Introduction

The identification of genomic alterations harbored in non-small cell lung cancer (NSCLC) patients, in particular in epidermal growth factor receptor (EGFR) gene, led to a milestone in the improvement of treatment choices with first- or second-generation EGFR tyrosine kinase inhibitor (TKI) therapy, the most common resistance mechanism involves the selection of a resistant clone carrying the exon 20 p.T790M point mutation. However, also for these patients, treated with a third-generation TKI (osimertinib) several mechanisms of acquired resistance are described. Here we report the case of a 68-year-old man with an EGFR exon 19 deletion treated with gefitinib in first line and osimertinib in second line besides on the presence of a p.T790M mutation, who developed an uncommon EGFR exon 20 p.E746_A750del point mutation at the progression to osimertinib, with the concomitant modification of the original sensitizing EGFR exon 19 deletion and the loss of p.T790M mutation.

Keywords: Epidermal growth factor receptor (EGFR); osimertinib; exon 20; fine needle aspiration (FNA); liquid biopsy; next generation sequencing (NGS)

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A750>P) in concomitance with an uncommon EGFR exon 20 point mutation (p.L792Q). Here we aim to describe and discuss this case in the landscape of literature data.

**Case presentation**

A 68-year-old man, with an history of smoking, showed dyspnea and abdominal pain in the liver region. Thus, a total body CT was performed and showed a mass (40 mm × 27 mm) in the right lung and two additional masses (26–71 mm) in the liver. On December 2013 a CT-guided fine needle aspiration (FNA) on a liver lesion was performed. The microscopic analysis showed poorly differentiated neoplastic cells and, according to immunophenotypical features (nuclear positivity for TTF-1), a diagnosis of NSCLC favor ADC was made. At the time of the diagnosis the patient presented liver metastasis, so the tumor was classified in stage IV.

The previous international guidelines [2013] from the College of American Pathologists (CAP), International Association for the Study of Lung Cancer (IASLC), and Association for Molecular Pathology (AMP) suggested that in case of newly diagnosed IIIB/IV NSCLC molecular tests were recommended to better define the therapeutic strategy (7). Consistent with these, on January 2014 we performed a fragment analysis assay (for exon 19 deletions) and a TaqMan based real-time polymerase chain reaction (RT-PCR—for exon 21 point mutations), following a previously validated protocol (8,9). The result was a 15 base pairs deletion in EGFR exon 19, confirmed by a Sanger sequencing and re-analyzed by a next generation sequencing (NGS) approach, which showed a p.E746_A750del. Besides on this result, the patient started a treatment with gefitinib. This latter had been carried out from January 2014 to June 2015. Due to progression disease with the increase of the previously described lesions and appearance of new lesions, on August 2015 the patient started a treatment with 4 cycles of carboplatin-paclitaxel. In October 2015 was requested at our Institution the assessment, on circulating tumor DNA (ctDNA) derived from liquid biopsy, of exon 20 EGFR resistance mutation (p.T790M) in order to administrate a third generation TKI in the osimertinib expanded access study (10). The EGFR mutational status assessment was performed by the previously validated SiRe® NGS panel on Ion Torrent Personal Genome Machine (PGM, Thermofisher, Waltham, Massachusetts, USA) (11). The patient showed the resistance point mutation in EGFR exon 20 (p.T790M) in concomitance with the exon 19 deletion (p.E746_A750del) and underwent a treatment with osimertinib. In May 2017 the patient underwent another liquid biopsy analysis due to the re-presentation of abdominal pain and asthenia. We identified a mutation switch respect to the initial EGFR exon 19 deletion (p.L747_A750>P), and the uncommon point mutation in EGFR exon 20 (p.L792Q), without the evidence of p.T790M (Figure 1).

**Discussion**

Treatment with first or second generation TKIs leads to the development of resistance mutations (e.g., p.T790M) which make consequently neoplastic cells responsive to the third generation TKIs (e.g., osimertinib) (5). In these patients, the ctDNA analysis could be a valid option to investigate the development of others EGFR gene mutations (12,13). However, even under treatment with third generation TKI, neoplastic cells can develop several resistance mechanisms (Tables 1,2) (6,14-23). The first described is the point mutation p.C797S in EGFR exon 20 (6). In the study from Thress et al., six patients showed, in plasma samples, the p.C797S in association with the an exon 19 deletion and the p.T790M mutation (6). Other known resistance mutations involve EGFR codon 796 (14,15). In fact, Zheng et al., by using a NGS approach, detected a p.G796D mutation whose mechanism is a conformational interference in the interaction between osimertinib and EGFR kinase domain (14). Also this patients, as reported in our case, lost the initial sensitizing mutation (p.L858R) and p.T790M after progression to osimertinib (14). Ou et al. reported in a single patient the development of different resistance mutations (p.G796S/R, p.L792F/H, p.C797S/G and V802F) all in trans with each other and in cis with the p.T790M (15). As in our case, Chen et al. identified ctDNA derived from three patients (n=2 plasma samples and n=1 pleural effusion), acquired resistance mutations on EGFR in codon 792 (L792F, L792Y and L792H) (16). Interestingly, all these alterations were in cis with the p.T790M and in trans with the p.C797S (16). In a case by Bersanelli et al. a novel p.L718Q EGFR point mutation was evaluated as a resistance mechanism against osimertinib, without the evidence of p.C797S or other known EGFR-independent mechanisms of resistance (17). Oztan et al. described other two cases (in tissue and blood samples respectively) in which an EGFR p.G724S was detected, with or without the p.T790M (18). Alternative mechanism of acquired resistance to osimertinib involved the BRAF
Figure 1 In the figure was reported the clinical evolution of patient’s disease in relation to EGFR mutational assessment. From the top to the bottom: (A) thoracic assessment by CT of the disease at diagnosis; (B) molecular characterization at diagnosis of the EGFR exon 19 deletion (p.E746_A750del) and (C) the absence of EGFR exon 20 resistant mutation (p.T790M); (D) thoracic assessment by CT after treatment with gefitinib showed the progression of the disease; (E) molecular evidence of the persistence of the EGFR exon 19 deletion (p.E746_A750del) and (F) the presentation of EGFR exon 20 resistant mutation (p.T790M); (G) thoracic assessment by CT after treatment with osimertinib showed another progression of the disease; (H) molecular evidence of the EGFR exon 19 deletion (p.L747_A750>P) in concomitance (I) with an uncommon EGFR exon 20 point mutation (p.L792Q) without p.T790M.

pathway, as shown by Ho et al. (19). In this study, by using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), they identified, on tissue sample, a BRAF p.V600E mutation (19); in particular, the first EGFR mutation (p.L858R) was lost or not yet detectable, after treatment with gefitinib and erlotinib, and the patient acquired a concomitant exon 19 deletion with p.T790M that can be detected also after progression to osimertinib in concomitance with the BRAF p.V600E (19). Furthermore, Planchard et al. described other mechanisms of resistance, in two different patients who underwent a therapy with a third generation TKI (20); the authors identified by comparative genomic hybridization (CGH) and fluorescent in situ hybridization (FISH), HER2 and MET amplification (20). In both cases these alterations were correlated with the loss of the EGFR p.T790M mutation (20). In another experience, Ou et al. also identified a MET amplification developed after treatment with osimertinib, and in accordance with Planchard et al., no evidence of this alteration was found before treatment (21). Another recent experience, reported by Knebel et al. showed, by using digital droplet PCR (ddPCR), an amplification of the EGFR exon 19 deleted allele without the evidence of other known resistance mechanisms to osimertinib (22). Ham et al. in two different cases, harboring two different EGFR sensitizing
mutation, identified a transition from adenocarcinoma to small cell carcinoma after osimertinib with the concomitant loss of the p.T790M mutation (23). As in our case, Ho et al. showed the switch of the initial sensitizing EGFR mutation (p.L858R) with another mutation (exon 19 deletion) (19). Instead, different from our experience, their case retain the p.T790M mutation with the acquisition of a concomitant BRAF p.V600E (19). The heterogeneity of EGFR mutational status has been described in literature, and also the possibility of arising of different clones after target treatment (24,25). Moreover, the development of an osimertinib resistance mutation (e.g., EGFR p.C797S)

### Table 1 EGFR resistance mechanisms against third generation TKIs and methodology adopted to their identification

| First author        | EGFR initial mutation (persistence at the progression to osimertinib) | First TKI adopted | Resistance mechanism described | Persistence at the progression to osimertinib of p.T790 | Methodology adopted |
|---------------------|---------------------------------------------------------------------|-------------------|-------------------------------|------------------------------------------------------|---------------------|
| Thress et al. (6)   | Exon 19del (Y)                                                      | NR                | p.C797S                       | Y                                                    | NGS                 |
| Zheng et al. (14)   | p.L858R (N)                                                        | Gefitinib         | p.G796D                       | N                                                    | NGS                 |
| Ou et al. (15)      | p.L858R (Y)                                                        | Erlotinib         | p.G796S/R, p.L792F/H, p.C797S/G, p.V802F | Y                                                    | HC NGS              |
| Chen et al. (16)    | Exon 19del (Y)                                                      | Gefitinib         | p.L792H/F, p.C797S/G/N, p.L718Q | Y                                                    | Targeted NGS        |
|                     | Exon 19del (Y)                                                      | Gefitinib         | p.L792H/F/Y, p.C797S          | Y                                                    |                     |
|                     | Exon 19del (Y)                                                      | Gefitinib         | p.L792F, p.C797S, p.P794S     | Y                                                    |                     |
| Bersanelli et al. (17) | p.L858R (Y)                                                         | Gefitinib         | p.L718Q                       | Y                                                    | NGS                 |
| Oztan et al. (18)   | Exon 19del (Y)                                                      | Erlotinib         | p.G724S                       | Y                                                    | CGP                 |
|                     | Exon 19del (Y)                                                      | Erlotinib         | p.G724S                       | N                                                    |                     |
| Pisapia, et al. (present study) | Exon 19del (N)                                                | Gefitinib         | p.L792Q                       | N                                                    | NGS                 |

TKI, tyrosine kinase inhibitor; HC NGS, hybrid capture based next generation sequencing; CGP, comprehensive genome profiling.

### Table 2 EGFR independent resistance mechanisms against third generation TKIs and methodology adopted to their identification

| First author        | EGFR initial mutation (persistence at the progression to osimertinib) | First TKI adopted | Resistance mechanism described | Persistence at the progression to osimertinib of p.T790 | Methodology adopted |
|---------------------|---------------------------------------------------------------------|-------------------|-------------------------------|------------------------------------------------------|---------------------|
| Ho et al. (19)      | p.L858R (N)                                                         | Gefitinib and erlotinib | p.V600E (BRAF)               | Y                                                    | MALDI-TOF MS        |
| Planchard et al. (20) | p.E746_A750del (Y)                                                | Gefitinib         | HER2 amplification            | N                                                    | CGH, FISH           |
|                     | p.L858R (Y)                                                        | Erlotinib         | MET amplification             | N                                                    |                     |
| Ou et al. (21)      | Exon 19del (NR)                                                    | Erlotinib         | MET amplification             | Y                                                    | CGP                 |
| Knebel et al. (22)  | Exon 19del (Y)                                                     | Erlotinib         | EGFR amplification            | Y                                                    | ddPCR               |
| Ham et al. (23)     | p. L858R (Y)                                                       | Erlotinib         | SCLC transformation           | N                                                    | NGS                 |
|                     | Exon 19del (Y)                                                     | Erlotinib         | SCLC transformation           | N                                                    |                     |

CGH, comparative genomic hybridization; CGP, comprehensive genome profiling; ddPCR, digital droplet polymerase chain reaction; FISH, fluorescent in situ hybridization; HC NGS, hybrid capture based next generation sequencing; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; NGS, next generation sequencing; NR, not reported; SCLC, small cell lung cancer; Y, yes; N, no.
with the concomitant loss of p.T790M and persistence of an EGFR sensitizing mutation allows the re-treatment with a first or second generation TKI, as recently reported by Chic et al. (26).

In conclusion, we report an uncommon mechanism of resistance to osimertinib, identified with a validated NGS approach, different from the classic point mutation p.C797S, which involved, in addition to the uncommon point mutation p.L792Q, the switch from the original sensitizing EGFR deletion (from p.E746_A750del to p.L747_A750>P) and the loss of p.T790M mutation.

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Footnote

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