Table 1 Patient characteristics

Characteristic	LCNEC	Possible LCNEC	*p* value
Number of patients	10	24	–
Age			
Median (range)	69 (57–83)	67 (57–78)	0.29
Gender			
Male	10	20	–
Female	0	4	
Smoking status			
Ever	10	23	–
Never	0	1	
ECOG-PS			
0	1	4	0.17
1	9	16	
2	0	4	
Stage			
IIIA	0	1	<0.01
IIIB	0	1	
IV	4	22	
Recurrence after surgery	6[a]	0	

LCNEC large cell neuroendocrine carcinoma of the lung, *ECOG-PS* Eastern Cooperative Oncology Group performance status

[a] pStage IB (4), pStage IIIA (2)

pericardium biopsy ($n = 1$), and transanal colon biopsy ($n = 1$). Positive rates in immunohistochemical staining for NE markers were as follows: NCAM was 10 (100 %) in LCNEC and 22 (92 %) in possible LCNEC, chromogranin A was 5 (50 %) in LCNEC and 12 (50 %) in possible LCNEC, and synaptophysin was 7 (70 %) in LCNEC and 16 (67 %) in possible LCNEC.

Patient characteristics are shown in Table 1. Age was similar in the LCNEC and possible LCNEC groups ($p = 0.29$). All LCNEC patients were male and ever smokers. Among the 24 possible LCNEC patients, 83.3 % were male and only 1 patient was a never smoker. Four possible LCNEC patients had Eastern Cooperative Oncology Group (ECOG) performance status (PS) 2, but no statistically significant difference in PS was found between the 2 groups ($p = 0.17$). There was a difference in stage between the 2 groups ($p < 0.05$). Among the 10 LCNEC patients, 4 patients had distant metastasis (stage IV) and 6 patients had pulmonary recurrence after surgery. All possible LCNEC patients had stage III or IV disease.

The chemotherapy regimens used are shown in Table 2. Most patients were treated with SCLC-based regimens such as platinum plus irinotecan or platinum plus etoposide. Four LCNEC patients and 11 possible LCNEC patients were treated with cisplatin plus irinotecan. Two LCNEC patients and 5 possible LCNEC patients were treated with platinum plus etoposide.

Table 2 Chemotherapy regimens

	LCNEC ($n = 10$)	Possible LCNEC ($n = 24$)
Cisplatin plus irinotecan	4	11
Cisplatin plus etoposide	0	1
Carboplatin plus etoposide	2	4
Carboplatin plus paclitaxel	4	4
Others	0	4[a]

LCNEC large cell neuroendocrine carcinoma of the lung

[a] Cisplatin plus paclitaxel (1), cisplatin plus docetaxel (1), cisplatin plus vinorelbine (1), carboplatin plus S-1 (1)

Table 3 Clinical response to first-line chemotherapy

Response	LCNEC	Possible LCNEC	*p* value
CR	1	1	
PR	6	12	
SD	1	7	
PD	2	3	
NE	0	1	
Response rate (%)	70	54	0.39
95 % CI	40–90	35–72	

LCNEC large cell neuroendocrine carcinoma of the lung, *CR* complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease, *NE* not evaluable, *CI* confidence interval

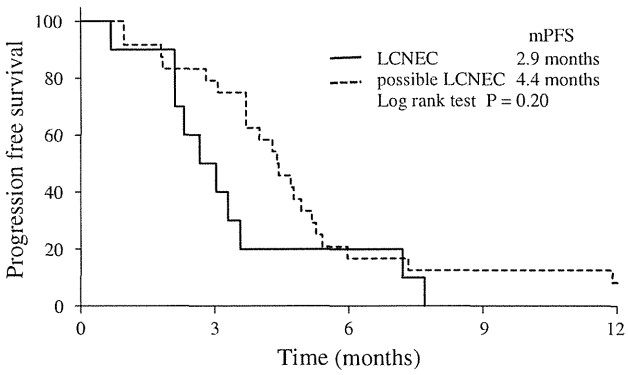

Fig. 1 Kaplan–Meier plot of progression-free survival (PFS) of patients with large cell neuroendocrine carcinomas (LCNEC) and possible LCNEC. The median PFS was 2.9 months in patients with LCNEC and 4.4 months in patients with possible LCNEC ($p = 0.20$)

The response rate was 70 % in LCNEC patients and 54 % in possible LCNEC patients (Table 3); and no statistically significant difference was found ($p = 0.39$).

The Kaplan–Meier curve for PFS is shown in Fig. 1. The median PFS was 2.9 months in the LCNEC group and 4.4 months in the possible LCNEC group ($p = 0.20$). The Kaplan–Meier curve for OS is shown in Fig. 2. The median

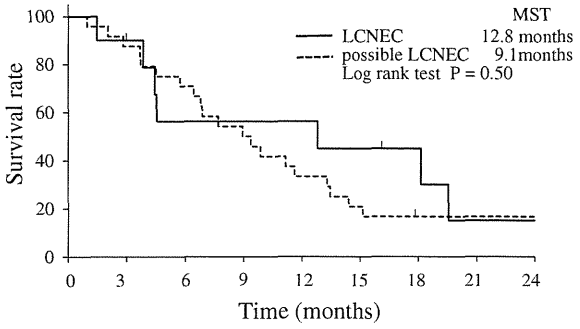

Fig. 2 Kaplan–Meier plot of overall survival of patients with large cell neuroendocrine carcinomas (LCNEC) and possible LCNEC. The median survival time (MST) was 12.8 months in patients with LCNEC and 9.1 months in patients with possible LCNEC ($p = 0.50$)

survival time (MST) was 12.8 months in the LCNEC group and 9.1 months in the possible LCNEC group ($p = 0.50$). In the present study, the median follow-up duration was 23.2 months.

Nine LCNEC patients and 15 possible LCNEC patients received second-line chemotherapy. Six LCNEC patients and 6 possible LCNEC patients were treated with amrubicin. Only 1 LCNEC patient who was treated with amrubicin showed a partial response.

Discussion

To the best of our knowledge, the present study is the first report comparing the efficacy of chemotherapy for LCNEC in patients diagnosed with LCNEC with that in patients diagnosed with possible LCNEC. In the present study, in the possible LCNEC group, the response rate was 54 % and the MST was 9.1 months. No statistically significant differences in the response rate and OS were found between the 2 groups.

Igawa et al. [7] evaluated 14 advanced possible LCNEC cases and showed that the response rate was 50 % and the MST was 10 months. In addition, Shimada et al. [8] analyzed 13 patients regarded as possible LCNEC with high-grade neuroendocrine carcinoma of the lung and reported that the response rate to first-line chemotherapy was 61 % and the MST was 12 months. These results were comparable to those of extensive disease (ED)-SCLC [7, 8] and to those in the possible LCNEC group in the present study. Resected LCNEC has been reported to be similar to SCLC in clinicopathological features and prognosis [5, 6].

Mazieres et al. [12] reported that 13 cases (72 %) of resected LCNEC relapsed with distant metastases, and 10 of these relapsed within 6 months. The 13 relapsed LCNEC cases were treated with platinum plus etoposide, and the response rate was 20 %. Other authors reported that the response rate of LCNEC was 50–59 % and the MST was 8–10.3 months, with most recurrences occurring after surgery [13, 14]. For LCNEC cases treated with cisplatin-based chemotherapy, the response rate was comparable to that of SCLC. Rossi et al. [15] reported that in 12 patients treated with platinum plus etoposide, the response rate was 50 % and the MST was 51 months, although 3 cases received radiotherapy in addition to chemotherapy. In previous studies, with 1 exception [12], the chemotherapeutic response of recurrent LCNEC was as good as that of SCLC [13–15]. In addition, Rossi et al. [15] reported that in another 15 patients treated with NSCLC-based regimens, the response rate was 0 % and the MST was 21 months. In the present study, an objective response was obtained in 4 of 6 LCNEC patients (66 %) who received platinum plus irinotecan or platinum plus etoposide, so-called SCLC-based regimens, and in 9 of 16 possible LCNEC patients (56 %) who received SCLC-based regimens. These results suggest that SCLC-based regimens might be effective for both LCNEC and possible LCNEC. In addition, the present study also indicated that treatment with paclitaxel-containing regimens might be effective for LCNEC and possible LCNEC. These anticancer drugs will be key to the treatment of LCNEC and possible LCNEC.

This study has several limitations. It was a retrospective study with an inherent potential for bias. Collection of clinical characteristics and treatment response data was retrospective and the follow-up interval for physical examinations was indefinite. The sample size was small. Therefore, future studies would benefit from investigating a much larger sample.

In conclusion, no statistically significant differences were found in the response rate, PFS, and OS between the LCNEC and possible LCNEC groups. These results suggest that possible LCNEC is similar to LCNEC in chemotherapeutic efficacy. In the future, a study of a larger series of LCNEC patients is mandatory to confirm the role of chemotherapeutic strategy.

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

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Molecular profiling of small cell lung cancer in a Japanese cohort

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ABSTRACT

Objectives: Advances in the molecular profiling of lung adenocarcinoma over the past decade have led to a paradigm shift in its diagnosis and treatment. However, there are very few reports on the molecular profiles of small cell lung cancers (SCLCs). We therefore conducted the present Shizuoka Lung Cancer Mutation Study to analyze genomic aberrations in patients with thoracic malignancies.
Materials and methods: We collected samples of SCLC from a biobank system and analyzed their molecular profiles. We assessed 23 mutations in nine genes (*EGFR*, *KRAS*, *BRAF*, *PIK3CA*, *NRAS*, *MEK1*, *AKT1*, *PTEN*, and *HER2*) using pyrosequencing plus capillary electrophoresis. We also amplified *EGFR*, *MET*, *PIK3CA*, *FGFR1*, and *FGFR2* using quantitative real-time polymerase chain reaction (PCR) and the fusion genes *ALK*, *ROS1*, and *RET* using reverse transcription PCR.
Results: Between July 2011 and January 2013, 60 SCLC patients were enrolled in the study. Samples included eight surgically resected snap-frozen samples, 50 formalin-fixed paraffin-embedded samples, and seven pleural effusion samples. We detected 13 genomic aberrations in nine cases (15%), including an *EGFR* mutation ($n = 1$, G719A), a *KRAS* mutation ($n = 1$, G12D), *PIK3CA* mutations ($n = 3$, E542K, E545K, E545Q), an *AKT1* mutation ($n = 1$, E17K), a *MET* amplification ($n = 1$), and *PIK3CA* amplifications ($n = 6$). *EGFR* and *KRAS* mutations were found in patients with combined SCLC and adenocarcinoma. No significant differences were detected in the characteristics of patients with and without genomic aberrations. However, serum neuron-specific enolase and progastrin-releasing peptide levels were significantly higher in patients without genomic aberrations than in those with aberrations ($p = 0.01$ and 0.04, respectively).
Conclusion: Genomic aberrations were found in 15% SCLC patients, with *PIK3CA* amplifications most frequently observed. To further our understanding of the molecular profiles of SCLC, comprehensive mutational analyses should be conducted using massive parallel sequencing.

1. Introduction

Lung cancer is the most common cause of cancer-related deaths, and small cell lung cancer (SCLC) accounts for approximately 12% of all lung cancers [1]. It follows a very aggressive course, with approximately 60–70% patients having disseminated disease at diagnosis. Although SCLC shows high sensitivity to chemotherapy and radiotherapy, the median survival time for extended-disease SCLC is 8–13 months, and the 2-year survival rate is only 5% [2].

Molecular abnormalities have been discovered in patients with non-SCLC over the last decade, and these discoveries have led to a paradigm shift in its diagnosis and treatment. For example, a relationship between activating epidermal growth factor receptor (*EGFR*) mutations and response to gefitinib was reported in 2004 [3,4]. Subsequently, a number of randomized studies showed that patients with activating *EGFR* mutations were highly responsive to

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EGFR tyrosine kinase inhibitors such as gefitinib and erlotinib [5–8]. Currently, it is essential that lung adenocarcinomas are classified on the basis of genomic aberrations to ensure that patients are treated with the appropriate molecular-targeted drugs [9,10]. Analyses of genomic aberrations and the development of new molecular-targeted drugs are ongoing for lung adenocarcinoma. In contrast, there have been few innovations in the treatment of SCLC, despite extensive basic and clinical research over the past 30 years.

There have been few molecular profiles of SCLC, and, till date, no molecular-targeted drugs have shown clinical activity against SCLC [11]. Identification of genomic aberrations linked to SCLC would facilitate the identification of potential therapeutic targets.

We conducted the present Shizuoka Lung Cancer Mutation Study to assess genomic aberrations in patients with thoracic malignancies. A biobank system was established in collaboration with a clinic pathology lab in July 2011. Mutational data were communicated to clinicians and utilized for assigning patients to appropriate therapy and/or enrolling them in clinical trials. Here we report the genomic aberrations identified in patients with SCLC in the Shizuoka study.

2. Materials and methods

2.1. Patients

We collected samples of SCLC from a biobank system and analyzed these to determine their molecular profiles. To evaluate the relationships between any genomic aberrations and patient characteristics, we collected patient demographic and clinical data from medical records. All patients who participated in this study provided their written informed consent.

Pathological diagnoses were made by institutional pathologists according to the 2004 World Health Organization classification based on morphology (uniform round to spindle-shaped small cells, sparse cytoplasm, high mitotic index, and necrotic areas). The diagnosis of SCLC was confirmed when necessary by immunohistochemical analyses of neuroendocrine markers (synaptophysin, chromogranin A, and CD56). And when it is difficult to diagnose samples as SCLC, we additionally performed immunohistochemistry with makers, such as CAM5.2, TTF-1 and Keratin. If more than 10% of a sample comprised adenocarcinoma, the patient was diagnosed with combined SCLC and adenocarcinoma. Surgically resected samples were macrodissected before nucleic acid extraction and tumor biopsy samples with 10% or more tumor cell component were tested for mutational profiling [12]. All of pleural effusion samples were confirmed that malignant cells were present in each pleural effusion by cytology and we analyzed the cytologically confirmed pleural effusion specimens subsequently.

Smokers were defined according to the Brinkman index (BI) as light (BI value < 600) or heavy (BI value ≥ 600) smokers. Limited stage-disease was defined as disease confined to one hemithorax, the ipsilateral supraclavicular fossa, or both. Disease not meeting these criteria was defined as extended-stage disease. Serum neuron-specific enolase (NSE) levels were measured using a solid-phase radioimmunoassay (RIA) method (SRL Inc., Tokyo, Japan), and progastrin-releasing peptide (Pro-GRP) levels were measured using an enzyme-linked immunosorbent assay (ELISA) kit (FUJIREBIO Inc., Tokyo, Japan).

2.2. Clinical genotyping

We developed a multiplexed tumor genotyping platform to assess 23 mutations in nine genes (*EGFR*, *KRAS*, *BRAF*, *PIK3CA*, *NRAS*, *MEK1*, *AKT1*, *PTEN*, and *HER2*), *EGFR*, *MET*, *PIK3CA*, *FGFR1*, and *FGFR2* amplifications, and *EML4-ALK*, *KIF5B-RET*, *CD74-ROS1*, and *SLC34A2-ROS1* fusion genes (Table 1).

Table 1
Multiplexed tumor genotyping panel.

Gene name	Position	AA mutant	Nucleotide mutant
EGFR	G719	G719	2155G > T/A
		G719A	2156G > C
	exon 19	Deletion	
	T790	T790M	2369C > T
	exon 20	Insertion	
	L858	L858R	2573T > G
	L861	L861Q	2582T > A
KRAS	G12	G12C/S/R	34G > T/A/C
		G12V/A/D	35G > T/C/A
	G13	G13C/S/R	37G > T/A/C
		G13D/A	38G > A/C
	Q61	Q61K	181C > A
		Q61R/L	182A > G/T
		Q61H	183A > T/C
BRAF	G466	G466V	1397G > T
	G469	G469A	1406G > C
	L597	L597V	1789C > G
	V600	V600E	1799T > A
PIK3CA	E542	E542K	1624G > A
	E545	E545K/Q	1633G > A/C
	H1047	H1047R	3140A > G
NRAS	Q61	Q61K	181C > A
		Q61L/R	182A > T/G
MEK1 (MAP2K1)	Q56	Q56P	167A > C
	K57	K57N	171G > T
	D67	D67N	199G > A
AKT1	E17	E17K	49G > A
PTEN	R233	R233*	697C > T
HER2	exon 20	Insertion	

2.3. Nucleic acid sample preparation

DNA samples were extracted from surgically resected tissues, body cavity fluids, and tumor biopsy sections using a QIAamp DNA mini kit (QIAGEN, Hilden, Germany) or a QIAamp DNA formalin-fixed paraffin-embedded (FFPE) tissue kit (QIAGEN). The DNA concentration was measured using a Quant-iT PicoGreen dsDNA assay kit (Invitrogen, Carlsbad, CA). Total RNAs were isolated with an RNeasy Mini kit (QIAGEN) and measured using a spectrophotometer (NanoDrop 2000C; Thermo Scientific, Wilmington, DE).

2.4. Pyrosequencing

Pyrosequencing was used to detect single base substitution-type mutations. An internal fragment of each gene was amplified by polymerase chain reaction (PCR) using primers specific for each gene and a PyroMark PCR kit (QIAGEN). The resulting PCR products were sequenced with the PyroMark Q24 (QIAGEN) pyrosequencer using PyroMark Gold Q96 reagents (QIAGEN) and sequencing primers specific for each gene.

2.5. Fragment analysis

Insertion/deletion-type mutations were identified by sizing the PCR-amplified products using capillary electrophoresis (QIAxcel, QIAGEN).

2.6. Gene copy number analysis

Copy number was evaluated by quantitative real-time PCR (qRT-PCR) performed on a StepOnePlus Real time PCR system (Applied

Biosystems) using SYBR® Premix Ex Taq™ II (Tli RNaseH Plus) (TAKARA BIO) and PCR primers for each gene. If the gene copy number from the samples was more than double that of the cell line known to be normal human genomic DNA, it was considered as evidence of amplification. Detailed methods are described previously [12].

2.7. Screening for transcripts of fusion genes

Fusion genes were detected by multiplex RT-PCR. Synthesis of cDNA templates was performed with total RNA (1 μg) using Oligo $(dT)_{12-18}$ Primer (Invitrogen) and Omniscript RT (QIAGEN) kits. *EML4-ALK* and *ROS1* fusion genes were detected according to the methods of Sun et al. [13] and Li et al. [14], respectively. Methods for the detection of *KIF5B-RET* fusions were kindly provided by Dr. Takashi Kohno (National Cancer Center, Tokyo).

2.8. Statistical analysis

All categorical variables were analyzed by the chi-square test or Fisher's exact test, as appropriate. Continuous variables, including tumor markers, were analyzed using the Mann–Whitney test. All *p*-values were reported to be two-sided, and values of <0.05 were considered statistically significant. All statistical analyses were performed using JMP version 9.0 software (SAS Institute Inc., Cary, NC, USA). Our study was approved by the Institutional Review Board.

3. Results

3.1. Patient characteristics

Between July 2011 and January 2013, SCLC samples from 60 patients were assessed for genomic aberrations. The patient characteristics are shown in Table 2. The median age (range) was 69 (43–82) years, and most patients were male (83%) and heavy smokers (80%). Only two patients were never-smokers. A total of 57 patients were diagnosed with SCLC, while three were diagnosed with combined SCLC and adenocarcinoma. Thirty-one patients had limited-stage disease and 29 had extended-stage disease. We analyzed eight surgically resected snap-frozen samples, 50 FFPE samples, and seven pleural effusion samples. Five patients provided two specimens: three provided both FFPE and surgically resected snap-frozen samples and two provided both FFPE and pleural effusion samples (Table 3).

Table 2
Patients characteristics that were analyzed in our study (overall, *N* = 60).

	N = 60	%
Median age (years)	69	
Range	43–82	
Gender		
Male	50	83
Female	10	17
Smoking status		
Never	2	3
Light (B.I. < 600)	10	17
Heavy (B.I. ≥ 600)	48	80
Histology		
Small cell carcinoma	57	95
Combined small cell carcinoma with adenocarcinoma	3	5
Disease extent		
Limited stage	31	52
Extended stage	29	48
Samples		
Surgically resected snap-frozen samples	8	
FFPE samples	50	
Pleural effusion	7	

Abbreviation: B.I., Brinkman index; FFPE, Formalin-fixed paraffin-embedded.

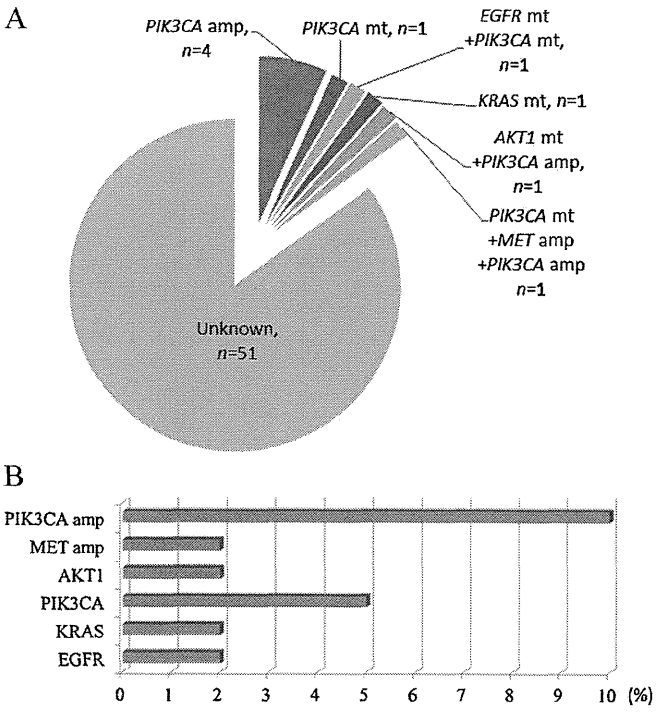

Fig. 1. Relative proportions of genomic aberrations in small cell lung cancer (*N* = 60). (A) Pie chart shows relative proportions of genomic aberrations. (B) Bar chart shows relative proportions of genomic aberrations. *Abbreviations*: mt: mutation; amp: amplification.

3.2. Genomic aberrations

We detected 13 genomic aberrations in nine cases (15%): an *EGFR* mutation (*n* = 1, G719A), a *KRAS* mutation (*n* = 1, G12D), *PIK3CA* mutations (*n* = 3; E542K, E545K, E545Q), an *AKT1* mutation (*n* = 1, E17K), a *MET* amplification (*n* = 1), and *PIK3CA* amplifications (*n* = 6; Fig. 1A and B).

Table 4 shows the individual characteristics of the SCLC patients who harbored genomic aberrations. Eight of the nine patients with genomic aberrations were male, and all were smokers. Two patients were diagnosed with SCLC combined with adenocarcinoma; an *EGFR* mutation was detected in one patient and a *KRAS* mutation in another. The patient with the *EGFR* mutation provided both FFPE and surgically resected snap-frozen samples, but the *EGFR* mutation was detected only in the snap-frozen samples. Genomic aberrations were detected in nine of the 50 FFPE samples, one of eight surgically resected snap-frozen samples, and none of the seven pleural effusion samples.

3.3. Comparison of patient characteristics and genomic aberrations

Patient characteristics are classified by genomic aberration status in Table 4. No significant differences in age, sex, disease extent at diagnosis, or smoking status were found between patients with and without genomic aberrations according to univariate analysis. However, serum NSE and Pro-GRP levels at diagnosis were significantly higher in patients without genomic aberrations than in those with genomic aberrations ($p = 0.02$ and $p = 0.04$, respectively).

Table 3
Patients characteristics that genomic aberrations were detected.

	Age	Gender	B.I.	Disease extent	TNM stage	Samples	Pathology	Genomic aberrations
1	73	Male	2760	LS	IA	FFPE	Small cell carcinoma	PIK3CA amp (3.14)
2	69	Male	1880	LS	IIA	FFPE	Small cell carcinoma	PIK3CA amp (4.42)
3	82	Male	1500	LS	IIIA	FFPE	Small cell carcinoma	PIK3CA amp (2.65)
4	58	Male	1000	ES	IV	FFPE	Small cell carcinoma	PIK3CA (E545K)
5	69	Male	940	LS	IIIA	FFPE	Small cell carcinoma	AKT1 (E17K), PIK3CA amp (2.49)
6	66	Male	840	ES	IIIB	FFPE	Small cell carcinoma	PIK3CA (E542K), MET amp (4.13), PIK3CA amp (3.62)
7	73	Male	795	LS	IIB	FFPE, snap-frozen samples	Small cell carcinoma combined with adenocarcinoma	EGFR (G719A), PIK3CA (E545Q)
8	74	Male	590	ES	IV	FFPE	Small cell carcinoma combined with adenocarcinoma	KRAS (G12D)
9	80	Female	500	LS	IIA	FFPE	Small cell carcinoma	PIK3CA amp (2.78)

Abbreviations: LS, limited stage; ES, extended stage; FFPE, formalin-fixed paraffin-embedded.

Table 4
Patients characteristics classified by genomic aberration status.

	Genomic aberration		*P* value
	Detected	Not detected	
N (%)	9 (15%)	51 (85%)	
Age at diagnosis (years)			0.26
Median	73	69	
Range	58–82	43–82	
Gender, *n* (%)			0.63
Male	8 (89%)	42 (82%)	
Female	1 (11%)	9 (18%)	
Disease extent at diagnosis, *n* (%)			0.32
Limited stage	6 (67%)	25 (49%)	
Extended stage	3 (33%)	26 (51%)	
Smoking status			0.78
Never	0	2	
Light (B.I. < 600)	2	8	
Heavy (B.I. ≥ 600)	7	41	
Serum neuron-specific enolase (NSE) level at diagnosis			0.02
n	9	48	
Median	14	37.1	
Range	7.8–34	6.4–334	
Serum pro-gastrin releasing peptide (Pro-GRP) level at diagnosis			0.04
n	8	47	
Median	75.5	738	
Range	43.1–1500	26.4–65900	

Abbreviation: B.I., Brinkman index.

4. Discussion

As per our knowledge, this was the first molecular profiling report of Asian patients with SCLC, wherein we detected genomic aberrations in 15% patients. *PIK3CA* amplifications were detected in 10% of all samples assessed, while *PIK3CA* mutations were detected in 5%. *PIK3CA* genomic aberrations were detected in eight of the nine patients with genomic aberrations. Recently, two independent comprehensive genomic studies of SCLC were published [15,16]. Peifer et al. [14] analyzed 99 SCLC specimens using 6.0 SNP array analyses and exome, transcriptome, and genome sequencing. They detected *TP53* and *RB1* alterations in 88% and 66% cases, respectively, *MYC* family member and *FGFR1* amplifications in 16% and 6% cases, respectively, and *CREBBP* and *EP300* and *PTEN* mutations in 18% and 10% cases, respectively. They did not detect any *PIK3CA* aberrations. Rudin et al. [15] analyzed 80 SCLC samples, including SCLC cell lines, using multiple exome sequencing, single genome analysis, genome-wide copy-number analysis, and whole-transcriptome sequencing and detected *TP53* and *RB1* mutations in 77% and 31% samples, respectively, a *SOX2* amplification in 27%, and a recurrent *RLF-MYCL1* fusion in 9%. In their study, *PIK3CA* mutation was detected in 2 of 30 primary SCLC tumor samples by exome capture followed by next generation sequencing (Rudin's report online methods). Recently, Umemura et al. undertook a comprehensive genomic analysis of SCLC in Japanese patients [17]. They analyzed 51 surgically resected SCLC samples using whole exome sequencing and copy-number analysis. Genetic alterations in the *PI3K* pathway (*PIK3CA*, *PTEN*, *AKT2*, *AKT3*, *RICTOR*, *mTOR*) were detected in 17 of 47 samples (36%). *PIK3CA* mutations were detected in three of the 47 samples (6%), which is consistent with the findings from our study.

Okudela et al. reported that *PIK3CA* amplification was detected in 1 of 3 samples (33.3%) and *PIK3CA* gene mutation was detected in

1 of 5 samples (20%) in Japanese patients with SCLC [18]. Although *PIK3CA* mutation is the major genomic aberration in Japanese SCLC patients, the larger study, such as our study and Umemura's report, detected it in approximately 5% of SCLC samples. Based on these results, there does not seem to be significant ethnic differences in the prevalence of *PIK3CA* mutation and *PIK3CA* mutation may be one of the major genomic alterations for the SCLC patients. The *PI3K* pathway plays a central role in cell proliferation and survival in human cancer [19]. The *PIK3CA* gene encodes a class IA PI3K catalytic subunit p110α and is frequently mutated in some of the most common human tumors [20]. Wojtalla et al. showed that approximately 25% primary SCLC tissue samples overexpress the PI3K isoform p110α [21]. They also reported that targeting PI3K p110α affected the proliferation of SCLC cells *in vitro* and *in vivo* and that p110α inhibition led to impaired SCLC tumor formation and vascularization *in vivo*. Many drugs targeting class IA PI3K have been developed [22], and preclinical studies have shown these to have potent antitumor activity. Some have led to a decrease in advanced solid tumors in phase I studies [23,24]; therefore, *PIK3CA* may be a suitable target for the treatment of SCLC.

EGFR and *KRAS* mutations were detected in the patients with combined SCLC and adenocarcinoma in our study. Tatematsu et al. analyzed 122 SCLC patients and detected *EGFR* mutations in 5 (4%) [25]. Their study included 15 combined subtype patients, and 20% of these had *EGFR* mutations. Compared with conventional SCLC, *EGFR* mutations are found significantly more frequently in the combined subtype. Fukui et al. retrospectively studied six patients with combined SCLC and adenocarcinoma and analyzed the *EGFR* mutation status in the microdissected SCLC and adenocarcinoma components of their resected samples [26]. In their report, one of six patients had a missense mutation in *EGFR* (L858R), and both the SCLC and adenocarcinoma components shared the same mutation. Gene mutation status in tissue samples from SCLC with other histology component remain an open question. Therefore it is necessary to perform microdissection in the future study. To the best of our knowledge, there has been no previous report of *KRAS* mutations in SCLC. In our study, a *KRAS* mutation was detected in one patient with combined SCLC and adenocarcinoma.

No significantly different characteristics were found between patients with and without genomic aberrations in the present study. Although the associations between serum tumor markers and genomic aberrations were unclear, serum NSE and pro-GRP levels at diagnosis were significantly lower in the patients with genomic aberrations. Pujol et al. reported that pro-GRP levels did not have any independent prognostic significance [27], while NSE levels have been shown to have better prognostic value [28]. We could not detect an association between prognosis and genomic aberration status (data not shown). Further studies are needed to clarify the relationships between genomic aberrations and serum tumor marker values.

In this study, genomic aberrations were detected in 18% FFPE samples and 13% surgically resected snap-frozen samples. The National Comprehensive Cancer Network (NCCN) guideline recommends that surgery should only be considered for patients with stage I SCLC. However, another report stated that only 5% patients with SCLC have true stage I SCLC [29]. Because surgery is not performed in most patients with SCLC, FFPE samples play a key role in detecting genomic aberrations. Kenmotsu et al. reported on the concordance between FFPE samples and surgically resected snap-frozen samples in multiplexed molecular profiling of lung cancers [30]. Complete concordance of driver mutations was shown for 65% FFPE and snap-frozen samples. These findings indicate that it may be better to analyze FFPE samples to identify SCLC molecular profiles and treat patients with molecular-targeted drugs such as PI3K inhibitors.

Our study had several limitations. First, we analyzed SCLC genomic aberrations using a nine-gene tumor genotyping panel, not a comprehensive panel. In addition, we did not include some known driver mutations such as *TP53* and *RB1* mutations in the panel. However, the objectives of our study were not only to assess the frequency of genomic aberrations but also to detect genomic aberrations that are treatable with targeted drugs, and our multiplexed tumor genotyping platform includes almost all known gene aberrations that are targeted by drugs. And detection of gene amplification may also require consideration of incorporating FISH for future studies. Second, we only analyzed 60 SCLC patients because we only began to analyze genomic aberrations in July 2011. However, other reports have also included a small number of samples. We continue to analyze SCLC samples and utilize the findings for targeted therapy of patients with SCLC.

5. Conclusions

In conclusion, genomic aberrations were found in 15% SCLC patients, with *PIK3CA* amplifications being frequently detected. We previously reported our massive parallel sequencing findings for non-SCLC [31], and we plan to undertake a similar analysis of SCLC samples. A larger study is necessary to further our understanding of the molecular profiles of SCLC.

Conflicts of interest

None of the authors have any financial or personal relationship with other individuals or organizations that could inappropriately influence this study.

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Original Article

Progression-free survival, post-progression survival, and tumor response as surrogate markers for overall survival in patients with extensive small cell lung cancer

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Abstract:

OBJECTIVES: The effects of first-line chemotherapy on overall survival (OS) might be confounded by subsequent therapies in patients with small cell lung cancer (SCLC). We examined whether progression-free survival (PFS), post-progression survival (PPS), and tumor response could be valid surrogate endpoints for OS after first-line chemotherapies for patients with extensive SCLC using individual-level data.

METHODS: Between September 2002 and November 2012, we analyzed 49 cases of patients with extensive SCLC who were treated with cisplatin and irinotecan as first-line chemotherapy. The relationships of PFS, PPS, and tumor response with OS were analyzed at the individual level.

RESULTS: Spearman rank correlation analysis and linear regression analysis showed that PPS was strongly correlated with OS ($r = 0.97$, $p < 0.05$, $R^2 = 0.94$), PFS was moderately correlated with OS ($r = 0.58$, $p < 0.05$, $R^2 = 0.24$), and tumor shrinkage was weakly correlated with OS ($r = 0.37$, $p < 0.05$, $R^2 = 0.13$). The best response to second-line treatment, and the number of regimens employed after progression beyond first-line chemotherapy were both significantly associated with PPS ($p \leq 0.05$).

CONCLUSION: PPS is a potential surrogate for OS in patients with extensive SCLC. Our findings also suggest that subsequent treatment after disease progression following first-line chemotherapy may greatly influence OS.

Key words:

Extensive small cell lung cancer, overall survival, post-progression survival, progression-free survival, tumor response

Lung cancer is one of the leading causes of cancer-related mortality worldwide. In 2007, 1.3 million people were diagnosed with lung cancer, 15-20% of whom were found to have small cell lung cancer (SCLC).[1,2] Overall survival (OS) is considered the most reliable endpoint in cancer studies, and when studies can be conducted to adequately assess survival, it is usually the preferred endpoint.[3] OS is a precise endpoint, is easy to measure, and can be documented by the date of death. Surrogate endpoints such as tumor response and progression-free survival (PFS) are also useful endpoints for phase II oncology clinical trials because they can be measured earlier and more conveniently. Events for these surrogate endpoints occur more frequently than do events for the main endpoints of interest, which are referred to as the true endpoints.

The effects of first-line chemotherapy on OS might be confounded by subsequent therapies. Indeed, PFS improvements do not necessarily result in an improved OS, as shown by recent randomized trials in patients with non-SCLC (NSCLC).[4] In recent years, a growing number of active compounds have become available as second- or third-line chemotherapy for breast, ovarian, and colorectal cancers[5-7], as well as advanced NSCLC. However, with respect to the treatment of SCLC, first-line chemotherapy is often beneficial for patients with poor performance status (PS), in contrast with NSCLC cases, albeit at the risk of serious toxic effects. SCLC is a distinct clinical and histological entity within the range of lung cancers. Only a few drugs are available for its treatment, and topotecan is currently the only drug approved for the treatment of relapsed SCLC patients in the United States.[8-10] Second-line treatment is an option in only a few patients, owing to rapid disease progression and poor PS.

Although PFS following first-line chemotherapy has not been validated as a surrogate endpoint for OS, post-progression survival (PPS) has been shown to be strongly associated with OS after first-line chemotherapy for advanced NSCLC.[11,12] Furthermore, it has been suggested that OS can be approximated as the sum of PPS and PFS.[3] Very few novel anticancer drugs have become available for extensive SCLC, and the relationship between PPS and OS in extensive SCLC remains unclear.

At the level of the individual patient, it is of interest to assess the effect of therapy administered after disease progression on survival. The validation of surrogate measures for OS after first-line therapy in individual patients with advanced NSCLC has been reported previously.[13] Further, the surrogate endpoint sometimes does not reflect the primary endpoint. The significance of PPS in SCLC also remains unclear at the level of the individual patient. Therefore, it is important to establish whether PFS, PPS, or tumor response could be valid surrogate endpoints for OS after first-line therapy in patients with extensive SCLC using individual-level data.

The first-line treatment of choice in extensive-stage SCLC remains 4 to 6 cycles of platinum combination chemotherapy.[1] Although many patients initially achieve clinical remission or disease control with first-line chemotherapy, most subsequently experience disease progression and eventually die of extensive SCLC. We examined first-line cisplatin and irinotecan combination chemotherapy because it is considered the standard first-line chemotherapy in these cases.[1] Previously, in a phase 3 study of extensive SCLC, first-line chemotherapy with irinotecan plus cisplatin was found to be more effective than etoposide/cisplatin (median survival of 12.8 months versus 9.4 months, $p = 0.002$).[14] The MST of patients with extensive SCLC was approximately 1 year. For extensive SCLC patients, OS is shorter and options for subsequent chemotherapy are limited.

In the present study, we analyzed the relationships of PFS, PPS, and tumor response with OS in patients with extensive SCLC at the individual level. The patients recruited to this study had only a limited number of options for subsequent-line chemotherapy. We also explored the prognostic value of baseline and tumor characteristics for PPS.

Methods

Patients

Between September 2002 and November 2012, 60 patients with extensive SCLC were treated with cisplatin and irinotecan as first-line chemotherapy and were enrolled in this study. The tumor response was not evaluated in 10 cases, and PFS data were censored in one case. These 11 patients were excluded from the analyses to maintain uniformity in patient background characteristics. Thus, data from 49 patients were analyzed. The study protocol was approved by the Institutional Review Board of Shizuoka Cancer Center (#.25-J91-25-1-3).

The patients in this study were treated with cisplatin (60 mg·m^{-2} day^{-1} for 1 day, followed by a pause of 28 days) and irinotecan (60 mg·m^{-2}day^{-1} on days 1, 8, and 15, followed by a pause of 28 days). This cycle was repeated every 28 days for a maximum of six courses.

The best overall response and maximum tumor shrinkage were recorded as tumor responses. Radiographic tumor responses were evaluated according to the Response Evaluation Criteria In Solid Tumors,ver. 1.1[15]: Complete response (CR), disappearance of all target lesions; partial response (PR), at least a 30% decrease in the sum of the target lesion diameters with the summed baseline diameters as a reference; progressive disease (PD), at least a 20% increase in the sum of the target lesion diameters with the smallest sum observed during the study serving as reference; and stable disease (SD), insufficient shrinkage to qualify as PR and insufficient expansion to qualify as PD. PFS was calculated from the start of treatment to the date of PD or death from any cause. OS was recorded from the first day of treatment until death or was censored on the date of the last follow-up consultation. PPS was recorded as the time from tumor progression until death or was censored on the date of the last follow-up consultation. In this study, we defined treatment-free interval (TFI) as the period from the date of completion of first-line treatment to the first relapse. When prophylactic cranial irradiation (PCI) was performed as first-line treatment, the date of completion was defined as the last day of these treatments. We defined sensitive relapse as TFI $\geq$ 90 days, based on the definition in several previous trials.[16,17]

Statistical analyses

To examine whether PFS, PPS, or tumor shrinkage was correlated with OS, we used Spearman rank correlation analysis and linear regression analysis. In order to identify possible prognostic factors for PPS, the proportional hazards model with a stepwise regression procedure was applied. Hazard ratios (HR) and 95% confidence intervals (CI) were estimated using this model. Because the HR is defined for a 1-unit difference, some factors were converted to an appropriately scaled unit. PPS values were compared using the log-rank test. A P value of ≤ 0.05 was considered significant for all tests. The two-tailed significance level was also set at 0.05. All statistical analyses were performed using JMP version 9.0 for Windows (SAS Institute, Cary, NC, USA).

Results

Patient characteristics and treatment efficacy

Of the 49 patients included in the analyses, 43 patients died; the median follow-up time was 14.0 months (range, 0.7-36.8 months). The characteristics of the 49 patients (median age, 63 years; range, 43-75 years) included in the present study are shown in Table 1. Target lesions were not evaluated in one of the cases. One, 38, 5, and 4 patients showed CR, PR, SD, and PD, respectively. The response rate was 79.6% and the disease control rate was 91.8%.

After progressing past first-line chemotherapy, 5 of the 49 patients did not receive further chemotherapy. The other 44 patients received subsequent chemotherapy after completing their first-line chemotherapy. Among the 49 patients, the median number of follow-up therapeutic regimens was 2 (range, 0-5 regimens). The chemotherapy regimens employed, after progressing past the first-line chemotherapy regimen, are shown in Table 2. Amrubicin was the most common second-line chemotherapy agent, and paclitaxel was the most common third-line chemotherapy agent.

The median PFS and OS were 5.5 months and 13.9 months, respectively [Figure 1a, 1b].

Table 1: Baseline patient characteristics

Characteristic	
Gender	
Male/female	44/5
Median age at treatment (years)	63 (43-75)
Performance Status (PS)	
0/1/≥2	13/32/4
Histology	
Small cell carcinoma/others	49/0
Stage	
IIIB/IV	0/49
Number of first-line chemotherapy courses	
1/2/3/4/5/6	1/4/3/38/2/1
Median (range)	4 (1-6)
Number of regimens after progression following first-line chemotherapy	
0/1/2/3/4/5	5/18/13/8/3/2
Median (range)	2 (0-5)
Median sum of target lesion diameters [mm] (range)	112 (29-287)
Prophylactic cranial irradiation	
Yes/No	3/46
Median treatment-free interval [days] (range)	68 (29-287)

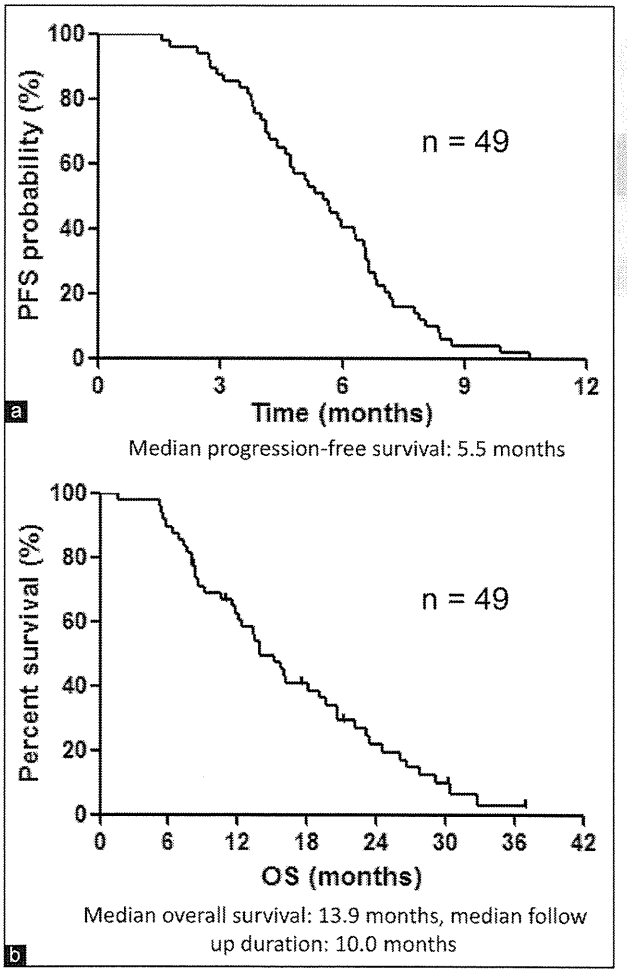

Figure 1: (a) Kaplan-Meier plots showing progression-free survival (PFS) (b) Kaplan-Meier plots showing overall survival (OS)

Relationship between OS and PFS, PPS, and tumor shrinkage

The relationship between OS and PFS, PPS, and tumor shrinkage is shown in Figure 2a, 2b, and 2c, respectively. PPS

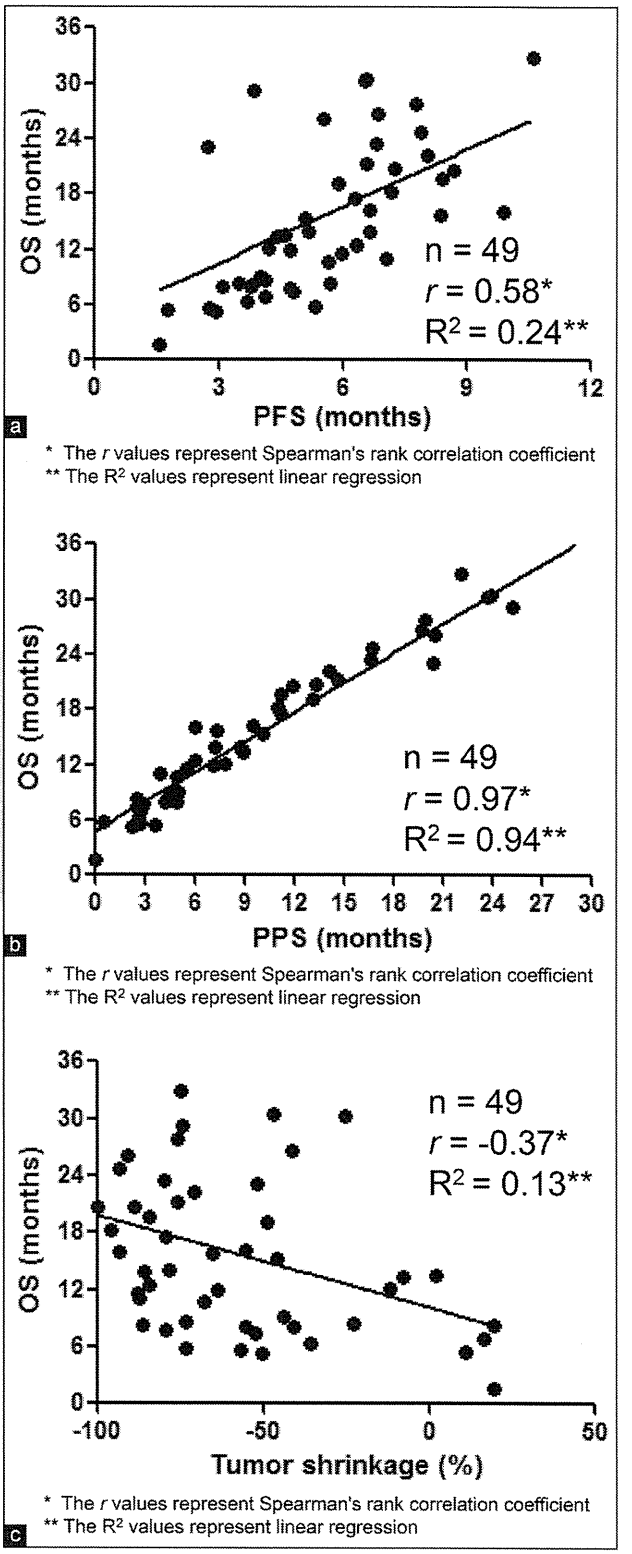

Figure 2: (a) Correlation between overall survival (OS) and progression-free survival (PFS) (b) Correlation between overall survival (OS) and post-progression survival (PPS) (c) Correlation between overall survival (OS) and tumor shrinkage

was strongly associated with OS ($r = 0.97$, $p < 0.05$, $R^2 = 0.94$), based on Spearman's rank correlation coefficient and linear regression, whereas PFS was moderately correlated with OS ($r = 0.58$, $p < 0.05$, $R^2 = 0.24$). Furthermore, tumor shrinkage was only weakly correlated with OS ($r = 0.37$, $p < 0.05$, $R^2 = 0.13$).

Factors affecting post-progression survival

PPS was strongly associated with OS. Therefore, the association between PPS and various clinical factors was assessed. In the univariate analysis [Table 3], PS at the end of first-line treatment, at the beginning of second-line treatment, and TFI (≥90/<90 days) as well as the best response at first-line treatment, the best response from the second-line treatment, and the number of regimens employed after progression beyond first-line chemotherapy were found to be associated with PPS ($p < 0.05$). Next, a multivariate analysis for PPS was conducted [Table 4]. This revealed that the best response after second-line treatment (non-PD/PD), and the number of regimens employed after progression following first-line chemotherapy were significantly associated with PPS ($p \leq 0.05$). The log-rank tests confirmed that PPS was significantly associated with the best response at second-line treatment (non-PD/PD), and the number of regimens employed ($p < 0.05$; Figure 3a and 3b). Based on the best response at second-line treatment, patients with non-PD had a median PPS of 13.1 months, which was longer than that of their counterparts, who had a median PD of 7.2 months (log-rank, $p = 0.05$; Figure 3a). According to the number of regimens employed after progression following first-line chemotherapy, the median PPS for those who were not administered additional regimens was 3.5 months; with 1 additional regimen, the median PPS was 5.5 months; and with ≥2 regimens, the median PPS was 14.1 months, (log-rank test, $p < 0.01$; Figure 3b). These results remained consistent after adjustment using the Cox proportional hazards models [Table 4].

Discussion

We examined the relationships of OS with PFS, PPS, and tumor shrinkage at the individual level in patients with extensive small cell lung cancer.PPS was strongly associated with OS, whereas PFS and tumor shrinkage were moderately and weakly correlated with OS, respectively. In addition, the best response to second-line treatment (non-PD vs. PD), and the number of regimens employed after progression following first-line chemotherapy, independently affected PPS.

Table 2: Chemotherapy regimens employed after progression following first-line chemotherapy

	Second-line	≥Third-line	Total
CDDP+irinotecan re-challenge	3	1	4
CDDP+VP16	2	1	3
CBDCA+VP16	2	4	6
CBDCA+PTX	0	3	3
Amrubicin	27	10	37
Topotecan	3	4	7
Paclitaxel	3	12	15
Irinotecan	0	2	2
Gemcitabine	3	7	10
Others	1	1	2

The validity of surrogate endpoints has been previously determined through meta-analyses.[18,19] In recent years,

Table 3: Univariate Cox regression analysis of baseline patient characteristics for post-progression survival

Factors	Post-progression survival		
	Hazard ratio	95% CI	*p* value
Gender	1.06	0.42-3.56	0.907
Age (years) at the beginning of first-line treatment	0.97	0.93-1.02	0.341
PS at the beginning of first-line treatment	1.20	0.70-2.05	0.490
Number of courses of first-line treatment administered	0.67	0.46-1.02	0.066
Sum of target lesion diameters	1.00	0.99-1.00	0.102
Best response at first-line treatment			
PR/non-PR	0.65	0.31-1.53	0.306
Non-PD/PD	0.22	0.08-0.77	0.021
PS at the end of first-line treatment	4.45	2.22-9.36	<0.001
Prophylactic cranial irradiation	0.81	0.28-3.39	0.738
Treatment-free interval (≥90/<90 days)	2.07	1.10-4.86	0.023
Age at the beginning of second-line treatment	0.96	0.92-1.01	0.196
PS at the beginning of second-line treatment	2.04	1.26-3.32	0.003
Best response following second-line treatment			
PR/non-PR	0.82	0.34-1.73	0.627
Non-PD/PD	0.48	0.24-0.92	0.028
Number of regimens after progression beyond first-line chemotherapy	0.50	0.35-0.70	<0.001

95% CI = 95% Confidence interval, PS = Performance status, PR = Partial response, PD = Progressive disease

Table 4: Multivariate Cox regression analysis of performance status (PS) at the end of first-line treatment, PS at the beginning of second-line treatment, best response at first-line treatment, best response at second-line treatment, and number of regimens employed after progression beyond first-line chemotherapy for post-progression survival

Factors	Post-progression survival		
	Hazard ratio	95% CI	*p* value
PS at the end of first-line treatment	1.81	0.60-6.10	0.29
PS at the beginning of second-line treatment	1.00	0.44-2.10	0.99
Best response at first-line treatment			
Non-PD/PD	0.50	0.14-2.34	0.34
Best response at second-line treatment			
Non-PD/PD	0.49	0.23-1.00	0.05
Number of regimens employed after progression beyond first-line chemotherapy	0.61	0.41-0.86	<0.01

95% CI = 95% Confidence interval, PD = Progressive disease

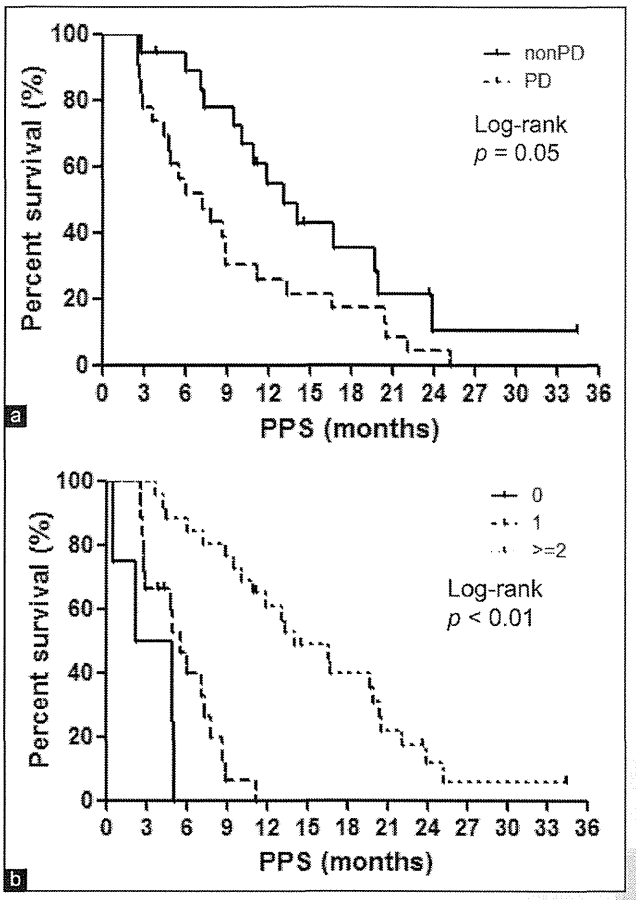

Figure 3: (a)Kaplan-Meier plots showing post-progression survival (PPS), according to the best response following second-line treatment Non-progressive disease (non-PD), median = 13.1 months; progressive disease (PD), median = 7.1 months. (b) Kaplan-Meier plots showing post-progression survival (PPS), according to the number of regimens after progression No further regimen, median = 3.5 months; 1 regimen, median = 5.5 months; 2 regimens, median = 14.1 months

biostatisticians have proposed a wide variety of measures for validating surrogate endpoints.[20,21] Although PFS is a potential surrogate endpoint for OS in extensive stage SCLC[22], its validity remains controversial. Broglio *et al.* recently focused on PPS, which they termed survival post progression (defined as OS minus PFS), in a hypothetical clinical trial setting under the assumption that treatment affected PFS but not PPS.[3] Recently, PPS was found to be strongly associated with OS after first-line chemotherapy for advanced NSCLC in a clinical trial[11,12], and we have previously reported the significance of PPS for advanced NSCLC based on an analysis of individual patients.[13]

In contrast with the findings of a previous study[22], we did not observe that PFS was a surrogate endpoint for OS in extensive stage SCLC, although PPS was not evaluated in the previous study. We analyzed our results pertaining to first-line therapy, which suggested that PFS and tumor response did not adequately reflect OS in such settings. We found that PFS was much shorter than PPS, and thus, PPS was closely related to OS — the relationship was linear. The fact that PPS accounted for the majority of OS suggests that the chemotherapy used was not sufficiently effective for PFS to be a significant component of OS. Thus, in clinical trials with patients expected to have a short PFS after first-line chemotherapy, for example those with extensive SCLC, as was the case in our study, factors that affect PPS need to be considered.

Based on trial-level data for advanced NSCLC, a long PPS is associated with a good PS and the use of first-line monotherapy with a molecular targeted agent.[11] Studies based on individual advanced NSCLC patients revealed that a long PPS was associated with the PS at the beginning of second-line treatment, the best response after second-line treatment (non-PD/PD), and the number of regimens employed after disease progression following first-line chemotherapy.[13] To date, however, no predictive factors for PPS in cases of extensive SCLC have been identified. We studied the prognostic value of baseline factors for PPS in individual patients. We found that the best response after second-line treatment, and the number of regimens employed after progression following first-line chemotherapy were strongly associated with PPS. Moreover, we confirmed the significance of these relationships using log-rank tests. Our findings suggest that patients for whom the disease has been controlled with second-line treatment achieve prolonged PPS after progression following first-line chemotherapy. These patients are also likely to be able to continue chemotherapy and achieve prolonged PPS, which is associated with a longer OS. The number of treatment regimens used after progression following first-line chemotherapy probably reflects the increasing number of available drugs, such as amrubicin, paclitaxel, and topotecan, which are available as second- or third-line chemotherapy for extensive SCLC. In fact, a number of different agents were used to treat our patients, as shown in Table 2.

This study has several limitations. First, the sample size was small. However, because relatively few extensive SCLC patients are treated with first-line cisplatin and irinotecan at our institution, this limitation is difficult to overcome, especially as the patients needed to have similar background characteristics. Nevertheless, our institution treats the relatively largest number of such cases, and the practice policy is largely unified simply because this is a single institution. There is of course some bias, but understanding the nature of this bias ensures that the results are still meaningful. In a future study, we will include a larger patient cohort, and more detailed examination is warranted. Second, we could not thoroughly evaluate treatments after progression following second-line chemotherapy, although only a few patients received third-line or subsequent chemotherapy. Third, the date on which a response was recorded was decided by each physician, which might have introduced variance in the PFS and tumor response rate. Fourth, chemotherapy regimens differ between Japan and the USA. In Japan, based on the results of a Japanese phase III trial[14], standard first-line chemotherapy for extensive SCLC currently is cisplatin combined with irinotecan. This combination is also described in the National Comprehensive Cancer Network guidelines as a suitable treatment option. Amrubicin is an effective second-line chemotherapy drug in a number of cancers including SCLC. In a phase III trial, it resulted in a significantly improved response rate compared to topotecan and also improved survival, especially in the subgroup of refractory patients.[23] On the basis of this trial,

amrubicin is now the standard second-line chemotherapy agent for extensive SCLC in Japan.

In conclusion, using individual patient data, PFS and tumor response were not found to be ideal surrogates for OS in patients with extensive SCLC who had limited options for subsequent chemotherapy. However, in these patients, PPS, rather than PFS, was strongly associated with OS. In addition, the best response after second-line treatment (non-PD/PD), and the number of regimens employed after disease progression following first-line chemotherapy were prognostic factors for PPS. Thus, the treatment course after progression following first-line chemotherapy greatly influences OS. We believe these findings justify further study to validate PPS as a surrogate marker of OS in patients with extensive SCLC.

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Role of surgical resection for patients with limited disease-small cell lung cancer

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ABSTRACT

Objectives: Although chemotherapy and radiotherapy are recommended for patients with limited disease small cell lung cancer (LD-SCLC), several series have reported favorable survival outcomes even in patients with stages II and III disease who underwent surgical resection. The purpose of this study is to compare the outcomes of the use of surgical resection to the other conventional non-surgical treatments in patients with LD-SCLC with respect to each clinical stage.
Materials and methods: We retrospectively reviewed 277 patients who received treatment for LD-SCLC and compared the outcomes of the use of surgical resection to the other conventional non-surgical treatments.
Results: The clinical stage was stage I in 50 cases (18%), stage II in 53 cases (19%) and stage III in 174 cases (63%). Eighty-eight patients received surgical resection and 189 patients were treated with non-surgical treatment. Surgery was performed in 44 patients (88%) with stage I, 27 patients (52%) with stage II and 17 patients (10%) with stage III disease. The five-year survival rates of the patients according to clinical stage were 58% in stage I, 29% in stage II and 18% in stage III. The five-year survival rates of the patients with and without surgical resection according to clinical stage were as follows: 62% and 25% in stage I ($p < 0.01$), 33% and 24% in stage II ($p = 0.95$), 18% and 18% in stage III ($p = 0.35$), respectively. In 44 propensity score-matched pairs with stages II and III disease, including matching for variables such as age, gender and the PS, the five-year survival rates was better in patients with surgical resection than in those without surgery ($p = 0.04$).
Conclusion: Surgical resection is effective for the patients with stage I LD-SCLC and some cases of stage II or III disease.

1. Introduction

Lung cancer continues to be the most common type of cancer, with approximately 1.6 million new cases diagnosed each year in the world [1]. This number is predicted to increase worldwide [1]. Small cell lung cancer (SCLC) represents 10–15% of all lung cancers, and the incidence of SCLC has been slowly decreasing over the past few years in the United States and Japan [2,3]. SCLC is one of the most aggressive cancers; therefore, more than 60% of SCLC is already extended disease at diagnosis, and stage I disease is diagnosed in less than 5% of the patients with SCLC [4]. On the other hand, due to the advances in new and more powerful diagnostic tools, such as chest computed tomography (CT) and positron emission tomography (PET), an increase in the detection of SCLC as small nodules is expected.

Generally, due to SCLC responds chemotherapy and radiotherapy, surgical treatment is considered to be an option for early stage SCLC, while its clinical benefit is considered to be limited in patients with more advanced disease [5,6]. The most recent National Comprehensive Cancer Network guidelines recommend that patients with SCLC that is clinical stage I (T1-2, N0) after a standard staging evaluation may be considered for surgical resection [5]. Furthermore, this guideline states that patients with disease exceeding T1–T2, N0 do not benefit from surgery [5]. The recommended treatment in cases of limited stage excess T1–T2, N0 with a good PS is chemotherapy with concurrent radiotherapy [5]. Similarly,

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Table 1
Characteristics of the patients.

Variable	Total (n = 277)	Surgery (n = 88)	Non-surgery (n = 189)	p-value
Age (range)	66 (38 to 89)	66 (43–83)	66 (38–89)	0.72
Gender				
Male	225 (81%)	72 (81%)	153 (81%)	0.86
Female	52 (19%)	16 (19%)	36 (19%)	
ECOG PS				
0	162 (58%)	68 (77%)	94 (50%)	<0.01
1	94 (34%)	18 (21%)	76 (40%)	
2, 3	21 (8%)	2 (2%)	19 (10%)	
cTNM stage				
Stage I	50 (18%)	44 (50%)	6 (3%)	<0.01
Stage II	53 (19%)	27 (31%)	26 (14%)	
Stage III	174 (63%)	17 (19%)	157 (83%)	
Treatment period				
1970s	36 (13%)	12 (14%)	24 (13%)	0.07
1980s	66 (24%)	26 (29%)	40 (21%)	
1990s	72 (26%)	27 (31%)	45 (24%)	
2000s	103 (37%)	23 (26%)	80 (42%)	

according to the American College of Chest Physicians guidelines, in patients with clinical stage I SCLC after a thorough distant and invasive mediastinal stage evaluation, surgical resection is suggested over non-surgical treatment based on grade 2C evidence [6]. On the other hand, several authors reported favorable results for surgical resection not only for stage I disease but also for more advanced disease [7–12].

In this study, we retrospectively compared the outcomes of the use of surgical resection compared to the other conventional non-surgical treatments in patients with LD-SCLC with respect to each clinical stage.

2. Materials and methods

2.1. Patients and methods

From 1974 through 2011, 605 consecutive patients were diagnosed with SCLC at the National Kyushu Cancer Center. Of those, 277 patients were treated for LD-SCLC. We retrospectively reviewed and analyzed the outcomes of these cases in terms of the role of surgical resection. Demographic, clinical and treatment data were abstracted from an institutional database that included all patients who had received treatment. The definition of LD-SCLC in this study was based on the International Association for the Study of Lung Cancer (IASLC) definition except for malignant pleural effusion or pleuritis carcinomatosa [13]. The institutional review board gave its approval for this study.

2.2. Diagnostic examinations

The diagnosis and staging procedure for the majority of patients was standardized to include bronchoscopy, laboratory parameters, CT of the chest and upper abdomen, brain CT or magnetic resonance imaging and a radionuclide bone scan and/or positron emission tomography with fluorine-18 fluorodeoxyglucose. Mediastinoscopy and endobronchial ultrasound mediastinal lymph nodes biopsies were performed as needed. Fifty-four patients (61%) in the surgical resection group received a pathological diagnosis prior to surgery. The TNM stage was determined according to the newly revised classification for lung cancer (American Joint Committee on Cancer seventh edition) [14].

2.3. Treatments

Surgical resection was performed for 88 patients, and included pneumonectomy (n = 10), lobectomy (n = 74) and limited resection (n = 4), such as wedge resection or segmentectomy. Chemotherapy was administered to 255 patients as the first-line treatment or in the adjuvant setting. The chemotherapy regimen most frequently administrated as an initial treatment was cisplatin and etoposide (PE) in 130 cases, followed by carboplatin and etoposide (CE) in 33 cases; vincristine, endoxan, mitomycin C and toyomycin (VEMT) in 30cases; cyclophosphamide, adriamycin and vincristine (CAV) in 24 cases and cisplatin and irinotecan (PI) in 10 cases. Other combinations were administrated to 10% (28 cases) of all patients. Irradiation of the primary tumor and mediastinal lymph nodes was performed for 161 patients with or without chemotherapy and surgical resection. The radiation dose given as the initial treatment was 30–75 Gy.

2.4. Statistical analysis

Comparisons of continuous and dichotomous variables between groups were performed with the Student's t-test and χ^2-test, respectively. The probability of survival was estimated using the Kaplan–Meier method. Differences in survival were evaluated by means of the log-rank test. An exploratory survival analysis such as a propensity matching analysis was added in the patients with stages II and III disease in order to balance the background of the patients. Patients with surgical resection in stages II and III disease were matched with those who received non-surgical therapy according to age, gender, ECOG PS and clinical stage. The analysis was conducted using SAS version 9.3 (SAS Institute, Cary, NC, USA). All p-values <0.05 were considered to be statistically significant.

3. Results

The age of the patients ranged from 38 to 89 years old (median, 66) and the patients included 225 males and 52 females (Table 1). The clinical stage was stage I in 50 cases (18%), stage II in 53 cases (19%) and stage III in 174 cases (63%). Thirty-six patients received treatment in the 1970s, 66 patients in the 1980s, 72 patients in the 1990s and 103 patients in the 2000s (Table 1). The distribution of treatments according to the clinical stage is shown in Table 2.

There were a total of 277 patients, 88 of whom underwent surgical resection and 189 of whom were treated with non-surgical treatments. Surgery was performed in 44 patients (88%)

Table 2
The initial treatment for LD-SCLC.

	c-stage I (n = 50)	c-stage II (n = 53)	c-stage III (n = 174)
Surgical treatment (n = 88)			
Surgery only	13(26%)	2(4%)	1(1%)
Surgery + chemotherapy	30(60%)	21(40%)	12(7%)
Surgery + chemoradiotherapy	1(2%)	4(8%)	4(2%)
Non-surgical treatment (n = 189)			
Chemotherapy only	1(2%)	6(11%)	30(17%)
Radiotherapy only	1(2%)	3(6%)	2(11%)
Chemoradiotherapy	4(8%)	17(32%)	125(72%)

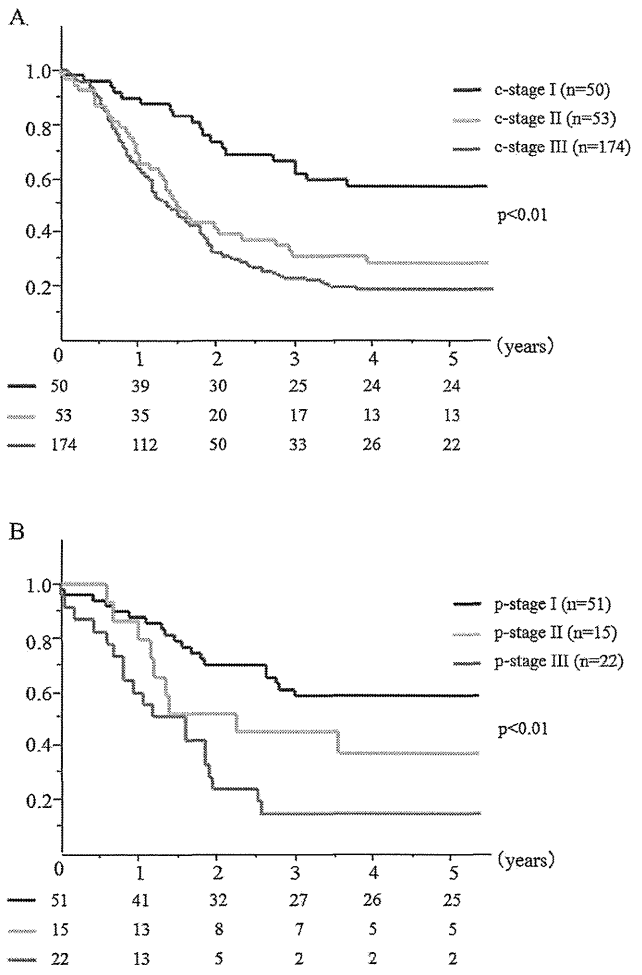

Fig. 1. The Kaplan–Meier curves of the overall survival according to (A) the clinical TNM stage and (B) the pathological TNM stage (seventh edition of the TNM).

in stage I, 27 patients (52%) in stage II and 17 patients (10%) in stage III. Twenty-seven patients in the surgical resection group received induction chemotherapy and two patients received induction chemoradiotherapy. Chemoradiotherapy was performed as the non-surgical treatment in 4 patients (8%) in stage I, 17 patients (32%) in stage II and 125 patients (72%) in stage III (Table 2). The agreement between the clinical and pathological stages of the patients who underwent surgery was as follows: 86% in stage I, 33% in stage II and 53% in stage III.

The median follow-up time for all cases was 16 months, and the median survival time (MST) for all cases was 18 months. The five-year survival rates of the patients according to clinical stage were 58% for stage I, 29% for stage II and 18% for stage III (Fig. 1A). The MST of the patients according to clinical stage was 75 months in the stage I cases, 18 months for stage II and 15 months for stage III (Fig. 1A). The five-year survival rates of the patients underwent surgery according to the pathological stage were 59% for those in stage I, 39% in stage II and 14% for those in stage III (Fig. 1B). The results of the Kaplan Meier analyses of patients according to the clinical stage and with or without surgical treatment are shown in Fig. 2. The five-year survival rates of the patients with or without surgical resection according to the clinical stage were as follows: 62% and 25% in stage I ($p < 0.01$), 33% and 24% in stage II ($p = 0.95$) and 18% and 18% in stage III ($p = 0.35$), respectively (Fig. 2A–C). A survival advantage related to surgery was observed in the patients with stage I disease, whereas in patients with stage II and stage III disease, no significant difference was observed in these groups (Fig. 2A–C). Comparison of long-term survival between the two groups after propensity matching analysis is shown in Fig. 2D. Forty-four pair patients were matched in each group. The five-year survival rates of the patients with or without surgical resection according to the analysis were as follows: 28% in surgical resection group and 11% in non-surgical group ($p = 0.04$). The propensity matching analysis demonstrated that the surgical resection group had a significant better survival than the non-surgical group in the cases of stage II and III LD-SCLC (Fig. 2D).

The five-year survival rates of the patients according to the treatment period were as follows: 20% in the 1970/1980s, 21% in the 1990s and 40% in the 2000s ($p < 0.01$) (Fig. 3).

4. Discussion

Two randomized prospective trials of surgery versus radiotherapy organized by the British Medical Research Council reported that surgery and radiotherapy were equally in effective for limited stage SCLC [15,16]. According to these reports, fewer than 2% of patients survived more than two years after the resection. Later, in 1994, Lad et al. reported the results of a randomized trial evaluating the role of surgery in limited-stage SCLC conducted by the Lung Cancer Study Group [17]. This study included 144 SCLC patients, all administrated chemotherapy followed by chest irradiation. The patients were then randomized to a surgery group or a non-surgery group. According to the report, no significant impact of surgery on survival was found, with the two-year survival rate being 20% for all cases [17]. Based on those studies, surgical treatment for SCLC is considered to be an option for early stage disease, but its clinical benefit is considered to be limited, especially for more advanced disease [15–17]. However, several decades have passed since these reports were published. During that time, several authors have reported the efficacy of surgical resection for LD-SCLC, especially when it is used as part of multidisciplinary therapy [7–12].

Recently, the role of surgery in SCLC has been analyzed using a large population database [12,18,19]. The Surveillance, Epidemiology, and End Results (SEER) database identified 14,179 patients with SCLC, including 863 patients who underwent surgery [12]. According to those results, the patients who underwent surgery had better survival rates than those who did not for both localized

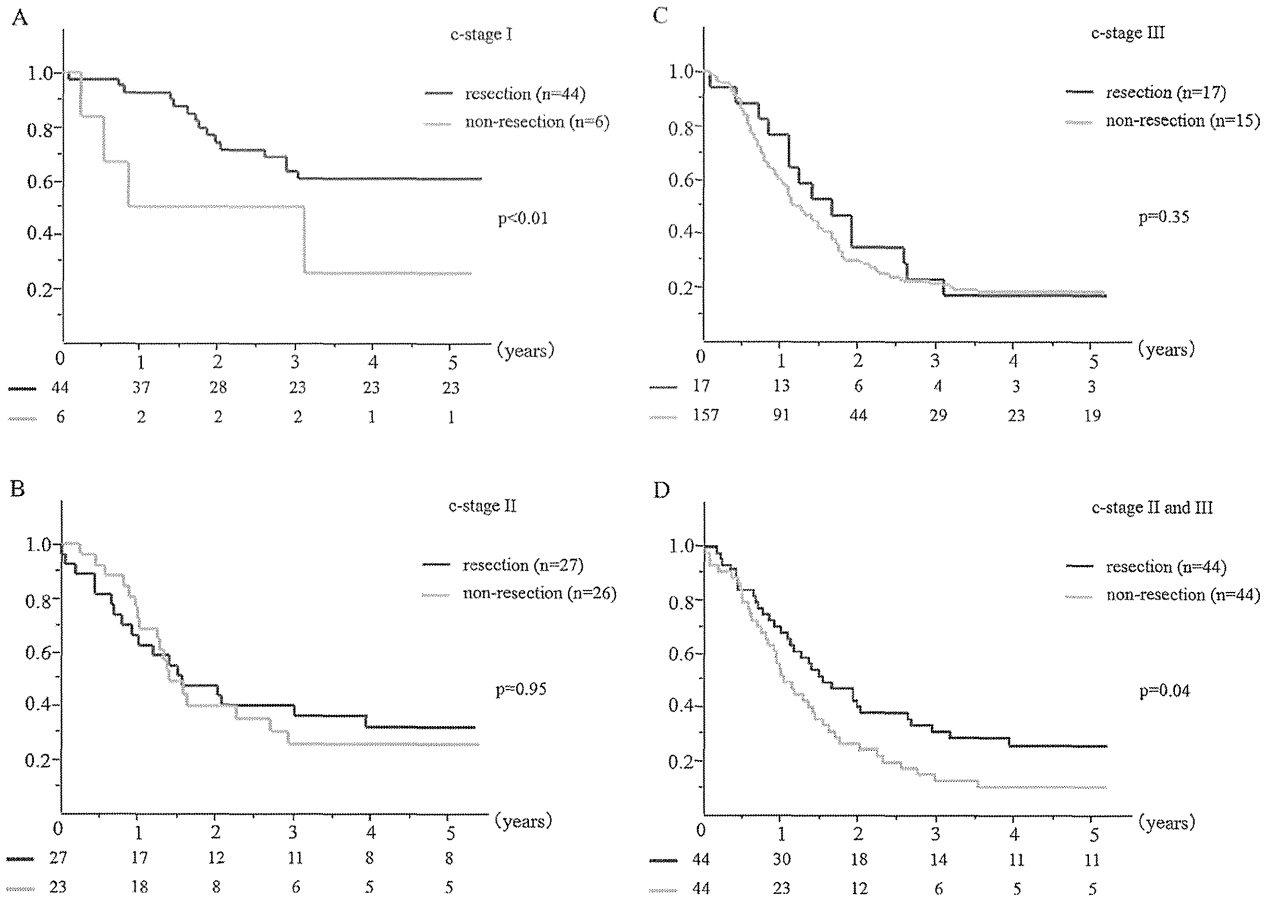

Fig. 2. The Kaplan–Meier curves of the overall survival of patients with or without surgical resection. (A) Stage I, (B) stage II, (C) stage III and (D) matched cohorts in stages II and III.

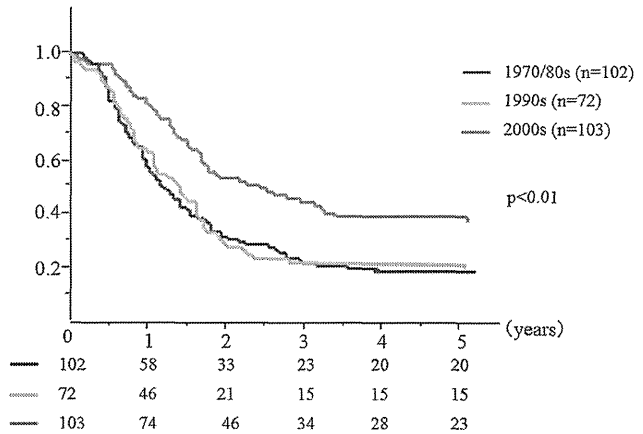

Fig. 3. The Kaplan–Meier curves of the overall survival according to the treatment period.

disease and regional disease, even in cases of N1 or N2 disease [12]. Another study using the SEER database reported that the five-year survival rate of the patients with stage I disease who underwent lobectomy was 50.3% and that of the patients who received external beam radiation alone was 14.9%, respectively [18]. The IASLC reported on 12,630 patients with SCLC, 349 of whom underwent surgical therapy [19]. According to the report, the five-year survival rates of the patients with pathological stages IA, IB, IIA, IIB, IIIA and IIIB disease were 56%, 57%, 38%, 40%, 12% and 0%, respectively [19]. The stages of the reports were classified using the seventh edition of the TNM grouping [19].

In the present study, we evaluated the outcomes of LD-SCLC patients reclassified using the TNM seventh edition. According to our results, the seventh edition of the TNM classification correctly reflected the prognosis of LD-SCLC. We also evaluated the outcomes of the patients with or without surgical resection according to the clinical stage. In this study, the use of surgery led to a satisfactory result in the patients with stage I disease, with a five-year survival rate of 62% for the surgical resection group and 25% for the non-surgical group. On the other hand, no significant benefit of surgical resection was observed in the patients with clinical stages II and III disease. Although the therapeutic strategy was not assigned randomly, more than 80% of the patients who underwent surgical resection also received chemotherapy or chemoradiotherapy. In addition, only three patients were treated with surgical resection alone in the clinical stages II and III group as the initial treatment. Although there was no difference in the overall survival between the patients treated with or without surgical resection in the overall cases, the propensity matching analysis demonstrated the efficacy of surgical resection in the patients with stages II and III LD-SCLC. In addition, the five-year survival rates according to the pathological stage were 59% in patients with stage I, 39% in those with stage II and 14% in those with stage III disease. Based on these results, some patients with stages II and III disease obtain a relatively good prognosis following surgical resection. One of the reasons for the differences in the outcome between clinical and

pathological stages was the existence of upstaged cases. In this series, 18 of the surgical cases were underestimated in terms of the clinical stage. The IASLC report analyzed the concordance between the clinical and pathological stages [19]. According to that report, 20% of the patients diagnosed with clinical stages I and II disease were upstaged, with pathological evidence of mediastinal lymph node metastases, and the five-year survival rate based on the pathological stage was better than that based on the same clinical stage [19]. In our series, some of the patients were not staged according to today's standards tools, such as PET or mediastinoscopy; therefore, the diagnosis of the clinical stage was less accurate than is now possible. The staging concordance using PET or PET/CT was reported to be 83–100% in the prospective setting [20–24]. In fact, the agreement between the clinical and pathological stages of all of the patients who underwent surgery was 87.5% in the 2000s in this study. Two decades have passed since the last prospective randomized trial evaluating the role of surgery was reported [17], and there have been new diagnostic tools and therapeutic techniques have developed during that time. In fact, our data suggested that the outcomes of treatment have been improved beginning in the 2000s. Similarly, Hanagiri et al. reported that the outcomes of the patients who received treatment for SCLC after the 1994, including surgery, improved compared to that before [25]. At any rate, it is currently uncertain whether all of the LD-SCLC cases except for those stage I disease are not indicated for surgical resection; therefore, further prospective studies might be considered to extend the indications for surgery for LD-SCLC based on the present diagnostic modalities and improved surgical techniques.

There are some limitations associated with this study. One of the limitations is the retrospective and non-randomized setting of this study. To compare the efficacy of surgical resection, it is important to evaluate the findings in a prospective and randomized setting. On the other hand, since few cases of limited disease are diagnosed each year, a prospective study would be difficult to carry out; therefore, it is important to accumulate retrospective data. Second, the sample size of this study was relatively small and the treatments were lacking in uniformity. However, to our knowledge, there have been few reports that have evaluated the outcomes of LD-SCLC cases restaged based on the TNM seventh edition as part of a single-institution study. Despite these several limitations, the present study reflects the actual clinical outcomes of LD-SCLC patients.

In conclusion, surgical resection provided a survival benefit for the patients with clinical stage I SCLC and some cases of stage II or III disease in this study. The outcomes of treatment for SCLC have been improved beginning in the 2000s. A further prospective study is warranted to clarify the possibility of extending the indications for surgical resection to curatively treat LD-SCLC in the present situation.

Conflict of interest

None.

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