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Pulmonary Large-Cell Neuroendocrine Carcinoma: Therapeutic Challenges and Opportunities

Review Article

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Abstract: Pulmonary large cell neuroendocrine carcinoma (P-LCNEC) is a rare, poorly differentiated, non-small cell malignancy within the spectrum of neuroendocrine tumors (NETs) of the lung. Despite sharing several similarities with small cell lung cancer (SCLC) in their clinical, immunohistopathological, genomic, and prognostic features, it is a distinct and biologically heterogeneous entity with challenging diagnostic and therapeutic requirements. Given the lack of prospective, randomized data to guide management, it is common practice to pursue thoracic surgery for resectable tumors according to the guidelines for non-small cell lung cancer (NSCLC) and implement systemic chemotherapy as early as at stage I, similar to the treatment of SCLC. However, important issues, such as the optimal timing and combination of therapeutic modalities, the most effective type of chemotherapy for advanced-stage disease, and the benefit from prophylactic cranial irradiation, remain debated. Accumulating evidence from retrospective, molecular profiling studies supports the existence of at least two P-LCNEC subtypes, most notably a SCLC-like and a NSCLC-like phenotype, which presumably underlie the observed differential sensitivity to platinum-based regimens and warrant further validation as predictive biomarkers of efficacy. Furthermore, several potentially actionable, driver molecular alterations have been identified, offering implications for personalized treatment approaches, including targeted therapies and immunotherapy. The current review discusses open questions on the diagnosis and management of P-LCNEC, as well as recent advances in its genomic and transcriptomic characterization that create promising therapeutic opportunities.

Keywords: *Pulmonary large cell neuroendocrine carcinoma (P-LCNEC) • diagnosis • treatment • molecular classification • biomarkers*

1. Introduction: an overview of a rare but distinct entity

Pulmonary large-cell neuroendocrine carcinoma (P-LCNEC) is a poorly differentiated malignant tumor consisting of large cells with neuroendocrine features confirmed by hematoxylin-eosin staining, immunohistochemistry, and/or electron microscopy (1). While previously considered a variant of large-cell carcinoma, since the 2015 revision of World Health Organization classification of lung cancer, it has been categorized within neuroendocrine tumors (NETs), along with typical and atypical carcinoids, as well as small cell lung cancer (SCLC), which is the other pulmonary high-grade neuroendocrine carcinoma (HGNEC) (2).

As a matter of fact, the latter entity shares several epidemiological, clinicopathological, and molecular characteristics with P-LCNEC, including a strong association with smoking, a male predominance, an aggressive biological behavior, a dismal prognosis, and some common genetic alterations such as *TP53* mutation and *MYC* family gene amplification (3). These commonalities have been the rationale for treating P-LCNEC similar to SCLC rather than non-small cell lung cancer (NSCLC).

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The integration of chemotherapeutic regimens designed for SCLC in the management of P-LCNEC was sparked by a European single-center, retrospective study involving 83 patients published back in 2005 and showing significantly better clinical outcomes with platinum-plus-etoposide combinations compared with traditional NSCLC-regimens, both in the metastatic and adjuvant setting, especially for stage I disease. This strategy was identified as the most important independent prognosticator for overall survival (OS) (4).

At present, however, there are no therapeutic recommendations by standard-setting organizations, such as European Society for Medical Oncology (ESMO) and US National Comprehensive Cancer Network (NCCN). The only exception comes from the 2017 update of the American Society of Clinical Oncology (ASCO) Practice Guidelines for the treatment of metastatic NSCLC, which includes the platinum-etoposide regimens as a first-line option, based on informal expert consensus (5).

P-LCNEC accounts for up to 3.5% of lung cancer in institutional surgical series, and less than 1% of all-stage cases in population-based registries in Europe and the United States (6-8). Its rarity underlies the lack of randomized controlled clinical trials, as well as the limited number and small sample size of prospective studies to guide therapy.

The current review will discuss open questions and challenges on the management of this rare, distinct malignancy, as well as recent advances in its molecular characterization that create promising therapeutic opportunities.

2. Challenges in diagnosis

Histological misdiagnosis is another important obstacle rating as high as 47%, which hampers the interpretation of retrospective case-series studies without confirmation by central pathology review (4). Distinguishing P-LCNEC from its mimickers—most notably SCLC, adenocarcinoma, and basaloid squamous cell carcinoma—can be quite challenging by light microscopy, especially on small biopsy specimens obtained on routine workup, because of considerable overlap in cytomorphology. The two most useful criteria for its differentiation from SCLC are the low nuclear-to-cytoplasmic ratio and the prominent presence of nucleoli, while strong immunoreactivity for at least one neuroendocrine marker can safely distinguish it from the other NSCLC types (3). Table 1 summarizes the key histopathological, immunohistochemical (IHC), and ultrastructural characteristics of P-LCNEC (1,3,9).

Nevertheless, approximately 6% of HGNECs carry a borderline phenotype that falls between those of P-LCNEC and SCLC, thus contributing to the frequently reported interobserver discordance in tumor classification (10). Furthermore, HGNECs can be a mixture of SCLC and P-LCNEC components, the latter comprising ≥10% of the tumor volume or may consist of either of the two in combination with adenocarcinoma and/or squamous-cell carcinoma (11).

Given the differential diagnostic difficulties that arise from cytologic and limited biopsy material, the term “possible P-LCNEC” was proposed for usage in the particular setting (12). A small retrospective study from Japan compared the clinical outcome to first-line platinum-based chemotherapy between patients with “possible P-LCNEC,” that is, diagnosed by small biopsies, versus those with “definite P-LCNEC” based on surgically resected specimens (13). There were no significant differences between the two groups in terms of overall response rate (ORR), progression-free survival (PFS), or OS, implying no impact of the diagnostic approach on treatment efficacy.

3. An insight to the disease’s natural history

Another critical issue that remains under question is the degree of resemblance between the two types of HGNEC in their natural history and how this justifies a therapeutic extrapolation from SCLC to P-LCNEC. First, it is worth mentioning that P-LCNEC presents more frequently at a peripheral location and at an early, potentially resectable stage (7,8,14). According to cancer registry data, its stage distribution resembles that of NSCLC rather than SCLC (7,8). Although this observation can be partially explained by underdiagnosis of advanced-stage P-LCNEC because of inadequate biopsy sampling, genetically engineered mouse models (GEMM) also suggest a more indolent dissemination of the tumor compared with SCLC (15). Moreover, unlike the latter, paraneoplastic manifestations are uncommon (1). On the other hand, the largest-to-date, registry-based, cohort study evaluating the distant spread of histologically confirmed P-LCNEC showed a quite similar metastatic pattern for the two types of HGNEC, with brain, liver, and skeleton being the three most commonly affected sites (8). However, while brain secondaries were significantly more prevalent in P-LCNEC than in SCLC, the reverse is applied for liver metastases. On the basis of the same report, the extent of mediastinal lymph node involvement was less prominent in P-LCNEC, even at stage IV.

Table 1. Key histopathological, immunohistochemical, and ultrastructural characteristics of P-LCNEC

• High grade: ▪ Mitotic activity of >10 mitoses per 10 HPF (2mm^2) ▪ Large areas of necrosis	• Cytologic features of NSCLC: ▪ Tumor cell size >3 times the diameter of resting lymphocytes ▪ Low nuclear-to-cytoplasmic ratio ▪ Polygonal shape ▪ Vesicular or fine nuclear chromatin ▪ Presence of nucleoli	• Neuroendocrine morphology/ cell arrangement: ▪ Palisading ▪ Organoid nesting ▪ Trabecular pattern ▪ Rosette-like structures • Strong IHC activity for ≥1 NE marker in ≥10% of tumor cells: chromogranin A, synaptophysin and/or NCAM/CD56 • Electron microscopy: NE granules

CD56, cluster of designation/ classification determinant 56; P-LCNEC, pulmonary large cell neuroendocrine cancer; HPF, high-power field; NSCLC, non-small cell lung cancer; NE, neuroendocrine; NCAM, neural cell adhesion molecule.

Literature data on the tumor's prognosis as compared with that of SCLC are conflicting (7,10,16,17). A potential pitfall in juxtaposing the clinical course and therapeutic outcome of the two HGNECs is the fact that, whereas P-LCNEC is staged by the TNM/AJCC system, SCLC has traditionally been classified and treated based on the simplified, dichotomous staging scheme by the Veterans' Administration Lung Group, which is less accurate at predicting prognosis, especially for lower-stage disease (18). Moreover, and in contrast to NSCLC, therapeutic strategy for SCLC is typically aggressive with the integration of chemotherapy and thoracic radiotherapy (TRT) as early as at stage IA and T1-2N1, respectively.

According to the aforementioned population-based study, which reflects the real-world practice of lung cancer treatment in a European country over a period of 10 years, P-LCNEC carried an overall adverse prognosis, with a median OS of 8.7 months for all stages combined (8). While the particular outcome measure lies between the ones reported for SCLC and non-neuroendocrine NSCLC, there were significant multivariable-adjusted differences in OS from SCLC depending on the disease stage. While patients with stages I and II P-LCNEC had a median OS almost double that of their SCLC counterparts (32.4 versus 17.8 months, respectively), the corresponding value in stage IV P-LCNEC was significantly lower than, although clinically comparable to, the one in extensive SCLC (4 versus 5.3 months, respectively).

To interpret these findings, we should keep in mind that the management approach was significantly different for the two HGNECs. In early-stage disease, surgery was applied more frequently to patients with P-LCNEC than to those with SCLC, with the reverse occurring with adjuvant chemotherapy. When survival analysis included only surgically treated cases, there was no significant difference between the two groups. On the other hand, a higher percentage of patients with stage IV P-LCNEC did not receive palliative chemotherapy. When the outcome comparison included only chemotherapy-treated cases, median OS for P-LCNEC was 7.7 months and statistically not different from that in SCLC. Of note, no information was available regarding the type of chemotherapy given.

On the basis of the same report, OS for early stage P-LCNEC was significantly worse comparatively with both adenocarcinoma and squamous cell carcinoma after 10 months of follow-up, even among patients treated with surgery and despite the fact that adjuvant chemotherapy was offered more frequently to those with P-LCNEC. Likewise, for stage IV disease, prognosis was significantly poorer compared with the two non-neuroendocrine types of NSCLC, even among patients treated with chemotherapy. Regarding stage III, around 31% of patients with P-LCNEC received chemoradiotherapy and another 21% had surgery. Although the therapeutic approach varied significantly between P-LCNEC and SCLC, the corresponding OS times were not different after multivariable adjustment (median, 12.6 versus 12.2 months, respectively). As compared with adenocarcinoma and squamous cell carcinoma, the distribution of applied treatment modalities and overall prognosis were similar.

4. Therapeutic approaches for early-stage and locally advanced disease

In the absence of robust evidence to guide the management of P-LCNEC, current daily practice tends to combine the standard treatment recommendations for NSCLC and SCLC, with surgery being pursued for stages I to III by analogy to resectable NSCLC and chemotherapy being strongly considered for as early as stage I, as in the case of SCLC (19). In a European, single-center, retrospective, cohort study of 70 patients with P-LCNEC, almost all of whom had undergone thoracic surgery in the context of a multimodality approach, those receiving postoperative radiotherapy, chemotherapy, or chemoradiotherapy, because of their higher pathologic stage or incomplete resection, had an outcome similar in terms of local PFS and OS to those

with earlier-stage disease treated only with surgery. Postoperative TRT was administered as indicated for NSCLC, while adjuvant chemotherapy was restricted to stages IIA to IIIA, using a regimen designed for SCLC in half of the cases. Noteworthy was the absence of a significant impact of the type of chemotherapy on outcome (20). On the basis of these findings, one may conclude that multimodal management of resectable P-LCNEC according to the guidelines for NSCLC, rather than SCLC, might be reasonable.

As for the setting of unresected, locally advanced disease, the limited available data from a European multi-institutional retrospective study indicate that platinum-etoposide chemotherapy with or without TRT displays only modest efficacy in P-LCNEC relative to SCLC (21). In an indirect comparison against 32 stage-matched SCLC controls, 16 patients with unresected stage III P-LCNEC treated as mentioned above had a significantly worse outcome in terms of ORR (44% versus 91%), PFS (median, 5.6 versus 8.9 months), and OS (median, 10.4 versus 17.6 months), with P-LCNEC histology identified as an independent unfavorable prognosticator. Although these results might imply a lower tumoral chemoradiosensitivity compared with SCLC, we should emphasize that in the group of patients with P-LCNEC, TRT was added to chemotherapy less frequently, and the two modalities were more often combined sequentially rather than concurrently.

5. Potential role of prophylactic cranial irradiation

Another controversial issue is the potential effect of prophylactic cranial irradiation (PCI), as extrapolated from the standard treatment principles for SCLC. According to the abovementioned report on the outcome of unresected stage III disease, the 18-month cumulative incidence of brain metastases was as high as 58% in the lack of PCI. The corresponding value for patients with metastatic disease was 48% (21). Although these figures might suggest a place for PCI in the management of advanced-stage P-LCNEC, there is no clear evidence to support this. A small-sized, European, single-center study retrospectively evaluated patients with stages IIIA–IV disease receiving PCI against their counterparts, all treated with platinum-plus-etoposide chemotherapy, with or without TRT. PCI demonstrated a significant PFS prolongation only in patients with stage III disease (median, 20.5 versus 12 months), and a trend for longer OS was observed in the total cohort (median, 33.4 versus 8.6 months) (22).

The benefit of PCI for P-LCNEC was also questioned by the aforementioned retrospective study of 70 patients treated primarily with surgery in the context of multimodal therapy (20). Although none of the three patients with locally advanced disease who received PCI had intracranial relapse, the detection rate of brain metastases in the total cohort over a median follow-up of 2 years was 25%, which is comparable with the one reported for NSCLC, rather than SCLC. Furthermore, patients with pathologic stage I disease had a significantly longer brain-metastases-free survival compared with those at more advanced stages. A low incidence of brain metastases not exceeding 15% was also reported by an Asian, single-center, retrospective analysis of 72 patients with P-LCNEC treated by surgery with or without adjuvant chemotherapy, most of whom had pathologic stages I–IIIA (23). Considering these observations, the role of PCI for early stages of P-LCNEC is probably subtle, in contrast to limited-stage SCLC, where such a therapeutic strategy is most beneficial.

6. (Neo)adjuvant strategies

On the other hand, there is a broad agreement among surgical series regarding the adverse prognosis of curatively resected early stage disease, suggesting a potential benefit of additional systemic therapy, similar to that of SCLC (23-25). An Asian, single-institutional, retrospective study revealed P-LCNEC histology as an independent, poor prognostic factor even for pathologic stage IA, with a hazard of death almost three times as high as that in other types of NSCLC (26). However, data on neoadjuvant and/or adjuvant chemotherapy are mostly retrospective and conflicting (6, 27-29).

In the most recent and largest-reported retrospective study of pathologic stage I P-LCNEC, derived from the US National Cancer Database, the addition of chemotherapy improved significantly the 5-year OS rate in both stages IA and IB disease to 59.4% versus 50.4% and 68.7% versus 44.7%, respectively, with the benefit confirmed by multivariable Cox regression and propensity-matched analysis (30). Cases excised with positive margins and/or treated with radiotherapy were excluded from the analysis. In the vast majority of patients, therapy was offered postoperatively, but there was no information regarding the specific regimens used or duration. The reduction of hazard of death was 45% across the whole cohort and most prominent for patients with stage IB, while sublobar resection was an independent adverse prognosticator.

Prospective data on adjuvant chemotherapy are only available from two small, Japanese, phase 2 studies

performed in 2006 and 2011, respectively, both using a SCLC-type regimen (31, 32). The first assessed the efficacy of only two cycles of cisplatin-plus-etoposide in 15 patients with completely resected stage I–IV disease (80% of whom with stages I–IIIA), while comparing outcomes with 23 matched historical controls from the same institution. Both the 5-year OS and disease-free survival (DFS) rates (88.9% versus 47.4% and 86.7% versus 34.8%, respectively) favored the study arm (31). In a following retrospective study of 72 patients, the same research team demonstrated a superiority of platinum-based adjuvant chemotherapy over no therapy and non-platinum regimens, as well as an independent prognostic effect on DFS by multivariable regression analysis, albeit not by propensity score analysis (23). The second pilot study recruited patients with stages I–IIIA HGNECs, including 23 with P-LCNEC. The adjuvant chemotherapy scheme consisted of four cycles of cisplatin-plus-irinotecan, a combination that is commonly used in Japan as an effective regimen for SCLC. At 3 years of follow-up, the OS rate was 85%, while the relapse-free-survival rate was 75%. Both the rate of treatment completion and toxicities were acceptable (32). On the basis of these promising results, there is an ongoing phase 3 clinical trial, aiming to demonstrate an OS superiority of irinotecan over etoposide in combination with cisplatin (33).

The role of neoadjuvant chemotherapy in resectable P-LCNEC is even less well defined. In the largest retrospective study testing the concept of perioperative chemotherapy, the ORR to induction platinum-based regimens among 22 patients was as high as 68%. Although in the total cohort of 100 patients, there was no significant association between outcome and the administration of neoadjuvant and/or adjuvant chemotherapy, when the analysis was restricted to stage IB–IIIA disease, there was a trend for OS improvement after adjustment for propensity score. Of note, the platinum/etoposide doublet was offered to about 25% of the patients in the neoadjuvant setting and, in most cases, in the adjuvant setting (28).

Back in 2005, a small, retrospective, cohort study from a single European institution provided preliminary evidence on the potential role of somatostatin analogs in the adjuvant setting of P-LCNEC with pathological tracer uptake on presurgical indium In-111 pentetreotide scintigraphy (34). Somatostatin receptors 2 and 5 were detected by polymerase chain reaction (PCR) in the surgical specimens of all cases with a positive scintigraphy, the latter performed routinely in the preoperative assessment and follow-up. Long-acting octreotide with or without adjuvant radiotherapy was offered to patients with stage IB–IIIA tumors until disease recurrence, exhibiting a favorable toxicity profile. With a median follow-up of 3 years, only 1 of 10 patients relapsed, in contrast to those having a negative somatostatin receptor scintigraphy and receiving no adjuvant systemic therapy, all of whom had disease recurrence. Despite being attractive as a therapeutic concept, no further relevant data have been reported since.

7. Treatment challenges in the metastatic setting

The topic dominating the discussion of the management of metastatic P-LCNEC is the optimal selection between chemotherapy regimens typically used in SCLC and those designed for NSCLC. Again, most data were derived from small retrospective studies, varying in case selection and pathologic confirmation and yielding conflicting results on which of the two strategies is superior (4, 35-37). Another limitation to the interpretation is that NSCLC-type regimens are frequently grouped in one category for statistical analysis, thus overlooking potential differences by specific therapy.

Only two phase 2 studies are available, both dated more than 5 years ago, with a multicenter and two-stage design, and assessing a SCLC-type regimen; one conducted in Europe and the other in Asia (38,39). In the first study, cisplatin was combined with etoposide, while in the second study, it was combined with irinotecan. Following central pathology review, around one-fourth of cases in both studies were reclassified as SCLC. Although ORR exceeded the predefined threshold in both trials, ranging from 34% to 47%, respectively, it was substantially lower than the ones reported by phase 3 trials of the corresponding regimens in extensive SCLC. By indirect comparison, the secondary efficacy results, including median PFS and OS, were also better with the irinotecan-including regimen used in the Asian study. Compared with the patients reclassified as having other tumor histology, those with confirmed P-LCNEC diagnosis had no significant differences regarding the outcome in either of the two studies, with the exception of OS, which was inferior in patients with P-LCNEC treated with cisplatin plus irinotecan (median, 12.6 versus 17.3 months). Neither of the two reports provided information on the type and efficacy of the subsequent-line therapies.

Therefore, P-LCNEC seems to display a distinct behavior from SCLC with respect to chemosensitivity in the metastatic setting. This notion is also supported by the largest-to-date retrospective series of pathologically confirmed metastatic P-LCNEC that included 128 cases

from the Netherlands Cancer and Pathology Registries (40). Despite limitations to the interpretation, the study showed a multivariably significant OS superiority for the cluster of platinum-based doublets containing gemcitabine, taxanes, and vinorelbine over the corresponding combinations with etoposide (median, 8.5 versus 6.7 months) and pemetrexed (median, 8.5 versus 5.9 months). Of note, the frequency of second-line treatment was not significantly different among the three chemotherapy groups. However, data on ORR and PFS were either incomplete or non-standardized. Regarding the observed poor therapeutic efficacy of pemetrexed, this might be explained by the previously reported, relatively high mRNA, and/or IHC expression levels of thymidylate synthase in P-LCNEC, which arguably confer resistance to the antimetabolite agent (41,42).

A consequently arising question is whether the addition of biological agents would enhance the efficacy of chemotherapy. According to the largest-to-date, retrospective, genomic profiling study of early and advanced-stage P-LCNEC in Japanese, chemotherapy-naïve patients, 15% of the tumors harbor key activating genetic alterations in the PI3K/AKT/mTOR pathway, including mutations and copy number gains (43). On the basis of the activity of everolimus in several types of NETs and the encouraging results of a phase 1 trial, a European, multicenter, phase 2 study evaluated the efficacy and safety of the mTOR inhibitor at a daily dose of 5 mg in 49 biomarker-unselected patients with metastatic P-LCNEC, initially combined with up to four cycles of carboplatin-and-paclitaxel as first-line treatment and later as maintenance monotherapy (44). Although the study ended prematurely because of low recruitment, the regimen scheme was well tolerated, and the outcome measures were comparable with or better than those reported in previous studies of platinum-based chemotherapy alone. The PFS rate at 3 months, which was the primary end point of the study, reached 76%.

However, a notable finding of two large, European, retrospective studies of pulmonary NET archival specimens was the significantly lower IHC expression of phosphorylated Akt and/or mTOR in P-LCNEC compared with typical and atypical carcinoids, implying a limited efficacy of mTOR inhibition in the former tumor type (45,46). We would, therefore, expect the outcome of metastatic P-LCNEC to improve by a tailored application of targeted therapies.

Unfortunately, actionable genetic alterations, such as *EGFR* and *BRAF* mutations or *ALK* translocations, have mainly been described in mixed P-LCNEC tumors with an adenocarcinoma component and in oncogene-driven lung adenocarcinomas transforming to P-LCNEC after exposure to targeted tyrosine-kinase inhibitors (TKIs) but are rarely detected in pure and de novo P-LCNEC (47-51). Furthermore, the predictive implications of such alterations remain controversial because there are only a handful of literature reports of P-LCNEC cases treated with the corresponding TKIs. While a number of these have shown encouraging results (50-54), there are speculations for intrinsic drug resistance in *EGFR*-mutant HGNECs through reduced translation and protein expression (49). Therefore, the cost-effectiveness of routine testing for genetic alterations, such as activating *EGFR* mutations, in pure P-LCNEC is under discussion. Screening immunostaining for the most frequent *EGFR* mutations, especially in non-smoking patients, might be a more rational and less-expensive approach (49).

On the other hand, in the first molecular study to include combined P-LCNEC and to analyze each component separately, it was interesting to observe a high concordance rate of key gene alterations, indicating that targeted agents would probably be effective for both tumor components (43).

8. Molecular characterization of a heterogeneous disease

Summing up our discussion in the previous sections, P-LCNEC is characterized by an aggressive behavior and a generally poor prognosis, but most aspects of its optimal management remain debated or unknown (55). It is presumably a distinct, albeit heterogeneous disease, which partially accounts for the discrepant findings among the limited relevant clinical studies. In the era of precision medicine, molecular profiling of this rare tumor would expectedly shed light on its biology, identify potentially actionable targets, and widen our treatment options. Several such studies of retrospective design have been reported during the past 5 years, offering opportunities for the development of more efficient therapeutic strategies (43, 56-63).

Despite their considerable heterogeneity regarding patient and disease characteristics, number and type of tumor specimens, sequencing technology, and platform, as well as comparison with matched normal tissue and/or other pulmonary NETs, there is general agreement in results showing, on one hand, genetic alterations that are unique to or significantly more prevalent in P-LCNEC, and, on the other hand, a genetic overlap with SCLC, including frequent biallelic inactivation of *TP53* and *Rb1*, a similarly high mutational burden and a transversion signature associated with exposure to tobacco. Key mutations in P-LCNEC are mostly clonal,

predominantly affect tumor suppressor genes and are frequently accompanied by loss of heterozygosity (64).

From a prognostic point of view, there is preliminary evidence that certain molecular events, such as *MYC* family gene amplification and *NFIB* overexpression, are associated with promoting metastasis and progressive disease (43, 65). Moreover, the copy number gains of the individual *MYC* family members were shown to be mutually exclusive, indicating an oncogenic driver role for each (43). On the other hand, gene expression and copy number analysis studies with limited numbers of P-LCNEC surgical specimens divided HGNECs into poor and good prognosis groups, as defined by OS, independent of histology of SCLC and P-LCNEC (66,67).

Of most importance, a proportion of P-LCNEC tumors exceeding 10% are shown to carry genetic alterations in at least one of the genes *KEAP1*, *STK11*, and *RAS*, which are commonly identified in NSCLC (64). Intriguingly, these are mutually exclusive with *RB1* alterations, the latter being the hallmark of SCLC. Furthermore, *CDKN2A* deletions, which lead to p16 inactivation and are extremely rare in SCLC, occur in up to 8% of P-LCNEC cases and in a mutually exclusive manner with *RB1* mutations (3,64,68). These observations point to the existence of at least two distinct subtypes of P-LCNEC, each sharing molecular features with the corresponding major histological type of lung cancer, that is, NSCLC against SCLC.

On the basis of an international multi-institutional, integrative genomic analysis of 60 fresh-frozen (FF), resected, pure P-LCNEC tumors, Fernandez-Cuesta *et al*. were the first to reveal two distinct subtypes: one characterized by alterations that are typical for SCLC, most notably the concurrent inactivation of *TP53* and *RB1* genes, and the other harboring inactivating *STK11* and *KEAP1* mutations, which are frequently observed in NSCLC (56). Similarly enough, in a U.S., single-institutional, targeted, next-generation sequencing (NGS) study of 45 formalin-fixed, paraffin-embedded (FFPE), resected, pure P-LCNEC tumors, Rekhtman *et al*. identified two major and one minor subsets: a SCLC-like and an NSCLC-like genotype comprising, respectively, 40% and 56% of the cases, as well as a carcinoid-like genotype, characterized by *MEN1* mutations and a low mutation burden. The three genomically defined subsets were enriched in P-LCNEC tumors with the corresponding morphologic and IHC features of SCLC, NSCLC, and highly proliferative carcinoids but could not be accurately predicted by routine pathological examination because of substantial overlap. Again, *TP53* and *RB1* coalterations, occurring in the SCLC-like subset, were mutually exclusive with alterations in *STK11* and also in *KRAS*, both observed in the NSCLC-like subset. Interestingly, IHC expression of protein RB1 could serve as a surrogate marker for the NSCLC-like subtype, even without genetic testing (61).

Together with the presence of shared mutations between the different components of mixed P-LCNEC tumors (43,69), the findings from the two abovementioned profiling studies suggest that, as an entity, P-LCNEC might represent an evolutionary trunk branching to either NSCLC or SCLC. Indeed, according to GEMM studies, the said tumor can derive from both neuroendocrine and non-neuroendocrine cells (69,70). Furthermore, biallelic *RB1* inactivation and *NOTCH* family gene loss have been described as potential mechanisms of transdifferentiation of non-neuroendocrine NSCLC toward the two HGNECs (63,64). It remains to clarify if the distinct molecular subtypes of P-LCNEC develop from specific cells of origin or through cellular plasticity processes.

9. Future opportunities for molecular profile-guided management

The therapeutic implications of the tumor subtyping are even more intriguing, as well as substantial for the daily clinical practice. In the largest-to-date, population-based cohort study with available data on the treatment outcome of patients with stage IV P-LCNEC, the efficacy of first-line platinum-based chemotherapy was retrospectively assessed across the two major, mutually exclusive, molecular subtypes, that is, SCLC- and NSCLC-like P-LCNEC, as defined by targeted NGS and IHC analysis of FFPE specimens (72). In resemblance to the findings of previous profiling studies, *RB1* mutations were present in 47% of the 79 cases available for NGS; they occurred mutually exclusively with those affecting *STK11* and, in their vast majority, co-existed with *TP53* alterations. Nevertheless, while RB1 protein expression was absent in almost all *RB1*-mutated tumors, it was also downregulated in about half of the wild-type ones, that is, in a total of 72% of the 109 cases available for IHC, therefore suggesting that immunostaining might be a more reliable marker for *RB1* inactivation. Furthermore, positive IHC expression for proteins RB1 and p16 was mutually exclusive.

Patient and treatment characteristics, including subsequent therapies, were similar between the two P-LCNEC subtypes. Although the mutational status of none of the evaluated genes had a significant impact on OS in the total study cohort, subgroup analysis of patients with *RB1* wild-type tumors showed a significant OS prolongation for those treated with platinum

doublets containing gemcitabine and taxanes over those receiving platinum combined with etoposide or pemetrexed (median, 9.6 versus 5.8 and 6.7 months, respectively). On the contrary, the type of chemotherapy did not significantly affect the OS in patients with *RB1*-mutated P-LCNEC. Identical associations of patients' outcome with the abovementioned regimens were demonstrated according to the status of Rb1 IHC expression, that is, significant OS differences only among patients with RB1 expressing tumors that favored the gemcitabine- and taxane-platinum doublets over the etoposide- and pemetrexed-based regimens (median, 9.6 versus 1.9 and 4.8 months, respectively). However, on multivariable regression analysis, only RB1 protein expression interacted significantly with the type of chemotherapy, implying its superiority over the mutational status of the gene as a predictive biomarker. Furthermore, although positive RB1 protein expression combined with p16 loss predicted for improved outcome with gemcitabine- and taxane-platinum chemotherapy, the absence of p16 expression did not have an additive predictive value (72).

Another candidate biomarker for chemosensitivity of P-LCNEC, but in the adjuvant setting, is the IHC expression of YAP1, the loss of which has emerged as a specific feature of pulmonary NETs (73). According to an Asian, multi-institutional, retrospective analysis of 71 surgically resected, stage I–IV HGNEC, 43% of which of P-LCNEC histology, all pure SCLC tumors were characterized by loss of YAP1 expression when compared with 60% of P-LCNEC tumors and only 3% of non-neuroendocrine NSCLC cases. Furthermore, positive immunostaining for YAP1 was significantly and inversely correlated with the expression of neuroendocrine markers, such as ASCL1. Univariable OS analysis of patients with HGNEC receiving adjuvant, predominantly platinum-based chemotherapy, showed that only those with YAP1-negative tumors had a significant benefit. On the other hand, the YAP1-status did not significantly affect the OS of patients treated only with surgery (74).

By comprehensively analyzing not only the genomic but also the transcriptional patterns of 75 stage I–IV, FF, pure and combined P-LCNEC tumors, a more recent, multi-institutional, molecular profiling study by George *et al*. identified two major mutually exclusive subsets, "type I" (37%) and "type II" (42%), each displaying an intriguing discordance between its genomic and transcriptomic profiles (63). Despite sharing *STK11* and *KEAP1* mutations with NSCLC, type I P-LCNEC exhibited an *ASCL1*^{high}/*DLL3*^{high}/*NOTCH*^{low} transcriptional signature that characterized the majority of SCLC tumors. However, notwithstanding similarities on neuroendocrine gene expression, type I P-LCNEC was distinguished from SCLC by upregulation of pathways involved in cellular respiration and metabolism, along with downregulation of those controlling cell cycle and mitosis. Conversely, while type II P-LCNEC resembled the majority of SCLC tumors in terms of concurrent *TP53 and RB1* mutations, it was transcriptionally distinct by expressing an *ASCL1*^{low}/*DLL3*^{low}/*NOTCH*^{high} signature and displaying upregulation of immune-related signaling pathways. The above findings contradict those of a previous, small profiling study that showed a correspondence between P-LCNEC genotyping and transcriptional phenotyping into SCLC- and NSCLC-like subsets based on *TP53* and *RB1* alteration patterns (75). Keeping in mind its retrospective design and enrichment for stage I–II tumors, the study by George *et al*. showed no significant association of the proposed molecular classification with either disease stage or patients' outcome.

Regarding its implications for systemic therapies, high *DLL3* expression in type I P-LCNEC might indicate susceptibility to the promising antibody–drug conjugate rovalpituzumab tesirine, which targets the corresponding non-canonical inhibitory Notch ligand (NCT02709889) (76). According to a retrospective study on FFPE primary P-LCNEC tumors from various sources, the rate of IHC detection of *DLL3* was as high as 84% (77). On the other hand, upregulation of immune-related pathways in type II P-LCNEC might predict better outcomes to novel immunotherapies. Lastly, despite the recent failure of the anti-Notch-2/3 monoclonal antibody tarextumab combined with platinum-based chemotherapy for chemotherapy-naïve extensive SCLC in a randomized, placebo-controlled, phase 2 study (78), targeted agents against either activating or inhibitory members of the Notch pathway appear as an attractive therapeutic strategy for the respective P-LCNEC subtypes.

10. Promising actionable targets and therapies

Besides tailored chemotherapy based on P-LCNEC subtyping, the implementation of personalized targeted therapy is also warranted. According to the abovementioned molecular profiling study by Rekhtman *et al*., two-thirds of P-LCNEC tumors harbored one or more potentially actionable genetic alterations, but these were detected significantly more frequently in the NSCLC-like compared with the SCLC-like subset. A notable example of such promising therapeutic targets was the tropomyosin-related kinase (Trk) receptors encoded by the *NTRK* family genes, the latter being altered in 19% of the total cohort (61).

Interestingly, in a previous, large, European, molecular study focusing on the *NTRK* status in primary NSCLC, including pulmonary NETs, mutations were restricted to P-LCNEC at a frequency of 31%, while in the case of mixed tumors, they were only detected in the P-LCNEC component (79). Although *NTRK* alterations identified in these two studies were non-fusion and involved *NTRK2* and *3* genes, there have been sparse reports of *NTRK*-rearranged P-LCNEC (56,63,80). Notwithstanding the lack of robust evidence for the oncogenic properties and predictive value of *NTRK* alterations other than fusion rearrangements, there are preclinical data supporting an important role of the Trk-B signaling pathway in tumorigenesis and invasiveness of P-LCNEC, relative to the other pulmonary NETs and NSCLC (81). Furthermore, promising antitumor activity of Trk inhibitors in P-LCNEC has been demonstrated by in vitro and in vivo studies (82,83).

As previously suggested by the limited clinical data available (44), the incorporation of targeted agents potentially synergizing with standard chemotherapy is another promising approach for advanced-stage P-LCNEC. The addition of the poly-ADP ribose polymerase (PARP) inhibitor veliparib to platinum-based regimens has been evaluated in patients with advanced-stage HGNEC and showed signals of efficacy as front-line therapy for extensive SCLC in a recent, randomized, phase 2 trial (84,85). While clinical assessment of such combinations in P-LCNEC is ongoing (NCT01642251, NCT02289690), a large, Asian, retrospective IHC analysis study of HGNEC FFPE specimens showed a strong PARP1 expression in as high as 68% of P-LCNEC tumors, although this proportion was significantly lower than the one in SCLC and the intensity was significantly higher in chemotherapy non-responding cases (86).

With the advent of novel immunotherapies in the management of both major histologic types of lung cancer, it is reasonable to consider their potential value for P-LCNEC and also to wonder how to predict their efficacy. Regarding the rate of positive IHC programmed cell death ligand 1 (PD-L1) expression in P-LCNEC, there is a wide variation in the literature that is partially attributed to inter-study differences in sample size, type of material, tumor and patient characteristics, as well as methodology and definition of PD-L1 positivity (87-91). Collectively, it seems that tumor cell membranous PD-L1 staining in P-LCNEC is at least comparable with that in SCLC but lower than that in NSCLC. In the aforementioned Dutch population-based cohort of patients with stage IV P-LCNEC receiving chemotherapy as first-line and, where applicable, second-line treatments, the prevalence of PD-L1 positive (≥1%) tumors was 16%, but only 5% had a PD-L1 expression of ≥50%. Interestingly, the rate of PD-L1 positivity did not differ significantly between the SCLC-like and NSCLC-like subgroups as defined by the *RB1* mutational status. Furthermore, a retrospective outcome analysis in the total cohort showed a favorable association of PD-L1 expression with OS (median, 8.9 versus 6.6 months) (92). On the contrary, a large, Asian, retrospective study of the prognostic value of PD-L1 staining in operable, early stage HGNEC failed to establish a significant association with either cancer-specific survival or OS in patients with P-LCNEC, in contrast to the favorable findings for SCLC that were significant by multivariable analysis (93).

As for the tumor mutational burden (TMB) in P-LCNEC, this has been shown by several retrospective studies to correlate with PD-L1 expression [63,94]. An interesting observation from a retrospective, targeted, NGS analysis of a small cohort of patients with stage IV P-LCNEC was that median TMB was significantly higher than the corresponding values in two large cohorts of unselected patients with NSCLC and SCLC. However, it did not differ significantly by the P-LCNEC subtype as defined by the *RB1* mutational status (95). Moreover, in the abovementioned NGS study by Rekhtman *et al*., both the SCLC-like and NSCLC-like subtypes of P-LCNEC exhibited a high TMB, which significantly exceeded the ones in their SCLC and NSCLC counterparts (61).

Although the predictive significance of the two immune-related biomarkers for P-LCNEC remains unclear, a few case series support the use of programmed cell death protein 1 (PD-1)- checkpoint inhibitors as second- and beyond second- line of therapy (96). In one of the largest such reports, most patients were treated with nivolumab after receiving first-line chemotherapy with platinum and etoposide. Considering that, in most cases, the PD-L1 status was unknown, the disease-control rate was encouragingly high as 70% and the median PFS was longer than 1 year (97).

11. Conclusions: where we stand and where we can go

P-LCNEC is a distinct, yet heterogeneous entity within the spectrum of HGNECs of the lung. Despite clinical and histological similarities with SCLC, it is marked by important differences in the genomic and transcriptional profiles, with plausible implications for treatment.

Most aspects of its optimal management remain unknown or debated, but in general, thoracic surgery is advocated for resectable tumors, while systemic chemotherapy should likely be implemented as early as in stage I. Unfortunately, no substantial therapeutic

advances have been achieved over the past years, with overall prognosis remaining poor.

Retrospective molecular profiling studies have recently revealed potentially actionable targets as well as tumor subsets with distinct biology that could underlie the observed differential sensitivity to platinum-based chemotherapy. Large prospective studies with central pathology review are warranted to validate these promising findings and to identify further predictive biomarkers that would guide a personalized management approach for both early and advanced stages.

As the conduct of randomized clinical trials is hampered by the rarity of the disease, the comparison of different treatment modalities and the establishment of novel therapeutic strategies would require the adoption of study designs for orphan diseases and the development of collaborative research networks.

12. Conflicts of interest

The author discloses no conflicts of interest related to this review.

13. Abbreviations (in order of appearance in text)

TP53, tumor protein p53 gene; *MYC*, v-myc avian myelocytomatosis viral oncogene homolog gene; *PI3K,* phosphoinositide 3-kinase gene*; AKT (PKB),* protein kinase B gene*; mTOR*, mechanistic target of rapamycin gene; *RB1*, retinoblastoma 1 gene; *KEAP1*, kelch like ECH associated protein 1 gene; *STK11*, serine/threonine kinase 11 gene; *RAS*, rat sarcoma viral oncogene homolog gene; *CDKN2A*, cyclin-dependent kinase inhibitor 2A gene; *MEN1,* menin 1 gene; *NOTCH*, Notch gene; *NFIB*, nuclear factor I B gene; *NTRK2/3*, neurotrophic receptor tyrosine kinase 2/3 genes; YAP1, Yes-associated protein-1; *ASCL1*, achaete-scute family bHLH transcription factor 1 gene; *DLL3*, delta-like Notch canonical ligand 3 gene.

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