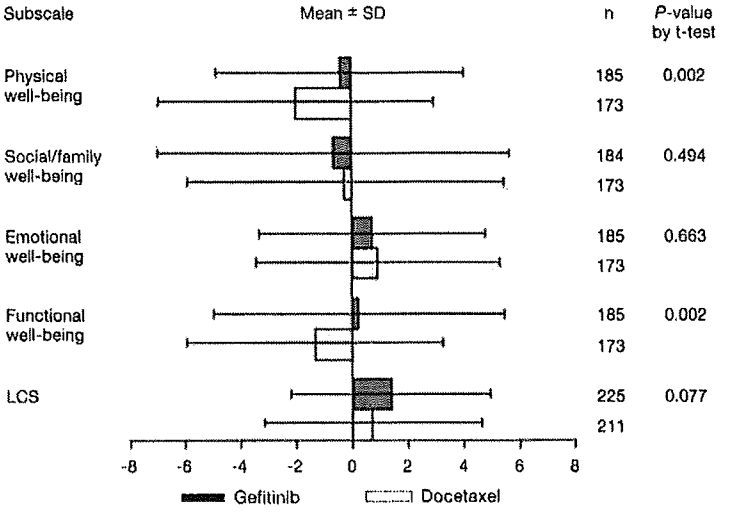

Figure 3. Mean change of mean score from baseline for Functional Assessment of Cancer Therapy-Lung subscales (assessable population).

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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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/).

K. Matsumoto and T. Arao contributed equally to this work.

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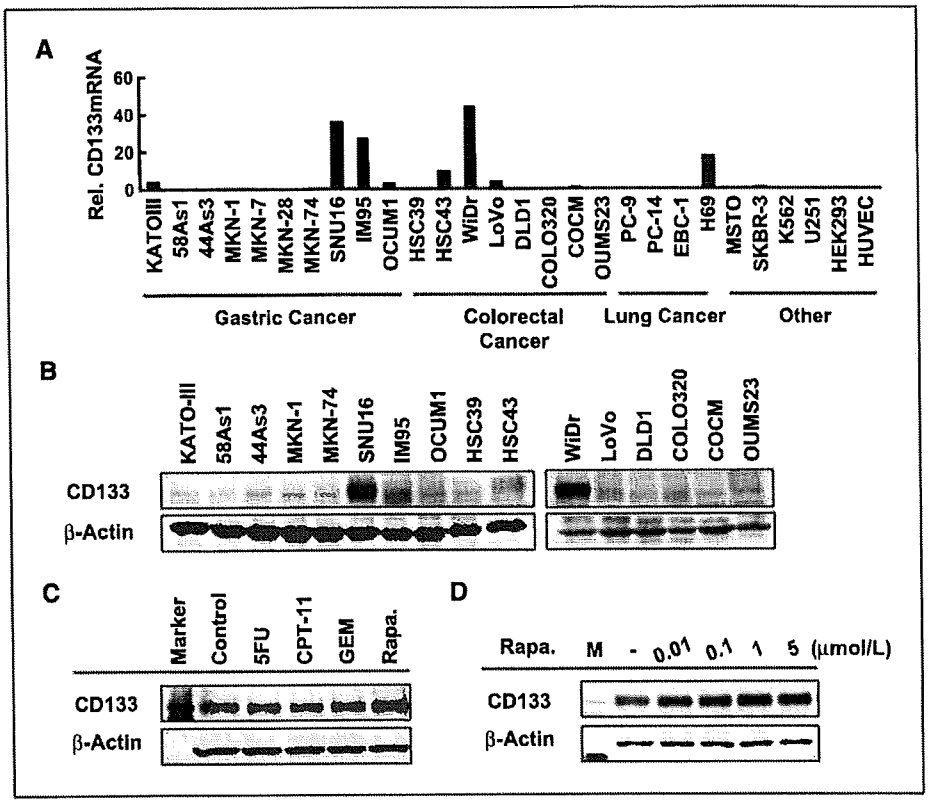

Figure 1. Rapamycin up-regulates CD133 expression. *A*, the mRNA expression levels of CD133 were examined using real-time RT-PCR in 26 cancer cell lines. *B*, the protein expressions of CD133 were determined using Western blotting in 16 gastric and colorectal cancer cell lines. *C*, Western blot of CD133 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

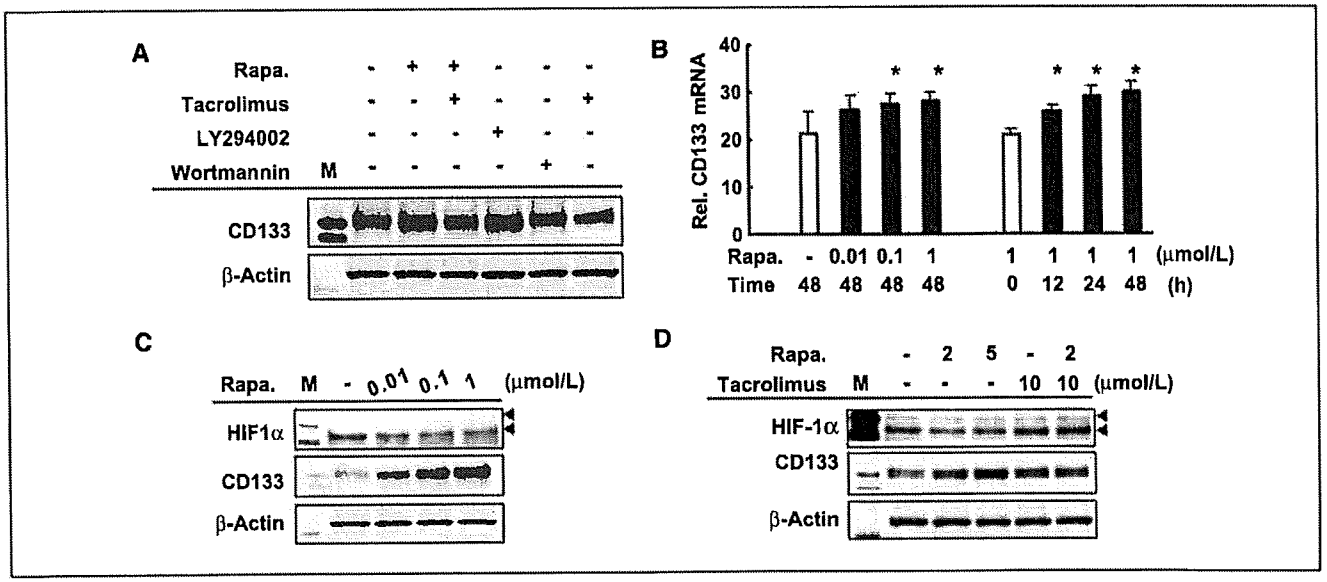

Figure 2. Rapamycin down-regulates HIF-1α expression and up-regulates CD133 expression at the transcriptional level. *A*, WiDr cells were exposed to rapamycin, the rapamycin-competitor tacrolimus, and the phosphoinositide-3-kinase inhibitors LY294002 and wortmannin for 48 h at concentrations of 10 μmol/L. 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

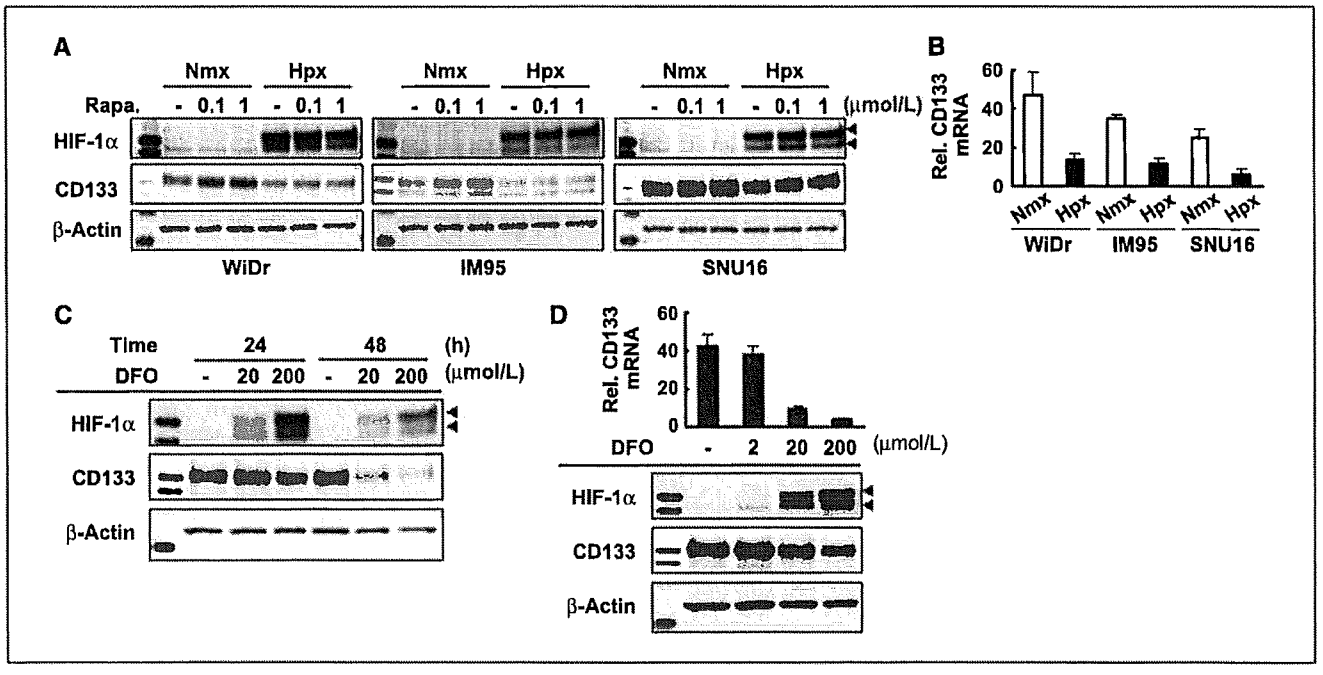

Figure 3. Induction of HIF-1α down-regulates CD133 expression in cancer cells. *A*, three gastrointestinal cancer cell lines were exposed to rapamycin under normoxic or hypoxic conditions for 24 h. Hypoxia induced HIF-1α expression and inversely down-regulated CD133 expression. *B*, hypoxia strongly 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.

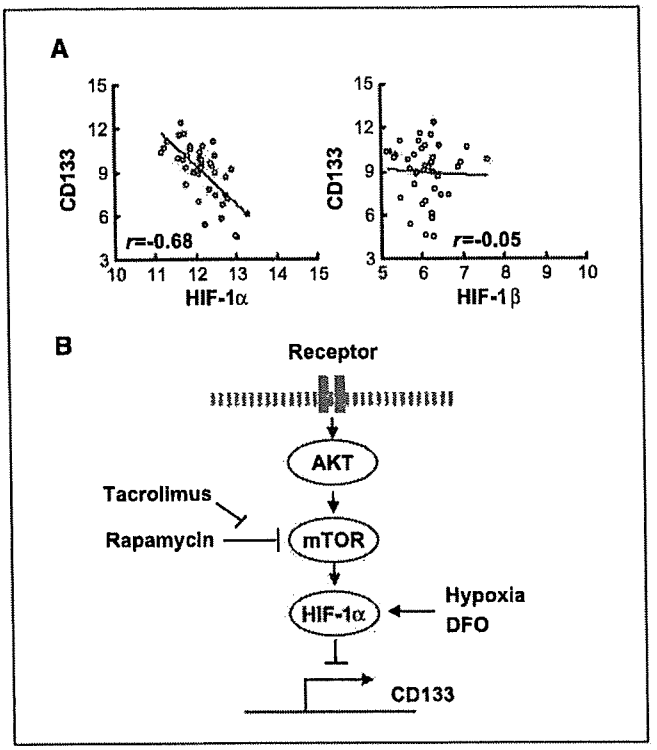

Figure 4. Inverse correlation between CD133 and HIF-1α in clinical samples of gastric cancer. *A*, the correlation between the expressions of CD133 and HIF-1α were analyzed in 40 clinical gastric cancer specimens using previously published microarray data. CD133 and HIF-1α were inversely correlated in 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.

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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

Kaoru Kubota, MD, PhD,† Seiji Niho, MD,* Sotaro Enatsu, MD, PhD,‡ Yoshihiro Nambu, MD, PhD,‡ Yutaka Nishiwaki, MD,* Nagahiro Saijo, MD, PhD,*§ and Masahiro Fukuoka, MD§*

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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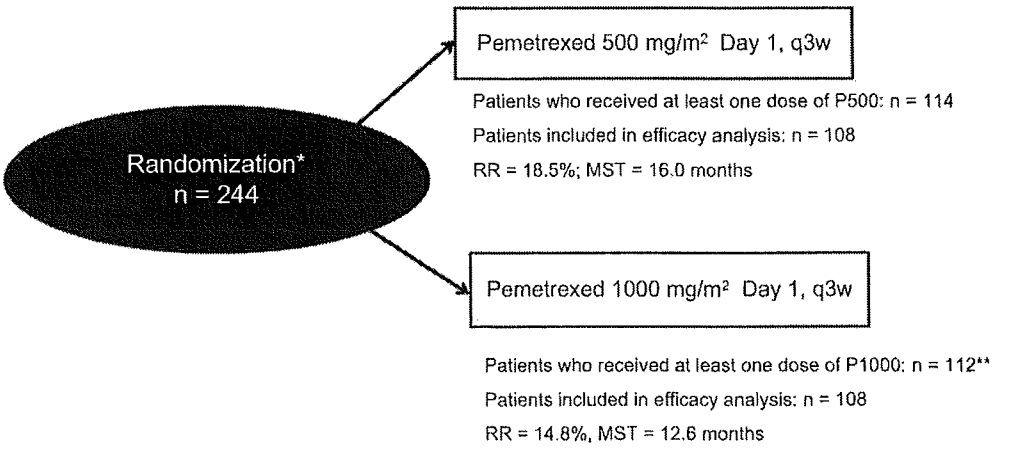

FIGURE 1. Trial design and efficacy data. From phase II randomized study.[8] NSCLC, non-small cell lung cancer; ECOG, Eastern Cooperative Oncology Group; PS, performance status; q, every; w, weekly; *n*, number of patients; RR, response rate; MST, median survival time. *Patients: stage IIIB/IV NSCLC, 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

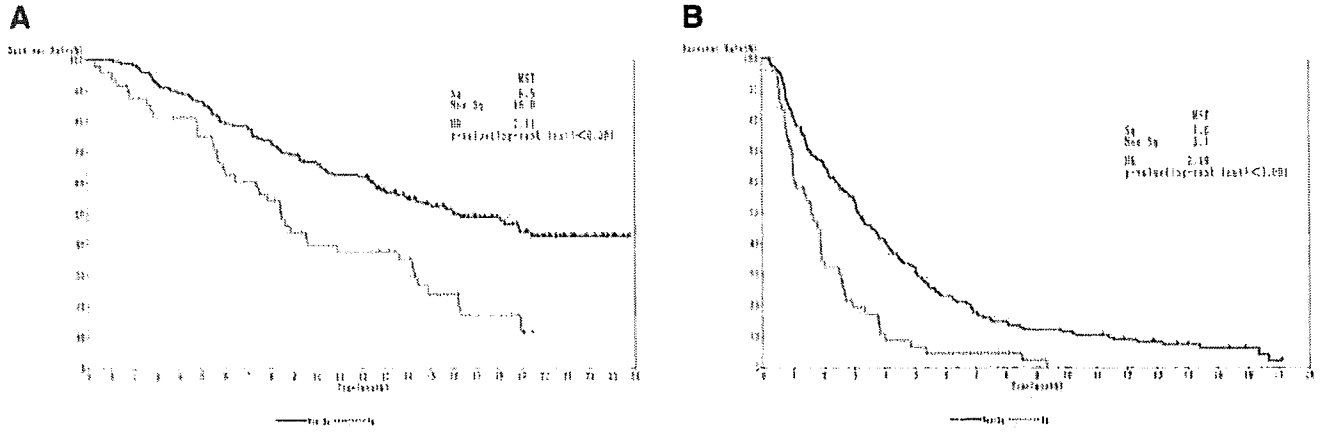

FIGURE 2. *A*, Kaplan-Meier curves for overall survival by histology. *B*, Kaplan-Meier curves for progression-free survival by histology. MST, median survival time.

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.

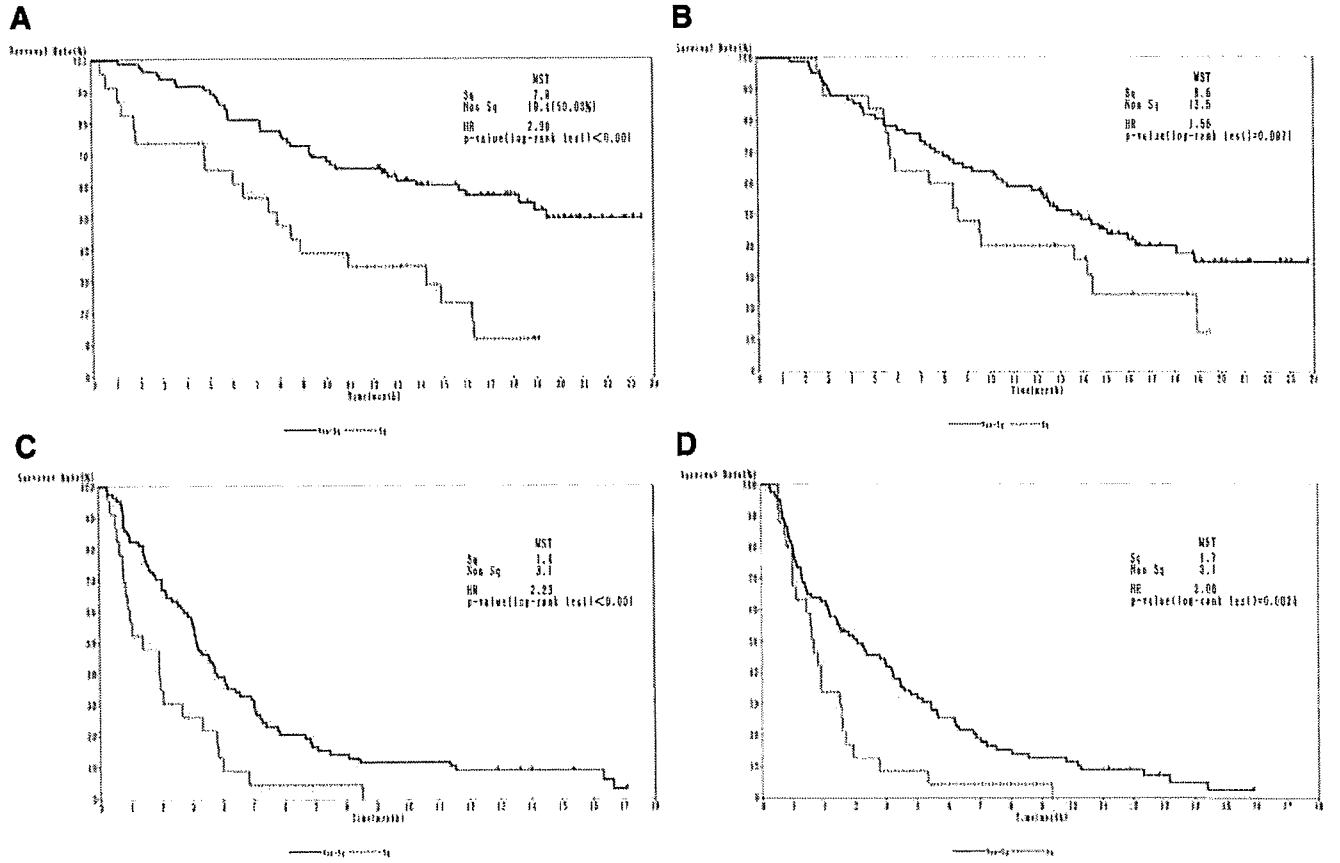

FIGURE 3. Kaplan-Meier curves for overall survival by dose and histology: (*A*) patients treated with pemetrexed 500 mg/m^2 and (*B*) patients treated with pemetrexed 1000 mg/m^2. Kaplan-Meier curves for progression-free survival by dose and histology: (C) patients treated with pemetrexed 500 mg/m^2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

Oshita et al. [5] prospectively evaluated the feasibility of cisplatin-based chemotherapy in patients aged 75 years or older. Only 10 (29%) out of the 34 patients fulfilled the eligibility criteria for the cisplatin-based regimen. Furthermore, the majority of these eligible patients had grade 4 neutropenia and infectious episodes requiring antibiotics. In another analysis of cisplatin pharmacokinetics, the area under the plasma concentration versus time curve (AUC) of the ultrafilterable and total plasma platinum increased with age, and this was an independent predictor of cisplatin pharmacokinetics [6]. Therefore, the administration of cisplatin is restricted to highly select elderly patients.

(Glycolate-*O,O'*)-diammine platinum (II) (nedaplatin) is a second-generation platinum analog synthesized by Shionogi & Co., Ltd. (Osaka, Japan). In the preclinical studies, nedaplatin is highly active against solid tumors and has higher aqueous solubility than cisplatin [7–9]. The emesis and nephrotoxicity of nedaplatin are substantially reduced, compared with those of cisplatin, and multiple days of hydration for renal protection are not required [10]. Dose-limiting toxicity (DLT) is thrombocytopenia, and recommended dose in Japanese patient ≤70 years is 100 mg/m^2 every 4 weeks. This agent is active against NSCLC, with a response rate of 20.5% for previously untreated patients [10]. In a pharmacokinetic analysis, thrombocytopenia was significantly correlated with renal function (i.e., creatinine clearance [Ccr]), and nadir platelet count could be predicted from the following formula [11]:

$$[\text{Nadir platelet count}]\,(/\text{mm}^3) = -64{,}264.7 + 2{,}783.4 \times [\text{Ccr}]\,(\text{mL/min})$$

We conducted a dose-finding and pharmacokinetic study of nedaplatin in elderly patients with NSCLC, stratified into two groups based on renal function. This study was conducted to determine the recommended dose, and evaluate the toxicity profiles, pharmacokinetics and antitumor activity.

Patients and methods

Eligibility

Patients with histologically and cytologically confirmed chemotherapy-naïve advanced or metastatic non-small cell lung cancer were eligible for this study. Other eligibility criteria included the following: (1) age ≥70 years; (2) Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1; (3) adequate bone marrow (white blood cell [WBC] count ≥4,000/mm^3, absolute neutrophil count [ANC] ≥2,000/mm^3, hemoglobin level ≥9.0 g/dL and platelet [PLT] count ≥100,000/mm^3), hepatic (serum total bilirubin level ≤1.5 mg/dL, serum asparatate aminotransferase [AST] level ≤100 IU/L and serum alanine aminotransferase [ALT] level ≤100 IU/L), renal (serum creatinine [Cr] level ≤1.5 mg/dL, creatinine clearance [Ccr] ≥40 mL/min) and pulmonary (PaO_2 ≥60 torr) functions.

The exclusion criteria were as follows: (1) symptomatic brain metastasis; (2) pleural or pericardial effusions and ascites requiring drainage; (3) serious pre-existing medical conditions such as uncontrolled infections, severe heart disease, uncontrolled diabetes and psychogenic disorders; and (4) hepatic B or C virus or human immunodeficiency virus infection.

Written informed consent was obtained from all the patients. This study was approved by the Institutional Review Board of the National Cancer Center.

Study design, dosage and dose escalation

This study was designed to determine the recommended dose of nedaplatin for elderly patients with advanced NSCLC, stratified into two groups based on renal function. The primary objective was to determine the recommended dose, and the secondary objectives were to evaluate toxicity profiles, pharmacokinetics and antitumor activity.

Patients were stratified into two groups based on their renal function at the time of study entry: Group A, Ccr ≥60 mL/min; and Group B, 40 ≤ Ccr < 60 mL/min. Ccr was measured on three consecutive days, and the mean value was used for stratification. Each Ccr was calculated using the following formula:

$$\text{Ccr (mL/min)} = [\text{urine volume (mL/min)} \times \text{urine creatinine (mg/dL)}]/\text{serum creatinine (mg/dL)}$$

In Group A, the initial dose of nedaplatin was 80 mg/m^2, and this was escalated to 100 mg/m^2. In Group B, the initial dose was 60 mg/m^2, and this was escalated to 80 and 100 mg/m^2. At least three to six patients were enrolled at each dose level, and the unacceptable dose was defined as the dose level at which >50% of the patients experienced DLT. The definition of DLT was as follows: (1) ≥grade 3 leukopenia, neutropenia or thrombocytopenia; (2) ≥grade 3 non-hematological toxicities except for alopecia, nausea and vomiting; (3) ≥grade 3 nausea and vomiting for ≥5 days. The recommended dose was defined as one dose level below the unacceptable dose level in each treatment arm.

Nedaplatin administration

Nedaplatin (Aqupla, (glycolate-*O,O'*)-diammine platinum (II); Shionogi Pharmaceutical Company, Osaka, Japan) was obtained commercially. Premedication, consisting of

3 mg of granisetron and 16 mg of dexamethasone diluted in 100 mL of 0.9% saline, was administered via a 30-minute intravenous (IV) infusion. The calculated doses of nedaplatin in both treatment groups were diluted in 300 mL of 0.9% saline and were administered using a 1-h IV infusion every 4 weeks. Following the nedaplatin administration, 500 mL of 0.9% saline was administered intravenously to provide minimal hydration.

Pretreatment and follow-up evaluation

On enrollment into the study, history and physical examination was performed. Complete differential blood cell count (including WBC count, ANC, hemoglobin and PLT), and clinical chemistry analysis (including serum total protein, albumin, bilirubin, Cr, AST, ALT, gamma-glutamyltransferase, and alkaline phosphatase) were performed. These above were performed at least twice a week throughout the study. Tumor measurement was planned every cycle, and antitumor response was assessed using the WHO standard response criteria. Toxicity was evaluated according to the National Cancer Institute common toxicity criteria (version 2.0).

PK study

Pharmacokinetic (PK) evaluations were performed in all patients during the initial cycle of treatment. Heparinized venous blood samples (7 mL) were taken before infusion, at 30 min and just before the end of infusion, as well as at 15 and 30 min and 1, 2, 3, 5, 7, 11, 23 and 47 h after the end of infusion.

Blood samples were centrifuged immediately at 4,000 rpm for 10 min. One milliliter of plasma was stored at −20°C or below in a polyethylene tube until the measurement of total plasma platinum (total-Pt) concentration. Residual plasma was transferred to an Amicon Centrifree tube (Amicon, Inc., Beverly, MA, USA) and centrifuged at 4,000 rpm for 20 min. Ultrafiltrate of the plasma was taken and stored at −20°C or below in a polyethylene tube until the measurement of the plasma-free platinum (free-Pt) concentration. The total-Pt and free-Pt concentrations were measured using flameless atomic absorption spectrometry, as previously reported [12].

The PK parameters were estimated using a nonlinear least-squares regression analysis (WinNonlin, Version 5.2; Bellkey Science, Inc., Chiba, Japan) with a weighting factor of $1/year^2$. The individual plasma concentration–time data were fitted to one-, two- and three-exponential equations using a zero-order infusion input and first-order elimination (corresponding to a one-, two- and three-compartment PK model). The model was chosen on the basis of Akaike's information criteria [13]. Fitted parameters (coefficients and exponent of exponential equations) were permitted in the computation of the following PK parameters: half life ($t_{1/2}$), area under the plasma concentration versus time curve (AUC), systemic clearance (CL), and volume of distribution at steady state (V_{dss}).

To assess the pharmacodynamic effect, percentage decrease was calculated in WBC, ANC or PLT according to the following formula:

$$\text{Percentage decrease} = [(\text{pretreatment count} - \text{nadir count})/(\text{pretreatment count})] \times 100.$$

These percentages were related to the AUC according to the sigmoid E_{max} model, as follows:

$$\text{Effect}(\%) = [E_{max}(\text{AUC})^k]/[\text{AUC}_{50}^k + \text{AUC}^k] \times 100.$$

A nonlinear least-squares regression using WinNonlin was used to estimate the AUC that produces 50% of the maximum effect (AUC_{50}) and the sigmoidicity coefficient (k).

Results

Patient characteristics

Between June 1996 and July 2001, 39 patients were stratified into two groups (22 in Group A and 17 in Group B) based on their renal functions at entry into the study (Table 1). They received a total of 83 cycles of therapy. The patients comprised 35 males and 4 females with good performance status, and the median age was 76 years in both treatment groups. All the patients were included in the toxicity evaluation. A total of 28 (72%) patients were included in the PK analysis and the remaining 11 (28%) were excluded because of insufficient PK samplings. Eight patients (two from Group A and six from Group B) had stage IIIA disease, but were not candidates for thoracic radiotherapy because of their poor pulmonary function. Six patients (five from Group A and one from Group B) received surgical resections for primary tumors. As much as 21 patients (54%, 12 from Group A and 9 from Group B) had squamous cell carcinoma. Nine patients (4 from Group A and 5 from Group B) received only one cycle of therapy because of progressive disease (PD) and 22 patients (12 from Group A and 10 from Group B) received two cycles of treatment. Among these 22 patients, partial response (PR), stable disease (SD) and PD were observed in 8, 10 and 4 patients, respectively. Five of eight patients with PR, two of ten with SD and one of four with PD received sequential thoracic radiotherapy for primary lesion following two cycles of treatment. Two of ten patients with SD and one of four with PD received palliative

radiotherapy for metastatic lesion. Two of four patients with PD received second-line chemotherapy. The remaining nine patients received supportive care according to the patients' request.

Toxicity

All the 39 patients were included in the toxicity evaluation. Major toxicities were hematological, such as leukopenia, neutropenia and thrombocytopenia, in both groups, and these hematological toxicities increased in severity with increased dose level of nedaplatin. In Group A, 1 (6.7%) out of the 15 patients treated at a dose level of 100 mg/m^2 had grade 3 neutropenia; this dose level was considered to be acceptable (Table 2). In Group B, three (50%) out of six patients treated at a dose level of 80 mg/m^2 had ≥grade 3 hematological toxicities (one with grade 3 neutropenia, another with grade 4 neutropenia and febrile neutropenia, and the other with grade 3 leukopenia, anemia and grade 4 thrombocytopenia). The patient with grade 4 thrombocytopenia required a platelet transfusion. At a dose level of 100 mg/m^2, three (60%) out of five patients had ≥grade 3 hematological toxicities (one with grade 3 leukopenia and neutropenia, another with grade 3 thrombocytopenia and grade 4 neutropenia, and the other with grade 3 leukopenia, thrombocytopenia and grade 4 neutropenia). These three patients had also febrile neutropenia. In Group B, a dose level of 100 mg/m^2 was considered to be unacceptable (Table 2).

Non-hematological toxicities, mainly nausea and anorexia, were generally mild in severity and were not dose limiting in either group (Table 3). Renal toxicity,

Table 1 Patient characteristics

	Group A (Ccr ≥60 mL/min)		Group B (40 ≤ Ccr < 60 mL/min)	
	No. of patients	Percentage	No. of patients	Percentage
Total patients enrolled	22	100	17	100
Assessable for toxicity	22	100	17	100
Assessable for PK analysis	15	68	13	76
Age, median (range), years	76 (70–82)		76 (70–78)	
Sex				
Male	19	86	16	94
Female	3	14	1	6
ECOG PS				
0	6	27	1	6
1	16	73	15	88
2	0	0	1	6
Stage				
IIIA	2	9	6	35
IIIB	4	18	6	35
IV	11	50	4	24
Postoperative recurrence	5	23	1	6
Pathological subtype				
Squamous cell carcinoma	12	54	9	53
Adenocarcinoma	9	41	8	47
P/D carcinoma	1	5	0	0
Dose of nedaplatin (mg/m^2)				
60	–	–	6	35
80	7	32	6	35
100	15	68	5	30
Treatment cycle				
Median (range)	2 (1–5)		2 (1–4)	
1 cycle	4	18	5	29
2 cycles	12	55	10	59
≥3 cycles	6	27	2	12

PK pharmacokinetics, *ECOG* Eastern Cooperative Oncology Group, *PS* performance status, *P/D carcinoma* poorly differentiated carcinoma

Table 2 Hematological toxicity

Group A (Ccr ≥60 mL/min)	Dose level (mg/m^2), (number of patients)									
	80 (n = 7) Grade					100 (n = 15) Grade				
Event	0	1	2	3	4	0	1	2	3	4
Leukopenia	6	1	0	0	0	12	1	2	0	0
Neutropenia	6	1	0	0	0	8	4	2	1[a]	0
Anemia	4	2	1	0	0	5	7	3	0	0
Thrombocytopenia	7	0	0	0	0	12	2	1	0	0
No. of patients with febrile neutropenia	0					0				
No. of patients with DLT	0					1				

Group B (40 ≤ Ccr < 60 mL/min)	Dose level (mg/m^2), (number of patients)														
	60 (n = 6) Grade					80 (n = 6) Grade					100 (n = 5) Grade				
Event	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
Leukopenia	5	1	0	0	0	2	1	2	1[a]	0	2	0	1	2[a]	0
Neutropenia	5	1	0	0	0	2	2	0	1[a]	1[a]	1	1	0	1[a]	2[a]
Anemia	4	1	1	0	0	3	1	1	1[a]	0	1	2	2	0	0
Thrombocytopenia	6	0	0	0	0	3	1	1	0	1[a]	2	1	0	2[a]	0
No. of patients with febrile neutropenia	0					1					3				
No. of patients with DLT	0					3					3				

[a] DLT

characterized as an increase in Cr, was also mild, and only one out of five patients treated at a dose level of 100 mg/m^2 in Group B had a grade 2 Cr increase. Considering the toxicity profiles, the recommended doses in Groups A and B were determined to be 100 and 80 mg/m^2, respectively.

Response and survival

The antitumor response was assessed in all the 39 patients (Table 4). Of the 39 patients who achieved PR, 13 had an overall response rate of 33%. Similar antitumor responses were observed in both treatment groups; that is, 6 (27%) of 22 and 7 (41%) of 17 patients had PRs in Groups A and B, respectively. Furthermore, 12 of the 13 patients with PRs in both groups had squamous cell carcinoma, and the response rate among patients with squamous cell carcinoma was 57%. Survival follow-up was completed in all the enrolled patients. The median survival time was 11.2 months (95% confidence interval: 7.7–14.6 months), and the 1-, 2- and 5-year survival rates were 46, 23 and 5%, respectively.

Pharmacokinetics

Pharmacokinetic analysis was performed using data from 28 (72%) of the 39 patients. The first patient enrollment in both treatment groups was started in 1996, and techniques of the sample centrifuging and measurement were not fully developed at the beginning of this pharmacokinetic study. Therefore, the remaining 11 patients (28%) were excluded for pharmacokinetic analysis. The mean plasma concentration–time profiles of total-Pt and free-Pt of nedaplatin are illustrated in Fig. 1. The plasma disappearances of total-Pt and free-Pt were biphasic, and the mean terminal half lives in all the assessable patients averaged 6.28 and 3.57 h, respectively. The C_{max} and AUC of the total-Pt and free-Pt tended to increase with the dose of nedaplatin. The AUCs of the total- and free-Pt at a dose of 100 mg/m^2 in Group A seemed similar to those at a dose of 80 mg/m^2 in Group B (Table 5), and there were no significant differences between these two treatment subgroups (P = 0.293 for total-Pt AUC and P = 0.336 for free-Pt AUC). Furthermore, the AUCs of free-Pt at the recommended doses in both groups (i.e., 100 mg/m^2 in Group A and 80 mg/m^2 in Group B) seemed also similar to that in patients aged 70 years or under who had been treated with 100 mg/m^2 of nedaplatin [14]. In the sigmoid Emax model assessing the pharmacodynamic effect of nedaplatin, the percentage decrease in the neutrophil counts were well correlated with the total-Pt (r = 0.652) and free-Pt (r = 0.723; Fig. 2).

Table 3 Non-hematological toxicity

Group A (Ccr ≥60 mL/min)	Dose level (mg/m^2), (number of patients)									
	80 (n = 7) Grade					100 (n = 15) Grade				
Event	0	1	2	3	4	0	1	2	3	4
Nausea	5	1	1	0	0	3	9	3	0	0
Vomiting	6	1	0	0	0	15	0	0	0	0
Anorexia	5	1	1	0	0	7	4	4	0	0
Diarrhea	6	1	0	0	0	14	1	0	0	0
Stomatitis	7	0	0	0	0	15	0	0	0	0
Hyperbilirubinemia	6	0	1	0	0	15	0	0	0	0
AST increase	6	1	0	0	0	13	2	0	0	0
ALT increase	6	1	0	0	0	13	2	0	0	0
ALP increase	7	0	0	0	0	15	0	0	0	0
Cr increase	7	0	0	0	0	15	0	0	0	0

Group B (40 ≤ Ccr < 60 mL/min)	Dose level (mg/m^2), (number of patients)														
	60 (n = 6) Grade					80 (n = 6) Grade					100 (n = 5) Grade				
Event	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
Nausea	1	4	1	0	0	1	3	2	0	0	1	1	3	0	0
Vomiting	6	0	0	0	0	5	1	0	0	0	5	0	0	0	0
Anorexia	4	2	0	0	0	1	3	2	0	0	1	1	3	0	0
Diarrhea	6	0	0	0	0	5	1	0	0	0	5	0	0	0	0
Stomatitis	6	0	0	0	0	6	0	0	0	0	5	0	0	0	0
Hyperbilirubinemia	6	0	0	0	0	6	0	0	0	0	4	0	1	0	0
AST increase	4	2	0	0	0	5	0	1	0	0	4	0	1	0	0
ALT increase	5	1	0	0	0	5	0	1	0	0	4	0	1	0	0
ALP increase	6	0	0	0	0	5	1	0	0	0	5	0	0	0	0
Cr increase	6	0	0	0	0	4	2	0	0	0	4	0	1	0	0

AST asparatate aminotransferase, *ALT* serum alanine aminotransferase, *ALP* alkaline phosphatase, *Cr* creatinine

Discussion

In this dose-finding study, we evaluated the toxicities, pharmacokinetics as well as antitumor activity, and determined the recommended doses of nedaplatin for elderly patients with advanced NSCLC based on renal function. The predominant toxicities were hematological, such as leukopenia, neutropenia and thrombocytopenia, in both groups. These hematological toxicities tended to increase in severity with the increased dose level of nedaplatin. Non-hematological toxicities were acceptable and those were not dose limiting in either group. The recommended dose was determined as 100 mg/m^2 every 4 weeks in elderly patients with a renal function of Ccr ≥ 60 mL/min, which is the same dose recommended for patients aged ≤70 years. On the other hand, for elderly patients with a renal function of 40 ≤ Ccr < 60 mL/min, the recommended dose was 80 mg/m^2 every 4 weeks. In this study,