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Annals of Case Reports

Servetto A, et al. Ann Case Rep: ACRT-187.

DOI: 10.29011/2574-7754/100087

Case Report

Dynamic Ultra-Deep Sequencing of Mutant Epidermal Growth Factor Receptor in a Non-Small Cell Lung Cancer Patient

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Citation: Servetto A, Marciano R, Malapelle U, Di Mauro C, Napolitano F, et al. (2018) Dynamic Ultra-Deep Sequencing of Mutant Epidermal Growth Factor Receptor in a Non-Small Cell Lung Cancer Patient. Ann Case Rep: ACRT-187. DOI: 10.29011/2574-7754/100087

Received Date: 15 June, 2018; **Accepted Date:** 25 June, 2018; **Published Date:** 02 July, 2018

Abstract

Introduction: Lung cancer still represents the main cause of death among malignant neoplasms, even though the increasing use of Epidermal Growth Factor Receptor (EGFR) Tyrosine Kinase Inhibitors (TKI) has significantly improved the survival of patient subgroups.

Case Description: In this study, we discuss the case of a 51 years old woman affected by lung adenocarcinoma harbouring deletion in the exon 19 of the EGFR gene. She was first treated with afatinib, a second generation irreversible EGFR TKI, but then the disease quickly progressed on the brain. Furthermore, the emergence of resistant T790M mutation in EGFR gene exon 20 was detected: as a result, the patient started treatment with the third-generation TKI, osimertinib. Particularly, we followed step by step the evolution of the tumor biology by performing an ultra-deep sequencing assay on both plasma and serum samples, analyzing each time the EGFR mutant allele rate.

Conclusions: Herein, we discuss the role of liquid biopsy in monitoring the cancer development through treatments of an EGFR mutated lung adenocarcinoma patient and suggest to further investigate the role of the mutation allele rates.

Keywords: Epidermal Growth Factor Receptor; Next generation sequencing; Non-Small Cell Lung Cancer; Tyrosine Kinase inhibitor

Introduction

In the last decade, molecular targeted therapies have reached clinically relevant results in patients affected by Non-small Cell Lung Cancer (NSCLC) harboring activating mutations in the Epidermal Growth Factor Receptor (EGFR) [1]. Among various mechanisms of resistance to first and second-generation EGFR-inhibitors, such as erlotinib, gefitinib and afatinib, the emergence of a secondary T790M mutation in the exon 20 of EGFR is the most common one, occurring in about 60% of cases [2]. A third generation EGFR-inhibitor, osimertinib, has prolonged survival of T790M mutated patients, progressing after first line EGFR-TKI therapy [3].

Obviously, this scenario shows the need to follow the dynamic changes which occur in the biological evolution of solid tumors, such as the acquisition of resistant mutations or new histological features. Invasive diagnostic techniques have been so far used to obtain tissue specimens, but they are charged with a number of possible complications and sometimes they cannot be performed because of poor performance status of NSCLC patients. Furthermore, the tissue derived from small biopsies or

cytologic samples is not often representative of the intratumor heterogeneity. In this background, the role of liquid biopsy seems to be an appealing alternative to overcome these difficulties. Many groups carried out the non-invasive detection of circulating free plasma DNA (cfDNA) collected from cancer patients with PCR-based assays, limited to detection of hotspot mutations [4]. Paweletz et al. firstly developed a bias-corrected targeted Next-generation Sequencing (NGS) approach for the identification of driver mutations and rearrangements in cfDNA from advanced NSCLC patients [4].

Herein we describe a NSCLC case report in which the clinical and biological changes have been dynamically evaluated through an ultra-deep sequencing assay aimed at identification of EGFR mutations in cfDNA. Written informed consent was obtained from the patient.

Methods

The immunohistochemically staining on lymph node was performed with anti-Thyroid Transcription Factor-1 (TTF-1) antibody (mouse monoclonal primary antibody, #8G7G3/1, Roche, Basel, Switzerland).

Circulating free DNA (cfDNA) were isolated with a standardized procedure, using a fully automated acid nucleic extraction system, QIAsymphony® DSP Virus/Pathogen Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. cfDNA was analyzed by a primers panel (SiRe®) covering 568 clinically relevant mutations in EGFR, KRAS, NRAS, BRAF, cKIT and PDGFRα genes, used in ultra-deep sequencing on a Personal Genome Machine (Termofisher, MA-USA), as we previously previously discussed [5].

Case Description

In November 2015, a 51 years old Caucasian female, never smoker, without significant comorbidities, came to our observation because of cough and fatigue: at the physical examination, she had a hard-right supraclavicular lymph node, increased in size. The excisional biopsy of this lymph node was performed and the histological and immunohistochemically examination identified it as a lung adenocarcinoma metastasis (Figure 1). After that, a positron emission tomography coupled with contrast-enhanced computed tomography (^{18}F-FDG-PET/CT) was performed, revealing a right upper lobe mass, metastases in both lungs, right parietal pleura involvement associated with pleural effusion, subcarinal, right supraclavicular and pectoral lymph nodes (Figure 2A). The EGFR mutational status was analyzed through a Taqman Derived Assay (TDA) [6] on lymph node specimen and revealed the deletion p.L747_T751 delLREAT in the exon 19 of the EGFR gene. To study the biologic profile of the patient's tumor and to follow its evolution during treatment, we periodically collected plasma and serum samples.

From those samples, two different aliquots of cfDNA were isolated. The first aliquot of cfDNA was analyzed by SiRe (see methods) [5]. All results obtained with our ultra-deep sequencing assay were confirmed on the second aliquot of cfDNA using Taqman Derived Assay (TDA) analysis [6] with concordance rate of 100%.

In December 2015, prior to drug administration, the mutation detected on tissue specimen was found in plasma and serum samples through NGS analysis, with a mutant allele rate of 4,9%. Thus, the patients started the treatment with afatinib 40 mg/die, orally.

Two months later, the blood sample analysis already showed a 2,7% decrease in the frequency of the EGFR mutant allele. At the first assessment, the CT scan demonstrated a partial remission of the disease and a reduction of right pleural effusion (Figure 2B). The blood sample related to the assessment - after three months of therapy - could not detect the EGFR mutant allele anymore.

The patient continued on treatment with afatinib until the end of June 2016, when a CT scan and a magnetic resonance imaging (MRI) showed multiple new millimetric brain lesions. In August 2016 she received a whole brain irradiation (30 Gy in 10 fractions). The patient continued the treatment with afatinib until October 2016, when the CT scan revealed severe disease progression, with increased dimension of the primary lung mass, new pulmonary metastases and anosteolytic lesion in the right iliac wing.

Between March and September 2016, plasma and serum samples were collected once a month and the analysis of cfDNA did not detect any EGFR mutant allele even when brain lesions appeared. The analysis was negative both with Taqman assay and ultra-deep NGS.

Nevertheless, blood samples analyzed in October 2016, after pulmonary progression (Figure 2C), revealed the recurrence of deletion of exon 19 of EGFR gene with a mutant allele rate of 1,3% and, overall, the emergence of a secondary T790M mutation in the exon 20, with a mutant allele percentage of 0,7%. As a consequence, in November 2016 the patient started treatment with osimertinib (80 mg/die, orally). After one month of treatment, the analysis of cfDNA detected none of the two EGFR mutations anymore and a CT scan performed in January 2017 revealed a partial remission of the disease, with size reduction of the right lung primary mass and of pulmonary metastases (Figure 2D). The patient is currently on treatment with osimertinib.

In Figure 3, the dynamic changes of LREAT and T790M EGFR mutated allele frequency, detected in periodically collected blood samples, are summarized.

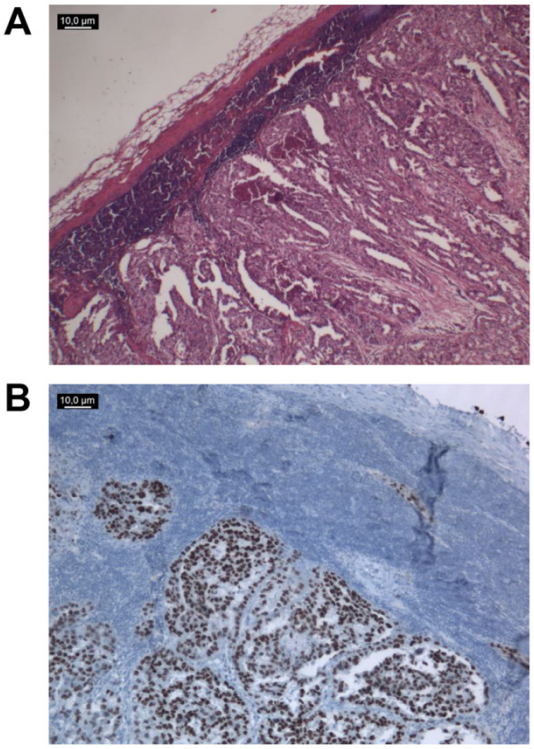
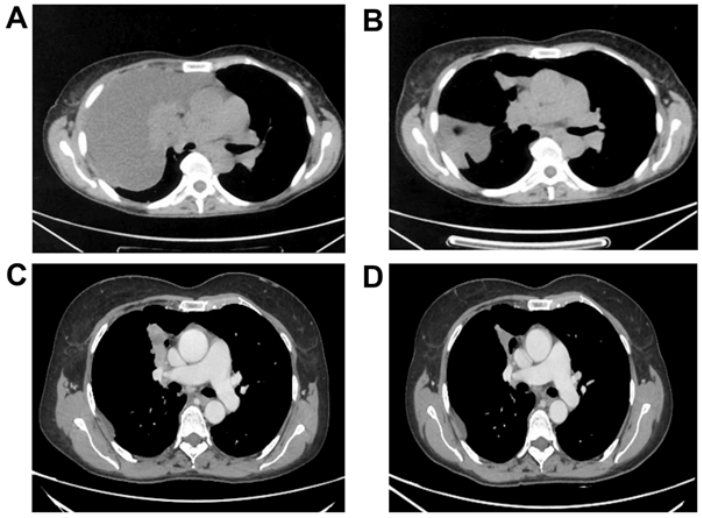


Figure 1: Histological examination of patient lymph node. A. Haematoxylin-eosin staining of the excisional biopsy showing malignant cells with abundant eosinophilic cytoplasm. **B.** Immunohistochemically staining for Thyroid Transcription Factor-1 (TTF-1) showing strong positive nuclear signal (4X magnification).



Figures 2(A-D): CT scans and in different moments of treatment. A. CT scans prior to treatment with afatinib and **B.** At partial remission; **C.** CT scans prior to treatment with osimertinib and **D.** at partial remission.

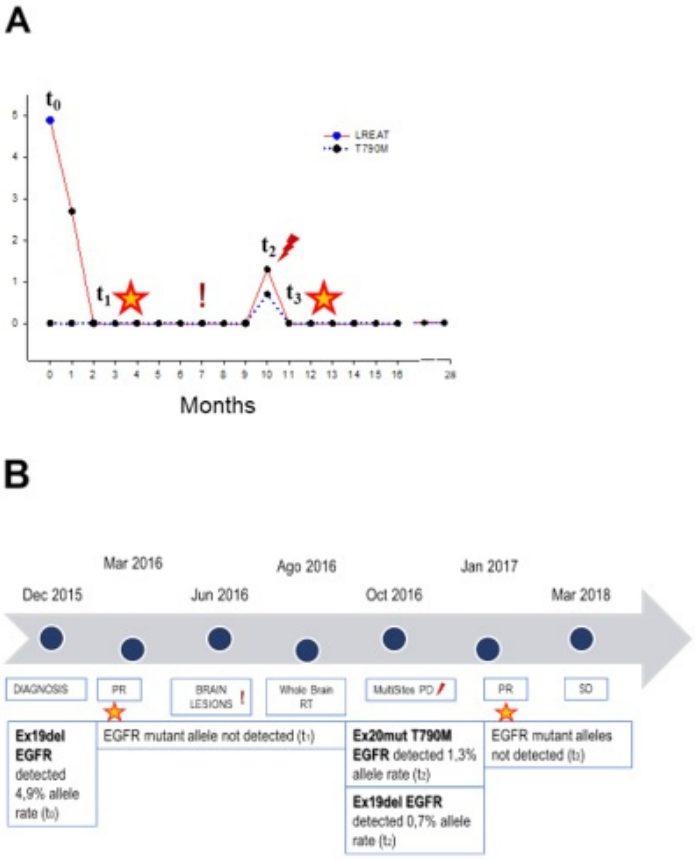


Figure 3: EGFR mutated allele frequencies detected on blood samples over the treatments. A. EGFR mutated allele frequency prior to treatment with afatinib (t_0) and at partial remission two months later (t_1); EGFR mutated allele frequency prior to treatment with osimertinib (t_2) and at partial remission (t_3). **B.** Timeline overview of treatments, responses and modification in allele frequency.

Discussion

In the management of lung cancer patients, the need to follow and detect the evolution of tumor biology has become a key-point. Liquid biopsy represents a revolutionary strategy in this setting, providing a non-invasive tool to find cfDNA released from cancer cells. First of all, blood sampling may depict the whole subclonal genetic landscape of disease in a single patient, because it is representative of the heterogeneity of different metastatic sites, overcoming the limited information obtained from single tumor biopsy [7]. Secondly, blood testing is certainly less invasive than tissue biopsy or fine needle aspiration.

In this case report, we described the NGS monitoring of EGFR mutant alleles burden and how it is related to clinical evolution of patient's disease. Particularly, both plasma and serum samples data anticipated the results showed in CT scans, mimicking the trend of neoplasm behavior. In fact, as expected, the progressive decrease in detection of blood ex19del EGFR mutant allele, after starting treatment with afatinib, predicted disease remission displayed in the first ^{18}F-FDG-PET/CT, performed in March 2016. Moreover, the drop-in detection of T790M EGFR mutant allele, one month after the start of therapy with osimertinib, anticipated the amelioration of the CT scan of January 2017.

However, our NGS testing did not reveal any EGFR mutant allele when the first clinical disease progression appeared, with the detection of isolated brain metastases. It can be speculated that this result could be due to blood-brain barrier, which could prevent cfDNA from entering the circulation, as suggested by Bettegowda and colleagues [8]. However, as the role of cfDNA keeps growing, further studies are needed to test this hypothesis.

To note, despite the patient having a good prognostic profile at diagnosis, with an exon 19 EGFR deletion [9] and a low mutant allele rate [8], still she had a short Progression Free Survival (PFS) of only six months, before the CT scan detected new brain lesions. In EGFR mutant NSCLC first line setting, median PFS for afatinib was eleven months [1]. Furthermore, Lee and colleagues, in their meta-analysis, demonstrated that although PFS is prolonged in all subgroups receiving EGFR TKIs compared with chemotherapy, the greatest benefits are observed in patients with exon 19 deletions, in general women and never smokers [10]. However, after that, Lee and colleagues pointed out that patients with exon 19 deletions starting on codon L747, as our patient, had a poorer median PFS than those starting on E746 [11]. The reasons for variable survival outcomes in response to EGFR TKIs are still unclear: more biological explanations are required.

Moreover, we analyzed our patient's mutant allele rates, following Bettegowda example in colon cancer patients, where a lower mutant allele rate seems related with better PFS and overall survival. In our case, this relationship was not found. Therefore, to better understand if allele mutant rates in EGFR mutated NSCLC can be used as a prognostic and/or predictive factor, more and wider studies are needed.

In conclusion, the widespread use of blood-based EGFR mutational testing, with different methods ranging from NGS to PCR, highlights the need to make available a unique, cheap and standardized test. In this context, NGS platforms could be more accurate than PCR in the detection of patients' mutational profile. Particularly, our ultra-deep sequencing method offers the chance to identify, in a single assay, the most clinically significant genomic mutations, meanwhile the PCR-based plasma genotyping assays is capable of revealing only hotspot mutations in coding regions. Despite all the progresses, our analysis systems can still fail: it would be interesting to know when, how and in which patient failure has to be expected. Moreover, the predictive and prognostic role of mutant allele rates in mutation-addicted cancers needs to be deeper investigated.

Acknowledgements

We thank the Associazione Italiana per la Ricerca sul Cancro (AIRC) for supporting these studies with Investigator Grant 2015-2017 (IG-15388) to RB. We thank the Consorzio Interuniversitario Nazionale per la Bio-Oncologia (CINBO).

We declare that we do not have any conflict of interest.

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