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Immunohistochemistry evaluation of biomarker expression in non-small cell lung cancer (Pharmacogenoscan study)

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ABSTRACT

Objectives: Platinum-based chemotherapy regimens are the standard treatment of non-small cell lung cancer (NSCLC). In this study, our objective was to identify tumor tissue protein biomarkers that might predict a benefit from these treatments.
Materials and methods: The Pharmacogenoscan study prospectively included consecutive chemotherapy-naive NSCLC patients at any stage between 2005 and 2010 at six hospitals in the Rhône-Alpes-Auvergne region of France. Of the 537 patients in the full analysis set, 460 had a complete histological diagnosis. We used the tumor tissue samples for an immunohistochemical evaluation of eight biomarkers: ERCC1, BRCA1, p53, p27kip1, class III β-tubulin (TUBB3), Bax, Fas, and FasL. We looked for associations between these biomarkers and the disease control rate (DCR) after 2/3 cycles of platinum-based chemotherapy, progression-free survival (PFS), and overall survival (OS).
Results: A tissue sample adequate for testing at least one biomarker was available for 289 patients. We found no significant association between biomarker expression levels and clinical or pathological variables; TUBB3 showed a trend toward higher expression in adenocarcinomas ($P = 0.005$). For none of the biomarkers were significant associations found between expression level and DCR, PFS, or OS. TUBB3-negative and FasL-negative tumors showed associations of borderline significance with higher DCR.
Conclusion: In a large cohort of patients with predominantly advanced or metastatic NSCLC, none of eight tested immunohistochemical biomarkers predicted the chemotherapy response or survival. Our data indicate limited usefulness of protein biomarkers in metastatic NSCLC and a need for further research based on molecular signatures of greater complexity.

Abbreviations: BRCA1, breast cancer susceptibility gene 1; CI, confidence interval; DCR, disease control rate; DNA, deoxyribonucleic acid; ERCC1, enzyme repair cross-complementation group 1; FasL, Fas ligand; HES, hematoxylin–eosin–saffron; IHC, immunohistochemistry; IQR, interquartile range; MIA, microtubule-interfering agents; NER, nucleotide excision repair; NSCLC, non-small cell lung cancer; OS, overall survival; PFS, progression-free survival; PS, performance status; TUBB3, class III β-tubulin.

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1. Introduction

Lung cancer is the leading cause of cancer-related death worldwide [1]. Non-small cell lung cancer (NSCLC) accounts for more than 85% of primary lung cancers and is advanced or metastatic at diagnosis in approximately 60% of cases [2]. When no druggable oncogenic molecular alterations have been identified in advanced NSCLC, platinum-based chemotherapy therefore remains the standard of care [3,4]. The most consistently identified prognostic variables include gender, poor performance status, weight loss, and number of metastatic sites [5]. However, some patients with adverse prognostic characteristics experience dramatic improvements after chemotherapy, suggesting tumor variability in susceptibility to chemotherapeutic agents. Selection of the most effective treatment strategy would benefit from the identification of biomarkers predicting susceptibility to the various available drugs.

Several tumor biomarkers associated with cell survival or death have been identified, chiefly in cohorts of patients with early-stage NSCLC. Enzyme repair cross-complementation group 1 (ERCC1) and the breast cancer susceptibility gene 1 (BRCA1) play a role in DNA repair [6,7]. The proteins p53 and $p27^{kip1}$ are involved in cell-cycle regulation [8,9]. Microtubule-interfering agents (MIAs) such as class III β-tubulin (TUBB3) are involved in mitotic kinesis [10]. The Fas/Fas ligand (FasL) system is a key regulator of apoptosis [11], and Bax promotes cell death [12]. Among these proteins, ERCC1 [4,13], $p27^{kip1}$ [14], TUBB3 [15–18], and Bax [19] have shown promise as predictive factors.

We designed the Pharmacogenoscan study to evaluate associations between the molecular profile of tumor and blood samples and the response to first-line chemotherapy for patients with NSCLC. More specifically, we sought to identify tumor proteins that predicted tumor response and patient survival.

2. Materials and methods

2.1. Patients and study design

The Pharmacogenoscan study was conducted in six hospitals in the Rhône-Alpes-Auvergne region of France to identify biological and histological factors associated with outcomes of patients with NSCLC. The primary study objective was to identify associations between biomarker expression levels and the disease control rate (DCR). The secondary objective was to identify biomarkers associated with patient survival. The study was approved by the Ethics Committee of the Grenoble University Hospital (NCT00222404) and patients signed an informed consent.

Consecutive patients ($n = 550$) with chemotherapy-naive NSCLC were included between July 2005 and August 2010. Inclusion criteria were NSCLC at any stage [20,21] and ECOG-performance status (PS) of 0–2. Patients had to be treated by platinum-based doublet chemotherapy as either neo-adjuvant treatment or first-line treatment for metastatic or recurrent disease. The response was first evaluated using RECIST 1.1 [22] after 2 or 3 chemotherapy cycles as decided by the investigator. No centralized review of response was planned. All clinical data were recorded prospectively. Missing data were rechecked by one of us (ACT) before database lock. As shown in the patient flow chart (Fig. 1), 537 patients were included in the full analysis set.

2.2. Tissue samples

The diagnosis of NSCLC was established using tissue samples in 460 (86%) patients and cytology samples in 77 (14%) patients. The tissue samples consisted of 337 (73%) endoscopic biopsies, 59 (12%) transthoracic biopsies or biopsies of metastases, and 64 (14%) biopsies obtained during mediastinoscopy. The paraffin blocks used for the diagnosis were collected from the pathology centers. Available hematoxylin–eosin–saffron stained (HES) slides were reviewed by one of us (EB) using a blind procedure.

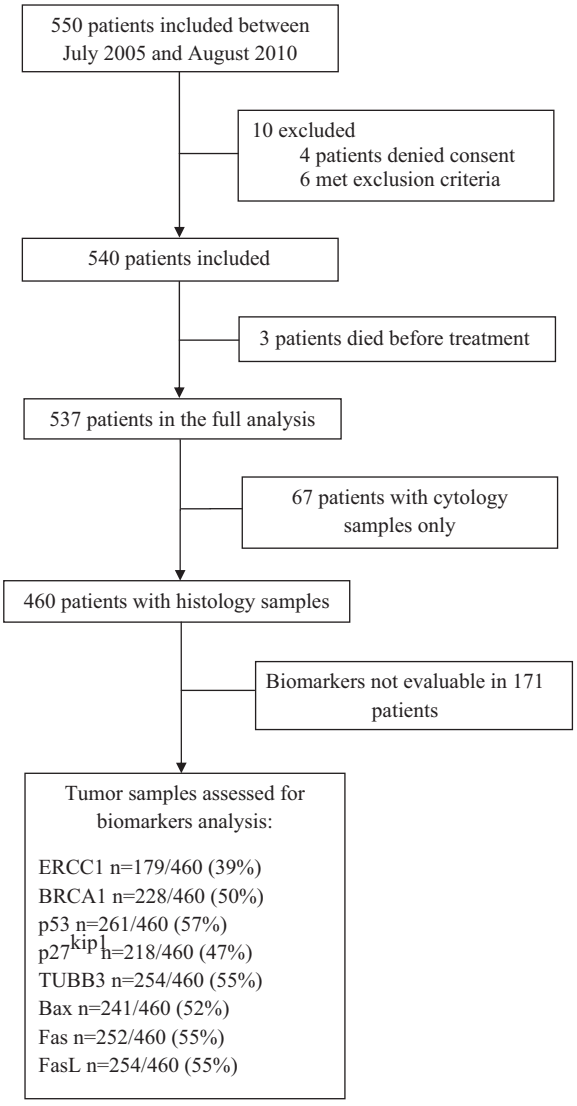

Fig. 1. Patient flow chart.

2.3. Immunohistochemistry

Immunohistochemical analyses were carried out at the pathology department of the Albert Michallon teaching hospital in Grenoble, France. Tissue sections were deparaffinized and rehydrated. The immunostaining techniques varied with the antibody used (Supplemental Table 1). For ERCC1, immunostaining was performed within 4 weeks after the tissue sections. Color development was with 3-3-diaminobenzidine. The slides were counterstained with Mayer's hematoxylin and mounted. Immunostaining characteristics with the localization of the stained cells and internal positive controls for each antibody are reported in Supplemental Table 2.

One of us (EB) evaluated all immunostainings under a light microscope at 400× magnification; this investigator was blinded to clinical data. The following eight markers were studied: ERCC1, BRCA1, p53, $p27^{kip1}$, TUBB3, Bax, Fas, and FasL. Staining intensity

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