Introduction

Among all lung primary pulmonary carcinomas, non-small cell lung cancer (NSCLC) accounts for about 75–80% of all cases of pulmonary malignancies (1) and radical surgery still remains the only treatment with curative intent. Different factors influence the best management of patients with NSCLC, including those associated with the patient (respiratory functions, performance status, past medical history) and/or related to the tumour characteristics (stage, molecular predictive biomarkers), including histology (or histological type) (2,3). Of note, epidemiologic works revealed a significant increase in the incidence of adenocarcinomas, particularly in women, from 1979 to 1998 (4,5), possibly due to smoking habit and a different cigarettes composition leading to greater exposure of cigarette carcinogens in the peripheral alveolar regions of the lungs (6).

In regards with histological tumour definition, clinicians generally subdivide lung cancer into two major groups: small cell lung cancer (SCLC) and NSCLC (7). This dichotomic classification has been considered sufficiently exhaustive for the management of patients with lung cancer up to the introduction of chemotherapy regimen with pemetrexed and/or bevacizumab, requiring the NSCLC subcategorization at least into squamous versus non-squamous cell carcinoma (8-11). In the meantime, the huge amount of molecular information derived from gene expression profiling and next generation sequencing studies evidenced several genetic alterations in lung cancer.
oncogenic drivers predicting efficacy using specific targeted molecules (small inhibitors and monoclonal antibodies), somehow limiting the role of tumour histotype (12-16).

Indeed, in the last decade the most important and effective changes in the therapeutic approach of patients with lung cancer are related to the discovery of molecular impairments in druggable oncogenic drivers and immunotherapy using humanized monoclonal antibodies blocking the programmed death (PD)-1/PD-ligand (PD-L)-1 checkpoint (12). In addition, the advent of liquid biopsy already permits to evidence key genetic alterations using highly sensitive methods to sequence DNA and RNA (13), preventing to obtain tissue samples using invasive procedures and facilitating the introduction of ongoing drugs against histology-agnostic genetic alterations (e.g., NTRKs) (Figure 1) (14-16). All these features are deeply challenging the role of classic lung tumor histologic definition and enrich the meaning of histology with additional molecular information (Figure 2).

Figure 1 A summarized landscape of lung cancer integrating predictive biomarkers, conventional histology and new available drugs from 1970 to 2020.

Figure 2 The ongoing multidisciplinary approach to lung cancer is deeply influenced by the introduction of liquid biopsy and NGS, somehow limiting the role of pathologists, bronchoscopists and radiologists. NGS, next-generation sequencing.
Table 1  Immunohistochemical expression of the most common primary antibodies used in primary lung cancer

<table>
<thead>
<tr>
<th>Antibody</th>
<th>ADC</th>
<th>SQC</th>
<th>SCLC/LCNEC</th>
<th>Carcinoid tumors</th>
<th>Metastasis</th>
</tr>
</thead>
<tbody>
<tr>
<td>TTF-1</td>
<td>~80%</td>
<td>Never</td>
<td>60–80%</td>
<td>Peripheral type</td>
<td>Endometrial cancer*</td>
</tr>
<tr>
<td>Napsin</td>
<td>~80%</td>
<td>Never</td>
<td>Never</td>
<td>Never</td>
<td>some renal cancer</td>
</tr>
<tr>
<td>CK7</td>
<td>Almost all</td>
<td>30–60%</td>
<td>&gt;50%</td>
<td>&gt;50%</td>
<td>several other primaries</td>
</tr>
<tr>
<td>p63</td>
<td>30%</td>
<td>Almost all</td>
<td>Almost never</td>
<td>Never</td>
<td>SQC from other sites urothelial carcinoma</td>
</tr>
<tr>
<td>p40</td>
<td>Almost never°</td>
<td>Almost all</td>
<td>Almost never</td>
<td>Never</td>
<td>SQC from other sites urothelial carcinoma</td>
</tr>
<tr>
<td>CK5/6</td>
<td>10%</td>
<td>Almost all</td>
<td>Almost never</td>
<td>Never</td>
<td>SQC from other sites urothelial carcinoma</td>
</tr>
<tr>
<td>CDX2</td>
<td>Enteric, colloid</td>
<td>Never</td>
<td>10–20%</td>
<td>Never</td>
<td>Colo-rectal ADC, ADC with enteric differentiation</td>
</tr>
<tr>
<td>CK20</td>
<td>Enteric, colloid, mucinous</td>
<td>Never</td>
<td>Never</td>
<td>Never</td>
<td>ADC with intestinal differentiation</td>
</tr>
<tr>
<td>ER/PgR</td>
<td>10–20%*</td>
<td>Never</td>
<td>Almost never</td>
<td>Sometimes</td>
<td>Breast, GYN tract</td>
</tr>
</tbody>
</table>

* TTF-1 clones SPT24 and SP141 may be expressed in several extra-pulmonary tumors (e.g., breast and gastric cancer, mesothelioma); °, ER/PgR are mainly expressed at low intensity in well-differentiated adenocarcinoma arising in women; °, p40 is focally expressed in a small subset of lung adenocarcinoma. ADC, adenocarcinoma; SQC, squamous cell carcinoma; SCLC/LCNEC, small cell lung cancer/large cell neuroendocrine carcinoma; TTF-1, thyroid transcription factor 1; CK, cytokeratin; CDX2, caudal type homeobox 2; GYN, gynaecologic tract; GI, gastrointestinal tract; CK, cytokeratin; ER, estrogen receptor; PgR, progesterone receptor.

Histology is no more limited to morphology

Histologic definition of lung cancer is based on criteria posed by the most recent 2015 WHO classification (17). Since more than two third of NSCLC are not resectable at diagnosis, histologic features derived from examination of surgically-excised tumors are not always translatable in cytology/small biopsy. In addition, limitation of histology-based therapies relies in the simple distinction between squamous cell versus non-squamous cell carcinoma as the mainstay for clinical management of patients with NSCLC.

Although recommended for NSCLC subtyping, the helpful role of immunohistochemistry in supporting morphology is somehow limited by the “aberrant” expression of some biomarkers (e.g., p63 reactivity in adenocarcinoma or CK7 in squamous cell carcinoma) (Table 1). In this setting, the best single marker for adenocarcinoma is TTF1 (clone 8G7G3/1) and the best single marker for squamous cell carcinoma is p40, realizing a two-hit, sparing material algorithm suitable for both cytology and small biopsy specimens (Figure 3) (Table 2) (18-24).

The list of primary lung cancers identified in the WHO classification comprises 3 main categories (adenocarcinoma, squamous cell carcinoma, neuroendocrine tumours) and poorly-differentiated or undifferentiated tumours lacking any differentiation at light microscope analysis or extensive immunostaining [e.g., large cell carcinoma (LCC) and sarcomatoid carcinoma].

Limitations of conventional histologic examination to disclose the original cell differentiation in poorly-differentiated/undifferentiated carcinomas are well-demonstrated by recent molecular studies in large cell neuroendocrine carcinoma (LCNEC), undifferentiated LCC [namely NSCLC, not otherwise specified (n.o.s.) on cytology and small biopsy] and sarcomatoid carcinoma.

LCNEC has been considered a variant of LCC in the previous 2004 WHO classification (2) of lung cancer and then included as poorly-differentiated carcinoma into the rubric of neuroendocrine tumours in the last classification (17). The diagnosis requires a clear-cut neuroendocrine differentiation at morphology and at the immunohistochemistry level. The prognosis is quite similar to that of SCLC (17). However, the overall survival at 5 years reported in literature is ranging from 13% to 51% in stage I, consistently suggesting that this challenging diagnosis depends on the application of rigorous pathologic criteria (25).

Indeed, next-generation sequencing (NGS) molecular analysis demonstrates that LCNEC is a biologically heterogeneous basket of tumours segregated in at least 3 main subgroups: SCLC-like (TP53+RB1 and MYCL amplification), NSCLC-like (lack of co-altered TP53+RB1 and mutations of STK11, KRAS, KEAP1)
**Table 2** Immunohistochemical primary antibodies indicating cell differentiation in lung cancer

<table>
<thead>
<tr>
<th>Histology</th>
<th>First choice</th>
<th>Second choice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenocarcinoma</td>
<td>TTF-1 (clone 8G7G3/1)</td>
<td>Napsin; CK7</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>p40</td>
<td>p63; CK5/6; desmocollin</td>
</tr>
<tr>
<td>NE tumors</td>
<td>chromogranin; synaptophysin</td>
<td>CD56</td>
</tr>
<tr>
<td>Others</td>
<td>CDX2 (adenocarcinoma, enteric and colloid variants)</td>
<td>SMARCA4 (undifferentiated SMARCA4-deficient carcinoma)</td>
</tr>
</tbody>
</table>

NE, neuroendocrine.

**Figure 3** A concise scheme illustrating the two-hits immunohistochemical algorithm in subtyping NSCLC using TTF-1 and p40 (magnification ×200). NSCLC, non-small cell lung cancer.

and carcinoid-like (*MEN1* mutations and low mutation burden). SCLC-like showed higher proliferative activity than NSCLC-like tumours (P<0.0001), while NSCLC-like LCNEC harboured distinctive genomic alterations, including mutations of NOTCH family genes regulating neuroendocrine differentiation (26).

In addition, Derks *et al.* (27) showed that LCNEC carrying a wild-type *RB1* gene or expressing the RB1 protein do better with NSCLC-like treatment (gemcitabine/Taxol) than with SCLC-chemotherapy (etoposide), confirming previous experiences (28).

Karlson *et al.* (29) investigated LCCs with (n=32) or without (n=41) neuroendocrine features using massive parallel sequencing for mutations in 26 cancer-related genes and gene fusions in *ALK, RET,* and *ROS1*. Based on immunostains, LCC without NE differentiation were subdivided in adenocarcinoma-like (TTF1/napsin +), squamous-like (CK5/p40) and “null” type. The most common alterations in LCC lacking NE features were *TP53* (83%), *KRAS* (22%), *MET* (12%) mutations, while *TP53*
(88%), STK11 (16%), and PTEN (13%) mutations were significantly higher in LCNEC, demonstrating that LCC with and without NE features follow different molecular pathways impacting in therapeutic decisions.

LCC is a non-small-cell carcinoma lacking morphologic differentiation of either adenocarcinoma or squamous cell carcinoma, basically representing a highly aggressive tumour with an end-stage cell differentiation (30,31). Rekhtman et al. (32) analysed 102 LCC with immunostaining (TTF-1 versus p40) and molecular gene alterations (EGFR, KRAS, BRAF, MAP2K1/MEK1, NRAS, ERBB2/HER2 mutations and ALK rearrangements versus PIK3CA and AKT1 mutations) for adenocarcinoma and squamous cell carcinoma, respectively. Of note, molecular alterations characteristic of adenocarcinoma occurred in tumours with immunoprofiles of adenocarcinoma or marker-null, but not in tumours with squamous immunoprofile (combined mutation rate 50% vs. 30% vs. 0%, respectively; P<0.001), whereas the sole PIK3CA mutation occurred in a tumour with squamous profile (5%). Then, the majority (80%) of LCC did represent poorly-differentiated forms of adenocarcinoma or squamous cell carcinoma.

Similarly, Driver et al. (33) reclassified 17 LCC as adenocarcinoma (9 cases with mutations in KRAS, EGFR, BRAF) and 8 as squamous cell carcinoma (PIK3CA, CDKN2A mutations) using NGS technology. Pelosi et al. (34) disected 30 LCC by unsupervised targeted next generation sequencing analysis demonstrating that 3 cases showing TTF1-/p40+ phenotype harboured TP53 only in keeping with a squamous cell lineage, while the others 90% featuring various phenotypical combinations of TTF1 and p40 comprised ATM, BRAF, CDKN2A, EGFR, ERBB4, FBXW7, FLT3, KRAS, NRAS, PIK3CA, PTPN11, RET, SMAD4, SMO, STK11, or TP53 mutations in keeping with adenocarcinoma lineage. Another study investigating lineage-specific immunomarkers, EGFR and KRAS mutations and ALK rearrangement in 121 LCC along the spectrum of variants provided by the 2004 WHO classification evidenced that all 47 LCNEC had a true neuroendocrine cell lineage without gene alterations, whereas all 24 basolaid and 2 lymphoepithelioma-like carcinomas showed squamous cell markers (35). Eighteen out of 22 clear cell carcinomas had glandular differentiation, with KRAS mutations in 39% of cases. Eighteen out of 20 undifferentiated LCC showed glandular differentiation upon immunohistochemistry, with exon 21 L858R EGFR mutation in one (5%) tumour, exon 2 KRAS mutation in eight (40%) tumours, and ALK translocation in one (5%). All 6 LCC of rhabdoid type expressed TTF-1 and/or CK7, 50% of which also harboured KRAS mutations (35). At the end, molecular alterations were restricted to LCC having an adenocarcinoma cell differentiation and stratification of LCC using immunohistochemistry and molecular analysis revealed a direct correlation between phenotypic and genotypic arrangements.

Sarcomatoid carcinoma is an umbrella term to indicate a group of poorly-differentiated/undifferentiated NSCLC showing sarcoma-like (giant and/or spindle cell component) or true sarcomatous (mainly chondrosarcoma, osteosarcoma and rhabdomyosarcoma) differentiation with or without a component of conventional NSCLC (17,36), then including different variants (pleomorphic, spindle cell, giant cell carcinomas, carcinosarcoma and pulmonary blastoma). Previous studies on relatively large series demonstrated that at immunohistochemistry thyroid transcription factor-1 (TTF-1) and cytokeratin 7 were positive in 55% and 70% of spindle and/or giant cell carcinomas and 43% and 63% in pleomorphic carcinomas, supporting the metaplastic histogenetic theory for these tumours, ancestrally starting from a conventional histology possessing a genetic EMT program (37). Even at molecular level, sarcomatoid carcinomas harbour mutations involving oncogenic drivers of adenocarcinoma or squamous cell carcinomas, including KRAS, EGFR, TP53, STK11, NOTCH1, NRAS, PI3KCA and BRAF (38-40). A significantly higher rate of c-MET skipping mutations in exon 14 and MET amplification have been reported in this rare histology (41,42).

Finally, histology has also a role in explaining primary or secondary resistance to tyrosine kinase inhibitors (TKI) in tumours harbouring mutations or rearrangements in the most common oncogenic drivers, but also in chemotherapy and immunotherapy (43-67). Indeed, a histologic change from adenocarcinoma to small cell or squamous cell carcinoma have been well-demonstrated in about 10% of EGFR mutated or ALK rearranged lung cancer (68,69).

More rarely, a sarcomatoid “transformation” due to activation of EMT has been described by Hsieh et al. (48) in 6 cases of adenocarcinoma (5 EGFR mutated, 1 ROS1 rearranged). Histologic change to sarcomatoid carcinoma in TKI resistant adenocarcinomas is accompanied by PD-L1 over-expression and c-MET gene alterations.

The pass to sarcomatoid histology has been previously demonstrated in EGFR-mutated cell lines of lung adenocarcinoma, and concurrent acquisition of other gene alterations (i.e., T790M EGFR or NKx2-4 mutation) was recently observed (49-52).
Histologic “transformation” has clearly a key therapeutic impact in predicting the urgent need for alternative therapies in NSCLC progressing on TKI.

**Histology-based chemotherapy**

While multivariate analyses on large cooperative groups on chemotherapy in advanced NSCLC stated that histotype is not a determinant factor of efficacy and have a little, if none, prognostic significance (7), alternative chemotherapeutic protocols have reported a different response rate in regards with lung cancer histology.

Tegafur-uracil in adjuvant setting improved overall survival of Japanese patients with stage I adenocarcinoma subtype (70). Again, Georgoulas et al. (71) revealed that the regimen including gemcitabine + docetaxel was significantly more effective in patients with adenocarcinoma histology than in non-adenocarcinomatous NSCLC. On the other hand, patients with non-adenocarcinoma NSCLC had a significant better response to cisplatinum + docetaxel than those with adenocarcinoma histology, then stating that histological type had an important predictive role at univariate and multivariate statistical analysis (71).

Another controversial lung tumour entity lacking standard chemotherapeutic protocols is LCNEC. As originally described by Travis et al. (72), LCNEC seems to have a very poor outcome, quite similar to SCLC with which LCNEC shares various genetic alterations (17,73). Recent retrospective studies have demonstrated a significant higher survival in patients receiving a SCLC-like chemotherapy protocol (platinum + etoposide/VP16) either in adjuvant and metastatic settings (28,74,75). In addition, a prospective study of adjuvant chemotherapy for pulmonary LCNEC by Iyoda et al. (75) confirmed that patients (n=15) undergoing cisplatin and VP16 after surgery had a significant survival improvement at 2 and 5 years. These results were subsequently confirmed in other works (26-28), particularly when LCNEC had SCLC-type alterations, namely TP53+RB1 co-mutation/loss (26).

The key role of histologic subtyping in the best choice of chemotherapeutic protocols has recently merged from a planned subgroup analysis of the JMEN phase III clinical trial comparing pemetrexed and gemcitabine in association with platinum. More in details, Scagliotti et al. (11) reported a survival advantage for cisplatin + pemetrexed over cisplatin + gemcitabine (11.8 versus 10.4 months) in patients with non-squamous NSCLC, with a more impressive result in patients with adenocarcinoma (12.6 versus 10.9 months; P=0.03). Of note, the analysis in the population with NSCLC n.o.s. failed to reveal a significant difference in survival. Based on this trial, histological subtyping was considered mandatory in planning a regimen with cisplatin + pemetrexed in chemo naive patients with non-squamous NSCLC, lacking targetable genetic alterations and/or not amenable to immunotherapy.

Nevertheless, another phase III study enrolling 436 patients did not demonstrate significant differences for quality of life and in overall survival between the two treatment arms (pemetrexed/carboplatin, 7.3 months versus gemcitabine/carboplatin, 7.0 months; P=0.63) with less hematologic toxicity and less need for supportive care (76).

In a phase II trial (10), 99 patients randomly assigned to bevacizumab or plus carboplatin or paclitaxel or carboplatin and paclitaxel alone, treatment with carboplatin and paclitaxel plus bevacizumab resulted in a higher response rate (31.5% vs. 18.8%), longer median time to progression (7.4 vs. 4.2 months) and a modest increase in survival (17.7 vs. 14.9 months). Bleeding was the most prominent adverse event and was major haemoptysis was associated with squamous cell histology, tumor necrosis and cavitation, and disease location close to major blood vessels. Then, patients with non-squamous cell histology appeared to represent a subset with improved outcome and acceptable safety risks. Based on this previous study, a randomized study by the Eastern Cooperative Oncology Group (ECOG) including 878 patients with recurrent or advanced non-small-cell lung cancer (stage IIIB or IV) compared chemotherapy with paclitaxel and carboplatin alone versus paclitaxel and carboplatin plus bevacizumab was designed, but squamous-cell carcinoma histology was a major parameter in excluding patients for enrolment (77).

So, non-squamous carcinoma histology has become a selective factor when using chemotherapeutic regimens comprising pemetrexed or bevacizumab (78) (Table 3).

**Histology, molecular biology and targeted therapy**

Several lung cancer oncogenic drivers acting even as targetable genes are specifically related to adenocarcinoma histology, namely EGFR, KRAS, BRAF, HER2, c-MET mutations and ALK, ROS1, RET or NTRK3 rearrangements (79).

In some way, these molecular alterations intrinsically possess a diagnostic value. In other words, the finding of the aforementioned genetic abnormalities consistently indicates that the analysed tumour has an adenocarcinoma histology,
although not in an absolute meaning (80-84) (Figure 4).

Indeed, sporadic reports have described cases of squamous cell carcinoma, SCLC/LCNEC, sarcomatoid carcinoma harbouring EGFR mutations or ALK and ROS1 rearrangements (85-87). Interestingly, clinical response to TKI in non-adenocarcinoma lung cancer with EGFR sensitive mutations is significantly lower than that observed in mutated adenocarcinomas and similar to chemotherapy (87).

Nevertheless, Cooperative Groups (88) have prospectively performed a genome-based diagnosis testing 5,145 lung cancer of different histology, successfully assigning a histopathologic type in 75% of cases. EGFR, KRAS, ERBB2, BRAF, STK11, ALK gene alterations and NKX2-1 amplification were significantly restricted to adenocarcinoma, whereas DDR2, FGFR3, NFE2L2 mutations and SOX2 or FGFR1 amplification in squamous cell carcinoma. MYCN amplification and RB deletion occurred in SCLC. These important results open to a molecular diagnosis providing a genetic diagnosis/histology and oncogenic driver alterations permitting specific tailored therapies.

In this revolutionary landscape dominated by identification of molecular gene alterations and specifically-related pharmaceutical agents using multigene panel by NGS broadly covering all types of lung cancer in routine practice, histology is losing its importance even in selecting molecular tests (89,90).

Indeed, molecular determinations can already override histology in poorly-differentiated NSCLC resulting particularly challenging at light microscopy and immunohistochemistry (e.g., adenocarcinomas

| Table 3 Therapeutic agents requiring predictive determinations in NSCLC |
|-------------------------|--------------------------|
| Agent                  | Predictive factor      |
| Bevacizumab            | Histology (non-squamous) |
| Pemetrexed             | Histology (non-squamous) |
| EGFR inhibitors        | EGFR mutation          |
| ALK inhibitors         | ALK rearrangement       |
| ROS1 inhibitors        | ROS1 rearrangement      |
| BRAF inhibitors        | BRAF (V600E) mutation   |
| PD-1/PD-L1 blockers    | PD-L1 ≥50% (first line with pembrolizumab) |
| NTRK inhibitors        | NTRKs rearrangements   |

NSCLC, non-small cell lung cancer.

Figure 4 A real-life case of a 51-non-smoker woman with lung cancer diagnosed on plasma-NGS after a negative transbronchial biopsy. The finding of EGFR mutation (or other targetable oncogenic drivers) has a double meaning, permitting a diagnosis of adenocarcinoma and targeted therapy with an EGFR inhibitor. NGS, next-generation sequencing.
with “squamoid” pattern, adenosquamous carcinoma or squamous cell carcinoma with pseudoglandular differentiation), finally revealing gene alterations specifically characterizing lung cancer histotypes (e.g., EGFR or KRAS mutations in adenocarcinoma or PI3KCA and miR-205 in squamous cell carcinoma) concomitantly having a therapeutic significance (91,92).

Several studies investigating gene expression profiles and massively sequencing tumor DNA have proposed a molecular classification of lung cancer secondary to evidence of histology-specific genetic alterations, then improving the diagnosis and treatments particularly in adenocarcinoma.

The seminal study by Bhattcharjee et al. (93) analyzed mRNA expression levels in 139 resected adenocarcinomas clustering expression data in distinct subclasses, evidencing 1 subset with high relative expression of neuroendocrine genes and poor prognosis and demonstrating the ability to discriminate primary lung adenocarcinomas from metastases of extra-pulmonary origin.

In comparison to conventional histologic classification, the added value of these studies is in highlighting the genetic complexity of lung cancer. In other words, dealing with a common squamous cell carcinoma, molecular analysis may reveal the hidden heterogeneity stratifying the tumor in different biologic entities. Wilkerson et al. (94) clustered squamous cell carcinomas at mRNA expression in at least 4 subtypes, named primitive, classical, secretory, and basal demonstrating the worst survival outcome of the primitive type (P<0.05) and the relationship between the different profiles and biological processes (primitive: proliferation; classical: xenobiotic metabolism; secretory: immune response; basal: cell adhesion).

Despite the literature clearly indicates that molecular alterations involving EGFR, KRAS, BRAF, HER2 and ALK or ROS1 rearrangements are basically detected in adenocarcinoma only or in combined adenocarcinoma (80,95-105), no study has been finalized at demonstrating the diagnostic role of mutational analysis in differentiating lung cancer histologic types.

Several studies have identified genomic alterations and actionable mutations in lung adenocarcinoma and even in squamous cell carcinoma and SCLC (106-109) demonstrating that just a limited number of somatically mutated genes overlap all three subtypes and possibly permitting the use of a set of genes to address lung cancer diagnosis.

The increase incidence of adenocarcinoma histotype among all primary lung cancers may more likely lead to multiple lesions in the lungs. The distinction between different primary tumors or intrapulmonary metastases has a fundamental clinical value influencing the diagnosis, the tumor stage and then the patient management. Since it is not always reliable to distinguish separate primary lung cancers from intrapulmonary metastasis on histology and immunostains, Chang et al. (110) investigated the value of molecular analysis by NGS in 76 tumor pairs from 60 patients. NGS classified tumor pairs into 51 definite separate primary cancers and 25 metastatic tumors evidencing discordant results with histology in 17 cases (22%), particularly in metastatic cancers (44% discordant). These results robustly support the diagnostic role of NGS to assist conventional histology and immunohistochemistry in defining primary versus metastatic multiple lung cancer in clinical practice.

**Molecular histology in liquid biopsy**

The diagnosis of lung cancer is generally based on identification of tumor cells at light microscope examination, but the ever increasing need to acquire tumor tissue to deeply investigate molecular profile in naive NSCLC, the mechanisms underlying secondary resistance to TKI during disease progression and intratumor heterogeneity is partially hampered by the minimal availability of re-biopsy (111-117).

In the era of targeted treatments guided by recognition of “druggable” oncogenic drivers, the advent of liquid biopsy providing a comprehensive genetic profile of lung cancer through analysis of circulating tumor DNA (ctDNA), circulating tumor cells or exosomes is a revolutionary approach over conventional sampling procedures (118,119). Liquid biopsy is recommended in the new College of American Pathologists (CAP)/International Association for the Study of Lung Cancer (IASLC)/Association for Molecular Pathology (AMP) guideline for molecular testing of patients with NSCLC (113).

According to the statement paper from the IASLC, Rolfo et al. (120) proposed the use of liquid biopsy (circulating cell-free tumor DNA in plasma) in case of biopsy containing insufficient tumor tissue or when tissue specimens are not obtainable by traditional procedures (suboptimal clinical condition of the patients, unfavorable tumor site, high risk of major complications). Tissue biopsy is generally more expensive than a blood draw, particularly when repeated analyses are required during disease progression on targeted
therapy. In addition, liquid biopsy has a shorter turnaround time and is more representative of the entire biology of metastatic NSCLC.

Zhang et al. (121) analyzed genetic alterations of 48 tissue biopsy and matched liquid biopsy from early-stage NSCLC using 546 genes capture-based NGS assay demonstrating a concordant setup, particularly in squamous cell carcinoma. Then, a model of 14 gene mutations (TP53, NOTCH3, IDH1, RET, ROS1, ALK, MET, MRE11A, ERCC3, CDC25C, RB1, ALK, NFE2L2, FBXW7) showed an overall accuracy of 90% in the training and testing set aimed at histologic subtyping.

In previous studies, plasma NGS (probe set including KRAS, EGFR, ALK, HER2, BRAF, NRAS, PI3K3CA, MET, MEK1, TP53 mutations and ALK, ROS1, RET rearrangements) performed in progressive NSCLC was able to identify gene mutations, amplification and rearrangements with a specificity of 100% and a sensitivity of 77% when compared with tumor tissue genotype (122). In addition, plasma NGS permitted to recognized actionable gene alterations (EGFR mutation and MET amplification) in patients with incomplete tissue genotyping without false positive results.

In another study, Müller et al. (123) investigated a cohort of 82 patients with non-squamous NSCLC by a massively parallel sequencing liquid biopsy assay covering 39 genes (NEOliquid) matching plasma and tissue samples. The concordance was 98%, the sensitivity 70% and the specificity 100%. Among discordant cases, some cases had a driver mutation only in plasma (IDH1, RET, MET mutations).

More recently, Aggarwal et al. (13) prospectively enrolled 323 patients with metastatic NSCLC routinely tested with plasma NGS using a 73-gene commercial platform. Among 94 patients with plasma NGS alone there were 31 targetable mutations (33%), while the 229 patients with plasma and tissue NGS, an actionable mutation was disclosed in 47 tissue biopsy (20.5%) and in 82 liquid biopsies (35.8%). The authors recommended an integration of plasma NGS testing into the routine practice, possibly increasing the spectrum of targetable mutations if compared with conventional tissue biopsy.

Finally, Zhou et al. (124) compared tumor DNA and cell-free DNA in 63 patients diagnosed as LCNEC by target-capture sequencing demonstrating that the mutation concordance was 90% and patients with LCNEC presenting a SCLC-like genomic subtyping (mutations or copy number loss in both RB1 and TP53) have a shorter overall survival and a superior response to etoposide-platinum chemotherapy.

Although liquid biopsy is still questionable in terms of sensitivity and not recommended to replace a diagnostic tissue biopsy, the aforementioned works consistently challenge this dogma (Figure 5).

**Histology-agnostic therapy**

Recently, the U.S. Food and Drug Administration has approved 2 drugs, pembrolizumab and larotrectinib, in a histology-agnostic setting. In other words, innovative clinical trials seem to favor the identification of targetable molecular alterations over conventional histology determining the tumor primary and histological tumor subtype (14,15,125).

Pembrolizumab, an anti-programmed cell death-1 (PD-1) monoclonal antibody (mAb), has received accelerated approval for the treatment of adult and pediatric patients with unresectable or metastatic solid tumors harboring microsatellite instability-high or deficient DNA mismatch repair. Similarly, nivolumab, another anti-PD-1 mAb, experienced an accelerated approval for adult and pediatric patients with microsatellite instability-high or deficient DNA mismatch repair metastatic colorectal cancer progressed after standard chemotherapy. Finally, larotrectinib, an oral and selective inhibitor of tropomyosin receptor kinases (TRK), demonstrated unprecedented efficacy on unresectable or metastatic solid tumors with neurotrophic tropomyosin receptor kinase (NTRK)-fusion proteins in adult and pediatric patients (14,15,125).

All these data represent a novel and revolutionary approach to cancer treatment based on biomarker biomarker-selected patients and characterized by high clinical efficacy, durable response and unselected patients’ population.

In other words, agnostic-histology development model of clinical trials together with the increasing accessibility to high-throughput genetic analysis (e.g., NGS) (126) and minimally invasive liquid biopsy could re-design the future role of conventional tissue biopsy and pathologists involved in oncology.

**Conclusions**

Despite the great efforts by an international panel of expert pulmonary pathologists of the WHO/IASLC in periodically developing new classification of lung tumours characterized
Figure 5 A modern management to lung cancer should take in consideration the simultaneously detection of diagnosis and actionable biomarkers performing liquid biopsy together with tissue biopsy.

Figure 6 The changing concept of histology in lung cancer, passing from a morphologic diagnosis at hematoxylin-eosin staining in the 2004 WHO, through the introduction of immunohistochemistry in 2015 up to the possible addition of genetic information in the future WHO classification.

by a good reproducibility and simplicity as well as clinical relevance, conventional histology will have a limited role in the future management of patients with lung cancer.

The concept of histology (or tumour histologic type) significantly changed in the last years with the overbearing entrance of molecular information. Nowadays, the modern meaning of histology should incorporate key genetic information permitting a more precise diagnosis, a correct tumour stage in pulmonary multiple cancers and molecularly guided targeted therapies (Figure 6).

The possibility to obtain robust histotype-related multigene data from liquid biopsy could partially replace the need for tumour tissue, dramatically introducing a novel and non-invasive paradigm in approaching patients with lung cancer.

Acknowledgments

None.

Footnote

Conflicts of Interest: The authors have no 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


Clinical Outcomes, J Thorac Oncol 2019;14:1784-93.


85. Berghethon K, ShawAT, Ou SHI, et al. ROS1 Rearrangements Define a Unique Molecular Class of Lung


123. Müller JN, Falk M, Talwar J, et al. Concordance between comprehensive cancer genome profiling in plasma and


doi: 10.21037/shc.2020.01.03

Cite this article as: Galli G, Rossi G. Lung cancer histology-driven strategic therapeutic approaches. Shanghai Chest 2020;4:29.