

Mutational profile by targeted next generation sequencing of non-small cell lung cancer in the Mexican population

Norma Hernández-Pedro, PhD,⁽¹⁾ Giovanni Soca-Chafre, PhD,⁽¹⁾ Carmen Alaez-Versón, PhD,⁽²⁾ Karol Carrillo-Sánchez, BsC,⁽²⁾ Alejandro Avilés-Salas, MD,⁽³⁾ Edgar Vergara, PhD,⁽⁴⁾ Oscar Arrieta, MSc.⁽⁴⁾

Hernández-Pedro N, Soca-Chafre G, Alaez-Versón C, Carrillo-Sánchez K, Aviles-Salas A, Vergara E, Arrieta O. Mutational profile by targeted next generation sequencing of non-small cell lung cancer in the Mexican population. *Salud Publica Mex.* 2019;61:308-317. <https://doi.org/10.21149/10113>

Abstract

Objective. Targeted next-generation sequencing (t-NGS) has revolutionized clinical diagnosis allowing multiplexed detection of genomic alterations. This study evaluated the profile of somatic mutations by t-NGS in Mexican patients with non-small cell lung cancer (NSCLC). **Materials and methods.** Genomic DNA was extracted from 90 lung adenocarcinomas and sequences were generated for a panel of 48 cancer genes. Epidermal Growth Factor Receptor (EGFR) mutations were detected in parallel by quantitative PCR. **Results.** The mutational profile of NSCLC revealed alterations in 27 genes, where TP53 (47.8%) and EGFR (36.7%) exhibited the highest mutation rates. EGFR Q787 mutations were present in 14 cases (15.6%), 10 cases had exon 19 deletions (11.1%), seven cases had L858R (7.8%). The mutational frequency for genes like EGFR, MET, HNF1A, HER2 and GUSB was different compared to caucasian population. **Conclusion.** t-NGS improved NSCLC treatments efficacy due to its sensitivity and specificity. A distinct pattern of somatic mutations was found in Mexican population.

Keywords: mutational analysis, DNA; adenocarcinoma; lung; DNA sequencing

Hernández-Pedro N, Soca-Chafre G, Alaez-Versón C, Carrillo-Sánchez K, Aviles-Salas A, Vergara E, Arrieta O. Perfil mutacional por secuenciación de nueva generación en cáncer de pulmón de células no pequeñas en población mexicana. *Salud Publica Mex.* 2019;61:308-317. <https://doi.org/10.21149/10113>

Resumen

Objetivo. La secuenciación dirigida de nueva generación (SNG) permite la detección múltiple de mutaciones. Este estudio evalúa el perfil de mutaciones somáticas por SNG en pacientes mexicanos con cáncer de pulmón de células no pequeñas (CPCNP). **Material y métodos.** Se aisló ADN de 90 muestras de pacientes con CPCNP y se analizaron 48 genes relacionados con cáncer. Las mutaciones del receptor del factor de crecimiento epidérmico (EGFR) se detectaron por PCR cuantitativa. **Resultados.** Se detectaron alteraciones en 27 genes. Las mutaciones más frecuentes fueron TP53 (47.8%) y EGFR (36.7%). En el gen EGFR, 14 casos fueron mutaciones Q787 (15.6%), 10 presentaron microdeleciones en el exón 19 (11.1%), y siete en L858R (7.8%). La frecuencia de mutación en EGFR, MET, HNF1A, HER2 y GUSB fue diferente en comparación con población caucásica. **Conclusión.** NGS modifica el tratamiento del paciente con CPCNP por su sensibilidad y especificidad para detectar mutaciones. La población mexicana presenta un perfil mutacional particular.

Palabras clave: análisis mutacional del ADN; adenocarcinoma; pulmón; secuencias de ADN

(1) Laboratorio de Medicina Personalizada, Instituto Nacional de Cancerología. Mexico City, Mexico.

(2) Laboratorio de Diagnóstico Genómico, Instituto Nacional de Medicina Genómica. Mexico City, Mexico.

(3) Departamento de Patología, Instituto Nacional de Cancerología. Mexico City, Mexico.

(4) Unidad Funcional de Oncología Torácica, Instituto Nacional de Cancerología. Mexico City, Mexico.

Received on: October 23, 2018 • Accepted on: April 5, 2019

Corresponding author: MSc. Oscar Arrieta. Instituto Nacional de Cancerología. Av. San Fernando 22, Sección XVI. 14080, Mexico City, Mexico.

E-mail: ogarrieta@unam.mx

Lung cancer (LC) is the most common cause of global cancer-related mortality, with over a million deaths each year.¹ Non-small cell lung cancer (NSCLC) represents 85% of the cases, the most frequent histological subtypes are adenocarcinoma, squamous cell carcinoma, and large cell carcinoma.^{2,3} NSCLCs are characterized by a unique pattern of genomic aberrations including mutations, amplifications, deletions, and rearrangements/fusions. Genetic profiling has identified driver mutations in over 80% of adenocarcinomas and approximately 47% of squamous cell carcinoma, many of which are relevant for clinical diagnosis and targeted therapy.^{4,5}

Currently, lung adenocarcinomas are treated on the basis of genomic aberrations to ensure better objective responses. Genomic testing of EGFR and ALK alterations is part of the standard diagnosis in NSCLC.⁶ Patients harboring EGFR mutations in advanced NSCLC benefit from receiving EGFR tyrosine kinase inhibitors (TKIs), like erlotinib, afatinib, gefitinib and osimertinib.⁷ Moreover, crizotinib has shown efficacy for patients with ALK-positive fusions. However, patients harboring sensitizing EGFR mutations develop TKIs resistance within one year.^{8,9}

Next-generation sequencing (NGS) has improved the diagnosis in NSCLC, and has increased the recognition of mutations like MET, BRAF and HER2 as novel targets for personalized therapies.¹⁰ Furthermore, around 37% of the patients receive targeted therapy based in genomic profile.¹¹ Recently, the evaluation of tumor mutational burden by NGS has been a useful predictor of response to treatment.¹²

The mutation profile of many potentially actionable NSCLC genes in the Mexican population of patients remains largely unexplored. Previous studies by our group have described different NSCLC mutations and their relationship with clinical characteristics, such as never-smokers, female gender, wood smoke exposure and prognosis.^{13,14} EGFR mutations frequency vary among ethnic groups with 50-60% incidence in Asian patients, 10-15% in Caucasians, and 25-30% in Hispanics, in this population is particularly associated with female gender and never-smoker.^{4,15} This molecular heterogeneity of NSCLC is particularly high in Latin American countries, including Mexico as shown by the Latin-American Consortium for the Investigation of Lung Cancer (CLICaP, by its acronym in Spanish).^{3,4} The aim of this study was to characterize the presence of potentially actionable mutations in NSCLC in Mexican patients by targeted NGS, and compare the mutation frequencies among other populations.

Materials and methods

Study population

A prospective, two-center study was conducted in patients with locally advanced NSCLC (clinical stage IIIA, IIIB) or oligometastatic disease (clinical stage IV) treated at the Thoracic Oncology Clinic of the *Instituto Nacional de Cancerología* (INCAN) and *Instituto Nacional de Enfermedades Respiratorias* (INER) from April 2015 to April 2018.

Eligible patients were over 18 years old with histologically confirmed locally advanced NSCLC (clinical stage IIIA and IIIB) or oligometastatic disease (clinical stage IV) according to the eighth edition lung cancer stage classification.¹⁶ All recruited patients were required to have a white blood cell count $\geq 3\ 000/\text{mm}^3$, platelets $\geq 100\ 000/\text{mm}^3$, hemoglobin $\geq 12\ \text{gr}/\text{dl}$, creatinine $\leq 1.5\ \text{mg}/\text{dl}$, total bilirubin levels ≤ 1.5 , transaminase levels (TGO, TGP) ≤ 2.5 the upper limit of normal (ULN), alkaline phosphatase < 5 lower ULN. Patients were excluded if they had a history of prior RT or CT at the primary site, were pregnant or lactating, those using anticoagulants in therapeutic doses, patients with concurrent malignant diseases. All patients signed informed consent. Clinical characteristics such as age, sex, smoking status, tumor stage, histology, metastasis and response to treatment were recorded in a database. The protocol was approved by the scientific committee and ethics committee (15/049/ICI and CEI/1023/15).

Samples

From 2015 to 2018, a total of 90 tumor clinical specimens were analyzed as fresh-frozen and formalin-fixed paraffin-embedded (FFPE) samples. Lung adenocarcinomas were classified by a pathologist according to histology as follows: low (lepidic), intermediate (acinar and papillary) and high grade (solid).

DNA extraction

FFPE samples were deparaffinized previous to DNA extraction. DNA was extracted from both FFPE and fresh frozen biopsies using the Genomic DNA Wizard kit (Promega). DNA quantity was evaluated using a Quantus fluorometer (Promega, Mannheim Germany), the minimum amount of DNA required was 50 ng. DNA quality was evaluated using a Nanodrop 2000 Spectrophotometer (Thermo Fisher Scientific Waltham, MA,

USA). DNA extracted from paraffin embedded tissues were analyzed by quantitative PCR (qPCR), with the FFPE QC Kit (Illumina) for detection of inhibitors prior to library preparation.

Library construction and Next-Generation Sequencing

The TruSeq Cancer Panel (Illumina, CA) for 212 amplicons and 48 cancer-related genes was employed as previously described.¹³ Quality control for concentration and size of genomic libraries was performed with a Quantus fluorometer and a 2100 Bioanalyzer (Agilent Technologies, CA, USA). Targeted sequencing was performed on a MiSeq instrument, with an average sequencing depth per base of 1000X.

Sequence analysis and variant calling

Sequences analysis and variant calling were performed using a bioinformatic pipeline developed for this project. The quality of FASTQ files generated in the sequencer was tested by the FASTQC software. High quality sequences were aligned with BWA against hg19 as human reference genome, and processed with the PICARD tools package, which prepares the alignment to work with the GATK program. The GATK program consists of several modules, the first is responsible for realigning the sites of the genome with high propensity to insertions or deletions. The second module recalibrates the quality of reads and alignments variant calls were made with the muTect software. Statistical filters were applied to the variants to distinguish actual mutations from any possible artifacts. The filtered variants were marked regarding their possible functional consequence by snpEff and Variant Studio. EGFR and KRAS mutations were analyzed in parallel by qPCR using the Rotor-Gene Q and the Scorpions and ARMS technologies.

Statistical tests

Statistical tests were performed using SPSS version 24 (SPSS Inc., Chicago, IL, USA). Continuous variables were summarized as arithmetic mean and standard deviation. Nominal variables were shown as ratios and 95% confidence intervals (CI). The association between categorical variables were assessed using χ^2 or Fisher exact tests, the Bonferroni correction was used for multiple comparisons. The Student t or Mann-Whitney U test were used for comparison of population means depending on data distribution. Progression free survival (PFS) and overall survival (OS) was calculated

by Kaplan-Meier method and compared between mutations using the Log Rank or Breslow tests. The multivariate analysis was based on Cox proportion hazard model. A p value <0.05 was considered significant in two-tailed tests.

Results

Demographic characteristics

The clinical-pathological characteristics of the patients are the following: female gender in 76.7% of the cases, median age of 64.5 years with a range of 33-81 years, and 66.7% of 60 years or older (table I). Smokers represented 38.9% of the patients, 43.3% had wood-smoke exposure. The performance status of the patients was predominantly ECOG 0-1 in 84.4%. All cases were adenocarcinomas, 73.3% at advanced stage (IV), predominantly with intermediate histological grade (50%). Metastatic NSCLC was present in 26.7% of the patients in contralateral lung, followed by bones (24.4%), pulmonary effusion (22.2%) and central nervous system (CNS) (18.9%). The carcinoembryonic antigen was elevated (≥ 10 ng/ml) in 47.8% of the patients. Chemotherapy was the treatment in 66.2% of the patients, while 33.8% received tyrosine kinase inhibitors (TKIs).

Somatic mutations

Mutations were found in 27 of 48 cancer-related genes sequenced (table II). TP53 mutations were detected in 43 patients (47.8%). In 36.7% of cases (33/90) mutations in the EGFR gene were found. The most frequent EGFR mutations were Q787 (15.6%), exon 19 deletions (11.1%), L858R mutation in exon 21 (7.8%), and T790M mutation in exon 20 (1.1%). Additional EGFR mutations were A750P and G719A (1.1%). Exon 19 deletions were identified in seven patients by qPCR, while by NGS ten cases were detected. Other mutations were identified, such as in KRAS, MET and PDGFRA (20%), HNF1A (14.4%), APC (12.2%) HER2 (11.1%) and MSH6 (10%). Alterations in lower frequency ($<10\%$) were found in PIK3CA, GUSB, ALK rearrangements, KSR1, KIT, STK11, FLT3, ERBB4, VHL, CTTN1, NOTCH1, GNAS, FGFR3, CDH1, BRAF, ABL1, FBXW7 and RB1.

There were concurrent mutations in TP53 and other genes, including EGFR in 51.2% of the patients ($p=0.006$), MET in 34.9% ($p=0.001$), KRAS in 27.9% ($p=0.073$), and PDGFRA in 39.5% ($p<0.001$); whereas TP53 mutations were mutually exclusive with HNF1 mutations in 93% of the cases ($p=0.054$), with APC in 76.7% ($p=0.002$) and HER2 in 79.1% ($p=0.005$).

Table I
CLINICAL CHARACTERISTICS OF LUNG ADENOCARCINOMA PATIENTS (N=90). MEXICO, 2018

Study Population	Characteristics	All (N=90) % (n/N)
Gender	Female	76.7 (69/90)
	Male	23.3 (21/90)
Age	Median (Range)	64.5 (33-81)
	<60 years	33.3 (30/90)
	≥60 years	66.7 (60/90)
Tobacco-smoking exposure and smoking index	Median (Range)	12.25 (0-120)
	Never-smokers	61.1 (55/90)
	Former-smokers	25.6 (23/90)
	Current-smokers	13.3 (12/90)
Wood-smoke exposure	Absent	56.7 (51/90)
	Present	43.3 (39/90)
ECOG PS	0-1	84.4 (76/90)
	2+	15.6 (14/90)
Disease stage	IIIB	26.7 (24/90)
	IV	73.3 (66/90)
	High	13.3 (12/90)
Histological grade	Intermediate	50.0 (45/90)
	Low	36.7 (33/90)
Metastasis		
Lung	Absent	72.2 (65/89)
	Present	26.7 (24/89)
Bone	Absent	74.4 (67/89)
	Present	24.4 (22/89)
Pulmonary effusion	Absent	76.7 (69/89)
	Present	22.2 (20/89)
CNS	Absent	80.0 (72/89)
	Present	18.9 (17/89)
Lymph nodes	Absent	90.0 (81/89)
	Present	8.9 (8/89)
Liver	Absent	90.0 (81/89)
	Present	8.9 (8/89)
Adrenal	Absent	93.3 (84/89)
	Present	5.6 (5/89)
Other	Absent	88.9 (80/89)
	Present	10.0 (9/89)
CEA	<10 ng/ml	43.3 (39/82)
	≥10 ng/ml	47.8 (43/82)

ECOG PS: Eastern Cooperative Oncology Group Performance Status; CNS: Central Nervous System, CEA: Carcinoembryonic Antigen

Table II
MOLECULAR PROFILE OF SOMATIC MUTATIONS IN MEXICAN PATIENTS WITH NSCLS (N=90). MEXICO, 2018

Gene	All (N=90) % (n/N)	
TP53	Negative	52.2 (47/90)
	Positive	47.8 (43/90)
EGFR	Negative	63.3 (57/90)
	Positive	36.7 (33/90)
EGFR exons	Exon 19 (Deletion)	11.1 (10/90)
	Exon 21 (L858R)	7.8 (7/90)
	Exon 20 (T790M)	1.1 (1/90)
	Exon 20 (Q787)	15.6 (14/90)
	Other	2.2 (4/90)
KRAS	Negative	80.0 (72/90)
	Positive	20.0 (18/90)
MET	Negative	80.0 (72/90)
	Positive	20.0 (18/90)
PDGFRA	Negative	80.0 (72/90)
	Positive	20.0 (18/90)
HNF1A	Negative	85.6 (77/90)
	Positive	14.4 (13/90)
APC	Negative	87.8 (79/90)
	Positive	12.2 (11/90)
HER2	Negative	88.9 (80/90)
	Positive	11.1 (10/90)
MSH6	Negative	90.0 (84/90)
	Positive	10.0 (9/90)
PIK3CA	Negative	91.1 (82/90)
	Positive	8.9 (8/90)
GUSB	Negative	91.1 (82/90)
	Positive	8.9 (8/90)
ALK fusions	Negative	87.8 (79/85)
	Positive	6.7 (6/85)
KSR1	Negative	94.4 (85/90)
	Positive	5.6 (5/90)
KIT	Negative	96.7 (87/90)
	Positive	3.3 (3/90)
STK11	Negative	96.7 (87/90)
	Positive	3.3 (3/90)
FLT3	Negative	98.7 (88/90)
	Positive	2.2 (2/90)
ERBB4	Negative	97.8 (88/90)
	Positive	2.2 (2/90)
VHL	Negative	97.8 (88/90)
	Positive	2.2 (2/90)

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CTNNB1	Negative	97.8 (88/90)
	Positive	2.2 (2/90)
NOTCH1	Negative	97.8 (88/90)
	Positive	2.2 (2/90)
GNAS	Negative	98.9 (89/90)
	Positive	1.1 (1/90)
FGFR3	Negative	98.1 (89/90)
	Positive	1.1 (1/90)
CDH1	Negative	98.9 (89/90)
	Positive	1.1 (1/90)
BRAF	Negative	98.9 (89/90)
	Positive	1.1 (1/90)
ABL1	Negative	98.9 (89/90)
	Positive	1.1 (1/90)
FBXW7	Negative	98.9 (89/90)
	Positive	1.1 (1/90)
RBI	Negative	98.9 (89/90)
	Positive	1.1 (1/90)

TP53: Tumor Protein 53, EGFR: Epidermal Growth Factor Receptor I, KRAS: Kirsten Rat Sarcoma Viral Oncogene Homolog, MET: Mesenchymal Epithelial Transition/Hepatocyte Growth Factor Receptor (HGFR), PDGFRA: Platelet-derived growth factor receptor alpha, HNF1A: Hepatocyte Nuclear Factor 1-Alpha, APC: Adenomatous Polyposis Coli protein, HER2: Epidermal Growth Factor Receptor 2, MSH6: MutS Homolog 6, PIK3CA: Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha, GUSB: Glucuronidase Beta, ALK: Anaplastic Lymphoma Kinase, KSR1: Kinase suppressor of Ras 1, KIT: V-Kit Hardy-Zuckerman 4 Feline Sarcoma Viral, STK11: Serine/Threonine kinase 11, FLT3: FMS-like Tyrosine Kinase 3, ERBB4: Epidermal Growth Factor Receptor 4, VHL: Von Hippel-Lindau tumor suppressor, CTNNB1: Catenin Beta 1, NOTCH1: Neurogenic Locus Notch Homolog Protein 1, GNAS: Guanine Nucleotide Binding Protein (G Protein) Alpha, FGFR3: Fibroblast growth factor receptor 3, CDH1: E-cadherin, BRAF: V-Raf Murine Sarcoma Viral Oncogene Homolog B1, ABL1: Abelson Murine Leukemia Viral Oncogene Homolog 1, FBXW7: F-Box And WD Repeat Domain Containing 7, RBI: Retinoblastoma-Associated Protein.

Associations between mutations and clinical characteristics

There was an association between EGFR ($p=0.005$) and HER2 ($p=0.026$) mutations with intermediate histological grade. MET ($p=0.046$), APC ($p=0.0051$) and PDGFRA ($p=0.009$) mutations were more frequently in women. MET ($p=0.012$) and HNF1A ($p=0.036$) were predominant in patients with pulmonary effusion. TP53 mutations were common in former smokers ($p=0.041$), while never smokers presented higher incidence of APC mutations ($p=0.030$) and PDGFRA alterations correlated with ECOG 0-1 ($p=0.042$).

Survival of NSCLC patients

Table III shows that better PFS was associated with ECOG-PS ≤ 1 , 11.0 vs. 2.4 months, ($p=0.025$); disease stage

IIIB compared to IV, 21.7 vs. 7.7 months, ($p=0.024$); lymph nodes absent 13.5 vs. 4.8 months ($p=0.020$); absence of bone metastasis, 15.7 vs. 5.5 months ($p=0.009$); and no APC mutations, 11.0 vs. 6.4 months ($p=0.057$). In the multivariate analysis, the only significant factor of poor prognosis for PFS was the presence of APC mutations (HR 3.1 [1.1-8.8], $p=0.032$).

Factors associated with OS in univariate analysis (table III) were smoking status where current smokers had a median OS of 21.2 months (95%CI 9.8-32.5), former smokers 46.8 months (95%CI not reached) and never smokers 10.2 months (95%CI 5.0-15.4), $p=0.027$. Another factor was ECOG-PS ≤ 1 , 19.5 vs. 1.3 months in, $p<0.001$. Multivariate analysis of OS showed that ECOG was the only independent factor with HR 2.9 (1.1-7.4), $p=0.021$.

According to data from the cBioportal database,^{17,18} we performed a comparison to determine differences in prevalence between the frequencies of somatic mutations in our population with respect to data for Caucasian patients. The Mexican population had a different prevalence of mutations in several genes including EGFR, MET, HNF1A, HER2 and GUSB (table IV). EGFR mutations were present in 36.7 vs. 17% in our population compared to Caucasians, while in MET gene the frequencies were 20 vs. 4%, HNF1A 14.4 vs. 2.7%, GUSB 8.9 vs. 0.5% ($p<0.001$), respectively, whereas in HER2 the mutation frequencies found were 11.1 vs. 2.2% ($p=0.003$).

Discussion

Lung cancer is the human neoplasm with the highest mutation rate after melanoma, with over 10 mutations/Mb for smokers.¹⁹ The presence of specific driver mutations has led to the development of targeted therapies for specific subsets of patients.^{20,21} In this study, we analyzed the mutation profile of NSCLC in the Mexican population, the association with clinical-pathological characteristics, therapeutic response and the contrast with other ethnic groups.

TP53 was the most frequently mutated gene in almost 50% of the patients and it was associated with former tobacco consumption. TP53 mutations had no prognostic value for OS of NSCLC patients. This tumor suppressor gene ranks first among the highly mutated genes in human cancers according to the lung cancer genome database.²² To date, TP53 is not a therapeutic target, nevertheless, it represents a prognostic factor of response. Recently, it has been shown that TP53 mutations correlate with resistance to chemotherapy, worse therapeutic responses and reduced OS of NSCLC patients depending

Table III
UNIVARIATE AND MULTIVARIATE ANALYSIS OF THE FACTORS ASSOCIATED WITH PROGRESSION-FREE SURVIVAL AND OVERALL SURVIVAL IN MEXICAN PATIENTS WITH NSCLC (N=90). MEXICO, 2018

		Progression-free survival				Overall survival			
		Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
		Mean, 95%CI	p-Value	HR (95%CI)	p-Value	Mean, 95%CI	p-Value	HR (95%CI)	p-Value
OVERALL		10.1 (4.0-16.2)				15.2 (9.3-21.1)			
Gender	Female	9.4 (5.3-13.5)	0.21			15.2 (5.9-24.5)	0.737		
	Male	21.7 (0.0-48.3)				15.7 (9.3-22.1)			
Age	<60 years	9.2 (4.6-13.9)	0.982			12.9 (2.5-23.3)	0.939		
	≥60 years	10.7 (2.2-19.2)				16.6 (8.5-24.7)			
Tobacco exposure	Never smoker	8.1 (3.4-12.8)	0.051	0.834 (0.50-1.3)	0.479	10.2 (5.0-15.4)	0.027	0.68 (0.43-1.0)	0.098
	Former smoker	21.7 (1.8-41.6)				46.8 (NR)			
	Current smoker	NR (NR)				21.2 (9.8-32.5)			
Wood-smoke exposure	Absent	11.0 (2.4-19.6)	0.131			21.1 (11.7-30.6)	0.103		
	Present	7.7 (2.4-12.9)				10.2 (1.2-19.2)			
ECOG PS	0-1	11.0 (4.4-17.5)	0.025	1.5 (0.59-4.25)	0.36	19.5 (13.5-25.5)	<0.001	2.9 (1.1-7.4)	0.021
	2+	2.4 (1.3-3.6)				1.3 (1.2-6.5)			
Disease stage	IIIB	21.7 (13.3-30.1)	0.024	2.3 (0.91-5.8)	0.07	21.2 (13.2-29.1)	0.322		
	IV	7.7 (3.2-12.2)				12.8 (6.4-19.2)			
Histological grade	High	16.0 (0.0-34.2)	0.556			12.1 (0.0-31.8)	0.512		
	Intermediate	6.4 (0.65-12.2)				15.5 (3.4-27.5)			
	Low	11.0 (4.9-17.0)				15.2 (3.8-26.6)			
Metastasis									
CNS	Absent	11.0 (3.4-18.5)	0.181			15.2 (7.0-23.4)	0.762		
	Present	5.2 (0.9-10.4)				13.6 (4.3-22.9)			
Lung	Absent	10.7 (4.9-16.5)	0.517			12.8 (5.0-20.5)	0.843		
	Present	4.8 (3.7-5.9)				16.6 (9.7-23.5)			
Pulmonary effusion	Absent	10.7 (2.9-18.4)	0.816			15.5 (9.6-21.3)	0.963		
	Present	9.4 (3.1-15.7)				10.2 (0.0-26.2)			
Lymph nodes	Absent	13.5 (6.5-20.4)	0.02	1.8 (0.62-5.1)	0.24	13.6 (6.4-20.7)	0.993		
	Present	4.8 (0.0-10.4)				15.2 (0.0-32.3)			
Liver	Absent	11.0 (4.1-17.9)	0.756			15.5 (9.2-21.7)	0.988		
	Present	5.7 (3.7-7.7)				9.7 (0.0-25.1)			
Bone	Absent	15.7 (8.3-23.1)	0.009	1.4 (0.69-2.9)	0.329	18.3 (9.2-27.5)	0.16		
	Present	5.5 (4.3-6.7)				8.6 (2.4-14.8)			
CEA	<10 ng/ml	18.3 (9.2-27.3)	0.167			15.7 (9.5-21.9)	0.37		
	≥10 ng/ml	10.7 (4.2-17.2)				21.2 (11.0-31.4)			
Chemotherapy	No	14.6(6.3-22.9)	0.503			20.8 (11.7-30.0)	0.152		
	Yes	87.1 (2.1-14.1)				12.9 (3.1-22.7)			
EGFR	Negative	13.5 (2.9-24.1)	0.492			12.8 (3.4-22.2)	0.43		
	Positive	9.2 (2.7-15.7)				17.7 (10.0-25.3)			
KRAS	Negative	10.7 (5.3-16.1)	0.946			15.7 (9.9-21.5)	0.934		
	Positive	5.2 (0.0-26.1)				7.5 (0.0-19.1)			
MSH6	Negative	11.0 (5.1-16.8)	0.063			15.2 (9.6-20.8)	0.792		
	Positive	6.4 (2.3-6.0)				22.1 (0.0-64.8)			
TP53	Negative	11.0 (2.7-19.22)	0.514			15.2 (5.4-25.0)	0.469		
	Positive	8.1 (1.7-14.5)				15.5 (7.0-23.9)			

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HER 2	Negative	10.7 (5.0-16.4)	0.628			15.2 (9.6-20.8)	0.438
	Positive	5.7 (3.5-7.9)				9.7 (0.0-24.9)	
HNF1A	Negative	9.2 (4.8-13.6)	0.765			13.6 (6.0-21.1)	0.621
	Positive	18.3 (8.1-28.5)				18.5 (8.9-28.2)	
PIK3CA	Negative	10.7 (4.7-16.9)	0.184			13.6 (7.3-19.8)	0.677
	Positive	4.8 (2.1-7.5)				19.5 (0.0-44.9)	
MET	Negative	13.5 (6.8-20.2)	0.183			15.5 (9.7-21.2)	0.668
	Positive	4.8 (3.3-6.3)				7.5 (2.6-12.4)	
APC	Negative	11.0 (4.2-17.8)	0.057	3.1 (1.1-8.8)	0.032	15.5 (9.6-21.3)	0.742
	Positive	6.4 (3.4-9.4)				7.5 (0.0-28.6)	
ALK FISH	Negative	10.7 (4.8-16.6)	0.993			15.7 (10.2-21.2)	0.515
	Positive	2.9 (0.0-20.4)				4.0 (1.7-6.36)	
PDGFRA	Negative	13.5 (5.6-21.4)	0.071			12.9 (7.3-18.6)	0.273
	Positive	4.8 (2.6-7.0)				22.1 (6.3-38.0)	
GUSB	Negative	10.7 (5.2-16.2)	0.893			15.2 (9.5-20.9)	0.374
	Positive	5.7 (3.1-8.3)				9.7 (NR)	

ECOG PS: Eastern Cooperative Oncology Group Performance Status; CNS: Central Nervous System; CEA: Carcinoembryonic Antigen; EGFR: Epidermal Growth Factor Receptor; KRAS: Kirsten Rat Sarcoma Viral Oncogene Homolog; MSH6: MutS Homolog 6; TP53: Tumor Protein 53; HER2: Epidermal Growth Factor Receptor 2; HNF1A: Hepatocyte Nuclear Factor 1-Alpha; PIK3CA: Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha; MET: Mesenchymal Epithelial Transition; APC: Adenomatous Polyposis Coli protein; ALK: Anaplastic Lymphoma Kinase; PDGFRA: Platelet-derived growth factor receptor alpha; GUSB: Glucuronidase Beta.

Table IV
COMPARISON BETWEEN HISPANIC AND CAUCASIAN
POPULATION WITH NSCLC. MEXICO, 2018

Genes	Hispanics N=90		Caucasians N=183		P Value*
	n	%	n	%	
TP53	43	47.8	93	51	0,636
EGFR	33	36.7	31	17	<0.001*
KRAS	18	20	49	27	0,221
MET	18	20	7	4	<0.001*
PDGFRA	18	20	15	8	0,005
HNF1A	13	14.4	5	2.7	<0.001*
APC	11	12.2	9	5	0,029
HER2	10	11.1	4	2.2	0,003*
MSH6	9	10	4	2.2	0,012
PIK3CA	8	8.9	7	4	0,084
GUSB	8	8.9	1	0.5	<0.001*

TP53: Tumor Protein 53; EGFR: Epidermal Growth Factor Receptor 1; KRAS: Kirsten Rat Sarcoma Viral Oncogene Homolog; MET: Mesenchymal Epithelial Transition/Hepatocyte Growth Factor Receptor (HGFR); PDGFRA: Platelet-derived growth factor receptor alpha; HNF1A: Hepatocyte Nuclear Factor 1-Alpha; APC: Adenomatous Polyposis Coli protein; HER2: Epidermal Growth Factor Receptor 2; MSH6: MutS Homolog 6; PIK3CA: Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha; GUSB: Glucuronidase Beta.

Chi-square or Fisher's Exact test (n≤5)

* Significant after Bonferroni correction

on disease stage and sequencing platforms. Likewise, the biological role of TP53 mutations can be different according to tumor histology and smoking history.²³

TP53 mutations were concurrent with mutations in EGFR, MET, KRAS, and PDGFRA, while they were mutually exclusive with mutations in HNF1, APC and HER2. Concurrent mutations in TP53 and EGFR are frequent in NSCLC and may have impact on response rate, nonetheless, these results are diverse depending on the therapeutic approach. Recently, a comprehensive study by the lung cancer mutation consortium showed the adverse effect of concomitant mutations in TP53-mutated patients with targeted treatments for alterations in EGFR, ALK and ROS1, by developing resistance to chemotherapeutic agents. Moreover, the alterations imposed by TP53/KRAS co-mutations on cell-cycle regulation, control of DNA replication and repair result in higher neoantigen expression of neoantigens including PD-L1 upregulation, thereby increasing tumor immunogenicity.^{24,25} Double-mutant tumors with TP53/KRAS co-mutations had significantly shorter OS and developed resistance to chemotherapy compared to wild type tumors. In contrast, TP53/KRAS co-mutation status was predictive of clinical benefit and better PFS in response to PD-1 immunotherapy.²⁶ The incidence of other TP53 co-mutations have been recently reported, nevertheless, their prognostic value remains unclear.²³

EGFR mutations are currently the main targetable oncogenic driver in the treatment of NSCLC patients, with improved response rates and less secondary effects than cytotoxic chemotherapy.²⁷ As previously reported, we found high prevalence of EGFR mutations, mainly exon 19 microdeletions and L858R point mutations in exon 21. In our study there was high concordance between results with qPCR based on Scorpions/ARMS technologies and the NGS platform in the detection of EGFR exon 19 deletions. EGFR mutations were associated with intermediate histological grade in advanced NSCLC. Consistently, a recent study indicated a correlation between moderately differentiated tumor grade and a higher frequency of EGFR mutations in metastatic lung adenocarcinomas. This modifies treatment selection, since patients with high grade or poorly differentiated tumors can be treated with chemotherapy or immunotherapy as first line treatment previous to obtaining the EGFR mutational status.²⁸

Other EGFR alterations found with low frequency were T790M mutation in exon 20 plus the A750P and G719A. Uncommon mutations are present in less than 10% of EGFR-mutant lung cancer and associated with high grade tumors, *i.e.* poorly differentiated, more aggressive phenotypes and could represent mechanisms of resistance to EGFR-TKI treatments.⁸ There are no targeted therapies for uncommon EGFR mutations, as their responses to TKIs are variable and the role in tumor biology is still unresolved.

In this study, MET exon 14 mutations were present in 20% of the patients, associated with female gender, pulmonary effusion and TP53 mutations. MET gene encodes a tyrosine kinase receptor that binds the hepatocyte growth factor. MET exon 14 codes for a portion of the juxtamembrane domain containing the binding site for ubiquitin ligases that participate in MET protein degradation. Mutations in MET exon 14 mutations cause exon 14 skipping, leading to constitutive signaling and oncogenicity.⁸ A recent study described a relationship between MET mutations, female gender and never smokers. Both MET mutations and amplifications show clinical benefit in NSCLC patients treated with TKIs not specifically designed for MET, having durable partial response with crizotinib and capmatinib and complete metabolic response with cabozantinib independently of histological subtype. Selective MET inhibitors have been developed such as tivantinib, onartuzumab and emibetuzumab with modest clinical benefits.^{8,29}

HER2 exon 20 mutations constitute 96% of the mutations in this gene and have been the most studied alterations in NSCLC. Conversely, other HER2 mutations have been described having prognostic value, including S310F/Y, D277G/H/V/Y and I655V.³⁰ Among

the HER2 mutations in our study, I655V (exon 17) was detected with high frequency. This amino acid change in the transmembrane region increases tyrosine kinase activity leading to oncogenic signaling. I655V is present in different malignancies including breast, gastric and lung cancer. It correlates with aggressive tumor phenotype, poor prognosis and risk of cardiotoxicity during trastuzumab treatment of breast cancer.^{8,31} However, there are few studies in lung cancer and without prognostic value.

In the present study, we found concurrence of HER2 mutations with intermediate histological grade and MET variants, but they were mutually exclusive with TP53 and EGFR mutations. There are no reports describing the association between HER2 mutations and tumor grade, although there is association with female gender, never smokers, adenocarcinomas, it has been reported that it confers a low sensitivity to traditional EGFR-TKIs.^{8,30} HER2 mutations in NSCLC have a prevalence of 4% and may have higher clinical impact than gene amplification. Preliminary results from selective inhibitors for HER2 exon 20 mutations such as poziotinib had marked radiologic and clinical response. Additionally, several pan-EGFR irreversible inhibitors such as afatinib, neratinib, and dacomitinib have shown activity in NSCLC patients with HER2 mutations.^{8,30,32}

Adenomatous polyposis coli (APC) is a tumor suppressor gene mutated in 80% of colon carcinomas and less frequently in other malignancies including liver, breast and lung cancer.³² In our study, we found APC mutations in NSCLC that correlated with never smokers, were mutually exclusive with TP53 mutations and predicted poor prognosis. APC is part of the B-catenin degradation complex in the Wnt pathway. APC mutations induce nuclear B-catenin accumulation leading oncogene activation. APC mutations have been associated with nonsmokers in colorectal cancer.³³ These mutations are generally insertions/deletions that modify frameshifts, introduce premature stop codons and loss of function via truncation of APC protein. Current therapies for APC loss in cancer inhibit signaling through the canonical Wnt/B-catenin pathway downstream of APC or aim to restore normal APC expression. APC and TP53 mutations occur early in the initiation of carcinogenesis, but they are not documented as mutually exclusive alterations.³⁴ Although these alterations alone may not be sufficient to have a significant impact on OS concomitant TP53 and APC mutations have been described as a more aggressive molecular phenotype with implications for worse prognosis in PFS/OS.⁴

There are differences in lung cancer incidence among different ethnic groups. While EGFR mutations appear in around 15% of North American and Euro-

pean patients, 40% of Asian patients, and between 2 to 14% of Afro-American patients, the frequency of EGFR mutations in Mexico is 27%.^{4,35} In our study, the prevalence of EGFR mutations was 36.7% compared to 17% in Caucasians. Other important differences in mutation frequency between the two populations were present in known oncogenic drivers of NSCLC such as MET and HER2, while the frequency of KRAS mutation was higher in Caucasians as expected although not statistically significant. The incidence of KRAS mutations in NSCLC in Latin America is approximately 14-17% as reported by the CLICaP.⁴ MET mutation profile also differs in type and frequency according to ethnicity. In our study, HER2 mutations were present in 11.1% of the Mexican patients in contrast to 2.2% in Caucasians and 3.9% in Asians. Similarly to EGFR mutations, HER2 mutations are associated with adenocarcinoma histology, female gender and never smokers and have favorable response to TKI and antibody treatments.²³ A higher incidence of HER2 mutations in our population opens the opportunity to improve response rates and overall survival with novel targeted therapies.

Conclusions

This study provides a profile of somatic mutations for NSCLC in the Mexican population. The main genomic alterations were present in TP53, EGFR, KRAS, MET, PDGFRA, HNF1A, APC, HER2 and MSH6. This mutation profile shows differences with other ethnic groups. Further studies are warranted to evaluate the germline molecular features underlying the relationship between ethnicity, somatic mutation rates, clinical responses and survival of NSCLC patients.

Declaration of conflict of interests. The authors declare that they have no conflict of interests.

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