1.4–43.5 months. All patients were Japanese women. The demographic characteristics of the present study population are presented in Table 2. Regarding hormonal status, 59% of patients were both estrogen and progesterone receptors negative. With regard to HER2 status, 86% of the patients were HER2 protein 3+ in immunohistochemistry and 14% were HER2-gene amplified in FISH.

The patients in the present study ailed from advanced disease. More than half of the patients (57%) had three or more metastatic organs. Approximately half of the patients had visceral metastasis of either the lung (49%) or liver (39%).

Table 2 Baseline patient and disease characteristics ($n = 49$)

Characteristics	No. of patients	%
Mean age (range)	54.3 (33–72)	
Performance status		
0	42	86
1	5	10
2	2	4
Estrogen receptor/progesterone receptor status		
+/+	9	18
+/–	9	18
–/+	2	4
–/–	29	59
HER2 status		
IHC 3+	42	86
FISH positive	7	14
No. of metastases		
Mean (range)	2.6 (1–5)	
1	8	16
2	13	27
3	28	57
Sites of metastases		
Lymph node	33	67
Lung	24	49
Bone	20	41
Liver	19	39
Chest wall/skin	19	39
Chemotherapeutic pretreatment	49	100
Adjuvant or neoadjuvant setting	24	49
Anthracyclines	20	41
Taxanes	12	24
Metastatic setting	47	96
1 prior regimen	8	16
2 prior regimens	17	35
Mean number of regimens (range)	2.7 (0–8)	
Anthracyclines	26	53
Taxanes	42	86
Trastuzumab	43	88

IHC Immunohistochemistry, *FISH* fluorescence in situ hybridization

Moreover, they had been heavily pretreated. Approximately 90% of the patients were pretreated with anthracyclines (42 of 49; 86%) and taxanes (43 of 49; 88%) in the adjuvant, neoadjuvant, and/or metastatic settings, and 88% (43 of 49) of the patients were pretreated with trastuzumab-containing regimens in the metastatic setting. The mean number of chemotherapeutic pretreatment regimens was 2.7 (range 0–8, median 2) in the metastatic setting.

Efficacy

Of the 49 patients, response was assessable in 44 patients. One patient achieved CR (2%), and seven patients achieved PR (14%). Therefore, ORR for capecitabine was 16% (95% CI: 7–30%). Moreover, 16 patients achieved SD, and of these, 15 achieved long SD (31%); hence, CBR for capecitabine was 47% (95% CI: 32–62%) (Table 3). Median TTF was 5.4 months (Fig. 1). Median overall survival (OS) has not been reached.

Safety (Table 4)

Grade 3 adverse events were observed in nine patients (18%). No grade 4 event was observed. Treatment interruption and/or individual dose adjustment of capecitabine was required in 15 patients (31%).

Table 3 Response to trastuzumab plus capecitabine ($n = 49$)

	No. of patients	%
Response		
Complete response	1	2
Partial response	7	14
Stable disease	16	33
(Long stable disease)	(15)	(31)
Progressive disease	21	43
Not evaluable	4	8
Objective response rate	8	16
Clinical benefit rate	23	47

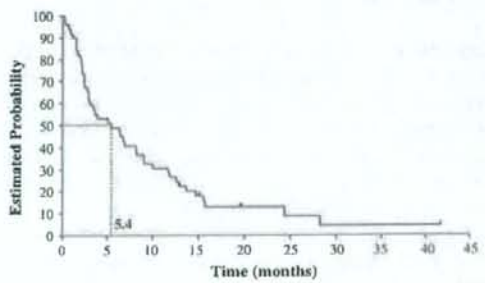

Fig. 1 Time-to-treatment failure ($n = 49$)

Table 4 Summary of adverse events worst by patient ($n = 49$)

	Total		Grade 1		Grade 2		Grade 3	
	No.	%	No.	%	No.	%	No.	%
Hand-foot syndrome	32	65	15	31	13	27	4	8
Fatigue	18	37	18	37				
Nausea	12	24	11	22	1	2		
Diarrhea	10	20	8	16	1	2	1	2
Anorexia	4	8	3	6	1	2		
Vomiting	4	8	4	8				
Interstitial pneumonia	1	2					1	2
Chronic heart failure	1	2					1	2
Leukopenia	27	55	20	41	7	14		
Neutropenia	13	27	7	14	6	12		
Anemia	14	29	8	16	4	8	2	4
Thrombocytepenia	1	2			1	2		
AST elevation	27	55	25	51	1	2	1	2
ALT elevation	15	31	10	20	4	8	1	2
Total bilirubin elevation	17	35	11	22	6	12		
Creatinine elevation	1	2	1	2				
All events	47	96	12	24	26	53	9	18

No grade 4 event was observed

Common adverse effects of the combination therapy were hand–foot syndrome, liver dysfunction, and bone marrow suppression. First, 32 patients had hand–foot syndrome (65%). This was classified as grade 3 in four patients (8%). Second, elevation of AST, ALT, and total bilirubin were noted in 27 (55%), 15 (31%), and 17 patients (35%), respectively. Grade 3 liver dysfunction occurred in one patient (2%). Third, effects of bone marrow suppression as leukopenia, anemia, and neutropenia were seen in 27 (55%), 14 (29%), and 13 patients (27%) at all grades; however grade 3 occurred in 2 patients (4%).

One patient suffered from grade 3 interstitial pneumonia, which improved after discontinuation of trastuzumab plus capecitabine.

Another patient without past medical history of cardiac dysfunction suffered symptomatic chronic heart failure (CHF), which improved after discontinuation of trastuzumab. She had been given doxorubicin in the neoadjuvant setting (total dose 300 mg/m^2). And also, she had been given trastuzumab in the metastatic setting for 1 year and 7 months. The interval between anthracycline and trastuzumab/capecitabine therapy was 2 year and 11 months.

Discussion

This retrospective study showed that the combination therapy of trastuzumab plus capecitabine is effective and safe for heavily pretreated patients with HER2-positive MBC. ORR was 16% and CBR was 47% (Table 3). Median TTF was 5.4 months (Fig. 1). Grade 3 adverse events were observed in 18% of the patients, but symptoms were improved after discontinuation of the therapy (Table 4).

Preclinical data investigating the combination of trastuzumab with 5FU showed that this combination was less effective than either drug alone, suggesting antagonism in vitro, whereas it may be synergic (cisplatin, thiopeta, etoposide) or additive (doxorubicin, paclitaxel, methotrexate, vinblastin) [20]. However, further studies indicated that trastuzumab and 5FU prodrug capecitabine had at least additive antitumor activity in in vivo models [21]. The reason for the discrepancy between the in vivo and in vitro results has not been clarified [21].

In the clinical setting, the combination therapy of trastuzumab plus capecitabine is effective for patients with HER2-positive MBC. In German multicenter phase II study of weekly trastuzumab with capecitabine (1,250 mg/m2 twice daily on days 1–14, tri weekly) in patients with pretreated MBC ($n = 27$), ORR was 45%, CBR was 68%, median progression-free survival time was 6.7 months, and median OS was 28 months [22]. Using the same treatment regimen as the German trial, this high ORR was mirrored in Chinese phase II study of the first-line therapy ($n = 43$), in which an ORR of 63% was recorded [23]. In Japanese phase II trial ($n = 27$), using the same regimen as the present study, ORR was 41%, median time to progression was 5.2 months, and median OS was 16.1 months [24]. In the present study, although ORR was inferior to these studies, tumor was

controlled for a relatively long time, considering the poor prognosis of the patients in the study population who had been heavily pretreated for the multiple metastases.

Furthermore, the combination therapy of trastuzumab plus capecitabine is well tolerated. The German trial showed that grade 3/4 adverse events were general pain (28%), motor dysfunction (16%), hand-foot-syndrome (16%), nausea (12%), anemia (8%), and leucopenia (4%) [22]. The Chinese trial showed that grade 3 hand–foot syndrome occurred in 9% and myelosupression occurred in 1% of patients [23]. The Japanese trial, same regimen as the present study showed no reports of grade 3/4 events [24]. Our results of adverse events are in the range of these prior studies.

The most clinically significant adverse event of trastuzumab was cardiac dysfunction. Patients ranging 2–5% who were treated with trastuzumab alone developed CHF [6, 7] and 0–2% of patients who were treated with trastuzumab plus non-anthracycline containing combination regimens developed CHF [9–11]. In the present study, grade 3 CHF was observed in one patient (2% Table 4), although approximately 90% of the patients pretreated with anthracycline (Table 2). Therefore, capecitabine added to trastuzumab does not increase CHF. Moreover, the clinically significant adverse events of capecitabine were hand-foot syndrome, liver dysfunction, and bone marrow suppression [14–18, 25, 26]. In the present study, the safety profile is not inferior to that seen in previous studies of capecitabine alone [14–18, 25, 26]. Therefore, trastuzumab added to capecitabine does not increase the adverse events of capecitabine.

In conclusion, the results of the present single-institute retrospective study confirm that the combination therapy of trastuzumab plus capecitabine is effective and tolerable in heavily pretreated patients with HER2-positive MBC.

References

1. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL (1987) Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science 235:177–182
2. Slamon DJ, Godolphin W, Jones LA, Holt JA, Wong SG, Keith DE, Levin WJ, Stuart SG, Udove J, Ullrich A, Press MF (1989) Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. Science 244:707–712
3. Pegram MD, Konecny G, Slamon DJ (2000) The molecular and cellular biology of HER2/neu gene amplification/overexpression and the clinical development of herceptin (trastuzumab) therapy for breast cancer. Cancer Treat Res 103:57–75
4. Toikkanen S, Helin H, Isola J, Joensuu H (1992) Prognostic significance of HER-2 oncoprotein expression in breast cancer: a 30-year follow-up. J Clin Oncol 10:1044–1048
5. Baselga J, Tripathy D, Mendelsohn J, Baughman S, Benz CC, Dantis L, Sklarin NT, Seidman AD, Hudis CA, Moore J, Rosen PP, Twaddell T, Henderson IC, Norton L (1996) Phase II study of weekly intravenous recombinant humanized anti-p185HER2 monoclonal antibody in patients with HER2/neu-overexpressing metastatic breast cancer. J Clin Oncol 14:737–744
6. Cobleigh MA, Vogel CL, Tripathy D, Robert NJ, Scholl S, Fehrenbacher L, Wolter JM, Paton V, Shak S, Lieberman G, Slamon DJ (1999) Multinational study of the efficacy and safety of humanized anti-HER2 monoclonal antibody in women who have HER2-overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease. J Clin Oncol 17:2639–2648
7. Vogel CL, Cobleigh MA, Tripathy D, Gutheil JC, Harris LN, Fehrenbacher L, Slamon DJ, Murphy M, Novotny WF, Burchmore M, Shak S, Stewart SJ, Press M (2002) Efficacy and safety of trastuzumab as a single agent in first-line treatment of HER2-overexpressing metastatic breast cancer. J Clin Oncol 20:719–726
8. Pegram MD, Lipton A, Hayes DF, Weber BL, Baselga JM, Tripathy D, Baly D, Baughman SA, Twaddell T, Glaspy JA, Slamon DJ (1998) Phase II study of receptor-enhanced chemosensitivity using recombinant humanized anti-p185HER2/neu monoclonal antibody plus cisplatin in patients with HER2/neu-overexpressing metastatic breast cancer refractory to chemotherapy treatment. J Clin Oncol 16:2659–2671
9. Marty M, Cognetti F, Maraninchi D, Snyder R, Mauriac L, Tubiana-Hulin M, Chan S, Grimes D, Anton A, Lluch A, Kennedy J, O'Byrne K, Conte P, Green M, Ward C, Mayne K, Extra JM (2005) Randomized phase II trial of the efficacy and safety of trastuzumab combined with docetaxel in patients with human epidermal growth factor receptor 2-positive metastatic breast cancer administered as first-line treatment: the M77001 study group. J Clin Oncol 23:4265–4274
10. Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, Fleming T, Eiermann W, Wolter J, Pegram M, Baselga J, Norton L (2001) Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med 344:783–792
11. Jahanzeb M, Mortimer JE, Yunus F, Irwin DH, Speyer J, Koletsky AJ, Klein P, Sabir T, Kronish L (2002) Phase II trial of weekly vinorelbine and trastuzumab as first-line therapy in patients with HER2(+) metastatic breast cancer. Oncologist 7:410–417
12. Burstein HJ, Kuter I, Campos SM, Gelman RS, Tribou L, Parker LM, Manola J, Younger J, Matulonis U, Bunnell CA, Partridge AH, Richardson PG, Clarke K, Shulman LN, Winer EP (2001) Clinical activity of trastuzumab and vinorelbine in women with HER2-overexpressing metastatic breast cancer. J Clin Oncol 19:2722–2730
13. Miwa M, Ura M, Nishida M, Sawada N, Ishikawa T, Mori K, Shimma N, Umeda I, Ishitsuka H (1998) Design of a novel oral fluoropyrimidine carbamate, capecitabine, which generates 5-fluorouracil selectively in tumours by enzymes concentrated in human liver and cancer tissue. Eur J Cancer 34:1274–1281
14. Talbot DC, Moiseyenko V, Van Belle S, O'Reilly SM, Alba Conejo E, Ackland S, Eisenberg P, Melnychuk D, Pienkowski T, Burger HU, Laws S, Osterwalder B (2002) Randomised, phase II trial comparing oral capecitabine (Xeloda®) with paclitaxel in patients with metastatic/advanced breast cancer pretreated with anthracyclines. Br J Cancer 86:1367–1372
15. Blum JL, Jones SE, Buzdar AU, LoRusso PM, Kuter I, Vogel C, Osterwalder B, Burger HU, Brown CS, Griffin T (1999) Multicenter phase II study of capecitabine in paclitaxel-refractory metastatic breast cancer. J Clin Oncol 17:485–493
16. Blum JL, Dieras V, Lo Russo PM, Horton J, Rutman O, Buzdar A, Osterwalder B (2001) Multicenter, phase II study of capecitabine in taxane-pretreated metastatic breast carcinoma patients. Cancer 92:1759–1768
17. Reichardt P, Von Minckwitz G, Thuss-Patience PC, Jonat W, Kolbl H, Janicke F, Kieback DG, Kuhn W, Schindler AE,

Mohrmann S, Kaufmann M, Luck HJ (2003) Multicenter phase II study of oral capecitabine (Xeloda®) in patients with metastatic breast cancer relapsing after treatment with a taxane-containing therapy. Ann Oncol 14:1227–1233

18. Fumoleau P, Largillier R, Clippe C, Dieras V, Orfeuvre H, Lesimple T, Culine S, Audhuy B, Serin D, Cure H, Vuillemin E, Morere JF, Montestruc F, Mouri Z, Namer M (2004) Multicentre, phase II study evaluating capecitabine monotherapy in patients with anthracycline- and taxane-pretreated metastatic breast cancer. Eur J Cancer 40:536–542
19. Wist EA, Sommer HH, Ostenstad B, Risberg T, Bremnes Y, Mjaaland I (2004) Oral capecitabine in anthracycline- and taxane-pretreated advanced/metastatic breast cancer. Acta Oncol 43:186–189
20. Pegram M, Hsu S, Lewis G, Pietras R, Beryt M, Sliwkowski M, Coombs D, Baly D, Kabbinavar F, Slamon D (1999) Inhibitory effects of combinations of HER-2/neu antibody and chemotherapeutic agents used for treatment of human breast cancers. Oncogene 18:2241–2251
21. Fujimoto-Ouchi K, Sekiguchi F, Tanaka Y (2002) Antitumor activity of combinations of anti-HER-2 antibody trastuzumab and oral fluoropyrimidines capecitabine/5′-dF^{d}Urd in human breast cancer models. Cancer Chemother Pharmacol 49:211–216
22. Schaller G, Fuchs I, Gonsch T, Weber J, Kleine-Tebbe A, Klare P, Hindenburg HJ, Lanker V, Hinke A, Bangemann N (2007) Phase II study of capecitabine plus trastuzumab in human epidermal growth factor receptor 2-overexpressing metastatic breast cancer pretreated with anthracyclines or taxanes. J Clin Oncol 25:3246–3250
23. Xu L, Song S, Zhu J, Luo R, Li L, Jiao S, Pan H, Tao M, Su Y, Liu D (2006) Capecitabine (X) + trastuzumab (H) as first-line treatment in patients (pts) with HER2-positive metastatic breast cancer (MBC): phase II trial results. Breast Cancer Res Treat 100(Suppl 1):2065a
24. Yamamoto D, Iwase S, Kitamura K, Odagiri H (2005) Multicenter phase II study of trastuzumab (H) and capecitabine (X) as first- or second-line treatment in HER2 over-expressing metastatic breast cancer (Japan Breast Cancer Study Group: JBCSG-003). J Clin Oncol 23(16S):802
25. Osako T, Ito Y, Takahashi S, Tokudome N, Iwase T, Hatake K (2007) Intermittent capecitabine monotherapy with lower dose intensity in heavily pretreated patients with metastatic breast cancer. Tumori 93:129–132
26. Oshaughnessy JA, Blum J, Moiseyenko V, Jones SE, Miles D, Bell D, Rosso R, Mauriac L, Osterwalder B, Burger HU, Laws S (2001) Randomized, open-label, phase II trial of oral capecitabine (Xeloda®) vs. a reference arm of intravenous CMF (cyclophosphamide, methotrexate and 5-fluorouracil) as first-line therapy for advanced/metastatic breast cancer. Ann Oncol 12:1247–1254

Efficacy and Safety of Two Doses of Pemetrexed Supplemented with Folic Acid and Vitamin B_{12} in Previously Treated Patients with Non-Small Cell Lung Cancer

Yuichiro Ohe,[1] Yukito Ichinose,[2] Kazuhiko Nakagawa,[3] Tomohide Tamura,[1] Kaoru Kubota,[4] Nobuyuki Yamamoto,[5] Susumu Adachi,[6] Yoshihiro Nambu,[7] Toshio Fujimoto,[7] Yutaka Nishiwaki,[4] Nagahiro Saijo,[4] and Masahiro Fukuoka[3]

Abstract **Purpose:** The objective of this study was to evaluate the efficacy and safety of two doses of pemetrexed supplemented with folic acid and vitamin B_{12} in pretreated Japanese patients with advanced non-small cell lung cancer (NSCLC).

Experimental Design: Patients with an Eastern Cooperative Oncology Group performance status 0 to 2, stage III or IV, and who received previously one or two chemotherapy regimens were randomized to receive 500 mg/m^2 pemetrexed (P500) or 1,000 mg/m^2 pemetrexed (P1000) on day 1 every 3 weeks. The primary endpoint was response rate.

Results: Of the 216 patients evaluable for efficacy (108 in each arm), response rates were 18.5% (90% confidence interval, 12.6-25.8%) and 14.8% (90% confidence interval, 9.5-21.6%), median survival times were 16.0 and 12.6 months, 1-year survival rates were 59.2% and 53.7%, and median progression-free survival were 3.0 and 2.5 months for the P500 and P1000, respectively. Cox multiple regression analysis indicated that pemetrexed dose was not a significant prognostic factor. Drug-related toxicity was generally tolerable for both doses; however, the safety profile of P500 showed generally milder toxicity. Main adverse drug reactions of severity grade 3 or 4 were neutrophil count decreased (20.2%) and alanine aminotransferase (glutamine pyruvic transaminase) increased (15.8%) in P500 and neutrophil count decreased (24.3%), WBC count decreased (20.7%), and lymphocyte count decreased (18.0%) in P1000. One drug-related death from interstitial lung disease occurred in the P500.

Conclusion: P500 and P1000 are similarly active with promising efficacy and acceptable safety outcomes in pretreated patients with NSCLC. These results support the use of P500 as a second- and third-line treatment of NSCLC.

Pemetrexed (LY231514; Alimta), a multitargeted antifolate, has shown antitumor activity as a single agent or in combination with other anticancer agents (1, 2). Pemetrexed at doses of 500 or 600 mg/m^2 has been evaluated in various clinical settings in a broad range of tumors including lung (non-small cell and mesothelioma), colorectal, gastric, pancreatic, head and neck, bladder, cervical, and breast cancers (3–13). In a randomized phase III trial that compared 3-week regimens of single-agent 500 mg/m^2 pemetrexed versus 75 mg/m^2 docetaxel in pretreated patients with non-small cell lung cancer (NSCLC), respective response rates (9.1% versus 8.8%) and median survival times (MST; 8.3 versus 7.9 months) did not differ between pemetrexed and docetaxel. However, fewer hematologic adverse effects, such as grade 3 or 4 neutropenia, febrile neutropenia, and neutropenic fever, were observed in patients treated with pemetrexed (3).

Myelosuppression is the predominant dose-limiting toxicity of pemetrexed as reported in phase I studies (14–16). A multivariate analysis identified the correlation between poor folate status (as indicated by elevated plasma homocysteine levels) and increased toxicity to pemetrexed, which led to the requirement that patients in all pemetrexed studies receive folic acid and vitamin B_{12} supplementation (2, 17). This has been shown to decrease toxicity to pemetrexed without compromising efficacy (18). Without supplementation, the maximum tolerated dose of pemetrexed, given every 3 weeks, has been shown to be 600 mg/m^2 in heavily pretreated patients (14); however, with supplementation, higher pemetrexed doses have been given without limiting side effects. In a Japanese phase I

Authors' Affiliations: [1]Department of Internal Medicine, National Cancer Center Hospital, Tokyo, Japan; [2]National Kyushu Cancer Center, Fukuoka, Japan; [3]Kinki University School of Medicine, Osakasayama, Japan; [4]National Cancer Center Hospital East, Kashiwa, Japan; [5]Shizuoka Cancer Center Hospital, Shizuoka, Japan; [6]Eli Lilly and Company, Oncology Platform Team, Indianapolis, Indiana; and [7]Eli Lilly Japan K.K., Lilly Research Laboratories Japan, Kobe, Japan

Received 12/11/07; revised 3/6/08; accepted 3/19/08.

Grant support: Eli Lilly and Company (study code: H3E-JE-NS01).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Note: The results of this study have been reported at American Society of Clinical Oncology, World Conference on Lung Cancer, and European Cancer Conference in 2007.

Requests for reprints: Yuichiro Ohe, Department of Internal Medicine, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan. Phone: 81-3-3542-2511; Fax: 81-3-3542-7006; E-mail: yohe@ncc.go.jp.

doi:10.1158/1078-0432.CCR-07-5143

study of pemetrexed that included folic acid and vitamin B_{12} supplementation, the maximum tolerated dose of pemetrexed was 1,200 mg/m^2 and recommended dose was 1,000 mg/m^2 given every 3 weeks (19). Pemetrexed pharmacokinetics in Japanese patients was not overtly different from those observed in Caucasian patients.

In view of these data, we conducted a randomized, phase II study that confirmed the efficacy and safety of a standard dose of pemetrexed (500 mg/m^2; P500) with that of a higher dose (1,000 mg/m^2; P1000), including folic acid and vitamin B_{12} supplementation, in previously treated NSCLC patients. The primary endpoint was evaluation of response rate. Secondary endpoints were assessments of response duration, progression-free survival (PFS), 1-year survival rate, MST, quality of life (QoL), and adverse events.

Materials and Methods

Patient selection. Men and women, between 20 and 75 years old, with a life expectancy of at least 12 weeks and histologically and/or cytologically confirmed advanced NSCLC were eligible for the study. In addition, all patients met the following inclusion criteria: stage III or IV disease, at least one target lesion, one or two prior chemotherapeutic regimens, an Eastern Cooperative Oncology Group performance status (PS) of 0 to 2, adequate bone marrow function (neutrophils ≥2,000/mm^3, platelets ≥100,000/mm^3, and hemoglobin ≥9.0 g/dL), hepatic function [total bilirubin within 1.5 times the upper normal limit, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) within 2.5 times the upper normal limit, and serum albumin ≥2.5 g/dL], renal function (serum creatinine ≤1.2 mg/dL and creatinine clearance ≥45 mL/min), and pulmonary function (functional oxygen saturation ≥92%).

Patients were excluded from the study for radiographic signs of interstitial pneumonitis or pulmonary fibrosis, serious or uncontrolled concomitant systemic disorders, active infections, the need for chronic administration of systemic corticosteroids, active double cancer and/or brain metastases, treatment with third-space fluid collections within 2 weeks of signing the informed consent or the need of such treatment, grade 3 or 4 toxicity, peripheral sensory neuropathy, previous pemetrexed therapy, unable or unwilling to take folic acid or vitamin B_{12} supplementation, or pregnant or breast-feeding.

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

Study design and sample size. This open-label multicenter study had response rate as the primary objective, and 244 patients were enrolled and 226 were allocated to either 500 mg/m^2 (P500) or 1,000 mg/m^2 (P1000) randomly.

The sample size was calculated to ensure that the response rate in each group exceeded 5%. Based on the results from previous study, assuming a 13% true response rate, 5% one-sided significance level for the test with exact probability based on binomial distribution, and 90% power, at least 107 patients in each treatment arm (total of 214) were necessary. Assuming a 10% dropout rate, 240 patients were planned for the study (actual: 244 patients).

The randomization was done by an independent registration center and was dynamically balanced for PS, previous platinum chemotherapy, disease stage, gender, time from prior chemotherapy to the enrollment, and hospital. Patients were balanced with respect to the study drug in each stratum for each prognostic factor using the minimization method.

Treatment plan. Pemetrexed was administered as an i.v., 10-min infusion on day 1 of a 21-day cycle. Patients were instructed to take orally 1 g/d of a multivitamin containing 500 μg folic acid from 1 week before day 1 of course 1 until 22 days after the last administration of pemetrexed. Vitamin B_{12} (1000 μg) was injected i.m. 1 week before day 1 of course 1 and repeated every 9 weeks until 22 days after the last administration of pemetrexed. Patients were discontinued from the study for disease progression, unacceptable adverse events, inadvertent enrollment, use of excluded concomitant therapy, a cycle delay of >42 days, or if the patient requested to discontinue the study.

Administration of pemetrexed was delayed if patients met any of the following criteria: neutrophils <2,000/mm^3, hemoglobin <9.0 g/dL, platelets <100,000/mm^3, AST/ALT >2.5 times the upper normal limit, total bilirubin >1.5 times the upper normal limit, serum creatinine >1.2 mg/dL, PS 3 or 4, or grade ≥3 nonhematologic toxicity (except for anorexia, nausea, vomiting, and fatigue). The dose of pemetrexed was decreased to 400 mg/m^2 in the P500 arm and to 800 mg/m^2 in the P1000 arm, if any of the following events occurred in the previous course: grade 4 leukopenia or neutropenia, grade ≥3 febrile neutropenia, thrombocytopenia, or platelet transfusion, grade ≥3 nonhematologic toxicity (except for grade 3 anorexia, nausea, vomiting, and fatigue), or AST/ALT increased. The pemetrexed dose was similarly reduced if initiation of the next course was postponed after day 29 due to drug-related adverse events. Patients who continued to show evidence of toxicity after reducing the pemetrexed dose were discontinued from the study.

Baseline and treatment assessments. Pretreatment assessments included chest X-ray, electrocardiogram, blood chemistry, urinalysis, pregnancy test, creatinine clearance, functional oxygen saturation, vital signs, PS, body weight, and use of prior therapies. Tumor size was examined using X-ray, computer tomography, or magnetic resonance imaging done within 28 days before the planned day of the first treatment. This was repeated about every 4 weeks after the first examination.

Tumor response rate was assessed as the percentage of patients in whom complete response (CR) and partial response (PR) were confirmed based on the best overall response of the tumor response evaluation. Response was evaluated according to the Response Evaluation Criteria in Solid Tumors (20). Objective tumor responses in all responding patients were evaluated by an external review committee given no information on the treatment groups.

Duration of overall response (CR + PR) was measured from the date of the first objective assessment of CR or PR until the date of progressive disease. PFS was measured from the date of registration (for the initiation of course 1) until the date of progressive disease or death. One-year survival rate was defined as the percentage of patients who survived for 1 year from the registration date. Survival was measured from the registration date to the date of death (regardless of cause).

QoL was assessed by the QoL Questionnaire for Cancer Patients Treated with Anticancer Drugs and the Functional Assessment of Cancer Therapy for Lung Cancer (Japanese version; refs. 21–23).

Assessments of QoL were done before treatment, before the second and third courses of chemotherapy, and 3 months after the start of treatment.

Adverse events were recorded throughout the study and after the last drug administration until signs of recovery were evident. All such events were evaluated according to the Common Terminology Criteria for Adverse Events version 3.0.

Statistical analysis. Efficacy measurements were done according to the guidelines for clinical evaluation methods of anticancer drugs. Efficacy analysis was done on patients who met all selection criteria and received at least one dose of pemetrexed. Safety analysis was done on patients who received at least one dose of pemetrexed.

Statistical tests were done to establish a pemetrexed response rate of >5%; 90% confidence intervals (CI) for the objective response rate were constructed for each arm. All survival curves for time-to-event variables were created using the Kaplan-Meier method; 95% CIs were calculated for each arm. Response rate, response duration, and PFS were compared between the two arms using the χ^2 test. Cox multiple regression analysis was done on all evaluable patients from two combined arms to

identify significant prognostic factors for survival. Covariates evaluated were pemetrexed dose, gender, age, PS, disease stage, histology, interval from prior chemotherapy to registration for the first treatment course, the number of prior chemotherapeutic regimens, and use of prior platinum chemotherapy. For the QoL analysis, distributions of subscales were summarized for each arm using descriptive statistics (mean, SD, minimum, median, and maximum). As a retrospective analysis for safety, major grade 3 to 4 drug-related adverse events were compared between the two arms using the χ^2 test.

Results

Patient disposition and characteristics. From October 2004 to October 2005, a total of 244 Japanese patients with advanced NSCLC were enrolled at 28 centers. Of the 244 patients enrolled, 226 were randomly assigned (114 to the P500 arm and 112 to the P1000 arm) at least 1 week before treatment after receiving folic acid and vitamin B_{12} supplementation. A total of 225 patients (114 in the P500 arm and 111 in P1000 arm) were evaluable for safety. Of these patients, 216 (108 in each arm) were evaluable for efficacy. Gender, age, PS, histology, stage, and prior platinum chemotherapy were well balanced across the two arms (Table 1).

Efficacy evaluation. Objective tumor response rates and durations of overall response are shown in Table 2. Of the 108 patients evaluable for efficacy in the P500 arm, 20 achieved PR for an objective response rate of 18.5% (90% CI, 12.6-25.8%); the median duration of response was 4.9 months (95% CI, 3.8-8.7 months). Of the 108 patients evaluable for efficacy in the P1000 arm, 16 achieved PR for an objective response rate of 14.8% (90% CI, 9.5-21.6%); the median duration of response was 3.0 months (95% CI, 2.8-6.1 months). As seen above, the lower limits of the 90% CI in both arms were >5%, showing a statistically significant objective response rate >5% in each of the arms. The differences between arms in response rate and response duration were not statistically significant ($P = 0.5839$ and 0.1740).

By October 2006, 125 of the 216 evaluable patients had died. The MST and 1-year survival rate were 16.0 months and 59.2% in the P500 arm and 12.6 months and 53.7% in the P1000 arm ($P = 0.1463$, log-rank test for survival; Fig. 1). Median PFS was 3.0 months (95% CI, 2.0-3.5 months) in the P500 arm and 2.5 months (95% CI, 1.8-3.2 months) in the P1000 arm ($P = 0.7139$, log-rank test).

Cox multiple regression analysis indicated that pemetrexed dose was not a significant prognostic factor; however, gender (female), PS (0), disease stage (III), histologic type (non-squamous cell carcinoma), and longer intervals from prior chemotherapy were shown to be good prognostic factors (Fig. 2). Of note, patients with non-squamous cell carcinoma had a longer MST compared with those with other histologic types (16.0 versus 9.3 months; $P = 0.00264$, Cox regression analysis). Pretreatment QoL assessments in both arms were relatively high and showed neither worsening nor improvement following pemetrexed treatment (Table 3).

Safety evaluation. A total of 225 patients (114 for P500 and 111 for P1000) were evaluable for safety. Leukopenia, neutropenia, lymphopenia, anemia, elevation of AST/ALT, lactate dehydrogenase, and rash were commonly reported; however, no grade 4 leukopenia or febrile neutropenia was observed (Table 4). Other grade 4 toxicities were uncommon. Gastrointestinal toxicities such as nausea, vomiting, and anorexia were mostly mild and more frequently reported in the P1000 arm. As a retrospective analysis for safety, major grade 3 to 4 drug-related adverse events were compared

Table 1. Patient characteristics

Variable	P500	P1000
Patients who were given at least one dose of pemetrexed	114	111
Gender		
Male	72	71
Female	42	40
Age, median (range)	61.0 (37-74)	62.0 (26-74)
Eastern Cooperative Oncology Group PS		
0	45	37
1	63	68
2	6	6
Histology		
Adenocarcinoma	79	82
Squamous cell carcinoma	25	26
Others	10	3
Disease stage		
III	22	22
IV	92	88
No. prior chemotherapies		
1	44	53
2	67	57
3	3	1
Prior platinum chemotherapy		
Yes	108	104
No	6	7
Interval from prior chemotherapy to registration for the first course starts (mo)		
<3	72	66
3	42	45

Table 2. Objective tumor response and median response duration

Variable	P500 (n = 108)	P1000 (n = 108)
Objective tumor response		
CR	0	0
PR	20	16
Stable disease	40	34
Progressive disease	48	58
Response rate (90% CI), %	18.50 (12.6-25.8)	14.80 (9.5-21.6)
Median response duration (95% CI), mo	4.9 (3.8-8.7)	3.0 (2.8-6.1)

between the two arms using the χ^2 test. Grade 3 or 4 anorexia was reported more frequently in the P1000 arm (10.8% versus 2.6%; P = 0.0284). Drug-related rash was observed in 67.5% and 80.2% of the patients treated with P500 and P1000, respectively. However, all severities were grade 1 or 2. Five of the P500 patients and 3 of the P1000 patients developed interstitial lung disease related to pemetrexed treatment that resulted in the death of one patient (P500 arm). The other 7 patients recovered from their illness after discontinuing the study drug. A total 16 (14.0%) patients in the P500 arm and 26 (23.4%) patients in the P1000 arm discontinued the treatment because of drug-related adverse events.

Dose administration. The median number of treatment courses completed in both arms was 3 (range, 1-24+). Eleven percent of patients in the P500 arm and 8% in the P1000 arm completed at least 10 courses. Dose reduction occurred in 20 (17.5%) patients in the P500 arm and 27 (24.3%) patients in the P1000 arm. The most frequent cause of dose reduction was ALT elevation. Relative dose intensities were 89.6% in the P500 group and 89.8% in the P1000 group.

Discussion

This phase II, randomized study is the first report on the efficacy and safety of a higher dose of pemetrexed (1,000 mg/m^2) in pretreated Japanese patients with NSCLC. Most patients (>50%) received two courses of prior chemotherapy, and the vast majority or patients (>90%) received prior platinum-based chemotherapy. The response data indicate promising tumor reduction activity and are noteworthy in pretreated patients. The survival data are also promising and better than those reported in second- and third-line settings and comparable with those reported in first-line settings (3, 24, 25). In the phase III study (3) comparing pemetrexed with docetaxel, the response

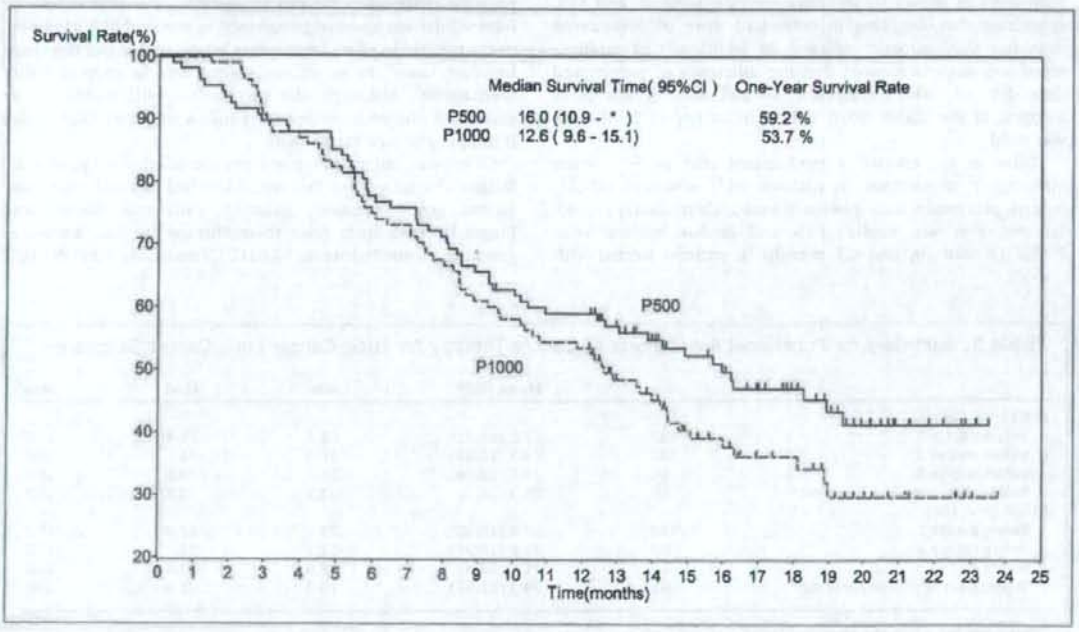

Fig. 1. Kaplan-Meier curve showing the overall survival for each arm. *Asterisk*, upper limit could not be calculated because of the censoring at the end of study period.

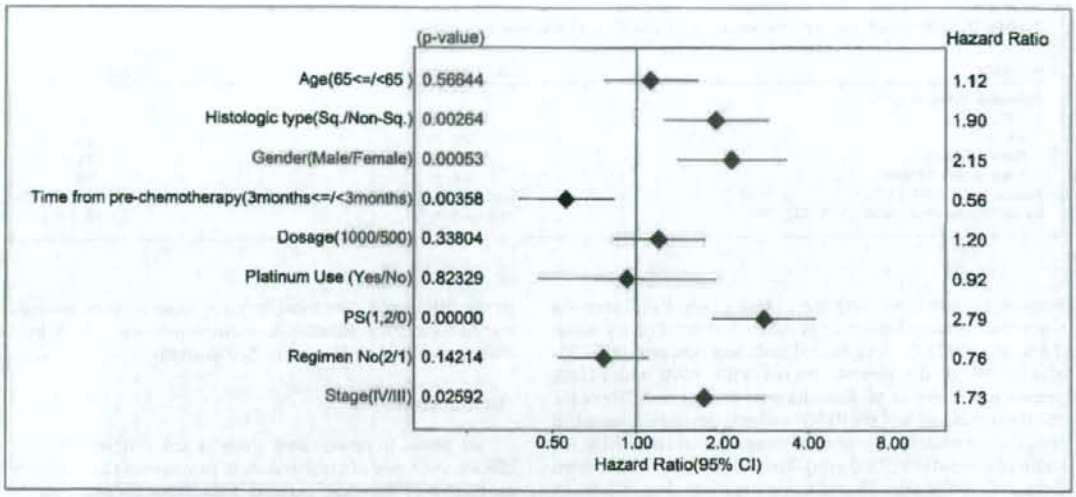

Fig. 2. Forest plot. Cox multiple regression analysis was done on all evaluable patients from two combined arms to identify significant prognostic factors for survival. Covariates evaluated were pemetrexed dose, gender, age, PS, disease stage, histology, interval from prior chemotherapy to registration for the first treatment course, the number of prior chemotherapeutic regimens, and use of prior platinum chemotherapy.

rate and median survival in the pemetrexed arm were 9.1% and 8.3 months, respectively.

Both P500 and P1000 with folic acid and vitamin B_{12} supplementation were similarly active in previously treated patients with NSCLC. All efficacy measures were similar in both arms as shown by the response rate, survival, and PFS, suggesting that doubling the standard dose of pemetrexed does not show superior efficacy. In addition, Cox multiple regression analysis showed that the difference of pemetrexed dose did not influence survival. Overall, toxicity was more frequent at the higher dose, although toxicity in both arms was mild.

Cullen et al. reported a randomized trial of 500 versus 900 mg/m^2 pemetrexed in patients with advanced NSCLC treated previously with platinum-based chemotherapy (26). The response rate, median PFS, and median survival were 7.1%, 2.6 months, and 6.7 months in patients treated with 500 mg/m^2 and 4.3%, 2.8 months, and 6.9 months in patients treated with 900 mg/m^2 pemetrexed, respectively. The higher dose did not improve survival more than the lower dose.

Dose intensification is not always accompanied by higher efficacy, such as in the case of docetaxel and cisplatin. One possible explanation for this in pemetrexed is that either the intracellular transport of pemetrexed is maximal at 500 mg/m^2 or the inhibition of target enzymes is saturated above this dose; however, there are as yet no *in vitro* data to support either mechanism. Although the mechanism still needs to be elucidated, the wide therapeutic window of pemetrexed makes it unique and safe for patients.

Of interest, our subgroup analysis identified some prognostic factors. The subgroups that were identified as good prognostic factors, gender (female), good PS, early-stage disease, and longer intervals from prior chemotherapy are well known as good prognostic factors for NSCLC. Of particular note, the MST

Table 3. Summary for Functional Assessment of Cancer Therapy for Lung Cancer Lung Cancer Subscale

	n	Mean (SD)	Min	Med	Max
P500 (*n* = 108)					
Before course 1	107	71.5 (18.81)	32.1	71.4	100
Before course 2	101	74.3 (16.68)	39.3	75	100
Before course 3	84	74.3 (18.08)	35.7	78.6	100
Registration of course 1 + 3 mo*	59	76.3 (18.1)	32.1	78.6	100
P1000 (*n* = 108)					
Before course 1	107	69.6 (18.52)	25	67.9	100
Before course 2	98	73.5 (17.21)	32.1	75	100
Before course 3	72	71.4 (18.4)	28.6	71.4	100
Registration of course 1 + 3 mo*	61	74.3 (18.62)	28.6	71.4	100

*Three months ±2 weeks after the day of registration for one course.

Table 4. Hematologic and nonhematologic toxicity evaluated by Common Terminology Criteria for Adverse Events version 3.0

	P500 (n = 114)				P1000 (n = 111)				P
	Grade (%)				Grade (%)				
	2	3	4	3/4/5	2	3	4	3/4/5	
Leukopenia	32.5	14.9	0	14.9	38.7	21.6	0	21.6	0.2582
Neutropenia	25.4	17.5	3.5	21.1	27.9	19.8	4.5	24.3	0.6695
Lymphopenia	28.9	9.6	2.6	12.3	30.6	16.2	1.8	18	0.31
Anemia	19.3	7	0.9	7.9	34.2	9	0.9	9.9	0.7667
Thrombocytopenia	0	0	0	0	8.1	0.9	0	0.9	NA
Febrile neutropenia	*	0	0	0	*	0	0	0	NA
Nausea	14	0	0	0	14.4	2.7	0	2.7	NA
Vomiting	7	0	0	0	11.7	1.8	0	1.8	NA
Anorexia	16.7	2.6	0	2.6	15.3	10.8	0	10.8	0.0284
Fatigue	3.5	0	0	0	1.8	0.9	0	0.9	NA
Diarrhea	2.6	0.9	0	0.9	1.8	1.8	0	1.8	0.9815
Constipation	1.8	0.9	0	0.9	5.4	0	0	0	NA
Rash	49.1	2.6	0	2.6	63.1	4.5	0	4.5	0.6903
Alopecia	0	*	*	*	0	*	*	*	NA
Pneumonitis	1.8	1.8	0	2.6†	0	2.7	0	2.7	1
AST	21.9	7.9	0	7.9	25.2	4.5	0	4.5	0.4375
ALT	17.5	16.7	0	16.7	32.4	7.2	0.9	8.1	0.8143

NOTE: Major grade 3 to 4 drug-related adverse events were compared between two arms using χ^2 test.
*Not indicated in Common Terminology Criteria for Adverse Events version 3.0.
†One patient died of drug-induced pneumonitis.

of patients with non-squamous cell carcinoma was significantly longer compared with that in patients with squamous cell carcinoma (16.0 versus 9.3 months; $P = 0.00264$). Pemetrexed induces its antitumor activity by inhibiting key enzymes related to the folate metabolism, such as thymidylate synthase. Studies of the tumor histology of adenocarcinoma progressive disease have reported lower-level expression of thymidylate synthase than squamous cell carcinoma (27). Good survival benefit in patients with non-squamous cell carcinoma by pemetrexed may be explained by lower levels of thymidylate synthase. Because MST was the subject of a subgroup analysis and survival was not a primary endpoint of this study, this finding should be considered exploratory requiring independent confirmation. However, if this finding of superior effectiveness in non-squamous cell carcinoma could be substantiated in future studies, it would be very useful. Indeed, histology could be a simple means of tailoring chemotherapy treatment.

In conclusion, although the recommended dose is P1000 with folic acid and vitamin B_{12} supplementation for Japanese patients, it has similar efficacy and safety with P500, the recommend dosage in rest of the world. These results support the use of P500 as a second- or third-line treatment of NSCLC.

Disclosure of Potential Conflicts of Interest

Authors have conflicts with Eli Lilly and company.

Acknowledgments

We thank the physicians who enrolled patients (Drs. Hiroshi Isobe, Akira Inoue, Yoshio Tomizawa, Akira Yokoyama, Shuichi Yoneda, Masaru Narabayashi, Masahiko Shibuya, Masahiro Tsuboi, Hiroaki Okamoto, Kenji Eguchi, Toshiyuki Sawa, Koji Takeda, Fumio Imamura, Shinzoh Kudoh, Masaaki Kawahara, Kaoru Matsui, Nobuyuki Katakami, Shunichi Negoro, Katsuyuki Kiura, Yoshihiko Segawa, Koichi Takayama, Mitsuhiro Matsumoto, and Takeshi Horai) and Michiyo Matsushima for assistance in the creation and submission of this article.

References

1. Walling J. From methotrexate to pemetrexed and beyond. A review of the pharmacodynamic and clinical properties of antifolates. Invest New Drugs 2006;24:37–77.
2. Hazarika M, White RM, Johnson JR, Pazdur R. FDA drug approval summaries: pemetrexed (Alimta®). Oncologist 2004;9:482–8.
3. Hanna N, Shepherd FA, Fossella FV, et al. Randomized phase III trial of pemetrexed versus docetaxel in patients with non-small-cell lung cancer previously treated with chemotherapy. J Clin Oncol 2004;22:1589–97.
4. Vogelzang NJ, Rusthoven JJ, Symanowski J, et al. Phase III study of pemetrexed in combination with cisplatin versus cisplatin alone in patients with malignant pleural mesothelioma. J Clin Oncol 2003;21:2636–44.
5. John W, Picus J, Blanke CD, et al. Activity of multitargeted antifolate (pemetrexed disodium, LY231514) in patients with advanced colorectal carcinoma: results from a phase II study. Cancer 2000;88:1807–13.
6. Cripps C, Burnell M, Jolivet J, et al. Phase II study of first-line LY231514 (multi-targeted antifolate) in patients with locally advanced or metastatic colorectal cancer: an NCIC Clinical Trials Group study. Ann Oncol 1999;10:1175–9.
7. Bajetta E, Celio L, Buzzoni R, et al. Phase II study of pemetrexed disodium (Alimta®) administered with oral folic acid in patients with advanced gastric cancer. Ann Oncol 2003;14:1543–8.
8. Miller KD, Picus J, Blanke C, et al. Phase II study of the multitargeted antifolate LY231514 (Alimta, MTA, pemetrexed disodium) in patients with advanced pancreatic cancer. Ann Oncol 2000;11:101–3.
9. Pivot X, Raymond E, Laguerre B, et al. Pemetrexed disodium in recurrent locally advanced or metastatic squamous cell carcinoma of the head and neck. Br J Cancer 2001;85:649–55.
10. Hanauske AR, Chen V, Paoletti P, Niyikiza C. Pemetrexed disodium: a novel antifolate clinically active against multiple solid tumors. Oncologist 2001;6:363–73.
11. Goedhals L, van Wiyk AL, Smith BL, Fourie SJ. Pemetrexed (Alimta, LY231514) demonstrates clinical activity in chemonaive patients with cervical cancer in a phase II single-agent trial. Int J Gynecol Cancer 2006;16:1172–8.
12. Miles DW, Smith IE, Coleman RE, Calvert AH, Lind MJ. A phase II study of pemetrexed disodium (LY231514) in patients with locally recurrent or metastatic breast cancer. Eur J Cancer 2001;37:1366–71.

13. Martin M, Spielmann M, Namer M, et al. Phase II study of pemetrexed in breast cancer patients pretreated with anthracyclines. Ann Oncol 2003;14:1246–52.
14. Rinaldi DA, Kuhn JG, Burris HA, et al. A phase I evaluation of multitargeted antifolate (MTA, LY231514), administered every 21 days, utilizing the modified continual reassessment method for dose escalation. Cancer Chemother Pharmacol 1999;44:372–80.
15. Rinaldi DA, Burris HA, Dorr FA, et al. Initial phase I evaluation of the novel thymidylate synthase inhibitor, LY231514, using the modified continual reassessment method for dose escalation. J Clin Oncol 1995;13:2842–50.
16. McDonald AC, Vasey PA, Adams L, et al. A phase I and pharmacokinetic study of LY231514, the multitargeted antifolate. Clin Cancer Res 1998;4:605–10.
17. Niyikiza C, Baker SD, Seitz DE, et al. Homocysteine and methylmalonic acid: markers to predict and avoid toxicity from pemetrexed therapy. Mol Cancer Ther 2002;1:545–52.
18. Scagliotti GV, Shin DM, Kindler HL, et al. Phase II study of pemetrexed with and without folic acid and vitamin B_{12} as front-line therapy in malignant pleural mesothelioma. J Clin Oncol 2003;21:1556–61.
19. Nakagawa K, Kudoh S, Matsui K, et al. A phase I study of pemetrexed (LY231514) supplemented with folate and vitamin B_{12} in Japanese patients with solid tumours. Br J Cancer 2006;95:677–82.
20. Therasse P, Arbuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors. J Natl Cancer Inst 2000;92:205–16.
21. Cella DF, Bonomi AE, Lloyd SR, Tulsky DS, Kaplan E, Bonomi P. Reliability and validity of the Functional Assessment of Cancer Therapy-Lung (FACT-L) quality of life instrument. Lung Cancer 1995;12:199–220.
22. Kurihara M, Shimizu H, Tsuboi K, et al. Development of quality of life questionnaire in Japan: quality of life assessment of cancer patients receiving chemotherapy. Psychooncology 1999;8:355–63.
23. Matsumoto T, Ohashi Y, Morita S, et al. The quality of life questionnaire for cancer patients treated with anticancer drugs (QOL-ACD): validity and reliability in Japanese patients with advanced non-small-cell lung cancer. Qual Life Res 2002;11:483–93.
24. Shepherd FA, Dancey J, Ramlau R, et al. Prospective randomized trial of docetaxel versus best supportive care in patients with non-small-cell lung cancer previously treated with platinum-based chemotherapy. J Clin Oncol 2000;18:2095–103.
25. Ohe Y, Ohashi Y, Kubota K, et al. Randomized phase III study of cisplatin plus irinotecan versus carboplatin plus paclitaxel, cisplatin plus gemcitabine, and cisplatin plus vinorelbine for advanced non-small-cell lung cancer: Four-Arm Cooperative Study in Japan. Ann Oncol 2007;18:317–23.
26. Cullen M, Zatloukal P, Sörenson S, et al. Pemetrexed for the treatment of advanced non-small cell lung cancer (NSCLC): results from a randomized phase III dose finding trial in patients who progressed following platinum-containing chemotherapy. J Thorac Oncol 2007;2:S316–7.
27. Ceppi P, Volante M, Saviozzi S, et al. Squamous cell carcinoma of the lung compared with other histotypes shows higher messenger RNA and protein levels for thymidylate synthase. Cancer 2006;107:1589–96.

Efficacy and Safety of Two Doses of Pemetrexed Supplemented with Folic Acid and Vitamin B_{12} in Previously Treated Patients with Non-Small Cell Lung Cancer

Yuichiro Ohe,[1] Yukito Ichinose,[2] Kazuhiko Nakagawa,[3] Tomohide Tamura,[1] Kaoru Kubota,[4] Nobuyuki Yamamoto,[5] Susumu Adachi,[6] Yoshihiro Nambu,[7] Toshio Fujimoto,[7] Yutaka Nishiwaki,[4] Nagahiro Saijo,[4] and Masahiro Fukuoka[3]

Abstract **Purpose:** The objective of this study was to evaluate the efficacy and safety of two doses of pemetrexed supplemented with folic acid and vitamin B_{12} in pretreated Japanese patients with advanced non-small cell lung cancer (NSCLC).

Experimental Design: Patients with an Eastern Cooperative Oncology Group performance status 0 to 2, stage III or IV, and who received previously one or two chemotherapy regimens were randomized to receive 500 mg/m^2 pemetrexed (P500) or 1,000 mg/m^2 pemetrexed (P1000) on day 1 every 3 weeks. The primary endpoint was response rate.

Results: Of the 216 patients evaluable for efficacy (108 in each arm), response rates were 18.5% (90% confidence interval, 12.6-25.8%) and 14.8% (90% confidence interval, 9.5-21.6%), median survival times were 16.0 and 12.6 months, 1-year survival rates were 59.2% and 53.7%, and median progression-free survival were 3.0 and 2.5 months for the P500 and P1000, respectively. Cox multiple regression analysis indicated that pemetrexed dose was not a significant prognostic factor. Drug-related toxicity was generally tolerable for both doses; however, the safety profile of P500 showed generally milder toxicity. Main adverse drug reactions of severity grade 3 or 4 were neutrophil count decreased (20.2%) and alanine aminotransferase (glutamine pyruvic transaminase) increased (15.8%) in P500 and neutrophil count decreased (24.3%), WBC count decreased (20.7%), and lymphocyte count decreased (18.0%) in P1000. One drug-related death from interstitial lung disease occurred in the P500.

Conclusion: P500 and P1000 are similarly active with promising efficacy and acceptable safety outcomes in pretreated patients with NSCLC. These results support the use of P500 as a second- and third-line treatment of NSCLC.

Pemetrexed (LY231514; Alimta), a multitargeted antifolate, has shown antitumor activity as a single agent or in combination with other anticancer agents (1, 2). Pemetrexed at doses of 500 or 600 mg/m^2 has been evaluated in various clinical settings in a broad range of tumors including lung (non-small cell and mesothelioma), colorectal, gastric, pancreatic, head and neck, bladder, cervical, and breast cancers (3-13). In a randomized phase III trial that compared 3-week regimens of single-agent 500 mg/m^2 pemetrexed versus 75 mg/m^2 docetaxel in pretreated patients with non-small cell lung cancer (NSCLC), respective response rates (9.1% versus 8.8%) and median survival times (MST; 8.3 versus 7.9 months) did not differ between pemetrexed and docetaxel. However, fewer hematologic adverse effects, such as grade 3 or 4 neutropenia, febrile neutropenia, and neutropenic fever, were observed in patients treated with pemetrexed (3).

Myelosuppression is the predominant dose-limiting toxicity of pemetrexed as reported in phase I studies (14-16). A multivariate analysis identified the correlation between poor folate status (as indicated by elevated plasma homocysteine levels) and increased toxicity to pemetrexed, which led to the requirement that patients in all pemetrexed studies receive folic acid and vitamin B_{12} supplementation (2, 17). This has been shown to decrease toxicity to pemetrexed without compromising efficacy (18). Without supplementation, the maximum tolerated dose of pemetrexed, given every 3 weeks, has been shown to be 600 mg/m^2 in heavily pretreated patients (14); however, with supplementation, higher pemetrexed doses have been given without limiting side effects. In a Japanese phase I

Authors' Affiliations: [1]Department of Internal Medicine, National Cancer Center Hospital, Tokyo, Japan; [2]National Kyushu Cancer Center, Fukuoka, Japan; [3]Kinki University School of Medicine, Osakasayama, Japan; [4]National Cancer Center Hospital East, Kashiwa, Japan; [5]Shizuoka Cancer Center Hospital, Shizuoka, Japan; [6]Eli Lilly and Company, Oncology Platform Team, Indianapolis, Indiana; and [7]Eli Lilly Japan K.K., Lilly Research Laboratories Japan, Kobe, Japan

Received 12/11/07; revised 3/6/08; accepted 3/19/08.

Grant support: Eli Lilly and Company (study code: H3E-JE-NS01).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Note: The results of this study have been reported at American Society of Clinical Oncology, World Conference on Lung Cancer, and European Cancer Conference in 2007.

Requests for reprints: Yuichiro Ohe, Department of Internal Medicine, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan. Phone: 81-3-3542-2511; Fax: 81-3-3542-7006; E-mail: yohe@ncc.go.jp.

doi:10.1158/1078-0432.CCR-07-5143

study of pemetrexed that included folic acid and vitamin B_{12} supplementation, the maximum tolerated dose of pemetrexed was 1,200 mg/m^2 and recommended dose was 1,000 mg/m^2 given every 3 weeks (19). Pemetrexed pharmacokinetics in Japanese patients was not overtly different from those observed in Caucasian patients.

In view of these data, we conducted a randomized, phase II study that confirmed the efficacy and safety of a standard dose of pemetrexed (500 mg/m^2; P500) with that of a higher dose (1,000 mg/m^2; P1000), including folic acid and vitamin B_{12} supplementation, in previously treated NSCLC patients. The primary endpoint was evaluation of response rate. Secondary endpoints were assessments of response duration, progression-free survival (PFS), 1-year survival rate, MST, quality of life (QoL), and adverse events.

Materials and Methods

Patient selection. Men and women, between 20 and 75 years old, with a life expectancy of at least 12 weeks and histologically and/or cytologically confirmed advanced NSCLC were eligible for the study. In addition, all patients met the following inclusion criteria: stage III or IV disease, at least one target lesion, one or two prior chemotherapeutic regimens, an Eastern Cooperative Oncology Group performance status (PS) of 0 to 2, adequate bone marrow function (neutrophils ≥2,000/mm^3, platelets ≥100,000/mm^3, and hemoglobin ≥9.0 g/dL), hepatic function [total bilirubin within 1.5 times the upper normal limit, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) within 2.5 times the upper normal limit, and serum albumin ≥2.5 g/dL], renal function (serum creatinine ≤1.2 mg/dL and creatinine clearance ≥45 mL/min), and pulmonary function (functional oxygen saturation ≥92%).

Patients were excluded from the study for radiographic signs of interstitial pneumonitis or pulmonary fibrosis, serious or uncontrolled concomitant systemic disorders, active infections, the need for chronic administration of systemic corticosteroids, active double cancer and/or brain metastases, treatment with third-space fluid collections within 2 weeks of signing the informed consent or the need of such treatment, grade 3 or 4 toxicity, peripheral sensory neuropathy, previous pemetrexed therapy, unable or unwilling to take folic acid or vitamin B_{12} supplementation, or pregnant or breast-feeding.

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

Study design and sample size. This open-label multicenter study had response rate as the primary objective, and 244 patients were enrolled and 226 were allocated to either 500 mg/m^2 (P500) or 1,000 mg/m^2 (P1000) randomly.

The sample size was calculated to ensure that the response rate in each group exceeded 5%. Based on the results from previous study, assuming a 13% true response rate, 5% one-sided significance level for the test with exact probability based on binomial distribution, and 90% power, at least 107 patients in each treatment arm (total of 214) were necessary. Assuming a 10% dropout rate, 240 patients were planned for the study (actual: 244 patients).

The randomization was done by an independent registration center and was dynamically balanced for PS, previous platinum chemotherapy, disease stage, gender, time from prior chemotherapy to the enrollment, and hospital. Patients were balanced with respect to the study drug in each stratum for each prognostic factor using the minimization method.

Treatment plan. Pemetrexed was administered as an i.v. 10-min infusion on day 1 of a 21-day cycle. Patients were instructed to take orally 1 g/d of a multivitamin containing 500 μg folic acid from 1 week before day 1 of course 1 until 22 days after the last administration of pemetrexed. Vitamin B_{12} (1000 μg) was injected i.m. 1 week before day 1 of course 1 and repeated every 9 weeks until 22 days after the last administration of pemetrexed. Patients were discontinued from the study for disease progression, unacceptable adverse events, inadvertent enrollment, use of excluded concomitant therapy, a cycle delay of >42 days, or if the patient requested to discontinue the study.

Administration of pemetrexed was delayed if patients met any of the following criteria: neutrophils <2,000/mm^3, hemoglobin <9.0 g/dL, platelets <100,000/mm^3, AST/ALT >2.5 times the upper normal limit, total bilirubin >1.5 times the upper normal limit, serum creatinine >1.2 mg/dL, PS 3 or 4, or grade ≥3 nonhematologic toxicity (except for anorexia, nausea, vomiting, and fatigue). The dose of pemetrexed was decreased to 400 mg/m^2 in the P500 arm and to 800 mg/m^2 in the P1000 arm, if any of the following events occurred in the previous course: grade 4 leukopenia or neutropenia, grade ≥3 febrile neutropenia, thrombocytopenia, or platelet transfusion, grade ≥3 nonhematologic toxicity (except for grade 3 anorexia, nausea, vomiting, and fatigue), or AST/ALT increased. The pemetrexed dose was similarly reduced if initiation of the next course was postponed after day 29 due to drug-related adverse events. Patients who continued to show evidence of toxicity after reducing the pemetrexed dose were discontinued from the study.

Baseline and treatment assessments. Pretreatment assessments included chest X-ray, electrocardiogram, blood chemistry, urinalysis, pregnancy test, creatinine clearance, functional oxygen saturation, vital signs, PS, body weight, and use of prior therapies. Tumor size was examined using X-ray, computer tomography, or magnetic resonance imaging done within 28 days before the planned day of the first treatment. This was repeated about every 4 weeks after the first examination.

Tumor response rate was assessed as the percentage of patients in whom complete response (CR) and partial response (PR) were confirmed based on the best overall response of the tumor response evaluation. Response was evaluated according to the Response Evaluation Criteria in Solid Tumors (20). Objective tumor responses in all responding patients were evaluated by an external review committee given no information on the treatment groups.

Duration of overall response (CR + PR) was measured from the date of the first objective assessment of CR or PR until the date of progressive disease. PFS was measured from the date of registration (for the initiation of course 1) until the date of progressive disease or death. One-year survival rate was defined as the percentage of patients who survived for 1 year from the registration date. Survival was measured from the registration date to the date of death (regardless of cause).

QoL was assessed by the QoL Questionnaire for Cancer Patients Treated with Anticancer Drugs and the Functional Assessment of Cancer Therapy for Lung Cancer (Japanese version; refs. 21–23).

Assessments of QoL were done before treatment, before the second and third courses of chemotherapy, and 3 months after the start of treatment.

Adverse events were recorded throughout the study and after the last drug administration until signs of recovery were evident. All such events were evaluated according to the Common Terminology Criteria for Adverse Events version 3.0.

Statistical analysis. Efficacy measurements were done according to the guidelines for clinical evaluation methods of anticancer drugs. Efficacy analysis was done on patients who met all selection criteria and received at least one dose of pemetrexed. Safety analysis was done on patients who received at least one dose of pemetrexed.

Statistical tests were done to establish a pemetrexed response rate of >5%; 90% confidence intervals (CI) for the objective response rate were constructed for each arm. All survival curves for time-to-event variables were created using the Kaplan-Meier method; 95% CIs were calculated for each arm. Response rate, response duration, and PFS were compared between the two arms using the χ^2 test. Cox multiple regression analysis was done on all evaluable patients from two combined arms to

identify significant prognostic factors for survival. Covariates evaluated were pemetrexed dose, gender, age, PS, disease stage, histology, interval from prior chemotherapy to registration for the first treatment course, the number of prior chemotherapeutic regimens, and use of prior platinum chemotherapy. For the QoL analysis, distributions of subscales were summarized for each arm using descriptive statistics (mean, SD, minimum, median, and maximum). As a retrospective analysis for safety, major grade 3 to 4 drug-related adverse events were compared between the two arms using the χ^2 test.

Results

Patient disposition and characteristics. From October 2004 to October 2005, a total of 244 Japanese patients with advanced NSCLC were enrolled at 28 centers. Of the 244 patients enrolled, 226 were randomly assigned (114 to the P500 arm and 112 to the P1000 arm) at least 1 week before treatment after receiving folic acid and vitamin B_{12} supplementation. A total of 225 patients (114 in the P500 arm and 111 in P1000 arm) were evaluable for safety. Of these patients, 216 (108 in each arm) were evaluable for efficacy. Gender, age, PS, histology, stage, and prior platinum chemotherapy were well balanced across the two arms (Table 1).

Efficacy evaluation. Objective tumor response rates and durations of overall response are shown in Table 2. Of the 108 patients evaluable for efficacy in the P500 arm, 20 achieved PR for an objective response rate of 18.5% (90% CI, 12.6-25.8%); the median duration of response was 4.9 months (95% CI, 3.8-8.7 months). Of the 108 patients evaluable for efficacy in the P1000 arm, 16 achieved PR for an objective response rate of 14.8% (90% CI, 9.5-21.6%); the median duration of response was 3.0 months (95% CI, 2.8-6.1 months). As seen above, the lower limits of the 90% CI in both arms were >5%, showing a statistically significant objective response rate >5% in each of the arms. The differences between arms in response rate and response duration were not statistically significant ($P = 0.5839$ and 0.1740).

By October 2006, 125 of the 216 evaluable patients had died. The MST and 1-year survival rate were 16.0 months and 59.2% in the P500 arm and 12.6 months and 53.7% in the P1000 arm ($P = 0.1463$, log-rank test for survival; Fig. 1). Median PFS was 3.0 months (95% CI, 2.0-3.5 months) in the P500 arm and 2.5 months (95% CI, 1.8-3.2 months) in the P1000 arm ($P = 0.7139$, log-rank test).

Cox multiple regression analysis indicated that pemetrexed dose was not a significant prognostic factor; however, gender (female), PS (0), disease stage (III), histologic type (non-squamous cell carcinoma), and longer intervals from prior chemotherapy were shown to be good prognostic factors (Fig. 2). Of note, patients with non-squamous cell carcinoma had a longer MST compared with those with other histologic types (16.0 versus 9.3 months; $P = 0.00264$, Cox regression analysis). Pretreatment QoL assessments in both arms were relatively high and showed neither worsening nor improvement following pemetrexed treatment (Table 3).

Safety evaluation. A total of 225 patients (114 for P500 and 111 for P1000) were evaluable for safety. Leukopenia, neutropenia, lymphopenia, anemia, elevation of AST/ALT, lactate dehydrogenase, and rash were commonly reported; however, no grade 4 leukopenia or febrile neutropenia was observed (Table 4). Other grade 4 toxicities were uncommon. Gastrointestinal toxicities such as nausea, vomiting, and anorexia were mostly mild and more frequently reported in the P1000 arm. As a retrospective analysis for safety, major grade 3 to 4 drug-related adverse events were compared

Table 1. Patient characteristics

Variable	P500	P1000
Patients who were given at least one dose of pemetrexed	114	111
Gender		
Male	72	71
Female	42	40
Age, median (range)	61.0 (37-74)	62.0 (26-74)
Eastern Cooperative Oncology Group PS		
0	45	37
1	63	68
2	6	6
Histology		
Adenocarcinoma	79	82
Squamous cell carcinoma	25	26
Others	10	3
Disease stage		
III	22	22
IV	92	88
No. prior chemotherapies		
1	44	53
2	67	57
3	3	1
Prior platinum chemotherapy		
Yes	108	104
No	6	7
Interval from prior chemotherapy to registration for the first course starts (mo)		
<3	72	66
3	42	45

Table 2. Objective tumor response and median response duration

Variable	P500 (n = 108)	P1000 (n = 108)
Objective tumor response		
CR	0	0
PR	20	16
Stable disease	40	34
Progressive disease	48	58
Response rate (90% CI), %	18.50 (12.6-25.8)	14.80 (9.5-21.6)
Median response duration (95% CI), mo	4.9 (3.8-8.7)	3.0 (2.8-6.1)

between the two arms using the χ^2 test. Grade 3 or 4 anorexia was reported more frequently in the P1000 arm (10.8% versus 2.6%; P = 0.0284). Drug-related rash was observed in 67.5% and 80.2% of the patients treated with P500 and P1000, respectively. However, all severities were grade 1 or 2. Five of the P500 patients and 3 of the P1000 patients developed interstitial lung disease related to pemetrexed treatment that resulted in the death of one patient (P500 arm). The other 7 patients recovered from their illness after discontinuing the study drug. A total 16 (14.0%) patients in the P500 arm and 26 (23.4%) patients in the P1000 arm discontinued the treatment because of drug-related adverse events.

Dose administration. The median number of treatment courses completed in both arms was 3 (range, 1-24+). Eleven percent of patients in the P500 arm and 8% in the P1000 arm completed at least 10 courses. Dose reduction occurred in 20 (17.5%) patients in the P500 arm and 27 (24.3%) patients in the P1000 arm. The most frequent cause of dose reduction was ALT elevation. Relative dose intensities were 89.6% in the P500 group and 89.8% in the P1000 group.

Discussion

This phase II, randomized study is the first report on the efficacy and safety of a higher dose of pemetrexed (1,000 mg/m^2) in pretreated Japanese patients with NSCLC. Most patients (>50%) received two courses of prior chemotherapy, and the vast majority or patients (>90%) received prior platinum-based chemotherapy. The response data indicate promising tumor reduction activity and are noteworthy in pretreated patients. The survival data are also promising and better than those reported in second- and third-line settings and comparable with those reported in first-line settings (3, 24, 25). In the phase III study (3) comparing pemetrexed with docetaxel, the response

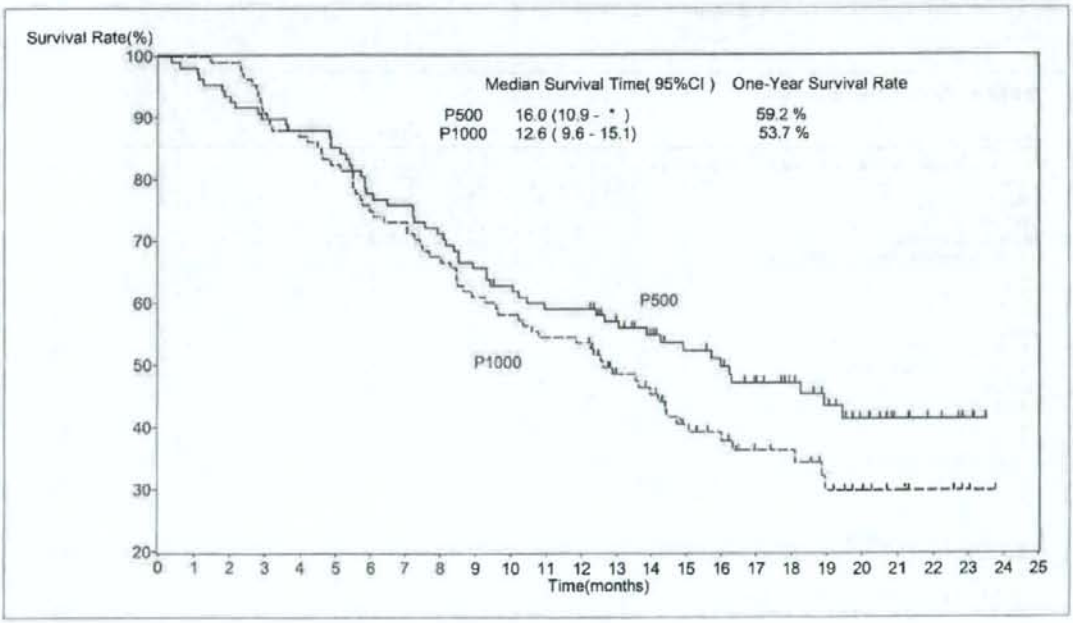

Fig. 1. Kaplan-Meier curve showing the overall survival for each arm. *Asterisk*, upper limit could not be calculated because of the censoring at the end of study period.

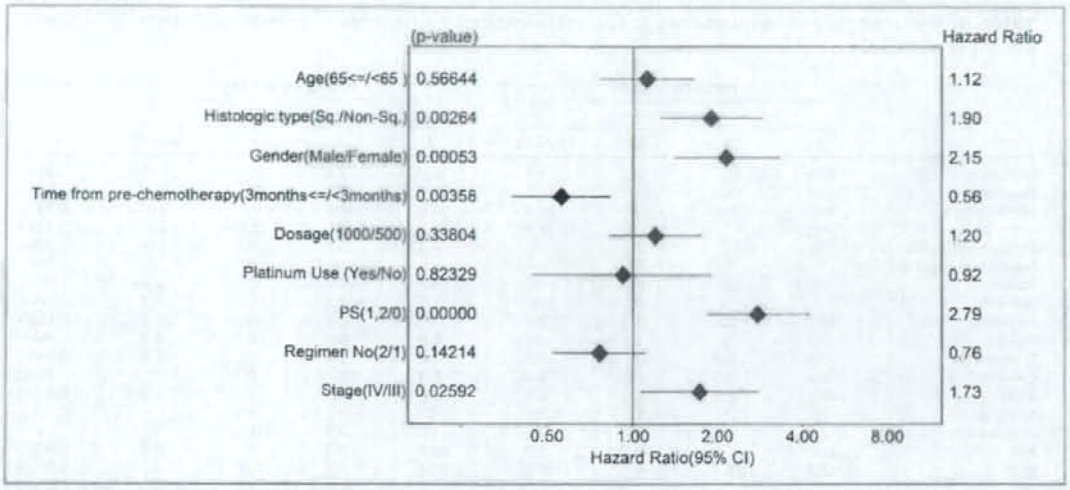

Fig. 2. Forest plot. Cox multiple regression analysis was done on all evaluable patients from two combined arms to identify significant prognostic factors for survival. Covariates evaluated were pemetrexed dose, gender, age, PS, disease stage, histology, interval from prior chemotherapy to registration for the first treatment course, the number of prior chemotherapeutic regimens, and use of prior platinum chemotherapy.

rate and median survival in the pemetrexed arm were 9.1% and 8.3 months, respectively.

Both P500 and P1000 with folic acid and vitamin B_{12} supplementation were similarly active in previously treated patients with NSCLC. All efficacy measures were similar in both arms as shown by the response rate, survival, and PFS, suggesting that doubling the standard dose of pemetrexed does not show superior efficacy. In addition, Cox multiple regression analysis showed that the difference of pemetrexed dose did not influence survival. Overall, toxicity was more frequent at the higher dose, although toxicity in both arms was mild.

Cullen et al. reported a randomized trial of 500 versus 900 mg/m^2 pemetrexed in patients with advanced NSCLC treated previously with platinum-based chemotherapy (26). The response rate, median PFS, and median survival were 7.1%, 2.6 months, and 6.7 months in patients treated with 500 mg/m^2 and 4.3%, 2.8 months, and 6.9 months in patients treated with 900 mg/m^2 pemetrexed, respectively. The higher dose did not improve survival more than the lower dose.

Dose intensification is not always accompanied by higher efficacy, such as in the case of docetaxel and cisplatin. One possible explanation for this in pemetrexed is that either the intracellular transport of pemetrexed is maximal at 500 mg/m^2 or the inhibition of target enzymes is saturated above this dose; however, there are as yet no *in vitro* data to support either mechanism. Although the mechanism still needs to be elucidated, the wide therapeutic window of pemetrexed makes it unique and safe for patients.

Of interest, our subgroup analysis identified some prognostic factors. The subgroups that were identified as good prognostic factors, gender (female), good PS, early-stage disease, and longer intervals from prior chemotherapy are well known as good prognostic factors for NSCLC. Of particular note, the MST

Table 3. Summary for Functional Assessment of Cancer Therapy for Lung Cancer Lung Cancer Subscale

	n	Mean (SD)	Min	Med	Max
P500 (*n* = 108)					
Before course 1	107	71.5 (18.81)	32.1	71.4	100
Before course 2	101	74.3 (16.68)	39.3	75	100
Before course 3	84	74.3 (18.08)	35.7	78.6	100
Registration of course 1 + 3 mo*	59	76.3 (18.1)	32.1	78.6	100
P1000 (*n* = 108)					
Before course 1	107	69.6 (18.52)	25	67.9	100
Before course 2	98	73.5 (17.21)	32.1	75	100
Before course 3	72	71.4 (18.4)	28.6	71.4	100
Registration of course 1 + 3 mo*	61	74.3 (18.62)	28.6	71.4	100

*Three months ±2 weeks after the day of registration for one course.

Table 4. Hematologic and nonhematologic toxicity evaluated by Common Terminology Criteria for Adverse Events version 3.0

	P500 (n = 114)				P1000 (n = 111)				P
	Grade (%)				Grade (%)				
	2	3	4	3/4/5	2	3	4	3/4/5	
Leukopenia	32.5	14.9	0	14.9	38.7	21.6	0	21.6	0.2582
Neutropenia	25.4	17.5	3.5	21.1	27.9	19.8	4.5	24.3	0.6695
Lymphopenia	28.9	9.6	2.6	12.3	30.6	16.2	1.8	18	0.31
Anemia	19.3	7	0.9	7.9	34.2	9	0.9	9.9	0.7667
Thrombocytopenia	0	0	0	0	8.1	0.9	0	0.9	NA
Febrile neutropenia	*	0	0	0	*	0	0	0	NA
Nausea	14	0	0	0	14.4	2.7	0	2.7	NA
Vomiting	7	0	0	0	11.7	1.8	0	1.8	NA
Anorexia	16.7	2.6	0	2.6	15.3	10.8	0	10.8	0.0284
Fatigue	3.5	0	0	0	1.8	0.9	0	0.9	NA
Diarrhea	2.6	0.9	0	0.9	1.8	1.8	0	1.8	0.9815
Constipation	1.8	0.9	0	0.9	5.4	0	0	0	NA
Rash	49.1	2.6	0	2.6	63.1	4.5	0	4.5	0.6903
Alopecia	0	*	*	*	0	*	*	*	NA
Pneumonitis	1.8	1.8	0	2.6[†]	0	2.7	0	2.7	1
AST	21.9	7.9	0	7.9	25.2	4.5	0	4.5	0.4375
ALT	17.5	16.7	0	16.7	32.4	7.2	0.9	8.1	0.8143

NOTE: Major grade 3 to 4 drug-related adverse events were compared between two arms using χ^2 test.
*Not indicated in Common Terminology Criteria for Adverse Events version 3.0.
[†]One patient died of drug-induced pneumonitis.

of patients with non-squamous cell carcinoma was significantly longer compared with that in patients with squamous cell carcinoma (16.0 versus 9.3 months; $P = 0.00264$). Pemetrexed induces its antitumor activity by inhibiting key enzymes related to the folate metabolism, such as thymidylate synthase. Studies of the tumor histology of adenocarcinoma progressive disease have reported lower-level expression of thymidylate synthase than squamous cell carcinoma (27). Good survival benefit in patients with non-squamous cell carcinoma by pemetrexed may be explained by lower levels of thymidylate synthase. Because MST was the subject of a subgroup analysis and survival was not a primary endpoint of this study, this finding should be considered exploratory requiring independent confirmation. However, if this finding of superior effectiveness in non-squamous cell carcinoma could be substantiated in future studies, it would be very useful. Indeed, histology could be a simple means of tailoring chemotherapy treatment.

In conclusion, although the recommended dose is P1000 with folic acid and vitamin B_{12} supplementation for Japanese patients, it has similar efficacy and safety with P500, the recommend dosage in rest of the world. These results support the use of P500 as a second- or third-line treatment of NSCLC.

Disclosure of Potential Conflicts of Interest

Authors have conflicts with Eli Lilly and company.

Acknowledgments

We thank the physicians who enrolled patients (Drs. Hiroshi Isobe, Akira Inoue, Yoshio Tomizawa, Akira Yokoyama, Shuichi Yoneda, Masaru Narabayashi, Masahiko Shibuya, Masahiro Tsuboi, Hiroaki Okamoto, Kenji Eguchi, Toshiyuki Sawa, Koji Takeda, Fumio Imamura, Shinzoh Kudoh, Masaaki Kawahara, Kaoru Matsui, Nobuyuki Katakami, Shunichi Negoro, Katsuyuki Kiura, Yoshihiko Segawa, Koichi Takayama, Mitsuhiro Matsumoto, and Takeshi Horai) and Michiyo Matsushima for assistance in the creation and submission of this article.

References

1. Walling J. From methotrexate to pemetrexed and beyond. A review of the pharmacodynamic and clinical properties of antifolates. Invest New Drugs 2006;24:37–77.
2. Hazarika M, White RM, Johnson JR, Pazdur R. FDA drug approval summaries: pemetrexed (Alimta®). Oncologist 2004;9:482–8.
3. Hanna N, Shepherd FA, Fossella FV, et al. Randomized phase III trial of pemetrexed versus docetaxel in patients with non-small-cell lung cancer previously treated with chemotherapy. J Clin Oncol 2004;22:1589–97.
4. Vogelzang NJ, Rusthoven JJ, Symanowski J, et al. Phase III study of pemetrexed in combination with cisplatin versus cisplatin alone in patients with malignant pleural mesothelioma. J Clin Oncol 2003;21:2636–44.
5. John W, Picus J, Blanke CD, et al. Activity of multitargeted antifolate (pemetrexed disodium, LY231514) in patients with advanced colorectal carcinoma: results from a phase II study. Cancer 2000;88:1807–13.
6. Cripps C, Burnell M, Jolivet J, et al. Phase II study of first-line LY231514 (multi-targeted antifolate) in patients with locally advanced or metastatic colorectal cancer: an NCIC Clinical Trials Group study. Ann Oncol 1999;10:1175–9.
7. Bajetta E, Celio L, Buzzoni R, et al. Phase II study of pemetrexed disodium (Alimta®) administered with oral folic acid in patients with advanced gastric cancer. Ann Oncol 2003;14:1543–8.
8. Miller KD, Picus J, Blanke C, et al. Phase II study of the multitargeted antifolate LY231514 (Alimta, MTA, pemetrexed disodium) in patients with advanced pancreatic cancer. Ann Oncol 2000;11:101–3.
9. Pivot X, Raymond E, Laguerre B, et al. Pemetrexed disodium in recurrent locally advanced or metastatic squamous cell carcinoma of the head and neck. Br J Cancer 2001;85:649–55.
10. Hanauske AR, Chen V, Paoletti P, Niyikiza C. Pemetrexed disodium: a novel antifolate clinically active against multiple solid tumors. Oncologist 2001;6:363–73.
11. Goedhals L, van Wyk AL, Smith BL, Fourie SJ. Pemetrexed (Alimta, LY231514) demonstrates clinical activity in chemonaive patients with cervical cancer in a phase II single-agent trial. Int J Gynecol Cancer 2006;16:1172–8.
12. Miles DW, Smith IE, Coleman RE, Calvert AH, Lind MJ. A phase II study of pemetrexed disodium (LY231514) in patients with locally recurrent or metastatic breast cancer. Eur J Cancer 2001;37:1366–71.

13. Martin M, Spielmann M, Namer M, et al. Phase II study of pemetrexed in breast cancer patients pretreated with anthracyclines. Ann Oncol 2003;14:1246–52.
14. Rinaldi DA, Kuhn JG, Burris HA, et al. A phase I evaluation of multitargeted antifolate (MTA, LY231514), administered every 21 days, utilizing the modified continual reassessment method for dose escalation. Cancer Chemother Pharmacol 1999;44:372–80.
15. Rinaldi DA, Burris HA, Dorr FA, et al. Initial phase I evaluation of the novel thymidylate synthase inhibitor, LY231514, using the modified continual reassessment method for dose escalation. J Clin Oncol 1995;13:2842–50.
16. McDonald AC, Vasey PA, Adams L, et al. A phase I and pharmacokinetic study of LY231514, the multitargeted antifolate. Clin Cancer Res 1998;4:605–10.
17. Niyikiza C, Baker SD, Seitz DE, et al. Homocysteine and methylmalonic acid: markers to predict and avoid toxicity from pemetrexed therapy. Mol Cancer Ther 2002;1:545–52.
18. Scagliotti GV, Shin DM, Kindler HL, et al. Phase II study of pemetrexed with and without folic acid and vitamin B_{12} as front-line therapy in malignant pleural mesothelioma. J Clin Oncol 2003;21:1556–61.
19. Nakagawa K, Kudoh S, Matsui K, et al. A phase I study of pemetrexed (LY231514) supplemented with folate and vitamin B_{12} in Japanese patients with solid tumours. Br J Cancer 2006;95:677–82.
20. Therasse P, Arbuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors. J Natl Cancer Inst 2000;92:205–16.
21. Cella DF, Bonomi AE, Lloyd SR, Tulsky DS, Kaplan E, Bonomi P. Reliability and validity of the Functional Assessment of Cancer Therapy-Lung (FACT-L) quality of life instrument. Lung Cancer 1995;12:199–220.
22. Kurihara M, Shimizu H, Tsuboi K, et al. Development of quality of life questionnaire in Japan: quality of life assessment of cancer patients receiving chemotherapy. Psychooncology 1999;8:355–63.
23. Matsumoto T, Ohashi Y, Morita S, et al. The quality of life questionnaire for cancer patients treated with anticancer drugs (QOL-ACD): validity and reliability in Japanese patients with advanced non-small-cell lung cancer. Qual Life Res 2002;11:483–93.
24. Shepherd FA, Dancey J, Ramlau R, et al. Prospective randomized trial of docetaxel versus best supportive care in patients with non-small-cell lung cancer previously treated with platinum-based chemotherapy. J Clin Oncol 2000;18:2095–103.
25. Ohe Y, Ohashi Y, Kubota K, et al. Randomized phase III study of cisplatin plus irinotecan versus carboplatin plus paclitaxel, cisplatin plus gemcitabine, and cisplatin plus vinorelbine for advanced non-small-cell lung cancer: Four-Arm Cooperative Study in Japan. Ann Oncol 2007;18:317–23.
26. Cullen M, Zatloukal P, Sörenson S, et al. Pemetrexed for the treatment of advanced non-small cell lung cancer (NSCLC): results from a randomized phase III dose finding trial in patients who progressed following platinum-containing chemotherapy. J Thorac Oncol 2007;2:S316–7.
27. Ceppi P, Volante M, Saviozzi S, et al. Squamous cell carcinoma of the lung compared with other histotypes shows higher messenger RNA and protein levels for thymidylate synthase. Cancer 2006;107:1589–96.

ANTICANCER RESEARCH *28*: 3855-3858 (2008)

Efficacy of Amrubicin for Non-small Cell Lung Cancer after Failure of Two or More Prior Chemotherapy Regimens

SATOSHI IGAWA[1], TOSHIAKI TAKAHASHI[1], YUKIKO NAKAMURA[1], ASUKA TSUYA[1], AKIRA ONO[1], TAKEHIRO SHUKUYA[1], HARUYASU MURAKAMI[1], MASAHIRO ENDO[2] and NOBUYUKI YAMAMOTO[1]

Divisions of [1]Thoracic Oncology, and [2]Diagnostic Radiology, Shizuoka Cancer Center, Shizuoka 411-8777, Japan

Abstract. *Background: No investigation of amrubicin monotherapy in pre-treated advanced non-small cell lung cancer (NSCLC) patients has yet been reported. Patients and Methods: The records were reviewed of NSCLC patients who had received amrubicin monotherapy between 2003 and 2007 with the following eligibility criteria: previously treated with at least two regimens including platinum and docetaxel for non-adenocarcinoma patients and platinum, docetaxel and epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKI) for adenocarcinoma patients. Amrubicin was administered to both groups at 35 mg/m^2 or 40 mg/m^2 for 3 consecutive days, every 3 weeks. Results: Thirty-nine patients were registered. The median number of prior chemotherapy regimens was three (range: 2 to 7). The median number of courses per patient was three (range one to 9). The toxicity profile was acceptable with no grade 3 or higher non-hematological toxicity. The overall response rate was 10.2%. The median survival time was 4.8 months. Conclusion: Amrubicin exhibits activity and acceptable toxicity as third or subsequent line of chemotherapy for advanced NSCLC.*

Combinations of platinum and the new agents developed in the 1990s are more useful against advanced non-small cell lung cancer (NSCLC) than old-generation combination chemotherapy and doublets of platinum and new-generation anticancer agents are now considered as standard chemotherapy regimens for advanced NSCLC (1, 2). However, among the patients receiving these as first-line chemotherapy, most present either NSCLC refractory to the chemotherapy or relapse after having been sensitive to the chemotherapy. Docetaxel has been considered a more reasonable standard therapy for NSCLC patients who experienced first-line failure compared with best supportive care (BSC) (3, 4). Equally importantly, two recent studies concerning the epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKI) reported that erlotinib exhibited significant overall survival benefit in a second- or third-line setting compared to placebo for unselected patients (5) and that gefitinib was not inferior to docetaxel in clinical efficacy for pre-treated patients with advanced NSCLC (6). Additionally, previous studies reported that adenocarcinoma histology was one of the predictive factors for response and survival in NSCLC patients treated with gefitinib (7, 8).

On the other hand, we have inevitably encountered difficulties in the treatment of patients with advanced NSCLC relapsed to second-line regimens such as docetaxel and EGFR-TKI. Whereas it is recognized that this patient population has been increasing, the insufficiency of a consistent approach to treatment for patients who have failed second-line therapy is apparent.

Amrubicin hydrochloride is a totally synthetic 9-aminoanthracycline and is metabolically activated to amrubicinol by a liver enzyme. Two phase II studies of amrubicin in patients with previously untreated advanced NSCLC successively demonstrated an overall response rate of 27.9% and 18.3% and median survival time of 9.8 months and 8.2 months (9, 10). Thus amrubicin was equivalent to newer agents such as taxanes, gemcitabine, vinorelbine and irinotecan in single-agent activity for NSCLC. These findings suggested that amrubicin might be a promising anti-tumor agent for the treatment of NSCLC. To our knowledge, no evaluation of such single-agent treatment in pre-treated advanced NSCLC patients has yet been reported. In the present study, the clinical efficacy of amrubicin for pre-treated patients with advanced NSCLC was investigated.

Correspondence to: Nobuyuki Yamamoto, Ph.D., Division of Thoracic Oncology, Shizuoka Cancer Center, 1007, Nagaizumi, Shizuoka 411-8777, Japan. Tel: +81 559895222, Fax: +81 55989 5783, e-mail: n.yamamoto@scchr.jp

Key Words: Amrubicin, NSCLC, chemotherapy failure, third-line treatment.

0250-7005/2008 $2.00+.40

Patients and Methods

Patient selection. This retrospective cohort study enrolled 39 patients previously treated for NSCLC between March 2003 and October 2007 at Shizuoka Cancer Center. Study participants were consecutively registered according to the following criteria: measurable disease target lesions on physical examination by chest X-ray, computed tomography (CT) of the chest and abdomen, or other procedures as indicated, including MRI of the head, positron-emission tomography (PET), or combined PET/CT; histologically confirmed advanced NSCLC and relapsed or progressive disease after first-line platinum-based chemotherapy; histologically confirmed adenocarcinoma and receiving each of EGFR-TKI and docetaxel following the first-line therapy; other histological subtypes of NSCLC and previously receiving docetaxel following the first-line therapy; age less than 80 years; Eastern Cooperative Oncology Group Performance Scale status (ECOG PS) of 2 or less; adequate bone marrow, hepatic, and renal function; no other serious disease and written informed consent.

Treatment methods. In a previous study amrubicin at a dose of 40 mg/m^2 showed efficacy and tolerability in previously treated small cell lung cancer (SCLC) patients (11). Furthermore, amrubicin at a dose of 35 mg/m^2 also exhibited significant activity as third-line chemotherapy against SCLC (12). Thus, a reduced dose of 35 to 40 mg/m^2 per day × 3 days was chosen for the present study in view of the history of previous chemotherapy in the patients. The treatment schedule comprised intravenous infusion of amrubicin in 50 ml normal saline over 5 minutes on days 1 to 3 every three weeks.

Before the start of treatment, the patients were required to have an absolute neutrophil count (ANC) of 1,500/mm^3 or more, a platelet count of 100,000/mm^3 or more, asparatate aminotransferase (AST) and alanine aminotransferase (ALT) values less than 3 times the maximum values of the normal range, and total bilirubin and creatinine values less than 1.5 times the maximum values of the normal range. The administration of granulocyte colony-stimulating factor (G-CSF) was permitted as a therapeutic intervention but was not mandatory as a prophylactic agent against the hematological toxicity of neutropenia. Subsequent doses were modified on the basis of the hematological and non-hematological toxicities at the discretion of the physician in charge. Peripheral blood and biochemistry examinations were repeated at least once a week after the initial evaluation.

Evaluation of response and toxicity. Tumor response was classified in accordance with the Response Evaluation Criteria for Solid Tumors. The patients were evaluated to determine the stage of their disease before the start of treatment and at the time of determination of disease progression or relapse, by complete medical history and physical examination, chest X-ray, CT of the chest and abdomen, and other staging procedures, such as MRI of the head and PET. The adverse events were recorded and graded using the National Cancer Institute Common Toxicity Criteria, Version 3.0 grading system (13).

Statistical analysis. Overall survival was measured from the first day of amrubicin treatment to the day of death or last follow-up. Progression-free survival was defined as the time between the initiation of treatment and failure (*i.e.* death or disease progression) or last follow-up. The time-to-event outcomes were compared using the log-rank test.

Table I. *Patient characteristics.*

No. of patients	39
Gender: female/male	15/24
Age (years): median (range)	60 (41-77)
Smoking history: +/–	24/15
ECOG PS: 0/1/2	14/17/8
Stage: IIIB/IV	6/33
Brain metastasis: +/–	10/29
Histology: Ad/SQ/Other	22/14/3
Number of prior regimens	
2/3/>=4	3/20/16

Ad: Adenocarcinoma, SQ: squamous cell carcinoma.

Table II. *Hematological toxicity by dose.*

	Pts	ANC		Hb		PLT		FN
Dose		G3	G4	G3	G4	G3	G4	G3
35 mg/m^2	28	8	6	3	0	2	2	2
40 mg/m^2	11	4	4	2	0	1	0	0
Total	39	12	10	5	0	3	2	2

ANC: Absolute neutrophil count, Hb: hemoglobin, PLT: platelets, FN: fibrile neutropenia.

Results

Patient characteristics. The characteristics of the patients with recurrent or refractory NSCLC enrolled in this study are listed in Table I. The majority (31 patients, 79%) had an ECOG PS of 1 or 0. The histological subtypes of NSCLC in the patients were: adenocarcinoma in 22 patients (56%), squamous cell carcinoma in 14 (36%), and other histological subtypes in 3 (8%). Amrubicin was administered at 35 mg/m^2 to 28 patients and at 40 mg/m^2 to 11 patients. For the whole study population, the best responses to prior therapy were: no complete response (CR); two (5%) partial response (PR); 15 (39%) stable disease (SD) and 22 (56%) progressive disease (PD).

Toxicity. In total 102 courses were given (median courses per patient, 3: range 1 to 9). All of these courses were included in the toxicity analysis. The principal toxicity of amrubicin monotherapy was neutropenia. The hematological toxicities in the 39 patients are summarized in Table II, which shows the highest level of toxicities in each patient. Grade 3 or higher neutropenia, grade 3 decrease in hemoglobin, and grade 3 or higher thrombocytopenia were observed in 22 patients (56%), 5 patients (13%) and 5 patients (13%), respectively. The incidence of grade 4 neutropenia was less frequent in patients receiving 35 mg/m^2 (21%) than in those receiving 40 mg/m^2 (36%). Febrile neutropenia was