case reports

Durable Response to Combined Osimertinib and Pralsetinib Treatment for Osimertinib Resistance Due to Novel Intergenic *ANK3-RET* Fusion in *EGFR*-Mutated Non–Small-Cell Lung Cancer

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Introduction

Although most cases of metastatic non–small-cell lung cancer (NSCLC) harboring *EGFR* (ex19del or L858R) mutations respond to the EGFR tyrosine kinase inhibitor (TKI) osimertinib, they inevitably become resistant to this drug. The extent of progression-free survival depends on intrinsic or acquired on-target/off-target mechanisms of osimertinib resistance.[1-3] In this respect, oncogenic fusions of receptor tyrosine kinases, detectable in 1%-10% of cases of acquired osimertinib resistance, are particularly relevant, as several of them are druggable by specific TKIs.[3] Yet, although osimertinib-resistant cases with acquired *ALK* or *ROS1* fusions have shown objective response (OR) to well-established ALK/ROS1-TKIs,[4,5] the treatment and outcome of patients acquiring *RET* fusions during treatment with osimertinib are more difficult to envisage because of variable and rare fusion partners and limited experience with RET-TKIs in this setting. In this study, we describe a patient with disseminated *EGFR*-mutated (*EGFR+*) NSCLC, who progressed on second-line osimertinib acquiring *ANK3-RET* fusion (not reported before in this setting) together with *MDM2* amplification and *PTEN* mutation. Despite these significant genomic coalterations, the patient has been displaying sustained ongoing OR to the osimertinib-pralsetinib combination for more than 12 months, providing clinical evidence for *ANK3-RET* fusion as important, effectively targetable mechanism of osimertinib resistance.

Case Report

The patient, a 62-year-old White female, never-smoker, underwent intracranial surgery for suspected pituitary adenoma. Histopathological assessment of the pituitary lesion and targeted next-generation sequencing (NGS) analysis of single nucleotide variants (SNVs), indels, and copy number variations (CNVs) across 161 unique cancer-associated genes using the Oncomine Comprehensive Assay v3 (Thermo Fisher Scientific, Waltham, MA) revealed instead metastatic pulmonary adenocarcinoma (immunohistochemically CK7+/TTF1+) with the *EGFR* ex19del p.E746_A750delELREA and negative programmed death ligand-1-expression. Moreover, total-body positron emission tomography-computed tomography (PET-CT) scan detected a tumor in the left lung's upper lobe and multiple metastases in both lungs, mediastinal lymph nodes, and L2 vertebra, corresponding to T3N2M1c. Figure 1 summarizes the systemic treatments used for the patient.

Stereotactic radiosurgery against the pituitary metastasis was followed by systemic erlotinib treatment. Progression of primary tumor and mediastinal lymph nodal metastases was observed 15 months later. Lymph nodal biopsy and DNA-NGS analysis revealed the original *EGFR* ex19del and a new *EGFR* mutation p.T790M. The patient started second-line osimertinib 80 mg once daily. Plasma cell-free DNA (cfDNA) testing (Oncomine Lung cfDNA NGS assay; Thermo Fisher Scientific) at 4, 7, and 13 months of this treatment showed no *EGFR* mutations. However, two liver metastases were observed after 15 months. DNA-NGS analysis of the biopsy from one of them showed persistent *EGFR* ex19del and p.T790M mutations, which concomitantly reappeared in plasma cfDNA. Additionally, two putative resistance mechanisms, *MDM2* amplification (19 gene copies) and the missense *PTEN* substitution p.C124S (oncogenic mutation in PTEN phosphatase catalytic domain, according to COSMIC[6] and OncoKB databases[7]), were identified in this metastasis. Hence, no further targeted treatment options were deemed possible. The patient received three cycles of carboplatin-pemetrexed while continuing osimertinib but could not tolerate more chemotherapy because of bone marrow toxicity.

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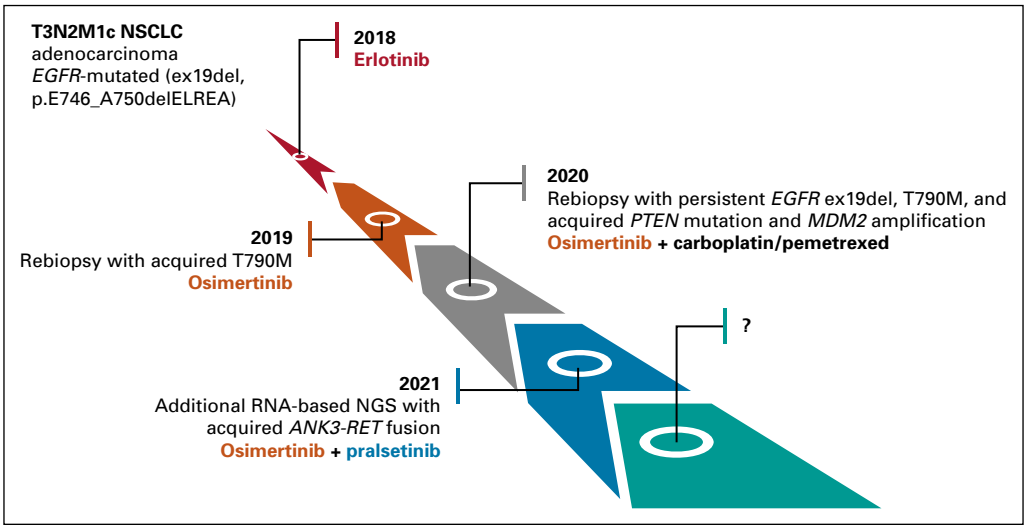

FIG 1. Tumor evolution illustrating multiclonality of the disease during progression and adopted treatments. As the patient is still responding to osimertinib-pralsetinib combination, the future molecular state of the disease remains to be explored (symbol ?). NGS, next-generation sequencing; NSCLC, non–small-cell lung cancer.

However, supplementary NGS analysis of RNA from the hepatic metastasis using the Archer FusionPlex Lung kit (ArcherDX Inc, Boulder, CO) showed *ANK3-RET* fusion on chromosome 10q with break points at positions chr10:61994446 and chr10:43612032, respectively (Fig 2). The Archer Analysis software v.6.2.1 predicted the fusion to be in frame and detected it in 71.5% of reads. According to COSMIC annotations,[6] both break points are outside the two genes' coding sequence (*RET*: chr10:43077027-43130351, positive strand; *ANK3*: chr10:60026298-60389730, negative strand). Furthermore, other genes are present on chromosome 10q between *ANK3* and *RET*, suggesting that the *ANK3-RET* fusion was intergenic and comprised *ANK3* exon 1-8 and *RET* exon 12-21 with completely preserved RET-kinase domain.

Retrospective NGS of RNA isolated from the pre-osimertinib biopsies (diagnostic pituitary biopsy and tumor rebiopsy at first intrathoracic progression) did not show the *ANK3-RET* fusion. This confirmed that the latter had been acquired during osimertinib treatment and could explain progression, as other *RET* fusions have been reported as mechanisms of acquired resistance to EGFR-TKIs.[4,8,9] Thus, RET-TKI treatment with pralsetinib 400 mg once daily combined with continuation of osimertinib 80 mg once daily was initiated. After 2 weeks, this combination was shortly paused because of

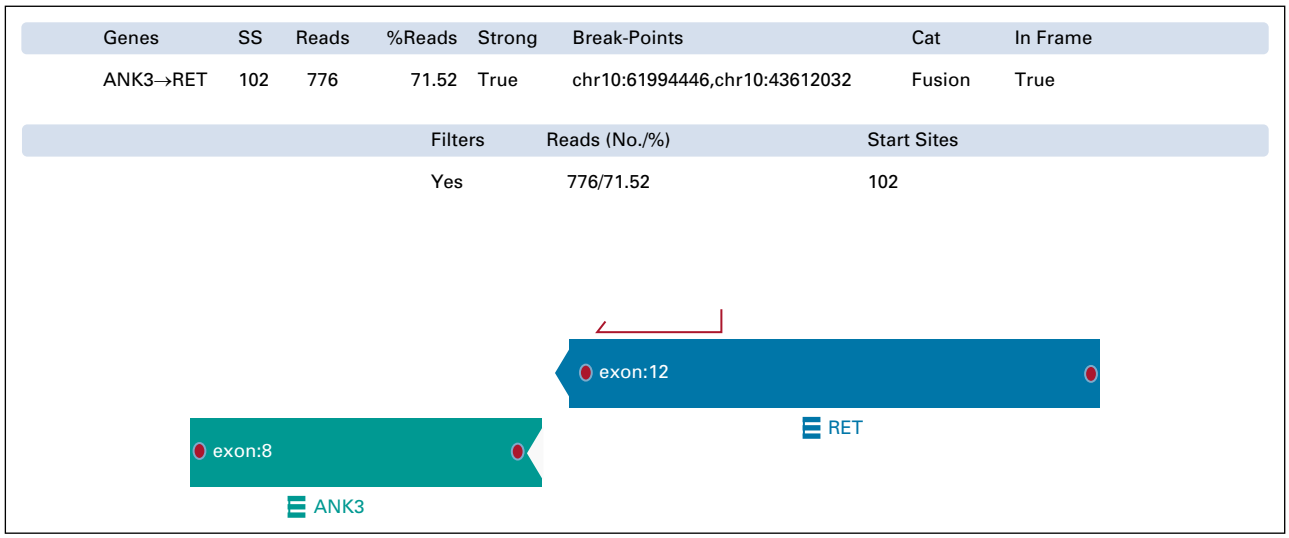

FIG 2. Intergenic *ANK3-RET* fusion on chromosome 10q (break point chr10:61994446, chr10:43612032) identified by RNA-NGS with an Archer FusionPlex Lung kit. SS, start sites.

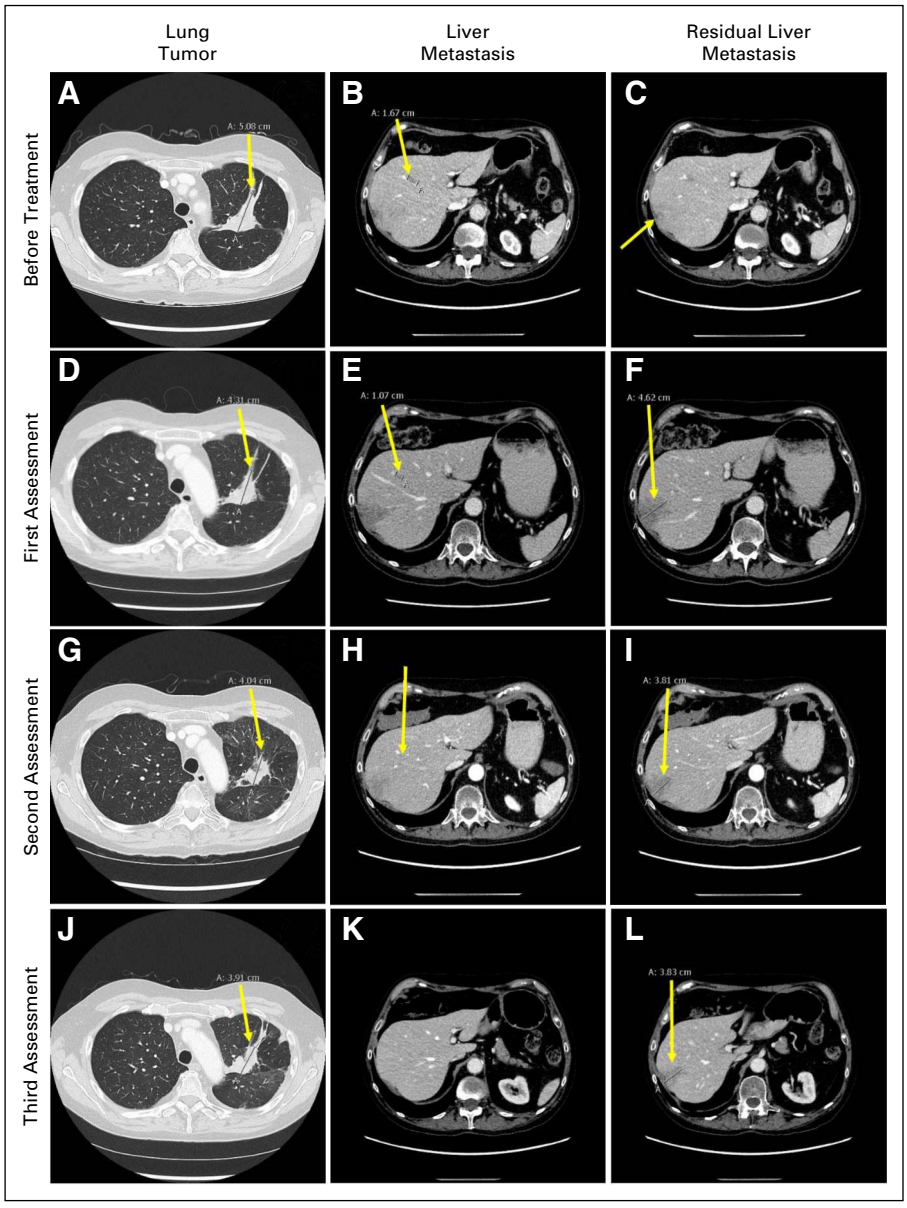

FIG 3. Response to combined treatment with osimertinib and pralsetinib. Before treatment: (A) lung tumor in left upper lobe (5.1 cm; arrow), (B) liver metastasis (1.7 cm; arrow), and (C) residual liver metastasis (nonmeasurable; arrow). First assessment: (D) lung tumor in left upper lobe (4.3 cm; arrow), (E) liver metastasis (1.1 cm; arrow), and (F) residual liver metastasis (4.6 cm; arrow). Second assessment: (G) lung tumor in left upper lobe (4.0 cm; arrow), (H) liver metastasis (nonmeasurable; arrow), and (I) residual liver metastasis (3.8 cm; arrow). Third assessment: (J) lung tumor in left upper lobe (3.9 cm; arrow), (K) liver metastasis (nonvisible), and (L) residual liver metastasis (3.8 cm; arrow).

interstitial pneumonia, which completely resolved on steroid therapy. The patient resumed the combined TKI treatment at reduced dose (osimertinib 40 mg once daily, pralsetinib 200 mg once daily) without experiencing additional adverse events. The first assessment performed 1 month later revealed a mixed response, with regression of the primary tumor, disappearance of one liver metastasis, and growth of the other one (Figs 3A-3C *v* Figs 3D-3F). At the second assessment 4 weeks later, further reduction of primary tumor and regression of both liver metastases were noticed (Figs 3G-3I). Stable disease (SD) in the chest and abdomen was observed after 5 months of treatment (Figs 3J-3L). The following assessments showed complete response in abdomen and slight progression of single pleural lesions, which were biopsied and displayed the *PTEN* mutation and *MDM2* amplification (34 gene copies), but not the pralsetinib-targeted *RET* fusion. Consequently, to control the pleural lesions, osimertinib dose was reincreased to 80 mg once daily, while pralsetinib 200 mg once daily was maintained. The last PET-CT, so far, performed after 12 months of treatment displayed persisting abdominal complete response and thoracic SD (data not shown). Accordingly, sequential plasma cfDNA did not show reappearance of *EGFR* mutations during the combination treatment.

The study was conducted according to the Declaration of Helsinki's guidelines and approved by the Danish Tumor Board Phase I and Lung Team Tumor Board (20200122) at Department of Oncology, Rigshospitalet, University of Copenhagen. Written informed consent for this report was obtained from the patient.

TABLE 1. Summary of Reported Cases Demonstrating Feasibility of Combined EGFR Tyrosine Kinase Inhibitor Plus RET Tyrosine Kinase Inhibitor Treatment for *EGFR*+ Non–Small-Cell Lung Cancer Patients With Acquired *RET* Fusions

Acquired *RET*-Fusion Type	Treatment Line (*EGFR* mutation type)	Treatment Used at Progression	Duration of Treatment	References
ANK3-RET	Second-line osimertinib (ex19del)	Osimertinib 40 mg once daily plus pralsetinib 200 mg once daily	12 months so far	Current report
CCDC6-RET	Third-line erlotinib (ex19del)	Cabozantinib 60 mg once daily plus erlotinib 150 mg once daily	3.5 months	9
CCDC6-RET	Second-line osimertinib (ex19del)	Osimertinib 80 mg once daily plus pralsetinib 300 mg once daily	4 months so far	9
NCOA4-RET	Second-line osimertinib (ex19del)	Osimertinib 80 mg once daily plus pralsetinib 400 mg once daily	4 months so far	9
CCDC6-RET	First-line osimertinib (ex19del)	Osimertinib 80 mg once daily plus selpercatinib 80 mg twice a day	6 months so far	13
CCDC6-RET (n = 5) *NCOA4-RET* (n = 4) *KIF5B-RET* (n = 2) *RUFY2-RET* (n = 1)	First-line or second-line osimertinib (11 with ex19del; one with L858R)	Osimertinib 80 mg daily (75%, range 40 mg once daily to 80 mg twice a day) plus selpercatinib 80 mg twice a day (92%, range 100 mg once daily to 120 mg twice a day)	Median 7.4 months so far	14
NCOA4-RET	First-line afatinib (L858R)	Afatinib 20 mg once daily plus cabozantinib 20 mg once daily	7 months so far	16

Discussion

Our finding of acquired *ANK3-RET* fusion as druggable mechanism of osimertinib resistance underlines the importance of including RNA-NGS for gene fusions in clinical practice to find resistance mechanisms and new targeted treatment options. Indeed, the use of complementary RNA-NGS in a prospective genomic profiling of 2,522 lung adenocarcinomas revealed 14% additional actionable gene fusions and *MET*ex14 alterations that had not been detected by DNA-NGS.[10] Since detecting *RET* fusions in plasma cfDNA is challenging, tumor rebiopsies, whenever feasible, should be performed in the setting of EGFR-TKI resistance to identify acquired fusions by RNA-NGS and histologic transformation. Progression on osimertinib due to acquired *RET* fusions has previously been reported and treated with different modalities, including continuing osimertinib beyond progression,[4,11] replacing it with the RET-targeting multi-TKI cabozantinib,[12] or combining osimertinib with RET-TKI (Table 1).[9,13,14] Although *RET* fusions develop more frequently during treatment with osimertinib than earlier-generation EGFR-TKIs,[4,11] a large-scale multicancer study identified patients with NSCLC acquiring *CCDC6-RET* (n = 10), *NCOA4-RET* (n = 4), or *TRIM24-RET* (n = 1) fusions at progression on first/second-generation EGFR-TKI, without though reporting any combined EGFR-TKI-RET-TKI therapy for these patients.[15] In another study including 3,505 *EGFR*+ NSCLCs, six cases acquiring *RET* fusions with *CCDC6*, *NCOA4*, or *TRIM24* during treatment with erlotinib or afatinib were detected.[16] One of these cases harboring *NCOA4-RET* fusion as resistance mechanism to afatinib responded with SD for 7 months to afatinib-cabozantinib combination therapy.[16] In a group of 41 patients progressing on second-line osimertinib, two with acquired *CCDC6-RET* and one with *NCOA4-RET* fusion were identified and treated with the osimertinib-pralsetinib combination obtaining an ongoing OR of 4 months.[9] Also combining osimertinib with another RET-TKI, selpercatinib, was effective for a group of *EGFR*+ NSCLC patients with acquired *NCOA4-RET*, *CCDC6-RET*, *KIF5B-RET*, or *RUFY2-RET* fusions, as the ORR was 50% and median duration of treatment 7.4 months.[14] Our case, after progressing on second-line osimertinib, acquired a *RET* fusion with *ANK3*, an unreported partner in this setting. After combining osimertinib with pralsetinib, we have obtained an ongoing OR that so far has been maintained for more than 12 months, which, to our knowledge, is the longest reported until now with osimertinib-RET-TKI combinations (Table 1).[13,14] The efficacy of these combinations against osimertinib resistance linked to *ANK3-RET* fusion needs confirmation in additional cases. Nonetheless, the observations summarized in Table 1 strongly indicate that combining osimertinib with a RET-TKI may counteract osimertinib resistance mediated by different *RET* fusions. It is also noteworthy that, after initial interruption of the osimertinib-pralsetinib therapy at standard dose because of pulmonary toxicity, we have been able to safely resume and continue the combination at reduced dose of both drugs for 9 months, still obtaining a durable OR. Afterward, we have reincreased osimertinib to standard dose to control the disease in the chest without causing toxicity. These results may be relevant for patients with acquired resistance mechanisms requiring dual targeted therapy with the risk of overlapping toxicity, as they may still benefit, at least for some time, from a dose reduction of the combined drugs.

As DNA-NGS identified *MDM2* amplification and *PTEN* mutation as putative mechanisms of osimertinib resistance and our patient's permanent steroid replacement for hypophysis insufficiency as well as *EGFR*+ NSCLC were contraindications for immunotherapy,[17] only chemotherapy could be offered to her. However, complementary RNA-NGS revealed the acquired *ANK3-RET* fusion, allowing further targeted treatment with combined osimertinib-pralsetinib. Hitherto, 48 different *RET* fusions have been reported in NSCLC,[18] both as de novo cancer drivers in *EGFR* wild-type cases and as acquired mechanisms of resistance to osimertinib and other EGFR-TKIs.[1,3,9,18,19] Notably, de novo *ANK3-RET* fusion has previously been identified in *EGFR* wild-type NSCLC, although without any information on response to RET-TKIs.[20,21] To our knowledge, this is the first report of this fusion as acquired osimertinib-resistance mechanism that can be effectively counteracted by combining pralsetinib with osimertinib continuation. Our report also adds clinically relevant information to the list of intergenic *RET* rearrangements in NSCLC.[18]

The *PTEN* mutation and *MDM2* amplification co-occurring in our patient may contribute to TKI resistance.[1] The *PTEN* substitution has not been described before under osimertinib treatment and may function as parallel resistance mechanism alongside the *RET* fusion, as the PTEN tumor suppressor inhibits the phosphatidylinositol 3-kinase/protein kinase B/mammalian target of rapamycin pathway. The concomitant high-level *MDM2* amplification may also promote osimertinib resistance, as MDM2 functions as an E3 ubiquitin ligase targeting the p53 tumor suppressor for proteolysis and antagonizing its transcriptional activity.[22] Indeed, coexisting *MDM2* amplification is associated with worse outcome in EGFR-TKI–treated *EGFR*+ NSCLC.[23] Neither *PTEN* mutation nor *MDM2* amplification were found in the diagnostic biopsy or the first rebiopsy (Fig 1); thus, we considered them as acquired TKI-resistance mechanisms that, together with the *ANK3-RET* fusion, reflect the clonal heterogeneity of advanced *EGFR*+ NSCLC at progression on TKIs.[24] Despite preclinical attempts to target MDM2 overexpression and PTEN inactivation,[25,26] these alterations remain undruggable in clinical practice. Yet, despite such coalterations, our patient's disseminated NSCLC has been exhibiting a prolonged, ongoing, clinically meaningful response to the pralsetinib-osimertinib combination, further revealing the *ANK3-RET* fusion as crucial, targetable mechanism of osimertinib resistance.

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PRIOR PRESENTATION

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DATA SHARING STATEMENT

The data from the study will be made available by the authors on request.

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Provision of study materials or patients: All authors
Collection and assembly of data: All authors
Data analysis and interpretation: All authors
Manuscript writing: All authors
Final approval of manuscript: All authors
Accountable for all aspects of the work: All authors

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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