15 patients to allow for dropouts. Subsequently, the response rate of crizotinib for patients with *ALK*-rearranged NSCLC was published.[20] We amended this study to test the null hypothesis of a threshold response rate of 45% for the study drug, based on the reported response rate of crizotinib.[21] We kept the expected response rate at 70%. Consequently, 41 patients were required to yield a statistical power of 90% with a two-sided significance of 5%. Allowing for dropouts, we identified the target sample size in this study as 45 patients. Considering the multiplicity of the analysis, we determined that the null hypothesis assessing 45 patients with the threshold response rate of 45% should be tested only when the null hypothesis assessing 15 patients with a threshold response rate of 25% was rejected.

We did the analysis by intent to treat. The decision as to whether to reject the null hypothesis that the response rate of 45% or less was based on whether the lower limit of the 95% CI estimated using the Clopper-Pearson method exceeded 45%. We estimated the proportion of patients who achieved disease control together with an estimate of the CI with the Clopper-Pearson method. Additionally, we did a pot-hoc subgroup analysis of response rate with regard to the age, sex, ECOG PS, body-mass index (BMI), number of previous chemotherapy regimens for metastatic disease, history of treatment with pemetrexed, types of ALK diagnostic method, and status of brain metastasis. All analyses were done with SAS version 9.2. This study is registered with the Japan Pharmaceutical Information Center, number JapicCTI-101264.

Role of the funding source

This study was designed and funded by the study sponsor (Chugai Pharmaceutical Co, Ltd) and monitored by a clinical research organisation (EPS Corporation). The clinical research organisation collected all data and the study sponsor did all data analysis and interpretation, with input from the authors and investigators. The initial draft of the report was reviewed and commented on by all authors, and by employees of Chugai Pharmaceutical Co, Ltd. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

The first patient identified with *ALK*-positive NSCLC was enrolled on Sept 10, 2010, and received their first dose on Sept 14, 2010. The last patient was enrolled on April 18, 2012, and received their first dose on April 18, 2012. Data cutoff for this report was July 31, 2012.

For both the phase 1 and phase 2 parts of this study, 436 patients were screened for ALK and 135 (31%) patients were identified as *ALK*-positive. 70 patients were enrolled and treated in either the phase 1 (24 patients) or the phase 2 portions (46 patients). The major reason for

	Patients	Dose-limiting toxicities
Fasting		
20 mg (twice daily)	1	None
40 mg (twice daily)	1	None
80 mg (twice daily)	1	None
160 mg (twice daily)	3	None
240 mg (twice daily)	3	None
300 mg (twice daily)	6	None
Non-fasting		
240 mg (twice daily)	3	None
300 mg (twice daily)	6	None

Table 2: **Dose escalation and dose-limiting toxicities in phase 1 (n=24)**

	Patients	T_{max} (h)	C_{max} (ng/mL)	C_{trough} (ng/mL)	AUC_{0-10} (ng·h/mL)
Fasting					
20 mg (twice daily)	1	4·00	25·5	19·6	220
40 mg (twice daily)	1	3·83	63·9	34·9	479
80 mg (twice daily)	1	2·00	150	105	1310
160 mg (twice daily)	3	4·61 (1·15)	300 (104)	214 (34)	2310 (598)
240 mg (twice daily)	3	3·33 (1·15)	385 (100)	262 (115)	2970 (937)
300 mg (twice daily)	6	3·99 (2·17)	575 (322)	463 (369)	4970 (3260)
Non-fasting					
240 mg (twice daily)	3	5·24 (1·13)	380 (83)	332 (79)	3300 (838)
300 mg (twice daily)	6	5·32 (1·58)	528 (138)	425 (150)	4220 (1190)

Data are individual values or mean (SD), unless otherwise stated. T_{max}=time to reach maximum concentration. C_{max}=maximum plasma concentration. C_{trough}=plasma concentration at trough. AUC_{0-10}=area under plasma-concentration time curve from 0–10 h.

Table 3: **Pharmacokinetic parameters of CH5424802 at steady state in the patients under fasting and non-fasting conditions (n=24)**

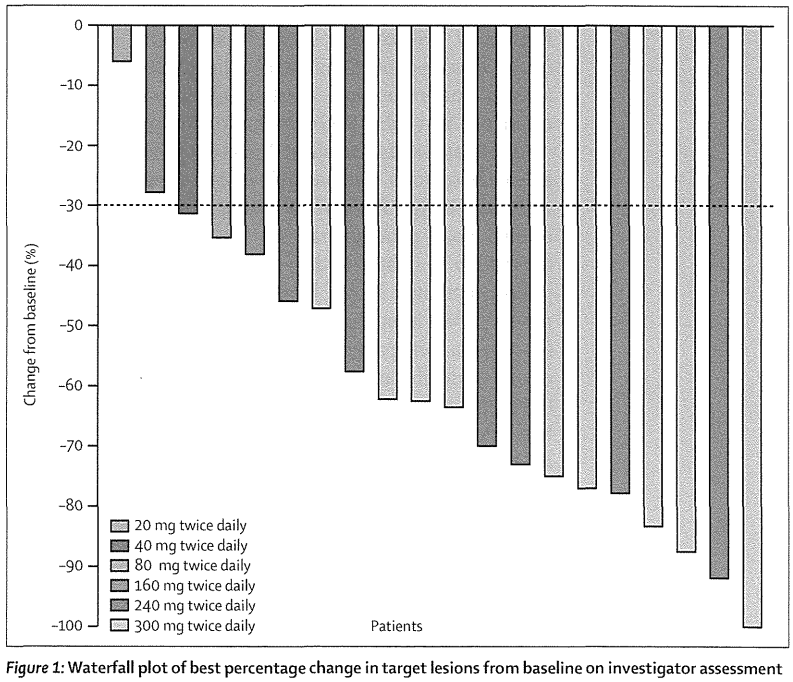

Figure 1: **Waterfall plot of best percentage change in target lesions from baseline on investigator assessment (20 patients with measurable lesions in phase 1)**

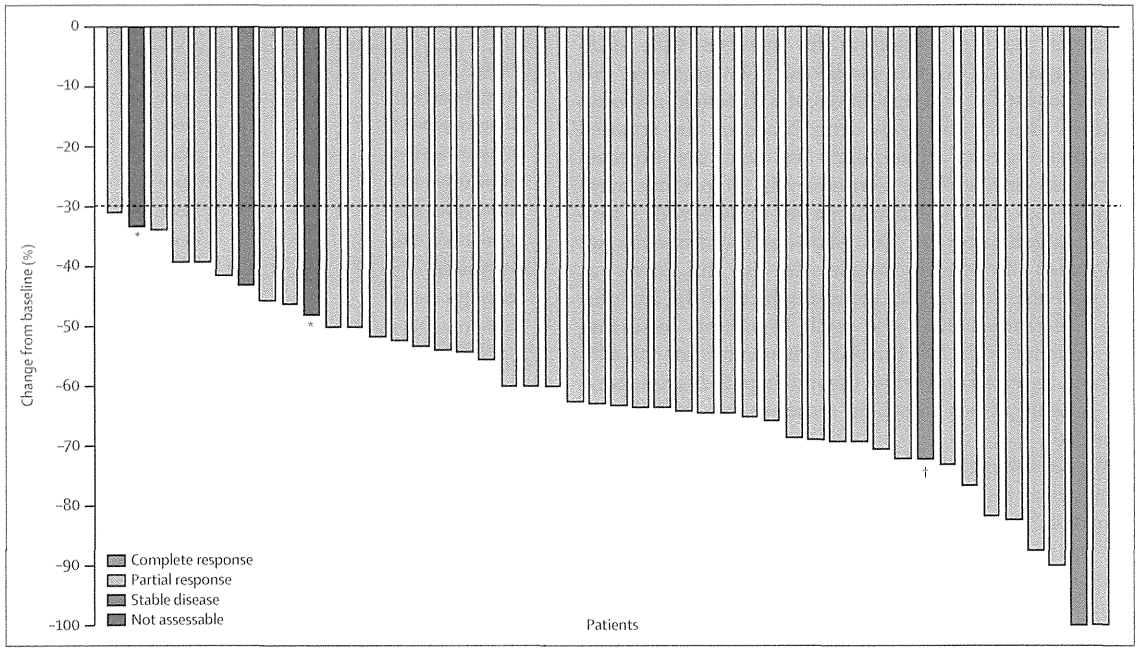

Figure 2: **Waterfall plot of best percentage change in target lesions from baseline based on independent review committee assessment (46 patients in phase 2)**
*Indeterminate response by early stopping because of safety reasons. †Classified as complete response according to the definition of Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 for patients for whom lymph nodes were identified as target lesions and which were reduced to less than 10 mm. These responses (complete response and partial response) were confirmed by subsequent scan.

exclusion of the other 65 ALK-positive patients was because of other eligibility criteria, or a reason not specified by investigators.

Table 1 summarises the baseline characteristics of patients enrolled in this study. In the phase 1 portion of the study, 15 patients were treated with CH5424802 under fasting conditions in six cohorts (20–300 mg twice a day), and nine were treated under non-fasting conditions in two cohorts (240 mg and 300 mg twice a day).

All 24 patients in the phase 1 part of the study completed at least two cycles, and had at least one adverse event while on study. Eight (33%) of 24 patients had grade 3 adverse events. Four patients had six adverse events that were deemed to be related to the study treatment—neutropenia (three patients, 13%), blood bilirubin increased (one patient, 4%), hypophosphataemia (one patient, 4%), and leucopenia (one patient, 4%). We noted no grade 4 adverse events or deaths at any dose level. We noted no DLTs up to the highest dose (300 mg twice a day; table 2). One patient had a dose reduction due to rash at a dose of 300 mg twice a day in the phase 1 portion, but no patient needed drug discontinuation because of adverse events. Thus, we did not identify the MTD in this study.

Blood samples were taken from all 24 patients. Table 3 shows the pharmacokinetics parameters at steady state after multiple dosing (day 21 in cycle 1). T_{max} was between 2·00 h and 4·61 h constantly throughout the dose range (20–300 mg twice daily), and the AUC_{0-10} increased in an approximately linear way within the dose range under the fasting condition. We compared the absorption of CH5424802 under fasting and non-fasting conditions at 240 mg and 300 mg twice daily. The plasma exposures at steady state were similar under fasting and non-fasting conditions, although it took longer to reach T_{max} under non-fasting conditions.

Of the 24 patients, all 20 (83%) patients with measureable lesions based on RECIST criteria and treated with CH5424802 showed tumour shrinkage and 17 (85%) of 20 patients had a partial response by investigator's assessment (figure 1). All 15 patients with measurable lesions treated at doses higher than 160 mg twice a day achieved a partial response (240 mg [six patients], and 300 mg [nine patients]). One patient (4%) with non-measurable lesions met the criteria of RECIST version 1.1 for a complete response. The mean duration of treatment was 11·8 months (range 3–18) with a median follow-up of 12·05 months (range 4·7–20·8). 16 (67%) patients enrolled during the phase 1 portion of this trial remained on study treatment as of July 31, 2012.

On the basis of these results, the planned highest dose (300 mg twice daily) was judged as acceptable to be the recommended dose in the phase 2 portion.

Of the 46 patients enrolled in the phase 2 portion of the trial (all of whom had measureable lesions), two patients (4·3%, 95% CI 0·5–14·8) achieved a complete response, 41 patients (89·1%, 76·4–96·4) had a partial response, and one patient (2·2%, 0·1–11·5) had stable disease by independent review committee assessment (figure 2). No

patient had progressive disease; two patients (4·3%) had an unknown response because of early withdrawal. Thus 43 patients (93·5%, 95% CI 82·1–98·6) had an objective response, and 44 (95·7%, 95% CI 85·2–99·5) achieved disease control. We noted no apparent differences in response when analysed by age, sex, ECOG PS, BMI, number of previous chemotherapy regimens for metastatic disease, history of treatment with pemetrexed, types of *ALK* test, and status of brain metastasis (data not shown).

Figure 2 shows a waterfall plot of the best percentage change in the size of target lesions from baseline. All patients had a reduction in tumour size of more than 30%. Response to treatment was noted early, and 30 (65%) of 46 patients reached the criteria for partial response within 3 weeks (cycle 1) and 40 (87%) patients did so within 6 weeks (cycle 2; figure 3).

The study is still ongoing; 40 (87%) of 46 patients remained on treatment as of data cutoff and more follow-up is needed for precise estimation of treatment duration and progression-free survival in the phase 2 portion. The median treatment duration as of data cutoff had already passed 7·1 months (range 1–11) with a median follow-up period of 7·6 months (3·4–11·3).

Of the 46 patients in the phase 2 portion, 15 (33%) patients had known brain metastases, of whom 12 (26%) had previous radiation for CNS metastases and three (7%) were clinically stable without symptoms at baseline. Seven patients had prolonged periods of disease control for more than 6 months on CH5424802 treatment (average 6·5 months, range 0·8–11·3). No progression of CNS lesions in any of the patients was noted by the time of data cutoff, although radiotherapy before treatment might have affected the natural history of brain disease. Of the patients with CNS lesions, 12 were on treatment at data cutoff, and three patients had discontinued treatment because of brain oedema, tumour haemorrhage, and progression of non-CNS tumour lesions. Two of the three patients who had baseline CNS lesion but no radiation continued the study medication for more than 300 days without progression of brain metastases.

Adverse events were recorded in all 46 patients included in the safety analysis. Grade 3 adverse events were reported in 17 (37%) patients, but no grade 4 adverse events or deaths were reported. Serious adverse events occurred in five (11%) patients (brain oedema, radius fracture, tumour haemorrhage, cholangitis sclerosing, and alveolitis allergic). Four (9%) patients discontinued treatment because of adverse events (brain oedema, tumour haemorrhage, interstitial lung disease, and sclerosing cholangitis), which were considered related to CH5424802 with the exception of brain oedema. 22 (48%) patients suspended treatment whithin the 21-day limit because of adverse events. No patients required dose reduction.

Table 4 shows treatment-related adverse events reported in 10% of patients or more. Treatment-related adverse events were noted in 43 (93%) of 46 patients. 12 (26%) patients had treatment-related grade 3 adverse events, including two patients each having decreased neutrophil count and increased blood creatine phosphokinase. Other treatment-related grade 3 adverse events were noted in one patient each only.

The most frequently reported treatment-related adverse events were dysgeusia, followed by increased aspartate aminotransferase (AST), increased blood bilirubin, increased blood creatinine, rash, constipation, and increased alanine aminotransferase (ALT; table 4). Almost all events were grade 1 or 2 (118 of 125 events, 94%).

All cases of dysgeusia were of grade 1 in nature and were not accompanied by loss of appetite. Increased blood bilirubin of grade 3 was noted in one patient, and other changes in laboratory values were limited to transient increases in AST and ALT and an increase in

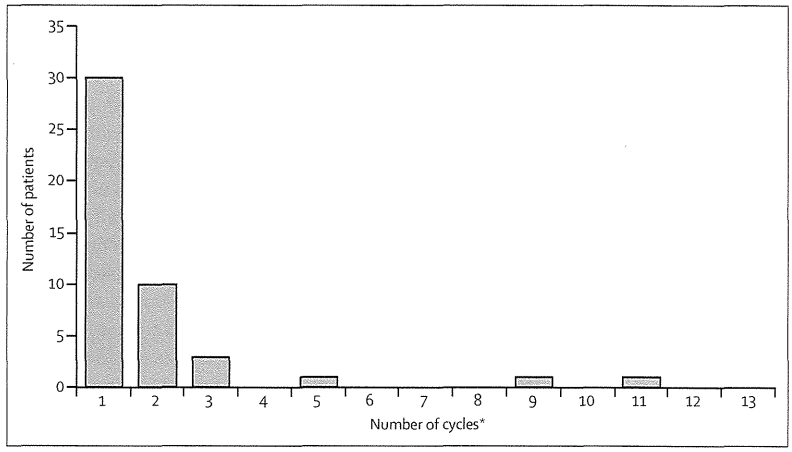

Figure 3: Number of patients who had tumour size reduction of 30% or more by treatment cycle in phase 2
*One cycle lasted 3 weeks.

	All grades	Grade 3
Dysgeusia	14 (30%)	0
Increased AST	13 (28%)	0
Increased blood bilirubin	13 (28%)	1 (2%)
Increased blood creatinine	12 (26%)	0
Rash	12 (26%)	1 (2%)
Constipation	11 (24%)	0
Increased ALT	10 (22%)	1 (2%)
Decreased neutrophil count	8 (17%)	2 (4%)
Increased blood CPK	7 (15%)	2 (4%)
Stomatitis	7 (15%)	0
Increased blood ALP	6 (13%)	0
Myalgia	6 (13%)	0
Nausea	6 (13%)	0

AST=aspartate aminotransferase. ALT=alanine aminotransferase. CPK=creatine phosphokinase. ALP=alkaline phosphatase.

Table 4: Treatment-related adverse events reported in 10% or more of patients enrolled in phase 2 (n=46)

Panel: Research in context

Systematic review

We searched PubMed for articles published in English until January, 2013 (no restriction for the starting date), with the search terms "ALK", "crizotinib", and "NSCLC". Although identified studies had small sample sizes, the effects of standard chemotherapy on *ALK*-rearranged non-small-cell lung cancer have been reported to be insufficient.[18] Crizotinib, a first-in-class ALK inhibitor, has been shown to be effective in patients with *ALK*-rearranged non-small-cell lung cancer.[6,23-25] While our study was underway, crizotinib was granted approval in the USA (on Aug 26, 2011), and subsequently in the EU and Japan. However, resistance to crizotinib-based treatment often develops within the first year after the start of treatment.[9]

Interpretation

Our phase 1–2 study suggests that CH5424802 is active and tolerable for treatment of patients with advanced *ALK*-rearranged non-small-cell lung cancer. ALK expression in normal tissue is very low[26] and might not be activated generally. CH5424802 is a selective ALK inhibitor and, therefore, allows a high exposure while limiting side-effects. The high proportion of patients achieving an objective response and the favourable effects on brain metastases suggest that CH5424802 is a promising ALK inhibitor. Investigation of CH5424802 in patients who are resistant to crizotinib is ongoing (NCT01588028).[27]

blood bilirubin of grade 1 or 2, and no case met Hy's law criteria[22] to suggest liver injury. The rash reported was clinically different from that caused by EGFR tyrosine kinase inhibitors, and limited to grade 1 or 2 in almost all patients. All increases in blood creatinine were grade 1 or 2. Visual disorders were rare with only visual impairment in one patient (2%), and blurred vision in another patient (2%), both of which were grade 1. Gastrointestinal toxic effects were mild, including nausea (six patients, 13%), diarrhoea (two patients, 4%), and vomiting (one patient, 2%). No cases of grade 3 nausea, diarrhoea, or vomiting were reported. All other adverse events were mild in severity.

Discussion

The results of this phase 1–2 study showed that CH5424802, given at a dose of 300 mg twice daily, is safe and active in patients with *ALK*-rearranged NSCLC. Almost 94% of patients achieved an objective response, and early reductions in tumour size of at least 30% were noted in most patients within the first 6 weeks. The proportion of patients who achieved an objective response noted here for CH5424802 is substantially higher than that of crizotinib (60·8% and 53%) in two separate early phase trials (panel).[6,23] Although median progression-free survival has not yet been reached, the median treatment duration at the time of data cutoff had already passed 7·1 months, and 40 of 46 patients remained on treatment.

The activity of CH5424802 could be explained by its potency and highly selective inhibitory effect on ALK. Whereas crizotinib is a multitargeted receptor tyrosine kinase inhibitor of *ALK*, *MET*, and *ROS1*, CH5424802 is highly selective for ALK without activity against MET and ROS1. In preclinical studies using Ba/F3 cells expressing the EML4-ALK fusion protein, CH5424802 showed more than two-fold higher potency than did crizotinib.[8,12] Moreover, the trough concentration of crizotinib given at the clinically recommended dose (250 mg twice daily) is reported to be 292 ng/mL,[28] whereas that of CH5424802 (at 300 mg twice daily) is 463 ng/mL, suggesting that sustained high blood concentrations can be achieved. Thus, sufficiently high exposure of CH5424802 was achieved in the clinical setting. Since ALK expression in normal adult tissues is extremely low,[26] the high selectivity for ALK might contribute to the better activity and safety profile of CH5424802 than crizotinib. On the other hand, there may be ethnic differences in pharmacokinetics of CH5424802 between Asian and non-Asian populations, as noted with crizotinib, which will be assessed in an ongoing phase 1–2 study in the USA (NCT01588028).[27]

Although most *ALK*-rearranged NSCLCs respond to treatment with ALK tyrosine kinase inhibitors, resistance to treatment with crizotinib often develops within the first year. This resistance is thought to be attributed to point mutations and amplification of the *ALK* fusion gene in a third of cases or activation of bypass signalling in other cases.[8,9] Most notably, the Leu1196Met aminoacid substitution has been shown to confer resistance to crizotinib, which corresponds to the gatekeeper mutations of *EGFR* (Thr790Met) and *BCR-ABL* (Thr315Ile), a mechanism of resistance to gefitinib and imatinib, respectively.[8,9] The fact that CH5424802 inhibits *EML4-ALK* Leu1196Met-driven cell growth[12] is another reason that CH5424802 could be more active than crizotinib. Currently, a clinical study assessing the activity of CH5424802 in patients who failed to respond to crizotinib-based treatment is ongoing (NCT01588028).[27]

Although limited by the small number of patients, and potential confounding by previous treatment with radiotherapy, CH5424802 seems to have activity in patients with CNS disease. In the three patients with CNS metastases but who did not receive brain irradiation, CNS lesions showed responses to treatment, which is encouraging considering almost half of patients treated with crizotinib have CNS relapse.[11]

In the present study, we did immunohistochemistry and FISH tests, and we deemed patients with double-positive results, or those confirmed by RT-PCR, as being positive for *ALK* fusion gene expression. By contrast, the crizotinib phase 1 trial[6,24] included patients who were positive by FISH test only, and later it was reported[29] that a higher response rate was noted in patients with double-positive

results, suggesting that there might have been patients with false-positive results by FISH test. Therefore, the difference in the diagnostic methods might contribute to the observed difference in the activity between the two drugs, and this should be explored in future studies.

CH5424802 was generally well tolerated with manageable adverse events. Although four patients discontinued treatment because of adverse events in this study, all 42 patients continued treatment with CH5424802 without any dose modification at the time of data cutoff. No adverse events specific to CH5424802 leading to discontinuation were identified either. Among 43 events in 22 patients with drug suspension, 24 events (56%) were due to the strict cycle initiation criteria. Since this is a first-in-human trial and safety profile of ALK inhibitors were not well known at the initiation of this study, strict cycle initiation criteria were defined, in addition to treatment suspension and dose reduction criteria. Patients with grade 2 non-haematological toxic effects or decreased neutrophil count suspended CH5424802 until they resolved to grade equal to or lower than 1 or grade at baseline at the initiation of each following cycle. Symptoms such as visual and gastrointestinal disorders (diarrhoea, vomiting, and nausea) that were frequently reported with crizotinib occurred at a low rate in this study. This could be related to the high selectivity of this compound to ALK kinase. The inhibitory activity against other kinases, such as MET and ROS1 by crizotinib, might be a reason for these side-effects of crizotinib.

Almost a third of the patients screened for ALK assessment were identified as *ALK* positive. This *ALK*-positive ratio is higher than that previously reported,[1] which might be due to bias by selecting patients with negative EGFR mutations, younger age, or non-smoking status. Limitations of this study can include a lack of any *EML4-ALK* mutational data. The study was also limited by a rather small enrolment and short follow-up period, and by its non-randomised nature.

Based on the results of the present study, CH5424802 could be an effective and safe option for the treatment of *ALK*-rearranged NSCLC. Further studies to confirm the efficacy of the drug and to assess its activity in patients resistant to crizotinib are ongoing.

Contributors

All authors contributed to data analysis, data interpretation, and writing of the report.

Conflicts of interest

TSe has received lecture fees and research funding from Chugai, Pfizer, and Novartis. KK has received lecture fees from Chugai, Pfizer, Novartis, and Astellas, and research funding from Chugai and Pfizer. MN has received lecture fees from Chugai and Pfizer, and research funding from Chugai, Pfizer, and Novartis. KN has received lecture fees and research funding from Chugai, Pfizer, Novartis, and Astellas. MM has received lecture fees from Chugai and Novartis, and research funding from Novartis. AI has received lecture fees and research funding from Chugai. TH has received lecture fees and research funding from Chugai, Pfizer, and Novartis. NY has received lecture fees from Chugai and Pfizer; research funding from Chugai, Pfizer, and Novartis; and advisory fee from Novartis. HY has received lecture fees from Chugai and Pfizer, and research funding from Chugai and Novartis. MH has received lecture fees from Chugai and Pfizer, and research funding from Chugai. YO has received lecture fees, research funding, and travel grants from Chugai, Pfizer, and Novartis. NN has received lecture fees and research funding from Chugai and Pfizer. KT has received lecture fees and research funding from Chugai and Nichirei, and advisory fee from Chugai and Nichirei. TSh and TTan are employees of Chugai Pharmaceutical Co, Ltd. TTam has received lecture fees from Chugai, Pfizer, and Novartis, and research funding from Chugai.

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ORIGINAL ARTICLE

Crizotinib versus Chemotherapy in Advanced *ALK*-Positive Lung Cancer

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ABSTRACT

BACKGROUND

In single-group studies, chromosomal rearrangements of the anaplastic lymphoma kinase gene (*ALK*) have been associated with marked clinical responses to crizotinib, an oral tyrosine kinase inhibitor targeting ALK. Whether crizotinib is superior to standard chemotherapy with respect to efficacy is unknown.

METHODS

We conducted a phase 3, open-label trial comparing crizotinib with chemotherapy in 347 patients with locally advanced or metastatic *ALK*-positive lung cancer who had received one prior platinum-based regimen. Patients were randomly assigned to receive oral treatment with crizotinib (250 mg) twice daily or intravenous chemotherapy with either pemetrexed (500 mg per square meter of body-surface area) or docetaxel (75 mg per square meter) every 3 weeks. Patients in the chemotherapy group who had disease progression were permitted to cross over to crizotinib as part of a separate study. The primary end point was progression-free survival.

RESULTS

The median progression-free survival was 7.7 months in the crizotinib group and 3.0 months in the chemotherapy group (hazard ratio for progression or death with crizotinib, 0.49; 95% confidence interval [CI], 0.37 to 0.64; P<0.001). The response rates were 65% (95% CI, 58 to 72) with crizotinib, as compared with 20% (95% CI, 14 to 26) with chemotherapy (P<0.001). An interim analysis of overall survival showed no significant improvement with crizotinib as compared with chemotherapy (hazard ratio for death in the crizotinib group, 1.02; 95% CI, 0.68 to 1.54; P=0.54). Common adverse events associated with crizotinib were visual disorder, gastrointestinal side effects, and elevated liver aminotransferase levels, whereas common adverse events with chemotherapy were fatigue, alopecia, and dyspnea. Patients reported greater reductions in symptoms of lung cancer and greater improvement in global quality of life with crizotinib than with chemotherapy.

CONCLUSIONS

Crizotinib is superior to standard chemotherapy in patients with previously treated, advanced non–small-cell lung cancer with *ALK* rearrangement. (Funded by Pfizer; ClinicalTrials.gov number, NCT00932893.)

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ANAPLASTIC LYMPHOMA KINASE (ALK) IS a validated tyrosine kinase target in several cancers, including non–small-cell lung cancer, anaplastic large-cell lymphoma, and pediatric neuroblastoma.[1-3] *ALK* rearrangements are found in approximately 5% of cases of non–small-cell lung cancer and define a distinct molecular subtype of lung cancer.[4-7] With an estimated 1.3 million new cases of non–small-cell lung cancer worldwide each year,[8] this translates into more than 60,000 patients with *ALK*-positive non–small-cell lung cancer annually.

Crizotinib is an oral small-molecule tyrosine kinase inhibitor targeting ALK, MET, and ROS1 tyrosine kinases.[1,9,10] In two single-group studies, crizotinib showed marked antitumor activity in patients with advanced *ALK*-positive non–small-cell lung cancer, with objective response rates of approximately 60% and a median progression-free survival of 8.1 months in one of the studies and 9.7 months in the other.[11,12] In contrast, standard single-agent chemotherapies in the general population of patients with non–small-cell lung cancer have been associated with response rates of 10% or lower and median progression-free survival of 2 to 3 months.[13-15]

To date, the activity of standard chemotherapy has not been established in *ALK*-positive non–small-cell lung cancer. Retrospective studies suggest that *ALK* rearrangements may be associated with enhanced sensitivity to pemetrexed-based chemotherapy, with durations of response similar to those observed with crizotinib.[16,17]

We conducted a randomized, controlled, open-label, phase 3 trial of crizotinib, as compared with standard chemotherapy in patients with advanced, previously treated *ALK*-positive non–small-cell lung cancer.

METHODS

PATIENTS

Patients were eligible for inclusion in the study if they had locally advanced or metastatic non–small-cell lung cancer that was positive for *ALK* rearrangements. *ALK* testing was performed centrally with the use of a break-apart fluorescence in situ hybridization assay, which has an analytic sensitivity of 100% (95% confidence interval [CI], 98 to 100) and specificity of 100% (95% CI, 97 to 100).[1] Other eligibility criteria included an age of at least 18 years, progressive disease after one prior platinum-based chemotherapy regimen, measurable disease as assessed with the use of Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1,[18] and an Eastern Cooperative Oncology Group (ECOG) performance status of 0, 1, or 2 (with 0 indicating 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[19]). Patients with stable brain metastases that had been treated previously or were untreated and asymptomatic were eligible. All patients provided written informed consent.

STUDY OVERSIGHT

The protocol was approved by the institutional review board or independent ethics committee at each participating site 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) together with the members of the PROFILE 1007 steering committee (see the Supplementary Appendix, available with the full text of this article at NEJM.org). The sponsor collected the data and analyzed them in conjunction with the authors. The corresponding author wrote all the drafts of the manuscript. All the authors made the decision to submit the manuscript for publication and vouch for the accuracy and completeness of the data and for the fidelity of this report to the study protocol. Editorial support was provided by a medical writer at ACUMED (New York), who was funded by the sponsor. 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 (250 mg twice daily) in a 3-week cycle or intravenous chemotherapy comprising either pemetrexed (500 mg per square meter of body-surface area) or docetaxel (75 mg per square meter) every 3 weeks. Patients who were randomly assigned to chemotherapy received pemetrexed unless their prior chemotherapy regimen contained pemetrexed or unless their tumor had predominantly squamous-cell histologic features. Patients were stratified according to ECOG performance status (0 or 1 vs. 2), the presence or absence of brain metastases, and prior or no prior therapy with epidermal growth factor receptor (EGFR) kinase inhibitors.

The primary end point was progression-free

survival, as assessed by independent radiologic review. Secondary end points included overall survival, response rate (rate of partial and complete responses), safety, and patient-reported outcomes. Treatment was continued until RECIST-defined disease progression was documented, unacceptable toxic effects developed, the patient withdrew from the study, or the patient died. Patients could continue treatment beyond RECIST-defined progression at the discretion of the investigator. Patients in the chemotherapy group with RECIST-defined progression were allowed to cross over to receive crizotinib as part of a separate study (ClinicalTrials.gov number, NCT00932451).

ASSESSMENTS

Patients underwent baseline tumor imaging, including brain and bone scanning. Tumor assessments were performed every 6 weeks until RECIST-defined disease progression. RECIST, version 1.1, was used to assess tumor responses; all scans were subject to central review by independent radiologists who were unaware of the group assignments.

Adverse events, which were classified and graded according to the Common Terminology Criteria for Adverse Events, version 4.0 (http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf), were assessed from the time the patient provided written informed consent until at least 28 days after the last dose of study drug was administered. Patient-reported symptoms, functioning, and global quality of life were assessed at baseline, on day 1 of every cycle, and at the end of treatment with the use of a validated questionnaire, the European Organization for Research and Treatment of Cancer quality-of-life questionnaire (QLQ-C30)[20] and its corresponding module for lung cancer (QLQ-LC13).[21] Scores on these questionnaires range from 0 to 100. For symptoms, higher scores indicate greater severity of symptoms; for global quality of life, higher scores indicate better global quality of life.

STATISTICAL ANALYSIS

We estimated that with a total of 217 progression events or deaths, the study would have 90% power to detect a 56% improvement in progression-free survival with crizotinib as compared with chemotherapy (i.e., median progression-free survival of 7.0 months vs. 4.5 months), at a one-sided alpha level of 0.025. Progression-free survival was defined as the time from randomization to progression of the disease, as assessed by means of independent radiologic review, or to death. The prespecified number of progression events or deaths was reached in March 2012; the date of data cutoff was March 30, 2012. One prespecified interim analysis of overall survival was performed at the time of the final analysis of progression-free survival. For the final survival analysis, we estimate that 241 events will be required for the study to have 80% power to detect a 44% increase in overall survival; this number of events is not projected to occur until 21 months after the time of data cutoff.

Efficacy end points were analyzed mainly in the intention-to-treat population. We used the Kaplan–Meier method to estimate progression-free survival and overall survival, one-sided stratified log-rank tests to compare survival curves between the two groups, and stratified Cox regression models to estimate hazard ratios. Response rates as assessed by means of independent radiologic review were compared between the treatment groups with the use of a two-sided stratified Cochran–Mantel–Haenszel test. We evaluated efficacy end points for pemetrexed and docetaxel separately in the as-treated population, which included patients who received at least one dose of study medication.

Patient-reported outcomes were evaluated in all treated patients who had completed a baseline assessment and at least one post-baseline assessment. Repeated-measures mixed-effects modeling was performed to compare the two groups with respect to the overall change from baseline scores on the QLQ-C30 and QLQ-LC13 scales. The time to deterioration was calculated as the time from randomization to the first increase of 10 points or more (indicating worsening condition) from baseline in scores for a composite end point of chest pain, dyspnea, or cough. The time to deterioration was estimated with the use of the Kaplan–Meier method and was compared between the two groups with the use of an unstratified log-rank test.

RESULTS

PATIENTS

From February 2010 through February 2012, a total of 4967 patients were screened, of whom 347 underwent randomization — 173 to crizotinib and 174 to chemotherapy (Fig. S1 in the Supplementary Appendix). The 347 patients who underwent ran-

domization comprised the intention-to-treat population. A total of 99 patients (57%) in the chemotherapy group received pemetrexed, and 72 (41%) received docetaxel. Three patients who were randomly assigned to the chemotherapy group and 1 who was randomly assigned to the crizotinib group did not receive the assigned study treatment.

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

Characteristic	Crizotinib (N=173)	Chemotherapy (N=174)
Age — yr		
Median	51	49
Range	22–81	24–85
Age distribution — no. (%)		
<65 yr	146 (84)	151 (87)
≥65 yr	27 (16)	23 (13)
Male sex — no. (%)	75 (43)	78 (45)
Race — no. (%)†		
White	90 (52)	91 (52)
Asian	79 (46)	78 (45)
Other	4 (2)	5 (3)
Smoking status — no. (%)‡		
Never smoked	108 (62)	111 (64)
Former smoker	59 (34)	54 (31)
Current smoker	5 (3)	9 (5)
Tumor histologic type — no. (%)§		
Adenocarcinoma	164 (95)	164 (94)
Non-adenocarcinoma	5 (3)	7 (4)
ECOG performance status — no. (%)¶		
0	72 (42)	65 (37)
1	84 (49)	95 (55)
2	16 (9)	14 (8)
Extent of disease — no. (%)		
Locally advanced	7 (4)	16 (9)
Metastatic	165 (95)	158 (91)
Presence of brain metastases — no. (%)	60 (35)	60 (34)

* There were no significant differences between the groups in any of the baseline characteristics listed here.
† Race was reported by the investigators.
‡ Data were missing for one patient in the crizotinib group.
§ Data were missing for seven patients: four in the crizotinib group and three in the chemotherapy group.
¶ An Eastern Cooperative Oncology Group (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 were missing for one patient in the crizotinib group.

At the time of data cutoff, the median follow-up for overall survival was 12.2 months in the crizotinib group and 12.1 months in the chemotherapy group.

The baseline characteristics of the patients were well balanced between the two study groups (Table 1). The majority of patients were younger than 65 years of age, had never smoked, and had adenocarcinoma of the lung — characteristics that were consistent with those of patients with *ALK*-positive non–small-cell lung cancer in prior studies.[22,23] The baseline characteristics of the patients according to the type of chemotherapy they received are shown in Table S1 in the Supplementary Appendix.

EFFICACY

Among the 347 patients in the intention-to-treat population, 227 had disease progression or died by the time of data cutoff. The median progression-free survival, as determined by independent radiologic review, was 7.7 months (95% CI, 6.0 to 8.8) in the crizotinib group, as compared with 3.0 months (95% CI, 2.6 to 4.3) in the chemotherapy group (hazard ratio for disease progression or death with crizotinib, 0.49; 95% CI, 0.37 to 0.64; P<0.001) (Fig. 1A). In subgroup analyses, there was significant improvement in progression-free survival with crizotinib as compared with pemetrexed (hazard ratio for disease progression or death, 0.59; 95% CI, 0.43 to 0.80; P<0.001) and as compared with docetaxel (hazard ratio for disease progression or death, 0.30; 95% CI, 0.21 to 0.43; P<0.001) (Fig. 1B). Progression-free survival was longer with crizotinib than with chemotherapy in patient subgroups defined according to baseline characteristics and stratification factors (Fig. S2 in the Supplementary Appendix).

In the intention-to-treat population, the response rate, as verified by means of independent radiologic review, was significantly higher in the crizotinib group than in the chemotherapy group: 65% (95% CI, 58 to 72) with crizotinib as compared with 20% (95% CI, 14 to 26) with chemotherapy (P<0.001) (Table 2). In the as-treated population, the response rate was higher with crizotinib than with either type of chemotherapy (Fig. S3 in the Supplementary Appendix): 66% (95% CI, 58 to 73) with crizotinib, as compared with 29% (95% CI, 21 to 39) with pemetrexed and 7% (95% CI, 2 to 16) with docetaxel. All the

differences in response rates between crizotinib and each type of chemotherapy were significant (P<0.001).

At the time of data cutoff, 96 deaths had occurred in the intention-to-treat population — 49 (28%) in the crizotinib group and 47 (27%) in the chemotherapy group — representing 40% of the total number of events required for the final analysis of overall survival. The median overall survival was 20.3 months (95% CI, 18.1 to not reached) with crizotinib and 22.8 months (95% CI, 18.6 to not reached) with chemotherapy (hazard ratio for death in the crizotinib group, 1.02; 95% CI, 0.68 to 1.54; P=0.54) (Fig. S4 in the Supplementary Appendix). Of the 174 patients who were randomly assigned to chemotherapy, 112 (64%) subsequently received crizotinib outside the study; 34 patients (20%) discontinued chemotherapy but did not receive crizotinib, including 13 patients who died either while receiving chemotherapy or before starting follow-up therapy (Table S2 in the Supplementary Appendix).

A total of 85 patients (49%) in the crizotinib group and 28 patients (16%) in the chemotherapy group were still receiving the study treatment at the time of data cutoff. More patients in the crizotinib group than in the chemotherapy group continued treatment beyond RECIST-defined progression of disease (58 vs. 17), and the duration of such therapy was longer with crizotinib than with chemotherapy (median, 15.9 weeks [range, 2.9 to 73.4] vs. 6.9 weeks [range, 6.0 to 42.0]).

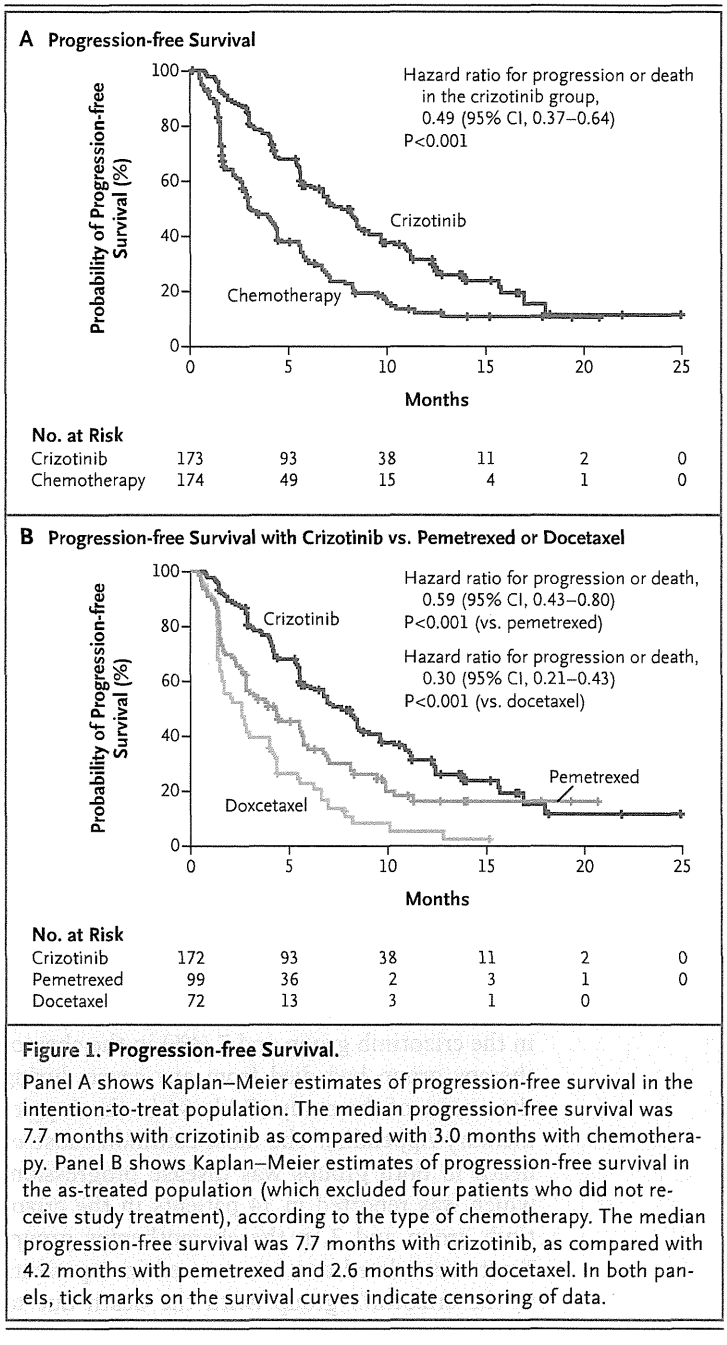

Figure 1. Progression-free Survival.

Panel A shows Kaplan–Meier estimates of progression-free survival in the intention-to-treat population. The median progression-free survival was 7.7 months with crizotinib as compared with 3.0 months with chemotherapy. Panel B shows Kaplan–Meier estimates of progression-free survival in the as-treated population (which excluded four patients who did not receive study treatment), according to the type of chemotherapy. The median progression-free survival was 7.7 months with crizotinib, as compared with 4.2 months with pemetrexed and 2.6 months with docetaxel. In both panels, tick marks on the survival curves indicate censoring of data.

SAFETY AND ADVERSE EVENTS

A total of 343 patients (the as-treated population) were included in the safety analysis. This analysis was not adjusted for the fact that patients in the crizotinib group received the assigned treatment for a longer duration than did patients in the chemotherapy group (median, 31 weeks vs. 12 weeks). The most common adverse events with crizotinib for which the incidence was at least 5% greater than that observed with chemotherapy were vision disorder (most frequently, visual impairment, photopsia, or blurred vision), diarrhea, nausea, vomiting, constipation, elevated liver aminotransferase levels, edema, upper respiratory infection, dysgeusia, and dizziness (Table 3). These events were mostly grade 1 or 2, with the exception of elevated aminotransferase levels, which were grade 3 or 4 in 27 patients (16%). The most common adverse events with chemotherapy for which the incidence was at least 5% greater than that observed with crizotinib were fatigue, alopecia, dyspnea, and rash (Table 3).

In the crizotinib group, grade 3 or 4 neutropenia occurred in 23 patients (13%), including 1 patient who had febrile neutropenia (Table S3 in the Supplementary Appendix). In the chemotherapy group, grade 3 or 4 neutropenia occurred in 33 patients (19%), including 16 patients who had febrile neutropenia.

Table 2. Summary of Responses in the Intention-to-Treat Population.*

Response	Crizotinib (N=173)	Chemotherapy (N=174)
Type of response — no. (%)		
Complete response	1 (1)	0
Partial response	112 (65)	34 (20)
Stable disease	32 (18)	63 (36)
Progressive disease	11 (6)	60 (34)
Could not be evaluated†	17 (10)	17 (10)
Rate of objective response — % (95% CI)‡	65 (58–72)	20 (14–26)
Duration of response — wk§		
Median	32.1	24.4
Range¶	2.1–72.4	3.0–43.6
Time to response — wk‖		
Median	6.3	12.6
Range	4.4–48.4	5.0–37.1

* 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 were indeterminate in 13 patients in each group and were not available owing to early death in 4 patients in each group.
‡ P<0.001 for the comparison between the two groups.
§ The duration of response was calculated from the date of the first documentation of partial or complete response to the date of RECIST-defined progression or death, with the use of the Kaplan–Meier method.
¶ This range takes into account only patients who had subsequent disease progression or who died.
‖ The time to response was calculated from the date of randomization to the date of the first documentation of a partial or complete response.

By the time of data cutoff, 25 patients (15%) in the crizotinib group and 7 (4%) in the chemotherapy group had died from any cause during the course of the study (Table S4 in the Supplementary Appendix). The most common cause of death in both groups was disease progression, which was reported in 14 patients in the crizotinib group and 3 in the chemotherapy group. Treatment-related deaths occurred in 3 patients in the crizotinib group (with the death due to ventricular arrhythmia in 1 patient and to interstitial lung disease or pneumonitis in 2 patients), and in 1 patient in the chemotherapy group (with the death due to sepsis). In addition, in the crizotinib group, hepatic dysfunction meeting the criteria for Hy's law (a serum bilirubin level ≥3 times the upper limit of the normal range in the absence of biliary obstruction or Gilbert's syndrome)[24] developed in 1 patient, who subsequently died of hepatic failure after the data cutoff date.

Overall, more adverse events of any cause were reported in the crizotinib group than in the chemotherapy group. This increase in all-cause adverse events was still apparent after events that occurred after RECIST-defined disease progression were excluded (Table S5 in the Supplementary Appendix). The incidence of treatment-related grade 3 or 4 adverse events was similar in the two groups (33% with crizotinib and 32% with chemotherapy), as was the incidence of treatment-related serious adverse events (12% and 14% in the two groups, respectively). Treatment-related adverse events leading to permanent discontinuation of the study drug occurred in 6% and 10% of patients in the two groups, respectively.

PATIENT-REPORTED OUTCOMES

Baseline scores on the QLQ-C30 and QLQ-LC13 are summarized in Table S6 in the Supplementary Appendix. There was a significantly greater overall reduction from baseline in the symptoms of alopecia, cough, dyspnea, fatigue, chest pain, arm or shoulder pain, and pain in other parts of the body with crizotinib than with chemotherapy (P<0.001 for all comparisons, without adjustment for multiple testing) (Fig. 2A). Patients treated with crizotinib also had a significantly greater delay in the worsening of symptoms. The median time to deterioration with respect to a composite end point of three symptoms — cough, dyspnea, or chest pain — was 5.6 months with crizotinib, as compared with 1.4 months with chemotherapy (hazard ratio with crizotinib, 0.54; 95% CI, 0.40 to 0.71; P<0.001) (Fig. 2B).

There was also a significantly greater overall improvement from baseline in global quality of life among patients who received crizotinib treatment than among those who received chemotherapy (P<0.001) (Fig. 2A). In particular, in the crizotinib group a statistically significant and clinically meaningful (≥10-point) improvement from baseline in global quality of life was observed in cycle 4, and a statistically significant (although <10-point) improvement from baseline in global quality of life was observed in cycles 2 through 12 and cycle 14. In contrast, in the chemotherapy group, no significant change from baseline in global quality of life was observed at any time point. Similarly, in all domains measuring functioning, except for the domain measuring cognitive functioning, there was a significantly greater overall improvement from baseline among patients

in the crizotinib group than among patients in the chemotherapy group (Fig. S5 in the Supplementary Appendix).

DISCUSSION

We conducted a prospective, randomized, phase 3 trial comparing crizotinib therapy with standard chemotherapy in patients with advanced *ALK*-positive non–small-cell lung cancer. As compared with standard second-line chemotherapy, treatment with crizotinib resulted in significantly longer progression-free survival, significantly higher response rates, a significant reduction in symptoms, and a significant improvement in global quality of life. In this study, crizotinib was more effective than either pemetrexed or docetaxel.

The efficacy of second-line docetaxel in patients with *ALK*-positive non–small-cell lung cancer was modest, a finding that was consistent with that in previous studies involving the general population of patients with non–small-cell lung cancer.[13,15] In contrast, the response rate to pemetrexed was higher than expected — 29%, as compared with 12.8% in the general population of patients with lung adenocarcinoma who had previously been treated with chemotherapy[13,25] — though the median progression-free survival among patients in our study who received pemetrexed was only 4.2 months. Thus, patients with *ALK*-positive non–small-cell lung cancer may have a higher response rate with pemetrexed than does the general population with non–small-cell lung cancer. However, the benefit of pemetrexed is less than that originally suggested in retrospective studies[16,17] and, importantly, less than that of crizotinib, as shown in this randomized trial.

In a prespecified interim analysis, overall survival was shown to be similar in the crizotinib and chemotherapy groups. This analysis was immature, and it is likely that it was confounded by the high crossover rate among patients in the chemotherapy group. Crossover has similarly complicated the analysis of overall survival in other randomized, phase 3 studies of EGFR kinase inhibitors in patients with advanced EGFR-mutant non–small-cell lung cancer.[26-28] Despite these limitations, the median overall survival among patients in this study from the time that second-line therapy was initiated was remarkably high, at longer than 20 months, suggesting that the addition of crizotinib either before or after second-line chemotherapy may contribute to improving survival. In contrast, in a small retrospective study, the median overall survival from the time of initiation of second-line therapy among patients with *ALK*-positive non–small-cell lung cancer who had not received crizotinib was 6 months.[29]

Both crizotinib and chemotherapy were associated with toxic effects that were primarily grade 1 or 2. Two important toxic effects that were associated with crizotinib were elevated aminotransferase levels and interstitial lung disease. Treatment-related elevation of aminotransferase levels of any grade was reported in 66 patients

Table 3. Adverse Events of Any Cause.*

Adverse Event	Crizotinib (N=172)		Chemotherapy (N=171)	
	Any Grade	Grade 3 or 4	Any Grade	Grade 3 or 4
	no. of patients (%)			
Vision disorder†‡	103 (60)	0	16 (9)	0
Diarrhea	103 (60)	0	33 (19)	1 (1)
Nausea§	94 (55)	2 (1)	64 (37)	1 (1)
Vomiting§	80 (47)	2 (1)	30 (18)	0
Constipation	73 (42)	4 (2)	39 (23)	0
Elevated aminotransferase levels†	66 (38)	27 (16)¶	25 (15)	4 (2)
Edema†	54 (31)	0	27 (16)	0
Fatigue	46 (27)	4 (2)	57 (33)	7 (4)
Upper respiratory infection†	44 (26)	0	22 (13)	1 (<1)
Dysgeusia	44 (26)	0	16 (9)	0
Dizziness†	37 (22)	1 (1)	14 (8)	0
Dyspnea†‖	23 (13)	7 (4)	32 (19)	5 (3)
Rash	15 (9)	0	29 (17)	0
Alopecia	14 (8)	0	35 (20)	0

* Adverse events are listed here if they were reported in 15% or more of patients in either treatment group and if there was at least a 5% difference between the two groups.
† This item comprised a cluster of adverse events that may represent similar clinical symptoms or syndromes.
‡ The category of vision disorder included (in descending order of frequency) visual impairment, photopsia, blurred vision, vitreous floaters, halo vision or photophobia, chromatopsia or diplopia, and reduced visual acuity.
§ The use of antiemetic agents was significantly higher in the chemotherapy group than in the crizotinib group (67% vs. 20%).
¶ Included is one case that met the criteria for Hy's law (a serum bilirubin level of ≥3 times the upper limit of the normal range in the absence of biliary obstruction or Gilbert's syndrome), with grade 5 hepatic failure occurring after the data cutoff date.
‖ One case of grade 5 dyspnea was reported in each treatment group (<1% of patients in each group).

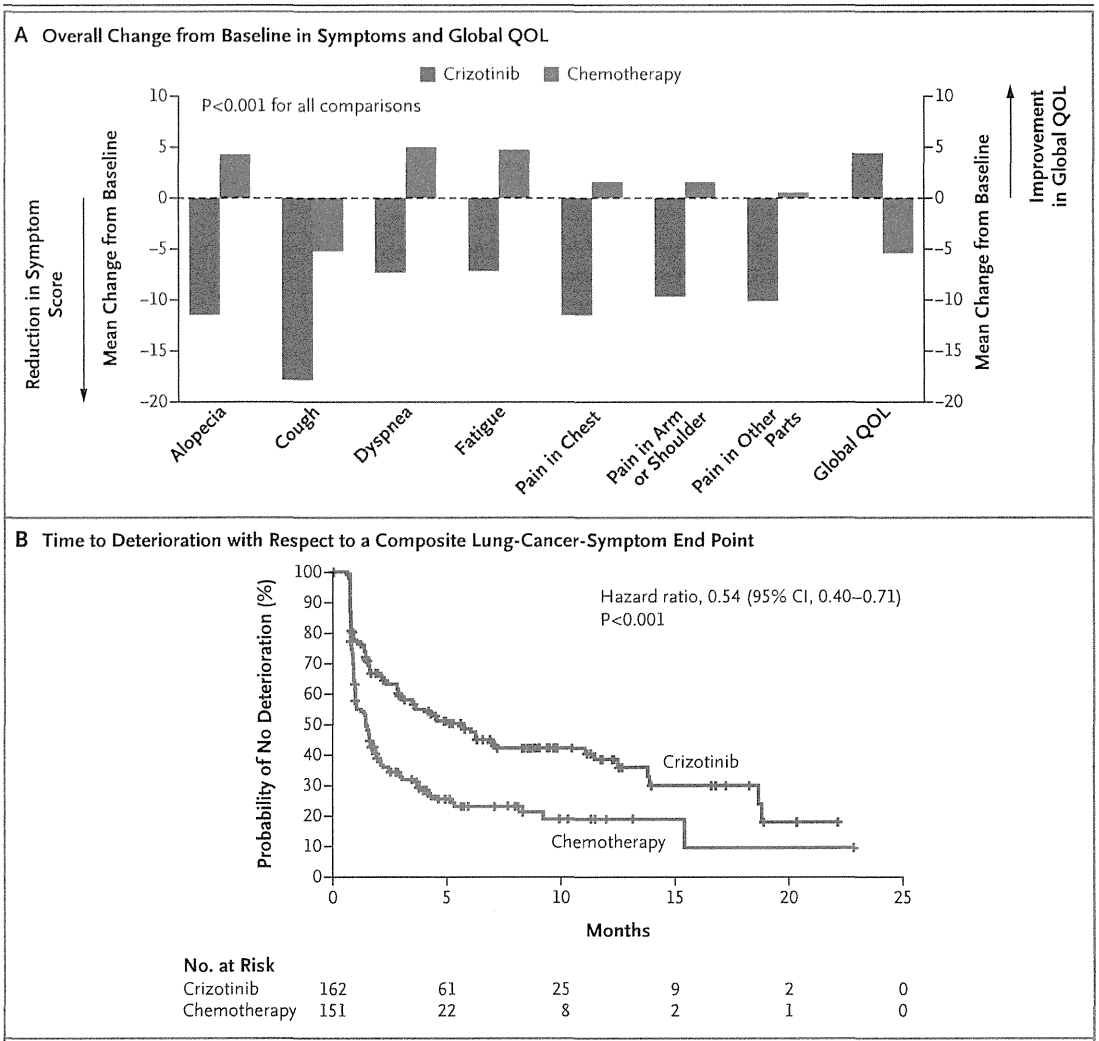

Figure 2. Patient-Reported Outcomes.

Patient-reported outcomes were assessed with the use of the European Organization for Research and Treatment of Cancer quality-of-life questionnaire (QLQ-C30) and the corresponding module for lung cancer (QLQ-LC13). The rates of completion of questionnaires ranged from 75% to 100% across cycles in both treatment groups. Panel A shows the overall change from baseline in the symptoms of alopecia, cough, dyspnea, fatigue, chest pain, arm or shoulder pain, and pain in other parts of the body, as well as in global quality-of-life (QOL) scores. Scores on the questionnaires range from 0 to 100. For symptoms, higher scores indicate greater severity of symptoms, and hence negative changes (downward bars) indicate improvement from baseline; for global quality of life, higher scores indicate better global quality of life, and hence positive changes (upward bars) indicate improvement from baseline. These analyses were based on a repeated-measures mixed-effects model with an intercept, treatment, treatment-by-time interaction, and subscale baseline score. The P values (which have not been adjusted for multiple testing) are for the between-group comparison of the overall change from baseline, as calculated with the use of repeated-measure analyses. Panel B shows Kaplan–Meier estimates of the time to deterioration with respect to a composite end point of three symptoms — cough, dyspnea, or chest pain. The median time to deterioration was 5.6 months with crizotinib and 1.4 months with chemotherapy.

(38%) in the crizotinib group, including 27 (16%) with grade 3 or 4 elevated levels; in 1 patient, concurrent elevations in bilirubin levels not related to cholestasis progressed to fatal hepatic failure. In two earlier studies of crizotinib, the incidence of elevated aminotransferase levels of grade 3 or 4 were lower, at 7% and 9%.[11,12] Although interstitial lung disease is much less common than elevated aminotransferase levels, it is a known and worrisome adverse event associated

with crizotinib. In this study, 3 patients in the crizotinib group (2%) had treatment-related interstitial lung disease of grade 3 or higher; two of the cases were fatal. Across all crizotinib studies, including this one,[11,12] the estimated incidence of treatment-related interstitial lung disease of grade 3 or higher is 1%, an incidence similar to that reported with EGFR kinase inhibitors in clinical studies.[30]

Although the incidence of treatment-related serious adverse events was similar in the crizotinib and chemotherapy groups, significantly more adverse events of any cause were observed in the crizotinib group. Two factors may have contributed to this finding. First, the duration of study treatment was significantly longer with crizotinib than with chemotherapy, and the safety analysis was not adjusted to take into account this difference in treatment durations. Second, significantly more patients in the crizotinib group continued treatment beyond RECIST-defined progression of disease, and the duration of such therapy was longer with crizotinib than with chemotherapy. These differences may have resulted in an imbalance between the two groups that could account in part for the increased incidence of all-cause adverse events seen with crizotinib (Table S5 in the Supplementary Appendix).

In conclusion, this study showed that crizotinib, as compared with chemotherapy, prolonged progression-free survival, increased response rates, and improved the quality of life in patients with advanced, previously treated *ALK*-positive non–small-cell lung cancer. The apparent lack of a survival benefit probably reflects the confounding effects of crossover, effects that have been observed in other randomized trials of molecularly targeted agents in patients with non–small-cell lung cancer.

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Epidermal growth factor receptor mutation analysis in previously unanalyzed histology samples and cytology samples from the phase III Iressa Pan-ASia Study (IPASS)☆

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ABSTRACT

Objectives: Epidermal growth factor receptor (*EGFR*) mutation testing is standard practice after lung adenocarcinoma diagnosis, and provision of high-quality tumor tissue is ideal. However, there are knowledge gaps regarding the utility of cytology or low tumor content histology samples to establish *EGFR* mutation status, particularly with regard to the proportion of testing performed using these sample types, and the lack of an established link with efficacy of treatment.
Methods: The randomized phase III Iressa Pan-ASia Study (IPASS; ClinicalTrials.gov identifier NCT00322452) of first-line gefitinib versus chemotherapy analyzed samples meeting preplanned specifications ($n = 437$ evaluable for *EGFR* mutation; $n = 261$ mutation-positive). This supplementary analysis assessed tumor content and mutation status of histology ($n = 99$) and cytology samples ($n = 116$) which were previously unanalyzed due to sample quality, type, and tumor content (<100 cells). Objective response rate (ORR) and change in tumor size with gefitinib treatment were assessed.
Results: *EGFR* mutation testing was successful in 80% and 19% of previously unanalyzed histology and cytology samples, respectively. Mutations were detected in 54 tumors previously described as mutation-unknown (histology, $n = 45$; cytology, $n = 9$). ORRs in mutation-positive cytology (83%) and histology (74%) subgroups were consistent with previous analyses (71%). Tumor size decrease was consistent across previously analyzed and unanalyzed samples (all mutation subgroups), with less consistency across ORRs in mutation-negative cytology (16%) and histology (25%) subgroups versus the previous analysis (1%).
Conclusions: Histology samples with low tumor content and cytology samples can be used for *EGFR* mutation testing; patients whose mutation status was confirmed using these sample types achieved a response to treatment consistent with those confirmed using high-quality histology samples. Better sample quantity/quality can potentially reduce false-negative results.

1. Introduction

Non-small-cell lung cancer (NSCLC) is traditionally treated using platinum-based chemotherapy [1,2]. Recently, the management of advanced lung adenocarcinoma has evolved, and use of molecular diagnosis to investigate driver mutations in tumor samples has become the most important step toward selecting the right agent for a patient's treatment [3].

The most established example is the use of epidermal growth factor receptor (*EGFR*) mutations as a predictive marker of tumor

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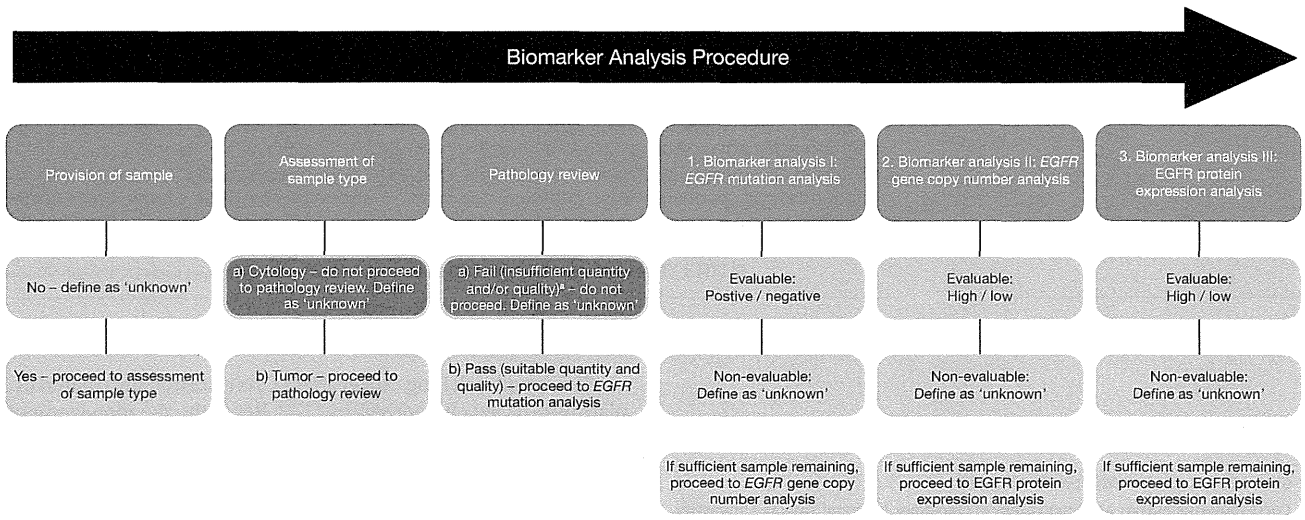
response to EGFR tyrosine kinase inhibitor (TKI) treatment. The first trial to confirm the utility of *EGFR* mutation as a predictor of anticancer efficacy was the Iressa Pan-ASia Study (IPASS), which investigated the outcomes of the overall study population ($n = 1217$) and subgroups (including those evaluable for *EGFR* mutation status [$n = 437$]) treated with gefitinib or carboplatin/paclitaxel [4,5]. IPASS demonstrated superior progression-free survival (PFS), objective response rate (ORR), symptom control, and quality of life with first-line gefitinib versus carboplatin/paclitaxel in patients with *EGFR* mutation-positive tumors. This finding was replicated in the smaller FIRST-Signal study [6]. Five additional phase III studies have subsequently reported significantly increased PFS with EGFR-TKIs (gefitinib, erlotinib, and afatinib) versus platinum-based chemotherapy in patients with *EGFR* mutation-positive tumors [7–11].

IPASS (overall population $n = 1217$) included exploratory objectives to investigate efficacy according to *EGFR* biomarker status (*EGFR* mutation, gene copy number, and protein expression) [4,5]. Collection of histology samples for biomarker analysis was not mandated; 85% of patients consented to donate their tumor. Samples were provided by 683/1217 patients (56%). Fukuoka et al. presented the IPASS exploratory biomarker data for 261 patients with *EGFR* mutation-positive tumors out of 437 evaluable patients (60%) [4].

The streamlined biomarker analysis process (Fig. 1) required all samples to meet stringent pre-specified thresholds for the number of tumor cells and sample quality/type, based on the higher cell requirements of fluorescent in situ hybridization (FISH) for gene copy number and immunohistochemistry (IHC) for protein expression. Prior to *EGFR* mutation analysis samples underwent central histopathological review, and samples were included in the biomarker analysis based on their quality, quantity, type, and tumor content (>100 cells) (Fig. 1). These criteria ensured quality results, reflecting the design of IPASS, determination of differential efficacy in biomarker positive/negative subgroups, limited data at the time regarding the predictive nature of the biomarkers, and extent of validation of the biomarker assays at the time IPASS was conducted (biomarker assays were not validated for cytology samples at that time). This approach provided a definitive answer regarding patients who derived most benefit in the clinical setting. While appropriate to answer the questions posed by the IPASS protocol, the *EGFR* mutation analysis threshold stringency was higher than would be employed for the diagnosis of patients in daily practice. Since IPASS reported, laboratories have gained experience of using existing *EGFR* mutation detection techniques on a spectrum of samples with varying tumor content and sample quality. Small biopsies and cytology samples make up ~30–80% of available diagnostic material, depending on diagnostic practices between different hospitals and countries [12], therefore their successful testing is paramount to ensure this sizeable proportion of patients are given the opportunity to receive optimal treatment. The percentage of mutation testing that occurs using cytology samples can be very variable however, and is currently not consistent across institutions or countries [13]. Smouse et al's retrospective review of *EGFR* sequencing over a two year period at a US hospital noted that only 12/239 (5%) specimens tested for *EGFR* mutation were cytological in origin [13], with focus given to the testing of high-quality tumor tissue samples. Conversely, Hagiwara et al. recently noted that ~40% of samples submitted for *EGFR* mutation testing across three major commercial test centers in Japan were of cytological origin [14], further commenting that this high percentage highlights that cytological samples are indispensable for testing all patients with advanced NSCLC.

The aim of the current study was to investigate whether cytology/histology samples that were not included in the IPASS pre-planned exploratory biomarker analyses could be used successfully to define *EGFR* mutation status and predict which patients were more likely to respond to EGFR-TKI treatment. We describe data generated from pathology review and mutation analysis of the previously unanalyzed histology samples and previously unanalyzed cytology samples, with the aim of testing the outcome of patients with NSCLC as per the study protocol, but by looking at the full spectrum of samples that are available from this population of patients. These data will help to inform the most appropriate thresholds for further trials, as well as the utility of samples received by diagnostic laboratories on a daily basis.



[a]Histology samples that failed pathology review due to insufficient tumor material for biomarker analysis (<100 tumor cells) or poor quality (inadequate fixation or a sample where accurate diagnosis was not possible) and were therefore not included in the main IPASS biomarker analyses. Previously unanalyzed samples included cytology samples and any samples that did not pass pathology review (highlighted in red).
EGFR, epidermal growth factor receptor; IPASS, Iressa Pan-ASia Study.

Fig. 1. The biomarker analysis process.

2. Materials and methods

2.1. Study design and patients

Full details of IPASS (ClinicalTrials.gov identifier NCT00322452) have been published previously [4,5]. Patients were eligible for inclusion into the study if they had histologically or cytologically confirmed stage IIIB or IV pulmonary adenocarcinoma (including bronchoalveolar carcinoma), were never-smokers (<100 cigarettes in their lifetime) or former light smokers (stopped smoking ≥15 years previously and smoked ≤10 pack-years), and had received no prior chemotherapy, biologic therapy, or immunologic therapy. Patients provided written informed consent with separate consent for the optional assessment of *EGFR* biomarkers. The study protocol was approved by independent ethics committees at each institution. Of 1217 randomized patients, 683 (56%) provided a sample for biomarker analysis. Tumor *EGFR* mutation status was evaluable for 437 patients (261 *EGFR* mutation-positive). Prior to *EGFR* mutation analysis samples underwent central histopathological review; only those considered suitable for the analysis of all exploratory biomarkers, including two methods requiring a specified cell number (*EGFR* gene amplification by FISH requiring 60 cells, and EGFR protein expression by IHC requiring 100 cells, for accurate scoring respectively), were included in the biomarker analysis (sample quality, type, and tumor content [>100 cells]) (Fig. 1). At the time of the original analysis, according to the protocol biomarker analyses were not performed for 215 samples: 116 cytology samples (biomarker analyses had not been validated for this sample type, as previously reported in the appendix of Fukuoka et al. [4]) and 99 histology samples (determined during pathology review not to meet pre-specified biomarker analysis thresholds regarding tumor content [>100 tumor cells] and sample quality/quantity [including samples with inadequate cellular morphology due to poor/inappropriate fixation]). The previously unanalyzed cytology and histology samples are the subject of this additional analysis.

The study was conducted in accordance with the Declaration of Helsinki, the International Conference on Harmonisation/Good Clinical Practice, applicable regulatory requirements, and AstraZeneca's policy on bioethics.

2.2. EGFR mutation analyses

EGFR mutation analyses were conducted at two central laboratories (Genzyme, Framingham, MA, USA and AstraZeneca Innovation Center China, Shanghai, China). *EGFR* mutation status of the previously unanalyzed samples was determined by analyzing paraffin-embedded archival histological and cytological cell blocks/smears. Sample tumor content was assessed (histopathological review) prior to categorization based on the number of tumor cells present; 0–9, 10–49, 50–99, and >100 cells. *EGFR* mutations were detected using an amplification mutation refractory system with *EGFR* mutation detection (Qiagen, Manchester, UK), as previously reported for IPASS [5]. Tumors were considered positive if ≥1 of 29 *EGFR* mutations was detected.

2.3. Statistical analyses

Statistical analyses were performed by AstraZeneca. Owing to the small numbers of evaluable cytology and previously unanalyzed histology samples, formal statistical testing was not appropriate. The ORR with exact 95% (Clopper–Pearson) confidence intervals (CIs) was calculated for *EGFR* mutation-positive and -negative cytology samples and *EGFR* mutation-positive and -negative previously unanalyzed histology samples.

Percentage change in tumor size was presented graphically (waterfall plots), with each patient's maximum percentage decrease in tumor size presented as a separate bar (largest increase to largest decrease).

3. Results

3.1. Patients

A total of 215 samples (99 histology; 116 cytology) were available but not analyzed in the main IPASS analysis (Fig. 2). Of the 99 histology samples, 79 (80%) were evaluable for *EGFR* mutations of which 45 (57%) were *EGFR* mutation-positive. Of these 45 patients with *EGFR* mutation-positive tumors, 27 (60%) had received gefitinib and 18 (40%) carboplatin/paclitaxel. Of the 116 cytology samples, 31 (19%) were evaluable for *EGFR* mutation of which nine (29%) were *EGFR* mutation-positive. Of these nine patients with *EGFR* mutation-positive tumors, six (67%) had received gefitinib and three (33%) carboplatin/paclitaxel. A total of 20 histology samples (20%) and 85 cytology samples (73%) were not evaluable for *EGFR* mutation status (insufficient DNA for mutation analysis or no material available for DNA extraction and subsequent analysis).

3.2. Analysis success and tumor cell number: cytology and histology samples that previously failed pathology review

Fig. 3 summarizes the number of evaluable and *EGFR* mutation-positive samples observed, according to tumor cell content. A total of 52 cytology samples (45%) had <100 tumor cells; eleven of these samples provided an evaluable *EGFR* mutation result, of which two (18%) were *EGFR* mutation-positive. A total of 64 cytology samples (55%) had >100 tumor cells; twenty of these samples provided an evaluable *EGFR* mutation result, of which seven (35%) were *EGFR* mutation-positive.

Data from the previously unanalyzed histology samples showed that 73 samples (74%) had <100 tumor cells, with 59 samples providing an evaluable *EGFR* mutation result; thirty (51%) were *EGFR* mutation-positive. A total of 26 histology samples (26%) had >100 tumor cells. These samples had previously been excluded from the main IPASS study on the basis that they did not meet the pre-specified thresholds regarding tumor content and sample quality/quantity (described in Section 2). Twenty samples provided an evaluable *EGFR* mutation result; 15 (75%) were *EGFR* mutation-positive.

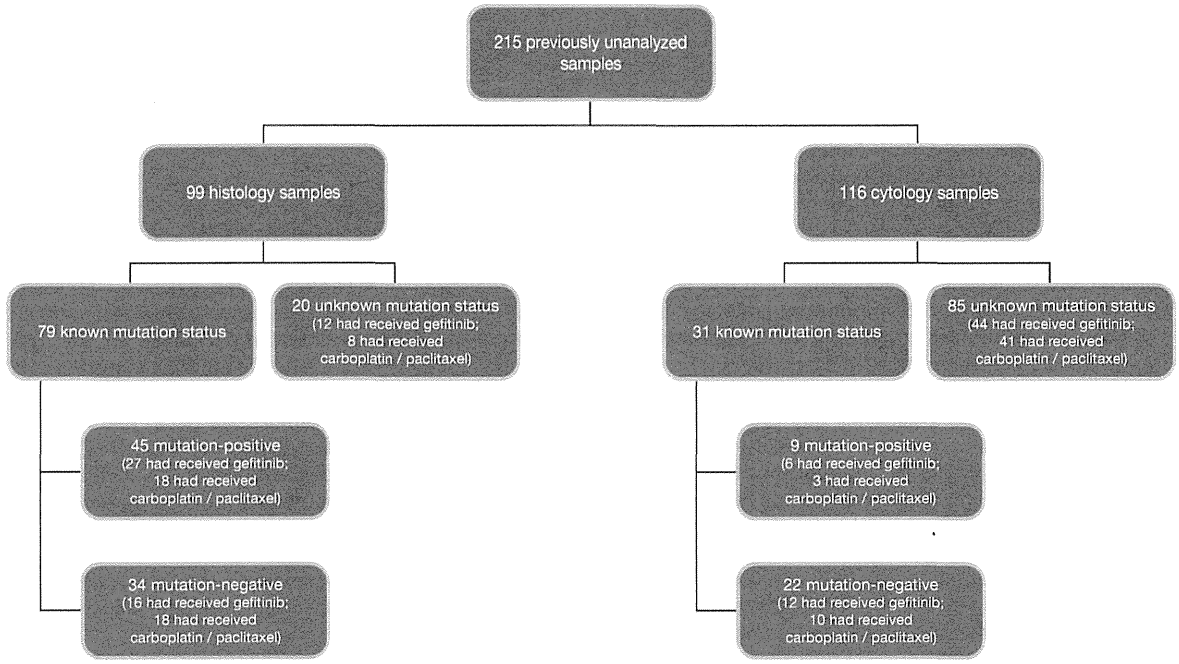
In total, therefore, *EGFR* mutation-positive tumors were detected in 54 patients which had previously been described as *EGFR* mutation-unknown.

3.3. Mutation subtype and frequency

Of the *EGFR* mutation-positive cytology samples, 5 (55.6%) were positive for exon 19 deletions and 4 (44.4%) were positive for exon 21 L858R. Of the *EGFR* mutation-positive histology samples, 22 (48.9%) were positive for exon 19 deletions, 18 (40%) for exon 21 L858R, and two (4.4%) for exon 18 G719S/A/C. A total of three samples were identified as having double mutations: two (4.4%) for exon 19 deletions and exon 21 L858R, and one sample (2.2%) for exon 18 G719S/A/C and exon 21 L861Q.

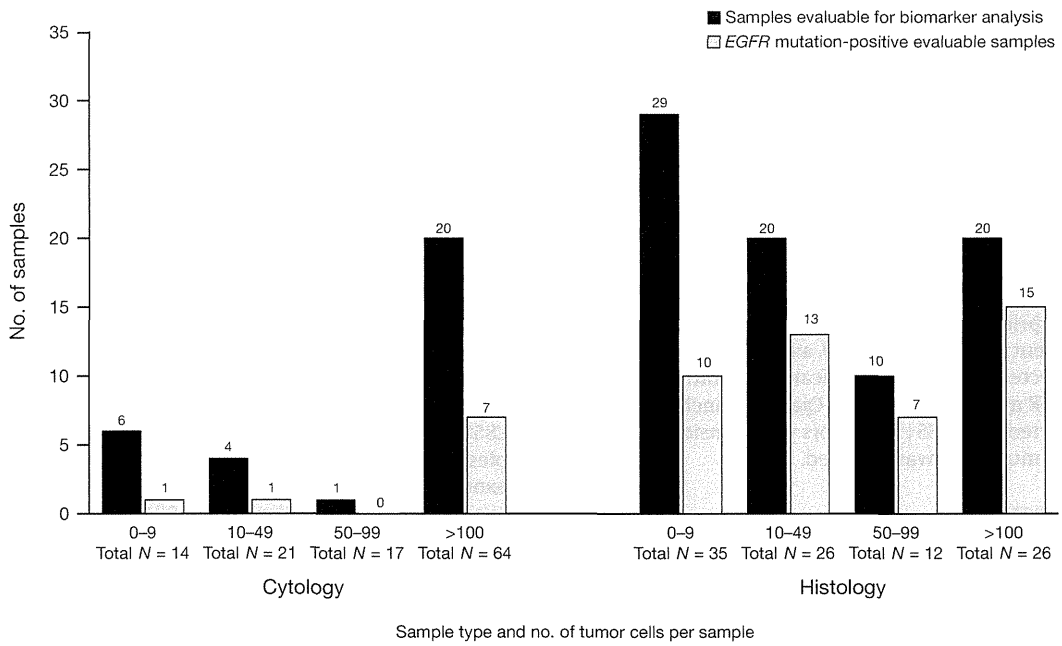
3.4. Efficacy

Data from the previously analyzed samples demonstrated the differential efficacy in terms of ORRs for patients with gefitinib, with 1% of patients ($n = 1/100$) having an objective response in the *EGFR* mutation-negative subgroup, 43% ($n = 167/386$) in the mutation-unknown subgroup, and 71% ($n = 94/132$) in the mutation-positive subgroup [4,5]. Note that in the previous analysis, the *EGFR* mutation-unknown subgroup consisted of 386



Among the 105 patients for whom tumor EGFR mutation status was unknown, the main reasons for unknown EGFR mutation status were insufficient DNA for analysis, no material available for analysis, inadequate fixation, and patient diagnosis unable to be confirmed.
EGFR, epidermal growth factor receptor.

Fig. 2. Sample disposition.



EGFR mutations were identified in both the previously unanalyzed histology and cytology samples, with a greater number of histology samples being evaluable for *EGFR* mutation status. *EGFR* mutation pick-up rate in the histology samples was also higher.
EGFR, epidermal growth factor receptor.

Fig. 3. Tumor cell content of the previously unanalyzed histology and cytology samples (intent-to-treat population).