TABLE 2. Summary of Docetaxel Therapy in the Two Groups

	Group P (N = 127)		Group NP (N = 100)		
Characteristics	**No.**	**(%)**	**No.**	**(%)**	***p***
Treatment administration					
Median (range)	3	1–17	3	1–13	0.596
Response to docetaxel therapy					
CR	0	(0)	1	(1.0)	0.256
PR	18	(14.2)	15	(15.0)	
SD	81	(63.8)	54	(54.0)	
PD	24	(18.9)	22	(22.0)	
NE	4	(3.1)	8	(8.0)	
CR/PR	18	(14.2)	16	(16.0)	0.702
CR/PR/SD	99	(78.0)	70	(70.0)	0.173
Median survival time, mo (95% CI)	10.9 (7.6–14.1)			11.1 (8.6–13.5)	0.567

CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; NE, not evaluable.

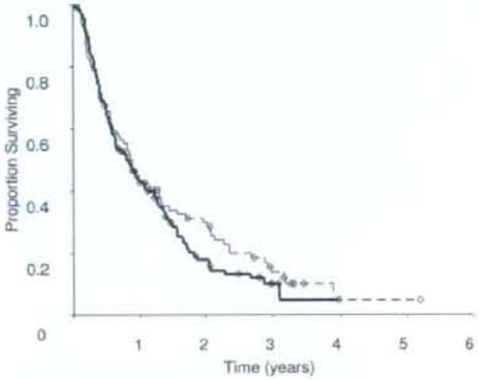

FIGURE 1. Overall survival classified by the previous chemotherapy regimens. Continuous line: carboplatin and paclitaxel (group P, $n = 123$); and dotted line: platinum and an agent other than paclitaxel (group NP, $n = 97$). Hazard ratio (95% confidence interval): 1.09 (0.81–1.47).

months) (Figure 1). The response rates to docetaxel in good and poor responders to previous chemotherapy were 21.8% and 9.4%, respectively, in group P ($p = 0.074$), and 25.0% and 12.0%, respectively, in group NP ($p = 0.164$). The overall survival did not differ between the good and poor responders (Figure 2).

The result of univariate and multivariate analysis of the response to the docetaxel are shown in Table 3. In the multivariate analysis adjusted for sex, age, PS at the start of docetaxel therapy, the response to previous chemotherapy significantly influenced the response to subsequent docetaxel therapy (odds ratio [OR]: 2.93; 95% CI: 1.28–6.72). The previous chemotherapy regimen (OR: 1.38; 95% CI: 0.63–3.01), and interval between the final administration of the previous chemotherapy and the start of docetaxel therapy (OR: 0.4; 95% CI: 0.86–1.02) were not found to be significant factors influencing the response to docetaxel therapy. The impact of the responses to the previous chemotherapy was denoted the same tendency in the analysis of each group (OR: 3.82; 95% CI: 1.09–13.5 for group P, and OR: 2.13; 95% CI: 0.67–6.70 for group NP). The result of univariate and multivariate analysis of the overall survival is shown in Table 4. Neither the response to nor the regimen used in the previous chemotherapy had significant impact. Interval between the final administration of the previous chemotherapy and the start of docetaxel therapy were statistically significant in the overall survival.

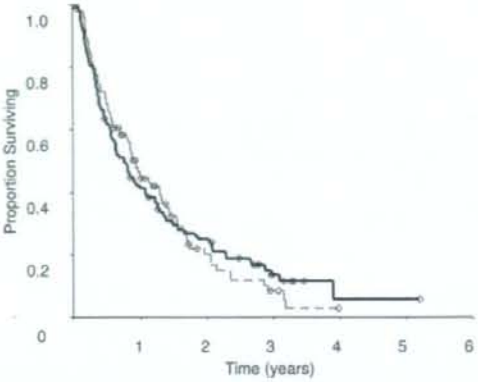

FIGURE 2. Overall survival classified by the responses to previous chemotherapy. Continuous line: SD/PD ($n = 118$); and dotted line: CR/PR ($n = 102$). Hazard ratio (95% confidence interval): 0.91 (0.68–1.23).

DISCUSSION

The purpose of this study was to evaluate the influence of previous chemotherapy on the efficacy of subsequent docetaxel chemotherapy. Above all, our major question was whether the regimen of previous chemotherapy, especially the use of paclitaxel, would have any influence on the subsequent docetaxel therapy. In previous studies, response to docetaxel therapy had no association with prior exposure to or the efficacy of paclitaxel therapy, but details about the paclitaxel treatment are not described in these reports.[6,7] In our study, by dividing patients according to the previous regimen received, we showed that the previous use of paclitaxel had no impact on the response to subsequent docetaxel therapy, and that the response to previous chemotherapy was associated with the response to, but not to the survival, after subsequent docetaxel therapy.

Although both paclitaxel and docetaxel are widely used, the influence of prior use of paclitaxel on the response to subsequent docetaxel therapy has not yet been thoroughly reviewed in cases of NSCLC. In the TAX320 study conducted by the Non-Small Cell Lung Cancer Study Group, 31% (114 of 373) of patients had a history of prior use of paclitaxel.[6] In that study, previous exposure to paclitaxel had

TABLE 3. Univariate and Multivariate Analyses of the Response to Docetaxel (N = 210)

	Univariate			Multivariate		
	OR	95% CI	*p*	OR	95% CI	*p*
Entire						
Response to previous chemotherapy (SD/PD vs CR/PR)	1.12	0.57–2.50	0.63	2.93	1.28–6.72	0.01
Regimen of previous chemotherapy (group P vs group NP)	0.84	0.40–1.75	0.84	1.38	0.63–3.01	0.421
Interval (with a 30-d increase)	0.97	0.91–1.05	0.48	0.94	0.86–1.02	0.14
Group P						
Response to previous chemotherapy (SD/PD vs CR/PR)	2.70	0.94–7.76	0.07	2.13	0.67–6.70	0.20
Interval (with a 30-d increase)	1.04	0.96–1.12	−0.39	1.01	0.92–1.11	0.06
Group NP						
Response to previous chemotherapy (SD/PD vs CR/PR)	2.37	0.78–7.19	0.13	3.82	1.09–13.5	0.04
Interval (with a 30-d increase)	0.88	0.75–1.02	0.10	0.84	0.69–1.01	0.80

Multivariate analysis was adjusted for sex, age, and performance status at the start of docetaxel.

OR, odds ration; HR, hazard ration; P, carboplatin and paclitaxel; NP, platinum and an agent other than paclitaxel; Interval, days between previous therapy and the start docetaxel chemotherapy; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

TABLE 4. Univariate and Multivariate Analyses of Overall Survival (N = 220)

	Univariate			Multivariate		
	HR	95% CI	*p*	HR	95% CI	*p*
Entire						
Response to previous chemotherapy (SD/PD vs CR/PR)	0.91	0.68–1.23	0.56	0.90	0.66–1.22	0.484
Regimen of previous chemotherapy (group P vs group NP)	1.09	0.81–1.47	0.57	0.88	0.65–1.20	0.43
Interval (with a 30-d increase)	0.97	0.94–0.99	0.01	0.96	0.94–0.99	0.01
Group P						
Response to previous chemotherapy (SD/PD vs CR/PR)	0.95	0.64–1.41	0.80	0.92	0.60–1.41	0.71
Interval (with a 30-d increase)	0.98	0.94–1.02	0.32	1.01	0.92–1.11	0.13
Group NP						
Response to previous chemotherapy (SD/PD vs CR/PR)	0.86	0.55–1.34	0.86	0.89	0.57–1.40	0.63
Interval (with a 30-d increase)	0.96	0.92–0.99	0.02	0.84	0.69–1.01	0.03

Multivariate analysis was adjusted for sex, age, and performance status at the start of docetaxel.

OR, odds ration; HR, hazard ration; P, carboplatin and paclitaxel; NP, platinum and an agent other than paclitaxel; Interval, days between previous therapy and the start docetaxel chemotherapy; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

no impact on the survival of patients who received docetaxel as second-line treatment; however, neither the data of survival nor the details of paclitaxel therapy have been described in the report. In a study comparing pemetrexed and docetaxel in 571 patients, 153 patients (25%) had received paclitaxel.[7] Although the results of the study showed that paclitaxel sensitivity/resistance in the first-line treatment did not predict any difference in the response between pemetrexed and docetaxel used for second-line treatment (details not shown), there were no data comparing the patients according to a history of previous use of paclitaxel.[7] In a study reassessing these data, 20% (113 of 571) of patients had previously received both paclitaxel and platinum, and the previous chemotherapy regimen had no influence on the overall survival.[12] However, the method used for the analysis, namely, assessment of the overall population treated with docetaxel or pemetrexed together, is inappropriate to evaluate the association of previous paclitaxel use with the efficacy of subsequent docetaxel therapy. Patients who had no history of prior taxane treatment were even excluded in some previous phase III studies comparing docetaxel with best supportive care or other agents as second-line treatment.[5,8] In this study, by comparing the patients according to the history of previous use of paclitaxel, we could show specifically that exposure to paclitaxel had no effect on efficacy of subsequent docetaxel therapy.

Although docetaxel and paclitaxel exert their activity via a similar mechanism of action, that is, by interfering with microtubular function and promoting tubulin polymerization and inhibiting the depolymerization of microtubules, the preclinical and clinical activity profiles of the two agents have been shown to exhibit some differences, with partial cross-resistance.[13] Preclinical studies have demonstrated docetaxel to be a 100-fold more potent than paclitaxel in inducing bcl-2 phosphorylation and apoptotic cell death, and the cellular uptake of docetaxel is known to be greater than that of paclitaxel, both of which lead to greater cytotoxic activity of docetaxel.[14] There has been a phase II study of docetaxel in breast cancer patients showing resistance to paclitaxel; objective responses were seen in 18% (8 of 44) of the patients, and the dose or efficacy of previous paclitaxel administration had no impact on the frequency of objective responses. This indicates that there was perhaps a partial cross-resistance between the two agents in patients of breast cancer.[15] Our study results indicate that this might also be the case in patients of NSCLC.

One of the tentative factors for better survival following second-line chemotherapy is the interval elapsed after the previous chemotherapy. This factor is a possible sign of efficacy of previous chemotherapy, but in the analysis of survival, it is difficult to distinguish whether this factor influences the response to chemotherapy or represents the characteristics of the disease in an individual. Therefore, the interval between two chemotherapy sessions has not been well established as a factor potentially influencing the response in previous studies on NSCLC patients.[5–8,16,17] Some of the studies showed that a longer interval from the last chemotherapy was significantly associated with increased survival.[7,12] In our study, interval between two chemotherapies was associated with the overall survival but not with response, which suggests that this factor have little influence on the antitumor activity of docetaxel therapy, but is representing the characteristics of the tumor.

Difference in the proportions of patients receiving surgery or radiation therapy between the two groups may be a big concern. These local therapies, however, should have only a small influence, if any, because all patients in this study had a metastatic disease at the time of recurrence and start of docetaxel therapy. Although responses to previous chemotherapy in patients treated with chemoradiotherapy could not be evaluated in the same way as the patients treated with chemotherapy alone, the response rates to previous chemotherapy did not differ between the groups P and NP (44.9% in group P, and 45.0% in group NP). Thus, we believe that these populations were appropriately included in our study.

In conclusion, the results of our study showed that docetaxel therapy was similarly active in patients with NSCLC, who had previously been treated with paclitaxel, and the response to previous chemotherapy was predictive of the response to subsequent docetaxel therapy. In the future, many promising agents, whether cytotoxic or molecule-targeted agents, may be developed for the second-line treatment of NSCLC. In the era of abundantly available agents, it will be meaningful to know which patients are likely to derive the most benefit from a particular agent. The results of this study are expected to be helpful for the selection of patients with advanced NSCLC who would benefit from docetaxel therapy.

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Importance of *UDP-glucuronosyltransferase 1A1**6* for irinotecan toxicities in Japanese cancer patients

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Abstract

Recent pharmacogenetic studies on irinotecan have revealed the impact of *UDP glucuronosyltransferase (UGT) 1A1*28* on severe irinotecan toxicities. Although the clinical role of *UGT1A1*6*, which is specifically detected in East Asian patients, in irinotecan toxicities is suggested, clear evidence remains limited. To examine the impact of **6*, the association of *UGT1A1* genotypes with severe irinotecan toxicities was retrospectively investigated in Japanese cancer patients. A significant **6*-dependent increase in the incidence of grade 3 or 4 neutropenia was observed in 49 patients on irinotecan monotherapy ($p = 0.012$). This study further clarifies the clinical importance of **6* in irinotecan therapy in East Asians.

Keywords: UGT1A1; Pharmacogenetics; Irinotecan; SN-38

1. Introduction

Irinotecan, an anticancer prodrug, is widely applied for a broad range of carcinomas, including colorectal and lung cancers. The active metabolite, SN-38 (7-ethyl-10-hydroxycamptothecin), a topoisomerase I inhibitor, is generated by hydrolysis of the parent compound by carboxylesterases [1]. SN-38 is subsequently glucuronidated by uridine diphosphate glucuronosyltransferase 1As (UGT1As) such as 1A1, 1A7, 1A9 and 1A10, to form the inactive metabolite, SN-38 glucuronide (SN-38G) [2–5]. Among the UGT

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isoforms, UGT1A1 is thought to be a predominant contributor to SN-38G formation [2,6]. The dose-limiting toxicities in irinotecan therapy are severe diarrhea and leucopenia [7], and lowered UGT activity is well correlated with severe irinotecan toxicities [8]. Since Ando et al. first reported the significant relevance of *UGT1A1*28* – a repeat polymorphism in the TATA box (−40_−39insTA) – to severe neutropenia/diarrhea [9], a number of clinical studies, primarily conducted in Caucasian patients, have shown associations between *UGT1A1*28* and lowered SN-38G formation or severe neutropenia/diarrhea [10–13]. Based on these findings, the Food and Drug Administration (FDA) of the United States approved a revision of the label for Camptosar (irinotecan HCl) (NDA 20-571/S-024/S-027/S-028), recommending "a reduction in the starting dose by at least one level of irinotecan for the *UGT1A1*28* homozygous patients". Subsequently, the clinical application of *UGT1A1*28* testing was put into practice for irinotecan therapy in the United States.

To implement personalized irinotecan therapy in Asian countries, the racial differences in *UGT1A1* polymorphisms among Caucasians, African-Americans, and Asians must be taken into consideration [14]. For East Asians, the frequency of **28* is one third of that of Caucasians or African-Americans, and another low-activity allele **6* [211G>A(G71R)], which is not detected in Caucasians or African-Americans, shows the same frequency as the **28* allele. Clinical studies in Japanese cancer patients have demonstrated that significantly low area under concentration-time curve (AUC) ratios of SN-38G to SN-38 are observed in patients having **6* and/or **28* [15–17], suggesting the necessity of typing **6* in addition to **28*. A recent report on Korean lung cancer patients who received a combination therapy of irinotecan and cisplatin, showed a significant association of **6* homozygotes with severe neutropenia [18]. However, data on the role of **6* in irinotecan toxicities is still limited in terms of the various irinotecan-containing regimens. In the first study by Ando et al. on Japanese cancer patients, the association of **6* with irinotecan toxicities was not evident, but a possible enhancement of **28*-related toxicities by **6* was suggested [9]. Other studies in Japanese patients showed an additive effect of **6* on the lowered UGT activity by **28* [15–17]. A significant association of the genetic marker "**6 or *28*" with severe neutropenia was also shown in our previous study, but due to a lack of **6* homozygotes in our patient population, the effect of **6* alone was not confirmed [17].

In this study, to further demonstrate the clinical importance of **6* alone, *UGT1A1* genotypes were determined using DNA extracted from paraffin-embedded specimens (non-cancerous tissues) from 75 Japanese cancer patients by the pyrosequencing method [19,20], and the associations between *UGT1A1* genotype and severe irinotecan toxicities and serum total bilirubin levels were retrospectively analyzed.

2. Materials and methods

2.1. Patients and irinotecan treatment

In a post-marketing surveillance study conducted by Daiichi Pharmaceutical Co., Ltd. (currently Daiichi Sankyo Co., Ltd., Tokyo, Japan), irinotecan was prescribed to 297 patients with various types of cancers from 1995 to 2000 at the National Cancer Center Hospital. The patients were selected through standard clinical practice according to the drug label for indications and contraindications. Methanol-fixed, paraffin-embedded archival tissue specimens, which were necessary for high-quality extraction of DNA greater than 2 kb in size [21], were available for 75 of the 297 patients and were analyzed in this study. Irinotecan was administered by intravenous 30-min infusion as a single agent or in combination chemotherapy at a dose of 60 mg/m^2 (weekly or biweekly), 100 mg/m^2 (biweekly), or 150 mg/m^2 (biweekly). Profiles of the patients in this study, including cancer type, treatment history, and regimens, are summarized in Table 1. The pre-treatment levels of serum total bilirubin were determined by a kit (VL T-BIL, Azwell Inc., Osaka, Japan) according to an enzymatic method using bilirubin oxidase [22]. Toxicities were monitored during irinotecan therapy and graded according to the Common Toxicity Criteria version 2 of the National Cancer Institute.

Because the samples in this study were residual specimens remaining after histopathological diagnosis in the hospital and not collected specifically for research purposes, the samples and their clinical information were anonymized in an unlinkable fashion according to the Ethics Guidelines for Human Genome/Gene Analysis Research by the Ministry of Education, Culture, Sports, Science and Technology, Ministry of Health, Labour and Welfare, and Ministry of Economy, Trade and Industry of Japan. This study was approved by the ethics committees of the National Cancer Center and the National Institute of Health Sciences.

2.2. DNA extraction from paraffin-embedded tissue sections and genotyping of UGT1A1 polymorphisms

Three sections (20 µm of pathologically normal tissues around tumors) were deparaffinized twice by treatment with 1.5 ml of xylene at room temperature. After centrifugations, the residual pellet was then washed twice with 1.5 ml of ethanol. Finally, the pellet was dried at 37 °C for 15 min. DNA extraction was performed using a QIAamp tissue kit (QIAGEN K.K., Tokyo, Japan) according to the manufacturer's instructions with some modifications. Briefly, 540 µl of ATL lysis buffer and 60 µl of proteinase K (Qiagen) were added to each pellet, mixed thoroughly, and incubated at 56 °C for 3 h with a rotator. Any remaining tissue debris was removed by centrifugation, and the resulting supernatant was used for the extraction. Twelve microliters of RNase A (100 mg/ml) was added to the supernatant and incubated for 2 min at room temperature. Next, 600 µl of buffer AL was added and mixed thoroughly, and the mixture was incubated at 70 °C for 10 min. Six-hundred microliters of ethanol was added to the solution and mixed well, followed by extraction of DNA using a Qiagen DNA extraction column. The DNA was eluted in a final elution volume of 150 µl. The yield was determined using a NanoDrop spectrophotometer (NanoDrop Technology, Inc, Rockland, DE, USA) and the size of the extracted DNA was checked by agarose gel electrophoresis.

Table 1
Profiles of cancer patients in this study

		No. of patients
Patients genotyped (Male/female)		75 (51/24)
Age		
Mean/range (y)	50.7/34–75	
Performance Status[a]	0/1/2	18/48/8
Previous treatment		
Surgery[a]	+/−	71/3
Chemotherapy[b]	+/−	63/10
Radiotherapy[b]	+/−	9/64
Combination therapy and tumor type [dose of irinotecan (mg/m^2)/(w or 2w)[c]]		
Irinotecan monotherapy	Lung (60/w or 100/2w)	4
	Stomach (100/2w or 150/2w)	5
	Colon (100/2w or 150/2w)	40
With cisplatin	Lung (60/w or 100/2w)	4
	Stomach (60/2w)	11
With mitomycin C (MMC)	Stomach (150/2w)	8
	Breast (120/2w)	1
With 5-fluorouracil (5-FU)	Colon (150/2w)	2
Available data on serum bilirubin levels		37

[a] Data from one patient is lacking.

[b] Data from two patients are lacking.

[c] Weekly or biweekly.

Genotyping of *UGT1A1**6* (211G>A, G71R), **28* (−364C>T, which is perfectly linked with −40_−39insTA in Japanese), and **60* (−3279T>G) were performed by pyrosequencing as described previously [19,20].

2.3. Association analysis and statistics

For association analysis, we focused on incidences of severe diarrhea and neutropenia (grade 3 or greater) observed during irinotecan-therapy. The incidence of severe diarrhea was very low, and the incidence of neutropenia was higher in combination therapy. Therefore, the association of neutropenia with *UGT1A1* genotypes was primarily evaluated in 49 patients with irinotecan monotherapy. As a parameter for in vivo UGT1A1 activity, serum total bilirubin levels taken at baseline from 37 patients were also used.

Statistical analysis for evaluation of the relationship between *UGT1A1* genotypes and severe neutropenia was performed using the chi-square test for trend using Prism version 4.0 (GraphPad Prism Software Inc., San Diego, CA). The gene-dose effect of the genetic marker "**6 or* **28*" on serum total bilirubin levels was analyzed using the Jonckheere–Terpstra (JT) test in the SAS system (version 5.0, SAS Institute, Inc., Cary, NC). The *P*-value of 0.05 (two-tailed) was set as a significant level. Multivariate logistic regression analysis on neutropenia (grade 3 or greater) was performed using JMP software (version 6.0.0, SAS Institute, Inc., Cary, NC), including variables for age, sex, body surface area, performance status, concomitant disease, history of adverse reaction, irinotecan dosage, dosing interval, and *UGT1A1* genotypes. The variables in the final model for neutropenia were chosen using the forward and backward stepwise procedure at the significance level of 0.1.

3. Results

3.1. UGT1A1 diplotypes/haplotypes

The diplotypes and haplotypes (**1*, **60*, **6* and **28*) of *UGT1A1* exon 1 were analyzed in 75 Japanese cancer patients (Table 1) and their frequencies were summarized (Table 2). The haplotypes were assigned according to our previous definition [15]. It should be noted that the **60* haplotype does not harbor the **28* allele (−40_−39insTA), but most of the **28* haplotype does harbor the **60* allele (−3279T>G). In this study, the **28* homozygote was not present, and the frequency of haplotype **28* (0.113) was slightly lower than that found in our previous study (0.138) [17]. In contrast, the frequency of haplotype **6* (0.213) was higher than that found in the previous study (0.167) [17].

Table 2
Frequencies of *UGT1A1* diplotypes (A) and haplotypes (B) for cancer patients in this study

		Frequency
(A) Diplotype	No. of patients (N = 75)	
*1/*1	21	0.280
*1/*60	9	0.120
*60/*60	2	0.027
*6/*1	14	0.187
*6/*60	8	0.107
*6/*6	4	0.053
*28/*1	12	0.160
*28/*60	3	0.040
*28/*6	2	0.027
*28/*28	0	0.000
(B) Haplotype[a]	No. of chromosomes (N = 150)	
*1	77	0.513
*60	24	0.160
*6	32	0.213
*28	17	0.113

[a] Haplotype definition follows the previous report [15]; **60*, −3279T>G without −40_−39insTA; **6*, 211G>A(G71R); **28*, −40_−39insTA.

3.2. Association of UGT1A1 genotypes with serum total bilirubin levels

Serum total bilirubin levels at baseline, a parameter of in vivo UGT1A1 activity, were available from 37 patients (treated by various regimens), and we analyzed their association with *UGT1A1* genotypes (Fig. 1). The median values of total bilirubin in **60/*1*, **28/*1* and **6/*1* heterozygotes were not significantly different from that of the wild type (**1/*1*). Higher median values were observed for the **6* homozygotes (**6/*6*) and the double heterozygotes of **6* and **28* (**6/*28*) than that of the wild type (**1/*1*), with increases of 1.9-fold and 2.2-fold, respectively. Since **6* and **28* are mutually independent and their reducing effects on UGT activity are equivalent [15,17], diplotypes were classified by the presence of "**6 or *28*" (indicated by "+" in Fig. 1). As shown in Fig. 1, a significant "**6 or *28*"-dependent increase in total bilirubin levels was observed ($p = 0.0088$, Jonckheere–Terpstra test).

3.3. Severe toxicities observed in this study

Incidences of severe diarrhea and neutropenia (grade 3 or greater) are shown in Table 3 for each irinotecan-containing regimen. Grade 3 diarrhea was observed in only 4 of the 75 subjects, and since the incidence of diarrhea was low (5.3%), an association analysis on diarrhea was not conducted. Regarding neutropenia, 26 patients experienced grade 3 or 4 neutropenia. Of these 26 patients, 90% experienced neutropenia within 2 months after starting irinotecan-therapy, and 70% within 2 weeks. Significant differences in neutropenia incidences were observed among the regimens used, and considerably high incidences were observed in the combination therapies. Accordingly, association of the *UGT1A1* genotypes with severe neutropenia was analyzed primarily in the patients who received irinotecan-monotherapy.

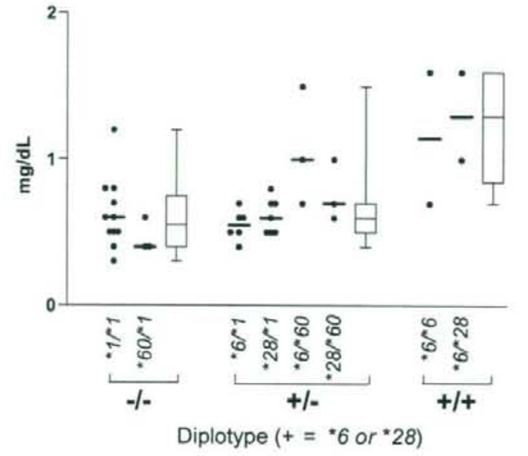

Fig. 1. Effects of *UGT1A1* genotypes on serum total bilirubin levels at baseline in Japanese cancer patients ($N = 37$). Each point represents a patient, and the median value of each diplotype is shown with a bar. All diplotypes are classified into −/−, +/−, and +/+ by the genetic marker, "*UGT1A1*6 or *28*", indicated by "+", and their distributions are shown by a box representing the 25–75 percentiles with a bar at the median and lines representing the highest and lowest values. A significant "**6 or *28*"-dependent increase in total bilirubin levels was observed ($p = 0.0088$, Jonckheere–Terpstra test).

Table 3
Severe toxicities observed in Japanese cancer patients

Treatment	Diarrhea[a]/total (%)	Neutropenia[b]/total (%)
Total patients	4/75 (5.3)	26/75 (34.7)
Irinotecan alone	1/49 (2.0)	6/49 (12.2)
With CDDP	2/15 (13.3)	11/15 (73.3)
With MMC	1/9 (11.1)	8/9 (88.9)
With 5-FU	0/2 (0.0)	1/2 (50.0)
P-value[c]	NS	<0.0001

[a] Grade 3.
[b] Grade 3 or 4.
[c] Chi-squre test.

3.4. Association of UGT1A1 genotypes with neutropenia

Since significant associations of *UGT1A1*6* and **28* with increased total bilirubin levels (decreased UGT-activity) were once again confirmed in this study, we assessed the clinical relevance of these haplotypes, focusing on the effect of **6* on severe neutropenia. In the 49 patients who received irinotecan monotherapy, the incidence of grade 3 or 4 neutropenia was _*6_-dependently increased ($p = 0.012$ in the chi-square test for trend). Namely, incidences of severe neutropenia in the _*6_ heterozygotes (_*6/*1_, _*6/*60_, and _*6/*28_) and homozygotes (_*6/*6_) were 2.3-fold and 15-fold higher, respectively, than that seen in the non-_*6_ bearing patients (_*1/*1_, _*60/*1_, _*28/*1_, and _*28/*60_) (Table 4). In this study, no _*28_ heterozygotes (_*28/*1_ and _*28/*60_) experienced any severe neutropenia, and there were no _*28_ homozygotes enrolled. Therefore, the effect of _*28_ could not be determined. For the _*60_-bearing patients without _*6 or *28_ (only heterozygote, _*60/*1_), one patient among six experienced severe neutropenia, and no significant _*60_-dependent increase was observed (data not shown). Although no statistically significant association of the _*28_ heterozygotes with severe neutropenia was confirmed in this study, the incidence of discontinuation of irinotecan monotherapy was higher in the _*28_-bearing patients (91%, $N = 11$) than that in the non-_*28_ subjects (79%, N = 38), while _*60_- or _*6_-dependent increased discontinuation rates were not found (data not shown). For the patients with cisplatin-combination therapy, a higher incidence of severe neutropenia was observed in the _*6_-bearing patients (_*6/*1_, _*6/*60_, and _*6/*6_) (100%, $N = 3$) than that in the non-_*6_ bearing subjects (_*1/*1_, _*60/*1_, _*60/*60_, and _*28/*1_) (66.7%, $N = 12$).

3.5. Multivariate analysis of neutropenia

In order to further clarify the clinical impact of _*6_ on irinotecan toxicities, multivariate logistic regression analysis on grade 3 or 4 neutropenia was conducted using variables, including _UGT1A1_ genotypes and patient background factors, described in Section 2. The final model revealed a significant association of _*6_ with the incidence of grade 3 or 4 neutropenia at an odds ratio of 5.87 (Table 5).

4. Discussion

The clinical application of the genetic test for _UGT1A1*28_ prior to irinotecan therapy has been in practice in the United States since 2005, which was based on cumulative evidence supporting the significant association of _*28_ with severe irinotecan toxicity [9–13]. Most of the evidence was obtained in Caucasian patients, where _*28_ is relatively frequent (30–40%) [14]. Although additive effects of another low activity allele, _*6_, which is specific for East Asians, has been also suggested [9,15–17], direct evidence in Japanese patients has remained limited. In this study, we clearly showed the significant correlation of _*6_ to grade 3 or 4 neutropenia in Japanese cancer patients who received irinotecan monotherapy. An increased incidence of severe neutropenia was also observed in the _*6_-bearing patients using cisplatin combination therapy. This finding is in accordance with a report on Korean lung cancer patients who received a combination therapy of irinotecan and cisplatin, which showed a significant association of _*6_ homozygotes with grade 4 neutropenia [18]. Since combination therapies using irinotecan may cause higher incidences of severe toxicities, the _UGT1A1_ polymorphisms should be carefully considered in regimens that include irinotecan.

Table 4
Association of _UGT1A1_ genotypes with severe neutropenia (grade 3 or 4) in irinotecan monotherapy

Diplotype[b]	Neutropenia[a]/total (%)	Effect of _*6_ (%)	
–/–	1/20 (5.0)	non-_*6_/non-_*6_	(3.4)
_*28/–_	0/9 (0.0)		
_*6/–_	3/16 (18.8)	_*6_/non-_*6_	(22.2)
_*6/*28_	1/2 (50.0)		
_*6/*6_	1/2 (50.0)	_*6/*6_	(50.0)
P-value[c]		0.012	

[a] Grade 3 or 4.

[b] "–" represents "_*1_ or _*60_".

[c] Chi-square test for trend.

Table 5
Multivariate logistic regression analyasis of severe neutropenia (grade 3 or 4) in irinotecan monotherapy

Variable	Coefficient	SE	_P_-value	Odds ratio	(95% Confidence limit)
UGT1A1*6	1.77	0.809	0.0289	5.87	(1.37–39.6)

$R^2 = 0.157$, Intercept = 3.15, $N = 49$.

Since the alleles _*6_ and _*28_ are mutually independent [15] and their effects on the UGT activities were shown to be equivalent, the usefulness of the genetic marker "_*6 or *28_" for personalized irinotecan therapies has been suggested [17]. This was also supported in the current study, which showed a "_*6 or *28_"-dependent increase in serum total bilirubin levels (Fig. 1). Because of the low frequency of _*28_ without homozygotes among our subjects, the influence of _*28_ on toxicities was not clearly demonstrated, as in the case of the Korean patients where the allele frequency of _1A1*6_ (23.5%) was much higher than that of _1A1*28_ (7.3%) [18]. However, in the current study, the double heterozygotes of _*6_ and _*28_ (_*6/*28_) showed increases in serum total bilirubin levels (Fig. 1). Moreover, a higher incidence of severe neutropenia in the _*6/*28_ patients was observed, although the patient number was small ($N = 2$) (Table 4). This finding also indicates the importance of "*6 or *28" in severe neutropenia, and in fact, a gene-dose effect of "*6 or *28" ($p = 0.04$ in the chi-square test for trend) and its significant contribution in multivariate analysis ($p = 0.0326$) were also confirmed (data not shown).

For the *60 haplotype (-3279T>G without -40_-39insTA), no association of *60 with severe neutropenia was observed in this study, which coincides with reports of other studies on Japanese cancer patients [17,23]. As for the *27 allele [686C>A(P229Q)], it was linked with the *28 allele and the haplotype was defined as the *28 subtype,*28c [15]. One *28c-heterozygous patient with irinotecan monotherapy showed no severe neutropenia, suggesting a small contribution of the *27 allele (data not shown).

In this study, the association between *UGT1A1* genotypes and antitumor activity was difficult to evaluate because of the small number of subjects stratified into each tumor type. Further clinical studies are needed to establish methods for selection of the appropriate regimen or dosage based on the *UGT1A1* genotypes, where a balance between toxicity and antitumor effect should be considered.

In conclusion, this study demonstrated the significant association of *UGT1A1*6* with severe irinotecan-mediated neutropenia. The current data also supported the usefulness of the genetic marker "*6 or *28" for personalized irinotecan therapy in Japanese, and likely East Asian, patients.

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Weekly Epoetin Beta Maintains Haemoglobin Levels and Improves Quality of Life in Patients with Non-Myeloid Malignancies Receiving Chemotherapy

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Objective: This study was aimed at investigating the effectiveness and safety of once-weekly epoetin beta for anaemic cancer patients receiving chemotherapy.

Methods: A total of 104 patients with a haemoglobin level of $\leq$11.0 g/dL were enrolled. Patients received a once-weekly subcutaneous dose of 36 000 IU epoetin beta for 12 weeks. If the increase in the haemoglobin level was $<$1.0 g/dL after 6 weeks, or a red blood cell transfusion was required between days 15 and 42, the dose of epoetin beta was increased to 54 000 IU from the subsequent week. The primary endpoint was the percentage of patients who achieved a haemoglobin increase of $\geq$2.0 g/dL; the haemoglobin response rate. Quality of life (QOL) was assessed using the Functional Assessment of Cancer Therapy-Anaemia (FACT-An) questionnaire.

Results: The haemoglobin response rate was 66.3% among the 98 patients (breast cancer: $n = 25$; malignant lymphoma: $n = 21$; ovarian cancer: $n = 20$; lung cancer: $n = 15$; other cancers: $n = 17$) assessable for a haemoglobin response. Thirty-nine patients (39.8%) required a dose escalation to 54 000 IU. At the end of the study, QOL assessable patients ($n = 96$) showed a mean improvement in the FACT-An total fatigue subscale score (FSS) of 0.3 points from baseline. Patients with a haemoglobin response had a mean change in the total FSS of +3.2, compared with −3.4 for patients without a haemoglobin response. No serious adverse event of epoetin beta was observed.

Conclusions: Epoetin beta administered at an initial dose of 36 000 IU once-weekly was well tolerated, with increased haemoglobin levels and improved QOL in anaemic cancer patients receiving myelosuppressive chemotherapy.

Key words: anaemia – erythropoietin – cancer – chemotherapy – quality of life

INTRODUCTION

Anaemia is a common complication of cancer patients undergoing chemotherapy. Symptoms of anaemia, including fatigue, palpitations, dizziness and dyspnea markedly reduce patient activity, resulting in impaired quality of life (QOL). In most cases, however, physicians hesitate to prescribe red blood cell (RBC) transfusions until the haemoglobin level is $<$8.0 g/dL, even if the patient has symptoms related to anaemia, such as fatigue. Although the safety of blood transfusion has improved in recent years, risks still remain, such as viral infections, graft versus host disease and haemolytic reactions.

In Europe and the United States, erythropoietin (EPO) agents have widely been used since the 1990s for the treatment of chemotherapy-induced anaemia. Although a three-times weekly dosing schedule was initially introduced (1–3), this schedule was inconvenient for outpatients. Several studies reported that once-weekly dosing of EPO increased the

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haemoglobin level and improved QOL in a manner comparable with those obtained by three-times weekly dosing (4,5).

Since EPO agents have not been approved for the treatment of chemotherapy-induced anaemia in Japan, we previously conducted a dose-finding study of weekly epoetin beta in patients with malignant lymphoma or lung cancer, resulting in a recommended weekly dose of 36 000 IU (6). In this prospective study, we investigated the haemoglobin response, the effects on QOL and the safety of once-weekly epoetin beta in anaemic patients with non-myeloid malignancies. We also investigated the effects of dose escalation to 54 000 IU in patients showing insufficient haemoglobin increase.

PATIENTS AND METHODS

PATIENT ELIGIBILITY

Inclusion criteria were as follows: (a) histological or cytological confirmation of non-myeloid malignancy diagnosis, (b) treatment with cyclic chemotherapy, (c) anaemia (haemoglobin level ≤ 11.0 g/dL) considered to be primarily chemotherapy-induced, (d) life expectancy of at least 4 months, (e) aged between 20 and 79 years, (f) Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0 to 2, (g) eligibility for the QOL questionnaire and (h) adequate hepatic and renal function.

Exclusion criteria included: (a) iron deficiency (mean corpuscular volume $<80\ \mu m^3$ or iron saturation $[\{Fe/(Fe+\text{unsaturated iron-binding capacity})\} \times 100] < 15.0\%$); (b) surgery scheduled during the study period; (c) EPO therapy within 4 weeks prior to the study; (d) documented haemorrhagic lesions; (e) pregnancy, breastfeeding or non-use of adequate birth control measures; (f) history of myocardial, pulmonary, cerebral infarction, serious drug allergy, uncontrolled hypertension, hypersensitivity to any EPO agent or any serious complication; and (g) tumor in the central nervous system.

STUDY DESIGN AND TREATMENT SCHEDULE

This multicentre, open-label study was conducted at 14 sites in Japan.

The protocol was approved by the institutional review board of the respective hospitals, and written informed consent was obtained from all patients who participated in the study.

The initial dose of epoetin beta (Chugai Pharmaceutical Co., Ltd, Tokyo, Japan) was 36 000 IU, and a once-weekly treatment was administered subcutaneously for 12 weeks. If the patient's haemoglobin level did not increase by ≥ 1.0 g/dL from baseline after 6 weeks of treatment, or an RBC transfusion was required between days 15 and 42, the dose of epoetin beta was increased to 54 000 IU weekly from the subsequent week. If the haemoglobin level increased to ≥ 14.0 g/dL, epoetin beta was discontinued until the haemoglobin level decreased to ≤ 12.0 g/dL, and was then restarted at two-thirds (24 000 IU or 36 000 IU) of the previous dose (36 000 IU or 54 000 IU). RBC transfusion was allowed at the discretion of the investigator during the study. An oral daily dose of 100–200 mg elemental iron was recommended if the mean corpuscular volume was $<80\ \mu m^3$ or the iron saturation was $<15.0\%$.

QOL was evaluated at baseline and week 12 using the Japanese Functional Assessment of Cancer Therapy-Anaemia (FACT-An) questionnaire (7,8), a well-validated instrument. In this study, the FACT-An total fatigue subscale, which consists of 13 fatigue related questions, was mainly analysed. The FACT-An total fatigue subscale scores (FSS) range from 0 to 52, with higher scores indicating less fatigue.

EVALUATION OF EFFICACY AND SAFETY

The American Society of Clinical Oncology/The American Society of Hematology guidelines (9) stipulate that the criteria for the haemopoietic effect should be an increase in haemoglobin level ≥ 1.0–2.0 g/dL in 6–8 weeks. Furthermore, there are reports (2,6), which showed that QOL is improved in patients with an increase in haemoglobin level of ≥ 2.0 g/dL.

The primary endpoint of the study was the percentage of patients achieving an increase in the haemoglobin level of ≥ 2.0 g/dL from the baseline between weeks 4 and 12, the haemoglobin response rate, excluding the data within 28 days after an RBC transfusion. The secondary endpoint was the change in FSS after 12 weeks of treatment. The percentage of patients receiving RBC transfusions between day 28 and the end of the study was also assessed. It was not expected that treatment with an EPO agent could influence transfusion requirements before day 28.

Adverse events (AEs) were assessed during the 12-week treatment period and during a 1-week observation period after the last dosing. Anti-erythropoietin antibodies were measured by the enzyme-linked immunosorbent assay and radio-immunoprecipitation (RIP) assay, and detection by either was judged as positive.

STATISTICAL ANALYSIS

We expected that 90 patients would need to be enrolled in the study to obtain a haemoglobin response rate of $70 \pm 10\%$ (95% confidence interval [CI]), as the primary endpoint.

Patients who received at least one dose of the study drug comprised the safety population. For efficacy analysis, the full analysis set (FAS) population was defined as eligible patients who received at least one dose of the study drug.

The changes in the haemoglobin level and FACT-An scores were calculated by subtracting each patient's baseline values from the last values. The rates of increase in haemoglobin before and after dose escalation were compared using a linear mixed-effects model. The potential factors influencing the change in FSS were examined by multiple regression analysis. Pearson correlation coefficients were calculated to assess the association between changes in the haemoglobin level and FACT-An scores.

RESULTS

DEMOGRAPHICS AND BASELINE CHARACTERISTICS

A total of 104 patients were enrolled in the study between February and November 2004. Five patients discontinued the study before the first dosing for the following reasons: patient eligibility criteria violation, $n = 3$; patient denial, $n = 1$; and disease progression, $n = 1$. Thus, 99 patients were administered epoetin beta. One patient was excluded because of non-compliance with the eligibility criteria, leaving 98 patients as the FAS population. Eighty-seven patients (88.8%) completed all 12 weeks of the study. Eleven patients (11.2%) withdrew from the study. The primary reasons for withdrawal were progressive disease and AEs.

The demographics and baseline characteristics of the FAS population are listed in Table 1. Common types of cancer were breast ($n = 25$), malignant lymphoma ($n = 21$), ovarian ($n = 20$) and lung ($n = 15$). The mean age was 58.4 years (range: 23–78), and the mean body weight was 50.7 kg (range: 31.7–74.0). Most of the patients had an ECOG PS of 0 or 1 and a tumour stage of III or IV. The main chemotherapeutic agents used during the study were platinum for lung and other types of cancer, anthracycline for malignant lymphoma, taxane for breast cancer and platinum plus taxane for ovarian cancer. All patients met the criterion that they should not be iron-deficient at the time of enrollment.

HAEMOGLOBIN RESPONSE

The mean change in the haemoglobin level from baseline to the end of the study was 2.47 g/dL (standard deviation [SD]: 2.09; range: -2.8 to 6.0), as shown in Fig. 1. Figure 1 shows the mean changes in haemoglobin levels by tumour type. The pattern of changes in haemoglobin level was similar for the different tumour types. The mean increase in the haemoglobin level in patients with and without an initial EPO level of ≥ 100 mIU/mL were 1.76 g/dL (SD: 2.60) and 2.50 g/dL (SD: 1.85), respectively.

The haemoglobin response rates, defined as the percentage of patients achieving an increase in haemoglobin level of ≥ 2.0 g/dL from the baseline between weeks 4 and 12, are listed in Table 2. The overall haemoglobin response rate was 66.3% (65 of 98 patients). The median time to the haemoglobin response was 56 days from the first dosing, analysed by the Kaplan–Meier method. The percentage of patients with a haemoglobin level of ≥ 12.0 g/dL between weeks 4 and 12 was 59.2% (58 of 98 patients).

The percentage of patients who required dose escalation to 54 000 IU was 39.8% (39 of 98 patients). In these patients, the haemoglobin level increased after dose escalation, and the change in the haemoglobin level was 1.23 g/dL (SD: 2.19) at the end of the study. The haemoglobin response rate was 33.3% (13 of 39 patients) in patients who required dose escalation. The rate of haemoglobin increase before and after dose escalation was 0.023 g/dL/week (Weeks 0–6) and 0.266 g/dL/week (Weeks 7–12), respectively ($P = 0.0055$).

For three patients, the drug treatment was discontinued when the haemoglobin level exceeded 14.0 g/dL, and was restarted at a dose of 24 000 IU when the haemoglobin level decreased to ≤ 12.0 g/dL.

QUALITY OF LIFE

Overall compliance in terms of the percentage of patients who completed the FACT-An was 100% at baseline and 97% (95 of 98 patients) at the end of the study. For three patients who dropped out due to progressive disease and were regarded as missing not at random, the scores at the end of the study were substituted with the minimum scores for all patients. Two patients were excluded from the evaluation of the change in the FSS because the responses to some items were missing.

The mean baseline FSS was 31.8 (SD: 11.4, $n = 98$) points. At the end of the study, the mean change from baseline was 0.3 (SD: 11.8, $n = 96$) points. The mean FSS change in the patients with progressive disease, as judged by each investigator, was -3.8 (SD: 16.7, $n = 15$) points (haemoglobin change: 2.4 g/dL). On the other hand, the mean change in patients without progressive disease was 1.9 (SD: 9.6, $n = 78$) points (haemoglobin change: 2.3 g/dL). These data indicated that progressive disease may be one of the independent variables affecting the change in FSS.

RELATIONSHIP BETWEEN HAEMOGLOBIN RESPONSE AND QOL SCORE

The results of a multiple regression analysis suggested that the change in the haemoglobin level ($P = 0.014$), the FSS at the initiation of dosing ($P < 0.0001$) and the PS at the end of the study ($P < 0.0001$) largely contributed to the change in the FSS. The correlation coefficient between the change in the FSS and the changes in the haemoglobin level was 0.280, indicating a significant correlation ($P = 0.006$, $n = 96$).

Patients who achieved an increase in the haemoglobin level of ≥ 2.0 g/dL experienced a 3.2-point mean change in FSS. On the other hand, patients who did not achieve an increase in haemoglobin level of ≥ 2.0 g/dL experienced a -3.4-point change (Fig. 2). There were no differences in the FSS at the initiation of dosing between patients with and without a change in haemoglobin level of ≥ 2.0 g/dL (32.0 versus 31.6). These data indicate that the change in FSS is dependent on the change in the haemoglobin level.

Concerning the relationship between the FSS at the initiation of dosing and the change in the FSS, patients with a baseline FSS of ≤ 36.0 reported greater improvement (mean ± SD: 1.6 ± 13.0) in the FSS at the end of the study (Table 3).

Table 1. Characteristics of the full analysis set population

	Characteristics	Total	Lung	Malignant Lymphoma	Breast	Ovarian	Other types
Sex	Male	27	11	10	0	0	6
	Female	71	4	11	25	20	11
Age (years)	Mean ± SD	58.4 ± 10.8	60.5 ± 10.5	56.5 ± 13.4	58.2 ± 9.0	54.4 ± 11.0	63.8 ± 8.0
	Range	23–78	41–78	23–74	39–77	30–75	40–76
ECOG performance status	0	48	1	9	14	13	11
	1	39	12	9	6	6	6
	2	11	2	3	5	1	0
Tumour stage	I	6	0	1	3	2	0
	II	17	1	4	7	4	1
	III	15	0	3	0	9	3
	IIIA	2	1	0	1	0	0
	IIIB	8	6	0	2	0	0
	IV	50	7	13	12	5	13
Treatment regimen	Platinum based	17	7	2	0	1	7
	Taxane based	28	5	0	19	3	1
	Anthracycline based	28	1	18	6	0	3
	Platinum, Anthracycline based	4	0	1	0	1	2
	Platinum,Taxane based	21	2	0	0	15	4
Weight (kg)	Mean ± SD	50.7 ± 8.2	53.8 ± 8.7	52.7 ± 9.9	47.9 ± 7.2	49.3 ± 6.6	50.9 ± 7.4
	Range	31.7–74.0	38.0–70.7	31.7–74.0	34.0–63.0	34.1–60.0	37.7–65.5
Haemoglobin (g/dL)	Mean ± SD	9.3 ± 1.4	9.6 ± 1.4	9.3 ± 1.4	9.4 ± 1.4	9.2 ± 1.6	9.1 ± 1.4
	Range	5.6–11.9	6.4–11.2	6.5–11.3	5.7–11.9	6.4–11.7	5.6–11.1
MCV (fL)	Mean ± SD	92.3 ± 6.5	89.0 ± 6.4	90.0 ± 5.4	91.9 ± 5.8	94.6 ± 7.5	95.7 ± 5.3
	Range	79.9–107.5	79.9–99.3	80–101	80.3–103.2	81.9–107.5	84–103.4
Reticulocyte (‰)	Mean ± SD	19.7 ± 16.4	20.8 ± 15.1	24.2 ± 24.1	18.0 ± 13.2	21.1 ± 15.6	14.1 ± 10.3
	Range	1–106	2–50	1–106	1–58	1–54	1.1–35.1
Transferrin saturation (%)	Mean ± SD	29.7 ± 22.3	22.4 ± 7.1	41.5 ± 30.6	21.9 ± 16.9	31.1 ± 24.3	30.5 ± 18.0
	Range	4.8–92.9	12.5–15.5	9.9–92.9	4.8–80.6	7.2–90.7	14.0–90.1
Serum endogenous erythropoietin (mIU/mL)	Mean ± SD	119.1 ± 310.5	64.3 ± 69.9	80.7 ± 108.0	88.4 ± 107.1	125.9 ± 144.8	252.0 ± 706.0
	Range	15.7–2970	15.7–234	17.3–399	16.7–472	23.2–578	20.4–2970
Baseline QOL:FACT-An							
Anemia subscale (0–80)	Mean ± SD	50.8 ± 14.5	47.0 ± 15.9	50.6 ± 11.7	47.1 ± 13.7	53.5 ± 11.1	56.7 ± 19.5
	Range	16–80	17–74	26–67	20–71	34–75	16–80
Fatigue subscale (0–52)	Mean ± SD	31.8 ± 11.4	29.6 ± 12.9	30.3 ± 10.6	29.7 ± 10.7	33.9 ± 8.7	36.5 ± 14.1
	Range	4–52	4–52	10–43	7–50	20–48	8–52

SD, standard deviation; ECOG, Eastern Cooperative Oncology Group; QOL, quality of life; FACT-An, Functional Assessment of Cancer Therapy-Anemia; MCV, mean corpuscular volume.

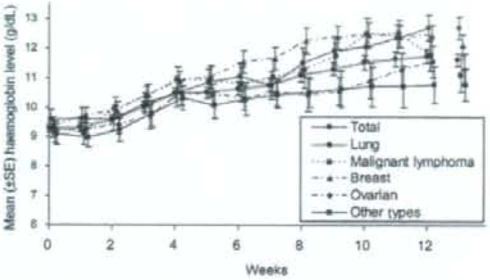

Figure 1. Change in haemoglobin level by tumor type. Mean weekly haemoglobin levels for the FAS population. Haemoglobin values within 28 days after RBC transfusion were excluded. FAS, full analysis set; RBC, red blood cell.

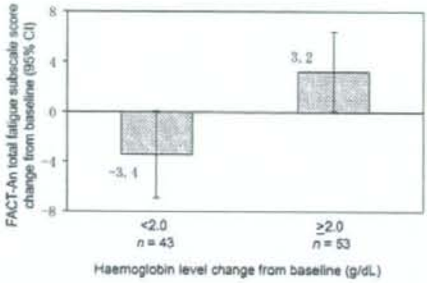

Figure 2. Changes in the FACT-An total fatigue subscale score by change in haemoglobin level. FACT-An, Functional Assessment of Cancer Therapy-Anaemia.

RBC TRANSFUSION REQUIREMENT

The percentage of patients who received RBC transfusions between day 28 and the end of the study was only 6.1% (6 of 98 patients). The mean pretransfusion haemoglobin level at the time of the first transfusion was 6.2 g/dL (range: 5.4–7.3 g/dL). The percentage of patients whose haemoglobin level had decreased to <8.0 g/dL or who received an RBC transfusion between day 28 and the end of the study was 20.4% (20 of 98 patients).

SAFETY

AEs reported by at least 20% of the patients are summarised in Table 4. Death as a result of disease progression was not reported as an AE. Adverse drug reactions reported by at least 5% of patients are listed in Table 5. Among the 133 events in 48 patients (48.5%) that were considered related to the study drug, Grade III events were headache, hypertension, diarrhea, decreased serum potassium, impaired consciousness, anorexia and decreased serum phosphate. Three events (3.0%) of hypertension were reported as possibly related to epoetin beta treatment. An antihypertensive drug was administered after the onset of hypertension in one patient, who had hypertension as a comorbidity before the study. One patient (65-year-old female with malignant lymphoma) experienced a thrombovascular event, a lacunar infarction, at week 6. This event was evaluated as being unrelated to epoetin beta and was attributed to aging.

The incidence and type of AEs in patients who required dose escalation did not differ from those in patients who did not.

In two patients with ovarian and gastric cancer, anti-erythropoietin antibodies were detected only by RIP assay.

Table 2. Haemoglobin response rate by baseline haemoglobin, tumour type and dose escalation

	%	*n*
Response rate*	66.3	65/98
Response rate by baseline haemoglobin, g/dL		
<10.0	68.8	44/64
≥10.0	61.8	21/34
Response rate by tumour type		
Lung	80.0	12/15
Malignant lymphoma	66.7	14/21
Breast	76.0	19/25
Ovarian	65.0	13/20
Other types	41.2	7/17
Response rate by dose escalation		
Yes	33.3	13/39
No	88.1	52/59

*All patients, including those receiving transfusions.

Table 3. Changes in the FACT-An total fatigue subscale score by baseline FSS and final PS

Time period	Baseline		End of treatment		
	n	Mean score (SD)	*n*	Mean score (SD)	Mean change from baseline (SD)
Total	98	31.8 (11.4)	96*	31.8 (13.5)	0.3 (11.8)
Baseline FSS					
≤36.0	62	24.8 (7.9)	62	26.5 (12.0)	1.6 (13.0)
>36.0	36	43.9 (4.0)	34*	41.5 (10.3)	−2.2 (8.8)
Final PS					
0	58	35.5 (11.3)	56*	37.4 (10.3)	2.4 (10.2)
1	28	27.4 (9.1)	28	29.0 (11.5)	1.6 (12.2)
2	4	19.3 (9.4)	4	11.8 (11.4)	−7.5 (7.9)
3	3	29.7 (15.9)	3	21.0 (7.2)	−8.7 (13.8)
4	5	25.7 (7.3)	5	6.4 (7.1)	−19.3 (6.4)

*Two patients missing FSS. Collected but could not be calculated.
FSS, FACT-An total fatigue subscale score; PS, performance status.

Table 4. Frequencies of adverse events ($n = 99$)

Event	n	%	Grade*				
			I	II	III	IV	V
Neutropenia	83	83.8	3	11	24	45	0
Leukopenia	78	78.8	2	16	41	19	0
Nausea	57	57.6	38	11	8	0	0
Thrombocytopenia	55	55.6	21	9	23	2	0
Lymphopenia	52	52.5	0	18	34	0	0
Anorexia	46	46.5	22	13	10	1	0
Fatigue	39	39.4	22	14	3	0	0
Vomiting	36	36.4	18	16	2	0	0
Diarrhea	33	33.3	23	6	4	0	0
Increased lactate dehydrogenase	32	32.3	25	6	1	0	0
Peripheral neuropathy	26	26.3	21	5	0	0	0
Fever	26	26.3	17	7	2	0	0
Constipation	24	24.2	3	13	7	1	0
Increased alanine aminotransferase	24	24.2	15	6	3	0	0
Alopecia	22	22.2	7	15	0	0	0

*National cancer institutes common toxicity criteria, version 2.0.

Table 5. Frequencies of adverse drug reactions ($n = 99$)

Event	n	%	Grade*				
			I	II	III	IV	V
Increased lactate dehydrogenase	10	10.1	9	1	0	0	0
Headache	7	7.1	6	0	1	0	0
Nausea	7	7.1	5	2	0	0	0
Rash	5	5.1	3	2	0	0	0
Back pain	5	5.1	5	0	0	0	0

*National cancer institutes common toxicity criteria, version 2.0.

Neutralisation of EPO activity was detected in neither patient, and the haemoglobin level was elevated after dosing with the study drug. The investigators judged that the antibody did not cause pure red cell aplasia.

When re-examined six months after the last observation, one of these patients (ovarian cancer) was antibody negative, whereas the other (gastric cancer) could not be re-examined, having died of the underlying disease.

DISCUSSION

Several studies have been conducted to assess the effects of EPO agents in anaemic cancer patients, and increased haemoglobin levels and improvement in QOL that correlated with the increased haemoglobin level were reported (1,10).

The objectives of our study were to investigate the effects of an initial once-weekly 36 000 IU dose of epoetin beta on haemoglobin levels and QOL in patients with non-myeloid malignancy undergoing chemotherapy. The criterion for a haemoglobin response, an increase in the haemoglobin level of ≥ 2.0 g/dL, was based on a report that symptoms of anaemia assessed by the FACT-An are improved in patients with a change in the haemoglobin level of ≥ 2.0 g/dL (2,6). According to this index, the haemoglobin response rate in the present study was 66.3% (65 of 98 patients). The increases in haemoglobin levels that were observed were independent of the tumour type or the baseline haemoglobin level. None of the investigators performed a randomised comparison of a dose increase versus an unchanged dose in EPO low responders. In the present study, there was an increase in the rate of haemoglobin increase after dose escalation to 54 000 IU, and the haemoglobin response rate for patients who required a dose escalation was 33.3% (13 of 39 patients).

The secondary endpoint, the change in the FSS, showed an increase of 0.3 points; however, in patients who showed an increase in the haemoglobin level of ≥ 2.0 g/dL, the FSS was increased by 3.2 points, which was significantly higher than the -3.4-point change in patients whose haemoglobin level increased by <2.0 g/dL. A 3.2-point increase is comparable with the 3 points considered to be a clinically significant change in FSS (11). In addition, the mean change in FSS for patients with progressive diseases (PD) was -3.8 points (median: -6.5 points, range: -37 to 35 points) even though correction of anaemia was observed. In total, excluding PD cases, a 1.9- point improvement was observed.

Investigating the relationship between the FSS at the initiation of dosing and the change in the FSS showed that greater improvements in FSS were observed in patients with lower FSS. The FSS before treatment with epoetin beta was 31.8 ± 11.4 points, which is higher than the scores (FSS: 22.1–29.7 points, change in FSS: 1.6–5.2 points) in cancer patients with anaemia reported in several randomised trials (1,10,12–14). Nevertheless, the mean initial haemoglobin level (9.3 g/dL) in the present study was equal to the levels in the other trials (9.2–10.1 g/dL). Since it has been reported that the FSS after treatment with an EPO agent is aggravated in patients with an FSS exceeding 36.0 at the initiation of dosing (15), the scores were analysed after stratification at 36.0. This resulted in improved scores (1.6 ± 13.0 points) for those patients with a baseline score of ≤ 36.0, when compared with patients with a score >36.0 (-2.2 ± 8.8 points). The results of a multiple regression analysis of the change in the FSS demonstrated that the change in the haemoglobin level, the FSS at the initiation of dosing and the PS at the end of the study were factors that largely contributed to the change in the FSS. A positive and significant association was observed between the degree of increase in the haemoglobin level and the degree of improvement in the FSS ($r = 0.280$, $P = 0.006$). It was comparable with the results ($r = 0.2879$, $P = 0.0002$; $r = 0.35$, $P = 0.001$ and $r = 0.2893$, $P < 0.0001$) of three other studies (1,10,16).

The RBC transfusion rate was only 6.1% (6 of 98 patients) between day 28 and the end of the study. As reported for once-weekly epoetin alfa administered to patients with various types of cancer (14), the transfusion rates between week 5 and the end of treatment were 14.5% (24 of 166 patients) for epoetin alfa and 29.3% (48 of 164 patients) for placebo. Furthermore, the mean pretransfusion haemoglobin levels for the first transfusion reported in the previous trial in the United States (7.9 and 7.8 g/dL, respectively) were higher than those (6.2 g/dL) in the present study in Japan. To evaluate the effect of EPO agents, the percentage of patients whose haemoglobin level had decreased to <8.0 g/dL or who received an RBC transfusion was considered to be a more objective index than the RBC transfusion rate in Japan, because RBC transfusion itself is prescribed at the discretion of the investigator and when the haemoglobin level is low.

Epoetin beta was well tolerated in the present study. Most of the AEs were consistent with the underlying disease or with the chemotherapy. Hypertension, which was judged to be related to epoetin beta was observed in three patients. It was alleviated either by no treatment or the administration of hypotensive agents. Lacunar infarction was also observed in one patient. A relationship to epoetin beta was ruled out, however, and this event was judged to be due to aging. Two recently published studies (17,18) targeting higher haemoglobin levels, in which survival was a primary endpoint, have raised concerns that EPO agents may have a negative impact on survival in cancer patients. A meta-analysis of 57 studies, including these two recent studies revealed an overall survival hazard ratio of 1.08 (95%CI: 0.99–1.18) and that uncertainties remain as to whether EPO agents affected survival (19). The FDA has provided new safety information on erythropoiesis-stimulating agents (ESAs), in which the target haemoglobin level is not to exceed 12 g/dL, because analyses of other studies in patients with cancer found a higher chance of serious and life-threatening adverse drug reactions or deaths with the use of ESAs (20). Although, in the present studies, there was no problem with safety when the haemoglobin level at which dosing was withheld was set at 14 g/dL, in consideration of FDA ALERTs, etc., we intend to investigate the use of lower values for target haemoglobin level and haemoglobin level at which dosing should be withheld.

In conclusion, once-weekly epoetin beta treatment increased the haemoglobin level and correspondingly improved the QOL in anaemic patients with non-myeloid malignancies receiving chemotherapy. Additionally, haemoglobin levels could be improved and controlled by once-weekly treatments at an initial dose of 36 000 IU followed by dose adjustment in the range of 24 000–54 000 IU.

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Conflict of interest statement

One of the authors, Hironobu Minami, receives honoraria from Chugai Pharmaceutical Co., Ltd. and Kirin Pharma Co., Ltd.

One of the authors, Yasuo Ohashi, consults on design and data analysis of clinical trials for Chugai Pharmaceutical Co., Ltd.

One of the authors, Nagahiro Saijo, holds stock option for Takeda Pharmaceutical Co., Ltd.

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Molecularly Targeted Therapy for Lung Cancer: Recent Topics

Many clinical trials of molecular target drugs have been done against advanced lung cancer, however, majority did not meet the primary endpoint. Positive studies of EGFR-TKI such as BR21 and Interest used unselected populations of non-small cell lung cancer. It was quite difficult to explain why they were positive. In the present review, the difficulties of clinical trial design in molecular target drugs were discussed based on the differences of the magnitude of antitumor activity and the target tumor cell population between cytotoxic drugs and molecular target therapy. **(J Lung Cancer 2008;7(1):1－8)**

Key Words: Lung cancer, Molecular target therapy, EGFR-TKI, Clinical trial

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The therapeutic efficacy of cytocidal anticancer drugs for lung cancer has reached a plateau(1～4), and it is extremely important to develop of new therapeutic agents. However, the majority of clinical trials of molecularly targeted drugs for lung cancer have yielded negative data, and the only drugs currently approved anywhere in the world are the EGFR-TKIs such as gefitinib and erlotinib and the anti-VEGF antibody, bevacizumab. Historically, matrix metalloprotease inhibitors(5), PKC α inhibitors, Ras kinase inhibitors(6), bexarotene, trastuzumab(7), etc.(8), have all been assessed with the prolongation of survival by simultaneous or consecutive use with cytocidal anticancer agents, but only negative data have been obtained (Table 1).

Table 1. Molecular Target-based Therapy in Lung Cancer

Specific target-based drugs	Combination	Results
Gefitinib (EGFR)	Y	Negative
	N	Negative, vs placebo
	N	Negative in Japanese, vs DTX Positive in Global, vs DTX
Erlotinib (EGFR)	Y	Negative
	N	Positive, vs placebo
Cetuximab (EGFR)	Y	Positive
Lonafarnib (ras)	Y	Negative
Bexarotene (RXR)	Y	Negative
Affinitac (PKC α)	Y	Negative
Sorafenib (Raf, VEGF etc)	Y	Negative
Trastuzumab	Y	Negative
Cetuximab	Y	Negative
Environment specific target-based drugs	**Combination**	**Results**
MMPI (Marimastat, Prinomastat)	Y	Negative
Bevacizumab	Y	Positive

N: No, Y: Yes

EGFR-TKIs

EGFR-TKIs are molecularly targeted drugs that selectively modify molecular biological abnormalities of tumor cells themselves(9～12). The amazing antitumor effect of EGFR-TKIs in cases in which platinum-taxane therapy failed attracted interest(13～16), but it was difficult to demonstrate that they contributed to any survival benefit(17～20). Erlotinib is used as second-line and third-line chemotherapy in cases of platinum-taxane failure, and it has shown a survival benefit in comparison with placebo in unselected non-small cell cancer(21). By contrast, it was impossible to show any overall survival benefit of gefitinib in a group of similar cases that were almost the same although the results were marginal(22,23), and while significant prolongation of survival time was observed in Asians (no Japanese were included) by post-study stratification, no difference in survival time at all from the placebo control group was observed in Caucasians. Moreover, four trials of standard chemotherapy (carboplatin+paclitaxel, gemcitabine+cisplatin) ±EGFR-TKI all yielded negative data(17~20), and in a comparative study with gefitinib as intensification chemotherapy for stage III non-small cell cancer the survival time of the gefitinib group was instead significantly poorer than in the control group(24). Adjuvant studies using EGFR-TKIs in resected cases was started in Japan and North America but case entry was poor, and it was stopped before completion(25).

Two comparative studies of docetaxel versus the EGFR-TKI gefitinib in cases in which platinum-taxane was ineffective yielded different results. Even though the response rate to gefitinib by the Japanese patients was higher than in the Western population, it was impossible to demonstrate non-inferiority versus docetaxel in the V15-32 study conducted in Japan(26). By contrast, non-inferiority was demonstrated in the Interest study conducted in a large number of cases in Western countries(27).

The majority of the results of these studies were not what the investigators expected (Table 2), and numerous questions have arisen.

1) In placebo-controlled studies in cases in which platinum-taxane therapy was ineffective, the ISEL study (gefitinib) was negative(22), whereas BR-21 (erlotinib) was positive(21). The efficacy of gefitinib was marginal, but no difference at all was observed in the Western subjects. Differences in dosage were stated as the reason, but that is not a satisfactory explanation.

2) Does not the fact that Intact I & II (gefitinib)(17,18) and Talent(19) & Tribute (erlotinib)(20) were all negative studies conflict with the evidence in BR-21 study. There is the explanation based on their effects on the cell cycle that anticancer drugs and EGFR-TKIs act antagonistically when administered simultaneously.

3) Non-inferiority versus docetaxel was demonstrated in the Interest study (gefitinib) even though the ISEL study (gefitinib) was negative. By contrast, although Japanese patients, who have a high response rate to EGFR-TKIs, were used as the study subjects of the V15-32 study (gefitinib), the docetaxel control group tended to have better survival at each time point of 10-12 months after the beginning of treatment.

Table 2. RCTs (Randomized Clinical Trials) of Erlotinib & Gefitinib

	Early	Stage III	Advanced	
Erlotinib	RADIANT (n=945, vs. placebo, *on going*)		First line TALENT (n=1172, CDDP/GEM± Erlotinib, *negative*) TRIBUTE (n=1059, CBDCA/PTX± Erlotinib, *negative*) SATURN (n=850, CT x 4 → vs. placebo, *on going*)	Relapsed BR.21 (n=731, vs. placebo, *positive*) TITAN (n=648, vs DTX, *on going*)
Gefitinib	BR.19 (n=1242, vs. placebo, *terminated*) Japanese trial (n=670, vs. placebo, *terminated*)	SWOG0023 (n=840, CRT→DTX→gefitinib, *terminated*)	First line INTACT1 (n=1093, CDDP/GEM± Gefitinib, *negative*) INTACT2 (n=1037, CBDCA/PTX± Gefitinib, *negative*)	Relapsed ISEL (n=1692, vs. placebo, *negative*) V15-32 (n=484, vs. DTX, *negative*) INTEREST (n=1466, vs. DTX, *positive*)