**Recent advances in the molecular landscape of lung neuroendocrine tumors**

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**Running head:** Recent advances in lung neuroendocrine tumors

**ABSTRACT**

 **Introduction:** Neuroendocrine tumors of the lung (Lung-NETs) make up a heterogenous family of neoplasms showing neuroendocrine differentiation and encompasses carcinoids and neuroendocrine carcinomas. On molecular grounds, they are considered two completely distinct and separate tumor groups with no overlap of molecular alterations nor common developmental mechanisms.

 **Areas covered:** Two perspectives were evaluated based on an extensive review and rethinking of literature: (i) the current classification as an instrument to obtaining clinical and molecular insights into the context of Lung-NETs; and (ii) an alternative and innovative interpretation of these tumors, proposing a tripartite separation into early aggressive primary high-grade neuroendocrine tumors (HGNET), differentiating or secondary HGNET, and indolent NET.

 **Expert commentary:** We herein provide an alternative outlook on Lung-NETs, which is a paradigm shift to current pathogenesis models and expands the understanding of these tumors.

**Key Words**: neuroendocrine; tumor; carcinoma; lung; pathogenesis; mutation; copy number variation; gene; Ki-67 index; evolution; genomic analysis

**1. Introduction**

The current World Health Organization (WHO) 2015 classification on lung neuroendocrine tumors (Lung-NETs) recognizes four histologic variants defined as typical carcinoid (TC), atypical carcinoid (AC), large cell neuroendocrine carcinoma (LCNEC) and small cell lung carcinoma (SCLC) [1,2]. Behaviorally, TC are low-grade tumors with favorable prognosis mostly treated by surgery alone, AC are intermediate-grade tumors with greater biological aggressiveness benefitting from multimodal therapy, and LCNEC and SCLC are fully fledged high-grade neuroendocrine tumors (HGNET) with poor prognosis despite use of chemotherapy and/or radiotherapy [1]. Diagnostic criteria for Lung-NETs have historically been developed on resection specimens and based on mitotioc count per 2 mm2, the amount of necrosis and a constellation of cyto-histologic and immunohistochemical details [1-4]. The resulting four-tier morphologic scheme leads to a three-tier epidemiologic, genetic, behavioral and clinical stratification of tumors, reflecting a continuum of neuroendocrine-differentiated tumors with increasing degrees of biological aggressiveness and gene alteration burden [1,2,5]. A joined expert consensus of the International Agency for Research on Cancer (IARC) and WHO has recently proposed a terminology change, according to which TC and AC are pulmonary NET G1 and NET G2, respectively, whereas LCNEC and SCLC are full-fledged pulmonary neuroendocrine carcinomas (NEC) of large cell-type and small cell-type, respectively [6]. A category of NET G3 as seen in the pancreas [7], which would represent the G3 variant of AC, is currently not recognized in either the lung or the thymus, which are the two major sources of thoracic neuroendocrine neoplasms (NENs), when the same defining criteria are used for classification [1]. However, several studies have suggested that G3 NET exist under a variety of different terms classified as LCNEC or SCLC [8-20]. An expert consensus proposal, which is similar to - albeit more exhaustive than – other classifications provided in the past [21-23], frames the diverse tumor categories under a common classification scheme [6]. NENs are still considered as monolithic and unrelated entities with no new insights into their developmental mechanisms [6]. In particular, it is repeatedly asserted that there is no a pathogenetic continuum among these tumors, that they have very different molecular profiles and that such grouping would not provide useful insights into the natural history of these neoplasms [1,6].

 A body of literature however shows that Lung-NETs are less uniform disease entities than it would seem from the current classification; a case mix of diversely behaving tumors can be observed within each tumor category when using different investigative tools to set defining criteria [24-29]. This holds particularly true for AC and LCNEC but, to lesser extent, also for TC and SCLC, and behavioral separations emerges within different patient subsets and conversely there is overlap between different tumor subtypes [24-32]. This bewildering situation causes uncertainty in clinical management of metastatic disease, when evaluating cytology/biopsy samples and/or demanding subtypes, such as AC and LCNEC: prediction of outcome and clinical decision-making are challenging by relying on morphology alone [5,24,25,30,31,33-39].

 The current view is that TC and AC on the one hand and full-blown neuroendocrine carcinomas (*i.e.,* LCNEC and SCLC) on the other hand represent two genetically unrelated tumor groups. A lowest somatic mutational rate (<1 per million base pairs) is found in the former and the higher somatic mutation rate (7-13 per million base pairs) in the latter [13,40-53]. This apparently sharp separation likely is a result of different pathogenesis, reflected in major differences in risk factors for carcinoids, including younger people, predominantly nonsmokers or light/intermittent smokers, relative prevalence in females, recurrent inactivation of MEN1 and other chromatin remodeling genes or epigenetic changes. Conversely, neuroendocrine carcinomas affect elderly people, current or former heavy male smokers and usually show *TP53/RB1* complete inactivation. The recognition of mutually almost exclusive molecular pathways strengthen the current interpretation on Lung-NETs [1,13,33,41,44,45,54,55].

 Differences in progenitor/stem cells could also help to explain why these tumors seem to be so far and unrelated to each other [13,40,42,44-46,48,49,54] and have different distribution inside the lung: SCLC and TC arise centrally in the major bronchi, AC centrally or peripherally, and LCNEC mostly peripherally [54,56]. Accordingly, TC and SCLC – and more occasionally LCNEC – are thought to variably derive from precursor/stem cells of the bronchial epithelium branch (including neuroepithelial bodies) with a neuroendocrine cell lineage through intermediate steps represented by neuroendocrine cell hyperplasia (NECH), diffuse idiopathic pulmonary neuroendocrine cell hyperplasia (DIPNECH) and neuroendocrine tumorlets for carcinoids and NECH or neuroepithelial bodies for SCLC and, occasionally, LCNEC [1,54,56]. Most LCNEC and, occasionally, SCLC are thought to originate from the same bronchoalveolar precursor/stem cells as non-small cell lung carcinomas (NSCLC) through an intermediate step of NSCLC with neuroendocrine differentiation (NSCLC-NED) [1,54,56]. This scheme of histogenesis does not conceptually allow any crossover between carcinoids and neuroendocrine carcinomas or NSCLC, which basically would remain separate tumor entities [57]. Combined variants described in up to one third of SCLC [58] and LCNEC [1] are interpreted as stemming from common precursor/stem cells capable of divergent differentiation [1,54,56] rather than resulting from the adaptive evolution/transformation of tumor cell clones [59]. All these considerations would confirm once again the sharp separation between carcinoids and HGNET, with no or minimal overlap in terms of morphology, histogenesis and pathogenesis along the spectrum of Lung-NETs.

 This rigid scheme, however, could be tempered by a more dynamic vision based on clinical and biological outcome by distinguishing early aggressive or primitive HGNET; differentiating, evolving or secondary tumors, and persistently indolent tumors. This tripartite separation would be the result of different risk factors responsible for a variety of molecular paths, in turn acting as the real determinants of the ultimate tumor fate [60-64]. This outlook could to some extent be even independent of progenitor/stem cell niches of origin, as it has been experimentally demonstrated that molecular mechanisms can program lung cancer formation by restricting tumor lineage, regardless of the cells of origin [65,66]. Therefore, different genetic/epigenetic drivers occurring stochastically at the onset of the natural history or accumulating over time in cancer stem/progenitor cells on the basis of different genetic programming and levels of cell distribution/patterning as a measure of cell disorder [67] might have the potential to shape the final morphology and clinical behavior of Lung-NETs. Consistent with this perspective, an *in silico* evaluation of 148 Lung-NETs resection specimens using next generation sequencing (NGS) [14], showed that most HGNET appeared to have developed from pre-existing carcinoids through sequential acquisition of different gene alterations, including a variety of mutations and copy number variations (CNVs) [68]. Furthermore, other recent studies [59] have allowed the neglected category of NSCLC-NED by the current 2015 WHO classification [1] to be revived as potential candidate or forerunner of HGNET, thus confirming that the evolutive spectrum in Lung-NETs is certainly broad and merits further unraveling [59,69].

 The clinical management of patients with Lung-NET is usually driven by histologic subtyping and tumor stage after adjusting for age, performance status and comorbidities, but outcomes still remain largely unsatisfactory [5,30,31,57,70]. As genetic/epigenetic alterations will control master regulators depending on cell lineage, affecting biological behavior, histologic features and growth characteristics, we speculate that tumor fate is determined at the onset of the tumor development, whether persistently indolent, early aggressive or destined to transform over time from low grade lesions into HGNET [59,68,71,72]. Therefore, we could outline three different scenarios in the landscape of Lung-NETs. i) The first refers to clinically early aggressive neuroendocrine carcinomas arising upon highly effective molecular mechanisms, which are responsible for highly effective *de novo* tumorigenesis mechanisms, with a very short natural history and no recognizable preinvasive lesions [73-76]. These early aggressive tumors will feature variably undifferentiated carcinomas, affect heavy smokers and will be predominantly diagnosed on biopsies because of extended disease at presentation (henceforth simply primary HGNET or P-HGNET). These P-HGNET are expected to account for 70-75% of Lung-NETs (13% of lung cancer), despite the decreasing incidence of SCLC worldwide [77,78]. ii) The second scenario regards more variably aggressive HGNET, which are likely to originate from a variety of molecular mechanisms and precursor lesions developing in transition via sequential alterations with a longer natural history [59,68]. These belatedly aggressive tumors may show many histologic features and primarily will be diagnosed on resection specimens because most patients are operable at the time of diagnosis (henceforth simply secondary or differentiating HGNET or S-HGNET) [68]. These S-HGNET, which are expected to account for 20-25% of Lung-NETs (6% of lung cancer), are likely to have contributed most to our knowledge of molecular alterations thus far accumulated in these tumors derived from surgical resections [13,40,42,44,46,48,49,52,53] with variable contribution of biopsies and/or tumor cell lines [43,45,47,50,51]. iii) The third scenario concerns clinically indolent Lung-NETs, mostly featuring TC or low malignant AC [28,79]; these mostly affect nonsmokers or light/intermittent smokers, younger people and women and have no or little propensity to progress even after partial removal (henceforth simply indolent NETs or I-NETs) [19,80]. These I-NET are mostly diagnosed on surgical specimens and account for 5% of Lung-NET (1% of lung cancer), making up a large mass of TC undergoing surgery [32]. The biological design for Lung-NET interpretation according to clinical behavior, pathogenesis, morphology and smoking habit is outlined in **Figure 1**.

 This review article has been written to provide an alternative interpretation to the current model of the Lung-NET spectrum, with special emphasis to outline features of HGNET. In particular, we would suggest that HGNET resembles a two-faced Janus where clinically distinct subsets of patients are tagged by their respective genetic traits, while morpholgical interpretation may be challenging. In this regard, Ki-67 protein immunohistochemistry (henceforth, simply Ki-67) – a widely agreed-upon marker of cell proliferation in Lung-NETs [81] – helps to unravel the complexity of HGNET by showing either diffuse nuclear decoration in P-HGNET, dual intra-tumor compartmentalization in S-HGNET or more uniform distribution in I-NETs. This alternative outlook is likely to be relevant to other types of cancer as a reflection of general mechanisms of tumorigenis. Another implication of this alternative outlook is that deciphering different biological mechanisms may open new avenues for innovative strategies of targeted therapy [82-84].

**2. Material and methods**

An overview of papers on the issue of Lung-NETs (not a systematic review or meta-analysis) was carried out until July 2018, after generating a list of key questions with regards to diagnosis, prognosis, classification, molecular alterations, pathogenesis, histogenesis and grading. Only articles dealing with the 1999, 2004 or 2015 WHO classifications have been included, reflecting more homogeneity in the use of diagnostic criteria for tumor definition [1,3,4]. This research was limited to the English literature available in PubMed®. Only published full-papers were taken into consideration, with some exceptions to abstracts when needed.

 We evaluated available literature under two perspectives: i) the current classification based on morphology as an instrumental tool to get clinical and molecular insights into the current state of the art on Lung-NETs, and ii) an alternative outlook on these tumors, with particular emphasis to a tripartite splitting into early aggressive P-HGNET, differentiating S-HGNET and indolent NET. The differential role of Ki-67 in carrying out this separation and the relevant differences in the genetic setting, developmental mechanisms, clinical attributes and pathologic features are also reappraised.

**3. The current understanding of lung neuroendocrine tumors**

 The diagnosis of Lung-NETs is a multistep process, which has been based on morphologic criteria [1-4,85,86]. The 2015 WHO classification has included all tumors with neuroendocrine morphology in a unique box of neoplasms [1] to improve the diagnostic recognition, eliminate the category of NSCLC-NED [69,87-90], reclassify SCLC and LCNEC as independent tumor entities [91-93] and stress the validity of the pathologic four-tier, clinical three-tier spectrum of Lung-NETs with progressive deterioration of prognosis [1,2].

 The wide lack of precise limits for mitotic count in LCNEC and SCLC (>10 mitoses per 2 mm2 with no uppr limit), some discretionality in recognizing large and small cells [1,94] and common genetic alterations may all contribute to explain why these tumor categories overlap so disappointly with each other [26,27,95]. In TC and AC, inter-observer variability on mitotic count and degree of necrosis contribute for low diagnostic reproducibility, which may in turn reduce the ability to distinguish entities with different behavior [29].

 The lack of morphologic connections across the spectrum of Lung-NETs apart from SCLC or LCNEC combined variants has been propsoed as evidence that carcinoids and full-fledged neuroendocrine carcinomas are tumor entities without inter-relatedness [1]. Combination of carcinoids, especially AC, and adenocarcinoma or squamous cell carcinoma or evidence of glandular differentiation in AC or neuroendocrine tumorlets are rare in the lung [96-101], as is the association of adenocarcinoma with AC [102] or small foci of DIPNECH [103]; in contrast combined variants account for up to one third of SCLC and LCNEC [1,58]. Intriguingly, genomic evaluation of cases with a morphological mix of adenocarcinoma and AC revealed a set of shared mutations in both morphological elements, but additional private mutations in either tumor component indicative of differential clonal evolution [102]. Pulmonary mixed neuroendocrine/non-neuroendocrine high-grade carcinomas have been found to be molecularly different from their pure counterparts when analyzed for cDNA quantification of ribonucleotide reductase, catalytic subunit M1 (RRM1), excision repair cross-complementation group 1 (ERCC1) and thymidylate synthase (TYMS), while topoisomerase IIa (TOP2A) was more expressed in the neuroendocrine components [104]. These findings have most often been ascribed to divergent plasticity/transdifferentiation of common ancestors or collision tumors, rather than the expression of evolving lesions in transition from preexisting NSCLC-NED. In these tumors, neuroendocrine traits would be acquired subsequently from non-neuroendocrine precursors, either spontaneously or pressured by therapy [59,71,72,96,105,106]

Immunohistochemistry for Ki67 is recommended in the 2015 WHO classification to avoid misdiagnosing carcinoids as SCLC in limited diagnostic material, especially when crush artifacts are present [81,107,108]. A number of reasons led to the conclusion that the time had not yet come for Ki-67 staining to be used in the classification of lung-NETs [1]. These include the imperfect correlation of Ki-67 with mitotic count results in overlap among adjacent diagnostic categories (TC and AC; AC and LCNEC; LCNEC and SCLC), purported troubles in inter-observer reproducibility [81] and the lack of an independent survival prediction in some studies [109,110]. Of note, Ki-67 has a wide range of expression even in LCNEC and SCLC, as does the mitotic count, suggesting that these tumor subtypes are biologically and behaviorally heterogeneous despite they are considered single tumor entities [1,81]. A recent grading proposal based on Ki-67, mitotic count and necrosis assessment on resection specimens was capable to effectively predict prognosis regardless of histology, with no or minimal overlap of 95% confidence intervals of the relevant survival curves [28]. Most importantly, this grading system showed that each prognostic category (G1 to G3) consisted of a mixture of diversely featuring tumors, with a more effective prognostic separation of AC, LCNEC and even SCLC. The goal of the study, however, was not to create an alternative substitute to morphology, but rather help clinicians to better manage Lung-NETs at the level of an individual patient’s cancer by identifying diversely behaving among similarly looking tumors [28].

Although KI-67 enumeration has been repeatedly accused of problems with reproducibility, this in principle is the same also for enumeration of mitoses, percentage of necrosis or indeed any repeated measurement [24,28,34,81]. It is emerging that the level of correspondence between biopsy samples and surgical specimens or different observers can be significantly improved when strict counting guidelines were applied also when using manual counting [111]. Of note, once hot spot regions have been identified in tumors being assessed, the count of Ki-67-positive cells per 2000 cells, 2 mm2 or the whole biopsy fragments minimized potential discrepancies irrespective of tissue sampling, biopsy size, intra-tumor heterogeneity and inter-observer variability; this was also independent of histological subtype [111]. These findings are methodologically important as they become are instrumental to the clinical utilization of Ki-67 in guiding the management of Lung-NETs patients, especially in the setting of a metastatic disease [24,35,81,112].

The current interpretation of Lung-NET pathogenesis supports the view that there are major differences in gene alterations between TC/AC and SCLC/LCNEC, with minor or no differences inside each tumor group [13,40,42-53,113]. Briefly, TC and AC were recently found to show recurrent alterations in mechanisms of epigenetic regulation (chromatin remodeling, SWI/SNF complex-dependent DNA packaging, histone methylation and acetylation), while SCLC exhibit recurrent mutations/deletions in cell cycle regulators (especially TP53 and RB1), gene amplifications (MYC family, SOX2) and alterations in mechanisms of neuroendocrine differentiation (NOTCH family). LCNEC are the most heterogeneous tumors on molecular grounds, with some of them resembling carcinoids, some overlapping with SCLC and some linking to NSCLC (especially adenocarcinoma but also squamous cell carcinoma) on the basis of their patterns of gene alterations [1,13,44,45,54]. A recent study by George et al on 75 cases of LCNEC found two main subgroups: type I, with high neuroendocrine expression (ASCL1high/DLL3high/NOTCHlow) and TP53 mutation similar to SCLC but with additional STK11/KEAP1 mutations and lack of RB1 inactivation; and type II, with low neuroendocrine expression (ASCL1low/DLL3low/NOTCHhigh), combined TP53 and RB1 mutations and an upregulation of immune-related pathways [44].

About 10-15% of human SCLC, SCLC cell lines and genetically engineered mouse models (GEMM) lack, or express at low levels, neuroendocrine markers and represent the ‘variant subtype of SCLC’. These tumors are characterized by distinctive morphological features (usually intermediate cells, sometimes resembling NSCLC or LCNEC) and molecular traits (downregulation of *ASCL1*, *NEUROD1, TTF1 and DLL3,* upregulation of *REST*, NOTCH and Hippo/TGFβ pathway and MYC amplification, with occurrence of epithelial mesenchymal transition via vimentin expression). These patients have a poorer response to chemo-radiotherapy but vulnerability to Aurora kinase inhibitors as compared to the high-neuroendocrine classical SCLC counterparts, while retaining and *TP53/RB1* biallelic inactivation [73,75,76,114-117]. In GEMM, conversion of a high-neuroendocrine classic subtype to a low-neuroendocrine variant has been described upon MYC amplification [117]. In these instances, NOTCH pathway and REST activation are documented in tumor cell subsets, both of which act as transcriptional repressors of neuroendocrine gene expression. These pathways provide a trophic/feeding microenvironment to classical SCLC cells [118] and reveal a high plasticity of cancer stem cells, with a pro-tumorigenic role in the development of SCLC and, to some extent, a linking to NSCLC precursors [59,76,118]. Interestingly, many of these variant lines were established from tumor tissue of inoperable SCLC patients with limited or extended disease at relapse after chemotherapy [119].

An interesting finding regards the intra-/inter-tumor molecular heterogeneity. In NSCLC genomic heterogeneity resulting from branched evolution is now accepted [120,121]. In contrast, SCLC shares most mutations between primary and metastatic foci suggesting a linear model of evolution [121]. Similar data in carcinoids are still lacking, because these tumors are rare and less prone to give metastases. An increment of proliferation has been documented at metastatic sites in the lung [15]. In this case, the authors felt that the term carcinoid remained appropriate despite mitotic rate overlapped with HGNET as the tumor retained neuroendocrine morphology and differentiation as well as RB1 expression [15]. The author’s decision however is arguable because it introduces an exception to current guidelines, which would recommend calling these carcinoid-like tumors as LCNEC or SCLC [1].

Much of our knowledge on molecular alterations of Lung-NETs has mostly been derived from the analysis of surgical specimens from single subtypes [13,40,42,44,46,48,49,52,53]. This is likely a reflection of technical limitations (enough amount of available DNA/RNA), the need to comply with tumor classification, and the greater availability of retrospective tumor series over prospective collection [1,5,122]. The source of data for SCLC [43,47,48,50,51] or LCNEC [45] is more heterogenous still: data have been merged from biopsy samples, tumor cell lines or experimental mouse. This is not a negligible issue in that investigational results derived from surgical specimens of either SCLC [46,49] or LCNEC [13,44,49,52,53] have been extrapolated to the entire population of patients including those where non-surgical material for either tumor type [43,45,47,48,50,51] was used for the diagnose. The premise was that early aggressive tumors (most often diagnosed on cytology/biopsy samples of primaries or metastases) were biologically the same tumors – just at a more advanced stage - as those undergoing complete surgical resection. Likewise, NGS studies on TC and AC have been performed mainly on surgical specimens [14,40,42], but these tumors may modify their proliferative status in metastatic foci in the lung [15] or elsewhere [11], either synchronous or metachronous. The level of inter-tumor genetic heterogeneity in these tumors is still largely unknown.

Another issue regards immunotherapy clinical studies aimed to re-activate the immune checkpoint PD1/PD-L1 by humanized monoclonal antibodies, which have not given encouraging results in Lung-NETs when using PD-L1 immunohistochemistry expression as prediction criterion [123]. Only a minority of LCNEC (likely those showing low expression of neuroendocrine markers and more akin to NSCLC) [44] express PD-L1, while carcinoids, SCLC and even ASCL1-positive adenocarcinoma with neuroendocrine differentiation are basically unreactive [124-128]. In keeping with the role of high tumor mutation burden in NSCLC in dictating better prognosis [129] or response to anti-PD1/PD-L1 treatment [130], more recent studies have been showing that efficacy of nivolumab plus ipilimumab was enhanced in SCLC patients with high tumor mutation burden [131]. Considering the inherent heterogeneity of LCNEC [13,44,45] and SCLC [59,73,76], only those tumors with high mutational burden leading to neo-antigen expression are likely to respond to immunotherapy. Once again, biology confirms that current diagnostic categories of LCNEC and SCLC are unsatisfactory and risk levelling differences either genetically, clinically or behaviorally by failure to separate biological entities.

**4. New concepts on lung neuroendocrine tumors**

Various findings support that each histologic variant of Lung-NETs is inherently heterogeneous but that this heterogeneity is not adequately predicted by morphology: expression of CD44, orthopedia transcription factor (OTP) or surviving (BIRC5), losses of 11q22.3-q25 and 9q34.11, and MEN1 mutation and/or deletion each identify subsets of patients with different clinical outcome in TC or AC [16-19]. Using expression microarrays HGNET consists of at least two patient populations with different prognosis, again independent of the classical histologic subtyping in SCLC and LCNEC [132].

Survival curves of Lung-NETs in published studies show that in all tumor categories relapses continue to occur over time. This is least frequent in TC [32], much more common in AC [79] and relentless in fully-fledged neuroendocrine carcinomas [28,133,134]. Most patients with LCNEC and SCLC die within the first two years after diagnosis, though for a minority of 10-40% there appears to be a survival plateau after five-year or more. This subgroup of diversely behaving tumors is again not predictable by morphological assessment [28,132-134]. There will be a bias in the case selection in these data sets: the studies have been carried out on resection specimens, which capture the large majority if not all TC and AC, presumably most LCNEC but only a minority of SCLC [1,122]. This is not surprising, because the greater the tumor biological aggressiveness, the greater the likelihood of diagnosis on cytology/biopsy samples only; *vice versa*, the lower the biological aggressiveness, the greater the likelihood of diagnosis on surgical specimens.

Few investigators have comparatively evaluated the entire spectrum of NETs by means of NGS, including full papers [14,135,136] or abstract communications [137]. Surprisingly, it has been found that common genetic traits may be shared by carcinoids and neuroendocrine carcinomas, although with different rates of prevalence [14,135-137]. Furthermore, other investigative techniques, e.g., comparative genomic hybridization [138,139] have also envisaged this possibility, as have studies that evaluated NETs arising in other anatomical location (e.g., the thymus, the pancreas or the gastrointestinal tract) [11,140,141]. It is tempting to speculate that in order to improve clinical management we will need to move from a histology-based analysis to a categorization based on molecular signatures, including the proliferation status of tumors [142]. Then for classification the premise becomes that phenotypic changes are likely to be driven by underlying gene alterations [16,18,35,41-44,48,49,56,57,113], but that morphology is only one aspect of the biology of the disease and that histologic subtypes may be expected to merge somewhat with each other.

 We know that tumor initiating stem cells are subjected to selection as a function of the type and timing of genetic, epigenetic and/or microenvironmental alterations/interactions with resulting clone and subclone evolution [143]. Further, in lung tissue, there are probably different niches of stem/progenitor cells distributed along the bronchial epithelial branches and the terminal respiratory unit that can both self-renew and produce distinct lineages of differentiated cells, with either neuroendocrine or non-neuroendocrine features [144-147]. It is however also possible that distinct molecular events can lead transformation and restrict tumor lineage regardless of the cell of origin [65,66]. Furthermore, tumor fate may be determined not only at the onset of its natural history and be tuned over time by therapeutic pressure and mechanisms of trans-differentiation [59,64,68,71,72,148]. Tumor formation may also be shaped by genetic/epigenetic reprogramming in more differentiated tumor cells and after the initial transformation. This influences the final hierarchical organization of tumors by altering chromatin structure, leading to expansion of intratumor cell heterogeneity upon reprogramming [149,150]. Defined cellular lineage may be associated with a unique susceptibility to malignant transformation when subjected to specific oncogenic insults and this has been captured in the term ‘cellular pliancy’. This concept emphasizes the critical role of cellular reprogramming in adult differentiated cells during tumorigenesis [151]. Interestingly, alterations in chromatin remodeling gene are shared by most Lung-NETs of either low-intermediate or high-grade, albeit affecting different genes and with varying prevalence. These including MEN1, ARID1A, EIF1AX, PSIP1, BREBBP, EP300, MLL, CHD7, SWI/SNF complex and MYCL [13,14,41,43,44,46-48]. Some alterations cut across the entire spectrum of NET even at different anatomical sites [14,138], for example MYC and MYCL [152], whose amplification has recently been documented in TC secondarily evolving to LCNEC [68]. The metastatic progression of RB1/TP53-inactivated SCLC upon NFIB amplification/overexpression or other alternative mechanisms suggest an intertumoral heterogeneity somewhat independent of the cells of origin or intrapulmonary location [84].

 An overview describing the evolution of cancer stem/progenitor cells, either native or reprogramming-induced, to I-NET, P-HGNET and S-HGNET and their potential collocation according to the natural history of Lung-NETs, is shown in **Figure 2**. It is tempting to speculate that lung cancer stem cells also independently of their anatomical location could give rise to primitive-looking neoplasms or P-HGNET as a function of risk factors [65,66,144]. This could happen regardless of intermediate “dysplastic” lesions (in humans, preinvasive lesions are not reported in SCLC or LCNEC, at variance with carcinoids or mouse models) [1,116-118], following NOTCH inactivation, neuroendocrine lineage induction upon INSM1 and then ASCL1 and bi-allelic inactivation of TP53 and RB1 [59,73,153]. P-HGNRT are particularly enriched in cancer stem cells [154] and encompass high-neuroendocrine classical SCLC, low-neuroendocrine SCLC variants or combined variants over a background of highly undifferentiated cancer cells. The malignancy of these early aggressive P-HGNET is exemplified by early route to widespread metastases, advanced stage at the time of diagnosis, accelerated clinical course with dismal prognosis, as classically expected for SCLC [1]. These P-HGNET are strongly associated with smoking habit and arise in the major bronchi as central lesions at the time of presentation, possibly also related to a niche of bronchial-based basal stem cells [144]. P-HGNET are not confined to the lung, but homologous tumors with exceedingly accelerated clinical course, TP53/RB1 inactivation as opposed to maintained DAXX/ATRX, down-regulation up to virtual disappearance of SSTR2 and uniformly high Ki-67 can also be traced in other NET-developing organs, such as the pancreas [155]. Because of their high malignant potential, these early aggressive P-HGNET are likely to predominate interval cancers in screening programs with low-dose computed tomography, which will not benefit from resection due to advanced stage at diagnosis [156-158]. P-HGNET would be thus the result of clonal/sub-clonal expansion/diversification of cancer stem cells early blocked in their capability to differentiate according to site-specific genetic, epigenetic and microenvironmental alterations as has been proposed for leukemogenesis [159] (**Figure 3**).

 In turn, S-HGNET are likely to arise from neuroendocrine or non-neuroendocrine-differentiated cancer stem cells, also independently of their anatomical position in the lung but as a function of risk factors [65,66,144], through a variety of gene alterations. Intermediate lesions will likely be carcinoids or their ancestors (hyperplastic neuroendocrine cells/neuroepithelial bodies) or NSCLC-NED [54,59]. The hallmark of these S-HGNET is to present with dual cell populations showing by different levels of cell proliferation in the same tumor [11,14,69]. Similarly to P-HGNET, S-HGNET but can be observed in other organs where they exhibit a great variety of histological features in pure or combined forms depending on the types of cell differentiation, either neuroendocrine or non-neuroendocrine [96]. Of note, NET harboring mixtures of carcinoid-like and high grade-appearing components within the same neoplastic mass have been reportedly described in the thymus [8,11] and gastrointestinal tract [140,141,160] as either anecdotal cases or small tumor series. Quite surprisingly, they are not mentioned in the lung [161] nor included in the current WHO classification as forerunners of HGNET [1]. Likewise, NSCLC-NED are not currently accepted as independent tumor entities 1 and combination of carcinoids with NSCLC are deemed to be only anecdotal occurrences [96-103], whereas tumor associations are allowed for SCLC or LCNEC among them or with NSCLC elements but not carcinoids [1,58].

 The assumption that S-HGNET feature exclusively neuroendocrine components derives from the observation that carcinoids and full-fledged neuroendocrine carcinomas may present with different prevalence rates of common genetic alterations, at several anatomical sites [14,19,111,135,138-141]. In a large series of 148 surgically resected Lung-NETs crossing its entire spectrum, Simbolo et al. have found that, beyond major differences among the four histologic variants, there were common gene alterations detectable in at least three different tumors, with a 40-gene signature consisting of 27 mutations and 13 CNVs [14]. Starting from this study and using an unsupervised *in silico* analysis, we identified six clusters, which could be organized spatially and temporally in two different groups, which would support an evolution of TC to LCNEC and of AC to SCLC [68]. In this study, TC could evolve to LCNEC with even a conversion of some SCLC-looking tumors to LCNEC, while AC were seen to precede SCLC on the basis of particular gene alterations. Briefly, these data suggest that the evolution of TC to LCNEC-featuring tumors happens through inactivation of TP53/RB1 upon deletion or mutation alongside MYC copy number gains and private KRAS mutation, in a clinical setting of predominantly male smokers. The recently described categories of carcinoids with augmented proliferation rate progression at metastatic sites [15,54], carcinoid-like LCNEC [11,13], NSCLC-like LCNEC harboring KRAS mutation [13,44,45] and the well-known low diagnostic reproducibility of LCNEC diagnosis towards either AC or SCLC and vice versa [26,27,162] support indeed these findings. The evolution of AC to SCLC-featuring tumors would reflect molecular alterations such as copy number gains at of, for example, TERT, SDHA and MYCL. Mutations in RICTOR (RPTOR independent companion of MTOR complex 2), which regulates hormone-dependent cell growth via AKT1 phosphorylation in a subgroup of SCLC [163], and TP53 and RB1 inactivation alongside private MEN1 deletion when AC were prevalent [16,18] were also found in predominantly male. The conversion of AC to SCLC-featuring tumors are consistent with reports of MYCL-amplified and TERT-overexpressing SCLC/LCNEC [44] or SCLC-like LCNEC as recently described by others [13]. Transition of TC to AC has also recently been documented upon chromothrypsis, as a further mechanism to explain the transformation of carcinoids to LCNEC- or SCLC-appearing tumors [14]. These two groups showed a steady increase of the mean values of Ki-67 consistent with the prevalent histologic subtype [68]. Most importantly, S-HGNET could be easily identified by an intra-tumor Ki-67 compartmentalization, whereas mitotic count and morphologic traits were by and large unhelpful because of close intermingling of high- and low-grade components (once again indicative of tumor cells in transition rather than in collision) [68].

 Neuroendocrine tumors with combined features ranging from well-differentiated carcinoid to poorly differentiated carcinoma have been described in the thymus [8,9] and the pancreas [140,160,164], but this did not led to a change in nomenclature of categorization. We can trace these *ante litteram* examples of neuroendocrine S-HGNET at different anatomical sites under several terms, such as (i) high-grade NE carcinoma with carcinoid morphology [10], (ii) secondary high-grade NET [11], (iii) well differentiated NET with a morphologically apparent high-grade component [160], (iv) poorly differentiated neuroendocrine carcinoma with regions demonstrating features of well differentiated neuroendocrine tumor [164], (v) transformed or mixed grade NET [160], (vi) well-differentiated NET with high-grade (G3) progression [140], (vii) carcinoid-like LCNEC with MEN1 mutation [12-14], (viii) carcinoid or NET with proliferation rate or Ki-67 progression at metastatic sites [15,165], (ix) progression of pulmonary carcinoid tumors [16-19], (x) neuroendocrine carcinoma with combined features ranging from well-differentiated (carcinoid) to small cell carcinoma [8], (xi) oat-cell carcinoma in transition from a carcinoid tumor [9], (xii) peripheral small-cell carcinoma of the lung resembling carcinoid tumor [20] and (xiii) the recent category of G3 NET in the pancreas [7,166]. As an aside, the latter diagnostic category [167] suffers from the same issues of morphologic reproducibility in the separation of well-differentiated G2 NET vs. G3 NET vs. poorly differentiated carcinoma as Lung-NETs [168,169], whereas Ki-67 successes to a greater extent, independently of anatomical location [111,170]. The concept of evolution of carcinoids to SCLC-appearing HGNET can be even traced in the old English literature on Lung-NETs thus confirming that this is an underdiagnosed but longer-known phenomenon [20,171]. A schematic representation on the development of Lung-NETs according to basal-like and luminal-type evolution of P-HGNET, S-HGNET and I-NET, respectively, is shown in **Figure 3**.

 At this point, the question is: how frequent is this phenomenon that never was described in the lung previously? We found that about 70% of HGNET fulfilled this tumor category (among 148 surgically resected specimens belonging to all histological variants), likely as a result of spontaneous natural history (none of the patients we studied underwent neoadjuvant therapies) [68]. These S-HGNET showing neuroendocrine components are different from P-HGNET by showing longer survival, clinical presentation of tumors being amenable of surgery and morphologic appearance, different precursor cancer stem cells and different molecular traits. Beyond the category of S-HGNET featuring neuroendocrine elements only, there are also other S-HGNET likely originating from NSCLC-related cancer stem cells/intermediate precursors undergoing neuroendocrine differentiation (NSCLC-NED) [59,69], which could evolve to secondary LCNEC- or SCLC-featuring lesions [59,69], in either pure or combined forms as currently classified [1].

 Increasing peripheral location in the lung of most LCNEC but even “operable” SCLC [1,54,133,172], organoid features and richness in cytoplasmic neuroendocrine/epithelial (keratins) markers of SCLC undergoing therapeutic resection (G. Pelosi, personal observations) and molecular [13]/phenotypical [173] similarities of LCNEC to NSCLC would support the view that S-HGNET as a whole are really evolving tumors in transition from pre-existing carcinoids or NSCLC or their relevant precursors. We could see these subtypes of S-HGNET as the result of luminal-type or differentiating tumors, which variably retain glandular (TTF1, napsin-A), squamous (p40) and neuroendocrine cell lineage (chromogranin A, synaptophysin, hormonal substances) markers. TTF1 amplification and/or expression has been documented in LCNEC [44] and SCLC [73,174], with a functional link to regulatory axis ASCL1/TTF-1/NFIB in parallel with neuroendocrine differentiation in SCLC contributing to its metastatic potential [175]. The meaning of TTF1 expression in LCNEC as a marker of either glandular or neuroendocrine differentiation requires further investigation. These S-HGNET are expected to run a better clinical course even when featuring apparently hopeless microscopic presentation of SCLC, especially if peripherally located where they also show an increased association with interstitial lung disease [176,177].

 Lastly, there would be a third category of Lung-NETs that in our study accounted for about 32% of 148 surgically resected tumors belonging to all histologic variants, mostly encompassing TC and AC, which apparently escaped any clustering by using the 40-gene signature [68]. These I-NET occurred predominantly in women, less smokers and younger patients with early stage of disease. It is known that carcinoids with indolent behavior are more frequent in women, nonsmokers and run a favorable clinical course [1,32] even after limited resection [1,32] or laser ablation in TC [80]. This also emphasizes that male gender is probably a risk factor for adverse survival of TC [32] or the development of life-threatening S-HGNET [68], as suggested by the better prognosis of SCLC in females [77]. I-NET would account for 5% of Lung-NETs as a whole and about 1% of lung cancer. I-NET are likely to derive from a neuroendocrine stem cell niche of preinvasive lesions (NECH or DIPNECH) through chromatin remodeling gene/epigenetic alteration mechanisms, are usually operable at the onset (diagnosis on resection specimens). They fulfill histological criteria for TC, low malignant AC with scant mitoses and no necrosis and present with low and uniform distribution of Ki-67 [111]. They do not express PD-L1 expression [128], whereas data on genetic heterogeneity between primary vs. metastases are still lacking likely due to the rarity of metastases in these tumors.

 Of note, the general outlook we herein describe is also reflected by other non-neuroendocrine tumors, where a dual pattern of tumorigenesis with basal-like and differentiating/luminal-like features may be envisaged, with different clinical aggressiveness. They include: i) the breast, with triple negative/basal-like vs luminal/Her-2 enriched tumors [143]; ii) the central nervous system, with primary vs. secondary glioblastoma [178]; iii) the urinary bladder, with high-grade non-papillary/in situ carcinoma vs. low- to high-grade papillary/noninvasive urothelial carcinoma [179]; iv) the endometrium, with high-grade non endometrioid carcinoma (type II, including serous high-grade and malignant mixed müllerian tumor/MMMT) vs. G1 to G3 endometrioid carcinoma (type I) [180]; v) the ovary/tubes, with type II carcinoma (serous high-grade, MMMT, transitional cell carcinoma) vs. type I carcinoma (low-grade serous, mucinous, endometrioid, Brenner tumor) [181].

**5. Differential traits of Lung-NETs**

 There are previous observations by Meder et al. confirming these models of P-HGNET and S-HGNET being derived from non-neuroendocrine cancer stem cells or NSCLC-NED, respectively, which were based on both experimental models and a clinical series of 35 SCLC, 28 extrapulmonary small cell carcinomas, 19 pulmonary LCNEC and 33 pulmonary adenocarcinomas [59]. However, the concept of S-HGNET derived from the evolution of pre-existing neuroendocrine tumors of lower grade was documented for the first time in the thymus [11] and in the largest series of Lung-NET thus far studied comparatively, encompassing all the four histologic variants to avoid selection biases [68]. Derks et al arrived at a similar conclusions in a recently published review article on the cells of origin in Lung-NETs [54]. Here the authors propose that TC and AC likely derive from normal neuroendocrine cells/neuroendocrine bodies through the intermediate steps of neuroendocrine cell hyperplasia and neuroendocrine tumorlets, also reappraising previous observations [182]. LCNEC would then arise from NSCLC through NSCLC-NED and, occasionally, from neuroendocrine cell hyperplasia or neuroendocrine bodies, whereas SCLC would arise from neuroepithelial cell hyperplasia and neuroendocrine bodies, NSCLC and/or NSCLC-NED [54]. Development of LCNEC or SCLC from NSCLC and/or NSCLC-NED could occur either spontaneously or induced by tyrosine kinase inhibitor treatment through mechanisms of trans-differentiation of EGFR-mutated adenocarcinoma to LCNEC- or SCLC-appearing tumors [54,71,72,183]. In turn, TC/AC could further progress to carcinoids with increased proliferative capabilities either in the lung [10] or metastatic sites [15]. According to this scheme, carcinoids, SCLC and, occasionally, LCNEC would stem from the bronchial epithelium branch in the larger airways, staying true to the neuroendocrine cell lineage [54]. Conversely, most if not all LCNEC would stem from the terminal respiratory unit in the smallest peripheral airways or even alveolar spaces through a non-neuroendocrine cell lineage [54]. It is perhaps noteworthy at this point that neuroepithelial bodies are thought to function as a component of the stem cell/progenitor niche incorporating club cell variants, which have been implicated in the development of neuroendocrine cell proliferations (hyperplasia and neoplasia) [145,184]. According to this scheme of histogenesis, there would be no crossover of different lesions along the entire spectrum of Lung-NETs, with TC/AC on the one hand and LCNEC/SCLC on the other hand making up two separate and unrelated tumor groups [57].

 This proposal is appealing as it simplifies the vision on Lung-NET development by a great deal. However, the lack of consensus on quantitative and qualitative criteria for diagnosing neuroendocrine cell hyperplasia and DIPNECH [185-187] as preinvasive lesions at risk to develop carcinoids [187,188] and the exclusively dimensional parameter (5-mm cut-off) for distinguishing neuroendocrine tumorlets from small carcinoids [1]. Therefore, caution is needed before attributing the histogenesis of Lung-NETs (and probably everywhere) so definitively. On the one hand, a rigid attribution of purported cells of origin is not strictly necessary, as the tumor ultimate fate and behavior of tumors could be rather driven by underlying gene signatures during the natural history of disease [60-65,148]. As already noted, chromothripsis mechanisms can induce development of AC from TC, regardless of the cells of origin [14], suggesting that even genetic/epigenetic alterations might be functionally relevant [60-65,148]. The role of the particular stem cell niches then be defined by risk factors, either exogenous or endogenous, by genetic, epigenetic and microenvironmental influences at the level of an individual patient’s tumor. An unexplored aspect of Lung-NETs is the duration of the preclinical phase of their natural history, when the tumor is born, develops, induces a microenvironment and expresses its biological potentials according to a continuous interplay with environmental and host organism factors. A small number of studies offer insights: tumor volume doubling time on imaging of AC was on average 79.6 months and six times shorter than that of TC [189] and that SCLC took 29 days to double, 2.8 years to reach one centimeter (time of diagnosis) and 3.2 years to reach 10 cm (time of death) [190], by and large the duration of the biological preclinical phase for Lung-NETs to develop still remains largely unclear.

**5.1 Summary of the main traits of P-HGNET**

- they account for 70-75% of Lung-NETs as a whole and about 13% of lung cancer;

*- de novo* or basal-like mechanisms of carcinogenesis with no intermediate/dysplastic lesions via devastating genetic alterations (see details in the legend of **Figure 3**). Even in situ lesions are present, as documented experimentally in mouse models but not in humans [73], they feature undifferentiated tumor cells;

- origin from cancer stem cells out of a neuroendocrine niche, with early differentiation block to model high-neuroendocrine classical SCLC (usually with primitive-appearing basal-like cells), low-neuroendocrine SCLC variants with lack or bare expression of neuroendocrine markers (usually intermediate to larger cells sometimes resembling NSCLC/LCNEC or high-grade hematologic malignancies) and combined variants (**Figure 3**);

- tumors enriched in cancer stem cells responsible for multidivergent differentiation highlighted by combined variants;

- epidemiologic association with heavy smokers, male patients and central location of tumors in close relationship with the largest airways;

- bi-allelic TP53 and RB1 inactivation, NOTCH pathway silencing, and neuroendocrine cell lineage activation by INSM1 and ASCL1 expression; SCLC variants downregulate neuroendocrine markers and their inducers and upregulate NOTCH signaling;

- even intra-tumor distribution of Ki-67, frequently approaching 100%;

- diagnosis on cytology/biopsy samples, as most of patients are metastatic at the time of diagnosis;

- solid to diffuse pattern of growth resembling small round cell tumors, NUT carcinoma or hematologic malignancies and featuring small, basal-like, undifferentiated cells with scant cytoplasm and dot-like paranuclear cytokeratin immunoreactivity (**Figure 4 A-B**); SCLC variants feature intermediate to larger cells; combined variants associate with NSCLC components in a background of diffuse pattern of growth (**Figure 4 E**);

- low mutational heterogeneity between primaries and related metastases, either synchronous or metachronous, indicative of highly effective carcinogenesis with low levels of conditional cell entropy [67];

- low or nihil expression of PD1/PD-L1 immune checkpoint;

- dismal prognosis with short clinical phase because of early metastasis formation, likely short preclinical phase of the natural history (**Figure 2**);

- short natural history as a function of devastating molecular alterations; substantial uselessness of screening programs based on CT scan to reduce lung cancer-related mortality;

This mechanistic model of *de novo* tumorigenesis is likely to hold true for other HGNET arising in extrapulmonary sites, as well as for other non-neuroendocrine tumor types, including high-grade non-papillary urothelial carcinoma/in situ carcinoma, tubal/ovarian high-grade serous carcinoma, breast triple negative/basal-like carcinoma, primary glioblastoma and uterine non-endometrioid carcinoma.

**5.2 Summary of the main traits of S-HGNET**

- they account for 20-25% of Lung-NETs as a whole and about 6% of lung cancer;

- evolution or transition carcinogenesis according to luminal-like/linear mechanisms of sequential acquisition of gene alterations over time, with possibility of intermediate/dysplastic lesions (neuroendocrine cell hyperplasia/DIPNECH, neuroepithelial bodies, carcinoids, NSCLC) via less devastating genetic alterations (see details in the legend of **Figure 3**);

- origin from cancer stem cells within a neuroendocrine niche as carcinoids or their precursors or non-neuroendocrine cancer stem cells acquiring neuroendocrine differentiation as NSCLC-NED, with allowed variable differentiation to model luminal-type/differentiating tumors (**Figure 3**);

- tumors enriched in more differentiated cancer stem/precursors or reprogrammed cells responsible for combination/mixture of diversely graded neuroendocrine components or NSCLC cell lineages where HGNET are thought to evolve through additional gene alterations from preexisting lesions (**Figure 3**);

- epidemiologic association with variably smoking patients, usually males and with preferential peripheral location of lesions;

- great variety of gene alterations with TP53→RB1 mono/bi-allelic inactivation, NOTCH inactivation, KRAS/LKB1/MEN1 mutation, MYC/MYCL/TERT/SDHA/RICTOR amplification and epithelial-mesenchymal transition;

- uneven distribution of Ki-67, with intra-tumor compartmentalization highlighting diversely graded tumor components (**Figure 5**);

- diagnosis on resection specimens, as most of patients are operable at the time of diagnosis; these tumors are likely to make up the large majority of HGNET undergoing surgical resection, where most molecular investigations have been thus far carried out;

- organoid patterns of growth with the greatest morphologic heterogeneity ever seen in lung cancer, such as mitotically active carcinoids, LCNEC resembling NSCLC or SCLC, genuinely appearing SCLC, combined variants or other demanding tumors histologically sometimes difficult to classify (**Figure 4 C**); tumor cells show clear neuroendocrine morphology with organoid aggregates, ribbons and trabeculae, variable amount of necrosis and IHC expression for epithelial and neuroendocrine markers (**Figure 4 D**); combined variant with NSCLC or non-NSCLC still exhibit an organoid pattern of growth by tumor cells (**Figure 4 F**);

- higher mutational heterogeneity between primaries and metastases, either synchronous or metachronous, indicative of less effective carcinogenesis mechanisms with higher levels of cell disorder/conditional entropy;

- higher expression of PD1/PD-L1 in a fraction of NSCLC-like LCNEC-appearing tumors;

- better prognosis (most patients will be resected) with longer clinical phase as a function of less devastating molecular alterations leading to belated metastasis formation; likely longer preclinical phase of the natural history (**Figure 2**);

- potential greater usefulness of screening programs based on CT scan to reduce cancer-related mortality.

This mechanistic model of tumorigenesis in transition according to a linear model is likely to hold true for other HGNET arising in extrapulmonary sites, as well as for other non-neuroendocrine tumor types, including papillary urothelial carcinoma; tubal/ovarian low-grade serous, mucinous, endometrioid and Brenner carcinoma; breast luminal A/B and Her-2 enriched carcinoma; secondary glioblastoma; uterine endometrioid carcinoma.

**5.3 Summary of the main traits of I-NET**

- I-NET are likely to derive from a neuroendocrine stem cell niche of preinvasive lesions (NECH or DIPNECH) through chromatin remodeling gene/epigenetic alteration mechanisms;

- I-NET would account for 5% of Lung-NETs as a whole and about 1% of all lung cancers;

- I-NET mostly encompass TC and low malignant AC (**Figure 6 A-B**), occur predominantly in women, less smokers and younger patients;

- I-NET present with early stage of disease at the time of diagnosis and favorable clinical course, even after limited resection;

- I-NET present with low and uniform distribution of Ki-67 (**Figure 6 C**) and do not express PD-L1;

- Data on genetic heterogeneity between primary vs. metastases of I-NET are still lacking.

**6. EXPERT COMMENTARY**

 Molecular characterization is expected to play an important role in the clinical handling of Lung-NETs, not only improving our knowledge on their biology, but also allowing better stratification of patients according to malignancy and identifying actionable therapy strategies. The dichotomic separation into primary/*de novo* and secondary/differentiating HGNET, as opposed to molecularly more stable and behaviorally indolent tumors (I-NET), supports an unconventional tripartite classification, which is likely to be consistent with general mechanisms of tumorigenesis as seen in neuroendocrine and non-neuroendocrine tumors of other organs. In particular, the type and timing of stochastic molecular alterations are the hallmarks of this innovative molecular approach to Lung-NET classification, which are able to dictate the ultimate histologic appearance and the clinical behavior. This distinction is likely to have direct implications in the clinical handling of Lung-NETs for personalized medicine at the level of an individual patient’s cancer [44,82,83,191]. This critical review of Lung-NETs, based on currently available literature data, emphasizes once again that is the tumor biology rather than the tumor morphology the key factor in the clinical decision making of these patients.

**7. FIVE-YEAR VIEW**

A general re-thinking of Lung-NETs is biologically and clinically warranted to unravel the clinical implications, improve classification and offer additional therapy strategies in different subsets of patients (the right drug, to the right patient, at the right time). Improved knowledge on the precise molecular mechanisms of primary and secondary HGNET, as well as I-NET, will be instrumental to design experimental and clinical studies, also by means of in vivo and in vitro models. This body of information will get new insights into the world of Lung-NETs, which are still too much addicted to morphology.

**8. KEY ISSUES**

The current 2015 WHO classification limits the biological and clinical interpretation of Lung-NETs, which, in the era of personalized therapies, makes rethinking in these tumors unavoidable.

Primary HGNET is a conceptually, biologically and clinically unitary category, resulting from highly efficient molecular mechanisms of tumorigenesis. This occurs in cancer stem cells out of a neuroendocrine niche;

Secondary HGNET is a conceptually unitary but biologically and clinically more heterogeneous category. SGHNET results from a variety of different precursors and activating molecular mechanisms, including genetic reprogramming;

Indolent-behaving NET is the third arm of this tripartite alternative outlook on Lung-NETs, which mostly feature TC and low malignant AC, with their own epidemiologic, genetic, pathologic, clinical and behavioral specific traits.

**FIGURE LEGENDS**

 **Figure 1.** A reinterpretation of Lung-NETs encompasses early aggressive primary high-grade neuroendocrine tumors (P-HGNET), less aggressive secondary high-grade neuroendocrine tumors (S-HGNET) and indolent NET (I-NET). Histologic features vary, especially in the category of S-HGNET, as does the prevalence of smoking which is an important risk factor especially in the category of P-HGNET. NETs stand for neuroendocrine tumors; LCNEC for large cell neuroendocrine carcinoma; SCLC for small cell lung carcinoma.

 **Figure 2.** Proposed derivation flowchart of Lung-NETs from transformed (yellow thunderbolts) cancer stem cells (CSC), precursor cells (PC) or reprogrammed differentiated cells (RDC) within local microenvironment (pericellular area) is shown. Indolent NET (I-NET) are low grade tumors with a long preclinical phase and low-malignant behavior resulting in long-term survival. Aggressive NET can be split into either primitive-appearing tumors represented by early aggressive primary high-grade neuroendocrine tumors (P-HGNET) that are characterized by short preclinical phase and accelerated clinical outcome with dismal prognosis, or evolving-appearing tumors represented by differentiating and belatedly less aggressive secondary high-grade neuroendocrine tumors (S-HGNET) that are characterized by variably longer preclinical phase and better survival with intermediate prognosis. NETs stand for neuroendocrine tumors; EA stands for early aggressive.

 **Figure 3.** Reconstruction of Lung-NET development according to basal-like/*de novo* or luminal-like/ differentiating mechanisms of tumorigenesis is depicted from cancer stem/progenitor or reprogrammed differentiated cells, to some extent also independent of the cells of origin as a function of risk factors and microenvironment influences. Basal-like/*de novo* carcinogenesis gives rise to primitive-appearing tumors cells, which precociusly become invasive with unapparent in situ carcinoma (red circles above basal membrane in black) leading to primary high-grade neuroendocrine tumors (P-HGNET) featuring invasive classical SCLC (red circles). Further alterations alongside any non-small cell lung carcinoma cell lineage cause combined variants of SCLC to form, whereas combined downregulation of TTF1 and neuroendocrine traits and upregulation of *REST*, NOTCH and Hippo/TGFβ pathway with MYC amplification are responsible for variant subtypes of SCLC to arise that are composed of intermediate (light blue polygons) or large cells (light blue circles with black nuclei). Luminal-like/linear carcinogenesis gives rise to organoid-appearing precursor lesions (pink circles), such as neuroendocrine hyperplasia or DIPNECH (green cells), able to further grow up alonside neuroendocrine features realizing NET-like secondary high-grade neuroendocrine tumors (S-HGNET), where LCNEC progressing from TC or its precursors and/or AC evolving to SCLC are observed (see text and reference Pelosi et al.[68] for details). Transformation of TC to AC (asterisk) may occur for chromotrypsis [14], as highlighted in the semi-transparent yellow box. Another intermediate lesion is represented by non-small cell lung cancer (NSCLC) and/or its precursors, where luminal-like differentiation leads to NSCLC-like S-HGNET formation, in pure or combined variants, through the intermediate step of NSCLC with neuroendocrine differentiation (light blue circle with yellow centers). Lastly, I-NET would result from luminal-like differentation of carcinoid-precursor lesions, likely different from those where originating carcinoids in transition are from (light gray box). TC stands for typical carcinoid; AC for atypical carcinoid; LCNEC for large cell neuroendocrine carcinoma; SCLC for small cell lung carcinoma.

 **Figure 4 A-F.** Histological details on high-grade neuroendocrine tumors are shown. Primary high-grade neuroendocrine tumors (P-HGNET) are supplied with diffuse pattern of growth, undifferentiated stem-like cancer cells (**A**) and dot-like paranuclear cytokeratin decoration (**B**). Secondary high-grade neuroendocrine tumors are characterized by organoid pattern of growth, trabecular aggregates of more differentiated cancer cells (**C**) and more abundant cytoplasmic decoration of cytokeratins (**D**). Combined variant of either tumor type, in either case with squamous cell component (reactivity for high weight molecular cytokeratins), repeat the same basic patterns of growth with either diffuse small-szed cancer cells (**E**) or organoid-appearing large-sized cells (**F**).

 **Figure 5 A-C.** Histological details on a case of secondary high-grade neuroendocrine tumors displaying a carcinoid-like organoid pattern with many mitoses (yellow asterisks) (**A**). Spindle small cells were also seen (**B**), which variably reacted for chrmohgranina A (**B**, inset). Ki-67 activity showed an inequivocal intra-tumor heterogeneity, with close intermingling of low-proliferating (**C**) and high-proliferating (**D**) cells. Tumor cells were also nuclearly decorated for p53 (**D**, inset).

 **Figure 6 A-C.** Histological details on indolent neuroendocrine tumors are shown. They fulfill the histological criteria for typical carcinoid (**A**) or atypical carcinoid with low mitotic count (≤3 mitoses per 2 mm2) and absence of necrosis (**B**), with an uniform distribution of low-stained Ki-67 labeling index (**C**).

**COMPLIANCE WITH EHICAL STANDARDS**

 This is only a review article on existing literature, which did not need any approval by Internal Review Board.

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**CONFLICTS OF INTEREST**

The authors declare that they have no conflicts of interest.

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