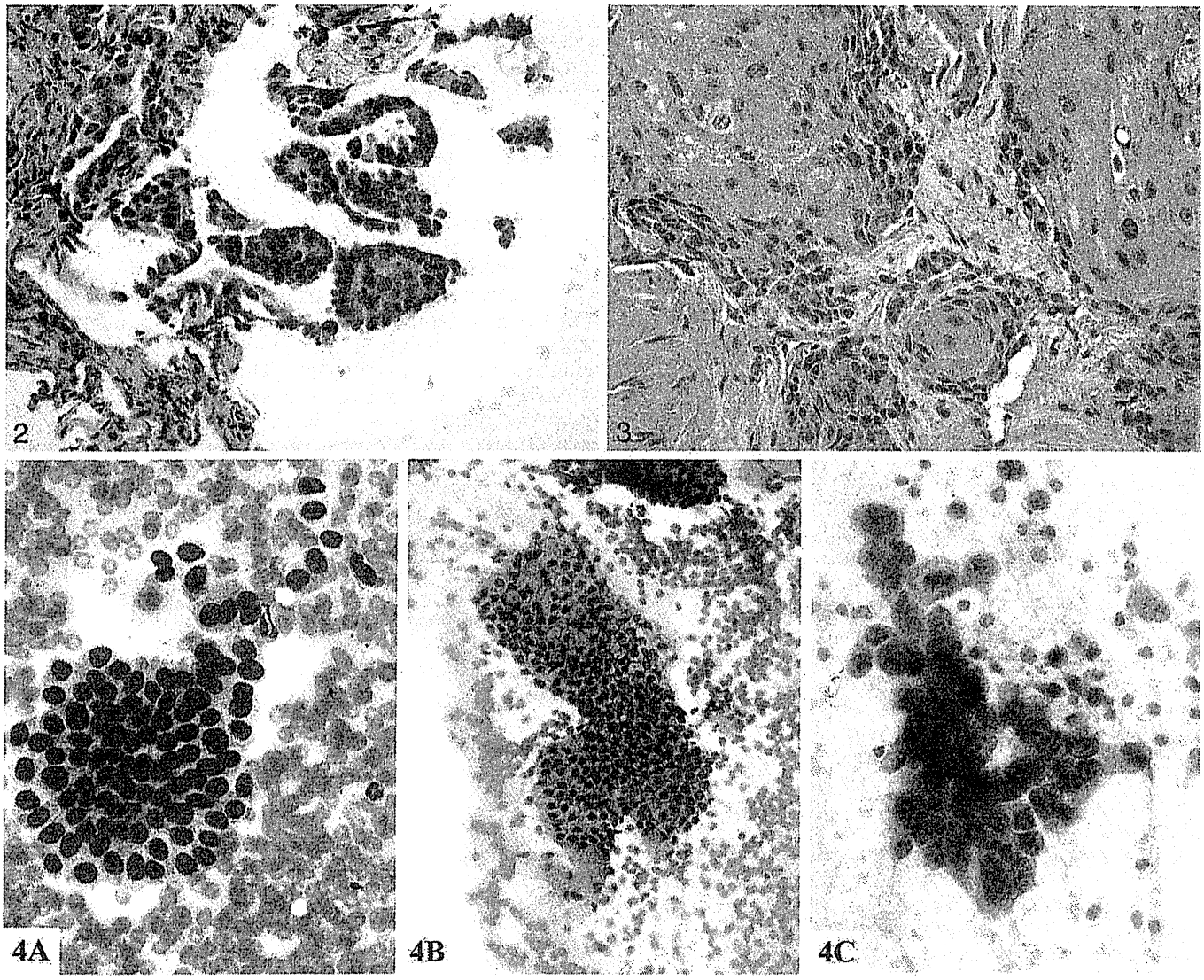


Figure 2. *Adenocarcinoma. This small biopsy shows fragments of adenocarcinoma with a papillary configuration (hematoxylin-eosin, original magnification X40).*

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]).*

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 adenocarci-

nomas. 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 immunohistochemistry (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]

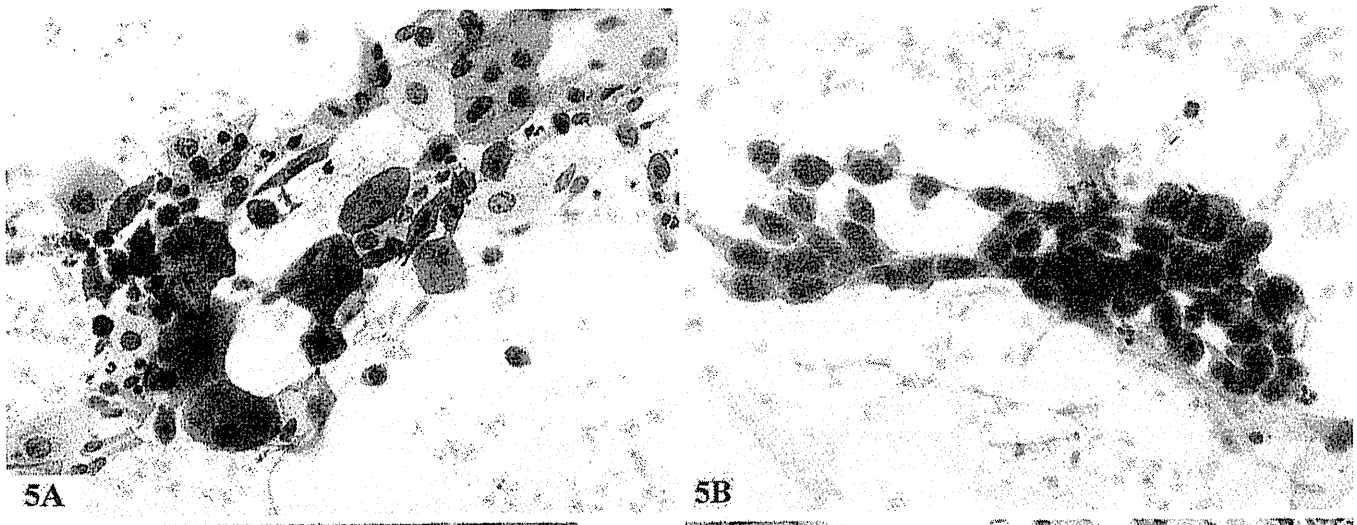
Marked reparative atypia may be mistaken for neoplasia, especially adenocarcinomas. In repair, benign epithelial cells share several morphologic attributes of malignant cells, such as enlarged nuclei and prominent nucleoli. Careful attention to details such as a low number of atypical cells vis-à-vis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

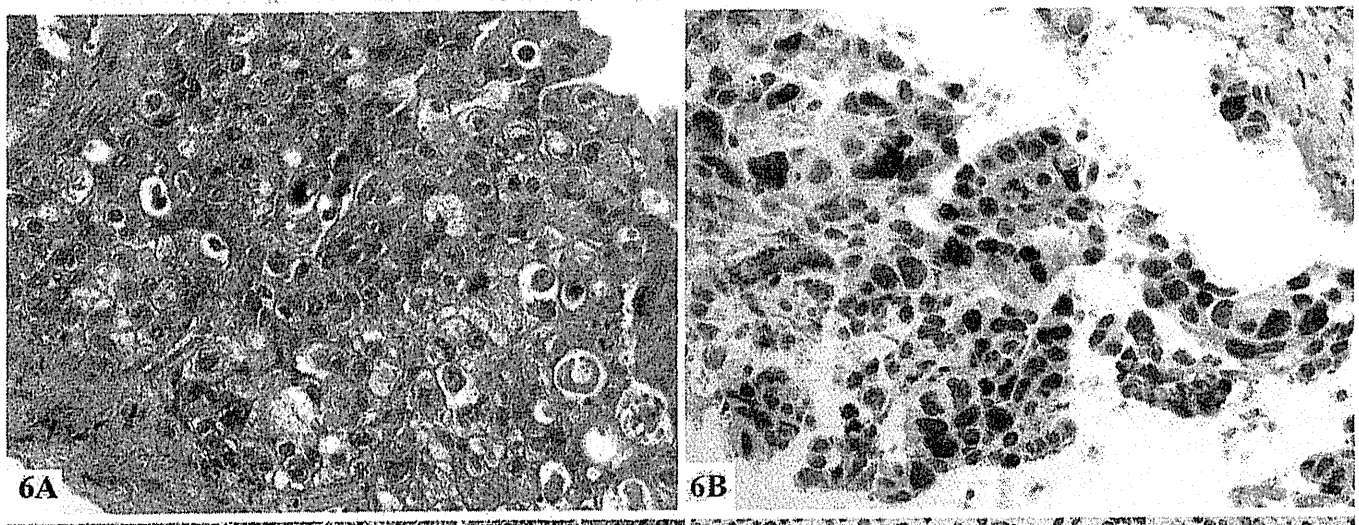
Grading of Lung Cancer in Small Biopsies and Cytology Specimens

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,

→

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 ×40 [A]; Diff-Quik, original magnification ×40 [B]).*





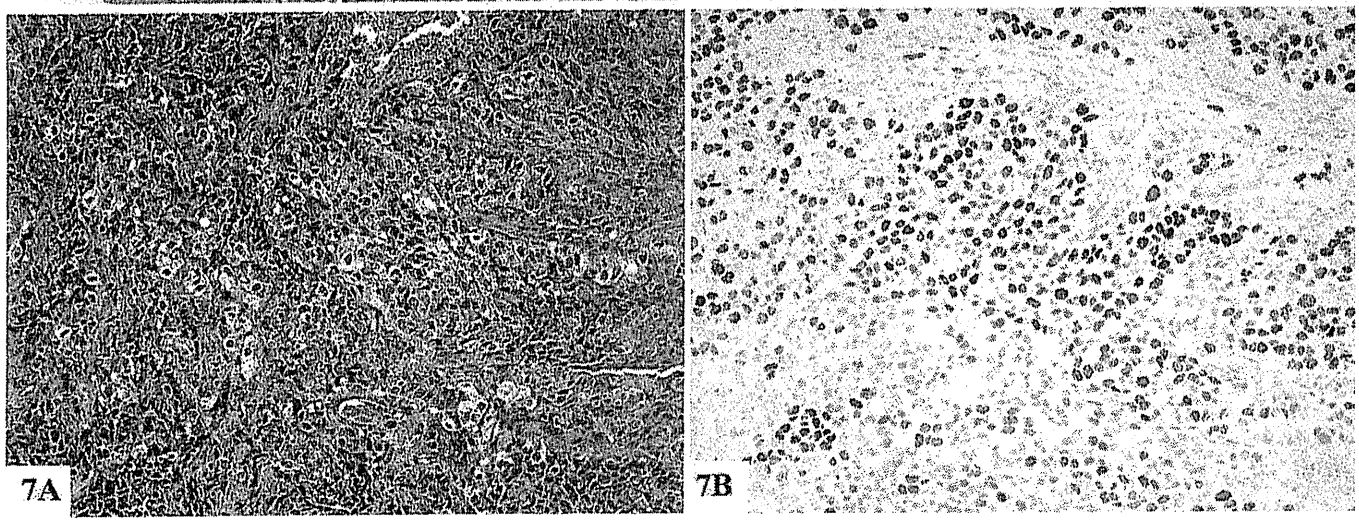


Figure 6. *Non–small cell lung carcinoma, favor adenocarcinoma. A, This tumor shows a solid pattern of growth with no clear squamous acinar, papillary, or lepidic growth and no intracytoplasmic mucin. The tumor was thought to have a pseudosquamous morphology and was initially diagnosed as a squamous 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 ×20 [A]; immunohistochemistry for TTF-1, original magnification ×40 [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]).*

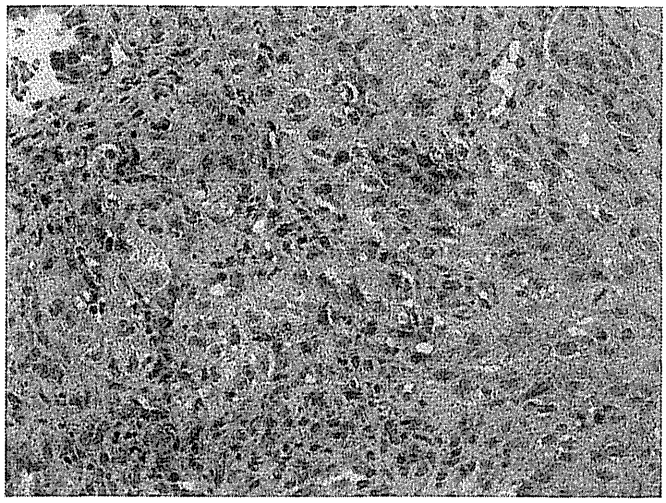


Figure 8. *Non–small cell carcinoma, not otherwise specified. This poorly differentiated carcinoma does not show any morphologic features of glandular or squamous differentiation, and both TTF-1 and p40 stains were negative (hematoxylin-eosin, original magnification ×20).*

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

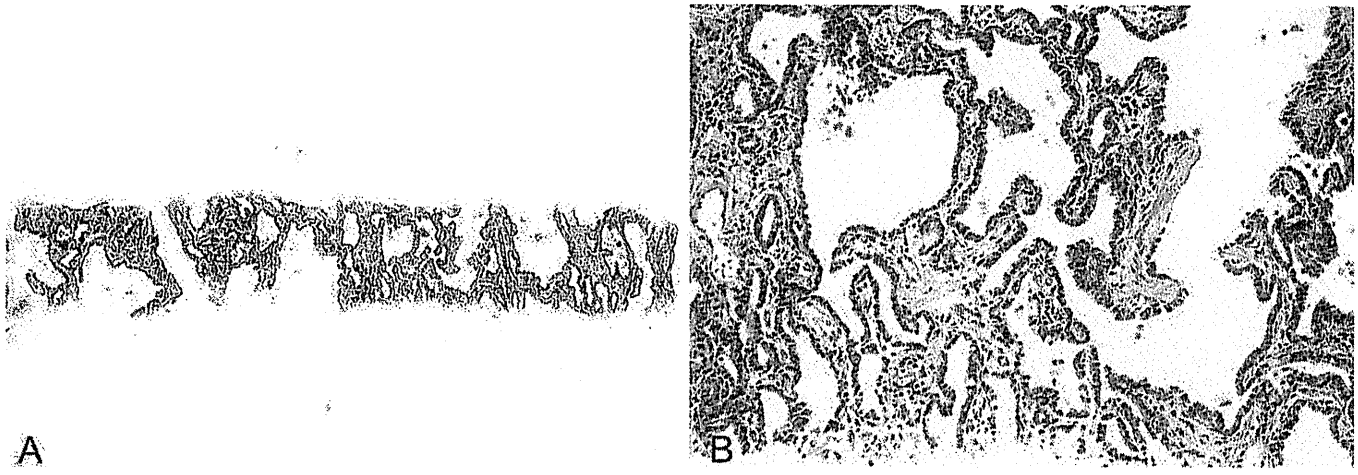


Figure 9. *Adenocarcinoma with lepidic pattern. A, This core biopsy shows an adenocarcinoma with a pure lepidic pattern. No clear invasive areas are identified. B, Atypical pneumocytes line the alveolar walls in a crowded manner consistent with a lepidic pattern of adenocarcinoma. The few structures 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]).*

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,

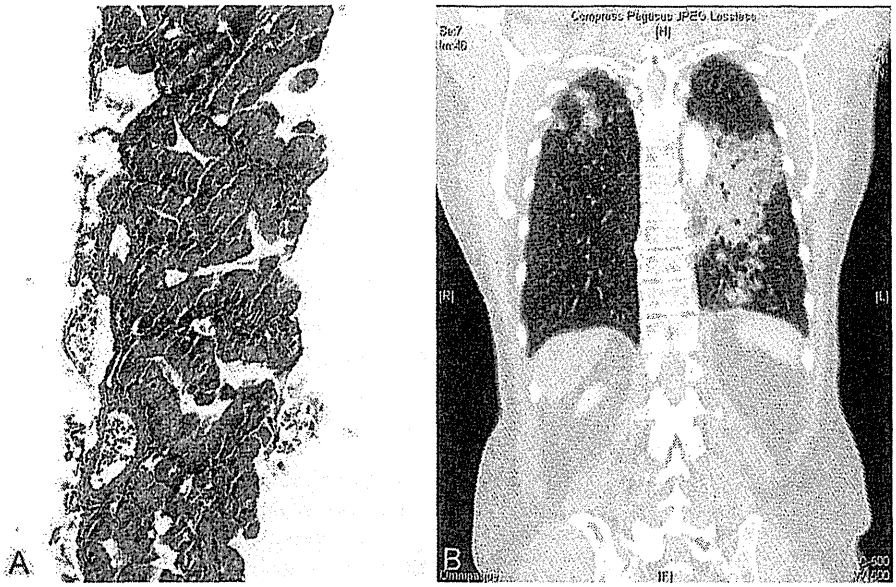


Figure 10. *Invasive mucinous adenocarcinoma. A, This adenocarcinoma is composed of columnar tumor cells with abundant apical mucin and small, basally oriented nuclei. Tumor cells line alveolar walls and are so crowded they form small papillary protrusions into some air spaces. B, The computed tomography 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).*

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

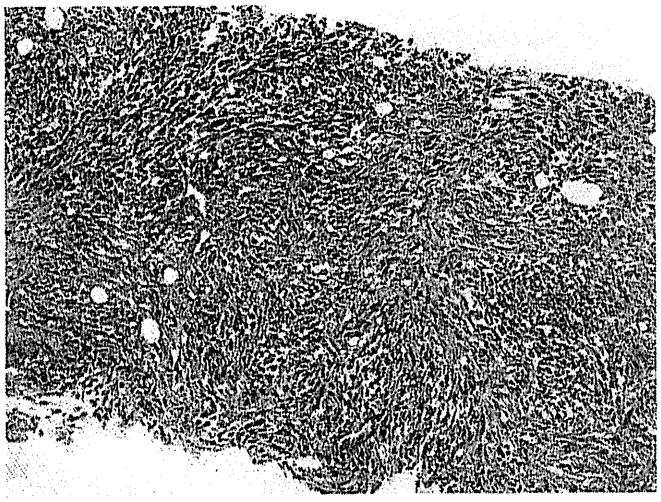


Figure 11. *Non–small cell carcinoma, favor sarcomatoid carcinoma. This poorly differentiated tumor consists of spindle-shaped cells in the pattern of a spindle cell carcinoma. The tumor stained positively for AE1/AE3 pancytokeratin and showed focal weak staining for thyroid transcription factor 1 (hematoxylin-eosin, original magnification ×20).*

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 neuroendo-

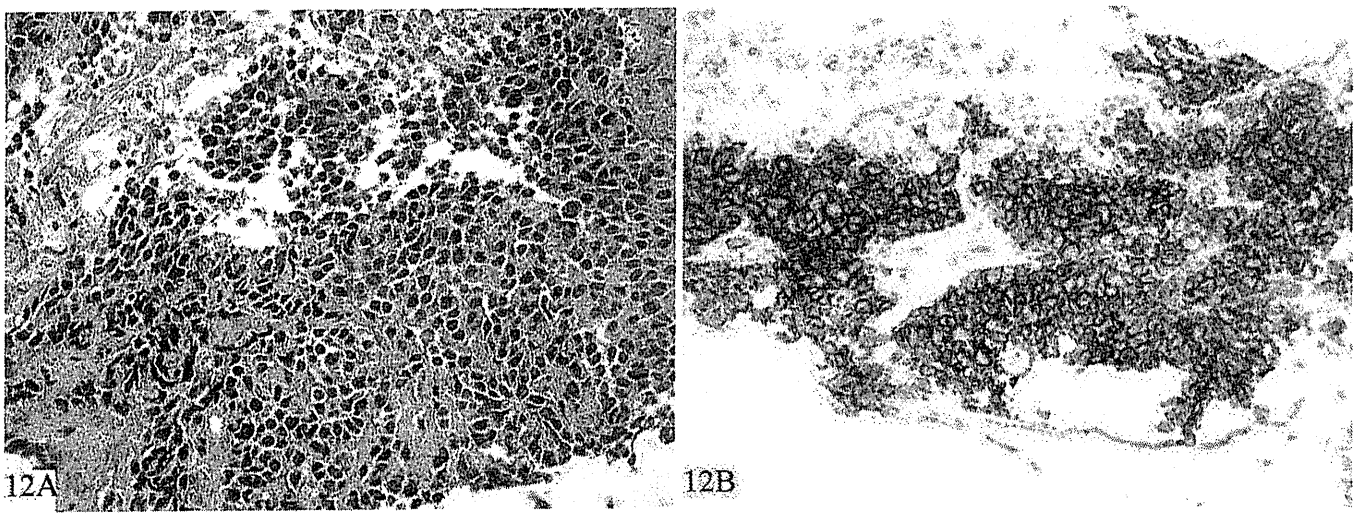


Figure 12. *Non–small cell carcinoma, favor large cell neuroendocrine carcinoma. A, This core biopsy shows a poorly differentiated carcinoma with neuroendocrine morphology consisting of organoid nesting arrangements of the tumor cells with some rosettelike structures. The tumor cells have relatively 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]).*

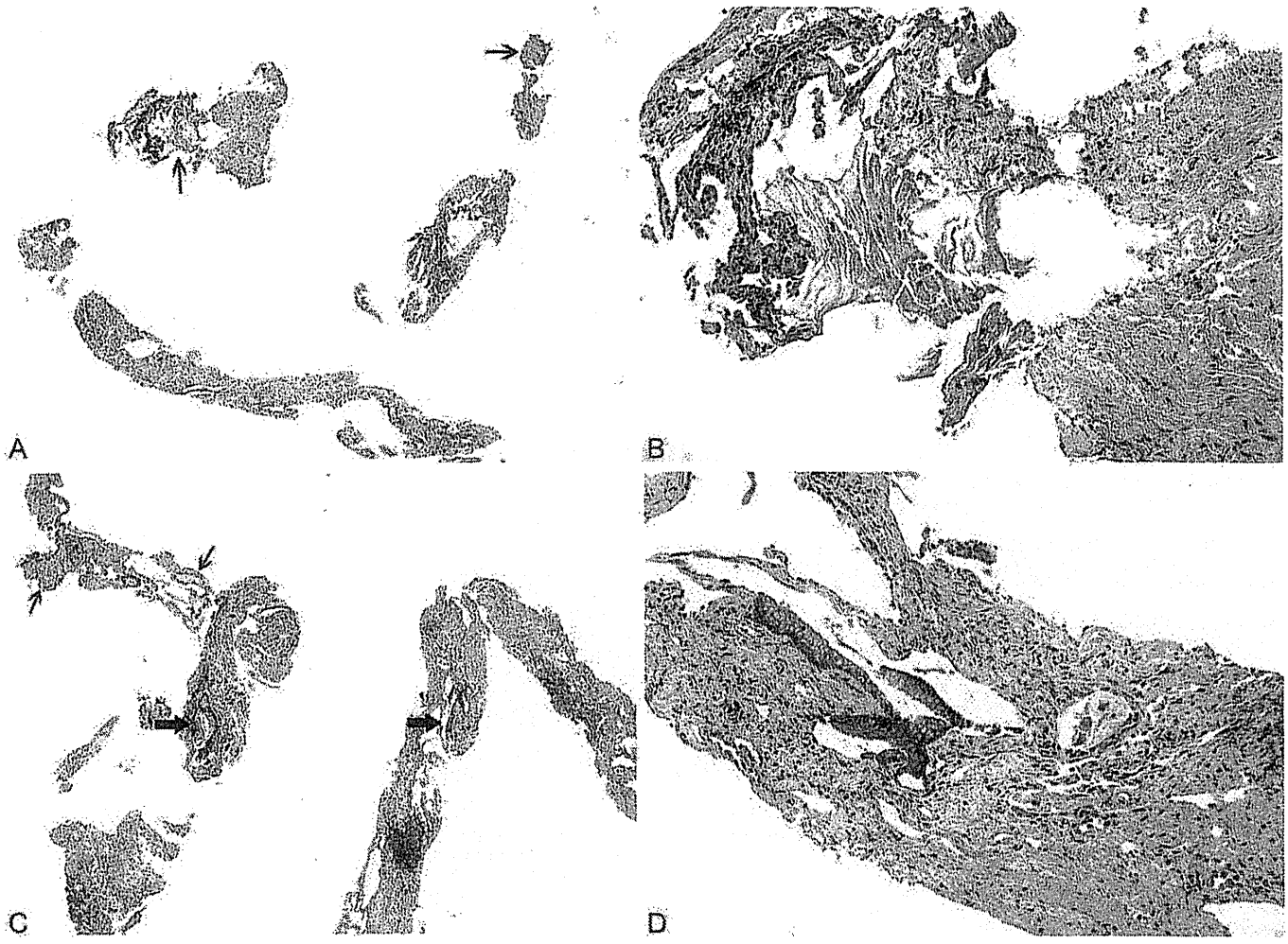


Figure 13. *Adenocarcinoma with colloid pattern. A, Initial core biopsy shows fibrous tissue and focal pools of mucin in air spaces (arrows), but no clear adenocarcinoma. B, Higher magnification shows pools of alveolar mucin, but no tumor cells can be seen. C, Deeper sections of same core show larger 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]).*

crine 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 immunostains 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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Diagnosis of Lung Adenocarcinoma in Resected Specimens

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

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• A new lung adenocarcinoma classification has been published by the International Association for the Study of Lung Cancer, the American Thoracic Society, and the European Respiratory Society. This new classification is needed to provide uniform terminology and diagnostic criteria, most especially for bronchioloalveolar carcinoma. It was developed by an international core panel of experts representing all 3 societies with oncologists/pulmonologists, pathologists, radiologists, molecular biologists, and thoracic surgeons.This summary focuses on the aspects of this classification that address resection specimens. The terms *bronchioloalveolar carcinoma* and *mixed subtype adenocarcinoma* are no longer used. For resection specimens, new concepts are introduced, such as adenocarcinoma in situ and minimally invasive adenocarcinoma for small solitary adenocarcinomas with either pure lepidic growth (adenocarcinoma in situ) and predominant lepidic growth with invasion of 5 mm or less (minimally invasive adenocarcinoma), to define the condition of patients who will have 100% or near 100% disease-specific survival, respectively, if they undergo complete lesion resection. Adenocarcinoma in situ and minimally invasive adenocarcinoma are usually nonmucinous, but rarely may be mucinous. Invasive adenocarcinomas are now classified by predominant pattern after using comprehensive histologic subtyping with lepidic (formerly most mixed subtype tumors with nonmucinous bronchioloalveolar carcinoma), acinar, papillary, and solid patterns; micropapillary is added as a new histologic subtype. Variants include invasive mucinous adenocarcinoma (formerly mucinous bronchioloalveolar carcinoma), colloid, fetal, and enteric adenocarcinoma.It is possible that this classification may impact the next revision of the TNM staging classification, with adjustment of the size T factor according to only the invasive component pathologically in adenocarcinomas with lepidic areas.

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A new lung adenocarcinoma classification has recently been published by the International Association for the Study of Lung Cancer (IASLC), the American Thoracic Society (ATS), and the European Respiratory Society (ERS).[1] This classification outlines multiple paradigm shifts that will impact pathologists in many aspects of the diagnosis and classification of lung adenocarcinoma. Unlike previous

Table 1. IASLC/ATS/ERS[a] Classification of Lung Adenocarcinoma in Resection Specimens

Preinvasive lesions
- Atypical adenomatous hyperplasia
- Adenocarcinoma in situ (≤3 cm, formerly BAC)
  - Nonmucinous
  - Mucinous
  - Mixed mucinous/nonmucinous
- Minimally invasive adenocarcinoma (≤3 cm lepidic-predominant tumor with ≤5 mm invasion)
  - Nonmucinous
  - Mucinous
  - Mixed mucinous/nonmucinous

Invasive adenocarcinoma
- Lepidic predominant (formerly nonmucinous BAC pattern, with >5 mm invasion)
- Acinar predominant
- Papillary predominant
- Micropapillary predominant
- Solid predominant with mucin production

Variants of invasive adenocarcinoma
- Invasive mucinous adenocarcinoma (formerly mucinous BAC)
- Colloid
- Fetal (low and high grade)
- Enteric

Abbreviation: BAC, bronchioloalveolar carcinoma.

[a] International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society.

Table 2. Adenocarcinoma In Situ

Diagnostic criteria
- A small tumor ≤3 cm
- A solitary adenocarcinoma
- Pure lepidic growth
- No stromal, vascular, or pleural invasion
- No pattern of invasive adenocarcinoma (such as acinar, papillary, micropapillary, solid, colloid, enteric, fetal,or invasive mucinous adenocarcinoma)
- No intraalveolar tumor cells present
- Cell type mostly nonmucinous (type II pneumocytes or Clara cells), rarely may be mucinous (tall columnar cells with basal nuclei and abundant cytoplasmic mucin, sometimes resembling goblet cells)
- Nuclear atypia is absent or inconspicuous
- Septal widening with sclerosis is common, particularly in nonmucinous adenocarcinoma in situ

Good practice points
- The tumor should be completely sampled. If desired, a small piece may be snap frozen for research if there is no solid component on CT or gross examination and there are no worrisome areas for invasion. This tissue may need to be examined by frozen section if invasion is suspected.
- Size may be underestimated on gross examination, so correlation with CT findings may be necessary to determine tumor size.
- If a solid component is present on CT or on gross examination, the lesion should be evaluated very carefully as this often correlates with an invasive component.
- For adenocarcinoma in situ, particularly mucinous adenocarcinoma in situ, great care must be taken to be sure the lesion is solitary and sharply circumscribed without miliary spread in adjacent lung parenchyma.
- The criteria for adenocarcinoma in situ can be applied in the setting of multiple tumors only if the other tumors are regarded as synchronous primary tumors rather than intrapulmonary metastases.

Abbreviation: CT, computed tomography.

Table 3. Minimally Invasive Adenocarcinoma

Diagnostic criteria
- A small tumor ≤3 cm
- A solitary adenocarcinoma
- Predominantly lepidic growth
- ≤5 mm invasive component in greatest dimension in any 1 focus
- Invasive component to be measured includes (1) any histologic subtype other than a lepidic pattern (such as acinar, papillary, micropapillary, solid, colloid, fetal, or invasive mucinous adenocarcinoma) or (2) tumor cells infiltrating myofibroblastic stroma
- Minimally invasive adenocarcinoma diagnosis is excluded if the tumor (1) invades lymphatics, blood vessels, or pleura or (2) contains tumor necrosis
- Cell type mostly nonmucinous (type II pneumocytes or Clara cells), rarely may be mucinous (tall columnar cells with basal nuclei and abundant cytoplasmic mucin, sometimes resembling goblet cells)

Good practice points
- Same good practice points from Table 1.
- If multiple microinvasive areas are found in 1 tumor, the size of the largest invasive area should be measured in the largest dimension and it should be ≤5 mm. The size of invasion is not the summation of all such foci if more than 1 occurs.
- If the manner of histologic sectioning of the tumor makes it impossible to measure the size of invasion, an estimate of invasive size can be made by multiplying the total percentage of the invasive (nonlepidic) components by the total tumor size.
- As most of the literature on the topic of adenocarcinoma in situ and minimally invasive adenocarcinoma deals with tumors ≤2 or 3 cm, there is insufficient evidence to support the notion that 100% disease-free survival can occur in such tumors >3.0 cm. These tumors should be classified as lepidic-predominant adenocarcinoma, suspect adenocarcinoma in situ, or minimally invasive adenocarcinoma.

World Health Organization (WHO) classifications,[2,3] with this effort a new approach to classification of small biopsy and cytology specimens is presented, and this is the topic of a separate article.[4] The present article is focused on resected specimens (Tables 1 through 3) and the impact of the new classification for pathologists in this setting, the topic primarily addressed in prior WHO classifications.

The frequent histologic heterogeneity of lung adenocarcinoma has presented difficult challenges for both pathologists and classification committees in developing a system that is clinically and biologically relevant. This new classification provides an approach to subtyping lung adenocarcinoma that provides a significant advance over previous classifications such as the 2004 WHO[3] and the Noguchi[5] classifications. First, in contrast to these historical classifications, the IASLC/ATS/ERS classification was developed by an international, multidisciplinary panel, allowing for confusing clinical and pathologic aspects of terminology and criteria to be identified and then addressed. For example, the term *bronchioloalveolar carcinoma* (BAC) was very confusing as it was used in several different ways in the revised classification[1] to encompass 5 different types of lung adenocarcinoma with dramatically different clinical and pathologic characteristics. Also, one of the limitations of previous classifications was the large number of tumors that fell into the "mixed subtype" (greater than 90%)[6] and "type C" (50%–60%)[5,7] categories in the 2004 WHO[3] and Noguchi[5] classifications, respectively, which provided little

Table 4. Summary of Pathology Recommendations Applicable to Resection Specimens

1. We recommend discontinuing the use of the term *bronchioloalveolar carcinoma* (BAC) (strong recommendation, low-quality evidence).
2. For small (≤3 cm), solitary adenocarcinomas with pure lepidic growth, we recommend the term *adenocarcinoma in situ*, which defines patients who should have 100% disease-specific survival if the lesion is completely resected (strong recommendation, moderate-quality evidence). Remark: Most adenocarcinomas in situ are nonmucinous, rarely are they mucinous.
3. For small (≤3 cm), solitary adenocarcinomas with predominant lepidic growth and small foci of invasion measuring ≤0.5 cm, we recommend a new concept of "minimally invasive adenocarcinoma" to define patients who should have near 100% disease-specific survival if the lesion is completely resected (strong recommendation, low-quality evidence). Remark: Most minimally invasive adenocarcinomas are nonmucinous, rarely are they mucinous.
4. For invasive adenocarcinomas, we suggest that comprehensive histologic subtyping be used to assess histologic patterns semiquantitatively in 5% increments, choosing a single predominant pattern. We also suggest that individual tumors be classified according to the predominant pattern and that the percentages of the subtypes be reported (weak recommendations, low-quality evidence).
5. In patients with multiple lung adenocarcinomas, we suggest comprehensive histologic subtyping in the comparison of the complex, heterogeneous mixtures of histologic patterns to determine if the tumors are metastases or separate synchronous or metachronous primary tumors (weak recommendation, low-quality evidence).
6. For nonmucinous adenocarcinomas previously classified as mixed subtype, where the predominant subtype consists of the former nonmucinous BAC, we recommend use of the term *lepidic-predominant adenocarcinoma* and discontinuing the term *mixed subtype* (strong recommendation, low-quality evidence).
7. In patients with early-stage adenocarcinoma, we recommend the addition of "micropapillary-predominant adenocarcinoma," when applicable, as a major histologic subtype owing to its association with poor prognosis (strong recommendation, low-quality evidence).
8. For adenocarcinomas formerly classified as mucinous BAC, we recommend they be separated from the adenocarcinomas formerly classified as nonmucinous BAC and, depending on the extent of lepidic versus invasive growth, that they be classified as mucinous adenocarcinoma in situ, mucinous MIA, or for overtly invasive tumors, as "invasive mucinous adenocarcinoma" (weak recommendation, low-quality evidence).
9. We recommend that the term *non–small cell lung carcinoma* (NSCLC) *not otherwise specified* (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: BAC, bronchioloalveolar carcinoma; MIA, minimally invasive adenocarcinoma.

opportunity to stratify patients according to subtypes with clinically and biologically meaningful correlations. Another limitation of these classifications was the understandable lack of recognition of micropapillary adenocarcinoma, which has emerged in recent years as an important poor prognostic subtype of lung adenocarcinoma in early-stage tumors.[8–10] Furthermore, both the 2004 WHO and Noguchi classifications lumped both mucinous and nonmucinous tumors, previously classified as BAC or Noguchi type A or B patterns, together under the same terminology, when these tumors have very different clinical, radiologic, pathologic, and molecular characteristics.[11–17]

This new classification is timely as it has been published in conjunction with 2 major advances in the lung cancer field where it can have a direct impact: (1) the finding by the National Lung Cancer Screening Trial that there is greater than 20% reduction in mortality in high-risk smokers[18] and (2) the concept of personalized medicine whereby histologic classification can determine therapeutic options for patients with lung cancer, although the latter concept is most applicable in the advanced lung adenocarcinoma setting.[19] As applied to resection specimens, this classification shows promise in stratifying patients for adjuvant therapy,[8,20] and it may ultimately impact the next revision of the TNM staging system by providing more accurate staging of multiple lung adenocarcinomas[21,22] and determining the size T factor according to the invasive size rather than total (invasive plus lepidic components) tumor size.[8,20,23] In both of these arenas, application of this new classification will increase the usefulness of information provided in pathology diagnoses, which will impact patient diagnosis and management.

The international multidisciplinary panel that developed this classification included pathologists, oncologists/respiratory physicians, radiologists, molecular biologists, and thoracic surgeons. It also was based on a systematic literature review to weigh evidence and make recommendations (Tables 4 and 5).[1,24] In this article, the evidence-based recommendations are listed with the strength of the recommendation and quality of the evidence according to the GRADE method (Table 4). Some research recommendations are also made in areas of uncertainty where further investigation is needed (Table 5). These tables include the recommendations taken from the main classification publications that are pertinent to the diagnosis of lung cancer in resection specimens.

DISCONTINUE TERM BRONCHIOLOALVEOLAR CARCINOMA

Many tumors diagnosed as BAC according to the 1999[2] and 2004[3] WHO classifications are now reclassified in the new classification into 5 different entities including (1) adenocarcinoma in situ (AIS) or solitary small noninvasive peripheral lung tumors, associated with a 100% 5-year survival if completely resected[5,8]; (2) minimally invasive adenocarcinomas (MIAs), which are associated with nearly 100% 5-year survival if completely resected[8,25,26]; (3) invasive adenocarcinomas with a lepidic component[27–31]; (4) invasive mucinous adenocarcinoma (former mucinous BAC)[14,27–30]; and (5) widespread advanced-stage adenocarcinomas with a lepidic component, which are associated with a very poor survival rate.[32] Owing to the widespread confusion from the multiple uses of the former *bronchioloalveolar carcinoma* term in the clinical and research arenas, the classification panel concluded that this term was no longer useful and possibly detrimental.[14,33–37]

Pathology Recommendation 1.—We recommend discontinuing the use of the term *bronchioloalveolar carcinoma* (BAC). Strong recommendation, low-quality evidence.

Throughout this article, the term *bronchioloalveolar carcinoma* (applicable in multiple places in the new classification) will be referred to as "former BAC." We understand this will

Table 5. Pathology Research Recommendations Applicable to Resection Specimens

1. Criteria for minimally invasive adenocarcinoma are based on limited published data and require further validation. Persistent questions include the following: What is the optimal method for measuring the size of the invasive component? Is 0.5 cm the best size cutoff? If multiple areas of invasion are present, should the greatest dimension of the largest invasive focus be used or the total size multiplied by the percentage of the invasive components? What should be the impact of scar size or prominent stromal desmoplasia and stromal inflammation on determining size of the invasive component? Should criteria for MIA be different for mucinous versus nonmucinous tumors?
2. Lepidic growth may also be composed of neoplastic cells with nuclear atypia resembling that of the adjacent invasive patterns. Whether there is any clinical implication is unknown, that is, it is not established if this is lepidic (non-invasive) growth or invasive carcinoma.
3. The level of reproducibility for identifying predominant histologic patterns is untested. In particular, how should the lepidic pattern be distinguished from other invasive patterns such as acinar and papillary?
4. Are tumors that meet criteria for minimally invasive adenocarcinoma associated with 100% disease-free survival if the invasive component is predominantly solid, micropapillary or if they show giant cell and spindle cell components that fail to qualify for a diagnosis of pleomorphic carcinoma?
5. What is the long-term follow-up for completely resected solitary mucinous minimally invasive adenocarcinoma? Can this be the initial presentation for multifocal invasive mucinous adenocarcinoma?
6. Does the micropapillary pattern have a similar poor prognostic significance in advanced stage as well as early stage tumors?
7. Is there any prognostic significance to the aggressive micropapillary or solid components when present in relatively small amounts if they do not represent the predominant pattern? If so, what percentage is needed for such significance?
8. The ability of pathologists to distinguish adenocarcinoma in situ from invasive disease at frozen section is not proven.
9. Currently, we cannot recommend any specific grading system. Further investigation is needed to determine whether the optimal grading system should include architectural versus nuclear assessment or both.

Abbreviation: MIA, minimally invasive adenocarcinoma.

be a major adjustment and suggest initially that when the new proposed terms are used, that they be accompanied in parentheses by "(formerly BAC)." This transition will impact, not only clinical practice and research, but also cancer registries' future analyses of registry data.

CLASSIFICATION FOR RESECTION SPECIMENS

The new proposed lung adenocarcinoma classification for resected tumors is summarized in Tables 1 through 3. Major changes include (1) the addition of AIS as a preinvasive lesion to join atypical adenomatous hyperplasia; (2) addition of MIA; (3) classification of invasive adenocarcinomas according to the predominant subtype after comprehensive histologic subtyping by semiquantitatively estimating the percentage of the various subtypes present in 5% increments; (4) use of the term *lepidic* for invasive adenocarcinomas that have a noninvasive component previously classified as BAC; (5) discontinuing the term *mixed subtype;* (6) introducing the term *invasive mucinous adenocarcinoma* for adenocarcinomas formerly classified as mucinous BAC, excluding tumors that meet criteria for AIS or MIA; (7) discontinuing the subtypes of clear cell and signet ring adenocarcinoma and recognizing these as a cytologic feature when any amount is present, however small; and (8) discontinuing the term *mucinous cystadenocarcinoma* and including this entity under the category of colloid adenocarcinoma.

PREINVASIVE LESIONS

In the 1999[2] and 2004[3] WHO classifications, atypical adenomatous hyperplasia was recognized as a preinvasive lesion for lung adenocarcinoma. This was based on multiple studies documenting these lesions as incidental findings in the adjacent lung parenchyma in 5% to 23% of resected lung adenocarcinomas,[38–42] as well as several molecular findings that demonstrated a relationship to lung adenocarcinoma, including clonality,[43,44] *KRAS* (Kirsten rat sarcoma) mutation,[45,46] *KRAS* polymorphism,[47] epidermal growth factor receptor (*EGFR*) mutation,[48,49] p53 expression,[50] loss of heterozygosity,[51] methylation,[52] telomerase overexpression,[53] eukaryotic initiation factor 4E (*eIF4E*) expression,[54] epigenetic alterations in the WNT pathway,[55] and fragile histidine triad (*FHIT*) expression.[56] Depending on the extensiveness of the search, atypical adenomatous hyperplasia lesions may be multiple in up to 7% of resected lung adenocarcinomas.[39,57]

A major change in this classification is the official recognition of adenocarcinoma in situ as a second preinvasive lesion for lung adenocarcinoma in addition to atypical adenomatous hyperplasia. In the category of preinvasive lesions, atypical adenomatous hyperplasia is the counterpart to squamous dysplasia, and adenocarcinoma in situ is the counterpart to squamous cell carcinoma in situ.

Atypical Adenomatous Hyperplasia

Atypical adenomatous hyperplasia is a localized, small (usually 0.5 cm or less) proliferation of mildly to moderately atypical type II pneumocytes and/or Clara cells lining alveolar walls and sometimes, respiratory bronchioles (Figure 1, A and B).[3,58,59] Gaps along the basement membrane are usually seen between the cells, which consist of rounded, cuboidal, low columnar, or "peg" cells with round to oval nuclei (Figure 1, B). There is a continuum of morphologic changes between atypical adenomatous hyperplasia and adenocarcinoma in situ.[3,58,59] A spectrum of cellularity and atypia occurs in atypical adenomatous hyperplasia. Although some have classified atypical adenomatous hyperplasia into low- and high-grade types,[53,60] such grading is not recommended.[3] Distinction between atypical adenomatous hyperplasia that is more cellular and cytologically atypical, and adenocarcinoma in situ, can be difficult histologically and impossible cytologically. The 0.5-cm size is not an absolute criterion; therefore, multiple characteristics, including size and architectural and cytologic features, are needed to separate lesions of atypical adenomatous hyperplasia that are more cellular and atypical from adenocarcinoma in situ.

Adenocarcinoma In Situ, Nonmucinous and/or Mucinous

Adenocarcinoma in situ (one of the lesions formerly known as BAC), is a localized small (≤3 cm) adenocarcinoma with growth restricted to neoplastic cells along

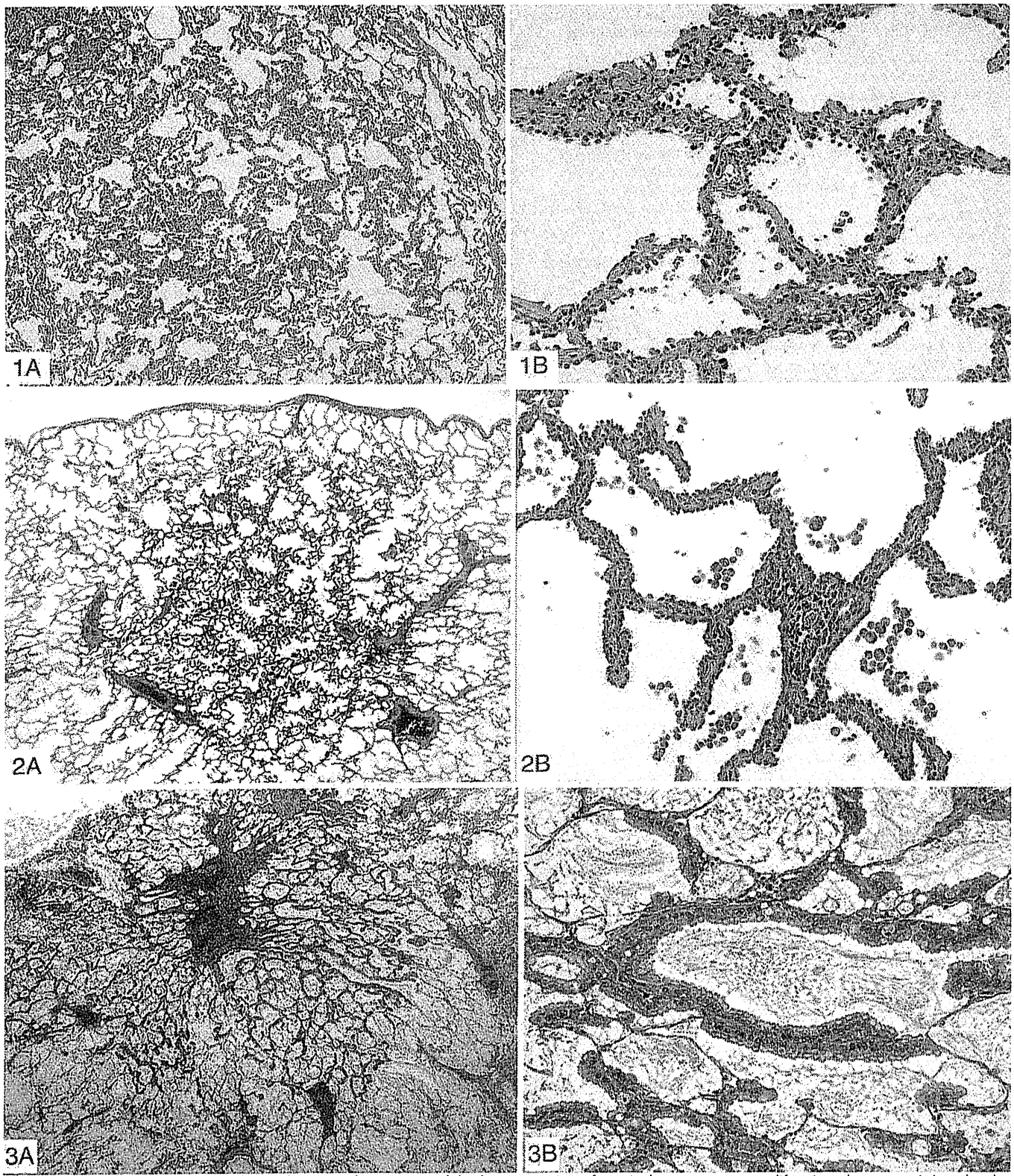

Figure 1. *Atypical adenomatous hyperplasia. A, This 3-mm nodular lesion consists of atypical pneumocytes proliferating along preexisting alveolar walls. There is no invasive component. B, The slightly atypical pneumocytes are cuboidal and show gaps between the cells. Nuclei are hyperchromatic and a few show nuclear enlargement and multinucleation (hematoxylin-eosin, original magnifications ×4 [A] and ×40 [B]).*

Figure 2. *Nonmucinous adenocarcinoma in situ. A, This circumscribed nonmucinous tumor grows purely with a lepidic pattern. No foci of invasion or scarring is seen. B, The tumor shows atypical pneumocytes proliferating along the slightly thickened, but preserved, alveolar walls (hematoxylin-eosin, original magnifications X4 [A] and X40 [B]).*

Figure 3. *Mucinous adenocarcinoma in situ (AIS). A, This mucinous AIS consists of a nodular proliferation of mucinous columnar cells growing in a purely lepidic pattern. Although there is a small central scar, no stromal or vascular invasion is seen. B, The tumor cells consist of cuboidal to columnar cells with abundant apical mucin and small, basally oriented nuclei (hematoxylin-eosin, original magnifications ×4 [A] and ×40 [B]). Reproduced with permission from Travis et al.*[1]

preexisting alveolar structures (lepidic growth), lacking stromal, vascular, or pleural invasion. Papillary or micropapillary patterns and intra-alveolar tumor cells are absent (Table 2). Adenocarcinoma in situ is subdivided into nonmucinous and mucinous variants. Virtually all cases of adenocarcinoma in situ are nonmucinous, consisting of type II pneumocytes and/or Clara cells (Figure 2, A and B). There is no recognized clinical significance to the distinction between type II or Clara cells; therefore, this morphologic separation is not recommended. The rare cases of mucinous adenocarcinoma in situ consist of tall columnar cells with basal nuclei and abundant cytoplasmic mucin; sometimes they resemble goblet cells (Figure 3, A and B). Nuclear atypia is absent or inconspicuous in both nonmucinous and mucinous adenocarcinoma in situ (Figures 2, B, and 3, B). Septal widening with sclerosis is common in adenocarcinoma in situ, particularly the nonmucinous variant.

Lesions that meet the criteria for adenocarcinoma in situ have formerly been classified as BAC according to the strict definition of the 1999[2] and 2004[3] WHO classifications and as type A and B adenocarcinoma according to the 1995 Noguchi classification.[5] Multiple observational studies on solitary lung adenocarcinomas with pure lepidic growth, smaller than either 2 or 3 cm, have documented 100% disease-free survival when the lesions are completely resected.[5,61–67] While most of these tumors are nonmucinous, 2 of the 28 tumors reported by Noguchi et al[5] as type A and B in the 1995 study were mucinous. Small size (≤3 cm) and a discrete circumscribed border are important to exclude cases with miliary spread into adjacent lung parenchyma and/or lobar consolidation, particularly for mucinous AIS. This is because the data that indicate 100% 5-year disease-free survival associated with resected AIS are mostly in series of tumors 2 cm or less, with some series including tumors up to 3 cm in diameter; moreover, there are few data regarding mucinous AIS.[5,61–67]

The criteria for AIS as well as MIA can be applied in the setting of multiple tumors only if the other tumors are regarded as synchronous primary tumors rather than intrapulmonary metastases.

Pathology Recommendation 2.—For small (≤3 cm), solitary adenocarcinomas with pure lepidic growth, we recommend the term *adenocarcinoma in situ*, which defines patients who should have 100% disease-specific survival if the lesion is completely resected (strong recommendation, moderate-quality evidence).

Remark: Almost all adenocarcinomas in situ are nonmucinous, rarely are they mucinous.

MINIMALLY INVASIVE ADENOCARCINOMA, NONMUCINOUS AND/OR MUCINOUS

Minimally invasive adenocarcinoma is a small, solitary adenocarcinoma (≤3 cm), with a predominantly lepidic pattern and invasion of 5 mm or less in greatest dimension in any one focus (Table 2).[25,26,68] It is usually nonmucinous (Figure 4, A through C) but rarely may be mucinous (Figure 5, A and B).[8] Minimally invasive adenocarcinoma is, by definition, solitary and discrete.

The invasive component to be measured in MIA is defined as follows: (1) histologic subtypes other than a lepidic pattern (ie, acinar, papillary, micropapillary, and/or solid) or (2) tumor cells infiltrating myofibroblastic stroma. Minimally invasive adenocarcinoma is excluded if the tumor (1) invades lymphatics, blood vessels, or pleura or (2) contains tumor necrosis. If multiple microinvasive areas are found in 1 tumor, the size of the largest invasive area should be measured in its greatest dimension and it should be 5 mm or less in size. The size of invasion is not the summation of all such foci, if more than 1 occurs. This approach was arbitrarily adopted from the approach recommended by the Collage of American Pathologists for measurement of the invasive component of breast cancers that have multiple foci.[69] If the manner of histologic sectioning of the tumor makes it impossible to measure the size of invasion, an estimate of invasive size can be made by multiplying the total percentage of the invasive (nonlepidic) components by the total tumor size. More investigation is needed to determine whether the diagnosis of MIA is best made by using percentage of the invasive component versus the single largest focus of invasion, as recommended in breast cancer.

Evidence for a category of MIA with 100% disease-free survival can be found in the 1995 article by Noguchi et al,[5] in which vascular and/or pleural invasion was found in 10% of the small solitary lung adenocarcinomas that otherwise met the former definition of pure BAC. Even these focally invasive tumors were associated with 100% disease-free survival.[5] Subsequent articles by Sakurai et al[70] and Suzuki et al[71] defined subsets of small lung adenocarcinomas associated with 100% disease-free survival by using scar size less than 5 mm and stromal invasion in the area of bronchioloalveolar growth, respectively. More recently, articles by Borczuk et al,[25] Yim et al,[26] and Maeshima et al[68] have described patients with MIA, defined similarly as in the aforementioned criteria, who have had near 100% disease-specific or very favorable overall survival. There are very limited data regarding mucinous MIA; however, this entity appears to exist. A mucinous MIA with a minor mixture of a nonmucinous component has been reported with no recurrence after 7.4 years.[8] The recent report by Sawada et al[12] of localized mucinous BAC may have included a few cases of mucinous AIS or MIA, but details of the pathology are not specific enough for certainty. A recent series of surgically resected solitary mucinous BACs did not document histologically whether focal invasion was present or not; therefore, AIS versus MIA status cannot be determined, but all 8 patients with tumors measuring 3 cm or less had 100% overall 5-year survival.[72] The diagnosis of AIS or MIA should not be made unless the lesion has a discrete circumscribed border; cases with miliary spread of small foci of tumor into adjacent lung parenchyma and/or with lobar consolidation should be excluded. Mucinous AIS or MIAs are extremely rare and these diagnoses need to be made with caution, as most tumors with this histologic appearance will be invasive mucinous adenocarcinomas (see below).

Also, it remains to be determined if patients with MIA will still have a 100% disease-free survival if the area of invasion shows a poorly differentiated component, such as solid or micropapillary adenocarcinoma, or if there is a giant and spindle cell component that does not meet criteria for pleomorphic carcinoma.

Pathology Recommendation 3.—For small (≤3 cm), solitary adenocarcinomas with predominant lepidic growth and small foci of invasion measuring 0.5 cm or less, we recommend the new concept of "minimally invasive adenocarcinoma (MIA)" to define patients who have near 100% disease-specific survival if

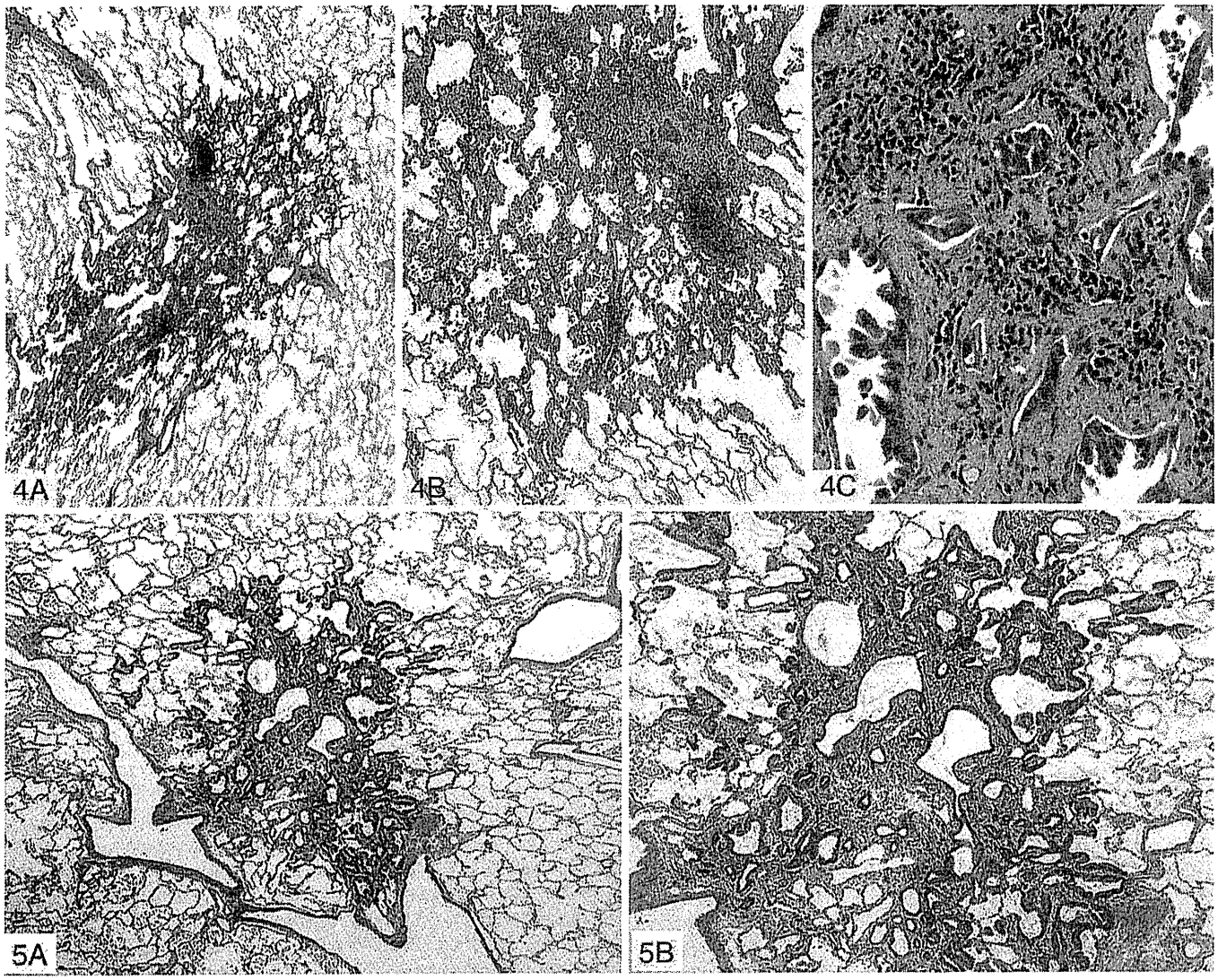

Figure 4. *Nonmucinous minimally invasive adenocarcinoma. A, This subpleural adenocarcinoma tumor consists primarily of lepidic growth with a small (<0.5 cm) central area of invasion. B, To the left is the lepidic pattern and on the right is an area of acinar invasion. C, These acinar glands are invading in the fibrous stroma (hematoxylin-eosin, original magnifications ×4 [A], ×10 [B], and ×40 [C]).*

Figure 5. *Mucinous minimally invasive adenocarcinoma (MIA). A, This mucinous MIA consists of a tumor showing lepidic growth and a small (<0.5 cm) area of invasion. B, The tumor cells consist of mucinous columnar cells growing mostly in a lepidic pattern along the surface of alveolar walls. The tumor invades the areas of stromal fibrosis in an acinar pattern (hematoxylin-eosin, original magnifications ×4 [A] and ×10 [B]). Reproduced with permission from Travis et al.*[1]

the lesion is completely resected (strong recommendation, low-quality evidence).

Remark: Most minimally invasive adenocarcinomas are nonmucinous, rarely are they mucinous.

TUMOR SIZE AND SPECIMEN PROCESSING ISSUES

The Entire Tumor Must Be Sampled for Diagnosis of AIS or MIA

The diagnosis of AIS or MIA cannot be firmly established without histologic sampling of the entire tumor. In a research setting, tissue procurement for frozen tissue banking is encouraged, but in potential AIS and MIA lesions, attention needs to be given to cases for which there is a need to examine the frozen sample histologically. For tumor procurement issues in AIS and MIA, see section on "Molecular-Histologic Correlations."

Radiologic-Pathologic Correlation for Tumor Size Assessment in Lepidic-Predominant Tumors

It can be difficult to appreciate tumor size on gross examination in lepidic-predominant tumors, and the size recorded by the prosector can underestimate actual tumor size. In such cases, it can be helpful to review computed tomography (CT) scans, which may more accurately demonstrate the size of the tumor, including the ground-glass versus solid components, which usually correspond to the lepidic versus invasive components histologically. If review of the CT reveals a discrepancy with the histologic findings, based on review of initial sections, further sampling of the gross specimen may be needed to make an accurate assessment of the extent of lepidic versus invasive components. An initial pathologic diagnosis of AIS or MIA may need to be reconsidered if the CT shows the tumor to be larger than 3 cm or to have a solid component

larger than 0.5 cm. Adenocarcinoma in situ will typically be a pure ground-glass nodule and MIA will have a predominant ground-glass component with a solid component that will typically be 5 mm or less in size. Both of these tumors should also measure 3 cm or less in total size.[1]

Suspected AIS or MIA Measuring Larger Than 3.0 cm

As most of the literature on the topic of AIS and MIA deals with tumors 2.0 or 3.0 cm or less, there is insufficient evidence to support the notion that 100% disease-free survival can occur with completely resected solitary tumors larger than 3.0 cm that are suspected to be AIS or MIA. Until data validate 100% disease-free survival for completely resected, solitary adenocarcinomas larger than 3.0 cm suspected of being AIS or MIA after complete sampling, the term *lepidic-predominant adenocarcinoma, suspect AIS or MIA* is suggested. If such a tumor larger than 3.0 cm has not been completely sampled, the term *lepidic-predominant adenocarcinoma* is best applied with a comment that an invasive component cannot be excluded.

Number of Sections to Submit for Overtly Invasive Adenocarcinomas

For overtly invasive adenocarcinomas, at least 1 section should be submitted per centimeter of the maximal tumor diameter. Additional sections may be helpful for tumors in which the extent of lepidic versus invasive growth is in question. It can be helpful to sample the interface between the tumor and adjacent nonneoplastic lung parenchyma to identify areas of tumor spread that may not be visible on gross examination.

Optimal Specimen Fixation

No effort was made in this IASLC/ATS/ERS classification to address optimal fixation of specimens for immunohistochemistry or molecular testing. However, 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) resected specimens should be sectioned at 5-mm intervals, and (3) specimens should be fixed for at least 6 hours, but not longer than 48 hours.[73,74] For lung cancer, no data have addressed specimen processing issues for immunohistochemistry or molecular testing as exist for breast cancer, so this is a topic that needs more study before specific recommendations can be made.

INVASIVE ADENOCARCINOMA

As the invasive adenocarcinomas represent more than 70% to 90% of surgically resected lung cases, one of the most important aspects of this classification is to present a practical method to address these tumors, which are often composed of a complex heterogeneous mixture of histologic subtypes. This complex mixture of histologic subtypes has presented one of the greatest challenges to classification of invasive lung adenocarcinomas. In recent years, multiple independent research groups[6,20,21,75–84] have begun to classify lung adenocarcinomas according to the most predominant subtype. This approach provides better stratification of the "mixed subtype" lung adenocarcinomas according to the 1999[2]/2004[3] WHO classifications and has allowed for novel correlations between histologic subtypes and both molecular and clinical features.[6,20,21,75–84]

In the revised classification, the term *predominant* is appended to all categories of invasive adenocarcinoma, as most of these tumors consist of mixtures of the histologic subtypes (Figure 6, A through C). This replaces the use of the term *adenocarcinoma, mixed subtype.* Semiquantitative recording of the patterns in 5% increments encourages the observer to identify all patterns that may be present, rather than focusing on a single pattern (ie, lepidic growth). This comprehensive histologic subtyping should be performed by review of all histologic sections of the tumor. Thus, this method provides a basis for choosing the predominant pattern. While most previous studies on this topic used 10% increments, using 5% increments allows for greater flexibility in choosing a predominant subtype when tumors have 2 patterns of relatively similar percentages; it also avoids the need to use 10% for small amounts of components that potentially may be prognostically important, such as micropapillary or solid patterns. Even though it is possible to have equal percentages of 2 prominent components, a single predominant component should be chosen. Recording of these percentages makes it clear to the reader of a report when a tumor has relatively even mixtures of several patterns versus a clear single predominant pattern. In addition, it provides a way to compare the histologic features of multiple adenocarcinomas (see below).[21] This approach may also provide a basis for architectural grading of lung adenocarcinomas.[78] A reproducibility study of classical and difficult selected images of the major lung adenocarcinoma subtypes, which were circulated among a panel of 26 expert lung cancer pathologists, documented κ values of 0.77 ± 0.07 and 0.38 ± 0.14, respectively.[85] A recent study of reproducibility for predominant pattern[86] showed moderate to good κ values of 0.44 to 0.72 for pulmonary pathologists. For untrained pathologists, κ values were expectedly lower, ranging from 0.38 to 0.47, but these improved to 0.51 to 0.66 after a training session, and reevaluation by the same reviewers led to very high κ values between 0.79 and 0.87.

The histologic subtypes of invasive lung adenocarcinomas encompass a spectrum of histologic patterns that represent a morphologic continuum rather than distinct entities. This concept helps to understand why in some cases it is difficult to distinguish between morphologic patterns, for example, lepidic versus acinar or papillary patterns and papillary versus micropapillary patterns. Nevertheless, since this classification was published, a growing number of studies of resected lung adenocarcinomas[8,20,77,78,84,87–90] have demonstrated its utility in identifying significant prognostic subsets and molecular correlations according to the predominant patterns.

Pathology Recommendation 4.—For invasive adenocarcinomas, we suggest comprehensive histologic subtyping be used to assess histologic patterns semiquantitatively in 5% increments, and then choosing a single predominant pattern Individual tumors are then classified according to the predominant pattern and the percentages of the subtypes are also reported (weak recommendation, low-quality evidence).

Histologic Comparison of Multiple Adenocarcinomas and Impact on Staging

Comprehensive histologic subtyping can be useful in comparing multiple lung adenocarcinomas in a single

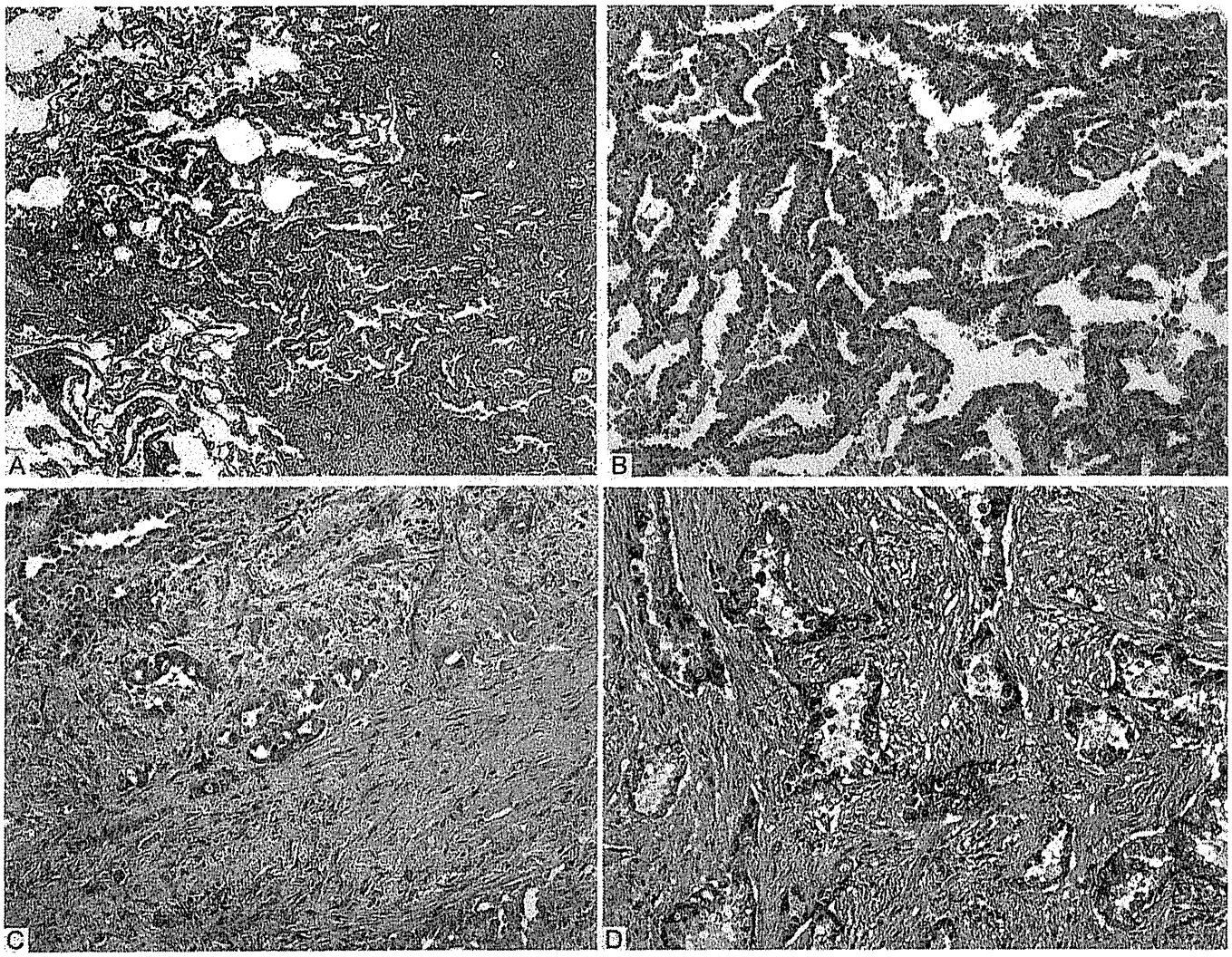

Figure 6. *Lepidic-predominant and acinar adenocarcinoma. A, Lepidic-predominant pattern with mostly lepidic growth (right) and a smaller area of invasive acinar adenocarcinoma (left). B, Lepidic pattern consists of a proliferation of type II pneumocytes and Clara cells along the surface of alveolar walls. C, Area of invasive acinar adenocarcinoma (same tumor as in A and B). D, Acinar adenocarcinoma consists of round to oval malignant glands invading a fibrous stroma (hematoxylin-eosin, original magnifications ×4 [A], ×20 [B and D], and ×10 [C].*

patient in order to distinguish multiple primary tumors from intrapulmonary metastases. This has a great impact on staging for patients with multiple lung adenocarcinomas. Recording the percentages of the various histologic types in 5% increments, not just the most predominant type, allows these data to be used to compare multiple adenocarcinomas, particularly if the slides of a previous tumor are not available at the time of review of the additional lung tumors.[21] In addition to comprehensive histologic subtyping, other histologic features of the tumors, such as cytologic (clear cell or signet ring features) or stromal (desmoplasia or inflammation) characteristics, may be helpful to compare multiple tumors.[21]

Pathology Recommendation 5.—In patients with multiple lung adenocarcinomas, we suggest that comprehensive histologic subtyping may facilitate comparison of the complex, heterogeneous mixtures of histologic patterns for determining if the tumors are metastases or separate synchronous or metachronous primary tumors (weak recommendation, low-quality evidence).

Lepidic-predominant adenocarcinoma typically consists of bland pneumocytic cells (type II pneumocytes or Clara cells) growing along the surface of alveolar walls with morphology similar to that defined in the above section on AIS and MIA (Figure 6, A and B). Invasive adenocarcinoma is present in at least 1 focus, measuring more than 5 mm in greatest dimension. Invasion is defined as (1) histologic subtypes other than a lepidic pattern (ie, acinar, papillary, micropapillary, and/or solid) and/or (2) myofibroblastic stroma associated with invasive tumor cells (Figure 6, C). The diagnosis of lepidic-predominant adenocarcinoma rather than MIA is made if the cancer (1) invades lymphatics, blood vessels, or pleura or (2) contains tumor necrosis. It is understood that lepidic growth can occur in metastatic tumors as well as in invasive mucinous adenocarcinomas. However, the specific term *lepidic-predominant adenocarcinoma* in this classification defines a nonmucinous adenocarcinoma that has lepidic growth as its predominant component, and these tumors are now separated from invasive mucinous adenocarcinoma. The term *lepidic-predominant adenocarcinoma* should not be used in the

context of invasive mucinous adenocarcinoma with predominant lepidic growth.

Lepidic growth may also be composed of neoplastic cells with nuclear atypia resembling that of the adjacent invasive patterns. Whether there is any clinical implication is unknown, that is, it is not established if this is in situ or invasive carcinoma. This point is made in the classification as a research recommendation to encourage further investigation of this issue (Table 5).

In the categories of mixed subtype in the 1999[2]/2004[3] WHO classifications and of type C in the Noguchi classification,[5] respectively, there was no assessment of the percentage of lepidic growth (former BAC pattern); therefore, in published series diagnosed according to these classification systems, most of the lepidic-predominant adenocarcinomas are buried among a heterogeneous group of tumors that include predominantly invasive adenocarcinomas. However, several studies[25,63,91–93] have shown that lepidic growth is associated with more favorable survival in cases of small solitary resected lung adenocarcinomas with an invasive component. Using this approach, several recent studies of early stage adenocarcinomas[8,20,84] demonstrated excellent outcome for these patients, with as high as 86% to 90% 5-year recurrence-free survival.

Pathology Recommendation 6.—For nonmucinous adenocarcinomas previously classified as mixed subtype, for which the predominant subtype consists of the former nonmucinous BAC, we recommend use of the term *lepidic-predominant adenocarcinoma* and discontinuation of the term *mixed subtype* (strong recommendation, low-quality evidence).

Acinar-predominant adenocarcinoma shows a majority component of glands, which are round to oval with a central luminal space surrounded by tumor cells (Figure 6, D).[3] The neoplastic cells and/or glandular spaces may contain mucin. Acinar structures also may consist of rounded aggregates of tumor cells with peripheral nuclear polarization with central cytoplasm without a clear lumen. Adenocarcinoma in situ with collapse may be difficult to distinguish from the acinar pattern. However, when the alveolar architecture is lost and/or myofibroblastic stroma is present, invasive acinar adenocarcinoma is considered present. Cribriform arrangements are regarded as a pattern of acinar adenocarcinoma.[94]

Papillary-predominant adenocarcinoma shows a major component of a growth of glandular cells along central fibrovascular cores (Figure 7, A and B).[3] This should be distinguished from tangential sectioning of alveolar walls in an area of lepidic adenocarcinoma. If a tumor has lepidic growth, but the alveolar spaces are filled with papillary structures, the tumor is classified as papillary adenocarcinoma. Myofibroblastic stroma is not needed to diagnose this pattern.

Micropapillary-predominant adenocarcinoma has tumor cells growing in papillary tufts (florets that lack fibrovascular cores; Figure 7, C and D).[3] These may appear detached and/or connected to alveolar walls. The tumor cells are usually small and cuboidal with minimal nuclear atypia. Ringlike glandular structures may "float" within alveolar spaces. Vascular and stromal invasion is frequent. Psammoma bodies may be seen.

The micropapillary pattern of lung adenocarcinoma was cited in the 2004 WHO classification[3] in the discussion, but there were too few publications on this topic to introduce it as a formal histologic subtype.[9,10,95] While most of the studies have used a very low threshold for classification of adenocarcinomas as micropapillary, including as low as 1% to 5%,[9,10] recent reports[8,20,84,87] have demonstrated that tumors classified as micropapillary, according to the predominant subtype, also have a poor prognosis similar to adenocarcinomas with a predominant solid subtype. All articles on the topic of micropapillary lung adenocarcinoma in patients with early-stage disease have reported data indicating this is a poor prognostic subtype.[8–10,75,78,96–103]

Additional evidence for the aggressive behavior of this histologic pattern is the overrepresentation of the micropapillary pattern in metastases compared to the primary tumors, where it sometimes comprises only a small percentage of the overall tumor.[78] The clinical significance of minor micropapillary components in primary lung adenocarcinomas that are not micropapillary predominant needs further study.

Pathology Recommendation 7.—For patients with early-stage adenocarcinoma, we recommend the addition of "micropapillary-predominant adenocarcinoma," when applicable, as a major histologic subtype owing to its association with poor prognosis in early-stage disease (strong recommendation, low-quality evidence).

Solid-predominant adenocarcinoma with mucin production shows a major component of polygonal tumor cells forming sheets that lack recognizable patterns of adenocarcinoma, that is, acinar, papillary, micropapillary, or lepidic growth (Figure 8, A through C).[3] If the tumor is 100% solid, intracellular mucin should be present in at least 5 tumor cells in each of 2 high-power fields, confirmed with histochemical stains for mucin (Figure 8, B).[3] Solid adenocarcinoma must be distinguished from squamous cell carcinomas and large cell carcinomas, both of which may show rare cells with intracellular mucin. Some solid adenocarcinomas have dense eosinophilic cytoplasm that resembles that of squamous cell carcinoma with a "pseudosquamous" morphology. Even in resection specimens, in poorly differentiated tumors that have a suggestion of squamous morphology (Figure 8, A) but lack clear squamous morphology, such as keratinization, pearls, or bridges, immunohistochemistry may be indicated with an adenocarcinoma marker such as thyroid transcription factor-1 (TTF-1) (Figure 8, C) and a squamous marker, such as p63 or the recently described p40, which is an isomer of p63 with greater specificity for squamous cell carcinoma.[104]

Neuroendocrine immunohistochemical markers should only be used in cases for which there is suspected neuroendocrine morphology. If neuroendocrine morphology is not suspected, neuroendocrine markers should not be used.

VARIANTS

Rationale for Changes in Adenocarcinoma Histologic Variants

Rationale for Separation of Invasive Mucinous Adenocarcinoma (Formerly Mucinous BAC) from Nonmucinous Adenocarcinomas.—Multiple studies[11,13–15,17,105–109] indicate that tumors formerly classified as mucinous BAC have major