Predictive Biomarkers and Personalized Medicine

Acquired Resistance to EGFR Tyrosine Kinase Inhibitors in EGFR-Mutant Lung Cancer: Distinct Natural History of Patients with Tumors Harboring the T790M Mutation

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Abstract

Purpose: Patients with epidermal growth factor receptor (EGFR)-mutant lung adenocarcinoma develop acquired resistance to EGFR tyrosine kinase inhibitors (TKI) after a median of 10 to 16 months. In half of these cases, a second EGFR mutation, T790M, underlies acquired resistance. We undertook this study to examine the clinical course of patients harboring the T790M mutation following progression on TKI.

Experimental Design: EGFR-mutant lung cancer patients with acquired resistance to EGFR TKIs were identified as part of a prospective rebiopsy protocol in which postprogression tumor specimens were collected for molecular analysis. Postprogression survival and characteristics of disease progression were compared in patients with and without T790M.

Results: We identified T790M in the initial rebiopsy specimens from 58 of 93 patients (62%, 95% CI: 52–72). T790M was more common in biopsies of lung/pleura tissue and lymph nodes than in more distant sites ($P = 0.014$). Median postprogression survival was 16 months (interquartile range = 9–29 months); patients with T790M had a significantly longer postprogression survival ($P = 0.036$). Patients without T790M more often progressed in a previously uninvolved organ system ($P = 0.014$) and exhibited a poorer performance status at time of progression ($P = 0.007$).

Conclusions: Among patients with acquired resistance to EGFR TKIs, the presence of T790M defines a clinical subset with a relatively favorable prognosis and more indolent progression. Knowledge of T790M status is therefore important both for the clinical care of these patients and for the optimal design and interpretation of clinical trials in this setting. *Clin Cancer Res; 17(6); 1616–22. ©2010 AACR.*

Introduction

The identification of epidermal growth factor receptor (EGFR)-sensitizing mutations in a subset of patients with lung adenocarcinoma has transformed the management of non–small cell lung cancer (NSCLC). This molecular subtype of lung cancer, diagnosed in approximately 20,000 patients in the United States each year, is the largest in a small group of cancers (including chronic myelogenous leukemia and gastrointestinal stromal tumor) which have been found to have profound sensitivity to single agent tyrosine kinase inhibitors (TKI). Multiple studies have shown that patients with EGFR-mutant lung cancer have an approximately 70% RECIST (Response Evaluation Criteria in Solid Tumors) response rate to EGFR TKIs (1–4). Prospective analyses have shown that presence of an EGFR-sensitizing mutation is the biomarker most strongly associated with progression-free survival benefit from first-line EGFR TKI treatment over chemotherapy (3, 5, 6).

Patients with EGFR-mutant lung adenocarcinoma develop progression of disease on TKI therapy after a median of 10 to 16 months (1, 3, 7). This condition has been described as "acquired resistance" to TKI, and the complexity of trial design and interpretation in this setting has led to recent publication of consensus clinical criteria for patient eligibility (8–11). Several groups studying the molecular biology of acquired resistance have found that at least 50% of cases are attributable to a secondary mutation, T790M, in exon 20 of EGFR (12–14). This mutation is felt to change the relative affinity of the mutant receptor in favor of ATP, largely abrogating the effect of the TKI (15). The T790M mutation has also occasionally been detected in tumor specimens from patients with no prior exposure to EGFR TKIs (3, 16–19), though the prevalence of

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Translational Significance

This analysis evaluates whether there is prognostic significance to the T790M mutation in patients with epidermal growth factor receptor (EGFR)-mutant lung cancer who have developed acquired resistance EGFR tyrosine kinase inhibitors such as erlotinib. Preclinical data have suggested that T790M mutant cell lines have indolent growth, but such an indolent behavior has not been previously shown clinically. In this analysis, we find that patients with T790M-mediated acquired resistance have relatively favorable progression characteristics and clinical outcomes, suggesting that this mutation identifies a subset of cancers with a distinct biology. Our findings indicate that T790M may be an important prognostic and mechanistic biomarker in patients with acquired resistance and mutation status should be routinely determined in patients enrolling in clinical trials for acquired resistance.

"de novo T790M" can vary depending on the detection method employed.

Although the detection of T790M in baseline specimens has been associated with a poorer outcome on TKI therapy (18), the association between T790M-related acquired resistance and clinical outcomes has not been systematically explored. Preclinical data have shown that EGFR-mutant cell lines that acquire the T790M mutation have a more indolent growth than parental cell lines (J. Chmielecki and colleagues, submitted; ref. 20), which could translate *in vivo* into a more indolent natural history. On the basis of this preclinical finding, we tested the hypothesis that patients with EGFR-mutant tumors and T790-mediated acquired resistance to EGFR TKIs would have a favorable natural history when compared with patients with acquired resistance lacking the T790M mutation.

Methods

Through a prospective rebiopsy protocol, consecutive patients with advanced EGFR-mutant lung adenocarcinoma were identified who had acquired resistance to EGFR TKI. Patients must have had response or durable stable disease (>6 months) on TKI followed by progression while receiving TKI. Eligibility was based on consensus criteria (8), with the exception that patients who received a TKI in combination with chemotherapy were considered eligible if their tumor harbored an EGFR mutation. Patients were also allowed to receive subsequent therapies in the interval since progression on TKI. Beginning in August 2004, patients with lesions amenable to biopsy were enrolled on a clinical trial to biopsy a growing site of disease (21). Additional patients from this period were included in the analysis if they possessed postprogression biopsy material obtained during a clinically indicated procedure, so long as they consented to a separate clinical trial allowing correlative tissue analyses.

Specimens were tested for the presence of an EGFR-sensitizing mutation, using a PCR-based assay previously described (22). Samples not found to harbor an EGFR-sensitizing mutation were excluded from this analysis. Testing for the T790M mutation was then carried out using standard methodologies (12, 23). For cases where additional tumor DNA was available, highly sensitive testing was also done using sequencing augmented by a locked nucleic acid (LNA) which suppresses amplification of the wild-type allele and allows detection of T790M when present in as little as 0.1% of DNA (23). Patients were classified as "T790M positive" or "T790M negative" on the basis of whether T790M was detected in their first post-progression biopsy specimen that was adequate for molecular testing. For patients with adequate tissue available, MET amplification was tested by FISH (24); cases were considered amplified if the ratio of MET to CEP7 was greater than 2:1.

Patient charts were retrospectively reviewed, in an Institutional Review Board–approved fashion, to summarize potentially relevant clinical characteristics and disease course. The association between the prevalence of the T790M mutation and clinical factors was tested using a Fisher's exact test. The date of progression was defined clinically on the basis of (i) sufficient growth of tumor in a known site of disease to make a clinician discuss alteration of therapy as documented in the patient's medical chart and/or (ii) imaging showing a new site of metastatic disease. Progression was identified as involving a new metastatic organ system if new metastases were identified within 1 week of date of progression. For the time to new metastasis analysis, the date of a new metastasis was defined as the date of the scan that documented metastatic involvement of a new organ system; patients without new metastases were censored at the date of last imaging evaluation. For survival analyses, patients were censored at date last known alive. All time-to-event outcomes were estimated using the Kaplan–Meier method and compared across groups using the log-rank test (for univariate analysis) and the Cox proportional hazards model (for multivariate analysis).

Results

We identified 127 patients with EGFR-mutant lung cancer and acquired resistance meeting eligibility criteria; 115 consented to the rebiopsy protocol, whereas 12 had consented to a separate protocol allowing correlative tissue analyses. Of these patients, 107 (84%) had adequate postprogression tissue available for complete mutation analysis. We then excluded 4 patients because they began an EGFR TKI as an adjuvant therapy while having no evidence of disease, 6 patients because they initially received an investigational EGFR TKI with known additional targets, and 4 patients who had progression in the central nervous system only, a condition which has been described to be
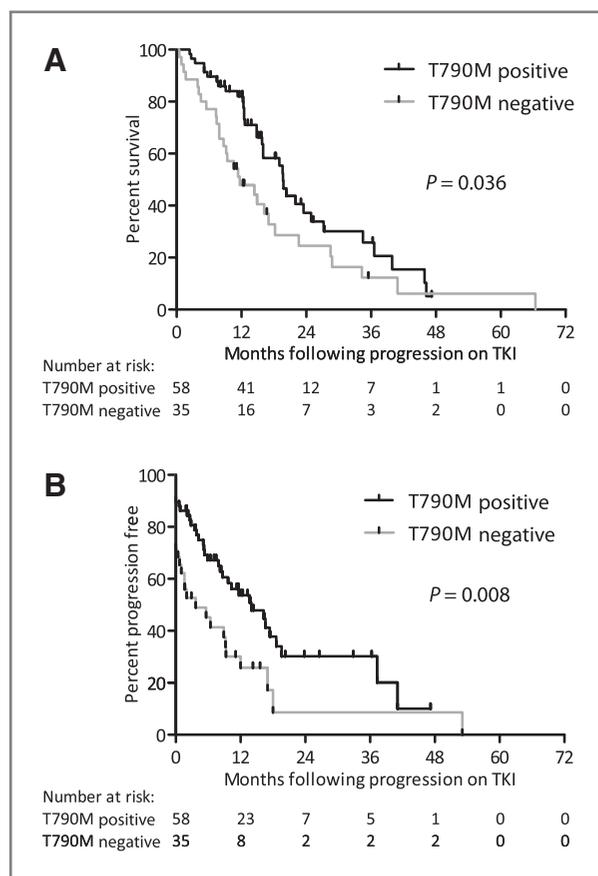
Table 1. Patient characteristics and T790M prevalence

Characteristics	n (% total)	% with T790M	P
Gender			
Male	33 (35)	64	1
Female	60 (65)	62	
Race			
Asian	17 (18)	59	0.79
Non-Asian	76 (82)	63	
Smoking history			
Never smoker	61 (66)	61	0.66
Former/current smoker	32 (34)	66	
Stage			
IV at diagnosis	71 (76)	61	0.62
Recurrent	22 (24)	68	
EGFR-sensitizing mutation			
Exon 19 deletion	70 (75)	63	1
Exon 21 point mutation	23 (25)	61	
Initial TKI received			
Erlotinib	64 (69)	66	0.36
Gefitinib	29 (31)	55	
Line of treatment with TKI			
First-line/maintenance	72 (77)	64	0.13
Second-line	11 (12)	36	
Third-line or later	10 (11)	80	
Site of biopsy			
Lung/pleura	67 (72)	66	0.014
Lymph node	8 (9)	75	
Other[a]	18 (19)	44	

[a]Liver ($n = 6$), bone ($n = 4$), brain ($n = 3$), adrenal ($n = 2$), uterine cervix, skin, peritoneum.

Figure 1. Postprogression survival (A) and time from progression until new metastasis (B), depending on T790M mutation status.

sensitive to TKIs when given in higher doses (25, 26). The remaining 93 patients comprised our group for analysis. To evaluate for selection bias, we compared our cohort with a reference cohort of all EGFR-mutant lung cancer patients with advanced disease treated at our institution (287 patients; ref. 27) and found no difference in median survival from date of advanced disease (40 vs. 37 months, $P = 0.24$; Supplementary Fig. 1).

Characteristics of the 93 patients are outlined in Table 1. Patients began TKI between February 1999 and August 2009 and developed progression between August 2003 and November 2009. Ninety of 93 biopsies (97%) were done on sites of growing disease. The median interval between date of progression and date of rebiopsy was 7 weeks (interquartile range = 3–21 weeks). Fifty-eight patients (62%, 95% CI: 52–72) had the T790M mutation detected in their initial postprogression biopsy; 35 patients (38%, 95% CI: 28–47) were found to be T790M negative. Seventy-seven percent of patients received erlotinib or gefitinib as part of their initial therapy. We continued EGFR TKI therapy following disease progression in addition to

subsequent therapies in 87% of patients (9). The T790M mutation was found at similar frequencies across multiple, different patient characteristics (Table 1), although the prevalence was higher in lung/pleura and lymph node specimens than in more distant sites ($P = 0.014$).

The median follow-up was 13 months following disease progression, and 67% of patients had died. Median postprogression survival for the entire cohort was 16 months (interquartile range = 9–29 months); comparison of postprogression survival from patients with and without T790M is shown in Figure 1A. Patients with T790M had a median postprogression survival of 19 months, longer than the 12-month median postprogression survival of patients without T790M ($P = 0.036$). Characteristics of disease progression were compared in patients with and without T790M (Table 2). Patients with T790M were more likely to progress in an existing site of disease rather than a new organ system ($P = 0.010$). Analysis of time to development of metastasis beginning with date of progression found earlier development of new metastatic organ system involvement in patients without T790M ($P = 0.008$; Fig. 1B). Patients without T790M were also found to have a lower Karnofsky performance status (KPS) at time of

Table 2. Survival and progression characteristics as a function of T790M mutation status

	T790M positive (*n* = 58)	T790M negative (*n* = 35)	*P*
Median postprogression survival, mo	19 (16–24)	12 (8–17)	0.036
KPS at progression			0.007
80%–90%	53 (91%)	26 (74%)	
60%–70%	5 (9%)	4 (11%)	
30%–50%	0 (0%)	5 (14%)	
Site of disease progression			0.014
New metastatic organ system involved	6 (10%)	11 (31%)	
Existing organ only	52 (90%)	24 (69%)	
Median time from progression to new metastatic organ involvement, mo[a]	14 (8–18)	4 (1–9)	0.008
TKI continued after progression			0.32
Yes	53 (91%)	29 (83%)	
No	5 (9%)	6 (17%)	
Treated with chemotherapy subsequently			0.31
Yes	47 (81%)	35 (71%)	
No	11 (19%)	10 (29%)	
Treated on clinical trial subsequently			0.39
Yes	28 (48%)	13 (37%)	
No	30 (52%)	22 (63%)	

[a]See Methods for detailed definition.

progression ($P = 0.007$). No differences in postprogression treatment were found in the two groups (Table 2). In multivariate analysis, there was no additional difference in postprogression survival based on T790M status when controlling for performance status and the presence of new metastatic disease at progression ($P = 0.27$).

To determine whether the difference in clinical course after progression was related to a different disease course prior to progression on TKI, we compared time to progression (TTP) on TKI in patients with and without T790M. This analysis relies on the hypothesis that the mechanistic and biological bases of resistance to therapy are phenomena that begin early in the course of treatment and thus can influence disease course long before clinical evidence of progression. Patients who developed T790M had a median TTP of 14 months, which was not significantly different from the median TTP of 11 months in patients who did not develop T790M ($P = 0.10$). Because it can be challenging to determine a specific "date of progression" in patients who have indolent progression on therapy, we conducted an exploratory analysis to evaluate the difference in overall survival from the beginning of TKI therapy in patients with and without T790M. Although this analysis could be biased because (a) it studies only the selected group of patients who were rebiopsied and (b) patient enrollment occurred after development of progression, it does allow a time-to-event analysis for which the start date (start of TKI treatment) and end date (date of death) are more firmly known. Considering these limitations, we found a significant difference in survival between the two subgroups, with a median survival of 39 months in patients who develop

T790M and 26 months in patients who do not ($P = 0.007$; Supplementary Fig. 2).

There were adequate MET FISH results for 38 of the 93 patients (41%). Four patients (11%, 95% CI: 3–25) were found to have MET amplification, a proportion that is lower than previously described (21%–22%) but the difference was not statistically significant ($P = 0.11$; refs. 24, 28). Three of the 4 patients additionally harbored T790M, including the 1 patient found to have high-level amplification (MET: CEP7 ratio > 3). The small number of patients with MET amplification precluded meaningful outcome analyses in this subset.

Discussion

Patients with EGFR-mutant lung cancer have a prolonged survival compared with the median survival of 12 months which is commonly reported for patients with advanced NSCLC (29). Although this may in part be due to durable responses to EGFR TKIs, it also is likely that these cancers have a distinct biology from that of EGFR wild-type lung cancers, with greater chemosensitivity and better surgical outcomes (3, 30). However, this remains a heterogeneous population: some patients with EGFR-mutant lung cancer do poorly after an initial response to TKI, whereas others can live for many years despite advanced disease. Our data suggest that detection of the T790M mutation after progression on TKI identifies a subset of EGFR-mutant lung cancer with a distinct natural history and longer survival, which is consistent with preclinical data showing that EGFR-mutant cell lines that acquire the

T790M mutation display indolent growth (20). If verified prospectively, the T790M mutation would be one of the first *acquired* molecular biomarkers with prognostic value in solid tumor oncology.

Our data suggest that patients with and without T790M after progression on TKI have very different clinical features of progression, likely driven by their different genotypes. The absence of T790M after progression, likely indicating some "other" resistance mechanism, is associated with earlier development of new metastatic sites of disease and a poorer performance status, contributing to the shorter survival of these patients. Alternatively, T790M-mediated resistance is more likely to occur in an existing site of disease and has also been found to be associated with indolent disease growth (J. Chmielecki and colleagues, submitted; ref. 20), characteristics that may explain the longer survival of these patients. Importantly, the vast majority of patients in our data set were maintained on chronic EGFR TKI therapy after developing resistance to prevent growth of TKI-sensitive clones, in addition to subsequent cytotoxic therapies intended to treat the subset of resistant clones (9). Although this is not standard practice at most institutions, we believe this strategy may help maintain the indolent characteristics of T790M-associated progression, though it may make our findings difficult to replicate with patient cohorts in which TKI was routinely discontinued on progression.

The existence of 2 biological subtypes of acquired resistance has important implications for clinical trials conducted in this setting (9–11), as these 2 populations likely require distinct treatment approaches. For example, T790M-positive patients may be more likely to maintain dependence on the EGFR signaling axis (31) and thus may benefit from newer strategies targeting EGFR and its signaling pathway (32, 33). This possible role for T790M at resistance as a predictive or prognostic biomarker is complicated by the need for rebiopsy to obtain tumor DNA, a strategy which can be logistically challenging, though we and others have found it feasible (21, 34–36). Technologies allowing minimally invasive determination of T790M status from peripheral blood specimens or cytologic specimens are in development and may simplify this process (18, 37, 38).

For patients without T790M, given their poorer prognosis, it is extremely important that we develop a better understanding of the molecular mechanisms of TKI resistance. MET amplification has been identified as one alternative mechanism of acquired resistance (24, 28); however, in the subset of our cohort tested by FISH, the prevalence was only 11% (with a single case of high-level amplification). Alternative pathways of TKI resistance, of which several have been hypothesized (39), are actively being investigated by multiple groups using high-throughput mutation testing, siRNA screens, and gene expression microarray analysis.

The positive prognostic implications of T790M in the acquired resistance setting differ from its negative prognostic role prior to treatment with TKI (18, 40). In pretreatment EGFR-mutant lung cancers, the reported prevalence of *de novo* T790M has varied widely (1%–38%; refs. 3, 17, 18, 40, 41), with the upper end of this range possibly reflecting false-positive results with some T790M assays (42). In the pretreatment setting, patients with no T790M detected would be expected to do better because they harbor only a sensitizing mutation and have not yet acquired a resistance mutation (16–18). In contrast, patients who are "T790M negative" in the resistant setting must harbor some other mechanism of resistance, leading to their poorer prognosis. Note that in our analysis, we included only patients who showed a clinical benefit on TKI; therefore, patients with a preexisting *de novo* T790M mutation and disease refractory to TKI treatment would not be included in our cohort. Analysis is ongoing to determine whether patients harboring the T790M after acquired resistance additionally harbor low levels of pretreatment; however, no such cases have yet been identified in our cohort (23).

One possible limitation of our data is that only a subset of all EGFR-mutant lung cancer patients had biopsy tissue available for molecular analysis after progression on TKI, leading to the exclusion of patients without adequate biopsies. One could hypothesize that poor prognosis patients would be unable to undergo rebiopsy and would be left out of our analysis, skewing our results. For example, only 25% of our cohort harbored EGFR exon 21 mutations rather than the expected 40% (1, 3), and these types of mutations have previously been found to be associated with poorer outcomes (2, 43). However, when we compared our cohort with a reference cohort of all EGFR-mutant lung cancer patients treated at the institution, we found no difference in median survival from date of advanced disease (Supplementary Fig. 1; ref. 27). We therefore believe that our sample accurately represents patients with EGFR-mutant lung cancer treated with erlotinib or gefitinib.

Because of the unique patient population analyzed in this study, accrual and analysis occurred over several years during which time molecular testing techniques have evolved. Initial testing techniques employing direct sequencing and fragment analysis detect mutant clones when present in at least 10% to 20% of DNA studied. With these techniques, false-negative results can occur when specimens contain significant amounts of stromal or inflammatory tissue. Our current testing mechanism, using direct sequencing augmented by an LNA which inhibits wild-type DNA amplification, can detect T790M when present in as little as 0.1% of DNA studied, significantly improving sensitivity of testing (23). This may explain why we have found a somewhat higher prevalence of T790M than has previously been reported (12, 35).

In conclusion, we find that patients with acquired resistance to gefitinib and erlotinib harboring the T790M mutation have a distinct natural history and that this mutation identifies a distinct subset of patients with a longer survival after progression and later development of new metastases. Our data suggest that knowledge of T790M status is important for the clinical care of these patients and also for the optimal design and interpretation of clinical trials in

this setting. A better understanding of the biological mechanisms of non–T790M-mediated acquired resistance is needed to develop treatments of this poorer prognosis population.

Disclosure of Potential Conflicts of Interest

M. Ladany: speakers' bureau, Genzyme, Sequenom; consultant, AstraZeneca. W. Pao: consultant, ownership interest, Molecular MD. G. J. Riley: consultant, AstraZeneca, Boehringer-Ingelheim, Bristol-Myers-Squibb. M.G. Kris: consultant, AstraZeneca, Boehringer-Ingelheim, Pfizer. V.A. Miller: consultant, Roche, Genentech, OSI.

References

1. Rosell R, Moran T, Queralt C, Porta R, Cardenal F, Camps C, et al. Screening for epidermal growth factor receptor mutations in lung cancer. N Engl J Med 2009;361:958–67.
2. Jackman DM, Miller VA, Cioffredi LA, Yeap BY, Jänne PA, Riely GJ, et al. Impact of epidermal growth factor receptor and KRAS mutations on clinical outcomes in previously untreated non-small cell lung cancer patients: results of an online tumor registry of clinical trials. Clin Cancer Res 2009;15:5267–73.
3. Mok TS, Wu Y-L, 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.
4. 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.
5. Fukuoka M, Wu Y, Thongprasert S, Yang C, Chu D, Saijo N, et al. Biomarker analyses from a phase III, randomized, open-label, first-line study of gefitinib (G) versus carboplatin/paclitaxel (C/P) in clinically selected patients (pts) with advanced non-small cell lung cancer (NSCLC) in Asia (IPASS). J Clin Oncol 2009;27:abstr 8006.
6. 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.
7. Janne PA, Wang XF, Socinski MA, Crawford J, Capelletti M, Edelman MJ, et al. Randomized phase II trial of erlotinib (E) alone or in combination with carboplatin/paclitaxel (CP) in never or light former smokers with advanced lung adenocarcinoma: CALGB 30406. J Clin Oncol 2010;27:abstr 7503.
8. Jackman D, Pao W, Riely GJ, Engelman JA, Kris MG, Jänne PA, et al. Clinical definition of acquired resistance to epidermal growth factor receptor tyrosine kinase inhibitors in non-small-cell lung cancer. J Clin Oncol 2010;28:357–60.
9. 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.
10. Sequist LV, Besse B, Lynch TJ, Miller VA, Wong KK, Gitlitz B, et al. Neratinib, an irreversible pan-ErbB receptor tyrosine kinase inhibitor: results of a phase II trial in patients with advanced non-small-cell lung cancer. J Clin Oncol 2010;28:3076–83.
11. Johnson ML, Riely GJ, Rizvi NA, Azzoli CG, Kris MG, Ginsberg MS, et al. Phase II trial of dasatinib for patients demonstrating acquired resistance to treatment with the epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) erlotinib (E) or gefitinib (G). J Thor Oncol 2009;4:S689.
12. 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.
13. Kobayashi S, Boggon TJ, Dayaram T, Jänne 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.
14. Kwak EL, Sordella R, Bell DW, Godin-Heymann N, Okimoto RA, Brannigan BW, et al. Irreversible inhibitors of the EGF receptor may circumvent acquired resistance to gefitinib. Proc Natl Acad Sci U S A 2005;102:7665–70.
15. Yun CH, Mengwasser KE, Toms AV, Woo MS, Greulich H, Wong KK, et al. The T790M mutation in EGFR kinase causes drug resistance by increasing the affinity for ATP. Proc Natl Acad Sci U S A 2008; 105:2070–5.
16. Inukai M, Toyooka S, Ito S, Asano H, Ichihara S, Soh J, et al. Presence of epidermal growth factor receptor gene T790M mutation as a minor clone in non-small cell lung cancer. Cancer Res 2006;66:7854–8.
17. Sequist LV, Martins RG, Spigel D, Grunberg SM, Spira A, Jänne PA, et al. First-line gefitinib in patients with advanced non-small-cell lung cancer harboring somatic EGFR mutations. J Clin Oncol 2008; 26:2442–9.
18. Maheswaran S, Sequist LV, Nagrath S, Ulkus L, Brannigan B, Collura CV, et al. Detection of mutations in EGFR in circulating lung-cancer cells. N Engl J Med 2008;359:366–77.
19. Girard N, Lou E, Azzoli CG, Reddy R, Robson M, Harlan M, et al. Analysis of genetic variants in never-smokers with lung cancer facilitated by an Internet-based blood collection protocol: a preliminary report. Clin Cancer Res 2010;16:755–63.
20. Chmielecki J, Foo J, Somwar R, Regales L, Vivanco I, Shen R, et al. EGFR T790M decreases growth potential of lung tumor cells with drug-sensitive EGFR mutant alleles in the absence of drug selection. In: AACR 100th Annual Meeting; 2009; Denver, CO. Abstract 4217.
21. Arcila ME, Riely GJ, Zakowski MF, Kris MG, Ladanyi M, Pao W, et al. Rebiopsy of patients (pts) with acquired resistance to epidermal growth factor tyrosine kinase inhibitors (EGFR-TKIs) in non-small cell lung cancer (NSCLC). J Clin Oncol 2009;27:abstr 8025.
22. 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.
23. Arcila ME, Oxnard GR, Khedoudja N, Riely GJ, Solomon SB, Zakowski M, 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 Can Res 2011;17:1169–80.
24. 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 U S A 2007;104:20932–7.
25. Jackman DM, Holmes AJ, Lindeman N, Wen PY, Kesari S, Borras AM, et al. Response and resistance in a non-small-cell lung cancer patient with an epidermal growth factor receptor mutation and leptomeningeal metastases treated with high-dose gefitinib. J Clin Oncol 2006;24:4517–20.
26. Clarke JL, Pao W, Wu N, Miller VA, Lassman AB. High dose weekly erlotinib achieves therapeutic concentrations in CSF and is effective in leptomeningeal metastases from epidermal growth factor receptor mutant lung cancer. J Neurooncol 2010;99:283–6.
27. Johnson ML, Sima C, Paik PK, Janjigian YY, Pao W, Kris MG, et al. Association of KRAS and EGFR mutations with survival in patients

with advanced lung adenocarcinoma. J Clin Oncol 2010;28:abstr 7541.

28. 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.

29. Sandler A, Gray R, Perry MC, Brahmer J, Schiller JH, Dowlati A, et al. Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. N Engl J Med 2006;355:2542–50.

30. Marks JL, Broderick S, Zhou Q, Chitale D, Li AR, Zakowski MF, et al. Prognostic and therapeutic implications of EGFR and KRAS mutations in resected lung adenocarcinoma. J Thorac Oncol 2008;3:111–6.

31. Godin-Heymann N, Ulkus L, Brannigan BW, McDermott U, Lamb J, Maheswaran S, et al. The T790M "gatekeeper" mutation in EGFR mediates resistance to low concentrations of an irreversible EGFR inhibitor. Mol Cancer Ther 2008;7:874–9.

32. Zhou W, Ercan D, Chen L, Yun CH, Li D, Capelletti M, et al. Novel mutant-selective EGFR kinase inhibitors against EGFR T790M. Nature 2009;462:1070–4.

33. Regales L, Gong Y, Shen R, de Stanchina E, Vivanco I, Goel A, et al. Dual targeting of EGFR can overcome a major drug resistance mutation in mouse models of EGFR mutant lung cancer. J Clin Invest 2009;119:3000–10.

34. Solomon SB, Zakowski MF, Pao W, Thornton RH, Ladanyi M, Kris MG, et al. Core needle lung biopsy specimens: adequacy for EGFR and KRAS mutational analysis. AJR Am J Roentgenol 2010;194:266–9.

35. Waltman BA, Dias-Santagata D, Cosper AK, Mino-Kenudson M, Borger DR, Fidias P, et al. SNaPshot multigene assay to detect mechanisms of acquired resistance to EGFR tyrosine kinase inhibitors (TKIs). J Clin Oncol 2010;28:abstr 7554.

36. Kim ES, Herbst RS, Lee JJ, Blumenschein G, Tsao A, Wistuba I, et al. Phase II randomized study of biomarker-directed treatment for non-small cell lung cancer (NSCLC): the BATTLE (Biomarker-Integrated Approaches of Targeted Therapy for Lung Cancer Elimination) clinical trial program. J Clin Oncol 2009;27:abstr 8024.

37. Kuang Y, Rogers A, Yeap BY, Wang L, Makrigiorgos M, Vetrand K, et al. Noninvasive detection of EGFR T790M in gefitinib or erlotinib resistant non-small cell lung cancer. Clin Cancer Res 2009;15:2630–6.

38. Brevet M, Arcila M, Ladanyi M. Assessment of EGFR mutation status in lung adenocarcinoma by immunohistochemistry using antibodies specific to the two major forms of mutant EGFR. J Mol Diagn 2010;12:169–76.

39. Engelman JA, Janne PA. Mechanisms of acquired resistance to epidermal growth factor receptor tyrosine kinase inhibitors in non-small cell lung cancer. Clin Cancer Res 2008;14:2895–9.

40. Rosell R, Molina MA, Costa C, Taron M, Bertran-Alamillo J, Mayo C, et al. Outcome to erlotinib in non-small cell lung cancer (NSCLC) patients (p) according to the presence of the EGFR T790M mutation and BRCA1 mRNA expression levels in pretreatment biopsies. J Clin Oncol 2010;28:abstr 7514.

41. Kris MG, Lau CY, Ang D, Brzostowski E, Riely GJ, Rusch VW, et al. Initial results of LC-MAP: an institutional program to routinely profile tumor specimens for the presence of mutations in targetable pathways in all patients with lung adenocarcinoma. J Clin Oncol 2010;28: abstr 7009.

42. Clennell D. Urgent Field Safety Notice 001. Manchester, UK: Diagnostic Innovations (dxsdiagnostics.com) [cited 2010 Jun 7]. Available from: http://www.swissmedic.ch/recalllists_dl/02354/Vk_20090603_14-e1.pdf.

43. 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.

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Acquired Resistance to EGFR Tyrosine Kinase Inhibitors in EGFR-Mutant Lung Cancer: Distinct Natural History of Patients with Tumors Harboring the T790M Mutation

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