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ORIGINAL REPORT

Role of Chemotherapy and the Receptor Tyrosine Kinases KIT, PDGFRα, PDGFRβ, and Met in Large-Cell Neuroendocrine Carcinoma of the Lung

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ABSTRACT

Purpose
Pulmonary large-cell neuroendocrine carcinoma (LCNEC) is a relatively uncommon, high-grade neuroendocrine tumor sharing several features with small-cell lung carcinoma (SCLC) but currently considered as a variant of non-SCLC and accordingly treated with poor results. Little is known about the optimal therapy of LCNEC and the possible therapeutic molecular targets.

Patients and Methods
We reviewed 83 patients with pure pulmonary LCNEC to investigate their clinicopathologic features, therapeutic strategy, and immunohistochemical expression and the mutational status of the receptor tyrosine kinases (RTKs) KIT, PDGFRα, PDGFRβ, and Met.

Results
LCNEC histology predicted a dismal outcome (overall median survival, 17 months) even in stage I patients (5-year survival rate, 33%). LCNEC strongly expressed RTKs (KIT in 62.7% of patients, PDGFRα in 60.2%, PDGFRβ in 81.9%, and Met in 47%), but no mutations were detected in the exons encoding for the relevant juxtamembrane domains. Tumor stage and size ($\geq$ 3 cm) and Met expression were significantly correlated with survival. At univariate and multivariate analysis, SCLC-based chemotherapy (platinum-etoposide) was the most important variable correlating with survival, both in the adjuvant and metastatic settings ($P < .0001$).

Conclusion
Pulmonary LCNEC represents an aggressive tumor requiring multimodal treatment even for resectable stage I disease, and LCNEC seems to respond to adjuvant platinum-etoposide–based chemotherapy. Patients who received this therapy had the best survival rate. Despite our failure in finding mutational events in the tested RTKs, the strong expression of KIT, PDGFRα, PDGFRβ, and Met in tumor cells suggests an important role of these RTKs in LCNEC, and these RTKs seem to be attractive therapeutic targets.

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INTRODUCTION

Large-cell neuroendocrine (NE) carcinoma (LCNEC) is the most recently recognized member of the family of pulmonary NE tumors and belongs, together with small-cell lung cancer (SCLC), to the group of high-grade NE tumors. First described by Travis et al,[1] LCNEC accounts for approximately 3% of all pulmonary malignancies.[2,3] Because there is no clear-cut evidence concerning the optimal treatment for LCNEC and because therapeutic approaches adopted for SCLC have not proved to be effective for patients with LCNEC, the new WHO classification of lung tumors has preferred to consider LCNEC as a subtype of large-cell carcinoma (LCC).[4] Nonetheless, the vast majority of previous studies,[5-16] with a few exceptions,[17,18] have found that LCNEC

predicted a poorer survival than expected for stage-matched non-SCLC (NSCLC), approaching the dismal outcome of SCLC.[2] In addition, there is an increasing body of evidence suggesting that LCNEC shares many similarities with SCLC on morphologic,[19,20] immunohistochemical,[21,22] and molecular grounds.[23-37] In line with other authors,[38,39] we recently found that a significant percentage of LCNECs overexpressed the KIT receptor tyrosine kinase (RTK),[40,41] a transmembrane type III tyrosine kinase encoded by the proto-oncogene c-*kit* and structurally related to platelet-derived growth factor receptors (PDGRF)[42]; however, we failed to find a significant expression in other NSCLCs and carcinoids.[40] The molecular pathway promoted by KIT is a well-known functional autocrine growth loop inducing and maintaining tumor cell proliferation and blocking apoptosis in SCLC,[43-45] but little is known about the role of RTKs in LCNEC. RTKs are key molecules in normal cellular differentiation, but they are commonly deregulated or mutated in human cancers and represent attractive molecular targets for alternative therapies using effective and safe selective inhibitors.[46] Briefly, PDGFRs occur as alpha and beta homodimers or an alpha/beta heterodimer, and the binding with the relevant ligand platelet-derived growth factor (occurring as a combination of subunits $\alpha\alpha$, $\alpha\beta$, and $\beta\beta$) results in homodimerization of the receptors and phosphorylation of specific tyrosine residues.[47] When activated, KIT and PDGFRs promote a cascade of intracytoplasmic signals that are essential to the regulation of cell growth of several cell lines, and the aberrant reactivation of these pathways is strongly involved in the carcinogenesis of different neoplasms, including lung cancer.[47] Met is another RTK serving as a high-affinity receptor for hepatocyte growth factor (HGF), a disulfide-linked heterodimeric molecule mainly produced by mesenchymal cells.[48,49] Signaling through the Met/HGF pathway has been shown to lead to tumor growth, angiogenesis, and the development of an invasive phenotype in several malignancies.[50] All these RTKs play an important role in lung cancer oncogenesis,[51-53] especially in SCLC, for which preclinical investigations demonstrated promising cytostatic results using selective RTK inhibitors.[54-60] The present study was undertaken to achieve more accurate insights on the clinicopathologic features of a large series of surgically resected LCNEC patients, focusing on the following two specific points: (1) the efficacy of different chemotherapeutic regimens in the treatment of this controversial entity and (2) the prognostic and possibly therapeutic value of the RTKs KIT, PDGFRα, PDGFRβ, and Met.

PATIENTS AND METHODS

Clinical and Pathologic Information

The files of the Sections of Pathologic Anatomy of the University of Modena and Reggio Emilia and of the St Maria Nuova Hospital of Reggio Emilia were searched for patients who underwent surgery for pulmonary LCC for whom the presence of NE features at morphologic examination were reported (such as organoid, trabecular, and/or basaloid growth pattern, nuclear palisading, and rosette-like structures). Patients who underwent surgery and who had a previous diagnosis of small-cell carcinoma of intermediate type according to the previous WHO classification of lung tumors[61] were also reviewed. Patients with other known primary tumors were excluded from the study. Overall, among 4,879 patients with primary surgically resected lung tumors diagnosed from January 1990 to December 2004, a total of 348 carcinomas were initially collected. All of the slides were then reviewed at a multihead microscope by three pathologists (G.R., A.C., and E.B.). Tumors were reclassified according to the criteria set by the new WHO lung tumors classification.[4] Briefly, LCNEC is defined as a tumor with the following histologic criteria: (1) NE morphology (organoid nesting, trabecular, rosette-like, and palisading patterns); (2) mitotic activity of more than 11 mitoses per 10 high-power fields (2 mm^2); (3) presence of necrosis (usually large areas); (4) cytologic features of NSCLC (cells of large size and polygonal in shape, low nuclear to cytoplasmic ratio, and vesicular or fine nuclear chromatin with frequent nucleoli; Fig 1A); and (5) strong immunoreactivity for at least one NE marker (chromogranin A, synaptophysin, or neural cell adhesion molecule [NCAM]/CD56; Figs 1B and 1C). After histologic review and immunohistochemical analysis, 139 of the carcinomas were reinterpreted as poorly differentiated adenocarcinomas (ADC), 95 were reinterpreted as poorly differentiated squamous cell carcinomas (SqC), 10 were reinterpreted as basaloid carcinomas, and six were reinterpreted as

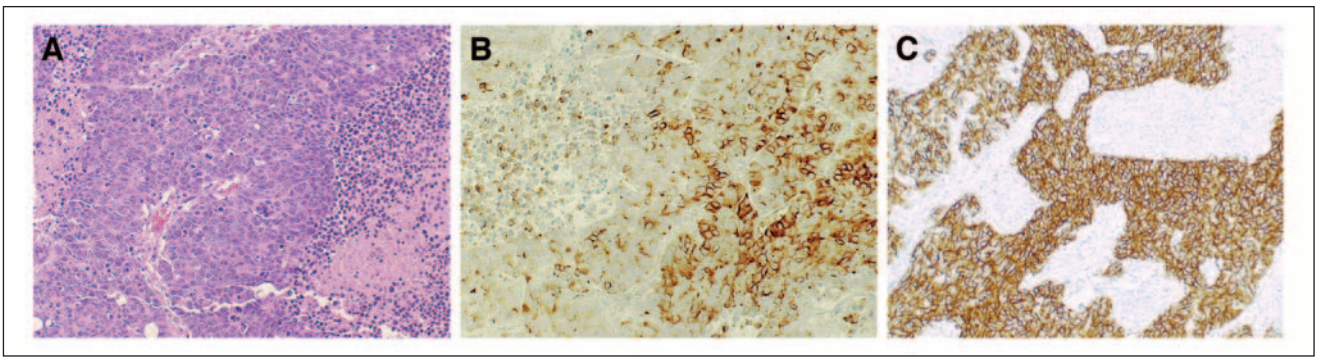

Fig 1. Large-cell neuroendocrine carcinoma is a high-grade tumor with neuroendocrine morphology, high mitotic rate, and large areas of necrosis (A, hematoxylin and eosin), showing positive staining for neuroendocrine markers (B, chromogranin; and C, neural cell adhesion molecule/CD56).

Table 1. Details of Antibodies Used for Immunohistochemistry

Antibody	Clone	Source	Dilution	Antigen Retrieval
Chromogranin A	mAb, DAK-A3	Dako, Glostrup, Denmark	1:100	MW*
Synaptophysin	pAb	Ventana, Tucson, AZ	1:100	MW
CD56	mAb, 123C3	NeoMarkers, San Ramon, CA	1:100	MW
CD117	pAb	Dako, Glostrup, Denmark	1:200	None
SCF	pAb	Santa Cruz Biotechnology, Santa Cruz, CA	1:40	MW
PDGFRα	pAb	Santa Cruz Biotechnology, Santa Cruz, CA	1:200	MW
PDGFRβ	pAb	Santa Cruz Biotechnology, Santa Cruz, CA	1:200	MW
Met	mAb, 8F11	Novocastra, Newcastle-upon-Tyne, UK	1:100	MW

Abbreviations: SCF, stem-cell factor; PDGFR, platelet-derived growth factor receptor; mAb, monoclonal antibody; pAb, polyclonal antibody; MW, microwave; UK, United Kingdom.
*Thirty minutes in 0.01 mol/L citrate buffer pH 7.8.

SCLC. Thus, 98 patients met the diagnostic criteria for LCNEC. However, 15 LCNECs were combined with other histotypes (10 with SCLC, reclassified as combined SCLC, four with ADC, and one with SqC) and then reclassified as combined LCNEC. Thus, 83 carcinomas presenting as pure LCNEC were finally selected for the current study. All these carcinomas consisted of a surgical specimen (two wedge resections, 80 lobectomies, and one pneumonectomy) and were routinely formalin fixed and paraffin embedded. A mean of 4.5 hematoxylin and eosin–stained slides (range, three to eight slides) per tumor were available. Clinical data were collected from pathologic reports, clinical charts, or referring physicians or directly from the patients' families. Patients who received chemotherapy in the adjuvant or metastatic setting were subdivided into the following two main groups: patients who received standard chemotherapy for SCLC (platinum + etoposide ± radiotherapy) and patients who received chemotherapy it for NSCLC (different combinations of platinum, gemcitabine, taxanes, and vinorelbine ± radiotherapy). The following data were recorded: age, sex, smoking habit, main clinical symptoms, tumor size, tumor location, stage, and follow-up (calculated from the date of surgery). Staging was evaluated according to the American Joint Committee on Cancer.[62]

Immunohistochemistry

For each patient, 4-μm–thick sections were obtained from a representative block. Sections were air dried overnight at 37°C and then deparaffinized in xylene and rehydrated through a decreasing concentration of alcohol to water. Endogenous peroxidase activity was blocked by immersion for 10 minutes with 3% hydrogen peroxide (H_2O_2) in methanol. Incubation with primary antibodies was accomplished with a modified streptavidin-biotin-peroxidase technique using an automated immunostainer (Ventana, Strasbourg, France); 3′-3diaminobenzidine was used as the chromogene, and Harris's hematoxylin was used as the counterstain. The antibodies used in the study and their technical characteristics are listed in Table 1. Negative and positive controls were included in each batch.

Table 2. Oligonucleotide Primers Used

Gene and Exons	Primer	Fragment Size (bp)	Annealing Temperature (°C)
c-kit			
Exon 9	Forward 5′-ATG CTC TGC TTC TGT ACT GCC-3′ Reverse 5′-CAG AGC CTA AAC ATC CCC TTA-3′	238	58
Exon 11	Forward 5′-CTA TTT TTC CCT TTC TCC CC-3′ Reverse 5′-TAC CCA AAA AGG TGA CAT GG-3′	193	53
PDGFRα			
Exon 12	Forward 5′-TCC AGT CAC TGT GCT GCT TC-3′ Reverse 5′-GCA AGG GAA AAG GGA GTC TT-3′	260	56
PDGFRβ			
Exon 12	Forward 5′-TAA TTC CTG GGG TTG GTC CTC-3′ Reverse 5′-AAC TTG AGT CCC CAC ACT GCC-3′	174	52
Exon 14	Forward 5′-GGG GCA GAA GAG TCA GAA TAG-3′ Reverse 5′GGA GTG TGC TGT TGT GCA AG-3′	300	63
Exon 18	Forward 5′-CCC AAA GCC CTT GAC ATG AAG-3′ Reverse 5′-ACT GGT CAG GAG GGA ATC TG-3′	274	63
c-met			
Exon 14	Forward 5′-TTC TGG GCA CTG GGT CAA AGT-3′ Reverse 5′-AAT GTC ACA ACC CAC TGA GGT-3′	282	58

Abbreviations: PDGFR, platelet-derived growth factor receptor; bp, base pair.

For each antibody, the percentage of positive cells and the intensity of staining (0, negative; 1+, weak; 2+, moderate; and 3+, strong) were recorded. A tumor was considered positive for NE markers if more than 10% of the neoplastic cells reacted with an intensity of 2+ or greater on the relevant subcellular localization (cytoplasmic for chromogranin and synaptophysin; cytoplasmic and membranous for NCAM/CD56). At least 30% of positive cells with at least 2+ intensity were recorded to achieve positivity for KIT, Met, PDGFRα, PDGFRβ, and stem-cell factor (SCF).

Mutational Analysis

Several 5-μm–thick sections obtained from a representative paraffin-embedded block were deparaffinized by xylene, and tumor DNA was extracted using a laser-capture microdissection method (LaserScissor-PRO300; Olympus, Tokyo, Japan). Microdissected tumor cells were subject to proteinase K treatment in an extraction buffer (50 mmol/L Tris-HCl, pH 8.0; 1 mmol/L EDTA; and 0.5% Tween-20) and then incubated overnight at 37°C. Polymerase chain reaction (PCR) was performed in 10-μL reactions containing 1.0 μL DNA, 10 mmol/L Tris-HCl (pH 8.3), 40 mmol/L KCl, 1.0 to 1.5 mmol/L $MgCl_2$, 200 mmol/L dNTP, 20 pM of each primer, and 0.25 U Platinum Taq polymerase (Invitrogen, Carlsbad, CA). PCR reaction was carried out on Uno II Thermoblock (Biometra, Gottingen, Germany). Initial denaturation at 94°C for 3 minutes was followed by 41 cycles and a final extension step (5 minutes at 72°C). The cycles included denaturation at 95°C for 1 minute, annealing at 55 to 58°C for 1 minute, and extension at 72°C for 2 minutes. The amplified DNA was electrophoresed on 1% low-melt agarose gel for 1 hour. The amplification products were then excised from the gel and purified by using Wizard PCR Preps-DNA Purification System (Promegar Corp, Madison WI) as indicated by the manufacturer's instructions. PCR products were then sequenced in both directions with BigDye Terminator (Applied Biosystems, Weiterstadt, Germany) sequencing kit using the same primers as used for PCR. PCR products were finally purified by Centri-Sep Spin Columns and subsequently analyzed using the ABI Prism 310 sequence analyzer (Applied Biosystems). The forward and reverse oligonucleotide primers used to amplify *c-kit* exons 9 and 11, *PDGFR*α exon 12, *PDGFR*β exons 12, 14, and 18, and *c-met* exon 14 are listed in Table 2.

Table 3. Clinicopathologic Characteristics of Patients With LCNEC

Characteristic	Patients (N = 83) No.	%
Sex		
Male	73	88
Female	10	12
Smoking habit		
Yes	80	96.4
No	3	3.6
Stage		
IA	16	19.3
IB	38	45.8
IIA	8	9.6
IIB	8	9.6
IIIA	10	12
IIIB	3	3.6
LNF involvement		
LNF negative	62	74.7
LNF positive	21	25.3
Tumor size		
≤ 3 cm	33	39.8
> 3 cm	50	60.2
Tumor site		
Central	19	22.9
Peripheral	64	77.1
First documented site of metastases, n = 54		
Brain	23	
Liver	12	
Bone	11	
Lung/mediastinum	5	
Adrenal gland	3	

Abbreviations: LCNEC, large-cell neuroendocrine carcinoma; LNF, lymph node.

Statistical Analysis

The correlation between clinicopathologic and immunohistochemical variables was performed using contingency table methods and tested for significance using the Pearson's χ^2 test. Survival curves were evaluated using the Kaplan-Meier method, and statistical significance was estimated by the log-rank test. Univariate and multivariate relative risks were calculated using Cox proportional hazards regression (SPSS version 10.0; SPSS Inc, Chicago, IL). A difference of $P < .05$ was considered as significant.

RESULTS

Clinical and Pathologic Findings

The most relevant clinicopathologic features are listed in Table 3. Patients consisted of 73 males and 10 females, with a median age at diagnosis of 67 years (mean, 64.8 years; range, 41 to 89 years). Eighty patients (96.4%) were smokers. The main symptoms were hemoptysis (n = 25), chest pain (n = 18), dyspnea (n = 13), compulsive cough (n = 13), and weight loss (n = 11); tumor was incidentally detected in only three patients. Fifty-four patients (65.1%) were pathologically staged as having stage I disease (16 patients with stage IA and 38 patients with stage IB), 16 patients (19.3%) had stage II disease (eight patients with stage IIA and eight patients with stage IIB), and the remaining 13 patients (15.7%) had stage III disease (10 with stage IIIA and three with stage IIIB). All the patients underwent surgical lymph node dissection, and 21 (25.3%) had lymph node involvement. Metastases were documented in 54 patients, with brain (n = 23), liver (n = 12), and bone (n = 11) resulting as the most commonly involved sites. In 64 patients (77.1%), the tumor was peripherally located, whereas it appeared as a central bronchial mass in 19 patients. Median tumor size was 4 cm (mean, 4.1 cm; range, 1 to 9 cm), and upper lobes were more commonly affected. Of the 83 patients, only 44 (53%) were initially classified as having LCNEC, whereas the other original pathologic diagnoses included poorly differentiated SqC and ADC in 13 and 12 patients, respectively, undifferentiated LCC in six

patients, intermediate-type small-cell carcinoma in six patients, and atypical carcinoid in the remaining two patients.

Fifty-seven patients (68.7%) died of the disease. Overall, median follow-up time was 17 months (mean, 25.35 months; range, 1 to 125 months). Median follow-up for stage I, II, and III LCNEC patients was 26, 24, and 11 months, respectively. The 5-year survival rate was 27.6% overall (33% in stage I patients, 23% in stage II patients, and 8% in stage III patients). Among clinicopathologic parameters, tumor stage (Fig 2) and size (< 3 *v* ≥ 3 cm; Fig 3) were the only factors significantly related to survival ($P = .0394$ and $P = .0039$, respectively). Twenty-eight patients underwent adjuvant chemotherapy, but the 13 patients who received an SCLC-based regimen presented with a significantly better survival than the patients who received drugs combinations (cisplatin + gemcitabine in eight patients, carboplatin + paclitaxel in four patients, and cisplatin + vinorelbine in three patients) that are more frequently used in NSCLC (median survival, 42 *v* 11 months, respectively; $P < .0001$; Fig 4). In particular, stage I LCNEC patients who received an SCLC-based adjuvant chemotherapy had the best prognosis ($P < .0001$; Fig 5). Even in metastatic disease, patients receiving SCLC-based chemotherapy (12 patients; three also received radiotherapy) had a significantly better survival than the 15 LCNEC patients who received therapeutic regimens for NSCLC (cisplatin + gemcitabine in 10 patients, carboplatin + paclitaxel in three patients, and gemcitabine only in two patients; six of these patients received radiotherapy; median survival, 51 *v* 21 months, respectively; $P < .0001$; Fig 6). In the metastatic setting, the response rate was 29%, but complete (n = 2) or partial (n = 4) responses to chemotherapy were observed only in patients receiving SCLC-based regimen.

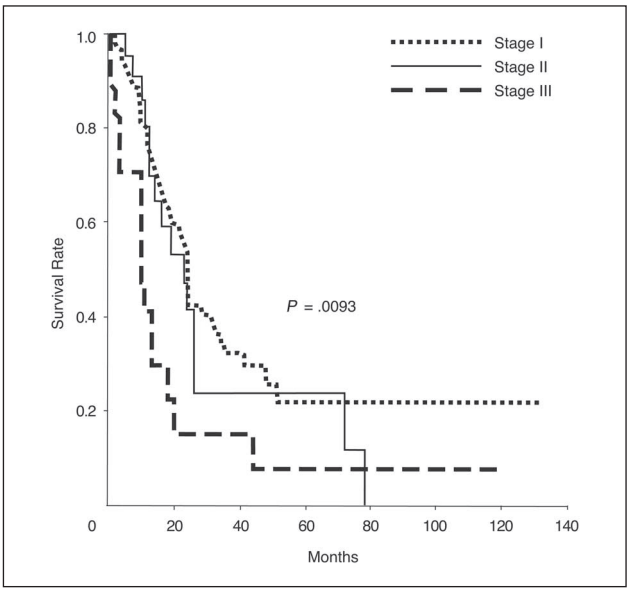

Fig 2. Kaplan-Meier curves for overall survival stratified according to tumor stage. The median overall survival times were 24, 23, and 10 months for patients with large-cell neuroendocrine carcinoma in stage I, II, and III, respectively.

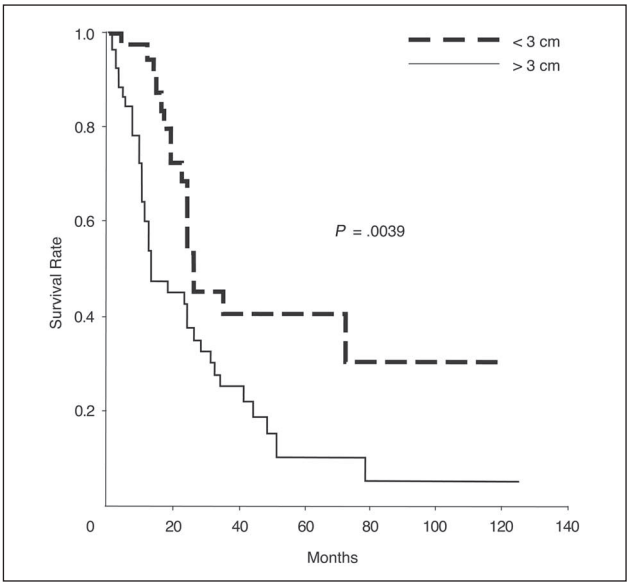

Fig 3. Kaplan-Meier curves for overall survival stratified according to tumor size. The median overall survival times were 24 and 13 months for patients with large-cell neuroendocrine carcinomas less than 3 cm and more than 3 cm, respectively.

Immunohistochemical and Molecular Findings

The distribution of NE markers and RTK expression is presented in Table 4. NCAM/CD56 was expressed in 77 LCNECs (92.8%), chromogranin was expressed in 54 LCNECs (65.1%), and synaptophysin was expressed in 44 LCNECs (53%). Among NE markers, only chromogranin expression was significantly correlated with tumor size ($P = .033$), and patients with chromogranin-positive LCNEC tended to have a lower stage of disease ($P = .078$).

Among RTKs (Fig 7), PDGFRβ was strongly expressed in 68 LCNECs (81.9%), KIT was expressed in 52 LCNECs (62.7%), PDGFRα was expressed in 50 LCNECs (60.2%), and Met was expressed in 39 LCNECs (47%). SCF was expressed in the cytoplasm of tumor cells in 47 LCNECs (56.6%), and all SCF-positive LCNECs coexpressed KIT. With regards to prognosis, Met was the only immunohistochemical marker significantly correlated with overall survival ($P = .0352$; Fig 8). No significant correlation was noted when RTK expression results were matched with survival and other clinicopathologic parameters (patient age < 65 *v* ≥ 65 years, tumor size, lymph node involvement, and disease stage).

At sequencing analysis, no mutational events were observed in the tested exons of different RTKs. Patients with Met-negative LCNEC who received adjuvant platinum + etoposide chemotherapy had the best survival rate (median, 103 months), whereas Met-positive LCNEC patients who underwent NSCLC-based adjuvant chemotherapy had the worst overall survival (median, 10 months; $P < .0001$).

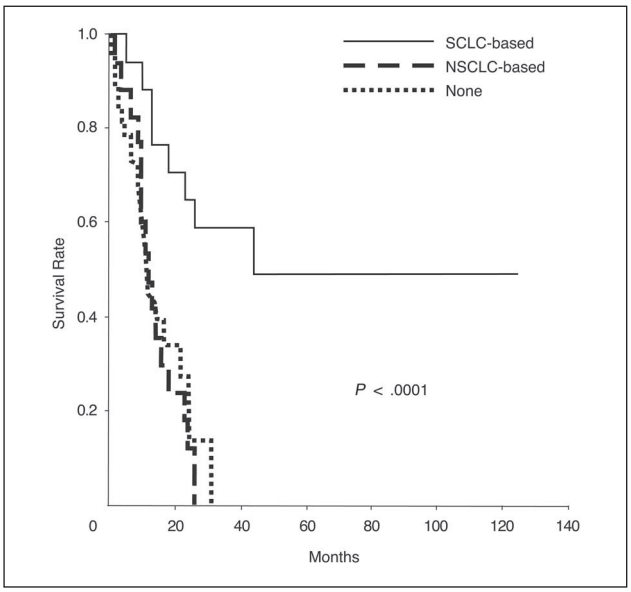

Fig 4. Kaplan-Meier curves for overall survival stratified according to different chemotherapeutic regimens in the adjuvant setting. The median overall survival times were 44, 12, and 12 months for patients with large-cell neuroendocrine carcinoma who received platinum plus etoposide, gemcitabine plus taxanes, and no adjuvant chemotherapy, respectively. NSCLC, non–small-cell lung cancer; SCLC, small-cell lung cancer.

In the multivariate Cox proportional hazards analyses listed in Table 5, tumor size remained significantly related to survival ($P = .013$), whereas disease stage and Met expression were not. However, SCLC-based chemotherapy, both in the adjuvant and metastatic setting, seemed to be the most important survival-related variable ($P < .0001$).

DISCUSSION

The advent of gene expression profiling investigations and the current availability of effective molecular targeted therapies in lung cancer have reinforced the key role played by the exact definition of lung tumor histotype in therapeutic strategies (eg, ADCs are more responsive to anti–epidermal growth factor receptor molecules gefitinib/erlotinib).[63] In addition, it is well known that there are several NSCLC histotypes related to a dismal outcome independently from the disease stage, such as sarcomatoid and basaloid,[4] that require a more aggressive therapeutic approach. In preparing this work, we focused on the following two major questions: (1) Can LCNEC be considered akin to SCLC with regards to patient outcome and chemotherapy response? (2) Is there a role for RTKs in future therapeutic strategies? Thus, we collected a large series of pure surgically resected LCNEC of the lung to better understand their clinical and biologic behavior and to test the role of several drugable RTKs in this poorly understood tumor entity. LCNEC is a relatively uncommon tumor, accounting for 1.7% of all resected primary lung cancers at our institutions, which is a figure that is intermediate between to the percentages reported by Jung et al[10] (1.6%) and Takei et al[11] (3%). LCNEC usually occurs in smokers, with a predominance in the male population and a median age of 65 years. According to Jung et al,[10] LCNEC features at computed tomography scan are nonspecific and indistinguishable to the features of conventional NSCLC and to the clinical manifestations characterized by the consistent lack of paraneoplastic syndromes. As rightly emphasized by several authors,[5,8,11] LCNEC is a poorly recognized and underdiagnosed entity that is frequently mistaken for poorly differentiated NSCLC, atypical carcinoid tumors, and intermediate cell–type SCLC. In our series, only 44 patients (53%) were originally correctly classified as having LCNEC, whereas 47% had a different diagnosis, with 31 patients being misdiagnosed as having other NSCLCs (13 SqCs, 12 ADCs, and six LCCs). This is mainly a result of the difficulty in recognizing NE morphology at light microscopy,[8] especially in cytologic samples or small biopsies. Because LCNEC had a significantly worse prognosis than stage-comparable conventional NSCLC,[2,5,8,11,13] a high index of suspicion and the use of appropriate NE markers may be of paramount importance for a correct diagnosis.[64,65]

The majority of previous studies showed a poor prognosis for LCNEC, although 5-year overall survival rates

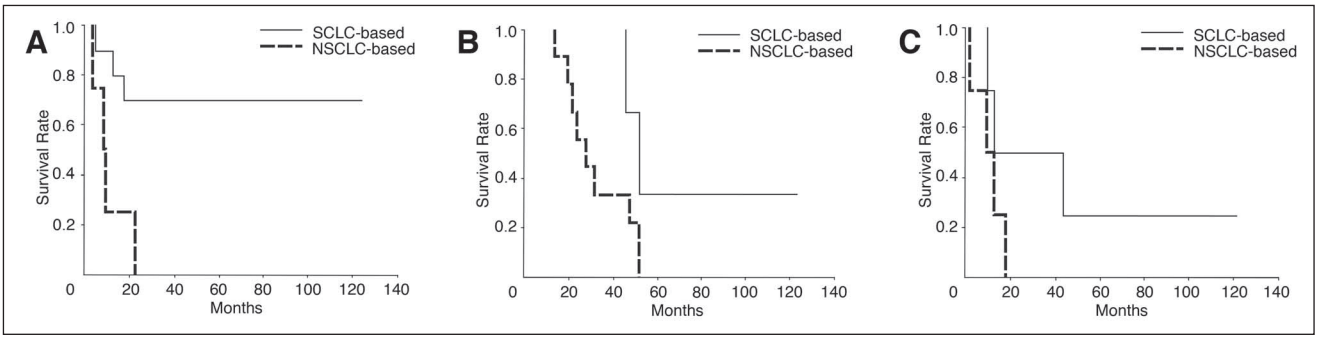

Fig 5. Kaplan-Meier curves for overall survival stratified according to chemotherapeutic protocols in the adjuvant setting and tumor stage. NSCLC, non–small-cell lung cancer; SCLC, small-cell lung cancer.

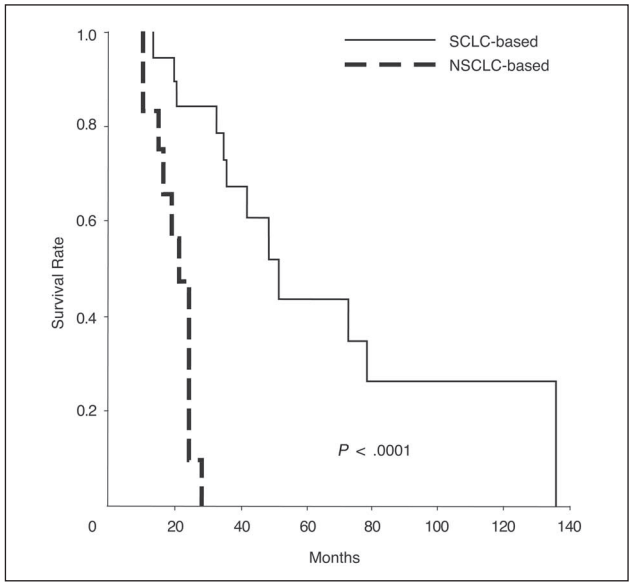

Fig 6. Kaplan-Meier curves for overall survival stratified according to different chemotherapeutic regimens in the metastatic setting. The median overall survival times were 51 and 21 months for patients with large-cell neuroendocrine carcinoma who received platinum plus etoposide and gemcitabine plus taxanes, respectively. NSCLC, non–small-cell lung cancer; SCLC, small-cell lung cancer.

ranged from 13% to 57% (Table 6).[5-16] In some studies, the broad survival range was mainly related to the enrollment of patients with atypical carcinoid tumors, combined SCLC/LCNEC, or LCC with NE differentiation or morphology, instead of enrollment of patients with only pure LCNEC.[7,13] In our series, LCNEC patients had a 5-year overall survival rate of 27.6% (33% in stage I patients). This figure is similar to the rate reported by Shepherd et al[68] in limited-stage resected SCLC patients and clearly worse than the rate observed in stage-comparable NSCLC patients, as previously observed.[2,5,8,11,13]

Several works have well demonstrated that LCNEC is similar to SCLC from morphology to molecular grounds. Marchevsky et al[20] showed a considerable overlap between the neoplastic elements of SCLC and LCNEC at morphometry, providing a good explanation for the frequent diagnostic disagreement between SCLC and LCNEC found by Travis et al.[19] At immunohistochemistry, LCNEC shows a clear-cut positivity for NE markers,[1-4] approximately half of which express TTF-1,[4,21] but unlike SCLC, LCNECs do not stain for high molecular weight cytokeratins 1, 5, 10, and 14.[22] In addition, SCLC and LCNEC show similar genetic changes that differentiate them from carcinoid tumors and NSCLC. Similarly to SCLC, LCNEC shows identical cell cycle protein abnormalities (high labeling index by Ki67 and loss of *Rb* and *p53* tumor-suppressor genes by mutational events),[23-29,34,37] high antiapoptotic activity (ie, increased bcl-2 levels),[23,25,29,30] and common chromosomal imbalances and genetic alterations by loss of heterozygosity at microsatellite analysis.[31-33,35,36] In contrast with conventional NSCLC, SCLC and LCNEC do not show mutational changes of the *k-ras-2* and *c-raf-1* genes.[24] More recently, Jones et al[69] demonstrated that SCLC and LCNEC were indistinguishable at gene expression profiling analysis, clustering together into two groups with different prognoses independently from histopathology. It is noteworthy that other previous mRNA expression profiling studies aimed at lung cancer classification identified subclasses of ADC and LCC displaying NE differentiation and associated with a poor outcome that were strikingly similar to SCLC.[70,71] It seems reasonable to suppose that this cluster of NSCLCs could be represented by LCNECs. Despite a convincing body of evidence suggesting that LCNEC is a high-grade NE malignancy biologically related to SCLC, it is still considered a variant of LCC and, therefore, accordingly treated. No studies have currently pointed out the optimal treatment of patients with LCNEC, and no evidence has been provided about whether these patients might draw a benefit from chemotherapeutic protocols for NSCLC or SCLC.[2-4] Given that there is no standard therapy for patients with LCNEC, the retrospective review of clinical data in our series revealed heterogeneous approaches in treatment regimens. Platinum-containing polychemotherapy is the most

Table 4. Expression Results of the Immunohistochemical Markers

	Patients (N = 83)	
Antibody	No.	%
Chromogranin		
Positive	54	65.1
Negative	29	34.9
Synaptophysin		
Positive	44	53
Negative	39	47
NCAM/CD56		
Positive	77	92.8
Negative	6	7.2
KIT		
Positive	52	62.7
Negative	31	37.3
SCF		
Positive	47	56.6
Negative	36	43.4
PDGFRα		
Positive	50	60.2
Negative	33	39.8
PDGFRβ		
Positive	68	81.9
Negative	15	18.1
Met		
Positive	39	47
Negative	44	53

Abbreviations: NCAM, neural cell adhesion molecule; SCF, stem-cell factor; PDGFR, platelet-derived growth factor receptor.

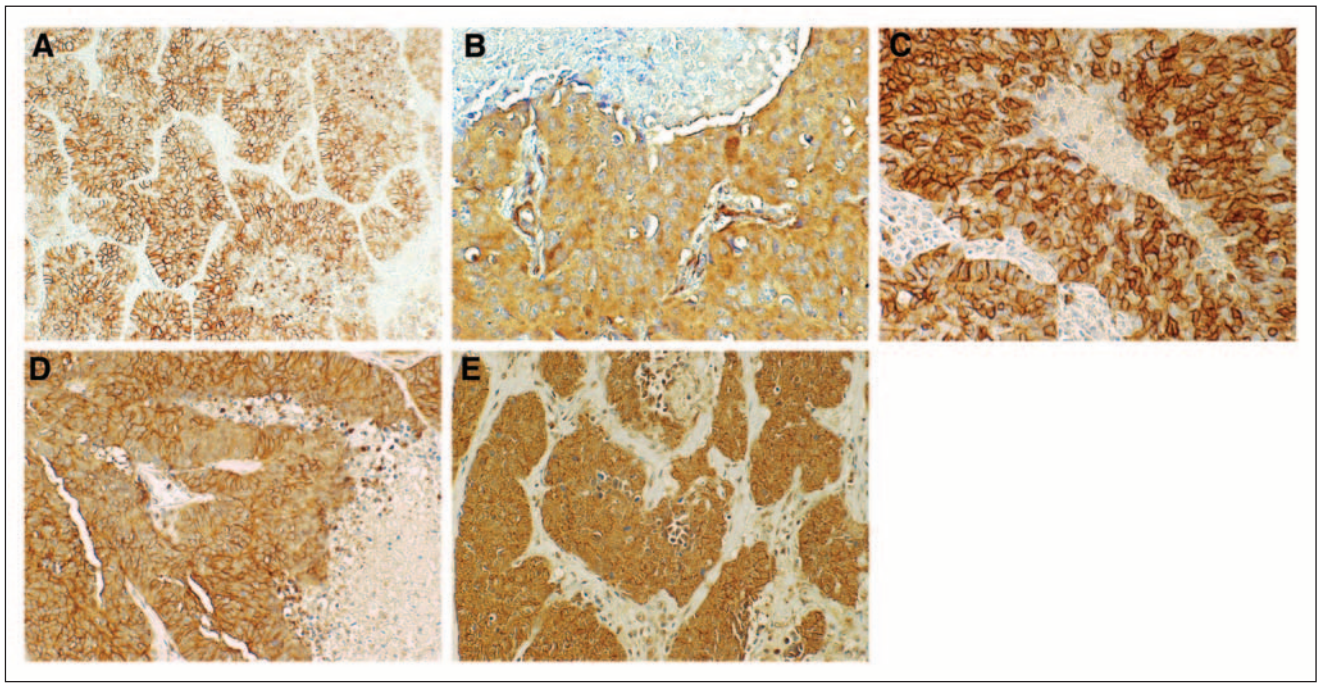

Fig 7. A large-cell neuroendocrine carcinoma expressing (A) KIT, (B) stem-cell factor, (C) PDGFRα, (D) PDGFRβ, and (E) Met.

effective regimen in lung cancer in general, but platinum plus etoposide still represents the mainstay of chemotherapy in SCLC. For this reason and on the basis of different therapeutic options in the treatment of SCLC[72] and NSCLC,[73] we identified the following two different groups of patients: patients who received standard chemotherapy for SCLC (platinum + etoposide ± radiotherapy) and patients who were treated as having NSCLC (different combinations of platinum/gemcitabine/taxanes/vinorelbine ± radiotherapy). In the literature, therapeutic data about LCNEC are limited to a few studies. Iyoda et al[74] reported a prolonged survival in patients with surgically resected stage I LCNEC receiving adjuvant chemotherapy based on a standard SCLC regimen. Similarly, Kozuki et al,[75] who investigated the treatment strategy in 12 LCNEC patients, concluded that the therapeutic approach used in SCLC is worthy of consideration because partial or complete responses were achieved in patients who received cisplatin plus etoposide with or without radiotherapy. Yamazaki et al[66] recently reported a response rate for LCNEC (50%) to cisplatin-based chemotherapy that was more similar to the rate observed in SCLC. Interestingly, Filosso et al[67] found promising preliminary clinical results using octreotide alone or in combination with radiotherapy in the adjuvant setting for patients with preoperative octreoscan-positive LCNEC.

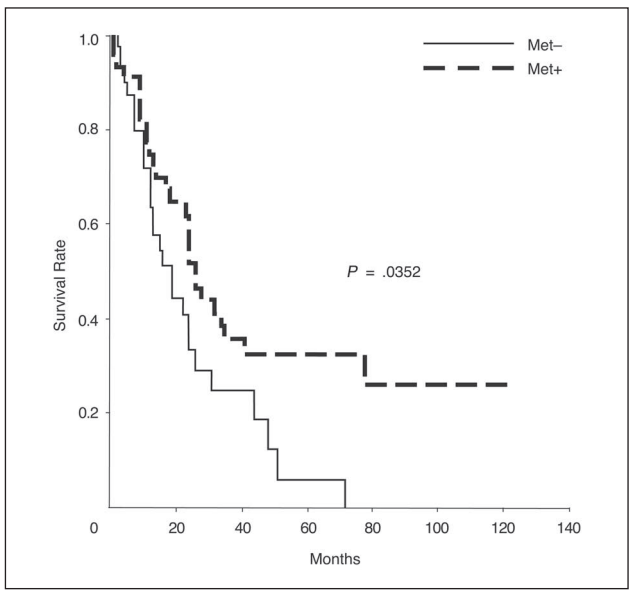

Fig 8. Kaplan-Meier curves for overall survival stratified according to Met expression. The median overall survival times were 18 and 24 months for patients with large-cell neuroendocrine carcinoma expressing or not expressing Met, respectively.

Our relatively homogeneous and large series of LCNEC patients demonstrated for the first time that, once completely resected, patients with LCNEC had a statistically significant benefit in terms of overall survival when they underwent adjuvant standard SCLC-based chemotherapy ($P < .0001$), especially patients with stage I disease. Moreover, drug combinations generally used for NSCLC were relatively ineffective. SCLC-based chemotherapy (± radiotherapy) seemed to be significantly more effective even in metastatic LCNEC ($P < .0001$). Although anecdotal, we

Table 5. Multivariate Cox Regression Analysis of Parameters Significantly Correlated at Univariate Analysis

Variable	β	P	Relative Risk	95% CI
Stage, II/III *v* I	.836	.029	2.308	1.089 to 4.892
Met, positive *v* negative	−.433	.152	0.648	0.358 to 1.173
Tumor size, > 3 cm *v* < 3 cm	.765	.013	2.150	1.176 to 3.932
Adjuvant chemotherapy, NSCLC based *v* SCLC based	2.742	.0001	15.524	5.046 to 47.757
Chemotherapy in metastatic setting, NSCLC based *v* SCLC based	3.069	.0001	21.529	6.920 to 66.972

Abbreviations: SCLC, small-cell lung cancer; NSCLC, non–small-cell lung cancer.

would like to mention that three patients with metastatic LCNEC who started chemotherapy with gemcitabine alone (n = 2) or carboplatin plus taxanes (n = 1) stopped therapy after a few cycles for progression of the disease. The patients then received a cisplatin plus etoposide regimen and achieved complete (n = 1) or partial response (n = 2). Of note, adjuvant chemotherapy in lung cancer seems to be associated with a significant improvement of survival in patients with NSCLC receiving postoperative chemotherapy, particularly in early stages.[76-78] Accordingly, our results seem to support the same considerations in patients with LCNEC but using chemotherapeutic compounds generally used in SCLC. Because of the limited number of patients receiving radiotherapy, we cannot draw any statistically proven conclusion about the value of radiotherapy in LCNEC.

RTKs are currently investigated for their possible role as important prognostic markers and as targets for alternative molecular therapies.[46,51-53,79] In agreement with other researchers,[38,39] we recently found that LCNEC overexpress KIT, an RTK deeply involved in SCLC, where KIT and its ligand SCF constitute a functional autocrine loop promoting tumor cell proliferation and blocking apoptosis.[42-44,79,80] Tamborini et al[81] recently discovered an autocrine loop between KIT overexpression and phosphorylation in the presence of SCF in SCLC. In this study, we also demonstrated that LCNEC tumor cells coexpressed KIT and SCF, evidencing that this tumor growth pathway acts similarly in both high-grade lung NE carcinomas. Most importantly, preclinical studies[54,55] revealed promising results related to in vitro and in vivo SCLC cell inhibition by the selective type III RTK inhibitor STI571 (imatinib mesylate), which is a 2-phenylaminopyrimidine derivative effective in chronic myeloid leukemia and GI stromal tumors.[82] Despite the lack of efficacy reported in a controversial phase II trial using imatinib in SCLC,[83] the potential benefit from targeted therapies against KIT-positive SCLC is far from being defined, and the value of combinations using chemotherapy and imatinib remains to be tested.[84] In addition, several other molecules (ie, SU11248, SU5416, and SU6597) acting against KIT, PDGFRα, PDGFRβ, and other RTKs or blocking Src-related RTKs seem to be providing promising preclinical results.[56,58-60,85]

Table 6. Overall Survival Reported in Literature for Patients With LCNEC

Study	No. of Patients	5-Year OS (%)	Note
Travis et al[6]	37	27	*
Dresler et al[7]	40	13 (18 in stage I)	
Iyoda et al[13]	77	32	*
Jiang et al[8]	22	44	†
Garcia-Yuste et al[9]	22	21	
Takei et al[11]	87	57 (67 in stage I)	‡
Mazieres et al[12]	18	27	
Paci et al[15]	48	21 (27 in stage I)	*
Casali et al[41]	33	51 (54 in stage I-II)	
Doddoli et al[18]	20	36	
Zacharias et al[17]	21	47	
Filosso et al[67]	20	35	
Yamazaki et al[66]	20	NA (35 at 1 year; 15 at 2 years)	
Battafarano et al[16]	45	30 (32 in stage I)	
Present study	83	27 (33 in stage I)	

Abbreviations: LCNEC, large-cell neuroendocrine carcinoma; OS, overall survival; SCLC, small-cell lung cancer; NA, not available; NSCLC, non–small-cell lung cancer.
*No significant difference between LCNEC and SCLC in OS and disease-free survival.
†Significant difference between LCNEC and NSCLC.
‡Significant difference between stage I LCNEC and NSCLC.

A few data have been reported in the literature concerning the role of PDGFRs in lung cancer.[47,86,87] In particular, Antoniades et al[86] reported aberrant in vivo coexpression of PDGFs and relevant receptors in tumor cells of SCLC and NSCLC, suggesting that this autocrine mechanism is upregulated in lung cancer.

Met, the product of the proto-oncogene *c-met*, is an RTK deeply involved in epithelial-mesenchymal interactions, commonly overexpressed in several solid tumors, including SCLC and NSCLC, and implicated in the development and progression of human cancers leading to tumor cell dissemination.[48-50] Basically, aberrant Met activation, by binding with its high-affinity ligand HGF/scatter factor or by autophosphorylation as a result of *c-met* mutations, provokes a cytoplasmic signals cascade, resulting in activation of multiple signal transducers (Grb2, Gab1, PI3K, STATs, ERK1/2, FAK, and PLC-γ).[48-50] In NSCLC, Met activation is associated with shortened survival.[44,48,49,88,89] Our study confirms that

Met was the only marker significantly correlated with overall survival at univariate analysis ($P = .0352$) and, thus, an important factor in selecting patients with LCNEC at high risk. Most importantly, several experimental works have reported that targeting Met in human cancer is possible using different strategies (ie, monoclonal antibodies and small competitive or noncompetitive molecules), leading to significant tumor cell growth inhibition.[90] Maulik et al[57] also demonstrated that the HGF/Met pathway is functional in SCLC cell lines and found tumor growth inhibition by apoptosis using geldanamycin, a small molecule indirectly interfering with Met. Constitutive intragenic gain-of-function mutations leading to ligand-independent RTKs are the best predictors of clinical response using targeted therapies in solid tumors, and Ma et al[91] recently demonstrated the presence of *c-met* mutations on the juxtamembrane domain in SCLC cell lines and tumor tissues. In our work, no mutations were identified in the exons encoding for the juxtamembrane domains of the tested RTKs. Thus, it is unlikely that the scenario seen in GI stromal tumors will be observed in LCNEC as well. Our results demonstrated that LCNECs are characterized by overexpression of several RTKs, evidencing their involvement in carcinogenesis of LCNEC.

Finally, as in SCLC, and in contrast with NSCLC,[92,93] LCNEC frequently shows overexpression for NCAM/CD56 (92.8% in our series), a member of the family of neural cell adhesion molecules. Apart from its diagnostic value as the most sensitive marker of NE differentiation in high-grade NE tumors,[94] CD56 seems to be a promising target against which is directed another novel compound, the immunoconjugate BB-10901, which was developed for the treatment of relapsed or refractory SCLC and other CD56-immunoreactive NE malignancies.[95]

In summary, our results confirm that LCNEC is a relatively uncommon, poorly recognized, and underestimated high-grade NE tumor that clinically and morphologically mimics conventional NSCLC but that is associated with a dismal outcome, even in early stage. Most importantly, for the first time, we convincingly demonstrated that adjuvant chemotherapy using an SCLC-based standard protocol is effective and significantly improves the survival of patients with LCNEC ($P < .0001$). Similar results were observed also in metastatic disease. From a more speculative viewpoint, LCNECs express the RTKs KIT, PDGFRα, PDGFRβ, and Met in a high proportion of paients, although no mutations were found in the relevant exons encoding for RTK juxtamembrane domains. Among these RTKs, only Met was significantly associated with patient survival at univariate analysis, but Met was not associated with patient survival at multivariate analysis. Prospective clinical studies on larger series of LCNEC are clearly mandatory to confirm current data, and the role of a therapy strategy with targeted RTK inhibitors deserves further investigation.

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Authors' Disclosures of Potential Conflicts of Interest

The authors indicated no potential conflicts of interest.

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