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REVIEW

# Tumors in the lung – morphologic features and the challenge of integrating biomarker signatures into diagnostics

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**Dedicated to Prof. Dres. Dres. h. c. Klaus Kayser.**

## Abstract

In the last decade, pathologic approaches concerning diagnosis and treatment of lung carcinomas have increasingly moved towards the implementation of molecular methods into the process of decision. In this study, an overview is given referring to the variety of tumors in the lung including common primary lung neoplasms and secondary tumors, and a modus operandi is presented which integrates immunology as well as molecular pathology within the process of finding correct diagnoses. Besides the conventional and approved methods and techniques leading to appropriate treatment including so-called targeted therapies, pathologist's work meanwhile depends on both histologic and molecular results. Since molecular techniques have increasingly entered the field of routine diagnostics, challenges and possibilities have changed and are still rapidly developing. The proceeding integration of molecular-biologic investigations into the process of diagnosing has changed the nature of diagnostics and will continuously grow in the near future. Only by obtaining a proper diagnosis, the optimal treatment of a patient can be assured, whereupon the knowledge of gene mutations and/or altered protein expression is crucial. By identifying those novel molecular target structures, the therapeutic spectrum is tremendously enlarged and will finally improve the patient's prognosis by personalized targeted therapies.

***Keywords*:** lung cancer, diagnosis, immunohistochemistry, EGF-R, RAS, molecular pathology.

## Introduction

The majority of tumors in the lung is malignant, either primaries or metastases. Only around 1% are benign. Lung cancer is the leading cause of cancer death in the world and its prognosis in general is poor. Patients with suspicion of lung cancer require a histologic diagnosis and disease staging before appropriate concepts of treatment can be established. The basis of any therapy is the exact diagnosis of the type and progression of the disease. Until recently, it was of paramount importance for clinicians to distinguish Small Cell Lung Carcinomas (SCLC) from Non-Small Cell Lung Carcinomas (NSCLC) on account of the different treatment options available. For the former the main strategy was a combined chemotherapy supported by local therapeutic measures, depending on the stage of the disease. The latter submits itself to surgery, radiology, and chemotherapy. A more precise description of the tumor given by pathologists in the past was often considered to be of no real relevance. Meanwhile, looking at the upcoming so-called targeted therapies, the diagnostic requirements for the pathologists are increasing and include molecular investigations like gene amplification or mutation analysis, which scrutinize the malignancy-related tissues on the nucleic acid level.

Four major types of neoplasms make up to 90% of primary lung neoplasms: adenocarcinomas, squamous cell carcinomas, small cell carcinomas, and undifferentiated large cell carcinomas. To maintain consistency in tumor diagnosis, patient therapy, and prognosis the use of and alignment to the standard international system of tumor classification is mandatory [1]. Besides conventional morphologic characterization, representing the sole basis of diagnostics over several decades, histochemical, immunological, genetic, and molecular-biologic techniques have expanded the diagnostic repertoire to a remarkable set of tools. Since immunologic and molecular techniques increasingly made their way into routine diagnostics, the challenges and prospects have changed and are still rapidly developing. Targeted therapies depend on both molecular and pathologic results. The proceeding integration of these modern techniques has begun to change the shape of diagnostics and will do so increasingly in the near future.

Pathologic-anatomic findings are the basis for primary diagnoses, allocation into groups according to main histopathologic features, and the postoperative pTNM stage determination of the tissue samples. The pathologists' appraisal comprises: (i) the classification of lung tumors (typing); (ii) assessment of tumor expansion in resected tissues (pathologic anatomic

staging); (iii) assessment of margins; (iv) assessment of tumor differentiation (grading); (v) assessment of regression grading; (vi) assessment of markers associated to prognosis and (vii) assessment of markers associated to therapy.

Histopathologic typing of lung tumors is performed according to the *WHO* criteria [1]. Frequently, the necessity occurs to use additional immunohistochemistry in order to give a reliable and precise tumor classification. Several more or less specific antibodies are useful in establishing or eliminating diagnoses of primary lung cancers; those used are dependent on the type of neoplasm suspected and the clinical situation encountered [2]. In the following, some examples are presented of features used, including proved and reliable markers (without any claim at all to be exhaustive), available for a few common malignant tumor groups.

## Immuno-/phenotypes

### Neuroendocrine neoplasms

In general, better differentiated neuroendocrine tumors, such as typical carcinoids, show more intensive staining with corresponding specific markers as listed in Table 1 than the more poorly differentiated neuroendocrine tumors such as SCLC.

**Table 1 –** ***Characteristics of neuroendocrine lung tumors and list of common and reliable markers (+positive and/or -negative staining) [1, 3–14]***

| Subtype | Marker+ (pos.) / Marker- (neg.) |
|---|---|
| ***Large cell neuroendocrine carcinoma*** | chromogranin+, synaptophysin+, NCAM/CD56+, TTF-1+/-, CK7+, CK high/lowMW+/-, AE1/AE3. |
| ***SCLC*** | TTF-1+, CD56 or other neuroendocrine marker+, CK+, CK lowMW+, AE1/AE3, CK highMW-, LCA-, MIB-1/Ki-67>50%. |
| ***Typical carcinoid*** | neuroendocrine marker+, TTF-1-, CK7, MIB1/Ki67<,<2mitoses/2sqmm (10HPF). |
| ***Atypical carcinoid*** | neuroendocrine marker+, TTF-1+/-, CK7+, MIB-1/Ki-67>, 2-10 mitoses/2sqmm and necroses. |

The intensity of the chromogranin reaction might roughly correlate with the number of neuroendocrine granules in the cytoplasm, demonstrated by electron microscopy. TTF-1 in tumor cells often provides important additional information. Apart from thyroid and lung tissue, TTF-1 expression has meanwhile been found also in malignant tumors of other provenance. Therefore, like with other markers, all results of immunohistochemistry always need to be interpreted with caution and restrictions [5]. Nonetheless, TTF-1 can help in discriminating lung neoplasms from metastases to the lung or in revealing distant metastasis of primary lung cancer. Most typical carcinoids (Figure 1A) appear as single neoplasms in the large bronchi immediately beneath the surface of an intact epithelium. Occasionally, they may occur in the periphery or/and as multiple discrete masses. Macroscopically they are intraluminal, yellow-tan, well demarcated, and they often invade the adjacent pulmonary parenchyma. They consist of uniform cells in variable patterns like trabecular, insular, solid, and spindle. Besides showing neuroendocrine markers, in addition to cytokeratins they might coexpress vimentin, the latter often in a punctate staining. Usually they are negative for TTF-1 and there is a negligible proliferation, using the marker MIB-1 directed against the nuclear Ki-67 antigen [15]. Many form distinct glands and show multidirectional differentiation with mucous granules in their apical cytoplasm. Metastases are uncommon and patients with typical carcinoids have excellent survival rates. Atypical carcinoids mostly occur in the periphery of the lung with an organoid appearance and show more pleomorphism, mitoses, as well as focal necroses, and metastases are common. The biological behavior of this tumor differs significantly from that of typical carcinoids and TTF-1 is frequently expressed. Large cell neuroendocrine carcinomas (Figure 1B) are most commonly located in the periphery or in the mid-lung field, and are usually bigger than 3 cm in maximum dimension. They are composed of proliferating, large tumor cells with large vesicular nuclei, prominent nucleoli and usually express the markers listed in Table 1. Small Cell Lung Carcinoma (SCLC) (Figure 1C) makes up to ca. 20% of common lung neoplasms and is usually located centrally with multifocal necrosis, shows lymphatic spread with metastases to regional lymph nodes, and immunohistochemically most of them express CK lowMW, and over 90% express TTF-1. P16 may be positive in TTF-1-negative SCLC [16]. Occasionally combined SCLCs are encountered composed of a SCLC with a NSCLC; the latter component should comprise at least 10% of the tumor. In conclusion, neuroendocrine lung neoplasms represent a diverse group of tumors showing neuroendocrine differentiation, ranging from low-grade typical carcinoids to highly malignant large cell neuroendocrine carcinomas and SCLCs.

### Squamous cell carcinomas (SCC)

Most of these tumors arise in the bronchial system, particularly segmental bronchi, and up to ca. one-third from small peripheral airways. Keratinization or intercellular bridges are the diagnostic morphologic features but are difficult or impossible to find in poorly differentiated tumors. In general, well-differentiated SCC tends to spread locally within the chest, whereas poorly differentiated ones metastasize to distant sites. (Tumors lacking either intercellular bridges or keratinization should be diagnosed as large cell carcinoma.) Moderately and poorly differentiated SCCs often form sheets, and individual tumor cells feature variations in size and shape (e.g. spindled or/and pleomorphic); atypical mitoses are common and necrosis can be extensive. SCCs stain for CK low/highMW, CK5/6/14 and/or p63 (Figure 2). Immunoreactivity for CK highMW increases with the degree of keratinization, staining for p63 shows an inverse relation to keratinization. Therefore, especially the latter are of interest in distinguishing a poorly differentiated pulmonary small cell carcinoma from a poorly differentiated squamous cell carcinoma [16], [17]. Otherwise, immunohistochemistry has a limited role in SCC diagnosis, since there is no single marker in

differentiating a primary SCC of the lung from non-pulmonary metastases. In addition, there are also some variants of SCC; pure forms are rare, but many SCCs contain areas with these particular patterns (e.g. papillary, clear cell, small cell and basaloid). Adeno-squamous lung cancer is a rare subtype containing components of both adenocarcinoma and squamous carcinoma, requiring a minimum of 10% of each component in the tumor [1].

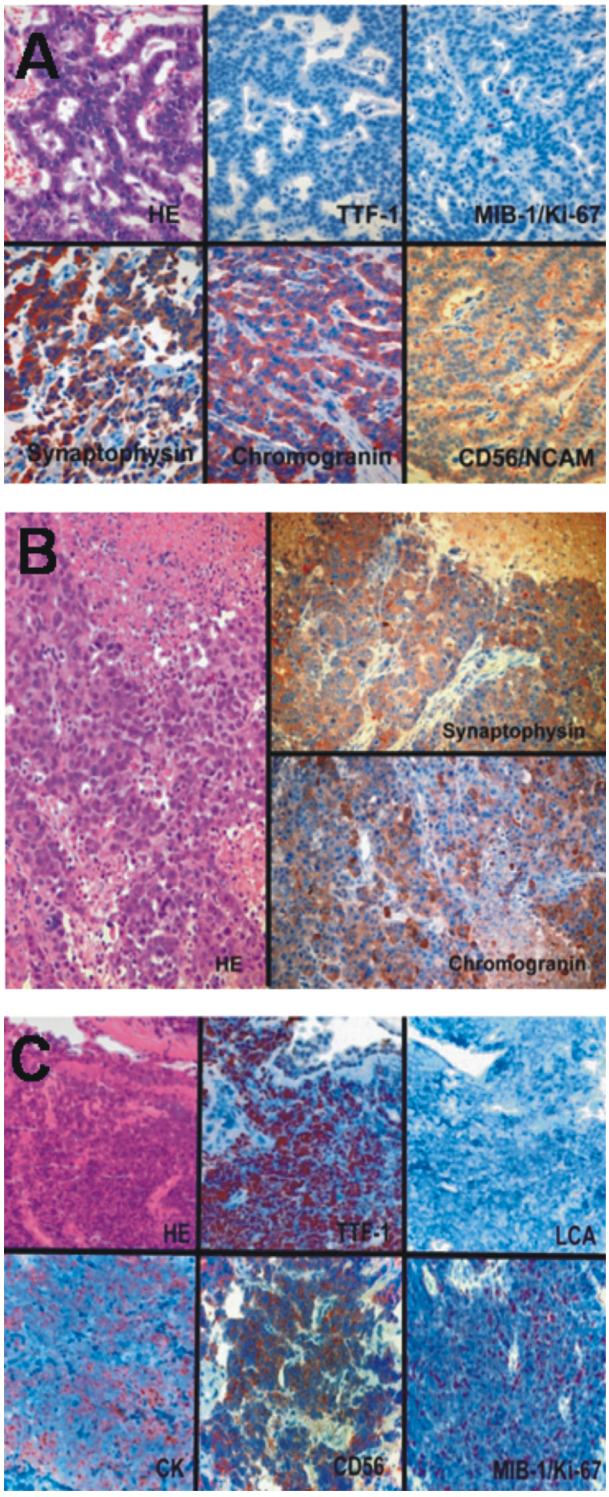


**Figure 1** – ***Neuroendocrine primary lung neoplasms (all: ×200): (A) Typical carcinoid tumor; (B) Large cell neuroendocrine tumor; (C) Small cell lung carcinoma.***

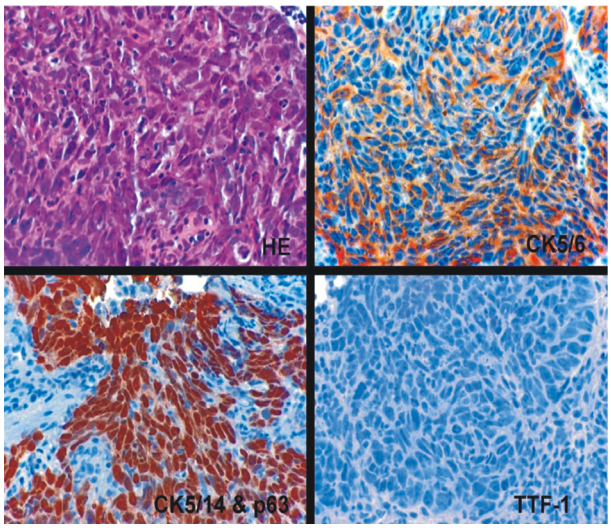


**Figure 2** – ***Poorly differentiated squamous cell carcinoma; besides negativity to TTF-1 there is also negativity to SP-A/B, and Napsin (all: ×250).***

## Adenocarcinomas (primaries of the lung and metastases as well as malignant mesothelioma)

Adenocarcinomas are glandular epithelial cancers and are the most common cell type of lung cancer; they are frequently heterogeneous in histology. Most adenocarcinomas are peripheral tumors and are thought to arise from Clara cells or type II pneumocytes. Major histologic subtypes include e.g. acinar, papillary, solid type with mucus, bronchioalveolar carcinoma, and adenocarcinoma with mixed subtypes. The latter is the most common histologic pattern, and over 80% of resected adenocarcinomas feature at least two and often three individual patterns.

Tumors usually feature solid or acinar components centrally and bronchioalveolar morphology at the periphery. Subtyping and grading of biopsy specimens are not recommended. In pulmonary adenocarcinomas it is common to see central scarring. The solid adenocarcinoma with mucous subtype is a poorly differentiated cancer and is confirmed by mucous stain in at least five tumor cells in each of two high-power fields (HPF–40×).

Papillary adenocarcinoma can be difficult to differentiate from bronchio-alveolar carcinoma (BAC); the distinction however is of great clinical importance as the first one has as comparable poorer prognosis, yet perhaps a greater likelihood of responding to epidermal growth factor receptor tyrosine kinase inhibitors (see later). Differentiating (papillary) adenocarcinomas of the lung from metastatic carcinoma of the thyroid (Figure 3A) and metastases of adenocarcinomas of other origin may require sophisticated immunohistochemical studies (Table 2).

BAC is a clinical, radiographic, and pathologically unique lung cancer. It demonstrates a pure lepidic growth without invasion of stroma, vessels, or pleura [18]. Three morphologic types are known: non-mucinous, mucinous, and mixed. Non-mucous BAC are

more likely to be solitary tumors, whereas mucinous tend to be multicentric; the latter grow often in a pneumonic pattern spreading through the air spaces.

**Table 2** – ***Selection of some proved markers in differential diagnosis of adenocarcinoma in the lung [2, 6, 12–14, 16, 22, 23, 39]***

| Organ/origin | Marker+ (pos.) / Marker- (neg.) |
|---|---|
| ***Lung*** | CK7+, CK20-, TTF-1+, SP-A/B+, GCDFP15-, HMB45-, melanA-, etc. |
| ***Breast*** | CK7+, CK20-, TTF-1-, GCDFP15+, HMB45-, melanA-, ER/PR+, etc. |
| ***Gastro-intestinal*** | CK7-, CK20+, TTF-1-, GCDFP15-, HMB45-, melanA-, CDX2+, etc. |
| ***Melanoma*** | CK7-, CK20-, TTF-1-, S-100+, GCDFP15-, HMB45+, melanA+, vimentin+, etc. |

Adenocarcinomas express many epithelial markers including various cytokeratins, epithelial membrane antigen (EMA), carcinoembryonic antigen (CEA), CD15/Leu M1, BER-EP4, and B72.3, staining fluctuates with tumor subtype and grade [19]. CK lowMW and TTF-1 expression patterns differ in non-mucinous (Figure 4A) and mucinous lung cancer (Figure 4B).

CK7 is expressed by most carcinomas of the first group while only rare cases express [20].CK20. About 80% show staining with TTF-1 [21]. Other useful markers are against surfactant proteins A, B, and Napsin [22–24] (Figure 4C).

Mucinous pulmonary adenocarcinomas include CK7 staining whereas TTF-1 marks less than 25% of these tumors and up to 90% of them are positive for CK20 [25]. The latter marker is readily used in combination with CDX2 [26] for detecting colorectal metastases (Figure 3B).

Metastatic neoplasms to the lung are the most common category of tumors found in the lung. If known, a history of a malignancy outside the lung is certainly helpful. Most common sources in approximate order of frequency are breast, being the most abundant one, followed by colon, stomach, pancreas, kidney (Figure 3A), melanoma, prostate, liver, thyroid, adrenal glands, and male and female genital tract (Figure 3C) [29].

By absolute numbers, adenocarcinomas far outnumber the other extrathoracic solid tumors that metastasize to the lung. Here an appropriate panel of markers together with clinical data is helpful. Considering the well known differential diagnostic problems in distinguishing malignant mesothelioma (MM) from primary pulmonary cancer and other malignancies, immunohistochemistry [2, 27] shows often a positivity for MM with markers for calretinin, WT-1 (Wilms Tumor), CK5/6, D2.40, BMA 120, and vimentin (Figure 5).

Especially, if clinical and radiological findings support a MM diagnosis in such a setting, a MM diagnosis may be established. The *WHO* recommendations of at least two positive and two negative IHC markers can be affirmed by our experience, as far as sensitivity is concerned. The marker choice has to be adapted to the specific differential diagnostic needs in each single case [29]. In difficult cases, it may be useful to refer to a second opinion.

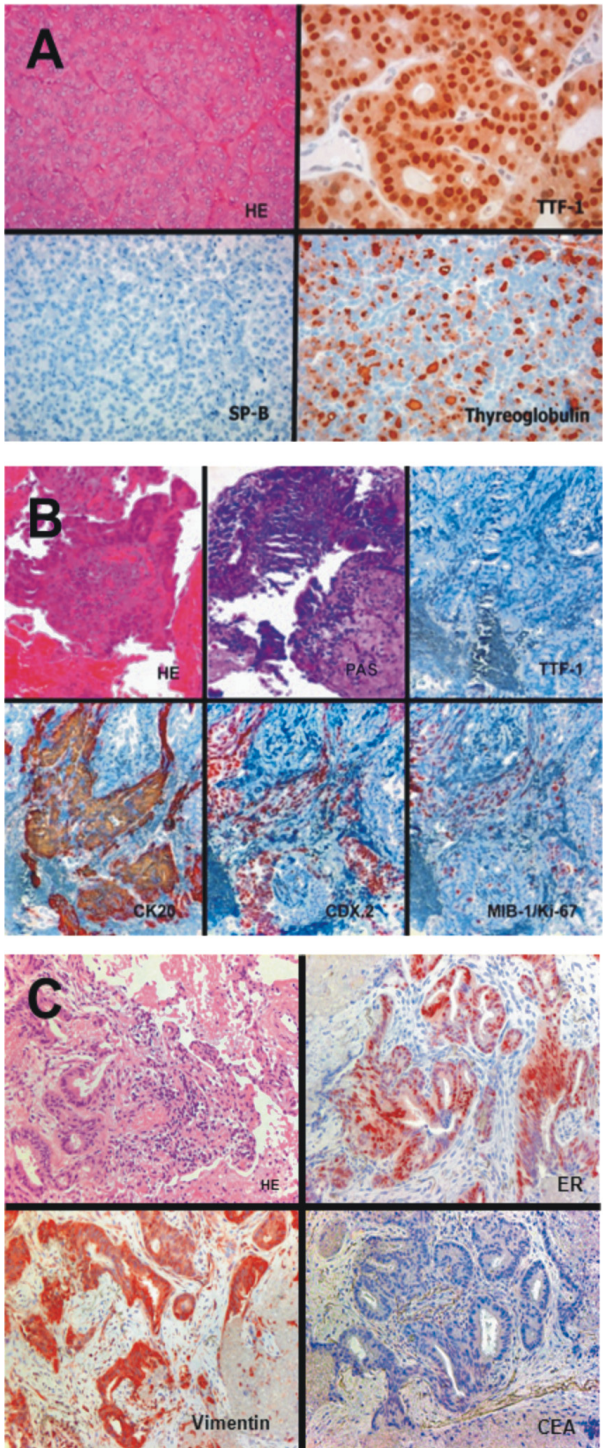


**Figure 3** – ***Metastases of adenocarcinomas to the lung: (A) Metastasis of a thyroid carcinoma; besides negativity to SP-B there is also negativity to Napsin, Sp-A, and CK7 (×200, respectively ×300); (B) Metastasis of a colon carcinoma; besides negativity to TTF-1 there is also negativity to CK7, SP-A/B, and Napsin (all: ×100); (C) Metastasis of an endometrial carcinoma; besides positivity to the estrogen receptor there is also a positivity to the progesterone receptor, and common markers for primary adenocarcinomas of the lung like TTF-1, SP-A/B and Napsin are negative (all: ×100).***

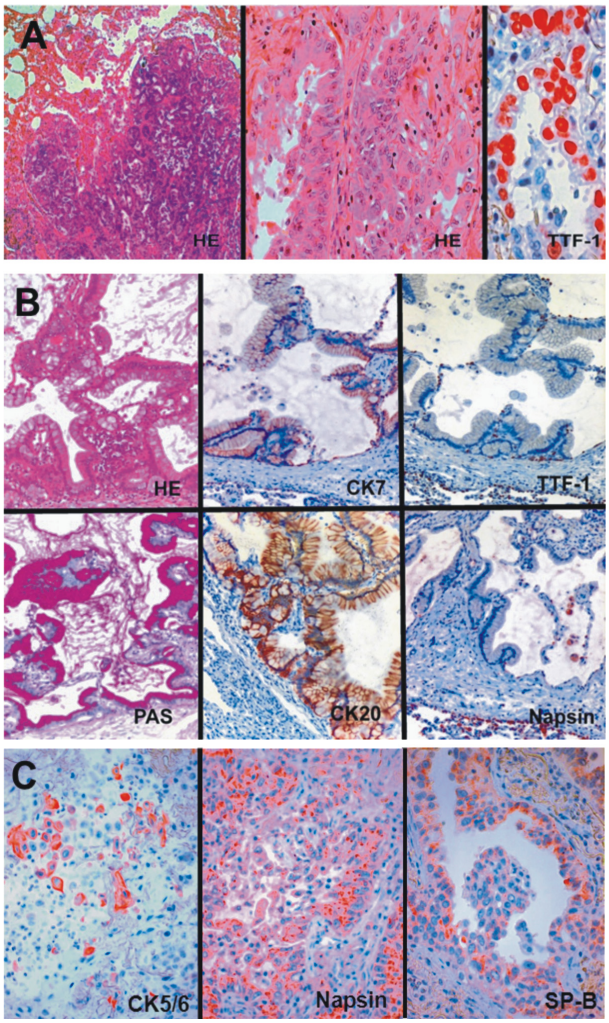


**Figure 4** – ***Primary adenocarcinomas of the lung: (A) Non mucinous pulmonary adenocarcinoma (×25, ×200, ×400); (B) Mucinous pulmonary adenocarcinoma; besides negativity for TTF-1 there is negativity to CK5/6, CK7, SP-A/B, and Napsin (all: ×200); (C) Pulmonary adenocarcinoma with some scattered tumor cells showing squamous cell features too (all: ×200).***

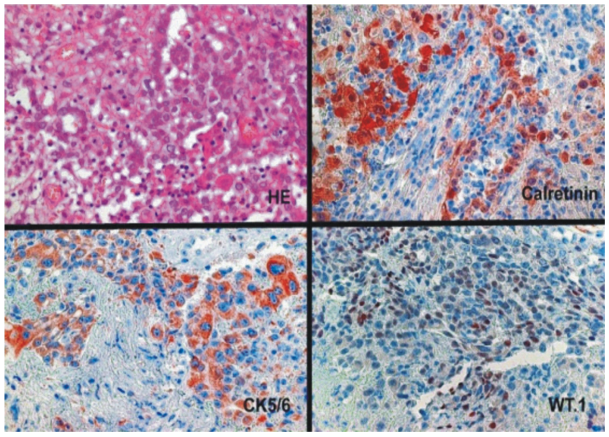


**Figure 5** – ***Malignant pleuramesothelioma (all: ×200).***

## Large cell carcinomas (LCC)

This group of neoplasms is defined by the *WHO*-classification as a NSCLC without light microscopic or histochemical evidence of glandular, squamous, or small cell differentiation [1]. LCCs often present as large peripheral, necrotic tumors. They are not a single tumor but rather a collection of poorly differentiated carcinomas that account for approximately 10% of all lung cancers. However, if NSCLCs were classified based on either ultrastructural or expression profile analysis then their number would diminish to about 1%. There are several variants of LCC, including large cell neuroendocrine carcinoma, basaloid carcinoma, clear cell carcinoma, lymphoepithelioma-like carcinoma and large cell carcinoma with rhabdoid phenotype. In diagnosing and delimiting it from metastases like, e.g., melanoma, immunohistochemistry may be helpful [6], [14], also depending on the clinical situation.

## Rare primary lung neoplasms

There is a variety of rare primary pulmonary tumors that are occasionally encountered by pathologists [31], but also common types of neoplasms with unusual morphology, that may cause diagnostic confusion [28] (Figure 6).

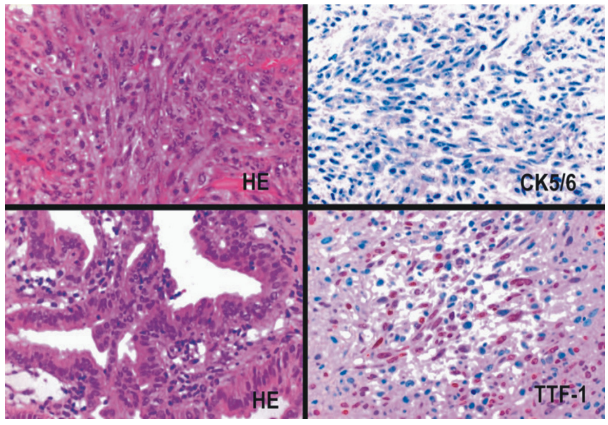


**Figure 6** – ***Pleuritis carcinomatosa of a primary adenocarcinoma of the lung with an additional rare spindle shape appearance of tumor cells in some areas positive for TTF-1 but negativity to CK5/6, calretinin, WT-1, and vimentin (all: ×200).***

Some examples are sarcomatoid carcinoma (carcinosarcoma, spindle cell carcinoma), pulmonary blastoma, and malignant hemangioendothelioma (intravascular bronchioloalveolar tumor-IVBAT). They are of epithelial derivation; in most cases, the spindle cells coexpress cytokeratins and vimentin, or occasionally other intermediate filaments like desmin or actin. In pulmonary blastomas [30] the epithelial component often forms glandular structures, and a spindle cell component, or forms occasionally squamous morulae respectively shows neuroendocrine differentiation. IVBATs have an epithelial appearance and characteristically they express endothelial markers like CD31, CD34, factor VIII antigen and are positive for vimentin, rarely for cytokeratins [31]. The lung also shows a wide spectrum of lymphoproliferative disorders, similar to those seen in lymph nodes with the same immunohistochemical markers applying. Primary sarcomas of the lung are rare; from clinical history, a metastasis has to be excluded. The immunophenotype is essentially the same to sarcomas that occur in soft tissue and other organs.

## Molecular diagnostics for stratification of lung cancer therapy

Currently, TNM staging, tumor typing, and grading are the most important tools we use to stratify patients for given therapies and to prognosticate the overall survival. Quite promising is the recent development of chemotherapeutics, specific for certain pathomechanisms of NSCLC. One way is by blocking neoangiogenesis [32] and thus stopping the supply with oxygen and nutrients (e.g. by the monoclonal antibody bevacizumab (Avastin®) directed against the binding site of the vascular endothelial growth factor – VEGF). Therefore, bevacizumab is considered as an angiogenesis inhibitor – a medication that works to prevent cancers from making new blood vessels. It appears to prolong survival in patients with advanced NSCLC [33]. The most common side effect is bleeding. For this reason, it is not used for those on blood thinners, who are coughing up blood, or have cancer that has spread to the brain due to the risk of bleeding in the brain. It is given as an intravenous therapy every two to three weeks. Another mechanism is to stop tumor growth by specifically blocking the transmission of signals via receptors of the tumor cell [34]. This receptor imparts signals into the cell via the enzyme tyrosine kinase, frequently present in tumor cells. First studies show that the epidermal growth factor receptor (EGF-R)–tyrosine kinase inhibitors (TKI) have a prominent and practical impact in NSCLC. The EGF-R-family [35] comprises transmembrane tyrosine kinase receptors and is composed of EGF-R (HER1), HER2, HER3, and HER4. When linked to their ligands, these receptors form autophosphorylated dimers, which activate intracellular cascades favoring proliferation, differentiation, apoptosis and angiogenesis. Several drugs target EGF-R and HER2, including small molecule TKI gefitinib (Iressa®), erlotininb (Tarceva®) and monoclonal antibodies cetuximab (Erbitux®) and trastuzumab (Herceptin®, targeting HER2) [36, 37]. Recently, application of small molecules (gefitinib and erlotinib) in targeted therapy in lung cancer has resulted in a remarkable response in about 10% of patients with NSCLC. Given as a daily pill, the most common side effects are a skin rash similar to acne, and diarrhea. Though the skin rash can be a cosmetic nuisance, those who develop a rash with erlotinib are more likely to be responding to therapy. Up till now the following predictive factors for a positive outcome in targeted therapies of NSCLC are meanwhile known: Mutations for EGF-R (exons 18, 19, 20, 21), FISH/IH positivity for EGF-R, non smokers, adenocarcinoma, (young) female sex, Asian descent, and negative Ki-Ras mutation status [38].

The Ras gene family encodes 21kDa proteins binding guanosintriphosphate (GTP) to form ras-GTP complexes, which activate transcription factors such as c-fos, c-jun, c-myc and DNA synthesis. Activating Ras mutations are mostly identified at codon 12 and induced by tobacco carcinogens. Ras mutations are detected more frequently in adenocarcinomas and large cell lung carcinomas and could be responsible for resistance to cytotoxic drug (cisplatin) or to TKI. Ki-Ras mutation and EGF-R mutations are mutually exclusive. Additionally, a recurrent fusion protein between echinoderm microtubule associated protein like 4 (EML4) and anaplastic lymphoma kinase (ALK), described in a subset of NSCLC with distinct clinical characteristics, could play a role in Ras activation [38]. This mutation could favor adenocarcinoma development in non-smokers. Less commonly, mutations can involve other EGF-R genes including HER2 (2% of NSCLC), HER4 (2%), BRAF (2%), and PI3KCA (4%). Patients who harbor this mutation do not benefit from EGFR TKIs and should be directed to trials of ALK-targeted agent.

Upcoming new details to our knowledge are both complicating the situation and providing new opportunities when it comes to predicting the selection for therapy. EGF-R respectively Ki-Ras mutation analyses are meanwhile incorporated into our diagnostic repertoire (Figure 7, A and B).

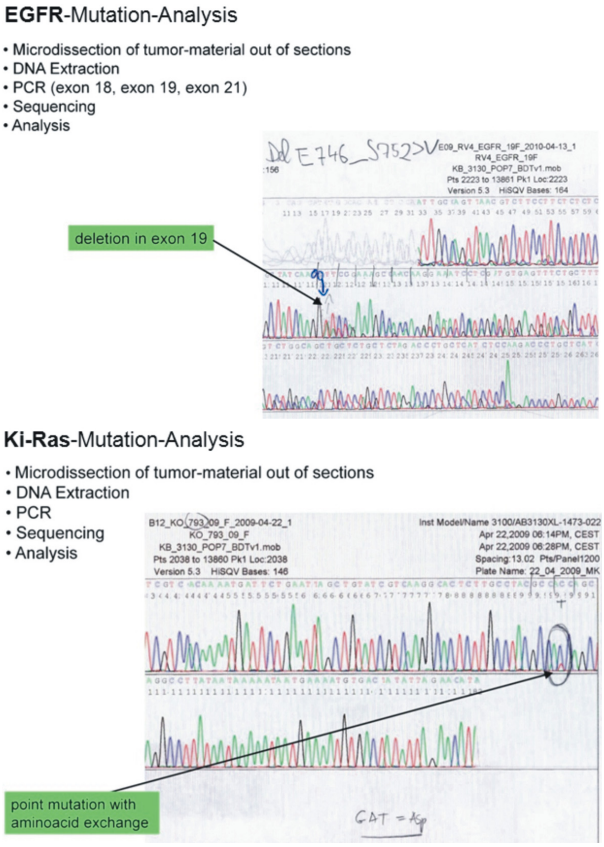


**Figure 7** – ***Molecular analyses: (A) EGF-R-Mutation-Analysis; (B) Ki-Ras-Mutation-Analysis.***

One of the real challenges that we face is trying to bring the utilization of the advancing knowledge in both the clinical and molecular area to impact on patient management. Large efforts are directed towards identifying potentially druggable molecular alterations. So far, we can identify therapeutic targets in only up to 20% of NSCLC with activating EGF-R mutations. Ca. 75% of patients with mutations of EGF-R in their tumors are susceptible for a TKI targeted therapy. The frequency for primary resistance against EGF-R-TKI is

more than 80%. Therefore, the majority of NSCLCs grows either independent of the EGF-R signaling pathway or was able to activate the pathway in a position downstream of the EGF-R. Targeted therapies in specific patient populations may reduce the need for chemotherapy and allows clinicians to tailor treatment to individual patients. In addition to the already known and practiced pathologic-anatomic assessment for classification of lung tumors, including typing, staging, grading, determination of resection margins and grading of regression meanwhile markers associated to prognosis and therapy are highly recommended [38]. Targeted cancer therapies may be used alone, in combination with other targeted therapies, or with other lung cancer treatments, such as chemotherapy, radiation therapy and Interventional Pulmonology. Thus, finally patients might benefit distinctly with increased response/survival rate, improved quality of life, because of less toxicity, and furthermore, expenses of non-effective treatment will be saved. Although results have been encouraging, much work remains to be done.

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