and SCLC is known to be challenging in some cases even with surgical specimens.

A previous study reported that the prognosis of patients with surgery alone for SCLC and LCNEC was poor; the 5-year survival was shown to be 35.7% for SCLC and 40.3% for LCNEC (4). However, several retrospective studies have described the favorable outcomes of clinical Stage I SCLC patients who underwent surgery followed by adjuvant chemotherapy (5). Based on these reports, surgery plus adjuvant chemotherapy is regarded as a standard therapy for clinical Stage I SCLC. Reports on LCNEC are very limited because it is still a new entity. However, post-operative chemotherapy has also been added as a standard therapy in practice for LCNEC because its prognosis after surgery alone is poor.

The Japan Clinical Oncology Group study, JCOG9101, which is a Phase II trial to evaluate the feasibility of etoposide and cisplatin (EP) for completely resected pathological Stage I–IIIA SCLC patients, demonstrated the sufficient feasibility of the EP regimen (6). The survival of each stage was better than that of pathological Stage I–III patients who were administered cyclophosphamide, doxorubicin and vincristine (CAV) in another prospective study (7). Thus, EP has been considered acceptable as a current standard post-operative adjuvant chemotherapy regimen for SCLC.

The only report of a prospective study on adjuvant chemotherapy in pathological Stage I–IV LCNEC revealed the favorable outcomes of EP (8). One retrospective review of adjuvant chemotherapy for LCNEC compared two major categories of regimens; one for a SCLC regimen, a combination of platinum and etoposide, and the other for NSCLC regimens, a combination of platinum and gemcitabine, taxanes or vinorelbine. The findings of this review showed that SCLC regimens significantly prolonged survival (median survival time 42 months versus 11 months, $P < 0.0001$) (9). Therefore, the EP regimen is regarded as a standard post-operative adjuvant therapy regimen for LCNEC in Japan.

JCOG9511, a Phase III trial comparing irinotecan plus cisplatin (IP) with EP in SCLC patients with extended disease (ED-SCLC), showed that survival was significantly longer in the IP arm than in the EP arm (12.8 months versus 9.4 months, $P = 0.002$ by the log-rank test) (10). However, all three randomized controlled trials conducted afterwards to confirm the superiority of IP failed to demonstrate a difference in survival between the two arms (11–13). On the other hand, a recent meta-analysis has suggested that overall survival may be superior with irinotecan plus platinum than with etoposide plus platinum (14). Therefore, IP is regarded as one of the standard treatment options for ED-SCLC patients and is also expected to be a promising regimen in adjuvant chemotherapy for completely resected HGNEC patients. Kenmotsu et al. (15) conducted a multicenter Phase II pilot study to evaluate the feasibility of IP in post-operative adjuvant chemotherapy for HGNEC patients, and showed that the proportion of completion of treatment and toxicities were acceptable.

Based on these backgrounds, we have commenced a multicenter randomized controlled trial to confirm the superiority of IP in terms of overall survival over EP as post-operative adjuvant chemotherapy for pathological Stage I–IIIA completely resected pulmonary HGNEC patients.

The JCOG Protocol Review Committee approved this study protocol in February 2013 and patient enrollment began in March 2013. Approval was obtained from the Institutional Review Board prior to starting patient accrual at each institution.

PROTOCOL DIGEST OF THE JCOG1205/1206

Objectives

The purpose of this study is to confirm the superiority of IP in overall survival over EP as post-operative adjuvant chemotherapy for pathological Stage I–IIIA completely resected pulmonary HGNEC patients.

Study Setting

A multi-institutional two-arm open label randomized Phase III study.

Endpoints

The primary endpoint is overall survival (OS) in all randomized patients. OS is defined as days from randomization to death from any cause, and it is censored at the last day when the patient is alive. The secondary endpoints are relapse-free survival (RFS), proportion of treatment completion, adverse events, serious adverse events and second malignancy. RFS is defined as days from randomization to relapse or death from any cause, and it is censored at the latest day when the patient is alive without any evidence of relapse.

Eligibility Criteria

Inclusion Criteria

(1) Pathologically proven high-grade neuroendocrine carcinoma (small cell carcinoma including combined small cell carcinoma, or large cell neuroendocrine carcinoma including combined large cell neuroendocrine carcinoma)
(2) Pathological Stage I–IIIA based on the seventh UICC-TNM classification (16).
(3) Pathologically proven R0, R1 (is) or R1 (cy+) based on the seventh edition of the General Rule for Clinical and Pathological Record of Lung Cancer by the Japan Lung Cancer Society (17)
(4) Aged 20–74-years-old
(5) ECOG performance status of 0 or 1
(6) Lobectomy or more extended surgery was performed
(7) ND 2a-1 or more extended lymph node dissection was performed
(8) Within 28–56 days after surgery
(9) No distant metastasis including brain metastasis
(10) No prior chemotherapy or radiotherapy for any cancers

(11) Adequate organ functions
(12) No diarrhea or intestinal obstruction
(13) Written informed consent

Exclusion Criteria

(1) Synchronous or metachronous (within 5 years) malignancy, except for carcinoma *in situ* or mucosal tumors curatively treated with local therapy
(2) Active infection requiring systemic therapy
(3) Body temperature $\geq$38°C
(4) Pregnant or lactating women or women of childbearing potential
(5) Severe mental disease
(6) Serious post-operative complications
(7) Patients receiving systemic steroid medication
(8) Poorly controlled diabetes mellitus or receiving the routine administration of insulin
(9) Poorly controlled hypertension
(10) Unstable angina within 3 weeks, or with a history of myocardial infarction within 6 months
(11) Positive serum HBs antigen or HCV antibody
(12) Positive serum HIV antibody
(13) Interstitial pneumonia, pulmonary fibrosis or severe emphysema

Randomization

After confirming the eligibility criteria, registration is made by telephone, fax or a web-based system to the JCOG Data Center. Patients are randomized to either arm A (EP) or arm B (IP) by the minimization method balancing the arms with institution, sex (male versus female), pathological stage (Stage I versus Stage II–IIIA) and pathological type (SCLC versus LCNEC).

Treatment Methods

Patients in the EP arm receive four courses of post-operative EP (etoposide, 100 mg/m^2/day, Day 1–3; cisplatin 80 mg/m^2/day, Day 1) repeated every 3 weeks. Patients in the IP arm receive four courses of post-operative IP (irinotecan, 60 mg/m^2/day, Day 1, 8, 15; cisplatin, 60 mg/m^2/day, Day 1) repeated every 4 weeks. When the leukocyte count is decreased to $<$3000/mm^3 or the platelet count to $<$100 000/mm^3 on the planned first day of both arms, the start of chemotherapy is delayed until the counts recover to 3000/mm^3 or more and 100 000/mm^3 or more, respectively. The administration of irinotecan is skipped on Day 8 and/or 15 when at least one of the following occurs; a leukocyte count $<$2000/mm^3, platelet count $<$100 000/mm^3, diarrhea Grade 1 or higher or a fever of 37.5°C or higher. The dose of etoposide and irinotecan in the subsequent cycles is reduced by 20 mg/m^2 and 10 mg/m^2 from the planned dose, respectively, when the leukocyte count is $<$1000 mg/m^2, platelet count is $<$20 000/mm^3 and/or Grade 3 non-hematologic toxicities (excluding hyponatremia and weight loss) develop. The dose of cisplatin is reduced by 20 mg/m^2 in the EP arm and 10 mg/m^2 in the IP arm when patients have serum creatinine $>$1.5 mg/dl, but not exceeding 2.0 mg/dl, Grade 2–3 peripheral motor or sensory neuropathy, myalgia, arthralgia or other Grade 3 non-hematologic toxicities (excluding hyponatremia and weight loss). The protocol treatment is terminated when serum creatinine exceeds 2.0 mg/dl or patients develop Grade 4 non-hematologic toxicities (other than hyperglycemia, hypernatremia, hyponatremia, hyperkalemia and hypokalemia). After completion of the protocol treatment, patients are observed without anti-cancer treatment until recurrence is detected.

Follow-up

All randomized patients are followed-up for at least 5 years after patient accrual is completed while analysis of the primary endpoint is conducted 3 years after accrual completion.

Chest X-rays are performed every 6 months for the first 5 years and every year afterwards. Tumor markers (CEA, NSE and ProGRP), enhanced computed tomography of the thorax and enhanced computed tomography or ultrasound of the upper abdomen are evaluated every 6 months for the first 3 years and every year from the fourth to the fifth year.

Study Design and Statistical Analysis

This randomized trial is designed to confirm the superiority of IP in terms of overall survival over EP as post-operative adjuvant chemotherapy for pathological Stage I–IIIA completely resected pulmonary HGNEC patients.

We assumed the 3-year survival with post-operative EP to be 70% and expected a 10% increase in the 3-year survival with post-operative IP. According to Schoenfeld and Richter's method (18), the sample size was calculated as 104 patients per arm with a one-sided alpha level of 5%, a power of 70%, an expected accrual period of 6 years and a follow-up period of 3 years. Eighty-eight events in total are expected. The total sample size was set at 220 patients to account for patients lost to follow-up. All statistical analyses will be conducted at the JCOG Data Center.

Interim Analysis and Monitoring

We plan to conduct two interim analyses, taking multiplicity into account using the Lan–DeMets method with the O'Brien and Fleming type alpha spending function (19). The first interim analysis will be conducted after half of the planned number of patients is enrolled and the second interim analysis after the planned patient accrual and their protocol treatment is completed. The Data and Safety Monitoring Committee (DSMC) of the JCOG will review the interim analysis reports independently from the group investigators and group statistician. If the superiority of the IP arm is demonstrated with a one-sided P value of the stratified log-rank test below an adjusted alpha level, the study will be terminated.

In-house monitoring will be performed every 6 months by the JCOG Data Center to evaluate and improve study progress, data integrity and patient safety.

UMIN Registration Number

This trial has been registered at the UMIN Clinical Trials Registry as UMIN000010298 [http://www.umin.ac.jp/ctr/index.htm].

Participating Institutions (from North to South)

Asahikawa Medical Center, National Hospital Organization Hokkaido Cancer Center, KKR Sapporo Medical Center, Miyagi Cancer Center, National Hospital Organization Sendai Medical Center, Tohoku University Hospital, Yamagata Prefectural Central Hospital, Ibaraki Prefectural Central Hospital and Cancer Center, Tochigi Cancer Center, National Nishigunma Hospital, Gunma Prefectural Cancer Center, Saitama Cancer Center, National Cancer Center Hospital East, Chiba University Graduate School of Medicine, National Cancer Center Hospital, Kyorin University Faculty of Medicine, Tokyo Medical University Hospital, Tokyo Metropolitan Cancer and Infectious Diseases Center Komagome Hospital, National Center for Global Health and Medicine, Cancer Institute Hospital of Japanese Foundation for Cancer Research, Juntendo University Hospital, Yokohama City University Medical Center, Kanagawa Cancer Center, Yokohama Municipal Citizen's Hospital, Niigata Cancer Center Hospital, Kanazawa University School of Medicine, Gifu Municipal Hospital, Shizuoka Cancer Center, Nagoya University School of Medicine, Aichi Cancer Center Hospital, National Hospital Organization Nagoya Medical Center, Aichi Cancer Center Aichi Hospital, Kyoto University Hospital, Osaka City University Hospital, Kinki University Faculty of Medicine, Osaka Prefectural Hospital Organization Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka Prefectural Hospital Organization Osaka Prefectural Medical Center for Respiratory and Allergic Disease, National Hospital Organization Kinki-Chuo Chest Medical Center, Osaka City General Hospital, Kobe City Medical Center General Hospital, Hyogo Cancer Center, Kurashiki Central Hospital, Okayama University Hospital, National Hospital Organization Kure Medical Center Chugoku Cancer Center, Hiroshima University Hospital, National Hospital Organization Yamaguchi-Ube Medical Center, National Hospital Organization Shikoku Cancer Center, National Kyushu Cancer Center, School of Medicine Fukuoka University, Nagasaki University Hospital, Kumamoto University Medical School, Kumamoto Chuo Hospital, Kumamoto Regional Medical Center Hospital and National Hospital Organization Okinawa Hospital

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Conflict of interest statement

None declared.

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Original contribution

A subset of small cell lung cancer with low neuroendocrine expression and good prognosis: a comparison study of surgical and inoperable cases with biopsy☆,☆☆,★

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Immunohistochemistry

Summary Patients with small cell lung carcinoma (SCLC) rarely demonstrate long-term survival. We previously reported that gene expression profiling identified a subset of SCLC with good prognosis in surgical cases. To find an easier way to routinely identify SCLC belonging to this subset, we conducted the present study with a hypothesis that neuroendocrine (NE) or basaloid (BA) phenotypes may influence prognosis. To confirm the subset, we used an array platform to analyze fresh samples. Because inoperable cases may differ from surgical cases, we enrolled 51 biopsy cases and 43 resected samples. To evaluate NE and BA phenotypes, we used NE (synaptophysin, chromogranin A, and CD56) and BA (p63 and CK34βE12) markers. To varying extents, expression profiling based on the array platform

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duplicated the subsets. For NE phenotypes, 77% of surgical cases and 100% of biopsy cases were positive for at least 1 marker. For BA phenotypes, only 19% of surgical cases were positive for at least 1 marker, whereas there were no positive biopsy cases. Cases undergoing surgery were categorized based on NE and BA immunoreactivity; 58% into NE+BA−, 19% into NE+BA+, 23% into NE−BA−, and 0 into NE−BA+ groups. NE− patients (n = 10) demonstrated a significantly better prognosis (P = .0306) than their NE+ counterparts (n = 33), whereas no survival difference was evident between the BA+ and BA− groups. Multivariate analyses showed that NE positivity was an independent prognostic factor. In conclusion, the SCLC subset with good prognosis is identified by low NE marker expression, which was found only in surgical cases.

1. Introduction

Small cell lung carcinoma (SCLC) accounts for about 15% of all lung cancers, and its high proliferative activity generally leads to early metastasis to lymph nodes and distant organs. It is known that, although more sensitive to chemotherapy and irradiation than non-SCLCs [1,5], SCLCs tend to recur in about 70% of cases [6]. Some cases that are initially misdiagnosed are only found to be SCLC after resection [7]. Including these cases, it has been found that stage I SCLC has only a 42% to 66% 5-year survival, which is much lower than for non-SCLCs [8,9]. These statistics reflect a disparate course, with some patients with SCLC surviving for a long time after therapy, whereas others appear insensitive to chemotherapy and irradiation, implying considerable heterogeneity.

SCLC has distinct histologic characteristics such as scant cytoplasm (high nucleocytoplasmic [N/C] ratio), ill-defined cell borders, finely granular nuclear chromatin, absent or inconspicuous nucleoli, round to spindle shaped, nuclear molding and rosette formation, extensive necrosis, and a high mitotic rate [10]. Surgically resected tumors show somewhat different histology such as larger cell sizes, occasional conspicuous nucleoli, and vesicular nuclear chromatin [7]. It is necessary to prove neuroendocrine (NE) differentiation by immunohistochemistry (IHC) or electron microscopy for the diagnosis of large cell NE carcinoma (LCNEC). However, according to the World Health Organization (WHO) classification [10], this is not mandatory for SCLC. Nevertheless, without IHC, it is sometimes difficult to differentiate an SCLC from a poorly differentiated non-SCLC composed of small-sized cells with a high N/C ratio, such as basaloid (BA) carcinoma. In such cases, immunohistochemical markers for BA cells are useful in distinguishing NE carcinomas from poorly differentiated squamous cell carcinomas and BA carcinomas [11,12]. It is important to distinguish such carcinomas from SCLC because they may have a better prognosis than SCLC, although BA carcinoma has a poorer prognosis than usual squamous cell carcinomas.

We previously identified a subset of SCLC with good prognosis by global gene expression profiling using custom-made complementary DNA (cDNA) microarrays [13], showing that differentially expressed genes included NE-related genes, implying that long-term survival is not simply a matter of chance. To further characterize this subset and define a more readily accessible technique, such as IHC, to identify this subset, we set out this study by hypothesizing that a degree of NE differentiation or a basal cell nature may be related to prognosis. Because this subset had been delineated in surgically resected cases and inoperable cases may possibly differ from surgical cases, we also enrolled inoperable cases using available biopsy materials. First, we confirmed the existence of the subset using another platform of gene expression profiling and then performed IHC with NE and basal cell markers for surgical and biopsied cases. We paid particular attention to excluding atypical carcinoids.

2. Materials and methods

2.1. Patients and tumor samples

Surgical samples of SCLC are scarce: during the period from January 1990 to December 2004, a total of 1568 lung cancers were resected surgically at the Cancer Institute Hospital, Japanese Foundation for Cancer Research, Tokyo, Japan. Among these cancers, only 56 cases (3.6%) were found to be SCLC by pathological examination of resected materials. In this study, we enrolled a total of 96 SCLC cases, which were composed of 45 surgical cases and 51 inoperable cases with only biopsy specimens available. Histologic diagnosis of SCLC was made according to the 2004 WHO classification [10], relying only on hematoxylin and eosin (H&E) staining. Cases with atypical histology were examined by a panel of Japanese expert pathologists organized by an NE tumor study group [9], supported by the Ministry of Health, Labour and Welfare. Also, a few of the atypical cases were presented at the Pathology Committee meeting of the International Association for the Study of Lung Cancer, held in Tokyo, Japan. Atypical carcinoids were carefully excluded, with special attention to mitosis and cell morphology. Excluding some SCLC cases with extensive degeneration due to induction therapy, or with insufficient tumor cells remaining after chemotherapy, 45 surgical tumors were used for this

analysis. Among these, fresh materials for 30 tumors were suitable for microarray gene expression analysis (18 were previously examined [13] and 12 were newly enrolled in this study), and for the remaining 15 cases, only paraffin blocks were available. Because for 2 cases among the 30, only fresh materials were available, no paraffin tissues from surgical materials being left for this study, tissues of 43 cases were used for immunohistochemical studies. In addition to the surgical cases, 51 patients who were inoperable and had undergone a biopsy between 1996 and 2006 were enrolled.

All tumors were pathologically staged according to the TNM classification system of the International Union Against Cancer [14] using resected materials. The clinical stages, serum level of markers (NSE, ProGRP, CEA, SCC, and CYFRA), and response rates to chemotherapy were investigated using medical records. Cumulative smoking was carefully surveyed and described with reference to the *smoking index* (SI), defined as the product of the number of cigarettes per day and duration in years. Cause of death was surveyed thoroughly using death certificates, and lung cancer–specific survival or overall survival was analyzed as appropriate. All tissues were collected with informed consent from patients, and the study protocol was approved by the Japanese Foundation for Cancer Research institutional review board.

2.2. RNA isolation and gene expression profiling

Fresh samples of 30 SCLCs were obtained at surgery. The tissues of resected tumors were grossly dissected and snap-frozen in liquid nitrogen typically within 15 minutes of removal. We always confirmed that fresh tumor tissues for RNA extraction actually contained viable SCLC cells, using frozen section diagnosis. Total RNA was extracted using an RNeasy Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. A 3-μg aliquot was used to generate ds-cDNA using a T7-Oligo (dT) primer, and the cDNA was transcribed into biotin-labeled cRNA using a GeneChip 3′ IVT Express Kit (Affymetrix, Santa Clara, CA, USA). Quality control of RNA and cRNA was performed using a bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). After fragmentation, each sample was hybridized to Affymetrix HG U133 plus 2.0, which covers 38 500 genes, 47 400 transcripts, and more than 54 000 probe sets, and was stained according to the manufacturer's instructions (Affymetrix). We used GeneChip Scanner 3000 for scanning and GeneChip Operating Software (GCOS; Affymetrix) for data output.

2.3. Array data analysis

Data were analyzed and visualized by use of R software (version 2.9.2; www.t-project.org). Before analysis, all data were log transformed and subjected to Robust Multichip Average normalization [15].

Unsupervised hierarchical clustering analysis was accomplished with standard Pearson correlations and the Ward method using 15 530 probe sets expressed above the background in at least 20% of the 30 samples and 100 or more expression signals. To identify genes that represent the most informative markers between 2 groups obtained from clustering analysis about SCLC, we focused on those with $P < .01$ by the Welch t test and log fold-change above 2.0 or below −2.0.

2.4. Procedures for tumor tissue arrays

Surgical specimens were fixed with 15% buffered formalin and embedded in paraffin. They were sectioned at 4-μm thickness and stained with H&E for histologic diagnosis. Tissue arrays were made from paraffin specimens as follows: 2 spots of the most representative tumor area were selected considering heterogeneity and cored in 2-mm diameter with a tissue-arraying instrument (Azumaya, Tokyo, Japan). In cases of combined SCLC, only SCLC components were chosen for coring. Core samples were retrieved from donor tissues and arrayed in a new paraffin block.

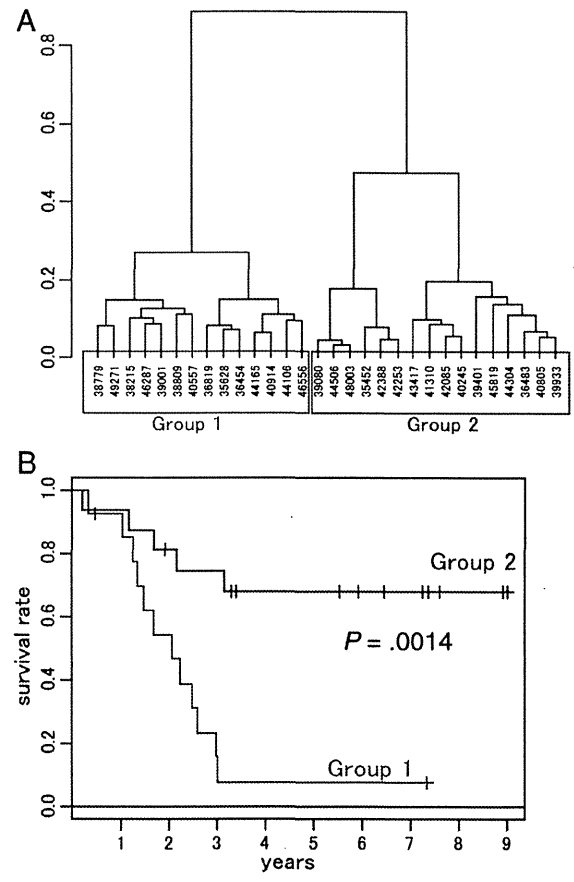

Fig. 1 A, Results of unsupervised hierarchical clustering of 30 SCLCs. B, SCLC-specific survival of groups 1 and 2. Note the better survival of group 2 as compared with group 1 ($P = .0014$).

Table 1 Patients characteristics of resected SCLC and LCNEC

Cases	Ref. no.	Diagnosis	Age (y)	Sex	SI	cT	cN	cM	Preoperative diagnosis	Induction chemotherapy	(reduction rate)	Operation	pT	pN
1	29635	SCLC	76	M	1500	2	2	0	SCLC	Yes	PR: 84%	Lobectomy	4	2
2	30017	SCLC	57	M	1050	4	2	0	SCLC	Yes	PR: 70%	Lobectomy	4	2
3	30156	SCLC	67	M	940	3	2	0	SCLC or LCC	None		Lobectomy	4	2
4	30323	SCLC	59	M	1435	2	1	0	SCLC	Yes	CR: scar+	Lobectomy	2	2
5	30865	SCLC	64	M	400	1	0	0	p/d ca	None		Lobectomy	1	2
6	31160	SCLC	68	M	1000	1	0	0	SCLC	None		Lobectomy	4	0
7	31401	SCLC	72	M	680	1	0	0	SCLC	None		Lobectomy	2	0
8	32658	SCLC	84	M	1200	1	0	0	Unconfirmed	None		Partial resection	1	1
9	33130	SCLC	46	M	940	1	0	0	SCLC or SQ	None		Lobectomy	2	0
10	33587	Combined SCLC + AD	59	M	1480	1	1	0	AD or LCC	None		Lobectomy	4	0
11	34802	SCLC	73	M	960	1	0	0	SQ	None		Lobectomy	1	0
12	34947	SCLC	54	M	680	1	0	0	SCLC	Yes	PR: 74%	Lobectomy	1	0
13	35452	Combined SCLC + AD		F	0	2	0	0	AD	None		Lobectomy	2	0
14	35628	SCLC	73	M	800	1	2	0	AD or SCLC	Yes	PR: 77%	Lobectomy	1	0
15	35996	SCLC	61	M	375	1	2	0	SCLC	Yes	PR: 79%	Lobectomy	4	2
16	36454	SCLC	66	M	300	1	1	0	SCLC	Yes	SD: 24%	Lobectomy	1	1
17	36483	Combined SCLC + Spindle	64	M	1470	3	0	0	SCLC	Yes	PR: 67%	Lobectomy	4	0
18	36819	SCLC	67	M	920	2	2	0	SCLC	Yes	CR: regrowth+	Lobectomy	1	2
19	38779	SCLC	57	M	1110	1	0	0	SCLC	Yes	SD: 26%	Lobectomy	1	1
20	38809	SCLC	79	M	1180	2	0	0	SCLC	None		Lobectomy	4	0
21	39001	Combined SCLC + AD	59	M	675	1	0	0	SCLC	None		Lobectomy	1	0
22	39080	SCLC	76	M	600	1	0	0	AD or SCLC	None		Segmentectomy	1	0
23	39401	SCLC[c]	67	M	2000	1	2	0	Unconfirmed	None		Partial resection	1	0
24	39933	SCLC	53	M	1050	2	1	0	SCLC	None		Pneumonectomy	4	2
25	40557	Combined SCLC + AS	74	F	0	2	1	0	NSCLC	None		Lobectomy	2	2
26	40805	SCLC	68	M	1440	2	0	0	p/d ca	None		Lobectomy	2	0
27	40914	SCLC	68	F	380	2	0	0	AD or SCLC	None		Lobectomy	4	1
28	41179	SCLC	65	M	2400	2	1	0	SCLC	Yes	PR: 51.3%	Lobectomy	4	1
29	41310	SCLC	64	F	700	1	0	0	carcinoma	None		Lobectomy	1	0
30	42085	SCLC	71	M	1060	1	0	0	SCLC or p/d ca	None		Lobectomy	1	1
31	42253	Combined SCLC + LCNEC	63	F	1880	3	0	0	SQ or SCLC	None		Lobectomy	4	0
32	43417	SCLC	74	M	200	2	1	0	NEC	None		Lobectomy	2	0
33	44106	SCLC	62	M	1175	1	0	0	SCLC	Yes	PR: 69.5%	Lobectomy	1	1
34	44165	Combined SCLC + LCC	70	M	1000	1	0	0	SCLC or LCC	None		Lobectomy	2	0
35	44304	Combined SCLC + LCC	63	M	3760	2	0	0	SCLC	None		Lobectomy	4	0
36	45819	SCLC	70	F	1000	2	0	0	SCLC	Yes	SD: 25.2%	Lobectomy	2	0
37	46287	SCLC	68	F	160	2	0	0	SCLC	Yes	PR: 73%	Lobectomy	4	0
38	49271	Combined SCLC + AD	80	M	840	2	0	0	AD	None		Lobectomy	2	2
39	50455	SCLC	70	M	1000	1	0	0	SCLC	None		Lobectomy	1	0
40	40245	SCLC	63	M	1660	2	0	0	SCLC	Yes	PR: 52.6%	Lobectomy	2	0
41	42388	Combined SCLC + AD	74	F	0	1	0	0	AD	No		Lobectomy	1	1
42	44506	SCLC	56	M	780	1	0	0	SCLC	Yes	PR: 50%	Lobectomy	1	0
43	48003	SCLC	68	F	270	1	0	0	SCLC	Yes	PR: 47%	Lobectomy	1	0

Table 1 (*continued*)

pM	p-Stage	Size (mm)	p	pm	v	ly	Adj-CTx	Reccurence	Treatment for recurrence: Regimen	Treatment for recurrence: Reduction status	Prognosis	Final follow-up (d)	Cause of death	Group
0	IIIB	22	1	1	1	1	Yes	Yes	CTx	PR	Dead	296	Pneumonia	N+B−
1	IV	50	0	2	1	0	Yes	Yes	CRTx	SD	Dead	737	Lung cancer	N+B+
0	IIIB	70	3	0	1	1	Yes	Yes	CTx	SD	Dead	568	Lung cancer	N+B+
0	IIIA	32	0	0	1	1	Yes	Yes	CRTx	CR	Dead	616	Lung cancer	N+B−
0	IIIA	16	0	0	0	1	Yes	None			Dead	732	Unknown	N+B−
0	IIIB	18	0	1	1	1	Yes	None			Alive	5825		N−B−
0	IB	33	0	0	1	0	Yes	Yes	Unknown[a]		Dead	617	Lung cancer	N+B+
0	IIA	20	2	0	1	1	None	Yes	Unknown[a]		Dead	209	Lung cancer	N+B−
0	IB	33	0	0	1	0	Yes	None			Alive	4103		N+B−
0	IIIB	26	0	1	1	1	None	None			Alive	4029		N+B−
0	IA	24	0	0	1	0	None	None			Dead	495	Mesentric embolism	N+B+
0	IA	22	0	0	1	0	Yes	Yes	CRTx + Op	PD	Dead	747	Lung cancer	N+B−
0	IB	45	0	0	1	0	Yes	None			Dead	2691	Unknown	N+B−
0	IA	20	1	0	1	0	None	None			Dead	174	Pneumonia	N+B−
0	IIIB	13	0	0	1	1	Yes	None			Alive	4072		N+B+
0	IIA	16	0	0	1	1	Yes	Yes	CTx	PD	Dead	373	Lung cancer	N+B−
0	IIIB	42	3	0	1	0	Yes	Yes	Unknown[a]		Dead	616	Lung cancer	N+B−
0	IIIA	25	0	0	1	1	Yes	Yes	CTx	PD	Dead	465	Lung cancer	N+B+
0	IIA	22	0	0	1	0	Yes	Yes	RTx	PR	Dead	948	Lung cancer	N+B−
0	IIIB	40	1-3[b]	1	1	1	None	Yes	RTx	CR	Dead	1098	Lung cancer	N+B−
0	IA	20	0	0	1	1	Yes	None			Alive	2682		N+B−
0	IA	19	0	0	1	0	None	Yes	CTx	SD	Dead	1157	Lung cancer	N+B−
0	IA	15	0	0	0	0	Yes	None			Alive	2167		N−B−
0	IIIB	58	3 (interlobe)	0	1	1	None	Yes	None[d]		Dead	77	Lung cancer	N−B−
0	IIIA	54	0	0	1	1	None	Yes	RTx	PD	Dead	120	Lung cancer	N+B+
0	IB	49	0	0	1	1	Yes	None			Alive	2623		N−B−
0	IIIB	48	1	1	1	1	Yes	Yes	RTx	CR	Dead	815	Lung cancer	N+B−
0	IIIB	11	0	1	1	1	None	None			Dead	312	Lung cancer	N+B−
0	IA	30	0	0	0	0	Yes	None			Dead	702	Respiratory failure	N−B−
0	IIA	20	0	0	1	1	Yes	None			Alive	3077		N−B−
0	IIIB	80	3	0	1	0	Yes	None			Alive	3037		N−B−
0	IB	80	1	0	1	0	Yes	None			Alive	2772		N−B−
0	IIA	15	2	0	1	1	Yes	Yes	CRTx	PR	Dead	1094	Lung cancer	N+B−
0	IIB	23	2	0	1	0	None	Yes	None[e]		Dead	620	Lung cancer	N+B−
0	IIIB	21	0	1	1	1	Yes	None			Alive	2363		N+B+
0	IB	32	1-3[b]	0	1	1	Yes	None			Dead	1203	Unknown	N+B−
0	IIIB	53	3	0	1	1	None	Yes	CRTx	PR	Dead	538	Lung cancer	N+B−
0	IIIA	31	0	0	1	0	Yes	Yes	Unknown[a]		Dead	760	Lung cancer	N+B−
0	IA	22	0	0	1	0	Yes	None			Alive	1330		N+B−
0	IB	32	1	0	0	1	Yes	None			Dead	1239	Other cancer	N−B−
0	IIA	30	0	0	1	1	No	Yes	RTx	SD	Dead	432	Lung cancer	N−B−
0	IA	9	0	0	0	0	No	Yes	RTx	PR	Dead	792	Lung cancer	N+B−
0	IA	11	0	0	0	0	No	None			Alive	2025		N+B−

2.5. Immunohistochemical analysis

Although histologic diagnosis was made based on H&E staining, immunohistochemical analyses were performed to characterize cells. Four-micrometer-thick tissue sections were mounted on silane-coated slides, routinely deparaffinized in xylene, and rehydrated through graded ethanol. For antigen retrieval, the slides were heated at 97°C for 40 minutes in citrate buffer at pH 6.0 or in EDTA buffer at pH 9.0. Immunohistochemical staining was performed using the EnVision+ DAB system with an autostainer (Dako, Glostrup, Denmark). Endogenous peroxidase activity was blocked with 3% hydrogen peroxide in methanol, and then each antibody was applied (Supplementary Table 1). We used antibodies for synaptophysin (SYP), chromogranin A (CGA), and CD56 as NE markers, as well as antibodies for p63 and high-molecular-weight cytokeratin (clone 34βE12 or K903) as basal cell markers (BA). Particular attention was paid to judgment of immunoreactivity in surgical materials because we intended to make a comparison between surgical and biopsy materials. Specifically, to avoid false-negative judgments in surgical materials, we always confirmed that positive control cells were correctly stained. Immunoreactivity was scored based on the percentage of cells that stained positively: negative, 0; less than 10%, 1+; 10% to 50%, 2+; and more than 50%, 3+. Only foci with SCLC morphology were evaluated if the case was diagnosed as combined with non-SCLC. The expression of each antibody in a tumor was defined as positive when 10% of the tumor cells or greater were stained (scores 2+ and 3+) and negative when less than 10% were stained (scores 0 and 1+). We defined cases with either positive p63 or CK34βE12 as belonging to the BA+ group and cases with any one of positive SYP, CGA, or CD56 as the NE+ group. Accordingly, all cases were divided into 4 groups: NE+BA−, NE+BA+, NE−BA+, and NE−BA−. Two independent observers (W. H. and Y. I.) pathologically reviewed all slides without any prior knowledge of patients, and discrepancies were resolved by joint discussion of the slides viewed with a multiheaded microscope.

2.6. Analysis of clinicopathological parameters

All analyses were performed using GraphPad PRISM software (ver 5.0b for Macintosh; GraphPad Software, San Diego, CA, USA) and SPSS software (ver 15.0; SPSS, Chicago, IL). We analyzed statistical correlations for clinicopathological features using the χ^2 test with Yate correction. Survival curves were delineated by Kaplan-Meier method, and survival difference was tested by the log-rank test using overall survival or cancer-specific survival, as appropriate. We also conducted univariate and multivariate analyses of the prognostic factors using the Cox proportional hazards model. All differences were considered statistically significant if $P < .05$.

3. Results

3.1. Gene expression analysis by microarray

To validate the results of the previous study with our cDNA microarray, 30 SCLCs were enrolled for the current study. The clinical characteristics of the enrolled cases were as follows: 21 men and 9 women; average age, 67 years; 27 (90%) were smokers; the median tumor size was 31 mm; and 14 (47%) were at p-stage I.

Unsupervised hierarchical clustering was performed with 15 431 of 54 000 probe sets on oligonucleotide array chips (Affymetrix HG U133 plus 2.0) expressed stably among all samples. The result of this clustering is shown in Fig. 1A. We obtained again 2 clusters, groups 1 and 2, and cases in group 2 had significantly better survival ($P = .0014$; Fig. 1B). We compared which genes were differently expressed in these 2 groups (Supplementary Table 2). Cases in group 2 highly expressed genes related to cell growth (G protein-coupled receptor, cyclin D1, *MYC*, etc), but many genes related to NE differentiation (*ASCL1*, *GRP*, *NCAM* [*CD56*], *CHGA*) were down-regulated.

3.2. Clinical characteristics of SCLC surgical patients and inoperable patients

As detailed in Table 1, for the surgical patients, the male/female ratio was 34:9, with a median age of 67 years (range, 46-84 years). Forty patients (95%) were smokers, with an average SI of 987. The median duration of follow-up was 24 months (range, 1-191 months). Among these, only 23 cases (53%) were definitely diagnosed as having an SCLC

Notes to Table 1

Abbreviations: p, pleural invasion; pm, intrapulmonary metastasis; v, vascular invasion; ly, lymphatic involvement; Adj-CTx, adjuvant chemotherapy; PR, partial response; CTx, chemotherapy; CRTx, chemoradiotherapy; RTx, radiotherapy; Op, Operation; LCC, large cell carcinoma; SD, stable disease; CR, complete response; p/d, poorly differentiated; ca, carcinoma; SQ, squamous cell carcinoma; AD, adenocarcinoma; NSCLC, non–small cell carcinoma, AS, adenosquamous cell carcinoma; NEC, NE carcinoma.

[a] Unknown means that the patient had treatment at another hospital.

[b] Invasion of visceral pleura was graded according to the report of Satoh et al [16]; p1-3 implies that a tumor extends to connective tissues between visceral and parietal pleural membranes.

[c] Double-synchronous primary carcinoma, SQ, and SCLC.

[d] Patients had best supportive care because of poor performance status.

[e] Patients had best supportive care because of his own decision.

Table 2 Immunoreactivity score and serum markers

Cases	Ref. no.	Immunoreactivity score								Serum markers					Group
		NE	SYN	CGA	NCAM	BA	p63	K903	Ki-67 (%)	CEA	SCC	CYFRA	NSE	ProGRP	
1	29635	1	3	3	3	0	1	0	90	3.9	1.1	–	–	–	N+B−
2	30017	1	3	3	3	1	3	0	90	6.4	0.5	–	–	–	N+B+
3	30156	1	3	1	1	1	3	1	100	4	1.1	––	–	–	N+B+
4	30323	1	3	3	3	0	0	0	80	3.1	0.3	–	9.6	–	N+B−
5	30865	1	3	3	3	0	0	0	70	2.3	–	–	–	–	N+B−
6	31160	0	1	0	1	0	0	1	80	7.1	0.5	–	6.7	–	N−B−
7	31401	1	3	0	3	1	1	2	80	4.2	0.7	–	4	–	N+B+
8	32658	1	3	3	3	0	1	1	100	3.9	2.1	–	–	–	N+B−
9	33130	1	3	1	3	0	0	0	60	11.2	–	–	–	–	N+B−
10	33587	1	3	0	3	0	1	0	70	23.9	–	–	–	–	N+B−
11	34802	1	3	1	3	1	3	3	80	4.1	0.9	–	–	–	N+B+
12	34947	1	3	2	3	0	1	0	70	0.8	0.7	–	8.8	–	N+B−
13	35452	1	3	3	3	0	0	1	80	5.5	–	–	4.6	–	N+B−
14	35628	1	3	3	3	0	1	1	100	1.3	4	–	3.2	–	N+B−
15	35996	1	3	3	3	1	2	0	80	1.1	–	–	1.9	–	N+B+
16	36454	1	3	3	3	0	0	0	100	3.2	1.2	–	7.2	–	N+B−
17	36483	1	0	1	3	0	1	0	80	3.2	1.4	–	5.1	–	N+B−
18	36819	1	3	3	3	1	2	0	80	5.4	0.2	–	14.3	–	N+B+
19	38779	1	3	3	3	0	0	1	90	2.1	0.2	–	7.6	70	N+B−
20	38809	1	3	3	3	0	0	0	90	6.7	0.4	–	8.6	–	N+B−
21	39001	1	3	3	3	0	0	1	100	2.3	–	1.3	6.4	29.9	N+B−
22	39080	1	3	3	3	0	1	1	70	8.4	–	–	–	–	N+B−
23	39401	0	0	1	1	0	0	1	90	3.6	2	–	–	–	N−B−
24	39933	0	0	0	1	0	0	1	70	5.7	0.4	4.5	6.7	17.2	N−B−
25	40557	1	3	3	3	1	3	3	100	2.1	0.4	3.5	7.5	20.5	N+B+
26	40805	0	0	0	1	0	0	0	100	3.5	0.7	1.8	6.5	25.5	N−B−
27	40914	1	3	1	3	0	1	0	100	15.1	0.7	2.4	6.6	72.5	N+B−
28	41179	1	3	3	3	0	1	1	70	3.1	1	–	3.8	151	N+B−
29	41310	0	0	1	1	0	1	0	70	6.3	0.3	1.8	4.9	35.3	N−B−
30	42085	0	1	0	0	0	1	1	80	3	0.6	1.3	2.1	20.8	N−B−
31	42253	0	0	1	0	0	0	0	90	5.2	0.4	4.7	13	26.4	N−B−
32	43417	0	1	0	1	0	0	1	90	1.7	–	2.3	10	26.6	N−B−
33	44106	1	3	3	3	0	1	0	100	2.3	0.7	–	–	78.3	N+B−
34	44165	1	3	3	3	0	1	1	90	5.4	1	–	–	–	N+B−
35	44304	1	0	1	2	1	0	3	80	3	0.4	–	–	18.6	N+B+
36	45819	1	3	2	1	0	1	1	100	3.9	–	–	–	49.6	N+B−
37	46287	1	3	3	3	0	0	0	90	5.3	–	–	23	638	N+B−
38	49271	1	3	3	3	0	1	1	100	18.8	0.7	–	6.9	56.2	N+B−
39	50455	1	3	3	3	0	0	0	90	2.6	0.6	–	–	32.4	N+B−
40	40245	0	0	0	0	0	0	0	80	3	0.2	–	12	24.7	N−B−
41	42388	0	0	0	0	0	0	0	70	4.2	–	–	–	–	N−B−
42	44506	1	3	3	3	0	0	0	90	1.9	–	1.2	2.8	16.4	N+B−
43	48003	1	2	3	3	0	0	0	70	0.8	0.9	1.4	–	267	N+B−
Positive ratio (%)		77	72	58	72	19	14	9	70-100	35	10	27	15	40	
N+: positive ratio (%)		100	94	75	94	24	18	12	86.1	33	9	20	11	62	
N−: positive ratio (%)		0	0	0	0	0	0	0	82	40	12	33	25	0	

NOTE. Reference values: CEA, 5.0 ng/mL; SCC, 1.5 ng/mL; CYFRA, 3.5 ng/mL; NSE, 10 ng/mL; ProGRP, 45.9 pg/mL.

before surgery. Most patients received a lobectomy and N2 lymph node dissection, except for 1 segmentectomy for a stage IA case, 1 pneumonectomy for stage IIB, and 2 partial resections. These 2 patients underwent partial resection because one was at a high risk (an advanced age and poor respiratory function) and the other had synchronous double-lung cancer with lobectomy performed for a larger tumor diagnosed as squamous cell carcinoma. Postoperatively, 25

cases were up-staged after identification of N or T factors. All information about the surgical patients is shown in Table 1, including invasion of visceral pleura, as graded for a previous study [16], and prognosis.

The characteristics of the biopsy group (n = 51) were as follows: median age, 67 years (range, 54-85 years); male/female ratio, 44:7; and 96% (43/45) having smoking history (average SI was 1152). One-year and 3-year survival rates were 57% and 2%, respectively. The serum level of ProGRP was higher than the reference value in 49 (96%) of 51 patients, and that of NSE was also higher in 27 (82%) of 33 patients.

Histologic review of resected materials confirmed that all cases were SCLCs according to the WHO classification, including 9 combined types as follows: 4 cases combined with adenocarcinomas, 1 with adenosquamous carcinoma, 1 with spindle cell carcinoma, 2 with large cell carcinoma, and 1 with LCNEC. Atypical cases were reviewed and agreed also by the pathology panel members of the Ministry of Health, Labour and Welfare study group as well as by some of the International Association for the Study of Lung Cancer pathology committee members. Tumors resembling SCLC such as Ewing sarcoma, poorly differentiated synovial sarcoma, lymphoma, squamous cell carcinoma composed of small-sized cells, and BA carcinoma were excluded, based on IHC results and/or close histopathologic observation.

3.3. NE and BA phenotypes in surgical and biopsied cases

Of the surgical patients, 31 (72%) were positive for SYN, 25 (58%) for CGA, 31 (72%) for CD56, 6 (14%) for p63, and 4 (9%) for CK34βE12. Percentages of NE marker positivity (58%-72%) were similar to the previous study based on surgery (57%-58% for SYN and CGA [7]). Immunoreactivity of BA markers might be explained by combined components with SCLC [12], although some cells were positive for both NE and BA markers. Interestingly, there were 8 patients (19%) positive for at least 1 BA marker, and 10 (23%) were negative for all NE markers (Table 2). According to these results, all cases could be classified into 4 subgroups: NE+BA− (n = 25; 58%), NE+BA+ (n = 8; 19%), NE−BA+ (n = 0), and NE−BA− (n = 10; 23%). Histologically, or using the Ki-67 index, it was difficult to distinguish among the 3 groups (Fig. 2; Table 2). When we compared immunoreactivity with several serum markers, the ProGRP value was significantly higher in the NE+ group, and no patients had an abnormal value in the NE− group (P = .023; Table 2), implying a good correlation of the NE phenotype between serum and tumors.

We examined concordance of classification by gene expression profiling and IHC phenotyping. Of 30 SCLC cases analyzed by gene expression profiling, 28 were successfully examined by IHC. All 12 cases classified to group 1 (poor prognosis group) by gene expression profiling fell into the NE+ group by IHC. Of the 16 cases classified to group

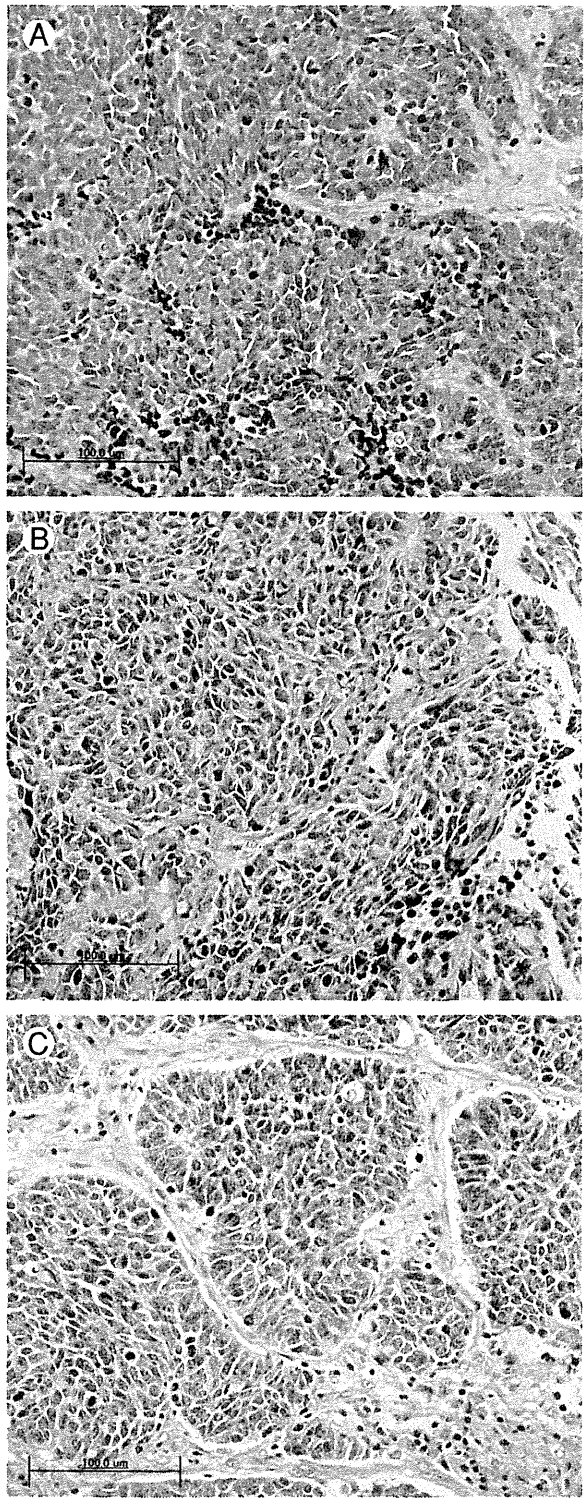

Fig. 2 Representative histologic pictures of SCLC subsets by NE differentiation and BA phenotypes (H&E, original magnification ×40). A, NE+BA−. B, NE+BA+. C, NE−BA−. Notably, there are almost no histopathologic differences among the 3 tumors, including mitosis counts.

Table 3 Comparison of clinicopathological features in the SCLC subgroups with/without NE and BA natures

Variable	No. of cases (n = 43)	NE markers		*P*	BA markers		*P*
		Negative (n = 10)	Positive (n = 33)		Negative (n = 35)	Positive (n = 8)	
Age (y)				.481			.7381
<60	10	1	9		9	1	
>61	33	9	24		26	7	
Sex				.718			.8666
Male	34	7	27		27	7	
Female	9	3	6		8	1	
Smoking status				.779			.9287
Never	3	1	2		2	1	
Smoker	40	9	31		33	7	
Tumor size (mm)				.818			.9044
≦30	25	5	20		21	4	
>30	18	5	13		14	4	
Lymph node metastasis				.616			.3605
Negative	25	7	18		22	3	
Positive	18	3	15		13	5	
Pathological stage				.687			.5953
I	17	5	12		15	2	
II-IV	26	5	21		20	6	
Combined subtypes	10	2	8		8	2	
AD	5	1	4		5	0	
SQ	0	0	0		0	0	
AS	1	0	1		0	1	
Spindle	1	0	1		1	0	
LCC	2	0	2		1	1	
LCNEC	1	1	0		1	0	
Induction CTx				.049			.9044
Negative	25	9	16		20	5	
Positive	18	1	17		15	3	
Adjuvant CTx				.56			.9303
Negative	14	2	12		12	2	
Positive	29	8	21		23	6	

Abbreviations: AD, adenocarcinoma; SQ, squamous cell carcinoma; AS, adenosquamous cell carcinoma; LCC, large cell carcinoma; CTx, chemotherapy.
NOTE. All were analyzed by χ^2 test with Yate correction.

2 (good prognosis group), 9 fell in the NE− group and the other 7 in the NE+ group. The concordance rates for groups 1 and 2 were 100% (12/12) and 56% (9/16), respectively.

For biopsy cases, all but 1 were positive for all the 3 NE markers and all were negative for the 2 BA markers. Only 1 patient was negative for CD56 and positive for SYN and CGA. As compared with surgical cases, therefore, the tumors of biopsy cases had a marked NE nature and lacked BA phenotypes.

3.4. Clinicopathological comparison between NE or BA expression and prognosis

We evaluated clinicopathological characteristics according to immunoreactivity for NE and BA markers (Table 3). Unfortunately, only 1 patient in the NE− group underwent induction chemotherapy, so we could not evaluate if the NE− tumors were chemosensitive or not. Rather, this indicated that tumors of the NE− group had almost no influence of chemotherapy and that their characteristics identified by IHC were innate, implying that the results of low NE expression were reliable. No factors showed any significant difference between the BA+ and BA− groups.

SCLC-specific survival curves of NE+/− and BA+/− groups are shown in Fig. 3. There was no difference based on the presence of BA phenotypes ($P = .28$; Fig. 3A), but NE phenotypes were critical for patient survival. In fact, the NE− group had a significantly better prognosis than did the NE+ group ($P = .03$; Fig. 3B). Among the 3 groups (NE+BA+, NE+BA−, NE−BA−), the NE−BA− group also showed a significant tendency toward a better outcome ($P = .036$; Fig. 3C).

3.5. Univariate and multivariate analyses of factors influencing prognosis

Thirty-three surgically treated patients underwent both lobectomy (single or bilobectomy) and platinum-based double chemotherapy (induction and/or adjuvant, ≥4 courses). We used this group with the same treatment condition to evaluate the factors influencing prognosis. Univariate analyses for

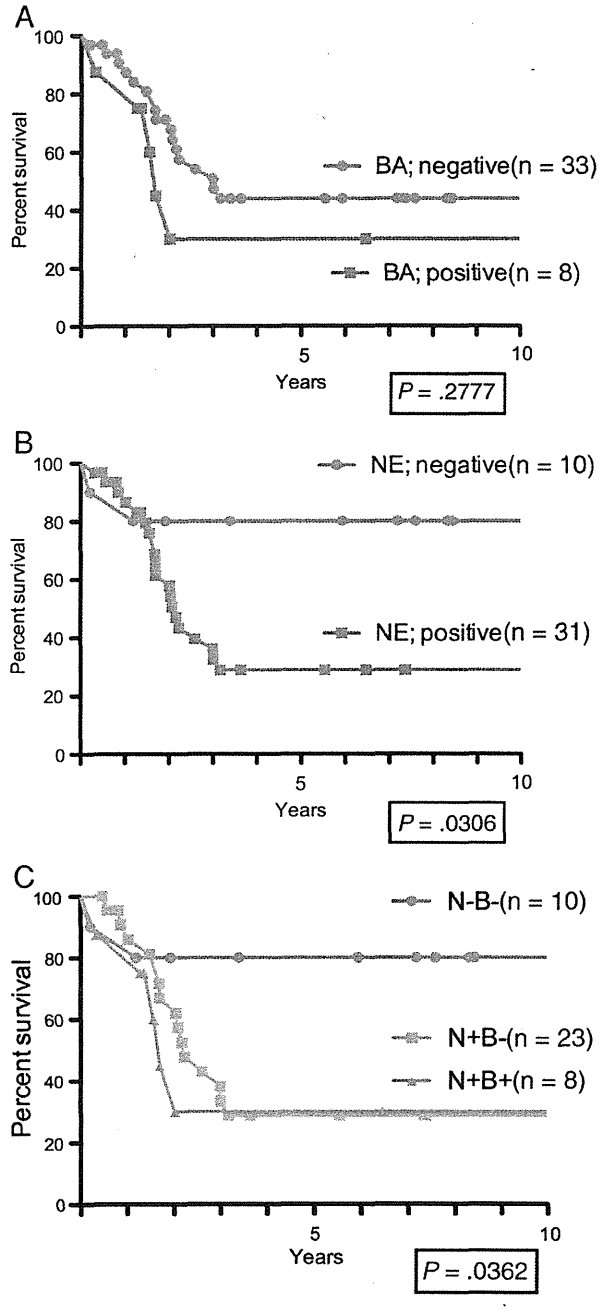

Fig. 3 SCLC-specific survival for patients with or without BA markers (A), $P = .278$, and NE markers (B), $P = .0306$. C, the NE− BA− group features a significantly better prognosis than the others.

overall survival showed that patients negative for NE markers tended to have good prognosis ($P = .047$; Table 4A). When using SCLC-specific survival, univariate analysis showed that both pathological stages ($P = .016$) and NE marker reactivity ($P = .012$) were significant markers for good prognosis. Age, SI, lymphovascular invasion, and BA marker immunoreactivity had no prognostic value. Multivariate analyses revealed that NE marker expression was the only independent factor influencing prognosis (Table 4B; risk ratio, 5.577; 95% confidence interval [CI], 1.172-26.524; $P = .031$). Multivariate analysis for SCLC-specific survival did not produce any significant results probably because the NE− group included no SCLC-specific deaths.

3.6. Induction chemotherapy and its effects on survival

Of the 43 surgical patients analyzed here, 17 (40%) underwent induction chemotherapy, and the reduction rate ranged from 24% to complete response, as detailed in Table 1. Because pretreatment might have some effect on prognosis, we performed survival analyses using 26 cases without pretreatment by comparing SCLC-specific survival between N+ (n = 17) and N− (n = 9) subgroups. As shown in Supplementary Fig. 1A, the survival of NE− subgroup was 3 times better than the NE+ subgroup. Although the difference was not significant ($P = .148$), this was probably due to the small number of cases. Furthermore, we compared SCLC death rates and survival difference of NE+ cases (n = 33) between those with induction chemotherapy (n = 16) and without (n = 17). They were 11 (69%) of 16 for cases with the pretreatment and 9 (53%) of 17 for cases without and were not significantly different. Also, as Supplementary Fig. 1B indicates, survival was not different between the 2 subgroups ($P = .19$), although the number of cases was larger than the analysis using non-pretreated cases. Based on these findings, we used all the cases including cases both with and without pretreatments for survival analysis.

4. Discussion

To our knowledge, this is the first report describing a hitherto unmarked SCLC subtype with a good prognosis, using substantial numbers of surgically resected cases. Here we demonstrated that the subtype can be detected by IHC alone using NE markers such as SYP, CGA and CD56. Previously, we identified the subtype by global gene expression profiling using cDNA microarrays. The current study, using oligonucleotide arrays (by Affymetrix), duplicated fairly well the subset with additional new cases. Also in this study, we focused on characterizing the SCLC subset by hypothesizing that low expression of NE-related proteins and/or a BA nature of tumor cells might explain differences from standard SCLCs.

In fact, BA carcinoma histologically resembles SCLC, and the BA pattern is a marker for worse prognosis for non-SCLC [17]. Our univariate and multivariate analyses reveal, however, that expression of NE markers is a prognostic factor, but the BA phenotype in terms of CK34βE12 and p63 protein expression has no effect on survival. The immunohistochemically defined obvious subtype of SCLC with a good prognosis comprised 23% of the surgically resected SCLC. Because there were no such cases in

Table 4 Univariate and multivariate analyses on factors influencing overall survival, based on all cases (n=96, surgery [n=45] and biopsy [n=51]). (A) analyses for SCLC-specific survival (B)

Parameters	A					B
	Univariate	Multivariate				Univariate
	P	*P*	Exp (coefficient)	Lower (95% CI)	Upper (95% CI)	*P*
Age (>60 y)	.900	.666	1.256	0.447	3.525	.618
Sex	.777					.303
Pathological stage (>I)	.135	.095	2.381	0.860	6.592	.016
Vascular invasion	.886					.174
Lymphatic invasion	.827					.534
NE marker	.047	.031	5.577	1.172	26.524	.012
BA marker	.777	.331	0.559	0.173	1.804	.208

inoperable patients, we could not perform a study using only biopsy materials.

According to the current WHO criteria for NE tumors, it is necessary to prove NE phenotypes for LCNEC diagnosis, but not for SCLCs. In the present study, approximately 80% of surgical tumors had NE phenotypes, largely consistent with the previous studies [7,18,20], and all the biopsy cases had obvious NE phenotypes proven by IHC. Although this fact suggests that the current WHO criteria work quite well, they are insufficient to distinguish the atypical SCLC subtype with a good prognosis, particularly for surgical cases.

Serum tumor markers including NSE, ProGRP, and CD56 are useful for clinical diagnosis of SCLC, and their immunohistochemical staining has been used for discrimination of NE tumors from others. However, their prognostic value has proved controversial [21,23]. In this study, we demonstrated immunohistochemical use for outcome prediction. Also, the NE marker levels in serum tended to be higher in the group with a poor prognosis. In fact, almost all the cases with elevated serum markers belonged to the poor prognosis group, as shown in Table 2. Because the number of cases with measured serum NE markers in the good prognosis group is limited, we should continue comparing the prognosis between groups with and without elevated values.

Chemosensitivity and radiosensitivity is crucial for SCLC treatment. Unfortunately, we were unable to determine if our NE− (negative) group was chemosensitive or not because none of the cases underwent induction chemotherapy or treatment of a recurrent tumor. We should further investigate sensitivity by accumulating more cases of this particular SCLC subtype.

Although it is difficult to distinguish histologically an SCLC subtype with a good prognosis, such a subtype may exist, which has distinct cellular and genetic characteristics. In our previous study [13], we performed an integrated analysis using clinical SCLC tumors and established SCLC cell lines. As a matter of fact, there were no cell lines that clustered together with the good prognosis subtype. Therefore, further studies may include establishing cell lines of this particular SCLC subtype.

Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.humpath.2014.01.001.

Acknowledgments

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Sublobar Resection for Lung Adenocarcinoma Meeting Node-Negative Criteria on Preoperative Imaging

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Background. This study evaluated the usefulness of sublobar resection for patients with clinical stage IA lung adenocarcinoma that met our proposed node-negative criteria: solid tumor size of less than 0.8 cm on high-resolution computed tomography or maximum standardized uptake value of less than 1.5 on [18F]-fluoro-2-deoxy-D-glucose positron emission tomography/computed tomography.

Methods. A multicenter database of 618 patients with completely resected clinical stage IA lung adenocarcinoma who underwent preoperative high-resolution computed tomography and [18F]-fluoro-2-deoxy-D-glucose positron emission tomography/computed tomography was used to evaluate the surgical results of sublobar resection for patients who met our node-negative criteria.

Results. No patient who met the node-negative criteria had any pathological lymph node metastasis. Recurrence-free survival (RFS) and overall survival (OS) rates at 5 years were significantly higher for patients who met the node-negative criteria (RFS: 96.6%; OS: 95.9%) than for patients who did not (RFS: 75.5%, $p < 0.0001$; OS: 83.1%, $p < 0.0001$). Among patients who met the node-negative criteria, RFS and OS rates at 5 years were not significantly different between those who underwent lobectomy (RFS: 96.0%; OS: 95.9%) and those who underwent sublobar resection (RFS: 97.2%, $p = 0.94$; OS: 95.9%, $p = 0.98$). Of 264 patients with T1b (2-cm to 3-cm) tumors, 106 (40.2%) met the node-negative criteria.

Conclusions. Sublobar resection without systematic nodal dissection is feasible for clinical stage IA lung adenocarcinoma that meets the above-mentioned node-negative criteria. Even a T1b tumor, which is generally unsuitable for intentional sublobar resection, can be a candidate for sublobar resection if it meets these node-negative criteria.

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Early-stage lung cancer, particularly lung adenocarcinoma, is now frequently being detected because of advanced radiographic techniques, such as high-resolution computed tomography (HRCT), and the widespread use of low-dose helical CT for tumor screening [1–3]. In a prospective randomized controlled study, the Lung Cancer Study Group reported that the outcomes of limited resections, such as segmentectomy and wedge resection, were inferior to those of standard lobectomy in patients with clinical T1 node-negative (N0) M0 non-small cell lung cancer (NSCLC) [4]. However, several studies have demonstrated the usefulness of sublobar resection for peripheral small-sized NSCLC [3, 5–10].

Theoretically, true N0 lung cancer can be treated by sublobar resection without nodal dissection when surgical margins are adequate. We previously reported that preoperative HRCT and [18F]-fluoro-2-deoxy-D-glucose (FDG) positron emission tomography/computed tomography (PET/CT) were useful for predicting N0 clinical stage IA lung adenocarcinoma [11].

The objective of this study was to evaluate the usefulness of sublobar resection for clinical stage IA lung adenocarcinoma that met our previously proposed N0 criteria: solid tumor size of less than 0.8 cm on HRCT or a maximum standardized uptake value (SUVmax) of less than 1.5 on FDG-PET/CT [11].

Patients and Methods

Patients

Between August 1, 2005, and June 30, 2010, we enrolled 618 patients with clinical T1 N0 M0 stage IA lung adenocarcinoma from 4 institutions in Japan (Hiroshima University, Kanagawa Cancer Center, Cancer Institute Hospital, and Hyogo Cancer Center). For this study, we retrospectively analyzed the data for all 618 patients in

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Abbreviations and Acronyms

CI	= confidence interval
CT	= computed tomography
F	= female
FDG	= [18F]-fluoro-2-deoxy-D-glucose
HR	= hazard ratio
HRCT	= high-resolution computed tomography
IRB	= Institutional Review Board
LI	= lymphatic invasion
LN	= lymph node
LNM	= lymph node metastasis
M	= male
N0	= node-negative
NSCLC	= non-small cell lung cancer
OS	= overall survival
PET	= positron emission tomography
PI	= pleural invasion
Pt	= patient
RFS	= recurrence-free survival
SUVmax	= maximum standardized uptake value
VI	= vascular invasion

this multicenter database. The database included patients who underwent preoperative staging using HRCT and FDG-PET/CT, followed by curative resection without neoadjuvant chemotherapy or radiotherapy, with a definitive histopathologic diagnosis of lung adenocarcinoma. Excluded were those with incompletely resected tumors (R1 or R2) and those with synchronous multiple tumors or previous lung operations. This database has been prospectively collected and maintained.

HRCT and FDG-PET/CT, followed by curative R0 resection, had been performed for all patients who were staged according to the TNM Classification of Malignant Tumours, 7th Edition [12]. Mediastinoscopy and endobronchial ultrasonography were not routinely performed because all patients had undergone preoperative HRCT and FDG-PET/CT. HRCT revealed less than 1-cm enlargement of mediastinal or hilar lymph nodes and FDG-PET revealed a SUVmax of less than 1.5 in these lymph nodes.

Segmentectomy was considered for patients with clinical stage IA tumors that could be completely resected with ample surgical margins. No lymph node metastasis was intraoperatively confirmed on rapid frozen sections for enlarged lymph nodes or lymph nodes that were suspected with disease in the thoracic cavity. In cases of apparent or suspected nodal metastasis, lobectomy was chosen. Systematic lymphadenectomy, including hilar and mediastinal node dissection, was performed during segmentectomy but not during wedge resection. Therefore, wedge resection was performed for tumors, of which a ground glass opacity component accounted for great majority on HRCT. All patients who had pathologically diagnosed lymph node metastases received four cycles of platinum-based chemotherapy after the operation. None of the study patients received adjuvant radiotherapy.

Patients were divided into two groups. One group included patients who met the N0 criteria of solid tumor size of less than 0.8 cm on HRCT or a SUVmax of less than 1.5 on FDG-PET/CT [11]. The other group included patients who did not meet these N0 criteria.

This multicenter study was approved by the Institutional Review Boards (IRBs) of Hiroshima University Hospital (IRB No. EKI-644), Kanagawa Cancer Center (IRB No. KEN-31), Cancer Institute Hospital (IRB No. 2008-1018), and Hyogo Cancer Center (IRB No. H20-RK-15). All IRBs waived the requirement for informed consent from individual patients for this retrospective review of a prospective database.

HRCT Acquisition

Chest images were acquired with 16-row multidetector CT independently of subsequent FDG-PET/CT examinations. For high-resolution tumor images, the following parameters were used: 120 kVp; 200 mA; 1- to 2-mm section thickness; 512- × 512-pixel resolution; 0.5- to 1.0-second scanning time; a high-spatial reconstruction algorithm with a 20-cm field of view; and mediastinal (level: 40 HU; width: 400 HU) and lung (level: −600 HU; width: 1,600 HU) window settings. Ground glass opacity was defined as a misty increase in lung attenuation that did not obscure underlying vascular markings. We defined solid tumor size as the maximum dimension of the solid component in the lung windows, excluding the ground glass opacity [13]. Radiologists from each participating institution reviewed the CT scans and determined the tumor sizes.

FDG-PET/CT Acquisition

Patients were instructed to fast for more than 4 hours before intravenous injection of 74 to 370 MBq of FDG, which was followed by a relaxation period of at least 1 hour before FDG-PET/CT scanning. Blood glucose levels were determined before the tracer injection to confirm a level of less than 150 mg/dL. Patients with blood glucose levels of 150 mg/dL or more were excluded from PET/CT imaging. For imaging, we used a Discovery ST (GE Healthcare, Little Chalfont, UK), Aquiduo (Toshiba Medical Systems Corp, Tochigi, Japan), or Biograph Sensation16 (Siemens Healthcare, Erlangen, Germany) integrated 3-dimensional PET/CT scanner.

Following a standard protocol, low-dose, nonenhanced CT images (2- to 4-mm section thickness) for attenuation correction and localization of lesions identified by PET were obtained from the head to the pelvic floor of each patient. Immediately after CT, PET covered the same axial field of view for 2 to 4 minutes per table position, depending on the condition of the patient and scanner performance.

An iterative algorithm with CT-derived attenuation correction was used to reconstruct all PET images with a 50-cm field of view. An anthropomorphic body phantom (NEMA NU2-2001; Data Spectrum Corp, Hillsborough, NC) was used to minimize variations in SUVs among the institutions. A calibration factor was evaluated by dividing the actual SUV by the gauged mean SUV in the

phantom background to decrease interinstitutional SUV inconsistencies. The final SUV used here is referred to as the revised maximum SUVmax. Radiologists from each institution determined the original SUVmax values.

Follow-Up Evaluations

All patients who underwent lung resection were followed up from the day of the operation. Postoperative follow-up procedures for the first 2 years included physical examination and chest roentgenography every 3 months and chest and abdominal CT examinations every 6 months. Subsequently, physical examination and chest roentgenography were performed every 6 months and chest CT examination was performed every year.

Statistical Analysis

Results are given as numbers (%) or medians, unless otherwise stated. A χ^2 test was used to compare frequencies for categoric variables. The Fisher exact test was used when sample sizes were small. Recurrence-free survival (RFS) was defined as the time from the date of the operation until the first event (relapse or death from any cause) or the last follow-up. Overall survival (OS) was defined as the time from the date of the operation until death from any cause or the last follow-up. The Kaplan-Meier method was used to analyze RFS and OS durations, and a log-rank test was used to compare differences in RFS and OS. We performed a Cox proportional hazards model to determine whether age (continuous), sex, solid tumor size (continuous), SUVmax (continuous), or surgical procedure influenced RFS. We only used preoperative potential confounding factors as variables because postoperative factors would never influence the decision for surgical procedure. SPSS 10.5 software (SPSS Inc, Chicago, IL) was used for statistical analysis. The level of statistical significance was set at a p value of less than 0.05.

Table 1. Clinicopathologic Features of Patients Who Did and Did Not Meet the Node-Negative Criteria

Variables[a]	Solid Tumor Size ≥0.8 cm and SUVmax ≥1.5 (n = 293)	Solid Tumor Size <0.8 cm or SUVmax <1.5 (n = 325)	*p* Value
Age, y	67.0 (37–84)	65 (31–89)	0.04
Male sex	137 (46.8)	135 (41.5)	
Whole tumor size (cm)	2.2 (0.8–3.0)	1.8 (0.6–3.0)	<0.001
Solid tumor size (cm)	1.8 (1.0–3.0)	0.4 (0–3.0)	<0.001
Clinical T			<0.001
T1a	135 (46.1)	219 (67.4)	
T1b	158 (53.9)	106 (32.6)	
SUVmax	3.0 (1.5–17.0)	0.9 (0–9.8)	<0.001
Adenocarcinoma in situ	5 (1.7)	92 (28.3)	<0.001
Procedure			<0.001
Lobectomy	246 (84.0)	137 (42.2)	
Sublobar resection	47 (16.0)	188 (57.8)	
Segmentectomy[b]	23 (7.8)	75 (23.1)	
Wedge resection	24 (8.2)	113 (34.8)	
Lymphatic invasion	87 (29.7)	5 (1.5)	<0.001
Vascular invasion	101 (34.5)	5 (1.5)	<0.001
Pleural invasion	62 (21.2)	5 (1.5)	<0.001
Lymph node metastasis	45 (15.4)	0 (0)	<0.001
N1	24 (8.2)	0 (0)	
N2	21 (7.2)	0 (0)	
Recurrence	57 (19.5)	2 (0.6)	<0.001

[a] Categoric data are shown as number (%) and continuous data as median (range). [b] Details of segmentectomy were right S1 in 4, S2 in 12, S3 in 3, S6 in 23, S8 in 5, S7 + 8 in 1, S8 + 9 in 3, S7 + 8 + 9 + 10 in 1, left S1 + 2 in 7, S3 in 3, S1 + 2 + 3 in 10, S1 + 2 + 3c in 1, S4 in 2, S5 in 1, S4 + 5 in 7, S6 in 10, S8 in 1, S9 in 3, and S6 + 8 + 9 + 10 in 1.

SUVmax = maximum standardized uptake value.

Results

The characteristics of the 325 patients who met our N0 criteria and the 293 patients who did not are summarized in Table 1. There were no 30-day postoperative deaths in this study population. The median follow-up period of censored patients after the operation was 42.9 months. The mean follow-up period after lobectomy and segmentectomy were 43.3 months ± 15.6 and 40.4 ± 14.7 months in the N0 criteria group ($p = 0.10$) and 43.8 ± 16.8 months and 40.1 ± 19.3 months in the non-N0 criteria group ($p = 0.39$), respectively. There were significant differences between the two groups with regard to age, whole tumor size, solid tumor size, clinical T factor, SUVmax, surgical procedure, pathologic invasiveness (lymphatic, vascular, and pleural invasion), lymph node metastasis, and recurrence.

Patients who met the N0 criteria had significantly fewer pathologically invasive tumors and underwent sublobar resection. Lymph node metastases were found in 45 of the 293 patients (15.4%) who did not meet the N0 criteria. Of 45 patients with lymph node metastasis, 1 was N2 after sublobar resection (S6 segmentectomy), 24 were N1 after lobectomy, and 20 were N2 after lobectomy. Two patients who met the N0 criteria had tumor recurrences (Table 2). One was a 57-year-old woman with a solid tumor size of 1.3 cm and an SUVmax of 1.2. Although she had undergone standard lobectomy and had no lymph node metastasis, mediastinal lymph node recurrence subsequently developed. The other patient was a 59-year-old man with a solid tumor size of 1.8 cm and an SUVmax of 1.4. He had undergone wedge resection without lymph node dissection, and multiple lung metastases without lymph node recurrence subsequently developed.

The 5-year RFS rate (96.6%) was significantly better for patients who met the N0 criteria than for patients who did not (75.5%, $p < 0.0001$; Fig 1A). The 5-year OS rate (95.9%) was also significantly better for patients who met the N0 criteria than for patients who did not (83.1%, $p < 0.0001$; Fig 1B).

Among the patients who met the N0 criteria, no significant difference was noted in the 5-year RFS rate

Table 2. Patients Who Met the Node-Negative Criteria and Developed Recurrences

Pt	Age	Sex	Whole Tumor Size	Solid Tumor Size	SUVmax	Procedure	LI	VI	PI	LNM	Recurrence Site	Outcome
1	57	F	1.4 cm	1.3 cm	1.2	Lobectomy	1	0	0	0	Mediastinal LN	24 m, dead
2	59	M	1.8 cm	1.8 cm	1.4	Wedge resection	0	0	0	0	Multiple lung	48.8 m, dead

F = female; LN = lymph node; LI = lymphatic invasion; LNM = lymph node metastasis; M = male; PI = pleural invasion; Pt = patient; SUVmax = maximum standardized uptake value; VI = vascular invasion.

between those who underwent lobectomy (96.0%) and those who underwent sublobar resection (97.2%, $p = 0.94$; Fig 2A). Similarly, the 5-year OS rate was not significantly different between patients who underwent lobectomy (95.9%) and those who underwent sublobar resection (95.9%, $p = 0.98$; Fig 2B). Of 164 patients with T1b tumors, 106 (40.2%) met the N0 criteria (Table 3). These patients rarely had pathologic invasiveness, and no recurrences developed.

In patients who did not meet the N0 criteria, the 5-year RFS rate was 63.9% for those who underwent segmentectomy and 77.7% for those who underwent lobectomy; this difference was not statistically significant ($p = 0.058$; Fig 2C). The 5-year OS rate for patients who underwent lobectomy (82.8%) and those who underwent sublobar resection (85.2%) was also not significantly different ($p = 0.69$; Fig 2D).

Multivariate Cox analysis including the preoperative factors and surgical procedures revealed that solid tumor size and SUVmax were independent prognostic factors for RFS, whereas age, sex, and surgical procedure were not (Table 4). In clinical T1b patients, SUVmax was an independent prognostic factor for RFS, whereas surgical procedure was not (Table 5).

Comment

The purpose of the current study was to assess the usefulness of sublobar resection for clinical stage IA lung adenocarcinoma that met our proposed N0 criteria. Patients who met our N0 criteria had fewer pathologically invasive tumors and fewer recurrences compared with those who did not meet these criteria. These results were consistent with those of our previous report [11].

Recurrences developed in 2 patients in this study who met the N0 criteria. Mediastinal lymph node recurrence developed in 1 patient after standard lobectomy, whereas multiple lung metastases without lymph node involvement after wedge resection without lymph node dissection were found in the other patient. We assumed that these patients would have had recurrences even if they had undergone standard surgical procedures.

Patients who met our N0 criteria had significantly better prognoses compared with those who did not. Therefore, clinical stage IA lung adenocarcinoma could be divided into two groups with different malignant behaviors and prognoses using solid tumor size on HRCT and SUVmax on FDG-PET/CT. These findings support our previous results that solid tumor size on HRCT and SUVmax on FDG-PET/CT were predictors of pathologic tumor invasiveness, lymph node metastasis, and prognosis [11, 13].

Among the patients who met the N0 criteria, we compared 5-year RFS and OS rates between those who underwent lobectomy and those who underwent sublobar resection. Patients who underwent sublobar resection had excellent prognoses, without any significant differences in RFS and OS rates compared with those

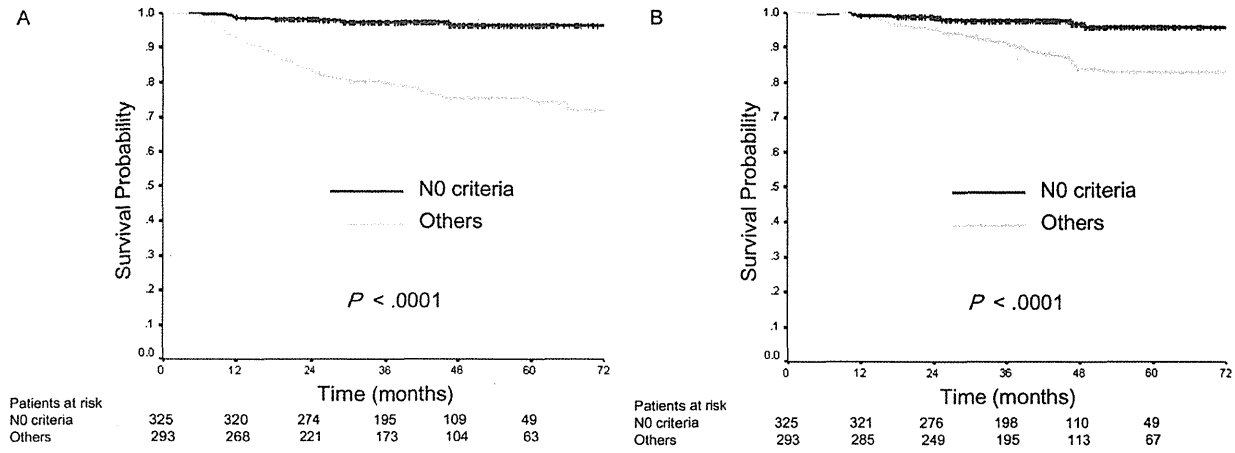

Fig 1. Recurrence-free survival (RFS) and overall survival (OS) curves are shown for patients who met the node-negative (N0) criteria (blue lines) and those who did not (yellow lines). (A) RFS at 5 years was significantly different between patients who met the N0 criteria (96.6%) and those who did not (75.5%, $p < 0.0001$). (B) OS at 5 years was significantly different between patients who met the N0 criteria (95.9%) and those who did not (83.1%, $p < 0.0001$).

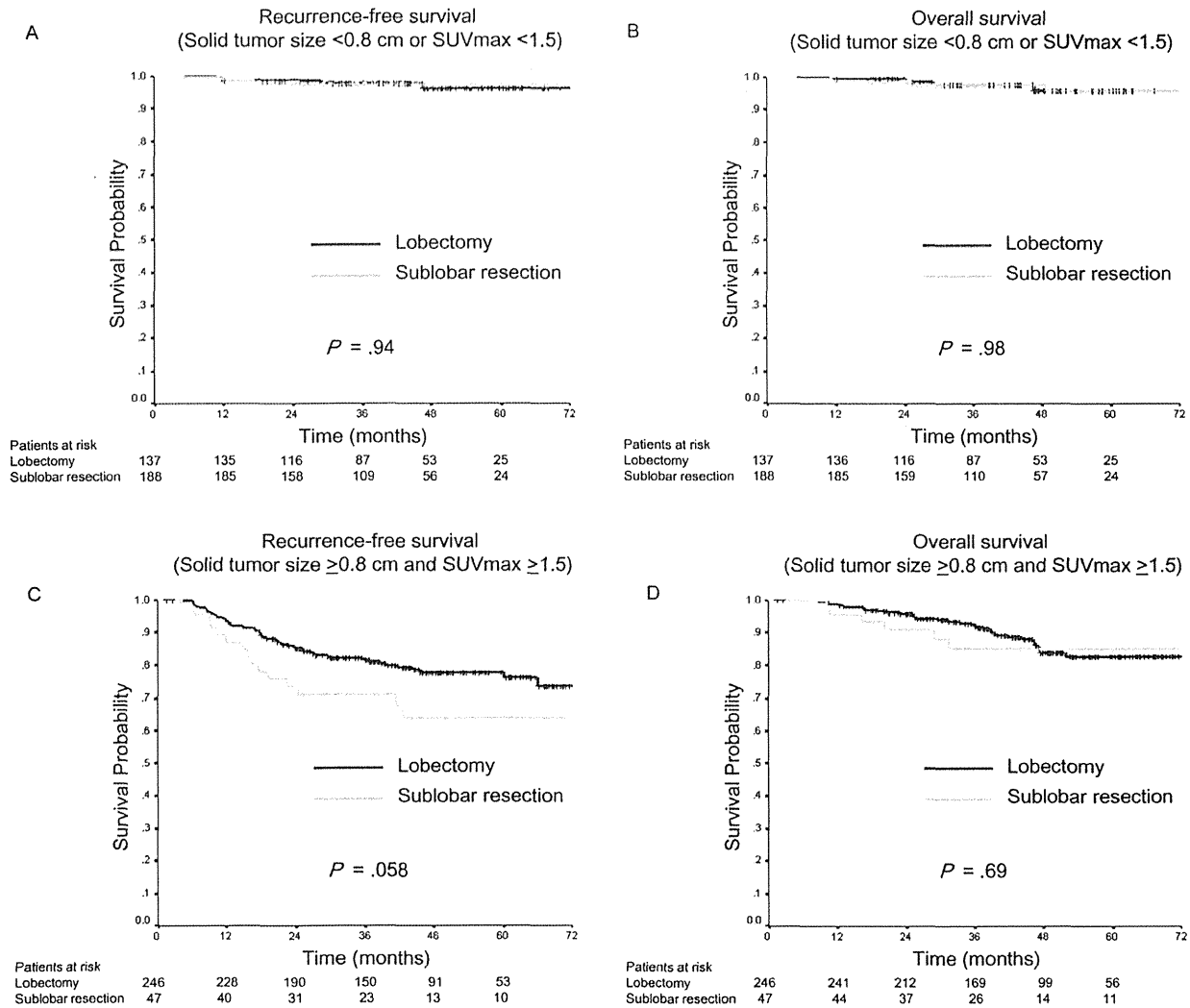

Fig 2. Recurrence-free survival (RFS) and overall survival (OS) curves are shown for patients who underwent lobectomy (blue line) or sublobar resection (yellow line) on the basis of the node-negative (N0) criteria. (A) For the group that met the N0 criteria, the RFS rate at 5 years was not significantly different between patients who underwent lobectomy (96.0%) and those who underwent sublobar resection (97.2%, p = 0.94). (B) For the group that met the N0 criteria, the OS rate at 5 years was not significantly different between patients who underwent lobectomy (95.9%) and those who underwent sublobar resection (95.9%, p = 0.98). (C) For the group that did not meet the N0 criteria, patients who underwent sublobar resection tended to have a worse RFS rate at 5 years (63.9%) than patients who underwent lobectomy (77.7%, p = 0.058). (D) For the group that did not meet the N0 criteria, there was no significant difference in the OS rate at 5 years between patients who underwent lobectomy (82.8%) and those who underwent sublobar resection (85.2%, p = 0.69). (SUVmax = maximum standard uptake value.)

who underwent lobectomy. For this study, we included segmentectomy and wedge resection as sublobar resections.

Actually, segmentectomy and wedge resection are considerably different procedures. The former can be used to approach hilar lymph nodes, whereas the latter cannot. However, patients who met our N0 criteria were considered not to have lymph node metastasis; therefore, systematic lymph node dissection did not appear to be necessary. Both procedures can be used for patients with solid tumor size of less than 0.8 cm on HRCT or a SUVmax of less than 1.5 on FDG-PET/CT. We should consider the surgical margin, and not lymph node dissection, when selecting the surgical procedure for patients with clinical stage IA lung adenocarcinomas that meet these N0 criteria.

Interestingly, approximately 40% of clinical T1b (2 to 3 cm) tumors in this study met the N0 criteria. Most research done in this area has generally not included patients with tumor sizes exceeding 2 cm for sublobar resection [3, 5–8]. However, these patients had T1b tumors with considerably low malignant potentials, and no recurrence developed. Therefore, even patients with T1b tumors that meet these N0 criteria can be candidates for