

Phase II trial of dacomitinib, a pan–human EGFR tyrosine kinase inhibitor, in recurrent glioblastoma patients with *EGFR* amplification

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Abstract

Background. We conducted a multicenter, 2-stage, open-label, phase II trial to assess the efficacy and safety of dacomitinib in adult patients with recurrent glioblastoma (GB) and epidermal growth factor receptor gene (*EGFR*) amplification with or without variant III (*EGFR*VIII) deletion.

Methods. Patients with first recurrence were enrolled in 2 cohorts. Cohort A included patients with *EGFR* gene amplification without *EGFR*VIII mutation. Cohort B included patients with *EGFR* gene amplification and *EGFR*VIII mutation. Dacomitinib was administered (45 mg/day) until disease progression/unacceptable adverse events (AEs). Primary endpoint was progression-free survival (PFS; RANO criteria) at 6 months (PFS6).

Results. Thirty patients in Cohort A and 19 in Cohort B were enrolled. Median age was 59 years (range 39–81), 65.3% were male, and Eastern Cooperative Oncology Group Performance Status 0/1/2 were 10.2%/65.3%/24.5%, respectively. PFS6 was 10.6% (Cohort A: 13.3%; Cohort B: 5.9%) with a median PFS of 2.7 months (Cohort A: 2.7 mo; Cohort B: 2.6 mo). Four patients were progression free at 6 months and 3 patients were so at 12 months. Median overall survival was 7.4 months (Cohort A: 7.8 mo; Cohort B: 6.7 mo). The best overall response included 1 complete response and 2 partial responses (4.1%). Stable disease was observed in 12 patients (24.5%: eight in Cohort A and four in Cohort B). Diarrhea and rash were the most common AEs; 20 (40.8%) patients experienced grade 3–4 drug-related AEs.

Conclusions. Dacomitinib has a limited single-agent activity in recurrent GB with *EGFR* amplification. The detailed molecular characterization of the 4 patients with response in this trial can be useful to select patients who could benefit from dacomitinib.

Key words

dacomitinib | *EGFR* | glioblastoma | high-grade glioma

Importance of the study

This study investigates safety and efficacy of dacomitinib, a pan-human epidermal growth factor receptor (HER) tyrosine kinase inhibitor in patients with relapsed GB. The importance of the study lies in 2 facts—first, the lack of effective therapeutic options in patients with relapsed GB who clearly have unmet needs; second, the antineoplastic agent is a pan-anti-HER related with successful anti-*EGFR* and anti-HER2 antitumor-targeted agents discovered in the last 15 years. Moreover, about 40% of GB cases carry amplification of the *EGFR* gene,

and half of these patients have *EGFRvIII* mutation leading to a constitutively activated truncated form of the receptor, and the activation of the *EGFR* pathway has been related with cell proliferation, migration, and invasiveness of GB cells. This background provided grounds for investigation of this anti-*EGFR* agent in patients with GB. However, after the negative results of this study, further investigation should take profit from biomarkers based on other *EGFR* alterations instead of *EGFR* gene amplification.

Glioblastoma (GB) is the most frequent primary CNS malignancy, with poor survival despite multimodal treatment with surgery, radiotherapy, and chemotherapy with temozolomide.¹ Little progress has been made over the past decade and there is an unmet medical need.

Around 40% of GB cases carry amplification of the epidermal growth factor receptor gene (*EGFR*), and half of these patients have the *EGFR* variant (v)III mutation, a deletion of exons 2–7 that generates a constitutive activation of the receptor tyrosine kinase domain.² Since activation of the *EGFR* pathway increases cell proliferation, migration, and invasiveness of tumor cells,^{3,4} inhibition of this pathway is an attractive therapeutic target in GB.

Several small molecules and antibodies directed against *EGFR* have been successfully used in different tumors with *EGFR* alterations, such as non-small cell lung cancer (NSCLC) and epidermoid head and neck cancers. However, targeted *EGFR* agents have not shown significant activity in patients with GB.^{5,6} To date, for patient selection, *EGFR* inhibitors have been tested in GB without requiring any specific *EGFR* alteration.

Dacomitinib is a second-generation, oral, irreversible, highly selective pan-human epidermal growth factor receptor (HER) small-molecule tyrosine kinase inhibitor. Interestingly, dacomitinib is active in erlotinib- and gefitinib-resistant *EGFR*-mutated NSCLC, suggesting that it could be more active than other *EGFR* inhibitors in patients with *EGFR*-amplified GBs.⁷ In addition, dacomitinib is unlikely to interfere with enzyme-inducing anti-epileptic drug therapies. Dacomitinib, as an inhibitor of other HER family members (dacomitinib inhibits not only *EGFR* but also HER2 and HER4), could avoid resistance from GB cells to *EGFR* inhibitor through the activation of HER2 or HER4. In fact, HER2 is commonly overexpressed in GB.⁸

Preclinical data suggest that dacomitinib has an effect on cell viability, self-renewal, and proliferation in *EGFR*-amplified GB cells. Moreover, systematic administration of dacomitinib strongly impaired the *in vivo* tumor growth rates in *EGFR*-amplified xenograft models.^{9,10} Unlike previous studies with other molecules,¹¹ the authors' work showed that dacomitinib was able to dephosphorylate the downstream effectors of *EGFR* *in vivo*. The effect is not decreased by the presence of *EGFR*-mutant isoforms, like *EGFRvIII*, although it was attenuated in the absence of phosphatase and tensin homolog (PTEN) activity.¹⁰ Taking into account these preclinical data and in an attempt to

improve the results with first-generation, reversible *EGFR* inhibitors, the Spanish Group for Research in Neuro-Oncology (GEINO) performed a phase II study of dacomitinib in patients with recurrent *EGFR*-amplified GB. The drug was tested in 2 independent cohorts with *EGFR* amplification: patients without *EGFRvIII* mutation (Cohort A) and patients with *EGFRvIII* mutation (Cohort B).

The aim of this multicenter, 2-stage, open-label, phase II clinical trial was to assess the efficacy and safety of dacomitinib in adult patients with recurrent GB with *EGFR* gene amplification with or without *EGFRvIII* mutation. Patients with or without the *EGFRvIII* mutation were analyzed independently because a different biological response to dacomitinib could be expected when *EGFRvIII* mutation was present as previously described in GB preclinical models.^{12,13}

Materials and Methods

Study Design and Patient Population

This was a 2-stage, open-label, phase II clinical trial of dacomitinib in patients with GB with *EGFR* amplification at first recurrence. Patients with *EGFR* amplification without the *EGFRvIII* mutation were enrolled in Cohort A and patients with *EGFR* amplification with *EGFRvIII* mutation were enrolled in Cohort B. Each cohort was analyzed independently.

Participants from 12 GEINO centers in Spain were enrolled between March 2012 and April 2015. All patients signed an informed consent form before the *EGFR* test and before enrollment.

The protocol was approved by the institutional review board at each participating site. This study was registered with ClinicalTrials.gov under identifier NCT01520870.

Patients over 18 years of age who had central review histologically confirmed recurrent GB with *EGFR* amplification (determined by fluorescence in situ hybridization [FISH] assay), also confirmed by central molecular pathology review, were eligible for the study. The assessment of the vIII mutation was made centrally by quantitative PCR.

Molecular analysis of *EGFR* could be performed before the patient fulfilled the rest of the inclusion criteria.

All patients must have progressive disease on MRI defined by Response Assessment in Neuro-Oncology

(RANO) criteria¹⁴ after the standard Stupp protocol.¹ The time interval for the start of treatment was at least 12 weeks from prior radiotherapy unless there was either histopathologic confirmation of recurrent tumor or new contrast enhancement on MRI outside of the radiotherapy treatment field.

Additional inclusion criteria were KPS ≥ 70 and adequate hematologic function (hematocrit $>28\%$, leukocytes $>3000/\mu\text{L}$, absolute neutrophil count >1500 cells/ μL , platelets $>100\,000$ cells/ μL), liver function (bilirubin $\leq 2\text{X}$ the upper limit of normal [ULN], aspartate aminotransferase [AST] and alanine aminotransferase [ALT] $\leq 2.5\text{X}$ ULN), and renal function (creatinine clearance >60 mL/min/1.73 m²). Women of childbearing potential and their partners had to use adequate contraception throughout the study period and for 12 weeks after its completion. Patients were not allowed to receive any other investigational agents or have a history of allergic reactions to compounds similar to dacomitinib.

Exclusion criteria were extracranial metastatic disease, Gliadel wafer treatment, severe intercurrent illness presentation, and HIV positivity with a combination antiretroviral therapy.

All patients were required to have pretreatment brain MRI within the 14 days before therapeutic treatment and to have received a stable steroid dose for ≥ 5 days.

Treatment

Dacomitinib was administered to all patients at an initial oral dose of 45 mg/day. The treatment was the same in both cohorts, Cohort A (*EGFR* amp/*EGFR*III⁻) and Cohort B (*EGFR* amp/*EGFR*III⁺). Therapy was continued until disease progression, significant clinical decline, unacceptable toxicity, or patient decision. Toxicity was graded using the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) v4.0.

Drug doses were withheld and/or reduced for intolerable grade 2 or grade 3–4 toxicity. A maximum of 2 dose-level reductions was permitted (30 mg, then 15 mg). Dacomitinib administration could be interrupted for a maximum of 14 days.

Study Procedures

Clinical evaluations were scheduled every 2 weeks to screen for toxicity and progression. Surveillance MRI was performed every 12 weeks to assess response to treatment according to RANO criteria.¹⁴ The baseline and follow-up scans for each patient were locally and centrally reviewed to determine the overall radiographic response (central review was done at the end of the follow-up). Clinical diagnosis of progressive disease was determined by progressive clinical decline due to the tumor progression.

Molecular Analysis

Fluorescence in situ hybridization

FISH assays were performed on formalin-fixed, paraffin-embedded (FFPE) sections of 4 μm thickness by using the

dual color probe LSI *EGFR*/chromosome 7 enumeration probe (CEP7) from Vysis (Abbott Laboratories). The pretreatment of the FFPE tissue and the hybridization protocol were performed following manufacturer's instructions.

Fluorescence signals were scored in accordance with the criteria described in previous reports.^{15,16} For each sample, only well-defined nuclei of at least 50 tumor cells in 4 tumor areas were analyzed, and the numbers of single-copy gene and control probe signals were scored. *EGFR* gene amplification was considered positive when the *EGFR* to CEP7 ratio was ≥ 2 , or the presence of *EGFR* gene clusters of ≥ 15 gene copies was detected in more than 10% of tumor cells.

Real-time reverse transcription PCR

Total RNA from FFPE tissues of *EGFR* amplified tumors was extracted using the RNeasy FFPE Kit (Qiagen) according to manufacturer's protocol. A total of 1 μg of RNA was reverse transcribed for the synthesis of cDNA using the PrimeScript RT kit (Takara Bio) following the manufacturer's instructions. Real-time PCR to amplify *EGFR*VIII and hypoxanthine-guanine phosphoribosyltransferase (*HPRT1*) as the control gene was performed on LightCycler 480 using the SYBR Green method (Roche). *EGFR*VIII and *HPRT1* primer sequences were derived from the Yoshimoto K report.¹⁷ Briefly, 2 μL of cDNA product was used as the template in a 10 μL PCR containing 2 μL of LightCycler FastStart DNA MasterPlus SYBR Green I (Roche) and 0.2 μM of each primer. All reactions were performed in duplicate. Amplification protocols were as follows: 95°C for 10 min; 40 cycles of 95°C/10 s, 60°C/10 s, and 72°C/10 s; and 95°C/10 s, 65°C/15 s, and 95°C with 0.06°C/s, 9 acquisitions per degree for melting curve analysis. Threshold cycle number (cross point) was automatically determined by LightCycler software.

High-resolution melting-curve analysis (HRM) and Sanger sequencing

Genomic DNA was extracted from FFPE sections of the GB patients who were enrolled in the clinical trial following manufacturer's recommendations (Qiagen). The hotspot mutations R132 and R172 of *IDH1* and *IDH2*, respectively, were amplified by PCR by using primers previously described.^{18,19} *IDH1* and *IDH2* mutations were screened by HRM analysis of the fluorescent melting curves of the amplified fragments on a LightCycler 480 following manufacturer's instructions. After HRM analysis, mutated samples were purified and Sanger sequenced using the same primers and the Big Dye Terminator v3.1 sequencing kit in an ABI 3130xl genetic analyzer (ThermoFisher).

Methylation-specific (MS) PCR

Extracted DNA (1 μg) from the FFPE tissue sections was subjected to bisulfite modification using the Epitect Bisulfite Kit (Qiagen) following manufacturer's recommendations. The methylation status of the promoter region of the O⁶-DNA methylguanine-methyltransferase gene (*MGMT*) was determined by MS-PCR by using a nested, 2-stage PCR approach and the primers previously

described by Palmisano and coworkers.²⁰ Positive and negative methylated DNA were obtained from normal lymphocytes of peripheral blood of healthy individuals. Methylated and unmethylated products were separated on a 3% agarose gel and examined under ultraviolet illumination.

Statistical Analysis

The primary endpoint was the rate of progression-free survival (PFS) at 6 months (PFS6) according to RANO criteria.¹⁴ Response was radiographically assessed by investigators and by an independent blinded radiologist.

Secondary endpoints were safety and tolerability of dacomitinib, overall survival (OS), and antitumor response assessed by RANO criteria.¹⁴

The sample size of each cohort was estimated according to the Simon 2-stage phase II design: P0 = 15% PFS6; P1 = 35% PFS6. To achieve an (α, β) error of (0.1, 0.1), a total of 32 patients for each cohort was required.

According to the Simon design, the primary endpoint would be considered not reached in each cohort if ≤ 2 patients were progression free at 6 months among the first evaluable 17 patients of each cohort, or if ≤ 7 patients were progression free at 6 months among 32 patients of each cohort in the second stage.

PFS6 and response rate were based on the proportion of patients known to have achieved that endpoint using the intention to treat concept. Median PFS and OS were calculated from the Kaplan–Meier curves. The time was measured

from registration date. All patients receiving treatment per protocol were included in the safety assessment. The analysis of toxicity was reported using CTCAE v4.0.

Results

Patient Disposition and Characteristics

Molecular screening was performed in 178 patients (121 had no *EGFR* amplification and 57 had *EGFR* amplification). Eight of these patients with *EGFR* amplification did not have disease progression when the study was closed and were not finally enrolled in the study. All the tissues from patients finally enrolled in the trial were obtained from the surgery at diagnosis.

Forty-nine patients were enrolled in the study: 30 in Cohort A (*EGFR* amp/*EGFR*vIII⁻) and 19 in Cohort B (*EGFR* amp/*EGFR*vIII⁺). Two patients in Cohort B were not evaluable for the primary endpoint (PFS6): one because the patient, with stable disease in the first MRI, decided not to continue treatment after 3 months. The other patient decided not to continue in the study after 1 month of treatment because he changed his place of residence. None of these patients had any grade 2 toxicities.

The demographic, molecular, and clinical baseline characteristics of the patients by each cohort are described in [Table 1](#). Detailed characteristics of each individual patient are displayed in Supplementary Table S1.

Table 1 Demographic, clinical, and molecular characteristics at baseline

	Cohort A (<i>EGFR</i> amp/ <i>EGFR</i> vIII ⁻) n = 30	Cohort B (<i>EGFR</i> amp/ <i>EGFR</i> vIII ⁺) n = 19	Total (n = 49)
Age, y, median (range)	62.5 (41–81)	52.0 (39–72)	59 (39–81)
Gender, n (%)			
Male	20 (66.7)	12 (63.2)	32 (65.3)
Female	10 (33.3)	7 (36.8)	17 (34.7)
ECOG-PS, n (%)			
0	3 (10.0)	2 (10.5)	5 (10.2)
1	19 (63.3)	13 (68.4)	32 (65.3)
2	8 (26.7)	4 (21.1)	12 (24.5)
MGMT methylation status, n (%)			
Methylated	9 (30)	1 (5.3)	10 (20)
Not methylated	7 (23.3)	10 (52.6)	17 (34.7)
Not determined	14 (46.6)	8 (42.1)	22 (44.9)
IDH1/2 mutations, n (%)			
<i>IDH1</i> mut	2 (6.6)	0	2 (4.1)
<i>IDH2</i> mut	0	0	0
<i>IDH1/2</i> not mutated	19 (63.3)	15 (79)	34 (69.4)
Not determined	9 (30)	4 (21)	13 (26.5)

ECOG-PS: Eastern Cooperative Oncology Group Performance Status.

Progression-free Survival at 6 Months

Progression-free survival rate at 6 months defined using RANO criteria was the primary endpoint. A Simon 2-stage design (PFS6 P0 = 15%; P1 = 35%; $\alpha = 0.1$; $\beta = 0.1$) required that at least 3 patients be progression free at 6 months in the first 17 evaluable patients in order to expand to a second step. This goal was achieved by Cohort A (*EGFR* amp/*EGFR*vIII⁻) but not by Cohort B (*EGFR* amp/*EGFR*vIII⁺), where only 1 patient was progression free at 6 months among the first 17 evaluable patients, and this cohort was closed after this first stage.

In Cohort A, only 4 of 30 patients were progression free at 6 months, and the study was closed at this point when the goal of 7 patients without progression at 6 months could not be reached in the second stage. The PFS6 was 13.3% (95% CI: 0–26.5%) and the median PFS was 2.7 months (95% CI: 2.3–3.2). The Kaplan–Meier curve of PFS in Cohort A is displayed in Fig. 1. Interestingly, the 3 patients without progression at 6 months were also progression free at 1 year and one of them was also progression free at 40 months of treatment, at the time of study closure.

As stated above, Cohort B was closed to enrollment in the first stage (19 patients were included with *EGFR* amp/*EGFR* vIII⁺ and 17 of them were evaluable). The PFS6 was 5.9% (95% CI: 0–18%) and the median PFS was 2.6 months (95% CI: 1.8–3.4). The Kaplan–Meier curve for Cohort B is shown in Fig. 1.

Globally, including both cohorts, the PFS6 was 10.6% (95% CI: 2%–19.6%), with a median PFS of 2.7 months (95% CI: 2.3–3.1).

Results obtained from the central radiological evaluation were very similar to those obtained from the local evaluation. In Cohort A, the PFS6 was 13.6% (95% CI: 0–31.4%) and the median PFS was 2.9 months (95% CI: 1.03–4.07), while in Cohort B the results were 5.9% (95% CI: 0–18%) and 2.4 months (95% CI: 1.9–2.8), respectively. Median PFS for both cohorts was 2.8 months (95% CI: 2.6–3.0).

Cases that Met the Primary Endpoint

Three patients in Cohort A were progression free at 6 months and at 1 year of treatment. In Cohort B only one patient had clear medical benefit, with a PFS of 12.6 months. Tissue from the surgery at diagnosis was available in 3 of these 5 cases with longer PFS and OS. *IDH1*, *IDH2*, and *MGMT* methylation status are shown in Table 2 together with the main demographic and clinical data of these long-term survivors. Neither *MGMT* methylation status nor the presence of isocitrate dehydrogenase (*IDH*) 1 and 2 mutations seems to preclude a clinical benefit with dacomitinib.

One patient on Cohort A had tumor progression after 27 months of treatment and a second resection was performed. Tissue from this second surgery was available and, interestingly, *EGFR* amplification was not found at this time.

Overall Survival

For Cohort A, the median OS was 7.8 months (95% CI: 5.6–10.1) and for Cohort B, 6.7 months (95% CI: 4.3–9.1). The

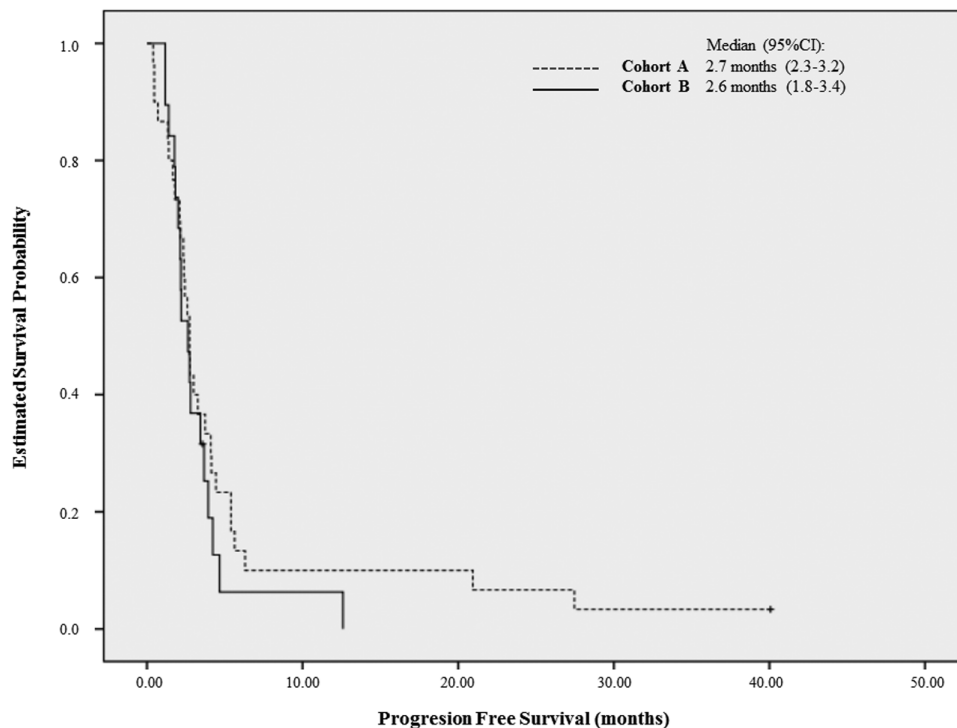


Fig. 1 Progression-free survival rate at 6 months for Cohort A (*EGFR* amp/*EGFR*vIII⁻) and Cohort B (*EGFR* amp/*EGFR*vIII⁺).

Table 2 Patients achieving progression-free status at 6 months

	Patient No 1	Patient No 16	Patient No 20	Patient No 23	Patient No 39
Age, y	59	61	68	65	59
Sex	Male	Female	Female	Male	Male
EGFR Status (Cohort)	<i>EGFR</i> amp/ <i>EGFR</i> vIII- (Cohort A)	<i>EGFR</i> amp/ <i>EGFR</i> vIII- (Cohort A)	<i>EGFR</i> amp/ <i>EGFR</i> vIII- (Cohort A)	<i>EGFR</i> amp/ <i>EGFR</i> vIII+ (Cohort B)	<i>EGFR</i> amp/ <i>EGFR</i> vIII- (Cohort A)
IDH1/<i>IDH2</i> status	Wild type	Wild type	<i>IDH1</i> mutation (R132H)	Tissue not available	Tissue not available
MGMT methylation status	Not methylated	Methylated	Methylated	Tissue not available	Tissue not available
Time from diagnosis to dacomitinib initiation (mo)	17	18	11	7	3
Time from the end of radiotherapy to dacomitinib initiation (mo)	14	14	9	3.5	1
Best response	SD	PR	CR	SD	SD
Time to disease progression (mo)	27.5	21	Progression-free after 40 mo	12.6	6
Follow-up (mo)	46.2	44	40	34.5	13
Outcome at the end of follow-up	Dead	Alive	Alive, on treatment	Dead	Dead
Surgery after progression to dacomitinib?	Yes. Loss of <i>EGFR</i> amplification.	No	No	No	No
Comments	—	Radiological evolution in Supplementary Figure S1	Radiological evolution in Supplementary Figure S1	—	—

*EGFR*amp/*EGFR*vIII-: *EGFR* amplification without *EGFR*vIII mutation.

*EGFR*amp/*EGFR*vIII+: *EGFR* amplification with *EGFR*vIII mutation.

SD: stable disease; PR: partial response. CR: complete response.

OS curve for both cohorts is shown in Fig. 2. For the total population, median OS was 7.4 months (95% CI: 5.6–9.2).

Response Rate

Four patients (3 in Cohort A and 1 in Cohort B) were not evaluable for this endpoint because of clinical decline before the radiologic assessment.

One patient in Cohort A achieved a complete response after 6 months of treatment. One patient in each cohort had a partial response as the best overall response (see Supplementary Figure S1). Eight patients from Cohort A and 4 patients from Cohort B had stable disease as the best overall response. However, most patients had

progressive disease in the first MRI performed. Central radiological evaluation was performed, subsequently validating and verifying all patient response.

The response rates are shown in Table 3.

Safety and Tolerability

During the study, 47 patients (96%) reported at least one adverse event (AE) potentially related to dacomitinib. Twenty (41%) patients experienced CTCAE grade 3 or 4. The most common AEs related to the study drug were: rash (82%), diarrhea (67%), fatigue (22.4%), and nausea (8.2%) (Table 4). One patient had ALT/AST grade 3 elevation.

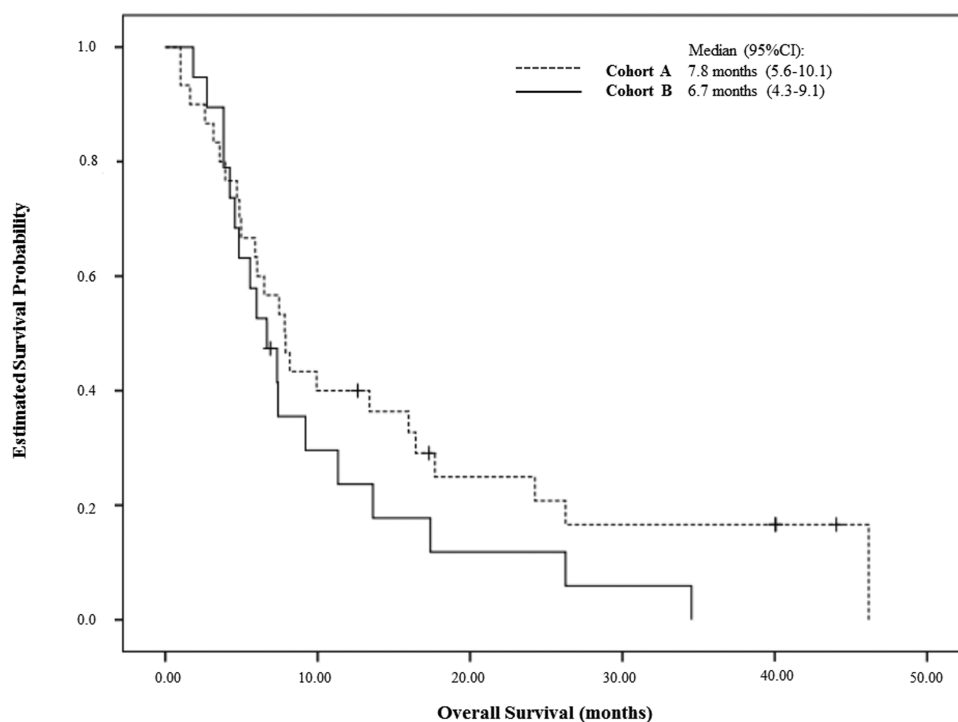


Fig. 2 Overall survival (mo) for Cohort A (*EGFR* amp/*EGFR*vIII-) and Cohort B (*EGFR* amp/*EGFR*vIII+).

Table 3 Best overall response

	Cohort A (<i>EGFR</i> amp/ <i>EGFR</i> vIII- n = 30, n (%))	Cohort B (<i>EGFR</i> amp/ <i>EGFR</i> vIII+ n = 19, n (%))	Total (n = 49) n (%)
Complete response	1 (3.3)	0 (0.0)	1 (2.0)
Partial response	1 (3.3)	1 (5.3)	2 (4.1)
Stable disease	8 (26.7)	4 (21.1)	12 (24.5)
Progressive disease	17 (56.7)	13 (68.4)	30 (61.2)
Not evaluable	3 (10.0)	1 (5.3)	4 (8.2)

*EGFR*amp/*EGFR*vIII-: *EGFR* amplification without *EGFR*vIII mutation.

*EGFR*amp/*EGFR*vIII+: *EGFR* amplification with *EGFR*vIII mutation.

A total of 16 dose reductions in 14 patients and 6 treatment interruptions in 5 patients were reported.

Discussion

Despite the rationale to target *EGFR* in GB, dacomitinib, a pan-HER tyrosine kinase inhibitor, has a limited

single-agent activity in recurrent GB patients with *EGFR* amplification. The response to *EGFR* inhibition in relapsed GB had also been disappointing previously with either erlotinib or gefitinib as first-generation oral *EGFR* inhibitors.^{5,6,21} The results of the European Organisation for Research and Treatment of Cancer randomized phase II trial, which included 110 patients (54 treated with erlotinib and 56 with chemotherapy), showed PFS6 of 12% for erlotinib and 24% for the control arm, with similar OS in both arms.²² Results with afatinib, a newer *EGFR* tyrosine kinase inhibitor that is able to irreversibly block *EGFR* and to inhibit *HER2* and *HER4*, were also negative in recurrent GB.²³

One of the major issues of the clinical trials with *EGFR* inhibitors is the fact that these drugs have been tested without any patient selection according to *EGFR* status. In fact, to date only one clinical trial developed by GEINO has evaluated an *EGFR* inhibitor after selecting patients by their *EGFR* status. In this study, erlotinib was tested in recurrent GB with expression of *EGFR*vIII and *PTEN* by immunohistochemistry. The study showed no significant activity, with a PFS6 of 20% and only one partial response.⁵

The results of our clinical trial show that dacomitinib has limited activity in recurrent GB, even though the drug was administered to a selected population with *EGFR* amplification. Indeed, the administration of dacomitinib did not meet the prespecified threshold for PFS6, neither in cases with *EGFR*vIII mutation nor in cases without this mutation. The observed antitumor activity with dacomitinib was comparable to that observed previously in trials with reversible *EGFR* tyrosine kinase inhibitors in nonmolecularly selected

Table 4 Summary of adverse events

Adverse Event by CTCAE 4.0	All Grades n (%)	Grade ≥ 3 n (%)
Patients with adverse events	47 (95.9)	20 (40.8)
Adverse events		
Rash	40 (81.6)	11 (22.4)
Diarrhea	33 (67.3)	3 (6.1)
Asthenia	11 (22.4)	2 (4.1)
Nausea/vomiting	4 (8.2)	0 (0.0)

recurrent GB. Erlotinib alone or in combination with chemotherapy achieved partial response rates of 6%–25% with modest impact on PFS or OS.^{24–26} Gefitinib in recurrent GB showed response rates of 0–13%, median PFS of 2 months, and PFS6 of 9%–13%.^{6,11,27,28}

Despite these disappointing global results, 4 patients had significant benefit from this treatment: 4 patients were progression free at 6 months, 3 were progression free after 12 months, and 1 patient was still progression free after 40 months of treatment.

In relation to toxicity, dacomitinib exhibited a profile consistent with previous reports,^{29–31} but this drug is clearly more toxic than erlotinib or gefitinib. The most frequently reported AEs with dacomitinib included rash and diarrhea.

In order to interpret our results, it is important to notice that intratumoral pharmacokinetic assessment of dacomitinib was not incorporated and it is not possible to determine whether insufficient intratumoral delivery was the cause of the poor therapeutic benefit.

This study has a few limitations. First, *EGFR* amplification, which has been used as a primary laboratory assessment, was tested in the primary tumor, and we have no evidence of the stability of this alteration in the recurrent GBs. However, the analysis of a cohort of 55 pairs of primary and recurrent GBs showed more than 80% of cases in which *EGFR* amplification remained stable.³² In any case, the presence of the amplification alone has not been found helpful in the use of other anti-*EGFR* agents in lung cancer or colorectal cancer. Instead, recent successes in this field have been based on the presence of point mutations associated with a constitutive activation of the pathway. However, these point mutations are not commonly found in GBs.

Other important limitations of our study are the small sample size and the nonrandomized design, which preclude drawing firm conclusions. Related to this, the 2-stage design has resulted in a very small sample size in the cohort of patients who could have provided the more interesting results: those with an *EGFR*VIII mutation.

Recent technological innovations have allowed the analysis of cancer genetics to be conducted on the single-cell level in every case. Parker et al,³³ for example, profiled 430 cells from 5 GBs and found that individual cells could be classified as different types of GB according to The Cancer Genome Atlas classification scheme. Other studies

confirmed the observation of heterogeneous amplification of *EGFR* and other receptor tyrosine kinases in GB.³⁴ GB tumor heterogeneity may be behind the lack of efficacy of targeted therapies in GB because the treatment would be only active in a group of tumor cells. Another feasible explanation could be the presence of other alterations that could activate the *EGFR* pathway downstream of the receptor. In fact, preclinical data suggest that the lack of PTEN renders the tumors less sensitive to dacomitinib¹⁰ and that phosphatidylinositol-3 kinase (PI3K) inhibitors synergize with *EGFR* inhibition. One of our patients with significant clinical benefit suffered a tumor regrowth that was removed. We did not find either *EGFR* amplification or other *EGFR* alterations in the relapse. This case suggests that a loss of the *EGFR* alterations, or a clonal selection of cells without *EGFR* modifications, may be a mechanism of secondary resistance to *EGFR* inhibitors in GB. However, the lack of tissue sample at the start of dacomitinib administration does not allow us to conclude that the loss of *EGFR* amplification was induced by the experimental treatment.

Long-term survival with dacomitinib was not related to *MGMT* promoter methylation status or the presence of *IDH1/2* mutations, showing that the benefit with the *EGFR* inhibitor was independent of known good prognosis factors.

Blood–brain barrier (BBB) penetrance remains a challenge in small-molecule–based anti-glioma therapy. Available information about the ability of dacomitinib to achieve adequate concentrations in brain parenchyma is scarce, since patients with brain metastases have been excluded from clinical trials in NSCLC and other tumors. However, preclinical studies conducted in mice suggest that dacomitinib can cross the BBB, since oral administration of dacomitinib 25 mg/kg is followed by an overall brain-to-plasma ratio (based on AUC₇₂ values) of 1.21, and rats receiving a single 4.98 mg/kg p.o. dose of [¹⁴C]dacomitinib achieved CNS and CSF radioequivalents over the time course of 2 to 48 hours (data not published/investigator brochure provided by Pfizer). Additionally, in vivo models of GB showed that dacomitinib inhibits tumor growth not only in patient-derived xenografts implanted into the flanks of immunodeficient mice but also in intracranial patient-derived xenografts.¹⁰ The results obtained in the flanks with strong downregulation of the phosphorylation of *EGFR* and its downstream target were confirmed in the intracranial models in the same study.

In conclusion, the administration of dacomitinib in recurrent GB and *EGFR* amplification showed minimal activity in both cases, with or without VIII mutation. The detailed molecular characterization of the 4 patients with response in this trial can help to narrow the possible predictive markers of dacomitinib effectiveness. Further studies should benefit from a more profound molecular profiling of *EGFR*/PI3K/PTEN status.

Supplementary Material

Supplementary material is available at *Neuro-Oncology* online.

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