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ORIGINAL ARTICLE

Role of circulating tumor DNA in the detection of sensitizing and resistance to epidermal growth factor receptor mutations in metastatic lung adenocarcinoma

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Summary

Purpose: *The EGFR (Epidermal Growth Factor Receptor) mutations may predict sensitivity and resistance to EGFR-TKIs (Tyrosine Kinases Inhibitors) in metastatic lung adenocarcinoma. The detection of these mutations is usually performed on tumor tissue samples. However, when a biopsy is not feasible or the amount of tissue is limited, circulating tumor DNA (ctDNA) may represent an alternative source for genotyping the tumor.*

Methods: *In the first phase of the study, the liquid biopsy was performed in newly diagnosed metastatic lung adenocarcinoma patients with and without EGFR mutations to evaluate the concordance between EGFR mutational analysis on ctDNA by real time PCR and on tissue. In the second phase it was performed in EGFR positive patients progressing after first or second generation TKIs in order to detect the T790M mutation.*

Results: *In the first phase, a 100% concordance between EGFR on ctDNA and tissue was revealed, leading to validation of the test. In the second phase, 44.8% of patients showed T790M positive result at liquid biopsy. Considering the rebiopsies performed in 31% of the cases, the overall positivity rate of T790M was 58.6%. Sensitivity and specificity were 76% and 75%, respectively. The median time to development of T790M mutation from the start of first line EGFR TKI was 244 days.*

Conclusions: *: Our experience confirms that liquid biopsy is a valid method to detect sensitizing and resistant EGFR mutations in patients with metastatic lung adenocarcinoma. Nevertheless, in the presence of negative ctDNA analysis, a rebiopsy should be performed whenever possible to confirm this result.*

Key words: *circulating tumor DNA, tissue biopsy, lung adenocarcinoma, sensitizing mutations, T790M mutation, real time PCR*

Introduction

Lung cancer is the second most commonly diagnosed tumor worldwide [1,2]. About 85% of the cases are represented by non-small cell lung cancer (NSCLC), of which adenocarcinoma is the more frequent histological subtype. Activating EGFR mutations occur in 10-15% of Caucasian patients and in 40-60% of South-East Asian patients with lung adenocarcinoma [3]: up to 90% of them have exon 19 deletions and L858 point mutations in exon 21 that show sensitivity to EGFR tyrosine kinase inhibitors (TKIs), while the remaining 10% are uncommon mutations (G19X, L861Q, S768I, EGFR fusions, exon 20 insertions) with limited or poor sensitivity to TKIs [4].

From 2009 todate, eight phase III studies demonstrated that first (gefitinib, erlotinib) and second

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(afatinib) generation EGFR TKIs were superior to chemotherapy in terms of response rate (RR) and progression-free survival (PFS) in mutated EGFR patients, while they did not show an overall survival (OS) advantage because of high cross-over rate from chemotherapy to EGFR TKI arms [5-16]. Unfortunately, after 6-13 months all patients treated with EGFR TKIs show progressive disease. In these cases the most common mechanism of acquired resistance is represented by the development of EGFR T790M mutation. It may occur in 50-60% of patients and consists of the substitution of threonine to methionine at amino acid position 790 in exon 20. Moreover, it enhances the ATP binding affinity of EGFR protein, reducing the efficacy of first and second generation EGFR TKIs [4,17]. Osimertinib is a third generation EGFR TKI able to overcome this mechanism of resistance. Two randomized phase 3 studies reported its efficacy both in second-line setting in comparison with chemotherapy (AURA 3 study) [18] and in first-line setting compared to gefitinib or erlotinib (FLAURA trial) [19]. Moreover, osimertinib showed a good safety profile and high efficacy in nervous central system metastases [19].

Therefore, the evaluation of T790M mutation is essential to identify the innate or acquired resistance and to select patients for the optimal sequence of therapies. The best source of material to assess T790M status is represented by tumor tissue, that derives from the biopsy performed at diagnosis or the re-biopsy at disease progression. However, biopsy procedures in NSCLC are often associated with a number of limitations, such as the insufficient tumor tissue, the location and size of tumor, the risk of complications, and the heterogeneity of the tumor [20,21]. Alternative sources of tumor DNA may be blood samples and also urine and cerebral spinal fluid, as recent data suggest [21]. In the blood, the tumor DNA may derive from cell tumor DNA (ctDNA), circulating tumor cells (CTCs) and exosomes [22,23]. In particular, ctDNA consists of small fragments of DNA at very low concentration (<10 ng/ml) and primarily derives from apoptosis and necrosis of cancer cells, although it may increase as a consequence of tissue trauma or inflammation [24]. The plasma concentration of ctDNA strictly depends on tumor type and burden [24]. Besides, its detection requires extremely sensitive analysis methods, such as real-time PCR, digital PCR, BEAMing (Beads, Emulsion, Amplification, Magnetics), and Whole Genome Sequencing (WGS) [24,25].

The procedure of using cell tumor DNA to detect genetic alterations is termed liquid biopsy [21,23,25,26]. It offers several advantages over tissue biopsy: it is safer for the patient, requires quicker turnaround time, provides information on the effect of the treatment and identify the development of resistance mechanisms, overcoming the bias of tumor heterogeneity [20,21, 24,27].

Table 1. Concordance between EGFR mutational analysis on tissue and ctDNA in the first phase of the study in order to validate the test

	Pyrosequencing		*Sequenom Mass Array*						*RT PCR*
	EGFR	*K-ras*	*EGFR*	*K-ras*	*N-ras*	*B-raf*	*PI3KA*	*DDR2*	*EGFR*
1	ex 19 del	-							ex 19 del
2	L858R	-							L858R
3	wt	G12D							wt
4	wt	wt							wt
5	ex 19 del	-							ex 19 del
6	ex 19 del	-							ex 19 del
7	wt	wt							wt
8	L858R	-							L858R
9	ex 19 del	-							ex 19 del
10			wt	G12D	wt	wt	wt	wt	wt
11	wt	G12C							wt
12			wt	G12C	wt	wt	wt	wt	wt
13			ex 19 del	wt	wt	wt	wt	wt	ex 19 del
14			wt	G13D	wt	wt	wt	wt	wt
15			wt	wt	wt	wt	wt	wt	wt
16			L858R	wt	wt	wt	wt	wt	L858R
17			ex 19 del	wt	wt	wt	wt	wt	ex 19 del
18			wt	wt	wt	wt	wt	wt	wt

The present study aimed to evaluate the reliability of liquid biopsy in the detection of EGFR mutations in NSCLC patients treated with EGFR TKIs in first- and second-line settings according to clinical practice.

Methods

The study included two different phases. In the first, a consecutive series of patients with newly diagnosed metastatic adenocarcinoma of the lung and known EGFR status underwent a liquid biopsy before starting the first line treatment with EGFR-TKIs or chemotherapy according to the EGFR mutational status. In this phase we evaluated the concordance between EGFR mutational analysis in blood and on tissue and we validated the method. The tissue analysis was performed on tumor samples obtained by small biopsies or fine needle aspirations: EGFR and K-ras mutations were detected by Pyrosequencing (Pyr) or Sequenom MassArray (SMA). Specifically, the multiplex, PCR, Myriapod Lung Status kit, based on MALDI-TOF SMA, allowed the identification of main mutations of EGFR (exons 18-21), KRAS (12, 13 and 61 codons), BRAF (466, 469, 594, 597 ad 600 codons), NRAS (12 and 61 codons), PI3KA and DDR2 genes. Only EGFR mutations were assessed on ctDNA by a RT PCR: the Easy EGFR, Diatech Pharmacogenetics, was the kit used to detect the main mutations of exons 18-21 of EGFR gene.

In the second phase, we performed a liquid biopsy only in patients with EGFR mutation detected on tissue analysis. Until April 2018, the blood analysis continued to be done at baseline in order to further confirm the validation of the test, and it was performed in all EGFR positive patients progressing after first or second generation TKIs to detect T790M resistance mutation in ctDNA. A tissue re-biopsy was performed when plasma T790M was negative. In addition, a tissue re-biopsy was also proposed to those T790M+ patients who had a site of disease easily accessible. As in the first phase, the degree of concordance between EGFR mutational analysis in blood and on tissue was evaluated. The T790M detection rate and the percentage of rebiopsy performed at disease progression following first-line treatment were also reported.

Written informed consent was obtained from all patients. The liquid biopsy consisted in 4 tubes of blood (7 mL each) containing EDTA as anticoagulant. Considering the short half-life of ctDNA, the plasma was separated from the blood cells by low-speed centrifugation (1200 x g for 15 min) at 4°C within 1 h after specimen collection. The liquid samples were stored at -80°C until ctDNA extraction or immediately analyzed, with the result available within 72 h.

Statistics

Descriptive statistics were used to report patient characteristics and results. The concordance between EGFR mutational analysis in blood and on tissue both before first-line treatment and after disease progression were reported as percentage. The detection rate of T790M and the number of rebiopsies performed at disease progression following first-line treatment were reported as percentage too. Sensitivity and specificity of T790M detection were calculated.

Results

Between April 2015 and April 2016, analysis of tumor tissue and on blood was performed in 18 patients (M/F=9/9) with a median age of 65 years (range 42-78). EGFR mutations were detected in 9/18 patients: 6 cases of exon 19 deletions (5 E746-A750 del, one E747-T751 del) and 3 cases of exon 21 substitution (L858R) (Table 1). Analysis of EGFR mutations on ctDNA showed a 100% concordance with tissue detection. Based on these data, the test was considered as internally validated, and became a standard method to detect EGFR mutations.

In the second phase of the study, data from a consecutive series of 36 patients was analyzed. These patients underwent liquid biopsies before and/or after treatment with EGFR TKIs, between April 2015 and December 2018 (Table 2). Among 19 patients (51.4%) evaluable for baseline EGFR expression on liquid biopsy, no patient showed T790M mutation. The EGFR mutation concordance between ctDNA and tissue was 79% (15/19),

Table 2. Patient and disease characteristics

Characteristics	*All patients (n=36) n (%)*
Age, years	
Median	69
Range	41-82
Sex	
Male	12 (33.4)
Female	24 (66.7)
Smoking history	
Never	24 (66.7)
Former	8 (22.2)
Current	4 (11.1)
Histology	
Adenocarcinoma	36 (100)
EGFR mutation	
Ex 19 deletion	25 (69.4)
L858R	10 (27.8)
L861Q	1 (2.8)
Stage	
IV	36 (100)
First line EGFR TKI	
Gefitinib	9 (25)
Erlotinib	18 (50)
Afatinib	9 (25)

Table 3. Concordance between tissue and liquid biopsy T790M detection

	T790M+	*T790M-*
Before first line treatment		
Tissue	0	19
Liquid biopsy	0	19
Concordance	100%	100%
After disease progression		
Tissue	5	3
Liquid biopsy	1	8
Concordance	20%	37.5%

One patient underwent rebiopsy at progression, but material showed poor cellularity for EGFR analysis

while in the remaining 4 patients it was not found (3 patients) or it was not evaluable (1 patient), due to inadequate tissue availability. At TKIs progression, liquid biopsy was performed in 29 patients with T790M detection in 44.8% of the cases (13 patients). Among these 29 patients a tissue rebiopsy was performed in 31% of the cases (9 patients) with 5 patients showing T790M mutation: the liquid biopsy was concordant only in one patient (Table 3). Therefore, the overall positivity rate (on tissue plus on plasma) of T790M was 58.6% (17/29 patients). Sensitivity and specificity were 76 and 75%, respectively.

Discussion

Our study confirmed the reliability of liquid biopsy in the detection of EGFR mutations in NSCLC patients treated with EGFR TKIs in first- and second-line setting according to clinical practice. Liquid biopsy represents one of the greatest achievements in thoracic oncology in recent years. In EGFR mutant lung cancers, ctDNA may be used to detect sensitizing mutations, to monitor the response to treatment and to identify resistance mechanisms as T790M [28-30]. The detection of EGFR mutations in ctDNA also depends on the burden of disease: a study including several cancer types reported a ctDNA detection rate of 82% in patients with stage IV disease in comparison with 47% in patients with stage I disease [31].

Moreover, other biological fluids such as saliva, urine and cerebrospinal fluid were also used to detect EGFR mutations [32].

The ctDNA may be detected by various assays that have different sensitivity, specificity ad concordance profiles. Traditional assays such as Sanger sequencing or Pyrosequencing are not sensitive enough to detect EGFR mutations in ctDNA, with a detection limit ranging from 1 to 10%. Therefore, alternative assays have been developed [33-35]. Cobas, Therascreen and amplification refractory mutation system (ARMS)/Scorpion assays have a detection limit of 1-3% [35,36], that may be lower to 2% with Peptide Nucleid Acid (PNA) mediated PCR and PNA-locked nucleid acid (PNA-LNA) [37]. Digital PCR and Beads Emultions Amplification and Magnetics (BEAM) can detect mutations in ctDNA at frequencies as low as 0.01%. [35,38,39].

In our study, we used a RT PCR to assess EGFR mutations in ctDNA, with results available within 72 h. A multicentric study demonstrated a robust interlaboratory performance and reproducibility of a RT PCR EGFR mutation test (cobas EGFR Mutation Test v2) to detect EGFR variants in plasma [40]. Besides, a prospective study used a RT PCR assay to screen EGFR mutations in ctDNA of advanced NSCLC patients, whose tissue was not available or insufficient for biomolecular characterization: 113 (11%) of 1033 of patients showed EGFR mutated at baseline; 105 were screened after progression to EGFR TKIs, maintaining sensitizing mutation in 56.2% of the cases and developing T790M in 35.2% [41]. In the first phase of our study we reported a 100% concordance between EGFR detection in blood and on tissue, therefore the test was validated, becoming a reliable method mainly when available tissue is insufficient for biomolecular analysis.

In the second phase, baseline liquid biopsy continued to be proposed before starting first-line treatment, reporting a concordance of 79% between ctDNA and tissue, while 29 patients underwent liquid biopsy after disease progression and 13 of them became T790M positive, with a detection rate of 44.8%. Considering the T790M positivity after rebiopsy, the overall positivity rate of T790M was 58,6% (17/29 patients), with high sensitivity and specificity (76 and 75%, respectively).

Our data are in line with both retrospective and prospective analyses of concordance between 790M detection in ctDNA and on tissue [42-44]. Therefore, the National Cancer Comprehensive Network (NCCN) and ESMO (European Society of Medical Oncology) guidelines recommend performing a rebiopsy if the plasma test is negative, while liquid biopsy represents an option when the disease progresses and tumor tissue is not available or the tissue biopsy is not feasible. [45,46]. The present paper confirms that only the plasma negativity for the T790M is insufficient to identify patients who could potentially benefit from osimertinib treatment.

Liquid biopsy has expected to have a role also in the identification of resistance mechanisms to third-generation EGFR TKIs [47].

Recent data suggests an important role of liquid biopsy also in the evaluation of ALK (Anaplastic Lymphoma Kinase) mutations and PDL1 (Programmed Death Ligand 1) expression in lung cancers. Specifically, ALK resistance mutations may be detected in ctDNA of patients treated with ALK inhibitors [48], while PDL1 status may be evaluated by using CTCs and circulating white blood cells considering the correlation with PDL1 status in tumor tissue [49]. Besides, ctDNA may be used to measure the tumor mutation burden, whose levels correlate with clinical benefit from immunotherapy [50].

A literature review of American Society of Clinical Oncology (ASCO) and the College of American Pathologists confirmed the clinical utility of ctDNA assay in lung cancer, while no evidence of clinical utility and validity of ctDNA was reported in most of cancers, particularly for screening, treatment monitoring, and detection of residual disease [51]. Therefore, the liquid biopsy has expected to give great impulse to translational research in lung tumors, providing a new source for cancer biomarkers and adding new dimensions in advanced clinical trials [52]. In particular, the identification of prognostic factors by liquid biopsy represents one of the most intriguing research areas: a recent study reported the association between CTCs and PFS in patients with EGFR T790M-positive NSCLC following disease progression on EGFR, TKI [53].

Our study presents a lot of limitations. First of all, the study was a monocentric experience, with high risk of selection bias. Second, the number of patients included in the analysis was low. In this view it is worth to note that the grade of concordance between blood and tissue analysis decreased over the years, inversely proportional to increase of patients included.

Third, we did not perform a direct comparison with other more sensitive assays: the RT PCR was the only assay available at our laboratory of molecular biology.

Finally, the role of rebiopsy needs to be underlined: the T790M mutations detection rate on liquid biopsy was less than 50%, and the overall positivity rate was almost 60% only with rebiopsy.

In conclusion, we suggest that a RT PCR performed on ctDNA can represent a valid method to detect sensitizing and resistance EGFR mutations in the blood of patients with advanced lung adenocarcinoma, both before and after treatment with first- and second-generation EGFR TKIs. In particular, RT PCR on ctDNA showed high sensitivity, specificity and concordance with tissue analysis: this is very relevant in clinical practice in the light of the introduction of osimertinib as first-line treatment, based on FLAURA study data.

The ctDNA better reflects the heterogeneity of metastatic lung adenocarcinoma and may be useful to disease monitoring during treatment. Moreover, the liquid biopsy represents a valid assay when the available tissue samples are insufficient for molecular analysis and a re-biopsy is difficult to perform.

Conflict of interests

All authors declare that they have no financial and/or personal relationships with other individuals or organizations that could inappropriately influence their work. All authors confirm that there are no conflicts of interests.

References

1. Torre LA, Siegel RL, Jemal A. Lung Cancer Statistics. Adv Exp Med Biol 2016;893:1-19.
2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. CA Cancer J Clin 2018;68:7-30.
3. Hsu WH, Yang JC, Mok TS, Loong HH. Overview of current systemic management of EGFR-mutant NSCLC. Ann Oncol 2018;1;29 (Suppl 1):i3-i9.
4. Westover D, Zugazagoitia J, Cho BC, Lovly CM, Paz-Ares L. Mechanisms of acquired resistance to first- and second-generation EGFR tyrosine kinase inhibitors. Ann Oncol 2018;1;29 (Suppl 1):i10-i19.
5. Mok TS, Wu YL, Thongprasert S et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med 2009;361:947-57.
6. Fukuoka M, Wu YL, Thongprasert S et al. Biomarker analyses and final overall survival results from a phase III, randomized, open-label, first-line study of gefitinib versus carboplatin/paclitaxel in clinically selected patients with advanced non-small-cell lung cancer in Asia (IPASS). J Clin Oncol 2011;29:2866-74.
7. Mitsudomi T, Morita S, Yatabe Y et al. Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): an open label, randomised phase 3 trial. Lancet Oncol 2010;11:121-8.
8. 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;62:2380-8.

9. Inoue A, Kobayashi K, Maemondo M et al. Updated overall survival results from a randomized phase III trial comparing gefitinib with carboplatin-paclitaxel for chemo-naive non-small cell lung cancer with sensitive EGFR gene mutations (NEJ002). Ann Oncol 2013;24:54-9.
10. Zhou C, Wu YL, Chen G et al. Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive nonsmall cell lung cancer (OPTIMAL, CTONG-0802): a multicentre, open-label, randomised, phase 3 study. Lancet Oncol 2011;12:735-42.
11. Zhou C, Wu YL, Chen G et al. Final overall survival results from a randomised, phase III study of erlotinib versus chemotherapy as first-line treatment of EGFR mutation-positive advanced non-small-cell lung cancer (OPTIMAL, CTONG-0802). Ann Oncol 2015;26:1877-83.
12. Rosell R, Carcereny E, Gervais R 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.
13. Wu YL, Zhou C, Liam CK et al. First-line erlotinib versus gemcitabine/cisplatin in patients with advanced EGFR mutation-positive non-small cell lung cancer: analyses from the phase III, randomized, open-label, ENSURE study. Ann Oncol 2015;26:1883-9.
14. Sequist LV, Yang JC, Yamamoto N et al. Phase III study of afatinib or cisplatin plus pemetrexed in patients with metastatic lung adenocarcinoma with EGFR mutations. J Clin Oncol 2013;31:3327-34.
15. Wu YL, Zhou C, Hu CP et al. Afatinib versus cisplatin plus gemcitabine for first-line treatment of Asian patients with advanced non-small-cell lung cancer harbouring EGFR mutations (LUX-Lung 6): an open-label, randomised phase 3 trial. Lancet Oncol 2014;15:213-22.
16. Yang JC, Wu YL, Schuler M et al. Afatinib versus cisplatin-based chemotherapy for EGFR mutation-positive lung adenocarcinoma (LUX-Lung 3 and LUX-Lung 6): analysis of overall survival data from two randomised, phase 3 trials. Lancet Oncol 2015;16:141-51.
17. Remon J, Steuer CE, Ramalingam SS, Felip E. Osimertinib and other third-generation EGFR TKI in EGFR-mutant NSCLC patients. Ann Oncol 2018;1,29 (Suppl 1):i20-i27.
18. Mok TS, Wu Y-L, Ahn M-J et al. Osimertinib or Platinum-Pemetrexed in EGFR T790M-Positive Lung Cancer. N Engl J Med 2017;376:629-40.
19. Soria JC, Ohe Y, Vansteenkiste J et al. Osimertinib in Untreated EGFR-Mutated Advanced Non-Small-Cell Lung Cancer. N Engl J Med 2018;378:113-25.
20. Oellerich M, Schütz E, Beck J et al. Using circulating cell-free DNA to monitor personalized cancer therapy. Crit Rev Clin Lab Sci 2017;54:205-18.
21. Goldman JW, Noor ZS, Remon J, Besse B, Rosenfeld N. Are liquid biopsies a surrogate for tissue EGFR testing? Ann Oncol 2018:29 (Suppl 1):i38-i46.
22. Qin A, Ramnath N. The “liquid biopsy” in non-small cell lung cancer - not quite ready for prime time use. Transl Cancer Res 2016;5 (Suppl 4):S632-5.
23. Buder A, Tomuta C, Filipits M. The potential of liquid biopsies. Curr Opin Oncol 2016;28:130-4.
24. Wan JCM, Massie C, Garcia-Corbacho J et al. Liquid biopsies come of age: towards implementation of circulating tumour DNA. Nat Rev Cancer 2017;17:223-38.
25. Diaz LA Jr, Bardelli A. Liquid biopsies: genotyping circulating tumor DNA. J Clin Oncol 2014;32:579-86.
26. Molina-Vila MA, Mayo-de-Las-Casas C, Giménez-Capitán A et al. Liquid Biopsy in Non-Small Cell Lung Cancer. Front Med (Lausanne) 2016;3:69.
27. Offin M, Chabon JJ, Razavi P et al. Capturing Genomic Evolution of Lung Cancers through Liquid Biopsy for Circulating Tumor DNA. J Oncol 2017;4517834.
28. Tang Y, Qiao G, Xu E, Xuan Y, Liao M, Yin G. Biomarkers for early diagnosis, prognosis, prediction, and recurrence monitoring of non-small cell lung cancer. Onco Targets Ther 2017;10:4527-34.
29. Cabanero M, Tsao MS. Circulating tumour DNA in EGFR-mutant non-small-cell lung cancer. Curr Oncol 2018;25 (Suppl 1):S38-S44.
30. Corcoran RB, Chabner BA. Application of Cell-free DNA Analysis to Cancer Treatment. N Engl J Med 2018;379:1754-65.
31. Bettegowda C, Sausen M, Leary RJ et al. Detection of circulating tumor DNA in early- and late-stage human malignancies. Sci Transl Med 2014;6:224ra24.
32. Li YS, Jiang BY, Yang JJ et al. Unique Genetic Profiles from Cerebrospinal Fluid Cell-free DNA in Leptomeningeal Metastases of EGFR-mutant Non-Small Cell Lung Cancer: A New Medium of Liquid Biopsy. Ann Oncol 2018;29:945-52.
33. Ansari J, Yun JW, Kompelli AR et al. The liquid biopsy in lung cancer. Genes Cancer 2016;7:355-67.
34. Huang WL, Chen YL, Yang SC et al. Liquid biopsy genotyping in lung cancer: ready for clinical utility? Oncotarget 2017;8:18590-608.
35. Kim E, Feldman R, Wistuba II. Update on EGFR Mutational Testing and the Potential of Noninvasive Liquid Biopsy in Non-Small-cell Lung Cancer. Clin Lung Cancer 2018;19:105-14.
36. Komiya K, Nakashima C, Nakamura T et al. Current Status and Problems of T790M Detection, a Molecular Biomarker of Acquired Resistance to EGFR Tyrosine Kinase Inhibitors, with Liquid Biopsy and Re-biopsy. Anticancer Res 2018;38:3559-66.
37. Kobayashi K, Naoki K, Manabe T et al. Comparison of detection methods of EGFR T790M mutations using plasma, serum, and tumor tissue in EGFR-TKI-resistant non-small cell lung cancer. Onco Targets Ther 2018;11:3335-43.
38. Takahama T, Sakai K, Takeda M et al. Detection of the T790M mutation of EGFR in plasma of advanced non-small cell lung cancer patients with acquired resistance to tyrosine kinase inhibitors (West Japan oncology group 8014LTR study). Oncotarget 2016;7:58492-9.
39. Kasahara N, Kenmotsu H, Serizawa M et al. Plasma epidermal growth factor receptor mutation testing with a chip-based digital PCR system in patients with advanced non-small cell lung cancer. Lung Cancer 2017;106:138-44.

40. Keppens C, Palma JF, Das PM et al. Detection of EGFR Variants in Plasma: A Multilaboratory Comparison of a Real-Time PCR EGFR Mutation Test in Europe. J Mol Diagn 2018;20:483-94.
41. Mayo-de-Las-Casas C, Jordana-Ariza N, Garzón-Ibañez M et al. Large scale, prospective screening of EGFR mutations in the blood of advanced NSCLC patients to guide treatment decisions. Ann Oncol 2017;28:2248-55.
42. Sacher AG, Paweletz C, Dahlberg SE et al. Prospective validation of rapid plasma genotyping for the detection of EGFR and KRAS mutations in advanced lung cancer. JAMA Oncol 2016;2:1014-22.
43. Sundaresan TK, Sequist LV, Heymach JV et al. Detection of T790M, the acquired resistance EGFR mutation, by tumor biopsy versus noninvasive blood-based analyses. Clin Cancer Res 2016;22:1103-10.
44. Oxnard GR, Thress KS, Alden RS et al. Association between plasma genotyping and outcomes of treatment with osimertinib (AZD9291) in advanced non-small-cell lung cancer. J Clin Oncol 2016;34:3375-82.
45. Novello S, Barlesi F, Califano R et al. Metastatic non-small-cell lung cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2016;27 (Suppl 5):v1-v27.
46. Ettinger DS, Wood DE, Aisner DL et al. NCCN Clinical Practice Guidelines in Oncology (NCCN guidelines). Non Small Cell Lung Cancer.V9.2017; http://www.nccn.org (25 June 2017, date last accessed).
47. Ricordel C, Friboulet L, Facchinetti F, Soria JC. Molecular mechanisms of acquired resistance to third-generation EGFR-TKIs in EGFR T790M-mutant lung cancer. Ann Oncol 2018;29 (Suppl 1):i28-i37.
48. Chen Y, Guo W, Fan J et al. The applications of liquid biopsy in resistance surveillance of anaplastic lymphoma kinase inhibitor. Cancer Manag Res 2017;9:801-11.
49. Ilié M, Szafer-Glusman E, Hofman V et al. Detection of PD-L1 in circulating tumor cells and white blood cells from patients with advanced non-small-cell lung cancer. Ann Oncol 2018;29:193-9.
50. Wauters E, Vansteenkiste J. Will liquid biopsies become our fluid transition to personalized immunotherapy? Ann Oncol 2018;29:11-3.
51. Merker JD, Oxnard GR, Compton C et al. Circulating Tumor DNA Analysis in Patients With Cancer: American Society of Clinical Oncology and College of American Pathologists Joint Review. J Clin Oncol 2018;36:1631-41.
52. Johann DJ Jr, Steliga M, Shin IJ et al. Liquid biopsy and its role in an advanced clinical trial for lung cancer. Exp Biol Med (Maywood) 2018;243:262-71.
53. Yang B, Zheng D, Zeng Y, Qin A, Gao J, Yu G. Circulating tumor cells predict prognosis following second line AZD9291 treatment in EGFR-T790M mutant non-small cell lung cancer patients. JBUON 2018;23:1077-81.