Lung neuroendocrine neoplasms (NENs) account for up to 25% of all lung cancers and can be subdivided into poorly differentiated, high-grade neuroendocrine carcinomas (NECs) including small cell lung carcinoma (SCLC, 20%) and large-cell neuroendocrine carcinomas (LCNEC, 3%), and well-differentiated neuroendocrine tumours (NETs, 2%) including the low- and intermediate-grade typical and atypical carcinoids, respectively (1). Each of these lung NEN subtypes shows different behaviour in terms of clinical presentation, prognosis and aetiology: whereas SCLC and LCNEC are smoking-related very aggressive, highly metastatic tumours with poor prognosis, typical and atypical carcinoids have thus far unclear association with smoking and are less aggressive, with longer survival expectancy (2). The distinction between lung NETs and NECs is crucial for the clinical management of these diseases; surgical resection is the treatment of choice for localized tumours—mostly NETs—while systemic treatment and, more recently, immunotherapy are preferred for disseminated NECs. However, cytotoxic agents are mostly ineffective in these tumours (3).

**Molecular landscape of lung NENs**

NECs and NETs have very distinct molecular characteristics and it is widely accepted that they are different diseases and not simply a continuum of neoplasms with a common pathogenesis (4,5): both typical and atypical pulmonary carcinoids show low mutation rate and frequent alterations in chromatin-remodelling genes (6,7) with very rare mutations in *TP53* and *RB1* genes. These latter two genes are, conversely, universally inactivated in SCLC (8,9). The molecular spectrum of LCNEC is more heterogeneous, with two well-distinguished large groups of samples: type-I LCNEC, with frequent alterations in *TP53*, *STK11* and *KEAP1*, and type-II LCNEC, with alterations in both *TP53* and *RB1* (10,11) (Figure 1). While the nomenclature given by Rekhtman and colleagues (NSCLC-like and SCLC-like LCNEC for type-I and type-II, respectively) is accurate in terms of mutational patterns, it does not reflect the expression patterns: type-I LCNECs have the mutational characteristics of NSCLCs, but expression profiles similar to SCLC, while type-II LCNECs have mutational characteristics of SCLC but low neuroendocrine expression profiles similar to NSCLCs (11). Recent studies on molecular subtypes of SCLC point to distinct expression profiles marked by the differential expression of key transcription factors (13) (Figure 1). With regard to the strong similarities of LCNEC and SCLC, and the difficulties in distinguishing these cancer types in cell culture and by histology, it remains to be understood how many of these SCLC subtypes may rather reflect similarities to LCNEC.
Evidence for grade-3 carcinoids in the lung

The distinction between NECs and NETs is currently based on morphologic characteristics, necrosis, and mitotic count (>10 per 2 mm$^2$ for NECs) (4). Ki-67 proliferation index is only recommended to separate lung NECs and NETs in non-resection specimens (14,15), with a threshold of 20% as the upper limit for NETs (4). Lung carcinoids correspond to the second most frequent type of NETs in the body (accounting for 30%), right after enteropancreatic NETs (50%) (16). In contrast to the lung, for the enteropancreatic system, a grade-3 category of NETs has recently been considered when the tumours present a Ki-67 index higher than 20%, or more than 20 mitoses/2 mm$^2$ along with well-differentiated morphology (17). A recent study by Rekhtman and colleagues on a series of 132 stage IV lung carcinoids has shown that, in the metastatic setting, lung carcinoids frequently exceed the mitotic count and Ki-67 index ceiling criteria for grade-2 NETs, suggesting that these tumours might be the counter partners of grade-3 enteropancreatic carcinoids (11); they called them carcinoid-like LCNEC (Figure 1). In another study, led by Simbolo and colleagues (12), using a 58-gene expression signature assessed in 35 atypical carcinoids and 32 LCNEC, the authors identified three clusters: C1 enriched for LCNEC, C2, which contained both LCNEC (C2a) and atypical carcinoids (C2b), and C3 enriched for atypical carcinoids. Based on their molecular characteristics, the C1 and C2a LCNEC-enriched clusters are likely to correspond to the previously described type-II and type-I LCNECs, respectively (10,11). Interestingly, atypical-carcinoid enriched cluster C3, in which MEN1 inactivation seem to play a major role, included four LCNEC samples, three of which also harboured MEN1 truncating mutations.

In a recent study, Alcala and colleagues performed integrative multi-omic analyses for a total of 257 lung NENs. Unsupervised Multi-Omics Factor Analysis (MOFA) (23) of expression and methylation data of lung carcinoids and LCNEC samples identified three robust clusters with different molecular and clinical characteristics: cluster Carcinoid A (enriched for typical carcinoids, 10-year overall survival above 80%), cluster Carcinoid B (enriched for atypical carcinoids, 10-year overall survival of 60%) and cluster LCNEC (10-year overall survival of 21%) (Figure 1). Interestingly, one of
the LCNEC samples fell into one of the carcinoid clusters, suggesting that this LCNEC sample shared the molecular characteristics of lung carcinoids (7).

Although these LCNEC cases with molecular characteristics of lung carcinoids remain anecdotal (n=7 in total), they might indeed correspond to the grade-3 carcinoids of the lung. Indeed, Ki-67 index assessment of the two carcinoid-like LCNECs identified by Rekhtman and colleagues showed that, although the Ki-67 index was relatively high for carcinoids (around 35%), it was relatively low when compared to that of type-I and type-II LCNECs (60% and 90%, respectively) (11).

**Supra-carcinoids: a new molecular entity of lung NENs**

One unexpected finding in the MOFA performed by Alcala and colleagues was the identification of six morphologically classified atypical carcinoids that fell into the molecular cluster of LCNEC. In addition to sharing the molecular features of LCNEC, they also present a poor survival (10-year overall survival of 33%). These samples were named supra-carcinoids (Figure 1), and they represented 17% of the atypical carcinoids in that series. The supra-carcinoids also showed distinct features, such as high levels of immune checkpoint receptors and ligands, and high neutrophil content (7), which might represent potential diagnostic and therapeutic candidates for this group of aggressive lung NETs.

Supra-carcinoids might also have been identified in the study from Simbolo and colleagues (12). Indeed, four of their atypical carcinoids fell into clusters C2a and C1, which were both enriched for LCNECs. This represents 13% of atypical carcinoids in their series, which is consistent with the 17% that Alcala and colleagues identified (7).

The discovery of supra-carcinoids suggests that the molecular link between lung NETs and NECs might be subtler than initially thought, especially between atypical carcinoids and LCNEC. In fact, over the past years, Pelosi and colleagues have been advancing the innovative idea in both the lung and the thymus that the progression or transition from lung NET to NEC, possibly through the accumulation of genetic anomalies, might be possible (14,24,25). To formally prove this hypothesis, in vitro and in vivo experimental models are needed. Dr. Talya Dayton in the lab of Hans Clevers is developing an organoid-based model of lung NETs that, if successful, could provide the perfect setting to test this hypothesis (personal communication).

**Open science to move forward with the study of these rare diseases: tumour maps**

The overall limitation to performing molecular studies on lung carcinoids and LCNEC is the limited number of samples of suitable quality to perform comprehensive genomic analyses of these rare diseases. The generally low number of samples included in each study makes it difficult to produce data from which to draw meaningful and reproducible conclusions. Thankfully, the overall field of research is moving towards open data, even if restrictions are still often present (26) and barriers and reluctance still exist (27,28). This allows researchers with few samples to answer a specific research question by performing comparative analyses with data that have already been published. For example, this approach led Alcala and colleagues to the discovery of the supra-carcinoids (7), through the integration of molecular data generated for 257 lung NENs in four different studies (6,8-10).

One additional step in the direction of more useful open science is the generation of so-called “tumour maps”, which provide an interactive way to explore the molecular data and allow easy statistical interrogation, including generating new hypotheses, but also locating samples from smaller studies into the molecular landscape (29). The placement of the data from a specific patient’s sample in a region of the map with other samples that have similar molecular characteristics might also assist clinicians in their diagnosis, prognosis and clinical management of that patient. As an example, we have recently added our comprehensive lung NEN molecular map to the UCSC Tumor Map portal (https://tumormap.ucsc.edu/?p=RCG_lungNENomics/LNEN, Gabriel et al. in preparation). Simbolo and colleagues (12) seem to have identified equivalent molecular groups of lung NENs as in previous studies (7,10). However, the variability introduced by (1) the fact that their samples were formalin-fixed paraffin-embedded tissue while the ones used to generate the lung NEN molecular map were snap frozen, and (2), the studies were based on different sequencing techniques, hampers the integration of both datasets. Indeed, data standardization remains an issue in this kind of approach (30).

**Conclusions**

Considerable progress has been made in the molecular characterization of lung NENs. In the context of LCNEC and carcinoids, these studies have led to the suggestion that
grade-3 enteropancreatic NETs might have their equivalent entity in the lung, and perhaps also in the thymus. Multimomic integrative analyses have identified a new molecular entity, the supra-carcinoids, whose full characterization warrants further investigation, not only including comprehensive molecular studies but also a central pathology review with detailed analysis of proliferation metrics. Finally, the above-mention observations further support the suggested molecular link between lung NECs and NETs. Further studies are nevertheless needed to assess the clinical implications of all of these discoveries.

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Footnote

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