Lung Cancer 115 (2018) 21–27

Contents lists available at ScienceDirect

Lung Cancer

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

Research paper

Primary resistance to osimertinib due to SCLC transformation: Issue of T790M determination on liquid re-biopsy

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ARTICLE INFO

Keywords:
EGFR
NSCLC
SCLC transformation
T790M
Liquid biopsy
ddPCR

ABSTRACT

Objectives: *EGFR* T790M mutation is the most common mechanism of resistance to first-/second-generation EGFR tyrosine kinase inhibitors (TKIs) in non-small cell lung cancer (NSCLC) and could be overcome by third-generation EGFR-TKIs, such as osimertinib. Liquid biopsy, a non-invasive technique used to test the presence of the resistant mutation, may help avoiding tissue re-biopsy. However, analysing only circulating-free DNA, information about other less frequent and coexisting resistance mechanisms may remain unrevealed.

Materials and methods: All patients reported in this series participated in the ASTRIS trial, a real world treatment study testing the efficacy of osimertinib (80 mg os die) in advanced T790M-positive NSCLC progressed to prior EGFR-TKI. Patients were considered eligible to osimertinib if T790M positive on tissue or plasma samples. In our patients, *EGFR* molecular testing on blood sample was conducted with digital droplet PCR (ddPCR).

Results: We report our experience of five patients treated with osimertinib after T790M detection on liquid biopsy that presented a disease progression at first tumor assessment mediated by SCLC transformation, as evidenced at tissue re-biopsies. All patients showed low ratio T790M/activating mutation in the blood before osimertinib (lower than 0.03). For three patients, *EGFR* mutational analysis was T790M-negative when re-assessed by using a less sensitive method (*therascreen*®) on the same liquid biopsy sample analysed by ddPCR before osimertinib therapy.

Conclusion: Although liquid biopsy is a relevant tool to diagnose T790M presence in NSCLC patients resistant to EGFR-TKI, in case of a low ratio T790M/activating mutation, tissue biopsy should be considered to exclude the presence of SCLC transformation and/or other concomitant resistance mechanisms.

1. Introduction

Non-small cell lung cancer (NSCLC) accounts for 85–90% of pulmonary malignancies and *EGFR* activating mutations are found in about 10–12% of lung adenocarcinomas arising in Caucasian patients [1]. EGFR tyrosine kinase inhibitors (EGFR-TKIs) such as gefitinib, erlotinib (first-generation compounds) and afatinib (second-generation) represent the standard of care as first-line treatment of advanced *EGFR*-mutated NSCLC [1]. However, after a median of 9–12 months of treatment, the majority of patients progress and various mechanisms of acquired resistance have been described [2], including the T790M mutation located in exon 20 of *EGFR*, which occurs in 50–60% of cases [2]. Other mechanisms of resistance include the activation of alternative signalling, including *MET* and *HER2* amplification, *BRAF*, *KRAS* and *PI3K* mutation and upregulation of *AXL* gene [2]. Histological transformation towards small cell lung cancer (SCLC) and transition to an epithelial-mesenchymal (EMT) phenotype represent less common mechanisms of acquired resistance.

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https://doi.org/10.1016/j.lungcan.2017.11.011
Received 30 July 2017; Received in revised form 1 November 2017; Accepted 13 November 2017

Findings of T790M mutation and SCLC transformation allow proper therapies: third-generation EGFR-TKI or appropriate chemotherapy, respectively. Osimertinib is the third-generation TKI compound in the most advanced stage of study having already obtained FDA and EMA approval for the treatment of *EGFR* T790M-positive NSCLC patients in progression to first- or second-generation TKI [3].

Currently, T790M testing could be performed using circulating-free DNA (cfDNA) and, if plasma genotyping is positive for resistance mutation, it may obviate the need for an invasive tissue re-biopsy. If plasma genotyping for T790M is negative, tissue re-biopsy is recommended considering the sub-optimal sensitivity (about 70%) of plasma testing [4]. Response rates and progression-free survival to osimertinib are similar whether T790M is detected in tissue or in plasma samples [3,4].

Despite the high concordance between T790M on liquid biopsy with tissue sample, some information about other potential resistance mechanisms, such as histological transformation in SCLC and EMT may be lost, leading to potential osimertinib failure when cfDNA is analysed only.

Here, we report our experience of five patients treated with osimertinib after T790M confirmation on liquid biopsy who present a primary resistance ultimately attributed to SCLC transformation.

2. Materials and methods

2.1. Patients

All patients reported in this series were enrolled in two different Institutions (University Hospital of Parma and Careggi Hospital of Firenze) to participate in the ASTRIS trial (NCT02474355 [5,6]), a real world treatment study testing the efficacy of osimertinib (80 mg os die) in advanced T790M-positive NSCLC that progressed to prior EGFR-TKI. Main inclusion criteria were: stage IIIB/IV NSCLC with T790M mutation, ECOG performance status 0–2, adequate bone marrow and organ function, absence of cardiac abnormalities at ECG. Main exclusion criteria were: prior treatment with osimertinib, previous history of interstitial lung disease, uncontrolled systemic disease and symptomatic brain metastases. After progression to first-line EGFR-TKI, patients were considered eligible to osimertinib if T790M positive on tissue or plasma samples. All patients signed informed consent form before any trial procedure and the trial was properly approved by local Ethics Committee of both Institutions. Enrolled patients underwent ophthalmological evaluation at baseline, blood tests plus urine analysis and physical examination every six weeks and radiological revaluation every 12 weeks.

Plasma samples for cfDNA analysis were collected from all patients before osimertinib initiation. Three out of five patients, referred to Parma University Hospital, were also enrolled in a local protocol of cfDNA monitoring in patients undergoing targeted therapies. In these patients, samples for cfDNA analysis were taken at baseline and at the same time-points of imaging revaluation.

2.2. Molecular analysis of EGFR mutational status

Baseline *EGFR* mutations were assessed as part of diagnostic procedure by validated methods including Sequenom (Diatech Pharmacogenetics®, Italy) or *therascreen*® *EGFR* RGQ PCR kit (Qiagen®, Valencia, CA, USA).

The analysis of *EGFR* T790M was performed on cfDNA. Six ml of blood were collected in EDTA and centrifuged twice for 10 min at 2000 × *g* within one hour after blood drawing; plasma samples were stored at −80 °C until analysis. cfDNA was extracted using a QIAmp Circulating nucleic acid kit (Qiagen®, Valencia, CA, USA) from 1 to 3 ml of plasma following the manufacturer's protocol and the DNA was eluted in 50 µl of buffer. The analysis of *EGFR* activating and resistance mutations on cfDNA was performed by a digital droplet PCR (ddPCR) and ddPCR Mutation Assay (BioRad®, Hercules, CA, USA) as previously described [7]. In three out of five patients, analyses on ctDNA were performed also with *therascreen*® *EGFR* RGQ PCR kit (Qiagen®, Valencia, CA, USA), following the manufacturer's instructions, as a comparative analysis.

Next-Generation Sequencing (NGS) was performed with TruSight Tumor 26 genes on the MiSeq platform (Illumina®, San Diego, CA) only for the liver biopsy of case 4 at the time of osimertinib progression, in order to look for EGFR T790M mutation with a highly sensitive method. In the same NGS panel was included also p53 gene (but not RB1) useful for the aim of this study.

2.3. p53 and Rb1 immunohistochemistry

Expression of p53 and Rb1 was evaluated with immunohistochemistry (IHC) in case 2 and 5.

With regard to the tissue sample from case 2, after fixation in formalin solution and inclusion in paraffin, sections of neoplastic tissue with a thickness 3–5 µm were stained with hematoxylin/eosin (H&E) for conventional evaluation. After deparaffinization and rehydratation, sections were treated with 3% hydrogen peroxidase for 5 min. For antigen retrieval, sections were treated with pH 9 Tris-EDTA buffer for 30 min in water-bath at 98 °C. The same method was used for case 5, after treatment of cytological sample decolorated from May-Grumwald Giemsa (MGG). Sections were stained with the following primary antibodies: anti-p53 (clone D0-7, Roche) and anti-Rb1 (clone Rb1, Dako, Agilent, Santa Clara, CA, USA). The sections were immunostained with the polymeric system Ultraview DAB Detection Kit (Ventana-Roche, Tucson, AZ, USA) in accordance with manufacturer specifications. Diaminobenzidine (DAB) was used for staining development and the sections were counterstained with haematoxylin. Negative controls consisted of substituting normal serum for the primary antibody.

3. Results

3.1. Patient's description

We report on a series of five patients (two females and three males) diagnosed with lung adenocarcinoma carrying an *EGFR* activating mutation that received first- or second-generation EGFR-TKI and, at resistance, were positive for *EGFR* T790M mutation in plasma samples. Nonetheless, all the patients progressed on osimertinib at first tumor assessment after 12 weeks or less, and tissue re-biopsies revealed a switch to SCLC histology. In four cases, re-biopsies were obtained after progression to osimertinib, while one patient was re-biopsied right after progression to first-line EGFR-TKI (i.e. before osimertinib administration). Clinico-biological characteristics representative of individual patients' disease course are summarized in Table 1.

3.1.1. Case 1

A never smoker 69-year-old male was symptomatic for increasing back pain. A Magnetic Resonance Imaging (MRI) evidenced multiple bone lesions. After radiological documentation of lung, adrenal, bone and lymph node lesions, a bronchoscopy allowed the diagnosis of lung adenocarcinoma, positive for *EGFR* exon 19 deletion (ex19del). The patient received palliative bone radiotherapy and subsequently started afatinib 40 mg os die, later reduced to 30 mg os die due to grade 3 skin rash. Positron Emission Tomography (PET) scan performed after three months showed significant response and therapy was continued until progression of disease, for a total amount of 10 months. Liquid biopsy was performed showing ex19del and T790M (Table 1). Osimertinib was then administered, being optimally tolerated. However, after two months, osimertinib was permanently discontinued because of further skeletal, hepatic, pulmonary and nodal metastatic spread. A new bronchoscopy was performed and histological assessment was consistent with SCLC; molecular analysis did not reveal any genetic