Thoracic Cancer

Open Access

Thoracic Cancer ISSN 1759-7706

ORIGINAL ARTICLE

Comprehensive genomic profiling of Brazilian non-small cell lung cancer patients (GBOT 0118/LACOG0418)

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Keywords
Co-occurring mutations; genomic profiling; non-small cell lung cancer; tumor mutational burden.

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[Correction added on 19 January 2021, after first online publication: the last author's name has been corrected.

Received: 25 September 2020;
Accepted: 25 November 2020.

doi: 10.1111/1759-7714.13777

Thoracic Cancer **12** (2021) 580–587

Abstract

Background: The aim of this study was to carry out a descriptive analysis of the somatic genetic profile and co-occurring mutations of non-small cell lung cancer (NSCLC) samples from patients tested with comprehensive genomic profiling (CGP).
Methods: This was a retrospective cross-sectional study of patients diagnosed with NSCLC from 2013 to 2018 in Brazil and whose samples were submitted to CGP (FoundationOne or FoundationACT) using either tumor or circulating tumor DNA (ctDNA) from plasma.
Results: We recovered 513 CGP results from patients, 457 (89.1%) of which were from tumors and 56 (10.9%) from plasma. The median age of patients was 64 years old, of which 51.6% were males. *TP53* mutations were identified in 53.6% of tumor samples, *KRAS* mutations in 24.2%, *EGFR* activating mutations were detected in 22.5%, *STK11* mutations in 11.6%, *PIK3CA* mutations in 8.8%, *ALK* rearrangements in 5.4%, *BRAF* mutations in 5.2%, and *ERBB2* alterations in 4.9%. The most commonly comutated gene was *TP53. TP53 p*.R337H was observed in 4.3% of samples and was associated with somatic mutations in *EGFR* and *ERBB2* ($P < 0.00001$). Tumor mutational burden (TMB) analysis was available for 80.5% of samples tested, and 5.5% of samples had high TMB (≥ 20 mutations/Mb).
In conclusion, this retrospective analysis of genomic data from NSCLC patients obtained by CGP showed that common abnormalities such as *EGFR* mutations and *ALK* rearrangements had similar frequency to those previously described by other groups using others strategies. Additionally, our data confirm an association between *TP53* p.R337H, supposedly germline in nature, and somatic mutations in genes of the HER family.

Key points

Significant findings of the study:

- This is the first report of the prevalence of driver mutations in Brazilian NSCLC patients using comprehensive genomic profiling (CGP).
- The frequency of the most common driver mutations in this population was similar to that previously described in Brazil.

What this study adds:

- *TP53* was the most commonly comutated gene across samples. *TP53* p.R337H was associated with somatic mutations in *EGFR* and *ERBB2*.
- Most samples had low TMB; only 5.5% of samples had high TMB.

Introduction

Lung cancer is the leading cause of cancer-related mortality worldwide.[1] Cancer driver mutations have been examined extensively and are the basis for modern precision therapy, and in addition patients diagnosed with advanced lung cancer may have multiple and sometimes rare genetic alterations.[2] Non–small cell lung cancer (NSCLC), regardless of histological subtype, is one of the most genetically diverse and deranged cancers, posing challenges for developing effective prevention, diagnostic and treatment strategies.[3,4]

Treatment selection was previously largely based on lung cancer classification in two broad categories: NSCLC or small cell lung cancer (SCLC). Nowadays, lung cancers are histologically subclassified and some undergo molecular profiling to determine the best treatment option for patients. The first genomic alterations reported to show sensitivity to specific targeted therapies in lung adenocarcinoma were *EGFR* mutations and *ALK* rearrangements.[5–8] More recently, other mutations have been identified as new therapeutic targets such as *BRAF* mutations or *ROS1* and *NTRK* rearrangements.[9–14] The role of nondisruptive *TP53* mutations in *EGFR* mutated lung adenocarcinoma patients has been previously explored and a prevalence close to 30%–50% has been described with a negative impact on prognosis, especially in those patients with exon 19 deletions.[13–15]

The access to comprehensive genomic tests in Brazil is still limited, and the prevalence of driver mutations and co-occurring genetic alterations among Brazilian NSCLC patients is not well known. Brazil is a country with large territorial extension and some degree of genetic background heterogeneity may exist in the population. Knowledge of the molecular profile of NSCLC in Brazil is extremely important to define better public health strategies. The objective of this study was to carry out a descriptive analysis of the somatic genetic profile of NSCLC in Brazilian patients and describe co-occurring mutations in samples tested with either tumor or circulating tumor DNA (ctDNA) profiling.

Methods

Study design

This cross-sectional study collected data from NSCLC samples tested in the period from 2013 to 2018. We retrospectively analyzed unidentified data from a Foundation Medicine database that comprised worksheets containing anonymous minimal clinical-pathological characteristics and comprehensive genomic profiling (CGP) results from either tumor (FoundationOne CDx) or plasma ctDNA from plasma (FoundationACT). The database was provided by Roche Pharmaceuticals and contained all genomic data, including tumor mutational burden (TMB). No patient had duplicated tumor samples analyzed. Patients or their relatives were not directly contacted and the researchers did not have access to the medical records of patients.

Ethical considerations

A waiver for informed consent was requested since all patients had previously signed an authorization for testing, and data were collected retrospectively through pathological reports review. No information capable of identifying patients was collected. This project was approved by the Ethics Committee of PUCRS on July 2020, approval number 3462050.

Statistical analysis

Statistical analyses were performed with information from 513 NSCLC samples. We described the molecular profile by using descriptive statistics of variables. Categorical variables were presented as total frequency and percentages and compared using Pearson's Chi-square test. All comparisons included two-tailed tests, with level of significance set at 5%.

All analyses were performed using SAS statistical software (version 9.4; SAS Institute, Inc. Cary, NC). The graphics were produced using Tableau Desktop version 2019.1.13.

Results

A total of 513 CGP results were analyzed, 457 (89.1%) from tumors and 56 (10.9%) from plasma ctDNA. Adenocarcinoma was the most common histological subtype (83.8%) followed by NSCLC not otherwise specified (NOS) (16.1%). Median age of patients at testing date was 64 years old (16–97) and 51.3% of patients were male.

Median number of mutated genes by sample was three (range 0–14) and most tumors had at least two (range 2–6) different types of mutations. The most common genomic alterations were single nucleotide variations (SNVs) (81.0%) followed by copy number variations (CNVs) (49.7%), frameshift mutations (31.4%), indels (19.3%), splice site mutations (19.1%), and rearrangements/fusions (12.5%).

TP53 mutations were identified in 53.6% of tumor samples, *KRAS* mutations in 24.2%, *EGFR* activating mutations were detected in 22.5%, *STK11* mutations in 11.6%, *PIK3CA* mutations in 8.8%, *ALK* rearrangements in 5.4%, *BRAF* mutations in 5.2%, *ERBB2* alterations in 4.9%, *MET* alterations in 4.7%, *RET* alterations in 2.4%, *ROS1* rearrangements in 2.2% and *NTRK* rearrangements in 0.6% (Fig 1). We also evaluated the frequency and distribution of mutations according to the available histological classification (adenocarcinoma x NSCLC) (Fig S1). We detected a higher frequency of *TP53* (45,45% × 33,59%) and *PI3K* (8,27% × 3,99%) mutations among the non-specified NSCLC cancer group when compared to the adenocarcinomas, and in the opposite direction, less frequent mutations in *KRAS* (9,09% × 17,64%) and *ERRB2* (0,83% × 3,83%).

A total of 55% of patients whose tumors bore an *EGFR* mutation were female. Among tumor samples with *EGFR* mutations, 37.0% had exon 19 deletion, 26.0% had exon

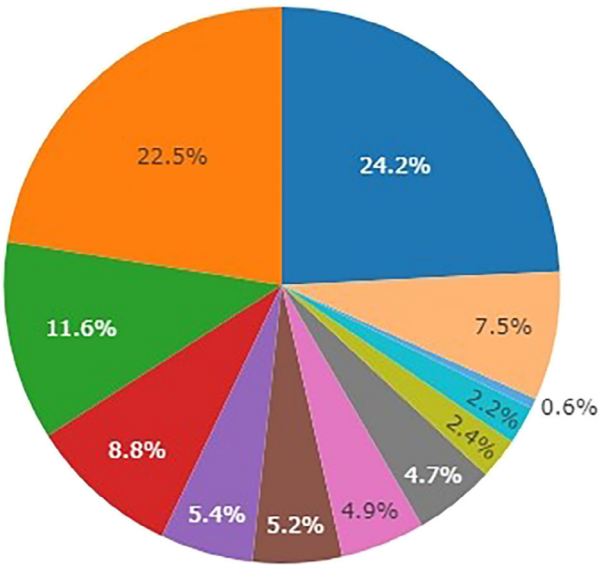
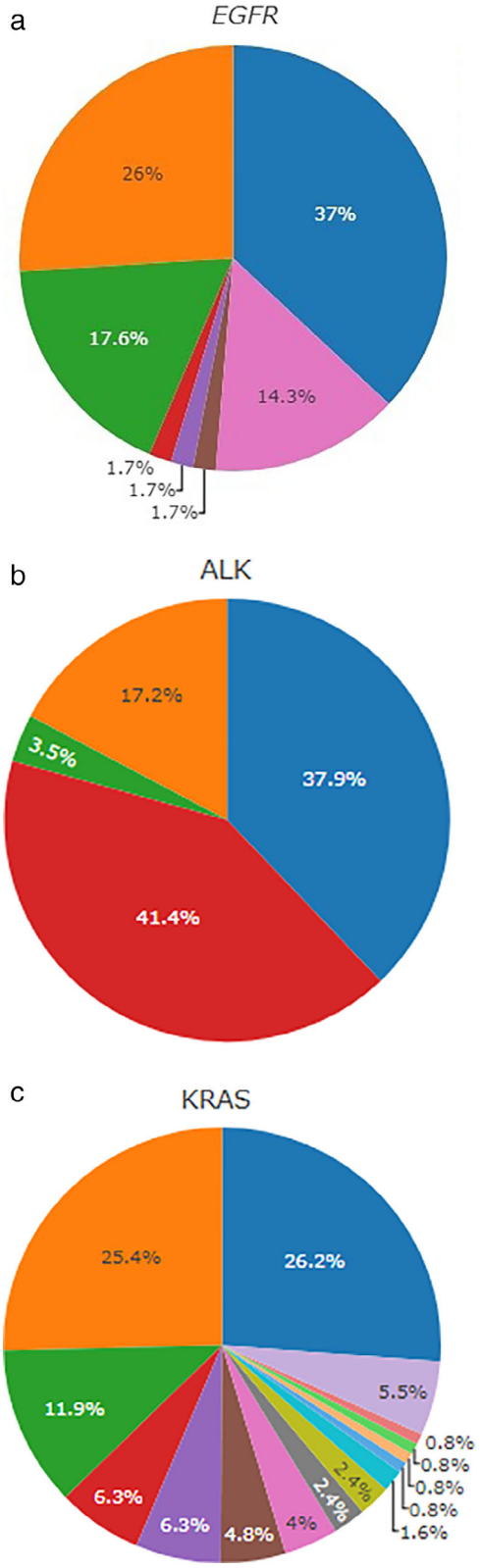


Figure 1 Frequency of driver mutations in Brazilian non-small-cell lung cancer (NSCLC). Mutation description was recovered from FoundationOne CDx and FoundationACT reports and calculated as a percentage of the total number of patients studied (*N* = 513) (■) KRAS, (■) EGFR, (■) STK11, (■) PI3K, (■) ALK, (■) BRAF, (■) ERBB2, (■) MET, (■) RET, (■) ROS, (■) NTRK, (■) Others.



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21 Leu858Arg substitutions (L858R), 17.6% had exon 20 insertions, 1.7% had T790M mutations, 1.7% had L861Q mutations and 1.7% had G719A mutations (Fig 2a). Two or more concurrent mutations in the *EGFR* gene were found in 13 tumor samples (10.9%): three samples with p.L858R and p.T790M; two samples with exon 19 deletion and p.T790M; one sample with p.V774M and p.H773L; one patient with p.G779C and p.L747_T751del (exon 19 deletion); one sample with p.L858R and p.E709K; one patient p.L858R and p.R108K, one patient with p.V774M and p.H773L, and one patient with p.L858R and p.L707F mutations. Two tumor samples had three concurrent mutations: one tumor with p.L833V, p.L858R and p.T790M and another tumor with p.C797S, exon 19 deletion, and p.T790M.

Among tumor samples with *ALK* rearrangements, variant 1 was found in 37.9%, followed by variant 3 in 17.2% (Fig 2b).

In relation to *KRAS* mutation, most alterations involved codons 12 and 13 (94.4%). p.G12V was the most frequent variant (26.2%), followed by p.G12C, observed in 25.4% of samples with *KRAS* mutation (Fig 2c). The majority of patients with *KRAS* mutation were male (57.1%) and median age was 67 years old.

zThe most common *BRAF* mutation was p.V600E detected in 50% of *BRAF* mutated samples. The majority of patients with *BRAF* mutations were female (53.5%) and median age in this group was 67 years old. *ERBB2* alterations were more common among male patients (57.7%), with a median age of 66 years old. The most common genomic alteration affecting the *ERBB2* gene was p.A775_G776INSYVMA (exon 20 insertion), corresponding to 26.9% of cases.

TP53 p.R337H mutation was observed in 22 samples (4.3%). They corresponded to 8.0% of all *TP53* gene alterations. Two patients harbored other variants in the R337 position (1 p.R337C and 1 p.R337L). 63.6% of patients bearing *TP53* p.R337H were 50 years old or younger.

Co-occurring mutations

In patients with *EGFR* mutations, the most commonly co-occurring mutations identified were in *TP53* (51.6%), *PIK3CA* (5.7%) and *CDK4* (3.8%) (Fig 3a). Among patients with *TP53* and *EGFR* co-occurring mutations, 12 patients (2.3%) bore *TP53* p.R337H. *TP53* p.R337H was also found

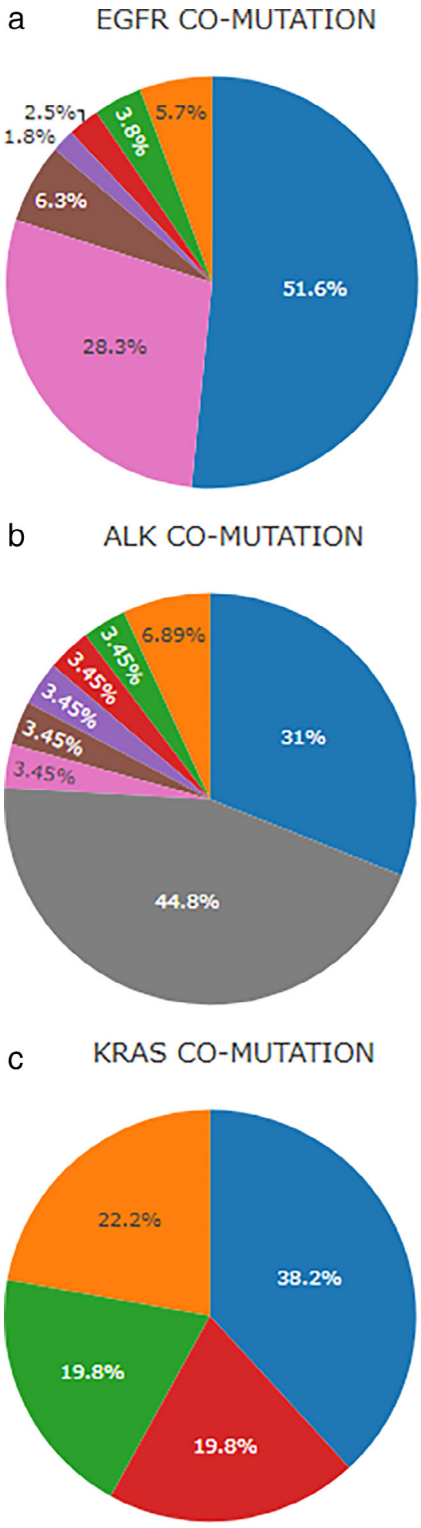

Figure 2 Distribution of *EGFR* mutations, *ALK* rearrangements and *KRAS* mutations subtypes. (**a**) Frequency of *EGFR* mutation subtypes (*N* = 119) (■) Exon 19 deletion, (■) L858R, (■) Exon 20 insertion, (■) T790M, (■) L861Q, (■) G719A, (■) Others. (**b**) Frequency of *ALK* fusion subtypes (*N* = 29) (■) Variant 1, (■) Variant 3, (■) Variant 2, (■) Others. (**c**) Frequency of *KRAS* mutation subtypes (*N* = 126) (■) G12V, (■) G12C, (■) G12D, (■) G12A, (■) G12S, (■) G13D, (■) G12R, (■) G13C, (■) Q61H, (■) Q61L, (■) A146V, (■) G12F, (■) G13R, (■) Q16K, (■) Amplification.

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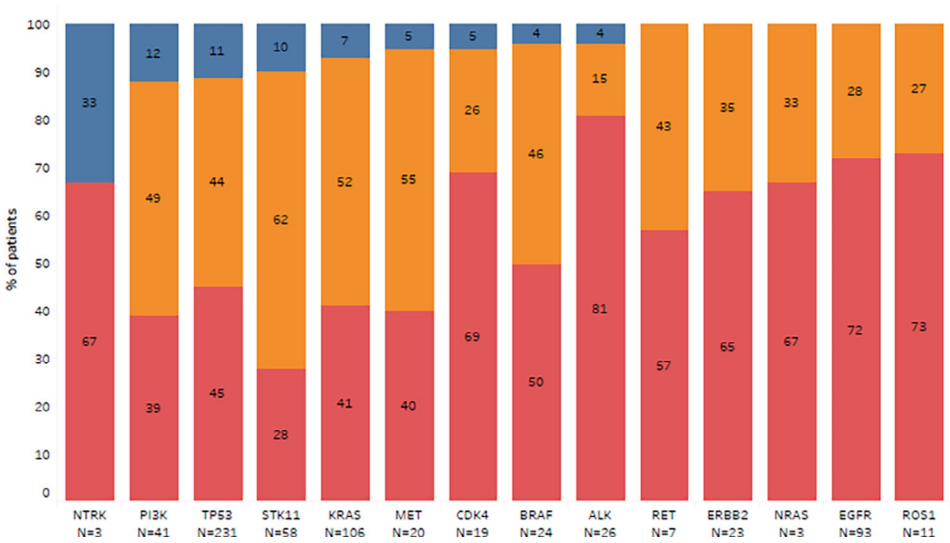

Figure 4 Distribution of TMB subgroups according to the type of driver mutation (N = 413) (■) high, (■) intermediate, (■) low.

in four (0.8%) patients whose tumors bore an *ERBB2* somatic genomic alteration. In patients with *ALK* rearrangements, co-occurring mutations were detected in 55.2% of tumor samples, *TP53* being the most prevalent, observed in 31%, followed by *PIK3A* detected in 6.9% (Fig 3b). Among *KRAS*-mutant samples, *TP53* co-occurring mutations were detected in 38.2% and *STK11* in 22.2% (Fig 3c).

A total of 12 samples with *TP53* p.R337H had concurrent *EGFR* (54.5%) and 16 samples had concurrent *ERBB2* (72.7%) mutations (Table S1). The association between *TP53* p.R337H and *EGFR* mutations was statistically significant (P = 0.0008) as well as between *TP53* p.R377H and *EGFR* plus *ERBB2* mutations combined (P < 0.0001) (Table S1). A total of 63.6% of these patients were 50 years old or younger (P < 0.0001) (Table S1). *EGFR* or *ERBB2* mutations altogether were observed in 28% of samples that bore a non-R337H *TP53* variant. The co-occurrence of *KRAS* p.G12C and *TP53 p.Y220C* was observed in one case. We did not observe other driver mutations frequently associated with *TP53* p.R337H (*AKT1* p.E17K in one case, *NF1* p.N1683FS*1 in one case, *PTEN* loss and *RB1* mutation 1 one case, and *KEL*, *MUTYH*, *VHL* and *RB1* mutations in one case).

Figure 3 Distribution of co-occurring mutations in NSCLC bearing *EGFR* mutations, *ALK* rearrangements and *KRAS* mutations. (**a**) Co-occurring genomic alterations in patients with *EGFR* mutation (N = 119) (■) EGFR + TP53, (■) EGFR + P13KA, (■) EGFR + CDK4, (■) EGFR + KRAS, (■) EGFR + MET, (■) Others, (■) No co-mutation identified. (**b**) Co-occurring genomic alterations in patients with *ALK* mutation (N = 29) (■) ALK + TP53, (■) ALK + P13KA, (■) ALK + CDK4, (■) ALK + ERBB, (■) ALK + KRAS, (■) ALK + NTRK, (■) ALK + STK11, (■) No comutation identified. (**c**) Co-occurring genomic alterations in patients with *KRAS* mutation (N = 126) (■) KRAS + TP53, (■) KRAS + STK11, (■) Others, (■) No comutation identified.

TMB

TMB analysis was available for 80.5% of samples tested and measured in mutation per megabase. A low TMB (<5 mutations/Mb) was found in 42.7%, an intermediate TMB (5–9 mutations/Mb in 32.4% and only 5.5% of samples had high TMB (≥10 mutations/Mb) The distribution of the tumor mutational burden according to the different driver genes studied is described in Fig 4.

Discussion

NSCLC is the leading cause of cancer death in Brazil and has a major impact on the public health system. In Brazil, many hurdles impair patients' access to expanded molecular testing, and consequently to the best available treatment. A better characterization of the genomic profile of NSCLC in Brazil is paramount to guide and improve the design of treatment strategies for NSCLC in the country.

This retrospective analysis of genomic data obtained by CGP from samples of Brazilian NSCLC patients showed that common abnormalities had, in general, similar frequency to those previously described by other groups using other strategies such as PCR based tests (*EGFR*) or immunohistochemistry and FISH (*ALK*).[16] For example, a study conducted in the South region of Brazil analyzed 619 lung tumor samples and identified *EGFR* mutations in 120 (19.2%), and ALK expression in 4.0%.[17] Another study that analyzed 262 lung adenocarcinomas samples detected *EGFR* mutations in 23% and *ALK* rearrangements in 7%.[18] Nevertheless, CPG-based strategies may provide more

accurate output since they assess all types of genetic mutations, copy number variations, and, in the case of gene fusions and rearrangements, the partner gene which might have predictive implications in the future.

Another study evaluated 173 NSCLC samples from patients who lived in the Northeast region of Brazil. ALK expression was detected in 10.4% of the samples, and 22.0% of the tumors harbored *EGFR* mutations. The most common *EGFR* mutation was an exon 21 L858R point mutation (in 45.5%), followed by an exon 19 deletion (in 36.3%). In this study, the authors did not describe TMB, mutations in other driver genes or co-occurring mutations. These results are somehow different from what we found in our study, with a higher than expected prevalence of *ALK* alterations.[19]

The knowledge that co-occurring mutations in addition to the classic driver mutations can largely modify the biology of the tumors and the response to treatment is rapidly consolidating. Co-occurring genomic alterations contribute to the heterogeneity of driver oncogene-defined NSCLC subgroups and can result in biologically important interactions. Selective pressure imposed by previous anticancer therapy can also substantially influence patterns of co-mutations.[20] No other published cohort from Brazil has described the frequency of genomic alterations in less common mutated genes like *RET* and *HER2* or the frequency of co-occurring genomic alterations.

Hu *et al.* evaluated the presence of mutations in *KRAS*, *NRAS*, *PIK3CA*, *BRAF*, and *HER2* as well as *ALK*, *ROS1*, and *RET* gene fusions in 320 patients who harbored *EGFR* activating mutations and received treatment with EGFR-tyrosine kinase inhibitor (TKIs). A total of 21 (6.6%) of the *EGFR* mutant samples had additional gene alterations, mutations in *PIK3CA* being the most common, followed by *EML4-ALK* rearrangements, *ERBB2* mutations, *RET* rearrangements, *ROS1* rearrangements and *KRAS* mutations. Those with isolated *EGFR* mutations had a significantly longer progression-free survival (PFS) compared to those with concurrent gene alterations; however, this condition did not have a significant impact in OS.[21]

The most frequently comutated gene we observed in association with mutations in *EGFR, ALK* and *KRAS* (the most frequently mutated NSCLC driver genes) was *TP53*, followed by *PIK3CA* in *EGFR*- and *ALK*-mutant samples, and *STK11* in *KRAS*-mutant genes. *TP53* mutations have been reported to be associated with shorter disease progression intervals in patients with *EGFR* or *ALK* mutated NSCLC treated with TKI.[14,21] Similarly, the comutation of *STK11* in *KRAS* mutated NSCLC has been reported to be associated with resistance to immune checkpoint inhibitors.[22]

The current study was based on an anonymized dataset from laboratory reports. Unfortunately, only limited clinical data were available, and we were unable to test the association between the genomic alterations we identified in this population and other variables such as smoking history, racial group, geographic location, stage or previous treatment.

To our knowledge, this is the first report of TMB from a Brazilian NSCLC cohort. We found that most tumors harbored low or intermediate TMB. High TMB was especially frequent among *TP53, STK11, PIK3CA* and *NTRK* mutant samples, while *ALK* and *ROS1* had the highest proportion of samples with low TMB. Singal *et al.* analyzed genomic and clinical data from 4064 NSCLC patients and found the same pattern of association between TMB and driver mutations as described herein.[23]

The great population heterogeneity and the diverse genetic background in the Brazilian population can lead to new findings. This is exemplified by the finding of a higher than expected frequency of *TP53* p.R337H in the present cohort. This variant represents a prevalent pathogenic germline *TP53* mutation frequently found in the South and Southeast of Brazil,[24] which seems to be associated with a higher frequency of somatic *EGFR* and *ERBB2* activating mutations in NSCLC in Brazil. Couto *et al.* identified germline TP53 R337H mutations in 8.9% of 45 unselected Brazilian NSCLC patients tested for *TP53* germline mutations.[25] Barbosa *et al.* also described a high frequency of *EGFR* somatic mutations (89%) among adenocarcinomas diagnosed in Brazilian carriers of germline *TP53* p.R337H mutation, while the frequency of *EGFR* mutations among unselected lung adenocarcinomas is around 25% in the country. The same authors described an association between *EGFR* somatic mutations and *TP53* p.R337H among unselected lung adenocarcinoma patients younger than 50 years old.[26] Following this publication, Mezquita *et al.* evaluated 22 NSCLC patients with Li-Fraumeni syndrome harboring diverse germline *TP53* variants and identified a somatic mutation in a driver gene in 90% (18 *EGFR* mutations and 1 *ROS1* fusion) of 21 samples analyzed.[27] Although we cannot ascertain that the aforementioned mutations identified in these patients are germline for sure based on the FoundationOne tests results, which is designed to assess somatic mutations, the authors inferred that they are probably germline in nature based on the fact that this variant (*TP53* p.R337H) is very rarely identified as a somatic mutation in NSCLC[28] and on data showing a high prevalence of this mutation (germline) in Brazil,[24] allied with the previously reported association with *EGFR* mutated NSCLC in Brazilian patients.[25,26] Besides, these mutations fulfill the criteria recently proposed to investigate germline origin from genomic profiling data.[29,30] The results of the present study reinforce the need to investigate *TP53* germline mutations in *EGFR* mutant NSCLC patients and to discuss genetic counseling,

mainly in individuals younger than 50 years old at diagnosis.

In conclusion, detailed analysis of NSCLC samples at the molecular level may provide relevant insights to improve the understanding of this disease and is paramount to establish personalized targeted therapy. The use of CGP-based testing may grant a deeper understanding of the prevalence of each specific driver mutation in the country and the identification of a larger repertoire of actionable mutations. At the same time, the detection of concurrent mutations may improve the prediction of response to targeted therapies.

This retrospective analysis of genomic data from Brazilian NSCLC patients obtained by CGP showed that common abnormalities such as *EGFR* mutations and *ALK* rearrangements had similar frequency to that described previously by other groups; nevertheless, here we describe for the first time the frequency of genomic alterations in other less common driver genes, comutations and the distribution of TMB in NSCLC tumor samples in Brazil. Additionally, we believe that our results strengthen the idea that there is an association between *TP53* p.R337H (probably germline) and somatic mutations in genes of the HER family (*EGFR* and *ERBB2*).

Acknowledgments

We thank Foundation Medicine, Inc. and Hoffmann La Roche for allowing access to the genomic data, for the scientific review, and financial support with medical writing and statistical analysis.

Disclosure

All authors declare that there are no conflicts of interest.

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Supporting Information

Additional Supporting Informationmay be found in the online version of this article at the publisher's website:

Table S1. Association of TP53 R337H mutation with EGFR mutation and age.
Table S2. Association between the presence of the *TP53* p. R337H mutation and mutations in EGFR, ERBB2 and age. The TP53 p.R337H is a putative germline mutation highly prevalent in the South and Southeast regions of Brazil. Associations between variables were tested with the Pearson's Chi-squared test.

