Published Ahead of Print on November 16, 2018 as 10.1634/theoncologist.2018-0254.

The Oncologist®

Global Health and Cancer

EGFR Mutation Testing: Changing Patterns of Molecular Testing in Brazil

Sofia Palacio,[a] Luciola Pontes,[b] Edna Prado,[c] Junaid Arshad,[a] Robert Ali,[a] Tony Piha,[d] Carlos Eduardo Bacchi,[e] Raja Mudad,[a] Gilberto Lopes[a]

[a]Department of Medicine, Miller School of Medicine and Sylvester Comprehensive Cancer Center, University of Miami, Miami, Florida, USA; [b]Hospital das Clínicas Instituto do Coração, São Paulo, Brazil; [c]Close-Up International, São Paulo, Brazil; [d]AstraZeneca, Lisbon, Portugal; [e]Laboratório Bacchi, Consultoria em Patologia, Botucatu, Brazil

Disclosures of potential conflicts of interest may be found at the end of this article.

Key Words. Lung adenocarcinoma • EGFR mutations • Brazil • Global health

Abstract

Background. In Brazil, cancer is the second most common cause of death. Most patients in resource-limited countries are diagnosed in advanced stages. Current guidelines advocate for *EGFR* mutation testing in all patients with metastatic adenocarcinoma. Tyrosine kinase inhibitors are recommended in patients with advanced or metastatic disease harboring sensitizing mutations. In Brazil, there are limited data regarding the frequency of *EGFR* testing and the changes in patterns of testing overtime.

Materials and Methods. This was an observational, retrospective study. We obtained deidentified data from a commercial database, which included 11,684 patients with non-small cell lung cancer treated between 2011 and 2016 in both public and private settings. We analyzed the frequency of *EGFR* mutation testing over time. We also directly studied 3,664 tumor samples, which were analyzed between 2011 and 2013. These samples were tested for *EGFR* mutations through an access program to tyrosine kinase inhibitors in Brazil.

Results. Overall, 38% of patients were tested for *EGFR* mutations; 76% of them were seen in the private sector, and 24% were seen in the public center. The frequency of testing for *EGFR* mutations increased significantly over time: 13% (287/2,228 patients) in 2011, 34% (738/2,142) in 2012, 39% (822/2,092) in 2013, 44% (866/1,972) in 2014, 53% (1,165/2,184) in 2015, and 42% (1,359/3,226) in 2016. *EGFR* mutations were detected in 25.5% of analyzed samples (857/3,364). Deletions in Exon 19 were the most frequent mutations, detected in 54% of patients (463/857).

Conclusion. Our findings suggest that the frequency of *EGFR* mutation in this cohort was lower than that found in Asia but higher than in North American and Western European populations. The most commonly found mutations were in Exon 19 and Exon 21. Our study shows that fewer than half of patients are being tested and that the disparity is greater in the public sector. ***The Oncologist*** 2018;23:1–5

Implications for Practice: These data not only indicate the shortage of testing but also show that the rates of positivity in those tested seem to be higher than in other cohorts for which data have been published. This study further supports the idea that awareness and access to testing should be improved in order to improve survival rates in lung cancer in Brazil.

Introduction

Lung cancer is the leading cause of cancer death worldwide for both men and women [1]. In Brazil, cancer is the second most common cause of death, and there were an estimated 27,330 new cases of lung cancer in 2014 [2]. Most patients in resource-limited countries are diagnosed in advanced stages of the disease in which targeted agents have changed the therapeutic landscape. Overexpression of the epidermal growth factor receptor (EGFR) is involved in non-small cell lung cancer pathogenesis by stimulation of downstream signal transduction that leads to cell proliferation and inhibition of apoptosis. Activating *EGFR* mutations confer sensitivity to tyrosine kinase inhibitors (TKIs) and are thus referred to as sensitizing mutations [3–5]. Four EGFR TKIs are available for the treatment of *EGFR*-mutated non-small cell lung cancer, including gefitinib, erlotinib, afatinib, and osimertinib (a fifth, icotinib, is also

Correspondence: Sofia Palacio, M.D., Department of Medicine, University of Miami Miller School of Medicine and Sylvester Comprehensive Cancer Center, 1120 NW 14th Streert, Suite 610N, Miami, Florida, USA. Telephone: 305-487-3669; e-mail: sofia.palacio@jhsmiami.org Received April 27, 2018; accepted for publication October 16, 2018. http://dx.doi.org/10.1634/theoncologist.2018-0254

Published Ahead of Print on November 16, 2018 as 10.1634/theoncologist.2018-0254.

available in Asia). Targeted therapy has markedly changed outcomes for patients with mutations. Several randomized clinical trials and meta-analyses have demonstrated an improvement in clinical efficacy of first-line treatment with EGFR TKIs compared with chemotherapy in patients whose tumors harbor activating *EGFR* mutations [6–10]. Current clinical guidelines advocate for universal testing of patients with advanced disease for *EGFR* mutations [11, 12].

Epidemiological studies have reported variable *EGFR* mutation prevalence according to ethnic background [5, 10, 13, 14]. The frequency of *EGFR* mutations varies from 27% to 60% in Asians, 8% to 13% in Europeans, 12% in Africans, 16% in white Americans, and from 20% to 25% in India. The most commonly reported mutations are deletions in Exon 19 and a point mutation in Exon 21 (L858R) [15]. Access to testing is often limited in the developing world. In the case of Brazil, there are limited data regarding the frequency of testing, the changes in patterns of testing over time, and whether differences exist across the public and private sectors.

A few studies have reported on the frequency of *EGFR* mutation in Brazilians with non- small cell lung cancer. One study reported the rates of *EGFR* mutations in a cohort of 207 Brazilian patients and their association with clinical and pathological characteristics. *EGFR* mutations were identified in 30.4% of patients. The most prevalent mutation was deletion in Exon 19 (60.3% of patients) followed by amino acid substitution L858R in Exon 21 in 27% of patients. Higher *EGFR* mutation rates were found in nonsmokers and patients with adenocarcinoma. There was no association with age, geographic location, or gender [16]. Another study found a lower rate of *EGFR* mutation of 21.6% in a cohort of 125 Brazilian patients. Deletions in Exon 19 (75%), followed by the point mutation of Exon 21 (25%), were also the most common mutations [17]. The frequency of *EGFR* mutations across different countries in Latin America has also been studied. The overall frequency of *EGFR* mutation was 33.2% in this larger cohort of 1,150 patients, but there was a large variability seen across different countries (Argentina, 19.3%; Colombia, 24.8%; Mexico, 31.2%; and Peru, 67%). In adenocarcinoma histology, the frequency was 26%. The researchers found a positive association with female gender, nonsmokers, and Mestizo/Indigenous ethnicity compared with white self-reported ethnicity. They postulated that the differences might be explained by different ethnic makeup of these countries. Argentina, for example, has a predominantly white population, with lower reported rates than Peru, where there have traditionally been higher rates of Asian migration [18].

Thus, the few published studies from Brazil have reported *EGFR* mutation frequency rates ranging from 12% to 30%, but most of these studies were small, and the only large patient cohort in Latin America did not include patients from Brazil. The data on frequency of testing are even more limited. Access to *EGFR* testing at the Brazilian National Cancer Institute increased from 40% to 85% from 2011 to 2013 [19]. Here, we report on the frequency of *EGFR* mutations in Brazil in a cohort of over 11,000 patients, as well as the rate of testing over 5 years. We also report on 3,364 patient samples that were tested directly for *EGFR* mutations. To our knowledge, this is the largest data set in the Brazilian population.

Materials and Methods

This was an observational, retrospective study involving practice patterns of over 2,000 cancer physicians in Brazil. We obtained deidentified data from a commercial database, which included 11,684 patients with non-small cell lung cancer treated between 2011 and 2016 in both public and private settings. We analyzed the frequency of *EGFR* mutation over time as well as the rate of *EGFR* mutation status in this cohort of patients.

Every 6 months the data administrators of a for-profit market intelligence company contacted over 400 physicians from a database that included approximately 2,000 cancer physicians and surveyed them on practice patterns. There was a screening questionnaire for physician selection. Physicians must have been in practice for at least 2 years, have treated a minimum of 30 patients per week, and have had no direct affiliation with pharmaceutical companies. On average 300 physicians answered each survey, providing deidentified data on approximately 18,000 patients whom they treated every semester. Physicians were paid a fee for participation and received feedback (final survey results). Every 6 months there was a natural switch of approximately 20% of the responding physicians. We obtained access to the lung cancer database, which reported on *EGFR* mutation testing patterns in the country over a 6-year period.

We also directly studied 3,664 tumor samples between 2011 and 2013. These samples were tested for *EGFR* mutations through an access program to TKIs. All patients had advanced or metastatic lung adenocarcinoma, and samples were taken from all states of Brazil. Clinical and pathological characteristics of all patients were obtained, and these data were deidentified. The Institutional Review Board at the University of Miami approved the research project.

All analyses were performed at two reference laboratories, as follows: after microdissection, DNA was isolated from serial sections of formalin-fixed, paraffin-embedded tumor tissue to obtain at least 70% tumor cells. Exons 18, 19, 20, and 21 of the *EGFR* gene were analyzed using Sanger sequencing. *EGFR* mutation rate was calculated, and its frequency was compared between clinical subgroups using the chi-square test. Data were summarized using descriptive statistics (mean, standard deviation, minimum, and maximum). *EGFR* mutation rate was calculated, and its frequency was compared between clinical subgroups using the chi-square test.

Results

In the commercial database of patients with non-small cell lung cancer treated in the country, 63% of patients had adenocarcinomas, 28% had squamous cell carcinoma, 5% had large cell cancer, and 3% had other histologies. Only patients with adenocarcinoma were analyzed, which included 11,684 patients from 2011 to 2016. Overall, 38% of patients in the commercial database were tested for

Published Ahead of Print on November 16, 2018 as 10.1634/theoncologist.2018-0254.

Table 1. Testing in the private and public sector

Year	Patients tested in the private sector, %	Patients tested in the public sector, %
2011	92	8
2012	80	20
2013	76	24
2014	74	26
2015	67	33
2016	68	32

Table 2. Frequency of EGFR testing among all patients with lung adenocarcinoma per year

Year	All patients with lung adenocarcinoma, *n*	Patients tested for *EGFR* mutation, *n* (%)
2011	2,228	287 (13)
2012	2,142	738 (34)
2013	2,092	822 (39)
2014	1,972	866 (44)
2015	2,184	1,165 (53)
2016	3,266	1,359 (42)

EGFR mutations. Of those tested, 76% of patients were seen in the private sector, and 24% were seen in the public center (Table 1). All patients had adenocarcinoma. The frequency of testing for *EGFR* mutations increased significantly over time (Table 2): 13% (287/2,228 patients) in 2011, 34% (738/2,142) in 2012, 39% (822/2,092) in 2013, 44% (866/1,972) in 2014, 53% (1,165/2,184) in 2015, and 42% (1,359/3,226) in 2016.

We directly tested 3,771 samples for *EGFR* mutations. Testing could not be reported for 407 patients, mostly because of an absence of tumor cells in the submitted material (39%) and inconclusive testing (52%), yielding 3,664 samples with adequate results. The median age of patients was 66 years (range, 18–97); 1,814 were male, and 1,957 were female. *EGFR* mutation was detected in 25.5% of samples (857/3364). Deletions in Exon 19 were the most frequent mutations, detected in 54% (463/857) of patients, followed by point mutations in Exon 21 in 28% (240/857 mutations), Exon 20 in 9.7% (83/857), and, less frequently, mutations in Exon 18 in 8.3% (71/857 mutations). Figure 1 shows the distribution of *EGFR* mutations. The health care providers ordering the test were mainly medical oncologists (88%, 3,289) or in pulmonary-critical care (5%, 191); 6% (237) were in other specialties.

No increase in the frequency of *EGFR* mutation detected was seen between the first 500 analyzed samples and the last 500, decreasing the possibility of phenotypic selection bias by physicians' referral ($p > .05$). We did not detect any differences in the rate of mutation across different regions in Brazil. For the patients who tested positive, 300 were male, and 557 were female.

Data on histologic subtypes were available for 1,472 samples, as follows: 1,086 patients (74%) had adenocarcinomas and 386 (26%) had carcinomas, not otherwise specified (NOS). No patients had squamous cell carcinoma, as

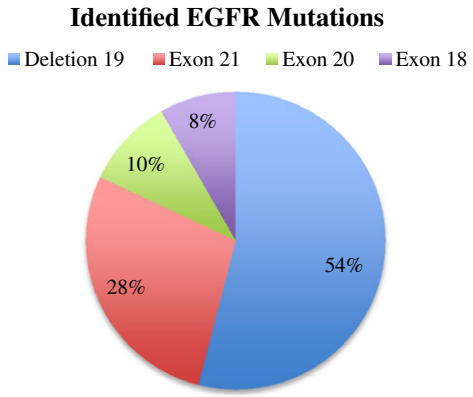

Figure 1. *EGFR* mutation distribution.

the program only accepted samples with nonsquamous, non-small cell lung cancer.

As shown in Figure 2, *EGFR* mutations were more common in patients with adenocarcinoma histology (30.5%) versus carcinoma NOS (10%; $p < .05$) and in women (30.2%) versus men (19%; $p < .05$).

Discussion

The frequency of *EGFR* mutations in non-small cell lung cancer varies across ethnic groups. Our findings suggest that the frequency of *EGFR* mutation in this cohort was lower than that found in Asia but higher than in North American and Western European populations, confirming findings seen in other Latin American countries. Brazil is the fifth largest country in the world, with a population of approximately 207 million people. It is also one of the most diverse, with a composition that includes different racial and ethnic groups, including Indigenous, European, African, and Asian. This heterogeneity might explain the high rate of *EGFR* mutations seen in this large database. Another reason might be the association between wood smoke exposure and *EGFR* mutations. Patients with wood smoke exposure have been found to be more frequently associated with *EGFR* receptor mutations, and although this particular variable was not captured in our study, wood smoke exposure is known to be higher in the developing world [20].

In our study, the most commonly found mutations were in Exon 19 and Exon 21, which were most likely to be L858R mutations. Unfortunately, data on all the specific mutations detected were not available. These findings are consistent with data from Asian (Exon 19, 60%, and Exon 21, 40%), European (Exon 19 deletion, 62.2%, and Exon 21, 37.8%), and Latin American populations [10]. Both of these mutations are activating and thus increase sensitivity to EGFR TKIs. Nonetheless, this study shows that less than 40% of patients are being tested. This disparity is greater in the public sector. Therefore, improvement in access to testing, as well as access to targeted EGFR therapy in those found to have sensitizing mutations, is essential in improving lung cancer outcomes in Brazil. Mutations were more common in individuals with adenocarcinoma histology and female gender, which has been previously described.

Several limitations of this study must be noted. Clinical data were only available on a subset of patients. Similarly,

Published Ahead of Print on November 16, 2018 as 10.1634/theoncologist.2018-0254.

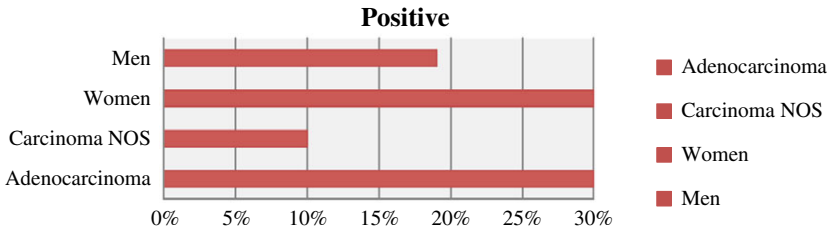

Figure 2. Characteristics of patients who tested positive for *EGFR* mutations. Abbreviation: NOS, not otherwise specified.

information on treatment selection and outcomes could not be obtained. Nonetheless, most of the reported literature has looked at frequency of mutations in small cohorts, which limits the interpretation of prevalence rates. To our knowledge this is the largest data analysis regarding changing practice patterns of molecular testing for lung adenocarcinoma over time in Brazil and Latin America and rates of *EGFR* mutation, and thus it likely reflects the true prevalence rate for *EGFR* mutations in the country. Although the frequency of testing has increased over the last 5 years, it is still below the current U.S. National Comprehensive Cancer Network guidelines recommendation that all patients with advanced disease be tested. This is especially relevant given the availability of TKIs in Brazil. There are also clear disparities between those treated in the public versus the private health care system. This study shows that over two thirds of the testing is conducted in the private sector and less than one third in the public sector. The disparity was even greater in the earlier years, when 80%–90% of testing was being conducted in the private sector. Two main factors are associated with this disparity. The first is that access to testing was initially through pharmaceutical companies mostly focused in the private sector. The second factor was that CONITEC, the National Committee for Technology Incorporation, initially denied approval of gefitinib and erlotinib for the Brazilian public health system. There was significant objection from advocacy groups, physicians, and industry, and these agents were finally approved in late 2013 [21]. However, despite TKI approval, the funding for testing and targeted therapy is still limited, and this certainly plays a role in the paucity of testing. Further understanding of these and other barriers to testing will hopefully lead to national strategies for universal implementation of molecular testing.

Conclusion

Given the increase in overall survival with the use of targeted agents in lung cancer, all patients with adenocarcinoma who have advanced or metastatic disease should be tested for *EGFR* mutations. Data regarding testing in resource limited countries is scant. To our knowledge this is the largest database describing the patterns of testing in Brazil. We report on the changes occurring in frequency of testing over time and the disparities in the public and private sector. Many challenges exist, as less than half of eligible patients are being tested despite EGFR drug availability in the country and higher rates of positivity than reported in other cohorts. Of those tested, the majority are in the private system, but most patients in the country receive care through the public health care system. Awareness is a first step in advancing access to testing, which in turn is essential to the improvement of lung cancer outcomes in Brazil.

Author Contributions

Conception/design: Sofia Palacio, Luciola Pontes, Junaid Arshad, Robert Ali, Tony Piha, Carlos Eduardo Bacchi, Raja Mudad, Gilberto Lopes
Provision of study material or patients: Edna Prado
Data analysis and interpretation: Sofia Palacio, Luciola Pontes, Junaid Arshad, Robert Ali, Tony Piha, Carlos Eduardo Bacchi, Raja Mudad, Gilberto Lopes
Manuscript writing: Sofia Palacio, Luciola Pontes, Junaid Arshad, Robert Ali, Tony Piha, Carlos Eduardo Bacchi, Raja Mudad, Gilberto Lopes
Final approval of manuscript: Sofia Palacio, Luciola Pontes, Edna Prado, Junaid Arshad, Robert Ali, Tony Piha, Carlos Eduardo Bacchi, Raja Mudad, Gilberto Lopes

Disclosures

The authors indicated no financial relationships.

References

1. Torre LA, Siegel RL, Jemal A. Lung cancer statistics. Adv Exp Med Biol 2016;893:1–19.

2. de Sa VK, Coelho JC, Capelozzi VL et al. Lung cancer in Brazil: Epidemiology and treatment challenges. Lung Cancer (Auckl) 2016;7:141–148.

3. Maemondo M, Inoue A, Kobayashi K et al. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. N Engl J Med 2010;362:2380–2388.

4. Lynch TJ, Bell DW, Sordella R et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. New Engl J Med 2004; 350:2129–2139.

5. Paez JG, Janne PA, Lee JC, et al. EGFR mutations in lung cancer: Correlation with clinical response to gefitinib therapy. Science 2004;304: 1497–1500.

6. Langer CJ. Epidermal growth factor receptor inhibition in mutation-positive non-small-cell lung cancer: Is afatinib better or simply newer? J Clin Oncol 2013;31:3303–3306.

7. Haaland B, Tan PS, de Castro G Jr et al. Meta-analysis of first-line therapies in advanced non-small-cell lung cancer harboring EGFR-activating mutations. J Thorac Oncol 2014;9: 805–811.

8. Mok TSK, Kim S, Wu Y et al. Gefitinib plus chemotherapy versus chemotherapy in epidermal growth factor receptor mutation-positive non-small-cell lung cancer resistant to first-line gefitinib (IMPRESS): Overall survival and biomarker analyses. J Clin Oncol 2017;35: 4027–4034.

9. Zhou C, Wu YL, Chen G et al. Final overall survival results from a randomised, phase III study of erlotinib versus chemotherapy as first-line treatment of EGFR mutation-positive advanced non-small-cell lung cancer (OPTIMAL, CTONG-0802). Ann Oncol 2015;26:1877–1883.

10. Rosell R, Moran T, Queralt C et al. Screening for epidermal growth factor receptor mutations in lung cancer. N Engl J Med 2009;361: 958–967.

11. National Comprehensive Cancer Network. Non-Small Cell Lung Cancer. Version 2.2018. https://www.nccn.org/professionals/physician_gls/pdf/nscl.pdf. Accessed February 15, 2018.

Published Ahead of Print on November 16, 2018 as 10.1634/theoncologist.2018-0254.

12. D'Addario G, Felip E; ESMO Guidelines Working Group. Non-small-cell lung cancer: ESMO clinical recommendations for diagnosis, treatment and follow-up. Ann Oncol 2009;20-(suppl 4):68–70.

13. Midha A, Dearden S, McCormack R. EGFR mutation incidence in non-small-cell lung cancer of adenocarcinoma histology: A systematic review and global map by ethnicity (mutMapII). Am J Cancer Res 2015;5:2892–2911.

14. Cote ML, Haddad R, Edwards DJ et al. Frequency and type of epidermal growth factor receptor mutations in African Americans with non-small cell lung cancer. J Thorac Oncol 2011;6:627–630.

15. Li AR, Chitale D, Riely GJ et al. EGFR mutations in lung adenocarcinomas: Clinical testing experience and relationship to EGFR gene copy number and immunohistochemical expression. J Mol Diagn 2008;10:242–248.

16. Bacchi CE, Ciol H, Queiroga EM et al. Epidermal growth factor receptor and KRAS mutations in Brazilian lung cancer patients. Clinics (Sao Paulo) 2012;67:419–424.

17. De Mello RA, Araújo A. Epidermal growth factor receptor mutation frequency and non-small cell lung cancer management: Implication for treatment choices. Clinics (Sao Paolo) 2012; 67:1349.

18. Lopes GL, Vattimo EFQ, de Castro Junior G. Identifying activating mutations in the EGFR gene: Prognostic and therapeutic implications in non-small cell lung cancer. J Bras Pneumol 2015; 41:365–375.

19. Montella T, Domingues P, Zukin M et al. Access to EGFR testing at the Brazilian National Cancer Institute (INCA): Impact of the reflex testing strategy. J Thorac Oncol 2014;9(suppl 3):S158–S159.

20. Arrieta O, Campos-Parra AD, Zuloaga C et al. Clinical and pathological characteristics, outcome and mutational profiles regarding non-small-cell lung cancer related to wood-smoke exposure. J Thorac Oncol 2012;7:1228–1234.

21. Strasser-Weippl K, Chavarri-Guerra Y, Villarreal-Garza C et al. Progress and remaining challenges for cancer control in Latin America and the Caribbean. Lancet Oncol 2015;16: 1405–1438.