

# Analysis of Predictive Biomarkers in Patients With Lung Adenocarcinoma From Southern Brazil Reveals a Distinct Profile From Other Regions of the Country

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## abstract

**PURPOSE** Adenocarcinoma is the most common histologic subtype of non–small-cell lung cancer, representing 40% of all diagnoses. Several biomarkers are currently used to determine patient eligibility for targeted treatments, including analysis of molecular alterations in *EGFR* and *ALK*, as well as programmed death-ligand 1 (PD-L1) protein expression. Epidemiologic data reporting the frequency of these biomarkers in Brazilian patients with lung adenocarcinoma (LUAD) are limited, and existing studies predominantly included patients from the southeast region of the country.

**MATERIALS AND METHODS** The goal of this study was to investigate the frequency of somatic mutations in the *EGFR*, *KRAS*, *NRAS*, and *BRAF* genes, *ALK*, and PD-L1 expression in a series of Brazilian patients diagnosed with LUAD predominantly recruited from centers in southern Brazil. Molecular analysis of the *EGFR*, *KRAS*, *NRAS*, and *BRAF* genes was performed by next-generation sequencing using DNA extracted from tumor tissue. Immunohistochemistry was used to detect *ALK* and PD-L1 expression.

**RESULTS** Analysis of 619 tumors identified *KRAS* mutations in 189 (30.2%), *EGFR* mutations in 120 (19.16%), and *BRAF* mutations in 19 (3%). Immunohistochemistry demonstrated *ALK* and PD-L1 expression in 4% and 35.1% of patients, respectively.

**CONCLUSION** To our knowledge, this is the first study investigating the molecular epidemiology of patients with LUAD from southern Brazil and the largest assessing the frequency of multiple predictive biomarkers for this tumor in the country. The study also reveals a distinct mutation profile compared with data originating from other regions of Brazil.

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## INTRODUCTION

Lung cancer (LC) is the leading cause of cancer mortality worldwide and responsible for 1.7 million deaths every year.<sup>1</sup> In Brazil, the National Cancer Institute estimated there would be 31,270 new patients with LC from 2018 to 2019, accounting for the second most common tumor type in the country. It is the leading cause of deaths among men, ahead of prostate cancer, and the second leading cause among women, only behind breast cancer. In southern Brazil in 2018, 5,350 and 3,110 new cases were estimated in men and women, respectively, which makes LC the third most frequent cancer in the region.<sup>2</sup>

Non–small-cell LC (NSCLC) accounts for approximately 85% of pulmonary neoplasm diagnoses.<sup>3,4</sup> Effective treatments remain scarce, considering that

the 5-year survival rate does not reach 20%, even in countries such as the United States.<sup>5</sup> In Brazil, this number is even lower, estimated at 16%.<sup>6</sup>

The use of predictive biomarkers allows therapeutic decisions to be based on tumor molecular profile.<sup>7</sup> For instance, certain somatic changes in the *EGFR*, *ALK*, *ERBB2*, and *BRAF* genes are substantial targets for tyrosine kinase inhibitors (TKIs).<sup>8</sup> In addition, new treatments for NSCLC using immune checkpoint inhibitors have recently been approved.<sup>9</sup> Its prescription depends on the expression of certain biomarkers on the tumor cell surface, such as the programmed death-ligand 1 (PD-L1) protein, a molecule in which the binding to its programmed death-1 receptor on T cells allows immune escape and tumor cell proliferation. The use of anti–programmed death-1/PD-L1

## ASSOCIATED CONTENT

### Data Supplement

Author affiliations and support information (if applicable) appear at the end of this article.

Accepted on July 30, 2019 and published at [ascopubs.org/journal/jgo](https://ascopubs.org/journal/jgo) on September 18, 2019; DOI <https://doi.org/10.1200/JGO.19.00174>

drugs blocks such binding and reactivates the patient's immune response.<sup>10</sup>

Although the molecular profile of predictive biomarkers in LUAD is already well documented in Europe, the United States, and some regions of Asia, there are few studies exploring these data in Latin America. In Brazil, only a few reports have been published since 2012, and all were essentially restricted to the southeast region. Thus, these data may not be representative of all regions in Brazil, given the differences in ancestry according to regions.<sup>11,12</sup>

On the basis of this information, the main goal of this study was to investigate the frequency of somatic alterations in *EGFR*, *KRAS*, *NRAS*, and *BRAF* genes by next-generation sequencing (NGS), as well as ALK and PD-L1 expression in a series of Brazilian patients diagnosed with LUAD. To our knowledge, this is the first study to include a large number of patients who were tested by a biomarker panel in southern Brazil. These results might be important for new public policies in the treatment of LUAD.

## MATERIAL AND METHODS

### Study Population

This was a retrospective study conducted by the Precision Medicine Program of the Hospital de Clínicas de Porto Alegre (HCPA) in Brazil, which enrolled a case series of patients with LUAD who underwent molecular testing from September 2016 to January 2019.

Samples from 619 individuals were obtained from different hospitals and clinics distributed in 22 centers located in the three states of the southern region of Brazil: Rio Grande do Sul (N = 516), Santa Catarina (N = 24), and Paraná (N = 74). The five remaining patients were obtained from Rio de Janeiro. All included patients had confirmed adenocarcinoma histology. The diagnostic slides and formalin-fixed, paraffin-embedded tissue blocks were retrieved and reviewed by pathologists with expertise in LC. This project was approved by the HCPA Research Ethics Committee (No. 18-0099) and registered under the Certificate of Presentation for Ethical Appreciation (No. 83557418.5.0000.5327).

### Tumor Selection and DNA Extraction

For all patients, 10- $\mu$ m thick sections representative of the tumor tissue were cut, and regions with a higher percentage of tumor cells were selected for DNA extraction. DNA from the tissue samples was extracted using the ReliaPrep FFPE gDNA Miniprep System (Promega, Madison, WI) according to the manufacturer's recommendations. After extraction, the DNA samples were quantified using the fluorescence method (Qubit 2.0 Fluorometer, Invitrogen, Carlsbad, CA), which provides an accurate DNA quantification.

Of the 799 NSCLC samples that were received by the Precision Medicine Program of the HCPA, 619 (77.4%) were considered suitable for NGS analysis on the basis of tumor cell content, DNA amount, and purity.

## Molecular Analysis by NGS

Molecular analysis of the *EGFR*, *KRAS*, *NRAS*, and *BRAF* genes was performed with an NGS platform (Ion Torrent PGM, server version 5.0; ThermoFisher Scientific, Waltham, MA). We used an AmpliSeq<sup>TM</sup> customized panel (ThermoFisher Scientific) to identify mutations in the *EGFR* gene (exons 18 to 21), *KRAS* (exons 2 and 3), *NRAS* (exons 2 and 3), and *BRAF* (exons 11 and 15). Data were analyzed on the bioinformatics platform Ion Torrent Suite and Ion Reporter version 5.0 with a minimum depth of 500x. Sequences NM\_005228.3 (*EGFR*), NM\_0033360.3 (*KRAS*), MM\_002524.3 (*NRAS*), and NM\_004333.4 (*BRAF*) were used as references. The test was performed using research-use-only reagents with internal validation.

### PD-L1 and ALK Analysis

For PD-L1 analysis, which included 202 patients, the immunohistochemistry (IHC) expression scores were based on the Interpretation Guide for Window PD-L1 (SP263) Assay Staining of NSCLC 2017 (Roche, Basel, Switzerland). The analysis considers the overall percentage of positive neoplastic cells in the sample of any intensity above the eventual observed background staining in the negative control slide showing a cytoplasmic membrane pattern (circumferential, discontinuous, or basolateral). A negative control slide with an antibody provided in the PD-L1 kit was used for all patients. A tissue sample with PD-L1 positivity was also used as a positive control. Positivity in immune cells was not considered for determining scores in the Window PD-L1 test.

Similarly, for the ALK assay, paraffin sections of the lesions from 350 patients were submitted to the IHC technique. Tissue sections were stained with the ALK antibody clone D5F3 (Roche), which is able to recognize ALK fusion proteins and EML4-ALK variant expression. The slides were analyzed using the Ventana BenchMark XT Automated System (Roche). All reactions had negative and positive controls on the blade itself.

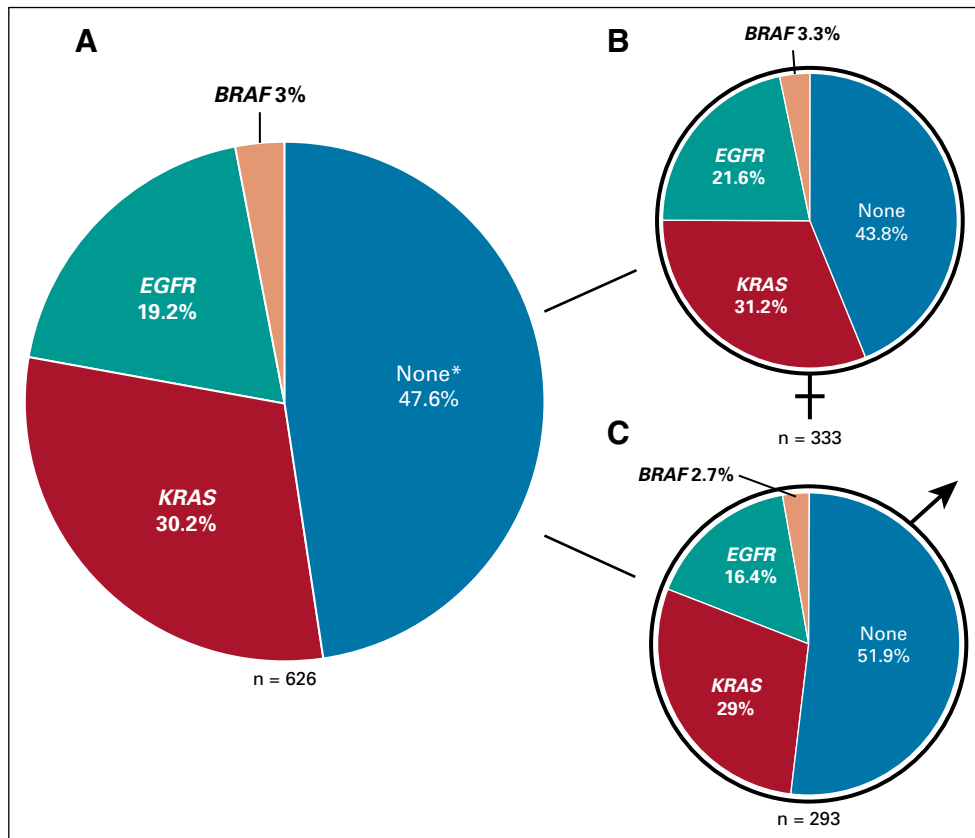
### Statistical Analysis

To verify any possible association of the predictive biomarkers with gender, age, and whether there was any statistically significant difference between our study and others performed in the Brazilian population, we performed  $\chi^2$  tests. The results were considered statistically significant when the *P* was < .05.

## RESULTS

### NGS Analysis of EGFR, KRAS, NRAS, and BRAF Genes

NGS results of 619 tumors revealed 189 patients (30.2%) with mutations in *KRAS*, 120 (19.16%) with mutations in *EGFR*, and 19 (3%) with mutations in *BRAF*. No *NRAS* mutations were identified, and seven patients harbored double mutations. In 298 patients (47.6%), we did not detect any alteration using our NGS panel (Fig 1).



**FIG 1.** Frequency of somatic mutations in *EGFR*, *KRAS*, and *BRAF* in lung adenocarcinoma tumors from patients in southern Brazil. (A) Female and male patients, (B) only female patients, and (C) only male patients. (\*) Not detected using our next-generation sequencing panel. It does not exclude the presence of alterations in other driver genes.

As expected, the frequency of *EGFR* mutations was 5.3% higher in women compared with men; however, this difference was not statistically significant ( $P = .096$ ). In contrast, the frequency of patients with no mutations detected by our panel was higher in men, with a statistically significant difference ( $P = .044$ ; Fig 1B and 1C; Data Supplement).

Among patients with *KRAS* mutations, p.(Gly12Cys) was the most frequent, accounting for 37% of the mutations found in this gene. This alteration was followed by p.(Gly12Val) and p.(Gly12Asp), found in 43 (22.75%) and 30 (15.9%) patients, respectively. Another 29 mutations were registered in exon 2 (15.3%). *KRAS* mutations in codon 61 of exon 3 were less frequently found in 17 patients (8.9%; Data Supplement).

Regarding the effect of *EGFR* mutations on TKI response, the majority was classified as sensitive ( $n = 98$ ; 81.67%). Most of the mutations were located in exon 19 ( $n = 57$ ; 47.50%), including the common deletion p.(Glu746\_Ala750del;  $n = 36$ ; 30.00%), followed by the point mutation p.(Leu858Arg;  $n = 35$ ; 29.17%) in exon 21. Resistance alterations were restricted to exon 20 ( $n = 15$ ; 12.50%). We also reported seven (5.83%) missense mutations of

unknown clinical significance, including four in exon 19 and two in exon 21, and one complex mutation p.(Glu709\_Thr710delinsAsp) in exon 18. In exon 20, there were 12 (10%) in-frame insertions and two (1.7%) duplications. Interestingly, one patient with no progression of disease was diagnosed with the p.(Thr790Met) mutation at a low frequency (3.2% of 883 sequence reads), which might be a case of primary resistance to first- and second-generation TKIs (Table 1; Fig 2).

Finally, among the 19 *BRAF* mutations, 13 (68.42%) were the well-known p.(Val600Glu) in exon 15. Another four mutations were located in exon 15, and two were located in exon 11, which also belongs to the kinase domain of the *BRAF* protein.

Although positive results of all predictive biomarkers were more commonly found in elderly patients ( $\geq 60$  years of age), only *KRAS* had a statistically significant difference ( $P = .044$ ; Data Supplement). The mean age at diagnosis of patients with *KRAS*, *EGFR*, and *BRAF* alterations was 67.5, 66.6, and 61.0 years, respectively.

#### PD-L1 and ALK IHC Analysis

PD-L1 staining by IHC was positive in 71 of the 202 analyzed tumors (35.1%). Approximately one fifth of tumors

**TABLE 1.** EGFR Mutations Identified in This Series of Patients and Grouped According to Their Sensitivity to Tyrosine Kinase Inhibitors

Sensitivity and Exon	Mutation	No. of Patients	Frequency. %
<b>Sensitive</b>			
Exon 18		5	4.17
	p.(Gly719Ala)	4	3.33
	p.(Gly719Ser)	1	0.83
Exon 19		57	47.50
	p.(Glu746_Ala750del)	36	30.00
	p.(Glu746_Arg748del)	3	2.50
	p.(Glu746_Thr751del)	1	0.83
	p.(Glu746_Thr751delinsAla)	1	0.83
	p.(Glu746Valfs*11)	2	1.67
	p.(Leu747_Pro753delinsSer)	2	1.67
	p.(Leu747_Ser752del)	1	0.83
	p.(Leu747_Thr751del)	6	5.00
	p.(Leu747fs)	1	0.83
	p.(Leu747Glnfs*16)	1	0.83
	p.(Thr751Lysfs*9)	1	0.83
	p.(Ser752_Ile759del)	1	0.83
	p.(Lys754fs)	1	0.83
Exon 20		1	0.83
	p.(Ser768Ile) <sup>a</sup>	1	0.83
Exon21		35	29.17
	p.(Leu858Arg)	35	29.17
Total		98	81.67
<b>Resistant</b>			
Exon 20		15	12.50
	p.(Met766_Ala767insAlaSerVal)	3	2.50
	p.(Ala767_Ser768insSerValAsp)	1	0.83
	p.(Ser768_Asp770dup)	2	1.67
	p.(Ser768_Val769insValAspAsn)	1	0.83
	p.(Val769_Asp770insCysVal)	1	0.83
	p.(Asp770_Asn771insGly)	1	0.83
	p.(Asp770_Asn771insGlyPhe)	1	0.83
	p.(Asp770_Asn771insPro)	1	0.83
	p.(Asn771_Pro772insProHis)	1	0.83
	p.(Asn771_Pro772insThr)	1	0.83
	p.(His773_Val774insAsnProTyr)	1	0.83
	p.(Thr790Met)	1	0.83
Total		15	12.50
<b>Unknown</b>			
Exon 18		1	0.83
	p.(Glu709_Thr710delinsAsp)	1	0.83

(Continued on following page)

**TABLE 1.** *EGFR* Mutations Identified in This Series of Patients and Grouped According to Their Sensitivity to Tyrosine Kinase Inhibitors (Continued)

Sensitivity and Exon	Mutation	No. of Patients	Frequency. %
Exon 19		4	3.33
	p.(Ala750Pro)	1	0.83
	p.(Ser752Phe)	3	2.50
Exon 21		2	1.67
	p.(Leu861Arg)	1	0.83
	p.(Leu861Gln)	1	0.83
Total		7	5.83

<sup>a</sup>Limited clinical data demonstrating the efficacy of TKIs.

(21.3%) had moderate expression, with positive staining noted between 1% and fewer than 50% of the cells. High expression of PD-L1, which is indicated by positive staining in 50% or more of the cells, was observed in 28 tumors (13.8%). ALK staining by IHC was positive in 14 of the 350 patients analyzed (4%), most of them identified in women (78.6%).

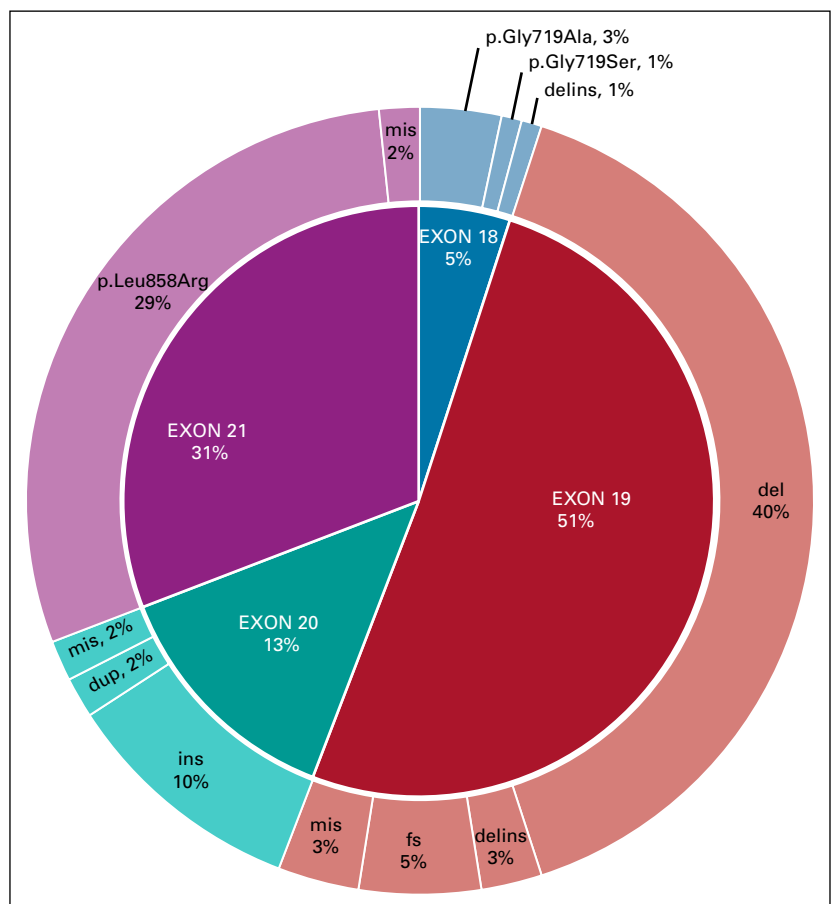
**DISCUSSION**

Although the prevalence of predictive biomarkers for molecular-targeted treatment in NSCLC has been established in several continents, including Asia, Europe, and

North America, only a few studies have been conducted in Latin America. Furthermore, most of the studies characterizing samples from patients with LC in Brazil have recruited patients from the southeast, which limits the true understanding of the scenario in the entire country. In accordance with this assumption, in our study, we identified a particular frequency pattern of predictive biomarkers compared with that observed in other Brazilian regions.

To our knowledge, the frequency of *EGFR* mutations (19.16%) was the lowest ever reported when considering

**FIG 2.** *EGFR* mutation frequencies according to their location in the gene. Del, deletions; delins, insertions/deletions; dup, duplications; fs, frameshifts; ins, insertions; mis, missense mutations.



**TABLE 2.** Comparison of Our Data With Four Previous Studies Conducted in Brazil

Alteration	First Author, No (%); <i>P</i>				
	Andreis (this study)	Bacchi <sup>13</sup>	de Melo <sup>14</sup>	Palacio <sup>15*</sup>	Leal <sup>16</sup>
<i>EGFR</i> variants (overall)	120 (19.1)	<b>64 (30.9); <math>6.9 \times 10^{-4}</math></b>	27 (20.6); .704	<b>857 (25.5%); <math>7.5 \times 10^{-4}</math></b>	108 (23.9); .058
<i>EGFR</i> – Exon 18	5 (4.1)	5 (7.9); .298	1 (3.7); .912	NA	2 (1.8); .311
<i>EGFR</i> – Exon 19 del/ins	57 (47.5)	38 (59.3); .125	8 (29.6); .091	NA	52 (48.1); .922
<i>EGFR</i> - L858R	35 (29.1)	17 (26.5); .708	3 (11.1); .052	NA	32 (29.6); .938
<i>EGFR</i> – Exon 20 del/ins plus T790M	15 (12.5)	3 (4.6); .089	1 (3.7); .184	NA	11 (10.2); .582
<i>EGFR</i> - unknown significance	8 (6.6)	1 (1.5); .089	<b>14 (51.8); <math>2.7 \times 10^{-9}</math></b>	NA	11 (10.2); .337
<i>KRAS</i> variants (overall)	189 (30.2)	<b>30 (14.5); <math>1 \times 10^{-5}</math></b>	33 (26.4); .396	NA	<b>90 (20.2); <math>2.7 \times 10^{-4}</math></b>

NOTE. Bold font indicates values with  $P < .05$  using the  $\chi^2$  test.

Abbreviations: del/ins, insertions and deletions; T790M, the p.(Thr790Met) point mutation; L858R, p.(Leu858Arg) point mutation; NA, not applicable.

\*Data on specific *EGFR* variants and *KRAS* were not available in this study.

those observed in four previous studies performed in Brazil (Table 2; Data Supplement).<sup>13-16</sup> In agreement with this observation, it is expected that the proportion of *EGFR*-mutated LUAD would decrease as the proportion of European ancestry increases in a given population. Several studies conducted in Europe showed a frequency of *EGFR* mutation of approximately 10%.<sup>17-19</sup>

In line with these observations, the prevalence of European ancestry in southern Brazil has been estimated at 80% to 90%, the highest among all Brazilian regions. African and Amerindian populations have a lower but no less important contribution.<sup>11,12</sup> Studies comparing the human leukocyte antigen allelic diversity in the region also confirmed a high similarity to Europeans and a significant difference from Asians or even Brazilian indigenous populations.<sup>20-23</sup> Together, this information supports the reason why the frequency of *EGFR* mutations in southern Brazil is lower than other regions of the country, but still higher than in Europe.

Considering the work of Bacchi et al,<sup>13</sup> which showed *EGFR* mutation in 30.9% of patients, it is important to note that the majority of the patients were from southeast and northeast Brazil. A recent and larger study performed in southeast Brazil also found a higher frequency of mutations in the gene (23.9%), but this difference was not statistically significant compared with our data ( $P = .058$ ). Although European ancestry was predominant in this series of patients, a relatively high proportion of them had Asian ancestry (7.3%),<sup>13</sup> a population in which the frequency of *EGFR* mutations may reach 60%.<sup>16,24</sup> Although de Melo et al<sup>14</sup> found a percentage of patients with *EGFR*-mutated disease similar to ours, more than half (51.8%) were variants of unknown therapeutic impact compared with only 6.6% in our study ( $P = 2.7 \times 10^{-9}$ ). Finally, the largest published study including data from *EGFR* molecular testing in Brazil (N = 3,364) also identified a higher and statistically significant mutation frequency (25.5%;  $P = 7.5 \times 10^{-4}$ ; Table 2; Data Supplement).<sup>15</sup> Interestingly, a study

conducted in Uruguay, a country geographically close to the southern region of Brazil, showed similar results regarding *EGFR* mutations (18.3%). The proportion of actionable alterations in exon 19 was similar (48.7% v 47.5%), but the missense substitution p.(Leu858Arg) in exon 21 was lower (22% v 29.17%;  $\chi^2, 1.025$ ;  $P = .311$ ). Our proportion of resistance mutations in exon 20 was also slightly higher (8.5% v 12.5%;  $\chi^2, 0.646$ ;  $P = .422$ ).<sup>25</sup>

Previous reports showed a statistically significant association between female patients and a higher prevalence of *EGFR* mutations,<sup>14,16,17,26</sup> which was not observed in our participants (Data Supplement). In addition, we reported six rare *EGFR* mutations of unknown therapeutic impact: p.(Glu709\_Thr710delinsAsp), p.(Ala750Pro), p.(Ser752Phe), p.(Leu861Arg), and p.(Leu861Gln), and p.(Ser768Ile). The efficacy of TKIs on tumors harboring these mutations is not well established, but some case reports suggest a benefit from treatment with first-generation TKIs.<sup>27-32</sup>

We also found *EGFR* alterations co-occurring in the same tumor in four patients. Two had the p.(Gly719Ala) coexisting with the p.(Ser768Ile) and the p.(Leu861Gln). Two other patients were carriers of two sensitive mutations in exon 19—p.(Glu746Valfs\*11) plus p.(Lys754fs) and p.(Leu747Glnfs\*16) plus p.(Thr751Lysfs\*9). Another three patients with coexisting resistance mutations were also reported. One had the *EGFR* alteration in exon 20 p.(Asp770\_Asn771insGly) plus *BRAF* p.(Val600Glu), another had a double mutation in *KRAS* (p.(Gly13Cys plus p.(Gln61His)), and one had the *KRAS* p.(Gly12Cys) plus the *BRAF* p.(Asp594Asn). Only p.(Gly719Ala) plus p.(Ser768Ile) was already reported in a Brazilian patient.<sup>14</sup> Our data are in accordance with other studies conducted in Brazil, which revealed that all *EGFR* and *KRAS* alterations were mutually exclusive.<sup>13,14,16,33</sup>

Regarding *KRAS* alterations, some studies reported mutations in tumors diagnosed at more advanced ages,<sup>26,34</sup>

which were confirmed in our participants (Data Supplement). The general frequency of such alterations identified in our study is notable (30.2%) and significantly higher than that observed in two previous Brazilian cohorts (Table 2; Data Supplement).<sup>13,16</sup> However, it is important to note that these studies used Sanger sequencing for the molecular analysis, a method that requires the presence of the mutant allele in at least 15% to 20% of tumor DNA. For comparison purposes, if we had excluded the positive patients with a mutant allele frequency below 15% and 20%, our *KRAS* mutation percentage would decrease from 30.2% to 18.21% and 15.49%, respectively. For *EGFR* mutations, the frequencies in these two scenarios also decreased, ranging from 14.85% to 13.57% when excluding samples with mutant allele frequencies less than 15% and less than 20%, respectively. These numbers are closer to the previous studies cited earlier and could indicate that our NGS panel is more sensitive in detecting these driver mutations in LUAD. In addition, actionable mutations would have been missed in our study if we used a cutoff of allele frequency at 15%, including seven sensitive deletions in exon 19, four p.(Leu858Arg) substitutions in exon 21 and five resistance mutations, four insertions in exon 20, and one p.(Thr790Met) point mutation.

We next focused on biomarkers with a lower mutation prevalence. To our knowledge, our study is the first to report *BRAF* mutations in Brazilian patients with LUAD, found in 3% of the participants. This prevalence is in agreement with results in non-Latin populations.<sup>33,35,36</sup> The European Medicines Agency and US Food and Drug Administration recently approved the combined use of dabrafenib and trametinib for the treatment of patients with NSCLC harboring the *BRAF* p.(Val600Glu) mutation.<sup>37</sup>

In Brazil, the only study to assess *ALK* expression was performed in the northeast region.<sup>38</sup> The authors found a much higher prevalence of *ALK*-positive tumors (13.3% compared with 4% in our study).<sup>38</sup> A recent report that included samples from nine countries in Latin America found that the prevalence of *ALK* rearrangements ranged from 4.1% (Colombia) to 10.8% (Peru). Argentina and Uruguay, which are more geographically close to the southern region of Brazil, had a frequency of 5.4% and 4.4%,<sup>39</sup> respectively, which is similar to our findings. Co-occurrence of *ALK* rearrangements with *EGFR* or *KRAS* mutations is rare, with only a few reports in the

literature.<sup>26,40-46</sup> We found two individuals with positive IHC for *ALK* protein who also harbored the *KRAS* variants p.(Gly12Val) and p.(Gly12Asp). Ulivi et al<sup>47</sup> reported that patients with *EML4-ALK* translocations co-occurring with *KRAS* point mutations had decreased responsiveness to crizotinib.

Alves da Silva et al<sup>38</sup> evaluated the PD-L1 expression in LC patients from the northeast region of Brazil and observed a similar proportion of positivity (40.5% v 35.1% in our study). The prevalence of tumors scoring from 1% to 50% or less and 50% or more was also similar between both studies (24% v 21.2% and 16.5% v 13.8%, respectively). These data reveal a much lower proportion of tumors with a staining score of 50% or more, due to a larger case series that had found high PD-L1 expression in 30.2% of the patients.<sup>48</sup>

Our study has some limitations. Because of its retrospective approach and the use of data from different private and public medical centers, we had to anonymize patients' information, including clinical data on treatment response, survival, and smoking status. However, we believe that this fact does not compromise the relevance of our results since the association between biomarkers and these clinical aspects has been widely studied.

In summary, to our knowledge, this is the first and largest study assessing the frequency of multiple predictive biomarkers for LUAD in Brazil. In addition, it reveals a unique pattern of mutation frequencies in different genes compared with data originating from other regions of the country. The frequency of *EGFR* mutations is the lowest found in Brazilian patients, possibly reflecting a higher proportion of individuals with European ancestry.<sup>11,12</sup> Our results also underscore the need to expand LUAD molecular testing in the Brazilian public health system, given that approximately 15% of patients with LUAD from the southern region would benefit from the use of TKIs. Araujo et al<sup>49</sup> estimated that fewer than half of the patients have their tumors submitted for molecular testing in Brazil. This number can be even lower in public health care institutions.<sup>49</sup> We expect that these data, together with results from other studies, will help to change this scenario and accelerate the implementation of new public policies for the treatment of LC in the country, on the basis of cost-effective analysis of our population.

## AFFILIATIONS

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## PRIOR PRESENTATION

Presented in part at Next Frontiers to Cure Cancer, São Paulo, Brazil, May 10 to 12, 2018; and International Congress of Genetics, Foz do Iguaçu, Brazil, September 10 to 14, 2018.

## SUPPORT

Supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico and Research Incentive Fund of Hospital de Clínicas de Porto Alegre (Grant no. 2018-0099). PhD scholarship awarded to T.F.A.

(Grant no. 140361/2018-9). All results presented here were performed by the Precision Medicine Program of Hospital de Clínicas de Porto Alegre, a diagnostic program that provides clinical services to AstraZeneca Brazil, Bristol-Myers Squibb, and Pfizer. Although the diagnostic tests were paid by these companies, none of them participated in or interfered with the design of the study, result analyses, and conclusions.

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#### AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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#### Vinícius Lorandi

**Travel, Accommodations, Expenses:** Astellas Pharma, MSD Oncology, Novartis, AstraZeneca, Genentech

#### Patricia Ashton-Prolla

**Research Funding:** AstraZeneca Brazil (Inst)

No other potential conflicts of interest were reported.

#### ACKNOWLEDGMENT

We thank the undergraduate students Julia Tsao Schein, from the Medicine School of Universidade Federal do Rio Grande do Sul, and Guilherme Danielski Viola, from the Institute of Biosciences of Universidade Federal do Rio Grande do Sul, for their collaboration.

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