Concurrent Small-Cell Transformation and Emergence of *Trans*-C797S and T790M Mutations Under Sequential Treatment With EGFR Inhibitors in Lung Adenocarcinoma

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INTRODUCTION

Epidermal growth factor receptor (*EGFR*) gene mutations are strong oncogenic drivers in a subset of non–small-cell lung cancer (NSCLC). Their inhibition with specific tyrosine kinase inhibitors (TKIs) represents a successful example of precision medicine.[1] Nevertheless, disease progression almost invariably occurs after 9-14 months of treatment with either gefitinib, erlotinib, or afatinib (first- and second-generation TKIs)[2-4] and after 19 months with osimertinib (a third-generation TKI)[5] because of the development of acquired therapeutic resistance.

Several mechanisms of TKI resistance have been reported, with different mechanisms for first-, second-,[6] and third-generation TKIs.[7] The acquisition of T790M mutation in exon 20 of *EGFR* represents the most frequent mechanism (more than 50% of patients) of disease progression with gefitinib, erlotinib, or afatinib, which can be overcome by osimertinib and other third-generation inhibitors. However, a frequent cause of third-generation TKI resistance is the development of mutations at the *EGFR* C797 codon, because they prevent binding to the EGFR active site.[8,9] In these patients, preclinical studies have shown that the C797S mutation can be found in the same (in *cis*) or different (in *trans*) T790M-mutated alleles, or in other patients the T790M mutation can be lost.[10]

In clinical practice, resistant mechanisms to different TKIs can be detected on disease progression through tissue rebiopsy. The isolation and analysis of circulating tumor DNA (ctDNA) through liquid biopsy is also recommended and widely used for identification of acquired resistance mutations of *EGFR*.[11]

However, not only target-dependent resistance mechanisms can occur. Other mechanisms involve bypass pathways, such as *MET* amplification, human epidermal growth factor receptor 2 amplification, *PIK3CA* mutation, and *BRAF* mutation.[12] Furthermore, epithelial to mesenchymal transition or histologic transformation to both squamous cell cancer[13] and small-cell lung cancer (SCLC; in 3%-15% of patients) have been described after treatment with first-, second-,[14,15] and third-generation TKIs.[7,16]

CLINICAL COURSE

A 75-year-old, nonsmoking white woman was diagnosed with stage IV lung adenocarcinoma in February 2017, with bilateral pulmonary lesions (Data Supplement), pleural effusion, and metastases to the thoracic lymph nodes, liver, adrenal gland, and bone. Based on an activating *EGFR* mutation (exon 19 deletion [del19]) detected from bronchoalveolar lavage cell block (Fig 1A), treatment consisted of gefitinib, which led to objective partial response of all lesions (Data Supplement). After 9 months, the patient experienced disease progression in the liver (Data Supplement), and a liquid biopsy revealed the acquisition of *EGFR* T790M mutation (Fig 1B; Table 1). She was then treated with osimertinib, obtaining stable disease with shrinkage of hepatic lesions (Data Supplement). Almost 12 months later, dyspnea worsened, with evidence of increased right pleural effusion and disease progression in the lung, lymph nodes, and liver (Data Supplement). A new liquid biopsy identified persistence of T790M plus the acquisition of C797S in *trans* conformation (Table 1; Fig 1-C). Two right thoracenteses and a talc pleurodesis were subsequently performed for treatment of recurrent pleural effusion; pleural biopsy revealed the presence of both adenocarcinoma and SCLC (Fig 1C), the latter with a high proliferation index (MIB-1, 100%). We also found neuroendocrine cells in pleural effusion, which continued to harbor the original *EGFR* exon del19, without T790M or C797S mutations (Table 1). Molecular analysis of both ctDNA in pleural fluid and pleural biopsy showed the coexistence of del19, T790M, and in *trans*-C797S, consistently with the simultaneous presence of SCLC and NSCLC in the tissue sample (Table 1).

The patient started systemic therapy with carboplatin monotherapy in January 2019, but a pulmonary

ASSOCIATED CONTENT

Data Supplement

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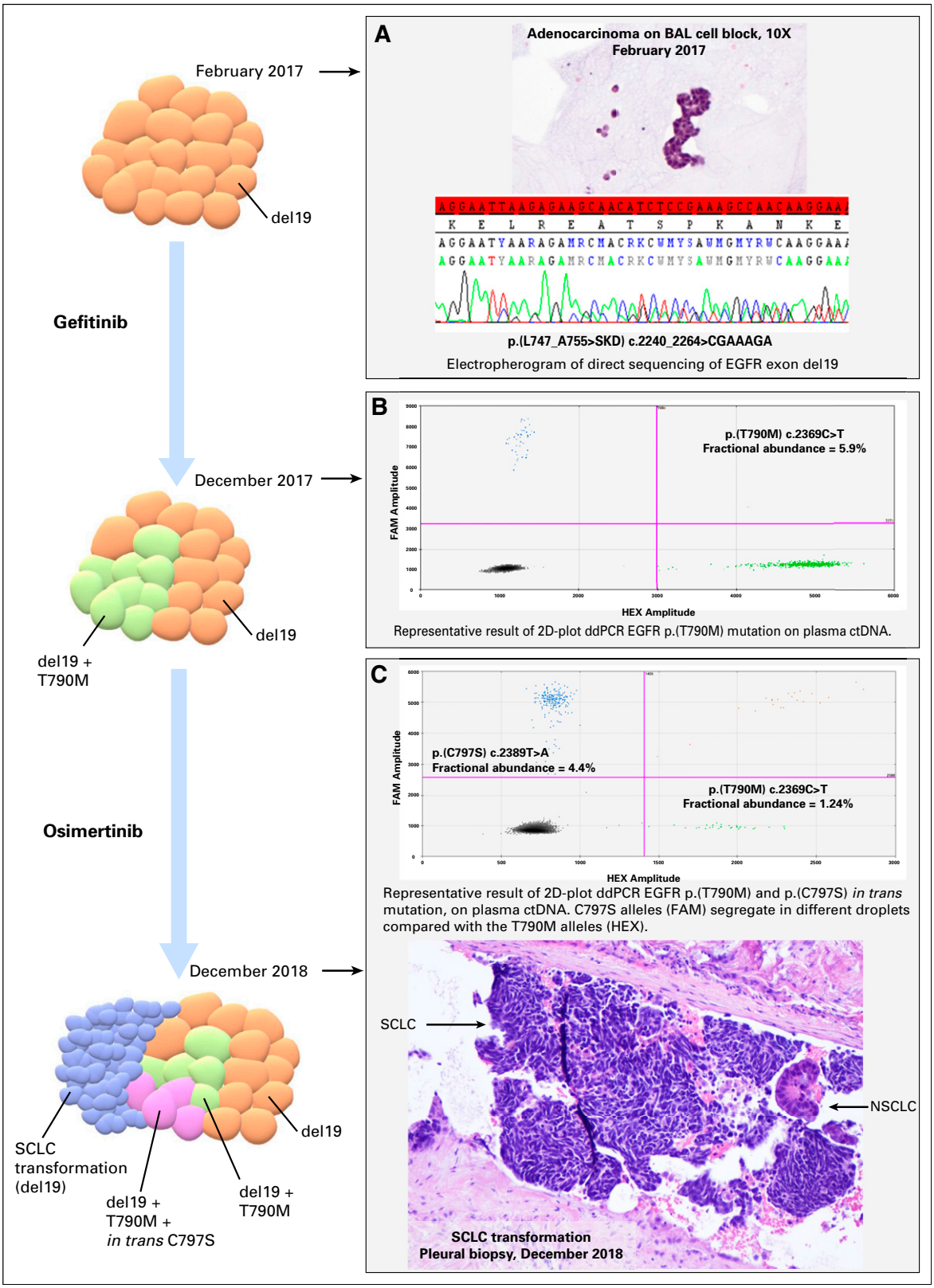

FIG 1. Synopsis of therapeutic history and histologic and molecular analysis of the present patient showing tumor evolution toward small-cell lung cancer (SCLC) transformation and accumulation of epidermal growth factor receptor (*EGFR*) genetic abnormalities. (A) Diagnosis of adenocarcinoma and detection of *EGFR* exon 19 deletion (del19). (B) Acquisition of T790M mutation. (C) Acquisition of C797S mutation on circulating tumor DNA (ctDNA) and evidence of SCLC transformation on pleural biopsy. BAL, bronchoalveolar lavage; ddPCR, droplet digital polymerase chain react; NSCLC, non–small-cell lung cancer.

TABLE 1. *EGFR* Mutational Status by ddPCR Analysis in Different Samples

Sample	*EGFR* Exon del19 p.(L747_A755>SKD) c.2240_2264>CGAAAGA	*EGFR* Exon 20 p.(T790M) c.2369C>T	*EGFR* Exon 20 p.(C797S) *in trans* c.2389T>A	T790M/del19 Ratio	C797S/del19 Ratio
Before Osimertinib					
Plasma (ctDNA) December 2017	Positive FA: 24.8%	Positive FA: 5.9%	Negative	0.24	/
After Osimertinib					
Cell block: neuroendocrine cells in pleural fluid December 2018	Positive FA: 54.5%	Negative	Negative	/	/
Plasma (ctDNA) December 2018	Positive FA: 59.7%	Positive FA: 1.24%	Positive FA: 4.4%	0.02	0.07
Pleural fluid (ctDNA) December 2018	Positive FA: 58.2%	Positive FA: 0.2%	Positive FA: 0.3%	0.003	0.005
Pleural tissue December 2018	Positive FA: 42%	Positive FA: 1.31%	Positive FA: 0.2%	0.03	0.004

Abbreviations: ctDNA, circulating tumor DNA; ddPCR, droplet digital polymerase chain reaction; del, deletion; EGFR, epidermal growth factor receptor; FA, fractional abundance.

embolism occurred 6 weeks later, and she died despite thrombolytic therapy.

DISCUSSION

In *EGFR*-mutated NSCLC, the development of mutations at the *EGFR* C797 codon, which usually occurs in *cis* with T790M and in < 5% of patients in *trans*,[9,16,17] is a frequent mechanism of resistance to third-generation TKIs because it prevents the drug from binding to the EGFR active site. Under therapeutic pressure exerted by osimertinib, it occurs in up to 24% of patients.[17]

Because the C797S mutation does not induce resistance to first-generation TKIs, a rechallenge with first-generation TKIs in combination with a third-generation TKI could be effective if the two mutations are in *trans* conformation[10] so they do not coexist in the same receptor (mutations in different alleles or different clones).[18] However, two case reports have demonstrated limited clinical benefit (progression-free survival [PFS] < 3 months),[19,20] and another report showed a PFS of 8 months with TKI combination treatment in addition to bevacizumab.[21] Ongoing clinical trials are also exploring this therapeutic option (ClinicalTrials.gov identifiers: NCT03333343, NCT03755102).

Histologic transformation to SCLC is another known mechanism of TKI resistance (reported in 15% of patients after receiving osimertinib).[16] In these patients, the original *EGFR* mutation is usually retained, but SCLCs lose their dependence on EGFR activity.[10,16] Non-*EGFR* resistance mechanisms to third-generation TKIs occur preferably with T790M loss.[16] Even after treatment with first- and second-generation TKIs, a low ratio of T790M/activating mutation could suggest the presence of other concomitant resistance mechanisms, such as an SCLC transformation.[22] Indeed, such a ratio could hypothetically indicate the proportion of cells that are T790M positive, assuming that every cancer cell harbors the original *EGFR*-activating mutation.[23] Chabon et al[24] observed a decrease of the T790M/activating mutation ratio during third-generation TKI therapy and a lower response in patients with a pretreatment ratio of ≤ 0.5. In the case of SCLC transformation, a favorable response to etoposide and platinum can be expected[25]; therefore, a rebiopsy should be performed when a histologic transformation is suspected (eg, aggressive progression).

In this case report, which describes a representative patient, different subclones harboring distinct resistance mechanisms coexisted. T790M mutation appeared after 9 months of treatment with gefitinib. The initial ratio of T790M/activating mutation was below the threshold of 0.05 and, according to the findings of Chabon et al,[24] the response to osimertinib was limited to a stable disease by RECIST criteria. This ratio deeply decreased (Table 1) at progression after 12 months of therapy with osimertinib when C797S appeared concomitantly with a small-cell transformation, after a total of 20 months of treatment with TKIs. At this time point, we observed a reduction of T790M/del19 ratio in ctDNA, which was similar to the C797S/del19 ratio (Table 1). Because neuroendocrine cancer cells retained *EGFR*-activating mutation, the increase in allelic frequency of *EGFR* del19 could be explained in our patient by the SCLC transformation. To our knowledge, this is the first study showing the coexistence of C797S in the rare *trans* conformation with T790M and SCLC transformation after osimertinib.

No tissue biopsy was performed in available case reports of TKI combination treatment in NSCLC acquiring C797S in the *trans* conformation; hence, the heterogeneous landscape of cancer evolution might not have been completely assessed, and more data are needed to understand the incidence of the co-occurrence of *trans*-C797S and SCLC transformation.

In conclusion, our case report underlines the importance of monitoring the evolution of the disease and of tissue rebiopsy for identifying heterogeneous resistance mechanisms, especially in patients with aggressive progression, to better define potential treatment modifications. A combination of different therapies might lead to clinical benefit in selected patients harboring different resistant subclones.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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