creatinine level of $\geqslant 1.5\,mg\,dl^{-1}$, appetite loss, diarrhoea, mucositis, nausea/vomiting of grade 2 or worse despite appropriate antiemetic therapy, and/or other grade 2 non-haematological toxicities other than body weight loss, alopecia, or hyponatraemia, the daily dose of S-1 was reduced from 120 to 100 mg, 100 to 80 mg, or 80 to 50 mg in the next cycle. If the patients experienced the above-mentioned toxicities after the dose reduction, then their daily dose of S-1 was reduced from 100 to 80 mg, or 80 to 50 mg. If a patient with a BSA of $<1.25\,m^2$ experienced the above toxicities at 50 mg, then the S-1 chemotherapy was terminated. If the adjuvant chemotherapy of DOC + CDDP was terminated after one or two cycles, then a shift to S-1 chemotherapy was allowed. However, these patients were not considered to have completed the protocol treatment.

**Safety assessment and follow-up.** For the toxicity assessment, blood samples were obtained before the start of each cycle. A chest X-ray examination was performed monthly throughout the study period. Toxicities were graded according to the Common Terminology Criteria for Adverse Events (CTCAE), version 3.0. A CT examination of the chest was performed at 1, 2, 3, and 5 years after the initiation of the protocol treatment.

**Study design and statistical analysis.** This trial was designed as a multicentre, prospective, single-arm, feasibility study and the study protocol was approved by the institutional review board of each participating institution. All the study data were managed by the TORG0809 data centre at Kitasato University Research Center for Clinical Pharmacology.

The primary end point of this study was feasibility, which was defined as the proportion of patients who had completed eight or more cycles of S-1 chemotherapy. If the lower 95% CI of this proportion was 50% or more, then the treatment was considered as feasible. If a patient received 75% or more of S-1 in a cycle, that is, 21 times per cycle, this patient was considered to have completed the treatment cycle. If 72 out of 120 patients (60%) completed the protocol treatment, then the 95% CI of the proportion of the treatment completion was 51.2–68.8%. Considering the possibility of ineligible patients, the sample size was set at 125 patients.

The secondary end points included adverse events, OS, RFS, and recurrence pattern. Because of the short follow-up period, we will report the OS and RFS data elsewhere. We plan to analyse the OS and RFS at 5 years after the last enrolment, as described in the study protocol. The statistical analysis was performed using SAS version 9.2 (SAS Institute, Cary, NC, USA).

This study was registered with the UMIN Clinical Trials Registry (number UMIN000001779).

## RESULTS

**Patient population.** A total of 131 patients were enrolled in this study between January 2009 and November 2010 from 20 institutions in Japan. One patient did not receive any protocol treatment at the patient's request. Another patient was enrolled as p-stage IIIA according to the UICC 7th edition; however, the p-stage corresponded to IIIB according to the UICC 5th edition, making this patient ineligible. This patient received three cycles of docetaxel plus cisplatin and two cycles of S-1 chemotherapy, and she was included in the safety analysis. A total of 129 patients were eligible (Figure 2). The patient characteristics are listed in Table 1. Sixty-four percent of the patients were male; the median age was 63 years. Seventy-eight percent of the patients had an adenocarcinoma histology.

**Treatment delivery and protocol compliance.** Overall, 114 patients received two cycles or more of DOC + CDDP. Of these, 67 patients (58.8%) required a dose reduction of DOC or CDDP. The most common reason for the dose reduction of DOC and CDDP was grade 4 neutropaenia ($n = 63$), followed by a fever of 38.0 °C or higher ($n = 16$). The dose of CDDP was reduced because of anorexia, nausea, and/or vomiting of grade 2 or worse for more than a week ($n = 16$) and an elevated serum creatinine level of $1.5\,mg\,dl^{-1}$ or more ($n = 6$).

In total, 109 patients (84.5%) completed three cycles of adjuvant chemotherapy consisting of DOC + CDDP (Table 2). The main reasons for the discontinuation of the adjuvant chemotherapy were toxicity ($n = 15$) and patient refusal because of toxicity ($n = 7$) (Table 3). One patient terminated the DOC + CDDP treatment after one cycle and completed eight cycles of S-1 chemotherapy. Another patient terminated the DOC + CDDP treatment after two cycles and received three cycles of S-1 chemotherapy.

One hundred and eight patients received S-1 chemotherapy. Of these, 34 patients (31.5%) required the interruption of S-1 during a treatment cycle. Thirty-one patients (28.7%) required a dose reduction of S-1. The majority of the reasons for the interruption

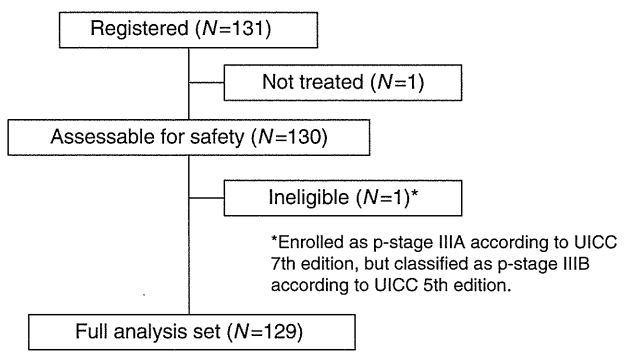


Figure 2. **CONSORT diagram.**

Table 1. Patient characteristics of 129 eligible patients

| Characteristic | Number of patients |
|---|---|
| **Sex** | |
| Male | 83 |
| Female | 46 |
| **Age (years)** | |
| Median | 63 |
| Range | 23–74 |
| **PS status** | |
| 0 | 107 |
| 1 | 22 |
| **Pathological stage[a]** | |
| IIA | 17 |
| IIB | 32 |
| IIIA | 80 |
| **Histological type** | |
| Adenocarcinoma | 100 |
| Squamous cell carcinoma | 25 |
| Others | 4 |

Abbreviations: PS = performance status; TNM = tumour-node-metastasis.
[a]Pathological stage was based on the Union Internationale Contre le Cancer fifth TNM edition.

or dose reduction of S-1 were appetite loss, diarrhoea, mucositis, or nausea/vomiting of grade 2 or worse ($n = 27$), followed by other non-haematologic toxicities of grade 2 or worse ($n = 20$).

One hundred and six patients (82.2%) completed three cycles of DOC + CDDP and subsequently switched to S-1 chemotherapy. Of these, 31 patients terminated the S-1 chemotherapy after receiving 3 or fewer cycles. A total of 66 patients (51.2%; 95% CI, 42.5–59.8%) completed 8 cycles or more of S-1 treatment (Table 2). The lower limit of the 95% CI for the completion rate was 42.5%, which was less than our previously defined criterion for treatment feasibility. The reasons for the discontinuation of the S-1 chemotherapy included toxicity ($n = 17$), patient refusal because of toxicity ($n = 15$), and recurrence ($n = 6$) (Table 3).

**Safety and toxicity.** The most common grade 3 or 4 toxicity experienced during the DOC + CDDP treatment was neutropaenia (78.5%) (Table 4). Ten patients (7.7%) developed febrile neutropaenia; however, all these patients recovered after receiving appropriate antibiotic therapy. Two patients experienced grade 3 or 4 allergic reactions to DOC during the first cycle, resulting in treatment termination.

Grade 3 or 4 toxicities during the S-1 chemotherapy included anaemia (7.3%), neutropaenia (3.7%), anorexia (3.7%), dyspnoea (1.8%), and infection with neutropaenia of grade 0–2 (1.8%). Febrile neutropaenia was not observed. One treatment-related death occurred during the study. This patient was a 63-year-old man. After two cycles of S-1 chemotherapy, he developed grade 3 fatigue. On day 36 of the second cycle of S-1, grade 3 dyspnoea was observed, and his $SpO_2$ was 92% in room air. A CT scan of the chest revealed bilateral diffuse ground-glass opacities. Prednisolone (80 mg day$^{-1}$; 1 mg kg$^{-1}$ per day) was administered, and an improvement in the opacities was observed. The prednisolone was tapered to 30 mg day$^{-1}$ for 6 weeks; however, multiple cavity lesions were visible on a chest CT image obtained 2 months after the initiation of the steroid therapy. Multiple abscesses at the neck, axilla, chest, and femur were noted, and the patient developed hypotension. *Nocardia* was isolated in blood and abscess samples, with a diagnosis of disseminated nocardiosis. Sulfamethoxazole/trimethoprim and antibiotics were administered and artificial ventilation therapy was performed. The patient was taken off the respirator once, but the pneumonitis recurred and disseminated intravascular coagulation also developed, leading to death.

Table 2. Treatment delivery in 129 eligible patients

| Treatment | Cycle | Number of patients | % | 95% Confidence interval |
|---|---|---|---|---|
| Docetaxel + cisplatin | 1 | 129 | 100 | |
| | 2 | 114 | 88.4 | |
| | 3 | 109 | 84.5 | |
| Maintenance chemotherapy using S-1 | 1 | 106 | 82.2 | |
| | 2 | 97 | 75.2 | |
| | 3 | 86 | 66.7 | |
| | 4 | 75 | 58.1 | |
| | 5 | 73 | 56.6 | |
| | 6 | 72 | 55.8 | |
| | 7 | 71 | 55 | |
| | 8 | 67 | 51.9 | |
| Completion | | 66 | 51.2 | 42.5–59.8 |

Table 3. Reason for discontinuation of the treatment

| Reasons | Docetaxel + cisplatin | Maintenance chemotherapy using S-1 |
|---|---|---|
| Recurrence | 1 | 6 |
| Toxicity | 15 | 17 |
| Patient refusal because of toxicity | 7 | 15 |
| Others | 0 | 2 |

## DISCUSSION

This feasibility study was designed to evaluate the tolerability, safety, and efficacy of single agent long-term administration of S-1 chemotherapy following three cycles of DOC plus CDDP in patients with completely resected stage II or IIIA NSCLC. Fifty-one percent of the patients (95% CI, 42.5–59.8%) completed three cycles of DOC plus CDDP and eight cycles or more of S-1 chemotherapy. The lower limit of the CI for this proportion was lower than the predefined criterion of 50%. Grade 3–4 haematologic toxicities were observed in 7.3% of patients, while grade 3–4 non-haematologic toxicities were observed in only 4%. However, grade 1–2 anorexia and/or fatigue were common, with rates of ~50–60%. S-1 was administered for 2 weeks with a 1-week rest. The long duration of S-1 administration might have been responsible for the low-grade but extended non-haematologic toxicities and might have been too intensive for patients especially after platinum-doublet chemotherapy. In a previous phase III study of adjuvant chemotherapy for gastric cancer with single agent of S-1, 78% of patients received S-1 for at least 6 months (Sakuramoto *et al*, 2007). Adjuvant chemotherapy of DOC + CDDP probably affected the compliance of S-1 chemotherapy negatively in our study. A modification of the treatment schedule for S-1 chemotherapy, such as a 2-week rest period rather than a 1-week rest period, might improve treatment compliance.

Efficacious treatment for advanced stage disease has been introduced and investigated in an adjuvant setting, such as bevacizumab plus platinum-doublet chemotherapy in patients with non-squamous cell carcinoma or erlotinib in patients with a mutated epidermal growth factor receptor gene. Recent phase III trials have demonstrated that switch maintenance chemotherapy consisting of pemetrexed or erlotinib, which were efficacious for second-line chemotherapy, prolonged the OS in patients with advanced NSCLC (Ciuleanu *et al*, 2009; Cappuzzo *et al*, 2010). Switch maintenance chemotherapy can be recognised as an early second-line chemotherapy. The purpose of adjuvant chemotherapy is to control micrometastasis and to prevent recurrence. Switch maintenance chemotherapy is considered to enhance the efficacy of adjuvant chemotherapy. Previous phase II trials have demonstrated that S-1 monotherapy produced a response rate of 7–14%, a median progression-free survival (PFS) of 3–4 months, and a median OS of 7–16 months as a second-line treatment for advanced NSCLC (Totani *et al*, 2009; Govindan *et al*, 2011; Shiroyama *et al*, 2011). Pemetrexed is effective against non-squamous NSCLC; on the other hand, S-1 is effective against both non-squamous and squamous NSCLC. A randomised trial comparing S-1 and docetaxel as a second- or third-line chemotherapy is now underway in Asia. Switch maintenance chemotherapy using S-1 is also being evaluated as a first-line chemotherapy for patients with advanced NSCLC in a phase II study (UMIN000003676). If promising RFS or OS data in this trial are obtained, then a prospective randomised trial will be warranted to compare adjuvant chemotherapy with or without single agent long-term administration of S-1 chemotherapy.

Table 4. Toxicity

| | Docetaxel + cisplatin ($n = 130$) | | | | | Maintenance chemotherapy using S-1 ($n = 109$) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Toxicity** | **Toxicity grade** | | | | | **Toxicity grade** | | | | | |
| | 1 | 2 | 3 | 4 | %3–4 | 1 | 2 | 3 | 4 | 5 | %3–5 |
| **Haematologic** | | | | | | | | | | | |
| Neutropaenia | 4 | 14 | 39 | 63 | 78.5 | 20 | 18 | 4 | 0 | 0 | 3.7 |
| Anaemia | 52 | 31 | 1 | 0 | 0.8 | 26 | 38 | 6 | 2 | 0 | 7.3 |
| Thrombocytopaenia | 30 | 6 | 0 | 0 | 0 | 35 | 0 | 0 | 0 | 0 | 0 |
| **Gastrointestinal** | | | | | | | | | | | |
| Anorexia | 55 | 47 | 22 | 0 | 16.9 | 43 | 21 | 4 | 0 | 0 | 3.7 |
| Vomiting | 23 | 20 | 5 | 0 | 3.8 | 11 | 5 | 1 | 0 | 0 | 0.9 |
| Diarrhoea | 35 | 11 | 15 | 0 | 11.5 | 19 | 3 | 1 | 0 | 0 | 0.9 |
| Mucositis | 12 | 4 | 0 | 0 | 0 | 23 | 7 | 0 | 0 | 0 | 0 |
| **Hepatic** | | | | | | | | | | | |
| AST | 14 | 5 | 2 | 0 | 1.5 | 25 | 5 | 0 | 0 | 0 | 0 |
| ALT | 25 | 9 | 1 | 0 | 0.8 | 24 | 4 | 0 | 0 | 0 | 0 |
| **Renal** | | | | | | | | | | | |
| Creatinine | 39 | 9 | 0 | 0 | 0 | 30 | 8 | 0 | 0 | 0 | 0 |
| **Neurologic** | | | | | | | | | | | |
| Neuropathy (sensory) | 9 | 4 | 0 | 0 | 0 | 19 | 2 | 2 | 0 | 0 | 1.8 |
| **Others** | | | | | | | | | | | |
| Hyponatraemia | 57 | — | 18 | 5 | 17.7 | 16 | — | 0 | 0 | 0 | 0 |
| Fatigue | 57 | 21 | 5 | 0 | 3.8 | 41 | 9 | 2 | 0 | 0 | 1.8 |
| Allergic reaction | 7 | 0 | 1 | 1 | 1.5 | 1 | 0 | 0 | 0 | 0 | 0 |
| Dehydration | 0 | 0 | 2 | 0 | 1.5 | 0 | 0 | 0 | 0 | 0 | 0 |
| Alopecia | 68 | 29 | 0 | 0 | 0 | 35 | 10 | 0 | 0 | 0 | 0 |
| Febrile neutropaenia | — | — | 10 | 0 | 7.7 | — | — | 0 | 0 | 0 | 0 |
| Infection with G3–4 neutropaenia | 0 | 3 | 5 | 0 | 3.8 | 0 | 0 | 0 | 0 | 0 | 0 |
| Infection with G0–2 neutropaenia | 0 | 3 | 2 | 1 | 2.3 | 1 | 3 | 1 | 0 | 1 | 1.8 |
| Pneumonitis | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0.9 |
| Dyspnoea | 0 | 1 | 0 | 0 | 0 | 8 | 2 | 2 | 0 | 0 | 1.8 |

Abbreviations: ALT = alanine aminotransferase; AST = aspartate aminotransferase; G = grade.

A recent phase III trial has also demonstrated that continuation maintenance chemotherapy consisting of pemetrexed prolonged the OS and PFS in patients with advanced non-squamous NSCLC. However, concurrent chemoradiotherapy consisting of pemetrexed plus CDDP followed by four cycles of pemetrexed did not improve OS over concurrent chemoradiotherapy consisting of etoposide plus CDDP in patients with stage III non-squamous NSCLC (PROCLAIM study). Up to four cycles of pemetrexed in the PROCLAIM study, comparable to S-1 chemotherapy in our study, might be unable to enhance curative treatment effect. We might have to distinguish strategy for stage IV disease from that for curative situations in completely resected stage II/III disease.

Combination chemotherapy consisting of DOC plus CDDP is a standard regimen for the treatment of patients with advanced NSCLC. A randomised trial demonstrated that DOC + CDDP resulted in a more favourable response rate and OS than vinorelbine (VNR) plus CDDP in chemo-naïve patients with advanced NSCLC. The median OS period was 11.3 months for patients treated with DOC plus CDDP and 10.1 months for patients treated with VNR plus CDDP. The hazard ratio was 1.183 (97.2% CI, 0.989–1.416) (Fossella *et al*, 2003). A higher incidence of grade 3–4 anaemia, nausea, and vomiting was observed in VNR + CDDP arm, compared with DOC + CDDP arm. Febrile neutropaenia occurred in <5% of patients in both regimens. Furthermore, the single agent DOC had a more favourable OS period than the single agent VNR in both first-line and second-line settings in patients with advanced NSCLC (Fossella *et al*, 2000). TORG0503 study demonstrated that >90% of patients completed three planned cycles of adjuvant chemotherapy in both DOC + CDDP and PTX + CBDCA arms. On the other hand, the most common regimen for adjuvant chemotherapy for pathological stage II or III NSCLC is VNR + CDDP, because most randomised trials, which resulted in positive results, adopted VNR + CDDP. Considering the promising results of clinical trials for advanced NSCLC, it might be reasonable to select DOC + CDDP as an adjuvant chemotherapy in patients with completely resected stage II or III NSCLC. Indeed, DOC + CDDP has been selected as one of the standard adjuvant chemotherapy regimens in ECOG1505 study, which is a randomised phase III trial of adjuvant chemotherapy with or without bevacizumab in patients with completed resected early-stage NSCLC (Wakelee *et al*, 2011). However, 7.7% of patients experienced grade 3 febrile neutropaenia

during the chemotherapy of DOC + CDDP in our study. Relatively high incidence of febrile neutropaenia could not support the use of adjuvant chemotherapy with DOC + CDDP as a new alternative. Four cycles of VNR + CDDP followed by long-term administration of S-1 might be a better strategy in a future study.

The treatment cycle for DOC plus CDDP was set at three because the actual median numbers of cycles delivered in previous phase III studies of adjuvant chemotherapy were three or four (Winton *et al*, 2005; Douillard *et al*, 2006), and a randomised study demonstrated that four cycles or more of platinum-based chemotherapy did not improve the OS in patients with advanced NSCLC (Smith *et al*, 2001). In the TORG0503 study, the number of treatment cycles for DOC plus CDDP or for PTX plus CBDCA as an adjuvant chemotherapy was also set at three, and a favourable 2-year RFS rate was observed (Ohira *et al*, 2011).

A previous randomised phase II study demonstrated that adjuvant chemotherapy with pemetrexed plus CDDP was safe and feasible with less toxicity and superior dose delivery compared with VNR + CDDP (Kreuter *et al*, 2013). Pemetrexed plus CDDP is considered as suitable for adjuvant chemotherapy because of relatively less toxic and promising antitumour activity in patients with non-squamous NSCLC. A randomised phase III study is underway comparing pemetrexed plus CDDP and VNR + CDDP in patients with completely resected stage II–IIIA non-squamous NSCLC in Japan. However, it is difficult to conduct a randomised phase III study of adjuvant chemotherapy in patients with NSCLC, because large sample size and long-term follow-up are needed. Therefore, a randomised phase II study containing control arm should be taken into consideration to select appropriate experimental treatment.

Aprepitant, a standard antiemetic drug for cisplatin therapy, was approved in December 2009 in Japan. As a result, ~20 patients did not receive aprepitant. If aprepitant had been available for all the enrolled patients, then the treatment compliance might have improved. Furthermore, 2 out of the 129 patients experienced grade 3 or 4 allergic reactions to DOC during the first cycle, resulting in treatment termination. Premedication for DOC + CDDP included dexamethasone only on day 1 in this study. The administration of dexamethasone on the day before the initiation of DOC + CDDP and an antihistamine on day 1 might be recommended in future clinical trials to prevent anaphylaxis in response to DOC.

In conclusion, the toxicity level of S-1 chemotherapy was acceptable, although the treatment completion rate did not meet our criterion for feasibility. A modification of the treatment schedule for S-1 chemotherapy, such as a 2-week rest period rather than a 1-week rest period, might improve treatment compliance. After referring to the results for OS and RFS, we would like to plan a randomised trial to investigate whether platinum-based chemotherapy followed by single agent long-term administration of S-1 chemotherapy improves survival in patients with completely resected stage II or III NSCLC.

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Case report

# An extremely rare case of small-cell lung cancer harboring variant 2 of the *EML4-ALK* fusion gene

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ABSTRACT

*Anaplastic lymphoma kinase* (*ALK*) fuses *echinoderm microtubule-associated protein-like 4* (*EML4*) to acquire a transforming activity in lung adenocarcinomas. However, the presence of an *EML4-ALK* fusion gene in other lung cancer histologies is an extremely rare phenomenon. A 43-year-old female was referred to our department due to dyspnea on effort and left back pain. Computed tomography (CT) showed a large mass in the upper lobe of the left lung and a massive left pleural effusion, while a CT-guided needle biopsy confirmed a diagnosis of small-cell lung cancer (SCLC). Surprisingly, the tumor was genetically considered to harbor the *EML4-ALK* fusion gene (variant 2). Although the patient underwent two regimens of cytotoxic chemotherapy for SCLC, she died approximately seven months after the administration of first-line chemotherapy. Our analysis of 30 consecutive patients with SCLC for *EML4-ALK* revealed that two patients, including the current patient and a patient we previously reported, harbored the *EML4-ALK* fusion gene.



## 1. Introduction

Oncogenic driver mutations, such as *epidermal growth factor receptor* (*EGFR*), *anaplastic lymphoma kinase* (*ALK*) and so on, have been shown to play essential roles in tumorigenesis, survival and proliferation in lung cancer, especially adenocarcinoma [1,2]. Driver mutations have attracted attention as potential targets of kinase inhibitors [2,3]. In addition to the molecular pathogenesis of lung adenocarcinomas, genetic insights into the pathogenesis of squamous cell carcinoma and small-cell lung cancer (SCLC) have recently been reported [4,5]. However, to the best of our knowledge, there is only one case of *echinoderm microtubule-associated protein-like 4* (*EML4*)-*ALK*-positive SCLC combined with adenocarcinoma, which we previously reported [6]. We herein report a genetically rare case of SCLC harboring an *EML4-ALK* fusion gene and describe the patient's clinical course.

## 2. Case report

A 43-year-old female ex-smoker of five pack-years was referred to our hospital due to dyspnea on effort and left back pain. A chest X-ray showed a large mass shadow in the left upper lung field and decreased transparency in the left lower lung field. Computed tomography (CT) revealed a large, irregular mass with a maximum diameter of 10 cm in the left upper lobe invading the 4th rib (Fig. 1A) and a massive left pleural effusion. Laboratory examinations revealed elevations in the levels of neuron specific enolase (NSE; 37.7 ng/ml) and pro-gastrin-releasing peptide (ProGRP; 1740 ng/ml), whereas no abnormalities were observed in other tumor markers. A CT-guided tumor biopsy was then performed, and the tumor was pathologically diagnosed as small-cell lung cancer (SCLC) with immunoreactivity to synaptophysin and CD56 (Fig. 2A and B), while no immunoreactivity against thyroid transcription factor-1 (TTF-1) was observed (Fig. 2C). The clinical stage was ultimately determined to be IV (cT3N0M1a: extensive disease). Multiplex reverse transcription-polymerase chain reaction (RT-PCR) and direct sequencing methods revealed the tumor to harbor variant 2 of the *EML4ALK* fusion gene (Fig. 2D), whereas no mutations of *epidermal growth factor receptor* (*EGFR*) or *TP53* were observed (data not shown). As the performance status of the patient

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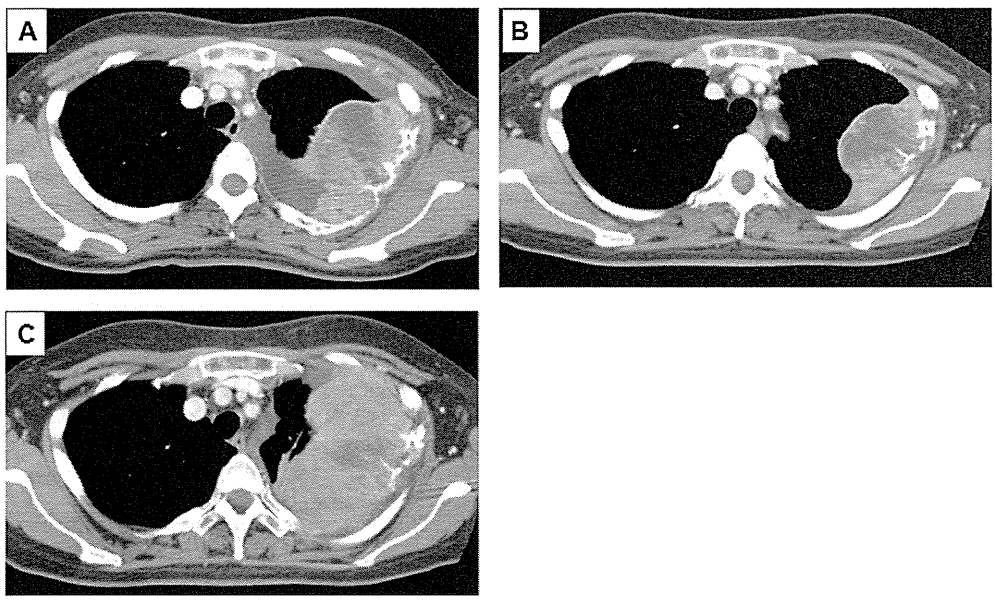


**Fig. 1.** Computed tomography showed a large mass invading the left 4th rib. (A) CT showed the mass approximately 2.5 (B) and four months (C) after the administration of first-line chemotherapy.

was 3, carboplatin (CBDCA) in combination with etoposide (VP-16) was administered as a first-line regimen with daily thoracocentesis of the pleural effusion. Since the PS improved from 3 to 0 following the administration of one cycle of CBDCA + VP-16, the patient underwent three cycles of cisplatin (CDDP) + VP-16. Although a partial response (PR) was achieved (Fig. 1B) and the levels of NSE and ProGRP decreased (9.9 and 409 ng/ml, respectively) after four cycles of chemotherapy, progressive disease was observed 1.5 months

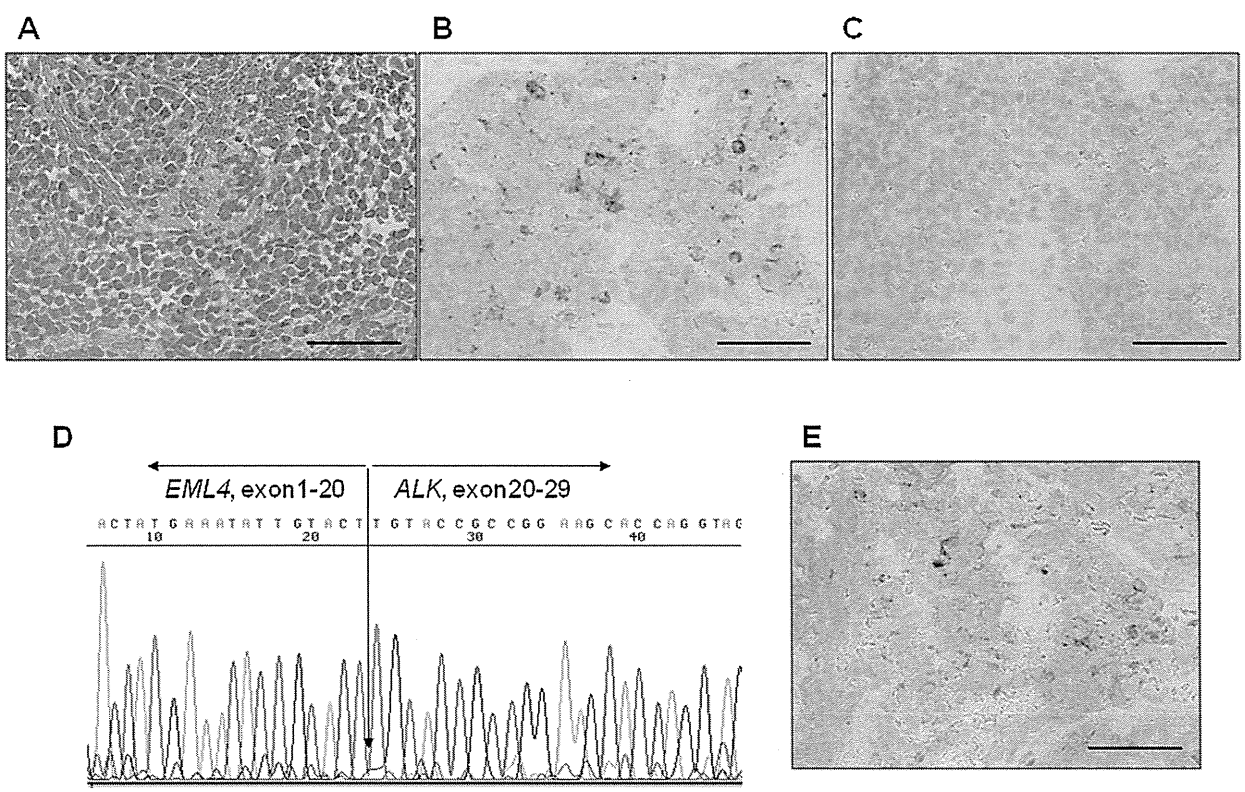


**Fig. 2.** (A) Microscopic findings of the tumor indicated small, round cells with abundant chromatin. (B) Immunohistochemistry using a specific antibody against synaptophysin (27G12, Novocastra) showed the tumor to be positively stained. (C) Immunohistochemistry using an antibody with specificity for thyroid transcription factor-1 (TTF-1; 8G7G3/1, Dako) showed that the tumor did not have immunoreactivity for TTF-1. (D) The direct sequencing method identified variant 2 of the *echinoderm microtubule-associated protein-like 4-anaplastic lymphoma kinase* (*ALK*) fusion gene. (E) Immunostaining using an antibody that specifically detects ALK (5A4, Nichirei) revealed immunopositivity of the tumor for ALK. Scale bar (A–C, E): 50 μm.

after the confirmation of a PR (Fig. 1C). Thereafter, a CT-guided biopsy was performed again, and the SCLC histology was reconfirmed. Furthermore, the presence of the *EML4-ALK* fusion gene was confirmed on immunohistochemistry (IHC) using an antibody that specifically detects ALK (Fig. 2E). Although amrubicin was then administered, the disease continued to progress. Approximately six months after the administration of the first-line chemotherapy, the patient was transferred to another hospital for hospice care and died 18 days after the transfer. Based on her clinical course, the progression-free survival (PFS) and overall survival (OS) from the administration of the first-line therapy were approximately four and seven months, respectively.

## 3. Discussion

Gene mutations in tyrosine kinases play essential roles in the pathogenesis of lung adenocarcinoma and have attracted much attention as potential therapeutic targets in the treatment of adenocarcinoma. The *ALK* gene has been shown to fuse the *EML4* gene, and as a consequence, to possess a transforming activity [1]. Importantly, tumors with the *EML4-ALK* fusion gene, the second most well-known tyrosine kinase in lung adenocarcinoma, can be successfully treated with ALK inhibitors [7]. Mutations of the *EGFR* gene in SCLC have already been identified (5/122: 4%) [8], and integrative genomic analyses have revealed mutations of tumor suppressor genes (TP53 and RB1), histone modifiers (MLL1) and so on in SCLC. However, to the best of our knowledge, there has been only one case of an SCLC patient harboring the *EML4-ALK* fusion gene [6]. In our previous case, fusion of the *ALK* gene to the *EML4* gene was intriguingly detected only in the SCLC component of the resected combined adenocarcinoma with SCLC. Although this previous patient harbored variant 1 of the *EML4-ALK* fusion gene, variant 2 of the fusion gene was identified in the current case. Based on these findings, there are considered to be multiple *EML4-ALK* variants in SCLC patients as well as adenocarcinoma patients. We analyzed 30 consecutive SCLC patients whose RNAs were available for RT-PCR and direct sequencing methods between April 2010 and March 2012. Two of the patients, the present patient and the patient we previously reported [6], were found to harbor the fusion gene. Although a positive reaction of IHC for the ALK protein expression without *ALK* fusion was reported to be found in a patient with SCLC [9], this does not apply to the current case because the fusion was detected using RT-PCR and direct sequencing methods. Furthermore, the possibility of the transformation of adenocarcinoma into SCLC, which is associated with the acquisition of resistance to EGFR-tyrosine kinase inhibitors (TKIs), should be taken into consideration [10]. However, this mechanism does not apply to the present patient, since no EGFR-TKIs were administered because of the absence of the *EGFR* mutations.

One of the limitations of the current case report is that the tumor was diagnosed to be SCLC by a biopsy sample. Although biopsy samples do not always reflect the exact histology of the whole tumor, and the absence of lymphadenopathy and *p53* mutations, which occur in more than 90% of all SCLCs [11], is relatively rare, the SCLC histology was confirmed by several findings in the present case. First, a CT-guided biopsy before and after the first-line chemotherapy diagnosed the tumor to be morphologically SCLC. Second, immunoreactivity of the tumor for synaptophysin and CD56 was observed. Third, the levels of tumor markers associated with SCLC, i.e., NSE and ProGRP, were elevated, while no elevation was observed in the levels of carcinoembryonic antigen and cytokeratin 19 fragment. Finally, combination chemotherapy with platinum plus VP-16, one of the standard regimens for patients with SCLC, led to a partial response. With regard to TTF-1 expression, TTF-1 was reported to be expressed in all adenocarcinomas harboring the *ALK* rearrangement [12], and TTF-1 expression was also observed in about 80% of SCLCs [13]; however, the current patient showed no expression of TTF-1, as shown in Fig. 2C, which was different from the results we previously reported in Ref. [6], and no definite correlation between TTF-1 expression and the *EML4-ALK* rearrangement in SCLC has been demonstrated so far. Although these findings show an apparently rare presentation of SCLC in the current patient, future studies would help to elucidate the characteristics of patients with SCLC harboring the *EML4-ALK* rearrangement.

Although SCLC manifests with aggressive features, such as rapid progression, these tumors are generally sensitive to chemotherapy. For first-line therapy, the response rate, median PFS and OS range from 67.5 to 84.4%, 4.7–6.9 months and 9.4–12.8 months, respectively [14,15]. Although the current patient achieved a PR after undergoing four cycles of platinum-based chemotherapy, the PFS and OS were much worse than those of historical controls. As a reason for the poor clinical course of the present patient, there is a possibility that the fusion gene affects sensitivity to chemotherapy. There have been two reports on chemosensitivity in patients with the *EML4-ALK* fusion gene [16,17]. Lee et al. reported that *ALK*-positive non-SCLC patients would benefit significantly from pemetrexed chemotherapy, whereas Takeda et al. demonstrated that the efficacy of first-line platinum-based chemotherapy does not depend on the presence or absence of the *EML4-ALK* fusion gene. Therefore, although the significance of *ALK*-positivity for chemosensitivity has yet to be clarified, *EML4-ALK* fusion may be involved in the sensitivity of platinum-based chemotherapy.

## 4. Conclusion

We herein reported a very rare case of SCLC in which the patient harbored variant 2 of the *EML4-ALK* fusion gene. Although the frequency and significance of the fusion gene in SCLC patients has not been determined, this phenomenon suggests that SCLC patients harboring the *EML4-ALK* fusion gene can be successfully treated with ALK inhibitors.

## Conflict of interest statement

Drs. Takenoyama, Shiraishi, Hirai, Yamaguchi, Seto and Ichionose have conflicts of interest with Pfizer, AstraZeneca and Chugai to disclose as shown in the attached file. The other authors have no conflicts of interest to declare.

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Case report

# The first case of lung carcinosarcoma harboring in-frame deletions at exon19 in the *EGFR* gene

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ABSTRACT

Mutations of the *epidermal growth factor receptor* (*EGFR*) gene play a critical role in carcinogenesis of lung cancer, particularly adenocarcinoma. However, to the best of our knowledge, no mutations of the *EGFR* in patients with lung carcinosarcoma have been identified. We herein report the case of a 61-year-old female referred for a detailed examination of a left pulmonary mass shadow. Although bronchoscopy was performed, it failed to lead to a diagnosis, and video-assisted thoracoscopic surgery was therefore carried out to diagnose the tumor. The pathology revealed biphasic features consisting of both adenocarcinoma and chondrosarcoma. Intriguingly, both the adenocarcinoma and chondrosarcoma components were proven to harbor an exon19 deletion in the *EGFR* gene. Although carcinosarcoma is a rare malignancy of the lungs, genetic analyses of oncogenic drivers, such as the *EGFR* gene, should be conducted.


## 1. Introduction

Carcinosarcoma of the lungs is a subtype of sarcomatoid carcinoma (SC) and a rare malignancy [1]. Although several reports have previously investigated genetic alterations in patients with SC [2–5], no mutations in oncogenic drivers of carcinosarcoma, including *epidermal growth factor receptor* (*EGFR*), have been identified. We herein report the case of a 61-year-old female referred for a detailed examination of a left pulmonary mass shadow. Although advanced left lung cancer with lumbar metastasis was clinically suspected, bronchoscopy failed to lead to a diagnosis, and surgery was therefore performed. The pathological examination revealed that the tumor was composed of a carcinomatous component and a sarcomatous component. Intriguingly, both components harbored the *EGFR* exon19 deletion. As far as we know, this is the first report of a patient with pulmonary carcinosarcoma harboring an exon19 deletion in the *EGFR* gene.

## 2. Case report

A 61-year-old female nonsmoker was referred to our department for a detailed examination of a persistent cough. A physical examination and laboratory tests, including measurements of the levels of tumor markers, showed no abnormalities. A chest X-ray revealed a mass shadow in the left lower lung field, and computed tomography (CT) showed a mass shadow with a maximum diameter of 3.2 cm (Fig. 1A) and a solitary lesion in the second lumbar vertebra (Fig. 1B, arrow) that was also observed on magnetic resonance imaging (Fig. 1C). Positron emission tomography/CT identified avid intake of [$^{18}$F]fluorodeoxyglucose in the left hilar lymph node (thin arrow) as well as pulmonary (thick arrow) and bone lesions (arrowhead) (Fig. 1D). Based on these findings, the patient was clinically suspected of having left lung cancer with lumbar metastasis according to the American Joint Committee on Cancer staging criteria (T2aN1M1b, Stage IV) [6]. Although bronchoscopy was performed, it failed to lead to a diagnosis, and video-assisted thoracoscopic surgery was therefore carried out to diagnose the tumor after obtaining the patient's informed consent. A solid mass with a diameter of approximately 3.2 cm was identified in the 9th segment of the left lung, although no pleural dissemination, malignant effusion or pulmonary metastases were observed.

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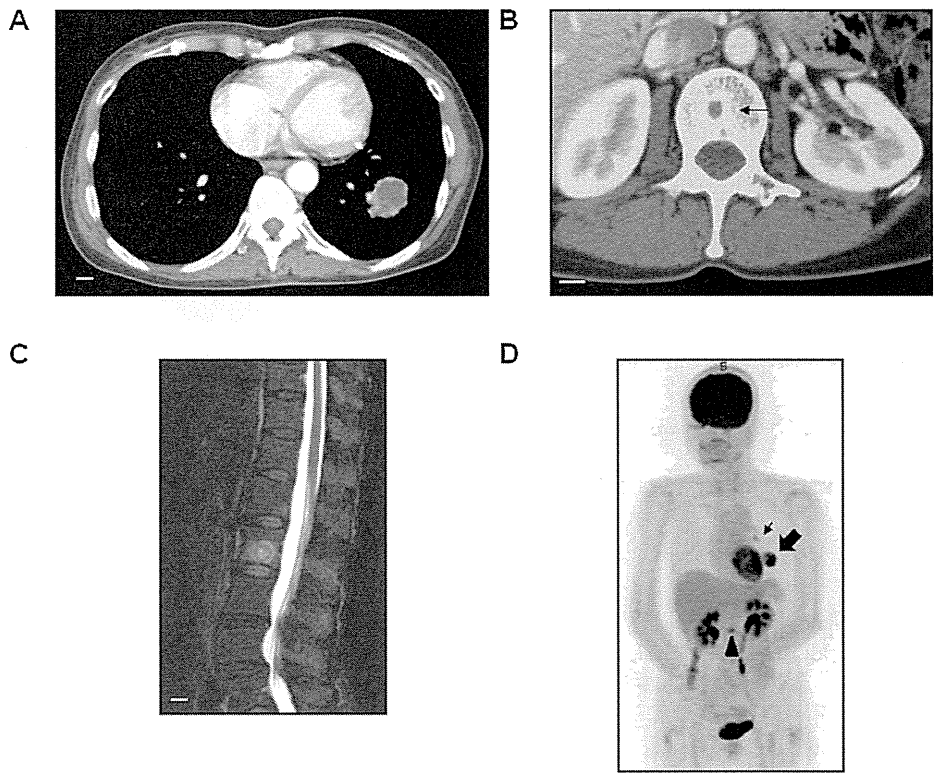


**Fig. 1.** Computed tomography (CT) showed a mass shadow in the left lower lobe (A) and a solitary lesion in the second lumbar vertebra (B, arrow) that was also observed on magnetic resonance imaging (C). Avid intake of [$^{18}$F]fluorodeoxyglucose in the left hilar lymph node (thin arrow) as well as pulmonary (thick arrow) and bone lesions (arrowhead) was identified on positron emission tomography/CT (D). Scale bar: 1.0 cm.

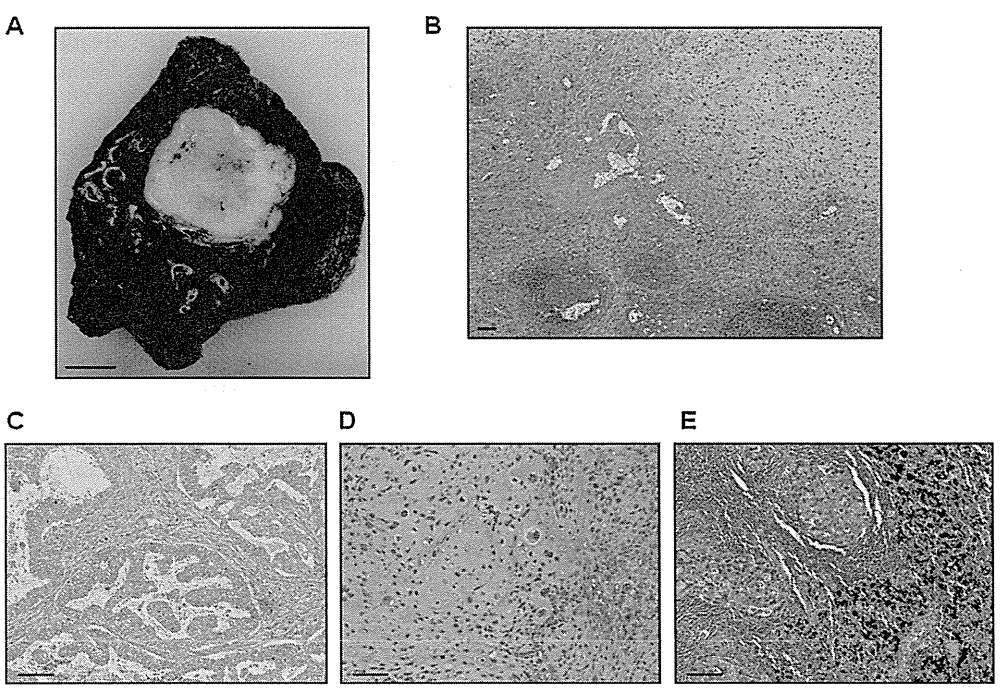


**Fig. 2.** The cut sections exhibited a gray, whitish component encircled with a yellow, whitish component (A). Microscopic findings of the tumor indicated biphasic features consisting of both adenocarcinoma (B and C) and chondrosarcoma (B and D). The #12l lymph node was metastasized by the adenocarcinoma component (D). Scale bar: 1.0 cm (A) and 100 μm (B–E).

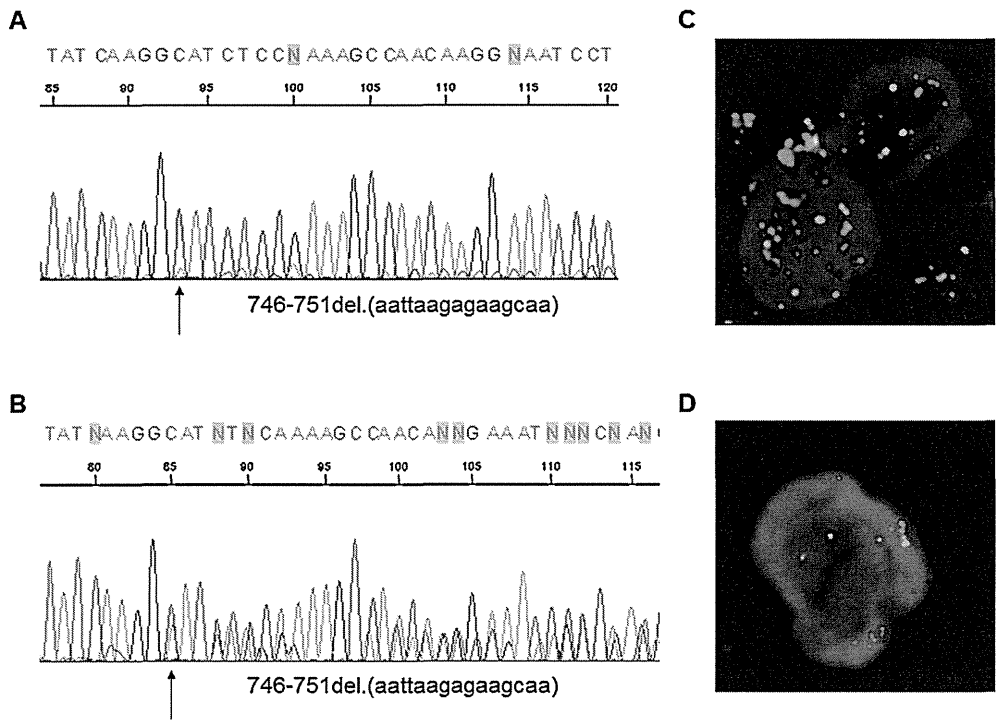


**Fig. 3.** Direct sequencing method showed that both the adenocarcinoma (A) and chondrosarcoma components (B) harbored an exon19 deletion in the *epidermal growth factor receptor* (*EGFR*) gene. Representative images of a fluorescence in situ hybridization assay showing the amplification of the *EGFR* gene in both the adenocarcinoma (C) and chondrosarcoma components (D). Orange color: *EGFR* DNA; green color: chromosome 7 control (*CEP7*) DNA.

Since the tumor was located in the proximal region of the 9th segment, it was impossible to perform partial resection, and left lower lobectomy with nodal resection was therefore completed. The cut sections exhibited a gray, whitish component encircled with a yellow, whitish component, with a maximum diameter of 3.7 cm (Fig. 2A). The pathological examination revealed that the tumor was composed of a carcinomatous component and a sarcomatous component (Fig. 2B). The carcinomatous component exhibited adenocarcinoma, papillary and acinar subtypes with focal neuroendocrine differentiation (Fig. 2C), whereas the sarcomatous component was composed of chondrosarcoma (Fig. 2D). In addition, metastasis of the adenocarcinoma component to the #12l lymph node was observed (Fig. 2E). Based on these findings, the patient was pathologically diagnosed with stage T2aN1M1b (Stage IV) lung carcinosarcoma.

Genetic analyses were performed to detect mutations in the *EGFR* gene and rearrangement of the *anaplastic lymphoma kinase* (*ALK*) gene. Microdissection was conducted to separate the two components, followed by reverse transcription-polymerase chain reaction (RT-PCR) and direct sequencing methods. Intriguingly, both the adenocarcinoma and chondrosarcoma components were proven to harbor an exon19 deletion in the *EGFR* gene (Fig. 3A: adenocarcinoma and Fig. 3B: chondrosarcoma). Furthermore, the same *EGFR* mutation was identified in the metastasized lymph node (data not shown). A fluorescence in situ hybridization assay identified the amplification of the *EGFR* gene in both components, predominantly in the adenocarcinoma component (Fig. 3C: adenocarcinoma and Fig. 3D: chondrosarcoma), which was perfectly in line with the finding that the signals of the mutant allele were more predominant than those of the wild-type in both components (Fig. 3A and B), although more so in the adenocarcinoma component than in the chondrosarcoma component. No rearrangement of the *ALK* gene was identified in either component. The patient was discharged with no complications nine days after the operation and is undergoing radiotherapy of the lumbar lesion. The patient approved this case report and the accompanying images for publication.

## 3. Discussion

Sarcomatoid carcinomas (SCs) are rare malignancies of the lungs, accounting for 1% or less of all lung tumors, and are known to behave in a more aggressive fashion than other histological subtypes of non-small cell lung cancer (NSCLC) [7,8]. The histologic subtypes of SC are divided into five groups, i.e., pleomorphic carcinoma, spindle cell carcinoma, giant cell carcinoma, carcinosarcoma and pulmonary blastoma [1]. Regarding carcinosarcomas, Koss et al. conducted a clinicopathologic analysis of a large series of 66 patients with these lesions [9]. Carcinosarcomas were identified predominantly in male smokers compared to females, and the median patient age was 65 years. The 5-year survival rate was 21.3%, which is similar to that reported by Martin et al. [8]. Additionally, the frequency of epithelial components was as follows: squamous cell carcinoma (46%), adenocarcinoma (31%) and adenosquamous carcinoma (19%). The sarcomatous components included rhabdomyosarcoma, chondrosarcoma, osteosarcoma and combinations of these elements. In the present female patient, chondrosarcoma was observed concomitantly with adenocarcinoma.

Mutations of oncogenic drivers, such as *EGFR* [10], *ALK* [11] and so on, have been identified, and molecular-targeted therapy has been proven to be effective for treating NSCLC patients with mutations in these genes [12]. As for SCs, several reports have previously investigated the *EGFR* mutation status [2–5]. Studies by Italiano and Pelosi reported that no mutations of the *EGFR* gene were observed in 22 and 23 cases of lung SC, respectively [2,3]. In contrast, there exist two reports identifying *EGFR* mutations in patients with lung SC [4,5]. Jiang et al. showed that nine of 32 patients with lung SC

harbored *EGFR* mutations. However, no patients with carcinosarcoma were included in that study. Furthermore, Leone et al. analyzed mutations of the *EGFR* gene in 23 cases of lung SC, and identified two patients who harbored *EGFR* mutations; both patients had exon19 in-frame deletions [5]. Importantly, the two patients with *EGFR* mutations cases both exhibited pleomorphic and giant cell carcinoma features (the author's reply). Accordingly, to the best of our knowledge, no mutations in the *EGFR* gene have been identified in patients with carcinosarcoma, and the current patient therefore represents the first case of lung carcinosarcoma with an *EGFR* mutation. Although SCs of the lungs are generally thought to be chemorefractory [13], the present patient would benefit more from EGFR-tyrosine kinase inhibitors, such as gefitinib and erlotinib, than cytotoxic chemotherapy. Furthermore, it may be feasible to administer gefitinib treatment because the metastatic lymph node involved in the adenocarcinoma component exhibited the *EGFR* mutation, indicating that the lumbar lesion was also assumed to be an adenocarcinoma harboring the *EGFR* mutation.

## 4. Conclusion

In summary, we herein reported the very rare case of a 61-year-old female patient with carcinosarcoma harboring an exon19 deletion in the *EGFR* gene. Although carcinosarcoma is a rare malignancy of the lungs, genetic analyses of oncogenic drivers, such as the *EGFR* gene, should be conducted.

## Conflict of interest statement

None declared.

## Acknowledgements

We thank Mrs. Yoko Takeda for performing the RT-PCR and direct sequencing methods, Mrs. Yuko Mitsuoka for conducting the immunohistochemistry and Dr. Brian T. Quinn for providing critical comments on the manuscript. Furthermore, we express special thanks to Drs. Alvaro Leone and Paolo Graziano for providing their data showing that the two patients with *EGFR* mutations in Reference 5 both exhibited pleomorphic and giant cell carcinoma features.

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ORIGINAL ARTICLE

# Phase I, dose-escalation study of AZD7762 alone and in combination with gemcitabine in Japanese patients with advanced solid tumours

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**Abstract**
*Purpose* AZD7762, a potent Chk1/Chk2 inhibitor, has shown chemosensitizing activity with gemcitabine in xenograft models.
*Methods* This open-label, Phase I, dose-escalation study evaluated the safety, pharmacokinetics (PK) and preliminary efficacy (RECIST) of AZD7762 alone and in combination with gemcitabine in Japanese patients with advanced solid tumours (NCT00937664). Patients received intravenous AZD7762 alone on days 1 and 8 of a 14-day cycle (cycle 0), followed by AZD7762 plus gemcitabine 1,000 mg/m$^2$ on days 1 and 8 of 22-day cycles, in ascending AZD7762 dose cohorts.
*Results* Twenty patients received AZD7762 at doses of 6 mg ($n = 3$), 9 mg ($n = 3$), 21 mg ($n = 6$) and 30 mg ($n = 8$). Dose-limiting toxicities occurred in 2/6 evaluable patients in the 30-mg cohort: one, CTCAE grade 3 elevated troponin T (cycle 0: AZD7762 monotherapy); one, neutropenia, thrombocytopenia, and elevated aspartate aminotransferase and alanine aminotransferase (cycle 1: combination therapy). The 30 mg dose was therefore regarded as non-tolerable. The most common adverse events (AEs) in cycle 0 (AZD7762 monotherapy) were bradycardia (50 %), hypertension (25 %) and fatigue (15 %). Overall, the most common AEs were bradycardia (55 %), neutropenia (45 %) and hypertension, fatigue and rash (30 % each). Grade ≥3 AEs were reported in 11 patients, the most common being neutropenia (45 %) and leukopenia (25 %). AZD7762 exposure increased approximately linearly. Gemcitabine did not appear to affect AZD7762 PK. There were no objective responses; five patients (all lung cancer) had stable disease.
*Conclusions* The maximum tolerated dose of AZD7762 in combination with gemcitabine, 1,000 mg/m$^2$ was determined as 21 mg in Japanese patients.

**Keywords** AZD7762 · Chk1 · Solid tumours · Japanese · Phase I · Safety

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## Background

Many tumour types are deficient in the $G_1$-DNA damage checkpoint pathway and instead are dependent on the S and $G_2$ checkpoints for DNA repair and cell survival [1]. S and $G_2$ cell-cycle arrest and repair in response to DNA damage are regulated by checkpoint kinases 1 and 2 (Chk1 and Chk2) [2–4]. These kinases have therefore been examined as potential targets for anticancer drug development; inhibition of these kinases would lead to death of tumour tissue without impacting the normal surrounding tissue that can still mediate DNA repair via a functioning $G_1$ checkpoint signalling pathway.

Inhibition of Chk1 signalling has been shown to impair DNA repair and increase tumour cell death in the presence of DNA-damaging agents [5]. Further, Chk1/Chk2 inhibitors can sensitize tumour models to DNA damage induced by chemotherapy and radiotherapy [6–8].

AZD7762 is a potent Chk1/Chk2 inhibitor that has shown chemosensitizing activity with a variety of DNA-damaging agents, including gemcitabine and irinotecan, in xenograft models [4, 8]. AZD7762 enhances the response to these DNA-damaging agents via different mechanisms of action, with gemcitabine arresting cells predominantly in S phase and irinotecan in $G_2$ [8]. Two Phase I, open-label, dose-escalation studies have investigated AZD7762 in Western patient populations; one in combination with irinotecan [9], and the other in combination with gemcitabine [10]. In combination with irinotecan, the maximum tolerated dose (MTD) was determined to be 96 mg during dose escalation; however, it became apparent during the dose-expansion phase that this dose could not be considered tolerable [dose-limiting toxicities (DLTs) occurred in three patients: left ventricular systolic dysfunction with troponin increase; troponin increase; cardiomyopathy] [9]. In combination with gemcitabine, the MTD of AZD7762 was 30 mg; however, this dose may be insufficient to fully inhibit Chk1 kinase activity and achieve significant clinical efficacy. Cardiac DLTs occurred in two patients in that study (asymptomatic elevated troponin I and myocardial ischaemia) [10]. Overall, the most common adverse events (AEs) in these two studies were diarrhoea, fatigue, nausea, neutropenia and pyrexia.

The aim of this study was to assess the safety, tolerability and pharmacokinetics (PK) of AZD7762 as monotherapy and in combination with gemcitabine in Japanese patients with advanced solid tumours.

## Methods

### Patients

Eligible patients were aged ≥20 and <75 years, with histologically or cytologically confirmed malignancies that were metastatic or unresectable for which standard therapies did not exist or were no longer effective. Patients were also required to have a World Health Organization (WHO) performance status of 0 or 1; absolute neutrophil count $>1.5 \times 10^9$/L, platelet count $>100 \times 10^9$/L or haemoglobin >9 g/L; serum total bilirubin <1.5 × upper limit of reference range (ULRR); alanine aminotransferase (ALT), aspartate aminotransferase (AST) or alkaline phosphate (ALP) <2.5 × ULRR (<5 × ULRR in the presence of liver metastases). Patient and tumour types needed to be suitable for weekly standard gemcitabine treatment; prior gemcitabine therapy was permitted. All patients provided written informed consent.

Specific cardiac-related exclusion criteria included troponin T Common Terminology Criteria for Adverse Events (CTCAE) grade ≥1 elevation; stage II, III, IV cardiac status (New York Heart Association classification); coronary heart disease or arteriosclerotic cardiovascular disease within the previous 6 months; second-degree heart block; resting left ventricular ejection fraction (LVEF) <55 %; PR interval >217 ms; prior anthracycline treatment and use of any potent negative inotropic drug. Other exclusion criteria included: severe or uncontrolled systemic disease; brain metastases or spinal cord compression; treatment with systemic chemotherapy ≤2 weeks prior to study entry; treatment with radiotherapy or major surgery ≤4 weeks prior to study entry; and the presence of any unresolved toxicity CTCAE grade ≥2 (excluding neurotoxicity and alopecia) from previous anticancer therapy.

### Study design

This study was a Phase I, open-label, dose-escalation study to assess the safety, tolerability, PK and preliminary tumour response of AZD7762 alone and in combination with gemcitabine (ClinicalTrials.gov identifier NCT00937664). In the first cycle (cycle 0), patients received a single dose of AZD7762 [60-min intravenous (iv) infusion] on days 1 and 8 of a 14-day cycle. In subsequent cycles (cycle 1 onwards), patients received the same dose level of AZD7762 (60-min iv infusion) plus gemcitabine 1,000 mg/m$^2$ (30-min iv infusion) on days 1 and 8 of 22-day cycles, with the AZD7762 infusion starting immediately following the completion of the gemcitabine infusion (Fig. 1). Following completion of cycles 0 and 1, patients could continue therapy providing they were continuing to benefit from treatment, in the opinion of the investigator.

The starting dose of AZD7762 was 6 mg. Dose escalation was decided, by the Dose Escalation Committee, following review of the safety, tolerability and PK data obtained from ≥3 evaluable patients during cycles 0 and 1. If one patient experienced a DLT, the cohort was expanded to six evaluable patients, and dose escalation could continue if no further patients experienced a DLT. If ≥2 patients experienced a DLT in the same cohort, the dose

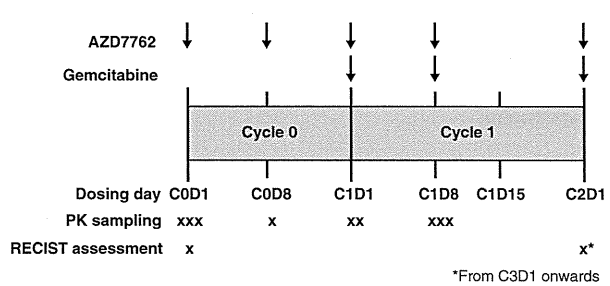


**Fig. 1** Study design. *C* cycle, *D* day, *RECIST* Response Evaluation Criteria in Solid Tumours. AZD7762 was administered immediately following gemcitabine infusion

was considered non-tolerable, dose escalation was stopped, and the previous lower dose was considered the MTD.

DLTs were treatment-related AEs that occurred in cycles 0 and 1 and were defined as follows. For cycle 0 (AZD7762 monotherapy), any grade ≥3 toxicity; grade ≥2 cardiac toxicity (except troponin T) lasting >24 h; grade ≥2 troponin T elevation (or grade 1 at ≥2 separate occurrences) in the absence of a well-defined concurrent event, such as sepsis or pulmonary embolism; grade ≥2 LVEF with a value representing a minimum 10-point decrease from baseline to a value <50 % or an absolute decrease of ≥16 % in a LVEF value that was above 50 % for ≥24 h post-dose. For cycle 1 (AZD7762/gemcitabine combination), grade ≥4 haematological toxicity (for neutropenia, grade ≥4 for >4 days or grade ≥3 neutropenia complicated with ≥38.5 °C fever); grade ≥3 non-haematological toxicity; grade ≥3 nausea, vomiting or diarrhoea for >24 h despite aggressive management; grade ≥3 ALT or AST elevation lasting >7 days in patients with liver metastases; grade ≥3 laboratory abnormalities at two consecutive timepoints within 2 days; grade ≥2 troponin T elevation (or grade 1 at ≥2 separate occurrences in cycle 1 or across cycles 0 and 1) in absence of a well-defined concurrent event, such as sepsis or pulmonary embolism; grade ≥2 LVEF with a value representing a ≥10-point decrease from baseline to a value <50 % or an absolute decrease of ≥16 % in a LVEF value that was above 50 % for ≥24 h.

The study was approved by the appropriate independent review boards and conducted in accordance with the Declaration of Helsinki, the International Conference on Harmonization and Good Clinical Practice and the AstraZeneca policy on bioethics [11].

### Study objectives

The primary objective of the study was to evaluate the safety and tolerability of AZD7762 alone and in combination with gemcitabine. Secondary objectives of the study were to determine the PK profile of AZD7762 when administered alone; to compare clearance rates of AZD7762 as monotherapy and in combination with gemcitabine; and to obtain preliminary evidence of efficacy.

### Assessments

Safety and tolerability were assessed throughout the study and until 30 days after the final dose of study treatment by evaluation of AEs (according to CTCAE, version 3.0), laboratory findings, vital signs and cardiac monitoring. Cardiac monitoring [echocardiogram (ECHO) and electrocardiogram (ECG)] was performed at baseline, on days 1 and 8 of cycles 0 and 1, on day 1 of cycle 2 and then routinely following the first dose and on the last week of each subsequent cycle or pre-dose on the first day of each cycle. If LVEF declined by ≥10 points from baseline to a value <50 %, or if there was an absolute decrease of ≥16 % in a LVEF value that was above 50 % for ≥24 h, assessment was repeated the following day. Cardiac troponin T measurements were performed pre-infusion, 4, 8, 24, 48 and 72 h after the single AZD7762 infusion (cycle 0, day 1); pre-infusion, 4 and 24 h after the second single AZD7762 infusion (cycle 0, day 8); pre-infusion, 4, 8, 24, 48 and 72 h after the start of the AZD7762 infusion (cycle 1, day 1); and thereafter, pre-infusion and 4 h after the start of the AZD7762 infusions (e.g. cycle 1, day 8; cycle 2, day 1).

Blood samples were collected for PK analysis in cycle 0 (AZD7762 monotherapy) at the following timepoints: pre-infusion, end of infusion (EOI), and then at 15 min, 1, 3, 5, 8, 24, and 48 h post-EOI, and pre-infusion on day 8. For cycle 1 (AZD7762 with gemcitabine), blood samples were collected 3–4 and 24 h post-EOI on day 1, and pre-infusion, EOI, and 15 min, 1, 3, 5, 8, 24 and 48 h post-EOI on day 8. Plasma AZD7762 concentrations were determined by high-performance liquid chromatography with tandem mass-spectrometric detection (HPLC MS/MS) by an independent laboratory (York Bioanalytical Solutions Ltd).

Tumour assessments were performed according to response evaluation criteria in solid tumours (RECIST, version 1.0 [12]) at baseline, between days 10 and 15 of cycle 0 (only if not previously obtained within 28 days of the first combination dose of AZD7762 and gemcitabine), day 1 of cycle 3, and subsequently after every three cycles of treatment.

### Statistics

No formal hypothesis testing was performed. Safety, tolerability PK and efficacy data are summarized using descriptive statistics.

## Results

### Patients

Between June 2009 and February 2011, 24 patients were enrolled, of which 20 patients (14 male, six female) received at least one dose of AZD7762 ($n = 3$, 6 mg; $n = 3$, 9 mg; $n = 6$, 21 mg; $n = 8$, 30 mg) and were evaluable for safety and PK analysis (Table 1); all 20 patients had measurable disease recorded at baseline and were evaluable for efficacy. The four patients excluded at screening were due to progression of brain metastases, increased troponin T, ground-glass patterns in chest X-ray

**Table 1** Patient demographics and baseline characteristics

| | AZD7762 dose | | | | |
|---|---|---|---|---|---|
| | 6 mg ($n = 3$) | 9 mg ($n = 3$) | 21 mg ($n = 6$) | 30 mg ($n = 8$) | Total ($n = 20$) |
| Gender, *n* (%) | | | | | |
| Male | 2 | 2 | 6 | 4 | 14 (70) |
| Female | 1 | 1 | 0 | 4 | 6 (30) |
| Median age, years (range) | 53 (52–69) | 69 (57–72) | 62 (47–72) | 55 (50–65) | 57 (47–72) |
| WHO PS, *n* (%) | | | | | |
| 0 | 1 | 0 | 2 | 5 | 8 (40) |
| 1 | 2 | 3 | 4 | 3 | 12 (60) |
| Local/metastatic sites, *n* (%) | | | | | |
| Local only | 1 | 0 | 1 | 0 | 2 (10) |
| Local + metastatic | 2 | 3 | 5 | 8 | 18 (90) |
| Primary tumour type, *n* (%) | | | | | |
| Lung | 1 | 2 | 4 | 7 | 14 (70) |
| Colorectal | 1 | 1 | 2 | 1 | 5 (25) |
| Pleura | 1 | 0 | 0 | 0 | 1 (5) |
| Prior therapy, *n* (%) | | | | | |
| Chemotherapy | 3 | 3 | 6 | 8 | 20 (100) |
| Surgery | 2 | 2 | 4 | 3 | 11 (55) |
| Radiotherapy | 2 | 1 | 1 | 6 | 10 (50) |
| Immunotherapy/hormone therapy | 0 | 0 | 0 | 0 | 0 (0) |
| Other | 1 | 1 | 3 | 5 | 10 (50) |

and use of a potent negative inotropic drug ($n = 1$ for each).

All patients had discontinued study treatment by 25 February 2011 (date of last patient, last visit); 13 patients discontinued due to disease progression, six patients due to AEs and one patient due to early termination of the overall AZD7762 development programme. Seventeen patients completed treatment cycles 0 and 1 and were eligible for DLT evaluation; one patient in the 21-mg cohort and two patients in the 30-mg cohort did not complete these treatment cycles.

## Safety and tolerability

The study was stopped prematurely due to discontinuation of the AZD7762 clinical development programme; nevertheless, results were sufficiently mature to determine the MTD in Japanese patients with advanced solid tumours.

The MTD of AZD7762 in combination with gemcitabine 1,000 mg/m$^2$ was determined to be 21 mg, based on the safety information from five evaluable patients at the time of study termination. DLTs were identified in two patients in the 30-mg-dose cohort. One cardiac DLT of grade 3 increased troponin T was recorded in one patient on day 1 of cycle 0 (AZD7762 monotherapy). Pre-dose, the patient had normal left ventricular contraction and ECG; post-dose ECHOs showed localized left ventricular dysfunction, a slight decrease in ejection fraction, and ECG showed T-wave abnormalities and left axis deviation, with suspected inferior ischaemia. Liver function and haematologically related DLTs (grade 3 increased ALT and AST, and grade 4 neutropenia and thrombocytopenia) were recorded in one patient between days 7 and 15 of cycle 1 (combination therapy; days 21 and 29 of the overall study). For both patients, the DLTs resolved following treatment discontinuation. The AZD7762 30 mg dose level was thus regarded as non-tolerable.

The median (range) duration of AZD7762 treatment was 47 days (23–52) in the 6-mg cohort, 65 days (24–72) in the 9-mg cohort, 26 days (15–113) in the 21-mg cohort and 45 days (1–176) in the 30-mg cohort. The AZD7762 mean dose intensity for the first three treatment cycles was >90 % for each cohort. The median (range) of gemcitabine treatment was 33 days (9–38) in the 6-mg cohort, 51 days (8–58) in the 9-mg cohort, 11 days (1–99) in the 21-mg cohort and 65 days (1–162) in the 30-mg cohort.

The most common AEs reported during cycle 0 (AZD7762 monotherapy) were bradycardia ($n = 10$, 50 %), hypertension ($n = 5$, 25 %) and fatigue ($n = 3$, 15 %) (Table 2). Overall, the most frequently reported AEs were bradycardia ($n = 11$, 55 %), neutropenia ($n = 9$, 45 %), fatigue ($n = 6$, 30 %), hypertension ($n = 6$, 30 %) and rash ($n = 6$, 30 %) (Table 2). Hypertension events were not associated with the cardiac toxicity observed in

**Table 2** Adverse events (any cause, all grades) occurring during AZD7762 monotherapy (≥2 patients) or AZD7762–gemcitabine combination therapy (≥4 patients)

| Adverse events, *n* (%) | AZD7762 dose | | | | |
|---|---|---|---|---|---|
| | 6 mg ($n = 3$) | 9 mg ($n = 3$) | 21 mg ($n = 6$) | 30 mg ($n = 8$) | Total ($n = 20$) |
| AZD7762 monotherapy | | | | | |
| Patients with any AE | 2 | 3 | 5 | 8 | 18 (90) |
| Bradycardia | 1 | 3 | 2 | 4 | 10 (50) |
| Hypertension | 0 | 1 | 2 | 2 | 5 (25) |
| Fatigue | 0 | 0 | 1 | 2 | 3 (15) |
| Dysgeusia | 0 | 0 | 0 | 2 | 2 (10) |
| ALT increased | 0 | 0 | 0 | 2 | 2 (10) |
| AST increased | 0 | 0 | 0 | 2 | 2 (10) |
| Palpitations | 0 | 1 | 0 | 1 | 2 (10) |
| Ventricular extrasystoles | 0 | 1 | 0 | 1 | 2 (10) |
| Diarrhoea | 1 | 0 | 0 | 1 | 2 (10) |
| AZD7762 and gemcitabine | | | | | |
| Patients with any AE | 3 | 3 | 5 | 8 | 19 (95) |
| Bradycardia | 1 | 3 | 3 | 4 | 11 (55) |
| Neutropenia | 2 | 1 | 2 | 4 | 9 (45) |
| Fatigue | 2 | 0 | 1 | 3 | 6 (30) |
| Hypertension | 0 | 1 | 3 | 2 | 6 (30) |
| Rash | 1 | 1 | 2 | 2 | 6 (30) |
| Diarrhoea | 1 | 1 | 0 | 3 | 5 (25) |
| Nausea | 0 | 1 | 0 | 4 | 5 (25) |
| Leukopenia | 2 | 0 | 1 | 2 | 5 (25) |
| Stomatitis | 0 | 1 | 2 | 1 | 4 (20) |
| Pyrexia | 2 | 0 | 1 | 1 | 4 (20) |
| ALT increased | 0 | 0 | 1 | 3 | 4 (20) |
| AST increased | 0 | 0 | 1 | 3 | 4 (20) |

*ALT* alanine aminotransferase, *AST* aspartate aminotransferase

this study, although three out of the five events that occurred during cycle 0 and three out of the six events that occurred during cycle 1 were considered related to AZD7762 treatment.

Grade ≥3 AEs were reported in 11 patients, the most common being neutropenia ($n = 9$, 45 %) and leukopenia ($n = 5$, 25 %) (Table 3). Only one grade ≥3 AE occurred during AZD7762 monotherapy; this was a DLT of increased troponin T. Grade 4 AEs were neutropenia ($n = 1$, 6 mg; $n = 1$, 21 mg; $n = 3$, 30 mg), leukopenia ($n = 1$ each, 6 mg and 30 mg), decreased blood sodium ($n = 1$, 21 mg) and thrombocytopenia ($n = 1$, 30 mg). Five patients experienced serious AEs (SAEs); these events were considered by the investigator to be treatment related in two patients [$n = 1$, 9 mg: interstitial lung disease; $n = 1$, 30 mg: increased ALT/AST, increased γ-glutamyl transpeptidase, leukopenia, neutropenia and thrombocytopenia (this patient was a DLT case)].

AZD7762 dose delays occurred in four patients due to AEs ($n = 1$, 6 mg; $n = 2$, 21 mg; $n = 1$, 30 mg); there were no AZD7762 dose reductions. Gemcitabine dose delays were also required in the same four patients ($n = 1$, 6 mg; $n = 2$, 21 mg; $n = 1$, 30 mg); a 75 % dose reduction of gemcitabine occurred in one patient in the AZD7762 6-mg cohort, due to an AE. Twelve AEs led to treatment discontinuation in six patients; all 12 AEs ($n = 1$, 9 mg, interstitial lung disease; $n = 2$ each, 21 mg and 30 mg, increased ALT/AST; $n = 1$, 30 mg, pneumonia; $n = 2$, both 30 mg, neutropenia; $n = 1$, 30 mg, leukopenia; $n = 1$, 30 mg, increased troponin T; $n = 1$, 30 mg, increased γ-glutamyl transpeptidase; $n = 1$, 30 mg, thrombocytopenia) were considered to be related to AZD7762 and included the two patients (one of which was a DLT case) with treatment-related SAEs previously mentioned. No patient died due to an AE.

A total of 12 patients had laboratory values of grade 3 or 4 as their worst grade during the treatment period. The most frequently recorded event was total and absolute neutrophil count ($n = 9$). Other frequently recorded events were reductions in leucocytes ($n = 5$).

**Table 3** Adverse events (any cause) of CTC grade ≥3 reported during cycle 0 (AZD7762 monotherapy) and cycle 1 onwards (AZD7762 and gemcitabine)

| Adverse events, *n* (%) | AZD7762 dose | | | | |
|---|---|---|---|---|---|
| | 6 mg ($n = 3$) | 9 mg ($n = 3$) | 21 mg ($n = 6$) | 30 mg ($n = 8$) | Total ($n = 20$) |
| AZD7762 monotherapy | | | | | |
| Patients with any grade ≥3 AE | 0 | 0 | 0 | 1 | 1 (5) |
| Troponin T increased | 0 | 0 | 0 | 1 | 1 (5) |
| AZD7762 and gemcitabine | | | | | |
| Patients with any grade ≥3 AE | 2 | 1 | 3 | 5 | 11 (55) |
| Neutropenia | 2 | 1 | 2 | 4 | 9 (45) |
| Leukopenia | 2 | 0 | 1 | 2 | 5 (25) |
| ALT increased | 0 | 0 | 1 | 1 | 2 (10) |
| AST increased | 0 | 0 | 1 | 1 | 2 (10) |
| Thrombocytopenia | 0 | 0 | 0 | 1 | 1 (5) |
| Hypophosphataemia | 0 | 0 | 0 | 1 | 1 (5) |
| Blood sodium decreased | 0 | 0 | 1 | 0 | 1 (5) |
| Haemoglobin decreased | 0 | 0 | 1 | 0 | 1 (5) |
| Diarrhoea | 0 | 1 | 0 | 0 | 1 (5) |
| Bacteraemia | 0 | 1 | 0 | 0 | 1 (5) |
| Febrile neutropenia | 1 | 0 | 0 | 0 | 1 (5) |

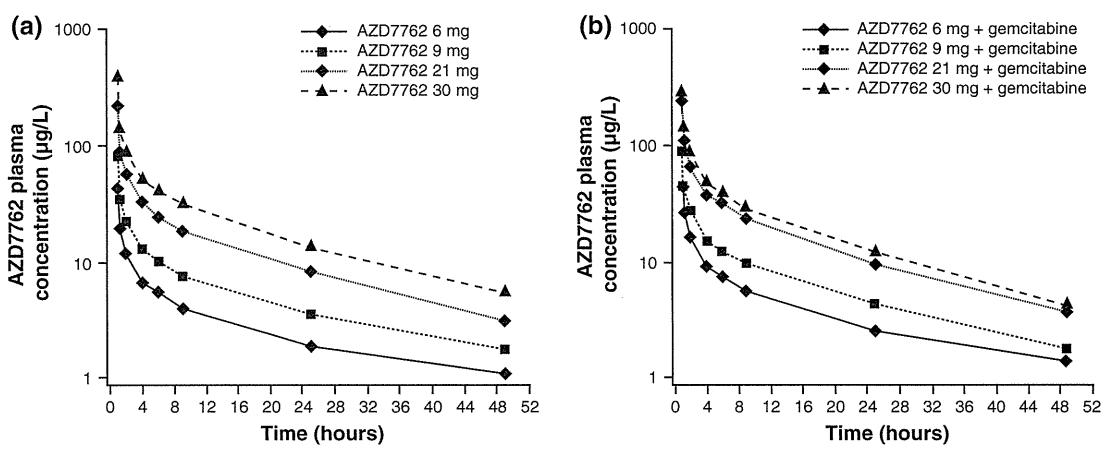


**Fig. 2** Geometric mean AZD7762 plasma concentration versus time **a** cycle 0, day 1 (AZD7762 monotherapy); **b** cycle 1, day 8 (AZD7762–gemcitabine combination therapy)

## Pharmacokinetics

Following a single-dose constant iv infusion, AZD7762 exposure [maximum AZD7762 plasma concentration ($C_{max}$), area under the plasma–concentration time curve (AUC)] increased in an approximately linear and dose-proportional manner, achieving $C_{max}$ approximately 1 h from the start of the infusion. At the EOI, the disposition of AZD7762 may be described as multi-phasic with an initial rapid decline (distribution phase) followed by a slower elimination phase (Fig. 2a; Table 4). The arithmetic mean half-life ($t_{½}$) ranged from 16.1 to 19.4 h and the geometric mean clearance (CL) from 22.0 to 32.7 L/h. AZD7762 was extensively distributed, with a mean volume of distribution at steady state ($V_{SS}$) of 382–601 L across all dose cohorts after single-dose administration.

The addition of gemcitabine did not appear to affect the PK of AZD7762 (Fig. 2b; Table 4). When administered as combination therapy, the $t_{½}$ of AZD7762 was 15.6–18.3 h and CL was 21.1–24.4 L/h. $V_{SS}$ ranged from 342–487 L across all dose cohorts after combination administration. Between-subject variability was

**Table 4** AZD7762 pharmacokinetics

| Parameters[a] | AZD7762 dose | | | |
|---|---|---|---|---|
| | 6 mg ($n = 3$) | 9 mg ($n = 3$) | 21 mg ($n = 6$) | 30 mg ($n = 8$) |
| AZD7762 monotherapy | | | | |
| $C_{max}$ (μg/L) | 43.8 (9.6) | 80.0 (33.3) | 221 (20.9) | 383.0 (26.2) |
| AUC (μg h/L) | 183.6 (25.1) | 349.3 (31.1) | 818.0 (21.0) | 1,361.2 (26.5) |
| CL (L/h) | 32.7 (25.1) | 25.8 (31.1) | 25.7 (21.0) | 22.0 (26.5) |
| $t_{½}^{b}$ (h) | 17.4 (6.0) | 19.4 (2.7) | 16.1 (3.0) | 16.2 (3.3) |
| $V_{SS}$ (L) | 601.2 (21.7) | 548.4 (23.5) | 429.1 (16.5) | 381.7 (25.5) |
| AZD7762 and gemcitabine combination therapy | | | | |
| $C_{max}$ (μg/L) | 45.3 (31.5) | 89.3 (33.1) | 247 (25.6) | 282.0 (23.8) |
| AUC (μg h/L) | 246.1 (38.6) | 412.7 (44.6) | 996.9 (28.5) | 1,236.1 (15.0) |
| CL (L/h) | 24.4 (38.6) | 21.8 (44.6) | 21.1 (28.5) | 24.3 (15.0) |
| $t_{½}^{b}$ (h) | 18.3 (8.5) | 16.8 (4.6) | 15.6 (4.5) | 16.8 (6.4) |
| $V_{SS}$ (L) | 487.4 (10.7) | 407.9 (12.0) | 341.5 (21.7) | 422.2 (42.2) |

$C_{max}$ maximum AZD7762 plasma concentration; *AUC* area under the plasma–concentration time curve; *CL* total body clearance; $t_{½}$ terminal elimination half-life; $V_{SS}$ volume of distribution at steady state

[a] Values are reported as geometric mean (coefficient of variation, %) unless otherwise stated

[b] $t_{½}$, arithmetic mean (SD)

low to moderate with the coefficient of variation ranging from 10 to 45 %.

### Efficacy

No objective responses were reported during the study. A best response of stable disease (≥8 weeks duration) was reported for five patients ($n = 1$, 9 mg; $n = 2$, 21 mg; $n = 2$, 30 mg), all of whom were diagnosed with lung cancer. The duration of stable disease ranged from 86 to 185 days across the dose cohorts. Of these patients with stable disease, two had received prior gemcitabine therapy (approximately 20 % tumour shrinkage was reported in one of these patients). There were four patients who had also previously received gemcitabine but did not achieve stable disease.

## Discussion

In this study, the MTD of AZD7762 in combination with gemcitabine 1,000 mg/m$^2$ was determined as 21 mg in Japanese patients. No DLTs were observed for the five patients at this dose level. Of the two DLTs in the 30 mg AZD7762 cohort, one of these was a cardiac DLT reported during AZD7762 monotherapy and the other was a liver function/haematologically related DLT during AZD7762 combination therapy. Both events resolved following discontinuation of treatment. It should be noted, however, that the MTD was established when at least two patients experienced a DLT in the same dose cohort. Although two DLTs occurred at the 30 mg dose, one of these was achieved with monotherapy and the other with combination therapy; it is therefore possible that AZD7762 could have been tolerated at the 30 mg dose alone.

Adverse cardiac effects have been reported in previous clinical studies of AZD7762 in Western populations [9, 10]. Given the emergence of these events, changes in vital signs were recorded as AEs in our study, even when unaccompanied by other clinical signs or symptoms. As a result, bradycardia and hypertension were recorded as the most common AEs during AZD7762 monotherapy. The mechanisms underlying AZD7762-induced cardiac toxicity are unknown; despite Chk1(−/−) mice experiencing embryonic lethality [13], there is no obvious cardiac defect. As part of its preclinical evaluation, AZD7762 displayed less than tenfold selectivity for kinases that were generally from the same family as Chk1, calcium/calmodulin-dependent protein kinases (CaM kinases) and src-like kinases [8]. Recent studies have revealed both the significance of distinct CaM kinases in maintaining various aspects of cardiac function including contractility [14], and the importance of CaM signalling to the action of ATPases considered to be 'critical' to heart muscle function in zebrafish [15].

Other common AEs during monotherapy were generally consistent with the previous studies in Western populations [9, 10], predominantly fatigue and gastrointestinal effects such as nausea and vomiting. The incidence and type of AEs with onset following the addition of gemcitabine were generally similar to those observed with AZD7762 monotherapy. However, there was a higher incidence of neutropenia and leukopenia with combination AZD7762 and gemcitabine compared with AZD7762 alone, consistent with the known side effects of gemcitabine [16–19]. The AE profile for AZD7762 in combination with gemcitabine is similar to that reported previously in a Western population receiving this combination treatment [10]. Similarly, of the reported CTCAE grade ≥3 AEs, the most frequent (neutropenia, leukopenia, increased ALT and AST) were consistent with the known side effects of gemcitabine [16–