# Genotyping Non-small Cell Lung Cancer (NSCLC) in Latin America

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**Introduction:** Frequency of mutations in EGFR and KRAS in non-small cell lung cancer (NSCLC) is different between ethnic groups; however, there is no information in Latin-American population.

**Methods:** A total of 1150 biopsies of NSCLC patients from Latin America (Argentina, Colombia, Peru, and Mexico) were used extracting genomic DNA to perform direct sequencing of EGFR gene (exons 18 and 21) and KRAS gene in 650 samples. In Mexico, Scorpions ARMS was also used to obtain a genetic profile.

**Results:** We report the frequency of mutations in EGFR and KRAS genes in four Latin-American countries ($n = 1150$). Frequency of EGFR mutations in NSCLC was 33.2% (95% confidence interval [CI] 30.5–35.9) (Argentina 19.3%, Colombia 24.8%, Mexico 31.2%, and Peru 67%). The frequency of KRAS mutations was 16.6% (95% CI 13.8–19.4). EGFR mutations were independently associated with adenocarcinoma histology, older age, nonsmokers, and absence of KRAS mutations. Overall response rate to tyrosine kinase inhibitors in EGFR-mutated patients ($n = 56$) was 62.5% (95% CI 50–75) with a median overall survival of 16.5 months (95% CI 12.4–20.6).

**Conclusions:** Our findings suggest that the frequency of EGFR mutations in Latin America lies between that of Asian and Caucasian populations and therefore support the genetic heterogeneity of NSCLC around the world.

**Key Words:** Non-small cell lung cancer, Mutation, EGFR, KRAS, Latin America.

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Subdividing non-small cell lung cancer (NSCLC) based on clinically relevant molecular alterations is a promising treatment strategy. The most commonly mutated genes in lung adenocarcinoma are KRAS and EGFR. Almost 90% of specific EGFR mutations for lung cancer comprise the leucine-to-arginine substitution at position 858 (L858R) and deletions in exon 19 that affect the conserved sequence LREA (delE746-A750).[1] Those mutations cause constitutive activation of the tyrosine kinase domain of the EGFR. Female patients, Asian ethnicity, nonsmokers, and those with adenocarcinoma have higher frequency of EGFR mutations.[2] KRAS mutation usually occurs at codon 12 (characterized by G-to-T transversions), occasionally at codon 13, and rarely at codon 61, which sustains the activation of the RAS signaling pathway.[3]

Activating EGFR mutations confer a special sensitivity to the tyrosine kinase inhibitors gefitinib and erlotinib,[2] but patients with KRAS mutations have a lower survival and response rate.[4] It is known that NSCLC patients harboring EGFR-dependent primary tumors can develop resistance because of new kind of mutations such as T790M for EGFR or cMET amplification.[3]

It has been shown that the frequency of mutations varies between ethnic groups; EGFR mutations: 15% of North American and European patients, 40% of Asian patients, and between 2% and 14% of Afro-American patients.[5–8] Frequency of KRAS mutation is around 30% in Caucasian population and 10% in East Asian subjects with lung adenocarcinoma.[9,10]

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The precise frequency of activating mutations in the Latin-American population has not been determined. Our group previously reported a high response rate (34%) in nonselected Mexican patients who progressed after chemotherapy, suggesting a higher frequency of EGFR mutations among Hispanics.

## PATIENTS AND METHODS

### Patient Selection

A large cohort of Latin American patients with histologically confirmed NSCLC (244 from Argentina, 322 from Colombia, 381 from Mexico, and 203 from Peru) were included; all gave their informed consent for testing, and the protocol was approved by local Institutional Review Boards.

### Tissue Attainment

Biopsies were taken using computed tomography-guided tru-cut or by bronchoscopy and were analyzed by the pathology departments of the different participating institutions for their histological diagnosis and quantification of neoplastic cellularity (>50%). Thereafter the tumor tissue was embebed in paraffin until DNA extraction.

### DNA Extraction

Genomic DNA was extracted by a standard procedure from areas of paraffin slides using the QIAamp DNAFFPE Tissue Kit (QIAGEN) according to the manufacturer's instructions.

### Mutational Analysis

Direct sequencing in patients from Argentina, Colombia, Mexico, and Peru was performed at each site. The kinase domain of the EGFR was amplified by polymerase chain reaction (PCR). Two separate PCRs, each with the corresponding pair of primers, were used to amplify the exons 19 and 21 of the EGFR genes. The codons 12 and 13 of the KRAS were amplified by PCR with specific primers. These PCR products were then subjected to direct sequencing using the same primers, and all mutations were confirmed by sequences originating from both the upstream and downstream primers. In Mexico, some mutations of EGFR (exons 18, 19, 20, and 21) and KRAS genes were detected by Therascreen RGQ PCR Kit (QIAGEN, Scorpions ARMS method), which combines two technologies, namely ARMS and Scorpions, to detect mutations using real-time PCRs, which was performed using a Rotor-Gene Q 5plex HRM (QIAGEN), according to the manufacturer's instructions.

### Statistical Analysis

For descriptive purposes, continuous variables were summarized as arithmetic means, medians, SDs, and categorical variables comprised proportions with 95% confidence intervals (95% CI). Inferential comparisons were carried out by Student's $t$ test or Mann-Whitney $U$ test according to the distribution of the data (normal and non-normal) determined by the Kolmogorov-Smirnov test. $\chi^2$ or Fisher's exact test was used to assess significance among categorical variables. Statistical significance was determined as $p < 0.05$ with a two-sided test. Statistically significant and borderline significant variables ($p < 0.1$) were included in multivariate logistic regression analysis. Progression-free survival was measured from day of starting treatment to the date of last follow-up visit and analyzed using Kaplan-Meier technique, whereas comparisons among subgroups were carried out with the log-rank test. For analysis of survival curves, all variables were dichotomized.

## RESULTS AND DISCUSSION

Herein, we report the frequency of mutations in EGFR and KRAS among Latin American countries including Argentina, Colombia, Mexico, and Peru. We included 1150 patients. Table 1 shows the clinical and pathological characteristics of the population. We found activating mutations in the EGFR gene in 382 of 1150 patients (33.2% [95% CI 30.5–35.9]). Our findings suggest that the frequency of EGFR mutations in Latin America is between that of Asian (40%) and European (15%) populations[5–7] and support the existence of ethnic variations in the frequency of these genomic alterations.

In our study, 48.4% patients had exon 19 deletion (DelEx19) and 49% have L858R mutation (Ex21) (Table 1). These mutations account roughly for 90% of activating EGFR mutations in NSCLC. These findings are consistent with the data from Asian (DelEx19 60% and Ex21 40%) and European populations (DelEx19 62.2% and Ex21 37.8%).[6] Interestingly, we detected a high frequency of EGFR mutations in patients from Peru (67%) as compared with other countries from the region, probably due to the Asian migration and aborigine predominance. It is important to highlight that in Peru, the relationship between DelEx19 and L858R is inverted, meaning that it is different to the other studied countries. Mexico had the second highest frequency of EGFR mutations (31.2%); data are consistent with already published response rate to erlotinib (34%) in nonselected lung cancer patients.[11]

The EGFR mutations show an independent association with adenocarcinoma histology ($p < 0.001$), older patients ($p = 0.002$), and nonsmokers ($p = 0.001$) (Table 2). These results are similar to previous reports and confirm the presence of a different biological pattern, opposite to that described for lung cancer induced by tobacco exposure.[12] The high frequency of EGFR mutations could be explained not only by ethnicity but also by the high frequency of women and nonsmokers, features suggesting a selection bias. However, smokers and men also display a higher frequency of EGFR mutations compared with previous non-Latin-American reports (24.6% and 29.5%, respectively) (Table 2). Our study shows similar results than a previous report showing that EGFR mutations were more frequent in older patients (69% in >57 years) than in the younger ones (39% in <57 years).[13]

Frequency of KRAS mutations at codons 12 and 13 was 16.6% (95% CI 13.8–19.4) among the 650 analyzed specimens (Table 1), similar data to that reported in Asian population.[9,10] We found higher frequency of EGFR mutations in patients without KRAS mutation (43.4% versus 4.6%, $p <$

**TABLE 1.** Patient Characteristics

| Variable | Argentina | Colombia | Mexico | Peru | Total |
|---|---|---|---|---|---|
| No. of patients | 244 | 322 | 381 | 203 | 1150 |
| Mean age ± SD | 61.3 ± 10.1 | 58.8 ± 12.8 | 59.8 ± 13.2 | 62.8 ± 10.9 | 60.3 ± 12.2 |
| Gender, *n* (%) | | | | | |
| Female | 140 (57.4) | 216 (67.1) | 215 (56.2) | 99 (48.8) | 670 (58.4) |
| Male | 104 (42.6) | 106 (32.9) | 164 (43.3) | 104 (51.2) | 478 (41.6) |
| Histology, *n* (%) | | | | | |
| Adenocarcinoma | 244 (100) | 263 (81.7) | 300 (79.6) | 203 (100) | 1010 (90.4) |
| LCC | | 6 (2.0) | 3 (0.8) | | 9 (0.8) |
| NOS/nondifferentiated | | 16 (5.5) | 14 (3.7) | | 30 (2.7) |
| SCC | | 8 (2.7) | 60 (15.9) | | 68 (6.1) |
| Smoking status, *n* (%) | | | | | |
| Smoker | 84 (41.4) | 72 (27) | 188 (49.7) | 69 (34) | 448 (42.6) |
| Never-smoker | 119 (58.8) | 195 (73) | 190 (50.3) | 134 (66) | 603 (57.4) |
| Ethnicity, *n* (%) | | | | | |
| Caucasian | 243 (100) | 51 (37) | 6 (1.6) | 6 (3) | 306 (31.7) |
| Mestizo/indigenous | | 86 (62.3) | 375 (98.4) | 197 (97) | 658 (68.2) |
| Black | | 1 (0.7) | 0 | 0 | 1 (.1) |
| EGFR sensitizing mutation status, *n* (%) | | | | | |
| Positive | 47 (19.3) | 80 (24.8) | 119 (31.2) | 136 (67) | 382 (33.2) |
| Negative | 197 (80.7) | 242 (75.2) | 262 (68.8) | 67 (33) | 768 (66.8) |
| Exon 18 (+) | 1 (2.1) | | 11 (9.2) | | 12 (3.1) |
| Exon 19 deletion (+) | 30 (63.8) | 54 (67.5) | 76 (63.9) | 25 (18.4) | 185 (48.4) |
| Exon 21 L858R (+) | 19 (40.4) | 26 (32.5) | 31 (26) | 111 (81.6) | 187 (48.9) |
| Exon 20 S768I (+) | 2 (4.2) | | 10 (8.4) | | 12 (3.1) |
| Complex mutations | 2 (4.2) | 0 | 11 (9.2) | 0 | 13 (3.4) |
| T790M basal, *n* (%) | | | | | |
| Positive | 2 (0.8) | 6 (5.2) | 8 (2.1) | | 16 (2.2) |
| Negative | 242 (99.2) | 108 (94.7) | 373 (97.9) | | 723 (97.8) |
| KRAS, *n* (%) | | | | | |
| Positive | | 35 (17.1) | 39 (16) | 34 (16.8) | 108 (16.6) |
| Negative | | 170 (82.9) | 204 (84) | 168 (83.2) | 542 (83.4) |

LCC, Large cell carcinoma; NOS, non small; SCC, squamous cell carcinoma.

0.001). Although most reports indicate that EGFR and KRAS mutations are mutually exclusive, suggesting the presence of different pathways of lung carcinogenesis, our findings show that KRAS mutation may coexist with EGFR mutation (4.6%), similar to a previous report.[14] We also found a higher frequency of KRAS mutations in smokers than in nonsmokers (20% versus 13.4%, $p = 0.029$) as reported previously.[15]

Information about treatment outcomes could be obtained from 56 EGFR-mutated patients treated in Mexico and Colombia; all received a reversible tyrosine kinase inhibitor in as first, second, third, and fourth lines in 26.7%, 35.7%, 26.8%, and 10.7%, respectively. We found a complete response rate in 7.1%, partial response rate in 55.4% (overall response rate 62.5%), and stable disease in 37.5%. Progression-free survival and overall survival were 15.1 (95% CI 12.4–17.9) and 16.4 months (12.4–20.6), respectively. No difference was found on overall survival between patients harboring DelEx19 (16.5 months [10.4–22.7]) or L858R (16.0 months [11.1–20.9]; $p = 0.612$); similar results were reported for patients treated with gefitinib as first-line therapy in patients with EGFR mutations (Supplemental Figure 1, Supplemental Digital Content 1, http://links.lww.com/JTO/A145).[16]

In conclusion, our findings suggest that the frequency of EGFR mutations in Latin America lies between that of Asian and Caucasian populations and complement growing evidence pointing to genetic heterogeneity of EGFR pathway in NSCLC among different ethnic groups. The increase in frequency of EGFR mutations in Asian and Latin American lung cancer patients compared with Caucasian ones could be explained by a common origin but may also suggest a possible genetic susceptibility to carcinogens present among these populations. Other factors such as wood smoke exposure and tuberculosis could be involved.[11] Our results confirm the need to consider ethnicity and geographical differences in designing future clinical trials.

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**TABLE 2.** Characteristics of Patients with EGFR Mutations

| | Argentina | | Colombia | | Mexico | | Peru | | Total | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Variable** | **Mutant** | ***p*** | **Mutant** | ***p*** | **Mutant** | ***p*** | **Mutant** | ***p*** | **Mutant** | ***p* (UA)** | ***p* (MA), OR (95% CI)** |
| Age (yr) | | | | | | | | | | | |
| ≤60 (%) | 10.8 | | 26.9 | | 28.9 | | 66.4 | | 30.1 | 0.026 | 0.002, 1.93 (1.3–2.9) |
| >60 (%) | 27.4 | 0.001 | 24.8 | 0.68 | 33.3 | 0.34 | 68.8 | 0.72 | 36.3 | | |
| Gender | | | | | | | | | | | |
| Female (%) | 25.7 | | 26.4 | | 38.6 | | 65.7 | | 36 | | |
| Male (%) | 10.6 | 0.003 | 21.7 | 0.36 | 22 | 0.001 | 68.3 | 0.69 | 29.5 | 0.022 | 0.97, 0.99 (0.65–1.5) |
| Histology | | | | | | | | | | | |
| Adenocarcinoma (%) | 19.3 | | 25.5 | | 37.3 | | 67.0 | | 35.8 | | |
| LCC (%) | | | 33.3 | | 0 | | | | 22.2 | | |
| NOS/nondifferentiated (%) | | | 18.8 | | 28.6 | | | | 23.3 | | |
| SCC (%) | | | 0 | 0.353 | 3.3 | <0.001 | | | 2.9 | <0.001 | <0.001, 0.079 (0.027–0.23) |
| Smoking status | | | | | | | | | | | |
| Smoker (%) | 10.1 | | 20.8 | | 20.2 | | 65 | | 24.6 | | |
| Never-smoker (%) | 34.5 | <0.001 | 26.7 | 0.32 | 41.6 | <0.001 | 67.9 | 0.69 | 41.6 | <0.001 | 0.001, 0.48 (0.3–0.74) |
| Ethnicity | | | | | | | | | | | |
| Caucasian (%) | | | 31.4 | | 0 | | 16.7 | | 20.9 | | |
| Mestizo/indigenous (%) | | | 25.6 | 0.631 | 31.7 | 0.096 | 68.5 | 0.016[a] | 41.9 | <0.001 | 0.001, 1.9 (0.075–0.52) |
| KRAS | | | | | | | | | | | |
| Positive (%) | | | 0 | | 12.8 | | 0 | | 4.6 | | |
| Negative (%) | | | 13.5 | 0.017 | 37.3 | 0.003 | 81 | <0.001 | 43.4 | <0.001 | <0.001, 0.50 (0.019–0.128) |

[a] Analyzed with Fisher's exact test.
UA, univariate analysis; MA, multivariate analysis, LCC, Large cell carcinoma; NOS, non small; SCC, squamous cell carcinoma.

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