

## Assessment of *EGFR* Mutation Status in Matched Plasma and Tumor Tissue of NSCLC Patients from a Phase I Study of Rociletinib (CO-1686)

Chris Karlovich<sup>1</sup>, Jonathan W. Goldman<sup>2</sup>, Jong-Mu Sun<sup>3</sup>, Elaina Mann<sup>1</sup>, Lecia V. Sequist<sup>4</sup>, Krzysztof Konopa<sup>5</sup>, Wei Wen<sup>6</sup>, Philipp Angenendt<sup>7</sup>, Leora Horn<sup>8</sup>, David Spigel<sup>9</sup>, Jean-Charles Soria<sup>10</sup>, Benjamin Solomon<sup>11</sup>, D. Ross Camidge<sup>12</sup>, Shirish Gadgil<sup>13</sup>, Cloud Paweletz<sup>14</sup>, Lin Wu<sup>6</sup>, Sean Chien<sup>6</sup>, Patrick O'Donnell<sup>6</sup>, Shannon Matheny<sup>1</sup>, Darrin Despain<sup>1</sup>, Lindsey Rolfe<sup>1</sup>, Mitch Raponi<sup>1</sup>, Andrew R. Allen<sup>1</sup>, Keunchil Park<sup>3</sup>, and Heather Wakelee<sup>15</sup>

### Abstract

**Purpose:** The evaluation of plasma testing for the *EGFR* resistance mutation T790M in NSCLC patients has not been broadly explored. We investigated the detection of *EGFR* activating and T790M mutations in matched tumor tissue and plasma, mostly from patients with acquired resistance to first-generation *EGFR* inhibitors.

**Experimental Design:** Samples were obtained from two studies, an observational study and a phase I trial of rociletinib, a mutant-selective inhibitor of *EGFR* that targets both activating mutations and T790M. Plasma testing was performed with the cobas *EGFR* plasma test and BEAMing.

**Results:** The positive percent agreement (PPA) between cobas plasma and tumor results was 73% (55/75) for activating mutations and 64% (21/33) for T790M. The PPA between BEAMing plasma and tumor results was 82% (49/60) for activating

mutations and 73% (33/45) for T790M. Presence of extrathoracic (M1b) versus intrathoracic (M1a/M0) disease was found to be strongly associated with ability to identify *EGFR* mutations in plasma ( $P < 0.001$ ). Rociletinib objective response rates (ORR) were 52% [95% confidence interval (CI), 31 – 74%] for cobas tumor T790M-positive and 44% (95% CI, 25 – 63%) for BEAMing plasma T790M-positive patients. A drop in plasma-mutant *EGFR* levels to  $\leq 10$  molecules/mL was seen by day 21 of treatment in 7 of 8 patients with documented partial response.

**Conclusions:** These findings suggest the cobas and BEAMing plasma tests can be useful tools for noninvasive assessment and monitoring of the T790M resistance mutation in NSCLC, and could complement tumor testing by identifying T790M mutations missed because of tumor heterogeneity or biopsy inadequacy. *Clin Cancer Res*; 22(10); 2386–95. ©2016 AACR.

<sup>1</sup>Clovis Oncology Inc., San Francisco, California. <sup>2</sup>University of California Los Angeles, Los Angeles, California. <sup>3</sup>Samsung Medical Center Sungkyunkwan University School of Medicine, Seoul, Korea. <sup>4</sup>Massachusetts General Hospital, Boston, Massachusetts. <sup>5</sup>Medical University of Gdansk, Gdansk, Poland. <sup>6</sup>Roche Molecular Systems, Pleasanton, California. <sup>7</sup>Sysmex Inostics GmbH, Hamburg, Germany. <sup>8</sup>Vanderbilt University School of Medicine, Nashville, Tennessee. <sup>9</sup>Sarah Cannon Research Institute, Nashville; Tennessee. <sup>10</sup>Institut Gustave Roussy, Villejuif, France. <sup>11</sup>University of Melbourne, Melbourne, Australia. <sup>12</sup>University of Colorado, Denver, Colorado. <sup>13</sup>Karmanos Cancer Institute/Wayne State University, Detroit, Michigan. <sup>14</sup>Dana-Farber Cancer Institute, Boston, Massachusetts. <sup>15</sup>Stanford University, Stanford, California.

**Note:** Supplementary data for this article are available at Clinical Cancer Research Online (<http://clincancerres.aacrjournals.org/>).

C. Karlovich, J. Goldman, J.-M. Sun, H. Wakelee, and K. Park contributed equally to this article.

**Corresponding Authors:** Jonathan W. Goldman, University of California Los Angeles, 2020 Santa Monica Blvd, Suite 600, Santa Monica, CA 90404. Phone: 310-633-8400; Fax: 310-633-8419; E-mail: JWGoldman@mednet.ucla.edu; and Heather Wakelee, E-mail: hwakelee@stanford.edu

**doi:** 10.1158/1078-0432.CCR-15-1260

©2016 American Association for Cancer Research.

### Introduction

The introduction of first-generation *EGFR* inhibitors (erlotinib, gefitinib) and subsequently afatinib for non-small cell lung carcinoma (NSCLC) patients with activating somatic mutations in *EGFR* has led to improved tolerability and efficacy compared with first-line chemotherapy. However, while patients have experienced objective response rates (ORR) of 60% to 70%, almost all ultimately develop resistance to therapy with an average progression-free survival (PFS) of 9 to 14 months (1–4). In approximately 60% of cases, resistance is mediated by the emergence of a second mutation, T790M, in the tyrosine kinase domain of *EGFR* (5). Rociletinib (CO-1686) is a novel, oral, irreversible tyrosine kinase inhibitor for the treatment of patients with mutant *EGFR* NSCLC that has demonstrated efficacy against the activating mutations (L858R and del19) and the acquired primary resistance mutation (T790M), while sparing wild-type *EGFR* (6). Promising clinical activity has recently been reported from an ongoing phase I/II trial of rociletinib with a 59% ORR in a heavily pretreated, centrally confirmed T790M mutation-positive patient population (7).

Precision medicine is a reality for NSCLC, with *EGFR* and anaplastic lymphoma kinase (*ALK*) testing now integrated into

### Translational Relevance

Several studies have demonstrated that *EGFR* mutation detection in the plasma of newly diagnosed NSCLC patients is feasible. However, detection of the acquired resistance mutation T790M in relapsed patient plasma has not been broadly assessed. Here we use two sensitive plasma testing methodologies to evaluate *EGFR* activating and T790M mutation detection in contemporaneously matched tumor and plasma, mostly from a phase I study of the third-generation *EGFR* inhibitor rociletinib. Plasma assays identified T790M resistance mutations missed by biopsy because of tumor heterogeneity or lack of adequate/available tumor tissue. Response rates for rociletinib were similar in T790M-positive patients whether identified by plasma or tumor tests (ORR: 44% vs. 52%). These data suggest plasma testing will be a useful complement to tumor testing, particularly in the setting of acquired resistance where tumor heterogeneity is likely to be greater and rebiopsy more challenging than in newly diagnosed patients.

the National Comprehensive Cancer Network (NCCN) and College of American Pathologists (CAP) guidelines (8, 9). However the acquisition of suitable tissue for molecular testing continues to be a challenge. In a recent phase IV trial of gefitinib in newly diagnosed patients with late-stage NSCLC, for example, approximately 15% of patients had inadequate tumor sample for molecular analysis, and a number of patients were poor candidates for biopsy due to comorbidities (10). In a later-line setting, the availability of suitable tumor tissue may be even more limited. The use of circulating tumor DNA (ctDNA) from plasma has shown promise in overcoming the challenges posed by tissue-based testing (11). In addition to being minimally invasive, the assessment of ctDNA has the potential to overcome sampling bias due to spatial and temporal genetic heterogeneity of tumor samples that has been observed for NSCLC and other cancers (12, 13).

Several published studies have demonstrated that the detection of *EGFR* mutations is feasible in plasma (1, 14–19). Technologies that have been evaluated include ARMS/Scorpions PCR, WAVE, digital PCR, PNA-clamp, and deep sequencing-based approaches. Performance is typically assessed by benchmarking plasma results against those of matched tumor tissue in patients. Most of these studies have been small and retrospective in nature. They have also focused on the detection of activating mutations rather than the acquired resistance mutation T790M as rebiopsy after initial therapy is not always feasible. It is in the context of acquired resistance to targeted therapies, where rebiopsy may not be readily attainable and tumor heterogeneity is likely greater than in a newly diagnosed setting, that the potential diagnostic utility of ctDNA may be greatest.

Here we report results from a large prospective series of matched tissue and plasma samples drawn from an ongoing phase I clinical trial of rociletinib and an observational study. Our primary objective was to assess detection of the T790M resistance mutation in patients with acquired resistance to first- and second-generation *EGFR* tyrosine kinase inhibitor therapy. We evaluated the cobas *EGFR* mutation test, a test platform based

on allele-specific PCR. We also tested a partially overlapping set of plasma samples for *EGFR* mutations using BEAMing (Beads, Emulsions, Amplification and Magnetics), a technology based on digital PCR, and compared the results to the cobas plasma test results. To investigate the diagnostic utility of plasma *EGFR* testing, we also evaluated ORR based on plasma *EGFR* status in the subgroup of phase I study patients who were treated at therapeutic doses of rociletinib.

## Materials and Methods

### Study design and patients

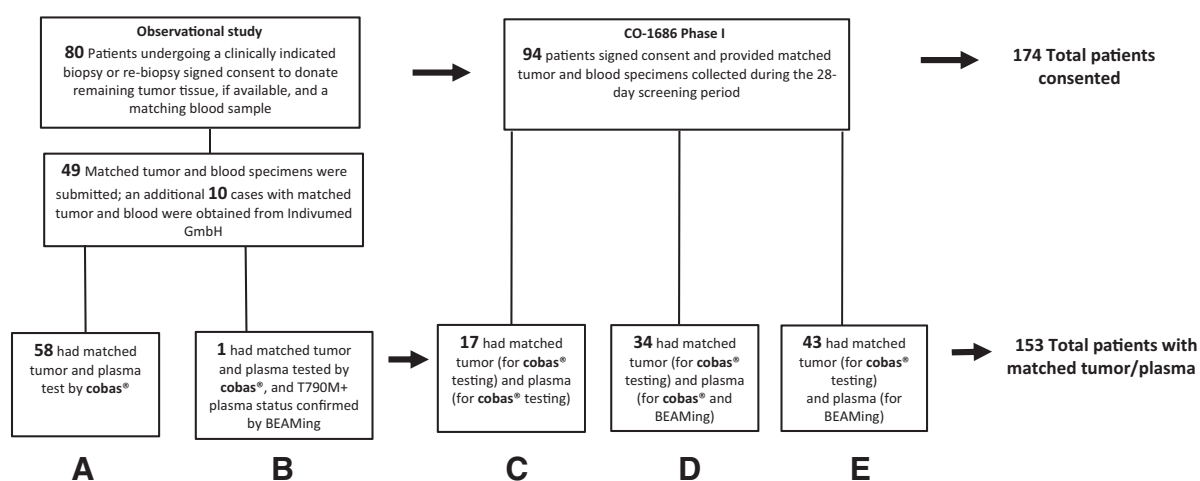
The observational, multicenter study sponsored by Clovis Oncology enrolled patients between April 2011 and June 2013 and was designed to prospectively collect matched blood and tumor tissue from newly diagnosed or relapsed patients with advanced (stage IIIB, IV) NSCLC. The observational study protocol allowed enrollment of patients without a requirement for documented evidence of an *EGFR* mutation. Data from local testing is incomplete; only central testing results are presented here. Eligible patients were undergoing, or had recently undergone, a clinically indicated biopsy or rebiopsy and signed an Ethics Committee/Institutional Review Board (EC/IRB)-approved consent prior to donating a blood sample and matched FFPE tumor tissue. Patients in the observational study did not receive rociletinib.

Clovis-sponsored trial CO-1686-008 (NCT01526928 known as TIGER-X) is an ongoing multicenter interventional study comprising a phase I dose-escalation part and a phase II part. All patients signed an EC/IRB-approved consent prior to any procedures. The primary objectives of phase I were to assess safety, tolerability, and pharmacokinetic parameters of rociletinib. Secondary endpoints included assessment of objective response rate (ORR) by RECIST (Response Evaluation Criteria in Solid Tumors) version 1.1, duration of response (defined as the time from first observation of response until RECIST-defined progression), and progression-free survival (PFS; defined as the time from first dose until RECIST-defined progression or death). On the basis of the dose–response relationship observed in the phase I study, therapeutic doses were defined as 900 mg twice daily free base formulation, and 500 mg twice daily and 625 mg twice daily HBr formulation. All patients enrolled in TIGER-X were required to have documented evidence of an *EGFR*-activating mutation in their medical history. Further details of the CO-1686-008 study design have been published previously (7).

### Sample collection and processing

Blood samples for cobas and BEAMing testing were collected in K2 EDTA tubes (up to four, 6-mL Vacuettes), processed into plasma within 30 minutes ( $1,800 \times g$  for 10 minutes at 18–23°C), and stored at –70°C or below before processing. For most patients, 2 mL of plasma were used for DNA purification and in no case was <1 mL plasma used. For BEAMing, DNA was extracted using the QIAamp DNA Circulating Nucleic Acid Kit according to the manufacturer's instructions. For the cobas plasma test, DNA was extracted as described previously (20). The leukocyte fraction of the blood was not collected, and germline *EGFR* status was not assessed.

For all phase I study patients, matched plasma was collected within 28 days of tumor biopsy. Plasma not obtained prior to biopsy was collected  $\geq 7$  days later to minimize risk of artificially elevating ctDNA levels following the biopsy procedure.



**Figure 1.**

Flow diagram of study design. A total of 174 patients were consented: 80 patients for the observational study; 94 patients for the phase I study. A total of 153 patients had matched tumor and plasma samples. Cobas plasma testing was performed on 110 patients from the observational and phase I studies (boxes A + B + C + D); BEAMing plasma testing was performed on 77 phase I patients (boxes D + E) and on one patient from the observational study (Box B) who was T790M-negative in tumor to confirm cobas plasma test T790M-positive status.

For all tissue specimens, DNA from one 5- $\mu$ m section was extracted for cobas *EGFR* testing. Tumor content was assessed by board-certified pathologists using hematoxylin and eosin (H&E)-stained sections. Tumor specimens were considered evaluable if the tumor content was  $\geq 10\%$ . If the tumor content was  $< 10\%$ , the specimen was still considered evaluable if tumor cells were present and macrodissection was performed, or if the mutation results were the same in both tumor and plasma (four cases for cobas test; two cases for BEAMing). Testing with the cobas *EGFR* tissue test was done at Roche Molecular Systems and at Carolinas Pathology Group.

#### *EGFR* mutation analysis

For all patient samples in both the observational and phase I studies, the cobas *EGFR* tissue test (Roche Molecular Systems, Inc.) was used to perform *EGFR* mutation analysis on DNA extracted from FFPE tumor tissue. All patients in the observational study and a subset of patients in the phase I study had ctDNA from plasma tested with the cobas *EGFR* plasma test. The cobas *EGFR* tissue and plasma tests have been described previously (21, 22). See Supplementary Methods for further details.

The principles and details of BEAMing (Symex Inostics GmbH) have been described previously (23). See Supplementary Methods for further details.

A comparative evaluation of additional *EGFR* testing platforms included OnTarget (Boreal Genomics) and droplet digital PCR (ddPCR; Bio-Rad) technologies. For OnTarget, target mutant sequences were first enriched using a hybridization-based approach. *EGFR* mutations were then identified from the enriched fraction using next-generation sequencing. Methodological details for OnTarget have been described elsewhere (24). For ddPCR, details of the assay design, primers and probes, and a detailed protocol have been described previously (25).

#### Statistical analysis

Correlation between ability to detect mutations in plasma and clinical characteristics was calculated using a logistic regression model with the following covariates: age (treated as a continuous

variable), sex (female or male), lines of previous therapy (treated as a continuous variable), smoking status (never vs. former smoker), Eastern Cooperative Oncology Group (ECOG) performance status (0 vs. 1), race (white vs. non-white), tumor burden (SLD treated as a continuous variable), and disease classification (M0/M1a vs. M1b). Tumor type (adenocarcinoma vs. other) and tumor stage (IIIB/IV vs. other) were not included in the analysis because the vast majority of patients were stage IIIB/IV with adenocarcinoma. The threshold for statistical significance for all analyses was considered to be  $P < 0.05$ .

## Results

### Patient characteristics

The patients described in the present analysis were enrolled in two separate studies: (i) an observational study of newly diagnosed and relapsed NSCLC patients and (ii) a phase I trial of rociletinib in patients who had received at least one prior *EGFR* inhibitor and had an *EGFR* activating mutation in their medical record, but who could have either T790M-negative, positive, or unknown status (7). The clinical characteristics of the patients are described in Supplementary Table S1.

A total of 174 patients provided consent for tissue and plasma testing ( $n = 94$  from the phase I study and  $n = 80$  from the observational study). We obtained usable matched tumor and plasma samples from 153 patients; 94 from the phase I and 59 from the observational study (Fig. 1). All patients from the phase I study had FFPE tumor tissue obtained after progression on their previous therapy and prior to initiating treatment with rociletinib. Baseline plasma blood draws were obtained within 4 weeks of the tissue biopsy. The majority of biopsy specimens (66/94, 70%) were core-needle biopsies (Supplementary Table S1).

For the observational study, 84% (60/71) of patients had matched plasma collected before therapy was initiated and within 60 days of tumor biopsy. Biopsies included tumor (55%), cytology (37%), and unknown (8%) specimen types. The majority of genotyping results were valid for both tumor and cytology specimens (Supplementary Table S1).

**Table 1.** Concordance between tumor and cobas plasma test *EGFR* status

	Cobas tumor test mutation status			Total
	Mutation positive	Mutation negative	Inadequate tissue	
Cobas plasma test				
Activating mutation				
Mutation positive	55	0	13	68
Mutation negative	20	24	3	47
Total	75	24	16	115 <sup>a</sup>
T790M				
Mutation positive	21	1	3	25
Mutation negative	12	61	12	85
Total	33	62	15	110
Activating mutations (tumor as reference)		<b>95% CI</b>		
PPA	73% (55/75)	(62%-83%)		
Negative percent agreement	100% (24/24)	(86%-100%)		
Overall percent agreement	80% (79/99)	(71%-87%)		
T790M (tumor as reference)		<b>95% CI</b>		
PPA	64% (21/33)	(45%-80%)		
Negative percent agreement	98% (61/62)	(91%-100%)		
Overall percent agreement	86% (82/95)	(78%-93%)		

Abbreviation: CI, confidence interval.

<sup>a</sup>Five patients had two activating mutations identified; each activating mutation was counted individually.**Assessment of matched plasma and tumor with the cobas EGFR mutation test**

A total of 110 patients had *EGFR* mutation status assessed in both plasma and tumor with the cobas *EGFR* mutation test (patient subsets A + B + C + D, Fig. 1), a qualitative test methodology based on allele-specific PCR (21). Of these, 75 cases were identified with *EGFR*-activating mutations in tumor specimens (Table 1), including 19 newly diagnosed patients from the observational study and 56 later line patients. The same activating mutation(s) was identified in 55 corresponding plasma samples tested with the cobas plasma test, yielding a positive percent agreement (PPA) of 73% with tumor as the reference sample type. Notably, there were no cases in which an activating mutation was found in plasma but not in tumor DNA. The two most common activating mutations, L858R and del19 mutations, had a similar PPA and NPA between plasma and tumor (Supplementary Tables S2 and S3). Ninety-three percent of activating mutations identified were either L858R or del19. We also identified patients with S768I, G719X, and L861Q mutations as well as exon 20 insertions (Supplementary Table S3). Five patients had compound activating mutations. For all patients, the same activating mutations were identified in tumor and plasma.

We then examined concordance for T790M mutation status by cobas testing in the same series of matched tumor and plasma DNA (Table 1). T790M mutations were identified in 33/110 tumor samples. In 21 of these, T790M was also identified in the corresponding plasma sample for a PPA of 64%. One patient had a T790M mutation identified in plasma but not tumor DNA. A replicate aliquot of plasma from this patient was also tested by BEAMing and was confirmed to be T790M-positive at a level of 29 molecules/mL.

**Baseline plasma *EGFR* assessment by BEAMing**

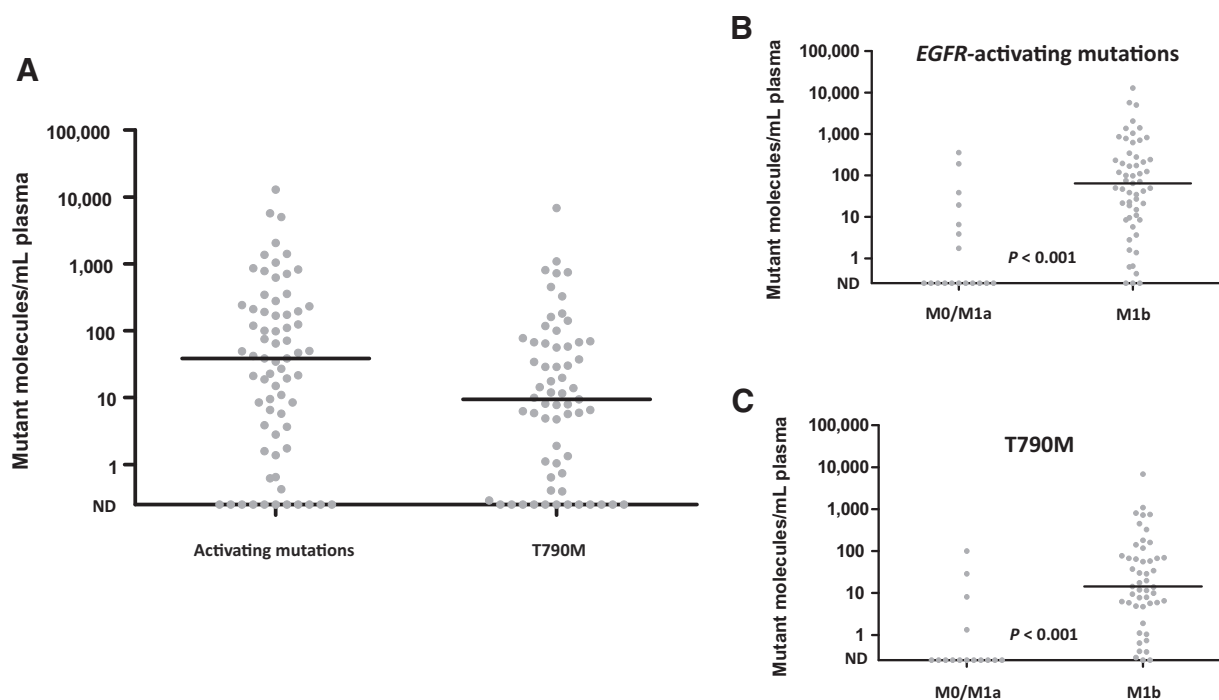
Seventy-seven patients from the phase I study, all having received at least one previous line of *EGFR* inhibitor therapy, had their plasma *EGFR* mutation status assessed by BEAMing (patient subsets D + E, Fig. 1), a quantitative method based on digital PCR followed by flow cytometry (23). Fourteen of these patients had a tumor biopsy that was inadequate for *EGFR* analysis, three were activating mutation-negative in tumor, and

18 were T790M-negative in tumor (Supplementary Table S4). The PPA between the BEAMing plasma test and cobas tumor test was 82% (49/60) for activating mutations and 73% (33/45) for T790M. There were nine patients with T790M mutations detected in plasma that were not detected in tumor and nine additional patients with T790M mutations in plasma who did not have a tumor sample adequate for molecular analysis. Therefore, overall, the BEAMing plasma test identified more T790M-positive patients (51) than did the tumor test (45) in the 77-patient subset. The median number of mutant molecules/mL in plasma was 38 (range, 0–12,872) for activating mutations and nine (range, 0–6,838) for T790M (Fig. 2A).

**Comparison of plasma mutation detection between different technology platforms**

There were 35 patients for whom we had both BEAMing and cobas *EGFR* plasma test results from replicate samples (patient subsets B + D, Fig. 1). The agreement between BEAMing and cobas tests in plasma was 86% (30/35) for activating mutations and 83% (29/35) for T790M (Supplementary Table S5). For activating mutations, there were three cases where the cobas test detected a mutation not identified by BEAMing and two cases where BEAMing detected a mutation not identified by cobas test. For T790M, there was one case where the cobas test detected a mutation not identified by BEAMing and five cases where BEAMing detected a mutation not identified by cobas test. Of note, all discordant cases that were positive by BEAMing and negative by cobas test occurred at  $\leq 6$  molecules/mL.

There were 8 cases among the 35 patients tested who were T790M-positive in tumor but T790M-negative in plasma by both BEAMing and cobas *EGFR* plasma tests. To investigate whether the inability to detect T790M in plasma in these cases might be a limitation resulting from the biology of NSCLC rather than the testing platform, we tested baseline plasma from 14 low-copy patients (<30 copies/mL by BEAMing) among the 35 patient subset, including 5 of the T790M tumor-positive/plasma-negative cases, by ddPCR and OnTarget (Boreal Genomics) technology platforms (Supplementary Table S6). The five T790M tumor-positive/plasma-negative cases were negative in plasma by all technology platforms.



**Figure 2.** *EGFR*-mutant molecules per mL at baseline in phase I patients tested by BEAMing. A, distributions of *EGFR*-activating mutation and T790M-mutant molecules in baseline plasma by BEAMing. Comparison of *EGFR*-activating mutation (B) and T790M (C) levels in plasma by M stage classification. Solid lines represent median values. For M0/M1a patients, the median values were 0 molecules/mL. The *P* values shown were derived from a Mann-Whitney test.

**Clinical characteristics that impact identification of *EGFR* mutations in plasma**

Previous studies have suggested that ctDNA levels in patient plasma are associated with tumor burden (26, 27). To assess this, we explored possible associations between *EGFR* mutation detection in plasma by BEAMing and tumor burden in the 77 phase I patients with a BEAMing plasma test result (patient subsets D + E, Fig. 1), 75 of whom had available target lesion data. No association was observed between tumor burden (sum of target lesions) and the ability to identify *EGFR* mutations in plasma (Supplementary Fig. S1).

However, TNM staging revealed that 11 of 13 T790M tumor-positive patients who did not have identifiable T790M mutations in plasma had M0 or M1a NSCLC. An M0 or M1a disease classification denotes there is no radiographic evidence of cancer beyond the thoracic cavity, whereas M1b staging is consistent with a spread to distant metastatic sites. We therefore interrogated whether a more general association might exist between M stage and the ability to identify mutations in plasma. A subgroup of the 77 phase I patients with a BEAMing plasma test result was *EGFR* mutation-positive and had known

M0/M1a/M1b status. Among 55 patients with M1b (extrathoracic metastatic) disease, 52 (95%) had activating mutations detected in plasma (Table 2). Conversely, among patients with M1a (intrathoracic metastases) only 39% (7/18) had *EGFR*-activating mutations identified in the plasma (M1a vs. M1b: *P* < 0.001). Similarly, T790M mutations were more commonly identified in the plasma of M1b versus M1a patients with a 96% detection rate for T790M in the plasma of M1b patients versus 27% for M1a (Table 2). M1b patients had higher levels of both activating mutations and T790M in plasma than did M1a patients (*P* < 0.001; Fig. 2B and C). We were able to confirm these initial observations in 72 patients, 34 of which overlapped the initial BEAMing dataset, using the cobas *EGFR* plasma test (*P* < 0.001; Supplementary Table S7).

We performed a logistic regression analysis to look for other clinical characteristics that might predict the ability to identify *EGFR* mutations in plasma including M stage, age, gender, race, performance status, smoking status, tumor burden, or lines of previous therapy. No variables other than M stage were associated with the ability to identify *EGFR* mutations in plasma (data not shown).

**Table 2.** *EGFR* mutation detection by BEAMing and NSCLC disease classification (*n* = 75)

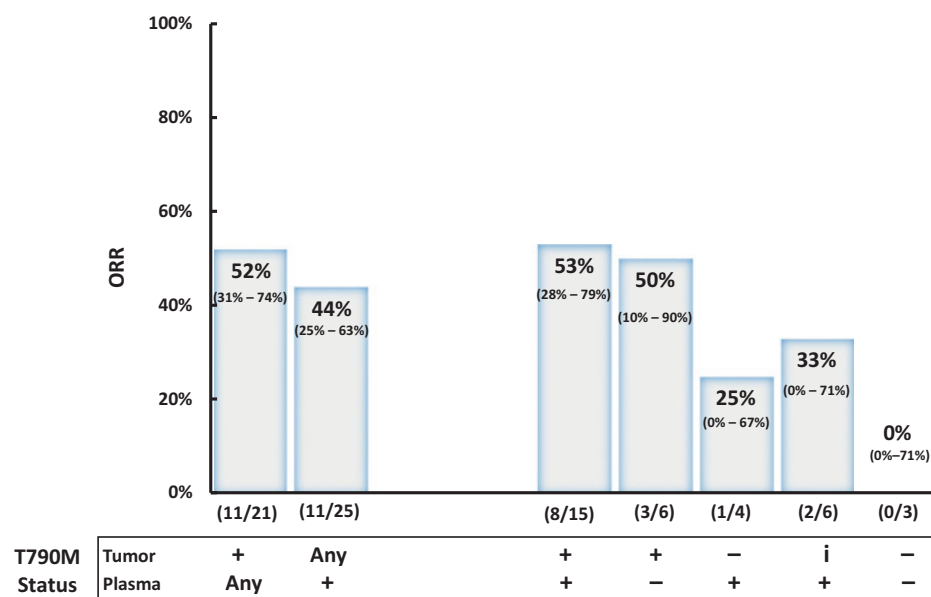
<i>EGFR</i> Mutation	Disease classification	Patients with mutation <sup>a</sup>	Subset with mutation in plasma	Percentage	<i>P</i> <sup>b</sup>
Activating mutations	M1a/M0	18	7	39%	<0.001
	M1b	55	52	95%	
T790M	M1a/M0	15	4	27%	<0.001
	M1b	49	47	96%	

<sup>a</sup>Includes patients with an *EGFR* mutation detected in tissue only, plasma only, or both tissue and plasma.

<sup>b</sup>Fisher exact test used for comparisons.

**Figure 3.**

Efficacy of rociletinib by tumor and plasma *EGFR* status. The percentages in each bar of the histogram represent ORRs with confidence intervals below in parentheses. The fraction of the total number of patients with objective responses is indicated in the lower part of each bar in parentheses. i, a subcategory of patients with biopsy inadequate for *EGFR* mutation analysis. To be evaluable for response, patients needed to have measurable disease at baseline and at least one postbaseline disease assessment.



#### Efficacy of rociletinib by tumor and plasma *EGFR* status

There were 34 patients from the phase I study with matched cobas tissue and BEAMing plasma results who were treated at therapeutic doses with rociletinib (500 or 625 mg twice daily HBr, 900 mg free base form with transition to 500 mg twice daily HBr). The ORR, defined as confirmed response assessed using RECIST 1.1, for patients with a T790M-positive tumor result ( $n = 21$ ), independent of plasma status, was 52% (Fig. 3). The ORR for patients with a T790M-positive plasma result ( $n = 25$ ), independent of tumor status, was 44%. For patients who were T790M-positive in both tumor and plasma ( $n = 15$ ), the ORR was 53%. Among six cases that were T790M tumor-positive/plasma-negative there were three responders. There were four patients who were T790M tumor-negative/plasma-positive and one had a partial response which included tumor shrinkage in the biopsied lesion. Three patients were negative for T790M in both tissue and plasma, and none of these responded. In addition, six patients identified as T790M-positive by the plasma test had biopsies that were inadequate for molecular analysis, and two of these had responses. The ratio of the level of T790M to activating mutation in plasma was found to be associated with depth of response to rociletinib as reported previously (13), with higher ratios generally corresponding to greater tumor shrinkage of target lesions ( $P = 0.004$ ; Supplementary Fig. S2).

#### Changes in plasma mutant *EGFR* in response to rociletinib

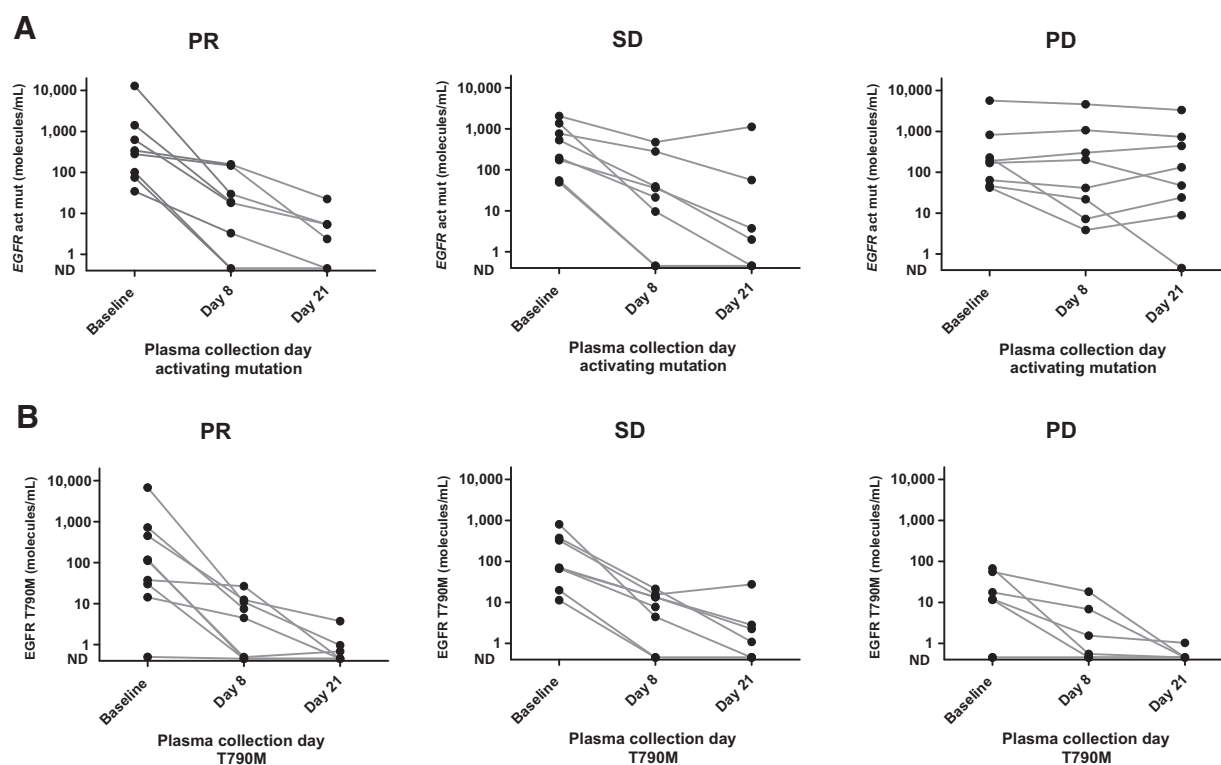
A small subgroup of patients from the phase I study ( $n = 24$ ) were evaluated for changes in *EGFR* mutation levels with rociletinib treatment based on BEAMing. Plasma was obtained just prior to dosing on day 1 (baseline), and at day 8 and day 21 of treatment. Patients were selected after response to rociletinib was known, to create approximately equal sized samples. Eight patients had a partial response (PR), eight patients had stable disease (SD), and eight patients had progressive disease (PD) as their best response. Three additional patients provided plasma at the time points assessed (two PR and one SD), but were excluded from the analysis because of either dose interruptions during the

assessment period or very low plasma *EGFR* titers (<10 copies) at baseline.

Patients with PR as best response experienced a rapid drop in *EGFR*-activating mutation levels, which fell to  $\leq 10$  molecules/mL by day 21 in all but one patient (Fig. 4A). In comparison, patients with PD as best response did not have significantly different mutant *EGFR* levels in plasma at day 1 (PR vs. PD at day 1:  $P = 0.51$ ), but had only modest decreases in activating mutation levels by day 21 (PR vs. PD at day 21:  $P = 0.01$ ). An exception was a PD patient who experienced a drop in *EGFR*-activating mutation levels to undetectable by day 21. This patient had progression due to the development of a brain lesion but 25% tumor shrinkage in target lesions, suggesting clinical benefit from rociletinib that is not reflected in the RECIST classification of PD. We also investigated early changes in plasma T790M in response to the introduction of rociletinib. In contrast to what was observed for the activating mutation, all but one patient experienced a drop in T790M plasma levels to  $\leq 10$  molecules/mL by day 21, regardless of clinical outcome (Fig. 4B).

## Discussion

In NSCLC and other cancers, molecular testing is becoming an integral part of patient management. However, tumor biopsies from NSCLC patients can present challenges for molecular analyses due to inadequate or insufficient sample material, tumor heterogeneity, or the presence of lesions that are inaccessible to needle biopsy in patients at risk for complications due to comorbidities. These challenges are accentuated in a later line setting because rebiopsy may not be feasible and tumor heterogeneity may be greater in patients with acquired resistance to targeted therapies. Previous studies have shown that the detection of mutations in blood, specifically in circulating tumor DNA (ctDNA) from plasma, can potentially overcome these limitations (1, 14, 16–18). Using a series of matched blood and plasma samples drawn from two studies, we have extended these observations to a large cohort of NSCLC patients with a focus on the



**Figure 4.** Changes in plasma-mutant *EGFR* in response to rociletinib. Changes in the *EGFR*-activating mutation (A) and T790M (B). Patients are categorized by best RECIST 1.1 response to rociletinib. Best response was achieved at first postbaseline imaging assessment (approximately study day 43) for all patients except in three cases of PR where it was achieved at the second postbaseline imaging assessment (approximately study day 85). Patients with highly similar mutation levels may appear to be represented by a single dot. Day 8 plasma was obtained on day 8 except for one patient with PD as best response (day 7). Day 21 plasma was obtained on day 20 or day 21 except for one patient with PR as best response (day 24).

acquired resistance mutation T790M where existing data from published studies is limited (25). A strength of our study is that >90% of all cases evaluated had matched tumor tissue and plasma prospectively collected within 60 days of one another.

With tissue as the reference method, the positive percent agreement (PPA) observed for activating mutations between plasma and tumor was 82% for BEAMing (49/60) and 73% for the cobas test (55/75). These results are similar to data for the cobas test from an analysis of 238 Asian lung cancer patients (22). They also compare favorably with two large studies that used allele-specific PCR (43%; 22/51; ref. 28) and (66%; 69/105; ref. 10), one that used protein-nucleic acid (PNA)-mediated PCR (59%; 97/164; ref. 1), and one that used a denaturing high-performance liquid chromatography (DHPLC) method (82%; 63/77; ref. 29). Higher sensitivities have been observed for blood with respect to tumor, but these have been in small studies of fewer than 20 patients (30, 31). With respect to detection of the T790M resistance mutation in plasma, we observed a slightly lower PPA than for activating mutations (73% for BEAMing, 64% for cobas test), reflecting the lower overall allelic burden of T790M seen in plasma relative to the activating mutation. BEAMing and cobas platforms are based on different technologies, and the rate of concordance we observed is impacted by both the biologic differences between tumor tissue and plasma as well as the technologies used for mutation detection.

The generally low level of plasma T790M observed in our phase I patients (median = 9 molecules/mL) reinforces a requirement for use of sensitive plasma detection methodologies in this patient population. Indeed, a significant fraction of patients with tumor *EGFR* mutations did not have mutations detected in plasma by either BEAMing or cobas tests. One explanation is that neither cobas nor BEAMing plasma assays have the required analytical sensitivity to identify low copy mutations. This seems unlikely given that BEAMing should identify as few as two *EGFR*-mutant molecules in a background of 10,000 genome equivalents (GE; ref. 23), while the cobas *EGFR* plasma test can identify *EGFR* mutations at a level of 0.1% (22). In addition, we assessed several of the *EGFR* plasma mutation-negative, tumor mutation-positive cases described here with two other sensitive methodologies based on digital PCR and ultra-deep sequencing, and were not able to identify plasma mutations with these approaches either. This suggests that it is not a limit of platform sensitivity, but rather the biologic features of NSCLC that affects our ability to identify plasma mutations. That is, some NSCLC patients, even on disease progression, may only shed very low quantities of DNA into the systemic circulation. Of note, the ability to detect ctDNA targets in plasma appears to depend on tumor type, with some cancers such as metastatic breast cancer having sensitivities approaching 100% using BEAMing while others, such as glioma and prostate cancer, may be <50% (32, 33).

One approach to circumvent the challenges posed by biology would be to simply use larger plasma input volumes. To this end, we are currently exploring the extent to which volumes greater than the 2 mL used in this study improve sensitivity. Another approach would be to identify favorable clinical characteristics that increase the likelihood that somatic alterations in ctDNA will be detected in plasma, and to use these clinical criteria to identify those patients most suitable for a plasma test. Tumor burden has previously been shown to be related to the level of ctDNA in plasma (27, 34). However, in our NSCLC cohort, we found only a trend toward association between tumor burden and the ability to identify plasma-mutant *EGFR*. Indeed, two tumor *EGFR* mutation-positive/plasma mutation-negative patients had among the highest tumor burden in our phase I study. A limitation of our study and other published studies is that the measurement of tumor burden was based exclusively on target lesions as measured by the sum of the longest diameters, and did not incorporate nontarget lesion data, which can be extremely challenging to quantitate.

In contrast, intrathoracic (M0/M1a) versus extrathoracic (M1b) disease status was a better predictor of ability to detect plasma-mutant *EGFR*. Over 90% of M1b patients with *EGFR* mutations (del19/L858R or T790M) identified in tumor biopsies had the corresponding mutations identified in plasma. A potential limitation of an *EGFR* plasma test based on ctDNA is the lower rate of mutation detection in M1a patients (39% for activating mutations, 27% for T790M by BEAMing in our study). Further prospective clinical studies are required to determine if and how M stage should be taken into account for plasma *EGFR* testing in the clinic. It is noteworthy that M1a patients constitute a minority (~25%–30%) of our advanced stage NSCLC clinical trial patient population. Tseng and colleagues also recently observed that detection sensitivity of del19 and L858R mutations was higher in M1b compared with M1a patients (35).

In published studies comparing tumor and plasma data, the tumor result has been considered the reference, and in some cases, even a gold standard. Indeed, all of the data reported in the current study was benchmarked to the tumor result. A shortcoming of using tumor as reference is that false negatives will invariably occur because of either low tumor cellularity or tumor heterogeneity. Heterogeneity of the T790M resistance mutation, for example, has been found both within and between individual lesions (36). In our study, there were nine cases where the T790M mutation was identified in plasma by BEAMing but not in the matched tumor tissue by cobas test (Supplementary Table S4). Eight of these were tested again using a second independent technology platform, either cobas plasma test or OnTarget, and all but two very low copy BEAMing plasma results were confirmed. This argues that the plasma mutations identified in these cases are true positives and the tumor results are false negatives. The cobas *EGFR* tissue test has a limit of detection for the T790M mutation of 2.0% (37). This is within *EGFR* testing guidelines which recommend T790M mutation detection in FFPE specimens in as few as 5% of tumor cells (9), and therefore the use of the cobas *EGFR* tissue test as comparator in this study was appropriate.

An additional consideration for a plasma test is how well it identifies potential responders to a targeted therapy compared with the standard tumor test. In this respect, the ORR for T790M-positive patients as identified by BEAMing was similar to that of

patients identified by the tumor test (44% and 52%, respectively). Plasma testing also identified three responders missed by the tumor test, further supporting its potential use as a complement to the tumor test. A drawback of this comparison is that it is based on a small number of plasma cases ( $n = 25$ ) with a likely bias toward tumor T790M-positive patients. Preliminary ORR data from a much larger cohort of patients from the combined phase I and phase II portions of the trial ( $n = 247$ ) were shown to be consistent with data presented here (38).

A further application of plasma mutant *EGFR* is as a pharmacodynamic marker in clinical trials (19). In our phase I study, the *EGFR*-activating mutation in plasma rapidly fell to low levels in patients who ultimately experienced a RECIST response while on treatment with rocicetinib. In contrast, patients with PD as best response had a much more modest decrease in plasma-activating mutation levels following the introduction of rocicetinib. Interestingly, T790M levels rapidly decreased in the plasma of almost all patients, regardless of clinical outcome. We hypothesize that the rapid decline in plasma T790M reflects potent activity against the T790M subclonal population of the tumor burden, even in patients who may have primary resistance to rocicetinib. Further investigation is ongoing to confirm this hypothesis.

In January 2015, the therascreen *EGFR* plasma test was approved for use with the first-generation *EGFR* inhibitor gefitinib as a companion diagnostic in the European Union. The high specificity and sensitivity of both cobas and BEAMing plasma *EGFR* tests suggests these tests, too, have clinical applications. For example, 23% (22/94) of patients from the phase I trial did not have a tumor tissue specimen that was adequate for molecular analysis, a figure similar to what has been observed for other clinical studies in NSCLC (10, 39). These patients could benefit from an *EGFR* blood test rather than be immediately subjected to a repeat biopsy. In addition, a reflex testing model where an *EGFR* blood test is performed first, followed by a biopsy if the blood test is negative, may be appropriate for some patients. A reflex testing model might be particularly well suited for patients with acquired resistance, where the challenges of tumor heterogeneity and rebiopsy are likely to be greater than for newly diagnosed patients. Both cobas and BEAMing tests are reliable, have short turnaround times of 5 to 10 days from sample receipt to report generation, and have been standardized and extensively validated. These factors and the favorable tumor/plasma comparison data from the current study demonstrate that plasma-based *EGFR* testing could be a viable complement to tumor-based testing, or an alternative where tumor tissue is not available, for the clinical management of NSCLC patients.

#### Disclosure of Potential Conflicts of Interest

C.A. Karlovich, P. O'Donnell, and L. Rolfe have ownership interest (including patents) in Clovis Oncology. J. Goldman, J.-C. Soria, B. Solomon, D.R. Camidge, and K. Park are consultants/advisory board members for Clovis Oncology. L.V. Sequist is a consultant/advisory board member for Ariad, Astra Zeneca, Boehringer Ingelheim, Clovis Oncology, Genentech, Merrimack, Novartis, and Taiho. L. Horn reports receiving a commercial research grant from Astra Zeneca, speakers bureau honoraria from Biodesix, and is a consultant/advisory board member for Bayer, Bristol-Meyers Squibb, Genentech, Merck, and Xcovery. S.M. Gadgeel reports receiving speakers bureau honoraria from Roche/Genentech and is a consultant/advisory board member for Boehringer Ingelheim, Novartis, Pfizer, and Roche/Genentech. H.A. Wakelee reports receiving commercial research grants from Astra Zeneca/MedImmune, Clovis Oncology, and Genentech/Roche and is a consultant/advisory board member for ACEA Biosciences, Clovis Oncology, and Genentech/Roche. No potential conflicts of interest were disclosed by the other authors.



## Authors' Contributions

**Conception and design:** C.A. Karlovich, J. Goldman, J.-C. Soria, S. Chien, M. Raponi, A.R. Allen, K. Park, H.A. Wakelee

**Development of methodology:** C.A. Karlovich, J. Goldman, W. Wen, P. Angenendt, C.P. Paweletz, L. Wu, S. Chien, K. Park

**Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.):** C.A. Karlovich, J. Goldman, J.-M. Sun, E. Mann, L.V. Sequist, K. Konopa, W. Wen, P. Angenendt, L. Horn, D.R. Spigel, J.-C. Soria, B. Solomon, D.R. Camidge, S.M. Gadgeel, C.P. Paweletz, K. Park, H.A. Wakelee

**Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis):** C.A. Karlovich, J. Goldman, J.-M. Sun, L.V. Sequist, W. Wen, D.R. Spigel, D.R. Camidge, S.M. Gadgeel, P. O'Donnell, D. Despain, L. Rolfe, M. Raponi, K. Park, H.A. Wakelee

**Writing, review, and/or revision of the manuscript:** C.A. Karlovich, J. Goldman, J.-M. Sun, E. Mann, L.V. Sequist, K. Konopa, W. Wen, P. Angenendt, L. Horn, D.R. Spigel, J.-C. Soria, B. Solomon, D.R. Camidge, S.M. Gadgeel, C.P. Paweletz, P. O'Donnell, S. Matheny, D. Despain, L. Rolfe, M. Raponi, A.R. Allen, K. Park, H.A. Wakelee

**Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases):** C.A. Karlovich, S. Matheny

**Study supervision:** C.A. Karlovich, S.M. Gadgeel, S. Matheny, L. Rolfe, M. Raponi, A.R. Allen, K. Park, H.A. Wakelee

## Acknowledgments

The authors thank the patients for their participation in the study and the study staff who were involved in collecting specimen material at the study sites. The authors also thank Borealis Genomics for their participation in the comparative platform study, and Andy Simmons and Thomas Harding for comments on the manuscript.

## Grant Support

This study was funded by Clovis Oncology, Sysmex Inostics, and Roche Molecular Systems.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received May 28, 2015; revised November 6, 2015; accepted November 11, 2015; published OnlineFirst January 8, 2016.

## References

- 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.
- 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.
- 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.
- Rosell R, Carcereny E, Gervais R, Vergnenegre A, Massuti B, Felip E, et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. *Lancet Oncol* 2012;13:239-46.
- Yu HA, Arcila ME, Rekhtman N, Sima CS, Zakowski MF, Pao W, et al. Analysis of tumor specimens at the time of acquired resistance to EGFR-TKI therapy in 155 patients with EGFR-mutant lung cancers. *Clin Cancer Res* 2013;19:2240-7.
- Walter AO, Sjin RT, Haringsma HJ, Ohashi K, Sun J, Lee K, et al. Discovery of a mutant-selective covalent inhibitor of EGFR that overcomes T790M-mediated resistance in NSCLC. *Cancer Discov* 2013;3:1404-15.
- Sequist LV, Soria JC, Goldman JW, Wakelee HA, Gadgeel SM, Varga A, et al. Rociletinib in EGFR-mutated non-small-cell lung cancer. *N Engl J Med* 2015;372:1700-9.
- Febbo PG, Ladanyi M, Aldape KD, De Marzo AM, Hammond ME, Hayes DF, et al. NCCN task force report: evaluating the clinical utility of tumor markers in oncology. *J Natl Compr Canc Netw* 2011;9:S1-32.
- Lindeman NI, Cagle PT, Beasley MB, Chitale DA, Dacic S, Giaccone G, et al. Molecular testing guideline for selection of lung cancer patients for EGFR and ALK tyrosine kinase inhibitors: guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology. *J Thorac Oncol* 2013;8:823-59.
- Douillard JY, Ostoros G, Cobo M, Ciuleanu T, Cole R, McWalter G, et al. Gefitinib treatment in EGFR mutated caucasian NSCLC: circulating-free tumor DNA as a surrogate for determination of EGFR status. *J Thorac Oncol* 2014;9:1345-53.
- Diaz LAJr, Bardelli A. Liquid biopsies: genotyping circulating tumor DNA. *J Clin Oncol* 2014;32:579-86.
- Gerlinger M, Rowan AJ, Horswell S, Larkin J, Endesfelder D, Gronroos E, et al. Intratumor heterogeneity and branched evolution revealed by multi-region sequencing. *N Engl J Med* 2012;366:883-92.
- Piotrowska Z, Niederst MJ, Karlovich CA, Wakelee HA, Neal JW, Mino-Kenudson M, et al. Heterogeneity underlies the emergence of EGFR T790 wild-type clones following treatment of T790M-positive cancers with a third generation EGFR inhibitor. *Cancer Discov* 2015;5:713-22.
- Kimura H, Kasahara K, Kawaiishi M, Kunitoh H, Tamura T, Holloway B, et al. Detection of epidermal growth factor receptor mutations in serum as a predictor of the response to gefitinib in patients with non-small-cell lung cancer. *Clin Cancer Res* 2006;12:3915-21.
- Maheswaran S, Sequist LV, Nagrath S, Ulluks 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.
- 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.
- Murtaza M, Dawson SJ, Tsui DW, Gale D, Forshew T, Piskorz AM, et al. Non-invasive analysis of acquired resistance to cancer therapy by sequencing of plasma DNA. *Nature* 2013;497:108-12.
- Newman AM, Bratman SV, To J, Wynne JF, Eclov NC, Modlin LA, et al. An ultrasensitive method for quantitating circulating tumor DNA with broad patient coverage. *Nat Med* 2014;20:548-54.
- Sorensen BS, Wu L, Wei W, Tsai J, Weber B, Nexø E, et al. Monitoring of epidermal growth factor receptor tyrosine kinase inhibitor-sensitizing and resistance mutations in the plasma DNA of patients with advanced non-small cell lung cancer during treatment with erlotinib. *Cancer* 2014;120:3896-901.
- Weber B, Meldgaard P, Hager H, Wu L, Wei W, Tsai J, et al. Detection of EGFR mutations in plasma and biopsies from non-small cell lung cancer patients by allele-specific PCR assays. *BMC Cancer* 2014;14:294.
- Lopez-Rios F, Angulo B, Gomez B, Mair D, Martinez R, Conde E, et al. Comparison of molecular testing methods for the detection of EGFR mutations in formalin-fixed paraffin-embedded tissue specimens of non-small cell lung cancer. *J Clin Pathol* 2013;66:381-5.
- Mok TS, Wu YL, SooLee J, Yu CJ, Sriuranpong V, Sandoval-Tan J, et al. Detection and dynamic changes of EGFR mutations from circulating tumor DNA as a predictor of survival outcomes in NSCLC patients treated with first-line intercalated erlotinib and chemotherapy. *Clin Cancer Res* 2015;21:3196-203.
- Diehl F, Li M, Dressman D, He Y, Shen D, Szabo S, et al. Detection and quantification of mutations in the plasma of patients with colorectal tumors. *Proc Natl Acad Sci U S A* 2005;102:16368-73.
- Thompson JD, Shibahara G, Rajan S, Pel J, Marziali A. Winnowing DNA for rare sequences: highly specific sequence and methylation based enrichment. *PLoS One* 2012;7:e31597.
- Oxnard GR, Paweletz CP, Kuang Y, Mach SL, O'Connell A, Messineo MM, et al. Noninvasive detection of response and resistance in EGFR-mutant lung cancer using quantitative next-generation genotyping of cell-free plasma DNA. *Clin Cancer Res* 2014;20:1698-705.
- Bidard FC, Weigelt B, Reis-Filho JS. Going with the flow: from circulating tumor cells to DNA. *Sci Transl Med* 2013;5:207ps14.
- Madic J, Piperno-Neumann S, Serois V, Rampanou A, Milder M, Trouiller B, et al. Pyrophosphorolysis-activated polymerization detects circulating

- tumor DNA in metastatic uveal melanoma. *Clin Cancer Res* 2012;18:3934–41.
28. Goto K, Ichinose Y, Ohe Y, Yamamoto N, Negoro S, Nishio K, et al. Epidermal growth factor receptor mutation status in circulating free DNA in serum: from IPASS, a phase III study of gefitinib or carboplatin/paclitaxel in non-small cell lung cancer. *J Thorac Oncol* 2012;7:115–21.
  29. Bai H, Mao L, Wang HS, Zhao J, Yang L, An TT, et al. Epidermal growth factor receptor mutations in plasma DNA samples predict tumor response in Chinese patients with stages IIIB to IV non-small-cell lung cancer. *J Clin Oncol* 2009;27:2653–9.
  30. He C, Liu M, Zhou C, Zhang J, Ouyang M, Zhong N, et al. Detection of epidermal growth factor receptor mutations in plasma by mutant-enriched PCR assay for prediction of the response to gefitinib in patients with non-small-cell lung cancer. *Int J Cancer* 2009;125:2393–9.
  31. Yung TK, Chan KC, Mok TS, Tong J, To KF, Lo YM. Single-molecule detection of epidermal growth factor receptor mutations in plasma by microfluidics digital PCR in non-small cell lung cancer patients. *Clin Cancer Res* 2009;15:2076–84.
  32. Higgins MJ, Jelovac D, Barnathan E, Blair B, Slater S, Powers P, et al. Detection of tumor PIK3CA status in metastatic breast cancer using peripheral blood. *Clin Cancer Res* 2012;18:3462–9.
  33. Bettegowda C, Sausen M, Leary RJ, Kinde I, Wang Y, Agrawal N, et al. Detection of circulating tumor DNA in early- and late-stage human malignancies. *Sci Transl Med* 2014;6:224ra24.
  34. Dawson SJ, Tsui DW, Murtaza M, Biggs H, Rueda OM, Chin SF, et al. Analysis of circulating tumor DNA to monitor metastatic breast cancer. *N Engl J Med* 2013;368:1199–209.
  35. Tseng JS, Yang TY, Tsai CR, Chen KC, Hsu KH, Tsai MH, et al. Dynamic plasma EGFR mutation status as a predictor of EGFR-TKI efficacy in patients with EGFR-mutant lung adenocarcinoma. *J Thorac Oncol* 2014;10:603–10.
  36. 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.
  37. Cobas<sup>®</sup> EGFR Mutation Test CE-IVD Package Insert: Roche Molecular Systems, Inc. Pleasanton, CA; 2013.
  38. Sequist LV, Goldman JW, Wakelee HA, Camidge DR, Yu HA, Varga A. Efficacy of rociletinib (CO-1686) in plasma-genotyped T790M-positive NSCLC patients. *J Clin Oncol* 33, 2015 (suppl; abstr 8001).
  39. Kim ES, Herbst RS, Wistuba II, Lee JJ, Blumenschein GR Jr, Tsao A, et al. The BATTLE trial: personalizing therapy for lung cancer. *Cancer Discov* 2011;1:44–53.