Hotta *et al.*[7] reported existing pulmonary fibrosis, poor ECOG PS, and prior irradiation as risk factors for ILD. Pre-existing pulmonary fibrosis and poor ECOG PS have also been shown to be associated risk factors for ILD in patients treated with either gefitinib or erlotinib.[8]

POLARSTAR was a large-scale surveillance study including all Japanese patients with NSCLC treated with erlotinib,[9] undertaken as a post-approval commitment in Japan to monitor safety and efficacy. The objectives were to obtain decisive information on the incidence of ILD, risk factors for developing ILD, and the efficacy of erlotinib. Here, we report the final analysis of the POLARSTAR surveillance study investigating the safety and efficacy of erlotinib treatment in Japanese patients with NSCLC.

Methods

Study design. All patients with unresectable, recurrent /advanced NSCLC who were treated with erlotinib in Japan between December 2007 and October 2009 were enrolled. Eligible patients receiving erlotinib (150 mg orally, once daily), from the 1027 institutions that could prescribe erlotinib, were monitored until erlotinib therapy termination or completion of 12 months of treatment. The study was approved by the relevant ethics committees and patients gave informed consent to participate in the analysis.

Assessments. Demographic and baseline data were collected for each patient, including age, gender, body mass index, tumor histology, ECOG PS, smoking history, and medical history (including hepatic dysfunction, renal dysfunction, cardiovascular disease, and lung disorders). Safety data were collected at 1, 6, and 12 months after the start of erlotinib therapy. All AE reports were collected and graded using the National Cancer Institute Common Terminology Criteria for AEs version 3.0 and coded using the Medical Dictionary for Regulatory Activities version 14.1 thesaurus terms.

Outcome measures. Primary endpoints were patterns of occurrence of ILD and risk factors for onset of ILD. Overall survival and PFS were secondary endpoints and were assessed according to the treating physician's standard clinical practice. The pattern of ADRs, excluding ILD, was an additional secondary endpoint.

Statistical analyses. The sample size determination is previously described.[9] Briefly, 3000 patients were to be enrolled to detect an AE in one case out of 3000 patients with at least a power of 95%; however, during enrolment, target accrual was increased to 10 000 patients by the Japanese Health Authority to further evaluate the safety and efficacy of erlotinib. The increased patient number allows high sensitivity regarding low-frequency ADRs. The safety population comprised all patients who received erlotinib and had case report form data available. The efficacy population comprised all patients included in the safety population, except those where erlotinib therapy was prescribed off-label (i.e. in the first-line setting) at the time of this study, or where a patient's therapeutic history was unknown.

Median PFS and OS were estimated using Kaplan–Meier methodology. Patients without data for the duration of the observation period or from the time of treatment initiation were excluded from the PFS analyses.

Statistical analyses were carried out using Statistical Analysis Software version 9.1 and 9.2 (SAS Institute, Cary, NC). Multivariate Cox regression analysis using a stepwise model was carried out to determine risk factors for ILD; occurrence of ILD was used as the dependent variable. Exploratory variables with $P > 0.05$ were not included in the final model. In the final step, additional multivariate analyses were carried out to investigate two-factor interactions; statistical significance was set at $P < 0.05$. This method is described in more detail in the interim analysis publication.[9]

To examine factors affecting poor prognosis in ILD, a stepwise, 5% significance level, multivariate logistic regression analysis was carried out with an analysis set of 310 patients in whom an ILD diagnosis was confirmed by the ILD Review Committee. The target variable was fatal ILD; exploratory variables included gender, age, primary lesion, histological type, smoking history, ECOG PS, honeycomb lung, non-metastatic lesions, and remaining normal lung. The exploratory variables were chosen by the results of a univariate analysis using ILD death as the target variable, with baseline characteristics and characteristics previously reported to affect poor ILD prognosis as the univariate exploratory variables.

Results

A total of 10 708 patients were enrolled in this study. Of these, 9909 patients were evaluated for the final safety analysis and 9663 patients were evaluated for the final efficacy analysis (Fig. 1). Baseline characteristics are shown in Table 1. Of note, more males than females were enrolled; the majority of patients had adenocarcinoma histology (80.9%) and most had ECOG PS 0–1 (74.0%).

Safety analysis. Adverse drug reactions were reported in 79.1% (7835/9909) of patients, the most common being skin disorders (67.4%), including rash (60.9%), diarrhea (21.5%), hepatitis, hepatic failure and hepatic function disorder (9.8%), eye disorders (3.3%) and hemorrhage (1.6%; Table 2). Median time to onset of ADRs was 9 days for rash, 8 days for diarrhea, 13 days for hepatitis, hepatic failure, and hepatic function

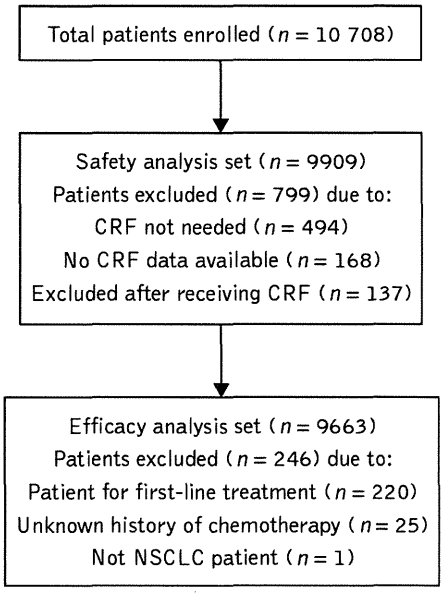

Fig. 1. Disposition of patients with unresectable, recurrent/advanced non-small-cell lung cancer who were treated with erlotinib in Japan between December 2007 and October 2009 and who were included in the final analysis. CRF, case report form; NSCLC, non-small-cell lung cancer.

Table 1. Baseline characteristics of patients with unresectable, recurrent/advanced non-small-cell lung cancer who were treated with erlotinib in Japan between December 2007 and October 2009

Characteristic	Patients, *n* (%) (*n* = 9909)
Gender	
Male	5300 (53.5)
Female	4609 (46.5)
Age	
<65 years	4466 (45.1)
65–74 years	3382 (34.1)
≥75 years	2059 (20.8)
Histology	
Adenocarcinoma	7950 (80.9)
Squamous cell	1285 (13.1)
Large cell	155 (1.6)
Other	438 (4.5)
ECOG PS	
0–1	7315 (74.0)
2–4	2576 (26.0)
Smoking history	
No	4366 (44.9)
Yes	5367 (55.1)
Number of previous treatment lines	
0	220 (2.2)
1	2481 (25.1)
2	2646 (26.8)
3	1993 (20.2)
4	1546 (15.6)
≥5	998 (10.1)
Previous gefitinib treatment	
Yes	4396 (44.7)
No	5446 (55.3)

ECOG PS, Eastern Cooperative Oncology Group performance status.

Table 2. Incidence of the most common adverse drug reactions (ADRs) in patients with unresectable, recurrent/advanced non-small-cell lung cancer who were treated with erlotinib in Japan between December 2007 and October 2009

ADR	All grades		Grade ≥3	
	Patients		Patients	
	n	%	*n*	%
ILD	429	4.3	257	2.6
Skin disorder				
Rash	6032	60.9	673	6.8
Dry skin	738	7.4	30	0.3
Pruritus	351	3.5	13	0.1
Paronychia	654	6.6	77	0.8
Hepatitis, hepatic failure, hepatic function disorder	976	9.8	183	1.8
Diarrhea	2133	21.5	137	1.4
Eye disorders	331	3.3	19	0.2
Corneal disorders	186	1.9	11	0.1
Hemorrhage	158	1.6	46	0.5
Gastrointestinal hemorrhage	39	0.4	20	0.2

ILD, interstitial lung disease.

disorder, 15 days for eye disorders, and 16 days for hemorrhage.

Interstitial lung disease. *Incidence.* Of the patients analyzed, 491 patients had 497 ILD-like events, of which 62 events were deemed non-ILD by the independent ILD Review Committee. In total, 429 (4.3%) patients were classified as having ILD (310 confirmed and reported by the ILD Review Committee, 119 patients not confirmed by the ILD Review Committee due to not having an evaluated image [*n* = 93], too difficult to distinguish from tumor progression [*n* = 4], and too difficult to distinguish from pneumonia due to insufficient evaluable images or clinical findings [*n* = 22] were still classified as ILD), with an overall mortality rate of 1.5% and a mortality rate of 35.7% in patients with ILD.

The majority of ILD cases (58.5%) were reported within 4 weeks of receiving erlotinib. The incidence of ILD (per 100 patient-weeks) was 0.63–0.81 within 4 weeks of the start of erlotinib treatment and 0.09–0.27 from 6 weeks after the start of erlotinib treatment (Fig. 2). Univariate analysis identified patients who were female, patients with non-adenocarcinoma histology, those with a period of treatment from initial NSCLC diagnosis to the start of treatment <360 days, concomitant or previous emphysema or COPD, concomitant or previous ILD, concomitant or previous lung infections, concomitant hepatic disorders, concomitant renal disorders, history of allergies, smoking history, ECOG PS 2–4, prior chest radiotherapy, pretreatment lactate dehydrogenase, and no previous treatment with gefitinib as risk factors for ILD development (Table 3). Age at start of treatment, body mass index, concurrent cardiovascular disorders, number of chemotherapy regimens and previous treatment with gemcitabine were variables that were not identified as risk factors from the univariate analysis. Multivariate analysis showed that concurrent/previous ILD (HR, 3.19), concurrent/previous emphysema or COPD (HR, 1.86), concurrent/previous lung infection (HR, 1.55), smoking history (HR, 2.25), and period from initial NSCLC diagnosis to the start of treatment (<360 days; HR, 0.58) were identified as significant risk factors for developing ILD by multivariate analysis (Table 3).

Outcomes of ILD. Of the confirmed cases of ILD, 75 (17.5%) patients fully recovered, 154 (35.9%) patients improved their condition, 32 (7.5%) patients did not recover, five (1.2%) patients had sequelae, 153 (35.7%) patients died, and 10 (2.3%) patients had unknown outcomes.

The outcome of ILD by CT image pattern was investigated in 283 patients out of the 310 patients deemed as having confirmed ILD by the independent ILD Review Committee. Diffuse alveolar damage-like pattern on CT was defined as abnormalities that showed non-segmental ground-glass attenuation or airspace consolidation with traction bronchiectasis and loss of volume. In the 63 patients with CT-DAD-like pattern, six (9.5%) patients recovered, 12 (19.1%) patients improved, three (4.8%) patients did not recover, one (1.6%) patient had residual ILD sequelae, and 41 (65.1%) patients died. In the 220 patients with a CT-non-DAD-like pattern, 37 (16.8%) patients recovered, 95 (43.2%) patients improved, 13 (5.9%) patients did not recover, one (0.5%) patient had residual ILD sequelae, 71 (32.3%) patients died, and three (1.4%) patients had unknown outcomes.

Fatal outcome of ILD. The multivariate logistic analysis identified ECOG PS 2–4 (OR, 2.45), ≤50% remaining normal lung area (OR, 3.12), and concomitant honeycombing with

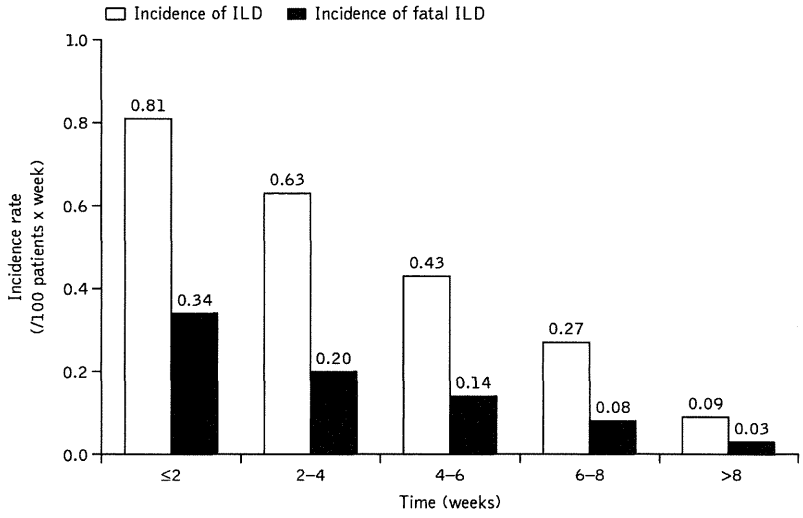

Fig. 2. Incidence rate of interstitial lung disease (ILD) stratified by time from start of erlotinib treatment to onset of ILD. The 34 patients without data for either the duration of observation or the time from the start of erlotinib treatment to the onset of ILD were excluded from the analysis. Value determined by dividing the number of patients developing ILD during the specified duration of observation by the patient-days during the observation period (total duration [number of days] of observation of all patients receiving erlotinib during the specified duration of observation).

Table 3. Cox regression univariate and multivariate analysis of factors affecting the incidence of interstitial lung disease (ILD) in patients with unresectable, recurrent/advanced non-small-cell lung cancer (NSCLC) who were treated with erlotinib in Japan between December 2007 and October 2009

Variables	Criterion variable	Evaluation variable	X^2 value	*P*-value	HR	95% CI
Univariate analysis						
Gender	Male	Female	76.3424	<0.0001	0.390	0.315–0.481
Age (years)	<55	≥55	2.257	0.133	1.256	0.933–1.692
Body mass index (kg/m^2)	<25	≥25	2.4468	0.1178	0.788	0.585–1.062
Histology	Adenocarcinoma	Non-adenocarcinoma	32.0958	<0.0001	1.847	1.494–2.283
Period from initial NSCLC diagnosis to the start of treatment	<360 days	≥360 days	20.1885	<0.0001	0.638	0.525–0.776
Concurrent/previous emphysema or COPD	No	Yes	85.1118	<0.0001	3.071	2.420–3.898
Concurrent/previous ILD	No	Yes	88.7072	<0.0001	3.862	2.915–5.116
Concurrent/previous lung infection	No	Yes	18.7152	<0.0001	1.979	1.453–2.697
Concurrent hepatic disorder	No	Yes	4.9716	0.0258	1.426	1.044–1.949
Concurrent renal disorder	No	Yes	9.1417	0.0025	1.611	1.183–2.195
Concurrent cardiovascular disorder	No	Yes	2.8576	0.0909	1.191	0.973–1.459
History of allergies	No	Yes	5.2846	0.0215	1.358	1.046–1.764
Smoking history	No	Yes	87.4412	<0.0001	2.896	2.318–3.620
ECOG PS	0–1	2–4	20.0203	<0.0001	1.620	1.311–2.001
Prior chest radiation therapy	No	Yes	11.9016	0.0006	1.431	1.167–1.753
Baseline lactate dehydrogenase†	–	–†	7.0077	0.0081	1	1–1
Number of chemotheraphy regimens for the primary diseases	–	–†	1.2809	0.2577	1.033	0.977–1.092
History of gemcitabine treatment	No	Yes	0.1141	0.7355	0.967	0.797–1.174
History of gefitinib treatment	No	Yes	38.7111	<0.0001	0.517	0.420–0.636
Multivariate analysis						
Concurrent/previous ILD	No	Yes	55.3796	<0.0001	3.187	2.349–4.325
Smoking history	No	Yes	34.1327	<0.0001	2.246	1.712–2.946
Concurrent/previous emphysema or COPD	No	Yes	20.704	<0.0001	1.860	1.424–2.431
Period from initial NSCLC diagnosis to the start of treatment	<360 days	≥360 days	19.3818	<0.0001	0.581	0.456–0.740
Concurrent/previous lung infection	No	Yes	6.5905	0.0103	1.550	1.109–2.165
ECOG PS	0–1	2–4	8.9467	0.0028	1.431	1.131–1.809
History of gefitinib treatment	No	Yes	5.3133	0.0212	0.729	0.557–0.954
Number of chemotherapy regimens†	–	–†	10.4136	0.0013	1.121	1.046–1.201

Objective variable: occurrence or non-occurrence of ILD. Explanatory variables: gender, age, body mass index, histological type, concurrent/previous emphysema or chronic obstructive pulmonary disease (COPD), concurrent/previous ILD, concurrent/previous lung infection, concomitant hepatic disorder, concomitant renal disorder, period from initial NSCLC diagnosis to the start of treatment, concomitant cardiovascular disease, history of allergies, smoking history, Eastern Cooperative Oncology Group performance status (ECOG PS), radiotherapy (chest), pretreatment lactate dehydrogenase, number of chemotherapy regimens for the primary disease, history of gemcitabine treatment, history of gefitinib treatment. †Analyzed as a continuous quantity. NSCLC, non-small-cell lung cancer; ILD, interstitial lung disease.; CI, confidence interval; HR, hazard ratio.

Table 4. Interstitial lung disease (ILD) poor prognosis risk factors from the final analysis results for Post-Launch All-patient-Registration Surveillance in Tarceva®-treated non-small-cell lung cancer patients (POLARSTAR)

Risk factors for ILD-related death	Criterion variable	Evaluation variable	X^2 value	*P*-value	OR	95% CI
ECOG PS 2–4	0–1	2–4	9.974	0.0016	2.45	1.41–4.27
≤50% normal lung area	>50	≤50	8.896	0.0029	3.12	1.48–6.58
Concomitant honeycombing	No	Yes	5.414	0.02	6.67	1.35–32.94

CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group performance status; OR, odds ratio.

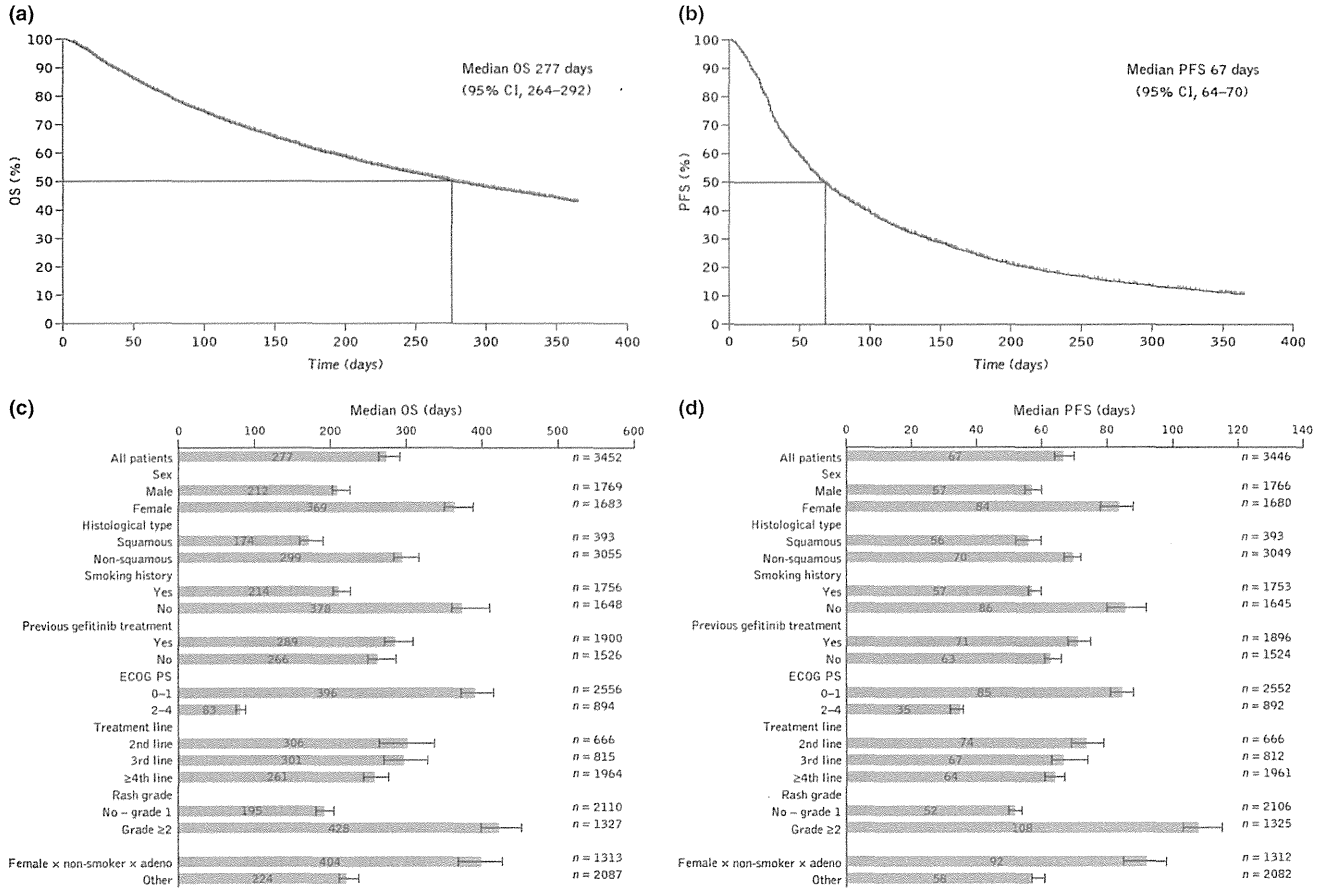

Fig. 3. (a) Overall survival (OS) and (b) progression-free survival (PFS) assessed by Kaplan–Meier methodology in the overall population of patients with unresectable, recurrent/advanced non-small-cell lung cancer who were treated with erlotinib in Japan between December 2007 and October 2009; (c) median OS and (d) PFS in patient subpopulations. CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group performance status.

interstitial pneumonia (OR, 6.67) as poor prognostic factors for ILD death (Table 4).

A total of 12 patients reported concomitant honeycombing and interstitial pneumonia; of these patients, nine patients died of ILD, two patients improved their condition, and one patient did not recover. Of those who died, eight were determined as having CT-non-DAD-like pattern on CT scan and the remaining patient was determined as having CT-DAD-like pattern.

Efficacy. Median OS was 277 days (95% CI, 264–292), with a 6-month survival rate of 62.6% and a 12-month survival rate of 42.8% (Fig. 3a). Median PFS was 67 days (95% CI, 64–70), with a 6-month progression-free rate of 25.8% and a 12-month progression-free rate of 10.6% (Fig. 3b). Compared with the overall population, median OS and PFS appeared to be longer in female patients, non-smokers, patients with ECOG PS 0–1, and patients with grade ≥2 rash (Fig. 3c,d).

Discussion

The development of drug-induced acute pulmonary disorders or interstitial pneumonia caused by EGFR TKIs is a common problem; this has particular importance in Japan, because a variety of evidence has suggested that Japanese populations are more vulnerable to these disorders. This large-scale POLARSTAR study provides further decisive information on this issue. Final data from the POLARSTAR study confirm that erlotinib has a well-characterized safety profile with proven efficacy in Japanese patients in routine clinical practice.

In the final analysis from POLARSTAR, the rates of ILD development and mortality in patients with ILD (4.3% and 35.7%, respectively) were comparable with the ILD-associated incidence rates of 3–5% and mortality rates of 27.9–50.0% previously reported among Japanese patients with NSCLC and ILD treated with gefitinib or erlotinib.[2,3,5,6,9] In the POLARSTAR analysis, it was shown that ILD onset was typically soon after initiation of erlotinib, with the highest incidence occurring during the first 4 weeks. Physicians should therefore monitor patients for the symptoms of ILD, which usually occur within 8 weeks of treatment initiation. These findings are further supported by those reported in Japanese NSCLC studies with gefitinib.[5,6]

The risk factors identified as significant primary risk factors (HR, ≥1.5) for ILD occurrence or exacerbation using a Cox proportional hazards multivariate analysis were concurrent/previous ILD, concurrent/previous lung infection, concurrent/previous emphysema or COPD, and smoking history. Cox proportional hazards multivariate analysis was selected for this assessment as the authors considered that a time-dependent analysis was needed, as there was no information regarding the ILD development point in the initial analysis. Concurrent /previous emphysema or COPD was newly identified as a significant primary risk factor for ILD occurrence when analyzed in 9909 patients compared with the result of the interim analysis of 3488 patients (Table 5).[9,10] As ILD is a collective term for a variety of different lung conditions, it is important to be careful not to misdiagnose conditions as ILD, as this will affect the risk factor analysis.

The period from initial NSCLC diagnosis to the start of treatment (<360 days) was not considered as a risk factor for ILD that needed to be highlighted at this time (HR, 0.58), as the clinical grounds for this factor were not clear. Stage of progression of primary disease or bias of observational period from initial NSCLC diagnosis to termination of treatment were speculated to be the reason; however, details of these reasons are uncertain. In contrast to this analysis, risk factors for ILD associated with gefitinib have been reported as ECOG PS ≥2, smoking history, concomitant interstitial pneumonia, and prior chemotherapy.[5,7,8]

The multivariate analysis identified ECOG PS 2–4, ≤50% remaining normal lung area and concomitant honeycombing with interstitial pneumonia as poor prognostic factors for ILD death in POLARSTAR. Many patients with idiopathic interstitial pneumonias have idiopathic pulmonary fibrosis or non-specific interstitial pneumonia, which have a heterogeneous natural progression, with some patients remaining stable for extended periods, while others show steady worsening of the condition.[11] Some patients with chronic idiopathic interstitial pneumonias, such as idiopathic pulmonary fibrosis and non-specific interstitial pneumonia, experience acute exacerbations characterized by suddenly progressive and severe respiratory failure, with new lung opacities and pathological lesions of DAD.[12] It should be noted that there are racial differences between Mongolians (including the Japanese) and Caucasians in the frequency of acute exacerbations.[13] In the POLARSTAR study, the outcome of ILD by CT image pattern was investigated in 283 patients out of the 310 patients deemed as having confirmed ILD by the independent ILD Review Committee. The mortality rate for ILD among patients who were deemed to have CT-DAD-like pattern was higher than that seen among patients who were deemed as having CT-non-DAD-like pattern (65.1% *vs* 32.2%, respectively). Those patients with honeycombing and interstitial pneumonia (n = 12) had a high risk of poor prognosis, regardless of their CT pattern. Therefore, physicians should be actively aware of the symptoms of ILD and it is suggested to carefully monitor for these symptoms by CT image or X-ray throughout the disease course. Once physicians recognize ILD, they should immediately discontinue the EGFR TKI and should take the necessary steps to manage the ILD.

The final efficacy results from POLARSTAR are in line with the results of our interim analysis of the study (Table 6).[9] The final efficacy results (median OS, 277 days; median PFS, 67 days) were also comparable with efficacy reported in previous clinical trials of erlotinib treatment. The BR.21 study reported median PFS of 2.2 months (67 days) versus 1.8 months (55 days) and OS of 6.7 months (203 days) versus 4.7 months (143 days) for erlotinib and placebo, respectively, in the second- or third-line setting.[1] Kubota *et al.* investigated second-line erlotinib in Japanese patients, resulting in a median PFS of 77 days and OS of 14.7 months (447 days).[2] In a sec-

Table 5. Comparison of the interstitial lung disease (ILD) analysis from the interim and final analysis results for Post-Launch All-patient-Registration Surveillance in Tarceva®-treated non-small-cell lung cancer patients (POLARSTAR)

Endpoint	Interim analysis (safety, n = 3488) (efficacy, n = 3453)	Final analysis (safety, n = 9909) (efficacy, n = 9663)
ILD analysis		
Patients with confirmed ILD, n (%)	158 (4.5)	429 (4.3)
ILD-related mortality rate, %	1.6	1.5
ILD-related mortality rate in ILD patients	34.8	35.7
Risk factors for ILD development, HR		
Previous/concurrent ILD	4.1	3.2
Previous/concurrent Emphysema or COPD	–	1.9
Previous/concurrent lung infection	2.0	1.6
Smoking history	3.0	2.2
ECOG PS 2–4	1.6	1.4
<360 days from diagnosis to treatment	–	0.58

COPD, chronic obstructive pulmonary disease; ECOG PS, Eastern Cooperative Oncology Group performance status; HR, hazard ratio.

Table 6. Comparison of the efficacy endpoints from the interim and final analysis results for Post-Launch All-patient-Registration Surveillance in Tarceva®-treated non-small-cell lung cancer patients (POLARSTAR)

Endpoint	Interim analysis (safety, n = 3488) (efficacy, n = 3453)	Final analysis (safety, n = 9909) (efficacy, n = 9663)
Efficacy endpoints		
Median OS, days	260	277
6-month OS rate, %	62.2	62.6
12-month OS rate, %	40.9	42.8
Median PFS, days	64	67
6-month PFS rate, %	23.7	25.8
12-month PFS rate, %	9.6	10.6

OS, overall survival; PFS, progression-free survival.

ond phase 2 study in Japanese patients with NSCLC, second-line erlotinib treatment resulted in median OS of 13.5 months (410 days).[3]

We acknowledge that there are several limitations of this study, including the fact that this was a single-arm observational study with no control group, and the lack of a strict observation period, unlike a clinical trial. The lack of information on *EGFR* mutation status is also considered a limitation as this is known to strongly affect the efficacy of erlotinib. The lack of patient selection criteria may also be seen as a limitation; however, this may mean that our study population was more representative of the actual Japanese population than would be the case in a clinical trial, especially because of the large patient population in this study. The information on EGFR TKI-associated ILD in this study is thought to be decisive; it provides valuable information for treatment considerations and monitoring in Japanese patients with *EGFR* mutant or wild-type lung cancer.

Healthcare providers should carefully observe patients during treatment with erlotinib to ascertain whether the patient has any of the risk factors detailed in this analysis. After suspicion of the onset of ILD and diagnosis by CT, it is important to follow the patient's status continuously and carefully monitor their risk level. The final safety and efficacy data from the large-scale POLARSTAR surveillance study confirm that erlotinib has a well-characterized safety profile with proven efficacy in Japanese patients; however, the risk of ILD should still be monitored.

Acknowledgments

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Disclosure Statement

KN, SK, YO, TJ, MA, NY, and MF have all participated as independent advisory board members for erlotinib, reimbursed by Chugai Pharmaceutical Co. Ltd. YO also has an immediate family member who is an employee of Chugai Pharmaceutical Co. Ltd. HA, YI, ME, TJ, MK, KK, FS, HT, AG, and YF have all participated as independent ILD Review Committee members for erlotinib, reimbursed by Chugai Pharmaceutical Co. Ltd. AS and TI are full-time employees of Chugai Pharmaceutical Co. Ltd. This trial was designed, funded, and monitored by Chugai Pharmaceutical Co. Ltd. Data were gathered, analyzed, and interpreted by Chugai with input from all authors. The corresponding author had full access to the relevant data and took full responsibility for the final decision to submit the report for publication. Although technically classed as a clinical trial, the POLARSTAR study was a non-interventional surveillance study analyzing all NSCLC patients receiving erlotinib in Japan, therefore it was not registered as a phase II/III clinical trial would be.

Abbreviations

ADR	adverse drug reaction
AE	adverse event
CI	confidence interval
COPD	chronic obstructive pulmonary disease
CT	computed tomography
DAD	diffuse alveolar damage
ECOG	PS Eastern Cooperative Oncology Group performance status
EGFR	epidermal growth factor receptor
HR	hazard ratio
ILD	interstitial lung disease
NSCLC	non-small-cell lung cancer
OR	odds ratio
OS	overall survival
PFS	progression-free survival
POLARSTAR	Post-Launch All-patient-Registration Surveillance in Tarceva®-treated NSCLC patients
TKI	tyrosine-kinase inhibitor

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ORIGINAL ARTICLE

First-Line Crizotinib versus Chemotherapy in *ALK*-Positive Lung Cancer

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ABSTRACT

BACKGROUND

The efficacy of the ALK inhibitor crizotinib as compared with standard chemotherapy as first-line treatment for advanced *ALK*-positive non–small-cell lung cancer (NSCLC) is unknown.

METHODS

We conducted an open-label, phase 3 trial comparing crizotinib with chemotherapy in 343 patients with advanced *ALK*-positive nonsquamous NSCLC who had received no previous systemic treatment for advanced disease. Patients were randomly assigned to receive oral crizotinib at a dose of 250 mg twice daily or to receive intravenous chemotherapy (pemetrexed, 500 mg per square meter of body-surface area, plus either cisplatin, 75 mg per square meter, or carboplatin, target area under the curve of 5 to 6 mg per milliliter per minute) every 3 weeks for up to six cycles. Crossover to crizotinib treatment after disease progression was permitted for patients receiving chemotherapy. The primary end point was progression-free survival as assessed by independent radiologic review.

RESULTS

Progression-free survival was significantly longer with crizotinib than with chemotherapy (median, 10.9 months vs. 7.0 months; hazard ratio for progression or death with crizotinib, 0.45; 95% confidence interval [CI], 0.35 to 0.60; P<0.001). Objective response rates were 74% and 45%, respectively (P<0.001). Median overall survival was not reached in either group (hazard ratio for death with crizotinib, 0.82; 95% CI, 0.54 to 1.26; P=0.36); the probability of 1-year survival was 84% with crizotinib and 79% with chemotherapy. The most common adverse events with crizotinib were vision disorders, diarrhea, nausea, and edema, and the most common events with chemotherapy were nausea, fatigue, vomiting, and decreased appetite. As compared with chemotherapy, crizotinib was associated with greater reduction in lung cancer symptoms and greater improvement in quality of life.

CONCLUSIONS

Crizotinib was superior to standard first-line pemetrexed-plus-platinum chemotherapy in patients with previously untreated advanced *ALK*-positive NSCLC. (Funded by Pfizer; PROFILE 1014 ClinicalTrials.gov number, NCT01154140.)

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Rearrangements of the anaplastic lymphoma kinase (*ALK*) gene are present in 3 to 5% of non–small-cell lung cancers (NSCLCs).[1,2] They define a distinct subgroup of NSCLC that typically occurs in younger patients who have never smoked or have a history of light smoking and that has adenocarcinoma histologic characteristics.[3-5]

Crizotinib is an oral small-molecule tyrosine kinase inhibitor of ALK, MET, and ROS1 kinases.[6] In phase 1 and 2 studies, crizotinib treatment resulted in objective tumor responses in approximately 60% of patients with *ALK*-positive NSCLC and in progression-free survival of 7 to 10 months.[7-9] In a randomized phase 3 trial involving patients with advanced *ALK*-positive NSCLC who had received previous platinum-based chemotherapy, crizotinib showed efficacy superior to that of single-agent second-line chemotherapy with either pemetrexed or docetaxel.[10] However, the efficacy of crizotinib as initial treatment for patients with newly diagnosed advanced *ALK*-positive NSCLC as compared with the existing standard-of-care, platinum-based double-agent chemotherapy,[11,12] is unknown.

We report the results of an ongoing international, multicenter, randomized, open-label, phase 3 study (PROFILE 1014) that compares crizotinib treatment with pemetrexed-plus-platinum chemotherapy with respect to efficacy, safety, and patient-reported outcomes in patients with previously untreated advanced *ALK*-positive NSCLC.

METHODS

PATIENTS

Patients were eligible for enrollment if they had histologically or cytologically confirmed locally advanced, recurrent, or metastatic nonsquamous NSCLC that was positive for an *ALK* rearrangement (as determined centrally with the use of a Vysis ALK Break Apart FISH Probe Kit [Abbott Molecular])[7,13] and if they had received no previous systemic treatment for advanced disease. Other eligibility criteria included an age of 18 years or older; measurable disease as assessed according to the Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1[14] (summarized in Table S1 in the Supplementary Appendix, available with the full text of this article at NEJM.org); an Eastern Cooperative Oncology Group (ECOG) performance status of 0, 1, or 2 (on a scale of 0 to 5, with 0 indicating that the patient is asymptomatic and higher numbers indicating increasing disability)[15]; and adequate hepatic, renal, and bone marrow function (as defined in the study protocol). Patients with treated brain metastases were eligible if the metastases were neurologically stable for at least 2 weeks before enrollment and the patient had no ongoing requirement for glucocorticoids. All patients provided written informed consent before enrollment.

STUDY OVERSIGHT

The protocol was approved by the institutional review board or independent ethics committee at each participating center and complied with the International Ethical Guidelines for Biomedical Research Involving Human Subjects, Good Clinical Practice guidelines, the Declaration of Helsinki, and local laws. The study was designed by the sponsor (Pfizer) and by members of the PROFILE 1014 steering committee (see the Supplementary Appendix). The sponsor collected and analyzed the data in conjunction with the authors, all of whom had full access to the data. The manuscript was written by the first two authors, with medical writing support from ACUMED (Tytherington, United Kingdom, and New York) funded by the sponsor. All the authors vouch for the accuracy and completeness of the data and for the fidelity of this report to the study protocol. The protocol and statistical analysis plan are available at NEJM.org.

STUDY DESIGN AND TREATMENT

Patients were randomly assigned, in a 1:1 ratio, to receive oral crizotinib, at a dose of 250 mg twice daily, or intravenous chemotherapy (pemetrexed, at a dose of 500 mg per square meter of body-surface area, plus either cisplatin, at a dose of 75 mg per square meter, or carboplatin, target area under the curve of 5 to 6 mg per milliliter per minute) administered every 3 weeks for a maximum of six cycles. The choice of platinum chemotherapy was made by the investigator. Randomization was stratified according to ECOG performance status (0 or 1 vs. 2), Asian or non-Asian race, and presence or absence of brain metastases. Treatment was continued until RECIST-defined disease progression, development of unacceptable toxic effects, death, or withdrawal of consent. Continuation of crizotinib beyond disease progression was allowed for patients who had been randomly assigned to crizotinib if the patient was perceived by the investigator to be having clinical benefit.

Patients in the chemotherapy group who had disease progression as confirmed by independent radiologic review could cross over to crizotinib treatment if safety screening criteria were met.

The primary end point was progression-free survival (the time from randomization to RECIST-defined progression, as assessed by independent radiologic review, or death). Secondary end points included the objective response rate, overall survival, safety, and patient-reported outcomes.

ASSESSMENTS

Tumor assessment was performed during screening (within 28 days before randomization), every 6 weeks during treatment, and at the post-treatment follow-up visits (which were scheduled every 6 weeks) until RECIST-defined progression. For patients who crossed over to crizotinib treatment or continued crizotinib treatment beyond progression, assessments continued to be performed every 12 weeks. Brain or bone lesions that were detected at the time of screening were evaluated in all subsequent tumor assessments (i.e., every 6 weeks). In all patients, brain and bone scanning was repeated every 12 weeks to monitor for new lesions. All scans were submitted for central independent radiologic review by radiologists who were unaware of the group assignments.

Adverse events were classified and graded according to Common Terminology Criteria for Adverse Events, version 4.0. Patient-reported outcomes were assessed with the use of the European Organisation for Research and Treatment of Cancer (EORTC) quality-of-life core questionnaire (QLQ-C30),[16,17] the corresponding lung cancer module (QLQ-LC13),[18] and the EuroQol Group 5-Dimension Self-Report Questionnaire (EQ-5D).[19]

STATISTICAL ANALYSIS

We estimated that with 229 events of progression or death, the study would have 85% power to detect a 50% improvement in progression-free survival with crizotinib versus chemotherapy (from 6 months to 9 months), at a one-sided alpha level of 0.025. The prespecified number of events for the primary end point was reached in November 2013; the data cutoff date was November 30, 2013. Efficacy end points were measured in the intention-to-treat population, which included all patients who underwent randomization. The Kaplan–Meier method was used to estimate time-to-event end points. Two-sided log-rank tests stratified according to baseline stratification factors were used for between-group comparisons of progression-free survival and overall survival; stratified Cox regression models were applied to estimate hazard ratios. As prespecified in the protocol, overall survival was also analyzed with the rank-preserving structural failure time model[20-22] to explore the effect of crossover to crizotinib in the chemotherapy group. All analyses in the chemotherapy group, with the exception of the analysis of overall survival, included only data collected before crossover to crizotinib. We used a two-sided stratified Cochran–Mantel–Haenszel test to compare the objective response rate between treatment groups. Safety evaluations were performed in the as-treated population, which included all patients who received at least one dose of study medication. Safety results were not adjusted for the shorter duration of treatment in the chemotherapy group. Patient-reported outcomes were evaluated in patients in the intention-to-treat population who also had a baseline assessment and at least one post-baseline assessment. Additional details of the statistical methods are provided in the Supplementary Appendix.

RESULTS

PATIENTS

Between January 2011 and July 2013, a total of 343 patients underwent randomization — 172 to crizotinib and 171 to chemotherapy (intention-to-treat population) (Fig. S1 in the Supplementary Appendix). Three patients underwent randomization but received no study treatment, leaving 340 patients in the as-treated population — 171 patients in the crizotinib group and 169 in the chemotherapy group (with 91 patients receiving pemetrexed–cisplatin and 78 receiving pemetrexed–carboplatin). At the time of data cutoff, the median duration of follow-up for overall survival was 17.4 months for patients assigned to crizotinib and 16.7 months for those assigned to chemotherapy. The baseline characteristics in the intention-to-treat population were well balanced between the groups (Table 1).

EFFICACY

The median progression-free survival was 10.9 months (95% confidence interval [CI], 8.3 to 13.9) among patients in the crizotinib group, as compared with 7.0 months (95% CI, 6.8 to 8.2) among patients in the chemotherapy group (hazard ratio

Table 1. Baseline Characteristics in the Intention-to-Treat Population.*

Characteristic	Crizotinib (N=172)	Chemotherapy (N=171)
Age — yr		
Median	52	54
Range	22–76	19–78
Male sex — no. (%)	68 (40)	63 (37)
Race — no. (%)†		
White	91 (53)	85 (50)
Asian	77 (45)	80 (47)
Other	4 (2)	6 (4)
Smoking status — no. (%)		
Never smoked	106 (62)	112 (65)
Former smoker	56 (33)	54 (32)
Current smoker	10 (6)	5 (3)
Histologic characteristic of tumor — no. (%)		
Adenocarcinoma	161 (94)	161 (94)
Nonadenocarcinoma	11 (6)	10 (6)
ECOG performance status — no. (%)‡		
0 or 1	161 (94)	163 (95)
2	10 (6)	8 (5)
Extent of disease — no. (%)		
Locally advanced	4 (2)	3 (2)
Metastatic	168 (98)	168 (98)
Time since first diagnosis — mo		
Median	1.2	1.2
Range	0–114.0	0–93.6
Brain metastases present — no. (%)	45 (26)	47 (27)

* There were no significant differences between the groups in any of the characteristics listed in this table.
† Race was self-reported.
‡ The Eastern Cooperative Oncology Group (ECOG) performance status was assessed at the time of screening; the score was not reported for one patient in the crizotinib group. Scores range from 0 to 5, with higher scores indicating increasing disability; an ECOG performance status of 0 indicates that the patient is fully active, 1 that the patient is ambulatory but restricted in strenuous activity, and 2 that the patient is ambulatory and capable of self-care but is unable to work.

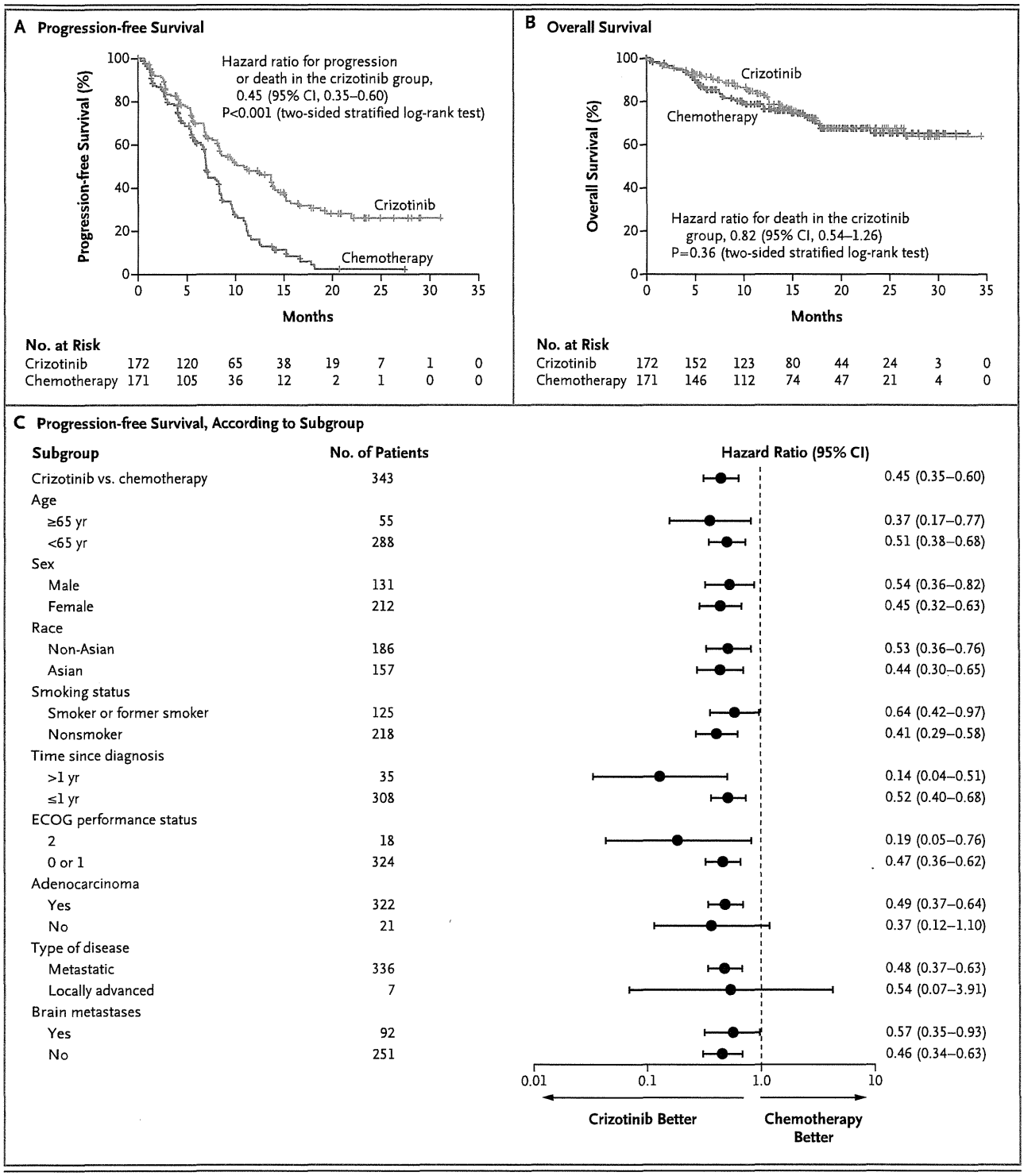
Figure 1 (facing page). Progression-free and Overall Survival.

Panel A shows Kaplan–Meier estimates of progression-free survival in the intention-to-treat population. There were 100 events of progression or death with crizotinib (89 progression events as assessed by independent radiologic review and 11 deaths without documented progression) and 137 events with chemotherapy (132 progression events as assessed by independent radiologic review and 5 deaths without documented progression). The median progression-free survival was 10.9 months with crizotinib as compared with 7.0 months with chemotherapy. The rate of progression-free survival at 18 months was 31% (95% CI, 23 to 39) in the crizotinib group and 5% (95% CI, 2 to 10) in the chemotherapy group. Panel B shows Kaplan–Meier estimates of overall survival in the intention-to-treat population. Because the rate of death from any cause at the time of data cutoff was relatively low (26%; 90 of the 343 patients who underwent randomization), the median overall survival was not reached in either group. Of the 171 patients randomly assigned to chemotherapy, 120 (70%) subsequently received crizotinib treatment. Of the 172 patients assigned to crizotinib, 21 (12%) subsequently received platinum-based chemotherapy. This analysis was not adjusted for crossover. Tick marks on the curves in Panels A and B indicate censoring of data. Panel C shows hazard ratios and 95% confidence intervals for the treatment effect on progression-free survival in subgroups of the intention-to-treat population defined according to prespecified stratification factors and baseline characteristics. Race was self-reported. Eastern Cooperative Oncology Group (ECOG) performance status scores range from 0 to 5, with higher scores indicating increasing disability; an ECOG performance status of 0 indicates that the patient is fully active, 1 that the patient is ambulatory but restricted in strenuous activity, and 2 that the patient is ambulatory and capable of self-care but is unable to work. Data for ECOG performance status were missing for 1 patient.

for progression or death with crizotinib, 0.45; 95% CI, 0.35 to 0.60; P<0.001) (Fig. 1A). The hazard ratio favored crizotinib across most subgroups defined according to stratification factors and other baseline characteristics (Fig. 1C).

The objective response rate was significantly higher with crizotinib than with chemotherapy (74% [95% CI, 67 to 81] vs. 45% [95% CI, 37 to 53], P<0.001) (Table 2). The median duration of response was 11.3 months and 5.3 months, respectively. The best percentage change from baseline in target lesions and the best overall response in individual patients are shown in Figure S2 in the Supplementary Appendix. Intracranial lesions progressed or new intracranial lesions developed in 25 patients in the crizotinib group and in 26 patients in the chemotherapy group (15% each).

There was no significant difference in overall survival between patients in the crizotinib group and those in the chemotherapy group at the time of the progression-free survival analysis (hazard ratio for death with crizotinib, 0.82; 95% CI, 0.54 to 1.26; P=0.36) (Fig. 1B) — probably owing to the relatively low rate of death from any cause



(26%; 90 of the 343 patients who underwent randomization) and the fact that 70% of the patients in the chemotherapy group crossed over to crizotinib treatment. The probability of 1-year survival was 84% (95% CI, 77 to 89) in the crizotinib group and 79% (95% CI, 71 to 84) in the chemotherapy group. After adjustment for crossover with the rank-preserving structural failure time model, the hazard ratio for death with crizotinib was 0.60 (95% CI, 0.27 to 1.42) as calcu-

Table 2. Response to Treatment in the Intention-to-Treat Population.*

Response	Crizotinib (N=172)	Chemotherapy (N=171)
Type of response — no. (%)		
Complete response	3 (2)	2 (1)
Partial response	125 (73)	75 (44)
Stable disease	29 (17)	63 (37)
Progressive disease	8 (5)	21 (12)
Could not be evaluated†	7 (4)	10 (6)
Objective response rate — % (95% CI)‡	74 (67–81)	45 (37–53)
Time to response — mo§		
Median	1.4	2.8
Range	0.6–9.5	1.2–8.5
Duration of response — mo¶		
Median	11.3	5.3
95% CI	8.1–13.8	4.1–5.8

* Tumor responses were assessed with the use of Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1, and were confirmed by independent radiologic review.

† Responses could not be evaluated in 4 patients in each group because of early death.

‡ P<0.001 for the comparison between the two groups. The 95% confidence interval was calculated with the use of the exact method based on the F distribution.

§ The time to tumor response was calculated from the date of randomization to the date of the first documentation of a partial or complete response as determined by independent radiologic review.

¶ The duration of response was calculated from the date of the first documentation of a partial or complete response to the date of RECIST-defined progression or death, with the use of the Kaplan–Meier method.

lated with the Wilcoxon test (Fig. S3A in the Supplementary Appendix) and 0.67 (95% CI, 0.28 to 1.48) as calculated with the log-rank test (Fig. S3B in the Supplementary Appendix), indicating that crossover may have confounded the results of the primary overall survival analysis.

Among patients randomly assigned to crizotinib, 74 of 89 patients with progressive disease (83%) continued to receive crizotinib beyond disease progression for a median of 3.0 months (range, 0.7 to 22.6). A total of 21 patients assigned to crizotinib (12%) subsequently received platinum-based chemotherapy. At data cutoff, 79 patients who had been randomly assigned to crizotinib (46%) and 62 patients assigned to chemotherapy who had crossed over to crizotinib (36%) were still receiving crizotinib therapy. Eighteen patients in the chemotherapy group who had progressive disease did not receive follow-up therapy with crizotinib; additional details are provided in the Supplementary Appendix. Other systemic therapies received during follow-up are listed in Table S2 in the Supplementary Appendix. The baseline characteristics of the patients and the efficacy outcomes in subgroup analyses of crizotinib versus individual chemotherapy regimens were similar to those in the analysis of the overall population (Table S3 and Fig. S4 in the Supplementary Appendix).

SAFETY AND ADVERSE EVENTS

The median duration of treatment was 10.9 months (range, 0.4 to 34.3) in the crizotinib group (a median of 16 cycles started [range, 1 to 50]) and 4.1 months (range, 0.7 to 6.2) in the chemotherapy group (a median of 6 cycles of chemotherapy started [range, 1 to 6]). The most common adverse events of any cause for which the incidence was at least 5 percentage points higher in the crizotinib group than in the chemotherapy group were vision disorder (occurring in 71% of the patients), diarrhea (in 61%), and edema (in 49%); and the events for which the incidence was at least 5 percentage points higher in the chemotherapy group than in the crizotinib group were fatigue (occurring in 38% of the patients), anemia (in 32%), and neutropenia (in 30%) (Table 3). Most adverse events in the two treatment groups were grade 1 or 2 in severity. Grade 3 or 4 elevations of aminotransferase levels occurred in 24 patients in the crizotinib group (14%) and in 4 patients in the chemotherapy group (2%), but these elevations were managed primarily with dose interruptions or dose reductions. Four hepatic events resulted in permanent discontinuation of treatment in the crizotinib group: three events involved elevated aminotransferase levels only (one event of grade 3 elevation of both alanine and aspartate aminotransferase levels and one event each of grade 2 and grade 3 elevation of the alanine aminotransferase level), and one event involved a grade 2 drug-induced liver injury that met the criteria for Hy's law[23] (elevated aminotransferase and total bilirubin levels without evidence of cholestasis [i.e., no elevated serum alkaline phosphatase level]) (see the Supplementary Appendix). An additional case that met the criteria for Hy's law occurred in a patient in the chemotherapy group after crossover to crizotinib. No deaths from hepatic dysfunction occurred. Grade 3 or 4 neutropenia occurred in 11% of patients in the

Table 3. **Adverse Events from Any Cause in the As-Treated Population.***

Adverse Event	Crizotinib (N=171)		Chemotherapy (N=169)†	
	Any Grade	Grade 3 or 4	Any Grade	Grade 3 or 4
	number of patients (percent)			
Higher frequency in crizotinib group				
Vision disorder‡	122 (71)	1 (1)	16 (9)	0
Diarrhea	105 (61)	4 (2)	22 (13)	1 (1)
Edema§	83 (49)	1 (1)	21 (12)	1 (1)
Vomiting	78 (46)	3 (2)	60 (36)	5 (3)
Constipation	74 (43)	3 (2)	51 (30)	0
Elevated aminotransferases§	61 (36)	24 (14)	22 (13)	4 (2)
Upper respiratory infection§	55 (32)	0	21 (12)	1 (1)
Abdominal pain§	45 (26)	0	20 (12)	0
Dysgeusia	45 (26)	0	9 (5)	0
Headache	37 (22)	2 (1)	25 (15)	0
Pyrexia	32 (19)	0	18 (11)	1 (1)
Dizziness§	31 (18)	0	17 (10)	2 (1)
Pain in extremity	27 (16)	0	12 (7)	0
Higher frequency in chemotherapy group				
Fatigue	49 (29)	5 (3)	65 (38)	4 (2)
Neutropenia§	36 (21)	19 (11)	51 (30)	26 (15)
Stomatitis§	24 (14)	1 (1)	34 (20)	2 (1)
Asthenia	22 (13)	0	41 (24)	2 (1)
Anemia§	15 (9)	0	54 (32)	15 (9)
Leukopenia§	12 (7)	3 (2)	26 (15)	9 (5)
Thrombocytopenia§	2 (1)	0	31 (18)	11 (7)
Similar frequency in the two treatment groups				
Nausea	95 (56)	2 (1)	99 (59)	3 (2)
Decreased appetite	51 (30)	4 (2)	57 (34)	1 (1)
Cough§	39 (23)	0	33 (20)	0
Neuropathy§	35 (20)	2 (1)	38 (22)	0
Dyspnea§	30 (18)	5 (3)	26 (15)	4 (2)

* Adverse events are listed here if they were reported in 15% or more of patients in either treatment group; rates were not adjusted for differences in treatment duration. Higher frequency indicates a difference of 5 percentage points or more between groups; similar frequency indicates a difference of less than 5 percentage points between groups.
† Only events that occurred before crossover to crizotinib are included.
‡ The category of vision disorder comprised a cluster of adverse events including (in descending order of frequency in the crizotinib group) visual impairment, photopsia, blurred vision, vitreous floaters, reduced visual acuity, diplopia, and photophobia.
§ This item comprised a cluster of adverse events that may represent similar clinical symptoms or syndromes.

crizotinib group and in 15% in the chemotherapy group, with no cases of febrile neutropenia reported with crizotinib and two with chemotherapy. Other grade 3 or 4 adverse events from any cause are shown in Table S4 in the Supplementary Appendix. Two patients (1%) in the crizotinib group had interstitial lung disease, resulting in permanent discontinuation of crizotinib treatment.

Adverse events from any cause that were associated with permanent discontinuation of treatment occurred in 12% of the patients in the

crizotinib group and in 14% of those in the chemotherapy group (before crossover); the corresponding rates of adverse events deemed by the investigator to be related to treatment that were associated with permanent discontinuation were 5% and 8%. One case of fatal pneumonitis, considered to be related to crizotinib treatment, occurred in a patient who had crossed over from chemotherapy. Grade 5 adverse events of any cause are shown in Table S5 in the Supplementary Appendix. With the exception of the fatal pneumonitis, described above, that occurred after crossover to crizotinib, no deaths were reported that were deemed by the investigators to be related to treatment.

PATIENT-REPORTED OUTCOMES

Baseline scores on the QLQ-C30, QLQ-LC13, and EQ-5D are summarized in Table S6 in the Supplementary Appendix. There was a significantly greater overall improvement from baseline in global quality of life among patients who received crizotinib than among those who received chemotherapy (P<0.001) (Fig. 2A, and see the Results section in the Supplementary Appendix for additional details). Crizotinib was also associated with a significantly greater overall improvement from baseline in physical, social, emotional, and role functioning domains (P<0.001) (Fig. 2A).

There was a significantly greater overall reduction from baseline with crizotinib than with chemotherapy in the symptoms of pain, dyspnea, and insomnia as assessed with the use of the QLQ-C30 (Fig. 2B) and in the symptoms of dyspnea, cough, chest pain, arm or shoulder pain, and pain in other parts of the body as assessed with the use of the QLQ-LC13 (Fig. 2C) (P<0.001 for all comparisons) (see the Results section in the Supplementary Appendix for additional details). Patients treated with crizotinib also had a significantly greater delay in the worsening of lung-cancer symptoms (a composite of cough, dyspnea, or pain in the chest) than did patients treated with chemotherapy (hazard ratio for worsening of symptoms with crizotinib, 0.62; 95% CI, 0.47 to 0.80; P=0.002; estimated probability of being event-free at 6 months, 38% vs. 22%) (Fig. S5 in the Supplementary Appendix). A significantly greater improvement from baseline was observed in EQ-5D general health status scores (as assessed with the use of a visual-analogue scale) with crizotinib than with chemotherapy (P=0.002).

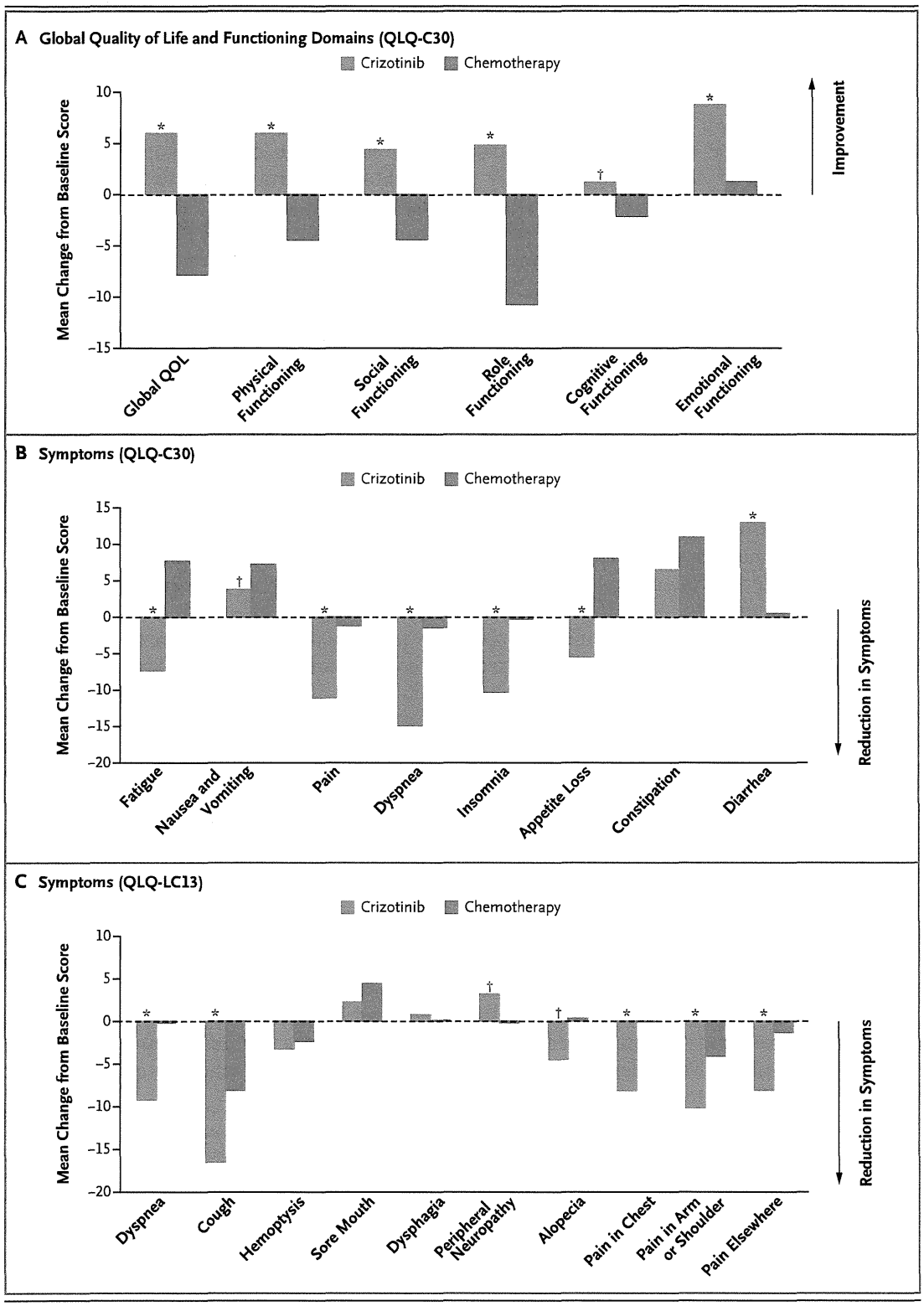
Figure 2 (facing page). Overall Change from Baseline in Global Quality of Life, Functioning Domains, and Symptoms.

Panel A shows the overall change from baseline in global quality of life (QOL) and functioning domains as assessed with the use of the European Organisation for Research and Treatment of Cancer Quality-of-Life Questionnaire (QLQ-C30). Panels B and C show the overall change from baseline in symptoms as assessed with the QLQ-C30 and the corresponding module for lung cancer (QLQ-LC13), respectively. Patient-reported outcomes were assessed at baseline, on days 7 and 15 of cycle 1, on day 1 of every subsequent cycle, and at the end of treatment. Scores on each scale ranged from 0 to 100. For global quality of life and functioning domains, higher scores indicate better global quality of life or functioning, and hence positive changes (upward bars) indicate improvement from baseline; for symptoms, higher scores indicate greater severity of symptoms, and hence negative changes (downward bars) indicate improvement from baseline. A change of 10 points or more is considered to be a clinically meaningful change. An asterisk indicates P<0.001, and a dagger P<0.05 for the comparison between treatment groups. In Panel C, the mean changes from the baseline score in dysphagia and in pain in the chest with chemotherapy were 0.10 and −0.05, respectively.

DISCUSSION

This study showed the superiority of first-line therapy with crizotinib over pemetrexed-plus-platinum chemotherapy in patients with previously untreated advanced *ALK*-positive NSCLC. Initial treatment with crizotinib significantly prolonged progression-free survival as compared with chemotherapy consisting of pemetrexed plus cisplatin or carboplatin. These results were independent of the type of platinum treatment administered, the performance status of the patient, the patient's race, and the presence or absence of brain metastases. Crizotinib treatment was also associated with a significantly higher response rate and significantly greater improvements in patient-reported measures of physical functioning, key lung-cancer symptoms (cough, dyspnea, chest pain, and fatigue), and global quality of life.

The standard of care for newly diagnosed NSCLC has generally been platinum-based double-agent chemotherapy,[11] except in the case of NSCLC that is positive for an epidermal growth



factor receptor (*EGFR*) mutation, for which randomized trials have shown superior efficacy of EGFR tyrosine kinase inhibitors over chemotherapy.[24-28] For tumors with nonsquamous histologic characteristics, cisplatin–pemetrexed has been shown to be superior to cisplatin–gemcitabine.[12]

Given that most advanced *ALK*-positive NSCLCs have nonsquamous histologic characteristics, pemetrexed in combination with cisplatin or carboplatin was selected as the standard chemotherapy for this trial. The efficacy of pemetrexed-based first-line chemotherapy has since been documented in *ALK*-positive NSCLC,[29,30] a finding that supports this selection. A potential limitation of our study was that pemetrexed was not continued beyond the planned six cycles of pemetrexed-plus-platinum chemotherapy, since this was not considered to be a standard approach when the study was initiated. However, in a study of patients without disease progression after four cycles of cisplatin–pemetrexed, maintenance pemetrexed therapy improved median progression-free survival over placebo by only 1.3 months (4.1 months vs. 2.8 months) from the start of maintenance therapy.[31] The way in which the use of maintenance pemetrexed therapy or other chemotherapy regimens would have affected the results in the control group of the current study is unclear.

The magnitude of the improvement in progression-free survival observed in the current study is similar to that observed in studies of *EGFR*-mutation–positive tumors treated with first-line EGFR tyrosine kinase inhibitors.[24-26] Although formal comparison across studies cannot be made, the efficacy of crizotinib in the first-line setting (median progression-free survival, 10.9 months; objective response rate, 74%) appeared to be greater than that seen with crizotinib in an otherwise similar patient population that had received previous treatment with platinum-based chemotherapy (median progression-free survival, 7.7 months; response rate, 65%).[10] Initiating crizotinib as first-line therapy in patients whose tumors test positive for *ALK* rearrangements maximizes the probability that these patients will benefit from ALK-directed therapy.

Overall survival did not differ significantly between the treatment groups at the time of this analysis, with a relatively small number of deaths reported (26%; 90 of the 343 patients who underwent randomization). As seen in randomized phase 3 studies of first-line EGFR tyrosine kinase inhibitors versus chemotherapy in *EGFR*-mutation–positive NSCLC, this finding is most likely attributable to the confounding effects of crossover treatment.[32] Of the 171 patients randomly assigned to chemotherapy, 120 received crizotinib treatment during follow-up for survival. It should be noted that the median survival had not been reached in either group, with a median follow-up of 17 months.

The safety profile of crizotinib was consistent with that reported earlier in patients with previously treated advanced *ALK*-positive NSCLC[10] and differed from that observed with chemotherapy. The incidence of adverse effects in the two treatment groups was probably affected by the fact that the duration of therapy with crizotinib was longer than that with chemotherapy and that crizotinib continued to be used in some patients beyond progression.[33] Discontinuations of therapy occurred in 5% of patients with crizotinib-related adverse events and in 8% of patients with chemotherapy-related adverse events. More serious potential adverse events previously reported with crizotinib were hepatotoxic and pulmonary toxic effects.[10] In the current study, grade 3 or 4 elevations of aminotransferase levels occurred in 14% of the patients in the crizotinib group and could be managed with dose interruptions or dose reductions. Two patients discontinued crizotinib therapy because of interstitial lung disease, and one case of fatal pneumonitis was reported in a patient who had crossed over from chemotherapy to crizotinib.

In conclusion, in patients with previously untreated *ALK*-positive NSCLC, crizotinib treatment was superior to pemetrexed-plus-platinum chemotherapy with respect to progression-free survival, objective response rate, reduction of lung-cancer symptoms, and improvement in quality of life.

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The anti-HER3 antibody patritumab abrogates cetuximab resistance mediated by heregulin in colorectal cancer cells

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ABSTRACT

We previously showed that tumor-derived heregulin, a ligand for HER3, is associated with both de novo and acquired resistance to cetuximab. We have now examined whether patritumab, a novel neutralizing monoclonal antibody to HER3, is able to overcome such resistance. Human colorectal cancer (DiFi) cells that are highly sensitive to cetuximab were engineered to stably express heregulin by retroviral infection, and the effects of cetuximab and patritumab on the resulting DiFi-HRG cells were examined. DiFi-HRG cells released substantial amounts of heregulin and showed resistance to cetuximab. Cetuximab alone inhibited EGFR and ERK phosphorylation in DiFi-HRG cells, but it had no effect on the phosphorylation of HER2, HER3, or AKT, suggesting that sustained AKT activation by HER2 and HER3 underlies cetuximab resistance in these cells. In contrast, patritumab in combination with cetuximab markedly inhibited the phosphorylation of EGFR, HER2, HER3, ERK, and AKT. The combination therapy also inhibited the growth of DiFi-HRG tumor xenografts in nude mice to a greater extent than did treatment with either drug alone. Activation of HER2-HER3 signaling associated with the operation of a heregulin autocrine loop confers resistance to cetuximab, and patritumab is able to restore cetuximab sensitivity through inhibition of heregulin-induced HER3 activation.

INTRODUCTION

Cetuximab, a chimeric human-mouse monoclonal antibody to the epidermal growth factor receptor (EGFR), has shown clinical efficacy in individuals with metastatic colorectal cancer (mCRC). However, a subset of mCRC patients fails to show an initial response (de novo resistance) to this agent, whereas others develop resistance after an initial response (acquired resistance). Well-established causes of de novo resistance to cetuximab include activating mutations in codon 12 or 13 of *KRAS* and in *BRAF* [1–4]. Various mechanisms responsible for acquired resistance to cetuximab in colorectal cancer have also been identified [5–7]. We previously established cetuximab-resistant cancer cells by exposing parental cells to increasing concentrations of cetuximab [8]. Analysis of these cells revealed that cell-derived heregulin confers cetuximab resistance through bypass signaling via HER2 (also known as ERBB2) and HER3 (also known as ERBB3). Heregulin is a ligand for HER3 and stabilizes the HER2-HER3 heterodimer [9]. We also found that high initial levels of serum heregulin protein and tumor heregulin mRNA were significantly associated with a poor clinical outcome in mCRC patients treated with cetuximab [8]. Furthermore, in patients who initially

achieved a partial response to cetuximab-based therapy, the serum concentration of heregulin after the development of clinical cetuximab resistance was significantly higher than that before treatment [8]. These preclinical and clinical data indicate that increased levels of heregulin are associated with both de novo and acquired resistance to cetuximab.

Patritumab (U3-1287) is a first-in-class, fully human monoclonal antibody directed to the extracellular domain (ECD) of HER3 that is currently in clinical development, as are other HER3-targeted antibodies such as MM-121 and LJM716 (MM-121 prevents ligand binding, whereas LJM716 specifically binds to an epitope formed by ECD domains II and IV in the closed conformation of HER3 [10]). Patritumab has been shown both to inhibit ligand-induced HER3 phosphorylation and to suppress the growth of pancreatic, non–small cell lung cancer, and colorectal cancer xenograft tumors [11, 12]. To identify strategies or agents capable of overcoming resistance to cetuximab induced by heregulin, we have now established sublines of the cetuximab-sensitive human colorectal cancer cell line DiFi that stably express heregulin derived from transfected cDNA. With the use of these cells, we investigated the effects of patritumab on cetuximab resistance mediated by cell-derived heregulin both *in vitro* and *in vivo*.

RESULTS

DiFi cells stably overexpressing heregulin show resistance to cetuximab

The human colorectal cancer cell line DiFi, which harbors wild-type alleles of *KRAS, BRAF*, and *PI3K*, is highly sensitive to cetuximab [13]. To investigate whether cell-derived heregulin might induce cetuximab resistance in DiFi cells, we established DiFi sublines that stably overexpress this protein (DiFi-HRG4, DiFi-HRG5, and DiFi-HRG6) or that stably harbor the corresponding empty vector (DiFi- Mock1) as a result of retroviral infection. Heregulin is a soluble growth factor that is synthesized as a transmembrane precursor molecule of 105 kDa. Cell surface proteases catalyze cleavage of the extracellular domain of this precursor, which is then released and functions as a ligand for HER3. Immunoblot analysis revealed the presence of the transmembrane form of heregulin in DiFi-HRG cells (with its abundance being greatest in DiFi-HRG4 cells), whereas no such band was detected in DiFi-Mock1 cells or the parental DiFi cells (Fig. 1A). Analysis of conditioned medium from these cell lines with an enzyme-linked immunosorbent assay (ELISA) also revealed the presence of substantial amounts of heregulin in the medium from all DiFi-HRG cell lines but not in that from DiFi-Mock1 or the parental cells (Fig. 1B). To assess the effect of cetuximab on cell growth, we exposed DiFi-HRG and DiFi-Mock1 cells to various concentrations of the drug for 5 days and then measured cell viability. All DiFi-HRG cell lines showed a reduced sensitivity to cetuximab compared with DiFi-Mock1 cells, with median inhibitory concentration (IC_{50}) values of > 100 μg/mL for the former cell lines and ~0.1 μg/mL for the latter (Fig. 1C). The DiFi-HRG cell lines also showed resistance to panitumumab, another antibody to EGFR (data not shown). These data thus suggested that DiFi-HRG cells are resistant to EGFR-targeted antibodies.

Heregulin maintains HER3 and AKT phosphorylation and survivin expression in the presence of cetuximab in DiFi-HRG cell lines

To investigate possible differences in signal transduction among the DiFi isogenic lines, we examined the effects of cetuximab (10 μg/mL) on EGFR, HER2, HER3, AKT, and extracellular signal–regulated kinase (ERK) phosphorylation (Fig. 2A). Immunoblot analysis revealed that cetuximab markedly inhibited the phosphorylation of all of these proteins in DiFi-Mock1 cells. In contrast, whereas cetuximab substantially reduced the level of EGFR and ERK phosphorylation in DiFi-HRG cells, it had little effect on the phosphorylation of HER2, HER3, or AKT. We next examined the effects of cetuximab on expression of the apoptosis-related proteins BIM (a proapoptotic BH3-only protein) and survivin (a member of the inhibitor of apoptosis, or IAP, family). We previously showed that inhibition of the MEK-ERK signaling pathway induces BIM expression, and that inhibition of the PI3K-AKT pathway suppresses survivin expression, with both of these effects being independently required for tyrosine kinase inhibitor (TKI)–induced apoptosis in lung cancer cells positive for *EGFR* mutation [14], breast cancer cells positive for *HER2* amplification [15], and gastric cancer cells positive for *MET* amplification [16]. Consistent with these observations, we found that cetuximab induced both up-regulation of BIM and down-regulation of survivin in DiFi-Mock1 cells, resulting in generation of the cleaved form of poly(ADP-ribose) polymerase (PARP), a characteristic of apoptosis (Fig. 2B). In contrast, in DiFi-HRG cell lines, whereas cetuximab induced BIM expression, it had little effect on the abundance of survivin or PARP cleavage (Fig. 2B), suggesting that sustained AKT signaling and survivin expression confer resistance to cetuximab in these cell lines.

The HER3 neutralizing antibody patritumab abrogates cetuximab resistance induced by heregulin

To investigate further the role of HER3 and heregulin in the resistance of DiFi-HRG cell lines to cetuximab, we exposed DiFi-HRG4 cells to cetuximab, the fully human HER3-targeted monoclonal antibody patritumab, or the combination of both agents. We found that neither antibody alone substantially affected cell proliferation, whereas the combination of both agents

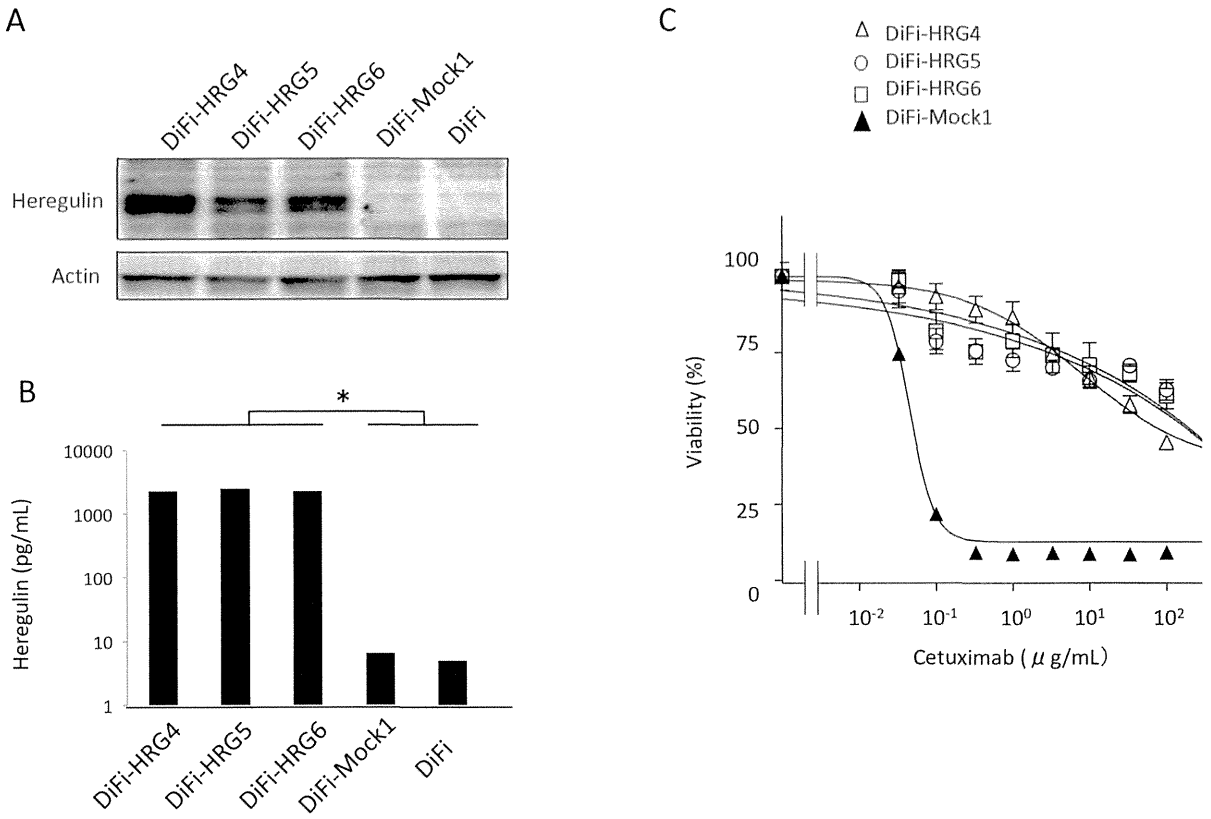

Figure 1: Characterization of DiFi isogenic cell lines. **(A)** DiFi isogenic cell lines (DiFi, DiFi-Mock1, DiFi-HRG4, DiFi-HRG5, and DiFi-HRG6) were cultured overnight in medium containing 10% serum and then incubated for 24 h in serum-free medium, after which the cells were lysed and subjected to immunoblot analysis with antibodies to heregulin and to β-actin (loading control). **(B)** Culture supernatants from cells cultured as described in Materials and Methods were assayed for heregulin with an ELISA. Data are means ± SE from three independent experiments. $^*P < 0.05$ (Student's *t* test) for comparison of each DiFi-HRG line with DiFi-Mock1 or DiFi cells. **(C)** Cells were treated with cetuximab at the indicated concentrations for 5 days, after which cell viability was assessed. Data are means ± SE from three independent experiments.

induced marked inhibition of cell growth (Fig. 3A). We next examined the effects of these antibodies on apoptosis in DiFi-Mock1 and DiFi-HRG4 cells. An annexin V binding assay revealed that cetuximab alone induced a substantial level of apoptosis in DiFi-Mock1 cells but not in DiFi-HRG4 cells (Fig. 3B, C), suggesting that the operation of a heregulin autocrine loop in these latter cells inhibits cetuximab-induced apoptosis. However, exposure of DiFi-HRG4 cells to the combination of patritumab (10 μg/mL) and cetuximab (10 μg/mL) resulted in a marked increase in the proportion of apoptotic cells (Fig. 3B, C), suggesting that patritumab sensitizes DiFi-HRG cells to cetuximab such that the extent of apoptosis induced by both antibodies in these cells is similar to that induced by cetuximab alone in DiFi-Mock1 cells.

We also examined the effects of patritumab alone or in combination with cetuximab on intracellular signaling. Immunoblot analysis showed that patritumab alone had little effect on such signaling in DiFi-Mock1 cells. In contrast, patritumab alone markedly inhibited the phosphorylation of HER3 and AKT, without affecting that of ERK, in DiFi-HRG4 cells (Fig. 3D). The combination of patritumab and cetuximab markedly attenuated the phosphorylation of EGFR, HER2, HER3, AKT, and ERK in DiFi-HRG4 cells (Fig. 3D). It also induced the cleavage of PARP in these cells to an extent similar to that observed in DiFi-Mock1 cells treated with cetuximab alone, and this effect was accompanied by both up-regulation of BIM and down-regulation of survivin expression (Fig. 3E). These results thus indicated that cetuximab resistance induced by heregulin is abrogated by patritumab through attenuation of AKT-suvivin signaling in DiFi-HRG4 cells.

Cell-derived heregulin induces cetuximab resistance and patritumab restores cetuximab sensitivity in tumor xenografts *in vivo*

To examine whether cell-derived heregulin induces cetuximab resistance as well as the efficacy of combined