CONCLUSIONS

The number of EPCs and the MVD in patients with predominantly solid adenocarcinomas were significantly higher than those in nonsolid adenocarcinoma patients. In particular, patients with solid adenocarcinoma may be the best candidates for antiangiogenic therapies against VEGF or EPCs among the various adenocarcinoma subtypes.

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Clinical features of unresectable high-grade lung neuroendocrine carcinoma diagnosed using biopsy specimens☆

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ABSTRACT

Background: The overall clinicopathological features or the optimal therapy for large cell neuroendocrine carcinoma (LCNEC) have yet to be defined, because LCNEC has not been studied in the same depth as had small cell lung carcinoma (SCLC) in both clinical and biological standpoints. The aim of this study was to elucidate the clinical features of high-grade neuroendocrine carcinoma (HGNEC)-probable LCNEC diagnosed by biopsy, and compare therapeutic efficacy with patients with SCLC.
Methods: We retrospectively examined the chart of total of 25 patients who underwent chemotherapy or chemoradiotherapy as initial therapy for a histologic diagnosis of HGNEC-probable LCNEC, using biopsy samples and compared their data with those of 180 patients with SCLC. We analyzed their responses to chemotherapy and/or radiation therapy and survival outcomes.
Results: In 25 patients with HGNEC-probable LCNEC, 18 patients initially received chemotherapy (17 (94%) of whom received platinum-based chemotherapy) with an overall response rate (ORR) of 61%. The remaining 7 patients received chemoradiotherapy with an ORR of 86%, and 12 of the 25 patients who received second-line chemotherapy had an ORR of 17%. A total of 101 patients with SCLC who initially received chemotherapy had an ORR of 63%, and 79 patients who initially received chemoradiotherapy had an ORR of 98%, and 102 of the 180 patients who received second-line chemotherapy had an ORR of 45%. The 1-year overall survival rate for patients with stage IV HGNEC-probable LCNEC ($n = 13$) and those with ED-SCLC ($n = 80$) was 34% and 49%, respectively ($p = 0.84$).
Conclusion: The overall response rate to initial treatment and the survival outcomes of HGNEC-probable LCNEC were comparable to those of SCLC, but the effectiveness of second-line chemotherapy appeared to differ between the 2 groups.

1. Introduction

Large cell neuroendocrine carcinoma (LCNEC) of the lung and small cell lung carcinoma (SCLC) are both now considered to be high-grade neuroendocrine carcinomas arising in the lung. Travis et al. [1] were the first to propose the term LCNEC in 1991, to describe cancer which exhibits neuroendocrine morphologic features such as rosette formation, organoid nesting, and palisading, large tumor cells (typically 3 times larger in diameter than a small resting lymphocyte) with a low nuclear/cytoplasmic ratio, numerous nucleoli, a high mitotic rate (>10 in 10 high-power fields), a large degree of necrosis, and immunohistochemical positive staining findings for 1 or more neuroendocrine markers [2]. The tumor cells of SCLC are round, oval, or spindle-shaped; usually less than the size of three small resting lymphocytes, and have scant cytoplasm, finely granular chromatin, and absent or inconspicuous nucleoli [2]. The morphologic features of LCNEC differ distinctly from those of SCLC by definition, however, distinguishing LCNEC from SCLC based on the tumor cell size and chromatin morphology may be difficult in some cases.

SCLC has poorer outcome, despite its marked chemosensitivity, enabling temporary remission in most SCLC patients because most tumors relapse after chemotherapy or chemoradiotherapy. The standard therapeutic strategy for SCLC has already been

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Table 1
Proposed criteria for diagnosis of pulmonary HGNEC-probable LCNEC using biopsy specimens.

1.	Solid tumor nesting without either acinar or squamous differentiation
2.	Moderate or marked cellular atypia
3.	Large cell size with low nuclear to cytoplasmic ratio or moderate to abundant eosinophilic cytoplasm
4.	Vesicular and/or coarsely granular nuclear chromatin
5.	Prominent nucleoli
6.	Positive immunostaining for one or more neuroendocrine markers (NCAM, chromogranin A, and synaptophysin)
7	Ki-67/MIB1 labeling index >40%

NCAM, neural cell adhesion molecule; HGNEC, high-grade neuroendocrine carcinoma; LCNEC, large cell neuroendocrine carcinoma.

established, and second-line chemotherapy has been recognized to be well-tolerated and effective in patients with chemotherapy-sensitive SCLC [3–7]. In contrast, the overall clinicopathological features or the standard treatment for LCNEC have yet to be defined, because LCNEC has not been studied in the same depth as had SCLC in both clinical and biological standpoints. Moreover, the incidence of the pre-therapeutic diagnosis of LCNEC in unresectable cases is unknown. Although obtaining a definitive diagnosis of LCNEC using small biopsy specimen is difficult, there is an urgent need to establish the diagnostic criteria for LCNEC. Therefore, instead of diagnosing LCNEC, we usually use the term "high-grade neuroendocrine carcinoma (HGNEC)-probable LCNEC" based on the proposed criteria (Table 1).

The aim of this study was to elucidate the clinical features of unresectable HGNEC-probable LCNEC (HG-pLCNEC) with those of SCLC, and compare their outcomes.

2. Patients and methods

2.1. Patient enrollment

From January 2002 through December 2009, we retrospectively examined the charts of total of 25 patients with a histologic diagnosis of HG-pLCNEC, using biopsy specimens. Diagnoses of HG-pLCNEC were all confirmed by pathological examination on biopsy specimens according to the modified criteria for the diagnosis of high-grade non-small cell neuroendocrine carcinoma using biopsy specimens proposed by Igawa et al. [8] (Table 1). All patients had undergone a minimum of 1 course of chemotherapy or chemoradiotherapy as initial therapy. Furthermore, the data of a total of 180 patients with histologically confirmed SCLC who had completed a minimum of 1 course of chemotherapy or chemoradiotherapy were examined as a control group. We used these criteria because the diagnostic criteria for LCNEC in the third edition of the World Health Organization (WHO) guidelines, which have been mainly established for cases of surgical specimens, and fulfilling the diagnostic criteria for LCNEC according to the WHO classification system is often difficult with biopsy specimens. We extracted the clinical data of patients from their medical records, all of whom had been given diagnoses of unresectable HG-pLCNEC or SCLC based on the results of pre-therapeutic evaluation including physical examination, chest radiography, computed tomography (CT) of the chest and abdomen, magnetic resonance imaging (MRI) of the brain, isotopic bone scan, and positron emission tomography (PET) or combined PET-CT. Their clinical disease staging was then reassessed according to the 7th edition of the International Union Against Cancer TNM classification system [9]. Data collection and analyses were approved by the institutional review board in December 2010, and the need to obtain informed consent from patients was waived due to the retrospective nature of the study.

2.2. Histopathology

We reviewed all the available pathology slides of biopsy specimens in this study. After fixing the specimens with 10% formalin and embedding them in paraffin, serial 4 μm sections were stained with hematoxylin–eosin (HE). The sections were reviewed by 2 observers (Y.S. and G.I.) and we classified HG-pLCNEC if they fulfilled all the relevant criteria as described above (Table 1). Immunohistochemical analysis was performed to confirm the neuroendocrine features of the specimens. Formalin-fixed paraffin sections were stained for a panel of neuroendocrine markers, including a polyclonal anti-chromogranin A antibody (Ventana, Arizona), anti-neural cell adhesion molecule (NCAM) antibody (Nippon Kayaku, Tokyo, Japan), and monoclonal anti-synaptophysin antibody (DAKO, Glostrup, Denmark). Immunohistochemically, neuroendocrine differentiation was considered to be positive if the tumor cells exhibited focal, patchy, or diffuse staining in the intracellular areas for one or more of these 3 antibodies. The anti-human Ki-67 antigen was identified by use of a monoclonal mouse anti-human Ki-67 (clone MIB1) antigen (DAKO, Glostrup, Denmark). Only nuclear immunostaining was considered to be positive. The labeling index of Ki-67/MIB1 in each tumor was estimated as a percentage of positive cells by counting from 100 to 1000 tumor cells.

2.3. Evaluation

Response criteria were evaluated according to the Response Evaluation Criteria for Solid Tumors (RECIST) guidelines [10]. Patients were evaluated to confirm disease progression or relapse by physical examination, chest radiography, and CT of the chest and abdomen. In some patients, we used PET-CT, MRI or bone scintigraphy to detect the extent of disease progression.

2.4. Statistical analysis

Survival curves were plotted according to the Kaplan–Meier method and compared using the log-rank test. Overall survival (OS) was measured from the first day of treatment to the date of death from any cause or the date on which the patient was last known to be alive. All tests were two-sided, and p-values less than 0.05 were considered to be represent statistically significant difference. We used Statview 5.0 software (SAS Institute Inc., Cary, NC) to perform statistical analysis.

3. Results

Overall, 25 patients were recognized to have tumors with histological characteristics consistent with HG-pLCNEC based on biopsy specimens. The typical microscopic appearances of the transbronchial biopsy specimens in the current study are shown in Fig. 1. The tumor cells showed a proliferation of polygonal cells, and a low nuclear–cytoplasmic ratio, with no differentiation of acinar or squamoid features (A). Positive immunostaining findings for NCAM antibody were observed (B), but findings for chromogranin A and synaptophysin were negative (data not shown). The diagnoses of 17 of 25 patients were obtained by transbronchial lung biopsy, and the diagnoses of the remaining 8 patients were obtained by CT-guided needle biopsy.

The characteristics of all the patients examined in this study are shown in Table 2. Among the 25 patients with HG-pLCNEC, the median age was 67 years (range 48–83 years), and 22 patients (88%) were men. Of the 25 patients, all (100%) were current or former smokers. Stage III B was noted in 7 patients (28%), and 13 patients (52%) had stage IV. In the patients with HG-pLCNEC, 18

Table 2
Patient characteristics.

Characteristics	Category	HGpL	%	SCLC	%
No. of patients		25		180	
Age	Median (range)	67 (48–83)		68 (28–84)	
Gender	Male	22	88	148	82
	Female	3	12	32	18
Smoking status	Ever	25	100	172	96
	Never	0	0	8	4
Clinical stage	I	0	0	2	1
	II	1	4	12	7
	IIIA	4	16	37	21
	IIIB	7	28	39	22
	IV	13	52	90	50
Initial therapy	CT	18	72	101	56
	CRT	7	28	79	44
Tumor marker					
NSE	Median (NL: <16 ng/ml) (range)	30 (10–273)		29 (3–585)	
ProGRP	median (NL: <46 pg/ml) (range)	234 (10–20,000)		488 (7–18,000)	

HGpL, high-grade neuroendocrine carcinoma probable large cell neuroendocrine carcinoma; SCLC, small cell lung carcinoma; CT, chemotherapy; CRT, chemoradiotherapy; NL, normal level; NSE, neuron-specific enolase; ProGRP, pro-gastrin-releasing peptide.

(72%) received chemotherapy, and 7 (28%) received chemoradiotherapy as initial treatment.

Among the 180 patients with SCLC, there were 99 patients with limited disease SCLC (LD-SCLC), and the number with extensive disease SCLC (ED-SCLC) was 81. The median age was 68 years (range 28–84 years), and 148 patients (82%) were men. Of 180 patients, 172 (96%) were current or former smokers. In the SCLC patients, 101 (56%) patients initially received chemotherapy, and 79 (44%) patients received chemoradiotherapy.

Of the 25 patients with HG-pLCNEC, 12 patients (48%) received second-line chemotherapy. The remaining 13 patients did not receive chemotherapy due to death from disease, adverse events caused by initial treatment, or no active treatment determination. Of the SCLC patients, 104 (58%) received second-line chemotherapy. A diagram of the tumor types and management in patients in this study is shown in Fig. 2.

Treatments and clinical response are summarized in Table 3. The regimens of initial treatment chemotherapies are listed in Table 3(a). Of 18 patients with HG-pLCNEC who initially received chemotherapy, 17 (94%) received platinum-based chemotherapy, and the 7 patients who had chemoradiotherapy received platinum-based chemotherapy and concurrent radiation of 45–60 Gy. Of the 101 patients with SCLC who underwent chemotherapy, the most frequently administered chemotherapy regimen was carboplatin and etoposide ($n = 42$), and the second most frequent was cisplatin and etoposide ($n = 23$).

Among the 18 patients with HG-pLCNEC initially receiving chemotherapy, 1 achieved a complete response (CR) and 10 achieved a partial response (PR), with an overall response rate (ORR) of 61% (Table 3(b)). One patient with CR and 4 patients with PR received cisplatin and irinotecan. There were 2 PRs observed in the patients treated with carboplatin and paclitaxel, and 1 PR was observed in each group of patients treated with either cisplatin and vinorelbine, cisplatin and docetaxel, cisplatin and amrubicin, or irinotecan alone. Among the 7 patients with HG-pLCNEC initially receiving initially chemoradiotherapy, 6 achieved PR, with an ORR of 86%. In the patients treated with cisplatin and vinorelbine, 3 PRs were observed while 2 PRs were observed in the patients treated with cisplatin and etoposide, and 1 patient achieved PR with carboplatin and etoposide.

Among the 101 patients with SCLC initially receiving chemotherapy, 2 achieved CR and 62 patients achieved PR, with an ORR of 63%. Among the 79 patients with SCLC initially receiving chemoradiotherapy 21 achieved CR and 56 patients achieved PR, with an ORR of 98%.

The regimens of second-line chemotherapies are listed in Table 3(c). The following chemotherapy regimens were used in 12 patients with HG-pLCNEC: amrubicin alone ($n = 4$), docetaxel alone ($n = 3$), cisplatin and irinotecan ($n = 3$), carboplatin and etoposide ($n = 1$), and cisplatin and irinotecan and etoposide ($n = 1$). In 102 patients with SCLC who received second-line chemotherapy, the frequent administered chemotherapy regimen was cisplatin and irinotecan ($n = 34$), and the second most frequently administered was amrubicin alone ($n = 18$).

Among the 12 patients with HG-pLCNEC receiving second-line chemotherapy, 2 patients achieved PR, with an ORR of 17% (Table 3(d)). One patients achieved PR in each group of patients treated with cisplatin and irinotecan, and carboplatin and paclitaxel. Among the 102 patients with SCLC receiving second-line chemotherapy, 4 achieved CR and 41 patients achieved PR, with an ORR of 45%. These results indicate that the effectiveness of second-line chemotherapy appeared to differ between HG-pLCNEC and SCLC patients in the present study, but the difference was statistically not significant ($p = 0.12$).

Fig. 3 shows the OS curves for the stage IV HG-pLCNEC and ED-SCLC groups. The 1-year OS rate for patients with stage IV HG-pLCNEC was 34%, and that for patients with ED-SCLC was 49%, with no statistically significant difference ($p = 0.84$).

4. Discussion

We set out to determine the clinical features of HG-pLCNEC and other related tumors diagnosed by biopsy specimens and compare these with those of SCLC. We also examined the efficacy of chemotherapy or chemoradiotherapy between HG-pLCNEC and that of SCLC. Little is known about the optimal treatment strategy of LCNEC because most publications concerning LCNEC are based on surgical materials, with limited cohort data [11–13]. From a treatment point of view, it is imperative to establish the appropriate definitive diagnostic criteria based on the examination of biopsy or cytologic specimens, and then evaluate the efficacy of chemotherapy or chemoradiotherapy for those patients with unresectable tumors.

It is often difficult to diagnose LCNEC with small biopsy specimens, because of the possibility of crushed remnants of tissue artifacts due to insufficient specimen size, and some morphological overlap regarding cell size or nucleus size between SCLC and LCNEC [14]. In order to resolve this histological ambiguity in neuroendocrine carcinoma cases with regard to a diagnosis of LCNEC

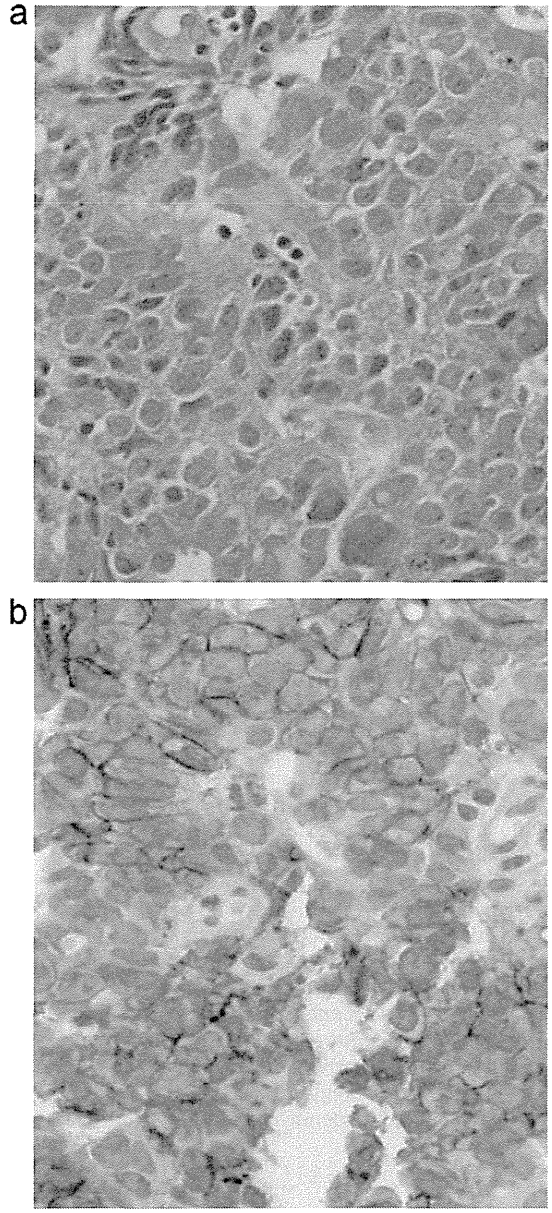
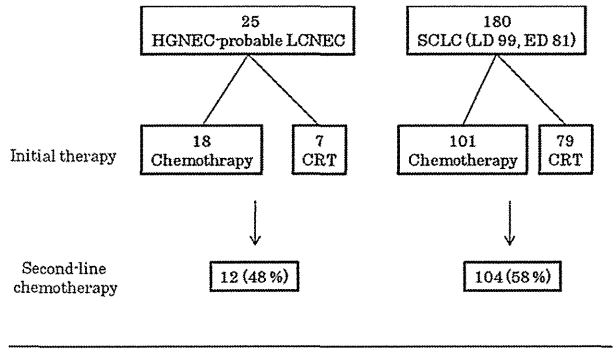


Fig. 1. A biopsy specimen diagnosed as HGNEC-probable LCNEC. (A) The histological features of HGNEC-probable LCNEC are shown with hematoxylin–eosin (HE) staining. The tumor cells are large, with a proliferation of polygonal cells, and have a low nuclear–cytoplasmic ratio, with no differentiation of acinar and squamoid features (400×). (B) Positive staining for neural cell adhesion molecule (NCAM).



HGNEC: high-grade neuroendocrine carcinoma, LCNEC: large cell neuroendocrine carcinoma, SCLC: small cell lung carcinoma, LD: limited disease, ED: extensive disease, CRT: chemoradiotherapy

Fig. 2. The characteristics of patients enrolled in this study.

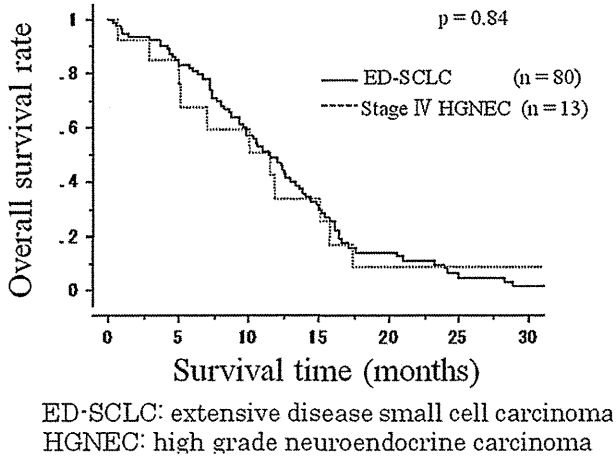


Fig. 3. Overall survival (OS) curve for stage IV HGNEC-probable LCNEC and ED-SCLC groups. The 1-year overall survival rate for patients with stage IV HGNEC-probable LCNEC was 34%, and that for patients with ED-SCLC was 49% ($p = 0.84$).

or SCLC, many researchers have performed immunohistochemistry or molecular analysis [15–18]. Hiroshima et al. reported that the frequencies of the expression of CD56, mASH1, TTF-1, and p16 were higher and that of NeuroD was lower in SCLC than in LCNEC in immunohistochemical analysis. The authors stated that LCNEC and SCLC are different morphologically, phenotypically, and genetically, although there are some overlapping features [15]. Nitadori et al. performed tissue microarray analysis of surgically resected LCNEC and SCLC specimens using 48 antibodies, and demonstrated that significant expression of CK7, CK18, E-cadherin, and β-catenin is more characteristic of LCNEC than of SCLC, suggesting that LCNEC and SCLC have a different biologic phenotype [17]. Ullmann et al. examined comparative genomic hybridization for LCNEC and SCLC, and reported that there were differences in the expression at 3q, 6p, 10q, 16q, and 17p [19]. On the other hand, Jones et al. demonstrated that cDNA microarrays gene expression profiles showed LCNEC was not differently clustered from SCLC, but different from large cell carcinoma or other NSCLC histology [20]. Although the clinicopathological features of LCNEC were similar to those of SCLC, and there is a histological ambiguity with regard to a diagnosis of LCNEC or SCLC, some biological behaviors of LCNEC were different from those of SCLC. Because there is actually the difficulty regarding the use of kinds of imuunohistochemical antibodies in daily practice, we have used the unique diagnostic criteria for HG-pLCNEC developed specifically for biopsy specimens by Igawa et al. [8]. However, lung cancer including LCNEC diagnosed by biopsy materials might not be representative of the whole tumor characteristics, particularly in heterogeneous cancers. Combinations with SCLC do occur, but such tumors are classified as combined variants of SCLC. Therefore, when using only biopsy materials for diagnosis, misdiagnosis may be unavoidable. The HG-pLCNECs examined in the present study might be mostly LCNECs and other related tumors, which included combined subtypes or other histological types, and excluded pure SCLC. This is one of the potential limitations of the present study.

To the best of our knowledge, there are few retrospective studies on the therapeutic efficacy of chemotherapy and/or radiation therapy for LCNEC [8,21,22], and this is the first study to examine

Table 3
Treatments and clinical response.

(a) Initial therapy and chemotherapy regimens

	HGpL	SCLC
No. of patients	25	180
Initial therapy		
Chemotherapy (%)	18 (72)	101 (56)
CDDP + CPT-11	8	21
CDDP + VNR	4	0
CBDCA + PTX	2	0
CBDCA + ETP	1	42
CDDP + DTX	1	0
CDDP + AMR	1	2
CPT-11	1	0
CDDP + ETP	0	23
Others	0	13
Chemoradiotherapy (%)	7 (28)	79 (44)
CDDP + VNR	3	0
CBDCA + ETP	2	5
CDDP + ETP	2	72
Others	0	2

(b) Clinical response after initial therapy

	HGpL (n = 25)		SCLC (n = 180)	
Initial therapy	Chemotherapy only	CRT	Chemotherapy only	CRT
No. of patients	18	7	101	79
CR	1	0	2	21
PR	10	6	62	56
SD	5	1	19	2
PD	2	0	12	0
NE	0	0	6	0
Response rate (%)	11/18 (61)	6/7 (86)	64/101 (63)	77/79 (98)

(c) Second-line chemotherapy regimens

	HGpL	SCLC
No. of patients	12	102
AMR	4	18
DTX	3	0
CDDP + CPT-11	3	34
CBDCA + ETP	1	12
CDDP + ETP + CPT-11	1	11
CDDP + ETP	0	10
Others	0	17

(d) Clinical response after second-line chemotherapy

Response	HGpL (n = 12)	SCLC (n = 102)	p-Value
CR	0	4	
PR	2	41	
SD	4	16	
PD	6	42	
NE	0	1	
Response rate (%)	2/12 (17)	45/102 (43)	0.12

HGpL, high-grade neuroendocrine carcinoma probable large cell neuroendocrine carcinoma; SCLC, small cell lung carcinoma; CDDP, cisplatin; CPT-11, irinotecan; VNR, vinorelbine; CBDCA, carboplatin; PTX, paclitaxel; ETP, etoposide; DTX, docetaxel; AMR, amrubicin; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; NE, not evaluable; CRT, chemoradiotherapy.

second-line chemotherapeutic efficacy. In the current study, the majority of patients with HG-pLCNEC were predominantly men, smokers, with elevated NSE and ProGRP values. These resemble the clinical features of those of SCLC, similarly to several previous reports regarding the clinicopathological characteristics of LCNEC [11,12]. We obtained a response rate to initial chemotherapy of 61% and that to chemoradiotherapy of 86% in patients with HG-pLCNEC, which was similar to those of SCLC. The survival of patients with stage IV HG-pLCNEC was also similar to that of ED-SCLC patients. Considered together, these results suggest that there was no statistically significant difference in the initial treatment efficacy between the HG-pLCNEC and SCLC groups. Some authors have reported no statistically significant difference in survival outcome between LCNEC and SCLC [11,13], whereas the survival of patients with surgically resected LCNEC is reported to be intermediate between that of atypical carcinoid and SCLC [23]. Many authors reported that survival in LCNEC was poorer than that in stage-matched NSCLC, and adjuvant therapy might be effective in cases of early stage LCNEC [24–27].

The present study showed that the ORRs of second-line chemotherapy were 17% and 45% for patients with LCNEC and SCLC, respectively. In patients with SCLC, the prognosis at relapse is poor, and response to second-line chemotherapy correlates with response to first-line therapy and also to the interval between first-line chemotherapy and disease progression. Second-line chemotherapy has been recognized to be well-tolerated and effective, with an ORR of 15–88% in patients with chemotherapy-sensitive SCLC [3–7]. The present study suggested that chemotherapeutic efficacy in patients with HG-pLCNEC might be lower than in those with SCLC, even though the chemotherapeutic regimens were heterogeneous. The number of patients with HG-pLCNEC in this study was too small to draw any definite conclusion in terms of differing benefits of chemotherapy regimens for NSCLC and SCLC, or a possible difference in second-line chemotherapeutic sensitivity between LCNEC and SCLC. However, although LCNEC is categorized as a NSCLC, molecular findings in SCLC and LCNEC showed some differences but much overlap, and overall clinicopathological features and the initial treatment response of LCNEC in our study or several published articles suggest that these tumors would be better classified as a high-grade neuroendocrine tumor comparable with SCLC, suggesting that chemotherapies using an SCLC-based standard protocol might be effective and significantly improves the survival of patients with LCNEC compared with those using a NSCLC-based protocol [12,24,26].

In conclusion, these results, although limited, that the clinical efficacy of initial chemotherapy and/or radiation therapy for patients with HG-pLCNEC is similar to that of SCLC, and there might be a different sensitivity to second-line chemotherapy between HG-pLCNEC and SCLC. Improved diagnostic criteria, specifically developed for biopsy specimens, are needed to analyze the biological behavior of LCNEC. Moreover, prospective additional studies in a larger series are clearly mandatory to confirm our data, and the role of a therapy strategy with SCLC-based regimens deserves sensitivity to chemotherapeutic agents and the optimal treatment protocol.

Conflict of interest statement

The authors declare no potential conflicts of interest regarding this study.

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CHEST

Original Research

LUNG CANCER

Predictive Factors of Pathologically Proven Noninvasive Tumor Characteristics in T1aN0M0 Peripheral Non-small Cell Lung Cancer

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Background: **We retrospectively analyzed preoperative factors that may predict pathologically invasive tumor characteristics, including lymph node involvement, and pleural and vessel invasion in patients with cT1aN0M0 peripheral non-small cell lung cancer (NSCLC), in an attempt to identify candidates for pulmonary resection less than lobectomy.**

Methods: **We reviewed the charts of 363 patients in whom cT1aN0M0 lung cancer in the lung periphery had been diagnosed or was suspected, based on high-resolution CT scan of 1- or 2-mm-slice intervals, within 1 month of surgical resection, and examined the relationships between preoperative clinical information and pathologic invasive tumor characteristics, corresponding to lymph node involvement and pleural and vessel invasion.**

Results: **Multivariate analysis showed that a tumor disappearance ratio (TDR) < 0.5, the presence of spiculation, and an absence of air bronchograms were statistically significant independent predictors of pathologic invasiveness. Most TDR ≥ 0.5 tumors were noninvasive (98.7%), and only one patient had a recurrence within 5 years after surgical resection. Of the tumors with a TDR ≥ 0.5 without spiculation, 98.3% were noninvasive, and all those patients remained recurrence-free for 5 years after surgery.**

Conclusion: **The combination of a TDR ≥ 0.5 and the absence of spiculation was highly predictive of noninvasive or minimally invasive NSCLC. Future studies should evaluate whether limited resection of these tumors provides acceptable outcomes.** ***CHEST 2012; 141(4):1003–1009***

Abbreviations: CEA = carcinoembryonic antigen; CIF = cancer with invasive factor; HRCT = high-resolution CT; non-CIF = cancer without invasive factor; NSCLC = non-small cell lung cancer; RFP = recurrence-free proportion; TDR = tumor disappearance ratio; TOM = tumor opacity on mediastinal window images

In the current TNM classification for lung cancer, T1 non-small cell lung cancers (NSCLCs) are subdivided into two groups according to tumor size: T1a (≤ 2 cm) and T1b (> 2 cm but ≤ 3 cm). Most cT1aN0M0 tumors, clinically ≤ 2 cm in greatest dimension but without lymph node or distant metastases, are asymptomatic and are detected by cancer screening. Today, these small peripheral lung cancers are encountered more frequently because of the widespread use of high-resolution CT (HRCT) scan in daily clinical practice and cancer screening.[1-3] Several studies have indicated that pulmonary resection less than lobectomy can be successful in such cases, based on preoperative radiologic findings, with reported outcomes comparable to those of lobectomy.[4-6]

However, Shimosato et al[7] reported that the presence of a fibrotic focus or scarring in NSCLC was significantly associated with lymph node metastases,

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pleural and vessel invasion, and unfavorable prognosis. In pulmonary resection less than lobectomy for small NSCLC lesions, these pathologically invasive tumor characteristics may lead to cancer-positive surgical margins and subsequent recurrence. In this study, we retrospectively analyzed preoperative factors that may predict pathologically invasive tumor characteristics, including lymph node involvement, and pleural and vessel invasion, in patients with cT1aN0M0 peripheral NSCLC, in an attempt to identify candidates for pulmonary resection less than lobectomy based on preoperative findings.

Materials and Methods

From January 2002 through December 2008, a total of 1,722 patients underwent surgical resection for primary lung cancer at our hospital. Of these, 511 patients were given diagnoses of or were suspected to have clinical T1aN0M0 (≤ 2 cm in maximal tumor dimension and absence of lymph node and distant metastases) lung cancer in the lung periphery, and none of them had received preoperative chemotherapy or radiotherapy. Preoperative evaluation included physical examination, chest radiography, CT scan of the chest and abdomen, and blood examination, including carcinoembryonic antigen (CEA). Among the 511 patients, 459 had undergone HRCT scan with 1- or 2-mm-slice intervals within 1 month before surgical resection. Of these, we excluded 96 pathologic Nx patients who did not undergo systematic node dissection. The remaining 363 patients undergoing systematic lymph node dissection were the subject of this study (Fig 1).

In cases of wedge resection or segmentectomy, either due to impaired pulmonary function or based on the radiologic diagnosis of minimally invasive disease, lavage cytology at the surgical margin was evaluated intraoperatively. All were confirmed to be negative.[8]

HRCT scans were evaluated by two observers (Y. S. and J. Y.), who were blinded to patient identification. The observers measured the maximal tumor dimension on both lung and mediastinal windows. Tumor disappearance ratio (TDR), vascular convergence, air bronchograms, pleural indentation, and spiculation were also reviewed on HRCT scan (Fig 2). TDR was defined as follows: On an HRCT scan slice, the maximal tumor dimension was measured on a lung setting (DL). On a mediastinal setting, the ground-glass area disappeared, leaving only the area of consolidation. The remaining maximal dimension of the consolidation area was measured (DM). TDR was calculated as 1 − DM/DL.[9] Any evaluation discrepancy between the observers was resolved by consensus. Interobserver variation in HRCT scan findings was quantified by the weighted κ coefficient of agreement.

We reviewed the medical records of each patient for clinicopathologic information, including age (divided into those ≥65 years and those <65 years), sex, smoking status, preoperative serum CEA level (dichotomized at the normal upper limit of 5 ng/mL), pathologic nodal involvement, vessel invasion (vascular invasion and lymphatic permeation), pleural invasion (as defined in the seventh edition of the *TNM Classification for Lung and Pleural Tumors*[10]), and histologic type. The histologic type was determined according to the third edition of the World Health Organization classification.[11] We used hematoxylin-eosin and Victoria blue van-Gieson stains for the evaluation of vessel and pleural invasion. We defined cases with one or more of the pathologic invasive factors vessel invasion, pleural invasion, or lymph node metastasis, as cancer with invasive factor (CIF), and those without any of these factors as cancer without invasive factor (non-CIF).

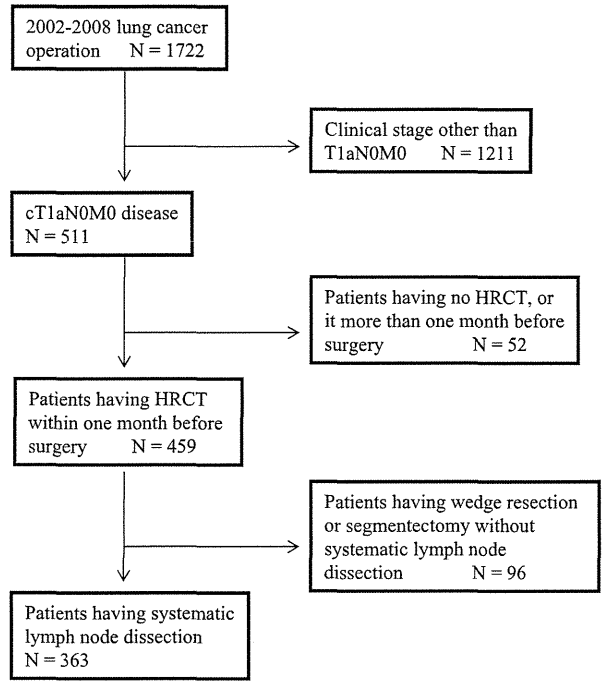


FIGURE 1. Patients included in this study. HRCT = high-resolution CT.

The length of the recurrence-free period was calculated in months from the date of resection to the date of the first recurrence or last follow-up. To calculate the recurrence-free proportion (RFP), patients who died without recurrence or who were known to have no recurrence at the date of last contact were censored. RFP curves were plotted according to the Kaplan-Meier method, and differences in variables were determined using the log-rank test. Categorical comparison was performed using the Pearson's χ^2 test. Multivariate analyses were performed using the Cox proportional hazards regression model. All tests were two sided, and P values $< .05$ were considered to represent a statistically significant difference. Statview 5.0 software (SAS Institute Inc) was used to perform the statistical analyses. Data collection and analyses were approved and the need to obtain written informed consent from each patient was waived by the institutional review board of National Cancer Center Hospital in August 2010.

Results

Clinicopathologic patient characteristics are summarized in Table 1. Of the 363 patients, 121 (33.3%) had CIF and 242 (66.7%) had non-CIF. Vessel invasion was identified in 97 patients (26.7%), pleural invasion in 50 patients (13.8%), and lymph node metastases in 31 patients (8.6%), respectively.

Table 2 shows RFP time according to the presence or absence of pathologic invasive factors. The median follow-up period was 3.5 years. The 3- and 5-year RFP time for patients with vessel invasion, pleural invasion, or lymph node metastasis was significantly less favorable than in patients without these factors.

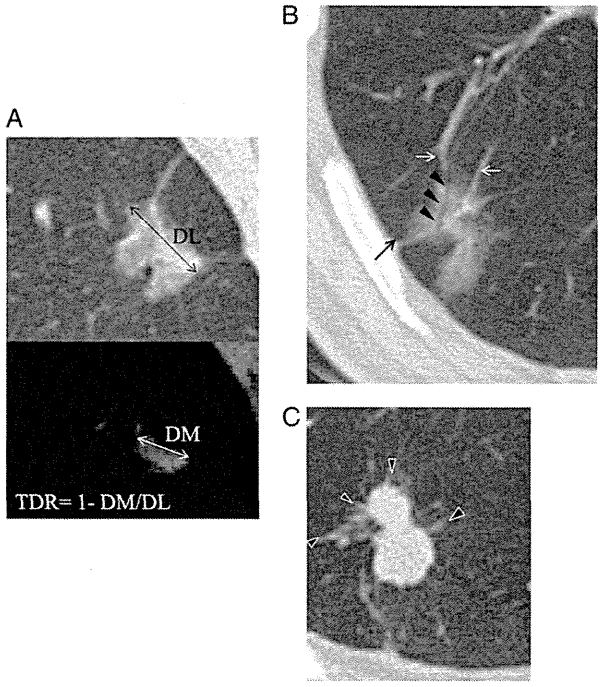


FIGURE 2. Preoperative HRCT scan images. A, TDR. B, Vascular convergence (white arrows), air bronchogram (arrowheads), pleural indentation (black arrow). C, spiculation (arrowheads). DL = maximal tumor dimension on lung setting; DM = maximal tumor dimension on mediastinal setting; TDR = tumor disappearance ratio. See Figure 1 legend for expansion of other abbreviation.

Table 1—*Clinicopathologic Characteristics (N = 363)*

Characteristic	CIF (%)	NON-CIF (%)	Total (%)
Overall	121 (33.3)	242 (66.6)	363 (100.0)
Age			
≥ 65 y	56 (15.4)	128 (35.3)	184 (50.7)
< 65 y	65 (17.9)	114 (31.4)	179 (49.3)
Sex			
Male	74 (20.4)	113 (31.1)	187 (51.5)
Female	47 (12.9)	129 (35.5)	176 (48.5)
Smoking status			
Ever smoker	84 (23.1)	125 (34.4)	209 (57.6)
Never smoker	37 (10.2)	117 (32.2)	154 (42.4)
CEA			
≥ 5 ng/mL	30 (8.3)	49 (13.5)	79 (21.8)
< 5 ng/mL	90 (24.8)	190 (52.3)	280 (78.2)
Extent of resection			
Segmentectomy	6 (1.7)	14 (3.9)	20 (5.5)
Lobectomy	115 (31.7)	228 (62.8)	343 (94.5)
Curability			
Complete	120 (33.1)	242 (66.6)	362 (99.7)
Incomplete	1 (0.3)	0 (0)	1 (0.3)
Histologic type			
Adenocarcinoma	91 (25.1)	210 (57.9)	301 (82.9)
BAC	0 (0)	25 (6.9)	25 (6.9)
Non-BAC	91 (25.1)	185 (51.0)	276 (76.0)
Squamous	15 (4.1)	26 (7.2)	41 (11.3)
Others	11 (3.0)	10 (2.8)	21 (5.8)
Vessel invasion			
Absent	24 (6.6)	242 (66.6)	266 (73.3)
Present	97 (26.7)	0 (0)	97 (26.7)
Pleural invasion			
Absent	71 (19.6)	242 (66.6)	313 (86.2)
Present	50 (13.8)	0 (0)	50 (13.8)
p-N status			
N0	90 (24.8)	242 (66.6)	332 (91.4)
N1	10 (2.8)	0 (0)	10 (2.8)
N2	20 (5.6)	0 (0)	20 (5.6)
N3	1 (0.3)	0 (0)	1 (0.3)
p-Stage			
I	89 (24.5)	241 (66.4)	330 (90.9)
II	9 (2.5)	0 (0)	9 (2.5)
III	24 (6.6)	0 (0)	24 (6.6)

BAC = bronchioloalveolar carcinoma; CEA = preoperative serum carcinoembryonic antigen level; CIF = cancer with invasive factors; non-CIF = cancer without invasive factors; squamous = squamous cell carcinoma.

In this study, the RFP curves of patients with CIF or non-CIF are shown in Figure 3. The RFP curve for patients with non-CIF (95.0% at 5 years) was significantly better than that for patients with CIF (64.5%) ($P < .001$).

We evaluated the relationships between CIF and preoperative clinical factors, including HRCT scan findings. The weighted κ coefficient for interobserver agreement in HRCT scan findings ranged from 0.62 to 0.90, suggesting good agreement between the observers. We found positive correlations among the following factors: sex, smoking status, TDR (divided at ≥ 0.5),[8] spiculation, pleural indentation, vascular convergence, and the presence of air bronchograms. In contrast, there was no correlation for age or preoperative serum CEA level (Table 3). For these statistically significant factors, multivariate logistic regression analysis was performed to determine the factors independently predictive of pathologic invasiveness. As shown in Table 4, a TDR < 0.5, the presence of spiculation, and the absence of an air bronchogram were shown to be statistically significant independent predictors of CIF. A TDR < 0.5 was shown to be a very strong predictor, with a risk ratio of CIF of 33.139.

Based on these findings, we investigated whether we could accurately identify patients with non-CIF who might be candidates for pulmonary resection less than lobectomy, by reversely combining these predictive factors (Table 5). Concerning single factors, a TDR ≥ 0.5 provided the best accuracy, with 77 of 78 patients (99%) with TDR ≥ 0.5 correctly predicted as non-CIF, and one patient with recurrence within 5 years after surgical resection. Among the two-factor combinations, a TDR ≥ 0.5 and the absence of spiculation provided the best accuracy, with 57 of 58 patients (98%) correctly predicted as non-CIF, and all patients in this group remained recurrence free for 5 years after surgery. The three-factor combination of a TDR ≥ 0.5, the absence of spiculation, and the presence of air bronchograms

Table 2—*Three- and Five-Year RFP of Patients According to Pathologic Invasive Factors*

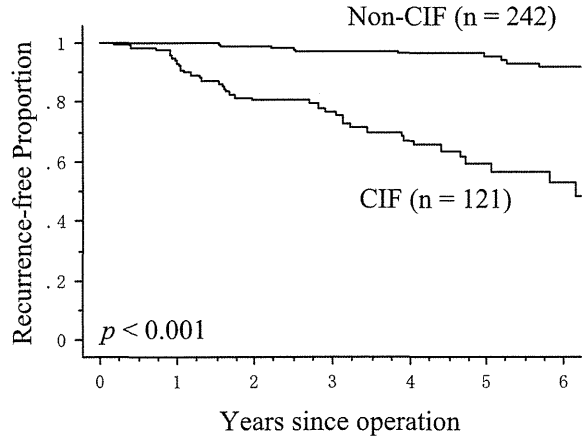
Factor	No. Patients	3-y RFP (%)	5-y RFP (%)	*P* Value[a]
Vessel invasion				
Absent	266	95.7	91.7	...
Present	97	77.7	66.4	<.001
Pleural invasion				
Absent	313	93.0	88.2	...
Present	50	76.6	62.9	<.001
Lymph node metastasis				
Absent	332	94.7	89.9	...
Present	31	46.6	24.9	<.001

RFP = recurrence-free proportion.
[a]Log-rank test.

was also accurate in predicting non-CIF, with 31 of 32 cases (97%) correctly predicted as non-CIF, and there was no patient with recurrence within 5 years after surgery. However, the number of patients who met the criteria was relatively small. The RFP curves of patients with the best two-factor combination, a TDR ≥ 0.5 and the absence of spiculation, and those of the other patients, are shown in Figure 4. The RFP rate of patients with a two-factor combination (100% at 5 years) was significantly better than those of the other patients (81.4%) ($P < .001$).

Discussion

We set out to determine those preoperative factors that may predict CIF in patients with cT1aN0M0



Patients at risk (n = 363)

Non-CIF	242	228	190	149	113	86	53
CIF	121	111	78	58	43	25	14

FIGURE 3. Recurrence-free proportion curves of patients with CIF or non-CIF. CIF = cancer with invasive factor; non-CIF = cancer without invasive factor.

peripheral NSCLC, to try to identify candidates for pulmonary resection less than lobectomy. The management of small lung cancers is of particular concern to modern thoracic surgeons, because some of these small cancers may be curatively managed by pulmonary resection less than lobectomy. Although determining the type of tumor suitable for pulmonary resection less than lobectomy remains controversial, most recent studies comparing lesser resection with lobectomy for selected patients with stage IA NSCLC demonstrated an equivalently favorable outcome.[4-6] However, several studies have reported that pathologic invasive features were not rare, even in patients with small NSCLC lesions,[12-17] raising the issue of patient selection for limited resection.

It has been reported that 6% to 19% of resected peripheral lung cancers of ≤ 2 cm in maximal dimension had lymph node involvement.[12,13,15-17] Several studies demonstrated that tumor size alone cannot be a reliable predictor of the biologic invasiveness/aggressiveness of lung cancer.[12,18,19] We previously evaluated resected peripheral lung cancers of all histologic types < 1 cm in maximal dimension, and 23% of the tumors displayed invasive features, representing vessel invasion or lymph node metastasis.[18] Shimosato et al[7] reported that seven of 13 resected peripheral lung cancers of < 2 cm in maximal dimension had an increased amount of collagenization or hyalinization in the central fibrotic focus of the tumor, and five of the seven patients were dead within 4 years after resection. In the current study, 31 patients (8.6%) of 363 evaluable patients exhibited lymph node involvement, 97 patients (26.7%) showed vessel invasion, and 50 patients (13.8%) showed pleural invasion. These findings clearly indicate that a considerable percentage of small NSCLC lesions harbor CIF.

Many retrospective studies have reported a correlation between pathologically invasive tumor characteristics and poor outcome in small-sized lung cancer patients following surgical resection.[12-17,19-23] In the present study, the 5-year RFP of patients with lymph node metastasis was 24.9%, whereas it was 61.7% for those with vessel invasion, and in those with pleural invasion it was 59.5%. Because of poor prognosis in these patients, it is imperative to preoperatively identify patients with non-CIF in indicating limited lung resection.

Tateishi et al[24] reported that potential preoperative predictors of vessel invasion in pulmonary adenocarcinomas were a lower proportion of nonsolid components, presence of spiculation and pleural indentation, male sex, and large tumor size. Matsuguma et al[16] reported that a lower proportion of areas of ground-glass opacities on HRCT scan was a strong predictor of lymph node metastasis and vessel invasion in cT1N0M0 adenocarcinoma patients. Hashizume et al[15]

Table 3—*Correlation Between Clinical Factors and Pathologic Invasiveness (N = 363)*

Factor	CIF (n = 121)	Non-CIF (n = 242)	*P* Value[a]
Age			
≥ 65 y	56	128	...
< 65 y	65	114	.235
Sex			
Male	74	113	...
Female	47	129	.009[b]
Smoking status			
Ever smoker	84	125	...
Never smoker	37	117	.001[b]
CEA			
≥ 5 ng/mL	30	49	...
< 5 ng/mL	90	190	.332
Extent of resection			
Segmentectomy	6	14	...
Lobectomy	115	228	.745
TDR			
≥ 0.5	1	77	...
< 0.5	120	165	< .001[b]
Spiculation			
Absent	22	110	...
Present	99	132	< .001[b]
Pleural indentation			
Absent	31	96	...
Present	90	146	.008[b]
Vascular conversion			
Absent	12	52	...
Present	109	190	< .001[b]
Air bronchogram			
Absent	66	78	...
Present	55	164	< .001[b]

TDR = tumor disappearance ratio. See Table 1 for expansion of other abbreviations.

[a]χ^2 test.

[b]Denotes signficance.

reported that no pathologic invasive findings or recurrence were found in patients with air-containing-type tumors, which represent tumors with TDR ≥ 0.5.

Several reports have demonstrated that these radiologic findings showed a correlation with pathologic invasiveness in small-sized lung adenocarcinomas. Saito et al[20] classified small pulmonary adenocarcinomas into two types according to HRCT scan findings. They defined air-containing-types as tumors with areas where tumor opacity on mediastinal window images (TOM) was ≤ 50% of those noted on lung window images, which corresponds to a TDR ≥ 0.5. The solid-density type was defined as having areas where TOM was > 50% of those noted on lung window images, which corresponds to a TDR < 0.5. They concluded that the pathologic components of TOM areas were markedly different between small adenocarcinomas with a TDR ≥ 0.5 and those with a TDR < 0.5. They also reported that survival was better in those with a TDR ≥ 0.5, which was consistent with our findings. These findings suggest that TDR in HRCT scan is strongly correlated with pathologic tumor characteristics.

Zwirewich et al[25] reported that spiculation most commonly correlated histologically with a desmoplastic response in a nodule, resulting in fibrotic strands radiating into the surrounding lung parenchyma. They also reported that direct infiltration of the tumor into the adjacent bronchovascular sheaths and lymphangitic extension corresponded to the radiologic coarse spiculation and thickening of the bronchovascular bundles around the tumors.[25] These findings support the concept that the presence of spiculation corresponds to vessel invasion and pathologic invasiveness.

To our knowledge, no reports have suggested a clear correlation between the presence of air bronchograms and tumor invasiveness/aggressiveness. However, Zwirewich et al[25] reported that psuedocavitations, which may also be depicted as air bronchograms, were observed more frequently among bronchioloalveolar carcinomas than among any other malignant lesions of the lung. Because pathologic invasive features are uncommon in bronchioloalveolar carcinomas, pulmonary cancers with air bronchograms on HRCT scan may be pathologically less invasive and may result in better outcome.

Based on these significant predictors of pathologic invasiveness, we investigated whether we could accurately identify patients with non-CIF who might be candidates for pulmonary resection less than lobectomy, by reversely combining these predictive factors.

Table 4—*Multivariate Analysis of Clinical Factors Predictive of Patients With CIF (N = 363)*

Factor	Risk Factors	Risk Ratio for CIF	95% CI	*P* Value[a]
Sex	Male	1.051	0.526-2.099	.889
Smoking status	Ever smoker	1.529	0.744-3.140	.248
TDR	< 0.5	33.139	4.414-248.810	< .001[b]
Spiculation	Present	2.315	1.189-4.510	.014[b]
Pleural indentation	Present	1.340	0.764-2.350	.307
Vascular conversion	Present	1.007	0.415-2.445	.988
Air bronchogram	Absent	2.825	1.703-4.687	< .001[b]

See Tables 1 and 3 for expansion of abbreviations.

[a]Logistic regression analysis.

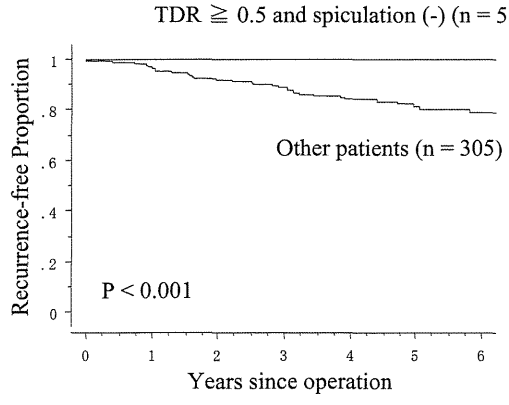
[b]Denotes signficance.

Table 5—*Preoperative Predictivity of Patients With Non-CIF*

Single Factor/Combination	Patients, No.	Patients With Non-CIF, No. (%)	5-y RFP, %
Single factor			
TDR ≥ 0.5	78	77 (99)	98
Spiculation (−)	132	110 (83)	91
Air bronchogram (+)	219	164 (75)	91
2 Factors			
TDR ≥ 0.5 + spiculation (−)	58	57 (98)	100
TDR ≥ 0.5 + air bronchogram (+)	51	50 (98)	97
Spiculation (−) + air bronchogram (+)	72	65 (90)	97
3 Factors			
TDR ≥ 0.5 + spiculation (−) + air bronchogram (+)	32	31 (97)	100

See Tables 1-3 for expansion of abbreviations.

For a single factor, a TDR ≥ 0.5 gave the best predictive accuracy, with 99% correctly predicted to be non-CIF; there was one patient with recurrence within 5 years after operation. When two of these factors were reversely combined (ie, TDR ≥ 0.5 and an absence of spiculation), this was highly predictive of non-CIF. All patients meeting the criteria survived 5 years without recurrence, and 98% had non-CIF. The three-factor combination of a TDR ≥ 0.5, the absence of spiculation, and the presence of air bronchograms was also highly accurate in predicting non-CIF, with 31 (97%) of 32 patients with non-CIF. The χ^2 test shows a strong correlation between a TDR ≥ 0.5 and the absence of spiculation ($P < .001$), but there is poor correlation between a TDR ≥ 0.5 and the presence of air bronchogram ($P = .303$), and between the absence of spiculation and the presence of air bronchogram ($P = .079$) (data not shown). If the presence of air bronchogram is uncorrelated with TDR ≥ 0.5 or the absence of spiculation, then we speculate that the number of patients who met all three factors was relatively small, suggesting that the combination of TDR ≥ 0.5 and the absence of spiculation is sufficient to be predictive of patients with non-CIF.



Patients at risk of recurrence (n = 363)

With 2-factor	58	56	48	43	35	28	15
Other patients	305	283	215	164	121	83	52

FIGURE 4. Recurrence-free proportion curves according to the two-factor combinations of a TDR ≥ 0.5 and the absence of spiculation. See Figure 2 legend for expansion of abbreviation.

There are several limitations to the present study. This study is retrospective and possible bias may exist. Although HRCT images were reviewed by two experienced observers who were blinded to patient identification, image evaluation is essentially subjective and our evaluation may lack reproducibility.

We retrospectively analyzed preoperative factors that might predict pathologically invasive tumor characteristics in cT1aN0M0 NSCLC patients. Our results showed that a TDR < 0.5, the presence of spiculation, and the absence of air bronchograms on HRCT scan were independent predictors of CIF and poor prognosis.

Conclusions

In conclusion, this study showed that detailed HRCT scan findings were predictive of CIF of small NSCLC lesions. However, the combination of a TDR ≥ 0.5 and the absence of spiculation was highly predictive of patients with non-CIF. Future studies should evaluate whether limited resection of these tumors provides acceptable outcomes.

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Dr Yoshida: contributed to the design and coordination of the study, revision of the article for important intellectual content, and reading and approval of the final manuscript.
Dr Hishida: contributed to the preparation of the manuscript and reading and approval of the final manuscript.
Dr Nishimura: contributed to the preparation of the manuscript and reading and approval of the final manuscript.
Dr Ishii: contributed to the preparation of the manuscript and reading and approval of the final manuscript
Dr Nagai: contributed to the preparation of the manuscript and reading and approval of the final manuscript.

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FOLFIRI Plus Bevacizumab 5 mg/kg Versus 10 mg/kg as Second-line Therapy in Patients with Metastatic Colorectal Cancer Who Have Failed First-line Bevacizumab Plus Oxaliplatin-based Therapy: A Randomized Phase III Study (EAGLE Study)

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We planned a multicenter randomized phase III study to evaluate the efficacy of appropriate dose of bevacizumab (5 or 10 mg/kg) with FOLFIRI in patients with advanced/metastatic colorectal cancer who have failed prior bevacizumab plus oxaliplatin-based therapy. The primary endpoint is progression-free survival. The secondary endpoints are the toxicity, response rate, time to treatment failure, overall survival, overall survival from the start of the first-line treatment and second progression-free survival (time duration from the initiation of the first-line treatment until progression after the protocol treatment). A total of 370 patients were considered to be appropriate for this trial.

Key words: bevacizumab – FOLIRI – irinotecan – beyond progression – advanced/metastatic colorectal cancer

INTRODUCTION

Age-adjusted prevalence of colorectal cancer (CRC) is the second largest percentage after that of gastric cancer in males and breast cancer in females in Japan (1). According to the CONCORD study, it is reported that Japanese men attain the first place and Japanese women attain sixth for a 5-year survival rate with CRC in the world (2). Japanese patient's clinical registered data from 1991 to 1994 by the Japanese Society for Cancer of the Colon and Rectum is superior to the same period's data from Survival Epidemiology and End Results and National Cancer Data Base for each of Stage I, II, III CRC, at most 20%.

It is estimated that the number of CRC patients will be 480 396 in 2015 and 512 225 in 2020 (1). It is also expected that the incidence of CRC will overtake that of breast cancer after 2010. Although CRC screening rates were improved, considerably large number of patients had a locally advanced or metastatic disease at the time of diagnosis. For patients with metastatic CRC, recommended first-line regimens by guidelines are FOLFOX or FOLFIRI (3,4) plus biological agents.

Bevacizumab (Avastin; Genentec, Inc., South San Fran cisco, CA), a recombinant, humanized monoclonal antibody that binds to and neutralizes vascular endothelial growth factor (VEGF) is one of the biological agents and was proved to improve overall survival (OS) and progression-free survival (PFS) in bevacizumab-naïve patients with metastatic CRC when administered to first- and second-line chemotherapy.

For patients with previously treated metastatic CRC, treatment results of FOLFIRI or FOLFOX as a second-line therapy were reported from the phase III study. PFS was 2.5 and 4.2 months, respectively (5). Treatment results of FOLFIRI plus bevacizumab at 5 mg/kg and FOLFOX plus bevacizumab at 5 mg/kg as a second-line treatment were reported from the phase II study. PFS was 7.8 and 5.3 months, respectively (6). In addition, the treatment result of FOLFOX4 plus bevacizumab at 10 mg/kg as a second-line therapy was reported from a randomized phase III study. OS as the primary objective was 12.9 months compared with 10.8 months of FOLFOX4 alone (HR, 0.66; $P < 0.0011$). PFS was 7.3 months, which is also significantly improved compared with 4.7 months of FOLFOX4 alone (HR, 0.61; $P < 0.0001$) (7). However, all of these treatments were examined for previously bevacizumab-naïve patients.

A key element of continuous administration of bevacizumab beyond progression is as shown below. In basic research, regrowth of tumor vessels are often observed soon after cessation of bevacizumab administration (8–10) and VEGF expression is identified across the board from the initial period of the tumor lifecycle (11). Several experimental studies have examined that the muMAb 4.6.1 antibody, mouse monoclonal precursor of VEGF inhibitors in CRC xenograft models prevents growth of tumor cells at metastatic sites dose dependently (12). In addition, the BRiTE study (13), one of the observational cohort studies in the USA provides supportive clinical data about the foregoing. Median OS were 12.6, 19.9 and 31.8 months in the no post-progressive disease (PD) treatment, chemotherapy without bevacizumab and chemotherapy with bevacizumab groups, respectively.

After adjustment for other prognostic factors, bevacizumab treatment beyond progression maintained a statistically significant effect on survival after PD, compared with no post-PD bevacizumab (HR, 0.49; 95% CI, 0.41–0.58; $P < 0.001$). In this study, the proportion of bevacizumab doses administered as the second-line therapy were 90.7% (5 mg/kg), 3.6% (7.5 mg/kg) and 2.3% (10 mg/kg). These results from the BRiTE study suggest that continuous VEGF inhibition with bevacizumab beyond initial PD could play an important role for prolonging survival of patients with metastatic CRC.

There are three major clinical questions to be solved about second-line biological agents in metastatic colorectal cancer. The first clinical question about the continuation of bevacizumab after exposure to bevacizumab treatment will be revealed from the results of the on-going trial 'AIO 0504'. The second clinical question about the drug selection between bevacizumab and anti-epidermal growth factor receptor antibodies with KRAS wild type after a first-line bevacizumab-containing regimen will also be answered by the on-going trial 'SPIRITT'.

On the other hand, the third clinical question about the optimal doses of bevacizumab as second-line treatment followed by a bevacizumab-containing regimen is still remains unsolved. The verified data indicates the efficacy of bevacizumab at 5 mg/kg/weekly (=10 mg/kg/biweekly) in the second-line setting followed by bevacizumab-naïve treatment (7). The recommended dose of bevacizumab is 5 mg/kg/weekly (=10 mg/kg/biweekly) in non-small cell lung cancer, breast cancer, renal cell cancer and second-line colorectal cancer (14–19), but 2.5 mg/kg/weekly (=5 mg/kg/biweekly) in the first-line CRC treatment. The dose of bevacizumab 2.5 mg/kg/weekly (=5 mg/kg/biweekly) could be lower than the recommended dose in the second-line CRC treatment.

Thus, it is necessary for us to investigate the effectiveness of high-dose bevacizumab for metastatic CRC.

Accordingly, we have conducted a randomized phase III study of FOLFIRI plus bevacizumab 5 mg/kg versus 10 mg/kg as second-line therapy in patients with metastatic CRC who have failed first-line bevacizumab plus oxaliplatin-based therapy (EAGLE study).

The study protocol was approved by the institutional review boards of each participating institution. The study met the ethical guidelines for clinical studies of the Health, Labor and Welfare Ministry in Japan, and was conducted in compliance with the Declaration of Helsinki. All patients provided written informed consent.

PROTOCOL DESIGN FOR EAGLE STUDY

Objective

A multicenter randomized phase III study of adding bevacizumab 5 or 10 mg/kg to FOLFIRI in advanced/metastatic CRC who have failed prior bevacizumab plus oxaliplatin-based first-line therapy.

Endpoint

The primary endpoint is PFS. The secondary endpoints are the toxicity, response rate, time to treatment failure, OS, OS from the start of the first-line treatment and second PFS (time duration from the initiation of the first-line treatment until progression after the protocol treatment). The progression will be evaluated on the basis of response evaluation criteria in solid tumors (RECIST) ver. 1.1.

Eligibility Criteria

Inclusion Criteria

(i) PD after chemotherapy with bevacizumab plus oxaliplatin-based therapy as the first-line treatment

(with measurable lesions in the RECIST criteria) or difficult to continue the first-line therapy due to the other reasons.
(ii) Oxaliplatin and bevacizumab were administered for more than four times in the first-line treatment.
(iii) Cytologically and/or histologically proven CRC.
(iv) Written informed consent.
(v) Aged 20 years old and above.
(vi) Eastern Cooperative Oncology Group performance status (ECOG PS) 0 or 1.
(vii) Life expectancy estimated $\geq$3 months.
(viii) Sufficient organ functions.

Exclusion Criteria

(i) Previous irinotecan treatment.
(ii) Administration of transfusion/hematopoietic factor or antithrombotic drug within 14 days.
(iii) Serious renal dysfunction.
(iv) Serious drug hypersensitivity or a history of drug allergy.
(v) Active concomitant malignancy.
(vi) Active infections.
(vii) Symptomatic or asymptomatic heart disease that is being treated at the time of registration to the trial.
(viii) History of thrombosis, interstitial pneumonia, pulmonary fibrosis or high-grade pulmonary emphysema.
(ix) Fresh hemorrhage from the digestive tube, intestinal tube paralysis, intestinal obstruction and peptic ulcer.
(x) Pleural effusion, peritoneal fluid and pericardial fluid.
(xi) Symptomatic brain metastasis.
(xii) History of mental disturbances or cerebrovascular accident.
(xiii) High blood pressure and diabetes that cannot be controlled.
(xiv) Uncontrolled diarrhea.
(xv) Serious non-healing wound and/or major surgical procedure within 4 weeks prior to enrolling in this trial.
(xvi) Traumatic fracture that has not been headed at the time of enrollment.
(xvii) Bleeding tendency and anti-platelet therapy (including aspirin and non-steroidal anti-inflammatory drugs).
(xviii) Pregnant women, possibly pregnant women, wishing to become pregnant and nursing mothers.
(xix) Needing treatment with atazanavir sulfate.
(xx) Paralyzed bowel.

Registration

Any medical institution that would like to participate could contact a secretariat at Epidemiological and Clinical Research Information Network (ECRIN) or publicly contact: Hideyuki Mishima at the Department of Surgery, National Hospital Organization Osaka National Hospital, Osaka, Japan.

Registration forms are sent from the ECRIN to the medical institution for registration.

Registered patients are allocated randomly into the FOLFIRI + 5 mg of bevacizumab arm (arm A) or the FOLFIRI + 10 mg of bevacizumab arm (arm B) at the data-center. For randomization, a minimization method or dynamic randomization is used with five balancing factors: baseline ECOG PS, number of metastasis ($2>$, $2\leq$), reason for a change in therapy to second-line treatment (PD in first-line treatment/non-PD), early recurrence within 6 months (during/after adjuvant treatment) and institutions.

Treatment Methods

FOLFIRI plus bevacizumab consists of bevacizumab at 5 mg/kg (or 10 mg/kg) as a 30-min infusion and *l*-leucovorin 200 mg/m^2 as a 2-h infusion, and concurrently irinotecan 150 mg/m^2 as an over 90-min infusion, followed by bolus fluorouracil (5-FU) 400 mg/m^2 within 15 min and 46-h infusion of 5-FU 2400 mg/m^2. Patients randomly assigned to arm A receive FOLFIRI plus bevacizumab 5 mg/kg. FOLFIRI plus bevacizumab 10 mg/kg is administered to patients randomly assigned to arm B. These treatments are repeated every 2 weeks until disease progression, unacceptable toxicity or patient choice.

Follow-Up

Disease progression and occurrence of new diseases are monitored by using abdominal radiography, abdominal computed tomography (CT) or magnetic resonance imaging, and thoracic CT, and by measuring levels of the tumor markers CEA and CA19–9 at the baseline and every 8 weeks during the treatment period (tumor marker levels are measured every 4 weeks). Blood tests and symptom checks (collecting adverse events) will be carried out throughout the treatment period. In case of dyspnea, arterial blood gases will be tested and chest X-ray test will be carried out. In case of arrhythmia, a 12 lead electrocardiogram will be carried out. The follow-up period is 1 year after the registration of the last patient.

Study Design and Statistical Analysis

The primary objective of this trial is to evaluate whether arm B (FOLFIRI plus 10 mg/kg of bevacizumab therapy) significantly improves PFS compared with arm A (FOLFIRI plus 5 mg/kg of bevacizumab therapy). The null hypothesis, if the PFS of both arms is equal, is tested by the stratified log-rank test with the balancing variables (except for the institutions) as the stratification factor. If arm B showed a statistically significant prolonging effect on PFS compared with the other arm, it is concluded that arm B is more

beneficial therapy. The overall significance level of the trial is set as 5% for the two-sided test.

PFS curves are depicted by the Kaplan–Meier method. Median PFS and the annual PFS rates are also estimated using the Kaplan–Meier method with the two-sided 95% confidence interval using the Greenwood formula (20). The stratified Cox proportional hazards model is used to assess the hazard ratio with Wald-type 95% confidence intervals for the treatment effect between both arms.

Median PFS of arm A in this trial is assumed to be 5.0 months based on previous studies (6,7) and it is considered as a clinically relevant prolongation if the median PFS of arm B is 7.0 months (risk reduction 30%). At the start of this trial, the planned sample size was 280 patients to detect 30% risk reduction with 80% power for a log-rank test comparing two survival curves with a two-sided significance level of 0.05, assuming an accrual time of 2 years and a follow-up time of 1 year (21). This calculation was carried out by employing nQuery Advisor 7.0 software (Statistical Solutions, Saugus, MA, USA). On 8 April 2011, an independent data monitoring committee of the EAGLE trial recommended that the statistical power be amended from 80 to 90% with the consideration of the promising enrollment of patients. As a result, 358 patients (330 events) will be needed to detect 90% power under the same assumption. Taking some dropouts into account, the sample size to be accrued was set at 370 patients in total.

THE EAGLE TRIAL GROUP

Principal investigator: H. Mishima (Osaka National Hospital, Osaka, Japan).
Promotion committee chairman: Y. Maehara (Graduate School of Medical Science, Kyushu University, Fukuoka, Japan).
Data and safety monitoring board: I. Hyodo (University of Tsukuba Graduate School of Comprehensive Human Sciences, Ibaraki, Japan), K. Muro (Aichi Cancer Center Hospital, Aichi, Japan) and T. Yoshino (National Cancer Center Hospital East, Chiba, Japan).
Data center: J. Sakamoto (Nagoya University Graduate School of Medicine, Aichi, Japan) and C. Abe (ECRIN, Kyoto, Japan).
Statistical advisor: K. Oba (Hokkaido University, Hokkaido, Japan).
Participating institutions: Approximately 150 Japanese institutions and hospitals are participating in this trial.

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Conflict of interest statement

None declared.

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CARDIOVASCULAR DISEASE RISK FACTORS AMONG RURAL KAZAKH POPULATION

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ABSTRACT

Cardiovascular diseases (CVDs) have remained a leading cause of mortality in Kazakhstan. The objectives of the present study were to estimate the prevalence of CVD risk factors (RFs) among the Kazakh population, and their ability to identify those CVD RFs. We interviewed 611 subjects aged 25–65 years using a structured self-administered questionnaire from April to July, 2008. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated to determine associations between CVD RFs and its correlations, such as socioeconomic status and level of knowledge of CVD RFs through a logistic regression model. Mean age of the respondents was 43.2 years, and 49.8% were male. Tobacco smoking, overweight (body mass index ≥25.0), hypertension (systolic blood pressure ≥140 mm Hg and diastolic blood pressure ≥90 mm Hg), and alcohol drinking were identified as important CVD RFs. Risk of overweight was greatest among the population aged 45–54 years, with an OR of 5.3 (95% CI=3.1–9.2). The overweight population was significantly associated with higher income (OR=1.6, 95% CI=1.1–2.4) and knowledge of RF (OR=1.7, 95% CI=1.2–2.4), with $p<0.05$. Only 25.0% of respondents had good knowledge about CVD RFs. Alcohol drinking was inversely related to the level of knowledge about CVD RFs (OR=0.7, 95% CI=0.5–0.9). We concluded that CVD RFs were very high among the Kazakh population, although their level of knowledge to identify those RFs was very low. Increasing knowledge about CVD RFs through awareness campaign activities can reduce CVD-related morbidity and mortality and ensure a better quality of life for the Kazakh population.

Key Words: Cardiovascular disease, Risk factors, Socioeconomic status, Rural, Kazakhstan

INTRODUCTION

Cardiovascular diseases (CVDs) are killing more and more people around the world, striking rich and poor alike[1)] and contributing significantly to the health costs in both developed and developing countries.[2)] Premature mortality as a result of CVD took second place in all countries of the Commonwealth of Independent States in a recent review, and was 3–4 times higher in Kazakhstan than in Western European countries.[1)] Continued morbidity, mortality and disability of people suffering from CVD, especially among the working population, greatly harms economic development and represents a high burden to society.[3,4)] CVD persists as a main killer, particularly

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