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MYC Amplification as a Potential Mechanism of Primary Resistance to Crizotinib in ALK-Rearranged Non-Small Cell Lung Cancer: A Brief Report[1,2]

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Abstract

INTRODUCTION: Translocations of the anaplastic lymphoma kinase (ALK) can be effectively targeted in advanced non-small cell lung cancer by ALK-TKI inhibitors including Crizotinib. However, the development of acquired resistance often limits the duration of these therapies. While several mechanisms of secondary resistance have been already identified, little is known about molecular determinants of primary resistance. In our brief report we investigated the tumor molecular profile of a patient who failed to respond to Crizotinib. *METHODS:* Fluorescence in situ hybridization (FISH) and next-generation sequencing (NGS) were run on tumor specimen as well as search and characterization of circulating tumor cells (CTCs) in the blood. Confirmation of clinical findings was achieved using a translational cell-line in vitro model. *RESULTS:* We identified the amplification of *MYC* as a potential new mechanism of primary resistance to ALK inhibition. Human EML4-ALK rearranged cells infected with a lentiviral vector carrying full-length human *MYC* cDNA were treated in vitro with crizotinib and alectinib. Overexpression of *MYC* overexpression was associated with a reduced sensitivity to both ALK-inhibitors. *MYC*-overexpressing clones displayed also increased levels of both cyclin D and E and their growth was reduced by using Cdk4/6 inhibitors such as Palbociclib. *CONCLUSIONS:* We postulate that the *MYC* gene may be implicated in the mechanism of primary resistance to ALK inhibitors. We also suggest potential *MYC*-directed inhibition strategies to overcome primary resistance in advanced ALK-rearranged NSCLC.

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Introduction

Non-small cell lung cancer (NSCLC) remains the leading cause of cancer-related mortality worldwide, accounting for approximately 18% of all cancer [1]. About 2–8% of NSCLC carry molecular alteration of the anaplastic lymphoma kinase (*ALK*), most commonly a fusion between the *ALK* and echinoderm microtubule-associated protein-like 4 (*EML4*) gene. Such *EML4-ALK* translocation leads to a constitutively activated protein kinase that is essential for transfor-

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mation [2,3]. Preclinical evidence has confirmed the activation of several downstream oncogenic pathways caused by EML4-ALK fusion protein, including PI3K, JAK/STAT and RAS/MEK/ERK [4].

Crizotinib was the first approved ALK inhibitor, based on an overall response rate of 57% in ALK positive patients [5]. Although the majority of patients experience rapid impressive response to these targeted therapies, the development of drug resistance is inevitable. Moreover, intrinsic (or primary) resistance may explain why very few patients fail to respond already from the beginning to ALK-inhibitor therapy.

Several mechanisms of acquired resistance have been reported, including secondary mutations within the ALK tyrosine kinase domain, amplification of the *ALK* fusion gene and the activation of alternative signaling pathways, such as EGFR, KIT and insulin-like growth factor receptor 1 [6]. By contrast, molecular mechanisms underlying innate primary resistance to ALK-inhibitors have not been thoroughly elucidated yet.

Analyzing the case of a patient with primary resistance to Crizotinib, we first discovered an amplification of *MYC* gene and then studied it in vitro as potential new mechanism of primary resistance to ALK inhibition.

Clinical Case

A 48-year-old, never smoker, woman was referred to our clinic with a diagnosis of ALK-rearranged NSCLC with axillary, lateral-cervical and mediastinal lymphadenopathies and multiple bone, lung and liver metastases, plus pleural effusion. She was symptomatic for cough and dyspnea on exertion, which was related to both the disease and a concomitant pulmonary embolism. Since at that time ALK-inhibitors were not registered in Italy for first-line therapy, she was started on a first-line chemotherapy with cisplatin plus pemetrexed every 3 weeks. After an initial clinical benefit, the patient's overall conditions deteriorated: a CT scan performed after 3 cycles of chemotherapy, showed a progressive disease with worsening of lung metastases and the development of pulmonary lymphangitic carcinomatosis. The patient was therefore rapidly switched to a second-line treatment with Crizotinib 250 mg BID, achieving a quick, albeit transient, improvement of overall conditions. The following CT scan showed a mixed objective radiological response with a shrinkage of mediastinal lymphadenopathies, improvement of bilateral lung lesions but significant worsening of the pulmonary lymphangitic carcinomatosis and pleural effusion (Figure 1). Crizotinib was not interrupted but approval for off-label Ceritinib was sought. However, her overall conditions rapidly deteriorated leading to hospital admission due to a dyspnea on minimal exertion. CT pulmonary angiography showed recurrence of pulmonary embolism and a significant further worsening of the lymphangitic carcinomatosis and right pleural effusion was assessed for cytology. The patient then started third-line treatment with ceritinib 750 mg OD but deceased few days later due to acute respiratory failure. This work has been carried out in accordance with the Code of Ethics of the World

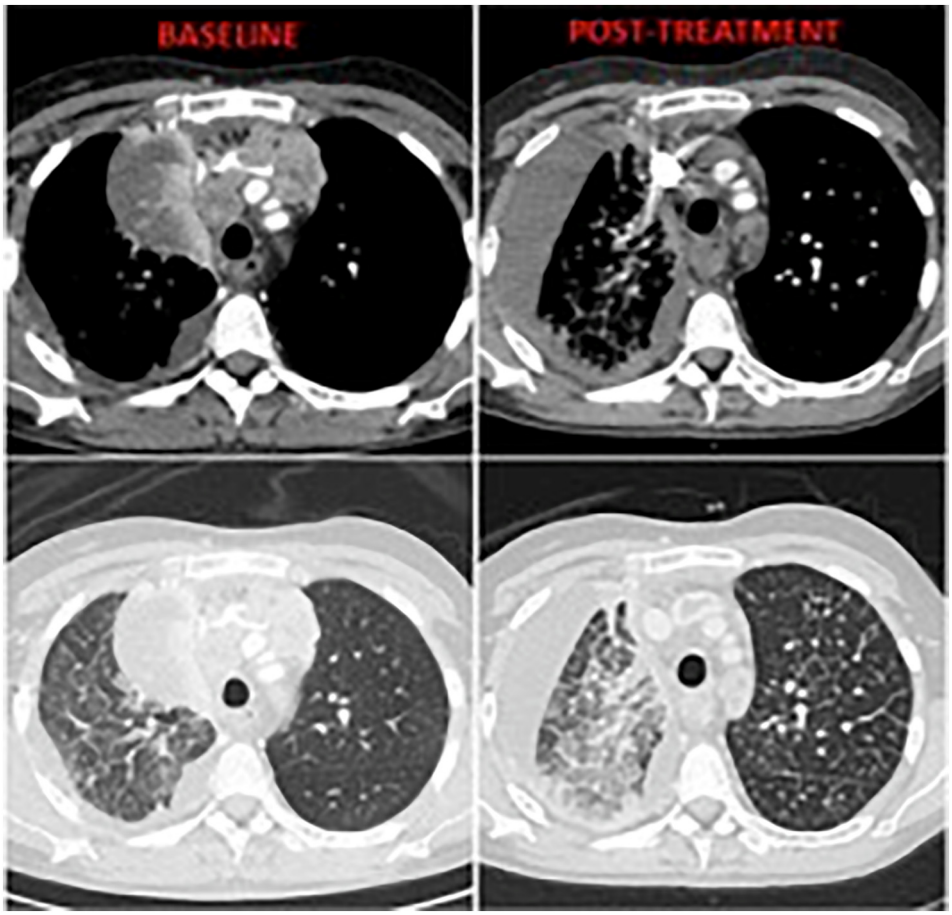

Figure 1. CT assessment before and after crizotinib treatment showing improvement in mediastinal lymphonodes enlargement with parallel worsening of pleural effusion and pulmonary interstitial disease.