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PHASE I STUDIES

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

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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].

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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 every cohort 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 $p27^{KIP1}$ (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 $p27^{KIP1}$. The number of Ki-67 and $p27^{KIP1}$ 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 $p27^{KIP1}$, 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 $p27^{KIP1}$.

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 ($AUC_{0-24,ss}$), peak plasma concentration at steady state ($C_{max,ss}$), the apparent total body clearance after extravascular administration at steady state (CL/F_{ss}), the apparent volume of distribution after extravascular administration at steady state (V_Z/F_{ss}), the terminal half-life at steady state ($t_{½,ss}$) and the accumulation ratio of C_{max} and AUC values at Days 1 and 21 ($R_{A,Cmax}$ and $R_{A,AUC}$). Time to peak plasma concentration at steady state ($t_{max,ss}$) 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

Table 1 Baseline clinical and demographic characteristics of patients

	Total patient population
Total treated	n (%)
	43 (100.0)
Gender	n (%)
Female	27 (62.8)
Male	16 (37.2)
Race	n (%)
Asian	5 (11.6)
Black	4 (9.3)
White	34 (79.1)
Age	years
Median (range)	61.0 (46.0–82.0)
Type of cancer	n (%)
Breast	8 (18.6)
Colorectal	11 (25.6)
Prostate	2 (4.7)
Thyroid	3 (7.0)
Other[a]	19 (44.2)
Number of prior therapies	n (%)
0	1 (2.3)
1	5 (11.6)
2	3 (7.0)
≥3	34 (79.1)

HER2 status was not known, as no tumor biopsies were analyzed by the central laboratory. The analysis of tumor biopsies was not mandatory

[a] Other cancers include: thyroid, esophageal, pancreatic, liver, lung, head and neck, prostate, gastric, and skin

DLTs occurred at 10 or 20 mg/day. At 40 mg/day, a 66-year-old female patient with colorectal cancer (CRC) developed a Grade 3 rash lasting 13 days, which resolved after dose reduction. In the 55 mg/day cohort, one patient developed a Grade 3 stomatitis. In the 65 mg/day cohort, three of the six patients experienced DLTs: diarrhea in one patient; dehydration and fatigue in another patient; and diarrhea, nausea, vomiting and dehydration in a further patient. Hence, the 55 mg/day dose was established as the MTD and was further evaluated in an expansion cohort, in which 14 patients, rather than the planned 12 patients, were treated. Of them, six patients developed DLTs including diarrhea (three patients), mucosal inflammation (one patient), dermatitis acneiform (one patient), and anorexia, dehydration and renal failure (one patient). Overall, seven of 20 patients treated at the MTD 55 mg/day developed an AE qualifying as a DLT during the first cycle, thus exceeding the preset limit in the protocol. Further enrolment into this dose cohort was discontinued. An expansion cohort below 55 mg/day was not evaluated.

Safety and tolerability

Overall, afatinib was tolerated with mainly mild or moderate (Grade 1 and 2) AEs. No Grade 4 or 5 AEs were observed. Table 3 summarizes all treatment-related AEs observed in the first 28-day cycle and in subsequent treatment cycles by dose level and CTCAE grade.

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

Table 2 Patient disposition and DLTs

	Afatinib dose					Total
	10 mg/day	20 mg/day	40 mg/day	55 mg/day	65 mg/day	
Patients treated, n^{total}	3	6	8	20	6	43 (100.0)
Patients who completed 1 cycle, n (%)	0 (0.0)	1 (16.7)	4 (50.0)	7 (35.0)	3 (50.0)	15 (34.9)
Patients who completed 4 cycles, n (%)	1 (33.3)	1 (16.7)	0 (0.0)	2 (10.0)	0 (0.0)	4 (9.3)
Patients who completed 6 cycles, n (%)	0 (0.0)	1 (16.7)	2 (25.0)	3 (15.0)	1 (16.7)	7 (16.3)
Patients treated in extension study, n (%)	–	–	–	–	–	4 (9.3)
DLTs occurring during the first treatment cycle[a]						
n (%)	–	–	1 (12.5)	7 (35.0)	3 (50.0)	11 (25.6)
			Rash (1 pt)	Stomatitis (1 pt)	Diarrhea (1 pt)	
				Diarrhea (3 pts)	Dehydration, fatigue (1 pt)	
				Mucosal inflammation (1 pt)	Dehydration, diarrhea, nausea, vomiting (1 pt)	
				Dermatitis acneiform (1 pt)		
				Anorexia, dehydration, renal failure (1 pt)		
Discontinued due to:						
DLT, n (%)	–	–	–	3 (15.0)	1 (16.7)	4 (9.3)
Other toxicity, n (%)	–	–	1 (12.5)	4 (20.0)	2 (33.3)	7 (16.3)
Disease progression, n (%)	3 (100.0)	2 (33.3)	3 (37.5)	8 (40.0)	1 (16.7)	17 (39.5)
Consent withdrawn, n (%)	–	–	–	1 (5.0)	–	1 (2.3)
Other reasons[b], n (%)	–	1 (16.7)	1 (12.5)	1 (5.0)	1 (16.7)	4 (9.3)

DLTs dose limiting toxicities, *pt* patient, *CTC* common terminology criteria

n = number of patients; n^{total} = total number of patients in each group

% based on n^{total}

[a] All DLTs listed were CTC Grade 3 except renal failure, which was CTC Grade 2

[b] Includes worsening of disease other than cancer, lost to follow-up, non-compliant and other

heavily pretreated cancer population. One patient with squamous-cell skin carcinoma experienced a transient partial response 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 %, respectively. 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 pharmacodynamic 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 inhibition of epidermal keratinocyte cell proliferation (Fig. 1a and b). This was accompanied by an increase in the total number of $p27^{KIP1}$-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. Therefore, 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

Table 3 Selected treatment-related AEs by treatment, highest CTCAE grade and preferred term

	Adverse events, n																							
Afatinib dose (mg/day)	10		20				40						55						65					
Grade[a]	1		1		2		1		2		3		1		2		3		1		2		3	
Cycle[b]	1	≥2	1	≥2	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	2	0	5	1	4	0	0	0	1	0	1	0	1	0
Vomiting	1	0	0	0	1	0	1	2	0	0	1	0	3	1	2	0	1	0	1	0	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	0	0
Diarrhea	0	0	0	0	0	0	2	0	1	0	1	0	9	0	4	0	5	0	2	0	2	0	2	0
Pruritus	1	0	0	0	0	0	1	1	0	0	0	0	2	0	2	0	0	0	3	0	0	0	0	0
Rash	1	0	0	0	0	1	3	0	1	0	1	0	10	0	3	1	0	0	3	0	1	0	0	0
Dry skin	0	0	1	0	0	0	0	0	0	0	0	0	4	3	0	0	0	0	2	0	0	0	0	0
Dermatitis acneiform	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	1	0	0	0	0	0	0	0
Epistaxis	0	0	2	0	0	0	0	0	0	0	0	0	5	2	0	0	0	0	1	0	0	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	0	0
Dehydration	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	2	0	0	0	0	0	2	0
Fatigue	0	0	0	0	0	0	0	0	0	0	1	0	0	0	4	1	0	1	1	0	0	0	1	0
Mucosal inflammation	0	0	0	0	0	0	0	1	1	0	0	0	4	1	1	0	1	0	1	0	0	0	0	0

AEs 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.

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 assessed in a 3-week-on/1-week-off schedule. 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

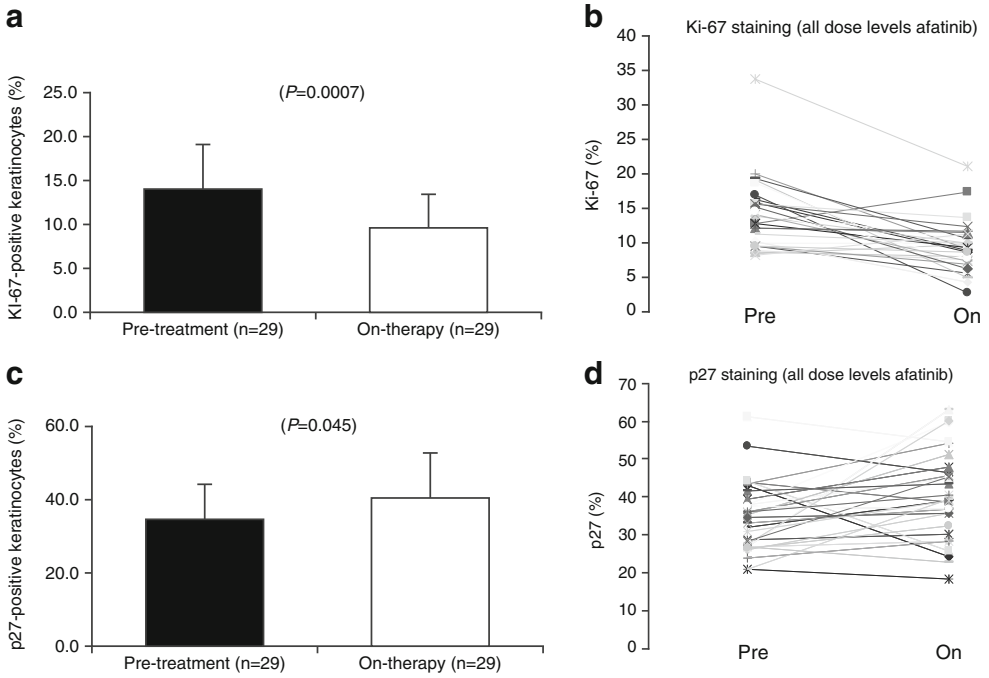

Fig. 1 Ki-67 and $p27^{KIP1}$ response in normal skin by dose. Afatinib treatment (all doses) resulted in a reduction in the number of Ki-67-positive epidermal keratinocytes expressed as a percentage of total keratinocytes assessed (mean±SD; on-therapy versus pretreatment samples) (**a**), and this reduction 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 $p27^{KIP1}$-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**)

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

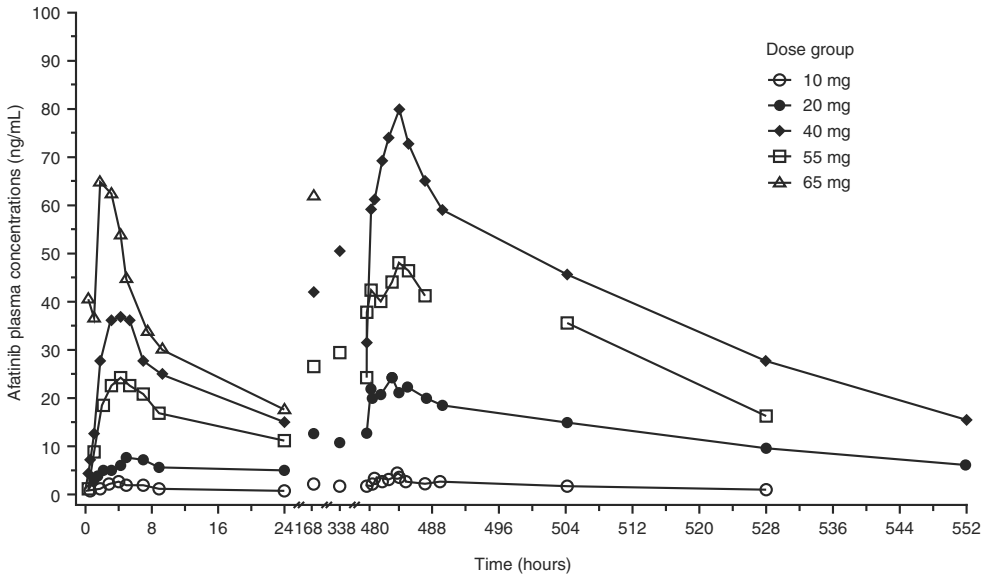

Fig. 2 Geometric mean drug plasma concentration–time profiles of afatinib after single and multiple rising oral administration of 10, 20, 40, 55 and 65 mg once daily afatinib tablets for 21 days in Treatment Period 1

Table 4 Geometric mean (and geometric coefficient of variation %) PK parameters of afatinib at steady-state (Day 21) after oral administration of 10 mg, 20 mg, 40 mg, 55 mg and 65 mg once daily afatinib tablets in Treatment Period 1

		Afatinib dose, once daily				
		10 mg	20 mg	40 mg	55 mg	65 mg
No. of patients		3	6	5	11	2[a]
$C_{max,ss}$	(ng/mL)	3.99 (83.7)	31.3 (94.1)	58.9 (83.7)	90.8 (67.8)[b]	18.0, 94.7
$C_{max,ss,norm}$	([ng/mL]/mg)	0.399 (83.7)	1.57 (94.1)	1.47 (83.7)	1.65 (67.8)[b]	0.277, 1.46
$AUC_{0-24,ss}$	(ng·h/mL)	63.7 (99.8)	455 (61.8)	908 (69.1)	1360 (68.1)[b]	355, 1270
$AUC_{0-24,ss,norm}$	([ng·h/mL]/mg)	6.37 (99.8)	22.8 (61.8)	22.7 (69.1)	24.7 (68.1)[b]	5.46, 19.5
$t_{max,ss}$[c]	(h)	4.00 (0.533–4.07)	5.00 (0.500–9.08)	3.00 (0.467–7.08)	3.00 (2.00–5.00)[b]	9.00, 2.00
$t_{½,ss}$	(h)	35.7 (33.1)	43.2 (37.9)	42.8 (35.9)	35.1 (14.1)	33.3, 25.8
CL/F_{ss}	(mL/min)	2620 (99.8)	732 (61.8)	734 (69.1)	653 (6.53)	3050, 856
V_z/F_{ss}	(L)	8080 (76.6)	2740 (66.8)	2720 (70.1)	2040 (62.0)[b]	8780, 1910
$R_{A,Cmax}$		1.36 (88.9)	2.06 (31.1)	1.88 (55.0)	2.35 (53.9)[b]	0.488, 1.15
$R_{A,AUC}$		1.81 (50.5)	3.07 (34.0)	2.27 (39.6)	2.90 (42.0)[b]	1.34, 1.50

PK pharmacokinetic, $C_{max,ss}$ maximum measured concentration of the analyte in plasma at steady-state, $C_{max,ss,norm}$ maximum measured concentration of the analyte in plasma at steady-state (dose-normalized), $t_{max,ss}$ time from dosing to the maximum concentration of the analyte in plasma at steady-state, $AUC_{0-24,ss}$ area under the concentration–time curve of the analyte in plasma over the respective time interval (hours) at steady-state; $AUC_{0-24,ss,norm}$ area under the concentration–time curve of the analyte in plasma over the respective time interval (hours) at steady-state (dose-normalized), $t_{½,ss}$ terminal half-life of the analyte in plasma at steady-state, CL/F_{ss} apparent clearance of the analyte in plasma following extravascular administration at steady-state, V_z/F_{ss} apparent volume of distribution during the terminal phase λ_z following an extravasular dose at steady-state, $R_{A,Cmax}$ accumulation ratio of C_{max} after multiple dose administration over a uniform dosing interval, $R_{A,AUC}$ accumulation ratio of AUC after multiple dose administration over a uniform dosing interval

[a] Individual values are displayed

[b] n=10

[c] Median and range are displayed

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 the expected range compared with other EGFR TKIs [20–23]. Analysis of the correlations between the PK and AE data in this population was not conducted because the PK data were too sparse.

Biomarker analysis of skin biopsies showed no changes in EGFR levels after 3 weeks of treatment with afatinib at any dose. This observation in benign skin biopsies is not necessarily unexpected and is similar to previous findings [11]. Treatment with afatinib resulted in significant inhibition of epidermal keratinocyte proliferation as judged by the Ki-67 index. This was accompanied by a significant increase in the number of $p27^{KIP1}$-positively stained epidermal keratinocytes, indicating an induction in differentiation of the epidermal keratinocytes assessed. In preclinical models, an induction of cellular differentiation has been correlated with the arrest of cellular growth associated with the inhibition of the EGFR pathway [24]. While these data indicate that treatment with afatinib results in modulation of EGFR signaling pathways, there was no change in levels of pMAPK, pAkt or EGFR. This observation may be due to: (i) a lack of inhibitory effect of afatinib on downstream 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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References

1. Arteaga CL (2002) Epidermal growth factor receptor dependence in human tumors: more than just expression? Oncologist 7(Suppl 4):31–39
2. Baselga J (2002) Why the epidermal growth factor receptor? The rationale for cancer therapy. Oncologist 7(Suppl 4):2–8
3. Hynes NE, Lane HA (2005) ERBB receptors and cancer: the complexity of targeted inhibitors. Nat Rev Cancer 5(5):341–354. doi:10.1038/nrc1609
4. Engelman JA, Janne PA (2008) Mechanisms of acquired resistance to epidermal growth factor receptor tyrosine kinase inhibitors in non-small cell lung cancer. Clin Cancer Res 14(10):2895–2899. doi:10.1158/1078-0432.CCR-07-2248
5. Nahta R, Esteva FJ (2006) Herceptin: mechanisms of action and resistance. Cancer Lett 232(2):123–138. doi:10.1016/j.canlet.2005.01.041
6. Li D, Ambrogio L, Shimamura T, Kubo S, Takahashi M, Chirieac LR, Padera RF, Shapiro GI, Baum A, Himmelsbach F, Rettig WJ, Meyerson M, Solca F, Greulich H, Wong KK (2008) BIBW2992, an irreversible EGFR/HER2 inhibitor highly effective in preclinical lung cancer models. Oncogene 27(34):4702–4711. doi:10.1038/onc.2008.109
7. Solca F, Dahl G, Zoephel A, Bader G, Sanderson M, Klein C, Kraemer O, Himmelsbach F, Haaksma E, Adolf GR (2012) Target binding properties and cellular activity of afatinib (BIBW 2992), an irreversible ErbB family blocker. J Pharmacol Exp Ther. doi:10.1124/jpet.112.197756
8. Solca F, Baum A, Guth B, Colbatzky F, Blech S, Amelsberg A, Himmelsbach F (2005) BIBW 2992, an irreversible dual EGFR/HER2 receptor tyrosine kinase inhibitor for cancer therapy. Proceedings, AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapeutics Philadelphia, PA 14–18 November 2005:118 (Abstract A244)
9. Lin NU, Winer EP, Wheatley D, Carey LA, Houston S, Mendelson D, Munster P, Frakes L, Kelly S, Garcia AA, Cleator S, Uttenreuther-Fischer M, Jones H, Wind S, Vinisko R, Hickish T (2012) A phase II study of afatinib (BIBW 2992), an irreversible ErbB family blocker, in patients with HER2-positive metastatic breast cancer progressing after trastuzumab. Breast Cancer Res Treat. doi:10.1007/s10549-012-2003-y
10. Hirsch FR, Varella-Garcia M, Cappuzzo F, McCoy J, Bemis L, Xavier AC, Dziadziuszko R, Gumerlock P, Chansky K, West H, Gazdar AF, Crino L, Gandara DR, Franklin WA, Bunn PA Jr (2007) Combination of EGFR gene copy number and protein expression predicts outcome for advanced non-small-cell lung cancer patients treated with gefitinib. Ann Oncol 18(4):752–760. doi:10.1093/annonc/mdm003
11. Eskens FA, Mom CH, Planting AS, Gietema JA, Amelsberg A, Huisman H, van Doorn L, Burger H, Stopfer P, Verweij J, de Vries EG (2008) A phase I dose escalation study of BIBW 2992, an irreversible dual inhibitor of epidermal growth factor receptor 1 (EGFR) and 2 (HER2) tyrosine kinase in a 2-week on, 2-week off schedule in patients with advanced solid tumours. Br J Cancer 98 (1):80–85. doi:10.1038/sj.bjc.6604108
12. Allred DC, Harvey JM, Berardo M, Clark GM (1998) Prognostic and predictive factors in breast cancer by immunohistochemical analysis. Mod Pathol 11(2):155–168
13. Agus DB, Terlizzi E, Stopfer P, Amelsberg A, Gordon MS (2006) A phase I dose escalation study of BIBW 2992, an irreversible dual EGFR/HER2 receptor tyrosine kinase inhibitor, in a continuous schedule in patients with advanced solid tumours. J Clin Oncol 24(18S):2074
14. Yap TA, Vidal L, Adam J, Stephens P, Spicer J, Shaw H, Ang J, Temple G, Bell S, Shahidi M, Uttenreuther-Fischer M, Stopfer P, Futreal A, Calvert H, de Bono J, Plummer R (2010) Phase I trial of the irrevisible ErbB1 (EGFR) and ErbB2 (HER2) kinase inhibitor BIBW 2992 in patients with advanced solid tumours. J Clin Oncol 28(25):3965–3972
15. Choong NW, Cohen EE (2006) Epidermal growth factor receptor directed therapy in head and neck cancer. Crit Rev Oncol Hematol 57(1):25–43. doi:10.1016/j.critrevonc.2005.06.002
16. Grunwald V, Hidalgo M (2003) Developing inhibitors of the epidermal growth factor receptor for cancer treatment. J Natl Cancer Inst 95(12):851–867
17. Arora A, Scholar EM (2005) Role of tyrosine kinase inhibitors in cancer therapy. J Pharmacol Exp Ther 315(3):971–979. doi:10.1124/jpet.105.084145
18. Herbst RS, Maddox AM, Rothenberg ML, Small EJ, Rubin EH, Baselga J, Rojo F, Hong WK, Swaisland H, Averbuch SD, Ochs J, LoRusso PM (2002) Selective oral epidermal growth factor receptor tyrosine kinase inhibitor ZD1839 is generally well-tolerated and has activity in non-small-cell lung cancer and other solid tumors: results of a phase I trial. J Clin Oncol 20(18):3815–3825
19. Hidalgo M, Siu LL, Nemunaitis J, Rizzo J, Hammond LA, Takimoto C, Eckhardt SG, Tolcher A, Britten CD, Denis L, Ferrante K, Von Hoff DD, Silberman S, Rowinsky EK (2001) Phase I and pharmacologic study of OSI-774, an epidermal growth factor receptor tyrosine kinase inhibitor, in patients with advanced solid malignancies. J Clin Oncol 19(13):3267–3279
20. Ranson M, Hammond LA, Ferry D, Kris M, Tullo A, Murray PI, Miller V, Averbuch S, Ochs J, Morris C, Feyereislova A, Swaisland H, Rowinsky EK (2002) ZD1839, a selective oral epidermal growth factor receptor-tyrosine kinase inhibitor, is well tolerated and active in patients with solid, malignant tumors: results of a phase I trial. J Clin Oncol 20(9):2240–2250
21. Burris HA 3rd, Hurwitz HI, Dees EC, Dowlati A, Blackwell KL, O'Neil B, Marcom PK, Ellis MJ, Overmoyer B, Jones SF, Harris JL, Smith DA, Koch KM, Stead A, Mangum S, Spector NL (2005) Phase I safety, pharmacokinetics, and clinical activity study of lapatinib (GW572016), a reversible dual inhibitor of epidermal growth factor receptor tyrosine kinases, in heavily pretreated patients with metastatic carcinomas. J Clin Oncol 23(23):5305–5313

22. Baselga J, Rischin D, Ranson M, Calvert H, Raymond E, Kieback DG, Kaye SB, Gianni L, Harris A, Bjork T, Averbuch SD, Feyereislova A, Swaisland H, Rojo F, Albanell J (2002) Phase I safety, pharmacokinetic, and pharmacodynamic trial of ZD1839, a selective oral epidermal growth factor receptor tyrosine kinase inhibitor, in patients with five selected solid tumor types. J Clin Oncol 20(21):4292–4302
23. Hidalgo M, Bloedow D (2003) Pharmacokinetics and pharmacodynamics: maximizing the clinical potential of Erlotinib (Tarceva). Semin Oncol 30(3 Suppl 7):25–33
24. Di Gennaro E, Barbarino M, Bruzzese F, De Lorenzo S, Caraglia M, Abbruzzese A, Avallone A, Comella P, Caponigro F, Pepe S, Budillon A (2003) Critical role of both p27KIP1 and p21CIP1/WAF1 in the antiproliferative effect of ZD1839 ('Iressa'), an epidermal growth factor receptor tyrosine kinase inhibitor, in head and neck squamous carcinoma cells. J Cell Physiol 195(1):139–150. doi:10.1002/jcp.10239
25. Sequist L, Yang J, C-H., Yamamoto N, O'Byrne K, Hirsh V, Mok T, Geater SL, Orlov S, Tasai C-M, Boyer M, Su W-C, Bennouna J, Kato T, Gorbunova V, Lee KH, Shah R, Massey D, Zazulina V, Shahidi M, Schuler M (2012) Phase III study of afatinib or cisplatin/pemetrexed in metastatic lung adenocarcinoma patients with epidermal growth factor receptor mutations. J Clin Oncol In press