Table 3 Univariate and multivariate analysis for overall survival

	Hazard ratio (95 % confidence interval)			
	Univariate analysis	*p*	Multivariate analysis	*p*
Group				
Stage IV group	1		1	
Postoperative group	0.323 (0.188–0.528)	<0.001	0.389 (0.220–0.657)	<0001
Type of EGFR mutation				
Exon 19 deletion	1		1	
L858R	1.276 (0.843–1.928)	0.248	0.920 (0.595–1.422)	0.708
Other	1.282 (0.630–2.384)	0.471	0.957 (0.456–1.850)	0.901
Sex				
Female	1		1	
Male	0.980 (0.639–1.471)	0.923	0.936 (0.555-1.564)	0.824
Age				
75≤	1		1	
<75	0.771 (0.496–1.240)	0.275	0.718 (0.453–1.172)	0.180
Performance status				
2–4	1		1	
0–1	0.342 (0.222–0.539)	<0.001	0.461 (0.272–0.787)	0.005
Smoking				
Previous/current	1		1	
Never	1.046 (0.695–1.611)	0.831	0.776 (0.469–1.307)	0.336
Chemotherapy before gefitinib				
Yes	1		1	
No	1.165 (0.775–1.786)	0.467	0.982 (0.611–1.595)	0.940
Number of metastatic organs				
Multiple	1		1	
Single	0.401 (0.264–0.560)	<0.001	0.442 (0.279–0.690)	<0.001

EGFR epidermal growth factor receptor

harboring EGFR mutations. Several types of cancer (such as ovarian cancer and renal cell carcinoma) are treated by surgical reduction of tumor burden in clinical practice [15, 18–20]. In addition, the efficacy of surgical reduction has been reported in selected patients with stage IV NSCLC [21–23]. Therefore, further clinical trials are warranted to develop and evaluate new treatment methods for patients with stage IV NSCLC harboring EGFR mutations.

In addition to postoperative recurrent disease, good PS and a single metastatic organ were independent favorable prognostic factors in our study. Good PS has been widely accepted as one of the most important favorable prognostic factors in lung cancer patients [10, 24–26]. Previous studies have reported that a number of metastatic organs were associated with survival [10, 16, 25] in accordance with the results presented in this study. Although age, gender, and smoking history have also been reported as prognostic factors in extensive stage NSCLC [10, 24–27], they were not significantly associated with survival in our study. However, these reports evaluated patients with NSCLC regardless of EGFR mutations, whereas our study included only patients harboring EGFR mutations of whom 70 % were female and 65 % had no history of smoking. Thus, this patient distribution may have influenced these results.

There were several limitations to our study. First, we retrospectively collected the data from a single institution. Second, the number of patients in the postoperative and stage IV group was imbalanced. Therefore, further multi-institutional studies are warranted to confirm our results.

In conclusion, PFS and OS were superior in patients with postoperative recurrent NSCLC harboring EGFR mutations treated by gefitinib than in those with stage IV disease. However, the RR of gefitinib treatment demonstrated no difference between the two groups. These results suggest that postoperative recurrent disease may be an independent prognostic factor, and should be considered as a stratification factor in clinical trials for NSCLC with EGFR mutations.

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Conflict of interest The authors declare that they have no conflict of interest.

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Original Article

Assessment of Mutational Profile of Japanese Lung Adenocarcinoma Patients by Multitarget Assays

A Prospective, Single-Institute Study

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BACKGROUND: Integration of mutational profiling to identify driver genetic alterations in a clinical setting is necessary to facilitate personalized lung cancer medicine. A tumor genotyping panel was developed and the Shizuoka Lung Cancer Mutation Study was initiated as a prospective tumor genotyping study. This study reports the frequency of driver genetic alterations in Japanese lung adenocarcinoma patients, and clinicopathologic correlations with each genotype. **METHODS:** Between July 2011 and January 2013, 411 lung adenocarcinoma patients admitted to the Shizuoka Cancer Center were included in this study with their written informed consent. Surgically resected tissues, tumor biopsies, and/or body cavity fluids were collected and tested for 23 hotspot sites of driver mutations in 9 genes (*EGFR*, *KRAS*, *BRAF*, *PIK3CA*, *NRAS*, *MEK1*, *AKT1*, *PTEN*, and *HER2*), gene amplifications in 5 genes (*EGFR*, *MET*, *PIK3CA*, *FGFR1*, and *FGFR2*), and *ALK*, *ROS1*, and *RET* fusions. **RESULTS:** Genetic alterations were detected in 54.3% (223 of 411) of all patients. The most common genetic alterations detected in this study were *EGFR* mutations (35.0%) followed by *KRAS* mutations (8.5%) and *ALK* fusions (5.0%). Concurrent genetic alterations were detected in 22 patients (5.4%), and *EGFR* mutations were observed in 16 patients as the most common partner for concurrent genetic alteration. Significantly more concurrent genetic alterations were observed in older patients. **CONCLUSIONS:** This is one of the largest reports of a prospective tumor genotyping study on Japanese patients with adenocarcinoma. These data suggest that mutational profiling data using a multimutational testing platform would be valuable for expanding the range of molecular-targeted therapeutics in lung cancer. *Cancer* 2014;120:1471–81. © *2014 American Cancer Society.*

KEYWORDS: lung adenocarcinoma, driver mutation, multimutational profiling, molecular-targeted therapeutics, personalized cancer medicine.

INTRODUCTION

Over the last decade, genetic alterations in oncogenic driver genes such as *KRAS*, *EGFR*, *HER2*, *PIK3CA*, *ALK*, *MET*, *AKT1*, *MEK1*, *BRAF*, *ROS1*, *RET*, and *NRAS* have been identified in lung adenocarcinoma, the most common histological type of lung cancer.[1-3] These findings have added a new dimension to the classification of lung adenocarcinoma, which now consists of molecular subgroups based on the mutational profile of the tumor, which is often used as a companion diagnostic tool for selecting molecular-targeted therapeutics.[1,4,5]

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Additional Supporting Information may be found in the online version of this article.

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In particular, the clinical use of epidermal growth factor receptor (EGFR)- and anaplastic lymphoma kinase (ALK)-targeted therapies has led to a paradigm shift in lung adenocarcinoma treatment.[6] *EGFR*-activating mutations are valid predictive biomarkers to identify patients who are likely to benefit from the EGFR-tyrosine kinase inhibitors (TKIs) gefitinib and erlotinib, which show response rates (RR) of 58% to 83% and progression-free survival times of 9 to 13 months.[6] Similarly, the ALK-TKI crizotinib shows promising clinical benefits, with an RR in excess of 60% and a progression-free survival of 8 to 10 months in *ALK* fusion–positive non–small cell lung cancer (NSCLC) patients.[6-8] The prevalence of those targetable oncogenic driver genetic alterations has been intensively investigated.[9-14] *EGFR*-activating mutations are found in 30% to 40% and 10% to 20% of patients with NSCLC in East Asia and North America, respectively, demonstrating that ethnicity plays a role in the prevalence of oncogenic mutations in this gene.[4] On the contrary, no clear ethnic difference has been recognized in the prevalence of *ALK*-positive NSCLC, which accounts for 1% to 7% of all NSCLC cases.[4] There are a number of ongoing clinical trials to assess the clinical efficacy of novel molecular-targeted therapeutics against tumors with oncogenic genetic alterations in genes such as *KRAS*, *BRAF*, *PIK3CA*, *MEK1*, *HER2*, *ROS1*, and *RET*.[1-3] Therefore, the integration of multimutational profiling into lung cancer clinical studies to determine the genotype of driver genetic alterations is necessary to further validate the effectiveness of molecular-targeted therapies and to assign patients to appropriate treatments.[4,5,9,10,13] In addition, the impact of ethnic differences on the prevalence of uncommon genetic alterations should be investigated and elucidated.

We developed a tumor genotyping panel to screen patients with lung cancer for genetic alterations relevant to novel molecular-targeted therapeutics in ongoing clinical trials.[1-3,15] (Supporting Table 1; see online supporting information). Multimutational analysis was implemented in the Shizuoka Lung Cancer Mutation Study, which is a new, prospective tumor genotyping study for patients with thoracic malignancies who have been admitted to Shizuoka Cancer Center. This is the first report on a prospective tumor genotyping study in Japan, which describes the frequency of driver genetic alterations in 411 Japanese patients with lung adenocarcinoma and clinicopathologic correlations with each genotype.

MATERIALS AND METHODS

Patients and Tissues

This study was approved by the Institutional Review Board of the Shizuoka Cancer Center (Ref #22-34-22-1-7). Between July 2011 and January 2013, written informed consent was obtained from 845 consecutive patients with a pathological diagnosis of lung cancer who were admitted to Shizuoka Cancer Center. Surgically resected tissue specimens were macrodissected by pathologists to enrich the tumor content. Tumor biopsy specimens containing 10% or more tumor content evaluated by hematoxylin-eosin staining were used for this study. Consequently, specimens from 411 patients with lung adenocarcinoma were considered adequate for mutational testing. Surgically resected tissues and tumor biopsies were snap-frozen on dry ice immediately after resection and stored at −80°C until use. Formalin-fixed paraffin-embedded (FFPE) specimens were sectioned with a thickness of 10 μm. Cells from body-cavity fluids (pleural or pericardial effusions) were isolated by density-gradient centrifugation with Lymphocyte Separation Media (MP Biomedicals, Irvine, Calif) and stored at −80°C to be used later. All the clinicopathologic information, including smoking history, used for this study was retrieved from the medical records of the patients.

Multimutational Profiling

Tumor genotyping panel (Supporting Table 1) was designed to assess 23 hotspot sites of genetic alterations in 9 genes (*EGFR*, *KRAS*, *BRAF*, *PIK3CA*, *NRAS*, *MEK1*, *AKT1*, *PTEN*, and *HER2*), gene amplifications in *EGFR*, *MET*, *PIK3CA*, *FGFR1*, and *FGFR2*, and *ALK*, *ROS1*, and *RET* fusions using pyrosequencing plus capillary electrophoresis, quantitative polymerase chain reaction (PCR), and reverse transcription PCR, respectively. These genetic alterations were selected by reference to articles listed in Supporting Table 1. Immunohistochemistry (IHC) and fluorescent in situ hybridization (FISH) were also used for the detection of *ALK* fusions. Detailed methods are described in the Supporting Methods (see online supporting information).

Statistical Analysis

Associations between each genotype and clinical characteristics were analyzed by a 2-sided Student *t* test and Fisher's exact test with GraphPad Prism 5 (GraphPad Software, Inc., San Diego, Calif), and by multivariate logistic regression analysis, which was done with JMP 9.0 (SAS Institute, Cary, NC). The significance level was set at $P < .05$.

TABLE 1. Demographics of Patients With Common Genetic Alterations

Characteristic	Overall Group		EGFR		KRAS		*ALK* Fusions [a]		PIK3CA		*EGFR* Amp		*MET* Amp		*HER2* Insertion		*PIK3CA* Amp		Concurrent Alterations	
Proportion	411	(100)	144	(35)	35	(9)	12	(5)	11	(3)	10	(2)	9	(2)	7	(2)	7	(2)	22	(5)
Sex																				
Female	158	(38)	**84**	**(58)**	8	(23)	3	(25)	2	(18)	4	(40)	3	(33)	3	(43)	2	(29)	10	(45)
Male	253	(62)	**60**	**(42)**	27	(77)	9	(75)	9	(82)	6	(60)	6	(67)	4	(57)	5	(71)	12	(55)
P value [b]			**<.0001**		NS		NS		NS		NS		NS		NS		NS		NS	
Age, y																				
Median (range) [c]	68	(29-89)	**69**	**(33-89) ***	69	(33-80)	**52**	**(29-85) ****	71	(55-82)	71	(57-85)	71	(40-79)	70	(58-82)	71	(50-74)	**71**	**(57-85) ***
>70	153	(37)	60	(42)	15	(43)	**1**	**(8)**	6	(55)	5	(50)	4	(44)	3	(43)	4	(57)	**13**	**(59)**
≦70	258	(63)	84	(58)	20	(57)	**11**	**(92)**	5	(45)	5	(50)	5	(56)	4	(57)	3	(43)	**9**	**(41)**
P value [b]			NS		NS		**.0344**		NS		NS		NS		NS		NS		**.0399**	
Stage																				
IA	62	(15)	**29**	**(20)**	8	(23)	**0**		1	(9)	1	(10)	0		**1**	**(14)**	0		1	(5)
IB	42	(10)	**19**	**(13)**	6	(17)	**0**		1	(9)	3	(30)	0		**4**	**(57)**	1	(14)	2	(9)
IIA	21	(5)	**8**	**(6)**	1	(3)	**1**	**(8)**	0		1	(10)	2	(22)	**0**		2	(29)	3	(14)
IIB	9	(2)	**2**	**(1)**	1	(3)	**0**		1	(9)	0		0		**0**		0		1	(5)
IIIA	50	(12)	**11**	**(8)**	4	(11)	**4**	**(33)**	2	(18)	1	(10)	1	(11)	**1**	**(14)**	3	(43)	4	(18)
IIIB	38	(9)	**7**	**(5)**	3	(9)	**2**	**(17)**	0		0		1	(11)	**0**		0		0	
IV	189	(46)	**68**	**(47)**	12	(34)	**5**	**(42)**	6	(55)	4	(40)	5	(56)	**1**	**(14)**	1	(14)	11	(50)
Early (I-II) vs Advanced (III-IV)*P* value [b]			**.0157**		NS		**.0308**		NS		NS		NS		**.0399**		NS		NS	
Smoking status [d]																				
Heavy smoker [e]	179	(44)	**29**	**(20)**	**24**	**(69)**	3	(25)	6	(55)	3	(30)	5	(56)	**1**	**(14)**	4	(57)	8	(36)
Light smoker [f]	98	(24)	**38**	**(26)**	**7**	**(20)**	5	(42)	3	(27)	3	(30)	2	(22)	**1**	**(14)**	0		7	(32)
Never-smoker	132	(32)	**77**	**(53)**	**3**	**(9)**	4	(33)	1	(9)	4	(40)	2	(22)	**5**	**(71)**	3	(43)	7	(32)
Smoker vs never-smoker *P* value [b]			**<.0001**		**.0017**		NS		NS		NS		NS		**.0381**		NS		NS	
Brinkman Index																				
Median (range) [c]	440	(0-3900)	**0 (0-3000) ******		**820 (0-2400) *****		**178 (0-820) ****		820 (0-2280)		185 (0-2080)		700 (0-3000)		**0 (0-800) ***		660 (0-1200)		275 (0-3000)	

Numbers in parentheses indicate percentages. Data in bold indicate statistically significant differences between genetic alteration-positive and wild-type genes. (**P* <.05; ***P* <.01; ****P* <.001; *****P* <.0001).
NS indicates not statistically significant.
[a] ALK fusion genes were tested in 238 patients.
[b] Fisher's exact test.
[c] Two-sided Student *t* test.
[d] No information about smoking history was available in 2 patients.
[e] Heavy smoker, Brinkman index ≧600.
[f] Light smoker, Brinkman index <600.

RESULTS

Patient Characteristics

Between July 2011 and January 2013, 502 adequate tissue samples for tumor genotyping were obtained from 411 lung adenocarcinoma patients, with 73 patients having multiple samples (Supporting Table 2). Table 1 and Supporting Table 3 show the summary of clinical characteristics of the patients. Patients had a median age of 68 years old (range = 29-89 years) and 38% were female. Stages I, II, III and IV were present in 25%, 7%, 21%, and 46%, respectively. One hundred thirty-two patients (32%) had never smoked before (never-smokers). Significant differences between females and males were observed in stage IA, IIIA, and IIIB, as well as heavy smokers and never-smokers, but not according to age, or in stage IB, IIA, IIB, and IV, and light smokers (Supporting Table 3).

Results of Multimutational Profiling

Genetic alterations were detected in 54.3% of all patients (Fig. 1A; Supporting Table 4). The most common genetic alterations were *EGFR* mutations (35.0%), followed by *KRAS* mutations (8.5%) and *ALK* fusions (5.0%), consistent with previous studies.[16] Mutation frequencies in all patients are shown in Figure 1A and Supporting Table 4. *ALK* fusions, *HER2* insertions, *MEK1* mutations, *KIF5B-RET*, and *CD74-ROS1* were mutually exclusive with other genetic alterations (Fig. 1B). There was no significant difference in the detection rate of genetic alterations among fresh-frozen tissues, FFPE tissues, and cells extracted from body cavity fluids ($P = .0940$; data not shown).

Concurrent Genetic Alterations

Concurrent genetic alterations were identified in 22 patients. *EGFR* mutations were observed in 16 patients as the most common partner for concurrent genetic alterations, (Fig. 1B; Supporting Table 5), indicating that *EGFR* mutations were not necessarily mutually exclusive with other driver genetic alterations. Among 18 patients with *PIK3CA* genetic alterations, which included 11 mutations and 7 amplifications, 11 patients (61%) had concurrent genetic alterations with other mutations in *EGFR*, *KRAS*, and *MET* (Fig. 1B; Supporting Table 5).

Clinicopathologic Correlations With Genotype

Clinicopathologic associations with genotype are shown in Tables 1 and 2. Patients with *EGFR* mutations were significantly more likely to be female. *ALK* fusion-positive patients were significantly younger than wild-type *ALK* patients (median, 52 versus 68; $P = .0052$), and this fusion was associated with advanced stage of disease. *HER2* insertions correlated with early-stage lung cancer. Never-smokers were significantly associated with *EGFR* mutations and *HER2* insertions, whereas a significant correlation between smoking history and *KRAS* mutations was observed. These clinicopathologic correlations with each genotype were confirmed by multivariate logistic regression analysis (Table 2) and were consistent with previous reports.[9,13,17,18] The number of concurrent genetic alterations was found to significantly increase with age (median, 71 versus 68, $P = .0450$, Table 1), and patients > 70 years of age had more concurrent genetic alterations than those who were 70 years of age or younger (Tables 1 and 2). Figure 2 represents the mutational profile based on smoking status. These profiles reflect the associations between genotype, especially *EGFR* and *KRAS*, and smoking status. Genetic alterations were detected in 40%, 58%, and 71% of heavy, light, and never-smokers, respectively, suggesting that never-smokers could potentially benefit from treatment with molecular-targeted therapies compared with smokers, especially heavy smokers.

A Case With Multiple Genetic Alterations Without a Smoking History

Our study included a 74-year-old male never-smoker patient with postoperative recurrence who harbored 5 different genetic alterations (Fig. 3A-C). At recurrence, the patient was initially wild-type *EGFR*, as determined by central laboratory testing. The patient then went on to receive a series of chemotherapeutic agents (Supporting Table 6). Tumor samples were tested for multiple genetic alterations upon his entry into this study and *EGFR* exon 19 deletion, *AKT1* mutation, and *PIK3CA* amplification were identified (Fig. 3A-C). *EGFR* exon 19 deletion, *EGFR* amplification, and *FGFR1* amplification were also detected using tumor cells isolated form pleural effusion (Fig. 3A-C). After the failure of fourth-line eribulin treatment, the patient was given erlotinib as fifth-line treatment and showed durable responses in lung and liver tumors (Fig. 3D; Supporting Table 6). What should be emphasized in this particular case is that if the patient fails erlotinib treatment, he has opportunities to enter clinical trials with anticancer agents that target other mutant genes present in the tumor, which was one of the pivotal aims of our study.

DISCUSSION

Tumor mutational profiling is critically needed for the facilitation of personalized medicine for lung adenocarcinoma, as well as for the development of molecular-targeted therapeutics. To address these needs, considerable efforts have been made to determine the prevalence of driver

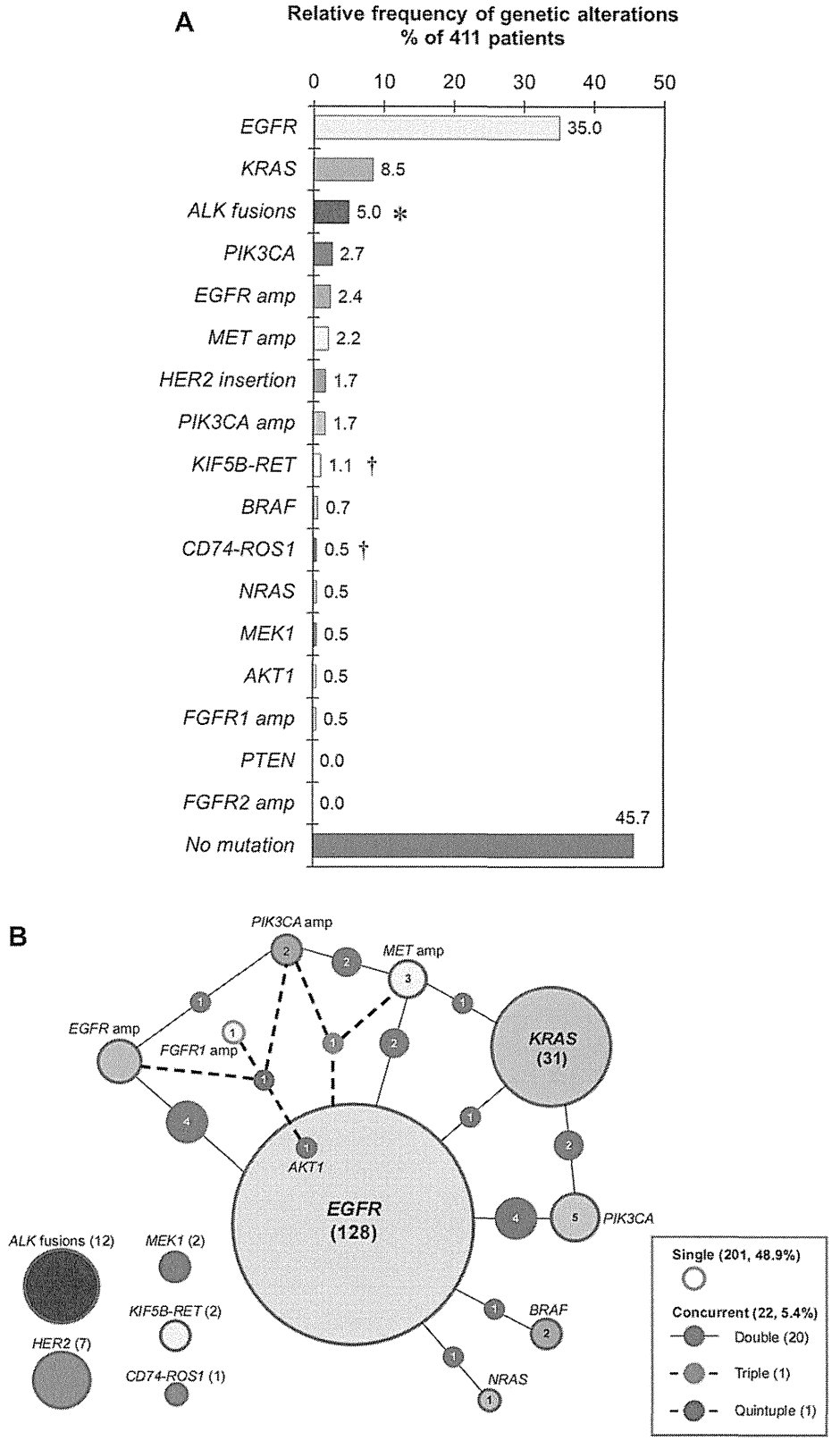


Figure 1. (A) Relative frequency of genetic alterations is shown for 411 patients with lung adenocarcinoma. Genetic alterations were detected in 54.3% (223/411) of all patients. **ALK* fusions were tested in 238 patients. †*ROS1* and *RET* fusions were tested in 182 patients for whom fresh frozen tissues and/or the body cavity fluids were available. (B) Concurrent genetic alterations were identified in 22 patients (5.4%). The dimension of each circle is proportionate to the frequency of each genetic alteration and concurrent genetic alteration. Lines that connect the circles indicate the identity of concurrent genetic alterations.

TABLE 2. Evaluation of Association Between Genotype and Clinicopathological Characteristics by Logistic Regression Analysis

Characteristic		*EGFR*	*KRAS*	*ALK* Fusions[a]	*HER2* Insertion	Concurrent Alterations
Sex	*P* value	**0.0156**	NS	NS	NS	NS
(female/male)	OR [95% CI]	1.99 [1.14-3.45]				
Age	*P* value	NS	NS	**0.0249**	NS	**0.0223**
(>70/≦70)	OR [95% CI]			0.15 [0.01-0.82]		2.81 [1.16-7.13]
Stage	*P* value	NS	NS	**0.0235**	**0.0371**	NS
(early/advanced)	OR [95% CI]			0.15 [0.01-0.80]	5.54 [1.11-41.14]	
Smoking status	*P* value	**0.0004**	**0.0036**	NS	**0.0101**	NS
(smoker/never-smoker)	OR [95% CI]	0.36 [0.21-0.63]	6.26 [1.76-29.89]		0.07 [0.01-0.52]	

Data in bold indicate statistically significant differences ($P < .05$).
Abbreviations: CI, confidence interval; NS, not statistically significant; OR, odds ratio.
[a] *ALK* fusion genes were tested in 238 patients.

genetic alterations in lung adenocarcinoma. Supporting Table 7 exhibits the comparison between the results of the present study and those of previous reports.[10-13] Kohno et al[11] recently reported the tumor mutational profile in Japanese lung adenocarcinoma patients. Comparatively, the overall detection rate of genetic alterations in our study was lower (48% in the current study versus 70% in Kohno et al[11]), especially in *EGFR* mutations (35% versus 53%). One of the most likely reasons for this difference is that we had significantly more smokers in our study (68% versus 51%, $P < .0001$), which probably reflects the characteristics of our local patient cohort. Importantly, there was no significant difference in the overall detection rate or frequency of *EGFR* mutations in never-smoker patients between these studies, which supports our hypothesis that differences in mutation rates were affected by the smoking status of the study cohort. Li et al[12] reported the mutational profile of Chinese never-smokers with lung adenocarcinoma and showed that 89% of patients had driver genetic alterations, which is a markedly high detection rate compared to our results. This may be due to their use of only archival, surgically resected tissues, on top of the fact that tissues were from an enriched cohort such as never-smokers.[12] There was no significant difference observed in the overall detection rate of mutations in surgically resected tissues from never-smoker patients between our study and Li et al[12] (data not shown). As stated above, our results appear to be different from previous Asian reports at first glance. However, our results on the detection of genetic alterations clearly hold up when adjusted based on the study cohort used for previous studies, indicating that our study was successful in reflecting the nature of the local patient population.

We also compared our data with 2 reports on prospective tumor genotyping studies conducted in North America[10,13] (Supporting Table 7). Our study and Johnson et al[10] had the same proportion of never-smoker patients (68% versus 66%); therefore, significant differences seen in the frequencies of *EGFR* and *KRAS* mutations between the 2 studies were certainly due to ethnic differences within the study cohorts.[10] Similar discrepancies in *EGFR* and *KRAS* mutations were also found between our study and Sequist et al.[13] There was no significant difference between our study and Johnson et al in overall detection rate (52% versus 54%, $P = 0.4272$).[10] *BRAF* mutations were also detected less frequently in our study (0.7% versus 1.8% in Sequist et al[13]; 2.1% in Johnson et al[10]). *BRAF* mutations have been reported to occur in approximately 3% of lung adenocarcinomas in North America.[19] In Chinese lung adenocarcinomas, *BRAF* mutations were detected in 3% of patients with a smoking history,[20] but were not found in never-smoker patients.[21] In our study, *BRAF* mutations were also detected only in patients with a smoking history (data not shown). Therefore, further investigation is needed to explore the association between *BRAF* mutations, smoking status, and ethnic differences in lung adenocarcinoma. The identification of *BRAF* mutations in lung adenocarcinoma is also important because patients with *BRAF* genetic alterations are highly likely to benefit from BRAF inhibitors.[22,23]

We identified 48 patients with uncommon genetic alterations in *PIK3CA*, *HER2*, *BRAF*, *NRAS*, *MEK1*, *AKT1*, *MET*, *FGFR1*, *ROS1*, and *RET* oncogenes. Clinical trials of novel anticancer agents targeting these genetic alterations are in progress.[1-3] Promising compounds for *HER2* insertions (detected in 1.7% of patients in our study; Fig. 1) are irreversible EGFR/HER2 TKIs such as afatinib, neratinib, and dacomitinib. In a phase 2 trial, afatinib alone showed promising activity in 2 of 5 patients with *HER2*-mutant lung adenocarcinoma.[24] Approved TKIs such as vandetanib, sunitinib, and sorafenib show activity against *RET* fusions,[11] including mutations that

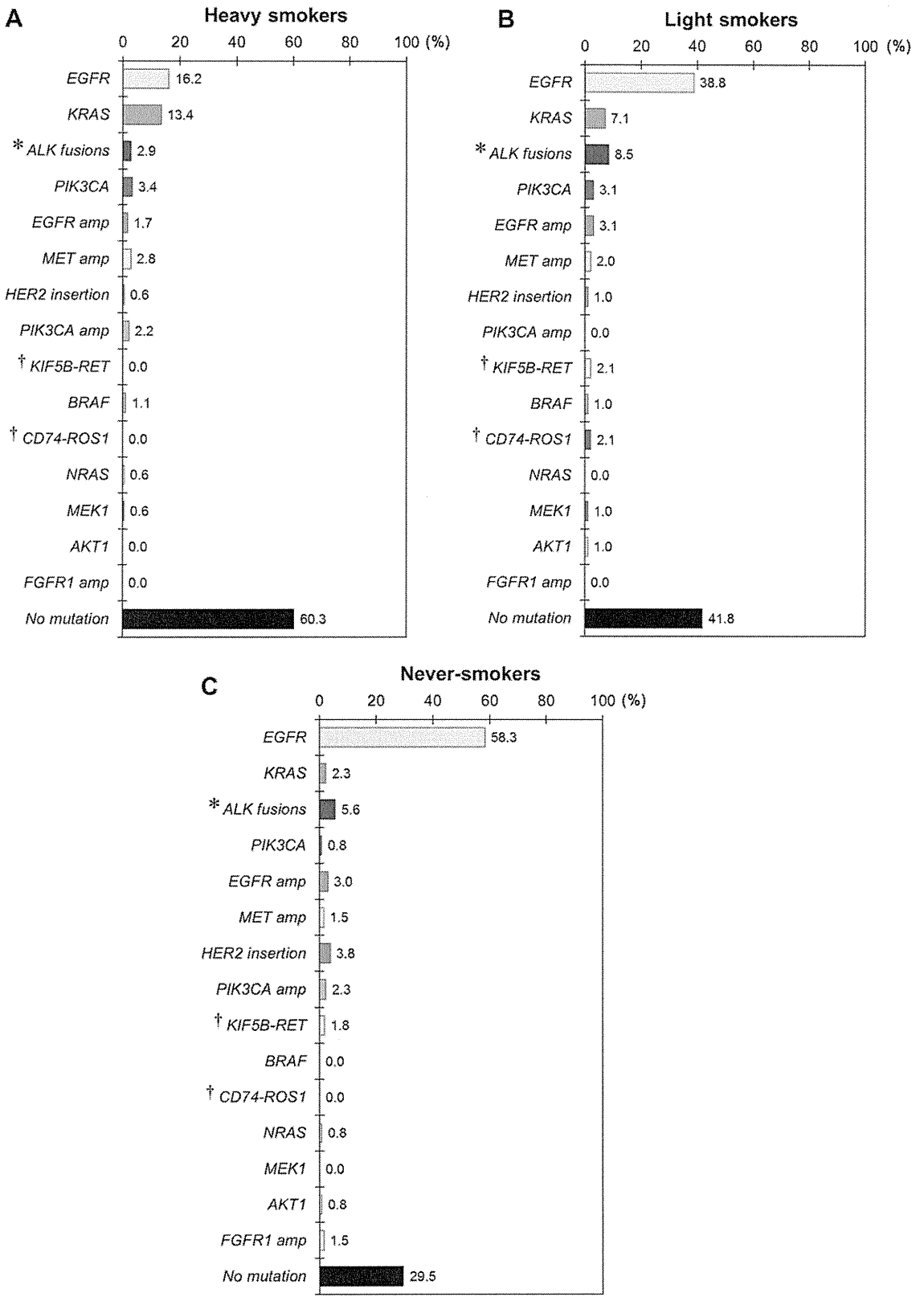


Figure 2. Relative frequency of genetic alterations is shown, based on smoking status in (A) heavy, (B) light, and (C) never-smokers. Genetic alterations were detected in 71 (39.7%) heavy smokers ($N = 179$), 57 (58.2%) light smokers ($N = 98$), and 93 (70.5%) never-smokers ($N = 132$). **ALK* fusions were tested in 238 patients. †*ROS1* and *RET* fusions were tested in 182 patients.

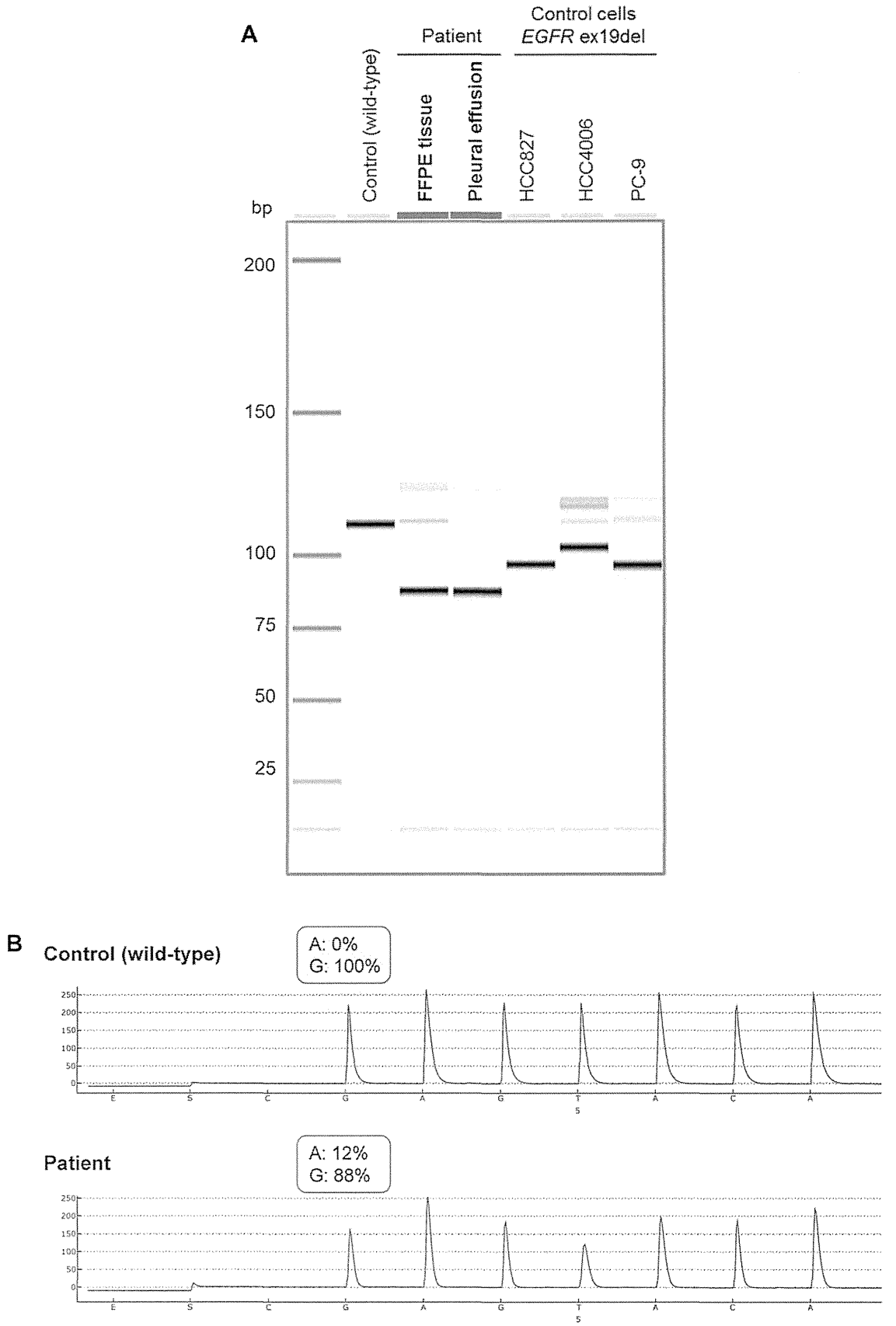
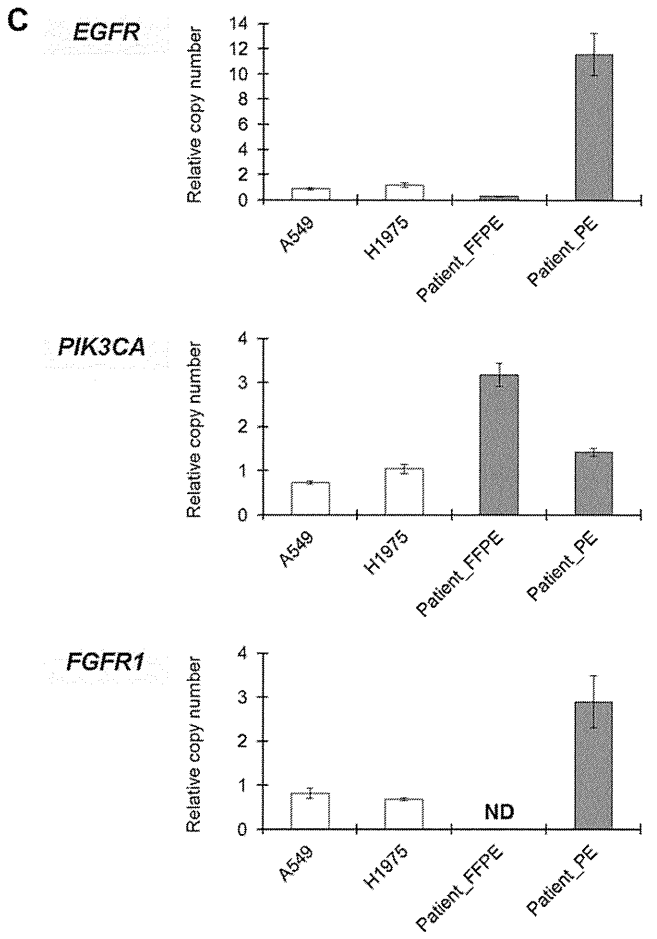


Figure 3. Results of mutational testing in a 74-year-old male never-smoker patient with lung adenocarcinoma. (A) *EGFR* exon 19 deletion was assessed by capillary electrophoresis. (B) *AKT1* mutation was detected with pyrosequencing. (C) Gene amplifications in *EGFR*, *PIK3CA*, and *FGFR1* were examined by qPCR using DNA extracted from surgically resected tissues (FFPE) and cells isolated from pleural effusion (PE). Each value is the average of triplicate measurements, and each error bar indicates the standard deviation (SD) in triplicate experiments. ND indicates "not detected." Lung adenocarcinoma cell lines A549 and H1975, which do not show amplifications in these genes (data not shown), were used as negative controls. (D) Computed tomographic (CT) scans of chest and abdomen were conducted at baseline and after 6 months of erlotinib therapy, and show significant shrinkage because of treatment.



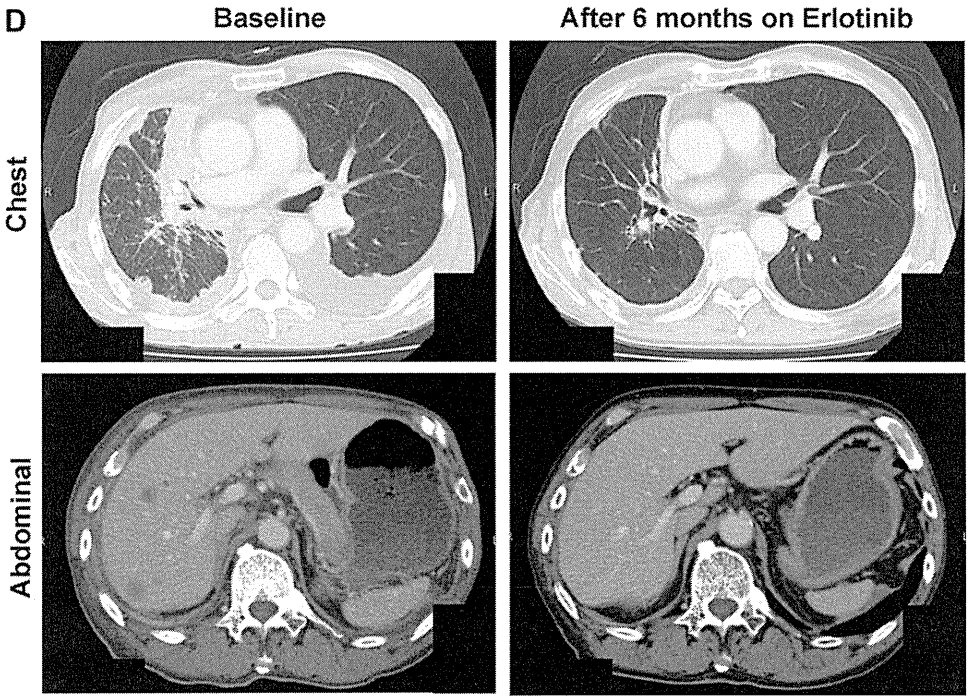


Figure 3. Continued.

were detected in 1.1% of our study cohort (Fig. 1). As well, a multi-institutional phase 2 clinical trial with vandetanib for *RET* fusion-positive patients is ongoing in Japan (UMIN000010095).[25] In order to enroll a sufficient number of patients for clinical trials of molecular-targeted agents against uncommon genetic alterations, multimutational profiling in routine clinical practice is crucial. As more molecular-targeted therapies are developed, there will be a need for more comprehensive and sensitive genotyping technologies with higher throughput to determine genotype using a limited amount of tissue. Next-generation sequencing (NGS) technology can allow us to further pursue this direction,[4] and we are currently working with this platform to identify mutations in NSCLC.[26]

Concurrent genetic alterations have been reported in 3% to 9% of lung adenocarcinoma by other groups.[10,14] Chaft et al[27] reported that 70% of lung adenocarcinoma patients with *PIK3CA* mutations in North America had coexisting genetic alterations, suggesting that *PIK3CA* mutations are one of the most common partners for concurrent genetic alterations regardless of ethnicity. However, appropriate therapeutic approaches for patients with coexisting oncogenic mutations have not been established. Our study included a 74-year-old male never-smoker patient with 5 different genetic alterations, including *EGFR* mutations, who showed durable responses to erlotinib treatment (Fig. 3D). This may be an unusual case, because patients with coexistence of *EGFR* mutations and *ALK* fusions do not necessarily respond to treatment with EGFR-TKIs.[28] These differential responses to EGFR-TKIs in patients with concurrent genetic alterations including *EGFR* mutation remain elusive, and presumably, some tumors may be driven by genetic alterations other than *EGFR*-activating mutations. In order to investigate the molecular and biological features of tumors with concurrent genetic alterations and to develop appropriate treatments, multimutational testing, combined with more comprehensive testing platforms such as next-generation sequencing technology, should be incorporated. Routine patient screening with these technologies would greatly facilitate the assessment of tumor heterogeneity.

There are limitations in this study. *ROS1* and *RET* fusions were tested only with reverse transcription PCR in 182 patients for whom fresh frozen tissues and/or the body cavity fluids were available. For future studies, implementation of FISH for detection of these fusions in FFPE tissues will be necessary. Detection of gene amplification may also require consideration of incorporating FISH for future studies.

To our knowledge, this is one of the largest tumor genotyping studies in lung adenocarcinoma conducted as a prospective single-institution trial in East Asia.[5] Our results revealed the frequency of genetic alterations in lung adenocarcinoma and identified clinicopathologic correlations with genotype, which reflect current practices in lung cancer clinics compared with reported retrospective studies.[11,12] We anticipate that multimutational analysis in lung cancer clinics will be crucial for the expansion of the range of molecular-targeted therapeutics available to treat this disease.

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CONFLICT OF INTEREST DISCLOSURES

The authors made no disclosures.

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A pilot study of adjuvant chemotherapy with irinotecan and cisplatin for completely resected high-grade pulmonary neuroendocrine carcinoma (large cell neuroendocrine carcinoma and small cell lung cancer)

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ABSTRACT

Background: Large cell neuroendocrine carcinoma (LCNEC) and small cell lung cancer (SCLC) are recognized as high-grade neuroendocrine carcinomas (HGNEC) of the lung. In patients with completely resected HGNEC, platinum-based adjuvant chemotherapy may be considered. However, the optimum chemotherapy regimen has not been determined. We conducted a multicenter single-arm phase II trial to evaluate irinotecan and cisplatin in postoperative adjuvant chemotherapy for HGNEC patients.

Patients and methods: Patients with completely resected stage I–IIIA HGNEC received four cycles of irinotecan (60 mg/m^2, day 1, 8, 15) plus cisplatin (60 mg/m^2, day 1). This regimen was repeated every 4 weeks. The primary endpoint was the rate of completion of chemotherapy (defined as having undergone three or four cycles), and secondary endpoints were the rate of 3-year relapse-free survival (RFS), rate of 3-year survival and toxicities.

Results: Forty patients were enrolled between September 2007 and April 2010. Patients' characteristics were: median age (range) 65 [45–73] years; male 85%; ECOG-PS 1 60%; LCNEC 57% and SCLC 43%; stage IA/IB/IIB/IIIA 32/35/8/5%; 95% received lobectomy. The rate of completion of chemotherapy was 83% (90% C.I.; 71–90%). The rate of overall survival at 3 years was estimated at 81%, and that of RFS at 3 years was 74%. The rates of overall survival and RFS at 3 years were 86 and 74% among 23 LCNEC patients, and 74 and 76% among 17 SCLC patients, respectively. Nineteen patients (48%) experienced grade 3 or 4 neutropenia, but only five patients (13%) developed febrile neutropenia. Two patients (5%) developed grade 3 diarrhea, and four patients (10%) had grade 3 nausea. No treatment-related deaths were observed in this study. All 40 specimens were also diagnosed as HGNEC by central pathological review.

Conclusions: The combination of irinotecan and cisplatin as postoperative adjuvant chemotherapy was feasible and possibly efficacious for resected HGNEC.

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1. Introduction

In 1991, Travis et al. proposed the classification of neuroendocrine tumor of the lung, including typical carcinoid, atypical carcinoid, large cell neuroendocrine carcinoma (LCNEC), and small cell carcinoma (SCLC) [1]. In addition, LCNEC and SCLC are recognized as high-grade neuroendocrine carcinomas (HGNEC) of the lung. LCNEC and SCLC share several histological features, including rosette formation, molding of nuclei, and lack of apparent glandular formation and keratinization [2,3].

LCNEC accounts for approximately 3% of all pulmonary malignancies, and SCLC accounts for 12%. In a large-scale, Japanese multi-institutional study of surgically resected pulmonary neuroendocrine tumors, there was no difference between LCNEC and SCLC in terms of overall survival. The survival curves were superimposed and the 5-year survival rates of surgically resected LCNEC and SCLC were 40.3 and 35.7%, respectively [4].

Retrospective analysis suggested that adjuvant chemotherapy using an SCLC-based standard regimen might be effective for LCNEC [5]. In patients with completely resected SCLC, platinum-based adjuvant chemotherapy may be considered [6,7]. The combination of cisplatin and etoposide as adjuvant chemotherapy is reported to be a feasible regimen and results in a favorable profile for SCLC [8]. However, the optimum chemotherapy regimen has not been determined. Combination chemotherapy with cisplatin and irinotecan is a standard treatment in Japan for extensive SCLC, and has been demonstrated to yield significantly longer overall survival than cisplatin and etoposide in the Japan Clinical Oncology Group Study 9511 [9]. Although LCNEC is now classified as non-small cell lung cancer (NSCLC) in WHO criteria, this combination has also been reported to be active for NSCLC [10]. Therefore, we conducted a multicenter phase II trial to evaluate irinotecan and cisplatin in postoperative adjuvant chemotherapy for completely resected HGNEC.

2. Patients and methods

2.1. Study design

This prospective phase II trial was conducted at 12 centers in Japan. It was approved by the institutional review boards of all participating centers, and all patients provided written informed consent. This study was registered at the UMIN Clinical Trial Registry (UMIN000001319).

2.2. Patients

Eligible patients were aged 20–74 years and histologically confirmed LCNEC and SCLC, completely resected, pathological stage IA, IB, IIA, IIB and IIIA. Patients were also required to have: the ability to start chemotherapy within 4–10 weeks after surgery; an Eastern Cooperative Oncology Group Performance Status (ECOG PS) of 0 or 1; no prior chemotherapy or radiotherapy; and adequate organ function (i.e., total bilirubin $\leq$1.5 mg/dL, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) <100 IU/L, serum creatinine $\leq$1.5 mg/dL, leukocyte count $\geq$4000/mm^3, hemoglobin $\geq$9.5 g/dL, platelets $\geq$100,000/mm^3, and PaO_2 at room air $\geq$70 torr). Patients without UGT1A1 polymorphisms (homozygous for *6 or *28, simultaneously heterozygous *6 and *28), associated with irinotecan-related severe toxicity, were included. Key exclusion criteria were: interstitial pneumonia or pulmonary fibrosis; watery diarrhea; and intestinal obstruction or paralysis.

2.3. Treatment

Patients received 60 mg/m^2 of cisplatin on day 1 and 60 mg/m^2 of irinotecan on days 1, 8, and 15, every 4 weeks, up to four cycles if neither unacceptable toxicity nor recurrence was observed. The administration of irinotecan on day 8 or 15 was skipped if a leukocyte count <3000/mm^3, platelets <75,000/mm^3, symptoms of infection, diarrhea within 24 h, and/or grade 3 nonhematological toxicities developed. In the event of grade 4 leukopenia or thrombocytopenia, grade 2 or 3 diarrhea, or grade 3 nonhematological toxicities except nausea, vomiting, hyponatremia, and creatinine, the dose of irinotecan at the next cycle was reduced to 50 mg/m^2.

When the next cycle of chemotherapy was started, each patient was required to meet the following criteria: ECOG PS of 0 or 1, leukocyte count $\geq$3000/mm^3, platelets $\geq$100,000/mm^3, total bilirubin $\leq$1.5 mg/dL, AST and ALT <100 IU/L, serum creatinine $\leq$1.5 mg/dL, no symptoms of infection, and no diarrhea within 24 h.

Recurrence evaluations with CT scans for chest and abdomen have been performed every 6 months until 3 years. In addition, systemic evaluation, with CT scans for chest and abdomen; with CT or MRI for head; with bone scintigraphy or PET, has been performed at 3 years.

2.4. Pathological review

Surgically resected specimens including hematoxylin-eosin stained sections and immunohistochemistry of neuroendocrine markers, which were selected by institutional pathologists, were centrally reviewed by seven expert pathologists (T.K., M.N., K.T., Y.I., K.I., G.I., and J.S.-X.) blind to clinical information. The pathology panel members performed an independent pathology review, and the final diagnosis was established by mutual agreement.

2.5. Statistical analysis

The primary endpoint was rate of completion of chemotherapy, which was defined as the rate of patients who underwent the planned three or four cycles of irinotecan and cisplatin. Secondary endpoints included rate of 3-year relapse-free survival (RFS), rate of 3-year survival, and toxicities. Efficacy and safety analyses were performed on all patients who received at least one dose of the study treatment. Adverse events (AEs) were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0.

In accordance with the minimax two-stage phase II study design by Simon, the treatment program was designed to refuse a completion rate of chemotherapy of 60% (P0) and to provide a significance level of .05 with a statistical power of 80% in assessing the feasibility of the regimen as an 80% completion rate (P1). The upper limit for first-stage drug rejection was eight completions in the 13 assessable patients; the upper limit of second-stage rejection was 25 completions within the cohort of 35 assessable patients.

Overall survival was defined as the interval between enrollment in this study and death or the final follow-up visit. The overall survival and RFS were estimated using the Kaplan–Meier analysis method.

3. Results

Forty patients were enrolled between September 2007 and April 2010, and all patients were eligible. The clinical data cut-off date was May 2013 for the analysis of efficacy, including overall survival and RFS.

Table 1
Patient characteristics (overall, $n = 40$).

	All	(%)	LCNEC	(%)	SCLC	(%)
Number of patients	40		23		17	
Gender						
Male	34	(85)	20	(87)	14	(82)
Female	6	(15)	3	(13)	3	(18)
Age, year						
Median	65		61		67	
(range)	(45–73)		(45–71)		(50–73)	
Performance status (ECOG)						
0	16	(40)	8	(35)	8	(47)
1	24	(60)	15	(65)	9	(53)
Surgical procedure						
Lobectomy	38	(95)	22	(96)	16	(94)
Pneumonectomy	1	(3)	1	(4)		
Segmentectomy	1	(3)			1	(6)
Pathological stage						
IA	13	(32)	3	(13)	10	(59)
IB	14	(35)	11	(48)	3	(18)
IIA	0		0		0	
IIB	7	(18)	6	(26)	1	(5)
IIIA	6	(15)	3	(13)	3	(18)

SCLC: Small cell lung carcinoma, LCNEC: Large cell neuroendocrine carcinoma.

Table 2
Treatment delivery of adjuvant chemotherapy.

Number of cycles	Number of patients	(%)
1	6	15
2	1	3
3	2	5
4	31	77

3.1. Patient characteristics

Table 1 summarizes the baseline characteristics of the 40 patients enrolled in this study. The median age was 65 years, and 85% of the patients were male. Histologically, SCLC and LCNEC were observed in 43 and 57%, respectively. Sixty-seven percent of the patients were diagnosed as pathological stage I. Forty-eight percent of LCNEC patients were diagnosed as pathological stage IB, and 59% of SCLC patients as pathological stage IA.

3.2. Treatment compliance

Thirty-three patients underwent the planned three or four cycles of planned adjuvant chemotherapy (Table 2). The rate of completion of chemotherapy was 83% (90% confidence interval (CI); 71–90%). However, seven patients received one or two cycles, because of adverse events in three patients (grade 3 diarrhea, cerebral hemorrhage, grade 2 enuresis) and treatment refusal in four patients. Nine patients experienced dose reduction, and 21 patients skipped administration of irinotecan. The dose intensity (the actual dose delivered as a proportion of the planned dose) was 74% for irinotecan and 87% for cisplatin.

3.3. Overall survival and recurrence-free survival

Overall survival and RFS data are shown in Fig. 1, with median follow-up for overall survival of 49 months. The rate of overall survival at 3 years was estimated at 81% (95% CI; 69–95%), and that of RFS at 3 years was 74% (95% CI; 61–90%). The rates of overall survival and RFS at 3 years were 86 and 74% among 23 LCNEC patients, and 74 and 76% among 17 SCLC patients, respectively (Fig. 2).

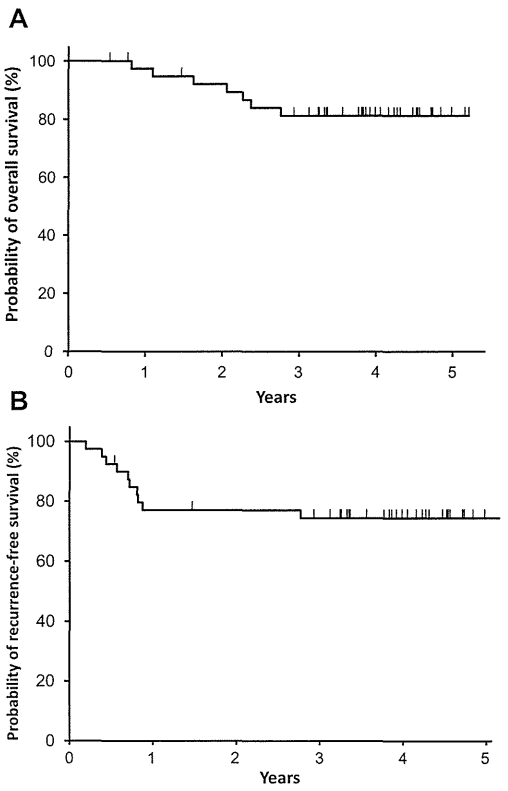

Fig. 1. (A) Overall survival curve including all eligible 40 patients. (B) Recurrence-free survival curve including all eligible 40 patients.

3.4. Safety and adverse events

Table 3 shows the incidence of AEs evaluated in all eligible patients. The most common toxicity was neutropenia. Nineteen patients (48%) experienced grade 3 or 4 neutropenia, but only five patients (13%) developed febrile neutropenia. Two patients (5%) developed grade 3 diarrhea, and four patients (10%) had grade 3 nausea. There were no treatment-related deaths in this trial.

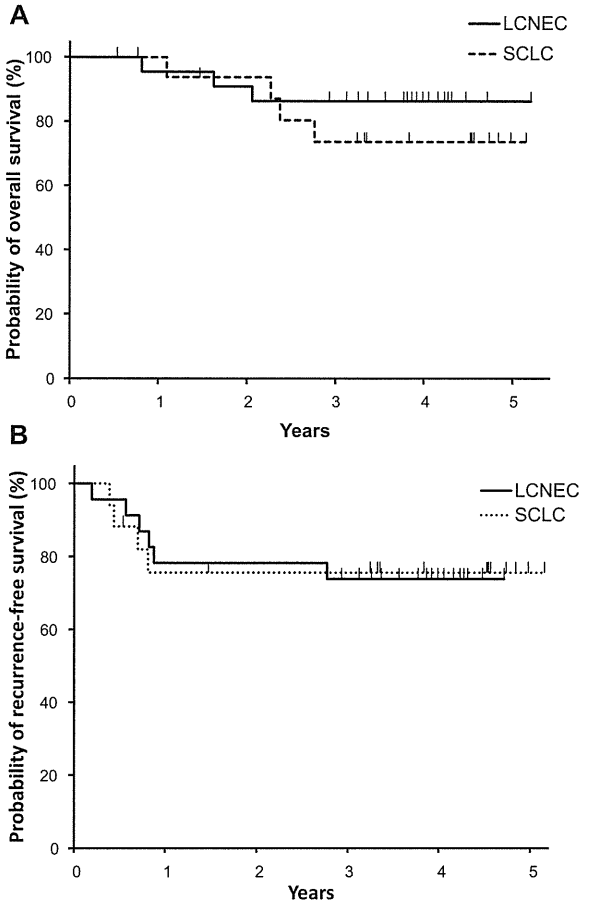

Fig. 2. (A) Overall survival curve for 23 large cell neuroendocrine carcinoma (LCNEC) patients and 17 SCLC patients. (B) Recurrence-free survival curve for 23 LCNEC patients and 17 small cell lung cancer (SCLC) patients.

3.5. Central pathological diagnosis

Pathological specimens for central review were available in all 40 patients. Twenty-eight specimens showed complete concordance of central pathological diagnosis among the seven expert pathologists. All 40 specimens were diagnosed as HGNEC at the central pathological review. There were two specimens that showed a difference between the institutional diagnosis and central pathological diagnosis. These specimens were diagnosed as LCNEC at each institution, and diagnosed as SCLC at the central pathological review.

Table 3
Treatment-related adverse events (overall, $n = 40$).

Toxicity	toxicity grade			%3–4
	2	3	4	
Leukocytes	18	7	0	18
Neutrophils	12	15	4	48
Hemoglobin	15	6	4	25
Platelets	2	0	0	0
Febrile neutropenia	–	5	0	13
Bilirubin	0	0	0	0
AST	0	0	0	0
ALT	1	0	0	0
Creatinine	0	0	0	0
Hyponatremia	0	6	0	15
Hypokalemia	0	4	0	10
Hyperkalemia	3	1	0	3
Nausea	8	4	–	10
Vomiting	4	2	0	5
Anorexia	12	4	0	10
Diarrhea	11	2	0	5
Fatigue	10	5	0	13
Constipation	3	0	0	0
Alopecia	7	–	–	0
Infection	2	0	0	0

AST: Aspartate transaminase, ALT: Alanine transaminase.

4. Discussion

Irinotecan and cisplatin showed acceptable toxicities and favorable feasibility as postoperative adjuvant chemotherapy for HGNEC of the lung. This study is the first prospective trial to evaluate the postoperative adjuvant chemotherapy of irinotecan and cisplatin for HGNEC. Although there have been no reports on a randomized trial of postoperative adjuvant chemotherapy for HGNEC, previous reports suggest the efficacy of postoperative adjuvant chemotherapy for very limited SCLC compared with surgery alone [11–15]. In addition, the guidelines of the European Society for Medical Oncology (ESMO) and American College of Chest Physicians (ACCP) recommend postoperative adjuvant chemotherapy for resected SCLC [7,16]. To our knowledge, there have been few prospective trials on postoperative adjuvant chemotherapy for resected SCLC [8,17], and only one trial for resected LCNEC [18]. In a phase II trial of adjuvant cisplatin and etoposide for resected SCLC, the 3-year survival rate was 61% [8]. In this study, the rate of overall survival at 3 years was estimated at 81%, and that of RFS at 3 years was 77%. Therefore, the combination of irinotecan and cisplatin could be effective.

The combination of irinotecan and cisplatin has been reported to be effective for extensive SCLC [9,19–21]. Retrospective analyses demonstrated that patients with advanced LCNEC who were treated with SCLC regimens, including irinotecan and cisplatin, had a better response rate and OS than those who were treated with non-small cell lung cancer (NSCLC) regimens [5,22–24]. Also, in the adjuvant setting, SCLC regimens are reported to be effective [5]. We conducted a phase II study of combination chemotherapy with irinotecan and cisplatin in 44 patients with advanced LCNEC, and the response rate and progression-free survival were 54.5% and 5.9 months, respectively [25].

In a phase II trial of adjuvant cisplatin and etoposide for resected SCLC, 77% of the patients underwent the planned three or four cycles of adjuvant chemotherapy [8]. Compliance of adjuvant chemotherapy for resected NSCLC showed that 48–74% of the patients completed the planned cycles [26–28]. In this study, 33 patients (83%) underwent the planned three or four cycles of adjuvant chemotherapy, and this compliance is comparable to these studies. The most common toxicity in our study was grade 3 or 4 neutropenia (48%), and grade 3 diarrhea was observed in only 5% of the patients. Toxicities were similar to previous reports of irinotecan and cisplatin in extensive SCLC [9,29]. Combination chemotherapy of irinotecan and cisplatin as adjuvant chemotherapy was safe with good compliance.

In conclusion, the combination chemotherapy of irinotecan and cisplatin as postoperative adjuvant chemotherapy was feasible and active in patients with resected HGNEC. This is the first prospective study of postoperative adjuvant chemotherapy for resected HGNEC. In Japan, a randomized phase III trial is ongoing to evaluate adjuvant chemotherapy of irinotecan and cisplatin, compared with etoposide and cisplatin, for completely resected HGNEC (Japan Clinical Oncology Group 1205/1206).

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Conflict of interest statement

The authors indicate no potential conflicts of interest.

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RESEARCH ARTICLE Open Access

Progression-free survival at 2 years is a reliable surrogate marker for the 5-year survival rate in patients with locally advanced non-small cell lung cancer treated with chemoradiotherapy

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Abstract

Background: In locally advanced Non-Small-Cell Lung Cancer (LA-NSCLC) patients treated with chemoradiotherapy (CRT), optimal surrogate endpoint for cure has not been fully investigated.

Methods: The clinical records of LA-NSCLC patients treated with concurrent CRT at Shizuoka Cancer Center between Sep. 2002 and Dec. 2009 were reviewed. The primary outcome of this study was to evaluate the surrogacy of overall response rate (ORR) and progression-free survival (PFS) rate at 3-month intervals (from 9 to 30 months after the initiation of treatment) for the 5-year survival rate. Landmark analyses were performed to assess the association of these outcomes with the 5-year survival rate.

Results: One hundred and fifty-nine patients were eligible for this study. The median follow-up time for censored patients was 57 months. The ORR was 72%, median PFS was 12 months, and median survival time was 39 months. Kaplan-Meier curve of progression-free survival and hazard ratio of landmark analysis at each time point suggest that most progression occurred within 2 years. With regard to 5-year survival rate, patients with complete response, or partial response had a rate of 45%. Five-year survival rates of patients who were progression free at each time point (3-months intervals from 9 to 30 months) were 53%, 69%, 75%, 82%, 84%, 89%, 90%, and 90%, respectively. The rate gradually increased in accordance with progression-free interval extended, and finally reached a plateau at 24 months.

Conclusions: Progression-free survival at 2 years could be a reliable surrogate marker for the 5-year survival rate in LA-NSCLC patients treated with concurrent CRT.

Keywords: Locally advanced non-small cell lung cancer, Chemoradiotherapy, Surrogate endpoint, Overall response rate, Progression-free survival

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Background

Lung cancer is the most common type of cancer, both worldwide and in Japan [1]. Non-small cell lung cancer (NSCLC) accounts for 80-85% of lung cancer cases, and approximately 30% of patients have unresectable, locally advanced disease at diagnosis [2]. In the 1990's, radiotherapy alone was recognized as the standard treatment, but its efficacy was insufficient [3]. Sause et al., reported that adding chemotherapy to radiotherapy brought further survival benefit [4]. A recent meta-analysis concluded that concurrent chemoradiotherapy (CRT) is state-of-the art treatment in this population [5,6].

The goal of CRT in locally advanced NSCLC (LA-NSCLC) is to cure. In the early period of treatment, tumor shrinkage is an indicator of efficacy. Although concurrent CRT provides a high rate of tumor response (60–70%), we should take into account that it does not always mean cure. Recent phase III trials of concurrent CRT reported that two-thirds of patients who experienced complete, or partial response eventually relapsed [7,8]. Another indicator of efficacy is progression-free survival (PFS). The Kaplan-Meier curves of PFS in LA-NSCLC showed the "infant mortality" type. This means that most progression occurred in the first 2 to 3 years. Therefore, we speculate that PFS rate at 2 years could be another candidate surrogate for cure.

Overall survival (OS) is the gold standard endpoint in phase III trials. However, it requires long-term follow-up, and a large number of patients. Overall response rate (ORR), median PFS, and PFS rate at specific time points were commonly adopted primary endpoints in phase II trials. However, their surrogacy for cure has not been fully investigated. The aim of this study is to search for the optimal surrogate marker of the 5-year survival rate in patients with LA-NSCLC treated with CRT.

Methods

Patient selection and treatment methods

We collected the clinical records of LA-NSCLC patients treated with concurrent CRT at Shizuoka Cancer Center between Sep. 2002 and Dec. 2009. The eligibility criteria of this study was as follows: (1) histologically or cytologically proven NSCLC; (2) chemoradiotherapy naïve; (3) age < 75 years; (4) Eastern Cooperative Oncology Group Performance Status (ECOG PS) of 0 to 2; and (5) treated with curative thoracic radiotherapy over 50Gy concurrent with platinum doublet chemotherapy.

Treatment comprised concurrent CRT and subsequent consolidation chemotherapy. Chemotherapy regimen was selected at investigator's discretion. The doses and schedules were in accordance with the published reports [7,9-12]. All patients were treated with a linear accelerator photon beam of 4 MV or more. The primary tumor and involved nodal disease were to receive at least 60 Gy in 2-Gy fractions over 6 weeks. Our radiation technique was based on elective nodal irradiation. The radiation fields contained the primary tumor, ipsilateral hilum, and mediastinal nodal areas from the paratracheal to subcarinal lymph nodes. The contralateral hilum was not included, and the supraclavicular areas were not routinely treated.

Assessment of outcomes and statistical analysis

Tumor response was classified in accordance with the Response Evaluation Criteria for Solid Tumors (RECIST), ver. 1.1. In almost all patients, tumor response was assessed every 2 courses of chemotherapy. After the treatment period, chest computed tomography (CT) was done every 2 to 3 months during the first year and at 3 to 6 month intervals thereafter. Positron emission tomography (PET) or PET-computed tomography (PET-CT) using 2-[^{18}F]-fluoro-2-deoxy-D-glucose (^{18}F-FDG) was performed at 6 to 12 month intervals if available. Magnetic resonance imaging (MRI) of the brain was performed only when clinical signs and symptoms suspicious for brain involvement were present. PFS was assessed from the first day of treatment with CRT to the earliest signs of disease progression as determined by CT or MRI imaging using RECIST criteria, or death from any cause.

The primary outcome of this study was to evaluate the surrogacy of ORR and PFS rate at 3-month intervals (from 9 to 24 months after the initiation of treatment) for the 5-year survival rate. Landmark analyses were performed to assess the association of these outcomes with the 5-year survival rate.

A p value of < 0.05 indicated statistical significance. The Kaplan-Meier method was used to estimate survival as a function of time. All the analyses were performed using JMP ver. 7 (SAS Institute Inc, USA) or R ver. 2. 15. 1. This retrospective analysis was approved by the institutional review board of Shizuoka Cancer Center.

Results

A total of 159 consecutive patients were enrolled in this retrospective study. Baseline characteristics of the patients are summarized in Table 1. Median age was 64 years, 79% of patients were male, 75% were heavy smokers, 56% had an ECOG PS of 0, 53% had adenocarcinoma, and 54% were stage IIIB. Treatment characteristics are shown in Table 2. The most common regimens were carboplatin (CBDCA) plus paclitaxel, and cisplatin (CDDP) plus S-1 (46 patients each), and the third most frequent regimen was CDDP plus vinorelbine (VNR) (41 patients). The median radiation dose was 60 Gy (range, 52–74). The median follow-up time for censored patients was 57 months. At the time of analysis, 89 patients (56%) had died and 114 patients (72%) showed disease progression.