factor receptors–tyrosine kinase inhibitors resulting in enhanced therapeutic response.[11–13] Additionally, a recombinant humanized monoclonal antibody inhibitor of vascular endothelial growth factor, bevacizumab (Avastin; Genentech, Inc, San Francisco, California), which has been found to be effective when used in combination with standard first-line chemotherapy, has been associated with life-threatening hemorrhage in lung SCC, particularly the cavitating variant. The addition of the antifolate agent pemetrexed to conventional chemotherapy provides increased efficacy in nonsquamous carcinomas, but not in SCC.[14] Therefore, histologic differentiation of NSCLCs is vital for improved treatment response and reduces adverse effect.[15]

In most cases, standard hematoxylin-eosin (H&E) evaluation provides sufficient information to classify NSCLC cases. However, accurate diagnosis can be limited in small biopsies or cytology specimens, in poorly differentiated neoplasms, and in cases where there is marked disruption of histologic architecture. A review of 303 primary lung cancer resections showed that 72% were diagnosed by biopsy or cytology, either alone or in conjunction.[16] In addition to limited tissue available for review, the concordance rates among pathologists vary significantly. In 2006, concordance rates among pathologists subtyping NSCLCs on H&E alone were as low as 81%.[17] Furthermore, NSCLCs represent primary lung malignancies with histologically indistinguishable counterparts in other organs. This leads to a potential diagnostic pitfall in both ACA and SCC due to the difficulty in differentiating a primary lung carcinoma from histologically identical lung metastasis with an unidentified primary source. Misdiagnosis in cases of unidentified primary lesions with metastasis to the lung can result in understaging and suboptimal treatment. Thus far, lung specific markers for SCC have been limited, whereas napsin A and thyroid transcription factor 1 (TTF-1) have proven to be useful in the diagnosis of primary lung ACAs.

A diagnosis of SCC is supported by presence of unequivocal cytoplasmic keratinization, squamous pearls, and desmosomes. A mucin stain is a useful adjunct to conventional H&E in diagnosis of ACA to demonstrate glandular differentiation or lepidic growth pattern. Lack of these features may render an accurate classification difficult on H&E stain, and the difficulty may be compounded by the limited tissue on fine-needle biopsies, which are being more frequently used for diagnosis.[17]

The implementation of immunohistochemistry has become a well-supported and accessible tool for the accurate diagnosis and typing of carcinoma, including primary lung carcinomas. Additionally, immunohistochemistry-derived immunophenotypes can allow for organ-specific differentiation of histologically indistinguishable primary and metastatic carcinomas. Given the therapeutic and prognostic information that stems from an accurate histologic typing of lung cancers, a number of immunohistochemical markers have been studied.[18] Most of the studies recommend use of panels of antibodies rather than a single antibody to increase the sensitivity and specificity. The panels have included TTF-1 with carcinoembryonic antigen for ACA and cytokeratin (CK) 5/6 and p63-desmoglein 3 (DG3) for SCC,[18–20] as well as TTF-1 and napsin A for ACA and p63 with CK5/6 for SCC.[21]

Thyroid transcription factor 1 is a 38-kDa homeodomain protein that shows nuclear-specific staining. It regulates gene expression in the thyroid, lungs, and diencephalon during embryogenesis. It is normally expressed in alveolar pneumocytes, Clara cells, ciliated respiratory epithelial cells, and basal cells of the lung.[22] The use of TTF-1 has been well established for differentiation between primary and metastatic ACA of the lung. Although TTF-1 is considered a relatively restricted marker with high sensitivity, the reported sensitivity for lung ACA has been as low as 54%.[23–30]

Napsin A is a newer antibody marker for pulmonary ACAs. It is a functional aspartate proteinase involved in the maturation of prosurfactant protein B in type II pneumocytes, and in the maturation of the biologically active surfactant protein B. This single chain protein is normally expressed in type II pneumocytes, alveolar macrophages, renal tubules, exocrine glands, and pancreatic ducts.[24,31] The role of napsin A in differentiating primary from metastatic ACA of the lung has been previously reported.[21,23,32–34] Although it may occasionally stain nonpulmonary ACAs, it is a highly useful marker in differentiating primary lung ACAs from SCCs.[33–35] Positive immunohistochemical stain shows intense granular cytoplasmic reactivity.[21,31,34,35]

p63 is a member of the p53 family. Located on chromosome 3q27–29, it is involved in the regular growth and development of epithelial tissue.[36] In normal tissues, p63 has been reported to be positive in basal cells of all squamous epithelia, in basal cells of urothelium, and in basal cells of prostate epithelium.[37,38] p63 is detectable in most SCCs of various primary sites, including SCCs of lung, with reported positivity of 80% to 97% in most studies.[39–43] In the lung, however, p63 has been shown to have some overlap in ACAs, with reported positivity in up to 18%.[37–40] To correctly interpret p63, only nuclear staining should be considered as positive.

Also from the p53 family, p40 appears to be a specific marker of squamous cell differentiation related to the nontransactivating isoforms of the p63 gene family of nuclear transcription factors.[44–46] The p63 gene encodes diverse messenger RNA isoforms, which are generated by the activity of 2 different promoters that leads to the accumulation of transactivating p63 isoforms (ΔNp63-p40), which may act as negative dominant agents that lead to the stimulation of cell proliferation, block apoptosis, and allow for unrestrained tumor cell growth.[46] There are very few reports of ΔNp63-p40 expression in lung cancers.[42,44,45]

Cytokeratins are the dominant, intermediate filament proteins of the epithelial cells. Cytokeratins 5 and 6 are related proteins that can be detected in normal cells, including breast myoepithelial cells, prostate basal cells, and the basal layer of the epidermis and salivary glands. Positive immunohistochemical staining displays a membranous staining pattern. Marson et al[25] reported positive staining in 100% of primary lung SCCs, which may be an overestimation in our experience.

Desmogleins are one of the major glycoproteins of the desmosomal structure found in epithelial cells. They are calcium-dependent adhesion molecules that belong to the cadherin superfamily and link to CK filaments via desmoplakins and plakoglobin. Therefore, their expression is also membranous and not cytoplasmic in epithelial cells. Furthermore, DG3 in particular has an important role in cellular adhesion of stratified epithelia such as squamous epithelium and is highly expressed in SCCs of the lung.[47–50] Savci-Heijink et al[20] described a sensitivity of 99% and specificity of 87% for SCCs in primary tumors from different