In the phase III Eastern Cooperative Oncology Group (ECOG) E4599 and Avastin in Lung trials, the addition of bevacizumab to platinum-based doublet chemotherapy resulted in significant improvements in the overall response rate and median progression-free survival (PFS) time (16,17). Bevacizumab has also been tested in Japanese patients with NSCLC. For example, in the Japanese phase II JO19907 trial, the addition of bevacizumab to paclitaxel and carboplatin resulted in a significant improvement in the median PFS time compared with paclitaxel and carboplatin alone (6.9 vs. 5.9 months; P=0.009) (18).

Although pemetrexed and bevacizumab have each demonstrated efficacy in patients with non-squamous NSCLC, less is known about their effects in combination. In a multicenter phase II trial, the combination of pemetrexed and carboplatin plus bevacizumab followed by maintenance pemetrexed and bevacizumab exhibited encouraging activity in chemotherapy-naïve patients with advanced non-squamous NSCLC (19). The response rate was 55% (95% CI, 41-69), the median PFS time was 7.8 months (95% CI, 5.2-11.5) and the median OS time was 14.1 months (95% CI, 10.8-19.6). The efficacy and safety of this regimen, however, was not assessed specifically in Japanese patients. The present study therefore evaluated the efficacy and safety of pemetrexed and carboplatin plus bevacizumab, followed by maintenance pemetrexed and bevacizumab, in Japanese patients with advanced non-squamous NSCLC.

## Patients and methods

*Patients.* The present study consisted of patients who were aged 20-75 years, with stage IIIB, stage IV or recurrent NSCLC, as confirmed histologically or cytologically, and who were naïve to chemotherapy. Each patient had at least one unidimensionally measurable lesion according to the Response Evaluation Criteria in Solid Tumors (20), an ECOG performance status of 0 or 1 (21), and adequate hematological, hepatic and renal functions, including a urine protein/creatinine level of ≤1.0 mg/dl and a creatinine clearance of >45 ml/min.

Exclusion criteria included the following: Histological evidence of a predominantly squamous cell cancer; a primary tumor in close proximity to a major vessel or with cavitation; a history of gross hemoptysis (≥2.5 ml); brain metastases or prior treatment for brain metastasis; uncontrolled pleural or pericardial effusion or ascites; a severe and uncontrolled complication; uncontrollable diabetes mellitus or hypertension (blood pressure, ≥150/100 mmHg); clinically significant cardiovascular disease, including unstable angina pectoris; pregnancy or lactation; a history of thrombotic or hemorrhagic disorder; regular use of aspirin (>325 mg/day); use of non-steroidal anti-inflammatory agents or other agents known to inhibit platelet function; radiation within 21 days of enrollment; major surgery within 28 days of enrollment; history of active double cancer; an unstable psychiatric disorder; or a decision of ineligibility provided by a physician.

The present study (trial no. UMIN000003387) was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines and approved by the institutional review board of Kansai Medical University (Hirakata, Japan). Patients were required to provide informed consent.

*Treatment plan.* The present study was a single-arm phase II trial of first-line pemetrexed, carboplatin and bevacizumab, followed by maintenance pemetrexed and bevacizumab. Eligible patients were administered pemetrexed (500 mg/m$^2$), carboplatin (area under the concentration-time curve, 6.0 mg/ml x min) and bevacizumab (15 mg/kg) intravenously every three weeks for four to six cycles, unless there was evidence of disease progression or intolerance to the treatment. Patients who achieved a complete response (CR), partial response (PR) or stable disease (SD) were subsequently administered maintenance therapy, consisting of intravenous pemetrexed (500 mg/m$^2$) and bevacizumab (15 mg/kg) every three weeks until there was evidence of disease progression or development of unacceptable toxicities.

All patients received oral folic acid (500 $\mu$g/day) and a vitamin $B_{12}$ injection (1,000 $\mu$g every nine weeks), beginning one to two weeks prior to the first dose of pemetrexed, carboplatin and bevacizumab, and continuing until three weeks after the last dose of the treatment.

*Endpoints.* The primary endpoint of this phase II trial was the response rate (RR). Secondary endpoints included PFS and OS times, time to response and safety.

*Assessment of objective response.* Prior to entering the study, a medical history was taken, and then the patients underwent a physical examination, measurements of any palpable lesions and an assessment of lesions by computed tomography. Tumor responses were assessed radiographically every four weeks for 12 weeks and every four to eight weeks thereafter until disease progression occurred. The disease status was assessed according to the Response Evaluation Criteria in Solid Tumors (20) and toxicities were graded according to the National Cancer Institute Common Toxicity Criteria, version 4.0 (22)

*Statistical analyses.* The response rate (RR) was set as the primary endpoint of the present study. The RR of platinum-based doublet chemotherapy for Japanese NSCLC patients has been reported to be ~30% (23). Also, the RR of cisplatin plus pemetrexed has been reported to be the same as that of cisplatin plus gemcitabine. In addition, the RR was recorded as 31 and 60.7%, respectively, in a Japanese phase II trial comparing carboplatin plus paclitaxel with carboplatin plus paclitaxel and bevacizumab. Therefore, it was expected that bevacizumab could increase the RR by 30% for the carboplatin plus pemetrexed regimen. Using the SWOG statistical tool (SWOG Statistical Center, Seattle, WA, USA), a total of 23 patients were evaluated to explain the present hypothesis, to disregard a RR of 30% and to provide a two-sided significance level of <0.1, with a statistical power of 90%, to assess the activity of the regimen as a 60% RR. Finally, a target sample size of 25 patients was chosen on the expectation that a proportion of patients would prove to be ineligible for the study. The main analysis of efficacy was conducted on the full analysis set, which was produced by omitting ineligible patients.

The time-to-event variables obtained from the Kaplan-Meier method were determined by log-rank tests. P<0.05 was considered to indicate a statistically significant difference. Statistical analyses were conducted by JMP software (version 9; SAS Institute Inc., Cary, NC, USA).

Table I. Patient characteristics.

| Parameters | Value |
|---|---|
| Median age years, range | 64 (40-74) |
| Gender, n (%) | |
| Male | 15 (65.2) |
| Female | 8 (34.8) |
| ECOG PS, n (%) | |
| 0 | 6 (26.1) |
| 1 | 17 (73.9) |
| Disease stage, n (%) | |
| IIIB | 3 (13.0) |
| IV | 20 (87.0) |
| Histology, n (%) | |
| Adenocarcinoma | 23 (100.0) |
| EGFR, n (%) | |
| Mutant | 3 (13.0) |
| Wild-type | 16 (69.6) |
| Unknown | 4 (17.4) |

EGFR, epidermal growth factor receptor; ECOG, Eastern Cooperative Oncology Group; PS, performance status.

Table II. Response (n=23).

| Parameters | n (%) |
|---|---|
| CR | 3 (13.0) |
| PR | 13 (56.5) |
| SD | 7 (30.4) |
| Progressive disease | 0 (0.0) |
| Response rate (CR+PR) | 16 (69.6) |
| Disease control rate (CR+PR+SD) | 23 (100.0) |

CR, complete response; PR, partial response; SD, stable disease.

## Results

*Patient characteristics.* Between March 2010 and January 2011, 26 newly diagnosed patients with advanced non-squamous NSCLC were enrolled in the present study at Kansai Medical University Hirakata Hospital (Hirakata, Osaka, Japan). Three patients were excluded, as they were diagnosed with brain metastasis prior to treatment. The ITT population therefore consisted of 23 patients, and their demographic and clinical characteristics are shown in Table I.

*Treatment.* The 23 eligible patients received a median of six cycles (range, four to six cycles) of induction chemotherapy, consisting of pemetrexed, carboplatin and bevacizumab. Of these patients, 13 (56.5%) received maintenance chemotherapy, consisting of pemetrexed and bevacizumab, for a median of four cycles (range, 0-27 cycles).

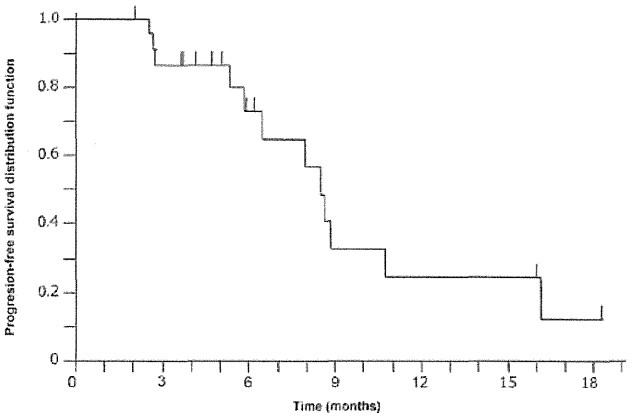


Figure 1. Kaplan-Meier curve for progression-free survival. The median progression-free survival time was 8.6 months (95% confidence interval, 5.9-10.9 months).

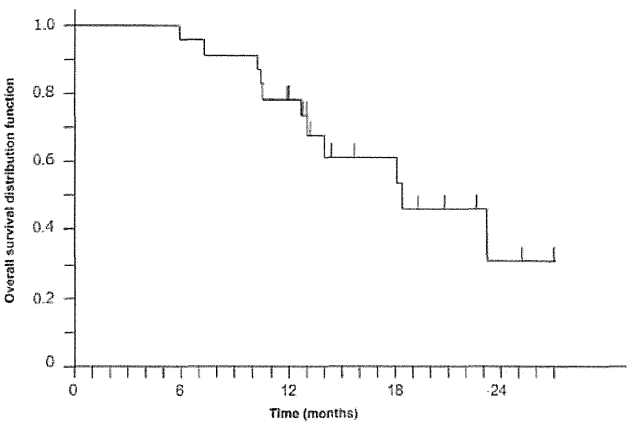


Figure 2. Kaplan-Meier curve for overall survival. The median overall survival time was 18.6 months (95% confidence interval, 12.9-24.8 months).

Reasons for discontinuation of induction chemotherapy included disease progression (n=3), unacceptable toxicity (n=3) and patient request (n=4). Reasons for discontinuation of the maintenance chemotherapy were disease progression (n=9) and unacceptable toxicity (n=3); one patient remains on maintenance chemotherapy at the present time.

Seven (30.4%) of the 23 patients had one or more dose reductions, two patients had two reductions each and two patients had more than two dose reductions each.

*Efficacy.* Of the 23 patients, three (13.0%) achieved a CR and 13 (56.5%) achieved a PR, making the RR 69.6% (95% CI, 47.1-86.8). The other seven patients (30.4%) achieved SD as their best response to therapy, making the disease control rate (DCR) 100% (Table II). The time to response was 1.2 months (95% CI, 0.72-1.93). At a median follow-up of 13.4 months (range, 5.9-27.4), the median PFS time was 8.6 months (95% CI, 5.9-10.9; Fig. 1) and the median OS time was 18.6 months (95% CI, 12.9-24.8 months; Fig. 2).

*Safety.* All adverse events (AEs) are listed in Table III. AEs of grade 3 or higher were observed in 15 patients (65.2%). During

Table III. Toxicities.

| Adverse events | Any grade, n (%) | Grade 3, n (%) | Grade 4, n (%) |
|---|---|---|---|
| Leukocytopenia | 12 (52.2) | 3 (13.0) | 0 (0.0) |
| Neutropenia | 11 (47.8) | 5 (21.7) | 2 (8.7) |
| Thrombocytopenia | 16 (69.6) | 2 (8.7) | 2 (8.7) |
| Anemia | 15 (65.2) | 1 (4.3) | 0 (0.0) |
| Hypoalbuminemia | 7 (30.4) | 0 (0.0) | 0 (0.0) |
| AST increased | 16 (69.6) | 2 (8.7) | 0 (0.0) |
| ALT increased | 17 (73.9) | 3 (13.0) | 0 (0.0) |
| Creatinine increased | 2 (8.7) | 0 (0.0) | 0 (0.0) |
| Blood bilirubin increased | 4 (17.4) | 0 (0.0) | 0 (0.0) |
| Blood K increased | 2 (8.7) | 0 (0.0) | 0 (0.0) |
| Fatigue | 11 (47.8) | 2 (8.7) | 0 (0.0) |
| Nausea | 12 (52.2) | 0 (0.0) | 0 (0.0) |
| Vomiting | 4 (17.4) | 0 (0.0) | 0 (0.0) |
| Anorexia | 14 (60.9) | 0 (0.0) | 0 (0.0) |
| Skin | 1 (4.3) | 0 (0.0) | 0 (0.0) |
| Hypertension | 3 (13.0) | 0 (0.0) | 0 (0.0) |
| Proteinuria | 11 (47.8) | 2 (8.7) | 0 (0.0) |
| Hemoptysis | 2 (8.7) | 0 (0.0) | 0 (0.0) |
| Venous thrombosis | 1 (4.3) | 0 (0.0) | 0 (0.0) |
| Mucositis oral | 4 (17.4) | 0 (0.0) | 0 (0.0) |
| Fever without neutropenia | 4 (17.4) | 0 (0.0) | 0 (0.0) |
| Alopesia | 4 (17.4) | 0 (0.0) | 0 (0.0) |
| Peripheral neuropathy | 4 (17.4) | 0 (0.0) | 0 (0.0) |
| Dysgeusia | 1 (4.3) | 0 (0.0) | 0 (0.0) |
| Pneumonitis | 1 (4.3) | 0 (0.0) | 0 (0.0) |
| Diarrhea | 3 (13.0) | 0 (0.0) | 0 (0.0) |
| Constipation | 9 (39.1) | 0 (0.0) | 0 (0.0) |

AST, aspartate aminotransferase; ALT, alanine transaminase.

cycles four to six of induction chemotherapy, the grade 3 and 4 hematological AEs included neutropenia (30.4%), thrombocytopenia (17.4%), leucopenia (13.0%) and anemia (4.3%), and the grade 3 and 4 non-hematologic AEs included liver damage (13.0%), fatigue (8.7%) and proteinuria (8.7%). Toxicity during the maintenance phase was minimal, with one patient each experiencing grade 3 leukocytopenia, anemia, fatigue and proteinuria. No patient experienced grade 3 or greater hypertension or venous thrombosis.

## Discussion

This single institution phase II study revealed that induction therapy with a combination of pemetrexed and carboplatin plus bevacizumab, followed by maintenance therapy with pemetrexed and bevacizumab, was effective and well-tolerated in chemotherapy-naïve Japanese patients with advanced non-squamous NSCLC. The RR was 69.6%, the DCR was 100%, the median PFS time was 8.6 months and the median OS time was 18.6 months. To the best of our knowledge, the present study is the first to evaluate the efficacy of this regimen in chemotherapy-naïve Japanese patients with advanced non-squamous NSCLC.

NSCLC was, until recently, considered to be a single disease, with first-line treatment of platinum-based doublet chemotherapy for patients with advanced disease. Carboplatin plus paclitaxel, the platinum-based doublet chemotherapy most frequently administered to patients with NSCLC, has been shown to exhibit an RR of 17%, a median PFS time of 3.1 months and a median OS time of 8.1 months (24).

Angiogenesis is important for tumor growth and metastasis, with the pro-angiogenic protein, VEGF, being a major regulator of angiogenesis in normal and malignant tissues (11,25,26). The overexpression of VEGF has been correlated with a poor prognosis in patients with NSCLC (12,13). Bevacizumab (Avastin; Genentech, South San Francisco, CA, USA) is a monoclonal antibody against VEGF that can impede tumor-associated angiogenesis. In a phase III randomized controlled trial, bevacizumab plus carboplatin and paclitaxel, followed by maintenance therapy with bevacizumab, was compared with carboplatin and paclitaxel in the treatment of patients with non-squamous NSCLC. The addition of bevacizumab improved the RR (35 vs. 15%), the median PFS time (6.2 vs. 4.5 months) and the median OS time (12.3 vs. 10.3 months) (16). Another phase III trial revealed that the efficacy of cisplatin plus pemetrexed was similar to that of cisplatin plus gemcitabine, a standard platinum-based doublet chemotherapy, but that cisplatin plus pemetrexed resulted in significantly fewer AEs (6). In addition, the OS time was longer with cisplatin plus pemetrexed compared with cisplatin plus gemcitabine in patients with non-squamous NSCLC, including those with adenocarcinoma (12.6 vs. 10.9 months) and large cell carcinoma (10.4 vs. 6.7 months). A more recent phase III randomized trial revealed that maintenance therapy with pemetrexed following first-line induction treatment with pemetrexed and cisplatin improved the PFS time in patients with advanced non-squamous NSCLC (7).

Taken together, these findings suggested that induction chemotherapy with platinum and pemetrexed plus bevacizumab, followed by maintenance pemetrexed and bevacizumab, would be effective in patients with advanced non-squamous NSCLC. Indeed, a phase II trial of carboplatin and pemetrexed plus bevacizumab, followed by maintenance pemetrexed and bevacizumab, exhibited a good RR (55%; 95% CI, 41-69), median PFS time (7.8 months; 95% CI, 5.2-11.5) and median OS time (14.1 months; 95% CI, 10.8-19.6) in Western patients with non-squamous NSCLC (19).

In a phase III trial, bevacizumab plus carboplatin and pemetrexed, followed by maintenance therapy with pemetrexed plus bevacizumab, significantly improved the PFS time in the treatment of patients with non-squamous NSCLC, although the OS time was not significantly improved (27). However, this regimen had not been tested in Japanese patients.

Japanese ethnicity has been reported to be a favorable prognostic factor for OS in NSCLC patients, with a higher RR and longer OS time in Japanese patients compared with Caucasian patients with NSCLC. Patients in the phase III ECOG E4599 trial who were treated with

carboplatin and paclitaxel with bevacizumab had an RR of 35% and a median OS time of 12.3 months (16), whereas those in the Japanese phase II JO19907 trial had an RR of 60.7% and a median OS time of 22.8 months (18). These findings suggested that the combination of carboplatin and pemetrexed plus bevacizumab, followed by maintenance pemetrexed and bevacizumab, would be effective and safe in Japanese patients with non-squamous NSCLC. Indeed, it was found in the present study that this regimen was effective in Japanese patients. The observed time to response in the present study was 1.2 months, similar to that observed in the JO19907 trial, suggesting that the addition of bevacizumab to chemotherapy results in rapid tumor size reduction. In addition, tumor-related symptoms were improved in the NSCLC patients who achieved a PR compared with SD following two cycles of first-line chemotherapy, thus improving patient quality of life.

This regimen was also demonstrated to be safe, as grade 4 hematological AEs were observed in only four patients, two each with neutropenia and thrombocytopenia, and grade 4 non-hematological AEs were not observed in any patients. AEs associated with bevacizumab, including hypertension and venous thrombosis, were also not observed.

In conclusion, treatment with induction chemotherapy, consisting of carboplatin and pemetrexed plus bevacizumab, followed by maintenance chemotherapy with pemetrexed and bevacizumab, was effective and tolerable in previously untreated Japanese patients with advanced non-squamous NSCLC, with a relatively short period to response. These results suggest that the regimen described in the present study should be tested in randomized, controlled trials of first-line treatment for Japanese patients with non-squamous NSCLC.

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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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*A complete list of the investigators in the PROFILE 1014 trial is provided in the Supplementary Appendix, available at NEJM.org.

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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.

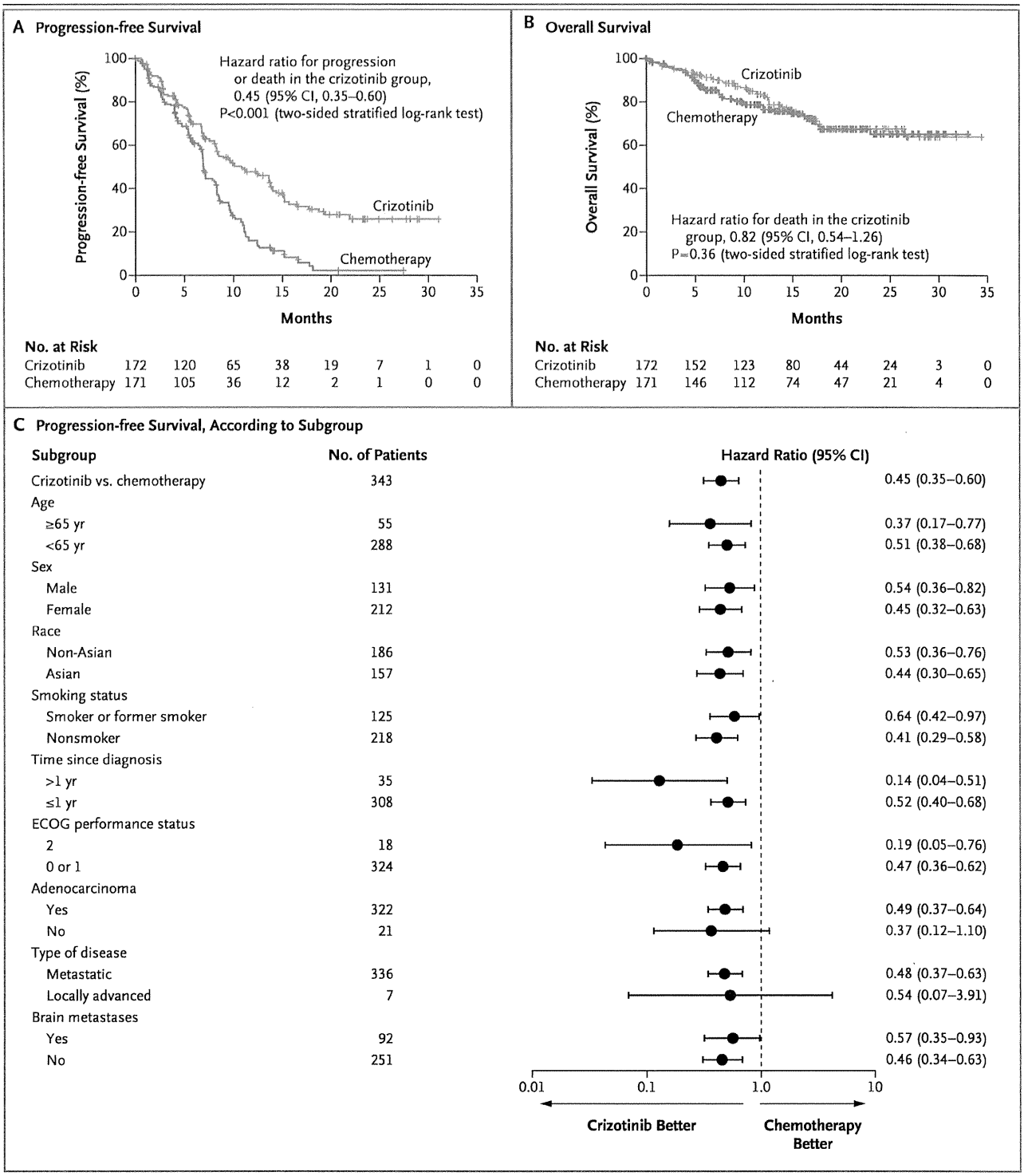
**Figure 1 (facing page). Progression-free and Overall Survival.**

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).

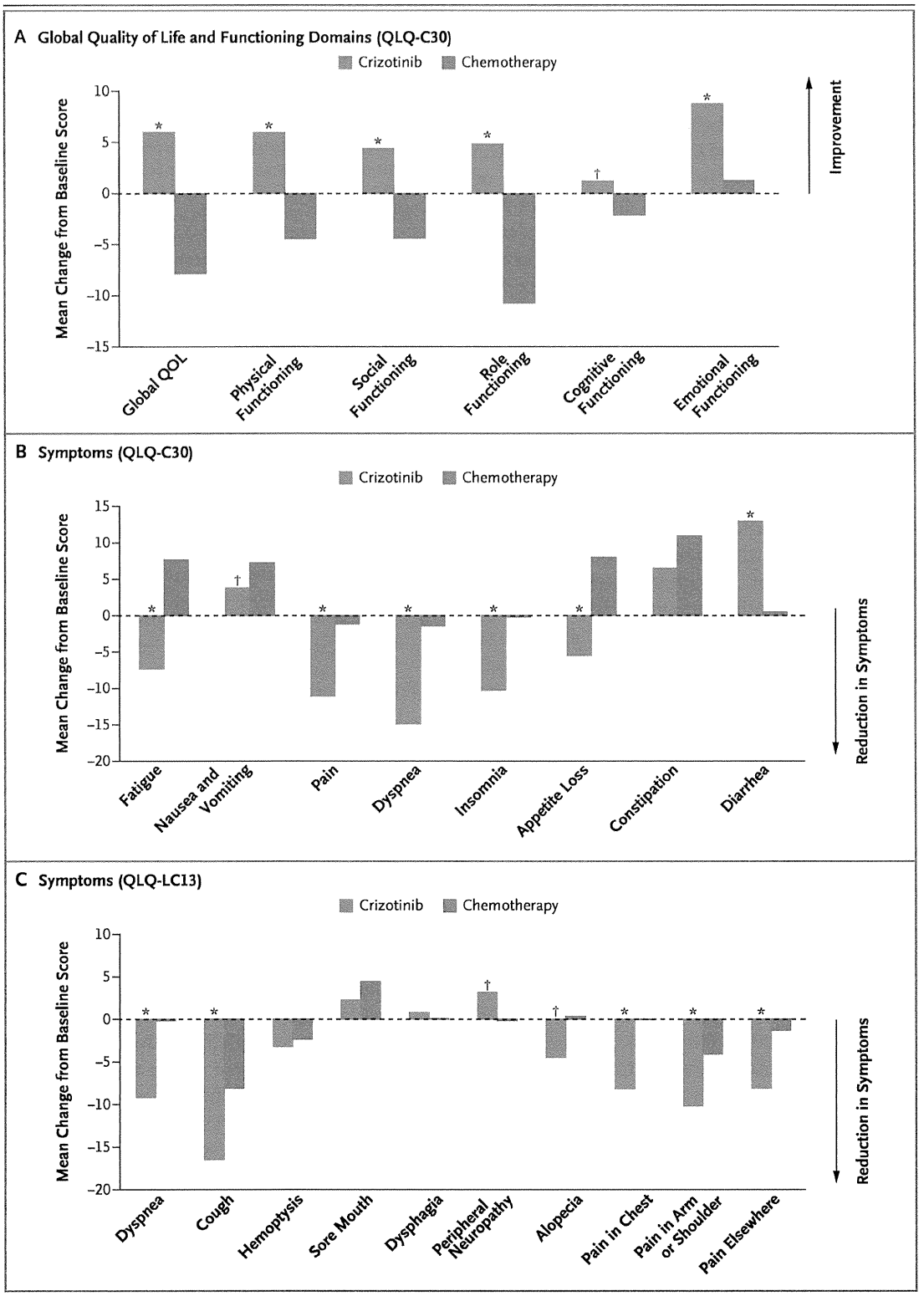
**Figure 2 (facing page). Overall Change from Baseline in Global Quality of Life, Functioning Domains, and Symptoms.**

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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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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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

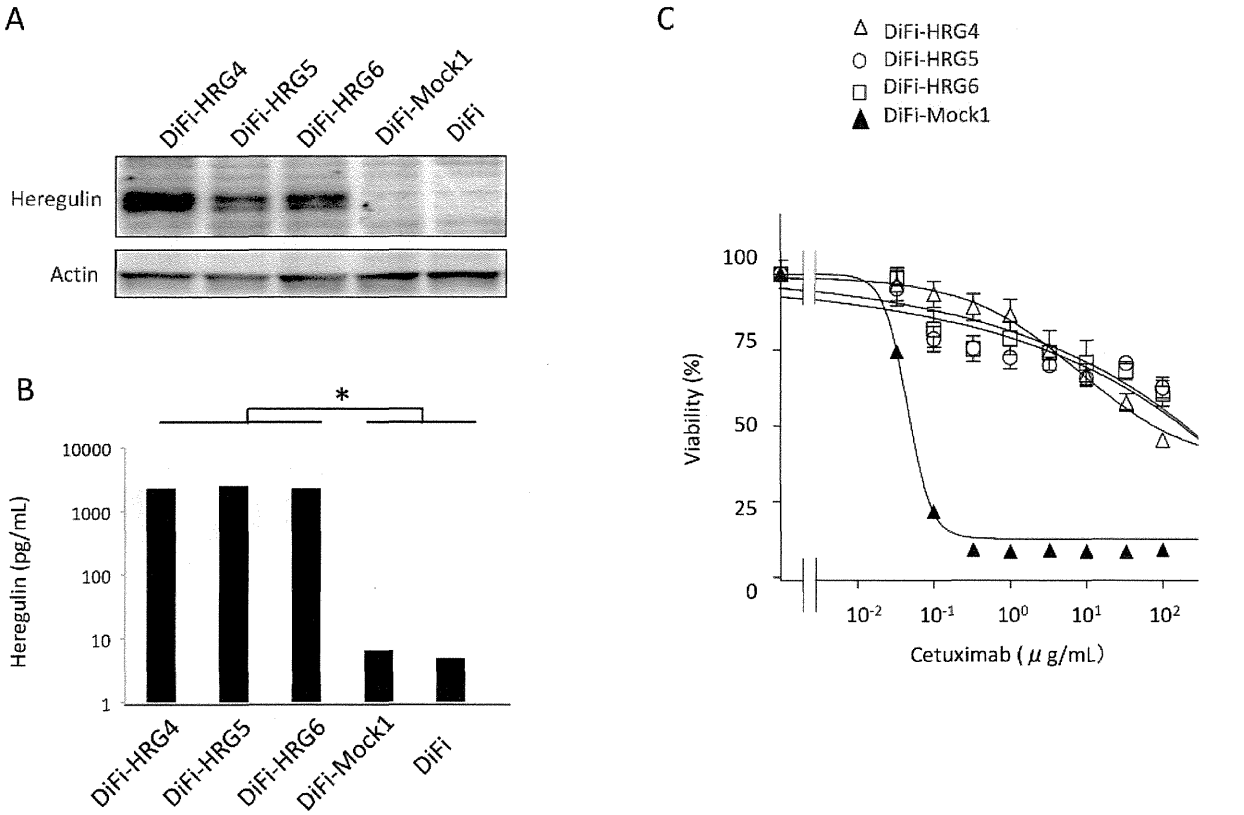


**Figure 1: Characterization of DiFi isogenic cell lines.** (**A**) DiFi isogenic cell lines (DiFi, DiFi-Mock1, DiFi-HRG4, DiFi-HRG5, and DiFi-HRG6) were cultured overnight in medium containing 10% serum and then incubated for 24 h in serum-free medium, after which the cells were lysed and subjected 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

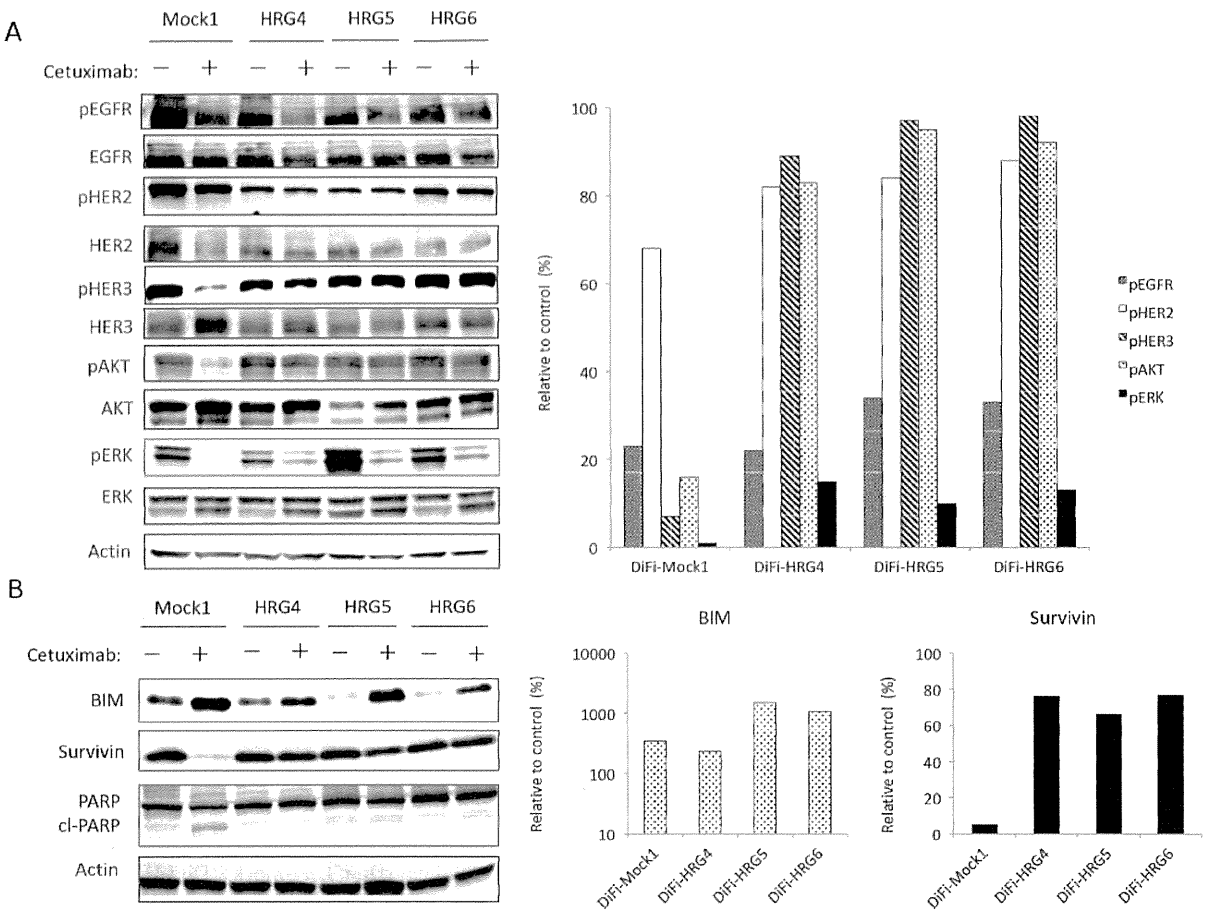


**Figure 2: Effects of cetuximab on intracellular signaling and the expression of apoptosis-related proteins in DiFi isogenic cell lines.** DiFi-Mock1, DiFi-HRG4, DiFi-HRG5, or DiFi-HRG6 cells were cultured overnight in medium containing 10% serum and then incubated for 6 h (**A**) or 24 h (**B**) in serum-free medium with or without cetuximab (10 μg/mL), after which cell lysates were prepared and subjected to immunoblot analysis with antibodies to phosphorylated (p) or total forms of the indicated proteins (left panels). A band corresponding to the cleaved (cl) form of PARP is indicated. The intensity of the bands corresponding to phosphorylated forms of EGFR, HER2, HER3, AKT, and ERK (A) or to BIM and survivin (B) was normalized by that of the corresponding total proteins or β-actin, respectively, and then expressed relative to the corresponding value for control cells not exposed to cetuximab (right panels).

treatment with patritumab and cetuximab *in vivo*, we injected nude mice with DiFi-Mock1 or DiFi-HRG4 cells to allow the formation of tumor xenografts. Whereas cetuximab alone markedly inhibited the growth of DiFi-Mock1 xenografts (Fig. 4A), DiFi-HRG4 xenografts were resistant to this drug (Fig. 4B). Patritumab alone had little effect on the growth of tumors formed by either cell line. However, the combination of cetuximab and patritumab induced substantial regression of DiFi-HRG4 xenografts (Fig. 4B). These results thus suggested that heregulin produced by colorectal cancer tumors harboring wild-type *KRAS* induces cetuximab resistance, and that combination therapy with cetuximab and patritumab overcomes such resistance *in vivo*.

## DISCUSSION

Resistance to cetuximab is a major problem in the treatment of colorectal cancer. Although various mechanisms of cetuximab resistance have been identified [1–7, 17–20], the optimal treatment strategies for mCRC patients who show resistance to this drug remain unclear. We previously showed that tumor-derived heregulin mediates cetuximab resistance in preclinical models [8]. High levels of heregulin were also associated with a poor clinical outcome in mCRC patients treated with cetuximab-based regimens [8]. Moreover, increased heregulin levels were observed in such patients after the development of clinical resistance to cetuximab-based

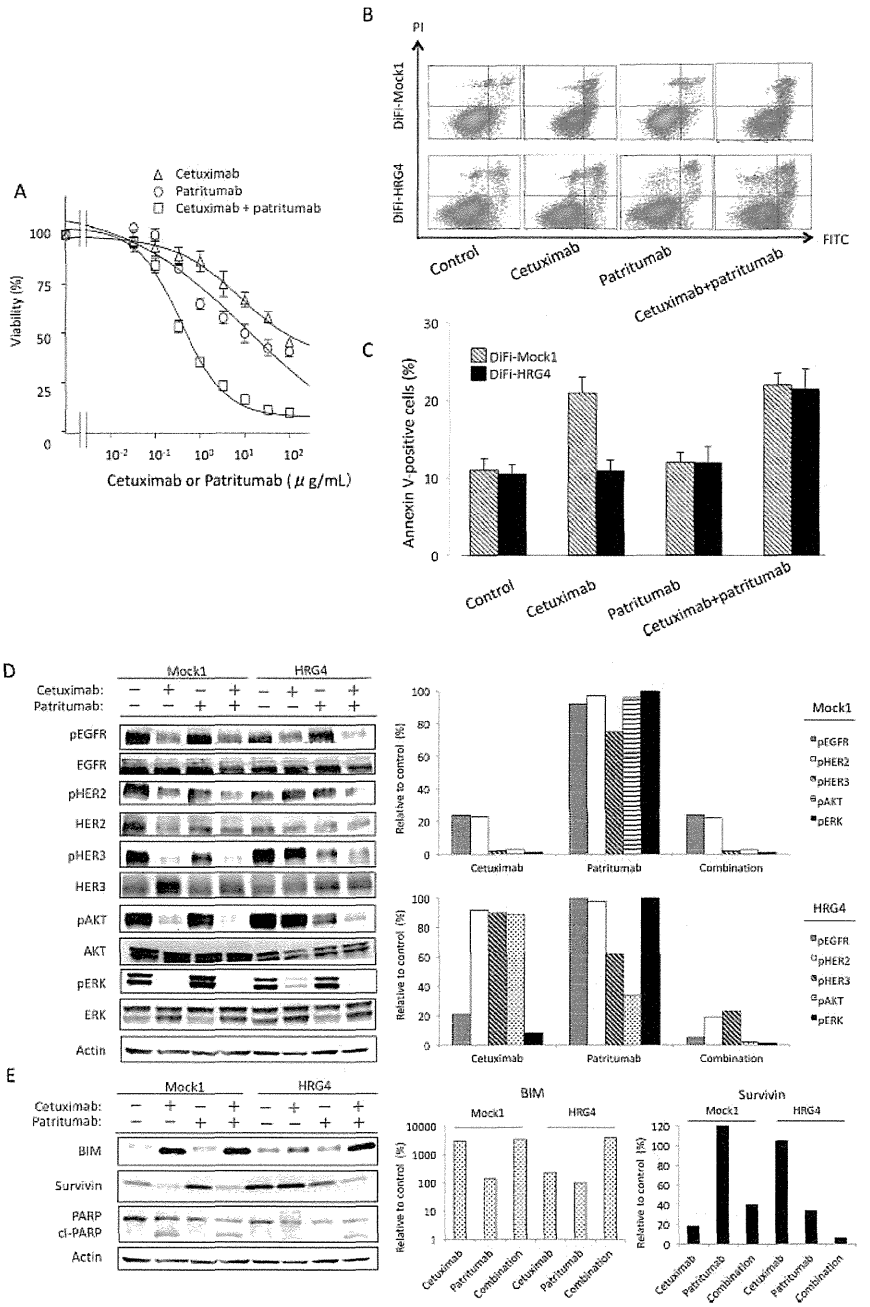


**Figure 3: Effect of patritumab on heregulin-mediated cetuximab resistance in DiFi-HRG cells *in vitro*.** (**A**) DiFi-HRG4 cells were incubated for 5 days with cetuximab alone, patritumab alone, or the combination of both drugs at the indicated concentrations, after which cell viability was assessed. Data are means ± SE from three independent experiments. (**B, C**) DiFi-Mock1 or DiFi-HRG4 cells were cultured overnight in medium containing 10% serum and then incubated for 48 h in the absence or presence of cetuximab alone (10 μg/mL), patritumab alone (10 μg/mL), or the combination of both drugs in serum-free medium, after which the number of apoptotic cells was determined by staining with propidium iodide (PI) and fluorescein isothiocyanate (FITC)–labeled annexin V followed by flow cytometry. Representative flow cytometric profiles are shown in (B), and quantitative data (means ± SE of three independent experiments) are shown in (C). (**D, E**) DiFi-Mock1 or DiFi-HRG4 cells were cultured overnight in medium containing 10% serum and then incubated for 6 h (D) or 48 h (E) in the absence or presence of cetuximab alone (10 μg/mL), patritumab alone (10 μg/mL), or the combination of both drugs in serum-free medium, after which cell lysates were prepared and subjected to immunoblot analysis with antibodies to the indicated proteins (left panels). The intensity of the bands corresponding to phosphorylated forms of EGFR, HER2, HER3, AKT, and ERK (D) or to BIM and survivin (E) was normalized by that of the corresponding total proteins or β-actin, respectively, and then expressed relative to the corresponding value for control cells not exposed to drug (right panels).