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Angiogenesis inhibition in high grade glioma

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Chapter 7.

Bevacizumab and dose-intense
temozolomide in recurrent high grade
glioma

Submitted

Bevacizumab and dose-intense temozolomide in recurrent high grade glioma

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Summary

Background: Angiogenesis inhibition is a rational treatment strategy for high- grade glioma (HGG). Combined anti-angiogenic therapy and chemotherapy could be beneficial, taking advantage of different mechanisms of anti-tumour activity of both therapies. We performed 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/m²), until clinical or radiological progression. Conventional and dynamic MRI were performed on day -4, 3 and 21, and until clinical or radiological progression.

Results: Overall response rate (20%), PFS6 (17.4%), median PFS (13.9 weeks) and median overall survival (17.1 weeks) were considerably lower compared to most other studies with bevacizumab containing regimens. The dynamic MRI parameters K^{trans} and rCBV decreased rapidly during the early phases of treatment, reflecting changes in vascularisation and vessel permeability but not of tumour activity. In addition, over 50% of patients showed oedema reduction and a reduced shift on T1 images.

Conclusions: Treatment with bevacizumab and TMZ is feasible and well tolerated but did not improve PFS6 and median overall survival.

Key words: Angiogenesis; Bevacizumab; Temozolomide; Recurrent Glioblastoma Multiforme; MRI.

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 for primary GBM is neurosurgery followed by 30 times 2 Gy irradiation combined with daily temozolomide (TMZ) chemotherapy (75 mg/m²), with 6 subsequent monthly adjuvant cycles of TMZ chemotherapy (150-200 mg/ m² daily for 5 days). With this regimen the median overall survival (OS) is 14.6 months [2].

In case of recurrent HGG, several drugs have demonstrated some activity, such as carmustine (BCNU), lomustine (CCNU), irinotecan (CPT-11) and carboplatin [3]. Unfortunately these therapies show limited response rates and, even when effective, the durability of response is modest. TMZ was demonstrated to increase survival as second-line therapy when compared with historical controls [4]. However, because most patients are now treated with TMZ as initial treatment, a rechallenge with TMZ at the same treatment schedule in second line is often not effective [5]. There is growing interest in alternative TMZ schedules, other than 150-200 mg/m² on 5 days of each 28-day cycle, especially regimens with continuous low-dose TMZ administration. Protracted TMZ regimens may deplete O6-methylguanine DNA methyltransferase (MGMT), an important factor in TMZ resistance [6], 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), have potential anti-angiogenic activity [7-12]. Several clinical studies with daily low-dose TMZ regimens in second line demonstrated responses in patients pretreated with TMZ [5,13-17].

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 [18]. Single-agent bevacizumab treatment showed an improved median OS of 9.7 months since latest recurrence [19]. The combination of anti-angiogenic therapy with chemotherapy is promising, as has been demonstrated by several phase II studies. Studies by Stark-Vance [20], Pope et al. [21], Vredenburgh et al. [22,23], Norden et al. [24], Guiu et al. [25], Cloughesy et al. [19], Kreisl et al. [26], Poulsen et al. [27], and Nghiemphu et al. [28] demonstrated that treatment with anti-VEGF monoclonal antibody bevacizumab in combination with the topoisomerase-1 inhibitor irinotecan in patients with relapsed HGG resulted in remarkably high response rates (RR) according to Macdonald criteria [29]: RR 43%-63%; 6 months progression-free survival (PFS6): 38%-46%; and 6 months OS (OS6): 72%-77%. These data are considerably better than historical controls treated with chemotherapy alone [30]. The underlying mechanisms of angiogenesis inhibition were explored in a study by Batchelor et al., where AZD2171 (a tyrosine kinase inhibitor of the VEGF receptor)

was administered as monotherapy in patients with relapsed GBM [31]. 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 anti-angiogenic strategies, bevacizumab with continuous dose-intense TMZ, in patients with relapsed HGG. Although in most studies in patients with high grade gliomas bevacizumab was administered every 2 weeks, in this study we have chosen for a once every 3 weeks schedule based on experience in other solid tumours and on pharmacokinetic characteristics of bevacizumab [32]. Primary end point of the study was PFS6. To quantify effects on tumour vasculature, patients were assessed with dynamic contrast-enhanced MRI (DCE-MRI) for vascular permeability of the tumour and with dynamic susceptibility contrast MRI (DSC-MRI) 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

Inclusion and exclusion criteria

The trial was performed at the Academic Medical Center of the University of Amsterdam. Eligible patients included adults with a life expectancy of more than 8 weeks, a histologically confirmed intra-cranial HGG (WHO grade III or IV), evidence of tumour recurrence at baseline MRI, Karnofsky Performance Score > 70%, and adequate recovery from prior treatment. An interval of at least 30 days from prior treatment was required, including surgical (re-)resection, radiotherapy and up to two chemotherapy regimens. Patients with reproductive potential had to use contraceptives. Additional eligibility criteria included satisfactory haematologic, renal and hepatic functions, at least 5 days prior to enrolment. Exclusion criteria included the use of any other anti-cancer therapy, any anticoagulant therapy, enzyme-inducing antiepileptic drugs, pregnancy, prior anti-angiogenesis treatments, prior thrombo-embolic events or serious concomitant systemic disorders (e.g. active infection or abnormal electrocardiogram indicative of cardiac disease). 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).

Study treatment

The experimental treatment consisted of continuous dose-intense TMZ (Temodal[®], daily 50 mg/m², orally, continuously), bevacizumab (Avastin[®], 10 mg/kg intravenously, 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. TMZ and dexamethasone (Oradexon[®])

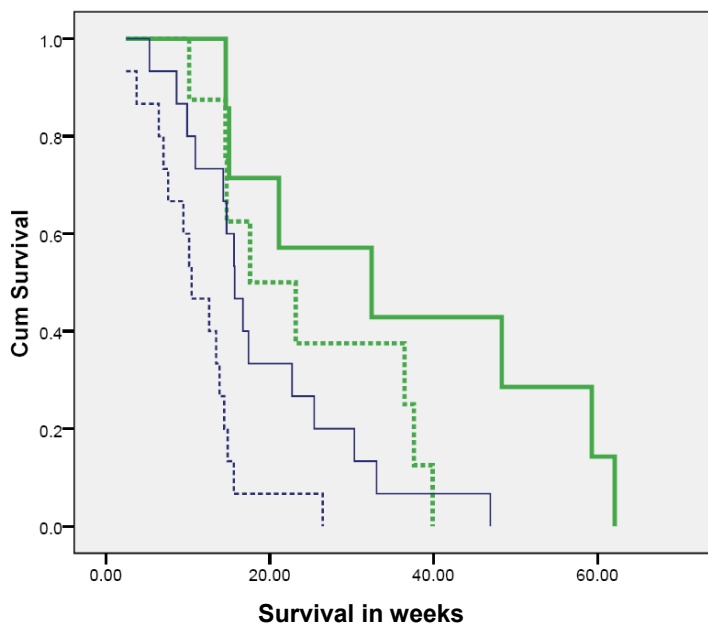


Figure 1 Kaplan-Meier curves for progression-free survival (PFS; dotted lines) and overall survival (OS; solid lines) of grade III (thick green line) and grade IV patients (thin blue line) (time in weeks).

were commercially obtained.

TMZ dose was delayed or reduced in case of grade 1 or greater haematological toxicity and grade 2 or greater non-haematological toxicity. Prior to each bevacizumab infusion patients were checked for hypertension (upper limit systolic 150 and diastolic 100 mmHg), proteinuria, and clinical signs of haemorrhage. Treatment of hypertension by calcium antagonists was allowed. The first dose of bevacizumab was administered intravenously during 90 minutes; subsequent doses were given during 30 minutes. Protocol treatment was discontinued when clinical or radiological disease progression was observed, and in case of grade 2 or greater central nervous system haemorrhage, grade 4 haematological and non-haematological toxicity, arterial or venous thrombosis, gastrointestinal perforation, wound dehiscence requiring medical or surgical intervention, or inability of the subject to comply with study requirements.

Study evaluations

Clinical study evaluations during treatment included medical interim history, physical exam (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 NCI Common Terminology Criteria for Adverse Events Version 3.0.

Radiological evaluation modalities included conventional T1 and T2 pre- and post-contrast MRI, diffusion weight imaging (DWI), DCE-MRI and DSC-MRI, serially performed 4 days prior to onset of therapy, on days 3, 21, every two months, and at relapse. Response was evaluated by neuro-radiologists on MRI T1 and T2 images, according to Macdonald et al. criteria [29], and for research purposes according to 3-dimensional net enhancing tumour volume (3D-NEV), based on Sorensen et al. [33].

Partial response (PR) was granted if contrasted T1

images decreased >50% in bi-dimensional enhancing tumour product together with a stable or decreased tumour size on T2. Progressive disease (PD) was defined as >25% increase in bi-dimensional enhancing tumour product or the appearance of new enhancing lesions on T1. Patients were defined as having stable disease (SD) when radiographic criteria for PR or PD were not met. PD evaluation to exclude patients was allowed from the second cycle of bevacizumab.

Dynamic MRI evaluations

Images were transferred to an off-line workstation for analysis. DCE-MRI and DSC-MRI images were analyzed either in a qualitative or a quantitative fashion. For the qualitative analysis we calculated the 3D-NEV, based on enhancing 3D tumour volume without non-enhancing tissue and cysts. For quantitative analysis, we calculated the volume transfer coefficient K^{trans} , as well as the perfusion parameter rCBV. Details of the calculations are given in the on-line appendix (Supplemental Methods section).

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., with PFS6 of 9% for grade IV patients [30].

Statistical analysis of PFS and OS was performed 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 (CI) are reported with 2-tailed probability values. The reported probability values in the Cox model are based on the Wald test, and a probability value < 0.05 was considered significant.

results

Patient characteristics

Between April 2007 and 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 2 patients bevacizumab was added after the first cycle to explore early (at day 3 and 21) MRI changes with dexamethasone and temozolomide only. These 2 patients received bevacizumab in the following cycles. Maximum number of cycles was 18, mean 5.5, 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**). The median interval between primary diagnosis and start of experimental treatment was 206 weeks for AA patients and 68 weeks for GBM patients. Five patients received second-line chemotherapy prior to inclusion, in 11 patients debulking surgery was performed more than once. No patients were

included within 2 months after resection; one patient was re-irradiated 2 months prior to experimental treatment. Dynamic MRI at sequential time points was completed in 21 patients (83 complete datasets in total).

Before reaching the primary endpoint of 6 months therapy, 18 of the 23 patients had PD on MRI, and experimental treatment was terminated. As of May 2009, 22 patients have died. Also as of May 2009, the only surviving patient developed progressive disease and is treated with other experimental regimens.

Toxicity

Four patients required TMZ dose reduction due to haematological toxicity grade 1-2 or fatigue grade 2. TMZ was discontinued for 1 week and reduced by 25% according to protocol after recovery of white blood cells and/or platelets. Opportunistic infections grade 1 (including oral *Candida* infections and skin infections) were observed and treated in 4 patients without reducing study medication. Three patients required antihypertensive medication because of hypertension grade 1. An intra-tumoral haemorrhage grade 1 without clinical symptoms was observed in one patient; bevacizumab was continued without problems. Two patients discontinued protocol treatment due to adverse events. One of them developed a deep venous thrombosis after the 4th cycle of bevacizumab and discontinued study treatment. One patient already considered progressive shortly after the 2nd bevacizumab infusion required hospitalisation due to an intra-tumoral haemorrhage grade 4, combined with thrombocytopenia grade 2 and followed by pulmonary embolism. A few days later this patient died, possibly related to trial medication. No adverse events related to wound healing were observed, despite 5 patients having a re-resection within 2 months prior to inclusion, and 4 patients being re-operated 6 weeks after the last bevacizumab infusion and exclusion.

Response rate

The diameter-based response assessment, bi-dimensional 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 day 3 and 21, but higher for AA than for GBM (**Table 2**). No objective responses were observed in the 2 patients without bevacizumab during the first 21-day treatment period.

The volume-based assessment (3D-NEV on T1 images, according to Sorenson et al.) revealed higher response rates compared to 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 to day 3; for grade IV tumours this was the other way around. No complete responses (CR) were documented according to either method.

Treatment Efficacy

Twenty three patients were evaluable for the primary study endpoint (**Table 2**). 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 to 16.8 weeks), for

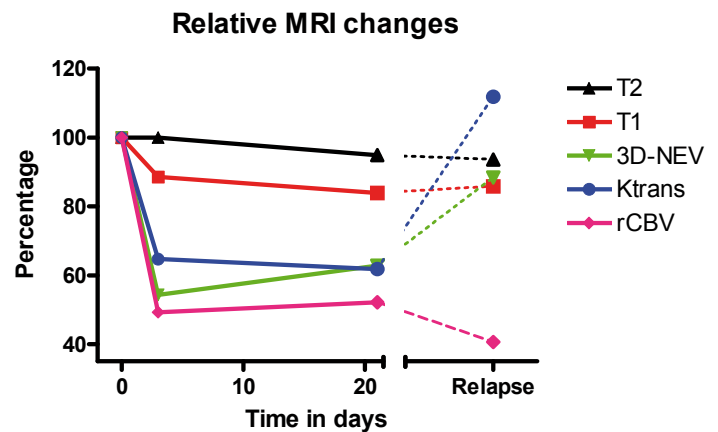


Figure 2 Relative percentage of median CE-T1, T2, 3D-NEV, rCBV and Ktrans of all dynamic scanned patients (n=21) at 4 time points: pre-treatment, day 3 and day 21, and during treatment at recurrence. After baseline measurement, all values (except those for T2) drop quickly as early as 72 hours after the start of experimental treatment, indicating a fast vascular response.

grade III and IV tumours 20.4 and 10.4 weeks, respectively (**Figure 1**). Median OS was 17.1 weeks (95% CI, 8.9 to 25.9 weeks), for grade III and IV tumours 32.4 and 15.7 weeks, respectively.

Using univariate analysis we evaluated patient characteristics for clinical outcome. Tumour grade was a strong prognostic factor for OS, with $p=0.004$ in favour of grade III patients (32.4 vs. 15.7 weeks median). Eighteen patients initially treated with corticosteroids had a shorter OS compared to 5 patients without need for corticosteroid treatment at inclusion (15.6 vs. 48.3 weeks median, $p=0.011$). Age under 55 years was a predictor for better OS (22.7 vs. 15.7 weeks median, $p=0.028$). Eleven patients with 2 or more surgical procedures had a higher OS (14.7 vs. 32.4 weeks median, $p<0.001$). In contrast, the number of prior chemotherapy regimens did not influence survival.

Changes of dynamic MRI parameters

During the first month 53% of the patients showed a decrease of more than 50% of the initial rCBV value (a measure of tumour blood volume, thus a mirror of vascularisation). Compared to baseline, the median decrease of rCBV on day 3 was 63% and 52% for grade III and IV tumours, respectively (**Supplemental table 2**). For K^{trans} (a measure of tissue permeability) an overall response rate of 56% was observed, defined as a decrease of more than 50% measured at day 3 and 21. The median decrease of K^{trans} on day 3 was 46% and 53% for grade III and IV tumours, respectively. rCBV and K^{trans} values at day 21 did not differ from day 3 values. At relapse, while treated with bevacizumab, rCBV levels remained low whereas K^{trans} increased to levels comparable to baseline (**Figure 2, Supplemental table 2**).

Predictive value of MRI responses and dynamic MRI parameters

Radiographic responses and the dynamic MRI parameters (rCBV and K^{trans}) were analysed for their predictive value on survival. No relationship was observed between early (day 3 and 21) radiographic responses (by Macdonald or Sorenson criteria) and disease outcome.

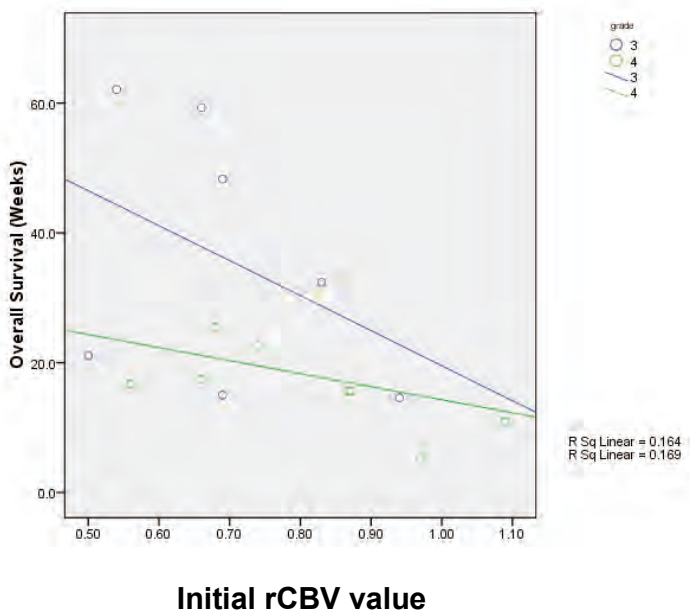
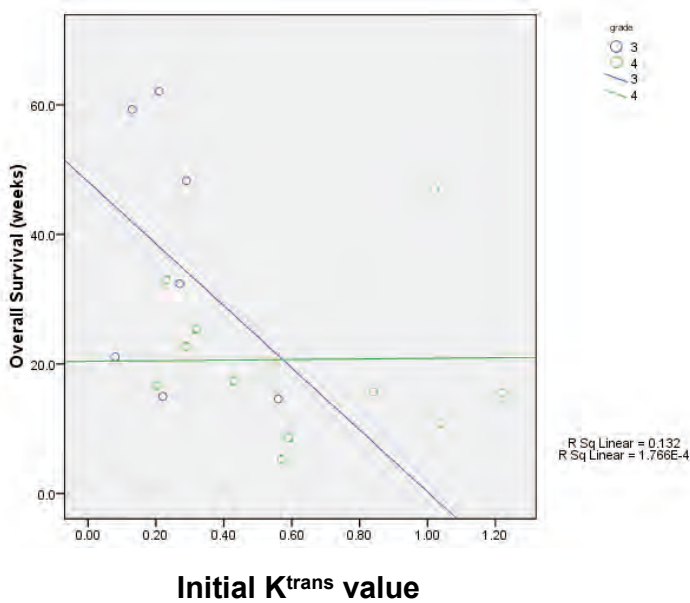


Figure 3 Overall survival (OS) in weeks versus initial Ktrans (A) and initial rCBV (B) values, prior to start of experimental treatment. For grade III patients, a higher Ktrans corresponds with lower OS. In all patients, but especially grade III patients, a higher rCBV corresponds with lower OS. Blue dots indicate grade III, and green dots grade IV patients. Trendlines are also shown.

A correlation was observed between initial K^{trans} values and survival ($p < 0.03$) for grade III (slope = 0.13), but not for grade IV tumours (slope = 0.00) (Figure 3a). In both groups survival correlated with rCBV ($p < 0.03$; grade III slope = 0.17; grade IV slope = 0.16, Figure 3b). Absolute or relative changes in rCBV and K^{trans} values by treatment did not correlate with survival.

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 2 patients at day 21. This phenomenon of oedema reduction occurred later than the rapidly observed

decrease in CE-T1 volume and rCBV and K^{trans} values at day 3. At relapse the oedema reduction persisted (Figure 2).

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 4). At relapse, during bevacizumab treatment, 3D-NEV increased towards baseline volume and K^{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 postmortem 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 performed 4 weeks earlier (Figure 5).

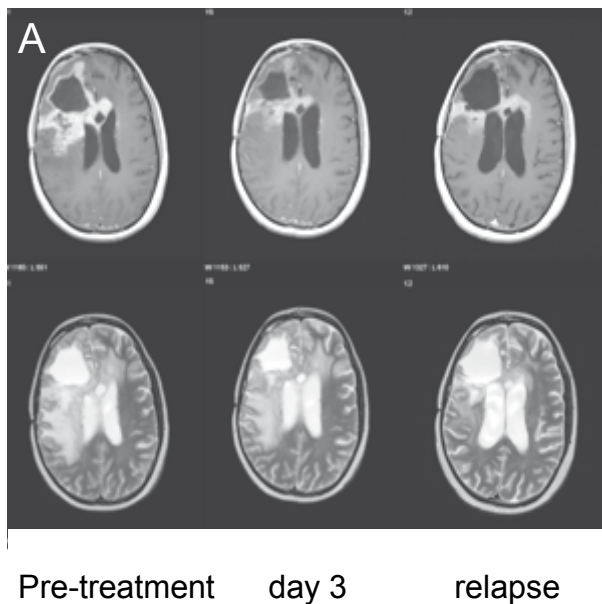
discussion

Response rates up to 57% have been reported in patients with relapsed HGG treated with combined bevacizumab and irinotecan [19,20,23-28,34]. These studies also demonstrated longer median PFS (14 - 24 weeks), and 6-months PFS (17% - 46%), compared with historical controls. Although some have reported a relatively long median OS (34 - 42 weeks) [19,23,34], Norden et al. demonstrated a longer median PFS (8 vs. 22 weeks), but not OS (39 vs. 37 weeks), when comparing recurrent GBM patients treated with anti-angiogenic therapy to conventional chemotherapy [35].

In the present study we evaluated the efficacy of the combination of bevacizumab and continuous dose-intense TMZ in relapsed HGG patients. Our data demonstrate that this treatment is feasible and well tolerated in heavily pre-treated patients with recurrent HGG. Overall, we observed major adverse events in 2 patients (deep venous thrombosis; intra-tumoral haemorrhage grade 4 with pulmonary embolism), both resulting in discontinuation of study treatment. These toxicity data are comparable with the reported side effects in previous studies with bevacizumab and irinotecan.

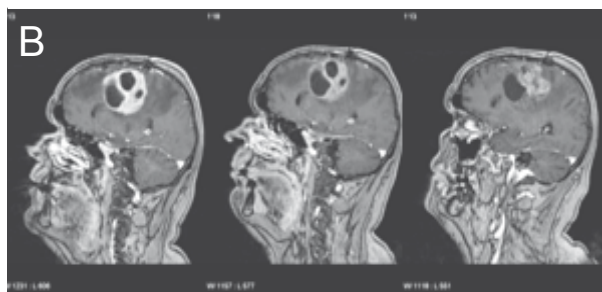
The response data (CE-T1, 20%) in our study are comparable to the ORR reported for single-agent therapy [19] but they are considerably lower than the reported response rates up to 57% in previous studies for bevacizumab combined with chemotherapy, i.e. irinotecan [23,26,27,34]. An explanation for this 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 Sorenson et al. [33], revealed a much higher decrease in enhancing volume. This 3-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 intra-tumoral cavities and necrotic

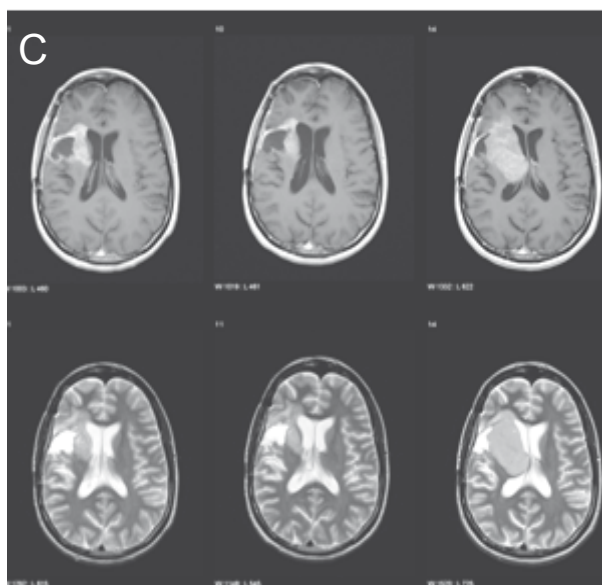


CE-T1

T2



CE-T1



CE-T1

T2

Figure 4 Three typical patients (a, b, c) scanned prior to 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.

tissue. Nevertheless, the rapid change in tumour-enhancing area or volume by anti-angiogenic therapy makes it highly improbable that this phenomenon represents a real anti-tumour 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 to the above-mentioned studies with bevacizumab and irinotecan. Because of the direct vascular effects and reduced permeability produced by anti-angiogenic 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 based on changes of enhancing tumour areas or volumes is difficult and not useful for the evaluation of anti-angiogenic therapy in HGG. Unlike progression-free survival, overall survival is the most robust endpoint, not affected by misinterpretations of anti-angiogenic 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 [19,23,24,27]. 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. resulting in 30 weeks for grade IV tumours treated with second-line chemotherapy alone [36].

Although we were not able to demonstrate obvious differences in patient characteristics of the included patients between our study and other studies, patient selection may be an explanation for our inferior results [37]. Additional factors may be the kind of chemotherapy combined with bevacizumab, and the schedule of bevacizumab administration. An alternative explanation for the negative results in this study may be that temozolomide actually negates the benefits of bevacizumab. The assumption that continuous dose-intense TMZ, in the present schedule, may be effective in TMZ pre-treated patients is not supported by the results of the present trial. Several preclinical/ and clinical observations suggest that the type of chemotherapy used in bevacizumab-containing regimens affects the anti-cancer effect. The initial enthusiasm amongst clinicians about the impressive bevacizumab-induced survival prolongation in patients with metastatic colorectal cancer, based on a randomized study comparing bolus 5-FU/irinotecan (IFL schedule) with and without bevacizumab [38], has been tempered considerably after the disappointing results of a randomized study comparing bevacizumab in an oxaliplatin schedule with infusional 5-FU [39]. Therefore, the kind of chemotherapy and schedule used in bevacizumab-containing regimens seems to be important for outcome.

Although the study of Mathieu et al. exploring bevacizumab and temozolomide in orthotopic glioma models suggests a beneficial effect of this combination [40], other preclinical studies with anti-angiogenic therapy do not. The study of Claes et al. demonstrates that the VEGFR-2 inhibitor vandetanib in intra-cerebral GBM xenografts antagonises the anti-tumour effect of TMZ [41]. In addition the study of Ma et al. shows that the anti-angiogenic compound TNP-470 leads to a reduced uptake of TMZ in intra-cerebral xenografts [42]. It is known that the intra-tumoral concentration of cytotoxic drugs is affected by vessel permeability, interstitial pressure in the tumour and, in case of brain tumours, also

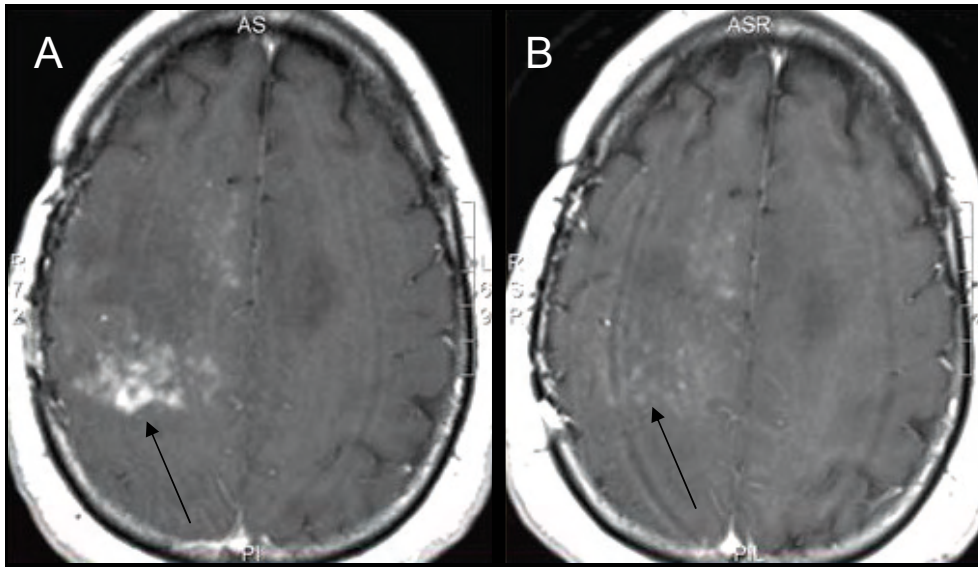


Figure 5 a) Pre-treatment CE-T1 MRI scan of a patient (PR) treated with bevacizumab and metronomic-dose TMZ; postmortem analysis showed an extensive infiltrative disease with tumour cells spreading into the contralateral hemisphere; b) during treatment, 4 weeks prior to CE-T1 MRI, the primary tumour site showed almost no contrast enhancement.

by the blood brain barrier (BBB) [43,44]. Several preclinical studies demonstrated that antiangiogenic therapy results in decreased interstitial tumour pressure and increased uptake of drugs, as has been demonstrated for irinotecan in human rectal carcinoma [38]. This may partially explain the superiority of combined treatment over bevacizumab alone in solid tumours. Because irinotecan as monotherapy has minimal activity in HGG, higher irinotecan concentrations in HGG in areas with persistently leaking BBB under antiangiogenic therapy may also contribute to the increased anti-tumour activity of irinotecan in HGG. Interestingly, Cloughesy et al. did not find additional value for combined irinotecan (OS 8.7 vs. 9.7 months) over single-agent bevacizumab [19]. To what extent decreased interstitial tumour pressure and changed uptake of drugs also plays a role in the concentration of temozolomide in HGG tumours is unknown, although the study of Ma et al. suggests that this is not the case [42]. Otherwise, a decrease in vessel permeability may even lead to lower TMZ concentrations in the tumour.

In the present study we measured K^{trans} (measure for permeability and/or blood flow) and rCBV (measure for tumour blood volume) at baseline and at various time points during treatment and at relapse (**Supplemental table 2**). Measurements at baseline and day 21 were performed just prior to the next bevacizumab administration. Compared to baseline, these two parameters were decreased considerably at day 3 and 21 thus supporting the evidence that bevacizumab rapidly affects permeability and blood flow, and is active during 3 weeks. Weak correlations were observed between initial rCBV (grade III) and K^{trans} (grade III and IV) values and survival, whereas the decrease in these values during treatment did not correlate with survival at all. Although these dynamic MRI values and changes are interesting and may offer insight into the activity of anti-angiogenic drugs on vessel permeability and blood flow, we were not able to observe a strong predictive value for an anti-tumour effect. These data are in agreement with the observation that also early responses on CE-T1, determined by anti-angiogenic therapy-induced vascular changes, are also not predictive for

outcome.

At relapse rCBV stayed low whereas K^{trans} increased dramatically. An explanation for this discrepancy at relapse is that the vascularisation status (rCBV) did not change, but that vessel permeability (K^{trans}) increased dramatically, and in a larger area. This effect may possibly be somewhat over-exaggerated, because here the timing of MR acquisition with respect to the last bevacizumab administration was less well defined compared to the time points at day 3 and day 21. Therefore, the mean time between last bevacizumab infusion and dynamic acquisition was larger, possibly leading to a decreased anti-permeability effect of bevacizumab.

New enhancing brain lesions were observed in 17% of our patients, more than observed in historical controls [24]. Other clinical studies also revealed that HGG recurs (mainly invasive) with more distant metastases when angiogenesis is inhibited [24,45].

conclusion

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 anti-angiogenic treatment is effective against the leaky bulk of the tumour, but not against invasive tumour components.

Furthermore, when the disappointing survival compared to TMZ mono-treatment (15.7 vs. 30 weeks) is taken into account, the reduced survival time may even indicate that anti-angiogenic 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 anti-angiogenic therapy) are necessary to define the role of this new treatment strategy and to overcome the problem of treatment bias in patients with HGG.

Table 1: patient 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			
Males	5	10	15
Females	3	5	8
% Males	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

Table 2: patient response

Characteristic	Value		
	Grade III	Grade IV	Overall
Total number of patients	8	15	23
CE-T1 ORR in %			
Day 3	37	7.7	19
Day 21	25	16.7	20
3D-NEV ORR in %			
Day 3	50	46	47
Day 21	37	58	50
PFS6 in %	37.5	6.7	17.4
OS6 in %	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)

ORR = overall response rate

PFS = progression-free survival

OS = overall survival

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Supplemental data

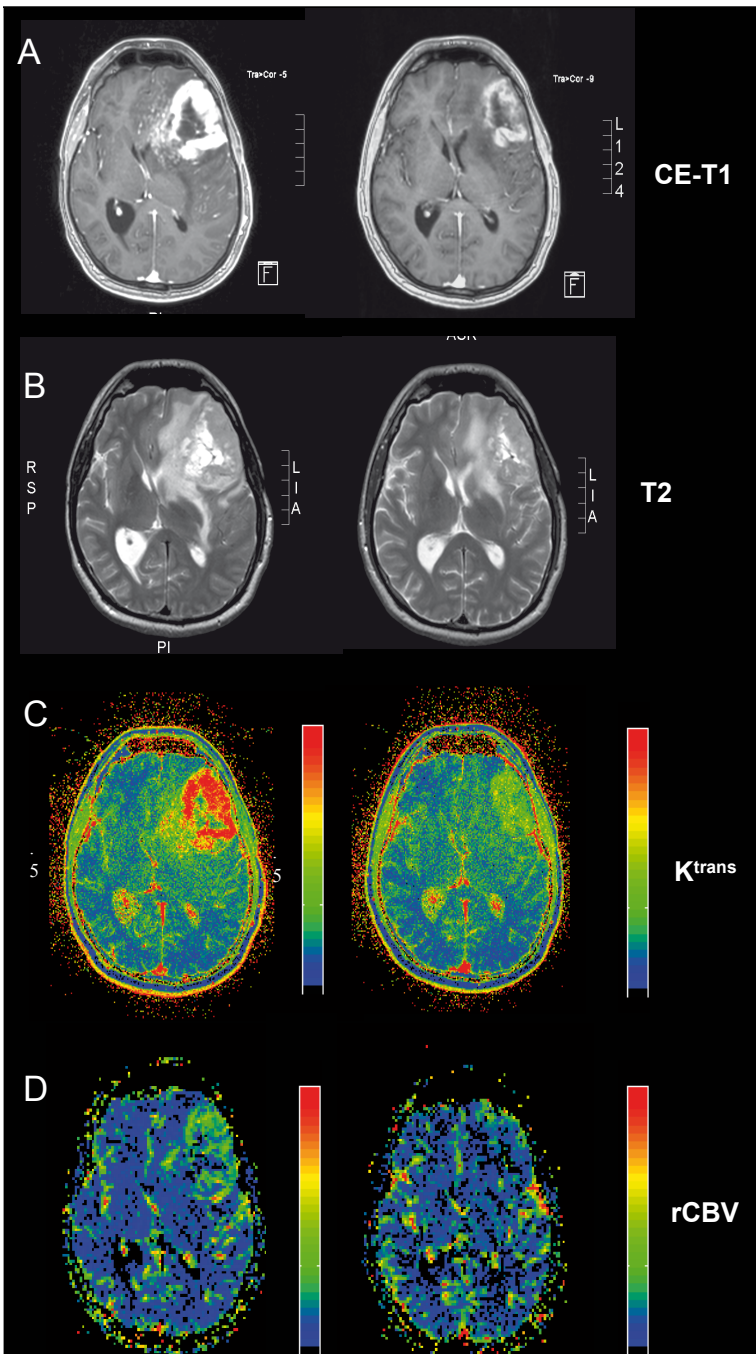
Supplemental Table 1: Previous treatment regimens

Previous treatment regimens	Value		
	Grade III	Grade IV	Overall
Total number of patients	8	15	23
Resection, chemo-irradiation (60Gy + TMZ), followed by 6 mo TMZ		12	12
Idem, followed by re-resection	4	2	6
Idem, followed by re-resection and 2 nd line chemotherapy	3		3
Resection, irradiation, followed by re-resection + TMZ, followed by 2 nd line chemotherapy	1	1	2

Supplemental Table 2: conventional and dynamic MRI responses

Characteristic	Value		
	Grade III	Grade IV	Overall
Total number of patients	8	15	23
T1 Cross-sectional product			
Day 0 value (%)	30.7 (100.0)	18.3 (100.0)	25.4 (100.0)
Range	4.6 - 71.6	4.2 - 56.2	4.2 - 71.6
Day 3	26.9 (87.6)	15.8 (86.3)	22.5 (88.6)
Range	1.9 - 51.8	2.8 - 40.9	1.9 - 51.8
Day 21	25.4 (82.7)	14.9 (81.4)	21.3 (83.9)
Range	1.4 - 54.8	2.3 - 41.0	1.4 - 54.8
At relapse	20.5 (66.8)	21.8 (119.1)	21.8 (85.8)
Range	3.3 - 62.3	5.7 - 59.0	3.3 - 62.3
T2 Cross-sectional product			
Day 0 value (%)	47.8 (100.0)	36.9 (100.0)	39.2 (100.0)
Range	11.0 - 138.8	19.0 - 65.0	11.0 - 138.8
Day 3	51.1 (106.9)	36.9 (100.0)	39.2 (100.0)
Range	8.8 - 138.8	19.0 - 58.1	8.8 - 133.0
Day 21	43.3 (90.6)	32.0 (86.7)	37.2 (94.9)
Range	10.3 - 136.4	12.8 - 56.2	10.3 - 136.4
At relapse	44.6 (93.3)	37.2 (100.8)	36.7 (93.8)
Range	5.9 - 155.9	16.3 - 77.0	5.9 - 155.9
T1 Net-Enhancing Volume			
Day 0 value (%)	21.3 (100.0)	16.4 (100.0)	16.4 (100.0)
Range	3.4 - 50.2	2.8 - 63.5	2.8 - 63.5
Day 3	8.3 (39.0)	8.9 (54.3)	8.9 (54.3)
Range	0.9 - 39.1	1.9 - 42.8	0.9 - 42.8
Day 21	11.6 (54.5)	9.7 (59.1)	10.3 (62.8)
Range	2.6 - 47.9	0.3 - 39.5	0.3 - 47.9
At relapse	15.7 (73.7)	14.1 (86.0)	14.5 (88.4)
Range	0.3 - 68.4	2.8 - 56.2	0.3 - 68.4
Maximum Enhancement			
Day 0 value (%)	0.74 (100.0)	0.74 (100.0)	0.74 (100.0)
Range	0.42 - 1.18	0.54 - 1.35	0.42 - 1.35
Day 3	0.50 (67.6)	0.56 (75.7)	0.56 (75.7)
Range	0.38 - 0.66	0.46 - 0.93	0.38 - 0.93
Day 21	0.55 (74.3)	0.55 (74.3)	0.55 (74.3)
Range	0.47 - 0.72	0.50 - 0.93	0.47 - 0.93
At relapse	0.54 (73.0)	0.60 (81.1)	0.59 (79.7)
Range	0.38 - 0.66	0.48 - 0.93	0.38 - 0.93

Characteristic		Value		
		Grade III	Grade IV	Overall
Tumour rCBV	Day 0 value (%)	0.68 (100.0)	0.86 (100.0)	0.69 (100.0)
	Range	0.50 - 0.94	0.56 - 1.09	0.50 - 1.09
	Day 3	0.25 (36.8)	0.41 (47.7)	0.34 (49.3)
	Range	0.12 - 0.68	0.14 - 0.92	0.12 - 0.92
	Day 21	0.33 (48.5)	0.42 (48.8)	0.36 (52.2)
	Range	0.10 - 0.93	0.16 - 0.74	0.10 - 0.93
At relapse	Range	0.23 (33.8)	0.32 (37.2)	0.28 (40.6)
	Range	0.13 - 0.38	0.18 - 0.60	0.13 - 0.60
Tumour K^{trans}	Day 0 value (%)	0.24 (100.0)	0.58 (100.0)	0.34 (100.0)
	Range	0.08 - 0.56	0.12 - 1.22	0.08 - 1.22
	Day 3	0.13 (54.2)	0.27 (46.6)	0.22 (64.7)
	Range	0.04 - 0.72	0.07 - 0.88	0.04 - 0.88
	Day 21	0.12 (50.0)	0.22 (37.9)	0.21 (61.8)
	Range	0.01 - 0.77	0.10 - 0.79	0.01 - 0.79
At relapse	Range	0.24 (100.0)	0.50 (86.2)	0.38 (111.8)
	Range	0.13 - 1.09	0.15 - 1.01	0.13 - 1.09



Supplemental Figure S1

a) CE-T1, b) T2, c) Colour maps of K^{trans} and d) colour maps of rCBV values for one patient during pre-treatment (left column) and during initial treatment phase (right column). Colour coded maps, K^{trans} values expressed in min^{-1} .