Lung Adenocarcinoma Patient Refractory to Gefitinib and Responsive to Crizotinib, with Concurrent Rare Mutation of the Epidermal Growth Factor Receptor (L861Q) and Increased *ALK/MET/ROS1* Gene Copy Number

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CASE REPORT

A 77-year-old never-smoker woman, with a history of ischemic-hypertensive cardiopathy and diabetes, experiencing cough and dyspnea (performance status 2), underwent a total-body computed tomography (CT) scan, which showed a large left pulmonary mass with ipsilateral parenchymal nodules and pleural effusion (Fig. 1*A*).

Pathological examination of a tumor cell block obtained from fine-needle aspiration of a 3-cm–sized nodule in the left lower lobe showed malignant epithelial cells with adenocarcinoma structure (Fig. 2). Sequencing analysis of epidermal growth factor (*EGFR*) exons 18 to 21 upon genomic DNA extracted from the same paraffin-embedded material (3500-Dx Genetic Analyzer; Applied Biosystem Life Technologies, Carlsbad, CA) showed CTG-CAG point mutation (pL861Q) in exon 21.

Dual-color, break-apart fluorescent in situ *hybridization* (FISH) for *ALK* at 2p23 (*ALK* FISH DNA Probe; Split Signal, Dako, Glostrup, Denmark) and *ROS1* at 6q22 (ZytoLight SPEC *ROS1* Dual Color Break Apart Probe; ZytoVision, Bremerhaven, Germany) and dual-color FISH for *MET*/CEP7 at 7q31 (ZytoLight SPEC *MET*/CEN 7 Dual Color Probe Zytovision) did not show any rearrangement, but an increased GCG was observed in 61%, 67%, and 72% cancer cells, with 2.6, 2.6, and 2.9 mean signals per cell, respectively (Fig. 2).

The patient was started on gefitinib, 250 mg/day (September 18, 2012). Although an initial clinical benefit was observed (performance status 1), symptoms did rapidly worsen in the fifth to seventh weeks, and a CT scan (November 7, 2012) showed progressive pleural disease and two brain metastasis (7 and 3 mm) (Fig. 1*B*: lung, Fig. 1*C*: brain).

Thus Gefitinib was stopped and given the patient's performance and comorbidities that did not allow to start chemotherapy, she was addressed to receive crizotinib 250 mg/twice a day on the basis of *ALK*, *MET*, and *ROS1* increased GCN. After 4 weeks on crizotinib, a significant improvement of symptoms (cough and dyspnea) and performance status (0–1) was obtained. Treatment was well tolerated, except for a grade 1 skin rash and increase of transaminases. The last CT scan (February 25, 2013; Fig. 1*D*) and clinical evaluation (March 5, 2013) still confirm a stable disease after 4 months of crizotinib.

DISCUSSION

Only few cases of concurrent *EGFR* mutations (prevalently exon-19 deletions) and *ALK* rearrangement are currently on record.[1] These coalterations seem even more anecdotal when accounting for *EGFR* rare point-mutations in exon 21, such as L861Q, and the presence of *ALK* GCG, whose clinical meaning is largely unknown. It is already known that patients with *EGFR* L861Q mutation may not have the same clinical benefit from gefitinib and erlotinib treatment as patients with *classical EGFR* alterations, but may respond better to irreversible second-generation tyrosine kinase inhibitors (TKIs). Differently from the well-validated role of *ALK* translocation, no clinical reports describing a potential relationship between gene copy (GC) gain involving concurrently *ALK*, *MET*, and *ROS1* genes and response to crizotinib have been reported to date. Although this cytogenetic event could have played a role in rapidly establishing resistance to *EGFR*-TKI, in this study we speculate that *ALK*, *MET*, and *ROS1* GC gain could have been responsible for the success of the treatment with the relevant inhibitor,[2] despite the lack of the classical gene translocations, and may be correlated with response to erlotinib. For example, we already know that *MET* amplification can be a mechanism of resistance to *EGFR*-TKIs and may predict response to crizotinib. Also, we would like to stress the need to accurately describe all cytogenetic alterations regarding *ALK*, *ROS1*, and *MET* genes when signing out the molecular pathology reports, to offer oncologists additional tools for deciding therapy. Although recent data have suggested that even patients with *ALK* GC gain could benefit from crizotinib treatment, this is the first evidence indicating that concurrent

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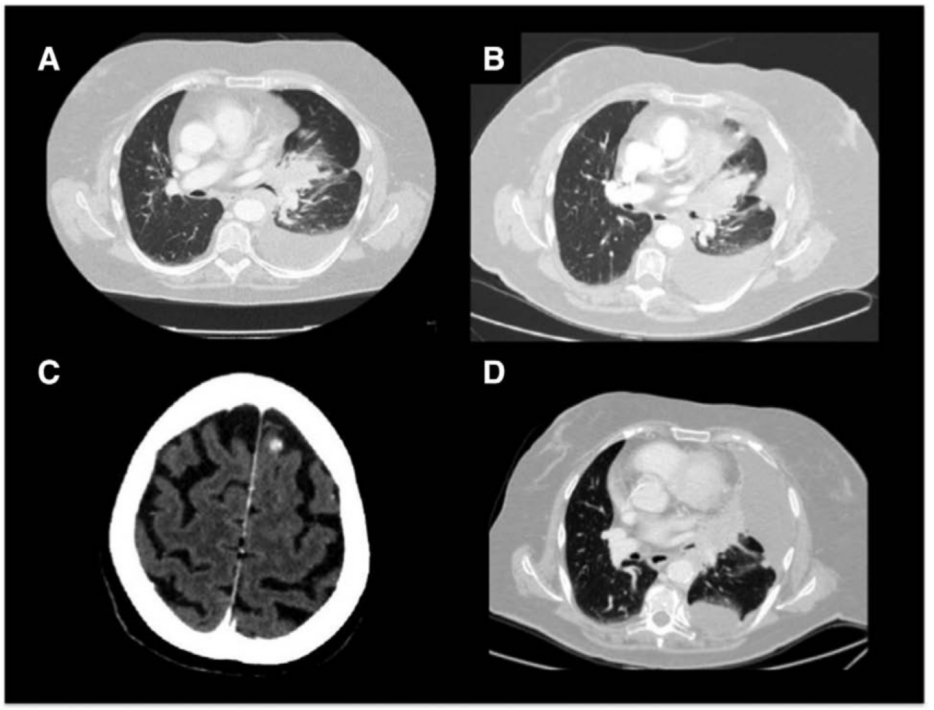

FIGURE 1. Radiological findings regarding different phases of the disease. *A*, Basal total-body computed tomography scan. *B–C*, Disease progression during treatment with gefitinib. *D*, Disease stability during treatment with crizotinib.

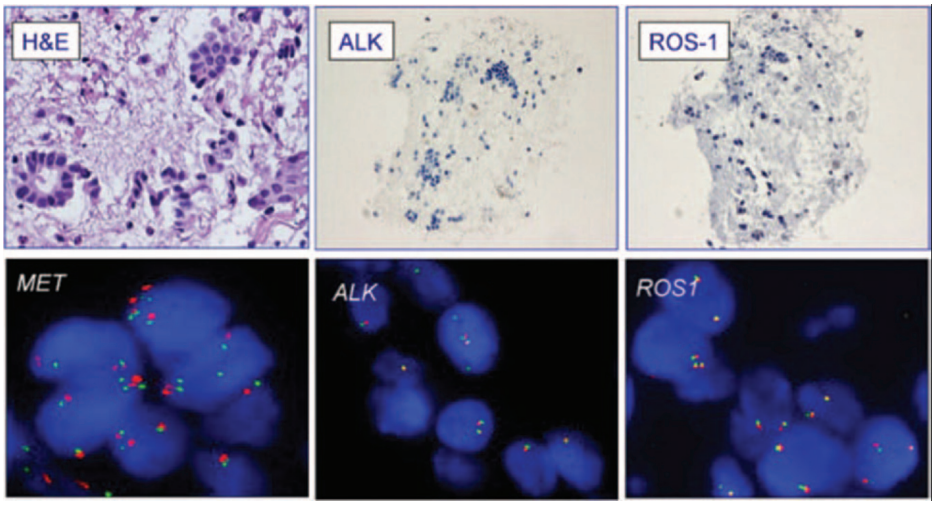

FIGURE 2. Cytologic examination showed an adenocarcinoma (H&E), which presented with an increased GCN for *ALK*, *MET*, and *ROS1* (immunohistochemistry and fluorescent in situ *hybridization* analysis). H&E, hematoxylin and eosin.

GC gain of *ALK*, *ROS1*, and *MET* may become a predictive biomarker of response to crizotinib beyond translocation or amplification, although further evidence is required to validate these preliminary data.

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