Pharmacokinetic and pharmacodynamic profiles of subcutaneous administration of continuous erythropoietin receptor activator in lung cancer patients with anemia induced by chemotherapy

TOSHIAKI TAKAHASHI[1], NOBUYUKI YAMAMOTO[1], TOMOHIDE TAMURA[2], HIDEO KUNITOH[2,5], YUTAKA NISHIWAKI[3] and SHUNICHI NEGORO[4]

[1]Division of Thoracic Oncology, Shizuoka Cancer Center, Shizuoka 411-8777; [2]Department of Internal Medicine, National Cancer Center Hospital, Tokyo 104-0045; [3]Thoracic Oncology Division, National Cancer Center Hospital East, Chiba 277-8577; [4]Thoracic Oncology, Hyogo Cancer Center, Hyogo 673-8558, Japan

Received March 12, 2011; Accepted July 15, 2011

DOI: 10.3892/ol.2011.398

Abstract. Continuous erythropoietin receptor activator (C.E.R.A.) is an innovative erythropoiesis-stimulating agent with unique erythropoietin receptor activity and a prolonged half-life. C.E.R.A. is currently in development for the correction of anemia and stable hemoglobin (Hb) control at extended administration intervals in patients with cancer who are receiving chemotherapy. The purpose of this pharmacological study was to evaluate the pharmacokinetic (PK), pharmacodynamic (PD) and safety profiles of C.E.R.A. administered subcutaneously once every 3 weeks (Q3W) in lung cancer patients with anemia induced by chemotherapy. This open-label, multicenter study recruited 46 patients. Entry Hb levels were not more than 11.0 g/dl. Five dose levels of C.E.R.A. (2.1, 4.2, 6.3, 9 and 12 μg/kg) were tested in sequential cohorts of 8-11 patients for 12 weeks. The mean values for C.E.R.A half-life ranged from 143 to 247 h. The maximum serum concentration (C_{max}) following the first administration of C.E.R.A. increased in proportion to the dose. The increase of Hb levels occurred in a dose-dependent manner. No serious adverse events reported as being related to C.E.R.A. were observed during the study period. Thrombovascular events were not observed in any patient. Anti-C.E.R.A antibodies were not detected in any patient. Thus, this pharmacological study confirmed the long half-life of C.E.R.A., thereby supporting subcutaneous administration of C.E.R.A. at the Q3W interval. PK and PD parameters demonstrated dose-proportionality over the range of doses tested in this study. Additionally, C.E.R.A. was generally well tolerated.

Correspondence to: Dr Toshiaki Takahashi, Division of Thoracic Oncology, Shizuoka Cancer Center, 1007 Shimonagakubo Nagaizumi-cho Sunto-gun, Shizuoka 411-8777, Japan
E-mail: t.takahashi@scchr.jp

Present address: [5]Department of Respiratory Medicine, Mitsui Memorial Hospital, Tokyo 101-8643, Japan

Key words: anemia, erythropoietin, chemotherapy, pharmacokinetic, pharmacodynamic

Introduction

Erythropoiesis-stimulating agents (ESAs) are commonly used to treat chemotherapy-induced anemia. The administration of these agents has been shown to be effective for treating anemia in patients who undergo chemotherapy. These agents are effective as they increase hemoglobin (Hb) concentrations and reduce or eliminate the need for red blood cell (RBC) transfusions, thus improving quality of life (QoL) (1-3). In anemic patients with cancer, ESAs were initially administered 3 times weekly, a schedule that had already proved effective in patients with renal anemia (4,5). Once-weekly (Q1W) administration with all ESAs has become the preferred treatment modality (6,7). Darbepoetin α has also been licensed for use once every 3 weeks (Q3W) in cancer patients with chemotherapy-induced anemia (8). Continuous erythropoietin receptor activator (C.E.R.A.) is an innovative agent with a prolonged half-life compared with that of epoetin α and epoetin β in healthy volunteers, and darbepoetin α in patients with peritoneal dialysis (9). C.E.R.A. is a chemically synthesized continuous erythropoietin receptor activator that differs from erythropoietin through the integration of amide bonds between amino groups and methoxy polyethylene glycol-succinimidyl butanoic acid (10,11). It has been developed to provide correction of anemia and to control Hb levels at extended administration intervals in patients with CKD on dialysis and not on dialysis (9). Moreover, C.E.R.A. is currently in development for the correction of anemia and stable control of Hb levels at Q1W and Q3W administration intervals in cancer patients with chemotherapy-induced anemia. In preclinical studies and studies in healthy subjects, C.E.R.A. had a lower systemic clearance and an increased elimination half-life compared with conventional ESAs, and superior potency *in vivo* with respect to the magnitude and duration of response (12,13). An exploratory Phase I-II dose-escalation study in anemic patients with multiple myeloma receiving myelosuppressive chemotherapy confirmed the long half-life

of C.E.R.A. Additionally, a dose-dependent increase in Hb response was observed with C.E.R.A. doses up to 8.0 μg/kg when administered Q3W by subcutaneous (SC) injection (14). Moreover, two Phase II dose-finding studies were carried out in anemic patients with aggressive non-Hodgkin's lymphoma and advanced non-small cell lung cancer (NSCLC) receiving myelosuppressive chemotherapy (15,16). A dose-dependent increase in Hb response was observed with C.E.R.A. doses up to 6.3 μg/kg when administered Q3W by SC injection. Notably, there was a trend to higher mean Hb increases and lower transfusion use in the Q3W groups as compared to the Q1W groups with respect to the NSCLC study. However, further dose-finding studies that use higher doses and allow dose escalation are required to determine the optimal C.E.R.A. Q3W dose regimen was administered in anemic cancer patients receiving chemotherapy as a limited effect was obtained even at the highest dose level of 6.3 μg/kg used in aggressive non-Hodgkin's lymphoma (15) and advanced non-small cell lung cancer (NSCLC) (16). Additonally, with regards to safety, neither dose-dependence in adverse events nor dose-limiting toxicity was observed at the dose level of 8.0 μg/kg (14).

This multicenter, open-label study was designed to evaluate the pharmacokinetic (PK)/pharmacodynamic (PD) properties and safety of five different dose levels of C.E.R.A. administered subcutaneously Q3W for up to 12 weeks in Japanese lung cancer patients with anemia induced by myelosuppressive chemotherapy.

Patients and methods

Patients. A total of 47 adult patients, aged ≥20 and <80 years at the time of registration, were recruited based on the following criteria: patient is diagnosed with lung cancer by tissue or cytological examination, receiving cyclic chemotherapy for ≤4 weeks as 1 cycle, and capable of undergoing chemotherapy within 3 days following the start of administration of the investigational medication. Patients were also required to have Hb levels ≤11.0 g/dl at the registration examination, a life expectancy of ≥4 months, Eastern Cooperative Oncology Group (ECOG) performance status grades of 0-2, a satisfactory mean corpuscular volume (MCV), and to meet transferrin saturation (Tsat), hepatic and renal function criteria [MCV 80 fl or higher, transferrin saturation [{Fe/(Fe + UIBC)}x100] of 15% or higher, total bilirubin value in serum: ≤2.0 mg/dl, AST [GOT], ALT [GPT]: 80 IU/l or lower, serum creatinine value: <2.0 mg/dl]. Exclusion criteria included transfusion within 4 weeks prior to the planned start of administration of the investigational medication; severe hypertension uncontrollable by pharmaceutical products; marked hemorrhagic lesions possibly affecting evaluation in the clinical study or presence of serious complications; pregnant or nursing women, who were premenopausal and tested positive for pregnancy in a pregnancy test; expression of lack of intention to use contraception; history/complication of cardiac infarction, pulmonary infarction or cerebral infarction (excluding asymptomatic cerebral infarction); and serious medication allergy including anaphylactic shock. Patients recruited had not received treatment with ESAs within the 4 weeks prior to registration. The design and conduct of the study complied with the ethical principles of good clinical practice, in accordance with the Declaration of Helsinki. The study was approved by an independent institutional review board at each cancer center, and all 47 patients provided written informed consent prior to enrollment.

Study medication. Three separate strength vials were available (200, 400 or 1000 μg/ml), each containing a 1 ml solution of C.E.R.A.

Study design. This was an open-label, multicenter, clinical pharmacology study that involved SC injections of C.E.R.A. over a 12-week treatment period, and a follow-up period for 3 weeks following the last administration of C.E.R.A. Patients were assigned sequentially according to increasing dose rotations to one of five groups, receiving C.E.R.A. at 2.1, 4.2, 6.3, 9 or 12 μg/kg. This was administered Q3W by SC injection for 12 weeks. If Hb levels recovered to >13.0 g/dl, treatment was discontinued. Chemotherapy and radiotherapy were used concomitantly during the period from the day of starting administration to the time of the last observation. Blood transfusions were performed concurrently in patients who did not show improvement in anemia and were judged clinically to require blood transfusion. Oral iron supplementation was administered daily during the administration period of the study medication if MCV was <80 fl or Tsat was <15%. Blood samples for detection of the C.E.R.A. antibody were collected prior to the first administration and within a maximum of 50 days following the final administration.

Serum assay. To determine PK parameters, blood samples were collected immediately prior to and 1, 8, 15, 22, 23-26, 27, 29, 32, 36, 43, 64 and 85 days following the initial administration of C.E.R.A. To investigate concentrations over time, samples were also collected immediately prior to the administration of each dose. Blood samples were allowed to stand at room temperature for 30 min and were then centrifuged at 4°C and 3,000 rpm for 10 min to separate the serum. The resulting serum was stored below -20°C until used for the measurement of serum C.E.R.A. concentrations. Serum concentrations of C.E.R.A. were measured by a validated enzyme-linked immunosorbent assay using a primary monoclonal antibody specific to C.E.R.A. that did not cross-react with endogenous erythropoietin, and a secondary polyclonal anti-immunoglobulin antibody coupled to horseradish peroxidase (Huntingdon Life Sciences, Alconbury, UK). The assay range was 150 to 4000 pg/ml. The inter-batch assay precision was 7.8 to 11.4%, and accuracy was -7.8 to -6.6%. The assay is specific to C.E.R.A. and does not detect human erythropoietin, and human erythropoietin does not interfere with the assay.

Pharmacokinetic analyses. Serum concentrations of C.E.R.A. were used to determine the maximum serum concentration (C_{max}) and time to maximum serum concentration (T_{max}). The $t_{1/2}$ was estimated for ln(2)/k, where the rate constant of elimination (k) was determined by linear regression of the logarithm of the serum concentration vs. time data in the post-distribution phase. The area under the concentration-time curve (AUC) following C.E.R.A. administration, from predose on day 22 until the last sampling time at which the

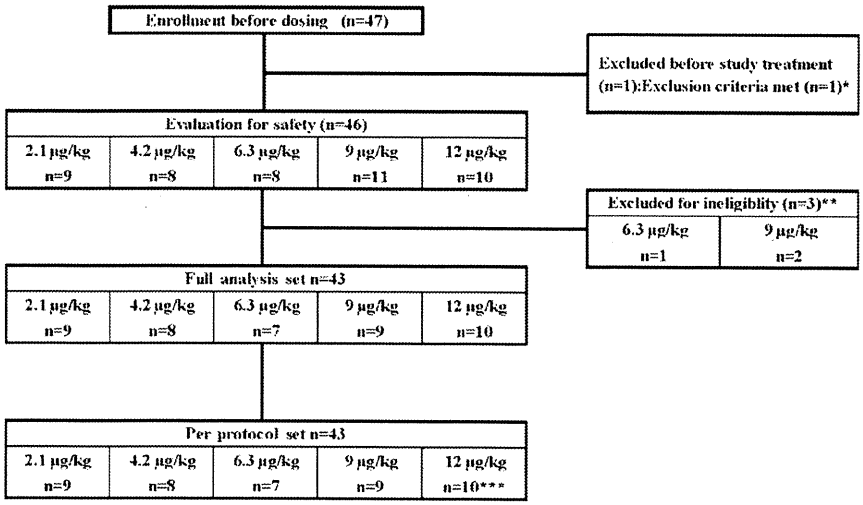

Figure 1. Patient conditions.*One patient did not receive study medication for the development of ileus. **One patient allocated to the 6.3 μg/kg dose group and two patients allocated to 9 μg/kg dose group were excluded from the data analyses caused by the defect of medication vials. ***One patient in the 12 μg/kg dose group was excluded from the data analyses following week 7, caused by a defect in medication vials.

concentration was measurable (day 43), was estimated by the linear trapezoidal rule.

Pharmacodynamic analyses. The PD parameters involved the change in Hb from the nadir value. The change in the Hb level from the nadir value was calculated by subtraction of the nadir value over the period weeks 1-4 from the Hb value at week 7. The increase in Hb produced by C.E.R.A. administration was estimated to occur over the 4 weeks following first administration of C.E.R.A. and certain patients were withdrawn from the study after 6 weeks (17). Moreover, to estimate the increase of Hb caused by C.E.R.A., the effect of chemotherapy on Hb was removed. Therefore, to evaluate the effect of C.E.R.A. on Hb levels, the change in Hb was calculated between the nadir values from weeks 1-4 and the values at week 7.

Safety. Safety endpoints included adverse events, clinical laboratory tests, vital signs, body weight, physical examination and ECGs. The intensities of adverse events and laboratory values were graded according to the National Cancer Institute/Common Terminology Criteria for Adverse Events v3.0 (CTCAE).

Statistical analysis. Mean, standard deviation, and the coefficient of variation of the PK parameters were calculated for each treatment. Mean, standard deviation, median, range and standard error are provided per day for Hb levels. Descriptive statistics were calculated using SAS (version 9.1.3). Figures were prepared with S-Plus (version 8.1).

Results

Patients. A total of 47 lung cancer patients were enrolled (Fig. 1). Treatment of one patient assigned to the 9 μg/kg dose group was discontinued due to the development of ileus prior to the first administration. The remaining 46 patients were treated with each dose of C.E.R.A. and were included in the safety populations. In total, 46 patients completed the study treatment period. However, one patient assigned to the 6.3 μg/kg dose group and two patients assigned to the 9 μg/kg dose group were excluded from the PK and PD analyses due to defects in the study medication, which included vial blistering and contents out of specification. The remaining 43 patients were included in the analysis of full analysis set and per protocol set populations. However, one patient in the 12 μg/kg dose group was excluded from the data analysis following week 7 due to a defect in the medication vial. Doses were held for a total of 25 patients. Consequently, these patients were not administered the full four doses of the study medication during the 12-week period. The reasons for discontinuation of the study medication were progressive disease, Hb levels >13 g/dl, Hb level elevation of ≥2.0 g/dl from previous treatment to next treatment and termination of concomitant chemotherapy.

Baseline characteristics and demographics are shown in Table I. Patients had received chemotherapy prior to the start of this study. A total of 11, 13, 25, 18 and 10% of patients had undergone surgery and 33, 50, 13, 18 and 20% of patients had undergone radiotherapy in the 2.1, 4.2, 6.3, 9 and 12 μg/kg dose groups, respectively. Median Hb levels ranged from 8.9 to 10.5 g/dl across all dose groups. The values for iron parameters in the 9 and 12 μg/kg dose groups at baseline were lower than those in the 2.1, 4.2 and 6.3 μg/kg dose groups.

Patients received at least one chemotherapy cycle within 3 days following the initial C.E.R.A. administration, which was most commonly platinum-based chemotherapy (the percentage of carboplatin or cisplatin treatments: 78, 63, 43, 78 and 40% in the 2.1, 4.2, 6.3, 9 and 12 μg/kg dose groups, respectively). Other common chemotherapies included amrubicin hydrochloride (11, 13, 29, 0 and 60% in the 2.1, 4.2, 6.3, 9, and 12 μg/kg dose groups, respectively) and docetaxel hydrate (11, 25, 29, 22 and 0% in the 2.1, 4.2, 6.3, 9 and 12 μg/kg dose groups, respectively) (Table II).

Overall, 22 patients received oral iron therapy. The percentage of patients receiving concomitant oral iron supplementation was higher in the 9 and 12 μg/kg dose

Table I. Summary of patient's baseline characteristics (range or %): safety population.

	C.E.R.A. dose group (μg/kg Q3W)				
	2.1 μg/kg n=9	4.2 μg/kg n=8	6.3 μg/kg n=8	9 μg/kg n=11	12 μg/kg n=10
Gender (%)					
Male	6 (67)	8 (100)	4 (50)	8 (73)	8 (80)
Female	3 (33)	0	4 (50)	3 (27)	2 (20)
Median age, years	59 (53-79)	67 (59-76)	64 (34-72)	67 (50-77)	71 (56-79)
Median body weight (kg)	60 (53-75)	56 (47-66)	58 (48-61)	56 (44-75)	52 (44-66)
ECOG PS (%)					
0	5 (56)	1 (12)	6 (75)	2 (18)	3 (30)
1	4 (44)	7 (88)	2 (25)	9 (82)	7 (70)
2	0	0	0	0	0
History of lung cancer					
Small cell	2 (22)	3 (38)	5 (63)	2 (18)	6 (60)
Non-small cell	7 (78)	5 (62)	3 (37)	8 (73)	4 (40)
Mixed type	0	0	0	1 (9)	0
Previous treatment (%)					
Chemotherapy	9 (100)	8 (100)	8 (100)	11 (100)	10 (100)
Surgery	1 (11)	1 (13)	2 (25)	2 (18)	1 (10)
Radiotherapy	3 (33)	4 (50)	1 (13)	2 (18)	2 (20)
Transfusion	0	1 (13)	1 (13)	0	0
Median Hb level (g/dl)	9.2 (7.7-10.5)	10.0 (9.3-10.8)	10.5 (8.8-10.9)	8.9 (7.2-10.4)	10.0 (8.1-11.8)
Median serum ferritin (ng/ml)	516 (85-800)	392 (147-771)	139 (10-2100)	340 (13-647)	286 (55-651)
Median serum Fe (μg/dl)	72 (33-111)	68 (22-75)	76 (19-123)	49 (19-70)	47 (15-69)
Median Tsat (%)	24.3 (11.0-46.3)	25.8 (10.0-35.4)	28.0 (6.7-42.7)	18.7 (10.0-31.0)	16.3 (8.4-27.6)

C.E.R.A, continous erythropoietin receptor activator; Hb, hemoglobin; Q3W, once every 3 weeks; PS, performance status; Tsat, transferrin saturation.

Table II. Summary of patient's baseline Hb level, reticulocyte counts and oral supplementation and combination chemotherapy (range or %): PPS population.

	C.E.R.A. dose group (μg/kg Q3W)				
	2.1 μg/kg n=9	4.2 μg/kg n=8	6.3 μg/kg n=7	9 μg/kg n=9	12 μg/kg n=10
Reticulocyte counts x10^4/mm^3	9.4 (3.3-12.5)	8.0[a] (4.3-15.0)	9.4 (3.3-12.5)	6.6 (3.2-11.2)	6.0 (2.6-10.4)
Oral iron supplementation during treatment (%)	3 (33)	3 (38)	3 (33)	8 (89)	5 (50)
Chemotherapy, n, (%)					
Platinums[b]	4 (44)	4 (50)	4 (44)	5 (55)	3 (30)
Platinums + taxanes		3 (33)	0	2 (22)	1 (10)
Non-platinums	2 (22)	3 (38)	2 (22)	2 (22)	6 (60)
Taxanes	1 (11)	2 (25)	1 (11)	2 (22)	0
Anthracyclines	1 (11)	1 (13)	1 (11)	0	6 (60)

C.E.R.A., continous erythropoietin receptor activator; Hb, hemoglobin; PPS, per protocol set; Q3W, once every 3 weeks. [a]In only one patient, the measurement was missed at baseline.[b]Platinum-based combination chemotherapy with the exception of taxanes.

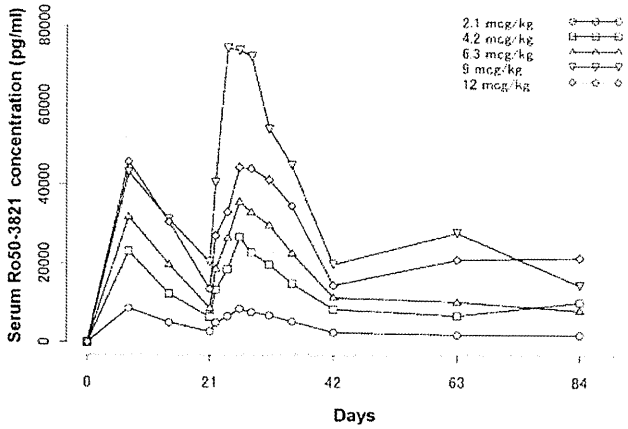

Figure 2. Serum C.E.R.A. concentration profiles (the mean ± SD).

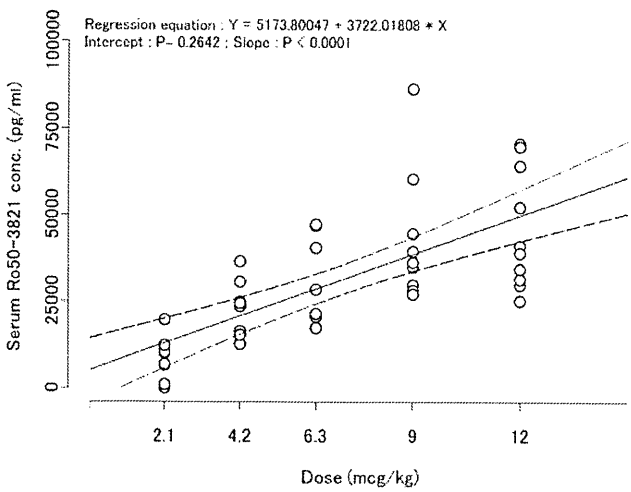

Figure 3. Correlation between dose and C_{max} following the initial dosing.

groups (89 and 50%, respectively) compared with the 2.1, 4.2 and 6.3 μg/kg dose groups (33, 38 and 43%, respectively) (Table II).

Pharmacokinetic analyses. Serum C.E.R.A. concentration-time profiles from weeks 1-13 are shown in Fig. 2. PK parameters were estimated following the second dosing and the correlation between dose and C_{max} was estimated following the initial dosing. PK parameter values are shown in Table III. Serum concentrations of C.E.R.A. following the second administration in the 2.1, 4.2, 6.3, 9 and 12 μg/kg dose groups reached C_{max} at 119 to 167 h (mean); the drug was then eliminated with half-lives ($t_{1/2}$) of 143 to 217 h, respectively (median). T_{max} values were similar among the dose groups. $T_{1/2}$ values were shown to be high across the dose groups and appeared to show no major differences among the dose groups.

AUC_{inf} in the 2.1, 4.2, 6.3, 9 and 12 μg/kg dose groups were 4060±2380, 12000±5580, 16100±7220, 30100±12500 and 23400±8080 ng•h/ml, respectively. C_{max} values were 9.09±5.26, 27.4±7.63, 38.9±13.9, 84.6±36.0 and 47.8±14.3 ng/ml, respectively.

Table III. PK parameters following the second dosing.

Parameter	Dose (μg/kg)	Mean ± SD	No.
C_{max} (ng/ml)	2.1	9.09±5.26	9
	4.2	27.4±7.63	8
	6.3	38.9±13.9	7
	9	84.6±36.0	9
	12	47.8±14.3	10
T_{max} (h)	2.1	146±43.1	9
	4.2	130±23.1	8
	6.3	119±43.6	7
	9	149±80.2	9
	12	167±42.6	10
AUC_t (ng*h/ml)	2.1	2730±1680	9
	4.2	8540±2430	8
	6.3	12100±4630	7
	9	23700±9470	9
	12	16200±5520	10
$t_{1/2}$ (h)	2.1	185 (152-276)	6
	4.2	217 (176-279)	8
	6.3	175 (165-182)	6
	9	143 (126-163)	7
	12	162 (159-237)	7
AUC_{inf} (ng*h/ml)	2.1	4060±2380	6
	4.2	12000±5580	8
	6.3	16100±7220	6
	9	30100±12500	7
	12	23400±8080	7
C_{min} (pg/ml)	2.1	2900±1680	8
	4.2	8460±4000	8
	6.3	11400±5480	7
	9	19900±15200	9
	12	14500±7130	10
C_{av} (ng/ml)	2.1	3.74±2.85	9
	4.2	8.92±2.22	8
	6.3	12.3±4.4	7
	9	27.4±13.4	9
	12	19.5±6.68	10
MRT (day)	2.1	15.9±5.87	6
	4.2	16.7±5.74	8
	6.3	15±5.59	6
	9	10.9±1.74	7
	12	15.5±6.89	7

$t_{1/2}$: median[Q1-Q3].

Exposure (AUC_{inf}, C_{max}) was increased in proportion to the dose from the 2.1 μg/kg to the 9 μg/kg dose groups. On the other hand, no such increases were observed between the 9 μg/kg and 12 μg/kg dose groups. The 95% confidence interval for the y-intercept of the regression line between dose and C_{max} was 0 (zero) (Fig. 3).

Table IV. Most common reported adverse events related to study treatment (in ≥10% of patients in any treatment group).

Adverse event, n (%)	2.1 μg/kg (n=9)	4.2 μg/kg (n=8)	6.3 μg/kg (n=8)	9 μg/kg (n=11)	12 μg/kg (n=10)	Total (n=46)
Potassium increase	2 (22.2)	3 (37.5)	-	1 (9.1)	2 (20.0)	8 (17)
Neutrophils decrease	2 (22.2)	3 (37.5)	1 (12.5)	-	-	6 (13)
WBC decrease	3 (33.3)	3 (37.5)	-	-	-	6 (13)
Lymphocytes decrease	1 (11.1)	3 (37.5)	1 (12.5)	-	-	5 (11)
Sodium increase	2 (22.2)	-	-	1 (9.1)	2 (20.0)	5 (11)
Diarrhea	1 (11.1)	-	1 (12.5)	-	3 (30.0)	5 (11)
Constipation	3 (33.3)	2 (25.0)	-	-	-	5 (11)
Headache	1 (11.1)	-	3 (37.5)	1 (9.1)	-	5 (11)

C.E.R.A., continuous erythropoietin receptor activator; Q3W, once every 3 weeks.

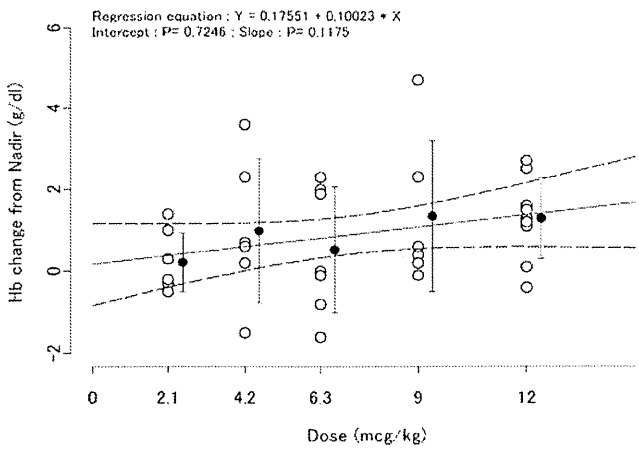

Figure 4. Correlation between the dose and change in Hb levels.

Photodynamic analyses. The change in Hb levels from the nadir value over the period weeks 1-4 to week 7 values is shown in Fig. 4. The changes in Hb levels were above 0 (zero) in the dosing groups and the slope between the dose of C.E.R.A. and the change in Hb was greater than 0 (zero) (Fig. 4).

Safety and tolerability. Q3W administration of C.E.R.A. was generally well tolerated across the dose groups. All of the patients reported at least one adverse event. Most adverse events were those expected in cancer patients receiving chemotherapy, and none occurred in a manner dependent on the C.E.R.A. dose. The percentage of patients reporting adverse events in which a causal correlation with the study medication cannot be completely excluded were 89, 75, 88, 64 and 40% in the 2.1, 4.2, 6.3, 9 and 12 μg/kg dose groups, respectively. The most common adverse events (incidence rate ≥10%) related to the study medication were potassium increase (17%), neutrophil decrease (13%), WBC decrease (11%), diarrhea (11%), constipation (11%) and headache (11%). A total of 10 patients experienced grade 3-4 adverse events related to the study treatment including WBC decrease, febrile neutropenia, neutrophil decrease, Hb decrease, platelet decrease, lymphocyte decrease, potassium increase and sodium decrease (Table IV). No clinically significant changes occurred from baseline in laboratory values and vital signs during the study period in the dose groups, or dose-dependent correlations with increased blood pressure. One patient in the 6.3 μg/kg dose group experienced grade 2 hypertension possibly associated with C.E.R.A. treatment from the 1st to the 14th day of the second administration. An antihypertensive medication was administered following the onset of hypertension. Blood pressure was then stabilized.

Serious adverse events were observed in one patient from each of the 4.2 and 6.3 μg/kg dose groups, and three patients in the 12 μg/kg dose group. Serious adverse events were evaluated as not related to the study medication.

Thrombovascular adverse events were not observed. Withdrawal of one patient in the 12 μg/kg dose group was attributed to disease progression and no mortality was reported during the study period. Moreover, no anti-C.E.R.A. antibodies were detected in any of the patients.

Discussion

Anemia is a frequent complication in patients with lung cancer who are administered chemotherapy (18,19). Anemia has a profound impact on QoL, with fatigue being one of its common symptoms (20-22). Furthermore, the widespread use of platinum-based chemotherapy contributes further to the development of anemia in patients with lung cancer (23).

C.E.R.A. is a chemically synthesized continuous erythropoietin receptor activator with a prolonged serum half-life that has been shown to be safe and effective for the treatment of chemotherapy-induced anemia when administered using Q1W or Q3W administration schedules (16).

This is the first study to examine the PK, PD and safety profiles of C.E.R.A. treatment subcutaneously Q3W in Japanese patients with lung cancer and anemia induced by chemotherapy. This study demonstrated that C.E.R.A. subcutaneously administered to Japanese lung cancer patients showed exposure in accordance with dose increase and a long half-life. Exposure (AUC_{inf}, C_{max}) following second administration increased with dose proportionality in the 2.1 to 9 μg/kg dose groups. On the

other hand, exposure was not increased across the 9 to 12 μg/kg dose groups. The reason for this non-linearity between dose and exposure may be due to the small number of patients, the verification of patient characteristics at baseline and the fact that the information was limited. It was considered to be reduced bioavailability from the SC injection site in the 12 μg/kg dose group, as the T_{max} values (an indicator of SC absorption rate) were similar among the dose groups and the $t_{1/2}$ values (indicator of elimination rate) also showed no major differences among the dose groups. A similar phenomenon was observed following SC injection of epoetin β (24). Nakagawa *et al* reported that C_{max} and AUC_{inf} following SC injection of epoetin β to lung cancer patients increased with dose-proportionality from the 9000 to 36000 IU dose groups and were similar between the 36000 and 54000 IU dose groups (25). These authors also suggested that declining bioavailability of epoetin β at higher doses is partially due to absorption following SC injection of this drug into the lymphatic system (26). The same mechanism may explain the reason for non-linearity between the dose of C.E.R.A. and levels of exposure, but further studies are required to clarify this non-linearity.

Median values for $t_{1/2}$ ranged from 143 to 217 h in this study. These results showed that the values for $t_{1/2}$ of C.E.R.A. were prolonged by 5-10 times compared with those reported for epoetin β in patients with lung cancer (24,25) or 2-4 times compared with those reported for darbepoetin α in patients with cancer (27).

Agoram *et al* suggested that the platinum-containing chemotherapy cycle count affected the clearance of darbepoetin α (28); thus, the PK parameters following the initial administration of C.E.R.A. were assessed to estimate the parameters under the same conditions of chemotherapy. Following the first C.E.R.A. administration, C_{max} showed dose-proportionality as the 95% confidence interval for the y-intercept of the regression line between dose and C_{max} was 0 (zero) (Fig. 3). These results suggested linear PKs of C.E.R.A. following SC administration, above the range of 2.1 to 12 μg/kg.

In addition, changes in Hb levels from the nadir value over the period weeks 1-4 to week 7 values were observed (Fig. 4). The change in Hb levels was above 0 (zero) in all dose groups and the slope of the line relating dose of C.E.R.A. to the change of Hb was over 0 (zero). These results suggest that the SC administration of C.E.R.A. above the range of 2.1 to 12 μg/kg Q3W increased Hb levels in these patients and these increases were somewhat dose-dependent. The Hb responses supported results previously observed in studies with C.E.R.A. in cancer patients following SC Q3W administration (15,16). Furthermore, the responses observed at doses of 9 and 12 μg/kg do not appear to have previously been reported.

All 46 patients receiving C.E.R.A. administration at least once were included in the safety analyses. C.E.R.A. was generally well tolerated across the dose groups, with adverse events that may be expected for patients with lung cancer receiving chemotherapy (e.g., neutrophil decrease), and these were similar to those reported for epoetin β (24). The most common adverse events were those expected in a cancer population receiving chemotherapy. Adverse events reported with regards to the study medication were observed in 32 (69.6%) patients. C.E.R.A. did not appear to have any adverse effects involving occurrence of thrombovascular events.

In conclusion, the dose proportionality observed regarding the PK and PD profiles and the good tolerability and safety profile in this study involving a small number of patients with lung cancer suggested that extended Q3W administration intervals are feasible in the clinic. However, further dose-finding studies may be required to determine the optimal C.E.R.A. dose regimen at Q3W in cancer patients with anemia induced by chemotherapy.

Acknowledgements

This study was funded by Chugai Pharmaceuticals Co. Ltd., Tokyo, Japan. Study medications were provided by Hoffmann-La Roche Inc. Responsibility for opinions, conclusions and interpretation of data lies with the authors.

References

1. Oberhoff C, Neri B, Amadori D, Perty KU, Gamucci T, Rebmann U and *et al*: Recombinant human erythropoietin in the treatment of chemotherapy induced anemia and prevention of transfusion requirement associated with solid tumors: a randomized, controlled study. Ann Oncol 9: 239-241, 1998.
2. Littlewood TJ, Bajetta E, Nortier JW, Vercammen E and Rapoport B: Epoetin Alfa Study Group: Effects of epoetin alfa on hematologic parameters and quality of life in cancer patients receiving nonplatinum chemotherapy; results of a randomized, double-blind, placebo-controlled trial. J Clin Oncol 19: 2865-2874, 2001.
3. Vansteenkiste J, Pirker R, Massuti B, Barata F, Font A, Fiegl M, Siena S, Gateley J, Tomita D, Colowick AB and Musil J: Double-blind placebo-controlled, randomized phase III trial of darbepoetin alfa in lung cancer patients receiving chemotherapy. J Natl Cancer Inst 94: 1211-1220, 2002.
4. Ludwig H, Fritz E, Kotzmann H, Hocker P, Gisslinger H and Barnus U: Erythropoietin treatment of anemia associated with multiple myeloma. N Engl J Med 322:1693-1699, 1990
5. Eshbach JW, Egrie JC, Dowing MR, Browne JK and Adamson JW: Correction of the anemia of end-stage renal disease with recombinant human erythropoietin. Results of a combined phases I and II clinical trial. N Engl J Med 316: 73-78, 1987.
6. Cazzola M, Beguin Y, Kloczko J, Spicka I and Coiffier B: Once-weekly epoetin beta is highly effective in treating anaemic patients with lymphoproliferative malignancy and defective endogenous erythropoietin production. Br J Haematol 122: 386-393, 2003.
7. Waltzman R, Croot C, Justice GR, Fesen MR, Charu V and Williams D: Randomized comparison of epoetin alfa (40,000 U weekly) and darbepoetin alfa (200 microg every 2 weeks) in anemic patients with cancer receiving chemotherapy. Oncologist 10: 642-650, 2005
8. Canon JL, Vansteenkiste J, Bodoky G, Mateos MV, Bastit L, Ferreira I, Rossi G and Amado RG: Randomized, double-blind, active-controlled trial of every-3-week darbepoetin alfa for the treatment of chemotherapy-induced anemia. J Natl Cancer Inst 98: 273-284, 2006.
9. Locatelli F and Reigner B: C.E.R.A. pharmacodynamics, pharmacokinetics and efficacy in patients with chronic kidney disease. Expert Opin Investig Drugs 16: 1649-1661, 2007.
10. Macdougall IC, Bailon P, Tare T and Pahlke W: CERA (Continuous Erythropoiesis Receptor Activator) for the treatment of renal anemia: an innovative agent with unique receptor binding characteristics and prolonged serum half-life. J Am Soc Nephrol 14: 769A (Abstract SU-PO 1063), 2003.
11. Macdougall IC: CERA (Continuous Erythropoietin Receptor Activator): a new erythropoiesis-stimulating agent for the treatment of anemia. Curr Hematol Rep 4: 436-440, 2005.
12. Haselbeck A, Reigner B, Jordan B, Pannier A and Glaspy J: Pre-clinical and Phase I pharmacokinetic and mode-of-action studies of CERA (Continuous Erythropoiesis Receptor Activator), an innovative erythropoietic agent with an extended serum half-life. Proc Am Soc Clin Oncol 22: 748 (Abstract 3006), 2003.

13. Dougherty FC, Reigner B, Jordan P and Pannier A: Continuous Erythropoiesis Receptor Activator (CERA) provides dose-dependent erythropoietic activity with a prolonged half-life in healthy volunteers. Ann Oncol 15: iii 157 (Abstract 592), 2004.
14. Dmoszynska A, Kloczko J, Rokicka M, Hellmann A, Spicka I and Eid JE: A dose exploration, phase I/II study of administration of continuous erythropoietin receptor activator once every 3 weeks in anemic patients with multiple myeloma receiving chemotherapy. Haematologica 92: 493-501, 2007.
15. Osterborg A, Steegmann JL, Hellmann A, Couban S, Mayer J and Eid JE: Phase II study of three dose levels of continuous erythropoietin receptor activator (C.E.R.A.) in anemic patients with aggressive non-Hodgkin's lymphoma receiving combination chemotherapy. Br J Haematol 136:736-744, 2007.
16. Hirsh V, Glaspy J, Mainwaring P, Manegold C, Ramlau R and Eid JE: Phase II study of two dose schedules of C.E.R.A. (Continuous Erythropoiesis Receptor Activator) in anemic patients with advanced non-small cell lung cancer (NSCLC) receiving chemotherapy. Trials 8: 8, 2007.
17. Osterborg A, Brandberg Y, Molostova V, Losava G, Abdalkadyrov K, Hedenus M and Messinger D: Randomized, double-blind, placebo-controlled trial of recombinant human erythropoietin, epoetin β, in hematologic malignancies. Journal of Clinical Oncology 20: 2486-2494, 2002.
18. Crawford J: Anemia and lung cancer. Lung Cancer 38: S75-S78, 2002.
19. Langer CJ, Choy H, Glaspy JA and Colowick A: Standards of care for anemia management in oncology; focus on lung carcinoma. Cancer 95: 613-623, 2002.
20. Cella D: Factors influencing quality of life in cancer patients: anemia and fatigue. Semin Oncol 25: 43-46, 1998.
21. Pirker R, Wiesenberger K, Pohl G and Minar W: Anemia in lung cancer: clinical impact and management. Clin Lung Cancer 5: 90-97, 2003.
22. Morère JF: Role of epoetin in the management of anemia in patients with lung cancer. Lung Cancer 46: 149-156, 2004.
23. Ludwig H, Van Belle S, Barett-Lee P, Birgegard G, Bokemeyer C, Gascón P, Kosmidis P, Krzakowski M, Nortier J, Olmi P, Schneider M and Schrijvers D: The European Cancer Anemia Survey (ECAS): a large, multinational, prospective survey defining the prevalence, incidence, and treatment of anemia in cancer patients. Eur J Cancer 40: 2293-2306, 2004.
24. Fujisaka Y, Tamura T, Ohe Y, Kunitoh H, Sekine I, Yamamoto N, Nokihara H, Horiike A, Kodama T and Saijo N: Pharmacokinetics and Pharmacodynamics of Weekly Epoetin Beta in Lung Cancer Patients. Jpn J Oncol 36: 477-482, 2006.
25. Nakagawa K, Ozaki T, Satoh T, Miyazaki M, Akashi Y, Terashima M, Fujisaka Y, Tamura T, Fukuoka M and Saijo N: Epoetin Beta Subcutaneous Administration to Lung Cancer Patients with Anemia; Results of Pharmacokinetics/Pharmacodynamics Study. Jpn J Lung Cancer 47: 313-322, 2007.
26. Tang L, Persky AM, Hochhaus G and Meibohm B: Pharmacokinetic aspects of biotechnology products. J Pharm Sci 93: 2184-2204, 2004.
27. Glaspy J, Henry D, Patel R and *et al*: Effects of chemotherapy on endogenous erythropoietin levels and the pharmacokinetics and erythropoietic response of darbepoetin alfa: a randomized clinical trial of synchronous versus asynchronous dosing of darbepoetin alfa. Eur J Cancer 41: 1140-1149, Epub 2005 Apr 8.
28. Agoram B, Heatherington AC and Gastonguay MR: Development and evaluation of a population pharmacokinetic-pharmacodynamic model of darbepoetin alfa in patients with nonmyeloid malignancies undergoing multicycle chemotherapy. AAPS J 8: E552-E563, 2006.

The Risk of Cytotoxic Chemotherapy-Related Exacerbation of Interstitial Lung Disease with Lung Cancer

Hirotsugu Kenmotsu, MD, Tateaki Naito, MD,* Madoka Kimura, MD,* Akira Ono, MD,* Takehito Shukuya, MD,* Yukiko Nakamura, MD,* Asuka Tsuya, MD,* Kyoichi Kaira, MD,* Haruyasu Murakami, MD,* Toshiaki Takahashi, MD,* Masahiro Endo, MD,† and Nobuyuki Yamamoto, MD**

Introduction: It is unknown what type of interstitial lung disease (ILD) has high risk for chemotherapy-related exacerbation of ILD. We investigated the risk of exacerbation of ILD for patients with lung cancer with ILD.

Methods: One hundred nine patients with lung cancer with ILD treated with cytotoxic chemotherapy at Shizuoka Cancer Center between August 2002 and April 2010 were retrospectively reviewed.

Results: On pretreatment computed tomography (CT) of the chest, 69 patients (63%) were identified with usual interstitial pneumonia (UIP) pattern, and 40 patients (37%) had non-UIP pattern. Patients with UIP pattern developed cytotoxic chemotherapy-related exacerbation of ILD more frequently than those with non-UIP pattern (30 versus 8%, $p = 0.005$). The incidence of grade 5 pulmonary toxicities was 9% in patients with UIP pattern, compared with 3% in those with non-UIP pattern. Multivariate analyses demonstrated that age (<70 years) and CT pattern (UIP) were significant independent risk factors for cytotoxic chemotherapy-related exacerbation of ILD. In small cell lung cancer, overall survival (OS) from the start of first-line chemotherapy was significantly shorter in UIP pattern than non-UIP pattern (median OS: 9 versus 16 months, $p = 0.0475$), whereas there was no significant difference in patients with non-small cell lung cancer (median OS: 12 versus 9 months, $p = 0.2529$).

Conclusions: Our results indicated that the incidence of exacerbation of ILD was significantly higher in patients with lung cancer with UIP pattern on CT findings than in those with non-UIP pattern. Therefore, great care is required when administering cytotoxic chemotherapy agents for patients with lung cancer with UIP pattern.

Key Words: Lung cancer, Interstitial lung disease, Usual interstitial pneumonia, Cytotoxic chemotherapy, Exacerbation.

(*J Thorac Oncol.* 2011;6: 1242–1246)

Divisions of *Thoracic Oncology, and †Diagnostic Radiology, Shizuoka Cancer Center, Nagaizumi-cho, Sunto-gun, Japan.

Disclosure: The authors declare no conflicts of interest.

Address for correspondence: Hirotsugu Kenmotsu, MD, Division of Thoracic Oncology, Shizuoka Cancer Center, 1007 Shimonagakubo Nagaizumi-cho, Sunto-gun, Shizuoka 411-8777, Japan. E-mail: h.kenmotsu@scchr.jp

ISSN: 1556-0864/11/0607-1242

Interstitial lung disease (ILD) is called diffuse parenchymal lung disease and is a diverse group of pulmonary disorders classified together because of similar clinical, radiological, physiological, or pathological features.[1] Preexisting ILD or idiopathic interstitial pneumonias (IIPs) are considered to be a risk factor for drug-related ILD.[2] A prospective large cohort study for gefitinib, an epidermal growth factor receptor tyrosine kinase inhibitor, has shown that preexisting ILD is not only a strong risk factor for gefitinib-related ILD but also a strong risk factor for cytotoxic chemotherapy-related ILD.[3] Cytotoxic chemotherapy agents, such as gemcitabine, docetaxel, and amrubicin, have been reported to develop severe ILD associated with cytotoxic chemotherapy.[4–6] Chemotherapy-related ILD is not common but is a potentially fatal complication of treatment for lung cancer.

Idiopathic pulmonary fibrosis (IPF) is a chronic progressive interstitial pneumonia of unknown cause limited to the lungs and associated with poor prognosis.[7,8] The American Thoracic Society (ATS) and European Respiratory Society (ERS) have defined IPF as clinical conditions characterized by progressive dyspnea and chronic cough, restrictive lung disease, and the histopathologic pattern of usual interstitial pneumonia (UIP).[7] In addition, in patients with IPF, the incidence of lung cancer is reported to be higher than in patients without IPF.[9–13]

In clinical practice, patients with lung cancer with ILD have been carefully treated with cytotoxic chemotherapy. Nevertheless, it is unknown what kind of chemotherapeutic agents are optimal for patients with lung cancer with ILD. In addition, it is also unknown what type of ILD has high risk for exacerbation of ILD.

To assess the risk of cytotoxic chemotherapy-related ILD, we retrospectively analyzed pretreatment computed tomography (CT) and investigated the clinical course of patients with lung cancer with ILD.

METHODS

The medical records of patients with lung cancer with ILD treated with cytotoxic chemotherapy at the Shizuoka Cancer Center between August 2002 and April 2010 were retrospectively reviewed. In this study, pretreatment CT of the chest was evaluated by one radiologist (M.E.) and two pulmonologists (H.K. and T.N.), who had no knowledge of

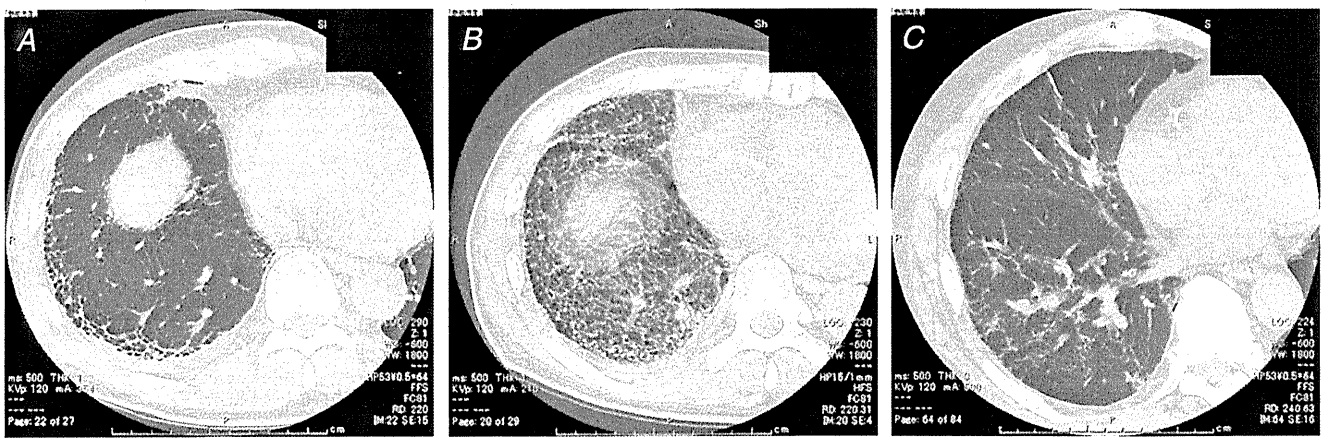

FIGURE 1. High-resolution computed tomography (HRCT) image of the chest. *A*, Pretreatment HRCT image of the chest showing subpleural distribution, honeycombing, traction bronchiectasis, and architectural distortion (UIP pattern). *B*, HRCT image of the chest showing ground-glass abnormality superimposed on pretreatment interstitial shadow (chemotherapy-related exacerbation of ILD). *C*, Pretreatment HRCT image of the chest showing patchy ground-glass opacity with reticulation, traction bronchiectasis, and architectural distortion (non-UIP pattern). UIP, usual interstitial pneumonia; ILD, interstitial lung disease.

the patient's outcome. The chest CT examinations were performed using multidetector-row CT machine at the end of suspended inspiration. CT images were reconstructed to 5-mm slice thickness, and thin section chest CT with 1 mm reconstruction thickness was also performed for evaluating primary tumor and ILD. ILD was diagnosed when the criteria of ground-glass opacity, consolidation, or reticular shadow in both lung fields were met. On the basis of CT characteristics, we classified the patients with ILD into two groups: UIP pattern and non-UIP pattern. Diagnosis of UIP pattern was based on CT features as defined by the International Consensus Statement of the ATS and ERS, showing subpleural distribution, honeycombing, traction bronchiectasis, and architectural distortion (Figure 1*A*).[7,14] All other cases, whose CT of the chest revealed ILD excluding the UIP pattern, were diagnosed as non-UIP pattern (Figure 1*C*).

Chemotherapy-related exacerbation of ILD was diagnosed on the basis of CT findings (bilateral ground-glass abnormality with or without focal consolidation, superimposed on pretreatment interstitial shadow) (Figure 1*B*).[15] Patients with apparent pulmonary infection, pulmonary embolism, or heart failure were excluded. Chemotherapy-related exacerbation of ILD was evaluated based on pneumonitis/pulmonary infiltrates by National Cancer Institute Common Terminology Criteria version 3.0—grade 3: symptomatic, interfering with activities of daily living, and oxygen indicated; grade 4: life-threatening; and grade 5: death. The patients who developed exacerbation of ILD within 1 year after thoracic radiotherapy and who received epidermal growth factor receptor tyrosine kinase inhibitor in the clinical course were excluded from chemotherapy-related exacerbation of ILD. To assess the incidence of exacerbation of ILD by treatment regimen, the duration between last administration of cytotoxic chemotherapy and the onset of exacerbation of ILD was defined as 4 weeks or less.

Univariate and multivariate analyses were performed to identify risk factors for the exacerbation of ILD associated with cytotoxic chemotherapy. All categorical variables were analyzed by the χ^2 test or Fisher's exact test, as appropriate. Multivariate analyses were performed using a logistic regression procedure to assess the relationship between various factors and exacerbation of ILD. Clinical evaluation of overall survival (OS) after the start of first-line chemotherapy was conducted by the Kaplan-Meier method to assess the time to death. The log-rank test was used to compare cumulative survival in each group. All p values were reported as two-sided, and values less than 0.05 were considered statistically significant. This study was approved by the institutional review board.

RESULTS

Patient Characteristics

One hundred nine patients were diagnosed with lung cancer with ILD and treated with cytotoxic chemotherapy. The characteristics of the patients are shown in Table 1. The median age was 69 years (range: 54–84 years), and almost all patients were smokers and men with good performance status. Histologically, adenocarcinoma, squamous cell carcinoma, and small cell lung cancer (SCLC) were observed in 33, 30, and 30%, respectively. Others included large cell carcinoma and undifferentiated non-small cell cancer. Stages III and IV were observed in 40 and 53%, respectively, and recurrence after surgical resection occurred in 7%. In SCLC, limited and extensive diseases were observed in 33 and 67%, respectively. On the basis of pretreatment CT of the chest, 69 patients (63%) were identified with UIP pattern, and 40 patients (37%) had non-UIP pattern. Although there were some imbalances between the two groups in terms of stage IV

TABLE 1. Patient Characteristics (Overall, n = 109)

	Total	UIP Pattern, n (%)	Non-UIP Pattern, n (%)	p
No. of patients	109	69	40	
Gender				0.117
Male	103	67 (97)	36 (90)	
Female	6	2 (3)	4 (10)	
Age (yr), median (range)	69 (54–84)	70 (55–84)	69 (54–80)	0.245
Smoking status				0.763
Never smoker	0	0	0	
Ex-smoker	47	29 (42)	18 (45)	
Current smoker	62	40 (58)	22 (55)	
Performance status (ECOG)				0.150
0–1	94	62 (90)	32 (80)	
2–3	15	7 (10)	8 (20)	
Histology				0.723
Adenocarcinoma	36	22 (32)	14 (35)	
Squamous cell carcinoma	33	21 (30)	11 (28)	
SCLC	33	20 (29)	13 (32)	
Others	8	6 (9)	2 (5)	
Clinical stages				0.044
IIIA and B	44	34 (49)	10 (25)	
IV	57	31 (45)	26 (65)	
Recurrence after surgical resection	8	4 (6)	4 (10)	
SCLC				0.314
Limited disease	11	8 (40)	3 (23)	
Extensive disease	22	12 (60)	10 (77)	

UIP, usual interstitial pneumonia; SCLC, small cell lung cancer; ECOG, Eastern Cooperative Oncology Group.

TABLE 2. Incidence of Cytotoxic Chemotherapy-Related Exacerbation of ILD

	No. of Patients (%)			
	Total, n (%)	UIP Pattern, n (%)	Non-UIP Pattern, n (%)	p
Overall	109	69	40	
Exacerbation of ILD	24 (22)	21 (30)	3 (8)	0.005
≥ Grade 3	22 (20)	20 (29)	2 (5)	0.003
Grade 3	5 (5)	4 (6)	1 (3)	
Grade 4	10 (9)	10 (14)	0	
Grade 5	7 (6)	6 (9)	1 (3)	

UIP, usual interstitial pneumonia; ILD, interstitial lung disease.

TABLE 3. Cytotoxic Chemotherapy Agents Considered to Cause the Exacerbation of ILD

	UIP Pattern		Non-UIP Pattern	
	No. of Patients Administered	Exacerbation of ILD (%)	No. of Patients Administered	Exacerbation of ILD (%)
Cisplatin	21	2 (10)	21	1 (5)
Carboplatin	40	5 (13)	19	0
Paclitaxel	31	1 (3)	14	0
Docetaxel	25	7 (28)	12	1 (8)
Etoposide	21	5 (24)	10	0
Vinorelbine	13	0	6	0
Gemcitabine	7	3 (43)	10	1 (10)
S-1	7	2 (29)	7	1 (14)
Irinotecan	6	2 (33)	6	0
Amrubicin	4	0	6	0
Pemetrexed	2	1 (50)	1	0

UIP, usual interstitial pneumonia; ILD, interstitial lung disease.

(p = 0.044), there were no significant differences in patient characteristics between both groups.

Incidence of Cytotoxic Chemotherapy-Related Exacerbation of ILD

Of the 109 patients with ILD, 24 (22%) developed cytotoxic chemotherapy-related exacerbation of ILD. In particular, patients with UIP pattern developed cytotoxic chemotherapy-related exacerbation of ILD more frequently than those with non-UIP pattern (30 versus 8%, p = 0.005; Table 2). In addition, the incidence of grade 3 or worse pneumonitis/pulmonary infiltrates was significantly higher in patients with UIP pattern than in patients with non-UIP pattern (29 versus 5%, p = 0.003). Almost all of the patients who developed grade 3 or worse pulmonary toxicities received corticosteroid therapy. Nevertheless, 9% of the patients with UIP pattern died because of exacerbation of ILD, whereas 3% of those with non-UIP pattern died.

The median time from last administration of cytotoxic chemotherapy to the diagnosis of the exacerbation of ILD was 17 days (range: 0–25 days). The incidence rate of exacerbation of ILD is shown in Table 3 for each agent; docetaxel (28%) or etoposide (24%) frequently led to exacerbation of ILD for patients with UIP pattern. On the other hand, the incidence of exacerbation of ILD was relatively low for vinorelbine or paclitaxel. Cisplatin or carboplatin was mainly administered with another agent, and it was difficult to assess the risk for ILD. In patients with SCLC, 63% of exacerbation of ILD occurred during the first-line chemotherapy, whereas in patients with non-small cell lung cancer (NSCLC) the corresponding proportion was 31%. In addition, only one patient received further chemotherapy after exacerbation of ILD.

The Risk of Cytotoxic Chemotherapy-Related Exacerbation of ILD

The results of the univariate analysis of risk factors for cytotoxic chemotherapy-related exacerbation of ILD are shown in Table 4. UIP pattern on CT was significantly associated with the exacerbation of ILD (p = 0.005). Multivariate analyses were performed using three variables (age, performance status, and CT pattern), and the results demonstrated that age (<70 years) (odds ratio [OR]: 2.75, 95% confidence interval: 1.03–7.93) and CT pattern (UIP) (OR:

TABLE 4. Univariate Analysis of Risk Factors Associated with Cytotoxic Chemotherapy-Related Exacerbation of ILD

	No. of Patients			
	Overall	Ex of ILD	Non-Ex of ILD	*p*
No. of patients	109	24	85	
Gender				—
Male	103	24	79	
Female	6	0	6	
Age (yr)				0.0897
<70	56	16	40	
≥70	53	8	45	
ECOG-PS				0.7043
0–1	94	20	74	
2–3	15	4	11	
Histology				0.7119
NSCLC	76	16	60	
SCLC	33	8	25	
CT pattern				0.0054
UIP	69	21	48	
Non-UIP	40	3	37	
Stage				0.3186
IIIA and B	44	7	37	
IV	57	14	43	
Recurrence after surgical resection	8	3	5	

UIP, usual interstitial pneumonia; Ex, exacerbation; ILD, interstitial lung disease; PS, performance status; NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer; CT, computed tomography; ECOG, Eastern Cooperative Oncology Group.

TABLE 5. Multivariate Analysis of Risk Factors Associated with Cytotoxic Chemotherapy-Related Exacerbation of ILD

Variable	Odds Ratio	95% CI	*p*
Age (<70 yr)	2.75	1.03–7.93	0.0495
ECOG-PS (2 and 3)	2.20	0.51–8.74	0.2653
CT pattern (UIP)	6.98	2.04–33.79	0.0053

PS, performance status; CT, computed tomography; UIP, usual interstitial pneumonia; ECOG, Eastern Cooperative Oncology Group.

6.98, 95% confidence interval: 2.04–33.79) were significant independent risk factors (Table 5).

Overall Survival

In this analysis, the median follow-up duration was 10.3 months. In SCLC, OS from the start of first-line chemotherapy was significantly shorter in patients with UIP pattern than those with non-UIP pattern (median OS: 9 versus 16 months, $p = 0.048$), whereas there was no significant difference in patients with NSCLC (median OS: 11 versus 9 months, $p = 0.334$).

DISCUSSION

In patients with IPF, the incidence of lung cancer is reported to be higher than in patients without IPF,[9–13] and IPF has been recognized to be an independent risk factor for lung carcinogenesis.[11] There are some reports that patients with lung cancer with preexisting ILD or pulmonary fibrosis have a high risk of developing exacerbation after anticancer therapy,[3,16–18] and the incidence of exacerbation of ILD was 20 to 24%.[16,17] It is very important to establish an optimal treatment, which is considered to be safe and effective, for patients with lung cancer with ILD or IPF.

To our knowledge, this is the first study to evaluate the risk of cytotoxic chemotherapy-related ILD based on pretreatment chest CT patterns. In clinical practice, patients with lung cancer with ILD have been carefully treated with cytotoxic chemotherapy. Nevertheless, it is unknown what type of ILD has a high risk for exacerbation of ILD. In this study, patients with lung cancer with UIP pattern on CT findings demonstrated a high risk of exacerbation of ILD, compared with those with non-UIP pattern. This result suggests that chest CT patterns could be a risk factor for the development of chemotherapy-related exacerbation of ILD. Although age (<70 years) was also shown to be a risk factor, these patients might tend to receive multiple drugs for longer periods than elderly patients.

As there have been few reports about chemotherapy for patients with lung cancer with ILD, the optimal agent remains controversial. From Japan, a prospective study to evaluate the safety and efficacy of weekly paclitaxel in combination with carboplatin for advanced NSCLC with IIPs was reported.[19] One of 18 patients enrolled in this prospective study developed exacerbation of IIPs. Our study also showed that carboplatin and paclitaxel were relatively safe for patients with lung cancer with ILD, for whom this regimen might be one of the optimal regimens for those patients. Our results suggested that vinorelbine might also be relatively safe for patients with ILD. Nevertheless, we could not completely rule out the influence of biopsy for lung cancer diagnosis, before chemotherapy and before radiotherapy. It is known that the long-term survival in IPF shows poor prognosis compared with non-IPF, such as nonspecific interstitial pneumonia and other subgroups of IIPs.[1] In this study, although the UIP pattern on CT was significantly associated with the exacerbation of ILD, in patients with NSCLC with UIP pattern OS was not significantly different from those with non-UIP pattern. On the other hand, OS was significantly shorter in patients with SCLC with UIP pattern than in those with non-UIP pattern, and the type of ILD might influence the prognosis of patients with SCLC with ILD. Sixty-three percent of exacerbation of ILD in patients with SCLC occurred during the first-line chemotherapy, and they could not receive subsequent chemotherapy. On the other hand, approximately 70% of exacerbation of ILD in patients with NSCLC occurred during the second or subsequent line of chemotherapy and completed first-line chemotherapy. Thus, the rate of failure in first-line chemotherapy might contribute to poor prognosis in SCLC.

A major limitation of this retrospective analysis was that the diagnosis of ILD was based on CT findings and not on histologic diagnosis. In addition, the diagnosis of exacerbation of ILD was also based on CT findings, and we could not confirm histologically the exacerbation of ILD. Although we tried to exclude infection by bacteriological examination

and heart failure by physical examination or echocardiography, we cannot completely exclude pulmonary infection, pulmonary embolism, or heart failure. Nevertheless, their clinical and radiological courses were consistent with exacerbation of ILD. It was reported that in clinical practice, surgical lung biopsies were performed in 8 to 12% of patients,[20] and the ATS/ERS consensus statement also described criteria for the clinical diagnosis of IPF.[7] Moreover, the ability of high-resolution computed tomography scanning to diagnose IPF has reported sensitivities of 43 to 78% and specificities of 90 to 97% for confident radiological diagnosis.[21–25] Thus, we consider that it is appropriate to diagnose IPF using the clinical and radiological findings in clinical practice. Further studies are needed to clarify the relationship between the radiological patterns and pathological patterns of ILD for patients with lung cancer.

In conclusion, our study indicated that in patients with lung cancer with UIP pattern on CT findings, the risk of exacerbation of ILD was significantly higher than in those with non-UIP pattern. In particular, greater care is required when administering cytotoxic chemotherapy agents for patients with lung cancer with UIP pattern on CT findings.

REFERENCES

1. King TE Jr. Clinical advances in the diagnosis and therapy of the interstitial lung diseases. *Am J Respir Crit Care Med* 2005;172:268–279.
2. Camus P, Kudoh S, Ebina M. Interstitial lung disease associated with drug therapy. *Br J Cancer* 2004;91(Suppl 2):S18–S23.
3. Kudoh S, Kato H, Nishiwaki Y, et al. Interstitial lung disease in Japanese patients with lung cancer: a cohort and nested case-control study. *Am J Respir Crit Care Med* 2008;177:1348–1357.
4. Pavlakis N, Bell DR, Millward MJ, et al. Fatal pulmonary toxicity resulting from treatment with gemcitabine. *Cancer* 1997;80:286–291.
5. Read WL, Mortimer JE, Picus J. Severe interstitial pneumonitis associated with docetaxel administration. *Cancer* 2002;94:847–853.
6. Yoh K, Kenmotsu H, Yamaguchi Y, et al. Severe interstitial lung disease associated with amrubicin treatment. *J Thorac Oncol* 2010;5:1435–1438.
7. American Thoracic Society/European Respiratory Society International Multidisciplinary Consensus Classification of the Idiopathic Interstitial Pneumonias. This joint statement of the American Thoracic Society (ATS), and the European Respiratory Society (ERS) was adopted by the ATS board of directors, June 2001 and by the ERS Executive Committee, June 2001. *Am J Respir Crit Care Med* 2002;165:277–304.
8. Bjoraker JA, Ryu JH, Edwin MK, et al. Prognostic significance of histopathologic subsets in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 1998;157:199–203.
9. Wiggins J, Strickland B, Turner-Warwick M. Combined cryptogenic fibrosing alveolitis and emphysema: the value of high resolution computed tomography in assessment. *Respir Med* 1990;84:365–369.
10. Matsushita H, Tanaka S, Saiki Y, et al. Lung cancer associated with usual interstitial pneumonia. *Pathol Int* 1995;45:925–932.
11. Hubbard R, Venn A, Lewis S, et al. Lung cancer and cryptogenic fibrosing alveolitis. A population-based cohort study. *Am J Respir Crit Care Med* 2000;161:5–8.
12. Le Jeune I, Gribbin J, West J, et al. The incidence of cancer in patients with idiopathic pulmonary fibrosis and sarcoidosis in the UK. *Respir Med* 2007;101:2534–2540.
13. Ozawa Y, Suda T, Naito T, et al. Cumulative incidence of and predictive factors for lung cancer in IPF. *Respirology* 2009;14:723–728.
14. Lynch DA, Godwin JD, Safrin S, et al. High-resolution computed tomography in idiopathic pulmonary fibrosis: diagnosis and prognosis. *Am J Respir Crit Care Med* 2005;172:488–493.
15. Collard HR, Moore BB, Flaherty KR, et al. Acute exacerbations of idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2007;176:636–643.
16. Isobe K, Hata Y, Sakamoto S, et al. Clinical characteristics of acute respiratory deterioration in pulmonary fibrosis associated with lung cancer following anti-cancer therapy. *Respirology* 2010;15:88–92.
17. Minegishi Y, Takenaka K, Mizutani H, et al. Exacerbation of idiopathic interstitial pneumonias associated with lung cancer therapy. *Intern Med* 2009;48:665–672.
18. Niho S, Goto K, Yoh K, et al. Interstitial shadow on chest CT is associated with the onset of interstitial lung disease caused by chemotherapeutic drugs. *Jpn J Clin Oncol* 2006;36:269–273.
19. Minegishi Y, Sudoh J, Kuribayasi H, et al. The safety and efficacy of weekly paclitaxel in combination with carboplatin for advanced non-small cell lung cancer with idiopathic interstitial pneumonias. *Lung Cancer* 2011;71:70–74.
20. The diagnosis, assessment and treatment of diffuse parenchymal lung disease in adults. Introduction. *Thorax* 1999;54(Suppl 1):S1–S14.
21. Flaherty KR, King TE Jr, Raghu G, et al. Idiopathic interstitial pneumonia: what is the effect of a multidisciplinary approach to diagnosis? *Am J Respir Crit Care Med* 2004;170:904–910.
22. Hunninghake GW, Zimmerman MB, Schwartz DA, et al. Utility of a lung biopsy for the diagnosis of idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2001;164:193–196.
23. Johkoh T, Müller NL, Cartier Y, et al. Idiopathic interstitial pneumonias: diagnostic accuracy of thin-section CT in 129 patients. *Radiology* 1999;211:555–560.
24. Raghu G, Mageto YN, Lockhart D, et al. The accuracy of the clinical diagnosis of new-onset idiopathic pulmonary fibrosis and other interstitial lung disease: a prospective study. *Chest* 1999;116:1168–1174.
25. Swensen SJ, Aughenbaugh GL, Myers JL. Diffuse lung disease: diagnostic accuracy of CT in patients undergoing surgical biopsy of the lung. *Radiology* 1997;205:229–234.

VOLUME 29 · NUMBER 21 · JULY 20 2011

JOURNAL OF CLINICAL ONCOLOGY ORIGINAL REPORT

Biomarker Analyses and Final Overall Survival Results From a Phase III, Randomized, Open-Label, First-Line Study of Gefitinib Versus Carboplatin/Paclitaxel in Clinically Selected Patients With Advanced Non–Small-Cell Lung Cancer in Asia (IPASS)

Masahiro Fukuoka, Yi-Long Wu, Sumitra Thongprasert, Patrapim Sunpaweravong, Swan-Swan Leong, Virote Sriuranpong, Tsu-Yi Chao, Kazuhiko Nakagawa, Da-Tong Chu, Nagahiro Saijo, Emma L. Duffield, Yuri Rukazenkov, Georgina Speake, Haiyi Jiang, Alison A. Armour, Ka-Fai To, James Chih-Hsin Yang, and Tony S.K. Mok

See accompanying editorial on page 2843; listen to the podcast by Dr Sequist on www.jco.org/podcast

From the Kinki University School of Medicine; AstraZeneca, Osaka, Japan; Guangdong General Hospital, Guangzhou; Cancer Hospital, Chinese Academy of Medical Sciences, Beijing; State Key Laboratory in Oncology in South China, Li Ka Shing Institute of Health Science and the Sir Y.K. Pao Center for Cancer, The Chinese University of Hong Kong, Hong Kong, China; Maharaj Nakorn ChiangMai Hospital, ChiangMai University, ChiangMai; Prince of Songkla University, Songkla; Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand; National Cancer Centre Singapore, Singapore; Tri-Service General Hospital; National Taiwan University Hospital and College of Medicine, Taipei, Taiwan; and AstraZeneca, Macclesfield, United Kingdom.

Submitted November 1, 2010; accepted February 14, 2011; published online ahead of print at www.jco.org on June 13, 2011.

Supported by AstraZeneca.

Presented in part at the 45th Annual Meeting of the American Society of Clinical Oncology, May 29-June 2, 2009, Orlando, FL; at the 13th World Conference on Lung Cancer, July 31-August 4, 2009, San Francisco, CA; and at the 35th European Society for Medical Oncology Congress, October 8-12, 2010, Milan, Italy.

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

Clinical Trials repository link available on JCO.org.

Corresponding author: Tony S.K. Mok, MD, The Chinese University of Hong Kong, 22D Union Court, 18 Fu Kin Street, Shatin, Hong Kong, China; e-mail: tony@clo.cuhk.edu.hk.

0732-183X/11/2921-2866/$20.00

DOI: 10.1200/JCO.2010.33.4235

ABSTRACT

Purpose
The results of the Iressa Pan-Asia Study (IPASS), which compared gefitinib and carboplatin/paclitaxel in previously untreated never-smokers and light ex-smokers with advanced pulmonary adenocarcinoma were published previously. This report presents overall survival (OS) and efficacy according to epidermal growth factor receptor (EGFR) biomarker status.

Patients and Methods
In all, 1,217 patients were randomly assigned. Biomarkers analyzed were *EGFR* mutation (amplification mutation refractory system; 437 patients evaluable), *EGFR* gene copy number (fluorescent in situ hybridization; 406 patients evaluable), and EGFR protein expression (immunohistochemistry; 365 patients evaluable). OS analysis was performed at 78% maturity. A Cox proportional hazards model was used to assess biomarker status by randomly assigned treatment interactions for progression-free survival (PFS) and OS.

Results
OS (954 deaths) was similar for gefitinib and carboplatin/paclitaxel with no significant difference between treatments overall (hazard ratio [HR], 0.90; 95% CI, 0.79 to 1.02; $P = .109$) or in *EGFR* mutation–positive (HR, 1.00; 95% CI, 0.76 to 1.33; $P = .990$) or *EGFR* mutation–negative (HR, 1.18; 95% CI, 0.86 to 1.63; $P = .309$; treatment by *EGFR* mutation interaction $P = .480$) subgroups. A high proportion (64.3%) of *EGFR* mutation–positive patients randomly assigned to carboplatin/paclitaxel received subsequent EGFR tyrosine kinase inhibitors. PFS was significantly longer with gefitinib for patients whose tumors had both high *EGFR* gene copy number and *EGFR* mutation (HR, 0.48; 95% CI, 0.34 to 0.67) but significantly shorter when high *EGFR* gene copy number was not accompanied by *EGFR* mutation (HR, 3.85; 95% CI, 2.09 to 7.09).

Conclusion
EGFR mutations are the strongest predictive biomarker for PFS and tumor response to first-line gefitinib versus carboplatin/paclitaxel. The predictive value of *EGFR* gene copy number was driven by coexisting *EGFR* mutation (post hoc analysis). Treatment-related differences observed for PFS in the *EGFR* mutation–positive subgroup were not apparent for OS. OS results were likely confounded by the high proportion of patients crossing over to the alternative treatment.

J Clin Oncol 29:2866-2874. © 2011 by American Society of Clinical Oncology

INTRODUCTION

The epidermal growth factor receptor (EGFR) represents an important signaling pathway that regulates tumorigenesis and cell survival and is frequently overexpressed in the development and progression of non–small-cell lung cancer (NSCLC).[1-4] EGFR tyrosine kinase inhibitors (TKIs) such as gefitinib (Iressa, AstraZeneca, Macclesfield, United Kingdom) are effective in the treatment of relapsed NSCLC,[5,6] with certain clinical subgroups deriving greater clinical benefit (adenocarcinoma histology,

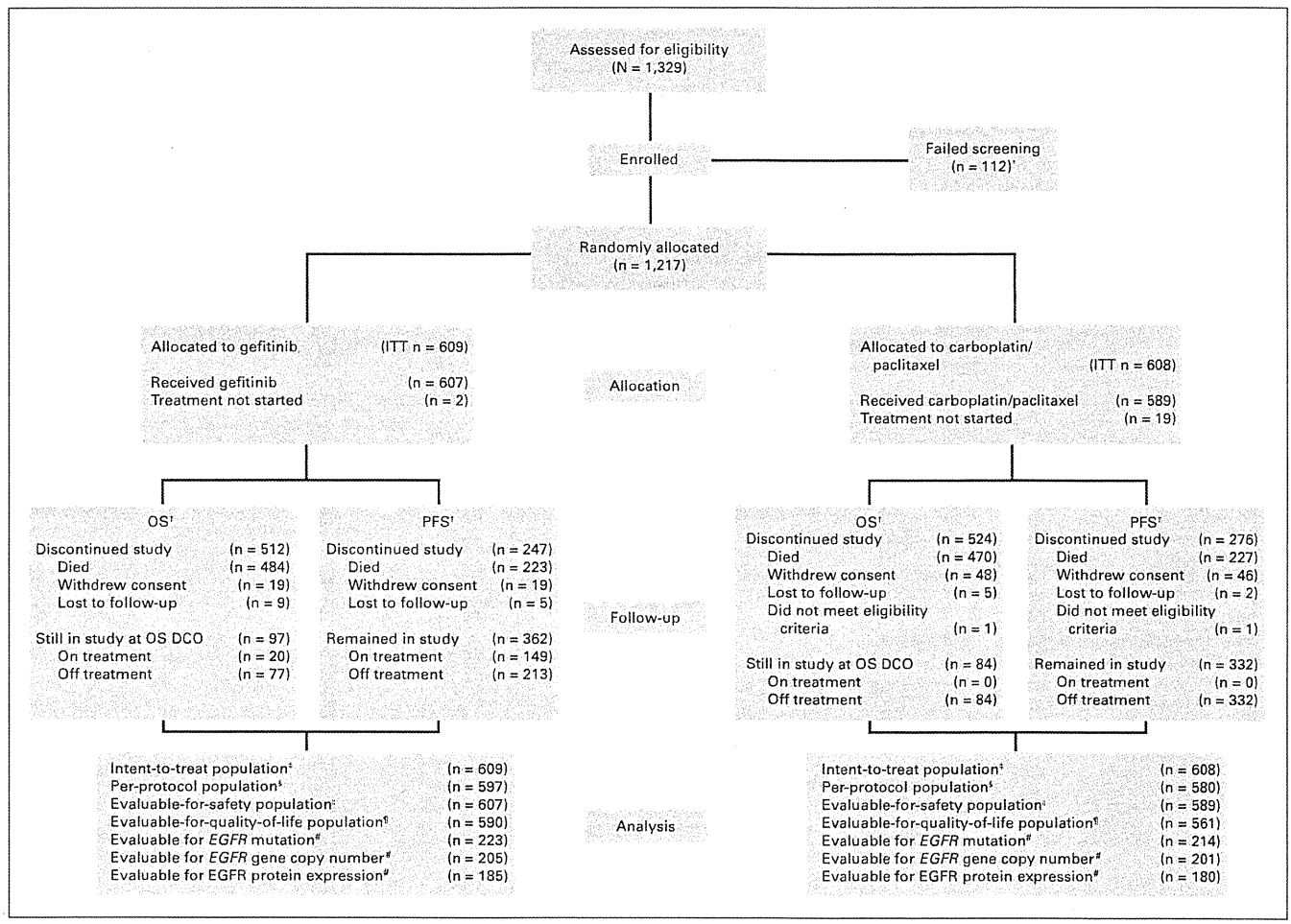

Fig 1. CONSORT diagram. (*) Among the 112 patients who failed screening, the main reasons for exclusion were abnormal serum creatinine (> 1.5 × upper limit of reference range)/creatinine clearance (≤ 60 mL/min) levels; untreated CNS metastases; or low neutrophil (< 2.0 × 10^9/L), platelet (< 100 × 10^9/L), or hemoglobin (< 10 g/dL) counts. (†) Cutoff dates: June 14, 2010, for overall survival (OS) and April 14, 2008, for progression-free survival (PFS). (‡) All patients who were randomly assigned to a study group were included in the intent-to-treat (ITT) analysis. (§) Patients who did not deviate substantially from the inclusion and exclusion criteria at entry or from the protocol were included in the per-protocol analysis. (‖) All patients who received at least one dose of study treatment were included in the safety analysis. (¶) All patients with a baseline and at least one postbaseline quality-of-life assessment that could be evaluated were included in the quality-of-life analysis. (#) All patients in the ITT population with an evaluable tumor sample. Of 683 patients (56%) who provided samples, 118 were cytology samples, and 128 were histologic samples of insufficient quality and were therefore not included in the main analysis. DCO, data cutoff; EGFR, epidermal growth factor receptor.

Asian ethnicity, female sex, and never-smoker status).[5-7] These subgroups are associated with a higher incidence of activating somatic mutations of the *EGFR* gene.[8-10] Optimization of anti-EGFR therapy depends on patient selection, and the exploration and identification of predictive biomarkers is important.

EGFR mutations, *EGFR* gene copy number, and EGFR protein expression are three EGFR-related biomarkers that have been studied in major clinical trials.[11-14] The significant overlap between EGFR biomarkers and limited availability of tumor samples in some studies made the interpretation of their individual predictive and prognostic values difficult.

Prolonged progression-free survival (PFS) and higher objective response rate (ORR) have been reported in patients with high *EGFR* gene copy number in single-arm and placebo-controlled randomized studies.[12,15-17] However, in the large phase III, randomized Iressa NSCLC Trial Evaluating Response and Survival Versus Taxotere (INTEREST) study with an active comparator, high *EGFR* gene copy number was not predictive for differential survival between gefitinib and docetaxel in patients with advanced NSCLC.[18]

The Iressa Pan-Asia Study (IPASS) is a phase III, randomized study of gefitinib versus carboplatin/paclitaxel in previously untreated never-smokers and light ex-smokers with advanced pulmonary adenocarcinoma in East Asia. As previously reported, IPASS exceeded its primary objective of noninferiority, demonstrating superiority of gefitinib relative to carboplatin/paclitaxel for PFS in this clinically selected population.[19] The treatment effect was not constant over time, driven by different outcomes according to mutation status. In the subgroup of patients with *EGFR* mutation–positive tumors, PFS was significantly longer for gefitinib versus carboplatin/paclitaxel (hazard ratio [HR], 0.48; 95% CI, 0.36 to 0.64; $P < .001$; median PFS, 9.5 *v* 6.3 months). Conversely, carboplatin/paclitaxel was superior in the *EGFR* mutation–negative subgroup (HR, 2.85; 95% CI, 2.05 to 3.98; $P < .001$; median PFS, 5.5 *v* 1.5 months); similarly, ORR significantly favored gefitinib and carboplatin/paclitaxel in the *EGFR* mutation–

Table 1. Summary of All Systemic Treatment After Discontinuation of Randomly Assigned Treatment in the Overall Population and in *EGFR* Mutation Subgroups (ITT population; data from OS data cutoff)

	Overall Population				*EGFR* Mutation Positive				*EGFR* Mutation Negative				*EGFR* Mutation Unknown			
	G (n = 609)		C/P (n = 608)		G (n = 132)		C/P (n = 129)		G (n = 91)		C/P (n = 85)		G (n = 386)		C/P (n = 394)	
Treatment	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Still on study treatment	20	3.3	0	0	3	2.3	0	0	1	1.1	0	0	16	4.1	0	0
None	190	31.2	230	37.8	29	22.0	37	28.7	21	23.1	25	29.4	140	36.3	168	42.6
Chemotherapy	393	64.5	251	41.3	99	75.0	61	47.3	69	75.8	44	51.8	225	58.3	146	37.1
Platinum-based†‡	363	59.6	55	9.0	90	68.2	13	10.1	65	71.4	10	11.8	208	53.9	32	8.1
C/P†‡	301	49.4	3	0.5	72	54.5	0	0	52	57.1	0	0	177	45.9	3	0.8
EGFR TKI	119	19.5	313	51.5	34	25.8	83	64.3	13	14.3	43	50.6	72	18.7	187	47.5
Gefitinib*†§	29	4.8	250	41.1	6	4.5	61	47.3	4	4.4	33	38.8	19	4.9	156	39.6
Erlotinib†§	71	11.7	83	13.7	16	12.1	31	24.0	9	9.9	7	8.2	46	11.9	45	11.4
Other EGFR TKI†§	33	5.4	35	5.8	15	11.4	12	9.3	2	2.2	5	5.9	16	4.1	18	4.6

NOTE. A patient may appear in more than one post-discontinuation treatment group. Patients may have received the same first- and second-line therapy. "None" is defined as patients who did not receive any form of cancer treatment after discontinuation of randomly assigned treatment. Radiotherapy, surgery, medical procedures, and other treatments were excluded.

Abbreviations: EGFR, epidermal growth factor receptor; ITT, intent-to-treat; OS, overall survival; G, gefitinib; C/P, carboplatin/paclitaxel; TKI, tyrosine kinase inhibitor.

*Non–study medication after discontinuation of randomly assigned study treatment.

†Patients may have also received other chemotherapy and/or EGFR TKIs during the study.

‡Excludes single platinum-based chemotherapy.

§Patients may have had more than one type of EGFR TKI and are counted once for each type received.

positive and *EGFR* mutation–negative subgroups, respectively.[19] A total of 1,038 of 1,217 patients consented to the preplanned exploratory biomarker analyses; 683 patients provided samples.

Early analysis of survival data (37% maturity) was presented in 2008.[19] Here we present the final results of the survival analyses and the results of the preplanned and post hoc analyses of the relationships between EGFR biomarkers (*EGFR* mutation, *EGFR* gene copy number, and EGFR protein expression) and clinical outcomes from IPASS.

PATIENTS AND METHODS

Study Design and Treatment

Full details of IPASS have been published previously.[19] Eligible patients had stage IIIB to IV pulmonary adenocarcinoma (including bronchoalveolar carcinoma), were either never-smokers (< 100 cigarettes in their lifetime) or light ex-smokers (stopped smoking ≥ 15 years previously and smoked ≤ 10 pack-years), and had received no prior chemotherapy or biologic or immunologic therapy.

Patients were randomly assigned 1:1 to gefitinib (250 mg/d) or carboplatin/paclitaxel (Paraplatin/Taxol, Bristol-Myers Squibb, Princeton, NJ; paclitaxel 200 mg/m^2 was given intravenously over 3 hours on day 1, immediately followed by carboplatin area under the serum concentration-time curve [AUC] 5.0 or 6.0 intravenously over 15 to 60 minutes in once every 3 weeks cycles for ≤ six cycles).

The primary objective of IPASS was noninferiority of gefitinib relative to carboplatin/paclitaxel in terms of PFS. ORR and overall survival (OS) were secondary end points. Evaluation of biomarker status (*EGFR* mutation, gene copy number, and protein expression) and efficacy of gefitinib versus carboplatin/paclitaxel were preplanned exploratory objectives. Post hoc analyses included clinical outcomes according to *EGFR* mutation subtype, *EGFR* gene copy number by *EGFR* mutation status, and clinical outcomes for patients with tumor *EGFR* gene high polysomy, and *EGFR* gene amplification. Correlation between *EGFR* mutation status and *EGFR* gene copy number was also investigated.

Patients provided written, informed consent with separate consent obtained for optional provision of tumor material for biomarker analyses. Study approval was obtained from independent ethics committees at each institution. The study was conducted in accordance with the Declaration of Helsinki, the International Conference on Harmonization/Good Clinical Practice, applicable regulatory requirements, and AstraZeneca's policy on bioethics.

Biomarker Analyses

Biomarker status was determined by analyzing paraffin-embedded archival tumor tissue in the following priority order: (1) *EGFR* mutation status, (2) *EGFR* gene copy number, (3) EGFR protein expression. Analyses were conducted at two central laboratories (Genzyme, Framingham, MA, and Quintiles-Lab in association with Peking Union Medical College Hospital, Beijing, China); scientists were blinded to clinical outcome and randomly assigned treatment. Samples underwent central histopathologic review; only those considered suitable for downstream biomarker analysis were progressed (on the basis of quality, sample source, and tumor content). If a patient provided more than one sample, the appropriate section was selected before database lock and analyzed on the basis of sample quality and largest area of tumor tissue.[20]

EGFR mutations were detected by using an amplification mutation refractory system with an *EGFR* mutation detection kit (DxS, Manchester, United Kingdom).[21,22] Patients were considered *EGFR* mutation positive if at least one of 29 *EGFR* mutations (Data Supplement) was detected. Additional validation for samples with T790M mutations was performed by using three methods: DNA sequencing, multithreaded electronic polymerase chain reaction sequencing, and an alternative amplification mutation refractory system assay (Data Supplement). *EGFR* gene copy number was measured by using fluorescent in situ hybridization and a previously published methodology.[15] High *EGFR* gene copy number was defined according to the University of Colorado Scoring System, which included both high polysomy (≥ four copies in ≥ 40% of cells; score 5) or gene amplification (presence of tight *EGFR* gene clusters and a ratio of gene/chromosome per cell ≥ two, or ≥ 15 copies of *EGFR* per cell in ≥ 10% of analyzed cells; score 6).[15] EGFR protein expression was assessed by immunohistochemistry by using the DAKO EGFR pharmDx kit (Dako, Glostrup, Denmark). Positive EGFR protein expression status was defined as having ≥ 10% of cells stained.

Statistical Analyses

The study statistician performed the statistical analyses at AstraZeneca. In the overall population and clinical subgroups, OS was analyzed by using a Cox proportional hazards model adjusted for the same covariates as for the primary PFS analysis (WHO performance status, 0 to 1 *v* 2; smoking history, never-smoker *v* light ex-smoker; and sex, female *v* male). The HR (gefitinib: carboplatin/paclitaxel) was estimated with 95% CIs and *P* values. Final analysis of OS was planned for when 944 deaths (78%) had occurred in the intent-to-treat (ITT) population, the same level of maturity as for PFS.

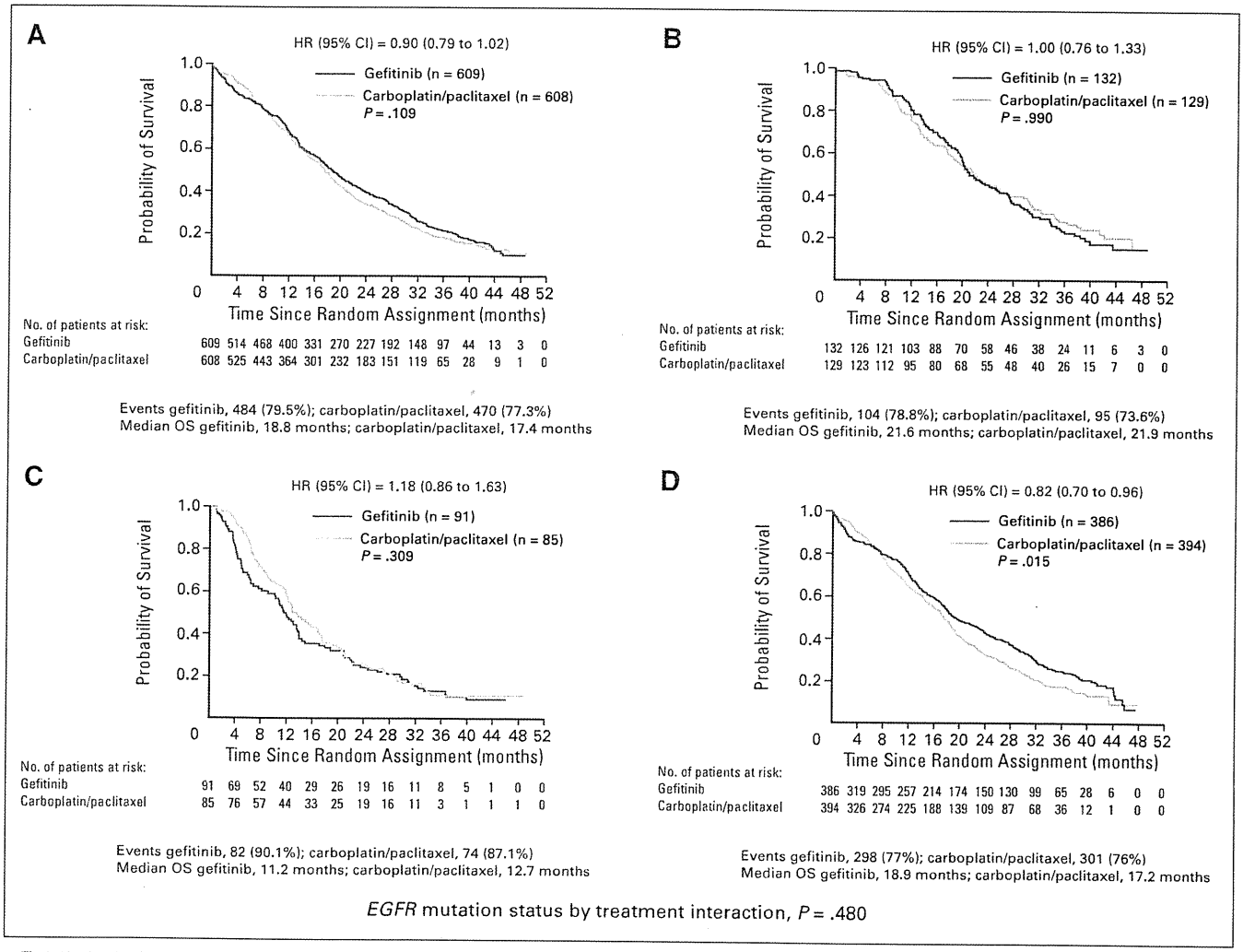

Fig 2. Kaplan-Meier curves for overall survival (OS) in the overall population and by epidermal growth factor receptor (*EGFR*) mutation status (intent-to-treat population). Hazard ratio (HR) < 1 implies a lower risk of death for patients treated with gefitinib. Cox analysis with covariates (performance status [0-1, 2], smoking history [never, light ex-smoker], and sex). (A) Overall population. (B) Patients with *EGFR* mutation–positive tumors. (C) Patients with *EGFR* mutation–negative tumors. (D) Patients with *EGFR* mutation status unknown tumors.

For each biomarker, patients were classified as positive, negative, or unknown. For each of these groups, HRs, 95% CIs, and *P* values were estimated for PFS and OS (by using a Cox proportional hazards model adjusted for the same covariates as for the primary PFS analysis in the ITT population). The biomarker status by randomly assigned treatment interaction was assessed individually for each biomarker for PFS and OS by using a Cox proportional hazards model adjusted for randomly assigned treatment, biomarker status (positive or negative), and the biomarker status by treatment interaction by using a 10% significance level to indicate potential predictive factors for gefitinib versus carboplatin/paclitaxel. When there were fewer than 20 events in a subgroup for PFS or OS, only descriptive summaries were produced. Odds ratios, 95% CIs, and *P* values were estimated for ORRs by using a logistic regression model adjusted for the same covariates as those used in the analysis of PFS in the ITT population.

RESULTS

Patients

Patient disposition is presented in Figure 1. Therapies received postdiscontinuation of randomly assigned treatment are listed in Table 1. Specifically, 83 (64.3%) of 129 patients with *EGFR* mutation–positive tumors randomly assigned to carboplatin/paclitaxel received subsequent EGFR TKIs.

OS (ITT Population)

The median duration of follow-up for OS was 17.0 months. At the time of data cutoff for OS (June 14, 2010), 954 patients (78%) had died (Fig 2A). In the overall population, OS was similar for gefitinib and carboplatin/paclitaxel with no significant difference between treatments (484 and 470 events, respectively; HR, 0.90; 95% CI, 0.79 to 1.02; $P = .109$; median OS for gefitinib, 18.8 months *v* 17.4 months for carboplatin/paclitaxel; Fig 2A). A consistent treatment effect was seen across all clinical subgroups (Fig 3C).

Biomarker Evaluations

Of 683 randomly assigned patients (56.1%) who provided samples for biomarker analysis, 118 were cytology samples, which were not included in the main analysis. The number of patients with an evaluable status was 437 (35.9%) for *EGFR* mutation, 406 (33.4%) for

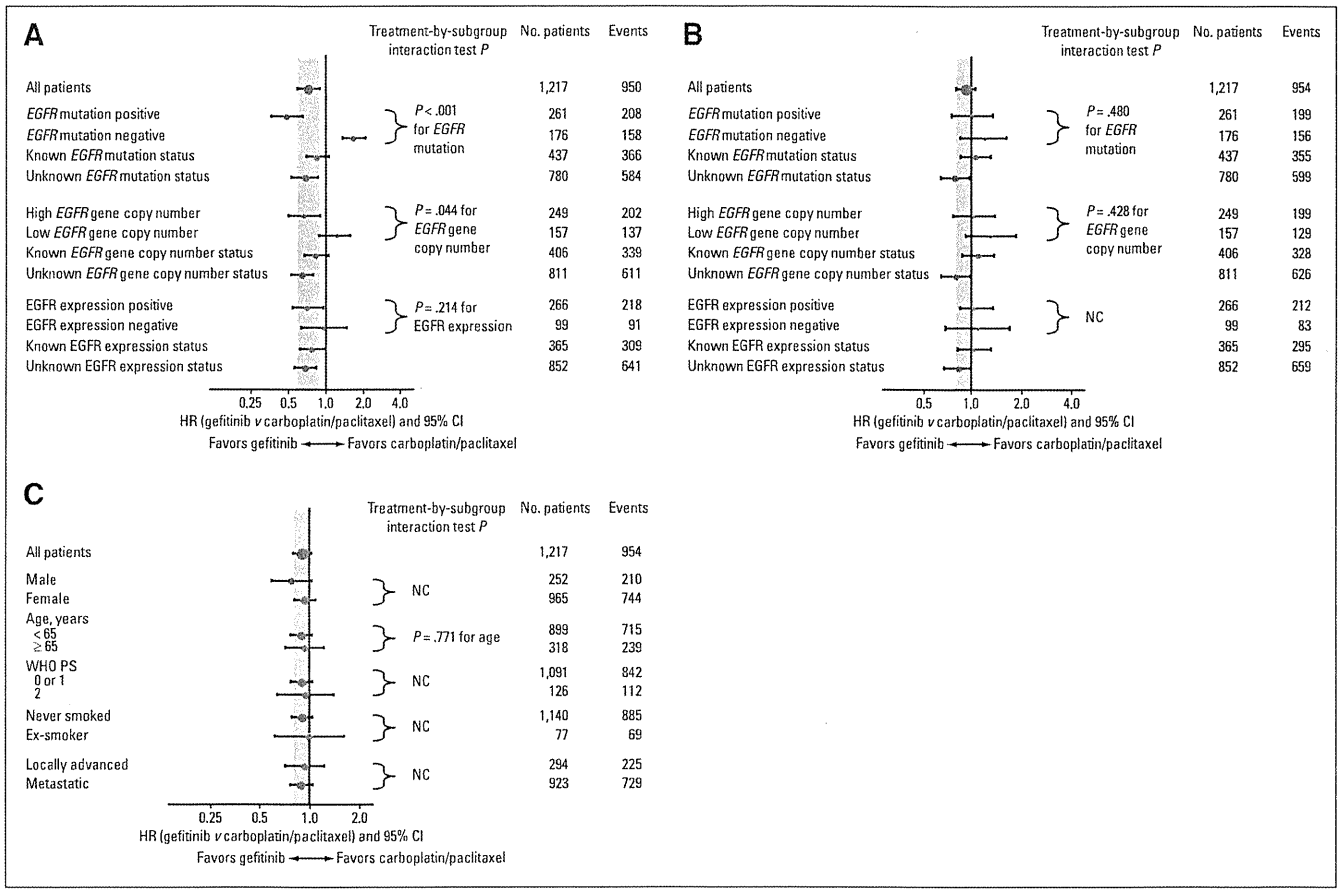

Fig 3. Forest plot of progression-free survival (PFS) and overall survival (OS) by epidermal growth factor receptor (EGFR) mutation status, gene copy number, and protein expression status (intent-to-treat population). (A) PFS by biomarker status. (B) OS by biomarker status. (C) OS by clinical subgroup. Hazard ratio < 1 implies a lower risk of progression or death for patients treated with gefitinib. The size of the point estimate reflects the number of events in the subgroup, with a larger circle indicating more events. Cox analysis with covariates (performance status [PS], 0 to 1 or 2; smoking history, never-smoker, light ex-smoker; and sex). OS by biomarker status; no formal adjustment for multiple testing was made, therefore, statistical significance at the traditional 5% level (95% CI < 1) cannot be claimed. Protocoled interaction tests were calculated only for OS and clinical subgroups if there was a significant interaction test for PFS. NC, not calculated.

EGFR gene copy number, and 365 (30.0%) for EGFR protein expression (Fig 1); the percentage of patients with a positive EGFR biomarker status was 59.7% (261 of 437), 61.3% (249 of 406), and 72.9% (266 of 365), respectively. A summary of EGFR biomarker status is presented in the Data Supplement.

The demographics, baseline characteristics, and efficacy results of patients with evaluable samples for assessment of *EGFR* mutation status, gene copy number, and protein expression were generally comparable with the ITT population (Table 2). There was a high degree of overlap between patients who were positive for all three biomarkers; 190 patients (78.5%) with high *EGFR* gene copy number also harbored an *EGFR* mutation; 132 patients were positive for all three biomarkers.

EGFR *Mutation Status*

Demographic and baseline characteristics by *EGFR* mutation status are shown in the Data Supplement. PFS results by *EGFR* mutation status have been previously published[19] (Fig 3A).

There was no differential treatment effect for OS by *EGFR* mutation (treatment by *EGFR* mutation interaction test $P = .480$). There was no significant difference in OS for gefitinib versus carboplatin/paclitaxel in the subgroups of patients with *EGFR* mutation–positive tumors (104 and 95 events, respectively; HR, 1.00; 95% CI, 0.76 to 1.33; $P = .990$; median OS, 21.6 *v* 21.9 months); *EGFR* mutation–negative tumors (82 and 74 events, respectively; HR, 1.18; 95% CI, 0.86 to 1.63; $P = .309$; median OS, 11.2 *v* 12.7 months), or mutation status unknown tumors (298 and 301 events, respectively; HR, 0.82; 95% CI, 0.70 to 0.96; $P = .015$; Figs 2B, 2C, 2D, and 3B). Postdiscontinuation treatments by *EGFR* mutation status are listed in Table 1.

EGFR *Gene Copy Number*

EGFR gene copy number was a predictive biomarker for the effect of gefitinib compared with carboplatin/paclitaxel on PFS (treatment by *EGFR* gene copy number interaction test $P = .044$; Fig 3A). In patients with high *EGFR* gene copy number (fluorescent in situ hybridization scores 5 and 6; n = 249), PFS was significantly longer with gefitinib versus carboplatin/paclitaxel (HR, 0.66; 95% CI, 0.50 to 0.88; $P = .005$). ORR also significantly favored gefitinib in these patients (58.9% *v* 44.8% for gefitinib *v* carboplatin/paclitaxel, respectively; odds ratio [OR], 1.79; 95% CI, 1.08 to 2.96; $P = .024$). Conversely, in

Table 2. Demographics, Baseline Characteristics, and Analysis Outcomes for Patients with Evaluable Tissue Samples for Each Biomarker Compared With the ITT Population

	Evaluable for *EGFR* Mutation Status* (n = 437)					Evaluable for *EGFR* Gene Copy Number Status* (n = 406)					Evaluable for EGFR Protein Expression Status* (n = 365)					ITT Population (n = 1,217)				
Variable	No.	%	HR	OR	95% CI	No.	%	HR	OR	95% CI	No.	%	HR	OR	95% CI	No.	%	HR	OR	95% CI
Demographic characteristic																				
Female	335	76.7				313	77.1				285	78.1				965	79.3			
Age < 65 years	326	74.6				303	74.6				262	71.8				899	73.9			
WHO PS 0 or 1	402	92.0				375	92.4				334	91.5				1,091	89.6			
Never-smoker	405	92.7				375	92.4				334	91.5				1,140	93.7			
Locally advanced	83	19.0				77	19.0				67	18.4				295	24.2			
Efficacy																				
PFS			0.85		0.69 to 1.06			0.83		0.66 to 1.03			0.79		0.62 to 0.99			0.74		0.65 to 0.85
ORR				1.21	0.83 to 1.78				1.31	0.88 to 1.95				1.43	0.94 to 2.18				1.59	1.25 to 2.01
OS			1.05		0.85 to 1.29			1.10		0.89 to 1.37			1.04		0.82 to 1.30			0.90		0.79 to 1.02

NOTE. Hazard ratio (HR) < 1 implies a lower risk of progression or death on gefitinib; odds ratio (OR) > 1 implies a greater chance of response on gefitinib.
Abbreviations: ITT, intent to treat; EGFR, epidermal growth factor receptor; PS, performance status; PFS, progression-free survival; ORR, objective response rate; OS, overall survival.
*Irrespective of whether positive or negative for each biomarker.

patients with low *EGFR* gene copy number (n = 157), PFS was numerically longer (HR, 1.24; 95% CI, 0.87 to 1.76; $P = .237$) and ORR was numerically higher (26.3% *v* 22.2%; OR, 0.80; 95% CI, 0.38 to 1.68; $P = .558$) with carboplatin/paclitaxel versus gefitinib.

A total of 190 patients (78%) with high *EGFR* gene copy number also harbored *EGFR* mutations. Of the 153 patients with low *EGFR* gene copy number, only 51 (33%) were also *EGFR* mutation positive. Post hoc analyses found that PFS was significantly shorter with gefitinib versus carboplatin/paclitaxel in patients with high *EGFR* gene copy number in the absence of a coexisting *EGFR* mutation (n = 55; HR, 3.85; 95% CI, 2.09 to 7.09), although patients with *EGFR* mutation achieved significantly longer PFS with gefitinib versus carboplatin/paclitaxel irrespective of whether they had high (HR, 0.48; 95% CI, 0.34 to 0.67; n = 190) or low (HR, 0.51; 95% CI, 0.25 to 1.04; n = 51) *EGFR* gene copy number (Figs 4A to 4D).

There was no differential treatment effect for OS by *EGFR* gene copy number (treatment by *EGFR* gene copy number interaction test $P = .428$). There was no significant difference in OS for gefitinib versus carboplatin/paclitaxel in patients with high *EGFR* gene copy number (104 and 95 events, respectively; HR, 1.03; 95% CI, 0.78 to 1.37; $P = .816$) or low *EGFR* gene copy number (67 and 62 events, respectively; HR, 1.30; 95% CI, 0.92 to 1.85; $P = .137$; Fig 3B).

EGFR Protein Expression

There was no differential treatment effect for PFS by EGFR protein expression (treatment by EGFR protein expression status interaction test $P = .214$; Fig 3A). PFS was significantly longer for gefitinib versus carboplatin/paclitaxel in patients with EGFR protein expression–positive tumors (HR, 0.73; 95% CI, 0.55 to 0.96; $P = .024$; n = 266). There was no significant difference in PFS between treatments in patients with EGFR protein expression–negative tumors (HR, 0.97; 95% CI, 0.64 to 1.48; $P = .893$; n = 99).

ORRs were similar between the gefitinib and carboplatin/paclitaxel groups for patients with either EGFR protein expression–positive (51.5% *v* 41.8%; OR, 1.49; 95% CI, 0.92 to 2.42; $P = .109$) or EGFR protein expression–negative (34.0% *v* 26.1%; OR, 1.44; 95% CI, 0.60 to 3.47; $P = .415$) tumors.

There was no significant difference in OS for gefitinib versus carboplatin/paclitaxel in patients with EGFR protein expression–positive (107 and 105 events, respectively; HR, 1.05; 95% CI, 0.80 to 1.37; $P = .731$) or EGFR protein expression–negative (46 and 37 events, respectively; HR, 1.09; 95% CI, 0.70 to 1.70; $P = .692$) tumors.

Activating EGFR *Mutation Type*

Of the 261 patients with *EGFR* mutation–positive tumors, 53.6% (n = 140) had tumors with exon 19 deletions, and 42.5% (n = 111) had exon 21 L858R mutations (Data Supplement); demography was generally similar between these groups (Data Supplement).

In post hoc analyses, PFS was significantly longer for gefitinib versus carboplatin/paclitaxel in both the exon 19 deletions (HR, 0.38; 95% CI, 0.26 to 0.56) and the exon 21 L858R mutation (HR, 0.55; 95% CI, 0.35 to 0.87; Figs 5A and 5B) subgroups. Within-treatment analysis indicated no significant difference in PFS with gefitinib in the exon 19 deletions versus exon 21 L858R mutation subgroup (HR, 0.78; 95% CI, 0.51 to 1.19). ORR was significantly higher with gefitinib (84.8%) versus carboplatin/paclitaxel (43.2%; OR, 7.23; 95% CI, 3.19 to 16.37) in the exon 19 deletions subgroup and higher (but not statistically significant) in the L858R subgroup (60.9% *v* 53.2%; OR, 1.41; 95% CI, 0.65 to 3.05).

DISCUSSION

Gefitinib showed similar OS to doublet chemotherapy with no significant difference in the overall population or in patients with *EGFR* mutation–positive or *EGFR* mutation–negative status. The significant treatment-related differences for PFS and ORR according to *EGFR* mutation status were not observed for OS. Although there may be other contributing factors, the subsequent treatments that patients received are likely to have confounded the true effect of the initial, randomized first-line treatment on OS. Of the *EGFR* mutation–positive subgroup randomly assigned to carboplatin/paclitaxel, 64.3% received EGFR TKIs postdiscontinuation. Fewer patients with unknown mutation status randomly assigned to carboplatin/paclitaxel received EGFR TKIs (47.5%) compared with patients with *EGFR* mutation–positive status (64.3%), which may potentially contribute to the numerical trend in favor of gefitinib in this subgroup; statistical significance at the traditional 5% level ($P < .05$) cannot be claimed because no adjustment was made for multiple testing. The First-SIGNAL study had a study design similar to that of IPASS[23] and

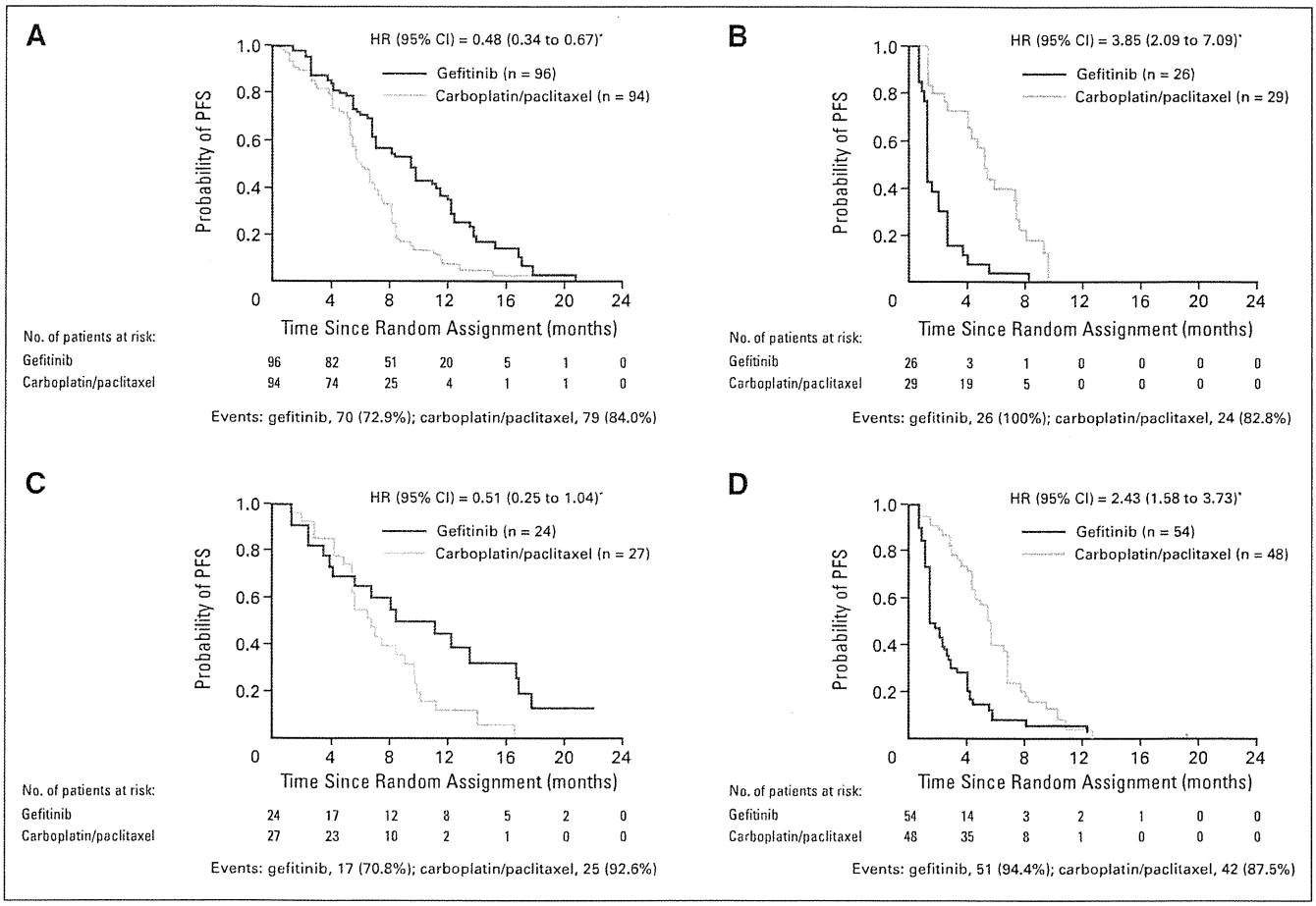

Fig 4. Kaplan-Meier curves for progression-free survival (PFS) by epidermal growth factor receptor (*EGFR*) mutation status and *EGFR* gene copy number. Hazard ratio (HR) < 1 implies a lower risk of progression/death for patients treated with gefitinib. (A) High *EGFR* gene copy number *EGFR* mutation–positive. (B) High *EGFR* gene copy number *EGFR* mutation–negative. (C) Low *EGFR* gene copy number *EGFR* mutation–positive. (D) Low *EGFR* gene copy number *EGFR* mutation–negative. (*) Cox analysis with covariates (performance status [0-1, 2], smoking history [never, light ex-smoker], and sex).

reported no significant difference in OS (primary end point) between gefitinib versus gemcitabine/cisplatin (overall population, 182 events; 59% maturity; mutation-positive HR, 0.82; 95% CI, 0.35 to 1.92; $P = .648$; median survival, 30.6 *v* 26.5 months, respectively). The randomized Japanese NEJ002 study also reported that OS did not differ significantly between gefitinib and carboplatin/paclitaxel in patients selected by *EGFR* mutation status (median survival, 30.5 *v* 23.6 months, respectively; $P = .31$), likely explained by treatment crossover.[24]

Although collection of tumor material was not mandatory or feasible in all patients, IPASS has the largest group of patients with *EGFR* mutation–positive tumors studied in a randomized controlled trial in NSCLC and has confirmed *EGFR* mutation to be the strongest predictive biomarker for the effect of gefitinib with a statistically significant interaction test for PFS. Patients with mutation-negative tumors have a poorer outcome in terms of PFS and ORR with gefitinib compared with carboplatin/paclitaxel, indicating that in the first-line setting, gefitinib should not be used in preference to doublet chemotherapy in patients with a negative mutation status.

Our findings were broadly consistent with those of previous first-line, single-arm studies of gefitinib in patients with *EGFR* mutation–positive tumors.[25-32] Recently, outcomes similar to those of IPASS among patients with *EGFR* mutation–positive tumors have been reported in two randomized phase III studies[24,33] comparing first-line gefitinib with doublet chemotherapy, with PFS as the primary end point. The NEJ002 study prospectively randomly assigned 230 patients with *EGFR* mutation–positive tumors to gefitinib or carboplatin/paclitaxel. PFS favored gefitinib over carboplatin/paclitaxel (PFS HR, 0.30; 95% CI, 0.22 to 0.41; $P < .001$; median PFS, 10.8 *v* 5.4 months; tumor response rate, 73.7% *v* 30.7%, respectively; $P < .001$).[24] The similarly designed West Japan Thoracic Oncology Group 3405 (WJTOG3405) study reported increased PFS with gefitinib over cisplatin/docetaxel in 172 patients with *EGFR* mutation–positive tumors (PFS HR, 0.49; 95% CI, 0.34 to 0.70; $P < .001$; median PFS, 9.2 *v* 6.3 months; 295 events; 95% maturity).[33] Tumor response rates (n = 117) were 62.1% and 32.2%. In the First-SIGNAL study, PFS (secondary end point) increased with gefitinib compared with gemcitabine/cisplatin in 42 patients with *EGFR* mutation–positive tumors (PFS HR, 0.61; 95% CI, 0.31 to 1.22; $P = .084$; median PFS, 8.4 *v* 6.7 months).[23] The OPTIMAL study compared erlotinib with gemcitabine/cisplatin in 154 patients with *EGFR* mutation–positive tumors and also reported a significant difference in PFS (HR, 0.16; 95% CI, 0.10 to 0.26; $P = .001$).[34] The similarly designed European Tarceva

