Original Article

ISSN: 1735-0344 *Tanaffos 2022; 21(3): 376-383*

TANAFFOS

T790M and Acquired Resistance of Epidermal Growth Factor Receptor to Tyrosine Kinase Inhibitors in Patients with Lung Adenocarcinoma

Hanifeh Mirtavoos-Mahyari [1], Azizollah Abbasi Dezfouli [1], Zahra Esfahani-Monfared [2], Adnan Khosravi [2], Sharareh Seifi [2], Kambiz Sheikhy [1]

[1] Lung Transplantation Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran, [2] Chronic Respiratory Disease Reseach Center, NRITLD, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Received: 10 August 2021
Accepted: 6 April 2022

Correspondence to: Mirtavoos-Mahyari H
Address: Lung Transplantation Research Center, NRITLD, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
Email address: hanifehmirmah@yahoo.com

Background: Activating mutations in the epidermal growth factor receptor (EGFR) are initially responsive to tyrosine kinase inhibitors (TKIs), but responses to TKIs is not permanent and drug resistance eventually happens for almost all patients. Subsequent studies found different resistance mechanisms, among which (EGFR) T790M mutation is the most important mechanism of TKI treatment failure. Using cell- free DNA (cfDNA) is a new way for diagnosing resistance mutations in EGFR. The aim of present study is to determine cfDNA-identified recurrence mutation rate and their association with clinical outcome in lung Adenocarcinoma patients.

Materials and Methods: Patients who were diagnosed with metastatic adenocarcinoma of the lung and acquired resistance to TKIs were enrolled. The incidence of T790M positivity, overall survival (OS) and median duration of TKI treatment before progression was calculated. Polymerase chain reaction (PCR) and sequencing were used to identify the T790M mutation in cfDNA.

Results: The incidence of T790M mutations was higher in men, younger cases (<59 years), in patients with *L858R* primary mutation and never smokers although they were not significantly different (P-values= 041, 0.316, 0.316 and 0.158, respectively). There was significant longer OS in the Del19 subgroup than the L858R subgroup (p = 0.014). In multivariable analysis, significant longer OS was associated with younger age (<59 years) and primary EGFR mutation exon 19 (P- values= 0.028 and 0.050, respectively).

Conclusion: T790M mutations frequency may differ by ethnicity, genetic factors and EGFR primary mutations. Detecting T790M mutations in plasma is considered as an indicator of treatment with third generation EGFR-TKIs.

Key words: Epidermal growth factor receptor; T790M; Cell-free DNAs; Non–small cell lung cancer

INTRODUCTION

Lung cancer is most leading cancer death worldwide (1). Development of molecularly targeted therapies has promising results in regard to lung cancer clinical outcomes. After epidermal growth factor receptor (EGFR) – a strong oncogene active in non-small cell lung cancer (NSCLC) - introduction, significant changes in clinical outcomes of NSCLC patients harboring such activated mutations of EGFR have occurred. Most important *EGFR* gene mutations occur in exons 18–21 (2). Thyrosin kinase inhibitors (TKIs) target the ATP-binding sites of the intracellular kinase domain of EGFR receptor and achive favorable primary clinical responses in practice (3). Patients with mutant *EGFR* who were treated by TKIs had

higher response rates and longer progression-free survival (PFS) compared to patients receiving traditional treatments (4). Unfortunately, responses to TKIs are not permanent and drug resistance eventually happens for almost all patients. The major cause of such resistance in 30-60% of cases is EGFR c.2369C>T (*T790M*) mutation (5-7). The *T790M* mutation site is in a GC-rich region of exon 20 (7). A structural change in the ATP binding pocket of the EGFR protein is created after *T790M* mutation (8). In presence of such mutations, third-generation TKIs, such as osimertinib (AZD9291), rociletinib, HM61713/BI1482694, ASP8273, and EGF816, which target EGFR *T790M*, has been used (7,9,10). Other possible gene alterations that are associated with resistance to TKIs are co-alterations in MET or mitogen-activated protein kinase (MAPK), phosphoinositol 3-kinase (PI3K), Wnt/β-catenin signaling pathways and in cell cycle (11).

Genetic alterations in tumor tissue after drug resistance is very challengeable in the availability of re-biopsy tissues in poor disease control patients, invasive nature of re-biopsy and high tumor vascularity. In these situations, liquid biopsy such as cell-free plasma DNA (cfDNA) is appropriate alternative test following TKI treatment failure (12,13). In addition, cfDNA has been used for disease progression and treatment efficacy monitoring, as well (7).

Lung cancer is the second main cause of death in Iran (1,14). Lung cancer incidence is relatively lower than other countries in Asia (15) or other parts of world (16). Exact data on the incidence of *T790M* or other recurrence mutations in Iranian patients are very scarce.

In the present study, we detected the *T790M* or other recurrence mutations using cfDNA from Iranian patients who had developed resistance to TKIs. Also, we investigated the relationship between the recurrence mutations status in cfDNA and clinical characteristics and prognosis. Additionally, few previous studies have assessed whether there was a discrepancy in EGFR TKI resistance mechanisms and primary *EGFR* exon mutations which was also investigated in our study.

MATERIALS AND METHODS

The present study was a retrospective, observational and single institute study. All experiments were performed in accordance with relevant guidelines and regulations. patients were considered eligible and enrolled in the present study if they met the following criteria: 1) the presence of histologically confirmed Adenocarcinoma, at Eastern Cooperative Oncology Group (ECOG) (17) performance status (PS) 0-2 , stageIV (by AJCC, 8th edition) (18), 2) presence of primary *EGFR* mutations at exons18-21 and TKIs treatment as first line therapy followed by progression during TKI treatment, 3) absence of de novo T790M mutation and finally, presence of clinically resistance to TKIs according to Jackman's criteria (19). We collected data from clinical records as follows: age at diagnosis, sex, smoking status, histology, disease stage, *EGFR* mutation status at initial diagnosis.

Sample collection

151 patients diagnosed with Lung Adenocarcinoma and treated with Erlotinib, who had demonstrated radiological disease progression after TKIs treatment failure were tested for T790M or other new recurrence *EGFR* mutations and 29 patients had recurrence mutations. Blood samples were collected within 14 days after the development of TKI acquired resistance. 10 mL of peripheral blood was collected into a cfDNA protection vacuum tube (AmoyDx, Xiamen, Fujian, China) containing a cell-free DNA protection reagent that promotes DNA stability for 7 days at 4–25 °C. Blood samples were transported to Genome-Nilou laboratory within 24 h. For DNA extraction, the blood samples were centrifuged at 2500×*g* for 10 min at 4 °C. The supernatant was transferred to a new tube and centrifuged at 15,800×*g* for 15 min at 4 °C. The supernatant (plasma) was stored at −80 °C. cfDNA from 1.5 mL plasma was extracted with a QIAamp Circulating Nucleic Acid kit according to the manufacturer's instructions (Qiagen, Hilden, Germany). *T790M* or other recurrence mutational status was determined by polymerase chain reaction (PCR) followed by sequencing

Survival analysis

PFS was defined as from date of registration in study to date of progression or death. Patients who were still alive at the time of analysis were censored. The mean ± standard deviation (SD) was calculated for continuous variables. All confidence intervals (CIs) for parameters to be estimated were constructed with a significance level of alpha=0.05 (a 95% confidence level). Never smoker was defined as person who has smoked less than 100 cigarettes in his/her lifetime (20). The analysis was "intention to treat" and included all enrolled patients regardless of subsequent withdrawal from treatment or deviation from the protocol. All the statistical tests were two-sided and P -value of less than 0.05 was considered statistically significant. IBM SPSS statistical software version 19 for Windows (IBM, Armond, NY, USA) was used for data analysis.The χ^2 test was used to analyze the relationships between the new recurrence *EGFR* mutation status in plasma extracted and several patient characteristics. The age at diagnosis was compared using the Student's **t**-test. Kaplan-Meier curves were used to analyze PFS and the log-rank test was used for comparisons. IBM SPSS statistical software version 19 for Windows (IBM, Armond, NY, USA) was used for data analysis.

RESULTS

A total of 151 Adenocarcinoma patients who were treated with first generation of TIKs (Erlotinib) were assigned to test T790M or other recurrence mutations at time of disease progression. Among them, in 29 (19.2%) patients, recurrence mutations were documented (Figure 1).

The mean age of study population in this study was 58.9±1.1 (median=60, range 38-79 years). A total of 15 patients (51.7%) were female, and rest of them (n=14, 48.3%) were male. Most of patients (n=20, 69%) were never smoker (Table 1). Baseline patient and disease characteristics of patients for study are shown in Table 1.

Table 1. Baseline Patient and Disease Characteristics of Patients for Study

Age(Mean± SD [a] , Range)	(58.9±1.1, range:38-79)
Sex	Male: n=14(48.3%) Female: n=15(51.7%)
Performance status [b]	1: n=10(34.5%) 2: n=19(65.5%)
Cigarette Smoking	Yes: n=9(31%) No: n=20(69%)
Primary mutated Exon	Exon 18: n=2(6.9%) Exon 19: n= 13(44.8%) Exon 21: n=14(48.3%)

A: standard deviation; b: Performance status was considered as Eastern Cooperative Oncology Group.

Recurrence mutations

The majority of cases had T790M mutatios, the Figure 2 shows the proportion of disease recurrence mutations.

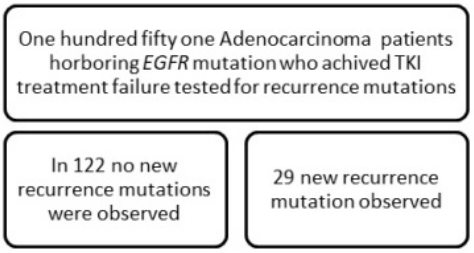

Figure 1. Study diagram

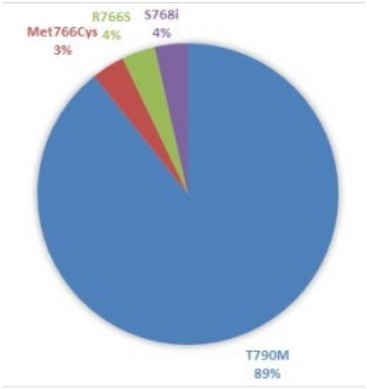

Figure 2. Proportion of disease recurrence mutations

Survival outcome

The mean PFS (first progression from initial diagnosis to TKI treatment failure) was 7.4± 4.6 months (median=7 months). Althogh the mean of PFS in paitents with *T790M* mutation at recurrence time was longer than cases harboring other recurrence mutations but statistically was not significant (7.8±0.91 vs. 5± 2.4 months, respectively, P-value=0.257)

During the follow-up, among 29 patients with new recurrence *EGFR* mutation , 12 (41.4%) of them expired.

The mean OS was 10.98± 1.6 months, overall. There was meaningful difference in mean OS times in exon 19 and *L858R* primary *EGFR* mutation subgroups (15.3±2.4 vs.7.5±1.4 months, P-value=0.014).

The mean OS was a little longer in patients harboring *T790M* mutation than cases with other mutations but no statistically significant difference was observed (10.8±1.8 vs. 9.9 months respectively, P-value=0.749).

The incidence of *T790M* mutations in the cfDNA tended to be higher in men, younger cases (<59 years), in patients with *L858R* and never smokers although they were not significantly different (P-values= 041, 0.316, 0.316 and 0.158, respectively). Mutation at time of disease recurrence and patients' list is shown in Table 2.

The relation between demographic characteristics (including: age, gender, primary mutant exon, recurrence mutation and smoking status) and OS was tested by uni and multivariate analysis (Table 3).

Table2. Patients' list related to T790M mutate status

	Sex	EGFR status at diagnosis	Recurrence mutation	Current status
1	M	Exon21 (L858R)	T790M	Alive
2	M	Exon19 deletion	T790M	Dead
3	F	Exon19 deletion	T790M	Alive
4	F	Exon21 (L858R)	T790M	Alive
5	F	L858R	T790M	Alive
6	F	Exon19 deletion	T790M	Alive
7	F	Exon19 deletion	T790M	Alive
8	M	L858R	T790M	Alive
9	M	Exon18 mutation	T790M	Alive
10	M	Exon19 deletion	T790M	Alive
11	M	Exon21 (L858R)	Exon 20 insertion(Met766-Cys775)	Alive
12	F	Exon21 (L858R)	T790M	Dead
13	M	Exon21 (L858R)	T790M	Alive
14	F	Exon19 deletion	S768I	Alive
15	M	Exon21 (L858R)	T790M	Dead
16	M	Exon18 mutation	T790M	Alive
17	M	Exon19 deletion	T790M	Alive
18	F	Exon21 (L858R)	T790M	Dead
19	M	Exon21 (L858R)	T790M	Alive
20	F	Exon19 deletion	T790M	Alive
21	F	Exon19 deletion	T790M	Alive
22	F	Exon19 deletion	R766C	Dead
23	F	Exon19 deletion	T790M	Alive
24	F	Exon19 deletion	D761Y	Dead
25	F	Exon21 (L858R)	T790M	Dead
26	M	Exon21 (L858R)	T790M	Dead
27	F	Exon21 (L858R)	T790M	Dead
28	M	Exon19 deletion	T790M	Dead
29	M	Exon21 (L858R)	T790M	Dead

Table 3. Association between demographic characteristics and OS

		Univariate analysis			P-value	Multivariate analysis		P-value
		N (%)	OR [a]	CI [b](95%)		OR	CI (95%)	
Gender	*Male*	8(61.5)	1	0.207-2.387	0.573	1	0.187-8.370	0.817
	Female	5(38.5)	0.714			1.252		
Age (years)	*>59*	5(38.5)	1	0.136-1.964	0.333	1	1.159-2.139	0.050*
	<59.	8(61.5)	0.517			0.159		
Primary exon mutation	*19*	6(46.1)	1	0.033-0.820	0.028*	1	0.004-0.703	0.026*
	21	7(61.7)	0.165			0.055		
Smoking status	*Non-smoker*	10 (77)	1	0.575-9.435	0.237	1	0.340-79.321	0.237
	Smoker	3(23)	2.228			5.189		
Recurrence mutation	T790M	11(84.6)	1	0172-11.472	0.450	1	0.025-17.451	0.803
	Other mutations	2(15.4)	1.406			0.660		

A: Odds ratio, b: CI: confidence interval.

DISCUSSION

As we know, the exact incidence of recurrence EGFR mutations in Iranian population is not known. This study is an attempt to find the frequency of most common resistance mutation (*T790M*) in Iranian patients. The rate of *T790M* mutation in present study was mostly near to other Asian countries. Also, we showed that the T790M mutation, which causes resistance to EGFR-TKIs, could be detected in cfDNA after TKI resistance. Interestingly, in our study 5 uncommon recurrence mutations were observed. Age at diagnosis and primary mutated EGFR exon were associated with OS.

Replacement of threonine instead of methionine at amino acid position 790 (*T790M*) is responsible for nearly half of new second-point recurrence mutation in *EGFR* gene (21). In addition to acquired *T790M* mutation, other mechanisms such as MET (met proto-oncogene) amplification(22), activation of insulin-like growth factor I receptor (IGF1R)(23) or transformation to small cell lung cancer (SCLC) have been reported. The frequency of the concomitant *T790M* mutation in TKI-resistant samples was reported 30-60% (5,6,7,24,25). Two Japanese studies reported the incidence of *T790M* mutations in similar clinical setting to a rate of 34.4%–38% (26,27). The proportion of *T790M* mutation in our study was near to Chen et al. (5) study. These frequency differences might be related to the ethnicity, difference in PCR steps, the method of mutation detection and the software which is used for analyzing the sequences, and also, the sequencer system. Also, Li et al. (28) hypothesized that selection pressure by EGFR TKIs may promote *KRAS* mutation and may cause the differences in the frequencies of *T790M* mutations across different studies.

Several previous studies (29-33) have described the *T790M* positivity rate between *Del19* and *L858R* mutations. In our study majority of *T790M* mutations were detected in patients with primary *L858R* mutations but as in other mentioned studies, this difference wasn't statistically significant.

Acquired mutations after TKI treatment failure other than *T790M*, were *T854A*, *D761Y* and *L747S*, are not common (25,34-36). Interestingly, in our study 5 uncommon recurrence mutations were observed.

Mean OS in our patients was shorter than other studies such as Zhang et al. (37). It may be due this fact that none of our patients with *T790M* mutation has been treated with third generation EGFR-TKIs. In this study, OS of the Del19 group tended to be longer than those of L858R group, which is compatible with previous studies. (38-40)

In our study as Ke et al. observed, patients harboring *T790M* mutation had longer OS before progression, comparing with patients with other recurrence mutations. It may indicate that resistance acquired through the *T790M* mutation might follow a more indolent course than clinical disease progression without the mutation (24).

Shorter OS in older patients in our series was in accordance to other studies (41, 42). In contrast, Wu et al. (43) showed poorer efficacy of EGFR-TKI treatment in younger patients (<50 years).

We do not have any information regarding alternative mechanisms of resistance to EGFR-TKI therapy, such as MET and EGFR amplification, small cell carcinoma transformation, and PIK3CA mutation and it was the most important limitations of the present study.

CONCLUSION

The cfDNA assay is a useful way for detecting T790M mutations in plasma, as an indicator for using third generation EGFR-TKIs. Also, T790M mutations frequency may differ by ethnicity, genetic factors and EGFR sensitizing mutations.

REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. ***CA Cancer J Clin*** 2020;70(1):7-30.
2. Ma C, Wei S, Song Y. T790M and acquired resistance of EGFR TKI: a literature review of clinical reports. ***J Thorac Dis*** 2011;3(1):10-8.

3. Babu Koyyala VP, Batra U, Jain P, Sharma M, Goyal P, Medisetty P, et al. Frequency of T790M mutations after progression on epidermal growth factor receptor tyrosine kinase inhibitor in metastatic non-small cell lung cancer in Indian patients: real-time data from tertiary cancer hospital. ***Lung India*** 2018;35(5):390-4.
4. Ohashi K, Maruvka YE, Michor F, Pao W. Epidermal growth factor receptor tyrosine kinase inhibitor-resistant disease. ***J Clin Oncol*** 2013;31(8):1070-80.
5. Chen YL, Lin CC, Yang SC, Chen WL, Chen JR, Hou YH, et al. Five Technologies for Detecting the *EGFR* T790M Mutation in the Circulating Cell-Free DNA of Patients With Non-small Cell Lung Cancer: A Comparison. ***Front Oncol*** 2019;9:631.
6. Kobayashi S, Boggon TJ, Dayaram T, Jänne PA, Kocher O, Meyerson M, et al. EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. ***N Engl J Med*** 2005;352(8):786-92.
7. Su KY, Tseng JS, Liao KM, Yang TY, Chen KC, Hsu KH, et al. Mutational monitoring of EGFR T790M in cfDNA for clinical outcome prediction in EGFR-mutant lung adenocarcinoma. ***PLoS One*** 2018;13(11):e0207001.
8. Nishikawa S, Kimura H, Koba H, Yoneda T, Watanabe S, Sakai T, et al. Selective gene amplification to detect the T790M mutation in plasma from patients with advanced non-small cell lung cancer (NSCLC) who have developed epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI) resistance. ***J Thorac Dis*** 2018;10(3):1431-9.
9. Sequist LV, Rolfe L, Allen AR. Rociletinib in EGFR-Mutated Non-Small-Cell Lung Cancer. ***N Engl J Med*** 2015;373(6):578-9.
10. Tan DS-W, Seto T, Leighl NB, Riely GJ, Sequist LV, Felip E, et al. First-in-human phase I study of EGF816, a third generation, mutant-selective EGFR tyrosine kinase inhibitor, in advanced non-small cell lung cancer (NSCLC) harboring T790M. ***ASCO Meet Abstr*** 2015;33(15_suppl):8013
11. Rath B, Plangger A, Hamilton G. Non-small cell lung cancer-small cell lung cancer transformation as mechanism of resistance to tyrosine kinase inhibitors in lung cancer. ***Cancer Drug Resist*** 2020;3(2):171-8.
12. Crowley E, Di Nicolantonio F, Loupakis F, Bardelli A. Liquid biopsy: monitoring cancer-genetics in the blood. ***Nat Rev Clin Oncol*** 2013;10(8):472-84.
13. Ho HL, Huang CC, Ku WH, Ho CL, Lin CH, Yu SL, et al. Liquid biopsy for detection of EGFR T790M mutation in nonsmall cell lung cancer: An experience of proficiency testing in Taiwan. ***J Chin Med Assoc*** 2019;82(6):473-6.
14. Saadat S, Yousefifard M, Asady H, Moghadas Jafari A, Fayaz M, Hosseini M. The Most Important Causes of Death in Iranian Population; a Retrospective Cohort Study. ***Emerg (Tehran)*** 2015;3(1):16-21.
15. Hassanipour S, Mokhtari A, Fathalipour M, Salehiniya H. The incidence of lung cancer in Iran: A systematic review and meta-analysis. ***World Cancer Res J*** 2017;4(4):e980.
16. Didkowska J, Wojciechowska U, Mańczuk M, Łobaszewski J. Lung cancer epidemiology: contemporary and future challenges worldwide. ***Ann Transl Med*** 2016;4(8):150.
17. Oken MM, Creech RH, Tormey DC, Horton J, Davis TE, McFadden ET, et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group. ***Am J Clin Oncol*** 1982;5(6):649-55.
18. Detterbeck FC, Boffa DJ, Kim AW, Tanoue LT. The Eighth Edition Lung Cancer Stage Classification. ***Chest*** 2017;151(1):193-203.
19. Jackman D, Pao W, Riely GJ, Engelman JA, Kris MG, Jänne PA, et al. Clinical definition of acquired resistance to epidermal growth factor receptor tyrosine kinase inhibitors in non-small-cell lung cancer. ***J Clin Oncol*** 2010;28(2):357-60.
20. DiFranza JR, Savageau JA, Rigotti NA, Fletcher K, Ockene JK, McNeill AD, et al. Development of symptoms of tobacco dependence in youths: 30 month follow up data from the DANDY study. ***Tob Control*** 2002;11(3):228-35.
21. Suda K, Onozato R, Yatabe Y, Mitsudomi T. EGFR T790M mutation: a double role in lung cancer cell survival? ***J Thorac Oncol*** 2009;4(1):1-4.
22. Guix M, Faber AC, Wang SE, Olivares MG, Song Y, Qu S, et al. Acquired resistance to EGFR tyrosine kinase inhibitors in cancer cells is mediated by loss of IGF-binding proteins. ***J Clin Invest*** 2008;118(7):2609-19.

23. Zhang X, Gureasko J, Shen K, Cole PA, Kuriyan J. An allosteric mechanism for activation of the kinase domain of epidermal growth factor receptor. ***Cell*** 2006;125(6):1137-49.
24. Ke EE, Zhou Q, Zhang QY, Su J, Chen ZH, Zhang XC, et al. A Higher Proportion of the EGFR T790M Mutation May Contribute to the Better Survival of Patients with Exon 19 Deletions Compared with Those with L858R. ***J Thorac Oncol*** 2017;12(9):1368-75.
25. 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(8):2240-7.
26. Hata A, Katakami N, Yoshioka H, Takeshita J, Tanaka K, Nanjo S, 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(24):4325-32.
27. Ko R, Kenmotsu H, Serizawa M, Koh Y, Wakuda K, Ono A, et al. Frequency of EGFR T790M mutation and multimutational profiles of rebiopsy samples from non-small cell lung cancer developing acquired resistance to EGFR tyrosine kinase inhibitors in Japanese patients. ***BMC Cancer*** 2016;16(1):864.
28. Li S, Li L, Zhu Y, Huang C, Qin Y, Liu H, et al. Coexistence of EGFR with KRAS, or BRAF, or PIK3CA somatic mutations in lung cancer: a comprehensive mutation profiling from 5125 Chinese cohorts. ***British Journal of Cancer*** 2014;110(11):2812-20.
29. Sun JM, Ahn MJ, Choi YL, Ahn JS, Park K. Clinical implications of T790M mutation in patients with acquired resistance to EGFR tyrosine kinase inhibitors. ***Lung Cancer*** 2013;82(2):294-8.
30. Kuiper JL, Heideman DA, Thunnissen E, Paul MA, van Wijk AW, Postmus PE, et al. Incidence of T790M mutation in (sequential) rebiopsies in EGFR-mutated NSCLC-patients. ***Lung Cancer*** 2014;85(1):19-24.
31. Hata A, Katakami N, Yoshioka H, Kaji R, Masago K, Fujita S, 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(11):1553-9.
32. Matsuo N, Azuma K, Sakai K, Hattori S, Kawahara A, Ishii H, et al. Association of EGFR Exon 19 Deletion and EGFR-TKI Treatment Duration with Frequency of T790M Mutation in EGFR-Mutant Lung Cancer Patients. ***Sci Rep*** 2016;6:36458.
33. Nosaki K, Satouchi M, Kurata T, Yoshida T, Okamoto I, Katakami N, et al. Re-biopsy status among non-small cell lung cancer patients in Japan: A retrospective study. ***Lung Cancer*** 2016;101:1-8.
34. Balak MN, Gong Y, Riely GJ, Somwar R, Li AR, Zakowski MF, et al. Novel D761Y and common secondary T790M mutations in epidermal growth factor receptor-mutant lung adenocarcinomas with acquired resistance to kinase inhibitors. ***Clin Cancer Res*** 2006;12(21):6494-501.
35. Bean J, Riely GJ, Balak M, Marks JL, Ladanyi M, Miller VA, et al. Acquired resistance to epidermal growth factor receptor kinase inhibitors associated with a novel T854A mutation in a patient with EGFR-mutant lung adenocarcinoma. ***Clin Cancer Res*** 2008;14(22):7519-25.
36. Costa DB, Halmos B, Kumar A, Schumer ST, Huberman MS, Boggon TJ, et al. BIM mediates EGFR tyrosine kinase inhibitor-induced apoptosis in lung cancers with oncogenic EGFR mutations. ***PLoS Med*** 2007;4(10):1669-79; discussion 1680.
37. Zhang S, Zhu L, Xia B, Chen E, Zhao Q, Zhang X, et al. Epidermal growth factor receptor (EGFR) T790M mutation identified in plasma indicates failure sites and predicts clinical prognosis in non-small cell lung cancer progression during first-generation tyrosine kinase inhibitor therapy: a prospective observational study. ***Cancer Commun (Lond)*** 2018;38(1):28.
38. Choi YW, Jeon SY, Jeong GS, Lee HW, Jeong SH, Kang SY, et al. EGFR Exon 19 Deletion is Associated With Favorable Overall Survival After First-line Gefitinib Therapy in Advanced Non-Small Cell Lung Cancer Patients. ***Am J Clin Oncol*** 2018;41(4):385-90.
39. Kim DW, Lee SH, Lee JS, Lee MA, Kang JH, Kim SY, et al. A multicenter phase II study to evaluate the efficacy and safety of gefitinib as first-line treatment for Korean patients with advanced pulmonary adenocarcinoma harboring EGFR mutations. ***Lung Cancer*** 2011;71(1):65-9.

40. Koyama N, Watanabe Y, Iwai Y, Kawamura R, Miwa C, Nagai Y, et al. Distinct Benefit of Overall Survival between Patients with Non-Small-Cell Lung Cancer Harboring EGFR Exon 19 Deletion and Exon 21 L858R Substitution. ***Chemotherapy*** 2017;62(3):151-8.
41. Yu X, Fan Y. Real-World Data on Prognostic Factors for Overall Survival in EGFR-Mutant Non-Small-Cell Lung Cancer Patients with Brain Metastases. ***J Cancer*** 2019;10(15):3486-93.
42. Gómez XE, Soto A, Gómez MA. Survival and prognostic factors in non-small cell lung cancer patients with mutation of the EGFR gene treated with tyrosine kinase inhibitors in a peruvian hospital. ***Am J Cancer Res*** 2019;9(5):1009-16.
43. Wu SG, Chang YL, Yu CJ, Yang PC, Shih JY. Lung adenocarcinoma patients of young age have lower *EGFR* mutation rate and poorer efficacy of EGFR tyrosine kinase inhibitors. ***ERJ Open Res*** 2017;3(3):00092-2016.