EGFR mutation in gefitinib-responsive small-cell lung cancer

Activating mutations within the tyrosine kinase domain of the epidermal growth factor receptor (EGFR) underlie responsiveness to gefitinib in non-small-cell lung cancer (NSCLC) [1–3]. To date, however, only a few EGFR mutations have been detected in other solid tumors [4]. We now describe a patient with gefitinib-responsive small-cell lung cancer (SCLC) who harbors a deletion in exon 19 of EGFR.

A 72-year-old woman with no history of smoking presented with a 2-week history of cough, dyspnea and intermittent hemoptysis. Computed tomography (CT) revealed a mass in the upper lobe of the right lung and a large metastatic mass in the liver. Bronchoscopic examination revealed a tumor occluding the right upper bronchus and a bronchoscopic biopsy was performed. Treatment with 250 mg of gefitinib once daily was initiated at the patient's request. Her symptoms improved rapidly, with CT performed 3 weeks after the initiation of gefitinib treatment revealing marked regression of both the primary lung tumor and the metastatic liver tumor. Histological examination of the transbronchial biopsy specimens showed that the tumor comprised small cells with round or oval nuclei (Figure 1A). The final pathological diagnosis was thus SCLC and was confirmed independently by three additional pathologists. Positive staining of the tumor cells for neural cell adhesion molecule (CD56), a sensitive and specific marker of neuroendocrine differentiation, supported the pathological diagnosis. Further immunohistochemical analysis revealed expression of EGFR in the tumor cells (Figure 1B). Direct sequencing of the region of EGFR that encodes the kinase domain (exons 18 to 21) in DNA isolated from tumor biopsy specimens identified a heterozygous in-frame 15-base pair deletion that resulted in the loss of amino acids 746 to 750 (delE746-A750) (Figure 1C). This mutation is identical to a previously described deletion in exon 19 of EGFR in NSCLC [1–3]. The mutation in the proband was detected in both sense and antisense sequences of the products of two independent polymerase chain reactions.

In contrast to NSCLC, EGFR expression has been reported to be low in SCLC. Gefitinib was recently shown to inhibit EGFR signaling in SCLC cell lines that express the receptor even at a low level [5], however, suggesting the presence of functional EGFRs in SCLC. As far as we are aware, ours is the first report of an EGFR mutation in a patient with SCLC, a finding that suggests that EGFR tyrosine kinase inhibitors may be a treatment option for a subset of SCLC tumors that express functional EGFRs.

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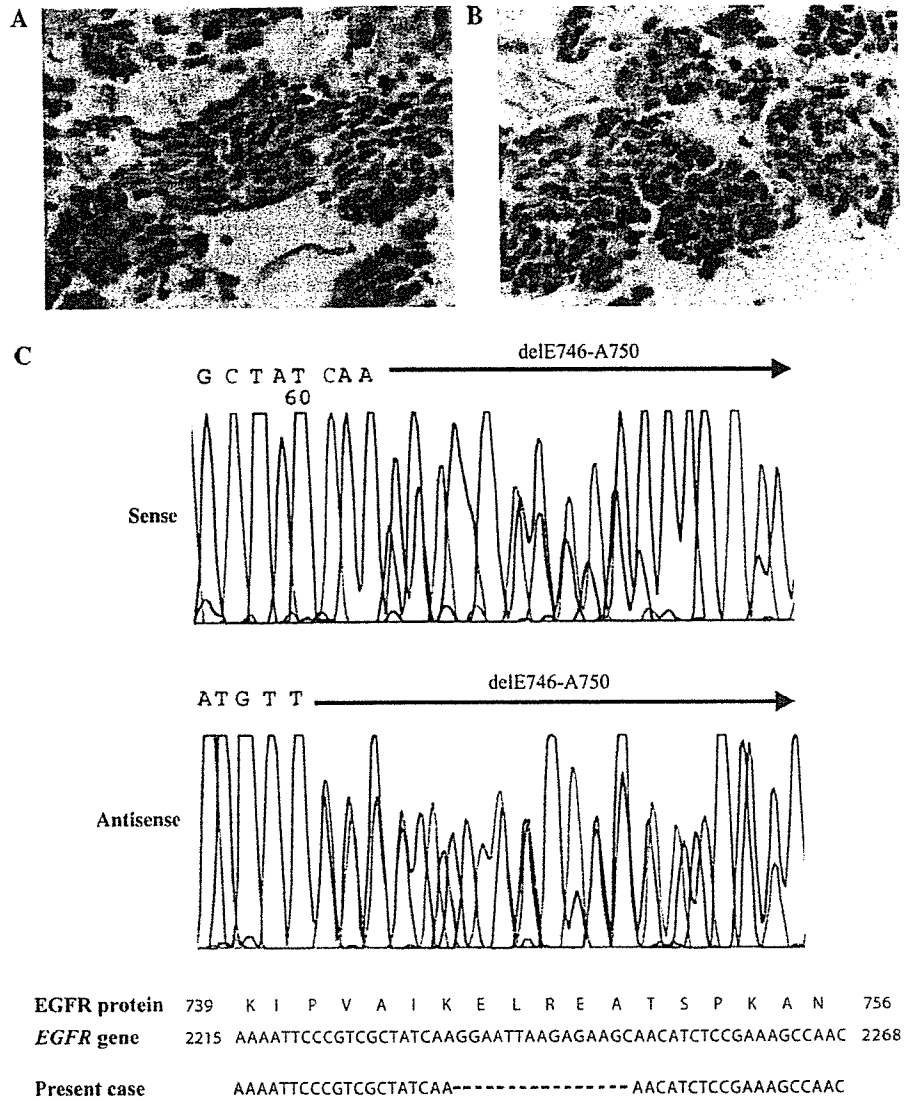

Figure 1. EGFR expression and mutation in tumor tissue at diagnosis of gefitinib-responsive SCLC. (A) Hematoxylin–eosin staining showed that the primary tumor was composed of small cells with round or oval nuclei and sparse cytoplasm. (B) Immunohistochemical analysis showed expression of EGFR in tumor cells. (C) Nucleotide sequencing of EGFR in tumor DNA revealed a heterozygous in-frame deletion within the region of the gene encoding the tyrosine kinase domain (double peaks).

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doi:10.1093/annonc/mdj114
Published online 15 December 2005

Cancer Chemother Pharmacol (2006) 58 (Suppl 1): s33–s37
DOI 10.1007/s00280-006-0313-7

Clinical development of EGFR-tyrosine kinase inhibitors in Japan

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Published online: 9 November 2006

Abstract Although the initial impact of the epidermal growth factor receptor-tyrosine kinase inhibitor (EGFR-TKI) gefitinib may have been less than spectacular in the field of non-small cell lung cancer (NSCLC), this EGFR-TKI does offer a therapy that, at least in the short term, markedly reduces tumors without bone marrow suppression including neutropenia and without causing severe nausea and vomiting even in NSCLC patients with the worst prognosis. This raises the possibility of putting the disease under control if only temporarily. Now we must be aware that overcoming gene mutation in lung cancer is the next significant milestone for new therapeutics. This report discusses clinical trials of EGFR-TKIs focusing on Japanese contributions to current knowledge, *EGFR* mutation, and future directions. A Japanese phase I clinical trial saw the first super-responders to gefitinib. Two randomized phase II trials identified Japanese, females, and those with adenocarcinoma of the lung as specific populations sensitive to gefitinib. Unexpectedly, in the context of first-line chemotherapy four phase III trials gave completely negative results for additional clinical benefit by EGFR-TKIs combined with standard chemotherapy. However, subset analysis suggested efficacy of this treatment strategy in nonsmokers and patients harboring activated-type *EGFR* mutations. In the settings of second-line and later therapy, two independent randomized placebo-controlled trials, BR.21 with erlotinib and ISEL with gefitinib, revealed better duration of overall survival, time to progression, and response rate in the EGFR-TKI versus control groups, although the result was nonsignificant in the latter study. Data suggesting that adenocarcinoma, Asian race, female, and nonsmoker are associated with better response to EGFR-TKI may be closely related with phenotype of *EGFR* mutations, making this parameter a "response predictive marker." On the other hand, some reports have stated that gene amplification of *EGFR* by FISH analysis shows better correlation with clinical benefit of EGFR-TKIs than that assessed by other means in large-scale phase III trials (BR21 and ISEL). Further validation of response predictive markers is needed. Recent studies of EGFR-TKIs in NSCLC provide novel biological insights and have given birth to the concept of patient selection for this disease. Further investigation of the biological significance of *EGFR* mutation and its validation as response predictive marker will lead to better treatments to come for NSCLC.

Keywords EGFR-TKI · Gefitinib · Erlotinib · *EGFR* mutation

This work was presented at the 21st Bristol-Myers Squibb Nagoya International Cancer Treatment Symposium, "Lung Cancer: Novel Therapy against Malfunctioning Molecules", 24–25 February 2006, Nagoya, Japan.

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Introduction

Epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs) have been clinically available for the treatment of nonsmall cell lung cancer

(NSCLC) for the past 4 years. In the course of clinical development of EGFR-TKIs, in comparison with conventional anticancer agents many unexpected findings were observed such as relating to tumor shrinkage, specific responder subsets, adenocarcinomatous disease, and gene mutation. Hence although knowledge concerning EGFR-TKIs and *EGFR* gene mutation is advancing in the laboratory setting, clinically it is unclear how we should use EGFR-TKIs in NSCLC and which patients might benefit most from these agents. In this review, clinical trials of EGFR-TKIs are recounted and a key factor for drug sensitivity, *EGFR* mutation, is discussed.

Clinical trials of EGFR-TKIs

Four phase I trials of EGFR-TKI including one Japanese study were performed in a total of 254 patients [4, 8]. These trials defined diarrhea and liver function test abnormality as dose-limiting factors. Five of 23 patients demonstrated partial responses (PRs) without dose–response tendency (Table 1). Toxicity profiles were quite different to those commonly observed with conventional anticancer agents. Ten percent of patients failed treatment at doses >600 mg/day and these early studies could not identify an optimal dosing schedule. Based on the results of phase I, the phase II IDEAL1 study was conducted in 210 previously treated advanced NSCLC patients in Japan, Australia, and Europe [1]. In this large-scale international study, a similar objective tumor response rate (20%) to those of previous studies was observed. There was no difference of clinical response between patients receiving 250 mg/day and those on 500 mg/day, whereas toxicity was more severe in the higher-dose group. Subset analysis revealed startling clinico-pathological subpopulations with especially high drug sensitivity to EGFR-TKI namely Japanese patients, females, nonsmokers, and those with adenocarcinoma (Table 2). In particular, Japanese females exhibited an overall response rate >50% in this analysis. For the first time, unlike conventional anticancer agents these results suggested that EGFR-TKIs are efficacious in specific subpopulations. While that phase II trial was ongoing, two large phase III trials in untreated NSCLC were begun in the USA and Europe [2, 3]. The rationale of these two clinical trials, INTACT1 and INTACT2, was based on preclinical studies that suggested synergistic effects of taxane plus gefitinib against cancer cells in vitro and in vivo. Hence, gefitinib or placebo was added onto standard chemotherapy regimens cisplatin/gemcitabine (INTACT1) and carboplatin/paclitaxel (INTACT2) [2, 3]. Both trials showed that there was no evidence for prolonged survival time with add-on gefitinib for either standard chemotherapy schedule. The same negative result was observed in another phase III trial using the same design with erlotinib as well as gefitinib [5]. However, in this trial subset analysis suggested enhanced efficacy of EGFR-TKI therapy among nonsmokers and those harboring activated-type *EGFR* mutations. Two subsequent studies of second-line and later treatment, BR.21 and ISEL, gave conflicting results for overall survival time, time to progression, and response rate: the former suggested additional benefit of add-on EGFR-TKI and the latter gave negative results [10, 11].

Table 1 Antitumor activity of gefitinib in Japanese phase I study

	Total	PR (%)
All cases	31	5 (16)
NSCLC	23	5 (22)
Histology		
Adenocarcinoma	19	5 (26)
Squamous cell carcinoma	4	0 (0)
Gender		
Male	15	1 (7)
Female	8	4 (50)

PR partial response

To clarify the clinical benefit of EGFR-TKIs in *EGFR* mutation-positive NSCLC, prospective phase II (WJTOG0403) and phase III (WJTOG3405) studies are now underway (Fig. 1). The results of these investigations aim to give us data that will enable us better to understand *EGFR* mutational status and whether mutant EGFR phenotype confers clinical benefit in patients.

EGFR mutation and drug sensitivity

To use gefitinib effectively in clinical settings we must first identify patient populations who respond well to this agent. As mentioned above, data from IDEAL1 revealed that gefitinib is highly effective in Japanese, females, adenocarcinomatous histology, good performance status (PS), and nonsmokers (Table 2). Since the target molecule of EGFR-TKIs is EGFR, some correlation between expression patterns of EGFR protein and clinical outcome was widely speculated. However, IDEAL1 and 2 found no correlation between these parameters clinically, questioning the concept of molecular-targeting drugs. However, the answer to this question was provided by the striking findings regarding *EGFR* gene mutations [7, 9]. These *EGFR* mutations, located on the ATP binding site (exon 19–21) of

Table 2 Overall survival by patient characteristics: IDEAL1

Characteristic	Evaluable (*n*)	MST, days (95% CI)	*P*-value[a]	ORR, % (*n*)
All patients	209	241 (205–276)		18.7 (39/208)
Dose				
250 mg/day	103	232 (161–318)	0.716	18.4 (19/103)
500 mg/day	106	243 (203–309)		19.0 (20/105)
Age				
<65 years	145	238 (198–284)	0.5598	19.4 (28/144)
>65 years	64	241 (188–371)		17.2 (11/64)
Gender				
Female	61	397 (261–439)	0.0025	34.4 (21/61)
Male	148	212 (161–243)		12.2 (18/147)
WHO PS				
0–1	182	268 (234–318)	<0.0001	21.0 (38/181)
2	27	83 (57–121)		3.7 (1/27)
Histology				
Adenocarcinoma	131	300 (236–371)	<0.0001	26.0 (34/131)
Other	78	198 (129–232)		6.5 (5/77)
Smoking history				
Yes	104	186 (127–241)	<0.0001	12.5 (13/104)
No	53	414 (357–534)		37.7 (20/53)

MST mean survival time, *ORR* overall response rate

[a] Log-rank test

EGFR tyrosine kinase domain, are missense or deletion mutations causing substitution or partial deficiency of amino acid. Based on the results of basic studies, structural changes of the ATP binding site were found to increase binding affinity for ATP and gefitinib. In other words, under physiological conditions *EGFR* mutations are activating mutations that constitutively increase tyrosine kinase activity, and it is speculated that signals via EGFR are thereby abnormally enhanced and have greater impact on malignant transformation such as cancer cell proliferation. Fortunately, since these mutations are thought to have more highly augmented binding affinity for gefitinib than for ATP, they may display overwhelmingly high sensitivity induced by EGFR-TKIs. What is surprising is the correlation between frequency of *EGFR* mutations and clinical antitumor effects. We compared mutation rates and projected response rates obtained from IDEAL 1 and 2 and from 154 subjects in the clinical study in which our institute participated, and found that the *EGFR* mutation was highly correlated with clinical response (Table 3). In addition, it was reported at the American Society of Clinical Oncology (ASCO) meeting 2005 that *EGFR* gene mutation is closely related to

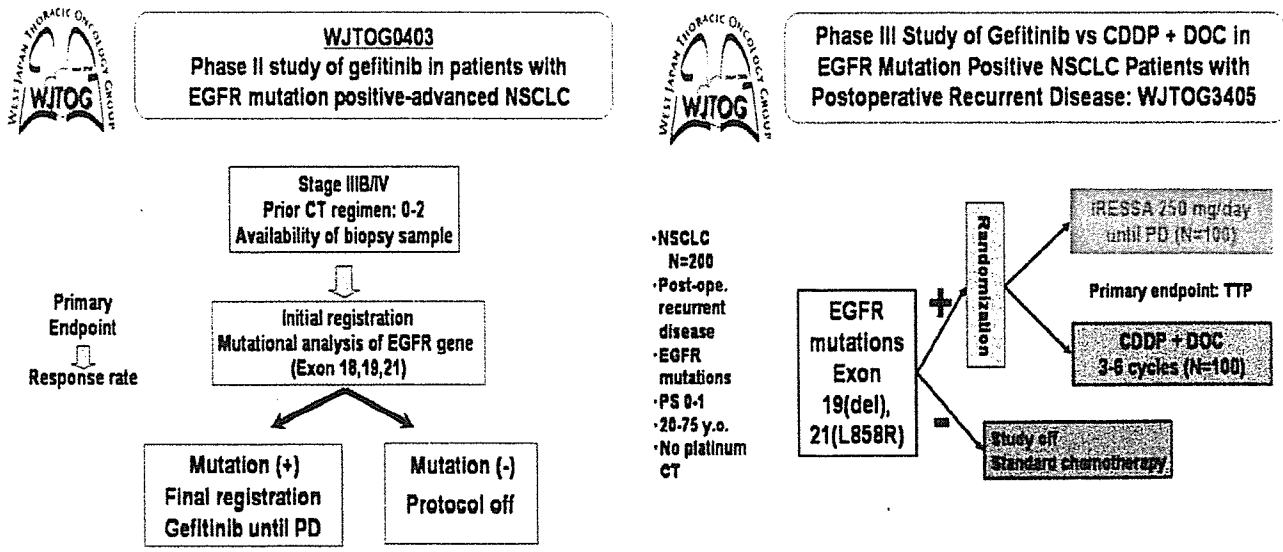

Fig. 1 Trial design of two ongoing prospective phase II (WJTOG0403) and phase III (WJTOG3405) studies investigating clinical benefit of EGFR-TKIs in *EGFR* mutation-positive NSCLC

Table 3 Estimated response rate (RR) for gefitinib and *EGFR* mutation in patients with NSCLC

Patient population	Estimated RR (%)	*EGFR* mutation (%)	
		Guillermo	Mitsudomi
Euro-American	10	2	–
Japanese	28	26	40
Japanese-adenocarcinoma	35	32	49
Female Japanese-adenocarcinoma	50	57	62

gefitinib sensitivity [6]. It is thought that the reason for the high response rate associated with Japanese race, female, adenocarcinoma, good PS, and nonsmokers is high frequency of *EGFR* mutations in these populations.

Future challenges

To establish clinical usage of EGFR-TKIs there are many issues to be addressed such as: (1) precisely identifying the site of *EGFR* mutations associated with drug sensitivity; (2) conducting a prospective clinical study of *EGFR* mutation and drug sensitivity; (3) establishing techniques to detect *EGFR* mutation precisely; (4) investigating efficacy of EGFR-TKI therapy in patients without *EGFR* mutations; (5) identifying patients responsive to EGFR-TKIs among those without *EGFR* mutations and clarifying the mechanism of action of EGFR-TKIs; and (6) clarifying mechanisms of EGFR-TKI resistance and developing drugs to overcome this resistance.

Combined use with conventional anticancer agents

Currently, gefitinib is the only EGFR-TKI available in Japan. How should we use gefitinib in combination with other anticancer agents? Large-scale clinical studies in Caucasian NSCLC patients indubitably have shown that concomitant use of conventional anticancer agents and gefitinib has no clinical usefulness in that patient population. Considering the association between gefitinib sensitivity and *EGFR* gene mutations, however, it seems too early to make a similar conclusion in Japanese patients in whom *EGFR* gene mutations might be more frequent. Therefore, it is important clinically to test gefitinib in Japanese patients concomitantly taking conventional anticancer drugs. In addition, in the context of combination therapeutic regimens not only simultaneous administration with conventional anticancer agents but sequential and maintenance therapies should be evaluated. To this end, the WJTOG phase III clinical trial is currently ongoing. Patients enrolled in this trial are divided into two groups: those taking three courses of two chemotherapeutic agents including one platinum-based drug followed by three courses of gefitinib, and the group on six courses of two drugs including one platinum drug alone. This trial, expected to terminate in April 2005, is aimed to show conclusively whether serial/sequential gefitinib therapy is useful in Japanese patients with NSCLC.

Conclusions

The advent of EGFR-TKIs convinced us that biological study of these agents in NSCLC could improve prognosis of these patients. Although the improvement elicited by gefitinib may be small so far, this agent does at least provide a new form of therapy that over the short term leads to markedly reduced tumor size without bone marrow suppression including neutropenia and no severe nausea and vomiting even in those patients with the worst prognosis. This raises the possibility of placing this rapidly fatal disease under some control. Doctors must be aware that making inroads towards understanding the implications of gene mutation in lung cancer will be a milestone for new therapeutics.

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British Journal of Cancer (2006), 1–6

www.bjcancer.com

Full Paper

A phase I study of pemetrexed (LY231514) supplemented with folate and vitamin B_{12} in Japanese patients with solid tumours

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The purpose of this study was to determine the maximum tolerated dose (MTD) and recommended dose (RD) of pemetrexed with folate and vitamin B12 supplementation (FA/VB_{12}) in Japanese patients with solid tumours and to investigate the safety, efficacy, and pharmacokinetics of pemetrexed. Eligible patients had incurable solid tumours by standard treatments, a performance status 0–2, and adequate organ function. Pemetrexed from 300 to 1200 mg m^{-2} was administered as a 10-min infusion on day 1 of a 21-day cycle with FA/VB_{12}. Totally, 31 patients were treated. Dose-limiting toxicities were alanine aminotransferase (ALT) elevation at 700 mg m^{-2}, and infection and skin rash at 1200 mg m^{-2}. The MTD/RD were determined to be 1200/1000 mg m^{-2}, respectively. The most common grade 3/4 toxicities were neutropenia (grade (G) 3:29, G4:3%), leucopenia (G3:13, G4:3%), lympopenia (G3:13%) and ALT elevation (G3:13%). Pemetrexed pharmacokinetics in Japanese were not overtly different from those in western patients. Partial response was achieved for 5/23 evaluable patients (four with non-small cell lung cancer (NSCLC) and one with thymoma). The MTD/RD of pemetrexed were determined to be 1200/1000 mg m^{-2}, respectively, that is, a higher RD than without FA/VB_{12} (500 mg m^{-2}). Pemetrexed with FA/VB_{12} showed a tolerable toxicity profile and potent antitumour activity against NSCLC in this study.

British Journal of Cancer advance online publication, 29 August 2006; doi:10.1038/sj.bjc.6603321 www.bjcancer.com

Keywords: antifolate; lung cancer; pemetrexed; pharmacokinetics; vitamin supplementation

Pemetrexed (LY231514, Alimta®, Eli Lilly and Company, IN, USA) is a novel antifolate (Taylor and Patel, 1992) that is approved in the United States and a number of European Union countries , for treatment of patients with malignant pleural mesothelioma (MPM) in combination with cisplatin, and non-small cell lung cancer (NSCLC) after prior chemotherapy as a single agent. *In vitro* experiments show that pemetrexed inhibits three enzymes in folate metabolism: thymidylate synthase (TS), dihydrofolate reductase (DHFR), and glycinamide ribonucleotide formyltransferase (GARFT) (Shih *et al*, 1998). Given the schedule dependency observed preclinically, three regimens were explored in phase I studies: (1) 0.2–5.2 mg m^{-2} daily for 5 days every 3 weeks (McDonald *et al*, 1998); (2) 10–40 mg m^{-2} weekly for 4 weeks repeated every 6 weeks (Rinaldi *et al*, 1995); and (3) 50–700 mg m^{-2} every 3 weeks (Rinaldi *et al*, 1999).

The third regimen (one dose every 3 weeks) was chosen for subsequent phase II studies because of its convenient administration, ability to give repeated doses, and occurrence of objective responses. The original maximum tolerated dose (MTD) and the recommended dose (RD) was 600 mg m^{-2}, but was decreased to 500 mg m^{-2} owing to toxicities experienced early in phase II studies. The initial phase I and II studies showed that myelosuppression was the principle drug-related toxicity, with a frequency of grade 3/4 neutropenia of 50% and grade 3/4 thrombocytopenia of 15% (Hanauske *et al*, 2001). Less than 10% of patients experienced gastrointestinal toxicities such as diarrhoea or mucositis. Although the prevalence of gastrointestinal toxicities and severe hematologic toxicities was low, these toxicities were associated with a high risk of mortality.

Infrequent severe myelosuppression with gastrointestinal toxicity has been observed not only for pemetrexed, but for the class of antifolates, including the DHFR inhibitor methotrexate (Morgan *et al*, 1990), the TS inhibitor raltitrexed (Maughan *et al*, 1999), and the GARFT inhibitor lometrexol (Alati *et al*, 1996; Mendelsohn *et al*, 1996). Clinical experience and nonclinical studies with methotrexate and lometrexol indicated that severe toxicity may be associated with nutritional folate status (Morgan *et al*, 1990; Alati *et al*, 1996; Mendelsohn *et al*, 1996). In fact, in the study of lometrexol, a significant effect of folate supplementation on toxicity was observed (Laohavinij *et al*, 1996). Based on these experiences, Niyikiza *et al* (2002a) investigated relationships between toxicity and baseline patient characteristics for early pemetrexed studies. They found total plasma homocysteine and methylmalonic acid levels to predict severe neutropenia and

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Received 15 May 2006; revised 24 July 2006; accepted 25 July 2006

thrombocytopenia, with or without grade 3/4 diarrhoea, mucositis, or infection. Homocysteine and methylmalonic acid are known as indicators of folate and vitamin B_{12} deficiencies (Rosenberg and Fenton, 1989; Savage *et al*, 1994). Thus, it was hypothesized that a patient's risk for severe toxicity could be reduced by decreasing the levels of homocysteine and methylmalonic acid with folate and vitamin B_{12} supplementation (FA/VB_{12}) (Niyikiza *et al*, 2002a).

FA/VB_{12} is now required for all patients participating in pemetrexed studies. Using this strategy, the pivotal phase III studies for MPM and NSCLC were successfully conducted with amelioration of severe drug-related toxicity (Niyikiza *et al*, 2002b; Vogelzang *et al*, 2003; Hanna *et al*, 2004).

One may expect that pemetrexed administration with supplementation would be more tolerable for patients and permit significant dose escalation above the current RD of 500 mg m^{-2}. Therefore, we conducted a phase I study to determine the MTD of pemetrexed with FA/VB_{12} for Japanese patients with solid tumours and to identify the RD for subsequent Japanese phase II studies. Our secondary objectives were to investigate the safety, antitumour effect, and pharmacokinetics of pemetrexed with supplementation in Japanese patients. A similar phase I study has been conducted outside Japan, but only preliminary data are available at this time (Hammond *et al*, 2003).

PATIENTS AND METHODS

Patient selection

Eligible patients had histologic or cytologic diagnosis of solid cancer that was incurable by standard treatments. Patients also must have been between 20 and 75 years of age, have an Eastern Cooperative Oncology Group (ECOG) performance status of 0–2, and have an estimated life expectancy of at least 3 months. Adequate organ function was required, which included bone marrow reserve (white blood cell count $4.0-12.0\times10^3$ mm^{-3}, platelets $\geqslant100\times10^3$ mm^{-3}, haemoglobin $\geqslant9.0$ g dl^{-1}, and absolute granulocyte count $\geqslant2.0\times10^3$ mm^{-3}), hepatic function (bilirubin $\leqslant1.5\times$ upper limit of normal, aspartate/alanine transaminase (AST/ALT) $\leqslant2.5\times$ upper limit of normal, and serum albumin $\geqslant2.5$ g dl^{-1}), renal function (serum creatinine $\leqslant$ upper limit of normal and Cockcroft and Gault creatinine clearance $\geqslant60$ ml min^{-1}), and lung function (PaO2 $\geqslant60$ torr).

Prior chemotherapy or hormone therapy was allowed if it was carried out $\geqslant14$ days before study entry ($\geqslant35$ days for nitrosourea or mitomycin-C). Previous radiotherapy was also allowed, but only if $\leqslant25\%$ of marrow was irradiated and if it was completed $\geqslant21$ days before study entry. Pretreated patients must have recovered from all toxicities before study entry. Prior surgery was allowed if patients recovered from the effect of the operation. Patients were excluded from this study for active infection, symptomatic brain metastasis, interstitial pneumonitis, or pulmonary fibrosis diagnosed by chest X-ray, serious concomitant systemic disorders incompatible with the study, clinically significant effusions, or the inability to discontinue aspirin and other nonsteroidal anti-inflammatory agents during the study.

This study was conducted in compliance with the guidelines of good clinical practice and the Declaration of Helsinki Principles, and it was approved by the local institutional review boards. All patients gave written informed consent before study entry.

Treatment

Pemetrexed was administered as a 10-min infusion on day 1 of a 21-day cycle. Patients remained on study unless they were discontinued because of disease progression, unacceptable adverse events, inadvertent enrollment, use of excluded concomitant therapy, cycle delay >42 days, or patient refusal.

Patients were instructed to take a daily 1 g multivitamin with 500 μg of folate beginning 1 week before day 1 of cycle 1 until study discontinuation. Vitamin B_{12} (1000 μg) was intramuscularly injected, starting 1 week before day 1 of cycle 1 and repeated every 9 weeks until study discontinuation.

Patients enrolled in pemetrexed clinical studies have received dexamethasone prophylactically to avoid pemetrexed-induced rash. As this was the first study of pemetrexed in Japanese patients and the incidence of the drug-induced rash in Japanese patients was unknown, the steroid was not to be administered prophylactically.

Dose escalation

In this study, 10 dose levels of pemetrexed, 300, 500, 600, 700, 800, 900, 1000, 1200, 1450, and 1750 mg m^{-2}, were to be examined with a starting dose of 300 mg m^{-2}. At dose levels from 300 to 1000 mg m^{-2}, three patients were to be treated initially. If no dose-limiting toxicities (DLTs) occurred during cycle 1, escalation proceeded to the next dose level. If 1 DLT occurred, three patients were added. If no additional DLTs were observed, escalation proceeded to the next dose level. At dose levels from 1200 to 1750 mg m^{-2}, six patients were to be treated at once. If two or more patients had DLTs at any dose level, dose escalation stopped, and this dose level was considered the MTD. The RD was then established by discussion with principal investigators, and the Efficacy and Safety Evaluation Committee.

A DLT was defined as the occurrence of one of the following toxicities during cycle 1: any grade 3/4 nonhematologic toxicity (except grade 3 nausea/vomiting and AST, ALT, or alkaline phosphatase elevation $<10\times$ upper limit of normal that returns to grade 0–1 by the beginning of cycle 2), grade 3/4 febrile neutropenia (<1000 mm^{-3} with $\geqslant38.0$°C), grade 4 leucopenia (<1000 mm^{-3}) or neutropenia (<500 mm^{-3}) lasting $\geqslant4$ days, thrombocytopenia ($<20\,000$ mm^{-3}), or thrombocytopenia ($\geqslant20\,000$ mm^{-3}) requiring platelet transfusion. A failure to start the second cycle by day 42 owing to toxicity was also considered a DLT. All toxicities were assessed according to National Cancer Institute-Common Toxicity Criteria (NCI-CTC) version 2.

Treatment assessments

Tumour response was assessed by the Response Evaluation Criteria in Solid Tumors (RECIST) criteria. Evaluable patients were subjected to CT or MRI measurement to determine the size of tumours at anytime at the discretion of investigators.

Pharmacokinetic analysis

Blood and urine were collected from each patient over a period of 72 h following administration in cycle 1. Blood samples were taken just before administration, at the end of infusion, and approximately 5, 15, 30 min and 1, 2, 4, 6, 8, 24, 48 and 72 h after the start of infusion. Urine was collected over the following time intervals: 0–4, 4–8, 8–12, 12–24, 24–36, 36–48, 48–60, and 60–72 h. Plasma and urine samples were analysed for pemetrexed at Taylor Technology Inc., Princeton, NJ, USA. Plasma samples were analysed using a validated liquid chromatography/electrospray ionisation-tandem mass spectrometry method that generated a linear response over the concentration ranges of 10–2000 ng/ml and 1000–200 000 ng/ml (Latz *et al*, 2006). Urine samples were analysed using a similar analytical technique (Chaudhary *et al*, 1999).

Pharmacokinetics were evaluated using noncompartmental methods (WinNonlin Professional Version 3.1; Pharsight Corporation, Cary NC, USA). Pharmacokinetic parameters determined

based on plasma concentration *vs* time data were maximum plasma concentration (C_{max}), elimination half-life ($t_{1/2}$), area under the plasma concentration *vs* time curve (AUC) from time 0 to infinity ($AUC_{0-\infty}$), volume of distribution at steady-state (V_{ss}) and plasma clearance (CL_p) (Rowland and Tozer, 1995). The fraction of drug excreted unchanged in urine (F_e) was calculated by dividing the cumulative amount of pemetrexed excreted unchanged in urine within 72 h (Ae_{0-72}) by the administered dose (Rowland and Tozer, 1995).

RESULTS

Patient disposition and characteristics

From October 2001 to September 2004, a total of 35 Japanese patients were enrolled and 31 were treated at four centres in Japan. Four patients were not treated owing to protocol criteria not met ($n=3$) and investigator decision ($n=1$). The majority of patients were male (65%), had an ECOG performance status of 1 (84%), were diagnosed with NSCLC (61%), and received prior chemotherapy (94%) (Table 1).

Table 1 Baseline patient characteristics

Parameter	N = 31
Sex, n (%)	
Male	20 (65)
Female	11 (35)
Age, years	
Median (range)	59 (31–74)
Mean (s.d.)	57 (11)
ECOG performance status, n (%)	
0	4 (13)
1	26 (84)
2	1 (3)
Diagnosis, n (%)	
Non-small cell lung cancer	19 (61)
Malignant pleural mesothelioma	7 (23)
Thymoma	2 (7)
Alveolar soft part sarcoma	1 (3)
Rectal cancer	1 (3)
Unknown primary cancer	1 (3)
Prior therapy, n (%)	
Surgery	14 (45)
Radiation	9 (29)
Chemotherapy	29 (94)

ECOG = Eastern Cooperative Oncology Group; s.d. = standard deviation.

Table 2 Dose escalation and DLTs

Dose ($mg\,m^{-2}$)	Number of patients	DLTs (*n*)
300	3	None
500	3	None
600	3	None
700	6	G3 ALT elevation (1)
800	3	None
900	4[a]	None
1000	3	None
1200	6	G3 infection (1); G3 rash (1)

ALT = alanine transaminase; DLT = dose-limiting toxicity; G3 = grade 3. [a]One patient was excluded for DLT analysis because of grade 3 hyperglycemia at the beginning of the study.

Dose escalation and dose-limiting toxicities

Three or six patients were enrolled at each dose level from 300 to 1200 $mg\,m^{-2}$, except the 900 $mg\,m^{-2}$ dose level (Table 2). At this dose level, one additional patient was enrolled because a patient was excluded from the DLT analysis. Before the dose initiation, this patient had grade 3 fasting hyperglycemia that was aggravated after the start of dosing. Therefore, this patient was rated as inappropriate for evaluation.

The first DLT was observed at the 700 $mg\,m^{-2}$ dose level. This 66-year-old woman with NSCLC experienced grade 3 ALT elevation. After an additional three patients were enrolled, no other DLTs were observed.

The next DLTs were observed at the 1200 $mg\,m^{-2}$ dose level, which enrolled six patients at once. One patient, a 72-year-old woman with MPM, had grade 3 infection at day 6 of cycle 1. Neutropenia was not simultaneously observed in this cycle. After 12 days, the event was resolved with antibiotics. This patient continued in study with dose reduction to 1000 $mg\,m^{-2}$. The other patient, a 68-year-old man with NSCLC, had grade 2 rash at day 5 of cycle 1. The severity of the event reached grade 3 at day 7. After 9 days from the occurrence, rash was resolved with dexamethasone and H_1-antihistamine. This patient continued in study without dose reduction. As two DLTs were observed, the 1200 $mg\,m^{-2}$ dose level was considered as the MTD. The RD for subsequent phase II studies was then evaluated to be pemetrexed 1000 $mg\,m^{-2}$. Both events were considered as drug-related events by investigators.

Safety

The safety evaluation was completed from data obtained from cycle 1–6 for all dose levels except 1200 $mg\,m^{-2}$ (cycle 1–3). These data were collected and analysed to evaluate safety when the MTD and RD were determined. The major toxicities observed in >50% of patients during all cycles evaluated for this report included rash, nausea, anorexia, fatigue, ALT elevation, AST elevation, lactate dehydrogenase elevation, leucopenia, neutropenia, lymphopenia, hematocrit decreased, haemoglobin decreased and erythropenia (Table 3). The most commonly reported grade 3/4 toxicity was neutropenia: nine patients (29%) had grade 3 neutropenia, and one patient (3%) had grade 4 neutropenia. Other grade 3/4 hematologic toxicities were grade 3 leucopenia in four patients (13%), grade 4 leucopenia in one patient (3%), grade 3 lymphopenia in four patients (13%), and grade 3 haemoglobin decreased in two patients (6%). The most commonly reported grade 3 nonhematologic toxicity was ALT elevation (four patients (13%)). Other grade 3 toxicities included AST elevation in one patient (3%), anorexia in one patient (3%), infection in one patient (3%), malaise in one patient (3%), and rash in one patient (3%) were observed. No grade 4 nonhematologic toxicities were reported.

The only serious adverse event was observed at the 900 $mg\,m^{-2}$ level. This 71-year-old man with NSCLC experienced grade 1 pyrexia at day 18 of cycle 3 and was hospitalized; however, the event was resolved the next day. The investigator did not consider it as a drug-related event. One patient at 900 $mg\,m^{-2}$ level discontinued treatment owing to adverse events (neutropenia, anorexia, and pyrexia). No deaths were observed during the study period or for 31 days after the last dose.

At the 900 $mg\,m^{-2}$ and higher dose levels, all patients had either grade 1/2 or grade 3/4 rash. At cycle 1, 25 patients experienced rash. Of these, 20 patients received corticosteroid. At or after cycle 2, corticosteroid treatment was given only for nine rash events, whereas rash events were observed in 20 cycles in cumulative total among patients. In addition, the severity of rash quickly improved or disappeared after administration of corticosteroid. Although the protocol allowed corticosteroid use for prevention of rash from cycle 2, only seven patients actually received the preventive treatment. Among those who did not receive the prophylactic

Table 3 Incidence of clinically relevant toxicities

	Dose ($mg\,m^{-2}$) (*n*)															
	Grade															
	300 (3)		500 (3)		600 (3)		700 (6)		800 (3)		900 (4)		1000 (3)		1200 (6)	
Toxicity	**1/2**	**3/4**	**1/2**	**3/4**	**1/2**	**3/4**	**1/2**	**3/4**	**1/2**	**3/4**	**1/2**	**3/4**	**1/2**	**3/4**	**1/2**	**3/4**
Hematologic																
Erythropenia	1	0	1	0	3	0	4	0	2	0	2	0	2	0	5	0
Hematocrit decreased	1	0	1	0	3	0	4	0	3	0	2	0	2	0	5	0
Haemoglobin decreased	2	0	2	0	2	0	3	0	2	0	1	1	2	0	4	1
Leucopenia	1	0	3	0	2	1	3	1	1	1	1	1	1	0	5	1
Lymphopenia	0	0	2	1	0	1	3	0	1	0	1	1	3	0	4	1
Neutropenia	1	0	1	2	1	2	3	2	0	2	1	1	2	0	2	1
Thrombocytopenia	0	0	2	0	1	0	2	0	2	0	2	0	1	0	2	0
Nonhematologic																
ALT elevation	0	0	2	0	2	0	2	3	3	0	1	1	1	0	5	0
AST elevation	0	0	3	0	2	0	4	1	3	0	3	0	2	0	5	0
Blood bilirubin increased	0	0	1	0	0	0	2	0	0	0	0	0	0	0	1	0
LDH elevation	0	0	3	0	3	0	5	0	3	0	2	0	1	0	4	0
Alopecia	0	0	0	0	2	0	2	0	1	0	2	0	0	0	0	0
Anorexia	0	0	1	0	3	0	5	0	3	0	0	1	3	0	4	0
Constipation	1	0	1	0	0	0	1	0	0	0	0	0	2	0	1	0
Diarrhoea	0	0	2	0	1	0	1	0	1	0	1	0	1	0	2	0
Fatigue	1	0	2	0	2	0	2	0	3	0	1	0	2	0	3	0
Infection	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	1
Nausea	2	0	3	0	3	0	5	0	3	0	2	0	2	0	5	0
Malaise	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0
Pruritus	0	0	0	0	2	0	2	0	1	0	0	0	1	0	2	0
Rash	3	0	2	0	3	0	5	0	2	0	4	0	3	0	5	1
Vomiting	2	0	3	0	2	0	3	0	1	0	1	0	1	0	0	0

ALT = alanine transaminase; AST = aspartate transaminase; LDH = lactate dehydrogenase.

corticosteroid, the incidence of a rash observed at, or after, cycle 2 was about one-third of the incidence observed in cycle 1.

Pharmacokinetic analysis

Mean dose-normalised pemetrexed plasma concentration *vs* time profiles following single doses of 300–1200 $mg\,m^{-2}$ pemetrexed are provided in Figure 1. This body surface area (BSA)-normalized dose range represents absolute doses of 414–2018 mg in Japanese patients with a mean BSA of 1.64 m^2 (range, 1.36–1.97 m^2).

Pharmacokinetic parameters for each dose group are summarised in Table 4. Lack of a monotonic trend in CL_p and V_{ss} between cohorts indicated that pemetrexed pharmacokinetics are consistent across dose groups. Consistency of pemetrexed pharmacokinetics across dose groups is also illustrated by the lack of systematic pattern across dose groups in the dose-normalised plasma concentration *vs* time profiles (Figure 1). The overall mean $t_{1/2}$ is approximately 2.74 h and was essentially similar across all dose groups (range, 2.28–3.62 h).

In this study, pemetrexed was primarily excreted unchanged in urine, which is consistent with its known elimination pathway (i.e., renal excretion). The F_e averaged 0.752 (range, 0.645–0.827). Mean F_e values were consistent across dosing cohorts.

Tumour response

In this study, 23 of the 31 patients were evaluable for response by RECIST criteria (Table 5). Partial responses (PRs) were observed in four patients with NSCLC (one patient each at 500, 700, 800, and 1200 $mg\,m^{-2}$) and one patient with thymoma at 500 $mg\,m^{-2}$. In addition, one patient with NSCLC at 500 $mg\,m^{-2}$ had a PR by the World Health Organization criteria, but was not evaluable via RECIST.

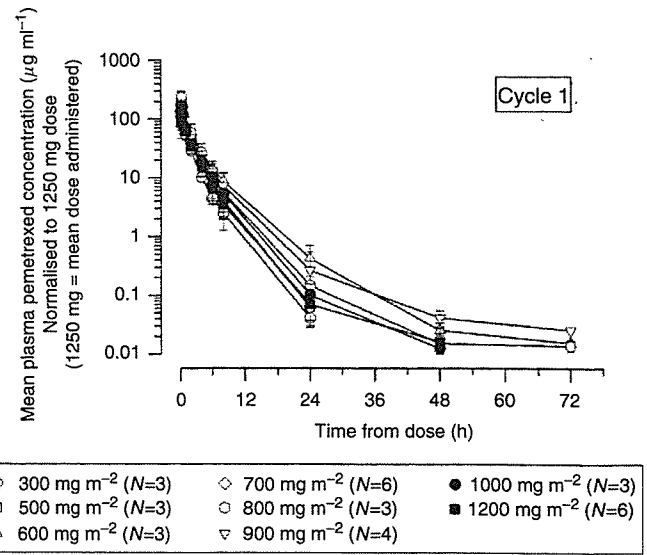

Figure 1 Mean dose-normalised pemetrexed plasma concentration–time profiles following single-dose administration in Japanese patients.

DISCUSSION

This is the first phase I study of pemetrexed in Japanese patients. The MTD for pemetrexed administered with FA/VB_{12} was 1200 $mg\,m^{-2}$ and determined the RD for subsequent phase II studies was 1000 $mg\,m^{-2}$.

In contrast with the previously determined MTD (600 $mg\,m^{-2}$) without vitamin supplementation (Rinaldi *et al*, 1999), our MTD

Table 4 Summary of pemetrexed pharmacokinetic parameters by dosing cohort arithmetic mean (CV%)

	Dose ($mg\,m^{-2}$) (*n*)							
Parameter	**300 (3)**	**500 (3)**	**600 (3)**	**700 (6)**	**800 (3)**	**900 (4)**	**1000 (3)**	**1200 (6)**
Dose (mg)	459 (12.4%)	783 (7.56%)	919 (8.28%)	1180 (8.06%)	1280 (16.5%)	1550 (5.47%)	1820 (7.04%)	1910 (6.71%)
C_{max}, ($\mu g\,ml^{-1}$)	58.2 (7.15%)	115 (19.1%)	178 (15.7%)	172 (9.30%)	240 (14.5%)	217 (7.05%)	269 (17.8%)	212 (13.2%)
$AUC_{0-\infty}$, ($\mu g\,h\,ml^{-1}$)	70.1 (7.04%)	158 (21.6%)	290 (12.5%)	250 (23.5%)	361 (17.0%)	388 (19.6%)	382 (6.55%)	337 (24.6%)
CL_p ($ml\,min^{-1}$)	109 (5.89%)	86.5 (32.5%)	53.0 (3.95%)	83.4 (27.7%)	61.4 (35.2%)	68.5 (20.0%)	79.3 (2.57%)	99.7 (24.7%)
V_{ss} (l)	13.5 (22.2%)	12.1 (20.1%)	11.5 (25.5%)	11.7 (20.0%)	10.6 (33.6%)	13.9 (31.7%)	14.4 (7.40%)	14.8 (9.41%)
$t_{1/2}$ (h)	2.28 (25.2%)	2.62 (3.29%)	3.62 (28.7%)	2.51 (3.91%)	2.93 (14.6%)	3.02 (17.8%)	2.67 (1.90%)	2.55 (10.9%)
F_e	0.659 (8.78%)	0.645 (8.34%)	0.788 (3.76%)	0.807 (10.1%)	0.705 (34.9%)	0.797[a] (5.11%)	0.648[a] (12.5%)	0.827[a] (7.58%)

CV% = coefficient of variation expressed as a percentage; C_{max} = maximum observed drug concentration; $AUC_{0-\infty}$ = area under the concentration versus time curve from zero to infinity; CL = total body clearance of drug after intravenous administration; V_{ss} = volume of distribution at steady state; $t_{1/2}$ = half-life associated with the terminal rate constant; F_e = fraction of dose eliminated unchanged in urine. [a]The numbers of patients in 900, 1000, and 1200 $mg\,m^{-2}$ were three, two, and five, respectively, owing to incompletion of urine collections for patients 209, 210, and 306.

Table 5 Antitumour activity by dose (RECIST)

		Evaluable ($n = 23$)				
Dose ($mg\,m^{-2}$)	**Number of patients**	**CR**	**PR[a]**	**s.d.**	**PD**	**NE**
300	3	0	0	2	0	1
500	3	0	2	0	0	0
600	3	0	0	1	0	0
700	6	0	1	3	1	0
800	3	0	1	0	1	1
900	4	0	0	2	0	1
1000	3	0	0	1	1	0
1200	6	0	1	2	1	0
Total	31	0	5	11	4	3

NSCLC = non-small cell lung cancer; CR = complete response; NE = not evaluated; PD = progressive disease; PR = partial response; s.d. = stable disease. [a]In addition, one NSCLC patient at 500 $mg\,m^{-2}$ had PR via WHO criteria.

increased by a factor of 2 whereas maintaining a tolerable safety profile. Niyikiza *et al* (2002a, b) conducted a multivariate analysis on 246 patients in phase II pemetrexed studies without vitamin supplementation, and the incidence of grade 4 neutropenia was 32% and grade 4 thrombocytopenia was 8% . Also 6% of patients had grade 3/4 diarrhoea, 5% had grade 3/4 mucositis, and a 5% incidence of drug-related death occurred. In contrast, our study had grade 4 neutropenia of only 3% (one patient) and no grade 4 thrombocytopenia. In addition, no grade 3/4 diarrhoea or mucositis, and no drug-related deaths were observed.

In the pivotal phase III study of NSCLC patients, those who received pemetrexed (500 $mg\,m^{-2}$) plus vitamin supplementation had a lower incidence of severe toxicities compared to those who received docetaxel (75 $mg\,m^{-2}$), including grade 3/4 neutropenia (5.3 *vs* 40.2%) and grade 3/4 diarrhoea (0.4 *vs* 2.5%) (Hanna *et al*, 2004).

Dose-dependency for toxicity of pemetrexed plus supplementation was further investigated to understand the effect of supplementation on safety. The patients in this study were divided into three groups by doses: low dose (300–600 $mg\,m^{-2}$ ($n = 9$)), middle dose (700–900 $mg\,m^{-2}$ ($n = 13$)), and high dose (1000 and 1200 $mg\,m^{-2}$ ($n = 9$)). Grade 1/2 toxicity such as erythropenia, lymphopenia, hematocrit decreased, ALT and AST elevation, and anorexia increased dose dependently from approximately 20–50% to approximately 75%. However, there was no obvious correlation between grade 3/4 toxicity and dose group. Therefore, high dose levels of pemetrexed with FA/VB_{12} is expected to be tolerable enough for patients.

In this study, severe rash was rarely observed even without the prophylactic corticosteroid. Although this result suggests that the steroid premedication for prevention of severe rash is no longer necessary for patients with pemetrexed treatment if the FA/VB_{12} is concomitantly conducted, it would be too early to conclude it as the data of patients untreated with the premedication are limited at this moment.

The pharmacokinetic results in our study were consistent with a phase I study of pemetrexed without vitamin supplementation in western patients by Rinaldi *et al* (1999) In that study, pemetrexed $t_{1/2}$ was 3.1 h; and CL was 85 ml/min (Rinaldi *et al*, 1999 and unpublished results). In our study, the $t_{1/2}$ of pemetrexed was about 2.7 h; and CL was 81.9 ml/min. Additionally, the F_e of pemetrexed was similar for Japanese patients (75% in our study) and western patients (78% in the Rinaldi study (Rinaldi *et al*, 1999)). These results indicate that pharmacokinetics of pemetrexed in Japanese patients are similar to those in western patients.

Although our study is the first phase I study to evaluate pemetrexed with FA/VB_{12} in Japanese patients, a similar phase I study has been conducted in western patients. In the preliminary results of that study, heavily pretreated patients had a MTD of 925 $mg\,m^{-2}$, and lightly pretreated patients had a MTD of 1050 $mg\,m^{-2}$ (Hammond *et al*, 2003). The comparison of these two studies suggests that the improved tolerability experienced by Japanese patients when pemetrexed is administered with FA/VB_{12} is not attributable to ethnic differences; rather, it is attributable to the vitamin supplementation.

In our phase I study, four NSCLC patients and one thymoma patient had PRs. Except for one, all of the patients with PR had $\geqslant 3$ prior chemotherapy regimens. The NSCLC patients with PRs received doses of pemetrexed higher than 500 $mg\,m^{-2}$, which is the approved dose for NSCLC treatment in a number of countries. Therefore, subsequent phase II studies using our RD of 1000 $mg\,m^{-2}$ with vitamin supplementation could show more prominent antitumour activity for cancer patients. To examine this hypothesis, a Japanese phase II study is being conducted, examining pemetrexed 500 or 1000 $mg\,m^{-2}$ every 3 weeks with full supplementation for patients with locally advanced or metastatic NSCLC. Clinical trials for other tumours, including MPM, are also ongoing. For the prophylactic corticosteroid, as severe rash was not frequently observed in this study, the steroid is not to be administered prophylactically in both currently on-going studies.

In conclusion, pemetrexed with FA/VB_{12} resulted in a tolerable toxicity profile. The MTD was 1200 $mg\,m^{-2}$. The RD was 1000 $mg\,m^{-2}$.

ACKNOWLEDGEMENTS

This study has been supported and funded by Eli Lilly Japan KK, Kobe, Japan.

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Phase I and Pharmacokinetic Study of Combination Chemotherapy Using Irinotecan and Paclitaxel in Patients with Lung Cancer

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The purpose of this study was to investigate the maximum tolerated doses, dose-limiting toxicities, efficacy, and pharmacokinetic profiles in the combination of irinotecan and paclitaxel. Eligibility criteria included age 75 years or younger, good performance status, adequate organ function, and unresectable non–small cell or extensive disease of small cell lung cancer. Irinotecan was administered on days 1 and 8 over 90 minutes, and paclitaxel was administered on day 8 over 3 hours after 90 minutes from the end of the irinotecan infusion. Irinotecan and paclitaxel were dose-escalated from 40 and 135 mg/m^2 and repeated every 4 weeks. The authors also administered a higher dosage with preventive granulocyte colony-stimulating factor support from day 9. Thirty-one patients were assessed for toxicities and responses. Dose-limiting toxicities were neutropenia and febrile neutropenia. The dose of irinotecan 60 mg/m^2 and paclitaxel 200 mg/m^2 with preventive granulocyte colony-stimulating factor support was tolerable and suitable for a phase II trial. Nine of 25 (36%) patients with non–small cell and all six patients with small cell carcinoma achieved partial response. The areas under the concentration versus time curves of irinotecan and its metabolites on day 8 were significantly higher than on day 1. This combination therapy must be planned only after careful consideration of the drug–drug interaction.

Key Words: Lung cancer, Irinotecan, Paclitaxel, Phase I, Pharmacokinetics.

(*J Thorac Oncol.* 2006;1: 226–230)

Chemotherapy for non–small cell lung cancer (NSCLC) has recently improved survival by using platinum compounds and new drugs (e.g., vinorelbine, gemcitabine, taxanes, and irinotecan).[1] Chemotherapy for extensive disease of small cell carcinoma (ED-SCLC) has also improved survival using cisplatin and irinotecan.[2] Although these regimens statistically improved survival, the benefits are far from satisfactory. There are comparatively few reports of nonplatinum regimens, and we do not have sufficient knowledge about these regimens regarding maximum tolerated doses (MTD), toxicities, responses, and pharmacokinetic profiles. However, irinotecan and paclitaxel have shown antitumor activity for both non–small cell and small cell carcinoma as a single agent.[3–6] This combination is also reported to have additive or supra-additive antitumor effects for lung cancer cells in vitro by using an isobologram.[7,8] Therefore, we conducted this combination phase I study to evaluate MTD, dose-limiting toxicities (DLTs), and pharmacokinetics in this combination therapy. We also evaluated the response rate and pharmacokinetic profiles.

Before planning this study, we performed this combination trial by another administration schedule.[9] In the prior trial, irinotecan was administered over 90 minutes on days 1, 8, and 15 and paclitaxel was given by infusion over 3 hours on day 2. Starting doses of irinotecan and paclitaxel were 50 and 135 mg/m^2, respectively. DLTs were neutropenia and febrile neutropenia, and MTD was the starting dose. Furthermore, most of the patients could not receive irinotecan on days 8 and 15 because of neutropenia. Although the neutropenia from this combination regimen was intolerable, an antitumor response was seen in the majority of the patients, suggesting that this combination might provide good antitumor activity and that an alternative administration schedule was needed to use these drugs. In this new trial, we therefore modified the administration schedule to escalate dose intensity while avoiding severe toxicities.

PATIENTS AND METHODS

Patient Selection

Patients with unresectable NSCLC or ED-SCLC were eligible for the trial. Pathologic confirmation and assessable lesions were necessary before study entry. Previous chemotherapy or radiotherapy, if given, must have been completed at least 4 weeks before entry. Other eligibility criteria included age 20 to 75 years, Eastern Cooperative Oncology Group performance status of 0 to 1, estimated life expectancy of at least 3 months, and adequate organ function defined as follows: white blood cell count greater than or equal to 4000 cells/μl, absolute neutrophil count greater than or equal to

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ISSN: 1556-0864/06/0103-0226

2000 cells/μl, platelet count greater than or equal to 100,000 cells/μl, serum creatinine less than or equal to 1.2 mg/dL, bilirubin less than or equal to 1.5 mg/dL, serum alanine aminotransferase (ALT) or aspartate aminotransferase (AST) less than twice the upper limit of normal, and PaO_2 greater than or equal to 60 mmHg. Patients with interstitial pneumonia, active infection, unstable cardiac disease, uncontrolled diabetes mellitus, pleural or cardiac effusion that required drainage, or symptomatic brain metastasis were ineligible. Our hospital institutional review committee approved this study, and all patients provided written informed consent.

Treatment

Irinotecan was administered on days 1 and 8 over 90 minutes, and paclitaxel was administered on day 8 over 3 hours after 90 minutes from the end of irinotecan infusion (Figure 1). All patients received premedication for paclitaxel and vomiting. The treatment was repeated every 4 weeks. The latter therapy was permitted using preventive granulocyte colony-stimulating factor (G-CSF) support from day 9 if patients experienced DLT of leukopenia or neutropenia and achieved partial response or stable disease on the previous course. The criteria for administration on day 8 were white blood cell count greater than or equal to 3000 cells/μl and other eligibility criteria before study entry. If patients did not clear this criteria for day 8, their treatment was cancelled and they were excluded from the evaluation of toxicities and responses.

Dose Escalation

The dose escalation schedule is shown in Table 1. Evaluation of DLTs for dose escalation was performed for the first course of chemotherapy. DLTs were defined using National Cancer Institute Common Toxicity Criteria (version 2.0)[10] as grade 4 neutropenia lasting 5 days or more, other grade 4 hematologic toxicities, neutropenic fever, or grades 3 and 4 toxicities in other organ systems except for nausea and vomiting. Three patients were assigned to each dose level. When all three patients did not experience DLT, we shifted to the next dose level. If one or two patients experienced DLT, an additional three patients were entered at the dose level before dose escalation. When at least three patients were found to have DLT, the dose was defined as the MTD. After the MTD was determined without preventive G-CSF support, we continued this study with preventive G-CSF support from day 9 until the recovery of neutropenia. We permitted the latter therapy by using preventive G-CSF support if patients who experienced DLT achieved stability or a partial response. Intrapatient dose escalation was not permitted. World Health Organization tumor evaluation criteria were used for tumor response evaluation.[11,12]

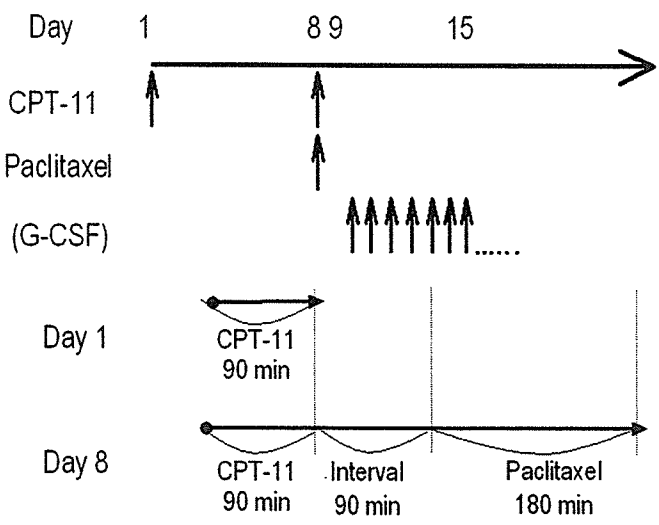

FIGURE 1. Treatment schedule of irinotecan and paclitaxel.

TABLE 1. Dose Escalation Schedule

Dose Level	CPT-11 (mg/m²)	Paclitaxel (mg/m²)
1	40	135
2	50	135
3	60	135
4	60	150
5	60	175
6	60	200

CPT-11, irinotecan.

Pharmacokinetic Analysis

Blood samples for pharmacokinetic analysis were obtained on days 1 and 8 in the first course. We collected samples by means of a peripheral venous catheter at the following times from the end of irinotecan infusion: 0, 15, 30, 90, 180, 240, 300, 420, 540, and 1410 minutes on day 1; and 0, 15, 30, 90, 180, 240, 270, 285, 300, 360, 420, 540, 630, and 1410 minutes on day 8, respectively. To analyze the pharmacokinetics of paclitaxel and the influence on the pharmacokinetics of irinotecan by paclitaxel, several processes were

TABLE 2. Patient Characteristics

Characteristic	Value
No. of patients enrolled	31
Median age (range) (yr)	62 (36–75)
Sex	
Male	23
Female	8
PS	
0	4
1	27
Prior chemotherapy	
Yes	2
No	29
Type of lung cancer	18
Adenocarcinoma	6
Squamous cell carcinoma	1
Large cell carcinoma	6
Small cell carcinoma	
Median no. of courses (range)	2 (1–5)

PS, performance status.

TABLE 3. Major Toxicities

	Level 1	Level 2	Level 3	Level 3' (G-CSF)	Level 4 (G-CSF)	Level 5 (G-CSF)	Level 6 (G-CSF)
No. of patients	3	6	3	2* + 1	6	6	6
Neutropenia							
G3	1	0	0	1	2	0	1
G4 (<5 days)	1	4	3	1	2	2	1
G4 (≥5 days)	0	0	0	0	0	0	0
Neutropenic fever	0	1	2*	0	1	1	1
AST or ALT							
G2	0	0	0	0	0	0	0
G3	0	1	0	0	0	0	0
Diarrhea							
G2	0	1	1	0	1	0	1
G3	0	0	0	0	0	1	0
DLT patients	0	2	2*	0	1	2	1

*Two patients who had neutropenic fever in level 3 were treated with preventive G-CSF support in second courses as level 3'. Level 3' was tolerable for them. G, National Cancer Institute Common Toxicity Criteria grade; DLT, dose-limiting toxicity.

added on day 8. Heparinized tubes were used, and the plasma was immediately separated by centrifugation and stored at –20°C until analysis. Plasma concentrations of irinotecan, its metabolites (SN-38 and SN-38G), and paclitaxel were measured using high-performance liquid chromatography on the reported conditions.[13,14]

The area under the plasma concentration-time curve (AUC) of irinotecan, its metabolites, and paclitaxel were calculated by the trapezoidal method with extrapolation to infinity using WinNonlin (version 1.1; Scientific Consulting, Inc., Apex, NC).

The AUC of irinotecan, SN38, and SN-38G on day 1 were compared with those on day 8 using paired *t* test and Wilcoxon matched-pairs signed ranks test. Clearance of paclitaxel was compared with reported data in monotherapy.

RESULTS

Patient Characteristics

Twenty-six men and eight women were enrolled in the study and were treated between March of 1999 and November of 2002 at Kinki University Hospital in Osaka, Japan. Two men in level 3 and one man in level 4 were excused because of the criteria for administration of day 8. One showed grade 3 elevation of ALT and ileus, another showed grade 2 elevation of ALT, and the other exhibited grade 2 rash. These patients were excluded from evaluation of toxicities and responses at each dose escalation. Finally, 31 patients were evaluated for their toxicities and responses, and blood samples were drawn on both day 1 and day 8 from 31 patients. The characteristics of the 31 patients are listed in Table 2.

Toxicities and Dose Escalation

Major toxicities are hematologic toxicities, diarrhea, and elevation of AST and ALT. Other nonhematologic toxicities are mild. Details are listed in Table 3. In level 2, one patient developed grade 3 liver dysfunction and the other developed neutropenic fever. In level 3, all patients developed grade 4 neutropenia and two of three patients developed neutropenic fever. Although level 3 had not reached the definition of MTD at this point, we judged that the dose of level 3 was probably MTD, and that further continuation of level 3 was dangerous. However, two patients who had neutropenic fever did not develop DLT in the second course of level 3 with preventive G-CSF support. We decided, therefore, to continue this study with preventive G-CSF support from level 3. One patient added to level 3 with preventive G-CSF support did not develop DLT. Most patients received second or later courses on schedule in each level. Although the schedules were delayed in a few patients, the reasons were not toxicities. This study was subsequently continued until level 6, and the dose did not reach the MTD with preventive G-CSF support. Although level 6 with G-CSF support was tolerable, this phase I study was discontinued because each dose was close to the recommended dose for monotherapy in Japan. We estimated that the recommended dose for phase II study was irinotecan 60 mg/m^2 (days 1 and 8) and paclitaxel 200 mg/m^2 (day 8) with preventive G-CSF support from day 9.

TABLE 4. Tumor Responses

Level	Patients	PR	SD	PD
1	3		3	
2	6	2 + 1*	2	1
3	4	1	1	2
4	6	0 + 3*	2	1
5	6	4 + 2*		
6	6	2	2	2

*Patients with ED-SCLC. †NSCLC (25 patients): PR, 9 (36%; 95% CI, 18–57%). ED-SCLC (6 patients): PR, 6 (100%; 95% CI, 61–100%). PR, partial response; SD, stable disease; PD, progressive disease; CI, confidence interval.

TABLE 5. Comparison of AUCs of Day 1 and Day 8

	CPT-11	SN-38	SN-38G
Average (μg/min/ml) ± SD			
Day 1	223.3 ± 73.6	5.92 ± 5.30	70.24 ± 70.40
Day 8 (with paclitaxel)	296.3 ± 92.0	8.31 ± 7.13	102.71 ± 123.14
Paired *t* test (*p* value)	<0.0001	0.0271	0.0136
Wilcoxon matched-pairs signed ranks test (*p* value)	<0.0001	0.0044	0.0001

SD, standard deviation; CPT-11, irinotecan.

Tumor Responses

Nine of 25 (36%) patients with NSCLC achieved partial response, and all six patients with ED-SCLC achieved partial response (Table 4).

Pharmacokinetics

Pharmacokinetic analyses were conducted on 31 patient blood samples. AUCs of irinotecan and its metabolites on day 8 were significantly higher than on day 1 (Table 5). Clearance of paclitaxel (day 8) was 14.3 ± 5.3 liters/hr/m^2.

DISCUSSION

Several other studies of this combination were reported.[15–17] Both paclitaxel and irinotecan were administered weekly in some studies, and patients were given paclitaxel on day 1 and irinotecan on days 1, 8, and 15 in some studies. DLTs and other major toxicities were hematotoxicities and diarrhea. These toxicities were similar to those in this study. Administration of irinotecan on day 8 or 15 was generally skipped in the weekly schedule, or administration of paclitaxel on day 1, because of hematotoxicities. This study schedule was designed to avoid skipping administration on day 8 and to elevate dose intensity and its efficacy by using G-CSF without any risky administration on day 15. Other studies did not increase the dosage with G-CSF and did not treat patients with ED-SCLC. This combination showed comparatively stronger hematologic toxicity than the other platinum combination regimens or nonplatinum regimens as indicated from our results and the other reports on this combination.

Platinum-based combinations with third-generation drugs are standard regimens in the treatment of advanced NSCLC.[1,18,19] However, a recent meta-analysis has reported that 1-year survival was not significantly prolonged when platinum-based therapies were compared with third-generation–based combination regimens.[20] Platinum-free doublet regimens are expected to offer improved survival without decreasing quality of life. Although this trial showed a response rate similar to other nonplatinum regimens, hematotoxicities were stronger than those of the other regimens. Therefore, this combination therapy might not be suitable for the treatment of NSCLC.

In the treatment of small cell lung cancer, the regimen of cisplatin and irinotecan ensures better survival than the regimen of cisplatin and etopside.[2] There have been very few reports of platinum-free doublet regimens based on third-generation drugs in small cell lung cancer. The response rate of this study regimen was noteworthy. Although the number of patients with small cell carcinoma was limited, all patients achieved partial response (95% confidence interval, 61–100%). This combination showed similar or better response than the combination of cisplatin and etoposide, and this regimen might be as effective as the combination of cisplatin and irinotecan. Therefore, this combination is proposed as an attractive regimen for small cell lung cancer chemotherapy.

In this trial, three persons were withdrawn from treatment by the criteria of day 8 and thus excluded from evaluation. We know from our previous study that this combination may cause severe neutropenia and that some patients occasionally show stronger toxicities for irinotecan than most. For example, it has been suggested that the polymorphism of UDP-glucuronosyltransferase might raise severe toxicities.[21,22] If only single administration of low-dose irinotecan produced toxicities that conflicted with the criteria of day 8, we can regard that patient as an anomaly regarding irinotecan. At this point, our administration schedule seems to be safe for this combination.

In the pharmacokinetic study, AUCs of irinotecan and its metabolites on day 8 were significantly higher than those of day 1. Clearance of paclitaxel was similar to that in many previously reported studies. We observed a 90-minute interval between irinotecan infusion and paclitaxel infusion to avoid severe drug interactions. We concluded that the mechanism of drug elimination is competitive because we had found indications of interaction from the pharmacokinetic investigation in our previous study. Irinotecan and its metabolite are mainly excreted by P-glycoprotein and cMORT in the liver, and paclitaxel or its vehicle (Cremophor EL) will compete in some stage of excretion. Noninterval administration of paclitaxel and irinotecan would heighten the AUC and the risk of toxicities. It has been advised in phase II trials that the administration time schedule of a phase I study be retained because it is very likely that the MTDs are different in each administration schedule. If the interval between irinotecan and paclitaxel administration is shorter or the order of administration is reversed, the possible pharmacokinetic interaction and toxicities might be much stronger. This combination therapy must be planned carefully with due consideration of the drug–drug interaction.

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ANTICANCER RESEARCH *26*: 2479-2486 (2006)

A Phase I Study of Irinotecan in Combination with Amrubicin for Advanced Lung Cancer Patients

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Abstract. *Background: A combination phase I study was conducted in a cohort of lung cancer patients to determine the maximum tolerated dose (MTD) and toxicities of irinotecan (CPT-11), a topoisomerase I inhibitor, in combination with amrubicin (AMR), a topoisomerase II inhibitor, and to observe their antitumor activities. Patients and Methods: Patients with lung cancer received AMR (35 – 40 mg/m^2 given intravenously over 5 min) for 3 consecutive days, and CPT-11 (50 – 60 mg/m^2 given intravenously over 90 min) after the completion of AMR infusion on days 1 and 8, every 3 weeks. Results: In total, eleven patients were enrolled in this study. The most frequent toxicities were bone marrow suppression, particularly leucopenia and neutropenia, followed by infection, diarrhea and pneumonitis. As a consequence of these toxicities, the MTD and the recommended dose could not be determined. There were two partial responses, which included one patient with small cell lung cancer (SCLC) who had previously received chemotherapy and the other with previously untreated non-small cell lung cancer (NSCLC). Conclusion: These data suggest that the combination of CPT-11 and AMR is not tolerated, as it mediates an unexpectedly strong myelosuppressive effect, and is inactive against both NSCLC and SCLC.*

Lung cancer is the leading cause of cancer deaths worldwide. In spite of the development of new anticancer agents, such as paclitaxel, docetaxel, irinotecan (CPT-11) and gemcitabine, the prognosis of lung cancer is still poor. New agents and new combination chemotherapy regimens are warranted in order to improve the outcome for lung cancer patients. The DNA topoisomerases are essential nuclear enzymes that catalyze the breakage and rejoining of DNA. There are two classes of DNA topoisomerases, type I (topo I) and type II (topo II), which alter the topology of single- and double-stranded DNA, respectively, and are concerned with genetic reactions including DNA replication, transcription and DNA repair (1). To date, several DNA topoisomerase inhibitors, including CPT-11, the anthracyclines and etoposide, have played an important role in lung cancer chemotherapy (2, 3). Moreover, some investigators have reported that the combination of topo I and topo II inhibitors resulted in a synergistic effect in preclinical studies (4). This synergistic effect may be related to their complementary functions. However, other investigators have reported, conversely, that inhibition of both topo I and topo II led to an antagonistic effect (5, 6). Thus, the inhibition of both topoisomerases seems to be a very attractive strategy in the context of lung cancer chemotherapy, although it is not clear whether the combination results in a synergistic, additive or antagonistic effect. Amrubicin (AMR) is a novel, totally synthetic, 9-aminoanthracycline derivative that inhibits topo II. It has more potent antitumor activity and less heart, liver and renal toxicities than doxorubicin, according to *in vivo* studies. Amrubicinol, the C-13 alcohol metabolite of AMR, which also inhibits topo II, has 10 to 100 times more antitumor activity than the parent compound. Based on preclinical study data, intravenous (*i.v.*) administration on 3 consecutive days every 3 weeks was recommended for use in a phase I/II study involving previously untreated advanced non-small cell lung cancer (NSCLC) patients. The dose-limiting toxicities (DLTs) were leucopenia, thrombocytopenia and gastrointestinal disturbance and the maximum tolerated dose (MTD) and the recommended dose (RD) for phase II studies were 50 mg/m^2/day and

Abbreviations: NSCLC, non-small cell lung cancer; ED-SCLC, extensive-disease small cell lung cancer; PS, performance status; topo I, topoisomerase I; topo II, topoisomerase II; CPT-11, irinotecan; AMR, amrubicin; MTD, maximum tolerated dose; DLT, dose-limiting toxicity; RD, recommended dose; MST, median survival time; JCOG, Japan Clinical Oncology Group; FACS, Four Arm Cooperative Study; AUC, area under the concentration-time curve; C_{max}, concentration$_{max}$.

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Key Words: Irinotecan, amrubicin, lung cancer.

45 mg/m^2/day, respectively. Two phase II studies for NSCLC showed response rates of 27.9% and 18.3%, while a phase II study for extensive-disease small cell lung cancer (ED-SCLC) had a response rate of 75.8% and a median survival time (MST) of 11.7 months. Based on these results, AMR seems to be active for both NSCLC and ED-SCLC.

CPT-11, a camptothecin derivative, is a semi-synthetic topo I inhibitor and is one of the most active drugs used in the treatment of NSCLC and SCLC (7, 8). Recently, the Japan Clinical Oncology Group (JCOG) indicated that the combination of cisplatin and CPT-11 allows for significantly better survival than the combination of cisplatin and etoposide for previously untreated ED-SCLC (9). Moreover, Kubota *et al.* recently reported the results of the Four Arm Cooperative Study (FACS), which showed that cisplatin plus CPT-11 had comparative activity to carboplatin plus paclitaxel, cisplatin plus gemcitabine and cisplatin plus vinorelbine for the treatment of advanced NSCLC(10). Therefore, in Japan, the combination of cisplatin and CPT-11 is considered to be one of the standard chemotherapy regimens for NSCLC and ED-SCLC.

With the aim of improving therapeutic effects, a phase I study of AMR and CPT-11, as a combined topo I/II-targeting chemotherapy regimen for advanced lung cancer, was conducted. The objectives of this phase I study were: (a) to determine the MTD of both drugs and the RD for phase II studies; (b) to evaluate the toxicity profile of this regimen; (c) to investigate the pharmacokinetics of CPT-11, SN-38, AMR and amrubicinol; and (d) to observe the antitumor activity.

Patients and Methods

Patient eligibility. Patients with histological or cytological confirmation of locally advanced or metastatic NSCLC or ED-SCLC, who had received either no prior chemotherapy or one previous chemotherapy regimen, were eligible. The eligibility criteria were as follows; (a) ≥20 but <75 years old; (b) Eastern Cooperative Oncology Group (ECOG) performance status (PS) 0-1; (c) adequate organ function [white blood cell count (WBC) ≥4000 μl^{-1}, neutrophil count ≥2000 μl^{-1}, platelet count ≥100,000 μl^{-1}, hemoglobin concentration ≥9.5 gdl^{-1}, serum total bilirubin ≤1.5 mgdl^{-1}, serum transaminase ≤2.5 x upper normal limits, serum creatinine ≤ upper normal limits, PaO_2 ≥ 60 mmHg]. At least 4 weeks had to have passed after the completion of prior therapy and the patients had to have recovered from any toxic effects of such therapy. The exclusion criteria comprised pulmonary fibrosis or interstitial pneumonitis with symptoms or apparent abnormalities on chest X-ray, massive pleural effusion, pericardial effusion, or ascites, pregnancy, lactation, symptomatic brain metastases, active concurrent malignancies, severe drug allergies, severe heart disease, cerebrovascular disease, uncontrollable diabetes mellitus, severe infection or active peptic ulcer. This study was performed at the Kinki University School of Medicine, Japan, and was approved by the Institutional Review Board. Written informed consent was obtained from all patients. This study was conducted in accordance with the Declaration of Helsinki.

Table I. *Dose modification schemes.*

Dose	Amrubicin (mg/m^2)	Irinotecan (mg/m^2)	No. of patients (courses)
-2	30	50	3(8)
-1	30	60	5(14)
1	40	50	3(11)

Pretreatment and follow-up studies. Prior to entry, a full history and physical examination were completed, including age, height, weight, PS, histological diagnosis, tumor stage, nature of previous treatment and presence of a complication. The pretreatment laboratory examinations included a complete blood cell count, differential WBC count, hemoglobin, platelet count, serum electrolytes, total protein, albumin, total bilirubin, transaminase, alkaline phosphatase, lactate dehydrogenase, BUN, creatinine, blood gas analysis and electrocardiogram. After the initiation of therapy, a complete blood cell count with a differential WBC count was performed at least twice a week. Blood chemistry profiles and chest X-rays were obtained weekly. The lesion measurements were performed during every second course at least. Toxicities were evaluated according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC) version 2 and tumor responses were assessed using the Response Evaluation Criteria in Solid Tumors (RECIST) guidelines (11).

Drug administration and dose escalation. The treatment schedule included AMR, diluted with 20 ml of 5% glucose fluid, given *i.v.* over 5 min for 3 consecutive days, and CPT-11 with 500 ml of normal saline, given *i.v.* over 90 min after the completion of AMR infusion on days 1 and 8, every 3 weeks. All patients were allowed to receive antiemetics with dexamethasone and granisetron. Granulocyte colony-stimulating factor (G-CSF) prophylaxis was not administered. Doses of CPT-11 on day 8 were given if the WBC count was >2,500 μl^{-1}, the platelet count was >75,000 μl^{-1}, no episode of diarrhea had been experienced, pneumonitis incidents were less than grade 2 and the other non-hematological toxicities were less than grade 3. The subsequent courses were started if the WBC count was >3,000 μl^{-1}, the platelet count was >100,000 μl^{-1}, serum total bilirubin ≤1.5 mgdl^{-1}, serum transaminase ≤2.5 x upper normal limits, no episode of diarrhea had been experienced and pneumonitis incidents were less than grade 2. The doses of both drugs were decreased by one dose level if DLTs occurred. In the case of the initial dose level, the dose reduction was not permitted and this study was canceled.

The dose escalations were performed as listed in Table I. Intra-patient dose escalation was not allowed. At least three patients were treated at each dose level, and three additional patients were entered at the same dose level if DLT was observed in one or two of the first three patients. The MTD was defined as the dose level at which three out of three patients, or more than three out of six patients experienced DLT. The definition of DLT was: (a) grade 4 neutropenia for more than 4 days, (b) grade 3 febrile neutropenia, (c) thrombocytopenia <20,000 μl^{-1}, (d) grade 3 non-hematological toxicity except for nausea/vomiting, appetite loss and pneumonitis, (e) more than grade 2 pneumonitis, (f) delay of administration of CPT-11 on day 8 over a week, or delay of subsequent courses over 2