case report

Acquired *ALK* and *RET* Gene Fusions as Mechanisms of Resistance to Osimertinib in *EGFR*-Mutant Lung Cancers

Michael Offin
Romel Somwar
Natasha Rekhtman
Ryma Benayed
Jason C. Chang
Andrew Plodkowski
Allan J.W. Lui
Juliana Eng
Marc Rosenblum
Bob T. Li
Gregory J. Riely
Charles M. Rudin
Mark G. Kris
William Travis
Alexander Drilon
Maria E. Arcila
Marc Ladanyi
Helena A. Yu

Author affiliations and support information (if applicable) appear at the end of this article.

Corresponding author: Helena A. Yu, MD, Memorial Sloan Kettering Cancer Center, 300 E 66th St, New York, NY 10065; e-mail: yuh@mskcc.org.

INTRODUCTION

Approximately 20% of patients with metastatic lung adenocarcinoma have somatic activating mutations in the epidermal growth factor receptor (EGFR) gene, *EGFR*.[1] Patients with *EGFR*-mutant lung adenocarcinomas have a 70% response rate to first-line EGFR–tyrosine kinase inhibitor (TKI) therapy (ie, erlotinib, gefitinib, or afatinib).[2] *EGFR* T790M is the dominant resistance mechanism to earlier-generation EGFR-TKIs.[3] Osimertinib is approved by the US Food and Drug Administration for the treatment of *EGFR*-mutant lung cancers that have an acquired *EGFR* T790M after failure of a previous EGFR-TKI[4] and now is approved in the first-line setting as well.[5]

Response to osimertinib eventually is followed by progression with known resistance mechanisms, including small-cell transformation[3,6]; acquired *EGFR* mutations, including G796/C797, L792 and L718/G719[7]; and non-*EGFR*–mediated resistance, including alterations/amplification in *MET*, *HER2*, *BRAF*, *MEK*, *KRAS*, and *PIK3CA*.[8-11] There have been rare reports of acquired fusions, including *RET*, *BRAF*, and *FGFR*, as mechanisms of resistance to EGFR-TKI.[12,13] This case series capitalizes on multimodality molecular analyses, which include next-generation sequencing (NGS) with Memorial Sloan Kettering Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT)[1,14] and ArcherDx (Boulder, CO)[15] platforms, immunohistochemistry (IHC), and fluorescent in-situ hybridization (FISH), to evaluate three cases of acquired resistance[16] to osimertinib: one acquired *RET* fusion and two *ALK* rearrangements.

ACQUIRED *ALK* REARRANGEMENTS IN *EGFR*-MUTANT LUNG CANCER AFTER OSIMERTINIB

Case 1

A 65-year-old woman, who was a former 9–pack-year smoker, presented with a 3 cm × 2 cm lingular primary mass, hilar adenopathy, and a liver lesion (staging: T2pN2cM1b). The biopsy revealed adenocarcinoma with a 15-bp *EGFR* exon 19 deletion (exon19del). The patient received erlotinib and continued this treatment for 19 months before progression occurred. Plasma circulating tumor DNA (ctDNA) and the tumor biopsy were positive for *EGFR* T790M; notably, NGS showed no *ALK* rearrangement. The patient transitioned to treatment with osimertinib and necitumumab (ClinicalTrials.gov identifier NCT02496663)[17] for 9 months until progression developed in the lungs. A biopsy noted an adenocarcinoma with IHC results positive for *ALK* and *EGFR* exon19del (Fig 1). NGS with ArcherDx and MSK-IMPACT confirmed an acquired *EML4-ALK* fusion (*EML4* exons 1 through 6 fused with *ALK* exons 20 through 29; c.667+516:*EML4*_c.3173-415:*ALK*inv) in addition to the *EGFR* exon19del and T790M mutations. The patient started combination treatment with osimertinib 80 mg daily and crizotinib 200 mg twice daily, remained on treatment with stable disease (by RECIST version 1.1), and continued to receive clinical benefit from treatment, with no report of toxicity. Subsequent imaging demonstrated oligoprogression in the target lesion with stable disease in nontarget lesions. The patient underwent radiation to the oligoprogressive site and has remained on combination therapy with continued disease control. (Figs 2A and 3B; Appendix Table A1).

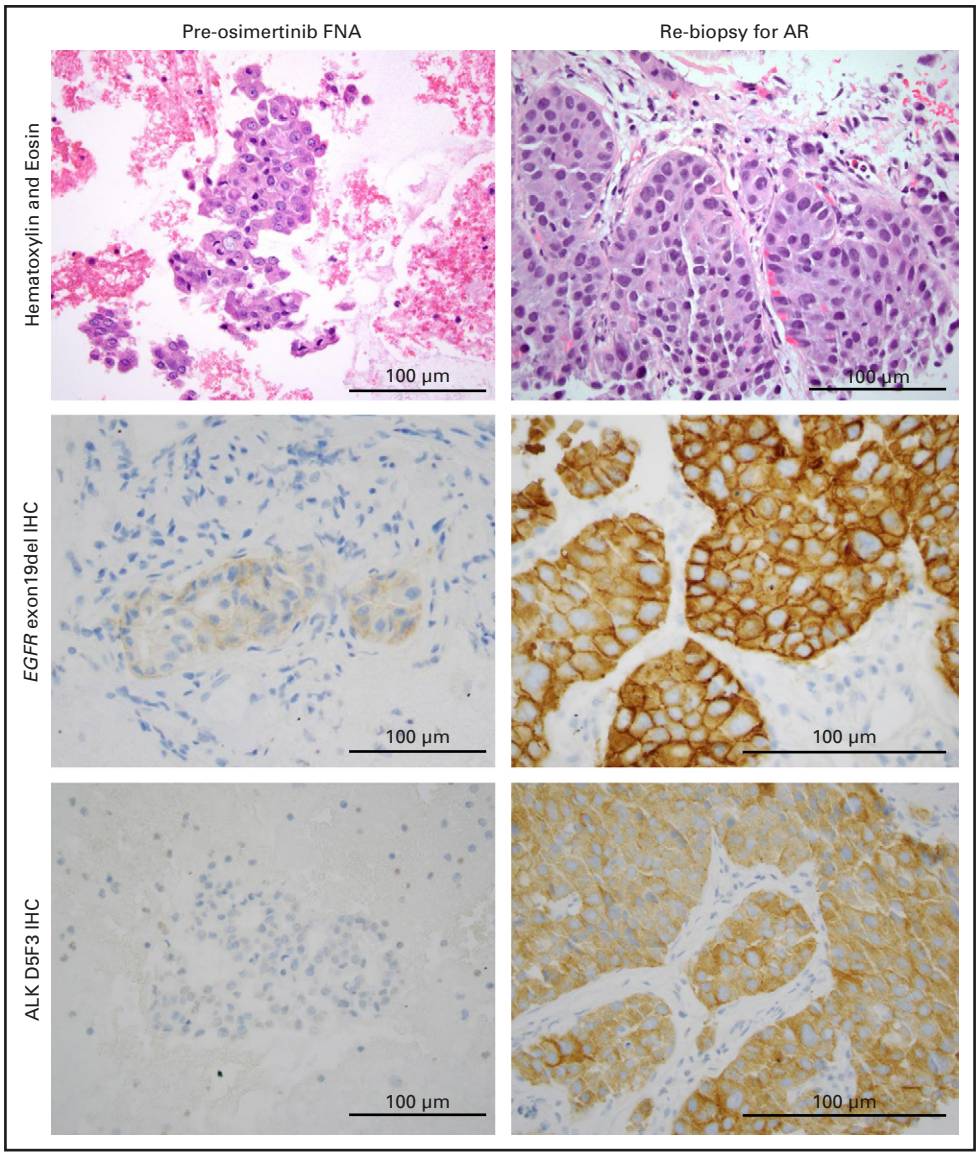


Fig 1. Representative immunohistochemistry (IHC) of the pre- and post-osimertinib biopsies for patient case 1 at ×20 magnification. The preosimertinib biopsy was a fine-needle aspiration (FNA) and was partially fragmented on staining, whereas the acquired resistance (AR) sample was a core-needle biopsy. The pretreatment *EGFR* exon19del staining was performed with epidermal growth factor receptor (EGFR)–E746; the post-treatment IHC stained more diffusely for *EGFR* exon19del, likely related to the *EGFR* amplification (fold change: 3.0) noted on next-generation sequencing with MSK-IMPACT. The post-treatment biopsy showed new staining for *ALK* (clone D5F3) compared with the pretreatment sample, which was negative. The IHC data suggest the presence of the *ALK* rearrangement and *EGFR* mutation within the same cell (however, this cannot be confirmed by targeted next-generation sequencing because of technical limitations).

Case 2

A 68-year-old woman, who was never a smoker, presented with a 9-cm right apical lung mass, right hilar lymphadenopathy, and a left temporal lobe mass (staging T4N1M1b). Biopsy of the lung mass demonstrated adenocarcinoma, and ctDNA showed *EGFR* L858R (insufficient tissue for IHC). She started treatment with erlotinib and continued it for 15 months until she experienced progression in a right supraclavicular lymph node. A biopsy revealed an *EGFR* T790M mutation on digital polymerase chain reaction, and NGS confirmed *EGFR* L858R and T790M mutations. (Of note, no *ALK* rearrangement was detected on NGS or FISH.) The patient started treatment with osimertinib and had an initial disease response, but oligoprogression occurred in the lung after 6 months. A lung biopsy found *EGFR L858R* mutation and *ALK* positivity on IHC. MSK-IMPACT confirmed the known *EGFR*

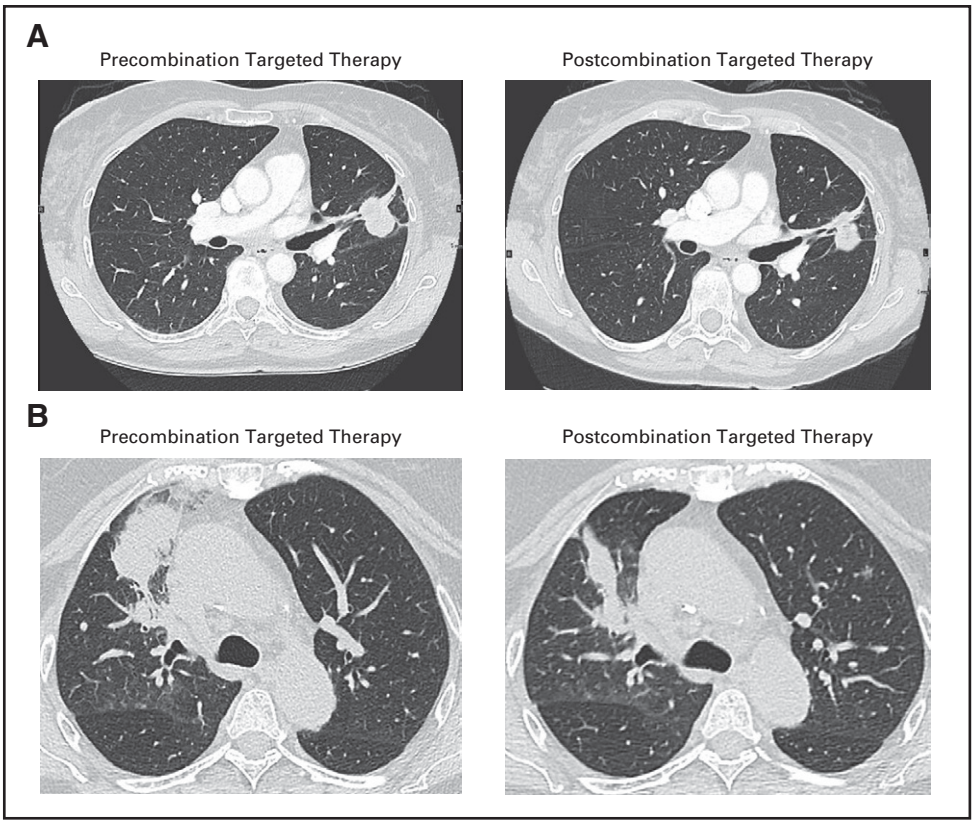


Fig 2. Representative radiologic images that illustrate the response to combination therapy in patients with acquired *ALK*-rearranged disease as evaluated by RECIST version 1.1. (A) Osimertinib and crizotinib in patient case 1 after one cycle (28 days) of treatment; image shows overall stable disease (0% best response). (B) Osimertinib and alectinib in patient case 2 after one cycle (28 days) of treatment; image shows regression of the dominant right upper lobe mass (25% reduction).

L858R and T790M mutations as well as the new *EML4-ALK* rearrangement (*EML4* exons 1 through 2 fused to *ALK* exons 20 through 29; c.208+4890:*EML4*_c.3173-373:*ALK*inv; Fig 3B; Appendix Table A2). The patient started treatment with alectinib 300 mg twice daily and osimertinib 80 mg daily; the first interval scan showed a decrease in the dominant right lung mass (25% reduction by RECIST version 1.1) as well as ongoing clinical benefit and no report of toxicity at the last follow-up. (Fig 2B).

ACQUIRED *RET* REARRANGEMENT IN *EGFR*-MUTANT LUNG CANCER AFTER OSIMERTINIB

Case 3

A 78-year-old man, who was never a smoker, presented with a 4-cm right lung mass and a liver lesion. A biopsy of the lung revealed adenocarcinoma; IHC was positive for *EGFR* L858R (staging T2aN2M1b), and this was confirmed by polymerase chain reaction. He started treatment with erlotinib and continued it for 29 months until progression developed in the lungs. A subsequent biopsy showed his original *EGFR* L858R and a newly acquired L747S mutation. He started combination treatment with carboplatin and pemetrexed along with palliative radiation to the right middle lobe. A repeat biopsy then showed an acquired *EGFR* T790M mutation in addition to *EGFR* L747S and L858R mutations, and he started osimertinib. The biopsy tissue was retrospectively analyzed with ArcherDx and was negative for gene fusion products. The patient developed oligoprogression after 16 months of osimertinib treatment and underwent local radiation. A right lung biopsy showed the known *EGFR* L747S and L858R mutations as well as a new *NCOA4-RET* fusion on MSK-IMPACT; the fusion was confirmed by ArcherDx. The patient has remained on osimertinib treatment as a result of the slow, asymptomatic progression (Fig 3A; Appendix Table A3).

EXPRESSION OF *RET* REARRANGEMENTS IN AN *EGFR*-MUTANT CELL LINE RESULTS IN OSIMERTINIB RESISTANCE

To determine if *RET* rearrangements confer resistance to osimertinib, we expressed two *RET* fusions in PC9 cells (del19 *EGFR*; ATCC, Manassas, VA) and generated stable cell lines using previously described techniques.[18] *CCDC6-RET* was chosen because of prior work,

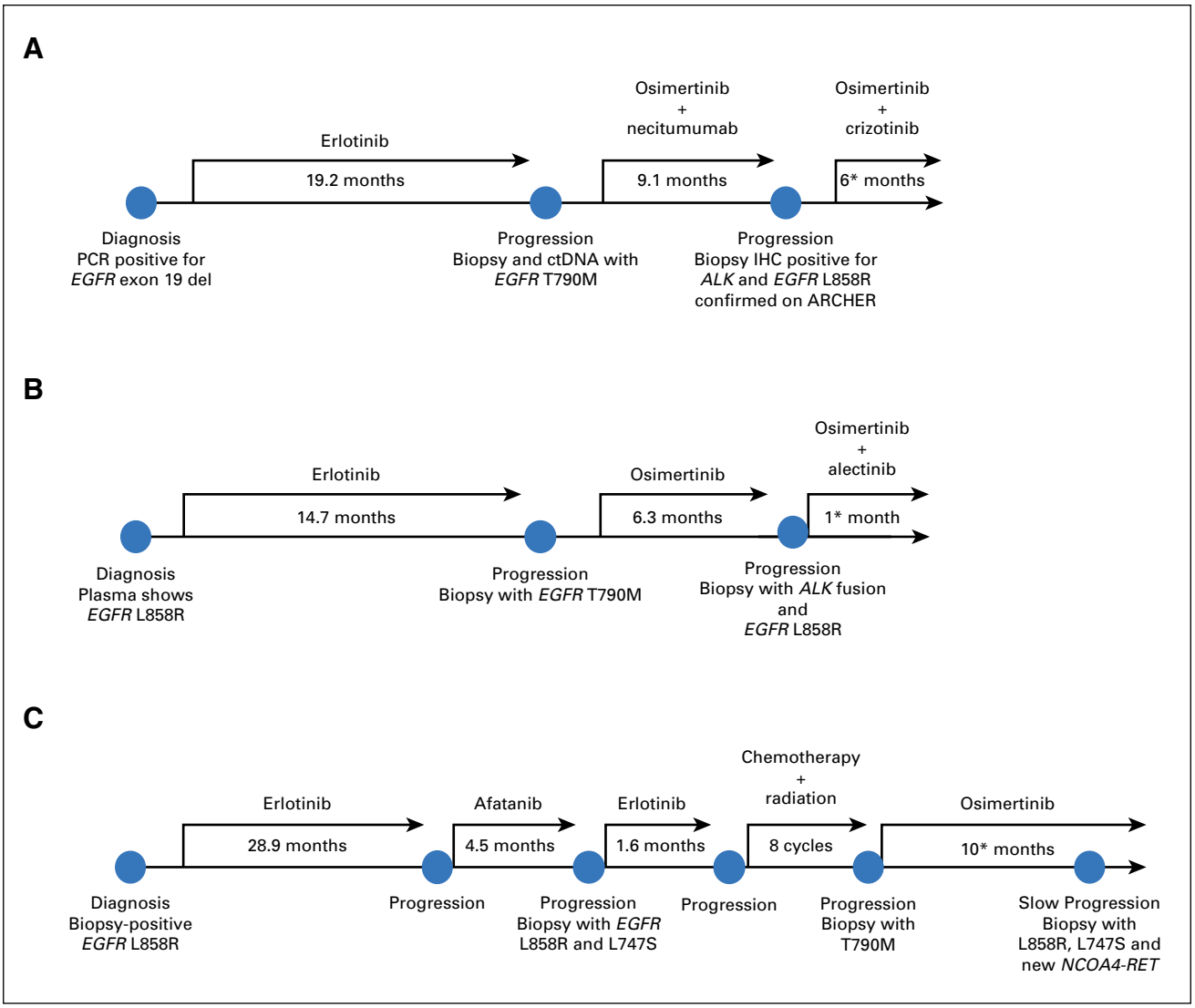
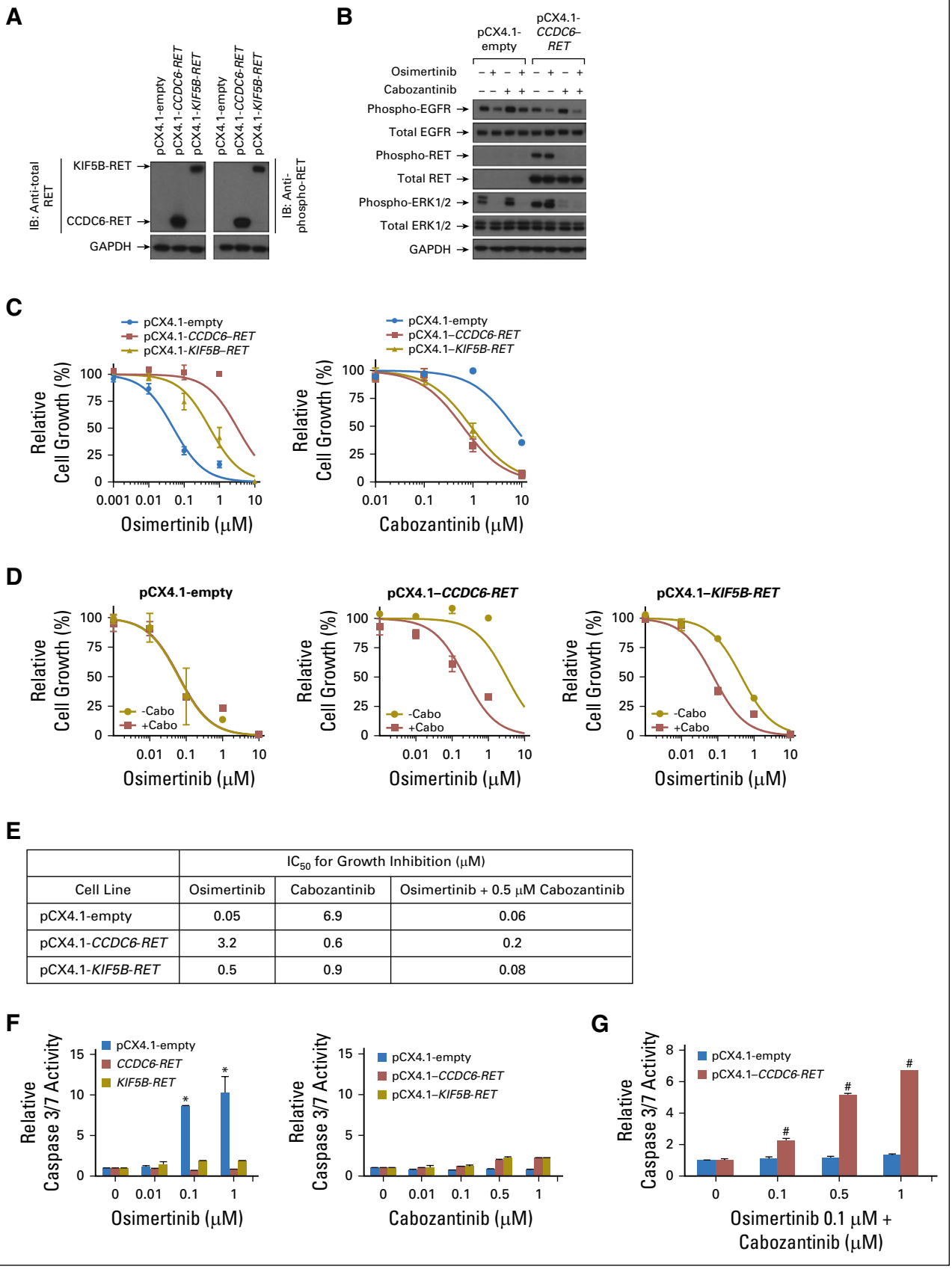


Fig 3. Description of clinical course and pertinent molecular and immunohistochemical findings for the three patient cases presented. (*) Treatment ongoing as of April 1, 2018.

which showed that the fusion partner did not influence *RET* activity in cell lines.[19,20] Each condition was assayed in triplicate in at least two independent experiments. The PC9 cells were used to assess the effect of *RET* fusions on sensitivity of growth and apoptosis (caspase 3/7 activity) in the presence of osimertinib and cabozantinib. Expression of RET and phosphorylated RET were confirmed via Western blot (Fig 4A). Osimertinib treatment reduced *EGFR* and *ERK1/2* phosphorylation in PC9-pCX4.1–empty cells (Fig 4B). However, *ERK1/2* phosphorylation was not sensitive to osimertinib treatment in PC9-pCX4.1–*CCDC6-RET* cells but was diminished with cabozantinib treatment (Fig 4B). The presence of a *RET* fusion did not alter the ability of osimertinib to inhibit *EGFR* phosphorylation (Fig 4B). These results suggest that *RET* rearrangements can cause bypass activation of growth-promoting pathways in cells that express oncogenic *EGFR*.

Growth of PC9 cells that stably expressed *CCDC6-RET* or *KIF5B-RET* rearrangements was at least 10-fold less sensitive to osimertinib than PC9-pCX4.1–empty cells were (50% inhibitory concentrations described in Fig 4E). PC9 cells that expressed *RET* fusions acquired sensitivity to cabozantinib (Figs 4C [right panel] and 4E) when compared with PC9-pCX4.1–empty cells. Sensitivity to osimertinib was restored



E

	IC_{50} for Growth Inhibition (μM)		
Cell Line	Osimertinib	Cabozantinib	Osimertinib + 0.5 μM Cabozantinib
pCX4.1-empty	0.05	6.9	0.06
pCX4.1-*CCDC6-RET*	3.2	0.6	0.2
pCX4.1-*KIF5B-RET*	0.5	0.9	0.08

when cells were treated with cabozantinib and osimertinib (Fig 4D). Expression of *RET* fusions in PC9 cells prevented osimertinib-induced activation of caspase 3/7, similar to the effect on growth rate (Fig 4F [left panel]). Treatment of control-group PC9 cells or PC9-*RET* cells with cabozantinib did not lead to activation of caspase 3/7 (Fig 4F [left panel]). However, a combination of cabozantinib and osimertinib led to caspase 3/7 activation in PC9-pCX4.1–*CCDC6-RET* cells (Fig 4G). In contrast, cabozantinib treatment did not have an additive effect on osimertinib-induced caspase 3/7 activity in PC9-pCX4.1–empty cells (Fig 4G). These results suggest that *RET* rearrangements can induce resistance to osimertinib in cells that have *EGFR* mutations and that response to osimertinib is restored when it is used in combination with cabozantinib.

In conclusion, osimertinib is now a first-line treatment for metastatic *EGFR*-mutant lung cancers.[5] Although potential resistance mechanisms to osimertinib have been identified, these have primarily been observed after later-line osimertinib in the setting of *EGFR* T790M mutations and have focused on ctDNA.[9-11] Many alterations identified as potential resistance mechanisms are seen concurrently with *EGFR* in pretreatment samples, including amplifications of *MET* and *HER2* as well as *PIK3CA* mutations, which makes the comparison with pretreatment tissue critical to identify truly acquired alterations.[13] As NGS becomes standard of care, it is feasible and fruitful to molecularly profile tumors broadly before treatment and at progression to identify acquired alterations that mediate resistance.

It remains unclear whether mechanisms of resistance to first-line osimertinib will differ from mechanisms of resistance to later-line osimertinib or earlier-generation EGFR-TKIs. Osimertinib is a potent mutant-EGFR inhibitor that inhibits *EGFR* T790M, so there is a potential to see novel on-target[21,22] and off-target[23-25] resistance mechanisms. This is corroborated by the low frequency (15% to 25%) of *EGFR* C797S and other acquired *EGFR* alterations after osimertinib use compared with 60% frequency of acquired *EGFR* T790M after use of earlier-generation EGFR-TKIs.[9,13,22] We may see new acquired alterations not previously seen or seen rarely with earlier-generation EGFR-TKIs. Our functional studies support the notion that acquired *RET* fusions can overcome the inhibitory effect of osimertinib by sustained activation of proliferation through MAPK signaling. Moreover, although cabozantinib can inhibit growth of *EGFR*- and *RET*-mutant/rearranged cell lines, a combination of osimertinib and cabozantinib induced apoptosis.

At our institution, among 174 patients with *EGFR*-mutant lung cancer in whom NGS was performed on tumor tissue after progression developed during treatment with erlotinib or afatinib, we found no *ALK* or *RET* fusions. To date, we have obtained biopsies from a small sample of patients (n = 14) after progression developed during osimertinib treatment; we found two *ALK* fusions and one *RET* fusion, which suggests a difference in the prevalence of these fusions in these two acquired resistance settings (Fisher's exact test $P < .001$). It is unknown whether this potential enrichment of acquired fusions is related to the more potent EGFR inhibition of osimertinib or to the later-line setting after multiple lines of EGFR inhibition. To our knowledge, this is the first report to document

Fig 4. Expression of *RET* fusions that induce resistance to osimertinib with a concomitant increase in sensitivity to cabozantinib. PC9 cells were infected with lentivirus that harbored an empty plasmid (pCX4.1-empty) or pCX4.1 with either *CCDC6-RET* or *KIF5B-RET* complementary DNA (cDNA), and cells that expressed the plasmids were selected to generate stable cell lines. (A) Cell extracts were immunoblotted (indicated by IB) for (left panel) total RET (left panel) or (right panel) phosphorylated RET. (B) Cell lines were treated with either osimertinib 0.05 μM or cabozantinib or 0.25 μM for 1 h; cell extracts were prepared and then immunoblotted for the indicated proteins. (C) Cells were treated with increasing concentrations (as indicated on the figure) of (left panel) osimertinib or (right panel) cabozantinib for 96 h, and then growth was determined. (D) Cells were treated with the indicated concentrations of osimertinib in the presence of cabozantinib 0.5 μM for 96 h, and then growth was determined. (E) 50% inhibitory concentration (IC_{50}) values were determined by nonlinear regression of growth data. (F) Cells were treated with the indicated concentrations of (left panel) osimertinib or (right panel) cabozantinib for 48 hours, and then caspase 3/7 enzymatic activity determined. (G) PC9 cells that expressed either pCX4.1-empty vector or pCX4.1–*CCDC6-RET* were treated with the indicated concentrations of cabozantinib in the presence of osimertinib 0.1 μM for 48 h, and then caspase 3/7 enzymatic activity was determined. Results are expressed as the fold change in caspase 3/7 activity compared with the corresponding cell line treated with osimertinib 0.1 μM alone. All experiments were conducted at least two times, and data represent the mean ± standard deviation. (*) $P < .05$ compared with untreated control. (†) $P < .05$ compared with the corresponding cell line treated with osimertinib 0.1 μM only. All data were analyzed by two-way Anova with the Tukey multiple comparison test. GAPDH, glyceraldehyde 3-phosphate dehydrogenase; –cabo, without cabozanitinib; +cabo, with cabozanitinib.

the clinical benefit of osimertinib combined with another agent to target an acquired mutation in a different oncogene. After the dominant resistance mechanisms to osimertinib are identified, prospective testing of combination therapies will be needed in the first-line setting to prevent or delay resistance and in the second-line setting to reverse or overcome acquired resistance.

DOI: https://doi.org/10.1200/PO.18.00126
Published online on ascopubs.org/journal/po on September 4, 2018.

AUTHOR CONTRIBUTIONS:

Conception and design: Michael Offin, Romel Somwar, Gregory J. Riely, Mark G. Kris, William Travis, Alexander Drilon, Maria E. Arcila, Marc Ladanyi, Helena A. Yu

Collection and assembly of data: Michael Offin, Romel Somwar, Natasha Rekhtman, Ryma Benayed, Jason C. Chang, Andrew Plodkowski, Allan J.W. Lui, Juliana Eng, Marc Rosenblum, Bob T. Li, Gregory J. Riely, Mark G. Kris, William Travis, Alexander Drilon, Maria E. Arcila, Helena A. Yu

Provision of study material or patients: Natasha Rekhtman, Juliana Eng, Bob T. Li, Charles M. Rudin, Mark G. Kris, William Travis, Helena A. Yu

Data analysis and interpretation: Michael Offin, Romel Somwar, Natasha Rekhtman, Allan J.W. Lui, Marc Rosenblum, Bob T. Li, Charles M. Rudin, Mark G. Kris, William Travis, Alexander Drilon, Maria E. Arcila, Marc Ladanyi, Helena A. Yu

Administrative support: Mark G. Kris

Financial support: Mark G. Kris

Manuscript writing: All authors

Final approval of manuscript: All authors

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The following represents disclosure information provided by authors of this manuscript. All relationships are considered compensated. Relationships are self-held unless noted. I = Immediate Family Member, Inst = My Institution. Relationships may not relate to the subject matter of this manuscript. For more information about ASCO's conflict of interest policy, please refer to www.asco.org/rwc or ascopubs.org/po/author-center.

Michael Offin
No relationship to disclose

Romel Somwar
Research Funding: Helsinn Healthcare
Travel, Accommodations, Expenses: Helsinn Healthcare

Natasha Rekhtman
No relationship to disclose

Ryma Benayed
No relationship to disclose

Jason C. Chang
No relationship to disclose

Andrew Plodkowski
No relationship to disclose

Allan J.W. Lui
No relationship to disclose

Juliana Eng
No relationship to disclose

Marc Rosenblum
No relationship to disclose

Bob T. Li
Consulting or Advisory Role: Roche, BIosceptre International, ThermoFisher Scientific, Mersana, Guardant Health
Research Funding: Roche (Inst), Genentech (Inst), Illumina (Inst), BioMed Valley Discoveries (Inst), AstraZeneca (Inst), GRAIL (Inst)

Gregory J. Riely
Research Funding: Novartis (Inst), Roche (Inst), Genentech (Inst), Millenium (Inst), GlaxoSmithKline (Inst), Pfizer (Inst), Infinity Pharmaceuticals (Inst), ARIAD (Inst)
Patents, Royalties, Other Intellectual Property: Patent application submitted covering pulsatile use of erlotinib to treat or prevent brain metastases (Inst)
Travel, Accommodations, Expenses: Merck Sharp & Dohme

Charles M. Rudin
Consulting or Advisory Role: Bristol-Myers Squibb, Abbvie, Seattle Genetics, Harpoon Therapeutics, Genentech, Roche, AstraZeneca

Mark G. Kris
Consulting or Advisory Role: AstraZeneca, Regeneron

William Travis
Speakers' Bureau: Genentech

Alexander Drilon
Consulting or Advisory Role: Ignyta, Loxo, TP Therapeutics, AstraZeneca, Pfizer, Blueprint Medicines, Genentech, Roche, Takeda, Helsinn Therapeutics, BeiGene

Maria E. Arcila
Consulting or Advisory Role: AstraZeneca
Travel, Accommodations, Expenses: AstraZeneca, Invivoscribe, Raindance Technologies

Marc Ladanyi
Honoraria: Merck (I)
Consulting or Advisory Role: National Comprehensive Cancer Network, Boehringer Ingelheim, AstraZeneca (Tagrisso RFP Advisory Committee)
Research Funding: Loxo (Inst)

Helena A. Yu
Consulting or Advisory Role: AstraZeneca
Research Funding: Astellas Pharma, Incyte, Lilly Oncology, Novartis, Daiichi, AstraZeneca

Affiliations

All authors, Memorial Sloan Kettering Cancer Center; **Michael Offin**, **Juliana Eng**, **Bob T. Li**, **Gregory J. Riely**, **Charles M. Rudin**, **Mark G. Kris**, **Alexander Drilon**, and **Helena A. Yu**, Weill Cornell Medical College, New York, NY; and **Allan J.W. Jui**, The Chinese University of Hong Kong, Sha Tin New Town, Hong Kong Special Administrative Region, China.

Support

Supported in part by the National Cancer Institute of the National Institutes of Health Grants No. T32 CA009207 and P30 CA008748.

REFERENCES

1. Jordan EJ, Kim HR, Arcila ME, et al: Prospective comprehensive molecular characterization of lung adenocarcinomas for efficient patient matching to approved and emerging therapies. Cancer Discov 7:596-609, 2017
2. Jänne PA, Wang XF, Socinski J, et al: Randomized phase II trial of erlotinib (E) alone or in combination with carboplatin/paclitaxel (CP) in never or light former smokers with advanced lung adnocarcinoma: CALGB 30604 trial. J Clin Oncol 30:2063-2069, 2012.
3. Yu H, Arcila ME, Rekhtman N, et al: Analysis of mechanisms of acquired resistance to EGFR TKI therapy in 155 patients with EGFR-mutant lung cancers. Clin Cancer Res 19:2240-2247, 2013
4. Mok TS, Wu YL, Ahn MJ, et al: Osimertinib or platinum-pemetrexed in EGFR T790M–positive lung cancer. N Engl J Med 376:629-640, 2017
5. Soria JC, Ohe Y, Vansteenkiste J, et al: Osimertinib in untreated EGFR-mutated advanced non–small-cell lung cancer. N Engl J Med 378:113-125, 2018
6. Sequist LV, Waltman BA, Dias-Santagata D, et al: Genotypic and histological evolution of lung cancers acquiring resistance to EGFR inhibitors. Sci Transl Med 3:75ra26, 2011
7. Yang Z, Yang N, Ou Q, et al: Investigating novel resistance mechanisms to third-generation EGFR tyrosine kinase inhibitor osimertinib in non–small-cell lung cancer patients. Clin Cancer Res 24:3097-3107, 2018
8. Wang Y, Li L, Han R, et al: Clinical analysis by next-generation sequencing for NSCLC patients with MET amplification resistant to osimertinib. Lung Cancer 118:105-110, 2018
9. Yang JC, Ahn MJ, Kim DW, et al: Osimertinib in pretreated T790M-positive advanced non–small-cell lung cancer: AURA study phase II extension component. J Clin Oncol 35:1288-1296, 2017
10. Chabon JJ, Simmons AD, Lovejoy AF, et al: Circulating tumour DNA profiling reveals heterogeneity of EGFR inhibitor resistance mechanisms in lung cancer patients. Nat Commun 7:11815, 2016
11. Piotrowska Z, Thress KS, Mooradian M, et al: MET amplification (amp) as a resistance mechanism to osimertinib. J Clin Oncol 35, 2017 (suppl; abstr 9020)
12. Iams W, Chae Y: Acquired resistance to osimertinib by CCDC6-RET fusion in a patient with EGFR T790M–mutant metastatic lung adenocarcinoma. J Thorac Oncol 12: S2249-S2250, 2017
13. Yu HA, Suzawa K, Jordan E, et al: Concurrent alterations in EGFR-mutant lung cancers associated with resistance to EGFR kinase inhibitors and characterization of MTOR as a mediator of resistance. Clin Cancer Res 24:3108-3118, 2018
14. Cheng DT, Mitchell TN, Zehir A, et al: Memorial Sloan Kettering-integrated mutation profiling of actionable cancer targets (MSK-IMPACT): A hybridization capture-based next-generation sequencing clinical assay for solid tumor molecular oncology. J Mol Diagn 17:251-264, 2015
15. Zheng Z, Liebers M, Zhelyazkova B, et al: Anchored multiplex PCR for targeted next-generation sequencing. Nat Med 20:1479-1484, 2014

16. Jackman D, Pao W, Riely GJ, et al: Clinical definition of acquired resistance to epidermal growth factor receptor tyrosine kinase inhibitors in non–small-cell lung cancer. J Clin Oncol 28:357-360, 2010

17. National Cancer Institute: Osimertinib and necitumumab in treating patients with EGFR-mutant stage IV or recurrent non–small-cell lung cancer who have progressed on a previous EGFR tyrosine kinase inhibitor (NCT02496663). https://clinicaltrials.gov/ct2/show/NCT02496663

18. Li GG, Somwar R, Joseph J, et al: Antitumor activity of RXDX-105 in multiple cancer types with RET rearrangements or mutations. Clin Cancer Res 23:2981-2990, 2017

19. Okamoto K, Kodama K, Takase K, et al: Antitumor activities of the targeted multi-tyrosine kinase inhibitor lenvatinib (E7080) against RET gene fusion–driven tumor models. Cancer Lett 340:97-103, 2013

20. Arai S, Kita K, Tanimoto A, et al: In vitro and in vivo anti-tumor activity of alectinib in tumor cells with NCOA4-RET. Oncotarget 8:73766-73773, 2017

21. Klempner SJ, Mehta P, Schrock AB, et al: Cis-oriented solvent-front EGFR G796S mutation in tissue and ctDNA in a patient progressing on osimertinib: A case report and review of the literature. Lung Cancer (Auckl) 8:241-247, 2017

22. Thress KS, Paweletz CP, Felip E, et al: Acquired EGFR C797S mutation mediates resistance to AZD9291 in non–small-cell lung cancer harboring EGFR T790M. Nat Med 21:560-562, 2015

23. Ou SI, Horn L, Cruz M, et al: Emergence of FGFR3-TACC3 fusions as a potential bypass resistance mechanism to EGFR tyrosine kinase inhibitors in EGFR-mutated NSCLC patients. Lung Cancer 111:61-64, 2017

24. Iams W, Chae Y.: Acquired resistance to osimertinib by CCDC6-RET fusion in a patient with EGFR T790M–mutant metastatic lung adenocarcinoma. J Thorac Oncol 12:S2249-S50, 2017

25. Ho CC, Liao WY, Lin CA, et al: Acquired BRAF V600E mutation as resistant mechanism after treatment with osimertinib. J Thorac Oncol 12:567-572, 2017

Appendix

Table A1. Detailed Molecular and Immunohistochemical Characterization of Patient Case 1 Presented

Timing of Molecular Test	Type of Molecular Test	Result
Diagnosis	Tissue PCR[a]	15–base pair *EGFR* exon 19 deletion detected
	IMPACT[b]	*EGFR* exon19 p.E746_A750del; TP*53* exon10 p.R337L; *ATR* exon41 p.D2331Y detected
Erlotinib progression	cfDNA[c]	*EGFR* T790M detected (0.79%)[d]
	Tissue PCR[c]	*EGFR* T790M detected (15.15%)[d]
	IHC[e]	ALK: negative; EGFR exon 19 deletion: positive
	IMPACT[b]	*EGFR* exon19 p.E746_A750del; *EGFR* exon20 p.T790M; *KMT2C* exon38 p.D2690N; *RAD51B* exon7 p.L209V detected
	ArcherDx[f]	No *ALK* fusion detected
	FISH[g]	No evidence of *ALK* rearrangement. Interphase/nuclear in situ hybridization: nuc ish(3′ALK,5′ALK)x2(3′ALK con 5′ALKx2)[0/100]
Osimertinib + necitumumab progression	IHC[e]	ALK: positive; EGFR exon 19 deletion: positive
	IMPACT[b]	*EGFR* exon19 p.E746_A750del; *EGFR* exon20 p.T790M; *TP53* exon4 p.D48Sfs*75; *EGFR* amplification (FC: 3.0); *NKX2*-1 amplification (FC: 2.7); *FOXA1* amplification (FC: 2.7); *CARD11* amplification (FC: 2.1); *PMS2* amplification (FC: 2.1); *RAC1* amplification (FC: 2.1); *ETV1* amplification (FC: 2.1); INHBA amplification (FC: 2.1); *IKZF1* amplification (FC: 2.1); MAX amplification (FC: 2.0); *RAD51B* amplification (FC: 2.0); *TSHR* amplification (FC: 2.0); *PRKD1* amplification (FC: 2.0); *NFKBIA* amplification (FC: 2.0); *CDKN2B* deletion (FC: -2.1); *CDKN2Ap16INK4A* deletion (FC: -2.1); *CDKN2Ap14ARF* deletion (FC: -2.1); *ATR* exon41 p.D2331Y; *NKX2*-1 exon2 p.P150L; *EML4* - *ALK* fusion (*EML4* exons 1-6 fused with *ALK* (exons 20-29) detected
	ArcherDx[f]	*EML4-ALK* fusion detected

[a]The specific mutations are detected by amplification of the corresponding exons by polymerase chain reaction (PCR), followed by a single base extension at the site of the point mutation. The single base extension product is detected by tandem mass-spectrometry on a Sequenom MassArray spectrometer (Sequenom, San Diego, CA).
[b]MSK-IMPACT (Integrated Mutation Profiling of Actionable Cancer Targets) was used to identify specific mutations in 468 genes.
[c]Digital PCR of cfDNA and/or tissue amplification of part of *EGFR* exon 20 in the presence of fluorescent probes specific to the wild-type and mutant alleles.
[d]Ratio of mutant allele/(mutant + wild-type allele).
[e]Immunohistochemistry (IHC) for pertinent proteins performed with the following clones: *EGFR*-exon19del (EGFR-E746): clone 6B6; *ALK*: clone D5F3.
[f]Archer FusionPlex Custom Solid Panel (Archer FusionPlex, Boulder, CO) uses the Anchored Multiplex PCR used to detect gene fusions in tumor samples consisting of 62 cancer-related genes previously reported to be involved in chromosomal rearrangements. Unidirectional gene-specific primers (GSPs) are designed to several targeted exons in 62 genes. GSPs in combination with adapters-specific primers amplify known and novel fusion transcripts. Enriched amplicons were sequenced on an Illumina MiSeq instrument.
[g]Interphase FISH analysis for *ALK* gene rearrangement using an LSI *ALK* break apart probe (Abbott Molecular, Chicago, IL) chromosome locus of the target gene: *ALK*, 2p23, cut off for normal variation for rearrangement: 10% for FFPE tissue.

Table A2. Detailed Molecular and Immunohistochemical Characterization of Patient Case 2

Timing of Molecular Test	Type of Test	Result
Diagnosis	cfDNA[a]	*EGFR* L858R detected.
Erlotinib progression	Tissue PCR[a]	*EGFR* exon 20 mutation p.T790M (22.75%)[b] detected.
	IHC[c]	EGFR-L858R: positive; ALK: negative.
	IMPACT[d]	*EGFR* exon20 p.T790M; *EGFR* exon21 p.L858R (c.2573T>G); *TERT* amplification (fold change: 2.0); *CDKN1A* gain (fold change: 1.8); *PIM1* gain (fold change: 1.8); *CCND3* gain (fold change: 1.8); *VEGFA* gain (fold change: 1.8); *CDKN2B* deletion (fold change: -2.7); *CDKN2Ap16INK4A* deletion (fold change: -2.7); *CDKN2Ap14ARF* deletion (fold change: -2.7); *APC* exon10 p.I413Pfs*40 (c.1237_1241delinsC); *NTRK3* (NM_001012338) exon8 p.D225Y (c.673G>T); *PTEN* exon2 splicing variant p.X27_ splice (c.80-1_80delGA); *PTPRD* exon36 p.A1391T (c.4171G>A) detected.
	FISH[e]	No evidence of *ALK* rearrangement. Interphase/nuclear in situ hybridization: nuc ish(ALKx2)(3′ALK sep 5′ALKx1)[2/100]/ (3′ALK,5′ALK)x3~4(3′ALK con 5′ALKx3~4)[8/100].
	ArcherDx[f]	Test failure, inadequate RNA extracted.
Osimertinib progression	IHC[c]	EGFR-L858R: positive; ALK: positive.
	IMPACT[d]	*EGFR* exon20 p.T790M (c.2369C>T); *EGFR* exon21 p.L858R (c.2573T>G); *CDKN1A* gain (fold change: 1.7); *PIM1* gain (fold change: 1.7); *CCND3* gain (fold change: 1.7); *VEGFA* gain (fold change: 1.7); *TERT* gain (fold change: 1.5); *CDKN2B* deletion (fold change: -2.3); *CDKN2Ap16INK4A* deletion (fold change: -2.3); *CDKN2Ap14ARF* deletion (fold change: -2.3); *APC* exon10 p.I413Pfs*40 (c.1237_1241delinsC); *NTRK3* exon8 p.D225Y (c.673G>T); *PTEN* splicing variant p.X27_splice (c.80-1_c.80delGA); *PTPRD* exon36 p.A1391T (c.4171G>A); *EML4 - ALK* fusion (EML4 exons 1-2 fused to ALK exons 20-29): c.208+4890:EML4_c.3173-373:ALKinv detected.
	ArcherDx[f]	Test failure, inadequate RNA extracted.

[a]Digital PCR of cfDNA and/or tissue amplification of part of EGFR exon 20 in the presence of fluorescent probes specific to the wild-type and mutant alleles.
[b]Ratio of mutant allele/(mutant + wild-type allele).
[c]Immunohistochemistry (IHC) for pertinent proteins performed with the following clones: *EGFR*-L858R: clone 43B2; *ALK*: clone D5F3.
[d]MSK-IMPACT (Integrated Mutation Profiling of Actionable Cancer Targets) was used to identify specific mutations in 468 genes.
[e]Interphase FISH analysis for *ALK* gene rearrangement using an LSI *ALK* break apart probe chromosome locus of the target gene: *ALK*, 2p23, cut off for normal variation for rearrangement: 10% for FFPE tissue.
[f]Archer FusionPlex Custom Solid Panel uses the Anchored Multiplex PCR used to detect gene fusions in tumor samples consisting of 62 cancer-related genes previously reported to be involved in chromosomal rearrangements. Unidirectional gene-specific primers (GSPs) are designed to several targeted exons in 62 genes. GSPs in combination with adapters-specific primers amplify known and novel fusion transcripts. Enriched amplicons were sequenced on an Illumina MiSeq instrument.

Table A3. Detailed Molecular and Immunohistochemical Characterization of Patient Case 3

Timing of Molecular Test	Type of Test	Result
Diagnosis	IHC[a]	EGFR-L858R: negative; EGFR-E746: negative.
	Tissue PCR[b]	*EGFR* L858R detected.
Erlotinib progression	ArcherDx[c]	No evidence of *RET* fusion.
	IMPACT[d]	*EGFR* exon19 p.L747S; *EGFR* exon20 p.T790M; *EGFR* exon21 p.L858R; *DDR2* exon15 p.R680H detected.
	Tissue PCR[b]	*EGFR* L858R; *EGFR* L747S (allele frequency: 0.49) detected.
	Tissue PCR[b]	*EGFR* L858R; *EGFR* L747S; *EGFR* T790M detected.
Osimertinib progression	IMPACT[d]	*EGFR* exon19 p.L747S (allele frequency: 0.26); EGFR exon21 p.L858R; *MDM2* Amplification (FC: 15.8); *CDK4* amplification; *DDR2* exon15 p.R680H; NCOA*4-RET* fusion: c.619-108NCOA4_c.2137-657RETdup detected.
	ArcherDx[c]	*NCOA4-RET* rearrangement: in-frame fusion between genes *NCOA4* Exon7 and *RET* Exon12 detected.

[a]Immunohistochemistry (IHC) for pertinent proteins performed with the following clone: *EGFR*-L858R: clone 43B2.
[b]The specific mutations are detected by amplification of the corresponding exons by polymerase chain reaction (PCR), followed by a single base extension at the site of the point mutation. The single base extension product is detected by tandem mass-spectrometry on a Sequenom MassArray spectrometer.
[c]Archer FusionPlex Custom Solid Panel uses the Anchored Multiplex PCR used to detect gene fusions in tumor samples consisting of 62 cancer-related genes previously reported to be involved in chromosomal rearrangements. Unidirectional gene-specific primers (GSPs) are designed to several targeted exons in 62 genes. GSPs in combination with adapters-specific primers amplify known and novel fusion transcripts. Enriched amplicons were sequenced on an Illumina MiSeq instrument.
[d]MSK-IMPACT (Integrated Mutation Profiling of Actionable Cancer Targets) was used to identify specific mutations in 468 genes.