Updated Frequency of EGFR and KRAS Mutations in NonSmall-Cell Lung Cancer in Latin America

The Latin-American Consortium for the Investigation of Lung Cancer (CLICaP)

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Introduction: Previously, we reported the frequency of epidermal growth factor receptor (EGFR) and KRAS mutations in nonsmall-cell lung cancer (NSCLC) patients in Latin America. The EGFR mutation frequency was found between Asian (40%) and Caucasian (15%) populations. Here, we report the updated distribution of NSCLC mutations.

Methods: A total of 5738 samples from NSCLC patients from Argentina (1713), Mexico (1417), Colombia (1939), Peru (393), Panama (174), and Costa Rica (102) were genotyped for EGFR and KRAS.

Results: The median patient age was 62.2 ± 12.3 years; 53.5% were women, 46.7% had a history of smoking, and 95.2% had adenocarcinoma histology. The frequency of EGFR mutations was 26.0% (95% confidence interval [CI], 24.9–27.1; Argentina, 14.4% [12.8–15.6]; México, 34.3% [31.9–36.7]; Colombia, 24.7% [22.8–26.6]; Peru, 51.1% [46.2–55.9]; Panamá, 27.3 [20.7–33.9]; and Costa Rica, 31.4% [22.4–40.4]). The frequency of KRAS mutations was 14.0% (9.1–18.9). In patients with adenocarcinoma, EGFR mutations were independently associated with gender (30.7% females vs. 18.4% males; $p < 0.001$), nonsmoker status (27.4% vs. 17.1%, $p < 0.001$), ethnicity (mestizo/indigenous, 35.3% vs. Caucasian, 13.7%, $p < 0.001$), and the absence of KRAS mutation (38.1% vs. 4.7%; $p < 0.001$). The overall response rate to EGFR tyrosine kinase inhibitors was 60.6% (95% CI, 52–69), with a median progression-free survival and overall survival of 15.9 (95% CI, 12.420.6) and 32 months (95% CI, 26.5–37.6), respectively.

Conclusion: Our findings support the genetic heterogeneity of NSCLC in Latin America, confirming that the frequency of EGFR mutations is intermediate between that observed in the Asian and Caucasian populations.

Key Words: Non small-cell lung cancer, Epidermal growth factor, Anaplastic lymphoma kinase, Mutation, Frequency.

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Although the overall incidence of cancer is lower in Latin America (age-standardized rate of 163 per 100,000) than in Europe (264 per 100,000) or the United States (300 per 100,000), the mortality burden is greater.[1] Lung cancer is the main cause of cancer-related death in Latin America,[2] although different regional trends in cancer types exist.

The recognition of a subgroup of patients with nonsmall-cell lung cancer (NSCLC) harboring mutations of the epidermal growth factor receptor (EGFR) gene that exhibit a favorable response to tyrosine kinase inhibitors (TKIs) has changed the way we view lung cancer over the last decade. The

90% of EGFR-activating mutations for lung cancer include the leucine-to-arginine substitution at position 858 (L858R) and deletions in exon 19.[3–6] The EGFR T790M mutation, which has been reported at a low frequency in untreated patients with stage IIIB/IV NSCLC (5.8%), has been detected in ~50% of patients who become resistant to EGFR TKIs. The classical phenotype associated with EGFR-activating mutations includes female sex, Asian ethnicity, nonsmoker status, and adenocarcinoma histology. The precise prevalence of activating mutations has been reported in different populations: 15% in patients from North America and Europe, 40% in patients from East Asia, 15% to 20% in African Americans patients, and 20% to 25% in patients from the Indian subcontinent.[7–11] Previously, we reported that the frequency of EGFR mutation was 33.2% in 1150 patients from Latin America (Argentina, 19.1%; Mexico, 31.2%; Colombia, 24.8%; and Peru, 67.0%).[3] In this report, we update the frequency of EGFR and KRAS mutations in Latin America, including data from Costa Rica and Panama.

PATIENTS AND METHODS

Patient Selection

All Latin-American patients (1713 from Argentina, 1417 from Mexico, 1939 from Colombia, 393 from Peru, 102 from Costa Rica, and 174 from Panama) exhibited histologically confirmed lung cancer. Brazil presented independently their results in Latin American Lung Cancer Conference 2014.[12] The protocol was approved by a central ethical and scientific committee in the National Cancer Institute in Mexico City (approval number 011/012/ICI, CB/678, "Frecuencia de Mutaciones, en el Gen del Receptor del Factor de Crecimiento Epidérmico, EGFR, en Latinos/Hispanos con Cáncer de Pulmón de Células No Pequeñas"). Informed consent authorizing implementation of detecting mutations of EGFR and Kras was independent for each country and in accordance with the provisions and guidelines of each institution. The analysis included patients from our previous report (1150 patients).[3]

Tissue Procurement

Biopsies were taken using transbronchial sampling, thoracoscopic segmental resections, or computed tomography-guided core needle biopsy and were analyzed by the pathology departments of the different participating institutions for their histological diagnosis and quantification of neoplastic cellularity (>50%). The sections were later embedded in paraffin until processing for DNA extraction. Genomic DNA was extracted by a standard procedure from areas of the paraffin slides using the QIAamp DNAFFPE Tissue Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions.

Mutational Analysis

Direct sequencing in patients from Argentina, Colombia, Peru, and Costa Rica was performed at each site. The kinase domain of EGFR was amplified by polymerase chain reaction (PCR). Two separate PCR reactions, each with the corresponding pair of primers, were used to amplify exon 19 (FWD 5′GCAATATCAGCCTTAGGTGCGGCTC3′ and RVS 5′CATAGAAAGTGAACATTTAGGATGTG3′) and exon 21 (FWD 5′CTAACGTTCGCCAGCCATAAGTCC3′ and RVS 5′GCTGCGAGCTCACCCAGAATGTCTGG3′) of the EGFR gene. Codons 12 and 13 of KRAS were amplified by PCR with specific primers (FWD 5′GGAGAGAGGCCTGCTGAAAATGAC3′ and RVS 5′TTGCTTCCTGTAGGAATCCTCTA3′). These PCR products were then subjected to direct sequencing using the same primers, and all mutations were confirmed by bidirectional sequencing originating from both the upstream and downstream primers. In México and Panama, mutations of the EGFR (exons 18, 19, 20, and 21) and KRAS genes were detected using the therascreen RGQ PCR Kit (QIAGEN, Scorpions amplification refractory mutation system [ARMS] method), which combines two technologies, namely the ARMS and quencher-tailed fluorescent primers or Scorpions, to detect mutations in real-time PCR reactions. Real-time PCR was performed using a Rotor-Gene Q 5plex HRM (QIAGEN) according to the manufacturer's instructions.[13,14] A similar procedure was performed in 75% of samples from Colombia using the cobas real-time PCR platform (Roche, Bogota, Columbia).

Statistical Analysis

For descriptive purposes, continuous variables were summarized as arithmetic means, medians, and standard deviations, and categorical variables were reported as proportions with 95% confidence intervals (95% CI). Inferential comparisons were performed using Student's t test. The χ^2 or Fisher's exact tests were used to assess significance among categorical variables. Statistical significance was determined as $p \leq 0.05$ with a two-sided test. Statistically significant and borderline significant variables ($p < 0.1$) were included in multivariate logistic regression analysis included the different countries. Progression-free survival and overall survival were measured from the day of EGFR TKI treatment to the date of progression or death and analyzed with the Kaplan Meier technique, whereas comparisons among subgroups were performed with the log-rank test.

RESULTS

The median age of all patients was 62.2 ± 12.3; 53.5% were women and 95.2% had adenocarcinoma histology. Of all patients, 46.7% had a history of smoking. With regard to ethnicity, 49% were self-reported Caucasian patients, and 50.8% were self-reported mestizo/indigenous ancestry (Table 1).

Molecular Genotyping of Patients

Among all patients, the frequency of EGFR mutations in NSCLC was 26.0% (95% CI, 23.8–28.2; Argentina, 14.4% [12.8–15.6]; México, 34.3% [31.9–36.7]; Colombia, 24.7% [22.8–26.6]; Peru, 51.1% [46.2–55.9]; Panamá, 27.3 [20.7–33.9]; and Costa Rica, 31.4% [22.4–40.4]).The frequency of KRAS mutations was 14.0% (9.1–14.9). The most frequent EGFR mutations were deletions in exon 19, 47.1%, followed by L858R in 37.3%. The basal T790M mutation was detected in 5.8% of EGFR-mutated NSCLCs; however, only 1.4% of all genotyped NSCLCs exhibited this mutation. We cannot assess whether these detected T790M mutations are associated with

TABLE 1. Demographic and Clinical Characteristics of All Patients

Variable	Argentina, N (%)	México, N (%)	Colombia, N (%)	Perú, N (%)	Costa Rica, N (%)	Panamá, N (%)	Total
Number of patients	1713 (29.9)	1417 (24.7)	1939 (33.8)	393 (6.8)	102 (1.8)	174 (3.0)	5738
Mean age ± SD	63.5 ± 10.5	60.5 ± 12.8	61.5 ± 13.6		65.9 ± 15	63.9 ± 12.6	62.2 ± 12.3
Gender							
Female (%)	748 (43.7)	505 (52.4)	1228 (63.3)	99 (48.8)	60 (58.8)	87 (50)	2727 (53.5)
Male (%)	965 (56.3)	458 (47.6)	711 (36.7)	104 (51.2)	42 (41.2)	87 (50)	2367 (46.5)
Histology							
Adenocarcinoma (%)	1713 (100)	1287 (90.8)	1807 (93.2)	203 (100)	97 (96.0)	162 (93.1)	5461 (95.2)
LCC (%)	0 (0)	8 (0.6)	7 (0.4)	0 (0)	1 (1.0)	1 (0.6)	17 (0.3)
NOS/ND (%)	0 (0)	30 (2.1)	84 (4.3)	0 (0)	2 (2.0)	6 (3.4)	122 (2.1)
SCC (%)	0 (0)	92 (6.5)	41 (2.1)	0 (0)	1 (1.0)	5 (2.9)	138 (2.4)
Smoking status							
Smoker[a] (%)	1184 (69.7)	242 (47.5)	467 (24.8)		61 (59.8)	75 (47.8)	2036 (46.7)
Never smoker[b] (%)	514 (30.3)	275 (52.5)	1416 (75.2)		41 (40.2)	82 (52.2)	2328 (53.3)
Ethnicity							
Caucasian (%)	1713 (100)	6 (0.5)	355 (36.2)	6 (3.0)			1977 (49)
Mestizo/Indigenous (%)	0 (0)	1228 (99.5)	619 (63.1)	197 (97.0)			2052 (50.8)
Asian (%)	0 (0)	1 (0.1)	0 (0)	0 (0)			1 (0.0002)
African Americans (%)	0 (0)	0 (0)	7 (0.7)	0 (0)			7 (0.2)
EGFR sensitizing mutation status							
Positive (%)	247 (14.4)	486 (34.3)	479 (24.7)	201 (51.1)	32 (31.4)	47 (27.3)	1491 (26.0)
Negative (%)	1466 (85.6)	931 (65.7)	1460 (75.3)	192 (48.9)	70 (68.6)	125 (72.7)	4247 (74.0)
KRAS							
Positive (%)		40 (15.9)	116 (12.9)	34 (16.8)			190 (14.0)
Negative (%)		210 (84.1)	785 (87.1)	168 (83.2)			1165 (86.0)

[a]Patients who have smoked more than 100 cigarettes in their lifetime.
[b]Patients who had never smoked cigarettes in their lifetime.
LCC, large cells cancer; ND, undifferentiated; NOS, not otherwise specified; SCC, squamous cells cancer.

clinical EGFR-TKI resistance. In patients harboring EGFR mutations, other less common mutations were reported in exon 18 (87 of 1124 patients, 7.7%), insertions in exon 20 (4 of 128 patients, 3.1%), in exon 20 S768I mutation (33 of 824 patients, 4.0%), in exon 21 L8161Q (6 of 468 patients, 1.3%), in exon 20 R831H (2 of 506 patients, 0.4%), and some point mutations in exon 21, T7847A, V834A, L858A, and L861G, 4 of 4768, 0.2% each one (Supplement 1, Supplemental Digital Content 1, http://links.lww.com/JTO/A795).

Clinical and Pathological Characteristics Associated with EGFR Mutations in Patients with Only Adenocarcinoma Histology

The frequency of the EGFR mutations in adenocarcinoma patients was 26.4%. EGFR mutations were associated with gender (30.7% in females vs. 18.4% in males; $p < 0.001$), self-reported nonsmoker status (27.4% vs. 17.1%; $p < 0.001$), self-reported ethnicity (mestizo/indigenous, 35.3% and Caucasian, 13.7%; $p < 0.001$), and the absence of KRAS mutation (38.1% vs. 4.7%, $p < 0.001$; Table 2). In the multivariate analysis, all these factors were associated (gender hazard ratio, 0.45 [95% CI, 0.36–0.56]; smoking history, 0.68 [0.550.83]; KRAS, 0.14 [0.060.35]; ethnicity, 0.47 [0.38–0.57], $p < 0.001$). However, multivariate logistic regression (including Countries/Regions) identified gender ($p < 0.001$), smoking history ($p < 0.001$), and regions ($p < 0.001$) to be independent predicted factor to EGFR mutation status (Supplement 2, Supplemental Digital Content 2, http://links.lww.com/JTO/A796).

Response to EGFR TKIs

Complete outcome information regarding EGFR TKI treatment was only available for 328 patients. The overall response rate in patients with EGFR-mutated NSCLC (n = 109) was 60.6% (95% CI, 52.10–69.09), with a progression-free survival of 15.9 months (95% CI, 12.4–20.6) and a median overall survival of 32 months (95% CI, 12.4–20.6; Fig. 1*A* and *B*). The overall response rate to EGFR TKIs for white/Caucasian was 58.3% (95% CI, 42.4–74.2) versus 65.5% (52.9–78.1) for mestizo/indigenous in patients with EGFR Mutations ($p = 0.492$).

DISCUSSION

We have demonstrated that a very significant proportion of NSCLCs from patients from Latin America with a Hispanic background harbor EGFR activating mutations and therefore constitute an important and very well-defined subgroup of patients with lung cancer. This information is important for both Latin-American countries and the United States; 16.3% of the US population is of Hispanic origin, and this proportion

TABLE 2. EGFR Mutation Frequency for Demographic and Clinical Characteristic Subgroups

		EGFR Mutation		
	N	Frequency (%)	95% CI	*P*
Country/Region				
Argentina	1713	247 (14.4	12.8–15.6	
Mexico	1417	472 (36.7	34.4–39.6	
Colombia	1939	456 (25.2	23.2–27.2	
Peru	393	201 (51.1	46.2–55.9	
Panama	174	41 (25.5	21.8–29.2	
Costa Rica	102	32 (32.7	23.4–41.9	<0.001
Gender				
Female	2589	794 (30.7)	29–32.4	
Male	2228	410 (18.4)	16.8–20	<0.001
Age				
≤60	1817	417 (22.9)	21–24.8	
>60	2567	592 (22.8)	21.6–24.6	0.757
Ethnic group				
Mestizo/indigenous	1891	668 (35.3)	33.2–37.5	
Caucasian	1942	267 (13.7)	12.2–15.3	
African Americans	6	0		<0.001
Smoking status				
Smoking	1953	333 (17.1)	15.4–18.7	
Never smoking	2188	600 (27.4)	25.4-29,3	<0.001
KRAS				
Positive	190	8 (4.7)	1.7–7.7	
Negative	1165	403 (38.1)	35.4–40.8	<0.001

is expected to increase in the coming years.[15] These differences could be partially explained by different genetic susceptibilities and exposure to several specific risk factors, such as wood smoke exposure (WSE). The problem of WSE is nearly exclusive to developing countries, and although not very well studied, it is estimated that up to 16% of households use wood as fuel for heating and cooking in some parts of Latin America.[2] It has been observed that WSE is an independent factor for increased EGFR mutation frequency and decreased KRAS mutation frequency.[16,17] The evident differences in the frequencies of EGFR mutations between the Caucasian and Asian populations are also present in other populations, including Turkey (42%),[18] India (33.3%),[19] Brazil (25.3%),[20] and Chile (22%),[21] where it appears to exist in an intermediate frequency between both populations.

Brazil recently published two abstracts evaluating the frequency of EGFR mutations in their population. In both abstracts, the reported EGFR mutation frequency was similar: 25.9% (847 of 3364)[22] and 27% (66 of 304; in these reports the detection of EGFR mutation was performed, in most cases using the Sanger sequencing). These results are comparable with those obtained by our analysis in the rest of Latin America.[12]

In our study, we observed an important heterogeneity in Latin America; as with previous studies, there was a strong positive relationship with female sex and negative relationship with the degree of tobacco exposure. Multivariate logistic analyses indicated a wide difference in mutation frequency by country/ethnicity regarding gender and smoking history (Supplement 2, Supplemental Digital Content 2, http://links.lww.com/JTO/A796). The EGFR mutation frequency was higher in Peru and lower in Argentina. Importantly, Peru has been a destination of Asian migration, whereas in Argentina, the majority of the population is of Caucasian origin. These findings suggest that lifestyle and genetics (smoking exposure history) and ethnicity are important in determining mutation frequency. Several reports show that polymorphism frequencies within EGFR gene are also influenced by smoke exposure and ethnicity.[23,24] Despite the high frequency of EGFR mutation in Asia, there is an important ethical heterogeneity manner similar to our study, there are differences in EGFR frequency in the mutation according to the country or regions.[25]

Another important difference in the Latin American population is the low frequency of KRAS mutation that could be explained by a lower frequency of smokers in our cohort and a high EGFR mutation frequency, which are mutually exclusive.[26] Previously, we have demonstrated that in patients with KRAS-mutated NSCLC, the response to treatment with chemotherapy and EGFR TKI is lower, which negatively impacts the overall survival.[26] Although, a recent report describes that the frequency of EGFR mutations is similar in US Hispanics compared with non-Hispanic whites.[27] This suggests important influence of lifestyle in EGFR mutation. Patel et al.[28] demonstrated that foreign-born Hispanic patients with NSCLC exhibited higher survival compared with non-Hispanic whites and US-born Hispanics. This observation could be explained by the high frequency of the EGFR mutation and the low frequency of the KRAS mutation in the Hispanic population. Recently, it was reported that several baseline clinical features (female sex, Asian or Hispanic ethnicity, lower stage, etc.) were independently associated with the frequency of mutation, which improved the cause-specific survival in patients <50 years of age. However, it appears that there are differences not only in molecular profiling but also in histological features. Hispanic patients diagnosed with NSCLC have a higher proportion of histologically favorable tumors, which are associated with improved survival in this population.[29] A limitation of our study was that the mutations were detected by two different methods (ARMS and Sequencing). However, standard to define the optimal testing method and specimen type required for the detection of EGFR mutations is not available. Some studies suggest that EGFR mutations are less detected in small biopsies from bronchoscopy regardless of the method used and where there is more tissue, and laboratory tests may be based on the experience of the laboratory but direct sequencing is highly recommended.[30] In other study, the sensitivity of direct sequencing and ARMS was 44.1% and 94.1%, respectively, for biopsy tumor tissues, but for surgery samples, sensitivity of direct sequencing and ARMS was 72.2% and 94.4%, respectively; ARMS has a higher sensitivity, and specific that sequencing of detection of EGFR mutation, and some report that the results for ARMS are more consistent with the efficacy of EGFR treatment.[31,32] Other important limitation is that our study is a retrospective cohort, and this leads to incomplete data about

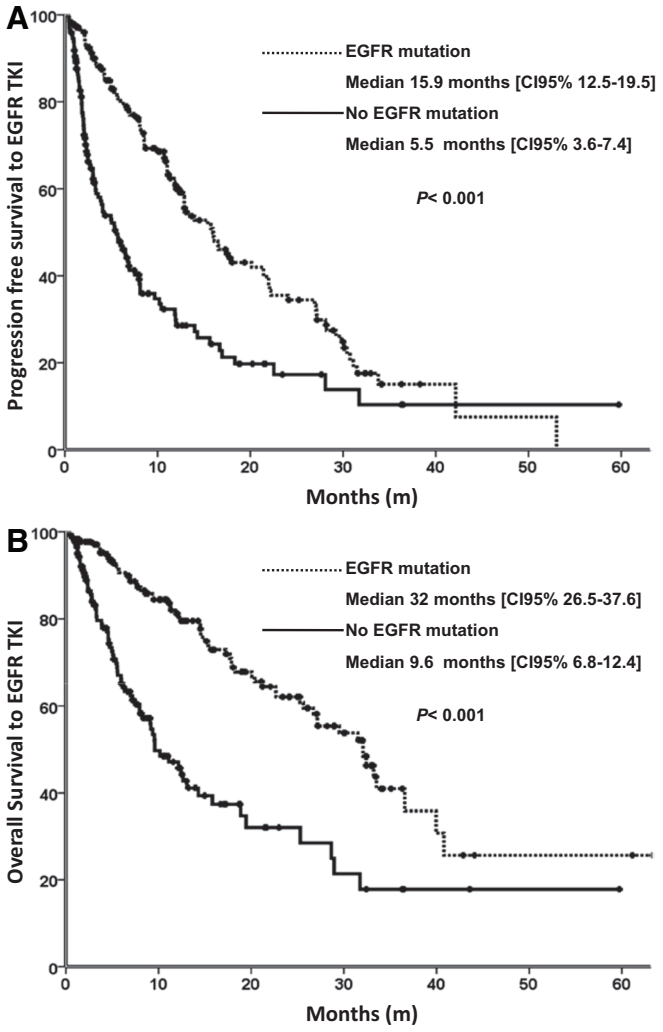

FIGURE 1. Progression free survival and overall survival to EGFR TKI.

the demographic characteristics, and we have a tendency to include patients with high possibility to harbor EGFR mutation, particular in Colombia and Peru.

In conclusion, this study represents the largest multinational effort to establish a genomic tumor profile in Latin America, which have a high frequency of EGFR mutations and a high variability in the frequency of EGFR mutation, and a low frequency of KRAS mutations compared with European/North American populations, possibly because of differences in ethnicity and other associated environmental risk factors for lung cancer. Our findings support the genetic heterogeneity of NSCLC around the world by confirming that the frequency of EGFR mutations in Latin America is intermediate between that observed in the Asian and Caucasian populations.

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