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Liquid Biopsy for Detection of T790M Mutation in Patient with EGFR-Mutant Non-Small Cell Lung Cancer with Leptomeningeal Metastases: A Case Report

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Abstract

Introduction: Liquid biopsy is increasingly used in management of Non-Small Cell Lung Cancer (NSCLC), especially when tissue availability tumor is limited. This especially true in the context of Leptomeningeal metastases. The circulating cell-free DNA from plasma or cerebrospinal fluid may provide genetic material needed for the diagnosis and management of patients with LM progression.

Case Report: We present the case of a 38-year-old Chinese female with a stage IVb NSCLC Epidermal Growth Factor Receptor (EGFR)- mutant with LM progression under Afatinib. A Droplet Digital PCR showed the presence of an EGFR exon 19 deletion and the T790M resistance mutation on both on plasma and CSF. Osimertinib resulted a rapid improvement in the clinical and neurological condition.

Conclusion: Analysis of cfDNA can provide valuable data for management of EGFR-mutant NSCLC progression under tyrosine kinase inhibitors.

Keywords: Liquid biopsy; EGFR-mutant NSCLC; Leptomeningeal metastases; T790M mutation

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Abbreviations

NSCLC: Non-Small Cell Lung Cancer; LM: Leptomeningeal Metastases; EGFR: Epidermal Growth Factor Receptor; ddPCR: Droplet Digital PCR; CSF: Cerebrospinal Fluid Analysis; IASLC: International Association for the Study of Lung Cancer; ESMO: European Society for Medical Oncology; cfDNA: Circulating Cell-Free DNA; FDG PET-CT: 18-Fluorodeoxyglucose Positron Emission Tomography-Computed Tomography; NGS: Next-Generation Sequencing; TKI: Tyrosine Kinase Inhibitors; CNS: Central Nervous System; CTC: Circulating Tumor Cells; PFS: Progression-Free Survival; NCCN: National Comprehensive Cancer Network; EMT: Epithelial-Mesenchymal Transition

Introduction

Management of Non-Small Cell Lung Cancer (NSCLC) increasingly relies on the detection of molecular abnormalities whose presence trigger specific treatments that generally result in a better quality of life or prognosis than conventional chemotherapy. However, availability of tumor tissue for analysis may be limited and a small biopsy cannot reflect tumor heterogeneity [1]. The application of liquid biopsy is increasingly common thus offering new possibilities to optimize patient care. Recently, the International Association for the Study of Lung Cancer (IASLC) and the European Society for Medical Oncology (ESMO) placed liquid biopsy as reference diagnostic method in a context of inaccessible tumor tissue [2,3]. This situation can be encountered in the context of Leptomeningeal Metastases (LM), occurring in 3% to 5% of advanced NSCLC patients and 9% to 10% of Epidermal Growth Factor Receptor (EGFR)- mutant NSCLC patients [4]. The Circulating Cell-Free DNA (cfDNA) from plasma or Cerebrospinal Fluid (CSF) may provide genetic material needed for the diagnosis and management of patients with LM progression [5]. However, evidence is lacking about how to use it in practice and the operational characteristics of this test. The choice of body fluid to analyze in the context of LM involvement in EGFR-mutant NSCLC is unclear. The following clinical case illustrates this context.

Case Presentation

A 38-year-old Chinese female presented with a 2-month history of back pain. She had no family

history of cancer and no smoking history. Suspicious metastatic bone lesions were highlighted on a dorsal spine radiography. The 18-Fluorodeoxyglucose Positron Emission Tomography-Computed Tomography (FDG PET-CT) demonstrated an FDG-avid hypermetabolic process within the left lower lobe associated with a left pleural effusion, disseminated pulmonary micronodules in both lungs, ipsilateral and contralateral mediastinal hilar lymphadenopathy and multifocal bone metastases. Gadolinium-enhanced brain magnetic resonance imaging revealed multiple cortical supratentorial lesions without signs of LM. Cytological analysis of the left pleural effusion by thoracic puncture led to the diagnosis of stage IVb lung adenocarcinoma [6]. Next-generation sequencing detected an EGFR exon 19 deletion (p.E746_A750del) from the pleural effusion. Afatinib was started. During 14 months, the patient showed an excellent tumor response with a complete extra-cerebral metabolic response and a partial intracranial response. She tolerated rather well Afatinib with some skin rash, nausea and vomiting. After 14 months of therapy, neurological symptoms (headache, increased in vomiting and a few weeks later, visual disturbances) appeared with a significant deterioration of her general condition. Gadolinium-enhanced brain magnetic resonance imaging revealed florid LM. A FDG PET-CT confirmed the complete extra-cerebral response (bone, pleural and lung). The patient was started on Dexamethasone. Given the absence of an accessible lesion for biopsy, plasma and CSF were collected. CSF cytology was collected twice by lumbar puncture and showed a white blood cell count of 17 per mm^3 (lymphocytosis of 66%) the first time and 16 per mm^3 the second time (lymphocytosis of 63%), respectively, but no malignant cells in both samples. There was a proteinorachia of 0.42 G/L the first time and of 0.62 G/L the second time (normal values 0.15 G/L to 0.45 G/L). A Droplet Digital PCR (ddPCR) was performed both on plasma and CSF confirming the presence of an EGFR exon 19 deletion and evidencing the T790M resistance mutation in exon 20. Osimertinib was started. A rapid improvement in the clinical and neurological condition was observed in the next weeks. Few months later, a complete cerebral and extra-cerebral response was observed with a complete regression of LM based on Gadolinium-enhanced brain magnetic resonance and FDG PET-CT, respectively. She has a sustained intracranial response for 24 months now.

Discussion

We observed in NSCLC a rapid evolution in the identification of targetable genetic markers for new therapies over the last decade. The development of liquid biopsies performed from different body fluids could revolutionize the genetic and molecular analyses for targeted therapies in lung cancer in the future. Many recent studies found a good concordance in mutational status between blood cfDNA and DNA from tissue biopsies [7,8]. Prabhash et al. [9] found 82.9% concordance between plasma and tissue testing. The sensitivity and specificity of Next-Generation Sequencing (NGS) were 68.4% and 90.1% in plasma and tissue, respectively. Several other studies investigated the diagnostic accuracy of cfDNA in NSCLC patients who progressed on prior EGFR Tyrosine Kinase Inhibitors (TKI), showing a very wide range of concordance rate with the tumor tissue analysis [3,10-12]. Some studies suggested that liquid biopsy would be more representative in terms of mutational analysis than tissue biopsy because of the heterogeneity in the tumor or metastatic sites and the risk of limited tissue samples by the volume and quality [13,14]. Liquid biopsy is a minimally invasive method associated with low morbidity and mortality, improving patient acceptability [15]. It also offers economic benefits being cheaper and faster than tissue biopsy, thanks to a reduction in turnaround time [16,17]. Moreover, tumor tissue is not always accessible for biopsy, as it is the case with LM metastases. Aside from initial detection of an EGFR mutation, cfDNA analysis by ddPCR is also effective in detecting the T790M resistance mutation after first- or second-generation EGFR TKI therapy [18,19] with comparable accuracy to conventional histologic or cytological samples [20,21]. A meta-analysis by Zhang et al identified 11 studies that provided diagnostic accuracy values of ddPCR for the detection of the EGFR T790M mutation in plasma cfDNA showing that it achieved a 70.1% sensitivity and 86.9% specificity [22]. The study of Oxnard et al. [23] confirmed the high positive predictive value of the T790M mutation plasma genotyping by ddPCR in NSCLC with acquired resistance to EGFR TKI, in coherence with previous studies [24,25]. In contrast, in the event of a negative result, its low negative predictive value cannot obviate the need for a standard tumor tissue biopsy [23]. An increasing number of reports were interested in Central Nervous System (CNS) progression including LM in EGFR-mutant NSCLC after first- or second-generation EGFR TKI treatment given its steadily increasing incidence [26]. Indeed, the survival of EGFR-mutant NSCLC patients is significantly prolonged [27] allowing more time for CNS metastases to develop, and the $1^{st}/2^{nd}$ generation EGFR TKI have poor penetration across the blood-brain barrier which may allow the development of CNS metastases [28]. LM involvement in lung cancer has a bad prognosis and is prone to clinically devastating complications [29]. In the case of CNS progression without extracranial progressive disease, obtaining a tissue biopsy is extremely difficult and yet absolutely necessary for detecting a targetable resistance mutation [30]. Moreover, it has been shown that, most likely due to the distinct brain microenvironment [31], the genomic alterations found in CNS metastases may differ from the primary tumors [32,33]. CSF cytology is currently the only minimally invasive method for molecular analysis of CNS progression. However, its analysis is only possible if neoplastic cells are present (which is not the case in this case report) and its sensitivity remains unknown despite multiple examinations [34]. Several studies have demonstrated that cfDNA has been shown to be present in the CSF of patients with brain tumors [35-38]. In 2020 a study of Chunhua Ma et al. [39] compared the molecular characteristics from CSF cfDNA with plasma cfDNA, plasma CTC (Circulating Tumor Cells), and brain tissue specimens in patients with brain metastases from NSCLC. They found that assessment of CSF cfDNA could provide a snapshot of what actually occurs in brain metastases and found a higher mutation detection rate in patients with LM than in those with brain parenchymal metastases. De Mattos-Arruda et al. [40] had already shown in 2015 that CSF cfDNA is more abundant and representative of CNS tumor genomic alterations than plasma, with a significantly higher sensitivity than plasma, probably due to the blood-brain barrier. These results were also confirmed in the particular case of LM progression [39,41,42], allowing to identify the presence of multiple resistance mechanisms emerging in the therapeutic course with TKI [43]. Such phenomenon can be explained by the fact that tumoral cfDNA constitutes a very small fraction of cfDNA in plasma due to the presence of DNA released by normal cells. In CSF, significantly fewer normal cells are present, resulting in an increase in the percentage of tumoral cfDNA [44]. The CSF cfDNA can also be used to monitor CNS progression as it follows the same trend as the variation in brain tumor burden [45]. These findings mean that liquid biopsy may allow the diagnosis of LM by mutation detection (as illustrated by this case report), in addition to clinical and gadolinium-enhanced brain magnetic resonance

imaging. In our case, the T790M mutation was detected in the CSF cfDNA by ddPCR method, corresponding to what is described in the literature. Moreover, the T790M mutation was also detected in the plasma by the same technique. Previous reports demonstrated that EGFR T790M are more likely to occur at extracranial sites [46,47]. These findings may suggest that taking two samples, CSF and plasma, may increase the chances of detecting the T790M mutation. Several studies have also shown that the use of repeated ddPCR-based cfDNA genotyping increased the sensitivity of resistance mutation detection [48,49]. Although EGFR TKI are the standard first-line treatment for EGFR mutant lung cancer, the development of acquired resistance limits Progression-Free Survival (PFS) to 10 to 12 months for first- and second generations TKI [50]. The acquisition of the T790M mutation in the EGFR gene is the most common resistance mechanism to 1st/2nd generation TKI, accounting for 50% to 60% of cases [50]. The third generation T790M mutant-selective EGFR TKI Osimertinib showed an efficacy superior to that of standard EGFR TKI in the first-line treatment of EGFR-mutant advanced NSCLC in terms of PFS (17.2 *vs.* 8.5 months) in the FLAURA trial [51]. Based on these results, the National Comprehensive Cancer Network (NCCN) guidelines included Osimertinib as a first-line treatment option, particularly in patients with EGFR-mutant lung cancer [52]. Thus, this particular case will naturally concern fewer patients in near future. Nevertheless, it should be highlighted that EGFR T790M is not the only mechanism of resistance to 1st/2nd generation EGFR TKI. As the treatment duration increases, the likelihood of the development of several secondary resistance mechanisms to EGFR TKI also increases [53,54]. Moreover, the occurrence of Epithelial-Mesenchymal Transition (EMT) phenotype is closely associated with the metastatic dissemination and treatment failure in lung cancer [55]. Liquid biopsy may identify very early in the time the expression of EMT markers [55]. The question of the best sample to analyze in case of LM progression will arise again in the future as new drugs devoted to the treatment of Osimertinib resistance mutations are developed. The performance of an NGS on a liquid biopsy will open the possibility to study the different mechanisms of resistance to TKI [56]. In case of LM progression EGFR-mutant NSCLC under TKI, the search for a resistance mutation using CSF cfDNA can be useful. Indeed, as Xi Wu et al. showed after analyzing the genetic profiling of cfDNA in CSF and plasma, the LM and extracranial lesions are thought to develop independently. To increase sensitivity repeating sampling of plasma and CSF cfDNA may be appropriate [57].

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