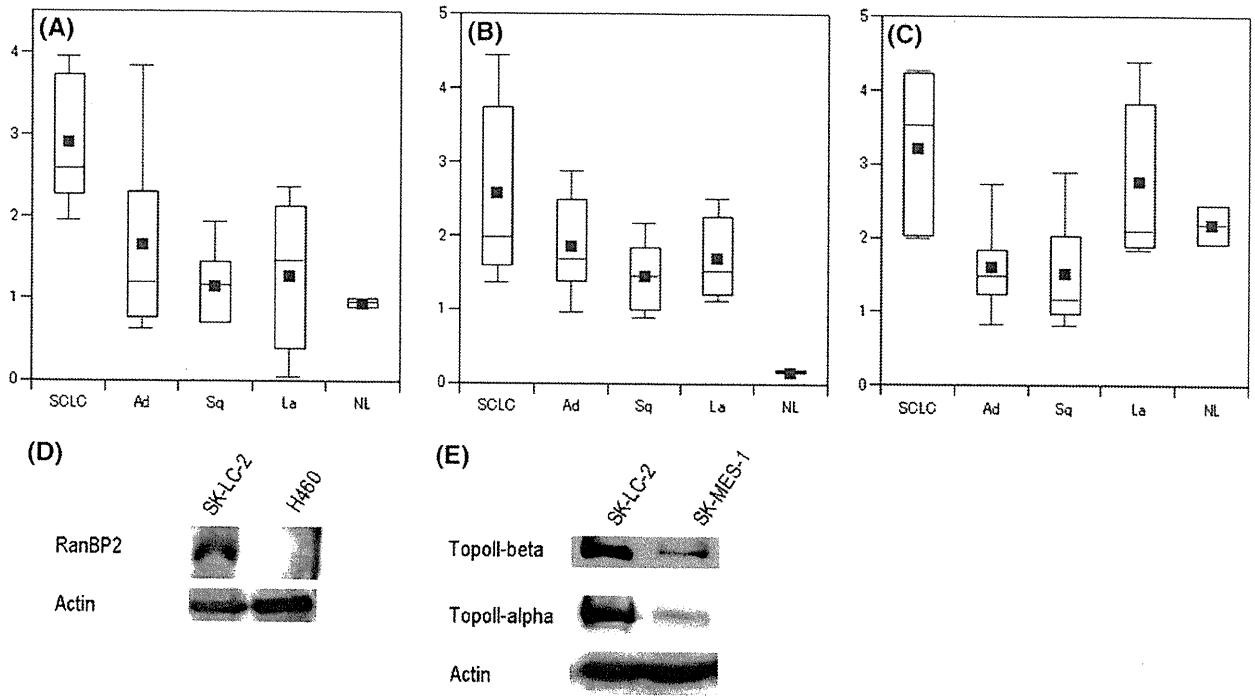

Fig. 2 Relative mRNA expression for (**a**) RanBP2, (**b**) TopoII-alpha and (**c**) TopoII-beta among histological subtypes and normal lung tissues, and protein expression for (**d**) RanBP2 and (**e**) TopoII isoforms in representative cell lines. **a** RanBP2 mRNA expression in SCLC was higher than those in the other histological subtypes of lung cancer. **b** TopoII-alpha mRNA expression levels in lung cancer cell lines were relatively higher compared to those in normal lung tissues. **c** The expression levels of TopoII-beta in lung cancer cell lines were similar to those in normal lung tissues. **d, e** Western blot analyses for RanBP2 and TopoII isoforms in two lung cancer cell lines representing high and low expression, respectively. The expression patterns of protein and mRNA were not different

although there were no significant differences in TopoII-alpha mRNA expression levels among four histological subtypes of lung cancer (Fig. 2b). On the other hand, the expression levels of TopoII-beta in lung cancer cell lines were similar to those of normal lung tissues, although relatively higher expression levels were observed in SCLC and large cell carcinoma (Fig. 2c). In addition, we checked TopoII-alpha and TopoII-beta protein expressions in two lung cancer cell lines, SK-LC-2 and SK-MES-1, representing high and low expression of the two TopoII isoforms, and found that protein expression patterns of these genes were not different with mRNA expression patterns (Fig. 2e).

There were weak but significant positive correlations between RanBP2 and TopoII-alpha mRNA expressions, between RanBP2 and TopoII-beta mRNA expressions and between TopoII-alpha and TopoII-beta mRNA expressions among 20 lung cancer cell lines ($r = 0.532$; $P < 0.05$, Fig. 3a and $r = 0.623$; $P < 0.05$, Fig. 3b, $r = 0.647$; $P < 0.01$, Fig. 3c, respectively). Chemosensitivity data were analyzed in relation to the mRNA expression levels of the RanBP2, TopoII-alpha, TopoII-beta genes using linear regression analysis. No significant associations were observed between the IC50 values of amrubicin and the mRNA expression levels of RanBP2 (Fig. 4a), TopoII-alpha (Fig. 4b) and TopoII-beta (Fig. 4c) among 20 cell lines.

Discussion

RanBP2 has been reported to be involved in both nucleocytoplasmic transport and mitosis and also act as a SUMO ligase for DNA TopoII and play a role in maintaining chromosome stability by recruiting TopoII to centromeres during mitosis [5]. In addition, RanBP2 hypomorphic mice are particularly sensitive to spontaneous and carcinogen-induced lung tumors, indicating that RanBP2 might play a potential tumor suppressor role in human lung cancer. Two previous studies reported that RanBP2 mRNA expression levels are substantially reduced in human non-SCLC [2, 8]. However, the present study showed that RanBP2 transcript levels were infrequently downregulated in human lung cancer cell lines compared with normal lung tissues, although there were statistically significant differences in the RanBP2 expression between SCLC and NSCLC. Consistent with our results, several lines of evidence from publicly available human gene expression data of the Oncomine

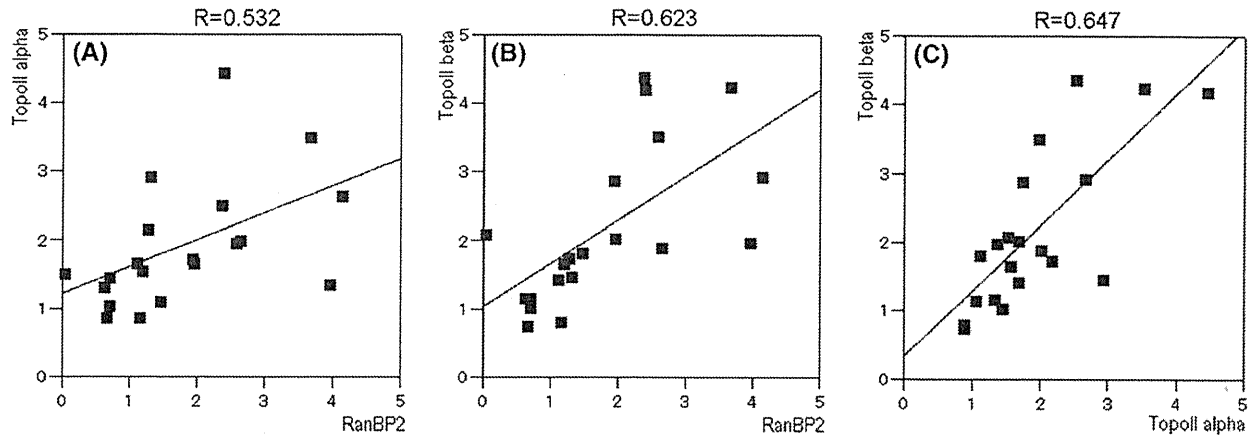

Fig. 3 Correlations between **a** RanBP2 and TopoII alpha mRNA expression, **b** RanBP2 and TopoII beta mRNA expression and **c** TopoII alpha and TopoII beta mRNA expression in lung cancer cell lines

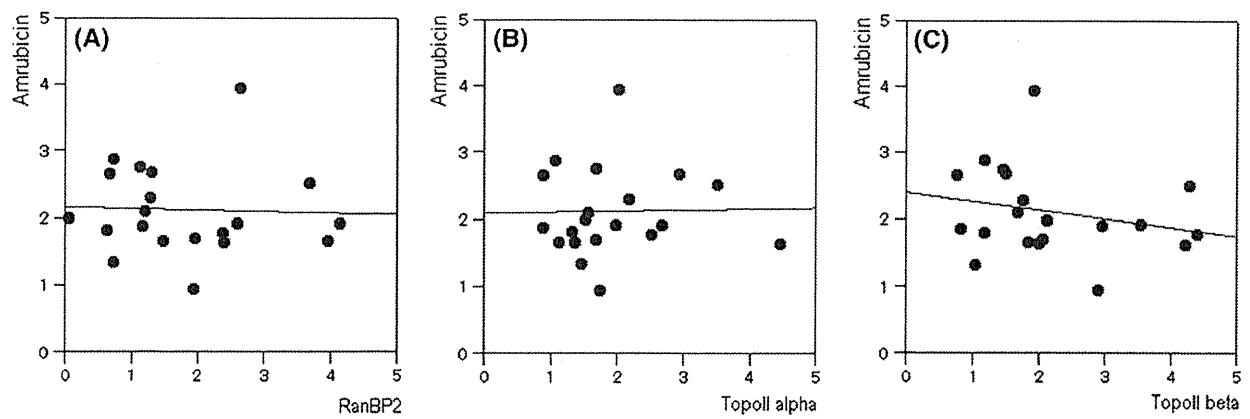

Fig. 4 Associations between relative mRNA expression for (**a**) RanBP2, (**b**) TopoII alpha and (**c**) TopoII beta and chemosensitivity of Amrubicin Log(IC50 in nM)

database (http://www.oncomine.com) and GEO profiles (http://www.ncbi.nlm.nih.gov/geo/) reported that RanBP2 mRNA expression levels are not reduced in NSCLC compared with normal lung tissues [3, 22, 26–28, 30]. In addition, there is a microarray study showing that RanBP2 expression levels are similar to those of our data in four overlapping lung cancer cell lines [9]. The concordance and discordance between our findings and previous works might be caused by the difference between cell lines and resected human lung tumors as well as the different experimental conditions used. Thus, further studies are warranted to establish the role of RanBP2 as a tumor suppressor gene in human lung carcinogenesis.

In RanBP2 hypomorphic murine embryonic fibroblasts (MEFs), formation of chromatin bridges in anaphase, a distinctive feature of cells with impaired DNA decatenation by mutation or chemical inhibition of TopoII-alpha [4], was observed, while spindle structure, kinetochore–microtubule interactions, and localization of kinetochore and spindle assembly checkpoint proteins appeared normal [5]. Therefore, the low expression of RanBP2 may have an analogous effect of TopoII inhibitors, although the inhibitors are able to cause an inevitable consequence of DNA damage at high doses [4, 21]. Then, we speculated that there might be an association between RanBP2 mRNA expression and chemosensitivity of a TopoII inhibitor, amrubicin and tested whether we could see it using human lung cancer cell lines. However, we did not find any associations, suggesting that cytotoxicity of amrubicin might come mainly from DNA damage response induced at high doses and that formation of chromatin bridges in anaphase caused by low expression of the RanBP2 gene might not have additional effects on amrubicin-induced DNA damage response.

The two isozymes, TopoII-alpha and TopoII-beta function to unknot and decatenate covalently closed circles of DNA, although functional differences of these isozymes

and their differential spliced variants as well as precise role of their homodimerization and heterodimerization are unknown [20, 21]. There are several lines of evidence indicating a close relationship between TopoII-alpha levels and drug sensitivity in cell lines made resistant to TopoII inhibitors [7, 17, 25], cell lines with reduced expression of TopoII [1] and a VP-16-resistant breast cancer cell line infected with adenovirus containing TopoII-alpha [32]. Another study has shown the relationship between TopoII expression and multidrug sensitivity including TopoII inhibitors using eight human lung cancer cell lines [10]. There is also some evidence that TopoII-beta may be related with resistance to TopoII inhibitors [6, 15]. However, we did not find any association between expression levels of TopoII isoforms and chemosensitivity of amrubicin. Consistent with our results, a previous report of unselected human lung cancer cell lines also showed no clear association between TopoII-alpha protein expression and in vitro sensitivity to TopoII inhibitors [31]. Another study also failed to show importance of the enzyme using a panel of cell lines [12]. Although the behavior of cell lines in vitro may differ from the in vivo situation, and depend on the experimental conditions, these contradictory findings may require further investigation.

Amrubicin is highly active and one of the most potent anticancer drugs against SCLC and NSCLC [14]. Among the toxicities, hematologic adverse events such as leukopenia and thrombocytopenia are frequent and dose-limiting factors. Although identification of molecular biomarkers with the potential to predict treatment outcomes is essential to eliminate the use of any ineffective agents and to avoid toxic side effects [16], the cellular response to amrubicin is still poorly understood. To predict drug response in lung cancer patients, integrated analyses such as array-based mRNA expression profile, epigenome profiles, proteome analysis would be needed.

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Phase II Study of Sequential Triplet Chemotherapy, Irinotecan and Cisplatin Followed by Amrubicin, in Patients with Extensive-Stage Small Cell Lung Cancer: West Japan Thoracic Oncology Group Study 0301

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Introduction: Combination chemotherapy of irinotecan, a topoisomerase I inhibitor, and cisplatin is a standard treatment in patients with extensive-stage small cell lung cancer (SCLC). Amrubicin, a novel 9-aminoanthracycline, inhibits topoisomerase II. We investigated a sequential triplet chemotherapy consisting of irinotecan and cisplatin followed by amrubicin in patients with extensive-stage SCLC.

Methods: Eligible patients were aged 20 to 70 years and had Eastern Cooperative Oncology Group performance status of 0 or 1, measurable lesions, and adequate organ functions. Chemotherapy consisted of irinotecan 60 mg/m^2 on days 1 and 8 plus cisplatin 60 mg/m^2 on day 1 every 3 weeks for three cycles and then amrubicin 40 mg/m^2 alone on days 1 to 3 every 3 weeks for three cycles.

Results: From September 2004 to September 2006, 45 patients were enrolled, 43 were evaluable for response and survival, and 44 were evaluable for toxicity. Twenty-eight patients (64%) completed the full planned chemotherapy. One patient achieved complete response and 33 had partial response for an overall response rate of 79%. Median progression-free survival was 6.5 months. Median overall survival was 15.4 months. Major toxicity was myelosuppression. Grade 3 or 4 neutropenia, anemia, thrombocytopenia, and febrile neutropenia occurred in 57%, 7%, 0%, and 7% of patients during irinotecan/cisplatin cycles and in 91%, 27%, 9%, and 15% of patients during amrubicin cycles, respectively.

Conclusions: The sequential triplet chemotherapy, irinotecan and cisplatin followed by amrubicin, is an effective and well-tolerated treatment in patients with extensive-stage SCLC. Further investigation of this treatment is warranted.

Key Words: Amrubicin, Small cell lung cancer, Sequential chemotherapy, Triplet chemotherapy.

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Small cell lung cancer (SCLC) accounts for approximately 15% of all lung cancers. Disease extension of SCLC is classified as limited stage or extensive stage. Limited-stage SCLC is defined as tumor confined to the hemithorax of origin, the mediastinum, and the supraclavicular lymph nodes, whereas extensive-stage SCLC as tumor spread outside these limits. For extensive-stage SCLC, chemotherapy is the mainstay of treatment. SCLC is highly sensitive to chemotherapy, with a response rate of 70% to 90% in first-line treatment. However, for most patients with extensive-stage SCLC, the disease recurs within several months, and the 5-year survival rate is less than 1%.[1] It is necessary to develop a new treatment for this serious disease.

Irinotecan, a derivative of camptothecin, inhibits topoisomerase I and shows strong antitumor effect for SCLC. The Japan Clinical Oncology Group conducted a randomized phase III trial (JCOG 9511) comparing irinotecan plus cis-

platin with etoposide plus cisplatin in patients with extensive-stage SCLC.[2] This trial was terminated early, because of a highly statistically significant difference in survival between the two arms. The median overall survival was 12.8 months in the irinotecan/cisplatin arm and 9.4 months in the etoposide/cisplatin arm ($p = 0.002$). In Japan, the combination of irinotecan and cisplatin is recognized as a standard treatment for extensive-stage SCLC.

Amrubicin, a novel 9-aminoanthracycline, inhibits topoisomerase II[3] and also shows strong antitumor effect for SCLC. The West Japan Oncology Group, formerly named the West Japan Thoracic Oncology Group (WJTOG), conducted a phase II study of amrubicin in previously untreated patients with extensive-stage SCLC.[4] In 35 patients treated, a response rate of 76% and a median overall survival of 11.7 months were shown. These figures compare favorably with standard doublet chemotherapy.

Some preclinical studies reported that a combination of topoisomerase I and II inhibitors shows a synergistic cytotoxicity.[5] For SCLC, a combination of this type, irinotecan and etoposide (a topoisomerase II inhibitor), was investigated clinically and showed promising results.[6,7] The similar combination of irinotecan and amrubicin is worthwhile to investigate.

Concurrent administration of a triplet combination requires dose reduction of each drug because of toxicities, especially myelosuppression. A sequential chemotherapy, i.e., a doublet followed by the other drug, can be used to avoid the need for dose reduction. In addition, Norton and Simon[8] presented a theoretical model describing the possibility of a sequential chemotherapy.

Therefore, we investigated a sequential triplet chemotherapy consisting of irinotecan and cisplatin followed by amrubicin in patients with extensive-stage SCLC (WJTOG 0301). The purpose of this study was to evaluate the efficacy and safety of this treatment.

PATIENTS AND METHODS

Patient Selection

Eligible patients were aged 20 to 70 years, had histologically or cytologically proven SCLC, extensive-stage disease, Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0 or 1, no prior chemotherapy, neither palliative radiation nor surgery of 14 days, measurable lesions, life expectancy of at least 2 months, and adequate organ functions (white blood cell [WBC] $\geq$4000/μL, neutrophil $\geq$2000/μL, platelet $\geq$100,000/μL, hemoglobin $\geq$10 g/dL, aspartate aminotransferase [AST] and alanine aminotransferase [ALT] $\leq$2 $\times$ upper limit of normal [ULN], total bilirubin $\leq$1.5 $\times$ ULN, creatinine $\leq$ULN, arterial partial pressure of oxygen $\geq$60 mm Hg, no abnormality requiring treatment on electrocardiogram, and left ventricular ejection fraction on echocardiogram $\geq$60%). Patients with any of the following conditions were excluded: symptomatic brain metastases, pleural or pericardial effusion requiring drainage, interstitial pneumonitis, active infection, watery diarrhea or ileus, active gastroduodenal ulcer, continuous administration of steroid or nonsteroidal anti-inflammatory drug, uncontrolled diabetes mellitus or angina pectoris, other active malignancy, and pregnancy or lactation.

All patients gave written informed consent. This study was approved by the institutional review boards at each participating institution.

Treatment Schedule

Chemotherapy consisted of irinotecan 60 mg/m^2 on days 1 and 8 plus cisplatin 60 mg/m^2 on day 1 every 3 weeks for 3 cycles and then amrubicin 40 mg/m^2 alone on days 1 to 3 every 3 weeks for three cycles. Irinotecan was administered as a 90-minute intravenous infusion, cisplatin as a 90-minute intravenous infusion with adequate hydration, and amrubicin as a 5-minute intravenous injection. Prophylactic administration of granulocyte colony-stimulating factor (G-CSF) was allowed at the discretion of the treating physician.

The minimum requirements for the administration of irinotecan and cisplatin were as follows: WBC $\geq$3000/μL, neutrophil $\geq$1500/μL, platelet $\geq$100,000/μL, AST and ALT $\leq$2.5 $\times$ ULN, total bilirubin $\leq$1.5 $\times$ ULN, creatinine $\leq$ULN, PS of 0 to 2, body temperature $\leq$37.5°C, no diarrhea, no interstitial pneumonitis, and other nonhematological toxicity $\leq$grade 2. The minimum requirements for administration of day-8 irinotecan were as follows: WBC $\geq$3000/μL, platelet $\geq$100,000/μL, body temperature $\leq$37.5°C, no diarrhea, no interstitial pneumonitis, and other nonhematological toxicity $\leq$grade 2. The minimum requirements for administration of amrubicin were as follows: WBC $\geq$3000/μL, neutrophil $\geq$1500/μL, platelet $\geq$100,000/μL, AST and ALT $\leq$2.5 $\times$ ULN, total bilirubin $\leq$1.5 $\times$ ULN, creatinine $\leq$1.5 $\times$ ULN, PS of 0 to 2, body temperature $\leq$37.5°C, no interstitial pneumonitis, and other nonhematological toxicity $\leq$grade 2.

If any of the following toxicities was observed, the doses of irinotecan, cisplatin, and amrubicin were reduced to 50, 50, and 35 mg/m^2, respectively: WBC $<$1000/μL, febrile neutropenia (neutrophil $<$1000 /μL), platelet $<$25,000 /μL, or grade 3 nonhematologic toxicity. If creatinine $>$ULN, the dose of cisplatin was reduced to 50 mg/m^2. If creatinine $>$2.0 mg/dL, the administration of cisplatin was discontinued. If grade 4 nonhematological toxicity or pneumonitis $\geq$grade 2 was observed, the study treatment was stopped.

Response and Toxicity Evaluation

Before treatment, a complete medical history was obtained, and physical examination was performed. The following examinations were carried out: complete blood count (CBC) with differential WBC count, blood chemistry, arterial blood gas analysis, urinalysis, electrocardiography, and echocardiography. Staging procedures consisted of chest radiograph, computed tomography (CT) of chest and upper abdomen, magnetic resonance imaging (MRI) or CT of brain, bone scintigraphy, and bone marrow aspiration. During treatment, CBC with differential WBC count, blood chemistry, and chest radiograph were examined at least once a week, and electrocardiography and CT and/or MRI for response evaluation were examined once a month. After treatment, chest radiograph was performed once a month, and CT and/or MRI were performed every 3 months.

Response was evaluated according to the Response Evaluation Criteria in Solid Tumors.[9] Extramural review of eligibility and response of all patients were performed. Toxicity was evaluated in accordance with the Common Terminology Criteria for Adverse Events, Version 3.0.[10]

Statistical Analysis

The primary end point of this study was response rate. Secondary end points were progression-free survival (PFS), overall survival, and toxicity. Survival curves were drawn using the Kaplan-Meier method.[11]

Assuming that a response rate of 90% would indicate potential usefulness, whereas a rate of 75% would be the lower limit of interest, with $\alpha = 0.05$ (one side) and $\beta = 0.20$, 38 patients were required. Allowing for a 15% loss to follow-up, enrollment of a total of 45 patients was planned.

RESULTS

Patient Characteristics

From September 2004 to September 2006, 45 patients were enrolled in this study. Two patients had limited-stage disease. One patient, who was able to receive thoracic radiation, was excluded from all analyses. The other patient, who was not able to receive thoracic radiation due to pleural dissemination, was included in analysis of toxicity and excluded from analysis of response and survival. Therefore, 43 patients were evaluable for response and survival, and 44 were evaluable for toxicity.

Patient characteristics are shown in Table 1. The median age was 63 years, 37 patients (84%) were men, and 31 patients (70%) had PS of 1. Thirty-nine patients (89%) had distant metastases. Frequent sites of distant metastases were brain, liver, bone, and adrenal gland. Of five patients without distant metastases, four had contralateral hilar lymph node involvement and one had pleural dissemination. No patient received prior treatment, including surgery and radiation.

TABLE 1. Patient Characteristics ($n = 44$)

Characteristic	n (%)
Sex	
Male	37 (84)
Female	7 (16)
Age (yr)	
Median (range)	63 (47–70)
ECOG performance status	
0	13 (30)
1	31 (70)
Distant metastases	
Present	39 (89)
Absent	5 (11)
Sites of distant metastasis	
Brain	10 (23)
Liver	10 (23)
Bone	10 (23)
Adrenal gland	10 (23)
Lymph node	7 (16)
Lung	6 (14)
Bone marrow	3 (7)
Other	3 (7)
Prior therapy	
None	44 (100)

ECOG, Eastern Cooperative Oncology Group.

TABLE 2. Treatment Delivery ($n = 44$)

Treatment Cycle	n (%)
Irinotecan/cisplatin	
Cycle 1	44 (100)
Cycle 2	40 (91)
Cycle 3	37 (84)
Amrubicin	
Cycle 1	33 (75)
Cycle 2	30 (68)
Cycle 3	28 (64)

TABLE 3. Tumor Response ($n = 43$)

	n (%)
Complete response	1 (2)
Partial response	33 (77)
Stable disease	1 (2)
Progressive disease	3 (7)
Not evaluable	5 (12)
Overall response	34 (79) (95% CI, 64–90)

CI, confidence interval.

Treatment Delivery

Of 44 patients, 37 patients (84%) received three cycles irinotecan/cisplatin and 28 patients (64%) completed the full planned chemotherapy, i.e., three cycles irinotecan/cisplatin followed by three cycles amrubicin (Table 2). Dose reduction of irinotecan/cisplatin and amrubicin was necessary in six and seven patients, respectively.

Response and Survival

Of 43 patients, 1 achieved complete response and 33 had partial response, for an overall response rate of 79% (95% confidence interval, 64–90%) (Table 3). Of the 33 partial responders, tumor shrinkage met partial response criteria during an irinotecan/cisplatin cycle in 30 patients and during an amrubicin cycle in 3. In the complete responder, tumor disappearance was achieved during an irinotecan/cisplatin cycle.

The survival curves are shown in Figure 1. The median PFS was 6.5 months (95% confidence interval, 4.9–7.4 months), with a 1-year survival rate of 8%. The median overall survival was 15.4 months (95% confidence interval, 11.7–18.0 months), with a 1-year survival rate of 61%.

Chemotherapy After Progression (Second-Line Treatment)

Thirty-five patients received chemotherapy after progression as follows: etoposide plus carboplatin in 10 patients;

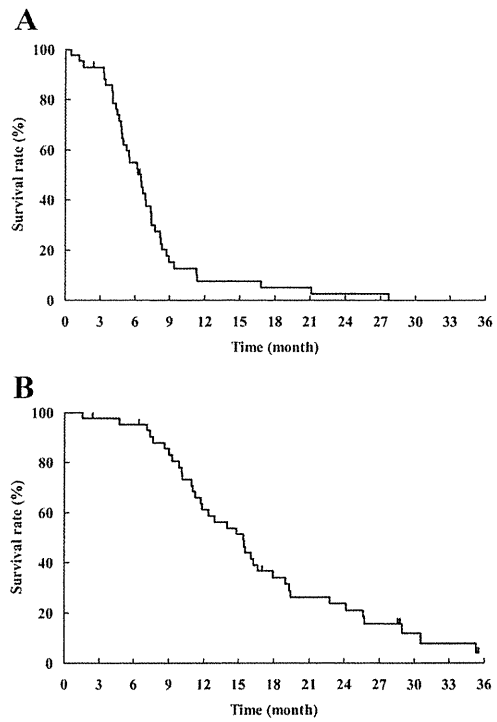

FIGURE 1. Survival curves (n = 43). *A*, Progression-free survival, median 6.5 months (95% confidence interval, 4.9–7.4 months), with a 1-year survival rate of 8%. *B*, Overall survival, median 15.4 months (95% confidence interval, 11.7–18.0 months), with a 1-year survival rate of 61%.

irinotecan plus cisplatin in 6; amrubicin in 5; topotecan plus carboplatin in 4; irinotecan plus amrubicin in 2; irinotecan in 2; and irinotecan plus etoposide, irinotecan plus carboplatin, etoposide plus cisplatin, etoposide, topotecan, and cyclophosphamide plus doxorubicin plus vincristine in 1 patient each.

Toxicity

Toxicities during irinotecan/cisplatin cycles are listed in Table 4. Of 44 patients, grade 3 or 4 leukopenia, neutropenia, anemia, thrombocytopenia, and febrile neutropenia occurred in 6 (14%), 25 (57%), 3 (7%), 0 (0%), and 3 patients (7%), respectively. G-CSF was administered in 12 patients (27%). One patient received transfusion of red blood cell concentrates. One patient (2%) developed grade 3 diarrhea. Grade 3 anorexia was observed in seven patients (16%).

Toxicities during amrubicin cycles are listed in Table 5. Of 33 patients, grade 3 or 4 leukopenia, neutropenia, anemia, thrombocytopenia, and febrile neutropenia occurred in 15 (45%), 30 (91%), 9 (27%), 3 (9%), and 5 patients (15%), respectively. G-CSF was administered in 20 patients (61%). One patient received transfusion of red blood cell concentrates and platelet concentrates, and two other patients received transfusion of red blood cell concentrates. Nonhematological toxicity was not common. One patient (3%) developed grade 3 pneumonitis. This patient was treated with steroid pulse therapy and recovered soon thereafter. No treatment-related death was observed.

TABLE 4. Toxicities During the Irinotecan/Cisplatin Cycle (n = 44)

	Grade					
	0	1	2	3	4	≥3
WBC	11	15	12	4	2	6 (14%)
Neutrophil	9	1	9	20	5	25 (57%)
Hemoglobin	3	23	15	3	0	3 (7%)
Platelet	24	19	1	0	0	0 (0%)
Febrile neutropenia	41	0	0	3	0	3 (7%)
AST/ALT	24	15	3	2	0	2 (5%)
Creatinine	35	7	2	0	0	0 (0%)
Nausea	14	14	12	4	0	4 (9%)
Vomiting	24	11	7	2	0	2 (5%)
Anorexia	11	19	7	7	0	7 (16%)
Fatigue	13	21	8	2	0	2 (5%)
Diarrhea	28	10	5	1	0	1 (2%)
Pneumonitis	44	0	0	0	0	0 (0%)
Infection	39	0	3	2	0	2 (5%)
Rash	37	6	0	1	0	1 (2%)

WBC, white blood cell; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

TABLE 5. Toxicities During the Amrubicin Cycle (n = 33)

	Grade					
	0	1	2	3	4	≥3
WBC	0	3	15	12	3	15 (45%)
Neutrophil	1	0	2	18	12	30 (91%)
Hemoglobin	0	5	19	5	4	9 (27%)
Platelet	13	13	4	0	3	3 (9%)
Febrile neutropenia	28	0	0	5	0	5 (15%)
AST/ALT	25	8	0	0	0	0 (0%)
Creatinine	30	3	0	0	0	0 (0%)
Nausea	18	12	3	0	0	0 (0%)
Vomiting	31	2	0	0	0	0 (0%)
Anorexia	17	12	3	1	0	1 (3%)
Fatigue	10	18	4	1	0	1 (3%)
Diarrhea	31	1	1	0	0	0 (0%)
Pneumonitis	31	1	0	1	0	1 (3%)
Infection	29	0	2	2	0	2 (6%)
Rash	30	2	1	0	0	0 (0%)

WBC, white blood cell; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

DISCUSSION

We performed a phase II study of sequential triplet chemotherapy consisting of irinotecan and cisplatin followed by amrubicin in patients with extensive-stage SCLC and demonstrated a response rate, median PFS, and median overall survival of 79%, 6.5 months, and 15.4 months, respectively. The primary end point of this study was response rate, and the expected and the threshold rates were set 90% and 75%, respectively. The actual response rate in this study (79%) was lower than the expected rate but higher than the threshold. JCOG 9511 reported a response rate, median PFS, and median overall survival of

irinotecan/cisplatin arm of 84%, 6.9 months, and 12.8 months, respectively.[2] Comparing this study with JCOG 9511, the response rate and PFS were similar, whereas overall survival was longer in this study. Taking the longer overall survival into consideration, the results of this study were regarded as promising. There is a possibility that the exclusion of PS 2 patients in this study, which were included in JCOG 9511, could have resulted in the longer overall survival. In addition, we could not find any specific trend that would show prolonged overall survival among second-line treatments.

Two randomized trials that compared irinotecan/cisplatin with etoposide/cisplatin were conducted mainly in North America as confirmation studies of JCOG 9511. One was reported by Hanna et al.[12] and the other was conducted by the Southwest Oncology Group (S0124).[13] Although JCOG 9511 showed survival advantage in the irinotecan/cisplatin arm over the etoposide/cisplatin arm, these North American trials did not show significant difference between the two arms. Irinotecan/cisplatin is a standard chemotherapy for SCLC in Japan, whereas etoposide/cisplatin remains standard in North America. It was reported that the response rate, median PFS, and median overall survival of irinotecan/cisplatin arm were 48%, 4.1 months, and 9.3 months in the trial by Hanna et al. and 60%, 5.7 months, and 9.9 months in S0124, respectively. This study showed better survival than the North American trials. However, great caution is needed when comparing this study with the North American trials. S0124 reported the possibility that inherent genetic differences might exist between the study populations, resulting in divergent outcomes with the same cytotoxic agents.[13] A similar suggestion was made for non-small cell lung cancer.[14] Population-related pharmacogenomics is important because the varied results for the same treatment could be attributed to ethnic differences.

Clinical studies of amrubicin for SCLC had been performed, in both first-line and second-line treatment, entirely in Japan.[15] The WJTOG study in first-line treatment reported a response rate of 76% and median overall survival of 11.7 months.[4] These figures compare favorably with standard doublet chemotherapy. Onoda et al.[16] conducted a phase II study of amrubicin in second-line treatment. They treated 16 patients with refractory disease and 44 patients with sensitive relapsed disease and demonstrated a response rate and median overall survival of 50% and 10.3 months in the refractory group and 52% and 11.6 months in the sensitive group, respectively. Furthermore, the North Japan Lung Cancer Study Group conducted a randomized phase II trial of amrubicin in comparison with topotecan in second-line treatment.[17] That trial showed a response rate and median PFS of 38% and 3.5 months for the amrubicin arm and 13% and 2.2 months for the topotecan arm, respectively. Multivariate analysis revealed that amrubicin has more influence than topotecan on overall survival. Amrubicin is one of the most promising new drugs for the treatment of SCLC.

The ECOG reported a phase III trial of topotecan versus observations after cisplatin and etoposide in extensive-stage SCLC.[18] They showed that four cycles of cisplatin/etoposide induction therapy followed by four cycles of topotecan improved PFS but failed to improve overall survival or quality of life in extensive-stage SCLC. Results of the North Japan Lung Cancer Study Group trial suggested that amrubicin is more effective than topotecan for SCLC. The ECOG trial failed to show survival benefit; however, it did show that amrubicin, instead of topotecan, has potential to lead to better survival in extensive-stage SCLC.

Bozcuk et al.[19] reported a meta-analysis of maintenance/consolidation chemotherapy in the management of SCLC. They analyzed 14 randomized trials, encompassing 2550 patients, and concluded that maintenance/consolidation chemotherapy improves survival in SCLC. Sequential amrubicin was stopped for three cycles in this study. If further cycles of amrubicin as maintenance treatment are given, PFS might be further prolonged.

The major toxicity of sequential amrubicin was myelosuppression, whereas nonhematological toxicity was not common. In the above-mentioned WJTOG study, amrubicin was administered at 45 mg/m^2 on days 1 to 3 as monotherapy.[4] To avoid severe myelosuppression in this study, amrubicin was decreased to 40 mg/m^2 on days 1 to 3 as sequential chemotherapy. This study confirmed that this dose of sequential amrubicin was feasible.

Kaneda et al.[20] reported a phase I study of irinotecan and amrubicin. They administered irinotecan on days 1 and 8 and amrubicin on days 1 to 3. They concluded that this combination was not tolerated because of severe myelosuppression. Although concurrent combination of irinotecan and amrubicin is not tolerable, this study showed that sequential combination of these drugs is tolerable. Irinotecan and amrubicin were administered without G-CSF support in both this study and the study by Kaneda et al.

In conclusion, the sequential triplet chemotherapy of irinotecan and cisplatin followed by amrubicin is an effective and well-tolerated treatment in patients with extensive-stage SCLC. Further investigation of this treatment is warranted.

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Randomized Phase 2 Dose-finding Study of Weekly Administration of Darbepoetin Alfa in Anemic Patients with Lung or Ovarian Cancer Receiving Multicycle Platinum-containing Chemotherapy

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Objective: This is the first clinical trial for Japanese to evaluate the dose–response and determine the clinically effective dose of darbepoetin alfa by weekly subcutaneously administration in anemic patients with lung cancer or ovarian cancer receiving chemotherapy.

Methods: Eligible patients were required to have anemia (hemoglobin level of ≤11.0 g/dl). Patients were randomized in a 1:1:1 ratio to receive darbepoetin alfa (1.0, 2.25 or 4.5 μg/kg) subcutaneously once a week for up to 12 weeks. The study drug was withheld from patients who had a hemoglobin level >15.0 g/dl (for men) or 14.0 g/dl (for women), and reinstated at 50% of the previous weekly dose when the hemoglobin level decreased to ≤13.0 g/dl. Quality-of-life assessments were conducted using the Japanese version of the Functional Assessment of Cancer Therapy-anemia (FACT-an) questionnaire.

Results: Hemoglobin response rate was 31.6%, 55.6% and 70.3% in 1.0, 2.25 and 4.5 μg/kg groups, respectively. The dosages of 2.25 and 4.5 μg/kg thus met the clinically effective dose criterion of at least 50% of patients achieving a hemoglobin response. The FACT-fatigue subscale had a high internal consistency with Cronbach's α score. Although no improvement in FACT-fatigue subscale score from baseline to the end of the treatment phase was confirmed for any dose group, there was a correlation between FACT-fatigue subscale score and hemoglobin concentration. Darbepoetin alfa appears to be well tolerated in this setting and no dose-dependent adverse events were observed.

Conclusions: Darbepoetin alfa alleviated anemia caused by platinum-based chemotherapy, and the dosage of 2.25 μg/kg was the lowest dose that met the clinically effective dose criteria when administered once weekly.

Key words: chemotherapy-induced anemia – erythropoietin – lung cancer – ovarian cancer – quality of life

INTRODUCTION

Anemia is a frequent complication in cancer patients receiving multicycle chemotherapy. Anemia is associated with a plethora of symptoms, including fatigue and dyspnea. Fatigue is the most frequently reported symptom in patients with cancer and has been found to have severe detrimental effects on their lives (1). The etiology of anemia is multifactorial (2–4). In particular, direct effects on the renal tubules by platinum-based compounds lead to a decrease in the production of erythropoietin (EPO), which is responsible for terminal differentiation, proliferation and survival of red

blood cell (RBC) precursors (5). If a patient with cancer develops severe or symptomatic anemia, RBC transfusions may be required, with their attendant risks. Acute transfusion reactions can occur, and although the blood supply is now safer with respect to infection than before, the risk of transmission of infectious agents still exists (6,7). In addition, there are some concerns that frequent RBC transfusions with allogeneic blood may adversely affect the immune system of patients with cancer, thereby increasing the tendency to develop infections and hastening the time to relapse or shortening survival (8).

Erythropoiesis-stimulating agents (ESAs), such as recombinant human EPO (rHuEPO) or darbepoetin alfa (DA), have provided another treatment option for anemic patients with cancer receiving chemotherapy and have been shown to reduce the need for transfusions in this setting (9,10). Previous studies have indicated that ESAs increase hemoglobin (Hb) concentration, relieve the symptoms of anemia, improve quality of life (QOL) and reduce transfusion requirements in patients with solid tumors (11) or lymphoproliferative malignancies (12–14).

DA is a unique EPO protein with higher sialic acid content, longer terminal half-life and higher biological activity than rHuEPO (15), allowing less frequent administration with a similar efficacy and safety profile (16–18). Previous studies of DA have demonstrated that it is effective for the treatment of anemia across a wide range of tumor types, with a similar dose–response curve observed in non-myeloid malignancies (19). Furthermore, in foreign countries, a Phase 3, randomized, double-blind, placebo-controlled study conducted on patients with lung cancer receiving chemotherapy confirmed that a DA starting dose of 2.25 μg/kg administered once weekly (QW) significantly reduced the percentage of patients who required an RBC transfusion and increased Hb concentrations compared with a placebo (10).

In Europe and the USA, ESAs have been widely used since the 1990s for the treatment of chemotherapy-induced anemia. However, they have not been approved yet in Japan. In this prospective study, we first planned a Phase 2 dose-finding study of QW dosing of DA in patients with lung or ovarian cancer who were expected to receive cyclic platinum-containing chemotherapy once every 3 or 4 weeks.

PATIENTS AND METHODS

Study Population

The protocol was approved by the institutional review boards of each of the 31 participating centers, and all patients gave written informed consent before any study-related procedures were carried out.

For entry into the study, patients were required to have been diagnosed with lung or ovarian cancer and expected to receive cyclic platinum-containing chemotherapy once every 3 or 4 weeks for at least two courses after enrollment. Eligible patients were 20–74 years of age and were required to have anemia (Hb level of $\leq$11.0 g/dl). Patients were required to have an Eastern Cooperative Oncology Group (ECOG) performance status of 0–2, and adequate hepatic and renal functions.

Patients were excluded if they were iron deficient; had primary or metastatic malignancy of the central nervous system; had a thrombotic tendency; had received more than three RBC transfusions within 4 weeks or any RBC transfusions within 2 weeks of randomization; were pregnant, breastfeeding or not using adequate birth control measures; or had a history of seizure disorders, active cardiac disease, uncontrolled hypertension, active infection or inflammation or a primary hematologic disorder as the cause of their present anemia.

Study Design and Treatment Schedule

This study was a Phase 2, multicenter, randomized, open-label, sequential dose-finding study (Fig. 1). DA (Kyowa Hakko Kirin Co., Ltd, Japan) was supplied in vials as a clear, colorless, sterile protein solution containing 500 μg/ml of the drug.

After registration, patients were randomized in a 1:1:1 ratio to receive DA (1.0, 2.25 or 4.5 μg/kg) subcutaneously once a week for up to 12 weeks, with a 2-week follow-up period after the last dose of DA. Randomization was performed using a central computerized system and was stratified to balance the treatment groups with respect to tumor type (lung cancer, ovarian cancer), Hb level ($<$9.0, 9.0 $\leq$ Hb level $<$ 10.0 and $\geq$10.0 g/dl) and treatment site. The patients received the first dose of DA on the first day of a chemotherapy cycle.

The study drug was withheld from patients who had an Hb level of $>$15 g/dl (for men) or 14 g/dl (for women), and reinstated at 50% of the previous weekly dose once the Hb concentration decreased to $\leq$13.0 g/dl. Patients with a serum ferritin concentration of $<$10 ng/ml or a serum transferrin saturation of $<$15% received iron therapy to prevent iron deficiency.

RBC transfusion policies were left to the discretion of the investigators, although RBC transfusions were recommended for patients with an Hb level of $\leq$8.0 g/dl or symptoms of anemia, regardless of the patient's Hb level.

Study Endpoints

The primary objective of this study was to determine the clinically effective dose (CED) of DA. The criteria for CED are shown in Table 1.

Efficacy was assessed using Hb endpoints and the incidence of RBC transfusions. The primary measure of efficacy was the percentage of patients achieving an Hb response, defined as an increase in Hb of $\geq$2.0 g/dl from the baseline

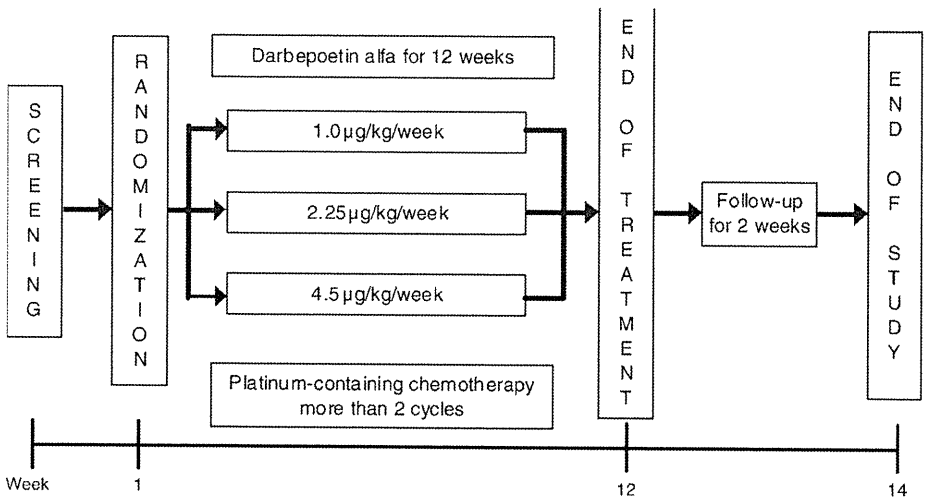


Figure 1. Study design and treatment schema. Darbepoetin alfa was administered once every week.

Table 1. Criteria for clinically effective dose

Efficacy
≥50% of patients achieve an Hb response
≤20% of patients in the safety analysis set experience a dose-limiting toxicity [treatment-related adverse events (>Grade 3 and SAE)]
Safety
≤20% of patients whose Hb concentration is >15.0 g/dl for men or >14.0 g/dl for women

Hb response: ≥2.0 g/dl increase over baseline in the absence of any red blood cell transfusions in the preceding 28 days; Hb, hemoglobin; SAE, serious adverse event.

in the absence of any RBC transfusions during the previous 28 days. The secondary efficacy endpoints were the change in Hb concentration from baseline during the treatment and the incidence of RBC transfusions.

QOL assessments were conducted at baseline, during 7–11 weeks, at the beginning of a chemotherapy course and at the end of a treatment phase after the initiation of DA administration. The Japanese version of the Functional Assessment of Cancer Therapy-anemia (FACT-an) questionnaire was used, which is composed of the FACT-general, a 20-item FACT-anemia subscale and 13 items of which make up the FACT-fatigue subscale.

The safety of DA was evaluated by monitoring adverse events, Hb level, changes in laboratory values and vital signs, and antibody formation resulting from DA administration.

Statistical Analysis

The efficacy analyses were conducted using a per-protocol set that included all patients who received seven or more doses of the study drug and at least two courses of platinum-containing chemotherapy, without major protocol deviations. The proportion of patients exhibiting an Hb response was estimated by subtracting the Kaplan–Meier estimate of the survivor function during week 1 until the end of treatment phase in the absence of an RBC transfusion during the previous 28 days with 95% confidence intervals (CIs), because of the anticipated withdrawal rate. The same analysis for patients in the FAS and analysis using a crude proportion were also performed as part of the sensitivity analysis. For secondary analysis, the percentage of patients exhibiting an Hb correction and patients who received at least one RBC transfusion were also estimated using the Kaplan–Meier method. Cronbach's α coefficient was calculated to assess the reliability of the FACT-an scales. Summary statistics by Hb levels were used to assess the validity of FACT-an scales.

Safety analyses were conducted on all patients who received at least one dose of the study drug. Adverse events were summarized by primary system organ class and by preferred term.

Baseline demographic and clinical characteristics were summarized by the summary statistics.

This study was determined to require a sample size of 120 patients (~40 patients in each dose cohort accounting for patients with drop-out). With 30 patients evaluated in each dose cohort, the proportion of Hb response could be estimated within a standard error of 0.09 if the true proportion is almost 50%.

RESULTS

Patient Demographics and Disposition

Of the 145 patients screened, 132 were enrolled into the study and randomized. Four patients withdrew from the study before receiving the first dose of the study drug. One

hundred and twenty-eight patients (42 patients in the 1.0 μg/kg group and 43 patients in each of the 2.25 and 4.5 μg/kg groups) received at least one dose of the study drug. Twenty-two patients (12 patients received less than seven doses of the study drug, 9 patients received less than two courses of platinum-containing chemotherapy and 1 patient did not have laboratory data after administration) were excluded from the efficacy evaluation due to protocol deviations. One hundred and six patients (33 patients in the 1.0 μg/kg group, 36 patients in the 2.25 μg/kg group and 37 patients in the 4.5 μg/kg group) were included for all efficacy endpoints. Demographic characteristics were similar among the groups, except for age (Table 2).

Efficacy

The proportion of patients that exhibited an Hb response is shown in Fig. 2. The Kaplan–Meier percentages of patients exhibiting an Hb response were 31.6% (95% CI = 14.3–48.9%), 55.6% (95% CI = 35.9–75.2%) and 70.3% (95% CI = 28.0–100.0%) in the 1.0, 2.25 and 4.5 μg/kg groups, respectively. Although there was no reduction in the median time to an Hb response at 4.5 μg/kg compared with 2.25 μg/kg (10 weeks for the 2.25 μg/kg group and 13 weeks for the 4.5 μg/kg group), the dosages of 2.25 and 4.5 μg/kg met the CED criterion that at least 50% of patients exhibited an Hb response.

The mean change in Hb level associated with administration of the various doses of DA was examined (Fig. 3). Although, in this study, there was no difference in the mean change in Hb concentration between the 2.25 and 4.5 μg/kg groups, a trend toward greater increases in Hb level with higher doses of DA was observed: the increase was 0.71 g/dl in the 1.0 μg/kg cohort compared with 1.71 g/dl in the 2.25 μg/kg and 1.72 g/dl in the 4.5 μg/kg cohorts at the end of the treatment phase.

Table 2. Patient characteristics at baseline (per-protocol set population)

	Darbepoetin alfa			Total ($n = 106$)
	1.0 μg/kg, $n = 33$	2.25 μg/kg, $n = 36$	4.5 μg/kg, $n = 37$	
Sex (*n*/%)				
Male	12 (36.4)	14 (38.9)	13 (35.1)	39 (36.8)
Female	21 (63.6)	22 (61.1)	24 (64.9)	67 (63.2)
Age (years)				
Mean (SD)	61.2 (9.9)	56.2 (10.2)	56.1 (12.8)	57.7 (11.2)
Body weight (kg)				
Mean (SD)	53.29 (9.68)	55.59 (9.64)	53.86 (9.36)	54.27 (9.51)
Primary disease (*n*/%)				
Lung	16 (48.5)	17 (47.2)	20 (54.1)	53 (50.0)
NSCLC	13 (39.4)	13 (36.1)	16 (43.2)	42 (39.6)
SCLC	3 (9.1)	4 (11.1)	4 (10.8)	11 (10.4)
Ovarian	17 (51.5)	19 (52.8)	17 (45.9)	53 (50.0)
ECOG PS (*n*/%)				
0	17 (51.5)	22 (61.1)	16 (43.2)	55 (51.9)
1	16 (48.5)	14 (38.9)	21 (56.8)	51 (48.1)
2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
>3/unknown	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Hb (g/dl)				
Mean (SD)	9.81 (1.27)	10.29 (0.98)	10.03 (1.07)	10.05 (1.11)
Hb < 9.0 (*n*/%)	7 (21.2)	4 (11.1)	6 (16.2)	17 (16.0)
9.0 ≤ Hb < 10.0 (*n*/%)	14 (42.4)	9 (25.0)	13 (35.1)	36 (34.0)
Hb ≥ 10.0 (*n*/%)	12 (36.4)	23 (63.9)	18 (48.6)	53 (50.0)
Endo-EPO (mIU/ml)				
Mean (SD)	98.56 (81.91)	57.15 (40.08)	66.41 (60.66)	73.27 (64.41)

Per-protocol set population: all patients who received seven or more doses of study drug and at least two courses of platinum-containing chemotherapy, without considerable protocol deviations; SD, standard deviation; NSCLC, non-small cell ling cancer; ECOG, Eastern Cooperative Oncology Group; PS, performance status; EPO, erythropoietin.

The Kaplan–Meier percentage of patients who received at least one RBC transfusion from week 5 to the end of the treatment phase was lower in the 2.25 μg/kg group [5.8% (95% CI = 0.0–13.7%)] than in the other groups [13.4% (95% CI = 1.1–25.8%) for 1.0 μg/kg group and 15.4% (95% CI = 0.7–30.1%) for 4.5 μg/kg group], although there was no significant difference.

Of the 128 patients, FACT-fatigue subscale score data were available for 127 (41 patients in the 1.0 μg/kg group and 43 patients in each of the 2.25 and 4.5 μg/kg groups). The Japanese version of the FACT-fatigue subscale score had a high internal consistency with Cronbach's α score, which was 0.908 at baseline and 0.932 at the end of the treatment phase. In this study, although no improvement in FACT-fatigue subscale score from baseline to the end of the treatment phase was observed for any dose group, FACT-fatigue subscale score was correlated with Hb concentration at the end of the treatment phase (Fig. 4). In addition, subscale score was also correlated with ECOG performance status score.

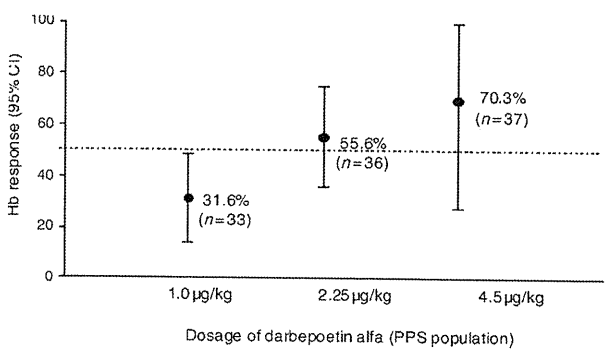
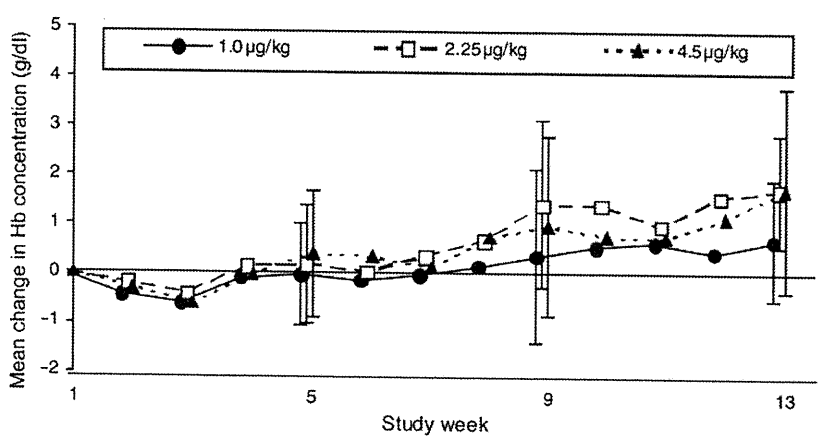


Figure 2. Kaplan–Meier rates of hemoglobin (Hb) response by treatment group [per-protocol set (PPS) population].

Safety

The incidence of adverse events that were considered by the investigators to be related to the study drug was similar among the cohorts: 15 patients (35.7%) in the 1.0 μg/kg group, 15 patients (34.9%) in the 2.25 μg/kg group and 15 patients (34.9%) in the 4.5 μg/kg group. The most frequently reported event was headache [one patient (2.4%) in the 1.0 μg/kg group, two patients (4.7%) in the 2.25 μg/kg group and three patients (7.0%) in the 4.5 μg/kg group]. Other treatment-related adverse events seen in two or more patients were sporadic in each dose cohort (Table 3). The treatment-related adverse events of Grade 3 or greater were angina, sudden hearing, adrenal hemorrhage, nausea, fatigue, increased blood pressure, increased blood uric acid, hypernatremia and prostate induration and each of them was observed in one patient. The incidences of serious adverse events and adverse events of Grade 3 or greater that were considered by the investigators to be related to the study drug were also similar in each dose cohort: three patients in each dose cohort (7.1% in the 1.0 μg/kg group, 7.0% in the 2.25 μg/kg group and 7.0% in the 4.5 μg/kg group). The incidence of adverse events regardless of relationship was at a level expected in a population of cancer patients receiving chemotherapy and occurred at a similar frequency within each dose cohort. The incidences of serious adverse events and adverse events of Grade 3 or greater were similar in each dose cohort.

The percentage of patients who exceeded the Hb thresholds (14.0 g/dl for women and 15.0 g/dl for men) was under 20%



	Week 5	Week 9	Week 13
1.0μg/kg (*n*=33)	−0.02±1.05 (*n*=31)	0.37±1.74 (*n*=27)	0.71±1.24 (*n*=20)
2.25μg/kg (*n*=36)	0.19±1.19 (*n*=36)	1.41±1.70 (*n*=27)	1.71±1.13 (*n*=17)
4.5μg/kg (*n*=37)	0.40±1.30 (*n*=37)	0.98±1.82 (*n*=24)	1.72±2.16 (*n*=15)

Figure 3. Mean change in Hb concentration from baseline to the end of the treatment phase in PPS population (mean ± SD).

in each cohort [one patient (2.4%) in the 1.0 μg/kg group, four patients (9.3%) in the 2.25 μg/kg group and six patients (14.0%) in the 4.5 μg/kg group].

Five patients (3.9%) [two patients (4.8%) in the 1.0 μg/kg group, two patients (4.7%) in the 2.25 μg/kg group and one patient (2.3%) in the 4.5 μg/kg group] died during the study, but none of the deaths were considered by the investigators to be related to the study drug. One venous thromboembolism, a renal vein thrombosis (Grade 1), was observed in one patient with ovarian cancer in 1.0 μg/kg group (2.4%). No anti-DA antibodies were detected in this population of patients receiving DA.

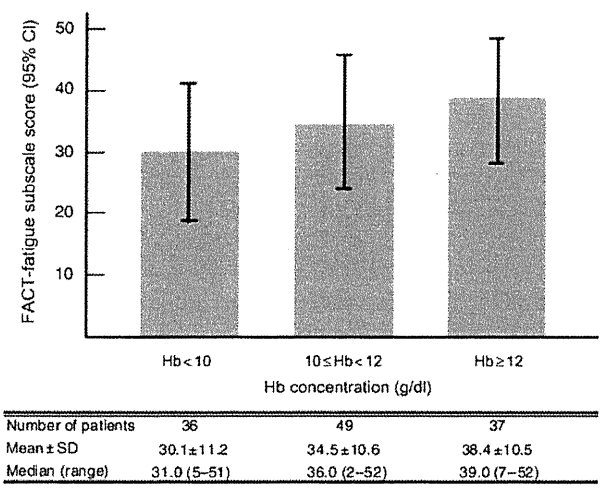


Figure 4. Correlation between FACT-fatigue subscale score and Hb concentration at the end of the treatment phase.

DISCUSSION

In this study, the proportion of patients who exhibited a ≥2.0 g/dl increase in Hb level from baseline was investigated. Dosages of both 2.25 and 4.5 μg/kg met the CED criterion, although there was no reduction in the median time to Hb response at 4.5 μg/kg group compared with 2.25 μg/kg group (10 weeks for the 2.25 μg/kg group and 13 weeks for the 4.5 μg/kg group). Meanwhile, in a study in the US study, there was an obvious dose-dependent increase in the percentage of patients exhibiting an Hb response at 4.5 μg/kg group compared with 2.25 μg/kg group (18). In this study, the median numbers of doses administered were 12, 10 and 9 in the 1.0, 2.25 and 4.5 μg/kg groups, respectively. The median number of doses in the 4.5 μg/kg group was smaller than that in the other groups irrespective of safety. There was no dose-dependent difference in the number of subjects not completing the study. This discrepancy in dose-dependency between the US study and this study may be related to the fact that the treatment duration in

Table 3. Adverse events related to study drug reported for two or more patients receiving darbepoetin alfa (safety analysis population)

Event (PT)	Darbepoetin alfa			Total ($n = 128$)
	1.0 μg/kg, $n = 42$	2.25 μg/kg, $n = 43$	4.5 μg/kg, $n = 43$	
Headache	1 (2.4)	2 (4.7)	3 (7.0)	6 (4.7)
Rush	2 (4.8)	2 (4.7)	1 (2.3)	5 (3.9)
Liver dysfunction	3 (7.1)	—	1 (2.3)	4 (3.1)
Back pain	1 (2.4)	1 (2.3)	1 (2.3)	3 (2.3)
Increased brood pressure	2 (4.8)	1 (2.3)	—	3 (2.3)
Urinary occult blood positive	—	1 (2.3)	2 (4.7)	3 (2.3)
Epigastric pain	—	—	2 (4.7)	2 (1.6)
Increased bilirubin	—	—	2 (4.7)	2 (1.6)
Constipation	—	2 (4.7)	—	2 (1.6)
Dizziness	1 (2.4)	—	1 (2.3)	2 (1.6)
Hypertension	—	1 (2.3)	1 (2.3)	2 (1.6)
Nausea	—	—	2 (4.7)	2 (1.6)
Peripheral edema	—	2 (4.7)	—	2 (1.6)
Melalgia	2 (4.8)	—	—	2 (1.6)
Palpitation	1 (2.4)	—	1 (2.3)	2 (1.6)
Fever	—	—	2 (4.7)	2 (1.6)
Positive urine protein	—	1 (2.3)	1 (2.3)	2 (1.6)

Values are expressed as *n* (%). PT, preferred term.

the 4.5 μg/kg group of this study was shorter than that for other groups. The incidence of RBC transfusions was assessed throughout the study. The period from week 5 to the end of the treatment phase in patients receiving at least one RBC transfusion was analyzed (14). The percentage of patients who received at least one RBC transfusion was lower in the 2.25 μg/kg group than in the other groups from week 5 to the end of the treatment phase, although there was no significant difference. It has been reported that once-weekly DA treatment reduced the percentage of patients receiving RBC transfusions (18). The enrollment of more subjects is considered necessary to assess the reduction in transfusion rate, because this study was designed to assess the percentage Hb response as the primary endpoint. Further large-scale studies focusing on RBC transfusion are needed in Japan.

ESAs have been shown to improve health-related QOL in several studies (20–22). A FACT-an questionnaire was used widely to evaluate cancer patients with anemia, but there are few Japanese reports of studies conducted using FACT-an. Therefore, in this study, the feasibility, reliability and validity of the FACT-an questionnaire were assessed. The collection rate of questionnaires was nearly 100%. FACT-fatigue showed a higher internal consistency (Cronbach's α score range = 0.908 and 0.932 before and after treatment) than other subscales. This internal consistency was consistent with previously reported results and other subscales as well (23). Investigation of the correlation between QOL score and Hb level with FACT-fatigue and FACT-an showed a trend of higher QOL score with increasing Hb level as well as a validation study of FACT (24). These results indicated that the use of the FACT-an questionnaire was a feasible, reliable and valid method of assessing anemia and fatigue in Japanese cancer patients.

In a US study, QOL score increased with increasing Hb concentration (18). In this study, no correlation between FACT-fatigue score and Hb concentration was found. Reasons may include that the QOL baseline score for Japanese patients is slightly higher than for others. A meta-analysis indicated that the baseline of FACT-fatigue is about 26, but in this study, the baseline is 36, which reflects less fatigue (25). A high baseline score may affect the efficacy's resistance to the change in QOL score. FACT-fatigue uses the minimum important difference (MID). MID is the 'smallest difference in score in the domain of interest that patients perceive as important, either beneficial or harmful, and that would lead a clinician to consider a change in the patient's management'. Because FACT-fatigue MID is already known as 3–4, characteristics may have been different between this study and those described in existing reports (26). This baseline difference in Japanese patients may cause difficulty for interpretation.

The results from this study suggest that DA is safe when administered to patients with anemia who are undergoing chemotherapy. The adverse event profile was dominated by findings, e.g. neutropenia, nausea, and vomiting, that are predictable in a population of patients with advanced malignancy receiving multicycle chemotherapy. No unexpected trends were noted in the incidence or severity of adverse events. Although the correlation between the rate of Hb concentration increase and adverse events was investigated, no relationship was apparent. Specifically, the incidence of hypertension and thrombotic events was reported to be associated with a rapid Hb concentration increase in patients with renal failure undergoing dialysis. In this study, the incidence of these complications in all patients was not associated with a rapid increase in Hb concentration. ESA-associated pure red cell aplasia cases have been reported, but almost all cases were observed among hemodialysis patients who received several months of one type of subcutaneously administered rHuEPO (Eprex; Johnson & Johonson, New Brunswick, NJ) (27). No evidence of antibodies to DA was detected for any patient in this study.

Several reports suggested that ESAs had a potential to increase the risk of mortality and/or disease control (28–35) and the negative safety signals were incorporated into the product labels in a boxed warning. It should also be noted that the recently published meta-analyses have indicated a negative impact of ESA use on mortality in cancer patients but the increases on mortality or disease progression were not detected in the patients with chemotherapy-induced anemia (36–39). Several non-clinical studies also have indicated that ESAs do not promote the tumor growth and improve chemotherapeutic outcome in cancer-bearing animals (40–42). Therefore, Aapro and Spivak (43) suggested that the benefit of ESAs outweighs their risks when used for labeled indication and guidelines. The impact of ESAs on mortality and/or disease progression could not be assessed since a long-term follow-up surveillance was not planned in this study. Therefore, further research is needed to clarify the increased risk of them in Japanese patients with chemotherapy-induced anemia.

In conclusion, DA was effective and well tolerated for the treatment of anemia in patients with lung or ovarian cancer receiving platinum-containing chemotherapy and dosages of DA 2.25 μg/kg/QW were the lowest dose that met the CED criteria. Therefore, dosage of DA 2.25 μg/kg/QW was determined as a recommended dose for randomized, placebo-controlled, Phase 3 trial in Japan.

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Conflict of interest statement

The author, Yukito Ichinose, receives honoraria from Kyowa Hakko Kirin Co., Ltd.

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Impact of smoking on lung cancer risk is stronger in those with the homozygous aldehyde dehydrogenase 2 null allele in a Japanese population

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The main lifestyle contributor to acetaldehyde exposure is the drinking of alcoholic beverages, but tobacco smoke also makes some contribution. Although acetaldehyde is associated with upper aerodigestive tract cancer risk, in accordance with genetically determined acetaldehyde metabolism, it is unclear whether lung cancer, a representative smoking-related cancer, is associated with acetaldehyde or genes impacting its metabolism. We conducted a case–control study to examine possible interaction between smoking and aldehyde dehydrogenase 2 (*ALDH2*) Glu504Lys polymorphism (rs671) on the risk of lung cancer in Japanese. Subjects were 718 lung cancer cases and 1416 non-cancer controls enrolled in the Hospital-based Epidemiologic Research Program at Aichi Cancer Center. Lifestyle factors, including smoking, were determined by self-administered questionnaire. We applied pack-years (PY; categorized into five levels: never, <15, <30, <45 and ≥45) as a marker of cumulative exposure to smoking. The impact of smoking, *ALDH2* genotype, and their interaction on lung cancer risk were assessed by odds ratio (OR) and 95% confidence interval adjusted for potential confounders. Adjusted ORs for PY <15, <30, <45 and ≥45 relative to never smokers among those with Glu/Glu or Glu/Lys were 1.39, 1.80, 3.44 and 6.25, respectively (P-trend = 1.4×10^{-30}). In contrast, ORs among Lys/Lys were 1.01, 10.2, 11.4 and 23.2, respectively (P-trend = 2.6×10^{-7}). Interaction between *ALDH2* genotype (Glu/Glu + Glu/Lys versus Lys/Lys) and cumulative smoking dose was statistically significant ($P = 0.036$) and was consistently observed in the analysis among never-drinkers (interaction $P = 0.041$). These results suggest that *ALDH2* Lys/Lys, a null enzyme activity genotype, modifies the impact of smoking on the risk of lung cancer.

Introduction

Alcohol consumption is an established risk factor for cancers of the head and neck, esophagus, colon and breast (1), an effect for which several biological mechanisms have been proposed (2,3). Interestingly, several recent reviews of epidemiologic studies have suggested a potential role for alcohol in carcinogenesis in the lung (4–6). Acetaldehyde, the first oxidative metabolite of ethanol, strongly impacts upper aerodigestive tract cancer via multiple mutagenic effects on DNA, suggesting that it may also play a role in carcinogenesis in the lung (7,8).

Acetaldehyde, which is also an ingredient in tobacco smoke (9–11), is oxidized into acetate by the aldehyde dehydrogenase (ALDH) enzymes. This oxidation is largely dependent on ALDH2 enzyme. The presence of a functional polymorphic site in *ALDH2* is known, namely 504Glu (*1: active)/504Lys (*2: null) (rs671: G>A). The *ALDH2* 504Lys allele is an inactive subunit, and thus, enzyme activity in individuals with the *ALDH2* Lys/Lys genotype is markedly limited compared with that of those homozygous for *ALDH2* 504Glu. Given that the *ALDH2* 504Lys alleles are clustered in East Asian populations, including Japanese, and their well-established impact on alcohol drinking behavior (12), we speculated that this polymorphism may affect lung cancer risk in Japanese in combination with drinking or smoking behavior. We were particularly interested in the possible interaction between this polymorphism and smoking-related acetaldehyde exposure.

Here, we evaluated the association between the *ALDH2* Glu504Lys polymorphism and the lung cancer risk in a case–control study in a Japanese population.

Materials and methods

Study population

The present subjects were aged 20–79 years and were enrolled between January 2001 and November 2005 in the framework of the second version of the Hospital-based Epidemiologic Research Program at Aichi Cancer Center (HERPACC). Details of the study design and subject characteristics have been described elsewhere (13,14). In brief, the second version of HERPACC was initiated at Aichi Cancer Center Hospital, Nagoya, Japan, in 2001. Information on lifestyle factors as well as a 7 ml blood sample was requested from all first-visit outpatients at our hospital, including cancer and non-cancer patients. Before first examination at our hospital, patients were asked about their lifestyle when healthy or before the current symptoms developed. Responses were systematically collected and checked by trained interviewers. Completed responses were obtained from 96.7% of 29 538 eligible subjects, of whom 50.7% donated a blood sample. Questionnaire data were loaded into the HERPACC database and periodically linked with the hospital cancer registry system to update cancer incidence. All participants gave written informed consent and the study was approved by the Ethics Committee of Aichi Cancer Center.

Cases and controls

Cases were 718 patients (423 adenocarcinomas, 127 squamous cell carcinomas, 66 small cell carcinomas, 49 large cell carcinomas, 14 others and 2 unknown) histologically diagnosed with lung cancer between January 2001 and 2005 at Aichi Cancer Center Hospital with no prior history of any cancer. Control subjects were randomly selected from first-visit outpatients who visited our hospital during the same period. A total of 7054 individuals who completed the questionnaire and provided blood samples and were confirmed not to have cancer according to the cancer registry, medical record and self-report were deemed potential controls. Eventually, 1416 controls were frequency matched with case, age and sex. In previous studies, we assessed the clinical diagnosis among non-cancer outpatients and confirmed that there were almost no abnormal findings or non-specific diseases among them (15). We also confirmed the feasibility of using non-cancer outpatients at our hospital as controls in epidemiological studies on the basis that their general lifestyles were accordant with those of a general population randomly selected from the electoral roll in Nagoya City, Aichi Prefecture (16).

Genotyping of ALDH2

DNA of each subject was extracted from the buffy coat fraction using BioRobot EZ1 and an EZ1 DNA Blood 350 ml kit (Qiagen, Tokyo, Japan) or DNA Blood mini kit (Qiagen). Genotyping for the *ALDH2* Glu504Lys

Abbreviations: ALDH2, aldehyde dehydrogenase 2; HERPACC, Hospital-based Epidemiologic Research Program at Aichi Cancer Center; OR, odds ratio; PY, pack-years.

