original article

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Stratified phase II trial of cetuximab in patients with recurrent high-grade glioma

B. Neyns[1]*, J. Sadones[1], E. Joosens[2], F. Bouttens[3], L. Verbeke[4], J.-F. Baurain[5], L. D'Hondt[6], T. Strauven[7], C. Chaskis[8], P. In't Veld[9], A. Michotte[10,11] & J. De Greve[1]

[1]*Department of Medical Oncology, UZ Brussel, Brussels;* [2]*Department of Medical Oncology, ZNA Middelheim, Antwerp;* [3]*Department of Radiation therapy, AZ St Lucas, Ghent;* [4]*Department of Radiation therapy, OLV Aalst, Aalst;* [5]*Department of Medical Oncology, Cliniques Universitaires St Luc, Brussels;* [6]*Department of Medical Oncology, Centre Hospitalier Notre-Dame et Reine Fabiola, Charleroi;* [7]*Department of Neurology, Sint-Augustinus, Antwerp, Departments of* [8]*Neurosurgery;* [9]*Experimental pathology;* [10]*Neurology and* [11]*Neuropathology, UZ Brussel, Brussels, Belgium*

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Background: To evaluate the antitumor activity and toxicity of single-agent cetuximab in patients with recurrent high-grade glioma (HGG) after failure of surgery, radiation therapy, and chemotherapy.

Patients and methods: In this two-arm, open-label, phase II study patients were stratified according to their *epidermal growth factor receptor* (*EGFR*) gene amplification status. Cetuximab was administered intravenously at a dose of 400 mg/m^2 on week 1 followed by weekly dose of 250 mg/m^2. The primary end point for this study was the response rate in both study arms separately.

Results: Fifty-five eligible patients (28 with and 27 without EGFR amplification) tolerated cetuximab well. Three patients (5.5%) had a partial response and 16 patients (29.6%) had stable disease. The median time to progression was 1.9 months [95% confidence interval (CI) 1.6–2.2 months]. Whereas the progression-free survival (PFS) was <6 months in the majority (n = 50/55) of patients, five patients (9.2%) had a PFS on cetuximab of >9 months. Median overall survival was 5.0 months (95% CI 4.2–5.9 months). No significant correlation was found between response, survival and *EGFR* amplification.

Conclusions: Cetuximab was well tolerated but had limited activity in this patient population with progressive HGG. A minority of patients may derive a more durable benefit but were not prospectively identified by *EGFR* gene copy number.

Key words: cetuximab, EGFR, glioblastoma

original article

introduction

Mutation and/or gene amplification of the *epidermal growth factor receptor* (*EGFR*) or *Her2/neu* gene (both members of the EGFR family of transmembrane tyrosine kinase receptors) is frequently found in human epithelial cancer cells [1, 2]. These acquired genetic alterations lead to hyperactivity of EGFR-regulated intracellular signaling pathways among which are the phosphoinositide 3-kinase (PI3K)/Akt and mitogen-activated protein kinases (MAPK) pathway. This results in an increase in cancer cell motility, invasion, and proliferation as well as an inhibition of apoptosis and induction of angiogenesis [1].

EGFR signaling can be inhibited with targeted monoclonal antibodies (mAbs) (e.g. cetuximab and panitumumab) or small-molecule tyrosine kinase inhibitors (smTKIs) [2]. mAbs have demonstrated activity in *KRAS* wild-type colorectal cancer, head and neck squamous cell carcinoma and non-small-cell lung cancer (NSCLC) [2, 3]. Across different tumor types, an increased *EGFR* gene copy number has been correlated with sensitivity to EGFR-targeted treatment and sensitivity to the Her2/neu-targeted mAb trastuzumab is strictly correlated with an increased gene copy number [4–6]. Activity of smTKIs is associated with the presence of specific point mutations within the ATP pocket of the EGFR as found in a small subgroup of patients with NSCLC [7, 8].

Patients with recurrent high-grade glioma (HGG) following prior surgery, radiation therapy (RT) and alkylating chemotherapy have a poor prognosis (median survivals are 39 and 30 weeks for patients with grade III and grade IV glioma, respectively) and no controlled trial has demonstrated to improve survival [9]. Among all cancers, an increased copy number of the *EGFR* gene has been observed most frequently in glioblastoma and anaplastic astrocytoma. Most often these amplified sequences are present in a very high copy number on 'double minute chromosomes' and they contain mutated *EGFR* sequences with the constitutively activated extracellular domain deletion mutant *EGFRvIII* being the most common [10, 11].

*Correspondence to: Prof. B. Neyns, Department of Medical Oncology, UZ Brussel, Laarbeeklaan 101, 1090 Brussels, Belgium. Tel: +3224776415; Fax: +3224776012
E-mail: bart.neyns@uzbrussel.be

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The *EGFR* gene copy number may vary substantially in individual cells of a glioma with *EGFR*-amplified cells, but the presence of the *EGFRvIII* mutant has been demonstrated in glioma stem cells [12]. The *EGFRvIII* mutant defines anaplastic astrocytoma with a worse prognosis and a clinically distinct subtype of glioblastoma [13–16]; in addition, high EGFR expression is an independent prognostic factor in newly diagnosed patients with glioblastoma treated with RT and temozolomide [17]. Therefore, EGFR represents a particularly attractive molecular target in patients with recurrent HGG.

Disappointing results were obtained with the EGFR inhibitors erlotinib and gefitinib throughout a number of phase II clinical trials in patients with recurrent glioblastoma or newly diagnosed glioblastoma [18–22]. Only a small subpopulation experienced durable disease control. However, interpreting these results one needs to consider the absence of activating mutations within the tyrosine kinase domain of *EGFR* in HGG and the uncertainty regarding the extent of *EGFR* inhibition in glioma tissues [23, 24]. Although a number of molecular markers (including phosphatase and tensin homology (PTEN), EGFRvIII and phosphorylated Akt) have been suggested to predict sensitivity, these observations have not been reproduced across studies and therefore remain of uncertain value today [19, 22, 25, 26].

The use of an mAb in the treatment of recurrent glioma seems counterintuitive because these large molecules do not readily cross an intact blood–brain barrier. Preclinical models, however, support the use of cetuximab (Erbitux®, Merck KGaA, Darmstadt, Germany), a chimeric IgG1 mAb that binds to EGFR with high specificity and affinity, as an active antiglioma agent and cetuximab is more efficient in inhibiting the EGFRvIII mutant as compared with the small-molecule EGFR inhibitors [27–29]. Phase I studies with a nonhumanized anti-EGFR mAb demonstrated the presence of mAb within resected glioma tissues [30, 31], and the EGFR-targeted mAb nimotuzumab and CD20-targeted mAb rituximab have demonstrated therapeutic activity against pediatric pontine glioma and primary central nervous system lymphoma, respectively [32–35]. Recently, patients with recurrent HGG responding to cetuximab treatment have been reported [36].

We hypothesized that the amplification status of the *EGFR* gene in HGG might determine the sensitivity to cetuximab, an IgG1 chimeric mAb directed against the *EGFR*, and therefore conducted a phase II clinical trial in which patients were stratified at baseline according to the amplification status of the *EGFR* gene determined by FISH (*EGFR* FISH).

patients and methods

study entry criteria and *EGFR* FISH

To be eligible, patients had to have histological documentation of supratentorial World Health Organization (WHO) grade III or IV glioma. In addition, determination of the *EGFR* gene copy number of the glioma cells by FISH in a single reference laboratory (UZ Brussel) was required. *EGFR* FISH results were classified according to published criteria [37]. This analysis was carried out on archival formalin-fixed, paraffin-embedded glioma tissue blocks using a commercially available kit (LSI *EGFR*/CEP 7 Dual Color Probe, Abbott Laboratories, Abbott Park, IL) according to the manufacturer's instructions. Histologic diagnosis was confirmed by central pathology review carried out by AM.

Patients were required to have demonstrated evidence of progressive disease (PD) on magnetic resonance imaging (MRI) of the brain following prior surgery, RT and chemotherapy (including temozolomide), a Karnofsky performance status (KPS) of ≥60% at study entry, and an absolute neutrophil count of 1500 cells/mm^3, a platelet count of 100 000 cells/mm^3, a white blood cell count of 3500 cells/mm^3, and a hemoglobin level of 9 g/dl. The total serum bilirubin level was required to be 1.5× the upper limit of normal and the alkaline phosphatase and aspartate aminotransferase/alanine aminotransferase levels were required to be 5× the upper limit of normal. The serum creatinine level was required to be <1.5× the upper limit of normal. Patients were required to have recovered from toxic effects of prior surgery, RT, and chemotherapy and may not have had either RT or a systemic drug therapy within 4 weeks of initiating cetuximab. Patients were required to be on a stable dose of corticosteroids for at least 2 weeks before initiating cetuximab.

This protocol was reviewed and approved by the institutional review board of each participating center, and all patients gave written informed consent before participation.

treatment plan

All patients received an initial dose of cetuximab 400 mg/m^2 administered i.v. during 120 min followed by weekly doses of cetuximab at 250 mg/m^2 during 60 min. Patients received premedication before the administration of cetuximab consisting of 10 mg oral cetirizine (or a similar oral antihistamine). Treatment was continued until disease progression, unacceptable toxicity or patient's refusal. Gadolinium-enhanced MRI of the brain and response assessments (based on Macdonald criteria for response evaluation in central nervous system glioma) were carried out at least every 8 weeks [38]. In the event of a grade 3 adverse event (AE), the dose of cetuximab was withheld until recovery to grade ≤2 or for a maximum of 2 weeks and resumed thereafter at the prior dose. For a second grade 3 event, cetuximab was reduced one dose level, and for a third event, cetuximab was reduced two dose levels (first to 200 mg/m^2, then to 150 mg/m^2). In the event of a hypersensitivity reaction of grade 1 or 2, the cetuximab infusion was stopped and the patient was treated as appropriate. The infusion could be resumed at 50% of the previous rate once the event resolved or decreased to grade 1 in severity. If a second hypersensitivity reaction was experienced, treatment was stopped and the patient was removed from the study. For a grade 3 or 4 hypersensitivity reaction, the patient was treated as appropriate and taken off study with no additional cetuximab treatment.

study design and statistical analysis

This was an open-label, nonrandomized, stratified, two-stage, phase II trial conducted at seven different Belgian medical centers. The primary objective of this study was to determine the tumor response rate for single-agent cetuximab separately in patients with *EGFR* gene-amplified and -nonamplified glioblastoma. We therefore conducted a stratified, two-arm, phase II, open-label, nonrandomized study according to a Simon's two-stage Minimax design (alfa 0.05 and beta 0.2), with separate decision rules for *EGFR* gene-amplified and -nonamplified patients. An accrual of response-assessable patients per subgroup was calculated for a total of 2 × 27 patients on the study. For each subgroup, 13 response-assessable patients were to be accrued to the first stage. If no responses were observed in that subgroup, no additional patients would be accrued, and it would be concluded that the true response rate is unlikely to be 5% for that subgroup. Otherwise, if at least one response was seen in the subgroup, accrual to that subgroup would continue to the second stage (total 27

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patients). If at least four responses were seen in a subgroup, we would conclude that cetuximab has sufficient activity for further study in this setting.

Secondary objectives were to determine the time to tumor progression (TTP), progression-free survival (PFS; from the date of recruitment to documented progression or death), and overall survival (OS) and safety within each subgroup and for the overall population. Demographic and baseline laboratory results were summarized using descriptive statistics for all treated patients. An exact two-sided 95% Clopper–Pearson confidence interval (CI) was computed for each response rate. For the survival and time to progression, Kaplan–Meier estimates were calculated; and 6-month survival percentages and medians with two-sided 95% CIs are reported. Worst toxicity grades (using National Cancer Institute—Common Toxicity Criteria, Version 3.0) per patient were tabulated for selected AEs and laboratory measurements.

results

patients

From May 2005 to December 2007, 114 glioma samples from 106 patients were screened with *EGFR* FISH. In four patients, no informative FISH test result could be obtained because of the poor quality of the archival tumor material. Consecutive enrollment of 27 patients without *EGFR* gene amplification was completed in March 2007 and enrollment of 28 patients with *EGFR* gene amplification was completed in November 2007. Patient demographics and disease characteristics are summarized in Table 1.

The patient study population with *EGFR* gene amplification on average was older (median 50 versus 57 years); more patients were diagnosed with *de novo* glioblastoma (79% versus 63%), had received upfront combination of radiation and temozolomide (86% versus 63%), and were recruited at first recurrence (57% versus 22%) as opposed to patients with nonamplified *EGFR* status who were more often treated at second or subsequent recurrences.

duration of treatment and dose intensity of cetuximab

At the time of this analysis (1 May 2008), a total of 815 cetuximab administrations were carried out. The median number of administrations per patient was 9 (range 2–86 administrations; only two patients received less than four administrations). Only two patients required one dose reduction of cetuximab (to 200 mg/m^2 weekly) because of treatment-limiting skin toxicity. The primary reason for discontinuing treatment was disease progression (48 patients).

efficacy

response rate. All patients who received any cetuximab on this trial were considered assessable for efficacy. Of the 55 patients treated, three obtained a partial response (PR) and 17 patients obtained a stable disease (SD) for a disease control rate (PR + SD) of 36% [95% exact CI 23% to 49%; based on investigator assessments]. As shown in Table 2, disease control rate was numerically superior in patients with *EGFR* gene amplification but the difference did not reach statistical significance. The extent of tumor regression was not clearly

Table 1. Baseline patient demographics and disease characteristics (n = 55)

	EGFR gene nonamplified (n = 27)	EGFR gene amplified (n = 28)	All patients, no. (%) (n = 55)
Gender			
Male	20	18	38 (69)
Female	7	10	17 (31)
Age, years			
Median	50	57	53
Range	32–73	38–69	32–73
Ethnicity			
Caucasian	26	27	53 (96)
African	1	1	2 (4)
KPS			
100	1	3	4 (7)
80–90	4	13	17 (31)
70–60	22	12	34 (62)
Histopathology at primary diagnosis			
De novo GBM	11	22	33 (60)
Secondary GBM	16	6	22 (40)
Prior diagnosis of low-grade or anaplastic glioma			
WHO grade 3 astrocytoma	6	6	12 (22)
WHO grade 3 oligoastrocytoma	5	0	5 (9)
WHO grade 2 astrocytoma	4	0	4 (7)
WHO grade 2 oligoastrocytoma	1	0	1 (2)
Surgery at diagnosis			
Complete	9	15	24 (44)
Partial	10	6	16 (29)
Stereotactic biopsy	7	4	11 (20)
Unknown extent of resection	1	3	4 (7)
Radiation and adjuvant chemotherapy			
RT with concomitant + adjuvant temozolomide	17	24	41 (74)
RT + adjuvant nitrosurea-based chemotherapy	3	1	4 (7)
RT only	7	3	10 (18)
No. of recurrences at initiation of cetuximab			
First	6	16	22 (40)
Second	13	9	22 (40)
Third	7	2	9 (16)
Fourth	1	1	2 (4)
EGFR gene copy number			
Amplified	0	28	28 (51)
Disomy	8	0	8 (15)
Low trisomy	9	0	9 (16)
High trisomy	1	0	1 (2)
Low polysomy	3	0	3 (5)
High polysomy	5	0	5 (9)
Not evaluable	1[a]	0	1[a] (2)

[a]This patient was referred as being nonamplified on EGFR FISH carried out at the referring center. The FISH test could not be repeated with success in the central laboratory.

EGFR, epidermal growth factor receptor; GBM, glioblastoma; KPS, Karnofsky performance status; WHO, World Health Organization; RT, radiation therapy.

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correlated with the duration of disease control as all three patients who obtained a PR experienced PD within 4 months from the initiation of cetuximab (Figures 1 and 2). An improvement or stabilization of disease-related symptoms was observed in patients experiencing disease control (investigator assessment). Patients with an initial diagnosis of low-grade glioma or anaplastic glioma without *EGFR* amplification seemed to benefit less as compared with patients with *de novo* glioblastoma or anaplastic astrocytoma with *EGFR* amplification (2 of 10 versus 18 of 45 patients experienced disease control).

Table 2. Efficacy of cetuximab in patients with and without EGFR gene amplification

	EGFR amplified (n = 28), no. of patients (%)	EGFR nonamplified (n = 27), no. of patients (%)	All patients, no. (%) (n = 55)
PR	2 (7.1)	1 (3.7)	3 (5.5)
SD	10 (35.7)	7 (25.9)	17 (30.9)
DCR	12 (42.9)[a]	8 (29.6)[a]	20 (36.4)
PD	16 (57.1)	19 (70.4)	35 (63.6)

[a]Fisher's exact test (two sided) $P = 0.4$.
EGFR, epidermal growth factor receptor; PR, partial response; SD, stable disease; DCR, disease control rate (=CR + PR + SD); PD, progressive disease; CR, complete response.

median time to progression, PFS, and OS. The median TTP was 1.9 months (95% exact CI 1.6–2.2 months) and the 6-month PFS percentage was 7.3% (95% exact CI 0% to 14%). The median OS from time of initiation of protocol therapy for the 55 treated patients was 5.0 months (95% exact CI 4.2–5.9 months) and the 6-month survival percentage was 37.9% (95% exact CI 25% to 90%). As demonstrated in Table 3 and Figure 3, PFS and OS were numerically superior in patients with *EGFR* gene amplification but this difference did not reach statistical difference. Five patients had a PFS of >9 months on cetuximab treatment.

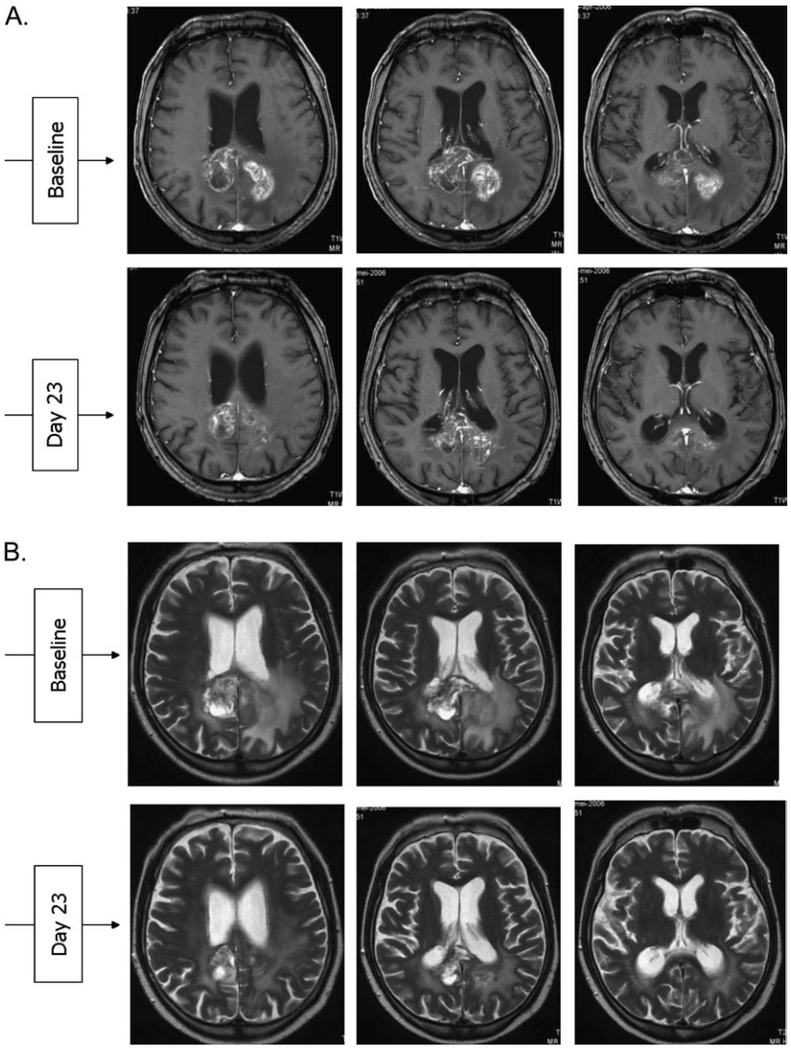

Figure 1. Baseline and post-treatment magnetic resonance imaging of a patient treated with cetuximab. Postcontrast axial (**A**) T1-weighted and (**B**) T2-weighted magnetic resonance scans in a patient with glioblastoma at baseline and at day 23 following the first administration of cetuximab.

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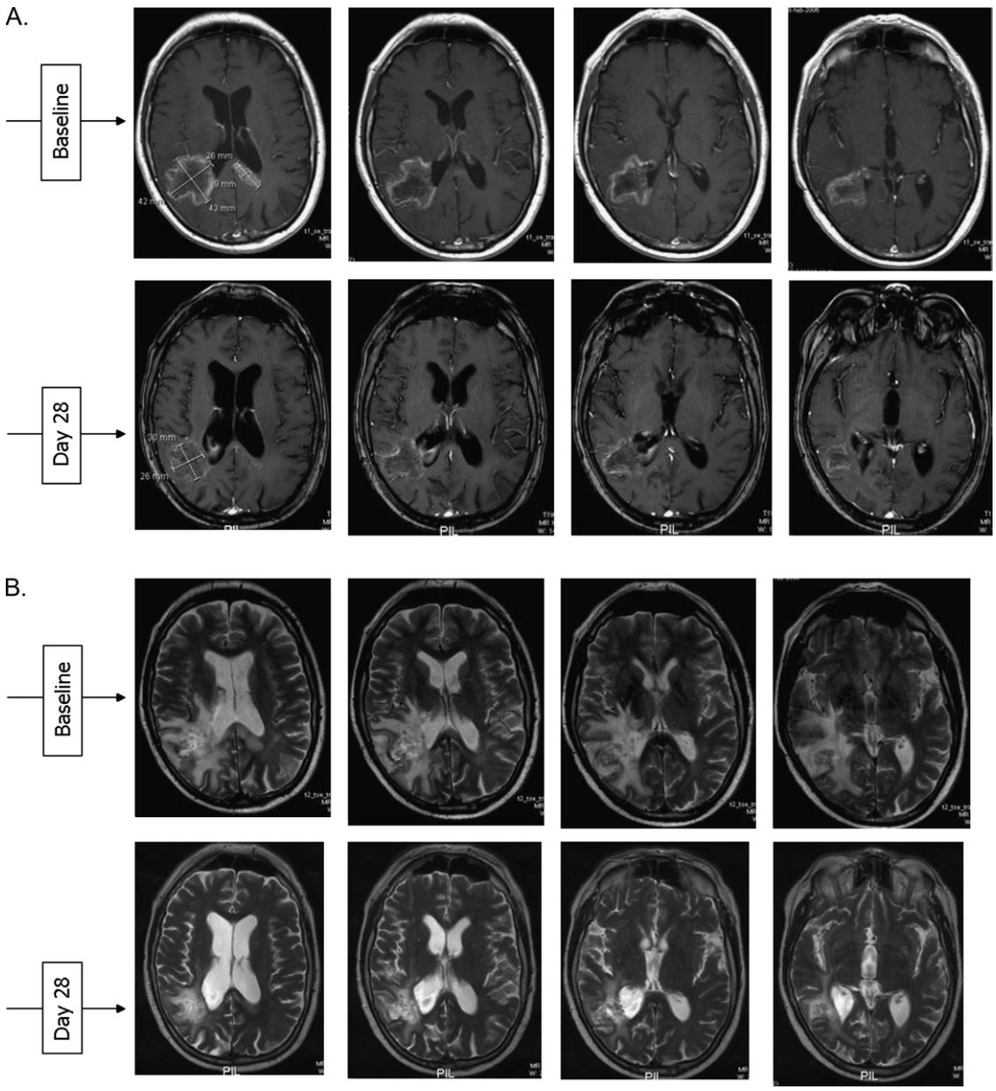

Figure 2. Baseline and post-treatment magnetic resonance imaging of a second patient treated with cetuximab. Postcontrast axial (**A**) T1-weighted and (**B**) T2-weighted magnetic resonance scans in a patient with glioblastoma at baseline and at day 28 following the first administration of cetuximab.

Table 3. Survival following cetuximab in patients with and without EGFR gene amplification

	EGFR nonamplified (n = 27)	EGFR amplified (n = 28)	All patients (n = 55)
Median PFS, months (95% CI)	1.8 (1.6–2.0)	1.9 (1.1–2.7)	1.9 (1.6–2.2)
6-Month PFS rate, % (95% CI)	11.1 (0–22)	7.1 (0–16)	7.3 (0–14)
Median OS, months (95% CI)	4.8 (3.7–5.9)	5.0 (3.9–6.1)	5.06 (4.2–5.9)
6-month OS rate, % (95% CI)	33.3 (15–51)	42.9 (24–61)	37.9 (25–50)

EGFR, epidermal growth factor receptor; PFS, progression-free survival; CI, confidence interval; OS, overall survival.

In an exploratory univariate analysis, patients who experienced grade 2 or 3 skin toxicity had a significantly better PFS and OS [median PFS was 1.7 months (95% CI 1.4–2.0) versus 2.9 months (95% CI 2.8–3.0) (log-rank P = 0.009) and the median OS was 4.6 months (95% CI 3.8–5.5) versus 9.7 months (95% CI 6.2–13.2) (log-rank P = 0.012) for patients who did not versus patients who did experience grade 2 or 3 skin toxicity, respectively]. No significant correlation was found between survival and age, KPS, initial WHO differentiation grade, gender, or the number of recurrences before the patient received cetuximab.

safety

Treatment was generally well tolerated and there were no grade 4 treatment-related AEs. Treatment-related toxicity data for all patients (n = 55) are summarized in Table 4.

Skin toxicity was the most frequently observed AE. Six patients (11%) developed an acne-like rash (folliculitis/

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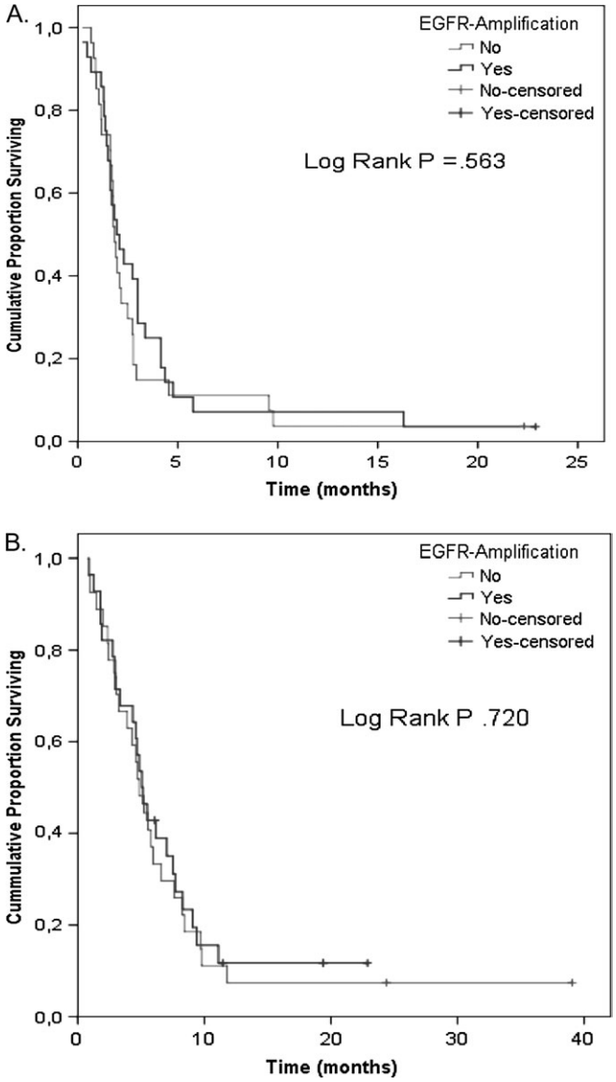

Figure 3. Kaplan–Meier curves of (**A**) progression-free survival and (**B**) overall survival according to epidermal growth factor receptor amplification status.

dermitis; grade 2 in four patients and grade 3 in two patients) that typically affected the face, scalp, and upper trunk. The intensity of this toxicity typically decreased when treatment was continued beyond 8 weeks and tended to fluctuate thereafter. Other skin toxic effects consisted of xerodermia (dry skin with exfoliation), paronichia (inflammation of the nail wall), fissures at the hands and/or feet, dermatitis of the eyelids, and increased facial hair growth. These side-effects were more often observed with prolonged treatment.

Three patients (5%) developed a subacute transient decrease in their consciousness that necessitated hospitalization within 24 h from their first cetuximab administration. All recovered within a few days and cetuximab could be readministered without repetition of the AE.

Hematologic toxicity was absent except for grade 2 or 3 thrombocytopenia which was observed in 10% of the patients.

Table 4. Cetuximab-related adverse events of CTCAEv3.0 grades 2 and 3 (worse toxicity per patient)

	No. of patients (%)	
	Grade 2	Grade 3
Skin toxicity	6 (11)	4 (7)
Thrombocytopenia	3 (5)	3 (5)
Infusion-related allergic reaction	1 (2)	0
Intratumoral hemorrhage	1 (2)	0
Diarrhea	1 (2)	0
Fatigue	3 (5)	0
Confusion/diminished consciousness	0	3 (5)
Fever	0	1 (2)

Based on a total of 815 administered treatment cycles [median number per patient 9 (range 2–86)].
CTCAEv3.0, Common Terminology Criteria for Adverse Events v3.0.

One patient experienced intratumoral hemorrhage (in the absence of thrombocytopenia or impaired coagulation) that was associated with a partial tumor response in the third week of treatment.

discussion

The results of this multi-institutional phase II trial indicate that cetuximab has a low single-agent activity in patients with recurrent HGG following surgery, RT, and chemotherapy. Two patterns of antitumor activity were observed. About 20%–30% of patients experienced disease control, with rapid regression of contrast-enhancing lesions in some, that was not sustained >5 months and seemingly did not translate into a marked improvement of survival. Additionally, a small percentage (5 of 55 patients, 9%) experienced a durable disease control (≥9 months) and improved OS as compared with the rest of the study population.

Of the five patients (9%) who had an outstanding favorable clinical evolution (PFS of ≥9 months), all except one had been diagnosed with radiological and clinical PD at >6 months after the administration of RT. It is therefore unlikely that they were diagnosed with pseudoprogression and therefore are considered to be true responders to cetuximab. The outcome of this small subgroup of patients might resemble the glioblastoma patients reported to derive a sustained benefit from the small-molecule *EGFR* inhibitors gefitinib or erlotinib [26]. No correlation was found in these five patients with the histology, WHO grade, or *EGFR* amplification status. We have attempted to characterize all tumors for additional molecular–genetic markers reported to correlate with the sensitivity to EGFR blockade in other studies (including EGFRvIII and PTEN). The accuracy and reproducibility of these immunohistochemical assays in our hands was, however, far below that of *EGFR* FISH and no reliable results could be obtained.

An improvement or stabilization of disease-related symptoms was observed in patients who experienced disease control. Patients with an *EGFR*-amplified glioma more frequently experienced disease control as opposed to patients

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without *EGFR* amplification (42.9 versus 29.6%) suggesting a correlation between the *EGFR* copy number and sensitivity to cetuximab. This difference, however, was not statistically significant and there was also an imbalance in baseline prognostic factors between both groups (the *EGFR*-amplified group consisted of more patients with '*de novo* glioblastoma', diagnosed at an older age, who were treated more frequently with combination radiotherapy and temozolomide upfront and received cetuximab more often at first recurrence because of progression during or early after administration of upfront combination therapy, but more patients in this group had a baseline KPS of >70%).

Treatment was generally well tolerated. The only frequent treatment-related toxicity was at the level of the skin. We observed the same correlation between the severity of the skin toxicity and survival as has been described in many other studies with cetuximab.

The median survival of our patient population was dismal and is illustrative of the aggressive nature of recurrent HGG that progresses despite surgery, radiation, and temozolomide chemotherapy. Although the disease control rate we observed was short lived in most patients, one needs to bear in mind the poor natural prognosis for these patients. The tumor response rate observed in this study is comparable to what has been observed with cetuximab as a single agent in the treatment of patients with previously treated NSCLC and the observed disease control rate (tumor response plus SD) with that observed in unselected patients with previously treated colorectal cancer [39–41]. Given the synergistic effect of EGFR inhibition with cetuximab and RT, further investigation of cetuximab in the upfront combination treatment of patients with newly diagnosed glioblastoma might be rewarding [18]. Early results of an ongoing phase II study on this combination for patients with newly diagnosed glioblastoma have been reported recently [42].

Recent insights into the biology of HGG revealed that multiple receptor tyrosine kinases are coactivated in these cancer cells and, in addition, a number of tumor suppressor genes such as the PTEN, p53, and p19 are lost [43–46]. Inhibitors targeting molecules within the PI3K pathway that themselves are not mutated, such as the mammalian target of rapamycin inhibitor temsirolimus, have been reported to have a variable activity across studies. Combination with EGFR-targeted agents is under investigation [47–49]. Proto-oncogenes that act within the PI3K- and MAPK-signaling pathways are involved by activating mutations including *PIK3CA, MET, NRAS, and BRAF* [50–53]. In analogy to the resistance to EGFR inhibitors observed in *KRAS* mutant colorectal and NSCLC, each of these mutations might be able to mediate resistance to EGFR inhibition in HGG. Evidence that the *MET* oncogene can mediate resistance to EGFR inhibition has recently been forwarded [43, 54]. Specific inhibitors targeting these oncogenes are under early clinical investigation. Besides *EGFR*, the frequency of mutation of these additional proto-oncogenes has been rather low and rational combination of targeted agents in glioblastoma might require individual genetic profiling, an objective that might become possible with the development of high-throughput genetic analysis [55–58].

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