Published in final edited form as:

Cancer Discov. 2018 December ; 8(12): 1529–1539. doi:10.1158/2159-8290.CD-18-1022.

Landscape of acquired resistance to osimertinib in *EGFR*-mutant NSCLC and clinical validation of combined EGFR and RET inhibition with osimertinib and BLU-667 for acquired *RET* fusion.

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Disclosure of Potential Conflicts of Interest:

Zofia Piotrowska has served as a compensated consultant or received honoraria for AstraZeneca, Ariad/Takeda, Novartis, AbbVie and Guardant Health, and receives institutional research funding from Novartis, Takeda, Spectrum and AstraZeneca. J.F. Gainor has served as a consultant or received honoraria from Novartis, BMS, Amgen, Agios, Regeneron, Oncorus, Genentech/Roche, Pfizer, Jounce, Incyte, Theravance, Ariad/Takeda, and Array. V.W. Zhu has received honoraria from AstraZeneca, Roche-Foundation Medicine, Roche/Genentech, and Takeda, and consulting fees from TP Therapeutics. J.J. Lin has received honoraria from Chugai and Boehringer-Ingelheim. R. Nagy and R. Lanman are employees and shareholders of Guardant Health. M. Mino-Kenudson has served as a compensated consultant for Merrimack Pharmaceuticals and H3 Biomedicine. A.J. Iafrate holds equity in ArcherDX. R.S. Heist has served as a consultant for Boehringer-Ingelheim, Tarveda and Novartis and receives research funding (to institution) from Celgene, Roche, Takeda, Peregrine, Novartis, Corvus, Incyte, Debiopharm, Mirati, AbbVie, Millenium, Daichii Sankyo, Agios and Exelixis. A.T. Shaw has served as a compensated consultant or received honoraria from Pfizer, Novartis, Genentech/Roche, Ignyta, Blueprint Medicines, LOXO, Daiichi-Sankyo, Ariad/Takeda, Chugai, Taiho Pharmaceuticals, EMD Serono, KSQ Therapeutics, Foundation Medicine, Natera, Guardant, and TP Therapeutics; and serves on the scientific advisory board of Blueprint Medicines. E.K. Evans, C. Clifford and B. Wolf are employees and shareholders of Blueprint Medicines. SH.I. Ou received honoraria from Pfizer, Roche/Genentech/Ignyta, Takeda/ARIAD, AstraZeneca, Foundation Medicine and is member of the Scientific Advisory Board of TP Therapeutics and has stock ownership in TP Therapeutics. A.N. Hata reports research funding from Novartis, Amgen and Relay therapeutics. LVS declares advisory relationships, honoraria or research funding from AstraZeneca, Blueprint Medicines, Novartis, Pfizer, Genentech, Boehringer Ingelheim, and Merrimack Pharmaceuticals. No potential conflicts of interest were disclosed by the other authors.

Please see supplemental methods for additional information.

Abstract

We present a cohort of 41 patients with osimertinib resistance biopsies, including two with an acquired *CCDC6-RET* fusion. While *RET* fusions have been identified in resistant *EGFR*-mutant NSCLC, their role in acquired resistance to EGFR inhibitors is not well described. To assess the biological implications of *RET* fusions in an *EGFR*-mutant cancer, we expressed CCDC6-RET in PC9 (*EGFR* del19) and MGH134 (*EGFR* L858R/T790M) cells and found that CCDC6-RET was sufficient to confer resistance to EGFR-TKIs. The selective RET inhibitors BLU-667 or cabozantinib resensitized CCDC6-RET-expressing cells to EGFR inhibition. Finally, we treated two patients with *EGFR*-mutant NSCLC and *RET*-mediated resistance with osimertinib and BLU-667. The combination was well-tolerated and led to rapid radiographic response in both patients. This study provides proof-of-concept that *RET* fusions can mediate acquired resistance to EGFR TKIs and that combined EGFR and RET inhibition with osimertinib/BLU-667 may be a well-tolerated and effective treatment strategy for such patients.

Keywords

EGFR; RET; Fusions; Osimertinib; NSCLC

INTRODUCTION

Osimertinib is a highly selective, CNS-penetrant, third-generation epidermal growth factor (EGFR) tyrosine kinase inhibitor (TKI) which nearly doubles progression-free survival (PFS) compared to first-generation EGFR TKIs and is now the standard front-line therapy for *EGFR*-mutant non-small cell lung cancer (NSCLC).[1] In addition, osimertinib remains the preferred second-line therapy for T790M-mediated resistance to first/second-generation EGFR TKIs.[2] Despite high initial response rates, however, patients typically develop acquired after about 1–2 years of treatment.

Mechanisms of osimertinib resistance are under active investigation but thus far have primarily been studied in the second-line, T790M-positive, setting because front-line use represents a more recent shift in the treatment paradigm. Prior studies demonstrated overlap between resistance mechanisms to osimertinib and to first/second-generation EGFR TKIs, including bypass pathway activation (e.g., *MET* amplification) and histologic transformation seen upon progression on all classes of EGFR inhibitors.[3–6] One notable exception is the *EGFR* T790M mutation, which develops in 50–60% of patients progressing on the older drugs, while for osimertinib T790M is a marker of sensitivity. Furthermore, *EGFR* C797S is recurrently observed in osimertinib-resistance, but not in resistance to first-generation drugs, as expected based on the drug-receptor binding characteristics.[7–9] However, the number of osimertinib-resistant cases reported to date remains limited and a significant proportion of osimertinib-resistant cases lack a clearly identified pathway driving resistance.[4]

Acquired fusions, including those involving *RET*, have recently been reported in a small number of patients progressing on osimertinib and other EGFR TKIs.[4,10–13] Historically, EGFR TKI resistance studies had not identified *RET* fusions, but this may have been due to the use of limited genotyping platforms that likely did not include *RET.* Fusions involving

RET, a recently-described driver oncogene in NSCLC, can be difficult to detect using standard next-generation sequencing (NGS) platforms. The functional role of *RET* and other fusions in EGFR TKI acquired resistance and the potential impact of RET-directed inhibitors in this population are unknown.

To characterize osimertinib resistance mechanisms including acquired fusion alterations, we analyzed tumor tissue or circulating tumor DNA (ctDNA) from a cohort of patients progressing on osimertinib. We also assessed the functional implications of *RET* fusions in *EGFR*-mutant cell line models and treated three patients with *EGFR*-mutant NSCLC and acquired *RET* fusions with combined EGFR and RET inhibition.

RESULTS

Osimertinib resistance cohort

Our study began as a survey of osimertinib resistance mechanisms among patients at Massachusetts General Hospital (MGH). A total of 41 patients with *EGFR*-mutant NSCLC were treated with single-agent osimertinib and underwent resistance assessment at progression between July 2014 and August 2018 (Table 1). There were 26 women and 15 men, median age 64 (range, 40–87). One patient received first-line osimertinib, 16 were treated in the second-line setting and 24 as third-line or later. All had T790M-positive disease pre-osimertinib except the front-line patient. Fifteen patients had received another third-generation EGFR TKI before osimertinib (rociletinib (12), nazartinib (2), ASP8273 (1)). The median duration of osimertinib treatment was 11.6 months (range, 1.0–32.7). To assess osimertinib resistance mechanisms, 17 patients had both a tissue biopsy and ctDNA analysis, 15 had tissue only and 9 had ctDNA only at clinical progression. Three patients had two distinct metastases sampled at osimertinib resistance.

Observed osimertinib resistance mechanisms

A total of 35 tissue biopsies among 32 osimertinib-resistant patients were analyzed (Figure 1). All had adenocarcinoma histology prior to osimertinib; two transformed to small cell lung cancer (SCLC) and one to squamous cell histology after progression on osimertinib. Molecular testing was performed on all cases, with the founder *EGFR* mutation detected in each specimen. Six (19%) patients had acquired *EGFR* C797S, each in *cis* configuration with T790M; seven (22%) developed *MET* amplification (defined as MET: centromere 7 ratio ≥ 2.2 by *FISH*). In 12 (38%) cases, T790M was not identified (11 previously T790M-positive) and no other resistance driver was detected, while in 3 (9%) cases T790M was maintained without an identified resistance mechanism.

Among 26 patients with ctDNA analysis at osimertinib resistance, the founder *EGFR* mutation was detected in 22 samples; the remaining 4 lacked detectable *EGFR* and therefore were uninformative for resistance mechanisms, which were also likely below the limit of detection (Figure 1). Resistance mechanisms detected via ctDNA were similar in spectrum to tissue samples with 7 (32%) C797S and 5 (23%) *MET* amplification (defined as mean plasma copy number ≥ 2.1). The number of samples with both tissue and informative ctDNA was too small for meaningful concordance analysis.

We observed intertumoral heterogeneity in all three patients who had two distinct metastatic foci biopsied. Two patients had C797S detected at one metastatic site while the other was C797 wild-type; the third had *MET* amplification detected within a pleural fluid cell block but had normal *MET* copy number in a coincident lung biopsy. In each case, no other putative resistance mechanism was identified in the second biopsy site.

One patient with plasma-only osimertinib resistance analysis (# 33) had both *CCDC6-RET* (mutant allele frequency, MAF, 1.9%) and TPM3-NTRK1 fusion (MAF 0.1%) detected in ctDNA (*EGFR* del19, MAF 14.2%). Given this finding, we used the MGH Solid Fusion Assay (SFA), an RNA-based anchored multiplex polymerase chain reaction (AMP), developed to identify fusion events in tissue biopsies and found 24/35 (69%) osimertinib-resistant tissue biopsies had sufficient tissue for analysis.[14] Among these, we detected a *CCDC6-RET* fusion in a progressing pleural metastasis in patient 1 and a *PCBP2-BRAF* fusion in a new liver metastasis which developed on osimertinib in patient 2 (Figure 1, Table 1). Additionally, patient 3 in our osimertinib-resistant cohort underwent NGS of a growing omental nodule at Foundation Medicine and an *AGK-BRAF* fusion was observed. In each case, there was concurrent T790M “loss” and no other resistance mechanisms identified in the tissue.

To broaden our cohort of *EGFR*-mutant NSCLC patients with acquired fusion events, we retrospectively ran the SFA on a subset of *EGFR*-mutant tissue biopsies obtained at MGH over the past ten years (Table 1). Many of these older biopsies were obtained upon progression on erlotinib, afatinib and gefitinib and did not originally undergo SFA. Among them, we identified one additional afatinib-resistant patient who had a *CCDC6-RET* fusion (#42, described in more detail below) and one patient (#43) with a *BAIAP2L1-BRAF* fusion detected after progression on chemotherapy/osimertinib. We also included one patient from the University of California Irvine (UCI) who acquired a *NCOA4-RET* fusion on FoundationOne NGS tissue testing obtained upon progression on first-line afatinib/cetuximab therapy (#44, described in further detail below).

CCDC6-RET expression in EGFR-mutant NSCLC cell lines confers resistance to EGFR inhibitors.

Next, we sought to determine whether gene fusions observed in the above patients are sufficient to cause acquired drug resistance. We initially focused on the *CCDC6-RET* fusion gene. CCDC6-RET expressing cell lines were generated by lentiviral infection of PC9 (*EGFR* del19) and MGH134 (*EGFR* L858R/T790M) cells (Figure S1). Cells expressing CCDC6-RET grew similarly to parental cells in the absence of EGFR inhibitor. When treated with osimertinib, PC9$^{CCDC6\text{-}RET}$ and MGH134$^{CCDC6\text{-}RET}$ cells continued to proliferate, in contrast to parental cells which showed a net decrease in cell viability (Figure 2A). Of note, the proliferation rate of CCDC6-RET expressing cells decreased in osimertinib, suggesting that *RET* activation does not fully compensate for EGFR signaling loss, although it is sufficient to drive acquired resistance.

We next examined the consequences of CCDC6-RET expression on downstream signaling pathway activation in PC9 and MGH134 cells. Compared to parental cells, which did not express detectable RET protein, phosphorylated RET was detected in both PC9$^{CCDC6\text{-}RET}$

and MGH134$^{CCDC6\text{-}RET}$ cells (Figure 2B, Figure S2A). CCDC6-RET expression alone did not lead to increased activation of downstream MAPK (phospho-ERK1/2) or PI3K (phospho-AKT) signaling at baseline, however RET, ERK1/2 and AKT phosphorylation was retained in the presence of afatinib or osimertinib in both PC9$^{CCDC6\text{-}RET}$ and MGH134$^{CCDC6\text{-}RET}$ cells (Figure 2B, Figure S2A). Thus, expression of the CCDC6-RET fusion is sufficient to confer resistance to EGFR-TKIs in *EGFR*-mutant NSCLCs.

Acquired resistance resulting from CCDC6-RET expression can be overcome by EGFR plus RET inhibition

Acquired resistance resulting from activation of other bypass signaling pathways can be overcome via dual pathway suppression.[15,16] To determine whether a similar strategy might overcome CCDC6-RET-mediated acquired resistance, we treated PC9$^{CCDC6\text{-}RET}$ cells with the selective RET inhibitor BLU-667[17] in the absence or presence of EGFR TKIs. Treatment with BLU-667 alone suppressed RET phosphorylation but did not decrease downstream ERK or AKT phosphorylation (Figure 2B). Combined treatment with BLU-667 and either osimertinib or afatinib completely suppressed both phospho-ERK and phospho-AKT and decreased cell viability to a similar level as parental cells treated with EGFR TKI (Figure 2C). Similar results were observed in MGH134$^{CCDC6\text{-}RET}$ cells (Figure S2). Additionally, PC9$^{CCDC6\text{-}RET}$ and MGH134$^{CCDC6\text{-}RET}$ cells were sensitive to EGFR TKI + cabozantinib, a multi-kinase inhibitor with RET activity (Figure S2, S3A, S3B). Taken together, these data demonstrate that acquired resistance resulting from the CCDC6-RET fusions can be overcome by dual EGFR plus RET blockade.

MEK but not BRAF inhibitors overcome acquired resistance resulting from PCBP2-BRAF fusion.

To expand our investigation beyond the *CCDC6-RET* fusion, we examined whether the novel *PCBP2-BRAF* fusion observed in patient 2 was driving resistance. We established a cell line (MGH845–1) from a core needle liver biopsy of the patient (Figure S4A, S4B) and confirmed the presence of the *PCBP2-BRAF* fusion gene and *EGFR* T790M loss (Figure S4C, S4D). Knock-down of BRAF in MGH845–1 using siRNAs targeting the *BRAF* coding sequence retained within the PCBP2-BRAF fusion had a modest effect on cell viability, and further sensitized cells to osimertinib (Figure S5A,B). Consistent with a prior report examining *de novo BRAF* fusions in melanoma[18], the MGH845–1 cells were sensitive to the MEK inhibitor trametinib but not to the RAF inhibitors dabrafenib or LXH245 (Figure S5C).

Treatment of EGFR-mutant acquired RET fusion-positive patients with EGFR plus RET inhibition

The preclinical results showing that combining EGFR and RET inhibitors can overcome resistance conferred by CCDC6-RET were sufficiently compelling to suggest patient treatment should be explored. The first MGH patient identified with an acquired *RET* fusion (Table 1; patient 42) was a 44-year-old man with del19 *EGFR*-mutant advanced NSCLC who received front-line cisplatin/pemetrexed, second-line afatinib (one year), then underwent a bronchoscopic biopsy of a growing lung lesion showing a *CCDC6-RET* fusion by SFA. Baseline tissue wasn't available for *RET* testing. He was treated with erlotinib 150

mg daily combined with off-label cabozantinib 60 mg daily. Scans after one month showed stable disease (RECIST 1.1), but subsequent scans after 2.5 months showed disease progression and prompted treatment discontinuation.[19] He had grade 1 diarrhea, rash, and AST elevation.

A 60-year-old woman with del19 *EGFR*-mutant advanced NSCLC (patient 1) received front-line afatinib (one year), acquired T790M, and was treated with osimertinib (18 months). She then underwent a pleural biopsy revealing a *CCDC6-RET* fusion via SFA. Baseline tissue was insufficient for SFA, but *RET* fluorescence *in situ* hybridization (FISH) was negative, suggesting the *CCDC6-RET* fusion was indeed acquired. Given the suboptimal response the first patient had using the multitargeted TKI cabozantinib and the successful experience with the selective RET TKI BLU-667 in NSCLCs harboring *RET* fusions as the primary oncogenic driver, we wrote an individual patient investigational new drug (IND) protocol for osimertinib plus BLU-667.[17] She began osimertinib 80 mg daily and BLU-667 200 mg daily, then increased BLU-667 to 300 mg after 2 weeks of treatment. Her dyspnea improved within days of therapy initiation. Scans after 8 weeks revealed a marked response with RECIST tumor shrinkage of 78% (Figure 3A), with a confirmed partial response seen on a follow-up imaging done after 16 weeks on treatment. The combination was well-tolerated with only grade 1 toxicities including fatigue, leukopenia, hypertension, xerostomia, and transaminitis. Treatment is ongoing at the time of this writing (4 months on treatment).

Finally, we collaborated with colleagues at UCI who identified a similar patient (Table 1, patient 44). A 67-year-old woman underwent surgery and adjuvant cisplatin/pemetrexed for a stage IIIA del19 *EGFR*-mutant lung adenocarcinoma, with subsequent recurrence. She received afatinib/cetuximab (2 years), then underwent a lung biopsy, which demonstrated an acquired *NCOA4-RET* fusion by FoundationOne NGS testing (not present in the pre-treatment biopsy). An individual IND protocol was again utilized. She took osimertinib 80mg daily and BLU-667 at 200 mg daily for 2 weeks, then 300 mg daily for 2 weeks, then ultimately escalated to 400 mg daily. Scans after 8 weeks also revealed a marked response with RECIST tumor shrinkage of 78% (Figure 3B). Grade 1 toxicities including fatigue, diarrhea, anemia, thrombocytopenia, and dysguesia, and grade 2 leukopenia and neutropenia were observed. Treatment is ongoing at the time of this writing (4 months on treatment).

DISCUSSION

Here we examine mechanisms of acquired resistance to osimertinib with a focus on *RET* fusions, demonstrating in engineered cell lines that they can mediate acquired resistance to *EGFR* TKIs and providing proof-of-principle clinical data that targeting this bypass track with a selective RET inhibitor like BLU-667 can be highly effective in patients. Both patients treated with osimertinib plus BLU-667 had rapid and impressive improvements in their cancer. This has immediate clinical implications for *EGFR*-mutant patients and suggests that testing for *RET* fusions should become part of standard panels used upon acquired EGFR resistance. Importantly, osimertinib and BLU-667 were well-tolerated in these two patients, and further study of this combination in additional patients is warranted.

The paradigm of testing for bypass track activation at acquired resistance to EGFR TKIs has precedence in *MET* amplification, a resistance mechanism first described in 2007.[15] Ten years later, the clinical validity of inhibiting EGFR plus MET in patients with *MET* amplification-driven resistance was demonstrated though the combination of osimertinib and the MET inhibitor savolitinib.[20] Prior EGFR plus MET TKI combinations were tested but success was limited, likely due to trial designs lacking a focus on true *MET* amplification as the resistance driver, as well as the poor tolerability of prior regimens built primarily on an erlotinib back-bone.[21–23] Just as osimertinib, a well-tolerated third-generation EGFR TKI, has led to better tolerated combinations with MET inhibitors, our experience suggests that we may see similar ease of building combination regimens for *RET*-mediated acquired resistance. The high RET selectivity of BLU-667 may also be a contributing factor to the tolerability of this combination. BLU-667 has been shown to be >15 times more potent on RET than any other kinase and >10 times more potent on RET than approved multi-targeted kinase inhibitors like cabozantinib.[17] The overall tolerability of osimertinib plus BLU-667 in both of our patients is an early sign of the high selectivity of BLU-667 and the feasibility of combining the two agents.

Pre-clinical modeling demonstrated that CCDC6-RET fusion expression resulted in sustained MAPK and PI3K signaling in the presence of EGFR inhibition, and in both models tested, was sufficient to cause EGFR TKI resistance. However, in both PC9$^{CCDC6\text{-}RET}$ and MGH134$^{CCDC6\text{-}RET}$ cells, EGFR TKIs exhibited partial activity in suppressing downstream signaling and slowing cell proliferation. While we cannot rule out the possibility that differences in expression levels of the CCDC6-RET fusion may contribute, these results suggest that CCDC6-RET may not fully recapitulate EGFR signaling such that resistant cells harboring this fusion retain partial dependency on EGFR signaling.

Other groups have also found *RET* fusions in *EGFR*-mutant patients with TKI resistance.[4,10–13] Reckamp and colleagues studied nearly 33,000 samples undergoing clinical plasma ctDNA testing at Guardant Health and identified 116 NSCLC patients with *RET* fusions, including 17 with co-occurring *EGFR* mutations.[10] Five *EGFR*-mutants had available information about their clinical course and all 5 had received prior first/second-generation TKI while three had also received osimertinib before the *RET* fusion was identified. Schrock and colleagues assessed over 3500 *EGFR*-mutant patients undergoing tissue sampling at Foundation Medicine for fusions and identified 19 patients with a *RET* fusion, including one afatinib-resistant L858R *EGFR*-mutant patient with an *NCOA4-RET* fusion, who had stable disease for 7 months on cabozantinib plus afatinib.[11] This patient anecdote is especially interesting in the context of the three patients treated with EGFR plus RET inhibitors we present here, as there are now at least two reported cases treated with cabozantinib that had stable disease as a best response, in stark contrast with two reported cases treated with BLU-667 and osimertinib that had dramatic and rapid responses.

With broad NGS panels steadily gaining popularity, we believe it is feasible for the oncology community to start testing for *RET* and other oncogene fusions in post-resistance *EGFR*-mutant biopsies. However, there are some noteworthy caveats. Translocation breakpoints may be present at any point in the genomic DNA and often occur in intronic regions, thus

focused NGS panels that examine only exons may miss these aberrations. Larger NGS libraries and alignment tools allowing mapping of DNA sequences to two different genomic sites can help overcome this obstacle. At MGH, our molecular pathology group has developed an RNA AMP technology to identify gene rearrangements without prior knowledge of the fusion partner.[14] This SFA can detect chimeric transcripts at the RNA-level which also enables prediction of the involved (transcribed) exons, typically fused at exon-intron junctions. In addition, SFA technology is compatible with the often short and fragmented nucleic acids input from formalin-fixed paraffin embedded specimens. We acknowledge that, while the SFA assay can identify (*RET*)-fusion partners by sequence, other technologies with specific advantages also exist. For example, FISH preserves the tissue context and enables gene fusion assessment on very small samples.

Our cohort adds to the growing body of knowledge about osimertinib acquired resistance. Acquired *RET* fusions should be considered a potentially actionable finding at osimertinib resistance but treatment options remain unclear for acquired *BRAF* fusions which will require more detailed mechanistic studies to unravel the complexities of RAF signaling in these patients. In addition to the fusion cases discussed, we observed C797S in 27% of patients, consistent with other experiences.[4] Since all cases were found in *cis* with T790M, there is not currently a targeted treatment strategy clinically available for these patients, though pre-clinical concepts are emerging.[24–26] In addition, we saw *MET* amplification in 24% of patients, which is encouraging given the promising treatment strategies available now for these patients.[20]

Our study is limited by its assessment of osimertinib primarily in the second (or beyond)-line T790M-positive setting; we acknowledge that our findings may not be directly applicable to patients who receive osimertinib for newly-diagnosed *EGFR*-mutant NSCLC. However, the patients we and others have identified with *RET* fusions after first/second generation EGFR TKIs lead us to believe that *RET* fusions will likely be recurrent findings after front-line osimertinib. Small numbers, especially only two patients treated with the osimertinib plus BLU-667, also limit our study. Further study of osimertinib plus BLU-667 will be needed to define clinical activity in a larger cohort of patients. Finally, 8 of the patients in our cohort were on osimertinib for less than 6 months prior to undergoing progression biopsies, and hence the findings in those cases may reflect an intrinsic resistance clone.

In conclusion, *RET* fusions are a *bona fide* acquired resistance mechanism among *EGFR*-mutant cancers and treatment with osimertinib plus BLU-667 may be a well-tolerated and effective therapy for this group.

METHODS

Patients

All sequential patients with *EGFR*-mutant NSCLC seen at MGH who underwent a tissue biopsy and/or ctDNA analysis after clinical progression on osimertinib and had sufficient tissue for molecular analysis were included. The sites of biopsy were selecting by the treating physician; progressing lesions were biopsied whenever feasible. We identified

additional patients with *EGFR*-mutant NSCLC and fusions detected by SFA, regardless of prior therapy. All patients provided signed informed consent under an Institutional Review Board (IRB)-approved protocol which allows chart review for research, NGS, and exploratory research on tissue biopsies. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Molecular testing of tissue biopsies

All osimertinib-resistant tissue biopsies were analyzed by CLIA-certified assays performed in the MGH Center for Integrated Diagnostics or Foundation Medicine using methods which have been described previously, including the MGH SNaPshot NGS panel, MGH SFA, FoundationOne NGS panel and FISH for *MET* and *EGFR* amplification[14,27]. SNaPshot uses AMP to detect single-nucleotide variants, insertions/deletions and copy number alterations in genomic DNA using the ArcherDX platform and Illumina NextSeq NGS. During this project, the SNaPshot assay platform was broadened from a 39-gene panel (NGS-V1) to a 91-gene panel (NGS-V2.) The SFA is an AMP-based platform for targeted fusion transcript detection using NGS. The list of genes covered by each assay is provided in Table S1. Tissue *MET* and *EGFR* amplification was tested by *FISH*, with amplification defined as a ratio of *MET* or *EGFR* to centromere 7 of > 2.2.

Plasma ctDNA testing

All plasma samples were analyzed by the Guardant360 NGS platform (Guardant Health, Redwood City, CA) as described previously.[28] Further details of the Guardant platform are available upon request.

Treatment with osimertinib plus BLU-667

Study of the osimertinib plus BLU-667 combination was conducted via single patient IND and clinical protocol (supplemental data) that was reviewed and approved by Food and Drug Administration and the local IRB of each site. Prior to treatment, written informed consent was obtained from each patient.

Cell culture

The PC9 and MGH134 cell lines have been previously described.[29] MGH845–1 cells were generated from a core needle biopsy of a liver metastasis from a patient progressing on osimertinib using methods that have been previously described.[16]

Generation of CCDC6-RET expressing cell lines

A *CCDC6-RET* fusion construct was synthesized by GenScript and ligated into the pLENTI6/V5-D-TOPO vector using the ViraPower Lentiviral Directional TOPO Expression Kit (Life Technologies). Lentivirus was generated by transfecting the pLENTI6 constructs and packaging plasmids into 293FT cells (Life Technologies). Virus production, collection, and infection were completed following the manufacturer's protocol. Transduced cells were selected in blasticidin (10–20 mg/mL) for one week.

Cell viability assay

For drug dose-response assays, cells were seeded into 96-well plates 24 hours before addition of drug. Cell proliferation was determined by CellTiter-Glo assay (Promega) 72–120 hours after adding drug, using standard protocols. For time-course experiments, multiple plates were seeded and drugged in identical fashion. At the indicated time points, plates were frozen at –80°C. All plates in an experiment were developed with CellTiter-Glo simultaneously. Luminescence was measured with SpectraMax i3× Multi-Mode Microplate Reader (Molecular Devices).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding: This study was supported by NCI grants 2R01CA137008 (LVS) and K08CA197389 (ANH), and funding from the American Cancer Society (ZP) and LUNGevity (ZP). Additional support was provided by Lung Strong, the Kevin Hoffman family, The Susanne E. Coyne fund, Targeting a Cure for Lung Cancer and Be A Piece of the Solution. Blueprint Medicines provided BLU-667 for both pre-clinical and clinical experiments, and ctDNA testing was supported by Guardant Health.

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STATEMENT OF SIGNIFICANCE

The role of *RET* fusions in resistant *EGFR*-mutant cancers is unknown. We report that *RET* fusions mediate resistance to EGFR inhibitors and demonstrate that this bypass track can be effectively targeted with a selective RET inhibitor (BLU-667) in the clinic.

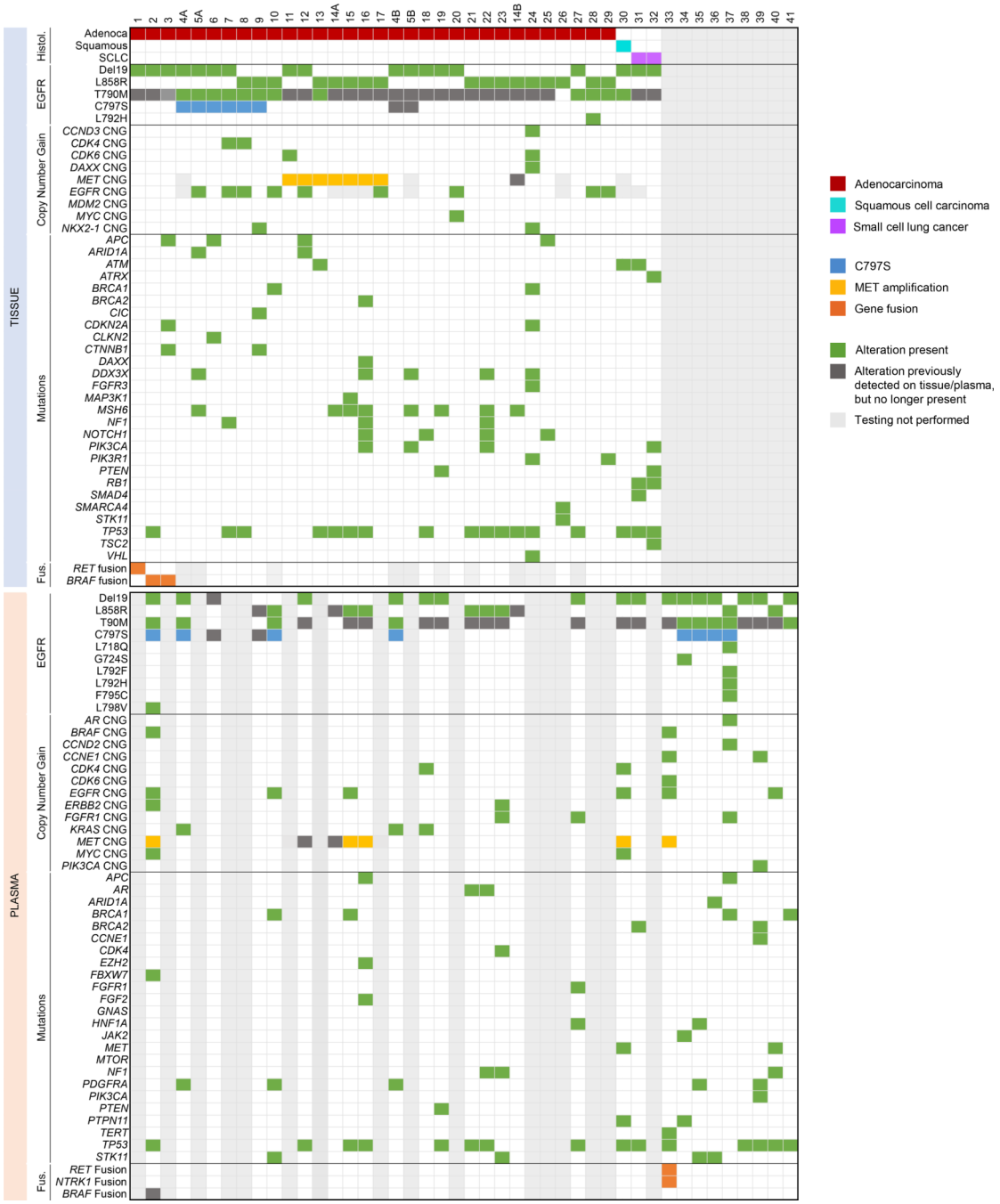

Figure 1. Summary of anatomic and molecular pathology findings from osimertinib-resistant cohort.

This heat map summarizes the findings of tissue (top) and ctDNA (bottom) analysis obtained at the time of clinical progression on osimertinib. Key resistance mechanisms are highlighted (see legend). Note that for patients with multiple tissue biopsies (4A/B, 5A/B, 14A/B), the same plasma results are shown below each tissue biopsy result.

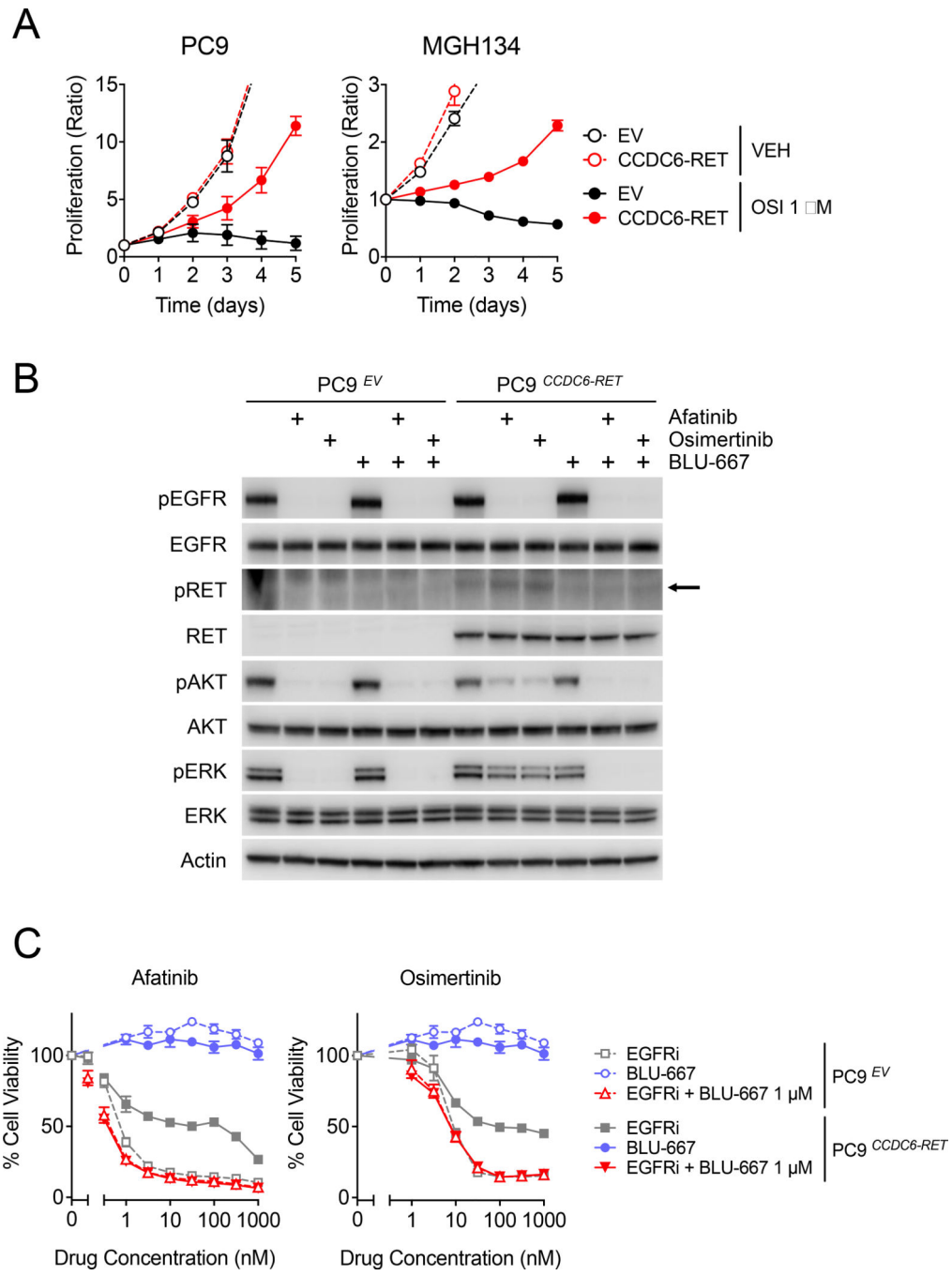

Figure 2. The CCDC6-RET fusion is sufficient for conferring resistance to EGFR-TKIs and can be overcome by combined EGFR and RET inhibition.

A, PC9 and MGH134 cells expressing the CCDC6-RET gene fusion or empty vector (EV) were treated with 1 μM osimertinib (OSI) or vehicle (VEH) and cell proliferation determined over the course of five days (ratio compared to the beginning of treatment). Data shown are the mean ± s.e.m. of three independent biological replicates. B, PC9^{EV} and PC9$^{CCDC6\text{-}RET}$ cells were treated with 100 nM afatinib, 1 μM osimertinib, BLU-667 or combinations for 6 hours and harvested for western blotting with the indicated antibodies. The arrow indicates the phospho-RET band. C, PC9^{EV} and PC9$^{CCDC6\text{-}RET}$ cells were treated

with BLU-667, or afatinib or osimertinib in the absence or presence of 1μM BLU-667 and cell viability was determined after 72 hours. The same BLU-667 data is replotted in both panels for comparison purposes. Data are shown as a percentage of vehicle treated control and are the mean ± s.e.m of three independent biological replicates.

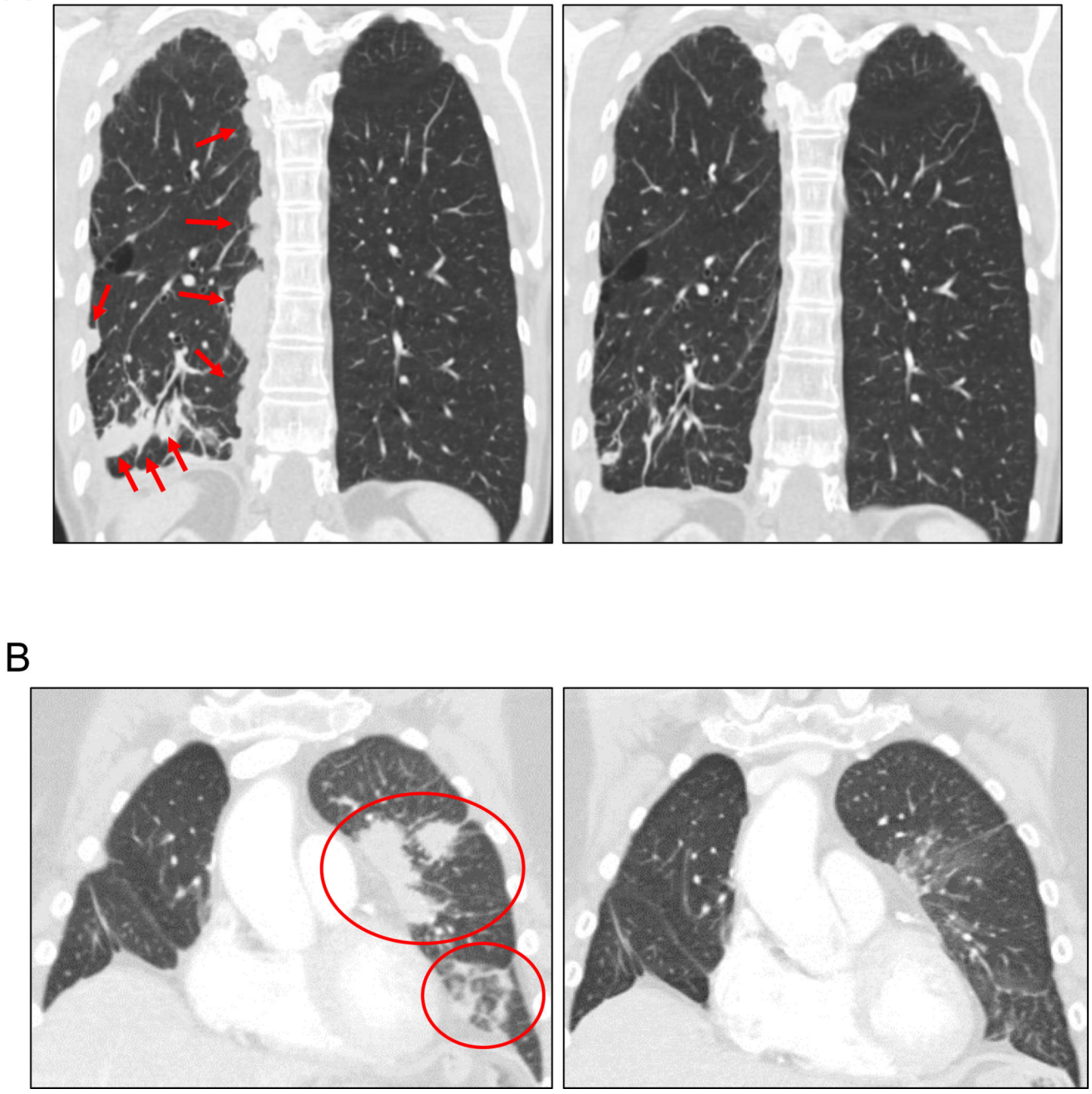

Figure 3. Responses observed in the two patients treated with osimertinib and BLU-667.

A. Treatment response of patient 1 to Osimertinib and BLU-667. Serial coronal contrast-enhanced computed-tomography images of the thorax demonstrate a right lower lobe lung mass and pleural nodularity (red arrows) seen at baseline (left) with partial response after 8 weeks of treatment with BLU-667 and osimertinib (right). B. Treatment response of patient 44 to osimertinib and BLU-667, with significant improvement in left upper and left lower lobe pulmonary opacities (right; circled) compared to baseline (left.)

Table 1.

Characteristics of the osimertinib-resistant cohort and the fusion-positive EGFR-mutant NSCLC patients.

CHARACTERISTICS OF THE OSIMERTINIB-RESISTANT COHORT (PATIENTS 1–41)	
Factor	**n(%)** ***unless otherwise noted***
Gender	
Male	15 (37)
Female	26 (63)
Age (years), Median (Range)	64 (40–87)
Founder *EGFR* Mutation	
Exon 19 Deletion	23 (56)
L858R	18 (44
Duration of Osimertinib Treatment (months)- Median (Range)	11.6 (1–32.7)
Prior lines of therapy	
0	1 (2)
1	16 (39)
2 or more	24 (59)
Treated with another 3rd gen EGFR TKI pre-osimertinib	
Rociletinib	12 (29)
Nazartinib	2 (5)
ASP8273	1 (2)
None	26 (63)
Type of Post-Osimertinib Biopsy	
Tissue only	15 (37)
Plasma only	9 (22)
Both tissue and plasma	17 (41)
Number of post-osimertinib tissue biopsies	
One	29 (91)
Two	3 (9)
CHARACTERISTICS OF THE PATIENTS WITH FUSION-POSITIVE *EGFR*-MUTANT NSCLC	

CHARACTERISTICS OF THE OSIMERTINIB-RESISTANT COHORT (PATIENTS 1–41)										
Factor						**n(%)** ***unless otherwise noted***				
Pt ID*	**Institution**	**T/P**†	**Testing**‡	**Acquired Fusion**	**Founder *EGFR* mutation**	**Treatment history prior to detection of fusion**	**T790M status**§	**Other** Molecular findings§	**Treatment after fusion detection**	**Response (RECIST 1.1)**
1	MGH	T	SFA	***CCDC6-RET***	Del19	1.Afatinib 2. Osimertinib	-	-	Osimertinib + BLU667	PR (−78%)
2	MGH	T	SFA	***PCBP2-BRAF***	Del19	1. Erlotinib 2. Carbo/Pem 3.Osimertinib	-	TP53	-	-
3	MGH	T	FO	***AGK-BRAF***	Del19	1. Erlotinib 2. Osimertinib	-	CTNNB1, APC, CDKN2A/B	-	-
33	MGH	P	G360	***CCDC6-RET+TPM3-NTRK1***	Del19	1. Erlotinib 2. Osimertinib	-	$EGFR^{Amp}$, $BRAF^{Amp}$, MET^{Amp}, $CKD6^{Amp}$, $CCNE1^{Amp}$, TP53, TERT	-	-
42	MGH	T	SFA	***CCDC6-RET***	Del19	1. Cisplatin/Pemetrexed 2. Afatinib	-	TP53	Afatinib + Cabozantinib	SD (−6%)
43	MGH	T	SFA	***BAIAP2L1-BRAF***	Del19	1. Erlotinib 2.Osimertinib 3.Carbo/ Pem, 4.Osimertinib/Gemcitabine	+	SMAD4, PTCH1, TP53	-	-
44	UC-Irvine	T	SFA	***NCOA4-RET***	Del19	1. Cisplatin/Pemetrexed (adjuvant) 2. Afatinib/Cetuximab	-	RNF43, CDKN2A	Osimertinib + BLU667	PR (−78%)

*Note: Patients 1–41 correspond to patients in the osimertinib-resistant cohort, with molecular findings shown in Figure 1. Patients 42, 43 and 44 are not included in Figure 1 because their biopsies were obtained at progression on therapies other than single-agent osimertinib.

†T- Tissue testing (from biopsies of progressing lesions); P- Plasma ctDNA testing (as indicated in next column)

‡Testing: SFA- MGH Solid Fusion Assay; FO- FoundationOne NGS Panel; G360- Guardant 360 ctDNA NGS Panel

§T790M and other molecular findings refer to the time of fusion detection.