Lung Cancer 93 (2016) 55–58

Contents lists available at ScienceDirect

Lung Cancer

journal homepage: www.elsevier.com/locate/lungcan

K-RAS mutations indicating primary resistance to crizotinib in *ALK*-rearranged adenocarcinomas of the lung: Report of two cases and review of the literature

Maria Cecilia Mengoli[a,*], Fausto Barbieri[b], Federica Bertolini[b], Marcello Tiseo[c], Giulio Rossi[a]

[a] *Unit of Pathology, University Hospital Policlinico, Modena, Italy*
[b] *Operative Unit of Oncology, University Hospital Policlinico, Modena, Italy*
[c] *Medical Oncology Unit, University Hospital, Parma, Italy*

ARTICLE INFO

Article history:
Received 19 October 2015
Received in revised form
28 December 2015
Accepted 4 January 2016

Keywords:
Lung
Adenocarcinoma
K-RAS
Crizotinib
ALK
Resistance

ABSTRACT

The paradigm of mutually exclusive alterations among oncogenic drivers in non-small-cell lung cancer (NSCLC) is challenged by the increasing evidence of detection of two or more driver alterations in the same tumor using highly-sensitive molecular assays. We report here two cases of *ALK*-rearranged adenocarcinomas harboring concomitant exon 2 *K-RAS* mutations (G13D and Q61H). The patients, a 49-year-old smoker man and a 59-year-old non-smoking woman, experienced a rapid disease progression and primary resistance to crizotinib. Search for similar cases in the literature reveals that concomitant *K-RAS* mutations and *ALK* rearrangement occur in a subset of NSCLC and seems to lead to resistance to crizotinib. Among 8 similar cases receiving crizotinib previously reported (4 in first line and 4 in second line), 1 had a partial response, 1 stable disease and 6 disease progression. One patient still had progression disease when switching to ceritinib. At the end, *K-RAS* mutations seem to represent a negative predictive marker in *ALK*-rearranged adenocarcinomas treated with ALK inhibitor.

1. Introduction

Anaplastic Lymphoma Kinase (*ALK*) gene rearrangement was identified in about 5% of non-small-cell lung cancer (NSCLC) patients, often characterized by adenocarcinoma (ADC) histotype, younger age and no or light-smoking history. The vast majority of these cases are wild-type for *Epidermal Growth Factor Receptor* (*EGFR*) and *Kirsten rat sarcoma viral oncogene homolog* (*K-RAS*) genes analysis [1,2]. *ALK*-positive adenocarcinomas are highly responsive to the triple ALK, c-MET, ROS1 inhibitor crizotinb in about 65% of cases [3,4]. The median clinical response is of about 10 months, but relapse inevitably occurs and secondary resistance is generally due to occurrence of ALK mutations, ALK gene amplification and activation of escape signalling pathways [5]. No consisting data are reported concerning the mechanisms leading to primary resistance in 30–40% of ALK-positive patients unresponsive to crizotinib [6,7]. Novel and more potent ALK-inhibitors, namely ceritinib, alectinib and brigatinib, have been developed to overcome primary or acquired resistance to crizotinib [6]. The concomitant occurrence of *K-RAS* mutations and *ALK* rearrangement have been recently described in a subset of adenocarcinomas, generally leading to primary resistance to crizotinib [7–13]. We report here two additional patients, a 49-year-old smoker male and a 59-year-old non-smoking woman, with advanced pulmonary adenocarcinoma harboring concomitant *K-RAS* mutations (G13D; Q61H) and *ALK* rearrangement experiencing rapid disease progression on chemotherapy and crizotinib. No *ALK* gene mutations were detected. The role of the "*undruggable*" *KRAS* mutations as negative predictive marker [14] in ALK rearranged adenocarcinoma is discussed.

2. Case report

2.1. Case 1

In September 2014, a 49-year-old man, heavy smoker presented to our Institution with seizures. CT and RM-brain imaging showed 2 frontal and a cerebellar metastatic lesions. Chest-CT revealed a

Abbreviations: EGFR, epidermal growth factor receptor; KRAS, Kirsten rat sarcoma viral oncogene homolog; ALK, Anaplastic Lymphoma Kinase; FISH, fluorescent in situ hybridization; IHC, immunohistochemistry; NSCLC, non-small-cell lung cancer.

* Corresponding author at: Unit of Pathology, University Hospital Policlinico, Via del Pozzo, 71-41124 Modena, Italy. Fax: +39 059 4224998.
E-mail address: cecilia.mengoli@gmail.com (M.C. Mengoli).

http://dx.doi.org/10.1016/j.lungcan.2016.01.002
0169-5002/

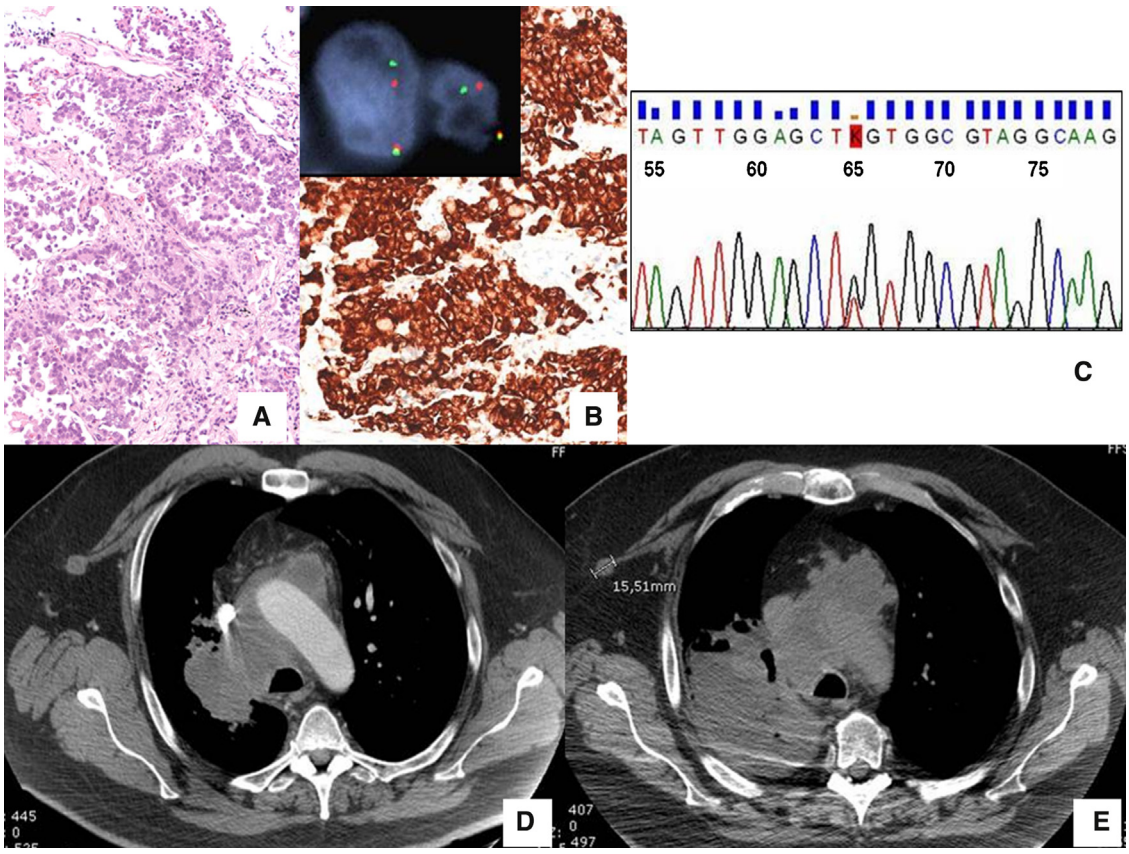

Fig. 1. Lung adenocarcinoma from Case 1 (A, hematoxylin-eosin stain) expressing ALK protein (B, immunohistochemistry clone D5F3) with gene rearrangement revealed by split of red and green probes at FISH assay (insert) and KRAS mutation (C, G13D, electropherogram). Chest CT scan at diagnosis (D) and on crizotinib therapy (E).

solid mass of the right upper lobe and enlarged mediastinal lymph nodes. A bronchial biopsy was performed and a diagnosis of primary pulmonary adenocarcinoma was made (stage IV according to the 7th AJCC/UICC tumor staging system) (Fig. 1). At immunohistochemistry (IHC), tumor cells were positive with TTF-1 (clone 8G7G3/1; Ventana Medical Systems, Inc., Tucson, AZ) and negative for p40 (clone BC28; Ventana). The assessment of *EGFR* (exons 18–21) and *K-RAS* (exon 2) mutations and determination of ALK by IHC and FISH were performed. The neoplasm had *ALK* rearrangement evidenced by ALK expression (clone D5F3, Ventana/Roche) at IHC, and FISH assay (Vysis LSI ALK Dual Color, Break Apart Rearrangement Probe; Abbott Molecular, Abbott Park, IL) with 20% of rearranged cells. The mutational analysis by MALDI-TOF revealed only *K-RAS* exon 2 mutation (G13D) among more than 200 analyzed mutations from 26 different genes (LungCarta® Panel v1.0, Agena Bioscience, San Diego, CA), further confirmed by Sanger's sequencing (Fig. 1). *EGFR* was wild-type. The patient received a first-line chemotherapy with cisplatin and pemetrexed. The brain lesions were treated with stereotactic radiotherapy. In November 2014, the patient experienced rapid worsening of performance status and progressive pulmonary and nodal disease. A second-line therapy with crizotinib was started, but the disease rapidly progressed with multiple lung, nodal, liver and bone metastases, and peritoneal carcinomatosis (Fig. 1). The patient died in December 2014, 4 months from diagnosis.

2.2. Case 2

In October 2014, a 59-year-old woman, never smoker presented with persistent cough, dysphonia and weight loss to our Institution. Chest X-rays and total-body CT revealed a solid mass of the left upper lung lobe and bilateral enlarged mediastinal lymph nodes. The bronchial biopsy showed a poorly-differentiated pulmonary adenocarcinoma (stage IIIB). At IHC, tumor cells were positive for TTF-1 and negative for p40. The assessment of *EGFR* (exons 18–21) and *K-RAS* (exon 2) mutations and determination of ALK by IHC and FISH were performed. Tumor cells did not express ALK at IHC, but 50% of *ALK*-rearranged cells were detected by FISH. The mutational analysis revealed also *K-RAS* exon 2 (Q61H) mutation. *EGFR* set-up was wild-type. The patient received chemotherapy with cisplatin and pemetrexed for 3 months without clinical response and progressive disease with an increasing number of pulmonary nodules. In April 2015, a second-line therapy with crizotinib was started, but the disease rapidly progressed with extensive lung involvement and the patient died 7 months from diagnosis.

3. Discussion

About 65% of *ALK*-rearranged lung adenocarcinomas respond to crizotinib and the occurrence of *ALK* mutations seems to represent the most important mechanism of secondary resistance [3–6]. However, few data are available concerning primary resistance and the presence of concurrent gene alterations in *ALK*-rearranged tumors has been poorly investigated [5–7].

We report here 2 cases of *ALK*-rearranged (both positive by FISH assay, while 1 positive and 1 negative by IHC) adenocarcinomas concurrently harboring exon 2 *K-RAS* mutations (G13D; Q61H) characterized by rapid disease progression and primary resistance to crizotinib. Similar cases in literature [7–13] reveal that concomitant *K-RAS* mutations and *ALK* rearrangement occur in a subset of NSCLC and seems to lead to resistance to crizotinib. Among 8 cases receiving crizotinib previously reported (4 in first line and 4 in second line), 1 had a partial response, 1 stable disease and 6 disease progression [7–9,11–13]. One patient still had progression dis-