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Phase I/randomized phase II study of afatinib, an irreversible ErbB family blocker, with or without protracted temozolomide in adults with recurrent glioblastoma

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Background. This phase I/II trial evaluated the maximum tolerated dose (MTD) and pharmacokinetics of afatinib plus temozolomide as well as the efficacy and safety of afatinib as monotherapy (A) or with temozolomide (AT) vs temozolomide monotherapy (T) in patients with recurrent glioblastoma (GBM).

Methods. Phase I followed a traditional 3 + 3 dose-escalation design to determine MTD. Treatment cohorts were: afatinib 20, 40, and 50 mg/day (plus temozolomide 75 mg/m^2/day for 21 days per 28-day cycle). In phase II, participants were randomized (stratified by age and KPS) to receive A, T or AT; A was dosed at 40 mg/day and T at 75 mg/m^2 for 21 of 28 days. Primary endpoint was progression-free survival rate at 6 months (PFS-6). Participants were treated until intolerable adverse events (AEs) or disease progression.

Results. Recommended phase II dose was 40 mg/day (A) + T based on safety data from phase I ($n = 32$). Most frequent AEs in phase II ($n = 119$) were diarrhea (71% [A], 82% [AT]) and rash (71% [A] and 69% [AT]). Afatinib and temozolomide pharmacokinetics were unaffected by coadministration. Independently assessed PFS-6 rate was 3% (A), 10% (AT), and 23% (T). Median PFS was longer in afatinib-treated participants with epidermal growth factor receptor (EFGR) vIII-positive tumors versus EGFRvIII-negative tumors. Best overall response included partial response in 1 (A), 2 (AT), and 4 (T) participants and stable disease in 14 (A), 14 (AT), and 21 (T) participants.

Conclusions. Afatinib has a manageable safety profile but limited single-agent activity in unselected recurrent GBM patients.

Keywords: afatinib, EGFRvIII, ErbB family, glioblastoma, temozolomide.

Temozolomide plus radiotherapy is standard treatment for newly diagnosed glioblastoma (GBM) patients.[1] Recent attempts to improve front-line treatment with bevacizumab[2,3] or cilengitide[4] have failed to improve survival when combined with standard treatment. Following front-line treatment, nearly all GBMs recur, and there are no effective treatments with durable benefit for recurrent GBM.[5] Recurrent GBM has an extremely poor prognosis, with a median overall survival (OS) of ~9 months and a 12-month OS of 14%.[5] Treatment failure is associated with the development of resistance to temozolomide and is primarily mediated by overexpression of specific proteins (O[6]-methylguanine methyltransferase [MGMT] and

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O[6]-alkylguanine-DNA-alkyl-transferase [AGAT]).[6] Nevertheless, temozolomide is well tolerated and may have activity despite prior exposure if novel dose schedules are used.[7,8] For example, protracted, low-dose (metronomic) temozolomide may deplete MGMT and AGAT[9] and restore temozolomide sensitivity in the recurrent setting.

Activation of the ErbB family of receptors, including the epidermal growth factor receptor (EGFR), can initiate downstream signaling pathways involved in cell growth and proliferation.[10] ErbB pathway regulation plays an important role in glioma progression, and certain germline *EGFR* polymorphisms may contribute to the glioma pathogenesis.[11] *EGFR* is amplified and overexpressed in ~50%–60% of GBMs, and multiple *EGFR* gene mutations occur in GBM tumors.[12,13] The EGFRvIII mutation is expressed in 30% of GBMs, including 41%–60% of those with EGFR amplification.[12] HER2 (ErbB2) is a possible low-penetrance gene candidate associated with GBM development.[11] The high frequency of EGFR pathway alterations in GBM has triggered interest in therapeutically targeting the ErbB family, including EGFR.

EGFR inhibition in vitro has activity against GBM; however, reversible EGFR tyrosine kinase inhibitors (TKIs) gefitinib and erlotinib have had limited impact on survival of recurrent GBM patients, either as monotherapy or in combination with other agents.[14–26]

Afatinib is a potent, orally bioavailable ErbB family blocker that irreversibly binds to the ATP binding pocket of the ErbB family of receptors, inhibiting the activity of EGFR (including the EGFRvIII variant), HER, and ErbB4 and blocks transphosphorylation of ErbB3.[27,28] Afatinib is active against ErbB family-driven tumors, including lung cancer.[29–31] In vitro, afatinib inhibits cells harboring mutations that are frequently found in GBM, including EGFRvIII and EGFR R108K.[28,32] Furthermore, unlike erlotinib and gefitinib, cytochrome P450 metabolism of afatinib is negligible.[33]

Phase I of this study aimed to establish the maximum tolerated dose (MTD) and pharmacokinetics (PKs) of afatinib plus temozolomide among recurrent malignant glioma patients. Phase II assessed the efficacy and safety of afatinib (± temozolomide) versus temozolomide monotherapy in patients with recurrent GBM.

Materials and Methods

Study Design and Patient Population

This was a multicenter, 2-part, phase I/II trial. Phase I was conducted in 9 centers and phase II in 26 centers, all in North America, between July 2008 and May 2011.

All patients were ≥18 years old and had recovered from previous surgery and chemotherapy. Phase I patients had histologically confirmed WHO grade 3/4 recurrent malignant glioma, KPS ≥60%, and were not restricted by number of prior progressions or salvage therapies.

Phase II patients had histologically confirmed WHO grade 4 malignant glioma at first recurrence after temozolomide chemoradiotherapy, bidimensionally measurable disease (tumor ≥10 mm in one diameter), and KPS ≥70%.

Exclusion criteria were: <12 weeks from radiotherapy; <2 weeks from surgery, chemotherapy, or investigational drugs; progressive disease (PD) or toxicity (Common Terminology Criteria for Adverse Events [CTCAE] Version 3.0 grade ≥3) with prior protracted temozolomide dosing; previous EGFR-targeted therapy or bevacizumab; ≥2 disease recurrences; or known interstitial lung disease.

The study was conducted in accordance with the Declaration of Helsinki, local laws, and the International Conference on Harmonisation of Good Clinical Practice Guideline, and it was approved by the relevant regulatory and independent ethics committees or institutional review boards. All participants provided written informed consent.

Treatments

Phase I followed a traditional 3 + 3 dose-escalation design, with continuous once-daily afatinib initiated at 20 mg/day and escalated to 40 and 50 mg/day. All participants received daily temozolomide (75 mg/m^2) for 21 days every 28-day cycle. The MTD was defined as the highest dose at which ≤1of 6 participants experienced dose-limiting toxicity (DLT). Additional participants were treated at the MTD to further evaluate safety. Treatment continued until disease progression, side effects requiring discontinuation, or withdrawal of consent.

Phase II participants were randomized (stratified by age [≥50 years vs <50 years] and KPS [70%–80% vs 90%–100%]) in a 1:1:1 ratio to receive: Arm 1, temozolomide monotherapy, 75 mg/m^2/day for 21 of 28 days; Arm 2, afatinib monotherapy at 40 mg/day; and Arm 3, afatinib at the recommended phase II dose plus temozolomide (75 mg/m^2/day for 21/28 days). A dose-reduction scheme was implemented for defined drug-related adverse events (CTCAE Version 3.0) including study drug interruption or modification. If the AE recovered to grade ≤1 within 14 days, treatment could be restarted at temozolomide, 50 mg/m^2 and afatinib, 30 mg. For a second occurrence of the same AE, treatment was restarted at temozolomide, 38 mg/m^2 and afatinib, 20 mg. If the AE occurred for a third time, treatment was discontinued. Similar dose-reduction and discontinuation schemes were implemented for the monotherapy arms.

Endpoints

Primary endpoints were occurrence of DLT in phase I and 6-month progression-free survival (PFS-6) rate in phase II.

Secondary endpoints in phase I included PKs and objective tumor response; secondary endpoints in phase II were objective tumor response, PFS, PKs, and molecular determinants of afatinib response.

Assessments and Definitions

A DLT was defined as an AE or laboratory abnormality that was considered study-regimen related, occurred during cycle 1, and met criteria for (i) hematologic toxicity (grade 4 neutropenia for >7 days; or grade 3/4 neutropenia associated with fever >38.3°C; or grade 3 thrombocytopenia; all other hematologic toxicities of grade ≥3 leading to an interruption of treatment with both study drugs for >14 days or until recovery to baseline or grade 1), or (ii) nonhematologic toxicity (grade ≥3 drug-related AEs, excluding grade ≥3 nausea or vomiting,

diarrhea, or rash without appropriate care) and; all other nonhematologic toxicities of grade ≥3 leading to an interruption of treatment with both study drugs for more than 14 days or until recovery to baseline or grade 1).

During phase II, response assessment was evaluated before odd cycles by both the investigator and an independent review committee (ICON Medical Imaging) according to Response Assessment in Neuro-Oncology (RANO) criteria.[34] PFS was defined as the duration from date of randomization to disease progression or death; for those without progression or death, PFS was defined as the date of last MRI or neurologic assessment showing no progression as the final time point for analyses.

Safety assessments included AEs (CTCAE Version 3.0), KPS, electrocardiography, left ventricular cardiac function, and standard hematological and chemistry laboratory tests.

In phase I, a PK profile (plasma samples before and 0.5, 1, 1.5, 2, 3, 4, 6, and 8 h after administration) for temozolomide were taken in the absence (day 1 of cycle 1) and presence (day 15 of cycle 1) of afatinib. Afatinib PKs were assessed in the presence (day 15 of cycle 1) and absence (day 28 of cycle 1) of temozolomide. In phase II, for participants receiving afatinib, samples were drawn pre- and 1-hour post dose on day 15 of cycles 2 and 3 only. Plasma samples were analyzed by a validated high-performance liquid chromatography tandem mass spectrometry method. Noncompartmental analysis of plasma concentration-time data was performed using WinNonlin Professional Network Version 5.2 software (Pharsight). Standard noncompartmental methods were used to calculate PK parameters as described previously.[35] Descriptive statistics were calculated using SAS Version 8.2 and 9.2 (SAS Institute).

For biomarker investigations, archived tumor samples were centrally analyzed by immunohistochemistry (IHC) for EGFR, EGFRvIII, phosphatase and tensin homolog (PTEN), P-AKT, and MGMT. In addition, EGFR and PTEN were analyzed by fluorescent in situ hybridization (FISH).

Sample Size Determination and Statistical Methods

Sample size for phase I assumed 6 participants per afatinib dose level plus an additional 6 participants at MTD. For phase II, 40 participants per afatinib ± temozolomide arm were expected to provide 87% probability of observing a PFS-6 ≥10% of that observed with temozolomide monotherapy.

Median PFS and PFS-6 values with 95% confidence intervals (CIs) were derived from Kaplan–Meier estimates with Greenwood estimates of variance. Fisher exact and log-rank tests were used to compare the overall response rate and PFS between the 3 treatment arms, respectively. PFS was assessed by age, baseline KPS, country, prior temozolomide treatment, and EGFRvIII status. EGFRvIII IHC intensity was scored as high positive (2+/3+ or 3+) or low positive (1+ or 2+).

Results

Phase I

Participant disposition and characteristics

Thirty-two participants received afatinib 20 mg/day ($n = 6$), 40 mg/day ($n = 8$), or 50 mg/day ($n = 18$), plus temozolomide (Table 1). Median duration of treatment was 29 days (range, 6–491 days); most participants (69%) received 1–2 cycles.

Table 1. Baseline demographics and characteristics of patients enrolled in the phase I study

	Afatinib Dose (mg daily) Plus Temozolomide 75 mg/m^2			
	20 ($n = 6$)	40 ($n = 8$)	50 ($n = 18$)	Total ($n = 32$)
Male sex, *n* (%)	4 (66.7)	6 (75.0)	10 (55.6)	20 (62.5)
Age, years, median (range)	49.5 (37.0–67.0)	50.0 (27.0–71.0)	50.0 (37.0–68.0)	50.0 (27.0–71.0)
WHO grade (%)				
Grade 3	2 (33.3)	3 (37.5)	6 (33.3)	11 (34.4)
Grade 4	4 (66.7)	5 (62.5)	12 (66.7)	21 (65.6)
Histological classification				
GBM	4 (66.7)	5 (62.5)	10 (55.6)	19 (59.4)
Anaplastic astrocytoma	0	0	3 (16.7)	3 (9.4)
Gliosarcoma	0	0	1 (5.6)	1 (3.1)
Anaplastic oligodendroglioma	1 (16.7)	3 (37.5)	2 (11.1)	6 (18.8)
Anaplastic oligoastrocytoma	0	0	1 (5.6)	1 (3.1)
Other	1 (16.7)	0	1 (5.6)	2 (6.3)
Time since diagnosis (months)				
Median (range)	38.0 (6.8–73.9)	11.8 (5.5–113.9)	15.9 (0.9–152.5)	15.9 (0.9–152.5)
KPS				
70%	1 (16.7)	1 (12.5)	1 (5.6)	3 (9.4)
80%	1 (16.7)	1 (12.5)	2 (11.1)	4 (12.5)
90%	2 (33.3)	4 (50.0)	11 (61.1)	17 (53.1)
100%	2 (33.3)	2 (25.0)	4 (22.2)	8 (25.0)
Prior bevacizumab treatment, *n* (%)	1 (16.7)	2 (25.0)	1 (5.6)	4 (12.5)

Abbreviations: GBM, glioblastoma.

Dose-limited toxicities and maximum tolerated dose

In the afatinib 20 mg dose cohort ($n = 6$) one participant experienced a DLT (grade 4 intracranial hemorrhage [ICH] while receiving enoxaparin). Another participant experienced grade 4 thrombocytopenia on day 36. Both participants recovered, but treatment was permanently discontinued. No DLTs occurred in the afatinib 40 mg dose cohort. One of 6 participants in the 50 mg afatinib dose cohort experienced a DLT (grade 3 diarrhea), but, 4 of 18 participants (22.2%) in the 50 mg/day expansion cohort experienced grade 3 AEs during cycle 2 (diarrhea, hypokalemia, pancreatitis, and generalized rash).

Based on the overall safety profile, afatinib 40 mg was the recommended phase II dose.

Safety

Although most AEs were grade 1/2, 17 of the 32 participants (53%) required dose reduction or discontinuation because of an AE. Treatment-related, grade ≥3 AEs that occurred among ≥5% of participants are summarized in Table 2.

Thirteen participants (40.6%) experienced a serious AE (SAE), including 2 (5.1%) that were related to study therapy: grade 4 ICH in the afatinib 20 mg/day plus temozolomide group (in a participant receiving concurrent enoxaparin) and grade 3 fatigue in the afatinib 50 mg/day plus temozolomide group. The afatinib 50 mg/day plus temozolomide group had the highest SAE frequency (55.6% vs 12.5% and 33.3% in the afatinib 40 mg/day and 20 mg/day groups, respectively). In the afatinib 50 mg/day plus temozolomide 75 mg/m^2 group, SAEs included infections (16.7%), gastrointestinal (5.6%), musculoskeletal, and connective tissue disorders (5.6% each). AEs that led to discontinuation were also higher in the afatinib 50 mg/day plus temozolomide group than the 20 mg/day and 40 mg/day groups (55.6%, 33.3% and 12.5%, respectively). There were no study-related deaths among phase I participants.

Pharmacokinetics

Afatinib PK parameters were comparable in the presence and absence of temozolomide (Supplementary data, Table S1), but geometric coefficients of variation were 58.0%–65.3%.

Temozolomide PK parameters were comparable in the presence and absence of afatinib, with geometric coefficients of variation between 24.1% and 39.7%.

Efficacy

One participant achieved a partial response (PR; duration, 284 days), and there were no complete responses (CRs). Stable disease was achieved by 4 participants in the afatinib 40 mg/day group, one participant in the 20 mg/day group, and 6 participants in the 50 mg/day group. The median PFS (months) was 0.95 (20 mg/day), 1.87 (40 mg/day), and 0.95 (50 mg/day).

Phase II

Participant disposition and characteristics

Of the 131 participants who were screened for phase II, 119 were randomized. Characteristics of the participants in each treatment group were comparable (Table 3). All participants had GBM, with 88.2% having primary GBM.

Table 2. Treatment-related adverse events at CTCAE grade ≥3 that occurred at an incidence of ≥5% in any treatment group in study phase I or II

	Phase I (Plus Temozolomide 75 mg/m^2)			Phase II		
	Afatinib 20 mg/day ($n = 6$)	Afatinib 40 mg/day ($n = 8$)	Afatinib 50 mg/day ($n = 18$)	Afatinib 40 mg/day ($n = 41$)	Afatinib 40 mg/day Plus Temozolomide 75 mg/m^2 ($n = 39$)	Temozolomide 75 mg/m^2 ($n = 39$)
Total with related AEs	2 (33.3)	0	6 (33.3)	9 (22.0)	14 (35.9)	8 (20.0)
Thrombocytopenia	1 (16.7)	0	0	0	1 (2.6)	1 (2.6)
Lymphopenia	0	0	1 (5.6)	0	0	4 (10.3)
Diarrhea	0	0	1 (5.6)	3 (7.3)	3 (7.7)	0
Vomiting	0	0	0	0	2 (5.1)	0
Pancreatitis	0	0	1 (5.6)	0	0	0
Fatigue[a]	0	0	1 (5.6)	2 (4.9)	5 (12.8)	2 (5.1)
Hypokalemia	0	0	2 (11.1)	0	0	0
Anorexia	0	0	1 (5.6)	0	0	0
Hemorrhage, intracranial	1 (16.7)	0	0	0	0	0
Rash/acne[a]	0	0	0	3 (7.3)	4 (10.3)	0
Rash, generalized	0	0	2 (11.1)	0	0	0
Pruritus, generalized	0	0	1 (5.6)	0	0	0
Decreased appetite	0	0	0	0	2 (5.1)	0

[a]Grouped terms (phase II part).
Abbreviations: CTCAE, Common Terminology Criteria for Adverse Events.

Table 3. Baseline demographics and characteristics of patients enrolled in the phase II study

	Afatinib 40 mg/day ($n = 41$)	Afatinib 40 mg/day Plus Temozolomide 75 mg/m^2 ($n = 39$)	Temozolomide 75 mg/m^2 ($n = 39$)	Total ($n = 119$)
Male sex, *n* (%)	27 (65.9)	21 (53.8)	25 (64.1)	73 (61.3)
Age, years, mean (SD)	56.6 ± 9.44	55.4 ± 11.02	56.9 ± 10.62	56.3 ± 10.3
Time since diagnosis (months)				
Median (range)	11.7 (4.0–57.8)	11.0 (4.6–122.8)	9.2 (3.6–70.6)	10.6 (3.6–122.8)
KPS, *n* (%)				
70%	9 (22.0)	12 (30.8)	9 (23.1)	30 (25.2)
80%	12 (29.3)	9 (23.1)	13 (33.3)	34 (28.6)
90%	17 (41.5)	15 (38.5)	12 (30.8)	44 (37.0)
100%	3 (7.3)	3 (7.7)	5 (12.8)	11 (9.2)
GBM, *n* (%)				
Primary	36 (87.8)	34 (87.2)	35 (89.7)	105 (88.2)
Secondary	5 (12.2)	5 (12.8)	4 (10.3)	14 (11.8)
Mean lesion size at baseline ± SD, mm^2	1045.4 ± 972.15	1326.2 ± 1055.58	1355.9 ± 1145.73	1239.8 ± 1058.97
Prior anticancer therapy, *n* (%)				
Chemotherapy	41 (100)	39 (100)	39 (100)	119 (100)
Surgery	41 (100)	39 (100)	39 (100.0)[a]	119 (100.0)
Radiotherapy	41 (100)	39 (100)	39 (100)	119 (100)

[a]One patient had a stereotactic biopsy at initial diagnosis.
Abbreviations: GBM, glioblastoma; SD, standard deviation.

Mean duration of treatment was 90.6 days (range, 2.0–518.0 days) with no major differences across study groups; the longest mean treatment duration was in the temozolomide monotherapy group (105.9 days; range, 2.0–469.0 days), and the shortest was in the afatinib monotherapy group (68.6 days; range, 7.0–370.0 days). As of March 2014, one participant remains progression-free (> 4 years) and remains on combination therapy.

Efficacy

The PFS-6 rate, median PFS, and disease control rates (DCR = CR + PR + SD) were significantly lower in the afatinib monotherapy arm compared with the temozolomide monotherapy arm, but these measures did not differ between the combination arm and the temozolomide monotherapy arm (Fig. 1 and Table 4).

There were no significant differences in OS across the study arms (Table 4).

Efficacy subgroups

The number of participants tested for specific correlative biomarkers is summarized in Supplementary data, Table S2. EGFRvIII expression status (assessable in 70 [59%] participants) was the only factor to suggest possible differences between treatment groups (Supplementary data, Fig. S3; Table 5); there was a nonstatistically significant trend between EGFRvIII expression and minimally improved outcome with afatinib therapy. However, these findings must be interpreted cautiously; definitive conclusions cannot be drawn due to the small number of participants assessed. In those with highly positive EGFRvIII status, median PFS was 3.35, 4.99, and 3.65 months in the afatinib, temozolomide, and combination groups, respectively, based on independent review data. In comparison, median PFS was shorter in participants who were negative for EGFRvIII in the afatinib and combination arms (0.99 and 1.05 months, respectively) but not the temozolomide arm (6.49 months). In total, 6 of 25 participants (24%) with EGFRvIII-positive tumors experienced durable disease stabilization (PFS ≥4 months: 2/8 afatinib, 3/13 afatinib plus temozolomide, and 1/4 temozolomide monotherapy). At the time of data cutoff, the longest PFS (9 months) was observed in a participant treated with afatinib plus temozolomide; however, an additional participant treated with afatinib plus temozolomide has subsequently been noted to remain progression free for 48 months.[36] Among participants with EGFRvIII-negative tumors, 5 of 19 (26%) experienced durable disease stabilization (2/5 afatinib plus temozolomide and 3/6 temozolomide monotherapy).

Among participants with EGFR amplification demonstrated by FISH analysis (there was no correlation with EGFRvIII status), a median PFS of 2.73 months was noted for afatinib plus temozolomide and 1.02 months for temozolomide monotherapy (HR = 0.74; Table 5). In the subgroup with IHC-identified PTEN loss, median PFS was 2.73 months with afatinib plus temozolomide, compared with 1.87 months with temozolomide monotherapy (HR = 0.96; Table 5).

Subgroup analysis of participants with highly positive EGFRvIII status and coexisting PTEN loss highlighted a median PFS of 2.96 months in those treated with afatinib (7/7), 3.65 months with afatinib plus temozolomide (11/13), and 9.03 months with temozolomide monotherapy (2/3).

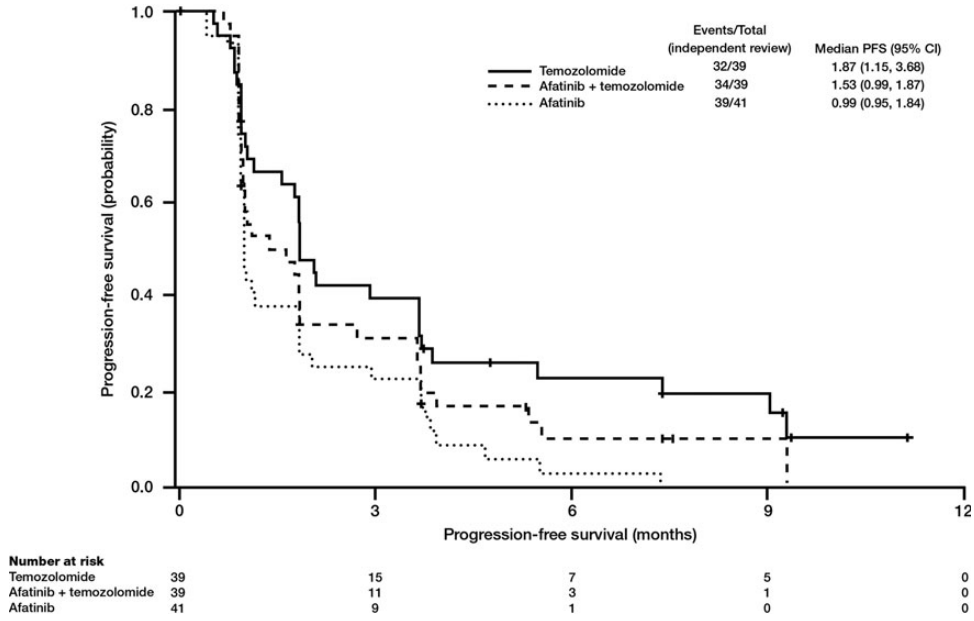

Fig. 1. Kaplan–Meier estimation of PFS in patients by treatment group (phase II part, by independent review). Abbreviations: CI, confidence interval; PFS, progression-free survival.

Table 4. Best overall response and survival outcomes in the phase II part of the trial (by independent review)

	Afatinib 40 mg/day ($n = 41$)	*P*-value[a]	Afatinib 40 mg/day Plus Temozolomide 75 mg/m^2 ($n = 39$)	*P*-value[a]	Temozolomide 75 mg/m^2 ($n = 39$)
PFS-6 rate, %	3	0.008	10	.148	23
Median PFS, months	0.99	0.032	1.53	.204	1.87
Median OS, months	9.8	0.386	8.0	.119	10.6
Disease control,[b] *n* (%) [95% CI]	15 (36.6) [22.1–53.1]	0.025	17 (43.6) [27.8–60.4]	.111	25 (64.1) [47.2–78.8]
Objective response, *n* (%) [95% CI]	1 (2.4) [0.1–12.9]	0.195	3 (7.7) [1.6–20.9]	>.99	4 (10.3) [2.9–24.2]
CR	0		1 (2.6)		0
PR	1 (2.4)		2 (5.1)		4 (10.3)
SD	14 (34.1)		14 (35.9)		21 (53.8)
Progressive disease, *n* (%)	23 (56.1)		17 (43.6)		13 (33.3)
Not evaluable, *n* (%)	3 (7.3)		5 (12.8)		1 (2.6)

[a]Versus the temozolomide 75 mg/m^2 group.
[b]Disease control was defined as CR + PR + SD.
Abbreviations: CI, confidence interval; CR, complete response; OS, overall survival; PFS, progression-free survival; PFS-6, 6-month progression-free survival; PR, partial response; SD, stable disease.

Pharmacokinetics

Afatinib gMean trough plasma concentrations were 19.8 ng/mL on Visit 1 of Cycle 2 and 19.6 ng/mL on Visit 1 of Cycle 3 in the afatinib monotherapy arm and 29.7 ng/mL and 20.2 ng/mL in the combination arm, respectively. Overall, moderate to high variability in plasma concentrations was observed as indicated by gCV values between 29.1% and 79.1%.

Safety

AEs occurred at a higher frequency in the afatinib arm (85.4%) and the combination arm (92.3%) compared with temozolomide arm (56.4%). Overall, 31 of 119 participants (26.1%) had an AE that led to study drug discontinuation and 13 (10.9%) had an AE that led to dose reduction. AEs leading to discontinuation were more frequent in the combination arm

Table 5. PFS in patients with EGFRvIII or EGFR and PTEN abnormalities

Biomarker	Patients *n* (%)	Median PFS, months (n with event/n[a])				
		Afatinib 40 mg/day	HR (95% CI)[b]	Afatinib 40 mg/day Plus Temozolomide 75 mg/m^2	HR (95% CI)[b]	Temozolomide 75 mg/m^2
EGFRvIII by IHC	70 (59)					
Highly positive	25 (21)	3.35 (8/8)	1.19 (0.30–4.79)	3.65 (11/13)	0.90 (0.24–3.40)	4.99 (3/4)
Negative	19 (16)	0.99 (7/8)	5.03 (1.16–21.76)	1.05 (4/5)	2.13 (0.47–9.59)	6.49 (4/6)
EGFR by FISH	68 (57)					
Gain	35 (29)	1.12 (9/9)	2.39 (0.88–6.50)	1.87 (13/16)	1.39 (0.56–3.42)	3.68 (8/10)
Amplification	30 (25)	0.99 (13/14)	1.27 (0.45–3.63)	2.73 (9/11)	0.74 (0.24–2.21)	1.02 (5/5)
PTEN by IHC	67 (56)					
Intact	16 (13)	0.97 (4/4)	20.1 (2.67–151.6)	1.63 (6/7)	3.90 (0.77–19.82)	7.39 (4/5)
Deletion	51 (430)	1.84 (18/19)	1.53 (0.67–3.50)	2.73 (17/21)	0.96 (0.42–2.18)	1.87 (9/11)
EGFRvIII (highly positive) and PTEN deletion	23 (19)	2.96 (7/7)		3.65 (11/13)		9.03 (2/3)

[a]Number of randomized patients for whom tumor samples were available and biomarker analysis was undertaken.
[b]Versus the temozolomide 75 mg/m^2 group.
Abbreviations: EGFR, epidermal growth factor receptor; FISH, fluorescence in situ hybridization; PFS, progression-free survival; PTEN, phosphatase and tensin homolog.

(35.9%) than in the temozolomide (23.1%) and afatinib (19.5%) arms. Of these, 28.2%, 10.3% and 4.9% were considered treatment related. AEs leading to dose reduction were also highest in the combination arm (17.9% vs 5.1% for temozolomide and 9.8% for afatinib). Rash/acne and diarrhea were the most common treatment-related AEs leading to dose reduction in the combination (7.7% and 5.1%) and afatinib arms (4.9% each), but were not experienced by participants in the temozolomide arm.

CTCAE grade ≥3 AEs were reported in 66.7% of the combination arm, 51.3% of the temozolomide arm and 43.9% of the afatinib arm. Treatment-related grade ≥3 AEs were higher in the combination arm (36%) than the afatinib (22%) or temozolomide (20%) arms (Table 2). The most frequent treatment-related grade ≥3 AEs in participants treated with afatinib were fatigue (9%), rash/acne (9%) and diarrhea (8%).

SAEs were reported in 6 (15.4%) participants in the temozolomide arm, 10 (24.4%) in the afatinib arm and 13 (33.3%) in the combination arm. Treatment-related serious AEs were reported for 2 participants (5.1%) in the combination arm (diarrhea [5.1%], vomiting [5.1%], acute pre-renal failure [2.4%], cerebral hemorrhage [2.4%], dehydration [2.4%]) and one participant (2.4%) in the afatinib arm (rash/acne). No treatment-related SAEs were reported in the temozolomide arm.

Among phase II participants, no deaths were considered to be treatment-related.

Discussion

This study showed that afatinib has limited single-agent activity in unselected participants with recurrent GBM, and the addition of afatinib to temozolomide did not improve PFS-6 rate or median PFS.

Temozolomide (plus radiation) constitutes standard care for newly diagnosed GBM1 and was selected for co-administration in this study due to evidence that some recurrent GBM patients respond to protracted temozolomide dosing.[7,8] We hypothesized that prolonged temozolomide exposure could resensitize tumor cells by depleting tumor-derived MGMT. Therefore, a dosing schedule of 75 mg/m^2 daily for 21/28 days was selected based on prior studies,[37] before data from RTOG 0525 were presented.[38]

Both afatinib and temozolomide exhibited safety profiles consistent with previous reports.[29,31,37] The most frequently reported AEs with afatinib included diarrhea and rash/acne, whereas fatigue, headache and vomiting were most common in participants receiving temozolomide. A higher frequency of AEs was noted in the combination group, but there were no AEs in this group that were inconsistent with the safety profile of either agent.

Pharmacokinetic parameters of afatinib and temozolomide were not affected by co-administration[39,40] indicating that there was no PK drug-drug interaction in the applied treatment schedule.

Despite the small numbers of participants involved in this trial, the observed antitumor activity was comparable with that observed for reversible EGFR TKIs administered on a continuous daily dosing schedule in GBM. Erlotinib alone and in combination with chemotherapy among recurrent adult malignant glioma patients achieved PR rates of 6%–25% with a modest impact on PFS or OS,[15–17,23,25,26] whereas phase II trials of gefitinib monotherapy in recurrent glioma reported response rates of 0%–13%, median PFS of 2 months and PFS-6 of 9%–13%.[14,18,19,24] Similarly, continuous lapatinib dosing has not demonstrated significant activity in GBM.[41] Moreover, EGFRvIII expression and PTEN loss did not predict a favorable subtype.[42]

Despite some conflicting data,[29–34,37] EGFRvIII overexpression appears to be a negative prognostic indicator in GBM.[43,44] However, a study validated in an independent data set found that co-expression of *EGFRvIII* and *PTEN* was significantly associated with response to erlotinib,[45] suggesting a potential subgroup effect.

PFS with afatinib monotherapy and combination therapy were higher in patients who tested highly immunoreactive for EGFRvIII compared with unselected patients. Those participants with EGFR amplification by FISH analysis and with PTEN loss on IHC also had modestly more durable responses with combination therapy than with temozolomide monotherapy; in those with PTEN loss, median PFS with combination therapy was 2.73 months compared with 1.87 months for temozolomide monotherapy. This pattern of response was not observed in participants with coexisting highly positive EGFRvIII and PTEN loss, although small sample size is a limitation. Nevertheless, these results suggest that afatinib may have higher efficacy in the presence of EFGRvIII indicating that further investigation may be warranted.

Perry, et al noted that response to protracted temozolomide dosing among recurrent GBM patients varied based on time of recurrence relative to prior adjuvant temozolomide dosing (PFS-6, 27.3% [early]; PFS-6, 7.4% [extended]; PFS,35.7% [rechallenge]).[7] Therefore, we compared PFS between the combination group and the temozolomide monotherapy group. We found no association between PFS and use of temozolomide; however, the results were limited by small sample size.

Several factors should be considered when interpreting our results. First, intratumoral PK assessment of afatinib's ability to inhibit EGFR/EGFRvIII was not incorporated into this study. As such, it is not possible to determine whether insufficient intratumoral delivery of afatinib contributed to the observed lack of therapeutic benefit. Afatinib does not cross the blood-brain barrier in healthy rats, and the extent to which it penetrates into the tumor-bearing brain has not been established. Second, at the time that this study was designed, initial disappointing results were emerging from trials evaluating first-generation, reversible EGFR inhibitors in patients with recurrent GBM.[14–19,23–26] In an attempt to improve upon on these disappointing data, this clinical trial was implemented on the basis of afatinib's ability to irreversibly block EGFR and its novel ability to effectively block EGFRvIII, despite the lack of preclinical evaluation against relevant orthotopic GBM models and confirmation of effective intratumoral CNS delivery. Third, there was no prospective enrichment based on EGFRvIII status; therefore, the sample size of participants who were highly positive for EGFRvIII was small. Fourth, although standard daily afatinib dosing was inactive for recurrent GBM participants, alternative dosing schedules such as pulsatile-increased dosing may be feasible, as observed in other cancer types.[35] Such a strategy is currently undergoing phase II evaluation in GBM partients (lapatinib plus temozolomide and standard radiation therapy [NCT01591577]; bevacizumab with standard radiation treatment [NCT01743950]).

Conclusions

Afatinib has limited single-agent activity in unselected patients with recurrent GBM. Moreover, the addition of afatinib to temozolomide did not improve the PFS-6 rate. Selected patient populations (eg, patients with high levels of EGFRvIII immunoreactivity, EGFR amplification, or PTEN loss) may have better responses and more durable PFS to afatinib; however, these results require prospective validation. There was no PK interaction between afatinib and temozolomide. The safety profile of afatinib was as expected, and AEs were effectively managed by dose reduction/interruption.

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Supplementary Material

Supplementary material is available online at *Neuro-Oncology* (http://neuro-oncology.oxfordjournals.org/).

Author Contributions

Concept and design: (D.A.R., J.C., D.D.E.; collection and assembly of data: DAR/LBN/WPM/JRP/WS/PK/SP/YF/JC/SW/DDE; Data Analysis and Interpretation: DAR/WPM/JRP/SP/AC/YF/JC/SW/DDE; Manuscript Writing: DAR/WPM/JRP/PK/DM/SP/AC/YF/JC/SW/DD; Final Approval of Manuscript: DAR/LBN/WPM/JRP/WS/PK/DM/SP/AC/YF/JC/SW/DDE; Provision of Study Materials or Patients: DAR/LBN/WPM/JRP/WS/DM/SP/YF/DDE.

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