points of 24, 48, and 72 h. The resultant cells were used for biological assays at the indicated time point after siRNA transfection. Stealth RNAi™ siRNA Negative Control Med GC Duplex (Invitrogen, Carlsbad, CA) was used as negative control throughout the experiment.

Lentivirus and infections

MISSION shRNA pLKO.1 constructs (Sigma-Aldrich, St. Louis, MO) were used to make self-inactivating shRNA lentivirus for *PAPPA* [sequence 5'-CCG GGG TGA CGG ATG GGA CAT ATT ACT CGA GTA ATA TGT CCC ATC CGT CAC CTT TTT TG-3' (clone NM_002581.3-3690s21c1)], and a non-target random scrambled sequence control (SHC002). For virus transduction, 2×10^6 NCI-H290 cells were incubated with lentivirus plus 5 μg/ml polybrene (CHEMICON International, Temecula, CA) for 24 h. The successfully transduced clones were identified by puromycin (Sigma-Aldrich, St. Louis, MO) selection.

MTT assay

Cell proliferation was measured by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) dye reduction method [26]. Briefly, the MPM cells (2×10^3/well) were seeded in 96-well plates and cultured for 72 h. After incubation, 50 μl of stock MTT solution (2 mg/ml; Sigma, St. Louis, MO) was added to each well, and the cells were then further incubated for 2 h. The media containing MTT solution were removed and the dark blue crystals were dissolved by adding 100 μl of DMSO. Absorbance was measured with a Sunrise Microplate Reader (Tecan Group, Männedorf, Switzerland) at test and reference wavelengths of 550 and 620 nm, respectively.

Orthotopic xenograft mouse model

Male severe combined immunodeficiency (SCID) mice aged 6-7 weeks (CLEA Japan, Osaka, Japan) were maintained under specific pathogen-free conditions throughout the study. Ethics approval for all animal experiments was obtained from Animal Care and Use Committee of The University of Tokushima. An orthotopic implantation model of human MPM was established as described previously [3-5, 26]. Briefly, 3×10^5 NCI-H290 cells in 100 μl of PBS were injected into the right pleural cavity of mice. The mice were sacrificed 21-23 days after tumor inoculation. The pleural tumors were carefully dissected and weighed; the pleural effusion was harvested using a 1-ml syringe, and the volume of the pleural effusion was measured.

Statistical analysis

The statistical significances of the differences were analyzed by Student's *t* test, one way ANOVA analysis, where applicable. The correlation efficient and the significance were calculated by Pearson's correlation analysis. The *P*-values less than 0.05 were considered significant in all experiments. Data analysis was carried out using GraphPad Prism version 5.01 (GraphPad Software, La Jolla, CA).

ACKNOWLEDGEMENTS

The authors thank Ms. Tomoko Oka for technical assistance. Y.N. receives research funding from Taiho Pharmaceutical.Co., Ltd. This work was supported in part by research grant from Taiho Pharmaceutical. Co., Ltd, KAKENHI (24390210, 22700882), a Grant-in-Aid for Scientific Research (Y.N.), and a Challenging Exploratory Research (S.K.) from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan.

REFERENCES

1. Robinson BW, Lake RA. Advances in malignant mesothelioma. N Engl J Med. 2005; 353: 1591-1603.
2. Ray M, Kindler HL. Malignant pleural mesothelioma: an update on biomarkers and treatment. Chest. 2009; 136: 888-896.
3. Li Q, Yano S, Ogino H, Wang W, Uehara H, Nishioka Y, Sone S. The therapeutic efficacy of anti-vascular endothelial growth factor antibody, bevacizumab, and pemetrexed against orthotopically implanted human pleural mesothelioma cells in severe combined immunodeficient mice. Clin Cancer Res. 2007; 13: 5918-5925.
4. Ikuta K, Yano S, Van TT, Hanibuchi M, Goto H, Li Q, Wang W, Yamada T, Ogino H, Kakiuchi S, Uehara H, Sekido Y, Uenaka T, Nishioka Y, Sone S. E7080, a multi-tyrosine kinase inhibitor, suppresses the progression of malignant pleural mesothelioma with different proangiogenic cytokine production profiles. Clin Cancer Res. 2009; 15: 7229-7937.
5. Van TT, Hanibuchi M, Goto H, Kuramoto T, Yukishige S, Kakiuchi S, Sato S, Sakaguchi S, Dat LT, Nishioka Y, Akiyama S, Sone S. SU6668, a multiple tyrosine kinase inhibitor, inhibits progression of human malignant pleural mesothelioma in an orthotopic model. Respirology. 2012; 17: 984-990.
6. Jakobsen JN, Sørensen JB. Review on clinical trials of targeted treatments in malignant mesothelioma. Cancer Chemother Pharmacol. 2011; 68: 1-15.
7. West SD, Lee YC. Management of malignant pleural mesothelioma. Clin Chest Med. 2006; 27: 335-354.
8. Wells A, Grahovac J, Wheeler S, Ma B, Lauffenburger D.

Targeting tumor cell motility as a strategy against invasion and metastasis. Trends Pharmacol Sci. 2013; 34: 283-289.

9. Lin TM, Galbert SP, Kiefer D, Spellacy WN, Gall S. Characterization of four human pregnancy-associated plasma proteins. Am J Obstet Gynecol. 1974; 118: 223-236.
10. Lawrence JB, Oxvig C, Overgaard MT, Sottrup-Jensen L, Gleich GJ, Hays LG, Yates JR III, Conover CA. The insulin-like growth factor (IGF)-dependent IGF binding protein-4 protease secreted by human fibroblasts is pregnancy associated plasma protein-A. Proc Natl Acad Sci USA. 1999; 96: 3149-3153.
11. Boldt HB, Conover CA. Pregnancy-associated plasma protein-A (PAPP-A): A local regulator of IGF bioavailability through cleavage of IGFBPs. Growth Horm IGF Res. 2007; 17: 10-18.
12. Gyrup C, Oxvig C. Quantitative analysis of insulin-like growth factor-modulated proteolysis of insulin-like growth factor binding protein-4 and -5 by pregnancy associated plasma protein-A. Biochemistry. 2007; 46: 1972-1980.
13. Pollak M. Insulin and insulin-like growth factor signalling in neoplasia. Nature Rev Cancer. 2008; 8: 915-928.
14. Hoang CD, D'Cunha J, Kratzke MG, Casmey CE, Frizelle SP, Maddaus MA, Kratzke RA. Gene expression profiling identifies matriptase overexpression in malignant mesothelioma. Chest. 2004; 125: 1843-1852.
15. Hoang CD, Zhang X, Scott PD, Guillaume TJ, Maddaus MA, Yee D, Kratzke RA. Selective activation of insulin receptor substrate-1 and -2 in pleural mesothelioma cells: association with distinct malignant phenotypes. Cancer Res. 2004; 64: 7479-7485.
16. Jacobson BA, De A, Kratzeke MG, Patel MR, Jay-Dixon J, Whitson BA, Sadiq AA, Bitterman PB, Polunovsky VA, Kratzke RA. Activated 4E-BP1 represses tumourigenesis and IGF-I-mediated activation of the eIF4F complex in mesothelioma. Br J Cancer. 2009; 101: 424-431.
17. King ER, Wong KK. Insulin-like growth factor: current concepts and new developments in cancer therapy. Recent Pat Anticancer Drug Discov. 2012; 7: 14-30.
18. Conover CA, Bale LK. Loss of pregnancy-associated plasma protein A extends lifespan in mice. Aging Cell. 2007; 6: 727-729.
19. Conover CA, Bale LK, Mader JR, Mason MA, Keenan KP, Marler RJ. Longevity and age-related pathology of mice deficient in pregnancy-associated plasma protein-A. J Gerontol A Biol Sci Med Sci. 2010; 65: 590-599.
20. Ryan AJ, Napoletano S, Fitzpatrick PA, Currid CA, O'Sullivan NC, Harmey JH. Expression of a protease-resistant insulin-like growth factor-binding protein-4 inhibits tumour growth in a murine model of breast cancer. Br J Cancer. 2009; 101: 278-286.
21. Boldt HB, Conover CA. Overexpression of pregnancy-associated plasma protein-A in ovarian cancer cells promotes tumor growth in vivo. Endocrinology. 2011; 152: 1470-1478.
22. Pan H, Hanada S, Zhao J, Mao L, Ma MZ. Protein secretion is required for pregnancy-associated plasma protein-A to promote lung cancer growth in vivo. PLoS One. 2012; 7: e48799.
23. Kalra N, Zhang J, Yu Y, Ho M, Merino M, Cao L, Hassan R. Efficacy of anti-insulin-like growth factor I receptor monoclonal antibody cixutumumab in mesothelioma is highly correlated with insulin growth factor-I receptor sites/cell. Int J Cancer. 2012; 131: 2143-2152.
24. Bang P. Serum proteolysis of IGFBP-3. Prog Growth Factor Res. 1995; 6: 285-292.
25. Liu Z, Klominek J. Chemotaxis and chemokinesis of malignant mesothelioma cells to multiple growth factors. Anticancer Res. 2004; 24: 1625-1630.
26. Van TT, Hanibuchi M, Kakiuchi S, Sato S, Kuramoto T, Goto H, Mitsuhashi A, Nishioka Y, Akiyama S, Sone S. The therapeutic efficacy of S-1 against orthotopically implanted human pleural mesothelioma cells in severe combined immunodeficient mice. Cancer Chemother. Pharmacol. 2011; 68: 497-504.
27. Lee TC, Zhang Y, Aston C, Hintz R, Jagirdar J, Perle MA, Burt M, Rom WN. Normal human mesothelial cells and mesothelioma cell lines express insulin-like growth factor I and associated molecules. Cancer Res. 1993; 53: 2858-2864.
28. Gordon GJ, Rockwell GN, Jensen RV, Rheinwald JG, Glickman JN, Aronson JP, Pottorf BJ, Nitz MD, Richards WG, Sugarbaker DJ, Bueno R. Identification of novel candidate oncogenes and tumor suppressors in malignant pleural mesothelioma using large-scale transcriptional profiling. Am J Pathol. 2005; 166: 1827-1840.
29. Friedrich N, Alte D, Völzke H, Spilcke-Liss E, Lüdemann J, Lerch MM, Kohlmann T, Nauck M, Wallaschofski H. Reference ranges of serum IGF-1 and IGFBP-3 levels in a general adult population: Results of the Study of Health in Pomerania (SHIP). Growth Horm IGF Res. 2008; 18: 228-237.
30. Su AI, Wiltshire T, Batalov S, Lapp H, Ching KA, Block D, Zhang J, Soden R, Hayakawa M, Kreiman G, Cooke MP, Walker JR, Hogenesch JB. A gene atlas of the mouse and human protein-encoding transcriptomes. Proc Natl Acad Sci USA. 2004; 101: 6062-6067.
31. Contois LW, Nugent DP, Caron JM, Cretu A, Tweedie E, Akalu A, Liebes L, Friesel R, Rosen C, Vary C, Brooks PC. Insulin-like growth factor binding protein-4 (IGFBP-4) differentially inhibits growth factor-induced angiogenesis. J Biol Chem. 2012; 287: 1779-1789.
32. Jadlowiec J, Dongell D, Smith J, Conover C, Campbell P. Pregnancy-associated plasma protein-A is involved in matrix mineralization of human adult mesenchymal stem cells and angiogenesis in the chick chorioallontoic membrane. Endocrinology. 2005; 146: 3765-3772.
33. Kawaguchi K, Murakami H, Taniguchi T, Fujii M, Kawata S, Fukui T, Kondo Y, Osada H, Usami N, Yokoi K, Ueda

Y, Yatabe Y, Ito M, Horio Y, Hida T, Sekido Y. Combined inhibition of MET and EGFR suppresses proliferation of malignant mesothelioma cells. Carcinogenesis. 2009; 30: 1097-1105.

34. Abe S, Morita Y, Kaneko MK, Hanibuchi M, Tsujimoto Y, Goto H, Kakiuchi S, Aono Y, Huang J, Sato S, Kishuku M, Taniguchi Y, Azuma M, Kawazoe K, Sekido Y, Yano S, et al. A Novel targeting therapy of malignant mesothelioma using anti-podoplanin antibody. J Immmunol. 2013; 190: 6239-6249.

35. Gunawardane RN, Sgroi DC, Wrobe CN, Koh E, Daley GQ, Brugge JS. Novel role for PDEF in epithelial cell migration and invasion. Cancer Res. 2005; 65: 11572-11580.

VOLUME 31 · NUMBER 27 · SEPTEMBER 20 2013

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

LUX-Lung 4: A Phase II Trial of Afatinib in Patients With Advanced Non–Small-Cell Lung Cancer Who Progressed During Prior Treatment With Erlotinib, Gefitinib, or Both

Nobuyuki Katakami, Shinji Atagi, Koichi Goto, Toyoaki Hida, Takeshi Horai, Akira Inoue, Yukito Ichinose, Kunihiko Koboyashi, Koji Takeda, Katsuyuki Kiura, Kazuto Nishio, Yoko Seki, Ryuichi Ebisawa, Mehdi Shahidi, and Nobuyuki Yamamoto

See accompanying editorial on page 3303 and articles on pages 3327 and 3342

Nobuyuki Katakami, Kobe City Medical Center General Hospital, Kobe; Shinji Atagi, National Hospital Organization Kinki-Chuo Chest Medical Center; Koji Takeda, Osaka City General Hospital; Kazuto Nishio, Kinki University, Osaka; Koichi Goto, National Cancer Center Hospital East, Chiba; Toyoaki Hida, Aichi Cancer Center Hospital, Nagoya; Takeshi Horai, The Cancer Institute Hospital of Japanese Foundation for Cancer Research, Tokyo; Akira Inoue, Tohoku University Hospital, Sendai; Yukito Ichinose, National Kyushu Cancer Center, Fukuoka; Kunihiko Koboyashi, Saitama International Medical Center, Saitama; Katsuyuki Kiura, Okayama University, Okayama; Yoko Seki and Ryuichi Ebisawa, Nippon Boehringer Ingelheim; Nobuyuki Yamamoto, Shizuoka Cancer Center, Shizuoka, Japan; and Mehdi Shahidi, Boehringer Ingelheim, Bracknell, United Kingdom.

Published online ahead of print at www.jco.org on July 1, 2013.

Supported by Boehringer Ingelheim and by Ogilvy Healthworld (for James Duggan who provided medical writing assistance).

Presented at the 47th Annual Meeting of the American Society of Clinical Oncology, Chicago, IL, June 3-7, 2011, and the 14th World Conference on Lung Cancer, Amsterdam, the Netherlands, July 3-7, 2011.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

Clinical trial information: NCT00711594.

Corresponding author: Nobuyuki Katakami, MD, PhD, Kobe City Medical Center General Hospital, 2-1-1 Minatojima Minami-machi Chuo-ku Kobe, 650-0047, Japan; e-mail: nkatakami@kcho.jp.

0732-183X/13/3127w-3335w/$20.00

DOI: 10.1200/JCO.2012.45.0981

ABSTRACT

Purpose
New molecular targeted agents are needed for patients with non–small-cell lung cancer (NSCLC) who progress while receiving erlotinib, gefitinib, or both. Afatinib, an oral irreversible ErbB family blocker, has preclinical activity in epidermal growth factor receptor (EGFR [ErbB1]) mutant models with EGFR-activating mutations, including T790M.

Patients and Methods
This was a Japanese single-arm phase II trial conducted in patients with stage IIIB to IV pulmonary adenocarcinoma who progressed after ≥ 12 weeks of prior erlotinib and/or gefitinib. Patients received afatinib 50 mg per day. The primary end point was objective response rate (complete response or partial response) by independent review. Secondary end points included progression-free survival (PFS), overall survival (OS), and safety.

Results
Of 62 treated patients, 45 (72.6%) were *EGFR* mutation positive in their primary tumor according to local and/or central laboratory analyses. Fifty-one patients (82.3%) fulfilled the criteria of acquired resistance to erlotinib and/or gefitinib. Of 61 evaluable patients, five (8.2%; 95% CI, 2.7% to 18.1%) had a confirmed objective response rate (partial response). Median PFS was 4.4 months (95% CI, 2.8 to 4.6 months), and median OS was 19.0 months (95% CI, 14.9 months to not achieved). Two patients had acquired T790M mutations: L858R + T790M, and deletion in exon 19 + T790M; they had stable disease for 9 months and 1 month, respectively. The most common afatinib-related adverse events (AEs) were diarrhea (100%) and rash/acne (91.9%). Treatment-related AEs leading to afatinib discontinuation were experienced by 18 patients (29%), of whom four also had progressive disease.

Conclusion
Afatinib demonstrated modest but noteworthy efficacy in patients with NSCLC who had received third- or fourth-line treatment and who progressed while receiving erlotinib and/or gefitinib, including those with acquired resistance to erlotinib, gefitinib, or both.

J Clin Oncol 31:3335-3341. © 2013 by American Society of Clinical Oncology

INTRODUCTION

Epidermal growth factor receptor (*EGFR* [ErbB1]) somatic mutations occur in 30% of patients with non–small-cell lung cancer (NSCLC) who are of East Asian ethnicity (eg, from Japan or Taiwan) compared with 8% of patients of other ethnicities (eg, from the United States or Australia).[1] The predictive significance of these mutations in NSCLC and the association with a considerable improvement in response and progression-free survival (PFS) with currently available tyrosine kinase inhibitor (TKI) therapy have been shown in several phase III trials.[2-7] Despite promising results, patients with NSCLC who harbor *EGFR* mutations will eventually experience disease progression as a result of the inevitable development of resistance mechanisms, in particular, the T790M mutation in exon 20, which is found in more than 50% of patients who received an EGFR TKI.[8,9] Currently, there are no treatments with proven efficacy for these patients; thus, there is an increased demand to develop novel molecular targeted agents.

Afatinib is an irreversible ErbB family blocker, the preclinical activity of which includes *EGFR*-mutant cell lines that have common mutations, including T790M.[10,11] Results from phase I/II trials have complemented these two preclinical studies, demonstrating the efficacy of afatinib in patients with NSCLC who harbor EGFR-activating mutations.[12] These trials also included a phase I study in Japan that suggested modest clinical activity of afatinib in such patients following progression on erlotinib, gefitinib, or both and identified the maximum-tolerated dose of afatinib as 50 mg.[13]

This phase II trial was conducted in Japan to evaluate the efficacy of 50-mg afatinib monotherapy in third- and fourth-line patients with NSCLC who had progressed while receiving erlotinib and/or gefitinib treatment.

PATIENTS AND METHODS

Study Design

This was a multicenter, single-arm, open-label phase II trial of afatinib monotherapy in patients with NSCLC who had progressed on currently available EGFR TKIs. The primary end point was objective response rate (ORR) by independent review according to Response Evaluation Criteria in Solid Tumors (RECIST) 1.0.[14] Secondary end points were time to ORR, duration of ORR, frequency and duration of clinical benefit (complete response [CR], partial response [PR], and stable disease [SD]), PFS, overall survival (OS), and disease control rate (DCR).

The study was conducted in line with the Declaration of Helsinki and the International Conference on Harmonization Good Clinical Practice guidelines and with the approval of each institutional review board. All patients provided written informed consent before study participation.

Study Population

Patients were required to have had at least 12 weeks of prior EGFR TKIs, which served as an enrichment strategy for patients with EGFR-activating mutations and subsequent acquired resistance mutations. Although *EGFR* mutation status at screening, including T790M status, was not required, mutation analysis was performed if adequate tumor tissue was available from existing specimens or by rebiopsy.

Patients were at least age 20 years, had an Eastern Cooperative Oncology Group performance status (ECOG PS) of 0 or 1, and a life expectancy of at least 3 months. Patients had to have either pathologically or cytologically confirmed stage IIIB to IV adenocarcinoma, with at least one tumor lesion measurable by computed tomography or magnetic resonance imaging. Patients who were incurable with radiotherapy and had received at least one, but not more than two, lines of chemotherapy (including at least one platinum-based regimen) were eligible. Following initial clinical benefit from chemotherapy, eligible patients should have had radiographically confirmed progression according to RECIST 1.0 following at least 12 weeks of erlotinib and/or gefitinib treatment. However, they should not have received either of these drugs within 2 weeks of starting afatinib nor should they have received any other investigational drug within 4 weeks before enrollment. Thoracic radiotherapy was not permitted nor was any radiotherapy permitted within 4 weeks before enrollment.

Patients were excluded if they had gastrointestinal disorders with diarrhea as a major symptom, significant cardiovascular disease, serious drug hypersensitivity, coelomic fluid retention, uncontrolled concomitant diseases, inadequate baseline organ function, additional significant malignancies diagnosed within the past 5 years, and brain tumors and/or brain metastases (symptomatic or requiring treatment).

Treatment

Patients received a single daily oral dose of afatinib at a starting dose of 50 mg 1 hour before food until progressive disease (PD), withdrawal of consent, or withdrawal due to adverse events (AEs). If patients experienced any grade $\geq$ 3 drug-related AE, as assessed by the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 3.0 or grade 2 diarrhea, nausea, or vomiting for $\geq$ 7 consecutive days despite appropriate supportive care, afatinib was stopped for up to 14 days. Following this and recovery to a grade $\leq$ 1 AE or baseline (whichever was higher), afatinib could be restarted with the dose reduced by 10 mg; this dose reduction could be repeated a second time. However, after a third occurrence, afatinib was discontinued. Treatment of tumor-related symptoms and AEs by medications such as antidiarrheals, antibiotics, analgesics, and antiemetics was allowed.

Efficacy Assessments

Baseline tumor assessments used computed tomography or magnetic resonance imaging scans of one to 10 target lesions at the initial screening. Patients who received at least one dose of afatinib and who had baseline disease measurable by RECIST were included in the efficacy analysis. ORR was measured by monitoring the same target lesions at 4, 8, and 12 weeks following the initial treatment and then every 8 weeks thereafter until study end. Patients were assigned by best response to one of the following RECIST categories: CR, PR, SD, or PD. Patients experiencing a CR or PR lasting for more than 4 weeks were defined as those with an ORR, whereas clinical benefit also included patients experiencing SD, which must have been observed after at least 6 weeks on the study. All imaging data were independently reviewed by a separate central evaluation committee, which consisted of two independent radiologists and a specialist for chest diseases, none of whom were involved in the study.

Safety and Tolerability Assessments

AEs defined by NCI-CTCAE version 3.0 were assessed during and after afatinib treatment.

Mutation Analyses

Molecular marker studies were performed on the majority of baseline primary tumors (by using tissue or serum samples or pleural effusion specimens). Only two tumor samples (pleural effusion specimen and tumor tissue) underwent rebiopsy at the time of disease progression with prior EGFR TKIs. At the central laboratory, tumor and serum samples were analyzed by the Scorpion amplification refractory mutation system method. By using tumor samples, K-ras codon 12/13 and exons 18 to 21 in the tyrosine kinase domain of the EGFR were analyzed by the direct sequencing method if there was a sufficient volume of DNA.

Acquired Resistance Criteria

Acquired resistance to erlotinib and/or gefitinib was defined by using the Jackman criteria: (1) being *EGFR* mutation positive, (2) having CR/PR to erlotinib and/or gefitinib or SD for at least 6 months with erlotinib and/or gefitinib, (3) receiving no erlotinib and/or gefitinib for less than 4 weeks, and (4) receiving no intervening chemotherapy.[15]

Statistical Analyses

A planned analysis (September 15, 2010) was performed 36 weeks after the initiation of afatinib treatment in the last entered patient, and a second planned analysis was done (February 14, 2011) to include mature efficacy data based on the independent review. A sample size of 60 patients was required to provide 94% power to detect statistically significant evidence of afatinib activity based on the assumption that the true response rate was $\geq$ 10%. The null hypothesis was a $\leq$ 1% ORR using an exact binomial test with a one-sided significance level of 0.025. Patients documented as having taken at least one dose of afatinib who had at least one response assessment were included in the primary analysis. Median PFS and OS calculations used Kaplan-Meier methods, and 95% CIs were calculated by using Greenwood's SE estimates.

RESULTS

Patient Population

Between June 16, 2009, and February 14, 2011, at 20 sites across Japan, 62 patients were entered onto the trial and received at least one dose of afatinib. At the second planned analysis, 58 patients (93.5%) had discontinued treatment because of PD (64.5%), AEs (25.8%), and

Table 1. Patient Demographics and Baseline Characteristics

Characteristic	Afatinib No.	Afatinib %
No. of patients	62	100
Sex		
Male	14	22.6
Female	48	77.4
Age, years		
Median	65.0	
Range	33-84	
Baseline ECOG PS		
0	29	46.8
1	33	53.2
Smoking history		
Never-smokers	43	69.4
< 15 pack-years and stopped > 1 year before diagnosis	7	11.3
Current or other ex-smoker	12	19.4
Clinical stage at screening		
IIIB	5	8.1
IV	57	91.9
EGFR mutation test*	56	90.3
Positive	45	72.6
Exon 19 deletion	22	35.5
Exon 19 deletion + L858R	1	1.6
Exon 19 deletion + T790M	1	1.6
Exon 19 deletion + other	1	1.6
L858R	15	24.2
L858R + T790M	1	1.6
L858R + other	3	4.8
L861Q	1	1.6
Negative	11	17.7
EGFR mutation unknown	6	9.7
No. of previous chemotherapy regimens		
1	52	83.9
2	10	16.1
Other previous anticancer therapies		
Surgery	15	24.2
Radiotherapy	21	33.9
Other	1	1.6
Best response to previous EGFR TKI		
CR	2	3.2
PR	38	61.3
SD	22	35.5
Previous EGFR TKIs		
Erlotinib only	7	11.3
Gefitinib only	49	79.0
Both erlotinib and gefitinib	6	9.7
Duration of previous EGFR TKI, weeks		
12 to < 24	3	4.8
24 to < 36	10	16.1
36 to < 48	13	21.0
≥ 48	36	58.1
Interval from discontinuation of EGFR TKI to start of afatinib, weeks		
< 4	52	83.9
4 to < 8	7	11.3
8 to < 12	2	3.2
≥ 12	1	1.6
Patients fulfilling Jackman et al[15] criteria of acquired resistance to prior EGFR TKI	51	82.3

Abbreviations: CR, complete response; ECOG, Eastern Cooperative Oncology Group; EGFR, epidermal growth factor receptor; PR, partial response; PS, performance status; SD, stable disease; TKI, tyrosine kinase inhibitor.
*Tumor tissue, pleural effusion specimens, or serum samples tested locally and/or by central laboratory.

refusal to continue treatment (3.2%). Four patients (6.5%) were continuing treatment and, as of February 8, 2012, one patient was still receiving afatinib. Mean total treatment time was 4.59 months (maximum treatment time, 16.3 months) for all 62 patients.

Patient demographics and baseline characteristics are provided in Table 1. The majority of patients were female (77.4%), 46.8% had an ECOG PS of 0, and 69.4% were never-smokers. Mutation testing was performed on 56 patients (90.3%), and 45 (72.6%) were determined to be *EGFR* mutation positive in their primary tumor according to local and/or central laboratory analyses (Appendix Table A1, online only). Acquired T790M was reported as a mutation sequence code in two patients (3.2%). No *KRAS* mutations were found among 12 patients with tissue sample test results.

The majority of patients (79.0%) had previously received gefitinib, 11.3% had received erlotinib, and 9.7% had received both. Patients had been on previous EGFR TKIs for a median of 57.5 weeks, and 95.2% had been on previous EGFR TKIs for at least 24 weeks. Approximately two thirds of patients (64.5%) had a response (PR/CR) to prior EGFR TKI therapy. The median interval from EGFR TKI discontinuation to afatinib treatment initiation was 3 weeks (range, 2 to 13 weeks). Fifty-one patients (82.3%) met the Jackman definition of having acquired resistance to erlotinib and/or gefitinib.

Antitumor Activity

Sixty-one patients were evaluable for tumor response (Table 2); one was excluded because of lack of evaluable tumor imaging data. Of 61 evaluable patients, five (8.2%; 95% CI, 2.7% to 18.1%) achieved a confirmed response, all of which were PRs, and 35 (57.4%) had SD for at least 6 weeks, with a DCR of 65.6% by independent review. Most responses were seen within 8 weeks of afatinib initiation. The mean duration of response was 24.4 weeks. Afatinib reduced the size of target lesions in 79% of all patients during the treatment period (Fig 1), with nine patients (16%) having at least a 30% reduction in tumor size. However, tumor size reduction did not last for more than 4 weeks in four of nine patients.

Median PFS was 4.4 months (95% CI, 2.8 to 4.6 months) by independent review (Fig 2A). The PFS data were mature, with 72.1% of patients having a PFS event at the time of the second planned analysis. Median OS was 19.0 months (95% CI, 14.9 months to not

Table 2. Overview of Response Rate by Independent Review

Response	Response Rate No.	Response Rate %	95% CI
Total No. of patients	61	100*	
DCR (CR, PR, or SD)	40	65.6	52.3 to 77.3
ORR (CR or PR)	5	8.2	2.7 to 18.1
CR	0	0.0	—
PR	5	8.2	—
SD	35	57.4	—
PD	17	27.9	—
Not evaluable	4	6.6	—

Abbreviations: CR, complete response; DCR, disease control rate; ORR, objective response rate; PD, progressive disease; PR, partial response; SD, stable disease.
*One patient with exon 19 deletion was excluded from the efficacy evaluation because of lack of evaluable tumor imaging data after the start of afatinib treatment.

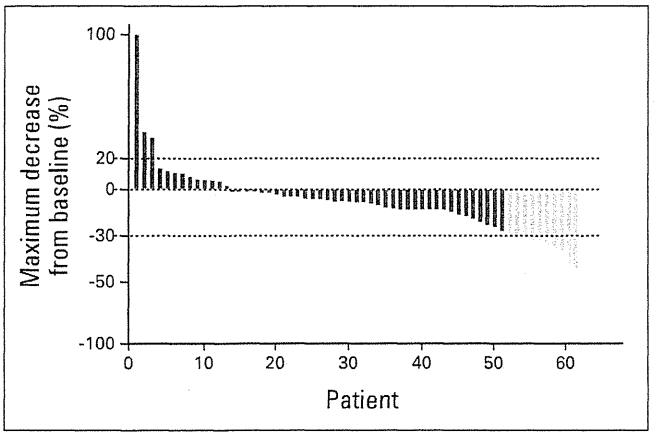

Fig 1. Waterfall plot of percent change from baseline in measurable tumor at the time of best response (by independent review). Data for patients with a decrease from baseline of 30% or more are shown in gold; data for patients with an increase from baseline of more than 100% to a decrease from baseline of less than 30% are shown in blue.

achieved; Fig 2B), with the probability of survival at 12 months estimated to be 73.0%; in addition, 34.4% of patients had an OS event. As of February 9, 2012, median OS was 18.4 months, and 63.9% of patients had an OS event.

Subgroup analysis of the efficacy data based on sex (women *v* men), ECOG PS (0 *v* 1), type of prior EGFR TKI (erlotinib *v* gefitinib), and the number of previous chemotherapy regimens (one *v* two) showed little variation in ORRs and DCRs (Appendix Table A2, online only). Efficacy data by mutation type were also similar among deletions in exon 19 (del19), L858R, and others (Table 3).

Patients meeting the Jackman criteria for acquired resistance had a median PFS of 4.4 months, PR of 5.9%, and DCR of 68.6%. Of the two patients with T790M mutations who underwent rebiopsy at the time of disease progression with prior EGFR TKI therapy, one patient harboring an L858R + T790M mutation had durable SD for 9 months, and the other patient with a del19 + T790M mutation had SD for 1 month. In *EGFR* mutation–negative patients, the ORR was 27% (three of 11), which was higher than in *EGFR* mutation–positive (4.5%; two of 44) or mutation-unknown (0%; zero of six) patients.

Safety and Tolerability

All 62 patients experienced an AE, with diarrhea and skin events being the most frequently reported (Table 4). Diarrhea occurred in all 62 patients, rash/acne in 57 patients (91.9%), and stomatitis in 53 patients (85.5%). Grade 3 diarrhea occurred in 37.1% of patients, and rash/acne occurred in 27.4% of patients. Loperamide use was capped at 8 mg per day for treatment of diarrhea (90.3% of patients received loperamide), and less than 10% of patients received systemic antibiotics for rash.

All patients received a starting dose of afatinib 50 mg per day, with 69.4% of patients requiring dose reduction to 40 mg per day, and 35.5% requiring further dose reduction to 30 mg per day. The most common AE leading to dose reduction was diarrhea, affecting 41.9% of patients. Treatment-related AEs leading to discontinuation of afatinib were experienced by 18 patients (29.0%) and were due to rash/acne (n = 7); decreased appetite (n = 3); diarrhea, interstitial lung disease, and stomatitis in two patients each; and dehydration, fatigue, nail effects, and pyrexia in one patient each. Four of these patients (three with rash, one with paronychia) had PD confirmed by tumor assessments at the same time as afatinib discontinuation due to AEs. Drug-related serious AEs occurred in 11.3% of patients, with diarrhea (6.5%) being the most common. Two interstitial lung disease-like AEs (grade 3 and grade 1) were considered to be related to study drug; in each case, the patient fully recovered after stopping afatinib. One on-treatment death as a result of hypoxia occurred after disease progression, which was not considered by the investigator to be drug related.

DISCUSSION

There is an increasing need to develop new molecular targeted agents that address the issue of resistance to erlotinib and/or gefitinib in patients with NSCLC who initially respond to treatment and then subsequently progress.[16] Previous phase II studies with criteria similar

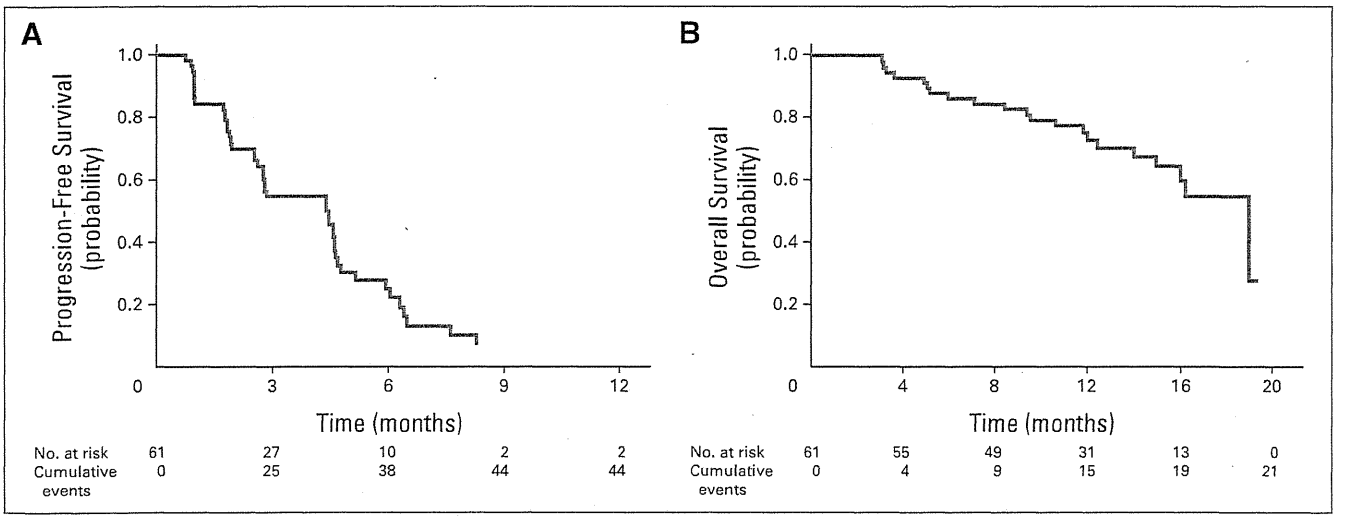

Fig 2. Kaplan-Meier plot of (A) progression-free survival by independent review and (B) median overall survival.

Table 3. Overview of DCR, ORR, and PFS by Mutation Type

	EGFR Mutation Type											
	Exon 19 Deletion				L858R				Other			
Response	No.	%	95% CI	Percentile	No.	%	95% CI	Percentile	No.	%	95% CI	Percentile
Total No. of patients	21	100*			15	100			8	100.0		
DCR (CR, PR, or SD)	14	66.7	43.0 to 85.4		10	66.7	38.4 to 88.2		5	62.5	24.5 to 91.5	
ORR (CR or PR)	1	4.8	0.1 to 23.8		1	6.7	0.2 to 31.9		0	0.0	0	
Median PFS, months	1.9			25th	1.9			25th	1.3			25th
	4.6				3.6				3.7			
	5.2			75th	5.3			75th	8.3			75th

Abbreviations: CR, complete response; DCR, disease control rate; EGFR, epidermal growth factor receptor; ORR, objective response rate; PFS, progression-free survival; PR, partial response; SD, stable disease.
*One patient who had an exon 19 deletion was excluded from the efficacy evaluation because of lack of evaluable tumor imaging data after the start of afatinib treatment.

to that of the current LUX-Lung 4 trial with prior failure of erlotinib and/or gefitinib and an enrichment strategy for patients with *EGFR* mutations by using XL-647, dasatinib, neratinib, and the combination of cetuximab plus erlotinib showed low ORR ranging from 0% to 3%.[17-20] The results of our trial demonstrated modest but noteworthy activity of afatinib in this difficult-to-treat population, with a median PFS of 4.4 months and an ORR of 8.2% (independent review).

As might be expected for a group of patients with NSCLC who derived significant benefit from prior erlotinib and/or gefitinib therapy, the study population was highly enriched (85%) for patients with *EGFR* mutations. This was further reflected in the patient demographics, with a large percentage of women and never-smokers. The trial was also highly enriched (82%) for patients meeting the Jackman criteria of acquired resistance, and the efficacy findings were similar in that subpopulation compared with the overall study population, with similar PFS results for the Jackman group of patients shown in LUX-Lung 1.[21] In the LUX-Lung 1 double-blind, placebo-controlled phase IIB/III study of afatinib plus best supportive care in patients with NSCLC who had progressed after prior chemotherapy and erlotinib and/or gefitinib treatment, a median PFS of 4.5 months was reported in those patients fulfilling the Jackman criteria for acquired resistance, which is consistent with the median PFS of 4.4 months reported in this trial.[21]

The estimated median OS of 19 months observed in this trial is of interest. However, nearly half the patients entering this trial were symptom-free with an ECOG PS of 0, and 72.6% had an EGFR-mutant tumor, suggesting the selection of a relatively good prognostic cohort despite their extensive pretreatment.

The Jackman criteria of acquired resistance to EGFR TKIs were fulfilled by 82% of the patients in this trial. The efficacy of afatinib in this subgroup of patients suggests that the clinical effect of afatinib is not merely due to re-exposure to another EGFR TKI, a phenomenon that was previously reported.[22] Although the literature reports that approximately 50% of the patients who develop acquired resistance to EGFR TKIs show secondary T790M mutation,[23] a relatively low incidence of T790M mutations was observed in this study. This may be due to the fact that tissue sampling was obtained before erlotinib and/or gefitinib exposure, and very few patients underwent rebiopsy.

The AEs observed in this phase II trial were consistent with the known safety profile reported for inhibitors of EGFR.[6] All patients experienced an AE considered to be drug related, with diarrhea, rash/acne, and stomatitis being the most common AEs. AEs were mostly managed by dose reduction and/or medical treatment. The rates of grade 3 diarrhea and rash/acne reported in this trial were similar to those of the LUX-Lung 2 phase II trial, in which a large proportion of patients (87%) were Asian.[24] In LUX-Lung 2 (afatinib 50 mg per day in first- and second-line patients whose tumors harbored *EGFR* mutations), diarrhea and rash/acne occurred in 94% of patients, with grade 3 diarrhea reported in 22% of patients and grade 3 rash/acne in 28% of patients.[24] The frequency and severity of AEs and treatment discontinuation due to AEs appears to be higher with afatinib compared with the historical data reported with erlotinib and gefitinib.[2-7,25] However, the early and proactive management of AEs, including dose

Table 4. All AEs for All Grades and NCI-CTCAE Grade 3 in ≥ 10% of Patients

	All Grades		Grade 3	
Preferred Term	No.	%	No.	%
No. of patients	62	100.0	62	100.0
Total with AEs	62	100.0	49	79.0
Diarrhea	62	100.0	23	37.1
Rash/acne	57	91.9	17	27.4
Stomatitis	53	85.5	6	9.7
Nail effect	43	69.4	7	11.3
Decreased appetite	38	61.3	3	4.8
Fatigue	25	40.3	5	8.1
Nausea	23	37.1	1	1.6
Vomiting	17	27.4	1	1.6
Weight decreased	17	27.4	0	0.0
Epistaxis	16	25.8	0	0.0
Lip effect	16	25.8	0	0.0
Ocular event	15	24.2	1	1.6
Dry skin	14	22.6	0	0.0
Dysgeusia	11	17.7	0	0.0
Dehydration	9	14.5	5	8.1
Nasal inflammation	8	12.9	0	0.0
Nasopharyngitis	7	11.3	0	0.0

NOTE. For all adverse events (AEs) listed, no grade 4 or grade 5 events occurred.
Abbreviations: AE, adverse event; NCI-CTCAE, National Cancer Institute–Common Terminology Criteria for Adverse Events.

reduction and the use of additional symptomatic therapies, could have been effective in our study, allowing patients who benefited from afatinib to continue on treatment as observed in the LUX-Lung 1 trial (afatinib 50 mg was the starting dose).[21] Proactive supportive management also has the potential to maintain quality of life by reducing the impact of AEs.

On the basis of the modest but noteworthy activity of afatinib observed in this trial in patients with NSCLC who have acquired resistance to erlotinib and/or gefitinib, additional studies to improve on the activity of afatinib in this setting are ongoing. In preclinical T790M tumor models, combined EGFR targeting with afatinib and cetuximab induced near CRs that were not seen with either agent alone or with a cetuximab plus erlotinib combination.[26] On the basis of these early observations, a phase IB trial is currently testing the combination of afatinib and cetuximab in a patient population similar to that of LUX-Lung 4. Preliminary results have shown that more than 90% of patients thus far have derived clinical benefit, including approximately 40% ORR in both T790M-positive and T790M-negative settings.[27,28]

To extend the investigation of afatinib in advanced NSCLC, the ongoing LUX-Lung 3 and LUX-Lung 6 randomized phase III studies are comparing the efficacy of first-line afatinib monotherapy with cisplatin and either pemetrexed or gemcitabine in white and Asian patients with NSCLC who are harboring *EGFR* mutations. Initial results from LUX-Lung 3 demonstrated a significant improvement in PFS of 11.1 months with afatinib compared with 6.9 months for chemotherapy.[29]

In conclusion, this phase II study conducted in Japan in a study population with NSCLC enriched for *EGFR* mutations showed modest but noteworthy efficacy of oral afatinib, an irreversible ErbB family blocker, in third- and fourth-line patients with NSCLC with acquired resistance to erlotinib and/or gefitinib. Further evaluation of the potential of afatinib in patients with advanced NSCLC will be addressed by the LUX-Lung phase III clinical trial program and the ongoing study of the afatinib plus cetuximab combination in the resistance setting.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) and/or an author's immediate family member(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

Employment or Leadership Position: Yoko Seki, Boehringer Ingelheim (C); Ryuichi Ebisawa, Boehringer Ingelheim (C); Mehdi Shahidi, Boehringer Ingelheim (C) **Consultant or Advisory Role:** Koichi Goto, Taiho Pharmaceutical (C), Ono Pharmaceutical (C); Akira Inoue, Boehringer Ingelheim (C); Nobuyuki Yamamoto, Boehringer Ingelheim (C) **Stock Ownership:** None **Honoraria:** Koichi Goto, Chugai Pharmaceutical, Ono Pharmaceutical; Kunihiko Koboyashi, AstraZeneca, Chugai Pharmaceutical, Taiho Pharmaceutical; Katsuyuki Kiura, AstraZeneca, Chugai Pharmaceutical, Pfizer **Research Funding:** Yukito Ichinose, Boehringer Ingelheim; Katsuyuki Kiura, Boehringer Ingelheim **Expert Testimony:** None **Other Remuneration:** None

AUTHOR CONTRIBUTIONS

Conception and design: Nobuyuki Katakami, Kazuto Nishio, Yoko Seki, Ryuichi Ebisawa, Mehdi Shahidi, Nobuyuki Yamamoto
Collection and assembly of data: Koichi Goto, Toyoaki Hida, Takeshi Horai, Akira Inoue, Yukito Ichinose, Kunihiko Koboyashi, Koji Takeda, Katsuyuki Kiura, Yoko Seki, Nobuyuki Yamamoto
Data analysis and interpretation: Nobuyuki Katakami, Shinji Atagi, Yoko Seki, Ryuichi Ebisawa, Mehdi Shahidi, Nobuyuki Yamamoto
Manuscript writing: All authors
Final approval of manuscript: All authors

REFERENCES

1. Shigematsu H, Lin L, Takahashi T, et al: Clinical and biological features associated with epidermal growth factor receptor gene mutations in lung cancers. J Natl Cancer Inst 97:339-346, 2005
2. Mok TS, Wu YL, Thongprasert S, et al: Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med 361:947-957, 2009
3. Mitsudomi T, Morita S, Yatabe Y, et al: Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): An open label, randomised phase 3 trial. Lancet Oncol 11:121-128, 2010
4. Maemondo M, Inoue A, Kobayashi K, et al: Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. N Engl J Med 362:2380-2388, 2010
5. Zhou C, Wu YL, Chen G, et al: Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): A multicentre, open-label, randomised, phase 3 study. Lancet Oncol 12:735-742, 2011
6. Ku GY, Haaland BA, de Lima Lopes G Jr: Gefitinib vs. chemotherapy as first-line therapy in advanced non-small cell lung cancer: Meta-analysis of phase III trials. Lung Cancer 74:469-473, 2011
7. Rosell R, Carcereny E, Gervais R, et al: Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): A multicentre, open-label, randomised phase 3 trial. Lancet Oncol 13:239-246, 2012
8. Kosaka T, Yatabe Y, Endoh H, et al: Analysis of epidermal growth factor receptor gene mutation in patients with non-small cell lung cancer and acquired resistance to gefitinib. Clin Cancer Res 12:5764-5769, 2006
9. Balak MN, Gong Y, Riely GJ, et al: Novel D761Y and common secondary T790M mutations in epidermal growth factor receptor-mutant lung adenocarcinomas with acquired resistance to kinase inhibitors. Clin Cancer Res 12:6494-6501, 2006
10. Li D, Ambrogio L, Shimamura T, et al: BIBW2992, an irreversible EGFR/HER2 inhibitor highly effective in preclinical lung cancer models. Oncogene 27:4702-4711, 2008
11. Solca F, Dahl G, Zoephel A, et al: Target binding properties and cellular activity of afatinib (BIBW 2992), an irreversible ErbB family blocker. J Pharmacol Exp Ther 343:342-350, 2012
12. Yap TA, Vidal L, Adam J, et al: Phase I trial of the irreversible EGFR and HER2 kinase inhibitor BIBW 2992 in patients with advanced solid tumors. J Clin Oncol 28:3965-3972, 2010
13. Murakami H, Tamura T, Takahashi T, et al: 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). Cancer Chemother Pharmacol 69:891-899, 2012
14. Therasse P, Arbuck SG, Eisenhauer EA, et al: New guidelines to evaluate the response to treatment in solid tumors: European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst 92:205-216, 2000
15. Jackman D, Pao W, Riely GJ, et al: Clinical definition of acquired resistance to epidermal growth factor receptor tyrosine kinase inhibitors in non-small-cell lung cancer. J Clin Oncol 28:357-360, 2010
16. Pao W, Chmielecki J: Rational, biologically based treatment of EGFR-mutant non-small-cell lung cancer. Nat Rev Cancer 10:760-774, 2010

17. Pietanza MC, Lynch TJ Jr, Lara PN Jr, et al: XL647: A multitargeted tyrosine kinase inhibitor—Results of a phase II study in subjects with non-small cell lung cancer who have progressed after responding to treatment with either gefitinib or erlotinib. J Thorac Oncol 7:219-226, 2012

18. Johnson ML, Riely GJ, Rizvi NA, et al: Phase II trial of dasatinib for patients with acquired resistance to treatment with the epidermal growth factor receptor tyrosine kinase inhibitors erlotinib or gefitinib. J Thorac Oncol 6:1128-1131, 2011

19. Janjigian YY, Azzoli CG, Krug LM, et al: Phase I/II trial of cetuximab and erlotinib in patients with lung adenocarcinoma and acquired resistance to erlotinib. Clin Cancer Res 17:2521-2527, 2011

20. Sequist LV, Besse B, Lynch TJ, et al: Neratinib, an irreversible pan-ErbB receptor tyrosine kinase inhibitor: Results of a phase II trial in patients with advanced non-small-cell lung cancer. J Clin Oncol 28:3076-3083, 2010

21. Miller VA, Hirsh V, Cadranel J, et al: Afatinib versus placebo for patients with advanced, metastatic non-small-cell lung cancer after failure of erlotinib, gefitinib, or both, and one or two lines of chemotherapy (LUX-Lung 1): A phase 2b/3 randomised trial. Lancet Oncol 13:528-538, 2012

22. Riely GJ, Kris MG, Zhao B, et al: Prospective assessment of discontinuation and reinitiation of erlotinib or gefitinib in patients with acquired resistance to erlotinib or gefitinib followed by the addition of everolimus. Clin Cancer Res 13:5150-5155, 2007

23. Workman P, Clarke PA: Resisting targeted therapy: Fifty ways to leave your EGFR. Cancer Cell 19:437-440, 2011

24. Yang JC, Shih JY, Su WC, et al: Afatinib for patients with lung adenocarcinoma and epidermal growth factor receptor mutations (LUX-Lung 2): A phase 2 trial. Lancet Oncol 13:539-548, 2012

25. Lee J, Park K, Kim S-W: A randomized phase III study of gefinitib (IRESSATM) versus standard chemotherapy (gemcitabine plus cisplatin) as a first-line treatment for never smokers with advanced or metastatic adenocarcinoma of the lung. Presented at the 13th World Conference on Lung Cancer, San Francisco, CA, July 31-Aug 4, 2009

26. Regales L, Gong Y, Shen R, et al: Dual targeting of EGFR can overcome a major drug resistance mutation in mouse models of EGFR mutant lung cancer. J Clin Invest 119:3000-3010, 2009

27. Horn L, Groen H, Smit E, et al: Activity and tolerability of combined EGFR targeting with afatinib (BIBW 2992) and cetuximab in T790M+ NSCLC patients. Presented at the 14th World Conference on Lung Cancer, Amsterdam, the Netherlands, July 3-7, 2011 (abstr O19.07)

28. Janjigian Y, Groen H, Horn L: Activity and tolerability of afatinib (BIBW 2992) and cetuximab in NSCLC patients with acquired resistance to erlotinib or gefitinib. J Clin Oncol 29:482s, 2011 (suppl; abstr 7525)

29. Yang JC-H, Schuler MH, Yamamoto N, et al: LUX-Lung 3: A randomized, open-label, phase III study of afatinib versus pemetrexed and cisplatin as first-line treatment for patients with advanced adenocarcinoma of the lung harboring EGFR-activating mutations. J Clin Oncol 30:480s, 2012 (suppl; abstr LBA7500)

Appendix

Table A1. Results of *EGFR* Mutation Testing Performed in Either a Local or Central Laboratory

	EGFR Mutation Test							
	Central Laboratory Using Tissue and Pleural Effusion Specimens		Central Laboratory Using Serum Samples		Local Laboratory*		Central or Local Laboratory†	
Mutation	No.	%	No.	%	No.	%	No.	%
No. of patients treated	62		62		62		62	
No. of patients with *EGFR* mutation test results	27	43.5	45	72.6	37	59.7	56	90.3
Positive	23	85.2	3	6.7	37	100.0	45	72.6
Exon 19 deletion	10	37.0	2	4.4	18‡	48.6	22‡	35.5
Exon 19 deletion + L858R	0	0.0	0	0.0	1	2.7	1	1.6
Exon 19 deletion + T790M	1	3.7	0	0.0	0	0.0	1	1.6
Exon 19 deletion + other	1	3.7	0	0.0	0	0.0	1	1.6
L858R	8	29.6	1	2.2	16	43.2	15	24.2
L858R + T790M	0	0.0	0	0.0	1	2.7	1	1.6
L858R + other	3	11.1	0	0.0	0	0.0	3	4.8
L861Q	0	0.0	0	0.0	1	2.7	1	1.6
Negative	4	14.8	42	93.3	0	0.0	11	17.7

Abbreviation: EGFR, epidermal growth factor receptor.
*Information on sample/specimen type unavailable.
†Results using tissue, pleural effusion specimens, or serum samples. If multiple data were available for a patient, positive data and/or more detailed data were selected.
‡Included one patient who was excluded from the efficacy analysis because the patient had no evaluable tumor imaging data after the start of afatinib treatment.

Table A2. Summary of DCR and ORR by Sex, ECOG PS, Previous Chemotherapy Regimens, and Type of Prior EGFR TKI

	DCR			ORR		
Variable	No.	%	95% CI	No.	%	95% CI
Sex						
Male (n = 14)	10	71.4	41.9 to 91.6	1	7.1	0.2 to 33.9
Female (n = 47)	30	63.8	48.5 to 77.3	4	8.5	2.4 to 20.4
Baseline ECOG PS						
0 (n = 29)	20	69.0	49.2 to 84.7	2	6.9	0.8 to 22.8
1 (n = 32)	20	62.5	43.7 to 78.9	3	9.4	2.0 to 25.0
No. of previous chemotherapy regimens						
1 (n = 51)	31	60.8	46.1 to 74.2	4	7.8	2.2 to 18.9
2 (n = 10)	9	90.0	55.5 to 99.7	1	10.0	0.3 to 44.5
Prior use of EGFR TKI						
Erlotinib (n = 7)	4	57.1	18.4 to 90.1	1	14.3	0.4 to 57.9
Gefitinib (n = 48)	32	66.7	51.6 to 79.6	4	8.3	2.3 to 20.0
Erlotinib and gefitinib (n = 6)	4	66.7	22.3 to 95.7	0		0

Abbreviations: DCR, disease control rate; ECOG, Eastern Cooperative Oncology Group; EGFR, epidermal growth factor receptor; ORR, objective response rate; PS, performance status; TKI, tyrosine kinase inhibitor.

Original Article

Phase 2 Study of S-1 and Carboplatin Plus Bevacizumab Followed by Maintenance S-1 and Bevacizumab for Chemotherapy-Naive Patients With Advanced Nonsquamous Non–Small Cell Lung Cancer

Yoshiko Urata, MD, PhD[1]; Isamu Okamoto, MD, PhD[2]; Masayuki Takeda, MD, PhD[3]; Yoshihiro Hattori, MD[1]; Keiko Okuno, MD[1]; Temiko Shimada, MD, PhD[1]; Takayasu Kurata, MD, PhD[2]; Hiroyasu Kaneda, MD, PhD[2]; Masaki Miyazaki, MD, PhD[2]; Masaaki Terashima, MD, PhD[2]; Kaoru Tanaka, MD, PhD[2]; Satoshi Morita, PhD[4]; Kazuhiko Nakagawa, MD, PhD[2]; Shunichi Negoro, MD[1]; and Miyako Satouchi, MD, PhD[1]

BACKGROUND: A previous phase 3 trial demonstrated noninferiority in terms of overall survival for combined S-1 (an oral fluoropyrimidine) and carboplatin compared with combined paclitaxel and carboplatin as first-line treatment for advanced non–small cell lung cancer (NSCLC). In the current study, the authors evaluated the efficacy and safety of combined S-1, carboplatin, and bevacizumab followed by maintenance with S-1 and bevacizumab in chemotherapy-naive patients with advanced nonsquamous NSCLC. **METHODS:** Patients received carboplatin (area under the concentration-time curve, 5 mg mL^{-1} per minute) and bevacizumab (15 mg/kg) on day 1 plus oral S-1 (80 mg/m^2 per day) on days 1 through 14 every 21 days for up to 6 cycles. For patients without disease progression, S-1 and bevacizumab were continued until disease progression or unacceptable toxicity developed. **RESULTS:** Forty-eight patients were enrolled in the phase 2 study; of these, 35 patients (72.9%) completed at least 4 cycles. Most toxicities of grade ≥3 were hematologic, and no increase in relative incidence associated with maintenance with S-1 and bevacizumab was observed. The objective response rate was 54.2% (95% confidence interval, 39.2%-68.6%), and the median progression-free survival was 6.8 months (95% confidence interval, 4.3-8.2 months). **CONCLUSIONS:** The regimen of combined S-1, carboplatin, and bevacizumab followed by maintenance with S-1 and bevacizumab was active and feasible as first-line treatment for advanced nonsquamous NSCLC. *Cancer* **2013;119:2275-81.**

KEYWORDS: bevacizumab; carboplatin; chemotherapy; maintenance; non–small cell lung cancer; S-1.

INTRODUCTION

Lung cancer is the leading cause of death related to cancer worldwide, with non–small cell lung cancer (NSCLC) accounting for 85% of lung cancer cases.[1] For individuals with advanced or metastatic NSCLC, platinum-based chemotherapy is the mainstay of first-line treatment[2,3] on the basis of the moderate improvement in survival and quality of life it affords compared with best supportive care alone.[4]

A phase 3 study, the Eastern Cooperative Oncology Group (ECOG) E4599 trial, demonstrated that bevacizumab, a humanized monoclonal antibody specific for vascular endothelial growth factor,[5] given with paclitaxel and carboplatin resulted in significant improvements in the overall response rate (ORR), progression-free survival (PFS), and overall survival (OS) compared with paclitaxel and carboplatin alone in individuals with advanced nonsquamous NSCLC.[6] A Japanese phase 2 study also indicated that bevacizumab in combination with paclitaxel and carboplatin improved the ORR and PFS compared with paclitaxel and carboplatin alone.[7] In a confirmatory phase 3 study (the Avastin in Lung [AVAiL] trial), the addition of bevacizumab to cisplatin and gemcitabine resulted in a significant improvements in the ORR and PFS.[8,9] These observations provide a rationale for combining bevacizumab with platinum-doublet chemotherapy in individuals with advanced nonsquamous NSCLC.

Corresponding author: Miyako Satouchi, MD, PhD, Department of Thoracic Oncology, Hyogo Cancer Center, 13-70 Kitaoji-cho, Akashi City, Hyogo 673-8558, Japan; Fax: (011) 81-78-929-2387; satouchi@hp.pref.hyogo.jp

[1]Department of Thoracic Oncology, Hyogo Cancer Center, Akashi, Japan; [2]Department of Medical Oncology, Kinki University Faculty of Medicine, Osaka-Sayama, Japan; [3]Department of Medical Oncology, Kishiwada City Hospital, Kishiwada, Japan; [4]Department of Biostatistics and Epidemiology, Yokohama City University Medical Center, Yokohama, Japan.

We thank all participating sites and investigators for their support with this clinical study.

DOI: 10.1002/cncr.28048, **Received:** October 23, 2012; **Revised:** January 5, 2013; **Accepted:** January 11, 2013, **Published online** April 4, 2013 in Wiley Online Library (wileyonlinelibrary.com)

S-1 (TS-1; Taiho Pharmaceutical Company Ltd., Tokyo, Japan) is an oral fluoropyrimidine agent that consists of tegafur (a prodrug of 5-fluorouracil), 5-chloro-2,4-dihydroxypyridine, and potassium oxonate.[10] It has demonstrated activity and acceptable tolerability in phase 2 studies of patients with advanced NSCLC.[11] Our previous phase 3 study demonstrated the noninferiority in terms of OS as well as a favorable toxicity profile for S-1 and carboplatin compared with standard paclitaxel-carboplatin as first-line treatment for patients with advanced NSCLC.[12] Thus, our results indicated that the combination of S-1 and carboplatin is a valid option for the first-line treatment of such patients.

In addition to the enhanced efficacy of cytotoxic chemotherapy observed with bevacizumab, preclinical studies indicate that the combination of bevacizumab with 5-fluorouracil derivatives results in increased antitumor activity.[13,14] Therefore, we have now performed a multicenter phase 2 study to evaluate the efficacy and safety of S-1 and carboplatin in combination with bevacizumab followed by maintenance therapy with S-1 and bevacizumab alone in chemotherapy-naive patients with advanced nonsquamous NSCLC.

MATERIALS AND METHODS

Eligibility

Eligible patients were required be aged ≥20 years and to have histologically or cytologically confirmed stage IIIB or IV nonsquamous NSCLC (diagnosed according to the seventh edition of the International Union Against Cancer cancer staging manual) or recurrence of this condition after surgery. The patients also were required to have measurable disease as defined by the Response Evaluation Criteria in Solid Tumors (RECIST) (version 1.1); adequate hematologic, hepatic, and renal function; an ECOG performance status of 0 or 1; and no tumor invasion into the trachea, main bronchi, or major vessels. Exclusion criteria included hemoptysis (>2.5 mL of red blood per episode), radiologic evidence of tumor invasion or tumor abutting of major blood vessels, evidence of a brain metastasis >1 cm, current or recent use of full-dose anticoagulants, medically uncontrolled hypertension or a history of thrombotic or hemorrhagic disorders, interstitial pneumonia recognized on a chest x-ray, supporting radiation therapy occupying the pulmonary region in the 3 months before enrollment, and major surgery within 28 days before enrollment. The study was performed according to good clinical practices and ethical principles outlined in the Declaration of Helsinki. The study protocol was approved by the institutional review board at each participating center, and all patients signed written informed consent forms before enrollment. The trial has been registered under University Medical Hospital Information Network (UMIN) Clinical Trials Registry Identifier UMIN 000003698.

Study Design and Treatment

The study was designed as a prospective, multicenter, single-arm phase 2 trial of first-line combination therapy with S-1, carboplatin, and bevacizumab followed by continuous maintenance therapy with S-1 and bevacizumab. The primary endpoint was treatment efficacy measured as the ORR in patients who received at least 1 course of the initial combination therapy. OS, PFS, the disease control ratio, and adverse events also were evaluated as secondary endpoints.

Patients received S-1 orally at a dose of 80 mg/m^2 per day on days 1 through 14 as well as carboplatin at a dose calculated to produce an area under the concentration-time curve of 5 mg mL^{-1} per minute and bevacizumab at a dose of 15 mg/kg as an intravenous infusion on day 1. The combination therapy was repeated every 21 days for up to 4 to 6 cycles unless there was evidence of disease progression or intolerance of the study treatment. After 4 to 6 cycles of treatment with S-1, carboplatin, and bevacizumab, patients who attained a complete response (CR), a partial response (PR), or stable disease (SD) continued to receive cycles of maintenance therapy with S-1 and bevacizumab every 21 days until they had evidence of disease progression or developed unacceptable toxicity. Administration of S-1 during the maintenance phase was interrupted if patients developed grade 4 neutropenia, a platelet count <50,000/μL, a serum creatinine concentration ≥1.5 mg/dL, a serum total bilirubin concentration ≥2.0 mg/dL, a putative infection with fever of at least 38°C, or severe diarrhea or stomatitis (grade 2 or higher). Treatment with S-1 and bevacizumab was resumed if the neutrophil count was ≥1500/μL, the platelet count was ≥100,000/μL, the serum total bilirubin concentration was <1.5 mg/dL, there was no infection with fever of at least 38°C, and diarrhea or stomatitis was grade 1 or lower.

Subsequent cycles of treatment were withheld until the following criteria were satisfied: neutrophil count, ≥1500/μL; platelet count, ≥100,000/μL; performance status, 0 to 2; serum total bilirubin concentration, ≤1.5 mg/dL; weight loss, grade 2 or lower abnormal electrolytes, peripheral nerve damage, and hepatotoxicity (based on aspartate aminotransferase, alanine aminotransferase, and total bilirubin levels); grade 3 or lower

hypertension; grade 2 or lower proteinuria; grade 0 hemoptysis or bleeding; and no infection with fever of at least 38°C. The study therapy was stopped if grade 2 or higher hemoptysis developed or if bleeding persisted after treatment of hemorrhage. Patients also were to be removed from the study if the next treatment cycle had not started within 42 days of the previous dosing as a result of toxicity.

Baseline and Treatment Assessments

Baseline evaluations included medical history, physical examination, electrocardiogram, ECOG performance status, and laboratory analyses. Computed tomography (CT) scans of the chest and abdomen, magnetic resonance imaging (MRI) studies of the brain, and bone scintigraphy or positron emission tomography (PET)-CT studies were performed for tumor assessment within 28 days of initiation of the study treatment. CT scans of the chest and abdomen were repeated every 2 cycles, brain MRI studies were repeated every 3 months or on the appearance of any neurologic symptoms, and bone scintigraphy or PET-CT studies were performed every 6 months or on the appearance of any bone-related symptoms. All treatment responses were defined according to RECIST version 1.1. If a patient had a documented a CR or a PR, then the respective response had to be confirmed 4 weeks later. A patient was considered to have SD if their response was confirmed and sustained for at least 6 weeks. PFS was calculated from the date of enrollment to the date of confirmation of progressive disease or the date of death from any cause. PFS from the start of maintenance therapy was calculated by subtracting the period of the initial combination therapy from overall PFS. OS was calculated from the date of initial treatment to the date of death from any cause. For patients with unknown death status, OS was censored at the last date the patient was known to be alive. Patients were assessed for toxicity according version 4.0 of the National Cancer Institute Common Toxicity Criteria.

Statistical Analysis

We assumed that an ORR of 50% for the study regimen in eligible patients would indicate potential usefulness, whereas an ORR of 30% would be the lower limit of interest. On the basis of these assumptions, our study was designed to have a power of 80% and a 1-sided level of type I error of 0.05, resulting in a requirement for 45 patients. A Simon 2-stage design (MiniMax) was adopted. Nineteen patients were to be initially assessed for response, and if more than 6 patients manifested a PR or a CR, then 26 additional patients would be added to the assessment. Efficacy and safety analyses were planned for patients who received at least 1 cycle of the treatment. PFS and OS were analyzed using the Kaplan-Meier method to estimate the median points with 95% confidence intervals (CIs).

RESULTS

Patient Characteristics

Between April 2010 and October 2011, a total of 48 patients with recurrent or newly diagnosed, advanced nonsquamous NSCLC were enrolled at 3 participating centers. The baseline characteristics of all assigned patients are provided in Table 1. The median age for the treated patients was 65.5 years (range, 35-77 years), 33 patients (68.8%) were men, 42 patients (87.5%) had adenocarcinoma histology, 40 patients (83.3%) had stage IV disease, and 16 patients (33.3%) were never-smokers. Epidermal growth factor receptor mutation status was evaluated in 42 patients (87.5%), and 4 individuals had activating mutations identified.

Treatment Delivery

Patient disposition is illustrated in Figure 1. Overall, 35 patients (72.9%) completed at least 4 cycles of S-1 and carboplatin combined with bevacizumab, and 29 patients (60.4%) were shifted to subsequent maintenance therapy with S-1 and bevacizumab and received a median of 4 cycles (range, 1-18 cycles). In the initial combination phase, 13 of 48 patients (27.1%) experienced dose

TABLE 1. Baseline Characteristics of the Study Patients, n = 48

Characteristic	No. of Patients (%)
Age: Median [range], y	65.5 [35-77]
Sex	
Men	33 (68.8)
Women	15 (31.3)
ECOG PS	
0	15 (31.3)
1	33 (68.8)
Disease stage	
IIIB	3 (6.3)
IV	40 (83.3)
Recurrence	5 (10.4)
Histology	
Adenocarcinoma	42 (87.5)
Large cell carcinoma	1 (2.1)
Others	5 (10.4)
Smoking status	
Never-smoker	16 (33.3)
Former or current smoker	32 (66.7)

Abbreviations: ECOG PS, Eastern Cooperative Oncology Group performance status.

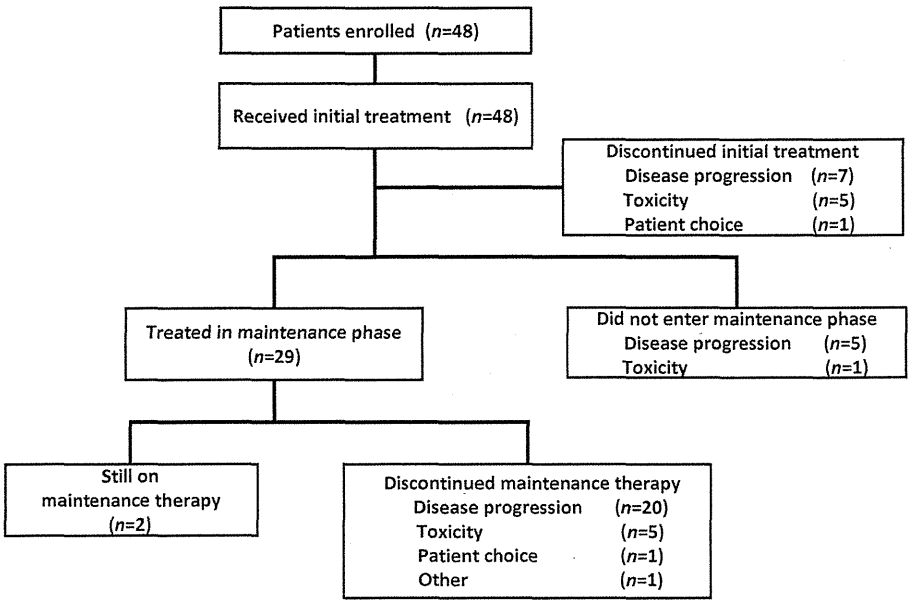

Figure 1. This is a Consolidated Standards of Reporting Trials (CONSORT) diagram for the current study.

TABLE 2. Treatment Outcomes for the Study Patients,, n = 48

Outcome	No. of Patients (%)
Complete response	0 (0)
Partial response	26 (54.2)
Stable disease	11 (22.9)
Progressive disease	11 (22.9)
Overall response rate [95% CI], %	54.2 [39.2-68.6]
Disease control rate [95% CI], %	77.1 [67.2-88]

Abbreviations: CI, confidence interval.

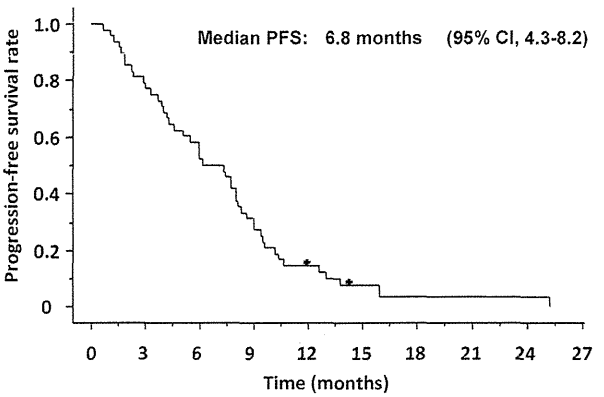

Figure 2. This Kaplan-Meier curve illustrates progression-free survival (PFS). CI indicates confidence interval.

reductions, and 34 patients (70.8%) experienced a treatment delay because of adverse events, mostly related to myelosuppression. Among the 29 patients who received maintenance therapy with S-1 and bevacizumab, 2 individuals (6.9%) underwent a dose reduction, and 20 individuals (69%) had a treatment delay.

Efficacy

Forty-eight patients were deemed eligible for evaluation of treatment response. Twenty-six patients attained a PR, and no patients attained a CR, yielding an ORR of 54.2% (95% CI, 39.2%-68.6%) (Table 2). Eleven patients (22.9%) had SD, yielding a disease control ratio (CR + PR + SD) of 77.1% (95% CI, 67.2%-88%). Eleven patients (22.9%) had progressive disease as their best response. At a median follow-up of 13.9 months (range, 2.0-27.5 months), the median PFS was 6.8 months (95% CI, 4.3-8.2 months) (Fig. 2), and the median OS was 22.8 months (lower limit of 95% CI, 13.1 months). Thirty-seven patients (77.1%) received subsequent chemotherapy regimens as poststudy treatment.

Safety Analysis

All 48 patients who received the study treatment were deemed eligible for safety analysis. The major adverse events for each treatment phase (initial and maintenance phases) are listed in Table 3. During treatment with the combination of S-1, carboplatin, and bevacizumab, grade

TABLE 3. Incidence of Adverse Events in the Study Patients

	No. of Patients (%)					
	Initial Treatment Phase, n = 48			Maintenance Phase, n = 29		
Adverse Event	All Grades	Grade 3	Grade 4	All Grades	Grade 3	Grade 4
Hematologic AEs						
Leukopenia	43 (89.6)	4 (8.3)	0 (0)	17 (58.6)	0 (0)	0 (0)
Neutropenia	44 (91.7)	12 (25)	3 (6.3)	16 (55.2)	4 (13.8)	0 (0)
Anemia	37 (77.1)	2 (4.2)	3 (6.3)	25 (86.2)	1 (3.4)	0 (0)
Thrombocytopenia	43 (89.6)	5 (10.4)	3 (6.3)	11 (37.9)	0 (0)	0 (0)
Nonhematologic AEs						
Fatigue	36 (75)	1 (2.1)	0 (0)	14 (48.3)	0 (0)	0 (0)
Appetite loss	31 (64.6)	3 (6.3)	0 (0)	12 (41.4)	0 (0)	0 (0)
Proteinuria	25 (52.1)	0 (0)	0 (0)	9 (31)	2 (6.9)	0 (0)
Nausea	21 (43.8)	0 (0)	0 (0)	9 (31)	0 (0)	0 (0)
Mucositis	17 (35.4)	0 (0)	0 (0)	9 (31)	1 (3.4)	0 (0)
Hypertension	14 (29.2)	4 (8.3)	0 (0)	5 (17.2)	0 (0)	0 (0)
Diarrhea	11 (22.9)	3 (6.3)	0 (0)	4 (13.8)	1 (3.4)	0 (0)
Hemoptysis	6 (12.5)	0 (0)	0 (0)	1 (3.4)	0 (0)	0 (0)
Intestinal perforation	2 (4.2)	0 (0)	1 (1.1)	1 (3.4)	0 (0)	0 (0)
Interstitial pneumonia	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Abbreviations: AEs, adverse events.

3 and higher hematologic toxicities included neutropenia (31.3%), thrombocytopenia (16.7%), anemia (10.5%), and leukopenia (8.3%). Among the 29 patients who continued with S-1 plus bevacizumab during the maintenance phase, 4 patients (13.8%) had grade 3 neutropenia, and 1 patient (3.4%) had grade 3 anemia during the period. Most nonhematologic adverse events were of mild to moderate intensity (grade 1 or 2); those that were grade 3 or higher during the initial phase of the study treatment included hypertension (8.3%), appetite loss (6.3%), diarrhea (6.3%), fatigue (2.1%), and intestinal perforation (2.1%); whereas those during the maintenance phase included proteinuria (6.9%), mucositis (3.4%), and diarrhea (3.4%). There were no clinically relevant bleeding events during either treatment phase for any patient, and there were no treatment-related deaths.

DISCUSSION

This multicenter phase 2 study is the first to evaluate the efficacy and safety of a new regimen, S-1 and carboplatin combined with bevacizumab followed by maintenance S-1 and bevacizumab, for first-line treatment of patients with advanced nonsquamous NSCLC. We observed that the combination was active, with an ORR of 54.2% (95% CI, 39.2%-68.6%), which met the primary objective of the study. At a median follow-up of 13.9 months, the median PFS was 6.8 months, and the median OS was 22.8 months. Our previous phase 3 study demonstrated that the combination of S-1 and carboplatin was not inferior relative to a standard paclitaxel-carboplatin regimen in terms of OS for chemotherapy-naive patients with advanced NSCLC.[12] The ORR for S-1 and carboplatin, however, was significantly lower than that for paclitaxel-carboplatin (20.4% vs 29%; $P = .019$). This difference in ORR also was apparent for patients who had nonsquamous NSCLC (18.8% vs 27.6%; $P = .027$).[15] It is believed that bevacizumab is targets the tumor vasculature, reducing interstitial pressure and increasing vessel permeability, thereby resulting in enhanced tumor sensitivity to cytotoxic chemotherapy.[16,17] Although there are limitations to comparisons of results among different studies, the ORR (54.2%) obtained in the current trial is indicative of increased antitumor activity of S-1 and carboplatin with the addition of bevacizumab.

Our trial also indicates a favorable toxicity profile for the study treatment. All toxicities were manageable with symptomatic treatment and dose reduction or interruption. In our previous phase 3 study, the combination of S-1 and carboplatin was associated with significantly lower rates of neutropenia, leukopenia, and febrile neutropenia compared with paclitaxel and carboplatin.[12] Consistent with these results, most patients enrolled in the current study had only mild hematologic toxicities, and no cases of febrile neutropenia occurred. Thus, no obvious exacerbation of chemotherapy-induced myelosuppression by the addition of bevacizumab to S-1 and carboplatin was apparent. There also was no increase in the relative incidence of hematologic toxicities associated with maintenance therapy with S-1 and bevacizumab. With regard to nonhematologic toxicities, there were no grade 3 or 4

toxicities encountered in >10% of patients throughout the study treatment. Mild or moderate fatigue and gastrointestinal adverse effects were the most frequent nonhematologic toxicities, consistent with findings from previous studies with combined S-1 and carboplatin.[12] Hypertension and proteinuria have been associated with bevacizumab administration[6,8] and also were observed in the current study. Two patients experienced grade 3 proteinuria in the maintenance period; however, both cases were fully reversible without dose reduction or cessation of treatment. Because clinically significant (grade ≥3) hemoptysis was observed in 0.9% to 1.9% of patients who received bevacizumab in the ECOG E4599 and AVAiL trials,[6,8] the lack of fatal bleeding events and treatment-related deaths in the current study also is noteworthy. Patients with brain metastases have been excluded from most clinical trials of bevacizumab for fear of intracranial hemorrhage.[6,8] However, recent data suggest that the risk of developing intracranial hemorrhage is independent of bevacizumab therapy in patients with NSCLC who have brain metastases.[18] Conversely, another study suggested that intracranial hemorrhage is more likely to become clinically symptomatic in larger (>2 cm) brain metastases.[19] On the basis of these data, patients with brain metastases that measured <1 cm were eligible for the current study. Indeed, 2 patients who had asymptomatic, small (<1 cm) brain metastases were enrolled in the study, but no intracranial hemorrhage was observed.

Bevacizumab is used in the maintenance setting for patients with advanced nonsquamous NSCLC on the basis of the results from the ECOG E4599 and AVAiL trials, in which bevacizumab monotherapy after induction therapy with bevacizumab plus platinum doublets was administered until patients developed disease progression.[6,8] More recently, a large phase 3 trial (PARAMOUNT) demonstrated that maintenance therapy with pemetrexed after induction therapy with pemetrexed and cisplatin resulted in a significant improvement in PFS and OS.[20] Furthermore, an ongoing ECOG phase 3 trial is comparing bevacizumab alone, pemetrexed alone, and the pemetrexed-bevacizumab combination for maintenance therapy after initial therapy with paclitaxel, carboplatin, and bevacizumab (National Clinical Trials identifier NCT01107626). Thus, extensive efforts are under way to examine the effects of the combination of bevacizumab and chemotherapy during the maintenance phase of treatment for advanced nonsquamous NSCLC. On the basis of the low level of toxicity accumulation for S-1,[21] we explored the efficacy and feasibility of continued maintenance therapy with S-1 and bevacizumab in the current study. The favorable tolerability profile of maintenance with S-1 and bevacizumab after induction therapy with S-1, carboplatin, and bevacizumab is reflected in out observation that 60% of patients were able to continue on S-1 and bevacizumab for a median of 4 cycles (range, 1-18 cycles). Among the 29 patients who received maintenance therapy with S-1 and bevacizumab, the median PFS of 8.2 months (95% CI, 7.4-9.6 months) from the beginning of induction treatment is encouraging and compares favorably with that of 6.9 months reported in the PARAMOUNT study for maintenance with pemetrexed monotherapy. Despite the limitations to comparisons of results from different studies, these data may stimulate further interest in the clinically relevant efficacy of maintenance therapy with S-1 and bevacizumab.

In conclusion, here, we have presented results from the first phase 2 study of combined S-1, carboplatin, and bevacizumab followed by maintenance therapy with S-1 and bevacizumab. Although our study was not randomized, the promising efficacy and favorable toxicity profile of the study treatment justify further development of regimens that contain S-1 and bevacizumab. A large randomized phase 3 trial comparing single-agent S-1 with docetaxel in previously treated patients with advanced NSCLC in Asian countries is currently under way (JPRN-JapicCTI-101155). We believe that further randomized trials are warranted comparing S-1, carboplatin, and bevacizumab with the current standard of care (paclitaxel, carboplatin, and bevacizumab) in previously untreated patients with advanced nonsquamous NSCLC.

FUNDING SUPPORT

No specific funding was disclosed.

CONFLICT OF INTEREST DISCLOSURES

The authors made no disclosures.

REFERENCES

1. Siegel R, Naishadham D, Jemal A. Cancer statistics 2012. *CA Cancer J Clin.* 2012;62:10-29.
2. Ohe Y, Ohashi Y, Kubota K, et al. Randomized phase III study of cisplatin plus irinotecan versus carboplatin plus paclitaxel, cisplatin plus gemcitabine, and cisplatin plus vinorelbine for advanced non-small-cell lung cancer: Four-Arm Cooperative Study in Japan. *Ann Oncol.* 2007;18:317-323.
3. Schiller JH, Harrington D, Belani CP, et al. Comparison of 4 chemotherapy regimens for advanced non-small-cell lung cancer. *N Engl J Med.* 2002;346:92-98.
4. Burdett S, Stephens R, Stewart L, et al. Chemotherapy in addition to supportive care improves survival in advanced non-small-cell lung cancer: a systematic review and meta-analysis of individual patient data from 16 randomized controlled trials. *J Clin Oncol.* 2008;28:4617-4625.
5. Ferrara N, Kerbel RS. Angiogenesis as a therapeutic target. *Nature.* 2005;438:967-974.

6. Sandler A, Gray R, Perry MC, et al. Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. *N Engl J Med.* 2006;355:2542-2550.
7. Niho S, Kunitoh H, Nokihara H, et al. Randomized phase II study of first-line carboplatin-paclitaxel with or without bevacizumab in Japanese patients with advanced non-squamous non-small-cell lung cancer. *Lung Cancer.* 2012;27:362-367.
8. Reck M, Von Pawel J, Zatloukal P, et al. Phase III trial of cisplatin plus gemcitabine with either placebo or bevacizumab as first-line therapy for nonsquamous non-small-cell lung cancer: AVAiL. *J Clin Oncol.* 2009;27:1227-1234.
9. Reck M, von Pawel J, Zatloukal P, et al. Overall survival with cisplatin-gemcitabine and bevacizumab or placebo as first-line therapy for nonsquamous non-small-cell lung cancer: results from a randomised phase III trial (AVAiL). *Ann Oncol.* 2010;21:1804-1809.
10. Shirasaka T, Nakano K, Takechi T, et al. Antitumor activity of 1 M tegafur-0.4 M 5-chloro-2,4-dihydroxypyridine-1 M potassium oxonate (S-1) against human colon carcinoma orthotopically implanted into nude rats. *Cancer Res.* 1996;56:2602-2606.
11. Kawahara M, Furuse K, Segawa Y, et al. Phase II study of S-1, a novel oral fluorouracil, in advanced non-small-cell lung cancer. *Br J Cancer.* 2001;85:939-943.
12. Okamoto I, Yoshioka H, Morita S, et al. Phase III trial comparing oral S-1 plus carboplatin with paclitaxel plus carboplatin in chemotherapy-naive patients with advanced non-small-cell lung cancer: results of a West Japan Oncology Group study. *J Clin Oncol.* 2010; 28:5240-5246.
13. Nukatsuka M, Saito H, Nakagawa F, et al. Combination therapy using oral S-1 and targeted agents against human tumor xenografts in nude mice. *Exp Ther Med.* 2012;3:755-762.
14. Shaked Y, Henke E, Roodhart J, et al. Rapid chemotherapy-induced acute endothelial progenitor cell mobilization: implications for antiangiogenic drugs as chemosensitizing agents. *Cancer Cell.* 2008;14: 263-273.
15. Yoshioka H, Okamoto I, Morita S, et al. Efficacy and safety analysis according to histology for S-1 in combination with carboplatin as first-line chemotherapy in patients with advanced non-small cell lung cancer: updated results of the West Japan Oncology Group LETS study [published online ahead of print February 4, 2013]. *Ann Oncol.* 2013.
16. Willett CG, Boucher Y, di Tomaso E, et al. Direct evidence that the VEGF-specific antibody bevacizumab has antivascular effects in human rectal cancer. *Nat Med.* 2004;10:145-147.
17. Dickson PV, Hamner JB, Sims TL, et al. Bevacizumab-induced transient remodeling of the vasculature in neuroblastoma xenografts results in improved delivery and efficacy of systemically administered chemotherapy. *Clin Cancer Res.* 2007;13:3942-3950.
18. Besse B, Lasserre S, Compton P, et al. Bevacizumab safety in patients with central nervous system metastases. *Clin Cancer Res.* 2010;16:269-278.
19. Srivastava G, Vishal R, Wallace S, et al. Risk of intracranial hemorrhage and cerebrovascular accidents in non-small cell lung cancer brain metastasis patients. *J Thorac Oncol.* 2009;4:333-337.
20. Paz-Ares L, de Marinis F, Dediu M, et al. Maintenance therapy with pemetrexed plus best supportive care versus placebo plus best supportive care after induction therapy with pemetrexed plus cisplatin for advanced non-squamous non-small-cell lung cancer (PARAMOUNT): a double-blind, phase 3, randomized controlled trial. *Lancet Oncol.* 2012;13:247-255.
21. Totani Y, Saito Y, Hayashi M, et al. A phase II study of S-1 monotherapy as second-line treatment for advanced non-small cell lung cancer. *Cancer Chemother Pharmacol.* 2009;64:1181-1185.

Lung Cancer 81 (2013) 97–101

Contents lists available at SciVerse ScienceDirect

Lung Cancer

journal homepage: www.elsevier.com/locate/lungcan

Efficacy and safety of weekly *nab*-paclitaxel plus carboplatin in patients with advanced non-small cell lung cancer☆

Miyako Satouchi[a,*], Isamu Okamoto[b], Hiroshi Sakai[c], Nobuyuki Yamamoto[d], Yukito Ichinose[e], Hironobu Ohmatsu[f], Naoyuki Nogami[g], Koji Takeda[h], Tetsuya Mitsudomi[i], Kazuo Kasahara[j], Shunichi Negoro[a]

[a] *Hyogo Cancer Center, Hyogo, Japan*
[b] *Kinki University Faculty of Medicine, Osaka, Japan*
[c] *Saitama Cancer Center, Saitama, Japan*
[d] *Shizuoka Cancer Center, Shizuoka, Japan*
[e] *National Kyushu Cancer Center, Fukuoka, Japan*
[f] *National Cancer Center Hospital East, Chiba, Japan*
[g] *National Hospital Organization Shikoku Cancer Center, Ehime, Japan*
[h] *Osaka City General Hospital, Osaka, Japan*
[i] *Aichi Cancer Center Hospital, Aichi, Japan*
[j] *Kanazawa University Hospital, Ishikawa, Japan*

ARTICLE INFO

Article history:
Received 15 January 2013
Accepted 24 February 2013

Keywords:
ABI-007
Abraxane
Carboplatin
Japanese
Nab-paclitaxel
Non-small cell lung cancer (NSCLC)
Squamous cell carcinoma (SCC)
Weekly

ABSTRACT

Purpose: In a large multicenter international phase III study (CA031) of *nab*-paclitaxel (*nab*-P, 130 nm albumin-bound paclitaxel particles) + carboplatin (C) vs solvent-based paclitaxel (sb-P) + C, conducted in 6 countries including Japan, *nab*-PC produced significantly higher overall response rate (ORR), primary end point compared with sb-PC, and acceptable safety profile. The aim of this analysis was to evaluate the efficacy and tolerability of *nab*-PC vs sb-PC in Japanese patients with advanced non-small-cell lung cancer (NSCLC) who were enrolled in the CA031 study.

Patients and methods: In the CA031 study, a total of 1052 patients were randomized to receive either *nab*-P 100 mg/m^2 weekly or sb-P 200 mg/m^2 every 3 weeks both in combination with C at area under the concentration-time curve (AUC) = 6 on day 1 of each 3-week cycle. This analysis included 149 Japanese patients with previously untreated stage IIIB or IV NSCLC.

Results: The baseline and histologic characteristics of patients were well balanced between the two arms. ORR was higher with *nab*-PC vs sb-PC (35% vs 27%; response rate ratio = 1.318). Progression-free survival (median 6.9 vs 5.6 months; hazard ratio [HR] = 0.845) and overall survival (median 16.7 vs 15.9 months; HR = 0.930) were better with *nab*-PC vs sb-PC. Of the grade ≥3 treatment-related adverse events, anemia and thrombocytopenia were more common in *nab*-PC arm, but sensory neuropathy was less common.

Conclusion: The *nab*-PC treatment yielded promising results regarding the efficacy endpoint, and it was generally well tolerated as first-line therapy for Japanese patients with advanced NSCLC.

1. Introduction

Non-small cell lung cancer (NSCLC) has a relatively poor prognosis despite continuous development of new therapeutic agents including molecular targeting agents. The choice of therapeutic agents varies widely, and in the recent strategy for the treatment of advanced NSCLC solvent-based paclitaxel (sb-P) plus carboplatin (C) remains an important treatment option [1,2], especially for patients with squamous cell carcinoma (SCC), in which beneficial treatment is limited [3–6]. However, due to the Cremophor EL excipient in sb-P, sb-P has been associated with the risk of developing severe toxicities including hypersensitivity reactions, which has been an important motivating factor in developing new formulations of paclitaxel [7–9].

Albumin-bound paclitaxel (*nab*-P) is a formulation of paclitaxel complexed with 130 nm albumin that is readily soluble in saline. The Cremophor EL-free medium enables administration of

☆ ClinicalTrials.gov Identifier: NCT00540514.

* Corresponding author at: Department of Thoracic Oncology, Hyogo Cancer Center, 13-70 Kitaoji, Akashi, Hyogo 673-8558, Japan. Tel.: +81 78 929 1151; fax: +81 78 929 2380.
E-mail address: satouchi@hp.pref.hyogo.jp (M. Satouchi).

0169-5002/$ – see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.lungcan.2013.02.020

nab-P in a shorter duration without the need for corticosteroid and anti-histamine premedication to prevent solvent-related hypersensitivity reactions.

A large multicenter international randomized phase III study (CA031) was conducted to compare the efficacy and safety of a weekly schedule of *nab*-P vs an every 3-week schedule of sb-P each in combination with C in patients with previously untreated stage IIIB or IV NSCLC [10]. This registration study for NSCLC was conducted in Australia, Canada, Japan, Russia, Ukraine, and the United States, and met its primary end point demonstrating a statistically significantly higher overall response rate (ORR), regardless of histology, for patients in the *nab*-PC arm compared to those in the sb-PC arm (33% vs 25%; response rate ratio = 1.313; $p = 0.005$), with acceptable safety profile. In addition, non-significant trends toward longer progression-free survival (PFS) (median 6.3 vs 5.8 months; hazard ratio [HR] = 0.902; $p = 0.214$) and overall survival (OS) (median 12.1 vs 11.2 months; HR = 0.922; $p = 0.271$) favoring *nab*-PC were observed. Based on the results of subgroup analyses of CA031, Socinski et al. reported that regional differences in characteristics might have played a role in the differences in survival outcomes. Furthermore, recent studies of inhibitors of epidermal growth factor receptor (EGFR) underlined benefits of investigating the presence of mutation in EGFR gene partly based on the differences of patient survival between Asian and Caucasian populations due to interethnic differences in the EGFR-mutant rate [11–16]. These results suggest the importance of the potential regional differences in the treatment of NSCLC.

We therefore performed a subgroup analysis of the Japanese patients who were enrolled in the CA031 study to evaluate the efficacy and toxicity profile of *nab*-PC vs sb-PC as first-line treatment of advanced NSCLC. Today the selection of chemotherapeutic strategy is greatly affected by histologic type. Exploratory subgroup analyses were also performed to reveal the potential clinical benefit of *nab*-PC, including efficacy outcomes, by histology.

2. Patients and methods

2.1. Patients

In the CA031 study comparing the *nab*-PC vs sb-PC, a total of 1052 patients, including 149 Japanese patients with 74 in the *nab*-PC arm and 75 in the sb-PC arm, with cytologically or histologically confirmed measurable previously untreated stage IIIB or IV NSCLC were enrolled (Fig. 1). Patients could have a prior adjuvant chemotherapy if >12 months had passed from the completion of the therapy. Patients were also required to satisfy the following criteria: Eastern Cooperative Oncology Group performance status (ECOG PS) of 1 or less, an estimated life expectancy exceeding 12 weeks. Patients were excluded if they had symptomatic brain metastasis or current active malignancy other than NSCLC, pre-existing peripheral neuropathy grade 2 or more according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 3.0. The study was approved by the institutional review board of each participating center and was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines of the International Conference on Harmonization. Written informed consent was obtained from all patients.

2.2. Treatment schedule

Patients were randomly assigned to either the *nab*-PC or the sb-PC arm. Randomization was stratified by disease stage (IIIB/IV), age (<70/≥70), gender, histology (squamous/adenocarcinoma/others) and geographic region (North America/Russia and Ukraine/Japan/Australia). Patients received a 21-day cycle of *nab*-PC or sb-PC treatment. *Nab*-P 100 mg/m^2 was given weekly (on days 1, 8, and 15) via a 30 min infusion, followed by C (AUC = 6) given on day 1, whereas sb-P 200 mg/m^2 was given every 3 weeks via a 3-h infusion, followed by C (AUC = 6)

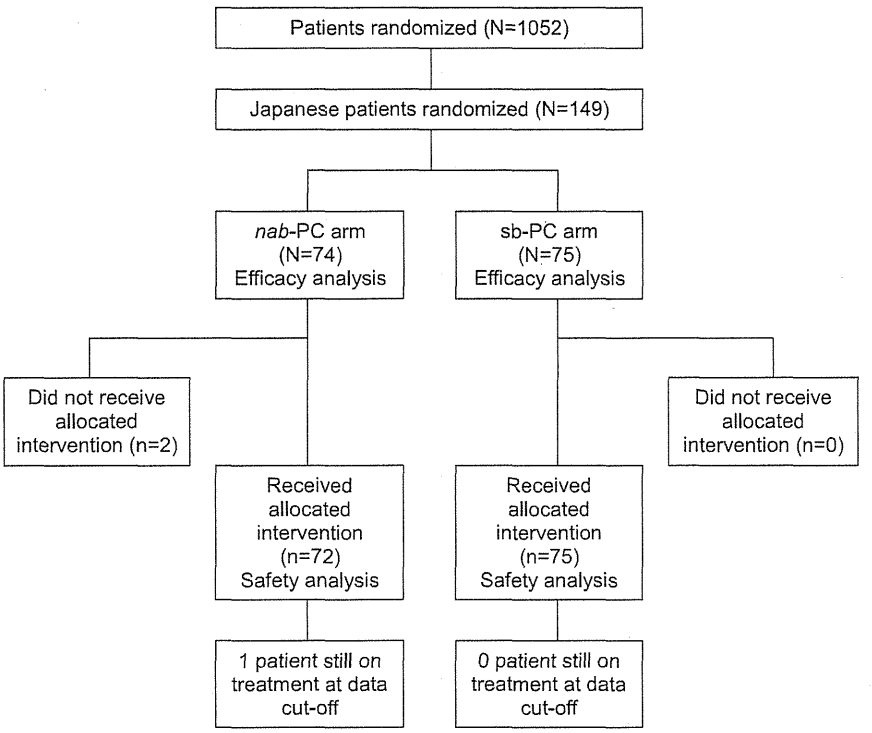

Fig. 1. CONSORT flow chart of the study.