Impact of Non–Small-Cell Lung Cancer-Not Otherwise Specified Immunophenotyping on Treatment Outcome

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Introduction: The vast majority of non–small-cell lung cancers (NSCLCs) presents as advanced disease, and histological diagnosis is widely based on small samples. The differential activity and toxicity profile of new cytotoxic and molecular-targeted therapies according to histotypes requires a precise subtyping of NSCLC. Immunohistochemistry (IHC) contributes to define the most probable histotype; however, the real impact of IHC characterization of NSCLC-not otherwise specified (NOS) in terms of outcome is not well established.

Methods: A large series of 224 advanced "nonsquamous" NSCLC diagnosed on small biopsy or cytological samples and homogeneously treated was retrospectively selected, all having adequate follow-up data available. Reviewed diagnoses resulted into two groups: adenocarcinoma (ADC) and NSCLC-NOS. The latter was further characterized by IHC (TTF-1, Napsin-A, p40, and Desmocollin-3) –identify a possible, most probable differentiation lineage.

Results: Sixty-seven percentage of cases were classified as ADC based on morphological examination only ("morphological ADC") and 33% as NSCLC-NOS. IHC profiling of NSCLC-NOS identified 43.2% of cases with an ADC immunophenotype ("NSCLC favor ADC"), 10.8% with a phenotype favoring squamous lineage, and 46% lacking differentiation features. Survival curves confirmed no difference in terms of outcome between the morphological ADC and the NSCLC favor ADC groups, while a significantly poorer outcome was found in the "null" group in terms of best response, progression-free survival or overall survival (OS).

Conclusion: Tumors with an IHC profile ADC-like had an OS comparable with that of morphological ADCs. These findings support the use of IHC to optimize lung cancer histological typing and therapy.

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The majority of non–small-cell lung cancers (NSCLCs) presents at advanced stage, and the histological definition is widely based on small biopsy or cytological samples. The differential activity and toxicity profile of new cytotoxic agents and molecular-targeted therapies according to different lung cancer histotypes[1,2] led to an increased need for a precise NSCLC subtyping,[3] and the differentiation between adenocarcinoma (ADC) and squamous carcinoma (SQC) is the minimum requirement. Unfortunately, in most cases, there are only limited amounts of tumor tissue obtained from primary or metastatic sites, generally through fine-needle aspiration cytology or tiny bronchoscopic biopsies, available for pathological examination. This may hamper the precise tumor definition, either because of scant viable cells or poor tumor differentiation.[4] In such a context, morphological diagnostic criteria could fail, particularly in undifferentiated cancers. The American Thoracic Society (ATS)/European Respiratory Society (ERS)/International Association for the Study of Lung Cancer (IASLC) guidelines recommend the use of immunohistochemistry (IHC) in biopsy samples when a precise morphology-based subtyping is not possible.[5] As a consequence, several studies proposed different panels of IHC markers, useful to identify the specific cell lineages. These IHC markers helped to distinguish SQC from ADC, not only in surgical material[6–8] but also in cytology[9] or biopsy samples.[10–13] Recently, our group demonstrated that a limited, four-marker panel (TTF-1, p63, Desmocollin-3, and Napsin-A) could narrow the percentage of unclassified NSCLC-NOS from 36% to 14%, thus contributing to refine lung cancer classification in fine-needle aspiration biopsies.[14]

However, the real impact on the patients' outcome of IHC-based subtyping of morphologically undifferentiated NSCLCs-not otherwise specified (NOS), compared with the behavior of cases having morphology-driven diagnoses, has not been established. In the present study, we retrospectively analyzed a consecutive series of patients with advanced NSCLC and a nonsquamous histological diagnosis (ADC and NSCLC-NOS), candidate for first-line treatment, for whom small biopsies or cytology specimens only were available. The group of lung cancers subtyped by an IHC marker panel was

correlated with two separate groups of morphology-only ADC and of NSCLC having a "null" phenotype (according to the markers used here), with respect to the response to treatment and outcome.

PATIENTS AND METHODS

Case Selection

A cohort of 224 consecutive patients with advanced NSCLC (IIIB and IV stages, UICC TNM 6th edition) diagnosed as nonsquamous NSCLC (ADC or NSCLC-NOS) on small biopsy or cytological samples and treated at the Thoracic Oncology Unit of San Luigi Hospital (Orbassano, Turin, Italy) from 2005 to 2010 was retrospectively selected. All considered specimens were obtained from chemotherapy-naive patients, who subsequently received first-line treatment; data on response and overall survival (OS) were available for all considered patients. Almost all patients received front-line platinum-based chemotherapy with/without experimental agents. Twelve patients with PS2 received single agent pemetrexed or erlotinib/gefitinib (n = 2). Forty-one patients were treated second-line (n = 22)/third-line (n = 19) erlotinib, according to the registration label. For institutional policy in that period of time, patients with ADC or any other type of NSCLC were not routinely checked for epidermal growth factor (EGFR) mutation or ALK translocation.

All pathological diagnoses were reviewed (L.R.) and segregated into two groups: (1) ADC based on morphology only and (2) NSCLC-NOS. An external unrelated pathologist (G.R.) reviewed all the cases of this latter group, confirming that they were all undifferentiated cases with no morphological criteria helpful to discriminate between adeno and squamous differentiation. Furthermore, those cases with cytological characteristics suggestive of neuroendocrine differentiation (large cell with homogeneous salt-and-pepper chromatin appearance, large nucleoli, abundant granular cytoplasm) were excluded from the series, and in those doubtful cases, IHC for neuroendocrine markers was performed to further exclude positive cases. The NSCLC-NOS group was further analyzed for a tissue sparing, minimalist IHC approach (as previously described)[14] to better characterize any residual differentiation lineage.

Immunohistochemistry

Five micrometer-thick serial sections were collected onto charged slides, dewaxed, rehydrated in pH 7.5 buffer, and processed for standard IHC staining. After blocking endogenous peroxidase activity in 0.3% hydrogen peroxide and methanol solution for 15 minutes, 5 μm-thick cell block sections were reacted for 40 minutes at room temperature with the nuclear markers TTF-1 (MoAb clone 8G7, 1/100) and p40 (MoAb clone BC28, prediluted) in a first run and with the cytoplasmic marker Napsin-A (MoAb clone TMU-Ad02, 1/100) and with the cell membrane desmosomal marker Desmocollin-3 (DSC3, MoAb clone DSC3, 1/30, overnight at 4°C). Slides were then incubated in a detection kit (EnVision Plus HRP; DakoCytomation, Glostrup, Denmark) according to the manufacturer's instructions, developing peroxidase activity with 3-3′-diaminobenzidine. Antigen retrieval was performed in a pressure cooker for 5 minutes at 125°C followed by a quick 10-second step at 90°C, using pH 8.0 ethylenediaminetetraacetic acid buffer for all primary antibodies, and pH 6.6 citrate buffer for DSC3. Finally, slides were counterstained with hematoxylin, dehydrated, and mounted. The specificity of all immunoreactions was double-checked by substituting the primary antibody with a nonrelated isotypic mouse immunoglobulin at a comparable dilution and with normal serum alone.

All histological bioptical or cytological cell blocks were used for immunohistochemical reactions. Normal bronchial epithelium and alveolar epithelium were used as internal controls for basal and glandular markers, respectively. TTF-1 and p40 were considered positive when a nuclear signal of any intensity was recorded; Napsin-A was considered positive when a finely granular intracytoplasmic signal was found; DSC3 was considered positive in case of weak linear membrane signal.

Statistical Analyses

Qualitative data were compared by Fisher's t test. OS was defined as the time between the date of diagnosis and the last follow-up and/or death, and progression-free survival (PFS) was calculated from the date of diagnosis to the date of clinical and/or radiological progression to the first-line treatment. Best response to therapy was recorded as complete response, partial response (PR), stable disease (SD), or progressive disease (PD), following Response Evaluation Criteria in Solid Tumors (RECIST) criteria.[15,16] Disease control rate (DCR) and response rate (RR) percentages were calculated on the basis of best responses. Survival estimates were calculated using the Kaplan-Meier's method and compared by the log-rank test. Cox's univariate survival analysis was performed to identify prognostic factors for both PFS and OS. All analyses were performed using the GraphPad PRISM 5 statistical software (Graphpad Software, Inc, San Diego, CA). All p values were based on two-sided test and considered as significant when less than 0.05, confidence intervals (CIs) at the 95% level.

RESULTS

Immunohistochemical Subtyping

Patients' characteristics are summarized in Table 1. After review, diagnoses were distributed as follows: 150 of 224 (67%) were ADC based on morphological examination only, while the other 74 of 224 (33%) were NSCLC-NOS (Fig. 1). After applying a panel of four markers (TTF-1, p40, DSC3, and Napsin-A), the NSCLC-NOS group was further divided as follows: on the one hand, 32 of 74 cases (43.2%) resulted TTF-1 and/or Napsin-A positive (and p40/DSC3 negative) and were subtyped as "NSCLC favor ADC" based on a glandular immunophenotype; on the other hand, 8 of 74 cases (11%) resulted p40 and/or DSC3 positive (and TTF-1/Napsin-A negative), being subtyped as "NSCLC favor SQC" according to a squamous phenotype. This group was subsequently excluded from the statistical analyses due to the small number of cases, and this study was designed for "nonsquamous" NSCLC to better understand the impact of diagnostic workup on therapeutic decision. Finally, 34 of 74 cases (46%) did not reveal any specific immunoprofile and were

