(Please see File S1). A total of 10,262 patients had been allocated randomly to 110 chemotherapy arms.

Study Characteristics

Table 1 lists the baseline characteristics of the trials. Trials were initiated between 1980 and 2006. The number of randomized patients and the proportion of patients with good PS increased over time (13.9 patient increase/year, $P<0.001$; and 1.32% increase/year, $P<0.001$, respectively; Figures 2A and 2B), whereas the proportion of male patients remained consistent (0.47% decrease/year, $P=0.114$; Figure 2C). In 19 trials that assigned PCI, it was planned that patients who achieved a complete response (CR) or CR/partial response (PR) after induction chemotherapy would receive PCI. Thirteen (25%) of the 52 phase III trials showed a statistically significantly difference in survival time. Of these, eight were in favor of the patient cohort that received the experimental therapy compared with the control group, while the remaining five were in favor of that in the control group.

Table 1. Characteristics of the 52 Randomized Trials.

Variable	Value
No. of trials	52
(No. of randomized patients in all trials 10262)	
No. of treatment arms	
2	47
3	4
4	1
Year of trial initiation	
Median (range)	1990 (1980–2006)
No. of randomized patients (%)	
<100	35
100–200	25
200–300	29
>300	11
Median (range)	158 (34–786)
Proportion of patients with good performance status† (%)	
<80	50
80–90	42
>90	8
Median percentage (range)	80 (35–100)
Male Patients (%)	
<80	54
80–90	35
>90	11
Median percentage (range)	75 (56–93)
Trials assigning PCI for those with CR or CR/PR to the initial chemotherapy	
Yes	37
No	63
Trials with a statistically significant difference in overall survival time (%)	
Yes	25
No	65
Not recorded	10

†Defined as a performance status of 0 or 1.
Abbreviations; PCI, prophylactic cranial irradiation; CR, complete response; PR, pertial response.
doi:10.1371/journal.pone.0007835.t001

Types of Chemotherapy Arms

There were 110 chemotherapy treatment arms in the 52 phase III trials (Table 2). Cisplatin-based regimens were the most frequently investigated. The PE regimen, currently considered as the standard treatment for patients with ED-SCLC, has increasingly been studied (Figure 1). As expected, the CAV alternating PE regimen was extensively examined in the 1980s, but this decreased in the 1990s.

Trends in Patient Survival

Data on patient survival were available from all 52 trials and 110 chemotherapy arms and analyzed by treatment arm. A scattergram of the two parameters (year of trial initiation and median survival time) revealed that the slope of the fitted line was 0.021, indicating a 0.021 month (0.63 day) increase in median survival time per year ($P=0.272$; Figure 3). Multiple regression analysis, adjusting for several confounding trial characteristics, also showed no significant association between the two parameters (regression coefficient for year of trial initiation $=0.011$, 95% confidence interval $=-0.36$–0.38, $P=0.950$; Table 3). In this setting, the proportion of patients with good PS was significantly associated with a favorable outcome. The multiple regression analysis also showed a significant influence of PCI setting on survival prolongation. This finding is partly supported by a recent report on the survival advantage of PCI in ED-SCLC patients who responded to initial chemotherapy [6].

Discussion

Our results demonstrate no significant improvement in patient outcomes over the years in phase III trials of systemic chemotherapy for ED-SCLC, with an increase of 0.021 months (0.63 days) per year (univariate analysis; $P=0.272$; Figure 3) confirmed in the multivariate model ($P=0.950$; Table 3). However, the proportion of patients with good PS and the trial design of assigning PCI for those with CR or CR/PR significantly influenced survival (Table 3).

The introduction of multiple drug regimens has been a great advance in the treatment of ED-SCLC; indeed, the CAV regimen yielded a survival time approximately twice as long as that of the single-agent therapy frequently used in the early 1970s [1,7]. However, the survival benefit from chemotherapy has reached somewhat of a plateau, even with the introduction of the PE regimen in recent clinical trials, as compared with the CAV regimen or CAV alternating PE [2,8,9,10]. In addition, most of newer antitumour agents introduced after PE (e.g., irinotecan and topotecan) failed to substantially prolong survival in the first-line setting over the standard PE regimen [11,12,13,14,15]. Thus, based on these findings, our main results demonstrate no significant improvement in survival since 1980. In contrast, a 1999 study showed a significant increase in overall survival time [3]. This difference in the time trend in overall survival is mainly attributable to differences in the study period (year of trial initiation: 1972–1994 vs. 1980–2006 in the earlier and present study, respectively; [3]).

In Figure 3, trials between 2000 and 2005 appeared to show extensive clustering with median survival time of around ten months. It would be attributable to some common characteristics among these trials, such as relatively uniformed chemotherapeutic regimens (cisplatin-based ones) and larger number of the registered patients. In contrast, there were other trial arms that yielded the

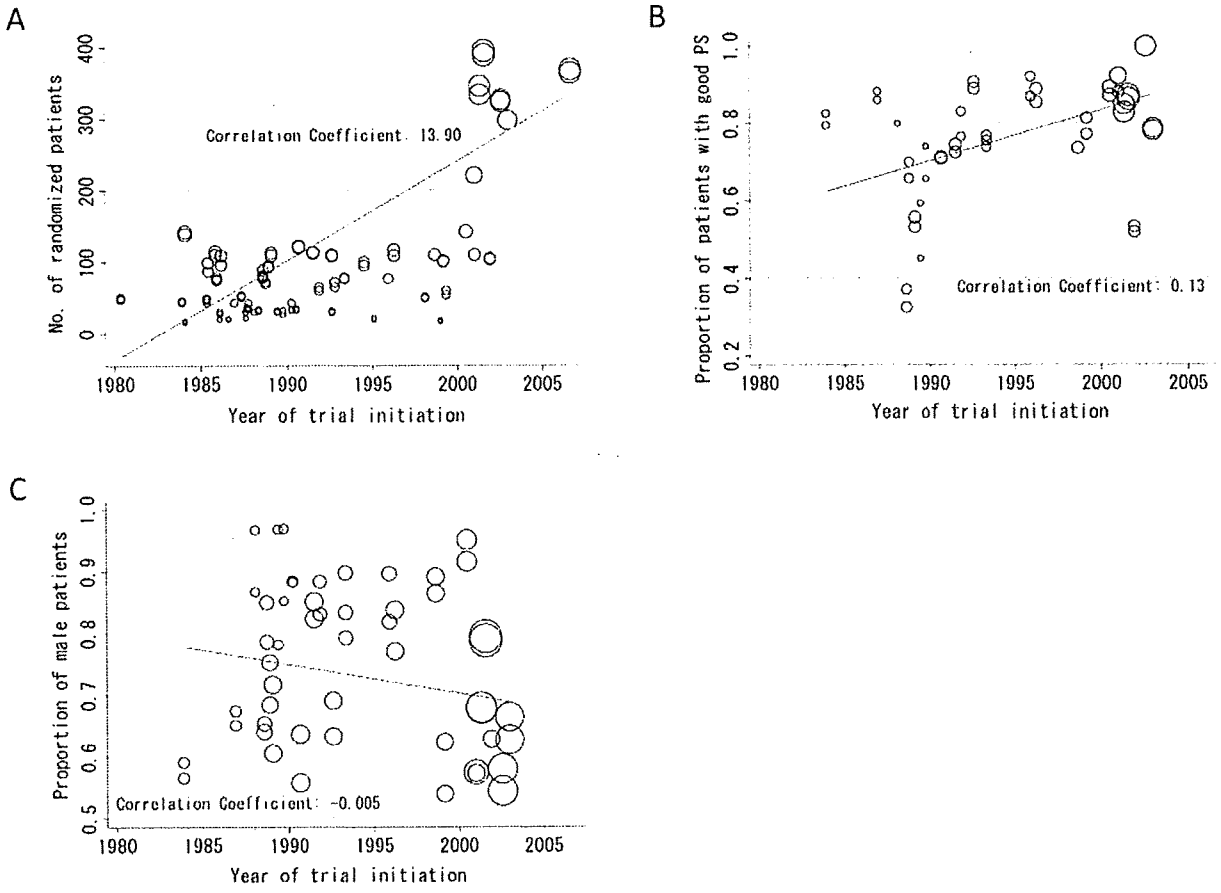

Figure 2. Trends in trial characteristics. These charts show the associations between year of trial initiation and number of randomized patients (A), proportion of patients with good PS (B), and proportion of male patients (C) in each trial. The size of solid circles represents data weighted on the basis of the number of randomized patients. Abbreviations: PS, performance status.
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longest versus shortest survival times (14–15 months versus 5–6 months). These included less number of the enrolled patients, which possibly resulted in a wide-range distribution in the Figure.

We investigated a similar issue previously [16], namely trends in prognosis over the years in chemo-naïve patients with advanced non-small cell lung cancer (NSCLC) enrolled in phase III trials.

Table 2. Types of Chemotherapy Arms and Treatment Outcomes (Per Treatment Arm).

Chemotherapy Arm	No. of Arms (%)	MST [range], months	
Total no. of arms	110	9.3	[4.9–14.5]
Platinum-based regimens	78 (70.9)	9.5	[4.9–14.5]
Cisplatin-based	64 (58.2)	9.6	[5.8–14.5]
CAV alternating PE	16 (14.5)	9.5	[5.8–14.5]
PE	16 (14.5)	9.4	[7.0–10.2]
Other Cisplatin-based	32 (29.1)	9.8	[6.7–12.8]
Nonplatinum regimens	32 (29.1)	8.5	[5.0–13.0]
CAV-based	10 (9.1)	9.1	[7.5–13.8]
Non-CAV-based combination therapy	19 (17.3)	8.2	[5.0–13.0]
Non-CAV-based monotherapy	3 (2.7)	8.3	[6.0–9.3]

Abbreviations: MST, median survival time; CAV, cyclophosphamide, doxorubicin, and vincristine; PE, cisplatin and etoposide.
doi:10.1371/journal.pone.0007835.t002

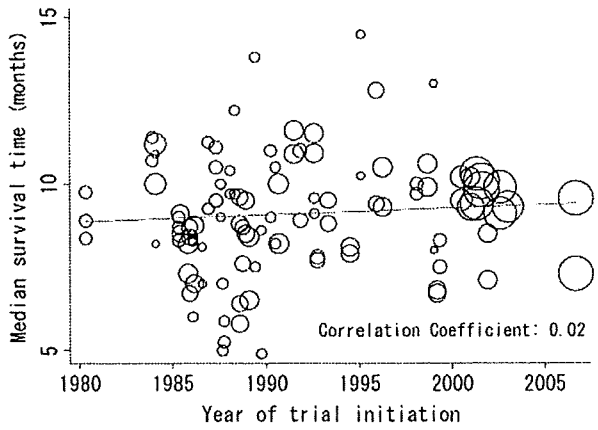

Figure 3. Relationship between year of trial initiation and median survival time. Analysis was weighted by the number of randomized patients. Each trial is represented by a circle; the size of each circle is proportional to the sample size of randomized patients in the given trial.
doi:10.1371/journal.pone.0007835.g003

Table 3. Multiple Stepwise Linear Regression Analysis of Overall Survival (Per Treatment Arm).

Factor	Regression Coefficient*	SE	P†
Year of trial initiation	Excluded		
Use of PE regimen (y or n)	Excluded		
Proportion of patients with good PS	6.65	1.30	<0.001
Proportion of male patients	Excluded		
Median age of patients	Excluded		
Design of the PCI setting (y or n)	2.14	0.742	0.009
Description of definition for ED (y or n)	Excluded		

*Threshold F values for entering and removing from the model were 0.05 and 0.10, respectively.
†$P<0.05$ was considered significant. This multivariate stepwise regression model excluded the factors "Year of trial initiation," "Use of PE regimen," "Proportion of male patients," "Median age of patients," and "Description of definition for ED" from the model.
Abbreviations: PE, cisplatin and etoposide; PS, performance status; PCI, prophilactic cranial irradiation; ED, extended disease.
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The analysis similarly revealed a very small increase in patient survival (3.61 days per year) but one that was statistically significant in the multiple regression model ($P<0.001$; ([16]). There may be several potential factors behind such differences in statistical results in SCLC and NSCLC settings. The most important is that new active agents such as taxanes appeared in the treatment of NSCLC [17,18] and few novel agents, including molecular-targeted agents, did in the treatment for SCLC [11,19,20,21] in these study periods. Another hypothesis is that advanced NSCLC might be more influenced than SCLC by lead time bias through early detection with improved imaging techniques, mainly because the growth rate of NSCLC is generally less rapid than that of SCLC throughout its natural history [22]. Progress in supportive care practices would lead to improvements in survival among patients with advanced NSCLC. Those with advanced NSCLC usually have less rapid disease progression and, thus, would likely benefit from its advancement. Finally, the statistical difference between our NSCLC and SCLC studies could have arisen from differences in sample size (number of trials), indicating that the current study may have lacked adequate power to accurately evaluate the association between the year of trial initiation and patient outcome.

The potential influence of second-line chemotherapy should also be considered in assessing the effect of first-line chemotherapy because it may contribute to recent improvements in survival [23]. The trials analyzed here rarely provided information about second-line treatment, and we can not assess its exact effect in this setting. There are few positive phase III trials of second-line treatments, and thus it is unlikely that such therapy can significantly confound patient prognosis after the initiation of first-line chemotherapy [24].

In conclusion, the results of our analysis suggest that, regardless of the reason, the survival of patients with ED-SCLC who were enrolled in phase III trials did not improve significantly over the years. Thus, the development of novel targets, newer agents, and comprehensive patient care will be essential in the future fight against lung cancer.

Supporting Information

File S1
Found at: doi:10.1371/journal.pone.0007835.s001 (0.05 MB DOC)

Author Contributions

Conceived and designed the experiments: KH. Performed the experiments: KH. Analyzed the data: IO KH. Wrote the paper: IO KH KK NO NT YF MT MT.

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Clinical Studies

Chemotherapy-induced neutropenia as a prognostic factor in advanced non-small-cell lung cancer: results from Japan Multinational Trial Organization LC00-03

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BACKGROUND: Neutropenia is a common adverse reaction of chemotherapy. We assessed whether chemotherapy-induced neutropenia could be a predictor of survival for patients with non-small-cell lung cancer (NSCLC).

METHODS: A total of 387 chemotherapy-naïve patients who received chemotherapy (vinorelbine and gemcitabine followed by docetaxel, or paclitaxel and carboplatin) in a randomised controlled trial were evaluated. The proportional-hazards regression model was used to examine the effects of chemotherapy-induced neutropenia and tumour response on overall survival. Landmark analysis was used to lessen the bias of more severe neutropenia resulting from more treatment cycles allowed by longer survival, whereby patients who died within 126 days of starting chemotherapy were excluded.

RESULTS: The adjusted hazard ratios for patients with grade-1 to 2 neutropenia or grade-3 to 4 neutropenia compared with no neutropenia were 0.59 (95% confidence interval (CI), 0.36–0.97) and 0.71 (95% CI, 0.49–1.03), respectively. The hazard ratios did not differ significantly between the patients who developed neutropenia with stable disease (SD), and those who lacked neutropenia with partial response (PR).

CONCLUSION: Chemotherapy-induced neutropenia is a predictor of better survival for patients with advanced NSCLC. Prospective randomised trials of early-dose increases guided by chemotherapy-induced toxicities are warranted.

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Keywords: non-small-cell lung cancer; chemotherapy-induced neutropenia; overall survival; tumour response; landmark analysis

Chemotherapy is the standard remedy for patients with advanced cancer and neutropenia is an important dose-limiting toxicity of anticancer agents. Several studies since the late 1990s have reported that neutropenia (or leukopenia) that occurs during chemotherapy is a predictor of significantly longer survival for patients with breast cancer (Saarto *et al*, 1997; Cameron *et al*, 2003). A recent study by Di Maio *et al* (2005) confirmed the positive correlation between chemotherapy-induced neutropenia and increased survival in a pooled analysis of three randomised trials, which included 1265 patients with advanced non-small-cell lung cancer (NSCLC). Pallis *et al* (2008) have also shown the association between chemotherapy-induced neutropenia and better clinical outcome for patients with NSCLC. In a prospective survey of oral fluoropyrimidine S-1 in 1055 patients with advanced gastric cancer, Yamanaka *et al* (2007) reported that patients with moderate (grade-2) neutropenia had the longest survival.

In light of these reports, we have analysed the associations between the extent of chemotherapy-induced neutropenia, overall survival and tumour response by reviewing data from a clinical trial of patients with advanced NSCLC.

MATERIALS AND METHODS

Patients and treatment

A total of 401 chemotherapy-naïve patients with NSCLC stage IIIB (positive pleural effusion) or stage IV (no brain metastases), who

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had Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, were enrolled in this randomised controlled trial (Japan Multinational Trial Organization LC00-03) between March 2001 and April 2005. Of 393 eligible patients, information regarding chemotherapy-induced neutropenia was not available for six patients. Thus, data from 387 patients were included in this analysis. These participants were divided into two groups by treatment. The experimental group (VGD arm, $n=192$) received three cycles of intravenous vinorelbine (25 mg/m^2) and gemcitabine (1000 mg/m^2) administered on days 1 and 8 of each 21 day cycle, followed by three cycles of single-agent intravenous docetaxel (60 mg/m^2) administered on day 1 of each 21 day cycle. The standard regimen (PC arm, $n=195$) consisted of six cycles of intravenous paclitaxel (225 mg/m^2) plus carboplatin (area under curve $=6$) infused on day 1 of each 21 day cycle. Details of dose modifications and reductions have been described previously (Kubota *et al*, 2008). The protocol permitted use of granulocyte-colony-stimulating factor (G-CSF) for patients with grade-3 neutropenia with fever or grade-4 leukopenia or neutropenia, but did not permit prophylactic use.

Statistical analysis

Neutrophil counts were recorded on day 1, 8 and 15 in each treatment cycle for all patients and neutropenia was categorised using the National Cancer Institute common terminology criteria for adverse events (CTCAE, version 2.0). Tumour response was assessed by the Response Evaluation Criteria in Solid Tumors (RECIST) Group criteria. Overall survival was defined as time from randomisation until death from any cause. To evaluate the prognostic impact of chemotherapy-induced neutropenia, we first identified the worst grade of neutropenia during treatment for each patient. Then, using the proportional-hazards regression model, we estimated hazard ratios for overall survival according to the worst grade of neutropenia, after adjustment for covariates.

The participants in the trial had advanced NSCLC and a considerable number of patients died during the treatment period. This can lead to serious bias and result in a false-positive association between chemotherapy-induced neutropenia and longer survival, because patients who die during treatment receive fewer cycles of chemotherapy and, therefore, have less chance of developing more severe neutropenia. To lessen this bias, we used landmark analysis, whereby patients who died within 126 days (i.e., six 21-day cycles) of starting chemotherapy were excluded.

Survival curves were estimated using the Kaplan–Meier method. All reported p values are two-tailed; a value below 0.05 was considered statistically significant. All analyses were performed using SAS version 9.1 (SAS Institute, Cary, NC, USA).

RESULTS

Incidence of neutropenia

Table 1 shows the grade of neutropenia according to treatment cycle of chemotherapy. A total of 275 of the 387 patients died. The median follow-up time for all patients was 393 days (range 19–1711). One hundred and fifty-five patients (40%) completed the planned six cycles of treatment and 308 patients (80%) had chemotherapy-induced neutropenia: 20 patients (5%) had grade 1, 38 (10%) had grade 2, 97 (25%) had grade 3 and 153 (40%) had grade 4 as the worst grade.

G-CSF use

Table 2 shows the use of G-CSF according to the worst grade of neutropenia. Prophylactic use was not permitted. Nevertheless, G-CSF was administered to 15 patients who did not have grade-3 or greater neutropenia, or grade-4 leukopenia, so these patients were excluded from the analysis.

Association between survival and chemotherapy-induced neutropenia

First, the association between the worst grade of neutropenia and the number of treatment cycles was evaluated. Patients who experienced more severe neutropenia received more cycles of chemotherapy (Table 3).

We then examined the causes of deaths that occurred within 126 days of the initiation of chemotherapy. Thirty-three patients died and lung cancer was the cause of death for 26 patients. Pneumonia, myocardial infarction, neutropenic sepsis and interstitial pneumonia resulting from previous radiation accounted for one death each. The causes of three deaths were unknown. Only one patient died from neutropenic sepsis through this clinical trial.

These data indicate that patients who had better outcomes could receive more cycles of treatment, resulting in higher incidence of chemotherapy-induced neutropenia. To lessen this bias, we used a landmark analysis, excluding the 33 patients who died and two patients who were lost to follow-up within 126 days of the initiation of chemotherapy. Thus, data from 337 patients were analysed: 162 patients in the VGD arm and 175 patients in the PC arm. Since the mean number of treatment cycles for patients who developed chemotherapy-induced neutropenia was still higher than that for patients who had no neutropenia (Table 3), we included the number of treatment cycles as a covariate in the multivariate analysis. Given the size of this trial, the patients were

Table 2 The use of G-CSF according to worst grade of neutropenia ($n=387$). Values indicate number (%) of patients

		Use of G-CSF	
Worst grade of neutropenia	***n***	**No**	**Yes**
Grade 0	79	70 (89)	9 (11)
Grade 1	20	19 (95)	1 (5)
Grade 2	38	33 (87)	5 (13)
Grade 3	97	65 (67)	32 (33)
Grade 4	153	25 (16)	128 (84)

Abbreviation: G-CSF, granulocyte-colony-stimulating factor.

Table 1 The incidence of neutropenia according to treatment cycle ($n=387$). Values indicate number (%) of patients

Treatment cycle	**1**	**2**	**3**	**4**	**5**	**6**	**1–6**
Number of patients	**387**	**350**	**300**	**242**	**181**	**155**	**387**
Grade 0	140 (36)	123 (35)	113 (38)	90 (37)	73 (40)	81 (52)	79 (26)
Grade 1	26 (7)	31 (9)	26 (9)	16 (7)	11 (6)	4 (3)	20 (5)
Grade 2	42 (11)	50 (14)	30 (10)	33 (14)	19 (10)	18 (12)	38 (10)
Grade 3	89 (23)	87 (25)	79 (26)	53 (22)	43 (24)	30 (19)	97 (25)
Grade 4	90 (23)	59 (17)	52 (17)	50 (21)	35 (19)	22 (14)	153 (40)
Grades 1–4	247 (64)	227 (65)	187 (62)	152 (63)	108 (60)	74 (48)	308 (80)

Table 3 Association between worst grade of neutropenia and number of treatment cycles received

	Number of treatment cycles			
	All patients ($n=372$)		Patients in landmark analysis ($n=337$)	
Worst grade of neutropenia	***n***	**Mean ± s.d.**	***n***	**Mean ± s.d.**
Grade 0	70	3.4 ± 1.9	55	3.9 ± 1.9
Grade 1	19	4.0 ± 1.7	18	3.9 ± 1.7
Grade 2	33	4.2 ± 1.9	28	4.6 ± 1.7
Grade 3	97	4.5 ± 1.6	90	4.7 ± 1.5
Grade 4	153	4.5 ± 1.7	146	4.6 ± 1.6

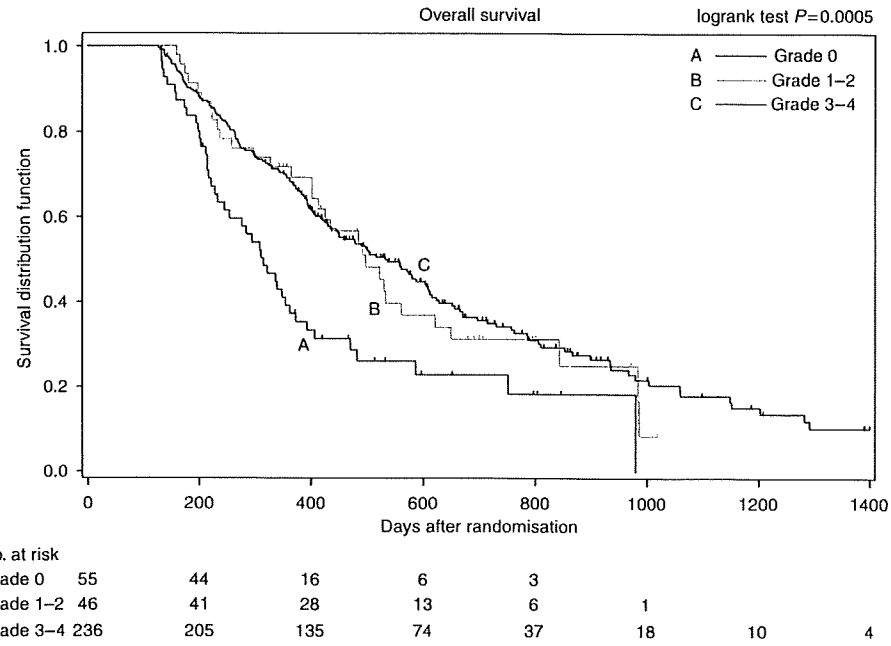

Figure 1 Kaplan–Meier survival curves according to the worst grade of chemotherapy-induced neutropenia (landmark time = 126 days).

distributed into three categories according to the worst grade of neutropenia: absent (grade 0), mild (grades 1 and 2) and severe (grades 3 and 4).

The median survival time was 10.5 months (95% confidence interval (CI) 8.2–12.4) for the grade-0 group ($n=55$), 16.6 months (95% CI 13.8–20.7) for the grade-1 to 2 group ($n=46$) and 17.8 months (95% CI 15.0–20.3) for the grade-3 to 4 group ($n=236$) (Figure 1). The baseline patient characteristics for the different groups are shown in Table 4. Using the proportional-hazards regression model to adjust for the imbalance of patient characteristics among groups, we estimated hazard ratios for overall survival according to the worst grade of neutropenia after adjustment for covariates (sex, smoking history, stage, ECOG performance status, weight loss, serum lactate dehydrogenase level, presence of bone, liver or skin metastases, pretreatment absolute neutrophil count and number of the treatment cycles as the known prognostic factors) (Paesmans *et al*, 1995; Pfister *et al*, 2004; Teramukai *et al*, 2009). Patients who had chemotherapy-induced neutropenia had lower risk of death than those who did not, although the difference between no neutropenia and grade-3 to 4 neutropenia was not significant. The adjusted hazard ratio compared with the grade-0 group was 0.59 (95% CI 0.36–0.97; $P=0.036$) for the grade-1 to 2 group, and that for the grade-3 to 4 group was 0.71 (95% CI 0.49–1.03; $P=0.072$) (Table 5). In both treatment arms, the proportion of patients who moved on from VGD or PC to second-line chemotherapy (e.g., because of progressive disease (PD)) was almost equal among the groups distributed by the grade of neutropenia.

We also estimated the hazard ratios for overall survival according to the combination of worst grade of neutropenia and best tumour response, after adjustment for the covariates listed above (Table 6). As a preliminary step, hazard ratios according to the best tumour response alone were calculated. The adjusted hazard ratio for stable disease (SD) compared with partial response (PR) as the best tumour response was 1.93 (95% CI, 1.39–2.67) and that for PD compared with PR was 3.31 (95% CI, 1.89–5.79). The adjusted hazard ratio compared with no neutropenia with PR was 0.29 (95% CI 0.11–0.80) for grade-1 to 2 neutropenia with PR; 0.44 (95% CI 0.21–0.92) for grade-3 to 4 neutropenia with PR; 0.78 (95% CI 0.33–1.87) for grade-1 to 2 neutropenia with SD and 0.80 (95% CI 0.38–1.70) for grade-3 to 4 neutropenia with SD. The hazard ratios did not differ significantly between the patients who developed neutropenia with SD and those who lacked neutropenia with PR.

Table 4 Baseline patient characteristics ($n = 337$)

	Grade 0 ($n = 55$)	Grade 1–2 ($n = 46$)	Grade 3–4 ($n = 236$)
Age (years)			
Median (range)	63 (45–78)	64 (43–81)	65 (33–81)
Sex			
Male (*n*) (%)	43 (78)	36 (78)	159 (67)
Female (*n*) (%)	12 (22)	10 (22)	77 (33)
Smoking history			
Current smokers (*n*) (%)	25 (45)	24 (52)	91 (39)
Former smokers (*n*) (%)	17 (31)	9 (20)	70 (30)
Non-smokers (*n*) (%)	11 (20)	10 (22)	65 (28)
Unknown (*n*) (%)	2 (4)	3 (7)	10 (4)
NSCLC stage			
IIIB (*n*) (%)	15 (27)	10 (22)	37 (16)
IV (*n*) (%)	40 (73)	36 (78)	199 (84)
ECOG performance status			
0 (*n*) (%)	16 (29)	16 (35)	112 (47)
1 (*n*) (%)	39 (71)	30 (65)	124 (53)
Weight loss			
< 5% (*n*) (%)	46 (84)	39 (85)	198 (84)
>5% (*n*) (%)	9 (16)	7 (15)	38 (16)
LDH			
Normal (*n*) (%)	40 (73)	32 (70)	172 (73)
High (*n*) (%)	15 (27)	14 (30)	64 (27)
Bone metastases			
No (*n*) (%)	42 (76)	36 (78)	170 (72)
Yes (*n*) (%)	13 (24)	10 (22)	66 (28)
Liver metastases			
No (*n*) (%)	52 (95)	43 (93)	217 (92)
Yes (*n*) (%)	3 (5)	3 (7)	19 (8)
Skin metastases			
No (*n*) (%)	54 (98)	45 (98)	233 (99)
Yes (*n*) (%)	1 (2)	1 (2)	3 (1)
Pretreatment neutrophil count			
Mean (per $mm^3 \pm$ s.d.)	5828 ± 2211	4968 ± 1732	4427 ± 2275

Abbreviations: ECOG = Eastern Cooperative Oncology Group; LDH = lactate dehydrogenase; NSCLC = non-small-cell lung cancer.

Table 5 Multivariate proportional-hazards regression analysis for associations between overall survival and worst grade of neutropenia ($n = 337$)

	Hazards ratio	95% CI	*P*-value
Neutropenia			
Grade 0	1	—	—
Grade 1/2	0.59	0.36–0.97	0.036
Grade 3/4	0.71	0.49–1.03	0.072
Sex			
Male	1	—	—
Female	0.75	0.53–1.06	0.104
Smoking history			
Non-/former smokers	1	—	—
Current smokers	1.67	1.23–2.28	0.001
Stage			
IIIB	1	—	—
IV	1.12	0.77–1.64	0.551
Performance status			
0	1	—	—
1	2.08	1.53–2.84	<0.0001
Weight loss			
<5%	1	—	—
⩾5%	1.06	0.74–1.50	0.765
Serum LDH			
Normal	1	—	—
High	1.64	1.20–2.25	0.002
Bone metastasis			
No	1	—	—
Yes	1.23	0.87–1.72	0.240
Liver metastasis			
No	1	—	—
Yes	1.62	1.02–2.60	0.043
Skin metastasis			
No	1	—	—
Yes	4.25	1.50–12.03	0.006
Neutrophil count			
< 4500/mm^3	1	—	—
⩾4500/mm^3	1.56	1.18–2.05	0.002
Number of treatment cycles			
1	1	—	—
2	0.94	0.48–1.84	0.866
3	1.07	0.58–1.96	0.838
4	0.88	0.48–1.61	0.674
5	0.59	0.29–1.21	0.151
6	0.58	0.34–1.01	0.054

Abbreviations: CI = confidence interval; LDH = lactate dehydrogenase.

DISCUSSION

It has been reported that haematological toxicity could be a measure of the biological activities of cytotoxic drugs. Many of us believe that administration of larger dose of chemotherapeutic agents over a defined period is more likely to result in success – the patient will have more chances to go into complete or partial remission, and this will improve survival (Luciani *et al*, 2009). However, several studies in the last decade have reported that larger doses of chemotherapy do not always improve prognosis (Stadtmauer *et al*, 2000; Möbus *et al*, 2007). Using a unique time-dependent approach to analyse data from a prospective survey of patients with advanced gastric cancer treated with oral fluoropyrimidine S-1, Yamanaka *et al* (2007) reported that survival was longest in patients who experienced grade-2 neutropenia as the worst grade.

Here we review data from a clinical trial of patients with advanced NSCLC. Patients who developed neutropenia showed longer survival than those who had no neutropenia. Furthermore, severe neutropenia (grade 3–4) was no better than mild neutropenia (grade 1–2) for prediction of overall survival. As a whole, these results are consistent with previous reports of the chemotherapy of NSCLC and gastric cancer (Di Maio *et al*, 2005; Yamanaka *et al*, 2007; Pallis *et al*, 2008), and strongly suggest that neutropenia *per se* is not important, but the use of neutropenia to reflect that an adequate dose has been given.

The dose of chemotherapeutic agents is usually determined on the basis of body surface area (BSA) or creatinine clearance; however, elimination of the agents will vary from patient to patient because of a variety of factors such as pharmacogenetic background (Friedman *et al*, 1999) and drug interactions (Relling *et al*, 2000). Variation in drug elimination may explain why some patients in this clinical trial experience severe toxicities or inadequate antitumour effects. Absence of neutropenia may mean that the doses of chemotherapeutic agents administered are not enough to produce the full antitumour effect. Gurney (2002)

Table 6 Multivariate proportional-hazards regression analysis for overall survival according to the worst grade of neutropenia and tumour response

Worst grade of neutropenia	Best tumour response		
	Partial response (PR)	Stable disease (SD)	Progressive disease (PD)
Grade 0	1 ($n=12$)	1.08 (0.47–2.48) ($n=25$)	1.04 (0.33–3.25) ($n=7$)
Grade 1/2	0.29 (0.11–0.80) ($n=16$)	0.78 (0.33–1.87) ($n=23$)	2.05 (0.60–7.03) ($n=5$)
Grade 3/4	0.44 (0.21–0.92) ($n=93$)	0.80 (0.38–1.70) ($n=95$)	1.56 (0.63–3.88) ($n=23$)
Grade 0–4	1 ($n=121$)	1.93 (1.39–2.67) ($n=143$)	3.31 (1.89–5.79) ($n=35$)

Abbreviation: CI = confidence interval. Values indicate hazards ratios (95% CIs).

pointed out a poor correlation between BSA and the pharmacokinetics of anticancer agents (Newell, 2002).

From this perspective, this association also suggests that neutropenia or other toxicities induced by chemotherapy can be used as an indicator for planning regimens tailored to individual patients. When we administer chemotherapy to patients, we prepare a schedule for administration of each agent. Then, after initiation of chemotherapy, we often reduce the planned doses of agents in the event of severe neutropenia or other toxicities, whereas we seldom increase the dose if a patient lacks such toxicities. However, increasing the doses of agents to induce mild or moderate neutropenia may be of benefit for patients who do not show haematological or major non-haematological toxicities in the first or second cycle of treatment.

We have previously confirmed that increased pretreatment neutrophil count is an independent negative prognostic factor (Teramukai *et al*, 2009), and we included it as one of covariates in the present study. Tumour-related leukocytosis (neutrophilia) is encountered occasionally in patients with NSCLC and has recently been demonstrated to be an important negative prognostic factor for overall survival and time to progression in patients with NSCLC (Mandrekar *et al*, 2006). Although autonomous production of G-CSF and granulocyte–macrophage-colony-stimulating factor (GM-CSF) by tumour has been identified in some cases, leukocytosis (neutrophilia) in NSCLC patients is not fully understood and is likely to be caused by a combination of factors. Considering the negative prognostic value of leukocytosis (neutrophilia), it can be hypothesised that a proportion of the patients who do not develop neutropenia during treatment may have a poorer prognosis because they may be potentially affected by tumour-related leukocytosis (neutrophilia) and protected from chemotherapy-induced neutropenia (Maione *et al*, 2009). However, the results of our analysis suggest that chemotherapy-induced neutropenia is a predictor independent of NSCLC-related leukocytosis, since the risk of death estimated by the proportional-hazards regression model was significantly lower in patients who had grade-1 to 2 chemotherapy-induced neutropenia after adjustment for covariates, including pretreatment neutrophil count.

We estimated hazard ratios for the overall survival for subgroups assigned by the combination of the worst grade of neutropenia and the best tumour response. Patients who experienced neutropenia with PR as the best tumour response showed lower risk of death than those with PR who lacked neutropenia. The hazard ratios did not differ significantly between the patients who developed mild or severe neutropenia with SD and those with PR who lacked neutropenia. There are some limitations to the assessment of tumour size using the RECIST method or other widely used methods of assessing tumour response to anticancer therapy. Lara *et al* (2008) reported the importance of how to interpret SD and introduced the concept of disease control rate. Results from the randomised trial (JMTO LC00-03) and this study add further evidence that the association between the RECIST response and overall survival may depend on the grade of neutropenia and that the RESICT response may not be a surrogate endpoint for overall survival of advanced NSCLC in the chemotherapy setting (Kubota *et al*, 2008). Further investigation into this association in a large-scale meta-analysis would be helpful to resolve the important question of whether tumour response to anticancer agents could be used as a surrogate for overall survival in patients with advanced cancer (Ichikawa and Sasaki, 2006).

In conclusion, we confirm that chemotherapy-induced neutropenia can predict survival for patients with advanced NSCLC. This association also suggests the possibility that neutropenia, or other chemotherapy-induced toxicities, can be used as indicators in setting up dosage regimens that are tailored for individual patients. Categorisation of patients according to drug elimination capacity may be useful in determining initial dosage regimens, with subsequent fine-tuning depending on the presence or absence of haematological and non-haematological toxicities during early cycles. Prospective randomised trials of early-dose increases guided by chemotherapy-induced toxicities are, therefore, warranted.

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original article

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A phase II dose-ranging study of palonosetron in Japanese patients receiving moderately emetogenic chemotherapy, including anthracycline and cyclophosphamide-based chemotherapy

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Background: The 5-HT_3 receptor antagonists (RAs) help maintain the standard of care, in various combinations with other agents, for prevention of chemotherapy-induced nausea and vomiting (CINV). Palonosetron is a new generation 5-HT_3 RA with indication not only acute but also delayed nausea and vomiting induced by moderately emetogenic chemotherapy (MEC). This study was carried out to determine the optimal dosage of palonosetron in combination with dexamethasone in patients in Japan.

Patients and methods: This study evaluated the efficacy and safety of palonosetron in patients receiving MEC combined with dexamethasone. Patients received single doses of 0.075, 0.25, or 0.75 mg of palonosetron before MEC. Dexamethasone was infused before palonosetron, at 20 mg for the patients receiving paclitaxel (Taxol) and 8 mg for the patients not receiving paclitaxel. The primary end point was complete response (CR: no emetic episodes and no rescue medication) in the acute phase (0–24 h).

Results: In total, 204 patients (88 men, 116 women; 96 with paclitaxel, 108 without paclitaxel) were assessable for efficacy. No dose–response relationship was observed regarding the CR rate in the acute phase. CR rates increased dose dependently for delayed (24–120 h) and overall (0–120 h) phases in patients receiving anthracyclines and cyclophosphamide combination (AC/EC, $n = 80$); however, the difference in CR rates among doses was not statistically significant. The most commonly reported adverse events related to palonosetron were constipation and headache, confirming the class safety profile.

Conclusion: This study indicates a statistically nonsignificant trend for the dose–response relationship for antiemetic protection in the delayed and overall phases in AC/EC patients (the regimen currently considered to be more emetogenic than MEC).

Key words: chemotherapy-induced nausea and vomiting, 5-HT_3 receptor antagonist, palonosetron

introduction

Chemotherapy-induced nausea and vomiting (CINV) are among the most common significant side-effects of cancer chemotherapy. CINV can become a major problem both for patients receiving highly emetogenic chemotherapy (HEC) and moderately emetogenic chemotherapy (MEC), especially in patients receiving a combination of anthracyclines and cyclophosphamide [1, 2]. Inadequate control of CINV can have a considerable negative impact on all aspects of patient quality of life and may lead patients to refuse to continue chemotherapy [1]. It is clear today that serotonin plays an important role in the development of CINV [3]. Control of acute nausea and vomiting improved significantly in the 1990s when 5-HT_3 receptor antagonists (RAs) were introduced into clinical practice. Combined with various agents, the 5-HT_3 RAs are now considered a standard of care [4–6]. The dose to be used varies in different settings. However, the effectiveness of

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previously developed 5-HT_3 RAs such as ondansetron, dolasetron, granisetron, and tropisetron, in preventing delayed nausea and vomiting (with symptoms occurring later than 24 h after chemotherapy) is considered less than optimal. In fact, patients still experience CINV when undergoing HEC or MEC even when multiple 5-HT_3 RAs are administered [7, 8].

Palonosetron is a new highly potent and selective 5-HT_3 RA. It has a receptor binding affinity that is ~100 times higher than previously developed 5-HT_3 RAs [9] and it has a significantly longer plasma elimination half-life, ~40 h [10] compared with other agents in this class [11, 12]. Results from three phase III trials [13–15] and one phase II study, conducted in the Western patients [16], indicated in a recommended dosage of 0.25 mg for palonosetron, administered as a single i.v. dose 30 min before chemotherapy. In the phase II study to determine the most appropriate dose of palonosetron (0.3–90 μg/kg) for patients receiving HEC, the two lowest effective doses were reported to be 3.0 and 10 μg/kg (reported to be equivalent to fixed doses of 0.25 and 0.75 mg per body, respectively) [16]. These two fixed doses of palonosetron were then compared with single i.v. doses of ondansetron 32 mg [15] and dolasetron 100 mg [14] in two phase III trials conducted in patients receiving MEC. In these trials, 0.25 mg of palonosetron was proven to control CINV with a clinically relevant better efficacy than ondansetron and dolasetron, at all times studied (acute, delayed, and overall phases). The difference in complete response (CR; no emesis and no rescue medication) rates between 0.25 mg palonosetron and comparator was around 15% in the delayed and overall phases. Both studies failed to show any advantage for patients who received 0.75 mg palonosetron. Of note, these studies included a minority of patients receiving corticosteroids. In contrast to the other 5-HT_3 RAs, palonosetron is given as a single injection to patients receiving MEC on the day of chemotherapy, to prevent CINV in the overall period following chemotherapy administration (1–5 days) [14, 15]. These data prompted the Food and Drug Administration (FDA) to grant palonosetron approval for the prevention of acute and delayed CINV in patients receiving MEC. Palonosetron is also approved for the prevention of CINV in European Union (EU) countries.

The current phase II, dose-ranging, randomized, double-blind, multicenter study was conducted on patients receiving MEC in Japan to identify the most effective dose of palonosetron when combined with fixed doses of dexamethasone. The additional objective was safety assessment, in the evaluated dose range.

patients and methods

patient selection

MEC was defined as chemotherapy based on the administration of a single agent (or a combination of agents) of emetogenicity level 3 or 4 of the National Comprehensive Cancer Network (NCCN) 2004 guidelines [17] [i.e. any dose of carboplatin, epirubicin, idarubicin, ifosfamide, or irinotecan; or cyclophosphamide (≤1500 mg/m^2), doxorubicin (≥20 mg/m^2), or cisplatin (<50 mg/m^2, infused over 1–4 h)]. Patients with diagnosis of cancer who were naive to chemotherapy and who satisfied the following inclusion criteria were scheduled to receive their first dose of MEC and enrolled in this dose-ranging study of palonosetron. Patients were required to have an Eastern Cooperative Oncology Group performance status (ECOG PS) of one or less, age between 20 and 79 years, and adequate bone marrow (white blood cell count ≥ 3000/mm^3), hepatic (serum aspartate aminotransferase and alanine aminotransferase levels < 100 IU/l each), and renal (creatinine clearance level estimated by the Cockcroft–Gault formula [18] ≥60 ml/min) function. Exclusion criteria included severe, uncontrolled, concurrent illness other than cancer; symptomatic brain metastasis; evidence of seizure disorder requiring anticonvulsants; pleural effusion or ascites that required drainage; gastric or intestinal obstruction; vomiting, retching, or ≥grade 2 nausea [National Cancer Institute—Common Terminology Criteria for Adverse Events v3.0 (CTCAE)]; corrected QT interval >450 msec on 12-lead electrocardiogram (ECG); known hypersensitivity to other 5-HT_3 RAs or dexamethasone sodium phosphate; no consent to practice adequate contraception; and participation in another study of investigational agents within 3 months. Patients were excluded if they were scheduled to receive level 4 or more emetogenic agents according to the NCCN 2004 guidelines or radiotherapy within the period of observation of efficacy (5 days). Administration of any antiemetics, sedatives, or corticosteroids (other than dexamethasone as a study medication) was not permitted within 24 h preceding palonosetron.

study design and treatment regimen

This was a phase II, randomized, double-blind, dose-ranging, multicenter study conducted in Japan from April to November 2005. Eligible patients were randomly assigned to receive a single i.v. dose of palonosetron of 0.075, 0.25, or 0.75 mg over 30 s, administered 30 min before the first dose of MEC or AC/EC regimen on day 1. Patients were stratified at randomization by gender and administration of paclitaxel (Taxol, Bristol-Myers K.K., Japan) using a minimization method. Dexamethasone 8 mg was also i.v. administered within 45 min before palonosetron administration. In case of MEC including paclitaxel, 20 mg dexamethasone, combined with 50 mg oral diphenhydramine and 20 mg i.v. famotidine or 50 mg i.v. ranitidine, was administered as premedication to prevent anaphylaxis at least 30 min before paclitaxel administration [19]. This study was approved by the Institutional Review Board at each participating institution and was conducted according to the Declaration of Helsinki. Written informed consent was obtained from each patient before they were enrolled in the trial.

efficacy parameters

The primary end point of this study was the proportion of patients who achieved a CR, defined as no emetic episode and no use of rescue medication during the first 24 h (acute phase) following administration of study chemotherapeutic agents. An emetic episode was defined as one episode of vomiting or a sequence of episodes in very close succession not relieved by a period of relaxation of at least 1 min, any number of unproductive emetic episodes (retching) in any given 5-min period, or an episode of retching lasting <5 min combined with vomiting not relieved by a period of relaxation of at least 1 min [15]. Secondary end points included CR rates from 24 to 120 h (delayed phase) and 0 to 120 h (overall phase); complete control (CC) rates, which was defined as no emetic episode, no need for rescue medication, and no more than mild nausea; time to treatment failure (first emetic episode or first need of rescue medication, whichever occurred first); number of emetic episodes; severity of nausea; and patient global satisfaction with antiemetic therapy as measured on a visual analogue scale.

study assessment procedures

Each consenting patient was screened for study eligibility within 7 days before being enrolled. Baseline assessment procedures included past medical history, vital sign measurements, concomitant medications, ECOG

PS, physical examination, 12-lead ECG, and laboratory tests (complete blood count with differential, blood chemistry, urinalysis, and estimated creatinine clearance). All patients were required to be hospitalized at least until completing assessment on day 2. In addition, follow-up assessment was conducted on day 8 (permissible range days 6–10) and on day 15 (days 14–20) for each patient. Assessment procedures included vital sign measurements, physical examination, 12-lead ECG, and laboratory tests. Evaluation of daily emetic episodes, severity of nausea, and patient global satisfaction until day 5 were reported by the patient in a diary. Use of rescue medication was recorded on each patient's medical chart. Safety was assessed using CTCAE until day 15. All adverse events were reported, irrespective of study medications.

statistical analyses

In this dose-ranging study of palonosetron, CR rates in acute phase were assumed to be 67%, 85%, and 85% in the 0.075, 0.25, and 0.75 mg palonosetron dose groups, respectively. This assumption was based on the following: (i) the CR rate in acute phase was reported to plateau at a palonosetron dose ≥0.25 mg in two earlier phase III studies of MEC in Western patients [14, 15]; (ii) concurrent use of dexamethasone (8 or 20 mg) in all patients in the present study would contribute a 10%–20% increase of CR rates [20]; and (iii) the CR rate in the lowest dose of 0.075 mg was estimated to be ~40% higher than the CR rate in the lowest dose group (24% in the 0.3 μg/kg dose group) of the preceding phase II study of HEC in Western patients [16] because the present study enrolled patients receiving MEC and all patients were administered dexamethasone with palonosetron. For the Cochran–Armitage trend test (contrast coefficient score setting: −2 at the 0.075 mg and 1 at the 0.25 and 0.75 mg dose groups), a sample size of 189 assessable patients was required to ensure a one-sided α level of 2.5% with a statistical power of 80%. Assuming five dropout patients per dose group, 204 patients were needed in this study.

Statistical analyses were carried out using SAS software version 8.0 (SAS Institute, Inc., Cary, NC). The Cochran–Armitage trend test was used to determine the significance of differences in dose–response parameters (i.e. CR or CC rates) between dose groups. The χ^2 test or Fisher's exact probability test was used to compare proportions of categorical variables. The difference in mean values of baseline characteristics was tested using one-way analysis of variance. The number of emetic episodes, severity of nausea, and patient global satisfaction were compared between dose groups using the Kruskal–Wallis test. Time-to-event distributions were calculated using the method of Kaplan and Meier, and differences between these distributions were assessed using the log-rank test.

Analyses of efficacy end points were carried out for the full analysis set (FAS) population, which was defined as those of patients receiving both palonosetron and level 3 or 4 emetogenic chemotherapy agents on day 1. Furthermore, additional efficacy analyses were carried out for the subgroup patients receiving combination chemotherapy of AC/EC, which is considered to be more emetogenic than MEC agents. Safety data for all patients receiving palonosetron were tabulated and summarized descriptively.

results

patient baseline demographics

We enrolled 211 patients in this study from 19 institutions. Patients were randomly assigned to one of the three palonosetron dose groups. Efficacy and safety analyses were carried out for the FAS population (67, 68, and 69 patients, respectively, in the 0.075, 0.25, and 0.75 mg palonosetron dose groups) because seven patients who had never received palonosetron were excluded (three patients each in the 0.075 and 0.25 mg dose groups and one patient in the 0.75 mg dose group).

Baseline demographic data and characteristics of patients in the FAS cohort are presented in Table 1. There were no differences between the groups in the distribution of patients by gender, age, height, weight, or ECOG PS. The most common types of tumor cancer were non-small-cell lung cancer (n = 108) followed by breast cancer (n = 82) and small-cell lung cancer (n = 9). The most common chemotherapeutic agents administered on day 1 were carboplatin (n = 112), paclitaxel (n = 96), cyclophosphamide (n = 83), epirubicin (n = 45), and doxorubicin (n = 36). Eighty patients received a combination of anthracyclines and cyclophosphamide (AC/EC). There were no differences in the proportion of cancer types or chemotherapy agents administered in individual palonosetron dose groups.

Table 1. Patient baseline demographics

	Palonosetron dose			P value
	0.075 mg (N = 67), n (%)	0.25 mg (N = 68), n (%)	0.75 mg (N = 69), n (%)	
Gender				
Male	31 (46.3)	28 (41.2)	29 (42.0)	0.822
Female	36 (53.7)	40 (58.8)	40 (58.0)	
Age (years)[a]	57.0 ± 11.2	58.2 ± 11.4	59.3 ± 10.2	0.474
Height (cm)[a]	160.5 ± 8.0	160.0 ± 7.6	157.7 ± 8.7	0.105
Weight (kg)[a]	60.1 ± 9.5	57.3 ± 8.9	56.8 ± 9.7	0.085
ECOG PS				
0	54 (80.6)	48 (70.6)	53 (76.8)	0.390
1	13 (19.4)	20 (29.4)	16 (23.2)	
Tobacco use				
Nonsmoker	32 (47.8)	38 (55.9)	34 (49.3)	0.642
Ex-smoker 180 days prior	13 (19.4)	9 (13.2)	17 (24.6)	
Ex-smoker within 180 days	10 (14.9)	12 (17.6)	14 (20.3)	
Current smoker	12 (17.9)	9 (13.2)	4 (5.8)	
Alcohol use				
None	31 (46.3)	27 (39.7)	34 (49.3)	0.414
Rarely	9 (13.4)	8 (11.8)	9 (13.0)	
Occasionally	11 (16.4)	13 (19.1)	11 (15.9)	
Regularly	16 (23.9)	20 (29.4)	15 (21.7)	
Cancer type				
Lung non-small cell	34 (50.7)	36 (52.9)	38 (55.1)	Not done
Breast	28 (41.8)	27 (39.7)	27 (39.1)	
Lung small cell	4 (6.0)	3 (4.4)	2 (2.9)	
Other	1 (1.5)	2 (2.9)	2 (2.9)	
Chemotherapy agent				
Carboplatin	37 (55.2)	37 (54.4)	38 (55.1)	Not done
Paclitaxel	32 (47.8)	32 (47.1)	32 (46.4)	
Cyclophosphamide	28 (41.8)	27 (39.7)	28 (40.6)	
Epirubicin	15 (22.4)	16 (23.5)	14 (20.3)	
Doxorubicin	11 (16.4)	12 (17.6)	13 (18.8)	

[a]Mean ± standard deviation.

ECOG PS, Eastern Cooperative Oncology Group performance status.

primary efficacy analysis

The CR rates in the acute phase were 85.1%, 82.4%, and 92.8%, respectively, for the 0.075, 0.25, and 0.75 mg palonosetron dose groups (Figure 1A). There was no significant dose–response relationship found with the Cochran–Armitage trend test (contrast coefficients: −2 at the 0.075 mg and 1 at the 0.25 mg and 0.75 mg dose groups; $P = 0.2499$), where age (<65 or ≥65 years) and gender were included as stratification factors because they were identified as covariates on blind review.

secondary efficacy analysis

For the delayed (24–120 h) and overall (0–120 h) periods, the CR rates increased in a dose-dependent way, although not with clinical relevance (62.7%, 66.2%, and 71.0% for the delayed and 59.7%, 64.7%, and 69.6% for the overall period in the 0.075, 0.25, and 0.75 mg palonosetron dose groups, respectively; Figure 1A), and a similar increase was observed in CR rate among the three dose groups for the cumulative periods (Table 2) as well as successive 24-h periods (24–48, 48–72, 72–96, and 96–120 h; data not shown). The CC rates for the acute, delayed, and overall periods were similar to those in the CR evaluation (data not shown). In addition, the number of emetic episodes, severity of nausea as assessed by the four-point Likert scale, and patient global satisfaction did not differ among the three palonosetron dose groups (data not shown).

Cumulative CR rates in patients who received a combination of anthracyclines (doxorubicin or epirubicin) and cyclophosphamide (AC/EC, $n = 80$) are shown in Figure 1B and Table 2. In this subgroup of patients who received less corticosteroids, a dose-dependent increase in CR rates was observed, with increases of >10%, showing better efficacy in the 0.75 mg dose group. These differences did not reach statistical significance. CR rates in patients receiving agents other than the AC/EC regimen, mainly carboplatin and paclitaxel, were 95.1%–100.0% in the acute phase and 76.2%–78.0% in the delayed phase (Figure 1C).

The time to treatment failure in the FAS population and AC/EC subgroup are shown in Figure 2A and B ($n = 203$ and $n = 80$, respectively). One patient in the 0.25 mg palonosetron dose group discontinued the study and was excluded from the FAS for this analysis. The median time to treatment failure in the FAS population was >120 h in all three dose groups, with first quartile times of 41.8, 44.3, and 72.2 h, respectively, in the 0.075, 0.25, and 0.75 mg palonosetron dose groups. In the AC/EC subgroup analysis, the median time to treatment failure was 36.2, 55.8, and >120 h, respectively, in the 0.075, 0.25, and 0.75 mg palonosetron dose groups. However, these differences did not reach statistical significance.

safety evaluation

Of the total 204 patients evaluated for safety, 67 (100%), 67 (98.5%), and 69 (100%) experienced at least one adverse event, in the 0.075, 0.25, and 0.75 mg palonosetron dose groups, respectively. Adverse drug reactions appeared in 22 (32.8%), 17 (25.0%), and 16 (23.2%) patients in the 0.075, 0.25, and 0.75 mg palonosetron dose groups, respectively. In addition, serious adverse events were reported in 10 (4.9%) patients, all of which were assessed as not related to palonosetron. A list of

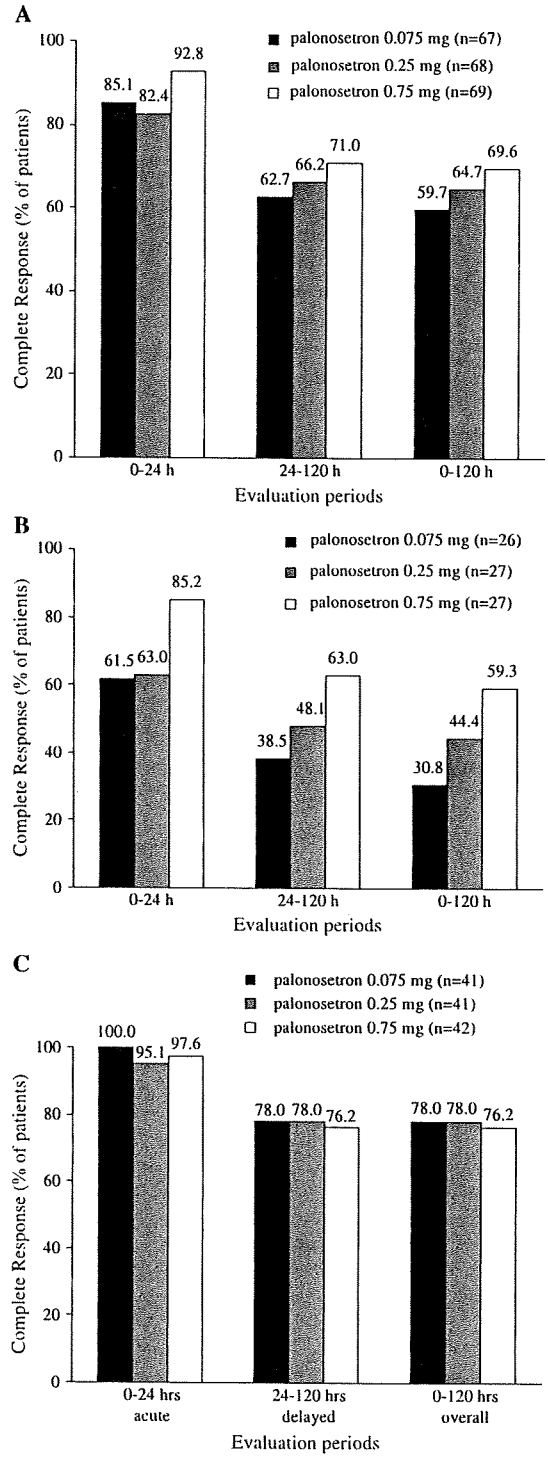

Figure 1. (A) Complete response rates after administration of moderately emetogenic chemotherapy in pooled patients population ($n = 204$). (B) Complete response rates in AC/EC subgroup ($n = 80$). (C) Complete response rates in non-AC/EC subgroup ($n = 124$). AC/EC, anthracyclines and cyclophosphamide combination.

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Table 2. Complete response rate in the FAS (A) and complete response rates in AC/EC subgroup (B)

Period (h)	*n*	%	95% CI	*n*	%	95% CI	*n*	%	95% CI
A									
	0.075 mg ($N = 67$)			0.25 mg ($N = 68$)			0.75 mg ($N = 69$)		
0–24	57	85.1	74.3–92.6	56	82.4	71.2–90.5	64	92.8	83.9–97.6
0–48	45	67.2	54.6–78.2	50	73.5	61.4–83.5	56	81.2	69.9–89.6
0–72	43	64.2	51.5–75.5	45	66.2	53.7–77.2	51	73.9	61.9–83.7
0–96	41	61.2	48.5–72.9	44	64.7	52.2–75.9	48	69.6	57.3–80.1
0–120	40	59.7	47.0–71.5	44	64.7	52.2–75.9	48	69.6	57.3–80.1
24–120	42	62.7	50.0–74.2	45	66.2	53.7–77.2	49	71.0	58.8–81.3
B									
	0.075 mg ($N = 26$)			0.25 mg ($N = 27$)			0.75 mg ($N = 27$)		
0–24	16	61.5	40.6–79.8	17	63.0	42.4–80.6	23	85.2	66.3–95.8
0–48	9	34.6	17.2–55.7	15	55.6	35.3–74.5	18	66.7	46.0–83.5
0–72	9	34.6	17.2–55.7	12	44.4	25.5–64.7	16	59.3	38.8–77.6
0–96	8	30.8	14.3–51.8	12	44.4	25.5–64.7	16	59.3	38.8–77.6
0–120	8	30.8	14.3–51.8	12	44.4	25.5–64.7	16	59.3	38.8–77.6
24–120	10	38.5	20.2–59.4	13	48.1	28.7–68.1	17	63.0	42.4–80.6

AC/EC, anthracyclines and cyclophosphamide combination; CI, confidence interval.

common treatment-related adverse events is given in Table 3. The most common adverse events related to palonosetron were constipation ($n = 20$) and headache ($n = 9$). Neither the incidence nor the severity of these events was dependent on dose. In this study, safety assessments were similar to what was reported in the safety profile observed in previous phase III trials [13–15] conducted in Western patients.

discussion

Palonosetron has been approved for CINV induced by MEC both in United States and EU at 0.25 mg i.v. Its peculiar characteristics are high affinity for the receptor and prolonged duration of action. The FDA approved palonosetron for both acute and delayed emesis (i.e. up to 120 h of observation).

The present dose-ranging study was conducted in patients receiving MEC in Japan. At the time this study was planned, the AC/EC regimens were considered MEC. Eighty of 204 patients (39.2%) received such regimens.

A slight but not clinically relevant dose–response relationship for antiemetic efficacy was observed in the FAS patient population between the three tested doses in the acute, delayed, and overall phases. Interestingly, in the subgroup of patients receiving AC/EC, the CR rates for the delayed and overall phases appeared to increase with dose and showed the highest efficacy in the 0.75 mg dose group. The lowest dose appeared to be suboptimal in the delayed (CR = 38.5%) and overall (CR = 30.8%) periods. The 0.75 mg dose appeared to be at least 20% more efficacious in the acute phase (CR = 85.2%) in comparison to 0.075 mg (CR = 61.5%) and 0.25 mg (CR = 63.0%). In the delayed period, it was ~15% better than the dose of 0.25 mg (CR, respectively, of 63.0% and 48.1%), while in the overall phase, CR accounted, respectively, for 59.3% and 44.4%, showing another difference of 15%. The difference of 15% between the two doses shows numerically higher CR rates for the 0.75 mg dose with overall safety similar

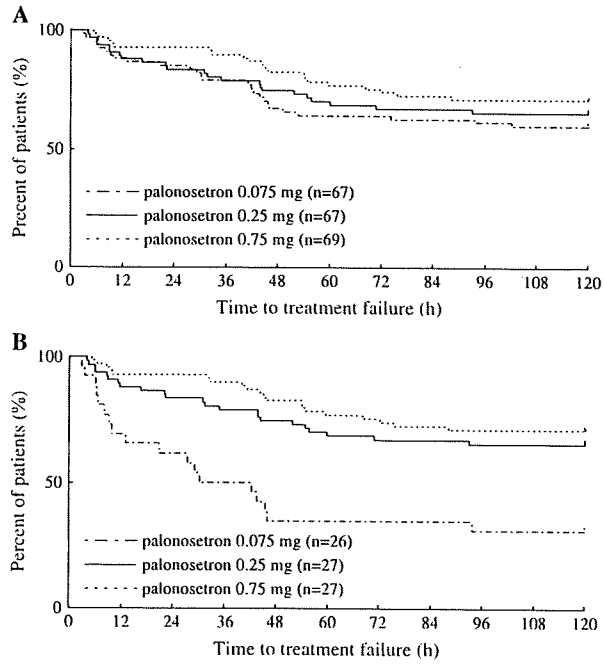

Figure 2. (A) Time to treatment failure of treatment of all patients ($n = 203$). (B) Time to treatment failure of treatment of AC/EC subgroup ($n = 80$). AC/EC, anthracyclines and cyclophosphamide combination.

to other dose groups and shows the same clinical difference found between palonosetron and comparators in the pivotal clinical phase III studies conducted in Europe and the United States, that included regimens of cyclophosphamide and/or anthracyclines [14, 15]. The highest dose level was shown to better protect from CINV those patients who received a regimen of AC/EC. On the other hand, CR rates in the patients receiving agents other than AC/EC showed no

Table 3. Common adverse events related to treatment

	Palonosetron dose											
	0.075 mg ($N = 67$), n (%)				0.25 mg ($N = 68$), n (%)				0.75 mg ($N = 69$), n (%)			
	Grade				Grade				Grade			
	1	2	3	4	1	2	3	4	1	2	3	4
Constipation	6 (9.0)	2 (3.0)	0	0	3 (4.4)	2 (2.9)	0	0	7 (10.1)	0	0	0
Headache	3 (4.5)	0	0	0	3 (4.4)	0	0	0	2 (2.9)	1 (1.4)	0	0
Rash	2 (3.0)	0	0	0	2 (2.9)	4 (5.9)	0	0	0	0	0	0
ALT increased	4 (6.0)	0	0	0	2 (2.9)	0	0	0	2 (2.9)	0	0	0
Blood bilirubin increased	2 (3.0)	0	0	0	2 (2.9)	1 (1.5)	0	0	2 (2.9)	0	0	0
ECG QT prolongation	3 (4.5)	1 (1.5)	1 (1.5)	0	1 (1.5)	0	0	0	0	0	1 (1.4)	0

ALT, alanine aminotransferase; ECG QT, electrocardiographic QT intervel.

significant difference among the three dose groups in the acute, delayed, and overall periods. This subgroup population included mainly patients receiving paclitaxel combined with carboplatin and they were given high-dose dexamethasone (20 mg), diphenhydramine, and histamine H_2-RA (famotidine or ranitidine) as premedication to prevent anaphylaxis. The CR rates in this subgroup were most likely influenced by the concomitant use of dexamethasone and diphenhydramine, which are also listed as antiemetic agents in the guidelines [21, 22]. This may also possibly have resulted in the absence of dose response through the efficacy evaluation period in this subgroup population.

Palonosetron was well tolerated in all the dose groups. Incidences, frequencies, intensities, and drug relationships of AEs appeared to be equally distributed among the three dose groups with no apparent relationship to dose. In another Japanese phase II study, conducted in patients receiving HEC, no differences were apparent in protection from CINV for 0.25 and 0.75 mg doses. Also in this trial, no differences in safety profile were evident between the two dose groups [23].

In conclusion, the trends for better efficacy and the excellent safety profile of palonosetron in this trial and the phase II HEC trial indicate that 0.75 mg could be the recommended dose of palonosetron for future studies in the Japanese population.

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ORIGINAL REPORT

Japanese-US Common-Arm Analysis of Paclitaxel Plus Carboplatin in Advanced Non–Small-Cell Lung Cancer: A Model for Assessing Population-Related Pharmacogenomics

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Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

Clinical Trials repository link available on JCO.org.

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ABSTRACT

Purpose
To explore whether population-related pharmacogenomics contribute to differences in patient outcomes between clinical trials performed in Japan and the United States, given similar study designs, eligibility criteria, staging, and treatment regimens.

Methods
We prospectively designed and conducted three phase III trials (Four-Arm Cooperative Study, LC00-03, and S0003) in advanced-stage, non–small-cell lung cancer, each with a common arm of paclitaxel plus carboplatin. Genomic DNA was collected from patients in LC00-03 and S0003 who received paclitaxel (225 mg/m^2) and carboplatin (area under the concentration-time curve, 6). Genotypic variants of CYP3A4, CYP3A5, CYP2C8, NR1I2-206, ABCB1, ERCC1, and ERCC2 were analyzed by pyrosequencing or by PCR restriction fragment length polymorphism. Results were assessed by Cox model for survival and by logistic regression for response and toxicity.

Results
Clinical results were similar in the two Japanese trials, and were significantly different from the US trial, for survival, neutropenia, febrile neutropenia, and anemia. There was a significant difference between Japanese and US patients in genotypic distribution for *CYP3A4*1B* ($P = .01$), *CYP3A5*3C* ($P = .03$), *ERCC1* 118 ($P < .0001$), *ERCC2* K751Q ($P < .001$), and *CYP2C8* R139K ($P = .01$). Genotypic associations were observed between *CYP3A4*1B* for progression-free survival (hazard ratio [HR], 0.36; 95% CI, 0.14 to 0.94; $P = .04$) and ERCC2 K751Q for response (HR, 0.33; 95% CI, 0.13 to 0.83; $P = .02$). For grade 4 neutropenia, the HR for ABCB1 3425C→T was 1.84 (95% CI, 0.77 to 4.48; $P = .19$).

Conclusion
Differences in allelic distribution for genes involved in paclitaxel disposition or DNA repair were observed between Japanese and US patients. In an exploratory analysis, genotype-related associations with patient outcomes were observed for *CYP3A4*1B* and *ERCC2* K751Q. This common-arm approach facilitates the prospective study of population-related pharmacogenomics in which ethnic differences in antineoplastic drug disposition are anticipated.

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INTRODUCTION

Results may vary between different clinical trials that evaluate the same treatment regimen for many reasons, including trial design, eligibility criteria, patient characteristics, and subtle alterations in the treatment regimens themselves. An additional explanation for divergence of outcomes is host-related genetic differences associated with ethnicity, which is particularly pertinent when trials that are performed in different parts of the world are compared.

More than 10 years ago, the Southwest Oncology Group (SWOG) established a collaboration with Japanese investigators of lung cancer to provide a forum for exchange of research data, to facilitate standardization of clinical trial design and conduct, and to establish areas for joint collaboration.[1] We hypothesized that outcome differences between trials performed in Japan and the United States that evaluated similar treatment regimens in advanced-stage, non–small-cell lung cancer (NSCLC) could be explained by population-related

pharmacogenomics. To evaluate this possibility, we prospectively designed three phase III trials, (Four-Arm Cooperative Study [FACS], LC00-03, and S0003), each with similar patient eligibility criteria, staging, and treatment with a common arm of paclitaxel plus carboplatin. We have reported previously that, despite this effort at trial standardization, differences in clinical outcomes were observed in Japanese versus US patients treated on these studies.[2,3] Herein, we report the results of a clinical and pharmacogenomic analysis that involved patients from two of the three clinical trials (LC00-03 and S0003), and we report implications for additional studies by using this clinical research approach in which population-related differences in drug disposition are anticipated.

METHODS

Patients

The clinical trial methodology employed was prospective design of three separate-but-equal, randomized, phase III trials in advanced-stage NSCLC, each with its own comparator regimens but linked by a common treatment arm of paclitaxel plus carboplatin. In FACS, patients were randomly assigned to a standard treatment in Japan (irinotecan plus cisplatin) versus experimental arms of paclitaxel plus carboplatin, gemcitabine plus cisplatin, and vinorelbine plus cisplatin. LC00-03 compared paclitaxel plus carboplatin to the nonplatinum regimen of sequential vinorelbine plus gemcitabine followed by docetaxel, whereas patients on S0003 were randomly assigned to paclitaxel plus carboplatin with or without the hypoxic cytotoxin tirapazamine.

Clinical results for the three trials have been previously presented and published separately.[4-6] Common elements of eligibility criteria are summarized here. All patients had histologically or cytologically confirmed chemotherapy-naïve NSCLC with stage IV (ie, no brain metastases) or selected stage IIIB disease (ie, positive pleural or pericardial effusion or multiple ipsilateral lung nodules); measurable or assessable disease, performance status (PS) of 0 or 1; and adequate hematologic, hepatic, and renal function. All patients gave written informed consent in accordance with institutional regulations, and each protocol was approved by the respective institutional review boards; trials were conducted with adherence to the Helsinki Declaration.

Treatment Schedule, Dose Modifications, and Toxicity Assessment

Study elements of S0003, FACS and LC00-03 were designed to be as similar as possible: each study contained a common arm of paclitaxel plus carboplatin, which was repeated on a 21-day schedule. In all three studies, carboplatin was dosed at an area under the concentration-time curve (AUC) of 6.0 mg/mL/min on day 1. Paclitaxel was dosed at 225 mg/m^2 in S0003 and LC00-03 and at 200 mg/m^2 in FACS because of regulatory requirements for this study; in each study, paclitaxel was delivered as a 3-hour infusion on day 1. Premedication to prevent paclitaxel-related allergic reactions were similar. Prophylactic granulocyte colony-stimulating factor was not utilized. A complete blood count and chemistries were performed on day 1 of each cycle. Dose modifications occurred as previously described.[4] Patients were evaluated every two cycles for objective response by using RECIST (Response Evaluation Criteria in Solid Tumors) criteria[7] Toxicity grading was performed in accordance with the National Cancer Institute Common Toxicity Criteria, version 2.0, in each study.[8]

DNA Extraction and Genotyping

Specimens were not available from FACS; therefore, this analysis compares pharmacogenomic results from LC00-03 with S0003. Whole-blood specimens were collected from consenting patients at the time of enrollment on to LC00-03 and S0003. For S0003, DNA was extracted from patient plasma by using the Gentra PureGene Blood Kit (Gentra, Minneapolis, MN) and the QIAamp DNA Blood midi kit (Qiagen, Valencia, CA), and DNA was reconstituted in a buffer that contained 10 mmol/L Tris (pH 7.6) and 1 mmol/L EDTA, as previously described.[9] For LC00-03, DNA was extracted from buffy coats by using the GenElute Blood Genomic DNA Kit (Sigma-Aldrich, St Louis, MO). Selected genotypic variants related to paclitaxel disposition (ie, the ABC transporter superfamily [multidrug resistance {MDR} transporter 1 P-glycoprotein, *ABCB1* 3435C→T], the pregnane X receptor (PXR, NR1I2-206 deletion), *CYP3A4* (*CYP3A4*1B* 392A→G, 5′ untranslated region), CYP3A5 (*CYP3A5*3C* 6986A→G, splice variant), *CYP2C8* (*CYP2C8*3* 416G→A, R139K) or to platinum-related DNA repair enzymes *ERCC1* (118C→T, silent) and ERCC2 (XPD, K751Q) previously reported to be of functional consequence were analyzed by polymerase chain reaction (PCR) or pyrosequencing, as previously described.[9-13] Briefly, PCR was conducted by using Amplitaq Gold PCR master mix (ABI, Foster City, CA), 5 pmol of each primer, and 5 to 10 ng of DNA. Pharmacogenetic analysis was conducted by using the Pyrosequencing hsAPSQ96 instrument and software (Biotage, Uppsala, Sweden). The genotype was considered variant if it differed from the Reference Sequence consensus sequence for the single-nucleotide polymorphism (SNP) position (http://www.ncbi.nlm.nih.gov/RefSeq/). The *ERCC1* polymorphism was analyzed by PCR restriction fragment length polymorphism, as previously described.[14]

Statistical Methods

Comparison of clinical results among the three trials was prospectively planned and was coordinated through the SWOG statistical center. Pharmacogenomic results were assessed by Cox model for progression-free survival (PFS) and overall survival and by logistic regression for response and toxicity, adjusted for sex and histology.[15] Comparisons of patient demographics, toxicity, and efficacy parameters were made, when applicable, from the available data sets, by two-sample *t* tests, log-rank tests, and Wilcoxon rank sum tests.

RESULTS

Clinical Results Summary

Clinical results are presented for all three trials to document similarities between the two Japanese trials compared with the US S003 trial, whereas pharmacogenomic information was derived only from LC00-03 and S0003. Table 1 summarizes characteristics of patients on the paclitaxel-plus-carboplatin arms of each of the three trials. The median ages and age ranges were similar, and there were no significant differences in sex, stage, or histology. In S0003, 3% of patients self-reported Asian heritage, not additionally specified. Toxicity, efficacy, and dose delivery comparisons are listed in Table 2, which compares S0003 versus FACS/LC00-03 when applicable. Grades 3 to 4 neutropenia and febrile neutropenia were comparable

Table 1. Patient Demographic and Clinical Characteristics

Characteristic	Trial: FACS (n = 145) No.	%	LC00-03 (n = 197) No.	%	S0003 (n = 184) No.	%	*P*
Age, years							.03*
Median	63		65		63		
Range	33-74		33-81		28-80		
Female sex	46	32	61	31	68	37	.42
Disease stage IV	117	81	162	82	161	87	.20
Nonsquamous tumor type	114	79	167	85	152	83	.17

Abbreviation: FACS, four-arm cooperative study.
*Two-sample *t* test to compare LC00-03 and S0003 data. Patient-level data not available for FACS.

Table 2. Toxicity Comparisons

Toxicity	Trial: FACS (n = 148) No.	FACS (n = 148) %	LC00-03 (n = 197) No.	LC00-03 (n = 197) %	S0003 (n = 184) No.	S0003 (n = 184) %	P
Neutropenia grades 3-4	130	88	137	70	70	38	< .0001
Febrile neutropenia grades 3-4	27	18	24	12	4	2	< .0001
Thrombocytopenia grades 3-4	16	11	14	7	12	6.5	.31
Anemia grades 3-4	22	15	16	8	12	7	.03
Neuropathy grades 2-4	25	17	32	16	30	16	.99

Abbreviation: FACS, four-arm cooperative study.

in FACS and LC00-03 and were significantly greater than in S0003. Anemia was more frequent in FACS compared with the two other trials (Table 2). Efficacy comparisons are summarized in Table 3. Response rates were similar between the three trials and ranged from 32% to 36%. Median PFS rates were 4.5, 6, and 4 months in FACS, LC00-03, and S0003, respectively. Median survival rates were higher in the Japanese studies at 12 and 14 months, versus 9 months in S0003, and 1-year survival was significantly higher in FACS and LC00-03 than in S0003 ($P = .0004$). Dose delivery, summarized in Table 4, was lower in FACS than in S0003 and LC00-03. Dose reductions were similar between LC00-03 and S0003. Dose reduction data were not available from FACS.

Pharmacogenomic Results

Table 5 lists allelic distributions of patients with common, heterozygous, and variant alleles in the Japanese (LC00-03) and US (S0003) trials. Fisher's exact test was used to determine whether allele distributions were different between the populations. There were significant differences between patients from Japan (LC00-03) and the United States (S0003) in genotype distribution for *CYP3A4*1B* ($P = .01$), *CYP3A5*3C* ($P = .03$), *ERCC1* 118 ($P < .0001$), *ERCC2* K751Q ($P < .001$), and *CYP2C8*3* ($P = .01$).

Across populations, genotypic correlations were observed between *CYP3A4*1B* for PFS (hazard ratio [HR], 0.36; 95% CI, 0.14 to 0.94; $P = .04$) and *ERCC2* K751Q for response (HR, 0.33; 95% CI, 0.13 to 0.83; $P = .02$). There were no other significant associations noted (Table 6). For grade 4 neutropenia, the HR for ABCB1 3425C→T was 1.84 (95% CI, 0.77 to 4.48; $P = .19$). The relationship between the ERCC2 polymorphism and patient response stems principally from US patients. All but one Japanese patient was homozygous for the common allele (A/A). Those who harbored one or more variant alleles were significantly more likely to respond to treatment compared with those who had the common genotype. The response rate for patients with variant alleles was 51% versus 19% for patients homozygous for the common allele $P = .004$). However, no differences were observed in overall survival when stratified by this locus.

In S0003 (ie, the US trial), there were seven African American patients who had specimens available for genotyping. African American patients accounted for all seven patients who were heterozygous or homozygous for the *CYP3A4*1B* allele (Table 5). Additionally, the three patients with the common allele for *CYP3A5*C* were African American.

Table 3. Efficacy Comparisons

Parameter	Trial: FACS (n = 145)	LC00-03 (n = 197)	S0003 (n = 184)	P
Response				.55
No.	47	73	61	
%	32	37	33	
PFS, months	4.5	6	4	.04*
MST, months	12	14	9	.0006*
1-year survival	51%	57%	37%	.0004

Abbreviations: FACS, four-arm cooperative study; PFS, progression-free survival; MST, median survival time.
*Log-rank test to compare LC00-03 and S0003. Patient-level data not available for FACS.

DISCUSSION

This report describes the culmination of a unique multinational and multistudy collaboration that explores the hypothesis that clinical differences in treatment outcomes between Japanese and US patients with NSCLC may be explained, in part, by pharmacogenomic factors. Potential differences in drug disposition related to ethnic variability in distribution of relevant single nucleotide polymorphisms are well recognized. To our knowledge, however, the current project represents the first attempt to prospectively incorporate study of this topic into a joint clinical trial design. To preplan such a multinational endeavor required a high level of collaboration and compromise among all participants, including, in the case of FACS, Japanese regulatory authorities. Nevertheless, this report demonstrates the overall feasibility of using a common-arm methodology to investigate this research topic, in which a single, prospectively planned, joint study cannot be conducted. Considering the limitations of the clinical and pharmacogenomic data sets generated in this effort, and considering the multiple comparisons generated, the results reported here should be viewed as exploratory only and as primarily useful for refining this common-arm model of multinational collaboration. Even so, the clinical results are remarkably consistent with those anticipated, in which expectations were for both improved efficacy and higher levels of toxicity in Japanese patients who received a similar treatment regimen. Observation of clinical differences despite reduced paclitaxel

