37	58	50
PFS6 (%)	37.5	6.7	17.4
OS6 (%)	62.5	20.0	34.8
PFS			
Median	20.4	10.4	13.9
Range	10.1–39.9	2.4–26.4	2.4–39.9
OS since latest recurrence			
Median	32.4	15.7	17.1
Range	14.6–62.1	5.3–46.9	5.3–62.1
OS since first diagnosis			
Median	206.6	68.1	104.6
Range	109–300	54.7–272.1	54.7–300

Time in weeks.

CE-T1, contrast-enhancing tumour product on T1 images; 3D-NEV, 3-dimensional net enhancing tumour volume; ORR, overall response rate; PFS6, 6-month progression-free survival; OS6, 6-month overall survival; PFS, progression-free survival; OS, overall survival.

MRI changes with dexamethasone and TMZ only. These two patients received bevacizumab in the following cycles. Maximum number of cycles was 18, mean 5.5 and median 4. Follow-up period lasted until May 2009.

All patients were previously treated with tumour resection followed by irradiation and TMZ after primary diagnosis and were progressive after first- or second-line therapy (supplemental Table S1, available at *Annals of Oncology* online). See supplemental data section (available at *Annals of Oncology* online) for toxicity results.

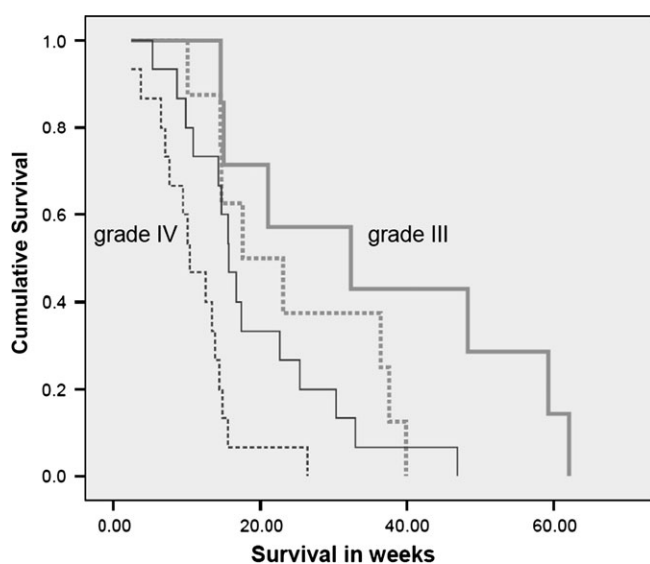
### response rate

The diameter-based response assessment, bidimensional cross-sectional contrast-enhancing tumour product on T1 images (CE-T1; according to Macdonald et al. criteria), revealed a comparable overall response rate (ORR) of 20% at days 3 and 21 but higher for AA than for GBM (Table 1). No objective responses were observed in the two patients without bevacizumab during the first 21-day treatment period.

The volume-based assessment [3-dimensional net enhancing tumour volume (3D-NEV) on T1 images, according to Sorensen et al.] revealed higher RRs compared with diameter-based response measurements, 47% at day 3 and 50% at day 21. Both methods demonstrated that in grade III tumours, there was a trend to a lower ORR on day 21 compared with day 3; for grade IV tumours, this was the other way around. No complete responses were documented according to either method.

### treatment efficacy

Twenty-three patients were assessable for the primary study end point (Table 1). The PFS6 was 17.4% (95% CI 1.6% to 33%), for grade III and IV tumours 37.5% and 6.7%, respectively. Median PFS was 13.9 weeks (95% CI 11.0–16.8 weeks), for grade III and IV tumours 20.4 and 10.4 weeks, respectively (Figure 1). Median OS was 17.1 weeks (95% CI 8.9–25.9



**Figure 1.** Kaplan–Meier curves of grade III (thick green line) and grade IV (thin blue line) patients for progression-free survival (dotted lines) and overall survival (solid lines) (time in weeks).

weeks), for grade III and IV tumours 32.4 and 15.7 weeks, respectively.

### oedema reduction

Although in 50% of the patients a reduction in oedema was observed on T2 images, a more than 50% reduction was only observed in two patients at day 21. This phenomenon of oedema reduction occurred later than the rapidly observed decrease in CE-T1 volume and relative cerebral blood volume (rCBV) and contrast transfer coefficient ( $K^{\text{trans}}$ ) values at day 3. At relapse, the oedema reduction persisted (Figure 2; supplemental Figures S1 and S2, available at *Annals of Oncology* online).

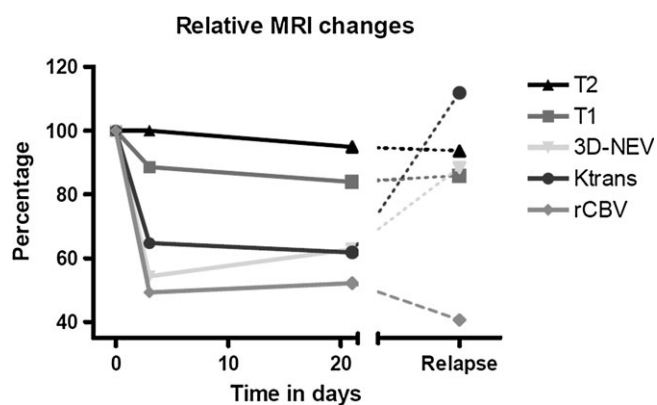
### patterns of relapse

Four patients (17%) acquired new contrast-enhancing lesions in other parts of the brain during treatment; all others showed local recurrences on MRI (Figure 3). At relapse, during bevacizumab treatment, 3D-NEV increased towards baseline volume and  $K^{\text{trans}}$  increased to levels higher than baseline (Figure 2).

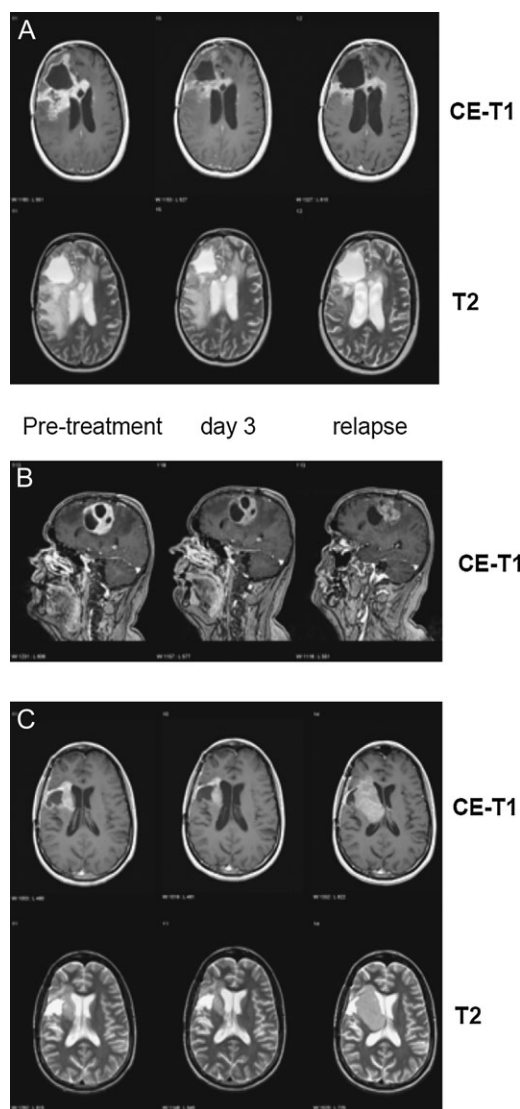
Four progressive patients were re-operated 6 weeks after the last bevacizumab infusion and resection specimens were collected for histological analysis. From one patient, we obtained post-mortem analysis 10 weeks after the last bevacizumab infusion. Histological reviewing revealed an extensively infiltrated disease present along pre-existing vasculature, whereas almost no contrast enhancement was visible on the CE-T1 MRI carried out 4 weeks earlier (supplemental Figure S3, available at *Annals of Oncology* online).

### discussion

The response data (CE-T1, 20%) in our study are comparable to the ORR reported for single-agent bevacizumab therapy [12]



**Figure 2.** Relative percentage of median CE-T1, T2, 3D-NEV, rCBV and  $K^{\text{trans}}$  of all dynamic scanned patients ( $n = 21$ ) at four time points: before treatment, day 3 and day 21 and during treatment at recurrence. After baseline measurement, all values (except those for T2) drop quickly as early as 72 h after the start of experimental treatment, indicating a fast vascular response. CE-T1, contrast-enhancing tumour product on T1 images; 3D-NEV, 3-dimensional net enhancing tumour volume; rCBV, relative cerebral blood volume;  $K^{\text{trans}}$ , contrast transfer coefficient; MRI, magnetic resonance imaging.



**Figure 3.** Three typical patients (A–C) scanned before treatment, at 3 days after start of treatment and at recurrence during treatment. A fast decrease of maximal enhancement, enhancing area, is observed after only 3 days of treatment; T2 response is slow but persistent.

but they are considerably lower than the reported RRs up to 57% in previous studies for bevacizumab combined with chemotherapy, i.e. irinotecan [16, 19, 20, 25]. An explanation for this may be that we administered bevacizumab every 3 weeks instead of every 2 weeks as in the other studies. Although the 3-weekly administration of bevacizumab is common practice in other solid malignancies, this schedule may be less effective in recurrent HGG.

The 3D-NEV, according to Sorensen et al. [26], revealed a much higher decrease in enhancing volume. This three-dimensional method is probably a more realistic approach to calculate changing tumour size than the cross-sectional product because it takes into account the irregular shape of tumours and excludes intratumoral cavities and necrotic tissue. Nevertheless, the rapid change in tumour-enhancing area or volume by antiangiogenic therapy makes it highly improbable that this phenomenon represents a real antitumour effect. This

is in line with the finding that no relation was observed between radiographic responses and disease outcome.

The PFS6 in our patients was also considerably lower compared with the above-mentioned studies with bevacizumab and irinotecan. Because of the direct vascular effects and reduced permeability produced by antiangiogenic agents, gadolinium-enhanced tumour areas are often ill-defined, faded and have a ‘smudged’ appearance. This complicates proper assessment of the underlying actual tumour mass and also the moment of tumour progression. Therefore, determination of the time to progression on the basis of changes of enhancing tumour areas or volumes is difficult and not useful for the evaluation of antiangiogenic therapy in HGG. Unlike PFS, OS is the most robust end point, not affected by misinterpretations of antiangiogenic therapy. In the present study, the median OS was 32.4 and 15.7 weeks for grade III and IV tumours, respectively. The reported median OS data in the various studies with bevacizumab and chemotherapy, selected for grade IV only, are less consistent and range from 28 to 42 weeks [12, 16, 17, 20]. The median OS of 15.7 weeks in our study for grade IV tumours compares unfavourably with these bevacizumab studies and is even worse compared with survival data from the meta-analysis of Wong et al. [27] resulting in 30 weeks for grade IV tumours treated with second-line chemotherapy alone.

New enhancing brain lesions were observed in 17% of our patients, more than that observed in historical controls [17]. Other clinical studies also revealed that HGG recurs (mainly invasive) with more distant metastases when angiogenesis is inhibited [17, 28].

## conclusions

In this trial, we investigated the combination treatment of bevacizumab and continuous dose-intense TMZ. This treatment is feasible and well tolerated but does not improve PFS6 and OS. Our dynamic imaging results may indicate that antiangiogenic treatment is effective against the leaky bulk of the tumour but not against invasive tumour components.

Furthermore, when the disappointing survival compared with TMZ mono-treatment (15.7 versus 30 weeks) is taken into account, the reduced survival time may even indicate that antiangiogenic treatment not only reduces vessel permeability for contrast-enhancing agents but may even reduce the activity of TMZ by limiting drug penetration. This study demonstrates that randomised trials (with and without antiangiogenic therapy) are necessary to define the role of this new treatment strategy and to overcome the problem of treatment bias in patients with HGG.

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