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ORIGINAL ARTICLE – OPEN ACCESS

Effect of Mutations Determined Via Liquid Biopsy on the Progress of the Disease in Advanced Lung Adenocancer

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ABSTRACT

Objective: This study aimed to examine the mutation panel studied with liquid biopsy in patients diagnosed with lung adenocancer and to investigate its relationship with survival.

Materials and Methods: The study comprised 24 patients diagnosed with lung adenocarcinoma between the ages of 18 and 80 who were metastatic and had not yet received treatment. Using the next-generation sequencing commercial kit 56G Oncology Panel, the cfDNAs (cell-free DNAs) isolated from the patient's blood were analyzed. SOPHiA DDM® (Saint-Sulpice, Switzerland) bioinformatics program was used to classify the detected genetic variants. The patients were followed for 3 years in terms of survival. For the statistical analysis, R Version 4.1.3 (https://rstudio.com/) and TURCOSA Analytical (https://turcosa.com.tr/) software was used.

Results: Mutation was found in liquid biopsy in 16 (66.7%) of the patients, and more than one mutation was detected in seven (29.1%) patients. The relationship between the variables and mortality was examined in the cases included in the study; there was a significant relationship between age and mortality (p=0.029), and mortality was increasing with aging. In the survival analysis, no statistically significant relationship was found between gender, smoking status, mutation status, and survival according to Kaplan–Meier graphs and log-rank tests.

Conclusion: Therefore, driver mutation was detected in 66.7% of the patients. Liquid biopsy may be essential in the progression of lung adenocarcinoma to detect the driver mutations, which are targets for treatments.

Keywords: EGFR, driver mutation, liquid biopsy, lung adenocarcinoma, non-small cell lung cancer, targeted therapy, somatic mutation

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INTRODUCTION

Lung cancer ranks first in cancer-related deaths worldwide for both sexes (1). Because of the high rates of mortality and morbidity, studies on the treatment and prevention of lung cancer are crucial. Recently, targeted therapies and immunotherapies have been emphasized due to the wide variety of chemotherapy drugs and their wide side effect profile. In this context, somatic genomic changes, known as "driver mutations," appear as the most useful biomarkers to predict the effectiveness of targeted therapy in advanced non-small cell lung cancer (NSCLC) (2). These mutations occur in genes that encode proteins important for cell growth and survival in cancer cells (3).

Oncogene dependence makes driver mutations a good biomarker in selecting patients for targeted therapies. As in other malignancies, pairing a specifically targeted drug with a driver mutation defined for a single patient provides reduced toxicity and increased therapeutic efficacy in lung adenocarcinoma; therefore, driver mutations are increasingly becoming a standard part of diagnostic procedures (3).

In patients with advanced-stage NSCLC, the tumor should be evaluated in terms of the presence of driver mutation as much as possible (4). Methods for screening NSCLC patients for driver mutations and other abnormalities are constantly evolving, and no single standard method is available for this. The method used should be cost effective, fast, and clinically applicable (3).

Although molecular diagnostic tests have traditionally been studied from biopsies taken from solid tumor tissue, blood-based tests—called liquid biopsies—are gaining in popularity. Liquid biopsies are less invasive and more cost effective and may be a potential method in cases with insufficient tumor tissue sample in biopsy for tissue sequencing. At the same time, during cancer, liquid biopsies may allow for monitoring molecular status, treatment response, and predicting or detecting relapse after adjuvant chemotherapy (5–8). The basic principle of liquid biopsy is based on the frequent presence of cell-independent circulating tumor DNA (ctDNA) and/or circulating tumor cells (CTC) in the blood of patients with lung cancer. Clinical practices have focused on isolating and detecting ctDNA rather than CTC in the blood (9, 10).

This study aimed to examine the mutation panel studied with liquid biopsy in patients with lung adenocarcinoma who applied to Erciyes University Faculty of Medicine Mehmet Kemal Dedeman Oncology Hospital and to investigate its relationship to survival.

MATERIALS and METHODS

Patient Selection

Fifty adenocarcinoma cases who applied to Erciyes University Faculty of Medicine Mehmet Kemal Dedeman Oncology Hospital between March 2019 and September 2019 were evaluated. Of these, 24 patients diagnosed with lung adenocarcinoma between the ages of 18 and 80 years, whose Eastern Cooperative Oncology Group (ECOG) performance scores were between 0 and 2, whose kidney and liver function tests were within normal ranges, and who had not received chemotherapy yet were accepted to the study. Regardless of the study, EGFR mutation was studied from the tissue at the time of diagnosis, and this information was also recorded. After obtaining the necessary consents (informed patient consent form) and obtaining permissions (Erciyes University Clinical Research Ethics Committee Approval was received on November 21, 2018, numbered 2018/583), the study was initiated. The patients were followed for 3 years in terms of survival.

Sample Collection and Study

The blood taken by vacutainer in Erciyes University Medical Genetics Department was put into special 10 ml streck tubes. After blood collection, the tube was inverted 10 times to mix the blood with the chemical thoroughly. Streck tubes were stored at room temperature (25°C); cfDNA (cell-free DNA) was isolated no later than 48 h after blood was taken.

The DNAs obtained were examined in terms of point mutations at Erciyes University Genome and Stem Cell Center using the next-generation sequencing commercial kit 56G Oncology Panel. With 56G Oncology Panel, *EGFR (3,7,15,18-21), CDKN2A (2), DNMT3A (23), DDR2 (18), TP53 (1-10), ABL1 (4-7), FGFR2 (5,7,8,11), AKT1 (3.6), NOTCH1 (26,27,34), ALK (23.25), APC (14), FGFR3 (7,9,12,14,16), ATM (8,9,12,17,26,34-36,39,50, 5456,6163), BRAF (11,15), STK11 (1, 4,6,8), CDH1 (3,8,9), CSF1R (7,22), CTNNB1 (3), ERBB2 (8,19-21), ERBB4 (3,4,6,7,8,9,15,23), EZH2 (16), FBXW7 (5.8-11), FGFR1 (4,5,7), FLT3 (11,14,16,20), KRAS (2,3,4), FOXL2 (1), VHL (1-3), PTPN11 (3,13), GNA11 (4,5), GNAQ (4,5), GNAS (8,9), HNF1A (3,4) HRAS (2,3), IDH1 (4), IDH2 (4), JAK2 (14, 16), JAK3 (4,13,16), KDR (6,7,11,19,21,26,2 7.30), KIT (2,9-11,13-15,17,18), MAP2K1 (2,3,6,7,11), MET (2, 11, 14,16,19), MLH1 (12), MPL (10), MSH6 (5), SMAD4 (3,4-6, 8,9,10-12), NPM1 (11), NRAS (2-4), PDGFRA (12,14,15,18), PIK3CA (2,5,7,8,10,14,19,21), PTEN (1-9), RB1 (4,6,8,10,11,14,17,18,20-23), RET (10,11,13,15,16), SMARCB1 (2,4,5,9), SMO (3,5,6,9,11), SRC (14), TSC1 (15)* mutations have been studied.

Evaluation of Mutations

Variants detected using the SOPHiA DDM® (Saint-Sulpice, Switzerland) bioinformatics program according to the American College of Medicine Genetics (ACMG) and Genomics and Molecular Pathology Association 2015 criteria were classified as pathogenic (P), likely pathogenic (LP), benign (B), likely benign (LB), or variant of unknown significance.

Table 1. Patient characteristics (n=24)

Variables	n	%
Age, Mean±SD	64.46±6.17	
Gender		
Female	8	33.3
Male	16	66.7
History of smoking		
With smoking history	15	62.5
Without smoking history	9	37.5
ECOG performance score		
ECOG 0	7	29.2
ECOG 1	11	45.8
ECOG 2	6	25.0
Mutation in liquid biopsy		
Positive	16	66.7
Negative	8	33.3
Multiple mutations in liquid biopsy		
Positive	7	29.2
Negative	17	70.8
TP53 mutation in liquid biopsy		
Positive	9	37.5
Negative	15	62.5

SD: Standard deviation; ECOG: Eastern Cooperative Oncology Group; TP53: tumor protein p53

Statistical Analysis

Histograms, q–q plot graphs and the Shapiro–Wilk test were used to evaluate the normality of the data. An independent sample t-test was used for continuous data and Fisher Exact Test for categorical data while evaluating the differences between groups. The data were summarized using frequency and percentages, and mean and standard deviations for categorical and numerical variables, respectively. To calculate overall survival probabilities and to make intergroup comparisons, Kaplan–Meier curves were created, and log-rank tests were applied. Analyses were made using R Version 4.1.3 (https://rstudio.com/) and TURCOSA Analytical (https://turcosa.com.tr/) programs; p values below 0.05 were considered statistically significant.

Supporting the Study

Our study was supported by the Scientific Research Project Unit of Erciyes University, with project number TTU-2019-8871.

RESULTS

Table 1 shows the patient characteristics. A mutation was detected in the liquid biopsy in a total of 16 (66.7%) of 24 patients. More than one mutation was detected in seven (29.1%) patients. A TP53 mutation was detected in nine (37.5%) of the patients.

Tissue samples of two patients were positive for an EGFR mutation, and both were EGFR exon 19 deletions. EGFR exon 18, 20, and 21 mutations were not determined in the tissue. In liquid biop-

Table 2. Somatic mutations detected in tissue and liquid biopsy

Mutation	Exon	Mutation type	Mutation location	Pathogenicity*	Number of positive patients in tissue biopsy	Number of positive patients in liquid biopsy
EGFR	19	Delesyon	p.(Glu746_Ala750del)	P	2	3
	18	Missense	p.(Gln701His)	LP	Not detected	1
	20	Missense	p.(Leu792Ile)	LP	Not detected	1
MET	16	Missense	p.(Asn1131His)	LP	Not studied	1
CDKN2A	2	Missense	p.(Leu130Gln)	LP	Not studied	1
SMAD4	4	Nonsense	p.(Ser144*)	P	Not studied	1
KRAS	2	Missense	p.(Gly12Asp)	P	Not studied	1
DNMT3A	23	Missense	p.(Arg882His)	LP	Not studied	1
DDR2	18	Missense	p.(Val770Leu)	LP	Not studied	1
FOXL2	1	Missense	p.(Phe112Ile)	LP	Not studied	1
RET	11	Missense	p.(Gly691Ser)	LB	Not studied	1
STK11	6	Frameshift	p.(Asp258Serfs*20)	P	Not studied	1
TP53	8	Missense	p.(Asp281His)	LP	Not studied	1
	8	Missense	p.(Arg267Trp)	LP	Not studied	1
	8	Missense	p.(Arg273Leu)	P	Not studied	1
	8	Missense	p.(Arg273His)	P	Not studied	1
	6	Nonsense	p.(Arg213*)	P	Not studied	1
	7	Missense	p.(Ser241Tyr)	P	Not studied	1
	5	Missense	p.(Tyr163Cys)	P	Not studied	1
	5	Missense	p.(Ile162Phe)	LP	Not studied	1
	4	Silent	p.(Tyr125=)	P	Not studied	1
	6	Missense	p.(Ser215Gly)	P	Not studied	1
	10	Splicing accepter	p.(?)	P	Not studied	1

EGFR: Epidermal growth factor receptor; MET: Mesenchimal epidermal transcription factor; CDKN2A: Cyclin-dependent kinase 2A; SMAD4: SMAD family member 4; KRAS: Kirsten rat sarcoma virüs; DNMT3A: DNA methyltransferase 3 alpha; DDR2: Discoidin domain reseptor 2; FOXL2: Forkhead transcription factor gene 2; RET: Ret proto-oncogene; STK11: Serine/threonine kinase 11; TP53: Tumor protein p53; P: Pathogenic; LP: Likely pathogenic; LB: Likely benign; *: Pathogenicity was determined according to the criteria of the American College of Medicine Genetics (ACMG) and Genomics and Molecular Pathology Association 2015

sy, an EGFR mutation was determined in five cases in total. Three of these were exon 19 deletions in EGFR. Besides two EGFR exon 19 deletion-positive patients in the tissue, one more EGFR exon 19 deletion was detected in liquid biopsy. In a liquid biopsy, also an EGFR exzon 18 p. (Gln701His) missense mutation was found in one patient, and EGFR exon 20 p. (Leu792Ile) missense mutation was detected in one patient. In our study, no exon 21 mutations were detected in liquid biopsy (Table 2).

Except for EGFR, point mutations detected in liquid biopsy in our study were MET, CDKN2A (cyclin-dependent kinase 2A), SMAD4, KRAS, DNMT3A (DNA methyl transferase 3A), DDR2 (discoidin domain receptor 2), FOX2L (forkhead transcription factor gene), RET, STK11 (serine threonine kinase), and TP53 mutations. Of these mutations, 11 different TP53 mutations were detected in nine (37.5%) different patients in total. BRAF, PIK3CA, NTRK, PTEN, AKT1, and HER2 mutations are known to have clinical significance in NSCLC but were not detected in our study.

When the relationship between the variables and mortality was examined in the cases included in the study, there was a significant relationship between age and mortality (p=0.029) and mortality was increasing with aging. No statistically significant difference was found between gender, smoking, mutation status, and mortality (Table 3).

In survival analyzes, according to Kaplan–Meier graphics and log-rank tests, males and females (p=0.060), smokers and nonsmokers (p=0.966), patients with and without somatic mutations (p=0.928), patients with and without somatic TP53 mutations (p=0.835), patients with and without multiple somatic mutations (p=0.678), patients with and without somatic EGFR mutations (p=0.852), and patients with EGFR exon 19 deletion and those without (p=0.775) were not statistically different in survival (Table 4) (Fig. 1).

DISCUSSION

Some advanced-stage NSCLCs have been found to have oncogenic activation of tyrosine kinases, the most significant of which are EGFR mutations, ALK, and ROS1 gene rearrangements. This has enabled the development of specific molecular therapies for patients. Moreover, the identification of these patient subgroups has led to an ongoing effort to identify biomarkers and treatments for patients with other advanced NSCLCs (3).

Table 3. Mortality rates for variables

Variables	Mortality				Total (n=24)		p
	Alive (n=5)		Dead (n=19)				
	n	%	n	%	n	%	
Age, Mean±SD	59.20±7.46		65.84±5.16			64.46±6.17	**0.029**
Sex							0.130
Female	0	0.0	8	42.1	8	33.3	
Male	5	100.0	11	57.9	16	66.7	
Smoke							0.999
Yes	3	60.0	12	63.2	15	62.5	
No	2	40.0	7	36.8	9	37.5	
Somatic mutation							0.631
Yes	4	80.0	12	63.2	16	66.7	
No	1	20.0	7	36.8	8	33.3	
Somatic p53 mutation							0.999
Yes	2	40.0	7	36.8	9	37.5	
No	3	60.0	12	63.2	15	62.5	
One more than somatic mutation							0.608
Yes	2	40.0	5	26.3	7	29.2	
No	3	60.0	14	73.7	17	70.8	
Somatic EGFR mutation							0.999
Yes	1	20.0	4	21.1	5	20.8	
No	4	80.0	15	78.9	19	79.2	
EGFR Exon19 deletion							0.521
Yes	1	20.0	2	10.5	3	12.5	
No	4	80.0	17	89.5	21	87.5	

EGFR: epidermal growth factor receptor; SD: Standard deviation. Statistically significant p values are written in bold

In advanced NSCLC, EGFR tyrosine kinase inhibitors, such as erlotinib, gefitinib, afatinib, and osimertinib, can be used for cases that have an EGFR mutation (11, 12). In the study of Colombino et al. (13), EGFR was studied in the pathology preparation, and 12.6% of the cases were positive for an EGFR mutation. Mutations in EGFR were found more frequently in nonsmokers and women, and exon 21 and exon 19 mutations were found more frequently than other EGFR mutations. In our study, EGFR exon 19 deletions were detected in two patients (8.3%) in tissue and three patients (12.5%) in a liquid biopsy from the plasma of 24 lung adenocarcinoma patients. Furthermore, exon 18 mutations in one patient and exon 20 mutations in one patient were determined via liquid biopsy. In total, an EGFR mutation was determined in five (21%) patients via liquid biopsy. Exon 21 mutations (found in other studies) were not detected in our study.

There are studies that show that the T790M mutation in EGFR exon 20 is associated with tyrosine kinase inhibitor (TKI) resistance. In a study conducted, when the cases with TKI resistance or relapsing diseases were examined, it was observed that 50% of these cases had the T790M mutation (14). There are studies linking acquired resistance to afatinib with the T790M mutation, and it has been stated that osimertinib treatment may be an option in patients who develop resistance (15). Therefore, in case of resistance or relapse under TKI therapy, it may be considered to investigate T790M mutation in EGFR exon 20. In our study, T790M mutation in EGFR exon 20 was not detected, the p. (Leu792Ile) missense mutation was detected in EGFR exon 20. The detected mutation was reported as possibly pathogenic according to ACMG and GMPD 2015 criteria. Further studies are required to show the relationship between the exon 20 p. (Leu792Ile) missense mutation and adenocarcinoma.

Some studies show that EGFR exon 18 deletion mutations and the G719X and E709X in EGFR exon 18 are more sensitive to second-generation tyrosine kinase inhibitors (afatinib or neratinib) (16). In our study, the p. (Gln701His) missense mutation, which was reported as possible pathogenic according to ACMG and GMPD 2015 criteria, was detected in EGFR exon 18. EGFR could not be studied in pathology in this patient because the tissue was insufficient. Detection of exon 18 mutations might be beneficial in terms of treatment decision and patient survival, and liquid biopsy may have a great advantage in detecting mutations in cases where tissue cannot be obtained or is insufficient, as in this case.

Table 4. Kaplan–Meier survival analysis (log-rank test)

Variables	Total number	Death toll	Overall survival time (months)		p
			Mean (CI)	Median	
Sex					0.060
Female	8	8	10.11 (8.00–12.28)	8.83 (5.00–12.67)	
Male	16	11	18.04 (11.45–24.62)	15.03 (1.31–28.75)	
Smoke					0.966
Yes	15	12	15.23 (9.18–21.28)	12.37 (2.98–21.75)	
No	9	7	15.43 (8.34–22.52)	10.17 (6.27–14.06)	
Somatic mutation					0.928
Yes	16	12	15.43 (9.48–21.39)	8.83 (3.80–13.86)	
No	8	7	14.95 (7.96–21.95)	13.10 (9.54–16.66)	
Somatic p53 mutation					0.835
Yes	9	7	14.55 (7.17–21.94)	8.83 (5.23–12.44)	
No	15	12	15.80 (9.85–21.76)	13.10 (7.08–19.12)	
One more than somatic mutation					0.678
Yes	7	5	16.66 (7.76–25.55)	10.93 (5.54–16.32)	
No	17	14	14.79 (9.37–20.21)	12.37 (4.97–19.76)	
Somatic EGFR mutation					0.852
Yes	5	4	14.06 (4.03–24.09)	8.83 (5.76–11.91)	
No	19	15	15.71 (10.45–20.96)	12.37 (8.20–16.54)	
EGFR Exon19 deletion					0.775
Yes	3	2	16.89 (2.84–30.94)	8.83 (6.54–11.13)	
No	21	17	15.15 (10.21–20.08)	12.37 (7.98–16.75)	

EGFR: Epidermal growth factor receptor; CI: Confidence Interval

There was no statistically significant difference in survival between patients with mutations and patients without mutations in our study. The small sample size may be the reason for this. KRAS, DDR2, DNMT3A, CDKN2A, STK11, and SMAD4 mutations, which are known to have clinical and prognostic significance in NSCLC, were detected, but survival could not be evaluated based on each mutation because the sample was small. Some data in the literature show that survival is lower in patients with a TP53 mutation (17, 18). Although there was no statistically significant difference between mutation status and survival in our study, the literature data necessitate examining the presence of these mutations and working in larger series.

Liquid biopsy is being used with increasing frequency, and it is a method with high sensitivity and predictive value (19). In our study, EGFR mutation was detected in more localization and more patients in liquid biopsy compared to tissue biopsy. The reason for this might be that the tissue biopsy material is insufficient or the conditions of sample collection and storage are not appropriate. In a liquid biopsy, the fact that the procedure is noninvasive and reproducible and that a large number of mutations can be evaluated in the same sample appears to be an advantage.

The development of resistance under TKI in patients with EGFR exon 20 T790M mutation is an important problem that affects the treatment response, and liquid biopsy was used instead of re-biopsy in studies to look at EGFR T790M in patients with suspected resistance. These authors noted that this was a suitable and easily applicable method in patients under treatment (15). There are studies showing that liquid biopsy can also be used to detect acquired mutations in other nonpulmonary malignancies instead of tissue biopsy (20).

Apart from plasma, liquid biopsy can be studied with the method of obtaining supernatant from pleural effusion in lung cancer patients (21). It seems to be advantageous that liquid biopsy can be studied from pleural effusion and can be an alternative option for patients in whom obtaining a tissue sample is difficult or impossible.

The limitations of our study include the small sample size, the limitation to examine only point mutations due to the nature of the kit used, and the inability to examine translocation and gene rearrangements. The advantages of our study include a homogeneous patient group and the ability to examine multiple mutations.

CONCLUSION

A somatic mutation was detected in 66.7% of the patients involved in our study. Therefore, somatic mutations should be investigated to manage lung adenocarcinomas. For detecting somatic mutations, liquid biopsy is a noninvasive and easily applicable method and can be repeated before, during, and after treatment. The use of liquid biopsy, which has the benefit of making it possible to identify drug sensitivity and resistance, is expected to spread and become standard procedure in the upcoming years.

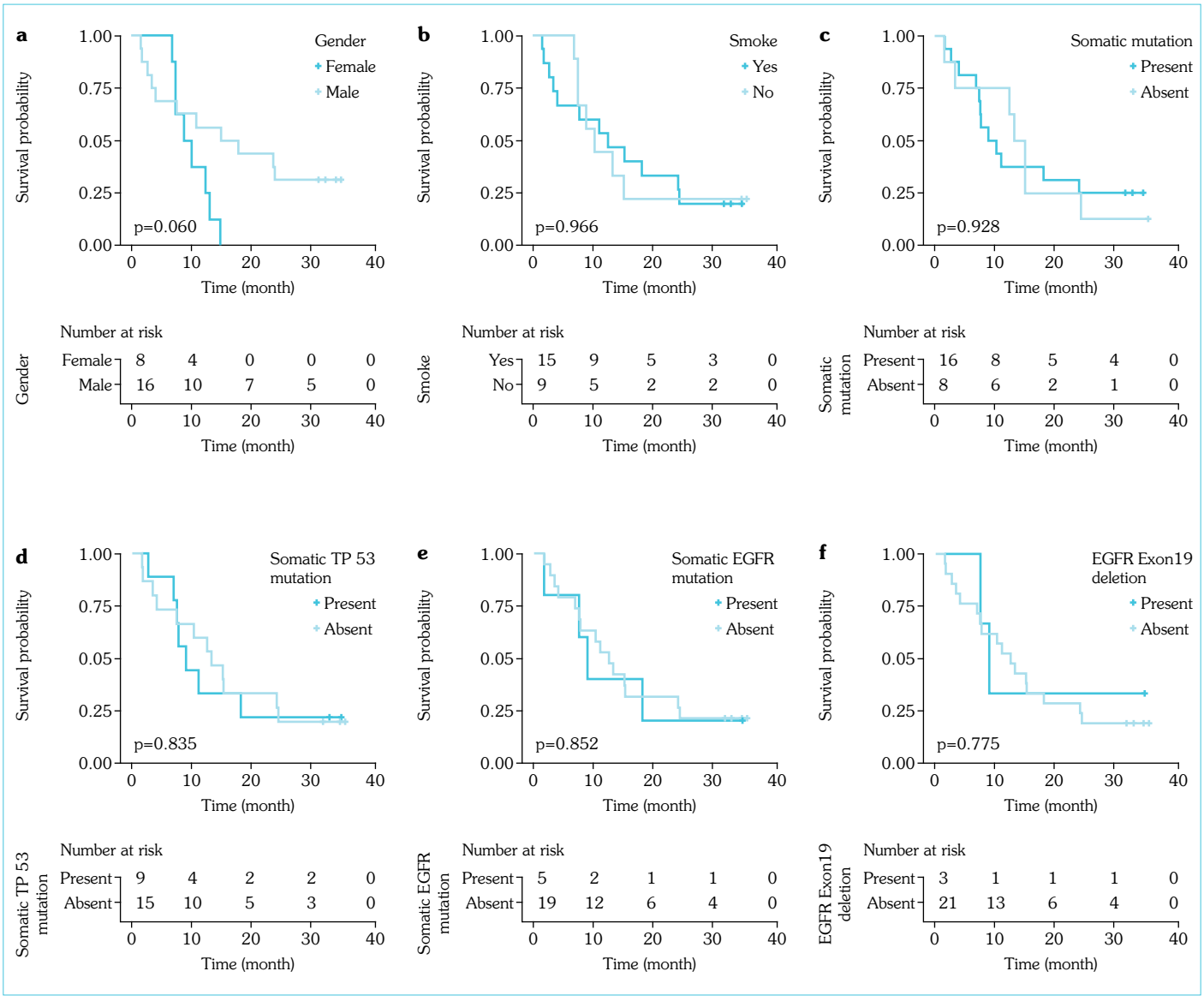

Figure 1. (a) Kaplan–Meier graph for sex. (b) Kaplan–Meier graph for smoking. (c) Kaplan–Meier graph for somatic mutation. (d) Kaplan–Meier graph for somatic p53. (e) Kaplan–Meier graph for somatic EGFR mutation. (f) Kaplan–Meier graph for EGFR Exon19 deletion

Ethics Committee Approval: The Erciyes University Clinical Research Ethics Committee granted approval for this study (date: 21.11.2018, number: 2018/583).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

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Author Contributions: Concept – ME, MIE, MÖ, AC; Design – ME, MIE, MÖ, AC; Supervision – ME, MIE, MÖ, AC; Resource – MÖ, ME; Materials – MÖ, ME; Data Collection and/or Processing – ME, MIE; Analysis and/or Interpretation – AC; Literature Search – ME, MIE, MÖ; Writing – ME; Critical Reviews – MÖ.

Conflict of Interest: The authors have no conflict of interest to declare.

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REFERENCES

1. Özsu S, Özlü T. Lung cancer epidemiology in Turkey. Current Chest Diseases Series 2013; 1(3): 7–11.
2. Dirican A, Dirican N, Ölmezoğlu A. Can liquid biopsy replace tissue biopsy in detecting target driver mutations in advanced non-small cell lung cancer? CBU-SBED 2016; 3(2): 165–8.
3. Uptodate. Personalized, genotype-directed therapy for advanced non-small cell lung cancer.Available from: URL: https://www.uptodate.com/contents/personalized-genotype-directed-therapy-for-advanced-non-small-cell-lung-cancer?search=https:%2F%2Fwww.%20uptodate.com%2Fcontents%2Fpersonalized-genotype-directed-therapy-for-advanced-non-small-cell%20lungcancer%3Fsearch%3Dpersonalized%2520genotype%2520directed%2520therapy%20%2520for%2520advanced%2520non%2520small%2520cell%2520lung%2520cancer%26source%3Dsearch_result%26selectedTitle%3D1~150%26usage_type%3Ddefault%26display_rank%3D1&source=search_result&se-

lectedTitle=1~150&usage_type=default&display_rank=1. Accessed Jun 1, 2019.
4. Lindeman NI, Cagle PT, Beasley MB, Chitale DA, Dacic S, Giaccone G, et al. Molecular testing guideline for selection of lung cancer patients for EGFR and ALK tyrosine kinase inhibitors: guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology. J Thorac Oncol 2013; 8(7): 823–59. [CrossRef]
5. Corcoran RB, Chabner BA. Application of cell-free DNA analysis to cancer treatment. N Engl J Med 2018; 379(18): 1754–65. [CrossRef]
6. Abbosh C, Birkbak NJ, Wilson GA, Jamal-Hanjani M, Constantin T, Salari R, et al. Phylogenetic ctDNA analysis depicts early-stage lung cancer evolution. Nature 2017; 545(7655): 446–51. [CrossRef]
7. Jamal-Hanjani M, Wilson GA, McGranahan N, Birkbak NJ, Watkins TBK, Veeriah S, et al. Tracking the evolution of non-small-cell lung cancer. N Engl J Med 2017; 376(22): 2109–21. [CrossRef]
8. Zugazagoitia J, Ramos I, Trigo JM, Palka M, Gomez-Rueda A, Jantus-Lewintre E, et al. Clinical utility of plasma-based digital next generation sequencing in patients with advance-stage lung adenocarcinomas with insufficient tumor samples for tissue genotyping. Ann Oncol 2019; 30(2): 290–6. [CrossRef]
9. Haber DA, Velculescu VE. Blood-based analyses of cancer: circulating tumor cells and circulating tumor DNA. Cancer Discov 2014; 4(6): 650–61. [CrossRef]
10. Oxnard GR, Paweletz CP, Sholl LM. Genomic analysis of plasma cell-free dna in patients with cancer. JAMA Oncol 2017; 3(6): 740–1.
11. Paliogiannis P, Attene F, Cossu A, Defraia E, Porcu G, Carta A, et al. Impact of tissue type and content of neoplastic cells of samples on the quality of epidermal growth factor receptor mutation analysis among patients with lung adenocarcinoma. Mol Med Rep 2015; 12(1): 187–91. [CrossRef]
12. Dong J, Li B, Lin D, Zhou Q, Huang D. Advances in targeted therapy and ımmunotherapy for non-small cell lung cancer based on accurate molecular typing. Front Pharmacol 2019; 10: 230. [CrossRef]
13. Colombino M, Paliogiannis P, Cossu A, Santeufemia DA, Sini MC, Casula M; Sardinian Lung Cancer (SLC) Study Group. EGFR, KRAS, BRAF, ALK, and cMET genetic alterations in 1440 Sardinian patients with lung adenocarcinoma. BMC Pulm Med 2019; 19(1): 209. [CrossRef]
14. Assi H, Tfayli A, Assaf N, Daya SA, Bidikian AH, Kawsarani D, et al. Prevalence of T790M mutation among TKI-therapy resistant Lebanese lung cancer patients based on liquid biopsy analysis: a first report from a major tertiary care center. Mol Biol Rep 2019; 46(4): 3671–6. [CrossRef]
15. Nakamura T, Nakashima C, Komiya K, Kitera K, Hirai M, Kimura S, et al. Mechanisms of acquired resistance to afatinib clarified with liquid biopsy. PLoS One 2018; 13(12): e0209384. [CrossRef]
16. Kobayashi Y, Togashi Y, Yatabe Y, Mizuuchi H, Jangchul P, Kondo C, et al. EGFR exon 18 mutations in lung cancer: Molecular predictors of augmented sensitivity to Afatinib or Neratinib as compared with First- or Third- Generation TKIs. Clin Cancer Res 2015; 21(23): 5305–13.
17. Bian C, Li Z, Xu Y, Wang J, Xu L, Shen H. Clinical outcome and expression of mutant P53, P16, and Smad4 in lung adenocarcinoma: a prospective study. World J Surg Oncol 2015; 13: 128. [CrossRef]
18. VanderLaan PA, Rangachari D, Mockus SM, Spotlow V, Reddi HV, Malcolm J, et al. Mutations in TP53, PIK3CA, PTEN and other genes in EGFR mutated lung cancers: Correlation with clinical outcomes. Lung Cancer 2017; 106: 17–21. [CrossRef]
19. Mok T, Wu YL, Lee JS, Yu CJ, Sriuranpong V, Sandoval-Tan J, et al. Detection and dynamic changes of EGFR mutations from circulating tumor DNA as a predictor of survival outcomes in NSCLC patients treated with first-line ıntercalated erlotinib and chemotherapy. Clin Cancer Res 2015; 21(14): 3196–203. [CrossRef]
20. Parikh AR, Leshchiner I, Elagina L, Goyal L, Levovitz C, Siravegna G, et al. Liquid versus tissue biopsy for detecting acquired resistance and tumor heterogeneity in gastrointestinal cancers. Nature Medicine 2019; 25: 1415–21. Erratum in: Nat Med 2019; 25(12): 1949. [CrossRef]
21. Guo Z, Xie Z, Shi H, Du W, Peng L, Han W, et al. Malignant pleural effusion supernatant is an alternative liquid biopsy specimen for comprehensive mutational profiling. Thorac Cancer 2019; 10(4): 823–31.