Table 1. Why do we need Asian guideline for lung cancer?

Difference in medical care for lung cancer
Systematic LN dissection versus sampling
Difference in Medical Care Insurance and economical situation
Ethnic difference of PGX
Evidence obtained specifically from Asian (Japanese) patients (trials)
UFT adjuvant (Stage 1B)
Gefitinib and erlotinib (advanced)
Irinotecan (small and non-small)
Difference in the selection of validated data
Histology: non-squamous versus squamous
Biomarker: ERCC1, RRM1, MSH2
Consolidation/maintenance therapy
Drug lag

between the US and Japan revealed that the US daily doses were higher than those in Japan for 33% of several cardiovascular and other drugs. In addition, ethnic differences are seen in regard to the molecular target, with the EGFR mutation rate being different, as well as drug metabolism and receptor sites.

Concerning molecular targeting, gefitinib monotherapy data can be compared between geographic regions on the basis of the IDEAL I and II Phase II studies (24,25), which were carried out in Japanese and non-Japanese populations, and in Americans, respectively. The patient characteristics were exactly the same in the three populations, but the response rate was significantly higher in the Japanese population, the median survival duration was also higher and the 1-year survival rate was double that of Americans. EGFR mutation in NSCLC was detected at a higher incidence in Asians than in non-Asians, by 32 to 6%. Moreover, the frequency of EGFR mutations was higher in every clinical subgroup, i.e. smokers, non-smokers, adenocarcinoma, males, females, etc., of East-Asian patients compared with non-East-Asian patients (1,26). Gefitinib is known to induce pulmonary toxicity. In Japanese studies, the frequency of gefitinib-induced interstitial lung disease (ILD) ranged from 3.5 to 5.8%, and the ILD mortality ranged from 1.6 to 3.6% (1). In contrast, the frequency of ILD was very low in the USA and other Asian countries, i.e. 0.36 and 0.34% (Table 2).

Irinotecan is another example of ethnic differences is in drug metabolism. Irinotecan is activated to SN-38 by carboxyesterase and then converted to SN-38G by beta-glucuronidase. UGT1A1 is an enzyme that converts SN-38 to SN-38G by glucuronidation. The UGT1A1 promoter shows polymorphism (4,5). When the UGT1A1 promoter has a genotype of 7/7, SN-38 glucuronidation is greatly decreased, and bilirubin glucuronidation is also somewhat decreased. Thus, patients with the 7/7 genotype show higher frequencies of toxicity, such as grade 4 leukopenia and/or grade 3 or higher diarrhea, compared with other UGT1A1 genotypes. In patients with the 7/7 genotype, the AUC of SN-38 is higher compared with other genotypes, while the SN-38G/SN-38 ratio is significantly lower. The distributions of the UGT1A1*28 promoter genotypes differ among racial groups. The 7/7 genotype was observed in only 3% of Japanese and Asian populations, whereas it was present at significantly higher rates of 17% in Canadians, 12% in Caucasians and 23% in Africans (3).

A common-arm analysis was performed to detect pharmacodynamic ethnic differences in paclitaxel plus carboplatin in the treatment of advanced NSCLC in Japan and the USA (27,28). Three trials were included in the analysis: the FACS, JMTO (LC00-03) and SWOG (S0003). The common arm was paclitaxel/carboplatin. The patient characteristics (age, gender and percentages of Stage IV and non-squamous cell carcinoma) were compared and were almost the same in the three studies. The toxicity of the treatment was analyzed with regard to the frequencies of neutropenia and febrile neutropenia, both of which were significantly higher in the Japanese population compared with the American population. When the same dose and same schedule were employed and the efficacy was analyzed, the response rate was almost the same in each of the studies. However, the median survival was 12 and 14 months in the two Japanese studies compared with 9 months in the American study (Tables 3 and 4). The 1-year survival rate was also higher in the Japanese populations compared with the American

Table 2. ILD by EGFR-TKI

	Number of patients	ILD (%)	ILD mortality (%)	Risk factors
WJTOG	1976	70 (3.5)	31 (1.6)	Male, smoker, pulmonary fibrosis
Prospective study of AZ	3322	193 (5.8)	75 (2.5%)	Poor PS, smoker, pulmonary fibrosis, prior CT
Okayama study group	330	15 (4.5)	8 (2.4)	
NCCH	112	6 (5.4)	4 (3.6)	
USA	~24 000	0.36	0.06	
AZ (Asian patient excluding Japanese)	53 150	0.34	0.11	
Korea	111	0		
China	31	0		

Table 3. Toxicity analysis

	FACS ($N = 145$)	LC00-03 ($N = 197$)	S0003 ($N = 186$)	*P*-value
Neutropenia (group 4), *N* (%)	102 (69)	106 (69)	48 (26)	<0.0001
Febrile neutropenia (groups 3–4), *N* (%)	26 (18)	38 (19)	6 (3%)	<0.0001

Gandara: ASCO 2004; Crowley: ASCO 2006; Gandara JCO 2009 (27).

Table 4. Efficacy

	FACS ($N = 145$)	LC00-03 ($N = 197$)	S0003 ($N = 182$)	*P*-value
Complete + partial response, *N* (%)	47 (32)	71 (36)	61 (34)	0.61
PFS (months)	4.5	6	4	NA
MST (months)	12	14	9	NA
1-year survival rate (%)	51	57	37	0.001

NA, statistical comparison not applicable.
Gandara: ASCO 2004; Crowley: ASCO 2006; Gandara JCO 2009 (27).

Table 5. Solution to drug lag in East Asia

Pharmaceutical companies
Simultaneous clinical development
Multinational clinical trial
Asian clinical trial
Investigations
Quick accrual of patients
Regulatories
Established high quality and speedy approval process
Regulatory harmonization and more collaborations among regulatory agencies

population: 51 and 57% versus 37%. Korean and Chinese trials have shown the same tendency.

Another very important factor is the lag time until drug approval. Comparison of Japan with the EU and the US shows that the average time from the first approval anywhere in the world until approval in each other country was about 500 days in the US and the UK, but over 1400 days in Japan. Looking at drug lag in East Asia shows that Taiwan and Korea were a little bit quicker than Japan and China for approval of some drugs. To solve this problem of drug lag in East Asia, it will be necessary for pharmaceutical companies to perform simultaneous clinical development in multiple countries, multinational clinical trials and Asian clinical trials. Also, investigators need to achieve quick accrual of patients, while the regulatory authorities need to establish high quality and speedy approval processes, and achieve regulatory harmonization and better collaboration among agencies (Table 5).

The National Comprehensive Cancer Network (NCCN) is an alliance of 21 of the world's leading cancer centers that is based in the USA. The NCCN promotes the importance of continuous quality improvement and creation of international and national clinical practice guidelines (10). The NCCN has international initiatives in Asia, including adaptation of NCCN Clinical Practice Guidelines in Oncology to create NCCN approved, translated and/or regionally adapted materials for national use. The process for such adaptation is that the NCCN authorizes selected groups to adapt its Practice Guidelines for national use. The participating countries select disease-specific representatives to review and suggest modifications to specific guidelines. Then the NCCN guidelines are circulated to multidisciplinary physicians in that country to determine where local practice is not concordant with the NCCN version. Regional meetings are held to agree on proposals, supported by data, for adaptation of the guidelines. A consensus for adaptation is approved by the NCCN, and the changes from the NCCN version are identified in the adaptation.

Asian consensus statements are intended as a reference and stepping stone for individual countries in Asia that do not yet have local editions of the NCCN guidelines so that they can develop their own guidelines. There have still been no pan-Asian guidelines developed for NSCLC. In general, the NCCN guidelines or national adaptations, or other recognized guidelines (e.g. ASCO, ACCP), are followed. Asian consensus statements are developed through the NCCN to help individual countries establish their own guidelines. As national NSCLC guidelines, Korea, China and Thailand adapted the NCCN guidelines. In Japan, the Japanese Society of Lung Cancer developed a Lung Cancer Practice Guideline in 2003 (13); this is different from the NCCN guidelines. China also has a Chinese Lung Cancer Management Guideline that is based on Chinese clinical practice and is used by most Chinese doctors. It was issued by the Chinese Society of Lung Cancer and is revised every 2 years. Hong Kong, India, Malaysia, Taiwan and Singapore have no NSCLC guideline (Table 6).

There are several differences between the NCCN version 2/2009 and the Korean NCCN 2008. For Stage IIIB resectable satellite lesions, the Korean NCCN guidelines specify the strategies for pN 0-1 and pN0. The therapy for recurrent and metastatic disease, chemotherapy for progressive disease and adjuvant chemotherapy regimens also differ between these guidelines. Comparison of the Korean NCCN guidelines and the ASCO guidelines shows that key differences exist in relation to Stage I disease and resected Stages I–IIIA. For Stage I, the Korean NCCN guidelines suggest adjuvant chemotherapy as an option, whereas it is not recommended in the ASCO guidelines (29). For resected Stages I–IIIA, the Korean NCCN guidelines suggest adjuvant radiotherapy when margins are positive, but it is not routinely recommended in the ASCO guidelines. The ASCO

Table 6. Current NSCLC guidelines in Asia

Pan-Asian guidelines
There are no pan-Asian guidelines developed for NSCLC
NCCN guidelines (or national adaptations of these) or other recognised guidelines (e.g. ASCO, ACCP) are generally followed
Asia Consensus Statements are developed through NCCN to help countries develop their own guidelines
National guidelines
Korea, Thailand: adaptation of NCCN guidelines
Japan: Japanese Society Lung Cancer developed Lung Cancer Practice guideline (2003)
China: adaptation of NCCN guidelines, Chinese LC Management Guideline
The following countries do not appear to have individual national guidelines
Hong Kong, India, Malaysia, Taiwan, Singapore

guidelines are very conservative and revised every 5 years, whereas the Korean NCCN guidelines are revised very frequently. Major institutions generally apply the Korean KCCN guidelines (11).

Regarding the current guideline for NSCLC in Japan, the background of its preparation includes such factors as that lung cancer is the number-one cause of death in Japan, the death rate due to lung cancer is increasing rapid, the cure rate is low at about 10–15%, there has been development of diverse diagnostic and treatment methods, and there is a need for a guideline that indicates standard medical care for lung cancer. The guideline should be evidence based, with scientific evidence obtained from clinical trials, should take into account the patients' requirements and preferences, and should also take into account physicians' professional experience and knowledge. As the method for development of a guideline, a systematic search of the published literature during the last 10–20 years should encompass PubMed, the Cochrane Review, Japanese medical journals, etc., critical and quantitative/qualitative evaluation of evidence, and scientific recommendations. Various key words are used to search the literature.

With regard to the history of development of a guideline for medical care of lung cancer in Japan, a study group was formed in 2001, with support from the Japanese Ministry of Health, Labour and Welfare (MHLW). The study group consisted of representatives from various Japanese medical societies, including the Japanese Society of Lung Cancer and the Japanese Society of Respiratory Disease. In 2003, the first 'Guideline for Medical Care in Lung Cancer (13),' also supported by grants from the MHLW, was developed. In 2005, the Guideline was revised by the Japanese Society of Lung Cancer. The contents of the guideline consisted of medical care (diagnosis and treatment modalities) and staging. The classification of the evidence level was similar to that for other guidelines. The highest level of evidence was (i) systematic review and meta-analysis of multiple randomized clinical trials. Subsequent levels consisted of (ii) more than one RCT, (iii) a non-RCT such as a Phase II study, (iv) an analytical-epidemiological study such as a cohort study or case-controlled study, (v) case reports and/or case series, and (vi) personal opinions of specialists or committee members. The recommendation levels consisted of (A) strongly recommended, (B) recommended, (C) not enough data for recommendation and (D) recommended not to do. Decision-making regarding the recommendation was based on the (A) evidence level, (B) amount of evidence and consistency, (C) hazard ratio (difference in efficacy), (D) clinical applicability and (E) evidence of toxicity and cost.

In the EBM guideline to chemotherapy for lung cancer, the recommendations regarding the roles of chemotherapy for advanced NSCLC are (i) chemotherapy in unresectable advanced NSCLC patients prolongs survival, improves QOL and is strongly recommended in this group of patients (Grade A recommendation) and (ii) chemotherapy in elderly, unresectable advanced NSCLC patients prolongs survival, improves QOL and is strongly recommended in this group of patients (Grade B recommendation). The recommendations regarding the target population for chemotherapy are (i) chemotherapy is recommended in patients less than 75 years old with a good performance status (PS 0, 1) (Grade A), (ii) chemotherapy is also recommended in patients more than 75 years old with a good PS (0, 1) (Grade B) and (iii) possibility of chemotherapy in PS 2 patients, but there is no evidence (Grade C). (underlining indicates a difference from Western guidelines.) There is the issue of use of gefitinib in patients with EGFR mutation, and the guideline thus needs to be revised.

The recommendations regarding the selection of anti-cancer drugs are (i) cisplatin-containing doublets are strongly recommended in patients less than 75 years old with a good PS (0, 1) (Grade A), (ii) drugs to be combined with cisplatin are irinotecan, vinorelbine, gemcitabine, paclitaxel and docetaxel (Grade A), and (iii) non-platinum doublets are recommended in patients who might be suffering from cisplatin-induced toxicity (Grade A). Questions remain regarding the use of gefitinib in patients with EGFR mutation and whether pemetrexed should be used, and the guideline thus needs to be revised.

The recommendation regarding the duration of chemotherapy is that first-line chemotherapy should consist of three to six courses (Grade B). But recently there has been development of the concepts of consolidation and maintenance therapy, so this recommendation also needs to be revised. For second-line chemotherapy (defined as chemotherapy for refractory or recurrent NSCLC after first-line chemotherapy), it is recommended that docetaxel be administered for refractory or recurrent NSCLC after first-line chemotherapy (Grade B). However, pemetrexed, erlotinib and gefitinib are now available, and this recommendation thus needs to be revised. With regard to molecular-target-based therapy, there is insufficient evidence for recommendation of EGFR/TKI in NSCLC (Grade C). However, positive results have since been obtained in EGFR-mutated NSCLC, and this description in the guideline thus also needs to be revised.

With regard to chemoradiotherapy (CRT) for locally advanced NSCLC, the recommendations are as follows: (i) CRT containing cisplatin is strongly recommended for inoperable, locally advanced NSCLC (Grade A); (ii) CRT is strongly recommended for patients with a good PS (0, 1) (Grade A); (iii) Chemotherapy should be given concurrently (Grade A); (iv) The dose of radiotherapy should be 60 Gy by usual fractionation (1.8–2.0 Gy/day) (Grade A); (v) there is no evidence for an effect of split-course radiotherapy on survival benefit, while there is not enough data for recommending not to split radiotherapy (Grade C); (vi) the chemotherapy regimen for concurrent CRT should be a platinum-containing doublet or triplet (Grade B). There is not enough data from large clinical trials regarding CRT-containing irinotecan, paclitaxel, docetaxel, vinorelbine and gemcitabine, and these drugs should be used only in clinical trials (Grade C). However, positive results have recently been obtained with paclitaxel and vinorelbine, and this description in the guideline thus also needs to be revised.

The recommendation with regard to adjuvant immunotherapy (postoperative) is that there is not enough evidence for an improved prognosis by using an immunostimulant. There is also no clear evidence for recommending use of an immunostimulant after surgery (Grade C). The recommendation with regard to preoperative chemotherapy in Stage I/II NSCLC is that there is not enough data to recommend preoperative chemotherapy (Grade C).

In addition to the guideline, since 2005 Japan has had a guidance for gefitinib prescription. The indication for gefitinib is inoperable or recurrent NSCLC. Gefitinib is not indicated for patients without prior chemotherapy, as adjuvant therapy, as maintenance therapy after CRT or in combination with anti-cancer drugs or radiotherapy. Gefitinib is recommended for the following patients: females, adenocarcinoma, non-smokers, Japanese (Asians) and patients with EGFR mutation.

Thus, Japan has an NSCLC guideline and a gefitinib guidance, but the reality is somewhat different. With regard to the market share of the first-line regimens for NSCLC in Japan, carboplatin/paclitaxel is number one, followed by gefitinib, which is surprising. As the second-line regimen, gefitinib is number one, followed by docetaxel. There is thus a discrepancy between the guidelines and actual clinical practice.

Based on the discussions among the study group members from various Asian countries, it seems difficult to establish a common guideline for NSCLC among Asian countries at the present time because of the differences in medical care in each country as well as the drug lag seen in some countries. Asian collaborative trials on treatment of NSCLC need to be started at an early date to generate Asian data.

EARLY-STAGE LUNG CANCER

Some differences are seen between Asia and Europe and the USA in regard to early-stage lung cancer. Based on clinical practice, it is found that the results of surgery for early-stage lung cancer are better in Asia than in the West. There are also differences with regard to the value of adjuvant chemotherapy. For example, for Stage I, adjuvant chemotherapy is not used in China, whereas in the US and Europe adjuvant chemotherapy is recommended for Stage IB lung cancer. One problem is how to treat patients with early-stage lung cancer with EGFR mutation, which occurs at a much higher incidence of about 30% in Asian populations. Asian clinical trials are needed to answer this.

LOCALLY ADVANCED NSCLC

In regard to locally advanced NSCLC, it is accepted that concurrent chemoradiation therapy (CRT) should be accepted as standard treatment. However, there are several questions regarding the drug to be used in Asian populations: the type of drug, dosage and schedule that will be suitable. As reported, chemotherapy toxicity is higher in Asian populations, but the response and survival are better than in the West. The radiation technique used in CRT has mostly been 3D conformal irradiation. However, this may not be possible in all Asian countries, so further investigation is needed regarding the radiation technique to be used concurrently with chemotherapy. Induction chemotherapy or CRT prior to surgery also needs to be studied in Asia, as does surgery for locally advanced NSCLC. A third point regarding locally advanced NSCLC is maintenance therapy, especially tyrosine kinase inhibitors (TKIs). Detrimental effects were reported in an American population administered maintenance TKI. However, because of the high incidence of EGFR mutation in Asians, it is not known whether maintenance therapy with TKIs will benefit the patient or not. In the West most population studies were based on PET CT, whereas in most Asian countries, especially Southeast Asia, the method is usually only CT scan. Thus, there are various problems remaining in Asian populations with regard to locally advanced NSCLC.

ADVANCED NSCLC

Three aspects of management of advanced NSCLC in the Asian region need to be addressed. First, there are some epidemiological differences, especially the incidence of NSCLC mortality. Second, there seem to be some differences in the etiological factors implicated in lung cancer in the East compared with the West. In the East, there are more cases that are not directly associated with smoking, meaning that lung cancer non-smokers are more prevalent, especially in East Asian women. Third, there is increasing evidence in support of major differences in treatment of advanced NSCLC in terms of the efficacy and toxicity, especially with TKIs. Asian patients derive much greater benefit from TKIs compared with Caucasian people. In fact, some of the Korean consensus guidelines suggest broader recommendation of TKIs even to patients with a poor performance status.

Cytotoxic agents are usually relatively or absolutely contraindicated for poor PS patients, but TKIs are much more convenient to administer and much less toxic than cytotoxic agents. Thus, TKIs can be recommended to a broader range of patients with a poor performance status. There are also recent data that indicate possible benefit from TKIs even in the first-line setting, without any prior chemotherapy.

In summary, there is mounting evidence of differences between Asian and Caucasian lung cancer patients in many aspects, including epidemiology, etiology and treatment outcomes and toxicities. Asia truly needs its own region-specific clinical trials to address each of these issues in regard to NSCLC.

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Conflict of interest statement

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Severe Interstitial Lung Disease Associated with Amrubicin Treatment

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Background: Amrubicin is a novel anthracycline agent that is well known to exert significant activity against small cell lung cancer (SCLC), but the adverse pulmonary effects of amrubicin are less well known. We investigated the incidence of acute interstitial lung disease (ILD) in SCLC patients who had been treated with amrubicin.

Methods: Medical records were used to retrospectively investigate a total of 100 cases of SCLC patients treated with single-agent amrubicin therapy at the National Cancer Center Hospital East between June 2003 and March 2008. The patients' radiographic records and clinical data were reviewed to identify patients who had developed acute ILD after being treated with amrubicin.

Results: After receiving amrubicin, seven of the 100 SCLC patients subsequently developed pulmonary infiltrates, and they were identified as cases of acute ILD associated with amrubicin. Of the seven patients who developed ILD, six were treated with corticosteroids, and the ILD improved in three of them, but the other three patients died of respiratory failure. The incidence of ILD was 33% (4/12) among the patients with pre-existing pulmonary fibrosis (PF) and 3% (3/88) among the patients without PF, and the difference between the two groups was statistically significant ($P = 0.0036$).

Conclusions: The results of this study indicated that amrubicin may cause severe ILD and that pre-existing PF was associated with a higher rate of ILD among SCLC patients treated with amrubicin. We recommend not administering amrubicin in the treatment of SCLC patients with pre-existing PF.

Key Words: Amrubicin, Interstitial lung disease, Toxicity, Small cell lung cancer, Chemotherapy.

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Amrubicin is a novel, totally synthetic 9-aminoanthracycline that is converted to an active metabolite, amrubicinol, as a result of reduction of its C-13 ketone group to a hydroxy group. Despite the similarity between the chemical structure of amrubicin and doxorubicin, amrubicin has a different mode of action. Amrubicin and amrubicinol are DNA topoisomerase II inhibitors, which exert their cytotoxic effects by stabilizing a topoisomerase II-mediated cleavable complex, and they are approximately 1/10 weaker than doxorubicin as a DNA intercalator. The in vitro cytotoxic activity of amrubicinol is 18 to 220 times more potent than that of its parent compound, amrubicin.[1,2] An in vivo comparison with doxorubicin showed that amrubicin has a more potent antitumor effect and lower toxic effects on the heart, which is a site of delayed toxicity with doxorubicin, and on the liver and kidneys.[3–5]

Amrubicin is a promising agent for the treatment of small cell lung cancer (SCLC).[6] Most patients with SCLC treated with standard chemotherapy, such as cisplatin plus etposide or cisplatin plus irinotecan, tend to experience a relapse within a year of the completion of treatment, and patients with relapsed SCLC historically have a poor outcome.[4,7] Some multicenter phase II trials in Japan or North America have shown that amrubicin has significant activity in patients with refractory or relapsed SCLC.[8,9] Randomized controlled trials with amrubicin for the treatment of SCLC patients are ongoing in the United States. The major toxicity of amrubicin is hematologic, and more than half of the patients treated with amrubicin develop grade 3 or 4 neutropenia. Nonhematologic toxicities, such as gastrointestinal toxicity or alopecia, are relatively mild. Surprisingly, several patients in Japanese phase II trials developed interstitial lung disease (ILD).[10,11] However, because the adverse pulmonary effects of amrubicin are less well known, in this study, we investigated the incidence of acute ILD in SCLC patients who had been treated with amrubicin.

PATIENTS AND METHODS

Medical records were used to retrospectively investigate a total of 100 consecutive cases of SCLC treated with single-agent amrubicin therapy at the National Cancer Center Hospital East between June 2003 and March 2008. The patients' radiologic reports and clinical data were reviewed to identify patients who had developed acute ILD after being treated with amrubicin. The study was approved by the institutional review board of our institution.

Three independent pulmonologists (K.Y., H.K., and Y.Y.) who had no knowledge of the patients' outcome diagnosed pre-existing lung conditions, i.e., pulmonary fibrosis (PF) and emphysematous change, based on the chest radiographic and

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computed tomographic (CT) findings before the start of amrubicin therapy. The diagnostic criteria for PF were a linear, ground-glass attenuation, or reticular shadows on chest radiographs and CT scans that were predominant in the lower zone of the lung. ILD was diagnosed on the basis of chest radiograph and CT findings (diffuse ground-glass opacity, reticular shadow, or consolidation without segmental distribution and honey-comb pattern), a serum lactate dehydrogenase (LDH) and/or KL-6, which is a mucin-like high-molecular-weight glycoprotein and shown to correlate well with the activities of several different kinds of interstitial pneumonia, elevation, and no evidence of underlying heart disease, infection, or lymphangitic carcinomatosis. Objective tumor response was assessed as complete response, partial response, stable disease ≥8 weeks, or progressive disease according to the Response Evaluation Criteria in Solid Tumors. Toxicity was graded by using the Common Terminology Criteria for Adverse Events version 3.0.

Univariate and multivariate analyses were performed to identify risk factors for ILD associated with amrubicin therapy. All comparisons between proportions were performed by the χ^2 test or Fisher's exact test, as appropriate. Multivariate analyses were performed using the logistic regression procedure to assess the relationship between several factors and the onset of ILD. *P* values less than 0.05 were considered statistically significant. Two-sided statistical tests were used in all analyses.

TABLE 1. Patient Characteristics

	Patients (n = 100)	
	N	%
Age (yr)		
Median	66	
Range	48–81	
Sex		
Female	17	17
Male	83	83
Performance status		
0/1	3/76	77
2/3	20/1	21
Smoking history		
Current/former smoker	98	98
Never smoker	2	2
No. of prior chemotherapy regimens		
1	43	43
2/3	51/6	57
Prior thoracic radiotherapy		
Yes	42	42
No	58	58
Pre-existing pulmonary fibrosis		
Yes	12	12
No	88	88
Pulmonary emphysematous change		
Yes	41	41
No	59	59
Amrubicin dose per square meter body surface area		
45 mg/m^2	37	37
40/35/30 mg/m^2	48/12/3	63

RESULTS

Patient Characteristics

The patients' characteristics are listed in Table 1. Their median age was 66 (range, 48–81) years, 17% of them were women, and 77% had an Eastern Cooperative Oncology Group performance status 0 and 1. Current smokers or exsmokers accounted for 98% of the patients, and emphysematous change was detected in 41% of the patients. Pre-existing PF was detected in 12% of the patients, but none of them had dyspnea. Amrubicin was used as a second-line treatment in 43% of the patients, and 57% had received two or more prior chemotherapy regimens. Amrubicin was diluted in 50 ml of normal saline and administered as a 5-minute daily intravenous injection at a dose of 30 to 45 mg/m^2 on 3 consecutive days, every 3 to 4 weeks.

Incidence and Outcome of ILD

After receiving amrubicin, 7 (7%) of the 100 SCLC patients developed pulmonary infiltrates in the absence of un-

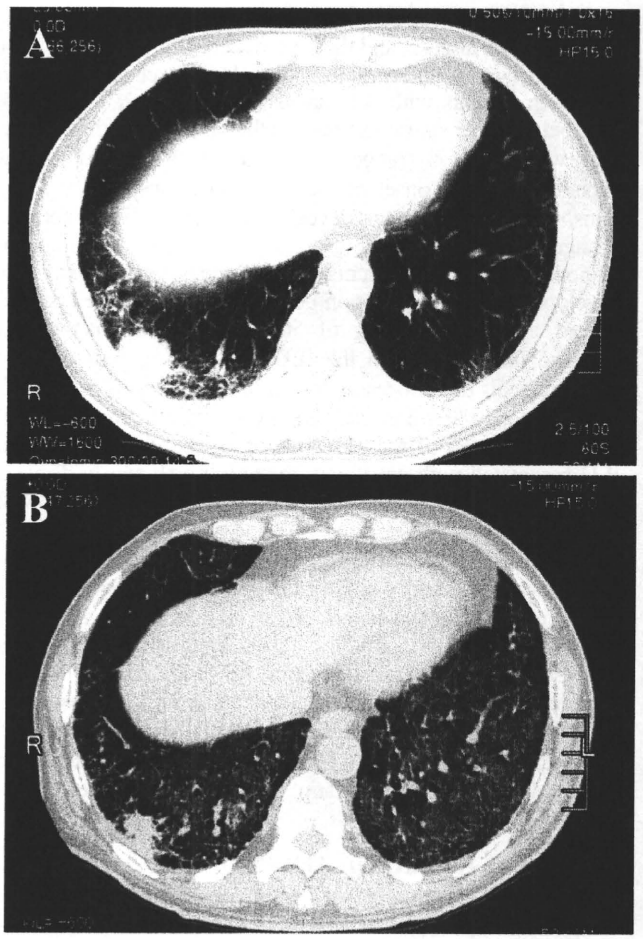

FIGURE 1. Computed tomography (CT) scans of the chest before and after treatment with amrubicin (patient 2 in Table 2). *A*, This CT scan of the chest before treatment with amrubicin shows a bilateral reticular shadow just beneath the pleura and a primary tumor in the right lower lobe. *B*, CT scan of the chest on day 17 of the first course of amrubicin therapy showing bilateral diffuse ground-glass opacities.

TABLE 2. Summary of Patients Who Developed Interstitial Lung Disease Associated with Amrubicin Therapy

No.	Age (yr)	Sex	PS	Smoking History	Prior Chemotherapy	Prior TRT	PF	Time to ILD From First AMR (d)	Initial Manifestations	ILD Status	Time to Death From Last AMR (d)
1	78	M	1	Yes	Carboplatin Etoposide	No	No	15 (day 15 in cycle 1)	Dyspnea, cough, hypoxemia	Died	23
2	53	M	2	Yes	Cisplatin Etoposide Irinotecan	No	Yes	17 (day 17 in cycle 1)	Dyspnea, fever, hypoxemia	Died	30
3	55	M	1	Yes	Cisplatin Etoposide Irinotecan	No	Yes	21 (day 21 in cycle 1)	Dyspnea, hypoxemia	Improved	—
4	70	M	1	Yes	Carboplatin Etoposide	No	Yes	22 (day 22 in cycle 1)	Dyspnea, fever, hypoxemia	Improved	—
5	64	M	1	No	Cisplatin Etoposide Irinotecan	Yes	No	43 (day 15 in cycle 2)	Cough, fever	Improved	—
6	62	M	1	Yes	Cisplatin Etoposide Irinotecan	No	Yes	72 (day 18 in cycle 3)	Dyspnea, hypoxemia	Improved	—
7	63	M	1	Yes	Carboplatin Etoposide	No	No	94 (day 21 in cycle 4)	Dyspnea, fever, hypoxemia	Died	36

PS, performance status; TRT, thoracic radiotherapy; PF, pre-existing pulmonary fibrosis; ILD, interstitial lung disease; AMR, amrubicin.

derlying heart disease, and they were identified as cases of acute ILD associated with amrubicin (Fig. 1). The characteristics of the seven patients with ILD are listed in Table 2. The median time between the start of amrubicin therapy and the diagnosis of ILD was 22 days (range, 15–94 days). All seven patients experienced acute onset or exacerbation of respiratory symptoms, and chest CT scans revealed the new diffuse interstitial changes in both lungs with ground-glass opacity and/or consolidation in all seven patients. Six of the seven patients who developed ILD, the exception being patient 6, received corticosteroid therapy consisting of 500 to 1000 mg methylprednisolone for 3 days, and the ILD improved in four of them. Three patients died of respiratory failure as a result of the ILD, but no autopsy was permitted in any of these three patients.

The results of the univariate analysis of risk factors for ILD associated with amrubicin therapy are shown in Table 3. The incidence of ILD associated with amrubicin was 33% (4/12) in patients with pre-existing PF and 3% (3/88) in patients without PF, and the difference in incidence between the two groups was statistically significant ($P = 0.004$). Based on the results of the univariate analysis, a multivariate analysis was performed using two variables (Pre-existing PF and LDH) and the results showed that pre-existing PF (odds ratio: 10.9, 95% confidence interval: 2.0–66.8) was a significant independent variable correlated with increased risk of ILD associated with amrubicin therapy ($P = 0.006$). LDH was not a significant independent variable (odds ratio: 3.3, 95% confidence interval: 0.44–66.3, $P = 0.30$).

TABLE 3. Relationship Between Clinical Variables and Interstitial Lung Disease Associated with Amrubicin Therapy

Variables	No. of Patients	Incidence of ILD (%)	*p*
Total	100	7	
Age			
<70 yr	65	7.7	>0.99
≥70 yr	35	5.7	
Sex			
Female	17	0	0.59
Male	83	8.4	
Performance status			
0/1	79	7.6	>0.99
2/3	21	4.8	
Smoking history			
Current/former smoker	98	6.1	0.13
Never smoker	2	50	
No. of prior chemotherapy regimens			
1	43	7	>0.99
2/3	57	7	
Prior thoracic radiotherapy			
Yes	42	2.4	0.23
No	58	10.3	
Pre-existing pulmonary fibrosis			
Yes	12	33.3	0.004
No	88	3.4	
Pulmonary emphysematous change			
Yes	41	9.8	0.69
No	59	5.1	
LDH			
High (more than upper limit of normal)	55	10.9	0.09
Normal	45	2.2	

ILD, interstitial lung disease; LDH, lactate dehydrogenase.

Efficacy of Amrubicin Therapy

The median number of cycles per patient was 2 (range, 1–6). The responses of all 100 patients were assessed, and the results showed a partial response in 32 patients, stable disease in 17 patients, and progressive disease in 51 patients. Thus, the overall response rate was 32% (32/100). The response rate of the chemotherapy-sensitive relapse (defined as relapse at an interval of ≥90 days after the completion of prior chemotherapy) group was 44% (18/41), which is higher than that in the refractory relapse (defined as relapse within 90 days after completion of prior chemotherapy) group (24% [14/59], $P = 0.034$). By contrast, the response rate of the group with pre-existing PF was 25% (3/12), as opposed to 33% (29/88) in the group without PF ($P = 0.74$).

DISCUSSION

Anticancer-agent-associated ILD is an important cause of respiratory failure during cancer chemotherapy.[12] Although the incidence of anticancer-agent-associated ILD seems low, more cases can be expected as increasing numbers of patients receive the new generations of anticancer agents, such as gemcitabine,[13] irinotecan,[14] docetaxel,[15] and gefitinib.[16] To our knowledge, this is the first review on the incidence of ILD in SCLC patients treated with amrubicin.

Amrubicin has already been tested as a treatment for advanced or relapsed SCLC in phase II trials and shown promising activity in Japan and North America. Yana et al.[11] reported finding that 1 (3%) of 33 previously untreated SCLC patients developed interstitial pneumonia after treatment with amrubicin. Inoue et al.[17] reported the results of a randomized phase II trial comparing amrubicin with topotecan in previously treated SCLC patients, and 1 (3.3%) of the 30 patients who received amrubicin had pneumonitis. No amrubicin-associated ILD was reported in two phase II trials of relapsed SCLC patients recently performed in the United States.[9,18] Based on the results of previous clinical trials, the risk of ILD seems to be around 0 to 3% in SCLC patients treated with amrubicin.

In this study, we found a relatively high incidence of ILD (7% of the patients) in SCLC patients treated with amrubicin, and it was higher than in previous clinical trials. The reason for the high incidence is thought to be the possibility of different background between the patients in the present and previous studies. Pre-existing PF has been reported to be the most significant risk factor for the development of anticancer-agent-associated ILD.[19] The patients in our study were treated with amrubicin as clinical practice and the incidence of pre-existing PF was 12%. In previous clinical trials, patients with pre-existing PF were ineligible and the incidence of pre-existing PF was unknown. We attempted to identify the risk factors for the development of amrubicin-associated ILD, and the results showed that pre-existing PF was associated with a significantly higher risk of amrubicin-associated ILD. In our study, six of the seven patients who developed amrubicin-associated ILD received corticosteroid therapy and the ILD improved in four of them. We speculate that patients who developed ILD may benefit partly from corticosteroids.

A major limitation of this study was that none of the patients diagnosed with amrubicin-associated ILD had undergone a lung biopsies during bronchoscopy and no autopsies were performed that would have enabled histologic confirmation of ILD. Therefore, we cannot completely exclude the possibility that the patients had developed lymphangitic carcinomatosis or other diseases and not ILD. However, because the clinical course and radiographic findings of these patients were consistent with drug-induced ILD, we made the diagnosis of amrubicin-associated ILD. In our study, only two patients underwent bronchoalveolar lavage culture. The bronchoalveolar lavage culture obtained from two patients showed no evidence of infection. The exact pathogenetic mechanism of amrubicin-associated ILD is unclear, and further investigation is needed to confirm this finding and evaluate associations between amrubicin-associated ILD and genetic or ethnic factors.

In conclusion, our findings indicated that amrubicin may cause severe ILD and that pre-existing PF was associated with a higher rate of amrubicin-associated ILD. We recommend not administering amrubicin in the treatment of SCLC patients with pre-existing PF. Physicians should have a caution and appropriate management to prevent the development of ILD when using amrubicin to treat patients with pre-existing PF.

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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

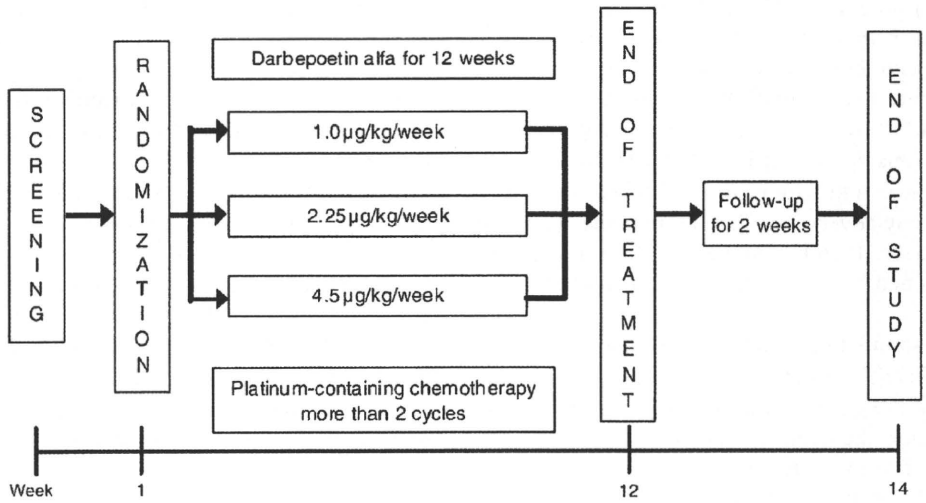


Figure 1. Study design and treatment schema. Darbepoetin alfa was administered once every week.

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.

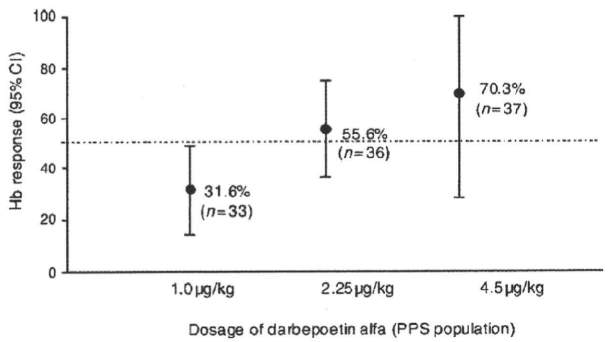
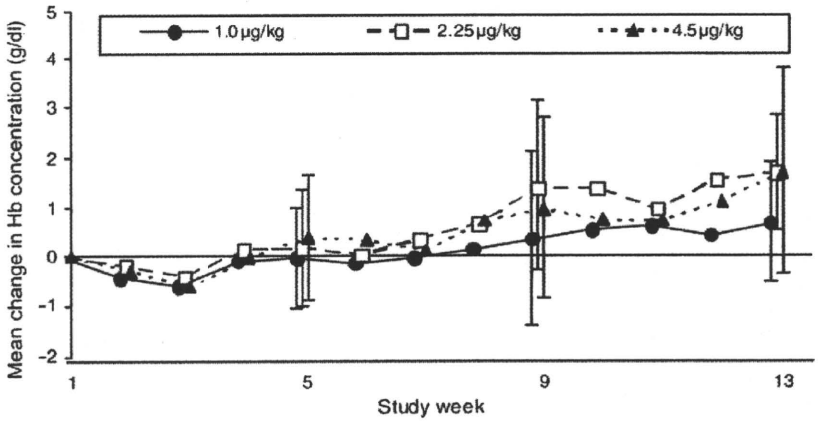


Figure 2. Kaplan–Meier rates of hemoglobin (Hb) response by treatment group [per-protocol set (PPS) population].

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
1.0 μg/kg (*n*=33)	−0.02 ± 1.05 (*n*=31)	0.37 ± 1.74 (*n*=27)	0.71 ± 1.24 (*n*=20)
2.25 μg/kg (*n*=36)	0.19 ± 1.19 (*n*=36)	1.41 ± 1.70 (*n*=27)	1.71 ± 1.13 (*n*=17)
4.5 μg/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.

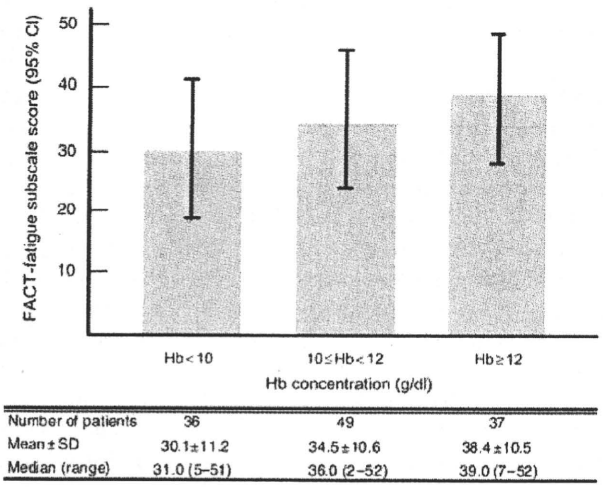


Figure 4. Correlation between FACT-fatigue subscale score and Hb concentration at the end of the treatment phase.

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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Individuals susceptible to lung adenocarcinoma defined by combined *HLA-DQA1* and *TERT* genotypes

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Adenocarcinoma (ADC) is the commonest histological type of lung cancer, and its weak association with smoking indicates the necessity to identify high-risk individuals for targeted screening and/or prevention. By a genome-wide association study (GWAS), we identified an association of polymorphisms in the 6p21.31 locus containing four human leukocyte antigen (HLA) class II genes with lung ADC risk. DQA1*03 of the *HLA-DQA1* gene was defined as a risk allele with odds ratio (OR) of 1.36 [95% confidence interval (CI) = 1.21–1.54, $P = 5.3 \times 10^{-7}$] by analysis of 1656 ADC cases and 1173 controls. DQA1*03 and the minor allele for a polymorphism, rs2736100, in *TERT*, another lung cancer susceptibility locus identified in recent GWASs on Europeans and Americans, were indicated to independently contribute to ADC risk with per allele OR of 1.43 (95% CI = 1.31–1.56, $P = 7.8 \times 10^{-16}$). Individuals homozygous both for the *DQA1*03* and minor *TERT* alleles were defined as high-risk individuals with an OR of 4.76 (95% CI = 2.53–9.47, $P = 4.2 \times 10^{-7}$). The present results indicated that individuals susceptible to lung ADC can be defined by combined genotypes of *HLA-DQA1* and *TERT*.

Introduction

Lung cancer is the leading cause of cancer-related deaths in the world. Adenocarcinoma (ADC) is the commonest histological type comprising ~40% of lung cancer cases among European, North American and Asian countries and is increasing in incidence (1). Development of ADC is more weakly associated with smoking than those of two other major histological types of cancer, squamous cell carcinoma (SQC) and small cell carcinoma (SCC) (1–3). Therefore, identification of high-risk individuals for lung ADC and targeted screening and/or prevention for these individuals will be a powerful way to reduce the number of lung cancer deaths in the world.

Recent genome-wide association studies (GWASs) with single-nucleotide polymorphism (SNP) array methodology have led to the identification of three loci associated with lung cancer risk, *CHRNA3/5* at chromosome 15q25.1, *TERT* and *CLPTM1L* at 5p15.33 and *BAT3-MSH5* at 6p21.33 (4–10). Among these loci, 5p15.33 was revealed as being a locus specifically associated with risk of ADC among major histological types of lung cancer (11). However, loci associated with lung ADC risk in Asians remain obscure. Here, we performed a GWAS on the risk of lung ADC in a Japanese population for 23 010 polymorphic microsatellite loci and identified *HLA-DQA1* at 6p21.31 as a novel locus associated with lung ADC risk. We further examined whether or not individuals susceptible to ADC can be defined by combined genotypes of *HLA-DQA1* and other lung cancer susceptibility loci described above.

Abbreviations: ADC, adenocarcinoma; CI, confidence interval; GWAS, genome-wide association study; HLA, human leukocyte antigen; HWE, Hardy–Weinberg equilibrium; LD, linkage disequilibrium; NCCH, National Cancer Center Hospitals; NNGH, National Nishi-Gunma Hospital; OR, odds ratio; PCR, polymerase chain reaction; SCC, small cell carcinoma; SNP, single-nucleotide polymorphism; SQC, squamous cell carcinoma.

Subjects and methods

Subjects

All the case and control subjects were Japanese and were enrolled in institutions in the Kanto area of Japan, an ~200 km diameter region containing Tokyo. This region is located in the middle of the main island in Japan, where homogeneity of the genetic background of individuals with several common diseases, including lung cancer, has been shown by a recent GWAS on population structure of Japanese (12).

The National Cancer Center Hospitals (NCCH) set consisted of 2343 lung cancer cases and 1173 controls (Table I). The cases were 1656 ADC, 390 SQC and 297 SCC cases. All ADC, SQC and SCC cases were enrolled in the NCCH from 1999 to 2008. All ADC, SQC and SCC cases, from whom informed consent as well as blood samples were obtained, were consecutively included in this study without any particular exclusion criteria. The participation rate was nearly 80%. All the cases were diagnosed by cytological and/or histological examinations according to WHO classification. The controls were 328 inpatients/outpatients of the NCCH, and 645 and 200 volunteers enrolled in Keio and Tokai Universities, respectively. The control NCCH subjects were selected with a criterion of no history of cancer from 1999 to 2007, whereas the 645 volunteers were the individuals with no known malignancies who offered blood on the occasion of a health check examination at Keio University in 2002 and 2003 (13). The 200 volunteers in Tokai University were healthy individuals enrolled from 2001 to 2003 as control subjects in a previous case–control study (14).

The National Nishi-Gunma Hospital (NNGH) sets were 84 ADC and 52 SQC cases and 145 controls who were enrolled in the NNGH from 1999 to 2003 (Table I). All ADC and SQC cases, from whom informed consent as well as blood samples were obtained, were consecutively included in this study without any particular exclusion criteria. The participation rate was nearly 80%. Controls were randomly selected from inpatients and outpatients with no history of cancer. Most of the controls had diseases other than lung cancer such as chronic obstructive pulmonary disease, pulmonary tuberculosis, bronchitis/pneumonia. Their characteristics were described in our previous studies (14–18).

Smoking histories of the subjects were obtained via interview using a questionnaire. Smokers were defined as those who had smoked at least one cigarette per day for 12 months or longer at any time in their life, whereas non-smokers were defined as those who had not. There were no individuals who had smoked less than one cigarette per day and/or for <12 months. Smoking exposure was represented by pack years, which was defined as the number of cigarette packs smoked daily multiplied by years of smoking.

Genomic DNA was extracted from whole-blood cells using a Blood Maxi Kit (Qiagen, Tokyo, Japan) according to the supplier's instructions. Genomic DNAs for 645 and 200 volunteers enrolled in Keio and Tokai Universities, respectively, were extracted from Epstein-Barr virus-transformed B-lymphocytes derived from the collected whole-blood cells (14,16).

Genome-wide association studies

The method of GWAS on microsatellite loci was described previously (14). Equal amounts of DNAs from 200 lung ADC cases and from 200 controls enrolled in Tokai University were mixed for the first set of case and control DNA pools, respectively. The second set of DNA pools was also prepared from another 200 ADC cases and 200 controls enrolled in Keio University. Fifty nanograms of pooled DNA was amplified by 40 cycles of polymerase chain reaction (PCR) in 96-well plates using a pair of PCR primers designed for amplifying fragments that include polymorphic microsatellite sequences. Allele frequencies in pooled DNA were estimated from the height of peaks:

Table I. Characteristics of study subjects

Category	Group	No	Age (mean ± SD)	Sex (% male)	Smoking habit (%)		Pack years of smokers (mean ± SD)
					Non-smoker	Smoker	
NCCH set	Case	2 3443	59 ± 9	65	34	66	51 ± 30
	ADC	1656	58 ± 9	56	46	54	43 ± 27
	SQC	390	62 ± 7	91	3	97	61 ± 29
	SCC	52	70 ± 9	90	6	94	62 ± 32
NNGH set	Case	136	68 ± 10	74	27	73	55 ± 29
	ADC	84	67 ± 10	64	39	61	48 ± 25
	SQC	52	70 ± 9	90	6	94	62 ± 32
	Control	145	64 ± 14	71	33	67	45 ± 35

the frequency of each allele was determined by dividing the height of each allele by the summed height of all alleles. The significance for difference in allelic distribution was evaluated by Fisher's Exact test, with the use of $2m$ (where m is the number of alleles).

The first set of case and control DNA pools was examined for differences in allelic distribution for 23 010 microsatellite markers, and the distribution for 1328 (5.8%) markers were judged as being significantly different by the criteria of $P < 0.05$ (first stage of GWAS in Table II). The inflation factor calculated by dividing the mean of the lower 90% of −log10 (P) values by the mean of the lower 90% of the expected values (19) for this screening was 0.639, indicating a deflation in the statistical tests (supplementary Table I is available at *Carcinogenesis* Online). However, in this screening, deduction of allele frequencies was affected by an inevitable experimental bias of the pooled DNA typing, i.e. 'shadow bands' in electrophoregrams due to slippages in the PCR reaction particularly for microsatellite markers containing repeat units of 2 bp, as reported previously (20). In fact, inflation factors for microsatellite markers containing repeat units of 3–6 bp were 0.919–1.022 (0.955 in total), i.e. deviations were within ±10% as have been observed in previous GWASs in which adequacy of the case–control matching (i.e. lack of a significant hidden population substructure) was indicated (4,8,9,19). Thus, the adequacy of the case–control matching was also indicated in the present screening with microsatellite markers containing repeat units of 3–6 bp. On the other hand, inflation factor for microsatellite markers containing repeat units of 2 bp was 0.520; therefore, the deflation described above was considered to be caused by misestimation in allele frequency in the screening with microsatellite markers containing repeat units of 2 bp. Therefore, among 1328 markers selected in the first stage of GWAS, 431 microsatellite markers with 3–6 bp units were further subjected to the second stage of GWAS.

The second set of DNA pools was examined for differences in allelic distribution for 431 microsatellite markers containing repeat units of 3–6 bp that passed the criteria of $P < 0.05$ in the first stage of GWAS. The distribution for 17 (3.9%) markers were significantly different by the criteria of $P < 0.05$ (second stage of GWAS in Table II). The inflation factor for the second stage screening was 1.010, indicating the adequacy of the case–control matching as well as the lack of differential genotyping of cases and controls (supplementary Table I is available at *Carcinogenesis* Online).

Next, individual typing was done on the 17 markers, which passed the criteria for the third stage, for 576 cases and 576 controls, consisting of 384 cases and 384 controls used in the first and second pooled DNA screening and an additional 192 cases and 192 controls from NCCH (third stage of GWAS in Table II). These 384 cases and 384 controls were consisted of two sets of 192 subjects, which were chosen from two sets of 200 subjects examined in the first and second GWAS stages, respectively, by simple random sampling. These analyses led to the identification of six loci, including D6S0067i, with differences in allelic distributions between the cases and controls with P-values <0.05 by the χ^2 test. The D6S0067i locus showed a P-value of 2.4×10^{-7}, whereas the other five showed P-values of 0.012–0.0011. A level of $P < 2.2 \times 10^{-6}$ was judged as significant by applying Bonferroni correction for multiple test (i.e. $P < 2.2 \times 10^{-6} = 0.05/23\,010$).

Genotyping of SNPs in the 6p21.31 locus

Five hundred and twenty-five cases and 525 controls, which were, respectively, chosen from the 576 cases and 576 controls examined in the third GWAS stage by simple random sampling, were subjected to SNP analysis. Twenty-nine SNPs were selected from the 450 kb region surrounding the D6S0067i locus based on the following criteria: (i) SNPs whose minor allele frequency in the Japanese population was >0.01 in the database of single nucleotide polymorphism (http://www.ncbi.nlm.nih.gov/projects/SNP/) and (ii) SNPs for which pre-designed or validated TaqMan probes were available from Applied Biosystems (Foster City, CA). Three other SNPs, rs1794282, rs3129763 and rs2187668, which showed significant associations with lung cancer risk in Europeans (8), were also examined. Thirty-two SNPs, in total, were genotyped using the TaqMan method according to the protocol for the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems).

Twenty-four SNPs located in exon 2 of the *DRB1*, *DQA1* and *DQB1* genes, which enable allele discrimination for *DRB1*, *DQA1* and *DQB1* at high-, low- and high-resolution levels, respectively, were genotyped by sequence-based typing methods recommended by the International Histocompatibility Working Group (http://www.ihwg.org/). In brief, exon 2 of the *DRB1* and *DQB1* genes was amplified by PCR with mixtures of allele-specific primers, whereas exon 2 of the *DQA1* gene was amplified with a set of common primers, and PCR products were directly sequenced using the ABI3700 DNA analyzer (Applied Biosystems). The location and alleles of the SNPs are described according to the database of major histocompatibility complex (http://www.ncbi.nlm.nih.gov/gv/mhc/). Based on the genotypes of 24 SNPs, alleles for *DRB1*, *DQA1*, *DQB1* and *DR-DQ* were determined, and alleles with frequencies >0.02 were subjected to the association study.

Statistical analyses

A Hardy–Weinberg equilibrium (HWE) test was performed using the SNPAlyze version 3 software (DYNACOM Co., Ltd, Chiba, Japan), and SNPs with a P-value for deviation >0.01 were considered to be in HWE as described (7). Calculation of the D' and R^2 values between SNPs and allele/haplotype estimation was performed by the expectation-maximization algorithm using the SNPAlyze version 3 software. The D6S0067i locus showed 19 polymorphic alleles in the same sets of cases and controls, and among them, alleles of 367 and 404 bp in sizes were significantly associated with an elevated risk for lung ADC [odds ratio (OR) = 1.60, $P = 9.9 \times 10^{-3}$ and OR = 1.42, 4.9×10^{-5}, respectively]. Therefore, for the calculation of the D' and R^2 values, genotypes for the D6S0067i polymorphism was expressed by presence or absence of these two alleles (supplementary Table II is available at *Carcinogenesis* Online).

Associations of SNPs/alleles with risks were digitized as crude ORs and ORs adjusted for gender, age and smoking with 95% confidence intervals (CIs) by unconditional logistic regression analysis using the JMP version 6.0 software (SAS Institute, Cary, NC). Variables used for adjustment in each test are described in the footnotes to supplementary Tables are available at *Carcinogenesis* Online. A level of $P < 0.05$ for an OR was judged as significant and that of $0.05 \leq P < 0.1$ was judged as marginal in association studies other than GWAS.

Genotyping of SNPs in the lung cancer susceptibility loci identified by previous GWASs

SNPs in the lung cancer susceptibility loci identified by previous GWASs were genotyped by the TaqMan method. Two intronic SNPs, rs2736100 and rs401681, in the *TERT* and *CLPTM1L* genes (4,21) were genotyped for the 5p15.33 locus against 2343 cases and 1173 controls (subjects of the NCCH set in Table I). Association results of the rs1051730 SNP in the *CHRNA3* gene for the 15q25.1 locus in a subset of the present study population were reported previously (22). Therefore, in this study, 1094 ADC cases and 236 controls that had not been examined in our previous study were genotyped (22). Eight SNPs in the 6p21.33 locus, consisting of rs3117582 and seven SNPs in linkage disequilibrium (LD) with this SNP in Europeans ($D' = 1$ in the HapMap database), were genotyped for 525 ADC cases and 525 controls used for the mapping stage (Table II).

Results and discussion

We performed a GWAS on the risk of lung ADC in a Japanese population for 23 010 polymorphic microsatellite loci. After a three-stage