Morphologic Features of CTCs Isolated using the MCA System

CTCs were counted, identified as being cytokeratin positive and CD45 negative, and as having a visible nucleus on the basis of analysis of fluorescent images. As can be observed in Figure 3, which shows a representative gallery of CTCs identified by image analysis, CTCs are larger than the surrounding leukocytes and often appear in clusters, defined here as contiguous groupings of cells containing 3 or more nuclei. Figure 4 shows a solitary CTC and a CTC cluster detected in one SCLC patient using the MCA system. Using the MCA system, CTC clusters were observed in 2 of the 22 NSCLC patients (Patient No. 13 and 21) and 4 of the 21 SCLC patients (Patient No. 31, 33, 34, and 43). May-Grünwald–Giemsa staining of the CTCs isolated using the MCA system revealed that they are characterized by a high N/C ratio, nuclear molding, and morphological similarity to primary tumor cells.

Discussion

ISET systems have been found to have higher CTC detection sensitivity than the CellSearch system in several cancers, including NSCLC [17,22] However, the pores of ISET filters, which are made of polycarbonate by track etching, are randomly placed within the systems at a nonuniform density. Unlike such track-etched polycarbonate filters, the size, geometry, and density of the microcavities in the MCA system assessed in the current study are precisely controlled to achieve specific cell separation according to differences in cellular size and deformability. Aligning cells on the MCA not only eases cell imaging by allowing for the scanning of specified areas with an automated fluorescence microscope but also enables reduction in the labor required for CTC counting [29,31]. As such, the MCA system provides a platform for the use of high-throughput imaging technologies that provide more rapid and less expensive data collection as well as CTC enumeration and advanced analysis of molecular phenomena, including fluorescence in situ hybridization for detection of tumor-specific genomic changes. Furthermore, the MCA is integrated with a miniaturized device so that enrichment of CTCs from blood, as well as staining and washing in the microfluidic assay, can be performed within one integrated device.

In the present study, CTCs isolated on the MCA were successfully stained with fluorescent-labeled antibodies that target tumor cell markers, and staining and washing were found to have little or no effect on the retention of tumor cells on the microcavities. Due to its very small size, the MCA system is portable, which, by enabling point-of-care CTC counting, eliminates the need to ship blood for testing under unfavorable shipment conditions and expedites clinical decision-making. These features, in addition to our recently developed procedure for isolating single cells from the MCA using microcapillaries, allow tumor cells to be recovered from the MCA for subsequent molecular analysis of CTCs [29].

In this blind comparison of use of the MCA system to that of the conventional CellSearch System for CTC enumeration in lung cancer patients, the MCA system was found capable of isolating various lung cancer cell lines spiked within whole blood at high levels of efficiency. However, the MCA system performed isolation of SCLC cell lines slightly less efficiently compared to that of NSCLC cell lines, indicating that small (<8 μm in diameter) cells of the SCLC cell lines might pass through the microcavities during blood filtration. In a previous study [31], we found that breast (MCF-7 and Hs578T), gastric (AGS and SNU-1), and colon (SW620) tumor cells lines that include EpCAM-negative tumor cells could be successfully recovered using the MCA system with greater than 80% efficiency. However, we also found that the efficiency of recovery of small cells (average diameter 11.6 μm) of the tumor cell line SW620 to be slightly less than that of other cell lines, as we did of the SCLC cell lines examined in this study.

The MCA system assessed in the present study was found to possess a higher detection sensitivity than the CellSearch system in NSCLC CTC enumeration, suggesting the superiority of size- and deformability-based isolation techniques compared to immuno-magnetic-based techniques. The poor sensitivity of CellSearch has been attributed to the low EpCAM expression in advanced NSCLC. However, one of the NSCLC patients assessed in the present study was found to be CTC positive using the CellSearch system but CTC negative using the MCA system, indicating that

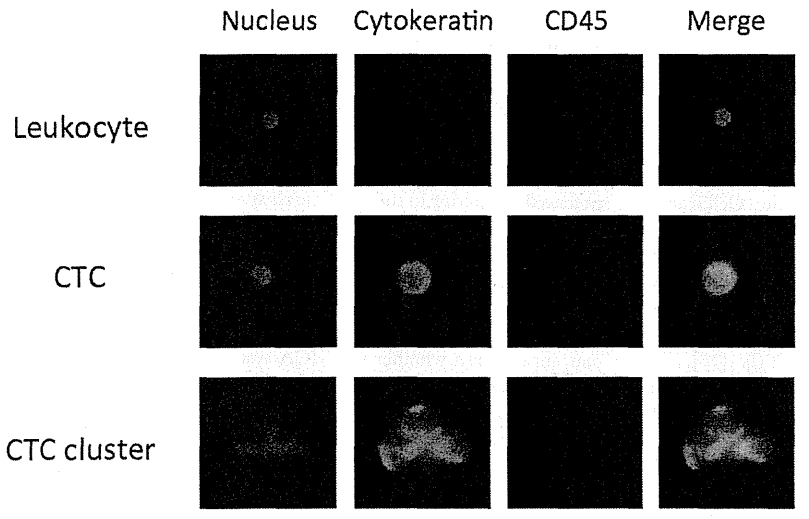

Figure 3. Gallery of cells captured on the MCA from blood of advanced lung cancer patients. Cells were stained with Hoechst 33342, FITC-labeled anti-cytokeratin antibody, and PE-labeled anti-CD45 antibody.
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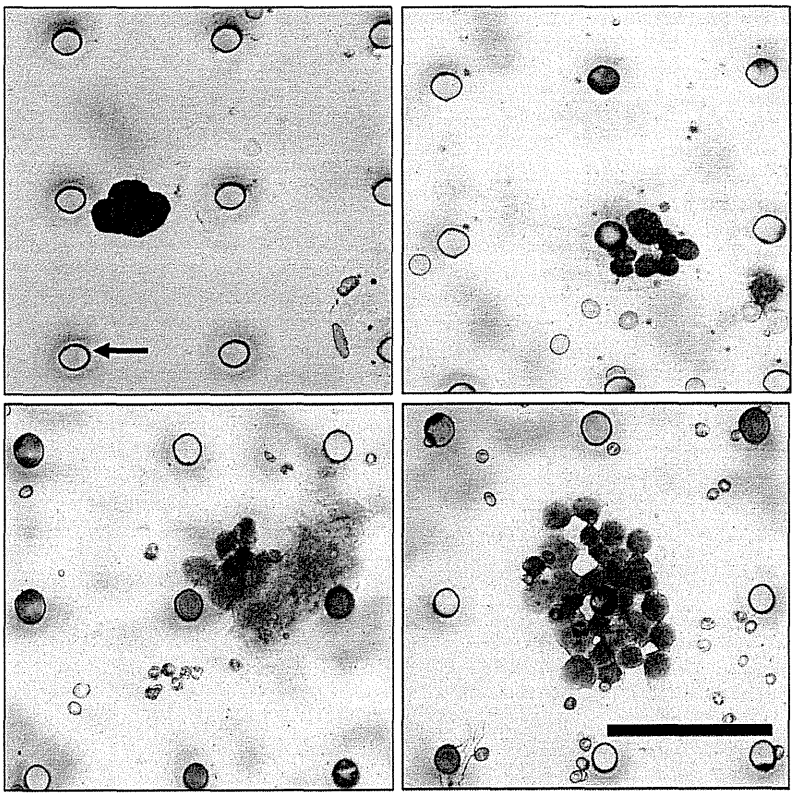

Figure 4. Gallery of CTCs captured on a transparent MCA from SCLC patient blood. May-Grünwald–Giemsa-stained cells showed a high nucleus–cytoplasm ratio and nuclear molding (×40). Black arrow indicates 9-μm microcavity. Scale bar = 60 μm.
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changes in EpCAM expression cannot solely account for the differences found between the two systems in NSCLC enumeration.

The CTC detection rate using the CellSearch system in SCLC patients was 67%, considerably higher than that in NSCLC patients and consistent with that found in previous studies [7,34–36]. Although the MCA system does not rely on EpCAM expression, which circulating SCLC cells have been reported to show high levels [37], in performing CTC isolation, its use was found to yield a high detection rate, indicating that it could be utilized for CTC detection in not only NSCLC but also SCLC patients. Nevertheless, the CTC counts of several patients were higher when analyzed using the CellSearch System compared to the MCA system, indicating that some small tumor cells in patient blood might flow through the microcavities, as described above. Previous research has suggested that immunomagnetic separation techniques lack the capacity to isolate large clusters, whereas use of size-based separation techniques leads to loss of small CTCs [17]. To address these problems, the shape of the microcavities in the MCA was modified to improve their efficiency in isolating small cells from tumor cells in whole blood in our recent study [38].

Observation of CTC clusters has been reported in various cancers, including lung cancer [23,24,39–42]. It is hypothesized that forming in clusters provides CTC cells with advantages over remaining solitary in terms of survival, proliferative capacity, and ability to form micrometastases. In this study, CTC clusters were isolated from both NSCLC and SCLC patients using the MCA system. Interestingly, the CTC-positive clusters were identified as having a small number of CTC cells by the CellSearch system but a large number by the MCA system. One reason why several SCLC patients were found to have a large CTC count when assessed by the MCA system may be that this system enables isolation of larger CTC clusters that cannot be isolated by immunomagnetic separation. Examination of this hypothesis requires further detailed analysis of the characteristics of CTC clusters, such as expression of epithelial markers and the presence of apoptotic cells within CTC clusters, which could be performed using the MCA system.

In conclusion, our results suggest that the MCA system is potentially superior to the CellSearch system in the CTC detection of lung cancer patients, with the former found capable of isolating significantly more CTCs and CTC clusters than the latter. The major limitation of this study was its examination of a small sample of patients with only one type of cancer. Further studies should thus examine larger cohorts of patients with various types of cancers to assess whether the MCA system is a more appropriate tool for CTC enumeration and characterization of metastatic tumors in patients with cancers other than lung cancer compared to other systems. We are currently planning the development of an automated MCA system that achieves robust, reliable, and reproducible sample processing for validation study using large cohorts of patients presenting at multiple institutes to assess the prognostic utility of CTC count in cancer patients.

Supporting Information

Figure S1 Comparison of cell recovery rate using the microcavity array (MCA) system and an isolation by size of epithelial tumor cell (ISET) filter. Non-small cell lung cancer cell line NCI-H358 was spiked into whole blood at a volume of 100 cells/mL to perform 3 separate tests of circulating cancer cell recovery using an MCA (pore size = 8 μm) and a track-etched polycarbonate ISET filter (pore size = 8 μm; Nucleopore).
(TIFF)

Figure S2 Bland–Altman plots of agreement between circulating tumor cell (CTC) test results obtained for non-small cell lung cancer (NSCLC; a) and small cell lung cancer (SCLC; b) patients using the CellSearch and microcavity array (MCA) systems. The solid horizontal line represents the mean difference and the dashed lines the limits of agreement (mean difference +/−2SD). In NSCLC, the mean difference was 50.1 (95%CI, 11.1 to 89.1), limits of agreement (-125.8 to 226.0) with the difference between systems becoming disproportionately greater with higher average CTC-count. In SCLC, the mean difference was 202.6 (95%CI, −116.7 to 521.9), limits of agreement (-1162.0 to 1567.2) with no bias observed between systems except for subjects with extremely high titer of CTCs.
(TIFF)

Table S1 Evaluation of sensitivity of microcavity array (MCA) system for circulating tumor cell (CTC) detection. Sensitivity testing was performed using artificial samples created by adding 1 and 3 cultured NCI-H358 cells to healthy donor blood samples. Individual cells were selected by micropipette under direct visualization, spiked into 7.5 mL aliquots of blood, and the resulting blood samples processed using the MCA system in 3 separate tests.
(DOC)

Author Contributions

Conceived and designed the experiments: MH H. Kenmotsu YK T. Yoshino TN TM NY. Performed the experiments: MH T. Yoshikawa RW SO. Analyzed the data: MH H. Kenmotsu YK T. Yoshikawa TN RW SO KM NY. Contributed reagents/materials/analysis tools: H. Kenmotsu TN T. Takahashi HM YN AT TS AO HA H. Kanbara KY NY. Wrote the paper: MH H. Kenmotsu YK T. Yoshino T. Yoshikawa TN T. Tanaka TM NY.

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ORIGINAL ARTICLE

Efficacy of bevacizumab-containing chemotherapy for non-squamous non-small cell lung cancer with bone metastases

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Abstract
Purpose Skeletal-related events (SREs) negatively affect the quality of life of patients with cancer. Vascular endothelial growth factor receptor (VEGFR)-targeted therapy is effective against bone metastasis in animal models, but the clinical efficacy of anti-VEGFR inhibitors against bone metastases remains unclear. Therefore, we aimed to investigate the efficacy of chemotherapy with bevacizumab, an anti-VEGF antibody, against bone metastases.
Methods We retrospectively reviewed consecutive patients with non-squamous non-small cell lung cancer who received first-line platinum-based chemotherapy with zoledronic acid at Shizuoka Cancer Center between 2007 and 2011.
Results Of 25 patients, 13 received bevacizumab-based chemotherapy (BEV group) and 12 received chemotherapy without bevacizumab (non-BEV group). The overall response (54 vs. 8 %, $p = 0.01$) and disease control (100 vs. 50 %, $p = 0.01$) rates were higher in the BEV group than in the non-BEV group. The bone-specific response (23 vs. 0 %, $p = 0.038$) and disease control (100 vs. 67 %, $p = 0.01$) rates were also higher in the BEV group. The median time to progression (TTP) for bone metastases was higher in the BEV group (13.7 vs. 4.3 months, $p = 0.06$), whereas that for overall disease was similar between the groups (5.7 vs. 2.6 months, $p = 0.17$). The proportions of patients with SREs were 23 and 50 % in the BEV and non-BEV groups, respectively ($p = 0.16$).
Conclusion Bevacizumab might potentiate the antitumor activity of chemotherapy against systemic disease and bone metastases, prolonging bone-specific TTP and reducing the incidence of SRE.

Keywords Bone metastases · Skeletal-related event · Bevacizumab · Chemotherapy

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Introduction

The incidence of bone metastases in patients with lung cancer is approximately 30–40 %, and the median survival time of patients with such metastases is 7 months [1]. A more recent retrospective review of 435 patients with non-small cell lung cancer (NSCLC) indicated an incidence of 24 % for skeletal metastases. In this review, most instances of skeletal metastases (66 %) were detected at the time of initial staging [2].

Patients with metastatic bone disease frequently experience osteoclast-mediated bone destruction, resulting in clinically important complications such as a fracture, the need for bone radiation or surgical therapy, spinal cord compression, or hypercalcemia [3, 4]. These complications, collectively known as skeletal-related events (SREs) [5–7], lead to pain and decreased quality of life [8]. Thus, SREs have a negative impact on the quality of life, performance status, and functioning of patients with cancer. In a Japanese retrospective review of 259 patients with NSCLC [9], 30 % of patients were found to have skeletal metastases during their clinical course, and 50 % of these patients had SREs. Among 135 stage IV patients, 41 % had

skeletal metastases at the initial staging, and 45 % had SREs.

Zoledronic acid has been used in patients with bone metastases because the drug can reduce the incidence of SREs and delay time to the first SRE [10]. Recently, the non-inferiority of denosumab to zoledronic acid in delaying the time to the first SRE was demonstrated [11]. However, we believe that the efficacy of these drugs cannot be insufficient. The efficacy of chemotherapy against bone lesions in patients with lung cancer has not been reported previously.

Bevacizumab, an anti-vascular endothelial growth factor (VEGF) agent, provides a clinical benefit when combined with platinum-based chemotherapy in first-line therapy against advanced non-squamous (non-Sq) NSCLC [12–14]. In particular, the response rate and progression-free survival (PFS) compared with those of non-bevacizumab-containing chemotherapy are improved by the addition of bevacizumab. Antitumor activity may be induced by the effects of bevacizumab on tumor vasculature, interstitial pressure, and blood vessel permeability, resulting in enhanced delivery of chemotherapy agents to tumor cells [15]. Nagengast et al. [16] demonstrated that bevacizumab distribution to the bone was similar as that to other organs in an ex vivo biodistribution model. Bäuerle et al. [17] reported that bevacizumab significantly inhibited osteolysis, surrounding soft tissue tumor growth, and angiogenesis in an experimental model of breast cancer bone metastasis as visualized on volumetric computed tomography (CT) and magnetic resonance imaging (MRI). Furthermore, the blocking of VEGF–VEGF receptor (VEGFR)-2 signaling inhibited bone metastasis in animal models of lung cancer [18]. Therefore, VEGF was suggested as a therapeutic target for bone metastasis [19]. Thus, we hypothesized that bevacizumab-containing chemotherapy could have some clinical benefit in patients with non-Sq NSCLC and bone metastases. We retrospectively investigated the efficacy of bevacizumab-containing chemotherapy and compared it to that of chemotherapy without bevacizumab in this study.

Patients and methods

Patients

We reviewed electronic medical records of consecutive patients who visited the Shizuoka Cancer Center between January 2007 and December 2011. In addition, electronically stored images were evaluated by a diagnostic radiologist. Eligible patients were pathologically diagnosed with non-Sq NSCLC, received platinum-based first-line chemotherapy, had bone metastases at the time of receiving chemotherapy, had at least 1 evaluable bone lesion according to the Revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) [20], and received zoledronic acid continuously. We permitted the inclusion of patients who received EGFR tyrosine kinase inhibitors before platinum-based chemotherapy. We selected carboplatin plus paclitaxel and carboplatin plus pemetrexed as the non-bevacizumab-containing chemotherapy regimens because we used only these regimens in combination with bevacizumab in our institution. The patients who received bevacizumab-containing chemotherapy comprised the "BEV group" and those who received chemotherapy without bevacizumab comprised the "non-BEV group".

Evaluation

We evaluated the objective response rate, disease control rate, time to progression of overall disease (TTP), time to progression of bone metastases (B-TTP), overall survival (OS), and proportion of patients with SREs. The response to chemotherapy was accessed according to RECIST criteria (version 1.1). At the initial staging, we performed chest and abdominal CT, brain MRI, and positron emission tomography (PET)–CT/bone scintigraphy. To ascertain disease progression or the relapse of overall disease and bone metastases, patients were evaluated by physical examination, chest radiography, and CT of the chest and abdomen. If bone metastases were detected at the initial staging, the patient was regularly followed up with radiography and CT. If progression of bone metastases was suspected, we additionally performed PET–CT, MRI, or bone scintigraphy, as required. Generally, all patients were evaluated for lesions during and approximately 6–8 weeks after the treatment period.

Time to progression was measured from the start of first-line chemotherapy to the date of an event of documented disease progression/recurrence or the last follow-up visit. B-TTP was measured from the start of first-line chemotherapy to the date of an event of documented progression of bone metastases and/or SRE or the last follow-up visit. Cases of TTP or B-TTP were censored under the following conditions: no progression or recurrence of overall disease or bone metastases and death. The incidence of SREs accounted for all events that occurred from the start of platinum-based chemotherapy to the date of first progression of overall disease or the last follow-up visit. SREs included a pathologic fracture, spinal cord compression, and the need for bone radiation or surgical therapy. OS was measured from the start of first-line chemotherapy to the date of death or the last follow-up visit.

Statistical analysis

All categorical variables, objective response rates, and incidences of SREs were analyzed and compared between the BEV and non-BEV groups using the χ^2 test or Fisher's exact test, as appropriate. The distributions of TTP, B-TTP, and OS were estimated using the Kaplan–Meier method, and the BEV and non-BEV groups were compared using the log-rank test. All p values were two-sided, and values less than 0.05 were considered statistically significant. All analyses were performed using JMP 9 software (SAS Institute, Cary, NC). This study was approved by the Institutional Review Board of Shizuoka Cancer Center.

Results

A total of 25 patients, 13 patients in the BEV group and 12 patients in the non-BEV group, were eligible for this retrospective study. Patient characteristics are shown in Table 1. In the BEV and non-BEV groups, the median ages of patients were 63 and 67 years, respectively. In total, 11 of 13 (85 %) patients in the BEV group and 9 of 12 (75 %) patients in the non-BEV group were men. The BEV group included 11 (85 %) current or ever smokers, and the non-BEV group included 7 (58 %) current or ever smokers. The numbers of patients with an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0–1 were 12 in the BEV group and 11 in the non-BEV group, and 1 patient in each group had an ECOG PS of 2. The EGFR status was not examined in 5 patients in the non-BEV group, but no statistically significant difference in EGFR status was found between the 2 groups ($p = 0.41$).

The administered chemotherapy regimens are shown in Table 1. In the BEV group, 6 patients were treated with carboplatin, paclitaxel, and bevacizumab, whereas 7 patients were treated with carboplatin, pemetrexed, and bevacizumab. In the non-BEV group, 11 patients received carboplatin plus paclitaxel, and 1 patient received carboplatin plus pemetrexed.

The response rates for overall disease were 54 % in the BEV group and 8 % in the non-BEV group ($p = 0.01$; Table 2). The disease control rates for overall disease were 100 % in the BEV group and 50 % in the non-BEV group ($p = 0.01$; Table 2). The response rates for bone metastases were 23 % in the BEV group and 0 % in the non-BEV group ($p = 0.038$; Table 3). The disease control rates for bone metastases were 100 % in the BEV group and 67 % in the non-BEV group ($p = 0.01$; Table 3).

The Kaplan–Meier curve for B-TTP is shown in Fig. 1. The median B-TTPs were 13.7 months in the BEV group and 4.3 months in the non-BEV group ($p = 0.06$). The Kaplan–Meier curve for TTP is shown in Fig. 2. The median TTPs were 5.7 months in the BEV group and 2.6 months in the non-BEV group ($p = 0.17$). Overall disease progression was observed in 12 of 13 patients in the BEV group and in all patients in the non-BEV group. The median OS was 6.6 months (range, 4.0–34.7 months) in the non-BEV group and this was not reached (range, 6.6 months-) in the BEV group ($p = 0.13$). In the present study, the median follow-up duration was 15.1 months.

Skeletal-related events occurred in 3 patients (23 %) in the BEV group and in 6 patients (50 %) in the non-BEV group (Table 4). The types of SREs were as follows: 3 instances of the need for bone radiation, 1 instance of spinal cord compression in the BEV group, and 5 instances of the need for bone radiation, 1 bone surgery, and 1 pathologic fracture in the non-BEV group.

Discussion

To the best of our knowledge, the present study is the first report to evaluate the bone-specific efficacy of

Table 1 Patients characteristics and chemotherapy regimens

		BEV	Non-BEV	*P* value
Number		13	12	–
Age	Median (range)	63 (35–75)	67 (40–76)	0.3255
Sex	M/f	11/2	9/3	0.5476
Smoking	Yes/no	11/2	7/5	0.1394
PS	0/1/2	3/9/1	0/11/1	0.9530
EGFR	Mt/wt/unknown	4/9/0	2/5/5	0.4054
Regimen of chemotherapy	CBDCA + PTX	–	11	–
	CBDCA + PEM	–	1	
	CBDCA + PTX + BEV	6	–	
	CBDCA + PEM + BEV	7	–	

Mt mutation, *Wt* wild type, *CBDCA* carboplatin, *PTX* paclitaxel, *PEM* pemetrexed, *BEV* bevacizumab

Table 2 Response and control rates for overall disease

Best response	BEV ($n = 13$)	Non-BEV ($n = 12$)	P value
PR	7	1	
SD	6	5	
PD	0	6	
Response rate	54 %	8 %	0.01
Disease control rate	100 %	50 %	0.01

PR partial response, *SD* stable disease, *PD* progressive disease

Table 3 Response and control rates for bone metastases

Best response	BEV ($n = 13$)	Non-BEV ($n = 12$)	P value
PR	3	0	
SD	10	8	
PD	0	4	
Response rate	23 %	0 %	0.04
Disease control rate	100 %	67 %	0.01

PR partial response, *SD* stable disease, *PD* progressive disease

chemotherapy in patients with bone metastases from NSCLC. In addition, it was important to evaluate the bevacizumab-mediated potentiation of chemotherapeutic efficacy against bone metastases. In the present study, in the BEV group, the response and disease control rates for bone metastases were 23 and 100 %, respectively, and the median B-TTP was 13.7 months.

Rosen et al. [10, 21] reported a Phase 3 trial of zoledronic acid. Among 254 patients who received zoledronic acid 4 mg, 124 patients (49 %) had NSCLC and 207 patients (82 %) received chemotherapy. The best bone response rate as per the original criteria was 8 %, and the disease control rate for bone metastases was 29 %. In this study, by using the RECIST guideline (version 1.1), the response rate for bone metastases was 0 % and the disease control rate for bone metastases was 67 % in the non-BEV group. In contrast, the response rate for bone metastases was 23 % and the disease control rate for bone metastases was 100 % in the BEV group. Although different bone lesion response criteria were used for the Phase 3 trial of zoledronic acid and this study, administration of bevacizumab-containing chemotherapy showed some potential for eliciting an effect on bone metastases. In the same Phase 3 trial, the median B-TTP of patients who received zoledronic acid 4 mg was 145 days, and the proportion of patients with at least 1 SRE over a period of 9 months was 38 %. In this study, the median B-TTPs were 130 days in the non-BEV group and 412 days in the BEV group. In terms of the proportion of patients with SREs, 50 % of patients in the non-BEV group and 23 % of patients in the BEV group had SREs until the first progression of overall disease or the last follow-up visit. These results suggest that bevacizumab-containing chemotherapy specifically controlled bone lesions as well as systemic lesions.

The antitumor activity of bevacizumab-containing chemotherapy is believed to be the result of enhanced chemotherapy delivery to tumor cells [15]. Bevacizumab distribution to bone was similar as that to other organs in ex vivo biodistribution analysis [16]. Inhibiting VEGF–VEGFR-2 signaling inhibited bone metastasis in animal models of lung cancer with bone metastasis [18]. Solares et al. [22] reported a patient with lung adenocarcinoma and bone metastases in whom a complete response was achieved with carboplatin, paclitaxel, and bevacizumab. Paule and Brion [23] reported that 2 patients with renal cell carcinoma (RCC) and bone metastases who were treated with the anti-VEGFR inhibitor sunitinib experienced long-term survival and stabilization of bone metastases. They concluded that VEGF-targeted agents such as sunitinib

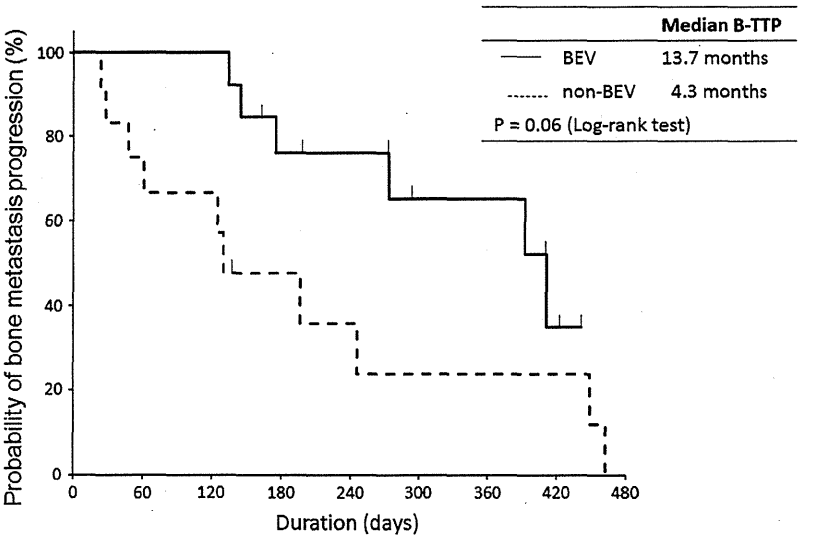

Fig. 1 Kaplan–Meier plot of time to progression of bone metastases (B-TTP) of patients who received chemotherapy containing bevacizumab (BEV group) or lacking bevacizumab (non-BEV group). The median B-TTPs were 13.7 months in the BEV group and 4.3 months in the non-BEV group ($p = 0.06$)

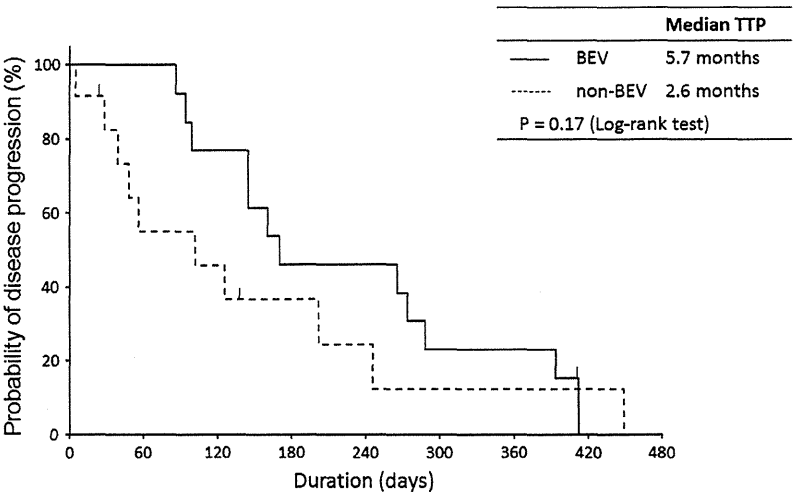

Fig. 2 Kaplan–Meier plot of time to progression of overall disease (TTP) of patients who received chemotherapy containing bevacizumab (BEV group) or lacking bevacizumab (non-BEV group). The median TTPs were 5.7 months in the BEV group and 2.6 months in the non-BEV group ($p = 0.17$)

Table 4 Proportion of patients with SREs until the first documented event of disease progression

	BEV $n = 13$	Non-BEV $n = 12$
SREs*	3 (23 %)	6 (50 %)
Radiation to bone	3	5
Surgery to bone	0	1
Spinal cord compression	1	0
Pathologic fracture	0	1

SRE skeletal-related events

* $P = 0.16$

may be effective treatments for bone metastases. Furthermore, a retrospective analysis reported that sunitinib plus bisphosphonates such as zoledronic acid and pamidronate improved the response rate, PFS, and OS in cases of RCC with bone metastases [24]. In our study, the response rates for bone metastases were 23 % in the BEV group and 0 % in the non-BEV group. These results might validate the clinical efficacy of bevacizumab-containing chemotherapy against bone metastases.

This study has several limitations. The sample size was small. This was a retrospective study with an inherent potential for bias. The collection of clinical characteristics and treatment response data was retrospective, and the follow-up interval for physical examinations was indefinite. Therefore, future studies are warranted to investigate larger sample sizes.

In conclusion, this study indicates that bevacizumab might potentiate the antitumor activity of chemotherapy against both systemic disease and bone metastases, thereby prolonging bone-specific TTP and reducing the incidence of SREs.

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Conflict of interest The authors declare that they have no conflict 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

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)

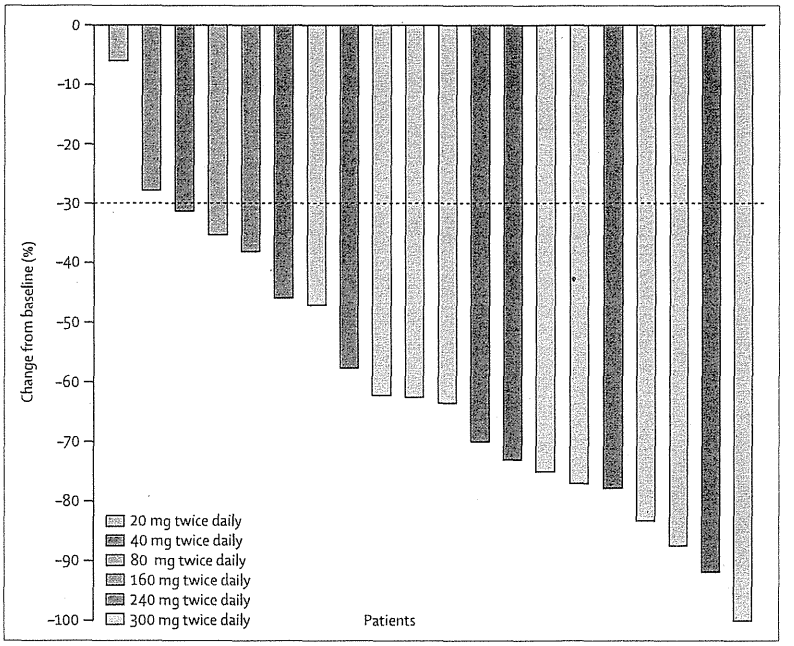

Figure 1: Waterfall plot of best percentage change in target lesions from baseline on investigator assessment (20 patients with measurable lesions in phase 1)

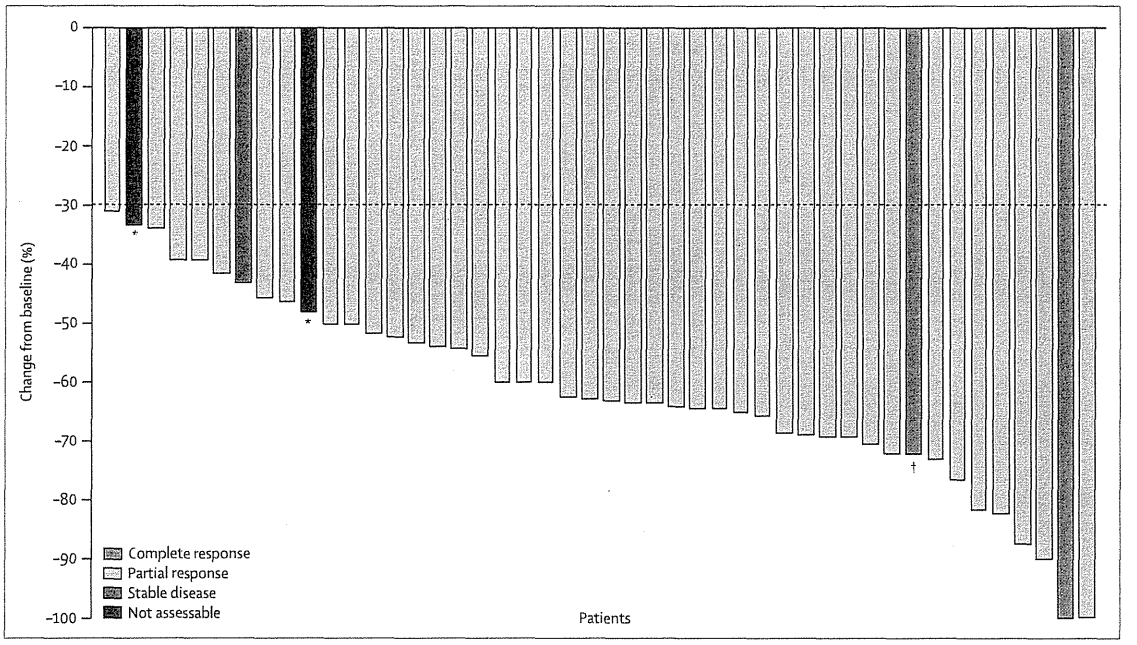

Figure 2: **Waterfall plot of best percentage change in target lesions from baseline based on independent review committee assessment (46 patients in phase 2)**
*Indeterminate response by early stopping because of safety reasons. †Classified as complete response according to the definition of Response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

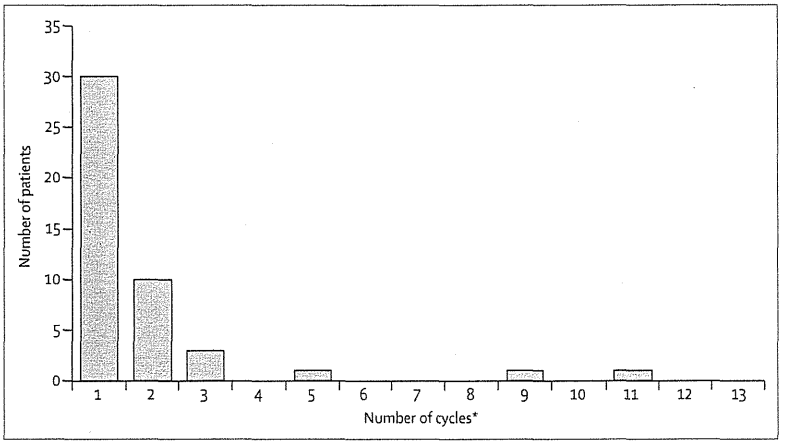

Figure 3: Number of patients who had tumour size reduction of 30% or more by treatment cycle in phase 2
*One cycle lasted 3 weeks.

	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)

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.[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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Randomized phase II trial of uracil/tegafur and cisplatin versus vinorelbine and cisplatin with concurrent thoracic radiotherapy for locally advanced unresectable stage III non-small-cell lung cancer: NJLCG 0601

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ABSTRACT

Introduction: The optimal chemotherapy with thoracic radiotherapy (TRT) for locally advanced non-small-cell lung cancer (NSCLC) remains to be established. This randomized phase II study of concurrent chemoradiotherapy was conducted to compare uracil/tegafur (UFT) and cisplatin with vinorelbine and cisplatin for stage III NSCLC.

Patients and methods: Patients with unresectable stage III NSCLC were randomized to receive UP (400 mg/m^2 UFT on days 1–14 and 29–42 and 80 mg/m^2 cisplatin on days 8 and 36) or NP (20 mg/m^2 vinorelbine on days 1, 8, 29, and 36 and 80 mg/m^2 cisplatin on days 1 and 29). TRT began on day 1 (total 60 Gy in 30 fractions).

Results: Of 70 enrolled patients, 66 were evaluable for efficacy and safety. The overall response rates were 80% (95% CI: 67–93%) and 71% (95% CI: 55–87%) for the UP arm and the NP arm. With a median follow-up of 20.2 months, the progression-free survival and median survival time were 8.8 and 26.9 months in the UP arm, and 6.8 and 21.7 months in the NP arm. The 2-/3-year survival rates were 51.0/34.3% and 46.9/33.4% for the UP arm and the NP arm, respectively. Grade 3/4 neutropenia occurred in 20% and 58% of patients in the UP and NP arms, respectively.

Conclusion: Combined with concurrent TRT, the UP arm achieved better efficacy and safety compared with the NP arm, suggesting it to be a promising candidate as a standard regimen for locally advanced NSCLC. Further evaluation of the UP arm is warranted.

1. Introduction

Lung cancer remains the leading cause of death related to cancer worldwide [1]. Non-small-cell lung cancer (NSCLC) accounts for 80% of lung cancer cases, and approximately 30% of NSCLC patients present with locally advanced disease [2].

In the 1980s, US cooperative groups showed that cisplatin-based, second-generation chemotherapy followed by thoracic radiotherapy (TRT) for stage III NSCLC improved median survival time (MST) and 5-year survival compared with TRT alone [3,4]. In the 1990s, two randomized studies that compared concurrent versus sequential cisplatin-based, second-generation chemoradiotherapy demonstrated that the concurrent approach provided superior survival outcome, although it was also associated with greater toxicity [5,6]. However, even in a meta-analysis, the superiority of concurrent chemoradiotherapy to sequential one was reported [7]. Thus, concurrent chemoradiotherapy is regarded as the standard treatment for locally advanced NSCLC.

During the last decade, platinum-based third-generation chemotherapies, such as paclitaxel, vinorelbine, gemcitabine,

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