Detection of EGFR-Activating and T790M Mutations Using Liquid Biopsy in Patients With EGFR-Mutated Non–Small-Cell Lung Cancer Whose Disease Has Progressed During Treatment With First- and Second-Generation Tyrosine Kinase Inhibitors: A Multicenter Real-Life Retrospective Study

Detection of EGFR-Activating and T790M Mutations by Liquid Biopsy in Patients with EGFR-Mutated Non-Small-Cell Lung Cancer Who Have Progressed to First- and Second-Generation Tyrosine Kinase Inhibitors: A Multicenter Real-Life Retrospective Study

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Declaration of Conflict of Interests

Marcello Tiseo has served on advisory boards and/or received speakers' fees for AstraZeneca, Pfizer, Eli-Lilly, BMS, Novartis, Roche, MSD, Boehringer Ingelheim, Otsuka, Takeda, Pierre Fabre. Research Grants from AstraZeneca and Boehringer Ingelheim. The other authors report no potential conflicts of interest.

Abstract

Objectives: In patients with advanced EGFR-mutated non-small-cell lung cancer (NSCLC) who have progressed to first- and second-generation tyrosine kinase inhibitors (TKIs), liquid biopsy is routinely used to evaluate the presence of EGFR T790M as an acquired resistance mechanism. The objective of this study was to assess a real-life picture of EGFR T790M detection in liquid biopsy samples.

Materials and Methods: Liquid biopsies performed between June 2016 and October 2018 for advanced EGFR-mutated NSCLC at progression to first- and second-generation TKIs were retrospectively evaluated in 5 Italian centers. Circulating tumor DNA was extracted from plasma and tested with different commercial kits. The detection rate in liquid biopsy samples and the patients' characteristics were correlated.

Results: One hundred and twenty patients were consecutively enrolled. The overall detection rate of T790M observed by liquid biopsy was 25.8%. Fifty-four of 89 (60.7%) patients with negative liquid biopsy results underwent tissue re-biopsy, and 56% were found to be positive for T790M. The overall rate of T790M positivity in the study cohort was 49.2%. Liquid biopsy performed before formal tumor progression according to the RECIST criteria was negative for T790M in all patients ($n = 21$; $P = 0.012$). T790M positivity was statistically significantly higher in cases of progression at extra-thoracic metastatic sites ($P = 0.008$) and, specifically, in the case of worsening bone disease ($P = 0.003$).

Conclusion: Our study shows that the detection of T790M-positive patients who have progressed to first- and second-generation TKIs in real-life was according to the literature. However, this result was obtained with a specific clinical course (repeat liquid biopsies and tissue re-biopsy), thus implying the necessity for multidisciplinary management.

Keywords: Non-small-cell lung cancer (NSCLC), EGFR, T790M mutation, Liquid biopsy, Real life

Introduction

Non-small-cell lung cancer (NSCLC), which accounts for about 85% of all lung cancers, is often diagnosed as advanced or metastatic disease, requiring systemic therapy.[1] About 12% of white patients with NSCLC (compared with almost 50% of patients of Asian ethnicity) harbor the activated epidermal growth factor receptor (EGFR), in most cases caused by deletion of exon 19 or point mutation of exon 21 L858R. The introduction of first- and second-generation tyrosine kinase inhibitors (TKIs) targeting EGFR (gefitinib, erlotinib, and afatinib) has improved outcomes in this patient population,[2–5] and EGFR TKIs are now the treatment of choice for patients with advanced NSCLC harboring EGFR-activating mutations.[1,6] Nonetheless, tumor cells inevitably develop acquired resistance after a median treatment time of 10–12 months. The secondary EGFR gene T790M point mutation, in exon 20, has been identified as a cause of disease progression in approximately 50%–60% of cases.[7,8] For T790M-positive patients, the third-generation EGFR TKI, osimertinib, is currently the only available therapeutic option; this compound is able to bind to and inhibit both the activating (Del19 or L858R) and resistance (T790M) mutations.[9] Osimertinib therapy resulted in improved survival over platinum-based chemotherapy in T790M-positive patients who had progressed after first- and second-generation EGFR TKIs.[10] Moreover, osimertinib was shown to have superior efficacy over standard first-generation EGFR TKIs in the treatment of previously untreated patients with EGFR-positive NSCLC with the common activating mutation[11] and was recently approved for first-line therapy in this category of patients. Therefore, in clinical practice, 2 potential scenarios are available for the treatment of patients with EGFR-mutated NSCLC: (1) osimertinib as first-line treatment followed by other non-EGFR TKI agents as second-line treatment; or (2) first-/second-generation TKIs followed by osimertinib only in EGFR T790M-positive patients.

Testing for EGFR mutations is thus mandatory at the progression of disease after first- and second-generation EGFR TKIs to assess the presence of the resistance T790M mutation. Molecular analysis of tissue biopsy specimens may be hampered by several factors, such as difficult access to tumor tissue samples by bronchoscopy or endoscopy, the low amount of material obtained, reduced tumor cellularity, and tumor heterogeneity, which would require biopsies of multiple sites. These issues, already significant at the time of the initial molecular characterization for first-line treatment, are even more troublesome at disease progression.

Liquid biopsy, an alternative method to molecularly characterize progression in patients with lung cancer and detect T790M mutation, is made possible by recent advances in studying circulating tumor DNA (ctDNA).[12–14] Liquid biopsy allows the analysis of ctDNA, a small fraction (1%–3%) of the patient's overall circulating cell-free DNA (cfDNA) in body fluids, with relative ease by means of a non-invasive blood sample. EGFR-activating and T790M mutations can be detected in ctDNA by real-time polymerase chain reaction (RT-PCR) or digital PCR (ddPCR) and next-generation sequencing techniques in serum or plasma.

However, a number of drawbacks, mainly associated with the characteristics of ctDNA and the detection limits of the technique, reduce the clinical sensitivity of liquid biopsy for the detection of EGFR mutations in plasma (around 60%), whereas a high specificity (80%–90%) has been reported in independent meta-analyses.[15–17] Despite these limitations, ctDNA from liquid biopsy samples was shown to be a surrogate marker for T790M in tumor tissue in clinical trials.[18–20] In a retrospective analysis reported by Oxnard et al.[19] in patients with acquired EGFR TKI resistance undergoing treatment with osimertinib, the sensitivity of plasma genotyping (BEAMing assay) for detection of T790M was 70%, suggesting that patients with T790M-negative plasma results still need a tissue re-biopsy to determine the presence or absence of the secondary mutation. However, the outcomes with osimertinib in T790M-positive patients by plasma or tissue-based assays were equivalent, supporting the use of liquid biopsy as a rapid diagnostic option in this population of patients, restricting re-biopsy only to T790M plasma-negative cases, as indicated in the guidelines.[1,6]

Outside the controlled clinical trials, recent analyses seem to confirm the feasibility and reliability of liquid biopsies for detection of EGFR mutations in patients within clinical practice,[21–23] although real-world data are still limited. The aim of our study was to describe the detection rate of the T790M molecular test performed by liquid biopsy in patients with NSCLC who have disease progression after first-line therapy with EGFR TKIs in a real-world setting.

Materials and Methods

Patients and Study Objectives

This multicenter retrospective observational study (DETECTION study) was carried out in 5 oncology and anatomic pathology centers in the Italian Emilia-Romagna region (Piacenza, Parma, Reggio Emilia, Modena, and Bologna) and included consecutive patients with advanced NSCLC treated with first- or second-generation EGFR TKIs who were tested by liquid biopsy at disease progression to TKI treatment between 1 June 2016 and 31 October 2018. Patients had to be >18 years old and have data available for clinical follow-up.

The main objective of the study was to assess the rate of detection of T790M mutation by liquid biopsy in a real-life population of patients with NSCLC in progression after EGFR TKIs. The following data were analyzed: (1) rate of T790M-positive patients identified by liquid biopsy; (2) the number of patients who underwent a repeat liquid biopsy if negative and the number of patients who underwent tissue re-biopsy; (3) concordance between plasma-based testing and tissue re-biopsy; (4) correlation between metastatic sites at the time of disease progression and detection of ctDNA.

Study Design and Data Collection

Participating centers entered data into a database and completed a survey on the operative procedures for performing a liquid biopsy. The EGFR molecular testing used to analyze the liquid biopsy specimens had to have similar sensitivity in all participating centers. The following data were extracted from medical records: patient characteristics (date of diagnosis, age at diagnosis, sex, Eastern Cooperative Oncology Group performance status, smoking status), disease history (disease stage at diagnosis, tumor histology, presence of EGFR-activating and T790M mutations, site of diagnostic biopsy). Outcome data for analysis were also collected from medical records, including the start and end dates of EGFR TKI therapy; type of response (complete response, partial response, stable disease, progression of disease [PD]); date of radiologic disease progression (according to the RECIST criteria), date of death, and survival outcomes (progression-free survival [PFS], overall survival [OS]). Outcome molecular data included date of liquid biopsy; result of liquid biopsy; and in patients with a negative ctDNA test result, the date and findings of

subsequent molecular analyses with liquid biopsy or tissue re-biopsy. Concordance between liquid biopsy and tissue re-biopsy was calculated in patients who underwent both procedures; concordance was defined when the same results for EGFR-activating and T790M mutations were observed for both liquid biopsy and tissue biopsy.

The study was conducted in accordance with the current revisions of the Declaration of Helsinki (Fortaleza, Brazil, 2013) and Italian laws on observational studies. Written informed consent for trial participation was obtained from all patients.

Data Analysis

Anonymous data were collected in the database by participating centers and treated according to Italian national laws and General Data Protection Regulations. Data were collected and analyzed by the Medical Oncology Unit of Parma University Hospital.

Standard descriptive statistics were used for analysis of the primary outcome (detection rate of T790M mutation by liquid biopsy); for secondary outcomes, explorative analyses were used to compare variables. Fisher's exact test was used to examine the differences in binary variables, and the Mann-Whitney U test was used to detect differences in continuous variables between groups of patients, given that the distribution of data was not normal (Kolmogorov-Smirnov test). Survival data (PFS and OS) were estimated from the beginning of first-line therapy with first- or second-generation TKI treatment using Kaplan-Meier curves. Log rank tests were used to determine the difference in survival between groups. A P value <0.05 was considered significant. SPSS Statistics v 25.0 (IBM) was used for all computational analyses.

Results

Patients

Within the study period, a total of 120 patients with NSCLC and EGFR mutation were diagnosed with disease progression to first-line treatment with first- or second-generation EGFR TKIs and enrolled in the study (Table 1). The median age was 67 years, 68.3% were female, 63% never smoked, and almost all had non-squamous histology (98.4%). Distribution of the metastatic site at the time of disease progression

was intra-thoracic (15.8%), extra-thoracic (28.4%), and both in 55.8% of patients. Deletion of EGFR exon 19 and L858R was present in 70 (58.3%) and 41 (34.2%) patients, respectively; uncommon mutations were reported in 9 patients (7.5%). Type of first-line TKI administered and treatment outcome are reported in Table 1. For the entire population, the overall response rate to first-line EGFR TKI treatment was 64.2%. Median PFS was 11.8 months (95% confidence interval [CI], 9.8–13.8) and median OS was 33.4 months (95% CI, 30.2–36.7).

Liquid Biopsy Procedures

T790M mutation testing practices were investigated at the local participating centers (Supplemental Table 1). There were no substantial differences in the liquid biopsy procedures between the centers; although the mutation testing techniques used (Easy EGFR, Diatech; Therascreen EGFR RGQ PCR kit, Qiagen; Cobas EGFR Mutation Test, Roche) were slightly different, their sensitivity was basically equivalent. Tests were performed on plasma samples (1–3 mL) collected in tubes containing EDTA in all cases. Samples were processed within 2 h (30 min in 1 center) by plasma separation and storage at −80°C. The test turnaround time from blood sampling to reporting ranged from a few hours (for urgent tests) to a maximum of 1 week.

Results of Liquid Biopsy

Among 120 patients who underwent EGFR mutational analysis by liquid biopsy, 23 patients (19.2%) were positive for both activating mutations and T790M (Act+/T790M+), 39 (32.5%) harbored activating mutations but not T790M (Act+/T790M−), and 58 patients (48.3%) were negative for both (Act−/T790M−) at first testing (Figure 1). Among the 97 T790M− patients, 40 underwent a second liquid biopsy, resulting in 13 Act+/T790M−, 22 Act−/T790M−, and 5 patients (12.5%) were detected as Act+/T790M+ (3 cases were double negative at the previous testing). Twelve T790M− patients at the second testing were submitted to a third plasma assay, and in 3 of them (25%), the test was positive for the T790M mutation (1 Act+/T790M− in both previous tests, 1 double negative, and 1 Act+/T790M− at the second test). Only 2 patients underwent a fourth liquid biopsy, both negative for T790M. Overall, a total of 31 of 120 patients (25.8%) were detected as T790M+ in all 4 rounds of liquid biopsy.

Results of Tissue Re-biopsy

Eighty-nine patients were negative for T790M mutation by liquid biopsy: of these, 54 (60.7%) underwent a tissue re-biopsy (Figure 2). Sites of re-biopsy were 26 lung, 7 liver, 11 pleura and/or pleura effusion, 3 bone, and 8 other sites (4 lymph nodes, 2 liquor, 1 bone marrow, and 1 peritoneum). Of the remaining 35 T790M− patients, the main reasons for not doing a tissue re-biopsy were as follows: the site of disease progression was not accessible in 11 patients, 11 had poor performance status and worsening clinical conditions, and other reasons for 13 patients.

Three tissue re-biopsies were not evaluable due to scarce material, and 1 patient was found to have small-cell lung cancer. Among the remaining 50 evaluable cases, 28 (56%) patients were Act+/T790M+, and 22 patients (20 Act+/T790M− and 2 Act−/T790M−) remained T790M− after tissue re-biopsy. In addition, 2 patients already identified as harboring T790M mutation by plasma-based testing underwent a tissue re-biopsy due to enrollment in a local clinical trial at the Parma center.

Considering all 52 evaluable patients submitted to both liquid and tissue re-biopsy, the concordance between the 2 methods was 46.1% (24 of 52), including 22 plasma-negative cases with corresponding T790M− tissue re-biopsy and 2 patients positive for both liquid and tissue biopsy.

The overall T790M mutation rate on tissue and plasma samples in the entire population was 49.2% (59 of 120 patients), including 31 patients identified by liquid biopsy and 28 patients T790M− by plasma testing who were positive at tissue re-biopsy. In particular, 44 of 59 (74.6%) T790M+ patients harbored deletion of EGFR exon 19 versus 14 (23.7%) positive for L858R mutation ($P < 0.001$). Patients who developed T790M mutation as a resistance mechanism had longer PFS to first- and second-generation TKIs than those who were negative for T790M mutation (median PFS of 13.3 months versus 10 months, $P = 0.066$). Fifty-eight T790M+ patients received osimertinib; only 1 patient did not receive osimertinib due to rapid worsening clinical condition.

Among the 56 patients who underwent tissue re-biopsy, resistance mechanisms other than T790M mutation were investigated in 8 patients only. Among Act+/T790M− patients, 4 cases harboring amplification of the MET gene (1 also presented HER2 polysomy), 1 case with amplification of HER2, and 1

case with HER2 polysomy were identified; in addition, 1 of the 2 Act−/T790M− patients showed transformation to squamous cell carcinoma. One additional case of MET amplification was detected among the 30 patients classified as Act+/T790M+ after tissue testing.

Timing of the Liquid Biopsy

Within the total population, 99 of 120 patients underwent the first liquid biopsy after radiologic disease progression (according to the RECIST criteria); the rate of detection of T790M mutation was 23.2% (23 patients) in this group, whereas 76.8% (76 patients) cases were negative. In contrast, none of the 21 patients tested before RECIST disease progression showed T790M positivity by plasma testing (Table 2); 9 were Act+/T790M− and 12 were Act−/T790M−. This comparison between the rates of T790M positivity according to the time of liquid biopsy (before or after RECIST progression) was statistically significant (P = 0.012).

In 12 of 21 patients tested before RECIST progression, liquid biopsy was retested (in 5 cases again before RECIST progression) within a median time of 83 days (range, 28–285 days); only 1 patient became Act+/T790M+ after 93 days (Act−/T790M− at first liquid biopsy performed before RECIST progression). In 4 of the remaining 11 T790M− patients, a third liquid biopsy was performed after RECIST progression within a median time from the first liquid biopsy of 147 days (range, 84–432 days); 2 patients became Act+/T790M+ after 84 and 432 days, respectively (1 Act+/T790M− in both previous tests, 1 double negative in the first test, and Act+/T790M− in the second test).

In general, considering the 8 patients with a positive liquid biopsy result after the first negative result, the median time for emergence of T790M mutation was 63 days (range, 9–91 days) for patients (n = 5) who were positive at the second liquid biopsy and 199 days (range, 84–432 days) for patients (n = 3) who were positive at the third liquid biopsy.

Correlations between Liquid Biopsy and Metastatic Sites

To investigate whether the number and/or localization of metastatic sites at the time of disease progression influenced T790M positivity, and the shedding status in general (defined as plasma positivity for

EGFR-activating mutation with or without T790M), the first liquid biopsy results were analyzed according to the presence of intra- and/or extra-thoracic metastases (solely brain, bone, or liver metastases) and the number of metastatic sites (<3 versus ≥3) detectable in the patients at the time of disease progression (Table 3). No statistically significant correlation was observed between T790M positivity and localization (intra- versus only extra-thoracic + both intra- and extra-thoracic), the number of metastatic sites, and the presence of metastases only in brain, bone, or liver at disease progression. With regard to the shedding status, the number of metastatic sites ≥3 and exclusively bone localization were significantly associated with positivity ($P = 0.01$ for both).

The same analysis was performed considering only the sites specifically associated with progression, both for T790M positivity and shedding status. A statistically significant correlation was found between T790M positivity and both metastatic localization (intra- versus only extra-thoracic + both intra- and extra-thoracic; $P = 0.008$) and bone metastases ($P = 0.003$). A similar correlation was also observed for the shedding status ($P = 0.001$ for metastatic localization and $P = 0.017$ for bone metastases).

Discussion

The primary aim of the DETECTION study was assessment of the EGFR detection rate by liquid biopsy in patients with NSCLC who had progressed to first-line TKIs in a real-world setting. The study stemmed from the observation that, in the clinical practice of the centers included in this project, the number of patients with NSCLC treated with second-line osimertinib therapy was lower than expected. This finding is in accordance with other real-world reports where only 25%–37% of patients received osimertinib as second-line therapy.[23–25] This might be related to the major challenges associated with access to mutational testing, with only 10%–30% of patients tested at disease progression.[23,26–28] Thus, liquid biopsy might emerge as an alternative non-invasive method for mutational analysis in this setting, overcoming the limitations of tissue re-biopsy and therefore improving access to second-line treatment.

Overall, our data show that, among a population of 120 patients, liquid biopsy led to the identification of T790M resistance mutation in about 25% of cases; in 8 patients, the assessment of positivity required 2 or more plasma-based tests. When feasible, tissue re-biopsy in patients with a negative liquid biopsy result detected the T790M mutation in a further 25% of cases, reaching a total T790M positivity rate

of 50%, as expected. In our cohort, many patients who were negative for T790M at the first liquid biopsy underwent multiple liquid tests: 40 patients had a second test, 12 a third, and 2 a fourth liquid biopsy. Although guidelines suggest performing a tissue re-biopsy after a negative liquid test,[1,6,29] in clinical practice, liquid biopsy is frequently repeated to avoid immediate invasive procedures waiting for clear radiologic or clinical progression, as also suggested by the Italian Association of Medical Oncology recommendation for liquid biopsy[30] and demonstrate in other recent Italian clinical experience.[31]

However, current literature data evaluating the performance of liquid biopsy in clinical practice are still limited. A comparison of our data with that from the study by Offin et al.[22] on 177 patients who progressed to first- and second-generation TKIs evaluated by liquid biopsy for EGFR-activating and T79M mutations may be useful. Among enrolled patients, liquid biopsy was positive in 32% (56 of 177) of cases, a slightly higher percentage than that observed in the DETECTION study (25%). The difference might possibly be related to the different technique used for liquid biopsy analysis, i.e., digital droplet PCR (ddPCR), a method with higher sensitivity than methods based on semi-quantitative PCR. In the same study, concordance between plasma and tissue tests was 80%, at variance from our data showing a concordance of 46.1%. Again, this discrepancy might be ascribed to the higher analytical sensitivity of ddPCR used by the American group.

Although it was not possible to assess the sensitivity of liquid biopsy in the DETECTION study because of the low number of patients undergoing both the plasma and tissue tests, our findings confirm that a tissue re-biopsy should always be performed in patients with a T790M negative plasma test, as recommended by the guidelines.[1,6] On the other hand, our real-word data show that front-line mutational analysis by liquid biopsy can detect at least 50% of T790M-positive patients, limiting tissue biopsy to the remaining 50%. However, the results of liquid biopsy are significantly influenced by the timing of the test, because all 21 patients undergoing the first plasma test before RECIST progression had a negative result, whereas 23 of 99 patients (23.2%) tested with liquid biopsy after RECIST progression were Act+/T790M+. Some of the T790M-negative patients became positive for plasma detection after RECIST progression (3 of 12). This finding underlines the futility of performing a liquid biopsy for T790M mutation before the onset of obvious clinical progression.

When the impact of the location and number of metastatic sites at the time of disease progression on plasma T790M positivity was investigated, a significant association between detection of T790M mutation by liquid biopsy and the presence of extra-thoracic ($P = 0.008$) and bone ($P = 0.003$) disease progression was found. These results are consistent with data reported by Passiglia et al.[32] who analyzed 10 studies to assess the diagnostic accuracy of liquid biopsy for T790M mutation in patients with intra- and extra-thoracic metastases and found a significantly lower sensitivity (50%) in patients with only intra-thoracic involvement compared with patients with extra-thoracic metastatic sites (79%). The DETECTION study results thus confirm that the M (metastasis) parameter should be considered as a clinical predictive factor of liquid biopsy diagnostic accuracy, regardless of the sensitivity of the PCR methodologies used. For the other metastatic sites, we found a statistically significant correlation with bone metastases, confirming the observations of Offin et al.[22]

Overall, our results suggest that shedding of ctDNA in plasma correlates with several factors, including RECIST progression and the involvement of extra-thoracic areas as sites of progression. This could at least partially explain the observations of some studies reporting a worse outcome after osimertinib treatment in patients classified as shedders (i.e., with detectable EGFR mutations in plasma) compared with non-shedders,[33] because the shedding of ctDNA seems to be more evident in patients with more advanced and widespread disease. The amounts of ctDNA are generally reported to be higher in patients with progression and increased tumor burden, as well as in those with extra-thoracic metastases, but are often low or even undetectable in patients with stable disease, with oligo-progression, or with exclusively cerebral or intra-thoracic metastases.[32,34]

The retrospective nature of the study and the blood tests collected from 5 different hospitals are the main limitations of the study. However, the selection of medical oncology centers with similar standard operative procedures in clinical care and laboratory execution should have limited these biases.

In conclusion, the results of the DETECTION study confirm the specificity of liquid biopsy for detection of T790M mutation by cfDNA analysis and emphasize the necessity of performing a tissue re-biopsy in all patients who have a negative plasma test result. However, although the rate of T790M positivity in our study was as expected (50%), in clinical practice, this result was achieved through a long and distinct

clinical course, requiring multiple liquid biopsies and, when possible, tissue re-biopsy in patients with negative plasma results. All these issues need to be accurately considered in the choice of first-line therapy, because osimertinib is currently available as first-line treatment, thus offering an effective therapeutic option at the beginning of the patient's clinical history. Using osimertinib in TKI-naive EGFR-mutated patients will inevitably reduce the access to ctDNA analysis for T790M detection in clinical practice but, on the other hand, liquid biopsy can be used to study the osimertinib resistance mechanism.

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Table 1. Patient population (N = 120) and clinical outcome

	Value[a]
Age (years), median (range)	67 (34–86)
Histology, *n* (%)	
Non-squamous	118 (98.4)
Squamous	1 (0.8)
Mixed	1 (0.8)
Sex	
Male	38 (31.7)
Female	82 (68,3)
Smoking status	
Smokers	5 (4.2)
Ex-smokers	39 (32.5)
Non-smokers	76 (63.3)
ECOG performance status	
0–1	113 (94.2)
2	7 (5.8)
Metastatic involvement	
Intra-thoracic	19 (15.8)
Extra-thoracic	34 (28.4)
Intra- + extra-thoracic	67 (55.8)
Metastatic site	
Liver	22 (18.3)
Bone	50 (41.7)
Adrenal	12 (10)
Brain	54 (45)
Lung	67 (55.8)
Pleura	37 (30.8)
Others	59 (42.2)
EGFR mutation at diagnosis	
Deletion of exon 19	70 (58.3)

L858R mutation	41 (34.2)
Rare and compound mutations	9 (7.5)
Type of tyrosine kinase inhibitor	
Gefitinib	61 (50.8)
Erlotinib	20 (16.7)
Afatinib	39 (32.5)
Best response	
CR + PR	77 (64.2)
SD	36 (30)
PD	6 (5)
NE	1 (0.8)
Progression-free survival (months), median (range)	11.8 (9.8–13.8)
Overall survival (months), median (range)	33.4 (30.2–36.7)

ECOG, Eastern Cooperative Oncology Group; CR, complete response; PR, partial response; SD, stable disease; PD, progression of disease; NE, not evaluable.

[a]Values are number (%) except where indicated otherwise.

Table 2. Timing of the first liquid biopsy and the results

	T790M		Overall	*P*
	Negative, *n* (%)	Positive, *n* (%)		
Liquid biopsy before RECIST progression				
No	76 (76.8)	23 (23.2)	99	0.012
Yes	21 (100)	0	21	
Overall	97 (80.8)	23 (19.2)	120	

Table 3. T790M positivity and shedding status according to the location and number of metastatic sites at progression and sites of disease progression

	T790M, *n* (%)		Overall	*P*	Shedding, *n* (%)		Overall	*P*
	Negative	Positive			Negative	Positive		
Metastatic sites at progression								
Location								
Intra-thoracic	18 (94.7)	1 (5.3)	19	0.12	13 (68.4)	6 (31.6)	19	0.08
Extra-thoracic ± intra-thoracic	79 (78.2)	22 (21.8)	101		45 (44.6)	56 (55.4)	101	
Only brain								
–	54 (81.8)	12 (18.2)	66	0.82	35 (53)	31 (47)	66	0.28
+	43 (79.6)	11 (20.4)	54		23 (42.6)	31 (57.4)	54	
Only bone								
–	61 (87.1)	9 (12.9)	70	0.06	41 (58.6)	29 (41.4)	70	0.01
+	36 (72)	14 (28)	50		17 (34)	33 (66)	50	
Only liver								
–	79 (80.6)	19 (19.4)	98	1	49 (50)	49 (50)	98	0.5
+	18 (81.8)	4 (18.2)	22		9 (41)	13 (59)	22	
Number								
<3	58 (85.3)	10 (14.7)	68	0.17	40 (58.8)	28 (41.2)	68	0.01
≥3	39 (75)	13 (25)	52		18 (34.6)	34 (65.4)	52	
Metastatic sites of progression								
Location								
Intra-thoracic	32 (97)	1 (3)	33	0.008	24 (72.7)	9 (27.3)	33	0.001
Extra-thoracic ± intra-thoracic	65 (74.7)	22 (25.3)	87		34 (39.1)	53 (60.9)	87	
Only brain								
–	84 (82.4)	18 (17.6)	102	0.3	48 (47.1)	54 (52.9)	102	0.6
+	13 (72.2)	5 (27.8)	18		10 (55.6)	8 (44.4)	18	
Only bone								

–	93 (84.5)	17 (15.5)	110	0.003	57 (51.8)	53 (48.2)	110	0.017
+	4 (40)	6 (60)	10		1 (10)	9 (90)	10	
Only liver								
–	93 (80.2)	23 (19.8)	116	0.6	56 (48.3)	60 (51.7)	116	1
+	4 (100)	0 (0)	4		2 (50)	2 (50)	4	
Number								
<3	79 (81.4)	18 (18.6)	97	0.8	51 (52.6)	46 (47.4)	97	0.07
≥3	18 (78.3)	5 (21.7)	23		7 (30.4)	16 (69.6)	23	

Figure 1. Flow diagram of the liquid biopsy results. Abbreviations: act, activating mutation; pts, patients.

Figure 2. Flow diagram of the tissue re-biopsy results.