Table 1. Patient Characteristics

Characteristic	BSA-Based Arm No. of Patients	%	Individualized Arm No. of Patients	%	P
Enrolled	30		29		
Eligible	30	100	29	100	
Age, years					.62
Median	61		62		
Range	52-73		45-73		
Sex					
Male	25	83	19	66	14
Female	5	17	10	34	
ECOG PS					
0	7	23	1	3	.08
1	22	73	26	90	
2	1	3	2	7	
Prior treatment					
None	4	13	4	14	99
Surgery	11	37	9	31	.65
Radiotherapy	13	43	10	34	49
Chemotherapy	21	70	18	62	52
Platinum-based regimens	20	67	16	55	.37
Site of disease					
Lung	23	77	28	97	.10
Liver	0	0	2	7	.24
Pleura	8	27	12	41	.23
Bone	7	23	9	31	.71
Extrathoracic lymph nodes	0	33	10	34	.93
Laboratory parameters					
ALB, g/L					.02
Median	38		35		
Range	26-45		24-44		
AAG, g/L					04
Median	1 00		1 25		
Range	0 28-2 15		0 64-2 54		
AST, U/L					67
Median	21		22		
Range	10-40		7-41		
ALT, U/L					88
Median	18		18		
Range	6-54		4-45		
ALP, U/L					03
Median	249		324		
Range	129-540		185-986		

Abbreviations: ECOG, Eastern Cooperative Oncology Group; PS, performance status, ALB, serum albumin; AAG, alpha-1-acid glycoprotein; ALP, serum alkaline phosphatase

AUC was significantly smaller in the individualized arm than in the BSA-based arm ($P < .01$; Fig 3).

PD

In both arms, neutropenia was the predominant toxicity related to docetaxel treatment, and 28 of 30 (93%) patients in the BSA-based arm and 25 of 29 (86%) patients in the individualized arm had grade 3 or 4 neutropenia.

Table 2. Docetaxel PK Parameters

Parameters	BSA-Based Arm (n = 30)	Individualized Arm (n = 29)
C_{max}, μg/mL	0.36-2.70	0.99-2.41
$t_{1/2}$ alpha*, minutes	9 2 ± 3 3	9 2 ± 2 7
$t_{1/2}$ beta*, hours	5.0 ± 4.8	7.4 ± 11.7
CL* L/h	37.6 ± 6.3	34.8 ± 7 1
CL* L/h/m^2	22.6 ± 3.4	22.1 ± 3.4
AUC		
Mean mg/L h	2 71	2.64
Range mg/L h	2.02-3.40	2.15-3.07
Median	2 65	2.66
SD	0.40	0.22

Abbreviations: PK, pharmacokinetic; BSA, body-surface area; CL, clearance; AUC, area under concentration-time curve; SD, standard deviation
*Data represent mean ± SD

Nonhematologic toxicities, such as gastrointestinal and hepatic toxicities (ie, hyperbilirubinemia, aminotransferase elevations), were mild in both arms.

PD effects shown as the percentage decrease in ANC are listed in Table 3. The percentage decrease in ANC for the BSA-based arm and individualized arm were 87.1% (range, 59.0 to 97.7%; SD, 8.7) and 87.5% (range, 78.0 to 97.2%; SD, 6.1), respectively, suggesting that the interpatient variability in the percentage decrease in ANC was slightly smaller in the individualized arm than in the BSA-based arm (Fig 4). The response rates between the two arms were similar; five of 30 (16.7%) and four of 29 (13.8%) patients

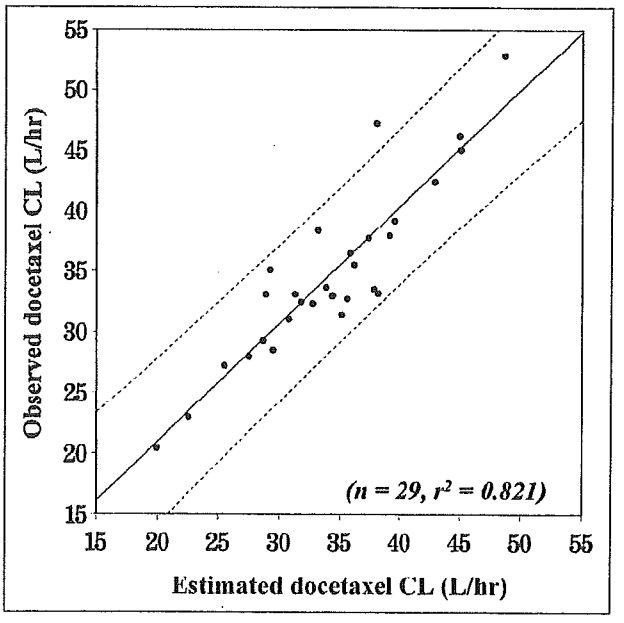

Fig 2. Correlation between the estimated and observed docetaxel clearance (CL) in the individualized arm (n = 29) (—) Linear regression line (r^2 = 0.821), (- - - -) 95% CIs for individual estimates

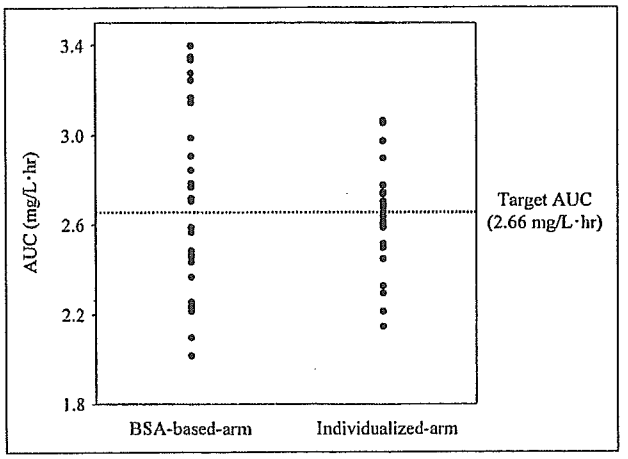

Fig 3. Comparison of area under the concentration-time curve (AUC) variability between the arms ($P < .01$; F test). BSA, body-surface area.

achieved a partial response in the BSA-based arm and individualized arm, respectively.

DISCUSSION

In oncology practice, the prescribed dose of most anticancer drugs is currently calculated from BSA of individual patients to reduce the interpatient variability of drug exposure. However, PK parameters, such as CL of many anticancer drugs, are not related to BSA.[2,39-43] Although PK parameters of docetaxel are correlated with BSA, individualized dosing based on individual metabolic capacities could further decrease the interpatient variability.[43]

CYP3A4 plays an important role in the metabolism of many drugs, including anticancer agents such as docetaxel, paclitaxel, vinorelbine, and gefitinib. This enzyme exhibits a large interpatient variability in metabolic activity, accounting for the large interpatient PK and PD variability. We have developed a novel method of estimating the interpatient variability of CYP3A4 activity by urinary metabolite of exogenous cortisol. That is, the total amount of 24-hour urinary 6-β-OHF after cortisol administration was highly correlated with docetaxel CL. We conducted a prospective randomized PK and PD study of docetaxel to evaluate whether the application of our method to individualized dosing could decrease PK and PD variability compared with BSA-based dosing.

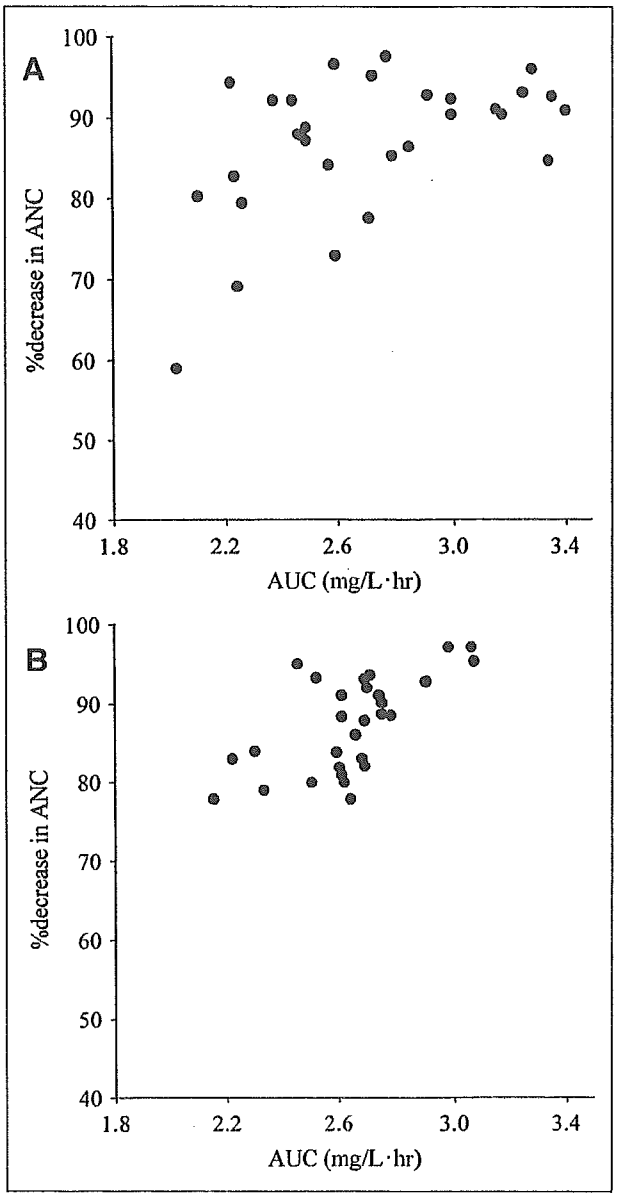

Fig 4. Correlation between area under the concentration-time curve (AUC) and percentage decrease in absolute neutrophil count (ANC) in each arm. (A) body-surface area–based arm; (B) individualized arm.

Table 3. Percentage Decrease in ANC

Parameters	BSA-Based Arm (n = 30)	Individualized Arm (n = 29)
Percentage decrease in ANC, %		
Mean	87.1	87.4
Range	59.0-97.7	78.0-97.2
Median	89.7	88.4
SD	8.7	8.1

Abbreviations: ANC, absolute neutrophil count; BSA, body-surface area; SD, standard deviation.

The study by Hirth et al[28] showed a good correlation between the result of the erythromycin breath test and docetaxel CL, and the study by Goh et al[29] showed a good correlation between the midazolam CL and docetaxel CL. In our study, we prospectively validated the correlation between docetaxel CL and our previously published method using the total amount of urinary 6-β-OHF after

cortisol administration in the individualized arm. As shown in Fig 2, the observed docetaxel CL was well estimated, and the equation for the estimation of docetaxel CL developed in our previous study was found to be reliable and reproducible. The target AUC in the individualized arm was set at 2.66 mg/L · h. This value was the mean value from our previous study, in which 29 patients were treated with 60 mg/m^2 of docetaxel. Individualized doses of docetaxel ranged from 37.4 to 76.4 mg/m^2 and were lower than expected.

The SD of AUC in the individualized arm was about 46.2% smaller than that in the BSA-based arm, a significant difference; this result seems to indicate that the application of our method to individualized dosing can reduce the interpatient PK variability. Assuming that the variability of AUC could be decreased 46.2% by individualized dosing applying our method, overtreatment could be avoided in 14.5% of BSA-dosed patients by using individualized dosing (Fig 5, area A), and undertreatment could be avoided in another 14.5% of these patients (Fig 5, area B). We considered that neutropenia could be decreased with patients in area A by individualized dosing. However, it is unknown whether the therapeutic effect of docetaxel could be improved in the patients in area B by individualized dosing because no significant positive correlation has been found between docetaxel AUC and antitumor response in patients with non–small-cell lung cancer.[43] In this study, seven of 30 (23.3%) and two of 30 (6.7%) patients in the BSA-based arm were included in area A and B, respectively (Figs 3 and 5).

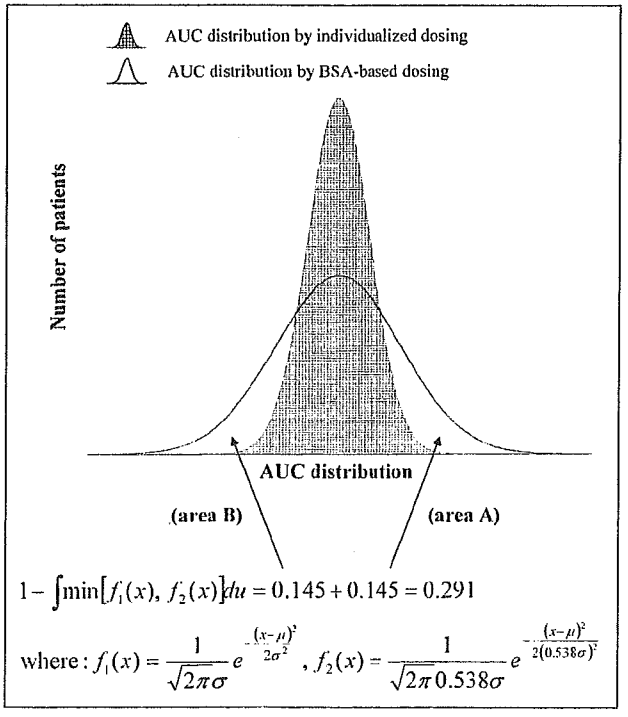

Fig 5. Simulated comparison of area under the concentration-time curve (AUC) distribution between body-surface area (BSA) –based dosing and individualized dosing when the variability of AUC is decreased 46.2% by individualized dosing applied using our method

As shown in Figure 4, the percentage decrease in ANC was well correlated with AUC in both arms, which was similar to previous reports.[37,43] It was also indicated that the interpatient variability in the percentage decrease in ANC was slightly smaller in the individualized arm than in the BSA-based arm; however, this difference was not significant. The response rates between the two arms were similar. Although the interpatient PK variability could be decreased by individualized dosing in accordance with our method, the interpatient PD variability such as toxicity and the antitumor response could not be decreased. Several reasons could be considered.

With regard to toxicity, the pretreatment characteristics of the patients in this study were highly variable. More than half of the patients in each arm had previously received platinum-based chemotherapy, and more than 30% had received radiotherapy. The laboratory parameters (ie, ALB, AAG, and ALP) were not balanced across the arms, although they were not included in the eligibility criteria (Table 1). These variable pretreatment characteristics and unbalanced laboratory parameters may have influenced the frequency and severity of the hematologic toxicity as well as the pharmacokinetic profiles. The antitumor effect may have been influenced by the intrinsic sensitivity of tumors, the variable pretreatment characteristics, and the imbalance in laboratory parameters. Non–small-cell lung cancer is a chemotherapy-resistant tumor. The response rate for docetaxel ranges from 18% to 38%,[5] and no significant positive correlation between docetaxel AUC and antitumor response has been found. We considered it quite difficult to control the interpatient PD variability by controlling the interpatient PK variability alone. Although we did not observe any outliers in either arm, such as the two outliers with severe toxicity observed in the study by Hirth et al,[28] our method may be more useful for identifying such outliers. If we had not excluded patients with more abnormal liver function or a history of liver disease by the strict eligibility criteria, the results with the two dosing regimens may have been more different, and the interpatient PD variability, such as the percentage decrease in ANC, may have been smaller in the individualized arm than in the BSA-based arm. Furthermore, the primary end point of this study was PK variability, evaluated by the SD of AUC in both arms, and the sample size was significantly underpowered to evaluate whether the application of our method to individualized dosing could decrease PD variability compared with BSA-based dosing.

For the genotypes of CYP3A4, several genetic polymorphisms have been reported (http://www.imm.ki.se/CYPalleles/); however, a clear relationship between genetic polymorphisms and the enzyme activity of CYP3A4 has not been reported. Our phenotype-based

individualized dosing using the total amount of urinary 6-β-OHF after cortisol administration produced good results. However, this method is somewhat complicated, and a simpler method would be of great use. We analyzed the expression of CYP3A4 mRNA in the peripheral-blood mononuclear cells of the 29 patients in the individualized arm. No correlation was observed between the expression level of CYP3A4 mRNA and docetaxel CL or the total amount of urinary 6-β-OHF after cortisol administration (data not shown).

In conclusion, the individualized dosing of docetaxel using the total amount of urinary 6-β-OHF after cortisol administration is useful for decreasing the interpatient PK variability compared with the conventional BSA-based method of dosing. This method may be useful for individualized chemotherapy.

Authors' Disclosures of Potential Conflicts of Interest

The authors indicated no potential conflicts of interest.

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Plasma MIP-1β levels and skin toxicity in Japanese non-small cell lung cancer patients treated with the EGFR-targeted tyrosine kinase inhibitor, gefitinib

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KEYWORDS
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Skin toxicity

Summary Gefitinib (Iressa®) is an orally active, selective EGFR tyrosine kinase inhibitor that blocks signal transduction pathways. Skin toxicity has been reported to be the major toxicity observed in patients treated with the EGFR-targeted tyrosine kinase inhibitors, such as gefitinib and erlotinib. Although the mechanisms underlying the development of the skin toxicity remain to be precisely clarified, immunological mechanisms are considered to be involved. We examined the correlations between the plasma levels of several cytokines and the risk of development of adverse events, especially skin toxicity, induced by the administration of gefitinib as first-line monotherapy in non-small cell lung cancer (NSCLC) patients.

Paired plasma samples were obtained from a total 28 patients of non-small cell lung cancer; the first before the initiation of gefitinib administration (250 mg/day) (24 patients) and the second 2 or 4 weeks after the initiation of treatment (23 patients). The plasma concentrations of 17 major cytokines were measured using a bead-based multiplex assay. The median concentrations of eight of these cytokines before the start of treatment ranged from 0.06 (IL-5) to 58.26 (MIP-1β) (μg/ml). The concentrations of the remaining nine cytokines were under the detectable limit (<0.01 μg/ml) in more than 50% of the samples. Comparisons of the levels before and after treatment showed no significant differences for any of the cytokines measured.

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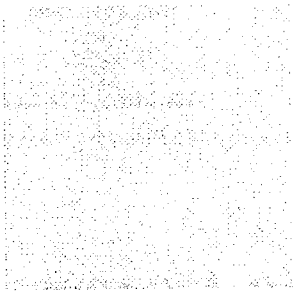

The MIP-1β levels were significantly lower in the patients with skin toxicity (16/24) as compared with those in the patients not showing any skin toxicity (59.1 ± 10.5 versus 119.0 ± 36.8; $p = 0.042$ by the two-sample t-test). The K-Nearest Neighbor Prediction ($K = 3$) showed the classification rate to be 75% for the prediction sets containing MIP-1β, IL-4 and IL-8. There were no significant associations between the levels of any of the cytokines measured and any other parameters, including the tumor response to the drug. In conclusion, the plasma MIP-1β level may be a useful predictor of the development of skin toxicity in patients receiving gefitinib treatment.

1. Introduction

The epidermal growth factor receptor (EGFR) has been found to be expressed, sometimes strongly, in a variety of solid tumors, including non-small cell lung cancer [1,2]. Recognition of the importance of the EGFR in tumor biology provides the rationale for the development of EGFR-targeted cancer therapies. Gefitinib (''Iressa'', ZD1839) is an orally active, selective EGFR tyrosine kinase inhibitor that blocks signal transduction pathways implicated in the proliferation and survival of cancer cells, and also other host-dependent processes that may promote cancer growth [3–5].

Gefitinib has been approved for use as a second-line drug for the treatment of non-small cell lung cancer in Japan, based on evidence collected from large-scale phase II trials (IDEAL 1 and IDEAL 2) [6,7]. In these studies, the adverse effects of gefitinib were mild as compared with those of other cytotoxic agents, and skin toxicity was the most frequently encountered of the adverse events. In some clinical studies, up to 90% of patients treated with gefitinib were reported to suffer from skin toxicity [8]. In others, development of skin toxicity necessitated the discontinuation of gefitinib treatment in some patients [9,10]. Recent publications have reported the development of skin toxicity in patients treated with the anti-EGFR antibody, cetuximab (''Erbitux'', IMC-C225), as well as in those treated with erlotinib (''Tarceva®'', OSI-774), which is an EGFR-targeted small molecule [11–14]. No clear preventive or curative treatment has been established for such drug-induced skin toxicity.

Cytokines mediate numerous physiological and immune reactions, which influence various biological activities, including tumor activity. Activated macrophages secrete many mediators which regulate host defenses by stimulating cellular immunity. Activated macrophages, which produce cytokines such as interleukin (IL)-12, tumor necrosis factor (TNF)-α and interferon (IFN)-α and IFN-β, are powerful activators of natural killer (NK) cells, which have been reported to exert cytotoxic activity against some tumors [15,16]. In non-small cell lung cancer patients, increased production of cytokines such as IL-2, 6, 8 and 10 has been shown to be associated with the response to treatment and survival [17–21]. Other solid tumors have also been shown to possess the ability to produce multiple cytokines [22–25]. These cytokines may act as autocrine growth factors regulating the proliferation and migration of endothelial, tumor, and immune cells. Correlations have been shown between endogenous cytokine levels and the phenotypic manifestations of cancers and prognosis of patients with solid tumors [24–26]. Skin toxicity is the most frequently encountered toxicity in patients treated with EGFR-targeted agents. Some studies have shown evidence of immune reactions in patients developing such skin toxicity, following the administration of other drugs besides the EGFR-targeted agents. In these studies, the levels of various cytokines were elevated after treatment in patients who showed skin toxicity [27–29].

We hypothesized that the serum levels of cytokines may be correlated with the clinical features of patients treated with gefitinib, including the tumor response and adverse effects, especially skin toxicity. To date, no direct comparisons have been made to determine the correlations between cytokine levels and the phenotypic manifestations in cancer patients treated with gefitinib. To investigate the relationship between the cytokine levels and the phenotypic manifestations of cancer in these patients, we measured the plasma concentrations of various cytokines and investigated the roles of these cytokines in NSCLC patients receiving gefitinib as first-line monotherapy.

2. Materials and methods

2.1. Patients and clinical trials

The present study was carried out as a correlative study in a multicenter clinical phase II trial of gefitinib monotherapy, between October 23, 2002, and August 3, 2003. The study was conducted with the

approval of the appropriate ethical review boards, and in accordance with the recommendations of the Declaration of Helsinki for biomedical research involving human subjects. Twenty-eight Japanese patients with histologically or cytologically proven stage IIIb or IV, chemotherapy-naïve NSCLC were enrolled in this trial. Histological subclassification was carried out according to the World Health Organization (WHO) classification (WHO, 1982). Staging was carried out according to the Fourth Edition of the UICC Tumour Node Metastases (TNM) classification. Gefitinib was administrated orally to all patients at a fixed dose of 250 mg daily. Tumor response was evaluated according to the ''Response Evaluation Criteria in Solid Tumours'' guidelines [30]. Patients were monitored for adverse events during each cycle of therapy, and these events were graded according to NCI-CTC, version 2.0.

2.2. Plasma collection

Blood samples from the 28 NSCLC patients were collected in heparinized tubes before and 14 or 28 days after the initiation of gefitinib administration. After centrifugation of the blood samples at 500 × *g* for 10 min, plasma samples were carefully collected from the top portion of the separated plasma. The separated plasma samples were stocked at −80 °C until use.

2.3. Cytokine assay

A panel of cytokines was measured in duplicate using the Bioplex protein assay kit (Bio-Rad Laboratories, Hercules, CA), in accordance with the instructions of the manufacturer. All samples were diluted by the addition of an equal amount of saline, and 15 μl of the diluted samples were used for this assay. The assay is a novel multiplexed, particle-based, flow-cytometric assay which utilizes anti-cytokine monoclonal antibodies linked to microspheres incorporating distinct proportions of two fluorescent dyes. The assay was customized to detect and quantify IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, IL-17, G-CSF, GM-CSF, TNF-α, IFN-γ, monocyte chemotactic protein-1 (MCP-1), and macrophage inflammatory protein-1 beta (MIP-1β). The minimum detectable limit for each of the cytokines was 0.01 μg/ml.

2.4. Statistical analysis

Comparisons of the plasma cytokine levels before and after treatment were carried out with Wilcoxon's signed-rank test, using the Stat View software package (version 5.0, SAS Institute Inc., Cary, NC). The correlations between the cytokine levels and the clinical manifestations were analyzed statistically using the two-sample *t*-test with a random variance model, which was performed using the R software package, version 1.9.0 (The R Foundation, http://www.r-project.org/). The patients were categorized into two groups, depending on the grades of the adverse events (Grade 0 versus >Grade 1). Cytokine values lower than the minimum detectable limit were represented as 0.001 pg/ml. When the significant differences were obtained in the two-sample *t*-test, predictive rates were calculated using the *K*-nearest neighbor prediction analysis ($K = 3$).

3. Results

3.1. Patients

A total 28 patients ware enrolled in this trial. The patients ranged in age from 44 to 87 years, with a median of 64 years, and the male:female ratio was 18:10. Plasma samples were collected before treatment from 24 (85.7%) patients and after treatment from 23 (82.1%) patients. All the patients were evaluated for the presence of drug-related adverse events (Table 1). Skin toxicity was the most frequently encountered drug-related adverse event; 71.4% of the patients receiving gefitinib showed skin toxicity.

3.2. Plasma cytokine levels in the lung cancer patients

The plasma levels of various cytokines in the patients are shown in Table 2. Scatter plots of the levels of individual cytokines are shown in Fig. 1. The levels of IL-2, IL-4, IL-7, IL-12, IL-17, IFN-γ, G-CSF, and GM-CSF in the plasma were lower than the minimal detectable limit (<0.01 μg/ml) in more than 50% of the patients. When the cytokine levels before and after treatment were compared, the MCP-1 levels were significantly higher in the

Table 1 Non-hematological toxicity

	0	1	2	3	4	Percentage of ≧ Grade1
Skin	8	13	5	2	0	71.4
Hepatitis	22	4	1	1	0	21.4
Pneumonitis	25	0	0	3	0	1.1
Diarrhea	18	7	2	1	0	35.7
Nausea	19	7	2	0	0	32.1

NCI-CTC version 2.0.

Table 2 Circulating cytokine levels (pg/ml)

	Pre	Post
Number of patients	24	23
IL-1β	0.09 (0–0.26)	0.02 (0–1.01)
IL-2	0 (0–0)	0 (0–11.58)
IL-4	0 (0–1.45)	0 (0–8.46)
IL-5	0.06 (0–0.85)	0.75 (0.03–1.77)
IL-6	16.45 (9.33–40.61)	23.01 (12.17–44.76)
IL-7	0 (0–0)	0 (0–1.88)
IL-8	7.41 (0–17.29)	8.1 (0–27.45)
IL-10	0.63 (0.11–1.65)	1.13 (0.39–1.96)
IL-12	0 (0–0)	0(0–0)
IL-13	0.24 (0–2.01)	1.21 (0–4.69)
IL-17	0 (0–0)	0 (0–0)
IFN-γ	0 (0–1.05)	0 (0–14.01)
TNF-α	0.74 (0–1.91)	1.02 (0.07–3.04)
G-CSF	0 (0–17.61)	0 (0–0)
GM-CSF	0 (0–0)	0 (0–0)
MCP-1[a]	0 (0–20.82)	37.45 (0–51.62)
MIP-1β	58.26 (25.49–95.0)	55.71 (32.65–121.42)

Values are expressed as median (interquartile range).
[a] Significant difference between pre and post.

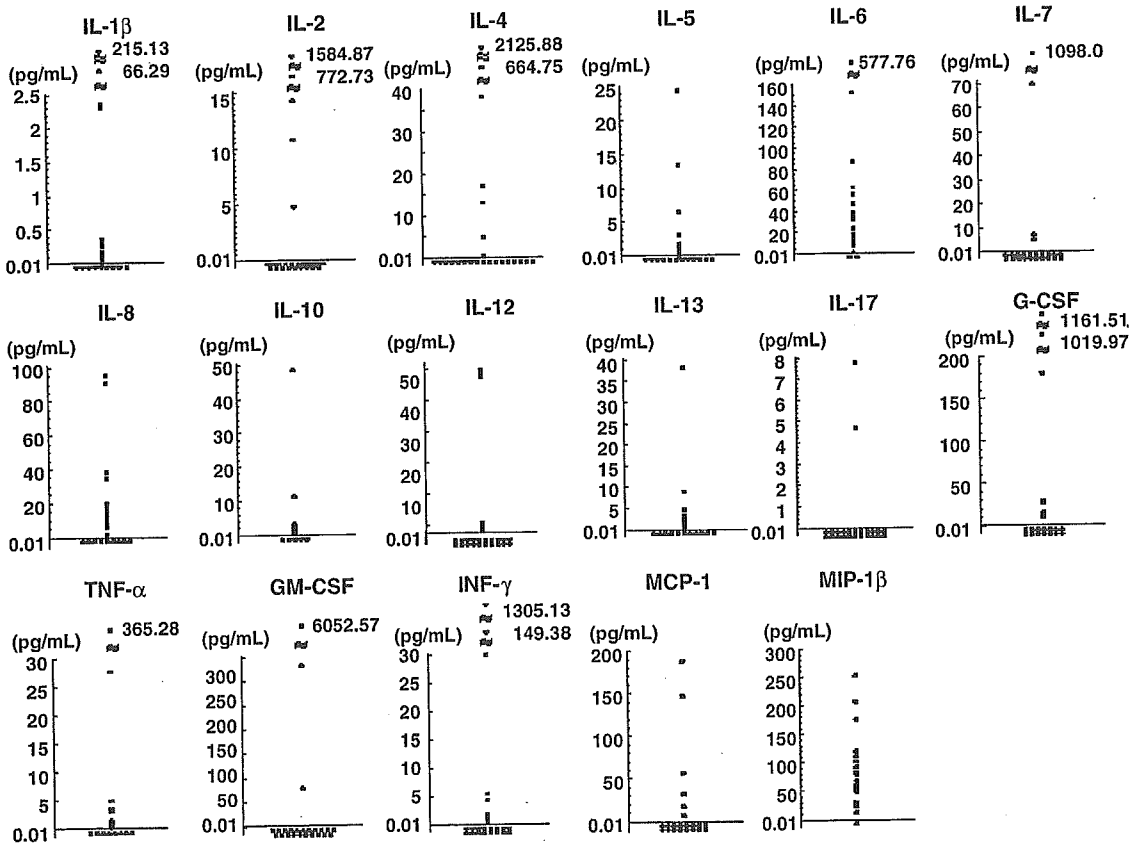

Fig. 1 Plasma concentrations of seventeen cytokines before the commencement of gefitinib in 24 patients. The plots under the line of 0.01 indicate levels lower than the measurement sensitivities.

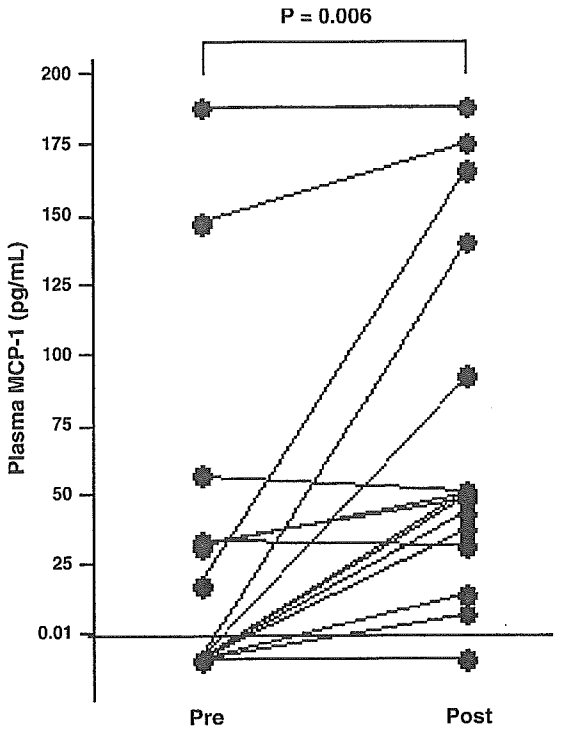

Fig. 2 Plasma concentrations of MCP-1 in 23 patients before and after gefitinib treatment. The differences between the values before and after treatment were significant ($p < 0.05$, paired t-test).

samples obtained after treatment than in those obtained before treatment ($p = 0.006$, by t-test, Fig. 2). There were no significant differences in the levels of any of the other cytokines measured.

3.3. Correlations between cytokine levels and the pharmacodynamic effects of gefitinib

The correlations between the cytokine levels and the clinical features of the patients, including the tumor response, symptomatic improvement, and the development of adverse events, were investigated using the two-sample t-test with a random variance model. There was no significant association between the levels of the various cytokines and the tumor response and symptomatic improvement in any of the patients. When the cytokine levels were comparatively analyzed depending on the grade of adverse events, the patients with skin toxicity (≧ Grade 1) showed significantly lower levels of MIP-1β as compared with those without skin toxicity (Grade 0) ($p = 0.042$, by two-sample t-test, Fig. 3). There was also a trend towards lower levels of IL-8 and IL-4, although the differences were not significant. In addition, the K-nearest neighbor prediction analysis ($K = 3$) showed the classification

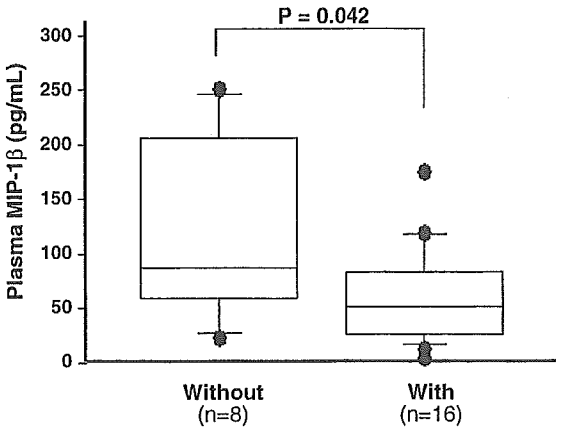

Fig. 3 Box-plots comparing the plasma concentrations of MIP-1β between patients with and without dermatitis; these samples were collected before the initiation of gefitinib. The patients were separated into two groups according to whether or not they developed dermatitis. Patients with skin toxicity showed significantly lower levels of MIP-1β as compared with those without skin toxicity ($p = 0.042$). The top and bottom quartiles and the mean values are depicted as box-plots. Bars indicate the 5th and 95th percentiles of the MIP-1β values.

rate to be 75% for the prediction sets containing MIP-1β, IL-4, and IL-8. There were no significant associations between the cytokine levels and any other parameters.

4. Discussion

The results of this study suggest that a correlation might exist between the serum levels of MIP-1β and the risk of development of skin toxicity during gefitinib administration, with lower levels of the cytokine being associated with a higher risk of appearance of the skin toxicity.

Skin toxicity has been reported to occur commonly in patients treated with EGFR-targeted agents, such as gefitinib, elrotinib, and cetuximab [6–14]. Numerous clinical studies have shown that skin toxicity is more frequently observed as compared to other toxicities during the administration of these drugs. Some studies have described the two major histological findings of the skin toxicity, as follows: presence of keratin plugs with microorganisms in dilated infundibula, and, purulent folliculitis surrounded by an infiltrate composed of lymphocytes and histiocytes, with the superficial portions of some follicles showing dense infiltration with neutrophilic granulocytes [9,31]. The skin toxicity induced by gefitinib has been reported to be similar to that induced by other EGFR-targeted agents, and is believed to result from direct

interference by the drug of the functions of EGFR signaling in the skin [32]. Since the blood levels of cytokines generally reflect the status of immune responses, these histological findings may suggest that the skin toxicity would be correlated with the plasma levels of some cytokines. On the other hand, in normal human skin, EGFR is expressed in the basal epidermal keratinocytes, sweat gland apparatus, and the hair follicle epithelium [33,34]. Therefore, the skin toxicity appears to be related to the mechanism of action of the EGFR-targeted agents and not to allergic reactions [35,36]. These characteristic changes, such as acneform eruptions and skin rashes, are probably secondary to an aberrant differentiation of suprabasal keratinocytes caused by EGFR inhibition.

The results in this study that lower level of plasma MIP-1β were correlated with skin toxicities. MIP-1β is a cysteine–cysteine chemokine that plays a role in inflammation and host defense mechanisms by interacting with its specific receptor CCR1, CCR5 and CCR8 [37,38]. MIP-1β is produced by monocytes, macrophages, lymphocytes and other cell types [39]. MIP-1β is closely related with inflammatory and immune responses. Then, we can arise two possible explanations to our evidence. Immune responses mediated by MIP-1β may play a rule in the healing process of keratinocytes damaged by EGFR-targeted agents. Another is that MIP-1β or its related factors may weaken the inhibiting power of the EGFR-targeted agents, although there is no supporting data for the speculations. Further studies are necessary to clarify the role of MIP-1β for cutaneous reactions.

In this study, 17 kinds of cytokine levels were measured using the bead-based multiplex assay. All of the cytokines were measurable with high sensitivity at once using 15 μl plasma sample volume. It is often difficult to obtain the tumor samples from the advanced non-small cell lung cancer patients. Then, the bead-based multiplex assay might be a useful assay system for biomarkers. This assay system is also able to be customized to detect phosphoproteines such as EGFR and ERK1/2 for the predictive marker for clinical response as the next step.

In conclusion, our results indicate that the plasma MIP-1β level may be a useful predictor of the risk of skin toxicity induced by EGFR-specific tyrosine kinase inhibitors.

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Dimerization and the signal transduction pathway of a small in-frame deletion in the epidermal growth factor receptor

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SPECIFIC AIM

A short, in-frame deletional mutant (E746-A750del) a major mutant form of EGFR in non-small cell lung cancer, and has been reported to be a major determinant of response to EGFR tyrosine kinase inhibitors such as gefitinib and erlotinib. However, the biological and pharmacological functions of mutational EGFR remain unclear. The aim of this study is to clarify whether it is constitutively active or not and whether alteration occurs downstream of the intracellular signaling.

PRINCIPAL FINDINGS

1. A short, in-frame deletional mutant (E746-A750del) induced dimerization and phosphorylation of EGFR without any ligand stimulation

To determine the biological functions of deletion mutant (E746-A750del) EGFR, we used the stable transfected cells of wild-type and deletion mutant of EGFR. Previously, we demonstrated that the 293(D) cells transfected with the deletional EGFR were hypersensitive to EGFR-targeted tyrosine kinase inhibitors such as gefitinib and ZD6474 as compared with the 293(W) cells transfected with wild-type EGFR. Dimerization and phosphorylation of EGFR in these cells were determined by using chemical cross-linker and by immunoblot analysis (**Fig. 1**). No expression of EGFR dimer or monomer was detected in the 293(M) cells. Increased dimerization and phosphorylation of the deletional EGFR with a molecular weight of ~400 kDa were detected without EGF stimulation in the 293(D) cells. When stimulated with the EGF, increased dimerized and phosphorylated EGFR were observed in the 293(W) cells, whereas no response of EGFR to EGF was observed in the 293(D) cells. The ratio of dimerized to monomeric EGFR in 293(W) and 293(D) cells was analyzed densitometrically (Fig. 1, right). The dimer/monomer ratio in the 293(W) cells was markedly increased (~3-fold) by addition of EGF. Under unstimulated conditions, the dimer/monomer ratio of the 293(D) cells was higher than that of the 293(W) cells and the ratio was unchanged by addition of EGF. These results suggest that the cells expressing the wild-type of EGFR responded to EGF for their dimerization and phosphorylation and that the deletional mutant of EGFR was dimerized and phosphorylated constitutively without any ligand stimulation.

2. p44/42 MAPK and AKT pathways are activated in the cells expressing deletional EGFR without ligand stimulation

We examined the phosphorylation status of p44/42 MAPK and AKT that are major downstream targets of EGFR in the transfectants. Even under unstimulated conditions, increased phosphorylation of p44/42 MAPK and AKT was observed in the 293(D) cells. In the 293(W) cells, increased phosphorylation of p44/42 MAPK and AKT was observed with the addition of EGF but p44/42 MAPK was remarkably phosphorylated. On the other hand, no increased phosphorylation of p44/42 MAPK and AKT was observed with the addition of EGF in the 293(D) cells. This result suggests that the p44/42 MAPK and AKT pathways are activated in cells expressing the deletional EGFR without ligand stimulation.

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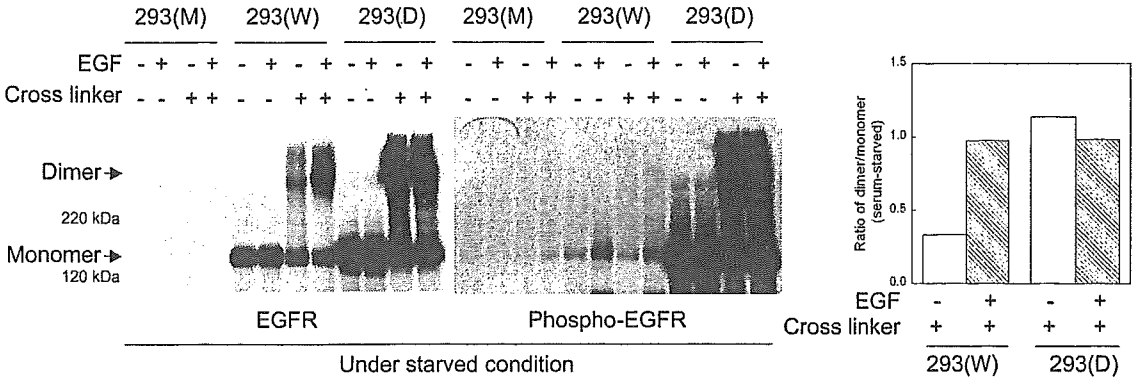

Figure 1. Dimerization and phosphorylation of wild-type EGFR and deletional EGFR A. The 293 cells transfected with the empty vector (293(M)), wild-type EGFR (293(W)), and deletional EGFR (293(D)) were treated with or without EGF (10 ng/mL) for 10 min after serum starvation. After two washes with ice-cold PBS(+), monolayer cells were incubated with the chemical cross-linking reagent BS^3 (1.5 mM) in PBS(+). Glycine (20 mM) was added for an additional 5 min to terminate the reaction. The lysates (twenty μg protein) were subjected to 2–15% SDS-PAGE followed by immunoblot analysis using anti-EGFR and anti-phospho-EGFR. Right panel: ratio of dimerized to monomeric EGFR.

3. Gefitinib inhibited the AKT signaling pathway more strongly than the p44/42 MAPK signaling pathway

We next determined the action of EGFR-targeted tyrosine kinase inhibitor gefitinib on downstream of deletional EGFR (**Fig. 2*A***). In the 293(W) cells, phosphorylation of p44/42 MAPK was not inhibited by exposure to a low dose of gefitinib (0.01 μM) but phosphorylation of AKT was inhibited by exposure to gefitinib (~70%, Fig. 2*C*). In contrast, exposure to gefitinib decreased phospho-EGFR in the 293(D) cells. Phosphorylation of AKT was completely inhibited by 0.01 μM gefitinib exposure (~99%, Fig. 2*C*), whereas inhibition of p44/42 MAPK phosphorylation was not remarkable in the 293(D) cells (~20%, Fig. 2*B*). These data suggest that gefitinib inhibited the AKT signaling pathway more strongly than the p44/42 MAPK signaling pathway in the cells expressing the deletion mutant EGFR.

4. AKT pathway was activated in the PC-9 cells expressing deletional EGFR intrinsically

To examine whether increased phosphorylation is also observed in the lung cancer cells intrinsically expressing deletional EGFR, we monitored the phosphorylation of EGFR and its related molecules in the PC-9 cells expressing deletional EGFR by using a beads-based mulitiplex assay. We found increased phosphorylation of EGFR and downstream molecules of AKT pathway including IκB-α in PC-9 cells. This finding is consistent with the result of the previous experiments with the 293(D) cells. It is suggested that AKT pathway is activated in the cells expressing deletional EGFR intrinsically.

CONCLUSIONS AND SIGNIFICANCE

To clarify the function of deletional EGFR, we used the cell transfectants with deletional EGFR [293(D)] that is

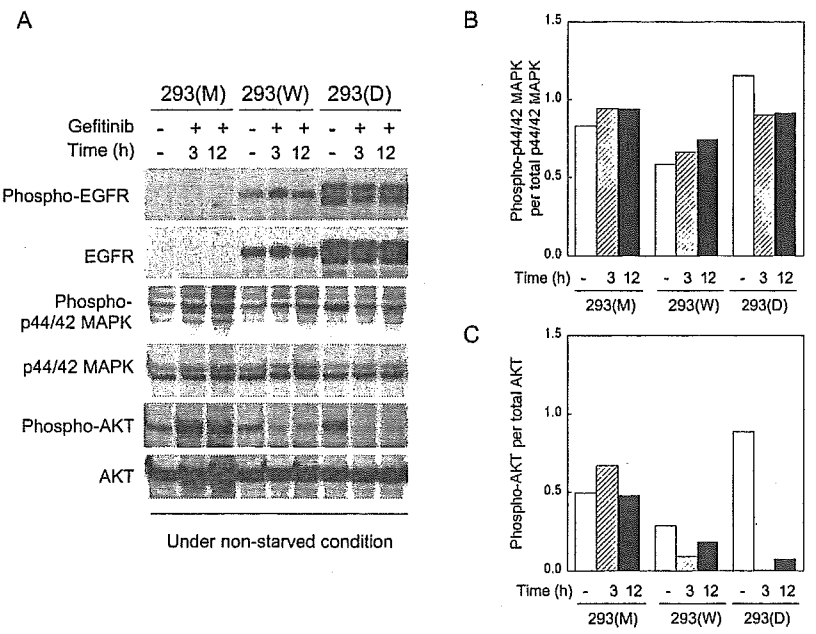

Figure 2. Effect of gefitinib on phosphorylation of EGFR, p44/42 MAPK, and AKT in the EGFR transfected 293 cells. *A)* The 293(M), 293(W), and 293(D) cells were incubated with gefitinib (0.01 μM) for 3 h or 12 h under nonstarved conditions. After two washes with ice-cold PBS(+), monolayer cells were lysed. Equivalent amounts of protein were separated by 2–15% gradient SDS-PAGE for EGFR or 10–20% for p44/42 MAPK, phospho-p44/42 MAPK, AKT, and phospho-AKT, then subjected to immunoblot analysis. *B)* Histogram of the degree of p44/42 MAPK activation expressed as phospho-p44/42 MAPK per total p44/42 MAPK. *C)* Histogram of the degree of AKT activation expressed as phospho-AKT per total AKT.

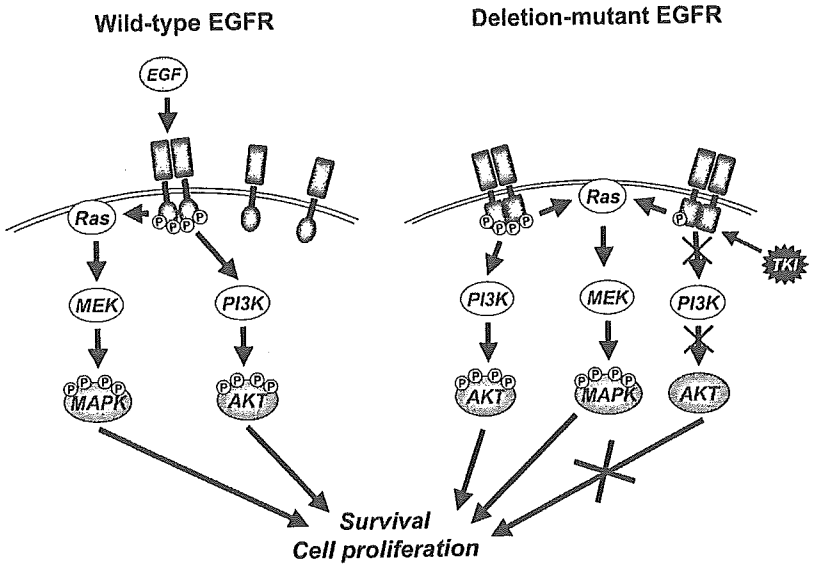

Figure 3. Function of deletional EGFR. Wild-type EGFR is dimerized and phosphorylated by EGF the wild-type EGFR and MAPK and AKT pathways are activated. The deletion mutant EGFR is dimerized and phosphorylated without EGF. Both MAPK and AKT pathways are activated; but phospho-AKT was inhibited by TKI predominantly in the cells expressing deletional EGFR. MEK, MAP kinase/extracellular regulated kinases; PI3K, phosphoinositide-3-kinase; TKI, tyrosine kinase inhibitors.

hypersensitive to tyrosine kinase inhibitors (e.g., gefitinib). We detected significantly higher levels of dimerization and phosphorylation of deletional EGFR without any ligand stimulation in the cells deletional EGFR. Increased phosphorylation of p44/42 MAPK and AKT was observed in the 293(D) cells. These results suggest that deletional EGFR is constitutively active. When the 293(D) cells were exposed to gefitinib (0.01 μM), AKT phosphorylation was completely suppressed, suggesting that deletional EGFR signaling inclines toward the AKT pathways. A summary of characteristics of deletional EGFR is shown in **Fig. 3.**

An additional experiment using a PC-9 lung cancer cell line intrinsically expressing deletional EGFR confirmed the gain of function of deletional EGFR and activated AKT signaling pathway.

Results from this study have provided the understanding for biological functions of deletional EGFR and cellular hypersensitivity to the EGFR-targeted tyrosine kinase inhibitor.

Now over 30 types of mutation have been reported in clinical lung cancer specimens. We will examine the biological function of other types of EGFR mutants differentially, with the aim of selecting clinically meaningful mutations. FJ

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A Phase I/II Study Comparing Regimen Schedules of Gemcitabine and Docetaxel in Japanese Patients with Stage IIIB/IV Non-small Cell Lung Cancer

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A Phase I/II Study Comparing Regimen Schedules of Gemcitabine and Docetaxel in Japanese Patients with Stage IIIB/IV Non-small Cell Lung Cancer

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Objective: Gemcitabine and docetaxel are non-platinum agents with activity in non-small cell lung cancer (NSCLC). This study was conducted to determine and evaluate the recommended regimen of gemcitabine–docetaxel and evaluated its efficacy and safety in chemonaive Japanese NSCLC patients.

Methods: In phase I, patients with stage IIIB/IV NSCLC were randomized and received either gemcitabine on days 1 and 8 plus docetaxel on day 1 or gemcitabine on days 1 and 8 plus docetaxel on day 8. The recommended regimen was the dose level preceding the maximum tolerated dose; once determined, patients were enrolled in phase II. Efficacy and toxicity were evaluated in all patients.

Results: Twenty-five patients were enrolled in phase I and six patients were given the recommended regimen; gemcitabine 1000 mg/m^2 on days 1 and 8 plus docetaxel 50 mg/m^2 on day 8. An additional 34 patients were enrolled into phase II and administered with the recommended regimen. The response rate was 32.2% [95% confidence interval (CI) 20.6–45.6%] overall and 30.0% (95% CI 16.6–46.5%) in patients with the recommended regimen (40 patients). Although grade 3 interstitial pneumonia was observed in two patients (5.0%) who received the recommended regimen, both recovered shortly after steroid treatment. No unexpected events were observed throughout this study.

Conclusions: Gemcitabine 1000 mg/m^2 on days 1 and 8 plus docetaxel 50 mg/m^2 on day 8 has comparable efficacy and more tolerable toxicities than previously reported platinum-based regimens. These results should be verified by a phase III study.

Key words: docetaxel – gemcitabine – non-small cell lung cancer

INTRODUCTION

Non-small cell lung cancer (NSCLC) is one of the most common malignant tumors, progresses in a short time period, has a bleak prognosis, and represents the leading cause of cancer death in the world. The number of patients with NSCLC is increasing, and most tumors are inoperable. Despite improvements in the detection and treatment of NSCLC, long-term survival is rare. Therefore, the development of new chemotherapy treatments is essential.

The use of single-agent and combination chemotherapy against NSCLC has been studied. Platinum-based regimens have shown high efficacy but at the cost of severe toxicities (1,2). Therefore, non-platinum agents such as gemcitabine, docetaxel, paclitaxel, irinotecan and vinorelbine have been developed and have proven their efficacies. Among the new agents, the combination of gemcitabine and docetaxel has emerged as one of the most promising, showing equivalent efficacy with, and less toxicity than, cisplatin-based chemotherapies (3).

Gemcitabine (2′-deoxy-2′,2′-difluorocytidine monohydrochloride) is a nucleoside antimetabolite against deoxycytidine. It is intracellularly metabolized to gemcitabine triphosphate,

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which inhibits DNA synthesis, and has shown potent cytocidal activity against solid tumors (4–8).

Docetaxel, an antineoplastic agent that acts on microtubules to promote formation of abnormal microtubule bundles, has also shown cytotoxicity (9–11). Gemcitabine and docetaxel have different mechanisms of action, but by combining them, there is the potential of synergistic antitumor activity (12).

Several studies have been conducted to evaluate the therapeutic benefits of gemcitabine and docetaxel (13–15). The efficacy of gemcitabine–docetaxel is similar to platinum-based regimens, but due to each drug's non-overlapping toxicities, their combination produces toxicities more tolerable than platinum-based regimens. Georgoulias et al. (16) compared gemcitabine 1100 mg/m^2 on days 1 and 8 plus docetaxel 100 mg/m^2 on day 8 with cisplatin 80 mg/m^2 on day 2 plus docetaxel 100 mg/m^2 on day 1 in 441 patients with NSCLC. They reported that the two regimens were equivalent in efficacy, but toxicities were more severe for the combination of docetaxel and cisplatin.

There has been no published report considering both administering dose and schedule for the combination of gemcitabine and docetaxel. Therefore, we conducted a phase I/II study to compare two schedules of gemcitabine–docetaxel in patients with NSCLC and determine the recommended regimen in phase II. We assessed the efficacy and safety in all 59 patients: the efficacy and detailed safety profile were also evaluated in 40 patients who were given the recommended regimen.

SUBJECTS AND METHODS

Eligibility Criteria

Japanese patients with histologically or cytologically confirmed unresectable TNM stage IIIB or IV NSCLC who met the following criteria were eligible for the study: suitable for first-line chemotherapy with no prior chemotherapy; measurable lesions that can be accurately measured in at least one dimension; aged 20–74 years; Eastern Cooperative Oncology Group (ECOG) performance status of 0–1; a life expectancy of at least 3 months; and adequate organ functions as indicated by white blood cell count $\geq 4.0 \times 10^9$/l, absolute neutrophil count $\geq 2.0 \times 10^9$/l, platelets $\geq 100 \times 10^9$/l, hemoglobin ≥ 9.5 g/dl, aspartate aminotransferase/alanine aminotransferase ≤ 2.5 times the upper limit of normal, total bilirubin ≤ 1.5 times the upper limit of normal, serum creatinine $\leq$ the upper limit of normal, PaO_2 in arterial blood ≥ 60 torr. If a patient had received radiotherapy during the 3 weeks before enrollment, the measurable disease had to be outside of the radiation port.

Patients were excluded from the study if they had radiologically and clinically apparent interstitial pneumonia or pulmonary fibrosis, intracavitary fluid retention requiring treatment, or grade 2–4 peripheral neuropathy or edema. Additional exclusion criteria included: superior vena cava syndrome; symptomatic brain metastasis; pregnancy or breast-feeding; active concurrent malignancy; any serious concurrent illness (e.g. uncontrolled diabetes mellitus, hepatopathy, angina pectoris, myocardial infarction within 3 months after onset, severe infection, or fever suggestive of severe infection); history of serious drug allergy; or any condition that, in the opinion of the investigator, disqualified the patient based on safety.

This study was conducted in accordance with the Declaration of Helsinki, Japanese Guidelines for Clinical Evaluation of Antineoplastic Agents (promulgated in February 1991) and good clinical practice. All patients who entered into this study were required to give written informed consent.

Study Design And Treatment

This was a multicenter, open-label, phase I/II study of gemcitabine and docetaxel in Japanese patients with advanced NSCLC.

In the phase I portion of this study, patients were randomized into two arms, each with a different treatment schedule. In both arms (Arm 1 and Arm 2), gemcitabine was administered in a 30-min infusion on days 1 and 8, every 21 days. In Arm 1, docetaxel was administered intravenously over at least 1 h on day 1; in Arm 2, docetaxel was given on day 8. The administration of docetaxel followed an intravenous infusion of dexamethasone 4 mg, and gemcitabine was given immediately after the docetaxel infusion.

Patients were discontinued from the study due to progressive disease; inability to initiate a treatment cycle even at 6 weeks after the start of the previous cycle; recurrence of a dose-limiting toxicity (DLT) after resumption of the study treatment at a reduced dose; occurrence of a serious adverse event or aggravation of a concomitant illness (e.g. interstitial pneumonia, pulmonary fibrosis, or severe infection) which caused rapid aggravation of disease and precluded continuation of the study treatment; patient's request to withdraw from the study; or any event that required discontinuation in the opinion of the investigator.

During study enrollment, the current approved maximum dosage of gemcitabine and docetaxel as single agents in Japan was 1000 mg/m^2 and 60 mg/m^2, respectively. In phase I, the sample size was determined to be six per cohort based on the conventional design of phase I clinical studies of antineoplastic agents. In this study, both arms were randomized according to a predetermined schedule, enrolled patients in cohorts of six, and were initially treated at dose level 1 (gemcitabine 1000 mg/m^2 and docetaxel 50 mg/m^2). For the first cycle of treatment, patients were treated on an inpatient basis; if their condition permitted, patients were treated on an outpatient basis thereafter. If fewer than 50% of the patients in dose level 1 experienced DLTs, patients were enrolled at dose level 2 (gemcitabine 1000 mg/m^2 and docetaxel 60 mg/m^2). If 50% or more of the patients in dose level 1 experienced DLTs, patients were enrolled at dose level 0 (gemcitabine 800 mg/m^2 and docetaxel 50 mg/m^2) (Fig. 1). The maximum tolerated dose (MTD) was defined as the dose level that produced any of the following DLTs (per the National Cancer Institute–Common

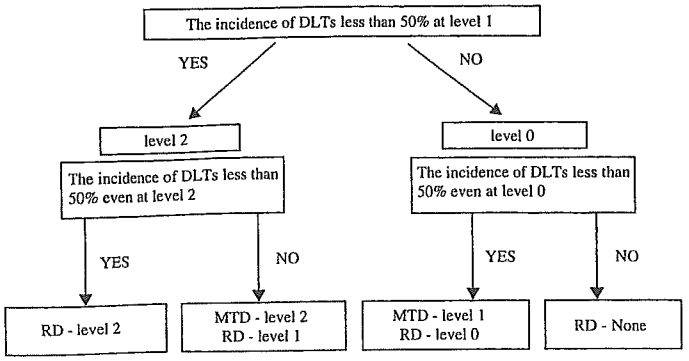

Figure 1. Recommended dosages in each arm. DLT, dose-limiting toxicity; RD, recommended dosage; MTD, maximum tolerated dose.

Toxicity Criteria scale) in 50% or more of patients during the first treatment cycle: grade 4 leukopenia or neutropenia persisting for at least 4 days; grade 3/4 neutropenia associated with a fever ≥38.0°C or infection; thrombocytopenia (<20 × 10^9/l) or need of a platelet transfusion; or grade 3/4 non-hematological toxicities (excluding nausea/vomiting, anorexia, fatigue and hypersensitivity). G-CSFs were administrated for the treatment of grade 4 neutropenia or grade 3 neutropenic fever. A DLT was also reported if any day-8 doses were omitted and dosing requirements were not satisfied until after day 15, or if the second cycle was delayed until after day 29 because the dosing requirements were not satisfied.

The recommended dose for phase II had to be determined from the arm that reached the highest dose level. If at dose level 2 the incidence of DLTs was less than 50%, the recommended dose was defined as dose level 2. The arm that reached the higher dose level reflected the recommended regimen for phase II. If the recommended dose level for the two arms was identical, the recommended regimen would be decided according to the following steps: (i) if frequency of DLTs was 0% in one arm and 33.3% or more in the other arm, the former was selected. If this did not occur, then (ii) if the dose intensity for evaluable patients in one arm was higher by 10% or more than the other arm, the arm with the higher dose intensity was selected. If this did not occur, then (iii) the arm with the fewer day-8 dose omissions in first and second cycles was selected. If the recommended dosage regimen still could not be decided, the sponsor (Aventis Pharma Japan and Eli Lilly Japan K.K.) and the coordinating investigator determined the recommended phase II regimen. If the MTD was dose level 0 in both arms, the study was terminated (Fig. 1).

The sample size for the recommended regimen was determined as follows. The response rate of this regimen and gemcitabine single agent was assumed to be 35 and 20%, respectively, in view of the response rates previously achieved (9,10,17,18). If the sample size of the recommended regimen was set as 40 patients, the probability for the one-sided 90% lower limit of response rate to exceed 20% was 82%. Thus, the target sample size in the recommended regimen including six patients in phase I was set at 40 patients.

The phase II study was conducted with 34 patients. Forty patients who were given the recommended regimen were evaluated for the efficacy and detailed safety profile: these patients consisted of six and 34 patients who entered into the study at phase I and II, respectively.

In this phase I/II study, patients received a minimum of two cycles of gemcitabine–docetaxel and up to four additional cycles.

Dose Modifications

During a cycle, dose modifications were not allowed. If not all of the following requirements were satisfied on either the day of treatment or the previous day, administrations of gemcitabine and docetaxel were delayed until the patient completely recovered. For gemcitabine and docetaxel doses administered on day 1 of Arm 1 or gemcitabine on day 1 of Arm 2, delays occurred for patients with an absolute neutrophil count <1.5 × 10^9/l, a platelet count <70 × 10^9/l, any grade 3/4 non-hematologic toxicities (except PaO_2), or PaO_2 <60 torr. When gemcitabine was given on day 8 of Arm 1, exceptions included leukopenia <2.0 × 10^9/l and an absolute neutrophil count <1.0 × 10^9/l, a platelet count <70 × 10^9/l, any grade 3/4 non-hematological toxicities. When gemcitabine was given on day 8 of Arm 2, exceptions included an absolute neutrophil count <1.5 × 10^9/l, a platelet count <70 × 10^9/l, any grade 3/4 non-hematological toxicities. If a patient developed a DLT, the subsequent doses were cancelled, and in the next cycle the patient could resume the study treatment at the next lower dose level. If a patient developed a DLT at dose level 0, gemcitabine 800 mg/m^2 and docetaxel 40 mg/m^2 were administered in the next cycle.

Baseline and Treatment Assessment

Assessments at baseline included tumor measurements by X-ray and computed tomography (CT) scan within 4 weeks before the day of starting the study treatment. Equally, grading performance status and physical examination were performed within a week; hematology, blood chemistries, urinalysis, arterial blood gas analysis and electrocardiogram were observed within 2 weeks.

After the start of treatment, tumor measurements were obtained every 2 weeks via X-ray and 4 weeks via CT scan. Tumor response was assessed with the World Health Organization (WHO) criteria. Safety assessments, including performance status, hematology, blood chemistries and urinalysis, were obtained weekly. Physical examination, arterial blood gas analysis and electrocardiogram were performed at any time. Adverse events were estimated according to National Cancer Institute–Common Toxicity Criteria version 2.0. All patients were assessed for efficacy and safety. An additional response rate was recorded for patients who received the recommended regimen in phase I and all phase II patients.