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Diagnosis of Lung Cancer in Small Biopsies and Cytology:

Implications of the 2011 International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society Classification

William D. Travis, MD, **Elisabeth Brambilla, MD**, **Masayuki Noguchi, MD**, **Andrew G. Nicholson, DM**, **Kim Geisinger, MD**, **Yasushi Yatabe, MD**, **Yuichi Ishikawa, MD**, **Ignacio Wistuba, MD**, **Douglas B. Flieder, MD**, **Wilbur Franklin, MD**, **Adi Gazdar, MD**, **Philip S. Hasleton, MD**, **Douglas W. Henderson, MD**, **Keith M. Kerr, MD**, **Iver Petersen, MD**, **Victor Roggli, MD**, **Erik Thunnissen, MD**, and **Ming Tsao, MD**

Department of Pathology, Memorial Sloan Kettering Cancer Center, New York, New York (Dr Travis); Service de Pathologie Cellulaire, Centre Hospitalier Universitaire de Grenoble, Grenoble, France (Dr Brambilla); the Department of Pathology, Institute of Basic Medical Sciences, University of Tsukuba, Tsukubashi, Japan (Dr Noguchi); the Department of Pathology, Royal Brompton Hospital, London, United Kingdom (Dr Nicholson); the Department of Pathology, Wake Forest University/Medicine, Winston-Salem, North Carolina (Dr Geisinger); the Department of Pathology and Molecular Diagnostics, Aichi Cancer Center, Nagoya, Japan (Dr Yatabe); the Department of Pathology, JFCR Cancer Institute, Tokyo, Japan (Dr Ishikawa); the Departments of Pathology and Thoracic/Head and Neck Medical Oncology, University of Texas MD Anderson Cancer Center, Houston (Dr Wistuba); the Department of Pathology, Fox Chase Cancer Center, Philadelphia, Pennsylvania (Dr Flieder); the Department of Pathology, University of Colorado Health Sciences Center at Fitzsimons, Aurora (Dr Franklin); the Department of Pathology, Hamon Cancer Center, UT Southwestern Medical Center, Dallas, Texas (Dr Gazdar); the Department of Pathology, Wythenshawe Hospital, Manchester, United Kingdom (Dr Hasleton); the Department of Pathology, Flinders Medical Centre, Adelaide, Australia (Dr Henderson); the Departments of Pathology, Aberdeen Royal Infirmary, and Pulmonary Pathology, Aberdeen University Medical School, Aberdeen, Scotland, United Kingdom (Dr Kerr); the Institute of Pathology, Universitätsklinikum, Friedrich-Schiller-University, Jena, Germany (Dr Petersen); the Department of Pathology, Duke University Medical Center, Durham, North Carolina (Dr Roggli); the Department of Pathology, VU Medical Center, Amsterdam, the Netherlands (Dr Thunnissen); and the Department of Pathology, Princess Margaret Hospital, Toronto, Ontario, Canada (Dr Tsao)

Abstract

The new International Association for the Study of Lung Cancer/American Thoracic Society/ European Respiratory Society lung adenocarcinoma classification provides, for the first time, standardized terminology for lung cancer diagnosis in small biopsies and cytology; this was not

Reprints: William D. Travis, MD, Department of Pathology, Memorial Sloan Kettering Cancer Center, 1275 York Ave, New York, NY 10021 (travisw@mskcc.org)..

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primarily addressed by previous World Health Organization classifications. Until recently there have been no therapeutic implications to further classification of NSCLC, so little attention has been given to the distinction of adenocarcinoma and squamous cell carcinoma in small tissue samples. This situation has changed dramatically in recent years with the discovery of several therapeutic options that are available only to patients with adenocarcinoma or NSCLC, not otherwise specified, rather than squamous cell carcinoma. This includes recommendation for use of special stains as an aid to diagnosis, particularly in the setting of poorly differentiated tumors that do not show clear differentiation by routine light microscopy. A limited diagnostic workup is recommended to preserve as much tissue for molecular testing as possible. Most tumors can be classified using a single adenocarcinoma marker (eg, thyroid transcription factor 1 or mucin) and a single squamous marker (eg, p40 or p63). Carcinomas lacking clear differentiation by morphology and special stains are classified as NSCLC, not otherwise specified. Not otherwise specified carcinomas that stain with adenocarcinoma markers are classified as NSCLC, favor adenocarcinoma, and tumors that stain only with squamous markers are classified as NSCLC, favor squamous cell carcinoma. The need for every institution to develop a multidisciplinary tissue management strategy to obtain these small specimens and process them, not only for diagnosis but also for molecular testing and evaluation of markers of resistance to therapy, is emphasized.

A new lung adenocarcinoma classification has recently been published under the joint sponsorship of the International Association for the Study of Lung Cancer (IASLC), the American Thoracic Society (ATS), and the European Respiratory Society (ERS).[1] This is 1 of 2 articles that highlight major pathology-related implications of the new classification, as there are many paradigm shifts that will impact pathologists in the diagnosis and management of specimens for lung cancer.[2] As there are very different issues related to small biopsies and cytology specimens (Tables 1 and 2; Figure 1) versus resection specimens, it seemed best to address these topics in 2 separate articles.

Because 70% of lung cancers are unresectable as patients present in advanced stages, small biopsy and cytology specimens are the primary method of diagnosis for the majority of lung cancer patients. Also, prior World Health Organization (WHO) classifications primarily addressed re-section specimens,[3,4] so they did not propose standardized terminology and criteria for small biopsies and cytology. Therefore, this article addresses one of the most important aspects of this classification. Although the IASLC/ATS/ERS classification primarily addressed lung adenocarcinoma, because no formal terminology or criteria were proposed for small biopsies and cytology, this classification provides for the first time a proposed set of terms and criteria for all major histologic types of lung cancer in these types of specimens.

MAJOR CHANGES IN PATHOLOGY ARE DRIVEN BY ADVANCES IN THORACIC ONCOLOGY

Largely driven by therapeutic advances, a revolution is taking place in the lung cancer field that has major implications for pathologic diagnosis and tissue management. The new IASLC/ATS/ERS classification was developed by an international multidisciplinary panel

including pathologists, medical oncologists, respiratory physicians, radiologists, molecular biologists, and thoracic surgeons to address some of these issues.[1] It also was based on a systematic review to weigh evidence and make recommendations (Table 3).[1,5] In this document, the evidence-based recommendations are listed with the strength of the recommendation and quality of the evidence according to the grades of recommendation, assessment, development, and evaluation method (Table 3).[6] In addition, some recommendations are provided for good clinical practice (Table 4). Some research recommendations are also made in areas of uncertainty (Table 5). For this article, we have selected the recommendations taken from the main classification publication that are pertinent to the diagnosis of lung cancer in small biopsy and cytology specimens.

Multidisciplinary Approach Is Required for Lung Cancer Diagnosis

Many of the new concepts presented in this classification are the direct result of the multidisciplinary approach, which includes clinicians, molecular biologists, radiologists, and surgeons and pathologists. One of the central proposals in this classification is that lung cancer diagnosis is now clearly a multidisciplinary problem. All specialists involved with the diagnosis of lung cancer patients need to work closely together to achieve the correct diagnosis and to obtain appropriate and sufficient tissue for molecular testing.

Each institution must have a multidisciplinary strategy that addresses how to best obtain these small specimens, how to process them in the pathology laboratory, how to preserve material for molecular testing, sending specimens to the molecular laboratory for expedited testing, and reporting the results in a pathology report. It is useful to have a multidisciplinary committee to develop this strategy and to keep lines of communication open in order to monitor issues as they arise in an ongoing fashion. Pathologists should take a leadership role in this process. Because there are widely varying institution-specific issues, this should be set up at a local level.

Personalized Medicine in Lung Cancer Is Driven by Histologic Cell Type and Genetics

Now that lung cancer therapy is becoming personalized for individual patients based on the histologic cell type and subtypes of lung cancer (adenocarcinoma versus squamous) and molecular status (ie, epidermal growth factor receptor [*EGFR*] mutation and anaplastic lymphoma kinase [ALK] rearrangement in adenocarcinoma), the pathologist's role and approach to lung cancer diagnosis in small biopsies and cytology has been affected dramatically. Specific therapies are selected for patients depending on the histologic diagnosis and the molecular status of the tumor. Understanding this new concept is essential for pathologists as they manage these specimens.

In particular, there have been 4 therapeutic advances for non–small cell lung carcinoma (NSCLC) since the 2004 WHO classification. These changes are directly tied to precise histologic classification. The first relates to tyrosine kinase inhibitors as first-line therapy in patients with advanced lung adenocarcinoma with *EGFR* mutations.[7–11] Second, adenocarcinomas with ALK rearrangements are responsive to crizotinib.[12–14] Third, patients with adenocarcinoma or NSCLC, not otherwise specified (NSCLC-NOS), are more responsive to pemetrexed than those squamous cell carcinoma.[15–17] Fourth, squamous cell

carcinoma is associated with life-threatening hemorrhage in patients treated with bevacizumab; therefore, it is contraindicated in lung cancer patients with this histology.[18]

Based largely on multiple phase III clinical trials,[7–11] the following clinical recommendation was made in the new classification.

Clinical Recommendation

In patients with advanced lung adenocarcinoma, we recommend testing for *EGFR* mutation (strong recommendation, moderate quality evidence).

Remarks: This is a strong recommendation because potential benefits clearly outweigh harms. This recommendation assumes that correct classification by *EGFR* mutation status is associated with important benefit based on randomized phase III clinical trials of EGFR tyrosine kinase inhibitor therapy that demonstrate a predictive benefit for response rate and progression-free survival, but not overall survival,[7–11] as well as subset analyses of multiple additional studies.

This clinical recommendation is listed in this document because of the major impact this has on the role for pathologists, not only in diagnosis but also in management of tissue for molecular testing. Now, not only do pathologists need to make a correct diagnosis, but also they need to manage the small amounts of cells and tissue in a manner that will preserve as much as possible for molecular testing.

Identification of New Molecular Targets in Lung Cancer Is a Rapidly Evolving Field

There are several examples of rapid advances occurring in the discovery of molecular targets for novel therapies in lung cancer.

An excellent example is the discovery that crizotinib is a clinically effective ALK inhibitor in patients with locally advanced or metastatic non–small cell lung cancer.[12,14] This was recently approved by the Food and Drug Administration for use in this setting: if the tumor is ALK positive as detected by a Food and Drug Administration–approved test or the Vysis ALK Break-Apart fluorescence in situ hybridization probe kit (Abbott Molecular, Des Plaines, Illinois).[12, 14] Other methods of detection such as immunohistochemistry show promise to be reliable methods of detecting ALK rearrangements,[19–21] but these need to be tested and validated in clinical trials. Although the Food and Drug Administration approval for crizotinib occurred after publication of the IASLC/ATS/ERS lung adenocarcinoma classification,[22] testing for ALK rearrangement is now part of molecular diagnostic testing for lung adenocarcinomas. The efficacy of crizotinib is now in need of further validation in phase III clinical trials. Anaplastic lymphoma kinase gene rearrangements are mostly found in lung adenocarcinomas lacking *EGFR* or Kirsten rat sarcoma (KRAS) mutations, and they are frequently thyroid transcription factor 1 (TTF-1) positive.[23,24]

ROS1 rearrangement was recently described in 1.7% of lung adenocarcinomas, and it appears to identify another subset of lung adenocarcinoma patients for whom there be an effective molecular targeted therapy.[25,26] ROS1 rearrangements are mutually exclusive with ALK rearrangements and also tend to occur in young never smokers with the histology of

adenocarcinoma. There does not appear to be an association with a specific histologic subtype. One patient had a near complete response to crizotinib.[26]

A frequent complication of EGFR tyrosine kinase inhibitor therapy is the development of acquired resistance through acquisition of *EGFR* T790M mutations, cMET amplification, dedifferentiation of the tumor with epithelial-mesenchymal transition, or development of a small cell carcinoma component.[27–30] For this reason, additional biopsies may be indicated in patients who have tumor progression after an initial response to tyrosine kinase inhibitor therapy. This phenomenon is also being observed with ALK inhibitors and is likely to occur with other molecular targeted therapies as well.[12]

There is also promise for lung squamous cell carcinoma with the recent discovery that fibroblast growth factor receptor 1 (FGFR1) amplification and discoidin domain receptor tyrosine kinase 2 (*DDR2*) mutations may render these patients sensitive to FGFR1 inhibition and dasatinib respectively.[31–33] Also, the Cancer Genome Atlas (TCGA) project sponsored by The National Cancer Institute has identified molecular alterations that may represent molecular targets in over 60% of squamous cell carcinomas of the lung.[34]

As a result of these advances, therapeutic decisions are now based on tumor typing by histology and/or cytology. This is leading to major changes in how pathologists diagnose lung cancer in small biopsy and cytology specimens. Therefore, pathologists need to make a greater effort to separate adenocarcinoma from squamous cell carcinoma; this includes a limited workup with special stains such as immunohistochemistry or mucin stains.[1,35] Although currently there is a rationale for molecular testing for *EGFR* mutation and ALK rearrangement in tumors classified as adenocarcinoma; NSCLC, favor adenocarcinoma; or NSCLC-NOS, it is anticipated that specific molecular tests will soon be recommended in squamous cell carcinomas, perhaps for FGFR-1 amplification or *DDR2* mutation.

These recent advances indicate that pathologists involved with lung cancer diagnosis need to pay close attention to the literature to be aware when molecular advances have reached the point of sufficient validation to be introduced into clinical practice. This is a challenge for practicing pathologists, because there are many new markers that are being recognized, but they may be neither ready nor suitable for routine clinical practice.

MAJOR CHANGES IN NEW CLASSIFICATION

Major changes in the approach to classification of lung cancer are introduced in the new IASLC/ATS/ERS classification compared with previous WHO classifications: (1) greater use of special stains to classify difficult cases further into adenocarcinoma or squamous cell carcinoma, (2) diagnosis using small samples, and (3) the need to manage tissue strategically for molecular studies. Several changes in terminology and introduction of new entities are addressed more fully in the second article, which focuses on classification of adenocarcinoma in resection specimens. These relate to the discontinuation of the terms bronchioloalveolar carcinoma and adenocarcinoma, mixed subtype, as well as the introduction of micropapillary as a new histologic subtype, the term lepidic pattern for the former bronchioloalveolar carcinoma growth pattern, and the specific term invasive mucinous adenocarcinoma for overtly invasive tumors previously classified as mucinous

bronchioloalveolar carcinoma.[1] The new concepts of adenocarcinoma in situ (AIS) and minimally invasive adenocarcinoma (MIA) are also addressed in more detail in the article on resection specimens.

New Terminology and Criteria for Classification of Major Lung Cancer Types in Small Biopsies and Cytology

The previous 1967, 1981, and 1999 WHO classifications addressed lung cancer classification based primarily on resection specimens.[4,36,37] Cytology was included for the first time in the 2004 WHO classification; however, practical issues of diagnosing lung cancer in small biopsies were not addressed.[3] Furthermore, because there was no clinical need to classify NSCLC further, the diagnosis of NSCLC without further specification was encouraged to avoid discrepancies with subsequent resected specimens. In small biopsies, the percentage of NSCLC cases diagnosed as NSCLC-NOS has been as high as 30% to 50%[38–40] and recent data from the Surveillance Epidemiology and End Results registry suggest the frequency of this diagnosis has been increasing.[41] For these reasons, until now, there have been no established standardized criteria or terminology for the diagnosis of lung cancer in small biopsies or cytology. However, the situation has changed because of the major implications of histology that impact the need for molecular testing and eligibility for specific therapies.

Expanded Use of Immunohistochemistry to Aid in Classification

In prior WHO classifications, lung cancer diagnosis was based mainly on light microscopy using routine hematoxylineosin–stained slides. The only special stain recommended in the 1967 and 1981 WHO classifications was mucin.[36,37] Immunohistochemistry was introduced for the first time in the 1999 WHO classification for 3 main tumors: (1) large cell neuroendocrine carcinoma, (2) sarcomatoid carcinomas, and (3) separation of malignant mesothelioma from carcinoma.[4] In the 2004 WHO classification, immunohistochemistry was preserved for these 3 tumors, but its usefulness was mentioned in the diagnosis of many other tumors as well.[3]

The reason for recommending only a few special stains in the 1999 and 2004 WHO classifications was to allow for widespread use of these classifications so they could be applied in parts of the world where these stains might not be available.[3,4] In the new classification, the concept of minimal stains is maintained. However, a new approach is introduced by recommending limited use of immunohistochemical and/or mucin stains for NSCLC-NOS cases that cannot be recognized as adenocarcinoma or squamous cell carcinoma definitively by light microscopy in order to try to classify these tumors further for clinical purposes. The reason for use of minimal stains is to preserve tissue for molecular studies. Methods that use substantial amounts of tissue to differentiate adenocarcinoma from squamous cell carcinoma, such as large panels of immunohistochemical stains, do not necessarily provide an advantage over routine light microscopy with a limited immunohistochemical workup.[42–45]

No effort was made in this IASLC/ATS/ERS classification to address optimal fixation of specimens for immunohistochemistry or molecular testing, although it is known that fixative

other than formalin may interfere with molecular testing. In particular, strong acids may denature DNA so that decalcification using strong acids may thwart definitive fluorescence in situ hybridization or DNA sequence testing. It may be reasonable to consider the recommendations of the American Society of Clinical Oncology guidelines for breast cancer regarding estrogen and progesterone receptor testing: (1) specimens should be placed in 10% neutral buffered formalin within 1 hour from tumor removal, (2) the tumor in resected specimens should be sectioned at 5-mm intervals, and (3) specimens should be fixed at least 6 hours, but not longer than 48 hours.[46,47] For lung cancer there are no data that have addressed specimen processing issues for immunohistochemistry or molecular testing such as exist for breast cancer, so this is a topic that needs more study before specific recommendations can be made.

NEW CRITERIA AND TERMINOLOGY FOR SMALL BIOPSIES AND CYTOLOGY

In this new classification, for the first time standardized criteria and terminology have been proposed that are specifically designed to apply to the pathologic diagnosis of lung cancer in small biopsies (bronchoscopic, needle, or core biopsies) and cytology. Criteria are proposed not only for adenocarcinoma but also for squamous cell carcinoma and tumors that in resection specimens might be classified as large cell carcinoma, large cell neuroendocrine carcinoma, adenosquamous carcinoma, and sarcomatoid carcinoma (Tables 1 and 2), because previous WHO classifications never addressed criteria for these tumors in small biopsies and cytology specimens.[1]

Tables 1 and 2 provide a comparison between the major lung cancer subtypes outlined in the 2004 WHO classification and the recommended terminology and criteria in the new classification.

Pathology Recommendation 1—For small biopsies and cytology, we recommend that NSCLC be further classified into a more specific histologic type, such as adenocarcinoma or squamous cell carcinoma, whenever possible (strong recommendation, moderate quality evidence).

Data Driving Need to Classify NSCLC Further Are Based Only on Light Microscopy

All current clinical trial data that justify the importance of the distinction between histologic types of NSCLC in advanced lung cancer patients are based upon light microscopy with or without mucin stains but not on the basis of immunohistochemical stains.[7–11,15–18,48]

Thus, the diagnosis for clinical work, research studies, and clinical trials should be recorded in a manner such that it is clear how the pathologist made the determination: based on light microscopy alone or light microscopy plus special studies.

Pathology Consideration for Good Practice—1. 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 based on light microscopy alone or if special stains were required.

If Light Microscopic Diagnosis Is Clearly Adenocarcinoma or Squamous Cell Carcinoma, Use These WHO Diagnostic Terms

Squamous cell carcinoma and adenocarcinoma should be diagnosed on biopsy and cytologic materials when the criteria for specific diagnosis of these tumor types in the 2004 WHO classification are met.[3] However, for tumors that do not meet these criteria, newly proposed terminology and criteria are outlined in Tables 1 and 2 and Figure 1.[1]

Adenocarcinoma or Squamous Cell Carcinoma Diagnosed by Morphology Alone

If clear squamous or adenocarcinoma differentiation is present by standard morphologic criteria,[3,49] a tumor can be diagnosed in small biopsies and cytology with the established terms *adenocarcinoma* (Figure 2) and *squamous cell carcinoma* (Figure 3).

Adenocarcinomas may manifest glandular differentiation by manifesting 1 or more architectural features of lepidic (formerly bronchioloalveolar), acinar, papillary, micropapillary or solid patterns. If these patterns are present, they can be mentioned in the report. Cytologically, adenocarcinoma differentiation can be expressed in several architectural patterns, including flat sheets or 3-dimensional cell balls, pseudopapillary aggregates or true papillae with central fibrovascular cores, cohesive clusters with acinar structures (Figure 4, A), “picket fence,” or “drunken honeycomb” (Figure 4, B).[49–51] In addition, individual tumor cells of adenocarcinoma typically have basophilic cytoplasm that may be homogeneous, distinctly granular, or foamy, and typically is translucent, often with cytoplasmic vacuoles (Figure 4, C). The nuclei are often situated eccentrically with chromatin that varies from finely granular and uniform to hyperchromatic and coarse with an irregular distribution. Most tumor cells have a single macronucleolus (Figure 4, C).

Squamous differentiation is manifest by 3 key morphologic features: keratinization, pearls, and intercellular bridges. Keratinization is also a distinctive feature in cytologic specimens, as the Papanicolaou stain keratinization appears orange to brilliantly yellow or red (Figure 5, A).[49–51] This needs to be distinguished from cytoplasmic eosinophilia induced by air drying. With the Romanowsky stain, keratinization manifests a characteristic robin's egg blue color. The cytoplasm has an opaque or dense, “hard” appearance and is less translucent than in adenocarcinomas and large cell carcinomas. Cells often have round to ovoid to elongated contours with sharply defined cell borders. Cells with long cytoplasmic tails and “tadpole” configurations may be seen. Nuclei are usually solitary, centrally situated, and hyperchromatic, with rectangular outlines and squared-off edges (Figure 5, B). Typically the chromatin is very dense, is homogeneous, and presents a pyknotic appearance. Nucleoli are not well developed.

When adenocarcinomas or squamous cell carcinomas are poorly differentiated, the defining morphologic criteria that allow for a specific diagnosis may be inconspicuous or absent. In these cases, immunohistochemistry or mucin stains may be necessary to make a more specific diagnosis. The introduction of molecular testing for *EGFR* and *KRAS* mutation

testing as well as routine use of immunohistochemistry has revealed that some adenocarcinomas have a “pseudosquamous” morphologic appearance. So the threshold for morphologic evidence of squamous differentiation should be high, and if there is any doubt, the diagnosis should be confirmed with immunohistochemistry. The mere presence of densely eosinophilic cytoplasm or sharp intercytoplasmic borders in the absence of frank keratinization, pearls, or intercellular bridges is insufficient for the diagnosis of squamous cell carcinoma. In fact, it is likely that many of the cases of *EGFR* mutation reported in squamous cell carcinoma may represent adenosquamous carcinomas or pseudosquamous adenocarcinomas that can be reclassified using the algorithm of special stains recommended herein.[52]

Judicious Use of Immunohistochemical Stains to Further Classify NSCLC-NOS Into NSCLC, Favor Adenocarcinoma, or NSCLC, Favor Squamous Cell Carcinoma

In those cases where a specimen shows NSCLC lacking either definite squamous or adenocarcinoma morphology, immunohistochemistry may refine diagnosis (Figure 1, step 2). To preserve as much tissue as possible for molecular testing in small biopsies, the workup should be as limited as possible.[43–45] Realizing that new markers are likely to be developed, we suggest the initial evaluation use only one adenocarcinoma marker and one squamous marker. At the present time, TTF-1 appears to be the single best marker for adenocarcinoma, and it provides the added value of serving as a pneumocyte marker that can help confirm a primary lung origin in 75% to 85% of lung adenocarcinomas.[45,53–55] Diastase–periodic acid-Schiff, mucicarmine, or Alcian blue/periodic acid–Schiff stains for mucin may also be of value. Until recently p63 was consistently reported as a reliable marker for squamous histology, and CK5/6 also can be useful.[40,56–64] A variety of other antibodies such as cytokeratin 7, 34βE12, and S100A7 are less specific and sensitive for squamous differentiation.[45,60,65] These data have been confirmed using resections where biopsies were originally interpreted as NSCLC,[59,60] and they also work on most needle aspirate specimens.[40,59]

The recent demonstration that the polyclonal p40 is a more specific marker than the monoclonal p63 (4A4) for squamous cell carcinoma with virtually no overlap in adenocarcinoma suggests this antibody may replace p63 as the best immunohistochemical squamous marker.[66–68] Although p63 is frequently positive in most nuclei of squamous cell carcinomas, it may show patchy and/or weak staining in 20% to 30% of adenocarcinomas. This immunophenotype, instead of being recognized as favoring lung adenocarcinoma, has been misinterpreted to favor squamous differentiation.[69] Thus a simple panel of TTF-1 and p40 may be able to classify most NSCLC-NOS cases, and this approach needs further validation.[66,67]

Another possible approach is use of cocktails of nuclear and cytoplasmic markers (TTF-1/cytokeratin 5/6 or p63/napsin A) may allow for use of fewer immunohistochemical studies of multiple antibodies.[62,70]

Cases positive for an adenocarcinoma marker (ie, TTF-1) and/or mucin with a negative squamous marker (ie, p40 or p63) should be classified as NSCLC, favor adenocarcinoma (Figure 6, A and B), and those that are positive for a squamous marker, with at least

moderate, diffuse staining, and a negative adenocarcinoma marker and/or mucin stains, should be classified as NSCLC, favor squamous cell carcinoma, with a comment specifying whether the differentiation was detected by light microscopy and/or by special stains (Figure 7, A and B). These 2 markers, TTF-1 and p40, are generally mutually exclusive.[45] If a case is positive for an adenocarcinoma marker such as TTF-1, the tumor should be classified as NSCLC, favor adenocarcinoma, despite any expression of squamous markers.[44,45,62,66] If TTF-1 reactivity is present in one population of tumor cells and another population is positive for squamous markers, this may raise the possibility of adenosquamous carcinoma, although this diagnosis can only be made based on a resection specimen.

If both TTF-1 and p40 are negative in a tumor that lacks clear squamous or glandular morphology, one may consider performing a cytokeratin stain to confirm that the tumor is a carcinoma. If a keratin stain is negative, further stains (ie, S100, CD45, or CD31) may be needed to exclude other tumors that might look epithelioid, such as melanoma, lymphoma, malignant mesothelioma, or epithelioid hemangioendothelioma.[42] Although primary lung adenocarcinomas can be TTF-1 negative, in this setting, one may perform additional immunohistochemical studies (ie, CDX-2, cytokeratin 20, estrogen receptor, or progesterone receptor) or suggest clinical evaluation to exclude a metastasis from other sites such as the colon or breast. Invasive mucinous adenocarcinomas or colloid adenocarcinomas are characteristically TTF-1 negative and can be CDX-2 positive, so clinical correlation is needed in such tumors to exclude a metastasis from other sites such as the pancreas or colon. Recent data suggests that mucin 6, Wilms tumor 1, and paired box gene 8 may be positive in a higher percentage of pancreatic, breast, and ovarian mucinous adenocarcinomas, compared with similar tumors of the lung.[71]

There may be cases where multidisciplinary correlation can help guide a pathologist in the evaluation of small biopsies and/or cytology specimens from lung adenocarcinomas. For example, if a biopsy showing NSCLC-NOS is obtained from an Asian, female never smoker with ground-glass nodules on computed tomography scans, the pathologist should be made aware of this information, as the tumor is more likely to be adenocarcinoma and to have an *EGFR* mutation. If tumor tissue is inadequate for molecular testing, there may be a need to rebiopsy the patient in order to perform testing that will guide therapy (Figure 1, Step 3).

NSCLC-NOS: If No Clear Differentiation by Morphology or Immunohistochemistry

There will remain a minority of specimens where the diagnosis remains NSCLC-NOS, as no differentiation can be established by routine morphology and immunohisto-chemistry (Figure 1, step 2, and Figure 8). In the setting of a tumor with a negative adenocarcinoma marker (ie, TTF-1) and only weak or focal staining for a squamous marker (ie, p40), it is best to classify the tumor as NSCLC-NOS rather than NSCLC, favor squamous cell carcinoma. These cases may benefit from discussion in a multidisciplinary setting as stated above (Figure 1, step 3).

Pathology Recommendation 2—We recommend that the term NSCLC-NOS be used as little as possible and we recommend it be applied only when a more specific diagnosis is not

possible by morphology and/or special stains (strong recommendation, moderate quality evidence).

Pathology Consideration for Good Practice—2. The term *non-squamous cell carcinoma* should not be used by pathologists in diagnostic reports. It is a categorization used by clinicians to define groups of patients whose tumors comprise several histologic types and who can be treated in a similar manner; in small biopsies/cytology, pathologists should classify NSCLC as adenocarcinoma, squamous cell carcinoma, NSCLC-NOS, or other terms outlined in Tables 1 and 2 or Figure 1.

NSCLC-NOS: When Morphology and/or Immunohistochemistry Are Conflicting

Rarely, small samples may show morphologic features of both squamous cell carcinoma and adenocarcinoma with routine histology or may show immunohistochemical expression of both squamous and adenocarcinoma markers; these should be termed as NSCLC-NOS with a comment recording the features suggesting concurrent glandular and squamous cell differentiation, specifying whether this was detected by light microscopy or immunohistochemistry. Because p63 expression can occur in up to one-third of adenocarcinomas,[40,45,72] in a tumor that lacks squamous cell morphology, virtually all tumors that show coexpression of p63 and TTF-1 are adenocarcinomas. Such coexpression has been reported frequently in *ALK*-positive adenocarcinomas.[24] It is possible the tumor may be an adenosquamous carcinoma, but that diagnosis cannot be established without a resection specimen showing at least 10% of each component. If TTF-1 and p40 or p63 positivity are seen in different populations of tumor cells, it is possible this may be more suggestive of adenosquamous carcinoma than if these markers are coexpressed in the same tumor cells.

Potential Errors in Small Samples From Respiratory Tract

Compared with resection specimens, both small biopsies and cytology samples from the lung suffer from greater inability to classify the subtype of carcinoma and to determine the presence of invasion accurately. However, such small specimens are also prone to the incorrect recognition of malignancy in general, resulting in false-negative and false-positive interpretations. One source estimates that such errors may occur in up to 15% of patients with a lung mass.[73]

For both cytology and biopsies, the most common reason for a false-negative diagnosis is sampling error by the clinician obtaining the specimen (eg, pulmonologists, radiologists). This may be reduced by on-site evaluation of small samples by a member of the pathology team.[74] The other major source of error is interpretation. Especially in cytology, false negatives may occur as sparse tumor cells are obscured by blood, inflammatory elements, and foreign material. In exfoliative samples, low-grade adenocarcinoma cells, especially those derived from AIS, may be mistaken for benign macrophages.[75]

Marked reparative atypia may be mistaken for neoplasia, especially adenocarcinomas. In repair, benign epithelial cells share several morphologic attributes of malignant cells, such as enlarged nuclei and prominent nucleoli. Careful attention to details such as a low number

of atypical cells vis-à-vis normal cells, delicate smooth nuclear membranes, and a lack of hyperchromatic chromatin should reduce the number of such false positives. However, this atypia may be striking, especially in association with inflammatory mass lesions, and in particular granulomatous inflammation.[76] Specific infections, for example *Aspergillus* sp, may cause striking atypia, resulting in incorrect diagnoses, especially of squamous cell carcinoma. It is well recognized that prior radiation and chemotherapy may produce alterations in benign cells that closely mimic carcinoma; here, a clinical history is paramount. Lymphoid cells, especially if crushed during forceps biopsies and smearing of cells, may simulate malignant elements; here the differential diagnosis usually revolves around small cell carcinoma. For decades, it has been recognized in exfoliative cytologic specimens that viral infections of the upper respiratory tract and benign reserve cell hyperplasia may cause confusion with squamous cell and small cell carcinomas, respectively. Still, this occasionally leads to an incorrect diagnosis of cancer.

Grading of Lung Cancer in Small Biopsies and Cytology Specimens

The IASLC/ATS/ERS lung adenocarcinoma classification did not make specific recommendations for grading of adenocarcinomas in small biopsies or cytology. Part of the reason for this is that even for resected adenocarcinomas, although data are emerging, there are no well established criteria as compared with other cancers such as prostate, breast, and kidney. The grade is inherent in some lung cancer diagnoses; for example, small cell carcinoma, large cell neuroendocrine carcinoma, and sarcomatoid carcinomas are poorly differentiated. Similarly, any NSCLC-NOS; NSCLC, favor adenocarcinoma; or NSCLC, favor squamous cell carcinoma will be poorly differentiated. Recent data that have demonstrated that architectural patterns are useful for grading adenocarcinomas are summarized in more detail in the article on adenocarcinoma in resected specimens.[2] Because of the issue of heterogeneity and sampling issues with small biopsies, there are few data regarding the prognostic significance of grading in these specimens. A recent study of liquid-based cytology specimens suggested that nuclear size, chromatin pattern, and nuclear contours could be combined in a scoring system that correlated with histologic grade and prognosis.[77] However, more data are needed with validation of the value of grading in small biopsies and cytology before this can be formally recommended.

Interpret Morphologic and Staining Patterns to Maximize Patient Eligibility for Therapies

Presently, the recommendation for *EGFR* mutation testing and candidacy for pemetrexed or bevacizumab therapy is for the diagnosis of (1) adenocarcinoma; (2) NSCLC, favor adenocarcinoma; or (3) NSCLC-NOS. For this reason, in most NSCLC, the primary decision pathologists need to focus on while interpreting small biopsies and cytology specimens is whether the tumor is a definite squamous cell carcinoma or NSCLC, favor squamous cell carcinoma, versus one of the above diagnoses. Thus, when morphology or immunohistochemical findings are equivocal, pathologists need to keep in mind that a diagnosis of squamous cell carcinoma or NSCLC, favor squamous cell carcinoma, will exclude them from histologically driven molecular testing or chemotherapy. In such a situation, it may be best to favor NSCLC-NOS, to allow the patient to be eligible for the therapeutic options mentioned above. Hopefully, more effective therapies, perhaps based on molecular targets, will become available for squamous cell carcinoma in the near future.

Pathology Consideration for Good Practice—3. The above strategy for the classification of adenocarcinoma versus other tumor type histologies and the terminology in Tables 1 and 2 and Figure 1 should be used in routine diagnosis as well as future research and clinical trials, so that there is uniform classification of disease cohorts in relation to tumor subtypes and data can be stratified according to diagnoses made by light microscopy alone versus diagnoses requiring special stains.

STRATEGIC USE OF PATHOLOGIC SPECIMENS FOR MOLECULAR STUDIES

Tissue Management for Molecular Studies Is Critical

A new responsibility for pathologists, in addition to making a correct diagnosis, is to manage these small biopsies and cytology specimens strategically so there is sufficient tissue preserved for molecular studies. Strategic use of small biopsy and cytology samples is important: use the minimum specimen necessary for an accurate diagnosis, in order to preserve as much tissue as possible for potential molecular studies (Figure 1).[42,43,51] This strategic approach should be multidisciplinary and requires pathologists to have good communication with the physicians who are obtaining the tissue samples (eg, interventional radiologist, surgeon, oncologist, pulmonologist, or cytopathologist). This ongoing dialogue can aid in making the best decision on how to obtain adequate tissue or cytology samples, not only for diagnosis but also for molecular testing. Methods that use substantial amounts of tissue to make a diagnosis of adenocarcinoma versus squamous cell carcinoma, such as large panels of immunohistochemical stains or molecular studies, may not provide an advantage over routine light microscopy with a limited immunohistochemical workup.[42–44]

Pathology Consideration for Good Practice—4. Tissue specimens should be managed not only for diagnosis but also to maximize the amount of tissue available for molecular studies.

Pathology Consideration for Good Practice—5. To guide therapy for patients with advanced lung adenocarcinoma, each institution should develop a multidisciplinary team that coordinates the optimal approach to obtaining and processing biopsy/cytology specimens to provide expeditious diagnostic and molecular results.

With the emerging importance of molecular diagnostics to guide therapy, a multidisciplinary approach is needed to establish a consistent strategy for obtaining and preserving tissue samples optimized to perform studies such as DNA sequence analysis, fluorescence in situ hybridization, and, in some settings, RNA-based studies. It is not possible to provide specific guidelines on how to do this in this current document, because of the wide variations in infrastructure and expertise from one institution to another. Still, this process begins with the method of obtaining tissue (fine-needle aspiration, core or transbronchial biopsy, surgical resection) and continues with the processing of the specimen in the pathology department, delivery of material for molecular analysis, and communication of the molecular results in pathology reports. As most critical molecular studies can be performed from formalin-fixed, paraffin-embedded tissue, there is a need for frozen samples

only for certain techniques, such as comparative genomic hybridization and gene expression profiling. An assessment of biopsy adequacy should be made in collaboration with the molecular laboratory, taking into account the specific platform used locally.

Small biopsies and/or cytologic samples including pleural fluids can be used for many molecular analyses.[51,78–90] *EGFR* and *KRAS* mutation testing are readily performed on these specimens.[51,78–82,84,86–89] Formalin-fixed, paraffin-embedded tissue samples can be used effectively for polymerase chain reaction–based mutation testing as well as for fluorescence in situ hybridization or chromogenic in situ hybridization testing for gene amplification, *ALK* rearrangement, and immunohistochemistry.

There are many different approaches to handling these small specimens that will vary greatly depending on individual laboratory workflow characteristics. The volume of tumor cells in biopsies may be small because of frequent prominent stromal reactions so that there may be scant material for molecular analysis, so a well–thought-out strategy in coordination with the histology and immunohistochemical laboratory technicians is important. A few approaches used in several laboratories are mentioned here, but there are many ways to do this. One approach is to cut 10 to 15 unstained slides from a paraffin block after the presence of tumor is identified in order to cut the block only once after initial hematoxylin-eosin staining, so that enough unstained slides are available for any required immunohistochemistry as well as molecular studies. It is useful for the histology technicians to understand the need for limited facing of the block and trying to save as many cuts of the tissue on unstained slides as possible. Another approach is to have biopsies with sufficient tumor placed into 2 separate blocks during specimen processing so one can be used for immunohistochemistry and the other for molecular studies.[43] Tumor-rich regions of paraffin blocks also may be cored using a 1-mm needle, avoiding the need for microdissection. Cells derived from clinical cytology smears can be analyzed for immunohistochemical and certain molecular studies, but it is far preferable if cell blocks are available.[51,91] Manual or laser-guided microdissection may enrich tumor cells for molecular studies. Each institution needs to consider the various options and choose what works best in its setting.

Cytology Is a Useful Diagnostic Method, Especially When Correlated With Histology

Cytology is a powerful tool in the diagnosis of lung cancer, in particular in the distinction of adenocarcinoma from squamous cell carcinoma.[92] In a recent study of 192 preoperative cytology diagnoses, definitive versus favored versus unclassified diagnoses were observed in 88% versus 8% versus 4% of cases, respectively.[51] When compared with subsequent resection specimens, the accuracy of cytologic diagnosis was 93%, and for the definitive diagnoses it was 96%. For the adenocarcinoma and squamous cell carcinoma cases, only 3% of cases were unclassified, and the overall accuracy was 96%. When immunohistochemistry was used, the accuracy was 100%.[51]

Whenever possible, cytology should be interpreted in conjunction with histology of small biopsies, as the 2 modalities are complementary.[40,51,93] In a recent study, the concordance between biopsy and cytology for adenocarcinoma versus squamous cell carcinoma was 93%.[93] However, when cytology was correlated with biopsy, the percentage of cases diagnosed as NSCLC-NOS was greatly reduced, to only 4%.[93] Factors that contribute the

greatest to difficulty in a specific diagnosis include poor differentiation, low specimen cellularity, and squamous histology.[51,93]

Pathology Consideration for Good Practice—6. When paired cytology and biopsy specimens exist, they should be reviewed together to achieve the most specific and concordant diagnoses.

Histologic Heterogeneity of Lung Cancer Is an Underlying Complexity

Because of histologic heterogeneity, small biopsy and/or cytology samples may not be representative of the total tumor, resulting in a discrepancy with the final histologic diagnosis in a resection specimen. However, combined histologic types that meet criteria for adenosquamous carcinoma comprise less than 5% of all resected NSCLCs.[3] The heterogeneity issue also makes it impossible to make the diagnosis of AIS, MIA, large cell carcinoma, or pleomorphic carcinoma in a small biopsy or cytology, because resection specimens are needed to make these interpretations. As invasion cannot be determined in cytologic samples and may not be evident in small tissues, the diagnosis of AIS and MIA cannot be made based on small specimens or cytology.

If a small biopsy shows a totally lepidic pattern of growth in the sample (Figure 9, A and B), the diagnosis should be adenocarcinoma with lepidic pattern, and a comment should be made that this could be from AIS, MIA, or an adenocarcinoma with a lepidic pattern, whether it is lepidic-predominant adenocarcinoma or an overtly invasive adenocarcinoma with a minor lepidic component. In such cases, correlation with computed tomography may be helpful. If the lesion is a pure ground-glass nodule no more than 3 cm in diameter, it is likely to be AIS. A ground-glass–predominant nodule with a solid component 0.5 cm in size or smaller is likely to be MIA. Lepidic-predominant adenocarcinoma is likely to show (1) a ground-glass–predominant ground-glass nodule and a solid component larger than 0.5 cm or (2) a ground-glass nodule larger than 3.0 cm.[1] As explained in the manuscript focused on the aspects of this classification that focus on resection specimens, most tumors formerly classified as mucinous bronchioloalveolar carcinoma have invasive areas, so the term proposed for these tumors is now invasive mucinous adenocarcinoma (Figure 10, A and B).[2] In small biopsies the term invasive mucinous adenocarcinoma can be used for most of these cases. Because very rare cases of mucinous AIS or MIA may occur, if a small biopsy from a mucinous adenocarcinoma shows a pure lepidic pattern from a tumor that is 3 cm or less in diameter by computed tomography, the term *mucinous adenocarcinoma with lepidic pattern* can be used if the biopsy does not show any invasive component, and a comment can be added that the tumor could represent mucinous AIS or MIA or invasive mucinous adenocarcinoma.

Histologic subtypes of adenocarcinoma are difficult or impossible to predict from cytologic specimens. Further, in smears from AIS, MIA, or lepidic-predominant adenocarcinoma, characteristic cellular attributes are often recognized, including uniform, round nuclei with grooves or pseudoinclusions and low nuclear to cytoplasmic ratios, but this is not specific; very similar changes may be seen in predominantly papillary adenocarcinomas.

The term large cell carcinoma has been used in some published clinical trials, but this diagnosis requires a resection specimen and cannot be made in small biopsies or cytology specimens, so it is not clear how these tumors were distinguished from NSCLC-NOS neoplasms.[16,17,94] Consistent use of the new terminology will hopefully obviate such confusion in future clinical trials.

Pathology Consideration for Good Practice—7. The terms AIS and MIA 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.

Pathology Consideration for Good Practice—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.

Preservation of Cell Blocks From Cytology Aspirates or Effusions for Molecular Studies

After sampling of effusions for microbiology and/or biochemistry, the remaining fluid should be evaluated for cytologic examination, and when tumor is identified, cell blocks should be prepared. Material derived from aspirates or effusions may have many more tumor cells than a concurrently obtained small biopsy, so any positive cytology samples should be preserved as cell blocks so that the tumor is archived for immunohistochemical and/or molecular studies.[40] Furthermore, these materials should be used judiciously in making the diagnosis to preserve as much material as possible for potential molecular studies.[40,89,90,95] In a recent study, material from cell blocks prepared from 128 lung cancer cytology specimens was suitable for molecular analysis for *EGFR* and *KRAS* mutations in 126 specimens (98%).[51]

Pathology Consideration for Good Practice—9. Cell blocks should be prepared from cytology samples including pleural fluids.

Distinction of Adenocarcinoma From Sarcomatoid Carcinomas

Specimens that show sarcomatoid features such as marked nuclear pleomorphism, malignant giant cells, or spindle cell morphology (Figure 11) should be preferentially regarded as adenocarcinoma or squamous cell carcinoma if features of glandular or squamous differentiation are clearly present, as this is apt to influence management. However, carcinosarcoma and blastoma are very difficult to diagnose in small specimens because of the limited ability to assess for mixed growth patterns. The diagnosis of pleomorphic carcinoma requires a resection specimen with a component of at least 10% spindle and/or giant cell carcinoma. Yet if a small biopsy shows what is probably an adenocarcinoma with pleomorphism, a comment should be made, for example, “NSCLC, favor adenocarcinoma, with giant and/or spindle cell features” (depending on which feature is identified), with a comment that this could be a pleomorphic carcinoma.

Pathology Consideration for Good Practice—10. In biopsies of tumors that show sarcomatoid features (marked nuclear pleomorphism, malignant giant cells, or spindle cell

morphology), these should initially be classified according to the guidelines above in relation to adenocarcinoma; NSCLC, favor adenocarcinoma; squamous cell carcinoma; or NSCLC favor squamous cell carcinoma if clear glandular or squamous features are present, as this is apt to influence management, with additional comment that giant and/or spindle cell features (depending on what feature) are present. If such features are not present, the term NSCLC-NOS should be used with comment on the sarcomatoid features.

Distinction of Adenocarcinoma From Neuroendocrine Carcinomas

Some cases of NSCLC may suggest neuroendocrine morphology; these should be assessed with neuroendocrine markers (CD56, chromogranin, and/or synaptophysin), so that a diagnosis of large cell neuroendocrine carcinoma (LCNEC) can be suggested. The term NSCLC, possible large cell neuroendocrine carcinoma, is usually the best term when this diagnosis is suspected, as it is difficult to establish a diagnosis of large cell neuroendocrine carcinoma on small biopsies. This situation may be changing as more core biopsies are obtained, making it possible both to identify the neuroendocrine morphology and to have sufficient tissue to do confirmatory immuno-stains for neuroendocrine markers (Figure 12). In those lacking neuroendocrine morphology, we recommend against using routine staining with neuroendocrine markers, as immunohistochemical evidence of neuroendocrine differentiation in otherwise definite adenocarcinoma and squamous cell carcinoma does not appear to affect prognosis[96,97] or treatment.

Pathology Consideration for Good Practice—11. Neuroendocrine immunohistochemical markers should be performed only in cases where there is suspected neuroendocrine morphology. If neuroendocrine morphology is not suspected, neuroendocrine markers should not be performed.

Variants of Invasive Adenocarcinoma in Small Biopsy and Cytology Specimens

The diagnosis of invasive mucinous adenocarcinoma,[98] as well as colloid,[99] fetal,[100] and enteric adenocarcinoma,[101] can be suspected based on small biopsy and cytology specimens if tumor is present. In some cases, initial hematoxylin-eosin sections may not be diagnostic, but deeper cuts, strategically made with extra unstained slides for potential molecular studies, may reveal a definitive diagnosis. For example, nondiagnostic alveolar mucin pools with a differential diagnosis of colloid pattern of adenocarcinoma versus mucus plugging in initial sections could be clearly adenocarcinoma with deeper sections (Figure 13). The detailed histologic characteristics of these tumors are addressed in the adenocarcinoma classification article focused on resection specimens, which are required to make a definitive diagnosis of these invasive adenocarcinoma variants.[2]

Structured Pathology Reports

The diagnosis of lung cancer in small biopsies and cytology specimens should have the following structure:

1. Pathologic or cytopathologic diagnosis according to the IASLC/ATS/ERS classification
2. Reporting of immunohistochemical and/or mucin stains

3. If appropriate, a comment about the differential diagnosis
4. If material has been submitted for molecular testing, this should be stated in a comment, specifying which block or slide is optimal for testing.

Although molecular studies may be pending, the surgical pathology and/or cytology report should not be delayed until after molecular test results are completed. However, ultimately those results should be reported in a pathology report or a molecular diagnostic pathology report. These results will need to be integrated in a multidisciplinary manner with clinical and radiologic correlation.

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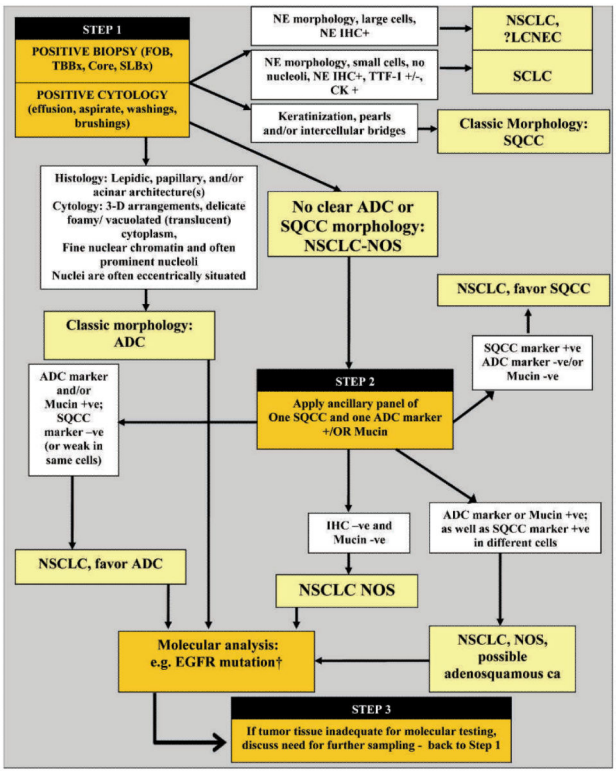

Figure 1.

Step 1: When positive biopsies (fiberoptic bronchoscopy [FOB] or transbronchial [TBBx], core, or surgical lung biopsy [SLBx]) or cytology (effusion, aspirate, washings, brushings) show clear adenocarcinoma (ADC) or squamous cell carcinoma (SQCC) morphology, the diagnosis can be firmly established. If there is neuroendocrine morphology, the tumor may be classified as small cell carcinoma (SCLC) or non–small cell lung carcinoma (NSCLC), probably large cell neuroendocrine carcinoma (LCNEC) according to standard criteria. If there is no clear ADC or SQCC morphology, the tumor is regarded as NSCLC, not otherwise specified (NOS). Step 2: NSCLC NOS can be further classified based on (1) immunohistochemical stains, (2) mucin (diastase–periodic acid-Schiff or mucicarmine) stains, or (3) molecular data. If the stains all favor ADC, with positive ADC marker(s) (ie, thyroid transcription factor 1 [TTF-1] and/or mucin positive) and negative SQCC markers, then the tumor is classified as NSCLC, favor ADC. If SQCC markers (ie, p63 and/or cytokeratin [CK] 5/6) are positive with negative ADC markers, the tumor is classified as NSCLC, favor SQCC. If the ADC and SQCC markers are both strongly positive in different populations of tumor cells, the tumor is classified as NSCLC-NOS, with a comment it may represent adenosquamous carcinoma. If all markers are negative, the tumor is classified as NSCLC-NOS. See text for recommendations on NSCLCs with marked pleomorphic and overlapping ADC/SQCC morphology. † Epidermal growth factor receptor (EGFR) mutation testing should be performed in (1) classic ADC; (2) NSCLC, favor ADC; (3) NSCLC-NOS; and (4) NSCLC-NOS, possible adenosquamous carcinoma. In these cases, if EGFR mutation testing is negative, testing for EML4-anaplastic lymphoma kinase (ALK) should be performed. In NSCLC-NOS, if either EGFR mutation or ALK rearrangements are

positive, the tumor is more likely to be ADC than SQCC. Step 3: If clinical management requires a more specific diagnosis than NSCLC-NOS, additional biopsies may be indicated. Abbreviations: ca, carcinoma; IHC, immunohistochemistry; NE, neuroendocrine; +, positive; −, negative; +/−, positive or negative; −ve, negative; +ve, positive.

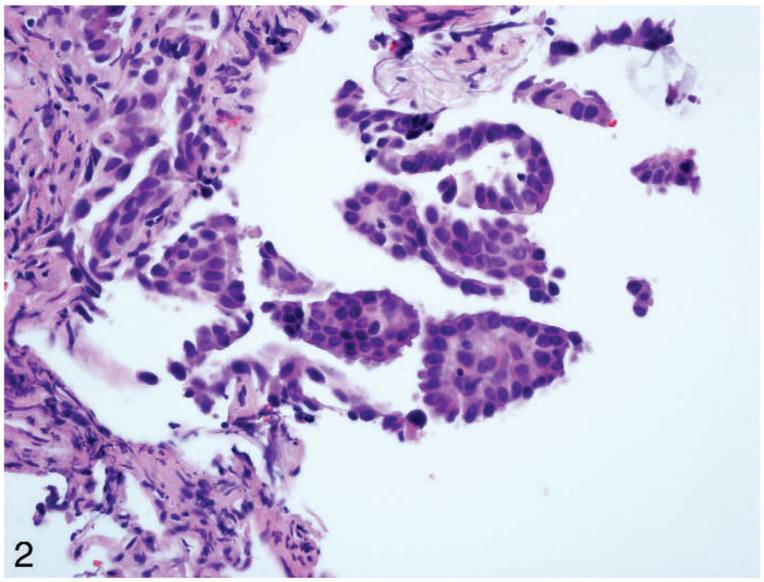

Figure 2.
Adenocarcinoma. This small biopsy shows fragments of adenocarcinoma with a papillary configuration (hematoxylin-eosin, original magnification ×40).

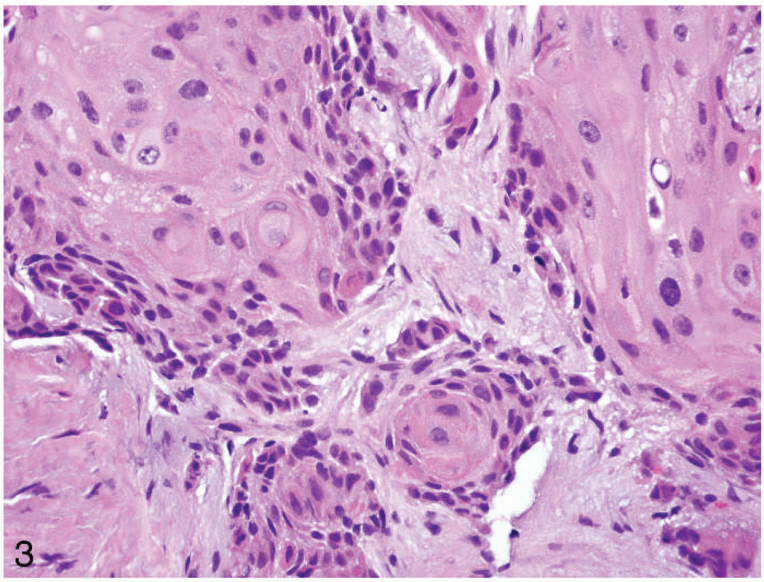

Figure 3.
Squamous cell carcinoma. This small biopsy shows squamous cell carcinoma with nests of tumor cells that have keratinization and pearls (hematoxylin-eosin, original magnification X20).

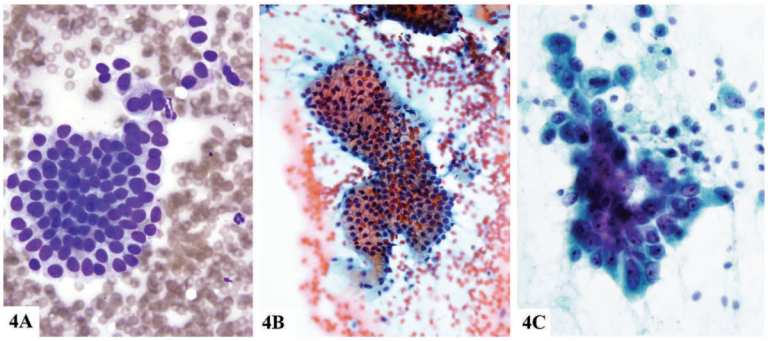

Figure 4.

Adenocarcinoma, cytology. A, A flat, cohesive sheet of rather uniform-appearing glandular cells is characterized by mild variability in nuclear sizes, inconspicuous nucleoli, very delicate cytoplasm, and a low level of disruption of polarity (nuclear crowding). B, This flat, cohesive sheet of uniform-appearing glandular cells has abundant clear cytoplasm filled with mucin and irregularly arranged nuclei in the “drunken honeycombing” pattern characteristic of invasive mucinous adenocarcinoma. C, A luminal space is surrounded by glandular cells with delicate cytoplasm and clearly malignant and often eccentrically located nuclei, each with a well-developed nucleolus. Note the mitotic figure (Papanicolaou, original magnification X40 [A]; Diff-Quik, original magnification X40 [B and C]).

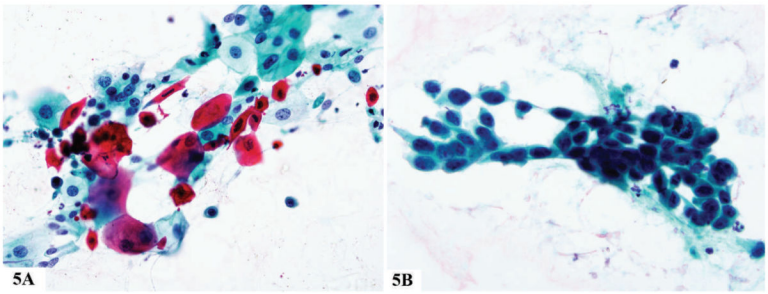

Figure 5.

Squamous cell carcinoma, cytology. A, Many of the tumor cells manifest cytoplasmic keratin as a dense, almost glassy red to orange coloration. Each cell houses a hyperchromatic nucleus, many of which possess jagged outlines. Nonkeratinized neoplastic cells with cyanophilic cytoplasm are also present. B, A flat mosaic sheet of malignant epithelial cells that are characterized by dense (or opaque) cyanophilic cytoplasm. Their nuclei are obviously hyperchromatic with small chromocenters and/or nucleoli. A mitotic figure is present (Papanicolaou, original magnification X40 [A]; Diff-Quik, original magnification X40 [B]).

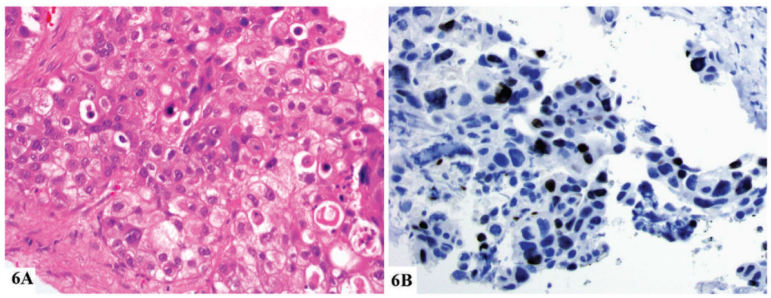

Figure 6.

Non–small cell lung carcinoma, favor adenocarcinoma. A, This tumor shows a solid pattern of growth with no clear squamous acinar, papillary, or lepidic growth and no intracytoplasmic mucin. The tumor was thought to have a pseudosquamous morphology and was initially diagnosed as a squamous cell carcinoma. B, A thyroid transcription factor 1 (TTF-1) stain is positive, favoring an adenocarcinoma. This tumor had an epidermal growth factor receptor exon 21 L858R mutation (hematoxylin-eosin, original magnification X20 [A]; immunohistochemistry for TTF-1, original magnification X40 [B]).

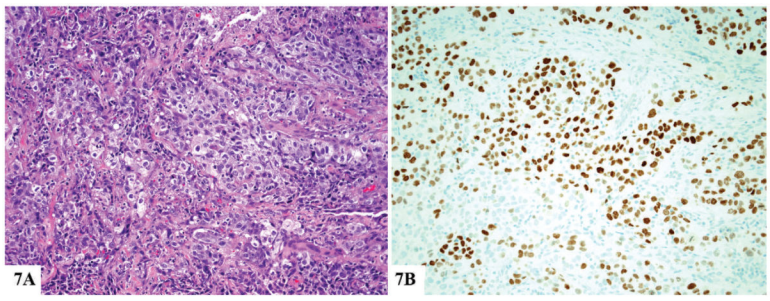

Figure 7.

Non–small cell lung carcinoma, favor squamous cell carcinoma. A, This biopsy shows a solid nest of tumor cells with no clear glandular or squamous differentiation. B, p40 shows strong nuclear staining (hematoxylin-eosin, original magnification ×20 [A]; immunohistochemistry for p40, original magnification ×40 [B]).

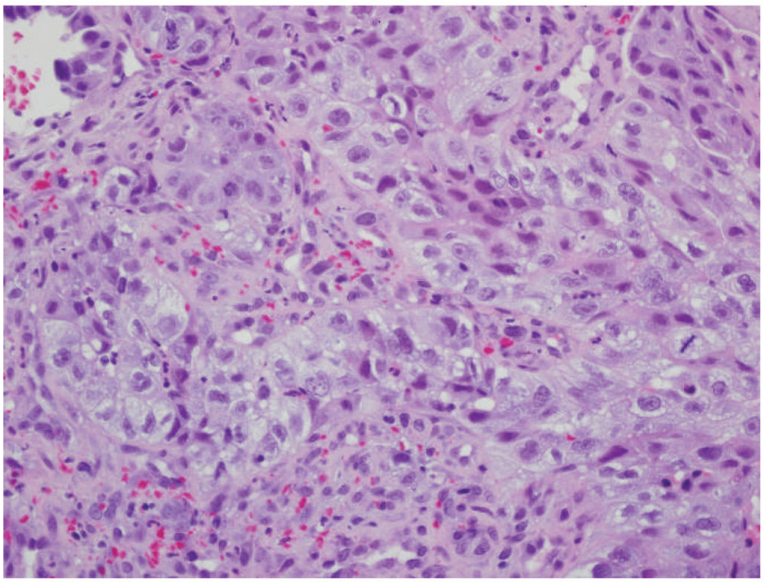

Figure 8.

Non–small cell carcinoma, not otherwise specified. This poorly differentiated carcinoma does not show any morphologic features of glandular or squamous differentiation, and both TTF-1 and p40 stains were negative (hematoxylin-eosin, original magnification ×20).

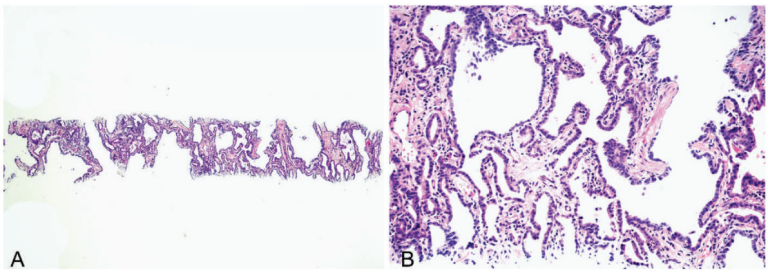

Figure 9.

Adenocarcinoma with lepidic pattern. A, This core biopsy shows an adenocarcinoma with a pure lepidic pattern. No clear invasive areas are identified. B, Atypical pneumocytes line the alveolar walls in a crowded manner consistent with a lepidic pattern of adenocarcinoma. The few structures that have a somewhat papillary or acinar appearance are most likely tangential cuts of alveolar walls rather than definite invasion. The differential diagnosis includes adenocarcinoma in situ, minimally invasive adenocarcinoma, and invasive adenocarcinoma with a lepidic component (hematoxylin-eosin, original magnifications ×4 [A] and ×40 [B]).

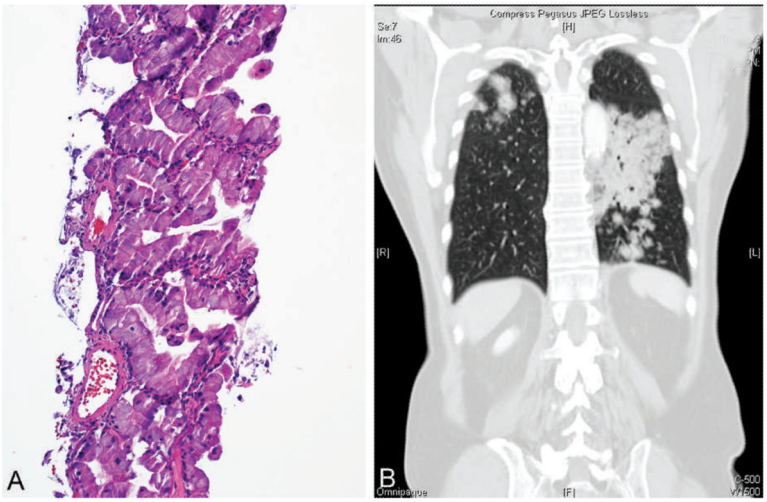

Figure 10.

Invasive mucinous adenocarcinoma. A, This adenocarcinoma is composed of columnar tumor cells with abundant apical mucin and small, basally oriented nuclei. Tumor cells line alveolar walls and are so crowded they form small papillary protrusions into some air spaces. B, The computed tomography scan from this patient shows bilateral nodules of consolidation with some air bronchograms, indicating this is not mucinous adenocarcinoma in situ or minimally invasive adenocarcinoma, but invasive mucinous adenocarcinoma (hematoxylin-eosin, original magnification ×20).

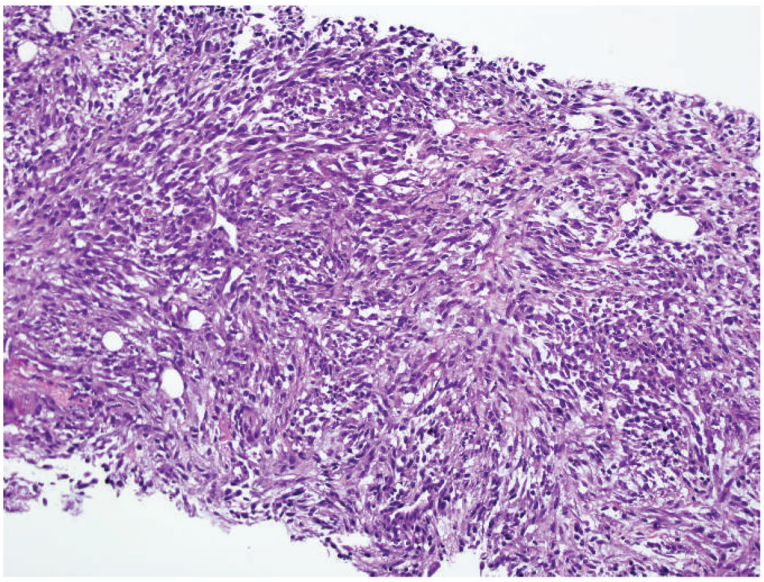

Figure 11.

Non–small cell carcinoma, favor sarcomatoid carcinoma. This poorly differentiated tumor consists of spindle-shaped cells in the pattern of a spindle cell carcinoma. The tumor stained positively for AE1/AE3 pancytokeratin and showed focal weak staining for thyroid transcription factor 1 (hematoxylin-eosin, original magnification ×20).

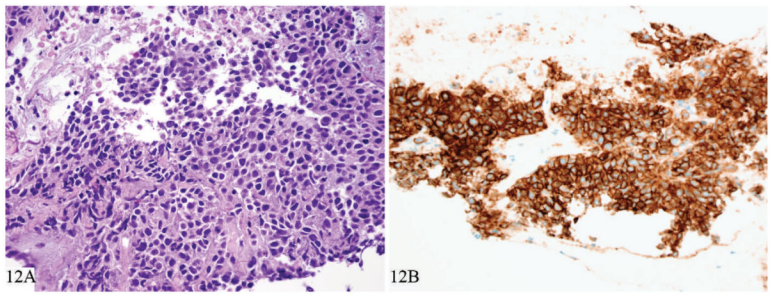

Figure 12.

Non–small cell carcinoma, favor large cell neuroendocrine carcinoma. A, This core biopsy shows a poorly differentiated carcinoma with neuroendocrine morphology consisting of organoid nesting arrangements of the tumor cells with some rosettelike structures. The tumor cells have relatively abundant cytoplasm and some nucleoli, suggesting a non–small cell carcinoma. B, The tumor cells stain strongly with the neuroendocrine marker CD56 showing a membranous pattern of staining (hematoxylin-eosin, original magnification ×20 [A]; CD56 immunostain, original magnification ×20 [B]).

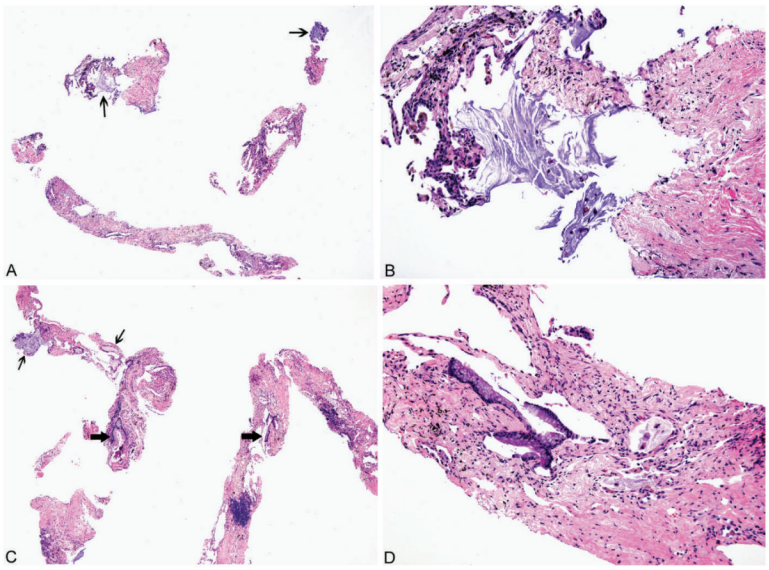

Figure 13.

Adenocarcinoma with colloid pattern. A, Initial core biopsy shows fibrous tissue and focal pools of mucin in air spaces (arrows), but no clear adenocarcinoma. B, Higher magnification shows pools of alveolar mucin, but no tumor cells can be seen. C, Deeper sections of same core show larger pools of mucin in air spaces (thin arrows), but in addition foci of adenocarcinoma are revealed (thick arrows). D, Along fibrotic connective tissue are glandular tumor cells with abundant apical mucin and small, basally oriented nuclei, diagnostic of adenocarcinoma. The overall pattern is suggestive of a colloid adenocarcinoma pattern (hematoxylin-eosin, original magnifications ×4 [A and C], ×10 [B], and ×20 [D]).

Table 1

Specific Terminology and Criteria for Adenocarcinoma, Squamous Cell Carcinoma, and Non–Small Cell Carcinoma, Not Otherwise Specified (NSCLC-NOS), in Small Biopsies and Cytology[a]

2004 WHO Classification, Including Updated IASLC/ATS/ERS Terminology	Morphology/Stains	IASLC/ATS/ERS Terminology
Adenocarcinoma	Morphologic adenocarcinoma patterns clearly present	Adenocarcinoma (describe identifiable patterns present)
Mixed subtype		
Acinar		
Papillary		
Solid		
Micropapillary		
Lepidic (nonmucinous)		Adenocarcinoma with lepidic pattern (if pure, add note: an invasive component cannot be excluded)
Lepidic (mucinous)		Invasive mucinous adenocarcinoma (describe patterns present; use term mucinous adenocarcinoma with lepidic pattern if pure lepidic pattern; see text)
No 2004 WHO counterpart; most will be solid adenocarcinomas	Morphologic adenocarcinoma patterns not present (supported by special stains, ie, +TTF-1)	Non–small cell carcinoma, favor adenocarcinoma
Squamous cell carcinoma	Morphologic squamous cell patterns clearly present	Squamous cell carcinoma
No 2004 WHO counterpart	Morphologic squamous cell patterns not present (supported by stains, ie, +p40)	NSCLC, favor squamous cell carcinoma
Large cell carcinoma	No clear adenocarcinoma, squamous or neuroendocrine morphology or staining pattern	NSCLC-NOS[b]

Abbreviations: IASLC/ATS/ERS, International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society; NSCLC, non–small cell lung carcinoma TTF-1, thyroid transcription factor-1; WHO, World Health Organization.

[a] Modified with permission from Travis et al.[1] The new IASLC/ATS/ERS international multidisciplinary lung adenocarcinoma classification. *J Thorac Oncol*. 2011;6(2):244–285.

[b] NSCLC-NOS pattern can be seen not only in large cell carcinoma but also when the solid, poorly differentiated component of adenocarcinoma or squamous cell carcinoma is sampled but does not express immunohistochemical markers or mucin.

Table 2

IASLC/ATS/ERS Classification for Small Biopsies/Cytology Comparing 2004 WHO Terms With New Terms for Small Cell Carcinoma, Large Cell Neuroendocrine Carcinoma (LCNEC), Adenosquamous Carcinoma, and Sarcomatoid Carcinoma[a]

2004 WHO Classification	Small Biopsy/Cytology: IASLC/ATS/ERS
Small cell carcinoma	Small cell carcinoma
LCNEC	Non–small cell carcinoma with NE morphology and positive NE markers, possible LCNEC
Large cell carcinoma with NE morphology	Non–small cell carcinoma with NE morphology (negative NE markers) Comment: This is a non–small cell carcinoma where LCNEC is suspected, but stains failed to demonstrate NE differentiation.
Adenosquamous carcinoma	Morphologic squamous cell and adenocarcinoma patterns present: non–small cell carcinoma, NOS (comment that adenocarcinoma and squamous components are present and this could represent adenosquamous carcinoma).
No counterpart in 2004 WHO classification	Morphologic squamous cell or adenocarcinoma patterns not present but immunostains favor separate glandular and adenocarcinoma components Non–small cell carcinoma, NOS (specify the results of the immunohistochemical stains and the interpretation) Comment: this could represent adenosquamous carcinoma.
Sarcomatoid carcinoma	NSCLC with spindle and/or giant cell carcinoma (mention if adenocarcinoma or squamous carcinoma are present)

Abbreviations: IASLC/ATS/ERS, International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society; NE, neuroendocrine; NOS, not otherwise specified; NSCLC, non–small cell lung carcinoma; WHO, World Health Organization.

[a]Reprinted with permission from Travis et al.[1] The New IASLC/ATS/ERS international multidisciplinary lung adenocarcinoma classification. *J Thorac Oncol.* 2011;6(2):244–285.

Table 3

Summary of Pathology Recommendations Applicable to Small Biopsy and Cytology Specimens

1. For small biopsies and cytology, we recommend that NSCLC be further classified into a more specific type, such as adenocarcinoma or squamous cell carcinoma, whenever possible (strong recommendation, moderate quality evidence).
2. We recommend that the term NSCLC-NOS be used as little as possible, and we recommend it be applied only when a more specific diagnosis is not possible by morphology and/or special stains (strong recommendation, moderate quality evidence).

Abbreviations: NSCLC, non–small cell lung carcinoma; NOS, not otherwise specified.

Table 4

Summary of Pathology Considerations for Good Practice Applicable to Small Biopsy and Cytology Specimens

1.	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 based on light microscopy alone or if special stains were required.
2.	The term *non–SQCC* should not be used by pathologists in diagnostic reports. It is a categorization used by clinicians to define groups of patients whose tumors comprise several histologic 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 outlined in Table 1 or Figure 1.
3.	The above strategy for classification of ADC versus other histologies and the terminology in Table 1 and Figure 1 should be used in routine diagnosis as well as future research and clinical trials, so that there is uniform classification of disease cohorts in relation to tumor subtypes and data can be stratified according to diagnoses made by light microscopy alone versus diagnoses requiring special stains.
4.	Tissue specimens should be managed not only for diagnosis but also to maximize the amount of tissue available for molecular studies.
5.	To guide therapy for patients with advanced lung ADC, each institution should develop a multidisciplinary team that coordinates the optimal approach to obtaining and processing biopsy/cytology specimens to provide expeditious diagnostic and molecular results.
6.	When paired cytology and biopsy specimens exist, they should be reviewed together to achieve the most specific and concordant diagnoses.
7.	The terms AIS and MIA 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.
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.	Cell blocks should be prepared from cytology samples including pleural fluids.
10.	In biopsies of tumors that show sarcomatoid features (marked nuclear pleomorphism, malignant giant cells, or spindle cell morphology), these should be initially classified as according to guidelines above in relation to ADC; NSCLC, favor ADC; SQCC; or NSCLC 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 NSCLC-NOS should be used, again with comment on the sarcomatoid features.
11.	Neuroendocrine immunohistochemical markers should be performed only in cases where there is suspected neuroendocrine morphology. If neuroendocrine morphology is not suspected, neuroendocrine markers should not be performed.

Abbreviations: ADC, adenocarcinoma; AIS, adenocarcinoma in situ; MIA, minimally invasive adenocarcinoma; NOS, not otherwise specified; NSCLC, non–small cell lung carcinoma; SQCC, squamous cell carcinoma.

Table 5

Pathology Research Recommendations Applicable to Small Biopsy and Cytology Specimens

1. It is unknown whether there is any added value provided by refining NSCLC-NOS via immunohistochemistry on small biopsies or cytology samples. This requires assessment in future trials using systemic therapy.
2. Additional markers for squamous or adenocarcinoma differentiation, such as desmoglein-3[102] or desmocollin[103] for squamous cell carcinoma or napsin A for adenocarcinoma[103], need further evaluation.

Abbreviation: NSCLC-NOS, non–small cell lung carcinoma, not otherwise specified.