Histopathology

Histopathology 2023 DOI: 10.1111/his.15058

REVIEW

Updates on lung neuroendocrine neoplasm classification

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Vocino Trucco G, Righi L, Volante M & Papotti M
(2023) *Histopathology*. https://doi.org/10.1111/his.15058

Updates on lung neuroendocrine neoplasm classification

Lung neuroendocrine neoplasms (NENs) are a heterogeneous group of pulmonary neoplasms showing different morphological patterns and clinical and biological characteristics. The World Health Organisation (WHO) classification of lung NENs has been recently updated as part of the broader attempt to uniform the classification of NENs. This much-needed update has come at a time when insights from seminal molecular characterisation studies revolutionised our understanding of the biological and pathological architecture of lung NENs, paving the way for the development of novel diagnostic techniques, prognostic factors and therapeutic approaches. In this challenging and rapidly evolving landscape, the relevance of the 2021 WHO classification has been recently questioned, particularly in terms of its morphology-orientated approach and its prognostic implications. Here, we provide a state-of-the-art review on the contemporary understanding of pulmonary NEN morphology and the potential contribution of artificial intelligence, the advances in NEN molecular profiling with their impact on the classification system and, finally, the key current and upcoming prognostic factors.

Keywords: artificial intelligence, classification, lung, molecular profile, neuroendocrine neoplasm

Introduction to lung neuroendocrine neoplasms: terminology and epidemiology

Lung neuroendocrine neoplasms (NENs) are defined as a family of heterogeneous pulmonary neoplasms showing neuroendocrine morphology and immunophenotype.[1,2] Despite the common neuroendocrine differentiation and neuroendocrine marker expression, members of this family differ significantly in terms of (1) morphology, (2) immunophenotype, (3) molecular profiles, (4) biological behaviour and (5) clinical behaviour. Four tumour entities belong to this variegated family: typical carcinoid (TC), atypical carcinoid (AC), small-cell lung carcinoma (SCLC) and large-cell neuroendocrine carcinoma (LCNEC).[1] In addition, combined neuroendocrine (NE) and non-NE carcinomas also exist.

Most recent data suggest that NENs account for 20% of primary lung neoplasms,[3] the vast majority of which are SCLC. SCLCs account for 15% of all primary lung malignancies, followed by LCNEC (3%) and lung carcinoids (2%), with a TC to AC ratio of 10:1. While SCLC and LCNEC tend to occur in older men with an history of heavy smoking, lung carcinoids tend to occur in women at a younger age, and they do not show a clearly defined association with smoking habits.[1] Relevantly, decreasing trends in smoking habits have translated into a steep decline of SCLC incidence, confirming the dangerous link between smoking and SCLC histotype.[4,5] During the first 10 years of the new millennium, a steady reduction of SCLC of 3.4% in males and 2.8% in females was observed in the United States, according to the Surveillance, Epidemiology and End Results program (SEER) (https://seer.cancer.gov), probably driven by an overall 7.7% reduction in smoking prevalence in that country, according to the World Health Organisation (WHO).[6]

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With the exception of SCLC, the annual incidence of other lung NENs has been steadily increasing, probably attributable to the progressive ageing of the population, to the improvements of diagnostic techniques[4] and the greater awareness of these entities in the broader medical community.[7,8]

Non-neoplastic conditions and pre-invasive neuroendocrine lesions of the lung

Neuroendocrine cell alterations in non-neoplastic and pre-invasive conditions represent a spectrum of morphological changes encompassing linear and nodular hyperplasia and tumourlets. Their clinical and morphological characteristics lack definitive criteria and frequently coexist in the same tissue sample. They may be isolated or develop in the context of diffuse idiopathic pulmonary neuroendocrine cell hyperplasia (DIPNECH). These lesions are postulated to be precursors of lung carcinoids, mainly those in a peripheral location, rather than of high-grade small- and large-cell neuroendocrine carcinomas, whose origin seems to be more complex (possibly also linked to non-NE cell types).[9]

Neuroendocrine cell hyperplasia determines an increased number of NE cells in the respiratory epithelium and is associated with many causative factors. In paediatric patients, neuroendocrine cell alterations are described in bronchopulmonary dysplasia and dysmaturity, respiratory distress syndrome, cystic fibrosis and cystic malformation, pulmonary hypertension and sudden infant death. In the adult population, alterations of the neuroendocrine cell compartment are generally associated with chronic obstructive diseases, smoking-related bronchiolar disease and pneumonia, or more generally to any condition leading to pulmonary injury and repair, as well as in interstitial inflammation and fibrosis.[10] Histopathological patterns of neuroendocrine cell hyperplasia are recapitulated into linear and nodular hyperplasia. The former is defined as an irregular overgrowth of triangular or flask-shaped neuroendocrine cells, located in close contact with the basal membrane of small or large airways, whereas the latter is made of small clusters of 10–20 neuroendocrine cells in contact with the basal membrane.

Tumourlets are proliferations of oval, round or spindle-shaped neuroendocrine cells with minimal atypia in the bronchial or bronchiolar walls with submucosal extension, having a size of less than 5 mm. They are usually incidental findings at light microscopy when a variety of pulmonary conditions are examined, including neoplastic conditions or bronchiectasis, chronic inflammation and fibrosis. Exceptionally, tumourlets can be associated with Cushing syndrome.[11] Tumourlets may also be encountered in the lung parenchyma surrounding carcinoid tumours (up to 8% in some series).[12]

DIPNECH may present with clinical symptoms or in asymptomatic patients. In the presence of symptoms, DIPNECH is most typically associated with constrictive bronchiolitis mimicking asthma.[13] Neuroendocrine cell hyperplasia presenting in asymptomatic patients is typically a pathological incidental finding in lung specimens resected for other reasons, or because lesions are detected on high-resolution CT and suspected to be metastases due to multiple bilateral lung nodules.[14]

Pathological definition of DIPNECH is the presence of a multifocal neuroendocrine cell hyperplasia and/or tumourlets involving small airways. Following the most recent WHO classification, DIPNECH diagnosis includes essential and desirable diagnostic criteria that combines a clinical and a pathological approach.[1] In pure pathological terms, the degree of neuroendocrine cell hyperplasia required to establish a diagnosis of DIPNECH has not yet been established. A proposal has been made to consider the presence of neuroendocrine cell hyperplasia in the epithelium of at least three separate bronchioles combined with at least three carcinoid tumourlets as the minimum diagnostic criteria for DIPNECH, but such criteria are not universally recognised and validated.[15]

In association with neuroendocrine cell proliferations, bronchiolar fibrosis with luminal narrowing or constrictive bronchiolitis are present. Single or multiple carcinoids may also be present,[16] strongly supporting the notion that the spectrum of neuroendocrine cell lesions, from neuroendocrine cell hyperplasia to carcinoids, represent a multistep model of progression. The clinical impact of DIPNECH in association with carcinoids is controversial, although some data suggest that multifocal pulmonary neuroendocrine proliferations represent a relevant adverse prognostic factor in carcinoid tumours, being associated with a higher risk of lymph node spread and tumour relapse.[17] Lastly, DIPNECH should be distinguished from neuroendocrine cell hyperplasia and tumourlets secondary to chronic lung disease (e.g. inflammation, granulomas, fibrosis or high altitude), as well as from localised reactive neuroendocrine cell hyperplasia associated with carcinoid tumours or other lung neoplasms.

DIPNECH should also be separated from localised neuroendocrine cell hyperplasia of infancy (NEHI).

The latter occurs in otherwise healthy infants in their first few months to 1 year. It presents with symptoms such as tachypnoea and intercostal retractions, which tend to improve over time, as opposed to DIPNECH, which occurs in adults during approximately the fifth or sixth decade of life and shows a slow, progressive clinical course.[18] Similar to DIPNECH, formal criteria for the diagnosis of NEHI are currently lacking. Two individual airways with at least 10% bombesin immunoreactive intra-epithelial cells is considered suggestive of NEHI, although failure to demonstrate positivity to bombesin does not exclude the diagnosis. In this setting, the correlation of pathological findings with clinical features is crucial to rule out other paediatric lung disorders associated with neuroendocrine cell hyperplasia.[18]

Classification of lung neuroendocrine neoplasms

Based on the grade of differentiation, the four entities TC, AC, SCLC and LCNEC can be separated into two classes: carcinoids/neuroendocrine tumours (NETs) and neuroendocrine carcinomas (NECs).[1] Within this framework, carcinoids correspond to well-differentiated NENs and include low- (i.e. TC) and intermediate-grade tumours (i.e. AC), while NECs correspond to high-grade carcinomas, and include SCLC and LCNEC[1] (Table 1). This binary classification framework has been promoted by the International Agency for Researchon Cancer (IARC)/WHO committee,[2] and has also been endorsed by the European Neuroendocrine Tumour Society (ENETS) and the International

Table 1. Present WHO terminology for neuroendocrine neoplasms (thoracic versus digestive)

Thoracic (WHO 5th edn, 2021)	Definitional criteria	Digestive (WHO 5th edn, 2019)	Definitional criteria
Typical carcinoid (NET, low-grade)	Well-differentiated neuroendocrine morphology, no necrosis, mitotic index < 2 mitoses/2 mm^2	NET (grade 1)	Well-differentiated neuroendocrine morphology, < 2 mitoses/2 mm^2 and Ki67 < 2%
Atypical carcinoid (NET, intermediate-grade)	Well-differentiated neuroendocrine morphology, necrosis (punctate) and/or mitotic index 2–10 mitoses/2 mm^2	NET (grade 2)	Well-differentiated neuroendocrine morphology, 2–20 mitoses/2 mm^2 and/or Ki67 3–20%
(Carcinoids with high mitotic/ proliferation index)	Well-differentiated morphology, mitotic index > 10 mitoses/2 mm^2 and/or Ki–67 > 30%	NET (grade 3)	Well-differentiated neuroendocrine morphology, > 20 mitoses/2 mm^2 and/or Ki67 > 20%
Large-cell neuroendocrine carcinoma	High-grade non-small- cell carcinoma with neuroendocrine morphology, NSCLC cytology (prominent nucleoli and/or moderate to abundant cytoplasm) and a mitotic count of > 10 mitoses/2 mm^2	NEC (small- and large-cell carcinoma)	Poorly differentiated neuroendocrine morphology, > 20 mitoses/2 mm^2 and/or Ki67 > 20%
Small-cell lung carcinoma	Small cells (usually less than the size of 3 resting lymphocytes) with scant cytoplasm, granular nuclear chromatin with absent or inconspicuous nucleoli and > 10 mitoses/2 mm^2		
Mixed* Combined SCLC or LCNEC + NSCLC (any %) Combined SCLC + LCNEC (≥ 10%)	Features of SCLC/LCNEC with a component of NSCLC (LCC, ADC, SCC or less commonly spindle and/or giant cell carcinoma) NSCLC combined with carcinoid is not included	Mixed –MiNEN†	Features of any possible NEN with a malignant non-neuroendocrine component (ADC, SCC); NEN combined with benign non-neuroendocrine component (i.e. adenoma) are not included

ADC, Adenocarcinoma; LCC, Large-cell carcinoma; LCNEC, Large-cell neuroendocrine carcinoma; MiNEN, Mixed neuroendocrine-non neuroendocrine neoplasm; NEC, Neuroendocrine carcinoma; NEN, Neuroendocrine neoplasm; NET, Neuroendocrine tumour; NSCLC, Non-small-cell lung cancer; SCLC, Small-cell lung carcinoma; SqC, Squamous cell carcinoma.

*At least 10% of LCNEC or large-cell carcinoma is required to diagnose combined SCLC/LCNEC or combined small-cell carcinoma with large-cell carcinoma. There is no threshold for the amount of other components including small-cell carcinoma.

†At least 30% of each component is required.

Association for the Study of Lung Cancer (IASLC) in the attempt to conceptually unify the lung NENs terminology with the WHO terminology for gastro-entero-pancreatic NENs.[19] The introduction of a common nomenclature for tumour entities occurring in different anatomical sites but belonging to the same family (i.e. NEN) not only reduces contradictions, but also favours comparisons across different classification systems.[2,19,20]

In addition to the novel binary framework, the category of 'combined tumours' has been retained in the spectrum of NENs. This class of neoplasms encompasses malignancies combining a NEN component with a non-small-cell lung cancer (NSCLC), similar to what occurs in the digestive tract (e.g. mixed neuroendocrine/non-neuroendocrine neoplasm, MiNEN), as well as combinations of a SCLC with LCNEC (classified as 'combined SCLC and LCNEC') (Table 1).[1,21,22]

The criteria used to allocate these neoplasms in the classification system were not changed. Since the 1999 classification, the diagnostic criteria include histological (i.e. morphological features/pattern of growth, mitotic count per 2 mm^2, presence/absence of necrosis) and cytological features (i.e. nuclear/cytoplasmic ratio, abundance of cytoplasm, chromatin, etc.) (see section below and Table 2).[1] Of these, tumour morphology is recognised to be the cornerstone to distinguish carcinoids/NETs from NECs, while it is not relevant to further subdivide carcinoids/NETs. By contrast, despite marking a watershed between NETs and NECs, mitotic criteria are conceptually not indispensable to make a diagnosis of NEC.[23]

Recently, this ironclad rule has started to erode as evidence of a grey zone between NETs and NECs has emerged. Within the group of carcinoids, one subtype has been recently described that is constituted by neoplasms classified as NETs based on their morphology, but showing a high mitotic count and/or proliferation index, usually with a heterogeneous pattern.[23,24] These tumours recapitulate NET G3 in the gastrointestinal tract and pancreas[25] and are characterised by a clinical behaviour intermediate between atypical carcinoids and LCNEC.[26,27] Moreover, despite specific molecular data being scarce, coexistence of molecular alterations common to both carcinoids and neuroendocrine carcinomas (including alterations involving different pathways such as the chromatin remodelling and the cell cycle) have been described.[28]

Conversely, another subclass includes NENs classified as LCNEC based on the mitotic count but having NET/carcinoid morphological and molecular features.[29,30] Indeed, as this grey zone continues to grow, further studies are needed: first, to determine how to best allocate these entities into a future classification; and secondly, and perhaps more importantly, to explore the possibility of a direct progression from carcinoids/NETs to NECs.[31] At present, the current WHO classification recommends classifying these grey-zone tumours with a mitotic index exceeding 10 per 2 mm^2 as LCNEC; however, it also advises to add a note stating the presence of histological features reminiscent of carcinoids/well-differentiated NETs and documenting the exact mitotic count and, possibly, the Ki-67 index.[1]

Lastly, another minor introduction in the classification system is worth mentioning: the term 'carcinoid NOS' (i.e. not otherwise specified). The use of this term is reserved to those clinical settings in which no clear distinction between TC and AC is reasonably feasible, such as evaluation of small biopsies, cytological specimens or metastatic tissues.[1,22,32] In fact, in these cases, accurate mitotic count or necrosis assessment can be challenging or not fully representative. For these reasons it is recommended to use the term 'carcinoid NOS' accompanied by a short note including the mitotic count, possible foci of necrosis and, desirably, Ki67 proliferation index.[1]

Morphological spectrum and immunophenotype

The main histological, cytological and immunohistochemical features of lung NENs are summarised in Table 2. At present, the defining parameters of lung NENs include morphological and cytological features, despite the growing utility and the exceptional advances of molecular pathology (see below).

In lung carcinoids, the prototypical morphology is the one of a well-differentiated NET (Figure 1). This translates into an overall organoid architecture with a huge number of possible different and frequently intermixed arrangements spanning from trabecular, rosette formation, insular to follicular or solid.[1,33] Similarly, a wide array of cell variants has also been described (e.g. polygonal, spindle, oncocytic, clear, melanin-laden, mucinous, etc.). Despite this interweaving of morphological aspects, TCs and ACs ultimately differ in mitotic index and the presence of necrosis (Figure 2).

Importantly, SCLC and LCNEC share their morphological features with the high-grade neuroendocrine carcinomas (NECs) occurring in other sites.[1,7] Compared to NETs, they are characterised by more

Table 2. Histological, cytological and immunohistochemical features of lung NENs (modified from Ref. 33)

	TC	AC	Carcinoids with high MI/PI	SCLC	LCNEC
Histological features					
Morphology (pattern of growth)	Organoid, trabecular, rosette formation, nested, insular	Organoid, trabecular, rosette formation, nested, insular	Organoid, trabecular, rosette formation, nested, insular	Sheet-like, diffuse	Organoid, trabecular, palisading
Mitotic count/ 2 mm^2	0–1	2–10	> 10¶	> 10 (generally 70–80)	> 10 (generally 50–60)
Proliferation index (Ki-67)*	< 10%	10–25%	> 30%¶	70–100%	25–80%
Necrosis	Absent	Focal, punctate	Focal, punctate	Geographic	Extensive
Cytological features					
Cell size	Variable	Variable	Variable	Small (usually less than the size of 3 resting lymphocytes)	Large
Nuclear chromatin	Finely granular texture (salt and pepper)	Finely granular texture (salt and pepper)	Finely granular texture (salt and pepper)	Finely granular texture, evenly distributed	Coarse to vesicular
Nucleoli	Occasional, small	Common, small	Common, small	Absent or inconspicuous	Common, large
Cytoplasm	Variable	Variable	Variable	Scarse	Abundant
Immunohistochemistry (IHC)					
Synaptophysin	+++	+++	+++	± to +†	+++
CgA	+++	++	+(+)	± to +†	+
INSM1	+++	+++	+++	+++†	+++
Rb‡	WT	WT	WT	Lost	WT (carcinoid-like and NSCLC-like) or lost (SCLC-like)**
p53§	WT	WT or (rarely) aberrant	WT	Aberrant	Aberrant**

IHC results are displayed on a scale from negative (−) to diffusely positive (+++); ±: positivity in up to 10% neoplastic cells.
AC, Atypical carcinoid; CgA, Chromogranin A; INSM1, Insulinoma-associated protein 1; LCNEC, Large-cell neuroendocrine carcinoma; Rb, Retinoblastoma; SCLC, Small-cell lung carcinoma; SSTR, Somatostatin receptor; TC, Typical carcinoid; WT, Wild-type.
*Ki-67 is quantified according to the percentage of nuclear-labelled cells over 2000 elements or per 2 mm^2.
†Aproximately 10% SCLC cases show negative to low expression of synaptophysin and other neuroendocrine markers, including INSM1.
‡Rb IHC staining is expressed as wild-type (i.e. strong and diffused nuclear positivity retained) or lost (i.e. no expression).
§p53 IHC staining is expressed as wild-type (i.e. patchy nuclear positivity) or aberrant (overexpression or null expression).
¶This tumour subset requires the presence of at least one of the two criteria, according to the 2021 WHO of thoracic tumours.
**According to the 2021 WHO of thoracic tumours, the use of Rb/p53 IHC to distinguish among LCNEC subtypes in routine clinical practice is currently not recommended.

extensive necrosis and a higher mitotic count. Even though an overall organoid architecture can still be recognised in LCNEC this is generally lost in SCLC, where a diffuse growth pattern is commonly encountered. Notwithstanding their different morphology, the key to distinguish LCNEC from SCLC lies in the cytological

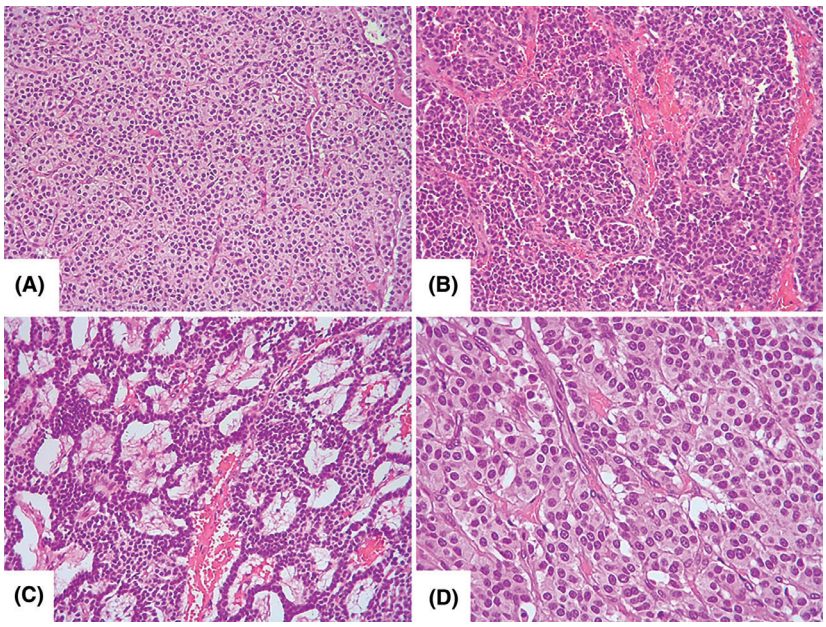

Figure 1. Architectural spectrum of typical carcinoids. Typical carcinoids (TC) are characterised by an overall organoid architecture with different arrangements spanning from solid (**A**) to alveolar (**B**), pseudo-glandular (**C**) or cord-like (**D**) [**A**,**B**,**C**, haematoxylin and eosin (H&E); **D**, H&E].

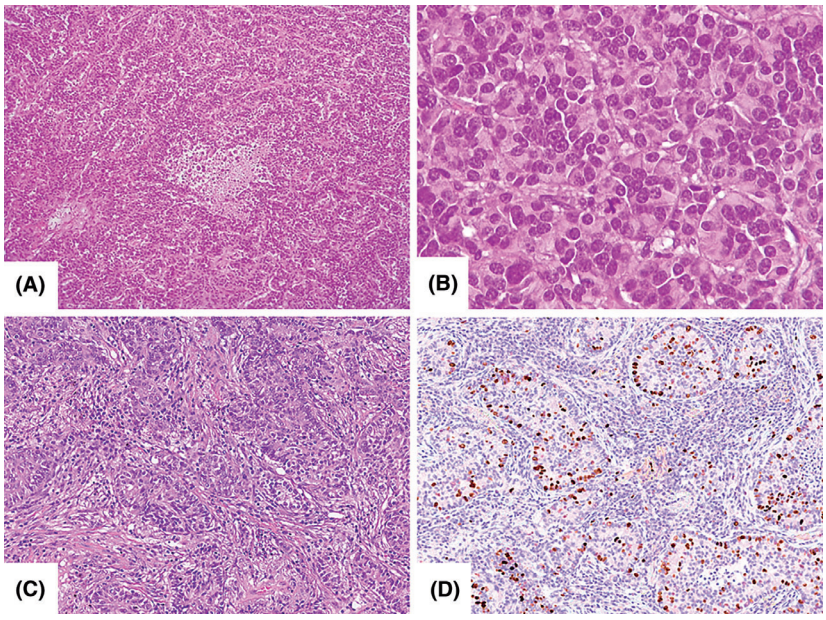

Figure 2. Histological features of atypical carcinoid and carcinoid with elevated proliferation index. An atypical carcinoid showing well-differentiated neuroendocrine morphology and a focus of necrosis (**A**) associated with mitotic figures (**B**). A case of carcinoid with high proliferation index showing a well-differentiated neuroendocrine morphology (**C**), indistinguishable from atypical carcinoid, but with a heterogeneous Ki-67 index, above 30% in hot-spots (**D**) [**A**, haematoxylin and eosin (H&E); **B**, H&E; **C**, H&E; **D**, Ki-67 proliferation index].

aspect: LCNEC is characterised by large cells with an abundant cytoplasm, coarse nuclear chromatin and prominent nucleoli (Figure 3), whereas SCLC is characterised by small cells with scant cytoplasm, finely granular nuclear chromatin and inconspicuous nucleoli[1] (Figure 4).

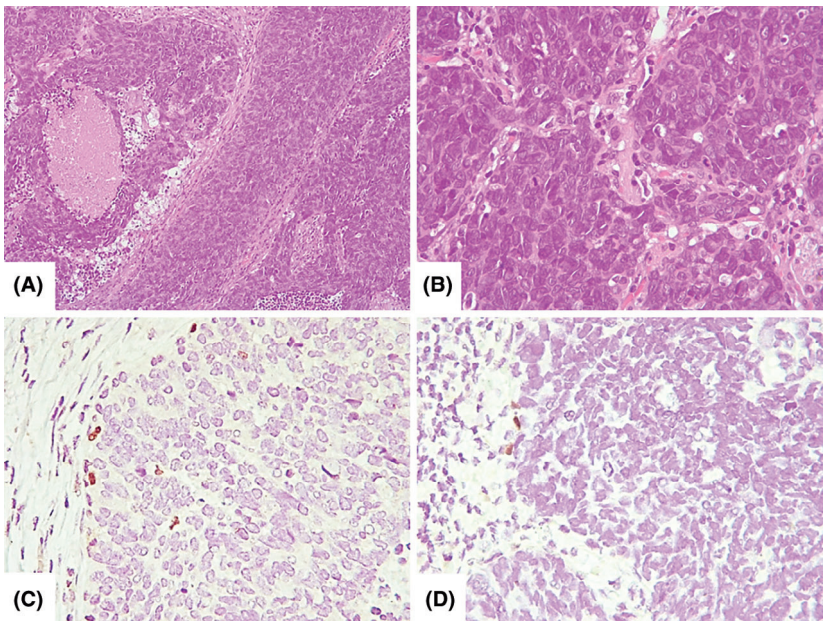

Figure 3. Histological and immunohistochemical characteristics of LCNEC. A case of SCLC-like LCNEC with organoid growth pattern with necrosis (**A**) and large cells with vesicular nuclei, occasional nucleoli and high mitotic index (**B**); the tumour shows concomitant total loss of Rb (**C**) and p53 expression (**D**) in the presence of isolated positive control cells [**A**, haematoxylin and eosin (H&E); **B**, H&E; **C**, Rb immunohistochemistry; **D**, p53 immunohistochemistry].

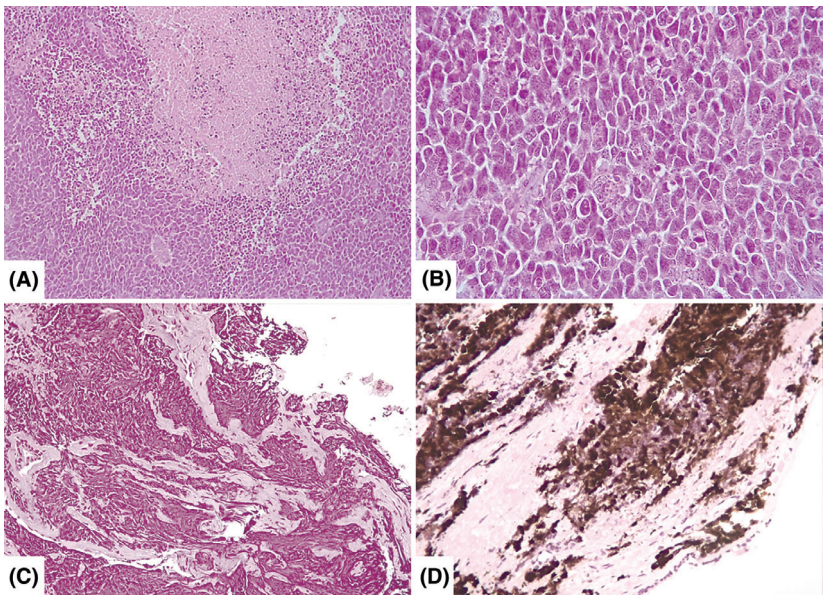

Figure 4. Histological and immunohistochemical characteristics of SCLC. SCLC grows in a diffuse pattern-less architecture with extensive necrosis (**A**), and is made of small cells with scant cytoplasm, dispersed nuclear chromatin and absence of nucleoli (**B**); extensive crushing artefacts with nuclear moulding are frequent in biopsy specimens (**C**); Ki-67 immunohistochemistry is very helpful in this context highlighting the very high proliferation index (**D**) [**A**, haematoxylin and eosin (H&E); **B**, H&E; **C**, H&E; **D**, Ki-67 immunohistochemistry].

In non-surgical material, the recognition of pathological features distinctive of the different histotypes may be concerning. Classically, pulmonary carcinoids/NETs are distinguished in TC and AC according to mitotic count and presence of punctuate necrosis, as mentioned previously. However, clinical reality (e.g.

a small or crush biopsy) does not always permit them to be clearly distinguished, and in these cases the term 'carcinoid not otherwise specified (NOS)' should be preferred. These rare cases do not portend relevant clinical implications at present, as the current surgical intervention for TC and AC is the same and a final differential diagnosis can be safely obtained *a posteriori* on the entire resected specimen.[1] This may change in the near future, and a pathologist may be required to tell them apart even on biopsy materials, as emerging surgical trends seem to favour a more conservative surgical approach in TC (i.e. segmentectomy), compared to ACs (conventional lobectomy).[34,35] In this setting, but also in the context of identifying prognostically divergent 'highly proliferating carcinoids', a novel differential diagnostic role for Ki-67 index has been foreseen, although it has not yet been endorsed by the international classifications.[1,36] Similarly, while the differential diagnosis between SCLC and LCNEC mainly relies upon morphology and different NE marker expression profiles (see below), in small biopsies with poor cellularity and/or crush artefacts, this distinction might be challenging.[37]

Lastly, as the boundary line between NETs and NECs is currently under discussion, so are the potential diagnostic tools that pathologists may use to identify such borderline cases. In this setting, where morphology clearly fails as a diagnostic and prognostic classifier, the remaining diagnostic clues are represented by an elevated mitotic count (> 10 mitoses/ 2 mm^2) and/or a higher than expected Ki-67 proliferation index (> 30%) (Figure 2). Unfortunately, the assessment of these two parameters is known to be operator-dependent,[38–40] such that the issue of borderline cases still remains a clinically relevant unmet need. In this context, the importance of immunohistochemistry (IHC) lies in confirming the NE differentiation in neoplasms whose morphology is already suggestive for it. Other than that, IHC is also widely utilised for differential diagnosis purposes and identification of prognostic biomarkers[33] (discussed in the appropriate sections below). As already stated, morphology alone can be sufficient in recognising the neuroendocrine phenotype of lung NENs, but current guidelines nonetheless require IHC for the confirmation of the diagnosis.[1] Of note, SCLC represents an exception, as it is generally considered a light microscope-based diagnosis and IHC is listed among desirable, but not essential, diagnostic criteria.[1] Conversely, evidence exists to discourage the use of IHC in the absence of morphological features suggestive for diagnosis,[40–42] as NE markers can be expressed by other neoplastic entities (e.g. up to 30% of NSCLC), and thus unnecessarily further complicate the differential diagnosis.[43]

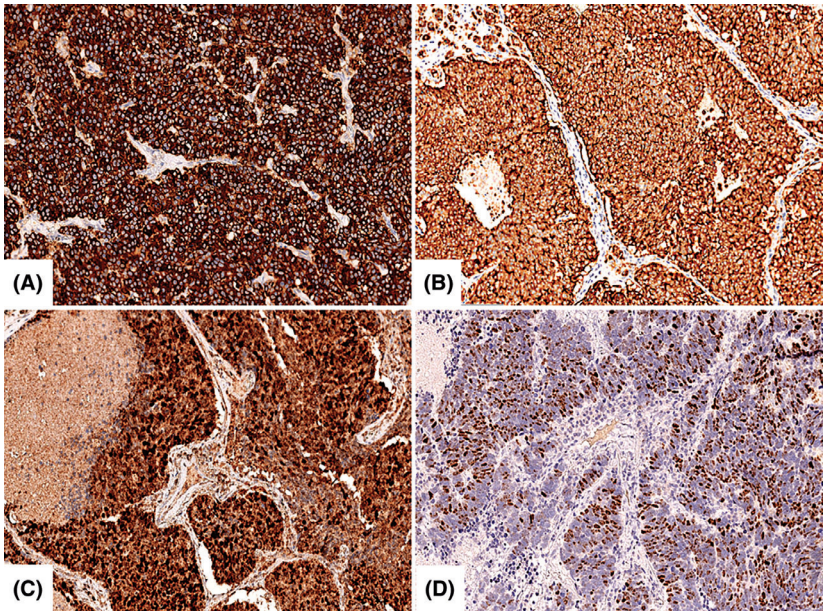

Figure 5. Patterns of expression of neuroendocrine markers in lung NENs. Strong and diffuse expression of chromogranin A in a case of typical carcinoid (**A**). Diffuse expression of synaptophysin in a case of atypical carcinoid (**B**); diffuse nuclear INSM1 expression in a case of LCNEC (**C**); ASCL1 (hASH1) nuclear expression in a case SCLC (**D**).

Immunophenotyping of lung NENs includes reactivity for low-molecular-weight cytokeratins[44] and markers of NE differentiation[45–47] (Figure 5). Among NE markers, chromogranin A, synaptophysin and INSM1 are considered the most sensitive and specific,[43] whereas CD56 positivity alone should be considered with caution to support neuroendocrine differentiation in the differential diagnosis of lung NENs, due to its low specificity. Despite this, a certain degree of heterogeneity across the various tumour entities exists. For example, carcinoids/NETs and LCNEC are strongly reactive for cytokeratins and all neuroendocrine markers;[1,44] conversely, due to their reduced cytoplasm and paucity of its neurosecretory granules, SCLC is often weakly and focally positive for these markers.[48,49] Moreover, 5–10% of SCLC may be completely negative for all NE markers defining the so called 'SCLC-variant type'.[50,51]

Finally, a few additional considerations about the classic and novel NE markers include:

i INSM1 has proved to be quite a sensitive and reliable NE marker; however, it is also positive in non-neoplastic (normal and reactive) NE tissue and in a few non-NE tumours.
ii Somatostatin receptor types 2 and 5 ($SSTR_2$ or $SSTR_5$) are often expressed in carcinoids and possibly in a subset of LCNEC, but at a weaker intensity.
iii Expression of the protein product of the *ASCL1* gene (hASH1), a marker of NE differentiation, is almost exclusively restricted to NECs, but it can also be identified in a small subset of lung carcinoids[52–54] (Figure 5).
iv POU2F3 (SKN-1a/OCT-11) is a recently reported SCLC marker, generally expressed in NE marker-negative cases.[55]
v Abnormal p53 (hyperexpression or null expression) is most frequently detected in NECs, and rarely in carcinoids.[56]
vi Loss of retinoblastoma 1 (*RB1*) protein expression is correct for NECs.[48]
vii Neurone-specific enolase (NSE) and protein gene product 9.5 (PGP9.5) are largely non-specific and their use in routine diagnostic practice is not advisable.[48]

Diagnostic reproducibility

Recently, the issue of diagnostic reproducibility has been raised, as the histological criteria (e.g. mitotic count and interpretation of Ki67 proliferation index, morphological evaluation, etc.) are intrinsically subjected to a high degree of interobserver variability. In this section, we will tackle the most important issues that may contribute to the low diagnostic reproducibility.

Mitotic count is one of the cornerstone parameters used to distinguish NENs, but its reproducibility has been shown to be incompletely satisfactory. A recent work by Swarts and colleagues on pulmonary carcinoids showed that the degree of interobserver variability among leading world experts was modest (kappa = 0.316), with the primary discordant factor being the estimated number of mitotic figures.[40] These data were further confirmed by Warth and colleagues., who found that among nine expert pathologists, the agreement on the differential diagnosis between TC and AC was rather poor (kappa = 0.213), and that this was due mainly to differences in reported mitotic count.[57] Indeed, mitotic figures are unevenly distributed across the tumour tissue, with large random effects, and unfortunately the current guidelines do not suggest any preferential method for mitotic counting. A possible solution for precise mitotic counting is IHC for phosphohistone H3 (PHH3), a marker of mitotic figures, which has been suggested to reduce interobserver variability in many tumour entities, including lung carcinoids,[58–62] but its use in routine grading requires validation.

Similarly, the implementation of Ki-67 labelling index to improve diagnostic reproducibility remains controversial.[1,57,63–65] Importantly, the major source of controversy is represented by the fact that the role of Ki-67 as diagnostic classifier of lung NENs has never been proven.[1] This, together with the absence of standardised evaluation methods and the issue of a high degree of interobserver variability,[66] akin to those observed for mitotic counts, have resulted in the guidelines suggesting the use of Ki-67% as a helpful, yet not essential, diagnostic tool.[1]

Important interobserver variations have been shown for the morphological assessment in NEC. For example, a study reported the agreement of LCNEC and SCLC morphological diagnoses to have been only fair (kappa = 0.4).[67] In fact, in this specific context, morphometric analysis revealed some transitional cell characteristics between LCNEC and SCLC, implying that the evaluation of cell size is also, to some extent, also arbitrary.[34,68,69]

In the attempt to overcome these issues, it is tempting to look with hope at the emerging IHC markers brought up by the continuous progress of molecular pathology (see below). An alternative and potentially appealing solution might be the reassessment of the prognostic impact of classical markers by means of artificial intelligence and digital image analysis, which could aid the pathologist in standardising the diagnosis, thus augmenting the diagnostic reproducibility.

Methods of artificial intelligence in diagnosisy

Artificial intelligence (AI) is a broad term defining a particular branch of computer science committed to design systems that process information and perform tasks in a similar, if not better, way than humans.[70] In the pathology field, where diagnostic infallibility represents the holy grail, the introduction of AI and specifically of digital image analysis (DIA) has acquired progressive interest as it promises to improve diagnostic accuracy, reproducibility and speed.[71] Evidence published in literature hitherto seem to justify these expectations, as developed machine- and deep-learning mechanisms have shown excellent performances in tasks such as image recognition and classification. In the field of diagnostics, such tasks confer to DIA an unique array of possible implications spanning from the analysis of tumour morphology to mitotic counting or quantification of theragnostic biomarkers.[72] It, therefore, comes as no surprise that some authors have proposed DIA as a solution for contemporary issues with diagnostic reproducibility.[68]

One of the possible benefits provided by DIA in diagnostic pathology involves the application of morphometric analyses for differential diagnosis purposes. In the field of thoracic pathology, this aspect has been extensively investigated in NSCLC,[73–75] whereas evidence regarding its application in NENs is more limited. Recently, Gonzalez and colleagues conducted a proof-of-concept study on fine-needle aspiration samples to demonstrate that a deep-learning algorithm can classify high-grade NECs according to their morphological characteristics. Although conducted on a small sample, results obtained were excellent both in terms of sensitivity and specificity (e.g. sensitivity = 1 and specificity = 0.875 for the Diff Quick® model).[76] More recently, Iliè and colleagues investigated the adoption of a convolutional neural network (CNN) for the differential diagnosis of surgically resected pulmonary NENs. In a head-to-head comparison, AI-driven algorithms distinguished the different entities with high accuracy (0.97 F1-score, 0.93 AUC), with a degree of sensitivity and specificity comparable to conventional assessment, but also showed a slightly higher agreement than that of pathologists, suggesting a beneficial role of this model in assisting pathologists in the diagnostic work-up.[77]

Besides supporting morphological analyses, DIA can also be employed for standard assessment and quantification of prognostic biomarkers (e.g. Ki-67 proliferation index and mitotic counting using PPH3-based IHC).[78] In these settings, positivity for Ki-67 or PHH3 is analysed through algorithms detecting nuclei by morphological shape and size and then classifying cells as positive or negative based on pixel colour and intensity.[79] Using digital quantification algorithms, the assessment of Ki-67 in NENs have shown similar, if not better, performances compared to manual counting in many studies,[78,80–82] including in those exclusively conducted on pulmonary NENs.[83–85] Similarly, computer-assisted mitotic count has also shown good performance in many tumour entities,[86,87] including NENs.[81]

These promising data notwithstanding, it is important to remember that the implementation of DIA algorithms in routine clinical practice is still far from being achieved, with further studies needed to validate standard models, define image storage policies and assess the pathologist's responsibilities.[88]

Molecular profile of lung NENs

In recent years, an astonishing progress has been made in unveiling the molecular spectrum of lung NENs. These findings have restructured the theories on the biological pathways of this entity and at the same time allowed new openings for actionable treatment strategies. Available evidence not only enriches the field with data on prevalence of the molecular alterations typical of each histological type, but also allows us to confirm the existence of an intermediate molecular class straddling the currently binary vision of NETs and NECs (corresponding to those borderline lesions not fitting into the classic morphological classification).[89]

Awareness that the current classification system is imperfect has led to an investigation into the molecular profile of NETs and NECs, using both supervised and unsupervised models of machine learning. Briefly, supervised models of machine learning leverage on labelled inputs and outputs aiming to predict outcomes or make inferences, while unsupervised models do not make use of labelled data and are aimed at understanding the architecture of the data, such as identifying clusters.[90] In this context, supervised models make use of preset morphological categories to investigate intraclass molecular heterogeneity, while the more disruptive unsupervised models are used to gain novel insights into molecular similarities, potentially offering novel insights that could be used to advance our current classification systems. We will first focus upon the insights from supervised models, followed by unsupervised models.

Importantly, evidence from the classic supervised models of molecular profiling suggest that there are

relevant interclass differences in intraclass molecular heterogeneity.

Lung NETs are characterised by a low mutation rate, with a slight increase from typical to atypical carcinoids, but overall lower than SCLC and LCNEC.[91,92] Approximately 50% of lung NETs harbour mutations in the chromatin remodelling and histone modification-related genes (e.g. *MEN1*, *EIF1AX* and *ARID1A*, etc.).[91,93] Of these, *MEN1* is the most frequently somatically mutated gene, found in 11–22% of carcinoids with a higher prevalence in ACs than TCs.[94,95] Other frequently mutated chromatin remodelling genes include genes belonging to the *SWI/SNF* complex (20%), *KMT2/MLL* (14%) and *PSIP1* (5%), with the latter found in NETs lacking *MEN1* gene alterations.[89,91]

Among lung NENs, LCNEC represent the most heterogeneous group in terms of molecular profile, encompassing three subclasses: SCLC-like LCNEC (40%), NSCLC-like LCNEC (55%) and carcinoid-like LCNEC (5%).[96] SCLC-like LCNEC is characterised by some molecular alterations typical of conventional SCLC, such as *RB1* and *TP53* inactivation, *MYCL1* amplification, *CREBBP*, *EP300* and *KMT2A* gene mutations, as well as *FGFR1* amplifications; however, they differ from conventional SCLC in their transcriptomic profile (*ASCL1*-low/*DLL3*-low/*Notch*-high profile in SCLC-like LCNEC versus *ASCL1*-high/*DLL3*-high/-*Notch*-low expression profile in conventional SCLC).[1,97] Conversely, NSCLC-like LCNEC share some molecular alterations with non-NE-tumours, such as *CDKN2A* deletion, *TTF1* amplifications, *KRAS*, *KEAP1* and *LKB1* mutations or alterations in other genes belonging to the *RAS* pathway.[97] Finally, the class of carcinoid-like LCNEC shares *MEN1* mutations with NETs.[96,98]

Conversely, SCLC is characterised by *TP53* and *RB1* gene bi-allelic inactivation and an extremely high mutation frequency. Other than *TP53* and *RB1*, frequently found that molecular alterations include *CREBBP*, *EP300* or *KMT2A* gene mutations, *NOTCH* gene inactivation and *MYC* amplification.[56] At the transcriptional level, four subclasses can be identified based on *ASCL1*, *NEUROD1* and *POU2F3* gene expression: *ASCL1* high (SCLC-A), *NEUROD1* high (SCLC-N), *POU2F3* high (SCLC-P) and a fourth subtype with no prevailing transcriptional signature (triple-negative SCLC). As the latter was found to be enriched for inflammatory genes such as those involved in the immune check-point system and human leucocyte antigens (HLAs) genes, this group was denominated SCLC-inflamed (SCLC-I).[99,100]

Of note, SCLC might also arise from NSCLC, as a result of acquired tumour resistance of lung adenocarcinomas harbouring targetable oncogene-driver mutations (i.e. *EGFR*, *ALK*, *ROS1*) and treated with tyrosine kinase inhibitors (TKI).[101] Interestingly, such tumours usually retain oncogene driver mutations. In addition, similarly to *de-novo* SCLC, these tumours gather *RB1* mutations during tumor progression or present them as co-alterations before treatment, evidence further supporting a major role of *RB1* in promoting SCLC oncogenesis.[102]

Recently, molecular studies have confirmed the existence of an overlapping class between NETs and NECs. This class, despite showing genetic alterations straddling between the two families of NENs, is counted as part of the NET spectrum, due to the presence of *MEN1* mutation typical of NETs.[96,103,104]

Considering that the above-mentioned supervised models of molecular profiling demonstrated a wide degree of intertumoral molecular, biological and clinical heterogenicity, unsupervised models of molecular profiling were used to gain novel insights into the genetic architecture of NENs. These included the intent of reclassifying them according to their molecular signature into novel categories able to more accurately predict the biological behaviour, the clinical outcome and as gene susceptibility to target-therapies[89,98] (Figure 6).

A speculative reappraisal of available molecular data on lung NENs has suggested the hypothesis that there may be three separate classes of NENs: primary neuroendocrine carcinomas, secondary neuroendocrine carcinomas and indolent neuroendocrine tumours. Although this view needs to be reconsidered by specific studies, it sounds meaningful from a clinical viewpoint.[9]

Primary neuroendocrine carcinomas are NENs characterised by severe gene alterations (e.g. bi-allelic inactivation of *RB1* and *TP53* and *NOTCH* silencing, etc.) which trigger a *de-novo* pathogenic mechanism with an early-maturation block in cancer stem cells of the neuroendocrine niche.

These lesions show an early malignant potential and aggressivity without the development of intermediate pre-invasive/dysplastic lesions. This group features undifferentiated tumour cells of the SCLC type, and account overall for 70–75% of NENs and 13% of all lung cancers.[98]

Conversely, secondary neuroendocrine carcinomas are lung NENs characterised by the sequential acquisition of less severe genetic alterations in cancer stem cells of a neuroendocrine niche or non-neuroendocrine cancer stem cells acquiring a neuroendocrine differentiation. According to this model, these lesions would probably be the result of a

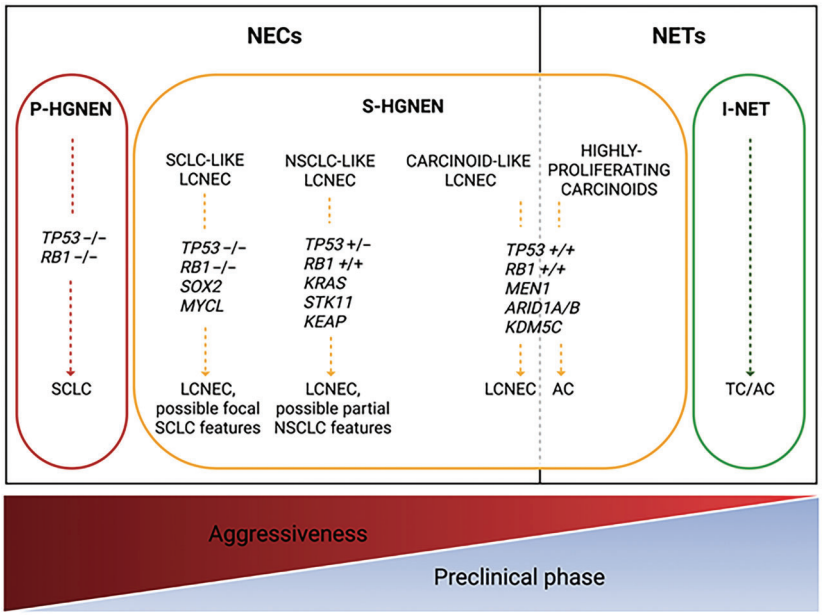

Figure 6. Unsupervised cluster model-based molecular classification of lung NENs. AC, atypical carcinoid; I-NET: indolent neuroendocrine tumour; NEC, neuroendocrine carcinomas; NET, neuroendocrine tumour; NSCLC, non-small-cell lung cancer; P-HGNEN, primary high-grade neuroendocrine neoplasm; S-HGNEN, secondary high-grade neoplasm; TC, typical carcinoid.

tumoral progression from pre-existing lesions (i.e. pre-invasive neuroendocrine lesions, NETs or NSCLC) through the acquisition of a wide range of possible genetic alterations (e.g. *TP53* → *RB1* mono/bi-allelic inactivation, *NOTCH* alteration, *KRAS*/*LKB1*/*MEN1* mutation, *MYC*/*MYCL*/*TERT*/*SDHA*/*RICTOR* amplification or epithelial–mesenchymal transition).[33,98]

Molecularly, this group of NENs is more heterogeneous in respect to primary ones, as would be expected from a transformed lesion, and may also show a greater morphological spectrum (e.g. SCLC-like, NSCLC-like and carcinoid-like). Therefore, ultimately this group includes LCNEC with its molecular subclasses (i.e. SCLC-like LCNEC, NSCLC-like LCNEC and carcinoid-like LCNEC, respectively).[96] Of note, the category of carcinoids with elevated proliferation (similar to grade 3 NET in the gastrointestinal tract) also merges into this group, as its molecular signature is superimposable to that of carcinoid-like LCNEC. Secondary neuroendocrine carcinomas account for approximately 20–25% of lung NENs and 6% of all lung cancers.

Finally, a third class was identified and termed indolent neuroendocrine tumours (I-NET). This group is characterised by molecular alterations that induce cancer cells to block at a later stage of differentiation without genetic segregation. Consequently, these tumours are enriched with differentiated cells and correspond to TCs or low proliferating ACs, accounting for 5% of lung NENs and approximately 1% of all lung cancers.[96]

Differential diagnosis

Owing to the important intra- and intertumoral diversity, NENs represent a true diagnostic challenge, especially in biopsy samples. In fact, primary lung NENs morphologically mimic numerous other tumours, and secondary localisations of NENs located elsewhere need to be considered in the differential diagnosis. As mentioned previously, IHC has a confirmatory value and needs to be used cautiously in differential diagnosis, as NENs can share NE markers with other tumours, even of non-NE nature. Lastly, NENs are characterised by an uneven intratumoral distribution of mitoses and necrosis with low interpathologist reproducibility.[1]

Considering the clinical presentation and the aggressive clinical course, as well as frequent surgical unresectability, the issue of a correct diagnosis of lung NECs on small samples (e.g. small biopsies or cytology) is of relevance. In this setting, several entities enter into the differential diagnosis with lung NECs. One entity is basaloid squamous cell carcinoma, whose morphology may overlap with that of

LCNEC and, occasionally, of SCLC. Albeit rarely, this neoplasm may also express some neuroendocrine markers (in particular CD56); however, a strong and diffuse expression of squamous markers (p40/p63, high-molecular-weight cytokeratin 34βE12) supports the diagnosis of basaloid squamous cell carcinoma.[1] Another entity is the SMARCA4-deficient undifferentiated tumour (SMARCA4-UT), which may show some morphological overlap with LCNEC and may also express NE markers (Figure 7), representing another relevant diagnostic challenge, especially in crush biopsy artefacts. In this context, complete loss of SMARCA4 (BRG1) expression is diagnostic, but it is important to remember that a marked reduction of SMARCA4 staining, rather than a complete loss, can be observed in approximately 25% of SMARCA4-UT.

Clinically, it is fundamental to distinguish the two entities, as SMARCA4-UT is highly resistant to chemotherapy and may benefit from the development of specific therapies targeted against the SWI/SNF complex.[105]

Moreover, in the differential diagnosis of SCLC, it is important to also consider a NUT carcinoma, a poorly differentiated carcinoma defined molecularly by the presence of *NUTM1* gene rearrangement. Similar to SCLC, NUT carcinoma typically grows in nests and sheets of small and monomorphic cells and may show necrosis. Additionally, expression of chromogranin, synaptophysin or even TTF1[106] might be observed, further complicating the differential diagnosis, particularly on small biopsies. In this setting, the presence of evenly spaced cells, the lack of nuclear moulding together with abrupt foci of keratinisation, characteristic of NUT carcinoma, should be promptly noted and used as a guide towards the correct diagnosis. Also, NUT carcinoma typically stains positive for pancytokeratin, p63, p40 and NUT antibody (clone C52B1), as opposed to SCLC.

Lastly, other frequent morphological mimickers of lung NECs include small round cell sarcomas, melanoma, lymphoma and Merkel cell carcinoma, for which however, a correct diagnosis can be easily reached through the implementation of different IHC markers.[1]

Within the lung, pulmonary carcinoids need to be distinguished from metastatic NETs, especially those deriving from the gastroenteropancreatic (GEP) area. Some morphological features (including more

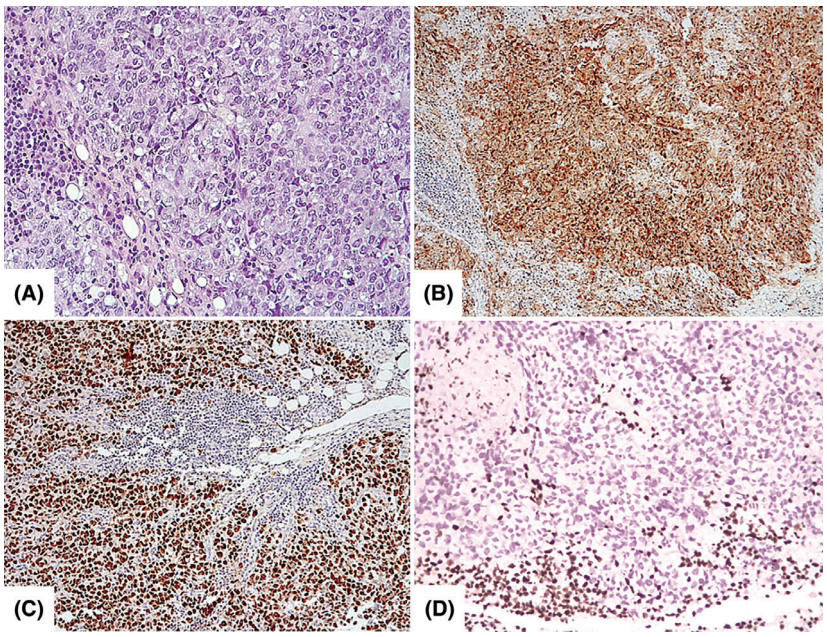

Figure 7. SMARCA4-deficient undifferentiated tumour: a potential diagnostic pitfall in the differential diagnosis of high-grade NEC. SMARCA4 undifferentiated tumour (SMARCA4-UT) grows in a solid pattern and is composed of intermediate to large cells characterised by relatively monotonous nuclei with vesicular chromatin and prominent nucleoli (**A**). Akin to high-grade NEC, synaptophysin expression can be prominent (**B**). In rare cases, TTF1 may be expressed (**C**). Complete loss of SMARCA4 (BRG1) nuclear staining is the clue for its diagnosis (**D**, small peritumoral lymphocytes serve as positive internal control) [**A**, haematoxylin and eosin (H&E); **B**, synaptophysin immunohistochemistry); **C**, TTF1 immunohistochemistry; **D**, SMARCA4 immunohistochemistry]. The diagnosis of SMARCA4-UT in the present cases was further supported by the negative staining for cytokeratins (CK7, CK20 and pan-CK using the AE1/AE3 clone) and the loss of SMARCA2/BRM nuclear expression (not shown in the figure).

common glandular structures in GEP NENs) and some immunomarkers may aid in guiding the diagnosis. Among the latter, orthopedia homeobox protein (OTP) has been suggested to favour a lung origin and to be useful in metastatic settings[107] while, conversely, the expression of CDX2 or PAX8 favours an EC-like cell and pancreatic origin, respectively. In the presence of compatible clinical manifestations, additional IHC markers such as gastrin or serotonin may also be used in this setting. Gastrin may support the presence of a gastrin-producing NET located in the gastric antrum or first segment of the duodenum, whereas serotonin is typically expressed in NETs localised in the appendix or jejuno-ileal tract and is rarely produced by lung NETs. The determination of the primary site of origin is even more important in the presence of metastatic (stage IV) NETs. In this setting it should be noted that stage IV lung carcinoids are somewhat infrequent compared to secondary GEP-NETs. To avoid the overdiagnosis of NECs pathologists should be aware that, in metastatic settings, pulmonary carcinoids/NETs may show a mitotic count and particularly a Ki-67 proliferation index significantly higher compared to their primary lesions, with Ki-67 sometimes exceeding even 30%.[108] Unfortunately, however, the exact prognostic and therapeutic implications of Ki-67 assessment in stage IV lung carcinoids are incompletely defined. Consequently, the WHO currently recommends to term such lesions 'metastatic carcinoid tumour NOS' without specifying TC or AC and invites reporting the Ki67 proliferative index togather with the mitotic count and the presence or absence of necrosis.[1]

Overall, an integrated approach must be used for the diagnosis of NENs, which is best made in referral centres.

Prognostic considerations and predictive markers

The prognostic implications of the 2021 WHO classification of lung NENs are not completely satisfactory. Recently, the molecular classification of lung NENs modelled by means of unsupervised molecular clustering was proposed as a powerful new tool not only to assist classification, but also as a prognostic and predictive tool.[98] This model is built upon the hypothesis that the more severe the molecular alteration, the greater is the tumour aggressiveness with consequent shorter preclinical phase and more malignant clinical course.[33] The three prognostically different molecular groups (primary neuroendocrine carcinomas, secondary neuroendocrine carcinomas and I-NET) have a progressively reducing burden of molecular alterations and a better prognosis. In addition to providing a potentially more harmonious and biologically driven prognostic classification, the molecular profiling of lung NENs has afforded the medical community with novel predictive markers.

Starting from pulmonary NETs, the alteration of *MEN1* indicates a poorer prognosis[34,109] and is similarly the loss of expression of OPT and CD44.[100,110] Of relevance, combined IHC positivity for these two markers [i.e. OTP (nuclear) combined with CD44 (cell membrane)] is also a predictor of recurrence-free survival.[110,111] Furthermore, expression of BIRC5, BUB1, IL20RA and KLK12 were found to be independent predictors of patient outcome.[112]

In SCLC, encouraging emerging data relate to the potential use of transcriptional subclass markers such as ASCL1, NEUROD1 and POU2F3 for therapy selection.[99] For example, ASCL1-dominant SCLC have been shown to be particularly chemosensitive compared to the other subclasses,[113] and delta-like canonical Notch ligand 3 (DLL3) targeted therapies are currently under investigation for this subclass.[114,115] Conversely, *POU2F3*-regulated SCLC shows a greater susceptibility to PARP inhibitors,[100] as well as to Aurora kinase A.[116] Moreover, increased *YAP1* gene expression was shown to correlate with a poorer prognosis, especially when associated with a wild-type expression of RB1,[34] and it has also been reported in association with an immune inflamed SCLC, leading to the assumption that YAP1 protein positivity could potentially identify SCLC cases that may benefit from immunotherapy.[117,118] Moreover, in SCLC, programmed cell death ligand 1 (PD-L1) inhibitors have been recently approved in combination with chemotherapy as a frontline treatment in extensive-stage SCLC, and clinical trials have yielded partially satisfactory results.[119–121] Evidence suggests that PD-L1 IHC expression fails to predict responses to immunotherapy,[118] indicating that further parameters are necessary to more effectively select potential candidates for immunotherapy.[122]

Relevant prognostic/predictive considerations for LCNEC are also in order. For example, as standard treatments of advanced stage LCNECs are not yet completely established, patients may be treated currently with a SCLC-like chemotherapy (i.e. platinum-etoposide-based) or a NSCLC-like chemotherapy protocol (i.e. gemcitabine/taxane/pemetrexed combined with platinum). However, recent evidence has emerged that wild-type *RB1* gene LCNECs (i.e. NSCLC-like NECs) respond better to a NSCLC-like

chemotherapy regimen compared to a SCLC-like chemotherapy,[123] with longer overall survival (9.6 versus 5.6 months).[124] Therefore, in this context the assessment of Rb IHC, which is easily assessable,[125] could be possibly used to predict response to a particular type of chemotherapy regimen. Different clones to detect Rb protein expression are available and applicable in the routine practice (including 4H1: Cell Signalling, Danvers, MA, USA; 13A10: Leica Biosystems, Wetzlar, Germany; 3C8 GeneTex: Alton Parkway Irvine, CA, USA).[34,125]

Additional targetable NSCLC molecular alterations include *EGFR* mutations and *ALK* gene rearrangements that, although rarely, might be present and targetable with FDA-approved tyrosine kinase inhibitors (TKI).[126,127] Finally, other markers potentially useful in target therapies include mammalian target of rapamycin (mTOR), PD-L1 and thymidylate synthase.[33,126]

Conclusions

Lung NENs are a heterogeneous group of pulmonary neoplasms characterised by different morphological, clinical and biological features. In an attempt to harmonise the classification of neuroendocrine neoplasms across different organ systems, the 2021 WHO classification of thoracic tumours has been updated by the introduction of a binary framework by which TC, AC, SCLC and LCNEC have now been separated into two classes: carcinoids/NETs and NECs. Additionally, the existence of a grey zone between NETs and NECs has been recognised and a novel subclass of NETs has been introduced (i.e. highly proliferating carcinoids).

At present, the diagnostic clues of lung NENs remain the histological and cytological features. However, issues pertaining to interobserver agreement of diagnostic criteria have led to proposals to either utilise immunohistochemical markers as a diagnostic aid or to introduce AI-derived digital image analysis to assist the pathologist, but validation studies are lacking.

From a molecular viewpoint, lung NETs are characterised by a low mutation rate, and in half of cases harbour mutations in the chromatin remodelling and histone modification-related genes, whereas NECs are usually characterised by *RB1* and *TP53* inactivation. The increased knowledge of the molecular background of lung NENs has also led to the identification of potential prognostic biomarkers. In the context of NETs, *MEN1* mutations and OTP loss have been associated with a poorer prognosis. Conversely, prognostic markers in NECs appear to be subclass-specific and include transcriptomic clusters in SCLC that are associated with different treatment responsiveness and *RB1* alterations in LCNEC that are predictive of response to chemotherapy.

In summary, lung NENs represent a promising field in evolution, where prospective studies are needed to refine the novel diagnostic approaches and to assess the clinical usefulness of the new molecular biomarkers which are currently emerging.

Acknowledgements

None.

Conflicts of interest

None to declare.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request; ethics approval was not applicable.

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