Mutation profile in liquid biopsy tested by next generation sequencing in Mexican patients with non-small cell lung carcinoma and its impact on survival

José Fabián Martínez-Herrera[1,2,3]^, Gisela Sánchez Domínguez[1]^, Juan J. Juárez-Vignon Whaley[4], Sonia Carrasco-Cara Chards[5], Claudia López Vrátný[6], Jordi Guzmán Casta[1], Rodrigo F. Riera Sala[1], Jorge A. Alatorre-Alexander[1], Alec Seidman Sorsby[7]^, Mayte Cruz Zermeño[7]^, Emilio Conde Flores[8], Rodrigo R. Flores-Mariñelarena[8], Carla P. Sánchez-Ríos[1]^, Luis M. Martínez-Barrera[1], Raquel Gerson-Cwilich[2], Patricio Santillán-Doherty[1,9], José C. Jiménez López[10], William López Hernández[1]^, Jerónimo R. Rodríguez-Cid[1,8]^

[1]Department of Thoracic Oncology, Instituto Nacional de Enfermedades Respiratorias, Dr. Ismael Cosío Villegas, Mexico City, Mexico; [2]Cancer Center, Medical Center American British Cowdray, Mexico City, Mexico; [3]Cancer Research Networking, Universidad Científica del Sur, Lima, Perú; [4]Centro de Investigación en Ciencias de la Salud (CICSA), FCS, Universidad Anáhuac México Campus Norte, Mexico City, Mexico; [5]Facultad de Medicina, Universidad Nacional Autónoma de México, Mexico City, Mexico; [6]Health Pharma Profesional Research, Mexico City, Mexico; [7]Facultad de Medicina, Universidad Panamericana, Mexico City, Mexico; [8]Oncology Center, Medica Sur Hospital, Mexico City, Mexico; [9]Medical Direction, Instituto Nacional de Enfermedades Respiratorias, Dr. Ismael Cosío Villegas, Mexico City, Mexico; [10]Universidad Nacional Autónoma de México, Mexico City, Mexico

Contributions: (I) Conception and design: JF Martínez Herrera, JJ Juárez-Vignon Whaley, JA Alatorre-Alexander, JR Rodríguez-Cid; (II) Administrative support: JF Martínez Herrera, JJ Juárez-Vignon Whaley, JA Alatorre-Alexander, JR Rodríguez-Cid; (III) Provision of study materials or patients: JF Martínez Herrera, JJ Juárez-Vignon Whaley, JA Alatorre-Alexander, JR Rodríguez-Cid; (IV) Collection and assembly of data: J Guzmán Casta, RF Riera Sala, RR Flores-Mariñelarena, W López Hernández (V) Data analysis and interpretation: JF Martínez Herrera, JJ Juárez-Vignon Whaley, JA Alatorre-Alexander, JR Rodríguez-Cid; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: José Fabián Martínez-Herrera, MD. Department of Thoracic Oncology, Instituto Nacional de Enfermedades Respiratorias, Dr. Ismael Cosío Villegas, Calzada de Tlalpan 4502, Belisario Domínguez Secc. 16, Tlalpan, 14080, Mexico City, Mexico; Cancer Center, Medical Center American British Cowdray, Mexico City, Mexico; Cancer Research Networking, Universidad Científica del Sur, Lima, Perú. Email: jfabianmd@gmail.com.

Background: Lung cancer represents a significant global health concern, often diagnosed in its advanced stages. The advent of massive DNA sequencing has revolutionized the landscape of cancer treatment by enabling the identification of target mutations and the development of tailored therapeutic approaches. Unfortunately, access to DNA sequencing technology remains limited in many developing countries. In this context, we emphasize the critical importance of integrating this advanced technology into healthcare systems in developing nations to improve treatment outcomes.

Methods: We conducted an analysis of electronic clinical records of patients with confirmed advanced non-small cell lung cancer (NSCLC) and a verified negative status for the epidermal growth factor receptor (*EGFR*) mutation. These patients underwent next-generation sequencing (NGS) for molecular analysis. We performed descriptive statistical analyses for each variable and conducted both univariate and multivariate statistical analyses to assess their impact on progression-free survival (PFS) and overall survival (OS). Additionally, we classified genetic mutations as actionable or non-actionable based on the European Society for Medical Oncology Scale of Clinical Actionability of Molecular Targets (ESCAT) guidelines.

^ ORCID: José Fabián Martínez-Herrera, 0000-0001-9894-2204; Gisela Sánchez Domínguez, 0009-0008-1528-5746; Alec Seidman Sorsby, 0000-0003-1301-9205; Mayte Cruz Zermeño, 0000-0001-7667-0893; Carla P. Sánchez Ríos, 0000-0003-1142-3175; William López Hernández, 0009-0002-7674-1641; Jerónimo R. Rodríguez-Cid, 0000-0001-8518-9065.

J Thorac Dis 2024;16(1):161-174 | https://dx.doi.org/10.21037/jtd-23-1029

Results: Our study included a total of 127 patients, revealing the presence of twenty-one distinct mutations. The most prevalent mutations were *EGFR* (18.9%) and Kirsten rat sarcoma viral oncogene homolog (*KRAS*) (15.7%). Notably, anaplastic lymphoma kinase (*ALK*) [hazard ratio (HR): 0.258, P<0.001], tumor mutation burden (TMB) (HR: 2.073, P=0.042) and brain magnetic resonance imaging (MRI) (HR: 0.470, P=0.032) demonstrated statistical significance in both the univariate and multivariate analyses with respect to PFS. In terms of OS, *ALK* (HR: 0.285, P<0.001) and *EGFR* (HR: 0.482, P=0.024) exhibited statistical significance in both analyses. Applying the ESCAT classification system, we identified actionable genomic variations (ESCAT level-1), including *EGFR*, *ALK*, breast cancer (*BRAF*) gene, c-ros oncogene 1 (*ROS1*), and rearranged during transfection (*RET*) gene, in 32.3% of the patients.

Conclusions: Our findings from massive DNA sequencing underscore that 32.3% of patients who test negative for the *EGFR* mutation possess other targetable mutations, enabling them to receive personalized, targeted therapies at an earlier stage of their disease. Implementing massive DNA sequencing in developing countries is crucial to enhance survival rates among NSCLC patients and guide more effective treatment strategies.

Keywords: Next-generation sequencing (NGS); non-small cell lung carcinoma (NSCLC); target mutations; progression-free survival (PFS); overall survival (OS)

Introduction

Background

Lung cancer, the leading cause of cancer-related deaths worldwide, accounted for an estimated 1,796,144 fatalities in 2020 (1). In the United States, there were an estimated 227,875 new cases in 2020, with 138,225 estimated deaths, resulting in a 5-year relative survival rate of 21.7%. In contrast, Mexico reported 7,588 new cases, along with 7,100 deaths, and a 5-year survival rate of 6.37% (1,2). Regrettably, lung cancer is frequently diagnosed at an advanced stage, leading to a bleak prognosis, and treatment typically relies on multiple chemotherapeutic regimens. Recent advancements in gene sequencing, facilitated by next-generation sequencing (NGS) and immunotherapy, have paved the way for personalized treatments, with the ultimate aim of enhancing overall survival (OS).

The inaugural nucleic acid sequencing, known as the Sanger method, was developed in 1970. Nowadays, cutting-edge technologies are referred to as NGS, which enables billions of sequencing reactions to occur simultaneously (3-5). The results are mapped to online genome databases, and specific genetic mutations are sought after and compared. NGS can sequence the entire genome within 24 hours, thus revolutionizing the field of oncology (6). NGS empowers physicians to pinpoint specific mutations in a given tumor and employ targeted drugs, as demonstrated in various trials such as NCI-MPACT, MOSCATO-01, and MOST (7).

While NGS is evolving into a valuable tool, it does come with certain limitations, including high costs, data interpretation challenges, and a limited amount of tumor sample obtained from the initial biopsy, often

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Highlight box

Key findings
- 32.3% of patients with negative epidermal growth factor receptor via reverse transcription-polymerase chain reaction maintain a target mutation to guide treatment.
- Next-generation sequencing (NGS) has a positive impact on progression-free survival (PFS) and overall survival (OS).

What is known and what is new?
- NGS allows physicians to identify specific mutations in a certain tumor and use targeted drugs, as studied in multiple trials such as the NCI-MPACT, MOSCATO-01, and MOST trials.
- Our study is the first descriptive and analytical study carried out in Mexican population correlating specific molecular mutations in patients with non-small cell lung cancer using NGS from liquid biopsy sample via a 62-gene panel and correlating their impact on PFS and OS.

What is the implication, and what should change now?
- NGS must be implemented in developing countries to guide treatment.

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necessitating rebiopsy, which is not always feasible (7). In such instances, liquid biopsy emerges as the sole option to detect molecular targets, particularly in non-small cell lung cancer (NSCLC) patients. It's crucial to note that the gold standard for lung cancer diagnosis remains tumor biopsy. Therefore, combining liquid biopsy with NGS has secured Food and Drug Administration (FDA) approval as a companion diagnostic test for patients with NSCLC, assisting in guiding treatment decisions (8-10). The analyte employed by NGS for liquid biopsies is circulating tumor DNA (ctDNA), extracted from plasma, and is the sole clinically approved analyte. It boasts high specificity and has undergone extensive study, particularly for assessing epidermal growth factor receptor (*EGFR*) status (11,12).

Rationale and knowledge gap

The combined use of NGS and liquid biopsy has primarily found application in the study of advanced-stage lung cancer, as plasma levels of ctDNA are more pronounced in these stages. Nevertheless, it has also been explored for screening and early detection in individuals at high risk to ensure appropriate treatment (11,13,14). In the context of *EGFR* mutations, liquid biopsy stands as a valid alternative to tissue samples for molecular testing, with the goal of administering tyrosine kinase inhibitors (TKIs). In fact, its application has yielded an overall response rate of 72% and a median progression-free survival (PFS) of 11 months (15,16). This approach also proves beneficial in cases where patients undergoing TKI treatment develop resistance mechanisms, especially through the emergence of the T790M mutation (17). The ongoing BFAST multi-cohort study, which assesses the relationship between NGS-based detection of anaplastic lymphoma kinase (*ALK*) mutations in blood and the effectiveness of targeted therapies, has substantiated the clinical advantages of *ALK* fusions in patients receiving alectinib, thus establishing NGS as a valuable tool for therapeutic decision-making in patients with advanced NSCLC and *ALK* mutations (18).

The therapeutic decision-making process relies on the identification of biomarkers in either tissue or blood samples. In the pursuit of streamlining and optimizing treatment, the European Society for Medical Oncology Scale of Clinical Actionability of Molecular Targets (ESCAT) was developed, classifying DNA mutations based on evidence-based medicine. Given the recent discovery of new targeted therapies tailored to specific genetic mutations in NSCLC management, the ESCAT classification system,

initially published in 2018, calls for an update to incorporate the insights offered by this classification. This article serves as an update on the ESCAT level 1 driver mutations identified within our population.

Objectives

❖ To delineate the mutational profile of patients diagnosed with NSCLC in the Mexican population through NGS.
❖ To perform an analysis of PFS and OS in patients diagnosed with advanced mutated adenocarcinoma NSCLC.

We present this article in accordance with the REMARK reporting checklist (available at https://jtd.amegroups.com/article/view/10.21037/jtd-23-1029/rc).

Methods

This was a descriptive, observational, retrospective study aimed at delineating the molecular mutations observed in patients with NSCLC of the adenocarcinoma subtype through NGS analysis of liquid biopsy samples obtained from Mexican patients, our group was able to access NGS via liquid biopsy through a financial requirement access, which did not include tissue evaluation by NGS. The study was conducted at the Instituto Nacional de Enfermedades Respiratorias – Ismael Cosio Villegas, located in Mexico City, within the Department of Thoracic Oncology. A comprehensive review of electronic clinical records spanning the last 5 years resulted in the identification of a total of 127 patients. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by Institutional Ethics Board Committee "Comité de Ética en Investigación Instituto Nacional de Enfermedades Respiratorias" (E08-19) and informed consent was taken from all the patients.

Patients with a confirmed NSCLC diagnosis were assessed through liquid biopsy samples, offering them the opportunity to receive targeted therapy based on the results. Before conducting a liquid biopsy, we determined the *EGFR* mutation status by analyzing tumor tissue samples with the Idylla™ real-time PCR method, using the Idylla™ *EGFR* Mutation Test. Patients without detectable *EGFR* mutations were subsequently subjected to liquid biopsy analysis via NGS prior to the initiation of first-line therapy. Our study concentrated on the molecular insights derived from the liquid biopsy results, which were processed using

Box 1 Genes explored by next-generation sequencing

ABL1	*AKT1*	*ALK*	*ARAF*	*BRAF*
BRCA1	*BRCA2*	*BTK*	*CCND1*	*CD274*
CDH1	*CDK4*	*CDK6*	*CDKN2A*	*CRKL*
CTNNB1	*DDR2*	*EGFR*	*ERBB2*	*ERRFI1*
ESR1	*EZH2*	*FGFR1*	*FGFR2*	*FGFR3*
FLT3	*FOXL2*	*GNA11*	*GNAQ*	*GNAS*
HRAS	*IDH1*	*IDH2*	*JAK2*	*JAK3*
KIT	*KRAS*	*MAP2K1*	*MAP2K2*	*MDM2*
MET	*MPL*	*MTOR*	*MYC*	*MYCN*
MYD88	*NF1*	*NPM1*	*NRAS*	*PDCD1LG2*
PDGFRA	*PDGFRB*	*PIK3CA*	*PTEN*	*PTPN11*
RAF1	*RET*	*ROS1*	*SMO*	*TERT*
TP53	*VEGFA*			

NGS technology provided by Foundation Medicine, a pharmaceutical and molecular diagnostics company. This comprehensive analysis explored more than 60 genes (*Box 1*), detecting a range of mutations, including insertions, deletions, and base substitutions.

Statistical analysis

Statistical analysis encompassed a descriptive examination of categorical variables, with their values expressed in terms of number and percentage. These variables included gender, age, smoking status, exposure to wood smoke, asbestos exposure, chronic obstructive pulmonary disease (COPD), type 2 diabetes mellitus, systemic arterial hypertension, Eastern Cooperative Oncology Group (ECOG) performance status, stage of diagnosis, presence of brain and liver metastases, clinical presentation, and mutation type. The impact of mutation type on PFS and OS was determined by calculating 95% confidence intervals (CIs) and P values. A multivariate logistic regression analysis was conducted. Interferential comparisons were facilitated through Student's *t*-distribution or Mann-Whitney *U* tests, depending on the data distribution as determined by the Kolmogorov-Smirnov test. The chi-square and Fisher's exact tests were utilized to assess the statistical significance of each categorical variable.

Survival analyses were performed using the log-rank test to compare outcomes between groups, with results deemed statistically significant if P≤0.05. Variables found to be significant or of borderline significance were integrated into the logistic regression analysis if the relationship exceeded a 10% threshold. Treatment response was evaluated according to Response Evaluation Criteria in Solid Tumours version 1.1 (RECIST v1.1) criteria and expressed as a percentage. PFS was calculated as the time elapsed from the initiation of treatment to the date of progression, while OS was measured from the date of diagnosis to the date of death. These survival analyses were conducted using the Kaplan-Meier technique, and group comparisons were made through the log-rank test. The relative risk for each profile was calculated, with the profile exhibiting the highest risk ratio being selected. Data analysis was performed using SPSS version 19 software.

Results

Population characteristics

A total of 127 patients diagnosed with advanced NSCLC, specifically of the adenocarcinoma subtype, were included in the analysis. These patients were treated at a Mexican national reference cancer center, and they all tested negative for the classic *EGFR* mutation via reverse transcription-polymerase chain reaction (rtPCR). Subsequently, they underwent NGS analysis using liquid biopsy samples. Among the entire population, there were 60 male patients (47.2%) and 67 females (52.8%), with an average age of 60 years, ranging from 27 to 87 years. The majority of patients were smokers (52.8%), with an average smoking index of 20.57±28.08. Additionally, 32.3% of patients had been exposed to asbestos, and 46.5% had a history of wood smoke exposure. In terms of associated comorbidities, only 9.9% had been diagnosed with COPD, 11.8% had type 2 diabetes mellitus, and 29.1% had chronic hypertension.

At the time of diagnosis, the functional status of the patients, as measured by the ECOG scale, indicated that 89.0% had an ECOG status of 0–1, while 11% had an ECOG status of 2. At the time of diagnosis, 100 patients were classified as stage IV (91.3%), and 17 patients (13.4%) presented with brain metastases, while 8 patients (6.3%) had liver metastases. Lung cancer patients exhibited a wide range of clinical symptoms; in this population, 78.7% presented with cough, followed by dyspnea (59.1%), chest pain (40.2%), weight loss (23.6%), and hemoptysis (15.7%). The complete demographic data for the population is presented in *Table 1*.

Table 1 Population characteristics

Characteristics	Values
Gender, n (%)	
Male	60 (47.2)
Female	67 (52.8)
Age, years, mean ± SD [range]	60.9±12.7 [27–87]
Smoking status	
Never-smoker, n (%)	60 (47.2)
Smoker, n (%)	67 (52.8)
Smoking index (pack years), mean ± SD	20.57±28.08
Exposure to wood smoke, n (%)	59 (46.5)
Asbestos exposure, n (%)	41 (32.3)
Comorbidities, n (%)	
COPD	12 (9.9)
T2D	15 (11.8)
SAH	37 (29.1)
ECOG, n (%)	
0–1	113 (89.0)
2	14 (11.0)
Stage, n (%)	
II–III	11 (8.7)
IV	116 (91.3)
Brain metastases, n (%)	17 (13.4)
Liver metastases, n (%)	8 (6.3)
Clinical characteristic, n (%)	
Chest pain	51 (40.2)
Dyspnea	75 (59.1)
Weight loss	30 (23.6)
Cough	100 (78.7)
Hemoptysis	20 (15.7)

SD, standard deviation; COPD, chronic obstructive pulmonary disease; T2D, type 2 diabetes; SAH, systemic arterial hypertension; ECOG, Eastern Cooperative Oncology Group.

Genetic mutations identified

One of the primary objectives of this study was to identify the most common mutations in patients, which were analyzed via NGS before the initiation of targeted therapy. Out of the 127 patients, 22 (17.3%) did not have an identifiable mutation. Among these 22 cases, 7 patients had variants of uncertain significance (VUS), while 15 patients had no identifiable mutations at all. The most prevalent mutation identified was *EGFR*, found in 24 patients (18.9%), followed by Kirsten rat sarcoma viral oncogene homolog (*KRAS*) in 20 patients (15.7%), tumor protein 53 (*TP53*) as the primary target mutation in 13 patients (10.2%), ALK in 11 patients (8.7%), and p110α subunit of phosphatidylinositol 3-kinases (*PIK3CA*) in 6 patients (4.7%). Other significant mutations included rearranged during transfection (*RET*) in 5 patients (3.9%), v-raf murine sarcoma viral oncogene homolog B1 (*BRAF*) in 4 patients (3.1%), breast cancer (*BRCA*)1/2 gene and human epidermal growth factor receptor 2 (*HER2*) in 3 patients each (2.4%), and cyclin dependent kinase inhibitor 2A (*CDKN2A*), isocitrate dehydrogenase 2 (*IDH2*), mesenchymal epithelial transition factor (*MET*), and myelocytomatosis (*MYC*), each identified in 2 patients (1.6%). Additional mutations, such as 'orphan' c-ros oncogene 1 (*ROS1*), telomerase reverse transcriptase (*TERT*), neuroblastoma RAS viral oncogene homolog (*NRAS*), neurofibromin type 1 (*NF1*), Janus kinase 2 (*JAK2*) gene, fibroblast growth factor receptor 2 (*FGFR2*), FGFR3, and fms-like tyrosine kinase 3 (*FLT3*), were each identified in a single patient (0.8%). A summary of all the mutations identified in the population can be found in *Figure 1*.

Thirteen patients were identified with *TP53* as the primary target mutation, while a total of 65 patients exhibited *TP53* mutations as co-mutations. From our population, 46 patients (36.2%) received targeted therapy, 69 patients (54.33%) received chemotherapy, and 12 cases (9.45%) did not receive any treatment. Patients who received targeted therapy did so from the moment of diagnosis and throughout the course of their disease (*Figure 2*).

Factors influencing PFS

The overall median PFS was 9 months (95% CI, 8.34–9.65) (*Figure 3*). Patients without any identified mutations had a median PFS of 8 months (95% CI, 7.08–8.91). Median PFS in months was analyzed for each identified mutation. *ALK* mutations exhibited the longest PFS at 23 months (95% CI, 18.68–27.31), followed by *FGFR* with 19 months (NA), *EGFR* with 15 months (95% CI, 13.12–16.87), and *MYC* with 11 months (NA). *BRCA* (95% CI, 5.19–14.80), *BRAF* (95% CI, 5.75–14.24), and *RET* (95% CI, 3.61–12.38) displayed a median PFS of 10 months, with *KRAS* (95% CI, 6.95–9.04), *TERT* (NA), *JAK2* (NA), *IDH2* (NA), and
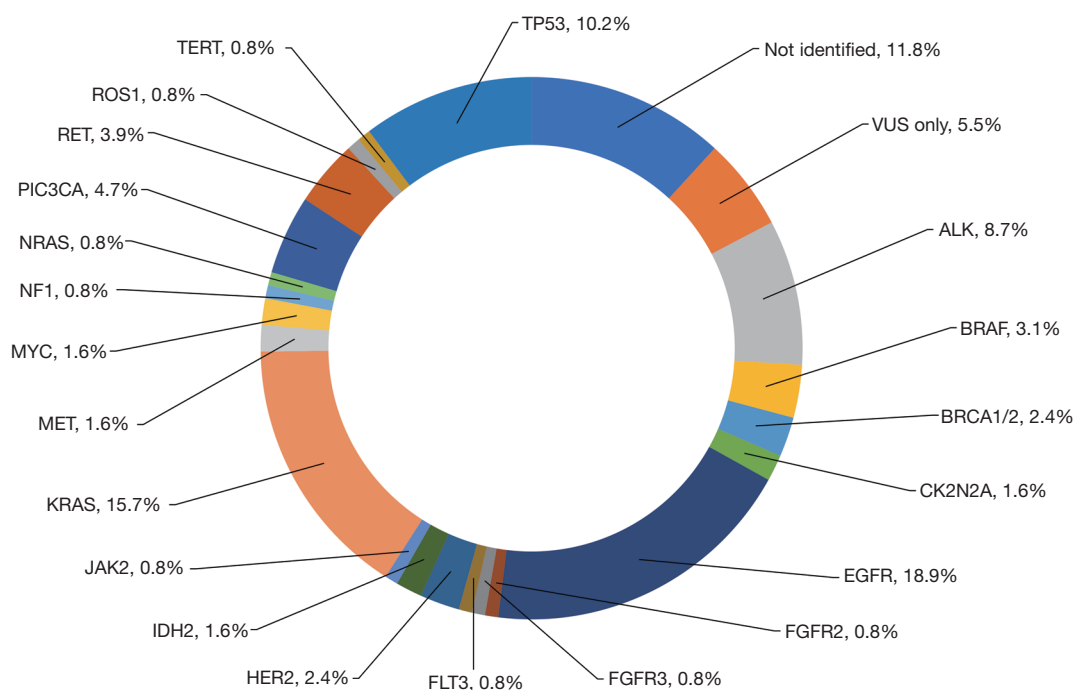
Figure 1 Genetic mutations identified in our population and their respective percentage.

HER2 (NA) following with an 8-month PFS. The mutations with lower PFS included *TP53* (95% CI, 1.34–10.52) and *PIK3CA* (95% CI, 1.34–12.65) with a 7-month PFS, while *MET* (NA) and *FLT3* (NA) had a 6-month PFS, and *NF1* (NA) a 5-month PFS, with *NRAS* (NA) displaying a 4-month PFS.

Notably, statistically significant differences were observed among the median PFS of mutations, with *ALK*, *EGFR*, *KRAS*, *FGFR*, and *MYC* being the most statistically significant. Worth mentioning, eight out of the eleven patients with *ALK* mutations received targeted therapy, and 14 out of 24 patients with *EGFR* mutations received the same treatment.

From the variables under study, a univariate analysis was conducted to identify the statistically significant variables for PFS. Subsequently, a multivariate analysis was performed, which included COPD, ECOG, brain MRI, tumor mutation burden (TMB), *ALK*, *EGFR*, *KRAS*, *FGFR*, *MYC*, and protein kinase B (*AKT*) (as an identified co-mutation, not a target mutation).

Although the previously mentioned variables yielded significant P values in the univariate analysis, *ALK* [hazard ratio (HR): 0.258, P<0.001], *EGFR* (HR: 0.538, P=0.041), TMB (HR: 2.073, P=0.042) and brain MRI (HR: 0.470, P=0.032) retained statistical significance in the multivariate

analysis. Additionally, ESCAT Level 1 mutations identified in our population included KRAS (HR: 1.37, P=0.24), while MET, RET, and BRAF did not reach significance in the univariate statistical analysis, potentially due to the relatively small population sample. The complete list of the univariate and multivariate analyses for each factor concerning PFS is provided in *Table 2*.

Factors influencing OS

The OS for the studied population stood at 14 months (95% CI, 12.43–15.56), boasting an impressive 1-year survival rate of 70% (*Figure 4*). Patients without identifiable mutations exhibited a median OS of 12 months (95% CI, 10.85–13.14). Among the analyzed mutations, *ALK* mutation took the lead with the highest median OS, standing at 35 months (95% CI, 25.28–44.71), closely followed by *EGFR* with 23 months (95% CI, 21.44–24.55). *FGFR* (NA) showed a respectable 19-month OS, while *MYC* (NA) demonstrated a commendable 16-month OS. Other mutations, such as *BRCA* (95% CI, 9.19–18.80), achieved a 14-month OS, whereas *BRAF* (95% CI, 6.14–19.86), *IDH2* (NA), and *HER2* (95% CI, 6.59–19.40) achieved a 13-month OS. *NF1*, *JAK2*, and *KRAS* displayed a 12-month OS, while *TP53* and *TERT* were associated with an 11-month OS. On the lower
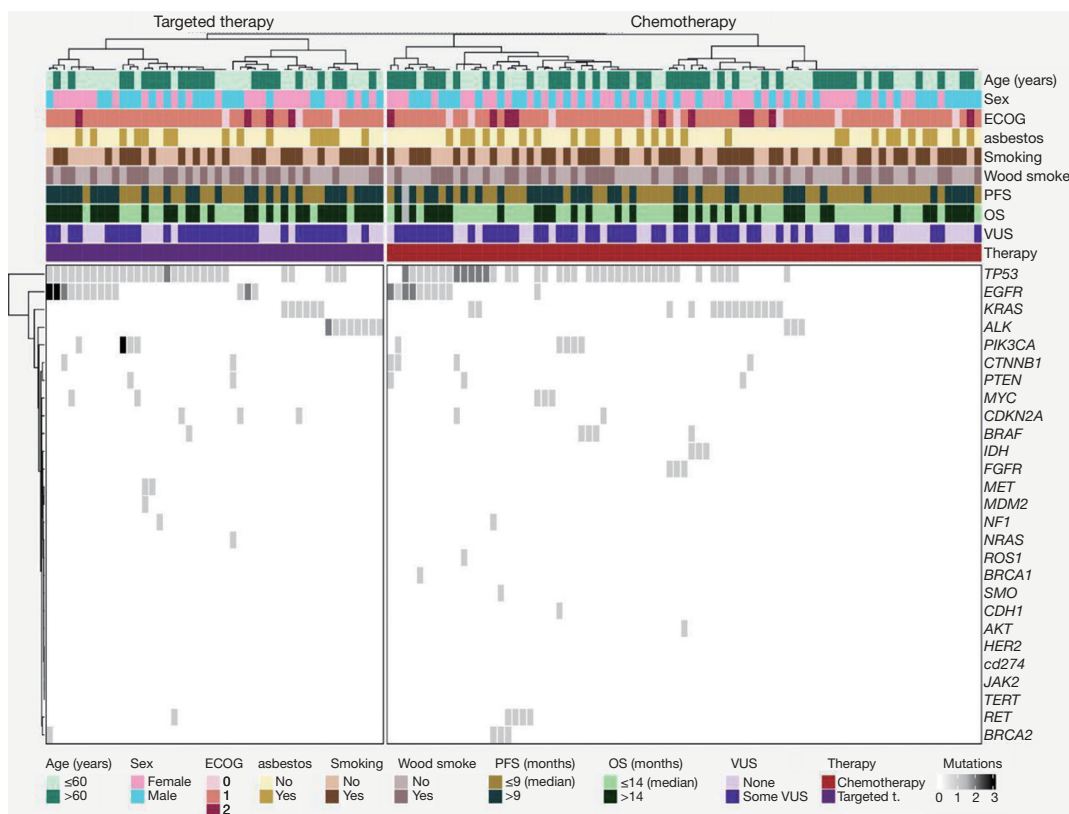
Figure 2 Heatmap describing our population characteristics based on the therapy received (targeted therapy *vs.* chemotherapy), including the genetic mutations present. ECOG, Eastern Cooperative Oncology Group; PFS, progression-free survival; OS, overall survival; VUS, variants of uncertain significance.
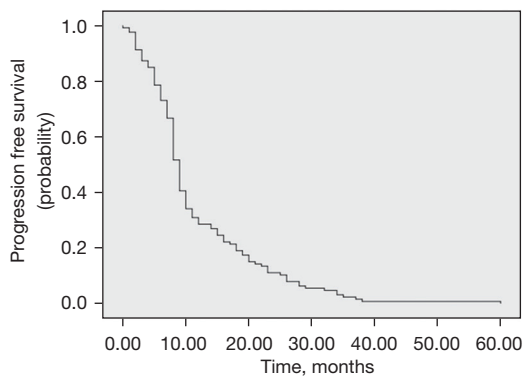
Figure 3 Kaplan-Meier curve demonstrating progression-free survival in our population.

end of the OS spectrum, *FLT3* presented a 10-month OS, while *PIK3CA* exhibited a 9-month OS. *MET*, on the other hand, displayed a 7-month OS, and *NRAS* was associated with a 4-month OS. *CDKN2A* had a mere 2-month OS.

The same analytical approach, as employed in the analysis of PFS, was applied to OS. This comprehensive analysis included variables such as COPD, dyspnea, thoracic pain, ECOG, PET-CT, brain MRI, TMB, *KRAS*, ALK, *EGFR*, *AKT*, *CTNNB1*, and *CD274* (with *AKT*, *CTNNB1*, and *CD274* being identified as co-mutations rather than target mutations).

While all the aforementioned factors demonstrated statistical significance in the univariate analysis, *ALK* (HR: 0.285, P<0.001) and *EGFR* (HR: 0.482, P=0.024) retained statistical significance in the multivariate analysis. It's essential to highlight those additional mutations found in our population, which hold significance, include *KRAS* (HR: 1.256, P=0.40). However, mutations like *RET*, *MET*, and *BRAF* did not achieve significance in the univariate analysis. It's crucial to note that this lack of statistical significance may be attributed to the relatively small sample size of the population. For a complete list of factors with their respective univariate and multivariate analysis outcomes, please refer to *Table 3*.

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Table 2 Univariate and multivariate analysis of significant characteristics/factors influencing PFS

Characteristics/factors	Univariate analysis		Multivariate analysis	
	Median (95% CI)	P value[†]	Hazard ratio	P value[‡]
Overall	9 (8.34–9.65)			
No. of pathogenic mutations	–	0.21	1.164 (0.970–1.397)	0.10
Target therapy	–	0.21	0.798 (0.559–1.141)	0.21
COPD	–	0.04	1.050 (0.483–2.283)	0.90
ECOG	–	0.04	1.315 (0.851–2.033)	0.21
Brain MRI	–	0.03	0.470 (0.236–0.938)	0.032
TMB	–	0.01	2.073 (1.025–4.191)	0.042
AKT	–	0.01	0.671(0.068–6.602)	0.73
KRAS	8 (6.95–9.04)	0.04	1.37 (0.810–2.32)	0.24
ALK	23 (18.68–27.31)	<0.001	0.258 (0.126–0.53)	<0.001
BRCA	10 (5.19–14.80)	0.19	–	–
ROS1	10 (NA)	0.42	–	–
EGFR	15 (13.12–16.87)	0.06	0.538 (0.297–0.975)	0.041
MYC	11 (NA)	0.01	0.463 (0.194–1.1)	0.081
NRAS	4 (NA)	0.20	–	–
BRAF	10 (5.75–14.24)	0.47	–	–
TP53	7 (1.34–10.52)	0.40	–	–
RET	10 (3.61–12.38)	0.50	–	–
PIK3CA	7 (1.34–12.65)	0.49	–	–
NF1	5 (NA)	0.19	–	–
FGFR	19 (NA)	0.005	0.878 (0.323–2.39)	0.79
MET	6 (NA)	0.10	–	–
TERT	8 (NA)	0.34	–	–
JAK2	8 (NA)	0.34	–	–
IDH2	8 (NA)	–	–	–
HER2	8 (NA)	0.28	–	–
FLT3	6 (NA)	–	–	–

[†], Pearson's correlation; [‡], Cox regression. NA, not available due to sample including only one patient. PFS, progression-free survival; CI, confidence interval; COPD, chronic obstructive pulmonary disease; ECOG, Eastern Cooperative Oncology Group; MRI, magnetic resonance imaging; TMB, Tumor Mutational Burden; AKT, protein kinase B; KRAS, Kirsten rat sarcoma viral oncogene homolog; ALK, anaplastic lymphoma kinase; BRCA, BReast CAncer gene; ROS1, 'orphan' receptor tyrosine kinase; EGFR, epidermal growth factor receptor; MYC, myelocytomatosis; NRAS, neuroblastoma RAS viral oncogene homolog; BRAF, v-raf murine sarcoma viral oncogene homolog B1; TP53, tumor protein 53; RET, rearranged during transfection; PIK3CA, p110α subunit of Phosphatidylinositol 3-kinases; NF1, Neurofibromin type 1; FGFR, fibroblast growth factor receptor; MET, mesenchymal epithelial transition factor; TERT, Telomerase reverse transcriptase; JAK2, Janus Kinase 2 gene; IDH2, isocitrate dehydrogenase 2; HER2, human epidermal growth factor receptor 2; FLT3, fms-like tyrosine kinase 3.
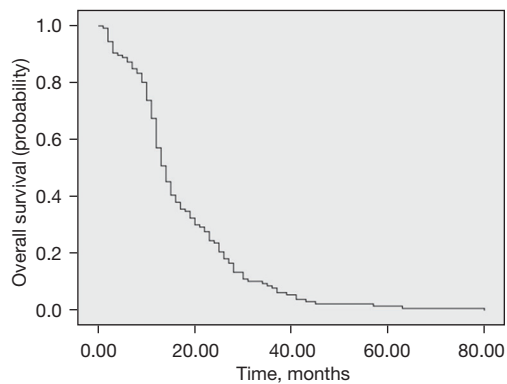
Figure 4 Kaplan-Meier curve demonstrating overall survival in our population.

Updating the ESCAT classification system

A distinctive contribution of this paper is the revision of the ESCAT classification system for NSCLC. The ESCAT system was initially published in 2018 in the Annals of Oncology. Our revision stems from the identification of ESCAT level 1 driver mutations within our study population. In recent years, substantial advancements have been made in the management of NSCLC patients, particularly in the realm of innovative treatments designed for specific target mutations. As such, we view it as paramount to recognize mutations categorized as ESCAT level 1 based on the ESCAT definitions. To accomplish this, we drew upon the National Comprehensive Cancer Network (NCCN) guidelines, insights gleaned from recent phase 1, 2, and 3 clinical trials, and findings from high-impact publications. Furthermore, we collaborated with certified medical thoracic oncologists from the National Institute of Respiratory Diseases in Mexico City. Our aim is to provide fellow clinicians with up-to-date information about mutations that already have available targeted therapies to guide treatment decisions. We also emphasize that NGS can unveil previously unidentified target mutations when compared to traditional rtPCR methods.

It's imperative to underline that ESCAT level 1 mutations are associated with validated drugs, providing a clear direction for treatment decisions. Our study population comprised 32.3% of patients harboring ESCAT level 1 mutations, despite previously testing negative for *EGFR* mutations through rtPCR. The mutations we identified encompassed a range of pathogenic mutations, including KRAS G12C, *ALK* fusion, *RET* fusion, *EGFR* deletion on exon 19, *EGFR L858R*, *EGFR* insertion on exon 20, *MET* splicing on exon 14, *BRAF V600E*, and *ROS1* fusion. Each of these mutations corresponds to specific pathogenic alterations, which can be deletions, insertions, or fusions, that categorize them as ESCAT level 1 (IA or IB).

In our population, we identified 3 cases with the *BRAF V600E* mutation, this mutation is notable as it shows excellent responses to target therapy. A similar scenario unfolded in cases involving *EGFR* mutations. Out of the 24 *EGFR*-positive cases identified, only 14 received targeted therapy. This situation is lamentable, not just within our country, but in numerous developing countries, where many patients are unable to bear the financial burden of these drugs, which could otherwise offer a more promising prognosis.

While 36% of our patients received targeted therapy, and 32% were identified with ESCAT level 1 driver mutations, the higher percentage of cases receiving targeted treatment can be attributed to scenarios in which patients classified as ESCAT level 2 became participants in research protocols that granted them access to targeted therapies. Conversely, those with ESCAT level 1 driver mutations who did not receive targeted therapy were impeded by their inability to afford the treatment, along with limitations stemming from our institution.

Discussion

Lung cancer remains the fourth leading cause of cancer-related mortality in Mexico, and regrettably, it is diagnosed at an advanced stage in 68.5% of cases (1,19). The integration of biomarkers into lung cancer management for treatment, prognosis, and diagnosis has become indispensable. However, even in developed nations, not all recommended biomarkers, which include *EGFR* mutations, *ALK* fusions, *ROS1* fusions, *BRAF V600E* mutations, *RET* fusions, *MET* amplification, *MET* exon-14 skipping variants, and *ERBB2* (*HER2*) mutations, are consistently tested. This discrepancy is often due to the unavailability of tumor tissue and the rapid deterioration of a patient's condition (20). As a result, patients with early mutations often initiate chemotherapy regimens (accounting for 19% of cases) instead of receiving targeted therapies at the outset of their disease (21).

Recent advancements in the identification of predictive biomarkers using models capable of providing a broader genomic view have yielded promising results in the management of NSCLC patients (22). For instance, the utilization of extensive DNA sequencing via liquid biopsy has proven as effective as direct tumor tissue analysis,

Table 3 Univariate and multivariate analysis of significant characteristics/factors influencing OS

Characteristics/factors	Univariate analysis		Multivariate analysis	
	Median (95% CI)	P value[†]	Hazard ratio (95% CI)	P value[‡]
Overall	14 (12.43–15.56)			
Number of pathogenic mutations	–	0.35	1.134 (0.916–1.404)	0.24
Target therapy	–	0.28	0.652 (0.446–0.952)	0.027
COPD	–	0.02	1.466 (0.654–3.29)	0.35
Dyspnea	–	0.02	1.373 (0.886–2.178)	0.17
Thoracic Pain	–	0.05	1.268 (0.840–1.912)	0.25
ECOG	–	0.01	1.398 (0.875–2.234)	0.16
PET-CT	–	0.07	0.859 (0.527–1.402)	0.54
Brain MRI	–	0.001	0.595 (0.272–1.303)	0.19
TMB	–	0.02	1.727 (0.834–3.575)	0.14
AKT	–	0.06	0.560 (0.057–5.507)	0.61
CTNNB1	–	0.06	1.651 (0.539–5.057)	0.38
CD274	–	<0.001	0.491 (0.049–4.904)	0.54
KRAS	12 (7.61–16.38)	0.04	1.256 (0.734–2.147)	0.40
ALK	35 (25.28–44.71)	<0.001	0.285 (0.141–0.574)	<0.001
BRCA	14 (9.19–18.80)	0.16	–	–
ROS1	11	0.30		
EGFR	23 (21.44–24.55)	0.009	0.482 (0.256–0.908)	0.024
MYC	16 (NA)	0.12	–	
NRAS	4 (NA)	0.14	–	–
BRAF	13 (6.14–19.86)	0.30	–	–
TP53	11 (8.65–13.34)	0.36	–	–
CDKN2A	2 (NA)	0.28	–	–
RET	11 (0–25.03)	0.30	–	–
PIK3CA	9 (2.99–15.00)	0.36	–	–
NF1	12 (NA)	0.24	–	–
FGFR	19 (NA)	0.07	0.751 (0.289–1.952)	0.55
MET	7 (NA)	0.26	–	–
TERT	11 (NA)	0.30	–	–
JAK2	12 (NA)	0.33	–	–
IDH2	13 (NA)	–	–	–
HER2	13 (6.59–19.40)	0.45	–	–
FLT3	10 (NA)	–	–	–

[†], Pearson's correlation; [‡], Cox regression. NA, not available due to sample including only one patient. OS, overall survival; CI, confidence interval; COPD, chronic obstructive pulmonary disease; ECOG, Eastern Cooperative Oncology Group; MRI, magnetic resonance imaging; PET-CT, positron emission tomography/computed tomography; TMB, tumor mutational burden; AKT, protein kinase B; CTNNB1, catenin beta 1; CD274, PDL1; KRAS, Kirsten rat sarcoma viral oncogene homolog; ALK, anaplastic lymphoma kinase; BRCA, BReast CAncer gene; ROS1, 'orphan' receptor tyrosine kinase; EGFR, epidermal growth factor receptor; MYC, myelocytomatosis; NRAS, neuroblastoma RAS viral oncogene homolog; BRAF, v-raf murine sarcoma viral oncogene homolog B1; TP53, tumor protein 53; CDKN2A, cyclin dependent kinase inhibitor 2A, RET, rearranged during transfection; PIK3CA, p110α subunit of Phosphatidylinositol 3-kinases; NF1, Neurofibromin type 1; FGFR, fibroblast growth factor receptor; MET, mesenchymal epithelial transition factor; TERT, Telomerase reverse transcriptase; JAK2, Janus Kinase 2 gene; IDH2, isocitrate dehydrogenase 2; HER2, human epidermal growth factor receptor 2; FLT3, fms-like tyrosine kinase 3.

offering a non-invasive method that exhibits high sensitivity for both NSCLC and solid tumors (23). Full cell-free DNA (cfDNA) analysis through NGS has demonstrated high specificity and strong concordance with tissue-based genotyping. In fact, the use of liquid biopsy, involving a 63-gene sequencing panel via NGS, as compared to real-world tissue analysis, offers sensitivity of 85% and specificity of 99.9% (24-26).

Our study stands out as the initial descriptive and analytical research carried out within the Mexican population to correlate specific molecular mutations in NSCLC patients. We employed NGS using a 62-gene panel to analyze liquid biopsy samples and explore their impact on both PFS and OS.

To describe the clinical characteristics of the entire population with the identified genetic mutations and the therapy received, a heatmap (*Figure 2*) was created. This heatmap visually represents the population, initially divided into those who received targeted therapy or chemotherapy, along with their respective clinical characteristics and identified genetic mutations.

A prior study conducted on 90 Mexican NSCLC patients using NGS unveiled *TP53* (47.8%) and *EGFR* (36.7%) as the most prevalent mutations (27). Another study conducted on 152 Asian patients, analyzing circulating DNA from lung cancer patients, revealed that the most common alterations in their population were *EGFR* at 46.05%, *ALK* at 7.24%, *KRAS* at 6.58%, and *PIK3CA* at 6.58%, respectively (28). This contrasts with a study conducted on 229 European patients, in which the most common alterations were *TP53* at 47.6%, *DNMT3A* at 33.2%, *EGFR* at 20.1%, and *KRAS* at 15.7% (29). In our series, *TP53* was identified as a sole mutation in 10.3% of patients (13 individuals), while it was found in association with other co-mutations in an additional 51.1% of cases. *EGFR* (24 cases), *KRAS* (20 cases), *ALK* (11 cases), and *ROS1* (1 case) represented other common mutations. These results underscore the diversity of mutations present in NSCLC patients within the Mexican population. Nevertheless, our data analysis did not reveal a correlation between these mutations and worse OS or PFS. This can be attributed to the fact that while *TP53* mutations are linked to poor prognosis, they do not predict the response to chemotherapy treatment (30,31). These mutations, on the other hand, are predictive of immunotherapy agents. Unfortunately, the majority of the population lacks access to these treatments. Notably, 34 out of the total 65 cases with *TP53* mutations had a history of tobacco smoking, aligning with findings in the existing literature (32).

An especially significant contribution of our research is the identification and classification of ESCAT level 1 mutations. In our study population, 32.3% (41 patients) presented an actionable genomic variation at ESCAT level 1. This percentage exceeds the reported 29% of NSCLC patients with potentially treatable mutations in prospective studies (33). This finding holds particular importance, underscoring that even in patients preselected as *EGFR*-negative, NGS can identify target mutations, guiding treatment decisions effectively.

In our study population, *EGFR* mutations were the most frequently mutated gene (18.9%), closely followed by *KRAS* (15.8%). Notably, our population, preselected for *EGFR* mutations, included a substantial proportion of resistance mutations to TKIs and other less common mutations. In a prior study conducted by our group, we demonstrated that 38.19% of cases in the Mexican population tested positive for *EGFR* mutations through rtPCR (19). Our results identified *EGFR* mutations in various forms, including six cases with mutations on exon 21 L858R, two of which had co-mutations with T790M. Additionally, there were 14 cases associated with mutations on exon 19, two cases with insertion mutations on exon 20, one case associated with a mutation in exon 18 and one with an impact on exon 7. Another study involving 153 NSCLC patients with deletion mutations on exon 19 discovered 22 distinct deletion types, indicating that unconventional sensitive mutations often elude detection by conventional studies such as rtPCR. Consequently, these patients may miss the opportunity to receive targeted therapy unless they undergo NGS (34). This scenario applies to a subset of the mutations identified in our patients, as some of these mutations may not meet the criteria for targeted therapies. One notable example is the situation involving exon 7, which does not encode for the tyrosine kinase receptor. Consequently, it becomes imperative to explore alternative treatment modalities. In our series, 13 patients with *EGFR* mutations became eligible for targeted therapy following NGS analysis. This underscores the utility of NGS in identifying less common sensitive mutations for which target therapies are available. Patients with these mutations can benefit from the latest generation of targeted therapies, such as amivantamab for insertion mutations on exon 20, where no treatment options were previously available, or its combination with lazertinib, which is effective for *EGFR-MET* mutations (35). The presence of *BRAF V600E* mutation, an uncommon mutational variant found in 1–4% of pulmonary

adenocarcinomas, is notable as it shows excellent responses to combination treatments with *BRAF* and *MEK* inhibitors, even being reported in studies involving isolated circulating tumor cells (36).

Among our study population, 15.8% had *KRAS* mutations, with 1.57% of these cases involving the p.G12C mutation. These findings diverge from prior studies, where the p.G12C mutation occurred in 13% of *KRAS* cases. This discrepancy can be attributed to the small sample size and racial distinctions. Among these patients, five cases had the p.G12C mutation, while eight cases had co-mutations with *TP53*. In an Italian study involving 194 NSCLC patients, NGS using the SiRe panel identified molecular alterations, with 5% of their cases exhibiting KRAS mutations and no relevant additional genes reported. Remarkably, the p.G12C alteration was found in 36% of their population (37). Other studies report *KRAS* mutations in 25% of NSCLC patients, with the p.G12C alteration occurring in 13%, highlighting the importance of identifying these mutations, as they respond well to target therapies, offering better disease control rates (80.6%) and prolonged PFS (38).

Our study underscores that utilizing NGS as a method to guide treatment in the Mexican population is a pivotal factor in improving patient survival. The need for new diagnostic tools with high sensitivity and specificity is paramount in identifying genetic mutations that enable patients to receive targeted therapies early in the course of their disease, potentially enhancing both OS and PFS. In developing countries such as Mexico, the transition to employing these novel technologies to guide treatment is an essential step towards providing appropriate and individualized care.

Conclusions

Massive DNA sequencing conducted in the Mexican population reveals that 32.3% of patients who tested negative for an *EGFR* mutation via rtPCR actually possess target mutations. This finding implies that these patients can initiate targeted therapy at an early stage of their disease, which is associated with prolonged survival. The implementation of massive DNA sequencing is proven to elevate survival rates among patients with non-small cell lung carcinoma. Therefore, the adoption of this technology is essential for guiding the treatment of lung cancer.

Acknowledgments

Footnote

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