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Challenging BRAF/EGFR co-inhibition in NSCLC using sequential liquid biopsies

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ABSTRACT

Objectives: There is some controversy surrounding the $BRAF^{V600E}$ mutation in patients with lung adenocarcinomas. Although the $BRAF^{V600E}$ mutation is sensitive to BRAF inhibitors, the efficiency of these inhibitors on patients harboring an $EGFR^{L858R/del19}$/$EGFR^{T790M}$/$BRAF^{V600E}$ pattern remains unknown.

Materials and methods: Here, we presented the case of a patient with initial response followed by progression on osimertinib. Resistance mutations ($EGFR^{T790M}$, $EGFR^{C797S}$, $BRAF^{V600E}$, *MET* amp and *HER2* amp) were assessed in the tissue or plasma DNA using NGS and digital droplet PCR at progression and during osimertinib treatment.

Results: Resistance to osimertinib coincided with the emergence of an additional tumor cell subpopulation carrying the known $BRAF^{V600E}$ resistance mutation. The patient exhibited two tumor subclones ($EGFR^{del19/T790M}$ and $BRAF^{V600E}$) that displayed distinct responses to successive tyrosine kinase inhibitors.

Conclusion: We report the first successful example of using sequential treatment with dabrafetinib/trametinib and osimertinib. Our finding provided that unique tumor biopsies deliver incomplete genetic information, and highlighted the complementary role of circulating tumor DNA to tissue biopsies and CT-scans to efficiently monitor response to osimertinib.

1. Case report

A 68-year-old female never-smoker received a diagnosis of stage-IV lung adenocarcinoma (cT2N3M1b) in October 2014. Testing of onco-driver mutational status at time of diagnosis in the initial bronchial biopsy identified an $EGFR^{del19}$ mutation (c.2235_2249del, p.Glu746_Ala750del). Four cycles of pemetrexed/cisplatin/bevacizumab were administered as first-line therapy. At progression, the patient was treated with afatinib, an oral tyrosine kinase inhibitor (TKI) sensitizing *EGFR* mutations (Fig. 1). After a first phase of partial clinical response according to the RECIST (Response Evaluation Criteria in Solid Tumors) V.1.1 criteria, computed tomography (CT) scan revealed a lung progression at 6 months. Afterward, the patient underwent four cycles of pemetrexed/carboplatin, four cycles of nivolumab and one cycle of docetaxel. However, the disease was not controlled as revealed by the detection of hepatic and bone lesions and progression of the chest lesions in January 2016 (Fig. 1). The bone lesions were therefore treated and responded well to radiotherapy. Next-generation sequencing (NGS) carried out on a subsequent hepatic biopsy showed the acquisition of an $EGFR^{T790M}$ mutation in addition to the $EGFR^{del19}$ mutation [1]. Osimertinib, an oral third-generation EGFR-TKI mutant active on the $EGFR^{T790M}$ mutation in non-small cell lung cancer (NSCLC), was then administrated to the patient (80 mg once daily). Detection of $EGFR^{T790M}$ mutation on circulating tumor DNA (ctDNA) was monitored at baseline and along the course of the treatment using highly sensitive droplet digital PCR (ddPCR). A control CT-scan performed 1 month after the treatment beginning showed a clear regression of the hepatic and lung lesions, associated with the absence of detection of $EGFR^{T790M}$ and $EGFR^{del19}$ mutations in plasma sample (Fig. 2, red and blue lines, respectively). In February 2017, after 10 months of treatment, copies of $EGFR^{del19}$ and $EGFR^{T790M}$ became detectable again in patient's liquid biopsy (71 and 11 copies/ml of plasma, respectively). This result anticipated a hepatic progression that was confirmed by CT-scan few weeks later (Fig. 1).

Resistance mechanisms to osimertinib involve bypass signaling pathways, including: *KRAS* mutations, *MET* amplification, *HER2* amplification, $EGFR^{C797S}$ mutation, and *BRAF* mutations. Non-invasive methods based on the detection of ctDNA to detect resistance and to

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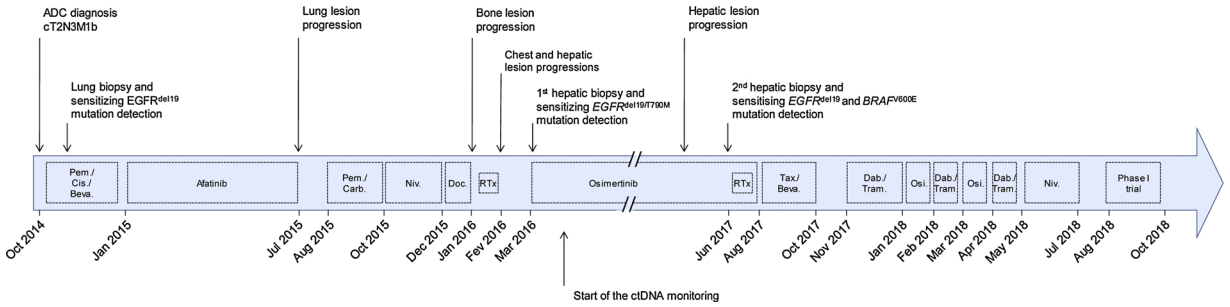

Fig. 1. Graphical overview of patient's medical history. Treatment and clinical information (biopsy and mestatsis progression) are indicated. Pem., pemetrexed; Cis., cisplatin; Beva., bevacizumab; Carb., carboplatine; Niv., nivolumab; Doc., docetaxel; RTx, radiotherapy; Tax., taxol; Dab., dabrafetinib; Tram., trametinib; Osi., osimertinib.

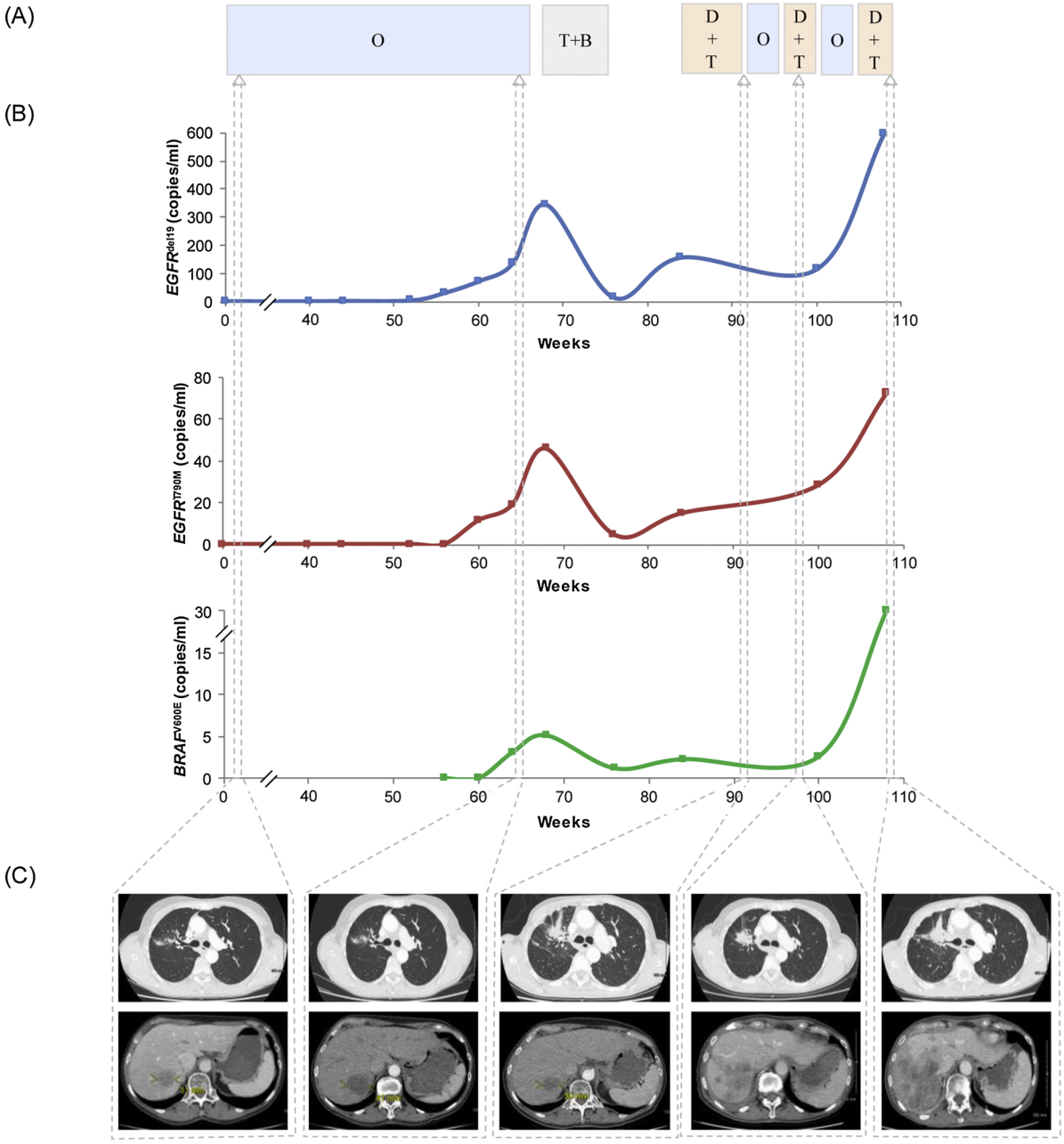

Fig. 2. Serial monitoring of *EGFR*^{del19}, *EGFR*^{T790M}, *BRAF*^{V600E} alterations in ctDNA in response to sequential TKI treatments. (A) TKI treatments administrated from the start of osimertinib are shown. (B) Level of *EGFR*^{del19} (blue line), *EGFR*^{T790M} (red line), *BRAF*^{V600E} (green line) mutations (copies/ml of plasma) are measured by ddPCR and followed over time. (C) Representative CT-scan images of the chest (top panels) and liver (bottom panels) at the indicated times of treatments are presented. D, dabrafetinib; T, trametinib; B, bevacizumab; O, osimertinib (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

rapidly reorient the therapeutic strategy in NSCLC [2]. Therefore, we carried out all these molecular alterations using dedicated ddPCR approaches. The presence of a *BRAF*^{V600E} mutation was detected at 3 copies/ml of plasma (Fig. 2, green line). This unexpected result prompted us to perform a second hepatic biopsy. Interestingly, NGS analysis confirmed the presence of the *BRAF*^{V600E} mutation, concomitantly with the detection of the *EGFR*^{del19} alteration. Of note, the *EGFR*^{T790M} mutation previously present in the first hepatic biopsy was not detectable in the second biopsy, while still present in the liquid biopsy samples, suggesting the development of distinct tumor subclones harboring different alterations. In addition, *ALK*, *RET* and *ROS1* translocations were all negative; and *MET* gene was not amplified in this sample. Due to disease progression in the liver, the patient underwent two cycles of taxol and bevacizumab. Notable tumor shrinkage was observed by CT-scan and was associated with a significantly decrease of mutated ctDNA (Figs. 1 and 2). However, a CT-scan performed one month later revealed progression with growth of the tumor in the liver, hence chemotherapy was discontinued. MEK inhibitor dabrafetinib (150 mg twice daily) combined to BRAF inhibitor trametinib (2 mg once daily), known to induce durable response and significant prolonged survival, was proposed [3,4]. After two months of treatment liver metastasis responded. However, since lung tumor still progressed and since no unacceptable toxicity was observed using tyrosine kinase inhibitors, alternation with osimertinib treatment was implemented.

In order to monitor the two distinct tumor subclonal populations and to optimize the different TKIs administration, a longitudinal analysis of the patient's ctDNA of the three molecular alterations (*EGFR*^{del19}, *EGFR*^{T790M}, *BRAF*^{V600E}) was carried out. CtDNA alteration measurements were strikingly linked to tomography. A differently response to TKI was observed with one cluster, sensitive to osimertinib, associated with lung disease, and a second cluster, sensitive to dabrafetinib and trametinib, linked to liver disease. Due to disease progression after 6 months of sequential treatment, nivolumab was reintroduced. After 4 cycles, a disease progression was observed. Based on the antigen-related cell adhesion molecule 5 (CEACAM5) positivity of more than 70% of the tumour cells, the patient was selected for an anti-CECAM5 directed phase I trial [5].

2. Discussion

BRAF mutation is detected in ˜2-3% of lung adenocarcinomas and can occur in tumors harboring an *EGFR* mutation [6,7]. *BRAF* mutation is also suspected to confer resistance to first- and third-generation TKIs. However, although patients that exhibited *BRAF*^{V600E} are sensitive to BRAF inhibitors [8], the efficiency of these inhibitors on patients harboring an *EGFR*$^{L858R/del19}$/*EGFR*^{T790M}/*BRAF*^{V600E} pattern remains unknown. Ho et al. reported the detection of a *BRAF*^{V600E} in the malignant effusion of a patient already harboring *EGFR*^{L858R} and *EGFR*^{T790M} in lung biopsies [9]. Unfortunately, although cells derived from this patient's effusion showed *in-vitro* sensitivity to BRAF inhibitors, the patient died one month after disease progression and did not received BRAF inhibitor treatment. More recently, Lin et al. have reported one patient displaying an acquired *BRAF* mutation (only 13 out of the 73 patients enrolled in the Aural study were available for a *BRAF*^{V600E} screening) [10]. However, this patient was no treated with a BRAF inhibitor. Here, we described the case of an *EGFR*$^{del19/T790M}$ patient who acquired resistance to osimertinib. This resistance was concomitant with the detection of an acquired *BRAF*^{V600E} mutation at a metastatic site. Interestingly, she displayed distinct responses to successive TKIs, demonstrating the defect of potentiation of both treatments. Osimertinib and BRAF and MEK inhibitors were not provide concurrently due to the absence of clinical data in the literature on objective response rate or on PFS of this drug combination. Of note, the regimen was tolerable and there was no added toxicity with the combination of osimertinib and dabrafetinib/trametinib compared to osimertinib given alone.

Finally, this report stresses the how critical it is to consider heterogeneity of cancer cell populations in targeted therapy–acquired resistance. Beyond this specific case, this study sketches a future where real-time information gleaned from tracking resistant clones along the treatment course could be clinically meaningful. Single tumor biopsies may provide incomplete genetic information and highlights how important and complementary are the ctDNA and CT-scan analysis to broaden the picture of the mutational pattern. Since *BRAF*^{V600E} mutation detection on liquid biopsy can impact the success of subsequent therapies, it should be routinely assayed for this mutation in samples from patients when they acquire resistance to osimertinib.

Liquid biopsy technologies that use comprehensive genomic profiling with robust gene datasets such as Guardant360 or FoundationOne Liquid may certainly help to detect all known/putative mechanisms of resistance to EGFR inhibitors. However, for other known and targetable alterations than *BRAF* and *EGFR*, we believe that prospective evidence on the use of liquid biopsy to achieve the best benefit for NSCLC patients in routine is still needed.

Conflict of interest statement

Authors declared no conflict of interest.

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