A Phase I, open-label, dose escalation study of afatinib, in a 3-week-on/1-week-off schedule in patients with advanced solid tumors

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Summary Background A Phase I study to determine the maximum tolerated dose (MTD) and pharmacokinetics of afatinib (BIBW 2992), a novel irreversible ErbB Family Blocker, administered orally once daily in a 3-week-on/1-week-off dosing schedule. Methods Patients with advanced solid tumors received single-agent afatinib at 10, 20, 40, 55 or 65 mg/day. Safety, antitumor activity, pharmacokinetics and pharmacodynamic modulation of biomarkers were assessed. Results: Forty-three patients were enrolled. Dose-limiting toxicities (DLTs) occurred in five patients in the dose escalation phase (1/8 at 40 mg/day; 1/6 at 55 mg/day; 3/6 at 65 mg/day). The MTD was established at 55 mg/day. In the expansion cohort at the MTD, 6 patients experienced a DLT in the first 28-day treatment period. The most frequent DLT was diarrhea. The most common adverse events were diarrhea, rash, nausea, vomiting and fatigue. Overall, the afatinib safety profile in a 3-week-on/1-week-off dose schedule was similar to that of our daily-continuous schedule. Afatinib displayed dose-dependent pharmacokinetics at doses up to and including 55 mg/day, with a terminal half-life
suitable for once-daily dosing. Signs of clinical antitumor activity were observed. In biopsies taken from clinically normal forearm skin, afatinib caused a reduced proliferation rate, with a concomitant increase in differentiation of epidermal keratinocytes. Conclusion Afatinib in a 3-week-on/1-week-off schedule showed a good safety profile. The MTD was 55 mg/day, although excess DLTs in the expansion cohort indicated that the 40 mg/day dose would have an acceptable safety profile for future studies. Dose cohorts between 40 and 55 mg/day were not examined in this study.

Keywords Afatinib · Pharmacokinetics · EGFR · HER2

Introduction

The ErbB Family receptor tyrosine kinases (TK) include the epidermal growth factor receptor (EGFR; ErbB1), the human epidermal growth factor receptor (HER2; ErbB2), ErbB3 and ErbB4 [1, 2]. EGFR and HER2 are important therapeutic targets [3] but resistance to both EGFR- and HER2-targeted therapies is frequently observed [4, 5]. Irreversible inhibition of receptor TKs, or inhibition of multiple receptors in the ErbB Family, may help to prevent or overcome resistant disease as observed in the clinic.

Afatinib is a novel, potent, small molecule ErbB Family Blocker that covalently binds and irreversibly blocks signaling through activated EGFR, HER2 and ErbB4 receptors, as well as the transphosphorylation of ErbB3 [6, 7]. Afatinib is thought to inhibit cancer-relevant ErbB Family dimers, regardless of receptor dimerization status. Irreversible binding possibly improves inhibition of tumor cell proliferation compared with reversible TK inhibitors (TKIs). In vitro studies have demonstrated that afatinib has superior activity to gefitinib and erlotinib in cells expressing EGFR-activating mutations, and superior In vivo antitumor activity in animal models compared with gefitinib and erlotinib [6]. In trastuzumab-resistant HER2-overexpressing breast and gastric cancer cell lines, as well as xenograft models [8], afatinib demonstrated antitumor activity. Results of Phase II studies in HER2-positive, trastuzumab-resistant patients with breast cancer were also encouraging [9].

This study was one of four Phase I studies conducted as part of the afatinib Phase I program to explore different dosing schedules of afatinib monotherapy in patients with solid tumors. This open-label dose-escalation study evaluated once-daily treatment with afatinib in a 3-week-on/1-week-off dose schedule in patients with advanced solid tumors. The primary objective was to determine the maximum tolerated dose (MTD) of afatinib. The pharmacokinetics (PK) and antitumor activity of afatinib were also evaluated, along with an assessment of afatinib’s pharmacodynamic modulation of biomarkers.

Patients and methods

Study design

This study was conducted in line with the Declaration of Helsinki (1996 version), the International Conference on Harmonization Tripartite Guidelines for Good Clinical Practice and local legislation, and was approved by all review boards at the individual participating sites. The starting dose was 10 mg/day afatinib and tablets were to be taken at the same time each morning under fasting conditions. Dose escalation was performed in cohorts of three patients and subsequently amended to yield six evaluable patients from 20–55 mg. If no dose-limiting toxicity (DLT) occurred during the first 28-day treatment period, doses were doubled in each new cohort until NCI Common Terminology Criteria for Adverse Events (CTCAE version 3.0) Grade ≥ 2 occurred in ≥ 1 patient/cohort. Thereafter, escalation steps of no greater than 35 % were allowed. The maximum tolerated dose (MTD) was defined as the dose of afatinib at which no more than one out of six patients experienced a DLT. Once the MTD was determined, the MTD cohort was expanded to a total of 18 evaluable patients to further evaluate safety and antitumor activity. A treatment cycle was defined as a 28-day period (3 weeks on afatinib followed by 1 week off [3-week-on/1-week-off]). After 6 treatment cycles, patients were entered into an extension study.

Eligibility

Male or female patients, aged ≥ 18 years, with confirmed advanced, non-resectable and/or metastatic solid tumors known to express EGFR and/or HER2, and not responsive to established treatments, were enrolled. Patients had to have a life expectancy of ≥ 3 months; an Eastern Cooperative Oncology Group (ECOG) performance score of 0–2; resolution of prior treatment-related adverse events (AEs) to Grade ≤ 1; recovery from surgery; and provision of written informed consent. Twelve additional
patients recruited at the MTD level were required to have measurable lesions. Exclusion criteria included compromised hematological, renal and liver function; active infectious disease; chronic diarrhea or gastrointestinal disorders; left ventricular ejection fraction (LVEF) CTC Grade ≥1; untreated or symptomatic brain metastases; and treatment with other investigational, EGFR- or HER2-inhibiting drugs within 4 weeks (8 weeks for trastuzumab).

Safety and tolerability assessments

All AEs were graded according to CTCAE version 3.0. DLTs were defined as the following AEs, if they occurred within the first 28-day treatment cycle: Grade 4 hematologic AEs; Grade 3 or 4 non-hematologic AEs (except untreated nausea, vomiting, or diarrhea); AEs of Grade ≥2 for LVEF or renal function; and persistent Grade ≥2 nausea and/or vomiting for ≥7 days despite optimal supportive care.

Antitumor activity and pharmacodynamic assessments

Objective tumor responses were evaluated according to Response Evaluation Criteria in Solid Tumors [RECIST 1.0] every 8 weeks from start of treatment. Pharmacodynamics, i.e., the modulation of expression of EGFR-associated biomarkers, including EGFR, phosphorylated mitogen-activated protein kinase (p-MAPK), phosphorylated Akt (pAkt) [10], Ki-67 (an indicator of cellular proliferation); and p27KIP1 (kinase inhibitory protein 1) were assessed by immunohistochemistry on skin punch biopsies (4 mm width × 4 mm depth) taken from the lateral aspect of the upper extremity, and tumor biopsies. Skin punch and tumor samples for pharmacodynamic assessment were taken just before the first dose of afatinib, and on Day 21 of the first treatment cycle. Skin biopsies were taken on Day 21 of the 3-week-on/1-week-off treatment regimen as it was anticipated that pharmacodynamic effects would be maximal or more pronounced at this time point, based on results from an earlier study where biopsies had been taken at 2 weeks in a 2-week-on/2-week-off regimen [11]. Specimens were immediately fixed in 10 % buffered neutral formalin for 16–24 h, and embedded in paraffin. Treatment effects of afatinib were assessed by counting ≥1000 epidermal keratinocytes and scoring those positively stained for Ki-67 and p27KIP1. The number of Ki-67 and p27KIP1 positive keratinocytes was expressed as a percentage of the total keratinocytes observed. The expression of pMAPK, pAkt and EGFR in epidermal keratinocytes was assessed as for Ki-67 and p27KIP1, with the percentage of pMAPK-, pAkt- and EGFR-positive keratinocytes expressed as a percentage of the total keratinocytes counted. In addition, the intensity of pMAPK, pAkt and EGFR staining was estimated using the Allred scoring system [12]. Paired t-tests were performed for both Ki-67 and p27KIP1.

PK sampling and data analysis

Blood samples (5 mL) for PK were collected prior to dosing and 0.5, 1, 2, 3, 4, 5, 7, 9 and 24 h after afatinib administration on Days 1 and at Day 21 of Cycle 1. Additionally, a PK sample was taken at 48 and 72 h after drug administration on Day 21 of Cycle 1. Additional trough PK samples were collected on Days 8 and 15 of Cycle 1. In patients receiving additional cycles, trough PK samples were collected on Days 8, 15 and 22 of each cycle. PK sample collection and analysis were performed according to previously published methods [11].

Non-compartmental analysis was conducted using WinNonlin® (Version 4.1, Pharsight, Mountainview, CA, USA). Standard non-compartmental methods were used to calculate the following PK parameters at steady-state: the area under the plasma concentration versus time curve from 0 to 24 h at steady state (AUC0–24,ss), peak plasma concentration at steady state (Cmax,ss), the apparent total body clearance after extravascular administration at steady state (CL/Fss), the apparent volume of distribution after extravascular administration at steady state (VZ/Fss), the terminal half-life at steady state (t½,ss) and the accumulation ratio of Cmax and AUC values at Days 1 and 21 (RA,Cmax and RA,AUC). Time to peak plasma concentration at steady state (tmax,ss) was reported as a median value.

Results

Patient disposition and exposure

Forty-three patients accrued from two study sites received afatinib. The first patient was enrolled in March 2004, and the last patient completed the follow-up visit in February 2006. Approximately two-thirds of patients were female, the mean age (male and female) was 61 years, and the majority of patients had received ≥3 lines of prior therapy (Table 1).

A total of 65 % of patients received more than one treatment cycle, and 16 % completed six cycles (Table 2). Four out of seven patients who completed six cycles were rolled over into an afatinib extension study.

DLTs and MTD

DLTs occurred in one patient in the dose escalation phase, and in six patients in the expansion cohort at the MTD (Table 2). All DLTs were Grade 3 in severity apart from the renal failure reported in the 55 mg/day group, which was Grade 2. No
Drug-related AEs were experienced by a total of 40 patients (93.0 %) during the course of the trial (38 within their first 28-day cycle). The most frequently reported drug-related AEs during the conduct of the entire trial included diarrhea (n=28 [65.1 %]), rash (n=25 [58.1 %]), nausea (n=18 [41.9 %]), vomiting (n=15 [34.9 %]), fatigue (n=9 [20.9 %]), anorexia (n=7 [16.3 %]), epistaxis and mucosal inflammation (n=10 [23.3 %] each), and stomatitis (11 [25.6 %]). No treatment-related Grade ≥2 AEs were observed in the 10 mg afatinib dose cohort. In patients who received afatinib at a dose of 40 mg/day, Grade 2 or 3 AEs included rash and nausea in two patients each, and folliculitis, dehydration, diarrhea, vomiting, dysuria, fatigue and mucosal inflammation in one patient each. There appeared to be a dose relationship for incidence and intensity of diarrhea. At dose levels below the MTD, only one patient experienced Grade 2 diarrhea. At the MTD, Grade 2 and 3 diarrhea occurred in 20 % and 25 % of patients. While no drug-related diarrhea was observed at 10 mg and 20 mg afatinib doses, 50 % of the patients developed diarrhea at 40 mg, 90 % at 55 mg, and the incidence increased to 100 % at 65 mg/day. No diarrhea episode was reported after the first 28-day cycle. In the majority of cases (80 %), diarrhea started within 1–7 days after afatinib treatment initiation. Only one patient treated below the MTD discontinued due to diarrhea; this patient received 40 mg.

Skin disorders were relatively mild at all dose levels (mostly Grade 1 or 2). There were single skin-related adverse events of Grade 3 in the 10–40 mg/day dose group and at the 55 mg/day dose level, respectively. In the majority of cases, skin events began 7–28 days after treatment initiation. Skin events were considered to be related to afatinib in most cases (91 %).

Fifteen patients (34.9 %) experienced a serious adverse event (SAE). The most common SAE was treatment-related diarrhea (six patients) in the 55 mg/day and 65 mg/day dose cohorts. Three deaths occurred during afatinib administration (one of unknown cause, one of myocardial infarction and one of progressive breast cancer). None were considered to be related to afatinib.

No patients had Grade ≥2 reductions in LVEF. Grade 3 aspartate aminotransferase elevations were only observed after discontinuation of the trial drug and associated with progression of disease in three patients with known liver metastases for CRC and breast cancer (BC).

Antitumor activity

There were no confirmed objective responses; however, some signs of antitumor activity were reported in this largely
heavily pretreated cancer population. One patient with squa-
mous-cell skin carcinoma experienced a transient partial re-
sponse with a decrease in tumor size of 31 %, but showed
progressive disease at the repeat evaluation 2 months later.
One patient with parotid carcinoma had tumor shrinkage of
13 % in Cycle 4, which was maintained until Cycle 6. This
patient was then enrolled into an extension study and received
treatment for a total of 322 days. Two more patients, one with
non-small cell lung cancer (NSCLC) and one with CRC,
showed a decrease in tumor size of 27 % and 14 %, respec-
tively. In addition, 8 patients had stable disease for at least
16 weeks and received at least 5 cycles of treatment.

Pharmacodynamics

A total of 29 paired skin samples (i.e., 29 pretreatment and
29 on-therapy skin specimens obtained from the clinically
normal forearm skin of the patient) were available for phar-
macodynamic studies. At doses of ≥ 55 mg/day, treatment
with afatinib significantly reduced the number of Ki-67-
positive cells in skin biopsies by 31 %, demonstrating inhibi-
tion of epidermal keratinocyte cell proliferation (Fig. 1a
and b). This was accompanied by an increase in the total
number of p27KIP1-positive epidermal keratinocytes, which
was about 16 % higher than pretreatment samples (Fig. 1c
and d). A similar trend was observed at lower doses. No
significant changes were observed in levels of pMAPK and
EGFR in normal skin punch biopsies between pretreatment
and on-therapy samples. While no significant difference in
the level of pAkt was observed between the pretreatment
and on-therapy paired skin biopsies in eight out of 29 paired
samples, there was a slight decrease in pAkt staining and in
nine out of 29 cases a slight increase. In the 12 remaining
cases, no treatment-induced changes were observed. There-
fore, the observed changes were probably due to biological
inter- and intra-patient variability.

Pharmacokinetics

Geometric mean (gMean) plasma concentration–time curves
of afatinib are displayed in Fig. 2. Afatinib exhibited similar
disposition kinetics after single and multiple dosing, which
could be described by at least bi-exponential disposition
kinetics. The gMean plasma concentrations on Days 1 and
21 increased with dose. Steady-state was reached within 8 days of once-daily dosing of afatinib, at the latest. At steady-state, both maximum plasma concentrations (C_{max,ss}) and exposure (AUC_{0-24,ss}), increased with the administered dose (Table 4). Peak plasma concentrations at steady-state were reached 3–5 h after dosing (median t_{max,ss} values). The gMean terminal half-life at steady-state was measured over a range between 35 and 43 h at Day 2. A moderate-to-high apparent total body clearance and a high volume of distribution were determined for afatinib (Table 4). The gMean accumulation ratios ranged from 1.36 to 2.35 when based on C_{max} values, and from 1.81 to 3.07 when based on AUC values. Moderate-to-high inter-patient variability for all PK parameters was detected over all groups.

### Discussion

The MTD of afatinib administered in a 3-week-on/1-week-off dose schedule was primarily determined to be 55 mg/day. However, due to the much higher occurrence of diarrhea at the 55 mg/day compared with the 40 mg/day dose, and the number of additional DLTs that were observed in the expansion cohort, the recommended dose for further studies using this schedule seems to be 40 mg/day, although dose cohorts between 40 and 55 mg/day were not examined in this study. Three other afatinib monotherapy Phase I studies using alternative dosing schemes have been performed in patients with solid tumors as part of the development program for afatinib; one study used a 2-week-on/2-week-off schedule [11], and two studies used a continuous dosing schedule [13, 14]. Based on the combined results from these four trials, 50 mg/day afatinib was established as the recommended Phase II dose for a continuous dosing schedule.

Afatinib was found to have an acceptable safety profile with no treatment-related Grade ≥4 AEs reported in any of the afatinib dose cohorts. As reported in other Phase I studies conducted with afatinib [11, 14], the most commonly reported treatment-related AEs were diarrhea, rash and nausea and were manageable with appropriate supportive care and dose reduction.

The adverse-event profile reported with afatinib was consistent with the safety profile of EGFR inhibitors [15–20]. In a Phase I dose-escalation study of the EGFR inhibitor gefitinib administered continuously in patients with solid tumors, DLTs observed were rash and diarrhea. The incidence of all grades of diarrhea appeared to be dose related and predominantly began during the first treatment period [18]. Incidence and severity of diarrhea also appeared to be related to dose during a Phase I investigation of erlotinib [19]; a pattern which was in agreement with results in this Phase I trial. Diarrhea was experienced within 1–7 days after starting afatinib treatment, with no

### Table 3

| Adverse events, n |
|-------------------|
| Afatinib dose (mg/day) | 10 | 20 | 40 | 55 | 65 |
| Gradea | 1 | 1 | 2 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| Cycleb | 1 | ≥2 | 1 | ≥2 | 1 | ≥2 | 1 | ≥2 | 1 | ≥2 | 1 | ≥2 | 1 | ≥2 | 1 | ≥2 | 1 | ≥2 | 1 | ≥2 |
| Nausea | 1 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 |
| Vomiting | 1 | 0 | 0 | 1 | 0 | 1 | 2 | 0 | 0 | 1 | 0 | 3 | 1 | 2 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 |
| Stomatitis | 0 | 0 | 2 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 2 | 2 | 1 | 0 | 1 | 0 | 2 | 0 | 0 | 0 |
| Diarrhea | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 1 | 0 | 1 | 0 | 9 | 0 | 4 | 0 | 5 | 0 | 2 | 0 | 2 | 0 | 2 |
| Pruritus | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 2 | 0 | 2 | 0 | 0 | 0 | 3 | 0 | 0 | 0 |
| Rash | 1 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 1 | 0 | 1 | 0 | 10 | 0 | 3 | 1 | 0 | 0 | 3 | 0 | 1 | 0 |
| Dry skin | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 3 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 |
| Dermatitis aciform | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| Epistaxis | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 2 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Anorexia | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 2 | 0 | 0 | 0 | 1 | 0 |
| Dehydration | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 2 | 0 | 0 | 0 | 0 | 2 |
| Fatigue | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 1 |
| Mucosal inflammation | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 4 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 |

| AEa adverse events, CTCAE Common Terminology Criteria for Adverse Events |
|-------------------|
a Worst CTCAE Grade  

b The cycle in which AEs started; Cycle length: 28 days; No treatment-related Grade ≥2 AEs were observed in the 10 mg dose cohort. No treatment-related Grade ≥4 AEs were reported in any of the afatinib dose cohorts.
discontinuations at lower doses indicating that appropriate management with early institution of supportive care and timely dose reductions are crucial to keep patients on afatinib while they are benefiting from treatment.

In agreement with other studies [11, 14], afatinib was absorbed moderately fast and displayed a terminal half-life that favors a once-daily dosing schedule (Fig. 2; Table 4). No deviation from dose linear PK was observed after a single dose or at steady-state either in this study or in studies using other dose ranges and schedules [11, 14]. A relatively high apparent total body clearance and volume of distribution were observed. Since the absolute bioavailability of afatinib in humans is unknown, these values should be treated with caution, although these data may

![Fig. 1](image1)

**Fig. 1** Ki-67 and p27KIP1 response in normal skin by dose. Afatinib treatment (all doses) resulted in a reduction in the number of Ki-67-positive epidermal keratinocytes expressed as a percentage of total keratinocytes assessed (mean±SD; on-therapy versus pretreatment samples) (a), and this reduction in the number of Ki-67-positive keratinocytes was also observed after afatinib treatment regardless of dose for each individual patient (each line represents results from a single patient) (b). Afatinib treatment resulted in an increase in the number of p27KIP1-positive keratinocytes expressed as a percentage of total keratinocytes assessed (mean±SD) in pretreatment and on-therapy samples (c). Similar effects were seen for the majority of individual patients (d).

![Fig. 2](image2)

**Fig. 2** Geometric mean drug plasma concentration–time profiles of afatinib after single and multiple rising oral administration of 10, 20, 40, 55 and 65 mg once daily afatinib tablets for 21 days in Treatment Period 1.
indicate that afatinib has a suitable elimination profile and a high tissue distribution. All PK parameters displayed moderate-to-high variability, although parameters were in high tissue distribution. All PK parameters displayed indicate that treatment with afatinib results in modulation of EGFR effectors pAkt and pMAPK; (ii) activation of alternate rescue pathways (re)activating these downstream effectors, or, (iii) methodological shortcomings including a low detection threshold or selection of inappropriate time-points to capture any conceived changes.

Although no confirmed responses were observed in this trial of heavily pretreated patients, antitumor activity of afatinib has been confirmed in multiple trials using afatinib at 40 mg/day or 50 mg/day in a daily-continuous dosing schedule. As a consequence, a continuous once-daily regimen is considered the optimal dosing for afatinib. This schedule is currently being assessed in ongoing Phase III trials in HER2-positive breast cancer, NSCLC, and head and neck squamous-cell carcinoma. Results from a completed Phase III trial in patients with advanced lung adenocarcinoma and EGFR mutations (LUX-Lung 3) have shown that afatinib is associated with prolongation of progression-free survival when compared with standard first-line doublet therapy [25].

In conclusion, afatinib in a 3-week-on/1-week-off schedule showed a tolerable safety profile. The MTD was defined per protocol to be 55 mg/day. However, the excess DLTs observed in the expansion cohort show that the 40 mg/day dose would have an acceptable safety profile for future studies. Dose cohorts between 40 and 55 mg/day were not examined in this study.
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