events were monitored up to the 28th day after the final dose, and assessed according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE version 4.0). When vision disorders occurred during this trial, an ophthalmological examination was done.

If a patient had thrombocytopenia or neutropenia of grade 4 or a non-haematological toxic effect of grade 3 or higher occurred, treatment with CH5424802 would be suspended until the toxic effects improved to grade 1 or lower, or the baseline grade. If the period of suspension was 14 days or less, treatment with CH5424802 could be resumed at the same dose level. If the period of suspension was longer than 14 days, treatment with CH5424802 would be resumed at a reduced dose. Treatment with CH5424802 would be discontinued permanently if treatment could not be resumed within 21 days of suspension. Additionally to these criteria, at the initiation of every cycle, treatment with CH5424802 would commence after it had been confirmed that all the following criteria were met (neutrophil count ≥1500 cells per μL [this criterion was amended so that patients with a neutrophil count ≥1000 cells per μL could receive the next cycle of treatment], platelet count ≥7·5×10⁴ cells per μL; non-haematological toxic effects of grade ≤1 or grade at baseline with exception of investigator's judgment).

| | Phase 1 (n=24) | Phase 2 (n=46) |
|---|---|---|
| Age, years | 42·5 (28–67, 39·0–60·0) | 48·0 (26–75, 37·5–54·5) |
| Sex | | |
| Female | 13 (54%) | 24 (52%) |
| Male | 11 (46%) | 22 (48%) |
| Smoking status | | |
| Never | 14 (58%) | 27 (59%) |
| Former | 10 (42%) | 18 (39%) |
| Present | 0 | 1 (2%) |
| Histological findings* | | |
| Adenocarcinoma | 22 (92%) | 46 (100%) |
| Squamous-cell carcinoma | 1 (4%) | 0 |
| Large-cell carcinoma | 1 (4%) | 0 |
| Clinical stage (at screening) | | |
| IIIB | 0 | 2 (4%) |
| IV | 14 (58%) | 31 (67%) |
| Postoperative recurrence | 10 (42%) | 13 (28%) |
| ECOG performance status | | |
| 0 | 9 (38%) | 20 (43%) |
| 1 | 15 (63%) | 26 (57%) |
| ALK diagnosis† | | |
| Immunohistochemistry and FISH | 22 (92%) | 39 (85%) |
| RT-PCR | 2 (8%) | 7 (15%) |
| EGFR status* | | |
| Wild-type | 22 (92%) | 41 (89%) |
| Mutation | 0 | 0 |
| Unknown | 2 (8%) | 5 (11%) |
| Previous chemotherapy regimens for metastatic disease | | |
| 0 | 0 | 1 (2%)‡ |
| 1 | 1 (4%)‡ | 21 (46%) |
| 2 | 10 (42%) | 9 (20%) |
| ≥3 | 13 (54%) | 15 (33%) |

Data are median (range, IQR) or number of patients (%). ECOG=Eastern Cooperative Oncology Group. FISH=fluorescence in-situ hybridisation. *Histological findings and EGFR status were reported by the investigator site. †ALK diagnosis was performed in two central reference laboratories (one for immunohistochemistry and FISH, and the other for RT-PCR). ‡Regarded as eligible for inclusion because relapse occurred within 6 months of completion of adjuvant chemotherapy.

*Table 1:* **Demographics and baseline characteristics**

## Pharmacokinetics

In the phase 1 portion of the study, we obtained 2 mL blood samples at pre-dose, 0·5 h, 1 h, 2 h, 4 h, 6 h, 8 h, 10 h, 24 h, 32 h, 48 h, and 72 h after single oral administration of CH5424802, and at pre-dose, 0·5 h, 1 h, 2 h, 4 h, 6 h, 8 h, and 10 h at steady state under fasting and non-fasting conditions. The blood samples were centrifuged at 1500–2000×g for 10 min at 4°C. The plasma samples were then stored at –70°C or less. We measured drug concentrations in plasma by the liquid chromatography-mass spectrometry and liquid chromatography-tandem mass spectrometry with limit of quantitation of 0·1 ng/mL.

## Statistical analysis

The primary endpoint of the phase 1 portion was DLT, MTD, safety, and pharmacokinetic parameters. The primary endpoint of the phase 2 portion was the proportion of patients who had an objective response, as determined by an independent review committee, which was to be confirmed by a subsequent scan. Secondary endpoints included safety, the proportion of patients who achieved disease control, progression-free survival, overall survival, and pharmacokinetic parameters.

In the phase 1 portion of the study, we did all statistical analyses in a descriptive manner; and we thus did no formal hypothesis testing. We analysed plasma CH5424802 concentrations with Phoenix WinNonlin Version 6.2 (Pharsight Corporation, Mountain View, CA, USA). We directly obtained the maximum plasma concentrations ($C_{max}$) from the plasma-concentration curves for every participant. We calculated the area under the plasma concentration-time curve (AUC) for every individual using the linear log trapezoidal method as implemented in Phoenix WinNonlin.

In the phase 2 portion of this study, initially, we used a threshold response rate of 25% for reference based on the response rate of a platinum doublet regimen that is a standard treatment for NSCLC,[18] and an expected response rate of 70% based on the response rate of the patients to crizotinib.[19] Since 12 individuals are necessary to yield a statistical power of 80% with a two-sided significance of 5%, we calculated a target sample size of

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)**

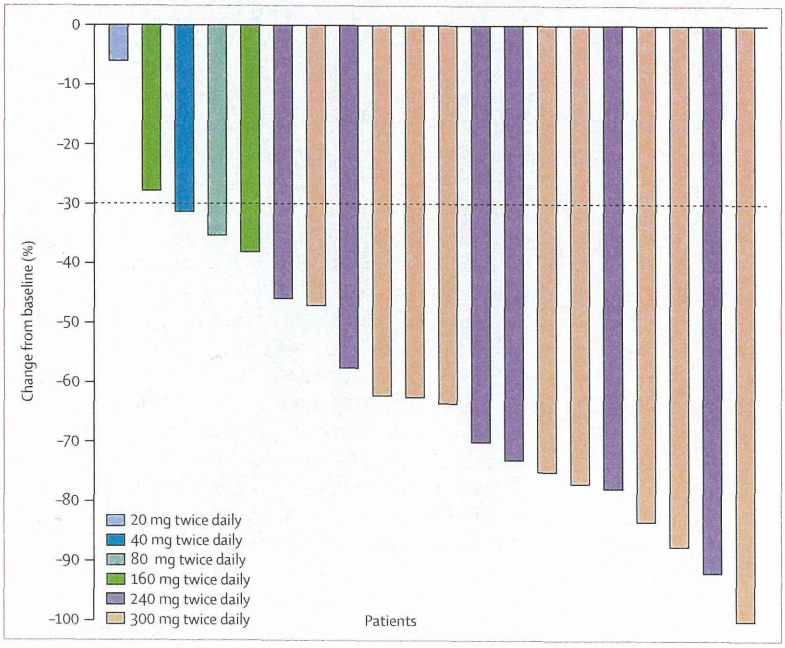


*Figure 1:* **Waterfall plot of best percentage change in target lesions from baseline on investigator assessment (20 patients with measurable lesions in phase 1)**

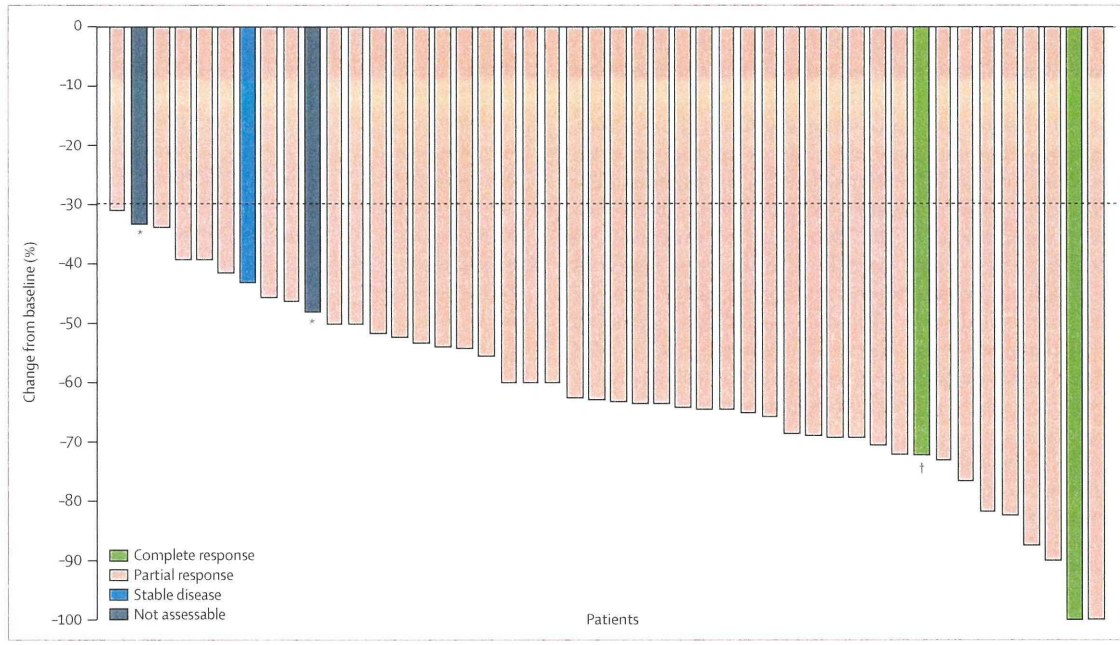


*Figure 2:* **Waterfall plot of best percentage change in target lesions from baseline based on independent review committee assessment (46 patients in phase 2)**
*Indeterminate response by early stopping because of safety reasons. †Classified as complete response according to the definition of Response 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.

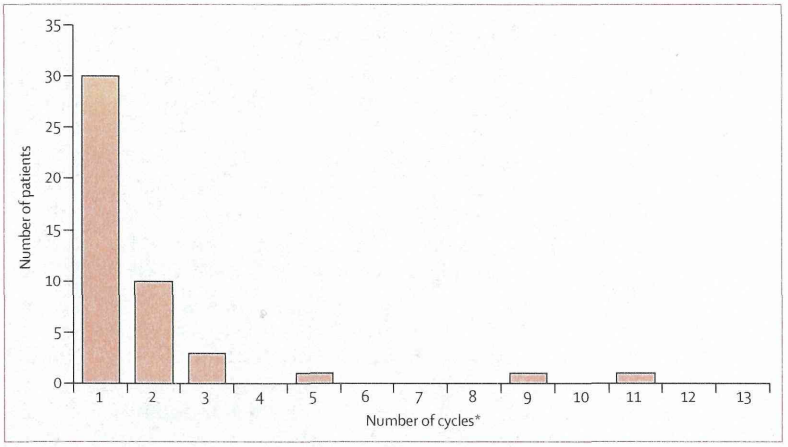


*Figure 3:* **Number of patients who had tumour size reduction of 30% or more by treatment cycle in phase 2**
*One cycle lasted 3 weeks.

| | 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)**

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

**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.

#### Acknowledgments

We thank the patients, their families, all of the investigators who participated in the study, and the central laboratory, SRL, that did the *ALK* rearrangement testing by RT-PCR method. Medical editorial assistance was provided by Rie Ishibashi and Damian Sterling from Nature Japan KK (Macmillan Medical Communications, Tokyo, Japan, funded by Chugai Pharmaceutical Co, Ltd).

#### References

1 Soda M, Choi YL, Enomoto M, et al. Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. *Nature* 2007; **448:** 561–66.
2 Soda M, Takada S, Takeuchi K, et al. A mouse model for EML4-ALK-positive lung cancer. *Proc Natl Acad Sci* 2008; **105:** 19893–97.
3 Webb TR, Slavish J, George RE, et al. Anaplastic lymphoma kinase: role in cancer pathogenesis and small-molecule inhibitor development for therapy. *Expert Rev Anticancer Ther* 2009; **9:** 331–56.
4 Christensen JG, Zou HY, Arango ME, et al. Cytoreductive antitumor activity of PF-2341066, a novel inhibitor of anaplastic lymphoma kinase and c-Met, in experimental models of anaplastic large-cell lymphoma. *Mol Cancer Ther* 2007; **6:** 3314–22.
5 Bergethon K, Shaw AT, Ou SH, et al. ROS1 rearrangements define a unique molecular class of lung cancers. *J Clin Oncol* 2012; **30:** 863–70.
6 Camidge DR, Bang Y-J, Kwak EL, et al. Activity and safety of crizotinib in patients with ALK-positive non-small-cell lung cancer: updated results from a phase 1 study. *Lancet Oncol* 2012; **13:** 1011–19.
7 Shaw AT, Yeap BY, Solomon BJ, et al. Effect of crizotinib on overall survival in patients with advanced non-small-cell lung cancer harbouring ALK gene rearrangement: a retrospective analysis. *Lancet Oncol* 2011; **12:** 1004–12.
8 Katayama R, Shaw AT, Khan TM, et al. Mechanisms of acquired crizotinib resistance in ALK-rearranged lung cancers. *Sci Transl Med* 2012; **4:** 120ra17.
9 Doebele RC, Pilling AB, Aisner DL, et al. Mechanisms of resistance to crizotinib in patients with ALK gene rearranged non-small cell lung cancer. *Clin Cancer Res* 2012; **18:** 1472–82.
10 Costa DB, Kobayashi S, Pandya SS, Yeo W-L. CSF concentration of the anaplastic lymphoma kinase inhibitor crizotinib. *J Clin Oncol* 2011; **29:** e443–45.
11 Otterson GA, Riely GJ, Shaw AT, et al. Clinical characteristics of ALK+ NSCLC patients (pts) treated with crizotinib beyond disease progression (PD): Potential implications for management. *Proc Am Soc Clin Oncol* 2012; **30** (suppl): abstr 7600.
12 Sakamoto H, Tsukaguchi T, Hiroshima S, et al. CH5424802, a selective ALK inhibitor capable of blocking the resistant gatekeeper mutant. *Cancer Cell* 2011; **19:** 679–90.
13 Choi YL, Soda M, Yamashita Y, et al. EML4-ALK mutations in lung cancer that confer resistance to ALK inhibitors. *N Engl J Med* 2010; **363:** 1734–39.
14 Takeuchi K, Soda M, Togashi Y, et al. RET, ROS1 and ALK fusions in lung cancer. *Nat Med* 2012; **18:** 378–81.
15 Takeuchi K, Choi YL, Togashi Y, et al. KIF5B-ALK, a novel fusion oncokinase identified by an immunohistochemistry-based diagnostic system for ALK-positive lung cancer. *Clin Cancer Res* 2009; **15:** 3143–49.
16 Sugawara E, Togashi Y, Kuroda N, et al. Identification of anaplastic lymphoma kinase fusions in renal cancer: Large-scale immunohistochemical screening by the intercalated antibody-enhanced polymer method. *Cancer* 2012; **118:** 4427–36.
17 Simon R, Freidlin B, Rubinstein L, et al. Accelerated titration designs for phase I clinical trials in oncology. *J Natl Cancer Inst* 1997; **89:** 1138–47.

18 Shaw AT, Yeap BY, Mino-Kenudson M, et al. Clinical features and outcome of patients with non-small-cell lung cancer who harbor EML4-ALK. *J Clin Oncol* 2009; **27:** 4247–53.

19 Kwak EL, Camidge DR, Clark J, et al. Clinical activity observed in a phase I dose escalation trial of an oral c-MET and ALK inhibitor, PF-02341066. *Eur J Cancer* 2009; **7** (suppl 3): 8.

20 Crinò L, Kim D, Riely GJ, et al. Initial phase II results with crizotinib in advanced ALK-positive non-small cell lung cancer (NSCLC): PROFILE 1005. *Proc Am Soc Clin Oncol* 2011; **29** (suppl): abstr 7514.

21 Xalkori (crizotinib) package insert. Initial US approval: August 2011 (revised: August 2011). Pfizer, NY, USA.

22 FDA. Guidance for industry. Drug-induced liver injury: premarketing clinical evaluation. http://www.fda.gov/downloads/Drugs/.../Guidances/UCM174090.pdf (accessed March 1, 2013).

23 Kim D-W, Ahn M-J, Shi Y, et al. Results of a global phase II study with crizotinib in advanced ALK-positive non-small cell lung cancer (NSCLC). *Proc Am Soc Clin Oncol* 2012; **30** (suppl): abstr 7533.

24 Kwak EL, Bang YJ, Camidge DR, et al. Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. *N Engl J Med* 2010; **363:** 1693–703.

25 Shaw AT, Kim DW, Nakagawa K, et al. Phase III study of crizotinib versus pemetrexed or docetaxel chemotherapy in patients with advanced ALK-positive non-small cell lung cancer (NSCLC) (PROFILE 1007). European Society for Medical Oncology 2012; Vienna, Austria; Sept 29–Oct 2, 2012; abstr 2862.

26 Morris SW, Kirstein MN, Valentine MB, et al. Fusion of a kinase gene, ALK, to a nucleolar protein gene, NPM, in non-Hodgkin's lymphoma. *Science* 1994; **263:** 1281–84.

27 A clinical study testing the safety and efficacy of CH5424802 in patients with ALK positive non-small cell lung cancer. http://www.clinicaltrials.gov/ct2/show/record/NCT01588028 (accessed Jan 21, 2013).

28 Bang Y-J, Kwak EL, Shaw AT, et al. Clinical activity of the oral ALK inhibitor PF-02341066 in ALK-positive patients with non-small cell lung cancer (NSCLC). *Proc Am Soc Clin Oncol* 2010; **28** (suppl): abstr 3.

29 Chihara D, Suzuki R. More on crizotinib. *N Engl J Med* 2011; **364:** 776–79.

*Annals of Oncology* 24: 1326–1331, 2013
doi:10.1093/annonc/mds629
Published online 30 December 2012

# Efficacy and safety analysis according to histology for S-1 in combination with carboplatin as first-line chemotherapy in patients with advanced non-small-cell lung cancer: updated results of the West Japan Oncology Group LETS study

H. Yoshioka[1], I. Okamoto[2*], S. Morita[3], M. Ando[4], K. Takeda[5], T. Seto[6], N. Yamamoto[7], H. Saka[8], S. Atagi[9], T. Hirashima[10], S. Kudoh[11], M. Satouchi[12], N. Ikeda[13], Y. Iwamoto[14], T. Sawa[15], Y. Nakanishi[16] & K. Nakagawa[2]

*[1]Department of Respiratory Medicine, Kurashiki Central Hospital, Kurashiki; [2]Department of Medical Oncology, Kinki University Faculty of Medicine, Osaka,; [3]Department of Biostatistics and Epidemiology, Yokohama City University Medical Center, Yokohama; [4]Center for Advanced Medicine and Clinical Research, Nagoya University Graduate School of Medicine, Aichi; [5]Department of Clinical Oncology, Osaka City General Hospital, Osaka; [6]Department of Thoracic Oncology, National Kyushu Cancer Center, Fukuoka; [7]Division of Thoracic Oncology, Shizuoka Cancer Center, Nagaizumi; [8]Department of Respiratory Medicine, National Hospital Organization, Nagoya Medical Center, Nagoya; [9]Department of Thoracic Oncology, National Hospital Organization, Kinki-chuo Chest Medical Center, Osaka; [10]Department of Thoracic Malignancy, Osaka Prefectural Medical Center for Respiratory and Allergic Diseases, Osaka; [11]Department of Respiratory Medicine, Osaka City University Medical School, Osaka; [12]Department of Thoracic Oncology, Hyogo Cancer Center, Akashi; [13]Department of Thoracic Surgery, Tokyo Medical University, Tokyo; [14]Department of Medical Oncology, Hiroshima City Hospital, Hiroshima; [15]Department of Respiratory Medicine and Oncology, Gifu Municipal Hospital, Gifu; [16]Research Institute for Diseases of the Chest, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan*

*Received 20 August 2012; revised 29 October 2012; accepted 2 November 2012*

**Background:** A phase III study (Lung Cancer Evaluation of TS-1) previously demonstrated noninferiority in terms of overall survival (OS) at interim analysis for carboplatin–S-1 compared with carboplatin–paclitaxel for first-line treatment of advanced non-small-cell lung cancer (NSCLC).

**Patients and methods:** A total of 564 patients were randomly assigned to receive either carboplatin on day 1 plus oral S-1 on days 1–14 or carboplatin–paclitaxel on day 1 every 21 days. Updated results and *post hoc* subgroup analysis according to tumor histology are presented.

**Results:** The updated analysis revealed a median OS of 15.2 months in the carboplatin–S-1 arm and 13.1 months in the carboplatin–paclitaxel arm, with a hazard ratio (HR) of 0.956 [95% confidence interval (CI) 0.793–1.151], consistent with the previous primary analysis. Median OS was 14.0 months in the carboplatin–S-1 arm and 10.6 months in the carboplatin–paclitaxel arm (HR 0.713; 95% CI 0.476–1.068) for patients with squamous cell carcinoma (SCC), with corresponding values of 15.5 and 13.9 months (HR 1.060; 95% CI 0.859–1.308) for those with non-SCC.

**Conclusions:** These results establish the efficacy and safety of carboplatin–S-1 in patients with advanced NSCLC regardless of tumor histology.

**Key words:** carboplatin, histology, non-small-cell lung cancer, S-1, squamous cell carcinoma

## introduction

Lung cancer is the leading cause of death related to cancer worldwide, with non-small-cell lung cancer (NSCLC) accounting for 85% of lung cancer cases [1]. Most NSCLC cases are categorized into two distinct histological subtypes: squamous cell carcinoma (SCC) and non-SCC. Treatment with pemetrexed–cisplatin was associated with a longer overall survival (OS) compared with that with gemcitabine–cisplatin in patients with non-SCC but not in those with SCC [2]. The addition of bevacizumab, a monoclonal antibody specific for vascular endothelial growth factor, to carboplatin and paclitaxel improved survival compared with chemotherapy alone in patients with non-SCC, but such treatment was contraindicated for patients with SCC because of an increased risk of fatal bleeding events [3–5]. Furthermore, the recent identification of oncogenic alterations, such as mutation of the epidermal growth factor receptor (EGFR) gene or the fusion of the genes for echinoderm microtubule-associated protein–like

*Correspondence to: Dr I. Okamoto, Department of Medical Oncology, Kinki University Faculty of Medicine, 377-2 Ohno-higashi, Osaka-Sayama, Osaka 589-8511, Japan. Tel: +81-72-366-0221; Fax: +81-72-360-5000; E-mail: chi-okamoto@dotd.med.kindai.ac.jp

4 (EML4) and anaplastic lymphoma kinase (ALK), and of the association of such gene alterations with a clinically relevant response to corresponding tyrosine kinase inhibitors (TKIs), has had a profound impact on the treatment of advanced NSCLC [6–10]. Almost all cases of NSCLC harboring *EGFR* mutations or *ALK* rearrangements are non-SCC, with adenocarcinomas being most common. Treatment options for non-SCC have thus increased, whereas the contribution of new drugs to the treatment of SCC has been minimal. The poor outlook for advanced NSCLC patients with SCC has prompted a search for new chemotherapeutic agents and combination regimens.

S-1 (TS-1; Taiho Pharmaceutical Co. Ltd., Tokyo, Japan) is an oral fluoropyrimidine anticancer agent that combines tegafur as the effector drug with two modulators, gimeracil, and oteracil potassium, in a molar ratio of 1 : 0.4 : 1 [11, 12]. We have recently completed a multicenter randomized phase III study comparing carboplatin and S-1 with standard carboplatin and paclitaxel combination therapy as first-line treatment in patients with advanced NSCLC [13]. The primary objective of the Lung Cancer Evaluation of TS-1 (LETS) study —determination of the noninferiority of carboplatin and S-1 compared with carboplatin and paclitaxel in terms of OS—was met at the planned interim analysis. On completion of the initially planned 2 years of follow-up, at which time an adequate number of events had been obtained, we updated the survival data of the LETS study. Given that histology (SCC or non-SCC) has recently become a key factor in the selection of chemotherapy regimens for the treatment of advanced NSCLC, we also assessed the efficacy and safety data according to the histological subtype of NSCLC by performing subgroup analyses that were not predefined in the study protocol but which address a clinically important issue.

## patients and methods

### patients

The design and results of the LETS study were published in 2010 [13]. In brief, the study group comprised patients aged 20–74 years who had a histopathologic diagnosis of stage IIIB or IV NSCLC, an Eastern Cooperative Oncology Group performance status of 0 or 1, and preserved functions of major organ systems. Patients had not previously received chemotherapy, and they were randomly assigned in a 1 : 1 ratio to receive carboplatin–S-1 or carboplatin–paclitaxel. In the carboplatin–S-1 group, carboplatin was given as a continuous i.v. infusion (area under the curve, 5) on day 1, and S-1 (80 mg/m$^2$ in two divided doses) was given orally on days 1–14. Treatment was repeated every 3 weeks for up to six cycles. Patients in the carboplatin–paclitaxel group received carboplatin (area under the curve, 6) and paclitaxel (200 mg/m$^2$) by continuous i.v. infusion on day 1 every 3 weeks. Treatment was repeated for up to six cycles. The primary end point was OS. Secondary end points were tumor response, safety, quality of life (QOL), and progression-free survival (PFS). Written informed consent was obtained from all patients before treatment, and the study protocol was approved by the institutional ethics committee of each of the participating institutions.

In this *post hoc* investigation, OS and PFS in the intention-to-treat population were determined from updated survival data. In addition, subgroup analyses were carried out to compare overall response rate (ORR), OS, and PFS between the treatment groups according to histological subtype (SCC versus non-SCC) of NSCLC. To assess the impact of post-study treatments with potential effects on survival, we analyzed the data according to treatment line and drugs administered (docetaxel and EGFR-TKIs). Treatment-related adverse events were also assessed according to each subgroup. QOL was assessed with the lung cancer subscale of Functional Assessment of Cancer Therapy-Lung (FACT-L) [14] and the neurotoxicity subscale of FACT/Gynecology Oncology Group-Neurotoxicity (FACT/GOG-Ntx) version 4 [15]. The maximum attainable scores on the lung cancer and neurotoxicity subscales were 28 and 44, respectively, with which a patient was considered to be asymptomatic. Patients were asked to complete each instrument at the time of enrollment and at 6 and 9 weeks after the initiation of treatment.

### statistical analysis

The definition of survival was similar to that used in the initial description of the LETS study [13]. OS was defined as the interval from the date of randomization until the date of death from any cause or the final date of follow-up. At the time of data cutoff, data on survivors and on patients who were lost to follow up were censored on the final date of follow-up. PFS was defined as the interval from the date of randomization until the date on which progressive disease was first confirmed by imaging or the date of death from any cause, whichever came first. If no events had occurred, data were censored at the most recent date of follow-up.

Survival curves in each treatment group and subgroup were estimated with the Kaplan–Meier method. The 95% confidence interval (CI) for median survival was calculated with the method of Brookmeyer and Crowley. A Cox proportional-hazards model was used to calculate the hazard ratio (HR) and CI and to examine the interaction effects between study treatment and subgroup. Longitudinal QOL data were analyzed with a linear mixed-effects model. All statistical analyses were carried out with SAS for Windows, release 9.2 (SAS Institute, Cary, NC). A $P$ value of $<0.05$ was considered statistically significant.

## results

### baseline characteristics

A total of 564 patients were enrolled into the phase III study, and 282 patients were treated in each of the carboplatin–paclitaxel and carboplatin–S-1 arms. At the time of the updated analysis, the median follow-up time was 33.4 months (range 2.1–43.6 months) and a total of 446 deaths (carboplatin–paclitaxel, $N = 219$; carboplatin–S-1, $N = 227$) had occurred. The median OS was 15.2 months (95% CI 12.3–17.8 months) in the carboplatin–S-1 group and 13.1 months (95% CI 11.7–14.9 months) in the carboplatin–paclitaxel group, with an HR for death of 0.956 (95% CI 0.793–1.151). The median PFS was 4.1 months (95% CI 3.8–4.7 months) in the carboplatin–S-1 group and 4.8 months (95% CI 4.3–5.2 months) in the carboplatin–paclitaxel group, with an HR for progression or death of 1.035 (95% CI 0.875–1.224). Of the 564 randomized patients in the phase III study population, 114 patients had SCC (carboplatin–paclitaxel, $N = 59$; carboplatin–S-1, $N = 55$) and 450 had non-SCC (carboplatin–paclitaxel, $N = 223$; carboplatin–S-1, $N = 227$). The CONSORT diagram for the study is shown in supplementary Figure S1, available at *Annals of Oncology* online. Baseline patient characteristics for both histological subtypes were generally well balanced between the treatment groups (Table 1).

**Table 1.** Patient demographics and characteristics according to histological subtype of NSCLC

| Characteristic | Squamous | | Nonsquamous | |
|---|---|---|---|---|
| | CBDCA–S-1 ($N = 55$) | CBDCA–PTX ($N = 59$) | CBDCA–S-1 ($N = 227$) | CBDCA–PTX ($N = 223$) |
| Age, median, years (range) | 66 (39–74) | 65 (43–74) | 64 (38–74) | 62 (36–74) |
| Sex, *N* (%) | | | | |
| Male | 48 (87.3) | 51 (86.4) | 169 (74.4) | 165 (74.0) |
| Female | 7 (12.7) | 8 (13.6) | 58 (25.6) | 58 (26.0) |
| ECOG PS, *N* (%) | | | | |
| 0 | 18 (32.7) | 14 (23.7) | 68 (30.0) | 77 (34.5) |
| 1 | 37 (67.3) | 45 (76.3) | 159 (70.0) | 146 (65.5) |
| Clinical stage, *N* (%) | | | | |
| IIIB | 20 (36.4) | 27 (45.8) | 48 (21.1) | 41 (18.4) |
| IV | 35 (63.6) | 32 (54.2) | 179 (78.9) | 182 (81.6) |
| Smoking status, *N* (%) | | | | |
| Smoker | 52 (94.5) | 56 (94.9) | 178 (78.4) | 174 (78.0) |
| Nonsmoker | 3 (5.5) | 3 (5.1) | 49 (21.6) | 49 (22.0) |

CBDCA, carboplatin; PTX, paclitaxel; ECOG, Eastern Cooperative Oncology Group; PS, performance status.

**Table 2.** Summary of OS, PFS, and response rate according to histological subtype of NSCLC

| | Squamous | | Nonsquamous | |
|---|---|---|---|---|
| | CBDCA–S-1 ($N = 55$) | CBDCA–PTX ($N = 59$) | CBDCA–S-1 ($N = 227$) | CBDCA–PTX ($N = 223$) |
| ORR, *N* (%) | 15 (27.3) | 20 (33.9) | 42 (18.5) | 61 (27.4) |
| Disease control rate, *N* (%) | 44 (80.0) | 45 (76.3) | 156 (68.7) | 162 (72.6) |
| Median PFS (months) | 4.37 | 4.87 | 4.14 | 4.77 |
| 95% CI | 3.65–5.79 | 3.98–5.72 | 3.65–4.77 | 4.18–5.23 |
| HR (95% CI) | 0.938 (0.642–1.371) | | 1.063 (0.881–1.282) | |
| Median OS (months) | 14 | 10.6 | 15.5 | 13.9 |
| 95% CI | 11.4–16.7 | 8.7–12.6 | 11.7–18.4 | 12.1–16.8 |
| HR (95% CI) | 0.713 (0.476–1.068) | | 1.060 (0.859–1.308) | |

## efficacy results based on histology

Efficacy results according to histological subtype of NSCLC are shown in Table 2. For the non-SCC cohort, ORR was significantly higher in the carboplatin–paclitaxel arm than in the carboplatin–S-1 arm (27.4% versus 18.5%; $P = 0.027$, chi-square test), with a response rate ratio of 0.680 (95% CI 0.4805–0.960), whereas the overall disease control (complete response + partial response + stable disease) rate was similar in both treatment groups (72.6% versus 68.7%, respectively; $P = 0.393$). The ORR was 33.9% and 27.3% ($P = 0.444$), with a response rate ratio of 0.805 (95% CI 0.460–1.408), for carboplatin–paclitaxel and carboplatin–S-1, respectively, in patients with SCC. No significant interaction was noted for ORR between histology and treatment ($P = 0.686$).

The median PFS was 4.8 months with carboplatin–paclitaxel and 4.1 months with carboplatin–S-1 in patients with non-SCC (HR 1.063; 95% CI 0.881–1.282). The median PFS was similar with carboplatin–paclitaxel or carboplatin–S-1 in patients with SCC (4.9 versus 4.4 months, respectively; HR 0.938; 95% CI 0.642–1.371). No interaction was observed between histology and treatment effect for PFS ($P = 0.547$).

Figure 1 shows Kaplan–Meier analysis of OS according to treatment arm for SCC and non-SCC subgroups. Patients with SCC experienced a longer median OS in the carboplatin–S-1 group than in the carboplatin–paclitaxel group (14.0 versus 10.6 months, respectively; HR 0.713; 95% CI 0.476–1.068). Patients with non-SCC assigned to carboplatin–S-1 had a median OS of 15.5 months, whereas those assigned to carboplatin–paclitaxel had a median OS of 13.9 months (HR 1.060; 95% CI 0.859–1.308). These data were suggestive of a positive interaction between histology and treatment of OS, but it did not achieve statistical significance ($P = 0.093$).

## safety results based on histology

Treatment-related adverse events according to histological subtype are shown in Table 3. Regardless of histology, carboplatin–S-1 was associated with a higher incidence of thrombocytopenia of grade 3 or 4 and a lower incidence of leukopenia, neutropenia, and febrile neutropenia of grade 3 or 4 compared with carboplatin–paclitaxel, consistent with the results previously reported for the intention-to-treat population [13].

## QOL results based on histology

In general, results for QOL were similar for both histological subtypes of NSCLC (Figure 2). In patients with SCC, the adjusted mean FACT-L scores at 6 and 9 weeks were 20.8 and 21.1, respectively, for carboplatin–S-1 and 21.0 and 20.8 for carboplatin–paclitaxel ($P = 0.723$ between treatment arms). In