The 2021 WHO Classification of Lung Tumors: Impact of Advances Since 2015

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ABSTRACT

The 2021 WHO Classification of Thoracic Tumours was published earlier this year, with classification of lung tumors being one of the chapters. The principles remain those of using morphology first, supported by immunohistochemistry, and then molecular techniques. In 2015, there was particular emphasis on using immunohistochemistry to make classification more accurate. In 2021, there is greater emphasis throughout the book on advances in molecular pathology across all tumor types. Major features within this edition are (1) broader emphasis on genetic testing than in the 2015 WHO Classification; (2) a section entirely dedicated to the classification of small diagnostic samples; (3) continued recommendation to document percentages of histologic patterns in invasive nonmucinous adenocarcinomas, with utilization of these features to apply a formal grading system, and using only invasive size for T-factor size determination in part lepidic nonmucinous lung adenocarcinomas as recommended by the eighth edition TNM classification; (4) recognition of spread through airspaces as a histologic feature with prognostic significance; (5) moving

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lymphoepithelial carcinoma to squamous cell carcinomas; (6) update on evolving concepts in lung neuroendocrine neoplasm classification; (7) recognition of bronchiolar adenoma/ciliated muconodular papillary tumor as a new entity within the adenoma subgroup; (8) recognition of thoracic SMARCA4-deficient undifferentiated tumor; and (9) inclusion of essential and desirable diagnostic criteria for each tumor.

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Keywords: Lung; Cancer; Pathology; Immunohistochemistry; Molecular pathology; Adenocarcinoma; Squamous cell carcinoma

Introduction

The 2021 World Health Organisation (WHO) Classification of Thoracic Tumours was published earlier this year, with classification of lung tumors being one of the chapters (Table 1). Previous WHO editions were published in 1967 and 1981, purely in relation to the lung, followed by the lung and pleura in 1999, and then the lung, pleura, thymus, and heart in 2004 and 2015. The 2015 WHO Classification book introduced many important changes, largely owing to the remarkable progress in understanding genetics and molecular-targeted therapies. Because of introduction of immunohistochemistry and molecular testing throughout the classification, many of the more sophisticated approaches to pathologic diagnosis have led to more precise pathologic and genetic classification of lung tumors allowing for better therapeutic strategies.

The principles of lung tumor classification remain those of using morphology first, supported by immunohistochemistry, and then molecular techniques. In 2015, there was particular emphasis on using immunohistochemistry to make classification more accurate. In 2021, there is greater emphasis throughout the book on advances in molecular pathology across all tumor types. Individual molecular abnormalities form part of the diagnostic criteria for a few of the rarer tumors, for example pulmonary myxoid sarcoma with EWSR1-CREB1 fusion but, although many molecular abnormalities do not yet impact on classification of specific tumor subtypes, they may affect patient management.

Major features within this edition are the following: (1) broader emphasis on genetic testing than in the 2015 WHO Classification; (2) a section entirely dedicated to the classification of small diagnostic samples; (3) continued recommendation to document percentages of histologic patterns in invasive nonmucinous adenocarcinomas, with utilization of these features to apply a

<table>
<thead>
<tr>
<th>Table 1. List of Lung Tumors in 2021 WHO Classification of Thoracic Tumors With ICD-O Codes</th>
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<td><strong>Epithelial tumors</strong></td>
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<tr>
<td>Papillomas</td>
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<tr>
<td>Squamous cell papilloma, NOS</td>
</tr>
<tr>
<td>Squamous cell papilloma, inverted</td>
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<tr>
<td>Glandular papilloma</td>
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<tr>
<td>Mixed squamous cell and glandular papilloma</td>
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<tr>
<td><strong>Adenomas</strong></td>
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<td>Sclerosing pneumocytoma</td>
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<tr>
<td>Alveolar adenoma</td>
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<tr>
<td>Papillary adenoma</td>
</tr>
<tr>
<td>Bronchiolar adenoma/ciliated muconodular papillary tumor</td>
</tr>
<tr>
<td>Mucinous cystadenoma</td>
</tr>
<tr>
<td>Mucous gland adenoma</td>
</tr>
<tr>
<td><strong>Precursor glandular lesions</strong></td>
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<tr>
<td>Atypical adenomatous hyperplasia</td>
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<tr>
<td>Adenocarcinoma in situ</td>
</tr>
<tr>
<td>Adenocarcinoma in situ, nonmucinous</td>
</tr>
<tr>
<td>Adenocarcinoma in situ, mucinous</td>
</tr>
<tr>
<td><strong>Adenocarcinomas</strong></td>
</tr>
<tr>
<td>Minimally invasive adenocarcinoma</td>
</tr>
<tr>
<td>Minimally invasive adenocarcinoma, nonmucinous</td>
</tr>
<tr>
<td>Minimally invasive adenocarcinoma, mucinous</td>
</tr>
<tr>
<td>Invasive nonmucinous adenocarcinoma</td>
</tr>
<tr>
<td>Lepidic adenocarcinoma</td>
</tr>
<tr>
<td>Acinar adenocarcinoma</td>
</tr>
<tr>
<td>Papillary adenocarcinoma</td>
</tr>
<tr>
<td>Microglandular adenocarcinoma</td>
</tr>
<tr>
<td>Solid adenocarcinoma</td>
</tr>
<tr>
<td>Invasive mucinous adenocarcinoma</td>
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<tr>
<td>Mixed invasive mucinous and nonmucinous adenocarcinoma</td>
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<td><strong>Colloid adenocarcinoma</strong></td>
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<tr>
<td>Fetal adenocarcinoma</td>
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<td>Adenocarcinoma, enteric type</td>
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<tr>
<td>Adenocarcinoma, NOS</td>
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<tr>
<td><strong>Squamous precursor lesions</strong></td>
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<tr>
<td>Squamous cell carcinoma in situ</td>
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<tr>
<td>Mild squamous dysplasia</td>
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<tr>
<td>Moderate squamous dysplasia</td>
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<tr>
<td>Severe squamous dysplasia</td>
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<tr>
<td><strong>Squamous cell carcinomas</strong></td>
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<tr>
<td>Squamous cell carcinoma, NOS</td>
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<tr>
<td>Squamous cell carcinoma, keratinizing</td>
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<tr>
<td>Squamous cell carcinoma, nonkeratinizing</td>
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<tr>
<td>Basaloid squamous cell carcinoma</td>
</tr>
<tr>
<td>Lymphoepithelial carcinoma</td>
</tr>
<tr>
<td>Large cell carcinomas</td>
</tr>
<tr>
<td>Large cell carcinoma</td>
</tr>
<tr>
<td>Adenosquamous carcinomas</td>
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<tr>
<td>Adenosquamous carcinoma</td>
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<tr>
<td>Sarcomatoid carcinomas</td>
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**Table 1. Continued**

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<thead>
<tr>
<th>Tumor Type</th>
<th>Code</th>
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<tr>
<td>Pleomorphic carcinoma</td>
<td>8022/3</td>
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<tr>
<td>Giant cell carcinoma</td>
<td>8031/3</td>
</tr>
<tr>
<td>Spindle cell carcinoma</td>
<td>8032/3</td>
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<tr>
<td>Pulmonary blastoma</td>
<td>8972/3</td>
</tr>
<tr>
<td>Carcinosarcoma</td>
<td>8980/3</td>
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<tr>
<td><strong>Other epithelial tumors</strong></td>
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<tr>
<td>NUT carcinoma</td>
<td>8023/3</td>
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<tr>
<td>Thoracic SMARCA4-deficient undifferentiated tumor</td>
<td>8044/3</td>
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<tr>
<td><strong>Salivary gland-type tumors</strong></td>
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<tr>
<td>Pleomorphic adenoma</td>
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<tr>
<td>Adenoid cystic carcinoma</td>
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<tr>
<td>Epithelial-myoepithelial carcinoma</td>
<td>8562/3</td>
</tr>
<tr>
<td>Mucoepidermoid carcinoma</td>
<td>8430/3</td>
</tr>
<tr>
<td>Hyalinizing clear cell carcinoma</td>
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<tr>
<td>Myoepithelioma</td>
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<tr>
<td>Myoepithelial carcinoma</td>
<td>8982/3</td>
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<tr>
<td><strong>Lung neuroendocrine neoplasms</strong></td>
<td></td>
</tr>
<tr>
<td>Precursor lesion</td>
<td>8040/0</td>
</tr>
<tr>
<td>Diffuse idiopathic neuroendocrine cell hyperplasia</td>
<td>8040/0</td>
</tr>
<tr>
<td><strong>Neuroendocrine tumors</strong></td>
<td></td>
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<tr>
<td>Carcinoid tumor, NOS/neuroendocrine tumor, NOS</td>
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</tr>
<tr>
<td>Typical carcinoid/neuroendocrine tumor, grade 1</td>
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</tr>
<tr>
<td>Atypical carcinoid/neuroendocrine tumor, grade 2</td>
<td>8249/3</td>
</tr>
<tr>
<td><strong>Neuroendocrine carcinomas</strong></td>
<td></td>
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<tr>
<td>Small cell carcinoma</td>
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<tr>
<td>Combined small cell carcinoma</td>
<td>8045/3</td>
</tr>
<tr>
<td>Large cell neuroendocrine carcinoma</td>
<td>8013/3</td>
</tr>
<tr>
<td>Combined large cell neuroendocrine carcinoma</td>
<td>8013/3</td>
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<tr>
<td><strong>Tumors of ectopic tissues</strong></td>
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<tr>
<td>Melanoma</td>
<td>8720/3</td>
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<tr>
<td>Meningioma</td>
<td>9530/0</td>
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<tr>
<td><strong>Mesenchymal tumors specific to the lung</strong></td>
<td></td>
</tr>
<tr>
<td>Pulmonary hamartoma</td>
<td>8992/0</td>
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<tr>
<td>Chondroma</td>
<td>9220/0</td>
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<tr>
<td>Diffuse lymphangiomatosis</td>
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<td>Pleuropulmonary blastoma</td>
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<tr>
<td>Intimal sarcoma</td>
<td>9137/3</td>
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<tr>
<td>Congenital peribronchial myofibroblastic tumor</td>
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<tr>
<td>Pulmonary myxoid sarcoma with EWSR1-CREB1 fusion</td>
<td>8842/3</td>
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<tr>
<td><strong>PEComatous tumors</strong></td>
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<tr>
<td>Lymphangioleiomyomatosis</td>
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<tr>
<td>PEComa, benign</td>
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<tr>
<td>PEComa, malignant</td>
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<tr>
<td><strong>Hematolymphoid tumors</strong></td>
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<tr>
<td>MALT lymphoma</td>
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<td>Diffuse large B-cell lymphoma, NOS</td>
<td>9680/3</td>
</tr>
<tr>
<td>Lymphomatoid granulomatosis, NOS</td>
<td>9766/1</td>
</tr>
<tr>
<td>Lymphomatoid granulomatosis, grade 1</td>
<td>9766/1</td>
</tr>
<tr>
<td>Lymphomatoid granulomatosis, grade 2</td>
<td>9766/1</td>
</tr>
</tbody>
</table>

Note: These morphology codes are from the ICD-O-3.2 (IACR [Internet]), Lyon (France): IARC; 2019. ICD-O-3.2; updated 2019 April 23. Available from: http://www.iarc.fr/index.php?option=com_content&view=article&id=149:icd-o-3-2&catid=80&Itemid=545. Behavior is coded /0 for benign tumors; /1 for unspecified, borderline, or uncertain behavior; /2 for carcinoma in situ and grade III intraepithelial neoplasia; /3 for malignant tumors, primary site; and /6 for malignant tumors, metastatic site. Behavior code /6 is not generally used by cancer registries. This classification is modified from the previous WHO classification, taking into account changes in our understanding of these lesions. Subtype labels are indented.


*Labels marked with a dagger have undergone a change in terminology of a previous code.

*Codes marked with an asterisk were approved by the IARC/WHO Committee for ICD-O at its meeting in October 2020.


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**Clinical Impact of Updates**

Lung cancer mortality is declining in the United States, and the major changes introduced in the 2011 International Association for the Study of Lung Cancer (IASLC)/American Thoracic Society (ATS)/European Respiratory Society (ERS) lung adenocarcinoma classification and 2015 WHO Classification have affected these improved patient outcomes by providing greater diagnostic accuracy and better therapeutic strategies through more efficient molecular and biomarker testing. Early data from the American Cancer Society reveal that in the United States, lung cancer accounted for nearly half of the overall decline in cancer deaths in the past 5 years, driving the record single-year drop of 2.5% from 2017 to 2018 for the second year in a row. For the two 5-year periods ending in 2013 and 2018, the pace of the...
annual decline in lung cancer mortality more than doubled from 2.4% to 5.5%.9 Although many factors contributed to these improved clinical outcomes, and data vary internationally,10 the fundamental principles introduced by the 2011 IASLC/ATS/ERS adenocarcinoma classification and 2015 WHO Classifications provided the foundation of many of these therapeutic advances by making accurate diagnoses and promoting molecular and biomarker testing for molecular targeted and immunotherapies.

Improved stratification of patient survival has also been revealed by using the eighth edition of the TNM staging classification for nonmucinous adenocarcinomas, where only the invasive size is used for T-factor size,11,12 which is based on accurate designation of architectural patterns using the WHO classification. Regarding surgical treatment, controversy persists on sublobar resection for small lung cancers less than or equal to 2 cm. Most importantly, a complete resection should be obtained to yield the best prognosis as life expectancy is reduced in resections that are not R0, including so-called uncertain resections.13 The recognition of STAS as a histologic feature within the WHO classification may be important in this field going forward.

Regarding systemic therapy, between 2013 and 2016, mortality from NSCLC decreased faster than its incidence, and this decrease has been associated with a substantial improvement in survival that corresponded to the timing of approval of targeted therapy. This reflects the impact of clinical implementation of EGFR- and ALK-inhibiting agents in patients whose tumors harbor specific genomic abnormalities.14 In addition, the impact of newly discovered druggable genetic drivers, such as ROS1, RET, NTRK1-3, BRAF, MET, and ERBB2, and that of immunotherapy and the implementation of early detection techniques are expected to further improve the 5-year survival rate of NSCLC in the next few years. Much of this remains predicated on the ability to classify accurately subtypes of NSCLC, with discrimination between squamous and nonsquamous nonsmall cell carcinomas being particularly important so that molecular testing, which includes programmed death-ligand 1 (PD-L1) testing and a selected panel of the abovementioned genes, is undertaken appropriately, although the types of tumors requiring testing will undoubtedly expand over time. Indeed, as more potential therapies become available and molecular pathology advances, so will the need for complete acquisition of molecular testing information before selecting the most appropriate treatment. This will likely include greater consideration of why immunotherapy treatments work so dramatically in some patients while not at all in others and how tumors which were initially sensitive to treatment can acquire resistance, such as through evolution of adenocarcinoma to small cell carcinoma after tyrosine kinase inhibitor therapy.

In contrast, mortality from SCLC has declined at a similar rate to decline in incidence. One study reveals that the decrease in SCLC mortality can be explained entirely by a decrease in incidence, as no improvement in survival was observed among patients with SCLC over time.14 Nevertheless, in the same period, significant advances have been made in understanding the molecular biology of SCLC, and although the morphologic criteria remain unchanged, with a new molecular classification proposed, this will hopefully be a driver in initiating new trials in specific subtypes of SCLC, paving potentially the way to a targeted approach, also in SCLC.15

**Small Diagnostic Samples**

Because 70% of lung cancers present in advanced stages and are unresectable, the diagnosis for these patients is based primarily on small biopsy and cytology specimens.16 Therefore, a new classification for lung cancer in small diagnostic samples was introduced in the 2011 IASLC/ATS/ERS Lung Adenocarcinoma Classification16 and adopted in the 2015 WHO Classification.7 These small tumor samples of primary or metastatic lung tumors are obtained through a variety of methods including fine-needle aspiration biopsies and exfoliative specimens, such as sputum, bronchial washings and secretions, bronchial brushings, and bronchoalveolar lavage. Depending on the local expertise of the physicians (pulmonologists, radiologists, surgeons, cytopathologists) who obtain the specimens, the optimal approach will vary from one institution to another. Confirmation of specimen adequacy at the time of the procedure can be aided by rapid on-site evaluation, including telepathology methods,17–21 although this is not essential. Molecular testing including next-generation sequencing can reveal high diagnostic yield in cytology samples with cell blocks or ethanol-fixed smears or liquid-based preparations.22,23 Obtainning multiple biopsy samples that can be put into different paraffin blocks and used separately for immunohistochemical staining versus molecular testing is helpful.

This workflow for managing small biopsy and cytology samples emphasizes the need for making an accurate diagnosis, including specific histologic typing of nonsmall cell carcinoma (NSCC) using ancillary techniques, such as immunohistochemistry,24–27 and highlighting the need for molecular testing.7,16 To spare as much tissue for molecular testing, it is recommended to use only a limited panel of immunohistochemical markers as well as mucin stains to diagnose and subtype NSCC.7
Recommended guidelines for good clinical practice (Table 2) and details of how to classify lung cancers in small biopsies are summarized in other publications and in Tables 3 and 4. It can be helpful to record the adenocarcinoma patterns present in small biopsies or cytology specimens, although unlike resection specimens, documenting relative percentages of the patterns is not recommended. In some clinical circumstances such as stereotactic body radiation therapy or thermal ablation therapy, the presence of a solid and/or micropapillary pattern in core biopsies from patients with lung adenocarcinomas is associated with poor outcomes.

In poorly differentiated NSCCs with a solid pattern where TTF-1 and/or mucin stains are positive but p40 is negative, the diagnosis is “NSCC, favor adenocarcinoma” (Table 3). In cases with both negative TTF-1 and p40, the diagnosis of adenocarcinoma can be supported if mucin stains are positive. It is also possible to identify features of adenocarcinoma on cytology specimens to allow for this diagnosis even if the biopsy result reveals a solid NSCC that is negative for TTF-1, p40, and mucin stains. Therefore, correlation between biopsy and cytology findings may be useful. Indeed, poorly differentiated adenocarcinomas can exhibit prominent eosinophilic cytoplasm suggesting keratinization (pseudosquamoid appearance) making it impossible to distinguish adenocarcinoma with a solid pattern from squamous cell carcinoma on morphology alone. Finally, although immunohistochemistry is required for NSCC lacking morphologic evidence of differentiation, the number of special stains should be minimized to maximize tissue available for molecular testing. In most tumors, staining only for TTF-1 and p40 will allow for a precise diagnosis of adenocarcinoma or squamous cell carcinoma (Fig. 1A and B). Criteria for diagnosis of “NSCC favor squamous cell carcinoma” and “NSCC NOS” are summarized in Table 3. NSCC-NOS may need a cytokeratin to confirm carcinoma and a limited immunohistochemical workup with stains to exclude a metastasis.

Certain diagnoses cannot be made definitively in small biopsies, such as large cell carcinoma, pleomorphic carcinoma, adenosquamous carcinoma, adenocarcinoma in situ (AIS), minimally invasive adenocarcinoma (MIA), and lepidic-predominant adenocarcinoma, as these require a resection specimen. Nevertheless, terminology for use when biopsy suggests one of these entities is provided in Table 4. More often than in the past, a definitive diagnosis of large cell NE carcinoma (LCNEC) can be made in small biopsies because larger tissues are obtained for molecular testing. These larger biopsies make it more readily possible to identify the clear NE morphology and staining with NE immunohistochemical markers required for the diagnosis.

### Table 2. Guidelines for Good Practice of Small Biopsies and Cytologic Preparations

1. For small biopsies and cytology, NSCC should be further classified into a more specific type, such as ADC or SQCC, whenever possible.
2. The term “non-small cell lung carcinoma-NOS (NSCLC-NOS)” should be used as little as possible, and only when a more specific diagnosis is not possible.
3. When a diagnosis is made in a small biopsy or cytology specimen in conjunction with special studies, it should be clarified whether the diagnosis was established on the basis of light microscopy alone or if special stains were required.
4. The term “non-squamous cell carcinoma (non-SQCC)” should not be used by pathologists in diagnostic reports. This categorization is used by clinicians to define groups of patients whose tumors comprise several histological types and who can be treated in a similar manner; in small biopsies/cytology, pathologists should classify NSCLC as ADC, SQCC, NSCLC-NOS, or other terms.
5. The above-mentioned classification of ADC versus other histologies and the terminology in Table 3 and 4 should be used in routine diagnosis, future research, and clinical trials, to ensure a uniform classification of disease cohorts in relation to tumor subtypes, stratified according to diagnoses made by light microscopy alone versus diagnoses requiring special stains.
6. When paired cytology and biopsy specimens exist, they should be reviewed together to achieve the most specific and concordant diagnosis.
7. The terms AIS and minimally invasive ADC should not be used for diagnosis of small biopsies or cytology specimens. If a noninvasive pattern is present in a small biopsy, it should be referred to as a lepidic growth pattern. Similarly, if a cytology specimen has the attributes of AIS, then the tumor should be diagnosed as an ADC, possibly with a comment that this may represent, at least in part, AIS.
8. The term large cell carcinoma should not be used for diagnosis in small biopsy or cytology specimens and should be restricted to resection specimens where the tumor is thoroughly sampled to exclude a differentiated component.
9. In biopsies of tumors that reveal sarcomatoid features (marked nuclear pleomorphism, malignant giant cells, or spindle cell morphology), these should be initially classified as mentioned previously in relation to ADC; NSCC, favor ADC; SQCC; or NSCC favor SQCC, as this is apt to influence management, with additional statement that giant and/or spindle cell features (depending on what feature) are present. If such features are not present, the term NSCC-NOS should be used, again with comment on the sarcomatoid features.
10. Neuroendocrine immunohistochemical markers should be performed only in cases where there is suspected neuroendocrine morphology.

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ADC, adenocarcinoma; AIS, adenocarcinoma in situ; IARC, International Agency for Research on Cancer; NOS, not otherwise specified; NSCC, nonsmall cell carcinoma; SQCC, squamous cell carcinoma
Small Biopsy Samples for Predictive Biomarker Testing

Current international guidelines on the treatment of NSCLC recommends routine testing of patient tumor samples for the presence of driver mutations/fusions in the EGFR, ALK, ROS1, BRAF, V600E, NTRK1-3, RET, KRAS, and MET genes33–35 and PD-L1 expression by immunohistochemistry.33,36 In at least two-thirds of patients who have advanced disease, testing is performed on the diagnostic core needle or cytology specimens.37,38 For molecular testing, studies have reported comparable results in the overall failure rate, mutation rate, or mutation type for EGFR testing performed on biopsies submitted for histology or cytology samples,39–41 although tumor cellularity can affect test-success or mutation rates,39,42,43 emphasizing the importance of preanalytical sample quality assurance step to ensure optimal testing.

Unlike genomic driver alterations which should be present in all tumor cells, PD-L1 protein expression is often heterogeneous within a tumor, and it could be dynamic and influenced by previous treatment.44
NSCLC, with a few exceptions, only the expression of PD-L1 on tumor cells (tumor proportion score; percentage of positive cells expressing membrane staining) is relevant as a predictive biomarker for immune checkpoint inhibitor (ICI) therapy.45 Trial data have revealed that PD-L1 evaluated on archival or fresh biopsy samples yielded comparable clinical outcomes.46 Aside from preanalytical issues, the most important criteria for sample adequacy are the presence of at least 100 assessable tumor cells for PD-L1 assessment. Despite being an imperfect marker to predict clinical benefit from ICI therapy, PD-L1 testing currently remains the most robust predictive marker for first-line ICI.47–50

<table>
<thead>
<tr>
<th>Terminology for Small Biopsies and Cytology Specimens</th>
<th>Terminology for Resection Specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small cell carcinoma</td>
<td>Small cell carcinoma</td>
</tr>
<tr>
<td>Nonsmall cell carcinoma with neuroendocrine morphology and positive neuroendocrine markers, possible large cell neuroendocrine carcinoma</td>
<td>Large cell neuroendocrine carcinoma</td>
</tr>
<tr>
<td>Morphologic squamous cell and adenocarcinoma patterns both present: nonsmall cell carcinoma-NOS</td>
<td>Adenosquamous carcinoma (if both components ≥10%)</td>
</tr>
<tr>
<td>Comment that adenocarcinoma and squamous components are present, and that this could represent adenosquamous carcinoma</td>
<td>Adenocarcinoma, squamous cell carcinoma, adenosquamous carcinoma, or large cell carcinoma with unclear immunohistochemical features</td>
</tr>
<tr>
<td>Morphologic squamous cell or adenocarcinoma patterns not present, but immunohistochemical stains favor separate squamous and adenocarcinoma components: nonsmall cell carcinoma-NOS</td>
<td></td>
</tr>
<tr>
<td>Specify the results of the immunohistochemical stains and the interpretation, and comment that this could represent adenosquamous carcinoma, but that diagnosis requires a resection specimen</td>
<td></td>
</tr>
<tr>
<td>Nonsmall cell carcinoma with spindle cell and/or giant cell carcinoma</td>
<td>Pleomorphic, spindle cell, and/or giant cell carcinoma</td>
</tr>
<tr>
<td>Mention if adenocarcinoma or squamous carcinoma is present. Comment that this could represent a pleomorphic carcinoma; however, that diagnosis requires a resection specimen.</td>
<td></td>
</tr>
</tbody>
</table>


IARC, International Agency for Research on Cancer; NOS, not otherwise specified.

Pathology Reports for Lung Cancer Diagnoses in Small Biopsies and Cytology Specimens

Several recommendations are made for reporting lung cancer diagnoses on the basis of small biopsies and cytology, including the following: (1) a pathologic or cytopathologic diagnosis according to the 2021 WHO Classification; (2) results of immunohistochemical and/or mucin stains; (3) a comment on the differential diagnosis (when appropriate); and (4) a statement whether any material has been submitted for molecular testing. It can be useful to specify which block was used and the percentage of viable tumor cells in the specimen should be documented either by the surgical pathologist or molecular team.

Figure 1. Immunohistochemistry in the subtyping of NSCC: favor adenocarcinoma. (A) This tumor is a poorly differentiated NSCC lacking any glandular or squamous morphology. (B) By immunohistochemistry, the tumor cells stain positively for TTF-1. NSCC, nonsmall cell carcinoma.
Adenocarcinoma

Overview of Changes

The subclassification of adenocarcinomas of the lung has largely remained unchanged since 2015 with invasive adenocarcinomas classified as MIA, invasive non-mucinous adenocarcinoma, invasive mucinous adenocarcinoma (IMA), colloid adenocarcinoma, fetal adenocarcinoma, or enteric-type adenocarcinoma. These are distinguished from the precursor glandular lesions atypical adenomatous hyperplasia or AIS. Invasive non-mucinous adenocarcinomas are the most common subtype of lung cancer and consist of malignant epithelial tumors with morphologic or immunohistochemical evidence of glandular differentiation and not fulfilling criteria for any other type of adenocarcinoma. Morphologic evidence of glandular differentiation consists of lepidic, acinar, papillary, or micropapillary growth patterns. In purely solid tumors, immunohistochemical (TTF-1 or Napsin A) or histochemical (e.g., a D-PAS stain) patterns. In purely solid tumors, immunohistochemical (TTF-1 or Napsin A) or histochemical (e.g., a D-PAS stain) evidence of adenocarcinomatous differentiation is required for diagnosis. For nonmucinous-part lepidic adenocarcinomas, measurement of invasion is now required for staging purposes (with only the size of the invasive component rather than total size contributing to the size T-factor), and a grading system has been introduced for invasive tumors (see subsequent discussion and Table 5). The percentage of each histologic pattern should be recorded in 5% to 10% increments to determine the predominant histologic pattern (subtype) and quantify any patterns to determine the tumor grade (see subsequent discussion). For measurement of invasion, any histologic subtype other than lepidic (acinar, papillary, micropapillary, solid, or less often colloid, enteric, fetal type, or IMA) or foci of tumor cells infiltrating myofibroblastic stroma is included in the assessment for tumors, including when the invasive component is not in a single measurable focus on one slide.

Table 5. Grading of Invasive Nonmucinous Adenocarcinomas

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (well differentiated)</td>
<td>Lepidic predominant with no or &lt;20% high-grade pattern*</td>
</tr>
<tr>
<td>2 (moderately differentiated)</td>
<td>Acinar or papillary predominant with no or &lt;20% high-grade pattern</td>
</tr>
<tr>
<td>3 (poorly differentiated)</td>
<td>Any tumor with &gt;20% high-grade pattern</td>
</tr>
</tbody>
</table>

*Micropapillary, solid, cribriform, and complex glandular patterns.


Grading of Invasive Nonmucinous Adenocarcinoma

Although the current classification of nonmucinous adenocarcinomas has consistently revealed good correlation with prognosis, a formal grading system for lung adenocarcinomas has been lacking. In general, lepidic predominant tumors have a better prognosis than acinar or papillary predominant adenocarcinoma, with micropapillary and solid-predominant tumors having the worst prognosis.51–56 A particular shortcoming in the prognostic value of the “predominant subtype” classification scheme is that “non-predominant” amounts of poor prognostic patterns, especially the micropapillary pattern, have been associated with an adverse outcome even when comprising only a small component of a tumor.57–59 As such, ongoing effort has been devoted to the development of a formal grading system to provide increased prognostic information that incorporates minor amounts of high-grade components, with a threetiered grading system for invasive nonmucinous adenocarcinomas proposed by the IASLC pathology committee.60 This proposed grading scheme is based on a combination of the predominant histologic pattern plus the worst pattern, particularly accounting for the high-grade patterns: micropapillary, solid, cribriform, and complex glandular patterns, if they account for at least 20% of the tumor (Figs. 2–4 and Table 5). This proposed IASLC grading scheme proved superior to models incorporating nuclear or cytologic grade, the presence of STAS, necrosis, or other negative prognostic features in a large validation cohort.60

Tumor STAS

STAS is defined as tumor cells within airspaces beyond the edge of the main tumor (Fig. 5).61 The following features favor artifactual spread of tumor cells over STAS: (1) randomly situated and ragged-edged clusters of tumor cells often at the edge of the tissue section or out of the plane of section of the tissue; (2) lack of continuous spread in airspaces from the tumor edge to the most distant airspace tumor cells; (3) pneumocytes or bronchiolar cells with benign cytologic features and/or presence of cilia; or (4) linear strips of cells that are lifted off alveolar walls.61 Using these criteria, separation of STAS from artifacts was highly reproducible (average kappa 0.857) in a study of selected images.62 The occurrence of STAS does not seem to be affected by gross specimen-handling procedures.63 STAS in adenocarcinoma is composed of three morphologic patterns, including micropapillary structures, solid nests, and discohesive single cells.61 STAS is associated with worse clinical outcome in resected lung adenocarcinoma and all investigated major histologic
lung cancer types. The association of STAS with worse prognosis seems stronger in patients undergoing limited resection compared with those with lobectomy. Because STAS is regarded as a pattern of tumor spread, it is not included in the total percentage of patterns or in tumor size for staging.

IMA and Mucinous Subtypes of AIS and MIA

Under the new WHO classification, invasive adenocarcinoma is divided into two main categories, namely nonmucinous and mucinous types, in addition to the rare variants. Although the nonmucinous type is more frequently encountered in clinical practice, the mucinous type still accounts for 3% to 10% of invasive adenocarcinomas. Historically, the term IMA was first proposed in the IASLC/ATS/ERS classification of adenocarcinoma to avoid confusion with mucinous bronchioloalveolar carcinoma, of which the primary definition was a noninvasive tumor. Indeed, although IMA can show a lepidic growth pattern, invasive patterns are always present. Nevertheless, many but not all IMAs are lepidic predominant. In small tumors less than or equal to 3 cm with similar morphology, mucinous subtypes of AIS or MIA (if the size of the invasive component is <5 mm) can be diagnosed. IMA is histologically defined as adenocarcinoma composed of goblet cell or columnar cell morphology. The immunophenotype is often characterized by CK7+, TTF-1-, CDX2 focal, CK20 focal, and HNF4α+. Differentiation overlapping with that of gastrointestinal epithelium owing to loss of function mutations in NKX2.1, the gene that encodes for TTF-1, is proposed as a possible pathogenesis. Genetically, this tumor is characterized by frequent KRAS mutations (62%–76%, mostly G12D and G12V), followed by NRG1 fusion, ERBB2 alterations (amplification and insertions), and other rare alterations.

Figure 2. Invasive nonmucinous adenocarcinoma. This acinar predominant adenocarcinoma has areas with a solid pattern (top right) comprising greater than 20% of the tumor, making it a grade 3 tumor.

Figure 3. Invasive nonmucinous adenocarcinoma. Micropapillary pattern. (A) Although there are acinar glands, because they are filled with micropapillary adenocarcinoma, this area of the tumor should be classified as micropapillary, not acinar adenocarcinoma. (B) Despite the papillary structures, this area of the tumor should be classified as micropapillary adenocarcinoma owing to the extensive micropapillary pattern that is also present.
(fusions of ALK, ROS1, NTRK1, BRAF, RET, FGFR2/3, and NRG2 and mutations of ERBB3 and BRAF), and low frequencies of EGFR and TP53 mutations, in contrast to invasive nonmucinous adenocarcinomas. Frequent multifocal, multilobar, and bilateral presentation is another characteristic of this tumor, and clonal relationship between these lesions has been found recently, suggesting IMA is susceptible to intrapulmonary spread.

Other Subtypes of Adenocarcinoma

Colloid, fetal, and enteric-type variants are rare subtypes. Colloid and enteric-type adenocarcinomas are proposed to have similar origin as pulmonary IMAs owing to identical molecular profiles with approximately half of the cases harboring KRAS mutations. The presence of more than 50% of the tumor revealing either pools of extracellular mucin or enteric morphology is one of the essential criteria for diagnosis of colloid and enteric adenocarcinoma respectively, if mixed with other patterns of conventional invasive nonmucinous adenocarcinoma. Distinction from metastasis is usually through clinical means, as both variants may be positive for intestinal markers (CDX2, CK20, and Villin) and negative or only focal and weakly positive for pneumocyte markers (TTF-1 and Napsin A).

Abnormalities in β-catenin and the WNT signaling pathway are crucial in the pathogenesis of low-grade or well-differentiated fetal adenocarcinomas making them molecularly distinct from conventional adenocarcinomas. High-grade fetal adenocarcinomas are often admixed with other patterns of adenocarcinomas, and greater than 50% high-grade morphology is required for their diagnosis. Lesser nuclear atypia, presence of morules, and nuclear/cytoplasmic beta-catenin staining distinguish low-grade from high-grade tumors. The latter behave aggressively and have poor prognosis.

Figure 4. Invasive nonmucinous adenocarcinoma. Cribriform (complex glandular) pattern: a cribriform or complex glandular pattern is classified as a high-grade pattern.

Figure 5. Tumor STAS. (A) This adenocarcinoma has tumor cells in airspaces beyond the edge of the main tumor, a feature associated with poor prognosis. (B) At high power, the atypical morphology distinguishes the cells from alveolar macrophages. STAS, spread through air spaces.
Molecular Pathology of Adenocarcinoma

The 2015 WHO classification (fourth edition) reported molecular abnormalities of adenocarcinomas in the section of “Somatic genetics.” The 2021 WHO Classification places molecular abnormalities in the sections covering “Etiology,” “Pathogenesis,” and “Diagnostic molecular pathology.” Many molecular changes have now been reported and are associated with pathogenesis, progression, and, importantly, treatment of adenocarcinoma.

Molecular characterization of lung adenocarcinoma identifies oncogenic driver mutations, some of which are relatively unique to lung adenocarcinoma (EGFR exon 19 deletions and exon 21 point mutations, EML4-ALK translocations) with several associated targeted therapeutic agents. Targetable alterations with matched approved agents include point mutations, in-frame deletions, and exon 21 point mutations, in particular EGFR mutations.103,93,94 and emerging targetability in ERRB297 and KRAS.98 Although these alterations are generally used in guiding therapy for patients with advanced stage,94 use of EGFR targeting has been expanding to patients with earlier stage.100 Other alterations that can be found in lung adenocarcinomas alone or in combination with other mutations, including TP53, STK11, and KEAP1, are not currently directly targetable but may be associated with tumor progression and resistance to ICI for STK11.101,102 Evaluation of tumors for clinically relevant alterations is listed as a desirable criterion in this 2021 WHO classification, highlighting its increasing integration into the classification hierarchy.

The frequency of these alterations in lung adenocarcinoma has been studied by sex, age, smoking status, and geographic regions. KRAS mutations, in particular transversion-type mutations, are found in cigarette smokers,103 whereas EGFR mutations and ALK, ROS1, and RET translocations are more likely found in light or never smokers. Other alterations such as TP53,104 NRAS,105 and MAP2K1106 are also more common in smokers; BRAF and MET are found in both smokers and nonsmokers; EGFR alterations are found more often in young patients and women, whereas ALK, ROS1, and RET alterations are more common in young patients but without sex predilection. In addition, it is evident that EGFR mutation is more frequent in East Asia and KRAS mutation in U.S./European populations.107 Other alterations have different frequencies as well, whereas some do not seem to vary geographically108-114 (Table 6). It is acknowledged that these epidemiologic associations are not absolute and as such should not determine testing.

Although histologic subtypes should not drive selection for molecular testing, some histologic patterns are more often associated with particular alterations. IMA115 and solid-predominant adenocarcinoma116 are most often KRAS-mutated tumors; specific translocations involving NRG1 are also found in IMA (see previous discussion). Well-differentiated fetal adenocarcinoma is associated with mutation in CTNNB1.82 Adenocarcinomas with a nonmucinous lepidic and papillary pattern and those that are TTF-1 positive are more likely to harbor EGFR mutations. ALK translocations and ROS1 and RET117,118 translocations are found with cribriform/solid and signet ring pattern.119,120

Oncogenic drivers are generally mutually exclusive within a particular tumor in the untreated setting. It is postulated that, when mutually exclusive, these drivers are early events in tumor formation; these alterations are found in early lesions, including atypical adenomatous hyperplasia121,122 and AIS,103,104 supporting this viewpoint. In addition, this mutual exclusivity of molecular alterations may provide supportive evidence for staging of independent primaries.76,123,124

Testing is also relevant to the first-line use of ICIs, which is precluded by the presence of EGFR mutations.125 As with EGFR mutation, ALK translocations preclude first-line therapy with ICIs.125 Although other oncogenic alterations may be associated with reduced response to ICIs, they do not affect decisions in first-line therapy with ICI.126 For example, alterations in STK11/LKB1 may also lead to PD-1 inhibitor resistance127 but do not currently preclude their first-line use.

<table>
<thead>
<tr>
<th>Gene Altered</th>
<th>East Asia (%)</th>
<th>USA/Europe (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGFR</td>
<td>40-59</td>
<td>5-19.4</td>
</tr>
<tr>
<td>ALK</td>
<td>3-7</td>
<td>3-6</td>
</tr>
<tr>
<td>ROS1</td>
<td>1-3</td>
<td>1-2</td>
</tr>
<tr>
<td>ERRB2</td>
<td>2-3</td>
<td>2-3</td>
</tr>
<tr>
<td>RET</td>
<td>1-2.2</td>
<td>1-2</td>
</tr>
<tr>
<td>BRAF V600E</td>
<td>0.5-1</td>
<td>2.3</td>
</tr>
<tr>
<td>Met ex14</td>
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<td>3</td>
</tr>
<tr>
<td>NTRK1/2/3</td>
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</tr>
<tr>
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</tr>
<tr>
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<td>1.2</td>
</tr>
<tr>
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<td>KEAP1</td>
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<td>15</td>
</tr>
<tr>
<td>PIK3CA</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>CTNNB1</td>
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<td>2.5</td>
</tr>
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<td>PTEN</td>
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<td>2</td>
</tr>
<tr>
<td>NF1</td>
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<td>1.9</td>
</tr>
<tr>
<td>TSC 1/2</td>
<td>&lt;2</td>
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</tr>
<tr>
<td>FGFR1/2</td>
<td>NA</td>
<td>0.7</td>
</tr>
</tbody>
</table>

ex14, exon 14; NA, not applicable; USA, United States of America.
NE Neoplasms

Framework of NE Tumor Classification

The 2021 WHO classification categorizes NENs of the lung as a single group of tumors, which includes low- and intermediate-grade typical carcinoid (TC) and atypical carcinoid (AC), respectively, and the high-grade NE carcinomas (NECs), including LCNEC and SCLC. Nevertheless, it is recognized that TC and AC are clinically, epidemiologically, histologically, immunohistochemically, and genetically very different from digestive and pancreatic NENs. Additionally, it is recognized that TC and AC may also be combined with LCNEC and is classified under the term “combined SCLC and LCNEC.” The classical terminology used for lung and thymus was, however, retained, where TC and AC generally correspond to the well-differentiated categories of NETs grades 1 and 2 and poorly differentiated LCNEC and SCLC correspond to grade 3 NEC.

The current classification also retains the categories of combined LCNEC and combined SCLC which may comprise up to 25% of the resected cases. Both categories include either LCNEC or SCLC combined with a NSCC component, most often adenocarcinoma or squamous cell carcinoma. A case revealing foci of both SCLC and adenocarcinoma would therefore be termed “combined SCLC and adenocarcinoma.” SCLC may also be combined with LCNEC and is classified under the term “combined SCLC and LCNEC.” In the lung, combined tumors generally occur in the setting of high-grade carcinomas and not in carcinoids. This differs from digestive and pancreatic NENs, where both high-grade and low-grade tumors can contain an exocrine glandular component, termed “mixed neuroendocrine/nonneuroendocrine neoplasm.” Although the overall nomenclature is essentially unchanged in the 2021 classification, there are several clinically relevant updates. These relate to the terminology for biopsy, cytologic, or metastatic specimens, to cell proliferation assessment and role of specific markers, and to the potential role of genetic data in the subtyping of NEN categories.

Carcinoid Not Otherwise Specified and Metastatic Carcinoids—Criteria and Terminology

The diagnostic criteria of pulmonary NEN subtypes are based on morphology alone, with mitoses and presence of necrosis representing the mainstay for classification. These criteria have been validated in surgical specimens, because most NENs undergo resection, with the exception of SCLC. Nevertheless, accurate mitotic counts and recognition of necrosis are often difficult in small biopsies or cytologic specimens, because the mitotic count can be heterogeneous. Therefore, the recommendation was made to use the term “carcinoid tumor not otherwise specified (NOS)” for small samples from primary or metastatic NETs and to record the mitotic count and the presence of any foci of necrosis (see criteria discussed subsequently) with a comment if the findings raise concern for AC. Although Ki-67 proliferation index is not an essential criterion in the 2021 WHO Classification, for carcinoid tumors, it is introduced as a desirable criterion, and it may be useful to incorporate this into the pathology reports. This is of importance especially when evaluating a metastatic carcinoid specimen, to support clinical decisions, because medical oncologists are accustomed to the use of Ki-67 index to stratify therapeutic options of progressing digestive and pancreatic NENs. Metastatic pulmonary (and thymic) carcinoids are treated with similar regimens and Ki-67 index may be requested in some circumstances. In metastatic carcinoids, the Ki-67 index may in rare cases exceed standard criteria, causing differential diagnosis problems with high-grade LCNEC and representing a challenge for therapeutic decisions.

Assessing Proliferation: Mitotic Count and Role of Ki-67.

As already detailed in the 2015 WHO Classification, a careful mitosis count is crucial, being the most important morphologic criterion for discriminating between TC and AC, and between carcinoids (NETs) and high-grade NECs. The current WHO edition recommends that mitoses should be counted in areas of highest activity and reported as number of figures per 2 mm² (not 10 high-power fields, to avoid discrepancies owing to the different microscope models). In addition, it was stated that in tumors with borderline values, near the expected cutoffs, three 2-mm² areas should be counted and the mean value reported; finally, only definite mitotic figures should be counted, excluding questionable ones, particularly those with features more characteristic of pyknotic cells, such as cytoplasmic eosinophilia, presence of nuclear membrane, and triangular or spiky nuclear shape.

The Ki-67 index is useful in separating carcinoids from high-grade tumors, including SCLC and LCNEC in
biopsies with extensive crush artifact. Nevertheless, no role for Ki-67 was otherwise accepted for NEN classification, given the absence of validated rules in lung tumors for its scoring, conflicting literature on its prognostic usefulness, and lack of reproducible cutoffs for TC versus AC and carcinoids versus LCNEC and SCLC. Currently published data report mean Ki-67 values generally below 5% for TC and ranging from 9% to 18% for AC, with rare carcinoid cases exceeding these values (see subsequent discussion). A Ki-67 index exceeding 30% is usually indicative of a high-grade LCNEC or SCLC.

Because of these controversial data, diagnostic criteria of pulmonary (and thymic) NENs remain different from those of their digestive and pancreatic NEN counterparts, where Ki-67 index is an integral part of the diagnostic parameters for stratifying NETs and carcinomas in three grades. Nevertheless, the current thoracic tumor classification mentions Ki-67 index determination among desirable criteria. However, its role in supporting clinical decisions remains to be defined.

Carcinoid Tumors With High Proliferation. In recent years, several papers have reported small series of NENs characterized by the well-differentiated, organoid morphology of carcinoids, but an elevated proliferative activity as documented by a mitotic count exceeding 10 per 2 mm², thus meeting the criteria for a diagnosis of LCNEC rather than carcinoid (Fig. 6A). These cases also had a high Ki-67 index (Fig. 6B), exceeding that usually observed in carcinoid tumors (>20% to 30%), particularly in their metastases, when those were available for testing. Although recognized in the 1999 WHO Classification, these cases are so uncommon, after 20 years and three subsequent WHO Classifications, the data are only sufficient to mention as an emerging observation rather than a formally recognized entity. The exact classification of these rare tumors with carcinoid morphology is still controversial. They correspond to the new entity called grade 3 NET in the digestive and pancreatic tracts, where these tumors seem to be more common. Evidence suggests that, in the lung, these share the molecular profile of carcinoids rather than that of LCNEC or SCLC. Because the clinical behavior and response to chemotherapy seems to be different from that of high-grade NEC, it is clinically relevant to identify these rare NEN subtypes. The current recommendation is to label such tumors as LCNEC (on the basis of the high mitotic count), but then add a note specifying the carcinoid morphology and the Ki-67 index, if available. More investigation of these tumors is needed with careful pathologic, clinical, and genetic data in the context of other NETs. This is an ongoing area of investigation within the IASLC pathology committee.

Combined SCLC and LCNEC

These can occur “de novo” or in adenocarcinomas, where a SCLC develops as a manifestation of resistance to specific therapies, such as EGFR tyrosine kinase inhibitors.

Nonsmall Cell Carcinoma With NE Differentiation

NE differentiation can be detected by immunohistochemistry in adenocarcinomas or squamous cell carcinomas lacking any NE morphology in 10% to 20% of cases. Nevertheless, this finding does not bear any

![Figure 6. Large cell neuroendocrine carcinoma, with carcinoid-like morphology. (A) This tumor has a carcinoid morphology with organoid nesting and finely granular nuclear chromatin. Nevertheless, the mitotic rate averaged 16 per 2 mm², which is greater than the upper threshold of mitotic counts for atypical carcinoid, resulting in the diagnosis of LCNEC. (B) The Ki-67 index was 20%. LCNEC, large cell neuroendocrine carcinoma.](image-url)
clinical impact,\textsuperscript{159,160} and the routine use of immuno-histochemistry in pathology practice is not recommended in the absence of a NE morphology.

**Emerging Genetic Data and Concepts**

Major advances are emerging from genetic and molecular studies, especially in the group of high-grade small and LCNECs. Although the current WHO Classification did not introduce a “molecular classification” of pulmonary NENs, the book highlights several areas that may inform the next edition. These include the following:

1. Although all NENs of the lung have been incorporated in a single spectrum of neoplasms since the 2015 WHO Classification, major clinical, epidemiologic, pathologic, and genetic differences are recognized between the carcinoid tumors (NETs) and the high-grade LCNEC and SCLC (NECs).

2. Even within each single NEN category, there is variation in molecular alterations, mostly in intermediate (AC) and high-grade (LCNEC and SCLC) types. High-grade carcinomas have more genetic alterations than carcinoids, including mutations and amplifications.

3. Carcinoids are characterized by chromatin-remodeling gene abnormalities in up to 40\% of cases and by \textit{MEN1}, \textit{PSIP1}, and \textit{ARID1A} gene mutations in a lower fraction of cases, in the absence of the classical SCLC gene alterations (\textit{TP53/RB1} mutations).\textsuperscript{149,161,162} In a comparative genomic and transcriptomic analysis, several molecular clusters were identified,\textsuperscript{163} and rare tumors having LCNEC morphology may also contain carcinoid-related mutations (\textit{MEN1}).\textsuperscript{149}

4. SCLC is consistently associated with biallelic \textit{TP53} and \textit{RB1} gene inactivation, but this does not mean that this tumor type is molecularly homogeneous. In fact, recent transcriptional data revealed intratumoral heterogeneity and identified at least four gene expression subtypes,\textsuperscript{164} two of them with predominant \textit{ASCL1} and \textit{NEUROD1} gene alterations and two others with anomalies of genes \textit{POU2F3} and \textit{YAP1}. The former two were associated with a prominent NE differentiation, whereas the latter, despite sharing a small cell morphology, do not express NE markers and rather correspond, at least in part, to the group of chemotherapy-resistant SCLCs. Clinical correlates with these subtypes are still incompletely established, but recent findings helped to better define these subgroups.\textsuperscript{165–168} In fact, it seems that the \textit{YAP1} subtype is not a separate group but overlaps with the other three. Conversely, a fourth SCLC subtype exists, defined by the absence of \textit{ASCL1}, \textit{NeuroD1}, and \textit{POU2F3} mutations (“triple negative” SCLC) with a marked “inflamed” profile, as defined by the expression of immune checkpoint molecules and T lymphocyte markers; this seems to be associated to resistance to SCLC chemotherapy regimens.\textsuperscript{167–170}

5. LCNECs have a heterogeneous molecular profile and at least three subgroups exist, the smallest of which encompasses \textit{MEN1} mutated cases that most probably represent carcinoids with high proliferation rates. The other two subgroups comprise most LCNECs and include a cluster with \textit{TP53} and \textit{RB1} gene mutations as observed in SCLC, and a subgroup with \textit{KRAS} and \textit{STK11/KEAP1} mutations, as that occurred in NSCLC. This subtyping may have relevant clinical implications, because NSCLC-like type can have a different response to classical SCLC chemotherapy regimens.\textsuperscript{149,157,161–163,171–173}

**Squamous Cell Carcinoma and NUT Carcinoma**

Only a few minor adjustments were made to the classification of squamous cell carcinoma. To discourage its use, “epidermoid carcinoma” is no longer recommended as a related terminology. Basaloid carcinoma remains a subtype of squamous cell carcinoma of the lung, but it is integrated within the same chapter together with the keratinizing and nonkeratinizing subtypes. Lymphoepithelial-like carcinoma, which in the fourth edition was under “other and unclassified carcinomas,” is now renamed as lymphoepithelial carcinoma and classified as a type of squamous cell carcinoma with diffuse positive staining for CK5/6, p40, p63, distinct syncytial growth pattern, variable lymphoplasmacytict infiltrate, and frequent association with Epstein-Barr virus (Fig. 7A). The demonstration of presence of EBER1 by in situ hybridization is regarded as desirable diagnostic criteria, as EBV-negative lymphoepithelial carcinoma occurs, especially among patients of European descent. In contrast, EBER1 positive staining is found in more than 90\% of cases among Asian patients (Fig. 7B).

Unlike lung adenocarcinoma, there has not been major discovery in the molecular characterization of lung squamous cell carcinoma. Therapeutically targetable driver mutation remains elusive for patients with squamous cell carcinoma, but they may benefit from anti-programmed cell death protein-1/PD-L1 therapy, alone or in combination with chemotherapy.\textsuperscript{33} Nevertheless, \textit{EGFR} and \textit{MET} mutations, and \textit{ALK} or \textit{ROS1} rearrangements, can occur in lung squamous cell carcinoma, especially among young never-smoking patients with squamous histology; thus, molecular testing for these driver mutations is essential in this clinical group.\textsuperscript{37}
NUT carcinoma is another tumor that usually, although not always, has squamoid differentiation and P40 positivity. Although it may be primary to the lung, it is discussed under thymic tumors as NUT carcinoma of the thorax. Relevant investigations for direct or indirect detection of NUTM1 gene rearrangements, including immunohistochemistry, should be considered in patients with malignant lung tumors that often have small cell morphology and may reveal evidence of squamoid morphology, especially when the clinical features are atypical for squamous cell carcinoma (young age, light or never-smoking history) (Fig. 8A and B).

### BA/CMPT and Its Differential Diagnosis

#### Definition and Key Histologic Features

BA/CMPT is a novel entity in the 2021 WHO Classification, recognized as a type of pulmonary adenoma. BA/CMPT was first described under the designation of CMPT. Recently, it was suggested that CMPT represents prominent mucinous/papillary morphology among lesions with broader morphologic spectrum recapitulating bronchiolar differentiation, thus prompting an umbrella term of BA to encompass the family of lesions with classic morphology or CMPT and those lacking prominent mucinous, papillary, and ciliated features. The defining features of BA/CMPT

![Figure 7. Lymphoepithelial carcinoma. (A) The tumor comprises sheets of morphologically undifferentiated NSCC. (B) There is diffuse positive nuclear staining for Epstein-Barr virus (EBER ISH stain). NSCC, nonsmall cell carcinoma.](image)

![Figure 8. NUT carcinoma. This NUT carcinoma has sheets and nests of poorly differentiated tumor cells with small squamous nests (arrows). (A) The inset reveals positive nuclear staining with immunohistochemistry for the NUT antibody. (B) Immunohistochemistry for P40 reveals diffuse positive staining.](image)
include nodular proliferation along the alveolar lung parenchyma of bland bilayered bronchiolar-type epithelium, containing a continuous layer of basal cells surrounding luminal cells. Luminal cells can range in the differentiation from those resembling proximal airways, and thus containing abundant ciliated and/or mucinous cells (Fig. 9A and B), and those resembling distal airways, and thus containing predominantly cuboidal, TTF-1-positive cells, with only scant or absent ciliated and mucinous cells (Fig. 9C and D). Although proximal-type lesions tend to be prominently papillary, distal-type lesions tend to be entirely flat or only focally papillary. The distinction of peripheral and proximal BA/CMPT is only proposed as an aid for recognizing their morphologic spectrum, but this distinction is not required for the diagnosis, in particular given that some lesions have a mixture of histologic features along the spectrum of proximal-distal differentiation.

Another common morphologic feature in BA/CMPT is the presence of micropapillary tufts containing ciliated cells.176–178 In addition, these lesions can have a limited discontinuous spread along alveolar walls, closely mimicking skip lesion found in IMA176–178 Central scarring and disruption of elastic fibers may be present. These findings should not be regarded as features of malignancy.
Although generally BA/CMPT can be diagnosed on hematoxylin and eosin, immunohistochemistry can be helpful, particularly in core biopsies. Basal cell markers (p63/p40 or CK5/6) are the key diagnostic aid by highlighting the presence of continuous basal layer (Fig. 9E).

Molecular Features

Molecularly, BA/CMPT exhibits a highly distinctive profile, characterized by a single driver alteration, most often involving BRAF V600E (Fig. 9F). Other reported alterations include noncanonical EGFR exon 19 deletions, EGFR exon 20 insertions, and alterations involving KRAS, HRAS, ALK, and AKT1. The spectrum of molecular alterations is overall similar in distal and proximal BAs (the latter corresponding to the classic CMPT), supporting their nosologic relationship.

Clinical Features

Clinically, BA/CMPT presents as incidental lesions, detected by computed tomography imaging as peripheral lung nodules, which may be solid or ground-glass, with some having cavitation. The lesions are typically small, most often measuring 0.5 to 1.5 cm. Patients are middle-aged to elderly, with a median age of 72 years, without a sex predilection. All patients with BA/CMPT reported to date have remained free of disease after surgical resection.

Differential Diagnosis

Given their benign nature, the most important differential diagnostic consideration for BA/CMPT is that of adenocarcinoma. In particular, for proximal-type BA/CMPT, with prominent mucinous features and peripheral skip lesions, the differential diagnosis includes IMA. The challenge of distinguishing CMPT/BA from adenocarcinoma, especially IMA, during intraoperative consultation has been highlighted in several studies, because the presence of cilia and basal cells may be subtle in frozen sections.

Proximal-type BA/CMPT could be morphologically indistinguishable from glandular/mixed papillomas, with the exception that the former is located in the alveolar lung parenchyma whereas the latter is endobronchial. A potential nosologic relationship between BA/CMPT and bronchial papilloma has been suggested, but it requires further study. Peripheral BA/CMPT is distinguished from peribronchiolar metaplasia by the fact that they present as discrete nodules.

SMARCA4-Deficient Undifferentiated Thoracic Tumor

This newly added entity to the fifth edition is a high-grade malignant neoplasm featuring undifferentiated or rhabdoid phenotype and deficiency of SMARCA4 (also known as BRG1), a key member of the BAF (SWI/SNF) chromatin-remodeling complex. The disease was previously known as “SMARCA4-deficient thoracic sarcoma.” Nevertheless, recent molecular evidence suggests its close genomic relationship with smoking-related NSCLC and most examples are thus increasingly reinterpreted as dedifferentiated/undifferentiated lung carcinoma. This has led to the decision that the entity be renamed and placed under the category of “other epithelial tumors of the lung” with some reservations that alternative pathogenetic pathways may exist in a minority of cases. Nonetheless, the WHO classification recognizes thoracic SMARCA4-deficient undifferentiated tumor (SMARCA4-UT) as a separate entity from conventional NSCLC with SMARCA4 deficiency because of distinct phenotype.

Thoracic SMARCA4-UT often affects young to middle-aged adults with a striking male predilection and heavy smoking history. The tumor presents as a large compressive mass in the mediastinum, hilum, lung, and/or pleura, associated with metastases. Because tumors with a similar phenotype could arise elsewhere (e.g., abdomen) and metastasize to the thorax, clinical correlation is mandatory. Thoracic SMARCA4-UT is a highly aggressive tumor, with a median overall survival of 4 to 7 months. They are generally nonresponsive to cytotoxic chemotherapy.

The histology consists of diffuse sheets of variably discohesive, large, round to epithelioid cells with vesicular chromatin and prominent nucleoli. The nuclei are relatively monotonous, with occasional cells displaying mild pleomorphism. Rhabdoid cells may be present, but they are not required for the diagnosis. Overall morphologic spectrum is reminiscent of that found in pediatric malignant rhabdoid tumors. Unequivocal epithelial architecture (e.g., glandular or squamous differentiation) should be absent, except rare, combined cases in which conventional NSCLC is juxtaposed. SMARCA4 (BRG1) immunohistochemical expression is lost or severely reduced. SMARCA2 (BRM) staining is lost in most cases, with many expressing CD34, SOX2, and/or SALL4. Cytokeratin is often expressed in a focal/weak manner and may be entirely negative, and claudin-4 is negative or only focally positive (Fig. 10A and B).

The tumor is driven by biallelic inactivation of SMARCA4, but sequencing is not necessary for the diagnosis if SMARCA4 deficiency is confirmed immunohistochemically. Up to 44% of the cases have additional
mutations in Kras, Stk11, and/or Keap1, which are common drivers of smoking-associated NSCLC. Most tumors also have genomic smoking mutation signatures and high tumor mutation burden, similar to NSCLC. Nevertheless, the transcriptional profiles of thoracic SMARCA4-UT are distinct from those of SMARCA4-deficient conventional NSCLC but similar to those of malignant rhabdoid tumor.

Approximately 5% of conventional NSCLC are deficient for SMARCA4; however, thoracic SMARCA4-UT is considered as a distinct entity from SMARCA4-deficient conventional NSCLC because it has significant histologic, immunohistochemical, clinical, and prognostic differences. SMARCA4-UT lacks epithelial architecture (e.g., glands) and diffuse strong keratin expression, unlike conventional NSCLC. Ancillary marker profiles (SMARCA2, Claudin-4, CD34, SALL4, and SOX2) can also be helpful for distinction, though none of these markers are entirely sensitive or specific. Importantly, routine SMARCA4 staining is not recommended in conventional NSCLC, but only in cases where SMARCA4-UT is suspected.

Other Tumors

Hyalinizing Clear Cell Carcinoma

In this edition of the classification, hyalinizing clear cell carcinoma has been added to the group of salivary gland-type tumors. Although rare, this is sufficiently well described within the airways to warrant inclusion. These tumors have EWSR1-ATF1 gene fusions and this book maintains the term “hyalinizing clear cell carcinoma” whereas the WHO Classification of Head and Neck Tumors has changed the name to just clear cell carcinoma, as it is important to maintain the distinction between this tumor and NSCLCs with clear cell morphology.

Melanoma and Meningioma

In the 2015 WHO Classification, a group termed “Tumors of ectopic origin” was created to encompass tumors more often presenting at other sites but very occasionally presented as primary lung tumors, possibly arising from nests of ectopic tissue. These were melanoma, thymoma, and meningioma. Any sample classified as one of the above-mentioned tumors in a lung biopsy warrants intense clinical review for an occult primary at more common sites, before assigning as a primary pulmonary tumor, even in the context of benign looking cytology (e.g., benign metastasizing meningioma), but rare primary cases are well described. In the 2021 WHO Classification, the category still remains, although thymomas are discussed in a separate chapter. Furthermore, for melanoma, recent data suggest that, even though there is no identifiable primary elsewhere, purported lung melanomas still carry a mutational signature characteristic of sun damage. This suggests that they may be metastatic from regressed primary skin tumors, although the possibility that some melanomas may arise from the lung airways is not completely excluded.

Inflammatory Myofibroblastic Tumor

Although Table 1 summarizes 10 mesenchymal tumors specific to the lung, the mesenchymal tumor where there have been most genetic advances is the inflammatory myofibroblastic tumor (IMT) which was placed in the “Mesenchymal Tumors of the Thorax” chapter. In contrast to extrapulmonary sites, fusions can be found in a high percentage of pulmonary IMTs. ALK rearrangements are most frequent, and most of these can be detected by immunohistochemistry with the D5F3 or 5A4 antibodies. If immunohistochemistry is negative for ALK, further search for fusions by

Figure 10. Thoracic SMARCA4-UT. (A) Thoracic SMARCA4-UT reveals diffuse proliferation of relatively uniform, variably discohesive, epithelioid cells with prominent nucleoli. (B) Immunohistochemistry reveals loss of SMARCA4 expression. SMARCA4-UT, SMARCA4-deficient undifferentiated tumor.
immunohistochemistry (with ROS1 or pan-NTRK antibodies) and additional molecular testing (such as fluorescence in situ hybridization, multiplex RNA sequencing, or NanoString Assay) may help to detect fusions in virtually all pulmonary IMTs.196

Future Work

This review highlights that, although there has been relatively little change in the morphologic definitions of most tumors, there has been significant increase in the intrinsic detail, particularly in relation to molecular advances. These data are the template for identifying future directions for work that might improve the classification, in terms of accuracy, reproducibility, and relevance to patient management. Examples include (1) validation of the proposed IASLC grading system of resected adenocarcinomas with development of grading for resected squamous cell carcinomas and NSCLC in small biopsies; (2) refining the classification of NENs with further study of ACs with high mitotic or proliferation rates; (3) better understanding of the clinical significance of the morphologic and genetic spectrum of both SCLC and LCNEC; and (4) expansion of the role of diagnostic molecular testing for more tumor types.

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Supplementary Data

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