12. Zatloukal P, Petruzelka L, Zemanova M, Kolek V, Skrickova J, Pesek M, et al. Gemcitabine plus cisplatin vs. gemcitabine plus carboplatin in stage IIIb and IV non-small cell lung cancer: a phase III randomized trial. *Lung Cancer* 2003;41:321–31.
13. Hotta K, Matsuo K, Ueoka H, Kiura K, Tabata M, Tanimoto M. Meta-analysis of randomized clinical trials comparing cisplatin to carboplatin in patients with advanced non-small-cell lung cancer. *J Clin Oncol* 2004;22:3852–9.
14. Ardizzoni A, Boni L, Tiseo M, Fossella FV, Schiller JH, Paesmans M, et al. Cisplatin- versus carboplatin-based chemotherapy in first-line treatment of advanced non-small-cell lung cancer: an individual patient data meta-analysis. *J Natl Cancer Inst* 2007;99:847–57.
15. Grigorescu AC, Draghici IN, Nitipir C, Gutulescu N, Corlan E. Gemcitabine (GEM) and carboplatin (CBDCA) versus cisplatin (CDDP) and vinblastine (VLB) in advanced non-small-cell lung cancer (NSCLC) stages III and IV: a phase III randomised trial. *Lung Cancer* 2002;37:9–14.
16. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. *Nephron* 1976;16:31–41.
17. Calvert AH, Newell DR, Gumbrell LA, O'Reilly S, Burnell M, Boxall FE, et al. Carboplatin dosage: prospective evaluation of a simple formula based on renal function. *J Clin Oncol* 1989;7:1748–56.
18. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, 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.
19. Langer C, Li S, Schiller J, Tester W, Rapoport BL, Johnson DH, et al. Randomized phase II trial of paclitaxel plus carboplatin or gemcitabine plus cisplatin in Eastern Cooperative Oncology Group performance status 2 non-small-cell lung cancer patients: ECOG 1599. *J Clin Oncol* 2007;25:418–23.
20. Lilenbaum RC, Herndon JE, 2nd, List MA, Desch C, Watson DM, Miller AA, et al. Single-agent versus combination chemotherapy in advanced non-small-cell lung cancer: the cancer and leukemia group B (study 9730). *J Clin Oncol* 2005;23:190–6.
21. Ando Y, Minami H, Saka H, Ando M, Sakai S, Shimokata K. Adjustment of creatinine clearance improves accuracy of Calvert's formula for carboplatin dosing. *Br J Cancer* 1997;76:1067–71.
22. Buccheri G, Ferrigno D, Tamburini M. Karnofsky and ECOG performance status scoring in lung cancer: a prospective, longitudinal study of 536 patients from a single institution. *Eur J Cancer* 1996;32A:1135–41.
23. Radzikowska E, Glaz P, Roszkowski K. Lung cancer in women: age, smoking, histology, performance status, stage, initial treatment and survival. Population-based study of 20 561 cases. *Ann Oncol* 2002;13:1087–93.
24. Pfister DG, Johnson DH, Azzoli CG, Sause W, Smith TJ, Baker S, Jr. American Society of Clinical Oncology treatment of unresectable non-small-cell lung cancer guideline: update 2003. *J Clin Oncol* 2004;22:330–53.
25. The Elderly Lung Cancer Vinorelbine Italian Study Group. Effects of vinorelbine on quality of life and survival of elderly patients with advanced non-small-cell lung cancer. *J Natl Cancer Inst* 1999;91: 66–72.
26. Ranson M, Davidson N, Nicolson M, Falk S, Carmichael J, Lopez P, et al. Randomized trial of paclitaxel plus supportive care versus supportive care for patients with advanced non-small-cell lung cancer. *J Natl Cancer Inst* 2000;92:1074–80.
27. Roszkowski K, Pluzanska A, Krzakowski M, Smith AP, Saigi E, Aasebo U, et al. A multicenter, randomized, phase III study of docetaxel plus best supportive care versus best supportive care in chemotherapy-naive patients with metastatic or non-resectable localized non-small cell lung cancer (NSCLC). *Lung Cancer* 2000;27:145–57.
28. Anderson H, Hopwood P, Stephens RJ, Thatcher N, Cottier B, Nicholson M, et al. Gemcitabine plus best supportive care (BSC) vs BSC in inoperable non-small cell lung cancer--a randomized trial with quality of life as the primary outcome. UK NSCLC Gemcitabine Group. Non-Small Cell Lung Cancer. *Br J Cancer* 2000;83:447–53.
29. Helbekkmo N, Aasebo U, Sundstrom SH, von Plessen C, Brunsvig PF, Bremnes RM; Norwegian Lung Cancer Study Group. Treatment outcome in performance status 2 advanced NSCLC patients administered platinum-based combination chemotherapy. *Lung Cancer* 2008;62: 253–60.
30. Kosmidis PA, Dimopoulos MA, Syrigos K, Nicolaides C, Aravantinos G, Boukovinas I, et al. Gemcitabine versus gemcitabine-carboplatin for patients with advanced non-small cell lung cancer and a performance status of 2: a prospective randomized phase II study of the Hellenic Cooperative Oncology Group. *J Thorac Oncol* 2007;2:135–40.
31. Taylor AE, Olver IN, Sivanthan T, Chi M, Purnell C. Observer error in grading performance status in cancer patients. *Support Care Cancer* 1999;7:332–5.
32. Gebbia V, Galetta D, De Marinis F. Non small cell lung cancer patients with ECOG PS2: unsolved questions and lessons from clinical trials. *Ann Oncol* 2005;16(Suppl 4):iv123–31.
33. Firat S, Byhardt RW, Gore E. Comorbidity and Karnofksy performance score are independent prognostic factors in stage III non-small-cell lung cancer: an institutional analysis of patients treated on four RTOG studies. Radiation Therapy Oncology Group. *Int J Radiat Oncol Biol Phys* 2002;54:357–64.
34. Frasci G, Lorusso V, Panza N, Comella P, Nicolella G, Bianco A, et al. Gemcitabine plus vinorelbine versus vinorelbine alone in elderly patients with advanced non-small-cell lung cancer. *J Clin Oncol* 2000;18:2529–36.
35. Inoue A, Kobayashi K, Usui K, Maemondo M, Okinaga S, Mikami I, et al. First-line gefitinib for patients with advanced non-small-cell lung cancer harboring epidermal growth factor receptor mutations without indication for chemotherapy. *J Clin Oncol* 2009;27:1394–400.

original article

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Quality of life and disease-related symptoms in previously treated Japanese patients with non-small-cell lung cancer: results of a randomized phase III study (V-15-32) of gefitinib versus docetaxel

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Background: This report describes quality of life (QoL) findings of a randomized study comparing gefitinib with docetaxel in patients with advanced/metastatic pretreated non-small-cell lung cancer.

Patients and methods: This open-label, phase III study randomized 490 Japanese patients to gefitinib (250 mg/day) or docetaxel (60 mg/m^2/3 weeks), with survival as the primary outcome. Preplanned QoL analyses included Functional Assessment of Cancer Therapy-Lung (FACT-L), Trial Outcome Index (TOI) and Lung Cancer Subscale (LCS) improvement rates, and mean change from baseline.

Results: Gefitinib showed statistically significant benefits over docetaxel in QoL improvement rates (FACT-L 23% versus 14%, $P = 0.023$; TOI 21% versus 9%, $P = 0.002$) and mean change from baseline score [mean treatment difference: FACT-L 3.72 points, 95% confidence interval (CI) 0.55–6.89, $P = 0.022$; TOI 4.31 points, 95% CI 2.13–6.49, $P < 0.001$], although differences did not meet the clinically relevant six-point change. There were no significant differences between treatments in LCS improvement rates (23% versus 20%, $P = 0.562$) or mean change from baseline score (0.63 points, 95% CI −0.07 to 1.34, $P = 0.077$).

Conclusions: Gefitinib improved aspects of QoL over docetaxel, with superior objective response rate and a more favorable tolerability profile and no statistically significant difference in overall survival (although noninferiority was not statistically proven).

Key words: docetaxel, gefitinib, non-small-cell lung cancer, quality of life

original article

introduction

Docetaxel is an established treatment of patients with previously treated advanced non-small-cell lung cancer (NSCLC) worldwide, including Japan; however, this is associated with typical cytotoxic side-effects including hematological toxicity, especially grade 3/4 neutropenia [1, 2]. Alternative agents with an improved tolerability profile, such as the epidermal growth factor receptor tyrosine kinase inhibitor (EGFR TKI) gefitinib, have been investigated in this setting [3–5].

In this randomized phase III study (V-15-32) comparing gefitinib versus docetaxel in previously treated Japanese patients with NSCLC, the primary objective (noninferiority of gefitinib versus docetaxel) was not statistically proven for overall survival (OS) [hazard ratio (HR) 1.12, 95.24% confidence interval (CI) 0.89–1.40], according to the predefined noninferiority criterion (upper CI for HR < 1.25) [6]. However, there were no statistically significant differences in OS ($P = 0.330$) or progression-free survival (PFS; $P = 0.335$) and gefitinib had a superior objective response rate (ORR) and a more favorable tolerability profile than docetaxel. Because of the significant

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burden of disease-related symptoms in patients with advanced NSCLC, improvements in health-related quality of life (QoL) and symptoms are an important additional parameter to guide treatment choice, particularly with the introduction of agents with better tolerability profiles. Here, we report in detail the QoL and symptom analyses of the V-15-32 study.

patients and methods

study design

This phase III study compared the effects of gefitinib versus docetaxel in Japanese patients with advanced/metastatic (stage IIIb/IV) or recurrent NSCLC who failed one or two chemotherapy regimens. Details of the study design and eligibility criteria have been published [6]. The primary end point was OS; the study aimed to show noninferiority of gefitinib versus docetaxel. Secondary end points were PFS, time-to-treatment failure, ORR, disease control rate, QoL, disease-related symptoms, safety, and tolerability.

The study was carried out in accordance with the Declaration of Helsinki and the International Conference on Harmonization/Good Clinical Practice (GCP), applicable regulatory requirements, and the AstraZeneca policy on Bioethics. The study protocol was approved by each institutional review board and written informed consent was obtained from all patients.

QoL assessments and analyses

The Functional Assessment of Cancer Therapy-Lung (FACT-L) questionnaire was used to assess QoL at baseline and every 4 weeks during study treatment until week 12. The FACT-L questionnaire is a validated, self-report questionnaire comprising physical, functional, social/family, emotional well-being subscales and Lung Cancer Subscale (LCS) [7]. The Trial Outcome Index (TOI), the sum of the physical, functional subscales, and LCS is reported to be a precise indicator of functional outcomes [7]. Disease-related symptoms were assessed weekly using the LCS. As previously reported [8], clinically relevant improvement was defined as change from baseline of ≥+6 for FACT-L or TOI or ≥+2 for LCS, on two visits at least 28 days apart. The assessable for LCS and assessable for QoL populations were subsets of the intent-to-treat (ITT) population with nonmissing baseline and one or more nonmissing post-baseline LCS and QoL assessments, respectively.

Preplanned analyses of FACT-L, TOI, and LCS scores included the following: mean change from baseline and 95% CI of the difference in mean change from baseline scores between the groups (based on the *t*-distribution; calculated as the difference between the mean overall patients on a treatment of the within-patient average change from baseline score); improvement, control (improvement or no change), and worsening rates and the odds ratio between treatments (with 95% CI and *P* value from a logistic regression model without covariates); and HR (gefitinib/docetaxel) for time to worsening (with 95% CI and *P* value using a proportional hazard model without covariates).

Supporting *post hoc* analyses of FACT-L, TOI, and LCS scores included the following: similar analyses using best change from baseline score instead of mean change; mean and best change from baseline for each subscale with two-sample *t*-test comparing treatments; mean and best change from baseline for individual questions; and correlation between mean change and best change from baseline and tumor response.

results

patients

Of 245 gefitinib and 244 docetaxel patients (one patient in the docetaxel arm was excluded due to GCP violation) in the ITT population, 185 (76%) and 173 (71%) patients, respectively, were assessable for QoL and 225 (92%) and 211 (86%) patients, respectively, were assessable for LCS. The demographic characteristics of the assessable for QoL and assessable for LCS populations (Supplemental Table 1, available at *Annals of Oncology* online) were representative of the overall study population [6].

QoL and disease-related symptoms at baseline

The baseline FACT-L, TOI, and LCS scores were similar between treatment groups (Table 1).

compliance and evaluability

Baseline compliance rates [(evaluable questionnaires during the treatment period)/(expected questionnaires) × 100] for gefitinib and docetaxel were high: 92% and 86%, respectively, for FACT-L and 93% and 87%, respectively, for LCS. During the first 12-weeks treatment, compliance rates for gefitinib and docetaxel were between 77% and 89% and 77% and 93%, respectively, for FACT-L completion and between 76% and 98% and 71% and 98%, respectively, for LCS completion, with smaller numbers of patients as time progressed as expected (Supplemental Table 2, available at *Annals of Oncology* online). Evaluability rates [(evaluable questionnaires during the treatment period)/(received questionnaires) × 100] were also high at between 88% and 100% (Supplemental Table 2, available at *Annals of Oncology* online).

QoL and symptom improvement

Significantly, more gefitinib-treated patients experienced a clinically relevant improvement in QoL (FACT-L and TOI) compared with docetaxel (Figure 1). There was no evidence of a difference between treatments in terms of symptom improvement rates measured by LCS (Figure 1).

Table 1. Baseline FACT-L, TOI, and LCS scores (assessable population)

Variable	Gefitinib			Docetaxel		
	n	Median (range)	Mean ± SD	*n*	Median (range)	Mean ± SD
FACT-L	185	98.5 (64.0–100.0)	98.7 ± 17.2	173	98.0 (49.3–138.0)	97.3 ± 17.5
TOI	185	58.4 (26.0–84.0)	58.0 ± 12.4	173	59.0 (28.0–82.0)	57.8 ± 12.6
LCS	225	19.0 (5.0–28.0)	19.4 ± 4.75	211	19.6 (5.0–28.0)	19.4 ± 4.91

SD, standard deviation; FACT-L, Functional Assessment of Cancer Therapy-Lung; TOI, Trial Outcome Index; LCS, Lung Cancer Subscale.

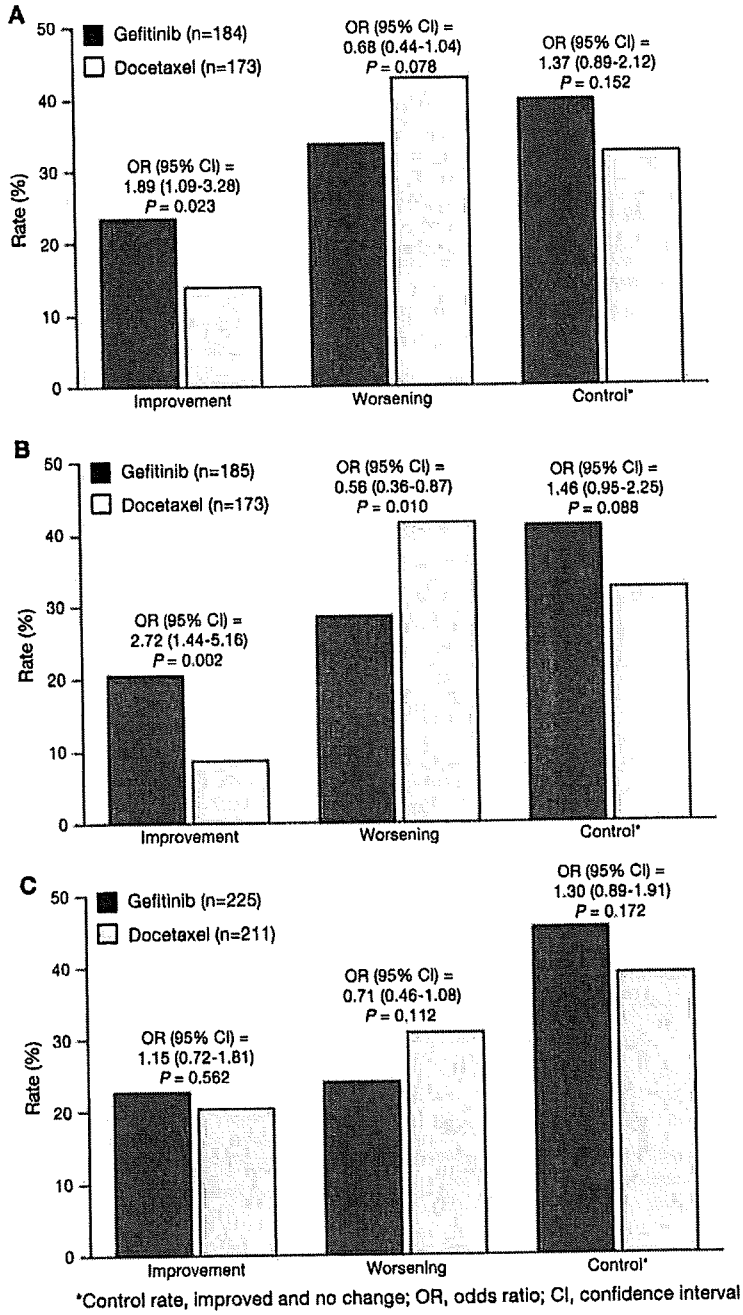

Figure 1. Improvement, worsening and control rates of (A) Functional Assessment of Cancer Therapy-Lung total, (B) Trial Outcome Index, and (C) Lung Cancer Subscale score (assessable population).

Time to worsening was significantly longer on gefitinib than docetaxel for TOI, numerically longer for FACT-L, and slightly longer for LCS (Figure 2).

Mean change from baseline for FACT-L, TOI, and LCS at each visit during the first 12 weeks of treatment is shown in Supplemental Figure 1 (available at *Annals of Oncology* online).

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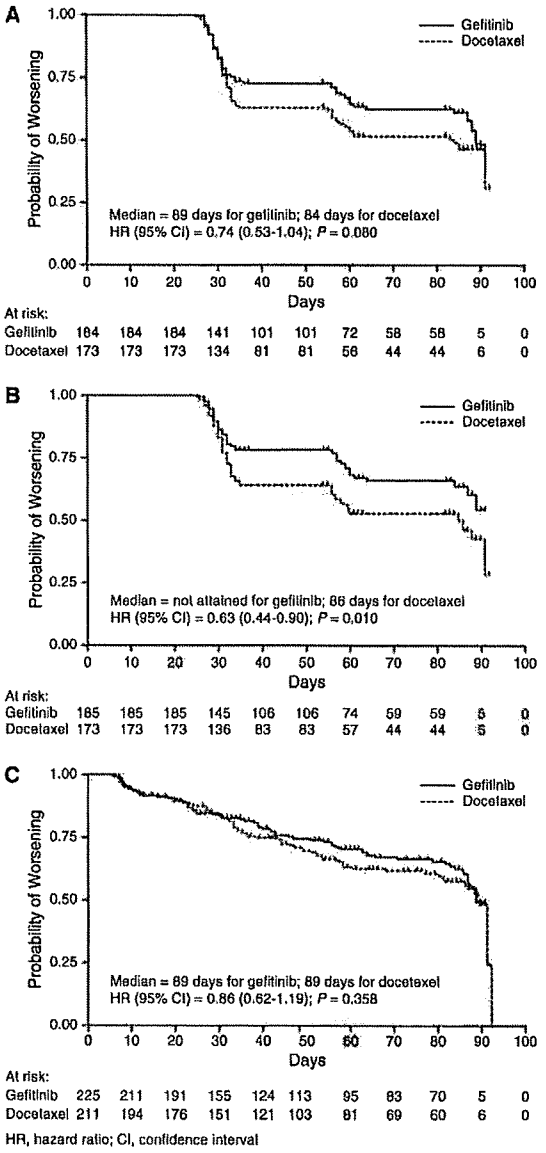


Figure 2. Time to worsening of (A) Functional Assessment of Cancer Therapy-Lung total, (B) Trial Outcome Index, and (C) Lung Cancer Subscale score (assessable population).

Statistically significant differences between treatments in mean change from baseline for QoL score (FACT-L and TOI) in favor of gefitinib were observed, but the differences did not meet the predefined, clinically relevant six-point change (FACT-L: 3.72 points, 95% CI 0.55–6.89, $P = 0.022$; TOI: 4.31 points, 95% CI 2.13–6.49, $P < 0.001$) (Table 2). There was no significant difference between treatments in mean change from baseline for LCS score (0.63 points, 95% CI −0.07 to 1.34, $P = 0.077$) (Table 2).

Table 2. Mean change during the first 12 weeks of treatment (assessable populations)

Variable	Gefitinib			Docetaxel			Difference	*P* value
	n	Mean	SD	*n*	Mean	SD	(95% CI)	by *t*-test
FACT-L	184	0.94	15.48	173	−2.78	14.96	3.72 (0.55 to 6.89)	0.022
TOI	185	0.81	10.22	173	−3.50	10.78	4.31 (2.13 to 6.49)	<0.001
LCS	225	1.38	3.58	211	0.75	3.89	0.63 (−0.07 to 1.34)	0.077

SD, standard deviation; CI, confidence interval; FACT-L, Functional Assessment of Cancer Therapy-Lung; TOI, Trial Outcome Index; LCS, Lung Cancer Subscale.

Post hoc analyses of mean change from baseline in the FACT-L subscales identified significant differences in favor of gefitinib over docetaxel in the physical ($P = 0.002$) and functional well-being subscales ($P = 0.002$) but not in the social/family ($P = 0.494$) or emotional well-being subscales ($P = 0.663$) (Figure 3).

In *post hoc* analyses, individual FACT-L questions with the largest differences between treatments in mean change from baseline (≥0.3 points difference of absolute value, all favoring gefitinib) were 'I am bothered by hair loss' (difference 2.03 points; question not included in calculating FACT-L, TOI, and LCS scores); 'I am content with the quality of my life right now' (0.47 points); 'I am forced to spend time in bed' (0.39 points); 'I am enjoying the things I usually do for fun' (0.33 points); 'I am sleeping well' (0.31 points); and 'I have a good appetite' (0.31 points). No question favored docetaxel by >0.21 points (Supplemental Table 3, available at *Annals of Oncology* online).

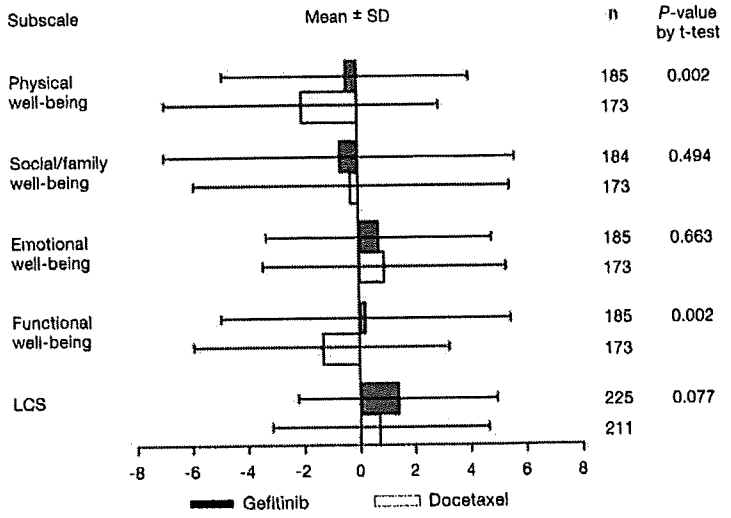
The results of *post hoc* analyses of best change from baseline score were consistent with the preplanned mean change from baseline score analyses.

QoL and symptom improvement by objective tumor response

Mean change from baseline in FACT-L, TOI, and LCS improved as best overall objective tumor response improved for both gefitinib and docetaxel (Supplemental Table 4, available at *Annals of Oncology* online). There was a higher correlation between changes and tumor response for gefitinib than docetaxel, which may be caused by more disperse distribution of objective tumor response for gefitinib. Similar results with slightly higher correlations were seen using best change from baseline.

discussion

In this randomized phase III study in previously treated advanced NSCLC, noninferiority of gefitinib versus docetaxel was not statistically proven for OS, although there were no statistically significant differences in OS or PFS between treatments. However, gefitinib demonstrated statistically significant benefits over docetaxel in QoL improvement rates and mean change from baseline QoL score (measured by



SD, standard deviation; LCS, lung cancer subscale

Figure 3. Mean change of mean score from baseline for Functional Assessment of Cancer Therapy-Lung subscales (assessable population).

FACT-L and TOI) in addition to superior ORR and a more favorable tolerability profile for gefitinib. *Post hoc* analyses showed that the biggest differences in favor of gefitinib were in the FACT-L physical and functional well-being subscales, the two subscales thought the most responsive to short-term changes [7]. Conversely, there were no significant differences between treatments in symptom improvement rates or mean change from baseline symptom score as measured by the LCS. In line with these results, time to worsening of QoL tended to be longer for gefitinib than docetaxel, significantly so for TOI. Further, *post hoc* analyses showed that there appeared to be a higher correlation between QoL and symptom changes and objective tumor response with gefitinib compared with docetaxel. Compliance and evaluability rates were high supporting the validity of these QoL data [9].

The QoL benefits seen in this study are consistent with other studies of gefitinib and docetaxel [3, 4, 10–13]. Docetaxel has demonstrated symptom relief including improvements in patient-rated pain scores ($P = 0.005$) and QoL with less deterioration in Lung Cancer Symptom Scale (LCSS) pain score ($P < 0.05$) in pretreated patients with advanced NSCLC compared with best supportive care [11]. Despite an improved tolerability profile with pemetrexed, no improvements were observed in QoL measurements compared with docetaxel in a phase III second-line setting in predominantly Western patients: symptom improvement rates (21% versus 22%, respectively, measured by LCSS) and rates of improvement or stabilization of anorexia (56% versus 61%), fatigue (55% versus 57%), cough (64% versus 64%), dyspnea (64% versus 60%), hemoptysis (70% versus 73%), and pain (64% versus 62%) were similar for pemetrexed and docetaxel [12]. In a phase II study in previously treated patients with advanced NSCLC (SIGN), QoL improvement rate of gefitinib was higher than docetaxel (34% versus 26%) and the mean change from baseline in FACT-L score was similar between the treatments (1.55 versus 0.39, $P = 0.63$) [10]. A larger international phase III study (INTEREST) with a very similar design to V-15-32 but in predominantly Western patients has established noninferior survival of gefitinib versus docetaxel in 1466 patients with pretreated advanced NSCLC [13]. Statistically significant benefits in QoL improvement rates for gefitinib over docetaxel were also observed in this study (FACT-L 25% versus 15%, $P < 0.0001$; TOI 17% versus 10%, $P = 0.0026$), with no significant difference between treatments in symptom improvement rates (LCS 20% versus 17%, $P = 0.1329$) [13]. Another EGFR TKI, erlotinib, was associated with QoL improvements [using the European Organization for Research and Treatment of Cancer QoL questionnaire (QLQ-C30)] compared with placebo [14] but no comparative data for erlotinib versus docetaxel exist.

In conclusion, gefitinib demonstrated statistically significant QoL benefits compared with docetaxel in the current study. From this study, we believe that treatment with gefitinib remains an effective treatment option with potential QoL advantages for previously treated Japanese patients with locally advanced/metastatic NSCLC.

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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/m^2) docetaxel in patients with advanced non-small-cell lung cancer. Cancer Chemother Pharmacol 2001; 48: 356–360.
2. 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: 2095–2103.
3. 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 2003; 21: 2237–2246.
4. 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 2003; 290: 2149–2158.
5. 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 2005; 366: 1527–1537.
6. Maruyama R, Nishiwaki Y, Tamura T et al. Phase III study, V-15-32, of gefitinib versus docetaxel in previously treated Japanese patients with non-small-cell lung cancer. J Clin Oncol 2008; 26: 4244–4252.
7. Cella DF, Bonomi AE, Lloyd SR et al. Reliability and validity of the Functional Assessment of Cancer Therapy-Lung (FACT-L) quality of life instrument. Lung Cancer 1995; 12: 199–220.
8. Cella D, Eton DT, Fairclough DL et al. What is a clinically meaningful change on the Functional Assessment of Cancer Therapy-Lung (FACT-L) questionnaire? Results from Eastern Cooperative Oncology Group (ECOG) Study 5592. J Clin Epidemiol 2002; 55: 285–295.
9. Osoba D, Bezjak A, Brundage M et al. Analysis and interpretation of health-related quality of life data from clinical trials: basic approach of The National Cancer Institute of Canada Clinical Trials Group. Eur J Cancer 2005; 41: 280–287.
10. 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 (stage IIIb or IV) non-small-cell lung cancer. Anticancer Drugs 2006; 17: 401–409.
11. Dancey J, Shepherd FA, Gralla RJ, Kim YS. Quality of life assessment of second-line docetaxel versus best supportive care in patients with non-small-cell lung cancer previously treated with platinum-based chemotherapy: results of a prospective, randomized phase III trial. Lung Cancer 2004; 43: 183–194.
12. 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: 1589–1597.
13. Kim ES, Hirsch V, Mok T et al. Gefitinib versus docetaxel in previously treated non-small-cell lung cancer: a randomised phase III trial (INTEREST). Lancet 2008; 372: 1809–1818.
14. Bezjak A, Tu D, Seymour L et al. Symptom improvement in lung cancer patients treated with erlotinib: quality of life analysis of the National Cancer Institute of Canada Clinical Trials Group Study BR.21. J Clin Oncol 2006; 24: 3831–3837.

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Priority Report

mTOR Signal and Hypoxia-Inducible Factor-1α Regulate CD133 Expression in Cancer Cells

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Abstract

The underlying mechanism regulating the expression of the cancer stem cell/tumor-initiating cell marker CD133/prominin-1 in cancer cells remains largely unclear, although knowledge of this mechanism would likely provide important biological information regarding cancer stem cells. Here, we found that the inhibition of mTOR signaling up-regulated CD133 expression at both the mRNA and protein levels in a CD133-overexpressing cancer cell line. This effect was canceled by a rapamycin-competitor, tacrolimus, and was not modified by conventional cytotoxic drugs. We hypothesized that hypoxia-inducible factor-1α (HIF-1α), a downstream molecule in the mTOR signaling pathway, might regulate CD133 expression; we therefore investigated the relation between CD133 and HIF-1α. Hypoxic conditions up-regulated HIF-1α expression and inversely down-regulated CD133 expression at both the mRNA and protein levels. Similarly, the HIF-1α activator deferoxamine mesylate dose-dependently down-regulated CD133 expression, consistent with the effects of hypoxic conditions. Finally, the correlations between CD133 and the expressions of HIF-1α and HIF-1β were examined using clinical gastric cancer samples. A strong inverse correlation ($r = -0.68$) was observed between CD133 and HIF-1α, but not between CD133 and HIF-1β. In conclusion, these results indicate that HIF-1α down-regulates CD133 expression and suggest that mTOR signaling is involved in the expression of CD133 in cancer cells. Our findings provide a novel insight into the regulatory mechanisms of CD133 expression via mTOR signaling and HIF-1α in cancer cells and might lead to insights into the involvement of the mTOR signal and oxygen-sensitive intracellular pathways in the maintenance of stemness in cancer stem cells. [Cancer Res 2009;69(18):7160–4]

Introduction

The CD133/prominin-1 protein is a five-transmembrane molecule expressed on the cell surface that is widely regarded as a stem cell marker. Growing evidence indicates that CD133 can be used as a cell marker for cancer stem cells or tumor-initiating cells in colon cancer, prostate cancer, pancreatic cancer, hepatocellular carcinoma, neural tumors, and renal cancer (1). Strict regulatory mechanisms governing CD133 expression are thought to be deeply related to inherent cancer stemness; however, such mechanisms remain largely unclear, especially in cancer cells. In brain tumors, the Hedgehog (2), bone morphogenetic protein (3), and Notch (4) signaling pathways have been implicated in the control of CD133+ cancer stem cell function.

Some investigators have shown a relation between hypoxia and CD133 expression in brain tissue. The percentage of CD133-expressing cells was found to increase in a glioma cell line cultured under hypoxic conditions (5), and mouse fetal cortical precursors cultured under normoxic conditions exhibited a reduction in CD133(hi)CD24(lo) multipotent precursors and the failure of the remaining CD133(hi)CD24(lo) cells to generate glia (6). With the exception of these studies in brain tissue, however, data on the expression of CD133 and the involvement of hypoxia and other signaling pathways in cancer cells remains limited.

Several reports have indicated that mTOR is a positive regulator of hypoxia-inducible factor (HIF) expression and activity (7), and the inhibition of HIF-mediated gene expression is considered to be related to the antitumor activity of mTOR inhibitors in renal cell carcinoma (8). We found that mTOR signaling was involved in CD133 expression in gastric and colorectal cancer cells. Thus, we investigated the regulatory mechanism of CD133 in cancer cells.

Materials and Methods

Reagents. 5-Fluorouracil, irinotecan (CPT-11), and rapamycin were purchased from Sigma-Aldrich. Gemcitabine was provided by Eli Lilly. Tacrolimus (LKT Laboratories), LY294002 and wortmannin (Cell Signaling Technology), and deferoxamine mesylate (DFO; Sigma-Aldrich) were purchased from the indicated companies.

Cell cultures and hypoxic conditions. All of the 28 cell lines used in this study were maintained in RPMI 1640 (Sigma) supplemented with 10% heat-inactivated fetal bovine serum (Life Technologies), except for LoVo (F12; Nissui Pharmaceutical), WiDr, IM95, and HEK293 (DMEM; Nissui Pharmaceutical), and Huvec (Humedia; Kurabo). Hypoxic conditions (0.1% O_2) were achieved using the AnaeroPouch-Anaero (Mitsubishi Gas Chemical) with monitoring using an oxygen indicator.

Real-time reverse transcription-PCR. The methods were previously described (9). The primers used for the real-time reverse transcription-PCR (RT-PCR) were as follows: CD133, forward 5′-AGT GGC ATC GTG CAA ACC TG-3′ and reverse 5′-CTC CGA ATC CAT TCG ACG ATA GTA-3′; glyceraldehyde-3-phosphate dehydrogenase (GAPD), forward 5′-GCA CCG TCA AGG CTG AGA AC-3′ and reverse 5′-ATG GTG GTG AAG ACG CCA GT-3′. GAPD was used to normalize the expression levels in the subsequent quantitative analyses.

Clinical samples. The mRNA expression levels of CD133, HIF-1α, and HIF-1β in gastric cancer specimens were obtained from previously published microarray data (9).

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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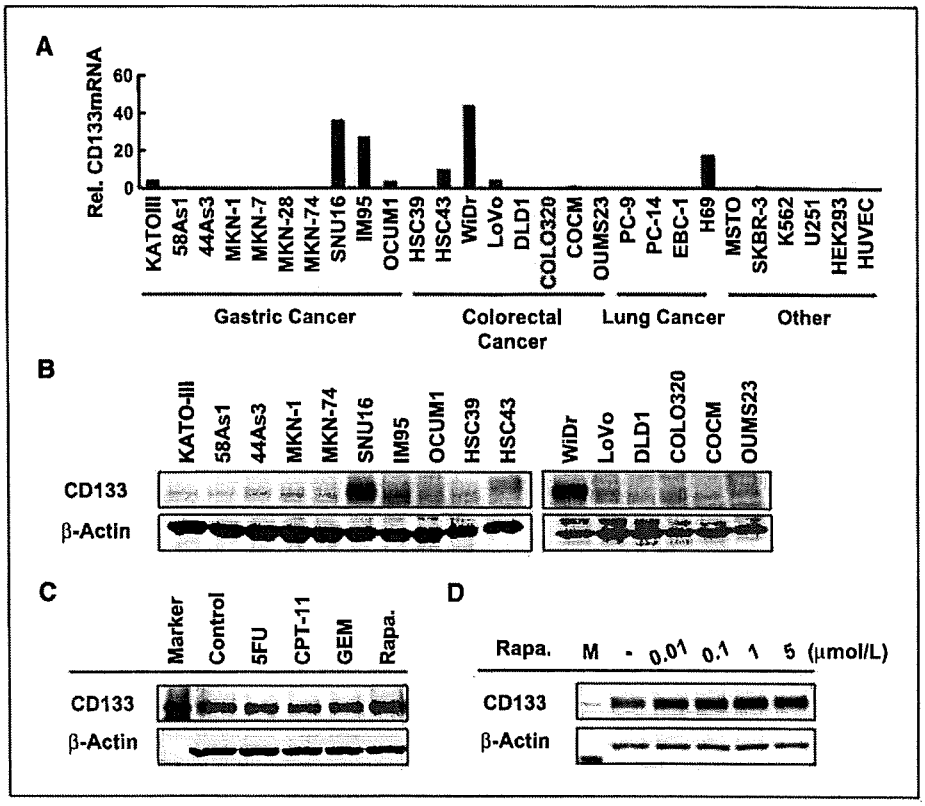

Figure 1. Rapamycin up-regulates CD133 expression. *A*, the mRNA expression levels of CD133 were examined using real-time RT-PCR in 26 cancer cell lines. *B*, the protein expressions of CD133 were determined using Western blotting in 16 gastric and colorectal cancer cell lines. *C*, Western blot of CD133 expression in WiDr cells exposed to cytotoxic drugs [1 μmol/L of 5-fluorouracil (*5-FU*), CPT-11, and gemcitabine (*GEM*)] and rapamycin (1 μmol/L) for 48 h. Note that only rapamycin up-regulates CD133 expression. *D*, WiDr cells were exposed to rapamycin at the indicated concentrations (0, 0.01, 0.1, 1, and 5 μmol/L) for 48 h. Rapamycin dose-dependently up-regulated CD133 expression. *Rel. CD133 mRNA*, normalized mRNA expression levels (CD133/GAPD × 10^4); *Rapa.*, rapamycin.

Immunoblotting. A Western blot analysis was performed as described previously (10). The experiment was performed in triplicate. The following antibodies were used: monoclonal CD133 antibody (W6B3C1; Miltenyi Biotec), rabbit polyclonal HIF-1α antibody (Novus Biologicals, Inc.), β-actin antibody, and HRP-conjugated secondary antibody (Cell Signaling Technology).

Results

Inhibition of the mTOR signal up-regulates CD133 expression in CD133-overexpressing gastrointestinal cancer cells. We examined the mRNA expression levels of CD133 in 26 cancer cell

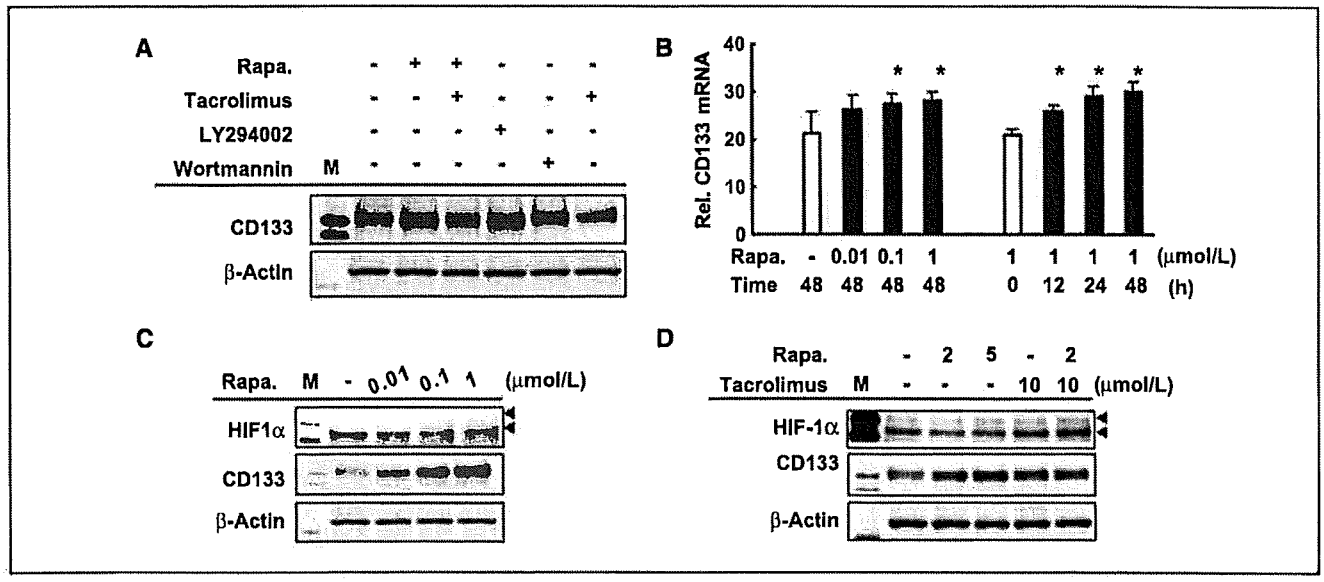

Figure 2. Rapamycin down-regulates HIF-1α expression and up-regulates CD133 expression at the transcriptional level. *A*, WiDr cells were exposed to rapamycin, the rapamycin-competitor tacrolimus, and the phosphoinositide-3-kinase inhibitors LY294002 and wortmannin for 48 h at concentrations of 10 μmol/L. The inhibition of mTOR signaling up-regulated CD133 expression. *B*, rapamycin up-regulated the expression of CD133 mRNA in WiDr cells in a time-dependent and dose-dependent manner. *Columns*, mean determined using real-time RT-PCR; *bars*, SD. *C* and *D*, rapamycin exposure and HIF-1α expression. WiDr cells were exposed to rapamycin with/without tacrolimus at the indicated concentration for 48 h. Rapamycin down-regulated HIF-1α expression and inversely up-regulated CD133 expression; these effects were canceled by tacrolimus. *Rel. CD133 mRNA*, normalized mRNA expression levels (CD133/GAPD × 10^4); *Rapa.*, rapamycin.

lines using real-time RT-PCR. Several gastric, colorectal, and lung cancer cell lines such as SNU16, IM95, HSC43, WiDr, and H69, overexpressed CD133 (Fig. 1*A*). The increased expression of CD133 protein was also confirmed in these cell lines (Fig. 1*B*). The mTOR inhibitor rapamycin, but not cytotoxic drugs (5-fluorouracil, CPT-11, and gemcitabine), increased the expression of CD133 in a dose-dependent manner in CD133-overexpressing WiDr cells (Fig. 1*C* and *D*). These results indicate that mTOR signaling is involved in the expression of CD133 in cancer cells.

Rapamycin down-regulated HIF-1α expression and up-regulated CD133 expression at the transcriptional level. To examine the signal transduction of rapamycin-induced CD133 expression, we used the rapamycin-competitor tacrolimus and the phosphoinositide-3-kinase inhibitors LY294002 and wortmannin. Tacrolimus (10 μmol/L) completely canceled the up-regulation of CD133 induced by rapamycin. The inhibition of phosphoinositide-3-kinase by LY294002 (10 μmol/L) and wortmannin (10 μmol/L) also up-regulated CD133 expression (Fig. 2*A*). Rapamycin up-regulated CD133 expression at the transcriptional level in a dose-dependent and time-dependent manner (Fig. 2*B*).

The inhibition of mTOR signaling is likely to lead to the down-regulation of the expression of certain molecules because the mTOR complex positively regulates the general translational machinery. Under the inhibition of mTOR signaling, HIF-1α, among several downstream molecules of mTOR, can activate transcription by acting as a repressor of specific transcription factors such as the MYC-associated protein X homodimer (11). Therefore, we focused on the possible role of HIF-1α in the regulation of CD133 expression. Rapamycin down-regulated HIF-1α expression but up-regulated CD133 expression (Fig. 2*C*). Meanwhile, tacrolimus canceled the effect of rapamycin on the expressions of HIF-1α and CD133 (Fig. 2*D*). These results suggest that the down-regulation of HIF-1α may mediate the up-regulation of CD133 expression in cancer cells. Up-regulation of CD133 expression by rapamycin was reproducibly observed in the CD133 high-expressing cell lines, but not in CD133 low-expressing cell lines (Supplemental Fig. S2).

Induction of HIF-1α down-regulates CD133 expression in cancer cells. Hypoxia mediates the stabilization of HIF-1α protein and enables its escape from rapid degradation, facilitating the up-regulation of HIF-1α expression (12). Hypoxia strongly induced HIF-1α expression, whereas CD133 expression was down-regulated in all three CD133-overexpressing cell lines (Fig. 3*A*). Rapamycin dose-dependently up-regulated CD133 expression under normoxic conditions, but no effect was seen under hypoxic conditions. We speculated that the effect of hypoxia on the induction of HIF-1α is much higher than the effect of rapamycin on the down-regulation of HIF-1α. The expression of CD133 mRNA was also strongly down-regulated under hypoxic conditions in all three cell lines (Fig. 3*B*) and in three additional cell lines (Supplemental Fig. S1).

In addition, DFO, a known HIF-1α activator, induced HIF-1α expression in a dose-dependent manner but down-regulated the expression of CD133 at both the mRNA and protein levels in WiDr cells (Fig. 3*C* and *D*), and in three additional cell lines (Supplemental Fig. S2). These results were consistent with those obtained under hypoxic conditions. Both hypoxia and DFO exposure markedly down-regulated CD133 expression, strongly suggesting that induction of HIF-1α results in the down-regulation of CD133 expression.

Inverse correlation between CD133 and HIF-1α in clinical samples. Finally, to address whether CD133 and HIF-1α expression are inversely correlated in clinical samples of gastric cancer

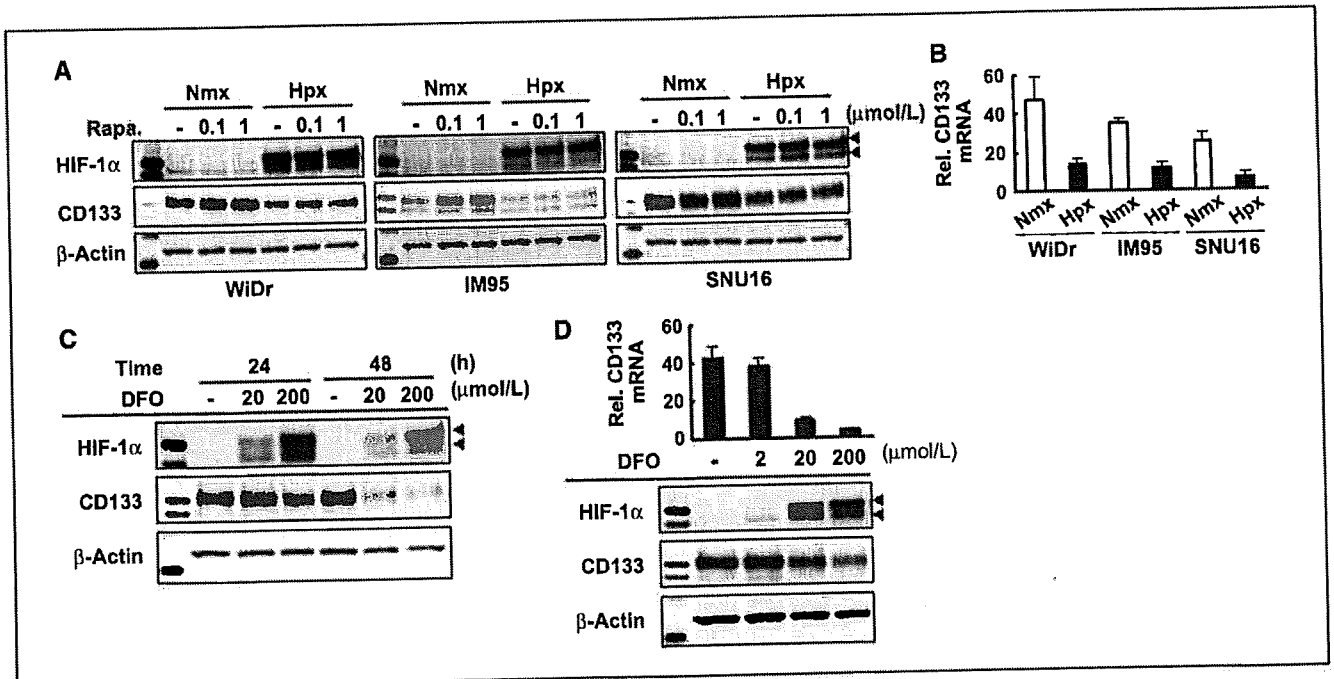

Figure 3. Induction of HIF-1α down-regulates CD133 expression in cancer cells. *A*, three gastrointestinal cancer cell lines were exposed to rapamycin under normoxic or hypoxic conditions for 24 h. Hypoxia induced HIF-1α expression and inversely down-regulated CD133 expression. *B*, hypoxia strongly down-regulated CD133 expression at the mRNA level. *Columns*, mean determined using real-time RT-PCR; *bars*, SD. *C*, DFO, a known HIF-1α activator, induced HIF-1α expression and down-regulated CD133 expression in WiDr cells. *D*, DFO induced these effects at both the mRNA and protein levels. Note that both hypoxia and DFO exposure had similar effects on HIF-1α induction and CD133 down-regulation. *Rel. CD133 mRNA*, normalized mRNA expression levels (CD133/GAPD × 10^4); *Rapa.*, rapamycin.

specimens, we examined the expression of these molecules using previously published microarray data (9). The expressions of CD133 and HIF-1α were inversely correlated in gastric cancer ($r = -0.68$; Fig. 4*A*), whereas the expressions of CD133 and HIF-1β were not ($r = -0.05$; Fig. 4*A*). These results are consistent with the *in vitro* findings in the present study.

Taken together, the present results suggest that an oxygen-sensitive intracellular pathway involving both HIF-1α and mTOR signaling may, at least in part, regulate CD133 expression in cancer cells (shown in the schema in Fig. 4*B*).

Discussion

Hypoxic conditions promote the proliferation of mammalian ES cells more efficiently than normoxia and are thought to be required for the maintenance of full pluripotency. Hematopoietic stem cells are located in the bone marrow, which is a physiologically hypoxic environment, and the survival and/or self-renewal of hematopoietic stem cells is enhanced *in vitro* if the cells are cultured under hypoxic conditions (13). Thus, accumulating data indicates that oxygen levels influence specific cell fates in several developmental processes; however, the effect of oxygen levels on cell differentiation is thought to be context-dependent (14). Our data on CD133 expression in response to hypoxia were different from the previous study shown in glioma (5). The discrepancy might be explained by (*a*) a different cellular context in glioma from the others, because CD133 expressions of all cell lines including the WiDr, IM95, SNU16, OCUM1, 44As3, and DLD-1 cells were reproducibly down-regulated by hypoxic condition (Supplemental Fig. S1; Fig. 3*B*), whereas the U251 cells failed to exhibit the down-regulation, and by (*b*) the different detection methods in our study (Western blot and quantitative real-time RT-PCR) from the previous report (flow cytometry for CD133-positive cells).

The detailed mechanism responsible for the repressive role of HIF-1α on CD133 expression is not fully understood; one possible explanation is raised by MYC, which is also known as c-Myc. HIF-1α binds to MAX and renders MYC inactive, and HIF-1 (homodimers of HIF-1α and HIF-1β) activates the expression of MXI1 (MAX interactor 1), which binds to MAX and thereby antagonizes MYC function (11). Recent reports have shown that HIF-1α inhibits MYC activity, which is thought to have implications for stem cell function (15, 16). Whether MYC directly activates CD133 transcription remains unclear; our preliminary data indicate that a MYC-inhibitor suppressed CD133 expression in WiDr cells.[4] Because the gene amplification of MYC and MYCN is frequently observed in many cancers, the relations among MYC, HIF-1α, HIF-1β, HIF-2, and CD133 should be investigated in future studies.

In conclusion, we showed that the inhibition of mTOR signaling up-regulated CD133 expression, whereas HIF-1α induction under hypoxic conditions or DFO exposure down-regulated CD133 expression in gastrointestinal cancer cells. Our findings show a novel regulatory mechanism for the expression of CD133 involving mTOR signaling and HIF-1α, and these findings may contribute to our understanding of the stemness character of cancer stem cells.

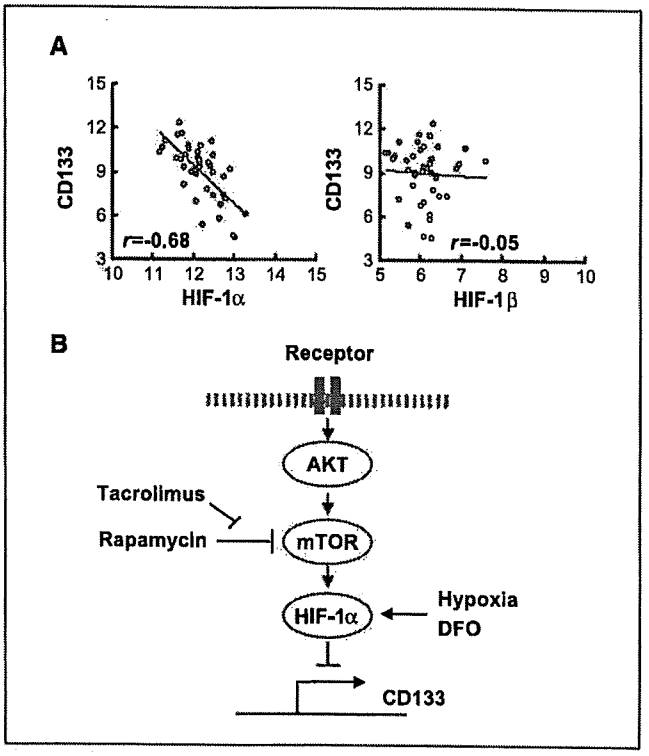

Figure 4. Inverse correlation between CD133 and HIF-1α in clinical samples of gastric cancer. *A*, the correlation between the expressions of CD133 and HIF-1α were analyzed in 40 clinical gastric cancer specimens using previously published microarray data. CD133 and HIF-1α were inversely correlated in gastric cancer ($r = -0.68$), whereas CD133 and HIF-1β were not ($r = -0.05$). *B*, proposed model depicting the involvement of mTOR signaling, HIF-1α, and CD133 expression. HIF-1α, a downstream molecule of mTOR, down-regulates CD133 expression at the transcriptional level in cancer cells.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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[4] Unpublished data.

References

1. Neuzil J, Stantic M, Zobalova R, et al. Tumour-initiating cells vs. cancer "stem" cells and CD133: what's in the name? Biochem Biophys Res Commun 2007;355:855–9.
2. Fan X, Matsui W, Khaki L, et al. Notch pathway inhibition depletes stem-like cells and blocks engraftment in embryonal brain tumors. Cancer Res 2006;66:7445–52.
3. Clement V, Sanchez P, de Tribolet N, Radovanovic I, Ruiz i Altaba A. HEDGEHOG-GLI1 signaling regulates human glioma growth, cancer stem cell self-renewal, and tumorigenicity. Curr Biol 2007;17:165–72.
4. Piccirillo SG, Reynolds BA, Zanetti N, et al. Bone morphogenetic proteins inhibit the tumorigenic potential of human brain tumour-initiating cells. Nature 2006;444:761–5.

5. Platet N, Liu SY, Atifi ME, et al. Influence of oxygen tension on CD133 phenotype in human glioma cell cultures. Cancer Lett 2007;258:286–90.
6. Chen HL, Pistollato F, Hoeppner DJ, Ni HT, McKay RD, Panchision DM. Oxygen tension regulates survival and fate of mouse central nervous system precursors at multiple levels. Stem Cells 2007;25:2291–301.
7. Hudson CC, Liu M, Chiang GG, et al. Regulation of hypoxia-inducible factor 1α expression and function by the mammalian target of rapamycin. Mol Cell Biol 2002; 22:7004–14.
8. Chiang GG, Abraham RT. Targeting the mTOR signaling network in cancer. Trends Mol Med 2007;13: 433–42.
9. Yamada Y, Arao T, Gotoda T, et al. Identification of prognostic biomarkers in gastric cancer using endoscopic biopsy samples. Cancer Sci 2008;99: 2193–9.
10. Takeda M, Arao T, Yokote H, et al. AZD2171 shows potent antitumor activity against gastric cancer over-expressing FGFR2/KGFR. Clin Cancer Res 2007;13: 3051–7.
11. Dang CV, Kim JW, Gao P, Yustein J. The interplay between MYC and HIF in cancer. Nat Rev Cancer 2008;8: 51–6.
12. Wouters BG, Koritzinsky M. Hypoxia signalling through mTOR and the unfolded protein response in cancer. Nat Rev Cancer 2008;8:851–64.
13. Danet GH, Pan Y, Luongo JL, Bonnet DA, Simon MC. Expansion of human SCID-repopulating cells under hypoxic conditions. J Clin Invest 2003;112:126–35.
14. Simon MC, Keith B. The role of oxygen availability in embryonic development and stem cell function. Nat Rev Mol Cell Biol 2008;9:285–96.
15. Koshiji M, Kageyama Y, Pete EA, Horikawa I, Barrett JC, Huang LE. HIF-1α induces cell cycle arrest by functionally counteracting Myc. EMBO J 2004;23: 1949–56.
16. Zhang H, Gao P, Fukuda R, et al. HIF-1 inhibits mitochondrial biogenesis and cellular respiration in VHL-deficient renal cell carcinoma by repression of C-MYC activity. Cancer Cell 2007;11:407–20.

Efficacy Differences of Pemetrexed by Histology in Pretreated Patients with Stage IIIB/IV Non-small Cell Lung Cancer

Review of Results from an Open-Label Randomized Phase II Study

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Introduction: Recent pivotal phase III studies in patients with advanced non-small cell lung cancer (NSCLC) consistently showed greater survival benefit of pemetrexed in patients with nonsquamous cell carcinoma histology (nonsquamous histology) compared with those with squamous cell carcinoma histology (squamous histology). To confirm the efficacy differences of pemetrexed by histologic type, we conducted an additional subgroup analysis of data from a Japanese randomized phase II study evaluating the efficacy and safety of pemetrexed 500 mg/m^2 (P500) and 1000 mg/m^2 (P1000) in patients with advanced NSCLC previously treated with chemotherapy. The efficacy and safety results of original phase II study have already been reported (Ohe et al., *Clin Cancer Res* 2008;14:4206–4212).

Methods: Objective response rates (ORRs), overall survival time, and progression-free survival time were analyzed by subgroup of histology, squamous, and nonsquamous, for the dose groups combined and separately.

Results: A total of 216 patients were evaluable for efficacy. One hundred sixty-eight patients had nonsquamous and 48 had squamous histology. ORRs were 20.8% and 2.1% ($p < 0.001$); median survival times (MST) were 16.0 and 8.5 months ($p < 0.001$); and median progression-free survival times (PFS) were 3.1 and 1.6 months ($p < 0.001$) for nonsquamous and squamous histology, respectively. In patients who were randomized to the P500 group, ORR were 23.5% and 0% ($p = 0.0062$); MST were 19.4 and 7.9 months ($p < 0.001$); and PFS were 3.1 and 1.4 months ($p < 0.001$) for nonsquamous and squamous histology, respectively. In patients who were randomized to the P1000 group, ORR were 18.1% and 4.0% ($p = 0.1113$); MST were 13.5 months and 8.6 months ($p = 0.0971$); and PFS were 3.1 and 1.7 months ($p = 0.0024$) for nonsquamous and squamous histology, respectively. There were no clinically relevant differences in the incidence of toxicities between histology groups.

Conclusions: This study showed the difference of pemetrexed efficacy by histologic type, and this result supports the treatment-by-histology effect observed in the past pivotal phase III studies. Higher dose of pemetrexed resulted in similar outcomes both in patients with nonsquamous histology and squamous histology. Pemetrexed is not as effective as alternative therapies for previously treated squamous histology; however, pemetrexed should be the key agent for the treatment of patients with nonsquamous histology.

Key Words: Pemetrexed, Non-small cell lung cancer, Nonsquamous, Squamous, Histology.

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Two-drug combinations of the third-generation agents (docetaxel, paclitaxel, gemcitabine, and vinorelbine) with a platinum compound have been considered the standard treatment option for advanced non-small cell lung cancer (NSCLC) based on several randomized studies.[1–3] Histology has not been consistently reported as prognostic or predictive for outcomes with cytotoxic cancer chemotherapy in advanced NSCLC, until publication of a large phase III study using cisplatin and pemetrexed.[4]

Pemetrexed is an inhibitor of thymidylate synthase, resulting in decreased thymidine necessary for pyrimidine synthesis, which is the primary mechanism of action.[5,6] Pemetrexed also inhibits dihydrofolate reductase and glycinamide ribonucleotide formyl transferase, the latter of which is a folate-dependent enzyme involved in purine synthesis. Unlike other classic antifolates, pemetrexed has a unique pyrrolopyrimidine nucleus and can inhibit multiple folate-dependent enzymes.

The phase III study comparing cisplatin plus gemcitabine with cisplatin plus pemetrexed in chemotherapy-naive patients with advanced NSCLC demonstrated noninferiority of cisplatin plus pemetrexed to cisplatin plus gemcitabine in the overall study population, with significantly less febrile neutropenia, anemia, thrombocytopenia, and alopecia favor-

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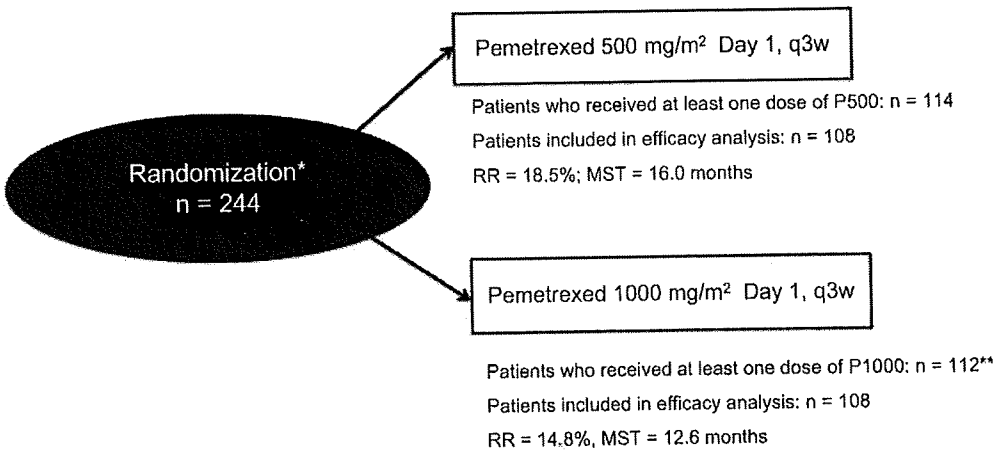

FIGURE 1. Trial design and efficacy data. From phase II randomized study.[8] NSCLC, non-small cell lung cancer; ECOG, Eastern Cooperative Oncology Group; PS, performance status; q, every; w, weekly; *n*, number of patients; RR, response rate; MST, median survival time. *Patients: stage IIIB/IV NSCLC, 1 to 2 prior chemotherapeutic regimens, and ECOG PS 0 to 2; Stratified by: gender, ECOG PS, disease stage, platinum use, time for prechemotherapy, and study site. **One patient was excluded from statistical analysis because the data of this patient was not available.

ing cisplatin plus pemetrexed.[4] This study showed that overall survival was statistically superior for cisplatin plus pemetrexed in patients with nonsquamous histology. In contrast, survival was shorter for cisplatin plus pemetrexed compared with cisplatin plus gemcitabine in patients with squamous cell carcinoma. This was the first phase III study in NSCLC that prospectively demonstrated survival differences for chemotherapy based on histologic type.

In the subgroup analysis of the phase III study, which compared pemetrexed alone with docetaxel in patients with NSCLC previously treated with chemotherapy, also demonstrated that overall survival was significantly longer for pemetrexed versus docetaxel in patients with nonsquamous histology, whereas conversely, survival was shorter for pemetrexed compared with docetaxel in patients with squamous histology.[7]

On the basis of these phase III results, we conducted an additional subgroup analysis of data from a Japanese phase II study, which randomized previously treated patients with NSCLC to pemetrexed 500 mg/m^2 (P500) or 1000 mg/m^2 (P1000) to further examine efficacy differences for pemetrexed by histology. The efficacy and safety results of original phase II study have already been reported[8]; Figure 1 shows the trial design and efficacy data of this phase II study. Of the 216 patients evaluable for efficacy (108 in each arm), response rates were18.5% (90% confidence interval, 12.6–25.8%) and 14.8% (90% confidence interval, 9.5–21.6%), median survival times (MSTs) 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. Drug-related toxicity was generally tolerable for both doses.

PATIENTS AND METHODS

Trial Design

We analyzed the data from the randomized, open-label, multicenter study[8] in which patients were registered through the central registration system. Two hundred forty-four patients with advanced NSCLC previously treated with chemotherapy at 28 medical institutions in Japan were registered between October 2004 and October 2005, and 226 patients were randomized to receive either pemetrexed 500 mg/m^2 (P500) or 1000 mg/m^2 (P1000) (Figure 1). The randomization was done by an independent registration center and was dynamically balanced for Eastern Cooperative Oncology Group performance status (PS), previous platinum chemotherapy, disease stage, gender, a 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. The primary end point was response rate, and the secondary end points included overall survival time, progression-free survival time, and incidence of toxicities.

The sample size was calculated to ensure that the response rate in each group exceeded 5%.[8] The study was conducted in accordance with the ethical principles stated in the Declaration of Helsinki after being approved by the institutional review board of individual hospitals. Primary results of this trial and further details regarding the study design and statistical analyses have been published previously.[8]

Patients and Treatment

Patients who satisfied all of the following criteria were included into the study[8]: age 20 to 75 years, performance status 0 to 2, stage III or IV diagnosed by images before the registration to this study, NSCLC confirmed by histology or cytology, at least one measurable tumor according to the Response Evaluation Criteria in Solid Tumor (RECIST criteria),[9] previously received one or two chemotherapy regimens for NSCLC, adequate organ function, life expectancy of at least 12 weeks, and written consent to participate in the study. Histologic subtypes outcome of NSCLC were examined in each institution.

Patients were randomly allocated to either pemetrexed 500 mg/m^2 (P500) arm or pemetrexed 1000 mg/m^2 (P1000) arm. Pemetrexed was administered as an intravenous, 10-minute 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 at least 7 days before the day 1 of cycle 1 until 22 days after the last administration of pemetrexed. Vitamin B_{12} (1000 μg) was injected intramuscularly, at least 7 days before the day 1 of cycle 1 and repeated every 9 weeks until 22 days after the last administration of pemetrexed.

Assessments

The antitumor effect of pemetrexed was evaluated based on the RECIST criteria. Response rate represented the percentage of patients whose best overall response had been either complete response or partial response. Survival time was defined as the period from the registered date of first administration until the date of death regardless of the causality with pemetrexed. Progression-free survival time was defined as the period from the registered date of first administration until the day on which progressive disease was determined or the date of death regardless of the causality with pemetrexed. All adverse events were graded based on the Common Terminology Criteria for Adverse Events, version 3.0.

Statistical Analysis

Of 226 patients enrolled in the study, the efficacy analysis included 216 patients who satisfied all the inclusion criteria, did not meet any of the exclusion criteria, and received at least one dose of pemetrexed. The safety analysis included 225 patients who received at least one dose of pemetrexed.

Efficacy and safety results were analyzed by histology for the dose groups combined and separately. Response rates, disease control rates, overall survival time, and progression-free survival time were compared between the histologic types (nonsquamous and squamous histology) for the P500 and P1000 arms combined and separately. Differences of response rates were compared by using Fisher's exact test. A Kaplan-Meier method was used to estimate overall survival time and progression-free survival time. Differences of time-to-event distributions by histology were compared using a log-rank test. A Cox proportional hazard model was used for hazard ratio estimation (squamous/nonsquamous histology). Two-sided significance level of 5% was used in all tests. In the safety analysis, number of deaths, serious adverse events,

TABLE 1. Characteristics of Patients

	Nonsquamous			Squamous		
Variable	**P500**	**P1000**	**Total**	**P500**	**P1000**	**Total**
Patients who received at least 1 dose of pemetrexed, *n*	89	85	174	25	26	51
Gender, *n* (%)						
Female	40 (44.9)	36 (42.4)	76 (43.7)	2 (8.0)	4 (15.4)	6 (11.8)
Male	49 (55.1)	49 (57.6)	98 (56.3)	23 (92.0)	22 (84.6)	45 (88.2)
Age (yr)						
Median	60	62	61	67	64	65
Range	37–74	26–74	26–74	58–74	50–74	50–74
ECOG PS, *n* (%)						
0	34 (38.2)	29 (34.1)	63 (36.2)	11 (44.0)	8 (30.8)	19 (37.3)
1	50 (56.2)	51 (60.0)	101 (58.0)	13 (52.0)	17 (65.4)	30 (58.8)
2	5 (5.6)	5 (5.9)	10 (5.7)	1 (4.0)	1 (3.8)	2 (3.9)
Disease stage, *n* (%)						
III	15 (16.9)	16 (18.8)	31 (17.8)	7 (28.0)	8 (30.8)	15 (29.4)
IV	74 (83.1)	69 (81.2)	143 (82.2)	18 (72.0)	18 (69.2)	36 (70.6)
No. of prior chemotherapy, *n* (%)						
1	32 (36.0)	39 (45.9)	71 (40.8)	12 (48.0)	14 (53.8)	26 (51.0)
2	54 (60.7)	45 (52.9)	99 (56.9)	13 (52.0)	12 (46.2)	25 (49.0)
3	3 (3.4)	1 (1.2)	4 (2.3)	0 (0.0)	0 (0.0)	0 (0.0)
Prior platinum, *n* (%)						
No	4 (4.5)	6 (7.1)	10 (5.7)	2 (8.0)	1 (3.8)	3 (5.9)
Yes	85 (95.5)	79 (92.9)	164 (94.3)	23 (92.0)	25 (96.2)	48 (94.1)
Interval from last prior chemotherapy, *n* (%)						
≥3 mo	31 (34.8)	34 (40.0)	65 (37.4)	11 (44.0)	11 (42.3)	22 (43.1)
<3 mo	58 (65.2)	51 (60.0)	109 (62.6)	14 (56.0)	15 (57.7)	29 (56.9)

n, number of patients; P500, pemetrexed 500 mg/m^2 arm; P1000, pemetrexed 1000 mg/m^2 arm; ECOG PS, Eastern Cooperative Oncology Group performance status.

grade 2 adverse events, and grade 3/4/5 adverse events were calculated separately for nonsquamous and squamous histology in each dose group.

RESULTS

Patient Characteristics

Patient characteristics are shown by histology and dose group (P500 or P1000) in Table 1. Total of 225 patients received pemetrexed 500 mg/m^2 or 1000 mg/m^2 at least once during the study. Baseline patient characteristics by histology were well balanced between the two dose groups.

TABLE 2. Summary of Efficacy Results by Histology

Variable	Nonsquamous (n = 168)	Squamous (n = 48)	p
Response rate (%)	20.8	2.1	<0.001[a]
Disease control rate (%)	57.1	29.2	<0.001[a]
Overall survival (median) (mo)	16.0	8.5	<0.001[b]
Progression-free survival (median) (mo)	3.1	1.6	<0.001[b]

[a] Fisher's exact test.
[b] Log-rank test.

Efficacy

Results of the efficacy analysis (response rate, disease control rate, overall survival time, and progression-free survival time) by histology for the dose groups combined are summarized in Table 2. Kaplan-Meier curves for overall survival time and progression-free survival time are shown in Figures 2*A*, *B*, respectively. Response rates in patients with nonsquamous and squamous histology were 20.8% (35/168) and 2.1% (1/48) ($p < 0.001$), and disease control rates in patients with nonsquamous and squamous histology were 57.1% (96/168) and 29.2% (14/48) ($p < 0.001$), respectively. MSTs in patients with nonsquamous and squamous histology were 16.0 and 8.5 months (hazard ratio, 2.11; log-rank test, $p < 0.001$), and median progression-free survival times were 3.1 and 1.6 months (hazard ratio, 2.19; log-rank test, $p < 0.001$), respectively.

Results of the efficacy analysis (response rate, overall survival time, and progression-free survival time) by histology for each dose group are summarized in Table 3. Kaplan-Meier curves for overall survival time and progression-free

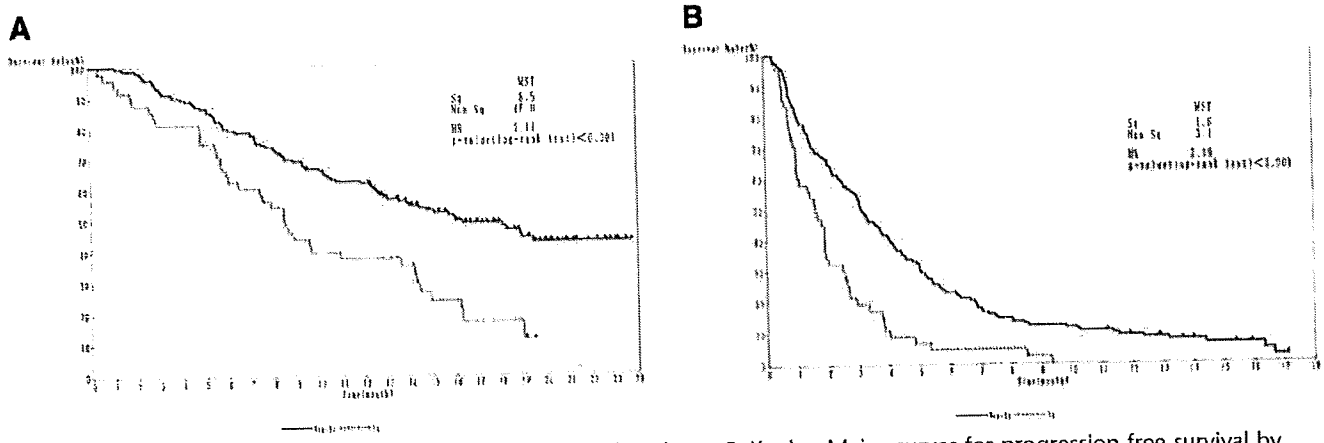

FIGURE 2. *A*, Kaplan-Meier curves for overall survival by histology. *B*, Kaplan-Meier curves for progression-free survival by histology. MST, median survival time.

TABLE 3. Summary for Efficacy Results by Dose and Histology

	P500			P1000		
	Nonsquamous (n = 85)	Squamous (n = 23)	p	Nonsquamous (n = 83)	Squamous (n = 25)	p
Response rate (%)	23.5	0.0	0.0062[a]	18.1	4.0	0.1113[a]
Disease control rate (%)	62.4	30.4	0.0088[a]	51.8	28.0	0.0419[a]
Overall survival (median) (mo)	19.4[b]	7.9	<0.001[c]	13.5	8.6	0.0971[c]
Progression-free survival (median) (mo)	3.1	1.4	<0.001[c]	3.1	1.7	0.0024[c]

Adjustment of multiplicity was not performed.
[a] Fisher's exact test.
[b] Survival rate was 50.03%.
[c] Log-rank test.
P500, pemetrexed 500 mg/m^2 arm; P1000, pemetrexed 1000 mg/m^2 arm.

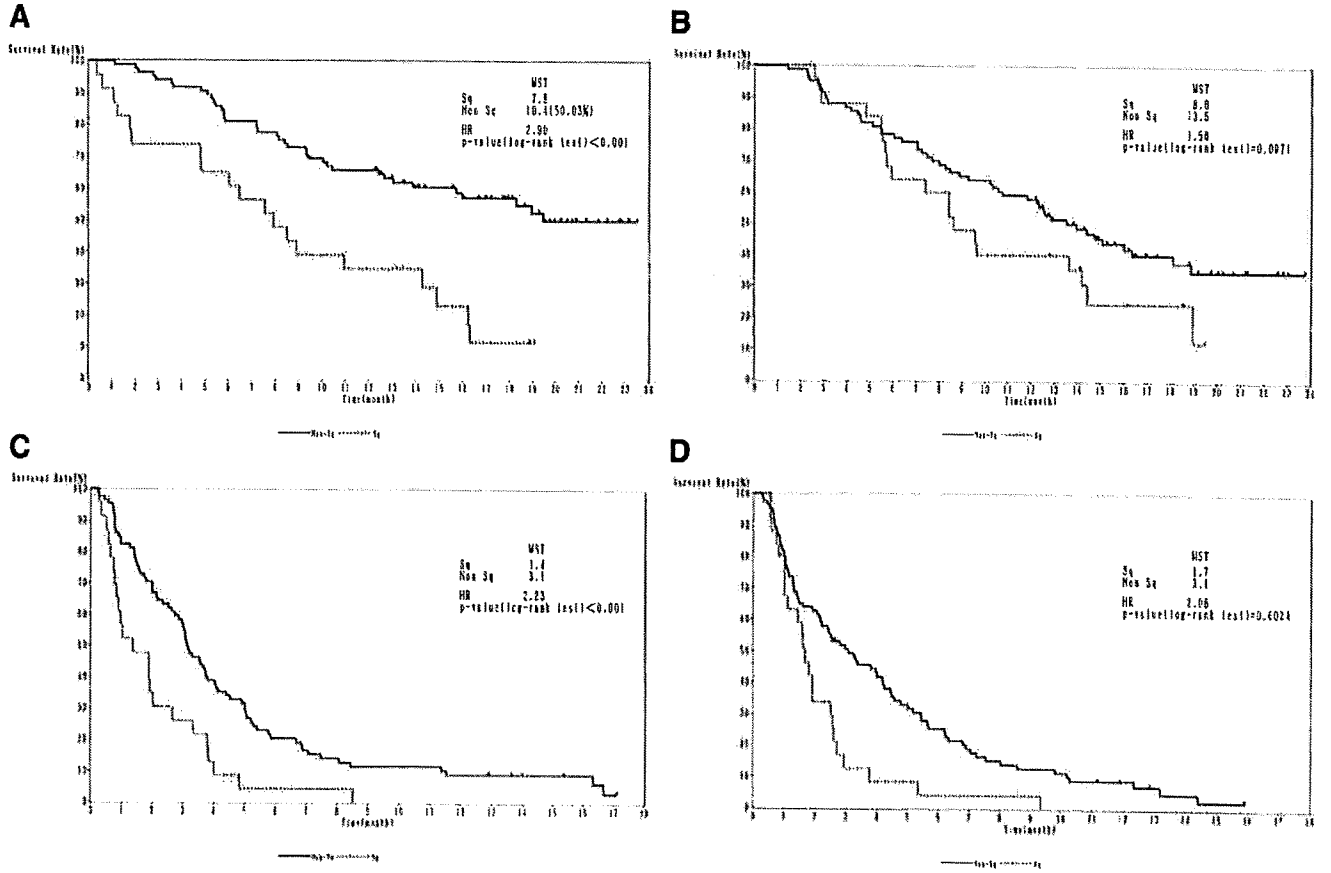

FIGURE 3. Kaplan-Meier curves for overall survival by dose and histology: (*A*) patients treated with pemetrexed 500 mg/m^2 and (*B*) patients treated with pemetrexed 1000 mg/m^2. Kaplan-Meier curves for progression-free survival by dose and histology: (*C*) patients treated with pemetrexed 500 mg/m^2 and (*D*) patients treated with pemetrexed 1000 mg/m^2. MST, median survival time.

survival time are shown in Figures 3*A–D*. Response rates of nonsquamous and squamous histology patients were 23.5% (20/85) and 0% (0/23) in P500 (p = 0.0062) and 18.1% (15/83) and 4.0% (1/25) in P1000 (p = 0.1113). Disease control rates of nonsquamous and squamous histology patients were 62.4% (53/85) and 30.4% (7/23) in P500 (p = 0.0088) and 51.8% (43/83) and 28.0% (7/25) in P1000 (p = 0.0419). In the P500 group, median overall survival time was 19.4 months in patients with nonsquamous histology (survival rate: 50.03%) and 7.9 months in patients with squamous histology patients (incidence of events: 50.00%) (hazard ratio, 2.90; log-rank test, $p < 0.001$). In the P1000 group, median overall survival time was 13.5 months in patients with nonsquamous histology and 8.6 months in patients with squamous histology (hazard ratio, 1.56; log-rank test, p = 0.0971). Median progression-free survival time was 3.1 months in patients with nonsquamous histology and 1.4 months in patients with squamous histology in the P500 group (hazard ratio, 2.23; log-rank test, $p < 0.001$). In the P1000 group, median progression-free survival time was 3.1 months in patients with nonsquamous histology and 1.7 months in patients with squamous histology (hazard ratio, 2.06; log-rank test, p = 0.0024).

Safety

The safety of pemetrexed 500 mg/m^2 and 1000 mg/m^2 has been reported by Ohe et al.[8] Major adverse events occurred in the study participants are shown by dose group (P500 and P1000) and histology in Table 4. Grade 3/4/5 pneumonitis regardless to causality with pemetrexed was observed in two nonsquamous and two squamous histology patients in the P500 group and one nonsquamous and two squamous histology patients in the P1000 group. Toxicities occurred in both dose groups were tolerable, and there were no clinically relevant differences in the incidence of toxicities by histology.

DISCUSSION

The results of subgroup analysis demonstrated efficacy differences of pemetrexed by histology in pretreated patients with advanced NSCLC. Objective response rate of pemetrexed was 20.8% in patients with nonsquamous histology and only 2.1% in squamous histology patients. Overall survival and progression-free survival were significantly better for patients with nonsquamous than squamous histology. MST of 16.0 months in nonsquamous histology patients is

TABLE 4. Major Hematologic and Nonhematologic Toxicity by Common Terminology Criteria for Adverse Events Version 3.0[a]

	P500				P1000			
	Nonsquamous (n = 89)		Squamous (n = 25)		Nonsquamous (n = 85)		Squamous (n = 26)	
	Grade 2	Grade 3/4/5	Grade 2	Grade 3/4/5	Grade 2	Grade 3/4/5	Grade 2	Grade 3/4/5
Leukopenia	36.0	13.5	20.0	20.0	40.0	27.1	34.6	3.8
Neutropenia	28.1	21.3	16.0	20.0	29.4	28.2	23.1	11.5
Lymphopenia	31.5	6.7	24.0	40.0	25.9	25.9	34.6	7.7
Anemia	19.1	5.6	28.0	20.0	40.0	8.2	19.2	15.4
Thrombocytopenia	0	0	0	0	8.2	0	7.7	3.8
Nausea	16.9	1.1	16.0	4.0	17.6	3.5	7.7	3.8
Vomiting	9.0	1.1	8.0	0	10.6	3.5	15.4	0
Anorexia	19.1	2.2	8.0	12.0	15.3	14.1	11.5	19.2
Fatigue	2.2	1.1	8.0	0	4.7	1.2	3.8	11.5
Diarrhea	3.4	1.1	0	0	3.5	2.4	3.8	0
Constipation	9.0	1.1	0	0	5.9	3.5	7.7	3.8
Rash	51.7	3.4	44.0	0	63.5	5.9	61.5	0
Alopecia	0	0	0	0	0	0	0	0
Pneumonitis	1.1	2.2	4.0	8.0	0	1.2	0	7.7
AST	27.0	6.7	4.0	12.0	29.4	5.9	11.5	0
ALT	20.2	19.1	8.0	12.0	35.3	9.4	23.1	3.8

The values are given in percentage.
[a] Major adverse events of grade 2 or grade 3/4/5 are shown irrespective of causal relationship with pemetrexed.
P500, pemetrexed 500 mg·m^2 arm; P1000, pemetrexed 1000 mg/m^2 arm; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

encouraging in this situation. The efficacy of pemetrexed for nonsquamous histology was shown in the recommended dose of 500 mg/m^2 and also in the higher dose of 1000 mg/m^2. Higher dose of pemetrexed resulted in similar outcomes both in patients with nonsquamous histology and squamous histology.

The difference in survival benefit of pemetrexed between the histologic types may in part be explained by a differential expression of thymidylate synthase, which is the primary mechanism of actions. In specimens from chemonaive patients with early-stage NSCLC, expression of thymidylate synthase was observed to be elevated in squamous histology compared with adenocarcinoma.[10] A preclinical study showed the overexpression of thymidylate synthase was associated with the decreased in vitro sensitivity of pemetrexed.[11] Translational studies are needed to evaluate biologic markers using clinical samples.

Pemetrexed was well tolerated in both the P500 and P1000 arms,[8] and also there were no clinically relevant differences in the toxicities between histologic groups. This is in contrast to vascular endothelial growth factor inhibitors, e.g., bevacizumab, which have an increased risk of life-threatening toxicities in patients with certain squamous cell lung tumors.

A randomized phase III trial designed to evaluate maintenance chemotherapy of pemetrexed versus placebo after platinum-based chemotherapy demonstrated that progression-free and overall survival were significantly longer with pemetrexed in patients with nonsquamous histology, whereas no treatment advantage was observed in patients with squamous histology.[12,13] This is the third phase III study to demonstrate efficacy differences by histology in the treatment of advanced NSCLC.

It has been regarded that two drug combinations of platinum agents with third generation agents have similar efficacy.[14,15] Gemcitabine-containing regimens showed significant longer progression-free survival than nongemcitabine-containing regimens in a meta-analysis.[16] Thus, cispaltin plus gemcitabine is one of the most active regimens for NSCLC. However, the randomized trial comparing cisplatin plus pemetrexed with cisplatin plus gemcitabine demonstrated statistically significant survival benefit favoring cisplatin plus pemetrexed in patients with nonsquamous histology. Considering the consistent results of other studies[4,17] using pemetrexed and favorable toxicity profile, cisplatin plus pemetrexed should be a reference regimen in future trials for patients with nonsquamous histology.

In conclusion, the results of subgroup analysis showed the difference of pemetrexed efficacy by histologic type, and this result supports the treatment-by-histology effect observed in the past pivotal phase III studies. Higher dose of pemetrexed resulted in similar outcomes both in patients with nonsquamous histology and squamous histology. Pemetrexed is not as effective as alternative therapies for previously treated squamous histology, however, pemetrexed should be the key agent for the treatment of patients with nonsquamous histology.

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REFERENCES

1. 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* 1995;311: 899–909.
2. 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;346:92–98.
3. 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 versus vinorelbine for advanced non-small-cell lung cancer: Four-Arm Cooperative Study in Japan. *Ann Oncol* 2006;18:317–323.
4. Scagliotti GV, Parikh P, von Pawel J, et al. Phase III study comparing cisplatin plus gemcitabine with cisplatin plus pemetrexed in chemotherapy-naive patients with advanced-stage non-small-cell lung cancer. *J Clin Oncol* 2008;26:3543–3551.
5. Shih C, Chen VJ, Gossett LS, et al. LY231514, a pyrrolo[2,3-d]pyrimidine-based antifolate that inhibits multiple folate-requiring enzymes. *Cancer Res* 1997;57:1116–1123.
6. Shih C, Habeck LL, Mendelsohn LG, Chen VJ, Schultz RM. Multiple folate enzyme inhibition: mechanism of a novel pyrrolopyrimidine-based antifolate LY231514 (MTA). *Adv Enzyme Regul* 1998;38:135–152.
7. Peterson P, Park K, Fossella F, et al. Is pemetrexed more effective in patients with non-squamous histology? A retrospective analysis of a phase III trial of pemetrexed vs docetaxel in previously treated patients with advanced non-small cell lung cancer (NSCLC) [abstract]. *Eur J Cancer* 2007;5(suppl):363–364.
8. Ohe Y, Ichinose Y, Nakagawa K, et al. Efficacy and safety of two doses of pemetrexed supplemented with folic acid and vitamin B12 in previously treated patients with non-small cell lung cancer. *Clin Cancer Res* 2008;14:4206–4212.
9. 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;92:205–216.
10. 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;107:1589–1596.
11. Sigmond J, Backus HH, Wouters D, Temmink OH, Jansen G, Peters GJ. Induction of resistance to the multitargeted antifolate pemetrexed (ALIMTA) in WiDr human colon cancer cells is associated with thymidylate synthase overexpression. *Biochem Pharmacol* 2003;6:431–438.
12. Ciuleanu TE, Brodowicz T, Belani CP, et al. Maintenance pemetrexed plus best supportive care (BSC) versus placebo plus BSC: a phase III study [abstract]. *J Clin Oncol* 2008;26:(May 20 suppl; abst 8011).
13. Belani CP, Brodowicz T, Ciuleanu T, et al. Maintenance pemetrexed (Pem) plus best supportive care (BSC) versus placebo (Plac) plus BSC: a randomized phase III study in advanced non-small cell lung cancer (NSCLC) [abstract]. *J Clin Oncol* 2009;27:18s (abst CRA8000).
14. Ardizzoni A, Boni L, Tiseo M, et al. Cisplatin- versus Carboplatin-based chemotherapy in first-line treatment of advanced non-small-cell lung cancer: an individual patient data meta-analysis. *J Natl Cancer Inst* 2007;99:847–857.
15. Douillard JY, Laporte S, Fossella F, et al. Comparison of Docetaxel- and Vinca alkaloid-based chemotherapy in the first-line treatment of advanced non-small cell lung cancer: a meta-analysis of seven randomized clinial trials. *J Thoracic Oncol* 2007;2:939–946.
16. Le Chevalier, Scagliotti G, Natale R, et al. Efficacy of gemcitabine plus platinum chemotherapy compared with other platinum containing regimens in advanced non-small-cell lung cancer: a meta-analysis of survival outcomes. *Lung Cancer* 2005;47:69–80.
17. 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;22:1589–1597.

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ORIGINAL ARTICLE

A dose-finding and pharmacokinetic study of nedaplatin in elderly patients with advanced non-small cell lung cancer

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Abstract
Purpose Nedaplatin is a second-generation platinum showing favorable activity against non-small cell lung cancer (NSCLC). Dose-limiting toxicity (DLT) is thrombocytopenia, predicted by creatinine clearance (Ccr). This study was conducted to determine the recommended dose, and evaluate the toxicities, pharmacokinetics and efficacy for elderly NSCLC patients.
Methods Patients ≥70 years were stratified into two groups based on renal functions: Group A, Ccr ≥ 60 and Group B, 40 ≤ Ccr < 60. The initial doses were 80 and 60 mg/m^2 in Groups A and B, respectively. The doses were escalated in 20-mg/m^2 increments to 100 mg/m^2 until DLT.
Results Chemotherapy-naïve 39 elderly patients (Group A/Group B: 22/17) received a total of 83 cycles. Major toxicities were hematological. In Group A, one of the 15 patients at 100 mg/m^2 experienced DLT (neutropenia) and the recommended dose was determined at 100 mg/m^2. In Group B, three of the five patients had DLTs (leukopenia, neutropenia, thrombocytopenia and febrile neutropenia) at 100 mg/m^2, and the recommended dose was determined at 80 mg/m^2. The percentage decreases of neutrophil were well correlated with total and free-Pt AUCs. Partial responses were observed in 13 (33%) of the 39 patients, and 12 of the 13 patients who responded had a squamous cell carcinoma.
Conclusions Nedaplatin was administered simply and feasibly by stratifying renal function and exerted favorable antitumor activity for elderly patients with NSCLC, especially on squamous cell carcinoma.

Keywords Nedaplatin · Dose-finding study · Pharmacokinetics · NSCLC · Elderly patient

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Introduction

The proportion of elderly patients with non-small cell lung cancer (NSCLC) is increasing [1]. At present, the first-line standard chemotherapy for non-elderly patients with advanced NSCLC is a platinum-based doublet regimen. The efficacy and feasibility of this strategy have been demonstrated in several randomized trials in patients with a good performance status and aged ≤70 years [2–4]. However, platinum-based doublet regimens are not always feasible for elderly patients. Age-related comorbidity and physiologic changes increase inter-individual pharmacokinetic variability, possibly leading to unacceptable severe toxicities. In particular, application of a cisplatin-based regimen to elderly patients is substantially restricted because of the risk of emesis, neurotoxicity and nephrotoxicity.