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Uncommon *EGFR* Exon 19 Mutations Confer Gefitinib Resistance in Advanced Lung Adenocarcinoma

To the Editor:

Recently, we read with interest the article by van der Wekken et al.[1] regarding the role of uncommon *EGFR* mutations. In this letter, we would like to share our experience of a case with a nonconservative double amino acid change that has not been described before. A 76-year-old never-smoker woman in good clinical conditions was referred to our Institution for a solid lesion in the left lung. Subsequent procedures, including positron emission tomography scan and fibrobronchoscopy, revealed a lung adenocarcinoma, confirmed by TTF-1 and CK7 immunohistochemistry positivity, apparently limited to the left upper lobe; therefore, the patient underwent radical left upper lobectomy. The surgical report revealed metastases in mediastinal lymph nodes and in the parietal pleura; the final stadiation was pT2b (G2), pN2, and pM1a for pleural involvement (stage IV). Consequently, we considered the patient eligible for systemic treatment. Sanger sequencing analysis revealed four consecutive genetic variants (GAA > GTT and TTA > CCA) in the exon 19 of *EGFR*, resulting in double amino acid substitution: E746V and L747P, respectively.

Despite limited and partially discordant literature data available on these variants,[1–3] we considered the patient eligible for treatment with gefitinib (250 mg/day), started in October 2013. Baseline computed tomography scan showed pleural effusion and sub-centimetric lesions in the lower left lobe; during treatment, no radiological response was observed (Fig. 1). In May 2014, progressive disease was defined because of increase of left pleural effusion and appearance of new mediastinal and left axillar lymphadenopathies and pulmonary lesions. As clinical conditions worsened, further treatments were limited to best supportive care until death, occurred in October 2014.

The poor outcome of the patient encouraged further investigations of this case: next generation sequencing did not disclose additional mutations, confirming our previous data on *EGFR* (Fig. 2*A*), and analysis in normal DNA pointed out the somatic origin of the variants.

Sanger sequencing of cDNA reported the transcription of these four mutations in approximately 50% of tumor cells (Fig. 2*B*), whereas protein analysis on tumor tissue revealed a marked phosphorylation of EGFR when compared with the correspondent normal tissue, confirming the activating role of this double amino acid substitution (Fig. 2*C*). Three-dimensional structure analysis of our mutated EGFR model revealed a rotation of approximately 32-degree of Helix A (K730-V745), mimicking the EGFR structure resulting from T790M-L858R mutations, which is characterized by a restored ATP affinity and gefitinib resistance.[4] This rotation determines a cleft allowing the protein to simultaneously bind one molecule of gefitinib and one molecule of ATP, in a favorable position to successfully undergo hydrolysis, as also demonstrated by our docking simulation (Fig. 3).

DISCUSSION

Here, we report the case of a patient affected by advanced lung adenocarcinoma harboring four novel consecutive missense mutations in the exon 19 of the *EGFR* gene that result in nonconservative double amino acid change (E746V-L747P).

Although tyrosine kinase inhibitors (TKIs) are generally recommended in *EGFR*-mutant lung adenocarcinoma, to date relatively little is known concerning the EGFR activation status related to rare mutations and their sensitivity to TKI.[1–3,5]

Our data confirmed the somatic origin of E746V-L747P genetic variants and their activating role on EGFR; structure prediction revealed a dramatic rearrangement of the N-terminal lobe of EGFR tyrosine kinase domain allowing ATP binding, irrespective of gefitinib. Indeed, docking simulations pointed out the high affinity of ATP to the double mutant in a position highly favorable for hydrolysis, resulting in TKI resistance.

The activating role of E746V-L747P mutations resulting in aberrant rearrangement of the EGFR structure, strongly supported by our data, confers weak TKI sensitivity and treatment failure.

This case highlights the importance of *EGFR* uncommon mutations on protein structure as a putative predictor of TKI efficacy.

Simona Coco, Anna Truini, and Irene Vanni contributed equally to this article.
Address for correspondence: Simona Coco, Lung Cancer Unit, IRCCS AOU San Martino-IST, Istituto Nazionale per la Ricerca sul Cancro, L. go R. Benzi 10, 16132, Genova, Italy. E-mail: simona.coco@hsanmartino.it
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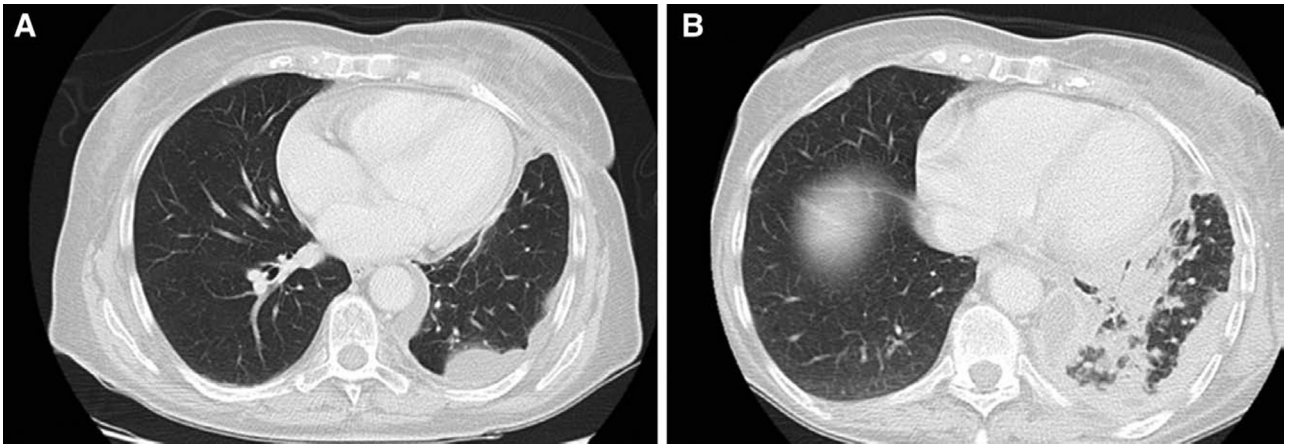

FIGURE 1. *A,* Computed tomography (CT)-scan showing left lower pleural effusion at baseline, although no measureable lesions were reported. *B,* CT-scan during gefitinib treatment revealed a progressive increase of the sub-centimetric nodules and increase pleural effusion.

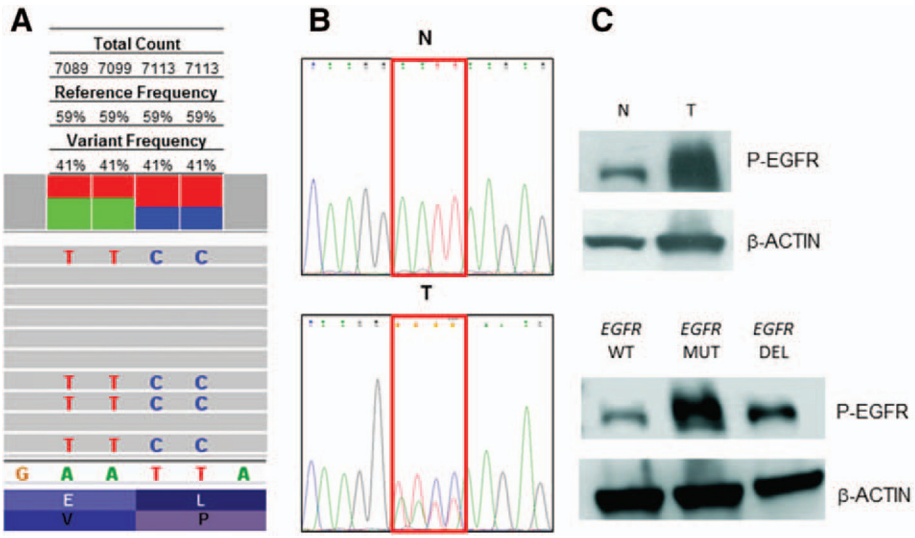

FIGURE 2. *A,* Next generation sequencing. Tumor DNA was sequenced by Ion PGM System (Life Technologies, Carlsbad, CA) using "Colon and Lung Cancer Research Panel" *v.*1. Integrative Genomics Viewer displays four consecutive heterozygous substitutions in the exon 19 of the *EGFR* (55242467, 55242468, 55242469, and 55242470). These genetic variants lead to double amino acid substitution E746V-L747P. *B,* Sanger sequencing of *EGFR* transcript. Total RNA from normal surrounding lung tissue (N) and adenocarcinoma (T) snap frozen samples were retrotranscribed and bidirectional sequencing using F:5′-TTGTGGAGCCTCTTACACCC-3′ and R:5′-TGTCTTTGTGTTCCCGGACA-3′ by ABI-Prism 3130 (Life Technologies). The electropherogram of T shows four consecutive mixed peaks compared with the N cDNA, confirming the mutations in approximately 50% of tumors cells. *C,* Western blot analysis of EGFR phosphorylation status. Upper panel: marked increase of EGFR phosphorylated form (EGFR-P) is observed in T compared with the matched N, confirming EGFR activation in exon 19 E746V-L747P sample. Lower panel: EGFR-P in T samples from three non–small-cell lung cancer tumors with a distinct *EGFR* mutational status: EGFR wild type (*EGFR* WT); exon 19 E746V-L747P (*EGFR* MUT), and exon19 delE746-A750 (*EGFR* DEL). Notably, exon 19 E746V-L747P tumor shows high EGFR-P.

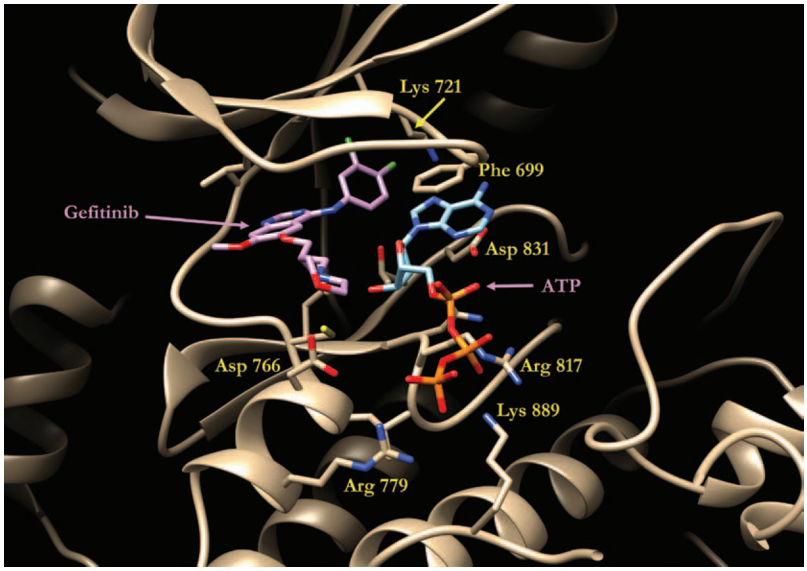

FIGURE 3. Predicted protein structure of the double mutant E746V-L747P. This structural rearrangement allows ATP (orange and blue chemical structure) binding, even in the presence of gefitinib (purple chemical structure), in a favorable position for hydrolysis. Visual analysis of the protein structures based on native EGFR alone, in complex either with tyrosine kinase inhibitor or ATP analogue, mutated EGFR with gefitinib from Protein Data Bank (http://www.rcsb.org/pdb/home/home.do. Accessed February 10, 2015; PDB: 4WRG, 4WKQ, 4HJO, 4G5J, 4I23, 3VJO, 4I22), was carried over using the program COOT and figure was drawn using the program Chimera.

Simona Coco, PhD
Anna Truini, MS
Irene Vanni, MS
Carlo Genova, MD
Lung Cancer Unit
IRCCS AOU San Martino-IST
Istituto Nazionale per la Ricerca sul Cancro
Genova, Italy

Camillo Rosano, PhD
Biopolymers and Proteomics Unit
IRCCS AOU San Martino-IST
Istituto Nazionale per la Ricerca sul Cancro
Genova, Italy

Maria Giovanna Dal Bello, PhD
Angela Alama, PhD
Lung Cancer Unit
IRCCS AOU San Martino-IST
Istituto Nazionale per la Ricerca sul Cancro
Genova, Italy

Roberta Venè, PhD
Molecular Oncology and Angiogenesis Unit
IRCCS AOU San Martino-IST
Istituto Nazionale per la Ricerca sul Cancro
Genova, Italy

Erika Rijavec, MD
Giulia Barletta, MD
Federica Biello, MD
Lung Cancer Unit
IRCCS AOU San Martino-IST
Istituto Nazionale per la Ricerca sul Cancro
Genova, Italy

Francesco Boccardo, MD
Dipartimento di Medicina Interna e Specialità Mediche (DIMI)
IRCCS AOU San Martino-IST
Istituto Nazionale per la Ricerca sul Cancro
Genova, Italy
UOC Clinica di Oncologia Medica,
IRCCS AOU San Martino-IST
Istituto Nazionale per la Ricerca sul Cancro
Genova, Italy

Francesco Grossi, MD
Lung Cancer Unit
IRCCS AOU San Martino-IST
Istituto Nazionale per la Ricerca sul Cancro
Genova, Italy

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MicroRNA Assays for Diagnosis Lung Cancer Biopsy

To the Editor:

The recent report on "MicroRNA Assays to Distinguish Squamous Cell Carcinoma from Adenocarcinoma in Lung Cancer Biopsies" is very interesting.[1] Patnaik et al.[1] noted that "histotypic microRNA assays can aid the subtyping of non-small-cell lung cancer biopsies as

Address for correspondence: Viroj Wiwanitkit, MD, Surin Rajabhat University, Surin, Thailand. E-mail: wviroj@yahoo.com
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