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Chemotherapy for pulmonary large cell neuroendocrine carcinomas: does the regimen matter?

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ABSTRACT Pulmonary large cell neuroendocrine carcinoma (LCNEC) is rare. Chemotherapy for metastatic LCNEC ranges from small cell lung carcinoma (SCLC) regimens to nonsmall cell lung carcinoma (NSCLC) chemotherapy regimens. We analysed outcomes of chemotherapy treatments for LCNEC.

The Netherlands Cancer Registry and Netherlands Pathology Registry (PALGA) were searched for patients with stage IV chemotherapy-treated LCNEC (2003–2012). For 207 patients, histology slides were available for pathology panel review. First-line platinum-based combined chemotherapy was clustered as "NSCLC-t", comprising gemcitabine, docetaxel, paclitaxel or vinorelbine; "NSCLC-pt", with pemetrexed treatment only; and "SCLC-t", consisting of etoposide chemotherapy.

A panel review diagnosis of LCNEC was established in 128 out of 207 patients. NSCLC-t chemotherapy was administered in 46% (n=60), NSCLC-pt in 16% (n=20) and SCLC-t in 38% (n=48) of the patients. The median (95% CI) overall survival for NSCLC-t chemotherapy was 8.5 (7.0–9.9) months, significantly longer than patients treated with NSCLC-pt, with a median survival of 5.9 (5.0–6.9) months (hazard ratio 2.51, 95% CI 1.39–4.52; p=0.002) and patients treated with SCLC-t chemotherapy, with a median survival of 6.7 (5.0–8.5) months (hazard ratio 1.66, 95% CI 1.08–2.56; p=0.020).

In patients with LCNEC, NSCLC-t chemotherapy results in longer overall survival compared to NSCLC-pt and SCLC-t chemotherapy.

Introduction

Pulmonary large cell neuroendocrine carcinoma (LCNEC) is a subtype of lung cancer with neuroendocrine morphology, neuroendocrine differentiation on immunohistochemistry, a high mitotic rate (>10 mitosis·2 mm^{-2}) and nonsmall cell cytological features [1]. LCNEC is rare and accounts for ~3% of all lung cancers, but the proportion of lung cancers diagnosed as LCNEC appears to be increasing [2]. Because the histological features of LCNEC overlap with nonsmall cell lung carcinoma (NSCLC) and occasionally with small cell lung carcinoma (SCLC), histological diagnosis can be difficult [3, 4].

Because of the difficulties in diagnosing LCNEC, and its rarity, the optimal systemic treatment has not been adequately established [5]. In the current European Society for Medical Oncology guidelines for NSCLC, no specific treatment for LCNEC is described [6]. In the American Society of Clinical Oncology (ASCO) guideline, either platinum–etoposide chemotherapy treatment (SCLC type) or the same regimen as for nonsmall cell nonsquamous carcinoma (NSCLC type) is advised for LCNEC. However, SCLC-type chemotherapy is considered by expert opinion to be most appropriate [7].

Several observations suggest that LCNEC should respond best to a SCLC-type treatment. For instance, recent studies show that the genomic profile of LCNEC corresponds closely with SCLC [8, 9]. In addition, we reported that the prognosis and metastatic pattern at diagnosis of LCNEC significantly overlaps with SCLC [2, 10]. However, important differences in the response to SCLC-type chemotherapy treatment for LCNEC and SCLC have been reported [5]. Two single-arm phase II trials in LCNEC (n=29 and n=30) showed an objective response rate (ORR) for etoposide or irinotecan combined with cisplatin ranging from 31% to 47% [11, 12], substantially lower compared to SCLC phase III trials evaluating etoposide–cisplatinum chemotherapy (ORR ≈66%) [13]. Because of the reported higher resistance to SCLC-type chemotherapy in LCNEC, some clinicians favour a NSCLC-type chemotherapy treatment.

Because of these perceived differences, we investigated the chemotherapy treatment of patients with metastatic LCNEC in the Netherlands from 2003 to 2012. Furthermore, we retrospectively correlated the overall survival and progression free survival (PFS) with chemotherapy type in patients with a panel-reviewed histological diagnosis of LCNEC.

Material and methods

Data sources and ethical regulations

Data were retrieved from the Netherlands Cancer Registry and Netherlands Pathology Registry (PALGA, the nationwide registry of pathology in the Netherlands [14]). The study was performed according to the cancer registry and pathology registry guidelines and national privacy regulations and approved by the medical ethical committee of the Maastricht University Medical Center (METC azM/UM 14-4-043, November 20, 2014).

Patient selection

All patients with a diagnosis of stage IV LCNEC recorded in either the cancer registry or the pathology registry between January 1, 2003 and December 31, 2012 were included. To select LCNEC from the cancer registry the International Classifications of Disease – Oncology 3rd edition code M8013 was used. Previously we have observed that a wide range of diagnostic terms are used to describe LCNEC [15]. To identify additional LCNEC cases in the cancer registry that had been diagnosed with alternative nomenclature, the additional diagnostic codes M8246 (neuroendocrine carcinoma) and M8574 (NSCLC with neuroendocrine differentiation) were included. Digital summaries of pathology reports retrieved from the pathology registry were screened for the diagnosis of LCNEC, as previously reported [15]. Patients diagnosed with metastatic LCNEC, including patients with tumours diagnosed with a nomenclature possibly referring to LCNEC, treated by chemotherapy retrieved from either of the national databases, were included. Data on the type of chemotherapy treatment was retrospectively updated in 2015 by qualified cancer registry data managers. Patients were excluded if details on chemotherapy were unavailable.

First, we analysed the type of chemotherapy in the selected patient study group (aim 1). We then performed a pathology review for all patients. Patients with a diagnosis based on cytology and patients for whom the original histopathological slides could not be retrieved were excluded. Overall survival and PFS were determined in patients with a panel-confirmed diagnosis of LCNEC (aim 2).

Conflict of interest: None declared.

Data collection

Collected data included stage (tumour, node and metastasis (TNM) stage 6 or 7) and time from diagnosis to death or last follow-up censored for 36 months of overall survival. PFS was calculated from date of diagnosis until first evidence of progression, death or last day of follow-up. Treatment data included chemotherapy subtype, number of chemotherapy cycles and second-line treatment. First-line chemotherapy was clustered into three groups, as follows. 1) "NSCLC chemotherapy type" (NSCLC-t), consisting of gemcitabine, docetaxel, paclitaxel or vinorelbine; 2) "pemetrexed NSCLC type" (NSCLC-pt), with pemetrexed treatment only; and 3) "SCLC type" (SCLC-t), consisting of etoposide chemotherapy. The platinum components were either cisplatin or carboplatin. Metastatic sites at diagnosis were retrieved from documented clinical data (cTNM). Pathology data included pathology history, pathological specimen type and diagnosis according to the digital pathology report summary.

Pathology revision

Tumour histology slides were collected and included at least one immunohistochemical (IHC) neuroendocrine stain (CD56/NCAM, chromogranin-A or synaptophysin) and a haematoxylin and eosin stained slide. Review was performed by three pathologists (E. Thunnissen, R. van Sulyen and M. den Bakker), who were blinded for clinical outcome and original diagnosis. IHC staining patterns for neuroendocrine markers, cytokeratins, TTF1 and p63 and Ki-67 (if available) were assessed by J. Derks and R. van Sulyen prior to the central review meetings. The assessors evaluated haematoxylin and eosin slides at the multihead microscope; information on IHC expression patterns was provided (J. Derks). LCNEC was established when at least two pathologists agreed on the diagnosis, referred to as panel-consensus LCNEC. World Health Organization (WHO) 2015 criteria were evaluated for all panel-consensus established diagnoses. Additional detailed pathology review information can be found in the online supplementary pathology data file.

Statistical analysis

The Chi-squared and Fisher exact tests were used to compare categorical data. Continuous variables were tested using the Mann–Whitney U-test and the median and interquartile range (IQR) reported. Overall survival and PFS censoring took place at the closing date (February 1, 2014). Overall survival was estimated according to the Kaplan–Meier method and tested using the log-rank test. Multivariate Cox regression analysis was performed including covariates significant at univariate analysis. Nonproportionality was visually assessed by log minus log plots. Two-sided p-values <0.05 were considered significant. Analyses were performed using SPSS (version 22 for Windows; Chicago, IL, USA).

Results
Population-based changes in chemotherapy treatment over time

Data from 1627 patients from the cancer registry and 1172 patients from the pathology registry were retrieved. 355 patients had stage IV disease treated with chemotherapy. After excluding patients for whom details of chemotherapy treatment could not be retrieved, chemotherapy treatment was analysed in 294 patients (figure 1). A complete overview of retrieved diagnoses and chemotherapy treatment is presented in online supplementary table S1. NSCLC-t chemotherapy treatment in LCNEC significantly decreased over time from 59% (2003–2009) to 31% (2010–2012) (p<0.001); NSCLC-pt chemotherapy type increased from 10% to 16% (p=0.29); and the SCLC type increased from 31% to 53% (p=0.002).

Panel-consensus diagnosed LCNEC

Histopathological slides were retrieved from 207 patients. In 128 patients LCNEC was diagnosed by consensus, with 108 cases meeting all required WHO 2015 criteria (online supplementary table S2) [16]. Patients with a panel-confirmed LCNEC diagnosis (n=128) had a median age of 65 (56–71) years, 59% were male and 67% were diagnosed by a (core) needle biopsy specimen (table 1). Metastases in the liver (53%), bone (27%) and nonmediastinal lymph nodes (22%) were most common. Metastases confined to a single organ were present in 48% of patients. A minimum of four chemotherapy cycles (median (IQR) 4 (2–4)) were administered in 62% of patients. Second-line chemotherapy was administered in 23% of patients. Patients with more than three metastases in different organs more frequently received SCLC-t chemotherapy. Overall, NSCLC-t chemotherapy was administered in 46% of patients, mainly platinum–gemcitabine (76% of NSCLC-t patients). NSCLC-pt and SCLC-t chemotherapy was administered in 16% and in 38% of patients, respectively. Characteristics of panel-consensus diagnosed LCNEC patients who fulfilled all required WHO criteria were not different and are described in online supplementary table S3.

Overall survival in panel-consensus diagnosed LCNEC by chemotherapy cluster

All but three patients died during the follow-up period. The median (95% CI) overall survival was 7.3 months (6.3–8.2 months). Patients treated with NSCLC-t chemotherapy had a median overall survival of 8.5 months (7.0–9.9 months), which was significantly longer than for patients treated with NSCLC-pt
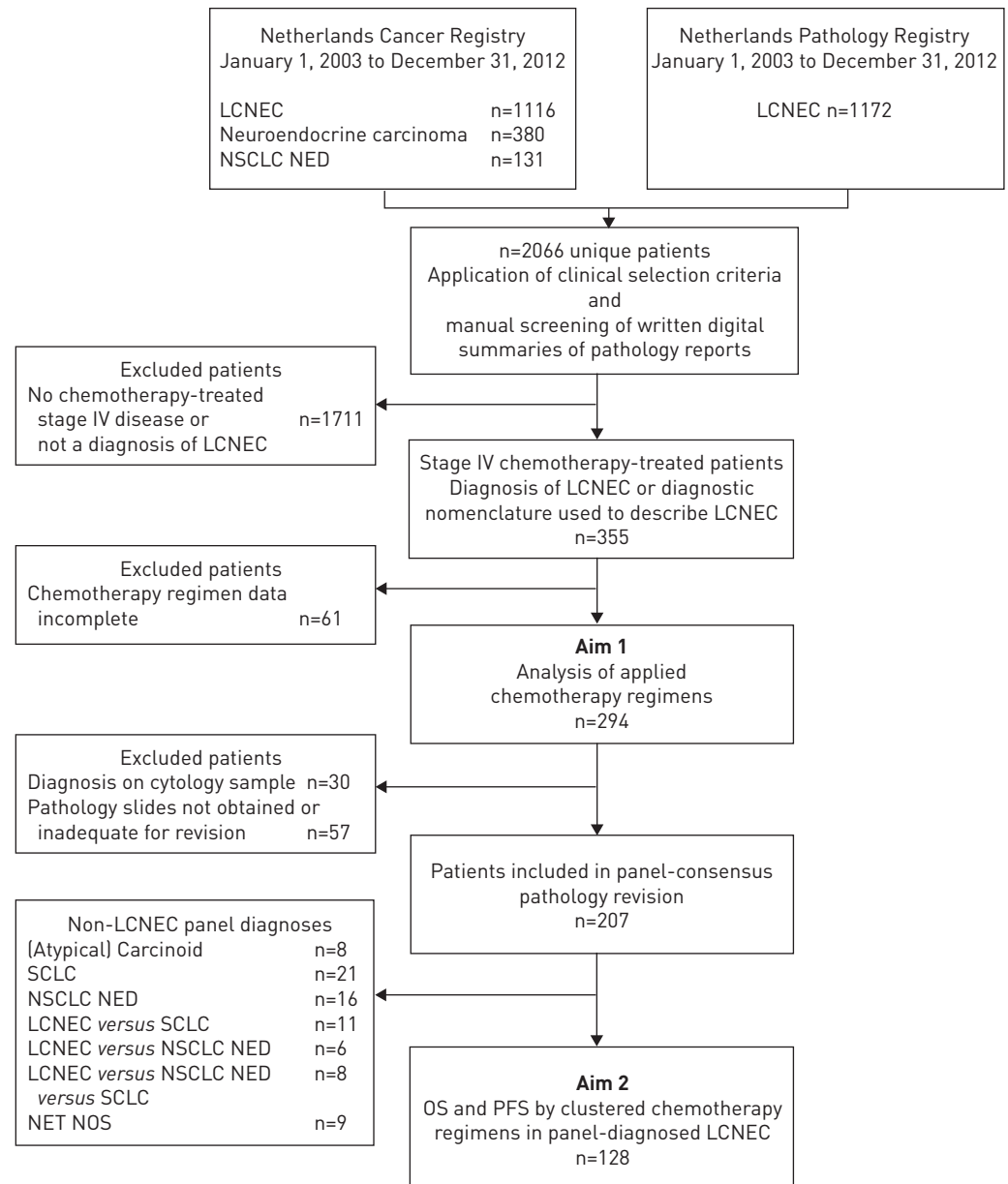
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┌─────────────────────────────────────┐        ┌─────────────────────────────────────┐
│   Netherlands Cancer Registry        │        │   Netherlands Pathology Registry     │
│ January 1, 2003 to December 31, 2012 │        │ January 1, 2003 to December 31, 2012 │
│                                      │        │                                      │
│ LCNEC                      n=1116    │        │          LCNEC n=1172                │
│ Neuroendocrine carcinoma   n=380     │        │                                      │
│ NSCLC NED                  n=131     │        │                                      │
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n=2066 unique patients
Application of clinical selection criteria and
manual screening of written digital
summaries of pathology reports

Excluded patients
No chemotherapy-treated stage IV disease or not a diagnosis of LCNEC   n=1711

Stage IV chemotherapy-treated patients
Diagnosis of LCNEC or diagnostic nomenclature used to describe LCNEC
n=355

Excluded patients
Chemotherapy regimen data incomplete   n=61

Aim 1
Analysis of applied chemotherapy regimens
n=294

Excluded patients
Diagnosis on cytology sample n=30
Pathology slides not obtained or inadequate for revision   n=57

Patients included in panel-consensus pathology revision
n=207

Non-LCNEC panel diagnoses
(Atypical) Carcinoid   n=8
SCLC   n=21
NSCLC NED   n=16
LCNEC *versus* SCLC   n=11
LCNEC *versus* NSCLC NED   n=6
LCNEC *versus* NSCLC NED   n=8
 versus SCLC
NET NOS   n=9

Aim 2
OS and PFS by clustered chemotherapy regimens in panel-diagnosed LCNEC
n=128

FIGURE 1 CONSORT (Consolidated Standards of Reporting Trials) diagram showing inclusion of patients and the performed pathology review. LCNEC: large cell neuroendocrine carcinoma; NSCLC NED: nonsmall cell lung carcinoma with immunohistochemically neuroendocrine differentiation; SCLC: small cell lung carcinoma; NET NOS: neuroendocrine tumour not otherwise specified; OS: overall survival; PFS: progression-free survival.

chemotherapy (5.9 months, 5.0–6.9 months; p=0.011), and significantly longer than patients treated with SCLC-t chemotherapy (6.7 months, 5.0–8.5 months; p=0.012) (figure 2a). In multivariate analysis, including the covariates significant at univariate analyses (sex, age, liver metastasis and number of organs with metastases at diagnosis) (online supplementary figure S2), results remained significant for NSCLC-t *versus* NSCLC-pt treatment (hazard ratio (HR) 2.51, 95% CI 1.39–4.52; p=0.002), and for NSCLC-t *versus* SCLC-t treatment (1.66, 1.08–2.56; p=0.020) (figure 3). Cisplatinum *versus* carboplatinum compounds did not have a significant effect on the treatment outcome data (online supplementary figure S3). Corresponding results for overall survival and PFS in 108 patients with LCNEC in whose tumour samples all WHO 2015 criteria were confirmed are described in online supplementary figures S3, S4 and S5.

Overall survival in panel-consensus LCNEC according to chemotherapy subtype
Patients treated with platinum–gemcitabine chemotherapy had a median overall survival (95% CI) of 7.8 months (5.9–9.6 months), which was significantly longer than for platinum–pemetrexed (5.9 months,

TABLE 1 Clinical characteristics of patients with panel-consensus diagnosed large cell neuroendocrine carcinoma

	Total cohort	Chemotherapy clusters			p-value	
		NSCLC-t	NSCLC-pt	SCLC-t	NSCLC-t *versus* NSCLC-pt	NSCLC-t *versus* SCLC-t
Patients	128 (100)	60 (46)	20 (16)	48 (38)		
Age years	65 (56–71)	64 (56–69)	70 (57–74)	63 (55–70)	0.24[#]	0.88[#]
Male	75 (59)	33 (55)	12 (60)	30 (63)	0.70	0.43
Number of organs with metastases					0.67[¶,+]	**0.012**[¶,+]
1	61 (48)	29 (48)	14 (70)	18 (38)		
2	44 (34)	24 (40)	5 (25)	15 (31)		
3	11 (9)	2 (3)	0 (0)	9 (19)		
>3	12 (9)	5 (8)	1 (5)	6 (13)		
Organ metastases at diagnosis						
Bone	34 (27)	14 (23)	5 (25)	15 (31)	0.88	0.34
Liver	68 (53)	30 (50)	10 (50)	28 (58)	1.00	0.39
Brain	17 (13)	7 (12)	2 (10)	8 (17)	0.57	0.81
Adrenal gland	21 (16)	9 (15)	2 (10)	10 (21)	0.57	0.43
Lung	16 (13)	10 (17)	2 (10)	4 (8)	0.47	0.20
Pleura	2 (2)	1 (2)	0 (0)	1 (2)	1.00[+]	1.00[+]
Lymph node	28 (22)	14 (23)	4 (20)	10 (21)	0.76	0.76
Nonclustered subtype of chemotherapy						
Gemcitabine	46 (36)	46 (76)				
Paclitaxel	7 (5)	7 (12)				
Docetaxel	6 (5)	6 (10)				
Vinorelbine	1 (1)	1 (2)				
Etoposide	48 (38)			48 (100)		
Pemetrexed	20 (16)		20 (100)			
Cycles of chemotherapy					0.30[§]	0.09[§]
1	18 (14)	6 (10)	2 (10)	10 (21)		
2	15 (12)	5 (8)	4 (20)	6 (13)		
3	14 (11)	6 (10)	3 (15)	5 (10)		
4	63 (49)	30 (50)	11 (55)	22 (46)		
>4	16 (13)	11 (18)	0 (0)	5 (10)		
Data missing	2 (2)	2 (3)	0 (0)	0 (0)		
Additional chemotherapy						
Second-line	29 (23)	13 (22)	4 (20)	12 (25)	0.88	0.68
Third-line	6 (5)	3 (5)	1 (5)	2 (4)	1.00[+]	1.00[+]

Data are presented as n (%) or median (interquartile range), unless otherwise stated. Bold type represents statistical significance. NSCLC: nonsmall cell lung carcinoma; NSCLC-t: NSCLC cluster of gemcitabine, paclitaxel, docetaxel and vinorelbine chemotherapy; NSCLC-pt: NSCLC cluster of pemetrexed chemotherapy; SCLC-t: small cell lung carcinoma cluster of etoposide chemotherapy. [#]: Mann–Whitney U-test; [¶]: compared ⩽2 organ metastases with >2 organ metastases; [+]: Fisher exact test; [§]: compared ⩽2 cycles *versus* ⩾3 cycles of chemotherapy, excluding unknown cases.

5.0–6.9 months; p=0.019) and for platinum–etoposide chemotherapy (6.7 months, 5.0–8.5 months; p=0.035) (figure 2b). In multivariate analyses overall survival for gemcitabine was superior to pemetrexed chemotherapy (HR 2.39, 95% CI 1.31–4.35; p=0.004) and a strong trend was observed compared to etoposide (1.54, 0.97–2.43; p=0.066) (figure 3). Paclitaxel-treated patients had a median overall survival of 8.7 months (95% CI 2.7–14.7 months), significantly longer than for pemetrexed chemotherapy (p=0.034), and a strong trend was observed for etoposide chemotherapy (p=0.057) (figure 2b). In multivariate analysis paclitaxel showed superior overall survival compared to pemetrexed chemotherapy (HR 4.04, 95% CI 1.46–11.22; p=0.007) and etoposide chemotherapy treatment (HR 2.60, 95% CI 1.07–6.35; p=0.035) (figure 3).

PFS in panel-consensus LCNEC according to chemotherapy subtype

Data on PFS were available in 119 patients; all except one patient progressed or died during the study period. The median PFS (95% CI) was 4.7 months (4.2–5.3 months). Only NSCLC-pt chemotherapy treated patients had a significantly worse PFS (4.1 months, 3.8–4.5 months; p=0.040) compared to patients treated with NSCLC-pt chemotherapy (figure 4a). Patients treated with gemcitabine chemotherapy had a significantly longer PFS of 5.2 months (4.1–6.2 months) compared to patients treated with NSCLC-pt chemotherapy (p=0.034) (figure 4b). All other comparisons of specific subtypes of chemotherapy showed no significant differences in PFS.
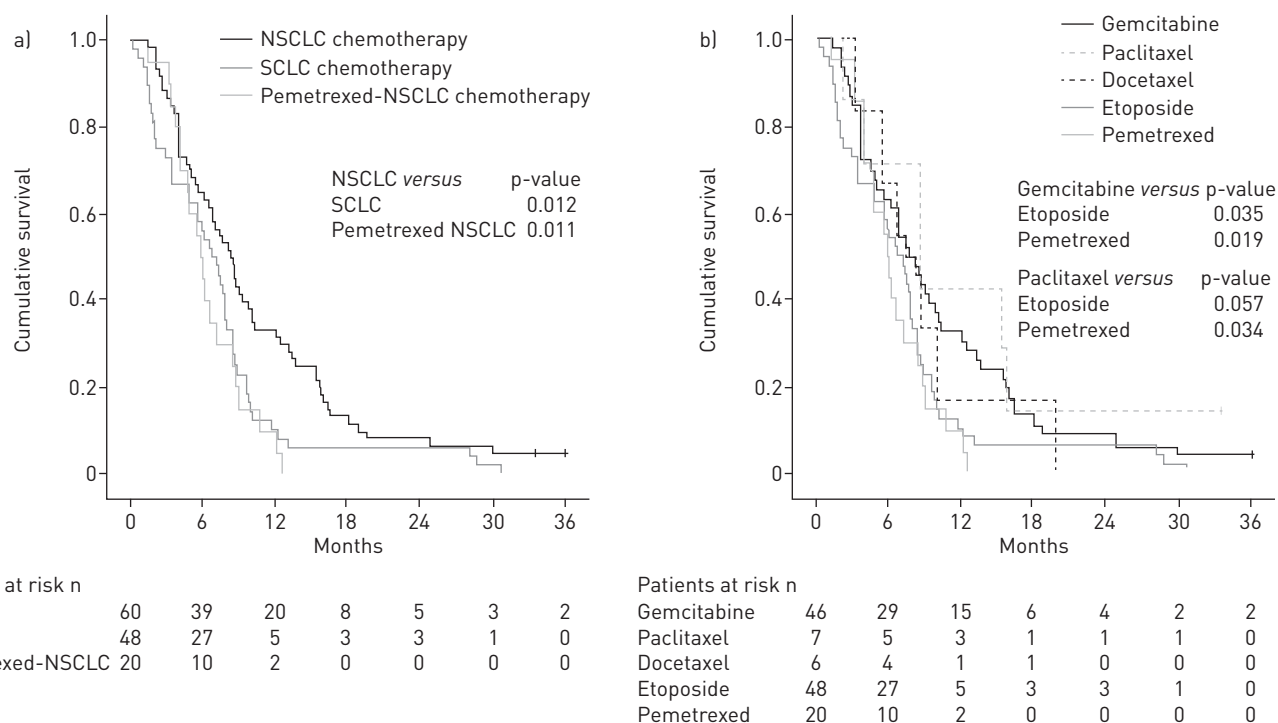
FIGURE 2 Overall survival in panel-consensus diagnosed large cell neuroendocrine carcinoma patients compared for a) chemotherapy clusters and b) subtypes of chemotherapy (excluding vinorelbine). n=128. NSCLC: nonsmall cell lung carcinoma regimen; SCLC: small cell lung carcinoma regimen.

Discussion

Patients treated with doublet combined chemotherapy for metastatic LCNEC have a poor survival and the optimal chemotherapy treatment for LCNEC remains unascertained. Here we report that patients treated with NSCLC-t chemotherapy, mainly gemcitabine, have superior overall survival compared with patients treated with NSCLC-pt chemotherapy. In addition, the combination of NSCLC-t regimens, excluding those containing pemetrexed, showed superior survival compared with etoposide (SCLC-t) chemotherapy. These results contrast with the advised treatment in the ASCO guideline [7].

Chemotherapy treatment for patients with LCNEC changed significantly between 2003 and 2012 in the Netherlands, with a decrease in NSCLC-t chemotherapy and an increase in SCLC-t chemotherapy. This observation corresponds with data accrued from a 2014 questionnaire survey circulated among 21 Dutch pulmonary physicians. In this survey the majority of physicians (80%) would treat LCNEC with SCLC chemotherapy (online supplementary figure S6). We were unable to find specific explanations why the treatment of LCNEC has changed. Treatment preferences may have been influenced by a study published in 2005 describing the favourable response of LCNEC to SCLC-type chemotherapy [17].

Several studies have evaluated chemotherapy in LCNEC, but the reported studies are heterogeneous in case selection and confirmation of the pathology diagnosis (table 2). Two phase II trials, both with pathology review, have been reported. A European trial [11] reported a median overall survival of 8.0 months (95% CI 3.7–7.9 months), a PFS of 5.0 months (95% CI 4.0–7.9 months) and an ORR of 34% in 29 patients treated with platinum–etoposide chemotherapy. In a Japanese trial [12], a median overall survival of 12.6 (95% CI 9.3–16.0) months, PFS of 5.8 (95% CI 3.8–7.8) months and an ORR of 47% was reported for treatment with platinum–irinotecan (n=30). In retrospectively evaluated cohorts of LCNEC patients, the reported ORR for platinum–etoposide chemotherapy ranged from 37% to 73% and overall survival ranged from 8.4 to 16.5 months [17–20]. Treatment outcomes for SCLC- and NSCLC-type chemotherapy for LCNEC has previously been evaluated; 27 patients showed an improved survival for platinum–etoposide chemotherapy compared to a combination of NSCLC regimens [17]. Conversely, evaluation of an additional 26 patients showed a significantly lower overall survival for platinum–etoposide chemotherapy compared to a combination of NSCLC regimens [19]. Because NSCLC regimens are frequently combined for analysis, there is a lack of data on subtype-specific overall survival and PFS. The reported ORRs for platinum combined with gemcitabine, docetaxel and paclitaxel are 41% (n=17), 77% (n=9) and 81% (n=11), respectively [18, 21].

Platinum–pemetrexed chemotherapy is advised as first-line treatment in patients with metastatic nonsquamous NSCLC [7]. However, platinum–pemetrexed chemotherapy showed inferior results compared
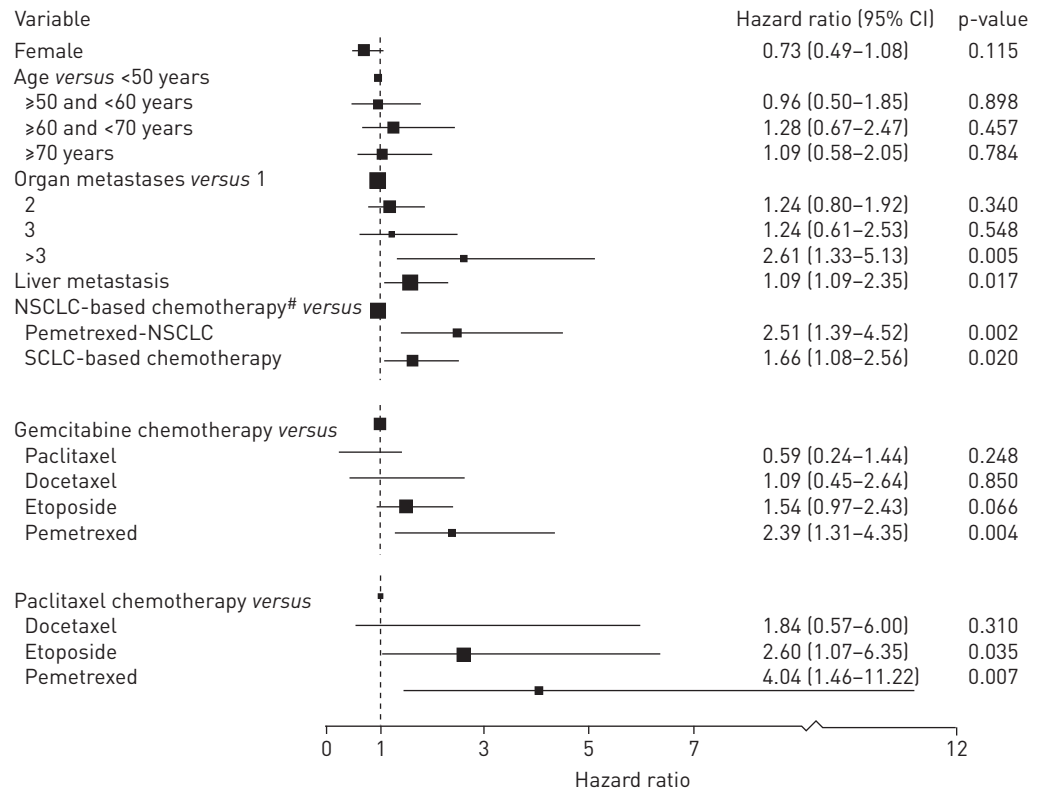
Variable	Hazard ratio (95% CI)	p-value
Female	0.73 (0.49–1.08)	0.115
Age versus <50 years		
≥50 and <60 years	0.96 (0.50–1.85)	0.898
≥60 and <70 years	1.28 (0.67–2.47)	0.457
≥70 years	1.09 (0.58–2.05)	0.784
Organ metastases versus 1		
2	1.24 (0.80–1.92)	0.340
3	1.24 (0.61–2.53)	0.548
>3	2.61 (1.33–5.13)	0.005
Liver metastasis	1.09 (1.09–2.35)	0.017
NSCLC-based chemotherapy# versus		
Pemetrexed-NSCLC	2.51 (1.39–4.52)	0.002
SCLC-based chemotherapy	1.66 (1.08–2.56)	0.020
Gemcitabine chemotherapy versus		
Paclitaxel	0.59 (0.24–1.44)	0.248
Docetaxel	1.09 (0.45–2.64)	0.850
Etoposide	1.54 (0.97–2.43)	0.066
Pemetrexed	2.39 (1.31–4.35)	0.004
Paclitaxel chemotherapy versus		
Docetaxel	1.84 (0.57–6.00)	0.310
Etoposide	2.60 (1.07–6.35)	0.035
Pemetrexed	4.04 (1.46–11.22)	0.007

FIGURE 3 Three multivariate models are presented for clustered chemotherapy, platinum–gemcitabine and platinum–paclitaxel chemotherapy in panel-consensus large cell neuroendocrine carcinoma. n=128. NSCLC: nonsmall cell lung carcinoma; SCLC: small cell lung carcinoma. #: excluding vinorelbine.

Patients at risk n

NSCLC	56	48	24	11	4	2
SCLC	43	31	13	3	1	0
Pemetrexed-NSCLC	20	16	3	0	0	0

Patients at risk n

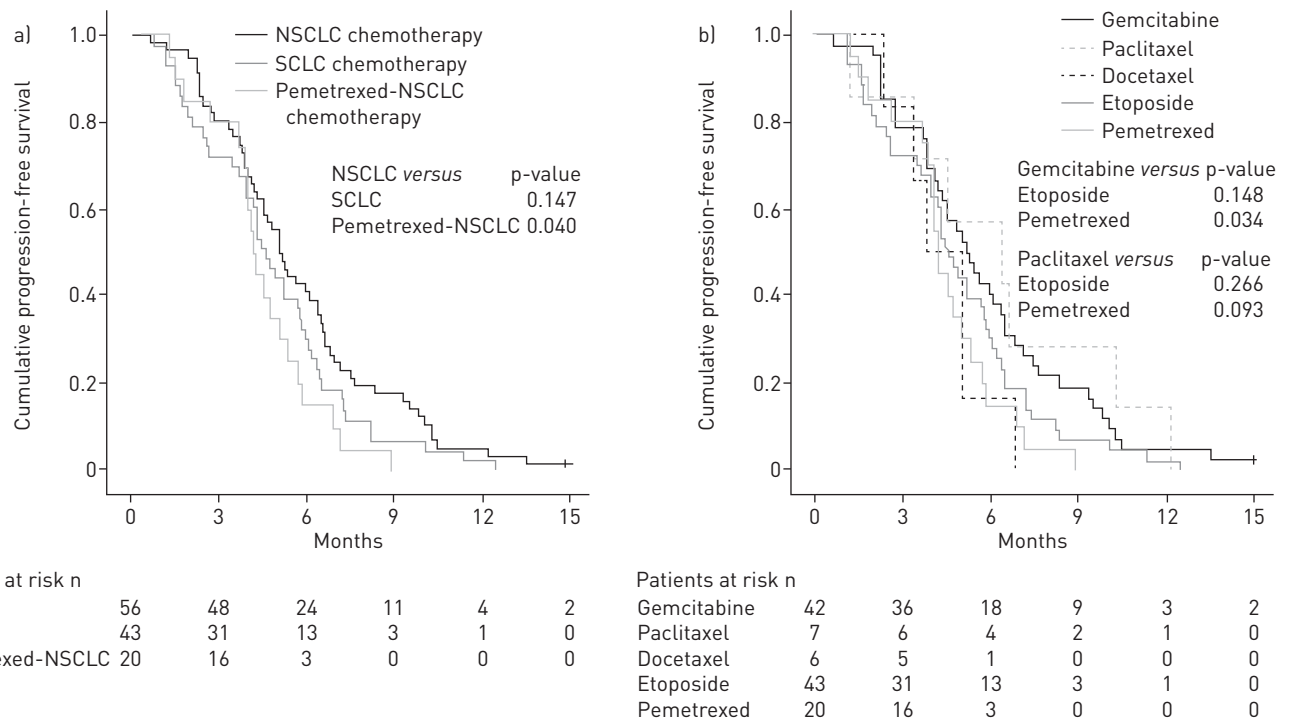|						
Gemcitabine	42	36	18	9	3	2
Paclitaxel	7	6	4	2	1	0
Docetaxel	6	5	1	0	0	0
Etoposide	43	31	13	3	1	0
Pemetrexed	20	16	3	0	0	0

FIGURE 4 Progression-free survival compared for a) chemotherapy clusters and b) subtypes of chemotherapy (excluding vinorelbine) in panel-consensus large cell neuroendocrine carcinoma. n=119. NSCLC: nonsmall cell lung carcinoma chemotherapy regimen; SCLC: small cell lung carcinoma chemotherapy regimen.

TABLE 2 Overview of response to chemotherapy in advanced-stage large cell neuroendocrine carcinoma disease

First author [reference]	Design	Panel review (pathologists n)	Inclusion period	NSCLC chemotherapy			SCLC chemotherapy		
				Patients n	ORR %	OS months	Patients n	ORR %	OS months
Le Treut [11]	P	Yes (?)	2004–2009				29	34	8.0
Niho [12]	P	Yes (6)	2005–2011				30	47	12.6
Metro [20]	R	No revision	Unclear				37	43	8.4
Naidoo [19]	R	Yes (3)	2006–2013	11[#]	0[#]	19.5	26[¶]	37	8.3
Sun [18]	R	Yes (?)	2001–2010	34[+]	50	9.2	11	73	16.5
Rossi [17]	R	Yes (3)	1990–2004	15	0	21	12	50	51
Fujiwara [21]	R	No (1)	1999–2006	9[§]	77		13	46	
Derks (present study)	R	Yes (3)	2003–2012	60 (NSCLC)		8.5	48	33	6.7
				20 (pemetrexed-NSCLC)		5.9			

Studies including patients treated with chemoradiotherapy are not shown. NSCLC: nonsmall cell lung carcinoma; SCLC: small cell lung carcinoma; ORR: objective response rate; OS: overall survival; P: prospective; R: retrospective. [#]: four patients were evaluated according to response evaluation criteria in solid tumours (RECIST), including temozolomide (n=2), pemetrexed (n=1) and platinum combined with everolimus (n=1); [¶]: 19 patients were evaluated according to RECIST criteria; [+]: including gemcitabine–platinum (n=17), taxane–platinum (n=4), tyrosine kinase inhibitor (n=2) and other platinum (n=11); [§]: taxane combined with platinum (n=7), taxane monotherapy (n=1) and platinum–vinorelbine (n=1).

to platinum–etoposide in SCLC [22], a tumour biologically closely related to LCNEC. The poor therapeutic response of pemetrexed may be due to the reported high expression of the thymidylate synthesis (TS) gene in LCNEC. Increased TS expression is suggested to be related to resistance to pemetrexed therapy [23–25]. The increased tendency for pemetrexed resistance coupled with the reported clinical observations suggest that pemetrexed should not be used in patients with LCNEC.

Molecular changes in LCNEC and SCLC have been described. SCLC is characterised by RB1 and TP53 gene mutations, whereas LCNEC was characterised by mutually exclusive RB1 and TP53 gene inactivation *versus* a combination of STK11/KRAS/KEAP1 gene mutations [9, 26]. In future studies it would be of interest to analyse these patterns to investigate whether the molecular background corresponds with responses to different chemotherapy regimens [9].

This study has several limitations. First, it is a retrospective study and chemotherapy data could not be retrieved in all patients. However, the exclusion of patients was random and not by selection, as evidenced by the similar overall survival and age range of excluded patients compared to the analysed patient cohort (online supplementary tables S4 and S5). Second, information on WHO performance score was lacking, and this may have confounded reported overall survival. We observed no differences in overall survival for treatment with cisplatinum or carboplatinum chemotherapy (online supplementary figure S2). Third, completion of chemotherapy cycles differed slightly between the NSCLC-t and SCLC-t treatments. Nevertheless, up to 62% of patients completed four or more cycles of chemotherapy and this was not significantly different between treatment groups. Fourth, the reported overall survival for chemotherapy-treated subtypes may have been confounded by strong therapeutic effects of second-line treatment. However, in the presented cohort the frequency of second-line treatment was relatively low (23%) and not statistically different among clustered chemotherapy subtypes (table 1). The frequency of second-line treatment is lower than reported in a Japanese phase II trial (86%) [12], but not much lower than reported for daily clinical practice in lung cancer (32%) [27]. Finally, data on PFS were obtained retrospectively and could not be formally evaluated by the RECIST (response evaluation criteria in solid tumours) criteria, as this was analysed in a real-world setting and not in a clinical trial. Response evaluation was not standardised and incomplete in 40% of patients; therefore, these data are not reported.

In conclusion, we present the largest series of patients with pathology-reviewed metastatic LCNEC to date, and show that NSCLC-t regimens, mainly platinum–gemcitabine chemotherapy, are superior to platinum–pemetrexed and platinum–etoposide treatment. These results need prospective evaluation, ideally in a randomised trial, in centrally confirmed LCNEC.

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