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STK11 and KEAP1 mutations in non-small cell lung cancer patients: Descriptive analysis and prognostic value among Hispanics (STRIKE registry-CLICaP)☆

Vladmir C. Cordeiro de Lima [a,1], Marcelo Corassa [a,1], Erick Saldanha [a,1], Helano Freitas [a,1], Oscar Arrieta [b,1], Luis Raez [c,1], Suraj Samtani [d,1], Maritza Ramos [b,1], Carlos Rojas [d], Mauricio Burotto [d], Diego F. Chamorro [e,f,1], Gonzalo Recondo [g,1], Alejandro Ruiz-Patiño [e,f], Luis Más [h], Lucia Zatarain-Barrón [b], Sergio Mejía [i], José Nicolas Minata [j], Claudio Martín [k], Juan Bautista Blaquier [g], Rodrigo Motta Guerrero [l], Carlos Aliaga-Macha [l], Carlos Carracedo [l], Camila Ordóñez- Reyes [e,f], Juan Esteban Garcia-Robledo [m], Luis Corrales [n], Carolina Sotelo [e,f], Luisa Ricaurte [o], Nicolas Santoyo [e,f], Mauricio Cuello [p], Elvira Jaller [e,f], July Rodríguez [e,f], Pilar Archila [e,f], Maritza Bermudez [e,f], Tatiana Gamez [e,f], Alessandro Russo [q], Lucia Viola [r], Umberto Malapelle [s], Diego de Miguel Perez [t], Christian Rolfo [t], Rafael Rosell [u], Andrés F. Cardona [e,f,v,*,1]

[a] *Thoracic Oncology Unit, A.C.Camargo Cancer Center, Sao Paulo, Brazil*
[b] *Thoracic Oncology Unit, National Cancer Institute (INCan), México City, Mexico*
[c] *Thoracic Oncology Department, Memorial Cancer Institute, Memorial Health Care System, Miami, FL, USA*
[d] *Medical Oncology Department, Bradford Hill Clinical Research Center, Santiago, Chile*
[e] *Foundation for Clinical and Applied Cancer Research - FICMAC, Bogotá, Colombia*
[f] *Molecular Oncology and Biology Systems Research Group (Fox-G), Universidad El Bosque, Bogotá, Colombia*
[g] *Thoracic Oncology Unit, Centro de Educación Médica e Investigaciones Clínicas (CEMIC), Buenos Aires, Argentina*
[h] *Medical Oncology Department, Instituto Nacional de Enfermedades Neoplásicas – INEN, Lima, Peru*
[i] *Clinical Oncology Department, Instituto de Cancerologia – Clinica las Americas - AUNA, Colombia*
[j] *Clinical Oncology Department, Hospital Italiano de Buenos Aires, Buenos Aires, Argentina*
[k] *Thoracic Oncology Unit, Instituto Alexander Fleming, Buenos Aires, Argentina*
[l] *Clinical Oncology Department, Centro Oncológico Aliada, Lima, Peru*
[m] *Division of Hematology/Oncology, Mayo Clinic, Scottsdale, USA*
[n] *Thoracic Oncology Unit, Centro de Investigación y Manejo del Cáncer – CIMCA, San José, Costa Rica*
[o] *Pathology Department, Mayo Clinic, Rochester, MN, USA*
[p] *Medical Oncology Department, Hospital de Clínicas, Universidad de la Republica –UdeLAR, Montevideo, Uruguay*
[q] *Medical Oncology Department, Azienda Ospedaliera Papardo, Messina, Sicilia, Italy*
[r] *Thoracic Oncology Unit, Fundación Neumológica Colombiana, Bogotá, Colombia*
[s] *Predictive Molecular Pathology Laboratory, Department of Public Health, University Federico II of Naples, Naples, Italy*
[t] *Center for Thoracic Oncology, The Tisch Cancer Institute Icahn School of Medicine, Mount Sinai, Mount Sinai Health System, One Gustave Levy Place, NY, USA*
[u] *Cancer Biology and Precision Medicine Program, Germans Trias i Pujol Research Institute (IGTP)/Dr. Rosell Oncology Institute (IOR) Quirón-Dexeus University Institute, Barcelona, Spain*
[v] *Direction of Research, Science and Education, Luis Carlos Sarmiento Angulo Cancer Treatment and Research Center (CTIC), Bogotá, Colombia*

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ABSTRACT

Background: Mutations in *STK11* (*STK11*^{Mut}) and, frequently co-occurring, *KEAP1* mutations (*KEAP1*^{Mut}) are associated with poor survival in metastatic Non-small Cell Lung Cancer (mNSCLC) patients treated with

☆ Latin American Consortium for the Investigation of Lung Cancer.

* Corresponding author at: Direction of Research, Science and Education, Luis Carlos Sarmiento Angulo Cancer Treatment and Research Center (CTIC), Bogotá, Colombia.
E-mail address: acardona@fctic.org (A.F. Cardona).

[1] These authors contributed equally to the study.

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immunotherapy. However, there are limited data regarding the prognostic or predictive significance of these genomic alterations among Hispanics.

Methods: This retrospective study analyzed a cohort of Hispanic patients (N = 103) diagnosed with mNSCLC from the US and seven Latin American countries (LATAM) treated with immune checkpoint inhibitors (ICI) alone or in combination as first-line (Cohort A). All cases were treated in routine care between January 2016 and December 2021. The main objectives were to determine the association of mutations in *STK11* or *KEAP1* in these patients' tumors with overall (OS) and progression-free survival (PFS), presence of *KRAS* mutations, tumor mutational burden (TMB), and other relevant clinical variables. To compare outcomes with a $STK11^{Wt}/KEAP1^{Wt}$ population, historical data from a cohort of Hispanic patients (N = 101) treated with first-line ICI was used, matching both groups by country of origin, gender, and Programed Death-ligand 1 (PD-L1) expression level (Cohort B).

Results: Most tumors had mutations only in *STK11* or KEAP1 (45.6%) without *KRAS* co-mutation or any other genomic alteration. Besides, 35%, 8.7%, 6.8%, and 3.9% were $KRAS^{Mut}$ + $STK11^{Mut}$, $KRAS^{Mut}$ + $STK11^{Mut}$ + $KEAP1^{Mut}$, $STK11^{Mut}$ + $KEAP1^{Mut}$, and $KRAS^{Mut}$ + $KEAP1^{Mut}$, respectively. Based on *KRAS* status, *STK11* alterations were associated with significantly lower PD-L1 expression among those with $KRAS^{Wt}$ ($p = 0.023$), whereas *KEAP1* mutations were predominantly associated with lower PD-L1 expression among $KRAS^{Mut}$ cases ($p = 0.047$). Tumors with $KRAS^{Mut}$ + $KEAP1^{Mut}$ had significantly higher median TMB when compared to other tumors ($p = 0.040$). For Cohort A, median PFS was 4.9 months (95%CI 4.3–5.4), slightly longer in those with $KEAP1^{mut}$ 6.1 months versus $STK11^{Mut}$ 4.7 months ($p = 0.38$). In the same cohort, PD-L1 expression and TMB did not influence PFS. OS was significantly longer among patients with tumors with PD-L1 $\geq$ 50% (30.9 months), and different from those with PD-L1 1–49% (22.0 months), and PD-L1 < 1% (12.0 months) ($p = 0.0001$). When we compared the cohorts A and B, OS was significantly shorter for patients carrying *STK1* [$STK11^{Mut}$ 14.2 months versus $STK11^{Wt}$ 27.0 months ($p = 0.0001$)] or *KEAP1* [$KEAP1^{Mut}$ 12.0 months versus $KEAP1^{Wt}$ 24.4 months ($p = 0.005$)] mutations. PD-L1 expression significantly affected OS independently of the presence of mutations in *STK11, KEAP1*, or *KRAS*. TMB-H favored better OS.

Conclusions: This is the first large Hispanic cohort to study the impact of *STK11* and *KEAP1* mutations in NSCLC patient treated with ICI. Our data suggest that mutations in the above-mentioned genes are associated with PD-L1 expression levels and poor OS.

1. 1.Introduction

Lung cancer (LC) is one of the leading causes of cancer-related deaths, with 2.2 million new cancer cases and almost 2 million deaths, and it is the second most diagnosed cancer worldwide in 2020, According to Global Cancer Statistics (GLOBOCAN) [1,2]. Most LC patients have metastasis at the time of diagnosis which reduces the 5-years survival to 6–8% [3,4]. Globally, lung cancer treatment aggregate cost represents a total of US 53 billion, and it is one of the main economic burdens to the health systems in developing countries [5,6].

This malignancy is broadly classified into two groups according to histology, Non-small Nell Lung Cancer (NSCLC), which represents 80–85% of LC cases, and Small Cell Lung Cancer (SCLC), which accounts for 15% of the total number of cases [7]. NSCLC can be subdivided in three major subtypes: adenocarcinoma (AC), squamous cell carcinoma (SCC), and large cell carcinoma. Additionally, NSCLC can be subclassified according to specific genomic alterations associated with cell of origin, malignant behavior, response to treatment, and prognosis [8] These molecular characteristics have changed the "one size fits all" treatment paradigm [9].

In non-squamous NSCLC patients, the most identified driver genes are V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (*KRAS)*, Epidermal Growth Factor Receptor (*EGFR)*, and Anaplastic Lymphoma kinase (*ALK)*. Novel treatments directed to these alterations (targeted therapy), like tyrosine kinase inhibitors (TKI), have rapidly become the new standard therapeutic approach in face of their high efficacy [10,11]. However, not every tumor possesses an actionable molecular alteration. In that scenario, immune checkpoint inhibitors (ICI) represent the cornerstone treatment, either alone or in combination with platinum-based chemotherapy, depending on PD-L1 expression [12,13].

Recent studies have proved that PD-L1 expression is not the only predictive biomarker for ICI response. Some molecular alterations are also associated with response to ICIs, mutations in serine/threonine kinase 11 (*STK11*)/liver kinase B1 (*LKB1*) and Kelch-like ECH-associated protein 1 (*KEAP1*) among them [14].

STK11 is an upstream activator of the AMP-activated protein kinase and a central regulator of the cellular microenvironment and glucose metabolism. Mutations in the *STK11* gene are reported in 5–30% of tumors from metastatic NSCLC (mNSCLC) patients [15,16]. On the other hand, *KEAP1* is the principal regulator of transcription of the nuclear factor erythroid-2-related factor (NRF2) which is involved in the cell response to oxidative stress and is a putative regulator of the stimulator of interferon genes (STING) pathway. Mutations in the gene (*KEAP1*) encoding KEAP1 have been reported in 10–30% of NSCLC [17,18].

Notably, *STK11* and *KEAP1* mutations have been associated with poor outcomes in NSCLC patients treated with ICI. According to the available literature, *STK11/KEAP1* mutated NSCLC is associated with an immunosuppressive tumor microenvironment which imposes a barrier to the action of some anti-PD-1/PD-L1 antibodies [19]. However, this effect has been only analyzed in the context of co-occurring *KRAS* mutations. It has been suggested that *STK11/KEAP1* mutations are prognostic rather than a predictive factor [20] Nevertheless, much of these data comes from studies in non-Hispanic white (NHW) populations, so the impact of *STK11* and *KEAP1* among Hispanics is not well established. To elucidate how *STK11/KEAP1* alterations affect the response and outcomes in Hispanic NSCLC patients, we conducted a multicenter retrospective cohort study including mNSCLC patients treated with ICI and determined the presence of molecular alterations in *STK11* and *KEAP1* and its association with survival outcomes.

2. Methods

2.1. Study design and patients

A multicenter, multinational retrospective cohort study of Hispanic patients (from Mexico, Colombia, Costa Rica, Argentina, Chile, Peru, Brazil, and United States residents) with advanced NSCLC treated with ICI as first-line and whose tumors underwent comprehensive genomic profiling (CGP) were included. All cases were treated homogeneously in routine care between January 2016 and December 2021 and were characterized as $STK11^{Mut}$ or $KEAP1^{Mut}$ (**Cohort A**) if they harbored loss-of-function alterations including nonsense, frameshift, insertion/deletion, splice site, or pathogenic missense mutations in these genes. To determine the pathogenicity of missense mutations, we reviewed all the identified alterations in the Catalogue of Somatic Mutations in Cancer (COSMIC) [21] To estimate differences in outcomes with an $STK11^{Wt}$

and *KEAP1*^{Wt} population (**Cohort B**), historical data from a case series of Hispanic patients (N = 101) treated in the same countries with first-line ICI was used, matching both groups by country of origin, gender, and PD-L1 expression level. Fig. 1 shows the distribution of the patients included in the study, and Table 1 shows their main clinical and pathological characteristics.

Objective response rate (ORR) to immunotherapy was assessed by blinded thoracic radiologists using Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1. Progression-free survival (PFS) was defined as the time from the start of immunotherapy to the date of disease progression or death, whichever occurred first. Patients who were alive without disease progression were censored at the date of their last adequate disease assessment. Overall survival (OS) was defined as the time from the start of immunotherapy to death. Patients who were still alive were censored at the date of the last contact. Patients were clinically and radiologically evaluated every 9 to 12-weeks.

Clinical, demographic, and molecular variables (including all mutations and Tumor Mutational Burden [TMB]) were stored in a centralized and anonymized database at the CLICaP/Foundation for Clinical and Applied Cancer Research (FICMAC, Bogotá, Colombia). All included patients provided signed informed consent. An Institutional Review Board and Privacy Board waiver was obtained to facilitate retrospective clinical-pathologic and molecular data (Lung Cancer-FICMAC/CLICaP Platform - Registration No. 2012/014, Kayre, Bogotá, Colombia).

2.2. Genomic profiling

Comprehensive genomic profiling was performed using FoundationOne® CDx in a reference laboratory accredited by the College of American Pathologists, with validated methods (Foundation Medicine, Cambridge, MA, http://www.foundationmedicine.com)[22] For all cases, the samples were collected in a reference center of each country and were subjected to the initial evaluation. Formalin-fixed paraffin-embedded samples were subjected to an assessment by a trained pathologist and subsequently microdissected to guarantee malignant sample representation. In the case of tissue nonavailability, CGP was conducted as a liquid biopsy to evaluate cell-free DNA in whole blood (FoundationOne® Liquid CDx or Guardant360) [23].

2.3. PD-L1 assessment and tumor mutational burden

The PD-L1 tumor proportion score (TPS) was determined by immunohistochemistry using the validated anti-PD-L1 antibodies: 22C3 (Dako North America Inc., Carpinteria, CA) and SP263 (Ventana, Roche Diagnostics, Indianapolis, IN). TMB, defined as the number of somatic, coding, base substitution, and indel mutations per megabase (Mb) of genome examined, was determined using commercial FoundationOne® CDx or Guardant 360 panels in paraffin-embedded tissue or liquid biopsy. TMB distributions were harmonized between the platforms by applying a standard transformation followed by standardization to Z-scores, as previously described [24] For the analysis, TMB-H was defined as ≥10 mutations/megabase [mut/Mb]) using the same cutoff used in the KEYNOTE-158 study [25].

2.4. Statistical analysis

Categorical and continuous variables were summarized descriptively using percentages and medians. The Wilcoxon-Rank Sum test and Kruskal-Wallis test was used to test for differences between continuous variables, and Fisher's exact test was used to test for associations between categorical variables. For data analysis, *STK11* and *KEAP1* mutations were grouped into four subgroups, including $KRAS^{Mut}$ + $STK11^{Mut}$, $KRAS^{Mut} + STK11^{Mut} + KEAP1^{Mut}$, $STK11^{Mut} + KEAP1^{Mut}$, and $KRAS^{Mut}$ + $KEAP1^{Mut}$. Forty-seven patients only had $STK11^{Mut}$ or $KEAP1^{Mut}$ plus other alterations different from *KRAS*. Kaplan-Meier methodology was used to estimate event-time distributions. Log-rank tests were used to test for differences in event-time distributions, and Cox proportional hazards models were fitted to obtain estimates of hazard ratios in univariate and multivariable analysis. The Cox proportional hazard model assumption was inspected by calculating the Schoenfeld residuals. All P values are two-sided, and confidence intervals are at the 95% level, with statistical significance defined as $P < 0.05$. All statistical analyses were performed using SPPS 23.0 (https://www.ibm.com/support/pages/spss-statistics).

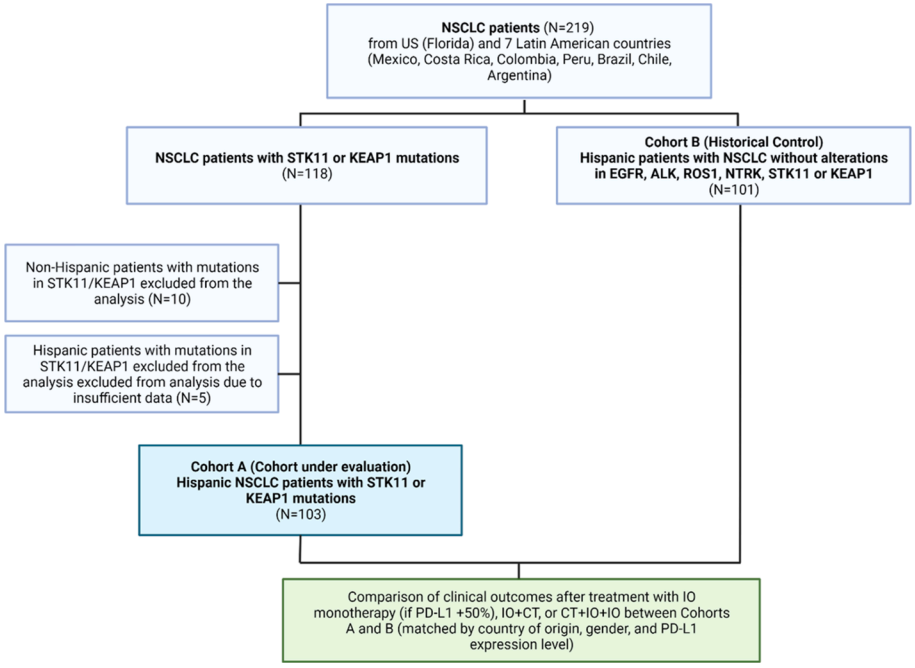

Fig. 1. Patient selection process and distribution in the study (cohorts proposed for the analysis) on STRIKE registry-CLICaP.

Table 1
Clinical characteristics of the patients included in the study considering the entire population, and patients in Cohort A (*STK11* or *KEAP1* mutated) and B (*STK11* or *KEAP1* wild type).

Clinical Characteristic	Combined cohort N = 204 (%)	Cohort A N = 103 (%)	Cohort B N = 101 (%)
Age, mean SD/median (range)	64.8 ± 10.7 /66 (r, 33–87)	62.7 SD ± 9.3 /68 (r, 42–87)	62.0 SD ± 11.4 /67 (r, 33–86)
Sex	103	61	42
Male	(50.5)101	(59.2)42	(41.6)59
Female	(49.5)	(40.8)	(58.4)
Smoking status	21	14	7
Current	(10.3)149	(13.6)75	(6.9)74
Former	(73.6)34	(72.8)14	(73.3)20
Never	(16.7)	(13.6)	(19.8)
Wood smoke exposure	13	6	7
Yes	(6.4)191	(5.8)97	(6.9)94
No	(93.6)	(94.2)	(93.1)
Histology	195		97
Adenocarcinoma	(95.6)4	98 (95.1)2	(96.0)2
AdenosquamousPoorly	(2.0)4	(1.9)2	(2.0)2
differentiated carcinoma	(2.0)	(2.4)	(2.0)
(NOS)	1	1	
ND	(0.4)	(0.6)	
Brain metastases (*at diagnosis*)	40	17	23
Yes	(19.6)164	(16.5)86	(22.8)78
No	(80.4)	(83.5)	(77.2)
***KRAS* mutation status**	84	49	35
Mutant	(41.2)120	(47.5)54	(34.3)66
Wild type	(58.8)	(52.4)	(65.3)
***STK11* mutation status**	90	90	
Mutant	(87.4)13	(87.4)13	–
Wild type	(12.6)	(12.6)	
***KEAP1* mutation status**	30	30	
Mutant	(29.1)73	(29.1)73	–
Wild type	(70.9)	(70.9)	
***TP53* mutation status**	53	53	
Yes	(51.5)50	(51.5)50	–
No	(48.5)	(48.5)	
ARID1A/B	3	3	
Yes	(2.9)100	(2.9)100	–
No	(97.1)	(97.1)	
Mutation combos	36	36	
KRAS + *STK11*	(35.0)9	(35.0)9	–
KRAS + *STK11* + *KEAP1*	(8.7)7	(8.7)7	
STK11 + *KEAP1*	(6.8)4	(6.8)4	
KRAS + *KEAP1*	(3.9)47	(3.9)47	
Only *STK11* or *KEAP1***	(45.6)	(45.6)	
ECOG performance status	182	88	94
0–1	(89.2)22	(85.4)15	(93.0)7
≥2	(10.8)	(14.5)	(7.0)
Line of therapy for PD-(L)1 inhibitor	204	103	101
1st	(100.0)	(100.0)	(100.0)
PD-L1 tumor proportion score		37	37
<1%		(35.9)42	(36.7)45
1–49%		(40.8)24	(44.6)19
≥50%		(23.3)	(18.8)

*Excluding *KRAS* (sotorasib and adagrasib are not available in Latin America and at the time of closing the database, neither in the US).
**Only *STK11* or *KEAP1* (without mutations in *KRAS* or *TP53*, but with other mutations without known prognostic significance).

3. Results

3.1. Patient population

In Cohort A, most of the patients included were men (59.2%) in the seventh decade of life (83.5%) with a smoking history (86.4%). Only 16% of subjects had de novo brain metastases in this group. According to their frequency, most patients only had mutations in *STK11* or *KEAP1* (45.6%) without *KRAS* or other genomic alteration. In addition, 35%, 8.7%, 6.8%, and 3.9% had $KRAS^{Mut}$ + $STK11^{Mut}$, $KRAS^{Mut}$ + $STK11^{Mut}$ + $KEAP1^{Mut}$, $STK11^{Mut}$ + $KEAP1^{Mut}$, and $KRAS^{Mut}$ + $KEAP1^{Mut}$, respectively. A trend for a higher rate of *de novo* brain metastases was found in those patients whose tumors had $STK11^{Mut}$ or $KEAP1^{Mut}$ only (p = 0.079). Tumors from patients/subjects who never smoked (N = 12) also showed a trend of a higher frequency of $STK11^{Mut}$ (p = 0.074).

Analyzing the impact of *STK11* and *KEAP1* mutation on PD-L1 expression, it was found that $STK11^{Mut}$ and $KEAP1^{Mut}$ were not more frequent among tumors with PD-L1 > 1% (p = 0.18). When analyzed by *KRAS* status, *STK11* alterations were associated with significantly lower PD-L1 expression among $KRAS^{Wt}$ tumors (p = 0.023), whereas *KEAP1* mutation was associated with lower PD-L1 expression predominantly among $KRAS^{Mut}$ tumors (p = 0.047). Only $KRAS^{Mut}$ + $KEAP1^{Mut}$ tumors had a significantly higher median TMB than the rest of tumors (p = 0.040). Individually, $STK11^{Mut}$ (p = 0.58) and $KEAP1^{Mut}$ (p = 0.77) were not associated with TMB-H, but $KRAS^{G12C}$ tumors were usually TMB-H (80%/p = 0.013). **Supplementary Table 1** details the variants found in *STK11, KEAP1, KRAS*, and *TP53* for Cohort A tumors.

3.2. Impact of STK11 and KEAP1 mutation status on clinical outcomes to PD-1(L1) inhibition (Cohort A)

In Cohort A, the overall response rate (ORR) for patients with alterations in *STK11* or *KEAP1* treated with ICIs in the first line was 20.4% (N = 21; complete response 4/3.9%, and partial response 18/17.5 %) and 41 patients achieved stable disease (39.8%). Median progression-free survival (PFS) was 4.9 months (95%CI 4.3–5.4) (Fig. 2**A**). PFS was slightly longer in patients with $KEAP1^{mut}$ [6.1 months (95%CI 4.1–8.0) vs. $STK11^{Mut}$ [4.7 months (95%CI 3.9–5.4); p = 0.38]. Presence of KRAS mutations did not affect PFS (p = 0.36). PD-L1 expression did not influence PFS [PD-L1 < 1%: 4.9 months (95%CI 3.8–5.9), PD-L1 1–49%: 4.7 months (95%CI 4.2–5.2), and PD-L1 ≥ 50%: 4.2 months (95%CI 3.7–4.6); p = 0.143] or ORR (p = 0.79) (Fig. 2**B**), even among high (≥50%) PD-L1 expressors (14/20 patients did not have a response; p = 0.81). TMB did not influence PFS (Fig. 2**C**) [4.2 months (95%CI 4.0–4.3) vs. TMB-L 4.9 months (495%CI 4.6–5.1); p = 0.43]. Among subgroups of co-mutations, PFS was significantly higher for patients whose tumors were $STK11^{Mut}$ + $KEAP1^{Mut}$ (especially when the $KRAS^{G12C}$ mutation was found) [8.6 months (95%CI 6.8–11.5); p = 0.005). The total number of co-mutations (>or < 3 co-mutations) (p = 0.28) and *TP53* mutations (p = 0.27) did not affect PFS.

Overall survival (OS) was 13.3 months (95%CI 10.3–16.2) (Fig. 2**D**), and slightly longer in $STK11^{Mut}$ patients (14.2 months, 95%CI 10.3–18.0) versus those who are $KEAP1^{Mut}$ (12.0 months, 95%CI 9.6–14.3; p = 0.59). *KRAS* mutations did not affect OS [$KRAS^{Mut}$ 13.3 months (95%CI 11.2–15.2) vs. $KRAS^{Wt}$ 14.0 months (95%CI 10.4–16.1); p = 0.55]. Although PD-L1 expression did not affect PFS, OS was significantly longer in patients with PD-L1 ≥ 50% [30.9 months (95%CI 18.6–43.3), PD-L1 1–49% 22.0 months (95%CI 16.5–28.8), and PD-L1 < 1% 12.0 months (95%CI 9.2–14.7); p = 0.0001) (Fig. 2**E**). Despite the immaturity of OS curves according to TMB (47 events), OS was significantly longer among TMB-H patients (p = 0.005) (Fig. 2**F**). OS was not associated with the different co-mutation subgroups (p = 0.79) neither with *TP53* mutations (p = 0.085).

3.3. Comparison of outcomes between cohorts a and B

When comparing OS between Cohorts A and B, we had 200 patients and 99 events. OS was significantly shorter for patients whose tumors carried mutations in *STK11* [$STK11^{Mut}$ 14.2 months (95%CI 10.3–18.0) vs. $STK11^{Wt}$ 27.0 months (95%CI 21.5–32.4); p = 0.0001] (Fig. 3**A**) and in *KEAP1* [$KEAP1^{Mut}$ 12.0 months (95%CI 9.6–14.3) vs. $KEAP1^{Wt}$ 24.4 months (95%CI 20.3–28.5); p = 0.005] (Fig. 3**B**). In contrast, the presence of KRAS mutations did not affect OS, being 25.0 months (95%CI

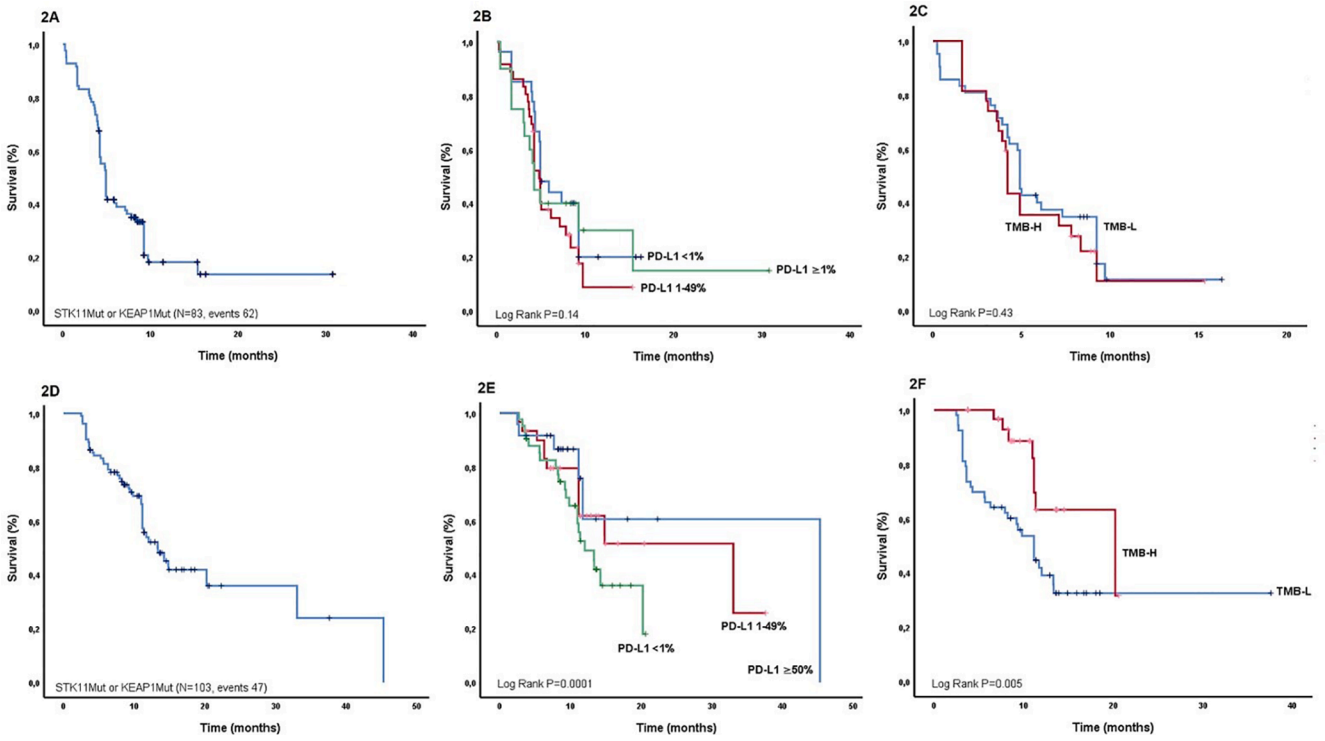

Fig. 2. Kaplan-Meier curves of patients trends. Patients median PFS for the included global population in the study **(2A)**; PFS according to the expression of PD-L1 expression **(2B);** PFS according to the TMB level discriminated as TMB-L and TMB-H **(2C)**; OS for the overall study population **(2D)**; OS discriminated according to the level and expression of PD-L1 **(2E)**; OS according to the TMB level, discriminated as TMB-L and TMB-H **(2F).**

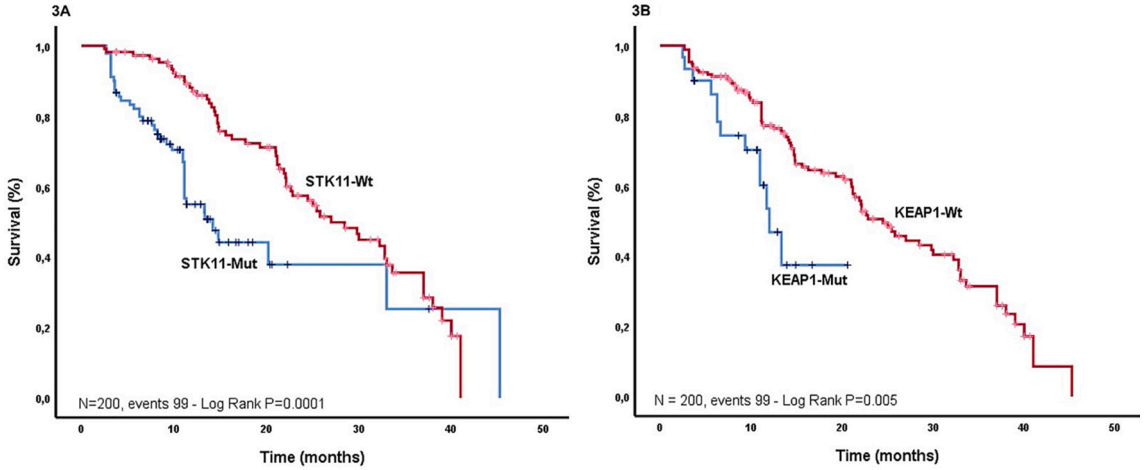

Fig. 3. Kaplan-Meier curves of the patiens of our study; OS according to the presence of mutations in STK11 **(3A)** or KEAP1 mutations **(3B)**.

15.9–34-1) and 22.1 months (95%CI 18.1–26.0) for the mutated and wild-type patients, respectively ($p = 0.83$) (Fig. 4**A**). On the other hand, the expression level of PD-L1 significantly affected OS independently of the presence of mutations in *STK11, KEAP1* or *KRAS*. Survival for patients whose tumors had PD-L1 < 1% was 16.2 months (95%CI 10.9–21.6), 27.0 months (95%CI 18.7–35.2) for the group with PD-L1 1–49%, and 37 months (95%CI 23.3–50.6; $p = 0.0001$) for high expressors (PD-L1 ≥ 50%) (Fig. 4**B**). TMB-H also favored better OS [TMB-H 20.2 months (95%CI 7.5–32.8) vs. TMB-L 11.1 months (95%CI 8.8–13.3); $p = 0.005$] (Table 2). In contrast, the subgroups of co-mutations did not affect OS ($p = 0.79$) (Supplementary Table 1).

Multivariate analysis (Table 2) showed that $STK11^{Mut}$ was an independent predictor of shorter PFS ($p = 0.02$). In contrast, TMB and PD-L1 expression were predictors of better PFS (HR 0.65 and 0.87, respectively). Regarding OS, in multivariate analysis, $STK11^{Mut}$ ($p = 0.001$) and $KEAP1^{Mut}$ ($p = 0.04$), and higher ECOG scores ($p = 0.001$) were associated with poor OS. In contrast, TMB ($p = 0.005$) and PD-L1 expression ($p = 0.004$) were predictors for better OS.

3.4. Adverse events

Information on toxicity was obtained in 72% of all patients and was directly related to the administration of immunotherapy in 40%. The main adverse events (AEs) during treatment were fatigue (40.3%), decreased neutrophil count (26.8%), nausea (23.5%), rash (17.6%), myalgia or arthralgia (11.7%), hypothyroidism (5.0%), diarrhea (3.3%), and liver toxicity (3.3%). Two patients had acute interstitial nephritis, one had diabetes, one had psoriasis, and one more developed adrenal

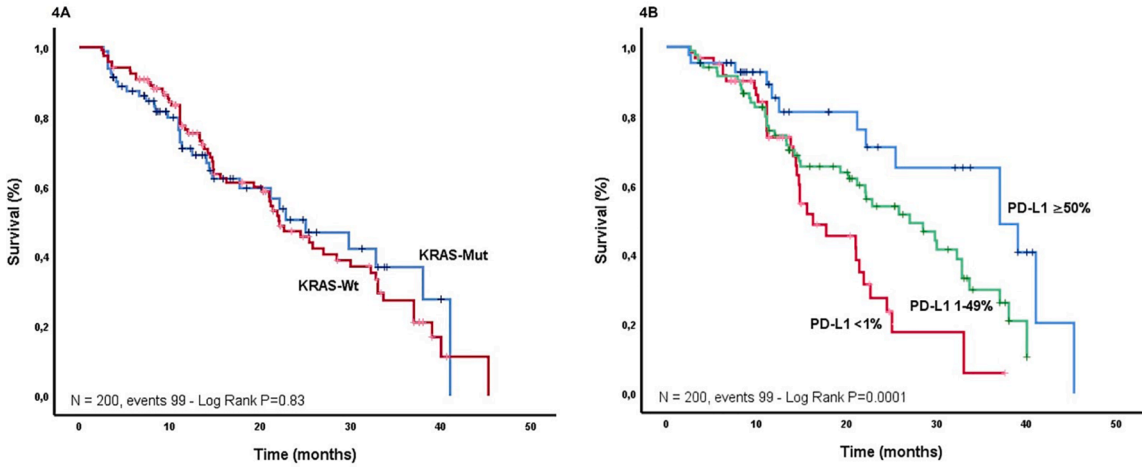

Fig. 4. Kaplan-Meier curves of the patients OS according to the presence of mutations in KRAS **(4A)** and according to the expression of PD-L1 (**4B**).

Table 2
Multivariable Cox regression for progression-free and overall survival in the combined cohort of *STK11* and *KEAP1* mutants.

	Multivariable Hazard Ratio PFS [95%CI]	P-value	Multivariable Hazard Ratio OS [95%CI]	P-value
STK11 (yes vs no)	1.31 [1.12–1.88]	0.02	1.33 [1.13–2.21]	0.001
KEAP1 (yes vs no)	1.27 [0.97–1.29]	0.32	1.19 [1.18–1.31]	0.04
Age (≥70 vs < 70)	1.39 [0.78–1.30]	0.53	1.36 [0.97–1.89]	0.09
Sex (male vs female)	0.78 [0.63–1.19]	0.47	1.22 [0.70–1.45]	0.82
ECOG PS (≥2 vs 0–1)	1.27 [0.72–1.38]	0.22	1.71 [1.32–3.12]	0.001
Smoking history (Former/ Smoker vs never)	1.18 [0.61–2.12]	0.65	0.82 [0.53–1.25]	0.80
*TMB score	0.65 [0.58–0.87]	0.0001	0.68 [0.67–0.98]	0.005
*PD-L1 expression	0.87 [0.78–0.91]	0.002	0.81 [0.80–0.97]	0.004

ECOG: Eastern Cooperative Oncology Group; PS: performance status; PD-L1: programmed death-ligand 1; TMB: tumor mutational burden *PD-L1 expression and TMB score were included as continuous variables in univariate and multivariable analysis. Hazard ratio and its 95%CI is per unit of PD-L1 tumor proportion score and TMB.

insufficiency. No association was observed between toxicity and the presence of mutations in *STK11, KEAP1*, or *KRAS*. **Supplementary Table 2** includes the AEs for cohorts A and B along with the degree of severity. **Supplementary Table 3** describes the treatment approach used for the cohorts of patients.

4. Discussion

It is fundamental to establish prognostic and predictive factors among patients with cancer, in order to optimize treatment. Tumor molecular alterations can modify survival outcomes following immunotherapy. As we mentioned before, alterations in *STK11* or *KEAP1* may have a prognostic rather than a predictive role in NSCLC patients treated with ICI; nevertheless, this has been adequately investigated in Hispanic populations. This study reinforces the prognostic role of *STK11/KEAP1* mutations among Hispanics as well.

Interestingly, we found that a considerable proportion of patients, almost 50% of them, had mutations on *STK11* or *KEAP1* without *KRAS* mutations; although, in the combined cohorts, a *KRAS* mutation rate reaches 40%. This result is consistent with much evidence establishing *KRAS* as one of the commonest drivers in NSCLC, with a mutation prevalence of 25–30% [26,27]. In our cohort, the presence of *KRAS* mutation was slightly higher than previously reported in the literature. Also, 86% of our cohort was classified as smokers or ex-smokers, and smoking is strongly associated with *KRAS* mutation [28].

Our cohort also showed a high frequency of co-occurring *STK11/KRAS* mutations. About two-thirds of our patients showed this co-mutation, which is slightly high compared to other cohorts that reported *STK11/KRAS* co-mutations ranging from 6.5 to 30% [29,30] *STK11/KEAP1* co-mutation in the presence of *KRAS* mutation was also relatively frequent. A marginal association was found between $STK11^{Mut}$ or $KEAP1^{Mut}$ ($p = 0.079$) and *the novo* brain metastasis. Current data shows that *STK11* mutations induce tumor microenvironment changes that promote angiogenesis and recruit an immunosuppressive cell infiltrate [31,32] Moreover, *STK11* alterations have been associated with poor tumor differentiation, increased invasive capacity, early lymph node metastasis, and advanced TNM stage [33]. All the molecular processes facilitated by *STK11* mutations increase the invasiveness of lung cancer cells and promote colonization of distant sites, which could explain the early development of brain metastasis found in association with mutation in *STK11*.

Regarding PD-L1 expression, we observed low tumor PD-L1 levels in two groups: tumors with wild-type *KRAS* plus *STK11* mutation and tumors with *KRAS* mutation plus *KEAP1* mutation. STK11 has been identified as a critical regulator of resistance to anti-PD-1/PD-L1 therapy. Alterations in this serine/threonine kinase are associated with low or absent PD-L1 expression, low lymphocyte tumor infiltration, and high production of myeloid-cell-attracting cytokines [34,35]. Interestingly, this was observed in the context of a normally functioning *KRAS*. Previous studies demonstrated that *KRAS* mutations are positively associated with increased PD-L1 expression in NSCLC, mainly adenocarcinoma. In our cohort, $KRAS^{WT}$ was not associated with a higher PD-L1 level in the context of $STK11^{Mut}$. That could suggest that the role of STK11 is more important or closely related to PD-L1 molecular interactions over *KRAS* gene [36,37] In contrast with previous studies, in our cohort, *KEAP1/KRAS* co-mutations were associated with lower PD-L1 expression. Some studies suggest that *KEAP1* alterations are associated with a better ICI response in NSCLC related to a higher PD-L1 expression. On the other hand, it seems to exist a positive association between *KEAP1* mutations and high TMB, as we also observed in this cohort [38,39].

Among patients treated with immune-checkpoint inhibitors, the ORR showed a higher value in comparison with other cohorts (≈7%) [30]. In addition, PFS was negatively affected by the presence of *STK11*

mutations. As other authors suggested, *STK11* alterations seem to be a negative predictive factor for anti-PD-1/PD-L1 therapy and they are associated with poor prognosis independent of therapy employed [14]. Besides a shorter PFS in the $STK11^{Mut}$ group, PD-L1 levels do not seem to have an association with PFS even taking in count the proposed role of STK11 and its association with PD-L1 expression. Our results suggest that in the context of *STK11* mutations, ICI therapy does not only target PD-L1 molecules but there are other interactions with the immune checkpoint inhibitor and the tumor microenvironment, and other immunetumor-infiltratingg cells [19]. In addition, when patients with $STK11^{Mut}$ + $KEAP1^{Mut}$ co-mutations were assessed (independent of KRAS status), we found a significant improvement in PFS. One explanation for this opposite effect over PFS could be that, in fact, *KEAP1* mutations can improve the response to ICI, and bypass the negative effect caused by *STK11* [39].

Additionally, comparing OS among ICI-treated patients, we found an opposite effect than observed in PFS. Patients with $STK11^{MUT}$ had better OS than $KEAP1^{MUT}$ patients. This finding could challenge the presence of *STK11/KEAP1* mutation in the context of its prognostic value because two outcome variables, PFS and OS, had opposite results. In this case, PD-L1 expression is related to a better OS. As we mentioned earlier, *STK11* could predict resistance to ICI due to this association with a cold immune microenvironment and low PD-L1 expression; we did not test the association of *STK11* mutations and PD-L1expression to establish if patients with a low PD-L1 expression were more commonly $STK11^{MUT}$ [40].

Finally, our study showed that *STK11* and *KEAP1* mutations conferred shorter OS in comparison with their wild-type counterparts, independently of *KRAS* status. As suggested in previous studies, mutations in the abovementioned genes impact the complex molecular pathways that make tumors resistant to ICI, but in this case, the data showed the effect of these mutations in Hispanic population. Despite that, PD-L1 expression affected OS irrespective of *STK11, KEAP1* or *KRAS* mutations. Our data suggest that event in the presence of STK11 or KEAP1 mutations, PDL-1 expression still hold some prognostic impact, although of lesser importance [41]. Moreover, high TMB was also associated with better OS in the context of ICI treatment. This has been proposed recurrently as a predictive factor of response to ICI on the premise that an elevated TMB results in a higher diversity of new tumor-related antigens that could activate a broader spectrum of specific cytotoxic T-cells thus improving antitumoral immunity [42].

We must discuss some limitations of our study. The first one is the small number of individuals. This makes it challenging to translate these results to large populations due to the ethnical heterogeneity and the molecular diversity of lung cancer among Hispanics. Another critical factor is the fact that this is a retrospective and observational study, so the strength of the associations hereby reported may be affected by the biases inherent to this type of study (recollection bias, time-lead bias). And, although we collected data on some of the most common mutations detected in NSCLC, many other molecular alterations/mutations have not been systematically included or reported.

In conclusion, our results provide some evidence that suggests that *STK11* and *KEAP1* mutations could be used as a prognostic factor in Hispanic patients under ICI treatment, and similarly to what has been reported elsewhere, these mutations were also associated with worse outcomes among these individuals.

CRediT authorship contribution statement

Vladmir C. Cordeiro de Lima: Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft. **Marcelo Corassa:** Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft. **Erick Saldanha:** Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft. **Helano Freitas:** Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft. **Oscar Arrieta:** Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Luis Raez:** Data curation, Investigation, Resources. **Suraj Samtani:** Data curation, Investigation, Resources. **Maritza Ramos:** Data curation, Investigation, Resources. **Carlos Rojas:** Investigation, Methodology, Validation. **Mauricio Burotto:** Data curation, Investigation, Resources. **Diego F. Chamorro:** Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Gonzalo Recondo:** Data curation, Investigation, Resources. **Alejandro Ruiz-Patiño:** Data curation, Investigation, Resources. **Luis Más:** Data curation, Investigation, Methodology, Resources. **Lucia Zatarain-Barrón:** Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft. **Sergio Mejía:** Data curation, Investigation, Methodology, Resources. **José Nicolas Minata:** Data curation, Investigation, Methodology, Resources. **Claudio Martín:** Data curation, Investigation, Methodology, Resources. **Juan Bautista Blaquier:** Data curation, Investigation, Methodology, Resources. **Rodrigo Motta Guerrero:** Data curation, Investigation, Methodology, Resources. **Carlos Aliaga-Macha:** Data curation, Investigation, Methodology, Resources. **Carlos Carracedo:** Data curation, Investigation, Methodology, Resources. **Camila Ordóñez- Reyes:** Formal analysis, Investigation, Resources. **Juan Esteban Garcia-Robledo:** Formal analysis, Investigation, Resources. **Luis Corrales:** Formal analysis, Investigation, Resources. **Carolina Sotelo:** Data curation, Investigation, Resources. **Luisa Ricaurte:** Data curation, Investigation, Resources. **Nicolas Santoyo:** Data curation, Investigation, Resources. **Mauricio Cuello:** Data curation, Investigation, Resources. **Elvira Jaller:** Data curation, Investigation, Resources. **July Rodríguez:** Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft. **Pilar Archila:** Data curation, Investigation, Methodology, Resources. **Maritza Bermudez:** Data curation, Investigation, Methodology, Resources. **Tatiana Gamez:** Data curation, Investigation, Methodology, Resources. **Alessandro Russo:** Data curation, Investigation, Methodology, Resources. **Lucia Viola:** Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft. **Umberto Malapelle:** Data curation, Investigation, Methodology, Resources. **Diego de Miguel Perez:** Data curation, Investigation, Methodology, Resources. **Christian Rolfo:** Data curation, Investigation, Methodology, Resources. **Rafael Rosell:** Data curation, Investigation, Methodology, Resources. **Andrés F. Cardona:** Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

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remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lungcan.2022.06.010.

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