Analysis of Tumor Specimens at the Time of Acquired Resistance to EGFR-TKI Therapy in 155 Patients with *EGFR*-Mutant Lung Cancers

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Abstract

Purpose: All patients with EGF receptor (*EGFR*)–mutant lung cancers eventually develop acquired resistance to EGFR tyrosine kinase inhibitors (TKI). Smaller series have identified various mechanisms of resistance, but systematic evaluation of a large number of patients to definitively establish the frequency of various mechanisms has not been conducted.

Experimental Design: Patients with lung adenocarcinomas and acquired resistance to erlotinib or gefitinib enrolled onto a prospective biopsy protocol and underwent a rebiopsy after the development of acquired resistance. Histology was reviewed. Samples underwent genotyping for mutations in *EGFR, AKT1, BRAF, ERBB2, KRAS, MEK1, NRAS* and *PIK3CA*, and FISH for *MET* and *HER2*.

Results: Adequate tumor samples for molecular analysis were obtained in 155 patients. Ninety-eight had second-site *EGFR* T790M mutations [63%; 95% confidence interval (CI), 55%–70%] and four had small cell transformation (3%, 95% CI, 0%–6%). *MET* amplification was seen in 4 of 75 (5%; 95% CI, 1%–13%). *HER2* amplification was seen in 3 of 24 (13%; 95% CI, 3%–32%). We did not detect any acquired mutations in *PIK3CA, AKT1, BRAF, ERBB2, KRAS, MEK1,* or *NRAS* (0 of 88, 0%; 95% CI, 0%–4%). Overlap among mechanisms of acquired resistance was seen in 4%.

Conclusions: This is the largest series reporting mechanisms of acquired resistance to EGFR-TKI therapy. We identified *EGFR* T790M as the most common mechanism of acquired resistance, whereas *MET* amplification, *HER2* amplification, and small cell histologic transformation occur less frequently. More comprehensive methods to characterize molecular alterations in this setting are needed to improve our understanding of acquired resistance to EGFR-TKIs. *Clin Cancer Res; 19(8); 2240–7. ©2013 AACR.*

Introduction

In patients with lung cancer whose tumors harbor activating EGF receptor (*EGFR*) mutations, treatment with EGFR-TKIs induces initial tumor shrinkage, with progression of cancer after a median of 8 to 16 months (1–4). Various mechanisms of resistance to erlotinib and gefitinib have been identified, and understanding these mechanisms is critical to developing treatment strategies in the acquired resistance setting. The most frequently reported mechanism of acquired resistance is the EGF receptor (*EGFR*) T790M point mutation within exon 20 (5, 6). Small cell histologic transformation has also been associated with the development of acquired resistance (7, 8). *MET* amplification and *HER2* amplification are also seen and illustrate the upregulation of parallel signaling pathways (9–11). Rare secondary *BRAF* mutations (12) have also been implicated. The frequency and overlap of these mechanisms of resistance is not well-characterized, as all reports have been in relatively small series of patients (8).

Rebiopsy of growing tumors at clinical progression has become increasingly important as the results may better predict prognosis (13, 14) or direct a change in therapy (7). Understanding why acquired resistance occurs is essential as new therapies focus on alternative means of EGFR inhibition and inhibition of parallel signaling pathways to prevent or circumvent resistance. To characterize the frequency of the various mechanisms of acquired resistance in a single population, we report the updated results of a prospective clinical trial initiated in 2004 to determine mechanisms of acquired resistance in patients with *EGFR*-mutant lung cancers who had an initial response to erlotinib or gefitinib (13).

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Translational Relevance

Responses to EGF receptor (EGFR) tyrosine kinase inhibitors (TKI) in *EGFR*-mutant lung cancer are limited by acquired resistance. The goal of this prospective study was to characterize the mechanisms of resistance by conducting repeat tumor biopsies at clinical disease progression. Previously published reports included small numbers of patients and do not adequately establish the frequency of the various mechanisms of resistance. This is the largest reported cohort of patients with *EGFR*-mutant lung cancer and acquired resistance to EGFR-TKI therapies that have had comprehensive mutational analysis on acquired resistance biopsy samples. Our findings indicate that second-site *EGFR* T790M mutations are the dominant mechanism of resistance identified. *MET* amplification, small cell histologic transformation, and *HER2* amplification are uncommon. There were no acquired mutations in other oncogenes identified. This protocol illustrates that postprogression tumor biopsies can be done on a large scale with minimal adverse events. Comprehensive tumor analysis at the time of resistance is important to develop new therapeutic strategies and also to inform patient care in the acquired resistance setting.

Materials and Methods

Patients

Patients had lung adenocarcinoma with a documented *EGFR* mutation, received treatment with single agent erlotinib or gefitinib, had either prolonged stable disease (>3 months) or a partial response to therapy, and developed radiographic progression while on EGFR-TKI. The primary objective of the study was to characterize mechanisms of acquired resistance to EGFR-TKI therapy. Biopsies were obtained in the least invasive manner possible and typically consisted of either a fine-needle aspiration (FNA) or core biopsy done with image guidance or rarely excisional biopsies. Fluid from malignant effusions was collected to create cell blocks. If a surgical procedure was clinically indicated, tissue from the procedure was obtained for analysis. Protocol #04-103 was approved by the Institutional Review Board of Memorial Sloan-Kettering Cancer Center (New York, NY), and all patients signed informed consent. Partial data from a subset of patients on this protocol have been reported previously (11, 13, 15).

Tissue analysis

All samples underwent histologic review. If required, additional diagnostic immunohistochemical stains were conducted at the discretion of the pathologist. For molecular analysis, gDNA was extracted from the tumor samples which included fresh, frozen, and formalin-fixed, paraffin-embedded (FFPE) tissue specimens. Cytologic samples were used to create cell blocks from which DNA was extracted. The initially identified sensitizing *EGFR* mutation was confirmed using previously described methods (16, 17).

Standard sequencing and/or fragment analysis were used to identify EGFR T790M using techniques previously described (15, 17). In July 2009, we began locked nucleic acid–based PCR sequencing to improve the sensitivity of EGFR T790M detection (18). Beginning in January 2009, a mass spectrometry–based mutation profiling assay (Sequenom) was used on all samples. This assay identifies 92 specific point mutations in 8 genes: *EGFR, BRAF, PIK3CA, AKT1, ERBB2, MEK1, NRAS*, and *KRAS*. If additional material was available on samples before 2009, locked nucleic acid–based sequencing and Sequenom analysis were conducted. For a subset of patients with available tissue, direct sequencing of *EGFR* exons 18 to 21 was also available. Available unstained FFPE tumor tissue was analyzed by a dual-color FISH assay using a MET/CEP7 probe cocktail (9, 15). A MET/CEP ratio was established on the basis of a count of at least 200 cells. Samples with a MET:CEP7 ratio greater than 2 were considered to have *MET* amplification (low amplification ≤ 3, high amplification > 3). Assessment of *HER2* gene copy number was also conducted on available unstained FFPE tumor tissue using the Vysis PathVysion *HER2* DNA Probe Kit (Abbott Laboratories) and scored according to previously published criteria (19, 20). Tumors were classified as amplified if the HER2/CEP17 ratio per cell was >2 or homogeneously staining regions with >15 copies in more than 10% of cells were present. At least 40 cells were analyzed for each case.

Statistical methods

Medical records were reviewed to obtain clinical information. Overall survival from the time of advanced cancer diagnosis and postprogression survival following the development of acquired resistance were calculated using Kaplan–Meier methodology, with patients censored if they were alive at the time of last follow-up, February 2012. Univariate comparisons between groups were conducted using the log-rank test.

To evaluate whether patients enrolled on the protocol were representative of patients with advanced non–small cell lung cancer (NSCLC), we assembled a reference cohort of all patients diagnosed with advanced NSCLCs at our institution during a similar time period (2004–2009), whose tumors harbored *EGFR* mutations, and were not enrolled in this acquired resistance protocol. The 2 groups were compared with respect to clinical characteristics using the χ^2 test.

Results

Enrollment and rebiopsy

From August 2004 to January 2012, 175 patients were enrolled and 162 underwent rebiopsy at the time of acquired resistance (see Table 1). Seven patients had biopsy samples with either insufficient tumor content for molecular analysis ($n = 5$) or no evidence of the previously present *EGFR* sensitizing mutation upon rebiopsy ($n = 2$). One lung core biopsy had insufficient tumor cells. One patient's core bone biopsy was inadequate for DNA analyses due to

Table 1. Procedures conducted

Procedures conducted	N = 162
Adrenalectomy	3
Brain resection	10
Lung resection	4
Lymph node excision	5
Autopsy	2
Biopsies	
Lung/chest Wall (core/FNA)	82 (64/18)
Bone (core/FNA)	9 (7/2)
Liver (core/FNA)	13 (10/3)
Lymph node (core/FNA)[a]	9 (2/7)
Other (core/FNA)[b]	9 (4/5)
Fluid	
Pleural	14
Peritoneal	1
Cerebrospinal fluid	1

[a]Inguinal, neck (n = 6), mediastinal (n = 3).
[b]Other includes biopsy of adrenal (n = 2), soft tissue (n = 2), omentum, spleen, kidney (n = 2), cervix.

Table 2. Clinical and molecular characteristics

Patient characteristics (N = 155)	
Age, y	
Median	57
Range	33–81
Sex, n (%)	
Female	100 (66)
Male	55 (35)
Race, n (%)	
White	113 (73)
Asian	30 (19)
Black	11 (7)
Other	1 (1)
Smoking history, n (%)	
Never smoker	107 (69)
Former smoker	48 (31)
Current smoker	0
Median pack-years	10
Range	0–96
Pretreatment EGFR mutation, n (%)	
Exon 19 deletion	103 (66)
Exon 21 L858R	46 (30)
Exon 21 L861Q	1
Other/double mutations	
Exon 19 deletion + T790M	1
Exon 19 deletion + PIK3CA	1
Exon 21 L861Q and exon 20 R776H	1
Exon 21 L833V and exon 21 H835L	1
Exon 21 L858R and exon 20 R776H	1

decalcification. Two patients had core biopsies of the lung with tumor evident where the sensitizing mutation could not be identified. Two samples, a brain resection and a pneumonectomy specimen, had no viable tumor cells due to tumor necrosis. Finally, one patient had a core biopsy of the adrenal gland that failed to sample the tumor, whereas the corresponding FNA revealed small cell transformation, but there was insufficient material for molecular testing. A total of 155 patients had biopsy samples that were sufficient for molecular analysis, including FNAs, core biopsies, surgical samples, and cytology from malignant effusions. There was only one serious adverse event, a pneumothorax requiring pigtail catheter placement.

Baseline clinical and molecular characteristics

Pretreatment clinical and molecular characteristics of the 155 patients are described in Table 2. Compared with a reference group of patients with EGFR-mutant lung cancers diagnosed in the same time period (data not shown), patients enrolled on the protocol were significantly younger, were more likely to be never smokers, had significantly fewer pack-years, and were more likely to have EGFR exon 19 deletions.

Prebiopsy course

The 155 patients began EGFR-TKI therapy from February 1999 to January 2011. One hundred ten patients (71%) were started on EGFR-TKI as first-line therapy. Twenty-four patients (15%) received EGFR-TKI as second-line therapy, and 9 patients (6%) as third- or fourth-line therapy. Twelve patients (8%) received EGFR-TKI as adjuvant or mainte-nance therapy after surgery and/or chemotherapy. The majority of patients (78%, 121 of 155) received single-agent

EGFR-TKI therapy, whereas 22% (34 of 155) were initially treated concurrently with EGFR-TKI and cytotoxic chemotherapy. The patients on this protocol had documented clinical progression on EGFR-TKI between March 2004 and December 2011. The median time from start of EGFR-TKI to clinical progression was 13 months with a range of 2 to 73 months. The time to progression on EGFR-TKI was similar for patients with EGFR exon 19 deletions versus EGFR L858R mutations, 15 and 17 months, respectively ($P = 0.99$). One patient with EGFR exon 19 deletion and EGFR T790M at baseline had a time to progression on EGFR-TKI of 2 months. Another patient with EGFR exon 19 deletion and a PIK3CA mutation had a time to progression of 5 months. No other patients had either EGFR T790M or PIK3CA mutations in their pre-TKI specimens. There was no difference in time to progression on EGFR-TKI for patients with secondary EGFR T790M mutations and patients with other mechanisms of resistance, 16 and 17 months, respectively ($P = 0.37$). Ninety-one percent (141 of 155) of patients continued their EGFR-TKI after evidence of clinical progression.

Findings at the time of acquired resistance

All 155 samples were tested for known mechanisms of acquired resistance. Not all analyses were conducted on all
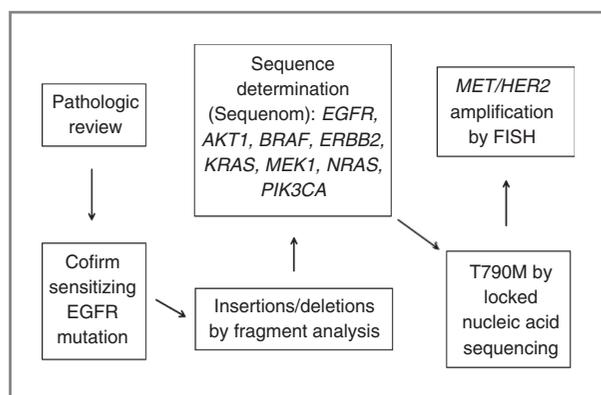
Figure 1. Assay prioritization.

specimens due to the limited amount of tissue available. Figure 1 illustrates the prioritization of analyses conducted. All specimens underwent pathologic review by a thoracic pathologist. Four of 155 had evidence of small cell histologic transformation [3%; 95% confidence interval (CI), 0%–6%]. Samples with small cell histology had further immunostains conducted including synaptophysin, chromogranin, and CD56 to confirm the diagnosis (Fig. 2). There was no evidence of small cell lung cancer in any of the pretreatment biopsy specimens. Morphologic changes consistent with epithelial-to-mesenchymal transition were not seen, although no immunohistochemical stains for vimentin or E-cadherin were conducted.

We identified a second-site EGFR T790M mutation in 98 of 155 samples (62%; 95% CI, 55%–70%). One patient had an acquired EGFR T854A mutation [observed incidence <1% (5% CI, <1%–4%]. In 88 patients, no acquired mutations were identified in PIK3CA, AKT1, BRAF, ERBB2, MEK1, NRAS, and KRAS (0%; 95% CI, 0%–4%).

Sufficient tissue was available for MET FISH analysis for 75 patients. Four of 75 patients tested had MET amplification, using the standard MET/CEP7 ratio of greater than 2 (5%; 95% CI, 1%–13%). Twenty-four patients had sufficient tissue available for HER2 FISH analysis. Two of 24 patients had evidence of HER2 amplification using the standard HER2/CEP17 ratio of greater than 2 (13%; 95% CI, 3%–32%). Baseline samples for the 4 MET-amplified and 2 HER-amplified cases were not available for FISH analysis. The frequency of the different mechanisms of resistance was similar when comparing patients with baseline EGFR L858R mutations versus EGFR exon 19 deletions. The relative percentages of the observations are illustrated in Fig. 3.

Overlap of mechanisms of resistance

Four percent of samples had more than one mechanism of resistance identified. Tumors from 2 patients had both small cell histologic transformation and EGFR T790M. One patient had small cell histologic transformation and MET amplification. In these patients showing small cell histologic transformation, there was no residual adenocarcinoma component in the biopsies studied, indicating that the

aforementioned acquired genetic alterations were present in the small cell carcinoma. Two patients had EGFR T790M and MET amplification by FISH; both molecular findings were identified in the same tumor samples. One patient had 2 acquired resistance samples; one sample was EGFR T790M–positive and the other had evidence of HER2 amplification. The autopsy specimen was EGFR T790M–positive but negative for HER2 amplification, and the adrenalectomy sample had evidence of HER2 amplification but was EGFR T790M–negative. The time to progression on EGFR-TKI for patients with 2 mechanisms of resistance ranged from 7 to 24 months.

Outcomes

Sixty-four percent (99 of 155) of patients have died as of February 2012. The median overall survival from diagnosis of stage IV disease was 3.8 years (95% CI, 3.1–5.1 years; Fig. 4). The median postprogression survival was 1.7 years (95% CI, 1.6–2.0 years; Fig. 4). In patients with EGFR T790M, the median postprogression survival was 1.9 years (95% CI, 1.6–2.6 years) and was 1.6 years (95% CI, 1.2–1.8 years) for those without EGFR T790M ($P = 0.015$; Fig. 5).

Discussion

To provide a more precise understanding of mechanisms of acquired resistance to EGFR-TKIs and their overlap in a single cohort, we report this prospective study of 155 patients. While the most commonly observed mechanism of resistance is EGFR T790M, our data show that tumors biopsied at the time of clinical acquired resistance to EGFR-TKIs display a spectrum of mechanisms, with rare overlap among mechanisms.

EGFR T790M is the most common mechanism of acquired resistance, seen in nearly two thirds of cases. MET amplification is uncommon and is less frequent than initially proposed (10). Acquired mutations in EGFR other than T790M, including T854A, D761Y, and L747S, are infrequent (21–23). Except for the one patient with a baseline PIK3CA mutation, no additional patients in this large series had a mutation in PIK3CA, AKT1, BRAF, HER2, MEK1, NRAS, and KRAS, which is consistent with another series that found no mutations in MEK1, NRAS, or KRAS, but did identify a secondary BRAF mutation in 2 of 195 acquired resistance samples (12). Point mutations in these genes including PIK3CA appear to be infrequent, having been occasionally reported in other series (8, 12). HER2 amplification may be a more common finding at the time of resistance, seen in 13% of cases in our series. Pretreatment samples were not available for HER2 FISH testing to confirm that amplification was not present before treatment. However, HER2 testing conducted on 99 untreated lung adenocarcinomas has been reported, with only 1 tumor (1%) displaying HER2 amplification pretreatment (11).

Small cell histologic transformation was also less frequent than previously reported (8). Interestingly, 3 of 4 cases of small cell histologic transformation occurred in
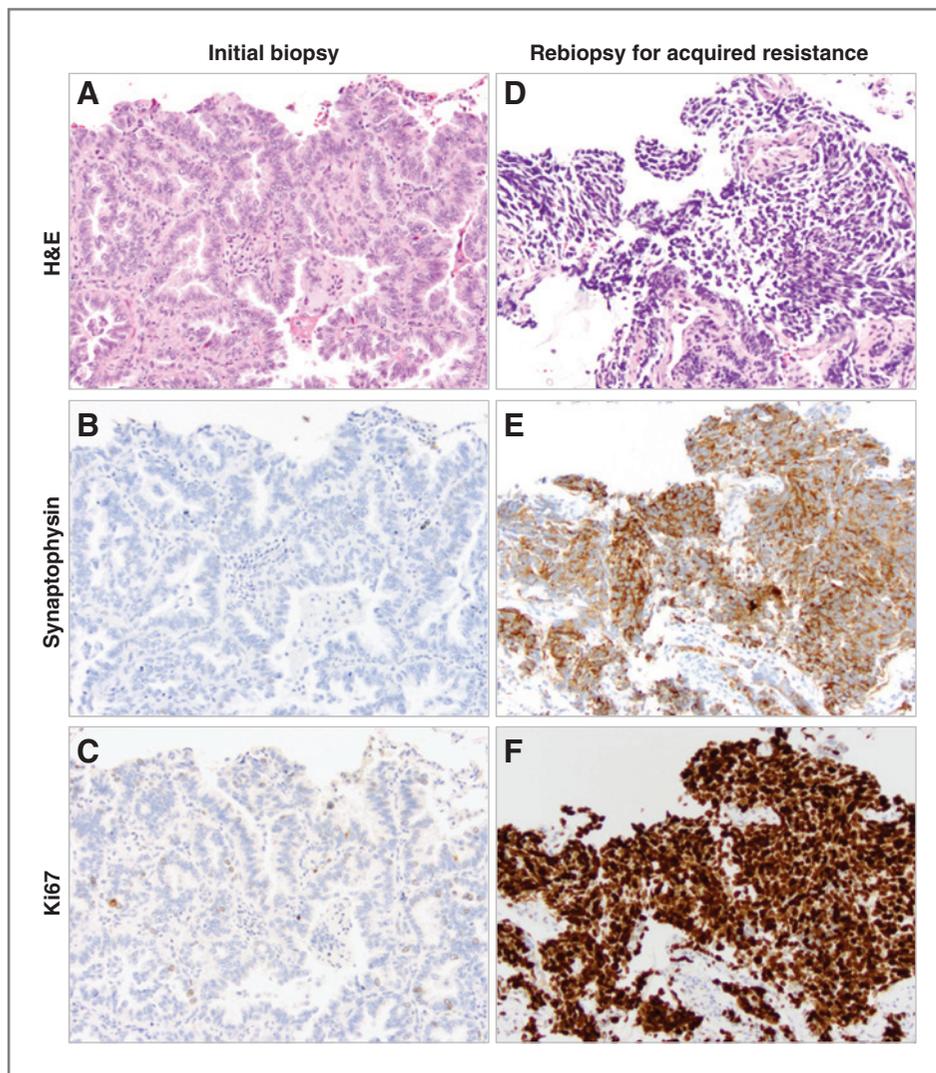
Figure 2. A patient's tumor tissue at initial lung biopsy (A–C) and biopsy at acquired resistance (D–F) with small cell histologic transformation. Hematoxylin and eosin stained material showing histologic changes from initial biopsy (A) to biopsy at acquired resistance (D) with immunohistochemical staining for CD56 and Ki67 on initial biopsy (B, C) and biopsy at acquired resistance (E, F).

addition to another mechanism of resistance. It is unclear if small cell transformation is merely associated with the development of resistance or is itself a causal mechanism.
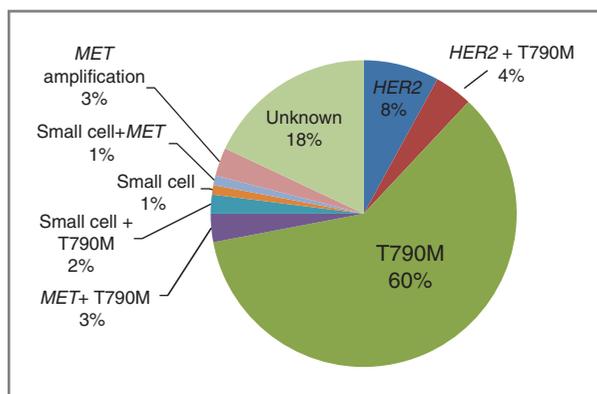
Figure 3. The relative frequencies of the various mechanisms of acquired resistance. Composite pie chart with percentages compiled from tests with varying denominators.

Small cell histologic transformation may be a phenomenon unique to TKI therapy as it has not been reported in patients who are treated and progress on cytotoxic chemotherapy, but rebiopsy data on such patients are limited. The patients with small cell histologic transformation all had a relatively aggressive clinical course as typically seen with small cell lung cancer. Two of the 4 patients received platinum-based doublet therapy and had partial responses after completion of 6 cycles of therapy. One patient received adjuvant cisplatin and etoposide after metastasectomy and had a 13-month disease-free interval before developing recurrent disease. One patient had a poor performance status and was treated with single-agent etoposide without response. Although the role of small cell histologic transformation in acquired resistance is presently unclear, histologic transformation is important to identify because it significantly alters our treatment recommendations.

MET amplification was seen concurrently with another mechanism of resistance in 3 of 4 cases. Two patients had both EGFR T790M and *MET* amplification and one patient
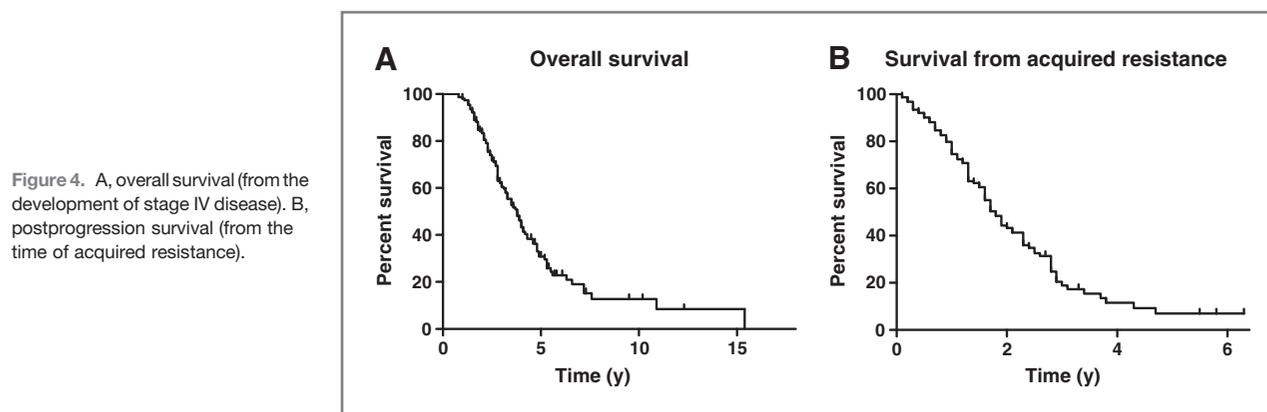
Figure 4. A, overall survival (from the development of stage IV disease). B, postprogression survival (from the time of acquired resistance).

had *MET* amplification and evidence of small cell transformation. This raises the question as to whether *MET* amplification alone is sufficient to induce resistance to EGFR-TKI therapy. Previous reports suggest a reciprocal relationship between EGFR T790M and *MET* amplification, with overlap of the 2 mechanisms becoming less frequent with increasing *MET* amplification ($\geq$4-fold; ref. 24). This reciprocal relationship was not seen in our cohort but is limited by the small number of patients with *MET* amplification. In 2 samples with EGFR T790 and *MET*, one had high level *MET* amplification (>10-fold) and one had moderate amplification (4-fold), and the sample with *MET* amplification only was determined by quantitative PCR and not quantified by FISH. Finally, one patient had different mechanisms of resistance, EGFR T790M and *HER2* amplification, identified in 2 distinct resistance samples with the same *EGFR*-sensitizing mutation. This suggests that either genetic alteration may be sufficient to impart clinical resistance, but different mechanisms of resistance may exist in distinct clones of a patient's cancer. Intratumor heterogeneity is a significant limitation of basing treatment decisions

on molecular analysis of a single tumor biopsy in the metastatic setting (25).

While many aspects of our findings are similar to other series, there are several differences. The median time to progression on EGFR-TKI of 13 months in our cohort is similar to previously published *EGFR*-mutant cohorts. (1–3) Despite this and other similarities to a similar cohort of patients with advanced *EGFR*-mutant lung cancers, these patients were somewhat younger with less tobacco exposure and were more likely to have *EGFR* exon 19 deletion. These factors have been associated with improved survival in previous studies (26–28) that may explain the patients' ability to enroll in a prospective trial at the time of development of acquired resistance.

The majority of patients (91%) continued EGFR-TKI therapy after clinical progression, sometimes in combination with chemotherapy. This treatment approach is consistent with preclinical data that continued EGFR inhibition is indicated due to the presence of a heterogeneous population of tumor cells, with varied sensitivity to EGFR inhibition (29) as well as clinical data showing rapid clinical progression (flare) after discontinuation of EGFR-TKI (30, 31). Recent retrospective analyses support the clinical observation of improved outcomes with continued EGFR-TKI therapy after radiologic progression (32–34). Prospective evaluation of continued EGFR-TKI after clinical progression will be important and will inform future clinical practice.

As this protocol opened, our understanding of the mechanisms of resistance has grown significantly, leading to some limitations in our analysis. Sufficient tissue from rebiopsies was not obtained for all the molecular tests currently conducted. Histologic analysis and testing for EGFR T790M was conducted on every sample, but FISH for *MET* and *HER2* and sequenom for other acquired oncogene mutations were conducted on only a portion of the samples. This limits the accuracy of the relative frequency of the various mechanisms we present and potentially underestimates the prevalence of overlap among the different mechanisms of resistance. Our analysis did not encompass alterations in autocrine or paracrine growth pathways that may also confer resistance (35, 36). Epithelial-to-mesenchymal transition has been described as a potential mechanism of resistance (8), although we did not note any
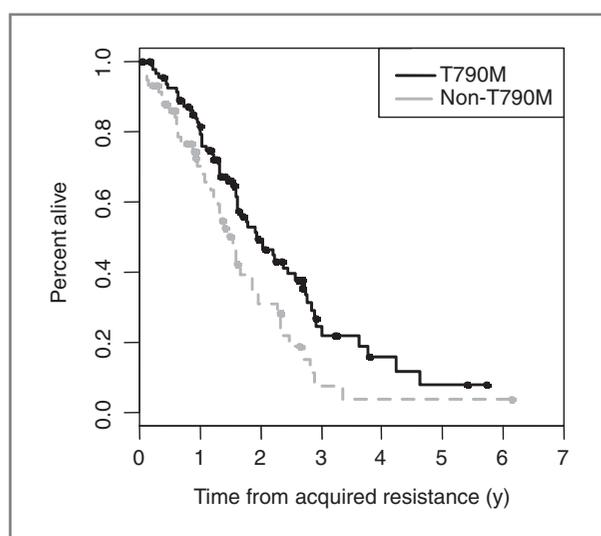

Figure 5. Postprogression survival for EGFR T790M+ patients and non-T790M+ patients.

evidence of spindle-like mesenchymal morphology on histologic review of our samples. However, we did not conduct immunohistochemistry for E-cadherin or vimentin which limits the conclusions that can be made. We confirmed the presence of the *EGFR*-sensitizing mutation in the pretreatment tumor sample of each patient included in this analysis, but we were unable to assess for *HER2* or *MET* amplification or conduct comprehensive genotyping on the majority of pretreatment specimens. Consequently, we cannot say with certainty that the genetic changes identified were acquired after treatment with an EGFR-TKI.

This prospective study confirms that standardized, stepwise molecular testing at the time of acquired resistance is feasible and safe. We plan to conduct comprehensive next-generation–based sequencing-based mutation profiling as well as protein and gene expression analysis on specimens where no mechanism of acquired resistance was identified. Other proposed mechanisms of acquired resistance to EGFR-TKI therapy including upregulated AXL and HGF expression and *MAPK1* amplification will need to be tested and validated in our larger dataset (37, 38). While the intent of these studies is to discover additional mechanisms of resistance to EGFR inhibition, the ultimate purpose is to better inform treatment decisions in the acquired resistance setting. Our growing knowledge of oncogenes and the molecular basis of resistance to kinase inhibition will allow for personalized treatment strategies in both the initial and resistance settings.

Disclosure of Potential Conflicts of Interest

Authors' Contributions

Grant Support

References

1. Mok TS, Wu YL, Thongprasert S, Yang CH, Chu DT, Saijo N, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med 2009;361:947–57.
2. Mitsudomi T, Morita S, Yatabe Y, Negoro S, Okamoto I, Tsurutani J, et al. Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): an open label, randomised phase 3 trial. Lancet Oncol 2010;11:121–8.
3. Maemondo M, Inoue A, Kobayashi K, Sugawara S, Oizumi S, Isobe H, et al. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. N Engl J Med 2010;362:2380–8.
4. Janne PA, Wang X, Socinski MA, Crawford J, Stinchcombe TE, Gu L, et al. Randomized Phase II trial of erlotinib alone or with carboplatin and paclitaxel in patients who were never or light former smokers with advanced lung adenocarcinoma: CALGB 30406 Trial. J Clin Oncol 2012;30:2063–9.
5. Kobayashi S, Boggon TJ, Dayaram T, Janne PA, Kocher O, Meyerson M, et al. EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. N Engl J Med 2005;352:786–92.
6. Pao W, Miller VA, Politi KA, Riely GJ, Somwar R, Zakowski MF, et al. Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. PLoS Med 2005;2:e73.
7. Zakowski MF, Ladanyi M, Kris MG. EGFR mutations in small-cell lung cancers in patients who have never smoked. N Engl J Med 2006;355:213–5.
8. Sequist LV, Waltman BA, Dias-Santagata D, Digumarthy S, Turke AB, Fidias P, et al. Genotypic and histological evolution of lung cancers acquiring resistance to EGFR inhibitors. Sci Transl Med 2011;3:75ra26.
9. Bean J, Brennan C, Shih JY, Riely G, Viale A, Wang L, et al. MET amplification occurs with or without T790M mutations in EGFR mutant lung tumors with acquired resistance to gefitinib or erlotinib. Proc Natl Acad Sci 2007;104:20932–7.
10. Engelman JA, Zejnullahu K, Mitsudomi T, Song Y, Hyland C, Park JO, et al. MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. Science 2007;316:1039–43.
11. Takezawa K, Pirazzoli V, Arcila ME, Nebhan CA, Song X, de Stanchina E, et al. HER2 amplification: a potential mechanism of acquired resistance to EGFR inhibition in EGFR mutant lung cancers that lack the second-site EGFR T790M mutation. Cancer Discov 2012;2:922–933.
12. Ohashi K, Sequist LV, Arcila ME, Moran T, Chmielecki J, Lin Y-L, et al. Lung cancers with acquired resistance to EGFR inhibitors occasionally harbor BRAF mutations but lack mutations in KRAS, NRAS, or MEK1. Proc Natl Acad Sci U S A 2012;109:E2127–33.
13. Oxnard GR, Arcila ME, Sima C, Riely GJ, Chmielecki J, Kris MG, et al. Acquired resistance to EGFR tyrosine kinase inhibitors in EGFR mutant lung cancer: Distinct natural history of patients with tumors harboring the T790M mutation. Clin Cancer Res 2011;17:1616–22.
14. Hata A KN, Katakami N, Yoshioka H, Takeshita J, Tanaka K, Nanjo S, et al. Rebiopsy of non-small cell lung cancer patients with acquired resistance to EGFR-TKI: comparison between T790M mutation-positive and -negative populations. J Clin Oncol 30, 2012 (suppl; abstr 7528).

15. Arcila ME, Oxnard GR, Nafa K, Riely GJ, Solomon SB, Zakowski MF, et al. Rebiopsy of lung cancer patients with acquired resistance to EGFR inhibitors and enhanced detection of the T790M mutation using a locked nucleic acid-based assay. Clin Cancer Res 2011;17:1169–80.

16. Pao W, Miller V, Zakowski M, Doherty J, Politi K, Sarkaria I, et al. EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib. Proc Natl Acad Sci U S A 2004;101:13306–11.

17. Pan Q, Pao W, Ladanyi M. Rapid polymerase chain reaction-based detection of epidermal growth factor receptor gene mutations in lung adenocarcinomas. J Mol Diagn 2005;7:396–403.

18. Arcila ME, Nafa K, Oxnard GR, Miller VA, Ladanyi M. Improved detection of the EGFR T790M mutation in lung cancer patients with acquired resistance to EGFR TKIs using a locked nucleic acid-based approach. J Mol Diagn 2009;11:Abstracts 655.

19. Cappuzzo F, Varella-Garcia M, Shigematsu H, Domenichini I, Bartolini S, Ceresoli GL, et al. Increased HER2 gene copy number is associated with response to gefitinib therapy in epidermal growth factor receptor-positive non-small-cell lung cancer patients. J Clin Oncol 2005;23: 5007–18.

20. Hirsch FR, Varella-Garcia M, McCoy J, West H, Xavier AC, Gumerlock P, et al. Increased epidermal growth factor receptor gene copy number detected by fluorescence in situ hybridization associates with increased sensitivity to gefitinib in patients with bronchioloalveolar carcinoma subtypes: a Southwest Oncology Group Study. J Clin Oncol 2005;23:6838–45.

21. Balak MN, Gong Y, Riely GJ, Somwar R, Li AR, Zakowski MF, et al. Novel D761Y and common secondary T790M mutations in epidermal growth factor receptor-mutant lung adenocarcinomas with acquired resistance to kinase inhibitors. Clin Cancer Res 2006;12:6494–501.

22. Bean J, Riely GJ, Balak M, Marks JL, Ladanyi M, Miller VA, et al. Acquired resistance to epidermal growth factor receptor kinase inhibitors associated with a novel T854A mutation in a patient with EGFR-mutant lung adenocarcinoma. Clin Cancer Res 2008;14:7519–25.

23. Costa DB, Halmos B, Kumar A, Schumer ST, Huberman MS, Boggon TJ, et al. BIM mediates EGFR tyrosine kinase inhibitor-induced apoptosis in lung cancers with oncogenic EGFR mutations. PLoS Med 2007;4:1669–79.

24. Suda K, Murakami I, Katayama T, Tomizawa K, Osada H, Sekido Y, et al. Reciprocal and complementary role of MET amplification and EGFR T790M mutation in acquired resistance to kinase inhibitors in lung cancer. Clin Cancer Res 2010;16:5489–98.

25. Gerlinger M, Rowan AJ, Horswell S, Larkin J, Endesfelder D, Gronroos E, et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. N Engl J Med 2012;366:883–92.

26. Riely GJ, Pao W, Pham D, Li AR, Rizvi N, Venkatraman ES, et al. Clinical course of patients with non-small cell lung cancer and epidermal growth factor receptor exon 19 and exon 21 mutations treated with gefitinib or erlotinib. Clin Cancer Res 2006;12:839–44.

27. Harichand-Herdt S, Ramalingam SS. Gender-associated differences in lung cancer: clinical characteristics and treatment outcomes in women. Semin Oncol 2009;36:572–80.

28. Radzikowska E, Glaz P, Roszkowski K. Lung cancer in women: age, smoking, histology, performance status, stage, initial treatment and survival. Population-based study of 20 561 cases. Ann Oncol 2002;13:1087–93.

29. Chmielecki J, Foo J, Oxnard GR, Hutchinson K, Ohashi K, Somwar R, et al. Optimization of dosing for EGFR-mutant non-small cell lung cancer with evolutionary cancer modeling. Sci Translat Med 2011;3: 90ra59.

30. Chaft JE, Oxnard GR, Sima CS, Kris MG, Miller VA, Riely GJ. Disease flare after tyrosine kinase inhibitor discontinuation in patients with EGFR-mutant lung cancer and acquired resistance to erlotinib or gefitinib: implications for clinical trial design. Clin Cancer Res 2011;17:6298–303.

31. Riely GJ, Kris MG, Zhao B, Akhurst T, Milton DT, Moore E, et al. Prospective assessment of discontinuation and reinitiation of erlotinib or gefitinib in patients with acquired resistance to erlotinib or gefitinib followed by the addition of everolimus. Clin Cancer Res 2007;13: 5150–5.

32. Goldberg SB, Oxnard GR, Digumarthy S, Muzikansky A, Jackman DM, Lennes IT, et al. Chemotherapy with erlotinib or chemotherapy alone in advanced NSCLC with acquired resistance to EGFR tyrosine kinase inhibitors (TKI). J Clin Oncol:30, 2012 (suppl; abstr 7524).

33. Oxnard GR, Lo P, Jackman DM, Butaney M, Heon S, Johnson BE, et al. Delay of chemotherapy through use of post-progression erlotinib in patients with EGFR-mutant lung cancer. J Clin Oncol 30, 2012 (suppl; abstr 7547).

34. Faehling M, Eckert R, Kamp TG, Kuom S, Spengler W. Treatment with EGFR tyrosine kinase inhibitors beyond progression in long-term responders to erlotinib in advanced non-small cell lung cancer: a case-control study of overall survival. J Clin Oncol 30, 2012 (suppl; abstr 7572).

35. Yano S, Wang W, Li Q, Matsumoto K, Sakurama H, Nakamura T, et al. Hepatocyte growth factor induces gefitinib resistance of lung adenocarcinoma with epidermal growth factor receptor-activating mutations. Cancer Res 2008;68:9479–87.

36. Yano S, Yamada T, Takeuchi S, Tachibana K, Minami Y, Yatabe Y, et al. Hepatocyte growth factor expression in EGFR mutant lung cancer with intrinsic and acquired resistance to tyrosine kinase inhibitors in a Japanese cohort. J Thorac Oncol 2011;6:2011–7.

37. Zhang Z, Lee JC, Lin L, Olivas V, Au V, LaFramoise T, et al. Activation of the AXL kinase causes resistance to EGFR-targeted therapy in lung cancer. Nat Genet 2012;44:852–60.

38. Ercan D, Xu C, Yanagita M, Monast CS, Pratilas CA, et al. Reactivation of ERK signaling causes resistance to EGFR kinase inhibitors. Cancer Discov 2012;2:934–947.

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