What clinicians are asking pathologists when dealing with lung neuroendocrine neoplasms?

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Abstract

Lung neuroendocrine tumors (NET) are currently classified in resection specimens according to four histological categories, namely typical carcinoid (TC), atypical carcinoid (AC), large cell neuroendocrine carcinoma (LCNEC) and small cell carcinoma (SCC). Diagnostic criteria have remained unchanged in the 2015 WHO classification, which has ratified the wide acceptance and popularity of such terminology in the pathologists' and clinicians' community. A unifying umbrella of NE morphology and differentiation has been recognized in lung NET, which has pushed to enter an unique box of invasive tumors along with diffuse idiopathic pulmonary NE cell hyperplasia (DIPNECH) as a pre-invasive lesion with a potential towards the development of carcinoids. However, uncertainties remain in the terminology of lung NET upon small samples, where Ki-67 antigen could play some role to avoid misdiagnosing carcinoids as high-grade NE tumors. Epidemiologic, clinical and genetic traits support a biological three-tier over a pathology four-tier model, according to which TC are low malignancy tumors, AC intermediate malignancy tumors and LCNEC/SCC high malignancy tumors with no significant differences in survival among them. Inconsistencies in diagnostic reproducibility, troubles in the therapy of AC and LCNEC, and limitations to histology within the same tumor category argue in favor of a global re-thinking of lung NET where a grading system could play a role. This review outlines three main key-questions in the field of lung NET: a) unbiased diagnoses, b) the role of Ki-67 and tumor grading, and c) management of predictive markers. Answers are still inconclusive, thus additional research is required to improve our understanding on lung NET.

Key words: neuroendocrine, tumor, carcinoid, large cell, small cell, diagnosis, immunohistochemistry, grading, Ki-67, prognosis, survival, predictive, molecular pathology

Approaching lung NET

The new 2015 WHO classification on lung neuroendocrine tumors (NET) [1] has substantially confirmed the four widely-agreed upon histological variants crystallized in the two previous editions of 1999 [2] and 2004 [3], namely typical carcinoid (TC), atypical carcinoid (AC), large cell neuroendocrine carcinoma (LCNEC) and small cell carcinoma (SCC). Remarkably, in this 2015 edition, these tumors have been pushed to enter a unique box of NE proliferations by moving LCNEC from the all-inclusive chapter of large cell carcinoma, and adding diffuse idiopathic pulmonary NE cell hyperplasia (DIPNECH) as a pre-invasive lesion with a potential towards the development of carcinoids [1]. There are several practical reasons why this traditional terminology of lung NET has been retained in the new 2015 WHO classification, which is the result of widely shared expert opinions according to the current state of the art [1, 4]. The term carcinoid, either typical or atypical, has been gaining wide popularity and diagnostic awareness among pathologists and clinicians while valuable alternatives are still lacking [1, 4]. Likewise, the other two histological variants, either LCNEC or SCC, are deemed to be full-blown high-grade carcinomas occurring in either pure or combined forms, which are almost relentlessly characterized by aggressive clinical behavior and dismal prognosis [1, 4-8].

There is general agreement that this four-tired histological classification is consistent with an operational three-tier prognostic scheme on the basis of epidemiological (age, sex, smoking habit), genetic (association with MEN1 syndrome and several other gene pathways), clinical (lymph node and distant metastases, association with paraneoplastic syndromes, type and response to therapy) and behavioral traits, which results in progressive grades of biological aggressiveness [1, 9-14]. Accordingly, TC is deemed to be a low-grade malignant tumor with longer life expectation and time to recurrence, AC an intermediate-grade malignant tumor with more aggressive clinical course, somewhat unpredictable clinical behavior and shorter time to recurrence, and LCNEC and SCC high-grade malignant tumors with dismal prognosis, challenging therapy options and, often, difficulties in reliably distinguishing from each others, either pathologically, genetically or clinically [1, 4, 8, 14-19].

As a function of cell differentiation and in keeping with the recent European Neuroendocrine Tumor Society (ENETS) guidelines [4] and the current WHO classification [1],

TC and AC as a whole are considered well-differentiated NE tumors because of their resemblance to the normal cell counterpart of the NE diffuse system or pre-invasive lesions, such as DIPNECH, as opposed to LCNEC and SCC, which are thought to make up a poorly differentiated tumor group [1, 20, 21]. As a matter of fact, TC and AC feature organoid growth patterns, typical to slight atypical cytology (even though they may uncommonly exhibit prominent nuclear pleomorphism) (**Figure 1**), absent to focal punctate necrosis, up to 10 mitoses per 2 mm^2 and consistent labeling for pan-NE markers, such as chromogranin A and synaptophysin, sometimes less intense and uneven in the setting of AC [1, 4, 12, 14]. On the contrary, SCC and LCNEC show solid growth patterns, extensive/geographic necrosis, mitotic count higher than 10 mitoses per 2 mm^2, and uneven labeling for pan-NE markers [1, 4, 12, 14]. Cytological criteria are then used to split SCC from LCNEC, although there is a considerable morphologic overlap between them making this separation quite subjective and difficult to carry out, with disappointingly low inter-observer diagnostic reproducibility [12, 15, 16, 22-25].

The molecular scenario of lung NET has been pushed to emerge by several studies confirming the assumption that there are two distinct groups in lung NET. As a matter of fact a dichotomous separation between low to intermediate malignancy tumors on the one hand (i.e., TC and AC) and high malignancy tumors on the other hand (i.e., SCC and LCNEC) is solidified by substantial differences in gene pathway alterations, levels of differentiation and cell derivation [8, 12, 26-32]. Accordingly, it is not surprising that common genetic traits may be shared by each of these two broad tumor categories, with TC/AC on the one hand and LCNEC/SCLC on the other hand exhibiting major differences in the somatic mutation rates and engagement of diverse gene pathways [8, 12, 26-33]. A further inherent molecular heterogeneity, however, is found within each histological variant on the basis of several functional and genetic biomarkers, which may identify different patient subsets with different prognosis [31, 34-37].

All these assumptions suggest the opportunity to reevaluate lung NET keeping in mind that all lung NET are malignant, that the malignancy rate has to be quantified for clinical purposes of personalized therapy, and that malignancy depends on several biological and functional factors, among which a grading system specifically devised for the lung could play a pivotal role [13]. The ultimate and ambitious goal is to improve our understanding in the field

of lung NET tumors, placing them into context for the best management practice of these patients.

Designing the article

A review of papers reported on the issues of lung NET with special reference to diagnosis, Ki-67, grading and predictive markers was performed until July 2015, taking advantage of a list of key questions for either subject. We limited our bibliography research to the English literature, apart from some historical papers published in other languages. Only full papers of peer-reviewed journals were considered. Research terms included carcinoid, typical, atypical, small cell, large cell, LCNEC, SCLC, intermediate, neuroendocrine, Ki-67, proliferation, grading, mitoses, count, necrosis, DNA, square millimeter, next generation, prognosis, survival, predictive factors, aggressiveness, therapy, targeted, sequencing, genome, exome, exon, genomic, landscape, portrait, whole, transcriptome, expression, high-throughput, thymidylate synthase, fluoropyrimidine therapy, excision repair cross-complementation 1 (ERCC 1), somatostatin receptors, mammalian target of rapamycin (m-TOR), and prediction. This article was not designed to make up an exhaustive overview on the current knowledge about lung-NET, but rather to critically reappraise and rethink these tumors in light of emerging issues and questions, which often arise among physicians who treat these tumors daily within operating multidisciplinary teams. The ultimate goal was to focus on practical aspects of the fascinating world of lung-NET to answer practical questions. Specifically, we have herein developed three main key-questions, which clinicians could like to ask pathologists whenever facing lung NET. They are relative to the need: a) to have more precise and unbiased diagnoses; b) to unravel the role of prognostic factors with particular emphasis to Ki-67 labeling index and tumor grading; and c) to use predictive markers in the clinical management of these tumor patients. The following exposition will follow these three main key-questions, in light of recently published papers.

Diagnosing lung NET

Diagnosis still remains the first but no longer the only task clinicians are requiring to pathologists whenever facing lung NET. Some entities proposed over time in the field of lung

NET may be considered milestones with direct and continuing integration to the current terminology, while other terms or taxonomy schemes are only a historical inheritance (at least according to recent guidelines [4] and WHO classification [1]). Diagnostic criteria for SCC - as we still know and currently rely on - date basically back to Azzopardi's publication of 1959 [38], where the appellation of oat cell carcinoma reappraised the previous concept of small-celled sarcoma by Barnard of 1926 [39]. The term AC was introduced in 1972 by Arrigoni [40] taking advantage of necrosis, increased mitoses, disorganized architecture and cell atypia to enucleate lung carcinoid patients characterized by more aggressive clinical course from the preexisting category of bronchial carcinoid/adenoma as authored by Hamperl in 1937 [41], who extended to the lung the entity initially recognized and described in the small intestine by Oberndorfer in 1907 [42, 43]. Subsequently, diagnostic criteria for AC were definitely outlined by Travis in 1998 [7], which retained both name and defining features with no remarkable changes in the subsequent three WHO classifications of 1999 [2], 2004 [3] and 2015 [1]. LCNEC as high-grade tumor intermediate behaviorally between AC and SCLC was authored by Travis in 1991 [44], which showed striking similarities to NE carcinoma of intermediate cell type described by Gould of 1983 [45]. In 1998, this entity was confirmed in its current diagnostic attributes [7] and inter-observer reproducibility [6], but survival rate was equaled to that of SCC.

Many other classifications and terminologies have been proposed over time on lung NET, whose detailed examination is beyond the scope of the current paper, by either introducing a concept of tumor grading [11, 13, 46-49], applying different thresholds to current defining criteria [50, 51] or extending to these tumors the same defining criteria as those used in the gastroenteropancreatic tract [21, 52-54]. These different proposals, however, have not gained wide acceptance yet because of the lack of clear clinical advantages over the last three WHO classifications on lung cancer, which represented the gold standard for these tumors. [1-3]. Suffice to say that lung NET have maintained the same terminology and defining criteria of the past 16 years, making them popular among pathologists and clinicians and justifying their application to the current clinical management. However, the diagnostic inter-observer reproducibility among the diverse categories of lung NET still remains an unanswered question [6, 15, 16, 23, 55-59], as well as difficulties in identifying different patient subsets with different clinical behavior within the same histological variant or stage of disease, or in

correctly diagnosing non-resection specimens [1, 4]. While it is reasonable that no classification is able to predict all exceptions, there are some open questions regarding the current taxonomy on lung NET, which we have been summed up in **Table 1**.

It is well established that the diagnosis of lung NET is a stepwise process [22], according to which NE architecture is recognized at first and then tumors are divided into four diagnostic categories on the basis of the number of mitoses per 2 mm^2 and the presence (and extent) of necrosis [1, 4]. Additional criteria include the demonstration of pan-NE markers upon immunohistochemistry (IHC) to split LCNEC from large cell carcinoma with NE morphology or more conventional non-small cell carcinomas and a constellation of cyto-morphologic features to separate LCNEC from SCLC [1]. A synopsis of diagnostic criteria in resection specimens as outlined by the recent 2015 WHO classification is reported in **Table 2**, while representative pictures of the four tumor categories according to these criteria are depicted in **Figure 2**.

Despite the presence of a general unanimous separation between the two ends of the lung NET spectrum, i.e. TC and SCLC, at least in surgical specimens, major diagnostic concerns emerge for adjacent categories whenever addressing boundary or gray zone tumors where the subjective application of defining criteria (mainly mitoses and necrosis) may encounter difficulties in their ultimate diagnostic attribution, i.e. TC vs. AC, AC vs. LCNEC, and LCNEC vs. SCLC [6, 15, 16, 23, 55-59]. Detailed studies on the clinico-pathologic features of these boundary or gray zone lung NET are still lacking, but it is well known that AC showing a number of mitoses comprised between 6 and 10 per 2 mm^2 run a worse clinical course [60], and that on the contrary about 15-20% of SCC or LCNEC patients experience long survival [7, 10, 13, 59, 61, 62]. There are a number of issues accounting for inconsistency between morphology and clinical behavior [6, 12, 63]. Difficulties in recognizing mitoses and necrosis in the group of TC and AC [55] and variability in assessing cell size and cytological features in the group of LCNEC and SCLC [6, 15, 16, 23, 57] may explain inconsistencies in the diagnostic reproducibility of lung NET. Additional criteria, such as the labeling for mitosis-specific marker anti-phosphohistone H3, have been proposed to objective subjectivities in mitosis assessment [64], but the experience in still limited and there are no objective methods to account for tumor cell necrosis. The use of Ki-67 antigen could simplify this evaluation of proliferation activity, but overlap existing

between adjacent tumor categories and quantification modalities [12, 29] prevented to exploit this marker as defining criterion of lung NET according to current guidelines [1, 4].

Recognition of lung NET, especially in non-resection samples, is recommended by using IHC for pan-NE (synaptophysin, chromogranin A, hASH1) and epithelial markers (cytokeratin pools), in keeping with recent guidelines and classifications [1, 4]. Worth mentioning is the application of always-negative-markers, such as high molecular weight cytokeratins [65, 66] or p40 [67], whereas p63 may be consistently expressed by SCC [68]. It should be kept in mind that many nuclear transcription factors used for differentiating NET arising in diverse anatomical sites, such as TTF-1, Islet-1, PAX-8 and CDX2, hold true for well-differentiated tumors only, i.e. TC and AC, as LCNEC and SCC can be associated with aberrant and illegitimate expression of these markers regardless of their origin (**Figure 3**).

Unraveling Ki-67 and tumor grading

As outlined above, current criteria for lung NET basically include mitosis count and necrosis [12], whilst tumor architecture, cell atypia, vascular invasion, lymph node metastases or immunohistochemistry profile do not play any role in this separation [14, 17]. However, some controversies still persist in their diagnostic reproducibility, so that searching for additional criteria more related to behavioral traits is clinically warranted.

Ki-67 antigen has been largely studied in lung NET [12, 25, 29, 69], with several features regarding technical issues, evaluation of results, diagnostic role, prognostic role (including tumor grading), and predictive role in therapeutic decisions being recently reviewed [69]. There are different options to quantify Ki-67 antigen in lung NET (the product of MKI67 gene mapping to 10q26.2 gene acting as a non-histone nuclear protein involved in all active stages of the cell cycle, but not in resting cells), most often carried out upon immunohistochemistry by using the clone MIB-1 and expressed as the percentage of positive tumor cells (labeling index, LI), i.e. manual counting, digital image analysis or eyeball estimation [69]. Most published investigations agreed on the opportunity of measuring Ki-67 LI in hot spot areas, taking into account all nuclear signals after visual scrutiny of the entire tumor area [69]. This would apply especially to TC or AC, whereas Ki-67 decoration is usually much more uniform in high-grade NE tumors. For practical purposes, Ki-67 LI should be calculated in surgical specimens by

counting at least 2000 consecutive tumors cells in hot spot fields at 40x magnification or 2 mm^2 for consistency with the histological classification, possibly in the same tumor areas as those used for assessing mitotic count [69]. In biopsy or cytology samples, in which the number of tumor cells may be lower than 2000 or the 2-mm^2 criterion not necessarily met, it could be reasonable to calculate Ki-67 LI on all tumor cells. For experienced pathologists, however, manual counting of Ki-67 LI upon visual inspection or eyeball estimation differs little from more sophisticated, time- consuming, or cumbersome methods [13, 70]. Although there are significant differences in the mean/median thresholds of Ki-67 LI amongst TC, AC, LCNEC and SCC [61, 62, 69, 71-74], some overlap existing between adjacent tumor categories prevented to establish a decisional role to this marker relative to histological classification [1, 17] simply because mitoses, necrosis and Ki-67 antigen look at different biological phenomena [69], albeit they are somewhat related to each other in terms of overwhelming behavioral impact [29]. Reproducibility studies on Ki-67 LI assessment revealed encouraging results [56, 75], with less than 1.5% of variability [75] and an out-performance of Ki-67 LI over mitotic count with regard to inter-observer agreement [56].

Ki-67 LI has a major value in distinguishing TC and AC from high-grade NET [71, 73, 74], especially when small crushed biopsy samples or cytology are dealt with (with a practical cut-off point of 25% to operate this distinction) [12, 13, 71, 73, 74], as well as in differentiating between lower and higher malignant NE tumors in resection specimens of TC and AC (with cut-off thresholds ranging from 4% to 5%) [13, 37, 54, 75-78] in keeping with pancreatic NE tumors [79, 80], albeit sometimes with a non-independent value upon multivariate analysis [75]. Although conceptually reasonable, few studies have so far addressed a role of Ki-67 LI in the prognostic stratification of poorly differentiated NE tumors in the lung [13], at variance with what has been proposed in other endocrine organs, such as the pancreas [81, 82].

Tumor grading is a way to unravel the inherent aggressiveness of tumors exactly as the temperature correlates with the thermal energy of a body according to its average status of molecular agitation. Just like temperature, grading should be an intensive property of tumors independent of, albeit correlated with, tumor stage. In other words, grading would define the level of biological recruitment of tumors, correlated with but not completely overlapping with cell differentiation, which alone cannot exhaustively anticipate biological

behavior of tumors. As a matter of fact it is possible to diversify subsets of patients with different life expectation in the histological categories of lung NET [32, 35, 36, 83]. This is the reason why grading systems based on the histological definition of disease may be not completely satisfying to take operational decisions in the clinical management of patients, especially in tumors where defining histological criteria are broader, such as AC or LCNEC [29].

Grading of lung NET according to histology/cell differentiation is inherently present in the current WHO classification scheme [1]. Accordingly, TC is low-grade malignant, AC intermediate-grade malignant, and LCNEC and SCLC high-grade malignant NET [1, 4, 11, 46, 48]. In particular, SCC and TC are so agreed-upon tumor entities in the lung to seem too reductive to simply call them G3 and G1 tumors, respectively. However, establishing a grading system in lung NET independent of histology could be clinically warranted in individual tumor patients for the personalized therapy requirements, in keeping with the lesson of GEP-NET. Naturally, this grading system should rely on different defining criteria in the lung compared to GEP-NET or other anatomical locations, as there are profound differences in biological behavior for tumors arising in different sites [29, 80, 84-86]. Such a system should hopefully be independent of staging to take clinical decisions also in the metastatic setting of disease in accordance with the biological characteristics of tumors. An innovative grading method in resection specimens has recently been proposed for lung NET, which jointly included Ki-67, mitotic rate and necrosis, each parameter being further tired according to three different expression levels independent at multivariate analysis (**Table 3**) [13]. In particular, G1 tumors were defined if at least 2 out of 3 parameters were at the level 1; G2 if at least 2 out of 3 parameters were at the level 2; and G3 if at least 2 out of 3 parameters were at the level 3. The combined assessment of these three parameters outperformed each individual parameter in predicting patient overall survival, resulting in a G1 to G3 grading system showing minimal overlap of 95% confidence intervals among these three defining categories. Interestingly, all TC clustered into the G1 category whilst a small fraction of SCC and LCNEC were classified into the G2 category in keeping with the clinical observation that a small fraction of these patients pursues an unexpected less aggressive clinical course despite histological diagnosis [36, 61, 62]. Importantly AC were split into all the three tumor grades reflecting the inherent behavioral heterogeneity of AC, some of which behave very similarly to TC whereas others are much

more aggressive, not diversely from poorly differentiated lung NET [60]. These findings are likely to reflect the subjective interpretation of AC vs. TC or LCNEC when morphology is the only discriminating factor [15, 16, 55]. Certainly many efforts will be needed for validating this grading proposal in lung NET by accruing independent tumor series in resection specimens, as well as for setting up a reliable grading system in small samples, which often are the only available material at the time of the initial diagnosis or in tumor metastases, where grading tumors could have clinical relevance [4] just like in GEP-NET [84-86].

Predicting in NET

According to recent guidelines, no molecular tests should currently be routinely carried out in lung NET, unless specifically required by study protocols (*Level of Evidence 4; Grade of Recommendation C*) [4]. However, an increasing body of knowledge is accumulating in lung NET about biomarkers with predictive value, which could modify the therapy of these tumors in near future. This holds true especially for TC and AC where treatment, when non directly surgical (as mainly happens), relies on multimodality approaches or non-conventional drugs, whilst LCNEC and SCLC are generally cured by exclusive chemo-radiotherapy [87, 88].

In this setting of predictive biomarkers, Ki-67 LI does not play a decisive role in lung NET beyond refining better diagnostic recognition in demanding cases, for instance when occurring severe crush artifacts in small tissue samples [71]. Ki-67 antigen is independent of or weakly associated with thymidylate synthase expression, an enzyme involved in DNA synthesis whose presence acts as a resistance factor to fluoropyrimidine therapy in tumors, including NET [89], as well as with excision repair cross-complementation 1 (ERCC 1) expression, a resistance factor against platinum-based chemotherapy in lung cancer [90], or mammalian target of rapamycin (m-TOR) signaling activation pathways, an attractive target for inhibitors such as everolimus [61]. Thus far, no randomized clinical trials have documented that establishing Ki-67 LI in lung NE tumors may help to guide the subsequent therapy, just like in NSCLC [91].

In lung NET, there are several potential predictive factors, which could become eligible for clinical trials, on which it is warranted to accumulate more information for better personalizing therapy [92], including antifolate chemotherapy, somatostatin receptors, m-TOR

signaling pathway molecules and a miscellaneous of other factors. According to the lesson of tamoxifen in breast cancer [93], it has been demonstrated that the thresholds of thymidylate synthase evaluated by adopting either mRNA quantitative PCR [89] or semiquantitative protein expression upon IHC [94] were significantly higher in LCNEC and SCC than AC and TC or NSCLC indicating a different level of responsiveness to fluoropyrimidine therapy with longer time to progression of 5-fluorouracil-treated lung NET patients with lower expression of this biomarker [89]. Somatostatin receptors are well-known targets for analogue drug therapy in GEP-NET [95, 96], but an emerging role is playing also in the control of non-surgical cases of well-differentiated lung NET due to their anti-proliferation activity and hormone secretion inhibition [4, 97]. Somatostatin receptors can be easily assayed by IHC on tumor sections and a reliable scoring system has also been devised, which accurately correlates with *in vivo* imaging upon octreoscan [62, 98], opening the way to its routine use especially in the setting of non-operable lung NET [99]. Targeting m-TOR pathway with specific inhibitors, such as everolimus, in lung well-differentiated NET results in anti-proliferative activity likely due to reduction of VEGF secretion and IGF1 signaling inhibition [100]. Molecules involved in the downstream m-TOR activation pathway, such as phosphorylated m-TOR, AKT, p70S6K and ERK1/2 (MAPK3/1) [101], can be all easily assayed by IHC upon tumor tissue sections [61]. Concurrent inactivation of m-TOR and PI3K pathways (for instance by using dual PI3K/mTOR inhibitor NVP-BEZ235) gave rise to more potent effects than everolimus alone in reducing the proliferation of human bronchial carcinoid cells, with resistant tumor cells displaying lower levels of mTOR, p70S6K, AKT and ERK1/2 [101]. These findings indicate that looking for these proteins may be useful to predict sensitiveness for high protein levels or resistance for low protein levels to synergic m-TOR and PI3K/m-TOR inhibitor treatment in well-differentiated NE proliferations, including carcinoids and DIPNECH [102, 103]. The pathway of m-TOR in lung NET is also related to energy and metabolism regulation by expression of GLUT1 and LAT1, the former being prevalent in high-grade NET with an inverse correlation with m-TOR and somatostatin receptor type 2 expression, the latter being prevalent in well-differentiated NET with direct correlation with somatostatin receptor type 2, survivin and angiopoietin II expression, independently of glucose or oxygen availability (Volante et al, manuscript in preparation). Additional molecular targets potentially useful in lung NET include c-

MET/phospho-cMET up-regulation via PAX5 activity in AC, SCC and LCNEC, where co-inhibition produced a synergistic effect in killing tumor cells, probably related to paxillin inactivation, which is a downstream target of activated c-MET involved in cell motility and tumor spread [104]. MET mutations are relatively rare in SCLC and others lung NET, they affect the juxtamembrane domain and are of no functional relevance as they do not influence c-Met phosphorylation, regardless of tyrosine kinase inhibitor treatment [105]. LCNEC patients present with variable c-KIT, Her-2/neu, VEGF PDGFR-alpha, PDGFR-beta protein overexpression but with no c-KIT or EGFR gene mutations or amplification [106], suggesting a negative prognostic factor for c-KIT expression [107, 108] and a potential therapeutic effect for anti-VEGF-, anti-c-KIT- and possibly anti-HER2-targeted agents in the treatment of these tumors [108, 109]. The same LCNEC show preferential expression of potential markers for cancer stem cells, including aldehyde dehydrogenase 1 family member A1, aldo-keto reductase family 1 members C1 and C3 and CD44 antigen, which could have diagnostic and prognostic implications in these tumors [110]. The differential expression of CD44, orthopedia transcription factor and menin, the product of MEN-1 gene, and 11q22.3-q25 deletion in TC and AC and of aurora B kinase and surviving in high-grade NE carcinomas may comprise therapeutic targets for these tumors [31, 83, 111], as well as identify subpopulations of patients within each tumor category with different life expectation allowing a better risk stratification for therapy purposes [31, 35, 83]. A better understanding of the entire landscape of molecular alterations in lung NET affecting either genetic or epigenetic mechanisms would hopefully lead to a molecular classification in part heralded by recent next generation sequencing studies [26, 28, 112, 113], where predictive biomarker assessment in the diverse tumor categories would help to identify different patient subpopulations suitable for personalized therapies.

Conclusion

Lung NET comprise a quite heterogeneous cluster of human malignancies with profound differences in the epidemiologic, genetic, pathologic and behavioral characteristics, which can cause a conundrum to the biological understanding of these lesions. Through an enlightened re-thinking of lung NET, pathologists should provide clinicians with better diagnostic refining of the diverse categories of lung NET with closer adherence to the clinical reality by means of an innovative concept of tumor grading. Additionally, they should clarify the meaning of Ki-67 LI in the practical clinical management of patients and offer expertise and knowledge about molecular, genetic and predictive (therapeutic) factors that could be meaningful for clinical purposes. The final goal is to unravel the inherent complexity of lung NET to finally increase our options of therapy in these tumor patients.

Figure legends

Figure 1. Typical carcinoid of the lung (no necrosis; 1 mitosis/2 mm^2) with nuclear pleomorphism in tumor cells: this feature is not *per se* diagnostic of atypical carcinoid.

Figure 2. Representative pictures of lung NET are shown according to the current 2015 WHO classification on resection specimens. Typical carcinoid is composed of trabecular arrangement of polygonal tumor cells with no necrosis and one mitotic figure only per 2 mm^2 (A), sometimes featuring spindle cell appearance especially in peripheral lung location (B). Atypical carcinoid exhibits at least 2 mitoses per 2 mm^2 and/or punctate necrosis (C), whereas large-cell neuroendocrine carcinoma shows organoid architecture with extensive necrosis (D), plentiful mitoses and with peripheral palisading (E). In turn, small cell carcinoma presents with small-sized tumor cells with very scant cytoplasm and innumerable mitotic figures (F).

Figure 3. This case of small cell carcinoma (A) showed high Ki-67 labeling index (B), faint and punctate positivity for cytokeratin pool (B, inset), strong and diffuse cytoplasmic decoration for synaptophysin (C) and scattered tumor cells positive for chromogranin A (C, inset). Unexpectedly, there was nuclear staining for transcription factors such as CDX-2 (D) and Islet-1 (E), whereas TTF-1 was diffusely positive as in most of these tumors (F).

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Table 1. Pros and cons about the current classification of pulmoanry neuroendocrine tumors

Pros	**Cons**
WHO terminology is widely agreed-upon, with wide clinics and pathology experience	Inconsistencies in tumor diagnostic reproducibility among different observers
	Troubles in the multimodality therapy of AC and LCNEC
TC, AC and SCLC/LCNEC are so distinctive tumors that it seems reductive to simply call them G1, G2 and G3	Any classification should be clinically useful, also in metastatic tumors or within the same tumor categories (e.g., TC or AC or LCNEC or SCC)
There are sharp differences in the therapy, prognosis and diagnosis of SCLC and TC	Epidemiologic, clinical & genetic data favor a 3-tier over a 4-tier classification scheme

TC: typical carcinoid; AC: atypical carcinoid; LCNEC: large-cell neuroendocrine carcinoma; SCC: small cell carcinoma

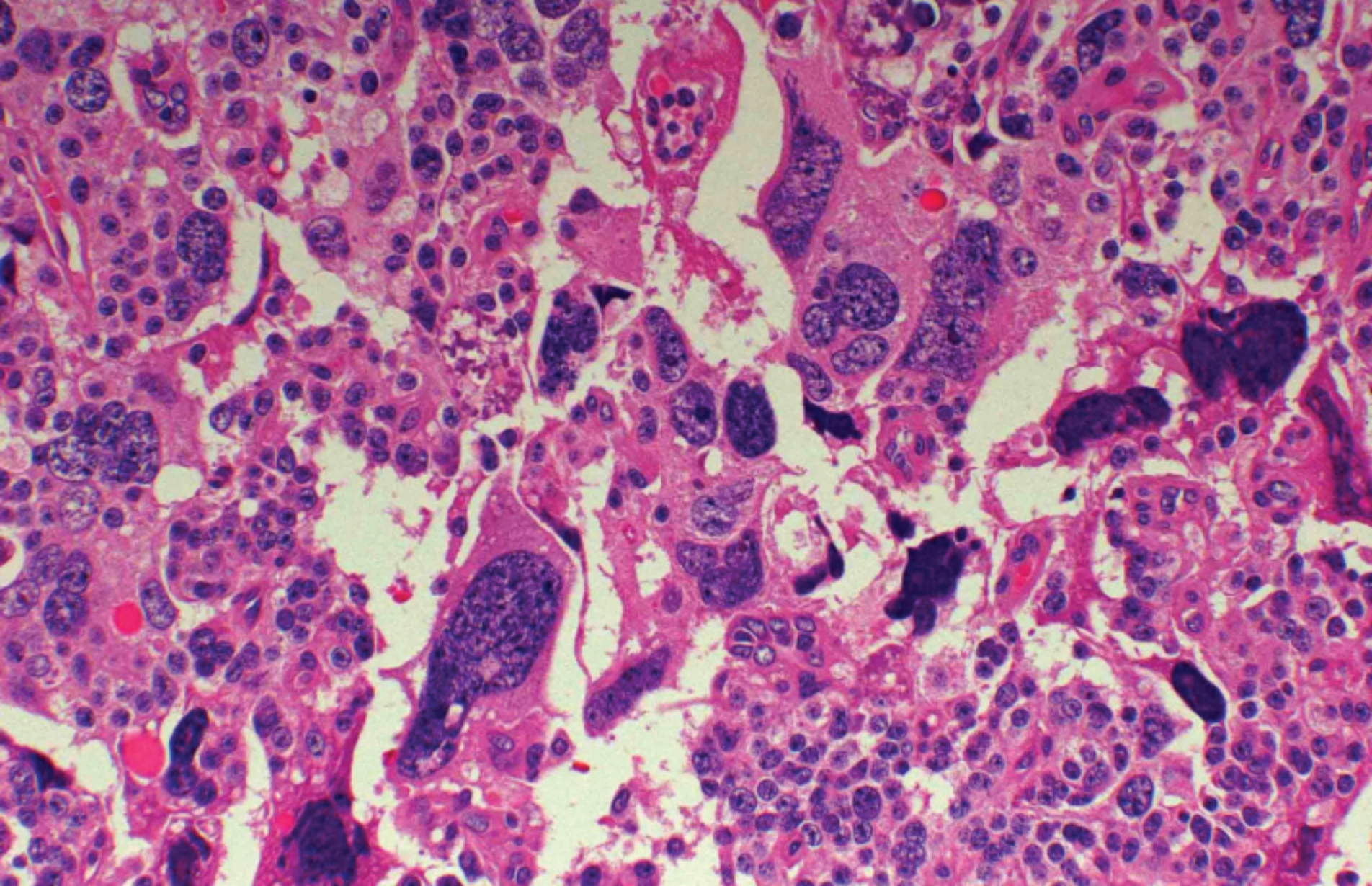
Table 2. Diagnostic criteria for lung neuroendocrine tumors according to WHO 2015 classification

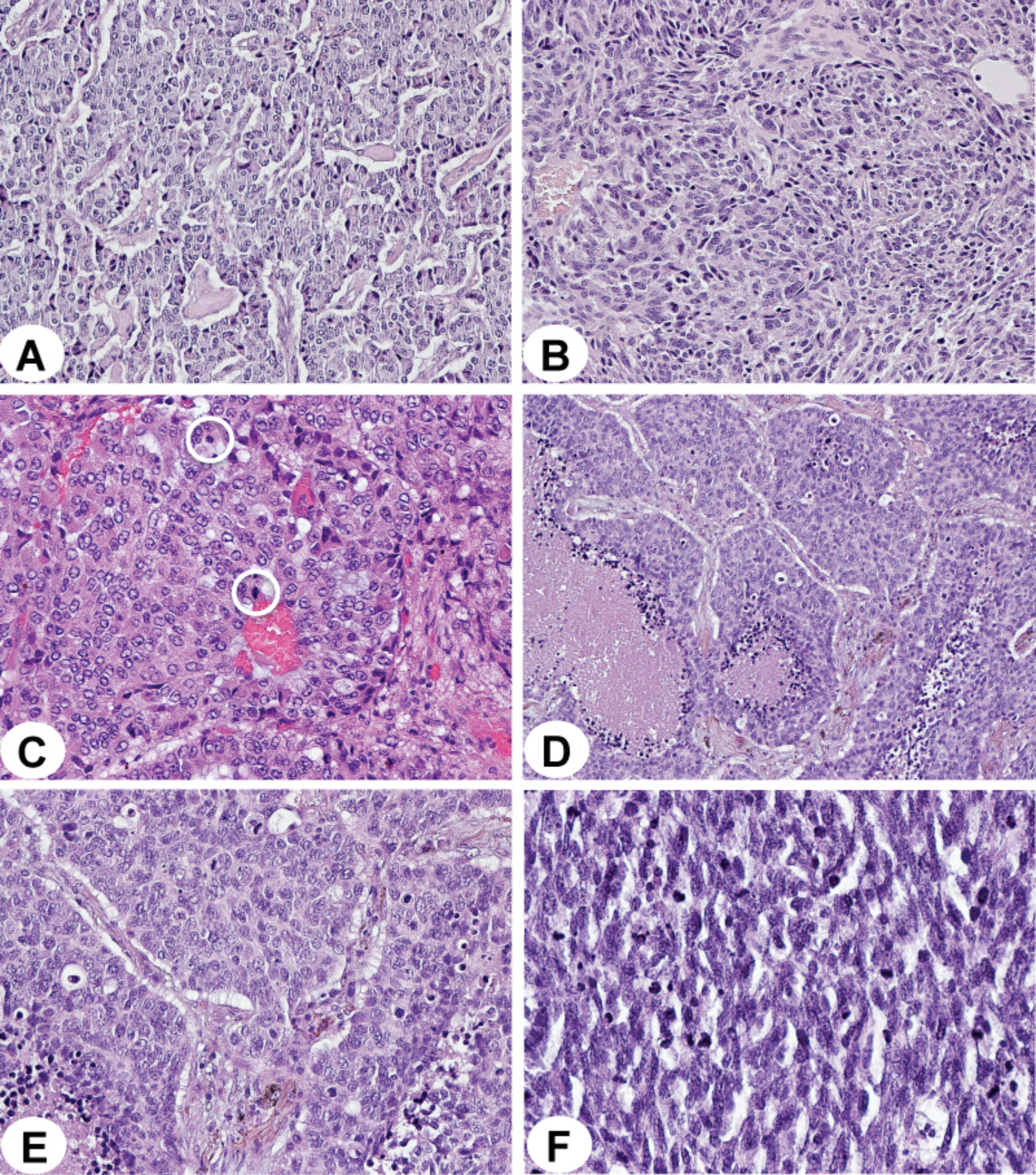
Variable	Typical carcinoid	Atypical carcinoid	Large-cell neuroendocrine carcinoma	Small-cell carcinoma
Neuroendocrine morphology	yes	yes	yes	yes
Cytologic criteria	no	no	yes	yes
Mitoses/2 mm^2	1	2-10	≥ 11	≥ 11
Necrosis	no	punctate	extensive	extensive
Combined variant	no	no	yes	yes
Ki-67 labeling index	up to 5%	up to 25%	40-80%	50-100%

Table 3. Grading system parameters in lung neuroendocrine tumors

Cut-off levels	Variable		
	Mitoses (10 HPF or 2 mm^2)	Ki-67 LI	Tumor necrosis
Level 1	2	< 4	absent
Level 2	>2 - 47	4 - 25	< 10%
Level 3	> 47	≥ 25	> 10%

Ki-67 LI: Ki-67 labeling index, percentage of immunoreactive tumor cells; for details on application of the grading system see the text and reference #13

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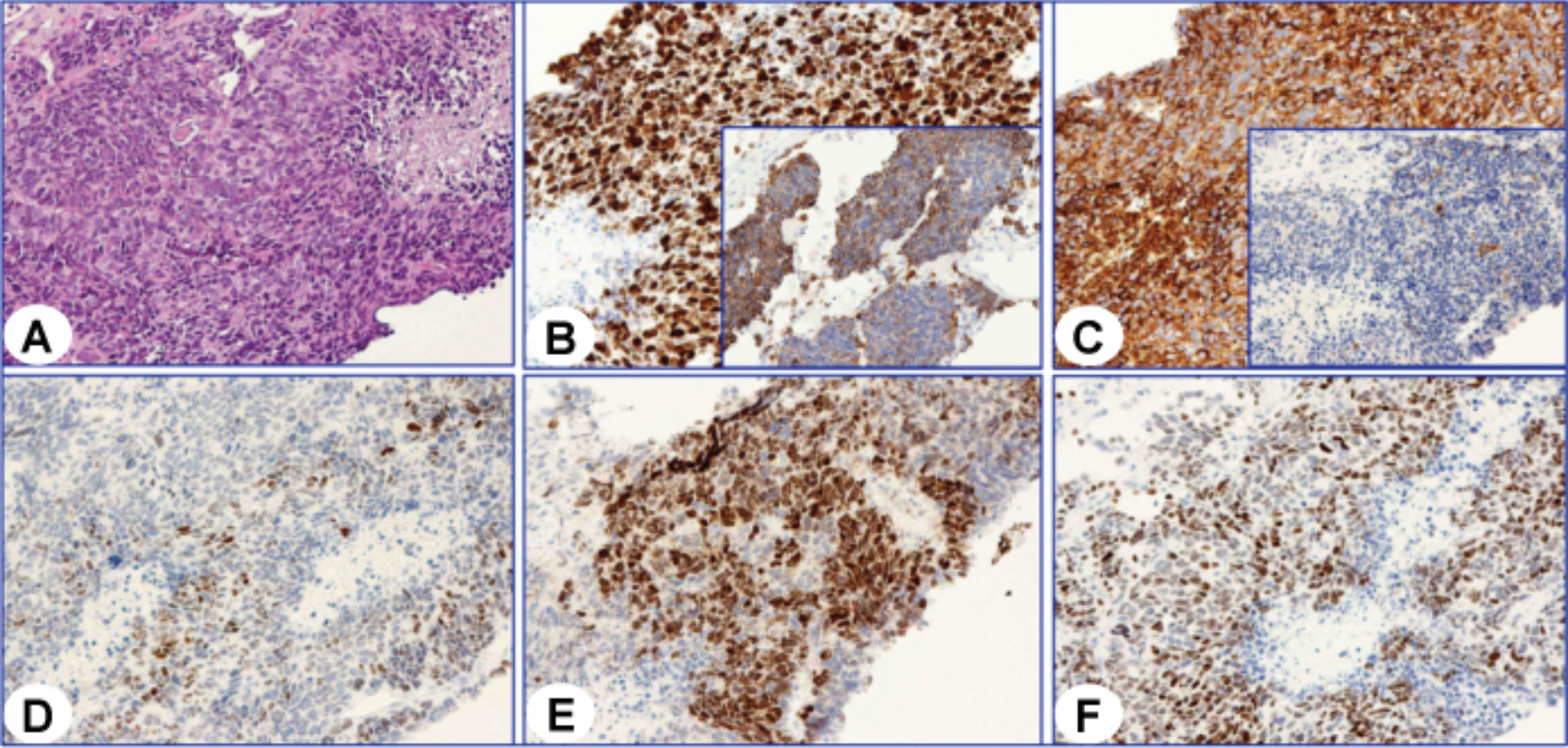
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