Cancer Science
Japanese Cancer Association JCA
Open Access

Rebiopsy for patients with non-small-cell lung cancer after epidermal growth factor receptor-tyrosine kinase inhibitor failure

Takahisa Kawamura,[1] Hirotsugu Kenmotsu,[1] Tetsuhiko Taira,[1] Shota Omori,[1] Kazuhisa Nakashima,[1] Kazushige Wakuda,[1] Akira Ono,[1] Tateaki Naito,[1] Haruyasu Murakami,[1] Keita Mori,[2] Takashi Nakajima,[3] Yasuhisa Ohde,[4] Masahiro Endo[5] and Toshiaki Takahashi[1]

[1]Division of Thoracic Oncology, Shizuoka Cancer Center, Shizuoka; [2]Clinical Research Center, Shizuoka Cancer Center, Shizuoka; [3]Division of Pathology, Shizuoka Cancer Center, Shizuoka; [4]Division of Thoracic Surgery, Shizuoka Cancer Center, Shizuoka; [5]Division of Diagnostic Radiology, Shizuoka Cancer Center, Shizuoka, Japan

Key words

Disease progression, epidermal growth factor receptor-tyrosine kinase inhibitors, non-small-cell lung cancer, rebiopsy, T790M mutation

Correspondence

Hirotsugu Kenmotsu, Division of Thoracic Oncology, Shizuoka Cancer Center, 1007 Shimonagakubo, Nagaizumi-cho, Suntou-gun, Shizuoka 411-8777, Japan.
Tel: +81-55-989-5222; Fax: +81-55-989-5783;
E-mail: h.kenmotsu@scchr.jp

Funding Information

No sources of funding were declared for this study.

Received January 18, 2016; Revised March 25, 2016; Accepted April 11, 2016

Cancer Sci **107** (2016) 1001–1005

doi: 10.1111/cas.12963

Although third-generation epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKI) can overcome T790M-mediated resistance in non-small-cell lung cancer (NSCLC), rebiopsy to confirm T790M status is occasionally difficult. We aimed to investigate the current tendency and the limitations of rebiopsy in clinical practice. This study included 139 consecutive NSCLC patients with *EGFR* mutations, who had experienced progressive disease (PD) after EGFR-TKI treatment. We retrospectively reviewed patient characteristics, tumor progression sites and rebiopsy procedures. Of 120 patients (out of the original 139) who were eligible for clinical trials, 75 (63%) underwent rebiopsy for 30 pleural effusions, 32 thoracic lesions, four bone, two liver, and seven at other sites. Rebiopsy procedures included 30 thoracocentesis, 24 transbronchial biopsies, 13 computed tomography (CT)-guided needle biopsies and 8 other procedures. Of the 75 rebiopsied patients, 71 (95%) were pathologically diagnosed with malignancy; and 34 (45%) had available tissue samples for *EGFR* analyses. Of the 75 biopsied patients, 61 (81%) were analyzed for *EGFR* mutation, using tissue or cytology samples; T790M mutations were identified in 20 (33%) of the 61 patients. Of the 120 patients, 45 (38%) did not undergo rebiopsy, because of inaccessible tumor sites (n = 19), patient refusal (n = 6) or decision of physician (n = 10). In conclusion, among patients with *EGFR* mutations who had PD after EGFR-TKI treatment, 63% underwent rebiopsy. Most rebiopsy samples were diagnosed with malignancy. However, tissue samples were less available and T790M mutations were identified less frequently than in previous studies. Skill and experience with rebiopsy and noninvasive alternative methods will be increasingly important.

Non-small-cell lung cancer (NSCLC) is the leading cause of cancer-related death worldwide. Although having limited efficacy, cytotoxic chemotherapies have been the primary therapeutic option for unresectable NSCLC. However, epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKI) have shown dramatic responses with more acceptable toxicity in patients with *EGFR*-mutant NSCLC.[1] However, patients with advanced NSCLC that harbors *EGFR*-sensitive mutations tend to develop progressive disease (PD) after a median response of 12 months, mainly due to acquired resistance to EGFR-TKI through secondary mutations.[2,3] The most frequently reported secondary mutation is the *EGFR* Thr790Met (T790M) point mutation within exon 20, which accounts for approximately half of acquired resistance to EGFR-TKI.[4–6] Recently, third-generation EGFR-TKI have been reported to be effective against T790M$^+$ NSCLC, and they are accessible through clinical trials.[7,8] We can register some of these clinical trials if rebiopsy tissue samples in PD lesions are available. However, performing rebiopsy to confirm T790M status is occasionally impossible, and obtaining tissue samples by rebiopsy remains challenging. In the present study we aim to evaluate the current status of rebiopsy at our institution and consider how to overcome the issues of rebiopsy in the clinical setting.

Patients and Methods

Patients. We initially screened 139 consecutive patients with NSCLC harboring *EGFR*-sensitive mutations who had experienced PD after any EGFR-TKI treatment at Shizuoka Cancer Center between January 2014 and March 2015. Both tissue sampling and cytologic specimen sampling were defined as rebiopsy. Patient characteristics, tumor progression sites and rebiopsy procedure were retrospectively reviewed from medical records. We also identified reasons for not undergoing rebiopsy in the non-rebiopsied patients. The primary objective of our study was to evaluate the frequency of rebiopsy and incidence of T790M mutation compared with previous studies.

The second objective was to identify factors that make rebiopsy difficult. These factors included "inaccessible lesions," which were defined as intracranial lesions or difficult target lesions (<20 mm) in tissue sampling. "Decision of physician" included patients who had other progressive lesions that required emergent radiation therapy (e.g. metastases in spinal cord and symptomatic brain metastases).

Processing method for pleural effusion cytology samples. Pleural effusion cytology samples were provided by Shizuoka Cancer Center. From 30 pleural effusion cytology samples collected from patients, 27 were diagnosed with NSCLC (adenocarcinoma) between January 2014 and March 2015 and confirmed by a pathologist to contain tumor cells. Samples were frozen within 10 and 30 min of sampling and stored at −80°C. Frozen samples were thawed at 37°C and refrozen rapidly to disrupt the cells and ensure an even distribution, then divided into equal aliquots in the laboratory. DNA was extracted at the laboratory using our own standard procedures and Cobas *EGFR* Mutation Test Kits (Roche Diagnostics K.K., Tokyo, Japan).

Epidermal growth factor receptor mutational analysis. Rebiopsies were conducted with various lesions at our institution. We used the Scorpion Amplification Refractory Mutation System (Scorpion ARMS method) in *EGFR* mutational analyses.[9] Some patients received rebiopsies on several occasions or at multiple lesions. In these cases, positive results of EGFR mutations or T790M mutation had priority to be adopted. No other acquired resistant molecular mechanisms (e.g. *MET* amplification) were examined in the present study.

Statistical analysis. Statistical analyses were performed using JMP 9 (SAS Institute, Cary, NC, USA), and the χ^2 and Mann–Whitney *U*-tests were used to evaluate differences in categorical and continuous variables between the two groups, respectively. $P < 0.05$ was considered significant. This retrospective study was approved by the institutional review board of Shizuoka Cancer Center.

Results

Rebiopsy rate after epidermal growth factor receptor-tyrosine kinase inhibitor failure. Among 139 patients who had experienced PD after EGFR-TKI treatment, 19 patients were ineligible for clinical trials because of poor performance status (PS; $n = 10$), comorbidity ($n = 7$), or because they were 85 and 87 years old ($n = 2$). Among 120 patients, tumor progression sites included 36 pleural effusion, 57 thoracic primary/metastatic lesions, 26 brain metastases, 21 bone metastases, 15 lymph node metastases, 7 hepatic metastases and 8 other lesions. Of the 120 remaining patients, 75 (63%) underwent rebiopsy. Individual characteristics of 120 patients included in this study are shown in Table 1. The rebiopsy and non-rebiopsy groups did not significantly differ in age, sex, smoking status, PS, *EGFR* mutation type or response to initial EGFR-TKI treatment. Anatomical sites of rebiopsy were as follows: 30 pleural effusion, 32 thoracic lesions, four bone lesions, two hepatic lesions and seven other lesions (pericardial effusion [$n = 2$], adrenal lesion [$n = 1$], skin lesion [$n = 1$], brain lesion [$n = 1$], leptomeningeal lesion [$n = 1$] and ascites [$n = 1$]). Rebiopsy procedures included 30 thoracocentesis, 24 transbronchial biopsies, 14 CT-guided needle biopsies and 7 other procedures (surgery of the bone lesion [$n = 1$] and brain lesion [$n = 1$], pericardiocentesis [$n = 2$], skin biopsy [$n = 1$], abdominocentesis [$n = 1$] and lumber puncture [$n = 1$]), as shown in Table 2. Of the 75 patients in the rebiopsy group, 71 (95%) were pathologically diagnosed with malignancy. Tissue samples for *EGFR* analyses were available in 34 (45%) of 75 patients, and *EGFR* mutational analyses were performed in 61 (81%) of 75 patients by using tissue or cytology samples. T790M mutations were identified in 20 (33%) of these 61 patients (Fig. 1). Subsequent chemotherapies after PD are shown in Table 3. Third-generation EGFR-TKI were administered significantly more frequently in the rebiopsy group (24%) than in the non-rebiopsy group (9%; $P = 0.04$). EGFR-TKI approved in Japan (gefitinib, erlotinib, afatinib) were administered significantly more frequently in the non-rebiopsy group (40%) than in the rebiopsy group (20%; $P = 0.01$).

Table 1. Individual baseline characteristics ($n = 120$ in total)

	Rebiopsy group ($n = 75$)	Non-rebiopsy group ($n = 45$)	*P*
Mean age (range) in years	68 (34–87)	71 (34–86)	0.23
Sex			
Male	26	13	0.51
Female	49	32	
Smoking status			
Smoker	32	19	0.96
Non-smoker	43	26	
Performance status			
0–1	55	33	1.00
2–4	20	12	
Metastatic site			
Brain	14	17	0.02
Bone	25	16	0.80
EGFR mutation			
Exon 19 deletion	45	25	0.77
Exon 21 L858R	27	17	
Others	3	3	
Response to initial EGFR-TKI treatment			
Complete response	0	0	0.76
Partial response	47	32	
Stable disease	22	11	
Progressive disease	2	1	

EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor.

Rebiopsy after first epidermal growth factor receptor-tyrosine kinase inhibitor failure. Of 67 patients who had experienced PD after initial administration of EGFR-TKI, 5 were judged as ineligible for clinical trials; 27 (44%) patients underwent rebiopsy, of whom 25 (93%) of the 27 patients were pathologically diagnosed with malignancy, using tissue or cytology samples. *EGFR* mutational analyses were performed for 23 (85%) of the 27 patients. T790M mutations were identified in 7 (30%) of the 23 patients before subsequent systemic therapy. T790M detection rates were similar between patients who underwent rebiopsy after their first EGFR-TKI therapy (30%) and those who were rebiopsied after their second or subsequent TKI therapies (35%, $P = 0.71$).

Rebiopsy for primary/metastatic lesions. Thirty patients underwent rebiopsy for pulmonary lesions by CT-guided needle biopsies or transbronchial biopsies: 18 patients for primary lesions and 13 patients for metastatic lesions. The detection rate of T790M mutations did not significantly differ between those rebiopsied for primary pulmonary lesions (4 of 18 patients [22%]) and those rebiopsied for metastatic lesions (including extrapulmonary lesions) (16 of 43 patients [37%]; $P = 0.26$).

Table 2. Anatomical progressive diseasesites and methods for rebiopsy (*n* = 75)

	n (%)
Rebiopsy sites	
Pleural effusion	30 (40)
Thoracic lesion	32 (43)
Bone lesion	4 (5)
Hepatic lesion	2 (3)
Others	7 (9)
Pericardial effusion	2 (3)
Adrenal lesion	1 (1)
Skin lesion	1 (1)
Brain lesion	1 (1)
Leptomeningeal lesion	1 (1)
Ascites	1 (1)
Rebiopsy methods	
Thoracocentesis	30 (40)
Transbronchial biopsy	24 (32)
CT-guided needle biopsy	14 (19)
Others	7(9)
Surgery (bone, brain)	2 (3)
Pericardiocentesis	2 (3)
Skin biopsy	1 (1)
Abdominocentesis	1 (1)
Lumber puncture	1 (1)

CT, computed tomography.

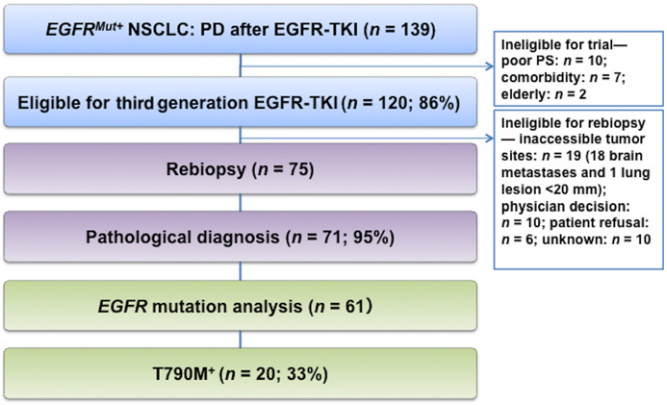

Fig. 1. Overview of rebiopsies among patients with non-small-cell lung cancer (NSCLC) treated with early-version epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKI). Patients were tested with the aim of enrolling them in trials for third-generation EGFR-TKI, which is notably effective against NSCLC with T790M mutations in the *EGFR* gene. $EGFR^{Mut+}$, EGFR mutation carrier; PD, progressive disease; PS, performance status.

Reasons for patients not undergoing rebiopsy. Among 120 patients eligible for clinical trials who had experienced PD after EGFR-TKI treatment between January 2014 and March 2015, 45 (38%) patients did not undergo rebiopsy because of inaccessible tumor sites (n = 19: 18 with brain metastasis and 1 with small pulmonary lesion), decision of physician (n = 10), patient refusal (n = 6) or unknown reasons (n = 10). Patients affected by "decision of physician" could not undergo rebiopsy even after completing emergent therapies (e.g. palliative radiotherapy for spinal cord compression or symptomatic brain metastases), mainly because of deterioration in PS.

Table 3. Subsequent treatments for non–small-cell lung cancer patients with PD after rebiopsy (*n* = 120)

	Rebiopsy group (%) *n* = 75	Non-rebiopsy group (%) *n* = 45	*P*
Subsequent regimens			
Third-generation EGFR-TKI	18 (24)	4 (9)	0.04*
Approved EGFR-TKI (gefitinib, erlotinib, afatinib)	15 (20)	18 (40)	0.01*
Chemotherapy other than EGFR-TKI	19 (25)	6 (13)	0.12
BSC or observation (including beyond PD)	23 (31)	17 (38)	0.42

*P<0.05. BSC, best supportive care; EGFR-TKI, epidermal growth factor receptor-tyrosine kinase inhibitor; PD, progressive disease.

Discussion

In this retrospective study, 63% of 120 consecutive patients with NSCLC who harbored *EGFR* mutations and had PD after EGFR-TKI treatment underwent rebiopsy, and the frequencies of T790M mutation at rebiopsy were 33% in the clinical setting at our institution. We conducted this retrospective study because of the increasing importance of rebiopsy after EGFR-TKI failure. Third-generation EGFR-TKI can solve the major problem of acquired resistance after EGFR-TKI treatments in NSCLC patients with *EGFR* sensitive mutations, by inhibiting both *EGFR* activating and resistance mutations while sparing wild-type *EGFR*.[10,11] In recent studies, third-generation TKI demonstrated promising response rates against tumors with acquired *EGFR* T790M.[7,8] Therefore, rebiopsies to detect T790M mutations are often required to enroll patients into clinical trials of third-generation EGFR-TKI. However, rebiopsy is occasionally challenging for both patients and physicians.

In our study, frequencies of rebiopsy for NSCLC patients harboring *EGFR* mutations who had PD after EGFR-TKI treatment seemed to be lower than in previous studies (60–86%).[12–14] These earlier studies may have been more selective than our study in terms of patient characteristics (e.g. more strict exclusion criteria) and rebiopsy methods (e.g. fewer cytological methods such as lumber punctures and thoracocentesis, and more tissue acquisition methods such as video-assisted thoracic surgery, lung core biopsy, CT-guided biopsy and transbronchial biopsy); our study might show rebiopsy frequencies in real clinical settings more precisely. One reason for the lower rebiopsy rate in our study is that our study included NSCLC patients with only inaccessible lesions (brain metastases or very small pulmonary lesions). In such cases, rebiopsy is not performed until other accessible lesions emerge. However, the rebiopsy may be limited to indolent cases because lung carcinoma often shows rapid growth. Another reason is decisions by physicians whose patients experienced PD. Indeed, patients who show PD as bone metastases or multiple brain metastases frequently need emergent radiation therapies to prevent concomitant symptoms, and physicians may hesitate to perform rebiopsy. In such cases, the decision to perform subsequent rebiopsies may depend on the efficacy of emergent therapies. In the present study, the T790M mutation frequency at rebiopsy (33%) was also low compared with previous studies (30–83%).[13,15–18] The incidence of T790M mutation in Japanese patients with *EGFR*-

mutant NSCLC following EGFR-TKI therapies might be lower than that in Caucasians.[15,16] T790M mutations are possibly induced by EGFR-TKI exposure, and EGFR-TKI treatment beyond PD or duration of EGFR-TKI before rebiopsy might be related to the incidence of T790M mutation. However, little data is available that supports these hypotheses.

Third-generation EGFR-TKI were administered after PD significantly more frequently in the rebiopsy group than in the non-rebiopsy group, while EGFR-TKI approved in Japan were administered significantly more frequently in the non-rebiopsy group (see Table 3). The result showed that some patients in the non-rebiopsy group could receive sequential EGFR-TKI therapies after PD: they will be able to receive third generation EGFR-TKI if better rebiopsy methods or alternatives to detect T790M mutation are developed. Skill and experience with rebiopsy, especially of transbronchial biopsies and CT-guided needle biopsies, will be also important in acquiring sufficient tissue samples. Yoon *et al.*[14] report that repeat biopsy by CT-guided needle biopsies can be performed safely with mild potential complications (such as pneumothorax and pulmonary hemorrhage) in patients with NSCLC. However, skilled experts are needed for CT-guided biopsies, especially for extrapulmonary lesions such as hepatic metastases and bone metastases, which can make rebiopsy especially challenging. Furthermore, cytology sampling, such as pleural effusion, pericardial effusion and cerebrospinal fluid, is often more easily available than tissue sampling under the presence of skilled experts; in the present study, tissue samples were available in only 45% of the patients. Liquid biopsies are an alternative for determining T790M mutation status among patients who are unable to undergo tissue rebiopsy, if they have sufficient diagnostic yields.[19,20] Some reports show that the concordance of T790M mutation status between liquid biopsy and conventional biopsy methods to be 50–65%.[20,21] Recently, osimertinib was approved in the EU according to blood-based test for circulating tumor DNA (ctDNA), and it might be meaningful for patients and physicians to have alternatives to detect T790M mutation. Our study also revealed that the T790M detection rate in primary lesions was not significantly higher than that in metastatic lesions. Therefore, we need not hesitate to perform rebiopsy, regardless of the rebiopsy sites.

This study is limited by its retrospective nature. Our results may also have been affected by our single-institution cohort and colleagues, and rebiopsy or EGFR mutation testing was performed based on the decision of each physician. Furthermore, acquired resistance is not strictly defined as Jackman *et al.*[22] propose in their analysis. Some patients received rebiopsies several times and their T790M mutation status changed heterochronously. The disappearance of T790M mutation may be due to low sensitivity of the *EGFR* mutation analysis method. However, the Scorpion ARMS method, which was used in the present study, showed reasonable sensitivity and specificity in previous reports.[9] Some reports also suggested that T790M was spatiotemporally heterogeneous due to selective pressure from EGFR-TKI, which might be another reason.[18,23,24] Finally, we did not evaluate other resistance mechanisms in these rebiopsies, such as *MET*-amplification, epithelial-to-mesenchymal transition, *BRAF* mutation, *HER2*-amplification and *PIK3CA*-mutations, as previously mentioned. 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.

In conclusion, 63% of the NSCLC patients harboring *EGFR* mutations who had PD after EGFR-TKI treatment underwent rebiopsy, and most of these rebiopsy samples were diagnosed with malignancy in the clinical setting at our institution. However, tissue samples were available for fewer than half of the patients and T790M mutations were identified in no more than 33% of the patients who underwent rebiopsy. Skill and experience with rebiopsy to obtain adequate tissue samples and non-invasive procedures, including analysis using cytology specimens and liquid biopsies, will be increasingly important in assessing NSCLC patients who develop PD after treatment with first-generation or second-generation EGFR-TKI.

Disclosure Statement

The authors have no conflicts of interest to declare.

References

1 Mok TS, Wu YL, Thongprasert S *et al.* Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *N Engl J Med* 2009; **361**: 947–57.
2 Maemondo M, Inoue A, Kobayashi K *et al.* Gefitinib or chemotherapy for non-small-cell lung cancer with mutated *EGFR*. *N Engl J Med* 2010; **362**: 2380–8.
3 Sun JM, Rampal S, Lee G *et al.* Real world impact of epidermal growth factor receptor mutation status on treatment patterns in patients with non-small-cell lung cancer. *Lung Cancer* 2013; **80**: 191–6.
4 Kobayashi S, Boggon TJ, Dayaram T *et al.* *EGFR* mutation and resistance of non-small-cell lung cancer to gefitinib. *N Engl J Med* 2005; **352**: 786–92.
5 Pao W, Miller VA, Politi KA *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.
6 Yano S, Yamada T, Takeuchi S *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**: 2011–17.
7 Jänne PA, Yang JC, Kim DW *et al.* AZD9291 in EGFR inhibitor-resistant non-small-cell lung cancer. *N Engl J Med* 2015; **372**: 1689–99.
8 Sequist LV, Soria JC, Goldman JW *et al.* Rociletinib in *EGFR*-mutated non-small-cell lung cancer. *N Engl J Med* 2015; **372**: 1700–9.
9 Goto K, Satouchi M, Ishii G *et al.* An evaluation study of *EGFR* mutation tests utilized for non-small-cell lung cancer in the diagnostic setting. *Ann Oncol* 2012; **23**: 2914–19.
10 Steuer CE, Khuri FR, Ramalingam SS. The next generation of epidermal growth factor receptor tyrosine kinase inhibitors in the treatment of lung cancer. *Cancer* 2015; **121**: E1–6.
11 Cross DA, Ashton SE, Ghiorghiu S *et al.* AZD9291, an irreversible EGFR TKI, overcomes T790M-mediated resistance to EGFR inhibitors in lung cancer. *Cancer Discov* 2014; **4**: 1046–61.
12 Chouaid C, Dujon C, Do P *et al.* Feasibility and clinical impact of rebiopsy in advanced non-small-cell lung cancer: a prospective multicenter study in a real-world setting (GFPC study 12-01). *Lung Cancer* 2014; **86**: 170–3.
13 Arcila ME, Oxnard GR, Nafa K *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.
14 Yoon HJ, Lee HY, Lee KS *et al.* Repeat biopsy for mutational analysis of non-small-cell lung cancers resistant to previous chemotherapy: adequacy and complications. *Radiology* 2012; **265**: 939–48.
15 Yu HA, Arcila ME, Rekhtman N *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.
16 Hata A, Katakami N, Yoshioka H *et al.* Rebiopsy of non-small-cell lung cancer patients with acquired resistance to epidermal growth factor receptor-tyrosine kinase inhibitor: comparison between T790M mutation-positive and mutation-negative populations. *Cancer* 2013; **119**: 4325–32.
17 Su KY, Chen HY, Li KC *et al.* Pretreatment epidermal growth factor receptor (*EGFR*) T790M mutation predicts shorter EGFR tyrosine kinase inhibitor

response duration in patients with non-small-cell lung cancer. *J Clin Oncol* 2012; **30**: 433–40.
18 Kuiper JL, Heideman DA, Thunnissen E *et al.* Incidence of T790M mutation in (sequential) rebiopsies in EGFR-mutated NSCLC-patients. *Lung Cancer* 2014; **85**: 19–24.
19 Diaz LA Jr, Bardelli A. Liquid biopsies: genotyping circulating tumor DNA. *J Clin Oncol* 2014; **32**: 579–86.
20 Karlovich C, Goldman JW, Sun JM *et al.* Assessment of EGFR mutation status in matched plasma and tumor tissue of NSCLC patients from a phase I study of rociletinib (CO-1686). *Clin Cancer Res* 2016; **22**: 2386–95.
21 Thress KS, Brant R, Carr TH *et al.* EGFR mutation detection in ctDNA from NSCLC patient plasma: a cross-platform comparison of leading technologies to support the clinical development of AZD9291. *Lung Cancer* 2015; **90**: 509–15.
22 Jackman D, Pao W, Riely GJ *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.
23 Sequist LV, Waltman BA, Dias-Santagata D *et al.* Genotypic and histological evolution of lung cancers acquiring resistance to EGFR inhibitors. *Sci Transl Med* 2011; **3**: 75ra26.
24 Hata A, Katakami N, Yoshioka H *et al.* Spatiotemporal T790M heterogeneity in individual patients with EGFR-mutant non-small-cell lung cancer after acquired resistance to EGFR-TKI. *J Thorac Oncol* 2015; **10**: 1553–9.