Keywords Panobinostat (LBH589) · HDAC inhibitor · Phase I study · Thrombocytopenia · Solid tumor

Introduction

Histone deacetylase (HDAC) inhibitors are a novel class of anticancer agents that inhibit HDACs involved in the deacetylation of histone and non-histone proteins [1, 2]. In humans, 18 HDACs have been identified and grouped into four classes according to their homology to yeast proteins [1–4]. Class I, II and IV HDACs contain a zinc (Zn) molecule in their active site [1–4]. Class I HDACs are localized within the cell nucleus and show high enzymatic activity toward histone substrates [1–4]. Class II HDACs act mainly on non-histone proteins [2]. Class IV HDAC has features of both class I and II [2]. Class III HDACs consist of the NAD-dependent sirtuin family, do not contain Zn and are not inhibited by any current HDAC inhibitors [1–4]. Cancer cells have high levels of expression of HDACs, resulting in hypoacetylation of histones [4]. In many tumor cell lines, inhibition or down-regulation of HDACs leads to cell-cycle arrest and differentiation, thereby inducing apoptosis [4]. HDAC inhibitors are broadly classified as class I specific inhibitors or pan-histone deacetylase (pan-HDAC) inhibitors against all class I, II and IV HDACs. Several HDAC inhibitors have been undergoing clinical development in recent years [1, 2]. Among the pan-HDAC inhibitors, vorinostat (suberoylanilide hydroxamic acid, SAHA) has been approved by the United States Food and Drug Administration (US FDA) for the treatment of cutaneous T-cell lymphoma (CTCL). More recently, romidepsin (depsipeptide, FK228), a class I specific HDAC inhibitor, was also approved by US FDA for CTCL.

Panobinostat (LBH589) is a potent pan-HDAC inhibitor [1, 2], belonging to the structurally new cinnamic hydroxamic acid class of compounds [1]. As a pan-HDAC inhibitor, panobinostat is at least 10-fold more potent than vorinostat in vitro [2]. As a result of promising preclinical data, Phase I and II clinical trials of intravenous and oral panobinostat have been conducted in patients with a wide variety of hematologic and solid tumors [1, 2].

Two phase I studies of panobinostat given intravenously on consecutive days have been performed in Western patients with solid or hematologic malignancies [5, 6]. However, because of unexpected severe cases of QT interval prolongation corrected for heart rate by Fridericia's formula (QTcF), the treatment schedule of one study was modified to once weekly on days 1, 8, and 15 of a 28-day cycle, and the other trial was terminated [5, 6]. Once-weekly treatment of panobisnostat reduced the occurrence of QTcF prolongation. However, many patients who were treated for a 28-day cycle at the maximum tolerated dose (MTD) of 20 mg/m^2 required dose delays and reductions due to thrombocytopenia on day 15 [5]. Therefore, for this study, a new dosing schedule wherein panobinostat was given on days 1 and 8 of a 21-day cycle was utilized.

This study was an open-label, multicenter Phase IA dose-escalation study of intravenous panobinostat. This will be the first reported evaluation of panobinostat on a new weekly schedule, given on days 1 and 8 of a 21-day cycle. The primary objective was to characterize the safety and tolerability of panobinostat as a single agent, including an assessment of the occurrence of dose-limiting toxicity (DLT) and the determination of MTD, in adult Japanese patients with advanced solid tumors. Secondary objectives included characterizing the pharmacokinetic profile of panobinostat and evaluating antitumor activity.

Materials and method

Eligibility criteria

Patients with histologically or cytologically confirmed, advanced solid tumors who had progressive disease that did not respond to available standard therapies or for whom no standard therapy was available were enrolled. Additional eligibility criteria for enrollment included: having at least one measurable or non-measurable lesion; being ≥20 years; having an Eastern Cooperative Oncology Group (ECOG) performance status of ≤2; having a life expectancy of ≥12 weeks; and having the following laboratory values: absolute neutrophil count ≥1,500/mm^3, hemoglobin ≥9 g/dl, platelets ≥100,000/mm^3, potassium ≥ the lower limit of normal (LLN) or correctable with supplements, total calcium (corrected for serum albumin) ≥ LLN or correctable with supplements, magnesium ≥ LLN or correctable with supplements, phosphorus ≥ LLN or correctable with supplements, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≤2.5×the upper limit of normal (ULN) or ≤5.0×ULN if liver metastases were present, serum bilirubin ≤1.5×ULN, serum creatinine ≤1.5×ULN or 24-h creatinine clearance ≥50 ml/min, and being clinically euthyroid (thyroid stimulating hormone and free T4 within their respective normal ranges). Patients with evidence of central nervous system tumors or metastases, any peripheral neuropathy of ≥ grade 2 according to the Common Terminology Criteria for Adverse Events (CTCAE), impaired cardiac function, or other uncontrolled medical conditions were excluded. Patients who were receiving medications potentially associated with prolongation of the QT interval that could not be discontinued or switched to a different medication before starting treatment with the study drug were also excluded. Women of childbearing potential must have had negative results from a pregnancy test

performed 7 days before administration of panobinostat. The protocol and informed consent form were approved by the Institutional Review Board before study initiation. Written informed consent was obtained from all patients. The study was designed and implemented in accordance with the protocol, the ICH Harmonized Tripartite Guidelines for Good Clinical Practice, the guidelines of the Japanese Ministry of Health, Labor and Welfare, and the ethical principles laid down in the Declaration of Helsinki.

Study design and treatment plan

Study design Patients were recruited into three cohorts according to the standard "3+3" method (Cohort 1: 10 mg/m^2, Cohort 2: 15 mg/m^2, Cohort 3: 20 mg/m^2). Decisions regarding dose escalation were based on the safety profile, particularly occurrence of DLT during the first cycle of treatment for each patient. The population for determination of MTD consisted of patients who received both scheduled doses during Cycle 1 and completed all safety evaluations for at least 21 days or patients who experienced a DLT during Cycle 1. Patients who did not meet these requirements (e.g. patients who received only one dose of panobinostat during cycle 1) were included in the full analysis but regarded as ineligible for the determination of MTD and replaced. An event would be classified as a DLT if any of the following criteria were met: CTCAE grade 3 or 4 neutropenia lasting for >7 days, neutropenic fever; CTCAE grade 3 thrombocytopenia lasting for >7 days, any CTCAE grade 4 thrombocytopenia; CTCAE grade 2 neurotoxicity lasting for >7 days, ≥CTCAE grade 3 neurotoxicity; ≥CTCAE grade 3 cardiac general adverse events; ≥CTCAE grade 3 vomiting or ≥ CTCAE grade 3 nausea despite the use of optimal antiemetics; ≥CTCAE grade 3 diarrhea despite the use of optimal antidiarrheal treatments; other CTCAE grade 3 adverse events (excluding alkaline phosphatase) with a duration >7 days or CTCAE grade 4 adverse events (excluding alkaline phosphatase); or any other adverse event requiring a dose delay of longer than 7 days from the next scheduled dosing date. If all three patients did not experience a DLT, a new cohort of three patients was enrolled at the subsequent dose level. If one of the three patients had a DLT, the cohort was expanded to include a total of six patients. If no additional DLT occurred, a new cohort of three patients was enrolled at the subsequent dose level. If two or more patients had a DLT at any dose level, the cohort would be stopped and the lower dose cohort immediately preceding the cohort where DLTs were observed would be expanded to six patients to confirm the safety of that dose as the MTD.

Treatment plan Patients were given panobinostat intravenously over the course of 30 min once daily on days 1 and 8 of a 21-day cycle. For administration, panobinostat was dissolved in a solution of 5% dextrose in water to an appropriate concentration to achieve the dose required by body surface area. The final volume of the solution was 50 ml.

The treatment of panobinostat was stopped if the patient had any ≥ CTCAE grade 2 cardiovascular toxicity or any ≥ CTCAE grade 3 toxicity. If panobinostat could not be administered on day 8 (+3 days) during a treatment cycle, no further attempt was made to administer panobinostat during that cycle. If the start of the next cycle was delayed by more than 7 days, treatment with panobinostat was discontinued.

Pharmacokinetics

Blood samples for pharmacokinetic analysis were collected from each patient during cycle 1. The samples were taken 0, 0.5, 0.75, 1, 2, 3, 5, 7, 24, 48, and 168 h after treatment on days 1 and 8.

Panobinostat concentrations in plasma samples were assayed using a validated liquid chromatography–tandem mass spectrometry assay. The lower limit of quantification was 0.5 ng/mL when 0.100 ml of human plasma was assayed. Panobinostat concentrations below the lower limit of quantification were treated as 0.0 ng/mL.

Noncompartmental analysis was employed to calculate pharmacokinetic parameters based on the panobinostat concentration–time data, using WinNonlin® Professional, version 5.2 software (Pharsight Corporation, Mountain View, CA). Areas under the time-concentration curves (AUCs) were calculated using the linear trapezoidal method.

Follow-up and clinical evaluation

Patients in whom treatment was interrupted or permanently discontinued due to an adverse event or laboratory abnormality were followed up at least once a week for 4 weeks and subsequently at 4-week intervals until resolution or stabilization of the event. All patients were followed up for adverse events 28 days after the last dose of panobinostat. Adverse events were monitored and recorded in accordance with CTCAE, version 3.0.

All potential sites of tumor lesions were assessed at baseline by radiologic techniques. Lesions were measured in accordance with the Response Evaluation Criteria in Solid Tumors (RECIST), version 1.0. Response was assessed ≤7 days before the completion of each cycle of chemotherapy, at the time of study treatment completion, and at other times at the discretion of the investigator. The best overall response in each patient was classified as complete response (CR), partial response (PR), progressive disease (PD), stable disease (SD), or unknown (UNK). An evaluation of PR or CR required that changes in tumor

Table 1 Patients' demographic and tumor characteristics

Demographic Variable/Tumor characteristics		N=14
Sex -n	Female	5
	Male	9
Baseline age (years)	Median (range)	63.0 (43–75)
Baseline weight (kg)	Median (range)	53.0 (44.2–71.2)
Primary site of cancer: n	Colon	3
	Stomach	2
	Gall bladder	1
	Lung	1
	Oesophagus	1
	Ovary	1
	Peritoneum	1
	Soft tissue	1
	Other	3
Tumor Histology/ Cytology: n	Adenocarcinoma	7
	Sarcoma	2
	Adenoid cystic carcinoma	1
	Melanoma	1
	Squamous cell carcinoma	1
	Undifferentiated carcinoma	1
	Other	1

measurements were confirmed by repeated assessments performed no less than 4 weeks after the criteria for the response had first been met.

Statistical analysis

Since platelet counts on day 7 or 8 influenced the occurrence of dose delays and reductions during the 21-day schedule, correlations between the percentage decrease in platelet counts on day 7 or 8 with dose and pharmacokinetic parameters were performed. All analyses were performed with Windows Version SAS® 9.2 software (SAS Institute Inc., Cary, North Carolina, USA).

Results

From July 10, 2008 through June 5, 2009, a total of 14 patients were enrolled (Table 1). Four patients who received a panobinostat dose of 20 mg/m^2 discontinued the study because of adverse events (n=2: adverse events were suspected to be related to the study drug in one patient and not related in the other) or withdrawal of consent (n=2: one was at cycle 2 and the other was at cycle 3). The remaining ten patients discontinued the study because of disease progression. The median duration of treatment was 109 days (range: 21–408).

Safety

After excluding two patients who received only one dose of panobinostat at 20 mg/m^2 during cycle 1 due to thrombocytopenia, the occurrence of DLT was assessed in the remaining 12 patients. During the study, one patient dosed at 20 mg/m^2 had drug-related liver function abnormality assessed as a DLT (grade 3 elevation of γ-glutamyl transpeptidase for >7 days). This adverse event occurred simultaneously with events of grade 3 elevation of AST and grade 2 elevation of ALT that resolved within 7 days. The two excluded patients recovered from thrombocytopenia within 7 days and continued the study drug treatment. General safety was assessed in all 14 patients. All 14 patients had one or more adverse event during treatment and the most common adverse events (>30% of the patients) were thrombocytopenia, leukopenia, neutropenia, nausea, stomatitis, vomiting, fatigue, fever, decreased appetite, hypoalbuminemia, and rash (Table 2).

Table 2 Adverse events (occurring >30% of the patients)

	10 mg/m^2 n=3		15 mg/m^2 n=3		20 mg/m^2 n=8	
	Any grade	Grade 3/4	Any grade	Grade 3/4	Any grade	Grade 3/4
Leukopenia	3	0	1	1	5	2
Neutropenia	2	0	1	1	5	3
Thrombocytopenia	3	1	3	2	8	5
Nausea	1	0	2	0	4	0
Stomatitis	2	0	1	0	3	0
Vomiting	1	0	1	0	3	0
Fatigue	2	0	2	1	3	1
Fever	0	0	1	0	4	0
Decreased appetite	1	0	2	1	5	0
Hypoalbuminemia	1	0	3	0	2	0
Rash	2	0	0	0	3	0

As for electrocardiographic abnormalities, one patient given 10 mg/m^2 had a >60 msec prolongation of the QT interval as compared with baseline value, but the corrected value (QTcF) remained within the normal range. The remaining 13 patients had no substantial electrocardiographic abnormalities.

All patients experienced ≥ grade 1 thrombocytopenia, with peaks reaching grade 3 in five patients and grade 4 in three patients (cycle ≥2). One of the three patients with grade 4 thrombocytopenia was in the 15 mg/m^2 cohort and the remaining 2 were in the 20 mg/m^2 cohort. One patient in each of these cohorts required platelet transfusions. Thrombocytopenia significantly depended on the dose and the platelet counts at baseline (Fig. 1a, rs=0.704, p=0.005; Fig. 1b, rs=0.798, p=0.0006 by Spearman's rank correlation coefficient, respectively). However, thrombocytopenia rapidly resolved within 8 days in all patients (Fig. 1c).

20 mg/m^2 was designated as the MTD in this study, without testing a higher dose. Although 20 mg/m^2 was considered acceptable as a starting dose for Japanese patients, three patients in the 20 mg/m^2 cohort required dose interruption or omission of the second dose of cycle 1 because of adverse events (primarily thrombocytopenia [n=2] and elevation of liver enzymes [n=1]); consequently, the relative dose intensity in the 20 mg/m^2 cohort was low (Table 3). As a result, further dose escalation was deemed inappropriate and the study was concluded.

Pharmacokinetics

Pharmacokinetic data was obtained in all 14 patients (Table 4). Plasma concentrations of panobinostat rapidly decreased after infusion, followed by the elimination phase. The half-life of the terminal phase was approximately 20 h. Systemic exposure of panobinostat increased in a dose dependent manner. The systemic clearance of panobinostat was moderate (40 to 60 L/h) for both days 1 and 8. The highest plasma concentration 168 h after administration was 2.3 ng/mL in the 15 mg/m^2 cohort. This value was less than 1/100 of the maximum plasma concentration (C_{max}).

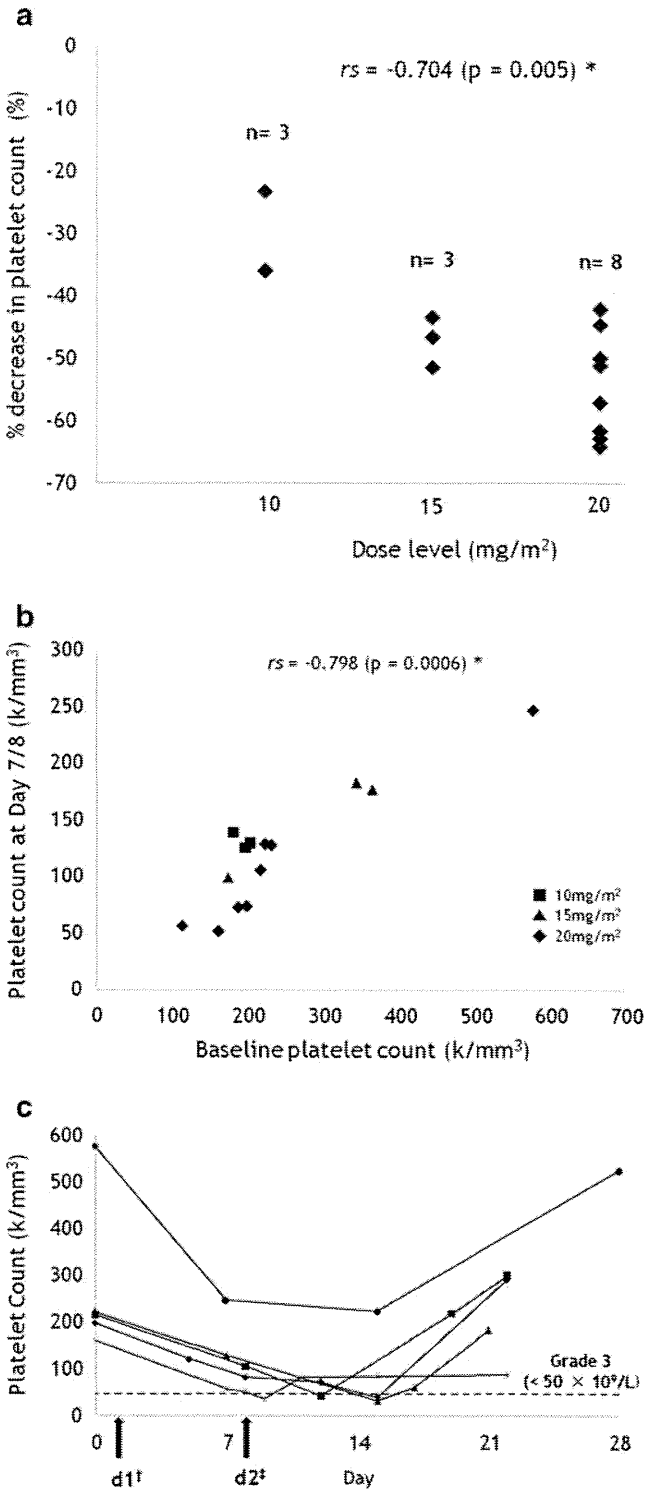

Fig. 1 **a**, percent decrease in platelet counts on day 7/8 according to dose level. The percent decrease was dose-dependent. **b**, scatter plot of baseline platelet counts versus platelet counts on day 7/8 during cycle 1. The platelet decrease appeared to correlate with the baseline counts. * *rs*: Spearman's rank correlation coefficient **c**, Time course of platelet counts during cycle 1 in five patients in the 20 mg/m^2 cohort who received both the 1st and 2nd doses. Three patients who received only the first dose during cycle 1 are excluded. (The second dose was not administered because of drug-related liver dysfunction in one patient and thrombocytopenia in the other two.) All patients recovered within 8 days after the second dose. The *dotted line* shows platelet count level of grade 3 (50×10^9/L). †d1: the 1st dose on day 1 of cycle 1 ‡d2: the 2nd dose on day 8 of cycle 1

Therefore, drug accumulation was considered negligible. Distribution of $AUC_{0\text{-}168}$ according to the dose level is shown in Fig. 2a. The severity of thrombocytopenia appeared to correlate with the $AUC_{0\text{-}168}$ (rs= −0.437, Fig. 2b) and the C_{max} (rs= −0.464).

Table 3 Exposure to panobinostat

		10 mg/m^2	15 mg/m^2	20 mg/m^2
Dose intensity (mg/day)	Mean±SD (range)	1.3±0.12 (1.2–1.4)	2.3±0.21 (2.0–2.5)	2.0±0.65 (1.3–3.0)
Relative dose intensity[a]	Mean±SD (range)	1.0±0.01 (1.0–1.0)	1.0±0.00 (1.0–1.0)	0.8±0.22 (0.5–1.0)

[a] Relative dose intensity = Actual dose intensity/Planned dose intensity

Efficacy

Stable disease for ≥4 months was observed in six patients (43%). No objective responses were confirmed. One female patient with ACC of the tongue had the longest exposure to panobinostat and received 10 mg/m^2 for 408 days (19 cycles). Another female patient with lung metastases from Ewing's sarcoma who was given 20 mg/m^2 had SD for 176 days, with a tumor shrinkage rate of 29.6% at the end of cycle 7.

Discussion

This phase I study of panobinostat in Japanese patients with advanced solid tumors demonstrated that the MTD of panobinostat given as an intravenous dose was 20 mg/m^2. Although only one patient experienced a DLT, the relative dose intensity in the 20 mg/m^2 cohort was low (0.8) and further dose escalation was deemed inappropriate. The result was consistent with a Western trial conducted in parallel, which had demonstrated that 20 mg/m^2 of i.v. panobinostat administered on Days 1, 8 of a 21-day cycle was recommended for future studies (unpublished data).

The most common adverse event was transient thrombocytopenia, which is a well-known class effect associated with HDAC inhibitors. Conventional cytotoxic agents usually cause bone marrow aplasia and megakaryocytopenia, whereas HDAC-inhibitors are thought to act via a distinct mechanism. Giver et al. observed an increase in

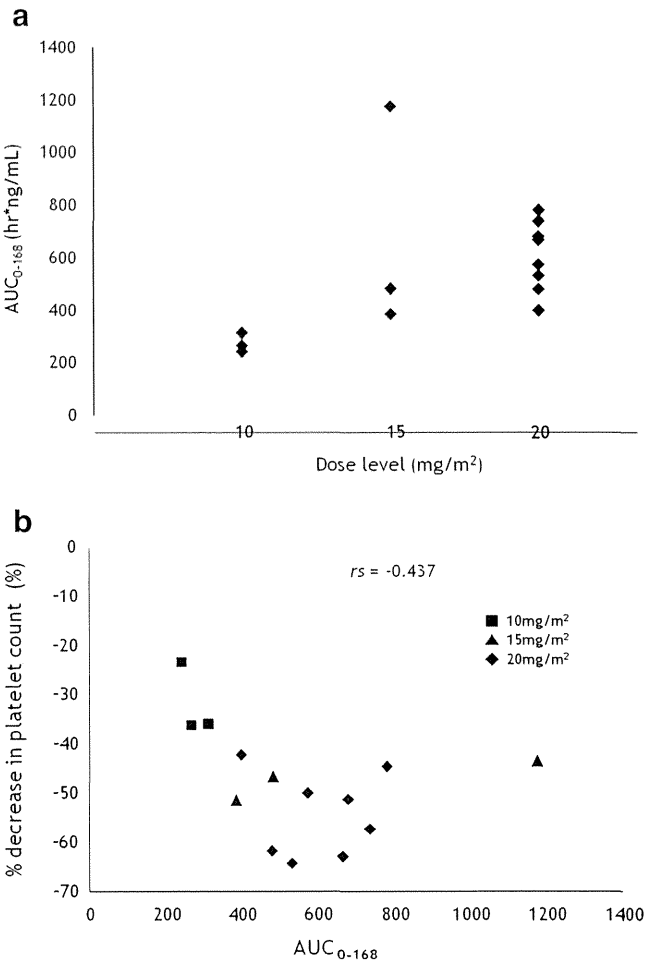

Fig. 2 **a**, scatter plot of $AUC_{0\text{-}168h}$ versus dose level. **b**, scatter plot of percent decrease in platelet counts on day 7/8 versus $AUC_{0\text{-}168h}$ during cycle 1. The percent decrease appeared to correlate with the AUC

Table 4 Summary of pharmacokinetic parameters according to initial dose cohort

dose level		t_{max} (hr)	C_{max} (ng/mL)	$AUC_{0\text{-}168h}$ (hr*ng/mL)	$AUC_{0\text{-}inf}$ (hr*ng/mL)	CL (L/hr)	$t_{1/2}$ (hr)
10 mg/m^2 n=3	Day 1	0.5 (0.5–0.5)	272.0±42.58	273.2±36.50	273.7±36.49	54.1±5.61	16.8±1.17
	Day 8	0.5 (0.5–0.5)	317.7±125.90	333.2±78.36	309.3±67.50	48.8±9.54	17.4±4.62
15 mg/m^2 n=3	Day 1	0.5 (0.5–0.5)	496.0±226.38	680.3±431.10	433.6±68.44*	53.7±5.54*	16.9±2.97[a]
	Day 8	0.5 (0.5–0.5)	308.7±158.53	503.0±156.96	441.3±140.24	57.7±15.91	17.9±1.26
20 mg/m^2 n=8	Day 1	0.5 (0.5–0.6)	492.8±147.80	605.1±132.14	606.8±132.20	54.0±12.78	18.5±2.12
	Day 8**	0.5 (0.5–0.6)	526.8±101.77	755.6±225.33	791.4±235.52	42.0±14.06	36.9±19.05

Means±SD except for t_{max}, given as the median (range)

t_{max}, time of C_{max}; C_{max}, maximum panobinostat concentration in plasma; $AUC_{0\text{-}168h}$, area under the plasma panobinostat time-concentration curve from 0 to 168 h; $AUC_{0\text{-}inf}$, AUC from 0 to infinity; CL, systemic clearance of panobinostat; $t_{1/2}$, half-life of the terminal phase

* n=2

** n=5

megakaryocytes in bone marrow during panobinostat-induced thrombocytopenia in mice. They also found that the thrombocytopenia rapidly resolved after panobinostat was discontinued and suggested that the paradoxical increase in megakaryocytes was due to the suspension of megakaryocyte maturation caused by panobinostat [7]. Similarly, Matsuoka et al. demonstrated that another HDAC inhibitor (FR235225) causes thrombocytopenia and simultaneously increases megakaryocytes in rat spleen models. It was also speculated that FR235225 delays the maturation of megakaryocytes [8]. Recently, Bishton et al. showed that HDAC inhibitor-induced thrombocytopenia is not due to myelosuppression or reduced platelet lifespan, but to decreased platelet release from megakaryocytes [9]. Such mechanisms may underlie the rapid resolution of panobinostat-induced thrombocytopenia after treatment was discontinued in patients in the 20 mg/m^2 cohort (Fig. 1c). Two patients in the 20 mg/m^2 cohort required omission of the second dose in cycle 1 because of thrombocytopenia. Given that thrombocytopenia was dose-dependent and seemed to correlate with the platelet count at the baseline, it is recommended that patients with relatively low platelet counts at baseline be closely monitored for platelet depletion and considered for dose omission and reduction, as indicated, during treatment with HDAC inhibitors such as panobinostat. In a phase I study of oral panobinostat in Japanese patients with advanced solid tumors or CTCL, rapid recovery of panobinostat-induced thrombocytopenia was also reported [10].

Although QTcF prolongation was an issue identified in previous dose-finding studies using daily intravenous treatment with panobinostat [6], there were no abnormal QTcF prolongation events in this study, suggesting that once-weekly treatment is safe. There were no other unexpected or previously undescribed panobinostat-associated toxicities. When compared with the Japanese oral panobinostat study, fewer gastrointestinal disorders (especially diarrhea) in this study were reported [10]. Only one patient who received 20 mg/m^2 experienced a DLT (grade 3 elevation of γ-glutamyl transpeptidase for >7 days).

The pharmacokinetics of intravenous panobinostat in this study were comparable to those in Western patients who received once-weekly treatment in a 28-day cycle [5], suggesting no interethnic difference in the pharmacokinetic profile of panobinostat. A weakness of this study was that no biomarker was used to study pharmacodynamics. Histone acetylation is often measured as an index of HDAC inhibitor activity. In a Western study of intravenous panobinostat, acetylation levels of histone H3 in peripheral blood mononuclear leukocytes were measured at 7-day intervals [5].

The assessment of antitumor activity was not a primary objective of our study; however, six of the 14 patients had stable disease lasting more than 3 months. One female patient with ACC of the tongue continued treatment for more than 1 year with no attenuation of antitumor effectiveness. This finding is consistent with the results of a study of vorinostat performed by the National Cancer Institute Organ Dysfunction Working Group. The group reported favorable anticancer activity in five patients with ACC [11]. These results suggest that HDAC inhibitors might be an effective therapy for ACC, a disease that is refractory to chemotherapy and has no standard treatment. In a female patient with Ewing's sarcoma, tumor shrinkage fell just short of a PR. Although the underlying mechanisms cannot be fully defined, preclinical studies have suggested that HDAC inhibitors suppress the expression of EWS-Fli 1 chimeric protein, which is encoded by the chromosomal translocation t(11;22)(q24;q12) found in most (85% to >90%) cases of Ewing's sarcoma [12–15].

In conclusion, the present study demonstrated that intravenous panobinostat administered once daily on days 1 and 8 of a 21-day cycle was relatively safe and potentially effective in patients with advanced solid tumors. Although the MTD was not determined as defined in the protocol, it is feasible to conclude that 20 mg/m^2 was the MTD and it could be reasonably recommended as the starting dose for phase II clinical trials.

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Chemoradiotherapy for Limited-disease Small-cell Lung Cancer in Elderly Patients Aged 75 Years or Older

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Background: As clinical trials for limited-disease small-cell lung cancer often exclude elderly patients due to comorbidities and a decline in organ function, the most suitable treatment for limited-disease small-cell lung cancer patients aged 75 years or older still remains unclear.
Methods: From July 2002 to June 2011, 20 consecutive patients aged 75 years or older, with Stage II to IIIB limited-disease small-cell lung cancer, were scheduled to be treated with concurrent or sequential chemoradiotherapy at the Shizuoka Cancer Center. We reviewed the medical charts of the patients and evaluated their characteristics, treatment compliance, toxicity and antitumor efficacy.
Results: Five patients were treated with concurrent chemoradiotherapy and the other 15 patients were scheduled to be treated with sequential chemoradiotherapy. Of these 15 patients, 12 were treated with four cycles of etoposide (80 mg/m^2, days 1–3, q3–4w) plus carboplatin (area under the curve 5, day 1, q3–4w), followed by thoracic radiotherapy. Of the five patients treated with concurrent chemoradiotherapy, discontinuation of chemotherapy/thoracic radiotherapy occurred in two patients due to toxicity and they suffered a prolonged decrease in performance status. Of the 12 patients treated with etoposide plus carboplatin followed by sequential thoracic radiotherapy, the response rate, median progression-free survival and median overall survival time were 91%, 244 and 601 days.
Conclusions: These results suggest that concurrent chemoradiotherapy is not feasible for all limited-disease small-cell lung cancer patients aged 75 years or older. The alternative of four cycles of etoposide plus carboplatin followed by thoracic radiotherapy is a candidate for the standard treatment of limited-disease small-cell lung cancer patients in this age group. A further trial is warranted to develop and evaluate the optimal treatment for elderly patients with limited-disease small-cell lung cancer.

Key words: small-cell lung cancer – limited-disease small-cell lung cancer – elderly – chemoradiotherapy – chemotherapy – radiotherapy – feasibility – efficacy

INTRODUCTION

Small-cell lung cancer (SCLC) accounts for 10–15% of all lung cancer cases, with individuals aged 70 years or older constituting 25–40% of SCLC patients (1,2). Limited-disease SCLC (LD-SCLC) is confined to one hemithorax and its regional lymph nodes, and can be treated using a

single radiation therapy port. Approximately 30–40% of all SCLC patients present with LD-SCLC (1,2). The proportion of elderly SCLC patients continues to increase with the growing geriatric population (1,3).

The combination of chemotherapy and radiotherapy, particularly etoposide plus cisplatin with early concurrent twice-daily thoracic radiotherapy (TRT), is regarded as the standard treatment for LD-SCLC, provided the patients are in a good general condition (4–6). However, many clinical trials for LD-SCLC have excluded elderly patients for reasons, such as comorbidities or a decline in organ function (7,8). Takada et al. (6) reported that etoposide plus cisplatin and concurrent TRT are more effective for the treatment of LD-SCLC than are etoposide plus cisplatin and sequential TRT, but patients aged 75 years or older were excluded from this trial.

Retrospective subset analyses of patients with LD-SCLC treated with etoposide plus cisplatin and concurrent early chemoradiotherapy (CRT) in Phase III trials have shown that severe hematological toxicity, pneumonitis of Grade 4 or more and treatment-related death occurred much more often among patients aged 70 years or older than among younger patients (9,10). Although the response rate and 5-year event-free survival rate did not significantly differ between these two subgroups, there was a trend for them to be worse in older patients, and significant difference in the 5-year overall survival rate favored patients younger than 70 years in one trial (9,10). These results suggest that this regimen is too toxic for elderly LD-SCLC patients and the most suitable method of treatment remains unclear.

The objective of our retrospective analysis was to discover the optimal treatment method for elderly patients with LD-SCLC aged 75 years or older. We compared the patient characteristics, treatment compliance, toxicity and antitumor efficacy between those undergoing concurrent and sequential CRT. Then, we focused on etoposide plus carboplatin and sequential TRT, as this is the most common method for treating elderly LD-SCLC patients in our institute, and evaluated their characteristics, treatment compliance, toxicity and antitumor efficacy of this regimen.

PATIENTS AND METHODS

Patient Selection

We reviewed 20 consecutive patients with Stage II–IIIB LD-SCLC, aged 75 years or older, whose treatment plan involved concurrent or sequential CRT at the Shizuoka Cancer Center between July 2002 and June 2011. The TNM stage was classified using TNM stage version 6 (11). Chest CT, abdominal CT, bone scintigram or FDG-PET, and brain magnetic resonance imaging (MRI)/CT were performed before treatment in all patients.

The inclusion criteria for concurrent or sequential CRT in our institution are generally as follows: a performance status (PS) of 0–2; white blood cell count, $\geq 3.0 \times 10^3/mm^3$; neutrophil count, $\geq 1.5 \times 10^3/mm^3$; platelet count, $\geq 1.0 \times 10^5/mm^3$; serum creatinine, ≤ 1.5 mg/dl; total bilirubin, ≤ 1.5 mg/dl and a transaminase level less than twice the upper limit of the normal value. The exclusion criteria were interstitial lung disease identified by a chest radiograph; the presence of malignant pleural or pericardial effusion prior to radiotherapy and serious complications, such as severe respiratory failure, active infectious diseases, serious heart diseases and poorly controlled hypertension/diabetes mellitus. The study protocol was approved by the institutional review board of Shizuoka Cancer Center.

Chemotherapy

The combination of etoposide (80 or 100 mg/m^2) on days 1–3 plus cisplatin (80 mg/m^2) on day 1, cisplatin (25 mg/m^2) on days 1–3, or carboplatin [area under the curve (AUC) 5] on day 1 were administered intravenously to elderly LD-SCLC patients every 3–4 weeks. The administered drug and its dose were determined by the physician in charge. The treatment cycles were repeated every 3–4 weeks for four cycles. The criteria for starting subsequent cycles of treatment in our institution are generally the same as the inclusion criteria for concurrent or sequential CRT mentioned in the 'Patient selection' section. If these criteria were not met, subsequent cycles were withheld until the noted abnormality had resolved. If there was no resolution of the abnormality after 7 weeks from the first day of the cycle, chemotherapy was stopped. Generally, the doses of etoposide and cisplatin or carboplatin were reduced or chemotherapeutic regimens were changed in the event of Grade 4 anemia, Grade 4 thrombocytopenia, prolonged Grade 4 leukopenia/neutropenia or Grade 3 or more severe non-hematological toxicity during the previous treatment cycle.

Radiotherapy

Generally, TRT was started concurrently in the first cycle of chemotherapy or sequentially after four cycles of chemotherapy in the elderly LD-SCLC patients. The timing and prescribed dose of TRT was determined by the physician in charge. All patients were required to undergo a chest CT to facilitate treatment planning. The primary tumor (gross tumor volume; GTV primary) was delineated in the pulmonary windows, and the nodal involvement (GTV node) was delineated in the mediastinal windows. The clinical target volume (CTV) included the GTV primary; GTV node; ipsilateral hilum and the elective mediastinum, for which the lower border was 3.0 cm below the carina up to 40 Gy in a once-daily fraction of 2 Gy per fraction or 30 Gy in twice-daily fractions of 1.5 Gy per fraction. Thereafter, CTV included the GTV primary and GTV node. The planning target volume was the CTV plus a margin to ensure that the planned dose was actually delivered to the CTV. The total planned dose was usually 50 Gy in a once-daily fraction or 45 Gy in twice-daily fractions. The initial field in the sequential arm was also based on the pretreatment tumor volume.

TRT was suspended if a patient experienced Grade 4 thrombocytopenia, radiation pneumonitis, fever caused by infection, a decrease in arterial oxygen pressure exceeding 10 mmHg or if a patient had difficulty swallowing a liquid diet. It was ensured that the normal lung volume receiving more than 20 Gy (V20) was $\leq$35% of the total lung volume. The maximum spinal cord dose was limited to 45 Gy in a once-daily fraction or 36 Gy in twice-daily fractions at any level.

After TRT, prophylactic cranial irradiation (PCI) was administered to patients with a complete or near-complete response represented by a scar-like shadow on a chest CT if the physician in charge judged the patient would benefit from PCI. The PCI consisted of 25 Gy/10 fr.

Evaluation of Efficacy and Toxicity

All the patients were evaluated for lesions approximately every 2 months by CT, MRI, bone scintigraphy or PET during the treatment period and every 3–6 months after treatment. The tumor response was evaluated in accordance with the response evaluation criteria in solid tumors (RECIST; version 1.0) (12). Adverse events were evaluated in accordance with the common terminology criteria for adverse events (CTCAE; version 3.0) (13).

Statistical Analyses

To evaluate the difference between concurrent CRT and sequential CRT, in relation to the patients' characteristics, the χ^2 test, Fisher's exact test and the Mann–Whitney U-test were performed. To analyze the PFS and OS, survival curves were drawn using the Kaplan–Meier method. The PFS was calculated from the date of initiation of the treatment to the date of detection of disease progression or the date of death from any cause. The PFS was censored at the date of the last visit for those patients who were still alive without any documented disease progression. PFS were compared between concurrent CRT and sequential CRT using the log-rank test. The OS was calculated from the date of initiation of the treatment to the date of death. The OS was censored at the date of the last visit for those patients whose deaths could not be confirmed. P values of <0.05 were considered to be statistically significant. All statistical analyses were performed by the application of JMP version 8.0 for Windows (SAS Institute Inc., Cary, NC, USA).

RESULTS

Characteristics and Treatment Methods of the 20 Patients Treated with Chemoradiotherapy

Twenty patients 75 years of age or older and with Stage II–IIIB LD-SCLC were scheduled to be treated with concurrent or sequential CRT at the Shizuoka Cancer Center. During the same period, seven patients 75 years of age or older and with Stage II–IIIB LD-SCLC were excluded by the inclusion/exclusion criteria of CRT. The reasons for exclusion were interstitial lung disease in six patients and renal failure in one patient. Tables 1 and 2 show the individual patients' characteristics, treatment methods and outcome of the patients treated with concurrent and sequential CRT. Of these patients, 80% were men and their median age was 77 years. Forty percent of the patients had a PS of 0 and the remaining a PS of 1. The majority of the patients were smokers and 80% were Stage IIIA or IIIB.

Five patients were treated with concurrent CRT and 15 were scheduled to be treated with sequential CRT. Of the five treated with concurrent CRT, two received TRT from the first cycle of chemotherapy and three received TRT from the second cycle of chemotherapy. From the beginning, two were scheduled to receive TRT from the second cycle after the confirmation of toxicity in the first cycle. The other patient was also scheduled to receive TRT from the second cycle if the symptom due to tumor compression had not recovered by chemotherapy only. Two patients received etoposide (80 mg/m^2, days 1–3) plus carboplatin (AUC 5, day 1), two were administered etoposide (100 mg/m^2, days 1–3) plus cisplatin (80 mg/m^2, day 1) and one received etoposide (80 mg/m^2, days 1–3) plus cisplatin (25 mg/m^2, days 1–3) as their chemotherapy regimen. Of these patients, one patient switched from etoposide (80 mg/m^2, days 1–3) plus cisplatin (25 mg/m^2, days 1–3) to etoposide (80 mg/m^2, days 1–3) plus carboplatin (AUC 5, day 1) from cycle 2 due to Grade 4 hyponatremia and Grade 3 anorexia.

Of the 15 patients scheduled to be treated with sequential CRT, 12 received etoposide (80 mg/m^2, days 1–3) plus carboplatin (AUC 5, day 1), two received etoposide (80 mg/m^2, days 1–3) plus cisplatin (25 mg/m^2, days 1–3) and one was administered etoposide (100 mg/m^2, days 1–3) plus cisplatin (25 mg/m^2, days 1–3) as chemotherapy. Two patients could not receive TRT due to discontinuation of treatment during the chemotherapy period.

The planned TRT doses were 45 Gy in twice-daily fractions and 1.5 Gy per fraction in 12 patients, 50 Gy in a once-daily fraction and 2 Gy per fraction in three patients, and the other radiation doses in three patients. PCI was performed in Patient #C-5 and #S-13.

Table 3 shows the individual patients' characteristics, past history and complications of the patients treated with concurrent and sequential CRT. Generally, past history and complications were fewer and less severe in concurrent CRT, especially in terms of cardiopulmonary diseases.

Comparison of Patient Characteristics, Response, PFS, Compliance and Adverse Events Between Concurrent CRT and Sequential CRT

In terms of patient characteristics, (gender, age, PS, stage), the difference in age between concurrent CRT and sequential CRT is significant (Mann–Whitney U-test $P = 0.041$).

Table 1. Individual patients' characteristics, treatment methods and outcome of the patients treated with concurrent chemoradiotherapy (CRT)

No.	Age (years)	Gender	PS	Stage	CTx	Response	RTx (timing)	RTx (Dose/Fr)	CTx compliance	RTx compliance	Failure site	PFS	OS
C-1	75	F	1	IIIA	CB(5)+ETP(80)2c	PR	From c2	39.6/22	Discontinuation +	Discontinuation +	WT	165	971
C-2	75	M	0	IIIA	CB(5)+ETP(80)3c	PR	From c2	44/22	Discontinuation +	4 days omission	Brain	547	1114
C-3	75	M	1	IIB	CD(80)+ETP(100)4c	PR	From c1	45/30	Dose reduction +	7 days omission	Brain	1790	2393+
C-4	76	M	1	IIB	CD(80)+ETP(100)4c	PR	From c1	45/30	Completed	2 days omission	Brain	214	2485
C-5	77	F	1	IIIB	CD(25)x3+ETP(80)1c→ CB(5)+ETP(80)3c	Near CR	From c2	45/30	Changed CTx regimen and dose reduction	Completed	Liver	201	359

No., number; PS, performance status; CTx, chemotherapy; RTx, radiotherapy; Fr, fraction; PFS, progression-free survival; OS, overall survival; F, female; M, male; CB, carboplatin; ETP, etoposide; c, cycle; CD, cisplatin; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; NE, not evaluable; NA, not available; WT, within the thorax; PF, progression-free.
The dose of carboplatin was indicated by area under the curve in parentheses.
The doses of etoposide and cisplatin were indicated by per body surface area in parentheses.

Patients tended to be female, have lower stage and have a poorer PS in concurrent CRT, although there is no significant difference.

All five patients treated with concurrent CRT exhibited a partial response (PR) and the response rate was 100%. Of the 15 patients treated with sequential CRT, 3 had a complete response (CR), 9 exhibited PR, 1 showed stable disease (SD), 1 developed progressive disease (PD) and 1 was not evaluable (NE). The response rate was 80%. The median PFS of concurrent and sequential CRT were 208 and 216 days, respectively (Fig. 1). There was no statistically significant difference between the PFS of the two treatment methods (log-rank $P = 0.9715$) and the two PFS curves almost overlapped each other.

Of the five patients treated with concurrent CRT, discontinuation of chemotherapy occurred in two (40%) and dose reductions were needed in two due to adverse events (40%). Moreover, discontinuation of radiotherapy occurred in one patient (20%) and omissions were needed in three (60%). Among the 15 patients treated with sequential CRT, 11 completed the whole treatment method without discontinuation, dose reduction and omission of chemotherapy/TRT. Dose reductions of chemotherapy were needed in two patients (13%), and one of the two patients was treated with etoposide (100 mg/m^2, days 1–3) plus cisplatin (25 mg/m^2, days 1–3). Discontinuation of chemotherapy occurred in two patients (13%) due to toxicities. Radiotherapy was completed without omission in all 11 patients who received sequential radiotherapy.

Table 4 shows the adverse events in patients treated with concurrent CRT and sequential CRT. Hematological toxicities, febrile neutropenia, fatigue and anorexia tended to be more frequent and severe in concurrent CRT than in sequential CRT. However, Grade 3 or more severe pneumonitis tended to be frequent in sequential CRT (four patients, 27%).

Patients' Characteristics, Tumor Response, PFS, OS and Toxicity in Patients Treated with Etoposide Plus Carboplatin Followed by Sequential TRT

Twelve patients were treated with etoposide plus carboplatin followed by sequential TRT. The number of male patients, 10 (83%), was larger than that of the female patients, and the median age of the patients was 79 years. Eight patients (67%) had a PS of 0 and the remaining a PS of 1. All were smokers, and 10 patients (83%) were Stage IIIA or IIIB and the remaining Stage IIA or IIB.

With regard to the tumor response, CR was achieved by three patients, PR by eight and one patient was NE. The response rate was 91%.

The median PFS and OS were 244 and 601 days, respectively (Fig. 2). The median follow-up duration was 496 days. In terms of the first failure site during and after CRT, nine patients (75%) had experienced disease relapse at the time of data analyses. Five (42%) and two (17%) patients

Table 2. Individual patients' characteristics, treatment methods and outcome of the patients treated with sequential CRT

No.	Age (years)	Gender	PS	Stage	CTx	Response	RTx (dose/Fr)	CTx compliance	RTx compliance	Failure site	PFS	OS
S-1	75	M	0	IIIA	CB(5)+ETP(80)4c	PR	45/30	Completed	Completed	PF	2754+	2754+
S-2	75	M	0	IIIA	CD(25)x3+ETP(80)4c	SD	45/30	Completed	Completed	Brain	137	578
S-3	75	M	0	IIIA	CD(25)x3+ETP(100)4c	PD	50/25	Dose Reduction +	Completed	WT	143	769
S-4	76	M	1	IIIB	CB(5)+ETP(80)4c	PR	45/30	Dose Reduction +	Completed	WT and liver	414	652
S-5	76	M	1	IIIA	CB(5)+ETP(80)4c	CR	45/30	Completed	Completed	Brain	137	257
S-6	77	M	1	IIA	CB(5)+ETP(80)4c	PR	45/30	Completed	Completed	PF	442+	442+
S-7	77	M	0	IIIB	CD(25)x3+ETP(80)3c	PR	NA	Discontinuation +	NA	WT	243	454
S-8	78	M	1	IIIA	CB(5)+ETP(80)4c	PR	59/32	Completed	Completed	Brain	181+	181+
S-9	78	M	0	IIIA	CB(5)+ETP(80)4c	PR	45/30	Completed	Completed	Brain	181	550+
S-10	80	F	1	IIIA	CB(5)+ETP(80)1c	NE	NA	Discontinuation +	NA	WT	70	316+
S-11	80	M	0	IIIB	CB(5)+ETP(80)4c	CR	45/30	Completed	Completed	Brain	152	258
S-12	81	F	1	IIB	CB(5)+ETP(80)4c	PR	50/25	Completed	Completed	PF	1892+	1892+
S-13	83	M	1	IIIB	CB(5)+ETP(80)4c	CR	45/30	Completed	Completed	Brain	269	327
S-14	83	F	1	IIIA	CB(5)+ETP(80)4c	Near CR	50/25	Completed	Completed	Liver and lung	408	415+
S-15	92	M	0	IIIA	CB(5)+ETP(80)4c	PR	45/30	Completed	Completed	WT	218	383

The dose of carboplatin was indicated by area under the curve in parentheses.
The doses of etoposide and cisplatin were indicated by per body surface area in parentheses.

Table 3. Individual patients' characteristics, past history and complications of the patients treated with concurrent CRT and sequential CRT

No	Age (years)	Gender	PS	Stage	Past history	Complications
C-1	75	F	1	IIIA	–	Osteoarthritis
C-2	75	M	0	IIIA	–	Anal stenosis
C-3	75	M	1	IIB	Gastric ulcer	COPD, prostatic hypertrophy
C-4	76	M	1	IIB	Gastric ulcer	–
C-5	77	F	1	IIIB	–	Hypertension, hyperlipidemia, osteoporosis
S-1	75	M	0	IIIA	–	Arrhythmia, prostate cancer
S-2	75	M	0	IIIA	–	Gastric ulcer, hypertension
S-3	75	M	0	IIIA	–	Prostatic hypertrophy, abdominal aortic aneurism
S-4	76	M	1	IIIB	Abdominal aortic aneurism	IHD, DM, hypertension
S-5	76	M	1	IIIA	Abdominal aortic aneurism	Aortic dissection
S-6	77	M	1	IIA	Laryngeal cancer, brain hemorrhage	Hypertension
S-7	77	M	0	IIIB	Gout, gastritis	Hypertension, prostatic hypertrophy
S-8	78	M	1	IIIA	Bladder cancer, brain hemorrhage	Hypertension
S-9	78	M	0	IIIA	ASO, IHD, gastric ulcer	–
S-10	80	F	1	IIIA	IHD, pneumothorax, gout, renal failure	COPD
S-11	80	M	0	IIIB	Rectal cancer	–
S-12	81	F	1	IIB	–	IHD
S-13	83	M	1	IIIB	Asthma, gastric ulcer, colon cancer	Hypertension
S-14	83	F	1	IIIA	Uterine cancer	Hypertension
S-15	92	M	0	IIIA	–	Reflux esophagitis, hypertension

COPD, chronic obstructive pulmonary disease; IHD, ischemic heart disease; DM, diabetes mellitus; ASO, arteriosclerosis obliterans.

experienced disease relapse outside the thorax and within the thorax, respectively. Two patients experienced disease relapse both within and outside the thorax. The most common first failure organ was the brain (five patients, 42%).

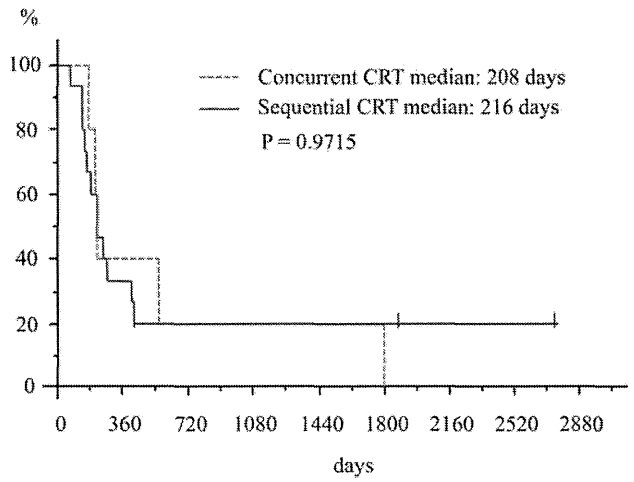

Figure 1. Kaplan–Meier curves for the progression-free survival (PFS) of patients aged 75 years or older treated with concurrent chemoradiotherapy (CRT) and sequential CRT are shown (concurrent CRT, red dashed line; sequential CRT, blue continuous line). The median PFS was 208 days in concurrent CRT and 216 days in sequential CRT. There was no statistically significant difference between the two groups (log-rank $P = 0.9715$).

Table 5 shows the adverse events in these 12 patients. Although there were moderate levels of hematological toxicities, gastrointestinal toxicities tended to be mild. It is noteworthy that Grade 3 or more severe pneumonitis occurred in four patients (33%).

DISCUSSION

Our investigation is important as it includes a considerable number of LD-SCLC patients aged 75 years or older who have been treated with CRT. Moreover, as this study documents a precise clinical course (i.e. treatment response, PFS, OS, treatment compliance and adverse events), it will enable physicians to determine the optimal treatment strategy for this category of patients.

Two previous research papers have detailed clinical course data in studies similar to ours. In one study, seven LD-SCLC patients aged 75 years or older were treated with etoposide plus cisplatin or carboplatin and with concurrent TRT (14). TRT treatment was delayed for more than 7 days in three of the seven patients. Three experienced Grade 3 or more severe febrile neutropenia, and three experienced

Table 4. Adverse events in patients treated with concurrent CRT and sequential CRT

	Concurrent chemoradiotherapy ($n = 5$)						Sequential chemoradiotherapy ($n = 15$)					
	Gr 1	Gr 2	Gr 3	Gr 4	≥Gr 3 (%)	All (%)	Gr 1	Gr2	Gr 3	Gr 4	≥Gr 3 (%)	All (%)
Leukopenia	0	0	3	2	100	100	1	6	8	0	53	100
Neutropenia	0	0	0	5	100	100	1	0	3	11	93	100
Anemia	0	4	1	0	20	100	3	7	2	0	13	80
Thrombocytopenia	2	2	1	0	20	100	6	3	3	1	27	87
Fatigue	1	1	1	0	20	60	7	2	0	0	0	60
Anorexia	2	1	1	0	20	80	6	5	0	0	0	73
Constipation	2	2	0	0	0	80	12	1	0	0	0	87
Nausea	2	2	0	0	0	80	6	1	0	0	0	47
Infection	0	2	0	0	0	40	1	1	1	0	7	20
Febrile neutropenia	0	0	3	0	60	60	0	0	2	0	13	13
Bilirubin	1	0	0	0	0	20	2	1	0	0	0	20
AST	0	0	0	0	0	0	2	0	0	0	0	13
ALT	1	0	0	0	0	20	3	0	0	0	0	20
Hyponatremia	2	0	0	1	20	60	4	0	1	1	13	40
Creatinine elevation	1	0	0	0	0	20	3	2	0	0	0	33
Pneumonitis	4	0	0	0	0	80	7	0	3	1	27	73
Esophagitis	1	3	1	0	20	100	5	4	0	0	0	60
Dermatitis	4	0	0	0	0	80	9	0	0	0	0	60
Eruption	2	0	0	0	0	40	1	1	0	0	0	13

Gr, grade; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

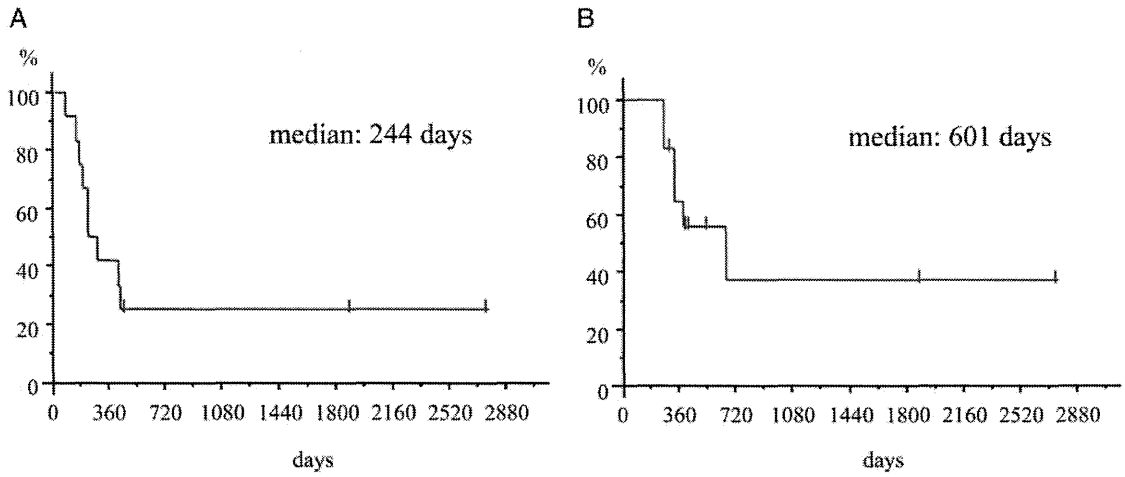

Figure 2. The Kaplan–Meier curve for the PFS (A) and overall survival (OS) (B) of 12 patients aged 75 years or older, treated with etoposide plus carboplatin followed by sequential thoracic radiotherapy is shown. The median PFS and OS were 244 and 601 days, respectively.

Table 5. Adverse events in patients treated by etoposide plus carboplatin and sequential radiotherapy, $n = 12$

	Gr 1	Gr 2	Gr 3	Gr 4	≥Gr 3 (%)	All (%)
Leukopenia	0	6	6	0	50	100
Neutropenia	0	0	3	9	100	100
Anemia	2	5	2	0	17	75
Thrombocytopenia	4	3	2	1	25	83
Fatigue	5	2	0	0	0	58
Anorexia	5	4	0	0	0	75
Constipation	9	1	0	0	0	83
Nausea	5	0	0	0	0	42
Infection	1	1	1	0	8	25
Febrile neutropenia	0	0	2	0	17	17
Bilirubin	1	1	0	0	0	17
AST	1	0	0	0	0	8
ALT	3	0	0	0	0	25
Hyponatremia	3	0	0	0	0	25
Creatinine elevation	2	2	0	0	0	33
Pneumonitis	5	0	3	1	33	75
Esophagitis	5	3	0	0	0	67

Grade 4 thrombocytopenia. One patient died due to radiation pneumonitis and this was judged as treatment-related death. In the second study, the outcome of elderly patients aged 70 years or older, five of whom were 75 years or older, who received early concurrent CRT with four cycles of etoposide plus cisplatin, was reported (15). Of the 12 patients in this report, 8 (67%) experienced Grade 3 or more severe febrile neutropenia. Of the five patients aged 75 years or older, three could not complete the four cycles of chemotherapy and all five experienced delayed TRT for more than 7 days. In our study, five patients received concurrent CRT and two could not complete the chemotherapy course due to toxicities. TRT was discontinued in one patient and another experienced delayed TRT for more than 7 days due to toxicities. These patients suffered from prolonged toxicities and their quality of life decreased for a long time. Moreover, it is speculated that fitter patients were treated by concurrent CRT and more fragile patients were treated by sequential CRT. Therefore, it is suggested that concurrent CRT is not feasible for all LD-SCLC patients aged 75 years or older. Moreover, a high frequency of discontinuation, dose reduction and omission of chemotherapy/TRT in concurrent CRT may lead to a similar PFS as that achieved with sequential CRT.

Based on the previous Phase III study which investigated chemotherapeutic regimen for elderly or poor-risk patients with ED (extensive disease)-SCLC (16) and the convenient administration schedule of carboplatin, etoposide (80 mg/m^2) on days 1–3 plus carboplatin (AUC 5) on day 1 followed by sequential TRT 45Gy in twice-daily fractions or 50 Gy in a once-daily fraction was the most frequently used treatment method for LD-SCLC patients aged 75 years or older in our institute. In our study, the major adverse events of etoposide plus carboplatin followed by sequential TRT were hematological toxicities, including neutropenia and thrombocytopenia. Gastrointestinal toxicities such as anorexia, nausea, vomiting and constipation were very mild. All of the toxicities were manageable and no treatment-related death occurred. The response rate, OS and PFS were satisfactory, when taking the patients' characteristics in our study and the results of the previous Phase II studies that evaluated CRT for LD-SCLC patients aged 70 years or older, into account (17, 18). However, as Grade 3 or more severe pneumonitis occurred in 4 of 12 patients (33%) similar to a retrospective subset analysis of LD-SCLC patients treated with etoposide plus cisplatin and concurrent early CRT in a Phase III trial (10), attention should be paid to the occurrence of radiation

pneumonitis. It may be appropriate to set the radiation field based on the tumor volume after induction chemotherapy to reduce the frequency and severity of radiation pneumonitis (19). On the other hand, the previous Phase III study have also shown etoposide plus split doses of cisplatin seems to be another standard chemotherapeutic regimen for elderly or poor-risk patients with ED-SCLC (16). Etoposide plus split doses of cisplatin on days 1–3 followed by sequential TRT could be a candidate for the standard treatment of LD-SCLC patients aged 75 years or older. However, because only three patients were treated by etoposide plus split doses of cisplatin on days 1–3 followed by sequential TRT, it is hard to lead a definitive conclusion in this study.

Our study has a few limitations. The intervals between evaluations for lesions in this study were not as accurate as those in a prospective study. The severity of non-hematological toxicities, in particular, may have been underestimated in the present study due to its retrospective nature. Patients were treated as inpatients during most of the treatment period, and the toxicity data were recorded in detail in the patients' medical records. The sample size in this study is not very large; therefore, it is difficult to reach a definitive conclusion. However, as it is not easy to collect data on a large number of LD-SCLC patients aged 75 years or older who have received CRT, this study may be useful for physicians trying to determine the optimal treatment strategy for LD-SCLC patients aged 75 years or older.

In conclusion, it is suggested that concurrent CRT is not feasible for all LD-SCLC patients aged 75 years or older. Etoposide (80 mg/m^2) on days 1–3 plus carboplatin (AUC 5) on day 1 followed by sequential TRT is one of the candidates for the standard treatment of these elderly LD-SCLC patients. A further prospective clinical trial is warranted to develop and evaluate the optimal treatment method for LD-SCLC patients aged 75 years or older.

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Conflict of interest statement

None declared.

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Large Cell Neuroendocrine Carcinoma of the Lung: Is it Possible to Diagnose from Biopsy Specimens?

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Objective: We have recently proposed new diagnostic criteria for high-grade non-small cell neuroendocrine carcinoma, i.e. possible large cell neuroendocrine carcinoma, in biopsy specimens and have started a clinicopathological comparative study of high-grade neuroendocrine carcinomas in an advanced stage. This study aimed to elucidate the usefulness of our diagnostic criteria for inoperable advanced large cell neuroendocrine carcinoma and to know the true incidence of large cell neuroendocrine carcinoma among lung cancers.
Methods: We reviewed all cancer lesions (1040 specimens) obtained by transbronchial lung biopsies in our hospital from 2002 to 2009 and selected 38 biopsy specimens that satisfied our diagnostic criteria for high-grade non-small cell neuroendocrine carcinoma. All 38 cases were clinicopathologically investigated and all biopsy specimens were precisely studied for their morphological characteristics.
Results: Clinicopathological information about the selected 38 cases was very similar to the clinicopathological characteristics of large cell neuroendocrine carcinoma reported. Of 38 cases, six were at Stage I, II or IIIA, underwent surgery, and the diagnosis was confirmed to be large cell neuroendocrine carcinoma using surgical tumor specimens. In the 38 biopsy specimens, features of neuroendocrine morphology such as organoid nesting, peripheral palisading and rosette formation were not frequent histological features and the majority of tumor cells contained nuclei with a fine chromatin pattern. Mitoses were difficult to find; however, immunohistochemical Ki-67/MIB1 labeling indices were quite useful for evaluating proliferative activity, which ranged from 43.4 to 99.0%.
Conclusions: Our study showed the diagnostic potential of using biopsy specimens for large cell neuroendocrine carcinoma, and we herein proposed more simplified diagnostic criteria for possible large cell neuroendocrine carcinoma in practical diagnostic use.

Key words: large cell neuroendocrine carcinoma – biopsy diagnosis – Ki-67 – neuroendocrine markers – small cell carcinoma

INTRODUCTION

Lung carcinoma is clinically classified into two categories, i.e. small cell lung carcinoma (SCLC) and non-SCLC (NSCLC), with regard to their response to chemoradiotherapy. In 1991, Travis et al. (1) separated a group of high-grade neuroendocrine carcinoma from NSCLC and proposed the new histological category of large cell neuroendocrine carcinoma (LCNEC), which was adopted into the WHO classification in 1999 (2) and retained in the 2004 WHO classification (3). The main criteria for diagnosing LCNEC are: (1) large

cell morphology; (2) high mitotic rate of more than 11 or greater/10 high power fields (HPFs); and (3) the detection of neuroendocrine morphology and immunohistochemical markers (1–3). However, it became clear that the clinical features of LCNEC are similar to those of SCLC, and the differential diagnosis between LCNEC and SCLC is quite difficult in some cases (4,5). The morphological and clinical similarities of these high-grade neuroendocrine carcinomas are problematic for clinicians and pathologists (1,4,5).

Although the role of chemotherapy in LCNEC is uncertain, Iyoda et al. (6) have reported that patients who received adjuvant chemotherapy had a better prognosis than patients who did not receive adjuvant chemotherapy. Moreover, recent advances in chemotherapy have elucidated that platinum-based adjuvant chemotherapy is effective and significantly improves the survival of patients with LCNEC compared with non-platinum-based adjuvant chemotherapy, suggesting that the initial treatment response of LCNEC to chemotherapy might be similar to that of SCLC (7–10). The results of chemotherapy in previous LCNEC studies were obtained by adjuvant chemotherapy after surgery and the diagnosis of LCNEC was made by using resected surgical materials. Not only for resectable LCNEC, but also for non-resectable LCNEC the pathological diagnosis should be made for selecting an appropriate chemotherapy. However, for the diagnosis of LCNEC in a biopsy specimen, the criteria of LCNEC cannot fit because, for example, the mitotic rate cannot necessarily be counted in 10 HPFs in a small necrosis-rich and/or crushed specimen. Thus, for the tumors that can possibly be LCNEC, another criterion for the diagnosis of possible LCNEC is required. Therefore, we focused attention on unresectable high-grade neuroendocrine carcinoma of the lung and aimed to perform a retrospective comparative study in order to know the chemotherapy response of LCNEC and SCLC (9). This comparative study used biopsy specimens instead of surgical specimens to diagnose LCNEC correctly and to differentiate LCNEC from SCLC. For this purpose, we modified the diagnostic criteria of LCNEC proposed by Travis et al. (1) and proposed new diagnostic criteria of high-grade non-small cell neuroendocrine carcinoma (HNSCNEC), which likely includes most LCNEC (9).

Using our criteria of HNSCNEC, at least two papers have been published so far and it was concluded that the efficacy of chemotherapy for unresectable LCNEC is comparable with that of SCLC (9,10). In this study, we morphologically reviewed all lung biopsy specimens obtained from our hospital from 2002 to 2009 and aimed to improve the criteria of HNSCNEC for better practice with using biopsy specimens and to estimate the true frequency of LCNEC in lung cancer.

PATIENTS AND METHODS

Biopsy Samples

From September 2002 to December 2009, transbronchial lung biopsy was performed on 1566 patients with lung tumor at Shizuoka Cancer Center Hospital. Using hematoxylin and eosin (H&E)-stained paraffin sections of these biopsy specimens, we histologically reviewed their diagnosis. Of 1566 biopsy cases, 1040 were evaluated to have carcinoma tissue, with a diagnosis of adenocarcinoma (518 cases), squamous cell carcinoma (318 cases), adenosquamous carcinoma (6 cases), SCLC (121 cases), large cell carcinoma (24 cases) and pleomorphic carcinoma (15 cases). Finally, we selected 38 HNSCNECs according to our criteria (9). Selected from the criteria shown in Table 1 (9), we used the following as essential conditions: no differentiation to squamous cell carcinoma or adenocarcinoma, positive immunostaining for at least one of neuroendocrine markers, large nuclear size with moderate or marked nuclear atypia, a Ki-67/MIB1 labeling index higher than 40%, and nuclear features (fine chromatin and/or prominent nucleoli) or one of the features of neuroendocrine morphology such as organoid nesting, peripheral palisading, rosettes and/or trabecular architecture.

We excluded poorly differentiated adenocarcinomas by positive periodic acid-schiff-alcian blue mucin staining after diastase digestion. We also excluded squamous cell carcinomas by p63 and keratin 5/6 immunopositivity. The immunohistochemical Ki-67 labeling index was the indicator of high-grade malignancy. Neuroendocrine carcinoma was defined by at least one positive neuroendocrine marker of chromogranin A, synaptophysin and neural cell adhesion molecule (NCAM) and by the neuroendocrine morphology.

Clinical information of these 38 patients was obtained from patients' records (Table 2).

Table 1. Applied criteria of high-grade non-small cell neuroendocrine carcinoma (HNSCNEC)

1. Solid tumor nesting without either acinar or squamous differentiation
2. Moderate or marked cellular atypia
3. Large cell size with low nuclear/cytoplasmic ratio or abundant cytoplasm
4. Vesicular and/or fine nuclear chromatin
5. Frequent nucleoli
6. Positive immunostaining for one or more neuroendocrine markers (NCAM, chromogranin A and synaptophysin)
7. Ki-67/MIB1 labeling index >40%
8. Frequent mitosis
9. Frequent massive necrosis
10. Intercellular space (cleft) with loose intercellular adhesion
11. Organoid nesting, basal palisading, rosettes and/or trabecular architecture

Proposed criteria for diagnosis of HNSCNEC using biopsy specimens (Table 2 of ref. (9)).

Immunohistochemistry

For immunostaining, 3 μm-thick sections were prepared from the formalin-fixed and paraffin-embedded tumor specimens. After deparaffinization and blocking of endogenous

Table 2. Clinical features of 38 biopsy cases used in this study

Case number	Age	Gender	Smoking	Tumor size on CT (cm)	Tumor margin on CT	Tumor location	Site of biopsy	T	N	M	Stage	Initial pathological diagnosis	Surgery	Status of patients (months)
1	74	Male	Yes	3.6 × 2.0	Non-lob	p	rt M	1	0	0	IA	LCNEC	Yes	NED (73)
2	70	Male	Yes	3.1 × 2.8	lob	p	lt U	2	0	0	IB	LCNEC	Yes	DOD (68)
3	63	Male	Yes	4.0 × 3.0	Non-lob	p	rt M	2	0	0	IB	Poorly diff. carcinoma	Yes	NED (5)
4	82	Male	Yes	3.8 × 2.8	lob	p	lt U	2	0	0	IB	High-grade NE carcinoma	Yes	AWD (17)
5	56	Male	Yes	3.8 × 3.6	lob	p	lt U	2	1	0	IIB	High-grade NE carcinoma	Yes	NED (14)
6	64	Male	Yes	2.4 × 1.9	lob	p	lt L	3	2	0	IIIA	High-grade NE carcinoma	Yes	NED (8)
7	57	Male	Yes	3.5 × 3.5	Non-lob	c	rt U	4	3	0	IIIB	Adenocarcinoma	No	NED (61)
8	72	Male	Yes	2.5 × 1.8	lob	p	rt L	1	3	0	IIIB	Poorly diff. adenocarcinoma	No	DOD (9)
9	67	Male	Yes	4.5 × 4.5	Non-lob	p	lt L	4	3	1	IV	Combined SCLC	No	DOD (8)
10[a]	71	Male	Yes	7 × 6 × 4.5	lob	p	lt L	2	1	1	IV	Non-small cell NE carcinoma	No	DOD (11)
11[a]	74	Male	Yes	3.9 × 2.7	Non-lob	c	rt U	2	3	1	IV	High-grade NE carcinoma	No	DOD (4)
12	75	Male	Yes	2.0 × 1.8	Non-lob	p	lt U	1	2	1	IV	LCNEC	No	NED (62)
13[a]	75	Male	Yes	4.5 × 3.6	lob	c	lt U	2	3	1	IV	LCNEC	No	DOD (3)
14	66	Male	Yes	4.8 × 2.6	Non-lob	p	lt L	2	2	1	IV	High-grade NE carcinoma	No	DOD (7)
15[a]	63	Male	Yes	2.5 × 2.1	Non-lob	p	rt U	1	3	1	IV	SCLC	No	DOD (17)
16	84	Male	Yes	8.7 × 5.0	lob	p	rt L	2	1	1	IV	LCNEC	No	LTF (3)
17	67	Male	Yes	2.6	Non-lob	p	rt U	4	0	1	IV	LCNEC	No	DOD (2)
18[a]	74	Male	Yes	6.5 × 6.0	lob	p	rt U	4	3	1	IV	High-grade NE carcinoma	No	DOD (9)
19	74	Male	Yes	7.5 × 6.0	lob	p	rt U	2	2	1	IV	LCNEC	No	DOD (3)
20	62	Female	Yes	5.3 × 4.3	Non-lob	p	rt L	2	3	1	IV	Large cell carcinoma	No	DOD (7)
21	70	Male	Yes	7.0 × 3.5	lob	p	rt L	4	3	1	IV	Large cell carcinoma	No	DOD (7)
22	76	Male	No	3.0 × 2.3	Non-lob	c	lt L	1	3	1	IV	Combined SCLC	No	DOD (11)
23	59	Male	Yes	8.0 × 5.5	Non-lob	c	rt U	4	3	1	IV	High-grade NE carcinoma	No	DOD (12)
24	73	Male	Yes	2.3 × 2.0	lob	p	rt U	4	0	0	IV	Poorly diff. adenocarcinoma	No	LTF (22)
25	64	Male	Yes	3.5 × 3.4	lob	c	lt L	4	3	1	IV	LCNEC	No	DOD (11)
26	77	Female	Yes	3.8 × 3.0	lob	p	lt L	3	1	1	IV	Large cell carcinoma	No	DOD (20)
27	62	Male	Yes	Unknown	Non-lob	c	rt MB	TX	NX	1	IV	High-grade NE carcinoma	No	LTF (1)
28	89	Male	Yes	4.7 × 3.7	Non-lob	p	rt M	2	3	1	IV	Large cell carcinoma	No	LTF (1)
29	67	Male	Yes	6.2 × 3.6	Non-lob	c	rt U	4	3	1	IV	High-grade NE carcinoma	No	DOD (3)
30	81	Male	No	Unknown	Non-lob	c	lt MB	1	0	0	IV	Large cell carcinoma	No[b]	DOD (16)
31	59	Female	Yes	1.5 × 1.3	lob	c	lt U	1	3	1	IV	LCNEC	No	DOD (7)
32	63	Male	Yes	5.2 × 5.0	lob	p	rt U	4	3	1	IV	Carcinoma	No	DOD (16)
33	69	Male	No	5.5 × 3.2	lob	p	lt U	4	2	1	IV	Large cell carcinoma	No	AWD (31)
34	65	Male	Yes	4.7 × 4.1	lob	p	lt U	4	1	1	IV	LCNEC	No	LTF (17)
35	76	Male	Yes	14.4 × 8.5	Non-lob	c	lt U	4	3	1	IV	High-grade NE carcinoma	No	DOD (2)
36	64	Male	Yes	9.6 × 4.6	Non-lob	c	lt L	4	3	1	IV	Poorly diff. carcinoma	No	DOD (22)
37	65	Female	Yes	2.4 × 1.5	lob	p	lt U	1	3	1	IV	LCNEC	No	DOD (17)
38	67	Male	Yes	6.1 × 4.9	lob	p	rt L	4	3	1	IV	High-grade NE carcinoma	No	DOD (2)

CT, computed tomography; non-lob, non-lobulated margin; p, peripherally located; rt, right; M, middle lobe; LCNEC, large cell neuroendocrine carcinoma; NED, no evidence of disease; lob, lobulated margin; lt, left; U, upper lobe; DOD, dead of disease; NE, neuroendocrine; AWD, alive with disease; L, lower lobe; c, centrally located; SCLC, small cell lung cancer; LTF, lost to follow-up; MB, main bronchus; TX, primary tumor cannot be assessed by imaging; NX, regional lymph nodes cannot be assessed.

[a]The case was examined in ref. (9).

[b]Patient with a history of lung cancer resection 7 years before.

peroxidase activity by 0.3% hydrogen peroxide in methanol, antigen retrieval was carried out using the conventional autoclave method (for 10 min at 121°C) with 0.01 M sodium citrate buffer (pH 6.0), if necessary. The details of the primary antibodies were as follows: NCAM (clone NCC-Lu-243; Nippon Kayaku, Tokyo, ×200), chromogranin A (code No. A0430; DAKO, Glostrup, Denmark, ×5000), synaptophysin (Cat No. 261-01; SIGNET, Dedham, MA, USA, ×100), Ki-67/MIB1 (clone MIB1, code No. M7240; DAKO, ×100), p63 (clone 4A4, Cat No. MS-1081-P; Lab Vision, Kalamazoo, MI, USA, ×500) and keratin 5/6 (code No. M7237; DAKO, ×100). The sections were incubated with the primary antibody for 30 min at 37°C and followed by the Dako EnVision+® detection system (code K4001; Dako Cytomation North America, Inc., CA, USA) and diaminobenzidine as the chromogen to visualize the antigens according to the manufacture's instructions. Negative control slides of each case without first antibody reaction and positive control slides of other normal organ tissue for each antibody were always used and stained simultaneously.

Histological Comparison Between Biopsy and Resected Specimens of Six Resected Cases

Six cases were resected after biopsy because they were in the early stages. We compared the histological findings of these six pairs of biopsy and surgical specimens.

RESULTS

Clinicopathological Findings

Clinicopathological information about the 38 HNSCNEC cases used in this study is shown in Table 2. By computed tomography (CT) imaging of these tumors, 20 tumors (52.6%) showed solid masses with a sharply lobulated margin and 26 tumors (68.4%) were located in the periphery of the lung. The patients' average age was 69.4 (range 56–89 years). Men predominated (M:F = 34:4) and 35 out of 38 patients were smokers (92.1%). Thirty patients (78.9%) were clinically at Stage IV at diagnosis and 32 (84.2%) were unresectable. Sixteen patients (42.1%) had intrapulmonary metastasis and 23 (60.5%) had distant metastasis at the time of diagnosis, in the brain (13 patients), bone (12 patients), liver (8 patients) and adrenal gland (6 patients). Six tumors at clinical TNM Stage I, II or IIIA were surgically resected and pathologically diagnosed as LCNEC from surgical specimens.

In 38 HNSCNEC cases, serum pro-gastrin releasing peptide (pro-GRP; normal value <46.0 pg/ml), carcinoembryonic antigen (normal value <5.0 ng/ml) and neuron-specific enolase (normal value <10.0 ng/ml) levels were elevated in 13 cases (13/37, 35.1%), 24 cases (24/37, 64.9%) and 27 cases (27/38, 71.1%), respectively.

Table 3. Morphological analysis of biopsies in 38 patients, of which 6 were resected and a diagnosis of LCNEC was made

Histological findings	Number of biopsies (%)	Number of biopsies in operated cases (%)
Fine nuclear chromatin	30 (78.9)	6 (100)
Intercellular clefts	21 (55.3)	4 (66.7)
Prominent nucleoli	18 (47.4)	2 (33.3)
Massive necrosis	17 (44.7)	2 (33.3)
Organoid nesting	14 (36.8)	1 (16.7)
Peripheral palisading	6 (15.8)	1 (16.7)
Rosette formation	6 (15.8)	1 (16.7)
Trabecular arrangement	5 (13.2)	0 (0)

Histological and Immunohistochemical Analyses

Initial pathological diagnoses of 38 HNSCNEC biopsy specimens were shown in Table 2. Eleven cases were finally diagnosed as LCNEC, and non-small cell neuroendocrine carcinoma (case No. 10) was most likely to be LCNEC. Eleven cases were given the diagnosis of high-grade neuroendocrine carcinoma, which seemed to be difficult to differentiate from SCLC. On the other hand, only three cases were diagnosed as SCLC including the combined type. Eleven cases were poorly differentiated carcinoma including large cell carcinoma and adenocarcinoma. These results indicated that two-thirds of HNSCNEC biopsy cases suggested to the pathologists the diagnosis of high-grade neuroendocrine carcinoma and one-third was histologically beyond the scope of neuroendocrine carcinoma.

All HNSCNECs were composed of NSCLC with large nuclei, three times larger than resting lymphocytes and abundant cytoplasm or a low nuclear/ cytoplasmic ratio. As shown in Table 3, morphological architectural characteristics of LCNEC proposed by Travis et al. (1) were reviewed in the 38 biopsy specimens used in this study. With regard to neuroendocrine morphology, organoid nesting (Fig. 1a) was the most frequently observed structure, but its frequency was only 36.8% of all specimens. Peripheral palisading, rosette formation (Fig. 1b) and trabecular arrangement were observed in only 6 (15.8%), 6 (15.8%) and 5 (13.2%) specimens, respectively. In other morphological characteristics of LCNEC, massive necrosis (Fig. 1c) was seen in 17 biopsy specimens (44.7%). Although most tumor cells contained nuclei with a fine chromatin pattern (Fig. 1d) and faint or visible nucleoli, prominent nucleoli (Fig. 1d, arrows) were observed in about half of all specimens (47.4%). Again, in about half of all biopsy specimens (55.3%), tumor cells had a distinct cell border with intercellular clefts and were often discohesive (Fig. 1b and d).

We counted mitotic figures in all 38 tumor specimens. Among them, 11 tumors lacked enough area for mitotic counting, that is to say, 9 HPFs or less. There were 7 tumors

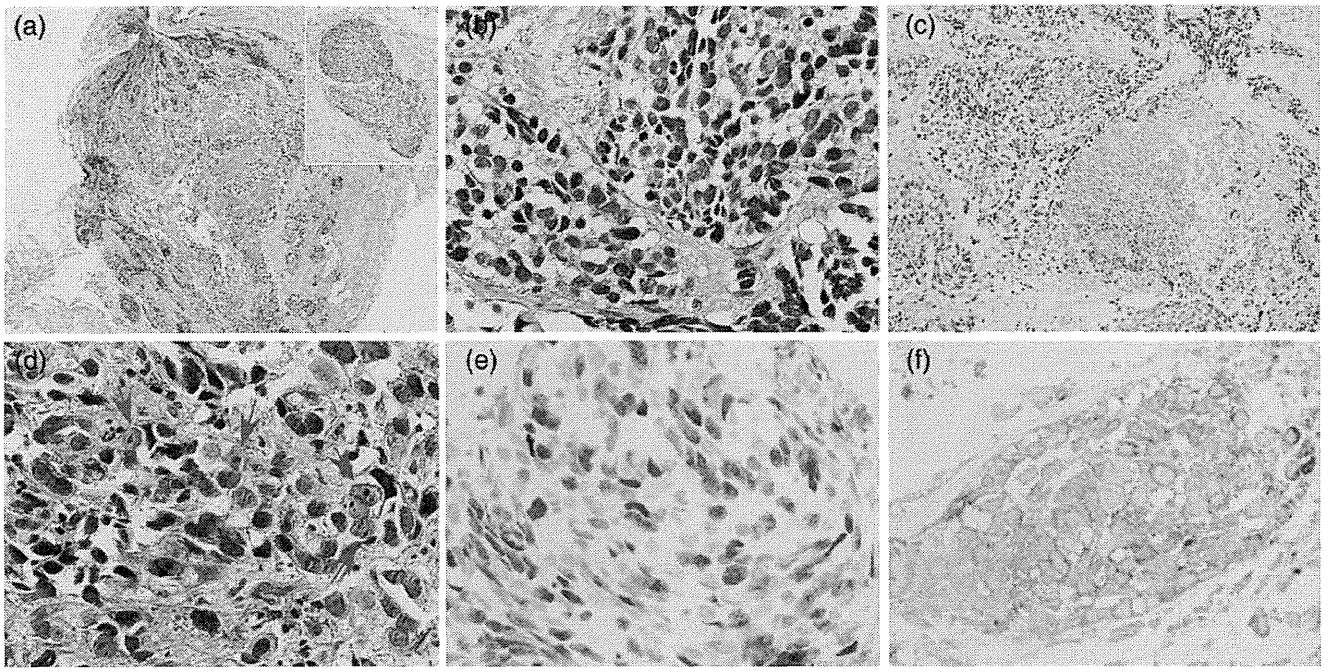

Figure 1. Characteristic morphological features of 38 biopsy specimens used in this study. In the low-powered view [a, hematoxylin and eosin (H&E), ×4], organoid nesting (a, inset) and massive necrosis (c, H&E, ×10) are recognized structure features in the biopsy specimen. In the high-powered view, tumor cells reveal special arrangements such as rosette formation (b, H&E, ×40), intercellular cleft (b) as well as nuclear characteristics such as a fine chromatin pattern (d, H&E, ×40) and prominent nucleoli (d, arrows, H&E, ×40). The immunohistochemistry shows high nuclear positivity of Ki-67 (e, ×40) and membrane positivity of the neural cell adhesion molecule (NCAM) (f, ×40) in the majority of biopsy specimens.

in which no mitotic figure was found, and another 7 tumors which had a high mitotic rate consistent with 11 or greater per 10 HPFs. In contrast to the mitotic counting, Ki-67/MIB1 labeling indices could be evaluated in all 38 tumor specimens (Fig. 1e), which ranged from 41.9 to 99.0% (median: 80.5%). The 7 biopsy specimens with 11 or more mitoses/10 HPFs showed high Ki-67/MIB1 labeling indices from 64.5 to 98.0% (median: 87.3%).

The results of the neuroendocrine immunophenotype in 38 HNSCNECs are shown in Table 4. NCAM (Fig. 1f) was the most frequently observed neuroendocrine marker (78.9%) among them. There were 12 (31.6%) triple marker-positive cases. No tumor was positive for chromogranin A alone.

As shown in Table 2, six patients underwent surgery after biopsy, and all resected tumors were pathologically examined and diagnosed as LCNEC. In case No. 1 (Table 2), for example, the biopsied specimen showed organoid nesting (Fig. 2a) and rosette formation, and tumor cells had abundant cytoplasm and crushed nuclei with a fine chromatin pattern (Fig. 2b). The immunohistochemistry elucidated NCAM (Fig. 2c) in addition to a high Ki-67/MIB1 labeling index (Fig. 2d). This biopsied tumor was pathologically diagnosed as strongly suggestive of LCNEC. As this tumor was peripherally located and clinically showed T1N0M0 (Stage IA), a right middle lobectomy was carried out after biopsy. Morphologically, the resected tumor was composed of large tumor cells with abundant cytoplasm, and showed fine or vesicular nuclei with prominent nucleoli and frequent mitoses (Fig. 3a and b). There was a necrotic area and tumor cells formed characteristic intercellular clefts and rosettes. Immunohistochemical results were almost identical to those of the biopsy specimen. Finally, the diagnosis of LCNEC was confirmed for this tumor.

Table 4. Distribution of immunohistochemical results of neuroendocrine markers in 38 biopsy specimens

Positively stained markers	Number of positive biopsy specimens (%)
NCAM	30 (78.9)
NCAM, SYN, CGA	12 (31.6)
NCAM, SYN	3 (7.9)
NCAM, CGA	1 (2.6)
NCAM alone	14 (36.8)
SYN	23 (60.5)
SYN, CGA	5 (13.2)
SYN alone	3 (7.9)
CGA	18 (47.4)
CGA alone	0 (0)

NCAM, neural cell adhesion molecule; SYN, syanptophysin; CGA, chromogranin A.

Histological details of six biopsy specimens with a confirmed diagnosis of LCNEC are shown in Tables 3 and 5. Although the sample size was small, morphological characteristics showed a similar tendency to those of other