Review Article

Lung cancer diagnosis

Giulio Rossi[1], **Paolo Graziano**[2]

[1]Pathologic Anatomy Unit, Department of Oncology, Fondazione Poliambulanza, Leonida Bissolati Street, Brescia, Italy; [2]Unit of Pathology, Fondazione IRCCS Casa Sollievo Della Sofferenza, San Giovanni Rotondo, Foggia, Italy

Contributions: (I) Conception and design: Both authors; (II) Administrative support: Both authors; (III) Provision of study materials or patients: G Rossi; (IV) Collection and assembly of data: Both authors; (V) Data analysis and interpretation: Both authors; (VI) Manuscript writing: Both authors; (VII) Final approval of manuscript: Both authors.

Correspondence to: Giulio Rossi, MD, PhD. Pathology Unit, Department of Oncology, Fondazione Poliambulanza, Leonida Bissolati Street, 57, 25122 Brescia, Italy. Email: giurossi68@gmail.com.

Abstract: Therapeutic management of lung cancer is mainly based on tumor histology and stage, patient performance status and predictive biomarkers for targeted therapies and immunotherapy. The pathologic diagnosis, based on the fifth edition of the World Health Organization classification bluebook, still remains a key issue in guiding the appropriate therapy and molecular determinations. The last 2021 edition emphasized the novel advances of molecular biology in various tumor types, including a subheading on the classification of different neoplasms on small samples. The use of the percentage of the histologic patterns and tumor grade in resected, invasive non-mucinous adenocarcinomas, and the determination of invasive dimension in pT definition when adenocarcinoma shows a lepidic component are further stressed. Introduction of spread through airspaces as a poor prognostic parameter of local tumor invasiveness, some evolving criteria in the classification of neuroendocrine tumors, and the recognition of new entities, as bronchiolar adenoma/ciliated muconodular papillary tumor and thoracic SMARCA4-deficient undifferentiated tumor were also reported. Finally, essential and desirable diagnostic criteria were added in each tumor entity. In this review, we concisely analyse the new findings introduced by the 2021 classification of lung tumors and discuss the diagnostic criteria applied to small biopsy/cytology, the role of immunostains and the minimal requirements for a diagnosis of lung cancer, finally underlining some tricks to appropriately handle tumor tissue aimed at implementing the yield of predictive molecular markers.

Keywords: Lung; histology; World Health Organization (WHO); classification; biomarkers

Received: 09 January 2022; Accepted: 24 March 2022; Published: 30 June 2022.

doi: 10.21037/jxym-22-1

View this article at: https://dx.doi.org/10.21037/jxym-22-1

Introduction

Lung cancer is a leading cause of cancer mortality worldwide and its diagnosis is still based on examination of cytology or biopsy. Lung cancer is broadly classified into two major groups, namely non-small cell lung carcinoma (NSCLC) and small cell lung carcinoma (SCLC), accounting for about 85% and 15% of all primary malignancies of the lung, respectively (1,2). Radical surgery is the only therapy with curative intent in lung cancer, but it is restricted to patients with good performance status and appropriate stage and histology of the tumor (3). NSCLC group includes several entities, mainly subdivided into adenocarcinoma, squamous cell carcinoma and large cell carcinoma, among which various histological variants are described (2).

From an epidemiologic viewpoint, adenocarcinoma is the most common histotype reaching an incidence of 50–60%, followed by squamous cell carcinoma (20–25%) and SCLC (15%). The increased prevalence of adenocarcinoma histology seems secondary to the increase smoking habit in women and to a different cigarette's composition leading to greater exposure of tobacco carcinogens in the peripheral compartment of the lungs (4,5). Of note, adenocarcinoma

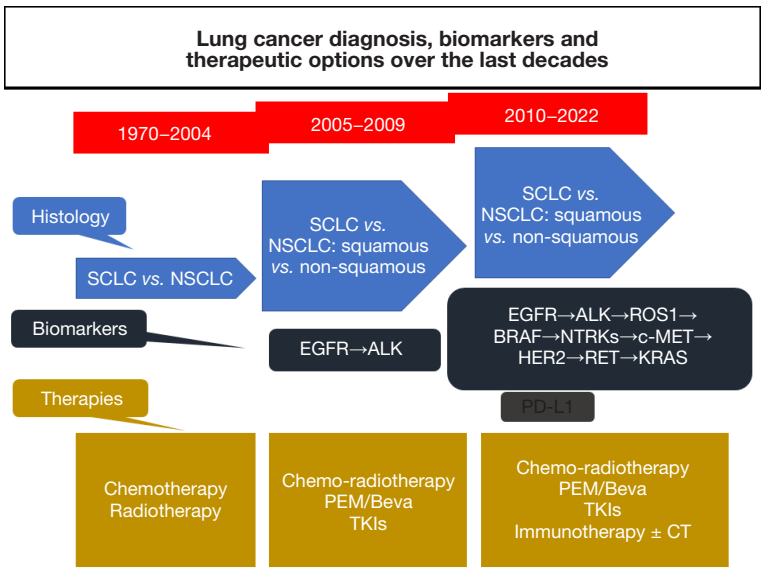

Figure 1 An updated landscape of lung cancer integrating histology, predictive biomarkers and available drugs (BEV, PEM, TKIs) in the last decades demonstrating a significant increase of complexity in terms of molecular genetic alterations and tailored targeted therapies. BEV, bevacizumab; CT, computed tomography; NSCLC, non-small cell lung carcinoma; PEM, pemetrexed; SCLC, small cell lung carcinoma; TKIs, tyrosine kinase inhibitors.

is by far the main histology in women and never smokers (2,4,6).

Basically, the distinction between SCLC and NSCLC, and squamous versus non-squamous cell carcinoma in the NSCLC subgroup is considered sufficiently exhaustive for the appropriate management of patients with lung cancer. However, the huge amount of information deriving from molecular studies in recent years has evidenced the close correlation of genetic alterations with lung tumor histology, introduced the advent of several effective drugs targeting oncogenic drivers, particularly in adenocarcinoma, and dramatically changed the diagnostic and therapeutic paradigm of the pulmonary neoplastic field (*Figure 1*) (7-10).

A new World Health Organization (WHO) classification

While the 2015 edition of the WHO classification has introduced the use of immunohistochemical markers for a more accurate definition of poorly differentiated NSCLC and a novel classification scheme of lung cancer diagnosis on small biopsies and/or cytology, the last 2021 WHO edition included new entities, namely SMARCA4-deficient undifferentiated carcinoma and a group of pulmonary adenomas comprising bronchiolar adenoma and ciliated muconodular papillary tumor (2). Some entities moved to a new tumor definition, as lymphoepithelial carcinoma (comprising EBV-positive and EBV-negative types) or the enteric type adenocarcinoma (*Table 1*).

Although the nomenclature of neuroendocrine tumors is not significantly changed from the previous 2015 scheme, the fifth edition mentioned some evolving concepts concerning the possibility of a limited subset of high-grade forms deriving from typical/atypical carcinoids and the appropriate use of labeling index by Ki67 (2).

The WHO board recognized the old concept of "spread through airspaces (STAS)" as an alternative invasive growth with a poor prognostic value (11,12).

Among adenocarcinomas, the authors recommended to report the rate of various patterns in invasive forms, the correlation of the pattern with the tumor grade and the need to calculate only the invasive size of the tumor in surgical specimens to define pathological T (pT) according to the TNM staging system. Of note, the lepidic-predominant and the minimally-invasive forms demonstrated the best prognosis, whereas the solid and micropapillary patterns showed the poorest outcome, although significantly associated with survival benefit from chemotherapy in the adjuvant setting (13).

An update of the genetic alterations discovered in

Table 1 Summary of the 2021 WHO classification of lung tumors

Tumor types	Precursor of invasive lesion	Variants	Notes
Papillomas	–	Squamous exophytic	Benign clinical behavior
		Squamous inverted	
		Glandular	
		Mixed squamous and glandular	
Sclerosing pneumocytoma (so-called sclerosing hemangioma)	–	Patterns: sclerotic, papillary, solid, hemorrhagic (frequently mixed with 2 or more patterns)	Benign clinical behavior Challenging diagnosis at frozen section
Adenomas	–	Alveolar	Benign clinical behavior
		Papillary	Challenging and/or deferring diagnosis at frozen section
		Bronchiolar/muconodular papillary ciliated tumor	
		Mucinous cystadenoma	
		Mucous gland adenoma	
Adenocarcinoma	Atypical adenomatous hyperplasia (AAH); in situ adenocarcinoma (AIS); minimally-invasive adenocarcinoma (MIA) (mucinous and non-mucinous)	Patterns: lepidic (grade 1), acinar (grade 2), papillary (grade 2), micropapillary (grade 3); solid (grade 3)	The tumor size is a key factor: AAH ≤5 mm; AIS ≤3 cm without invasive features; MIA ≤3 cm with stromal invasion ≤5 mm
		Mucinous	
		Fetal (low/high grade)	
		Enteric	
		Colloid	
Squamous cell carcinoma	Dysplasia; in situ squamous carcinoma	Keratinizing	–
		Non-keratinizing	
		Basaloid	
Large cell carcinoma	–	–	Negative squamous (p40, CK5/6), glandular (TTF-1, napsin) and neuroendocrine (chromogranin, synaptophysin, CD56) markers of differentiation
Adenosquamous carcinoma	–	–	The squamous and adenocarcinoma components (at least 10%, each) should be evident on morphologic examination
Sarcomatoid carcinoma	–	Spindle cell carcinoma	At least 10% of both component in biphasic tumors
		Giant cell carcinoma	
		Spindle and giant cell carcinoma	
		Pleomorphic carcinoma (a conventional NSCLC with a spindle and/or giant cell component)	
		Carcinosarcoma (a conventional NSCLC with a heterologous sarcoma component: osteo/chondro/leio/rhabdomyo/angio-sarcoma)	
		Blastoma	

Table 1 (*continued*)

Table 1 (*continued*)

Tumor types	Precursor of invasive lesion	Variants	Notes
Other carcinomas	–	Lymphoepithelial carcinoma	–
		NUT carcinoma	
		SMARCA4-deficient undifferentiated carcinoma	
Neuroendocrine tumors	Diffuse idiopathic pulmonary neuroendocrine cell hyperplasia (DIPNECH) in a subset of carcinoid tumors	Typical carcinoid	Mitotic count × mm^2, necrosis, cell features
		Atypical carcinoid	Labeling index by Ki67 is particularly helpful in small biopsy with crushed artifacts
		Large cell neuroendocrine carcinoma	
		Small cell carcinoma	
		Combined SCLC/LCNEC	
		Combined SCLC or LCNEC with NSCLC	
Tumors from ectopic tissue	–	Melanoma	–
		Thymoma	
		Meningioma	
		Germ cell tumors	
Mesenchymal tumors	–	Hamartoma	–
		Chondroma	
		PEComa	
		Inflammatory myofibroblastic tumor	
		Lymphangioleiomyomatosis	
		Vascular tumors (epithelioid hemangioendothelioma, angiosarcoma, Kaposi's sarcoma)	
		Solitary fibrous tumor	
		Synovial sarcoma	
		Pleuropulmonary blastoma	
		Intimal sarcoma	
		Myxoid sarcoma with EWSR1-CREB1 translocation	
Lymphomas and histiocytic neoplasms	–	Extranodal marginal zone lymphoma of the mucosa-associated lymphoid tissue (MALT)	–
		Diffuse large B cell lymphoma	
		Intravascular large B cell lymphoma	
		Lymphomatoid granulomatosis	
		Langerhans cell histiocytosis	
		Erdheim-Chester disease	
Metastasis	–	–	–

LCNEC, large cell neuroendocrine carcinoma; NSCLC, non-small cell lung carcinoma; PEComa, perivascular epithelioid cell tumor; SCLC, small cell lung carcinoma; WHO, World Health Organization.

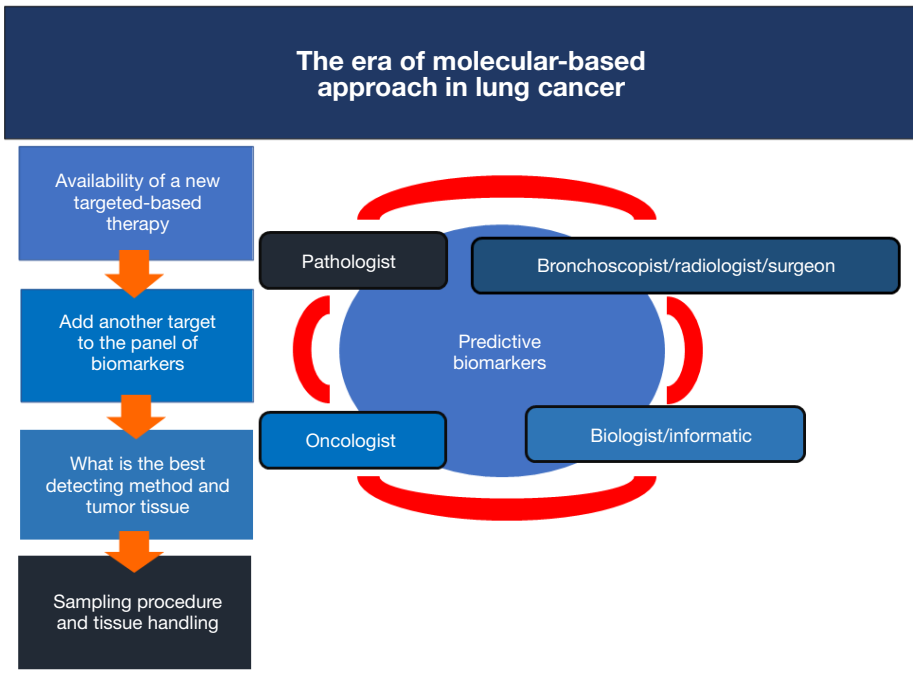

Figure 2 The need for predictive biomarkers is secondary to availability of targeted therapies, but it still requests sampling of adequate tumor tissue using a multidisciplinary approach to lung cancer involving pathologists, bronchoscopists/radiologists/surgeons, oncologists and biologists/bioinformatics (computed tomography/positron emission tomography).

different lung cancers with a diagnostic and/or predictive role has been implemented, as well as the diagnostic rules in the diagnosis of lung cancer when facing with cytology or small biopsies (2,14).

Diagnosis on cytology and small biopsy

The main source of tumor tissue derives from a multidisciplinary approach requiring a close collaboration between pathologist, pneumologist, radiologist, thoracic surgeon and molecular biologist to obtain an adequate material aimed at permitting a reliable diagnosis and determination of predictive molecular markers. Thus, even in the era of liquid biopsy, tumor tissue handling is the gold standard (*Figure 2*) (2,14,15). Pathological diagnosis still plays a key role in the management of lung cancer, but two third of patients present with unresectable disease and the diagnosis may be obtained only on small biopsy or cytology. Nevertheless, there are several modalities to achieve appropriate tumor tissue, mainly deriving from bronchoscopic [bronchial and transbronchial biopsy with/without cryoprobe, endobronchial ultrasound (EBUS)-guided transbronchial fine needle aspiration (FNA) or transesophageal endoscopic ultrasonography (EUS) FNA] or radiologic [computed tomography (CT)-guided biopsy or FNA] procedures. By contrast, exfoliative cytology or bronchoalveolar lavage are poorly informative and inadequate in this setting (14-17).

The diagnosis of lung cancer actually requires 2 integrated levels, namely a detailed histological typing and mandatory molecular tests, in order to tailor the most effective therapy in each patient. The fifth edition of the WHO classification of lung tumors (2) reinforced the concept previously introduced in the fourth edition, providing a practical workflow based on a careful examination of morphologic characteristics on hematoxylin-eosin and, if necessary, the subsequent use of a limited panel of immunohistochemical markers with/without mucin stains. Nevertheless, a diagnosis of adenocarcinoma or squamous cell carcinoma may be established on morphology alone in presence of glandular formation or keratinization (*Figures 3*,*4*) (2,18). The main suggested panel of immunohistochemical markers consist of TTF-1 (possibly using the clone 8G7G3/1) and p40 for adenocarcinoma and squamous cell carcinoma differentiation, respectively (*Table 2*, *Figure 5*) (19-23). Other immunostains may be

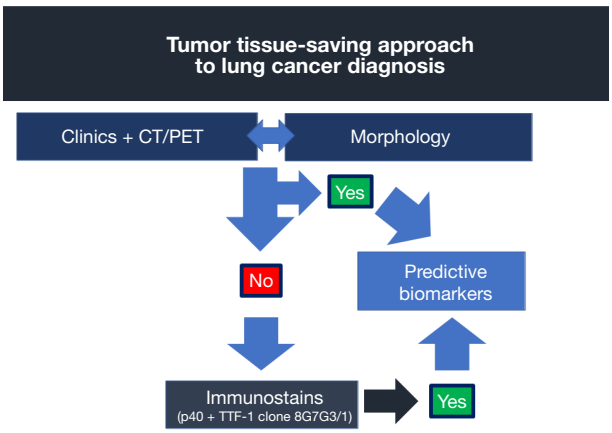

Figure 3 A tumor-tissue saving practical approach to lung cancer diagnosis based on integration of clinics, imaging and morphology, eventually limiting immunohistochemistry to a two-hits panel including TTF-1 and p40. CT, computed tomography; PET, positron emission tomography.

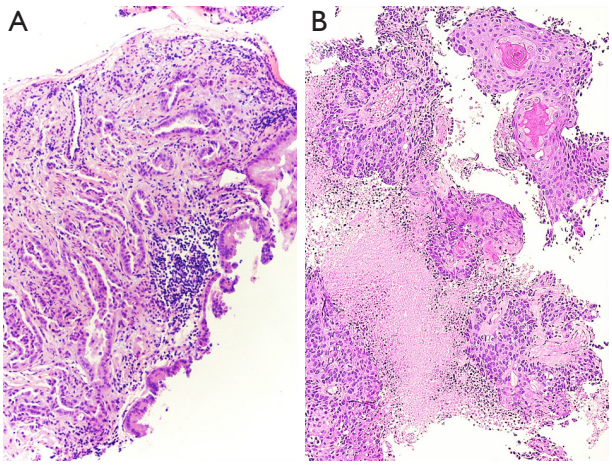

Figure 4 Real-life case of lung biopsies morphologically demonstrating an invasive adenocarcinoma with overt glandular differentiation (A, hematoxylin-eosin staining ×180) and an invasive squamous cell carcinoma with squamous pearls and necrosis (B, hematoxylin-eosin staining ×180). In these cases, immunostains are not necessary.

Table 2 Preferable organization of immunostains demand in NSCLC subtyping

Morphology	Features	TTF-1 (clone 8G7G3/1) and/or mucin staining	p40	Diagnosis
Adenocarcinoma	Gland structures, papillary/ pseudopapillary formations, tridimensional aggregates of nucleated cells, mucin secretion	Not necessary	Not necessary	Adenocarcinoma; if possible, report the predominant growth pattern
Squamous cell carcinoma	Keratinization, squamous pearls formation, intercellular bridges, bright orange cytoplasm and less prominent nucleoli	Not necessary	Not necessary	Squamous cell carcinoma; if possible, state the presence of obvious keratinization or not
NSCLC, poorly differentiated	Solid growth of large cells lacking glandular or squamous differentiation	Positive	Negative	NSCLC, favoring adenocarcinoma
NSCLC, poorly differentiated	Solid growth of large cells lacking glandular or squamous differentiation	Negative	Positive (usually diffuse)	NSCLC, favoring squamous cell carcinoma
NSCLC, poorly differentiated	Solid growth of large cells lacking glandular or squamous differentiation	Positive	Positive (usually few positive nuclei)	NSCLC, favoring adenocarcinoma
NSCLC, poorly differentiated	Solid growth of large cells lacking glandular or squamous differentiation	Negative	Negative	NSCLC, not otherwise specified (NOS); additional immunostaining workup could be considered after evaluation of clinical and imaging findings (particularly in patients with previous malignancy or other suspected primaries)

NSCLC, non-small cell lung carcinoma.

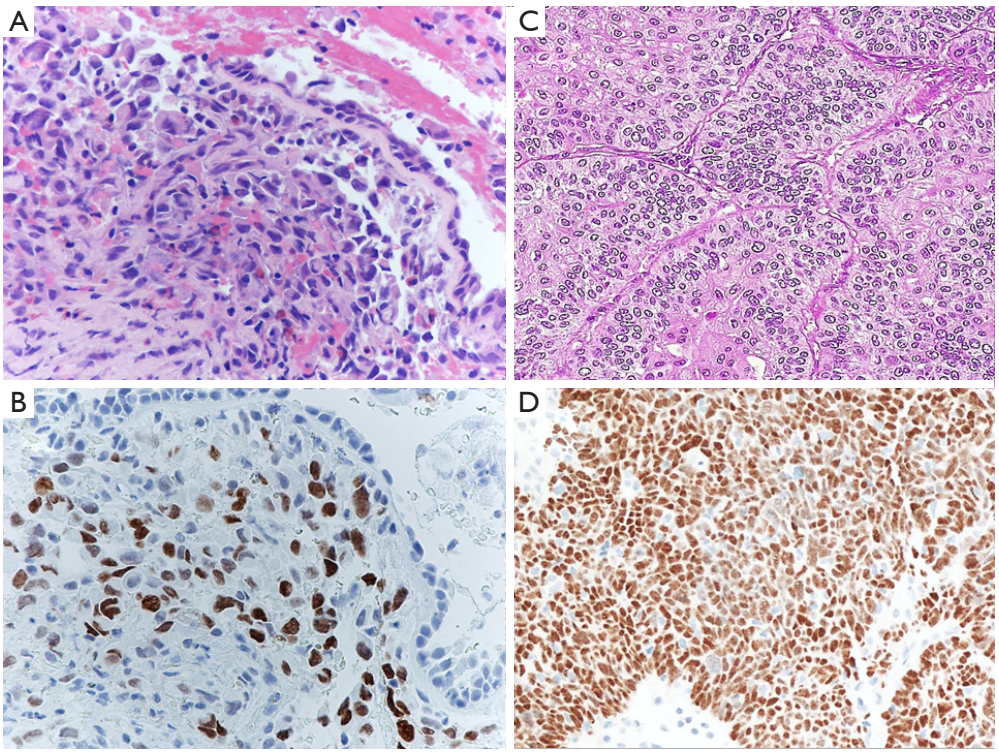

Figure 5 Poorly differentiated NSCLC (A and C, hematoxylin-eosin staining ×200) favoring adenocarcinoma (B, immunohistochemistry with TTF-1 ×200) and squamous cell carcinoma (D, immunohistochemistry with p40 ×200) after immunostaining. NSCLC, non-small cell lung carcinoma.

Table 3 Essential immunostains used in NSCLC subtyping

Cell differentiation	Preferable	Others
Adenocarcinoma	TTF-1 (clone 8G7G3/1)	Napsin; CK7 (positive in about 30% of squamous cell carcinomas)
Squamous cell carcinoma	p40	p63 (positive in about 20% of adenocarcinomas); CK5/6 (positive in 10% of adenocarcinomas); desmocollin-3 (low sensitive in high-grade forms)
Neuroendocrine neoplasms	Chromogranin; synaptophysin	CD56 (too sensitive and also staining some high-grade NSCLC)
Other useful markers	NUT (NUT-carcinoma)	SMARCA4 deficiency may be observed in a subset of NSCLC
	SMARCA4 (undifferentiated SMARCA4-deficient carcinoma)	CDX2 should be used with caution, particularly in patients with previous history of intestinal neoplasms
	CDX2 (enteric-type and colloid variants of adenocarcinoma)	

NSCLC, non-small cell lung carcinoma.

used with caution in case of double negativity with TTF-1 and p40 (*Table 3*). Pathologists should be aware that primary antibodies used in determining NSCLC subtyping may be positive in extrapulmonary malignancies. On the other hand, some non-pulmonary immunostains are expressed in primary lung cancer (*Tables 4,5*, *Figures 6,7*) (24-29).

The aim of this approach is to limit at minimum the rate of NSCLC not otherwise specified (NOS), possibly less than

Table 4 Extrapulmonary neoplasms of primary antibodies used in NSCLC subtyping

Primary antibody	Staining in extra-pulmonary tumors
TTF-1	Gynaecological neoplasms (not infrequent from gastrointestinal tract and breast with clone SPT24); thyroid neoplasms (including medullary carcinoma); extra-pulmonary high-grade neuroendocrine carcinomas from various sites; brain tumors
Napsin	Renal cell carcinoma; clear cell carcinoma of the ovary
p40	Squamous cell carcinomas from other sites (skin, uterine cervix, urothelium, myoepithelial cells)
CK5/6	Mesothelioma; squamous cell carcinomas from other sites (skin, uterine cervix, urothelium, myoepithelial cells)

NSCLC, non-small cell lung carcinoma.

Table 5 Extrapulmonary primary antibodies expressed in primary lung cancer

Primary antibody	Staining in extra-pulmonary tumors
CDX2	High-grade neuroendocrine carcinomas; colloid adenocarcinoma; enteric-type adenocarcinoma
CK20	Colloid adenocarcinoma; enteric-type adenocarcinoma
ER	Non-mucinous adenocarcinoma; neuroendocrine tumors

ER, estrogen receptors.

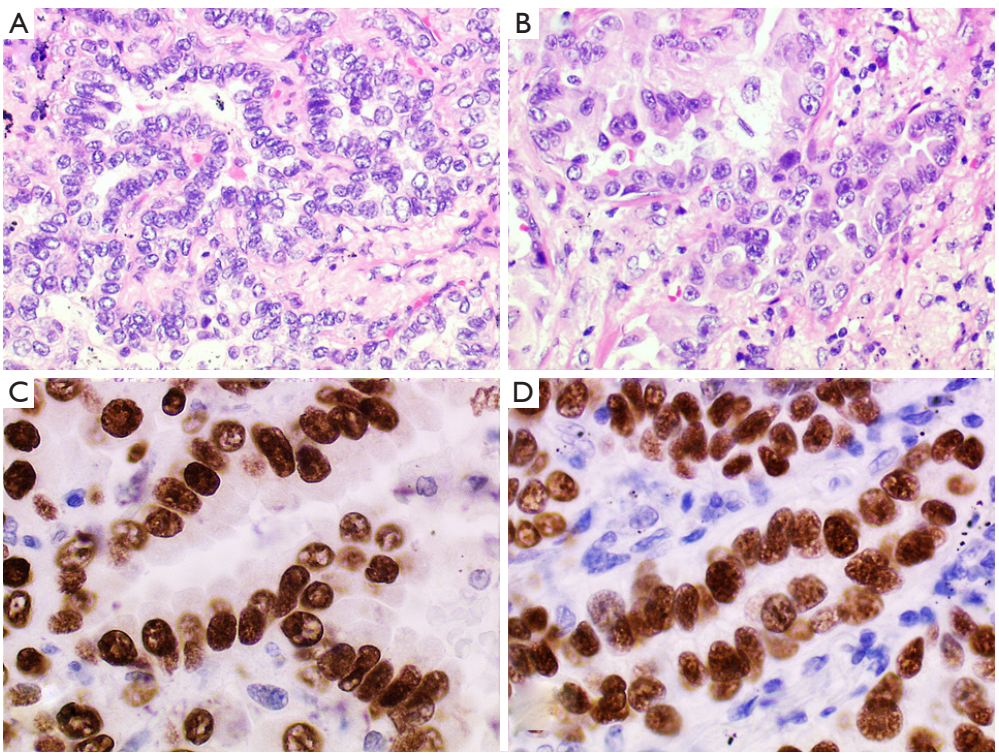

Figure 6 A primary adenocarcinoma of the lung showing lepidic (A, hematoxylin-eosin staining ×250) and acinar (B, hematoxylin-eosin staining ×250) patterns aberrantly expressing estrogens (C, immunohistochemistry ×300) and progesterone (D, immunohistochemistry ×300) receptors.

	ADK mucinous Goldstein *et al. Am J Clin Pathol* 2001;116:319-325	ADK colloid Rossi *et al. Am J Surg Pathol* 2004;28:442-452	ADK enteric type Inamura *et al. Am J Surg Pathol* 2005;29:660-665
CK7	+	+/–	+/–
CK20	+/–	+/–	–/+
TTF-1	–/+	+/–	–/+
CDX2	–	+	+/–

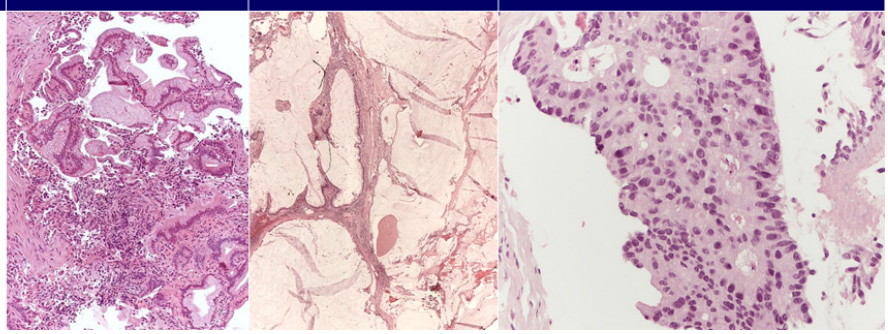

Figure 7 A summary of immunohistochemical expression of CK7 and TTF-1 (markers generally positive in lung primary carcinomas) and CK20 and CDX2 (markers commonly expressed in intestinal carcinomas) in 3 different types of lung primary adenocarcinomas with mucinous/intestinal differentiation (hematoxylin-eosin staining ×200).

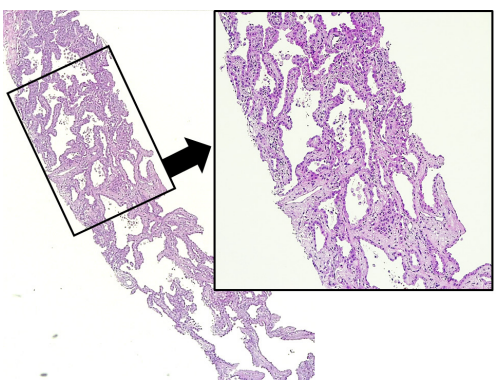

Figure 8 A generous transthoracic biopsy of a pulmonary ground-glass opacity, histologically demonstrating an adenocarcinoma with lepidic pattern lacking invasive features. In this case, a diagnosis of adenocarcinoma with lepidic pattern is correct. The definitive diagnosis requires a careful examination of the surgical specimen, possibly representing a non-mucinous *in situ* or minimally invasive adenocarcinoma or an invasive adenocarcinoma with lepidic pattern (hematoxylin-eosin staining ×100 on the left and ×200 on the right).

5% in the routine practice. The diagnosis of "non-squamous cell carcinoma" in case of a primary NSCLC lacking TTF-1 and p40 is essentially a clinical term and the WHO classification recommend to avoid this nomenclature (2). Nevertheless, since p40 is basically always expressed in squamous cell carcinomas (19,22) and its absence consistently supports the clinically-fruitful diagnosis of "non-squamous cell carcinoma" for practical purposes.

Since some lung malignancies are extremely heterogeneous from morphology to molecular grounds or require a complete examination of the entire tumor, the diagnosis of large cell carcinoma, adenosquamous carcinoma, sarcomatoid carcinomas, large cell neuroendocrine carcinoma (LCNEC), *in situ* and minimally invasive adenocarcinomas should be restricted to surgical specimens (*Figure 8*) (2).

However, in particularly lucky samples demonstrating a coexistent glandular and squamous cell differentiation, a diagnosis of adenosquamous carcinoma may be suggested even in small biopsy or cytology (*Figure 9*). Analogously, a limited specimen demonstrating a NSCLC with obvious

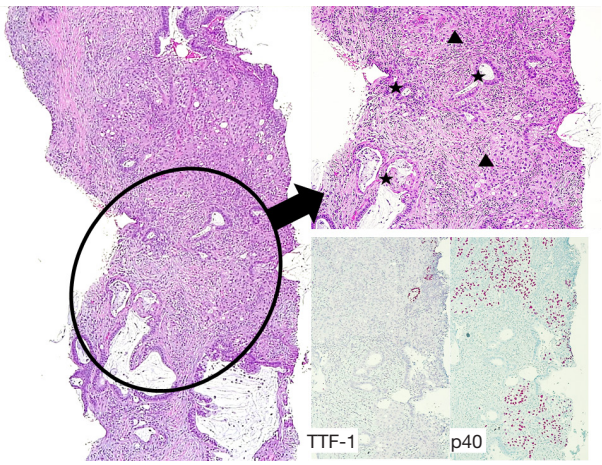

Figure 9 A generous transthoracic biopsy of a solid lung nodule histologically showing a biphasic tumor consisting of an invasive adenocarcinoma (black stars) strikingly intermingled with a poorly-differentiated squamous cell carcinoma (black triangles). At immunohistochemistry, the adenocarcinoma lacked both TTF-1 expression (probably due to the mucinous features; note the entrapped positive red nuclei of alveoli as positive internal control) and p40, whereas the squamous cell carcinoma resulted strongly and diffusely immunoreactive for p40 (hematoxylin-eosin staining ×100 on the left and ×200 on the right top; immunohistochemistry with TTF-1 and p40 ×80).

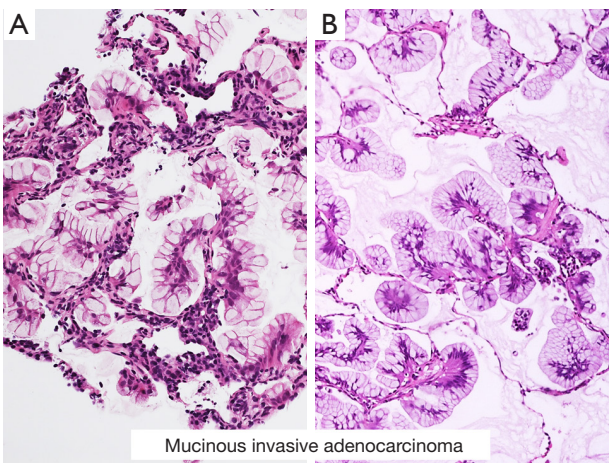

Figure 10 Some lung tumors have a homogeneous appearance permitting a reliable diagnosis even in small biopsy, as mucinous invasive carcinoma. (A) Transbronchial biopsy; (B) surgical specimen. Hematoxylin-eosin staining ×250.

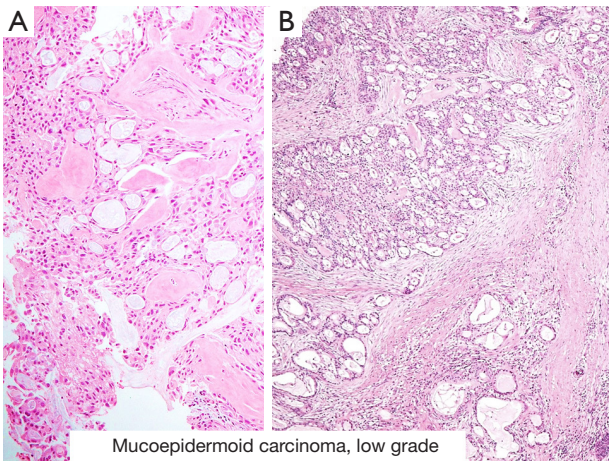

Figure 11 Mucoepidermoid carcinoma is another primary salivary gland-type lung tumor with homogeneous appearance on bronchial biopsy (A, hematoxylin-eosin staining ×200) and surgical specimen (B, hematoxylin-eosin staining ×100).

neuroendocrine morphology (peripheral nuclear palisading, solid organoid growth, rosette-like formations) and consistent expression of neuroendocrine markers should suggest a diagnosis of LCNEC (30).

The possibility of a sarcomatoid carcinoma could be also suggested when a malignant spindle, giant, blastomatous or sarcomatous heterologous component is present in a small sample concomitantly showing a part of conventional NSCLC (2,31).

Some other malignancies generally show a more homogeneous morphology on resection specimens and should be more consistently diagnosed in small biopsy or cytology, namely SCLC, mucinous invasive adenocarcinoma, salivary gland tumors and carcinoid tumors (*Figures 10,11*).

Report of lung cancer: minimal requirements

The conventional diagnosis of lung cancer is mainly performed in small biopsies and cytology and needs to be accompanied with some clinical data, including personal data of the patient, smoking habit, disease's stage, radiologic characteristics (central *vs.* peripheral nodule, solid *vs.* ground-glass, unique *vs.* multiple, metastatic sites), past medical history (e.g., previous neoplasms if any and what

primary), serum markers if detected, the phone and email address of the referring clinician.

On the other hand, the pathologic report should include the following data:

(I) Type of sample (cytology, biopsy, cell block) and interventional procedure (bronchial/transbronchial with/without cryoprobe, EBUS/EUS, CT-guided transthoracic approach);
(II) Adequacy evaluation: inadequate; adequate for diagnosis only; adequate for diagnosis and molecular biomarkers;
(III) Histological or cytopathological tumor type according to the fifth edition of the WHO classification (the prevalent pattern in case of adenocarcinoma);
(IV) Results of immunohistochemical and/or mucin stains, if used: a comment on the confidence level of the diagnosis (low *vs.* high) and the possible differential diagnosis, if useful;
(V) In case of reflex molecular testing, it is necessary to indicate the tumor tissue selected for molecular determinations (cytology, biopsy, cell block), the tumor/normal tissue rate of the selected tissue, the approximate percentage/number of viable tumor cells, the presence of necrosis, the use of microdissection, the use of decalcification.

Tissue handling

The first driver of lung cancer treatment is an accurate histologic definition. Indeed, histology has a key role in selecting chemotherapy with/without immunotherapy in wild-type NSCLC and in guiding the determination of targetable oncogene alterations (32). According to the National recommendations, several genes (e.g., *EGFR*, *BRAF*, *KRAS*, *HER2*, *c-MET* mutations, *ALK*, *ROS1*, *NTRKs* and *RET* rearrangements) need to be tested concomitantly in order to define the best first line approach, but the limited availability of tumor tissue requires a wise handling of small biopsy and cytology aimed at minimizing the failure rate in molecular testing. Pathologists are called to maximize this effort even using in-house tricks in collaboration with pulmonologists/radiologists, technicians and molecular biologists. Some examples are briefly mentioned in the following subheadings.

Limiting the staining panel for NSCLC subtyping

According to the fifth edition of the WHO classification of lung tumors (2), morphology on good-quality hematoxylin-eosin-stained slide is entirely sufficient to define histology in the great majority of cases (at least two thirds of routine cases in our personal experiences). In case of a poorly-differentiated NSCLC, pathologists should limit the immunohistochemical diagnostic panel at a minimum, including TTF-1 (possibly using the clone 8G7G3/1) and p40. Neuroendocrine markers should be tested only in carcinoma with overt neuroendocrine morphology, since some highly-sensitive and poorly-specific markers (e.g., NSE, PGP9.5, CD56) not infrequently stain adenocarcinomas and squamous cell carcinoma (2). Knowledge of imaging studies and past medical history of the patient are helpful finding in correctly addressing the differential diagnosis and preventing unnecessary immunostains then saving tumor tissue in favor of molecular determinations.

Finally, the knowledge of the patient's eligibility for surgical resection could also allow to avoid further immunostains in case of poorly-differentiated NSCLC on small or cytology specimen and to defer precise histotype definition to the analysis of the surgical tumor sample.

Cell block preparation

In situ (immunohistochemistry, in situ hybridization) and extractive molecular techniques tend to work preferably on different types of samples (cytology *vs.* biopsy) (*Figure 12*). Then, it may be crucial to arrange a cell block from all cytologic specimens (e.g., effusions, FNA) concurrently with smeared cytology. Preparation of cell block is a technique used to obtain and concentrate small fragments of tissue starting from a cytologic samples creating a unique pellet of cells. In this way, it is possible to appreciate the architecture of the tissue and provide multiple serial sections of various thickness especially dedicated to in situ ancillary analyses. Cell blocks may be archived as biopsies or used for tissue microarray construction. There are several methods to prepare cell block with various yields. Plasma thrombin, direct clotting, gel-based, collodion bag preparations are the most common, while buffered formalin is the most commonly used fixative (33-35). Optimization of cell block preparation requires a close collaboration with the physicians involved in sampling the tumor and a great skill of the pathologists and technicians. When cell block is prepared during rapid-on site evaluation (ROSE), the first smeared slides (air-dried for Diff-Quick and alcohol fixed for Papanicolaou stains) should be used to state the

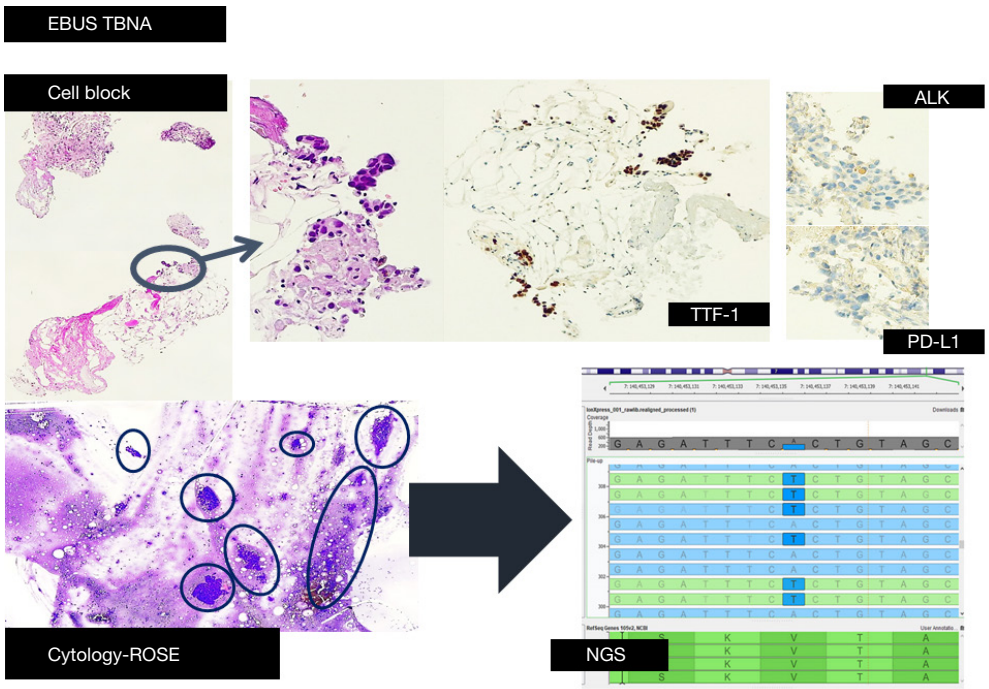

Figure 12 Tumor tissue for molecular markers should be treated differently in regards with the different techniques. In this endobronchial ultrasound-transbronchial needle aspiration, the tissue obtained from cell-block is used for in situ molecular determinations (immunohistochemistry for TTF-1, ALK and PD-L1), while formalin-free smeared cytology originally analyzed for rapid-on site evaluation is the perfect material for extractive methods as next-generation sequencing (images on the top from left to right: hematoxylin-eosin staining ×40, ×100, and then immunohistochemistry ×100; image on the bottom May-Grunwald-Giemsa ×100). EBUS, endobronchial ultrasound; NGS, next-generation sequencing; TBNA, transbronchial needle aspiration; ROSE, rapid-on site evaluation.

adequacy of the sample and the presence (and amount) of tumor cells, while all the remaining material should be dedicated to cell block preparation.

An alternative preparation of cell block, namely "cytoscrape", may be obtained from previously smeared FNA cytology, decolorizing and gently scraping tumor cells off the slides to perform cellular clots and then proceeding to cell block preparation (36).

Tumor enrichment by microdissection

Pathologists should mark and select tumor cells in the slides to enrich at maximum the sample for molecular testing achieving high-quality molecular testing. Necrosis, bloody areas, mucous plugs and inflammatory infiltrate should be eliminated or reduced at minimum from the selected material for molecular methodologies. Tumor cell microdissection from conventional smeared cytology, cell block or biopsy facilitates enrichment of tumor material due to an increased number of tumor cells reaching a very high tumor/normal cell ratio, possibly higher than 30% to minimize false-negative molecular results. Tumor cell microdissection should enter into the skills equipment of pathologists through the identification and selective isolation of tumor cells from H&E-stained slides examined at light microscope (*Figure 13*) (37,38).

High-throughput sequencing analysis

Hybrid-capture-based next-generation sequencing identifies a higher number of targetable gene alterations, then influencing treatment decisions in close to half of the patients if compared to other techniques.

Even more targeted therapies are continuously approved and next-generation sequencing can screen a large number of gene mutations/amplifications/rearrangements in a limited tumor tissue, then preventing the necessity to consume tumor tissue to perform immunohistochemistry and/or fluorescence

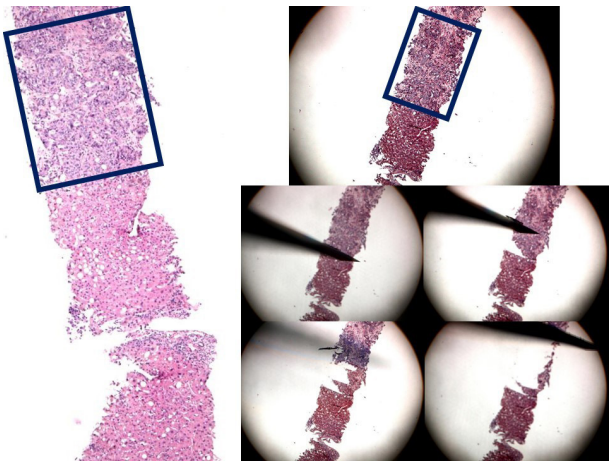

Figure 13 Manual microdissection (note the needle under the light microscope) of the neoplastic component in a liver biopsy partially involved by metastatic lung adenocarcinoma, enriching the tumor cell percentage and the tumor-to-normal tissue ratio (hematoxylin-eosin staining ×100 of the left, then ×50 at the right top and ×40 on the bottom).

in situ hybridization testing. Thus, the introduction of next-generation sequencing technologies in routine practice might allow a comprehensive characterization of all current and ongoing targetable genomic alterations (mutations, amplifications and gene fusions) from relatively limited source of tumor tissue and saving money if compared to conventional step-by-step molecular analysis (39,40).

Next-generation sequencing also enables to discover a portion of NSCLC patients with concomitant targetable gene alterations or druggable secondary resistance-related genetic changes, eventually permitting alternative treatments or enrollment in available clinical trials.

Standardized operative procedures

All the samples should be transferred to the pathology laboratory as soon as possible and biopsies or cell blocks should be fixed in buffered formalin for a period of time between 8 at minimum and 48 hours at maximum. Strategies to maximize tissue availability mainly involve pathologists and technicians, since minimally invasive sectioning requires experienced technicians with specific skills trained to cut appropriately the paraffin-embedded blocks in order to have the complete surface of the bioptic fragments on the initial slides without losing relevant material for diagnostic ancillary techniques and molecular testing (16,17). Although no relevant data are published in literature, a continue and direct communication between dedicated pathologists and technicians is very precious in optimizing tumor tissue handling in real-life practice.

Since re-cuts should be kept at the minimum, another internal smart option is to cut multiple (approximately 20) serial unstained sections and keep them stored until a preliminary diagnosis has been rendered and ancillary testing is requested. Sparing extra unstained sections at the first cutting session might to avoid tissue waste, especially if the amount of tissue is low, shortening turnaround times (5–10 days), saving tissue and reducing costs (16,17).

Multiple blocks from multiple biopsies/single biopsy

The pathology laboratory generally receives multiple small biopsies in a single container from interventional bronchoscopist or radiologist during invasive procedures in patients with suspected lung cancer. Separation of the fragments into multiple paraffin-embedded blocks acceptably increases the laboratory staff workflow, maximizing the probability of successful molecular testing, consuming less tumor surface in a unique slide and saving tissue with the aim to dedicate separate blocks for diagnostic immunostains (1 block), predictive molecular analysis (1 block) or tumor tissue for centralized investigation in clinical trial (1 block).

Since PD-L1 staining is characterized by intratumoral heterogeneity, in case of multiple biopsies it is essential to obtain the initial H&E-stained slide plus PD-L1 immunostaining collecting all the fragments in a unique block, then separating the bioptic fragments in dedicated different blocks as previously mentioned (41).

Reflex testing

Once diagnosed an advanced/metastatic NSCLC, up-front reflex testing ordering molecular biomarkers and/or preparing tumor tissue for molecular predictive markers may significantly decrease turnaround time and improving the detection rates of targeted gene alterations (42,43). Moreover, incorporating reflex biomarker testing into a diagnostic algorithm for NSCLC at the level of the pathology laboratory should be implemented at maximum, in agreement with the National Health System regulations.

Tissue decalcification

Decalcification alters nucleic acids and bone biopsy of

metastatic NSCLC requiring decalcification fixatives should be avoided whenever possible (44). In case of bone biopsy, it is a good practice to warn the laboratory and address the material directly to the pathologist and/or the technician involved in preparing the tumor tissue for molecular biomarker analysis, separating non-osseous component by palpation in an additional formalin-fixed paraffin-embedded block.

Final key messages

The diagnosis of lung cancer should be performed in accordance to the fifth WHO classification criteria (2). Definition of histological type of lung tumors is still useful for different aspects: (I) basic separation of SCLC from NSCLC has remarkable influence in considering surgery and medical treatments; (II) NSCLC subtyping significantly impact in guiding molecular biomarkers determination and relevant targeted treatments; (III) some patterns of adenocarcinoma (micropapillary and solid) could receive a clinical benefit from adjuvant chemotherapy; (IV) presence of STAS has a prognostic role directly correlating with recurrence and metastasis.

Apart from conventional diagnosis of lung cancer, the efficacy of novel targeted therapies in NSCLC has dramatically changed the role of the pathologist, actually leading to a structured classification of lung cancer in small biopsy/cytology samples and to a modern approach deeply focused in maximizing tumor tissue optimization for ensuring predictive molecular testing at first line treatment.

Acknowledgments

Funding: None.

Footnote

Provenance and Peer Review: This article was commissioned by the Guest Editors (Umberto Malapelle and Giancarlo Troncone) for the series "Predictive Molecular Pathology in Lung Cancer" published in *Journal of Xiangya Medicine*. The article has undergone external peer review.

Conflicts of Interest: Both authors have completed the ICMJE uniform disclosure form (available at https://jxym.amegroups.com/article/view/10.21037/jxym-22-1/coif). The series "Predictive Molecular Pathology in Lung Cancer" was commissioned by the editorial office without any funding or sponsorship. The authors have no other conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

References

1. Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin 2021;71:209-49.
2. WHO Classification of Tumours Editorial Board. Thoracic Tumours. 5th edition. Lyon: IARC Press, 2021.
3. Birim O, Kappetein AP, van Klaveren RJ, et al. Prognostic factors in non-small cell lung cancer surgery. Eur J Surg Oncol 2006;32:12-23.
4. Tyczynski JE, Bray F, Parkin DM. Lung cancer in Europe in 2000: epidemiology, prevention, and early detection. Lancet Oncol 2003;4:45-55.
5. Boffetta P, Pershagen G, Jöckel KH, et al. Cigar and pipe smoking and lung cancer risk: a multicenter study from Europe. J Natl Cancer Inst 1999;91:697-701.
6. Tsai LL, Chu NQ, Blessing WA, et al. Lung Cancer in Women. Ann Thorac Surg 2021. [Epub ahead of print].
7. Camidge DR, Doebele RC, Kerr KM. Comparing and contrasting predictive biomarkers for immunotherapy and targeted therapy of NSCLC. Nat Rev Clin Oncol 2019;16:341-55.
8. Aggarwal C, Thompson JC, Black TA, et al. Clinical Implications of Plasma-Based Genotyping With the Delivery of Personalized Therapy in Metastatic Non-Small Cell Lung Cancer. JAMA Oncol 2019;5:173-80.
9. Hierro C, Matos I, Martin-Liberal J, et al. Agnostic-Histology Approval of New Drugs in Oncology: Are We Already There? Clin Cancer Res 2019;25:3210-9.

10. Yan L, Zhang W. Precision medicine becomes reality-tumor type-agnostic therapy. Cancer Commun (Lond) 2018;38:6.
11. Gross DJ, Hsieh MS, Li Y, et al. Spread Through Air Spaces (STAS) in Non-Small Cell Lung Carcinoma: Evidence Supportive of an In Vivo Phenomenon. Am J Surg Pathol 2021;45:1509-15.
12. Kadota K, Nitadori JI, Sima CS, et al. Tumor Spread through Air Spaces is an Important Pattern of Invasion and Impacts the Frequency and Location of Recurrences after Limited Resection for Small Stage I Lung Adenocarcinomas. J Thorac Oncol 2015;10:806-14.
13. Tsao MS, Marguet S, Le Teuff G, et al. Subtype Classification of Lung Adenocarcinoma Predicts Benefit From Adjuvant Chemotherapy in Patients Undergoing Complete Resection. J Clin Oncol 2015;33:3439-46.
14. Travis WD, Brambilla E, Noguchi M, et al. Diagnosis of lung cancer in small biopsies and cytology: implications of the 2011 International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society classification. Arch Pathol Lab Med 2013;137:668-84.
15. Rossi G, Bargellini I, Bonifazi M, et al. Optimized tumor sampling and processing by a multidisciplinary approach for an accurate diagnosis in non-small cell lung cancer. EMJ Oncol 2019;7:90-9.
16. Thunnissen E, Kerr KM, Herth FJ, et al. The challenge of NSCLC diagnosis and predictive analysis on small samples. Practical approach of a working group. Lung Cancer 2012;76:1-18.
17. Dietel M, Bubendorf L, Dingemans AM, et al. Diagnostic procedures for non-small-cell lung cancer (NSCLC): recommendations of the European Expert Group. Thorax 2016;71:177-84.
18. Rossi G, Tiseo M, Cavazza A, et al. Is immunohistochemistry always required to diagnose lung cancer? Adv Anat Pathol 2013;20:327-33.
19. Rossi G, Pelosi G, Barbareschi M, et al. Subtyping non-small cell lung cancer: relevant issues and operative recommendations for the best pathology practice. Int J Surg Pathol 2013;21:326-36.
20. Yatabe Y, Dacic S, Borczuk AC, et al. Best Practices Recommendations for Diagnostic Immunohistochemistry in Lung Cancer. J Thorac Oncol 2019;14:377-407.
21. Rekhtman N, Ang DC, Sima CS, et al. Immunohistochemical algorithm for differentiation of lung adenocarcinoma and squamous cell carcinoma based on large series of whole-tissue sections with validation in small specimens. Mod Pathol 2011;24:1348-59.
22. Pelosi G, Fabbri A, Bianchi F, et al. D (delta)Np63 (p40) and thyroid transcription factor-1 (TTF1) immunoreactivity upon small biopsies or cell blocks for typing non-small cell lung cancer: a novel two-hit, sparing-material approach. J Thorac Oncol 2012;7:281-90.
23. Bishop JA, Teruya-Feldstein J, Westra WH, et al. p40 (ΔNp63) is superior to p63 for the diagnosis of pulmonary squamous cell carcinoma. Mod Pathol 2012;25:405-15.
24. Matoso A, Singh K, Jacob R, et al. Comparison of thyroid transcription factor-1 expression by 2 monoclonal antibodies in pulmonary and nonpulmonary primary tumors. Appl Immunohistochem Mol Morphol 2010;18:142-9.
25. Ordóñez NG. Value of thyroid transcription factor-1 immunostaining in tumor diagnosis: a review and update. Appl Immunohistochem Mol Morphol 2012;20:429-44.
26. Stelow EB, Yaziji H. Immunohistochemistry, carcinomas of unknown primary, and incidence rates. Semin Diagn Pathol 2018;35:143-52.
27. Gomez-Fernandez C, Mejias A, Walker G, Nadji M. Immunohistochemical Expression of Estrogen Receptor in Adenocarcinomas of the Lung. Appl. Immunohistochem. Mol. Morphol. 2010;18:137–41.
28. Rossi G, Murer B, Cavazza A, et al. Primary mucinous (so-called colloid) carcinomas of the lung: a clinicopathologic and immunohistochemical study with special reference to CDX-2 homeobox gene and MUC2 expression. Am J Surg Pathol 2004;28:442-52.
29. Sonzogni A, Bianchi F, Fabbri A, et al. Pulmonary adenocarcinoma with mucin production modulates phenotype according to common genetic traits: a reappraisal of mucinous adenocarcinoma and colloid adenocarcinoma. J Pathol Clin Res 2017;3:139-52.
30. Baine MK, Sinard JH, Cai G, et al. A Semiquantitative Scoring System May Allow Biopsy Diagnosis of Pulmonary Large Cell Neuroendocrine Carcinoma. Am J Clin Pathol 2020;153:165-74.
31. Baldovini C, Rossi G, Ciarrocchi A. Approaches to Tumor Classification in Pulmonary Sarcomatoid Carcinoma. Lung Cancer (Auckl) 2019;10:131-49.
32. Davidson MR, Gazdar AF, Clarke BE. The pivotal role of pathology in the management of lung cancer. J Thorac Dis 2013;5 Suppl 5:S463-78.
33. Saqi A. The State of Cell Blocks and Ancillary Testing: Past, Present, and Future. Arch Pathol Lab Med 2016;140:1318-22.
34. van der Heijden EH, Casal RF, Trisolini R, et al. Guideline

for the acquisition and preparation of conventional and endobronchial ultrasound-guided transbronchial needle aspiration specimens for the diagnosis and molecular testing of patients with known or suspected lung cancer. Respiration 2014;88:500-17.
35. Kossakowski CA, Morresi-Hauf A, Schnabel PA, et al. Preparation of cell blocks for lung cancer diagnosis and prediction: protocol and experience of a high-volume center. Respiration 2014;87:432-8.
36. Nga ME, Lim GL, Barbro N, et al. Successful retrieval of fine-needle aspiration biopsy material from previously stained smears for immunocytochemistry: a novel technique applied to three soft tissue tumors. Mod Pathol 2005;18:728-32.
37. Aisner DL, Rumery MD, Merrick DT, et al. Do More With Less: Tips and Techniques for Maximizing Small Biopsy and Cytology Specimens for Molecular and Ancillary Testing: The University of Colorado Experience. Arch Pathol Lab Med 2016;140:1206-20.
38. Roy-Chowdhuri S, Aisner DL, Allen TC, et al. Biomarker Testing in Lung Carcinoma Cytology Specimens: A Perspective From Members of the Pulmonary Pathology Society. Arch Pathol Lab Med 2016;140:1267-72.
39. Rozenblum AB, Ilouze M, Dudnik E, et al. Clinical Impact of Hybrid Capture-Based Next-Generation Sequencing on Changes in Treatment Decisions in Lung Cancer. J Thorac Oncol 2017;12:258-68.
40. Dall'Olio FG, Conci N, Rossi G, et al. Comparison of Sequential Testing and Next Generation Sequencing in advanced Lung Adenocarcinoma patients - A single centre experience. Lung Cancer 2020;149:5-9.
41. Pruneri G, De Braud F, Sapino A, et al. Next-Generation Sequencing in Clinical Practice: Is It a Cost-Saving Alternative to a Single-Gene Testing Approach? Pharmacoecon Open 2021;5:285-98.
42. Anand K, Phung TL, Bernicker EH, et al. Clinical Utility of Reflex Ordered Testing for Molecular Biomarkers in Lung Adenocarcinoma. Clin Lung Cancer 2020;21:437-42.
43. Lim C, Tsao MS, Le LW, et al. Biomarker testing and time to treatment decision in patients with advanced nonsmall-cell lung cancer. Ann Oncol 2015;26:1415-21.
44. Vanderlaan PA, Yamaguchi N, Folch E, et al. Success and failure rates of tumor genotyping techniques in routine pathological samples with non-small-cell lung cancer. Lung Cancer 2014;84:39-44.

doi: 10.21037/jxym-22-1
Cite this article as: Rossi G, Graziano P. Lung cancer diagnosis. J Xiangya Med 2022;7:16.