Is Histologic Subtype Significant in the Management of NSCLC?

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Abstract: Histologic subtype has emerged as a potential prognostic and/or predictive factor in advanced non-small cell lung cancer (NSCLC). Several studies support the importance of differentiating between squamous and non-squamous NSCLC in terms of efficacy of chemotherapeutics (e.g. pemetrexed) or treatment-related toxicities (e.g. bevacizumab). In addition, molecular markers and gene profiles have been correlated with histologic subtype. This review examines the emerging clinical significance of histologic type in the management of NSCLC, discusses caveats in accurate histologic diagnosis, and reviews biomarkers with potential predictive value for NSCLC chemotherapeutics.

Keywords: Lung cancer, histology, adenocarcinoma, squamous cell carcinoma.

INTRODUCTION

Lung cancer is the leading cause of cancer-related mortality worldwide [1] Based on pathologic features, lung cancers are classified into non-small cell lung cancer (NSCLC), which accounts for about 85% of cases, and small cell lung cancer (SCLC) that accounts for approximately 15% of cases. NSCLC is divided into three major histologic subtypes, adenocarcinoma, squamous cell carcinoma (SCC) and large cell carcinoma. SCC used to be the most common histologic subtype of NSCLC; however, in recent years there has been a significant increase in the proportion of adenocarcinoma cases with a corresponding decline in proportion of SCC cases.

The majority of patients with lung cancer present with advanced stage disease, therefore, systemic therapy plays a major role in their management. However, the benefit from systemic therapy is modest. The median survival of patients with advanced NSCLC ranges from 9-12 months and the median progression-free survival (PFS) from 4 to 6 months. In recent years, many new agents have been incorporated into the armamentarium against NSCLC. These agents include bevacizumab, a monoclonal antibody targeting vascular endothelial growth factor (VEGF), erlotinib, an epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI) and pemetrexed, a novel antifolate. Recent data has suggested that the efficacy or toxicity of these agents may be influenced by the histologic subtype of NSCLC. We review the data regarding differences in management according to histologic subtype and discuss the pitfalls of histologic classification in NSCLC.

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HISTOLOGY AS A PREDICTIVE FACTOR OF TREATMENT SAFETY

Preclinical and correlative studies in NSCLC showed that the degree of tumor-associated angiogenesis correlates with disease progression and serves as a marker of unfavorable survival outcome [2-4] The most important pro-angiogenic factor is the vascular endothelial growth factor (VEGF). Bevacizumab is a fully humanized monoclonal antibody which binds VEGF-A. The principal mechanism of action of bevacizumab appears to be through angiogenesis inhibition, which results in a more mature vasculature that is thought to facilitate the delivery of chemotherapeutic agents,[5, 6] which may explain why bevacizumab acts synergistically with cytotoxic or other targeted agents. The addition of bevacizumab to carboplatin and paclitaxel was first assessed in a randomized phase II trial.[7] In this trial, patients who received high dose bevacizumab (i.e. 15 mg/kg every 3 weeks) had a significantly higher time-to-progression (TTP) and a trend towards superior OS and response rate (RR) compared to the chemotherapy alone group. However, six patients out of 65 treated on either low- or high-dose bevacizumab experienced a major life-threatening hemorrhage that resulted in four deaths. Four of these events occurred in the 13 patients with SCC (31%), whereas all were noted in centrally located tumors, and 5 in tumors with cavitation or necrosis [7]. Due to the potential for increased risk of life-threatening and fatal episodes of pulmonary hemorrhage in patients with SCC, a subsequent phase III study of carboplatin/paclitaxel with or without bevacizumab (E4599) excluded patients with SCC. E4599 showed that the addition of bevacizumab to carboplatin and paclitaxel increases the survival of selected patients with advanced NSCLC [8] This trial established the combination of carboplatin, paclitaxel, and bevacizumab as a standard first-line treatment for advanced non-squamous cell NSCLC.

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There is emerging data with sorafenib, a multi-kinase inhibitor including VEGF receptor tyrosine kinase, in patients with advanced NSCLC. A phase III study (ESCAPE) evaluated the addition of sorafenib to carboplatin and paclitaxel as first-line treatment in all histologic types of NSCLC was closed early following a pre-planned interim analysis showing that the primary endpoint of a superior survival outcome for the sorafenib arm could not be achieved. More importantly, there was a strong indication of a deleterious effect of sorafenib in patients with squamous histology (214 patients or 24% of total accrual) who achieved a median OS of 8.9 months in comparison with 13.6 months for similar patients treated with chemotherapy alone. The incidence of toxicity was more evident in SCC patients given that 9 out of 13 fatal pulmonary hemorrhages were observed in the SCC subset. However, 5 cases occurred in the sorafenib arm and 4 in the placebo arm [9].

It is possible that centrally located and necrotic or cavitated tumors have an increased risk for major hemoptysis during treatment with bevacizumab as shown in the study by Johnson *et al*. [7] SCC tumors usually arise centrally and in proximity with large vessels. Moreover, SCC has a greater tendency to cavitate as compared to adenocarcinoma. These factors could potentially explain the higher incidence of hemoptysis in patients with SCC treated with bevacizumab. Sandler *et al.* analyzed risk factors for pulmonary hemorrhage in the context of E4599 and suggested that baseline tumor cavitation may be a risk factor, however, the number of events were small and there was not enough power to detect statistically significant differences [10].

HISTOLOGIC SUBTYPE AS PROGNOSTIC FACTOR IN NSCLC

Several studies have investigated the potential correlation of histology with the prognosis of patients with NSCLC. Finkelstein *et al.* reported that among 893 patients with metastatic NSCLC enrolled in seven phase III studies conducted by ECOG, large cell carcinoma was associated with better 1-year survival, regardless of treatment [11]. The potential correlation of histology with the disease prognosis was also stated in a study by Okamoto *et al*. The rate of survival of patients with completely resected NSCLC was dependent on the histological subtype of the carcinoma, as well as on the stage the carcinoma had progressed to. Patients with stage I adenocarcinoma had a better prognosis after complete resection than those with stage I squamous cell carcinoma. On contrary, patients with stage II squamous cell carcinoma had a better prognosis after complete resection than those with stage II adenocarcinoma [12] .

Hirsch *et al.* [13] reviewed clinical trials conducted over the last 25 years evaluating systemic therapy in advanced NSCLC. Of 408 reviewed studies, only 32 referred to the association between the histology and clinical outcome; 18 studies used cytotoxic agents and 14 tyrosine kinase inhibitors (TKIs). It is noteworthy that the results of these studies do not concur regarding the NSCLC histological subtype with the most favorable prognosis. Most of the studies concluded that adenocarcinomas had better outcome compared with SCC. However it is unclear if histology was a predictive or prognostic factor. Veronesi *et al.* compared a four-drug regimen with cisplatin/etoposide in NSCLC. Patients with SCC histology had better RR than those with adenocarcinoma when treated with the cisplatin/etoposide regimen [14, 15]. In a randomized phase II study which compared three different treatment regimens, patients with SCC histology patients had a significantly better RR than those with non-squamous NSCLC. The OS and the estimated 2-year survival were also in favor of patients with SCC but the difference was not statistically significant. Sculier *et al.* on behalf of the International Association for the Study of Lung Cancer (IASLC) International Staging Committee (ISC), studied a large database that included both patients who had received chemotherapy and patients who had not received chemotherapy. In a multivariate analysis of prognostic factors for survival, SCC histology was identified as independent prognostic factor [16]. In another study, the combination of cisplatin plus etoposide with sequential or concurrent radiotherapy (RT) significantly prolonged the overall survival (p=0.04) in patients with SCC in comparison with patients with non-squamous NSCLC [17] In a phase III study by Georgoulias *et al.* cisplatin plus docetaxel was compared with gemcitabine plus docetaxel. Although no difference in PFS or OS was observed between the two treatment groups, patients with adenocarcinoma had higher RR with gemcitabine/docetaxel (43% versus 28%), whereas patients with other histologies responded better to cisplatin/docetaxel (23% versus 40%) [18]. In an older phase III study that compared cyclophosphamide, epirubicin and cisplatin alternating every 4 weeks with methotrexate, etoposide and lomustine with best supportive care, it was shown that OS increased with chemotherapy in adenocarcinoma patients, whereas SCC patients had similar OS independentiof treatment [19] A recent meta-analysis of 9 randomized trials (n=2,968) evaluated the efficacy of cisplatin versus carboplatin-based chemotherapyd. Histology was found to predict a lower RR and a higher risk of mortality for patients with non-SCC treated with carboplatin-containing regimens; however, no differences were observed in patients with SCC [20].

A subset analysis of an adjuvant phase III study of cisplatin/vinorelbine versus observation only (ANITA trial) showed that the 5-year survival rate was similar in SCC and non-SCC in each treatment arm (51.6% versus 50.7% for the chemotherapy arm and 43.7% versus 41.4% for the observation arm, respectively). Adenocarcinomas in the observation arm were found to have a very poor prognosis which was reversed by the administration of adjuvant chemotherapy. The overall survival benefit with chemotherapy was 8.6% at 5 years, whereas the benefit for patients with adenocarcinomas and SCC was 13.9% and 7.9%, respectively [21].

HISTOLOGIC TYPE AS PREDICTOR OF PEMETREXED EFFICACY

The differential therapeutic efficacy based on histologic subtype is most well documented for pemetrexed. In a phase III study, 1725 patients with advanced or metastatic NSCLC were randomly assigned to receive cisplatin/gemcitabine (control arm) or cisplatin/pemetrexed. A pre-specified analysis by histology was part of the study design. Although no difference in efficacy was observed between the two arms, patients with non-squamous histology had a survival benefit when treated with cisplatin/pemetrexed versus cispaltin/gemcitabine, while the reverse was observed in patients with SCC histology [22] (Table **1**). Similar observations were made in a retrospective

analysis of a phase III trial that compared pemetexed to docetaxel for the second-line therapy of NSCLC [23] as well as in a phase III trial of maintenance therapy with pemetrexed versus placebo in advanced NSCLC [24] (Table **1**). In the latter study by Ciuleanu *et al.* 663 patients with stage IIIB or IV NSCLC who had not progressed on four cycles of platinum-based chemotherapy were randomly assigned (2:1 ratio) to receive pemetrexed (500 mg/m^2 day 1) plus best supportive care (n=441) or placebo plus best supportive care (n=222) until disease progression. Pemetrexed maintenance resulted in improved PFS (p< 0.0001) and OS (p= 0.002). However, a survival benefit was not evident in patients with SCC histology [24]. A combined survival analysis of those three randomized, phase III pemetrexed-based studies in NSCLC according to histology was performed (Table **1**) [25]. Treatment-by-histology interactions were statistically significant in all three studies for OS (p=0.002, 0.001 and 0.033, respectively), indicating that patients with non-SCC achieved longer OS with pemetrexed than with comparator regimen, while the reverse was observed in SCC patients [25] Based on these observations that demonstrate that pemetrexed is an inferior treatment choice for patients with SCC of the lung, the FDA has restricted its use to non-SCC NSCLC. On the other hand, pemetrexed-based regimens should be favored for the treatment of patients with adenocarcinomas.

Other retrospective analyses have been conducted to assess if the efficacy of agents other than pemetrexed are influenced by histologic subtype. In a subset analysis of a phase III trial comparing a platinum-based combination with either vinorelbine or docetaxel, both arms reported similar results in terms of ORR, time to tumor failure (TTF) and OS. However, adenocarcinoma diagnosis predicted better response to chemotherapy for the vinorelbine-treated patients [26]. An analysis of the SWOG database for the outcomes of platinum based chemotherapy in combination with vinca alkaloid or taxane agent by NSCLC histologic subtype, no difference in OS or PFS was evident [27]. Similarly, in a retrospective analysis of ECOG study (E1594), no significant difference in the OS and PFS was shown among different NSCLC histologic subtypes [28].

EGFR-TKIS IN ADVANCED NSCLC

Several studies with EGFR-TKIs in NSCLC have shown that female sex, Asian origin, never-smokers and adenocarcinoma histology can predict response to treatment [29-31]. Two independent reports in 2004 described EGFR activation mutations and their association with responsiveness to treatment with EGFR-TKIs in NSCLC [32, 33]. Subsequent large retrospective series confirmed the initial observations [34-43]. Marchetti *et al.* showed that SCC did not harbor EGFR mutations in contrast with adenocacinoma (6%) and bronchioalveolar carcinoma (BAC, 22%) [44]. The presence of EGFR mutations in adenocarcinomas may explain the demonstrated better outcome of patients with that histologic subtype after treatment with EGFR-TKIs. However, a double-blind, randomized, phase III study of maintenance erlotinib versus placebo following non-progression with 1st-line platinum-based chemotherapy in patients with advanced NSCLC (SATURN trial) showed that erlotinib-treated patients had longer PFS (HR-0.71) and overall survival (HR-0.81) irrespectively of histology, smoking status, race and EGFR expression [45]. On the basis of that study, erlotinib approved by FDA for use in the maintenance setting. Accordingly, the BR.21 trial, which tested erlotinib in the second-line setting, showed increased PFS and OS with erlotinib versus placebo regardless of EGFR status [30, 45]. However, in both SATURN and BR.21, patients with tumors with high EGFR protein expression or high EGFR copy number had longer survival (PFS in SATURN and OS in BR.21) when treated with erlotinib versus placebo, whereas survival differences in patients with EGFR negative or low copy number tumors were not statistically significant. A phase III study conducted in East Asia evaluated gefitinib versus chemotherapy (carboplatin and paclitaxel) as front line therapy for a select group (adenocarcinoma) of patients with advanced NSCLC. Selection criteria included clinical features that predicted for high rate of benefit from gefitinib, such as never smoking or light smoking status. In this study, patients harboring EGFR mutations who received gefitinib had significantly longer PFS (HR-0.48) than those who were randomized to chemotherapy; in contrast, patients with wild type EGFR had a significantly inferior PFS (HR-2.85) when treated with gefitinib [46]. Thus, these data suggest that in the front line setting, patients whose tumors do not have EGFR mutations should not be treated with EGFR-TKIs. Moreover, it is apparent that EGFR mutations should be used to select patients for EGFR-TKI treatment. .

Table 1. Overall and Progression-Free Survival by Histologic Subtype in Phase III Trials of Pemetrexed in NSCLC

Study Design	N	Phase	Overall Survival		PFS	
			Squamous	Non Squamous	Squamous	Non Squamous
First Line [10] Cisplatin/pemetrexed *vs* Cisplatin/ gemcitabine	862 863	III	9.4 *vs* 10.8 p= 0.05	11.8 *vs* 10.4 p= 0.005	4.4 *vs* 5.5 HR=1.36	5.3 *vs* 4.7 HR= 0.90
Second Line [82] Pemetrexed *vs* Docetaxel	283 288	III	6.2 *vs* 7.4 p= 0.018	9.3 *vs* 8.0 p= 0 .048	-	-
Maintenance [24] Pemetrexed *vs* Placebo	481 182	III	9.9 *vs* 10.8 p=NS	15.5 *vs* 10.3 p= 0.002	2.8 *vs* 2.6 p= 0.039	4.5 *vs* 2.6 p <0.0001

A fusion oncogene (EML4-ALK) that plays a significant role in NSCLC, as a key driver of tumorigenesis, was discovered in 2007 [47]. This oncogene is generated in chromosome 2p by the fusion of echinoderm microtubule-associated protein-like (EML4) to the intracellular kinase domain of anaplastic lymphoma kinase (ALK). In a study of 141 screened tumors from selected patients with two or more of the following characteristics; adenocarcinoma, Asian, female sex, and light or never smoking history, the incidence of EML4-ALK was 13% and of EGFR mutations 22%.[48] Compared to patients with EGFR mutant and wild type patients for either EML4-ALK or EGFR, patients with EML4-ALK were more likely to be men and younger. Similarly with mutant EGFR tumors, the EML4-ALK was strongly associated with never/light smoking and adenocarcinoma histology. EML4-ALK patients had a longer median OS compared with wild type patients. The identification of this molecular abnormality in a small subset of patients with NSCLC is significant, since these patients did not respond to EGFR-TKIs and they likely benefit from ALK inhibitors [48]. EML4-ALK and EGFR mutations are mutually exclusive and occur in similar patient groups (e.g. adenocarcinoma, never/light smokers), therefore, it is important for oncologists to be alert about not only EGFR status but EML4-ALK as well [48-50].

Caveats in Histologic Diagnosis

Establishing histologic subtype of NSCLC on cytology specimens can be particularly challenging since there is often insufficient tumor material for evaluation, which may result in a diagnosis of NSCLC, not otherwise specified (NOS). A retrospective population-based study of 175,298 NSCLC patients diagnosed histologically or cytologically from the California Cancer Registry from 1989 to 2006, reported that the incidence of carcinoma NOS among NSCLC cases increased over time [51] Cytologically diagnosed NSCLC was associated with significantly worse OS in comparison to histologically diagnosed NSCLC. In the same analysis, cytological diagnosis was found as an independent unfavourable prognostic factor for the patients with stage IV NSCLC [51]. Whether NSCLC, NOS is a distinct entity remains controversial but it may represent a significant proportion (ranging from 15% to 30%) of NSCLC. Moreover, the term NSCLC, NOS may include poorly differentiated or undifferentiated tumors that have the poorest survival among major NSCLC histologies and lower survival benefit from chemotherapy when it compared to adenocarcinomas.

Although there is a high level of consistency among pathologists on differentiating SCLC from NSCLC, the sub-classification of NSCLC is more challenging [52]. It is well documented that lung cancer is a heterogenous disease and that fact is apparent in the pathological classification of many tumours as 'mixed tumours' (e.g., adenosquamous, SCLC and NSCLC components). Obviously, the diagnostic methods which are used may vary between the different countries and laboratories and significantly depend on the pathologist experience and reliability. So far, the histologic subtyping of the NSCLC is primarily designated by tumor cell morphology.

A prospective study evaluated the reproducibility of histologic diagnosis among different pathologists [53]. Suboptimal agreement in H&E diagnosis of squamous versus non-squamous histologic subtypes of NSCLC was noted. Of interest was that higher level of agreement was achieved among expert lung pathologists than community pathologists. The kappa coefficient among the expert pathologists was 0.64; while for the community pathologists, it was 0.41 (kappa coefficient over 0.7 defines good agreement). Moreover, this particular study underlined the need of confirmatory, additional special stains and the use of new markers for more accurate diagnosis of the NSCLC [53].

Immunohistochemical (IHC) stains can assist the pathologist in assigning the histologic subtype of NSCLC. Currently, thyroid transcription factor (TTF-1), cytokeratin 7 (CK7) and surfactant proteins A1, B and C are primarily used to distinguish adenocarcinoma from other NSCLC subtypes [54-56] On the other hand, cytokeratin 5, 6, 13 and 17 (CK 5, 6, 13 17) and p63 gene amplification or overexpression are associated with SCC [57]. Finally, we underscore the potential difficulties in conducting IHC analysis in cytologic material because of insufficient tumor sample.

MOLECULAR MARKERS ASSOCIATED WITH CHEMOTHERAPY EFFICACY

The role of various genes in influencing therapeutic efficacy of chemotherapeutic agents for NSCLC is under investigation. Differences in target gene expression may account for differential chemosensitivity between histologic subtypes of NSCLC [58]. In this context, we review the potential role of thymidylate synthase (TS), excision repair cross complementation group 1 (ERCC1) and ribonucleotide reductase subunit M1 (RRM1).

Thymidylate Synthase (TS)

Thymidylate synthase is an enzyme which is involved in DNA biosynthesis through its involvement in the folate metabolism and is a target enzyme for antifolate agents, such as pemetrexed. Higher intratumoral expression of TS mRNA has been correlated with decreased response rate to 5-fluorouracil in various cancers [59-63]. Higher levels of TS have been reported in SCC in comparison to adenocarcinoma. However, the role of TS in NSCLC remains controversial [64-66]. For example, in a study with 160 patients with early stage NSCLC (stage I) who did not undergo adjuvant chemotherapy, high levels of TS correlated with prolonged OS [67]. On the contrary, in another adjuvant study, SCC patients were found to have significantly higher expression of TS (gene or protein) compared with adenocarcinoma patients[58]. The higher expression of TS in SCC can explain the superior efficacy of pemetrexed in non-SCC patients [22,23]. Also, the high expression of TS in SCLC may explain the poor activity of pemetrexed in SCLC [68, 69].

Excision Repair Cross Complementation Group 1 (ERCC1)

The excision repair cross complementation group 1 (ERCC1) is a member of the nucleotide excision repair pathway. This pathway is involved in DNA damage repair and is considered to have both prognostic and predictive value [70, 71]. ERCC1 repairs the formed adducts between

platinum compounds and DNA molecules, hence, overexpression of ERCC1 is presumed to lead to resistance to platinum agents.[72] Simon *et al.* evaluated the effect of intratumoral ERCC1 expression on OS in 51 patients with NSCLC and reported that median survival was significantly prolonged in patients with high ERCC1 expression compared to patients with low ERCC1 expression.[73] Similarly, in a retrospective analysis of patients with available tumor specimens from the International Adjuvant Lung cancer Trial (IALT), patients with ERCC1 negative tumors had significantly higher survival with cisplatin-based adjuvant chemotherapy (p= 0.002), in contrast with patients with ERCC1 positive tumors who did not benefit (p= 0.40). Conversely, among patients randomized to observation, those with ERCC1 positive tumors had significantly higher survival than those with ERCC1 negative tumors (p= 0.009) [74]. In addition, Zheng *et al.* showed that in early stage NSCLC patients treated with surgery alone, high levels of ERCC1 and RRM1 correlated with longer survival [75]. Finally, it should be mentioned that despite the fact that an increasing number of studies investigate the role of ERCC1 in the treatment selection and its impact in the clinical outcome, the validity of the used techniques for its assessment remains controversial [76].

Ribonucleotide Reductase Subunit M1 (RRM1)and Cytidine Deaminase (CDA)

Ribonucleotide reductase regulates substrate specificity and activity of ribonucleotide reductase subunit 1, which catalyzes deoxynucleotide production and is a major cellular determinant of gemcitabine (2,2 difluorodeoxycytidine) efficacy. Similarly to ERCC1, it has shown potential prognostic significance in early stage NSCLC [74, 75]. A prospective study in previously untreated patients with advanced NSCLC with good performance status was designed to assess the feasibility and efficacy of selecting double-agent chemotherapy based on tumoral *RRM1* and *ERCC1* expression. Four different doublets were available containing cisplatin, docetaxel, gemcitabine or vinorelbine. Patients' selection based on the intratumoral expression of *RRM1* and *ERCC1* resulted in promising clinical outcome with a RR of 44%, disease control rate (PR and SD) of 88%, a 1-year survival of 59% and a median OS of 13.3 months. These results compared favorably with historical control data.

Finally, gene mutations in cytidine deaminase (CDA), which is involved in gemcitabine metabolism, have been associated with increased toxicity and low activity of gemcitabine [77-80]. Although there is emerging data from preclinical and clinical studies**,** the precise role of CDA polymorphism remains under investigation [81].

CONCLUSIONS

As a growing number of therapeutic agents is now available for the treatment of NSCLC, their optimal application has become increasingly important. Histology has emerged as an important determinant of therapeutic choice for agents, such as pemetrexed. It is therefore imperative that every effort is made to determine the histologic subtype. However, differences in tumor morphology, such as histologic subtype, may be driven by genetic alterations. The expression of TS and other biomarkers may be of predictive value. It is expected that further advances in the understanding of cancer biology will contribute to better tailored and individualized therapies.

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