ONCOLOGY

Next-generation sequencing as a valuable tool for mutational spectrum in advanced-stage NSCLC patients

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Abstract

Background and aim. Lung cancer remains one of the most threatening malignancies, ranking as the second most diagnosed cancer, and it continues to be the leading cause of cancer-related deaths worldwide. Challenges persist with late diagnosis and the high mutational burden characteristic of lung cancer.

Methods. Our study focuses on identifying the mutational spectrum of a cohort of advanced-stage non-small cell lung cancer (NSCLC) patients using a minimally invasive method through blood collection. To analyze the mutational landscape of these patients, we employed plasma DNA for the next-generation sequencing (NGS) cancer panel Ion Torrent, which contains 50 of the most mutated genes in lung cancer. All protocols for extraction, quality and quantity control, and library preparation follow the manufacturer's rules. Bioinformatics analysis was performed to select pathogenic mutations versus non-pathogenic-benign ones.

Results. This approach is particularly valuable for patients in advanced stages (III and IV, n=10) of lung adenocarcinoma and lung squamous cell carcinoma, who lack surgical options and limited therapeutic avenues. The comprehensive sequencing analysis revealed that nine of the ten lung cancer patients carried a TP53 mutation. Also, several other mutations exist in various cases, showing heterogeneous profiling.

Conclusions. Our findings demonstrate the potential of liquid biopsies in providing crucial genetic insights that can guide personalized treatment strategies, improving the management and outcomes for patients with advanced lung cancer.

Keywords: Next-generation sequencing, NSCLC, biomarkers

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Introduction

Lung cancer remains a significant health issue worldwide, with a higher incidence each year and, unfortunately, a high mortality rate that follows the incidence. With the discovery of new targeted therapies and immunotherapy, we could have expected an improvement in decreasing the mortality rate. Even more, this *Faust* event occurs in both sexes and continues to grow in smokers as well as non-smokers. Non-small cell lung cancer (NSCLC) accounts for approximately 85% of all lung cancer cases. This histological classification, including adenocarcinomas and squamous cell carcinomas, remains one of the world's leading causes of cancer-related death despite advances in diagnosis and treatment. Due to their broad heterogeneity, innovative approaches are required to manage and treat NSCLC effectively.

In NSCLC, mutational profiling, a comprehensive analysis of genetic alterations within tumor cells, has emerged as a pivotal tool [1,2]. Mutations, deletions, amplifications, and other genetic

modifications are identified as coordinating the disease evolution up to extensive progression and metastasis [3]. These alterations influence cancer cells' progression and tumor microenvironment behaviors, and treatment response is also impacted, particularly immunotherapy [4]. Actual treatments can target specific mutations in a patient's tumor by understanding the genetic landscape of the tumor. Personalized and effective treatments can be achieved through the comprehensive status of the mutational profiling [5,6].

Next-generation sequencing (NGS) technology has revolutionized mutational profiling, enabling the simultaneous examination of numerous genes with high precision and efficiency [2]. NGS platforms can detect a variety of genetic alterations, including single nucleotide variations (SNVs), insertions and deletions (Indels), copy number variations (CNVs), and structural rearrangements [7]. The purpose of this comprehensive profiling is to identify actionable mutations that can be targeted by specific drugs, such as tyrosine kinase inhibitors (TKIs) and immunotherapies [3].

Mutational profiling also allows us to understand resistance mechanisms to therapies, thereby guiding the development of new therapeutic strategies and combinations [8]. Molecular profiling is critical in advancing precision oncology and offering hope for improved survival and quality of life for patients with this challenging disease as our understanding of the disease's molecular underpinnings expands [7,9]. Therefore, deciphering the mutational profiling of NSCLC is essential to lung cancer management. By elucidating the genetic alterations that drive cancer progression, this approach enables personalized treatment strategies that improve patient outcomes [9]. As technology advances and our understanding of NSCLC's molecular landscape deepens, mutational profiling will remain at the forefront of efforts to combat this prevalent and deadly disease [3].

Our study focuses on identifying the mutational profiling of a cohort of NSCLC patients using a minimally invasive method through blood collection. This method, also associated with liquid biopsy, is increasingly considered a pertinent solution for patients in advanced stages. We investigated our cohort using plasma DNA to determine the mutational spectrum in advanced-stage NSCLC patients using an NGS cancer panel on an Ion Torrent PGM machine.

Methods

Patients

Selection of NSCLC Patients and Tissue Procurement. The criteria for our study were the presence of an NSCLC tumour stage IIIB/IVA, B – in male or female patients with access to a blood sample, from which we extracted serum and plasma. We selected ten patients diagnosed with NSCLC (non-small cell lung cancer) for whom informed consent was obtained. The study was approved by the the Ethical Committee of Iuliu Hatieganu University of Medicine and Pharmacy no. 438/24.11.2016 and no. 231/ 24.06.2020. All blood samples were obtained during clinical enrolment, then immediately prepared for serum and plasma and stored at −80 °C. All samples were collected at the clinician's recommendation.

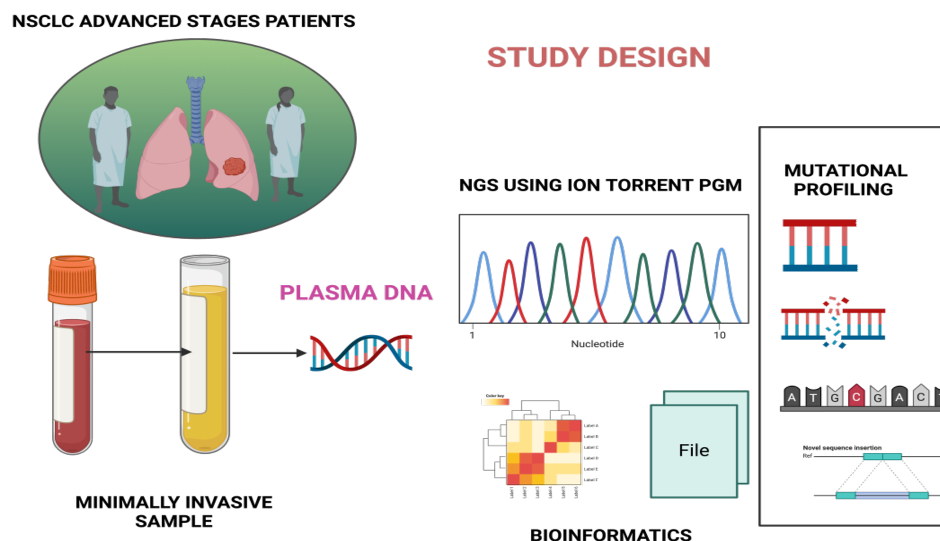


Figure 1. Study design for mutational spectrum in NSCLC patients (LUAD and LUSC).

Table I. Clinical and histopathological information of patients enrolled for NGS mutational profiling.

Case no.	Sex	Age	Diagnosis	Staging	Metastasis
Case1	M	69	lung squamous cell carcinoma	IV	Mediastinal adenopathy
Case 2	F	44	lung adenocarcinoma	IIIB	Pulmonary, hepatic, left adrenal gland
Case 3	F	64	lung squamous cell carcinoma	IV A	Pleural
Case 4	M	65	lung adenocarcinoma	IV	Pulmonary, mediastinal adenopathy
Case 5	M	60	lung squamous cell carcinoma	IVB	Hepatic
Case 6	M	79	lung adenocarcinoma	IV	Right adrenal gland, hepatic, right axillary node,
Case 7	M	69	lung adenocarcinoma	IV	Bone, brain, leptomeningeal carcinomatosis
Case 8	M	51	lung adenocarcinoma	IV	Bone
Case 9	F	62	lung adenocarcinoma	IIIB	Laterocervical lymph node metastasis
Case 10	F	62	lung squamous cell carcinoma	IV	Pulmonary mediastinal adenopathy supraclavicular

Table II. Target genes in the Ion AmpliSeq Hotspot Panel version 2.

GENE	GENE	GENE	GENE	GENE
ABL1	EGFR	GNAS	KRAS	PTPN11
AKT1	ERBB2	GNAQ	MET	RB1
ALK	ERBB4	HNF1A	MLH1	RET
APC	EZH2	HRAS	MPL	SMAD4
ATM	FBXW7	IDH1	NOTCH1	SMARCB1
BRAF	FGFR1	JAK2	NPM1	SMO
CDH1	FGFR2	JAK3	NRAS	SRC
CDKN2A	FGFR3	IDH2	PDGFRA	STK11
CSF1R	FLT3	KDR	PIK3CA	TP53
CTNNB1	GNA11	KIT	PTEN	VHL

Clinical and histopathological information was collected for all patients (Table I). All methods and experimental protocols were performed per the relevant guidelines of the hospital's ethics committee, and all patients were granted informed consent. The study design is presented in figure 1.

DNA extraction

DNA from plasma (cfDNA) was extracted from 1200 µl using the MagMax Cell-Free DNA Kit (ThermoFisher Scientific) and the manufacturer's protocol. The DNA concentrations were evaluated using the Qubit HS DNA kit and the Qubit 2.0 fluorometer. The DNA concentrations ranged between 2.5 and 94.7 ng/µl.

Next Generation Sequencing using Ion Torrent PGM

20 ng of genomic DNA was used for sequencing library synthesis using Ion Ampliseq Cancer Panel Primer Pool v2 (ThermoFisher Scientific, Waltham, Massachusetts, United States) and Ion Ampliseq Library kit 2 (ThermoFisher Scientific, Waltham, Massachusetts, United States). The obtained libraries were quantified using Qubit 2.0 and the Qubit HS dsDNA kit (ThermoFisher Scientific, Waltham, Massachusetts, United States) and diluted to 100pM for template synthesis. After template synthesis, the libraries were sequenced using the Ion Torrent Personal Genome Machine (ThermoFisher Scientific, Waltham, Massachusetts, United States). We sequenced four libraries on a 316 Ion Chip (ThermoFisher Scientific, Waltham, Massachusetts, United States) using the Ion PGM HighQ View Sequencing kit (ThermoFisher Scientific, Waltham, Massachusetts, United States). The NGS data were analyzed using the sequencer's software for trimming and alignment, and for secondary analysis, we used the Ion Reporter v5 software. The evaluated genes are presented in table II.

cBioPortal Dataset Analyses

cBioPortal (http://cbioportal.org) uses data from the Gene Expression Omnibus (GEO) and The Cancer Genome Atlas (TCGA) to profile. It includes a wide range of data such as DNA copy numbers, DNA methylation, mRNA and microRNA expression levels, and nonsynonymous mutations [10,11]. In this study, the cBioPortal database was utilized to analyze genetic variations of ATM, BRAF, CSF1R, ERBB4, FGFR3, FLT3, JAK3, KDR, KIT, PIK3CA, SMAD4, SMARCB1, STK11, and TP53 in NSCLC patients was determined using the online cancer genomics platform cBioPortal.

The STRING database

The STRING database. The STRING database (Search Tool for the Retrieval of Interacting Genes; available at http://string-db.org/) [12] is widely recognized for its comprehensive coverage and user-friendly interface,

making it a valuable resource for exploring protein-protein interactions. In our study, we independently constructed gene networks for selected mutated genes by importing gene symbols into the database. We specifically focused on interactions relevant to Homo sapiens, ensuring that only those with a confidence score greater than 0.9 were included, providing a reliable basis for our analysis of gene interactions within this biological context. This database was also utilized to identify significantly enriched pathways, and the expression sites associated with these pathways were subsequently analyzed.

Results

Clinicopathological characteristics of the cohort. Table I lists the main characteristics of the patients included in the study cohort. The patients in the next-generation sequencing study were between 44 and 79 years old, with a mean age of 62.5 years. Sixty per cent of the cases were males, and forty per cent were females. We investigated six lung adenocarcinomas and four lung squamous cell carcinomas. For this study, we only selected patients with stage III and IV at diagnosis. We performed hematoxylin-eosin staining for all cases for diagnostic and histological type confirmation in figure 2.

Mutation alteration in NSCLC. All samples were sequenced and compared with the reference genome. We focused on identifying any modification that might impact the malignant phenotype of lung cancer patients for 10 cases before any therapy and 3 of the cases post-treatment (Table II). Figure 3 summarizes the data on the number of mutations correlated with the mutations in specific genes for the ten cases before treatment. Our data show that some genes, like CSFR1, are mutated in all samples, and others, like CSF1R, FLT3, and TP53, are mutated in most samples. TP53 expresses the most mutations from all genes, followed by KDR and PIK3CA. In the analyzed cases, post-treatment was observed to maintain the same mutational pattern.

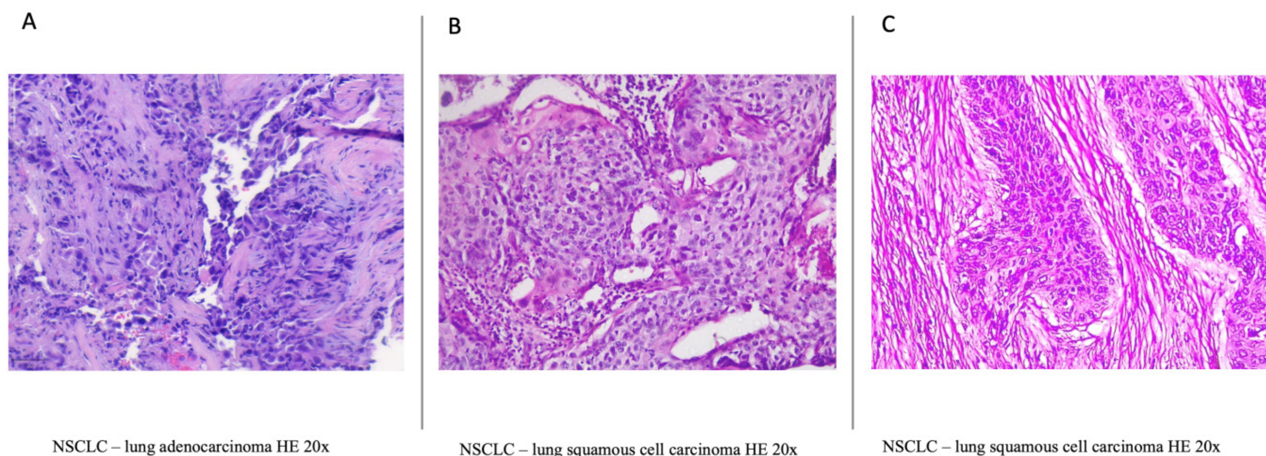


Figure 2. Hematoxylin–eosin staining of lung cancer tissues: (a) Lung adenocarcinoma at 20× magnification; (b) Lung squamous cell carcinoma at 20×; (c) Lung squamous cell carcinoma at 20×.

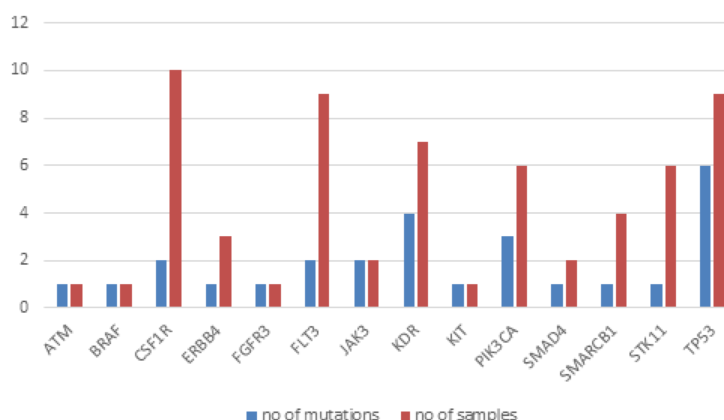


Figure 3. Graphical representation of the number of mutations correlated with the presence in specific genes for the ten cases before treatment.

Table III. Mutational pattern evaluated using Ion Torrent technology for the 10 cases before treatment and 3 cases posttreatment.

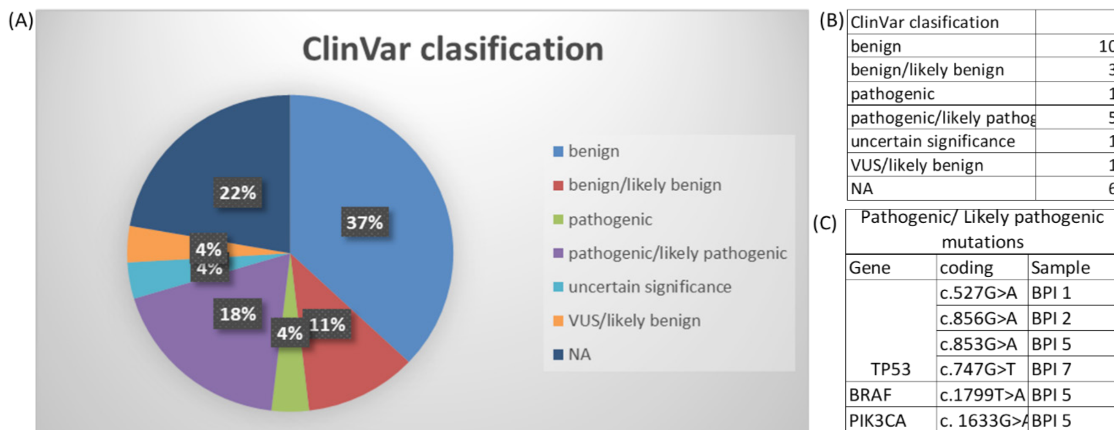
Sample	Locus	Genes	Exon	Coding	ClinVar
Case 1	chr4:1806131	FGFR3	Exon 9	c.1150T>C	Benign/Likely benign
	chr4:55980239	KDR	intron 6	c.798+54G>A	
	chr5:149433595	CSF1R	Exon 21	c.*37AC>C	
	chr5:149433597	CSF1R	Exon 21	c.*35C>TC	
	chr11:108200916	ATM	intron 49	c.7308-25T>G	
	chr13:28610183	FLT3	intron 10	c.1310-3T>C	Benign
	chr17:7578368	TP53	Exon 5	c.527G>A	Pathogenic
	chr17:7579472	TP53	Exon 4	c.215C>G	Benign
	chr19:1220321	STK11		c.465-51T>C	Benign
	chr22:24176287	SMARCB1	intron 5	c.1119-41G>A	Benign
Case 2	chr3:178917005	PIK3CA	intron 2	c.352+40A>G	Benign
	chr5:149433595	CSF1R	Exon 21	c.*37AC>C	
	chr5:149433597	CSF1R	Exon 21	c.*35C>TC	
	chr17:7577070	TP53	Exon 8	c.856G>A	Pathogenic/Likely pathogenic
	chr17:7579472	TP53	Exon 4	c.215C>G	Benign
	chr18:48586344	SMAD4	intron 8	c.955+58C>T	Benign
	chr19:1220321	STK11		c.465-51T>C	Benign
Case 3	chr3:178917005	PIK3CA	intron 2	c.352+40A>G	Benign
	chr5:149433595	CSF1R	Exon 21	c.*37AC>C	
	chr5:149433597	CSF1R	Exon 21	c.*35C>TC	
	chr13:28610183	FLT3	intron 10	c.1310-3T>C	Benign
	chr17:7579472	TP53	Exon 4	c.215C>G	Benign
	chr17:7579473	TP53	Exon 4	c.214C>G	Uncertain significance
	chr19:1220321	STK11		c.465-51T>C	Benign
	chr19:17945708	JAK3	Exon 16	c.2152G>C	VUS/Likely bening
Case 4	chr4:55946354	KDR	intron 29	c.3849-24C>A	
	chr4:55980239	KDR	intron 6	c.798+54G>A	
	chr5:149433595	CSF1R	Exon 21	c.*37AC>C	
	chr5:149433597	CSF1R	Exon 21	c.*35C>TC	
	chr13:28602292	FLT3	intron 16	c.2053+23A>G	Benign
	chr13:28610183	FLT3	intron 10	c.1310-3T>C	Benign
	chr17:7579472	TP53	Exon 4	c.215C>G	Benign
	chr22:24176287	SMARCB1	intron 5	c.1119-41G>A	Benign
Case 5	chr3:178936091	PIK3CA	Exon 10	c.1633G>A	Pathogenic/Likely pathogenic
	chr4:55962545	KDR	intron 18	c.2615-37_2615-36insC	
	chr4:55972974	KDR	Exon 11	c.1416A>T	benign
	chr4:55980239	KDR	intron 6	c.798+54G>A	
	chr5:149433595	CSF1R	Exon 21	c.*37AC>C	
	chr5:149433597	CSF1R	Exon 21	c.*35C>TC	
	chr7:140453136	BRAF	Exon 15	c.1799T>A	Pathogenic/Likely pathogenic
	chr13:28610183	FLT3	intron 10	c.1310-3T>C	Benign
	chr17:7577070	TP53	Exon 8	c.853G>A	Pathogenic/Likely pathogenic
	chr17:7579472	TP53	Exon 4	c.215C>G	Benign
Case 6	chr2:212812097	ERBB4	intron 3	c.421+58A>G	Benign
	chr3:178917005	PIK3CA	intron 2	c.352+40A>G	Benign
	chr3:178927410	PIK3CA	Exon 7	c.1173A>G	Benign
	chr4:55980239	KDR	intron 6	c.798+54G>A	
	chr5:149433595	CSF1R	Exon 21	c.*37AC>C	
	chr5:149433597	CSF1R	Exon 21	c.*35C>TC	
	chr13:28602292	FLT3	intron 16	c.2053+23A>G	Benign
	chr13:28610183	FLT3	intron 10	c.1310-3T>C	Benign
	chr17:7579472	TP53	Exon 4	c.215C>G	Benign
	chr18:48586344	SMAD4	intron 8	c.955+58C>T	Benign

Table III. Mutational pattern evaluated using Ion Torrent technology for the 10 cases before treatment and 3 cases posttreatment.

Sample	Locus	Genes	Exon	Coding	ClinVar
Case 7	chr4:55962545	KDR	intron 18	c.2615-37_2615-36insC	
	chr4:55972974	KDR	Exon 11	c.1416A>T	benign
	chr4:55980239	KDR	intron 6	c.798+54G>A	
	chr5:149433595	CSF1R	Exon 21	c.*37AC>C	
	chr5:149433597	CSF1R	Exon 21	c.*35C>TC	
	chr13:28610183	FLT3	intron 10	c.1310-3T>C	Benign
	chr17:7577528	TP53	Exon 7	c.747G>T	Pathogenic/Likely pathogenic
	chr17:7579472	TP53	Exon 4	c.215C>G	Benign
	chr19:1220321	STK11		c.465-51T>C	Benign
Case 8	chr2:212812097	ERBB4	intron 3	c.421+58A>G	Benign
	chr3:178917005	PIK3CA	intron 2	c.352+40A>G	Benign
	chr3:178927410	PIK3CA	Exon 7	c.1173A>G	Benign
	chr4:55593464	KIT	Exon 10	c.1621A>C	Benign/Likely benign
	chr4:55962545	KDR	intron 18	c.2615-37_2615-36insC	
	chr4:55980239	KDR	intron 6	c.798+54G>A	
	chr5:149433595	CSF1R	Exon 21	c.*37AC>C	
	chr5:149433597	CSF1R	Exon 21	c.*35C>TC	
	chr13:28610183	FLT3	intron 10	c.1310-3T>C	Benign
	chr17:7579472	TP53	Exon 4	c.215C>G	Benign
	chr19:1220321	STK11		c.465-51T>C	Benign
	chr22:24176287	SMARCB1	intron 5	c.1119-41G>A	Benign
Case 9	chr4:55962545	KDR	intron 18	c.2615-37_2615-36insC	
	chr5:149433595	CSF1R	Exon 21	c.*37AC>C	
	chr5:149433597	CSF1R	Exon 21	c.*35C>TC	
	chr13:28610183	FLT3	intron 10	c.1310-3T>C	Benign
Case 10	chr2:212812097	ERBB4	intron 3	c.421+58A>G	Benign
	chr3:178917005	PIK3CA	intron 2	c.352+40A>G	Benign
	chr3:178927410	PIK3CA	Exon 7	c.1173A>G	Benign
	chr5:149433595	CSF1R	Exon 21	c.*37AC>C	
	chr5:149433597	CSF1R	Exon 21	c.*35C>TC	
	chr13:28610183	FLT3	intron 10	c.1310-3T>C	Benign
	chr17:7579472	TP53	Exon 4	c.215C>G	Benign
	chr19:1220321	STK11		c.465-51T>C	Benign
	chr19:17945696	JAK3	Exon 16	c.2164G>A	Benign/Likely benign
	chr22:24176287	SMARCB1	intron 5	c.1119-41G>A	Benign
Case 3 posttreatment	chr3:178917005	PIK3CA	intron 2	c.352+40A>G	Benign
	chr5:149433595	CSF1R	Exon 21	c.*37AC>C	
	chr5:149433597	CSF1R	Exon 21	c.*35C>TC	
	chr13:28610183	FLT3	intron 10	c.1310-3T>C	Benign
	chr17:7579472	TP53	Exon 4	c.215C>G	Benign
	chr19:1220321	STK11		c.465-51T>C	Benign
	chr19:17945708	JAK3	Exon 16	c.2152G>C	VUS/Likely bening
Case 4 posttreatment	chr4:55946354	KDR	intron 29	c.3849-24C>A	
	chr4:55980239	KDR	intron 6	c.798+54G>A	
	chr5:149433595	CSF1R	Exon 21	c.*37AC>C	
	chr5:149433597	CSF1R	Exon 21	c.*35C>TC	
	chr13:28602292	FLT3	intron 16	c.2053+23A>G	Benign
	chr13:28610183	FLT3	intron 10	c.1310-3T>C	Benign
	chr17:7579472	TP53	Exon 4	c.215C>G	Benign
	chr22:24176287	SMARCB1	intron 5	c.1119-41G>A	Benign
Case 6 posttreatment	chr2:212812097	ERBB4	intron 3	c.421+58A>G	Benign
	chr3:178917005	PIK3CA	intron 2	c.352+40A>G	Benign
	chr3:178927410	PIK3CA	Exon 7	c.1173A>G	Benign
	chr4:55980239	KDR	intron 6	c.798+54G>A	
	chr5:149433595	CSF1R	Exon 21	c.*37AC>C	
	chr5:149433597	CSF1R	Exon 21	c.*35C>TC	
	chr13:28602292	FLT3	intron 16	c.2053+23A>G	Benign
	chr13:28610183	FLT3	intron 10	c.1310-3T>C	Benign
	chr17:7579472	TP53	Exon 4	c.215C>G	Benign
	chr18:48586344	SMAD4	intron 8	c.955+58C>T	Benign

After establishing parameters for filtering significant mutations in the targeted sequencing experiment, we filtered out the synonymous mutations as they are filtered from most of the cancer genome studies. The selected mutations were classified as benign, likely benign, neutral, likely pathogenic, or pathogenic according to the FATHMM score and ClinVar interpretation.

Mutations are then interpreted using ClinVar, which classifies them as benign (37%), likely benign (11%), pathogenic (4%), and VUS/likely benign (4%). Figure 4A presents the benign mutations. The pathogenic mutations are summarized in Table III, and the pathogenic mutated genes are part of Figures 4B and 3C.

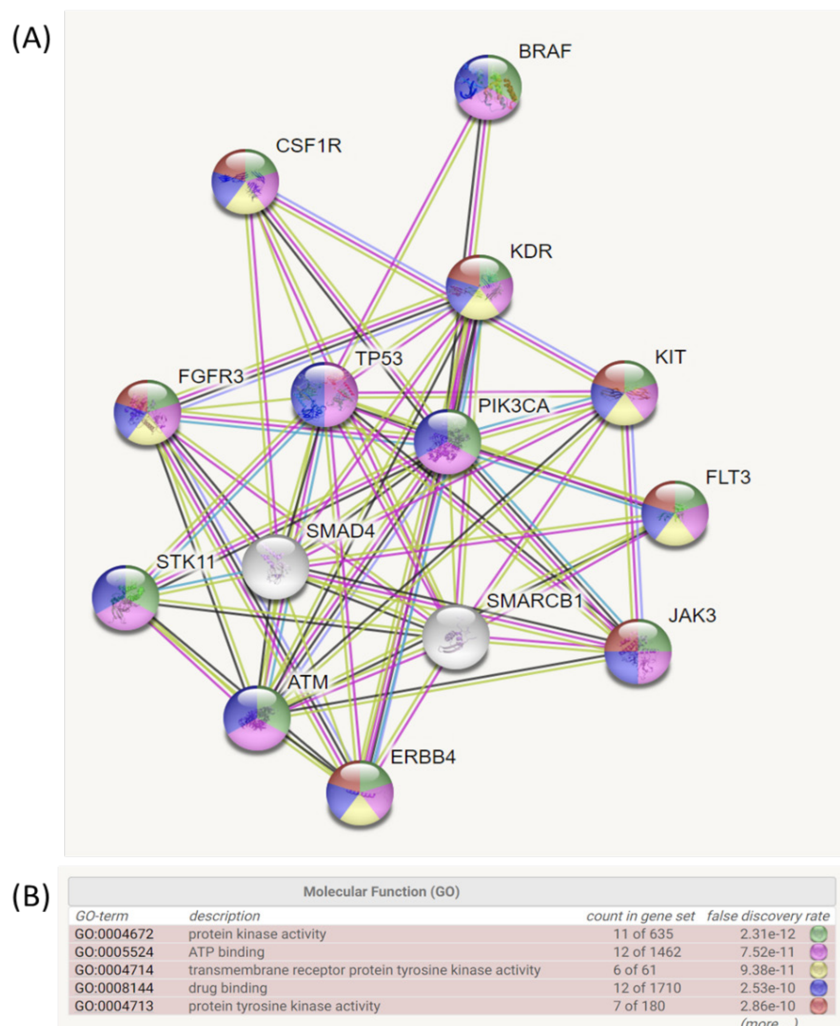


ClinVar clasification	
benign	10
benign/likely benign	3
pathogenic	1
pathogenic/likely pathog	5
uncertain significance	1
VUS/likely benign	1
NA	6

Pathogenic/ Likely pathogenic mutations		
Gene	coding	Sample
	c.527G>A	BPI 1
	c.856G>A	BPI 2
	c.853G>A	BPI 5
TP53	c.747G>T	BPI 7
BRAF	c.1799T>A	BPI 5
PIK3CA	c. 1633G>A	BPI 5

Figure 4. ClinVar mutation classification in NSLCL liquid biopsy. (A) ClinVar classification of the mutation in the analyzed cases from liquid biopsy, color legend presents each specific type of the mutation with specific %; (B) summarization of the main type of the mutations (C) pathogenic mutation identified in samples.

Figure 5. Analysis of gene mutations using the Oncomine database in NSCLC- TCGA data set, gene mutation frequencies and types. The red bars indicate gene amplifications, blue bars are deep deletions, green bars are missense mutations, and grey bars indicate truncating mutations or a different kind of alteration, as indicated in the legend.



Molecular Function (GO)				
GO-term	description	count in gene set	false discovery rate	
GO:0004672	protein kinase activity	11 of 635	2.31e-12	
GO:0005524	ATP binding	12 of 1462	7.52e-11	
GO:0004714	transmembrane receptor protein tyrosine kinase activity	6 of 61	9.38e-11	
GO:0008144	drug binding	12 of 1710	2.53e-10	
GO:0004713	protein tyrosine kinase activity	7 of 180	2.86e-10	

(more ...)

Figure 6. String networks for genes mutated gene in NSCLC. (A) String network for the selected mutated genes (B) Enrichment analysis, emphasis on the main molecular functions related to the frequently mutated genes.

Gene mutations in CBioPortal database for NSCLC

We analyzed the genetic variation of the selected genes based on the NSCLCG sample data from the TCGA database via the cBioPortal database. The results are presented in figure 5. As anticipated, TP53 was the gene with the most frequent alterations across all three datasets. It exhibited an overall alteration frequency of 58% in the samples analyzed from these datasets. STK11 is also frequently observed mutation in lung cancer (17%), contributing significantly to the molecular landscape of the disease.

Pathway analysis of detected somatic mutations

The most frequent mutations were integrated into STRING to emphasise their interconnection (Figure 6A). We conducted GO functional annotation and KEGG pathway enrichment analysis for the selected genes, most of which have protein kinase and drug-binding activity (Figure 6B).

Discussion

Our study aimed to identify the mutational spectrum of a cohort of advanced-stage NSCLC patients using a minimally invasive method through blood collection [13]. By leveraging plasma DNA and employing a next-generation sequencing (NGS) cancer panel, we comprehensively analyzed lung cancer's genetic landscape in these patients. This approach is precious for patients in advanced stages (III and IV), where surgical options are unavailable and therapeutic choices are limited [14].

Liquid biopsy, through the analysis of plasma DNA, offers a significant advantage over traditional tissue biopsy, especially in late-stage lung cancer patients [13,15]. Traditional biopsies can be invasive, risky, and sometimes unfeasible due to the patient's health condition or tumor localization. In contrast, liquid biopsy is minimally invasive, reduces patient discomfort, and can

be performed repeatedly to monitor disease progression and treatment response [16]. This is crucial for late-stage patients who often require ongoing assessment to tailor their therapeutic regimens effectively [13,14,16].

While our study demonstrates the promise of liquid biopsy, there are limitations. The tumor burden and the shedding rate of tumor DNA into the bloodstream can influence the sensitivity and specificity of plasma DNA analysis. Additionally, specific mutations present at low frequencies might be missed, necessitating complementary diagnostic approaches in some cases [14].

Future research should focus on improving the sensitivity of liquid biopsy techniques and validating their clinical utility in larger, more diverse cohorts. Longitudinal studies tracking patients over time will be essential to understand the full potential of liquid biopsy in managing lung cancer.

Several mutations have been detected in EGFR, HER2, PIK3CA, BRAF, KRAS, and TP53 genes [17]. Notably, the genes ATM, BRAF, CSF1R, ERBB4, FGFR3, FLT3, JAK3, KDR, KIT, PIK3CA, SMAD4, SMARCB1, STK11, and TP53 in NSCLC patients were proven to have similar mutations to those identified in TCGA data sets, as shown by similar data with those observed from TCGA.

Our data show that some patients could be treated with at least one targeted drug based on their ‚druggable genes. For instance, in the present study, vemurafenib was identified as a treatment for patients with the BRAF mutation. Additionally, the anti-epidermal growth factor receptor drug cetuximab has been highly successful in treating metastatic lung cancer [18,19].

TP53 was the most frequently mutated gene, and the highest number of mutations was found in nearly all patients (90%), probably due to the advanced stages used for the present analysis. According to the lung cancer genome database, this tumor suppressor gene is the most commonly mutated gene in human cancers [20]. Although TP53 is not currently a therapeutic target, it is a prognostic factor for treatment response. Recent studies indicate that TP53 mutations are linked to chemotherapy resistance, poorer therapeutic responses, and reduced OS in NSCLC patients, varying by disease stage and sequencing methods [21-23]. Additionally, the biological impact of TP53 mutations can differ based on tumor histology and smoking history [23,24].

TP53, EGFR and *STK11* mutations are major determinants of the tumor immune profile [25]. STK11 mutations in NSCLC have significant prognostic implications, as these mutations can alter the tumor microenvironment by affecting the presence and activity of tumor-infiltrating immune cells. This alteration can impact the effectiveness of immunotherapy, potentially leading to varied responses in patients [26].

Targeted sequencing offers the advantage of focusing on specific genomic regions of interest, thereby providing higher depth and accuracy at a lower cost. It is limited by its inability to detect novel or unexpected mutations outside the targeted areas, potentially missing relevant genomic alterations [27]. NGS platforms for ctDNA analysis, such as the FDA-approved Guardant360 and FoundationOne Liquid CDx, offer comprehensive mutation profiling for lung cancer liquid biopsies. Guardant360 and FoundationOne Liquid CDx are liquid biopsy platforms that have received FDA approval for detecting genetic mutations in cancer patients into the clinic. Guardant360 covers 55 genes, focusing on providing actionable information for a select number of common mutations and associated therapies. In contrast, FoundationOne Liquid CDx's coverage of 311 genes offers a more extensive profile; both platforms have a similar turnaround time and use NGS technology, ensuring high-quality, reliable results.

By leveraging these technologies, clinicians can detect a broad range of genetic alterations, including those in genes not typically covered by smaller panels, thereby improving the chances of identifying effective targeted therapies and enhancing patient outcomes [28].

Conclusions

In conclusion, our study underscores the feasibility and clinical relevance of using plasma DNA and NGS for mutational profiling in late-stage lung cancer patients. This minimally invasive method facilitates the identification of actionable mutations and supports ongoing disease monitoring and personalized treatment strategies. As we advance towards more precise and individualized cancer care, liquid biopsy stands out as a transformative tool, particularly for patients with limited therapeutic options, especially in cases where tissue biopsies are not feasible or insufficient for advanced molecular analysis.

Many somatic mutations in this tumor class have the potential to lead to various targeted therapies for cancer patients. Thus, identifying genetic factors influencing treatment response is crucial for developing next-generation medicines targeting patients ‚druggable' alterations.

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