Phase I/II study of amrubicin, a novel 9-aminoanthracycline, in patients with advanced non-small-cell lung cancer

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Summary

Purpose: Amrubicin is a novel, totally synthetic 9-aminoanthracycline. The present phase I/II study was performed to define its maximum-tolerated dose (MTD), efficacy and toxicity in the treatment of previously untreated patients with advanced non-small-cell lung cancer (NSCLC). *Patients and Methods*: Chemonaive patients were required to have cytologically or histologically proven measurable NSCLC, an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0 to 2, and adequate organ functions. Amrubicin was administered by daily intravenous injection for 3 consecutive days every 3 weeks. *Results*: In a phase I study, four patients were enrolled at dose level 1 (40 mg/m^2/day) and four at dose level 2 (45 mg/m^2/day). No dose limiting toxicity (DLT), which was defined as toxicity consisting of grade 4 neutropenia and leukopenia lasting four days or more, and grade 3 or 4 toxicity other than neutropenia, leukopenia, anorexia, nausea/vomiting, and alopecia, was observed at these dose levels. Subsequently, at dose level 3 (50 mg/m^2/day), 3 of 5 patients experienced DLTs (leukopenia, neutropenia, thrombocytopenia, or gastrointestinal complications). The MTD and recommended dose (RD) were determined to be 50 mg/m^2/day and 45 mg/m^2/day, respectively. Three partial responses (PRs) were achieved in 13 patients (response rate, 23.1%) in a phase I study. In a phase II study, 15 patients were assessable for efficacy and toxicity at the RD, and four PRs were obtained (response rate, 26.7%). The major toxicities were leukopenia and neutropenia, while non-hematologic toxicities were mild. The overall response rate in the combined patient population of the phase I/II study was 25.0% (7 PRs in 28 patients), with a 95% confidence interval of 10.7% to 44.9%. *Conclusion*: Amrubicin exerted promising antitumor activity on NSCLC with acceptable toxicity.

Introduction

Amrubicin is a novel, totally synthetic 9-aminoanthracycline, (+)-(7S, 9S)-9-acetyl-9-amino-7-[(2-deoxy-β-D-erythro-pentopyranosyl)oxy]-7,8,9,10- tetrahydro-6,11-dihydroxy-5,12-naphthacenedione hydrochloride, and is similar to doxorubicin in chemical structure, as shown in Figure 1 [1]. Amrubicin showed more potent antitumor activity than doxorubin on several human tumor xenografts implanted in nude mice [2]. Its toxic profile was qualitatively similar to that of doxorubicin in terms of acute toxicities [3], but amrubicin rarely caused delayed-type toxicity as observed with doxorubicin, especially cardiotoxicity [4, 5]. In an early phase II study of single-dose intravenous injection of 120 mg/m^2 every 3 weeks, amrubicin exhibited promising antitumor activity

on non-small-cell lung cancer (NSCLC) with a response rate of 25% (95% confidence interval, 8.7% to 49.1%) [6].

A major characteristic of amrubicin that is closely associated with the efficacy and toxicity is that it is converted to an active metabolite, amrubicinol, via reduction of its C-13 ketone group to a hydroxy group. The *in vitro* cytotoxic activity of amrubicinol was almost equipotent to that of doxorubicin, and 20 to 220 times more potent than that of its parent compound, amrubicin [7]. The *in vivo* antitumor activity of amrubicin was closely related to the tumor concentration of amrubicinol [8]. In addition, the experimental data have shown that amrubicin yields greater efficacy in daily treatment for 5 consecutive days than in a single treatment, due to accumulation of greater amounts of amrubicinol in tumor tissues [9].
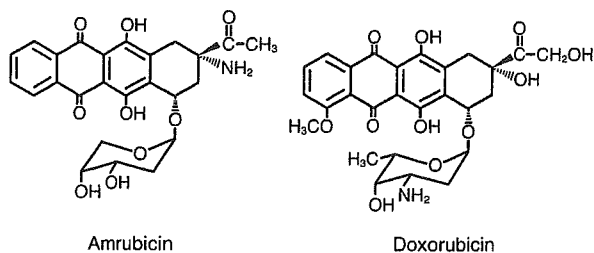
Figure 1. Chemical structures of amrubicin and doxorubicin

These data suggest that amrubicin may exert more potent effect against NSCLC in the divided treatment schedule than in the single-dose treatment schedule.

In addition, it has been reported that epirubicin, the same anthracycline derivative as amrubicin, could be administered at higher doses in 3-day consecutive treatment every 3 weeks than in single-dose treatment every 3 weeks, and consequently the high dosage of epirubicin in the former treatment schedule resulted in a higher response rate, compared with standard dosages of epirubicin in the latter treatment schedule, in previously untreated patients with advanced NSCLC [10].

In the present phase I/II study, therefore, daily treatment for 3 consecutive days every 3 weeks was chosen as the divided treatment schedule, and the efficacy and safety of amrubicin were evaluated in previously untreated patients with advanced NSCLC.

Patients and methods

Patient eligibility

This study involved patients with histologically or cytologically confirmed unresectable NSCLC in stages IIIA, IIIB, and IV. Eligibility criteria included no prior treatment, measurable lesions, an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0 to 2, an estimated life expectancy of at least 2 months, and age less than 75 years. Adequate organ function was required and defined as: white blood cell (WBC) count $\geq 4,000/\mu L$, platelet count $\geq 100,000/\mu L$, hemoglobin level ≥ 10 g/dL, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≤ 2 times the upper limit of normal, serum creatinine level $\leq$ normal limit, and electrocardiography (ECG) within normal limits.

The following patients were excluded: those with symptomatic brain metastasis or bone metastasis accompanying pain, those with plural fluid retention requiring treatment like drainage, those with continuous long term treatment with non-steroidal anti-inflammatory agents, glucocorticoids, or morphine derivatives, those with serious complications or other active cancer, and those judged by the investigators to be inappropriate for the study. Pa-

tients who were pregnant, breast-feeding, or taking inadequate contraceptive precautions were also ineligible. All eligible patients were required to provide signed informed consent prior to entering this study. The individual investigational review board at each institution approved the treatment protocol.

Drug administration

Amrubicin (Sumitomo Pharmaceuticals Co., Ltd, Osaka, Japan) was supplied as a freeze-dried powder in vials containing 20 mg each, reconstituted in 20 mL of physiological saline or 5% glucose solution, and administered intravenously over 5 minutes on 3 consecutive days every 3 weeks. At least 2 cycles were instituted, except in case of disease progression, unacceptable toxicity or patient refusal.

Dose levels

The phase I study was started at a dosage of 40 mg/m²/day to determine the dose limiting toxicity (DLT), maximum-tolerated dose (MTD) and recommended dose (RD) of amrubicin given on 3 consecutive days (120 mg/m²/course). The starting dosage was set at the same dosage per cycle as that used in the early phase II study for NSCLC in which amrubicin was given once every 3 weeks [6], because experimentally, amrubicin could be administered at a higher total dosage in the divided treatment schedule than in the single treatment schedule [10].

The dosage of amrubicin was escalated by 5 mg/m²/day (15 mg/m²/course). At least four patients were entered at each dose level until the MTD was reached. The dose escalations were determined based on the tolerability observed during the first 3 weeks of treatment as follows. The dose at which none or one patient experienced a DLT was escalated, and the MTD was the dose at which at least two patients developed a DLT, i.e., the dose at which at least 2/4, 2/5 or 2/6 patients experienced a DLT. Dosages were not escalated for individual patients.

The following phase II study was performed at the RD estimated in the phase I study.

Definition of DLT, MTD, and RD

DLT was defined as toxicity consisting of grade 4 neutropenia and leukopenia lasting four days or more, and grade 3 or 4 toxicity other than neutropenia, leukopenia, anorexia, nausea/vomiting, and alopecia. MTD was defined as the dose level at which at least one-third of patients experienced a DLT. The RD was chosen as the dose one-level lower than the MTD.

Adjustment of dosage and schedule modification

The treatment was repeated if the WBC count recovered to $\geq 3,000/\mu L$ and the platelet count recovered to $\geq 100,000/\mu L$. In incomplete recovery, the treatment was delayed until the WBC count recovered to $\geq 3,000/\mu L$ and the platelet count recovered to $\geq 100,000/\mu L$. If the WBC count and platelet count did not recover within 5 weeks after administration of amrubicin, the trial was discontinued. If the WBC nadir was $<1,000/\mu L$ for ≤ 3 days, or $\geq 1,000/\mu L$ and the platelet nadir was $\geq 50,000/\mu L$, the treatment was conducted at the same dosage as the previous course. If the WBC nadir was $<1,000/\mu L$ for ≥ 4 days and/or the platelet nadir was $<50,000/\mu L$, the dosage was reduced by 5 mg/m²/day from the dosage of the previous course.

Treatment evaluation

Before treatment, all patients underwent medical history review, physical examination, hematology and serum biochemistry tests, urinalysis, ECG, and baseline tumor measurements (e.g. chest radiography, computed tomography (CT) scan, bone scintigraphy, abdominal CT, brain CT). All measurable and assessable lesions were evaluated within 2 weeks before start of treatment.

Complete and differential blood cell counts, platelet counts, and hematocrit values were obtained two times a week as a rule, and biochemical data [AST, ALT, alkaline phosphatase, LDH, total bilirubin, BUN, creatinine, serum bilirubin, albumin, total protein, and electrolytes (Na, K, Cl, and Ca)], and urinalysis findings (protein, glucose, urobilinogen, and occult blood), were recorded weekly. ECG was performed every treatment cycle.

Subjective symptoms and objective signs were checked daily for 5 consecutive days from the start of treatment in each cycle, and thereafter ad libitum.

Response and toxicity evaluation

Response was assessed according to the "Criteria for the evaluation of the clinical effects of solid cancer chemotherapy" of the Japan Society for Cancer Therapy [11], which is almost equal to the World Health Organization criteria [12]. A complete response (CR) was defined as the disappearance of all lesions. A partial response (PR) was defined as a reduction by 50% or more in the size of lesions measurable in two dimensions, objective improvement in any evaluable lesions, and no new lesions. CR and PR required response durations of at least four weeks. No change (NC) was defined as lesions unchanged (a reduction of <25% or an increase of <25% in the size of lesions) for at least four weeks. Progressive

disease (PD) was defined as failure, with an increase of $\geq 25\%$ in the size of lesions and appearance of new lesions. The Kaplan-Meier product-limit method was used to estimate the survival time.

Toxicity grading was recorded based on the side effect record form in the "Criteria for the evaluation of the clinical effects of solid cancer chemotherapy" of the Japan Society for Cancer Therapy [11], which is almost equal to the World Health Organization criteria [12]. For toxicity items that were not included on the record form, only their presence or absence was recorded, without grading.

Results

Patient characteristics

Thirteen patients were entered in the phase I study, and subsequently 17 patients in the phase II study, between November 1992 and September 1994. Of the 13 patients entered in the phase I study, 4 were treated at dose level 1 (40 mg/m²/day × 3), 4 at level 2 (45 mg/m²/day × 3), and 5 at level 3 (50 mg/m²/day × 3); all were assessable for efficacy and safety.

In the phase II study, 15 of 17 patients were assessable for efficacy and safety; 2 of them were ineligible because one had suffered from serious complications of pneumonitis and arrhythmia, a deviation against the inclusion criteria in the protocol, and another had been treated without registration prior to the study.

The characteristics of the eligible patients are listed in Table 1.

Phase I study

Toxicity. Hematologic toxicity is shown in Table 2. Dose-related leukopenia and neutropenia were noted. At dose level 1 (40 mg/m²), one patient experienced grade 4

Table 1. Characteristics of eligible patients

Characteristic	No. of patients	
	Phase I study	Phase II study
No. of patients entered	13	17
No. of eligible patients	13	15
Gender(Male/Female)	8/5	10/5
Median age, years (range)	69 (45–74)	65 (29–72)
ECOG performance status		
0/1/2	5/3/5	1/12/2
Histology		
Squamous cell carcinoma	5	6
Adenocarcinoma	7	8
Large cell carcinoma	1	1
Stage (IIIA/IIIB/IV)	2/1/10	1/3/11

Table 2. Hematologic toxicity of amrubicin in phase I study

	Grade of toxicity (No. of patients)											
	40 mg/m² (n = 4)				45 mg/m² (n = 4)				50 mg/m² (n = 5)			
Toxicity	1	2	3	4	1	2	3	4	1	2	3	4
Hemoglobin, decrease	1	0	1	0	2	1	1	0	2	1	2	0
Leukopenia	1	1	1	1	1	0	3	0	0	0	3	2
Neutropenia	0	1	1	1	0	1	0	3	0	0	0	5
Thrombocytopenia	1	0	0	0	0	1	1	0	3	0	1	1

Table 4. Efficacy of amrubicin in phase I study

	No. of patients						
Dose	Total	CR	PR	NC	PD	ORR (%)	95% CI (%)
40 mg/m²	4	0	1	1	2	25.0	
45 mg/m²	4	0	2	1	1	50.0	
50 mg/m²	5	0	0	5	0	0.0	
Total	13	0	3	7	3	23.1	5.0–53.8

Abbreviation: CR, complete response; PR, partial response; NC, no change; PD, progressive disease; ORR, overall response rate (CR + PR); 95% CI, 95% confidence interval

neutropenia and leukopenia, which did not last for 4 days or longer. At dose level 2 (45 mg/m²), three of four patients also experienced grade 4 neutropenia, lasted 4 days or longer in only one. At this dose level, no grade 4 leukopenia was observed. Dose-limiting leukopenia and neutropenia lasting for more than 4 days were seen in two and in all five patients at dose level 3 (50 mg/m²), respectively. Grade 3 or 4 hemoglobin decrease and thrombocytopenia each occurred in two patients at the highest dose level. Three patients required blood transfusion or platelet transfusion or both.

As shown in Table 3, non-hematologic toxicities observed frequently in this study were anorexia, nausea/vomiting, fever, diarrhea and alopecia, but no grade 3 or 4 toxicity was seen at dose level 1 or 2. On the contrary, at dose level 3, grade 3 or 4 toxicity was noted in three of five patients; grade 3 nausea/vomiting and melaena and grade 4 hematemesis in one patient each. Because the grade 3 melaena and grade 4 hematemesis were noted in

two patients who had received indomethacin or diclofenac sodium over 50 days, these episodes were considered to be associated with the long-term treatment of nonsteroidal anti-inflammatory agents. Therefore, the criteria for entry into the study were revised in the subsequent studies to exclude patients who had been treated with nonsteroidal anti-inflammatory agents for a long period. There was no toxicity to renal or cardiac function but a mild effect on hepatic function was observed. As uncommon toxicities, two episodes of grade 1 vitreous floaters occurred at 40 and 45 mg/m², and one episode of grade 1 eruption occurred at 50 mg/m².

Based on the above results, the MTD and RD of amrubicin in a 3-day consecutive administration were determined as 50 mg/m² (150 mg/m²/course) and 45 mg/m² (135 mg/m²/course), respectively. The DLTs were leukopenia, neutropenia, thrombocytopenia and digestive dysfunction including nausea/vomiting, melaena, and hematemesis.

Table 3. Non-hematologic toxicity of amrubicin in phase I study

	Grade of toxicity (No. of patients)											
	40 mg/m² (n = 4)				45 mg/m² (n = 4)				50 mg/m² (n = 5)			
Toxicity	1	2	3	4	1	2	3	4	1	2	3	4
Stomatitis	0	0	0	0	0	0	0	0	1	1	0	0
Anorexia	2	1	0	-[a]	1	0	0	-[a]	0	2	0	-[a]
Nausea/vomiting	2	0	0	-[a]	3	0	0	-[a]	1	1	1	-[a]
Diarrhea	3	0	0	0	1	0	0	0	1	0	0	0
Fever	1	0	0	0	0	1	0	0	1	4	0	0
Alopecia	1	0	0	-[a]	1	3	0	-[a]	2	3	0	-[a]
Melaena	0	0	0	0	0	0	0	0	0	0	1	0
Hematemesis	0	0	0	0	0	0	0	0	0	0	0	1
AST, increase	1	0	0	0	1	0	0	0	2	0	0	0
ALT, increase	1	0	0	0	1	0	0	0	2	0	0	0
ALP, increase	0	0	0	0	1	0	0	0	0	0	0	0
BUN, increase	0	0	0	0	0	0	0	0	1	0	0	0

Abbreviation: AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; BUN, blood urine nitrogen. [a]No grading.

Efficacy. Antitumor response is shown in Table 4. One of four patients (25.0%) at dose level 1 (40 mg/m²) and two of four patients (50.0%) at dose level 2 (45 mg/m²) showed PR. At dose level 3 (50 mg/m²), three patients discontinued treatment after the first cycle because of toxicity, and none of five patients responded. In total, three of the 13 patients had PR, an overall response rate of 23.1%. One of five patients with squamous cell carcinoma (20.0%) and two of seven with adenocarcinoma (28.6%) responded.

Phase II study

Efficacy. In the phase II study, amrubicin was administered daily for 3 consecutive days at 45 mg/m², which was the RD determined in the phase I study. The responses to amrubicin in patients with previously untreated NSCLC are shown in Table 5. Of 15 patients, four (26.7%) achieved PR. Of these responders, one patient (1/6, 16.7%) had a histology result indicating squamous cell carcinoma and three (3/8, 37.5%) had adenocarcinoma.

Table 5. Efficacy of amrubicin in phase II study

| Histology | No. of patients | | | | | ORR (%) | 95% CI (%) |
	Total	CR	PR	NC	PD		
Adenocarcinoma	8	0	3	3	2	37.5	
Squamous cell	6	0	1	5	0	16.7	
Large cell	1	0	0	1	0	0.0	
Total	15	0	4	9	2	26.7	7.8–55.1

Abbreviation: CR, complete response; PR, partial response; NC, no change; PD, progressive disease; ORR, overall response rate (CR + PR); 95% CI, 95% confidence interval.

Table 6. Hematologic toxicity of amrubicin in phase II study

| Toxicity | No. of pts. | Grade (No. of pts.) | | | | ≥ Grade 3 | |
		1	2	3	4	No. of pts.	%
Hemoglobin, decrease	15	4	3	3	1	4	26.7
Leukopenia	15	2	5	5	3	8	53.3
Neutropenia	15	0	4	3	8	11	73.3
Thrombocytopenia	15	0	1	3	1	4	26.7

Table 7. Non-hematologic toxicity in phase II study

| Toxicity | No. of pts. | Grade (No. of pts.) | | | | ≥ Grade 3 | |
		1	2	3	4	No. of pts.	%
Stomatitis	15	3	0	0	0	0	0.0
Anorexia	15	8	3	0	—[a]	0	0.0
Nausea/vomiting	15	9	2	0	—[a]	0	0.0
Diarrhea	15	3	0	0	0	0	0.0
Fever	15	0	3	1	0	1	6.7
Phlebitis	15	2	0	0	0	0	0.0
Alopecia	15	4	5	0	—[a]	0	0.0
Peripheral neuropathy	15	0	1	0	0	0	0.0
ECG abnormalities	12	0	4	0	0	0	0.0
Arrhythmia	15	0	1	0	0	0	0.0
Palpitation	15	0	1	0	0	0	0.0
Pneumonia	15	0	1	0	0	0	0.0
AST, increase	15	3	0	0	0	0	0.0
ALT, increase	15	3	0	0	0	0	0.0
Total bilirubin	15	4	0	0	0	0	0.0
Proteinuria	15	1	0	0	0	0	0.0

Abbreviation: AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; BUN, blood urine nitrogen.
[a]No grading.

The two studies of phase I and II were combined, and the overall data were analyzed for response. Of 28 patients, seven achieved PR, accounting for an overall response rate of 25% (95% confidence interval, 10.7% to 44.9%). Median survival time was 9.1 months (95% confidence interval, 6.8 months to 12.1 months), and 1-year and 2-year survival rates were 35.7% (95% confidence interval, 18.0% to 53.5%) and 12.1% (0% to 24.6%), respectively.

Toxicity. Hematologic toxicity was common, as shown in Table 6. In particular, neutropenia and leukopenia developed in all patients, with grade 3 or 4 leukopenia at 53.3% and neutropenia at 73.3%. Hemoglobin decrease and thrombocytopenia were also frequently noted, but these were less severe, compared with leukopenia and neutropenia. Grade 3 or 4 hemoglobin decrease and thrombocytopenia were each observed in four patients (26.7%). Blood transfusion was required by two patients, and platelet transfusion by one.

Non-hematologic toxicity seen in the phase II study is summarized in Table 7. Stomatitis, anorexia, nausea/vomiting, diarrhea, fever and alopecia were commonly observed, but there were no grade 3 or 4 episodes except for one of grade 3 fever (6.7%). AST, ALT and total bilirubin levels, which were the referenced indices of hepatic function, were slightly increased, but no effect was seen on BUN or serum creatinine levels, the indices of renal function. There were four patients (33.3%) with abnormal ECG, showing nonspecific decreases in T-wave

level without ST change. Other effects on cardiac function were palpitation and arrhythmia, occurring in one patient each. No patient had reactions such as abnormal visual system (i.e., myodesopsia), eruption, melaena, or hematemesis, all observed in the phase I study.

Discussion

The present study was performed as a 3-day consecutive administration every 3 weeks, on the basis of encouraging experimental findings that amrubicin exerted more potent antitumor activity on human tumor xenografts implanted in nude mice in the divided treatment schedule than in the single treatment schedule [9]. When given on 3 consecutive days every 3 weeks, amrubicin achieved an overall response rate of 25% (7PRs in 28 patients) in previously untreated patients with advanced NSCLC. It has been reported that amrubicin also demonstrated an overall response rate of 25% (5 PRs in 20 patients) in an early phase II study which was conducted in chemotherapy-naöve patients by single-dose intravenous injection of 120 mg/m² every 3 weeks [6]. The data, therefore, indicate that there was no difference in the response rate between two clinical studies conducted under different treatment schedules, but the scales were too small to evaluate which of the two treatment schedules is superior; single-dose treatment or 3-day consecutive treatment, because only 20 or 28 patients were enrolled into each study. Subsequent, larger scale clinical studies are needed for confirmation.

Currently, NSCLC is treated with newer agents such as taxanes, gemcitabine, vinorelbine, and irinotecan, in combination with cisplatin and carboplatin, and these agents have single-agent reproducible response rates of more than 20% for NSCLC [13, 14]. Amrubicin showed response rates of more than 20% in two clinical studies conducted independently and under differing treatment schedules, as described above. These reproducible results strongly suggest that amrubicin is an anticancer agent with promising single-agent activity on NSCLC, comparable to the newer agents for NSCLC in efficacy, and further clinical trials are warranted to evaluate it. In addition, amrubicin is different from other newer agents in mode of action [15], in that it is an potent inhibitor of topoisomerase II, so that amrubicin is expected to play an important role in combination therapy, differently from other agents.

The major toxicity of amrubicin was hematologic, and especially neutropenia and leukopenia were remarkable. In the phase II study, 53.3% and 73.3% of patients experienced grade 3 and 4 leukopenia and neutropenia, respectively. On the other hand, non-hematologic toxicity such as anorexia, nausea and vomiting, diarrhea, fever, and alopecia was frequently observed, but relatively mild; grade 3 or 4 episodes were not seen other than in one patient (6.7%) who experienced grade 3 fever.

As noteworthy toxicity, grade 3 melaena and grade 4 hematemesis were noted in one patient each in the phase I study, although these episodes were not observed in the clinical trials using single-bolus treatment [6, 16]. These toxicities were considered to be associated with the long-term treatment of nonsteroidal anti-inflammatory agents, because these two patients had received indomethacin or diclofenac sodium for more than 50 days. The criteria for entry into the study was therefore revised to exclude patients who had been treated with nonsteroidal anti-inflammatory agents for a long period, and thereafter such episodes have not been experienced. As uncommon toxicity, two episodes of grade 1 myodesopsia and one episode of grade 1 eruption occurred in a phase I study, but these episodes were not observed in the subsequent phase II study.

In a phase II study, 4 patients (33.3%) experienced ECG abnormality, showing nonspecific decreases in T-wave level without ST change. Other effects on cardiac function were palpitation and arrhythmia, which occurred in one patient each. All these effects seemed to be different from cardiomyopathy caused by cumulative doses of doxorubicin, but these data show that amrubicin might affect cardiac function in a different manner from doxorubicin. Therefore, careful observation might be needed concerning the effects of amrubicin on cardiac function in subsequent clinical studies.

Appendix

Amrubicin has showed reproducible response rates of 18.3% (11/60) and 27.9% (17/61) in two subsequent phase II studies when used as single agents in previously untreated patients with advanced NSCLC. Amrubicin, therefore, is considered to be comparable to newer agents such as paclitaxel, docetaxel, gemcitabine, vinorelbine, and irinotecan in efficacy for NSCLC. The clinical study of amrubicin in combination with other agents, in particular cisplatin, is currently planned.

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Non-small cell lung cancer: radiation therapy for locoregional recurrence after complete resection

Abstract
Background. We investigated patterns of failure after radical radiation therapy in relation to the radiation field in patients with postsurgical locoregional recurrence of non-small cell lung cancer.
Methods. Between 1992 and 2002, 31 patients with locoregional recurrence were treated with radiation therapy. At the time of radiation therapy, the sites of recurrence were the bronchial stump, the regional lymph nodes, the chest wall, and both the regional lymph nodes and the chest wall in 7, 20, 3, and 1 patient, respectively. The prescribed dose was 60 Gy in 30 fractions over 6 weeks in all patients.
Results. The response rate was 87%. The overall 1-year, 2-year, and 4-year Kaplan-Meier survival rates were 61%, 30%, and 15%, respectively, and the median survival time was 14 months. Locoregional relapse with or without distant metastasis occurred in 15 patients (in-field, 7; marginal, 7; out-field, 1), and distant metastasis alone occurred in 7 patients. The sites of marginal relapse were the upper margin in two patients, the ipsilateral margin in one patient, the contralateral margin in one patient, and the lower margin in three patients, respectively (in one patient, the data for marginal relapse overlapped). In all patients with relapse on the lower margin, the mediastinal lymph nodes were dissected at the initial surgery.

Conclusion. Postoperative recurrent non-small cell lung cancer showed distinctive features: the response rate was high, and the incidence of marginal relapse was also high, as in small cell lung cancer. The incidence of lower marginal relapse was high, in contrast to that in surgery-naive patients.

Key words Non-small-cell lung cancer · Radiation therapy · Surgery · Recurrence

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Introductions

Stereotactic radiotherapy is rapidly spreading as a definitive treatment for stage I non-small cell lung cancer.[1] However, until recently, surgery has been a standard treatment for patients with early stage non-small cell lung cancer. After surgery, 5%–20% of patients develop locoregional recurrence as the first site of the failure.[2-5] For locoregional recurrence, radiation therapy is the treatment of choice, and several reports have shown that 2- and 5-year survival is comparable to those for radiation therapy alone in patients with primary stage III non-small cell lung cancer.[6-8] Therefore, we have treated these patients with radical radiation therapy when possible.

To investigate the role of radical radiation therapy in this patient population, the data were reviewed for a single institution. In particular, patterns of failure in relation to the radiation field were investigated.

Patients and methods

Eligible for the current analysis were patients with locoregional recurrence of non-small cell lung cancer after curative surgery. Patients with distant metastasis or contralateral hilar lymph node metastasis were excluded from this analysis. Between 1992 and 2002, 31 eligible patients were treated with radical radiation therapy in our

Table 1. Characteristics of patients

Characteristics	Number of patients
Sex	
Male	26
Female	5
Age (median, 68 years; range, 44–83 years)	
<70 years	16
≥70 years	15
Histology	
Squamous cell carcinoma	20
Adenocarcinoma	9
Other	2
ECOG performance status	
0–1	26
2	4
3	1
Surgery	
Lobectomy	24
Pneumonectomy	6
Wedge resection	1
Recurrence site	
Stump	7
Regional lymph node	
N2	13
N3	8
Peripheral	4
Longest diameter of recurrent tumor	
8–19 mm	3
20–39 mm	14
40–59 mm	12
60–85 mm	2

ECOG, Eastern Cooperative Oncology Group
Recurrence sites overlap in one patient

Table 2. Agents in chemotherapy

Characteristics	Number of patients
Cisplatin + vindesine	1
Gemcitabine + paclitaxel	1
Carboplatin + paclitaxel	1
Cisplatin + vinorelbine	1
Docetaxel	1
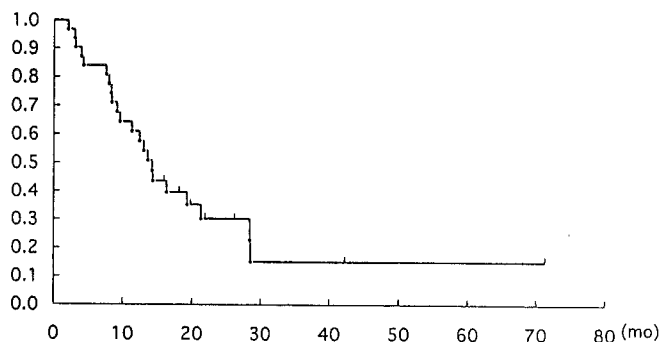
Fig. 1. Kaplan-Meier survival curve

institution. Oral informed consent was obtained from all patients.

Initial surgery was lobectomy in 24 patients (78%), pneumonectomy in 6 patients (19%), and wedge resection in 1 patient (3%). The mediastinal lymph nodes were dissected in 23 patients (74%). The median interval between initial surgery and radiation therapy was 15 months (range, 4–61 months).

Characteristics of the patients at the time of radiation therapy are summarized in Table 1. Recurrence was histologically diagnosed in 20 patients (65%). In other patients, obvious enlargement of the tumor was confirmed by postoperative follow-up computed tomography (CT). The sites of recurrence were the bronchial stump, the regional lymph nodes, the chest wall, or both the regional lymph nodes and the chest wall in 7 (23%), 20 (64%; N2, 12; N3, 8), 3 (10%), and 1 (3%; N2) patient, respectively. The longest diameter of the recurrent tumor, measured on CT, is also presented in Table 1.

Irradiation was performed with 10MV photons from a linear accelerator. Lung density correction was not performed. The prescribed dose was 60Gy in 30 fractions over 6 weeks in all patients. The radiation field contained the ipsilateral hilar lymph nodes and the mediastinal lymph nodes (from the subcarinal lymph nodes to the upper mediastinal lymph nodes) in 26 (84%) and 18 (58%) patients, respectively. Elective mediastinal irradiation was often omitted in patients with supraclavicular lymph node metastasis alone, or in patients who had undergone pneu-

monectomy. In these patients, the radiation field contained the recurrent tumor and margins of more than 20mm. When the initial radiation field contained the spinal cord, off-cord (i.e., the spinal cord was outside the field) oblique boost fields were used after initial irradiation with a dose of 30Gy or 40Gy. Chemotherapy was performed sequentially or concurrently in five patients. The agents are listed in Table 2.

Survival was calculated using the Kaplan-Meier method, and the differences between the curves were analyzed using the generalized Wilcoxon method. Tumor response to irradiation was evaluated with CT. A complete response (CR) was defined as 100% regression of the tumor, and a partial response (PR) was defined as more than 50% regression of the tumor, when evaluated 0–6 months after irradiation.

Results

One patient could not receive the full dose of radiation therapy owing to the presence of a bronco-esophageal fistula. The overall 1-year, 2-year, and 4-year Kaplan-Meier survival rates were 61%, 30%, and 15%, respectively, and the median survival time was 14 months (Fig. 1). The response rate was 87% (CR, 23%; PR, 64%).

The median survival times according to various prognostic factors are summarized in Table 3. The median survival time among patients with recurrence in the bronchial stump and the regional lymph nodes was 16 months and 13 months, respectively (generalized Wilcoxon, $P = 0.28$). No correlations were found between survival and extent of initial surgery, tumor histology, or radiation field.

Locoregional relapse with or without distant metastasis occurred in 15 patients, and distant metastasis alone occurred in 7 patients. Local relapse was subgrouped accord-

Table 3. Median survival time (MST) according to various prognostic factors

Factor	MST (months)	P
Age		
<70	16	0.06
≥70	9	
Histology		
Squamous cell carcinoma	14	0.66
Adenocarcinoma	14	
Performance status		
0–1	16	0.01
2–3	4	
Surgery		
Lobectomy	14	0.85
Pneumonectomy	14	
Recurrence site		
Stump	16	0.11
N2	9	
N3	19	
Radiation field (mediastinum)		
Yes	12	0.07
No	21	

P value for the recurrence site was between the stump and N2 lymph node metastasis
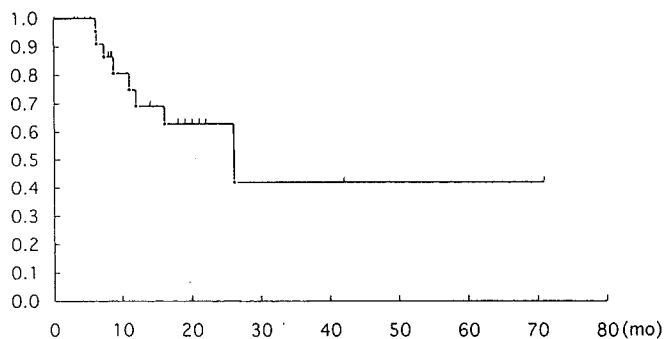
Fig. 2. Kaplan-Meier curve of the in-field control

ing to in-field relapse, marginal relapse, or out-field relapse, that is, relapse with respect to the radiation field (marginal relapse was defined as locoregional relapse at the edge of the radiation field). In-field relapse, marginal relapse, and out-field relapse occurred in seven, seven, and one patient, respectively (the out-field relapse was ipsilateral hilar lymph node metastasis; the lymph nodes had not been contained in the radiation field). The sites of marginal relapse were the upper margin in two patients, the ipsilateral margin in one patient, the contralateral margin in one patient, and the lower margin in three patients, respectively (in one patient, the data for marginal relapse overlapped). In four of the seven patients with marginal relapse, the radiation field contained the mediastinal lymph nodes. In all patients with relapse on the lower margin, the mediastinal lymph nodes were dissected at the initial surgery. The 2-year and 4-year in-field control rates were 62% and 41%, respectively (Fig. 2).

Discussion

There are several reports on the role of radiation therapy in the treatment of patients with postoperative locoregional recurrent non-small cell lung cancer. Although the number of patients was not large in the current study, the prescribed dose was uniform and patterns of recurrence in relation to the radiation field were investigated.

In surgery-naive patients, marginal relapse after radiation therapy occurred in 4% and 16% of patients with non-small cell lung cancer and with small cell lung cancer, respectively, in our institution.[9,10] However, the presented results showed that marginal relapse occurred in 23% of the patients with postoperative locoregional recurrent non-small cell lung cancer. A narrow radiation field did not cause the frequent marginal relapse since among 18 patients with the conventional radiation field, which contained the mediastinal lymph nodes, marginal relapse occurred in 22%. Furthermore, the response rate was 87%, which is higher than the usual response rate in surgery-naive non-small cell lung cancer. These features were similar rather to those of small cell lung cancer. However, causes for the distinctive features are unclear; invasively spread tumors might be specific to this population, or the nature of the tumor may have been changed by surgery.

In patients with surgery-naive small cell lung cancer, marginal relapse frequently occurs on the upper margin of the radiation field.[10] However, in the current study, the incidence of lower marginal relapse was high. In all patients with lower marginal relapse, the mediastinal lymph nodes were dissected. Therefore, a change in lymphatic circulation by surgery is considered to have caused the lower marginal relapse.

The median survival time of 14 months and the 2-year survival of 30% are comparable to results for radiation therapy alone in patients with surgery-naive locally advanced non-small cell lung cancer.[11,12] Therefore, radiation therapy is considered to play a role in the treatment of postoperative recurrent non-small cell lung cancer. However, the role of radiation therapy will be changed by progress in surgical techniques or in imaging techniques used for diagnosis, such as positron emission tomography.[13,14]

In conclusion, postoperative recurrent non-small cell lung cancer showed distinctive features: the response rate was high, and the incidence of marginal relapse was also high, similar to those of small cell lung cancer. The incidence of lower marginal relapse was high, in contrast to that in surgery-naive patients.

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A Phase I/II Study Comparing Regimen Schedules of Gemcitabine and Docetaxel in Japanese Patients with Stage IIIB/IV Non-small Cell Lung Cancer

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Objective: Gemcitabine and docetaxel are non-platinum agents with activity in non-small cell lung cancer (NSCLC). This study was conducted to determine and evaluate the recommended regimen of gemcitabine–docetaxel and evaluated its efficacy and safety in chemonaive Japanese NSCLC patients.

Methods: In phase I, patients with stage IIIB/IV NSCLC were randomized and received either gemcitabine on days 1 and 8 plus docetaxel on day 1 or gemcitabine on days 1 and 8 plus docetaxel on day 8. The recommended regimen was the dose level preceding the maximum tolerated dose; once determined, patients were enrolled in phase II. Efficacy and toxicity were evaluated in all patients.

Results: Twenty-five patients were enrolled in phase I and six patients were given the recommended regimen; gemcitabine 1000 mg/m^2 on days 1 and 8 plus docetaxel 50 mg/m^2 on day 8. An additional 34 patients were enrolled into phase II and administered with the recommended regimen. The response rate was 32.2% [95% confidence interval (CI) 20.6–45.6%] overall and 30.0% (95% CI 16.6–46.5%) in patients with the recommended regimen (40 patients). Although grade 3 interstitial pneumonia was observed in two patients (5.0%) who received the recommended regimen, both recovered shortly after steroid treatment. No unexpected events were observed throughout this study.

Conclusions: Gemcitabine 1000 mg/m^2 on days 1 and 8 plus docetaxel 50 mg/m^2 on day 8 has comparable efficacy and more tolerable toxicities than previously reported platinum-based regimens. These results should be verified by a phase III study.

Key words: docetaxel – gemcitabine – non-small cell lung cancer

INTRODUCTION

Non-small cell lung cancer (NSCLC) is one of the most common malignant tumors, progresses in a short time period, has a bleak prognosis, and represents the leading cause of cancer death in the world. The number of patients with NSCLC is increasing, and most tumors are inoperable. Despite improvements in the detection and treatment of NSCLC, long-term survival is rare. Therefore, the development of new chemotherapy treatments is essential.

The use of single-agent and combination chemotherapy against NSCLC has been studied. Platinum-based regimens have shown high efficacy but at the cost of severe toxicities (1,2). Therefore, non-platinum agents such as gemcitabine, docetaxel, paclitaxel, irinotecan and vinorelbine have been developed and have proven their efficacies. Among the new agents, the combination of gemcitabine and docetaxel has emerged as one of the most promising, showing equivalent efficacy with, and less toxicity than, cisplatin-based chemotherapies (3).

Gemcitabine (2'-deoxy-2',2'-difluorocytidine monohydrochloride) is a nucleoside antimetabolite against deoxycytidine. It is intracellularly metabolized to gemcitabine triphosphate,

which inhibits DNA synthesis, and has shown potent cytocidal activity against solid tumors (4–8).

Docetaxel, an antineoplastic agent that acts on microtubules to promote formation of abnormal microtubule bundles, has also shown cytotoxicity (9–11). Gemcitabine and docetaxel have different mechanisms of action, but by combining them, there is the potential of synergistic antitumor activity (12).

Several studies have been conducted to evaluate the therapeutic benefits of gemcitabine and docetaxel (13–15). The efficacy of gemcitabine–docetaxel is similar to platinum-based regimens, but due to each drug's non-overlapping toxicities, their combination produces toxicities more tolerable than platinum-based regimens. Georgoulias et al. (16) compared gemcitabine 1100 mg/m^2 on days 1 and 8 plus docetaxel 100 mg/m^2 on day 8 with cisplatin 80 mg/m^2 on day 2 plus docetaxel 100 mg/m^2 on day 1 in 441 patients with NSCLC. They reported that the two regimens were equivalent in efficacy, but toxicities were more severe for the combination of docetaxel and cisplatin.

There has been no published report considering both administering dose and schedule for the combination of gemcitabine and docetaxel. Therefore, we conducted a phase I/II study to compare two schedules of gemcitabine–docetaxel in patients with NSCLC and determine the recommended regimen in phase II. We assessed the efficacy and safety in all 59 patients: the efficacy and detailed safety profile were also evaluated in 40 patients who were given the recommended regimen.

SUBJECTS AND METHODS

ELIGIBILITY CRITERIA

Japanese patients with histologically or cytologically confirmed unresectable TNM stage IIIB or IV NSCLC who met the following criteria were eligible for the study: suitable for first-line chemotherapy with no prior chemotherapy; measurable lesions that can be accurately measured in at least one dimension; aged 20–74 years; Eastern Cooperative Oncology Group (ECOG) performance status of 0–1; a life expectancy of at least 3 months; and adequate organ functions as indicated by white blood cell count $\geq 4.0 \times 10^9$/l, absolute neutrophil count $\geq 2.0 \times 10^9$/l, platelets $\geq 100 \times 10^9$/l, hemoglobin ≥ 9.5 g/dl, aspartate aminotransferase/alanine aminotransferase ≤ 2.5 times the upper limit of normal, total bilirubin ≤ 1.5 times the upper limit of normal, serum creatinine $\leq$ the upper limit of normal, PaO$_2$ in arterial blood ≥ 60 torr. If a patient had received radiotherapy during the 3 weeks before enrollment, the measurable disease had to be outside of the radiation port.

Patients were excluded from the study if they had radiologically and clinically apparent interstitial pneumonia or pulmonary fibrosis, intracavitary fluid retention requiring treatment, or grade 2–4 peripheral neuropathy or edema. Additional exclusion criteria included: superior vena cava syndrome; symptomatic brain metastasis; pregnancy or breast-feeding; active concurrent malignancy; any serious concurrent illness (e.g. uncontrolled diabetes mellitus, hepatopathy, angina pectoris, myocardial infarction within 3 months after onset, severe infection, or fever suggestive of severe infection); history of serious drug allergy; or any condition that, in the opinion of the investigator, disqualified the patient based on safety.

This study was conducted in accordance with the Declaration of Helsinki, Japanese Guidelines for Clinical Evaluation of Antineoplastic Agents (promulgated in February 1991) and good clinical practice. All patients who entered into this study were required to give written informed consent.

STUDY DESIGN AND TREATMENT

This was a multicenter, open-label, phase I/II study of gemcitabine and docetaxel in Japanese patients with advanced NSCLC.

In the phase I portion of this study, patients were randomized into two arms, each with a different treatment schedule. In both arms (Arm 1 and Arm 2), gemcitabine was administered in a 30-min infusion on days 1 and 8, every 21 days. In Arm 1, docetaxel was administered intravenously over at least 1 h on day 1; in Arm 2, docetaxel was given on day 8. The administration of docetaxel followed an intravenous infusion of dexamethasone 4 mg, and gemcitabine was given immediately after the docetaxel infusion.

Patients were discontinued from the study due to progressive disease; inability to initiate a treatment cycle even at 6 weeks after the start of the previous cycle; recurrence of a dose-limiting toxicity (DLT) after resumption of the study treatment at a reduced dose; occurrence of a serious adverse event or aggravation of a concomitant illness (e.g. interstitial pneumonia, pulmonary fibrosis, or severe infection) which caused rapid aggravation of disease and precluded continuation of the study treatment; patient's request to withdraw from the study; or any event that required discontinuation in the opinion of the investigator.

During study enrollment, the current approved maximum dosage of gemcitabine and docetaxel as single agents in Japan was 1000 mg/m^2 and 60 mg/m^2, respectively. In phase I, the sample size was determined to be six per cohort based on the conventional design of phase I clinical studies of antineoplastic agents. In this study, both arms were randomized according to a predetermined schedule, enrolled patients in cohorts of six, and were initially treated at dose level 1 (gemcitabine 1000 mg/m^2 and docetaxel 50 mg/m^2). For the first cycle of treatment, patients were treated on an inpatient basis; if their condition permitted, patients were treated on an outpatient basis thereafter. If fewer than 50% of the patients in dose level 1 experienced DLTs, patients were enrolled at dose level 2 (gemcitabine 1000 mg/m^2 and docetaxel 60 mg/m^2). If 50% or more of the patients in dose level 1 experienced DLTs, patients were enrolled at dose level 0 (gemcitabine 800 mg/m^2 and docetaxel 50 mg/m^2) (Fig. 1). The maximum tolerated dose (MTD) was defined as the dose level that produced any of the following DLTs (per the National Cancer Institute–Common
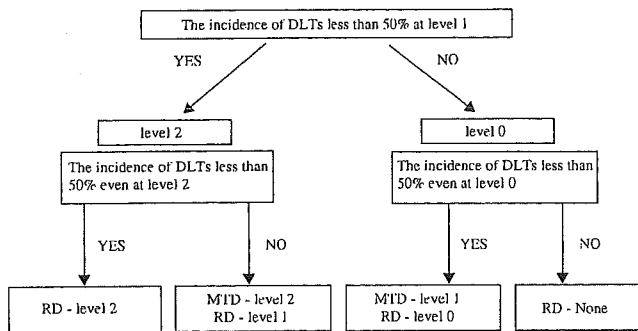
Figure 1. Recommended dosages in each arm. DLT, dose-limiting toxicity; RD, recommended dosage; MTD, maximum tolerated dose.

Toxicity Criteria scale) in 50% or more of patients during the first treatment cycle: grade 4 leukopenia or neutropenia persisting for at least 4 days; grade 3/4 neutropenia associated with a fever $\geqslant 38.0°C$ or infection; thrombocytopenia ($<20 \times 10^9/l$) or need of a platelet transfusion; or grade 3/4 non-hematological toxicities (excluding nausea/vomiting, anorexia, fatigue and hypersensitivity). G-CSFs were administrated for the treatment of grade 4 neutropenia or grade 3 neutropenic fever. A DLT was also reported if any day-8 doses were omitted and dosing requirements were not satisfied until after day 15, or if the second cycle was delayed until after day 29 because the dosing requirements were not satisfied.

The recommended dose for phase II had to be determined from the arm that reached the highest dose level. If at dose level 2 the incidence of DLTs was less than 50%, the recommended dose was defined as dose level 2. The arm that reached the higher dose level reflected the recommended regimen for phase II. If the recommended dose level for the two arms was identical, the recommended regimen would be decided according to the following steps: (i) if frequency of DLTs was 0% in one arm and 33.3% or more in the other arm, the former was selected. If this did not occur, then (ii) if the dose intensity for evaluable patients in one arm was higher by 10% or more than the other arm, the arm with the higher dose intensity was selected. If this did not occur, then (iii) the arm with the fewer day-8 dose omissions in first and second cycles was selected. If the recommended dosage regimen still could not be decided, the sponsor (Aventis Pharma Japan and Eli Lilly Japan K.K.) and the coordinating investigator determined the recommended phase II regimen. If the MTD was dose level 0 in both arms, the study was terminated (Fig. 1).

The sample size for the recommended regimen was determined as follows. The response rate of this regimen and gemcitabine single agent was assumed to be 35 and 20%, respectively, in view of the response rates previously achieved (9,10,17,18). If the sample size of the recommended regimen was set as 40 patients, the probability for the one-sided 90% lower limit of response rate to exceed 20% was 82%. Thus, the target sample size in the recommended regimen including six patients in phase I was set at 40 patients.

The phase II study was conducted with 34 patients. Forty patients who were given the recommended regimen were evaluated for the efficacy and detailed safety profile: these patients consisted of six and 34 patients who entered into the study at phase I and II, respectively.

In this phase I/II study, patients received a minimum of two cycles of gemcitabine–docetaxel and up to four additional cycles.

DOSE MODIFICATIONS

During a cycle, dose modifications were not allowed. If not all of the following requirements were satisfied on either the day of treatment or the previous day, administrations of gemcitabine and docetaxel were delayed until the patient completely recovered. For gemcitabine and docetaxel doses administered on day 1 of Arm 1 or gemcitabine on day 1 of Arm 2, delays occurred for patients with an absolute neutrophil count $<1.5 \times 10^9/l$, a platelet count $<70 \times 10^9/l$, any grade 3/4 non-hematologic toxicities (except PaO_2), or PaO_2 <60 torr. When gemcitabine was given on day 8 of Arm 1, exceptions included leukopenia $<2.0 \times 10^9/l$ and an absolute neutrophil count $<1.0 \times 10^9/l$, a platelet count $<70 \times 10^9/l$, any grade 3/4 non-hematological toxicities. When gemcitabine was given on day 8 of Arm 2, exceptions included an absolute neutrophil count $<1.5 \times 10^9/l$, a platelet count $<70 \times 10^9/l$, any grade 3/4 non-hematological toxicities. If a patient developed a DLT, the subsequent doses were cancelled, and in the next cycle the patient could resume the study treatment at the next lower dose level. If a patient developed a DLT at dose level 0, gemcitabine 800 mg/m² and docetaxel 40 mg/m² were administered in the next cycle.

BASELINE AND TREATMENT ASSESSMENT

Assessments at baseline included tumor measurements by X-ray and computed tomography (CT) scan within 4 weeks before the day of starting the study treatment. Equally, grading performance status and physical examination were performed within a week; hematology, blood chemistries, urinalysis, arterial blood gas analysis and electrocardiogram were observed within 2 weeks.

After the start of treatment, tumor measurements were obtained every 2 weeks via X-ray and 4 weeks via CT scan. Tumor response was assessed with the World Health Organization (WHO) criteria. Safety assessments, including performance status, hematology, blood chemistries and urinalysis, were obtained weekly. Physical examination, arterial blood gas analysis and electrocardiogram were performed at any time. Adverse events were estimated according to National Cancer Institute–Common Toxicity Criteria version 2.0. All patients were assessed for efficacy and safety. An additional response rate was recorded for patients who received the recommended regimen in phase I and all phase II patients.

RESULTS

PATIENT CHARACTERISTICS

Between July 2000 and July 2002, 59 chemonaive patients (43 male, 16 female) with NSCLC were enrolled in phase I and II portions from the five hospitals after approval by the IRB. Twenty-five patients were enrolled in the phase I portion of the study, and 34 patients were enrolled in phase II. Baseline patient characteristics for all patients and patients who received the recommended regimen are summarized in Table 1.

PHASE I

Twenty-five patients were enrolled into the phase I portion of the study. The number of patients treated and the DLTs observed in the first cycle at each dose level of gemcitabine and docetaxel are shown in Table 2.

In Arm 1, 50% of patients had DLTs at dose level 1 and dose level 0, therefore Arm 1 could not be the recommended regimen: there were 2/6 and 3/6 patients who achieved partial response (PR) at dose level 1 and 0 in Arm 1, respectively.

In Arm 2, no DLT was observed at dose level 1: 3/6 patients achieved PR. At dose level 2, one patient discontinued due to progressive disease; therefore, one patient was added. However, another patient discontinued due to grade 3 hypersensitivity (not a DLT). In this regimen, two DLTs had already been observed in five other patients, but the sponsors (Aventis Pharma Japan and Eli Lilly Japan K.K.) and investigators decided not to add one more patient to dose level 2 in Arm 2 in consideration of patients' safety. PRs were observed in 2/7 patients at dose level 2 of Arm 2.

Therefore, the recommended regimen was determined as gemcitabine 1000 mg/m^2 on days 1 and 8 plus docetaxel 50 mg/m^2 on day 8 due to the incidence of DLT.

DOSE ADMINISTRATION

In Arm 1, a total of 49 cycles were accomplished. One case delayed the date of administration on day 1 (defined as more than 8 days) as a matter of convenience; seven and four cases delayed their dates of administration on day 8 (defined as more than 1 day) because of adverse events and non-medical reasons, respectively; and four cases could not be treated on day 8 because of adverse events. In Arm 2, including phase I and II portions, a total of 145 cycles were accomplished. Four and five cases delayed their dates of administration on day 1 because of adverse events and non-medical reasons, respectively; 21 and nine cases delayed their dates of administration on day 8 because of adverse events and non-medical reasons, respectively; and two cases could not be treated on day 8 because of

Table 1. Baseline characteristics

Patient characteristics	All patients ($n = 59$), n (%)	Patients who received the recommended regimen ($n = 40$), n (%)
Gender		
Male	43 (72.9%)	26 (65.0%)
Female	16 (27.1%)	14 (35.0%)
Age		
Median	62	64
Range	38–74	38–74
ECOG performance status		
0	5 (8.5%)	2 (5.0%)
1	54 (91.5%)	38 (95.0%)
Stage		
IIIB	14 (23.7%)	8 (20.0%)
IV	33 (55.9%)	23 (57.5%)
Postsurgical recurrence	12 (20.3%)	9 (22.5%)
Histological type		
Adenocarcinoma	34 (57.6%)	25 (62.5%)
Squamous cell carcinoma	19 (32.2%)	14 (35.0%)
Large cell carcinoma	5 (8.5%)	1 (2.5%)
Other	1 (1.7%)	0 (0%)
Prior therapy		
None	45 (76.3%)	29 (72.5%)
Surgery	13 (22.0%)	11 (27.5%)
Radiotherapy	0 (0%)	0 (0%)
Radiotherapy and surgery	1 (1.7%)	0 (0%)

ECOG, Eastern Cooperative Oncology Group.

Table 2. Phase I dose-limiting toxicities

Dose level	GEM/DOC (mg/m^2)	Arm 1	Arm 2
0	800/50	3/6 patients: • G3 ALT increased • G1 fever, G3 neutropenia • G2 infection, G3 neutropenia	N/A
1	1000/50	3/6 patients: • G3 infection, G3 neutropenia • G4 neutropenia, G1 fever, G3 infection • G3 neutropenia, G2 infection, G3 arrhythmia, G3 diarrhea	0/6 patients
2	1000/60	N/A	2/5 patients: • G3 ALT increased • G2 fever, G3 neutropenia

GEM, gemcitabine; DOC, docetaxel; G, grade; ALT, alanine aminotransferase; N/A, not applicable.

adverse events. The most common adverse event for a dose delay was neutropenia.

EFFICACY

All 59 patients were involved in the analysis for efficacy, and 19 of 59 patients achieved PR for an overall response rate of 32.2% [95% confidence interval (CI) 20.6–45.6%]. Of the 40 patients who received the recommended regimen in either phase I or phase II, 12 patients achieved PRs for a response rate of 30.0% (95% CI 16.6–46.5%).

The median time to progressive disease in all 59 patients was 111 days (95% CI 71–154 days). Median survival time was 11.9 months (95% CI 7.0–15.0 months), with 1-year survival rate at 47.1% (95% CI 34.0–60.2%).

SAFETY

All 59 patients were evaluable for safety. Grade 3 and 4 drug-related toxicities observed in all 59 patients are shown in Table 3. Grade 3 and 4 drug-related toxicities observed in 40 patients who received the recommended regimen are also shown in Table 4.

In all 59 patients, grade 3 and 4 neutropenia were observed in 19 (32.2%) and 20 (33.9%) patients, respectively. Grade 3 and 4 leukopenia were observed in 24 (40.7%) and four (6.8%) patients, respectively. Grade 3 non-hematological toxicities included infection in four patients (6.8%), anorexia in four patients (6.8%), and nausea, diarrhea, rash and constipation in three patients (5.1%) each. After starting docetaxel administration, grade 3 interstitial pneumonia was reported in three patients (5.1%), all of whom recovered shortly after steroid treatment; grade 4 anaphylaxis was reported in two patients (3.4%). There were no toxic deaths.

DISCUSSION

In this phase I/II study, we examined the activity and tolerability of gemcitabine and docetaxel. In phase I, the recommended regimen was determined as gemcitabine 1000 mg/m^2 on days 1 and 8 plus docetaxel 50 mg/m^2 on day 8. The response rate of all 59 patients was 32.2% (95% CI 20.6–45.6%). When re-evaluated in the 40 patients who received the recommended regimen, the response rate was 30.0% (95% CI 16.6–46.5%). Although the number of patients was limited, Arm 1 (docetaxel on day 1) had a numerically better response: for the 12 patients in Arm 1, five PRs were recorded for a response rate of 42%. However, Arm 1 had more toxicities than the docetaxel on day-8 schedule.

Overall, the toxicity associated with the gemcitabine–docetaxel regimen was manageable. In Arm 1, five patients (42%) had grade 3/4 neutropenia supervened with infection or fever, while only one patient (9%) had grade 3 neutropenia with infection or fever in Arm 2. This indicated that docetaxel was better tolerated on day 8 than on day 1 in a 21-day cycle. It is speculated that the influence of time to nadir of neutropenia is different in each agent: 14–20 days with gemcitabine and 9 days with docetaxel. The time to recover from nadir is

Table 3. NCI–CTC grade 3/4 toxicities (*n* = 59)

Toxicities	Grade 3		Grade 4	
	n	%	*n*	%
Hematological toxicities				
Leukopenia	24	40.7	4	6.8
Neutropenia	19	32.2	20	33.9
Lymphopenia	10	16.9	0	0.0
Hemoglobin decreased	4	6.8	0	0.0
Thrombocytopenia	1	1.7	0	0.0
Thrombocytosis	1	1.7	0	0.0
Non-hematological toxicities				
ALT increased	5	8.5	0	0.0
Infection	4	6.8	0	0.0
Anorexia	4	6.8	0	0.0
Nausea	4	6.8	0	0.0
Diarrhea	3	5.1	0	0.0
Interstitial pneumonia	3	5.1	0	0.0
Rash	3	5.1	0	0.0
Constipation	3	5.1	0	0.0
AST increased	2	3.4	0	0.0
Fatigue	2	3.4	0	0.0
Vomiting	2	3.4	0	0.0
Hyperglycemia	1	1.7	0	0.0
Hyponatremia	1	1.7	0	0.0
Allergic reaction	1	1.7	0	0.0
Vasovagal reaction	1	1.7	0	0.0
Body temperature decrease	1	1.7	0	0.0
Weight increase	1	1.7	0	0.0
Hypotension	1	1.7	0	0.0
Pneumonia	1	1.7	0	0.0
Arrhythmia	1	1.7	0	0.0
Edema	1	1.7	0	0.0
Neuropathy peripheral	1	1.7	0	0.0
Anaphylaxis	0	0.0	2	3.4

NCI–CTC, National Cancer Institute–Common Toxicity Criteria version 2.0; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

7–8 days with gemcitabine and 8 days with docetaxel. This could explain why docetaxel on day 8 was better tolerated.

Meta-analysis studies have reported that cisplatin-based regimens produce a significant survival benefit in NSCLC (20–23), improve median survival time by 6–8 weeks and 1-year survival rate from 15% to 25% when compared with the best supportive care (24). But studies with platinum-based combinations have also reported severe toxicities, so the deterioration of patients' quality of life is a major problem to be solved (3).

New effective non-platinum-based therapies have been used in various combinations in recent years, and the combination of gemcitabine and docetaxel has been established as one of the

Table 4. NCI–CTC grade 3/4 toxicities (*n* = 40, recommended regimen)

Toxicities	Grade 3		Grade 4	
	n	%	*n*	%
Hematological toxicities				
Leukopenia	13	32.5	2	5.0
Neutropenia	12	30.0	11	27.5
Lymphopenia	5	12.5	0	0.0
Hemoglobin decreased	2	5.0	0	0.0
Thrombocytopenia	1	2.5	0	0.0
Thrombocytosis	1	2.5	0	0.0
Non-hematological toxicities				
ALT increased	2	5.0	0	0.0
Diarrhea	2	5.0	0	0.0
Infection	2	5.0	0	0.0
Interstitial pneumonia	2	5.0	0	0.0
Rash	2	5.0	0	0.0
Fatigue	2	5.0	0	0.0
Nausea	2	5.0	0	0.0
Vomiting	2	5.0	0	0.0
Hyperglycemia	1	2.5	0	0.0
Hyponatremia	1	2.5	0	0.0
AST increased	1	2.5	0	0.0
Allergic reaction	1	2.5	0	0.0
Vasovagal reaction	1	2.5	0	0.0
Anorexia	1	2.5	0	0.0
Body temperature decrease	1	2.5	0	0.0
Weight increase	1	2.5	0	0.0
Hypotension	1	2.5	0	0.0
Pneumonia	1	2.5	0	0.0
Edema	1	2.5	0	0.0
Constipation	1	2.5	0	0.0
Peripheral neuropathy	1	2.5	0	0.0
Anaphylaxis	0	0.0	2	5.0

NCI–CTC, National Cancer Institute–Common Toxicity Criteria version 2.0; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

well-examined regimens. In recent studies using gemcitabine–docetaxel in NSCLC, response rates of 25–50% (19,25–29) and time-to-progression of disease of 106–132 days (31,32) have been reported. Georgoulias et al. (16) reported that the gemcitabine–docetaxel and docetaxel–cisplatin regimens they compared were equivalent in efficacy, but toxicity was severe in the latter. While docetaxel–cisplatin regimens showed severe toxicities of grade 3 anemia (5%), grade 3/4 neutropenia (13%/21%), grade 3 nausea/vomiting (10%) and grade 3 diarrhea (8%), gemcitabine–docetaxel regimens had grade 3/4 anemia (1%/1%), grade 3/4 neutropenia (11%/11%), grade 3 nausea/vomiting (2%) and grade 3/4 diarrhea (2%/1%) in 441 patients. However, the difference of efficacy

and safety by the administration schedule and dosage of gemcitabine and docetaxel has not been well documented.

There are some studies that have examined the efficacy and safety of the same schedule as the recommended regimen in our study, namely gemcitabine on days 1 and 8 plus docetaxel on day 1. In these studies dosages were various: gemcitabine was 800–1100 mg/m² and docetaxel was 60–100 mg/m² (18,19,27–30). Response rates in these studies also varied from 16 to 38%, which indicates that the response rate of the recommended regimen in our study (30.0%) was clinically meaningful because the dosage of docetaxel (50 mg/m²) in our study is less than that in any other studies. This might have contributed to the relatively mild toxicities of our recommended regimen.

In another study (26), a high response rate (50.0%) was achieved in patients with another administering schedule: gemcitabine 1000 mg/m² on days 1 and 10 plus docetaxel 80 mg/m² on day 1, administered every 21 days. The most common treatment-related toxicity was myelosuppression. Grade 3/4 leukopoenia and neutropenia occurred in only six (18%) and eight (24%) patients, respectively.

The median survival was 11.9 months in our study, being slightly better than the result from the median survival of the phase III study with gemcitabine and cisplatin, which was 8.7–9.1 months (33,34). This result suggests that the regimen we selected in the phase II portion of this study is comparable in survival with the cisplatin-based regimen.

In conclusion, the combination of gemcitabine 1000 mg/m² on days 1 and 8 plus docetaxel 50 mg/m² on day 8 is suggested to be better tolerated and has equivalent efficacy to cisplatin-based therapy. These results should be verified by a phase III study in Japanese patients.

CONCLUSION

In this phase I/II study, we studied the activity and tolerability of gemcitabine and docetaxel in Japanese patients. The combination of gemcitabine 1000 mg/m² on days 1 and 8 plus docetaxel 50 mg/m² on day 8 is suggested to be well tolerated and has equivalent efficacy to cisplatin-based therapy.

Acknowledgments

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Hepatoma-derived growth factor as a prognostic marker in completely resected non-small-cell lung cancer

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Abstract. Hepatoma-derived growth factor (HDGF), unrelated to hepatocyte growth factor, is a heparin-binding protein originally purified from human hepatoma HuH-7 cells. HDGF exhibits mitogenic activities for certain hepatoma cells, fibroblasts and vascular smooth muscle cells, and angiogenic activities through nuclear targeting. Recently, HDGF was found to be a mitogen for lung epithelial cells *in vitro* and *in vivo*. This suggests that HDGF may play a critical role in the development and progression of lung cancer. We investigated, immunohistochemically, the relationship between HDGF expression and clinicopathological variables, and the prognostic significance of HDGF in 102 patients with completely resected non-small-cell lung cancer (NSCLC: 70 adenocarcinomas and 32 squamous cell carcinomas). To address the mechanism of action of HDGF, we evaluated the contribution of HDGF to tumor cell proliferation and intratumor angiogenesis using anti-Ki-67 and anti-CD31 antibodies, respectively. HDGF expression was strongly detected in the nucleus of cancer cells; the HDGF-labeling index (LI) was 20-95% (median 64.5%). There was no significant association between HDGF-expression level and clinicopathological variables. Patients with NSCLC showing a high HDGF-LI (≥65%) had significantly worse overall and disease-free survivals than those with NSCLC showing a low HDGF-LI. Multivariate analysis revealed that HDGF is a significant independent prognostic factor, more powerful than pathological stage. Moreover, HDGF expression correlated with Ki-67-LI and intratumor microvessel density. We consider HDGF as a useful prognostic marker for patients with completely resected

NSCLC and it may play a critical role in the pathobiology of lung cancer through its mitogenic and angiogenic activities.

Introduction

Hepatoma-derived growth factor (HDGF), unrelated to hepatocyte growth factor (HGF) produced by non-parenchymal cells, is a secretory heparin-binding protein that was purified from the conditioned medium of human hepatoma HuH-7 cells, and its cDNA was cloned from HuH-7 cells (1,2). HDGF represents a new family of growth factors called HDGF-related proteins (HRPs), including HRP1, HRP2, HRP3, HRP4 and p52/p75/lens epithelium-derived growth factor (LEDGF) (3). These proteins have in common the following characteristics: i) homology in the N-terminal amino acids [termed homologous to the amino terminus of HDGF (hath) region] containing a PWWP domain, which is suspected to play a role in cell growth and differentiation possibly by DNA binding, ii) bipartite nuclear localization signals, and iii) lack of signal peptides (3-5). Recent studies have shown that HDGF is an exogenous mitogen for HuH-7, Swiss 3T3 fibroblasts (2), endothelial cells (6-8), and vascular smooth muscle cells (9,10), and that nuclear targeting of HDGF is essential for its mitogenic activity (10,11).

As for roles of HDGF in tumor pathobiology, HDGF stimulates *in vitro* proliferation of hepatoma cells such as HuH-7, and antisense oligonucleotides of HDGF can suppress it (12). *In vivo*, HDGF induces tumorigenesis of NIH3T3 cells in nude mice through its angiogenic activity (7) and may also play an important role in the development and progression of hepatocellular carcinoma in humans and rodents on the basis that HDGF expression is higher in hepatoma cells than in the adjacent non-cancerous tissues (13).

Although HDGF was originally identified in hepatoma cells, HDGF and its mRNA are expressed in various normal adult tissues, including lung tissue (2,6,14). HDGF may be involved in fetal lung development (15). Recently, Mori *et al* (14) reported that HDGF is also a mitogen for lung epithelial cells *in vitro* and *in vivo*. Taken together, these findings suggest that HDGF may play a critical role in the development and progression of lung cancer.

The most common cancer in Japan today is lung cancer. Lung cancer was the leading indication for general thoracic

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surgery (~43%) and more than 20,000 patients were operated on at Japanese institutions in 2002 (16). Non-small-cell lung carcinomas (NSCLC) represent 98% of all operable cases of lung cancer, and they are still associated with a poor prognosis, even when operable. Many molecular markers of prognosis have been studied, although the critical cause for the poor prognosis of patients with NSCLC remains to be determined.

In the present study, we investigate immunohistochemically the relationship between HDGF expression and clinicopathological variables and the prognostic significance of HDGF in NSCLC patients who underwent complete resection. Additionally, to address the mechanism of action of HDGF on lung cancer biology, we evaluated the contribution of HDGF to tumor cell proliferation and intratumor angiogenesis.

Materials and methods

Patients and tumors. Among patients with primary lung carcinoma who were operated on at the Osaka Prefectural Medical Center for Respiratory and Allergic Diseases (Osaka, Japan) from 1994 through 1997, one hundred and two patients underwent complete resection for adenocarcinoma (n=70) or squamous cell carcinoma (n=32) without previous chemotherapy or radiotherapy, and adequate paraffin-embedded tissue sections were available. These patients had no other form of malignancy. Tumor specimens were fixed in 10% formaldehyde solution, embedded in paraffin and microscopically examined after hematoxylin and eosin (HE) staining. Histological classification of tumors was based on the World Health Organization criteria. Visceral pleural involvement was classified according to the Japan Lung Cancer Society (17) as follows: P0, the tumor does not penetrate the elastic layer of the visceral pleura; P1, the tumor penetrates the elastic layer but is not exposed on the pleural surface; P2, the tumor is exposed on the pleural surface but does not involve adjacent anatomic structures; and P3, the tumor involves adjacent anatomic structures (18). A tumor larger than 3 cm in diameter or a P2 tumor of any size was defined as T2 classification. All tumors were staged according to the TNM pathological classification of the American Joint Committee on Cancer (AJCC) and the International Union Against Cancer (UICC) (19): 40 stage I (23 cases in stage IA and 17 cases in stage IB), 21 stage II (3 cases in stage IIA and 18 cases in stage IIB), 35 stage III (26 cases in stage IIIA and 9 cases in stage IIIB) and 6 stage IV (patients with a metastatic nodule in the ipsilateral non-primary-tumor lobe of the lung). The patients (69 men and 33 women) were between 40 and 80 years of age (mean 64 years) and grouped according to age as being either <70 or ≥70 years old. Smoking status was 0-232 (median 44.5) pack-year, and patients were divided into 2 groups: those who smoked <40 pack-year and those who smoked ≥40 pack-year. Survival was calculated from the day of surgery, and follow-up of the 102 patients ranged from 4.1 to 108.9 (median 61.3) months; 54 patients (52.9%), without exception, died of recurrence or metastasis of lung cancer during follow-up. Our study was carried out with the approval of the ethical committee of the Osaka Prefectural Medical Center for Respiratory and Allergic Diseases.

Immunohistochemical examination. Immunohistochemical staining for HDGF was performed essentially as previously

described (7,12,14,20). The paraffin sections (4 μm thick) were deparaffinized, microwaved in 10 mmol/l citrate buffer (pH 6.0) and then immersed in methanol containing 0.3% hydrogen peroxide. Slides were blocked with normal goat serum and incubated with a 1:5,000 dilution of rabbit polyclonal IgG raised against C-terminus (231-240) of the human HDGF sequence for 30 min at room temperature. After washing the sections twice with phosphate-buffered saline, they were incubated with peroxidase-conjugated goat anti-rabbit immunoglobulin (Envision; Dako, Glostrup, Denmark) for 30 min at room temperature. After washing, diaminobenzidine tetrahydrochloride (DAB) solution was applied. The sections were then counterstained in hematoxylin. Specificity of the anti-HDGF antibody (Ab) had been previously demonstrated by Western blot analysis using recombinant human HDGF (14). Weak staining of smooth muscle cells and endothelial cells of blood vessels was used as the internal positive control. Negative controls were treated in the same way, but anti-HDGF Ab was replaced by non-immune rabbit serum. HDGF was detected mainly in the nucleus of cancer cells more strongly than in that of smooth muscle cells, and weakly in the cytoplasm of some cancer cells. HDGF immunoreactivity was judged positive when HDGF staining in the nucleus of tumor cells was equivalent to or stronger than that in the nucleus of smooth muscle cells. HDGF-labeling index (LI) was expressed as the proportion of cancer cells with positive HDGF nuclear reactivity.

Immunohistochemical staining for Ki-67 nuclear antigen was performed using a mouse monoclonal anti-human Ki-67 antigen Ab (MIB-1, DAKO) according to the manufacturer's instructions. Ki-67-LI was expressed as the proportion of Ki-67-positive cancer cells. For evaluation of HDGF- and Ki-67-LI, more than 1,000 cancer cells were counted in at least 5 representative areas without necrosis in each section. Intratumor angiogenesis was assessed by counting the microvessels detected with CD31 staining using a mouse monoclonal anti-human CD31 Ab (JC/70A, DAKO) according to the manufacturer's instructions. Intratumoral microvessel density (MVD) was calculated as the average value of microvessels/mm² using the criteria previously described elsewhere (21,22). After the area of highest vascularization was identified by scanning sections at low power, individual microvessel counts were determined at magnification x200 (0.95 mm² area) in 3 different fields under an Olympus microscope (Tokyo, Japan). All values determined by slide examination were presented by the median of scores evaluated by 3 investigators (Teruo Iwasaki, Yoshiaki Takada and Kunimitsu Kawahara).

Statistical analysis. The relationship between HDGF expression and clinicopathological variables [age, sex, smoking, tumor size, pathological stage, T-factor (classification), N-factor (classification), pleural involvement, vascular involvement, lymphatic involvement, histological type and degree of differentiation] was analyzed by the χ^2-test. The significance of differences in Ki-67-LI and MVD was tested by Student's t-test. The Kaplan-Meier method was used to estimate overall and disease-free survival as a function of time, and survival differences were analyzed by the log-rank test. Factors potentially related to overall and disease-free survival were analyzed by the Cox proportional-hazards model. For all