

Clinical Response to Crizotinib Retreatment After Acquisition of Drug Resistance

Case Report

A 39-year-old Japanese male former smoker presented at our hospital with several months of history of an occasional dry cough. He was found to have stage IV (cT4, N2, M1b) adenocarcinoma of the lung with bone metastasis. He received six courses of first-line chemotherapy with cisplatin and pemetrexed. His best response was a partial response, and after 7 months of first-line treatment, he showed evidence of progressive disease, with an increase in the size and number of pulmonary metastases as well as pleural fluid accumulation (Fig 1A). Mutation analysis of biopsied tumor tissue revealed that the tumor was wild type for the epidermal growth factor receptor (*EGFR*) gene. However, fluorescent in situ hybridization analysis with break-apart probes for the anaplastic lymphoma kinase gene (*ALK*) revealed the presence of an *ALK* rearrangement. As a second-line treatment, therefore, crizotinib was administered orally at a dose of 250 mg twice per day. Marked tumor regression became apparent (Fig 1B), and this response persisted for 4 months. Given that crizotinib toxicities were mild and manageable, the drug was administered without pause or dose reduction. After this time, however, pulmonary metastases again developed, and crizotinib was discontinued. The patient was enrolled

onto a phase I clinical trial of new agents, but treatment was withdrawn after 4 weeks because of disease progression. Docetaxel was then administered as fourth-line chemotherapy, but the patient was hospitalized again after 8 weeks with obstructive pneumonitis as a result of disease progression (Fig 2A). Retreatment with crizotinib (500 mg daily) commenced at the patient's request 5 months after completion of the initial crizotinib treatment. One month later, a significant response had been achieved (Fig 2B), and this response persisted for 2.5 months.

Discussion

Despite the great benefits of crizotinib treatment for *ALK* rearrangement-positive non-small-cell lung cancer (NSCLC), all such treated patients ultimately develop drug resistance, which has been observed with other effective small-molecule tyrosine kinase inhibitors (TKIs) such as those for *EGFR*. Although multiple clinical trials have studied therapies for acquired TKI resistance, no published results have led to a change in clinical practice.¹ The current standard practice for such patients is to switch to conventional cytotoxic chemotherapy. We now describe a pronounced antitumor effect of crizotinib retreatment in a patient with *ALK* rearrangement-positive NSCLC who initially responded to this drug but subsequently showed tumor progression. A recent study found that NSCLC cells that harbored an activating *EGFR* mutation and acquired resistance to

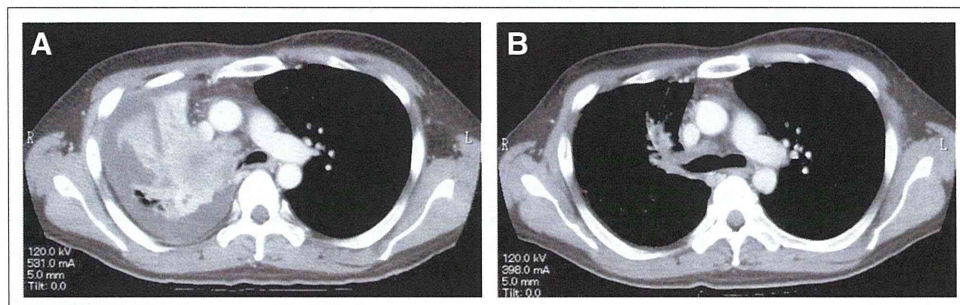


Fig 1.

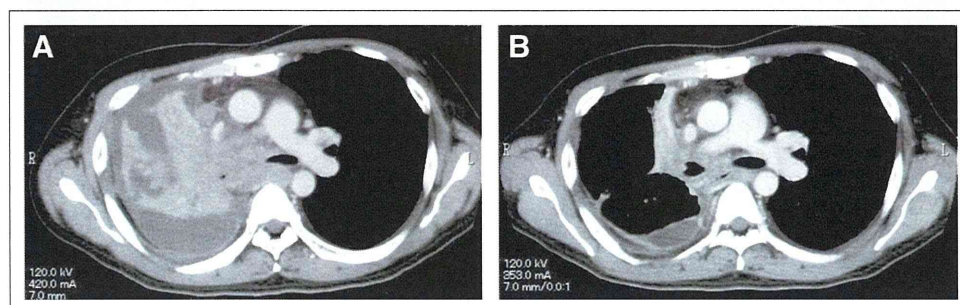


Fig 2.

EGFR-TKIs as a result of a secondary *EGFR* mutation (T790M) regained sensitivity to these drugs after drug withdrawal, with this renewed sensitivity being associated with a reduction in the proportion of cells harboring the T790M mutant allele.² Similar to these *in vitro* findings, withdrawal of treatment with an EGFR inhibitor after the development of drug resistance in patients with *EGFR* mutation-positive NSCLC resulted in the restoration of tumor sensitivity to subsequent treatment with either the same or a different EGFR inhibitor.³⁻⁵ We recently established an ALK-TKI-resistant NSCLC cell line positive for the echinoderm microtubule-associated protein-like 4 gene (*EML4*)–*ALK* transforming fusion gene by exposing the parental H3122 cells to increasing concentrations of ALK-TKI, and we found that the resistant cells regained sensitivity to ALK-TKI treatment after culture in drug-free medium.⁶ These observations may be explained by a selection process in which the withdrawal of a TKI results in a reduction in the fraction of TKI-resistant tumor cells, rendering the tumor sensitive to retreatment with the TKI. Taken together, they provide a rationale for temporary cessation of treatment after the development of ALK-TKI resistance in patients with *EML4-ALK*-positive NSCLC to allow the tumor to regain drug sensitivity. Our experience with the present case further supports such a strategy. Additional clinical evaluation of such an approach is thus warranted.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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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

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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-naïve 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 study⁷ comparing survival outcomes in crizotinib-treated patients enrolled in the phase 1 trial and crizotinib-naïve 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

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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-naïve 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 design¹⁷ 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 pre-defined 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 $\geq 7.5 \times 10^4$ 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 \times 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. AUC₀₋₁₀=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)

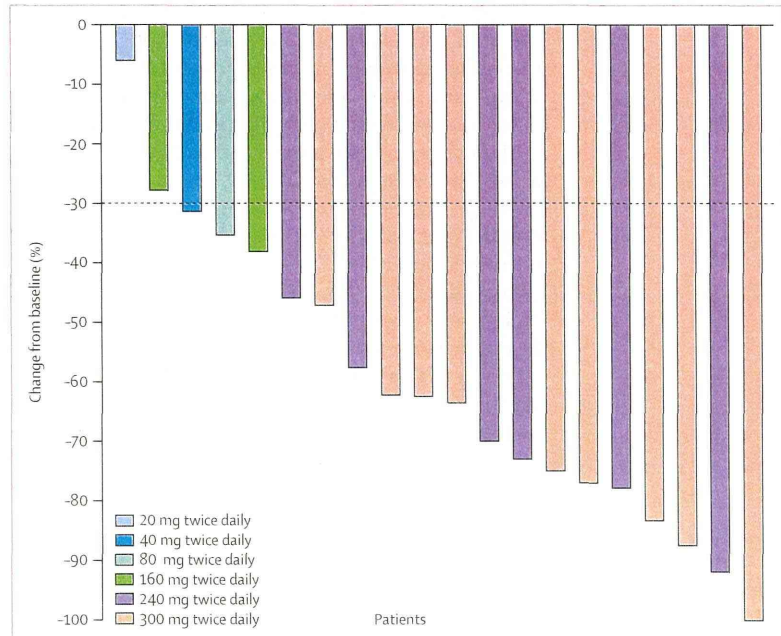


Figure 1: Waterfall plot of best percentage change in target lesions from baseline on investigator assessment (20 patients with measurable lesions in phase 1)

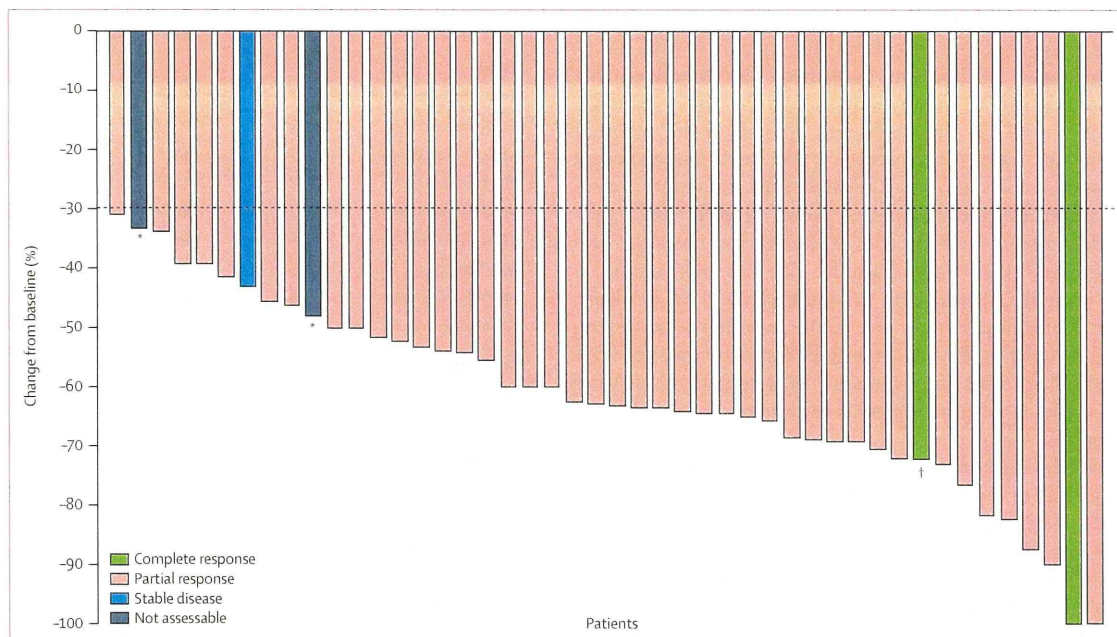


Figure 2: Waterfall plot of best percentage change in target lesions from baseline based on independent review committee assessment (46 patients in phase 2)
 *Indeterminate response by early stopping because of safety reasons. †Classified as complete response according to the definition of Response Evaluation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

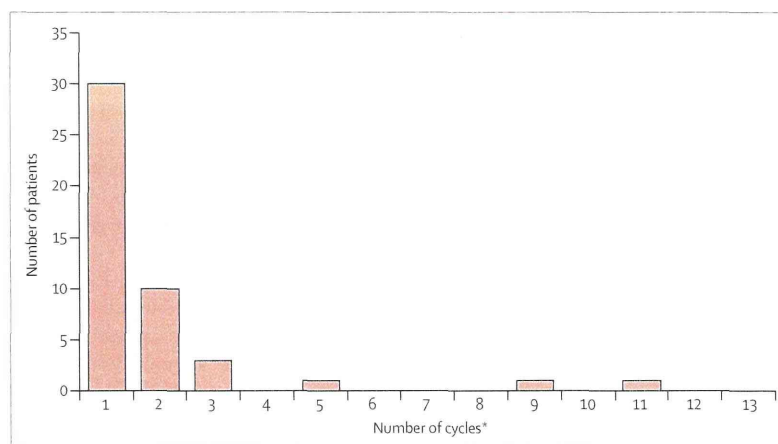


Figure 3: Number of patients who had tumour size reduction of 30% or more by treatment cycle in phase 2
*One cycle lasted 3 weeks.

	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=creatinine phosphokinase. ALP=alkaline phosphatase.

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

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

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.^{5,23-25} 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 low²⁶ 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 criteria²² 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 growth¹² 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 reported²⁹ 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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