Precision Medicine and Imaging

Landscape of EGFR-Dependent and -Independent Resistance Mechanisms to Osimertinib and Continuation Therapy Beyond Progression in *EGFR*-Mutant NSCLC

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Abstract

Purpose: Osimertinib was initially approved for T790M-positive non–small cell lung cancer (NSCLC) and, more recently, for first-line treatment of *EGFR*-mutant NSCLC. However, resistance mechanisms to osimertinib have been incompletely described.

Experimental Design: Using cohorts from The University of Texas MD Anderson Lung Cancer Moonshot GEMINI and Moffitt Cancer Center lung cancer databases, we collected clinical data for patients treated with osimertinib. Molecular profiling analysis was performed at the time of progression in a subset of the patients.

Results: In the 118 patients treated with osimertinib, 42 had molecular profiling at progression. T790M was preserved in 21 (50%) patients and lost in 21 (50%). EGFR C797 and L792 (26%) mutations were the most common resistance mechanism and were observed exclusively in T790M-preserved cases. MET amplification was the second most common alteration (14%). Recurrent alterations were observed in 22 genes/

pathways, including PIK3CA, FGFR, and RET. Preclinical studies confirmed MET, PIK3CA, and epithelial-to-mesenchymal transition as potential resistance drivers. Alterations of cell-cycle genes were associated with shorter median progression-free survival (PFS, 4.4 vs. 8.8 months, *P* = 0.01). In 76 patients with progression, osimertinib was continued in 47 cases with a median second PFS (PFS2) of 12.6 months; 21 patients received local consolidation radiation with a median PFS of 15.5 months. Continuation of osimertinib beyond progression was associated with a longer overall survival compared with discontinuation (11.2 vs. 6.1 months, *P* = 0.02).

Conclusions: Osimertinib resistance is associated with diverse, predominantly EGFR-independent genomic alterations. Continuation of osimertinib after progression, alone or in conjunction with radiotherapy, may provide prolonged clinical benefit in selected patients. *Clin Cancer Res; 24(24); 6195–203. ©2018 AACR.*

See related commentary by Devarakonda and Govindan, p. 6112

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Introduction

Osimertinib is a third-generation tyrosine kinase inhibitor (TKI) targeting EGFR (1), with significant efficacy against both classical *EGFR*-sensitizing mutations (such as exon 19 deletion or L858R mutation) and EGFR resistance T790M mutation (2). In a randomized phase III trial (3), osimertinib significantly improved both progression-free survival (PFS) and objective response rate when compared with platinum-pemetrexed chemotherapy for T790M-positive non–small cell lung cancer (NSCLC) after progression on first- or second-generation EGFR TKIs. Moreover, when compared with erlotinib or gefitinib in the first-line setting for *EGFR*-mutant NSCLC, osimertinib showed a significant PFS benefit in a phase III study (FLAURA trial; NCT02296125; ref. 4). Despite the increasing role of osimertinib for treatment of NSCLC, there is limited data regarding resistance mechanisms to this agent. Reports on individual cases or clinical series have demonstrated that *EGFR* exon 20 tertiary mutations, including C797S

Translational Relevance

The mechanisms underlying resistance to osimertinib, a third-generation EGFR inhibitor, have been investigated by several other groups. Here, we characterized the molecular landscape of osimertinib resistance in non–small cell lung cancer and clinical practices that might prolong osimertinib benefit. We found that in contrast to first-generation EGFR inhibitors, resistance mechanisms to osimertinib are predominantly EGFR independent, including MET amplification, PI3K pathway activation, and epithelial–mesenchymal transition. EGFR-dependent resistance occurs more frequently in T790M-preserved cases through C797/L792 mutations, whereas EGFR mutations can also occur in EGFR-T790M clonal depletion settings. Therefore, treatment strategies for osimertinib-resistant patients will require tailoring for these diverse subgroups. At the time of progression, continuation of osimertinib alone or in combination with local consolidative therapy was associated with clinical benefit in selected patients. Our analysis of molecular profiles and clinical outcomes of osimertinib-treated lung cancers underscored the importance of EGFR-independent resistance mechanisms to osimertinib and provided potential treatment strategies for prolonging clinical benefit in selected patients.

(5–7), MET amplification (8), ERBB2 amplification (9), small cell transformation (10), and epithelial–mesenchymal transition (EMT; refs. 10, 11), are possible drivers of resistance. Most recently, several groups reported that a subset of cases lost T790M at the time of progression (6, 12–14).

A comprehensive understanding of resistance mechanisms to osimertinib is needed in order to develop strategies to overcome osimertinib resistance. Here, we analyzed a cohort of 118 patients with advanced EGFR-mutant NSCLC treated with osimertinib at The University of Texas MD Anderson Cancer Center (MDACC) and Moffitt Cancer Center and Research Institute (MCC). We evaluated clinical and molecular data and described features that are associated with differential outcome. We also identified known and novel potential resistance mechanisms upon osimertinib progression.

Materials and Methods

Study population

Retrospective analyses were performed at MDACC and MCC. We queried The University of Texas MD Anderson Lung Cancer Moon Shot GEMINI database (MDA protocol number 13-0589), a prospective database for patients with advanced NSCLC, for patients treated with osimertinib from January 2014 to October 2017. We queried the Moffitt electronic health record, Clinical Genomic Action Committee database (an internal initiative for tumor genomic profiling across multiple tumor types), and pyrosequencing database for NSCLC patients with EGFR T790M mutation between January 2011 and December 2017 and isolated patients who were treated with osimertinib. Information on patient demographics, previous lines of therapy, survival, and current status was collected until February 2018 (MDACC) and January 2018 (MCC), when the dataset was locked for the outcome analysis. Written-informed

consents were obtained from the patients, and the studies were conducted in accordance with ethical guidelines including the Declaration of Helsinki and U.S. Common Rule. The study was approved by the Institutional Review Boards at MDACC and University of South Florida.

Statistical analysis

Time-to-treatment failure (TTF) on prior EGFR TKI was defined as time from initiation of first EGFR TKI until discontinuation of treatment due to progressive disease (PD) or toxicity. PFS 1 (PFS1) was defined as time from starting osimertinib until PD or death. For patients who continued treatment beyond progression, PFS2 was defined as time from starting osimertinib until second PD or death (15). Overall survival 1 (OS1) was defined as time from starting osimertinib until death from any cause. OS from diagnosis (OS Dx) was defined as time from diagnosis of recurrent or metastatic EGFR-mutant NSCLC until death. Patients alive at last follow-up were censored for both OS analyses. The Kaplan–Meier method was used to estimate PFS1, PFS2, OS1, and OS Dx. Exploratory subgroup analysis included cell-cycle gene alterations (e.g. CDK4/6 amplification), TP53 and T790M mutational status (previously associated with worse outcomes for EGFR TKIs; ref. 16), and central nervous system (CNS) metastasis at the time of starting osimertinib. Between-group differences were assessed through the log-rank test. HRs and 95% confidence intervals (CI) were assessed with Cox proportional hazards model. Multivariate analysis was performed by the Cox regression method.

Genomic profiling

Genomic profiling data were collected through test reports or medical charts. At MDACC, Molecular Diagnostics Labs-MDACC (MDL, 50-gene panel, Supplementary Methods) was used for tissue samples and digital-droplet PCR for blood samples. Biodesix GeneStrat, Pyrosequencing of EGFR gene, and Moffitt Illumina TruSight Tumor 26 (TST26) sequencing (Supplementary Methods) were used at MCC. Commercially available next-generation sequencing platforms such as FoundationOne (Foundation Medicine) and Guardant[360] (Guardant Health) were used at both sites (Supplementary Methods).

Cell lines, drug treatment, and Western blotting

HCC827, HCC4006, and H1975 cells were maintained in RPMI with 10% FBS. Western blot, drug treatment, and cell proliferation measurements were previously described (Supplementary Methods).

Results

Patient characteristics and efficacy of osimertinib

A total of 118 patients met the study criteria (Consort diagram shown in Supplementary Fig. S1). Median age was 63 years, 72% were female, 68% were never smokers, and 95% received previous EGFR TKI (erlotinib: 78%). Median TTF on prior EGFR TKI was 14 months (Table 1). Median follow-up on osimertinib was 13 months, with 63% alive at the time of analysis. Median PFS1 was 8.4 months (95% CI, 6.7–10.7; Fig. 1A), and median OS1 was 25.2 months (95% CI, 17.5–29.2; Supplementary Fig. S2). Three patients developed treatment-related pneumonitis, and 2 patients developed treatment-related cardiotoxicity with decreased ejection fraction.

On exploratory subgroup analysis, CNS metastasis prior to osimertinib was associated with inferior outcome (PFS1, 10.4 vs.

4.6 months; HR 1.9; 95% CI, 1.2–3.0, log-rank $P = 0.01$; Supplementary Fig. S3). Seven patients with asymptomatic CNS diseases deferred brain radiation, among those, osimertinib CNS disease control rate was 86% (6/7), with 1 patient developing

Table 1. Patient demographics and clinical characteristics

Patients	118
Age, y	
Median	63
Range	36–88
Gender, N (%)	
Male	33 (28)
Female	85 (72)
Smoking status, N (%)	
Never	80 (68)
Former	38 (32)
Current	0 (0)
Histology, N (%)	
Adenocarcinoma	116 (98)
Squamous cell carcinoma	1 (1)
Mixed	1 (1)
Disease stage, N (%)	
Recurrent	21 (18)
Metastatic	97 (82)
Performance status, N (%)	
0–1	78 (66)
2	5 (4)
3–4	2 (2)
NA	33 (28)
CNS disease, N (%)	
Yes	31 (26)
No	85 (72)
NA	2 (2)
Previous lines of therapy, N (%)	
0	6 (5)
1	45 (38)
2	36 (30)
3	15 (13)
$\geq$4	16 (14)
Previous EGFR TKI treatment, N (%)	
Erlotinib	92 (78)
Other	20 (17)
None	6 (5)
TTF on previous TKI, months	
Median	14
Range	3–88
Previous cytotoxic chemotherapy, N (%)	
Yes	54 (46)
No	64 (54)
Previous immunotherapy, N (%)	
Yes	16 (14)
No	102 (86)
Overall response rate, N (%)	
Deep response	1 (1)
Overall response	59 (50)
Stable disease	31 (26)
PD	24 (20)
NA	3 (3)
Cause for PFS1, N (%)	
Disease progression	76 (64.4)
Death	11 (9.3)
No event	31 (26.3)
Site of progression, N (%)	
Systemic	66 (87)
CNS	7 (9)
Both	3 (4)
Status at analysis, N (%)	
Alive	74 (63)
Dead	44 (37)

Abbreviation: y, years.

leptomeningeal disease. Cell-cycle gene alterations at the time of PD were associated with worse outcome when compared with wild-type (PFS1, 8.8 vs. 4.4 months; HR 2.8; 95% CI, 1.2–6.4, log-rank $P = 0.01$, Fig. 1D; Supplementary Methods), whereas TP53 mutation and T790M loss were not (Supplementary Fig. S4)

Subsequent treatment after progression on osimertinib

Osimertinib was continued beyond PD in 62% of patients (47/76). Median PFS2 was 12.6 months (95% CI, 8.3–15.5; Supplementary Fig. S5), with 10 patients (21%) without an event at data cutoff (Table 2). Among the osimertinib-continued patients, 21 (45%) received palliative radiation for oligometastatic progressing lesions (Table 2), which was the most common practice in this cohort. There was a trend for improved outcomes in the radiated population compared with nonradiated patients (PFS2, 15.5 vs. 8.2 months; HR, 0.5; 95% CI, 0.3–1.0, log-rank $P = 0.05$, Supplementary Fig. S6) with PD occurred in 62% (13/21) of radiated and 77% (20/26) of nonradiated patients. Of the 21 patients who received radiotherapy, the most commonly radiated sites were lung, mediastinal lymph nodes, bone, and brain. Two patients received radiation only to the brain (one with stereotactic radiosurgery, also known as GammaKnife, the other with whole brain radiation therapy). Eight had radiation to bone metastases, 8 to the lung, and 5 to lymph nodes, with three 3 having radiation to more than one organ. One case received radiation to a hepatic lesion. Majority of the cases (13/21, 62%) had radiation only to one site (Supplementary Table S1).

For 1 patient (2%), bevacizumab was added to the treatment regimen, and best response was stable disease (Pt. 38, Supplementary Table S2) for 8 months. Two patients developed C797S mutation and were treated with osimertinib and first-generation inhibitors. The combination was well-tolerated. One patient had disease progression after 4 months (gefitinib), and the other died from influenza pneumonia without response assessment (erlotinib). Twenty-six patients discontinued osimertinib at the time of progression, and 24 (92%) received additional lines of therapy. The most commonly used agents were PD-1/PD-L1 checkpoint inhibitors (10/24; 41.7%) and chemotherapy (8/24; 33.3%; Table 2). When compared with osimertinib-continued patients, the discontinued group had shorter survival (OS, 11.2 vs. 6.1 months; HR, 0.45; 95% CI, 0.2–0.9, log-rank $P = 0.02$, Fig. 1C). This difference is likely a result of multiple contributing factors, including difference in tumor biology, extent of disease progression, and physician/patient preference.

With median follow-up of 39 months, OS Dx for the entire cohort was 71.9 months (95% CI, 51.4 – 92.1; Fig. 1B), a strikingly long survival compared with median OS of 19 to 28 months with first- or second-generation EGFR TKIs (17–19).

Potential mechanisms of resistance to osimertinib

In our cohort, 6 patients received osimertinib as their first-line treatment, including four cases with inferred germline T790M due to near 50% allelic frequency and two with baseline T790M (Supplementary Table S3). Genomic profiling at the time of progression was available for 42 patients (Supplementary Table S2). Majority of the genetic tests (32/42) were obtained at the time of first progression (PFS1) or at both first and second progression (3/42; Supplementary Table S4). Of the acquired T790M cases, 47% (19/40) preserved and 53% (21/40) lost T790M at progression (Fig. 2A). Both T790M-preserved and -loss groups had similar frequency with next-generation sequencing–
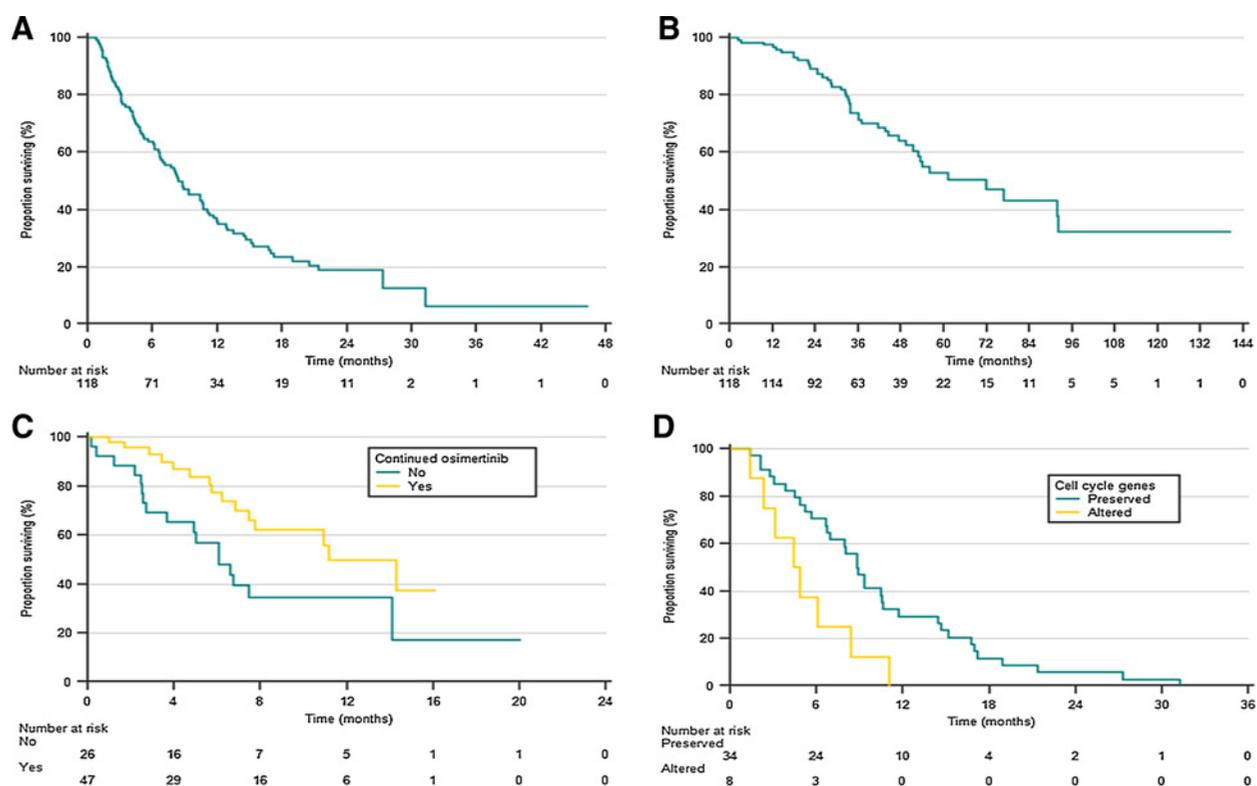
Figure 1.
Kaplan–Meier estimates of survivals for osimertinib-treated patients and subgroups. **A,** PFS1 8.8 months (95% CI, 6.7–10.7). **B,** OS Dx was 71.9 months (95% CI, 51.4–92.1). **C,** OS1 continued versus discontinued osimertinib 11.2 months versus 6.1 months (HR, 0.45; 95% CI, 0.2–0.9, log-rank $P = 0.02$). **D,** PFS1 in patients with preserved and altered cell-cycle gene alterations, 8.8 months versus 4.4 months (HR, 2.8; 95% CI, 1.2–6.4, log-rank $P = 0.01$).

based paneled tests (14/19 vs. 15/21, $\chi^2 P = 0.87$). In 1 patient, two genetic profiling results (4 weeks apart) showed discrepancy between T790M status (Pt. 4, Supplementary Table S2).

In the T790M-preserved group, previously defined osimertinib-resistant *EGFR* mutations (11/19; C797S: 8; C797G: 1; L792H: 2; refs. 20, 21) were the most common resistance mechanisms. These previously defined osimertinib-resistant *EGFR* mutations were exclusively observed in T790M-preserved cases; this association was statistically significant (11/19 vs. 0/21, $P < 0.001$, Fisher exact test; Fig. 2B vs. C). Other than C797S/L792 mutations, we observed other acquired *EGFR* mutations (Fig. 2A–C; Supplementary Table S5). Among those, 2 co-occurred with germline T790M (Pts. 20, 21, Supplementary Table S2), and 6 were acquired after osimertinib. We performed *in silico* predictions (Combined Annotation Dependent Depletion score, CADD) to determine their potential oncogenic function. All variants had CADD $\geq$ 20, which indicated high likelihood of biological function (among the 1% most deleterious mutations for the *EGFR* gene, Supplementary Table S5). The four cases where acquired *EGFR* mutations emerge when T790M was lost were particularly interesting, as some of the resulted changes might be overcome by first- and/or second-generation EGFR inhibitors. L718 is located within the p-loop and directly interacts with bound inhibitors. Substitution of leucine for glutamine was shown to result in steric hindrance reducing binding ability of third-generation EGFR TKI, such as WZ4002 (20), and lead to resistance. However, *in vitro* work has also demonstrated that

L718Q may still respond to first- and second-generation EGFR inhibitors, especially when T790M is lost, as is the case for the patient No. 29 (20). G724S also resides in the p-loop, and previous studies have shown that this mutation results in resistance to osimertinib in the presence of T790M (22). Other mutations shown to prevent inhibitor stabilization include L844V where the shortening of amino acid prevents hydrophobic interactions with WZ4002 (20). Similarly, we predict that V834L which results in a larger hydrophobic amino acid at V834 may result in steric hindrance of the drug near the anisole (methoxybenzene) group.

MET amplification has been reported as a mechanism of resistance to gefitinib (8) and osimertinib (23). In our cohort, it was identified in T790M-preserved (5/19) and T790M-loss (1/21) cases, and the second most common resistance mechanism after *EGFR* tertiary mutations (5/42, 14%). We cultured HCC827 cells (*EGFR Del745_760*) in erlotinib to develop EGFR TKI resistance and identified one clone (ER2) having increased c-Met protein expression and *MET* copy-number gain (Fig. 3A and B). This clone was resistant to osimertinib (Supplementary Fig. S7A) without resistant mutations in the *EGFR* exon 20 (Supplementary Fig. S7C), but exquisitely sensitive to c-Met inhibitors (tepotinib, EMD Serono; Fig. 3C) and crizotinib (Supplementary Table S5), suggesting that c-Met inhibition can overcome *MET* amplification–mediated osimertinib resistance. Among *MET*-amplified patients, *CDK6* (4/5) and *BRAF* amplification (3/5) were common events (Fig. 2A; Pts. 1, 4, 5, 9, and 15,

Table 2. Treatment beyond progression on osimertinib

Continued osimertinib beyond progression (total 76), N (%)	
Yes	47 (62)
No	26 (34)
Not evaluable	3 (4)
Additional treatment with osimertinib continuation (total 47), N (%)	
Radiation	21 (45)
Cryoablation	1 (2)
Osimertinib 160 mg/day	1 (2)
Bevacizumab	1 (2)
Erlotinib	1 (2)
Gefitinib	1 (2)
Nivolumab	1 (2)
None	20 (43)
Cause for PFS2 (total 47), N (%)	
Disease progression	33 (70)
Death	4 (9)
No event	10 (21)
Additional treatment without osimertinib (total 26), N (%)	
Yes	24 (92)
No	2 (8)
Types of additional treatment without osimertinib (total 24), N (%)	
PD-1/PD-L1 checkpoint inhibitors	10 (41.7)
Chemotherapy	8 (33.3)
Phase I/II clinical trials	3 (12.5)
TKI	2 (8.3)
Radiation	1 (4.2)

Supplementary Table S2). Because of the relative proximity of the three genes in chromosome 7q (*CDK6* at Chr7q21.2, *MET* at Chr7q31.2, and *BRAF* at Chr7q34), it is possible that these amplifications arose from a single genomic event.

The 21 cases with T790M loss had more diverse genetic alterations. One tumor with preexisting *RB1/TP53/PIK3CA* alterations underwent neuroendocrine transdifferentiation (Pt. 27, Supplementary Table S2). Two cases acquired *PIK3CA E418K/E542K* and *E542K/E545K* mutations (Pts. 35 and 40, Supplementary Table S2), two cases acquired KRAS alterations, one with *CDK4/KRAS/MDM2* amplification and the other with *KRAS Q61R* mutation (Pts. 22 and 39, Supplementary Table S2), one had *FGFR3/FGFR19* amplification (Pt. 32, Supplementary Table S2), one had *MET* amplification (Pt. 36; FISH ratio: 7.4, Supplementary Table

S2), and one had ERBB2 amplification (Pt. 40, Supplementary Table S2). The remaining cases had unknown resistance mechanisms (Fig. 2A; Supplementary Table S2).

We overexpressed *PIK3CA E545K*, *BRAF*, and *FGFR2b* in HCC4006 cells (*EGFR Del747_749, A750P*) and evaluated response to osimertinib. We found that *PIK3CA E545K* overexpression confers moderate resistance to osimertinib (IC$_{50}$ increased from 25 nmol/L to 67 nmol/L, $P < 0.01$), whereas *BRAF*, *FGFR2b*, and *PDGFR* overexpression did not significantly alter osimertinib sensitivity (Fig. 3D). The data suggest that acquired *PIK3CA* hotspot mutations may contribute to osimertinib resistance, and that *BRAF*, *FGFR*, or *PDGFR* amplifications may be "passenger" events instead of "drivers," or may not be able to promote resistance alone without additional concurrent alterations.

There were 14 cases without potential resistance genetic drivers, 12 in T790M-preserved group, which highlights our limited understanding of T790M-negative resistance mechanisms. One case with preexisting *TP53* mutation and *RB1* loss displayed neuroendocrine transformation (Pt. 27; Supplementary Table S2). EMT has also been indicated as one mechanism of resistance to EGFR TKIs (10). We observed an *AXL* mutation in one case with acquired T790M/C797S (Pt. 12; Supplementary Table S2; ref. 24), suggesting association with EMT. In cell lines, we observed E-cadherin loss and vimentin gain in HCC827 EGFR TKI–resistant cells (HCC827-ER1; Fig 3A) and in H1975 osimertinib-resistant cells (H1975-OR3 and OR13, Fig. 3E; Supplementary Fig. S7B), indicating that EMT could be a potential resistance mechanism to osimertinib. Stress hormone–induced beta-adrenergic pathway activation has recently been identified as a driver of resistance to first-generation TKIs and can be overcome by beta-adrenergic blockade (ref. 25; Supplementary Fig. S7D); therefore, we hypothesize that stress hormones may induce resistance in osimertinib-treated lung cancers. In HCC827 cells, norepinephrine (10 μmol/L) significantly increased proliferation in the presence of osimertinib (from 10 to 50 nmol/L), suggesting resistance (red line, Fig. 3F). Propranolol (1 μmol/L), a nonselective beta-adrenergic blocker, restored osimertinib sensitivity in HCC827 cells (gray line, Fig. 3F), supporting that stress
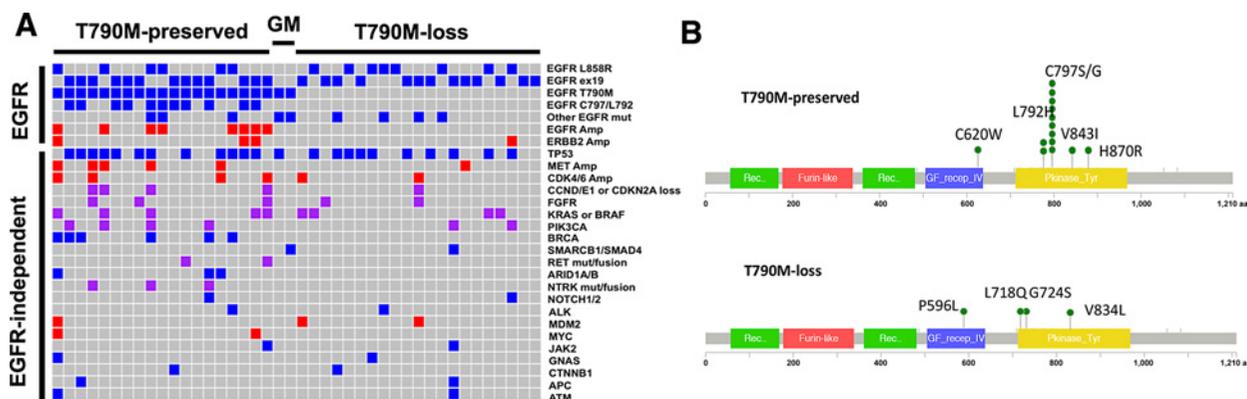
Figure 2.
Recurrent genomic alterations associated with osimertinib resistance. **A,** Co-occurring mutations for 42 cases having genetic profiling at progression to osimertinib. Blue box, mutations; red box, amplification; purple box, mutation and/or amplification. Amp, amplification; GM, germline T790M; mut, mutation. **B,** EGFR tertiary mutations in T790M-preserved cases (top) and in T790M-loss cases (bottom).

Le et al.
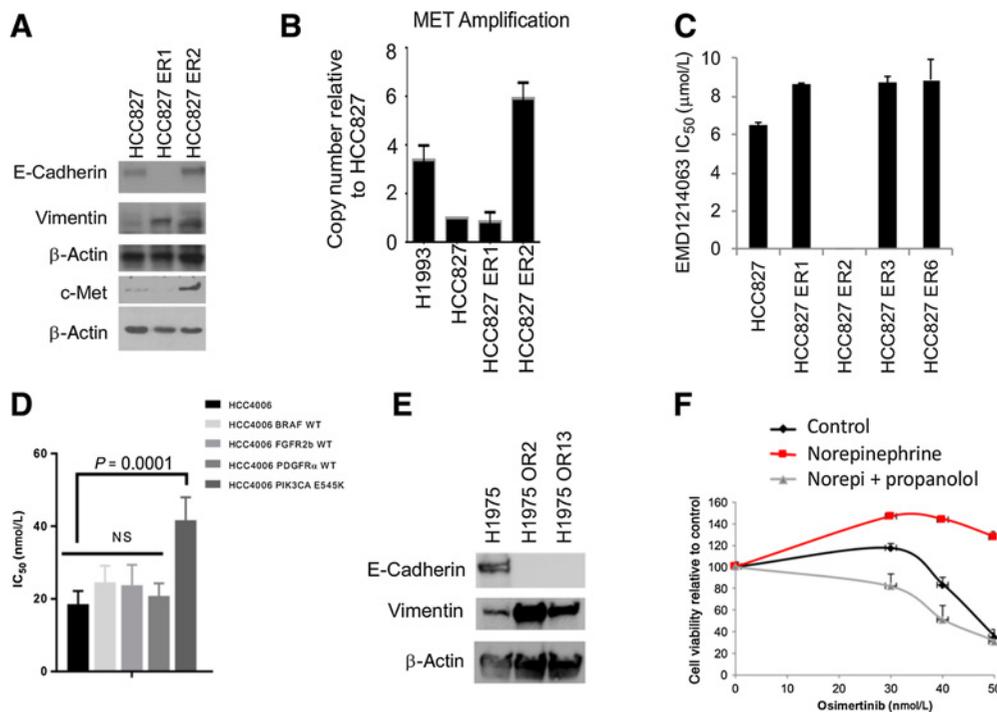
Figure 3.
EGFR-independent potential mechanisms of resistance. **A,** HCC827 parental cells and cells with acquired resistance to EGFR inhibitors were tested for protein levels of E-cadherin, vimentin, and c-Met. **B,** HCC827 parental cells and cells with acquired resistance to EGFR inhibitors were evaluated for MET copy-number changes. H1993 cells were used as positive control. **C,** MET small-molecule inhibitor (EMD 1214063) IC_{50} in HCC827 parental cells and cells with acquired resistance to EGFR inhibitors. **D,** Osimertinib IC_{50} to HCC4006 cells with overexpression of BRAF, FGFR2b, PDGFRa, and PIK3CA E545K. NS, not significant; WT, wild type. **E,** H1975 parental cells and cells with acquired resistance to osimertinib (OR2 and OR13) were tested for protein levels of E-cadherin and vimentin. **F,** Norepinephrine (1 μmol/L) increases HCC827 cell proliferation in the presence of osimertinib, and propranolol (1 μmol/L) overcomes such resistance.

hormones induce proliferation in the presence of osimertinib. Furthermore, such resistance can be overcome through beta-adrenergic blockade in *EGFR*-mutant *T790M*-negative lung cancer cells. Taken together, these preclinical data provide evidence that EGFR-independent mechanisms of resistance observed for first- and second-generation EGFR TKIs (*MET* amplification, *PIK3CA* mutation, EMT, and beta-adrenergic activation) may promote osimertinib resistance as well.

Platform-matched samples in pre- and post-osimertinib treatment are especially valuable to understand acquired genetic events that lead to clinical resistance. Among the 42 cases, we identified 18 cases having platform-matched profiling before and at the time of progression to osimertinib treatment (Supplementary Table S7 and Supplementary Fig. S8), 11 by Guardant[360], 1 by FoundationOne, and 6 by MD Anderson Molecular Diagnostic Laboratory test (MDL, Supplementary Methods). Although small, this platform-matched paired sample cohort is most informative for understanding acquired resistance mechanisms. Of the 18 cases, 9 (50%) preserved T790M with 6 acquired *EGFR C797S/L792H* mutations and one with concurrent *EGFR* amplification (Pt. 10). One patient acquired *NCOA4-RET* fusion as well as *EGFR* and *CDK4* amplification (Pt. 19), which might represent different resistant clones. Only two cases with preserved T790M did not acquire new resistant genetic alterations. Nine cases lost the T790M-resistant clones at the time of progression. Four did not acquire any new genetic alterations. One case lost T790M but acquired *EGFR*

V834L (Pt. 34). One case acquired coamplification of *FGF3/FGF19/EMSY* at chromosome 11q13.3-13.5 locus (Pt. 32). One acquired *PIK3CA E418K/E542K* mutations (Pt. 35); one acquired *TSC1 E1044fs*, and one with *ERBB2 R340Q*. The 18 platform-matched cases represent the common resistance mechanisms seen in our 42 cases cohort, confirming that the T790M-preserved cases frequently use EGFR reactivation or bypass pathway for resistance, whereas T790M-loss cases engage in EGFR-independent mechanisms for resistance.

We also investigated cases in which there was a discrepancy in the next-generation sequencing results from blood ctDNA and tumor biopsies, to determine whether these may represent somatic mutations from hematopoietic stem cells instead of NSCLC tumor cells, otherwise known as clonal hematopoiesis (26). There were four cases having both Guardant[360] tests for more than one time point, and a tissue test (Pts. 28, 35, 39, and 42). In three of these cases, mutations' potential resulting from clonal hematopoiesis was observed: *TP53 C135W* in Pt. 28, *JAK2 V617F* in Pt. 35, and *MYC N353S* in Pt. 42 (Supplementary Table S7). Both *JAK2 V617F* and *TP53* mutations are known to be common mutations in clonal hematopoiesis (27). The data are consistent with the recent report from Circulating Cell-Free Genome Atlas study showing that up to 54% of the somatic mutations detected in the blood samples from lung cancer patients were from hematopoietic cells' somatic mutations, but not from tumors (28). This real-world experience highlighted that thoracic oncologists need to be

aware of the phenomenon of clonal hematopoiesis when interpreting blood biopsy reports for lung cancer patients.

Discussion

In this study, we reviewed a cohort of patients with advanced NSCLC treated with osimertinib at MDACC and MCC. Patients who continued osimertinib beyond progression had improved clinical outcomes compared with those who discontinued it. Due to the nature of retrospective analysis, this result may be affected by selection bias, where physicians had to choose discontinuation of osimertinib in cases with obvious progression and clinical deterioration. Nevertheless, the practice of continuation of TKI beyond progression is supported by previous reports of patients with indolent and small volume progression benefiting from continuation of TKIs (15, 29). The biological mechanism underlying this observation is hypothesized to be that in the case of localized disease progression, the drug may continue to suppress the majority of tumor cells; furthermore, compensatory pathways may be upregulated during treatment, and drug withdrawal may therefore result in a "tumor flare." In our cohort, about half of cases that continued osimertinib also received local consolidation radiation to the progressing sites. This practice is supported by randomized phase II trials showing that aggressive local consolidation therapy (LCT) increases time to progression compared with maintenance or observation in patients with oligometastatic NSCLC (30). The rationale for this approach is that local consolidation therapies decrease the resistant subclone tumor burden, whereas continuation of osimertinib suppresses sensitive cells. We are currently evaluating the role of LCT in *EGFR*-mutant lung cancers treated with osimertinib in a randomized phase II study (NCT03410043).

We then analyzed genomic profiling data at the time of osimertinib progression. We found that cell-cycle gene alterations (*CDK4/6* or *CCND/E1* amplifications, or *CDKN2A* loss) were associated with worse outcome (31), highlighting the need for novel therapeutic options for these patients. In our cohort, we identified both known and novel resistance mechanisms to osimertinib that, based on prior studies, may be divisible into three groups: (1) reactivation of EGFR pathway through tertiary mutations; (2) activation of known bypass signaling pathways, such as *MET* or *ERBB2* amplifications, or *PIK3CA* mutation; and (3) alterations that likely promote "rewiring" and diminished dependence on EGFR or bypass signaling (e.g., EMT, SCLC, etc.). In our cohort, tertiary *EGFR* mutations were the most common resistance mechanism (11/42, 26%). As for bypass signaling, *MET* amplification was observed in both T790M-preserved and T790M-loss cases, and the second most common resistance mechanism (6/42, 14%). Other than *MET*, we also observed cases with amplification or mutations in other possible driver oncogenes, including *PIK3CA*, *BRAF*, *FGFR*, and *PDGFR*. In our preclinical models, *PIK3CA* hotspot mutation, but not *BRAF*, *FGFR*, and *PDGFR* overexpression alone, was found to contribute to osimertinib resistance. Whether *PIK3CA* mutations determine treatment response to EGFR TKIs remains controversial (32, 16, 33, 34, 35). The role of PI3K pathway activation in osimertinib-treated resistance *EGFR*-mutant lung cancer warrants further evaluation. Other than oncogene driver–mediated resistance, we showed that neuroendocrine and mesenchymal cellular rewiring and cytokine changes in the tumor microenvironment can also affect tumor cell's sensitivity to therapy. We found that epinephrine reduces the sensitivity of *EGFR*-mutant cells to osimertinib *in vitro*. Beta-adrenergic blockade with propranolol reversed this resistance phenotype. These data highlighted the importance of understanding T790M-negative resistance mechanisms, with a focus on cytokine-mediated changes.

Our real-world study has several limitations due to its retrospective nature. In the clinical outcome analysis, the disease progression was based on physician assessment and not specific formal criteria. Therefore, the benefit of continuation of osimertinib beyond progression must be validated in future prospective studies, several of which are ongoing. In the genetic profiling analysis, in some cases different platforms were used for the pretreatment and postprogression profiling; however, analysis of the platform-matched cases shows similar results as the overall analysis. In addition, the majority of the patients in our cohort received osimertinib as second or later lines of therapy (Table 1), with only 6 patients with baseline EGFR T790M or germline T790M receiving osimertinib as the first-line treatment (Supplementary Table S3). As osimertinib is increasingly being used as first-line therapy, additional studies will be needed to determine whether similar alterations are associated with osimertinib resistance in the first-line, TKI-naïve setting.

Despite these limitations, our cohort is one of the largest analyzing both real-world clinical outcomes of osimertinib-treated *EGFR*-mutant NSCLC and genetic profiles associated with resistance. We showed that many patients with EGFR-mutant NSCLC might have prolonged benefit from osimertinib beyond their initial progression event, especially when radiation was used at progression, suggesting a potential role for local consolidative therapy in this setting. Furthermore, we showed that T790M loss is common in osimertinib-resistant cases and that these cases demonstrated a different pattern of resistance compared with the T790M-preserved cases. Specifically, in most of the T790M-preserved cases, resistance is associated with continued EGFR activation through known resistance tertiary mutations (e.g., C797S) or activation of bypass signaling pathways, whereas resistance in T790M-loss cases occurs through diverse and predominantly EGFR-independent mechanisms. Given this pattern, and the effectiveness of osimertinib at suppressing T790M-positive subclones, it is conceivable that resistance to osimertinib in the first-line setting will also be associated with a greater frequency of EGFR-independent mechanisms. These issues will require further study; nevertheless, given the increasing role for osimertinib in the treatment of *EGFR*-mutant advanced NSCLC, these data represent an important step for understanding resistance to this agent and developing subsequent treatment strategies.

Disclosure of Potential Conflicts of Interest

and is a consultant/advisory board member for AstraZeneca, Novartis, Genentech, Spectrum, and Boehringer Ingelheim. No potential conflicts of interest were disclosed by the other authors.

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Landscape of EGFR-Dependent and -Independent Resistance Mechanisms to Osimertinib and Continuation Therapy Beyond Progression in *EGFR*-Mutant NSCLC

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