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Somatic Mutation Profiling by NGS-Based Liquid Biopsy in Advanced Non- Small Cell Lung Cancer: Frequency, Clinical Correlates, and Prognostic Significance

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Abstract

Background: Liquid biopsy-based next-generation sequencing (NGS) enables non-invasive, comprehensive mutational profiling in advanced non-small cell lung cancer (NSCLC). However, data on multi-gene cfDNA panels in Turkish populations remain limited.

Methods: One hundred consecutive stage IV NSCLC patients (42 newly diagnosed, 58 with progression/recurrence) who underwent cfDNA-based NGS analysis between January and November 2019 were retrospectively analyzed. Somatic mutations across 19 genes were assessed using the GeneRead QIAact Lung DNA UMI Panel (Qiagen) on the GeneReader platform.

Results: Pathogenic mutations were detected in 25 patients (25%), yielding 34 PMs across seven genes. EGFR was most frequently mutated (52.9%), followed by KRAS (20.5%) and PIK3CA (14.5%). Multimetastatic patients had significantly higher cfDNA levels (10.0 ± 3.9 vs. 3.8 ± 1.0 ng; $p = 0.01$) and shorter OS (17.2 ± 5.5 vs. 49.5 ± 5.7 months; $p < 0.001$). Among EGFR wild-type patients with progression, resistance mutation carriers had markedly shorter OS (16.5 ± 0.5 vs. 48.7 ± 6.6 months; $p = 0.001$). KRAS G12C was the predominant KRAS variant (71%).

Conclusions: NGS-based liquid biopsy effectively identifies actionable and resistance-associated mutations in advanced NSCLC. cfDNA concentration correlates with metastatic burden. The high prevalence of KRAS G12C and EGFR T790M underscores the clinical utility of multi-gene cfDNA panels for guiding personalized treatment.

1. INTRODUCTION

Lung cancer remains the leading cause of cancer-related mortality worldwide, with approximately 2.1 million new diagnoses and 1.8 million deaths reported annually [1]. More than half of patients are diagnosed between ages 55 and 74, and the overall 5-year survival rate remains approximately 19% [2, 3]. Cigarette smoking is the most prominent etiological factor, with approximately 85% of patients having a history of smoking [4].

Non-small cell lung cancer (NSCLC) accounts for 80–85% of all cases and has become a paradigm for precision oncology [5]. Numerous actionable driver mutations have been identified, including EGFR mutations (15–40%), KRAS mutations (15–25%), and alterations in PIK3CA, BRAF, NRAS, ALK, ROS1, and MET [5, 6, 7]. Targeted therapies—including EGFR tyrosine kinase inhibitors (TKIs), crizotinib for ALK/ROS1 rearrangements, and dabrafenib for BRAF V600E mutations—have substantially improved progression-free survival in molecularly defined subgroups [5]. However, acquired resistance inevitably emerges [8].

Tissue biopsy remains the diagnostic standard but is limited by procedural invasiveness, tumor inaccessibility, and inability to capture intratumoral heterogeneity [9, 10]. Liquid biopsy, based on circulating cell-free DNA (cfDNA) analysis, has emerged as a minimally invasive alternative enabling real-

time monitoring of somatic mutations and resistance [10]. Next-generation sequencing (NGS) platforms offer simultaneous multi-gene profiling from cfDNA with molecular barcoding for accurate low-frequency variant detection [11].

In this study, we aimed to determine the frequency and distribution of somatic mutations detected by NGS-based liquid biopsy across 19 cancer-related genes in stage IV NSCLC patients, and to evaluate their associations with metastatic burden, cfDNA levels, treatment resistance, and overall survival.

2. MATERIALS AND METHODS

2.1. Study Design and Patient Selection

This retrospective observational study included 100 consecutive stage IV NSCLC patients between January and November 2019 at the Department of Medical Genetics, Ondokuz Mayıs University, Samsun, Turkey. The cohort comprised 42 newly diagnosed and 58 progressive/recurrent patients from the Central and Eastern Black Sea regions. Patients were classified by metastatic burden: no distant metastasis (n = 18), oligometastatic (1–3 distant sites; n = 71), and multimetastatic (≥ 3 sites; n = 11) [12].

2.2. cfDNA Isolation and Quantification

Peripheral blood (30 mL) was collected into two PAXgene Blood ccfDNA tubes (PreAnalytiX GmbH). cfDNA was isolated using the QIAamp Circulating Nucleic Acid Kit with QIAvac 24 Plus (Qiagen) and quantified by Qubit dsDNA HS Assay on a Qubit 3.0 Fluorometer (Thermo Fisher Scientific).

2.3. NGS Library Preparation and Sequencing

Samples with cfDNA ≥ 2 ng/μL were processed using the GeneRead QIAact Lung DNA UMI Panel (Qiagen), covering hotspot regions of 19 genes (AKT1, ALK, BRAF, DDR2, EGFR, ERBB2, ESR1, FGFR1, KIT, KRAS, MAP2K1, MET, NRAS, NTRK1, PDGFRA, PIK3CA, PTEN, RICTOR, ROS1). Samples below threshold were processed with the GeneRead QIAact Actionable Insights Tumor Panel. Library preparation included UMI-tagged adapter ligation and target enrichment. Sequencing was performed on the GeneReader platform (Qiagen).

2.4. Bioinformatic Analysis

Quality parameters (Q-score ≥ 25, coverage ≥ 500×) were assessed using QCI Analyze. Variants with allele fraction ≥ 1% were retained [13, 14]. Pathogenicity was determined using QCI Analyze, COSMIC, ClinVar, ACMG/AMP guidelines, and in silico tools (MutationTaster, PROVEAN, VarSome, SIFT).

2.5. Statistical Analysis

Analyses were performed using SPSS 15.0. Kruskal–Wallis test with Bonferroni-corrected Mann–Whitney U ($p \leq 0.016$), Kaplan–Meier survival analysis with log-rank test ($p \leq 0.05$).

2.6. Ethics

he study protocol was approved by the Clinical Research Ethics Committee of Ondokuz Mayıs University (Approval No.: B.30.2.ODM.0.20.08/861; Date: 14 November 2019). The research was carried out in accordance with the guidelines of the Clinical Research Ethics Committee of Ondokuz Mayıs University and the principles of the Declaration of Helsinki (2013 revision). The present study is a retrospective analysis of de-identified clinical and molecular data obtained from 100 consecutive patients who had been referred to our center for clinical liquid biopsy (cfDNA next-generation sequencing) testing as part of their routine oncologic workup; clinical information was retrieved from the Nukleus Hospital Information System and from the archive of the Department of Medical Genetics of Ondokuz Mayıs University. In view of this retrospective design and the exclusive use of de-identified data, the ethics committee formally waived the requirement for additional written informed consent for research use of the data.

3. RESULTS

3.1. Patient Characteristics

The study included 100 stage IV NSCLC patients. Demographics and clinical characteristics are summarized in Table 1.

Table 1
Baseline Demographics and Clinical Characteristics
(N = 100)

Characteristic	n (%)
Sex	
Male	66 (66.0)
Female	34 (34.0)
Age, years	
Mean ± SD	63.7 ± 9.7
Median (range)	65 (37–86)
Histopathology	
Adenocarcinoma	80 (80.0)
Squamous cell carcinoma	15 (15.0)
NSCLC-NOS	5 (5.0)
Stage	
IV	100 (100)
Clinical status	
Newly diagnosed	42 (42.0)
Progressive/recurrent	58 (58.0)
Smoking status (n = 66)	
Never-smoker	29 (43.9)
Smoker (mean 44 pack-years)	37 (56.1)

NSCLC-NOS, non-small cell lung cancer not otherwise specified.

3.2. Metastatic Burden, cfDNA Levels, and Overall Survival

At enrollment, 82 patients had distant metastases. The distribution of metastatic sites is shown in Table 2. cfDNA levels and OS data stratified by metastatic burden are presented in Table 3. Multimetastatic patients had significantly higher cfDNA levels (10.0 ± 3.9 vs. 3.8 ± 1.0 ng; $p = 0.01$) and shorter OS (17.2 ± 5.5 vs. 49.5 ± 5.7 months; $p < 0.001$).

Table 2
Distribution of Distant Metastatic Sites at Enrollment

Metastatic site	n	%
Bone	34	34.0
Contralateral lung	27	27.0
Adrenal glands	24	24.0
Brain	16	16.0
Pleura	15	15.0
Liver	11	11.0
Pericardium	1	1.0
No distant metastasis	18	18.0

Some patients had metastases at multiple sites simultaneously.

Table 3
cfDNA Concentration and Overall Survival Stratified by Metastatic Burden

Metastatic group	n	cfDNA (ng) mean ± SD	OS (months) mean ± SD	Status
No distant metastasis	18	3.8 ± 1.0	—	All alive
Oligometastatic (1–3 sites)	71	4.4 ± 0.4	49.5 ± 5.7	—
Multimetastatic (≥ 3 sites)	11	10.0 ± 3.9	17.2 ± 5.5	—
p-value		**p = 0.01**	**p < 0.001**	

 Multimetastatic vs. no distant metastasis (Mann–Whitney U test). Kruskal–Wallis test across all groups. OS, overall survival.

3.3. Somatic Mutation Landscape

Pathogenic mutations were detected in 25/100 patients (25%), yielding 34 PMs across seven genes (Table 4, Fig. 3). Mean VAF was 8.3% (range 1–71%). Of 34 PMs, 23 (68%) were SNVs, 5 (15%) deletions, 1 duplication, 2 insertions, and 3 (9%) indels. Among 25 mutation-positive patients, 17 (68%) carried a single PM and 8 (32%) carried multiple PMs.

Table 4
Distribution of 34 Pathogenic Mutations by Gene

Gene	Patients n (%)	Mutations n (%)	Most common variant(s)
EGFR	12 (12)	18 (52.9)	Exon 19 del (33%), T790M (22%), L858R (17%)
KRAS	7 (7)	7 (20.6)	G12C (71%), G13C (14%), G60D (14%)
PIK3CA	4 (4)	5 (14.7)	S553fs*7 (40%), E545A, E542K, P381fs*11
BRAF	1 (1)	1 (2.9)	G469A
KIT	1 (1)	1 (2.9)	V559F
NRAS	1 (1)	1 (2.9)	Q61H
RAF1	1 (1)	1 (2.9)	S257L
Total	25 (25)	34 (100)	

Percentages in the "Mutations" column refer to proportion of total 34 PMs. Gene names in italics per HUGO nomenclature.

3.4. EGFR Mutations

Twelve patients (12%) harbored 18 EGFR PMs across exons 19, 20, and 21 (Fig. 4). Exon 19 deletions were most frequent (33%), followed by T790M (22%) and L858R (17%). All four T790M-positive patients had progressed on erlotinib (mean 9 months) and were alive at analysis (mean OS 18 ± 6 months).

Table 5
Clinical Characteristics of Patients with EGFR T790M/T790A Resistance Mutations

Pt	Resistance mutation	Sensitizing mutation	Sex	Age	cfDNA (ng)	TKI duration (months)	TKI agent	Metastatic sites
22	T790M	L858R	M	65	8.0	6	Erlotinib	Lung, Bone
88	T790M	L858R	F	52	2.0	12	Erlotinib	Lung
95	T790M	Ex19 del	M	67	4.75	9	Erlotinib	Adrenal, Bone
98	T790M	L858R	F	63	2.5	9	Erlotinib	Liver, Bone
97	T790A	L858R	F	73	1.6	24	Erlotinib	None

All patients had adenocarcinoma histology. Mean TKI duration: 9 months (excluding patient 97 on TKI for 24 months). M, male; F, female.

3.5. KRAS and Other Gene Mutations

Seven patients (7%) harbored KRAS PMs—G12C predominated (5/7, 71%). PIK3CA mutations were found in 4 patients (5 PMs). Single PMs were detected in BRAF (G469A), KIT (V559F), NRAS (Q61H), and RAF1 (S257L).

3.6. Progressive/Recurrent Disease and Resistance

Among 58 progressive/recurrent patients, 13 (22%) had PMs on liquid biopsy. Ten (77%) harbored resistance mutations (Fig. 5). Among wtEGFR patients, resistance mutation carriers had significantly shorter OS (16.5 ± 0.5 vs. 48.7 ± 6.6 months; p = 0.001).

Table 6
Overall Survival in EGFR Wild-Type Progressive/Recurrent Patients by Resistance Mutation Status

Subgroup	**n**	**OS (months) mean ± SD**	**p-value**
Resistance mutation positive	4	16.5 ± 0.5	
EGFR A767_V769dup	1		
PIK3CA P381fs*11	1		
NRAS Q61H	1		
KRAS G12C	1		
Resistance mutation negative	25	48.7 ± 6.6	
			p = 0.001

Log-rank test. OS, overall survival. All patients were on conventional chemotherapy prior to liquid biopsy.

3.7. Smoking Status

Among 29 never-smokers, EGFR mutations predominated (92%). Among 37 smokers, PIK3CA (43%) and KRAS (43%) predominated (Fig. 6).

4. DISCUSSION

This study evaluated the clinical utility of NGS-based liquid biopsy using a 19-gene cfDNA in 100 consecutive stage IV NSCLC patients from Turkey's Central and Eastern Black Sea region. The overall pathogenic mutation (PM) detection rate was 25%, which is notably lower than rates reported in several comparable studies. Büyükşimşek et al. reported a 42% detection rate using a similar cfDNA-NGS approach in a Turkish cohort [15], while Ottestad et al. identified somatic mutations in 52% of advanced NSCLC patients in Norway [16]. Sabari et al. documented a 45.7% mutation detection rate US cohort [17],

and a recently Yang et al. reported detection rates exceeding 60% using a comprehensive 168-gene panel [18]. More recently, Doval et al. reported a 65% detection rate in the first Indian liquid biopsy experience [28]. These discrepancies likely reflect methodological differences: our stringent 1% minimum allele frequency (MAF) threshold, the narrower 19-gene panel scope, and the high proportion of patients (58%) on active systemic therapy at the time of sampling—a factor known to suppress ctDNA shedding through treatment-induced tumor cell depletion [29]. A 2025 systematic review by Jahani et al. confirmed that NGS provides the most comprehensive mutational profiling among cfDNA technologies but emphasized that detection sensitivity is highly dependent on panel breadth, sequencing depth, and bioinformatic filtering thresholds [19].

EGFR mutations were detected in 12% of our cohort, consistent with the reported 10–17% frequency in Turkish tissue-based studies. Calibasi-Kocal et al. reported an overall EGFR mutation frequency of 16.6% in 409 Turkish NSCLC patients [30], while a more recent single-center analysis by Aksakal et al. found EGFR mutations in 11.5% of 182 Turkish NSCLC cases [31]. These rates align with European NSCLC populations (7–20%) rather than Asian populations (40–60%), a pattern consistent with ethnicity-based variation extensively documented by Midha et al. in a global EGFR mutation map [20]. Our cfDNA-based detection rate of 12% is slightly lower than tissue-based Turkish studies, which is expected given the known sensitivity gap between plasma and tissue genotyping. A meta-analysis by Yu et al. demonstrated concordance rates of 60–80% between cfDNA and tissue EGFR testing, with sensitivity losses primarily attributable to low tumor shedding and subclonal heterogeneity [32]. Nevertheless, the 2024 NCCN guidelines (v5.2024) have elevated plasma cfDNA testing from a salvage option to an equivalent alternative for initial genotyping, reflecting accumulating evidence that plasma NGS identifies clinically actionable targets with sufficient reliability [22].

Detection of the EGFR T790M resistance mutation in four patients who had progressed on erlotinib (mean TKI duration 9 months) underscores the value of liquid biopsy for non-invasive resistance monitoring. T790M emerges as the dominant acquired resistance mechanism in approximately 50–60% of patients treated with first- or second-generation EGFR TKIs [8, 33]. All T790M-positive patients in our cohort were alive at the time of analysis (mean OS 18 ± 6 months), reflecting the availability of effective subsequent therapy with osimertinib, a third-generation EGFR TKI. The FLAURA trial demonstrated that first-line osimertinib achieves a median OS of 38.6 months versus 31.8 months with earlier-generation TKIs (HR 0.80; $p = 0.046$) [21], while the AURA3 trial established osimertinib as standard second-line therapy in T790M-positive NSCLC with a median PFS of 10.1 months versus 4.4 months for platinum–pemetrexed [34]. Moreover, the FLAURA2 trial recently demonstrated that the addition of chemotherapy to first-line osimertinib further extended median PFS by 8.8 months compared with osimertinib monotherapy (HR 0.62; $p < 0.0001$) [35]. These evolving data reinforce the clinical imperative of cfDNA-based T790M surveillance to identify patients eligible for osimertinib and to guide sequential treatment strategies.

KRAS mutations were detected in 7% of patients, with G12C constituting the predominant variant (5/7, 71%). This allelic distribution is consistent with large-scale genomic profiling studies reporting that G12C

accounts for approximately 40% of all KRAS mutations in lung adenocarcinoma, making it the most prevalent KRAS subtype in Western populations [36]. The predominance of G12C in our cohort aligns with the known association between this specific transversion mutation and tobacco carcinogen exposure, as the majority of our KRAS-mutant patients were current or former smokers. Notably, real-world prevalence data indicate significant geographic variation in KRAS G12C frequency, ranging from 8.9–19.5% of NSCLC cases in the United States and 9.3–18.4% in Europe to 1.4–4.3% in Asia [37]. Our finding of 5% KRAS G12C prevalence in a Turkish Black Sea cohort provides novel population-specific data that contributes to the growing global map of KRAS G12C distribution. With the recent regulatory approval of selective KRAS G12C inhibitors, cfDNA-based identification of this variant has become directly actionable, and all seven KRAS-mutant patients in our cohort — including the five harboring G12C — represent candidates who could benefit from targeted therapy or clinical trial enrollment [37]. These findings underscore the importance of extending liquid biopsy panels beyond EGFR to capture the full spectrum of therapeutically relevant alterations.

PIK3CA mutations were identified in four patients (5 PMs), making it the third most frequently mutated gene in our cohort. PIK3CA alterations were predominantly found in smokers (43% of mutations in the smoking subgroup), consistent with prior reports associating PIK3CA mutations with tobacco exposure and squamous histology in NSCLC [38]. The frameshift variant S553fs*7, detected in two patients, and the well-characterized hotspot mutations E545A and E542K represent genomic alterations with potential implications for treatment stratification, as PIK3CA mutations have been associated with resistance to EGFR-TKI therapy and may modulate sensitivity to immune checkpoint inhibitors [26]. Single pathogenic mutations in BRAF (G469A), KIT (V559F), NRAS (Q61H), and RAF1 (S257L) further illustrate the genomic heterogeneity captured by multi-gene cfDNA panels. Although individually rare, these variants collectively accounted for 11.8% (4/34) of all detected pathogenic mutations and may have clinical implications for treatment selection, including eligibility for basket trial enrollment. Large-scale cfDNA profiling studies have similarly reported a long tail of low-frequency but potentially actionable mutations beyond the canonical EGFR and KRAS drivers, reinforcing the value of broad panel-based approaches over single-gene assays in advanced NSCLC [18]. Taken together, these findings demonstrate that liquid biopsy captures a clinically meaningful mutation spectrum that extends well beyond EGFR, enabling comprehensive molecular characterization from a single blood draw.

Total cfDNA concentration showed a significant positive correlation with metastatic burden in our cohort: multimetastatic patients (≥ 3 sites) had substantially higher cfDNA levels (10.0 ± 3.9 ng) compared with oligometastatic (4.4 ± 0.4 ng) and non-metastatic (3.8 ± 1.0 ng) groups ($p = 0.01$). This finding is consistent with the landmark observations of Newman et al., who demonstrated that ctDNA levels correlated with tumor volume in NSCLC using CAPP-Seq technology [23], and with Zhu et al., who reported that elevated cfDNA independently predicted shorter OS in advanced NSCLC [24]. More recently, a 2021 study by Li et al. confirmed that baseline cfDNA positively correlates with tumor burden and that cfDNA kinetics (the ratio of post-treatment to baseline cfDNA) can predict treatment response with high accuracy [39]. A 2025 prospective real-world study further validated ctDNA tumor fraction ≥ 1% as a robust prognostic biomarker: patients above this threshold had a median OS of 17.6 months compared

with not reached for those below [40]. Taken together, these data support the dual utility of cfDNA as both a qualitative tool for mutation detection and a quantitative biomarker for disease burden stratification and prognostic assessment.

Among 58 progressive/recurrent patients, 13 (22%) harbored pathogenic mutations on liquid biopsy, and resistance mutation carriers among wtEGFR patients had markedly shorter OS (16.5 ± 0.5 vs. 48.7 ± 6.6 months; p = 0.001). This prognostic dichotomy is consistent with prior studies demonstrating the adverse impact of acquired resistance mutations on survival. Zhao et al. reported that concurrent KRAS and PIK3CA mutations confer significantly worse OS in advanced NSCLC [25], while Ludovini et al. showed that multiple co-occurring oncogenic drivers in cfDNA are independently associated with poor clinical outcomes [26]. The detection of resistance-associated mutations (EGFR A767_V769dup, PIK3CA P381fs*11, NRAS Q61H, and KRAS G12C) in four progressive wtEGFR patients—all of whom had substantially shorter survival—highlights the clinical importance of liquid biopsy in identifying resistance mechanisms that would be missed without molecular profiling. The 2025 LIBRA study extended this concept further by demonstrating that ctDNA-based molecular progression precedes radiographic progression by a median of 4.9 weeks, suggesting a potential window for preemptive treatment modification [27]. Longitudinal ctDNA monitoring studies, including a 2023 machine-learning-based model from the IMpower150 trial, have confirmed that ctDNA dynamics robustly predict survival independently of radiographic response, supporting the integration of serial cfDNA assessment into routine clinical practice [41].

The mutation landscape in our cohort was strongly influenced by smoking status: EGFR mutations predominated in never-smokers (92% of mutations in 29 never-smokers), while PIK3CA (43%) and KRAS (43%) mutations predominated among 37 current/former smokers. This dichotomy replicates the well-established molecular epidemiology of NSCLC, where EGFR mutations are enriched in never-smokers, females, and adenocarcinoma histology, while KRAS mutations are strongly associated with tobacco carcinogenesis [6,20,47]. The concentration of EGFR mutations in never-smokers within our Black Sea cohort is clinically important because it suggests that patients in this demographic subgroup should be prioritized for reflex liquid biopsy testing at diagnosis, even when tissue availability is limited. Furthermore, the mutual exclusivity of EGFR and KRAS mutations observed in our cohort is consistent with the established paradigm of distinct oncogenic driver pathways in NSCLC [43].

Our study has several notable strengths. First, it represents one of the few liquid biopsy studies from Turkey’s Black Sea region, providing population-specific mutation frequency data for an underrepresented geographic area. Second, the consecutive enrollment of 100 patients with comprehensive clinical annotation—including metastatic burden stratification, smoking status, and long-term survival follow-up—enhances the clinical relevance of our findings. Third, the use of a UMI (unique molecular identifier)-based NGS panel with molecular barcoding minimizes sequencing artifacts and provides reliable low-frequency variant detection, addressing one of the key technical limitations of earlier cfDNA studies [11, 19]. Finally, our cohort included both newly diagnosed (42%) and

progressive/recurrent (58%) patients, enabling evaluation of liquid biopsy utility across different clinical scenarios.

Several limitations should be acknowledged. First, the absence of concurrent tissue genotyping precluded assessment of plasma–tissue concordance, which is recognized as a critical quality metric for liquid biopsy validation studies [9, 32]. Second, the modest cohort size (n = 100) limits the statistical power for subgroup analyses, particularly for rare mutations such as BRAF, KIT, and NRAS. Third, the 19-gene panel does not assess gene amplifications (MET, HER2), gene fusions (ALK, ROS1, RET, NTRK), or copy number alterations—all of which are therapeutically relevant in NSCLC and recommended for routine testing by current NCCN guidelines [22]. Fourth, the 1% MAF threshold, while reducing false positives, may have caused underdetection of subclonal mutations present at very low allele frequencies, particularly in patients receiving systemic therapy. Fifth, the potential confounding effect of clonal hematopoiesis of indeterminate potential (CHIP) was not systematically evaluated through matched white blood cell sequencing, which could lead to false-positive somatic mutation calls, especially for variants in genes such as PIK3CA [44]. Future studies incorporating larger cohorts, concurrent tissue and plasma genotyping, broader gene panels including fusion detection, serial longitudinal sampling, and CHIP filtering through paired leukocyte sequencing will be necessary to fully characterize the clinical utility of cfDNA-based precision oncology in this population.

In conclusion, NGS-based liquid biopsy using a 19-gene cfDNA panel effectively identified actionable somatic mutations in 25% of stage IV NSCLC patients from Turkey's Black Sea region, with EGFR (12%), KRAS (7%), and PIK3CA (4%) as the most frequently mutated genes. cfDNA concentration correlated with metastatic burden, and resistance mutation status independently predicted shorter survival in progressive patients. The high prevalence of KRAS G12C (71% of KRAS mutations) and EGFR T790M (22% of EGFR mutations) underscores the clinical utility of multi-gene cfDNA panels for identifying patients eligible for targeted therapies including osimertinib, sotorasib, and adagrasib. These findings support the integration of liquid biopsy into routine clinical practice for NSCLC patients in Turkey, complementing tissue-based molecular testing to enable timely, personalized therapeutic decision-making.

Declarations

Author Contribution

Ç.D. and Ü.A. contributed to study conceptualization, patient data collection, NGS data analysis, genetic data interpretation, clinical–genomic correlation, and drafting and finalizing the manuscript. B.Y., G.D., C.A., and M.S. contributed to oncological diagnosis and patient evaluation, collection of oncological data, comparison of oncological findings with genetic analysis results, and critical review of the manuscript draft. All authors read and approved the final manuscript.

Data Availability

The datasets generated and/or analyzed during the current study are not publicly available due to patient privacy and ethical restrictions but are available from the corresponding author on reasonable request.

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Figures

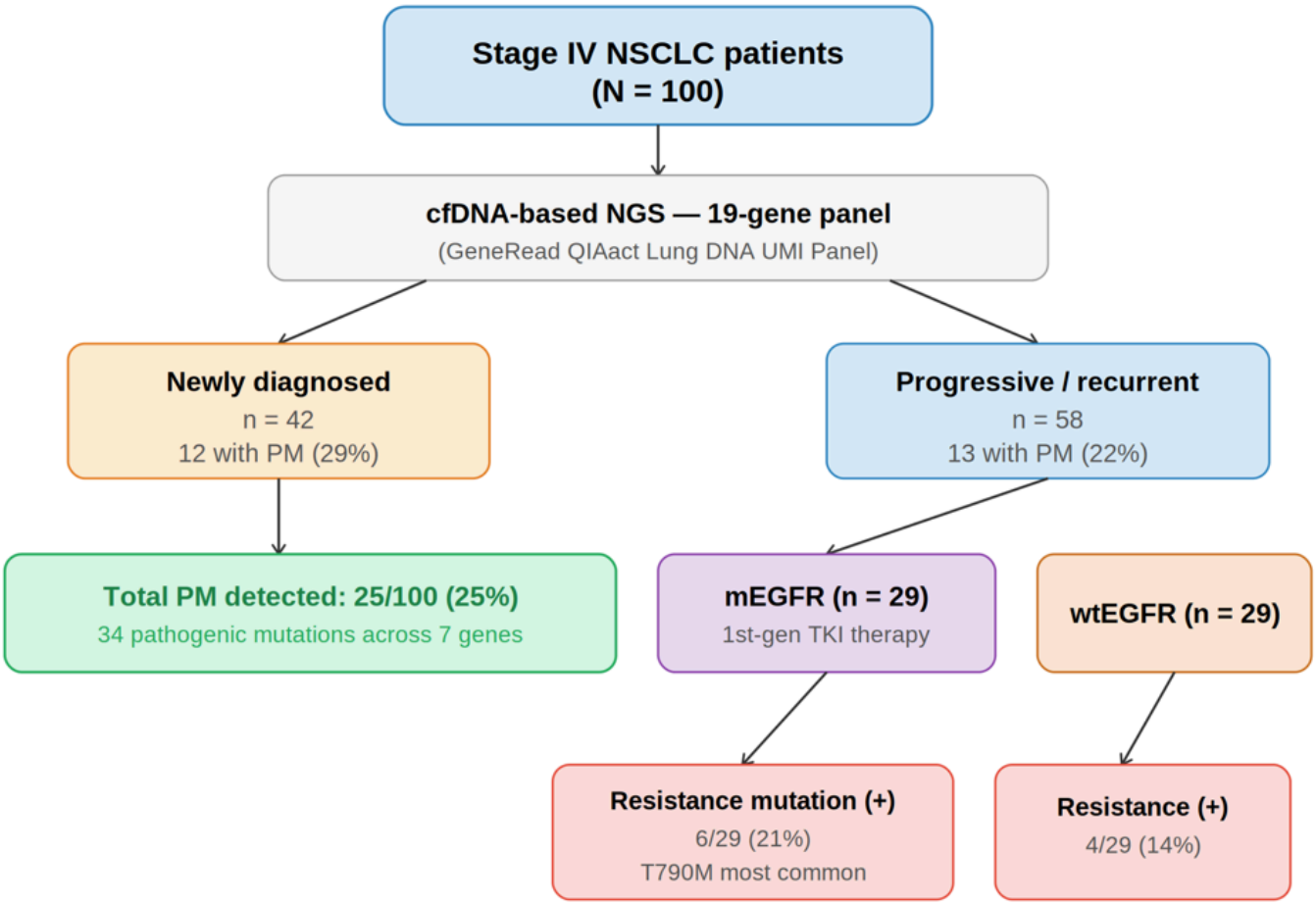


Figure 1. Study flowchart and patient classification schema.

Figure 1

Study flowchart and patient classification schema. One hundred stage IV NSCLC patients were classified as newly diagnosed (n = 42) or progressive/recurrent (n = 58). Progressive/recurrent patients were further stratified by prior EGFR mutation status (mEGFR vs. wtEGFR). Pathogenic mutations were detected in 25% of the total cohort. PM, pathogenic mutation; mEGFR, EGFR-mutant; wtEGFR, EGFR wild-type; TKI, tyrosine kinase inhibitor.

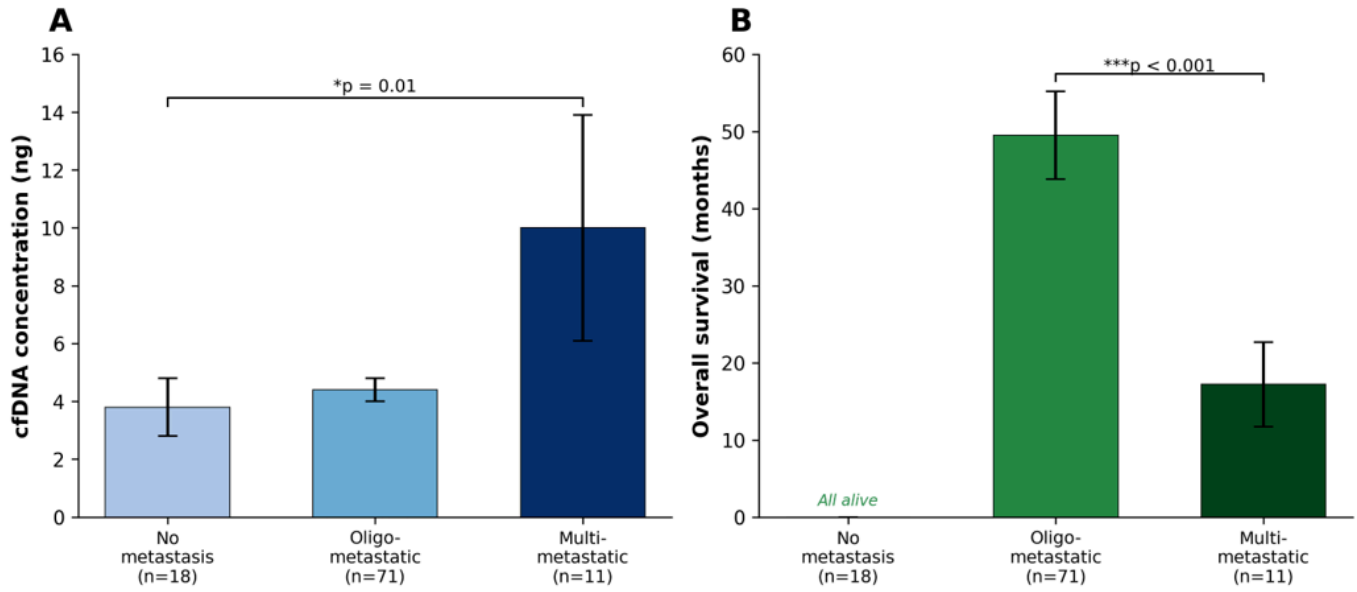


Figure 2

cfDNA concentration and overall survival stratified by metastatic burden. (A) Mean cfDNA concentration was significantly higher in multimetastatic patients compared with those without distant metastasis (10.0 ± 3.9 vs. 3.8 ± 1.0 ng; p = 0.01). (B) Mean overall survival was significantly shorter in multimetastatic patients compared with oligometastatic patients (17.2 ± 5.5 vs. 49.5 ± 5.7 months; p < 0.001). All patients without distant metastasis were alive at analysis. Error bars represent standard deviation.

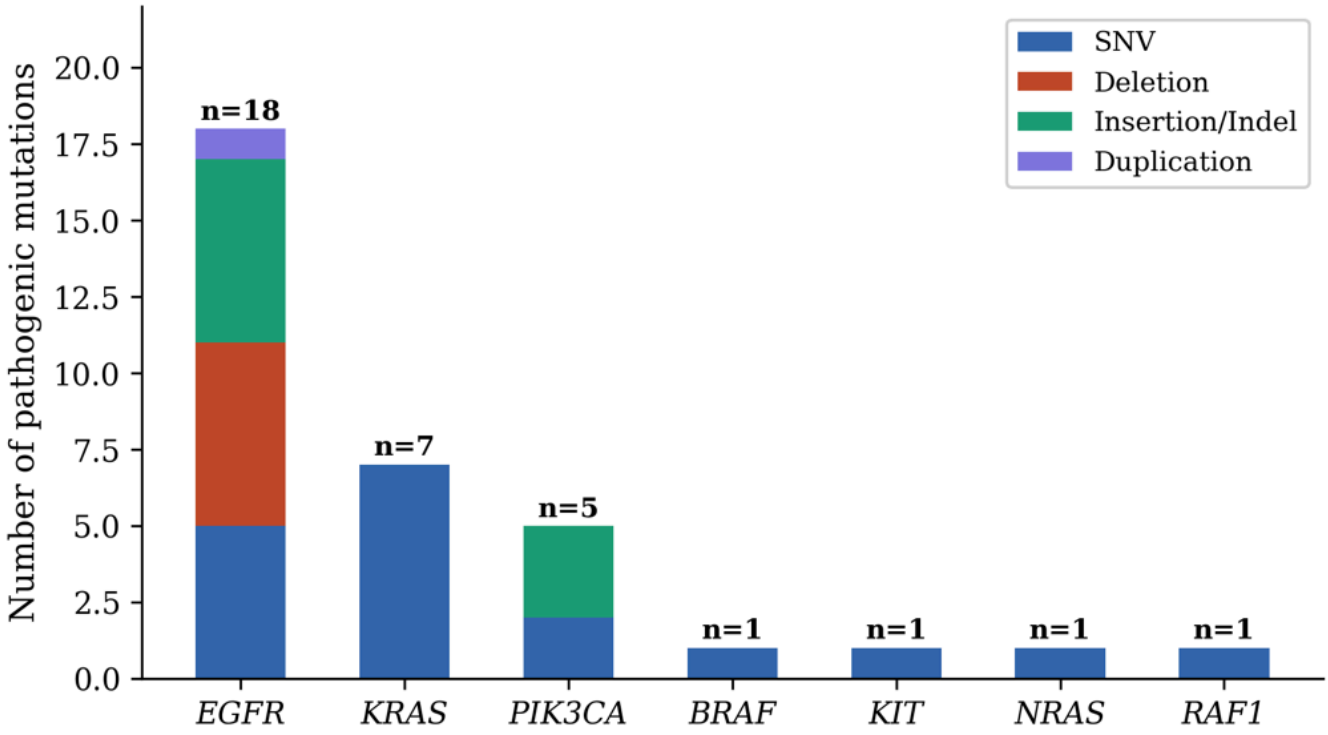


Figure 3

Distribution of 34 pathogenic mutations across seven genes, stratified by mutation type. EGFR was the most frequently mutated gene (n = 18 mutations, 52.9%), followed by KRAS (n = 7, 20.6%) and PIK3CA (n = 5, 14.7%). Mutation types included single nucleotide variants (SNV), deletions, insertions/indels, and duplications. SNV, single nucleotide variant.

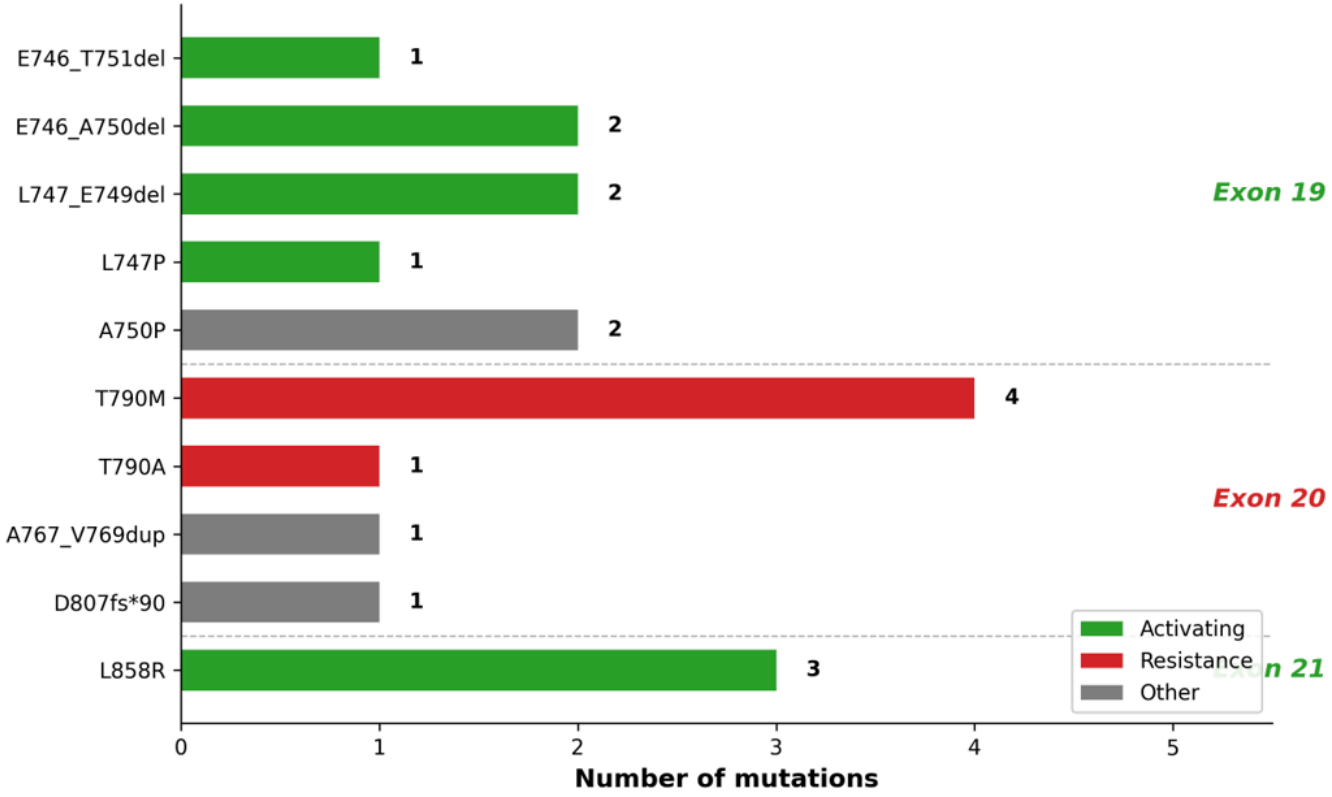


Figure 4

EGFR mutation spectrum (n = 18 mutations in 12 patients) by variant and exon location. Activating mutations (exon 19 deletions, L858R) are shown in green, resistance mutations (T790M, T790A) in red, and other variants in gray. Exon 19 deletions were the most common activating alteration (33%), followed by T790M (22%) as the predominant resistance mutation.

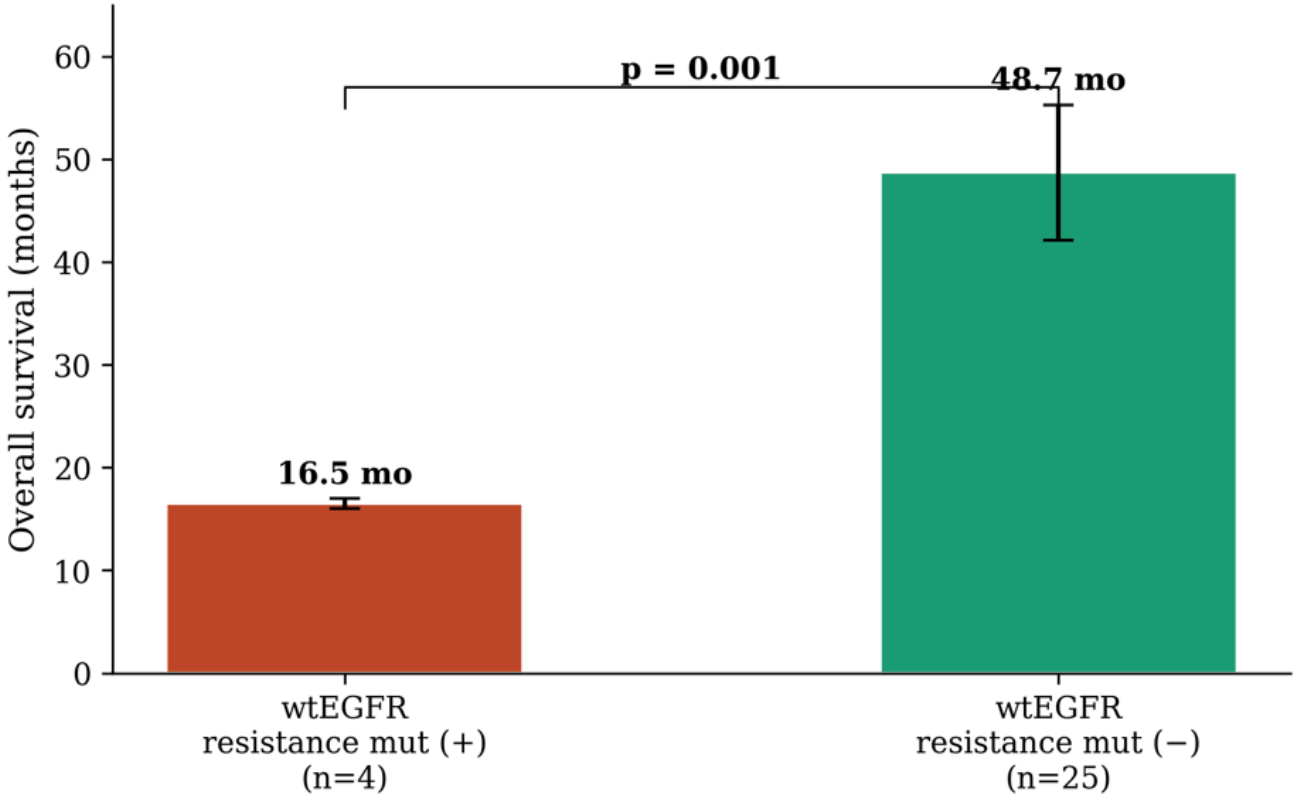


Figure 5

*Overall survival comparison in EGFR wild-type progressive/recurrent patients by resistance mutation status. Patients harboring resistance mutations (n = 4) had significantly shorter mean OS compared with mutation-negative patients (16.5 ± 0.5 vs. 48.7 ± 6.6 months; p = 0.001, log-rank test). Resistance mutations included EGFR A767_V769dup, PIK3CA P381fs*11, NRAS Q61H, and KRAS G12C.*

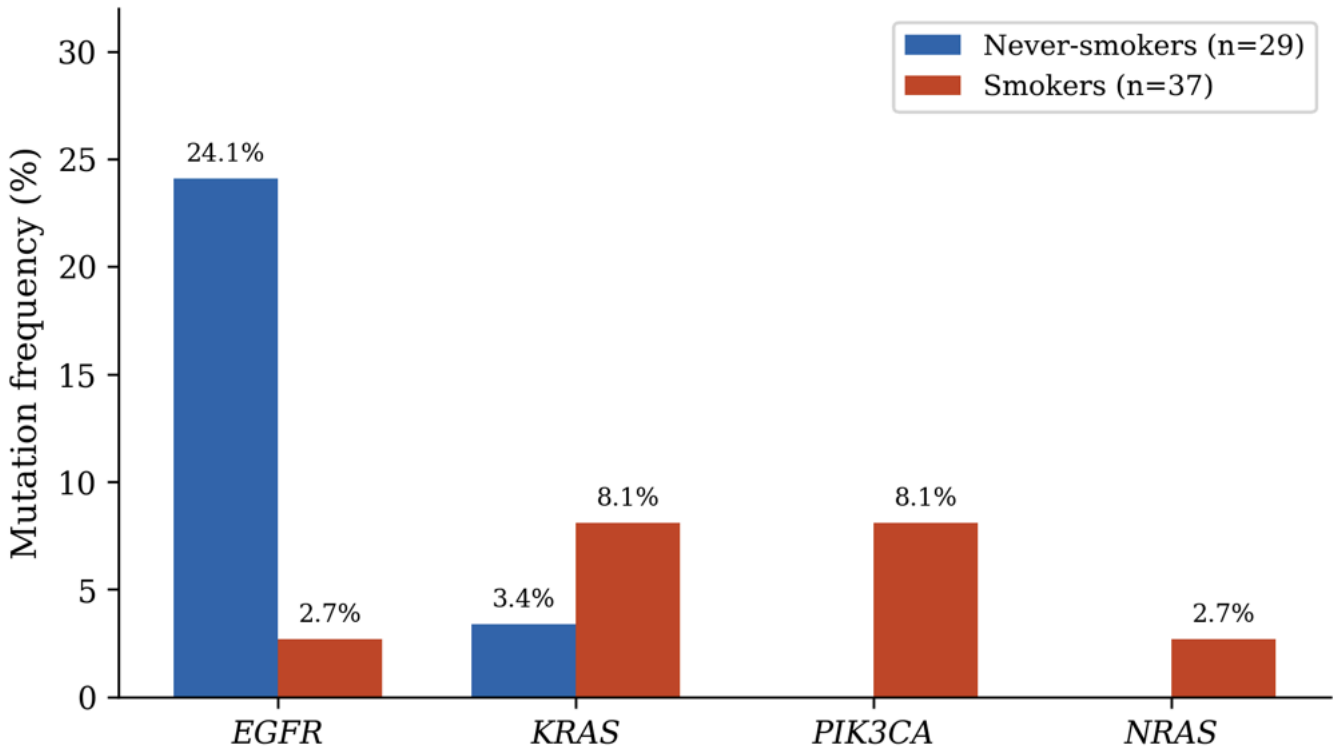


Figure 6

Gene-specific pathogenic mutation frequencies stratified by smoking status. Among never-smokers (n = 29), EGFR mutations predominated (24.1%), while KRAS and PIK3CA mutations were absent or rare. Among smokers (n = 37), KRAS (8.1%) and PIK3CA (8.1%) were the most commonly mutated genes, while EGFR mutations were rare (2.7%). No PIK3CA mutations were detected in never-smokers.