Cancer Chemother Pharmacol
DOI 10.1007/s00280-011-1738-1

ORIGINAL ARTICLE

Phase I study of continuous afatinib (BIBW 2992) in patients with advanced non-small cell lung cancer after prior chemotherapy/erlotinib/gefitinib (LUX-Lung 4)

H. Murakami · T. Tamura · T. Takahashi · H. Nokihara · T. Naito · Y. Nakamura · K. Nishio · Y. Seki · A. Sarashina · M. Shahidi · N. Yamamoto

Received: 17 January 2011 / Accepted: 31 August 2011

Abstract
Purpose This Phase I study determined the maximum-tolerated dose (MTD) of afatinib (Afatinib is an investigational compound and its safety and efficacy have not yet been established) (BIBW 2992; trade name not yet approved by FDA), an irreversible inhibitor of epidermal growth factor receptor (EGFR)/human epidermal growth factor receptor (HER)1 and 2, up to a dose of 50 mg/day in advanced non-small cell lung cancer (NSCLC), to establish the recommended dose for Phase II.

Methods Patients with advanced NSCLC who had received prior platinum-doublet chemotherapy and/or erlotinib/gefitinib therapy, or who were ineligible for, or not amenable to, treatment with established therapies, received oral afatinib once daily. The MTD was determined based on dose-limiting toxicities (DLTs); other assessments included safety, pharmacokinetic profile, antitumour activity according to response evaluation criteria in solid tumours and EGFR/HER1 mutation analysis where possible.
Results Twelve evaluable patients were treated at doses of 20–50 mg/day. One DLT was observed at 50 mg/day in Course 1 (Grade 3 mucositis). The most frequent drug-related adverse events were diarrhoea, dry skin, stomatitis, rash, paronychia and anorexia; most were Grade 1 or 2. Six out of 12 patients had tumour size reductions; durable stable disease was achieved in three patients including one with EGFR/HER1 exon 19 and T790 M mutations. Peak plasma concentrations of afatinib were reached 3–4 h after administration and declined with a half-life of 30–40 h. Afatinib 50 mg/day was well tolerated with an acceptable safety profile during Phase I.
Conclusion Recommended dose for Phase II was defined as 50 mg/day for Japanese patients; the same as for non-Japanese patients.

Keywords Phase I · Afatinib · BIBW 2992 · Epidermal growth factor receptor · Tyrosine kinase inhibitor · Non-small cell lung cancer

All authors have contributed equally to the development of the manuscript, and all authors are in agreement with the content of the manuscript.

Clinical trial registration: NCT00711594 (http://clinicaltrials.gov).

H. Murakami · T. Takahashi · T. Naito · Y. Nakamura · N. Yamamoto (✉)
Division of Thoracic Oncology, Shizuoka Cancer Center, 1007, Shimonagakubo Nagaizumi-cho Sunto-gun, Shizuoka 411-8777, Japan
e-mail: n.yamamoto@scchr.jp

T. Tamura · H. Nokihara
National Cancer Center Hospital, Tokyo, Japan

K. Nishio
Kinki University School of Medicine, Osaka, Japan

Y. Seki
Nippon Boehringer Ingelheim, Tokyo, Japan

A. Sarashina
Nippon Boehringer Ingelheim, Kobe, Japan

M. Shahidi
Boehringer Ingelheim, Bracknell, UK

Introduction

Despite the availability of a variety of conventional anti-cancer agents, non-small cell lung cancer (NSCLC)

Published online: 10 November 2011

remains a leading cause of cancer death worldwide. However, increased understanding of the mechanisms underlying cancer development has led to rational approaches to drug development and new treatment agents designed to specifically target these mechanistic pathways [1]. The epidermal growth factor receptor (EGFR or ErbB) tyrosine kinase family is one of the most extensively studied signal transduction networks and is known to promote cancer cell proliferation and tumour invasion [2]. The ErbB receptor family consists of four receptor tyrosine kinases, which includes EGFR (also known as ErbB1 or human epidermal growth factor receptor [HER]1), HER2 (neu/ErbB2), HER3 (ErbB3) and HER4 (ErbB4) [2, 3]. Hyperactivation of the ErbB signalling network has been observed in a variety of malignancies [2, 4] and represents an attractive option for targeted therapy in patients with NSCLC, as overexpression of EGFR/HER1 has been detected in 40–80% of NSCLC tumours [5, 6]. Indeed, the small molecule, reversible, EGFR/HER1 tyrosine kinase inhibitors (TKIs), gefitinib and erlotinib, demonstrate selectivity for EGFR/HER1 and are associated with anti-tumour activity in NSCLC [7–10]. Unfortunately, resistance to reversible TKIs such as gefitinib and erlotinib develops in all patients. This has been attributed to clonal selection of tumour cells, which exhibit resistance mechanisms such as additional mutations in EGFR/HER1, for example T790 M, that renders gefitinib and erlotinib ineffective inhibitors of EGFR/HER1 kinase activity, or by amplification of the hepatocyte growth factor receptor (MET) oncogene, another receptor tyrosine kinase [11–13]. Thus, there is a need for improved targeted therapies that can overcome the mechanisms associated with resistance.

Afatinib (BIBW 2992) is a novel, next-generation, irreversible TKI that selectively targets EGFR/HER1 (half-maximal inhibitory concentration [IC_{50}] 0.5 nM) and HER2 (IC_{50} 14 nM) [14]. Irreversible binding of afatinib to the target receptor is an attractive feature and may help to overcome the issue of resistance. Furthermore, afatinib is thought to inhibit all cancer-relevant EGFR/HER1- and HER2-containing dimers [14]. In vitro studies have shown that afatinib inhibits the anchorage-independent proliferation of NSCLC cell lines irrespective of the EGFR/HER1 mutational status [14] and has demonstrated antitumour activity in NSCLC models in vivo [14]. Afatinib has also shown superior activity to gefitinib and erlotinib in T790 M models in vivo [14].

Data from Phase I/II trials have demonstrated the efficacy of afatinib in patients with NSCLC harbouring EGFR/HER1-activating mutations [15, 16]. This small-scale, open-label, uncontrolled Phase I/II trial was planned to specifically estimate the efficacy of afatinib in patients with advanced NSCLC. An assessment of overall safety data from four previous Phase I trials in non-Japanese patients [17–20] established a recommended Phase II dose of 50 mg/day for continuous daily dosing of afatinib [20]. Based on this experience, treatment groups receiving higher than 50 mg were not included in this study to ensure the safety of Japanese patients. The Phase I step of this study was, therefore, performed to determine the maximum-tolerated dose (MTD) at dose levels of up to 50 mg/day (i.e. recommended Phase II dose in non-Japanese patients) and to determine the recommended dose for the Phase II step in Japanese patients. Here, we report the Phase I findings.

Materials and methods

Study design

This was a Phase I/II, open-label, multicentre trial conducted in Japan. Here, we report the findings from the Phase I part of this trial, which followed a dose-escalation design. The primary endpoint of this study was to assess the safety of afatinib based on the incidence of dose-limiting toxicities (DLTs) and the incidence and intensity of adverse events (AEs). This study was conducted according to the Declaration of Helsinki and in accordance with the Guideline for Good Clinical Practice. Written informed consent was obtained from all participants.

Study population

Eligible patients were adults ($\geq$20 and $\leq$74 years) with pathological confirmation of NSCLC with tissue or cytological diagnosis who had previously received platinum-doublet chemotherapy and/or erlotinib/gefitinib therapy or who were ineligible for, or not amenable to, treatment with established therapies. Patients were required to have a life expectancy of at least 3 months and an Eastern Cooperative Oncology Group (ECOG) performance score of 0 or 1. Patients were also required to have fully recovered from all therapy-related toxicities (except for alopecia) from previous chemo-, hormone-, immuno- or radiotherapies to Common Terminology Criteria for Adverse Events (CTCAE) Grade $\leq$1 and from previous surgery. All patients must have terminated prior chemo-, hormone-, immuno- or radiotherapy 4 weeks before enrolment. Patients with significant gastrointestinal disorders with diarrhoea as a major symptom, e.g. Crohn's disease, malabsorption or CTCAE Grade >2 diarrhoea of any aetiology at the time of enrolment, were excluded from study entry. Additional exclusion criteria included current or previous history of distinct/suspected pulmonary fibrosis or interstitial lung disease determined by the chest radiographic findings, brain tumour and/or brain metastases, active double cancer, a history of

uncontrolled cardiac disease, coelomic fluid retention requiring treatment or uncontrolled concomitant disease (such as diabetes mellitus and hypertension). Sexually active patients unwilling to use a medically acceptable method of contraception during the trial, and pregnant or breast-feeding women, were also excluded.

Dose escalation

Afatinib was administered orally in continuous daily dosing based on a 28-day treatment course and was continued until disease progression or intolerable toxicity. Dose escalation followed a 3 + 3 escalation scheme; a minimum of three patients were treated per dose level with expansion to six patients if a DLT was observed in Course 1. If no patient experienced a DLT, dose escalation was continued. The starting dose was 20 mg/day with escalation to 40 mg/day and then 50 mg/day. Dose escalation was discontinued when the upper limit (50 mg) was reached or MTD was determined.

Concomitant medications

Anti-diarrhoeal drugs, such as loperamide, were permitted to manage diarrhoea, and patients with CTCAE Grade $\geq$2 nausea and/or vomiting were permitted anti-emetic therapy. Patients who experienced CTCAE Grade $\geq$2 diarrhoea, nausea and/or vomiting for 7 days or more, despite supportive care, were required to stop afatinib treatment until recovery (CTCAE Grade $\leq$1).

Study assessments

The safety and tolerability of afatinib was assessed by changes in the incidence and severity of AEs according to CTCAE version 3.0 and by physical examination, vital signs (including twelve-lead electrocardiogram) and laboratory parameters.

All toxicities were graded using CTCAE version 3.0. A DLT was defined as a drug-related CTCAE Grade 3 or 4 non-haematological toxicity (except for transient electrolyte abnormality), or Grade 4 drug-related haematological toxicity. Additional DLTs included: CTCAE Grade $\geq$2 decrease in cardiac left ventricular function; CTCAE Grade $\geq$3 nausea and/or vomiting or persistent CTCAE Grade $\geq$2 nausea and/or vomiting for $\geq$7 days, despite anti-emetic medication; and CTCAE Grade $\geq$3 diarrhoea or persistent CTCAE Grade $\geq$2 diarrhoea for $\geq$7 days, despite anti-diarrhoeal medication, such as loperamide. The MTD was defined on the basis of DLTs observed during the first treatment course (4 weeks) and was a dose $\leq$50 mg once daily, at which no more than 33% of patients experienced a DLT.

Tumour response was assessed according to response evaluation criteria in solid tumours. Target lesions were defined as either having a complete response, partial response, stable disease or progressive disease. Patients were assessed at screening and then at the end of every treatment course.

EGFR/HER1 mutation analysis was performed where possible. Tumour biopsies and surgical material from prior biopsy sampling or surgery were collected from respective sites (pathology departments) during the screening period. Initial diagnostic tumour specimens and/or tissue material obtained at disease recurrence after initial EGFR/HER1 TKI treatment was considered adequate. If multiple biopsies were available for individual patients, the most recent and/or the most appropriate biopsy material was requested. Tumour material and a single serum sample collected during the screening period were centrally analysed for EGFR/HER1 mutations using the Scorpion Amplified Refractory Mutation System™ and Direct Sequencing. The genome DNA solution was analysed using a DxS EGFR/HER1 Mutation Test Kit. A Sequence Detection System (Applied Biosystems, ABI PRISM 7700) was used to detect mutations in EGFR/HER1. A Genetic Analyzer (Applied Biosystems, ABI PRISM 3100) was used to analyse the nucleic acid sequence of the EGFR/HER1 gene.

Pharmacokinetic sampling and data analysis in Course 1

Blood samples for the evaluation of pharmacokinetic (PK) parameters were collected at pre-dose, 0.5, 1, 2, 3, 4, 5, 7 and 9 h, and 24 h after dosing on Days 1, 28 and 48, and 72 h after dosing on Day 28. Pre-dose blood samples to determine trough plasma concentrations were collected on Days 8, 15 and 22 before drug administration. Afatinib plasma concentrations were determined by validated high-performance liquid chromatography coupled to tandem mass spectrometry. Non-compartmental PK parameters were determined using WinNonlin®.

Statistical analyses

All patients who received at least one dose of afatinib (treated set) were included in the efficacy and safety analyses. Safety, efficacy and PK characteristics were analysed in an exploratory and descriptive manner.

Results

Patient population

In total, 13 patients were enrolled in this study, and 12 patients were treated (five men and seven women); three

patients were treated with afatinib 20 mg/day, three patients were treated with afatinib 40 mg/day and six patients were treated with afatinib 50 mg/day. The median (range) duration of afatinib exposure was 68.5 (28–370) days. Patient demographics and clinical characteristics are summarized in Table 1. Patients were heavily pre-treated with an average of three and a half previous chemotherapy regimens. All patients eventually discontinued treatment. Ten patients discontinued study medication because of disease progression, one patient discontinued owing to an AE not related to afatinib and one patient withdrew consent. EGFR/HER1 mutation analysis was conducted for 11 patients: five patients were found to have EGFR/HER1 mutations, of which four had double mutations including the T790 M mutation (see Table 1).

Table 1 Patient demographic characteristics

Characteristic	Afatinib
Age (years): median (range)	62.5 (39–67)
Men/Women	5/7
ECOG score:	
0	8
1	4
Smoking history:	
Non-smoker	7
Ex-smoker	5
EGFR/HER1 mutation status:	
Del 19 + T790 M (tissue)/NA (serum)	1
Del 19 (tissue)/Del 19 + T790 M (serum)	1
NA (tissue)/Del 19 + T790 M (serum)	1
NA (tissue)/L858R + T790 M (serum)	1
NA (tissue)/S768I (serum)	1
Wild (tissue)/Wild (serum)	1
NA (tissue)/Wild (serum)	5
NA (tissue)/NA (serum)	1
Tumour histology	
Adenocarcinoma	10
Squamous cell carcinoma	1
Squamous/adenocarcinoma	1
Number of metastasis sites: median (range)	4.0 (0–11)
Number of prior chemotherapy (range)	3.5 (1–8)
Prior therapies:	
Surgery	3
Radiotherapy	5
Prior erlotinib and/or gefitinib	8
Clinical stage at screening:	
IIIB	1
IV	11
Starting dose of afatinib (mg):	
20	3
40	3
50	6

ECOG Eastern Cooperative Oncology Group, *EGFR* Epidermal growth factor receptor, *HER* Human epidermal growth factor, *NA* not applicable

Safety

All twelve treated patients were evaluable for safety, and all experienced at least one drug-related AE (Table 2). The most frequent drug-related AEs observed in more than 40% of the patients were diarrhoea, dry skin, stomatitis, rash, paronychia and anorexia. Only one patient (afatinib 20 mg/day group) experienced an AE leading to discontinuation of study medication. This patient developed bile duct cancer (active second primary cancer; serious AE), which occurred in Course 1, and was considered unrelated to the study medication. Two additional patients experienced serious AEs of mucosal inflammation and enteritis, respectively; both were experienced in patients receiving afatinib 50 mg/day, were considered related to the study medication and were resolved following study drug discontinuation.

Four patients required a one-step dose reduction of afatinib related to AEs (one patient in the 40 mg/day dose group and three patients in the 50 mg/day dose group). Adverse events necessitating dose reduction included rash, paronychia, mucosal inflammation, diarrhoea and enteritis.

Only one DLT was reported during Course 1; Grade 3 mucosal inflammation in a patient receiving afatinib 50 mg/day that resolved following dose interruption followed by reduction. Two further DLTs were reported after Course 1 in patients receiving afatinib 50 mg/day; one patient experienced Grade 3 enteritis in Course 4 and one patient experienced Grade 3 diarrhoea in Course 2. Both events resolved following dose reduction.

No clinically significant changes were noted in clinical laboratory parameters, vital signs, electrocardiograms and left ventricular function.

Efficacy

All twelve treated patients were evaluable for response. No complete responses or partial responses were reported. Six out of twelve patients had tumour size reductions; details of the patients experiencing tumour reduction are shown in Table 3 and Fig. 1, which illustrate the maximum tumour size reduction of individual patients by mutation status. Nine patients reported a best overall response as stable disease, with three achieving prolonged stable disease. One patient, a 64-year-old woman with an adenocarcinoma of the lung diagnosed 2.9 years ago and resistant to gefitinib and erlotinib, was progression-free for 310 days and had a maximum tumour size reduction of −7.7% (stable disease), despite the presence of T790 M resistance mutations.

Table 2 Treatment-related AEs occurring at a rate >10% of the total population by dose and highest CTCAE Grade ≥2

Adverse event[a]	Afatinib dose, n						All doses ($n = 12$), n (%)
	20 mg ($n = 3$)		40 mg ($n = 3$)		50 mg ($n = 6$)		
	Grade 2	Grade 3	Grade 2	Grade 3	Grade 2	Grade 3	All grades
Diarrhoea	0	0	2	0	3	1	10 (83.3)
Dry skin	1	0	1	0	2	0	9 (75.0)
Stomatitis	0	0	1	0	1	0	7 (58.3)
Rash	1	0	3	0	2	0	7 (58.3)
Paronychia	0	0	1	0	2	0	6 (50.0)
Anorexia	0	0	0	0	1	0	5 (41.7)
Nausea	0	0	1	0	2	0	3 (25.0)
Acne	0	0	0	0	1	0	3 (25.0)
Mucosal dryness	0	0	0	0	0	0	3 (25.0)
Mucosal inflammation	0	0	0	0	2	1	3 (25.0)
Blood urine present	0	0	0	0	0	0	3 (25.0)
Weight decreased	1	0	0	0	2	0	3 (25.0)
Pharyngitis	0	0	0	0	0	0	2 (16.7)
Leucopoenia	0	0	0	0	0	0	2 (16.7)
Conjunctivitis	0	0	0	0	0	0	2 (16.7)
Epistaxis	0	0	0	0	0	0	2 (16.7)
Oropharyngeal discomfort	0	0	0	0	0	0	2 (16.7)
Vomiting	0	0	0	0	1	0	2 (16.7)
Nail disorder	0	0	0	0	1	0	2 (16.7)
Pruritus	0	0	0	0	0	0	2 (16.7)
Fatigue	0	0	0	0	0	0	2 (16.7)
Malaise	0	0	0	0	0	0	2 (16.7)

AE adverse event, *CTCAE* Common terminology criteria for adverse events

[a] Preferred terms

Response was maintained for more than 11 courses before progressive disease (see Table 3). The median number (range) of courses was 2.5 (1–18).

Pharmacokinetics

Peak plasma concentrations of afatinib were reached at 3–5 h after drug administration and subsequently declined with at least in a biphasic manner (Fig. 2). In some patients, double-peak plasma concentration–time profiles of afatinib were observed. The PK parameters of afatinib are summarized in Table 4. Median t_{max} and $t_{max,ss}$ of afatinib were approximately 3–4 h. The area under the curve $(AUC)_{0-24}$ and C_{max} values of afatinib increased with increasing doses for all doses on Day 1 and all doses except 50 mg at steady state (Table 4). The geometric mean (gMean) values of the apparent total clearance were large and ranged from 799 to 1,200 mL/min on Day 1 and from 538 to 827 mL/min on Day 28. Afatinib exhibited a high apparent volume of distribution ranging from 1,880 to 2,710 L on Day 28. The terminal half-life ($t_{1/2}$) ranged from 14.8 to 37.9 h on Day 1 and from 33.5 to 40.4 h on Day 28. The gMean values of accumulation ratios (single dose vs. steady state) based on the AUC and on C_{max} were between 1.96 and 3.97, and 1.63 and 4.41, respectively. Steady state was reached at around Day 8.

Discussion

The aim of the Phase I stage of this study was to estimate the MTD of afatinib, up to 50 mg/day, in patients with advanced NSCLC and to determine the recommended dose for Phase II evaluation. In this study, afatinib 50 mg administered as an oral, once-daily continuous dose, was well tolerated with an acceptable safety profile. Whilst DLTs occurred in three of the six patients treated with afatinib 50 mg, only one patient in treatment Course 1, the treatment course in which MTD was defined, experienced a DLT. Furthermore, no DLTs resulted in study discontinuation, and all three patients who experienced DLTs continued to receive afatinib at a lower dose.

Table 3 Profiles of patients with tumour size reduction

Age (sex)	Histology	Previous treatment	Afatinib dose (daily; mg)	Month/ courses on study	Best response of targets (%)	*EGFR/HER1* mutation status	
						Tissue	Serum
67 (f)	Squamous adenocarcinoma	1. Cisplatin + amrubicin	20	2	−14.5	NA	NA
64[a] (f)	Adenocarcinoma	1. Cisplatin + TS-1 2. Gefitinib 3. Erlotinib 4. Gefitinib	20	11	−7.7	Del 19 + T790 M	NA
57[a] (f)	Adenocarcinoma	1. Cisplatin + gemcitabine 2. Nimotuzumab	40/30	18	−24.6	NA	Wild type
61[a] (f)	Squamous cell carcinoma	1. Carboplatin + paclitaxel 2. Gefitinib 3. Erlotinib	50/40/30	3	−15.3	NA	Wild type
65[a] (m)	Adenocarcinoma	1. Cisplatin + docetaxel 2. Docetaxel 3. Gefitinib 4. Gefitinib + gemcitabine 5. Gefitinib	50/40	12	−20.8	NA	Wild type
67[a] (f)	Adenocarcinoma	1. Gefitinib 2. Carboplatin + gemcitabine 3. Erlotinib 4. TS-1	50/40	3	−2.4	NA	Del 19 + T790 M

EGFR Epidermal growth factor receptor, *HER* Human epidermal growth factor receptor, *NA* not applicable

[a] Never smoker

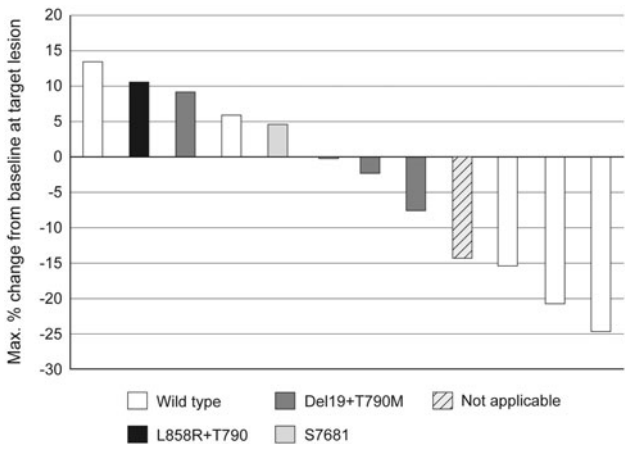

Fig. 1 Maximum tumour size reduction of individual patients by mutational status

The tolerability profile of afatinib 50 mg/day reported here in Japanese patients was similar to that previously observed in the non-Japanese population; diarrhoea and skin-related AEs were predominant and were manageable with a combination of appropriate concomitant medication and dose reduction. Therefore, continuous oral administration of afatinib at a starting dose of 50 mg once daily together with a tolerability-adapted, dose-reduction scheme and supportive care, was deemed an appropriate dosing regimen for Japanese patients in the Phase II step of this trial. Given that this patient population has already been previously treated with a reversible EGFR/HER1 TKI, dose intensity could be crucial, and the proposed dosing regimen will give these patients the opportunity of treatment with the highest possible dose for the longest duration.

Importantly, preliminary signs of efficacy were observed in this heavily pre-treated population. Although no partial or complete responses were observed, six out of 12 patients had tumour size reductions, with three achieving prolonged stable disease. This included one patient with a mutation in EGFR/HER1 exon 19 (T790 M), who remained progression-free for 11 months. This patient had previously received both gefitinib and erlotinib treatment.

Although the number of patients included in this study was limited, there appeared to be no relationship between mutation status and the maximum percentage change in target lesion from baseline or days on study. These findings support the use of afatinib as a potential novel treatment option for patients with advanced NSCLC and tumours harbouring EGFR/HER1 mutations, even after previous treatment with reversible EGFR/HER1 TKIs. The findings reported here are also in agreement with previous findings

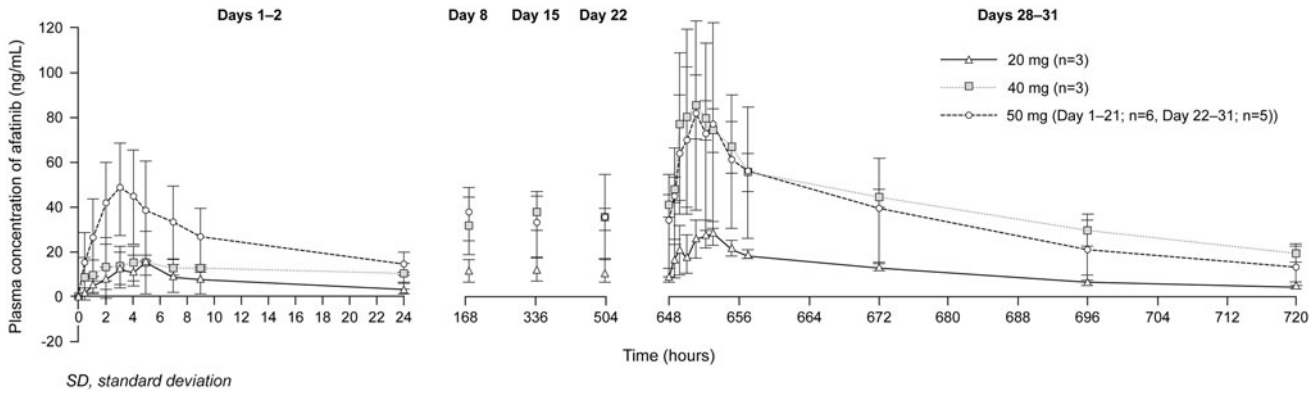

Fig. 2 Arithmetic mean (± standard deviation) plasma concentration–time profiles of afatinib after single and multiple oral administration over 28 days in Course 1

Table 4 Pharmacokinetic parameters of afatinib after multiple oral administration of afatinib 20, 40 and 50 mg once daily

Course 1	Day 1			Day 28 (steady state)		
Parameter (unit)	20 mg ($n = 3$) gMean (gCV [%])	40 mg ($n = 3$) gMean (gCV [%])	50 mg ($n = 6$) gMean (gCV [%])	20 mg ($n = 3$) gMean (gCV [%])	40 mg ($n = 3$) gMean (gCV [%])	50 mg ($n = 5$) gMean (gCV [%])
AUC_{0-24} (ng h/mL)	147 (84.5)	299 (6.0)[b]	539 (59.0)	409 (16.5)	1,240 (9.7)	1,010 (71.5)
$AUC_{0-24\ norm}$ ([ng h/mL]/mg)	7.33 (84.5)	7.47 (6.0)[b]	10.8 (59.0)	20.5 (16.5)	31.0 (9.7)	20.1 (71.5)
C_{max} (ng/mL)	12.4 (101)	18.9 (45.8)	44.4 (60.6)	26.9 (24.9)	83.3 (30.1)	66.8 (71.6)
$C_{max,\ norm}$ ([ng h/mL]/mg)	0.620 (101)	0.473 (45.8)	0.887 (60.6)	1.34 (24.9)	2.08 (30.1)	1.34 (71.6)
t^{a}_{max} (h)	3.9 (3.0–5.0)	4.1 (2.0–9.0)	3.0 (2.0–5.0)	4.0 (2.9–5.0)	3.0 (2.0–4.0)	3.0 (1.0–5.0)
$t_{½}$ (h)	21.3 (63.1)	37.9 (24.9)[b]	14.8 (20.0)	38.5 (14.4)	40.4 (11.9)	33.5 (22.2)
CL/F (mL/min)	1,200 (39.5)	799 (19.5)[b]	1,030 (55.9)	814 (16.5)	538 (9.7)	827 (71.5)
V_z/F(L)	2,200 (122.0)	2,620 (5.2)[b]	1,320 (62.8)	2,710 (30.3)	1,880 (3.8)	2,400 (80.6)

gCV geometric coefficient of variation; *gMean* geometric mean; *AUC* area under the curve; *CL* clearance

[a] median (range), [b] $n = 2$

in non-Japanese patients, in which afatinib has demonstrated activity in patients with advanced NSCLC and EGFR/HER1 mutations [16, 21]. However, it should be considered that EGFR/HER1 mutations were not identified in all patients in this study, and in three cases, mutations were identified from serum samples rather than tumour samples.

Pharmacokinetic analysis revealed that plasma concentrations of afatinib peaked at 3–4 h after administration and declined with a half-life of 30–40 h at steady state. The accumulation ratio based on the AUC values was approximately 2–4. Afatinib exhibited high apparent volume of distribution, which indicates a high tissue distribution of the drug. However, the values of the apparent volume of distribution should be interpreted with caution, as the absolute bioavailability of afatinib in humans is unknown. Steady state was considered to have been reached on Day 8 (7 days after the start of drug administration). Although dose proportionality was not evaluated statistically in this study owing to the limited number of patients, exposure of afatinib generally increased with increasing doses, and there was no obvious deviation from a dose-proportional increase in exposure. This is in agreement with findings from previous trials, which have shown no obvious deviation from dose proportionality in the dose range of 10–160 mg of afatinib [17–19, 22].

Comparison of the PK parameters obtained from previous Phase I studies in non-Japanese cancer patients suffering from advanced solid tumours [17–19, 22] to those in Japanese patients reported here revealed that the PK of afatinib in Japanese patients can be considered comparable to those in non-Japanese patients. Comparison of the individual AUC and C_{max} values of Japanese and non-Japanese patients showed that although the AUC and C_{max} values tended to be higher in Japanese patients than in non-Japanese patients at some doses, most values in Japanese were within the same range of those in non-Japanese (Fig. 3). T_{max} and $t_{1/2}$ values reported here in Japanese

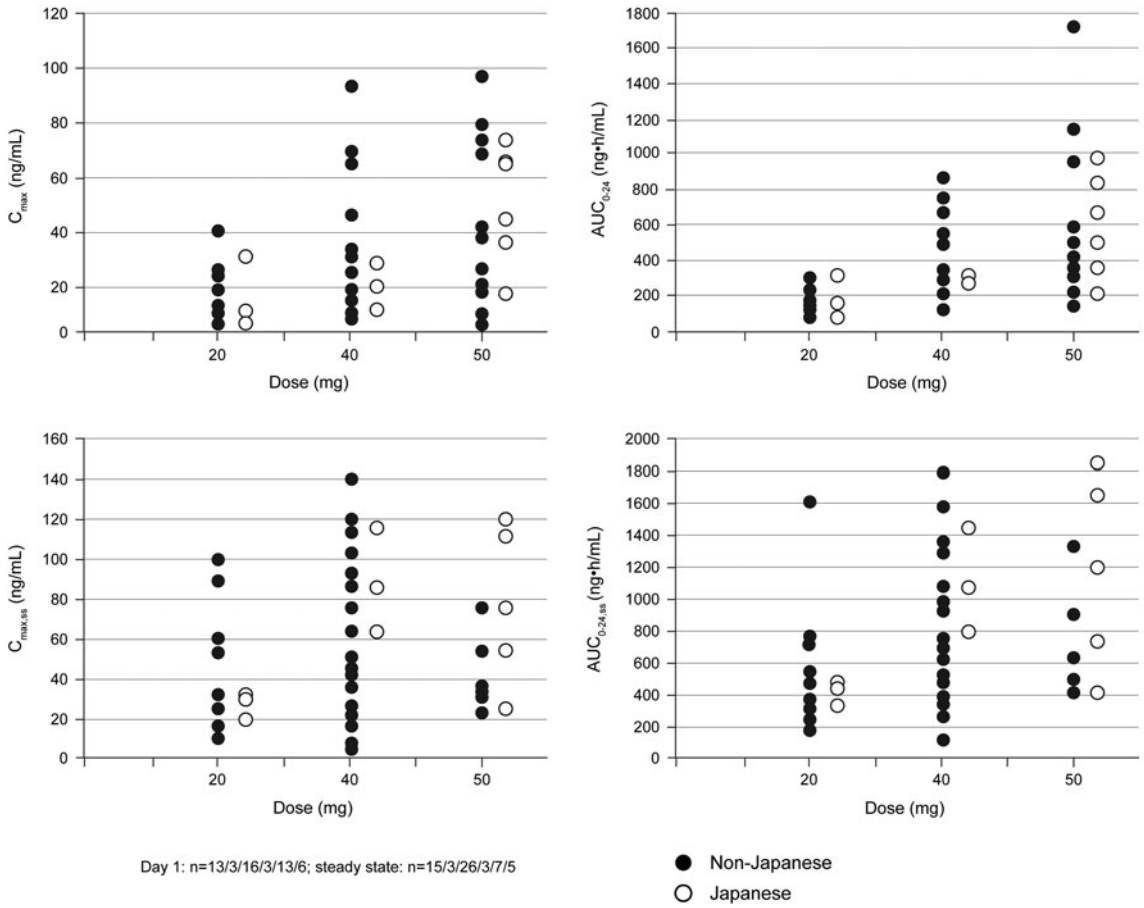

Fig. 3 Comparison of pharmacokinetic parameters between Japanese and non-Japanese patients. Comparison of pharmacokinetic parameters showed that although the area under the curve and C_{max} values tended to be higher in Japanese patients than in non-Japanese patients at some doses, most values in Japanese were within the same range of those in non-Japanese

patients were also within the same range as those in non-Japanese patients. Whilst we cannot rule out that pharmacogenomic differences between Japanese and non-Japanese patients may have an effect on the pharmacodynamic profile of afatinib, no such observations were made in this study, and the mechanism by which pharmacogenomic differences in patient populations may exert an effect on the pharmacodynamics of afatinib remains to be clearly established.

In conclusion, the recommended dose for Phase II study in Japanese patients is 50 mg/day. Further evaluation of afatinib in NSCLC patients who have been previously treated with erlotinib and/or gefitinib in the Phase II part of this trial is currently being conducted. Furthermore, a Phase III trial with afatinib in an enriched population of TKI-naïve NSCLC patients is currently ongoing.

Acknowledgments This study was supported by Boehringer Ingelheim. The authors acknowledge the editorial assistance of Ogilvy Healthworld. Boehringer Ingelheim provided financial support for this assistance.

Conflict of interest Haruyasu Murakami, Tomohide Tamura, Toshiaki Takahashi, Hiroshi Nokihara, Tateaki Naito, Yukiko Nakamura, Kazuto Nishio, Nobuyuki Yamamoto: nothing to disclose. Yoko Seki, Akiko Sarashina, Mehdi Shahidi: employee of Boehringer Ingelheim.

References

1. Winer E, Gralow J, Diller L, Karlan B, Loehrer P, Pierce L, Demetri G, Ganz P, Kramer B, Kris M, Markman M, Mayer R, Pfister D, Raghavan D, Ramsey S, Reaman G, Sandler H, Sawaya R, Schuchter L, Sweetenham J, Vahdat L, Schilsky R, Blayney D, Lichter A (2008) Clinical cancer advances 2008: major research advances in cancer treatment, prevention, and screening–a report from the American Society of Clinical Oncology. J Clin Oncol 27(5):812–826. doi:10.1200/JCO.2008.21.2134
2. 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
3. Salomon DS, Brandt R, Ciardiello F, Normanno N (1995) Epidermal growth factor-related peptides and their receptors in human malignancies. Crit Rev Oncol Hematol 19(3):183–232. doi:1040842894001441

4. Arteaga CL (2002) Epidermal growth factor receptor dependence in human tumors: more than just expression? Oncologist 7(Suppl 4):31–39
5. Johnson BE, Jackman D, Janne PA (2006) Impact of EGFR mutations on treatment of non-small cell lung cancer. Cancer Chemother Pharmacol 58(Suppl 7):5–9
6. Doroshow JH (2005) Targeting EGFR in non-small-cell lung cancer. N Engl J Med 353(2):200–202. doi:10.1056/NEJMe058113
7. Giaccone G, Rodriguez JA (2005) EGFR inhibitors: what have we learned from the treatment of lung cancer? Nat Clin Pract Oncol 2(11):554–561
8. Fukuoka M, Yano S, Giaccone G, Tamura T, Nakagawa K, Douillard JY, Nishiwaki Y, Vansteenkiste J, Kudoh S, Rischin D, Eek R, Horai T, Noda K, Takata I, Smit E, Averbuch S, Macleod A, Feyereislova A, Dong RP, Baselga J (2003) Multi-institutional randomized phase II trial of gefitinib for previously treated patients with advanced non-small-cell lung cancer (The IDEAL 1 Trial). J Clin Oncol 21(12):2237–2246
9. Paez JG, Janne PA, Lee JC, Tracy S, Greulich H, Gabriel S, Herman P, Kaye FJ, Lindeman N, Boggon TJ, Naoki K, Sasaki H, Fujii Y, Eck MJ, Sellers WR, Johnson BE, Meyerson M (2004) EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science 304(5676):1497–1500
10. Shepherd FA, Rodrigues Pereira J, Ciuleanu T, Tan EH, Hirsh V, Thongprasert S, Campos D, Maoleekoonpiroj S, Smylie M, Martins R, van Kooten M, Dediu M, Findlay B, Tu D, Johnston D, Bezjak A, Clark G, Santabarbara P, Seymour L (2005) Erlotinib in previously treated non-small-cell lung cancer. N Engl J Med 353(2):123–132. doi:10.1056/NEJMoa050753
11. 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
12. Turke AB, Zejnullahu K, Wu YL, Song Y, Dias-Santagata D, Lifshits E, Toschi L, Rogers A, Mok T, Sequist L, Lindeman NI, Murphy C, Akhavanfard S, Yeap BY, Xiao Y, Capelletti M, Iafrate AJ, Lee C, Christensen JG, Engelman JA, Janne PA (2010) Preexistence and clonal selection of MET amplification in EGFR mutant NSCLC. Cancer Cell 17(1):77–88. doi: 10.1016/j.ccr.2009.11.022
13. Maheswaran S, Sequist LV, Nagrath S, Ulkus L, Brannigan B, Collura CV, Inserra E, Diederichs S, Iafrate AJ, Bell DW, Digumarthy S, Muzikansky A, Irimia D, Settleman J, Tompkins RG, Lynch TJ, Toner M, Haber DA (2008) Detection of mutations in EGFR in circulating lung-cancer cells. N Engl J Med 359(4):366–377. doi:10.1056/NEJMoa0800668
14. 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
15. Plummer R, Vidal L, Li L, Shaw H, Perrett R, Shahidi M, Amelsberg A, Temple G, Calvert H, de Bono J (2006) Phase I study of BIBW 2992, an oral irreversible dual EGFR/HER2 inhibitor, showing activity in tumours with mutated EGFR. Eur J Cancer Suppl 4(12):174
16. Yang C-H, Shih J, Su W, Hsia T, Sequist LV, Chang G, Calvo R, Cong XJ, Shahidi M, Miller VA (2010) A phase II study of BIBW 2992 in patients with adenocarcinoma of the lung and activating EGFR/HER1 mutations (LUX-LUNG2). Ann Oncol 21(Suppl8):viii122–viii161 (abstract 367PD)
17. 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
18. 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
19. Lewis N, Marshall J, Amelsberg A, Cohen RB, Stopfer P, Hwang J, Malik S (2006) A phase I dose escalation study of BIBW 2992, an irreversible dual EGFR/HER2 receptor tyrosine kinase inhibitor, in a 3 week on 1 week off schedule in patients with advanced solid tumors. ASCO Meet Abstr 24 (18_suppl):3091
20. 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
21. Plummer R, Vidal L, Perrett R, Spicer J, Stopfer P, Shahidi M, Temple G, Futreal A, Calvert H, de Bono J (2007) A Phase I and pharmacokinetic (PK) study of BIBW 2992, an oral irreversible dual EGFR/HER2 inhibitor. Eur J Cancer Suppl 5(4):108
22. Spicer J, Calvert H, Vidal L, Azribi F, Perrett R, Shahidi M, Temple G, Futreal A, De Bono J, Plummer R (2007) Activity of BIBW2992, an oral irreversible dual EGFR/HER2 inhibitor, in non-small cell lung cancer (NSCLC) with mutated EGFR. J Thorac Oncol 2(8):S410