Histotype in Non-Small Cell Lung Cancer Therapy and Staging: The Emerging Role of an Old and Underrated Factor

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Abstract: Therapeutic management of lung cancer is mainly based on a dichotomic distinction between small cell (SCLC) and non-small cell lung cancer (NSCLC), tumour stage and patient performance status. However, crossing the recent data emerging from molecular studies of gene expression profiling, from the new 2004-WHO histopathological classification of lung tumours as well as from clinical trials with newl targeted therapies against EGFR (gefitinib/erlotinib/cetuximab), it seems that a better definition of tumour histotype in NSCLC might somehow be helpful in predicting clinical response and patient outcome. In addition, lung tumours histotype may deeply influence the tumour stage when assessing parameters (*i.e.*, pulmonary atelectasis, pleural invasion, tumour dimension) defining the current lung tumours staging system. Thus, in this review we analyze the possible future role of histotype as an important influencing factor in the clinical management of patients with NSCLC.

Keywords: Lung, histotype, cancer, WHO, classification, EGFR.

Non-small cell lung carcinoma (NSCLC) accounts for about 75-80% of all cases of pulmonary malignancies [1]. Radical surgery still remains the only therapy with curative intent in NSCLC. Several factors may influence the selection of patients who undergo tumour surgical excision and/or medical treatments, as follows: 1. factors related to the patient's clinical background (pulmonary function and performance status); 2. factors related to the tumour (stage and histology) [2].

Neoadjuvant therapy (chemotherapy ± radiotherapy) may significantly lead to downstage locally advanced unresectable NSCLC (stages IIIA and IIIB), but most recently the attention of the oncologists focused on the promising results of clinical trials using platinum-based chemotherapy in adjuvant setting [3,4].

In histological tumour definition, clinicians generally subdivide lung cancer into two major groups: small cell lung carcinoma (SCLC) and NSCLC. This latter group includes several tumour entities, mainly subdivided into adenocarcinoma, squamous cell carcinoma and large cell carcinoma, together with their histological variants [1,5,6].

Basically, this dichotomic classification is considered sufficiently exaustive for the management of patients with lung cancer. However, the amount of molecular information deriving especially from gene expression studies on lung cancer has evidenced the key role of tumour histotype, resulting in a good correlation among molecular findings, lung tumour histology and prognosis [7-10].

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Another important point on lung tumour histotype derives from epidemiologic studies performed from 1979 to 1998, demonstrating a significant increase in the incidence of adenocarcinomas, especially in women [11-15]. This is mainly due to the increase smoking habits in women [15] and a different cigarettes composition leading to greater exposure of tobacco smoke carcinogens in the peripheral lung [16].

1. HISTOTYPE AND PROGNOSIS

Among all the epithelial organs, the lungs are the anatomic site in which primary carcinomas show the broadest spectrum of morphologic features.

This fact is further highlighted by the extreme heterogeneity at histopathological examination, but also supported by the molecular findings on lung cancer gene expression profiling studies [1,5,6,7-9].

The most recent 2004-WHO classification [1,6] recognized and introduced several entities and variants of lung tumours based on several published works providing additional and consistent information in patients treatment associated with specific tumour morphologic characteristics.

At a first glance, reading the list of pulmonary lesions included in this classification, it appears quite evident that there are lung tumour entities for which complete surgical resection is curative without other additional therapy, and lung tumours intrinsically associated with a clinical aggressive behaviour and a dismal prognosis when compared with conventional stage-matched NSCLC. Intuitively, these tumours should be possibly treated with some benefit using additional treatments, in particular chemotherapy. Indeed, several recently appeared clinical trials using platinum-based chemotherapy in adjuvant setting have shown well-demonstrated survival advantages in patients with NSCLC [3,4]. So, it is desirable that lung cancers associated with the

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worse prognosis could represent the best candidates to receive a post-surgical chemotherapeutic treatment in order to have the best chance to control the disease after complete tumour resection.

As summarized in Table **1**, among malignant tumours with good prognosis, salivary gland-type tumours (such as mucoepidermoid carcinoma) (Fig. **1A**) and typical carcinoid (Fig. **1B**) usually occur in children or non-smoking people younger than those with conventional NSCLC [1,5,17,18]. These tumours have a central bronchial location frequently leading to prompt discovery after recurrent antibiotic-resistant pneumonitis and/or lobar atelectasis [1,5].

Table 1. Non-Small Cell Lung Cancer Histotypes Showing a Well-Recognized Prognostic Value

Good Prognosis*	Poor Prognosis^
Salivary gland-type tumors	Basaloid carcinoma
Typical carcinoid	LCNEC
Fetal adenocarcinoma	Large cell carcinoma with rhabdoid features
Mucinous/colloid adenocarcinoma	Sarcomatoid carcinoma
Non-mucinous BAC	Signet-ring cell adenocarcinoma
	Solid adenocarcinoma with mucin production
	Papillary/micropapillary adenocarcinoma

Abbreviations: BAC, bronchioloalveolar carcinoma; LCNEC, large cell neuroendocrine carcinoma.
*Complete resection is generally curative.
^Chemotherapy is noteworthy of consideration after radical surgery.

Mucinous/colloid carcinoma (Fig. **1C**), fetal adenocarcinoma (Fig. **1D**) and non-mucinous bronchioloalveolar carcinoma (BAC) (Fig. **1E**) are considered well-differentiated variants of adenocarcinoma with peculiar morphologic clues [1,5,6,19-24].

All these lesions are peripherally-located but characterized respectively by large pools of mucus with neoplastic cells having a goblet and/or signet ring cell apperance and an intestinal differentiation at immunohistochemistry (nuclear positivity for CDX2) [19,20], the presence of neoplastic glands resembling fetal lung tubules in pseudoglandular stage or secretory-type endometrium with morules [21,22], and a prominent lepidic growth along the alveoli of non-mucinous cells with Clara cell or type II cell differentiation, but lacking invasion of interstitial stromal tissue, vessels and/or lymphatics and pleural surface [1,23]. Interestingly, Zell *et al.* [24] demonstrated a significant survival advantage in patients with BAC diagnosed after the introduction of the morphologic criteria of the WHO classification independently from smoking status or tumour stage at diagnosis.

Conversely, there are several pulmonary neoplastic entities that are strongly associated with an aggressive behaviour, for which post-operative chemotherapy could offer some benefit in the disease growth control.

The great majority of patients with NSCLC for which histotype alone is by definition a negative prognosis predicting factor are represented by current and heavy smokers.

Basaloid carcinoma, as first described by Brambilla *et al.* [25,26], is a variant of large cell carcinoma with *vague* neuroendocrine morphologic features (organoid or solid nodules growth pattern with peripheral palisading and anastomoting trabeculae) (Fig. **1F**) coupled with the immunophenotype of squamous cell carcinoma (positivity for high-molecular weight cytokeratins 1,5,10,14 and/or p63, but negativity for TTF-1) [27]. Stage I and II basaloid carcinomas have a worse prognosis than conventional squamous cell carcinoma even when poorly differentiated [26].

Large cell neuroendocrine carcinoma (LCNEC) is another variant of large cell carcinoma, first defined by Travis *et al.* [1,6,28], showing a clear-cut neuroendocrine differentiation at morphology (Fig. **1G**) and at immunohistochemistry/electron microscopy. Prognosis is poor and similar to that of SCLC [29-34]. A significantly worse prognosis was reported for patients with stage I LCNEC when compared with stage-stratified conventional NSCLC [29,35]. Overall survival at 5 years ranges from 13% to 51%, possibly depending on the pathologic criteria used to detect this entity [33].

Large cell carcinoma with rhabdoid features (Fig. **1H**), first decribed by Colby *et al.* in 1995 [5], is a very rare tumour characterized by large cells with abundant cytoplasm and a rounded cytoplasmic inclusion [36-39]. Rhabdoid features may also be observed as a minor component of adenocarcinoma or sarcomatoid carcinoma and seems to be associated with a very aggressive clinical course (the median survival of published cases with available follow-up was 5 months) [36-39].

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 [1,6], then including pleomorphic carcinoma (Fig. **1I**), spindle cell carcinoma, giant cell carcinoma (Fig. **1J**), carcinosarcoma and pulmonary blastoma. Sarcomatoid carcinomas are usually advanced tumours at diagnosis, and, even when completely resected, the 5 years' survival in early stages (stage I) is about 20% [40-46].

Adenosquamous carcinoma is a carcinoma showing a double component (of at least 10%) consisting of a squamous cell carcinoma and of an adenocarcinoma deeply intermingled or with a separate (back-to-back) growth pattern (Fig. **1K**) [1,5,6]. Again, adenosquamous carcinoma showed a poor prognosis after complete surgical resection. Some authors found that stage I adenosquamous carcinoma had the same prognosis of patients with stage IIIA conventional NSCLC and a particularly significant higher frequency of pleural invasion [47-49]. The cumulative survival at 5 years is about 18.5% [50].

Solid adenocarcinoma with mucin is a variant of adenocarcinoma, characterized by a solid growth pattern consisting of sheets of polygonal cells without presence of glandular differentiation but with a substantial presence of intracytoplasmic mucin deposition (Fig. **1L**) [1,5,6].

Riquet *et al.* [51] in a review study of a large series of squamous cell carcinomas and adenocarcinomas found that solid adenocarcinoma with mucin component was associated

with a significant poorer survival rate (36.8% at 5 years survival) than that observed in squamous cell carcinoma (50.2%) or in adenocarcinoma without areas of solid adenocarcinoma with mucin deposition (58.1%).

Signet-ring cell carcinoma is a mucin-producing variant of adenocarcinoma characterized by the presence of a neoplastic proliferation of cells with abundant intracytoplasmic mucin accumulation displacing the nucleus at the periphery of the cells then leading the appearance of a "signet-ring" (Fig. **1M**) [1,5,6]. The morphology in primary lung tumours is similar to that observed in signet-ring cell adenocarcinomas occurring in more ordinary locations, such as the stomach, but markers of pulmonary primary (cytokeratin 7 and TTF-1) are commonly positive [52,53]. The prognosis is dismal. Recently, Tsuta *et al.* [54] found that 5 years' survival of adenocarcinomas with a signet-ring cell component > 50% is significantly poorer than that observed in patients with adenocarcinoma without signet-ring cell component (20.4% *versus* 52.7%).

Another variant of adenocarcinoma associated with a bad outcome is the papillary/micropapillary subtype.

This entity is characterized by the presence of papillary and micropapillary projections without fibrovascular core coming off from larger papillary structures with a single layer of cells covering a fibrovascular core (Fig. **1N**) [1,55-61]. The loss of adhesion molecules network seems to be a possible molecular mechanism leading to extensive release of cancer cells into the airways and lymphatics [61].

Mucinous type BAC is a mucin-rich low-grade tumour entity generally characterized by a bland-looking cytology (Fig. **1O**) but also by a massive aerogenous spread leading to satellite nodules and inexorably to lobar and subsequently extensive lung consolidation (pneumonia-like appearance) [1,5,62-64]. It is almost impossible to perform a complete resection of this sort of neoplasm, and the pulmonary recurrences are the rule. Since chemotherapy is uneffective, mucinous BAC represents the lung tumour with the worse prognosis among of the low-grade lung tumours [5,65]. Of note, the overall incidence of such lung tumours is not insignificant,reaching at least one fifth of all pulmonary malignancies.

2. HISTOTYPE AND STAGE

While SCLC is still subdivided in limited or extensive disease by clinical staging and treated with multimodal chemo-radiotherapy [66,67], tumour staging in NSCLC (according to the recent AJCC guidelines) is the most important parameter in establishing the therapeutic strategy [68,69]. While clinical staging is mainly based on radiologic work-up, the pathological stage ("pT, pN, pM") relies on accurate macroscopic and histopathological examination at microscope assessed on pulmonary surgical resection [70]. A recent study by Lopez-Encuentra *et al.* [71] found a lower diagnostic accuracy of clinical staging as compared with pathological accuracy, showing a high rate of downstaging with a good degree of comparison only in stage I.

Pathological staging information is indeed essential for oncologists, and relies not only on objectionable criteria (tumour size; invasion of visceral and parietal pleura as well as the adjacent structures- chest wall, diaphragm, mediastinal pleura, pericardium, mediastinum, heart, great vessels, trachea, esophagus, vertebral body, carina-; presence of tumour cells in pleural effusion), but also on subjective or inconsistent features (presence of atelectasis or obstructive pneumonitis extending to the hilar region– pT2 - or to the entire lung- pT3; involvement of main bronchus with tumour 2 cm or more distal to the carina- pT2- less than 2 cm distal to the carina but without involvement of the carina- pT3). In particular, even when pathological examination is performed by an expert pulmonary pathologist, these latter features are very difficult to be diagnosed and rather need to be recognized by radiologists [72]. So, pathological report may downstage the lung tumour based on the lack of recognition of atelectasis and distance to carina.

In addition, histotype may have some role in determining tumour stage. It is well known that squamous cell carcinoma has a central location involving the main lobar bronchus in the majority of cases [1,5]. By contrast, adenocarcinoma and large cell carcinoma are more frequently located at the periphery of the lung [1,5]. Then, it is implicit that squamous cell carcinoma should involve the carina or that atelectasis/obstructive pneumonitis present more often than adenocarcinoma due to the tumour growth occluding the lobar bronchus. However, this fact is unlikely defined in a clear-cut way in the pathological report, somehow leading to a lung tumour downstaging. On the other hand, adenocarcinoma histotype is most often associated with visceral pleura invasion. In this way pT2 squamous cell carcinoma is basically determined by tumour size greater than 3 cm, but not by atelectasis/obstructive pneumonitis or distance to carina, while pT2 adenocarcinoma determined by pleural invasion is more accurately detected on histopathological examination. Having said that, it is also important to underline that pathologists not infrequently are aware of a squamous cell carcinoma infiltrating soft tissues (mainly adipose tissue) around the main bronchus also involving the surgical resection margin (not the bronchial margin but that sketched by the neighbouring soft tissues) as evidenced by the presence of tissues cauterization artefacts. It is difficult to accurately determine the staging value of this latter finding. Anatomically, soft tissues in this area should be considered as mediastinum tissue/mediastinal pleura, then leading to a pT3/pT4 tumor. Nevertheless, this is a controversial and open question, probably never dealt with in literature and possibly requiring further considerations on large series of such cases.

Again, tumour stage determined solely on the presence of multiple nodules in the same (pT4) or different (pM1) pulmonary lobe is a histotype-related finding, being almost exclusively observed in patients with adenocarcinoma showing a bronchioloalveolar pattern [73,74].

At this point, it is more reasonable to introduce blood vessel/lymphatic invasion as a more objective factor to determine pT2 stage instead of atelectasis/obstructive pneumonitis. Blood vessel/lymphatic invasion is almost uniformely accepted as a prognostic factor worsening patients' survival [75] and it is significantly associated with other parameters predicting dismal outcome such as pleural invasion or pN+ and tumour grade differentiation [75].

Given the renewed interest of the oncologists in adjuvant chemotherapy that actually seems to offer some benefit in

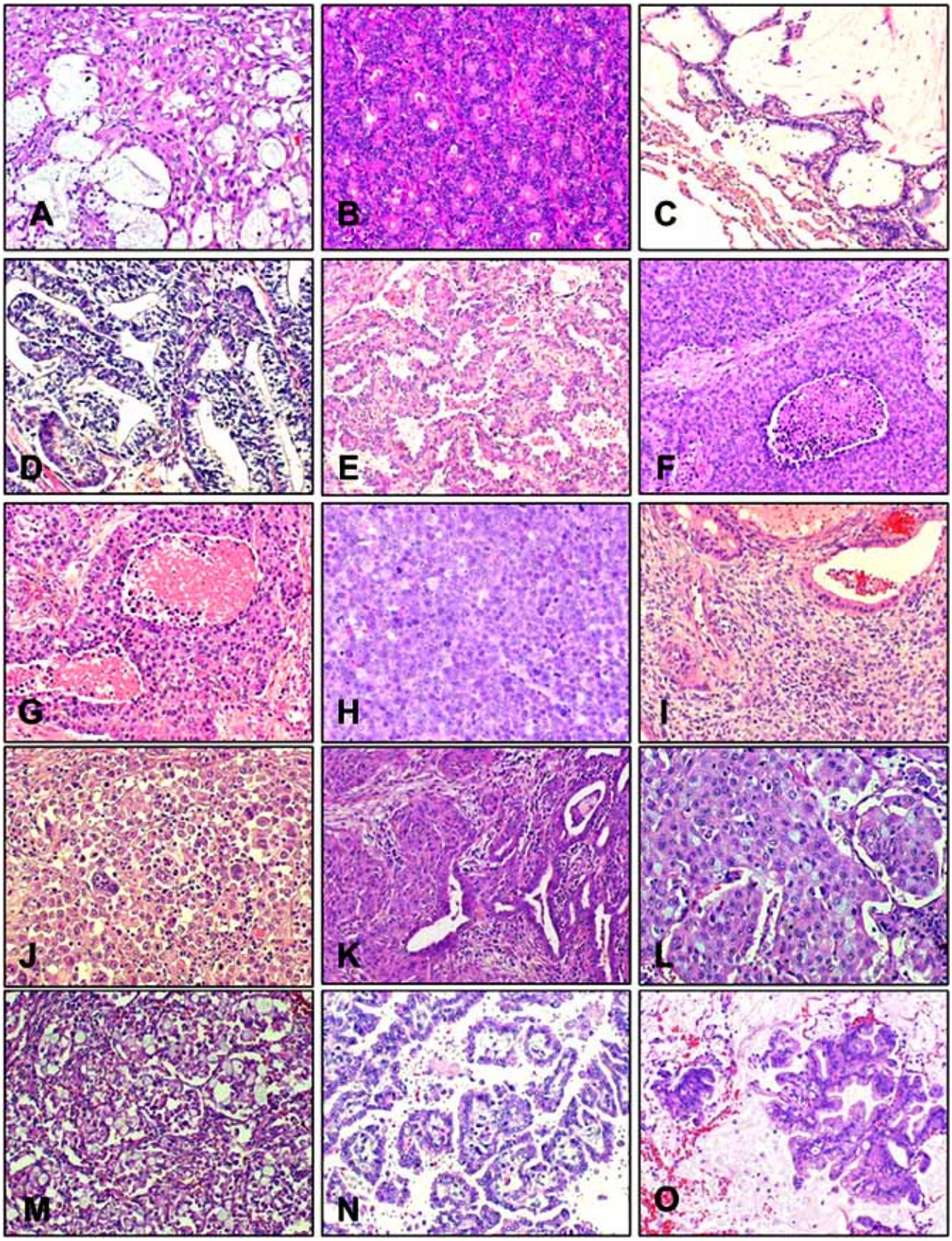

Fig. (1). Examples of NSCLC subtypes at hematoxylin-eosin stain associated with an intrinsic prognostic value, as follows: mucoepidermoid carcinoma (**A**), typical carcinoid (**B**), mucinous/colloid adenocarcinoma (**C**), fetal adenocarcinoma (**D**), non-mucinous type BAC (**E**), basaloid carcinoma (**F**), LCNEC (**G**), large cell carcinoma with rhabdoid features (**H**), pleomorphic carcinoma (**I**), giant cell carcinoma (**J**), adenosquamous carcinoma (**K**), solid adenocarcinoma with mucin production (**L**), signet-ring cell adenocarcinoma (**M**), papillary/micropapillary adenocarcinoma (**N**), mucinous type BAC (**O**).

disease-free and overall survival particularly in patients with stage IB NSCLC, it is mandatory to reconsider the parameters determining pT2 stage in the near future [74]. Marginally, several recent works have highlighted the need for further refinement of tumour size as pT influencing factor [77,78] showing that a different tumour size cut-off (<2 cm; between 2 and 5, and >5 cm) may determine a more reliable clinico-pathological parameter in establishing NSCLC patients' survival. Besides, the heterogeneity of pT2 stage, which comprises the great majority of resected NSCLC, is not sufficiently recognized by the current criteria, which should therefore be revised in the next future [76].

Finally, there is a great attention to early stage lung cancer detection by helical CT scanning into the programs of lung cancer screening. As expected, the great majority of these screen-detected cancers are small-sized adenocarcinomas and may pose some problems in differential diagnosis between pre-malignant (atypical adenomatous hyperplasia), pre-invasive (bronchioloalveolar adenocarcinoma) and invasive (acinar adenocarcinoma) glandular lesions [79]. Since non-invasive or minimally-invasive adenocarcinomas have a survival next to 100%, a correct diagnosis has an important clinical significance in this setting [80,81].

Table **2** summarized the key points related to the histotype features involving the lung tumour's stage.

Table 2. Summary of Major Histotype-Related Features Influencing Tumor Stage

Squamous cell carcinoma and other centrally-located tumors (salivary gland type tumors, adenoid cystic carcinoma, carcinoids) are more often associated with:
• lobar atelectasis/obstructive pneumonia
• involvement of the main bronchus and carina
• involvement of soft tissue of the main lobar bronchus for which no clear-cut significance/value has been established (pT3 ?)
• no pleural involvement
Adenocarcinoma and other peripherally-located tumors (large cell carcinoma variants) are more often associated with:
• pleural involvement
• absence of lobar atelectasis/obstructive pneumonia
• no involvement of the main bronchus and/or carina and/or soft tissue of the main lobar bronchus

3. HISTOTYPE AND CHEMOTHERAPY

Data from 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 [82]. This lead to a change in chemotherapeutic protocols, so that a more homogeneous use of chemotherapy concerning drugs schedules in lung oncologic field is currently accepted worldwide [83,84]. It is now universally accepted that chemotherapy for NSCLC should include platinum-derivatives associated with taxanes, gemcitabine or vinorelbine.

However, there are some experiences in literature reporting a different response rate in lung tumour histotype [85,86].

At least in Japanese people, tegafur-uracil in adjuvant setting seems to improve overall survival of patients with stage I adenocarcinoma subtype [85]. Again, Georgoulias *et al.* [86] found that the chemotherapeutic regimen including gemcitabine and docetaxel had a significantly higher response rate in patients with adenocarcinoma histology than in those of the group with non-adenocarcinomatous tumours. By contrast, patients with non-adenocarcinoma histology had a significant greater response rate to cisplatinum and docetaxel than those with adenocarcinoma. Histology, in these studies [85,86], then appeared as a main predictive factor for response both at univariate and multivariate analysis. Interestingly, the authors suppose that this fact should be related to the higher frequency of *k-ras* mutations observed in adenocarcinoma subtype, with *k-ras* mutations providing a high sensitivity to gemcitabine in human tumour cells [87,88].

Another interesting issue concerns the role of chemotherapy in BAC. Response rate to chemotherapy in this subset of lung tumours is significantly lower (6-11%) than that expected for conventional NSCLC (20-40%) as reported by Shiller *et al.* [83] and more recently by Scagliotti *et al.* [89]. These latter authors [89] in a phase II trial on 19 advanced BACs with paclitaxel alone found a limited efficacy, as supported by a response rate ranging from 5.6% to 11.1%, a median survival of 8.6 months and a median progression free survival of 2.2 months.

Although the median survival in patients with BAC is better than that observed in patients with other NSCLC, the response rate to chemotherapy is very low [64,65] and this may be due to the low doubling replication time and cytoproliferative activity of BAC cells. Response rate in SWOG 9714 study was 14% using paclitaxel alone in advanced stage BAC patients [64]. Recently, we performed a retrospective studies on 51 cases of "pure" BACs reviewed according to the recent criteria of the 2004-WHO classification of lung tumours and found a partial response in only 1 among 16 patients undergoing platinum-based chemotherapy (Rossi & Marchioni, *personal unpublished observations*). In particular, we noted no response to chemotherapy and high frequency of *k-ras* mutations in absence of *EGFR* mutations in the mucinous variant (12 out 12), while *EGFR* and *k-ras* mutations characterized 34% and 13%, respectively, of the non-mucinous type.

As suggested by Marchetti *et al.* [90], it seems plausible that the concept of BAC includes an heterogeneous subset of lung adenocarcinomas and the mucinous type actually represents a biologic entity separate from other subtypes of adenocarcinoma.

Since mucinous BAC seems to lack *EGFR* mutations [91] and more frequently occur in smokers, we argue that it should be important to have a detailed classification of tumour (possibly with central pathologic review) in ongoing and future studies on EGFR inhibitors in lung cancer, and BAC in particular.

Another controversial lung tumour entity for which no standard chemotherapeutic protocols were defined is LCNEC. First defined by Travis *et al.* [28], LCNEC seems to be correlated to a very dismal outcome akin to that of SCLC, with which also shares several molecular features [11,33,34]. Although retrospectively, recent clinicopathologic studies have evidenced a significant better survival in patients undergoing chemotherapy using SCLC-based

regimens in adjuvant and metastatic setting [33,92-95]. In addition, a prospective work of adjuvant chemotherapy for pulmonary LCNEC by Iyoda *et al.* [96] confirmed that patients (15 cases) receiving cisplatin and VP16 after surgery had a significant improvement of their prognosis (overall survival rate at 2 and 5 years of 88.9%).

4. HISTOTYPE AND TARGETED THERAPIES

EGFR/HER1 is a receptor tyrosine kinase deeply involved in lung cancerogenesis and represents a molecular tumour target for selective small molecule inhibitors, namely gefitinib and erlotinib. Recent phase II and III trials have demonstrated partial responses in about 10% of unselected patients with chemoresistant progressive NSCLC [97].

Responses were higher in female sex, never-smokers, asian ethnicity and adenocarcinoma histotype [98,99], particularly when showing bronchioloalveolar features. Among biologic factors predicting clinical response, the presence of *EGFR* mutations occurring in the exons 19 and 21 encoding for the kinase domain of the protein were correlated with dramatic response to drugs as well as to clinicopathologic predicting factors [100-103]. Of note, patients with squamous cell carcinoma harboring *EGFR* mutations did not have a significant response to gefitinib [101].

In addition, several studies highlighted that adenocarcinoma with BAC features at histology appeared as one of the most useful and valuable predicting factor of response to anti-EGFR therapies [103-107].

Mutations in *HER2/neu* or *ErbB2* have been recently reported in a limited subset of patients with NSCLC. Again, never-smokers, femal gender and adenocarcinomas were significantly associated with these mutations [107, 108].

Estrogen and progesterone receptors are overexpressed in a not insignificant number of NSCLC possibly representing targets for endocrine therapies. The upregulations of these receptors is more frequent in the adenocarcinoma histotype [109].

VEGF is another possible tumor target in NSCLC which can be inhibited using the recombinant, humanized monoclonal antibody Bevacizumab. This antibody, either in combination with erlotinib or carboplatin and paclitaxel, seems to improve overall and time to progression survival in patients with advanced or recurrent NSCLC [110,111]. However, these clinical trials demontrated that squamous cell carcinoma histotype was associated with a significantly higher occurrence of fatal pulmonary hemorrhage, then leading the investigators to exclude patients with squamous cell carcinoma from these trials [110,111].

The key points concerning the histotype-related features possibly influencing lung cancer therapies are summarized in Table **3**.

5. CONCLUSIONS

Despite the great efforts by an international panel of expert pulmonary pathologists of the WHO/IASLC in developing the new classification of lung tumours, characterized by a good reproducibility and simplicity as well as clinical relevance, histotype seems to remain an underrated factor in the management of patients with lung cancer. However, although this is particularly true in oncologic clinical trials testing the efficacy of different chemotherapeutic regimens, the advent of targeted therapies using anti-EGFR or anti-VEGF molecules/antibodies coupled to the key role of adenocarcinoma subtype as one of the main successful response predictors, seems to have awaken up the attention of physicians on histology.

Table 3. Summary of the Major Histotype-Related Features Influencing Tumor Therapy

Chemotherapy
• Gemcitabine is more effective in adenocarcinomas
• Lack of chemotherapeutic protocols for mucinous BAC and mucin-producing tumors in general
Targeted therapies
• Adenocarcinoma histotype is one of the main clinicopathological predicting factor of efficacy when using gefitinib or erlotinib
• Patients with squamous cell carcinoma should be excluded from therapies with anti-VEGF bevacizumab due to the high rate of haemorrhagic events

Furthermore, there are several NSCLC entities that are associated with a particularly poor survival rate, thus requiring more aggressive treatments and possibly dedicated clinical trials aimed at identifying the optimal therapeutic approach.

Finally, NSCLC subtypes may be strongly related to a specific anatomic location in the lungs impacting on tumour stage (*e.g.*, the great majority of squamous cell carcinomas is centrally-sited and adenocarcinoma or large cell carcinoma are mainly peripheral). This evidences critical key issues in staging system of NSCLC, possibly suggesting future debate which may lead to important changes in the identification of lung cancer staging system (*i.e.*, introduction of other objective data such as vessel/lymphatic invasion, different tumour size cut-off, abolition of atelectasis/obstructive pneumonitis as pT2/pT3 staging feature, consideration for tumour infiltration of soft tissue around the main bronchi).

Practically speaking, clinicians should reconsider the factor "histotype" in the ongoing therapeutic approach of NSCLC, at least for the following aspects: 1. in the impossibility to perform histotype-based clinical trials, patients with a NSCLC associated with a particularly aggressive outcome should be the preferable candidates for adjuvant chemotherapy; 2. adenocarcinoma has the higher incidence among NSCLC and seems to represent a sort of "epidemic" tumor in the near future, particularly in women; 3. distinction between adenocarcinoma and non-adenocarcinoma subtype among NSCLC should be performed in order to obtain a better selection of patients undergoing targeted-therapies with EGFR inhibitors or anti-VEGF; 4. mucus-producing adenocarcinomas are strongly correlated to *K-RAS* mutations, chemoresistance and poor response to anti-EGFR therapies; 5. adenocarcinoma subtype and the relevant variants are generally peripherally-located and then most often associated with pleural and lymphatic invasion (lymphatic vessels are particularly numerous in pleural/subpleural areas) and T4/M1 stages due to multiple neoplastic nodules; 6. the broader spectrum of chemotherapeutic drugs of third generation and of molecular targeted-therapies in the treatment of NSCLC will require a more patient-tailored approach and then a striking collaboration between physicians and pathologists,

these latter being also deeply engaged in determination of biomarkers (EGFR, K-RAS, HER2, pharmacogenetic markers as RRM-1 or ERCC-1) positively predicting the efficacy of the adopted therapies.

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