nitrogen stream at approximately 40°C to remove the solvent. The residue was suspended in ammonium acetate aqueous solution (containing formic acid)/acetonitrile (for aprepitant) or methanol/water (for dexamethasone) for use in LC/MS/MS.

The pharmacokinetic parameters of aprepitant and dexamethasone were calculated by non-compartment analysis using WinNonlin Professional® software Ver.4.0.1 (Pharsight Corporation, Mountain View, CA, USA). The maximum plasma concentration (C_{max}), time to maximum plasma concentration (t_{max}), and area under the plasma concentration–time curve from 0 to 24 h post-dose ($AUC_{0-24\ h}$) were calculated for aprepitant, and the C_{max}, area under the plasma concentration–time curve from 0 to infinity ($AUC_{0-\infty}$), $t_{1/2}$, total clearance (CL_{tot}), and volume of distribution at steady state (V_{ss}) were calculated for dexamethasone.

Assessment of substance P

Before administration of aprepitant on days 1–5, venous blood was collected in an ethylenediaminetetraacetic acid (EDTA)/aprotinin-treated tube from each subject and inverted to mix. After blood was immediately centrifuged at 1,500*g* (approximately 3,000 rpm) for 10 min at 4°C, 0.5 mL of plasma was stored frozen at –20°C. The plasma substance P concentration was measured by enzyme immunoassay (EIA).

Statistical analysis

To assess ethnic differences in the pharmacokinetics of aprepitant, the C_{max} and $AUC_{0-24\ h}$ at a dose of 125 mg in Japanese cancer patients were compared with those in non-Japanese cancer patients [12] by calculating the geometric mean ratio (Japanese/non-Japanese) and its 90% confidence interval for each parameter.

To assess the validity of adjusting the dose of dexamethasone in the 125/80 and 40/25 mg groups, the exposure levels of dexamethasone were compared. The C_{max} and $AUC_{0-\infty}$ of dexamethasone in each group were used to calculate the geometric mean ratio (125/80 mg group/40/25 mg group) and its 90% confidence interval for each parameter. In addition, the clearance of dexamethasone in each group was compared with that calculated in the absence of aprepitant in Japanese cancer patients [10].

For substance P, the plasma concentration on each measurement day was used to assess the change on day 2 and thereafter, and these changes were evaluated by paired *t* test.

This study was designed and funded by Ono pharmaceutical Co., Ltd. and Merck & Co., Inc., the manufacturer of aprepitant.

Results

Patients

A total of 20 patients (10 in the 125/80 mg group and 10 in the 40/25 mg group) were included. Patients' characteristics are shown in Table 1. There were 18, 1, and 1 patients with non–small cell lung cancer, small-cell lung cancer, and mesothelioma, respectively. All were treated with at least the highly emetogenic chemotherapeutic agent cisplatin ($\geq$70 mg/m^2). The two groups were generally similar in age, sex, height, and body weight.

Pharmacokinetics of aprepitant

All 20 enrolled patients were included in the pharmacokinetic analysis. The pharmacokinetic parameters of aprepitant are shown in Table 2. In the 125/80 mg group, the $AUC_{0-24\ h}$ on days 1 and 5 increased out of proportion to the dose, compared with that in the 40/25 mg group.

The geometric mean ratio and its 90% confidence interval (CI) of the C_{max} and $AUC_{0-24\ h}$ of aprepitant in Japanese cancer patients to non-Japanese cancer patients was 1.09 (0.79–1.52) and 1.12 (0.87–1.45), respectively,

Table 1 Characteristics of patients

Characteristics	125/80 mg regimen $n = 10$	40/25 mg regimen $n = 10$
Male/female (*N*)	6/4	7/3
Age (years)		
Mean (S.D.)	59.7 (6.7)	63.6 (5.9)
Range	47–71	55–72
Height (cm)		
Mean (S.D.)	161.16 (9.91)	161.24 (12.97)
Range	147.0–179.5	139.2–177.3
Weight (kg)		
Mean (S.D.)	55.72 (10.28)	56.86 (14.17)
Range	42.2–76.6	42.4–82.7
Primary cancer diagnosis (*N*)		
Non–small cell lung cancer	9	9
Small-cell lung cancer	1	0
Mesothelioma	0	1
Chemotherapy regimen (*N*)		
Cisplatin + gemcitabine	3	5
Cisplatin + tegafur/gimeracil/oteracil	2	2
Cisplatin + vinorelbine	2	2
Cisplatin + etoposide	2	0
Cisplatin + docetaxel	1	1

Table 2 Summary of the pharmacokinetics of aprepitant on days 1 and 5

Day	Parameter	125/80 mg regimen	40/25 mg regimen
1	C_{max} (ng/mL)	2,210 ± 870	536 ± 105
	T_{max} (h)	7.0 (3.0–9.0)	3.0 (2.0–9.0)
	$AUC_{0-24\ h}$ (ngh/mL)	30,000 ± 8,700	6,360 ± 1,350
5	C_{max} (ng/mL)	3,070 ± 850	453 ± 109
	T_{max} (h)	3.0 (2.0–9.0)	3.0 (2.0–3.0)
	$AUC_{0-24\ h}$ (ngh/mL)	46,000 ± 17,100	5,420 ± 1,680

Mean ± SD, T_{max} median (range)

C_{max}, maximum plasma concentration, T_{max}, time to maximum plasma concentration, $AUC_{0-24\ h}$ area under plasma concentration–time curve from 0 to 24 h post-dose

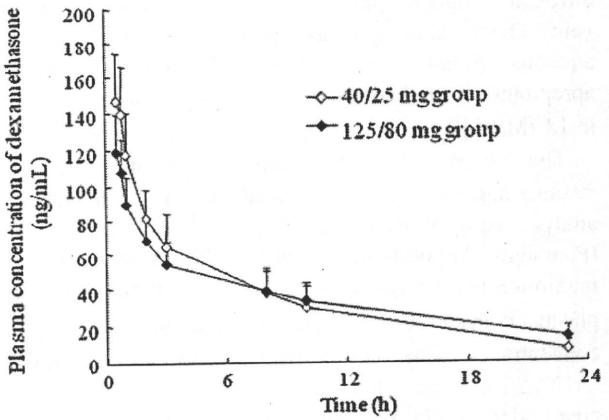

Fig. 1 Time profile of plasma dexamethasone concentration in each treatment group (day 1). Mean + SD ($n = 10$)

showing little differences between Japanese and non-Japanese groups in the pharmacokinetics of aprepitant.

Pharmacokinetics of dexamethasone

For dexamethasone, the pharmacokinetic parameters and time profile of plasma concentration on day 1 in the 125/80 and 40/25 mg groups are shown in Table 3 and Fig. 1, respectively. The geometric mean ratio (90% CI) of C_{max} and $AUC_{0-\infty}$ of dexamethasone on day 1 in the 125/80 mg group to the 40/25 mg group was 0.83 (0.73–0.94) and 1.15 (0.88–1.50), respectively, showing that although the C_{max} tended to be high in the 40/25 mg group, the $AUC_{0-\infty}$was similar between the two treatment groups. To verify the dose reduction of dexamethasone in cancer patients who receive emetogenic cancer chemotherapy in combination with aprepitant, we compared the clearances of dexamethasone in this study with that obtained from Japanese cancer patients in the absence of aprepitant at a dose of 12 mg on day 1 (13.3 L/h) [10]. In the 125/80 and 40/25 mg groups (dexamethasone at a dose of 6 and 8 mg on day 1, respectively), the clearance of dexamethasone was 6.48 and 10.0 L/h, respectively. That is, the clearances of dexamethasone in the 125/80 and 40/25 mg groups decrease by approximately 52 and 25%, respectively. These results demonstrate the validity of reducing the dose of dexamethasone by 50 and 25% in the 125/80 and 40/25 mg groups, compared with the dose of dexamethasone in the absence of aprepitant.

Table 3 Pharmacokinetic parameters of dexamethasone in each treatment group (on day 1)

Parameter	125/80 mg regimen (Dexamethasone 6 mg)	40/25 mg regimen (Dexamethasone 8 mg)
C_{max} (ng/mL)	121 ± 17	147 ± 27
AUC_{0-t} (ngh/mL)	823 ± 213	838 ± 253
$AUC_{0-\infty}$ (ngh/mL)	1,020 ± 300	899 ± 287
$t_{1/2}$ (h)	9.6 ± 2.4	5.7 ± 1.4
CL_{tot} (L/h)	6.48 ± 2.50	10.0 ± 4.1
V_{ss} (L)	74.6 ± 14.3	65.5 ± 11.7

Mean ± SD

C_{max} maximum plasma concentration, AUC_{0-t}, area under plasma concentration–time curve from 0 to the last measurable concentration, $AUC_{0-\infty}$, area under plasma concentration–time curve from 0 to infinity, $t_{1/2}$ elimination half-life, CL_{tot} total clearance, V_{ss} volume of distribution at steady state

Evaluation of plasma substance P

The time profile of plasma substance P concentration after administration of chemotherapeutic agents in all 20 patients (days 1–5) is shown in Fig. 2. The substance P

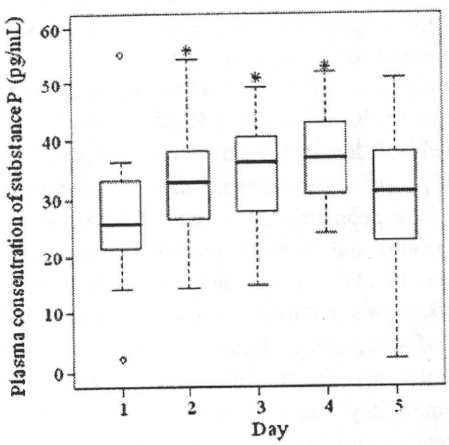

Fig. 2 Time profile of plasma substance P concentration ($n = 20$). *Top bar* highest value in the range of quartile × 1.5; *lower bar* lowest value in the range of quartile × 1.5; *top of box* upper quartile; *bottom of box* lower quartile; middle bar: median value; *circles* outliers $*P < 0.05$ compared with baseline (day 1) concentration

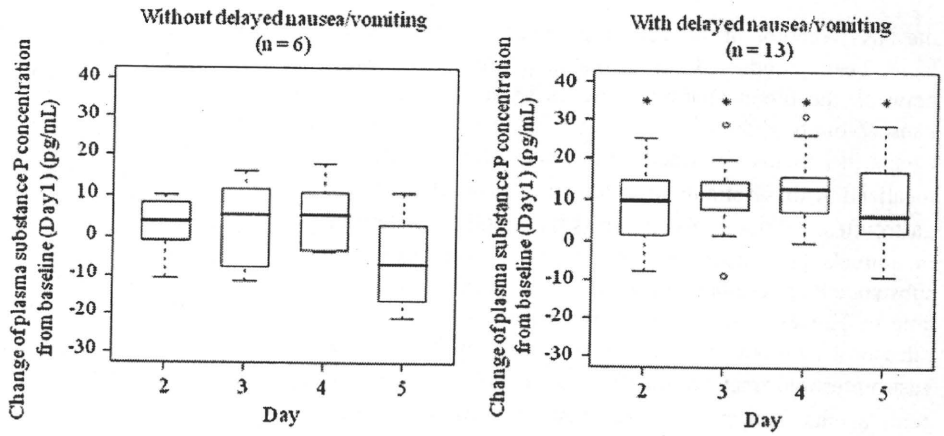

Fig. 3 Change in plasma substance P concentration ($n = 19$). *Top bar* highest value in the range of quartile × 1.5; *lower bar* lowest value in the range of quartile × 1.5; *top of box* upper quartile; *bottom of box* lower quartile; *middle bar* median value; *circles* outliers $^*P < 0.05$ between days 2 and 5 compared with baseline (day 1)

concentration significantly increased on days 2–4, compared with that on day 1 (baseline) ($P < 0.05$, paired t test).

There was no difference in the change in the plasma substance P concentration between the 125/80 and 40/25 mg groups (data not shown). The change in substance P concentration in plasma from baseline (the concentration before the start of treatment with aprepitant) in patients with or without delayed nausea/vomiting is shown in Fig. 3. One patient had missing data for the substance P concentration on day 1, and so we analyzed the change in substance P concentration from baseline (day 1) in 19 patients. In patients with delayed nausea/vomiting, substance P concentration increased significantly between days 2 and 5 compared with baseline (day 1) ($P < 0.05$, paired t test). On the other hand, in patients without delayed nausea/vomiting, the increase in substance P concentration on days 2–5 was not statistically significant.

Discussion

In this study, the pharmacokinetics of aprepitant and dexamethasone were determined in Japanese cancer patients receiving emetogenic chemotherapeutic agents. There were no differences in the pharmacokinetics of aprepitant between Japanese and non-Japanese cancer patients. In addition, we showed the validity of dose adjustment of dexamethasone used in combination with aprepitant (i.e., reducing the dose of dexamethasone by 50% when combined with 125 mg of aprepitant). We also found that the blood concentration of substance P, which is deeply involved in the pharmacological effects of aprepitant, increased after administration of chemotherapeutic agents.

In the present study, the geometric mean ratio of the C_{max} and $AUC_{0-24\ h}$ in Japanese cancer patients to non-Japanese cancer patients was 1.09 and 1.12, respectively, indicating no ethnic differences in the pharmacokinetics of aprepitant. In the aprepitant 125/80 mg group, more than dose-proportional increase in $AUC_{0-24\ h}$ occurred on both days 1 and 5, compared with that in the 40/25 mg group. Aprepitant is primarily metabolized by CYP3A4 [13], and this more than proportional increase in the $AUC_{0-24\ h}$ of aprepitant may reflect saturated metabolism of aprepitant via CYP3A4 as previously reported in healthy non-Japanese volunteers [14].

In this study, granisetron hydrochloride and dexamethasone sodium phosphate were concomitantly used as standard antiemetic therapy. Aprepitant–dexamethasone interaction causes the increase in plasma dexamethasone concentration [9], and it has been suggested that this drug interaction may also cause a slight increase in the incidence of infection-related serious adverse events [15]. Since the AUC of dexamethasone (p.o.) has been shown to increase approximately two times after administration of aprepitant at a dose of 125 mg on day 1 in healthy adults [9], the dose of dexamethasone has to be reduced by 50% when used in combination with 125 mg of aprepitant. While oral dexamethasone was used in the report by McCrea et al. [9], this was the first full pharmacokinetic study of intravenous administration of dexamethasone when used in combination with aprepitant in cancer patients actually receiving chemotherapeutic agents. In the 125/80 mg group, the clearance of intravenous dexamethasone decreased by approximately 52% from that calculated in the absence of aprepitant, justifying a 50% dose reduction of intravenous dexamethasone used in combination with 125 mg of aprepitant in cancer patients as McCrea et al. demonstrated in healthy adults [9]. And the results from this full pharmacokinetic study also supported a report using a population pharmacokinetics model by Nakade et al. [10] that the clearance of intravenous dexamethasone used in combination with aprepitant at a dose of 125 mg decreased by 47.5% of that in the absence of aprepitant.

While aprepitant may exert its antiemetic effect during chemotherapy, by inhibiting the binding of substance P to

the NK_1 receptor in the vomiting center [1], few studies have been conducted to investigate the relationship between the blood pharmacokinetics of substance P and nausea/vomiting during treatment with chemotherapeutic agents in humans. Substance P has been shown to be co-localized with serotonin in enterochromaffin cells in the gastrointestinal tract [16] and cross the blood–brain barrier in animals [17]. These reports raise the possibility that substance P of peripheral origin may act centrally to induce emesis. However, it is still not shown whether exocytotic release of substance P from enterochromaffin cells in the gastrointestinal tract occurs after administration of emetogenic agents. This study showed that the plasma substance P concentration significantly increased on days 2–4 after administration of chemotherapeutic agents. It was also shown that the plasma substance P concentration significantly increased only in patients with delayed nausea/vomiting. These results, as well as the report from Higa [18], support the possibility that the elevation of the plasma substance P concentration by emetogenic chemotherapeutic agents may be involved in the pathogenesis of CINV, especially in the delayed phase. The plasma substance P concentration ranged from 0 to 1,608 pg/mL in a report by Higa et al. [18] and from 2–55 pg/mL in the present study. The cause of this difference is unknown, but may be attributed to different assay kits used to measure the substance P concentration (Higa et al. used R&D systems, and we used Cayman Chemical).

In conclusion, this study demonstrated similar plasma pharmacokinetics of aprepitant in Japanese and non-Japanese, the validity of reducing dexamethasone dose, and the existence of increased substance P concentration in patients receiving highly emetogenic cancer chemotherapy. Further studies are required to clarify whether measurement of the plasma pharmacokinetics of substance P may be a clinically meaningful marker for CINV in patients receiving emetogenic agents.

Conflict of interest None.

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Comparison of chemotherapy for unresectable pulmonary high-grade non-small cell neuroendocrine carcinoma and small-cell lung cancer

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ABSTRACT

Background: Pulmonary large cell neuroendocrine carcinoma (LCNEC) shares several features with small cell lung carcinoma (SCLC). Most histologic diagnoses of LCNEC are currently obtained by surgical specimens. While the diagnosis of LCNEC by biopsy specimens is challenging, a definitive diagnosis of this highly malignant tumor is critical in unresectable cases to determine the optimal therapeutic strategy. The objective of this study was to assess the efficacy of chemotherapy for unresectable high-grade non-small cell neuroendocrine carcinoma (HNSCNEC) called by us, which likely includes most LCNECs except for combined types, and to compare the efficacy of chemotherapy for HNSCNEC, with that for extended disease SCLC (ED-SCLC).

Methods: Between September 2002 and October 2007, we reviewed 14 patients with HNSCNEC, which was defined using biopsy specimens according to histological and immunohistological criteria proposed by us. We simultaneously evaluated the clinical response to the chemotherapy and survival time of the 14 HNSCNEC and 77 ED-SCLC patients.

Results: The chemotherapy regimens in the 14 patients with unresectable HNSCNEC were platinum-based combination regimens or irinotecan or vinorelbine or docetaxel alone. The chemotherapy regimens in the 77 patients with ED-SCLC were platinum-based combination regimens. We assessed an objective response rate, a one-year survival rate, and median survival time as 50% (7/14), 34% and 10 months, respectively, in the 14 HNSCNEC patients, and as 53% (41/77), 48% and 12.3 months, respectively, in the 77 ED-SCLC patients.

Conclusion: The clinical efficacy of chemotherapy for unresectable HNSCNECs, including most LCNECs, is comparable to that for ED-SCLC.

1. Introduction

In the 1970s, pulmonary neuroendocrine tumors were histologically classified into three categories, i.e., carcinoid, atypical carcinoid, and small cell lung carcinoma (SCLC). In 1991, Travis and colleagues proposed a fourth category: pulmonary neuroendocrine tumors. With this classification, large cell neuroendocrine carcinoma (LCNEC) was regarded as an entity distinct from typical carcinoid, atypical carcinoid, and small cell lung carcinoma (SCLC) [1]. In 1999, the World Health Organization (WHO) defined LCNEC as a variant of large cell carcinoma [2].

LCNEC has specific morphological features and a phenotype that identifies it as a neuroendocrine tumor. Several of these characteristics, such as organoid nesting, palisading, rosette-formation, and frequent mitotic figures, can be observed using light microscopy. The nuclei of LCNEC tumor cells can be differentiated from those of small cell carcinoma by the presence of vesicular or fine chromatin and/or frequent nucleoli. To confirm a neuroendocrine phenotype diagnosis, an immunohistochemical analysis using markers such as chromogranin A, synaptophysin, and neural cell adhesion molecule (NCAM) is required.

At present, most LCNECs are diagnosed using surgically resected specimens and rarely or never using biopsy or cytology specimens. Almost all publications concerning resected LCNECs are based on retrospective analyses of surgical specimens [3–7]. The incidence of the pre-therapeutic diagnosis of LCNEC in unresectable cases is unknown. Therefore, the overall clinico-pathological features of

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Table 1
Criteria for diagnosis of large cell neuroendocrine carcinoma (1999, WHO [2]).

1. A tumor with a neuroendocrine morphology (organoid nesting, palisading, rosettes, trabeculae)
2. High mitotic rate: 11 or greater per 2 mm^2 (ten HPFa), a median of 70 per 2 mm^2 (ten HPFa)
3. Necrosis (often large zones)
4. Cytologic features of a NSCLC: large cell size, low nuclear to cytoplasmic ratio, vesicular or fine chromatin, and/or frequent nucleoli. Some tumors have fine nuclear chromatin and lack nucleoli, but qualify as NSCLC because of large cell size and abundant cytoplasm
5. Positive immunohistochemical staining for one or more NE markers (other than neuron specific enolase) and/or NE granules by electron microscopy

HPFa: high power field; NSCLC: non-small cell lung carcinoma.

Table 2
Proposed criteria for diagnosis of high-grade non-small cell neuroendocrine carcinoma (HNSCNEC) 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 abundant cytoplasm
4. Vesicular and/or fine nuclear chromatin
5. Frequent nucleoli
6. Positive immunostaining for one or more neuroendocline markers (NCAM, chromogranin A, and synaptophysin)
7. Ki-67/MIB1 labeling index > 40% [10,11]
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

LCNEC have not yet been completely defined. In the case of surgical specimens, diagnostic criteria for LCNEC have been established and are described in Table 3 of "Introduction of Histological Typing of Lung and Pleural Tumours" (Third Edition, Springer, 1999) [2] (reprinted in Table 1). For small biopsy specimens, however, a diagnosis of LCNEC that fully meets the criteria described in Table 1 is often difficult. Therefore, instead of diagnosing LCNEC, we have devised and proposed a set of criteria for diagnosing high-grade non-small cell neuroendocrine carcinoma (HNSCNEC) using small biopsy specimens (Table 2), based on the conventional criteria for LCNEC (Table 1). The first seven items are obligatory criteria, and the latter four items are facultative. Our HNSCNEC classification likely includes most LCNECs and large cell carcinomas with a neuroendocrine phenotype. The examination of a larger series of surgically resected LCNECs, along with preoperative biopsy specimens, would enable the validity of diagnoses of HNSCNEC based on biopsy specimens to be confirmed. Although we believe that HNSCNEC and LCNEC are, by definition, similar, evidence of such similarities is not yet available. As previous studies have reported that the response of LCNECs to chemotherapy is similar to the response of small cell carcinoma, rather than the response of NSCLCs [8,9], we compared the chemotherapeutic responses of HNSCNECs and ED-SCLCs in the present study.

2. Patients and methods

2.1. Criteria for diagnosing HNSCNEC (Figs. 1 and 2)

The criteria proposed for diagnosing high-grade non-small cell neuroendocrine carcinoma (HNSCNEC) using biopsy specimens were as follows (Table 2): (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 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/or synaptophysin), (7) Ki-67/MIB1 labeling index > 40% [10,11], (8) frequent mitosis, (9) frequent massive necrosis, (10) intercellular space (cleft) with loose intercellular adhesion, and (11) organoid nesting, basal palisading, rosettes, and/or trabecular architecture. When findings (1) to (7) were observed in a biopsy specimen, the patient was diagnosed as having a large cell carcinoma (with solid tumor nesting without either acinar or squamous differentiation, moderate or marked cellular atypia, large cell size with low nuclear to cytoplasmic ratio or abundant cytoplasm, vesicular and/or fine nuclear chromatin, and frequent nucleoli). Positive neuroendocrine markers confirmed a neuroendocrine nature. Moreover, a Ki-67/MIB1 labeling index > 40% indicated a high-grade tumor [10,11]. Tumors meeting these criteria were diagnosed as HNSCNEC and were most likely either LCNECs or a related tumor. The presence of one or more of findings (8), (9), and/or (11), which are included in the 1999 WHO LCNEC criteria (Table 1), were regarded to reinforce the possibility of LCNEC, although these findings are often absent in small transbronchial biopsy specimens. Transthoracic core aspiration biopsy specimens and specimens from metastatic lesions are usually large enough to enable a diagnosis based on the WHO LCNEC criteria. Finding (10) (intercellular space (cleft) with loose intercellular adhesion) is very common and enables LCNEC to be distinguished from classical large cell carcinoma, as described in our previous paper [3] discussing surgical cases of LCNEC. Thus, the first seven findings in Table 2 must always be present for the diagnosis of HNSCNEC, which likely includes LCNECs and large cell carcinoma with a neuroendocrine phenotype, although combined LCNECs, including combined small cell carcinoma and LCNEC and combined LCNEC and adenocarcinoma/squamous cell carcinoma/classical large cell carcinoma [2], may not be diagnosable using small biopsy specimens since one component of the combined histology can be easily missed.

Although there are no previous reports which compared the HNSCNECs in preoperative or pretreatment biopsy specimens with the diagnosis in surgical specimens or in autopsy, we have, for the present, six surgically resected cases, in which HNSCNEC had been diagnosed by biopsy before treatment.

2.2. Immunohistochemistry

Formalin-fixed paraffin sections were stained using a panel of neuroendocrine markers including an anti-neural cell adhesion molecule (NCAM) antibody (Zymed Technology Invitrogen, South San Francisco, CA), a polyclonal anti-chromogranin A antibody (DAKO, Glostrup, Denmark), and a monoclonal anti-synaptophysin antibody (SIGNET, Denver, CO). Neuroendocrine differentiation was identified by positive immunohistochemical staining for one or more of NCAM, chromogranin A, and synaptophysin. Immunostaining for each neuroendocrine marker was judged as positive when over 30% of the tumor cells were stained. The anti-human Ki-67 antigen was identified by use of a monoclonal mouse anti-human Ki-67 (clone MIB1) antigen (code No. M7240, DAKO Cytomation Denmark A/S). Only nuclear immunostaining was regarded as positive. The labeling index of the Ki-67/MIB1 in each tumor was estimated as percentage of positive cells by counting of 100–1000 tumor cells. All immunostaining results were determined by the consensus of at least two observers of R.W., II and T.K.

2.3. Patient selection

A total of 14 patients with a histologic diagnosis of pulmonary HNSCNEC made between September 2002 and October 2007 were enrolled in this retrospective study. As well, a total of 77 patients with histologically and clinically confirmed ED-SCLC who had received a platinum-based chemotherapy regimen such as cisplatin/irinotecan, cisplatin/etoposide, or carboplatin/etoposide were enrolled. None of the patients had received prior radiotherapy

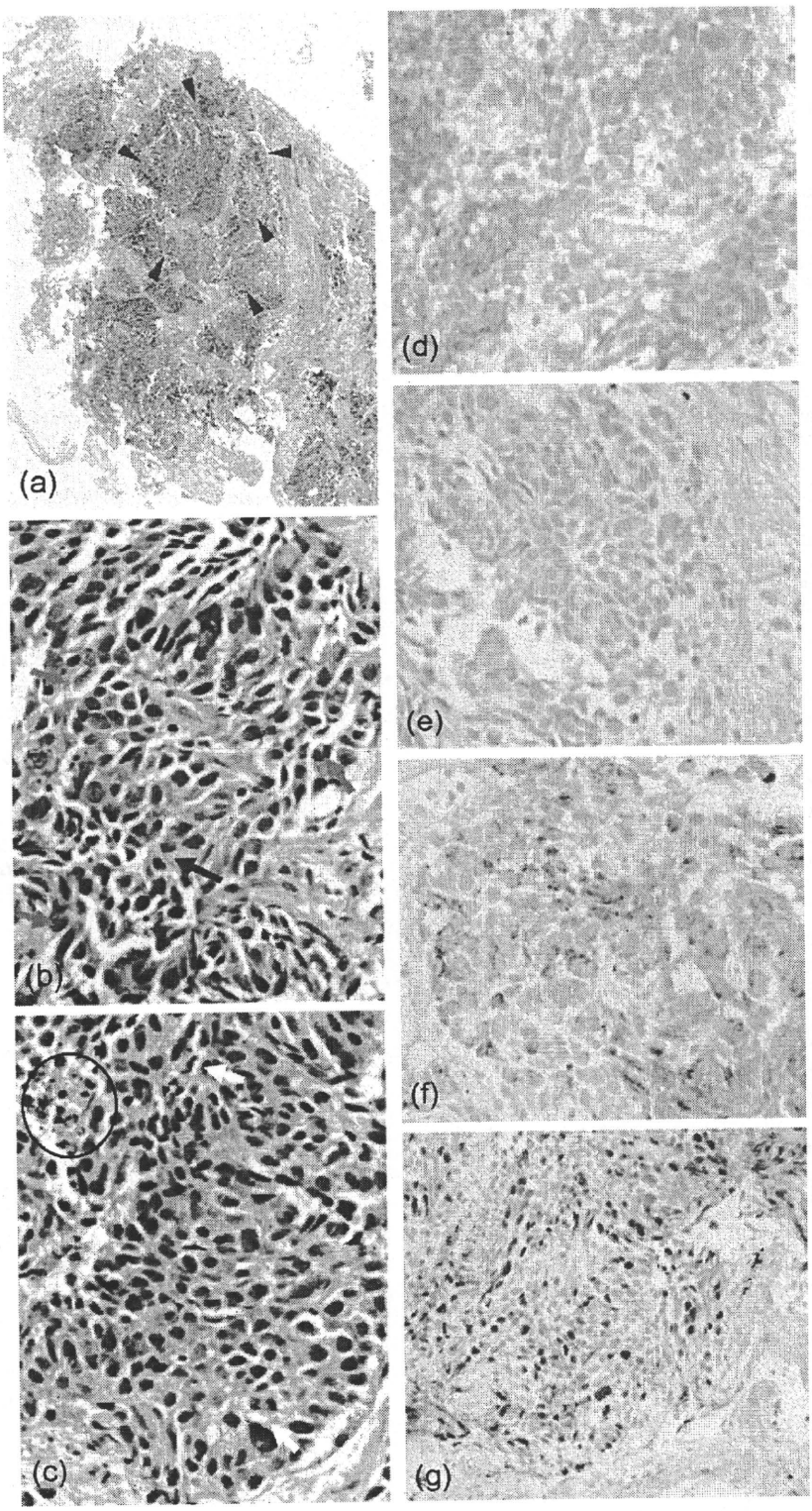

Fig. 1. A biopsy specimen diagnosed as HNSCNEC indicates histology of evident carcinoma which is composed of fused large organoid nests (red arrowheads) in which atypical polygonal cells proliferate, with no differentiation to acinar or squamoid features (a: ×4; b and c: ×40). Atypical cancer cells are dyscohesive to each other (green arrows), tend to form encircled and moulded cell arrangement, namely showing a rosette-like structure (red arrow), and have scattered mitoses (white arrow). Nucleoli are not obvious. In some areas, small aggregates of necrotic cells are observed (inside the circle). Immunohistochemically many cancer cells show positivity for NCAM (membranous, d, ×40), synaptophysin (granular, e, ×40), chromogranin A (granular, f, ×40). Ki-67/MIB1 labeling index is over 50% (g, ×20). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

or chemotherapy, and all the patients had been diagnosed as having unresectable HNSCNEC or ED-SCLC based on the results of chest radiography and computed tomography examinations of the chest and abdomen as well as other procedures such as magnetic resonance imaging (MRI) of the head and positron emission tomography (PET) or combined PET/computed tomography (PET-CT).

2.4. Evaluations

Tumor response was classified according to the Response Evaluation Criteria for Solid Tumors [12]. Patients were evaluated to determine the stage of their disease before treatment and to confirm whether their disease had progressed or relapsed using a complete medical history and physical examination including a chest radiograph, CT of the chest and abdomen, and other staging procedures such as MRI and PET.

2.5. Statistical analysis

Survival curves were calculated using the Kaplan–Meier method and were compared using a log-rank test between the HNSNEC and ED-SCLC groups. Overall survival was measured from the first day of treatment to the day of the last follow-up (cut-off) or death. The objective response rates were compared using a Fisher exact probability test. All the analyses were performed using SPSS ver. 2 (SPSS Inc., Chicago).

3. Results

Overall, 14 patients treated between September 2002 and October 2007 were recognized as having tumors with histopathological characteristics consistent with HNSCNEC, based on biopsy examinations. Among the biopsy specimens obtained in the 14 cases, we obtained ten specimens via transbronchial lung biopsy (Figs. 1 and 2) and four specimens via biopsy from a metastatic lesion (in the colon, liver, brain and adrenal gland, respectively).

We have, for the present, six surgically resected cases, in which HNSCNEC had been diagnosed by biopsy before treatment. Among these cases, five tumors were confirmed to be pure LCNEC (Figs. 3 and 4, and the other one to be combined LCNEC and small cell carcinoma by examination of surgical specimens.

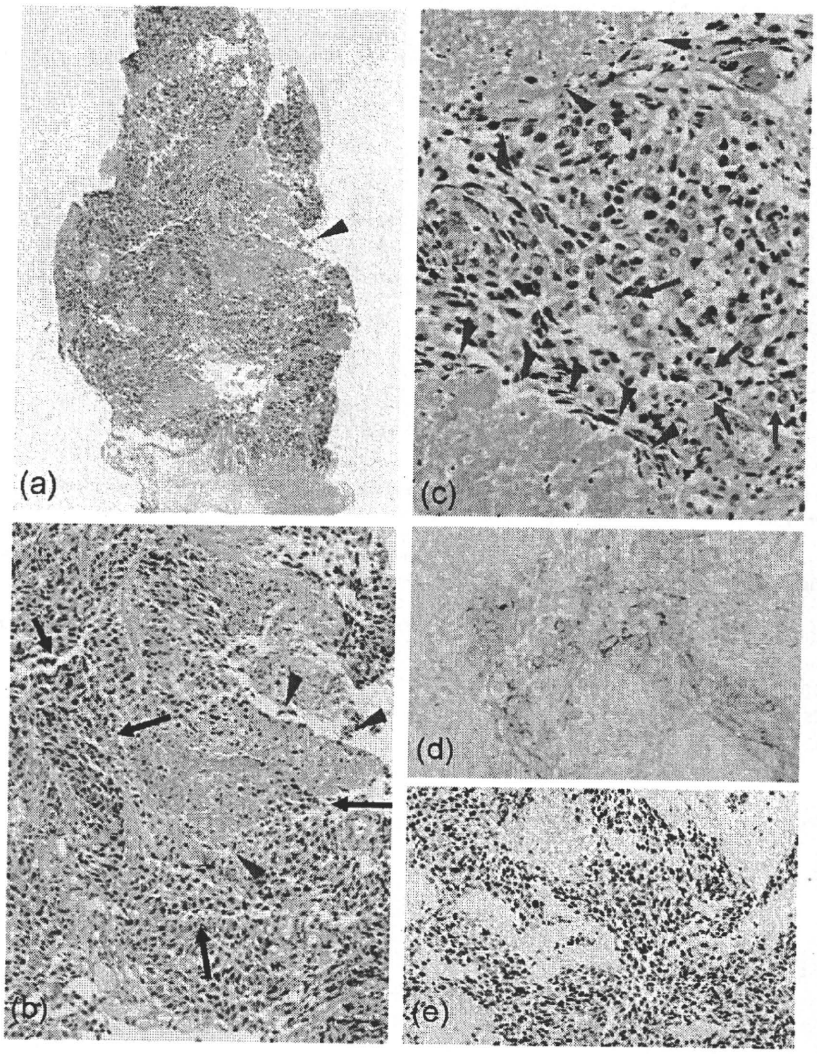

Fig. 2. A biopsy specimen diagnosed as HNSCNEC from another case shows confluent proliferation of cancer cells with remarkable necrotic foci (red arrowheads). Although organoid nesting is not obvious, polygonal cancer cells are dyscohesive (black arrows), and have prominent nucleoli (green arrows) (c, ×40). Immunohistochemically there is membranous positivity for NCAM (d, ×40), negativity for synaptophysin and chromogranin A (data not shown), and high labeling index of Ki-67/MIB1 over 90% (e, ×20). Magnification number in each parentheses indicates magnification of objective lens. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

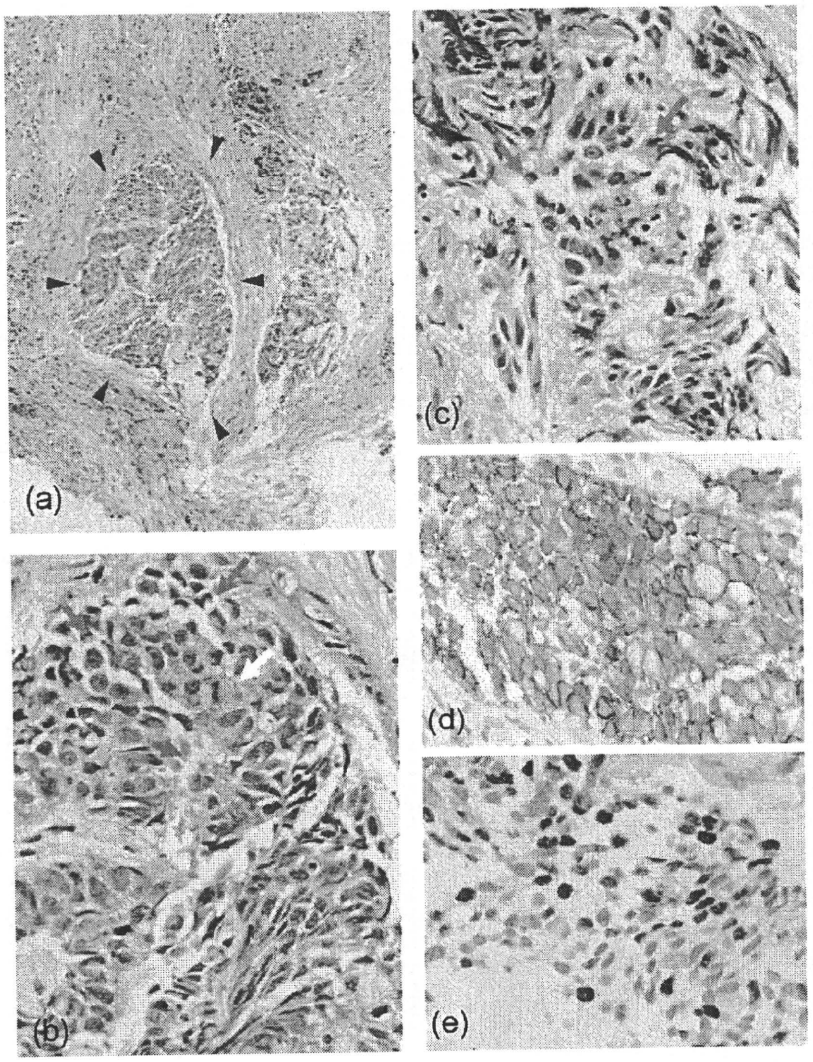

Fig. 3. A biopsy specimen before surgery was diagnosed as HNSCNEC (same figure) and the surgical specimen of the same patient revealed LCNEC (shown in Fig. 4). There was an organoid-like tumor-cell nest (a, ×10, red arrowheads) comprising large cells with abundant cytoplasm. Mitotic figures are rare (b, ×40, white arrow). Another tumor cell island was less organoid and lacking palisading arrangement in periphery. Note less cohesion of tumor cells and intercellular clefts in both nests (green arrows). The tumor cells are positive for NCAM (d, ×40), negative for chromogranin A and synaptophysin (not shown). The Ki-67/MIB1 labeling index is 62% (e, ×40). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

The characteristics of the patients are listed in Table 3. Among the 14 patients, the mean age was 70 years (range, 57–81 years), 13 patients (92%) were men, and 13 patients (92%) were current or former smokers. Two patients had stage IIIB disease, eleven patients had stage IV disease, and one patient had a postoperative recurrent case. The performance status (PS) of the patients was either PS 0 or 1 ($n=13$) or PS 2 ($n=1$). The following chemotherapy regimens were used: (i) cisplatin/irinotecan ($n=7$), (ii) carboplatin/etoposide ($n=2$), (iii) carboplatin/paclitaxel ($n=1$), (iv) cisplatin/paclitaxel ($n=1$), (v) irinotecan alone ($n=1$), (vi) vinorelbine ($n=1$), and (vii) docetaxel alone ($n=1$).

Table 3
Patient characteristics in HNSCNEC ($n=14$) and ED-SCLC ($n=77$) groups.

	HNSCNEC	ED-SCLC
Age, median (range)	70 (57–81)	69 (51–86)
Male/female	13/1	62/15
Smoking	13/1	67/10
Staging IIB/IV postop.	2/11/1	–
Performance status		
0–1/≧2	13/1	51/26
Regimens		
IP/CE/CP/DTX	7/2/1/1	
PP/CPT-11/VNR	1/1/1	
PE/IP/CE		16/27/34

IP, cisplatin/irinotecan; PE, cisplatin/etoposide; CE, carboplatin/etoposide; CP, carboplatin/paclitaxel; PP, cisplatin/paclitaxel VNR, vinorelbine; CPT-11, irinotecan.

Seventy-seven cases of ED-SCLC were treated between September 2002 and October 2007. Fifteen patients were women, and 62 were men (80%); their median age was 69 years (range, 51–86 years), and 67 patients (86%) were current or former smokers. The patients had a PS of either PS 0 or 1 ($n=51$) or PS 2 ($n=26$). The following chemotherapy regimens were used: cisplatin/irinotecan ($n=27$), cisplatin/etoposide ($n=16$), or carboplatin/etoposide ($n=34$). The characteristics of the ED-SCLC cases are listed in Table 3.

Among the 14 patients with HNSCNEC, seven patients achieved a partial response (PR), with an overall response rate of 50% (Table 4). Four PRs were observed among the patients treated with cisplatin/irinotecan, and one PR was observed in each group of patients treated with cisplatin/paclitaxel, carboplatin/etoposide, or carbo-

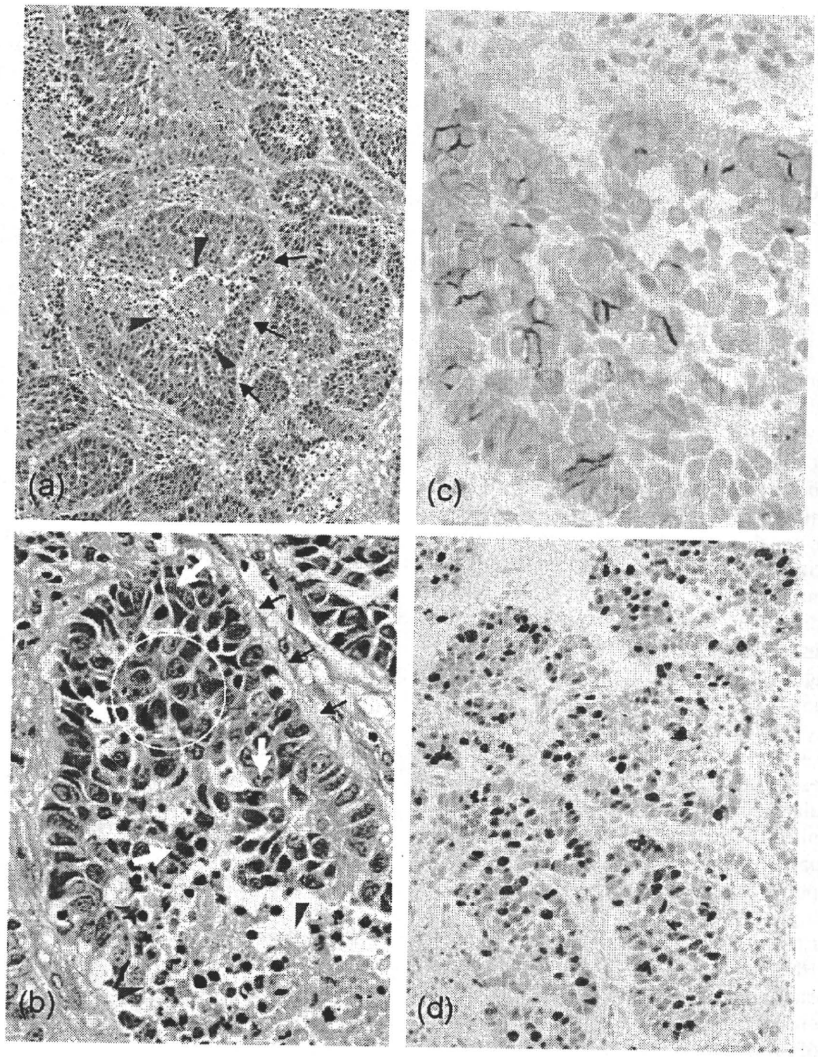

Fig. 4. A surgically resected specimen diagnosed as LCNEC. This patient was diagnosed as HNSCNEC by prior biopsy (shown in Fig. 3). We can find the histology typically characteristic of LCNEC in these two H&E pictures (a and b); central necrosis (a, ×10; and b, ×40, red arrowheads), peripheral palisading arrangement (a and b, blue arrows), rosette formation (b, white circle), and frequent mitoses (b, white arrows). Note that tumor cells are less cohesive, not like the pattern of adenocarcinoma. The results of immunostaining were the same as the biopsy specimen (c, ×40, positive for NCAM). The other neuroendocrine markers such as chromogranin A and synaptophysin were negative (not shown). The Ki-67/MIB1 labeling index was 56% (d, ×20). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

platin/paclitaxel. We evaluated overall survival using the data of 13 cases with HNSCNEC except one case with postoperative recurrence. The median survival time and one-year survival rate (as of treatment enrollment) were 10 months and 34%, respectively (Fig. 5).

Among the 77 patients with ED-SCLC, four CRs and 37 PRs were observed, with an overall response rate of 53% (Table 4). The median

Table 4
Clinical responses in HNSCNEC ($n = 14$) and ED-SCLC ($n = 77$) groups.

	HNSCNEC	ED-SCLC
CR		4
PR	7	37
SD	5	20
PD	1	13
NE	1	3
RR (%)	50%	53%

Evaluated according to RECIST Guideline.
RR, response rate; CR, complete response; PR; partial response; SD, stable disease; PD; progressive disease; NE, not evaluable.

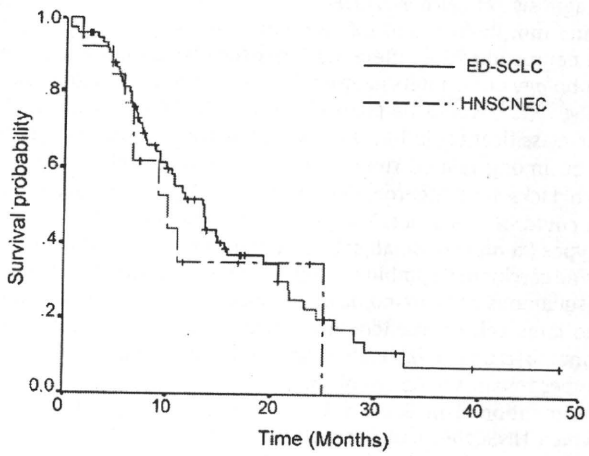

Fig. 5. Kaplan–Meier plot of overall survival of patients with HNSCNEC and patients with ED-SCLC.

survival time and one-year survival rate (after treatment) were 12.3 months and 48%, respectively (Fig. 5). No statistically significant differences were found in the objective response rates between the HNSCNEC and ED-SCLC groups ($p = 0.82$); similarly, no significant differences were found in overall survival between the HNSCNEC and ED-SCLC groups ($p = 0.68$). Thus, these results indicate that the response rate of HNSCNEC to various regimens of chemotherapy seems to be comparable to that of ED-SCLC.

4. Discussion

Two important results were obtained in this study. First, biopsy specimens were used to diagnose 14 cases of unresectable lung cancer as HNSCNEC, a category that likely includes most LCNECs and other related tumors. Until now, the diagnosis of LCNEC has mostly been made using surgically resected specimens and rarely or never by biopsy or cytology specimens alone. The architectural arrangement of tumor cells is often extremely difficult or almost impossible to appreciate using small tumor specimens, and diagnoses of biopsy or cytology specimens are usually limited to either non-small cell carcinoma, large cell carcinoma, poorly differentiated carcinoma, or, at best, suspected neuroendocrine carcinoma. Therefore, few details are known regarding the clinical efficacy of chemotherapy for patients with unresectable LCNEC and related tumors, and the establishment of diagnostic criteria for these tumors based on the examination of biopsy or cytology specimens alone is an urgent task. The difficulty in diagnosing LCNEC based on hematoxylin and eosin (H&E)-stained biopsy sections resides in the poorly differentiated states of such tumors. Organoid architectures (organoid nesting, trabecular, palisading or rosette-like growth patterns) enabling the recognition of neuroendocrine features may be scarce or absent [3], and pathologists may have difficulty making a diagnosis using small, imperfect specimens, such as dry specimens or specimens with crushing artifacts. Thus, we devised a series of pathological diagnostic criteria for high-grade non-small cell neuroendocrine carcinoma (HNSCNEC) that could be used with both routine H&E and immunostained sections of biopsy specimens. H&E sections can be used to identify massive necrosis, nuclear and cellular atypia, an abundance of cytoplasm, mitotic figures, intercellular incohesiveness, and, if discernible, some features of neuroendocrine morphology such as organoid nesting, basal palisading, rosettes and/or trabeculae. Immunostaining for Ki-67/MIB1 was used to evaluate whether a high-grade tumor was present, and immunostaining for NCAM, synaptophysin and chromogranin A were used to evaluate the neuroendocrine differentiation.

If a biopsy specimen fulfills our proposed criteria (Table 2), a diagnosis of either LCNEC, which has both a neuroendocrine morphology and differentiation, or large cell carcinoma with neuroendocrine differentiation, which lacks neuroendocrine morphology but exhibits neuroendocrine markers upon immunostaining [2,13], would be plausible, although the incidence of the latter classification is likely to be much lower than that of the former among related tumors [13]. Classic large cell carcinoma, which lacks both neuroendocrine morphology and differentiation, could be ruled out. The possible misdiagnosis of combined subtypes (combined small cell carcinoma and large cell neuroendocrine carcinoma, combined large cell neuroendocrine carcinoma and squamous cell carcinoma or adenocarcinoma, and lastly combined large cell neuroendocrine carcinoma and classic large cell carcinoma) is unavoidable, although the true incidence of combined subtypes remains to be established.

As mentioned in Section 3, all six surgically resected cases, in which HNSCNEC had been diagnosed by biopsy before treatment, were confirmed to be pure LCNEC or combined LCNEC and small cell carcinoma. At present, we are making an on-going multi-institutional study on comparison of diagnosis of more than 30 cases of both by biopsy and surgical specimens of the same patients, and there is increasing evidence that most LCNECs and their related tumors are included in the HNSCNEC category.

The clinical importance of this paper is that the chemotherapeutic responsiveness and survival of the patients with unresectable HNSCNEC was similar to those of ED-SCLC patients treated during the same period. Although seven of 11 cases except for cases with the monotherapy were responsive to chemotherapy and the rate (63.6%) was higher than that of ED-SCLC, there was no significant difference between them.A previous study reported an objective response rate to platinum-based chemotherapy of 64% in chemo-naive patients with unresectable LCNEC, which is somewhat higher than the chemotherapy response rates of other histological subtypes of NSCLC and appears to be comparable to that of SCLC [8]. In the above-mentioned study, five patients of post-operative recurrence and 15 patients who were found to have histological characteristics consistent with the diagnosis of LCNEC by autopsy had received cisplatin-based chemotherapy.

Platinum-doublet regimens, especially platinum-etoposide, have been reported to be significantly correlated with favorable survival in both an adjuvant setting and in metastatic cases with LCNEC [9]. The results of these previous studies seem to agree with the results of our study. Equally important, the fact that a male predominance (92%) and a high rate of smokers (92%) were seen in the HNSCNEC group, similar to the rates reported for LCNEC (85–90% and 50–99%, respectively) [4,5,6,14], suggests that most LCNECs are included among HNSCNECs.

In conclusion, the results of this study suggest that the clinical efficacy of chemotherapy for unresectable HNSCNECs, which likely includes most LCNECs, is comparable to that of ED-SCLC. However, because of the retrospective design and the small sample size of this study, we could not arrive at satisfactory and definitive conclusion. At present, we are making a multi-institutional study to examine a large series of specimens and to confirm whether most LCNECs are included in the HNSCNEC category.

Conflict of interest

None declared.

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Clinical assessment of patients with advanced non-small-cell lung cancer eligible for second-line chemotherapy: A prognostic score from individual data of nine randomised trials ☆

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ABSTRACT

Purpose: Knowledge of prognostic factors for advanced non-small-cell lung cancer (NSCLC) patients eligible for second-line treatment is scarce. The aim of this study was to assess the prognostic role of a number of routinely collected clinical variables and to provide a summary index to discriminate patients according to probability of survival.

Methods: Individual data from nine randomised trials of second-line treatment in advanced NSCLC were analysed. Primary end-point was overall survival (OS). Cox model, stratified by trial, was used for multivariate analyses, and a prognostic index was provided and validated according to an internal/external procedure.

Results: Out of 1239 patients, 1197 patients (97%) had complete information. Median OS was 7.4 months. At multivariate analysis, prognosis was significantly influenced by gender (worse in males), performance status (PS), tumour histology (worse in squamous and other histology *versus* adenocarcinoma), stage (worse in IV *versus* IIIB), type of previous treatment (worse for patients pretreated with platinum) and response to first-line (worse for patients

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not obtaining objective response). Prognostic score values ranges from 0 to 14. When three categories were derived, median overall survival values were equal to 11.6, 7.5 and 3.0 months for best (<5), intermediate (5-9) and worst (>9) category, respectively.

Conclusion: Prognosis of patients eligible for second-line treatment of advanced NSCLC is significantly conditioned by gender, PS, histology, stage, previous use of platinum and response to first-line. A prognostic score was derived that discriminates well subjects with a relatively more favourable prognosis and those with very short life expectancy.

1. Introduction

Patients who experience disease progression during or after first-line treatment for advanced non-small-cell lung cancer (NSCLC) have a limited life expectancy. The aims of second-line treatment are palliation of symptoms, benefit in quality of life and prolongation of survival. However, the impact of treatment on the natural history of the disease is modest. Until 10 years ago, there was actually no high-level evidence supporting the efficacy of second-line treatment, although chemotherapy was often offered to patients in good clinical conditions. In recent years, the efficacy of several drugs in the second-line setting has been demonstrated in phase III trials, and second-line treatment is now a standard of care.[1]

Due to the availability of new drugs approved for second- and third-line treatment of advanced NSCLC, the length of time spent by patients receiving active anti-cancer treatment has recently increased. However, many patients with advanced NSCLC who receive second-line treatment are near the end of life. In a retrospective review of patients treated for advanced NSCLC in a community oncology setting, nearly half of the patients had received chemotherapy in the last month of life, and one patient out of five received treatment in the last 2 weeks.[2] This can be partially explained by the increased demand for additional treatment by patients and their relatives, who are unable to recognise the futility of further therapy and the inevitability of death from progressive NSCLC. However, it may also be due to physicians' inability to correctly predict life expectancy, and this emphasises the importance of correctly identifying prognostic factors for patients who are potentially eligible to receive second-line treatment.

While the prognostic factors of patients receiving first-line chemotherapy have been extensively described, much less information is available about the prognostic factors in patients who are candidates to further treatment after first-line failure. Prognostic factors are not necessarily predictive of treatment efficacy, but their identification may help the treating physician in determining the likelihood of clinical benefit of further therapy and in identifying patients with a very limited life expectancy.[3] Furthermore, a better definition of prognostic factors in the second-line setting would be important when planning and interpreting the results of future clinical trials in advanced NSCLC.

The aim of our study was to evaluate the prognostic role of baseline patient characteristics (age, gender, performance status [PS]), tumour characteristics (histology, stage) and characteristics of first-line treatment (use of platinum, best response obtained) in patients with advanced NSCLC eligible for second-line treatment, and to produce a summary prognostic index. With this objective, we analysed individual patient data (IPD) of patients enrolled in nine randomised trials conducted in the setting of second-line treatment.

2. Patients and methods

Data used for this analysis had been previously collected for two IPD meta-analyses of randomised trials performed in the setting of second-line treatment of advanced NSCLC.[4,5] The first meta-analysis collected data of five trials[6–10] by comparing weekly *versus* every 3 week administration of docetaxel.[4] The second meta-analysis collected data of six trials[11–16] by comparing single-agent *versus* doublet chemotherapy.[5]

Out of 11 trials potentially available, 2 trials[8,10] were excluded from this analysis of prognostic factors because of missing information on one or more variables.

The variables considered in this analysis can be divided into patient characteristics (age, gender and PS), tumour characteristics (stage and histology) and characteristics of first-line treatment (use of platinum-based chemotherapy and best objective response).

2.1. Statistical analysis

Only patients with complete information on study variables were included in the analysis.

The primary end-point was overall survival (OS), defined as the time between the date of randomisation and the date of death, or the last date of follow-up for censored patients.

In order to describe the impact of baseline characteristics on OS, survival curves were drawn with the Kaplan–Meier product limit method. Statistical analysis was performed by using the Cox proportional hazards model, stratified by trial, including age (older than 70 *versus* younger), gender (male *versus* female), PS (1 *versus* 0; 2 *versus* 0), histology (squamous *versus* adenocarcinoma; other histology *versus* adenocarcinoma), tumour stage (IV *versus* III B), type of first-line (platinum-based *versus* other) and objective response to first-line (no *versus* yes) as covariates. The proportional hazard assumption was tested using graphical methods and was adequately met for all analyses. Results are reported as hazard ratio (HR) of death with 95% confidence intervals (CI). Two-tailed p values were determined with the use of a likelihood-ratio test, and values less than 0.05 were considered statistically significant.

The log-hazard rates obtained from the Cox model were used to derive weighting factors of a prognostic index, aimed to identify differential risks of death. Coefficients estimates were 'normalised' dividing by the smallest one and rounding the resulting ratios to the nearest integer value. The concordance C-index statistic proposed by Pencina *et al.*[17] was adopted as a measure of discriminating power allowing for stratification as proposed by the Fibrinogen Studies Collaboration.[18] Possible overfitting bias was assessed by using the internal–external cross-validation (IECV) approach[19] on the C-Index statistic.

Analyses were performed with S-PLUS software (S-PLUS 6.1 Professional, release 1; Insightful Corporation, Seattle, WA, USA).

3. Results

3.1. *Patients characteristics*

Details about treatment arms, period of accrual and number of patients of the 9 trials are reported in Table 1. Out of 1239 patients, 1197 (97%) had complete information about prognostic factors and were included in this analysis. Their baseline characteristics are depicted in Table 2. Median age was 61 years (range 26–84). The majority of the patients were males (77.7%) and had a good PS (0 or 1 in 87.1%) As expected, there was a significant association between gender and tumour histology: tumours were squamous in 37% of males compared to 14% of females, while adenocarcinomas were more common among women than men (64% and 43%, respectively). Women were younger than men: median age was 58 and 62 years, respectively. There were no gender-related differences in terms of baseline PS.

Most patients had previously received a first-line platinum-based treatment (84.3%). This was obviously driven by inclusion criteria of the trials: previous platinum-based treatment was actually mandatory in six trials,[7,10,11,14–16] not mandatory in four trials,[6,8,9,13] while one trial was dedicated to patients not previously treated with platinum.[12] Overall, 44% of patients had obtained objective response to first-line treatment: this proportion varied significantly in the different trials ranging from 32% to 63%. The higher proportion of responders was recorded in the Japanese trial (61%), and in the Dutch trial that selected patients progressing more than 3 months after completion of first-line platinum-based chemotherapy (63%).[15,16]

3.2. *Outcome and prognostic factors*

Overall, 956 deaths were recorded (80%), with median OS in the whole population equal to 7.4 months. Six-month survival was 57.9%, and 1-year survival was 29.3%.

Table 1 – Trials included in the analysis.

First author (reference)	Treatment arms	Accrual (years)	Number of patients	
			Randomised	Eligible for analysis of prognostic factors
Gridelli[6]	Arm 1: Docetaxel 75 mg/m^2 every 3 weeks Arm 2: Docetaxel 33.3 mg/m^2 weekly for 6 weeks, then 2 weeks of rest	2000–2002	220	220
Gervais[7]	Arm 1: Docetaxel 75 mg/m^2 every 3 weeks Arm 2: Docetaxel 40 mg/m^2 weekly for 6 weeks, then 2 weeks of rest	2000–2001	125	125
Lai[9]	Arm 1: Docetaxel 66 mg/m^2 every 3 weeks Arm 2: Docetaxel 33 mg/m^2 weekly for 2 weeks, then 1 week of rest	1999–2002	47	47
Georgoulias[11]	Arm 1: Irinotecan 300 mg/m^2 day 1 every 3 weeks Arm 2: Gemcitabine 1000 mg/m^2 day 1 and 8 + irinotecan 300 mg/m^2 day 8 every 3 weeks	1999–2001	147	134
Georgoulias[12]	Arm 1: Cisplatin 80 mg/m^2 day 1 every 3 weeks Arm 2: Cisplatin 80 mg/m^2 day 8 + irinotecan 110 mg/m^2 day 1, 100 mg/m^2 day 8 every 3 weeks	1999–2002	139	118
Wachters[13]	Arm 1: Docetaxel 75 mg/m^2 day 1 every 3 weeks Arm 2: Docetaxel 60 mg/m^2 day 1 + irinotecan 200 mg/m^2 day 1 every 3 weeks	2000–2003	108	103
Gebbia[14]	Arm 1: Docetaxel 33.3 mg/m^2 day 1, 8, 15 every 4 weeks Arm 2: Docetaxel 30 mg/m^2 day 1, 8, 15 every 4 weeks + gemcitabine 800 mg/m^2 (or vinorelbine 20 mg/m^2) day 1, 8 every 4 weeks Arm 3: Docetaxel 30 mg/m^2 day 1, 8, 15 every 4 weeks + capecitabine 1300 mg/m^2 days 5–18 every 4 weeks	2005–2006	84	84
Takeda[15]	Arm 1: Docetaxel 60 mg/m^2 day 1 every 3 weeks Arm 2: Docetaxel 60 mg/m^2 day 8 + gemcitabine 800 mg/m^2 day 1, 8 every 3 weeks	2002–2003	130	128
Smit[16]	Arm 1: Pemetrexed 500 mg/m^2 day 1 every 3 weeks Arm 2: Pemetrexed 500 mg/m^2 day 1 every 3 weeks + carboplatin AUC5 day 1 every 3 weeks	2005–2007	240	238

Table 2 – Characteristics of the patients eligible for the analysis (n = 1197).

Age, n (%)		
Median, years (range)	61	(26–84)
Younger than 70 years	988	(82.5%)
Older than 70 years	209	(17.5%)
Gender, n (%)		
Male	930	(77.7%)
Female	267	(22.3%)
Performance status, n (%)		
0	334	(27.9%)
1	709	(59.2%)
2	154	(12.9%)
Tumour stage, n (%)		
IIIB	213	(17.8%)
IV	984	(82.2%)
Histologic type, n (%)		
Squamous	380	(31.7%)
Adenocarcinoma	568	(47.5%)
Other	249	(20.8%)
Type of first-line treatment, n (%)		
Platin-based	1009	(84.3%)
Other	188	(15.7%)
Objective response to 1st line, n (%)		
Yes	527	(44.0%)
No	670	(56.0%)

Kaplan–Meier curves of OS according to baseline patient characteristics (age, gender and PS) are shown in Fig. 1. Kaplan–Meier curves of OS according to tumour characteristics (stage and histology) and characteristics of previous treatment (type of chemotherapy and objective response) are reported in Fig. 2.

In the Cox model stratified by trial, all the covariates were independently prognostic, with the exception of age. Prognosis was worse in males than in females, with a HR of death 1.23 (95% CI 1.04–1.45). As compared with PS 0 patients, HR was 1.36 (95% CI 1.16–1.59) for PS 1 patients and 3.01 (95% CI 2.41–3.76) for PS 2 patients. Compared to patients with adenocarcinoma, risk of death was higher for subjects with both squamous tumours (HR 1.18, 95% CI 1.01–1.38) and other histology (HR 1.49, 95% CI 1.26–1.77). Stage IV was associated with a worse prognosis compared to stage IIIB (HR 1.28, 95% CI 1.07–1.53). Both type of previous treatment and response obtained with first-line were predictive of prognosis: HR of death was 1.49 (95%CI 1.14–1.93) for patients who had received platinum-based first-line chemotherapy, and 1.25 (95% CI 1.10–1.44) for those who had not achieved an objective response. Results of multivariate analysis are summarised in Table 3.

3.3. *Prognostic index*

All the covariates showing independent prognostic role in the Cox model were included in the prognostic index. Table 4

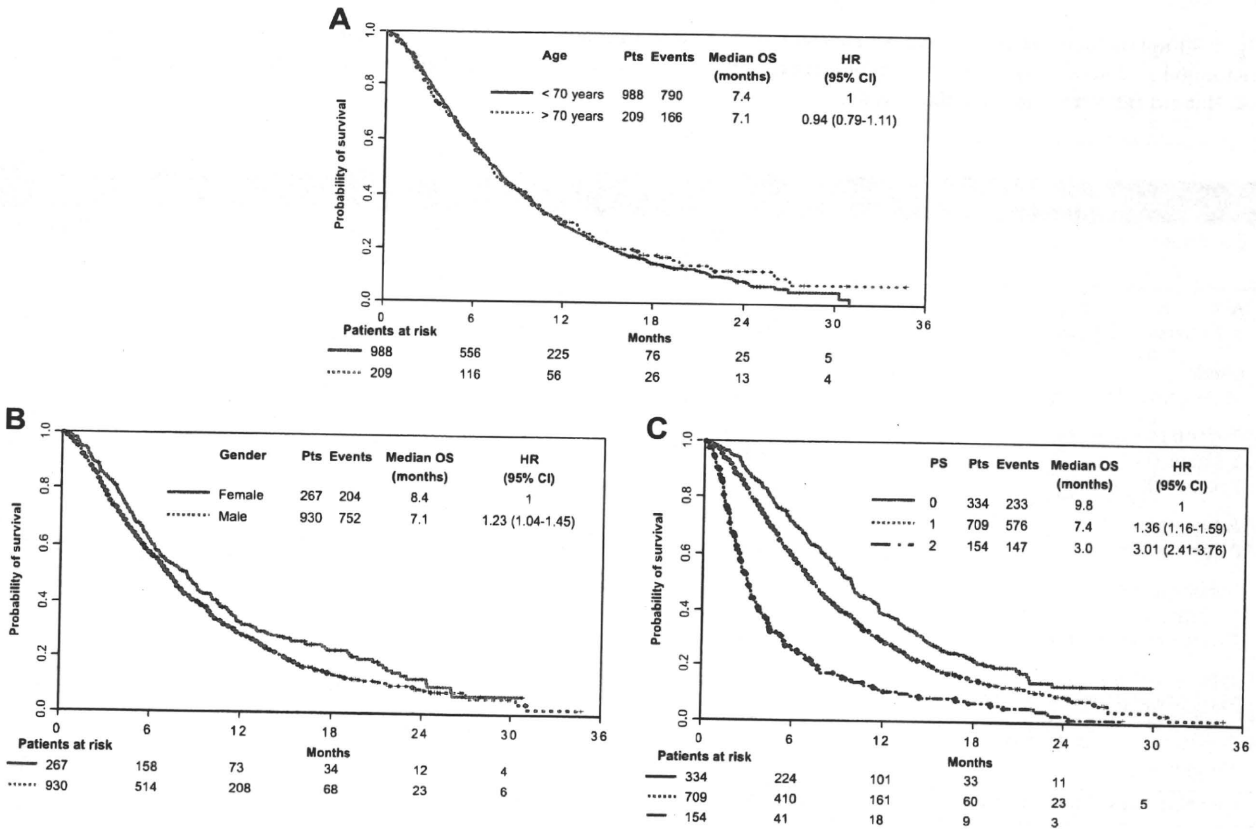

Fig. 1 – Kaplan–Meier curves of overall survival (OS) according to patient characteristics (panel A: age; panel B: gender; and panel C: performance status). HR: Hazard ratio from multivariate analysis.

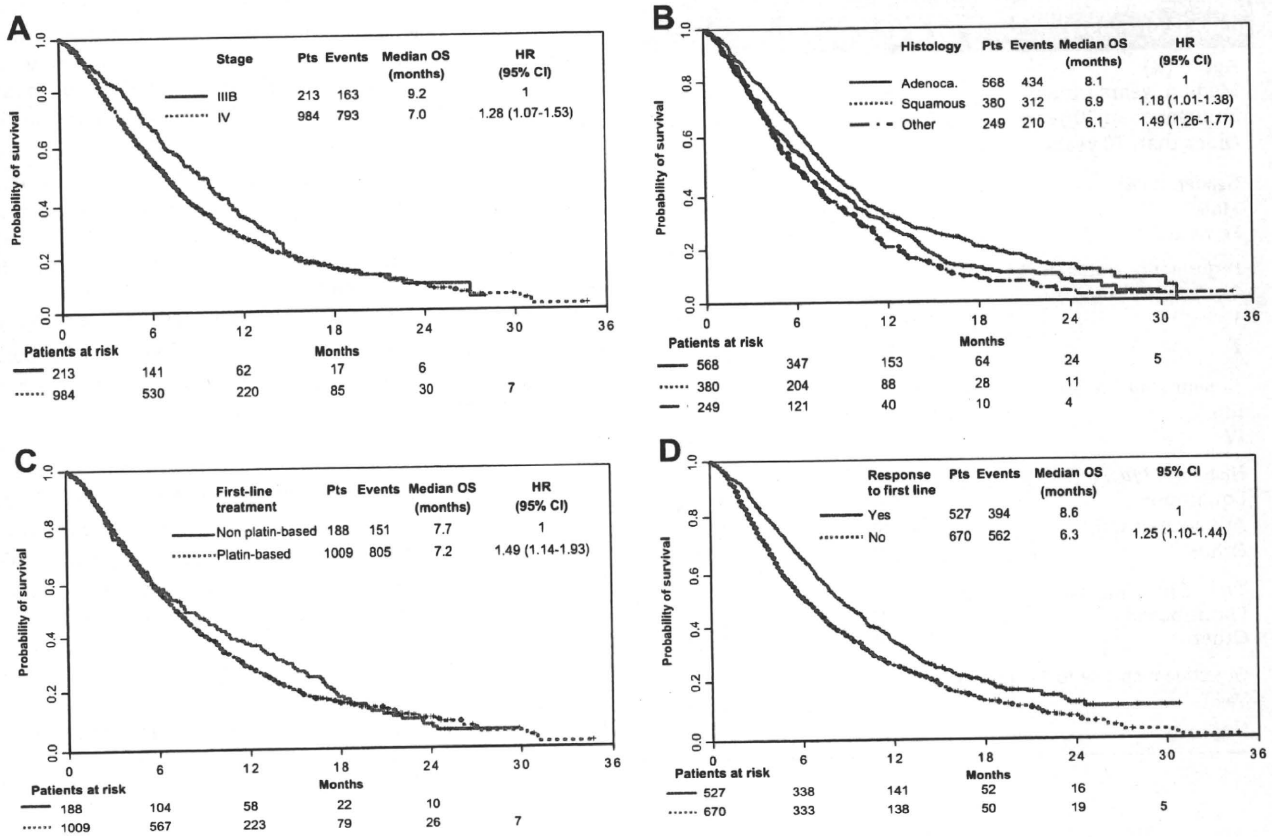

Fig. 2 – Kaplan–Meier curves of overall survival according to tumour characteristics (panel A: tumour stage and panel B: histology) and according to characteristics of first-line treatment (panel C: use of platinum and panel D: objective response). HR: Hazard ratio from multivariate analysis.

Table 3 – Multivariate analysis: Cox model (n = 1197).

Covariate	Hazard ratio of death	95% confidence limits	P-value[a]
Age			
≫70 *versus* <70 years	0.94	0.79–1.11	0.400
Gender			
Male *versus* female	1.23	1.04–1.45	0.013
Performance status			<0.001
1 *versus* 0	1.36	1.16–1.59	
2 *versus* 0	3.01	2.41–3.76	
Tumor stage			
IV *versus* IIIb	1.28	1.07–1.53	0.006
Histologic type			<0.001
Squamous *versus* adeno	1.18	1.01–1.38	
Other *versus* adeno	1.49	1.26–1.77	
Type of first-line			
Platin-based *versus* other	1.49	1.14–1.93	0.003
Objective response to first-line			
No *versus* yes	1.25	1.10–1.44	0.001

a P-values were determined with the use of a likelihood-ratio test. Cox model was stratified by trial.

shows the scores based on the HRs in the Cox model. The C-index was estimated to be 0.626 (CI: 0.605, 0.647) and 0.643 (CI: 0.619, 0.667) for the Cox model and the prognostic index, respectively.

Table 4 – Definition of the scoring system.[a]

	Points			
	0	1	2	7
Gender	Female	Male		
Performance Status	0		1	2
Tumour stage	IIIb	IV		
Histologic type	Adenocarcinoma	Squamous	Other	
Type of first-line	Without platinum		Platin-based	
Objective response to first –line	Yes	No		

a The coefficients estimates (i.e. the logarithm of hazard ratios) were 'normalised' by dividing by the smallest one and rounding the resulting ratios to the nearest integer value.

The possible overfitting biases estimated by the IECV approach[19] were approximately equal to 1.8% and 1.9% for the Cox model and the score, respectively, and were both not statistically significant suggesting that generalisability of the score is well supported by the data.

The outcome of patients according to the prognostic score is shown by dividing patients into three categories. Cutoffs were chosen at approximately equal distance along the range of values: <5 (best), 5–9 (intermediate) and >9 (worst). Such three-category score exhibited a C-index estimate equal to 0.706 (CI: 0.67, 0.741). The associated overall raw survival estimates are depicted in Fig. 3. Median survival was 11.6, 7.5 and 3.0 months for the best, intermediate and worst category, respectively.

Kaplan–Meier curves of overall survival according to three risk categories in the nine analysed trials are reported in Fig. 4.

4. Discussion

This prognostic analysis was conducted in 1197 patients receiving second-line chemotherapy for advanced NSCLC and showed that PS, gender, histology, stage, use of a platinum-based first-line and best response to previous chemotherapy are independent prognostic factors. All these variables are easily collected being part of the minimum baseline evaluation for patients candidate to second-line treatment in clinical practice.

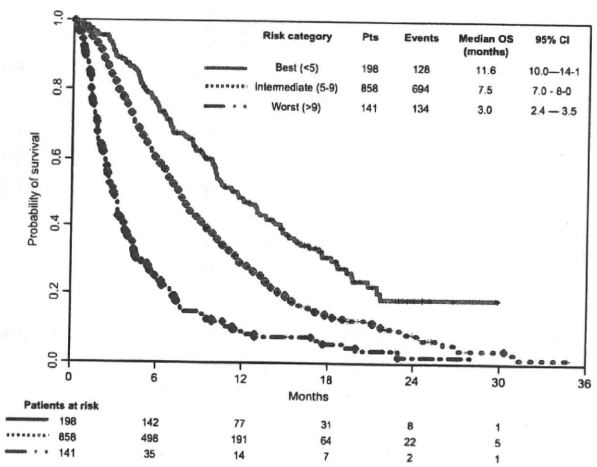

Fig. 3 – Kaplan–Meier curves of overall survival according to three risk categories based on the prognostic score.

Although the trials considered in our analysis are necessarily only a fraction of all the trials conducted in this setting, to our knowledge this is the largest database of patients receiving second-line chemotherapy for advanced NSCLC. Importantly, all patients were enrolled in randomised trials, and information about baseline characteristics and outcome was collected prospectively. We recognise that treatments received were heterogeneous among the different trials, but the two meta-analyses showed no significant difference in efficacy between treatment arms (weekly *versus* every-3-week docetaxel[4] and single-agent *versus* combination chemotherapy[5]), and the Cox model was stratified by trial.

There are several interesting points that deserve some comment.

Interestingly, most of the characteristics with prognostic role are similar to the ones influencing the outcome in first-line treatment. First of all, similarly to what is commonly described in first-line treatment, PS shows a very strong association with outcome of these patients. Life expectancy of subjects who fail first-line therapy appears to be largely dependent on their clinical condition at the beginning of second-line. In our series, despite the potential positive selection bias due to eligibility for a clinical trial, median survival of PS 2 patients was lower than 3 months compared to that of PS 1 patients and PS 0 patients which was more than 7 months and nearly 10 months, respectively. In our model, PS 2 is by far the worst prognostic characteristic, and it is relevant that only patients with PS 2 (7 points) can totalise a prognostic score higher than 9, which is the worst category. Patients with PS 2 are unfit, but are generally considered candidates for further treatment in clinical practice. Little evidence has been produced on the efficacy of second-line chemotherapy compared to best supportive care in poor PS patients, and PS 2 patients probably derive a modest absolute benefit, if any, from treatment. The BR.21 trial compared erlotinib *versus* placebo as second- or third-line in patients considered to be no longer eligible for chemotherapy showing a significant improvement in overall survival with erlotinib.[20] According to subgroup analysis, the benefit associated with erlotinib appears similar in unfit patients (PS 2 or 3) compared to that in patients with better PS, and there was no evidence of significant interaction between treatment efficacy and PS.[20] Although this may suggest avoiding chemotherapy and preferring biologic agents in these patients, in a randomised trial by comparing docetaxel

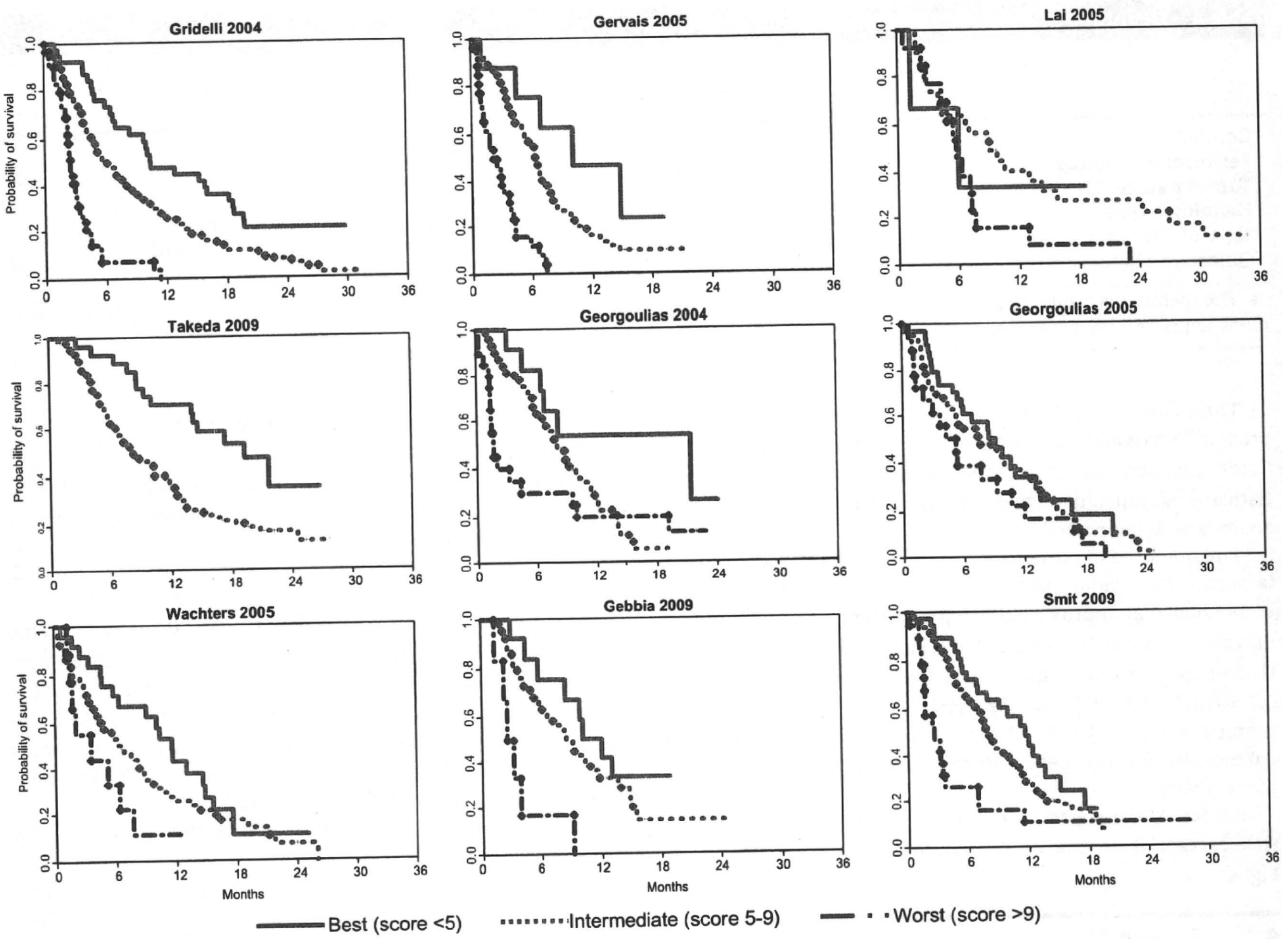

Fig. 4 – Kaplan–Meier curves of overall survival according to three risk categories based on the prognostic score in each of the nine trials considered in the analysis.

with gefitinib, as second-line treatment, there was actually no evidence of better outcome with the biologic agent compared to chemotherapy in PS subgroups.[21] The decision about second-line treatment for patients with poor PS should be based on careful evaluation of the expected toxicity profile considering that the absolute benefit in terms of survival is probably modest.

Treatment of advanced NSCLC has been traditionally independent from histologic subtypes. Recently, this concept has been destabilised by some evidence suggesting differential efficacy of pemetrexed according to tumour histology, both in first- and second-line setting.[22,23] Despite adenocarcinoma being associated with a higher chance of obtaining objective response with Epidermal Growth Factor Receptor (EGFR) inhibitors, tumour histology did not show a significant predictive role for efficacy of erlotinib compared to that of placebo in the BR.21 trial,[20] and there was no significant interaction between treatment and histology in the INTEREST trial comparing gefitinib to docetaxel.[21] Of course, due to trials design, our data cannot explore the predictive role of histology, but the present analysis shows that histotype, besides the suggested predictive role, has some prognostic impact on survival. Similar data have been recently presented in chemotherapy-naïve patients, where adenocarcinoma was associated with a 3-month advantage in overall survival compared to squamous tumours.[24] In our series, compared to adenocarcinoma, prognosis appears to be slightly worse for squamous tumours, and worse for other histotypes (large cell, mixed and undifferentiated) that are currently pooled together with adenocarcinoma, under the definition of non-squamous tumours.

In our analysis, prognosis was significantly better for female patients. Median overall survival was 8.4 months in women, and 7.1 months in men. This gender difference is consistent with a number of previous publications, at various stages of disease.[25,26] In this series, adenocarcinoma was indeed more common in women than in men. However, the significant prognostic impact of gender at multivariate analysis shows that the better outcome of female patients is not explained – at least not entirely – with the difference in terms of histotype.

We found that two characteristics of previous treatment were independently associated with prognosis: use of platinum-based first-line chemotherapy and best response obtained with first-line treatment. In particular, patients who had previously received platinum-based chemotherapy show a worse prognosis compared to patients who have not received platinum compounds. We can argue that the margin of further benefit associated with treatment appears to be