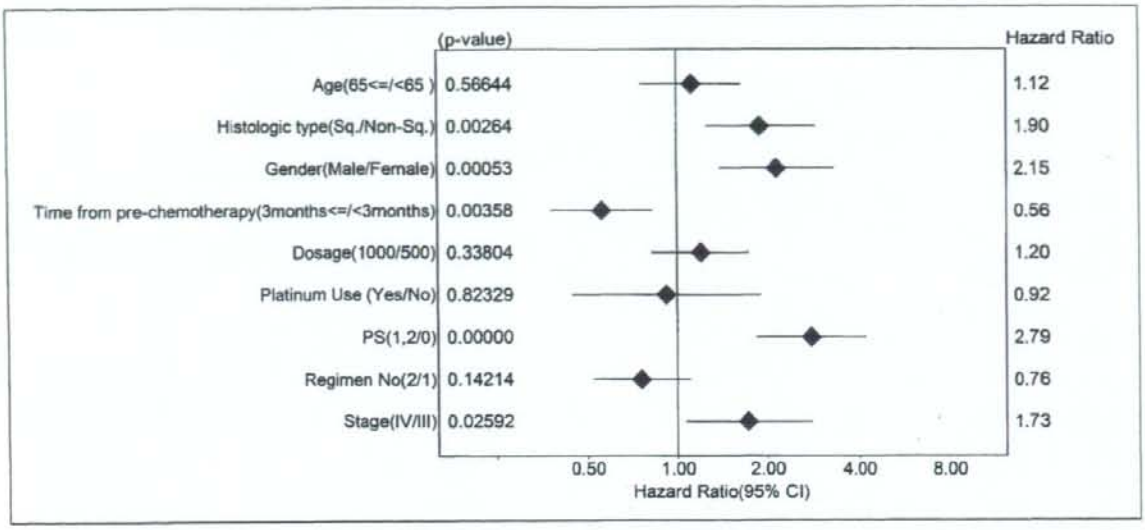

Fig. 2. Forest plot. Cox multiple regression analysis was done on all evaluable patients from two combined arms to identify significant prognostic factors for survival. Covariates evaluated were pemetrexed dose, gender, age, PS, disease stage, histology, interval from prior chemotherapy to registration 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.

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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;14:1245–52.
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.

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JOURNAL OF CLINICAL ONCOLOGY ORIGINAL REPORT

Phase III Study, V-15-32, of Gefitinib Versus Docetaxel in Previously Treated Japanese Patients With Non–Small-Cell Lung Cancer

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ABSTRACT

Purpose
This phase III study (V-15-32) compared gefitinib (250 mg/d) with docetaxel (60 mg/m^2) in patients (N = 489) with advanced/metastatic non–small-cell lung cancer (NSCLC) who had failed one or two chemotherapy regimens.

Methods
The primary objective was to compare overall survival to demonstrate noninferiority for gefitinib relative to docetaxel. An unadjusted Cox regression model was used for the primary analysis.

Results
Noninferiority in overall survival was not achieved (hazard ratio [HR], 1.12; 95.24% CI, 0.89 to 1.40) according to the predefined criterion (upper CI limit for HR ≤ 1.25); however, no significant difference in overall survival (P = .330) was apparent between treatments. Poststudy, 36% of gefitinib-treated patients received subsequent docetaxel, and 53% of docetaxel-treated patients received subsequent gefitinib. Gefitinib significantly improved objective response rate and quality of life versus docetaxel; progression-free survival, disease control rates, and symptom improvement were similar for the two treatments. Grades 3 to 4 adverse events occurred in 40.6% (gefitinib) and 81.6% (docetaxel) of patients. Incidence of interstitial lung disease was 5.7% (gefitinib) and 2.9% (docetaxel). Four deaths occurred due to adverse events in the gefitinib arm (three deaths as a result of interstitial lung disease, judged to be treatment related; one as a result of pneumonia, not treatment related), and none occurred in the docetaxel arm.

Conclusion
Noninferiority in overall survival between gefitinib and docetaxel was not demonstrated according to predefined criteria; however, there was no statistically significant difference in overall survival. Secondary end points showed similar or superior efficacy for gefitinib compared with docetaxel. Gefitinib remains an effective treatment option for previously treated Japanese patients with NSCLC.

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INTRODUCTION

In Japan, patients with advanced non–small-cell lung cancer (NSCLC) who fail first-line platinum-based therapy often receive second-line docetaxel.[1,2] However, docetaxel has been associated with significant levels of toxicity, especially grades 3 to 4 neutropenia (40% to 67% and 63% to 73% for docetaxel 75 mg/m^2 and 60 mg/m^2, respectively).[1-4] In North America and in European countries, docetaxel,[3,4] pemetrexed,[2] and erlotinib[5] are approved second-line treatments for NSCLC.[3,6]

In phase II trials (IDEAL 1 and 2), the epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor gefitinib (Iressa; AstraZeneca, London, United Kingdom) 250 mg/d showed response rates of 12% to 18% and median survival of 7.0 to 7.6 months in patients who had pretreated advanced NSCLC.[7,8] A subset of Japanese patients in IDEAL 1 demonstrated a higher response rate (27.5%) and longer median survival (13.8 months) compared with the overall population.[9] A phase III study (Iressa Survival Evaluation in Lung Cancer) in patients who had previously treated refractory NSCLC

showed that gefitinib was associated with a nonsignificant trend toward improved overall survival versus placebo.[10] Preplanned subgroup analyses demonstrated a statistically significant increase in survival for gefitinib compared with placebo in patients of Asian origin (hazard ratio [HR], 0.66; 95% CI, 0.48 to 0.91; $P = .010$; median survival, 9.5 *v* 5.5 months) and in never-smokers (HR, 0.67; 95% CI, 0.49 to 0.92; $P = .012$; median survival, 8.9 *v* 6.1 months).[10,11]

Reported here is the first phase III study to compare the effects of targeted therapy (gefitinib) with chemotherapy (docetaxel) on overall survival in Japanese patients with advanced/metastatic (stages IIIB to IV) or recurrent NSCLC who failed one or two chemotherapy regimens.

METHODS

Study Design

This multicenter, randomized, open-label, postmarketing clinical study (V-15-32) compared gefitinib with docetaxel in Japanese patients who had pretreated, locally advanced/metastatic (stages IIIB to IV) or recurrent NSCLC. Patients were randomly assigned by using stratification factors of sex (female *v* male), performance status (PS; 0 to 1 *v* 2), histology (adenocarcinoma *v* others), and study site.

The primary end point was overall survival, and the study aimed to show noninferiority of gefitinib versus docetaxel. Secondary end points were progression-free survival (PFS), time to treatment failure, objective response rate (ORR), disease control rate (DCR), quality of life (QoL), disease-related symptoms, safety, and tolerability.

A late protocol amendment included exploratory end points, such as EGFR gene copy number, protein expression, and mutation status of tumor tissue.

Patients

Patients age 20 years or older were eligible if they had the following: histologically or cytologically confirmed NSCLC (stages IIIB to IV) not amenable to curative surgery or radiotherapy, or postoperative recurrent NSCLC; failure of prior treatment with one or two chemotherapy regimens ($\geq$ 1 platinum-based regimen); life expectancy of 3 months or greater; WHO PS 0 to 2; and measurable disease by Response Evaluation Criteria in Solid Tumors (RECIST). To improve recruitment, the protocol was amended approximately 6 months after study initiation to allow patients without measurable lesions to participate. This was not expected to greatly impact the primary end point.

Treatment

Gefitinib 250 mg/d was administered orally; docetaxel was administered every 3 weeks as a 1-hour intravenous infusion of 60 mg/m^2 (ie, the approved dose in Japan). Patients received treatment until disease progression, intolerable toxicity, or discontinuation for another reason. Poststudy treatment was at physician and patient discretion; a switch to other study treatment was prohibited unless requested by the patient.

Assessments

Overall survival was assessed from date of random assignment to date of death as a result of any cause, or data were censored at the last date the patient was known to be alive. Tumor response by RECIST was performed at baseline, every 4 weeks for the first 24 weeks, and every 8 weeks thereafter. Complete response (CR) or partial response (PR) was confirmed on the basis of two consecutive examinations that were at least 28 days apart. Investigator assessment of best overall tumor response was used for the primary analysis; sensitivity analyses were performed with independent response evaluation committee assessment. PFS was defined as the time from random assignment to the earliest occurrence of disease progression or death from any cause; patients who had not progressed or died at data cutoff were censored at last tumor assessment. QoL was assessed with the FACT-L questionnaire at baseline and every 4 weeks during study treatment until week 12. The FACT-L total score and trial outcome index (TOI; sum of FACT-L physical well-being + functional well-being + additional concerns subscales) were calculated. Disease-related symptoms were assessed weekly with the FACT-L lung cancer subscale (LCS). Improvement was defined as an increase from baseline of at least six points for FACT-L or TOI, or an increase of at least two points for LCS, on two visits that were at least 28 days apart. Adverse events (AEs) were monitored and graded according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC; version 2.0). Routine laboratory assessments were performed. EGFR gene copy number was determined by fluorescent in situ hybridization (FISH).[12] EGFR mutations were assessed by direct sequencing of exon 18 to 21 of chromosome 7. EGFR protein expression was measured by immunohistochemistry with the DAKO EGFR pharmaDxTM kit (DAKO, Glostrup, Denmark).[10]

Statistical Analysis

The primary overall survival analysis was conducted in the intent-to-treat (ITT) population by estimating the HR and two-sided 95.24% CI for gefitinib versus docetaxel, derived from a Cox regression model without covariates (significance level adjusted because of interim analysis). Noninferiority was to be concluded if the upper CI limit was $\leq$ 1.25. Superiority was concluded if the upper CI limit was less than 1. A total of 296 death events were required for 90% power to demonstrate noninferiority, with the assumption that gefitinib had better overall survival than docetaxel (median survival, 14 *v* 12 months[4]), and the study plan was to recruit 484 patients.

Robustness of the primary conclusion was assessed by supportive analyses in the per-protocol population and by using a Cox regression model with covariate adjustment for sex (male *v* female), PS (0 or 1 *v* 2), tumor type (adenocarcinoma *v* other), smoking history (ever *v* never), number of prior chemotherapy regimens (1 *v* 2), age at random assignment ($<$ 65 years *v* $\geq$ 65 years), time from diagnosis to random assignment ($<$ 6 *v* 6 to 12 *v* $>$ 12 months), and best response to prior chemotherapy (CR/PR *v* stable disease [SD] *v* progressive disease not assessable/unknown).

Preplanned subgroup analyses were performed on the basis of these covariates. Subgroups were first assessed for evidence of randomized treatment effect by subgroup interactions, to ensure that outcomes between subgroups were likely to be different; then, the subgroups for which evidence existed were examined further.

For PFS, the HR and its 95% CI for gefitinib versus docetaxel were calculated for the population that was assessable for response (defined as patients with $\geq$ 1 measurable lesion at baseline by RECIST) by using a Cox regression model without covariates. Supportive analyses were performed in the ITT population by using a model adjusted for covariates. Overall survival and PFS were summarized with Kaplan-Meier methods.

The ORR (proportion of CR + PR) and the DCR (proportion of CR + PR + SD $\geq$ 12 weeks) were estimated in the assessable-for-response population and were compared between treatments by generating an odds ratio and a 95% CI from a logistic regression model that included covariates.

The exploratory analysis of biomarker subgroups was performed with similar methods to the overall and clinical subgroup analyses when possible.

RESULTS

Patients

From September 2003 to January 2006, 490 patients were randomly assigned from 50 institutes. In the ITT population, 245 patients were randomly assigned to gefitinib, and 244 patients were randomly assigned to docetaxel; one patient was excluded because of a Good Clinical Practice violation (Fig 1). Treatment groups were generally well balanced for baseline demographics (Table 1), except for some small imbalances in smoking history (7% fewer never-smokers and 10% more ex-smokers in the gefitinib arm). The overall population was representative of an advanced, pretreated NSCLC population in a clinical trial setting in Japan. The median (range) duration of treatment for gefitinib was 58.5 (4 to 742) days and, for docetaxel, was 3 (1 to 12) cycles.

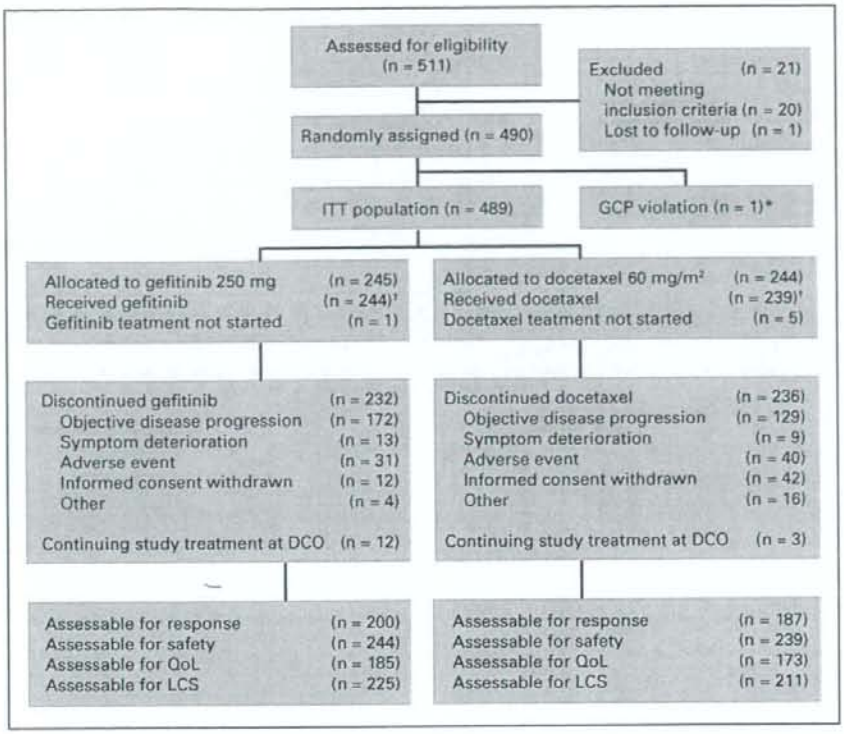

Fig 1. Study flow. (*) Allocated to the docetaxel group. (†) The safety analysis, conducted according to treatment received, was performed on this population. ITT, intent to treat; GCP, Good Clinical Practice; DCO, data cutoff date for overall survival (October 31, 2006); QoL, quality of life; LCS, Lung Cancer Subscale.

Poststudy, 36% of gefitinib-treated patients received subsequent docetaxel, and 40% received no other therapy except for gefitinib; 53% of docetaxel-treated patients received subsequent gefitinib, and 26% received no other therapy except for docetaxel.

Survival

At data cutoff for overall survival (October 31, 2006), overall mortality was 62.6%, and median follow-up was 21 months. Noninferiority in overall survival was not achieved (HR, 1.12; 95.24% CI, 0.89 to 1.40) according to the predefined criterion (upper CI limit for HR $\leq$ 1.25). However, no statistically significant difference in overall survival was apparent ($P = .330$; Fig 2A).

A supportive Cox analysis, which took into account imbalances in known prognostic factors, showed an HR of 1.01 (95% CI, 0.80 to 1.27; $P = .914$), which suggested that a demography imbalance that favored docetaxel may have had some impact on the primary, unadjusted, overall survival result.

The median survival and the 1-year survival rates were 11.5 months and 47.8%, respectively, for gefitinib and were 14.0 months and 53.7%, respectively, for docetaxel.

PFS

There was no significant difference between treatments in PFS in the unadjusted analysis (HR, 0.90; 95% CI, 0.72 to 1.12; $P = .335$); median PFS was 2.0 months with both treatments (Fig 2B). Similar PFS results were obtained from supportive Cox regression analysis adjusted for covariates (HR, 0.81; 95% CI, 0.65 to 1.02; $P = .077$).

Tumor Response

For ORR, gefitinib was statistically superior to docetaxel (22.5% *v* 12.8%; odds ratio, 2.14; 95% CI, 1.21 to 3.78; $P = .009$; Table 2). Gefitinib was similar to docetaxel in terms of DCR (34.0% *v* 33.2%; odds ratio, 1.08; 95% CI, 0.69 to 1.68; $P = .735$). The primary ORR results that were based on investigator judgment were generally consistent with those obtained from independent response evaluation committee assessment.

Symptom Improvement and QoL

Gefitinib showed statistically significant benefits compared with docetaxel in QoL improvement rates (FACT-L: 23.4% *v* 13.9%; $P = .023$; TOI: 20.5% *v* 8.7%; $P = .002$; Table 2), but there were no significant differences between treatments in LCS improvement rates (22.7% *v* 20.4%; $P = .562$).

Subgroup Analyses

Survival outcomes were generally consistent across subgroups, with the exception of best response to prior chemotherapy (treatment by subgroup interaction test $P = .017$). For patients with best response to prior chemotherapy of progressive disease, overall survival was numerically longer on gefitinib than on docetaxel, whereas patients with a best response of SD had significantly longer survival on docetaxel than on gefitinib (HR, 1.58; 95% CI, 1.09 to 2.27; $P = .015$; Fig 3A). However, the result was not supported by the PFS (Fig 3B) or ORR results in this subgroup, which favored gefitinib.

Table 1. Baseline Patient Characteristics in Intent-to-Treat Population

Characteristic	Patients per Arm: Gefitinib (n = 245) No.	%	Docetaxel (n = 244) No.	%
Age, years				
≤ 64	138	56.3	135	55.3
≥ 65	107	43.7	109	44.7
Sex				
Male	151	61.6	151	61.9
Female	94	38.4	93	38.1
WHO performance status				
0	85	34.7	93	38.1
1	149	60.8	141	57.8
2	11	4.5	10	4.1
Smoking status				
Ever	174	71.0	157	64.3
Never	71	29.0	87	35.7
Histology				
Adenocarcinoma	192	78.4	188	77.0
Squamous cell carcinoma	37	15.1	41	16.8
Other	16	6.5	15	6.2
Time from diagnosis to random assignment, months				
< 6	70	28.6	60	24.6
6-12	99	40.4	96	39.3
> 12	76	31.0	87	35.7
Disease stage at diagnosis				
IIIB	47	19.2	50	20.5
IV	159	64.9	150	61.5
Recurrent	39	15.9	44	18.0
Number of prior chemotherapy regimens				
1	212	86.5	201	82.4
2	33	13.5	42	17.2
Best response to previous chemotherapy				
CR/PR	113	46.1	106	43.4
SD	91	37.1	101	41.4
PD/NA/unknown	41	16.7	37	15.2
Target lesions at baseline				
Yes	201	82.0	187	76.6
No	44	18.0	57	23.4

Abbreviations: CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; NA, not assessable.

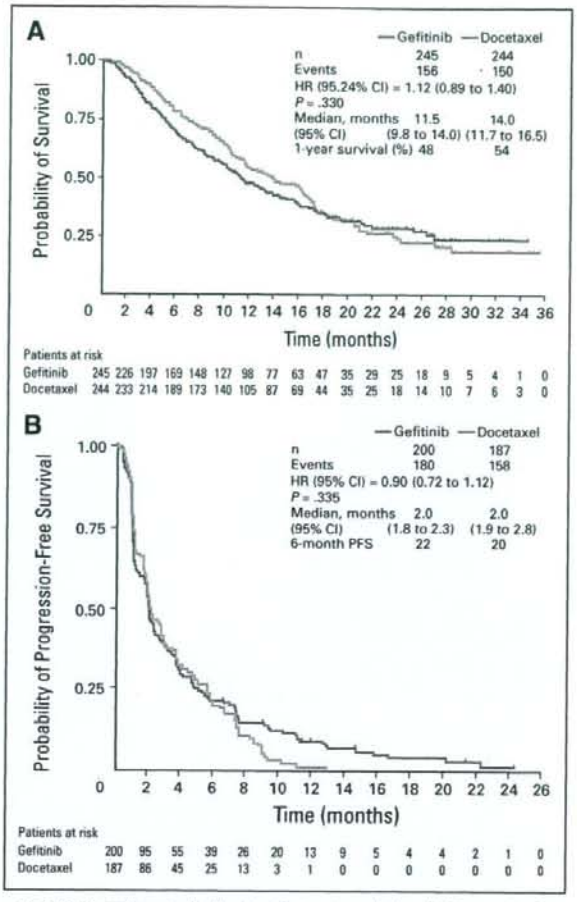

Fig 2. (A) Overall survival in the intent-to-treat population; (B) Progression-free survival (PFS) in the assessable-for-response population. HR, hazard ratio.

Safety

Gefitinib was associated with fewer dose interruptions or delays than docetaxel (26% *v* 52%, respectively). There were no clinically relevant differences in the frequencies of serious AEs or discontinuations of study treatment as a result of AEs between treatment groups (Table 3). Fewer NCI-CTC grades 3 to 4 AEs occurred with gefitinib compared with docetaxel (40.6% *v* 81.6%). There were four deaths as a result of AEs in the gefitinib arm (three as a result of interstitial lung disease that was considered by the investigator to be treatment related; one as a result of pneumonia that was not considered treatment-related), and none in the docetaxel arm.

The most common AEs with gefitinib were rash/acne (76.2%) and diarrhea (51.6%), and the most common AEs with docetaxel were neutropenia (79.5%) and alopecia (59.4%; Table 4). There was a higher incidence of grades 3 to 4 neutropenia with docetaxel (73.6%) compared with gefitinib (8.2%). Interstitial lung disease events occurred in 5.7% (n = 14) and 2.9% (n = 7) of patients who received gefitinib and docetaxel, respectively (Table 3).

Biomarkers

Of the 74 EGFR biomarker samples provided, 53 to 60 were assessable (depending on biomarker). Because of the late protocol amendment, these samples were from long-term survivors who were recruited early or from patients who were recruited later in the study. Compared with the overall study population, this subgroup was over-representative of some stratification factors on both treatment arms: good PS, females, never-smokers, greater than 12 months from diagnosis to random assignment, and best response to prior chemotherapy of CR/PR. There were insufficient events to allow meaningful evaluation of overall survival in relation to biomarker status, and the PFS and ORR data should be interpreted with caution.

Thirty-one (54.4%) of 57 patients had EGFR mutation–positive tumors, and 42 (70.0%) of 60 had EGFR FISH–positive tumors. There

Table 2. Response Rates and Improvement Rates

Rate	Treatment Arm: Gefitinib — Total No. of Assessable Patients	Gefitinib — %	Treatment Arm: Docetaxel — Total No. of Assessable Patients	Docetaxel — %	Analysis — OR	Analysis — 95% CI	Analysis — P
Response*	200		187				
Overall		22.5		12.8	2.14	1.21 to 3.78	.009
Disease control		34.0		33.2	1.08	0.69 to 1.68	.735
Improvement							
FACT-L	185	23.4	173	13.9	1.89	1.09 to 3.28	.023
TOI	185	20.5	173	8.7	2.72	1.44 to 5.16	.002
LCS	225	22.7	211	20.4	1.15	0.72 to 1.81	.562

Abbreviations: OR, odds ratio; FACT-L, Functional Assessment of Cancer Therapy—Lung (Japanese version 4-A, which includes two additional Japan-specific questions in the subscale on social/family well-being); TOI, trial outcome index; LCS, lung cancer subscale.
*Overall response rate consists of complete response plus partial response rates. Disease control rate consists of the complete response plus partial response rates plus those with stable disease for at least 12 weeks.

was a high degree of overlap between EGFR mutation and clinical characteristics (eg, high frequency in females, in those with adenocarcinoma, and in never-smokers). EGFR mutation–positive patients appeared to have better PFS than EGFR mutation–negative patients on both treatments (gefitinib-positive *v* gefitinib-negative HR, 0.33; 95% CI, 0.11 to 0.97; 17 events; docetaxel HR, 0.15; 95% CI, 0.04 to 0.57; 15 events). In addition, EGFR FISH–positive patients appeared to have better PFS than EGFR FISH–negative patients on both treatments (gefitinib-positive *v* gefitinib-negative HR, 0.75; 95% CI, 0.28 to 1.98; 18 events; docetaxel HR, 0.45; 95% CI, 0.14 to 1.41; 16 events). There were no clear PFS differences between gefitinib and docetaxel in any biomarker subgroups, although the number of events was small and the CIs for the HRs were wide. PFS could not be assessed for EGFR protein expression because of the small number of events in the expression-negative group. For EGFR mutation–positive patients, the ORR was 67% (six of 9 patients) with gefitinib administration and 46% (five of 11 patients) with docetaxel administration. For EGFR FISH–positive patients, the ORR was 46% (five of 11) with gefitinib administration and 33% (six of 18) with docetaxel administration. For EGFR expression–positive patients, the ORR was 36% (five of 14) with gefitinib administration and 31% (four of 13) with docetaxel administration. There were no responses among EGFR mutation–negative, or EGFR FISH–negative, patients, and there was one response (13%) of eight EGFR expression–negative patients who received docetaxel.

DISCUSSION

V-15-32 is the first phase III study to compare gefitinib versus docetaxel in previously treated Japanese patients who have advanced NSCLC. Both gefitinib and docetaxel demonstrated efficacy and tolerability, and findings were consistent with previous experience for both agents in Japan.

Although noninferiority in overall survival for gefitinib versus docetaxel was not proven, there was no statistically significant difference between the two treatments. The original statistical assumption was that gefitinib would have 20% longer survival than docetaxel; hence, the relatively small sample size for a noninferiority study. However, since the study was initiated, data from postmarketing experience in Japan (the SIGN study[13]) and substantial switching to the alternative study treatment on progression in V-15-32 indicated that it would be more likely that gefitinib and docetaxel had similar overall survival. With the assumption of equal survival, the chance (power) of showing noninferiority with this study size is reduced to 48%. The median survival with gefitinib 250 mg/d in our study was consistent with previous experience in Japan (11.5 *v* 13.8 months for Japanese subset of IDEAL 1).[9] Docetaxel demonstrated a longer median survival in V-15-32 (14.0 months) compared with previous Japanese studies (7.8 to 9.4 months).[1,4,14]

In line with increasingly available therapy for NSCLC since the trial was designed and with standard practice in Japan, a large proportion of patients received additional anticancer therapy after discontinuation of the randomly assigned study treatment. Crossover was greater than initially expected, and differences in the number and types of patients who received these poststudy treatments complicated interpretation of survival results. A greater proportion of patients who received docetaxel received poststudy therapy compared with those who received gefitinib. Imbalances in the use of gefitinib after chemotherapy have been reported recently in a phase III study of Japanese patients with lung cancer who were treated with docetaxel and have been cited as a possible explanation for the prolonged median survival seen with docetaxel.[15] INTEREST (Iressa NSCLC Trial Evaluating Response and Survival against Taxotere), a worldwide phase III trial that is comparing gefitinib with docetaxel in pretreated patients who have advanced NSCLC recently demonstrated that gefitinib had statistically noninferior survival to docetaxel.[16] In contrast to V-15-32, INTEREST was larger (1,466 patients) and had subsequent therapies that were well-balanced between treatment arms.

Secondary end points, largely unaffected in this study by subsequent therapy, provided further evidence of the clinical efficacy of both gefitinib and docetaxel in Japanese patients. PFS was similar with gefitinib and docetaxel, and ORR was statistically significantly improved with gefitinib. The ORR in V-15-32 with gefitinib (22.5% *v* 12.8% with docetaxel) was consistent with a subset analysis from IDEAL 1 in Japanese patients (27.5%).[3,8,9]

A number of patient subgroups (including females, patients with adenocarcinoma, and never-smokers) have been reported

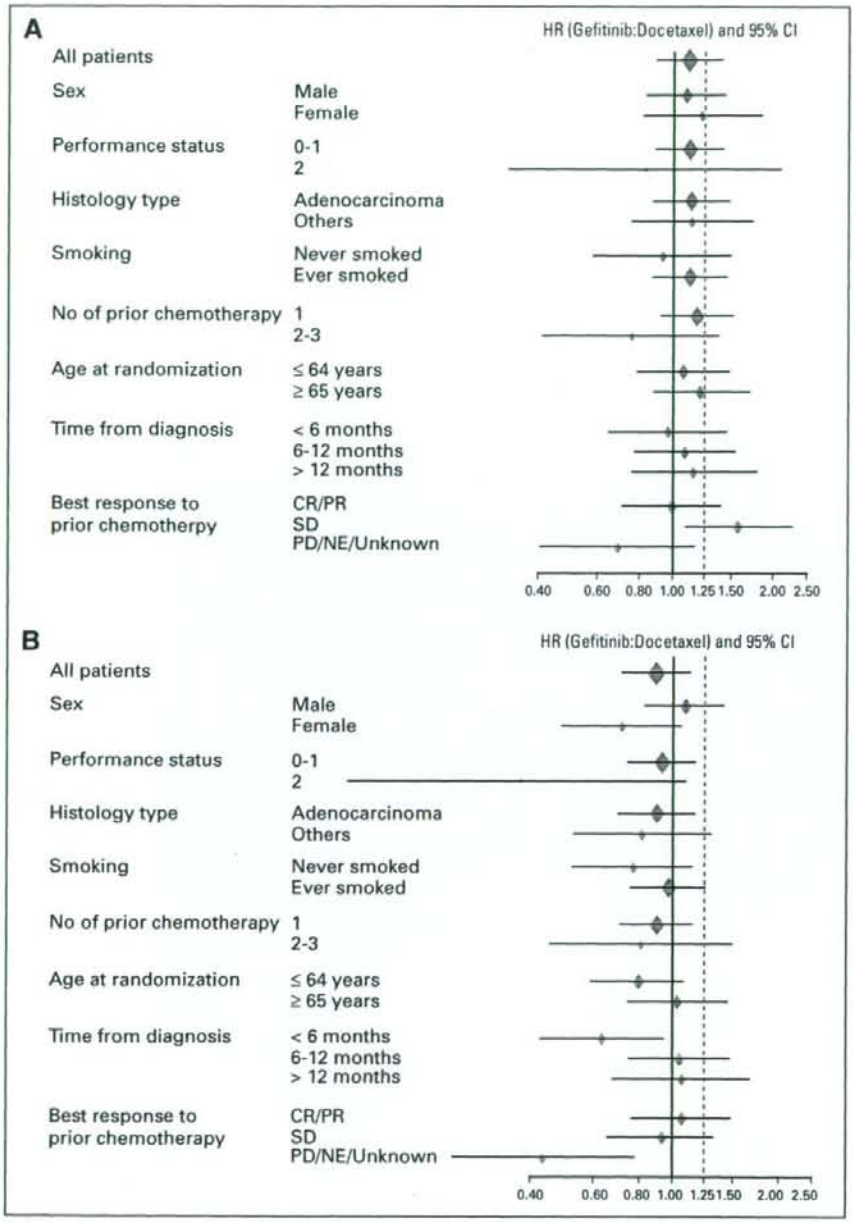

Fig 3. Forest plots of (A) overall survival and (B) progression-free survival that compare treatment groups within clinically relevant subgroups. HR, hazard ratio; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; NE, not assessable.

previously to experience improved clinical benefit with gefitinib.[2,4,7,8,10] Subgroup analyses in this study should be interpreted with caution, as the primary objective was not met, some subgroups were small, and there were imbalances in poststudy treatments. In between-treatment comparisons, no statistically significant overall survival benefit was found for gefitinib compared with docetaxel in any subgroup. However, when post hoc, within-treatment comparisons were performed, females, never-smokers, and patients with adenocarcinoma (and also patients with poor PS and > 12 months since diagnosis) had significantly longer survival than their opposite subgroups on both gefitinib and docetaxel ($P < .001$ for females *v* males, adenocarcinoma *v* others, and never-smokers *v* ever-smokers on both treatments). It appears that the subgroups typically associated with a gefitinib benefit were seen but that they also did well on docetaxel. However, the rate of subsequent gefitinib prescription in the docetaxel arm was high in

Table 3. Summary of Adverse Event Data in the Assessable-for-Safety Population

Category*	Patients			
	Gefitinib (n = 244)		Docetaxel (n = 239)	
	No.	%	No.	%
Adverse events	242	99.2	236	98.7
Treatment-related adverse events	233	95.5	233	97.5
Treatment discontinuation because of an adverse event	33	13.5	42	17.6
NCI-CTC adverse event grades 3 to 4	99	40.6	195	81.6
Serious adverse events	42	17.2	34	14.2
Death as a result of a serious adverse event	4	1.6	0	0
ILD events	14	5.7	7	2.9

Abbreviations: NCI-CTC, National Cancer Institute Common Toxicity Criteria; ILD, interstitial lung disease.
*Participants with multiple events in the same category are counted only once in that category. Participants with events in more than one category are counted once in each of those categories.

these subgroups (eg, approximately two-thirds of docetaxel never-smokers and females had gefitinib as their first poststudy treatment); for PFS and ORR, which are largely unaffected by subsequent treatment, the benefit in these subgroups remained for gefitinib but not for docetaxel, which suggested that poststudy treatments are confounding the interpretation of overall survival in the subgroups.

AEs in our study were consistent with those previously observed, and the most commonly reported AEs were rash/acne and diarrhea for gefitinib and neutropenia for docetaxel. Docetaxel demonstrated a

Table 4. Most Common Adverse Events

Adverse Event	Occurrence by Treatment Arm							
	Gefitinib (n = 244)				Docetaxel (n = 239)			
	Total		Grades 3 to 4		Total		Grades 3 to 4	
	No.	%	No.	%	No.	%	No.	%
Rash/acne*	186	76.2	1	0.4	73	30.5	1	0.4
Diarrhea	126	51.6	5	2.0	67	28.0	2	0.8
Dry skin	90	36.9	0	0.0	13	5.4	0	0.0
Constipation	69	28.3	14	5.7	74	31.0	6	2.5
Anorexia	68	27.9	10	4.1	119	49.8	17	7.1
Nausea	61	25.0	5	2.0	92	38.5	9	3.8
Abnormal hepatic function†	59	24.2	27	11.1	13	5.4	2	0.8
Stomatitis	55	22.5	0	0.0	42	17.6	0	0.0
Nasopharyngitis	50	20.5	0	0.0	32	13.4	0	0.0
Pruritus	42	17.2	0	0.0	15	6.3	0	0.0
Vomiting	41	16.8	4	1.6	41	17.2	3	1.3
Fatigue	36	14.8	1	0.4	107	44.8	6	2.5
Paronychia	33	13.5	1	0.4	2	0.8	0	0.0
Insomnia	32	13.1	0	0.0	20	8.4	0	0.0
Neutropenia‡	24	9.8	20	8.2	190	79.5	176	73.6
Pyrexia	24	9.8	1	0.4	51	21.3	1	0.4
Alopecia	19	7.8	0	0.0	142	59.4	0	0.0
Leukopenia	18	7.4	15	6.1	136	56.9	94	39.3
Headache	12	4.9	1	0.4	25	10.5	0	0.0
Edema§	11	4.5	0	0.0	30	12.6	2	0.8
Myalgia	8	3.3	0	0.0	25	10.5	0	0.0
Dysgeusia	7	2.9	0	0.0	37	15.5	0	0.0
Febrile neutropenia	4	1.6	2	0.8	17	7.1	17	7.1

NOTE. The most common adverse events were considered those that occurred in ≥ 10% of the study population or occurred with > 5% difference between treatments.
*Includes MedDRA high-level terms of rashes, eruptions and exanthems; and of acnes and preferred terms of rash pustular, dermatitis, dermatitis exfoliative, and dermatitis exfoliative generalized.
†Includes MedDRA preferred terms of hepatic function abnormal, alanine aminotransferase increased, aspartate aminotransferase increased and liver disorder.
‡With the exception of one treatment-related adverse event, all other instances of neutropenia reported with gefitinib were in patients who had switched to docetaxel 60 mg/m^2 or other chemotherapy and were reported within the 30-day reporting period. In these other instances, no causal relationship was assigned by the investigator.
§Includes MedDRA preferred terms of edema, edema peripheral, face edema, eyelid edema, and macular edema.

typically high incidence of neutropenia (79.5%) and febrile neutropenia (7.1%) compared with gefitinib (9.8% and 1.6%, respectively). These neutropenia levels that accompanied docetaxel treatment are consistent with previously reported studies in Japanese patients (95.4%[1] and 81.5%[4]). The incidence of interstitial lung disease reported in this study with gefitinib (5.7%) is consistent with that reported in the Japanese postmarketing study (5.8%).[17]

Although the patient numbers were too small for firm conclusions, the biomarker data from this study suggest that EGFR mutation–positive or EGFR FISH–positive patients have a greater response to both gefitinib and docetaxel compared with EGFR mutation– or FISH–negative patients. The gefitinib data are consistent with several previous reports.[18] The docetaxel data provide potential new information about EGFR biomarkers and chemotherapy; this has not been consistently seen before, because there are only a few small studies in the literature, and they have conflicting results.[19] Hence, it is difficult to say conclusively that EGFR mutation or EGFR FISH–positivity predict for docetaxel as well as gefitinib benefit.

Although the study did not prove noninferior survival for gefitinib compared with docetaxel in this patient population, the clinical efficacy and tolerability of gefitinib 250 mg/d in Japanese patients who had NSCLC, reported here, is consistent with the clinical experience reported to date, and gefitinib remains an effective treatment option for previously treated Japanese patients who have locally advanced/metastatic NSCLC.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

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REFERENCES

1. Mukohara T, Takeda K, Miyazaki M, et al: Japanese experience with second-line chemotherapy with low-dose (60 mg/m2) docetaxel in patients with advanced non–small-cell lung cancer. Cancer Chemother Pharmacol 48(5):356-360, 2001
2. 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 22:1589-1597, 2004
3. 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 18:2095-2103, 2000
4. Nakamura Y, Kunitoh H, Kubota K, et al: Retrospective analysis of safety and efficacy of low-dose docetaxel 60 mg/m2 in advanced non–small-cell lung cancer patients previously treated with platinum-based chemotherapy. Am J Clin Oncol 26:459-464, 2003
5. Shepherd FA, Rodrigues PJ, Ciuleanu T, et al: Erlotinib in previously treated non–small-cell lung cancer. N Engl J Med 353:123-132, 2005
6. Fossella FV, Devore R, Kerr RN, et al: Randomized phase III trial of docetaxel versus vinorelbine or ifosfamide in patients with advanced non–small-cell lung cancer previously treated with platinum-containing chemotherapy regimens. J Clin Oncol 18:2354-2362, 2000
7. Kris MG, Natale RB, Herbst RS, et al: Efficacy of gefitinib, an inhibitor of the epidermal growth factor receptor tyrosine kinase, in symptomatic patients with non-small cell lung cancer: A randomized trial. JAMA 290:2149-2158, 2003
8. Fukuoka M, Yano S, Giaccone G, et al: Multi-institutional randomized phase II trial of gefitinib for previously treated patients with advanced non–small-cell lung cancer. J Clin Oncol 21:2237-2246, 2003
9. Nishiwaki Y, Yano S, Tamura T, et al: [Subset analysis of data in the Japanese patients with NSCLC from IDEAL 1 study on gefitinib]. Gan To Kagaku Ryoho 31:567-573, 2004
10. Thatcher N, Chang A, Parikh P, et al: Gefitinib plus best supportive care in previously treated patients with refractory advanced non-small-cell lung cancer: Results from a randomised, placebo-controlled, multicentre study (Iressa Survival Evaluation in Lung Cancer). Lancet 366:1527-1537, 2005
11. Chang A, Parikh P, Thongprasert S, et al: Gefitinib (IRESSA) in patients of Asian origin with refractory advanced non–small-cell lung cancer: Subset analysis from the ISEL study. J Thorac Oncol 1:847-855, 2006
12. Cappuzzo F, Hirsch FR, Rossi E, et al: Epidermal growth factor receptor gene and protein and gefitinib sensitivity in non–small-cell lung cancer. J Natl Cancer Inst 97:643-655, 2005
13. Cufer T, Vrdoljak E, Gaafar R, et al: Phase II, open-label, randomized study (SIGN) of single-agent gefitinib (IRESSA) or docetaxel as second-line therapy in patients with advanced (stages IIIB to IV) non-small-cell lung cancer. Anticancer Drugs 17: 401-409, 2006
14. Non-Small-Cell Lung Cancer Collaborative Group: Chemotherapy in non–small-cell lung cancer: A meta-analysis using updated data on individual patients from 52 randomised clinical trials. BMJ 311:899-909, 1995
15. Kudoh S, Takeda K, Nakagawa K, et al: Phase III study of docetaxel compared with vinorelbine in elderly patients with advanced non–small-cell lung cancer: Results of the West Japan Thoracic Oncology Group Trial (WJTOG 9904). J Clin Oncol 24: 3657-3663, 2006
16. Douillard J-Y, Kim ES, Hirsh V, et al: Phase III, randomized, open-label, parallel-group study of oral gefitinib (IRESSA) versus intravenous docetaxel in patients with locally advanced or metastatic non–small-cell lung cancer who have previously received

platinum-based chemotherapy (INTEREST). Eur J Cancer 5:2, 2007 (suppl)

17. Yoshida S: The results of gefitinib prospective investigation. Med Drug J 41:772-789, 2005

18. Lynch TJ, Bell DW, Sordella R, et al: Activating mutations in the epidermal growth factor receptor underlying responsiveness of non–small-cell lung cancer to gefitinib. N Engl J Med 350:2129-2139, 2004

19. Sequist LV, Joshi VA, Janne PA, et al: Response to treatment and survival of patients with non–small-cell lung cancer undergoing somatic EGFR mutation testing. Oncologist 12:90-98, 2007

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Appendix

The Appendix is included in the full-text version of this article, available online at www.jco.org. It is not included in the PDF version (via Adobe® Reader®).

Phase II Study of Combination Therapy with S-1 and Irinotecan for Advanced Non–Small Cell Lung Cancer: West Japan Thoracic Oncology Group 3505

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Abstract **Purpose:** To evaluate the efficacy and toxicity of combination therapy with the oral fluoropyrimidine formulation S-1 and irinotecan for patients with advanced NSCLC.

Experimental Design: Chemotherapy-naive patients with advanced NSCLC were treated with i.v. irinotecan (150 mg/m^2) on day 1 and with oral S-1 (80 mg/m^2) on days 1 to 14 every 3 weeks.

Results: Fifty-six patients (median age, 63 years; range, 40-74 years) received a total of 286 treatment cycles (median, 5; range, 1-15). No complete responses and 16 partial responses were observed, giving an overall response rate of 28.6% [95% confidence interval (95% CI), 17.3-42.2%]. Twenty-four patients (42.9%) had stable disease and 12 patients (21.4%) had progressive disease as the best response. The overall disease control rate (complete response + partial response + stable disease) was thus 71.4% (95% CI, 57.8-82.7%). Median progression-free survival was 4.9 months (95% CI, 4.0-6.4 months), whereas median overall survival was 15 months. Hematologic toxicities of grade 3 or 4 included neutropenia (25%), thrombocytopenia (3.6%), and anemia (3.6%), with febrile neutropenia being observed in four patients (7.1%). The most common nonhematologic toxicities of grade 3 or 4 included anorexia (14.3%), fatigue (8.9%), and diarrhea (8.9%). There were no deaths attributed to treatment.

Conclusions: The combination of S-1 and irinotecan is a potential alternative option with a favorable toxicity profile for the treatment of advanced NSCLC. This nonplatinum regimen warrants further evaluation in randomized trials.

Non–small cell lung cancer (NSCLC) is the leading cause of death related to cancer worldwide (1). Platinum-based chemotherapy is the standard first-line treatment for advanced NSCLC based on the moderate improvement in survival and quality of life it confers compared with best supportive care alone (2–4). The poor outlook even for patients with advanced NSCLC who receive such treatment has prompted a search for new chemotherapeutic agents and combination regimens.

S-1 is an oral fluorinated pyrimidine formulation that combines tegafur, 5-chloro-2,4-dihydroxypyridine (CDHP), and potassium oxonate in a molar ratio of 1:0.4:1 (5). Tegafur is a prodrug that generates 5-fluorouracil (5-FU) in blood largely as a result of its metabolism by cytochrome P450 in the liver. CDHP increases the plasma concentration of 5-FU through competitive inhibition of dihydropyrimidine dehydrogenase, which catalyzes 5-FU catabolism (6). CDHP also attenuates the cardiotoxic and neurotoxic effects of 5-FU by reducing the production of fluoro-β-alanine, the main catabolite of 5-FU (7, 8). Oxonate reduces the gastrointestinal toxicity of 5-FU. After its oral administration, oxonate becomes distributed selectively to the small and large intestine, where it inhibits the phosphorylation of 5-FU to fluoropyrimidine monophosphate catalyzed by orotate phosphoribosyltransferase within gastrointestinal mucosal cells, thereby reducing the incidence of diarrhea (9). In a phase II trial of S-1 as a single agent for treatment of advanced NSCLC, a response rate of 22% and a median survival time of 10.2 months were obtained in 59 patients without prior chemotherapy (10). Few severe gastrointestinal or hematologic adverse events were reported (10). Moreover, a phase II trial of S-1 plus cisplatin in advanced NSCLC patients revealed a response rate of 47% and a median survival time of 11 months (11).

Irinotecan is an inhibitor of DNA topoisomerase I. It has shown activity as a single agent in first-line chemotherapy for advanced NSCLC (12). Weekly administration of irinotecan

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Translational Relevance

Non–small cell lung cancer (NSCLC) is the leading cause of cancer-related deaths worldwide. The dismal outlook for patients with advanced NSCLC treated with available therapies has prompted a search for new and more effective chemotherapeutic agents and combination regimens. S-1 is a new oral fluorinated pyrimidine formulation that combines tegafur, 5-chloro-2,4-dihydroxypyridine, and potassium oxonate and has been found to exhibit marked antitumor activity in recent clinical trials with cancer patients, including those with NSCLC. We have now examined the therapeutic efficacy and toxicity of the combination of S-1 and irinotecan in chemotherapy-naive patients with advanced NSCLC. We found this drug combination to be active, with a response rate of 28.6%, median progression-free survival of 4.9 months, and median overall survival of 15 months, values that compare favorably with those reported for phase III studies of standard platinum-based doublet chemotherapy. Furthermore, toxicities were manageable, and in most instances, treatment could be continued in the outpatient setting. Our data indicate that the combination of S-1 and irinotecan is a promising alternative for treatment of advanced NSCLC. This nonplatinum regimen warrants further evaluation in randomized trials.

(100 mg/m^2) for 3 weeks followed by 1 week of rest yielded a response rate of 20.5% and a median survival time of 10.6 months in 132 patients with advanced NSCLC (13).

S-1 and irinotecan have both shown single-agent activity against a wide range of solid tumors, including NSCLC, and the combination of these two agents has manifested synergistic effects in tumor xenograft models *in vivo* (14). A phase I study examined administration of irinotecan at a dose of 150 mg/m^2 on day 1 and of S-1 at 80 mg/m^2 per day from days 1 to 14 of a 21-day cycle (15); it found no difference in pharmacokinetic variables for the two drugs relative to the expected values for S-1 or irinotecan administered as single agents. A subsequent phase II study in patients with advanced colorectal cancer showed that this combination was well tolerated and had marked antitumor activity (16). The safety or effectiveness of the combination of S-1 and irinotecan in patients with advanced NSCLC has not previously been reported.

We now present the results of a multicenter phase II trial of S-1 in combination with irinotecan for patients with previously untreated advanced NSCLC. The aims of this study were to determine the objective tumor response rate, overall and progression-free survival, and toxicity profile for such treatment.

Materials and Methods

Patient eligibility. The criteria for patient eligibility included a diagnosis of NSCLC confirmed either histologically or cytologically, clinical stage IV or IIIB (including only patients with no indications for curative radiotherapy, such as those with malignant pleural effusion, pleural dissemination, malignant pericardial effusion, metastatic lesions in the same lobe of the primary lesion, or involvement of contralateral mediastinal or hilar lymph nodes), measurable disease, no prior chemotherapy, an age range of 20 to 74 y, an Eastern Cooperative Oncology Group performance status of 0 or 1, and a projected life expectancy of at least 3 mo. Other eligibility criteria for organ function included a leukocyte count of ≥3,000/mm^3, a neutrophil count of ≥1,500/mm^3, a platelet count of ≥100,000/μL, a serum bilirubin concentration of ≤1.5 mg/dL, serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels of ≤2.5 times the upper normal limit, a normal serum creatinine level, and either a partial pressure of arterial oxygen of ≥65 torr or a peripheral oxygen saturation of ≥92%. Main exclusion criteria included active concomitant of any malignancy, symptomatic brain metastasis, interstitial pneumonia, watery diarrhea, obstructive bowel disease, heart failure, uncontrolled diabetes mellitus, active infection, and a past history of drug allergy. Written informed consent was obtained from all patients, and the study protocol was approved by the institutional ethics committee of each of the participating institutions.

Study design and treatment. This was a multicenter, open-label, single-arm, phase II study. The primary end point of the study was the response rate, which determined the sample size. We chose a 35% response rate as a desirable target level and a 20% response rate as uninteresting with an α error of 0.05 and a power of 0.8, resulting in a requirement for 50 patients. Allowing for a patient ineligibility rate of 10%, we planned to enroll 55 patients.

Each treatment cycle consisted of the oral administration of S-1 (40 mg/m^2) twice daily for 2 wk, with a 90-min i.v. infusion of irinotecan (150 mg/m^2) on day 1 followed by a drug-free interval of 1 wk. S-1 was available as capsules containing 20 or 25 mg of tegafur. Patients were assigned based on body surface area to receive one of the following oral doses of S-1 twice daily: 40 mg (body surface area < 1.25 m^2), 50 mg (1.25 ≤ body surface area < 1.50 m^2), or 60 mg (body surface area ≥ 1.50 m^2). Courses of treatment were repeated every 21 d until the occurrence of tumor progression or unacceptable toxicity, refusal of the patient, or a decision by the physician to stop treatment.

If laboratory variables changed after the start of treatment so that they no longer met the eligibility criteria for the study, subsequent courses of treatment were withheld until the abnormality had resolved. If the abnormality had not resolved within 43 d, the patient was excluded from the study. The doses of both S-1 and irinotecan were reduced in the event of any of the following toxicities during the previous treatment cycle: neutropenia of grade 4 for >7 d, febrile neutropenia, thrombocytopenia of grade ≥4, and nonhematologic toxicity of grade ≥3. S-1 was reduced in subsequent courses from 60, 50, or 40 mg twice daily to 50, 40, and 25 mg twice daily, respectively. The dose of irinotecan was reduced by 25 mg/m^2 for subsequent courses. Once lowered, the doses of S-1 and irinotecan were not increased.

Evaluation. Tumor response was assessed according to the Response Evaluation Criteria in Solid Tumors (17). Tumors were measured by computed tomography within 2 wk before the first cycle of treatment and then every 4 wk. Patients were evaluable for response if they had a baseline exam and at least one follow-up exam and had received at least one cycle of treatment. A central radiological review was done to determine the eligibility of patients and the response to treatment. Response was confirmed at least 4 wk (for a complete or partial response) or 6 wk (for stable disease) after it was first documented. Progression-free survival was defined as the time from registration until objective tumor progression or death. Patients whose disease had not progressed at the time of discontinuation of the study treatment continued to be assessed until progression was documented. If a patient died without documentation of disease progression, the patients was considered to have had tumor progression at the time of death, unless there was sufficient documented evidence to conclude otherwise. Overall survival was defined as the time from registration until death from any cause. Progression-free and overall survival as well as the 1-y survival rate were estimated by the Kaplan-Meier method.

Table 1. Characteristics of the 56 eligible patients

Characteristic	No. patients
Median age, y (range)	63 (40-74)
Sex	
Male	46 (82%)
Female	10 (18%)
Performance status (ECOG)	
0	20 (36%)
1	36 (64%)
Stage	
IIIB	16 (29%)
IV	40 (71%)
Histology	
Adenocarcinoma	30 (54%)
Squamous cell carcinoma	21 (38%)
Adenosquamous cell carcinoma	1 (1.8%)
Large cell carcinoma	1 (1.8%)
NSCLC, not specified	3 (5.4%)

Abbreviation: ECOG, Eastern Cooperative Oncology Group.

Adverse events were graded according to the National Cancer Institute Common Toxicity Criteria (version 3). All patients who received one dose of chemotherapy were assessable for toxicity. A clinical and laboratory assessment was done at least every 2 wk.

Results

Patient characteristics. Between February and June 2006, a total of 59 patients were enrolled in the study at the 14 participating centers. Three patients did not receive treatment: one patient withdrew her consent, and two patients had a fall before treatment onset that resulted in a reduction in performance status. These three patients were thus not included in the analysis. The remaining 56 patients (46 men and 10 women) were eligible for the current analysis and their characteristics are summarized in Table 1. Their median age was 63 years, with a range of 40 to 74 years. Histologic analysis revealed that 30 patients (54%) had adenocarcinoma and 21 patients (38%) had squamous cell carcinoma. Forty patients (71%) had stage IV disease and the other 16 patients had stage IIIB disease (including 12 patients with malignant pleural effusion).

Treatment administered. Patients received a median of five cycles of treatment (range, 1-15), with 37 patients (66%) completing at least four cycles. Overall, 286 cycles of chemotherapy were delivered. The mean relative dose intensities of S-1 and irinotecan were 91% and 98%, respectively. Dose reductions were uncommon and were necessary according to the study protocol in only eight cycles (2.8% of total cycles) because of diarrhea in three patients, anorexia in two patients, vomiting in two patients, and an increase in serum ALT and AST levels in one patient. Treatment administration was delayed for at least 1 week because of toxicity in 12 cycles (4.2% of total cycles); the major causes of delayed administration were insufficient bone marrow function (six cycles with a leukocyte count of $<3{,}000/mm^3$ and one cycle with a platelet count of $<100{,}000/\mu L$) and nonhematologic toxicity (two cycles with fever in the absence of neutropenia, two cycles with an increase in serum ALT and AST levels, and one cycle with diarrhea).

Table 2. Overall response rate (Response Evaluation Criteria in Solid Tumors criteria) by independent radiologic assessment

Response	No. patients (%)
Complete response	0 (0)
Partial response	16 (28.6)
Overall response	16 (28.6; 95% CI, 17.3-42.2)
Stable disease	24 (42.9)
Disease progression	12 (21.4)
Not evaluable	4 (7.1%)

Response and survival. Four patients were not evaluable for response: three patients withdrew from the study after one treatment cycle and one patient did not have a measurable target lesion. There were 16 partial responses and no complete responses, yielding an overall response rate of 28.6% (Table 2). Twenty-four patients (42.9%) had stable disease, yielding an overall disease control rate (complete response + partial response + stable disease) of 71.4% [95% confidence interval (95% CI), 57.8-82.7%]. Twelve patients (21.4%) had progressive disease as the best response.

All 56 treated patients were assessable for progression-free survival and overall survival. With a median follow-up time of 14.9 months (range, 1.4-20.1 months), 25 patients were still alive. The progression-free survival curve is shown in Fig. 1; the median progression-free survival was 4.9 months (95% CI, 4.0-6.4 months). The curve for overall survival is shown in Fig. 2; the median overall survival time was 15 months (95% CI could not be estimated) and the 1-year survival rate was 63% (95% CI, 50-75%). No correlation was apparent between overall survival and sex, age, histology, disease stage, or smoking status.

Toxicity. The adverse events observed for all 56 treated patients are summarized in Table 3. The most frequently observed hematologic toxicity of grade 3 or 4 was neutropenia (14 cases, 25%). Four patients (7.1%) developed febrile neutropenia. Anemia or thrombocytopenia of grade 3 or 4 was less frequent, each occurring in 3.6% of patients. Nonhematologic toxicities were generally mild in intensity. The most common nonhematologic toxicities of grade 3 or 4 were anorexia (14.3%), fatigue (8.9%), diarrhea (8.9%), vomiting (3.6%), and an increase in serum ALT or AST levels (3.6%). Treatment was discontinued because of toxicity in only two of

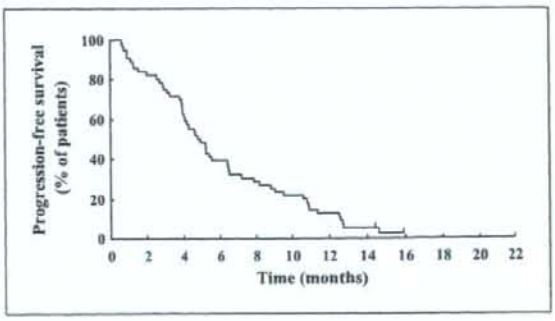

Fig. 1. Kaplan-Meier analysis of progression-free survival for all 56 treated patients.

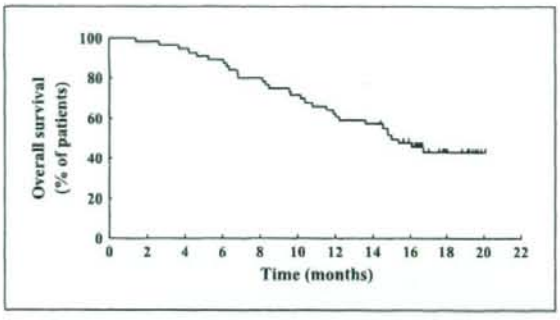

Fig. 2. Kaplan-Meier analysis of overall survival for all 56 treated patients.

the 56 patients (3.6%): in one patient because of pneumonitis (grade 3) and in the other because of prolonged anorexia (grade 3) and fatigue (grade 3). The patient with pneumonitis developed fever with hypoxemia after the fourth course of treatment. A computed tomographic scan of the chest revealed new ground-glass opacities distributed diffusely in both lungs. The patient responded well to steroid therapy and improved. No treatment-related deaths were observed.

Discussion

Platinum-based doublet chemotherapy is the standard of care for most patients with advanced NSCLC (2–4). However, there continues to be reluctance on the part of both patients and treating physicians to accept the toxicity of platinum-based therapy given the associated small gain in survival. Active therapies with improved toxicity profiles are clearly needed in this setting. Since the introduction of active third-generation agents (docetaxel, paclitaxel, gemcitabine, vinorelbine, and irinotecan), many clinical trials have been undertaken to evaluate nonplatinum regimens based on these drugs in the hope that platinum analogues could be eliminated from the treatment of advanced NSCLC. A recent meta-analysis showed that these newer nonplatinum regimens are valid options for the treatment of advanced NSCLC because of their shown activity and good toxicity profiles (18). Currently, however, there is no single best treatment regimen for advanced NSCLC.

As first-line chemotherapy for advanced NSCLC, the oral fluoropyrimidine formulation S-1 administered as a single agent showed a response rate of 22% and a median survival time of 10.2 months with toxicities that were generally mild (10). Combinations of S-1 with other active agents with a different mechanism of action are being investigated with the aim of achieving a greater clinical benefit. Irinotecan and fluoropyrimidines were shown not to induce cross-resistance in both experimental and clinical settings (19). Preclinical studies have also found that the combination of irinotecan and 5-FU has antitumor activities that are additive to synergistic (20). Furthermore, a possible molecular mechanism for synergistic cytotoxicity of S-1 and irinotecan has been suggested by the observation that irinotecan reduces thymidylate synthetase activity in tumor xenografts and thereby facilitates the antitumor effect of S-1 (14). Recent phase II studies have shown that combination treatment with S-1 and irinotecan is highly active with acceptable toxicity in patients with advanced colorectal cancer or gastric cancer (16, 21). However, the activity of this combination in patients with NSCLC has not previously been documented.

We have now assessed the efficacy and safety of combined treatment with S-1 and irinotecan in patients with previously untreated advanced NSCLC. We found the combination to be active, with a response rate of 28.6%, median progression-free survival of 4.9 months, median overall survival of 15 months, and 1-year survival rate of 63%. Previous phase III studies of platinum-based doublets for the treatment of advanced NSCLC showed response rates of 17% to 33%, a median time to progression or progression-free survival of 3 to 5 months, and a median overall survival time of 7 to 14 months (22–25). Although there are limitations to comparisons of the results from different studies, the efficacy data in our study compare favorably with those reported in these previous phase III studies of platinum-based doublets.

The S-1-irinotecan regimen was well tolerated in the patients of the present study. With regard to hematologic toxicity, neutropenia of grade 3 or 4 occurred in only 25% of all treated patients without the prophylactic administration of granulocyte colony-stimulating factor. Anemia and thrombocytopenia of grade 3 or 4 were each observed in only two patients (3.6%). These results compare favorably with the toxicity profiles reported for platinum-based combinations in previous studies with NSCLC patients, in which higher frequencies of neutropenia (~80%), anemia (~20%), and thrombocytopenia (~23%) of grade 3 or 4 were observed (22–24). The only nonhematologic toxicity of grade 3 or 4 encountered in >10% of patients in the present study was anorexia (14.3%). Although irinotecan and S-1 have each been shown to increase the frequency of severe diarrhea, the incidence of diarrhea of grade 3 in the present study was only 8.9%, consistent with the findings of a recent phase II study of the combination of S-1 and irinotecan administered according to the same doses and schedule in patients with advanced colorectal cancer (16).

Table 3. Toxicity for all 56 treated patients according to the National Cancer Institute Common Toxicity Criteria (version 3)

Toxicity	Grade				Grade ≥3 (%)
	1	2	3	4	
Leukopenia	9	10	5	0	8.9
Neutropenia	1	7	12	2	25.0
Anemia	31	19	1	1	3.6
Thrombocytopenia	23	2	2	0	3.6
Febrile neutropenia	NA	NA	4	0	7.1
Anorexia	25	10	8	0	14.3
Fatigue	18	12	4	1	8.9
Diarrhea	12	11	5	0	8.9
Nausea	27	11	1	0	1.8
Vomiting	12	4	2	0	3.6
Stomatitis	7	6	0	0	0
Rash	8	6	0	0	0
Hyperbilirubinemia	12	6	0	0	0
Elevation of AST/ALT	18	3	2	0	3.6
Elevation of creatinine	2	1	0	0	0
Pneumonitis	1	0	1	0	1.8

Abbreviation: NA, not applicable.

Thus, both hematologic and nonhematologic toxicities were generally manageable, and in most instances, treatment could be continued in an outpatient setting, resulting in a median of five treatment courses (range, 1-15).

In conclusion, we have presented the results of the first phase II study of the combination of S-1 and irinotecan for the treatment of chemotherapy-naive patients with advanced NSCLC. This regimen yielded a response rate, progression-free survival, and overall survival similar to or better than those previously reported for platinum-based regimens. In addition, this regimen was well tolerated and could be administered in an outpatient setting. Given its efficacy and favorable toxicity profile, the combination of S-1 and irinotecan is a promising alternative for treatment of advanced NSCLC and a feasible nonplatinum option to which molecularly targeted agents can be added. The chemotherapy regimens of S-1 plus platinum derivatives have been studied (11). We are currently conducting a randomized phase III trial comparing carboplatin/S-1 with carboplatin/paclitaxel for chemonaive advanced NSCLC. We firmly believe that further trials comparing S-1 plus irinotecan with platinum-based doublet chemotherapy (perhaps carboplatin/S-1) are warranted.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer statistics, 2007. CA Cancer J Clin 2007;57:43–66.
2. Chemotherapy in non-small cell lung cancer: a meta-analysis using updated data on individual patients from 52 randomised clinical trials. Non-small Cell Lung Cancer Collaborative Group. BMJ 1995;311:899–909.
3. Clinical practice guidelines for the treatment of unresectable non-small-cell lung cancer. Adopted on May 16, 1997 by the American Society of Clinical Oncology. J Clin Oncol 1997;15:2996–3018.
4. Socinski MA, Crowell R, Hensing TE, et al. Treatment of non-small cell lung cancer, stage IV: ACCP evidence-based clinical practice guidelines (2nd edition). Chest 2007;132:277–89S.
5. Shirasaka T, Nakano K, Takechi T, et al. Antitumor activity of 1 M tegafur-0.4 M 5-chloro-2,4-dihydroxypyridine-1 M potassium oxonate (S-1) against human colon carcinoma orthotopically implanted into nude rats. Cancer Res 1996;56:2602–6.
6. Takechi T, Nakano K, Uchida J, et al. Antitumor activity and low intestinal toxicity of S-1, a new formulation of oral tegafur, in experimental tumor models in rats. Cancer Chemother Pharmacol 1997;39:205–11.
7. Robben NC, Pippas AW, Moore JO. The syndrome of 5-fluorouracil cardiotoxicity. An elusive cardiopathy. Cancer 1993;71:493–509.
8. Kato T, Shimamoto Y, Uchida J, et al. Possible regulation of 5-fluorouracil-induced neuro- and oral toxicities by two biochemical modulators consisting of S-1, a new oral formulation of 5-fluorouracil. Anticancer Res 2001;21:1705–12.
9. Shirasaka T, Shimamoto Y, Fukushima M. Inhibition by oxonic acid of gastrointestinal toxicity of 5-fluorouracil without loss of its antitumor activity in rats. Cancer Res 1993;53:4004–9.
10. Kawahara M, Furuse K, Segawa Y, et al. Phase II study of S-1, a novel oral fluorouracil, in advanced non-small-cell lung cancer. Br J Cancer 2001;85:939–43.
11. Ichinose Y, Yoshimori K, Sakai H, et al. S-1 plus cisplatin combination chemotherapy in patients with advanced non-small cell lung cancer: a multi-institutional phase II trial. Clin Cancer Res 2004;10:7860–4.
12. Fukuoka M, Niitani H, Suzuki A, et al. A phase II study of CPT-11, a new derivative of camptothecin, for previously untreated non-small-cell lung cancer. J Clin Oncol 1992;10:16–20.
13. Negoro S, Masuda N, Takada Y, et al. Randomised phase III trial of irinotecan combined with cisplatin for advanced non-small-cell lung cancer. Br J Cancer 2003;88:335–41.
14. Takiuchi H, Narahara H, Tsujinaka T, et al. Phase I study of S-1 combined with irinotecan (CPT-11) in patients with advanced gastric cancer (OGSG 0002). Jpn J Clin Oncol 2005;35:520–5.
15. Yamada Y, Yasui H, Goto A, et al. Phase I study of irinotecan and S-1 combination therapy in patients with metastatic gastric cancer. Int J Clin Oncol 2003;8:374–80.
16. Goto A, Yamada Y, Yasui H, et al. Phase II study of combination therapy with S-1 and irinotecan in patients with advanced colorectal cancer. Ann Oncol 2006;17:968–73.
17. Therasse P, Arbuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst 2000;92:205–16.
18. D'Addario G, Pintilie M, Leighl NB, Feld R, Cerny T, Shepherd FA. Platinum-based versus non-platinum-based chemotherapy in advanced non-small-cell lung cancer: a meta-analysis of the published literature. J Clin Oncol 2005;23:2926–36.
19. Vanhoefer U, Harstrick A, Achterrath W, Cao S, Seeber S, Rustum YM. Irinotecan in the treatment of colorectal cancer: clinical overview. J Clin Oncol 2001;19:1501–18.
20. Houghton JA, Cheshire PJ, Hallman JD II, et al. Evaluation of irinotecan in combination with 5-fluorouracil or etoposide in xenograft models of colon adenocarcinoma and rhabdomyosarcoma. Clin Cancer Res 1996;2:107–18.
21. Inokuchi M, Yamashita T, Yamada H, et al. Phase I/II study of S-1 combined with irinotecan for metastatic advanced gastric cancer. Br J Cancer 2006;94:1130–5.
22. Schiller JH, Harrington D, Belani CP, et al. Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer. N Engl J Med 2002;346:92–8.
23. Fossella F, Pereira JR, von Pawel J, et al. Randomized, multinational, phase III study of docetaxel plus platinum combinations versus vinorelbine plus cisplatin for advanced non-small-cell lung cancer: the TAX 326 study group. J Clin Oncol 2003;21:3016–24.
24. 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.
25. Hotta K, Fujiwara Y, Matsuo K, et al. Recent improvement in the survival of patients with advanced nonsmall cell lung cancer enrolled in phase III trials of first-line, systemic chemotherapy. Cancer 2007;109:939–48.

Radiosensitizing Effect of YM155, a Novel Small-Molecule Survivin Suppressant, in Non–Small Cell Lung Cancer Cell Lines

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Abstract

Purpose: Survivin, a member of the inhibitor of apoptosis protein family, is an attractive target for cancer therapy. We have now investigated the effect of YM155, a small-molecule inhibitor of survivin expression, on the sensitivity of human non–small cell lung cancer (NSCLC) cell lines to γ-radiation.

Experimental Design: The radiosensitizing effect of YM155 was evaluated on the basis of cell death, clonogenic survival, and progression of tumor xenografts. Radiation-induced DNA damage was evaluated on the basis of histone H2AX phosphorylation and foci formation.

Results: YM155 induced down-regulation of survivin expression in NSCLC cells in a concentration- and time-dependent manner. A clonogenic survival assay revealed that YM155 increased the sensitivity of NSCLC cells to γ-radiation *in vitro*. The combination of YM155 and γ-radiation induced synergistic increases both in the number of apoptotic cells and in the activity of caspase-3. Immunofluorescence analysis of histone γ-H2AX also showed that YM155 delayed the repair of radiation-induced double-strand breaks in nuclear DNA. Finally, combination therapy with YM155 and γ-radiation delayed the growth of NSCLC tumor xenografts in nude mice to a greater extent than did either treatment modality alone.

Conclusions: These results suggest that YM155 sensitizes NSCLC cells to radiation both *in vitro* and *in vivo*, and that this effect of YM155 is likely attributable, at least in part, to the inhibition of DNA repair and enhancement of apoptosis that result from the down-regulation of survivin expression. Combined treatment with YM155 and radiation warrants investigation in clinical trials as a potential anticancer strategy.

Survivin is a 16.5-kDa member of the inhibitor of apoptosis protein (IAP) family. It blocks the mitochondrial pathway of apoptosis by inhibiting caspases (1, 2) and regulates cell division through interaction with the proteins INCENP and Aurora B (3). It is abundant in many types of cancer cells but not in the corresponding normal cells (4–6). High levels of survivin expression in cancer cells are associated with poor patient prognosis and survival as well as with resistance to therapy and an increased rate of cancer recurrence (7–9). Survivin has therefore become a therapeutic target and potentially important prognostic marker for many tumor types, including non–small cell lung cancer (NSCLC; refs. 7, 10). Molecular antagonists of survivin including antisense oligonucleotides, and dominant negative mutants have been shown to induce apoptosis in cancer cells *in vitro* and *in vivo* as well as to enhance chemotherapy-induced cell death (11–13). Although antisense oligonucleotides and ribozymes can be engineered to be highly specific for survivin, they may be difficult to deliver in the clinical setting.

YM155, a small imidazolium-based compound, was identified by high-throughput screening of chemical libraries for inhibitors of the activity of the survivin gene promoter in a reporter assay (14). This compound specifically inhibits the expression of survivin at both the mRNA and protein levels and exhibits pronounced anticancer activity in preclinical models (14). An advantage of YM155 compared with previously investigated suppressors of survivin expression (15–20) is that it is active in the subnanomolar range. Pharmacokinetic analysis also revealed that YM155 was highly distributed to tumor tissue in tumor xenograft models *in vivo* (14). YM155 is thus an attractive candidate drug for cancer therapy, and clinical trials of YM155 in single-agent therapy are currently under way for some types of cancer.

Glioblastoma cells that overexpress survivin were found to be less responsive to radiation than survivin-negative cells in a preclinical model (21). Clinically, high levels of survivin expression have been associated with an increased risk of local treatment failure after radiochemotherapy in patients with rectal cancer (9). These observations suggest that survivin plays

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Translational Relevance

Survivin is a potentially important molecular target for cancer therapy. Reflecting the many mechanisms that seem to regulate survivin expression, diverse approaches have been evaluated for targeting survivin in experimental models. YM155 is a novel small, imidazolium-based compound that specifically inhibits survivin expression in various types of cancer cell lines *in vitro*. In addition, YM155 has been shown to distribute preferentially to tumor tissues rather than to plasma as well as to exert pronounced antitumor activity in tumor xenograft models *in vivo*. The use of YM155 as a single agent in phase I clinical trials did not reveal significant toxicity. Although phase II studies of YM155 use as a single agent for certain types of cancer are currently under way, the effects of YM155 in combination with radiation have not been reported. We now show that inhibition of survivin expression by YM155 sensitizes tumor cells to radiation *in vitro* and *in vivo*. Therefore, our preclinical results provide a rationale for future clinical investigation of the therapeutic efficacy of YM155 in combination with radiotherapy.

a role in resistance to radiotherapy. Indeed, suppression of survivin expression with the use of antisense oligonucleotides or ribozymes has been shown to increase the radiosensitivity of cancer cells *in vitro* (20, 22–26). We have now examined the effects of the combination of YM155 and radiation on NSCLC cell lines *in vitro* and *in vivo*.

Materials and Methods

Cell culture and reagents. The human NSCLC cell lines NCI-H460 (H460) and Calu6 were obtained from the American Type Culture Collection. The cells were cultured under an atmosphere of 5% CO_2 at 37°C in RPMI 1640 (Sigma) supplemented with 10% fetal bovine serum. YM155 (Astellas Pharma, Inc.) was dissolved in DMSO.

Immunoblot analysis. Cells were washed twice with ice-cold PBS and then lysed in a solution containing 20 mmol/L Tris-HCl (pH 7.5), 150 mmol/L NaCl, 1 mmol/L EDTA, 1% Triton X-100, 2.5 mmol/L sodium PPi, 1 mmol/L phenylmethylsulfonyl fluoride, and leupeptin (1 μg/mL). The protein concentration of lysates was determined with the Bradford reagent (Bio-Rad), and equal amounts of protein were subjected to SDS-PAGE of a 15% gel. The separated proteins were transferred to a nitrocellulose membrane, which was then exposed to 5% nonfat dried milk in PBS for 1 h at room temperature before incubation overnight at 4°C with rabbit polyclonal antibodies to human survivin (1:1000 dilution; R&D Systems), to human c-IAP1 (1:1,000 dilution; MBL International), to human XIAP (1:1,000 dilution; Cell Signaling), to human STAT3 (1:1,000 dilution; Cell Signaling), or to β-actin (1:500 dilution; Sigma), or with mouse monoclonal antibodies to human p53 (1:1,000 dilution; Santa Cruz Biotechnology). The membrane was then washed with PBS containing 0.05% Tween 20 before incubation for 1 h at room temperature with horseradish peroxidase–conjugated goat antibodies to rabbit (Sigma) or mouse (Santa Cruz Biotechnology) IgG. Immune complexes were finally detected with chemiluminescence reagents (Perkin-Elmer Life Science).

Clonogenic survival assay. Exponentially growing cells in 25-cm^2 flasks were harvested by exposure to trypsin and counted. They were diluted serially to appropriate densities and plated in triplicate in 25-cm^2 flasks containing 10 mL of complete medium in the presence of 50 nmol/L YM155 or vehicle (final DMSO concentration of 0.1%; we confirmed that this DMSO concentration did not affect the proliferation of NSCLC cell lines). After incubation for 48 h, the cells were exposed at room temperature to various doses of γ-radiation with a ^{60}Co irradiator at a rate of −0.82 Gy/min. The cells were then washed with PBS, cultured in drug-free medium for 10 to 14 d, fixed with methanol:acetic acid (10:1, v/v), and stained with crystal violet. Colonies containing >50 cells were counted. The surviving fraction was calculated as: (mean number of colonies)/(number of inoculated cells × plating efficiency). Plating efficiency was defined as the mean number of colonies divided by the number of inoculated cells for nonirradiated control cells. The surviving fraction for combined treatment was corrected by that for YM155 treatment alone. Cell survival was corrected according to the equation $S = 1 - (1 - f)^{1/N}$, where S is the single-cell survival rate, f is the measured surviving fraction, and N is multiplicity, which was defined as the average number of cells per microcolony at the time of radiation and which ranged from 2.4 to 6.7 for the cell lines studied under the described conditions. The dose enhancement factor was then calculated as the dose (Gy) of radiation that yielded a surviving fraction of 0.1 for vehicle-treated cells divided by that for YM155-treated cells (after correction for drug toxicity).

Detection of apoptotic cells. Cells were fixed with 4% paraformaldehyde for 1 h at room temperature, after which a minimum of 1,000 cells per sample was evaluated for apoptosis with the use of the terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) technique (*In situ* Cell Death Detection Kit; Boehringer Mannheim).

Assay of caspase-3 activity. The activity of caspase-3 in cell lysates was measured with the use of a CCP32/Caspase-3 Fluometric Protease Assay Kit (MBL). Fluorescence attributable to cleavage of the DEVD-AFC substrate was measured at excitation and emission wavelengths of 390 and 460 nm, respectively.

Immunofluorescence staining of γ-H2AX. Cells were grown to 50% confluence in two-well Lab-Tec Chamber Slides (Nunc) and then cultured for 48 h in the presence of 50 nmol/L YM155 or vehicle before exposure to 3 Gy of γ-radiation. At various times thereafter, they were fixed with 4% paraformaldehyde for 10 min at room temperature, permeabilized with 0.1% Triton X-100 for 10 min at 4°C, and exposed to 5% nonfat dried milk for 10 min at room temperature. The slides were washed with PBS and then incubated at room temperature first for 2 h with mouse monoclonal antibodies to histone γ-H2AX (Upstate Biotechnology) at a dilution of 1:300 and then for 1 h with Alexa 488–labeled goat antibodies to mouse IgG (Molecular Probes) at a dilution of 1:700. The slides were mounted in fluorescence mounting medium (Dako Cytomation), and fluorescence signals were visualized with a confocal laser-scanning microscope (Axiovert 200M; Carl Zeiss) equipped with the LSM5 PASCAL system (Carl Zeiss). Three random fields each containing ≈50 cells were examined at a magnification of × 100. Nuclei containing ≥10 immunoreactive foci were counted as positive for γ-H2AX, as previously described (27), and percentage of positive cells was calculated.

Evaluation of tumor growth **in vivo.** All animal studies were done in accordance with the Recommendations for Handling of Laboratory Animals for Biomedical Research compiled by the Committee on Safety and Ethical Handling Regulations for Laboratory Animal Experiments, Kyoto University. The ethical procedures followed met the requirements of the United Kingdom Coordinating Committee on Cancer Research guidelines (28). Tumor cells (2×10^6) were injected s.c. into the right hind leg of 6-week-old female athymic nude mice (BALB/c nu/nu). Tumor volume was determined from caliper measurement of tumor length (L) and width (W) according to the formula $LW^2/2$. Treatment was initiated when the tumors in each group of animals achieved an average volume of ~200 to 250 mm^3. Treatment groups (each containing eight mice) consisted of vehicle control (physiologic saline), YM155 alone, vehicle plus radiation, and YM155 plus radiation. Vehicle or YM155 at a dose of 5 mg/kg of body mass was administered over 7 consecutive days (days 1-7) with the use of an implanted micro-osmotic pump (Alzet model 1003D; Durect). Mice in the radiation groups received 10 Gy of γ-radiation from a cobalt irradiator either as

a single fraction on day 3 of drug treatment or fractionated over 5 consecutive days (days 3 to 7); the radiation was targeted to the tumor, with the remainder of the body shielded with lead. Growth delay (GD) was calculated as the time required to achieve a 5-fold increase in volume for treated tumors minus that for control tumors. The enhancement factor was then determined as: $(GD_{combination} - GD_{YM155})/GD_{radiation}$.

Statistical analysis. Data are presented as means ± SD or SE and were compared with the unpaired Student's t test. A P value of <0.05 was considered statistically significant.

Results

Inhibition of survivin expression in NSCLC cells by YM155. We first examined the effect of YM155 on survivin expression in human NSCLC cell lines by immunoblot analysis. Treatment of H460 or Calu6 cells with YM155 at 1 to 100 nmol/L for 48 hours inhibited survivin expression in a concentration-dependent manner (Fig. 1A). In contrast, YM155 had no effect on the abundance of other members of the IAP family including XIAP and c-IAP1 (Fig. 1A), suggesting that YM155 specifically inhibits survivin expression in the NSCLC cell lines. The mechanism by which YM155 inhibits survivin expression remains to be elucidated. Previous observations have shown that p53 and signal transducer and activator of transcription 3 (STAT3) regulate survivin expression at the transcriptional level (29). We therefore examined the effect of YM155 on the abundance of p53 and STAT3 in NSCLC cell lines. YM155 showed no marked effect on the amounts of p53 and STAT3 in H460 or Calu6 cells (Fig. 1A), suggesting that the inhibition of survivin expression by YM155 is independent of these transcriptional regulators. Monitoring of the time course of survivin expression in cells exposed to 50 nmol/L

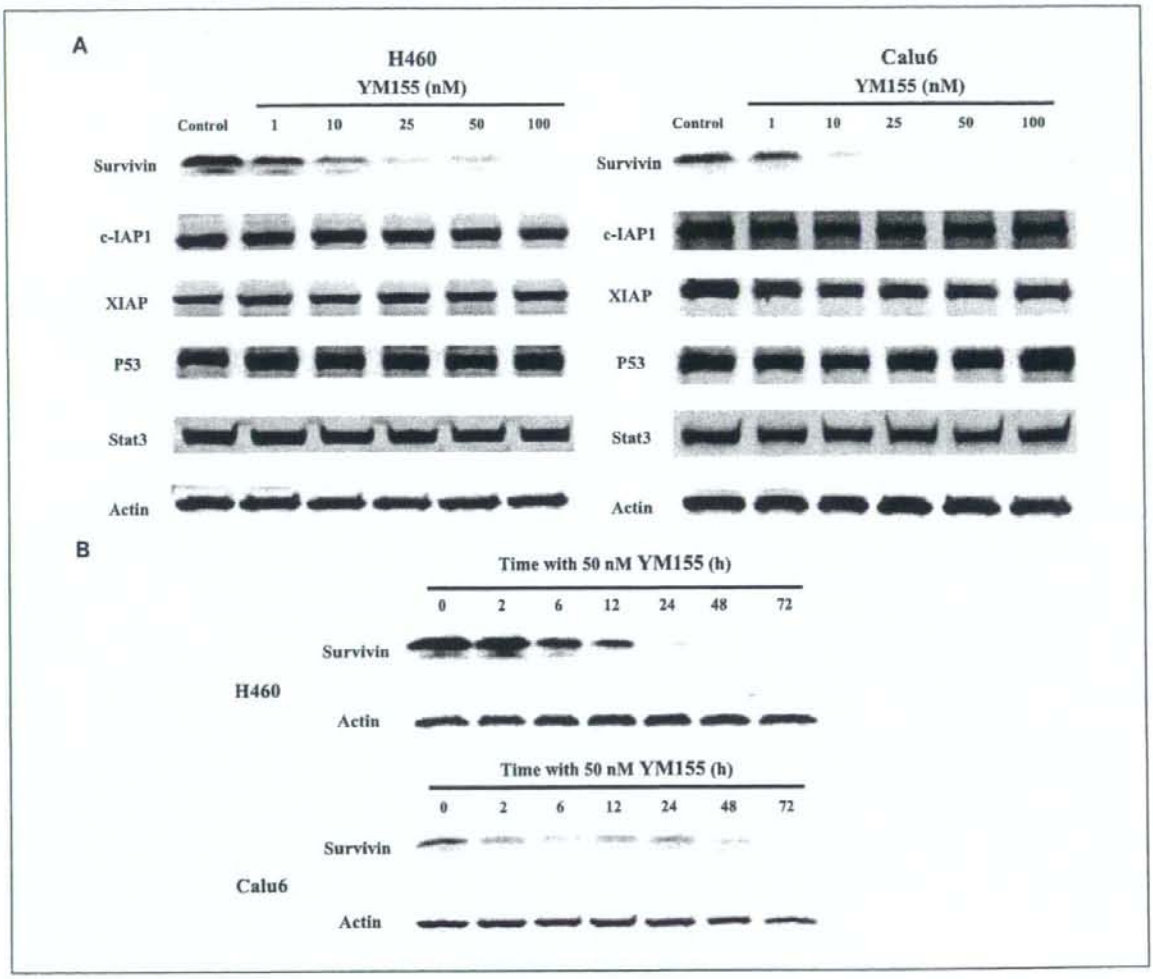

Fig. 1. Effect of YM155 on survivin expression in human NSCLC cells. *A*, H460 or Calu6 cells were incubated in the absence (control, 0.1% DMSO) or presence of various concentrations (1, 10, 25, 50, or 100 nmol/L) of YM155 for 48 h. Cell lysates were then prepared and subjected to immunoblot analysis with antibodies to survivin, to c-IAP1, to XIAP, to p53, to STAT3, or to β-actin (loading control). *B*, H460 or Calu6 cells were incubated with 50 nmol/L YM155 for the indicated times, after which cell lysates were subjected to immunoblot analysis with antibodies to survivin or to β-actin.