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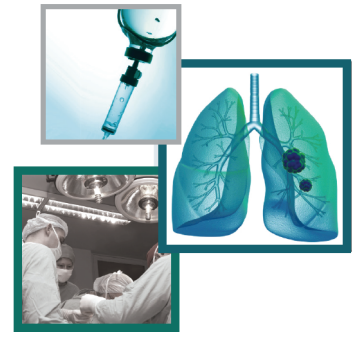
Lung Cancer Management



# Are neuroendocrine negative small cell lung cancer and large cell neuroendocrine carcinoma with WT RB1 two faces of the same entity?

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## Practice points

- Similarity between some subtypes of small cell lung cancer (SCLC) and large cell neuroendocrine carcinoma (LCNEC) can be even higher than between SCLC and LCNEC in general.
- Neuroendocrine markers negative/low RB1 WT SCLC-Y subtype and neuroendocrine markers negative/low WT RB1, WT KEAP1, WT STK11 LCNEC subtype are highly similar and might be in fact the same entity.
- Unanimous agreement between expert pathologists is around 50% in differentiating between SCLC and LCNEC, indicating high level of diagnostic variability.
- Patients with neuroendocrine markers negative/low WT RB1, WT KEAP1, WT STK11 LCNEC subtype might be candidates for clinical trial-based treatment with CDK4/6 inhibitors.
- Lung anatomic locations could be important information to record along with genomic information and neuroendocrine markers staining.
- About 10% of tumors diagnosed as SCLC are RB1 WT, such tumors may have sensitivity to treatments which are not applicable to majority of SCLC with inactivated RB1, therefore, confirming RB1 status should be an important consideration.

Until recently, small cell lung cancer (SCLC) was described as SCLC and SCLC variant, based upon cellular morphology and loss of neuroendocrine markers in the SCLC variant. However, based on recent research advances, driven in part by the increase in comprehensive genomic data, it has become clear that there are multiple SCLC subtypes including an ASCL1 and NEUROD1 low, YAP1 high (SCLC-Y) subtype enriched for WT RB1. Comparing morphological and other features of this SCLC subtype to neuroendocrine negative RB1, KEAP1, STK11 WT LCNEC raises a number of important questions with diagnostic and therapeutic implications.

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Small cell lung cancer (SCLC) is an aggressive form of lung cancer with limited therapeutic options, a very high mortality rate and is characterized, in most cases, by neuroendocrine features. SCLC accounts for approximately 15% of lung cancers. The majority of SCLC are genetically characterized by bi-allelic inactivation of RB1 (~90%) and TP53 (~98%) tumor suppressor genes [1–6]. The prevailing hypothesis is that inactivation of RB1 in SCLC leads to increase in cellular proliferation due to loss of cell cycle control and inactivation of TP53 prevents oncogene-induced senescence. SCLC diagnosis is commonly based on morphological features of biopsy or cytology samples. A panel of neuroendocrine markers (CHGA, NCAM1, SYP) may also be utilized [7,8]. In a noticeable fraction of cases, SCLC is present along with other lung cancer subtype(s) such as: large cell neuroendocrine carcinoma (LCNEC), large cell carcinoma (LCC), adenocarcinoma and/or squamous cell carcinoma [9].

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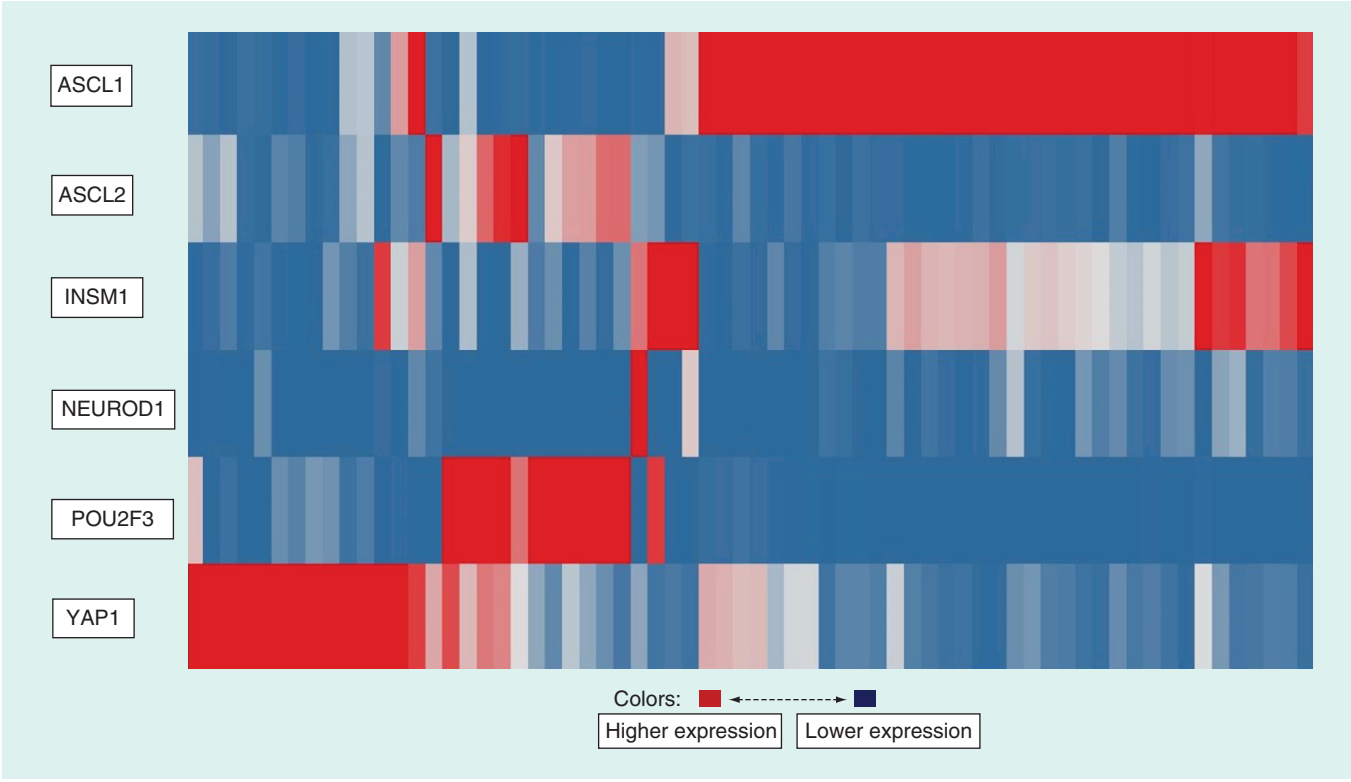


**Figure 1. Gene expression clustering of large cell neuroendocrine carcinoma by key 'small cell lung cancer' transcription factors.**

Currently, SCLC is divided into four subtypes: ASCL1 high (SCLC-A), NEUROD1 high (SCLC-N), POU2F3 high (SCLC-P) and YAP1 high (SCLC-Y) subtype enriched for WT RB1 [10–13]. SCLC-Y subtype has low or absent expression of ASCL1, NEUROD1 and other neuroendocrine markers and accounts for approximately 5–10% SCLC tumors [11,12]. The SCLC-Y subtype is enriched for CCND1 amplification and CDKN2A inactivation, these alterations may play similar role to RB1 inactivation resulting in cell cycle control defects [11].

LCNEC is a relatively rare lung cancer, accounting for approximately 3% of lung cancer cases. In general, LCNEC is characterized by neuroendocrine morphology and markers; similarly to SCLC, patients with LCNEC have a poor prognosis [14]. About 90% of LCNEC have bi-allelic TP53 inactivation, ~40% have bi-allelic RB1 inactivation and ~40% have KEAP1 and/or bi-allelic STK11 inactivation; bi-allelic RB1 inactivation is generally mutually exclusive with KEAP1/STK11 bi-allelic inactivation [15,16]. From genetic alterations, prospective LCNEC with bi-allelic RB1 and TP53 inactivation are considered 'SCLC like' and LCNEC with KEAP1/STK11 bi-allelic inactivation are considered 'NSCLC like'. About 4–8% of LCNEC are ASCL1 and NEUROD1 low with WT RB1, WT KEAP1, WT STK11 and bi-allelic TP53 inactivation. It is interesting to note that clustering gene expression LCNEC data from George *et al.* using key 'SCLC' transcription factors results in the gene expression pattern shown in Figure 1 which is very similar to one observed in SCLC [10,16].

Thus, there are noticeable genetics, genomics and phenotypical similarities between the RB1 WT SCLC-Y subtype and the WT RB1, WT KEAP1, WT STK11 LCNEC subtype, where each subtype displays low or absent expression of ASCL1 and NEUROD1. In order to provide a more detailed background for comparison, some of the key features of neuroendocrine negative (NE-) SCLC are listed in Table 1. For comparison purposes, features of classical neuroendocrine positive (NE+) SCLC are also listed. Properties of LCNEC listed in Table 1 are generic and not necessarily specific to neuroendocrine negative (NE-) LCNEC subtype with WT RB1, WT KEAP1, WT STK11; it is possible that such LCNEC subtype has morphological properties even more similar to neuroendocrine negative (NE-) SCLC.

In general, SCLC are mostly located in central lung location and LCNEC in peripheral or midzone lung location [17–19]. A recent study from Zhou *et al.* suggests the possibility of differences between LCNEC in pe-

Table 1. Morphological properties of large cell neuroendocrine carcinoma, neuroendocrine negative small cell lung cancer and classical neuroendocrine positive small cell lung cancer.

| Feature | LCNEC | SCLC NE- | SCLC NE+ |
|---|---|---|---|
| Mostly smokers | Yes | Yes | Yes |
| Median age | ~67 years | ~65 years | ~65 years |
| Cell size | Typical of NSCLC | Larger than classical SCLC | Small |
| Cytoplasm | Abundant | More plentiful than classical SCLC | Scarce |
| Nucleoli | Prominent | Prominent | Not prominent |
| Mitotic rate | High | High | High |
| Nuclear chromatin | Less uniform | More uniform | More uniform |
| Cell borders | More distinct | Less distinct | Less distinct |

LCNEC: Large cell neuroendocrine carcinoma; NE: Neuroendocrine; NSCLC: Non-small-cell lung carcinoma; SCLC: Small cell lung cancer.

ripheral versus central locations [20]. Unfortunately, data regarding tumor location in the lung is limited for the neuroendocrine-negative SCLC subtype and for neuroendocrine-negative LCNEC with WT RB1, KEAP1, STK11.

There is currently no definitive marker(s) for LCNEC and diagnosis is based on exclusion. In part due to this and significant variabilities in LCNEC and SCLC phenotypes, there is limited agreement among pathologists on the LCNEC diagnosis. Ha *et al.* indicated that, among five expert thoracic pathologists, unanimous agreement in the diagnosis of LCNEC vs SCLC was achieved in only 40% of cases, other studies indicate similar levels of unanimous agreement between expert pathologists in SCLC and LCNEC diagnosis [21–23]. As can be seen from Table 1, neuroendocrine negative SCLC and LCNEC have a number of similarities and potential differences that are rather subtle, which makes it even more difficult to achieve agreement between pathologists.

In the Nicholson *et al.* study, out of 100 surgical biopsies or resections with a diagnosis of SCLC, 28 showed evidence of an NSCLC component in addition to SCLC, with LCC being the largest mixture component present in 16 cases of combined SCLC. Out of these 16 SCLC/LCC combined cases, 6 had LCNEC subtype of LCC [24]. In most instances of NSCLC combined with SCLC there is strong evidence of clonality between SCLC and NSCLC components, likely indicating a common precursor cell in such cases [25–29]. Pulmonary neuroendocrine cells (PNECs) and perhaps common pulmonary stem cells are potential cells of origin for SCLC and LCNEC [30–32].

Keeping in mind a likely common cell of origin and very similar genetics, genomics and morphology of the RB1 WT SCLC-Y subtype and the WT RB1, WT KEAP1, WT STK11 LCNEC subtype with low or absent expression of ASCL1 and NEUROD1, it is tempting to suggest that perhaps these two lung cancer subtypes are in-fact two faces of the same entity. If this is indeed the case, there are important diagnostic and therapeutic implications. From a diagnostic prospective, it should be important to note that such tumors share SCLC and LCNEC properties. Such acknowledgment has a potential therapeutic implication in relation to CDK4/6 inhibitors. Due to their lack of functional RB1, the clear majority of SCLC models are insensitive to the CDK4/6 inhibitors [33–36]. In contrast, some SCLC models with functional RB1 are sensitive to the CDK4/6 inhibitors [11,12,36,37]. Following these preclinical studies, a clinical trial of a CDK4/6 inhibitor in RB1 WT SCLC is being developed. Based on the discussion in this perspective, patients diagnosed with LCNEC subtype with WT RB1, WT KEAP1, WT STK11 and loss of neuroendocrine markers may benefit from being considered eligible for such clinical trial.

## Future perspective

We hope that analysis presented in this article would help to increase awareness about highly similar subtypes of SCLC and LCNEC and potential clinical implications which might became even more relevant in future. We also hope our work would help to rejuvenate efforts on collecting information on lung anatomic locations of SCLC, LCNEC (and perhaps other lung tumors) in addition to genomic information and neuroendocrine markers staining. It is possible that paying attention to lung location such as: peripheral, midzone or central along with genomic information and neuroendocrine status may help to better characterize lung malignancies.

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No writing assistance was utilized in the production of this manuscript.



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