Cancer Center, Japan. The majority of eligible participants in this study were residents of Chiba and East Tokyo, and all were of Japanese nationality. Personal and clinical data from patients who participated in the Lung Cancer Database Project at the National Cancer Center Hospital East (NCCH-E) and the National Cancer Center Research Institute East were used in the current study. The database includes information on demographic factors, physical symptoms, psychological factors, and lifestyle factors (diet, smoking, etc) obtained from self-reported questionnaires and medical information from the patients' medical charts and blood, DNA, and urine specimens. All patients who were enrolled in the current study had primary lung cancer that was newly diagnosed with histologic or cytologic confirmation at the Thoracic Oncology Division of the NCCH-E, Japan, from September 1997 to June 2000. All patients provided their written informed consent prior to enrolment in this project. Unmatched controls were newly recruited individuals from the population with no history of cancer or other tumors who visited the Thoracic Oncology Division of NCCH-E from March 2002 to May 2003 and were confirmed as cancer-free by appropriate examinations (chest computed tomography scans, bronchofibroscopy, video-assisted thoracoscopic biopsy, etc). The major reasons for visiting the hospital were suspicions of lung cancer on chest x-ray or sputum cytology at their annual medical check-up or referral from other hospitals. Epidemiologic data were collected by personal interview. All individuals in the control group completed the same standardized questionnaire that was completed by the Lung Cancer Database Project participants, including detailed demographic information, history of cancer, occupational and residential history, and detailed information regarding alcohol and tobacco consumption. All participants provided their written consent.

Sample Collection and DNA Extraction

Four milliliters of peripheral venous blood were collected into heparinized tubes. Genomic DNA was purified from peripheral blood lymphocytes using a DNA isolation kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions and was stored at 80°C.

Polymorphism Analysis

ADH_3 and $ALDH_2$ genotyping was performed by using the polymerase chain reaction-restriction fragment-length polymorphism (PCR-RFLP) method. To prevent the amplification of closely related ADH_1 and ADH_2 genes, samples initially were digested with the *Nla*III restriction enzyme (TOYOBO, Osaka, Japan). A 145-base pair (bp) section of the ADH_3 gene was amplified by PCR using 200 ng of predigested genomic DNA with primers (sense, 5′-GCTTTAAGAGT AAATATTCTGTCCCC-3′; antisense, 5′-AATCTACCTCT zTTCCGAAGC-3′). The PCR product obtained in this manner then was digested directly with restriction enzyme *Ssp*I (TOYOBO). After polyacrylamide gel electrophoresis, ADH_3 alleles were visualized by ethidium bromide and were photographed under ultraviolet light. The ADH_3^1 allele produced fragments of 67 bp, 63 bp, and 15 bp; and the ADH_3^2 allele produced fragments of 131 bp and 15 bp.

A 134-bp fragment of the $ALDH_2$ gene was amplified by PCR according to a slightly modified method of Harada et al.[12] One hundred fifty nanograms of genomic DNA were mixed with 5 pmol of each primer (sense, 5′-CAAATTACAGGGTCAAGGGCT-3′; antisense: 5′-CCACACTCACAGTTTTCTCTT-3′) in a total volume of 50 μL that contained 50 μM deoxynucleotide triphosphate, 1.5 mM $MgCl_2$, and 1 U Taq DNA polymerase; Takara Shuzo, Kyoto, Japan). Thirty-five cycles (denaturation at 94°C for 15 seconds, annealing at 58°C for 1 minute and 30 seconds, and polymerization at 72°C for 30 seconds) were performed using a GeneAmp PCR system 9600 (PerkinElmer, Oak Book, Ill). After purification, each PCR product was digested with *Mbo*II (TOYOBO), electrophoresed on a 20% polyacrylamide gel, stained with ethidium bromide, and photographed. The $ALDH_2^1$ allele produced fragments of 125 bp and 9 bp, and the $ALDH_2^2$ allele produced fragments of 134 bp.

The *CYP2E1* genotypes ascribed to the *Rsa*I site in the 5′-flanking region also were identified as RFLPs by PCR. Genomic DNA (100 ng) was subjected to PCR with each primer (sense, 5′-ATCCACAAGTG ATTTGGCTG-3′; antisense, 5′-CTTCATACAGACCCTC TTCC-3′). PCR was performed for 35 cycles under the following conditions: 1 minute at 95°C for denaturation, 1 minute at 55°C for primer annealing, and 1 minute at 72°C for primer extension. The 412-bp fragment was digested with *Rsa*I (TOYOBO). The products that were yielded were fragments with 360 bp and 50 bp for c1/c1; 360 bp, 50 bp, and 410 bp for c1/c2; and 410 bp for c2/c2 detected by electrophoretic analysis in 5% polyacrylamide gels.

Statistical Analysis

Patient characteristic (see Table 1) were compared with characteristic in the control group by using the Student *t* test or the chi-square test. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were obtained by unconditional logistic regression analy-

TABLE 1
Baseline Characteristics of Lung Cancer Cases and Controls

Characteristic	No. (%) Cases (n = 505)	Controls (n = 256)	P for difference
Mean age SD, y	64.8 8.3	63.5 10.2	.06*
Sex			
Men	360 (71.3)	126 (49.2)	
Women	145 (28.7)	130 (50.8)	<.0001†
Smoking status			
Never	140 (27.7)	129 (50.4)	
Past	97 (19.2)	64 (25)	
Current	268 (53.1)	63 (24.6)	<.0001†
Smoking amounts, pack-years			
Past			
<27	35 (36.1)	32 (50)	
27	62 (63.9)	32 (50)	.08†
Current			
<40	71 (26.5)	30 (47.6)	
40	197 (73.5)	33 (52.4)	.001†
Alcohol drinking habit, times/wk			
Seldom	116 (23)	118 (46.1)	
2	43 (8.5)	42 (16.4)	
3–6	96 (19)	22 (8.6)	
Daily	250 (49.5)	74 (28.9)	.0001†
Alcohol amounts, g/day			
0	120 (23.8)	119 (46.5)	
<31.6	154 (30.5)	65 (25.4)	
31.6	231 (45.7)	72 (28.1)	.0001†

SD indicates standard deviation.
* Determined using the Student *t* test.
† Determined using the chi-square test.

sis. In our regression models, we adjusted ORs for potential confounding variables, including age, sex, smoking status (never, past, current) or amounts smoked (pack-years) and alcohol consumed (none, light, heavy). Because differences in the amount of alcohol consumed (ethanol, in gram per day) were very large, we divided those who drank into 3 categories: nondrinkers, light drinkers (31.6 g per day), and heavy drinkers (>31.6 g per day). The amount of tobacco smoke exposure was calculated as pack-years (usual amount per day/20 × overall duration [years] of use). Participants were considered current smokers if they smoked up to 1 year before the date of diagnosis in the case group or up to the date of the interview for the control group. The average amount of daily ethanol intake was calculated in grams. Calculation of this value was based on an average ethanol content of 4-volume% in beer, 15-volume% in Japanese sake (rice wine), 25-volume% in Japanese spirits (syochu), 12-volume% in wine, and 40-volume% in spirits. Drinking frequency was assessed as 5 categories: less than once a week, 1 or 2 days a week, 3 or 4 days a week, 5 or 6 days a week, and daily. Categorical variables were compared with the chi-square test. ORs and 95% CIs were calculated by using logistic regression analysis adjusting for age, sex, smoking, and drinking. The Mantel extension test was used to evaluate linear trends across categories of alcohol consumption that were divided into 4 categories by quartiles for control. Resulting P values <.05 (2-tailed) were considered statistically significant. All statistical analyses were performed using the SAS statistical software package (SAS Institute Inc., Cary, NC).

RESULTS

In total 510 patients with lung cancer (cases) and 260 healthy controls participated in this study. Because of the lack of DNA samples or information on lifestyle, 9 participants were eliminated. Table 1 summarizes the baseline characteristics of selected variables for the lung cancer cases and controls. Age distribution was similar in both groups (mean, 64.8 years and 63.5 years, respectively); however, the cases were more likely than the controls to be men (71.3% and 49.2%), to be current smokers (53.1% and 24.6%) and heavy smokers, and to consume more alcohol. The proportions of those who consumed >31.6 g per day of ethanol and of daily drinkers were 45.7% and 49.5%, respectively, for cases and 28.1% and 28.9%, respectively, for controls. The median values from the control group for the 2 smoking amount categories were used as the cut-off values. The 3 categories of alcohol consumption were lifetime nondrinker, below the median intake, and above the median intake.

The frequency of *ADH*$_3$, *ALDH*$_2$, and *CYP2E1* genotypes and ORs among lung cancer cases and controls are presented in Table 2. After adjustment for age, sex, smoking amount, and amount of alcohol consumed, the ORs for individuals with the *ADH*$_3$, *ALDH*$_2$, and *CYP2E1* variant alleles, compared with individuals who were homozygous for the common allele, were 1.01, 0.73, and 0.93, respectively. Thus, there were no significant differences in the frequencies of any genotypes between cases and controls. The OR for carriers of the *CYP2E1* c2/c2 genotype, compared with the c1/c1 genotype, was 4.66 (P <.05). This genotype is not in Hardy-Weinberg equilibrium in the control population, the observed frequency is most likely an underestimate, and the finding of an association with lung cancer is most likely a false-positive result.

Without taking these genotypes into consideration, a direct association between alcohol consumption and lung cancer occurrence can be derived, as

TABLE 2
The Frequency of Alcohol Dehydrogenase 3, Aldehyde Dehydrogenase 2, and Cytochrome P450 2E1 Genotypes and Odds Ratios Among Lung Cancer Cases and Controls

Genotype	No. (%)		OR	
	Cases (n = 505)	Controls (n = 256)	Crude	Adjusted*
ADH_3				
C/C	459 (90.9)	227 (88.7)	1	1
C/V	44 (8.7)	29 (11.3)	0.75 (0.46–1.23)	0.71 (0.40–1.16)
V/V	2 (0.4)	0 (0)	—	—
C/V and V/V	46 (9.1)	29 (11.3)	0.78 (0.48–1.28)	0.74 (0.44–1.24)
$ALDH_2$				
C/C	319 (63.2)	134 (52.3)	1	1
C/V	168 (33.3)	108 (42.2)	0.65 (0.48–0.90)†	0.73 (0.52–1.03)
V/V	18 (3.6)	14 (5.5)	0.54 (0.26–1.12)	0.75 (0.35–1.59)
C/V and V/V	186 (36.8)	122 (47.7)	0.64 (0.47–0.87)†	0.73 (0.53–1.02)
CYP2E1				
C/C	300 (59.4)	147 (57.4)	1	1
C/V	175 (34.7)	106 (41.4)	0.81 (0.59–1.11)	0.83 (0.60–1.15)
V/V	30 (5.9)	3 (1.2)	4.90 (1.47–16.32)†	4.66 (1.36–16.0)†
C/V and V/V	205 (40.6)	109 (42.6)	0.92 (0.68–1.25)	0.93 (0.68–1.29)

OR indicates odds ratios; ADH_3, alcohol dehydrogenase 3; C, common allele; V, variant allele; $ALDH_2$, aldehyde dehydrogenase 2; *CYP2E1*, cytochrome P450 2E1.

* ORs were adjusted for age, sex, smoking amounts (pack-years), and alcohol amounts (ethanol: mg per day).

† $P < .05$.

shown in Table 3. Drinking was classified as none, light (31.6 g per day) or heavy (>31.6 g per day). When adjusted for age, sex, and smoking amounts, drinking imposed a significantly greater risk of lung cancer occurrence. The ORs for the light drinkers and heavy drinkers, compared with nondrinkers, were 1.76 and 1.95, respectively (P for trend = .012). Thus, the risk of lung cancer increases as the amount alcohol consumed increases.

ORs for developing lung cancer in association with the ADH_3, $ALDH_2$, and *CYP2E1* genotypes also are presented in Table 3. Similar to what was observed in all participants taken together, an increased risk for developing lung cancer also was observed among individuals who were homozygous for the common allele ADH_3^{1-1}. However, because there were too few ADH_3 variant allele carriers to analyze any association between alcohol consumption and lung cancer risk for this allele, it was inappropriate to compare the ADH_3^2 and ADH_3^{1-1} genotypes.

The adjusted OR for the $ALDH_2^{1-1}$ group was 0.75 (95% CI, 0.39–1.42) in light drinkers and 0.46 (95% CI, 0.20–0.99) in heavy drinkers. In contrast, individuals with the $ALDH_2^2$ allele had a significantly greater risk of lung cancer; light drinkers had a 3.6-fold increased risk, and heavy drinkers had a 6.2-fold increased risk compared with nondrinkers (P for trend < .0001). These results indicate that, in individuals with the $ALDH_2$ variant allele, continuous alcohol consumption is a strong risk factor for lung cancer.

The OR for the *CYP2E1* c1/c1 genotype was 1.81 (95% CI, 0.97–3.38) for light drinkers and 1.67 (95% CI, 0.86–3.21) for heavy drinkers. For individuals with the *CYP2E1* c2 allele, the OR was 1.74 (95% CI, 0.91–3.35) for light drinkers and 2.56 (95% CI, 1.16–5.65) for heavy drinkers (P for trend = .005). These results may indicate that individuals with the *CYP2E1* variant allele are in a high-risk group for lung cancer in heavy drinkers.

It must be emphasized that, because of differences in distribution according to sex between cases and controls, we analyzed relative risks only in men (Table 4). For baseline characteristics among men, higher consumption of alcohol and more smoking were observed, as expected. Regarding associations between alcohol consumption and lung cancer risk, drinking was associated with an increased risk of developing lung cancer in all participants. The adjusted OR for the light and drinkers, compared with nondrinkers, was 6.54 (95% CI, 3.13–13.7) and 6.58 (95% CI, 3.28–13.2), respectively. However, in individuals with active $ALDH_2^{1-1}$ genotypes, there was no association between alcohol consumption and lung cancer risk. In individuals with the inactive $ALDH_2^2$ alleles, the risk for lung cancer was 6.8-fold (95% CI, 2.72–17.1) for light drinkers and 9.3-fold (95% CI, 3.72–23.4) for heavy drinkers compared with nondrinkers (P for trend <.0001). The risk in men who were heavy drinkers was much greater compared with women and those who carried the active $ALDH_2^{1-1}$ genotype.

In individuals with the c2 allele, the risk of lung cancer for light drinkers (OR, 8.31; 95% CI, 2.67–25.9) and for heavy drinkers (OR, 9.93; 95% CI, 3.39–29.1) was increased compared with individuals who were homozygous for the *CYP2E1* c1 allele and compared with the risks in all men. However, it should be noted that, because of the low incidence of homozygosity for variant allele in the control group, statistical power was limited in this instance. Similar assessments also were made in women, but no significant associations between any genotype and lung cancer risk were observed (data not shown).

Table 5 shows the distribution of the ADH_3, $ALDH_2$, and *CYP2E1* genotypes according to tumor histology. The frequency of the ADH_3^2 allele for all histologic types was similar to the frequency observed in controls. The frequency of the $ALDH_2^2$ allele for squamous cell carcinomas, small cell carci-

TABLE 3
Odds Ratios of Developing Lung Cancer for Alcohol Dehydrogenase 3, Aldehyde Dehydrogenase 2, and Cytochrome P450 2E1 Genotypes Stratified by Drinking Amounts

			Drinkers						
	Nondrinkers		31.6 g/Day			>31.6 g/Day			
Genotype	No.*	Reference	No.*	OR (95% CI)†	P	No.*	OR (95% CI)†	P	P for trend‡
All	120/119	1	154/65	1.76 (1.12–2.75)	.014	231/72	1.95 (1.19–3.21)	.0085	.012
ADH_3									
C/C	112/105	1	141/60	1.59 (0.99–2.55)	.054	206/62	1.88 (1.10–3.21)	.02	.025
C/V and V/V	8/14	1	13/5	4.31 (0.912–20.38)	.065	25/10	3.28 (0.742–14.55)	.12	.17
$ALDH_2$									
C/C	57/41	1	99/39	0.75 (0.39–1.42)	.37	163/54	0.46 (0.2– 0.99)	.049	.03
C/V and V/V	63/78	1	55/26	3.63 (1.76–7.46)	.0005	68/18	6.15 (2.77–13.65)	<.0001	<.0001
CYP2E1									
C/C	72/61	1	95/36	1.81 (0.97–3.38)	.061	133/50	1.67 (0.86–3.21)	.13	.31
C/V and V/V	48/58	1	59/29	1.74 (0.91–3.35)	.097	98/22	2.56 (1.16–5.65)	.02	.005

OR indicates odds ratio; 95% CI, 95% confidence interval; *ADH_3*, alcohol dehydrogenase 3; C, common allele; V, variant allele; *$ALDH_2$*, aldehyde dehydrogenase 2; *CYP2E1*, cytochrome P450 2E1.
* The number of cases/number of controls.
† ORs were adjusted for age, sex, and smoking amount (pack-years).
‡ The Mantel extension test.

TABLE 4
Odds Ratios of Developing Lung Cancer for Alcohol Dehydrogenase 3, Aldehyde Dehydrogenase 2, and Cytochrome P450 2E1 Genotypes Stratified by Drinking Amounts Among Men

			Drinkers						
	Nondrinkers		31.6 g/Day			>31.6 g/Day			
Genotype	No.*	Reference	No.*	OR (95% CI)†	P	No.*	OR (95% CI)†	P	P for Trend‡
All	17/31	1	120/36	6.54 (3.13–13.65)	<.0001	223/59	6.58 (3.28–13.22)	.0001	<.0001
ADH_3									
C/C	15/27	1	110/34	6.14 (2.83–13.29)	<.0001	201/49	7.27 (3.44–15.36)	.0001	<.0001
C/V and V/V	2/4	1	10/2	23.31(1.41–286.0)	.028	22/10	5.43 (0.63–47.09)	.12	.47
$ALDH_2$									
C/C	5/2	1	72/16	1.47 (0.25–8.67)	.67	158/42	1.10 (0.20–6.23)	.91	.29
C/V and V/V	12/29	1	48/20	6.82 (2.72–17.13)	<.0001	65/17	9.33 (3.72–23.39)	.0001	<.0001
CYP2E1									
C/C	10/14	1	77/24	5.22 (1.95–13.94)	.0003	125/42	4.71 (1.85–12.05)	.0012	.08
C/V and V/V	7/17	1	43/12	8.31 (2.67–25.89)	.0001	98/17	9.93 (3.39–29.09)	.0001	<.0001

OR indicates odds ratio; 95% CI, 95% confidence interval; *ADH_3*, alcohol dehydrogenase 3; C, common allele; V, variant allele; *$ALDH_2$*, aldehyde dehydrogenase 2; *CYP2E1*, cytochrome P450 2E1.
* Values shown represent the number of cases/number of controls.
† OR were adjusted for age, sex, and smoking history (pack-years).
‡ Mantel extension test.

nomas, and other histologic types was similar to that observed in controls. However, the *$ALDH_2^2$* allele was significantly less common in patients with adenocarcinomas than in controls (36.1% vs 47.7%; $P = .018$). In contrast, the *CYP2E1* c2/c2 genotype was more common in patients with adenocarcinomas (5.8%) and small cell carcinomas (9.8%) than in controls (1.2%).

In this study, we observed that alcohol consumption was an independent risk factor for lung cancer after adjusting for the influence of smoking (P for trend = .012). Although we assumed that individuals who had the *ADH_3^{1-1}* genotype were at greater risk for lung cancer compared with individuals who had the *ADH_3^2* allele, there was no evidence of an association between lung cancer and the *ADH_3* genotype

TABLE 5
Distribution of Alcohol Dehydrogenase 3, Aldehyde Dehydrogenase 2, and Cytochrome P450 2E1 Genotype According to Histologic Findings

	No. (%)				
		Histologic type			
Genotype	Control group (n = 256)	Adenocarcinoma (n = 330)	Squamous cell (n = 100)	Small cell (n = 51)	Other (n = 24)
ADH_3					
C/C	227 (88.3)	297 (90)	91 (91)	48 (94.1)	23 (95.8)
C/V	29 (11.7)	31 (9.4)	9 (9)	3 (5.9)	1 (4.2)
V/V	0 (0)	2 (0.6)	0 (0)	0 (0)	0 (0)
P for difference*		.35	.52	.25	.28
$ALDH_2$					
C/C	134 (52.3)	211 (63.9)	54 (54)	36 (70.6)	18 (75)
C/V	108 (42.2)	104 (31.5)	45 (45)	13 (25.5)	6 (25)
V/V	14 (5.5)	15 (4.6)	1 (1)	2 (3.9)	0 (0)
P for difference*		.018	.17	.056	.083
CYP2E1					
C/C	147 (57.4)	197 (59.7)	59 (59)	31 (60.8)	13 (54.2)
C/V	106 (41.4)	114 (34.6)	37 (37)	15 (29.4)	9 (37.5)
V/V	3 (1.2)	19 (5.8)	4 (4)	5 (9.8)	2 (8.3)
P for difference*		.0067	.19	.001	.04

ADH_3 indicates alcohol dehydrogenase 3; C, common allele; V, variant allele; $ALDH_2$, aldehyde dehydrogenase 2; *CYP2E1*C, cytochrome P450 2E1.
* Chi-square test for comparison with controls.

in any analysis. Because the enzyme activity of $ALDH_2^2$ is extremely low, acetaldehyde accumulates after alcohol intake. We could not demonstrate any association of $ALDH_2$ genotypes with the risk of lung cancer after adjusting for smoking and the amount of alcohol consumed. However, we observed that individuals who had the $ALDH_2^2$ allele were at a significantly greater risk of lung cancer because of alcohol consumption, although there was a significant trend for lower levels of alcohol consumption in individuals who had the $ALDH_2^{1-1}$ genotype (P for trend = .03). We hypothesized that not only the differences in blood acetaldehyde concentrations but also the differences in enzyme activity on tobacco-specific carcinogens contribute to carcinogenesis. However, we produced no evidence that lung cancer risk is related to possession of the *CYP2E1* c2/c2 genotype or that the *CYP2E1* genotype modifies lung cancer susceptibility related to alcohol intake.

DISCUSSION

The control population for this study was recruited from the visitors to the NCCH-E. The majority of patients had false-positive chest x-rays at their annual check-up and had normal chest computed tomography scans, and they were not suffering from any respiratory illness. Furthermore, their family medical histories were similar to those expected in the ordinary Japanese population, although the number of current smokers among both men (42.9%) and women (6.9%) may have been somewhat lower than the average (46.8% and 11.1%, respectively, for 2003 according to the Announcement of the Ministry of Health, Labor, and Welfare). For these reasons, we believe that our control group was not at greater risk of cancer occurrence compared with the regular Japanese population. Moreover, it was not necessary to take into account any biases stemming from the selective inclusion only of consenting participants, because the great majority of both patients and controls agreed to participate in the study.

The data from the control group showed that individuals who had the $ALDH_2$ wild-type genotype consumed more alcohol than individuals who had the variant genotype. This may suggest that genetic polymorphisms of alcohol-metabolizing enzymes influence drinking habits, because consumption may be limited by the unpleasant reactions caused by the accumulation of acetaldehyde in individuals with $ALDH_2$ variant genotypes. Nonetheless, habitual drinking can increase consumption because of increased microsomal acetaldehyde-oxidizing system activation, further promoting the oxidation of acetaldehyde. The association between drinking habit and ADH_3 and *CYP2E1* genotypes remains uncertain.

Regarding correlations between smoking and drinking habits, the coexistence of smoking and

drinking increased the risk of lung cancer compared with nondrinkers who never smoked, particularly the OR for heavy smokers (>37 pack-years) and drinkers, which was 8.4 (95% CI, 2.3–30.2; $P = .0012$) in the light drinkers and 7.0 (95% CI, 2.1–23.4) in the heavy drinkers (data not shown).

The involvement of alcohol in lung cancer etiology has been controversial, although many epidemiologic studies have suggested positive associations between different parameters of alcohol consumption and lung cancer risk. In the current study, we have demonstrated that drinking is a strong risk factor for lung cancer that is dose-dependent and is stronger in men than in women. This same tendency was observed even in the genotype analysis, but none of the results indicated a significant association between lung cancer and drinking in women. Furthermore, no associations were observed between peripheral lung adenocarcinoma, drinking, and genotypes of alcohol metabolite-related enzymes in women.

The question of ethnicity in the distribution of the polymorphisms of these alcohol metabolite-related enzyme genes always must be considered. The ADH_3^2 allele is present in almost 60% of whites but is far more rare (5–10%) in Japanese. In contrast, the $ALDH_2^2$ allele is found only in Asians. The *CYP2E1* c2 allele is present in 35% to 56% of Japanese and Chinese, and in 2% to 5% of whites. In the current study, the frequency of variant alleles of each polymorphism was 9.9% for ADH_3, 40.5% for $ALDH_2$, and 41.3% for *CYP2E1*. This is consistent with previous studies in Japanese and other Asians.

We observed that the risk for lung cancer was increased significantly by alcohol consumption in a dose-dependent fashion in individuals with the $ALDH_2^2$ alleles. Previously, some Japanese studies also showed a strong genetic and environmental interaction between $ALDH_2^2$ and alcohol intake for the risk of developing esophageal and upper aerodigestive tract cancer.[18–21] In contrast, for individuals with the $ALDH_2^{1-1}$ genotype, there was an inverse association between alcohol consumption and the risk of lung cancer. These results suggest that increased acetaldehyde concentrations from a reduction in acetaldehyde oxidation caused by the presence of the $ALDH_2^2$ allele contribute to the development of lung cancer. Significantly higher blood acetaldehyde concentrations after drinking in individuals with the ADH_3^1 or $ALDH_2^2$ allele have been reported compared with the concentrations in individuals who lacked these alleles,[11,29] and it has been demonstrated that breath acetaldehyde levels are proportional to blood acetaldehyde levels. Indeed, Muto et al.[30] and Jones[31] observed significantly higher acetaldehyde levels in the breath from individuals with the $ALDH_2^2$ allele than in those without that allele. Therefore, exposure to higher concentrations of acetaldehyde in the lower respiratory tract may play a critical role in alcohol-related carcinogenesis. Regarding the influence of smoking, when adjusted for age, sex, and amount of alcohol consumed, the risks for developing lung cancer in current smokers were 1.5-fold greater for those with the inactive $ALDH_2$ genotype (data not shown) compared with nonsmokers. The lung cancer risk for individuals with the $ALDH_2^2$ allele was not increased further by smoking.

Although there have been some reports of a significant association between the ADH_3^1 allele and some types of upper aerodigestive tract cancer, this association has been controversial.[16,17,32–34] We failed to observe an association between ADH_3 gene polymorphisms and the development of lung cancer, most likely because of the limited statistical power from the low frequency of the variant allele in the Japanese population.

Several investigations[24,31,35,36] have indicated that the *CYP2E1* c2 allele is associated with susceptibility to some types of cancer. However, other investigators reported that carriers of the c2 allele had decreased susceptibility to a number of cancers[25–27,37] and reported no association between *CYP2E1* genotypes and cancer.[23,28,38] Discrepancies among these results may be caused by several factors, including differences in study design, sample size, and the populations' ethnicity. Statistical power usually is very limited in studies of the white population because of the extreme rarity of variant genotypes. Although *CYP2E1* enzyme activity is induced by certain chemicals, such as ethanol, large interindividual variation has been observed in its constitutive activity as well as after induction. Watanabe et al.[39] and Hayashi et al.[15] reported that the *Rsa*I variant c2 allele produced higher enzyme activity than the c1/c1 genotype in Japanese individuals, although this finding is itself controversial.[40–42] Highly activated *CYP2E1* induced by alcohol may play a more important role in the metabolic activation of several tobacco-specific procarcinogens, including various nitrosamines. It has been suggested that these low-molecular-weight carcinogens are associated with the development of peripheral adenocarcinoma. This finding is consistent with the results from our analysis of *CYP2E1* presented in Table 5. However, the *CYP2E1* c2/c2 genotype is not in Hardy-Weinberg equilibrium in the control population, the observed frequencies most likely are underestimates, and these findings of

an association with histologic type most likely are false-positive results. In our analysis of *$ALDH_2$*, the incidence of adenocarcinoma was high among individuals who had the wild-type genotype. Although a high incidence of squamous cell carcinoma was not observed, this result may imply that carcinogenesis caused by acetaldehyde occurs more in cancers other than adenocarcinoma as well as in esophageal and upper aerodigestive tract cancers.

A previous hospital-based study that was conducted in Japan failed to identify any association between the *RsaI* polymorphism and lung cancer, even when the analysis was stratified according to different histologic type.[28] A more recent study indicated that there was a significant decrease in overall lung cancer risk associated with the possession of at least 1 copy of the *CYP2E1 RsaI* variant allele, whereas there was no association between the *CYP2E1 RsaI* polymorphism and the histologic type of lung cancer.[27] However, none of the previous studies had adjusted for risk according to alcohol consumption levels, which strongly influence the activity of this enzyme. In the current study, we demonstrated that there is a difference between individuals who have the *CYP2E1 RsaI* c2/c2 genotype compared with individuals who have the common c1/c1 genotype, with an adjusted OR of 4.66 (95% CI, 1.36–16.0) for the former group. Because of the low incidence of homozygosity in controls, the genotype distribution was not in Hardy-Weinberg equilibrium in our control population. The increased lung cancer risk among individuals with the *CYP2E1* c2/c2 genotype likely was a false-positive result.

A correlation between the amount of alcohol consumed, genetic polymorphisms in the alcohol metabolite-related enzymes, and the stage of lung cancer was not observed in the current study, and we could not confirm that these factors were related to the aggressiveness of lung cancer. Furthermore, no associations were identified between the location of the primary cancer, the amount of alcohol consumed, and the genotype of these enzymes or between the risk for lung cancer and the type of alcoholic beverage consumed.

In summary, we report a significant association between amounts of alcohol consumed and susceptibility to lung cancer and that the risk of lung cancer in individuals with *$ALDH_2$* variant alleles, but not with *ADH_3* or *CYP2E1* variant alleles, apparently was enhanced more by alcohol intake than in individuals with common genotypes. Moreover, to our knowledge, this is the first report documenting an association between lung cancer and genetic polymorphisms of alcohol metabolite-related enzymes. Because the sample size was relatively small for the investigation of effects stratified by each genotype, the current findings should be confirmed in large-scale studies with greater statistical power.

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Phase II trial of carboplatin and paclitaxel in non-small cell lung cancer patients previously treated with chemotherapy

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Summary The purpose of this phase II trial was to evaluate the efficacy and toxicity of carboplatin plus paclitaxel in the treatment of advanced non-small cell lung cancer (NSCLC) previously treated with chemotherapy. Patients with a performance status (PS) of 0 or 1 who had received one or two previous chemotherapy regimens for advanced NSCLC were eligible. Paclitaxel 200 mg/m^2 was infused over 3 h and followed by carboplatin (area under the curve 6) infusion over 1 h, once every 3 weeks. Thirty patients were enrolled. A complete response was observed in 1 patient and a partial response in 10 patients, for an overall response rate of 36.7%. The median time to progression was 5.3 months. The median survival time was 9.9 months, and the 1-year survival rate was 47%. Hematological toxicity in the form of grade 3/4 neutropenia occurred in 54%, but grade 3 febrile neutropenia developed in only 3%. Non-hematological grade 3 toxicities were less frequent. There were no treatment-related deaths. The combination of carboplatin plus paclitaxel is an active and well-tolerated regimen for the treatment of NSCLC patients who have previously been treated with chemotherapy and have a good PS.

1. Introduction

Lung cancer remains a major cause of death from cancer in many countries. More than half of all patients diagnosed with non-small cell lung cancer (NSCLC) have advanced stage IIIB or IV disease at presentation, and patients with advanced NSCLC are candidates for systemic chemotherapy. Platinum-based chemotherapy is considered the standard first-line treatment for patients with advanced NSCLC, and prolongs survival, palliates symptoms, and improves quality of life [1,2]. Many patients with good performance status (PS) when progression occurs after first-line chemotherapy are suitable candidates for second-line chemotherapy [3].

The taxanes are an important class of new agents for the treatment of advanced NSCLC. Paclitaxel, in combination with carboplatin, is the most common regimen

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used as first-line chemotherapy for advanced NSCLC, and this combination has a more favorable toxicity profile and is more convenient to administer than other platinum-based regimens [4,5]. Docetaxel has been investigated more extensively than any other agent for second-line treatment of advanced NSCLC, and the results of two randomized phase III trials of second-line chemotherapy in patients with advanced NSCLC demonstrated that docetaxel monotherapy significantly improved survival compared with best supportive care or other single agents (vinorelbine or ifosfamide) [6,7].

Belani et al. recently reported that results of a phase III trial comparing a carboplatin plus paclitaxel regimen with a cisplatin plus etoposide regimen for first-line treatment of advanced NSCLC [8]. Carboplatin plus paclitaxel yielded a higher response rate (23% versus 15%), time to progression (121 days versus 111 days), and overall quality of life benefit than cisplatin plus etoposide, but the median survival time was better in the cisplatin plus etoposide arm than in the carboplatin plus paclitaxel arm (274 days and 233 days, respectively [$P = 0.086$]). The authors reported that a substantially greater proportion of patients in the cisplatin plus etoposide arm received second-line chemotherapy with a taxane-containing regimen than in the carboplatin plus paclitaxel arm, and suggested that treatment with taxanes in a second-line setting may have had an impact on the survival in their study. Remarkably, more than half of the regimens that were used in the second-line setting of their study consisted of paclitaxel alone or carboplatin plus paclitaxel, not docetaxel. While the efficacy of paclitaxel-containing regimens as first-line chemotherapy for advanced NSCLC has been established in many randomized phase III trials [9], the data on the efficacy of paclitaxel-containing regimens in second-line settings are limited [10,11].

Based these considerations we conducted a phase II trial to evaluate the efficacy and toxicity of carboplatin plus paclitaxel in the treatment of advanced NSCLC previously treated with chemotherapy.

2. Patients and methods

2.1. Eligibility criteria

The inclusion criteria were: pathologically confirmed advanced NSCLC patients with measurable disease who had received one or two previous chemotherapy regimens for their disease. Patients were required to submit evidence of failure of prior chemotherapy. Patients who were previously treated with carboplatin or paclitaxel were excluded if the best response was progressive disease (PD). Patients who had received prior radiotherapy were eligible provided that at least 30 days had elapsed between the completion of radiotherapy and entry into the study. Patients were also required to be 20–75 years of age, have an Eastern Cooperative Oncology Group PS of 0 or 1, and have adequate organ function as indicated by the following parameters: absolute neutrophil count $\geq 1500\,mm^{-3}$, platelet count $\geq 100{,}000\,mm^{-3}$, hemoglobin $\geq 9.0\,g/dl$, AST and ALT $\leq 2.0 \times$ the institutional upper normal limits, total bilirubin $\leq 1.5\,mg/dl$, creatinine $\leq 1.5\,mg/dl$, $PaO_2 \geq 65$ Torr. Exclusion criteria were: uncontrolled pleural or pericardial effusion, active concomitant malignancy, prior irradiation to areas encompassing more than a third of the pelvis plus spine, active infection, myocardial insufficiency or myocardial infarction within the preceding 6 months, uncontrolled diabetes mellitus or hypertension, any other condition that could compromise protocol compliance, pregnancy and/or breast-feeding. All patients were required to provide written informed consent before entry into the study. The study was approved by the institutional review board of our instituion.

2.2. Treatment plan

Treatment was started within a week of entry into the study. Patients received paclitaxel $200\,mg/m^2$ diluted in 500 ml of 0.9% saline as a 3-h intravenous infusion followed by carboplatin (area under the curve [AUC] 6; Calvert formula) diluted in 250 ml of 5% glucose as a 1-h intravenous infusion, every 3 weeks. All patients were premedicated with dexamethasone (24 mg i.v.), famotidine (20 mg i.v.), and diphenhydramine (50 mg orally) 30 min before the paclitaxel infusion to prevent a hypersensitivity reaction. A 5-HT3-receptor antagonist was intravenously administered as an antiemetic before carboplatin. Therapy was continued for at least two cycles unless the patient experienced unacceptable toxicity or had PD. The maximum number of cycles of chemotherapy was six. In the event of grade 4 leukopenia or thrombocytopenia or of grade 3 neutropenic fever, the dose of carboplatin and paclitaxel was reduced to AUC 5 and $175\,mg/m^2$, respectively, in the following cycle of chemotherapy. The next cycle of chemotherapy was started if the neutrophil count was $\geq 1500\,mm^{-3}$, the platelet count $\geq 100{,}000\,mm^{-3}$, AST and ALT $\leq 100\,IU/l$, total bilirubin $\leq 2.0\,mg/dl$, creatinine $\leq 1.5\,mg/dl$, PS 0 or 1, and the patient was afebrile.

Pretreatment evaluation included a medical history, a physical examination, vital signs, height and body weight, PS, complete blood count, biochemical studies, arterial blood gas analysis, electrocardiogram, chest radiograph and computed tomography scan (CT), abdominal ultrasound or CT, and brain magnetic resonance imaging or CT. A complete blood count, biochemical studies, and chest radiograph were performed weekly during the first cycle of chemotherapy, and 2 weekly starting with the second cycle.

2.3. Response and toxicity assessment

Objective tumor response was assessed as complete response (CR), partial response (PR), stable disease ≥ 8 weeks (SD), or PD according to the Response Evaluation Criteria in Solid Tumors. Measurable lesions were defined as lesions whose longest diameter was $\geq 2\,cm$. Imaging studies were repeated every 4 weeks until the objective tumor response was confirmed. All responses were reviewed by an independent radiologist. Toxicity was graded using National Cancer Institute-Common Toxicity Criteria version 2.0.

2.4. Statistical analysis

The primary endpoint of this study was the response rate, defined as the proportion of patients whose best response was CR or PR among all enrolled patients in the intent-to-treat analysis. The secondary end points were toxicity and overall and progression-free survival (PFS) from the date of enrollment in this study.

According to Simon's minimax two-stage phase II study design, the treatment program was designed for a minimal response rate of 5% and to provide a significance level of 0.05 with a statistical power of 80% in assessing the activity of the regimen according to a 20% response rate. The upper limit for first-stage drug rejection was no response in 13 evaluable patients. The upper limit for second-stage drug rejection was three responses in 27 evaluable patients. Overall survival time was defined as the interval between enrollment in this study and death or the most recent follow-up visit. PFS was defined as the interval between enrollment in this study and the first documented PD, death, or the most recent follow-up visit. Survival was estimated by the Kaplan–Meier analysis method. All comparisons between proportions were performed by Fisher's exact test.

3. Results

3.1. Patient characteristics

Between October 2002 and November 2003, 30 patients were enrolled in this study, and their characteristics are shown in Table 1. Twenty-six (87%) patients were men, and 21 (70%) patients had adenocarcinoma. Median age was 60 years. The majority of the patients (93%) had received prior platinum-based chemotherapy, and seven (23%) patients had received two prior chemotherapy regimens. The platinum-based chemotherapy regimens that had been used were: cisplatin plus vinorelbine ($n=26$), cisplatin plus gemcitabine ($n=1$), and carboplatin plus gemcitabine ($n=1$). There were 15 (50%) responders to any of the prior chemotherapy regimens and 12 of them had experienced a response (CR/PR) to cisplatin-based chemotherapy. Twenty-one (70%) patients had a treatment-free interval of 3 or more months since the final dose of the prior chemotherapy regimen.

A total of 94 cycles of chemotherapy were administered, and the median number of cycles per patient was three (range, 1–6). Four patients had received only one cycle of treatment either because of toxicity (two patients, grade 3 rash), the patient's refusal (one patient), or PD (one patient).

3.2. Response and survival

Two patients were not evaluable for response because the protocol treatment had been terminated because of toxicity (grade 3 rash) during the first cycle of chemotherapy, and they subsequently received further chemotherapy without PD. There was 1 CR and 10 PRs among the 30 patients, and the objective response rate in the intent-to-treat analysis was 36.7% (95% confidence interval [CI], 19.9–56.1%) (Table 2). Treatment outcomes of all patients are listed in

Table 1 Patient characteristics

Characteristic	No. of patients (%)
Patients enrolled	30
Sex	
Male	26
Female	4
Age, years	
Median	60
Range	39–75
ECOG performance status	
0	7
1	23
Stage	
IIIB	11
IV	19
Histology	
Adenocarcinoma	21
Squamous cell carcinoma	7
Large cell carcnioma	2
Prior treatment	
Platinum-based chemotherapy	28 (93)
Docetaxel	5 (16)
Chest radiotherapy	4 (13)
No. of prior chemotherapy regimens	
1	23
2	7

Table 3. The response rate of patients who experienced a response (CR/PR) to prior cisplatin-based chemotherapy was 43% (6/14), as opposed to 23% (3/13) among the non-response patients ($P=0.41$). The response rate of the patients who had received one prior chemotherapy regimen was 39% (9/23), as opposed to 28% (2/7) among the patients who had received two regimens ($P>0.99$). According to the treatment-free interval since the final dose of the prior chemotherapy regimen, the response rate of patients whose interval was 3 months or more was 33% (7/21), com-

Table 2 Treatment efficacy ($n=30$)

	No. of patients	%
Response		
Overall response rate	11	36.7
Complete response	1	3.3
Partial response	10	33.3
Stable disease	12	40
Progressive disease	5	16.7
Not evaluable	2	6.7
Survival		
Median (months)	9.9	
1 year (%)	47	
Progression-free survival		
Median (months)	5.3	

Table 3 Treatment outcomes of all patients

Patient No.	Prior first-line therapy		Prior second-line therapy		Time from last therapy (months)	CBDCA + PTX, best response	PFS (months)	Survival (months)
	Regimen	Best response	Regimen	Best response				
1	CDDP + VNR	SD	DOC	PD	1.8	SD	1.4	25.2
2	CBDCA + GEM	NE	Gefitinib	PD	0.8	PR	3.8	8.8
3	CDDP + VNR	SD	—		6.8	SD	7.6	18.1
4	CDDP + GEM	PR	—		9.5	PR	7.5	33.8+
5	CDDP + VNR	SD	—		4.8	SD	2.8	7.0
6	CDDP + VNR + DOC + RT	PR	—		6.0	PR	8.0	21.6
7	GEM + VNR	SD	—		23.0	PD	1.2	7.8
8	CDDP + VNR + RT	PR	—		13.6	SD	6.7	25.0+
9	CDDP + VNR	SD	—		5.0	SD	2.1	3.7
10	CDDP + VNR	SD	—		5.0	PD	1.2	6.7
11	CDDP + VNR	PR	—		8.9	NE	1.1	3.3
12	CDDP + VNR	SD	Gefitinib	CR	1.9	SD	6.3	6.3
13	CDDP + VNR	PR	—		5.4	NE	1.0	13.4
14	CDDP + VNR	PR	—		1.7	SD	4.8	5.7
15	CDDP + VNR + RT	PR	—		9.3	SD	5.0	15.7
16	CDDP + VNR	SD	—		2.8	PR	3.7	15.8
17	CDDP + VNR	SD	DOC + GEM	SD	3.8	SD	5.3	21.6+
18	CDDP + VNR + DOC + RT	PR	—		3.9	SD	4.5	9.0
19	CDDP + VNR	PR	—		12.9	PR	9.4	16.0
20	CDDP + VNR	PR	—		11.5	CR	24.8+	24.8
21	CDDP + VNR	PD	—		1.1	PR	9.2	23.6+
22	CDDP + VNR	SD	DOC	SD	4.5	PD	2.3	5.5
23	Gefitinib	SD	—		0.9	PR	8.8	12.7
24	CDDP + VNR	PR	—		11.1	PR	5.3	10.2
25	CDDP + VNR	PR	Gefitinib	PR	4.4	PR	5.5	9.9
26	CDDP + VNR	NE	—		11.7	PR	7.0	12.2
27	CDDP + VNR	PR	—		5.4	SD	6.2	9.4
28	CDDP + VNR	SD	—		0.8	PD	1.4	2.5
29	CDDP + VNR	PR	—		4.4	PD	0.2	8.4
30	Gefitinib	PD	CDDP + VNR	PD	0.9	SD	3.1	3.3

CBDCA, carboplatin; PTX, paclitaxel; PFS, progression-free survival; CDDP, cisplatin; VNR, vinorelbine; GEM, gemcitabine; DOC, docetaxel; RT, chest radiotherapy; SD, stable disease; NE, not evaluable; PR, partial response; PD, progressive disease; CR, complete response.

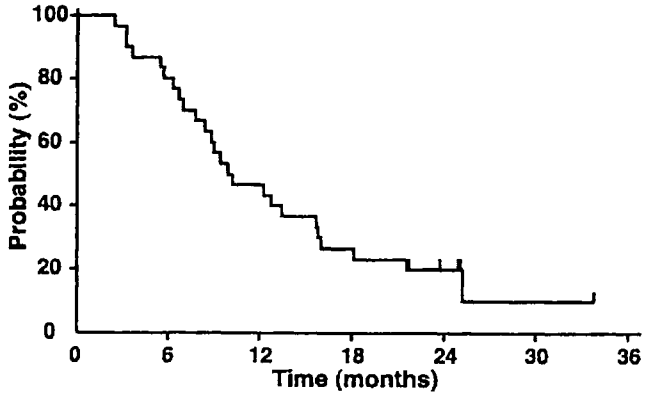

Fig. 1 Kaplan–Meier curve for overall survival.

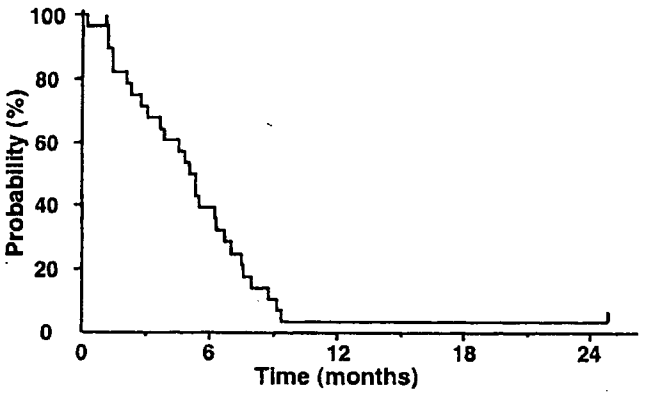

Fig. 2 Kaplan–Meier curve for progression-free survival.

pared with 44% (4/9) in patients in whom it was less than 3 months ($P = 0.68$).

The median follow-up time was 24 months. The median survival time (MST) was 9.9 months (range, 2.5–33.8 months), and the 1-year survival rate was 47% (95% CI, 29–65%). The median PFS was 5.3 months. The Kaplan–Meier curve for overall survival and for PFS is shown in Figs. 1 and 2, respectively. Nineteen patients (63%) received at least one subsequent chemotherapy regimen, and their regimens are shown in Table 4. Fourteen of them were treated with gefitinib, and a PR was achieved in three of them.

3.3. Toxicity

The common toxicities associated with carboplatin plus paclitaxel are listed in Table 5. Grade 3/4 neutropenia occurred in 54% of the patients in our study, but grade 3 febrile neutropenia developed in only 3%. Grade 3/4 anemia and thrombocytopenia were observed in five patients (16%) and two patients (13%), respectively. Non-hematological grade 3 toxicities were less frequent. Grade 3 hyponatremia was observed in five (16%) patients, but they were all asymptomatic. Grade 2 neuropathy occurred in 33% of the patients. There were no treatment-related deaths.

Table 4 Post-study chemotherapy

Regimen	No. of patients	Responder (%)
Gefitinib	14	3 (21)
Docetaxel	9	0
Gemcitabine plus vinorelbine	1	0

4. Discussion

Docetaxel, pemetrexed, and erlotinib have been approved for second-line treatment of advanced NSCLC on the basis of the results of phase III trials [6,7,12,13]. Hanna et al. reported a phase III study comparing 3-weekly pemetrexed 500 mg/m^2 with 3-weekly docetaxel 75 mg/m^2 as second-line treatment for advanced NSCLC. The overall response rate with pemetrexed and docetaxel was 9.1% and 8.8%, respectively, and MST was 8.3 months and 7.9 months, respectively. Although efficacy in terms of the outcome as measured by survival time and response rate was similar for both treatments, the pemetrexed group experienced less grades 3–4 hematological toxicity and alopecia of all grades [12]. In the trial reported by Shepherd et al. 731 NSCLC patients previously treated with chemotherapy were randomized to receive either erlotinib at a dose of 150 mg daily or placebo, and the response rate in the erlotinib group was 8.9%. MST was 6.7 months in the erlotinib group and 4.7 months in the placebo group ($P < 0.001$). The results of their trial showed that erlotinib significantly prolonged the survival of patients with advanced NSCLC who had previously been treated with chemotherapy [13]. Despite the positive results of these phase III trials, the response rate of advanced NSCLC to second-line chemotherapy remains low, and the life expectancy of advanced NSCLC patients remains short. Alternative effective chemotherapy option is needed for second-line treatment of advanced NSCLC.

The combination of carboplatin plus paclitaxel has proved effective as one of the standard platinum-based doublet regimens for first-line treatment of advanced NSCLC [4,5,14]. However, since the efficacy of carboplatin plus paclitaxel used in a second-line setting had hardly been assessed, in the present study we evaluated the efficacy and toxicity of carboplatin plus paclitaxel in the second- or third-line treatment of advanced NSCLC. The results in the 30 patients with advanced NSCLC previously treated with chemotherapy indicated that the combination of carboplatin plus paclitaxel yielded an objective response rate of 36.7% and an MST of 9.9 months, with a 1-year survival rate of 47%. Our study had not included patients who were treated with the platinum/taxane combination chemotherapy. Most of the toxicity observed in our study was hematological. Grade 3/4 neutropenia, anemia, or thrombocytopenia occurred in 54, 16, or 13% of the patients in our study, respectively. Hematological toxicity of carboplatin plus paclitaxel used in first-line treatment for Japanese patients with advanced NSCLC has been reported that grade 3/4 neutropenia, anemia, or thrombocytopenia occurred in 88, 15, or 11% of the patients [15]. The toxicity observed in our study appeared similar to that of carboplatin plus paclitaxel, which was administered as the first-line treatment, although the number of patients in our study was not large. The combination of carboplatin plus paclitaxel seems to be effective and tolerable, not only as first-line therapy for advanced NSCLC but

Table 5 Hematological and non-hematological toxicity ($n=30$)

Toxicity	NCI-CTC Version 2.0, grade							
	0–1		2		3		4	
	n	%	*n*	%	*n*	%	*n*	%
Leukopenia	11	37	10	33	9	30	0	0
Neutropenia	10	33	4	13	14	47	2	7
Anemia	7	23	18	60	3	10	2	7
Thrombocytopenia	27	90	1	3	2	7	0	0
Febrile neutropenia	29	97	–		1	3	0	0
Nausea	27	90	3	10	0	0	–	
Fatigue	30	100	0	0	0	0	0	0
Neuropathy	20	67	10	33	0	0	0	0
Arthralgia	21	70	8	27	1	3	0	0
Rash	28	93	0	0	2	6	0	0
Infection	29	97	0	0	1	3	0	0
Arrhythmia	29	97	0	0	1	3	0	0
Alopecia	21	70	9	30	–		–	
AST/ALT	29	97	1	3	0	0	0	0
Hyponatremia	25	83	–		5	17	0	0

as second-line therapy as well if the patients had not been previously treated with the platinum/taxane combination chemotherapy.

Hotta at al. reported a meta-analysis based on abstracted data to compare the effect of carboplatin-based chemotherapy with that of cisplatin-based chemotherapy on overall survival, response rate, and toxicity in the first-line treatment of patients with advanced NSCLC [16]. The results indicated that combination chemotherapy consisting of cisplatin plus a third generation agent produced a significant survival benefit compared with carboplatin plus a third generation agent, although the toxicity profiles of the two modalities were quite different. Recently, Pignon et al. reported a pooled analysis from five randomized clinical trials of cisplatin-based chemotherapy in completely resected NSCLC patients [17]. Their analysis suggested that adjuvant cisplatin-based chemotherapy improved survival in patients with NSCLC. Based on the results of their meta-analysis, cisplatin-based chemotherapy should be recommended as first-line therapy for patients with advanced NSCLC. Moreover, in view of the results of our own study, we speculate that the combination of carboplatin plus paclitaxel may be suitable as second-line treatment for advanced NSCLC patients who had experienced progression after first-line cisplatin-based chemotherapy.

Care must be exercised in interpreting the favorable outcome in our study. One concern is that it was a single-institution phase II study, and therefore patient selection may have influenced the outcome. The responders to any of the prior chemotherapy regimens accounted for 50% of the 30 patients enrolled in this study, and about 80% of the patients had received only one prior chemotherapy regimen. The selection criteria, such as an ECOG PS of 0 or 1, may also have contributed to this favorable outcome. Another concern is that our study had included only five patients who were previously treated with chemotherapy using taxanes. Therefore, the efficacy of carboplatin plus paclitaxel as the secondary therapy after chemotherapy using taxanes is not clear. A further randomized study is warranted to be able to draw definitive conclusions about our results.

Conflict of interest statement

None declared.

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Multidisciplinary Treatment for Advanced Invasive Thymoma with Cisplatin, Doxorubicin, and Methylprednisolone

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Background and Objectives: Advanced invasive thymomas are not usually manageable by surgical resection and radiotherapy. We reviewed our experience with a multidisciplinary approach and evaluated chemotherapy in the treatment of invasive thymoma.

Patients and Methods: Seventeen consecutive patients with invasive thymoma were treated with multimodality therapy consisting of chemotherapy, surgery, and/or radiotherapy. Four patients had stage III disease with superior vena cava invasion, nine had stage IVa disease, and four had stage IVb disease. The chemotherapy regimen consisted of cisplatin, doxorubicin, and methylprednisolone (CAMP). Chemotherapy was administered in a neoadjuvant setting to the 14 patients and in an adjuvant setting to the remaining three patients. Surgical resection was intended in all patients. After those treatments, chemotherapy and/or radiation therapy were performed.

Results: All but one of the 14 patients with induction chemotherapy responded to the CAMP therapy, and the response rate was 92.9%. Seven of these patients underwent complete remission after surgical resection and chemoradiotherapy, and the others underwent partial remission. All three patients treated with surgical resection and then chemotherapy with or without radiotherapy also achieved complete remission. Tumor progression after multimodality therapy occurred in 10 patients. After retreatment, eight of these patients were alive at the time of analysis, with a median survival time after recurrence of 30 months. The 5- and 10-year overall survival rates for all patients were both 80.7%. The major side effect of CAMP therapy was acceptable neutropenia.

Conclusions: CAMP therapy was highly effective for invasive thymomas, and the multimodality therapy containing this chemotherapy brought about good disease control in the majority of patients. We believe that this multidisciplinary treatment with CAMP therapy, surgery, and radiotherapy is a justifiable initial treatment for patients with advanced invasive thymoma. Furthermore, appropriate treatments are essential for the long-term survival of patients with recurrences after multimodality therapy.

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In patients with thymoma, surgical resection with or without radiation therapy has been advocated as the treatment of choice for early-stage diseases.[1–3] Nevertheless, advanced-stage diseases such as tumors with great vessel invasion, pleural and/or pericardial dissemination, lymph node involvements, or distant metastases are difficult to manage by surgery and radiotherapy, and the treatment strategy for those diseases remains controversial.[4,5]

Chemotherapy has been shown to have significant antitumor activity against unresectable, recurrent, or metastatic thymomas.[6–9] Recently, multimodality therapy using chemotherapy has been examined in the treatment of advanced thymomas.[10–12] Investigators have demonstrated that combined-modality therapy can improve outcomes for advanced thymoma patients. Nevertheless, the chemotherapy regimens and treatment schedules in these studies were varied, and an optimal treatment strategy has not yet been determined. Furthermore, although it is well known that thymoma has a slow-growing nature and a late recurrent tendency, few reports contained longer follow-up data or results of retreatment of recurrences.[13–15]

To improve the outcome of patients with advanced invasive thymomas, we have conducted a study of multimodality therapy including chemotherapy. Here, we report the results with a longer follow-up.

PATIENTS AND METHODS

From February 1988 to September 2003, 38 patients with thymoma were referred to our hospital. Their clinical characteristics are shown in Table 1. Of these patients, 17 consecutive patients with advanced invasive thymoma, (four patients with stage III disease, nine with stage IVa disease, and four with stage IVb disease) including four patients with recurrent tumor, were enrolled in the study of multimodality therapy including chemotherapy, surgery, and/or radiotherapy. In all but three patients, pathologic diagnosis of thymoma was obtained by thoracotomy, transthoracic needle biopsy, or fiberoptic bronchoscopic biopsy before initiation of treatment. Among the patients without pretreatment histologic diagnosis, one patient had multiple recur-

TABLE 1. Profile of Patients with Thymoma

Sex	
Male	17
Female	21
Age (yr)	
Median (range)	57 (25–75)
World Health Organization tumor type	
A	2
AB	6
B1	3
B2	22
B3	5
Masaoka stage	
I	15
II	4
III	6
IVa	9
IVb	4

rent pleural tumors after surgical treatment and chemotherapy for thymoma, and the remaining two had anterior mediastinal mass suspected invasive thymoma on computed tomography (CT) that were located at unsuitable places for needle biopsy. Clinical staging was determined by the medical history and physical examination, chest radiography, and chest CT. Other imaging modalities such as magnetic resonance imaging, echocardiography, or venography were performed when indicated. The staging was based on the Masaoka staging system.[16] All patients gave written informed consent for the study.

The treatment strategy of the multimodality therapy was as follows: (a) If a tumor of stage III with invasion to the great vessels or stage IV disease was distinctly demonstrated on diagnostic imaging at the initial staging, induction chemotherapy was conducted. After three or four cycles of the chemotherapy, surgical resection was attempted when the residual tumor was found, and consolidation chemotherapy and/or radiotherapy were given. (b) When stage IV disease was found on operation despite a clinically earlier stage, surgery for debulking the tumor was attempted. After that, chemotherapy was administered as a postsurgical adjuvant treatment, and then radiation therapy was applied if indicated.

The chemotherapy regimen consisted of cisplatin (20 mg/m^2 per day, continuous infusion on days 1 through 4), doxorubicin (40 mg/m^2 intravenously on day 1), and methylprednisolone (1000 mg/day intravenously on days 1 through 4 and 500 mg/day intravenously on days 5 and 6) (CAMP). Treatment cycles were repeated every 21 to 28 days. Prophylactic granulocyte colony stimulating factor was not routinely used. Surgery was intended through a median sternotomy in all patients. Resection was defined as complete (R0) if all gross disease was removed and if all surgical margins were free of the tumor. An incomplete resection meant that the surgical margins were microscopically positive (R1) or that gross residual tumors (R2) were left at the end of the operation. Radiation therapy was administered to the mediastinal or residual tumor areas using opposite anterior and posterior parallel fields and doses of more than 50 Gy. When malignant pericardial effusion was noted during the operation, whole mediastinal irradiation was carried out.

The patients were evaluated with CT for response after induction chemotherapy and completion of the multimodality treatment. A complete remission (CR) was defined as the complete disappearance of all objective evidence of disease on CT for at least 4 weeks. A partial remission (PR) was defined as a decrease of at least 50% in the sum of the product of the perpendicular diameter of measurable lesions for at least 4 weeks. Disease progression was defined as an increase of at least 25% in tumor size or new lesions. All other circumstances were classified as no change (NC).

Survival was measured from the first day of treatment until death or the last date of the follow-up (March 31, 2004). The survival curves were calculated according to the Kaplan–Meier method, and comparisons among the curves were made by means of the log-rank test. The median follow-up time of all patients (n = 17) was 54 months (range, 2–193 mo), and median follow-up time of surviving patients (n = 14) was 62 months (range, 6–193 mo).

RESULTS

Of the 17 patients, eight were women and nine were men, ranging in age from 25 to 72 years (median, 51 yr) (Table 2). Pretreatment pathologic diagnoses were obtained in 14 patients, and the tumor histology of the remaining three patients (patients 15–17) was revealed after chemotherapy and surgical treatment. Histologic types of the thymoma were B2 tumor in 14 patients and B3 tumor in three patients, according to the World Health Organization classification.[17] All four patients who were diagnosed as having stage III disease were found to have a tumor with superior vena cava invasion on diagnostic imaging. Nine patients with stage IVa disease had pleural tumor dissemination and/or pericardial effusion, and four with stage IVb disease had pulmonary metastasis or lymph node involvement.

A summary of treatments and outcomes is listed in Table 3. CAMP therapy was administered in a neoadjuvant setting to 14 patients (Figures 1 and 2). One complete response and 13 partial responses were obtained, with an overall response rate of 92.9% (95% confidence interval [CI], 66.1–99.8%). After chemotherapy, nine patients underwent surgical resection of the residual tumor with curative intent. However, R0 resection was performed in only two patients, R1 resection in one patient, and R2 resection in six patients. Postsurgical radiotherapy was performed in eight patients. Among the remaining four patients, one complete responder for CAMP therapy had no additional treatment. Two partial responders received radiotherapy because of the unresectable tumor, and the other one refused further treatment.

Three patients (patients 1, 2, and 11) who were categorized at the initial staging as having stage I to III disease were found on operation to have stage IVa disease with pleural dissemination or malignant pericardial effusion. The patients underwent resection of the main tumor and extended

TABLE 2. Characteristics of Patients with Advanced Invasive Thymoma

Patient No.	Age (yr)	Sex	Histology	Disease Stage	Site of Disease
1	40	M	B2	IVa	Pleural dissemination
2	59	F	B2	IVa	Pericardial effusion, pericardium, aorta, lung
3	72	M	B2	IVa	Pericardial effusion, pericardium, SVC, lung
4	63	M	B2	IVb	Mediastinal lymph nodes, pleural effusion
5	38	F	B2	III	SVC
6	33	M	B2	IVa	Pleural dissemination, lung
7	65	F	B2	IVb (rec)	Pulmonary metastasis, pleural dissemination
8	66	F	B2	IVb (rec)	Pulmonary metastasis
9	62	F	B2	III	SVC
10	56	M	B3	IVa (rec)	Pleural dissemination
11	29	M	B2	IVa	Pleural dissemination, pericardium, lung
12	49	M	B3	IVa	Pleural dissemination, pericardium, pulmonary artery
13	51	F	B2	III	SVC, lung
14	62	F	B3	IVa	Pleural dissemination
15	25	M	B2	IVa (rec)	Pleural dissemination
16	29	M	B2	IVb	Pulmonary metastasis
17	62	F	B2	III	SVC

Rec, recurrent case; SVC, superior vena cava.

TABLE 3. Summary of Treatments

Patient No.	Previous Treatment	Cycles of CAMP Therapy	Response to CAMP Therapy	Subsequent Treatment	Total Response	Sites of Tumor Progression	Progression-Free Survival (mo)	Treatment for Recurrences	Overall Survival (mo)
1	S (R2)	4	NA		CR	Pleura	61	S (R0)	193+
2	S (R2)	4	NA	RT	CR		180		180+
3		4	PR	S (R1), CAMP × 2, RT	CR	Pleura, lung	45	RT	180+
4		4	PR	S (R2), RT	PR	Pericardium	11	CT[1]	13
5		4	PR	S (R0), RT	CR		169		169+
6		2+CT[2]	PR	S (R2), RT	PR	Pleura	17	CT[2]	18
7		2	PR		PR		2		2
8		3	CR		CR	Pulmonary metastasis	7	S (R0)	88+
9		2	NC	S (R2), RT	PR	Primary site	42	RT	72+
10		4	PR	RT	CR	Pleura	32	RT	67+
11	S (R2)	4	NA		CR	Pleura	24	CAMP × 2, S (R0)	56+
12		4	PR	RT	PR		54		54+
13		4	PR	S (R0)	CR		43		43+
14		4	PR	S (R2), RT	CR	Pleura	23	CAMP × 4	37+
15		4	PR		PR	Pleura	18	CAMP × 4, S (R0)	29+
16		4	PR	S (R2), RT	CR		9		9+
17		4	PR	S (R2), RT	PR		6		6+

CR, complete remission; CT[1], CDDP+VLB+BLM; CT[2], CPA+ADM+VCR+prednisone; NA, not assessable; NC, no change; PR, partial remission; R0, complete resection; R1, microscopically incomplete resection; R2, macroscopically incomplete resection; RT, radiation therapy; S, surgery.

thymectomy combined with a partial resection of the pericardium, parietal pleura, and/or lung. Even after the resection, patients 1 and 11 retained numerous miliary pleural tumors in the hemithorax, and patient 2, with malignant pericardial effusion, had a residual mass on the aortic arch. These patients received four cycles of CAMP therapy after surgery, and only patient 2 underwent subsequent whole mediastinal radiation therapy.

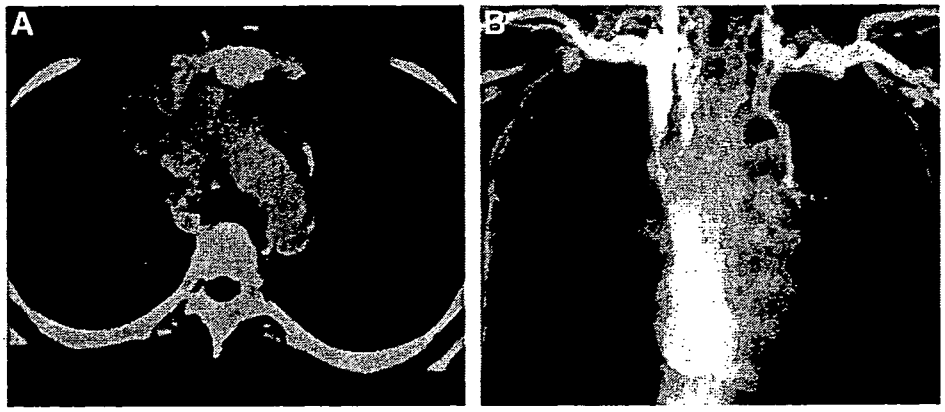

FIGURE 1. Patient 5 before chemotherapy. (*A*) CT scan showing a large anterior mediastinal tumor invading the superior vena cava. (*B*) Venous phlebogram illustrating an almost complete obstruction of the superior vena cava at the level of the junction of bilateral brachiocephalic veins.

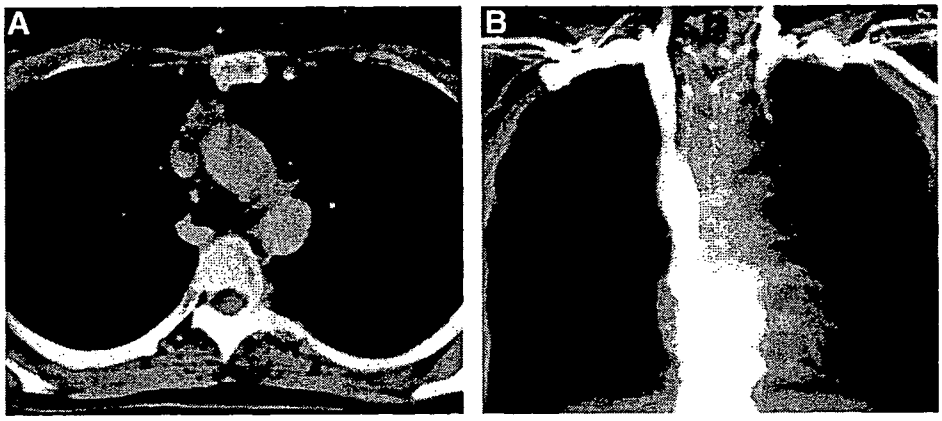

FIGURE 2. Patient 5 after four cycles of induction chemotherapy. (*A*) CT scan revealing considerable shrinkage of the tumor. (*B*) Venous phlebogram demonstrating the marked improvement of superior vena cava obstruction.

After completion of the multimodality therapy, 10 patients achieved CR and seven achieved PR; the overall remission rate was 100%. Tumor progression after treatment was observed in six (60%) of 10 CR patients and in four (57%) of seven PR patients, with a median progression-free survival of 24 months (range, 7–61 mo). The remaining six patients (four CR patients and two PR patients), 35% of the total population, had no tumor progression six to 180 months after the initiation of the multimodality therapy.

Treatment for recurrences was performed in all 10 patients. Complete surgical resection for the recurrences with or without preoperative CAMP therapy was accomplished in four patients. Patients 1 and 15 underwent an extrapleural pneumonectomy for pleural dissemination. Patient 8, who had recurrence after extrapleural pneumonectomy for the primary tumor, had a wedge lung resection for pulmonary metastasis, and patient 11 received a partial pleurectomy. For patients with unresectable recurrent tumors, radiotherapy was performed in three patients, and chemotherapy was performed in three patients whose tumors were unsuitable for radiotherapy. Two of the patients treated with chemotherapy died during the retreatment, one from recurrent tumor and the other from fulminant rhabdomyolysis.[18]

The 5- and 10-year overall survival rates of all patients were both 80.7% (95% CI, 60.9–100%) (Fig. 3). The survival curves according to stages of disease are shown in Figure 4. The 10-year survival rates of patients with stage III and stage IVa disease were 100 and 88.9% (95% CI, 68.4–100%), respectively. In stage IVb, the 5-year survival rate was 37.5% (95% CI, 0–93.6%), and only patient 8 survived for more than 5 years after CAMP therapy and resection for recurrence. In the 10 patients with recurrence, the median survival time and 5-year survival rate after retreatment were 30 months (range, 1–132 mo) and 30.0% (95% CI, 1.6–58.4%), respectively.

Toxicity of CAMP Therapy and the Multidisciplinary Treatment

The side effects of CAMP therapy are shown in Table 4. Seventy-one cycles were administered (median, four cycles;

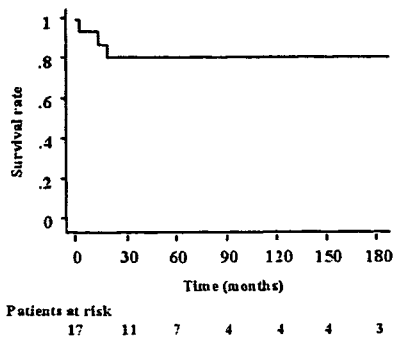

FIGURE 3. Overall survival of patients with advanced invasive thymoma who were treated with the multimodality therapy.

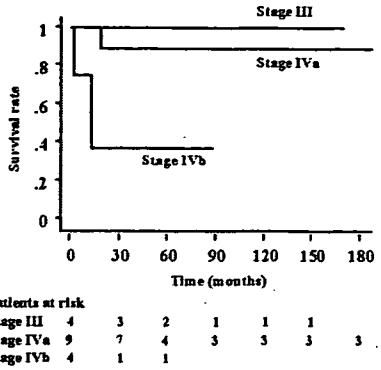

FIGURE 4. Survival according to the Masaoka staging system. In univariate analysis, there was a significant difference between stage IVa and stage IVb disease ($p = 0.036$), but there were no significant differences between stage III and stage IVa disease ($p = 0.564$) and stage III and IVb disease ($p = 0.123$).

TABLE 4. Toxic Effects of Cisplatin, Doxorubicin, and Methylprednisolone Therapy

NCI-CTC grade (%)	0	1	2	3	4	5
Leukocytes	14	12	39	27	8	
Neutrophils	10	9	21	34	26	
Hemoglobin	75	12	11	3		
Platelets	55	36	6	3		
Nausea/vomiting	31	26	36	5	1	
Infection	92	3		3	1	1

range, two to eight cycles), and the major adverse effects were leukopenia and neutropenia. Although 60% of cycles were associated with grade 3 or 4 neutropenia, almost all patients in the study received no granulocyte colony stimulating factors or no dose reduction of all three drugs. Treatment delays (median, 1 wk; range, 1–6 wk) were performed in eight patients because of neutropenia and patients' wishes. Chemotherapy-related death occurred in patient 7. She had multiple pulmonary metastases and pleural recurrences complicated with myasthenia gravis, pure red cell aplasia, and hypogammaglobulinemia. She died of pneumonia after the second cycle. Another peculiar complication of tumor lysis syndrome developed in patient 6, with a huge thymoma of predominantly lymphocytic type during the first cycle.[18]

After CAMP therapy and surgical treatment, mild cardiac dysfunction was observed in two patients (patients 2 and 3[19]) who received whole mediastinal irradiation because of malignant pericardial effusion. No other severe complications were encountered.

DISCUSSION

Complete surgical resection is considered essential in the treatment of thymomas, even for advanced diseases and recurrences.[1–3] Nevertheless, 20 to 40% of patients who undergo surgery for thymoma receive incomplete resection or biopsy alone.[1–3] Moreover, at the initial staging, some lesions are regarded as unresectable; these are usually advanced stage III or stage IV diseases, which are treated with chemotherapy and/or radiotherapy.[6–9]

We originated this aggressive multimodality therapy in February 1988 to improve the survival of patients with advanced or recurrent thymoma. In our study, eligible patients were limited to those with stage III lesions with great vessel invasion, stage IV lesions, or recurrences, because those tumors are not usually manageable by surgery and radiotherapy and are associated with unsatisfactory outcomes.[1–5] Our original chemotherapy regimen for invasive thymoma was designed from single-agent responsiveness for thymoma, which showed that cisplatin, doxorubicin, and corticosteroids had been the most active drugs.[20] Chemotherapy was not only administered in a neoadjuvant setting but also in a postsurgical adjuvant setting, because the initial stagings have not always been accurately estimated, even with CT and magnetic resonance imaging.

Neoadjuvant chemotherapy for invasive thymoma has been attempted in the treatment of locally advanced diseases because of the effectiveness of combination chemotherapy.[10–15] The chemotherapy regimens administered have been diverse, but almost all have included cisplatin and doxorubicin/epirubicin. The reported response rates have been documented to be 69 to 100%, and some patients receiving the treatment have had complete histologic remission. After induction chemotherapy for advanced tumors, the complete resection rates were around 70%. Of patients receiving the multimodality therapy using induction chemotherapy for locally advanced invasive thymoma, 5-year overall survival rates were reported to be between 55 and 95%,[13–15] because the study populations and treatment strategies were different.

In our 14 patients with neoadjuvant therapy, the response rate of CAMP therapy was 92.9%, which was better than or comparable with those of previous reports.[6–15] However, only two patients underwent complete resection, and seven underwent incomplete resection. The other tumors were interpreted as being unresectable after induction chemotherapy. Even after postsurgical radiotherapy, four patients without complete resection remained in PR, and two of them had a short survival. Our low complete resection rate is considered to be a result of the far advancement of the tumors: 13 of 17 patients had stage IV disease and/or recurrent tumors. Furthermore, CT was still incapable of predicting the possibility of performing a radical excision of the tumors after induction chemotherapy.

Patients undergoing incomplete resection or biopsy have been reported to show a significantly shorter survival than those with complete resection.[1–3] Blumberg et al.[2] reported that survival rates in patients with partial resection had been documented at 70 and 28% for 5 and 10 years, and 38 and 24% for biopsy, respectively. All three of our patients who had stage IV disease and were treated with surgery and then adjuvant chemotherapy with or without radiotherapy had distinct residual tumors after the operation. After the adjuvant therapy, two patients had pleural recurrences, but only after disease-free intervals of more than 5 and 2 years, respectively. In the remaining patient, postoperative CAMP therapy