Clinical Cancer Research

Phase I Study to Assess the Combination of Afatinib with Trastuzumab in Patients with Advanced or Metastatic HER2-Positive Breast Cancer

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Abstract

Purpose: The HER2 mAb, trastuzumab, is a standard therapy for patients with HER2-positive breast cancer before acquired resistance. Afatinib, an irreversible, oral, small-molecule ErbB family blocker, shows clinical activity in trastuzumab-refractory HER2-positive breast cancer.

Experimental Design: This phase I study used a 3+3 dose escalation to determine the MTD of oral once-daily afatinib in combination with the recommended dose of intravenous trastuzumab (4 mg/kg week 1; 2 mg/kg/wk thereafter). Adult women with confirmed advanced/metastatic HER2-positive breast cancer were eligible.

Results: Of 18 patients treated, 16 received daily afatinib 20 mg and two 30 mg. Overall, 4 of 13 and 2 of 2 patients receiving afatinib 20 mg and 30 mg, respectively, experienced dose-limiting toxicity (DLT; all CTCAE grade 3 diarrhea). Most frequent treatment-related adverse events were diarrhea (94%), rash (56%), and fatigue (56%). Overall, pharmacokinetic profiles of afatinib and trastuzumab in combination were consistent with the known characteristics of each alone. Overall, objective response and disease control rates were 11% and 39%, respectively, with median progression-free survival 111.0 days (95% confidence interval, 56.0–274.0).

Conclusions: The MTD of afatinib was 20 mg daily combined with the recommended weekly dose of trastuzumab, with 1 of 6 patients showing DLTs in the dose escalation. However, additional DLTs occurred in the dose-expansion phase meaning that this MTD cannot be recommended for phase II development without strict diarrhea management. There was no evidence suggesting relevant pharmacokinetic drug–drug interactions. Signs of clinical activity were seen in trastuzumab-resistant HER2-positive breast cancer, suggesting further investigation with optimal diarrhea management is warranted. *Clin Cancer Res; 21(12); 2737–44. ©2014 AACR.*

See related commentary by Sledge and Pegram, p. 2663

Introduction

HER2 (ErbB2) is a member of the ErbB family of receptor tyrosine kinases, which also includes EGFR (ErbB1), ErbB3, and ErbB4. Amplification of HER2 leads to increased receptor homo- and heterodimerization, and subsequent activation of downstream signaling pathways associated with cell proliferation, differentiation, survival, and angiogenesis (1). HER2 is amplified and overexpressed in 15% to 20% of breast cancers (2). Trastuzumab, a HER2-targeting mAb, has become the mainstay of therapy in the palliative and adjuvant or neoadjuvant settings for patients with HER2-positive breast cancer since its first approval in 1998 (3). However, HER2-positive breast cancer may be intrinsically, or may become, resistant to trastuzumab in around 70% of cases (4). In these patients, further treatment options are limited (5). Several potential factors influencing resistance to trastuzumab have been proposed, including: compensatory cross-talk with other receptors (6, 7); a truncation known as p95-HER2, a splice variant missing exon 16 of the HER2 receptor (7); mutations in or increased activation of the PI3K/AKT pathway (7, 8); PTEN deficiency (8); the upregulation of Rac1 (9); and overexpression of mucin-4 (MUC4) or MUC1 (10).

Afatinib is an anilinoquinazoline-derived, irreversible, oral, small-molecule ErbB family blocker that inhibits EGFR, HER2 and ErbB4, and ErbB3 transphosphorylation. Afatinib is currently approved in several countries in Asia, the United States, the European Union, and elsewhere for the treatment of patients with locally advanced or metastatic non–small cell lung cancer with *EGFR* tyrosine kinase mutations; specific indications vary between countries. Afatinib's potential to block irreversibly all cancer-relevant ErbB family receptor homo- and heterodimers makes it a candidate for mediating sensitivity in trastuzumab-resistant breast cancer. Indeed, afatinib has demonstrated clinical activity in advanced solid tumors and trastuzumab-refractory

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Translational Relevance

"Vertical" inhibition of HER2 by combining a HER2 mAb and a HER2-directed tyrosine kinase inhibitor is increasingly recognized as an effective treatment option for patients with advanced/metastatic HER2-positive breast cancer. This dose-finding study provides the first data on the pharmacokinetics, safety, and preliminary efficacy of the combination of afatinib, an irreversible ErbB family blocker, and trastuzumab in patients with advanced/metastatic HER2-positive breast cancer, almost all of whom had previously progressed on trastuzumab. Although pharmacokinetic data indicate drug–drug interactions to be unlikely and the combination pharmacologically feasible, dose escalation was limited by diarrhea at a 20 mg daily dose of afatinib with weekly trastuzumab. Optimum management of the adverse event of diarrhea with this combination of targeted therapies is essential. Early indications of antitumor activity with the combination merit further assessment of afatinib and trastuzumab with prompt and optimal management/prevention of diarrhea.

HER2-positive breast cancer (11, 12). A phase II study of afatinib conducted in patients with heavily pretreated metastatic breast cancer that had progressed on trastuzumab reported that 46% of patients achieved clinical benefit, which was associated with a median PFS of 15.1 weeks (11). However, on the basis of the results of trials such as EGF104900, "vertical" HER2 blockade, via a mAb combined with a tyrosine kinase inhibitor (TKI), is increasingly recognized as generally more effective than HER2-directed monotherapy for the treatment of HER2-positive metastatic breast cancer (13–15). However, no preclinical data on afatinib plus trastuzumab are available. This phase I study was undertaken to determine the MTD (primary endpoint), safety, pharmacokinetics, and antitumor activity of afatinib given in combination with trastuzumab to patients with a confirmed diagnosis of advanced or metastatic HER2-positive breast cancer.

Materials and Methods

Patient population

Women 18 years old or over with advanced or metastatic breast cancer–overexpressing HER2 (immunohistochemistry 3+ or 2+ with positive gene amplification by FISH) were eligible to participate. Prior treatment with trastuzumab or lapatinib (in the adjuvant or metastatic settings) was permitted but not required. Other eligibility criteria included: measurable disease, life expectancy of at least 3 months, and an Eastern Cooperative Oncology Group performance status of 0, 1, or 2, and adequate organ function. Patients were excluded from participation if they had received radiotherapy within 2 weeks of enrollment or chemo-, hormone-, or immunotherapy within 4 weeks of first drug administration, were currently receiving EGFR-targeting therapy or HER2-inhibiting drugs, or if they had a left ventricular ejection fraction (LVEF) <50%, known interstitial lung disease or active brain metastases.

Study design and treatment

This was a multicenter, open-label, phase I, dose-escalation study, conducted at five specialist cancer centers in the United Kingdom. Patients received oral afatinib once daily in combination with the recommended weekly intravenous dose of trastuzumab for breast cancer [starting at 4 mg/kg (week 1) followed by repeated doses of 2 mg/kg] in 28-day treatment cycles. Each patient was initially treated for one treatment cycle and could continue on therapy in the absence of disease progression or unacceptable toxicity.

The planned afatinib dose-escalation tiers were 20, 40, and 50 mg once daily. A 30-mg dose tier was added by a protocol amendment implemented in response to adverse events (AE) associated with afatinib observed in the 20 mg cohort. Dose escalation followed a standard 3+3 design with MTD determination based on dose-limiting toxicities (DLT; defined below) observed in the first treatment cycle. If no patients experienced a DLT at a given dose level, 3 patients were enrolled at the next dose level. Observation of DLT in 1 of 3 patients prompted enrollment of a further 3 patients at that dose level. Dose escalation continued if no further patients experienced a DLT in that cohort. The MTD was defined as the highest dose at which no more than 1 of 6 patients experienced any DLTs during the first cycle. Provision was made to expand the MTD level by up to 12 evaluable patients, such that the cumulative total of dose escalation and MTD expansion was a maximum of 18 patients treated at the MTD. If 4 or more patients at the expanded MTD experienced DLT, the dose was to be deescalated.

DLTs were defined as AEs considered by the investigators to be related to study treatment and meeting any of the following criteria according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version 3.0: grade 4 hematologic toxicities; grade 3 or 4 nonhematologic toxicity (except untreated nausea, vomiting, or diarrhea); grade 2 or higher worsening of cardiac left ventricular function or renal function; or grade 2 or higher diarrhea, nausea, or vomiting (persisting for 7 days or more despite supportive treatment). DLTs arising in subsequent cycles were analyzed separately. Patients who experienced DLT stopped treatment and resumed at a lower dose of afatinib following recovery to grade 1 or baseline within 14 days. A maximum of two afatinib dose reductions were allowed according to a prespecified schedule; however, the dose of afatinib could not be reduced below 20 mg, and patients experiencing DLT at the first dose level did not resume treatment.

Study conduct

This study was carried out according to the Declaration of Helsinki, the International Conference on Harmonization Harmonized Tripartite Guideline for Good Clinical Practice requirements and relevant local guidelines. Written informed consent was obtained before each patient's participation. Trial registration ID: NCT00950742.

Endpoints and assessments

The primary endpoint was the MTD of afatinib combined with trastuzumab. Secondary endpoints were the incidence and intensity of AEs (according to CTCAE), pharmacokinetic parameters of afatinib and trastuzumab when administered in combination, objective tumor response [i.e., complete response (CR) or partial response (PR)], best overall objective response (OR), disease control (DC), and progression-free survival (PFS). A first analysis was planned when all patients in the study had been evaluated for at least 6 months, had withdrawn from the study, or had died (whichever occurred first).

Safety assessments

Safety was assessed by monitoring AEs, laboratory values, and physical examinations throughout the study. Patients were required to report AEs throughout the study; these were graded according to CTCAE version 3.0. A serious AE (SAE) was defined as any AE that resulted in death, was immediately life-threatening, resulted in persistent or significant incapacity, required or prolonged patient hospitalization, resulted in a congenital defect, or was deemed serious for any other reason linked to a significant hazard. Physical examinations, including vital signs, laboratory evaluations, 12-lead electrocardiogram, and cardiac LVEF assessment by echography or multigated acquisition (MUGA) scan, were performed at screening and repeated throughout the study period (at weekly intervals except for echo/MUGA, which was performed at baseline and at 4 weeks). Medical judgment determined whether there was a relationship between an AE or SAE and study medication.

Pharmacokinetics assessments

Plasma and serum samples for pharmacokinetic analysis of afatinib and trastuzumab were taken before trastuzumab infusion and afatinib intake and again at the end of trastuzumab infusion on days 1, 8, 15, 22, and 29. On day 1, afatinib was taken at the end of the trastuzumab infusion to allow collection of comparative single-agent pharmacokinetic data. On each day, two samples were taken (one before and one immediately after the end of trastuzumab infusion), apart from on day 15 when, in addition to the before and immediately after trastuzumab infusion samples, six more samples were taken at 2, 3, 4, 5, 6, and 8 hours after the start of trastuzumab infusion. The samples relevant to trastuzumab pharmacokinetic analysis were each of the before and immediately after samples on each day (10 samples overall). The samples relevant to afatinib pharmacokinetic analysis included all of the before trastuzumab samples on each day, plus all of the samples on day 15 (12 samples overall). Afatinib plasma concentrations were determined using a validated high-performance liquid chromatography–mass spectroscopy assay at the Department of Drug Metabolism and Pharmacokinetics, Boehringer Ingelheim Pharma GmbH and Co. KG, Biberach, Germany. Trastuzumab concentrations in human serum were determined by a validated ELISA at QPS, Groningen, the Netherlands. Afatinib pharmacokinetic parameters in the presence of trastuzumab were compared with historical data from other monotherapy studies. To determine whether afatinib had any impact on the pharmacokinetic characteristics of trastuzumab, a simulation of trastuzumab serum concentrations was performed, according to a model described in the literature (16), and then compared with observed values.

Efficacy assessments

Tumor lesions were measured using CT or MRI within the 4 weeks preceding baseline and then every 8 weeks after start of treatment. Response [CR, PR, stable disease (SD), or progressive disease (PD)] was assessed according to RECIST version 1.1, with best response recorded from the start to the end of treatment. Disease control rate was defined as SD for at least 6 weeks, or CR/PR.

Statistical analysis

All data were summarized using descriptive statistics. No formal statistical inferences were planned or performed.

Results

Patient population

Between September 2009 and September 2013, this study enrolled a total of 25 patients of whom 18 were treated (6 patients consented then failed screening; 1 patient was found to have rapidly progressive disease on a baseline CT scan and other therapy was thought to be more appropriate). Sixteen patients received afatinib daily doses of 20 mg and 2 received afatinib daily doses of 30 mg. Patient demographics and clinical characteristics are shown in Table 1. Overall, the median age was 59.5 years (range, 39–80 years). Seventeen patients had previously been treated with trastuzumab, 4 patients with lapatinib (all of whom had also received trastuzumab), and 1 patient had previously received pertuzumab following trastuzumab therapy.

DLTs and MTD

Only 1 of the first 3 patients enrolled into the afatinib 20 mg cohort experienced DLT (grade 3 diarrhea; Table 2). Therefore, according to the 3+3 design, an additional 3 evaluable patients were enrolled at the 20-mg dose level, none of whom experienced DLT. Dose escalation thereafter proceeded to the afatinib 30-mg level; however, the first 2 patients enrolled at this dose level experienced DLT (both grade 3 diarrhea; Table 2). Dose escalation was therefore stopped. As only 1 of 6 patients enrolled had DLT, the MTD of the combination was defined per the trial protocol as afatinib 20 mg plus the recommended weekly dose of trastuzumab for breast cancer, and the dose level expanded to enroll a total of 16 patients, 13 of whom were evaluable for MTD determination. A further 3 patients in the expansion cohort experienced DLT (all grade 3 diarrhea, Table 2), taking the total number of patients with DLT at the 20 mg afatinib dose level to four, and thereby fulfilling the requirement for dose deescalation. Doses of afatinib lower than 20 mg could not be tested and the trial was therefore closed.

Adverse events

All patients had at least one AE. The most frequently reported AEs [by Medical Dictionary for Regulatory Activities (MedDRA) preferred term] were diarrhea ($n = 17$, 94%), nausea and rash (both $n = 11$, 61%), and decreased appetite and fatigue (both $n = 10$, 56%). AEs leading to discontinuation of study medication were reported for 7 (39%) patients, all of whom were treated with 20 mg afatinib once daily. SAEs occurred in 3 (17%) patients, all from the 20 mg afatinib cohort, which included 1 patient with diarrhea (drug related), 1 patient with acute renal failure (not drug related), and 1 patient who had a fatal pulmonary embolism (not drug related). The most common drug-related AEs (Table 3) at either dose (20 and 30 mg) were diarrhea ($n = 17$, 94%), rash and fatigue (both $n = 10$, 56%), nausea ($n = 9$, 50%), and skin fissures and decreased appetite (both $n = 8$, 44%). The majority of drug-related AEs were CTCAE grades 1 and 2. Of the 17 patients who experienced drug-related diarrhea, 7 of 15 patients in the 20 mg afatinib group and both patients in the 30 mg afatinib group had grade 3 events. Other grade 3 drug-related events comprised one decreased ejection fraction and one decreased hemoglobin value in the 20 mg afatinib group, and one elevated blood alkaline phosphatase in the 30 mg afatinib group. The patient with grade 3 decreased ejection fraction was enrolled into the study with an LVEF of 49%, which was later recorded as an important protocol violation; the

Table 1. Baseline demographics and characteristics of patients

	20 mg afatinib + trastuzumab	30 mg afatinib + trastuzumab	Total
Treated set, *n* (%)	16 (100)	2 (100)	18 (100)
Median (range) age, y	60.5 (39–80)	52.5 (48–57)	59.5 (39–80)
ER status (% negative/positive)	56/44	0/100	50/50
PgR status (% negative/positive)	88/13	50/50	83/17
Median (range) number of metastatic sites	2.0 (1–4)	2.5 (2–3)	2.0 (1–4)
Metastatic sites, *n* (%)			
Liver	7 (44)	2 (100)	9 (50)
Lung	5 (31)	2 (100)	7 (39)
Peritoneum	0 (0)	0 (0)	0 (0)
Brain	1 (6)	0 (0)	1 (6)
Other	13 (81)	1 (50)	14 (78)
Previous anticancer therapy, *n* (%)			
Surgery	15 (94)	1 (50)	16 (89)
Chemotherapy	13 (81)	2 (100)	15 (83)
Radiotherapy	12 (75)	2 (100)	14 (78)
Median (range) number of prior systemic therapy regimens			
Cytotoxic	3 (0–5)	2 (2–2)	2.5 (0–5)
Trastuzumab	1 (0–5)	1 (1–1)	1 (0–5)
Lapatinib	0 (0–1)	0 (0–0)	0 (0–1)
Endocrine	0.5 (0–4)	2.5 (1–4)	1 (0–4)
Other[a]	0 (0–1)	0.5 (0–1)	0 (0–1)

Abbreviations: ER, estrogen receptor; PgR, progesterone receptor.
[a]Pertuzumab or investigational drug.

subsequent grade 3 event was related to both study drugs. No drug-related AEs of grades 4 or 5 were reported.

Pharmacokinetics

There were 17 patients from whom data were available for pharmacokinetic analysis. Afatinib reached steady state by day 8 of treatment at the latest, and this was maintained until the end of the assessment period. The pharmacokinetic parameters for afatinib following once-daily 20 mg dosing with weekly trastuzumab infusions of 2 mg/kg are shown in Table 4. The variability of pharmacokinetic parameters in terms of the geometric coefficient of variance (gCV%) was high. Trough plasma concentration of afatinib remained stable throughout the observation period, with the exception of 1 patient who had an approximately 3.5-fold increase in trough plasma concentrations in comparison with the geometric mean (gMean) values on day 15 (data not shown). However, the afatinib trough plasma concentrations on days 8 and 22 were similar to the respective gMeans.

The results of the comparison between observed and simulated trastuzumab pharmacokinetic values are shown in Table 5. The observed median trastuzumab concentrations were well captured within the respective 95% prediction intervals for all pharmacokinetic parameters. When comparing these observed values with the respective median of the individual 100 percentiles from each of the simulated studies, the deviation was between −20.4% and −4.9% for all pharmacokinetic parameters, including maximum plasma concentration (C_{max}), on day 1, which represented trastuzumab exposure in the absence of afatinib (control). The 25th and 75th percentiles of the observed concentrations were also within the respective 95% prediction intervals, except for C_{max} on day 1 and plasma concentration before infusion (C_{pre}) on day 8.

Efficacy

The unconfirmed overall OR rate was 11% ($n = 2$, both PRs); a further 5 (28%) patients achieved a best response of SD (Supplementary Table S1). For the 2 patients with an OR, times to response were 57 and 58 days, and durations of response were 29 and 35 days. The DC rate was 39% ($n = 7$), with a median duration of DC of 111.0 days [95% confidence interval (CI), 85.0–388.0; interquartile range, 92–388]. The last patient to remain on treatment had a duration of DC of 1,270 days. Median duration of PFS was also 111.0 days (95% CI, 56.0–274.0). Best tumor shrinkage for 8 patients with evaluable baseline and on-treatment post-baseline tumor measurements is illustrated in Fig. 1.

Treatment duration

The mean duration of exposure to study medication was 155 days. In total, 9 (50%) patients were treated for more than one treatment cycle. For these patients, the mean duration of exposure to study medication was 287.0 days (median, 112 days; range, 57–1,288). At the final analysis, all patients had discontinued

Table 2. DLTs in cycle 1

Dose levels				
Trastuzumab (mg/kg weekly)	Afatinib (mg/d)	Patients treated, *n*	Patients with DLT in cycle 1, *n*	DLTs
2[a]	20 (dose-escalation cohort)	8[b]	1	Diarrhea (grade 3)
2[a]	20 (MTD expansion cohort)	8[c]	3	Diarrhea (grade 3)
2[a]	30	2	2	Diarrhea (grade 3)

[a]Starting dose 4 mg/kg.
[b]Of whom six were evaluable.
[c]Of whom seven were evaluable.

Table 3. Drug-related AEs occurring in ≥10% patients in combined 20 mg ($n = 16$) and 30 mg ($n = 2$) afatinib once-daily dose groups, overall, and by maximum CTCAE grade

	Afatinib once daily + trastuzumab			
MedDRA preferred term	**All, *n* (%)**	**Grade 1, *n* (%)**	**Grade 2, *n* (%)**	**Grade 3, *n* (%)**
Patients with drug-related AEs	17 (94)	2 (11)	4 (22)	11 (61)
Diarrhea	17 (94)	2 (11)	6 (33)	9 (50)
Rash	10 (56)	7 (39)	3 (17)	0 (0)
Fatigue	10 (56)	6 (33)	4 (22)	0 (0)
Nausea	9 (50)	7 (39)	2 (11)	0 (0)
Skin fissures	8 (44)	5 (28)	3 (17)	0 (0)
Decreased appetite	8 (44)	6 (33)	2 (11)	0 (0)
Oral pain	7 (39)	6 (33)	1 (6)	0 (0)
Mouth ulceration	6 (33)	4 (22)	2 (11)	0 (0)
Dry skin	5 (28)	4 (22)	1 (6)	0 (0)
Epistaxis	5 (28)	5 (28)	0 (0)	0 (0)
Cheilitis	4 (22)	3 (17)	1 (6)	0 (0)
Dyspepsia	4 (22)	4 (22)	0 (0)	0 (0)
Dysgeusia	3 (17)	3 (17)	0 (0)	0 (0)
Nasal discomfort	3 (17)	2 (11)	1 (6)	0 (0)
Vomiting	3 (17)	3 (17)	0 (0)	0 (0)
Abdominal pain	2 (11)	2 (11)	0 (0)	0 (0)
Abdominal pain upper	2 (11)	2 (11)	0 (0)	0 (0)
Ageusia	2 (11)	2 (11)	0 (0)	0 (0)
Decreased ejection fraction	2 (11)	0 (0)	1 (6)	1 (6)
Dermatitis acneiform	2 (11)	2 (11)	0 (0)	0 (0)
Dry mouth	2 (11)	2 (11)	0 (0)	0 (0)
Foreign body sensation in eyes	2 (11)	2 (11)	0 (0)	0 (0)
Mucosal inflammation	2 (11)	1 (6)	1 (6)	0 (0)
Nasal congestion	2 (11)	2 (11)	0 (0)	0 (0)
Pain in the extremities	2 (11)	2 (11)	0 (0)	0 (0)
Rhinorrhea	2 (11)	2 (11)	0 (0)	0 (0)
Stomatitis	2 (11)	1 (6)	1 (6)	0 (0)

treatment due to PD ($n = 8$), DLTs or other AEs ($n = 7$), or refusal to continue taking study medication ($n = 3$). One patient from the 20 mg cohort completed 45 cycles of treatment (1,288 days) with SD.

Discussion

This study evaluated the MTD, safety, pharmacokinetics, and efficacy of afatinib in combination with trastuzumab in patients with advanced or metastatic HER2-positive breast cancer. The MTD for daily oral afatinib when combined with weekly trastuzumab infusions was 20 mg. However, when the 20 mg cohort was expanded, 4 of 13 patients experienced DLTs, which according to the protocol required a lower dose tier to be examined. Because there was no possible lower dose tier, due to the lack of availability of afatinib in a dose formulation below 20 mg, further recruitment into the study was stopped.

Table 4. Pharmacokinetic parameters for afatinib following multiple once-daily oral administrations of 20 mg afatinib and trastuzumab weekly infusions 2 mg/kg[a]

Afatinib pharmacokinetic parameter	***N***	**gMean**	**gCV%**
$AUC_{0-8,ss}$, ng·h/mL	10	116	73.6
$C_{max,ss}$, ng/mL	10	17.9	80.9
Day 22 $C_{pre,ss}$, ng/mL	11	9.82	69.4
Day 29 $C_{pre,ss}$, ng/mL	8	9.15	64.7
$t_{max,ss}$, h[b]	11	4.25	0.750–7.02

Abbreviations: $AUC_{0-8,ss}$, area under the plasma concentration–time curve from time point 0 to 8 hours after dose administration at steady state; ss, steady state; $t_{max,ss}$ = time to reach C_{max} at steady state.
[a]Starting dose 4 mg/kg.
[b]For $t_{max,ss}$, the median and range (min–max) are given.

All DLTs observed were diarrhea (all grade 3), which is a known AE associated with afatinib (17). The incidence of diarrhea in one phase II afatinib monotherapy study in patients with HER2-positive metastatic breast cancer was 90.2% ($N = 41$) for afatinib 50 mg (11), whereas in this study, in which a lower dose of afatinib was combined with trastuzumab, the incidence was 94% ($N = 18$). In the Neo ALTTO study the combination of lapatinib and trastuzumab was associated with a higher rate of grade 3 diarrhea than that seen with trastuzumab alone (21% vs. 2%, respectively; ref. 18); diarrhea is common with lapatinib monotherapy (18, 19).

AEs of diarrhea can be effectively managed with antidiarrheal medication. This was recognized in the protocol for this study, which required loperamide to be available to patients at all times and allowed dosing up to 20 mg per day, in accordance with current clinical practice for the management of the gastrointestinal AEs associated with afatinib (17). Also, in the course of this study, addition of codeine phosphate to antidiarrheal therapy (which was not a protocol recommendation) showed positive effects in some patients. Use of antidiarrheal medication during the study was required to be recorded; however, not all patients who had diarrhea reported use of antidiarrheal medication. Thus, it is possible that management of diarrhea was suboptimal in some patients. Proper and prompt management of diarrhea with loperamide, as recommended in afatinib studies, combined with patient education regarding compliance with antidiarrheal therapy and dietary measures, may improve tolerance of the combination of afatinib with trastuzumab.

On the basis of the metabolic and pharmacokinetic characteristics of afatinib and trastuzumab, a pharmacokinetic drug–drug interaction is considered unlikely. Afatinib undergoes minimal

Table 5. Comparison of observed and simulated trastuzumab serum concentrations across afatinib dose cohorts

Pharmacokinetic variable	*N*	Percentile	Observed value	Simulated value[a]	Deviation[b] (%)
Day 1 C_{max}, ng/mL	8	25th	91,300	63,200 (42,800–87,200)	−30.8
		50th (median)	98,400	79,000 (57,000–106,000)	−19.7
		75th	114,000	98,800 (70,800–137,000)	−13.2
Day 8 C_{pre}, ng/mL	8	25th	27,200	17,600 (10,500–26,500)	−35.0
		50th (median)	29,800	23,700 (15,800–34,200)	−20.4
		75th	36,700	31,300 (21,100–44,900)	−14.8
Day 15 C_{pre}, ng/mL	7	25th	30,200	19,900 (11,600–31,100)	−34.1
		50th (median)	30,900	26,800 (15,300–41,200)	−13.4
		75th	37,000	35,400 (21,900–53,700)	−4.2
Day 15 C_{max}, ng/mL	7	25th	71,800	53,100 (36,600–76,600)	−26.0
		50th (median)	74,400	66,400 (47,100–93,500)	−10.8
		75th	79,800	81,900 (60,500–117,000)	2.7
Day 29 C_{pre}, ng/mL	5	25th	34,100	26,200 (13,400–45,500)	−23.2
		50th (median)	36,300	34,500 (19,100–56,100)	−4.9
		75th	38,500	44,200 (25,200–75,000)	14.9
Day 29 C_{max}, ng/mL	3	50th (median)[c]	83,000	75,700 (45,500–127,000)	−8.8

[a]Median of the individual percentiles derived from each of the 1,000 simulated studies together with the 2.5th and 97.5th percentiles (= 95% prediction interval) in parentheses.
[b]The deviation from the observed value was calculated as: (median of all simulated values–observed value)/observed value.
[c]Results for the 25th and 75th percentiles not shown due to the low number of patients.

metabolism, and is excreted via the enterohepatic system. The *in vitro* metabolic profile of afatinib and pharmacokinetic data suggest no interaction between afatinib and cytochrome P450 substrates (20). Trastuzumab has a half-life of up to 28.5 days (21) and has shown additive effects with other agents, including taxanes and vinorelbine (22, 23). The overall pharmacokinetic profiles observed in this study were consistent with the known characteristics of afatinib and trastuzumab (12, 24), and there was no evidence that trastuzumab had a relevant effect on the pharmacokinetics of afatinib or vice versa. gMean pharmacokinetic parameters after multiple administration of afatinib once daily in the presence of trastuzumab were similar to those following multiple administrations of afatinib as monotherapy in solid tumors, and in keeping with experience detailed in a recent meta-analysis (25). Furthermore, the findings from the comparison between observed and simulated trastuzumab pharmacokinetic values indicate that any pharmacokinetic interaction between afatinib and trastuzumab is unlikely. Therefore, the incidence of diarrhea with afatinib and trastuzumab combination therapy in this study is more likely the result of a pharmacodynamic interaction than a pharmacokinetic interaction.

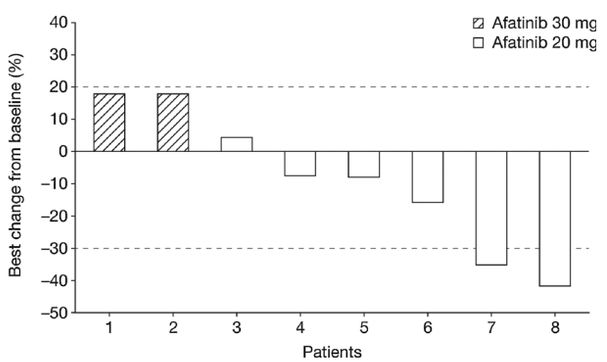

Figure 1.
The Waterfall plot of target lesions, sum of diameters best change from baseline (%) for patients with evaluable baseline and on-treatment post-baseline tumor measurements. Ten patients did not have evaluable baseline and on-treatment post-baseline tumor measurements.

Despite the small population size and trastuzumab-resistant/-refractory disease at study entry [17/18 patients (94%) had progressed following trastuzumab treatment], there were indications of clinical antitumor activity for the combined therapy of afatinib plus weekly trastuzumab (OR rate, 11%). The last patient to remain on treatment completed 45 cycles of treatment, which was relatively well tolerated, and had a best response of SD associated with a duration of DC of 1,270 days. Before study entry, this patient had already received three lines of chemotherapy, as well as trastuzumab and pertuzumab, all with a best response of SD.

Previously, afatinib monotherapy was shown to be clinically active in HER2-positive metastatic breast cancer: in an open-label, single-arm, phase II study of afatinib in 41 patients following trastuzumab failure, PR was achieved in 4 patients and SD in 8 patients (maintained for at least four cycles; ref. 11). Other TKIs are under investigation in this setting. Neratinib is a potent irreversible TKI that blocks signal transduction through EGFR, HER2, and HER4. In a nonrandomized phase II trial, 136 patients were treated with neratinib monotherapy. The OR rate was 24% in the 66 patients who had received prior trastuzumab treatment and was 56% for patients with no prior trastuzumab treatment (26). A phase III study of neratinib in combination with capecitabine versus lapatinib and capecitabine is underway in women with HER2-positive advanced breast cancer (ClinicalTrials.gov identifier: NCT01808573).

Dual HER2 blockade with the combination of trastuzumab and a TKI, such as lapatinib or afatinib, alone or in combination with chemotherapy, is also a therapeutic option for trastuzumab-refractory metastatic breast cancer (14). The reversible TKI lapatinib has shown activity in metastatic breast cancer in combination with trastuzumab. This combination has now received approval in Europe for adult patients with breast cancer whose tumors overexpress HER2 (ErbB2), with hormone receptor–negative metastatic disease that has progressed on prior trastuzumab

in combination with chemotherapy. Lapatinib and trastuzumab demonstrated a significant 4.5-month median overall survival advantage compared with lapatinib monotherapy in patients with heavily pretreated HER2-positive metastatic breast cancer (13). However, reversible multitargeted TKIs are also limited by the development of resistance, although afatinib's irreversible ErbB family blockade may provide a strategy to overcome this limitation.

The inability to escalate afatinib to doses achieved in other studies due to excessive DLTs of diarrhea did not permit recommendation of a phase II dose. However, further assessment of afatinib with trastuzumab is warranted in HER2-positive advanced cancers with an optimal management/prevention of diarrhea. A new phase I study is currently ongoing with the aim of determining the MTD of afatinib in combination with three-weekly trastuzumab in HER2-overexpressing cancer and assessing the efficacy of the combination MTD dosage (ClinicalTrials.gov identifier: NCT01649271).

Conclusions

The MTD of afatinib was 20 mg daily in combination with the recommended weekly dose of trastuzumab. This MTD cannot be recommended for phase II development without strict diarrhea management; however, lower doses could be explored if formulation allowed. Signs of clinical activity were seen in trastuzumab-resistant HER2-positive breast cancer, suggesting that further investigation with optimal diarrhea management is warranted.

Disclosure of Potential Conflicts of Interest

R. Plummer reports receiving other commercial research support from Boehringer Ingelheim. No potential conflicts of interest were disclosed by the other authors.

Disclaimer

The authors were fully responsible for all content and editorial decisions, were involved at all stages of article development, and have approved the final version.

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References

1. Yarden Y, Sliwkowski MX. Untangling the ErbB signalling network. Nat Rev Mol Cell Biol 2001;2:127–37.
2. Ross JS, Slodkowska EA, Symmans WF, Pusztai L, Ravdin PM, Hortobagyi GN. The HER-2 receptor and breast cancer: ten years of targeted anti-HER-2 therapy and personalized medicine. Oncologist 2009;14:320–68.
3. Nielsen DL, Kumler I, Palshof JA, Andersson M. Efficacy of HER2-targeted therapy in metastatic breast cancer. monoclonal antibodies and tyrosine kinase inhibitors. Breast 2013;22:1–12.
4. Arribas J, Baselga J, Pedersen K, Parra-Palau JL. p95HER2 and breast cancer. Cancer Res 2011;71:1515–9.
5. Nahta R, Esteva FJ. Trastuzumab: triumphs and tribulations. Oncogene 2007;26:3637–43.
6. Nahta R, Hung MC, Esteva FJ. The HER-2-targeting antibodies trastuzumab and pertuzumab synergistically inhibit the survival of breast cancer cells. Cancer Res 2004;64:2343–6.
7. Rexer BN, Arteaga CL. Intrinsic and acquired resistance to HER2-targeted therapies in HER2 gene-amplified breast cancer: mechanisms and clinical implications. Crit Rev Oncog 2012;17:1–16.
8. Shojaei S, Gardaneh M, Rahimi SA. Target points in trastuzumab resistance. Int J Breast Cancer 2012;2012:761917.
9. Dokmanovic M, Hirsch DS, Shen Y, Wu WJ. Rac1 contributes to trastuzumab resistance of breast cancer cells: Rac1 as a potential therapeutic target for the treatment of trastuzumab-resistant breast cancer. Mol Cancer Ther 2009;8:1557–69.
10. Wong AL, Lee SC. Mechanisms of resistance to trastuzumab and novel therapeutic strategies in HER2-positive breast cancer. Int J Breast Cancer 2012;2012:415170.
11. Lin NU, Winer EP, Wheatley D, Carey LA, Houston S, Mendelson D, et al. 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 2012;133:1057–65.
12. Yap TA, Vidal L, Adam J, Stephens P, Spicer J, Shaw H, et al. Phase I trial of the irreversible EGFR and HER2 kinase inhibitor BIBW 2992 in patients with advanced solid tumors. J Clin Oncol 2010;28:3965–72.
13. Blackwell KL, Burstein HJ, Storniolo AM, Rugo HS, Sledge G, Aktan G, et al. Overall survival benefit with lapatinib in combination with trastuzumab for patients with human epidermal growth factor receptor 2-positive metastatic breast cancer: final results from the EGF104900 Study. J Clin Oncol 2012;30:2585–92.
14. Kumler I, Tuxen MK, Nielsen DL. A systematic review of dual targeting in HER2-positive breast cancer. Cancer Treat Rev 2014;40:259–70.
15. Piccart-Gebhart MJ, Holmes AP, Baselga J, De Azambuja E, Dueck AC, Viale G, et al. First results from the phase III ALTTO trial (BIG 2–06; NCCTG [Alliance] N063D) comparing one year of anti-HER2 therapy with lapatinib alone (L), trastuzumab alone (T), their sequence (T->L), or their combination (T+L) in the adjuvant treatment of HER2-positive early breast cancer (EBC). ASCO Meeting Abstracts 2014;32:LBA4.

16. Bruno R, Washington CB, Lu JF, Lieberman G, Banken L, Klein P. Population pharmacokinetics of trastuzumab in patients with $HER2^{+}$ metastatic breast cancer. Cancer Chemother Pharmacol 2005;56:361–9.
17. Yang JC, Reguart N, Barinoff J, Kohler J, Uttenreuther-Fischer M, Stammberger U, et al. Diarrhea associated with afatinib: an oral ErbB family blocker. Expert Rev Anticancer Ther 2013;13:729–36.
18. Baselga J, Bradbury I, Eidtmann H, Di Cosimo S, de Azambuja E, Aura C, et al. Lapatinib with trastuzumab for HER2-positive early breast cancer (NeoALTTO): a randomised, open-label, multicentre, phase 3 trial. Lancet 2012;379:633–40.
19. Blackwell KL, Burstein HJ, Storniolo AM, Rugo H, Sledge G, Koehler M, et al. Randomized study of lapatinib alone or in combination with trastuzumab in women with ErbB2-positive, trastuzumab-refractory metastatic breast cancer. J Clin Oncol 2010;28:1124–30.
20. Stopfer P, Marzin K, Narjes H, Gansser D, Shahidi M, Uttereuther-Fischer M, et al. Afatinib pharmacokinetics and metabolism after oral administration to healthy male volunteers. Cancer Chemother Pharmacol 2012;69:1051–61.
21. Harris K, Washington CB, Lu J-F, Lieberman G, Lu J-F, Mass R, et al. A population pharmacokinetic (PK) model for Herceptin (H) and implications for clinical dosing. Proc Am Soc Clin Oncol 2002;21:123a (abstr 488).
22. Konecny G, Pegram MD, Beryt M, Untch M, Slamon DJ. Therapeutic advantage of chemotherapy drugs in combination with Herceptin against human breast cancer cells with HER-2/neu overexpression. Breast Cancer Res Treat 1999;57:114 (abstr 467).
23. Pegram MD, Konecny GE, O'Callaghan C, Beryt M, Pietras R, Slamon DJ. Rational combinations of trastuzumab with chemotherapeutic drugs used in the treatment of breast cancer. J Natl Cancer Inst 2004;96:739–49.
24. Cobleigh MA, Vogel CL, Tripathy D, Robert NJ, Scholl S, Fehrenbacher L, et al. Multinational study of the efficacy and safety of humanized anti-HER2 monoclonal antibody in women who have HER2-overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease. J Clin Oncol 1999;17:2639–48.
25. Wind S, Schmid M, Erhardt J, Goeldner RG, Stopfer P. Pharmacokinetics of afatinib, a selective irreversible ErbB family blocker, in patients with advanced solid tumours. Clin Pharmacokinet 2013;52:1101–9.
26. Burstein HJ, Sun Y, Dirix LY, Jiang Z, Paridaens R, Tan AR, et al. Neratinib, an irreversible erbb receptor tyrosine kinase inhibitor, in patients with advanced ErbB2-positive breast cancer. J Clin Oncol 2010;28:1301–7.