CTV, aggressive high-dose PRT could become a legitimate treatment for a certain population of patients with unresectable HCC for whom there is no standard treatment available other than TACE or liver transplantation.

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Genome-Wide Association Study on Overall Survival of Advanced Non-small Cell Lung Cancer Patients Treated with Carboplatin and Paclitaxel

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Purpose: Our goal was to identify candidate polymorphisms that could influence overall survival (OS) in advanced non-small cell lung cancer (NSCLC) patients treated with carboplatin (CBDCA) and paclitaxel (PTX).

Methods: Chemotherapy-naïve stage IIIB or IV NSCLC patients treated with CBDCA (area under the curve = 6 mg/mL/min) and PTX (200 mg/m^2, 3-hour period) were eligible for this study. The DNA samples were extracted from peripheral blood mononuclear cells before treatment, and genotypes at approximately 110,000 gene-centric single-nucleotide polymorphisms (SNPs) were obtained by Illumina's Sentrix Human-1 Genotyping BeadChip. Statistical analyses were performed by the log-rank test and Cox proportional hazards model.

Results: From July 2002 to May 2004, 105 patients received a total of 308 cycles of treatment. The median survival time (MST) of 105 patients was 17.1 months. In the genome-wide association study, three SNPs were associated significantly with shortened OS after multiple comparison adjustment: rs1656402 in the *EIF4E2* gene (MST was 18.0 and 7.7 months for AG [n = 50] + AA [n = 40] and GG [n = 15], respectively; $p = 8.4 \times 10^{-8}$), rs1209950 in the *ETS2* gene (MST = 17.7 and 7.4 months for CC [n = 94] and CT [n = 11] + TT [n = 0]; $p = 2.8 \times 10^{-7}$), and rs9981861 in the *DSCAM* gene (MST = 17.1 and 3.8 months for AA [n = 75] + AG [n = 26] and GG [n = 4]; $p = 3.5 \times 10^{-6}$).

Conclusion: Three SNPs were identified as new prognostic biomarker candidates for advanced NSCLC treated with CBDCA and PTX. The agnostic genome-wide association study may unveil unexplored molecular pathways associated with the drug response, but our findings should be replicated by other investigators.

Key Words: Advanced non-small lung cancer, Carboplatin, Paclitaxel, Genome-wide association study, Single-nucleotide polymorphisms.

(*J Thorac Oncol.* 2011;6: 132–138)

Lung cancer is the leading cause of cancer death in Japan and worldwide for both men and women.[1] Non-small cell lung cancer (NSCLC) accounts for approximately 85% of lung cancer cases. Several third-generation agents are available for the treatment of NSCLC, including docetaxel, paclitaxel (PTX), gemcitabine, and vinorelbine, and the combination of one of these agents with a platinum compound has been considered the standard treatment option for advanced NSCLC.[2–9]

Despite these advances, survival prospects still remain disappointingly low for most patients. To seek further improvements in response rate and survival time, the conventional treatment approach to NSCLC is beginning to shift toward the application of specific strategies and techniques, such as pharmacogenomics to tailor treatment to individual patients.[10,11]

To identify the clinical predictors of outcome, it is critically important to observe individual differences in drug response and the role of genetic polymorphisms that are relevant to the pathways of drug metabolism and/or the biology of drug responses. However, genetic polymorphisms that are associated with overall survival (OS) or antitumor effect have not yet been fully elucidated.

With this as background, this prospective study employed a genome-wide association study (GWAS) to identify candidate polymorphisms that could influence OS in advanced NSCLC patients treated with carboplatin (CBDCA) and PTX. Possible associations with toxicities and pharma-

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Disclosure: Dr. Minami has received honororia from Bristol-Myers Squibb KK. The other authors declare no conflicts of interest.

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ISSN: 1556-0864/11/0601-0132

cokinetic (PK) parameters were also tested to complement our previous candidate gene approach focusing on CYP3A4[12] and CYP2C8.[13]

PATIENTS AND METHODS

Patient Recruitment and Treatment Schedule

Patients with histologically and/or cytologically documented NSCLC were eligible for participation in the study and treated with CBDCA and PTX at the National Cancer Center Hospital and National Cancer Center Hospital East. Each patient had to meet the following criteria: clinical stage IIIB or IV, no prior chemotherapy, no prior surgery and/or radiotherapy for the primary site, age older than 20 years, and Eastern Cooperative Oncology Group performance status[14] between 0 and 2. This study was approved by the Ethics Review Committees of the National Cancer Center and National Institutes of Health Sciences, and written informed consent was obtained from all patients before study entry.

One hundred five patients received 200 mg/m^2 of PTX (Bristol-Myers K.K., Tokyo, Japan) over a 3-hour period followed by carboplatin at a dose calculated to produce an area under the concentration time curve of 6.0 mg/mL/min on day 1, with the cycle being repeated every 3 weeks. In addition, to prevent hypersensitivity reactions, all patients received short-term premedication including dexamethasone, ranitidine, and an antiallergic agent (diphenhydramine or chlorpheniramine maleate).

Monitoring, Response and Toxicity Evaluation, and Follow-Up

A complete medical history and data on physical examinations were recorded before the CBDCA and PTX combination therapy. Complete blood cell and platelet counts as well as blood chemistry were measured once a week during the first 2 months of the treatment. Response was evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST), except that tumor markers were excluded from the criteria. Toxicity grading criteria in National Cancer Institute Common Toxicity Criteria Version 2.0 were used to evaluate toxicity. Patients were followed by direct evaluation or resident registration until death or up to 5 years after treatment. OS was calculated from the date of patient enrollment in this study to the date of death or the last follow-up.

Pharmacokinetic Sampling and Analysis

For PTX PK analysis, 5 ml of heparinized blood was sampled before the first PTX administration and at 0, 1, 3, and 9 hours after the termination of the infusion. The area under the curve (AUC) and clearance (CL m^{-2}) were calculated by a curve fitting method using the model of two compartments with constant infusion using WinNonlin ver. 3.3 (Pharsight Corporation, Mountain View, CA). The PK data were used in our previous pharmacogenetic analyses.[12,13]

DNA Extraction and Genotyping

Whole blood was collected from patients at the time of enrollment, and DNA was extracted from peripheral lymphocytes using a proteinase-K phenol chloroform method or Qiagen FlexiGene DNA isolation kit (QIAGEN Inc., Valencia, CA). All samples were assayed with the Illumina Infinium Human-1 BeadChip (Illumina Inc., San Diego, CA), which assays 109,365 gene-centric single-nucleotide polymorphisms (SNPs). If a genotyping call rate on all SNPs was found to be less than 95%, the sample was excluded from the analysis.

Statistical Analysis

As a quality control for genotyping, Hardy-Weinberg equilibrium testing was applied. To estimate the association between OS and genotypes, hazard ratios (HRs) and 95% confidence intervals were calculated using univariate or multivariate Cox proportional hazards models[15,16] and assessed using the log-rank test. Survival curves were drawn using the Kaplan-Meier method.[14] Statistical significance level was set to 0.05, two sided, after Holm's adjustment for a multiple testing.[17] All statistical analyses were performed with the use of SAS software, version 9.1.3 (SAS Institute Inc., Cary, NC). All statistical analyses were planned before the study.

RESULTS

Patient Characteristics, Survival, Response, and Toxicity

From July 2002 to May 2004, 239 patients treated with PTX were enrolled. Among them, 110 chemotherapy-naïve advanced NSCLC patients treated with CBDCA (AUC = 6 mg/mL/min) and PTX (200 mg/m^2, 3-hour period) were eligible in this study, but five patients were excluded from the analysis because genotyping data were not available. Their characteristics are shown in Table 1. All patients were followed up for more than 2.5 years, and the median follow-up time among censored observations was 38 months (range, 27–46 months), with 89 patients deceased (85%) as of November 2006. The median survival time (MST) of the 105 patients was 17.1 months (95% confidence interval: 15.0–18.7) (Figure 1). The 1- and 3-year survival probabilities were 68% and 16%, respectively.

Of the 105 patients, changes in tumor measurements were partial response in 43 (41%) patients, stable disease in 47 (45%), progressive disease in 11 (10%), and not evaluated in 4 (4%). There were no cases with a complete response.

All patients were evaluated for toxicity. Hematologic toxicity and nonhematologic toxicity are summarized in Table 2. Grade 3 or 4 nonhematologic toxicity occurred in 15

TABLE 1. Patient Characteristics

Assessable patients	105
Gender (male/female)	76/29
Age, median (range)	61 (29–80)
PS (0/1/2)	20/82/3
Stage (IIIB/IV)	46/59
No. of treatment cycles	
Mean	2.93
Range	1.0–6.0

PS, performance status.

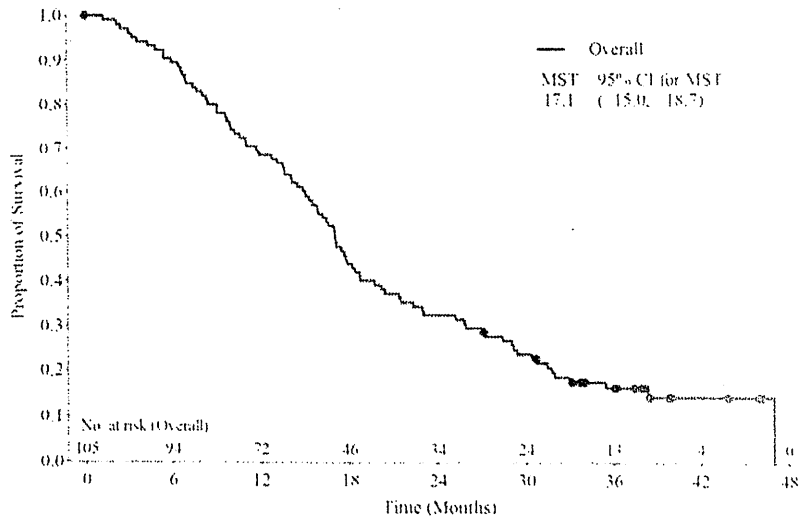

FIGURE 1. Kaplan-Meier plot for overall survival.

TABLE 2. Incidence of Hematologic and Nonhematologic Toxicities After the First Cycle

Toxicity	Grade 1	Grade 2	Grade 3	Grade 4	Total
Leukopenia	40	34	9	0	101
Neutropenia	8	22	39	18	105
Anemia	73	16	2	0	105
Thrombocytopenia	16	3	0	0	102
Febrile neutropenia	0	0	5	0	105
Nausea	7	3	0	0	105
Vomiting	8	4	3	0	105
Diarrhea	5	6	0	1	105
Arthralgia	58	12	2	0	105
Myalgia	47	10	1	0	105
Hyperbilirubinemia	33	10	0	0	105
AST (GOT) increase	38	1	0	0	105
ALT (GPT) increase	38	3	1	0	105
ALP increase	32	5	0	0	105
Neuropathy, sensory	65	6	1	0	105
Neuropathy, motor	1	0	0	1	105

AST, aspartate transaminase; GOT, glutamic oxaloacetic transaminase; ALT, alanine aminotransferase; GPT, glutamate pyruvate transaminase; ALP, alkaline phosphatase.

TABLE 3. Univariate Analysis of Patients' Characteristics

	Overall Survival		
Variable	Crude HR	95% CI for HR	*p*
Age			
≥65 vs. <65	1.12	0.72–1.71	0.61
Gender			
Male vs. female	2.06	1.26–3.39	0.0039
PS			
2 vs. 0–1	7.68	2.28–25.8	0.0010
Stage			
IV vs. IIIB	1.19	0.78–1.83	0.40
No. of cycles	0.92	0.74–1.13	0.42
Tumor response			
PR vs. PD	0.199	0.098–0.403	<.0001
NC vs. PD	0.216	0.108–0.434	<.0001

CI, confidence interval; HR, hazard ratio; PR, partial response; PD, progressive disease; NC, no change.

(14%) patients, suggesting that nonhematologic toxicity was generally mild; but grade 4 motor neuropathy occurred in one patient and grade 4 diarrhea occurred in another. On the other hand, grade 3 or 4 hematologic toxicity occurred in 57 (53%) patients. Grade 4 neutropenia occurred in 18 (17%) patients. Febrile neutropenia (grade 3) occurred in five patients.

Effects of Patients' Background on Overall Survival

The effects of patients' background on OS were analyzed as summarized in Table 3. The effects of gender, Eastern Cooperative Oncology Group performance status, and tumor response showed significant associations with OS, but age, stage, and number of cycles did not show a significant association.

Pharmacogenomic Analyses

Table 4 lists 10 SNPs, showing the least *p* values for log-rank test. The following three SNPs were associated significantly with shortened OS after multiple comparison adjustment: rs1656402 in the *EIF4E2* gene (MST for AG [$n = 50$] + AA [$n = 40$] and GG [$n = 15$] were 18.0 and 7.7 months, respectively; $p = 8.4 \times 10^{-8}$, HR = 4.22 [2.32–7.66]), rs1209950 in the *ETS2* gene (MST for CC [$n = 94$] and CT [$n = 11$] + TT [$n = 0$] were 17.7 and 7.4 months, respectively; $p = 2.8 \times 10^{-7}$, HR = 4.96 [2.52–9.76]), and rs9981861 in the *DSCAM* gene (MST for GG [$n = 75$] + AG [$n = 26$] and AA [$n = 4$] were 17.1 and 3.8 months, respectively; $p = 3.5 \times 10^{-6}$, HR = 16.1 [5.38–51.2]). In Figure 2, the Kaplan-Meier plots were drawn with subjects stratified into subgroups according to each significant polymorphism in either dominant or recessive model. Two (rs1656402 and rs9981861) of these significant SNPs were associated with tumor response and AUC 6α-,C3′-*p*-dihydroxy-PTX as shown

TABLE 4. Ten SNPs Associated with OS in GWAS

		SNP Information			Patients						
Chr #	Rs #	Gene Symbol	Genotype	Frequency	Total	Events	MST (95% CI)	HR (95% CI)	p^a	p^b	p^c
2	rs1656402	*EIF4E2*	AA	0.145	40	37	15.6 (13.5–17.0)	Ref	8.4×10^{-8}	4.5×10^{-7}	0.0046
			AG	0.461	50	37	24.4 (18.6–30.3)	0.42 (0.26–0.67)			
			GG	0.393	15	15	7.69 (5.95–12.7)	2.73 (1.46–5.10)			
21	rs1209950	*ETS2*	CC	0.938	94	78	17.6 (16.2–21.4)	Ref	2.8×10^{-7}	6.5×10^{-5}	0.015
			CT	0.059	11	11	7.39 (4.86–10.2)	4.96 (2.52–9.76)			
			TT	0.002	—	—	—	NA			
21	rs9981861	*DSCAM*	AA	0.652	75	61	17.8 (15.3–21.4)	Ref	3.5×10^{-6}	9.2×10^{-7}	0.050
			AG	0.314	26	24	16.5 (2.14–18.1)	1.33 (0.82–2.15)			
			GG	0.034	4	4	3.78 (2.14–7.69)	18.0 (5.78–56.2)			
2	rs10496036	*RTN4*	GG	0.701	84	70	17.6 (15.9–21.4)	Ref	2.4×10^{-5}	0.00063	1.00
			AG	0.270	18	2	14.1 (9.63–19.6)	1.52 (0.87–2.62)			
			AA	0.030	3	0	4.30 (2.43–5.95)	22.2 (5.72–86.2)			
6	rs1547633		GG	0.678	69	60	16.9 (13.6–18.3)	Ref	2.3×10^{-5}	7.7×10^{-6}	1.00
			GT	0.283	33	26	21.4 (16.2–27.0)	0.76 (0.48–1.21)			
			TT	0.039	3	3	3.58 (3.02–4.30)	29.7 (6.47–136)			
6	rs1570070	*IGF2R*	GG	0.553	66	57	18.2 (15.8–21.4)	Ref	2.2×10^{-5}	0.00010	1.00
			GA	0.388	33	27	16.4 (11.4–17.7)	1.01 (0.63–1.62)			
			AA	0.059	4	4	4.67 (2.17–7.39)	10.5 (3.85–28.9)			
7	rs2711095		GG	0.655	70	59	17.3 (15.9–19.6)	Ref	2.3×10^{-5}	5.0×10^{-5}	1.00
			AG	0.303	30	25	17.3 (11.7–27.0)	1.33 (0.88–2.00)			
			AA	0.042	5	5	5.39 (1.25–9.63)	10.2 (3.8–27.1)			
16	rs4313828	*CNTNAP4*	AA	0.947	99	83	17.4 (15.8–20.4)	Ref	2.2×10^{-5}	8.2×10^{-5}	1.00
			AG	0.050	6	6	7.51 (3.22–9.92)	7.12 (2.87–17.6)			
			GG	0.003	—	—	—	NA			
6	rs894817	*IGF2R*	AA	0.560	65	56	18.3 (15.8–22.3)	Ref	2.8×10^{-5}	0.00012	1.00
			AG	0.379	36	29	16.2 (10.2–17.7)	1.09 (0.69–1.71)			
			GG	0.061	4	4	4.67 (2.17–7.39)	14.3 (4.57–44.9)			
7	rs959494	*SCIN*	AA	0.659	70	56	17.5 (15.9–21.4)	Ref	3.1×10^{-5}	0.00043	1.00
			AG	0.299	30	28	16.0 (8.44–20.3)	1.53 (0.97–2.42)			
			GG	0.042	4	4	5.08 (2.43–9.07)	12.0 (3.97–36.7)			

[a] p values were calculated by univariate Cox proportional hazards model.
[b] p values were calculated by multivariate Cox proportional hazards model including gender and PS as covariates.
[c] p values were adjusted for multiple testing by using the Holm's method.
MST, median survival time; CI, confidence interval; HR, hazard ratio.

in Supplementary Tables 1 (http://links.lww.com/JTO/A43) and 2 (http://links.lww.com/IGC/A24), respectively.

The following PK parameters were measured in this study: AUC PTX (h*/μg/mL), AUC 6-α-hydroxy-PTX (6-α-OH-PTX) (h/μg/ml), AUC C3′-*p*-hydroxy-PTX (3′-*p*-OH-PTX) (h*/μg/mL), AUC 6α-,C3′-*p*-dihydroxy-PTX (diOH-PTX) (h*/μg/mL), AUC Cremophor EL (μl*/h/mL), CL PTX (L/h/m^2). However, no significant association was detected between the PK parameters and the SNPs by a multiple testing correction (data not shown). For reference, we showed the results of association between top 10 SNPs and PK parameters in Supplementary Table 2. This GWAS neither detected a statistically significant association with any of the grade 3/4 adverse reactions (data not shown), probably due to their low incidence, except for neutropenia (Table 2).

DISCUSSION

Cytotoxic chemotherapy continues to be the mainstay for initial treatment of patients with advanced NSCLC. Individualizing chemotherapy to deliver the most active and least toxic agent to each patient could provide an important improvement in patient care.[11] Previous pharmacogenetic studies have identified biomarkers for survival of patients with advanced NSCLC treated with platinum-based chemotherapy.[18–22] Among these are the *XRCC1*, *XRCC3*, and *XPD* genes, which play an important role in DNA repair.[23–28] Similar to previous studies of platinum-based chemotherapy, Gurubhagavatula et al.[18] observed a trend toward decreased survival for patients with variant *XPD* or *XRCC1* genotype and improved survival for patients with variant *XRCC3* genotype.

These genetic polymorphisms were identified by candidate gene approach, which relies on an a priori selection of small numbers of candidate genes based on the existing information or hypothesis. Although successful in several examples, this candidate gene approach may not be able to capture all the genetic factors, which influence a drug response in a complex interplay with multiple unknown as well

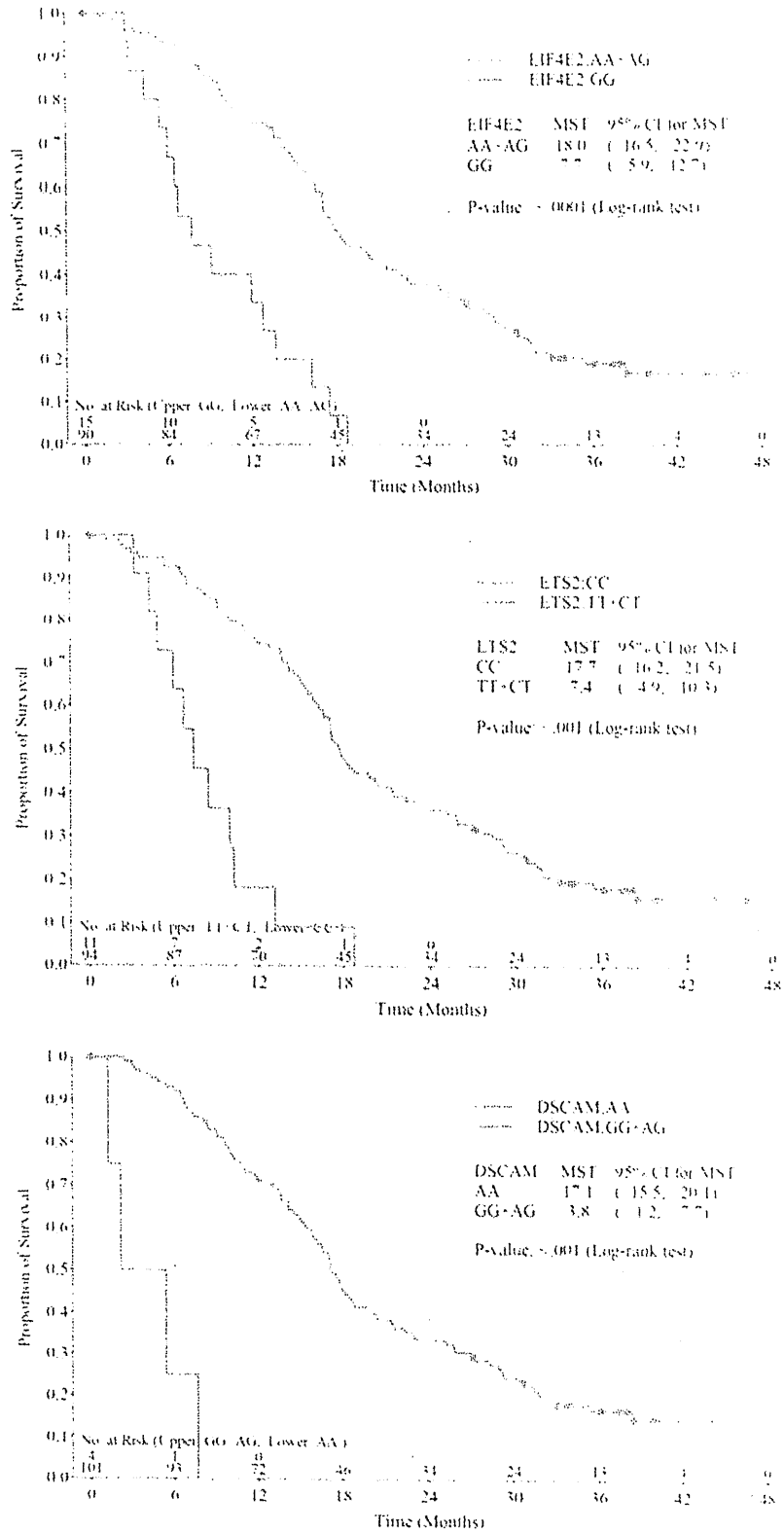

FIGURE 2. Overall survival stratified for the single-nucleotide polymorphism genotype.

as known factors such as disease phenotypes, genetic factors, and the variability in drug target response. GWAS, which makes no assumptions about the genomic location of the causal variants but surveys the whole genome,[29,30] is expected to complement the candidate gene approach. According to our findings from a gene-centric GWAS, three poly-

morphisms were associated with shortened OS in advanced NSCLC with CBDCA and PTX. The three SNPs have not been previously investigated for an association with NSCLC risk or drug response. On the other hand, the SNPs implicated in the prognosis of NSCLC by the previous candidate gene approach[18] were not detected in the GWAS, because the Human-1 BeadChip does not harbor the identical SNPs analyzed before and/or their p values were not sufficiently small in the context of the genome scan.

The first candidate SNP for the OS association, rs1656402, is in the third intron of the gene, *EIF4E2*, encoding for the translational factor eukaryotic initiation factor 4E, which is a central component in the initiation and regulation of translation in eukaryotic cells. Through its interaction with the 5′ cap structure of mRNA, eIF4E functions to recruit mRNAs to the ribosome.[31–34] Prototypical eIF4E-2 is expressed ubiquitously,[33,35] but in metastatic tumors, its expression was increased,[36] suggesting that eIF4E-2 plays an active role in the prognosis of NSCLC.

The second candidate SNP is located at the 4321 bp upstream of the *ETS2* gene. The Ets family of transcription factors includes important downstream targets in cellular transformation. For instance, alteration of Ets activity has been found to reverse the transformed phenotype of ras-transfected mouse fibroblasts and of several human tumor cell lines. It has been reported that Ets factor activity can strongly influence the transformed and invasive phenotype of a human prostate tumor cell line.[37]

The third candidate rs9981861 is in the 31st intron of the 33-exon *DSCAM* gene, which encodes Down syndrome cell adhesion molecule, a member of the immunoglobulin superfamily. The gene was cloned from the Down syndrome region on chromosome 21q22 and found to be expressed widely in the developing nervous system.[38] Mouse *DSCAM* has been shown to mediate arborization of neurite processes and spacing of neuronal cell bodies.[39,40] Expression of the *DSCAM* gene has been upregulated in small cell lung cancer compared with NSCLC.[41]

Because a GWAS is based on a linkage disequilibrium (LD) mapping of a disease locus by use of SNPs as markers, the particular SNPs per se identified in this study may not be functionally responsible for the observed effect on survival time. In fact, LD maps drawn by the HapMap data around the three SNPs indicate that at least the SNPs of the *EIF4E2* and *ETS2* genes are embedded in extended LD blocks (Supplementary Figure 1, http://links.lww.com/IGC/A25); it may be then difficult to narrow down the regions of interest further for these SNPs by statistical genetics alone, at least in the Asian population.

In summary, a hypothesis-free GWAS detected previously unrecognized associations between polymorphisms of the three genes and shortened OS in advanced NSCLC treated with CBDCA and PTX. Additionally, these three SNPs on the three genes were significant after a multiple testing adjustment. In considering a multiple testing problem, we assume the existence of about 10,000 linkage disequilibrium blocks within 100,000 gene-centric SNPs, which are concentrated in about 2% of the human genome (i.e., average interval of two SNPs is 600 bp). It follows that the p value cutoff is set at 5.0×10^{-6} if the Bonferroni correction is applied. However, in the first screening, such correction for a multiple testing is often too conservative, failing to detect many drug-response SNPs; therefore, we showed top 10 SNPs in Table 4. In addition, to facilitate the second screening or replication studies by other investigators, statistics of association between OS, PK parameters, toxicity, and all SNPs analyzed in this study are available at Genome Medicine Database of Japan (http://gemdbj.nibio.go.jp).

The ultimate goal of this work is better clinical management of patients after the assessment of genotype risk on OS. To this end, however, we need to identify genetic polymorphisms that can differentiate patients' response and outcome to different chemotherapeutic agents. Although our work may contribute as the first step to establish such a predictive factor, especially the survival-related SNPs that also influence pharmacokinetics, the current single-arm prospective study does not provide definite evidence of pharmacogenomic profiling for a platinum-based chemotherapy. Several targeted therapies for NSCLC are in clinical development, and it is hoped that this line of pharmacogenetic studies will eventually help clinicians to choose platinum or nonplatinum doublets as the first-line regimen, for instance. Further studies of NSCLC would stratify patients according to the SNP status to tailor treatment to individual patients. The results of a single association study should be validated by independent studies by other investigators as well as biologic functional analyses.

ACKNOWLEDGMENTS

Supported by the Program for the Promotion of Fundamental Studies in Health Sciences from National Institute of Biomedical Innovation (ID 05-41).

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British Journal of Cancer (2011), 1–6

www.bjcancer.com

Full Paper

Randomised, phase III trial of epoetin-β to treat chemotherapy-induced anaemia according to the EU regulation

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BACKGROUND: Erythropoietin-stimulating agents (ESAs) effectively decrease the transfusion requirements of patients with chemotherapy-induced anaemia (CIA). Recent studies indicate that ESAs increase mortality and accelerate tumour progression. The studies also identify a 1.6-fold increased risk of venous thromboembolism. The ESA labelling was thus revised in Europe and the United States in 2008. This is the first randomised, phase III trial evaluating the efficacy and safety of epoetin-β (EPO), an ESA, dosed in accordance with the revised labelling, which specifies that ESAs should be administered to CIA patients with a haemoglobin level of $\leqslant 10\,g\,dl^{-1}$ and that a sustained haemoglobin level of $>12\,g\,dl^{-1}$ should be avoided.

METHODS: A total of 186 CIA patients ($8.0\,g\,dl^{-1} \leqslant$ haemoglobin $\leqslant 10.0\,g\,dl^{-1}$) with lung or gynaecological cancer were randomised to receive EPO 36 000 IU or placebo weekly for 12 weeks.

RESULTS: The proportion of patients receiving transfusions or with haemoglobin $<8.0\,g\,dl^{-1}$ between week 5 and the end of the treatment period as the primary end point was significantly lower in the EPO group ($n=89$) than in the placebo group ($n=92$; 10.0% *vs* 56.4%, $P<0.001$). The proportion receiving transfusions was significantly lower in the EPO group (4.5% *vs* 19.6%, $P=0.002$). Changes in quality of life were not different. No significant differences in adverse events – for example, the incidence of thromboembolic events was 1.1% for each group – or the 1-year overall survival were observed between groups.

CONCLUSION: Weekly EPO administered according to the revised labelling approved by the European Medicines Agency is effective and well tolerated for CIA treatment. Further investigations are needed on the effect of ESAs on mortality.

British Journal of Cancer advance online publication, 29 September 2011; doi:10.1038/bjc.2011.395 www.bjcancer.com

Keywords: chemotherapy-induced anaemia; erythropoietin; survival

Anaemia is a common adverse event in cancer patients receiving chemotherapy, particularly in patients with lung and gynaecological cancers (Ludwig *et al*, 2004; Ohe *et al*, 2007; Katsumata *et al*, 2009). Several of the symptoms associated with anaemia, such as fatigue, syncope, palpitations and dyspnoea, reduce patient activity and have a profound effect on the quality of life (QOL) (Bokemeyer *et al*, 2007). Red blood cell (RBC) transfusion is one of the available treatments for anaemia. However, RBC transfusion is associated with a risk of volume overload, infection of unknown virus and transfusion reactions. And in Japan, blood transfusion therapy is problematic because of an increasing demand for blood products and a scarcity of blood supply arising from the declining birth rate and ageing population.

In Europe and the United States, erythropoiesis-stimulating agents (ESAs) have been used since 1993 for the treatment of chemotherapy-induced anaemia (CIA). The ESAs increase haemoglobin levels and reduce the need for RBC transfusion (Littlewood *et al*, 2001; Österborg *et al*, 2002). Since 2003, several studies have suggested that ESAs are associated with increased mortality and/or tumour progression in cancer patients when administered with a target haemoglobin level of $>12\,g\,dl^{-1}$ (Hedenus *et al*, 2003; Henke *et al*, 2003; Leyland-Jones *et al*, 2005; Overgaard *et al*, 2007; Wright *et al*, 2007; Smith *et al*, 2008; Thomas *et al*, 2008). Accordingly, the risks of ESAs have been investigated by regulatory authorities (Juneja *et al*, 2008) and, in response to these investigations, the labelling of ESAs in Europe and the United States was revised in 2008. A recent meta-analysis of ESAs has suggested that the increase in mortality in ESA-treated cancer patients undergoing chemotherapy is less pronounced than in those patients undergoing other anticancer treatments such as radiotherapy or no anticancer treatment (Bohlius *et al*, 2009a, b). Similarly, another meta-analysis indicated that when used within current European Organisation for Research and Treatment of Cancer (EORTC) treatment guidelines, the ESA epoetin-β (EPO) had no negative impact on survival and tumour progression (Aapro *et al*, 2008a). However, the risks of ESAs have also been shown to be independent of haemoglobin levels and dosing (Bennett *et al*, 2008; Bohlius *et al*, 2009a, b), and these meta-analyses were not able to verify that the risks of ESAs were completely eradicated by adherence to the new labelling.

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Received 23 May 2011; revised 5 September 2011; accepted 8 September 2011

The purposes of this study were to evaluate the efficacy and safety of EPO for the treatment of CIA with a dosing strategy according to the current labelling approved by the European Medicines Agency (EMA) (inclusion haemoglobin level criteria $\leqslant 10\,g\,dl^{-1}$, ceiling haemoglobin level $= 12\,g\,dl^{-1}$). We previously conducted a dose-finding study of once-weekly EPO in CIA patients with malignant lymphoma or lung cancer, and recommended a weekly dose of 36 000 IU based on our results (Morishima *et al*, 2006).

PATIENTS AND METHODS

Patient eligibility

Inclusion criteria were as follows: (1) lung or gynaecological cancer; (2) receiving platinum-based chemotherapy and expected to receive at least two additional cycles of chemotherapy; (3) CIA ($8.0\,g\,dl^{-1} \leqslant$ haemoglobin level $\leqslant 10.0\,g\,dl^{-1}$); (4) age between 20 and 79 years; (5) Eastern Cooperative Oncology Group performance status (PS) of 0–2; and (6) adequate hepatic and renal function.

Exclusion criteria included: (1) iron-deficiency anaemia (serum transferrin saturation (TSAT) $<15\%$ or mean corpuscular volume (MCV) $<80\,\mu m^3$); (2) ESA therapy within 8 weeks or RBC transfusion within 4 weeks before the study; (3) surgery scheduled during the study period; (4) previous radiation therapy to the pelvis; (5) documented haemorrhagic lesions; (6) history of myocardial, pulmonary or cerebral infarction; (7) uncontrolled hypertension; (8) history of hypersensitivity to ESA; (9) serious drug allergy; and (10) tumour in the central nervous system.

Study design and treatment

This multicentre, randomised, double-blind, placebo-controlled, phase III study was conducted at 37 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. Patients were randomised 1 : 1 to receive EPO 36 000 IU or placebo subcutaneously once a week for up to 12 weeks. Epoetin-β and placebo were supplied by Chugai Pharmaceutical Co., Ltd (Tokyo, Japan). Participants in the study and investigators (outcome assessors) were blinded toward treatment allocation. Randomisation was conducted by a contract research organisation (CRO) that was independent from the investigators. The randomisation was carried out by a central registration system and was stratified by tumour type, PS, haemoglobin level and institution using a dynamic balancing method. The randomisation table was kept sealed and stored until a database lock by the CRO. Analysis methods were determined before the database lock.

If the haemoglobin level increased to $>12.0\,g\,dl^{-1}$ at any time during the study, administration was discontinued until the haemoglobin level decreased to $\leqslant 11.0\,g\,dl^{-1}$, and was then restarted at two-thirds of the previous dose (24 000 IU). If the planned cycle of chemotherapy was completed or discontinued, treatment was withheld at 6 weeks after day 1 of the final chemotherapy cycle. A daily dose of 100–200 mg elemental iron was administrated if TSAT fell to $<15\%$ or MCV fell to $<80\,\mu m^3$. The RBC transfusion was allowed at the discretion of the investigator during the study.

Evaluation of efficacy and safety

The primary end point of this study was the proportion of patients receiving RBC transfusion or with a haemoglobin level $<8.0\,g\,dl^{-1}$ between week 5 and the end of the treatment period (EOTP). The secondary end points were the proportion of patients receiving RBC transfusion between week 5 and the EOTP, change in haemoglobin level and QOL from baseline to the EOTP. QOL was evaluated using the Japanese Functional Assessment of Cancer Therapy-Anaemia (FACT-An) questionnaire (Yoshimura *et al*, 2004). In this study, the FACT-An total fatigue subscale, which consists of 13 fatigue-related questions, was the principal means of analysis. The FACT-An total fatigue subscale scores (FSS) range from 0 to 52, with higher scores indicating less fatigue.

Safety end points included adverse events, tumour progression and death (during the treatment phase and 1-year follow-up period). Adverse events were assessed according to the National Cancer Institute Common Toxicity Criteria, ver. 3, translated by the Japan Clinical Oncology Group. The presence of neutralising antibodies to EPO was assessed at baseline and the EOTP.

Statistical analysis

The sample size of 160 patients (including an anticipated withdrawal rate of 40%, mainly because of completing or discontinuing the planned cycle of chemotherapy) was calculated to yield 80% power to significantly detect a 25% reduction (from 45 to 20%) in the primary end point, the proportion of patients receiving RBC transfusion or with a haemoglobin level $<8.0\,g\,dl^{-1}$ between week 5 and the EOTP. Statistical testing was conducted using a two-sided significance level of $P = 0.05$. The study was not powered for QOL as a secondary efficacy end point. Patients who received at least one dose of the study drug comprised the safety population. For efficacy analysis, ineligible patients were excluded from the safety population, resulting in the full analysis set (FAS) population. The proportion of patients receiving RBC transfusion or with a haemoglobin level $<8.0\,g\,dl^{-1}$ was estimated by the Kaplan–Meier method. The requirement for RBC transfusion was compared using the χ^2 method. Changes in the haemoglobin level and FSS between groups were compared using Student's *t*-test.

RESULTS

Demographics and baseline characteristics

A total of 186 patients were enroled in the study between June and December 2008, and 181 (89 EPO and 92 placebo) of these were eligible for efficacy evaluation (the FAS population). Five patients were excluded because of discontinuation before the first dosing for the following reasons: withdrawal of patient consent ($n = 2$), chemotherapy regimen cancelled ($n = 1$), patient eligibility criteria violation ($n = 1$) and a positive result in the skin test to EPO ($n = 1$). In all, 51 (57%) patients in the EPO group and 55 (60%) in the placebo group completed 12 weeks of the study. Elemental iron was administrated in 40 patients (45%) in the EPO group and 32 (35%) in the placebo group. The demographics and baseline characteristics of the FAS population were well balanced (Table 1). The range of haemoglobin levels at screening was $8.0–10.0\,g\,dl^{-1}$, whereas those at baseline (1–17 days after the screening) ranged from 7.2 to $11.4\,g\,dl^{-1}$. The main chemotherapeutic regimen for both lung and gynaecological cancer was carboplatin-paclitaxel therapy.

Transfusion-related and haemoglobin end points

The proportion of patients receiving RBC transfusion or with a haemoglobin level $<8.0\,g\,dl^{-1}$ between week 5 and the EOTP was significantly lower in the EPO group than the placebo group (10.0%; 95% confidence intervals (CIs) in the EPO group, 3.4–16.6 *vs* 56.4%; 95% CI in the placebo group, 45.4–67.4%, $P<0.001$; Figure 1). Fewer patients received RBC transfusions between week 5 and the EOTP in the EPO group (4 of 89 patients, 4.5%) than in the placebo group (18 of 92 patients, 19.6%, $P = 0.002$). The range of pretransfusion haemoglobin levels at the time of the first transfusion was $5.3–8.1\,g\,dl^{-1}$.

Table 1 Characteristics of full analysis population

	EPO ($n=89$)	Placebo ($n=92$)
Sex		
Male	47	40
Female	42	52
Age (years), median (min–max)	67 (40–79)	63.5 (44–79)
Weight (kg), median (min–max)	53.5 (35–102)	52.8 (37.4–78.1)
Tumour		
Small cell lung cancer	20 (22.5)	22 (23.9)
Non-small cell lung cancer	40 (44.9)	38 (41.3)
Ovarian cancer	19 (21.3)	19 (20.7)
Other	10 (11.2)	13 (14.1)
ECOG performance status		
0	42 (47.2)	41 (44.6)
1	45 (50.6)	50 (54.3)
2	2 (2.2)	1 (1.1)
Haemoglobin ($g\,dl^{-1}$), median (min–max)	9.4 (8.1–11.4)	9.3 (7.2–11.4)
Transferrin saturation (%), median (min–max)	25.1 (5.4–97.6)	24.1 (6.4–99.4)
Serum endogenous erythropoietin ($mIU\,ml^{-1}$), median (min–max)	43 (7.78–577)	43.6 (10.5–320)

Abbreviations: EPO = epoetin-β; ECOG = Eastern Cooperative Oncology Group.

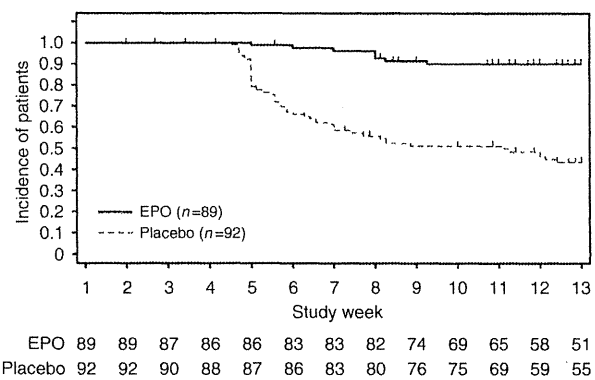

Figure 1 Time to RBC transfusion or haemoglobin level $<8.0\,g\,dl^{-1}$.

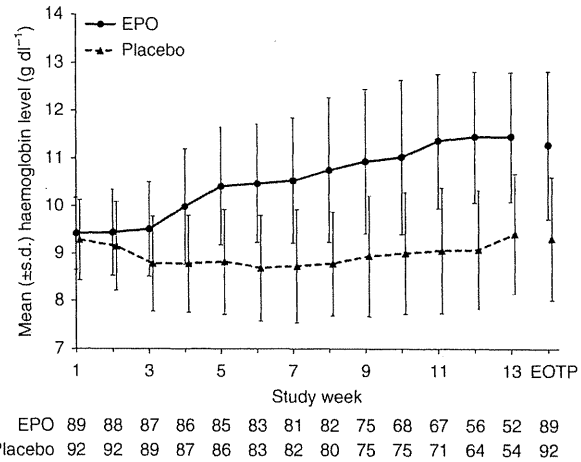

Figure 2 Change in haemoglobin level by treatment group. Abbreviation: EOTP = end of treatment period.

Table 2 Incidence of adverse events

	EPO ($n=89$)		Placebo ($n=92$)	
	No. of patients	%	No. of patients	%
Adverse events	88	98.9	92	100.0
Common adverse events				
Neutropenia	82	92.1	74	80.4
Leucopenia	81	91.0	77	83.7
Thrombocytopenia	61	68.5	55	59.8
Lymphocytopenia	44	49.4	52	56.5
Anorexia	43	48.3	50	54.3
Nausea	43	48.3	46	50.0
Adverse drug reactions	37	41.6	28	30.4
Common adverse drug reactions				
Constipation	6	6.7	2	2.2
Increased blood pressure	5	5.6	3	3.3
Diarrhoea	5	5.6	1	1.1

Abbreviation: EPO = epoetin-β.

The mean change in haemoglobin level from baseline to the EOTP in the EPO group ($1.9\,g\,dl^{-1}$) was significantly higher than that in the placebo group ($0.0\,g\,dl^{-1}$, $P<0.001$). Figure 2 shows the mean changes in haemoglobin levels throughout the study in both groups. The mean nadir haemoglobin level between week 5 and the EOTP was $9.7\,g\,dl^{-1}$ in the EPO group and $7.9\,g\,dl^{-1}$ in the placebo group ($P<0.001$).

The percentage of patients with a haemoglobin level $>12.0\,g\,dl^{-1}$ after dosing, and whose administration was halted, was 50% in the EPO group and 2% in the placebo group.

QOL

Overall compliance in terms of the percentage of patients who completed the FACT-An questionnaire was 98.3% (178 of 181) at baseline and 93.9% (170 of 181) at the end of the study. The mean baseline FSS was 35 points in the EPO group and 33 points in the placebo group. The mean changes in FSS from baseline to the EOTP in the EPO group were higher than in the placebo group, but these changes did not achieve statistical significance (0.30 *vs* −0.99, $P=0.387$).

Safety

A total of 181 patients received study treatment and were included in the safety analysis. The overall incidence of adverse events was similar between the two groups (99% EPO and 100% placebo). There were 120 adverse events (in 37 patients) related to the study drug (adverse drug reactions) in the EPO group and 78 (in 28 patients) in the placebo group. Of these adverse drug reactions, constipation (6.7%), increased blood pressure (5.6%) and diarrhoea (5.6%) were reported by at least 5% of patients in the EPO group (Table 2). In all, 8 patients (14 events) in the EPO group and 17 patients (21 events) in the placebo group experienced serious adverse events. Of these, 5 events (acute respiratory distress syndrome, pneumonia, pulmonary embolism, neutropenia and thrombocytopenia) were considered to be related to EPO. As a thromboembolic event, one pulmonary embolism was observed in the EPO group. It was not associated with higher haemoglobin

level (the haemoglobin level at the onset was 9.4 g dl^{-1}). In the placebo group, haemorrhagic cerebral infarction (asymptomatic; no treatment was required) occurred in one patient. The proportion of patients who experienced tumour progression during the treatment period was similar in both groups (27.0% in the EPO group and 26.1% in the placebo group). No neutralising antibodies to EPO were detected.

Survival

One patient in the EPO group died during the active study period. Follow-up survival data for all 181 patients who received the study drug were gathered through December 2009, at which time the median follow-up period was 54 weeks after the first dose of study drug. The 1-year overall survivals were 58.7% (95% CI, 48.4–69.1%) and 63.4% (95% CI, 53.4–73.3%) in the EPO and placebo groups, respectively ($P=0.560$, by the log-rank test), and the hazard ratio (HR) was 1.15 (95% CI, 0.72–1.85).

DISCUSSION

Erythropoietin-stimulating agents, one of the treatment options for anaemia, raise haemoglobin levels, reduce the proportion of patients requiring transfusions and improve QOL (Littlewood *et al*, 2001; Österborg *et al*, 2002; Boogaerts *et al*, 2003; Iconomou *et al*, 2003). However, recent meta-analyses on QOL have shown that ESAs induce a statistically significant but not clinically meaningful improvement of fatigue as measured with FACT-Fatigue (Tonelli *et al*, 2009; Minton *et al*, 2010). The ESAs have been approved for the treatment of CIA, and are widely used in the United States and Europe. The EPO is approved and marketed in Europe but not in the United States.

In recent years, however, several randomised clinical trials using ESAs (Hedenus *et al*, 2003; Henke *et al*, 2003; Leyland-Jones *et al*, 2005; Overgaard *et al*, 2007; Wright *et al*, 2007; Smith *et al*, 2008; Thomas *et al*, 2008) and meta-analyses (Bennett *et al*, 2008; Bohlius *et al*, 2009a, b) have raised concerns about the negative impact on overall survival and tumour progression. Such safety issues regarding the use of ESAs in cancer patients have been discussed by regulatory authorities in the United States and Europe for several years (Juneja *et al*, 2008). To minimise the risks, both regulatory authorities have revised the labelling for ESAs and restricted their use in cancer patients. One of the restrictions in the United States is not to administer ESAs to patients with potentially curable cancers. Based on the decisions made by the EMA, the current labelling information specifies that ESAs should be administered to cancer patients with CIA whose haemoglobin level is $\leqslant 10$ g dl^{-1} and that a sustained haemoglobin level of >12 g dl^{-1} should be avoided. The present study was the first to evaluate the efficacy and safety of ESAs when dosed in accordance with the current labelling approved by the EMA in a randomised, double-blind, placebo-controlled manner. The inclusion criterion with regard to haemoglobin level was 8.0–10.0 g dl^{-1}, and the median baseline haemoglobin level was 9.4 g dl^{-1} in the EPO group and 9.3 g dl^{-1} in the placebo group. If the haemoglobin level increased to >12.0 g dl^{-1} during the study period, the study drug was discontinued until the haemoglobin level decreased to $\leqslant 11.0$ g dl^{-1}.

The results of this study demonstrated that once-weekly EPO administration significantly reduced the proportion of patients requiring RBC transfusions or having a haemoglobin level <8.0 g dl^{-1} after 4 weeks of treatment (10.0% *vs* 56.4%, $P<0.001$) and also reduced the proportion of patients requiring RBC transfusions (4.5% *vs* 19.6%, $P=0.002$); however, the dosing strategy in this study was conservative compared with those of previous studies (Boogaerts *et al*, 2003; Iconomou *et al*, 2003; Fujisaka *et al*, 2006; Morishima *et al*, 2006; Nakagawa *et al*, 2007; Aapro *et al*, 2008a, b; Suzuki *et al*, 2008; Bohlius *et al*, 2009a, b; Tsuboi *et al*, 2009). The relatively low percentage of patients receiving transfusions in both groups reflects the fact that most physicians hesitate to prescribe transfusions, preferring to monitor the situation until anaemia symptoms become remarkable. The pretransfusion haemoglobin levels at the time of the first transfusion in the current study were in the range of 5.3–8.1 g dl^{-1}.

EPO was well tolerated in this study. The incidence and types of adverse events were similar between the EPO and placebo groups. Previous meta-analyses have indicated that the use of ESAs leads to an increased risk of thromboembolic events (relative risk (RR) 1.67; 95% CI, 1.35–2.06 (Bohlius *et al*, 2006) and RR 1.57; 95% CI, 1.31–1.87 (Bennett *et al*, 2008)). In the current study, one pulmonary embolism was observed during treatment with EPO, but no death due to thromboembolic events was reported.

The results of the latest Cochrane meta-analysis using individual patient data from 53 ESA trials were recently published in the *Lancet* (Bohlius *et al*, 2009a, b). In this report, subgroup analysis of data from chemotherapy-treated patients (10 441 patients in 38 trials) indicated that the increase in mortality associated with ESAs was less pronounced in this population (HR for death during the active study periods = 1.10; 95% CI, 0.98–1.24, $P=0.12$; HR for overall survival = 1.04; 95% CI, 0.97–1.11, $P=0.263$) than in patients undergoing other anticancer treatments such as radiotherapy, radiochemotherapy or no anticancer treatment (HR 1.33–1.53). However, none of the studies included in the Cochrane meta-analysis used ESAs in accordance with the revised labelling indications (baseline haemoglobin levels, target and ceiling and so on). Although the current study was not designed and not powered to show that EPO did not increase mortality in this dosing scheme and that EPO was safe, the number of patients who died during the study period was one in the EPO group and none in the placebo group. The 1-year overall survival in the EPO group was 58.7% (95% CI 48.4–69.1%) and that in the placebo group was 63.4% (95% CI 53.4–73.3%; log-rank, $P=0.560$). There have been considerable debates as to the mechanism by which ESAs increase the risk for mortality (Fandrey and Dicato, 2009). One possible explanation is that aggressive dosing with ESAs to achieve higher target haemoglobin levels (not recommended in the revised labelling information) can cause adverse effects. The FDA has requested that a prospective randomised controlled trial of the use of ESAs be carried out, assessing their safety at haemoglobin levels of <12 g dl^{-1}. Such a trial is currently ongoing in patients with non-small cell lung cancer undergoing chemotherapy.

In conclusion, the findings from this study provide new evidence that ESAs are effective and well tolerated when used within the revised labelling indications by the EMA, with the limitation that we did not formally search for thromboembolic events. However, it is important that ESAs be used in accordance with the labelled indications. In addition, the risk of thromboembolic events and possible negative effects on survival should be carefully weighed against the benefits of ESA treatment in patients with CIA, taking into account the patients' comorbidities and the conditions under which they are treated. Further investigations are needed on the effect of ESAs on mortality.

ACKNOWLEDGEMENTS

This study was supported by Chugai Pharmaceutical Co., Ltd, Tokyo, Japan. We thank Yutaka Ariyoshi of the Aichi Cancer Center Aichi Hospital, a remunerated consultant of Chugai Pharmaceutical Co., Ltd, for providing advice on this manuscript. The following institutions participated in this study: Osaka Medical College Hospital, Iwate Medical University School of Medicine, Aichi Cancer Center Aichi Hospital, Tokyo Medical

University, Osaka City University Hospital, Shizuoka Cancer Center, Saitama Cancer Center, Okayama University Hospital, Kinki University Hospital, Nagoya Daini Red Cross Hospital, Aichi Cancer Center Hospital, Tokyo Metropolitan Cancer and Infectious diseases Center Komagome Hospital, Kyushu University Hospital, National Cancer Center Hospital, Tochigi Cancer Center, Hyogo Cancer Center, National Kyushu Cancer Center, Gihu Municipal Hospital, Tohoku University Hospital, Niigata University Medical & Dental Hospital, Sapporo Medical University Hospital, Niigata Cancer Center Hospital, Kanazawa University Hospital, Hokkaido University Hospital, Fujita Health University Hospital, Cancer Institute Hospital of Japanese Foundation for Cancer Research, National Hospital Organization Nagoya Medical Center, Nippon Medical School Hospital, Nagoya University Hospital, Kurashiki Central Hospital, Osaka Medical Center for Cancer and Cardiovascular Diseases, Yokohama Municipal Citizen's Hospital, National Cancer Center Hospital East, Tsukuba University Hospital, National Hospital Organization Shikoku Cancer Center, National Hospital Organization Hokkaido Cancer Center and Kurume University Hospital.

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JJCO Japanese Journal of Clinical Oncology

Jpn J Clin Oncol 2011;41(1)25–31
doi:10.1093/jjco/hyq163
Advance Access Publication 27 August 2010

A Phase II Study of Cisplatin and Irinotecan As Induction Chemotherapy Followed by Concomitant Thoracic Radiotherapy with Weekly Low-dose Irinotecan in Unresectable, Stage III, Non-Small Cell Lung Cancer: JCOG 9706

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Received July 7, 2010; accepted July 28, 2010

Objective: It is important to identify optimal regimens of cisplatin-based, third-generation chemotherapy and thoracic radiotherapy for patients with unresectable, Stage III, non-small cell lung cancer.

Methods: Patients with unresectable, Stage III non-small cell lung cancer were treated with the following regimen: cisplatin 80 mg/m^2 on days 1 and 29, with irinotecan 60 mg/m^2 on days 1, 8, 15, 29, 36, and 43 and 30 mg/m^2 on days 57, 64, 71, 78, 85 and 92. Thoracic radiotherapy was started on day 57 at 2 Gy/day (total 60 Gy).

Results: From February 1998 to January 1999, 68 patients were enrolled. Grade 3/4 toxicities during induction chemotherapy primarily included neutropenia (73.5%) and diarrhea (20.6%), while Grade 3/4 toxicities during concomitant thoracic radiotherapy with irinotecan consisted of neutropenia (18.4%), esophagitis (4.1%) and hypoxia (6.5%). There was one treatment-related death due to radiation pneumonitis. The response rate was 64.7% (95% confidence interval, 52.2–75.9%). The median survival time was 16.5 (95% confidence interval, 12.6–19.8) months. The 1- and 2 year survival rates were 65.8% (95% confidence interval, 54.4–77.1%) and 32.9% (95% confidence interval, 21.6–44.1%), respectively. Overall, only 36 (56%) completed both the scheduled chemotherapy and thoracic radiotherapy.

Conclusions: Induction chemotherapy with cisplatin plus irinotecan followed by low-dose irinotecan and concomitant thoracic radiotherapy was feasible according to the prespecified decision criteria in this study for patients with unresectable Stage III non-small cell lung cancer. We did not decide to select this regimen for further investigations because approximately half of the patients completed the scheduled treatment.

Key words: cisplatin – irinotecan – chemoradiotherapy – non-small cell lung cancer

INTRODUCTION

In unresectable Stage III non-small cell lung cancer (NSCLC), several randomized trials have shown that combinations of chemotherapy and thoracic radiotherapy (TRT) have improved survival compared with radiotherapy alone (1–4). It is important to identify optimal regimens of combined chemotherapy and radiotherapy and to evaluate the feasibility and efficacy of such combinations.

Irinotecan (CPT-11) is an antitumor agent that inhibits the nuclear enzyme topoisomerase I (5). CPT-11 has played a significant role in the development of chemotherapy for NSCLC since the initial reports of its efficacy as a single agent (6,7). Combination chemotherapy of CPT-11 and cisplatin (CDDP), which is also a commonly used agent for NSCLC, is a promising regimen for NSCLC, since its high antitumor activity and manageable toxicity have been reproducibly reported (8,9). One critical but uncommon toxicity of CPT-11 was reported to be pulmonary toxicity (7), and it is necessary to clarify how the chemotherapy regimen should be combined with TRT in patients with Stage III NSCLC.

In addition to combined chemoradiotherapy using full dose of anticancer drugs, concomitant treatment with low doses of anticancer drugs as radiosensitizers has also been investigated in patients with Stage III NSCLC. Schaake-Koning et al. (10). reported that daily low-dose CDDP combined with TRT improved the local control of tumors in a randomized study. CPT-11 has also been investigated as a radiosensitizer (11), and a phase I/II study of weekly administration of low dose CPT-11 combined with TRT has been reported (12). Esophagitis, pneumonitis and diarrhea were the dose-limiting toxicities of weekly irinotecan combined with TRT. The maximum tolerated dose and the recommended dose were 60 and 45 mg/m^2/week, respectively.

Therefore, in order to improve therapeutic outcomes in patients with unresectable Stage III NSCLC, a phase II study of a regimen of two courses of CDDP plus CPT-11 as induction chemotherapy, followed by TRT with weekly low-dose CPT-11 administration, was conducted. The recommended dose of CPT-11 with concomitant TRT was reconsidered and set at 30 mg/m^2/week in order to avoid radiation pneumonitis in this study setting.

PATIENTS AND METHODS

Patient Selection

Patients with histologically or cytologically confirmed, unresectable, Stage III NSCLC who had not received cancer therapy were enrolled in this study. Staging for entry criteria was performed according to the lung cancer staging system of the International Union against Cancer. Staging procedures included chest X-ray, computed tomography (CT) scan of the chest, CT scan or magnetic resonance imaging of the brain, CT scan or ultrasound of the abdomen and isotope bone scanning. *N*-status was mainly based on size criteria on the chest CT scan. Patients with pleural or pericardial effusions were excluded from the study. Each patient was required to meet the following criteria: Eastern Cooperative Oncology Group performance status (PS) of 0 or 1; $\leq$70 years of age; predicted area of radiation field less than half of one lung; adequate hematological, pulmonary, renal and hepatic functions, i.e. white blood cell (WBC) count $\geq$4000/μl and $\leq$12 000/μl, hemoglobin level $\geq$9.5 g/dl, platelet count $\geq$100 000/μl, PaO_2 $\geq$70 torr, %DLco $\geq$60%, serum creatinine level no higher than the upper limit of normal, serum total bilirubin level $\leq$1.5 mg/dl and serum glutamic oxaloacetic transaminase and glutamic pyruvic transaminase levels less than twice the upper limit of normal. Patients with synchronous or metachronous malignancy, uncontrolled heart failure or diabetes, interstitial pulmonary fibrosis or chronic obstructive pulmonary disease that restricts thoracic radiation, or a history of myocardial infarction in the last 3 months were excluded from the study. Female patients who were pregnant or lactating when chemotherapy was to be given were also excluded. All patients gave their written informed consent.

Treatment Schedule

The treatment schema was shown in Fig. 1. After enrollment in the study, the patients received chemotherapy consisting of intravenous infusion of 80 mg/m^2 of CDDP on day 1 and 60 mg/m^2 of CPT-11 on days 1, 8 and 15. The induction chemotherapy was repeated 4 weeks after the start of the first course, as long as the patients had recovered sufficiently from toxicity. The induction chemotherapy of CPT-11 and CDDP was to be performed for two courses, unless unacceptable toxicity or disease progression occurred.

CPT-11 on day 8 or 15 was skipped if the WBC count was <3000/μl, the platelet count was <100 000/μl, or Grade 2 or higher diarrhea or abdominal pain developed. During chemotherapy, if the WBC count fell to <2000/μl or the neutrophil count dropped to <1000/μl, daily granulocyte colony-stimulating factor (G-CSF) was administered subcutaneously until the WBC count increased to $\geq$10 000/μl or was no longer clinically indicated. Radiotherapy with concomitant use of G-CSF was contraindicated. When the second course of CDDP plus CPT-11 was started, each patient was required to meet the following

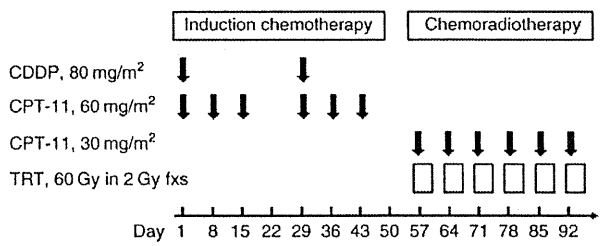

Figure 1. Treatment schema for this study.

criteria: WBC count $\geq$4000/μl, platelet count $\geq$100 000/μl, serum creatinine level no higher than the upper limit of normal and no episodes of diarrhea. If the second course was delayed 2 weeks or more due to toxicity, chemotherapy with CDDP plus CPT-11 was terminated, and only radiotherapy was given. Based on toxicities during the first course of chemotherapy, the doses of CDDP and CPT-11 were reduced by 20 and 10 mg/m^2, respectively, for febrile neutropenia with Grade 4 leucopenia or neutropenia, Grade 4 thrombocytopenia and Grade 3 or 4 non-hematological toxicity.

Criteria for starting radiotherapy with weekly CPT-11 administration were the same as those mentioned above as the entry criteria of this study. Patients who developed Grade 4 diarrhea during induction chemotherapy with CPT-11 and CDDP were off-treatment. If the same criteria were not fulfilled at 6 weeks after initiation of the second course of chemotherapy, CPT-11 administration was terminated. In that case, only radiotherapy was given.

Four weeks after the start of the second course of CPT-11 plus CDDP, weekly CPT-11 with concomitant TRT was started. The initial opposing anterior–posterior treatment fields encompassed the primary tumor, the bilateral mediastinal lymph nodes and the ipsilateral hilar nodes. The supraclavicular nodes were included within the field when there was clinical evidence of their involvement. A 1.5-cm tumor-free margin was required. The fraction size delivered was 2.0 Gy, 5 days per week. Thus, the total radiation dose was 60 Gy in 30 fractions over 6 weeks. The methods for spinal block and boost after the first 40 Gy deliveries were left to the discretion of the treating radiation oncologist. The patients also received intravenous CPT-11 weekly up to six times with TRT concurrently. CPT-11 was dosed to 30 mg/m^2/week and administered intravenously for 60–90 min immediately before the chest irradiation.

During chemoradiotherapy, if the WBC count fell to $<$2000/μl or the neutrophil count fell to $<$1000/μl, radiotherapy was suspended. After neutropenia improved to Grade 1 or 0, radiotherapy could be restarted. If the platelet count fell to $<$75 000/μl, radiotherapy was suspended until recovery from thrombocytopenia. If patients had a fever of 38°C or higher, chemoradiotherapy was suspended until they were afebrile. If Grade 3 or 4 radiation-related esophagitis was seen, chemoradiotherapy was suspended but could be started again when this toxicity improved to Grade 1 or 0. If the PaO_2 level decreased by 10 torr or more compared to the baseline value, chemoradiotherapy was suspended, and if it returned to baseline, treatment could be restarted carefully. CPT-11 was not administered if the WBC count fell to $<$3000/μl, the platelet count fell to $<$100 000/μl, or Grade 2 or more diarrhea developed on the treatment day.

Treatment Evaluation

Tumor response was evaluated according to the World Health Organization response criteria (13). Response was confirmed by extramural review in this study. Toxicity was evaluated once a week according to the Japan Clinical Oncology Group (JCOG) toxicity criteria (14). The complete blood cell count was checked twice a week. Routine blood chemistry, arterial blood gas and chest radiographs were checked at least once a week until the patient had apparently recovered from all acute toxic effects after the completion of the treatment. After completion of treatment, response was evaluated every 6 months until disease progression or for 2 years, by chest CT, brain MRI or CT, abdominal CT or ultrasound.

Study Design and Statistical Methods

This trial was designed as a multicentre, prospective, single-arm, phase II study and the study protocol was approved by the Clinical Trial Review Committee of JCOG before study activation and the institutional review board of each participating institution before patient enrollment. After pre-treatment staging and eligibility evaluation, patients were registered at the JCOG Data Center by facsimile or by telephone. The study was performed by the JCOG Lung Cancer Study Group and all study data were managed by the JCOG Data Center.

The primary endpoint of this study was the %2 year survival in all eligible patients. The overall survival (OS) was measured from the date of patient registration to the date of death due to any cause. If a patient was alive at the final follow-up, OS was censored at the last contact date. The OS was estimated using the Kaplan–Meier method and the confidence interval (CI) of survival proportion was calculated by Greenwood's formula. The expected and threshold levels of the %2 year OS were set to be 33.0 and 18.9%, which were derived from the expected and threshold value of median survival 15 and 10 months with an assumption of exponential distribution, respectively. With one-sided alpha of 0.10 and beta of 0.10, the planned total sample size for eligible patients was 62. Considering ineligible patients, 65 patients were to be registered with a 1.5 year entry period and a 2 year follow-up period. The analyses were carried out using the SAS release 8.1 (Carry NC.).

RESULTS

Patients' Characteristics

A total of 68 patients from 14 institutions were enrolled in this study between February 1998 and January 1999. The patients' characteristics are listed in Table 1. The patients included 52 men and 16 women, with a median age of 63 (range, 46–70) years. The histological classifications included adenocarcinoma in 34 patients and squamous cell carcinoma in 33. ECOG PS was 0 in 22 patients and 1 in 46. Twelve patients with more than 5% weight loss over 6 months were included. Twenty-eight patients were in Stage IIIA and 40 were in Stage IIIB. All 68 patients were eligible and evaluable for both efficacy and safety.

Table 1. Patients' characteristics

	Number (%)
Enrolled	68
Eligible	68
Age (years)	
Median	63
Range	46–70
Sex	
Male	52 (76.5)
Female	16
PS (ECOG)	
0	22
1	46
Weight loss	
≥5%	12 (17.6)
<5%	50
Unknown	6
Smoking	
Never	10 (14.7)
Ever	58
Histology	
Adenocarcinoma	34
Squamous cell carcinoma	33
Large cell carcinoma	1
Clinical stage	
IIIA	28
IIIB	40
T-stage	
T1	9
T2	23
T3	11
T4	25
N-stage	
N0	2
N1	6
N2	46
N3	14

ECOG, Eastern Cooperative Oncology Group.

Treatment Delivery and Protocol Compliance

Of the 68 patients enrolled in the study, 38 (55.9%) completed both the scheduled chemotherapy and the radiotherapy. In 18 patients, induction chemotherapy was terminated due to disease progression (4 patients) and unacceptable toxicity (14 patients). Four patients developed disease progression during induction chemotherapy with CDDP plus CPT-11. One patient had stable disease; however, the predicted area of the radiation field exceeded half of one lung. In seven patients, Grade 3 or 4 diarrhea and Grade 4 ileus persisted, whereas in another patient, an allergic skin reaction developed and resulted in termination of induction chemotherapy. Six patients had prolonged toxicity during the induction chemotherapy and could not receive radiotherapy.

Forty-nine patients received TRT according to the protocol and 38 patients received the complete 60 Gy of radiation with weekly CPT-11. Radiotherapy could not be completed for 11 patients; the median dose was 48 (range, 12–56) Gy. The reason for not receiving radiotherapy was disease progression in two patients and toxicity in nine patients.

Toxicity

Toxicity was assessed throughout induction chemotherapy, during CPT-11 concurrent with TRT treatment, and during the follow-up period until 2 years after the date of enrollment. Table 2 presents the toxicity during the induction chemotherapy with CPT-11 plus CDDP and Table 3 shows the acute toxicity for the chemoradiotherapy.

Grade 3 or 4 neutropenia and diarrhea, which were common toxicities during the CPT-11-containing chemotherapy regimen, occurred in 73.5 and 20.6% of the patients, respectively, during the induction chemotherapy. Eleven patients had Grade 2/3 esophagitis during chemoradiotherapy, and it caused termination of the therapy in only one patient. Pulmonary toxicity, which is listed as decreasing PaO_2 or dyspnea in Table 3, developed during the chemoradiotherapy. In nine patients, radiotherapy was terminated due to development of any grades of radiation pneumonitis. There was one treatment-related death due to radiation pneumonitis. This patient had completed the induction chemotherapy of CDDP plus CPT-11 without serious toxicity and moved to the chemoradiotherapy phase. After a radiation dose of 32 Gy, hypoxia and dyspnea developed. The protocol treatment was terminated and the patient was treated with appropriate medication and supportive care. This patient died due to this toxicity at 39 days after the last treatment day.

Response, Survival and Recurrence Pattern

Of the 68 patients, 6 achieved complete responses and 38 achieved partial responses, giving a response rate of 64.7% (95% CI, 52.2–75.9%).

Figure 2 shows the OS curve of all patients enrolled in this study. After follow-up for 26 months after the last enrollment, the median survival time (MST) was 16.5 (95% CI, 12.6–19.8) months. The 1- and 2 year survival rates in the 68 patients were 65.8% (95% CI, 54.4–77.1%) and 32.9% (95% CI, 21.6–44.1%), respectively. For the primary endpoint, the lower limit of 80% CI, which corresponds to one-sided alpha 0.1, of %2 year survival was 25.5% (greater than the prespecified threshold value 18.9%). The overall progression-free survival rate was 26.5% (95% CI,

Table 2. Toxicity during the induction chemotherapy phase

Item	N	JCOG grade					%3–4
		0	1	2	3	4	
Leukopenia	68	4	17	23	21	3	35.3
Neutropenia	68	3	0	15	29	21	73.5
Anemia	68	8	21	24	15	–	22.1
Thrombocytopenia	68	56	8	2	2	0	2.9
Bilirubin	67	53	–	12	2	0	3.0
GOT	68	47	17	3	1	0	1.5
GPT	68	37	22	7	2	0	2.9
ALP	67	42	23	1	1	0	1.5
Creatinine	68	55	13	0	0	0	0
PaO_2	53	29	22	2	0	0	0
Hypercalcemia	67	63	4	0	0	0	0
Hypocalcemia	67	64	2	1	0	0	0
Hyponatremia	68	21	30	10	6	1	10.3
Hypokalemia	68	36	15	12	5	0	7.4
Nausea/vomiting	68	6	22	34	6	–	8.8
Diarrhea	68	13	24	17	10	4	20.6
Stomatitis	68	65	3	0	0	0	0
Esophagitis	68	67	0	1	0	0	0
Infection	68	50	8	7	2	1	4.4
Dyspnea	68	63	1	2	2	0	2.9
Fever	68	45	9	13	0	1	1.5
Neuropathy	68	67	1	0	0	–	0
Constipation	68	44	21	3	0	0	0
Eruption	68	61	2	4	1	0	1.5
Alopecia	68	11	48	9	–	–	–

JCOG, Japan Clinical Oncology Group; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; ALP, alkaline phosphatase.

Table 3. Acute toxicity during the chemoradiotherapy phase

Item	N	JCOG grade					%3–4
		0	1	2	3	4	
Leukopenia	49	4	10	23	12	0	24.5
Neutropenia	49	12	13	15	6	3	18.4
Anemia	49	1	13	25	10	–	20.4
Thrombocytopenia	49	47	2	0	0	0	0
Bilirubin	49	46	–	3	0	0	0
GOT	49	37	11	1	0	0	0
GPT	49	32	14	2	1	0	2.0
ALP	49	37	11	1	0	0	0
Creatinine	49	47	2	0	0	0	0
PaO_2	46	14	21	8	2	1	6.5
Hypercalcemia	48	45	3	0	0	0	0
Hyponatremia	49	32	12	5	0	0	0
Hypokalemia	49	36	11	2	0	0	0
Nausea/vomiting	49	22	20	7	0	–	0
Diarrhea	49	33	13	3	0	0	0
Stomatitis	49	46	3	0	0	0	0
Esophagitis	49	7	31	9	2	0	4.1
Infection	49	42	5	2	0	0	0
Dyspnea	49	47	1	1	0	0	0
Fever	49	28	5	16	0	0	0
Neuropathy	49	48	1	0	0	–	0
Paralysis	49	48	0	0	1	0	2.0
Constipation	49	38	9	2	0	0	0
Eruption	49	45	3	1	0	0	0
Alopecia	49	11	30	8	–	–	–

JCOG, Japan Clinical Oncology Group; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; ALP, alkaline phosphatase.

16.0–37.0%) at 1 year and 13.2% (95% CI, 5.2–21.3%) at 2 years, with a median of 8.8 months (95% CI, 6.8–10.4 months).

The sites of initial failure are shown in Table 4. The primary tumor inside the radiation field was the site of initial failure in 20 patients, while distant metastasis was the cause of failure in 34 patients. In 10 patients, including all three patients who achieved a complete response, there was no evidence of recurrent disease.

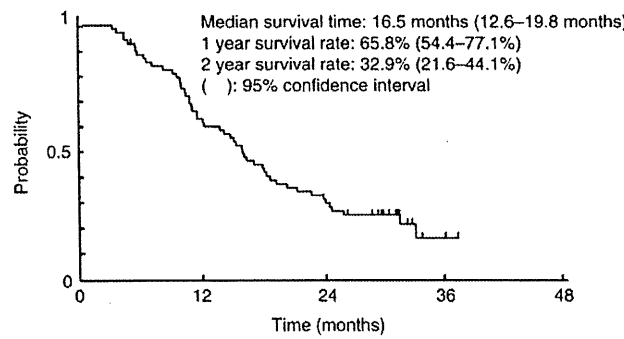

Figure 2. Kaplan–Meier survival curve including all 68 eligible patients.

DISCUSSION

This multi-center, phase II study demonstrated that two cycles of induction chemotherapy with CDDP plus CPT-11, followed by concomitant TRT with weekly low dose CPT-11, was effective with acceptable toxicity in patients with locally advanced NSCLC. The MST was 16.5 months (95% CI, 12.6–19.8 months) and the 1- and 2 year survival

Table 4. Pattern of failure

Site of initial failure	Patients (*n*)	%
Inside radiation field	20	29.4
Outside radiation field	34	50.0
Overlap above	4	5.9
Response continued	10	14.7
Unknown	0	0

rates in the 68 patients were 65.8% (95% CI, 54.4–77.1%) and 32.9% (95% CI, 21.6–44.1%), respectively. The primary endpoint, the %2 year survival, was met with the prespecified decision criteria in this study.

Although some investigators have reported that concurrent administration of full-dose chemotherapy and TRT is possible, it is considered difficult for many regimens, especially with third-generation agents, such as CPT-11, which are difficult to use at their full doses for concurrent chemoradiotherapy because of the high incidence of toxicity. Therefore, for concurrent chemotherapy with TRT, these chemotherapeutic agents have been used at reduced doses in several reported clinical studies (15–18). In the Japanese trial of Furuse et al. (19), the concurrent combination of TRT with full-dose mitomycin, vindesine and CDDP (MVP), which was an old chemotherapy regimen, was considered to have the best efficacy for locally advanced NSCLC. We conducted a randomized, phase III trial comparing third-generation chemotherapeutic agents of paclitaxel or CPT-11 plus carboplatin with the MVP regimen, with early concurrent TRT in patients with Stage III NSCLC (20). When we selected the investigational arms, the strategy of early concurrent TRT with reduced low-dose chemotherapeutic agents followed by consolidation chemotherapy was considered to be better without obvious clinical evidence. Although the present results are promising, we did not decide to select this strategy of induction chemotherapy followed by concomitant TRT with CPT-11.

Recently, some articles have shown that addition of induction chemotherapy before concurrent chemoradiotherapy increases toxicity and provided no survival benefit (17,18). In the present study, only 49 among 68 patients could receive TRT according to the protocol, although the radiotherapy should be a key treatment option for patients with locally advanced NSCLC. Furthermore, of the 49 patients, 38 (56%) completed 60 Gy of TRT with weekly CPT-11. This lower treatment delivery due to early disease progression and unacceptable toxicity was thought to be critical disadvantages of the strategy for induction chemotherapy followed by chemoradiotherapy. Further studies to investigate the role of induction chemotherapy followed by chemoradiotherapy may not be necessary until more active anticancer drugs appear.

In view of toxicity management, neutropenia and diarrhea were considered to be common toxicities requiring careful management during combination chemotherapy using CPT-11. Pneumonitis and esophagitis induced by CPT-11 and concomitant TRT have been considered serious, but they were easily manageable in the present study. Under the investigator's careful observation, the concurrent TRT with CPT-11 was sometimes terminated due to radiation pneumonitis. In the present study, one patient died due to radiation pneumonitis; this incidence of fatal radiation pneumonitis was considered acceptable. Esophagitis was very mild and less toxic in Japanese patients than in Western patients.

In conclusion, induction chemotherapy with CPT-11 plus CDDP, followed by concomitant TRT with weekly low-dose CPT-11, was feasible and effective with acceptable toxicity according to the prespecified decision criteria in this study. However, lower TRT delivery in this treatment strategy was a critical disadvantage in treatment for patients with locally advanced NSCLC. We did not decide to select this regimen for further investigations.

Acknowledgements

The authors are indebted to Ms Miyuki Niimi and Ms Mieko Imai for data management, Dr Naoki Ishizuka for statistical analysis and Dr Isamu Saito and Dr Taro Shibata for helpful comments to the manuscript (JCOG Data Center), and Dr Yoshiro Tanio (Osaka General Medical Center, Osaka), Dr Harumichi Ikegami (Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka), Dr Minoru Takada (Rinku General Medical Center, Izumisano), Dr Kyong-Yob Min (Osaka Medical College, Ibaragi), Dr Kazuya Higashino (Hyogo Medical College, Nishinomiya), Dr Kenji Eguchi, (National Hospital Organization Shikoku Cancer Center, Matsuyama) and Dr Jun Araki (Sasebo Municipal Hospital, Sasebo) for their contributions to this study.

Funding

This work was supported in part by Grants-in-Aid for Cancer Research and for the Second-term Comprehensive 10 year Strategy for Cancer Control from the Ministry of Health, Labour and Welfare.

Conflict of interest statement

None declared.

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