Clinicopathologic Characteristics, Treatment Outcomes, and Acquired Resistance Patterns of Atypical *EGFR* Mutations and *HER2* Alterations in Stage IV Non–Small-Cell Lung Cancer

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Abstract

The outcomes for atypical *EGFR* and *HER2* alterations remain underexplored. We performed a single-center retrospective study to determine unique clinical characteristics, treatment outcomes, and resistance mutations among this subgroup. Patients with atypical *EGFR* and *HER2* alterations have a unique metastatic phenotype with higher rate of synchronous lung and bone metastases, different proportion of resistance mutations relative to typical *EGFR* mutations, and poorer responses to targeted and immunotherapies. Novel approaches are urgently needed.

Background: The clinicopathologic characteristics, acquired resistance patterns, and outcomes among patients with atypical *EGFR* mutations and *HER2* alterations remain underexplored. **Patients and Methods:** A single-center retrospective review was conducted. Oncogenes assessed include typical *EGFR* (t-EGFR; exon 19 del and L858R), atypical *EGFR* (a-EGFR; G719X, exon 20, L861Q), *HER2* (exon 19, exon 20, amplifications), gene fusions (*ALK*, *ROS1*, *RET*), *RAS* (*KRAS*, *NRAS*), and *RAF* (*BRAF* V600E). Progression-free survival (PFS), overall survival (OS), disease control rate, and objective response rate (Response Evaluation Criteria in Solid Tumors 1.1) were collected. **Results:** Among 570 patients, we found 55 a-EGFR mutations (13 G719X, 38 exon 20, 4 L861Q) and 31 HER2 alterations (2 exon 19 mutations, 27 exon 20 insertions, 2 amplifications). Patients with *EGFR* and *HER2* alterations had increased lung and bone metastases relative to patients with gene fusions, *RAS*/*RAF* mutations, and no identified driver oncogenes ($P < .001$). Patients with *EGFR* exon 20 insertions had a median PFS to EGFR tyrosine kinase inhibitors (TKIs) of 5 months and an OS of 16 months—significantly worse than exon 19 del and L858R (Bonferroni correction; $P < .001$), but not G719X or L861Q. Relative to t-EGFR mutations, T790M and *MET* amplification occurred less frequently as acquired resistance mechanisms among a-EGFR samples ($P < .001$). Ten patients with a-EGFR mutations and HER2 alterations received single-agent immune checkpoint inhibitors (ICIs) with no radiographic responses and a median PFS of 2 months. **Conclusion:** *EGFR* and *HER2*-mutated NSCLC have a high rate of synchronous lung and bone metastases. Patients with a-EGFR mutations have inferior responses to EGFR-directed therapies with lower rates of acquired T790M and *MET* amplification. Responses to ICIs are uniformly poor. Novel therapeutic approaches are needed.

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Introduction

The identification of activating mutations in the epidermal growth factor receptor (*EGFR*) gene and the development of tyrosine kinase inhibitors (TKIs) that selectively target these mutations has revolutionized the management of patients with non–small-cell lung cancer (NSCLC).[1-5] Most clinical trials evaluating EGFR TKIs have only included patients harboring typical drug-sensitive *EGFR* mutations, specifically exon 19 deletions and the exon 21 L858R substitution.[6-16] Collectively, these mutations (hereafter referred to as typical EGFR mutations; t-EGFR) represent approximately 80% to 85% of all *EGFR* mutations.[1-4,17,18] The remaining 15% to 20% of *EGFR* mutations (hereafter referred to as atypical; a-EGFR) are largely composed of point mutations (G719X and L861Q) or exon 20 insertion mutations.[17-23]

The *ERBB2* gene (encoding the HER2 protein) is also a proto-oncogene that can activate downstream signaling through PI3K-AKT and MEK-ERK pathways.[24] In contrast to other members of the ErbB family, no ligand has been described for HER2, which is activated by either homodimerization or heterodimerization with other members within the ErbB family. HER2 alterations have been identified as oncogenic drivers in 2% to 3% of NSCLC.[25-27] The most common HER2 alteration is a 12 bp in-frame insertion (encoding YVMA) in exon 20. Preclinical studies have shown that mutations in the kinase domain result in constitutive phosphorylation and activation of the HER2 receptor.[26-30] Additionally, increased signalling through *ERBB2/HER2* gene amplification is another important HER2 alteration, though importantly, gene amplification may be defined in different ways (eg, absolute mean copy number per cell or the ratio of gene to the centromere on the same chromosome). As a continuous variable, the threshold for defining positivity has not yet been established in NSCLC.[31]

Patients with t-EGFR mutations have high objective response rates (ORR) and meaningful progression-free survival (PFS) to first-generation reversible EGFR TKIs (eg, erlotinib, gefitinib),[9,10,12,14] second-generation irreversible TKIs (eg, afatinib, dacomitinib),[6,11,15,16] and third-generation irreversible TKIs (eg, osimertinib).[8,13] In contrast, patients with a-EGFR and HER2 alterations have experienced variable efficacy with EGFR- and HER2-directed therapies.[21,23,25,26,32-34] The role of immune checkpoint inhibitors (ICIs) alone or with chemotherapy in patients with *EGFR* and *HER2*-mutated NSCLC continues to evolve.[29,30,35-38]

In this retrospective study, we assessed the clinicopathologic characteristics of patients with a-EGFR mutations and HER2 alterations, treatment outcomes of patients with a-EGFR mutations treated with TKIs, acquired resistance patterns among a-EGFR mutations, outcomes of patients with HER2 alterations treated with HER2-directed therapy, and outcomes of patients with a-EGFR mutations and HER2 alterations treated with ICIs (either as single agent or in combination with platinum doublet chemotherapy).

Patients and Methods

Patients and Clinical Characteristics

All patients with NSCLC (according to the American Joint Committee on Cancer (AJCC) 8th edition of the tumor, node, metastasis classification system) evaluated at University of Colorado from June 2009 to March 2019 were eligible for assessment. Patients were identified through a database that included all patients treated through the University of Colorado health system through an institutional review board–approved protocol. The patient population contained a mix of patients who were diagnosed and treated within the University of Colorado health system and patients who were treated elsewhere and subsequently referred to our institution.

Outcomes data and imaging results were collected by retrospective chart review. We captured age, sex, smoking status, histology, tumor grade, clinical stage, and metastatic sites. EGFR and HER2 subtypes were recorded and included t-EGFR mutations (exon 19 deletion, L858R), a-EGFR mutations (G719X, exon 20 insertions, L861Q), and HER2 alterations (exon 19 mutations, exon 20 insertions, and *HER2* gene amplifications defined as *HER2/CEP17* $\geq$ 3). Where available, we identified comutations and programmed death ligand 1 (PD-L1) expression at diagnosis.

We performed a formal analysis to look for statistically significant differences in the patterns of metastatic spread between oncogene groups including mutations within the ErbB family (*EGFR* and *HER2*), gene fusions (*ALK*, *ROS1*, and *RET*), mutations within the Ras/Raf pathway (K-RAS, N-RAS, and BRAF V600E), and other mutations (no identifiable oncogene driver). Metastatic sites considered for this analysis included the lung (contralateral lung metastases), pleura (either pleural plaques or malignant effusions), brain, bone, liver, and the adrenal glands.

Only responses and clinical outcomes from the first EGFR- or HER2-directed therapy that each patient received were considered. EGFR agents considered for this study included first-generation TKIs (erlotinib, gefitinib), second-generation TKIs (afatinib, dacomitinib), and third-generation TKIs (osimertinib). Patients with a-EGFR mutations enrolled onto clinical trials were excluded from this retrospective review. Outcomes from ICIs (pembrolizumab, nivolumab, or atezolizumab) as single agents or in combination with chemotherapy (using KEYNOTE-189 regimen) were captured. PFS was defined as time from initiation of therapy to time of radiographic progression (as defined by Response Evaluation Criteria in Solid Tumors [RECIST] 1.1) or death. Overall survival (OS) was defined as time from initiation of therapy to time of death.

Given variability in follow-up, we captured RECIST responses on the basis of first scan after starting EGFR- or HER2-directed therapy. ORR represents the sum of partial response (PR) and complete response (CR). Disease control rate (DCR) represents the sum of stable disease (SD), PR, and CR. In defining acquired resistance to EGFR TKI, we utilized criteria proposed by Jackman et al.[39] When available, resistance mutations at the time of progression were captured utilizing combination of circulating tumor DNA (Guardant Health, Redwood City, CA) or tissue biopsy samples for only those patients with an objective ORR or a PFS greater than 6 months. Patients with a secondary cancer receiving active cytotoxic treatment, nonmetastatic NSCLC, or incomplete molecular or clinical documentation were excluded.

Molecular Methods

Molecular testing was conducted via Clinical Laboratory Improvement Amendments–certified laboratories and was

Table 1 Clinicopathologic Characteristics of Patients With Atypical EGFR Mutations and HER2 Alterations

Characteristic	a-EGFR (N = 55)	HER2 (N = 31)
Sex		
Male	17 (31)	10 (32)
Female	38 (69)	21 (68)
Race		
White	39 (71)	20 (65)
Black	3 (5)	0
Asian	7 (13)	1 (3)
Hispanic	0	2 (6)
Other	6 (11)	8 (26)
Age (y), median (range)	62 (38-91)	62 (47-80)
Smoking Status (pack-years)		
Never	32 (58)	23 (76)
Light (≤10)	7 (13)	3 (8)
Heavy (>10)	16 (29)	5 (16)
Histology		
Adenocarcinoma	53 (96)	30 (97)
Adenosquamous	1 (2)	1 (3)
Squamous	1 (2)	0
Stage at Diagnosis		
I	4 (8)	0
II	2 (4)	1 (3)
III	2 (4)	3 (10)
IV	47 (85)	27 (87)
Brain metastases[a]	16 (27)	9 (29)

Data are presented as n (%) unless otherwise indicated. All disease was staged by magnetic resonance imaging.
Abbreviation: a-EGFR = atypical EGFR.
[a]Brain metastases at time of stage IV disease.

performed using laboratory assays that were independently validated. Over the course of time of this study, the testing approach for standard-of-care laboratory testing evolved, and therefore testing was performed using a variety of assay platforms. These approaches included the following: Sanger sequencing of relevant targeted regions, single nucleotide base extension assay (SNaPshot), real-time polymerase chain reaction, targeted next-generation sequencing (NGS) using a 26-gene panel (TruSight; Illumina, San Diego, CA), or Archer VariantPlex/FusionPlex Solid Tumor library preparation kit (ArcherDx, Boulder, CO) with raw sequence data analyzed by Archer Analysis 4.1.1.7 software (ArcherDx).

For internally tested cases, fluorescence in-situ hybridization (FISH) for *MET* and *HER2* (*ERBB2*) was performed on 4 ± 1 μm thick formalin-fixed, paraffin-embedded tumor sections. FISH was performed by manual slide processing technique on neutral formalin-fixed, paraffin-embedded tissue that was pretreated with proteinase K, then hybridized with the PathVysion *HER2* DNA Probe kit (Vysis *HER2*/neu (17q11.2) SpectrumOrange and Vysis 17 centromere SpectrumGreen; Abbott Molecular, Des Plaines, IL). The *MET* FISH assays were performed with laboratory-developed reagents or commercial reagents encompassing the genomic sequences of *MET*. CEP7 was used as a control probe to define the relative CNG. This sample was considered positive for *MET* amplification if *MET/CEP7* ≥ 3 and *HER2* amplification if the *HER2/CEP17* ≥ 3.

For liquid biopsies, we used the Guardant360 circulating tumor DNA assay (Guardant Health, Redwood City, CA). Approximately 50 to 100 ng of DNA is extracted from plasma, and 73 genes are used to identify somatic genomic alterations utilizing massively parallel sequencing of amplified target genes via Illumina HiSeq 2500 or Illumina NextSeq 500 platforms using hg19 as the reference genome. The limit of detection for single-nucleotide variants is 0.1%.[40] PD-L1 expression was assessed using a combination of the PD-L1 IHC 22C3 pharmDx and the Ventana PD-L1 (SP263) assays with expression categorized according to the tumor proportion score (percentage of tumor cells with membranous PD-L1 staining).[41]

Statistical Analysis

Descriptive statistics summarizing patient characteristics included the median, frequency, and percentage for categorical variables. Group comparisons of patient characteristics were performed using the Fisher exact test for categorical variables. Student *t* test or analysis of variance were used for continuous variables. Survival analysis was performed by Kaplan-Meier estimates using a log-rank test to assess for differences between subgroups. We addressed multiple statistical testing by adjusting the familywise error rate using the Bonferroni method. $P \leq .05$ was defined as statistically significant. All statistical analyses were performed by GraphPad Prism 6.00 for Mac (GraphPad Software, La Jolla, CA) and JMP Pro 14.3.0 for Mac (SAS Institute, Cary, NC).

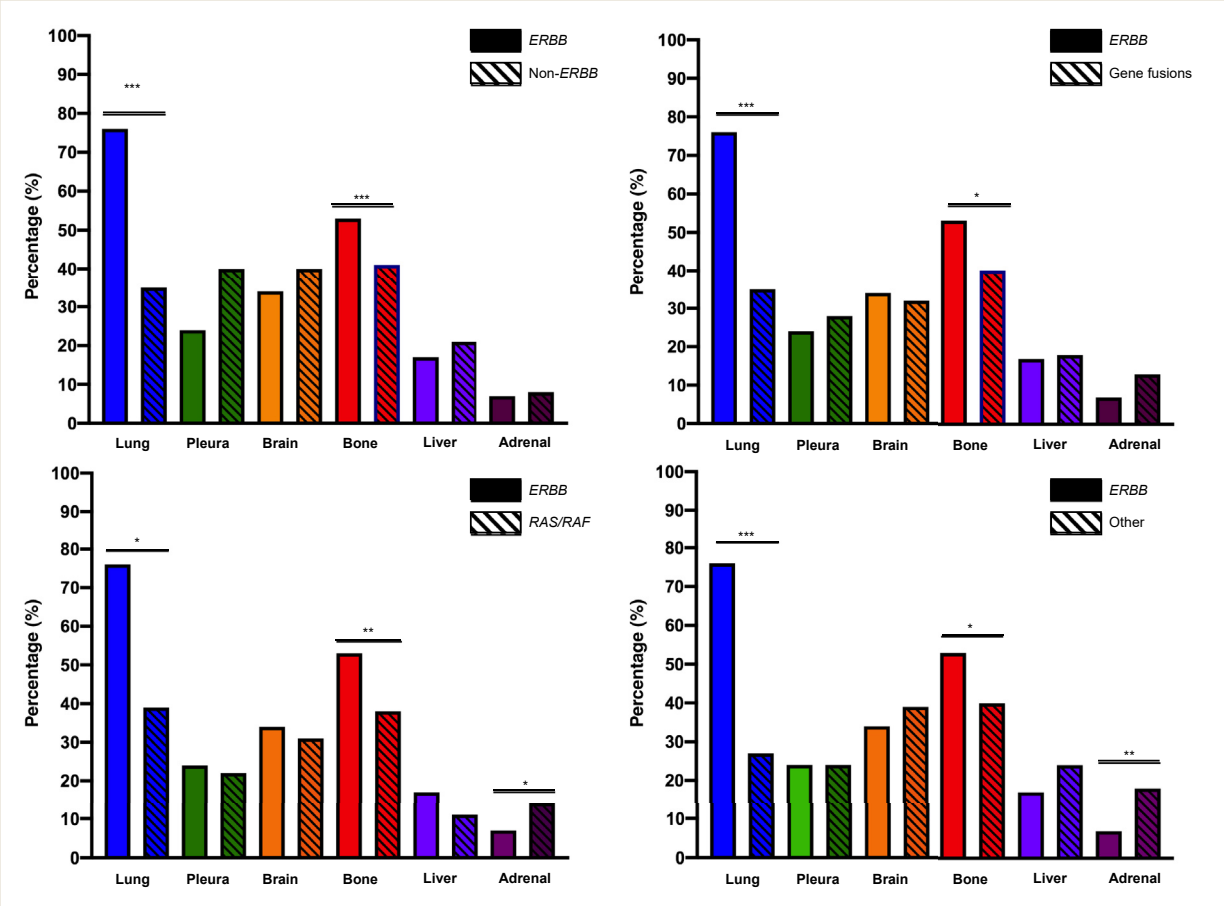
Results

Clinicopathologic Characteristics of Patients With Atypical EGFR Mutations and HER2 Alterations

A total of 603 patients seen at the University of Colorado Cancer Center with stage IV NSCLC from 2009 to 2019 were considered for this study. Of these, 33 were excluded and 570 patients were included for further analysis, as shown in the CONSORT diagram (Supplemental Figure 1 in the online version). We identified 55 a-EGFR mutations (13 G719X, 38 exon 20 insertions, 4 L861Q) and 31 HER2 alterations (2 exon 19 mutations, 27 exon 20 insertions, 2 gene amplifications). No patient with a *HER2* exon 20 insertion had synchronous *HER2* amplification (*HER2/CEP*17 ≥ 3). Baseline clinical characteristics are listed in Table 1. The median age of patients with a-EGFR and HER2 alterations was 62 years for both groups (range, 38-91 years). There was a predominance of female patients with a-EGFR mutations (38/55, 69%) and HER2 alterations (21/31, 68%). The majority of patients with a-EGFR mutations (32/55, 58%) and HER2 alterations (23/31, 75%) were never smokers.

The distribution of metastatic sites in stage IV disease by oncogene is shown in Supplemental Table 1 in the online version. Within the ErbB family (ie, *EGFR* and *HER2*), there was an increased incidence of lung metastases (112/220, 76%, vs. 116/322, 35%; $P < .002$) and bone metastases (117/220, 53%, vs. 124/322, 40%; $P < .001$) relative to other metastatic sites. We then compared metastatic sites across oncogene families. The incidence of lung and bone metastases within the ErbB family was significantly higher than gene fusions, mutations within the Ras/Raf pathway, and mutations without an identified oncogene driver

Figure 1 Distribution of Metastatic Sites Based on Driver Oncogene. Lung and Bone Metastases Were Significantly More Common Within the ErbB Family (ie, *EGFR* and *HER2*) Relative to Gene Fusions, Mutations in the Ras/Raf Pathway, and Mutations Without an Identified Oncogene Driver



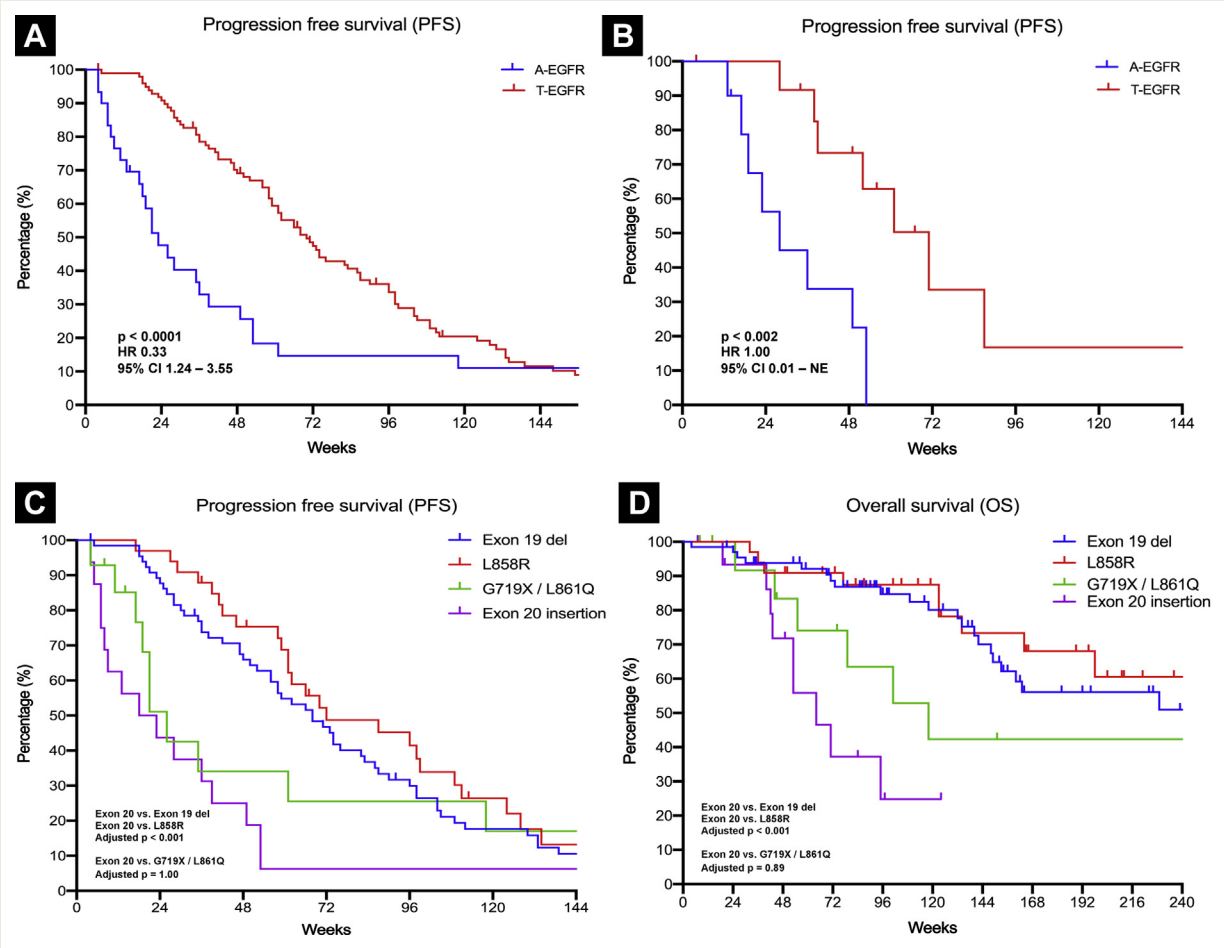
($P < .001$; Figure 1). A decreased incidence of adrenal metastases was noted within the ErbB family when compared to mutations within the Ras/Raf pathway ($P = .03$) and other mutations ($P = .001$), but not when compared to gene fusions ($P = .842$). To assess whether these patterns of metastatic spread varied within the ErbB family, we compared *EGFR* mutations to *HER2* alterations and found no significant differences in the incidence of lung or bone metastases (Supplemental Table 2 in the online version).

Treatment Outcomes in Patients With a-EGFR Mutations Receiving EGFR-Targeted Therapies

The distribution of EGFR TKIs evaluated in this study is shown in Supplemental Table 3 in the online version. These reflect the first EGFR TKI that each patient received. Both a-EGFR and t-EGFR cohorts received a median of one prior systemic therapy before initiating therapy with an EGFR TKI. The median PFS was significantly worse among patients with a-EGFR mutations compared to t-EGFR mutations (6 vs. 17.5 months; $P < .0001$; hazard ratio [HR] = 0.33; 95% confidence interval [CI], 0.22-0.50; Figure 2A). This difference in PFS was maintained even when we restricted our analysis to patients who first received afatinib or osimertinib (respectively, 7 vs. 15.5 months; $P < .002$; HR = 0.81; 95% CI, 0.08-8.23; Figure 2B). Median PFS across EGFR subtypes was as follows: exon 19 del (17 months), L858R (18 months), G719X/L861Q (7 months), and exon 20 insertion (5 months). Median OS across EGFR subtypes was as follows: exon 19 del (63 months), L858R (73 months), G719X/L861Q (30 months), and exon 20 insertion (16 months). After Bonferroni correction, patients with *EGFR* exon 20 insertions had a significantly worse PFS and OS to first EGFR TKI relative to patients with exon 19 del and L858R (adjusted $P < .001$), but not G719X/L861Q (adjusted $P = 1.00$; adjusted $P = .89$; Figure 2C and D).

First posttreatment imaging after treatment was available for 16 patients with a-EGFR mutations. RECIST responses are shown as a waterfall plot in Figure 3A. The ORR and DCR based on the first EGFR TKI received is shown in Supplemental Table 4 in the online version. Among the 10 patients who received erlotinib, the ORR was 10% and the DCR was 50%. The DCR by a-EGFR mutation was as follows: G719X (2/4, 50%), L861Q (2/3, 66%), and exon 20 insertion (1/3, 33%). Only 3 patients (19%) had PR to EGFR-directed therapies, 2 of whom were patients with exon 20 insertion mutations receiving afatinib. The PFS of a-EGFR mutations to different generations of TKIs is shown in a swimmer plot in Figure 3B. No patient with an *EGFR* exon 20 insertion who received erlotinib had a PFS greater

Figure 2 Treatment Outcomes for Patients With Atypical EGFR Mutations and Typical EGFR Mutations Treated With EGFR TKIs. Only Responses to First EGFR TKI Received Were Considered. Log-rank Test Was Used to Compare PFS and OS Between 2 Groups. (A) PFS Among Patients With a-EGFR Mutations Compared to t-EGFR Mutations. (B) PFS Among Patients With a-EGFR Mutations Compared to t-EGFR Mutations When Only Afatinib and Osimertinib Were Included. (C) PFS to EGFR TKIs Separated out by EGFR Subtype: Exon 19 Deletion, L858R Mutations, G719X/L861Q, and Exon 20 Insertions. Median PFS Across EGFR Subtypes Was as Follows: Exon 19 Deletion (17 Months), L858R (18 Months), G719X/L861Q (7 Months), Exon 20 Insertion (5 Months). After Bonferroni Correction, Exon 20 Insertions Had Significantly Worse PFS Relative to Exon 19 Del and L858R ($P < .001$), but Not G719X/L861Q ($P = 1.00$). (D) Median OS Across EGFR Subtypes Was as Follows: Exon 19 Del (63 Months), L858R (73 Months), G719X/L861Q (30 Months), and Exon 20 Insertion (16 Months). After Bonferroni Correction, Exon 20 Insertions Had Significantly Worse OS Relative to Exon 19 Del and L858R ($P < .001$), but Not G719X/L861Q ($P = .89$)



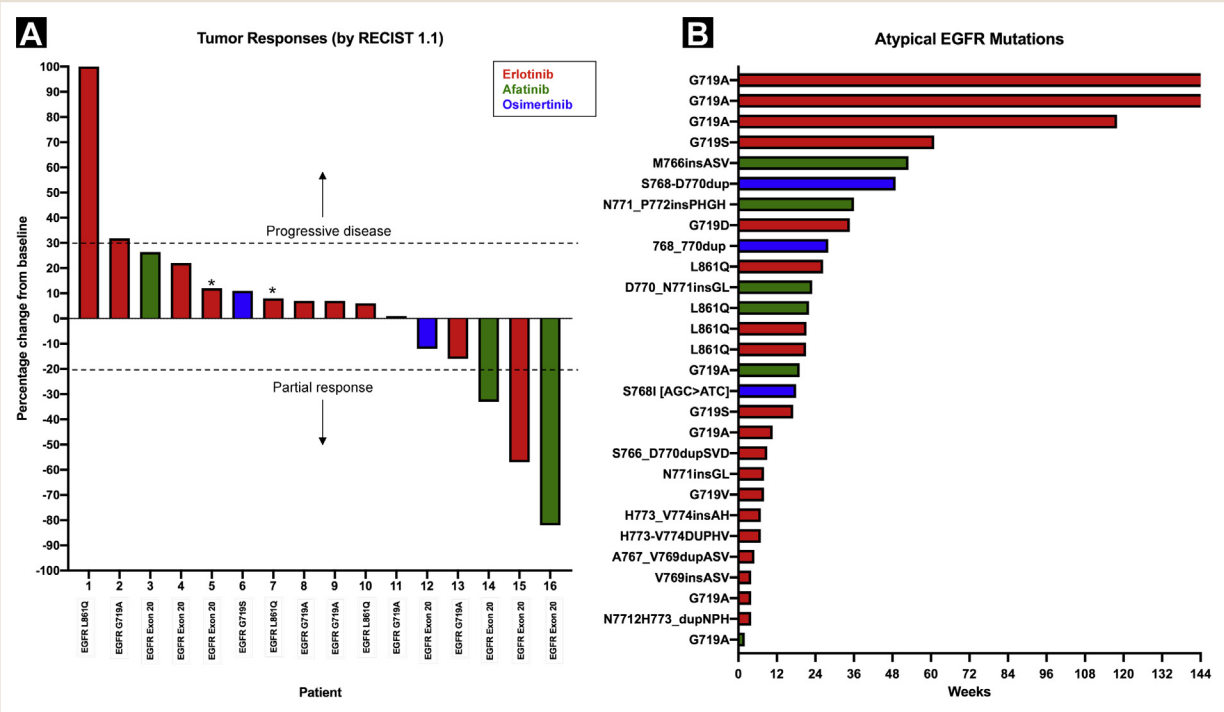
Abbreviations: a-*EGFR* = atypical *EGFR*; OS = overall survival; PFS = progression-free survival; t-*EGFR* = typical *EGFR*; TKI = tyrosine kinase inhibitor.

than 6 months (the median PFS for a-EGFR mutations in our series). In contrast, 5 patients with EGFR exon 20 insertion mutations who received afatinib or osimertinib had a median PFS greater than 6 months. All 4 patients with *EGFR* L861Q insertions had a PFS ranging from 5 to 7 months. There was notable heterogeneity in the PFS benefit among the EGFR G719X group independent of amino acid pair. Forty percent (4/10) of patients with an *EGFR* G719X mutation had a PFS of greater than 12 months while an equal percentage of patients had a PFS of 3 months or less. Twenty-one patients underwent comutation testing at diagnosis, and the percentage of *TP53* gene mutations was as follows: G719X (3/7, 48%), exon 20 insertion (7/10, 70%), and L861Q (3/4, 75%) with no significant differences between each group.

Acquired Resistance Mechanisms Among Patients With Atypical EGFR Mutations Receiving EGFR-Targeted Therapies

One hundred twenty-nine patients with *EGFR* mutations who had serial molecular testing and clinical follow-up available for review were considered for this analysis. Of the 129 patients, 65 patients with *EGFR* mutations underwent testing for acquired resistance having met the criteria of prior objective response (n = 9) or PFS ≥ 6 months (n = 56). Acquired resistance was assessed using utilizing circulating tumor DNA (via Guardant) or tissue testing (via Archer VariantPlex or FusionPlex). Patients with a-EGFR mutations underwent testing for resistance mutations at a significantly lower rate than patients with t-EGFR

Figure 3 Waterfall and Swimmer Plots for Patients With Atypical EGFR Mutations Receiving EGFR TKI Therapy. (A) RECIST Responses to First Posttreatment Imaging After Initiating Therapy With EGFR TKI. (B) Swimmer Plot Demonstrating PFS of a-EGFR Mutations Separated out by Different Generations of TKI. * Indicates That Patient Had New Lesions Detected on First on Treatment Scan, Consistent With Progressive Disease



Abbreviations: PFS = progression-free survival; RECIST = Response Evaluation Criteria in Solid Tumors; TKI = tyrosine kinase inhibitor.

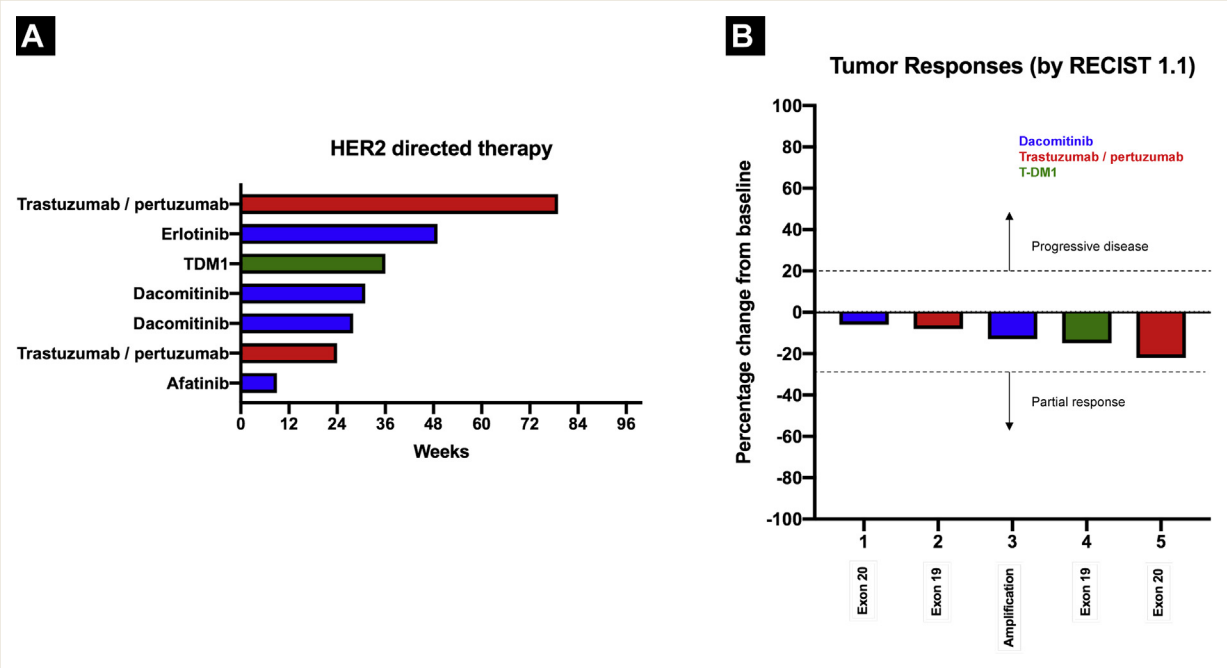
mutations (respectively, 9/55, 16%, vs. 56/74, 76%; $P < .001$). The distribution of acquired resistance mutations across *EGFR* mutations and method for detecting acquired resistance is shown in Supplemental Table 5 in the online version. Among the 65 patients tested for acquired resistance, 59 patients received erlotinib, gefitinib, or afatinib-TKIs where the T790M acquired resistance mutation has been observed. The T790M acquired resistance mutation occurred at a significantly lower rate among patients with a-EGFR mutations relative to t-EGFR mutations (respectively, 2/7, 29%, vs. 39/52, 75%; $P < .001$). The percentage of T790M by EGFR subtype was exon 19 del (26/34, 76%), L858R (13/18, 72%), and G719X (2/7, 33%). T790M was only seen in patients with G719X mutations and did not occur in 2 patients with L861Q and exon 20 mutations.

Among the 65 patients tested for acquired resistance, *HER2* and *MET* amplification assessed using FISH occurred in 66% (6/9) of a-EGFR and 55% (31/56) of t-EGFR samples. *MET* amplification as an acquired resistance mechanism was significantly higher among patients in the t-EGFR group (respectively, 5/31, 16%, vs. 0/9; $P < .001$). Only one patient (G719S mutation) with response to EGFR-directed therapy developed *HER2* amplification as an acquired resistance mechanism, though this was detected through circulating tumor DNA (Guardant) and not using FISH. No identifiable resistance mechanism was found in 66% (6/9) a-EGFR and 20% (11/56) t-EGFR tested samples, though 50% (3/6) a-EGFR and 54% (6/11) t-EGFR samples were not assessed for *HER2* or *MET* amplification via FISH. Only one patient with a *HER2* exon 20 insertion was tested for acquired resistance (via NGS from a tissue biopsy) at time of disease progression while receiving afatinib, with no identifiable resistance mechanism found.

Treatment Outcomes in Patients With HER2 Alterations Receiving Targeted Therapies

Nineteen patients with *HER2* alterations had clinical follow-up data. Thirty-seven percent (7/19) of these patients received HER2-directed therapy (which includes TKIs, HER2 monoclonal antibodies, and HER2 antibody–drug conjugates) during their treatment. Patients received a median of 2 lines of systemic therapy (eg, cytotoxic chemotherapy or chemoimmunotherapy combinations) before receiving their first HER2-directed treatment. A swimmer plot demonstrating the PFS of 7 patients with HER2 alterations who received initial HER2-directed therapies is provided in Figure 4A. Four patients received TKIs, 2 patients received trastuzumab with pertuzumab, and 1 patient received trastuzumab emtansine (T-DM1). Five patients had measurable lesions by RECIST, and all had SD based on the first posttreatment scan while receiving HER2-directed therapies (Figure 4B). Patients who received HER2-directed therapies had a higher OS relative to those who received cytotoxic chemotherapy (respectively, 65 vs. 29 months), although this was not statistically significant ($P = .1574$; HR = 0.30; 95% CI, 0.05-1.73).

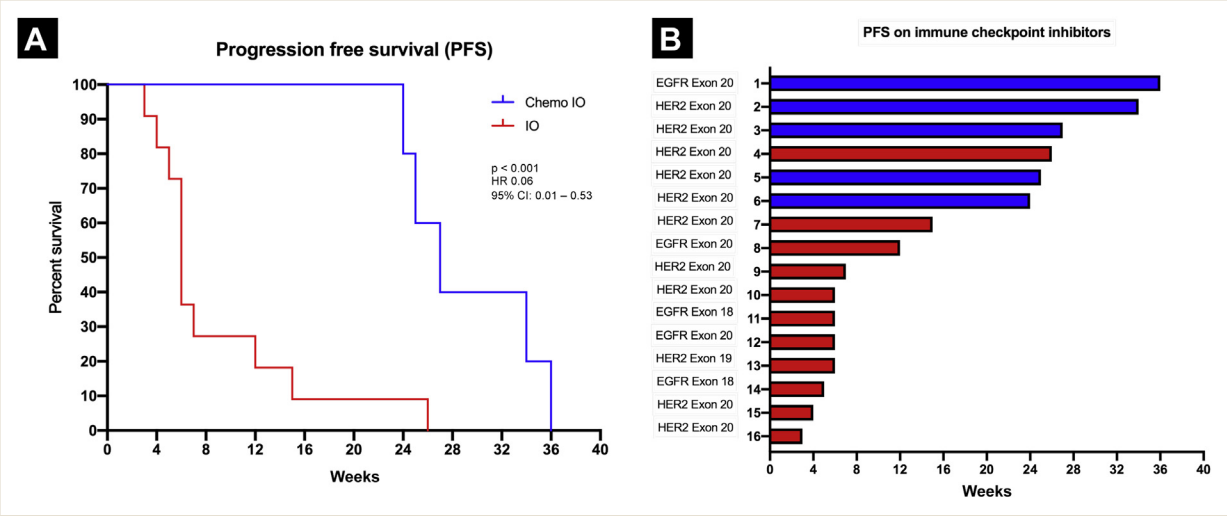
Figure 4 Swimmer and Waterfall Plots for Patients With *HER2* Alterations. (A) Swimmer Plot of 7 Patients With *HER2* Alterations Separated by Initial *HER2*-directed Therapy. (B) Waterfall Plot of First Posttreatment Responses Among 5 Patients With Measurable Lesions Who Received *HER2*-directed Therapies



Treatment Outcomes in Patients With Atypical EGFR and HER2 Alterations Receiving Immune Checkpoint Inhibitors

Sixteen patients with a-EGFR mutations (5/16, 31%) and HER2 alterations (11/16, 69%) received ICIs during their treatment course. PD-L1 expression was available in 8 patients prior to receiving ICIs. PD-L1 $\geq$ 50% was seen in 66% (2/3) of a-EGFR samples and 60% (3/5) of HER2 samples. The majority of patients were never smokers (13/16, 81%), with a median pack-year history of zero pack-years. Thirty-one percent (5/16) of patients received

Figure 5 Outcomes of Patients With Atypical EGFR and HER2 Alterations Treated With ICIs. (A) Patients Who Received ICI With Platinum Doublet Chemotherapy (Using KEYNOTE-189 Regimen) Had Significantly Prolonged PFS Relative to Those Who Received Single-agent ICI. (B) Swimmer Plot of Patients With a-EGFR Mutations and HER2 Alterations Receiving ICI Therapy



Abbreviations: a-EGFR = atypical EGFR; ICI = immune checkpoint inhibitor; PFS = progression-free survival.

ICIs with platinum chemotherapy using the KEYNOTE-189 regimen,[42] pathway (K-RAS, N-RAS, and BRAF V600E), and other mutations (no identifiable oncogene driver).Ten patients had posttreatment imaging available for review. No objective responses to single-agent ICI were seen in either the a-EGFR (0/4) or the HER2 (0/6) group. This poor response to single-agent ICI was seen even in 3 patients with PD-L1 $\geq$ 50%. Partial responses were seen in 2 patients with EGFR exon 20 and HER2 exon 20 insertion mutations who received ICI with platinum chemotherapy using the KEYNOTE-189 regimen.[42] Patients treated with ICIs had received a median of 2 prior systemic therapies. Patients with a-*EGFR* and *HER2* alterations who received ICIs with platinum doublet chemotherapy had significantly prolonged PFS relative to those who received ICIs as monotherapy (respectively, 7 vs. 2 months; $P <$ .001; HR = 0.06; 95% CI 0.01-0.53; Figure 5).

Discussion

Atypical *EGFR* and *HER2* alterations are activating oncogenes in NSCLC with unique clinical features, varied responsiveness to current TKI therapy, and poor response to single-agent ICI therapy. There was a predominance of female patients with a-EGFR mutations and HER2 alterations, similar to what is seen among patients with t-EGFR mutations. While the majority of patients in the a-EGFR and HER2 groups were never smokers, a notable percentage of these patients (29% and 16%, respectively) were heavy smokers ($\geq$ 10 pack-years). There has been a growing appreciation that different oncogenes can have different patterns of metastatic spread,[43] and it appears that NSCLC with mutations in the ErbB family (ie, *EGFR* and *HER2*) have a tropism for lung and bone metastases. This metastatic phenotype was statistically significant when compared to patients with gene fusions, mutations in the Ras/Raf pathway, and with no identifiable oncogene drivers. There were no differences in the incidence of lung or bone metastases when *EGFR* and *HER2* mutations (both within the ErBB family) were compared to each other. Our findings are consistent with other studies that report an increased incidence of lung metastases in patients with t-EGFR and HER2 activating mutations.[44-46] The increased incidence of bone metastases within the ErbB family deserves mention. Given the propensity of bone metastases among patients with mutations in the ErbB family, the role of osteoclast inhibitors in conjunction with targeted therapy in reducing skeletal fracture risk remains unexplored and is being currently evaluated in the context of an ongoing clinical trial (ClinicalTrials.gov, NCT03958565).

Consistent with multiple other studies, patients with a-EGFR had significantly worse PFS to EGFR TKIs relative to patients with t-EGFR mutations.[19,23,33,34,47] This inferior response was seen even among patients who received only afatinib and osimertinib (respectively, 7 vs. 15.5 months; $P <$ .002; HR = 0.81; 95% CI, 0.08-8.23). Patients with *EGFR* exon 20 insertions and G719X/L861Q mutations had a median PFS of 5 months and 7 months to EGFR TKIs, though only exon 20 insertions had a statistically inferior PFS to exon 19 del and L858R after using Bonferroni adjustment ($P <$ .001).

Single-nucleotide variants in exon 18 (G719X) and exon 21 (L861Q) have been reported to confer a moderate degree of sensitivity to EGFR TKIs.[23,33,48,49] A post hoc analysis of NEJ002 study data, which compared first-line gefitinib to carboplatin/paclitaxel for advanced NSCLC with activating *EGFR* mutations, identified 10 patients with a-EGFR mutations (7 G719X, 3 L861Q). The median PFS for patients with EGFR G719X and L861Q mutations was significantly shorter than patients with t-EGFR mutations who received gefitinib (2.2 vs. 11.4 months; $P <$ .0001), but this did not differ among patients who received platinum doublet chemotherapy (5.9 vs. 5.4 months; $P =$ .847).[50] A limitation of the NEJ002 analysis was the imbalance in the number of G719X/L861Q mutations (n = 10) compared to t-EGFR mutations (n = 215). In contrast to the post hoc analysis of the NEJ002 study, a retrospective analysis by Passaro et al[23] included patients who received both first-generation EGFR TKI (erlotinib, gefitinib) and second-generation TKI (afatinib). Patients in this study with G719X/L861Q mutations had a numerically lower (but not statistically significant) median PFS of 12.3 months and an OS of 31 months compared to patients with t-EGFR mutations, consistent with data in our study. We found that there was notable heterogeneity among patients with G719X mutations, with 40% (4/10) deriving a PFS benefit to erlotinib that was greater than 12 months, while another 40% demonstrating progression within 3 months of initiating erlotinib therapy. One possibility for this observation could be that acquired T790M occurs at lower rate among G719X mutations relative to sensitizing mutations such as exon 19 del and L858R, and that alternative patterns of acquired resistance may emerge in this subgroup. Another possibility could be related to the presence of a *TP53* gene mutation (a known negative predictive factor),[3,5] which was detected in 48% of patients with G719X mutations in our series.

Exon 20 insertions account for approximately 5% of *EGFR* mutations and are associated with poor response to first-generation EGFR TKIs.[22,34,47] In a combined post hoc analysis of uncommon *EGFR* mutations in the LUX-Lung 2, LUX-Lung 3, and LUX-Lung 6 trials, patients with exon 20 insertions had a PFS of 2.7 months and an ORR of 8.7% to afatinib.[50] Our study showed that afatinib may result in a higher ORR in patients with EGFR exon 20 insertions (2/4, 50%), though our sample sizes are small and thus limit the strength of our conclusions. In our series, no patient with an *EGFR* exon 20 insertion had a PFS of greater than 3 months when treated with erlotinib, but all 5 patients who received either afatinib or osimertinib had a PFS greater than 6 months (the median PFS for a-EGFR mutated NSCLC treated with an EGFR TKI in our series). There are ongoing clinical trials assessing the efficacy of different TKIs in patients with *EGFR* exon 20 insertion mutations such as osimertinib (ClinicalTrials.gov, NCT03414814), poziotinib (NCT03066206), tarloxotinib (NCT03805841), TAK-788 (NCT02716116), and JNJ-372 (NCT02609776).

Acquired resistance remains a major clinical challenge in the management of oncogene-driven NSCLC. Patients with a-EGFR mutations underwent resistance testing at a significantly lower rate than patients with t-EGFR mutations. Among 59 patients who received erlotinib, gefitinib, or afatinib, the percentage of T790M by EGFR subtype was as follows: exon 19 del (76%), L858R (72%), and G719X (40%). T790M was only seen in patients with G719X and did not occur in 2 patients with L861Q and exon 20 insertions. *MET* amplification (defined as *MET/CEP7* $\geq$ 3) was seen as an acquired resistance mechanism in 16% (5/31) of t-EGFR samples, but was not seen in the a-EGFR group. No identifiable

resistance was seen in 66% of patients with a-EGFR mutations. One possibility is that current EGFR-directed therapies ineffectively suppress cancer growth among patients with a-EGFR mutations. Therefore, the selective pressure towards developing acquired resistance mutations is lower than that for t-EGFR mutations. It is worth noting that among a-EGFR mutations with acquired resistance, only half of these cases were tested for *HER2* or *MET* amplification via FISH, and therefore the incidence of gene amplification as a potential resistance mechanism remains underexplored.

There is growing appreciation of HER2 alterations in NSCLC and increasing interest in the development of HER2-targeted therapies. In our series, 37% of patients with HER2 alterations received HER2-directed therapy (which included TKIs, HER2 monoclonal antibodies, and HER2 antibody—drug conjugates) during their treatment. All patients with measurable lesions had SD on their first on-treatment scans. Patients who received HER2-directed therapies had numerically longer median survival relative to patients who received cytotoxic chemotherapy (65 vs. 29 months), although this did not meet threshold for statistical significance. Given our small sample size, we were unable to differentiate responses to HER2-directed therapies based on specific insertion mutations, though this is an area of importance. Afatinib has been explored in the management of patients with *HER2* exon 20 insertions, with mixed results.[51] In one retrospective series, the ORR to afatinib was 100% (n = 4),[25] though this was not replicated in a prospective phase 2 Niche trial of 13 patients, with only one patient experiencing a PR.[52] Recent data suggest that pyrotinib, an irreversible pan-HER receptor TKI, demonstrated a superior antitumor effect within a PDX model relative to afatinib ($P = .047$) and T-DM1 ($P = .013$). Among 15 patients in the phase 2 cohort, pyrotinib had an ORR of 53.3% (8/15) and a PFS of 6.4 months.[53]

The efficacy of ICIs in patients with a-EGFR mutations and HER2 alterations also remains underexplored. A subgroup analysis of the IMpower150 trial reported the efficacy of ICIs in combination with platinum doublet chemotherapy and bevacizumab for patients with *EGFR* mutations and *ALK* gene fusions,[37] though this finding was only significant among patients with t-EGFR mutations. In our series, no patient with a-EGFR or HER2 alterations had a RECIST response to single-agent ICI. Patients with a-EGFR and HER2 alterations who received ICI with platinum doublet chemotherapy (using the KEYNOTE-189 regimen) had significantly improved PFS relative to single-agent ICI (7 vs. 2 months; $P < .001$; HR = 0.06; 95% CI 0.01-0.53). These poor single-agent ICI responses are similar to those reported in a large cohort study by Mazieres et al[29] that found that the PFS to single-agent ICI ranged from 1.4 to 2.8 months depending on EGFR mutation subtype, and 2 to 3.4 months among those with HER2 alterations.[29] Increased smoking status positively correlated with improved PFS to single-agent ICI among patients with HER2 alterations ($P = .04$) and trended toward significance among those with EGFR mutations ($P = .06$).[29] It also remains an open question as to whether there are subtle differences in the efficacy between the KEYNOTE-189 and IMpower150 regimens among patients with a-EGFR mutations and HER2 alterations.

There are several limitations to our study. This was a retrospective single-institution study and was therefore prone to selection bias. Given variability in follow-up among patients in this study, we performed RECIST measurements according to the first on-treatment scan, not best treatment response. It is possible that our response rates were skewed toward measuring SD. Nonetheless, patients with sensitizing mutations will typically demonstrate radiographic responses by the first on-treatment scan,[41,54] and we thought that this was a close surrogate for best response to targeted therapy. We were unable to control for multiple confounders like age, performance status, prior lines of therapy, and brain metastases when making PFS and OS comparisons across EGFR subtypes because of our small sample size. Given that our objective was to assess clinical outcomes of patients with a-EGFR and HER2 alterations with currently available targeted agents, we excluded patients who were first treated with agents in clinical trials, and we acknowledge that this may potentially bias our selection of patients. A limited number of patients underwent testing for acquired resistance, tempering our ability to draw strong conclusions regarding mechanisms of resistance, though this is an area ripe for future exploration. Finally, our sample size limited our ability to assess the impact of smoking status and PD-L1 expression on ICI outcomes in patients with a-EGFR and HER2 alterations, though this is an important area of investigation.

Conclusion

Our study has several important findings of clinical relevance. Alterations within the ErbB family (ie, EGFR and HER2) appear to have a unique metastatic phenotype characterized by a higher rate of synchronous lung and bone metastases compared to gene fusions, mutations within the Ras/Raf pathway, or mutations without an identifiable driver. Patients with a-EGFR mutations receiving afatinib and osimertinib have an inferior PFS relative to patients with t-EGFR mutations, with exon 20 insertions having the worst PFS and OS to current EGFR directed therapies. In our series, patients with a-EGFR mutations were much less likely to undergo testing for acquired resistance. A large percentage of patients with no identified resistance mutations did not undergo subsequent testing for *HER2* or *MET* amplification. Elucidating differences in acquired resistance mechanisms between a-EGFR and t-EGFR mutations is an area ripe for further investigation. Finally, patients with a-EGFR mutations and HER2 alterations have uniformly poor responses to single-agent ICI, even with high PD-L1 expression. Novel therapies are urgently needed for patients with these mutations, as current outcomes remain poor.

Clinical Practice Points

- Most clinical trials exploring EGFR-directed therapies have only included patients with typical drug-sensitive *EGFR* mutations. Atypical EGFR mutations, representing only 15% to 20% of *EGFR* mutations, are less well described. Likewise, HER2 alterations have been identified as oncogenic drivers in 2% to 3% of NSCLC.
- We found that mutations within ErbB family (ie, *EGFR* and *HER2*) appear to have a unique metastatic phenotype characterized by higher rate of synchronous lung and bone metastases compared to gene fusions, mutations within the Ras/Raf pathway, or those without an identifiable oncogene driver.
- Patients with a-EGFR mutations have a worse PFS and OS relative to patients with t-EGFR mutations, even when afatinib and osimertinib are used in the first-line setting.

- Patients with a-EGFR mutations were much less likely to undergo resistance testing. Acquired T790M occurred at a lower rate among patients with a-EGFR mutations treated with first and second generation EGFR TKIs. *MET* amplification was not seen among patients with a-EGFR mutations, though only half of samples were tested for *MET* and *HER2* amplification.
- Survival was numerically (though not statistically) improved for patients with HER2 alterations who received HER2-directed therapies over cytotoxic chemotherapy.
- Patients with a-EGFR mutations and HER2 alterations have uniformly poor ORRs and PFS to single-agent immunotherapy.

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Supplemental Data

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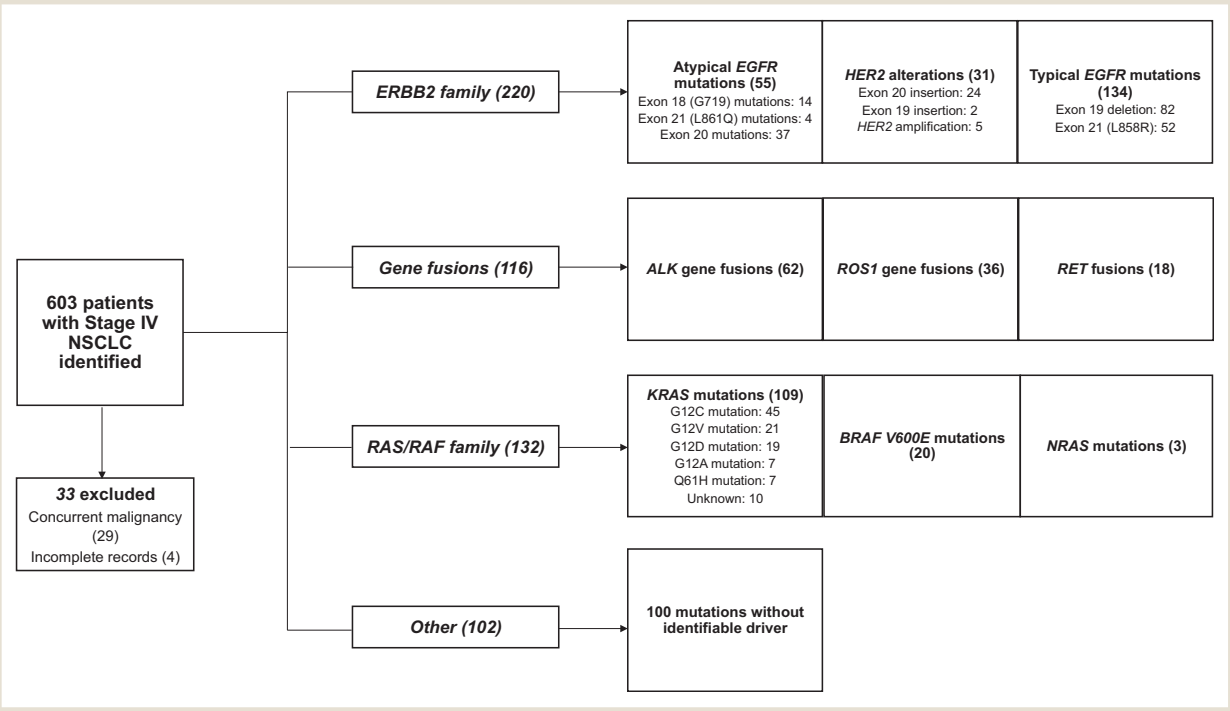
Supplemental Figure 1 CONSORT Diagram With Schema for Patient Inclusion and Exclusion



Abbreviation: CONSORT = Consolidated Standards of Reporting Trials.

Supplemental Table 1 Distribution of Metastatic Sites for Stage IV Disease by Oncogene

Site	a-*EGFR* (N = 55)	*HER2* (N = 31)	t-*EGFR* (N = 134)	*ALK* (N = 62)	*ROS1* (N = 36)	*RET* (N = 18)	*RAS* (N = 112)	*BRAF* V600E (N = 20)	Other (N = 102)
Lung	36 (65)	20 (65)	56 (42)	27 (44)	8 (22)	6 (33)	44 (39)	8 (40)	28 (27)
Pleura	22 (40)	7 (23)	46 (34)	26 (42)	13 (36)	7 (39)	21 (19)	8 (40)	24 (24)
Brain	16 (29)	10 (32)	43 (32)	20 (32)	10 (28)	2 (11)	39 (35)	4 (10)	40 (39)
Bone	30 (55)	19 (61)	68 (51)	28 (45)	12 (33)	8 (44)	41 (37)	9 (45)	41 (40)
Liver	15 (27)	1 (3)	21 (16)	14 (23)	7 (19)	3 (17)	13 (12)	2 (5)	24 (24)
Adrenal	2 (4)	2 (6)	11 (8)	5 (8)	2 (6)	2 (11)	13 (15)	2 (5)	18 (18)

Data are presented as n (%) unless otherwise indicated.
Abbreviations: a-*EGFR* = atypical *EGFR*; t-*EGFR* = typical *EGFR*.

Supplemental Table 2 Incidence of Individual Metastatic Sites Among *EGFR* Mutations and *HER2* Alterations

Site	*EGFR* (N = 189) (N)	*HER2* (N = 31) (N)	*EGFR* (%)	*HER2* (%)	*P*
Lung	112	20	59	65	.693
Pleura	75	7	40	23	.07
Brain	69	10	37	32	.691
Bone	117	19	62	61	1.00
Liver	37	1	20	3	.02
Adrenal	2	2	4	6	1

Supplemental Table 3 Targeted Therapies Used in Patients With Atypical *EGFR* Mutations and *HER2* Alterations

Therapy	First Targeted Therapy	Prior Chemotherapy
Atypical *EGFR* (N = 30)		
Erlotinib	20 (66%)	4/18 (22%)
Afatinib	6 (20%)	4/6 (66%)
Osimertinib	4 (13%)	1/4 (25%)
HER2 Alterations (N = 7)		
Erlotinib	1 (14%)	0/1
Afatinib	1 (14%)	1/1 (100%)
Dacomitinib	2 (28%)	2/2 (100%)
T-DM1	1 (14%)	1/1 (100%)
Trastuzumab/pertuzumab	2 (28%)	1/2 (50%)

Supplemental Table 4 ORR and DCR of Patients With Atypical *EGFR* Mutations Based on First *EGFR* TKI Received

RECIST 1.1 Response	All TKI (N = 16)	First-Generation Erlotinib (N = 10)				Second-Generation Afatinib (N = 4)				Third-Generation Osimertinib (N = 2)			
		All (N = 10)	G719X (N = 4)	L861Q (N = 3)	Exon 20 (N = 3)	All (N = 4)	G719X (N = 1)	L861Q (N = 0)	Exon 20 (N = 3)	All (N = 2)	G719X (N = 1)	L861Q (N = 0)	Exon 20 (N = 1)
CR	0	0	0	0	0	0	0	—	0	0	0	—	0
PR	3 (19)	1 (10)	0	0	1 (33)	2 (50)	0	—	2 (66)	0	0	—	0
SD	7 (44)	4 (40)	2 (50)	2 (66)	0	1 (25)	1 (100)	—	0	2 (100)	1 (100)	—	1 (100)
PD	6 (37)	5 (50)	2 (50)	1 (33)	2 (66)	1 (25)	0	—	1 (100)	0	0	—	0
ORR	3 (19)	1 (10)	0	0	1 (33)	2 (50)	0	—	2 (66)	0	0	—	0
DCR	10 (63)	5 (50)	2 (50)	2 (66)	1 (33)	3 (75)	1 (100)	—	2 (66)	2 (100)	1 (100)	—	1 (100)

Data are presented as n (%).
Abbreviations: CR = complete response; DCR = disease control rate; ORR = objective response rate; PD = progressive disease; PR = partial response; SD = stable disease; TKI = tyrosine kinase inhibitor.

Supplemental Table 5 Distribution of Acquired Resistance Mutations Across *EGFR* Mutations and Method for Detecting Acquired Resistance

EGFR Mutation	TKI Before Testing	Liquid/Tissue	FISH Testing	T790M	*MET*	Other Mechanisms
Exon 19 del	• Erlotinib 31/36 (86%) • Afatinib 2/36 (6%) • Osimertinib 3/36 (8%)	• Liquid: 6/36 (17%) • Tissue: 26/36 (72%) • Both: 4/36 (11%)	21/36 (58%)	26/36 (72%)	4/36 (11%)	
L858R	• Erlotinib 18/20 (90%) • Afatinib 1/20 (5%) • Osimertinib 2/20 (10%)	• Liquid: 5/20 (25%) • Tissue: 13/20 (65%) • Both: 2/20 (10%)	10/20 (50%)	13/20 (65%)	1/20 (5%)	*NCOA/RET* fusion
G719X	• Gefitinib 1/6 (17%) • Erlotinib 3/6 (50%) • Afatinib 1/6 (17%) • Osimertinib 1/6 (17%)	• Liquid: 2/6 (33%) • Tissue: 2/6 (33%) • Both: 2/6 (33%)	5/6 (83%)	2/6 (33%)	0/6	*HER2* amplification
L861Q	• Erlotinib 1/1 (100%)	• Liquid: 1/1 (100%) • Tissue: 0 • Both: 0	0/1	0/1	0/1	—
Exon 20 insertion	• Erlotinib 1/2 (50%) • Osimertinib 1/2 (50%)	• Liquid: 1/2 (50%) • Tissue: 1/2 (50%) • Both: 0	1/2 (50%)	0/2	0/1	—

Abbreviations: FISH = fluorescence in-situ hybridization; TKI = tyrosine kinase inhibitor.