Departament de medicina/Universidad Autónoma de Barcelona

Pablo Martínez Rodríguez

ALK rearrangement in a selected population of advanced non small cell lung cancer patients. FISH and inmunohistochemistry diagnostic methods, prevalence and clinical outcomes

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INDEX

1. ABSTRACT

ALK rearrangement represents a novel molecular target in a subset of non small cell lung cancers (NSCLC). Our aim is to explore fluorescence in situ hibridation (FISH) and inmunohistochemistry (IHC) as diagnostic methods, prevalence and clinical outcomes of ALK rearrangement patients in a selected population of NSCLC. Methods: patients with NSCLC previously screened for EGFR mutation at our institution bettween June 2006 and January 2010 were selected. ALK rearrangement was identified by using FISH and the value of IHC (D5F3 monoclonal antibody-mAb) was explored. For IHC ALK protein expression positivity was defined as tumor-specific staining of any intensity in ≥10% of the tumor cells. Results: 92 patients were identified. Data is available for 71 patients: median age was 61 years (range 36-83), 80% were adenocarcinomas, 7% squamous and 13% NOS carcinomas. 51% patients were female. All were caucasian. 32% of the patients were never smokers and 30% former smokers. 6 (8.5%) patients were ALK rearranged positive by FISH, 9 (12.7%) were EGFR mutant, and 56 (78.8%) were wild type (WT/WT) for both ALK and EGFR. ALK rearrangements and EGFR mutations were mutually exclusive. ALK rearranged patients tend to be younger than EGFR mutated or WT/WT patients (median age of 53, 59 and 62 years, respectively). Patients with ALK positive tumors were predominantly never smokers (67%) and adenocarcinomas (83.4%) with equal distribution for sex. ALK positive and EGFR mutant patients have a better survival than WT/WT patients (p=0.003 and p=0.03). All patients with ALK FISH negative tumors were negative for ALK IHC. Out of 6 patients positive for ALK FISH, 4 were also positive for ALK IHC, 1 negative and in the other there was not enough tissue to perform the analysis. Conclusions: The prevalence of ALK rearrangement is 8.5% in a caucasian selected population of NSCLC. ALK positive patients have different clinical features and a better prognostic than EGFR WT and ALK negative patients. IHC with D5F3 mAb against ALK is a promising method for detecting ALK rearranged NSCLC patients

2. INTRODUCTION

Lung cancer is the most frequent cause of cancer-related death worldwide, accounting for more than 1 million deaths per year.[i] Although cytotoxic chemotherapy remains the mainstay of treatment for the majority of patients with advanced non small cell of cancer (NSCLC),[ii] [iii] identification of specific genetic lesions that drive the proliferation of cancer cells have led to the development of new target therapies in a subset of patients with NSCLC[iv] [v]. Actually, EGFR (epidermal growth factor receptor) TKI´s (tyrosine kinase inhibitors) , gefitinib or erlotinib, are an option for newly diagnostic patients with NSCLC harboring activating mutations in the EGFR TK domain[vi] [vii] . In recent years, Anaplastic lymphoma kinase (ALK) rearrangement, predominantly with EML4 gene, has been identified as an oncogenic event in a subset of NSCLC patients [viii]. ALK translocation results in the constitutively expression of the tyrosine kinase domain of ALK protein, which results in tumor development and growth. The oncogenic dependence of this event is demonstrated on the basis that aboling ALK kinase activity reverses the malignant pattern and growth. More recently, results of a phase 1 trial evaluating an ALK inhibitor, crizotinib, in patients with ALK positive NSCLC demonstrated encouraging results [ix]. Clinical trials with crizotinib and other

ALK inhibitors in this subset population of ALK positive NSCLC patients are now ongoing.

Initial reports have shown that ALK positive NSCLC patients used to have an adenocarcinoma histology, even with signet ring cell pattern. ALK positive patients were predominantly non/light smokers and youngers than overall NSCLC patients[x]. These same clinical and pathological features are present also in patients with EGFR mutations, but both genetic events seems to be mutually exclusive[xi]. In unselected patients with NSCLC the prevalence of ALK positivity range from 1% to 7% [xii], but more than 30% in patients selected by clinical and genetic features as EGFR WT, adenocarcinoma and never smokers [x]. Its prevalence in a selected European population of NSCLC patients it is not yet known.

However, as ALK has been identified in a subset of patients with NSCLC and its particular characterictics has been better elucidated, definition of ALK positivity remains a challenging issue. First reports on the prevalence of EML4-ALK rearrangements used RT-PCR for detecting patients with this molecular profile, usually as a retrospective analyses of resected specimens from patients with NSCLC[viii xi]. However, this method is unable to detect unknown EML4-ALK variants or rearrangements with other partners different from EML4. Fluorescence in situ hibridation (FISH) with a break apart probe to ALK is the diagnostic method for selecting patients in crizotinib trials. This method allows detecting ALK translocations, no matters the partner or the variant, but ALK positivity definition by FISH and its restricted use to resection or biopsy specimens are limitations[xiii]. Inmunoshistochemistry (IHC) has also been explored. IHC analyses with previous antibodies against ALK protein used in hematologic malignances have shown poor sensitivity in detecting this protein in patients with NSCLC, probably due to the lower protein levels expressed compared to hemathologic malignances with ALK rearrangements. New high sensitivity monoclonal antibody D5F8 seems to have accuracy enough to identify patients in a more worldwide reproducibility manner [xiv], as all patients in which there was tissue enough in phase 1 crizotinib trial previously selected by FISH positivity were also positive by IHC, whereas only two of three parts of that patients were positive by RT-PCR. FISH-negative samples and normal lung tissues did not express ALK protein by IHC[ix].
The aim of this study is to explore the prevalence of ALK positivity in an European cohort of selected NSCLC patients by FISH, define its clinical features and outcomes and explore IHC as diagnostic method for NSCLC testing for ALK.

3. **MATERIAL AND METHODS**:

Patients

All included patients had received treatment or consultation from the Medical Oncology Service at Vall d´Hebron University Hospital. Since to, all non small cell

lung cancer patients previously screened for EGFR status were selected. EGFR mutational analyses had been performed based on a medical case per case indication, taking into account gender, histology and smoking history but without fixed parameters. Medical records were revised and basal clinicopathological features, treatments and outcomes were recorded. If tissue available for ALK analyses, patients were first tested by FISH and, subsequently, by IHC. An institutional ethic committee approved protocol.

ALK FISH and ALK IHC testing

Unstained slides from formalin-fixed, paraffin-embedded (FFPE) tumor samples from biopsies or cell blocks reconstructed from cytology or, if not other tissue available, slides from cytology were then analyzed. To identify ALK rearrangements, FISH was performed by using a break-apart probe to ALK (Vysis LSI ALK Dual Color, Break Apart Rearrangement Probe; Abbott Molecular). Samples were deemed to be FISH-positive if more than 15% of scored tumor cells had split ALK5´and 3´probe signals or had isolated 3´signals. For inmunohistochemistry analyses, rabbit monoclonal antibody (mAb) D5F3 was applied. Samples were deemed to be IHC-positive if a tumor-specific staining of any intensity in ≥10% of the tumor cells were present.

Statistical analyses

Unless otherwise specified, for the analyses of clinical and molecular markers on the patient samples, the Fisher´s exact test was used to assess correlation between categorical variables and Student´s t test was used to assess association between the distributions of treatment outcome. All reported p values are two-sided unless otherwise specified, and we considered a test as statistically significant if $p \leq 0.05$. To compare the correlation between FISH and IHC to detect ALK positive patients we used a kappa method

4. RESULTS

Between May 2006 and January 2010 99 patients previously screened for EGFR mutations and with tissue available for ALK analyses by FISH were identified. Data is available for 71 patients.

Of the 71 tumor samples screened, 9 patients (12.7%) harbored an activating EGFR mutation, 6 patients (8.5%) were ALK positive and 56 patients (78.8%) were wild type and negative for EGFR and ALK.

Basal characteristics of the patients are summarized in table 1. Patients were predominantly adenocarcinomas (80%), never/former smokers (62%) and females (51%).

Consistent with previous studies EGFR mutation and ALK positivity were mutually exclusive (table 2).

ALK positive patients tend to be younger (53 years) than EGFR mutant (59 years) or EFGR WT/ALK negative (62 years) patients. There was not a gender prevalence for ALK positive patients (3 males and 3 females). All ALK positive patients had a non squamous histology, 5 of 6 patiemts were adenocarcinoma and the other had a NOS NSCLC. EGFR mutant patients were also predominantly adenocarcinomas but most of them were women.

We also explored the best clinical response with an EGFR TKI or platinum based chemotherapy regimen in patients with ALK positive, EGFR mutant or EGFR WT/ALK negative metastatic disease. As expected, ALK positive patients treated with erlotinib had no objective responses; compared with a 75% of responses for EGFR mutant patients and no responses for EGFR WT/ALK negative patients. Responses to first line platinum doublet were 25% for ALK positive patients, 60% for EGFR mutant (patients and 40% for EGFR WT/ALK negative.

At the time of review, median follow-up of patients with metastatic NSCLC was 9.5 months. We analyzed overall survival (OS) of patients according to ALK and EGFR genotype. The median OS were 4.5 months for EGFR WT/ALK negative, and had been not reached for ALK positive and EGFR mutant patients. Four of the 6 ALK positive patients included in this analyses had received crizotinib as part of their treatment at sometime in the course of their disease.

Finally, we performed an ALK IHC with the D5F8 antibody in the 64 patients in which there was material still available. All of 59 patients negative for ALK by FISH were also negative for IHC. Of five ALK FISH positive patients tested for IHC, 4 were positive, one negative.

5. DISCUSSION

ALK activation had been identified as a driver oncogene alteration in a subset of NSCLC patients. This genetic alteration , mostly associated with rearrangements in the same chromosome arm with EML4 gene, is an example of oncogenic dependence. ALK positive patients had a predominantly clinicopathological features as adenocarcinoma histology, never/light smoking history and younger age at diagnosis. Developement of new drugs targeting this alteration, consisting in constitutively activation of ALK kinase domain, led to impressive tumor responses in this subset of patients.

In our report, we select a subset of patients treated or having consultation at our institution on the basis that previously determination of EGFR status would let as to identify a population of patients more suitable to harbor an ALK alteration, as both population has similar clinical features.

In this cohort, the prevalence of EGFR mutations were 12.7%, which is close to the frequency reported by the Spanish lung cancer group in a similar population. ALK positivity was 8.5%, as expected by publications of other investigator in this population of patients and being the first ALK prevalence report in a cohort of predominantly metastatic and clinically selected european NSCLC patients.

ALK positive patients tend to be younger than EGFR mutant and EGFR WT/ ALK negative patients without a gender preference. However, the vast majority were adenocarcinoma and never/former smokers but this characteristics could be a bias due to the basal clinical selection to perform EGFR analyses.

As previously reported, ALK positive patients had no responses to EGFR TKI and had a similar benefit from chemotherapy. Although previous reports have suggest that pemetrexed could be the preferred drug for ALK patients[xv], due to the small size of our ALK population we were not capable to perform the analyses.

In the survival analyses, ALK positive and EGFR mutant patients (median survival not reached in both groups) have a significant improve in survival compared to EGFR WT/ALK negative patients (median survival 4.5 months) ($p=0.033$ and $p=0.003$, respectively). The median survival in the global cohort was 9.5 months, which is in the normal range of a metastatic serie of patients. However, this cohort could be enriched by patients with poor performance status or more comorbidities than general population of lung cancer patients. Reasons for this hypothesis are that patients suitable to be included in a trial had EGFR tested in a central laboratory or patients with good performance were included in more trials requiring tissue for biomarkers analyses, not being available for this study. This is encouraged suggested by the poor median survival in the EGFR WT/ALK negative group (4.5 months).

IHC exploratory analyses with D5F3 antibody was performed in 64 patients. All patients with ALK FISH negative tumors were negative for ALK IHC. Out of 6 patients positive for ALK FISH, 4 were also positive for ALK IHC, one negative and in the other there was not enough tissue to perform the analysis. The reason for one false negative result has to be elucidate but a possible explanation could be the heterogeneity of the tumor, as previously suggested by other investigators[xvi]. However, this antibody seems to have enough accuracy to asses a positive result for selecting patients to treatment with ALK inhibitors.

6. CONCLUSSIONS

ALK positive NSCLC represents a 8.5% of patients in a clinical selected population. ALK positive patients have a clinical and pathological features useful to select patients more suitable to harboring this genetic alteration. At least, in the era of ALK inhibitors this biomarker analyses seems to be relevant for prognosis. IHC with D5F3 antibody seems to be accuracy enough for selecting patients for treatment with ALK inhibitors.

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