CH5424802 (RO5424802) for patients with *ALK*-rearranged advanced non-small-cell lung cancer (AF-001JP study): a single-arm, open-label, phase 1–2 study

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Summary

Background Currently, crizotinib is the only drug that has been approved for treatment of *ALK*-rearranged non-small-cell lung cancer (NSCLC). We aimed to study the activity and safety of CH5424802, a potent, selective, and orally available ALK inhibitor.

Methods In this multicentre, single-arm, open-label, phase 1–2 study of CH5424802, we recruited ALK inhibitor-naive patients with *ALK*-rearranged advanced NSCLC from 13 hospitals in Japan. In the phase 1 portion of the study, patients received CH5424802 orally twice daily by dose escalation. The primary endpoints of the phase 1 were dose limiting toxicity (DLT), maximum tolerated dose (MTD), and pharmacokinetic parameters. In the phase 2 portion of the study, patients received CH5424802 at the recommended dose identified in the phase 1 portion of the study orally twice a day. The primary endpoint of the phase 2 was the proportion of patients who had an objective response. Treatment was continued in 21-day cycles until disease progression, intolerable adverse events, or withdrawal of consent. The analysis was done by intent to treat. This study is registered with the Japan Pharmaceutical Information Center, number JapicCTI-101264.

Findings Patients were enrolled between Sept 10, 2010, and April 18, 2012. The data cutoff date was July 31, 2012. In the phase 1 portion, 24 patients were treated at doses of 20–300 mg twice daily. No DLTs or adverse events of grade 4 were noted up to the highest dose; thus 300 mg twice daily was the recommended phase 2 dose. In the phase 2 portion of the study, 46 patients were treated with the recommended dose, of whom 43 achieved an objective response (93·5%, 95% CI 82·1–98·6) including two complete responses (4·3%, 0·5–14·8) and 41 partial responses (89·1%, 76·4–96·4). Treatment-related adverse events of grade 3 were recorded in 12 (26%) of 46 patients, including two patients each experiencing decreased neutrophil count and increased blood creatine phosphokinase. Serious adverse events occurred in five patients (11%). No grade 4 adverse events or deaths were reported. The study is still ongoing, since 40 of the 46 patients in the phase 2 portion remain on treatment.

Interpretation CH5424802 is well tolerated and highly active in patients with advanced *ALK*-rearranged NSCLC.

Funding Chugai Pharmaceutical Co, Ltd.

Introduction

A fusion tyrosine kinase gene comprising the *EML4* gene and the *ALK* gene has been identified in non-small-cell lung cancer (NSCLC) with inversion of chromosome 2p. Mouse 3T3 fibroblasts expressing *EML4-ALK* had increased transforming activity and tumorigenicity.[1] Transgenic mice expressing *EML4-ALK* fusion gene in lung alveolar epithelial cells were generated and exhibited development of adenocarcinoma in lungs shortly after birth,[2] suggesting that the *EML4-ALK* fusion gene could be a driver mutation for NSCLC and serve as a promising candidate for a therapeutic target.[1,3] Therefore, the introduction of new ALK inhibitors is expected to improve the treatment of patients with *ALK*-rearranged NSCLC.[3]

So far, crizotinib, a multi-targeted receptor tyrosine kinase inhibitor of *ALK*, *MET*, and *ROS1* oncogene,[4,5] is the only agent that has been approved for *ALK*-rearranged NSCLC in the USA, European Union, Japan, and other countries. In the phase 1 trial of crizotinib in patients with *ALK*-rearranged NSCLC, 87 of 143 evaluable patients had an objective response (60·8%, 95% CI 52·3–68·9). Median progression-free survival (PFS) was 9·7 months.[6] In a retrospective study[7] comparing survival outcomes in crizotinib-treated patients enrolled in the phase 1 trial and crizotinib-naive controls screened during the same period, crizotinib therapy was associated with better survival. However, resistance to crizotinib occurs by a number of mechanisms, including *ALK* gene alterations, such as *ALK* point mutations and copy number gain, and activation of bypass signalling through activation of other oncogenes.[8,9] Additionally, poor penetration of crizotinib across the blood–brain barrier is thought to be associated with a higher incidence of brain involvement if relapse occurs.[10] In the crizotinib phase 2 trial, the most common site for single organ disease progression was the brain.[11]

CH5424802 (RO5424802; Chugai Pharmaceutical Co, Ltd, Tokyo, Japan) is a novel, highly selective oral ALK inhibitor. In-vitro kinase assays showed that this compound selectively inhibits ALK. CH5424802 also shows high anti-tumour activity both in vitro and in vivo against tumour cell lines with some type of *ALK* gene alteration, such as NSCLC and anaplastic large-cell lymphoma lines harbouring an *ALK* fusion gene and a neuroblastoma line harbouring amplified *ALK* gene. More importantly, CH5424802 yielded potential anti-tumour activity against the gatekeeper Leu1196Met mutation in *EML4-ALK*,[12] which has been identified in tumour cells refractory to crizotinib.[13]

We report the results of a phase 1–2 study of CH5424802 (AF-001JP study) that was designed to identify the maximum tolerated dose (MTD) and pharmacokinetic parameters of the drug, and subsequently to assess its activity and safety in ALK inhibitor-naive patients with *ALK*-rearranged NSCLC.

Methods

Study design and patients

This study was a multicentre, single-arm, open-label, phase 1–2 trial (AF-001JP). Patients were eligible if they were aged 20 years or older; had histologically or cytologically confirmed advanced or metastatic *ALK*-rearranged stage IIIB, IV, or recurrent NSCLC; had an Eastern Cooperative Oncology Group performance status (ECOG PS) of 0 or 1; had measurable lesions as defined by Response Evaluation Criteria in Solid Tumors (RECIST; version 1.1) (for the phase 2 portion only); received two or more (phase 1 portion) or one or more (phase 2 portion) previous chemotherapy regimens; and had adequate haematological, hepatic, and renal function. We excluded patients who had received previous treatment with any ALK inhibitor. Other exclusion criteria included symptomatic brain metastases or brain metastases requiring treatment, history of serious cardiac dysfunction, clinically significant gastrointestinal abnormality that would affect the absorption of the study drug, and pregnant or lactating women.

To identify whether patients were positive for *ALK* fusion gene expression, formalin-fixed paraffin-embedded sections from previous diagnostic or surgical procedures were sent to the laboratory in the Cancer Institute, Japanese Foundation for Cancer Research, Tokyo, Japan, and screened using anti-ALK immunohistochemistry with iAEP method (ALK Detection Kit, Nichirei Bioscience, Tokyo, Japan).[14–16] In patients who were positive by immunohistochemistry, the fluorescence in-situ hybridisation (FISH) test was subsequently done for confirmation. An experienced pathologist (KT) judged these tests. Additionally, we did a multiplex RT-PCR method (SRL, Tokyo, Japan) on samples of cells or frozen cancer tissue sections. We deemed patients to be positive for *ALK* fusion gene expression when either FISH or RT-PCR showed positive results.

In this study, patients gave written informed consent for *ALK* assessment by a central laboratory. If tumours were confirmed to be *ALK* positive, patients signed another informed consent form for enrolment into this trial. Patients participating in the study were treated at 13 hospitals in Japan. The study was approved by the institutional review board at each participating institution, and done in accordance with the Declaration of Helsinki and Good Clinical Practices.

Procedures

In the phase 1 portion of this study, patients received CH5424802 orally twice daily (once in the morning and once in the evening) in an open-label, sequential-cohort, dose-escalation study. We did the dose escalation with an accelerated titration design[17] under fasting conditions from 20 mg to 300 mg twice daily. We determined a dose of 300 mg twice daily as the highest planned dose on the basis of the available safety information about the additive formulation in Japan. Patients fasted for 2 h before administration and 1 h after administration. We predefined dose-limiting toxicities (DLTs) as a treatment-related adverse event that occurs during the DLT assessment period (from day 1 to day 3 in cycle 0 and from day 1 to day 21 in cycle 1) and met any of the following criteria: grade 4 thrombocytopenia, grade 4 neutropenia continuing for 4 days or more, non-haematological toxic effects of grade 3 or worse (excluding transient electrolyte abnormalities and diarrhoea, nausea, or vomiting that recovers to grade 2 or lower with appropriate treatment), and events that required suspension of treatment for at least 7 days. The recommended dose was to be determined after taking into consideration tumour response in addition to the MTD, safety, and pharmacokinetic parameters under fasting conditions. While this fasting part was ongoing with DLT assessment in the cohort of patients given 300 mg twice daily, we amended the study to conduct a non-fasting part at doses of 240 mg and 300 mg twice daily by a traditional 3+3 design. We assessed the effect of food by comparing results under fasting and non-fasting conditions at both doses in the two groups of patients.

In the phase 2 portion of this study, patients received CH5424802 at the recommended dose identified in the phase 1 portion of the study orally twice a day (once in the morning and once in the evening). The patients fasted for 2 h before administration and 1 h after administration. Treatment was continued in 21-day cycles until disease progression, intolerable adverse events, or withdrawal of consent.

Tumours were assessed every cycle until four cycles and every two cycles thereafter, with RECIST version 1.1. In the phase 2 portion, tumour assessment from brain to pelvis at baseline was mandatory. Tumour assessment in this trial was done with CT scans for chest and abdomen; with CT or MRI for head, neck, and pelvis; and with bone scintigraphy, PET, x-ray, CT, or MRI for bone. Adverse

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)**

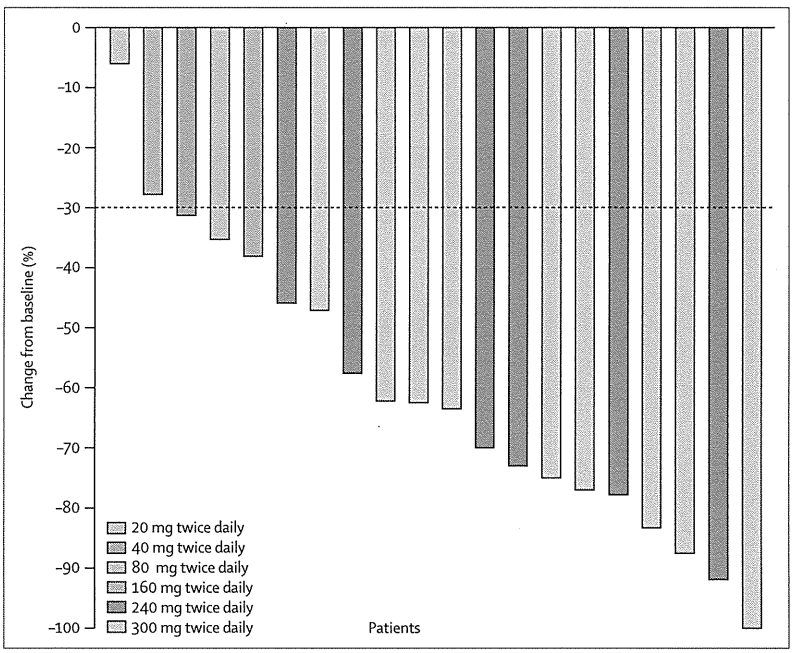

Figure 1: **Waterfall plot of best percentage change in target lesions from baseline on investigator assessment (20 patients with measurable lesions in phase 1)**

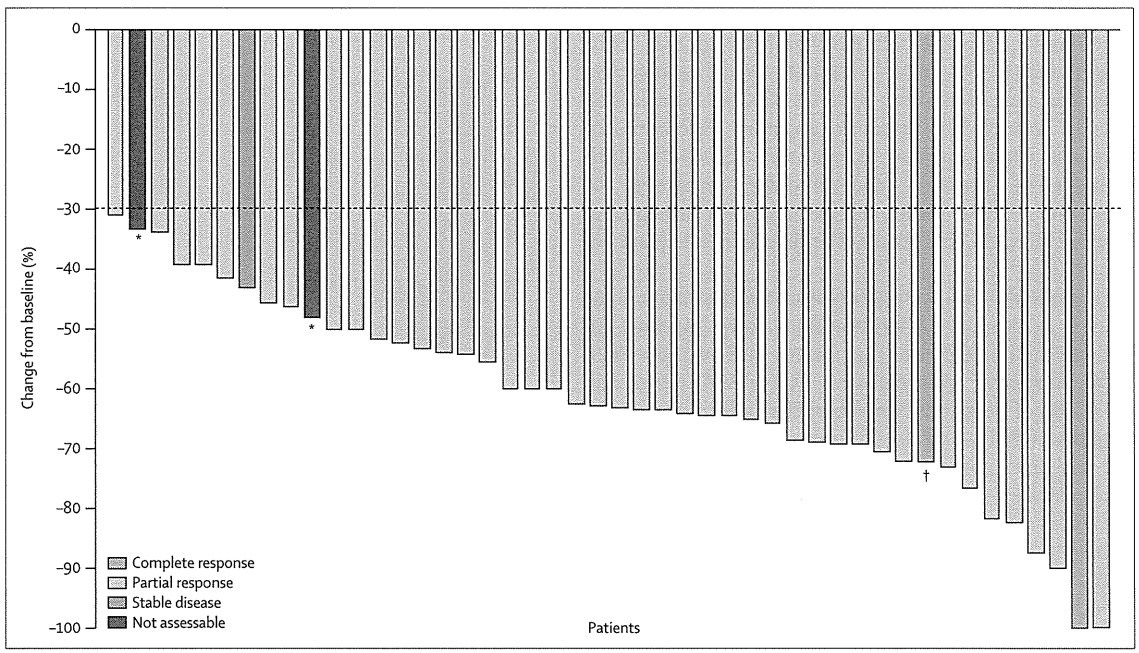

Figure 2: **Waterfall plot of best percentage change in target lesions from baseline based on independent review committee assessment (46 patients in phase 2)**
*Indeterminate response by early stopping because of safety reasons. †Classified as complete response according to the definition of Response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

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

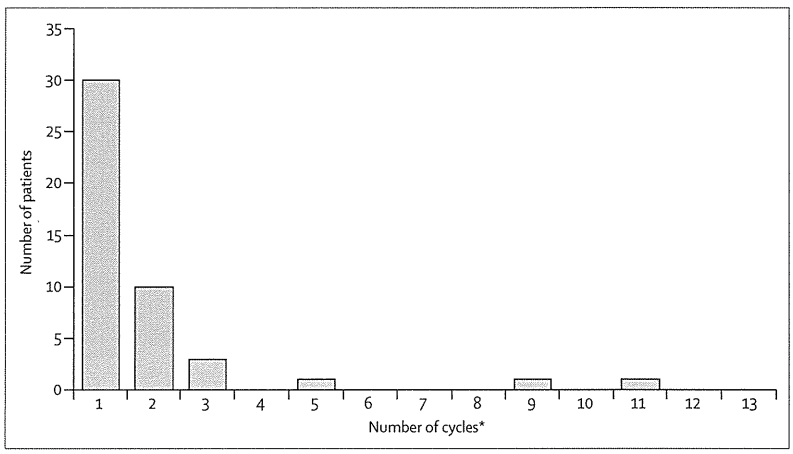

Figure 3: **Number of patients who had tumour size reduction of 30% or more by treatment cycle in phase 2**
*One cycle lasted 3 weeks.

	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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Histology and Smoking Status Predict Survival of Patients with Advanced Non–Small-Cell Lung Cancer

Results of West Japan Oncology Group (WJOG) Study 3906L

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Introduction: Smoking status is one of the prognostic factors in advanced non–small-cell lung cancer (NSCLC). Currently, adenocarcinoma (Ad) histology is considered a predictive factor in advanced NSCLC. We investigated the correlation between histology or smoking status and survival of NSCLC patients receiving chemotherapy.

Methods: We retrospectively reviewed clinical data from stage IIIB or IV NSCLC patients who started first-line chemotherapy at affiliated institutions of West Japan Oncology Group from 2004 to 2005. We also collected information on pack-years of cigarette smoking and years since cessation. Overall survival was compared using log-rank test, and Cox regression analysis was used to identify independent prognostic factors.

Results: In total, 2542 consecutive patients were enrolled at 40 institutions. Of those, 71 were excluded because of unknown smoking history. The median overall survival of nonsmoking Ad patients (593 days) was longer than that of smoking Ad, nonsmoking non-Ad, and smoking non-Ad patients (384, 374, and 319 days, respectively; $p < 0.001$). In Cox regression with sex, age, stage, performance, and treatment as covariates, we found significant interaction ($p = 0.039$) between histology (Ad/non-Ad) and smoking status (smoker/nonsmoker); smoking conferred a hazard ratio of 1.34 (95% confidence interval, 1.15–1.55) in Ad, but only 0.99 (0.75–1.31) in non-Ad. Higher pack-years and shorter period since cessation were significantly associated with poorer survival in Ad ($p < 0.001$), but not in non-Ad ($p \geq 0.434$).

Conclusion: Ad histology is associated with better prognosis, and only smoking status had a prognostic impact in Ad.

Key Words: Non–small-cell lung cancer, Histology, Adenocarcinoma, Smoking status.

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This study is registered with University Hospital Medical Information Network-Clinical Trial Registry (UMIN-CTR) (http://www.umin.ac.jp/ctr/index.htm umin.ac.jp/ctr; identification number UMIN000001263).

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Lung cancer is the leading cause of cancer-related mortality in Japan, and the rest of the world, with more than one million people dying from it each year. Non–small-cell lung cancer (NSCLC), which accounts for nearly 80% of all lung cancers, comprises several histological types, including adenocarcinoma (Ad), squamous cell carcinoma (Sq), and large-cell carcinoma (La). NSCLC had been treated as a single disease because of similar therapeutic effects of conventional chemotherapeutic agents. In the last few decades, however, treatment with new drugs, such as epidermal

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growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs), bevacizumab, and pemetrexed revealed that tumor histology has profound impact on the benefits of a variety of chemotherapy or targeted-therapy regimens for advanced NSCLC.[1–4] Thus, histology came to be considered a predictive factor for the effectiveness of specific chemotherapy in patients with advanced NSCLC. However, there is no previous report on histology as a prognostic factor, that is, a variable determining survival irrespective of the chemotherapy regimen administered.

Previous studies showed that cigarette smoking is an independent prognostic factor in patients with NSCLC,[2,5–7] but a dose–response relationship between the quantity of smoking and survival has not been established. Although Yelena et al.[6] noted that patients who had smoked up to 15 pack-years had a longer survival than those with more than a 15 pack-year history, other cutoff points for the amount of cigarette smoking have not been considered. In addition, the relationship between smoking and survival was not investigated with respect to differences in NSCLC histological subtypes, and the studies that did evaluate survival in Sq versus non-Sq patients did not reach a firm conclusion.[7,8] However, Kawaguchi et al.[8] showed that Ad had better prognosis than Sq in never-smokers, but not in ever-smokers, suggesting that the prognostic impact of cigarette smoking may differ among histologic subtypes in NSCLC.

We hypothesized that Ad histology and lower smoking status would result in better overall survival (OS) in advanced NSCLC. To test this hypothesis, we investigated the impact and possible interaction of histology and smoking status on survival of advanced NSCLC patients receiving chemotherapy in the clinic.

PATIENTS AND METHODS

Study Patients

We sent case report forms to 40 affiliated institutions of West Japan Oncology Group, and requested them to provide demographic and clinical data from medical records for all patients with stage IIIB or IV NSCLC, who started first-line systemic chemotherapy between January 1, 2004 and December 31, 2005. Patients who had a relapse after surgery or radiotherapy were excluded. The case report forms were submitted by the participating institutions during the period from September 2008 to January 2009. This study was approved by the institutional review board of each participating institution.

Demographic and Clinical Variables

We obtained the following baseline demographic and clinical information from the case report forms: age, sex, histology, disease stage, Eastern Cooperative Oncology Group performance status (PS), smoking status, type of first-line chemotherapy, number of treatment regimens, and the year in which first-line chemotherapy was started. Disease stage was determined according to the tumor, node, metastasis system.[9] Staging classification was performed by physical examination, chest–abdominal computed tomography, brain magnetic resonance imaging, bone scan, and positron emission tomography if necessary. Patients were categorized into nonsmokers and smokers according to smoking status. Nonsmokers were defined as those who had smoked less than 100 cigarettes. Among smokers, exsmokers were defined as those who had quit smoking 1 year or more before diagnosis, and current smokers as those who continued their smoking habit at diagnosis. Pack-years of smoking were calculated by multiplying the number of packs (20 cigarettes in one pack) smoked per day by the number of years smoked, and categorized as less than 10, 10 to 19, 20 to 29, 30 to 39, 40 to 49, 50 to 59, and 60 or more. Years of smoking cessation were categorized as 1 to 4, 5 to 9, 10 to 14, 15 to 19, and 20 or more. Type of first-line chemotherapy was categorized into platinum-based combination, nonplatinum combination, and single-agent chemotherapy. Because the only approved EGFR-TKI for the treatment of inoperable or recurrent NSCLC in Japan before October 2007 was gefitinib, we collected information on gefitinib usage during the observation period and noted the starting day of gefitinib treatment. OS was calculated from the start of first-line chemotherapy to the date of death. Patients still alive were censored as of the last known follow-up.

TABLE 1. Patient Characteristics

Parameter	Ad (n = 1731)	Non-Ad (n = 740)	p
Men/women	1056/675	641/99	<0.001
Smoking status			<0.001
Nonsmoker	659	79	
Exsmoker	300	165	
Current smoker	772	496	
Stage IIIB/IV	444/1287	271/469	<0.001
PS			0.002
0	546	206	
1	873	402	
2	191	96	
3	90	25	
4	31	11	
Histology			—
Sq	—	516	
La	—	71	
Others	—	153	
Chemotherapy			0.181
Single-agent	354	137	
P doublet	1306	571	
Non-P doublet	71	32	
Regimen			<0.001
1	536	285	
2	445	201	
3	322	115	
≥4	428	139	
Gefitinib Y/N	959/772	146/594	<0.001

Ad, adenocarcinoma; PS, performance status; Sq, squamous cell; La, large cell; P, platinum; Y, yes; N, no.

Statistical Analysis

Demographic and clinical variables were compared among groups according to lung cancer histology, using the χ^2 test. The primary endpoint of this study was OS. Survival curves were calculated by the Kaplan–Meier method and compared using the log-rank test. Prognostic importance of histology and smoking status were analyzed using the Cox regression analysis adjusted for sex, age, disease stage, PS, type of first-line chemotherapy, and the year in which first-line chemotherapy was started. For detection of possible interaction between histology and smoking status, the terms of interaction of the two variables were evaluated by the likelihood ratio test. Because gefitinib was the preferred choice in patients with Ad, another Cox regression analysis was performed, in which patients were censored at the start of gefitinib administration, and the results were compared with the original Cox analysis. Significance level was set at a p value of 0.05. Statistical analyses were performed with SAS version 9.2 software (SAS Institute, Cary, NC).

RESULTS

Between January 1, 2004 and December 31, 2005, 2542 consecutively treated patients were enrolled at 40 institutions. Of these, 71 were excluded because of unknown smoking history. The characteristics of the study population, categorized into Ad and non-Ad, are listed in Table 1. There were 1731 Ad and 740 non-Ad patients (29.9% and 70.1%, respectively). Among them, we confirmed 1346 and 599 deaths in Ad and non-Ad patients, respectively. There were significantly more women (39.0% in Ad versus 13.4% in non-Ad) and nonsmokers (38.1% in Ad versus 10.7% in non-Ad) in the Ad group than in the non-Ad group. Patients who received single-agent chemotherapy accounted for approximately 20% of the study population. Compared with combination regimens, single-agent chemotherapy was associated with old age (63.6 years for combination regimens versus 71.1 years for single-agent chemotherapy), high proportions of female patients (29.3% versus 40.0%), nonsmokers (27.8% versus 34.0%), stage IV (69.4% versus 78.3%), and PS 0 to 1 (60.9% versus 87.1%). The proportion of Ad histology was not significantly different between single-agent and combination regimens (72.1% and 69.5%, respectively). The OS was 464 days in Ad compared with 326 days in non-Ad ($p < 0.001$; Fig. 1*A*). Between Ad and non-Ad, which was divided into Sq and La, Ad had significantly better survival than the other two histological groups (Sq, 341 days; La, 254 days; $p < 0.0001$; Fig. 1*B*). With regard

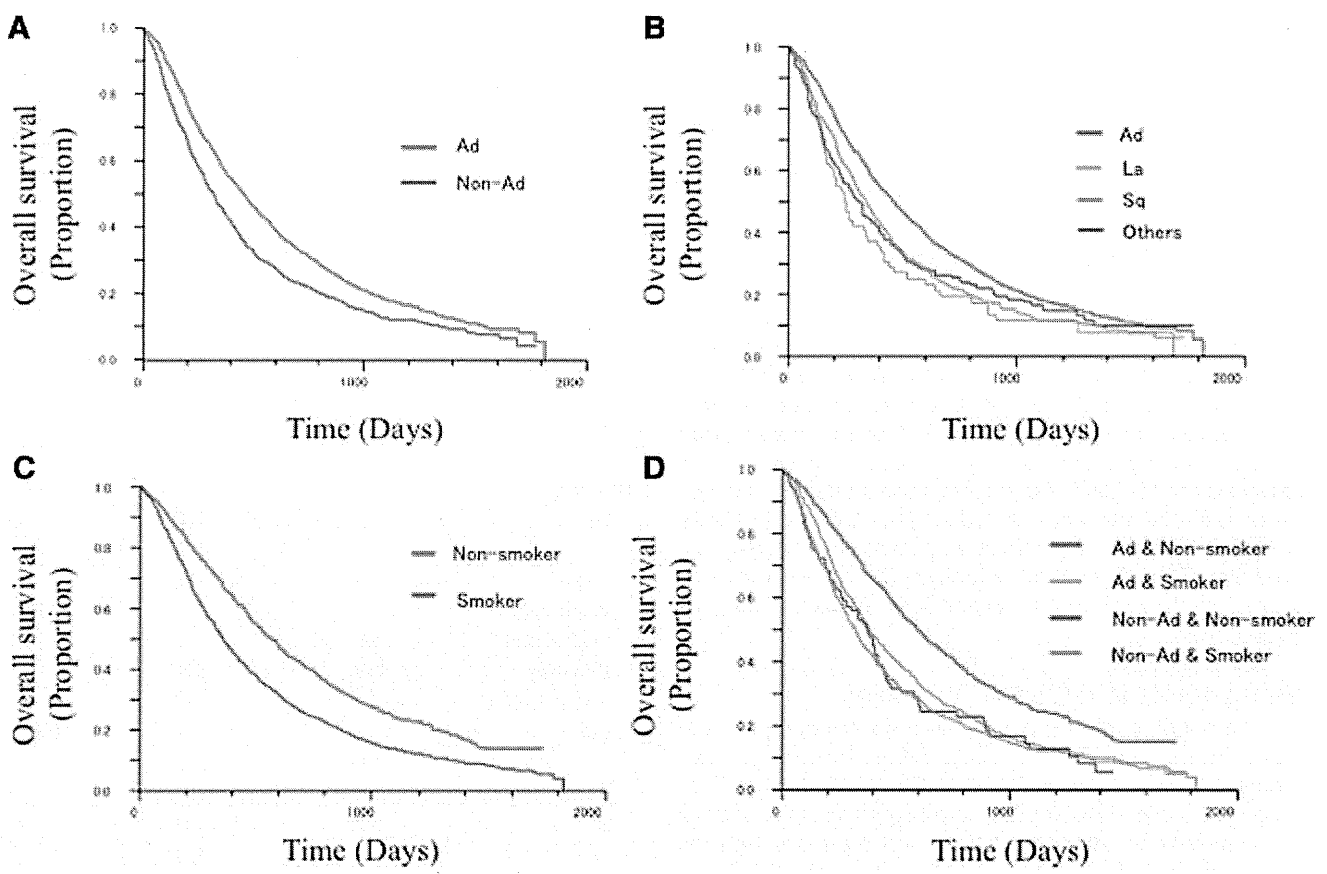

FIGURE 1. Kaplan–Meier plots of overall survival for patients classified according to histology type as (*A*) Ad and Non-Ad; histologic subtype as (*B*) Ad, La, Sq, and others; smoking status as (*C*) smokers and nonsmokers; and combination of smoking status and histology as (*D*) Ad and nonsmoker, Ad and smoker, Non-Ad and nonsmoker, and Non-Ad and smoker. Ad, adenocarcinoma; La, large cell; Sq, squamous cell.

TABLE 2. Survival Analysis by Cox Proportional Hazards Model (n = 2471)

Parameter	HR	95% CI	p
Sex			
Women	1		
Men	1.342	1.168–1.541	<0.001
Age yrs	1.007	1.002–1.012	0.005
Smoking status			
Nonsmoker	1		
Exsmoker	1.178	0.997–1.391	0.054
Current smoker	1.335	1.155–1.543	<0.001
Clinical stage			
Stage IIIB	1		
Stage IV	1.505	1.358–1.669	<0.001
PS			
0	1		
1	1.609	1.446–1.790	<0.001
2	2.229	1.910–2.601	<0.001
3	3.048	2.455–3.785	<0.001
4	5.487	3.864–7.790	<0.001
Histology			
Ad	1		
Sq	1.143	1.015–1.286	0.028
La	1.542	1.182–2.011	0.001
Others	1.397	1.159–1.683	<0.001
Chemotherapy			
Single-agent	1		
Non-P doublet	0.842	0.657–1.080	0.175
P doublet	0.793	0.699–0.899	<0.001

HR, hazard ratio, CI, confidence interval; PS, performance status; Ad, adenocarcinoma; Sq, squamous cell; La, large cell; P, platinum.

to smoking status, nonsmokers (568 days) had significantly longer survival than smokers (358 days; $p < 0.0001$; Fig. 1*C*). In a combined analysis of smoking status and histology, the median OS of Ad in nonsmokers was longer than that of Ad in smokers, non-Ad in nonsmokers, and non-Ad in smokers (593, 384, 374, and 319 days, respectively; $p < 0.001$; Fig. 1*D*). In Cox regression analysis, sex, age, smoking status, disease stage, PS, histology, and chemotherapy showed a statistically significant prognostic impact on survival (Table 2). When the interaction between histology (Ad/non-Ad) and smoking status (smoker/nonsmoker) was included in the Cox model, significant interaction was observed ($p = 0.039$); smoking conferred a hazard ratio (HR) of 1.34 (95% confidence interval [CI], 1.15–1.55) in Ad, in contrast to 0.99 (0.75–1.31) in non-Ad. In detailed analyses that excluded the 104 patients (current smokers, 89; unknown, 15) with unknown amount of cigarette smoking, shorter period since cessation showed a significant trend for poorer survival in the whole population ($p < 0.001$). This trend was also observed in Ad ($p < 0.001$; Table 3), but not in non-Ad ($p \geq 0.434$; Table 3). When non-Ad patients were divided into Sq and La or others, the trend p was 0.534 in Sq and 0.165 in La or others. The prognosis became significantly worse with higher pack-years of cigarette smoking in the whole population and Ad ($p < 0.001$; Table 3), but no significance was not achieved for the non-Ad group ($p = 0.519$; Table 3). When non-Ad patients were divided into Sq and La or others, the trend p was 0.798 in Sq and 0.380 in La or others. The prognostic impact of histology and smoking status remained significant in the Cox regression analysis, in which patients were censored at the start of gefitinib administration; positive smoking history, Sq histology, and La or other histology conferred an HR of 1.51 (95% CI, 1.21–1.88), 1.22 (95% CI, 1.06–1.41), and 1.59 (95% CI, 1.32–1.93), respectively. The negative prognostic impact of shorter period since cessation and pack-years of cigarette smoking was also essentially unchanged ($p < 0.001$ in both).

DISCUSSION

The consensus report of prognostic factors in NSCLC at the 1990 International Association for the Study of Lung Cancer Workshop showed that histology was not a prognostic factor for advanced NSCLC.[10] Our study is the first report to reveal that histology is a significant prognostic factor for advanced NSCLC. Importantly, we showed that Ad patients have the longest survival of all three histological groups (Ad, Sq, and La). Ad is the most common histological subtype of lung cancer in nonsmokers,[11] who have been reported to have a better prognosis than smokers.[12–14]

Smoking has been described as a prognostic factor in lung cancer. Although multiple studies have demonstrated the negative effects of smoking in patients with NSCLC, most included a heterogeneous population comprising patients with all stages and types of lung cancer.[5] In contrast, our study cohort consisted exclusively of patients with advanced NSCLC treated with first-line chemotherapy. We showed that smoking status is an independent prognostic factor for survival in those patients. Similar data have been shown in former studies.[2,5] However, those reports did not show whether smoking conferred any survival impact for advanced NSCLC irrespective of histological subtypes. In our study, only Ad histology had significant interaction with smoking status or smoking index and prognosis. A higher level of smoking was related to shorter survival in Ad patients, whereas smoking level and survival were not associated in non-Ad patients. Although the proportion of non-Ad patients was 29.9% of the total, the observed number of deaths in this study yielded a statistical power of more than 80% for detecting an HR of 1.5 at the 5% significance level in both Ad and non-Ad patients. Others have found that Ad histology is a significant prognostic factor in separate multivariate analysis for never-smokers in advanced NSCLC.[8] Yelena et al.[6] showed that high cigarette smoking, as measured in pack-years, is associated with decreased survival after diagnosis of stage IIIB/IV NSCLC. However, the patients of that study received a wide variety of therapies, raising the possibility that the outcomes might have been the result of distinct therapeutic responses. Although we only assessed the prognostic value of smoking status at diagnosis, assessment of smoking status at a later point, that is, at the time of treatment, would also have been of interest to determine whether cessation at the time of diagnosis leads to improved survival.

TABLE 3. Hazard Ratios According to Quantitative Aspects of Smoking

	Ad			Non-Ad		
	HR	95% CI	*p*	HR	95% CI	*p*
Years after cessation	(*n* = 1731)			(*n* = 740)		
Current	1.492	1.271–1.750	<0.001	1.204	0.849–1.707	0.297
Exsmoker 1–4 yr	1.438	1.114–1.857	0.005	1.101	0.733–1.653	0.643
Exsmoker 5–9 yr	1.549	1.101–2.180	0.012	1.228	0.700–2.155	0.474
Exsmoker 10–14 yr	1.127	0.783–1.621	0.520	1.235	0.680–2.245	0.488
Exsmoker 15–19 yr	1.199	0.761–1.890	0.433	1.410	0.712–2.794	0.325
Exsmoker ≥20 yr	0.873	0.834–1.203	0.407	1.103	0.662–1.837	0.706
Trend *p*	<0.001			0.434		
Pack-yr	(*n* = 1665)			(*n* = 702)		
<10	1.267	0.899–1.785	0.176	1.196	0.535–2.672	0.662
10–19	1.118	0.801–1.561	0.513	0.963	0.512–1.812	0.908
20–29	1.346	1.048–1.729	0.020	1.368	0.887–2.109	0.157
30–39	1.345	1.071–1.689	0.011	0.954	0.624–1.458	0.827
40–49	1.370	1.096–1.712	0.006	1.128	0.763–1.669	0.546
50–59	1.483	1.164–1.890	0.001	1.238	0.828–1.851	0.298
≥60	1.595	1.312–1.939	<0.001	1.135	0.791–1.628	0.491
Trend *p*	<0.001			0.519		

[a] Nonsmokers were set as the reference category.
Ad, adenocarcinoma; HR, hazard ratio; CI, confidence interval.

In agreement with the findings of another study,[15] we also found that a large proportion of Ad patients were nonsmoking. The prognostic difference between Ad in never-smokers and smokers may suggest that both are different disease entities. Of note, tumor-mutational frequencies and spectra suggest differences between smokers and nonsmokers.[16,17] However, significant differences in the frequency of somatic mutations in oncogenes such as *EGFR* and *KRAS* have been observed between smoking and nonsmoking lung cancer patients.[11] *EGFR* mutations, clinical predictors of EGFR-TKI therapeutic benefits, are more frequently found in nonsmoking Ad patients.[11] In another study, *EGFR* mutations were identified in nonsmokers (51%), former smokers (19%), and current smokers (4%).[18] Moreover, the incidence of *EGFR* mutations decreased with increasing number of pack-years of cigarette smoking.[18] However, *KRAS* mutations, predicting poor survival and resistance to EGFR-TKI, are more frequently found in smoking Ad patients. Interestingly, *EGFR* and *KRAS* mutations are mutually exclusive.[11]

Currently, therapeutic options other than EGFR-TKIs (e.g., bevacizumab and pemetrexed) are available in Japan. Still, NSCLC subtypes have been showing variable response rates and adverse events.[2,4,19,20] Non-Sq histology, especially Ad, is currently the NSCLC subtype with broader and more efficacious treatment options. At the time of this study, however, the only approved therapeutic agent for NSCLC in Japan was gefitinib. Unfortunately, we did not investigate *EGFR* mutation status. However, genetic background could possibly predict response to gefitinib. Along with its retrospective nature, this was a limitation of our study. However, we found that the treatment choice was made on the basis of clinical background, and we were unable to conclude whether or not gefitinib contributed to better survival under unknown *EGFR* mutation status. Hence, we suggest that decision-making based on clinical information alone is inappropriate. Both the V15-32 study[21] and the Iressa Survival Evaluation in Lung Cancer (ISEL) study[22], support our observations. Furthermore, the IRESSA Pan-Asia Study (IPASS) study,[23] conducted under the hypothesis that EGFR-TKI would be effective in clinically selected patients, confirmed the strong predictive value of *EGFR* mutations for the response of Ad to gefitinib.

This retrospective study has a few other limitations as well. First, information on smoking was not obtained from the interview or the self-administered questionnaire. Smoking data can be inaccurate, particularly when collected retrospectively. Second, we did not collect data on the procedures for histological diagnosis. The basis for pathological diagnosis is important because cytological assessment alone may lead to underdiagnosis of specific histologic types.

In conclusion, this survey demonstrated that Ad histology is associated with better prognosis, and that smoking status has a prognostic impact only in patients with Ad.

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Recurrence of thymic neuroendocrine carcinoma 24 years after total excision: A case report

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Abstract. A 77-year-old male presented with chest pain in March 2012. The individual had undergone surgery for an anterior mediastinal tumor 24 years earlier and the pathological diagnosis was that of a thymoma. The patient underwent a medical check-up every 6 months for the next 20 years. However, ~3 years following the final check-up, sudden chest pain was reported and the patient was referred again. Computed axial tomography revealed a mediastinal mass adjacent to the left lung, pericardium and sternum. There was no apparent invasion to the adjacent structures. The patient underwent surgical resection following a diagnosis of recurrent thymoma. A posterolateral thoracotomy was performed under video-assisted thoracoscopy. Severe adhesions were observed around the tumor, which appeared to invade the left lung and pericardium, but not the chest wall. The tumor was extirpated in combination with partial resection of the left lung and pericardium. The pathological diagnosis of the tumor was of a well-differentiated neuroendocrine carcinoma (NEC) of the thymus. The specimen that was excised 24 years earlier was re-examined by a pathologist and was reported to exhibit the same histology. Primary NECs of the thymus are rare among anterior mediastinal tumors and the 5-year survival rate is ~30%. The present case study reports a case of a thymic NEC and describes the pathological and clinical features.

Introduction

Thymic neuroendocrine carcinomas (NECs) are rare and have been estimated to account for 2-4% of all anterior mediastinal tumors (1). Local and distal metastases frequently develop following surgical excision of these tumors (2-4). A previous study by Fukai *et al* showed that recurrence occurred 4-99 months after surgery (3) and recurrence after as long as 9 years has been described (5,6). However, to the best of our knowledge, no studies have discussed the development of recurrence >20 years after total excision. Although the optimal therapeutic modality for the treatment of recurrent disease has not been determined, more aggressive treatment, including re-excision of recurrent tumors, may be required to reduce the incidence of local recurrence and distant metastasis and to improve survival. The current study presents a rare case of a recurrent neuroendocrine tumor in the thymus developing 24 years after total excision. Written informed consent was obtained from the patient.

Case report

Clinical presentation. A 77-year-old male was referred for an evaluation of an acute onset of chest pain. The patient had undergone a thymectomy via a median sternotomy for an anterior mediastinal tumor 24 years previously. The pathological diagnosis was of a World Health Organization (WHO) type B3 thymoma classified as pathological stage I due to the absence of capsular invasion (Masaoka classification). Regular medical check-ups had been performed twice a year for 20 years after the surgery and had been completed without evidence of recurrence. However, ~3 years after the final check-up, a sudden onset of left-sided chest pain was reported and the patient was referred again. Laboratory examinations revealed elevated C-reactive protein levels (3.53 mg/dl), but no other abnormal levels of any tumor markers, including neuron specific enolase. Computed tomography (CT) revealed an irregularly enhanced tumor in the anterior mediastinum with a maximum size of ~3 cm (Fig. 1A). Positron emission tomography/CT scans revealed increased ^{18}F-fluorodeoxyglucose uptake in the mass (maximum standard uptake value, 3.35), although no abnormal uptake indicative of distant metastases was observed (Fig. 1B).

Surgery. Surgery was performed under the diagnosis of a suspected recurrent thymoma. A posterolateral thoracotomy was performed under video-assisted thoracoscopy. Severe adhesions were observed around the tumor, which appeared

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Key words: thymic neuroendocrine carcinoma, recurrence, long-term survival, surgery

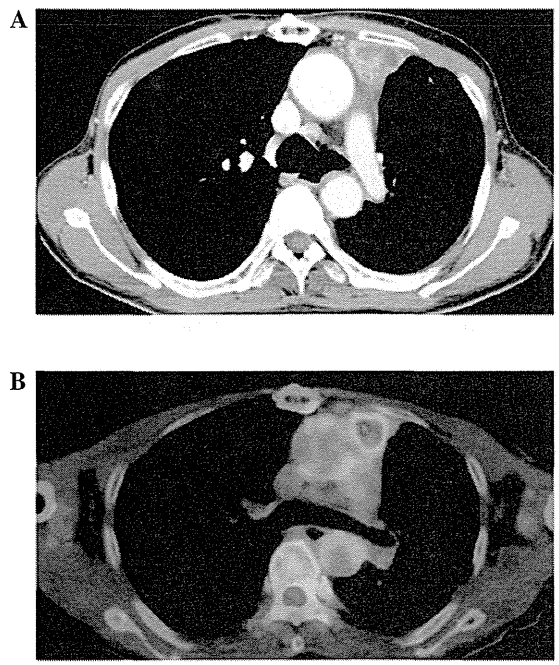

Figure 1. Imaging observations. (A) CT revealing an anterior mediastinal tumor with a maximum size of ~3 cm. (B) PET/CT scan revealing abnormal uptake of ^{18}F-fluorodeoxyglucose in the tumor. CT, computed tomography; PET, positron emission tomography.

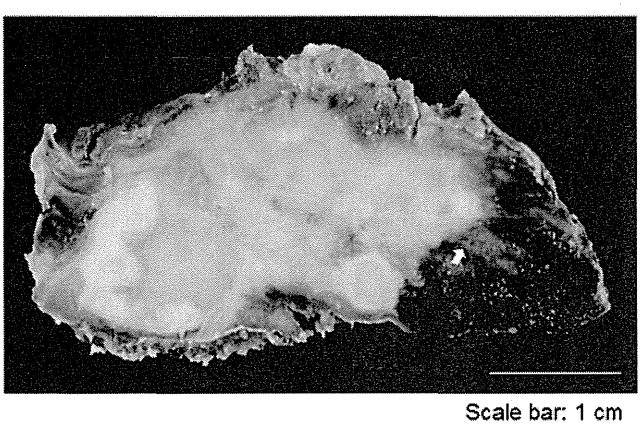

Figure 2. Macroscopic appearance of the cut sections of the tumor revealing a yellowish-white mass invading the left upper lobe (arrow).

to have invaded the left upper lung and pericardium, while no pleural dissemination was observed. Therefore, the tumor was extirpated in combination with partial resection of the left upper lung and pericardium, and the excised pericardium was repaired using a polytetrafluoroethylene sheet. The tumor was found to be a yellowish-white solid mass invading the lung (Fig. 2).

Histopathology. Histopathologically, atypical carcinoid cells were observed to be arranged in sheets or small nested patterns accompanied by necrosis and lymphoid infiltration invading the surrounding adipose tissue and lungs, while extremely few mitotic cells were observed (Fig. 3A). An immunohistochemical analysis revealed that the tumor exhibited immunoreactivity to neuroendocrine markers, including chromogranin A (Fig. 3B).

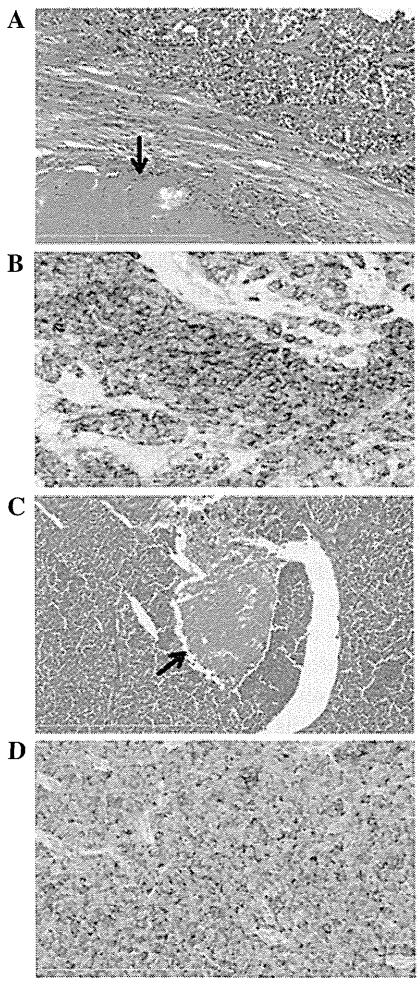

Figure 3. Microscopic features and immunohistochemical observations. (A) HE staining demonstrating that the tumor consisted of atypical carcinoid cells proliferating in sheets or small nested patterns accompanied by necrosis (arrow). (B) Immunoreactivity to chromogranin A. (C) HE staining and (D) immunohistochemistry of the specimen that was resected 24 years earlier demonstrating the same pathological observations of necrosis (arrow) and immunoreactivity to chromogranin A (original magnification, x200). HE, hematoxylin and eosin.

Based on these observations, the tumor was diagnosed as a well-differentiated NEC (atypical carcinoid, due to the presence of necrosis). The surgical margin of the lung was affected by the cancer cells. Retrospectively, the specimen that had been excised 24 years previously was re-examined and was reported to exhibit the same histology, HE results and immunoreactivity to the neuroendocrine markers as the present tumor (Figs. 3C and D).

There were no post-operative complications. Although the surgical margins were positive for cancer cells, no medical intervention was administered due to the patient's age and the invasiveness of radiation and chemotherapy.

Discussion

Thymic NEC is a rare type of neoplasm arising in the thymus, accounting for 2-4% of all anterior mediastinal tumors (1). This form of neoplasm has long been confused with thymoma, although Rosai and Higa described thymic NEC as a separate entity from thymoma in 1972 (7). Thymic

NECs are predominantly or exclusively composed of neuroendocrine cells and must be distinguished from other typical thymic carcinomas with small numbers of neuroendocrine cells (8). Thymic NECs are divided into two groups, well- and poorly-differentiated, depending on the degree of tumor differentiation. The former group contains typical and atypical carcinoids classified according to the presence of necrosis and/or the number of mitotic cells, while the latter group includes large cell NEC and small cell carcinoma. This categorization is significant in that the prognosis of a well-differentiated NEC is improved compared with that of a poorly-differentiated NEC (8). In the present case, well-differentiated neuroendocrine cells were accompanied by necrotic components.

Local recurrence and distant metastasis develops frequently following surgical excision of thymic NECs (2-4). Wang *et al* previously reported that local recurrence or distant metastasis developed 15-60 months after surgery in 4/5 (80%) patients. In these cases, the sites of relapse included the chest wall, regional lymph nodes, bones and lungs (2). In addition, Fukai *et al* reported that distant metastases developed in 10/13 (76.9%) of patients who underwent total tumor resection, despite the absence of local recurrence (3). The study also reported intervals of 4-99 months between surgery and recurrence, comparable to that reported by Tiffet *et al* (22-83 months) (4). A study by Economopoulos *et al* identified recurrence in one case 9 years after surgery (5). However, to the best of our knowledge, there are no reports of any cases of recurrent thymic NEC relapsing 10-20 years after surgery. Therefore, the present case involves the longest period of time between the recurrence of thymic NEC and surgery. The optimal therapeutic modality for the treatment of recurrent disease has not been determined. However, due to the aggressive nature of tumors prone to recur or metastasize even following total excision, more aggressive treatments, including routine adjuvant chemotherapy and re-excision of recurrent tumors, as performed in the present case, may be required to reduce the incidence of local recurrence and distant metastasis, and therefore improve survival.

In conclusion, this study presents a case of a surgically-excised thymic NEC recurring >20 years after the initial excision. Thoracic oncologists must be aware that thymic NECs may recur ≥20 years after surgical treatment.

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FULL PAPER

BJC

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Keywords: S-1; long-term; adjuvant chemotherapy; non-small cell lung cancer

Feasibility trial for adjuvant chemotherapy with docetaxel plus cisplatin followed by single agent long-term administration of S-1 chemotherapy in patients with completely resected non-small cell lung cancer: Thoracic Oncology Research Group Study 0809

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Background: We conducted a multicentre feasibility study for single agent long-term S-1 chemotherapy following docetaxel plus cisplatin in patients with curatively resected stage II–IIIA non-small cell lung cancer.

Methods: Patients received three cycles of docetaxel (60 mg m^{-2}) plus cisplatin (80 mg m^{-2}) and then received S-1 (40 mg m^{-2} twice daily) for 14 consecutive days with a 1-week rest for >6 months (maximum, 1 year). The primary end point was feasibility, which was defined as the proportion of patients who completed eight or more cycles of S-1 chemotherapy. If the lower 95% confidence interval (CI) of this proportion was 50% or more, then the treatment was considered as feasible. The sample size was set at 125 patients.

Results: One hundred and thirty-one patients were enrolled, of whom 129 patients were eligible and assessable. In all, 109 patients (84.5%) completed 3 cycles of docetaxel plus cisplatin and 66 patients (51.2%, 95% CI: 42.5–59.8) completed 8 or more cycles of S-1 treatment. Grade 3/4 toxicities during the S-1 chemotherapy included anaemia (7.3%), neutropaenia (3.7%), and anorexia (3.7%).

Conclusion: The toxicity level was acceptable, although the results did not meet our criterion for feasibility. Modification of the treatment schedule for S-1 chemotherapy might improve the treatment compliance.

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Primary surgery is the standard of care for resectable clinical stage I or II non-small cell lung cancer (NSCLC). The 5-year survival rate for patients with clinical stage IB and stage II surgically resected NSCLC was ~66% and 50%, respectively. The majority of patients with recurrences have distant metastases, indicating that systemic micrometastases are common in patients with completely resected NSCLC. To control distant micrometastasis and to improve patients' survival, adjuvant chemotherapy has been examined in patients with completely resected NSCLC of pathological stage I–III. Several randomised studies and meta-analyses have demonstrated that cisplatin-based adjuvant chemotherapy improved the overall survival (OS) in patients with pathological stage IB to III NSCLC (Arriagada *et al*, 2004; Hotta *et al*, 2004; Winton *et al*, 2005; Douillard *et al*, 2006; Pignon *et al*, 2006). However, the absolute increase in survival was only 4% at 5 years. Thus, new treatment strategies or drugs are needed to improve the clinical outcome in patients with resectable NSCLC.

A randomised phase III study demonstrated that adjuvant chemotherapy with uracil-tegafur (UFT) improved survival among patients with completely resected pathological stage I adenocarcinoma of the lung. The 5-year OS was 88% in the UFT group and 85% in the control group (hazard ratio 0.71, 95% confidence interval (CI) 0.52–0.98) (Kato *et al*, 2004). S-1 is an oral anticancer agent comprises tegafur, gimeracil (an inhibitor of dihydropyrimidine dehydrogenase, which degrades fluorouracil), and oteracil (which inhibits the phosphorylation of fluorouracil in the gastrointestinal tract, thereby reducing the gastrointestinal toxicity of fluorouracil) in a molar ratio of 1 : 0.4 : 1 (Shirasaka *et al*, 1996). S-1 is approved for the treatment of NSCLC as well as gastric, colorectal, head and neck, breast, pancreatic, and biliary tract cancer in Japan. In a phase II trial, S-1 monotherapy produced a response rate of 22% as a first-line treatment in patients with advanced NSCLC (Kawahara *et al*, 2001). S-1 is believed to have a stronger antitumour activity against NSCLC than UFT, since UFT monotherapy produced a response rate of only 6% in another phase II study (Keicho *et al*, 1986). A randomised phase III trial demonstrated that S-1 plus carboplatin (CBDCA) was non-inferior in terms of OS, compared with paclitaxel plus CBDCA, in patients with advanced NSCLC (Okamoto *et al*, 2010). Another randomised phase III trial also demonstrated that S-1 plus CDDP was non-inferior in terms of OS, compared with docetaxel plus CDDP, in patients with advanced NSCLC (Katakami *et al*, 2012). Previous phase II trials demonstrated that S-1 monotherapy produced a response rate of 7–14% as a second-line treatment for advanced NSCLC (Totani *et al*, 2009; Govindan *et al*, 2011; Shiroyama *et al*, 2011).

Recent phase III trials have demonstrated that switch maintenance chemotherapy consisting of pemetrexed or erlotinib prolonged the OS of patients with advanced NSCLC who showed no signs of progression after four cycles of platinum-based chemotherapy (Ciuleanu *et al*, 2009; Cappuzzo *et al*, 2010). Continuation maintenance with pemetrexed also prolonged the OS in patients with non-squamous NSCLC in another randomised trial (Paz-Ares *et al*, 2012a,b). Maintenance chemotherapy has thus received considerable attention.

The Thoracic Oncology Research Group (TORG) conducted a randomised phase II study comparing docetaxel (DOC) plus CDDP with paclitaxel (PTX) plus CBDCA as an adjuvant chemotherapy in patients with completely resected stage IB to IIIA NSCLC (TORG 0503). This study showed that DOC plus CDDP had a promising activity with a favourable 2-year recurrence-free survival (RFS) rate (74.1% *vs* 72.5%, respectively) (Ohira *et al*, 2011). Taking these rationales into consideration, we conducted a feasibility study for adjuvant chemotherapy consisting of DOC plus CDDP followed by single agent long-term S-1 chemotherapy in patients with completely resected NSCLC (TORG 0809).

PATIENTS AND METHODS

Patient population. Patients were required to have completely resected stage II or IIIA (according to the Union Internationale Contre le Cancer (UICC) fifth TNM edition) NSCLC, an age of 20–74 years, and an ECOG performance status (PS) of 0 or 1. Other criteria included a PaO_2 at room air $\geqslant$70 torr or an SpO_2 at room air $\geqslant$95%, and adequate organ function (i.e., total bilirubin $\leqslant$1.2 mg dl^{-1}, AST and ALT $\leqslant$100 IU l^{-1}, serum creatinine $\leqslant$1.2 mg dl^{-1}, creatinine clearance $\geqslant$60 ml min^{-1}, leukocyte count $\geqslant$4000 per mm^3 and $\leqslant$12 000 per mm^3, neutrophil count $\geqslant$2000 per mm^3, haemoglobin $\geqslant$10.0 g dl^{-1}, and platelets $\geqslant$100 000 per mm^3). Patients were required to start the protocol treatment within 10 weeks after surgical resection.

Key exclusion criteria were a lack of recovery from surgical complications; active infection; interstitial pneumonia as determined using computed tomography (CT) of the chest; acute cardiac infarction within 6 months; uncontrolled heart disease, liver dysfunction, or diabetes mellitus; grade 2 or worse peripheral neuropathy; active concomitant malignancy; pregnancy or breast-feeding; a history of hypersensitivity to drugs including polysorbate-80; and the concurrent use of flucytosine. Patients who had undergone a pneumonectomy were also excluded. All the patients were required to provide written informed consent.

Treatment plan. The treatment schema is shown in Figure 1. Treatment was started within 1 week after enrolment in the study. Patients received adjuvant chemotherapy with DOC (60 mg m^{-2}, day 1) and CDDP (80 mg m^{-2}, day 1) every 3–4 weeks for up to three cycles. After the completion of adjuvant chemotherapy with DOC plus CDDP, if the leukocyte count was $\geqslant$3000 per mm^3, the neutrophil count was $\geqslant$1500 per mm^3, the platelet count was $\geqslant$100 000 per mm^3, the AST and/or ALT level was $\leqslant$100 IU l^{-1}, the total bilirubin level was $\leqslant$1.5 mg dl^{-1}, the serum creatinine level was $<$1.5 mg dl^{-1}, and all other non-haematological toxicities were grade 1 or better with the exception of alopecia, body weight loss, and hyponatraemia, then the patients were treated with oral S-1 at a dose of 40 mg m^{-2} twice daily for 14 consecutive days, followed by a 1-week rest. The actual dose of S-1 was selected as follows: patients with a body surface area (BSA) of $<$1.25 m^2 received 80 mg daily; those with a BSA of 1.25 m^2 or more but $<$1.5 m^2 received 100 mg daily; and those with a BSA of 1.5 m^2 or more received 120 mg daily. If the serum creatinine level was 1.2 mg dl^{-1} or more but $<$1.5 mg dl^{-1} before the initiation of S-1 chemotherapy, then the S-1 dose was reduced to a lower level. This 3-week cycle was repeated for 6 months (maximum, 1 year) if neither unacceptable toxicity nor tumour recurrence was observed. In the event of a leukocyte count of $<$2000 per mm^3, a platelet count of $<$75 000 per mm^3, an AST and/or ALT level of $\geqslant$100 IU l^{-1}, a total bilirubin level of $\geqslant$2.5 mg dl^{-1}, a serum

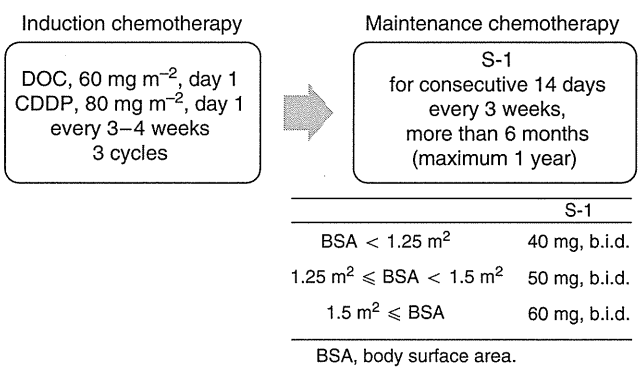

Figure 1. **Treatment schema for this study.**