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Liquid Biopsy Testing for the Management of Patient with Non-Small Cell Lung Cancer Carrying a Rare Exon-20 EGFR Insertion

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Abstract

Increasing evidence suggests that liquid biopsy might play a relevant role in the management of metastatic non-small cell lung cancer (NSCLC) patients. Here, we show how the Molecular Tumor Board (MTB) in our cancer center employed liquid biopsy to support therapeutic decisions in a patient with NSCLC carrying a rare EGFR mutation. A 44-year-old woman, never-smoker with an EGFR, ALK, and ROS1-negative lung adenocarcinoma and multiple brain metastases received systemic therapy and surgery before being referred to our Institute. The MTB suggested NGS testing of tumor biopsy that revealed a rare exon-20 EGFR insertion (p.His773dup; c.2315_2316insCCA) and EGFR amplification. The MTB recommended treatment with erlotinib and follow-up with liquid biopsy, by using both cell-free DNA (cfDNA) and circulating tumor cells (CTCs). An increase of EGFR mutation levels in cfDNA revealed resistance to treatment about 6 months before clinical progression. Extremely low levels of EGFR p.T790M were detected at progression. Based on preclinical data suggesting activity of osimertinib against EGFR exon-20 insertions, the MTB recommended treatment with brain and bone radiotherapy and osimertinib. A dramatic reduction of EGFR mutation levels in the cfDNA was observed after 4 weeks of treatment. The PET scan demonstrated a metabolic partial remission that was maintained for 9 months. This case supports the evidence that liquid biopsy can aid in the management of metastatic NSCLC. It also suggests that treatment with osimertinib might be a therapeutic option in patients with EGFR exon-20 insertions when a clinical trial is not available.

Key words: cell-free DNA; liquid biopsy; circulating tumor DNA, non-small cell lung cancer; osimertinib.

Key Points

- Next-generation sequencing testing of tumor tissue from patients with lung adenocarcinoma may reveal rare *EGFR* mutations that are not detected by routine diagnostic methods.
- Monitoring response to EGFR tyrosine kinase inhibitors by liquid biopsy testing may provide relevant information for therapeutic decisions in selected patients.

Introduction

Genomic profiling has a critical role in the management of patients with advanced non-small cell lung cancer (NSCLC). According to current recommendations, NSCLC patients with advanced disease should be tested at least for EGFR and BRAF mutations and ALK/ROS1 rearrangements.[1] More recently, drugs targeting MET exon-14 skipping mutations and RET fusions have become available for NSCLC patients.[2] Indeed, driver genetic alterations that offer the potential for therapeutic intervention through registered drugs or clinical trials have been identified in over 50% of lung adenocarcinoma.[3] Based on this evidence, the European Society of Medical Oncology (ESMO) recommended that patients with lung adenocarcinoma should be tested with multigene, next-generation sequencing (NGS)

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panels.[2] In fact, targeted sequencing allows for optimization of the tissue available for molecular analyzes, which is often limited in patients with NSCLC.[4]

NGS testing might also reveal rare variants not detected by routine diagnostic techniques. In this respect, EGFR activating mutations have been detected in 10%-20% of Caucasians and approximately 50% of Asian NSCLC patients.[5-7] A series of small deletions in exon 19 and the point mutation L858R in exon 21, account for 80%-90% of EGFR mutations in NSCLC and are usually associated with sensitivity to EGFR tyrosine kinase inhibitors (TKIs).[8] Uncommon, rare variants account for the remaining 10%-20% EGFR mutations detected in NSCLC patients.[9,10] Rare EGFR mutations may remain undetected if the EGFR test is limited to the most frequent mutations. Furthermore, for many rare mutations, no data are available on their biological role and on the response to TKIs.[9-11]

Tumor tissue testing is the gold standard for genomic profiling. Liquid biopsy and in particular the analysis of circulating cell-free DNA (cfDNA) represents an alternative for biomarker testing in patients with inadequate or insufficient tissue samples.[8] Real-time-PCR or droplet digital PCR (ddPCR) is widely used for EGFR mutation testing of cfDNA.[12] More recently, several studies have demonstrated that cfDNA sequencing with NGS can provide a genomic profiling of NSCLC similar to tissue testing.[13,14] Because small tissue biopsy or limited cytological samples are only available for the majority of lung cancer patients in an advanced stage, cfDNA testing might represent a valuable alternative approach for genomic profiling when tissue is not adequate for full genotyping. Evidence suggests that liquid biopsy might also play a relevant role in monitoring the molecular evolution of the disease and in the assessment of the response to therapy.[12,15] However, the use of liquid biopsy to guide the treatment of patients with advanced disease is currently not a standard in NSCLC. This is partly linked to the difficulty in interpreting the results of the cfDNA test, with regard in particular to the application of the results of the analysis to the clinical management of the individual patient.

Here, we describe the case of an NSCLC patient with a rare EGFR exon-20 insertion in which liquid biopsy was used to guide the treatment choices by the Molecular Tumor Board (MTB).

Patient Story

In July 2015, a 44-year-old woman, never smoker, without comorbidities was diagnosed with a lung adenocarcinoma of the right superior lobe with multiple brain metastases. Quite surprisingly, EGFR, ALK, and ROS1 status were not determined on the tumor biopsy. The patient underwent brain radiotherapy (30 Gy) followed by 6 cycles of chemotherapy with cisplatin/pemetrexed with partial remission (Figure 1). In March 2016, she underwent right superior lobectomy (stage pT2N2). EGFR, ALK, and ROS1 testing of the surgical specimen revealed no genetic alterations as assessed by standard diagnostic methods, that is, Sanger sequencing of EGFR exons 18, 19, and 21 and immunohistochemistry and/or fluorescent in situ hybridization for ALK and ROS1. CT total body after surgery was negative and follow-up was planned.

In September 2016, the patient had a lung progressive disease and was referred to our center. The patient was enrolled in the SENECA trial of second-line therapy with weekly docetaxel plus nintedanib.[16] The patient received 6 cycles of therapy with stable disease, followed by 10 cycles of nintedanib as maintenance treatment. In November 2017, a further lung progressive disease was observed.

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Genomic Profiling at Progression from Chemotherapy and Treatment Decision

At progression of the disease, the patient was referred to the institutional MTB. Based on the characteristics of the patient (female, never smoker) and the good Performance Score (PS1), the MTB recommended enrollment in the SCRIGNO trial, in which patients are tested on either tissue or liquid biopsy with NGS panels. The aim of this trial is to search for actionable mutations that allow the administration of genomically driven therapies, through approved drugs or enrollment in clinical trials.

NGS testing of the surgical tumor specimen was performed with the Oncomine Focus Assay (Thermofisher) that targets single-nucleotide variants (SNVs), indels, copy number variations, and gene fusions in 52 cancer-related genes. The analysis revealed the presence of an exon-20

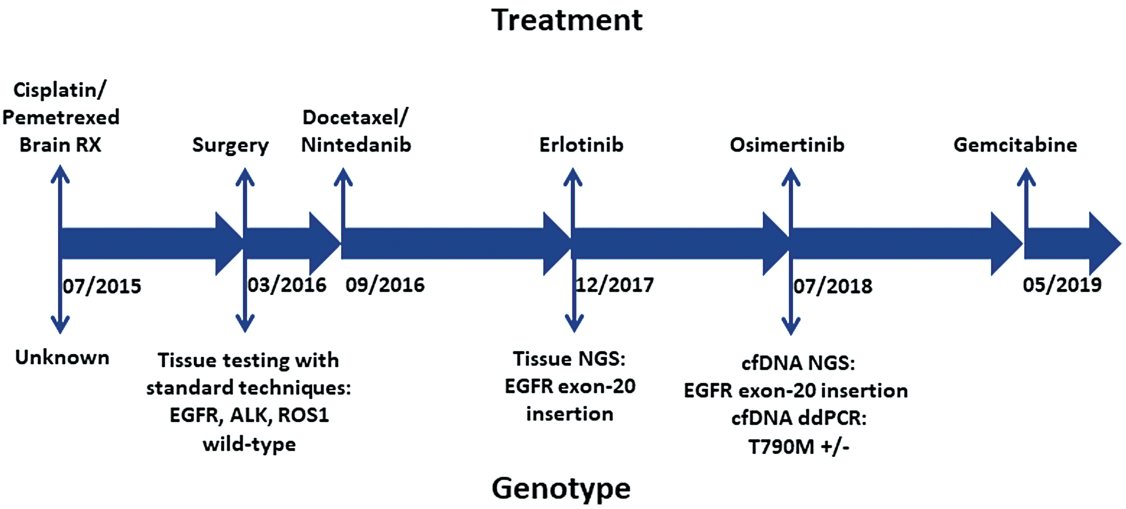

Figure 1. Timeline of the treatments received by the patient, the corresponding progression-free survival and the genotype data.

EGFR insertion (p.His773dup; c.2315_2316insCCA; variant allelic frequency (VAF) 52%) and EGFR amplification (Copy Number Variation 5.27). This rare EGFR mutation is not detected by most routine diagnostic methods. In this regard, our finding highlights the importance to perform genomic profiling using targeted sequencing that can reveal the presence of variants at low frequency, thus increasing the possibility of therapeutic intervention with targeted agents. Importantly, detection of driver genetic alterations is also relevant for the possible negative interaction with immunotherapy.[17,18]

The MTB discussed the different therapeutic options. The EGFR p.His773dup variant is still classified as a variant of unknown significance (www.oncokb.org, last accessed December 31, 2021). However, the presence of co-amplification of the EGFR gene and the high allelic frequency suggested that this genomic alteration was likely to be the main driver of the lung tumor of our patient. EGFR exon-20 insertions are relatively resistant to first-, second-, and third-generation TKIs and novel compounds with increased sensitivity are being explored.[10,11,19] Lack of response to a combination of cetuximab and afatinib in an NSCLC patient carrying the EGFR p.His773dup insertion has been reported in a paper published in 2018, after the discussion of this case in the MTB.[20]

The MTB recommended treatment with erlotinib based on different factors. Although specific information for the EGFR p.His773dup variant was not available, anecdotal responses have been observed in patients with EGFR exon-20 insertions treated with either first- or second-generation TKIs.[9,10] The patient's wish was to receive the least toxic treatment possible and from this point of view the toxicity profile of the first-generation TKIs is more favorable than that of afatinib.[21] No clinical trial with agents with higher sensitivity for EGFR exon-20 insertions was available in our Institute. Finally, in a visit carried out before the NGS test, erlotinib was proposed as a possible in-label third-line therapy. However, given the low probability of response, the MTB also recommended a monthly monitoring with liquid biopsy, to monitor response to treatment and identify early recurrence of the disease.[12] In this respect, it must be emphasized that a number of new treatment strategies have been reported for lung cancer patients with EGFR exon 20 insertions, either with new EGFR TKIs such as poziotinib or mobocertinib, or bispecific antibodies, for instance, amivantamab, which may represent a new standard of care in the coming future for these patients.[22]

In December 2017, the patient started erlotinib 150 mg/day as third-line therapy based on the recommendation of the MTB. Monthly cfDNA testing was performed with the Oncomine Lung cfTNA Assay, which covers SNVs and short indels in 11 genes and selected gene fusions and amplifications.[23] In addition, circulating tumor cells (CTC) were counted with the CellSearch System (Menarini Sylicon Byosistems), although the clinical value of CTC count and the best method for CTC evaluation in NSCLC need still to be defined.[24]

The EGFR exon-20 insertion was not detectable in the cfDNA in December 2017, before starting the treatment with erlotinib (Figure 2). A slight increase of the levels of the EGFR mutation was observed starting in February 2018 (VAF 0.37%), while the patient showed stable disease. No additional variants were detected in the genes covered by the panel at this time point neither in the tests performed throughout the clinical history of this patient.

The case was discussed again in the MTB on the request of the treating physician. A literature search confirmed that the persistence of EGFR mutation or increase in EGFR mutation

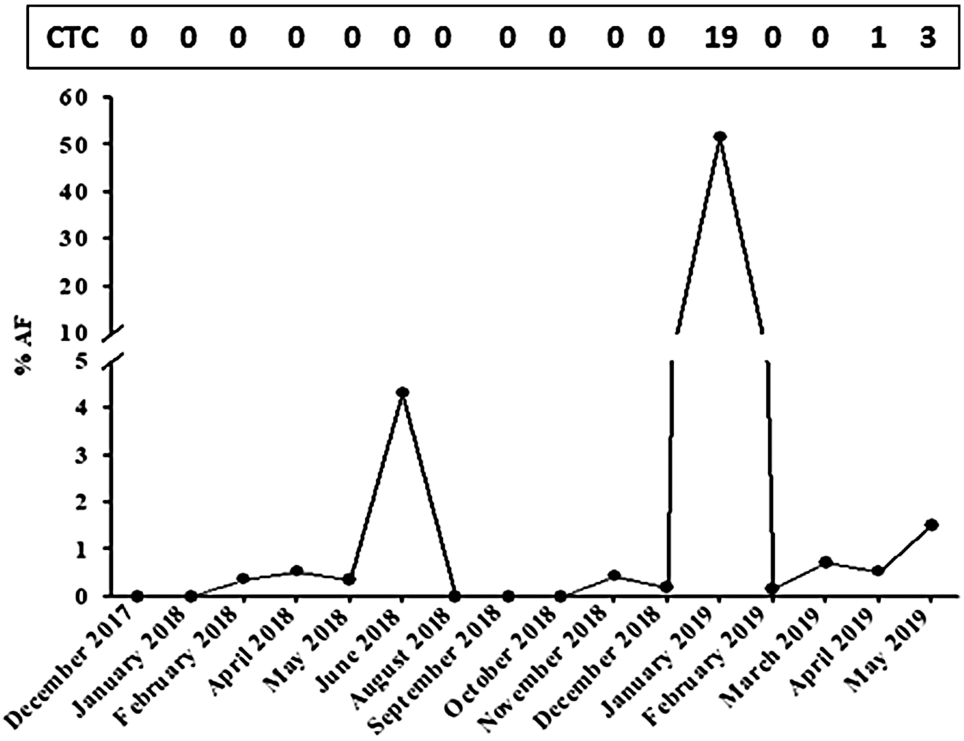

Figure 2. Analysis of EGFR mutations with the Oncomine Lung cfTNA Assay and of circulating tumor cells (CTC) with the CellSearch System at different time points. The variant allelic frequency (VAF) of the exon 20 EGFR insertion p.His773dup and the CTC count are shown.

levels in cfDNA during treatment with EGFR TKIs almost always predicts the development of resistance and the progression of the disease.[25-28] However, increased levels of cfDNA might precede weeks or months the clinical progression of the disease. In some cases, the increase in EGFR mutation levels was observed hundred days before clinical progression.[26,27] Based on the available data, the MTB recommended to continue erlotinib treatment, but also a more intensive clinical and radiological follow-up of the patient, to detect clinical progression before deterioration of performance status.

The EGFR mutation levels were quite stable in April (VAF 0.53%) and May 2018 (VAF 0.36%). In June 2018, a more significant rise of EGFR mutation levels (VAF 4.31%) coincided with a symptomatic progression of disease on lung, bone, adrenal, and brain (Figures 2 and 3A and B). The patient was referred again to the MTB.

Genomic Profiling at Progression from Erlotinib and Treatment Decision

The MTB discussed the results of genomic profiling of cfDNA. While NGS revealed only the presence of the EGFR exon-20 insertion, ddPCR showed the presence of a single droplet positive for the p.T790M variant with a VAF of 0.2%. Although the NGS panel that we used for testing has a sensitivity up to 0.1%, this limit is significantly affected by the quality and quantity of cfDNA that is possible to add to the sequencing reaction.

According to the standard operative procedures of the laboratory, samples are considered positive when at least two droplets carrying the specific mutation are detected. Therefore, the positivity of the sample for the p.T790M was at least doubtful. In addition, the VAF of the p.T790M was only 0.2% versus 4.31% of the exon-20 insertion. In this regard, some studies found that response to osimertinib can occur also when the levels of the p.T790M are very low, while others suggest that a low ratio p.790M/sensitizing mutation might indicate the presence of additional mechanisms of resistance to first-/second-generation TKI and are associated with a low probability of response to osimertinib.[29-31] Given the difference in the VAF of the EGFR exon-20 insertion versus the p.T790M and the doubts on the true positivity of the p.T790M test, the MTB concluded that it was unlikely that the resistance to erlotinib was driven by the p.T790M.

However, the MTB reasoned that the patient never really responded to erlotinib and that progression could be due to primary resistance to this drug. In this respect, osimertinib has shown in preclinical models an activity higher than first-generation TKIs on EGFR exon-20 insertions.[32] In agreement with these findings, preclinical studies suggested that selected EGFR exon-20 insertions might be sensitive to osimertinib.[33] Response to high-dose osimertinib of NSCLC patients carrying EGFR exon-20 mutations has been also reported.[34]

Based on the above-summarized findings and taking into account the favorable toxicity profile of osimertinib, the MTB suggested treatment with brain and bone radiotherapy followed by osimertinib (80 mg/day). The patient started osimertinib in July 2018. The liquid biopsy demonstrated on August 2018 a dramatic reduction of the levels of the EGFR exon-20 insertion after 4 weeks of treatment with osimertinib and the PET/TC scan showed a metabolic partial remission in September 2018 (Figure 3C and D; Supplementary Figure 1). These findings confirmed that monitoring levels of EGFR mutations in the cfDNA can predict response to EGFR TKI. Importantly, several studies have demonstrated that dynamic liquid biopsy testing can provide prognostic and predictive information also in non-oncogene-addicted patients receiving treatment with either chemotherapy or immune therapy.[35,36]

Patient Follow-up

The levels of cfDNA increased again in November 2018. A peak of CTCs (n.19) associated with a huge increase in EGFR mutation levels (VAF 51.6%) was observed in January 2019, but it was not associated with any symptom or sign of clinical progression. The levels of both CTCs and cfDNA declined in the next months to increase again in May 2019, when a clinical and radiological progression of the disease occurred in the lung. The patient died in October 2019.

Although the response to osimertinib was relatively limited in our patient, a 9-month progression-free survival in fourth-line therapy for metastatic NSCLC with standard-dose osimertinib can be considered as a good therapeutic effect. Therefore, treatment with osimertinib in patients with EGFR exon-20 insertions might be a therapeutic option when a clinical trial is not available. More importantly, this case demonstrates that liquid biopsy testing can provide important information for the management of patients with rare EGFR mutations whose response to currently available inhibitors is not known.

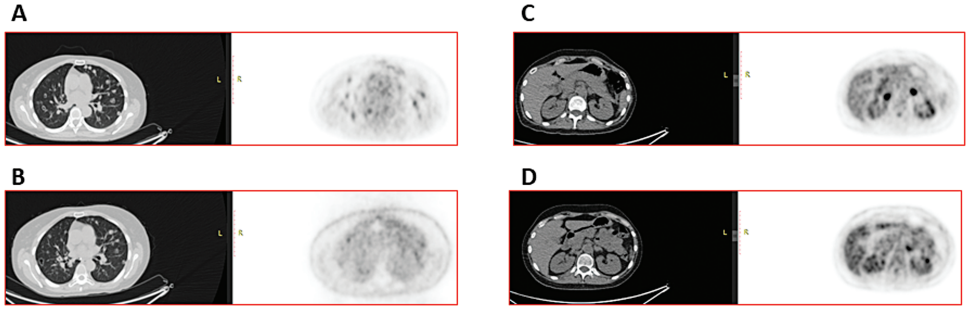

Figure 3. Positron emission tomography (PET)/Computed tomography (CT) scan at progression following treatment with erlotinib on June 2018 (**A** and **C**) and after 2 months of therapy with osimertinib in September 2018 (**B** and **D**). Lung (A and B) and adrenal (C and D) details are shown.

Supplementary Material

Supplementary material is available at *The Oncologist* online.

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Conflict of Interest

Alessandro Morabito: Roche, Takeda, Pfizer, Boehringer Ingelheim, AstraZeneca, MSD Oncology, Bristol Myers Squibb (H); **Nicola Normanno:** Roche, Boehringer Ingelheim, AstraZeneca, MSD Oncology, Bristol Myers Squibb, Merck, Qiagen, ThermoFisher, Illumina (H). The other authors indicated no financial relationships.

(C/A) Consulting/advisory relationship; (RF) Research funding; (E) Employment; (ET) Expert testimony; (H) Honoraria received; (OI) Ownership interests; (IP) Intellectual property rights/inventor/patent holder; (SAB) Scientific advisory board.

Author Contributions

Conception/design: A.M. and N.N. **Provision of study material or patients:** All authors. **Collection and/or assembly of data:** A.M., An.M., A.M.R., R.P., M.G., A.D.L., R.D.C., C.P., S.L., and N.N. **Data analysis and interpretation:** A.M., A.M.R., A.D.L., and N.N. **Manuscript writing:** A.M., A.D.L., and N.N. **Final approval of manuscript:** All authors.

Data Availability

The data underlying this article will be shared at reasonable request to the corresponding author.

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