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Original Article

EGFR Mutations and PD-L1 Expression in Early-Stage Non-Small Cell Lung Cancer: A Real-World Data From a Single Center in Brazil

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Abstract

Background: Targeted and immunotherapies are currently moving toward early-stage settings for patients with non-small cell lung cancer (NSCLC). Predictive biomarkers data are scarce in this scenario. We aimed to describe the frequency of *EGFR* mutations and PD-L1 expression levels in early-stage non-squamous patients with NSCLC from a large, single Brazilian oncology center.

Methods: We retrospectively evaluated patients with NSCLC diagnosed at an early-stage (IB to IIIA-AJCC seventh edition) at Barretos Cancer Hospital (n = 302). *EGFR* mutational status was assessed in FFPE tumor tissues using distinct methodologies (NGS, Cobas, or Sanger sequencing). PD-L1 expression was evaluated by immunohistochemistry (clone 22C3) and reported as Tumor Proportion Score (TPS), categorized as <1%, 1-49%, and ≥50%. We evaluated the association between *EGFR* mutational status and PD-L1 expression with sociodemographic and clinicopathological parameters by Fisher's test, qui-square test, and logistic regression. Survival analysis was assessed by the Kaplan-Meier method and Cox regression model.

Results: *EGFR* mutations were detected in 17.3% (n = 48) of cases and were associated with female sex, never smokers, and longer overall and event-free survival. PD-L1 positivity was observed in 36.7% (n = 69) of cases [TPS 1-49% n = 44(23.4%); TPS ≥50% n = 25(13.3%)]. PD-L1 positivity was associated with smoking, weight loss, and higher disease stages (IIB/IIIA).

Conclusion: The frequencies of EGFR mutations and PD-L1 positivity were described for early-stage non-squamous patients with NSCLC. These results will be essential for guiding treatment strategies with the recent approvals of osimertinib and immunotherapy in the adjuvant setting.

Key words: lung cancer; precision medicine; biomarkers; Brazil.

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Implications for Practice

Recent approvals of targeted therapies and immunotherapy for non-metastatic patients with NSCLC have paved the way for the tailored treatment in the adjuvant setting. Herein, we described the frequency of *EGFR* mutations and the PD-L1 positivity in early-stage patients with NSCLC from a single Brazilian center. In this early-stage series, the frequency of *EGFR* mutations was 17.3% and the PD-L1 positivity was 36.7%. Early-stage *EGFR*m and PD-L1-positive patients could be eligible for adjuvant treatment with osimertinib and immunotherapy treatments. Our results can expand the knowledge for the oncologists about the early-stage NSCLC related to *EGFR* and PD-L1.

Introduction

Lung cancer is one of the most frequent cancers diagnosed worldwide and is the first cause of cancer-related death in many countries.[1] The observed high mortality rate is directly influenced by the late diagnosis of this disease. In Brazil, approximately 30% of patients with non-small cell lung cancer (NSCLC) are diagnosed at early stages and hence treated with curative intent surgery, whether or not followed by adjuvant chemotherapy and/or radiotherapy.[2] Although the number of early-stage patients with NSCLC that may benefit from curative intent treatment is currently scarce, it is projected to increase in the near future due to the uptake of lung cancer screening programs.[3,4] Early-stage patients with NSCLC experience high recurrence rates, exhibiting a 5-years overall survival (OS) rate of approximately 30%-50%.[5-7] Thus, adjuvant therapy is needed to reduce the risk of disease recurrence or death for resected patients with NSCLC.[8] Adjuvant cisplatin-based chemotherapy is currently considered the standard of care, however, at 5 years, the overall survival (OS) benefit is only 5%.[8,9]

Over the last years, targeted therapies have dramatically improved survival of patients whose tumors harbor somatic driver oncogenes, such as the mutant Epidermal Growth Factor Receptor gene (*EGFRm*). EGFR tyrosine kinase inhibitors (EGFR-TKis) are currently the standard of care for *EGFRm* NSCLC with advanced disease.[10] Recently, osimertinib (a third-generation EGFR-TKi) was approved by the Food and Drug Administration (FDA) and Agência Nacional de Vigilância Sanitária (ANVISA) as an adjuvant treatment for resected NSCLC with *EGFR* mutation. Studies from all over the world have reported a wide range of *EGFR* mutation frequency in distinct ethnic groups, associated with genetic ancestry and disease stage.[11-16] However, most of these studies enrolled patients with advanced-stage NSCLC, and data for early-stage setting patients remain lacking, particularly in Latin-America.[17]

In addition to targeted therapies, immunotherapy has also improved the outcome of patients with NSCLC with advanced disease. Programmed death-ligand 1 (PD-L1) expression remains the only biomarker used in clinical practice for predicting response to anti-PD-(L)1 therapies.[18-20] Approximately a quarter of patients with NSCLC have high PD-L1 expression (≥50%)[19]-with no actionable alterations-and are more likely to benefit from these therapies. Patients with advanced NSCLC *EGFR*m with high PD-L1 expression did not respond to pembrolizumab, which is an anti-PD-1 antibody.[21] Similarly to targeted therapies, immunotherapies are now being assessed in various nonmetastatic settings. In 2017, the FDA approved durvalumab as consolidation therapy, after concomitant chemoradiotherapy, for unresectable stage III NSCLC. Neoadjuvant durvalumab combined with radiotherapy has also emerged as a promising approach, yielding good pathological response rate in early-stage NSCLC.[22] The FDA and Brazilian Health Regulatory Agency (ANVISA) have also approved atezolizumab for adjuvant treatment after surgery and platinum-based chemotherapy in NSCLC patients diagnosed at stages II to IIIA whose tumors express PD-L1 in ≥1% of tumor cells.[23]

The frequency of *EGFR* mutations and PD-L1 expression levels has been recently explored in admixture populations, including Brazilian patients with NSCLC. However, these studies focused exclusively on patients with advanced-stage disease.[11,12,24-26] Herein, we evaluated the frequency of *EGFR* mutations and PD-L1 expression in an early-stage NSCLC series from a single center in Brazil and explored the association between these biomarkers with patients' clinicopathological features.

Patients and Methods

Study Oversight

The institutional review board approved the study protocol (CAAE 05744712.3.0000.5437) and waived the need for written informed consent from patients, because of the retrospective nature of the study. The study was performed according to relevant guidelines and regulations.

Study Population

This study comprises a retrospective screened series from a 2599-patients pre-existing cohort diagnosed with non-small cell lung cancer at the Barretos Cancer Hospital, between 2006 and 2020. The Barretos Cancer Hospital is one of the most prominent non-profit cancer centers in Latin America with fully free assistance to cancer pateints, being part of the Brazilian Health System (SUS), with approximately 6000 daily consultations.[27,28]

Patients with histologically confirmed NSCLC patients with clinical stages IB-IIIA (AJCC 7th edition) were included. For resected patients (most cases), the pathological stage was used. For those who did not undergo surgery, the clinical stage was used for the analyses. As per institutional guidelines, all patients were staged with computed tomography (CT) scans or PET/CT and brain magnetic resonance imaging (MRI) to exclude metastases. Mediastinoscopy was performed when indicated. According to the General Personal Data Protection Law, patients' data were stored and managed on Research Electronic Data Capture (REDCap) system.[29]

Molecular Analysis: *EGFR* Mutational Status

All eligible cases (n = 315) were qualified for molecular analysis, but 13 cases were excluded due to screening failure, and 5 cases were excluded due to unsuccessful sample retrieval (FFPE availability) (Supplementary Fig. S1). Thus, FFPE samples that qualified for molecular analysis included 297 patients genotyped by NGS (n = 71; 23.9%), Realtime Cobas-Roche (n = 85; 28.6%) and Sanger Sequencing (n = 141; 47.5%).

Next-Generation Sequencing

The mutational analysis for EGFR hotspots regions (exons 18, 19, 20, and 21) was performed in a subset of cases (n = 71) using NGS, as previously described.[30] Briefly, targeted sequencing was performed using the TruSight Tumor 15 panel (Illumina, USA). Sample DNA libraries were prepared using 10 ng of frozen tissue genomic DNA as input, following the manufacturer's sample preparation protocols. The TruSight Tumor 15 panel covers the hotspot regions of 15 high-risk cancer-associated genes, namely: *AKT1*, *GNA11*, *NRAS*, *BRAF*, *GNAQ*, *PDGFRA*, *EGFR*, *KIT*, *PIK3CA*, *ERBB2*, *KRAS*, *RET*, *FOXL2*, *MET*, and *TP53*. The Qubit dsDNA HS assay kit (Thermo Fisher Scientific, Eugene, OR, USA) was used to quantify the enriched libraries on the Qubit 2.0 Fluorometer (Thermo Fisher Scientific). Amplification quality assessment was performed using 2% agarose gel electrophoresis. Individual samples were diluted to the molarity of 2 nM with the buffer provided by the reagent kit and then pooled in a batch of 8 samples. Up to 8 pM of the pooled library was submitted to cluster generation on the flow cell. Paired-end sequencing (2 × 150) using MiSeq Reagent Kit v3 in a MiSeq sequencer platform (Illumina) was performed.

Data demultiplexing and FASTQ file generation were performed by the BaseSpace Sequence Hub platform (Illumina). Alignment, variant calling, and annotation steps were performed using the Sophia DDM version v5.4.2.5. (SOPHiA GENETICS, Saint Sulpice, Switzerland) software. The hg19 human reference sequence was used for alignment (EGFR:NM_005228.5). Variant calling was focused on exonic nonsynonymous alterations that had a read depth of at least 500 × and a variant allele frequency (VAF) no lower than 3%. Any variant that did not fit these parameters was filtered out. Annotation was also manually curated for pathogenicity using the ClinVar database.[31]

Real-Time PCR for *EGFR* Testing

The mutational analysis for *EGFR* hotspot regions (exons 18, 19, 20, and 21) in a subset of cases (n = 85) was analyzed by real-time PCR using the COBAS platform (Roche). First, we used the Cobas DNA Sample Preparation Kit (Roche) for manual sample preparation followed by the Cobas z 480 analyzer for automated amplification and detection following Cobas EGFR Mutation Test v2 kit (Roche), according to manufacturer's instructions.

Sanger Sequencing

The mutational analysis for *EGFR* hotspot regions (exons 18, 19, 20, and 21) in a subset of cases (n = 141) was analyzed by PCR, followed by direct sequencing, as previously described.[11,32] Direct sequencing was carried out using BigDye Terminator v3.1 Cycle Sequencing kit (ThermoFisher Scientific), and sequencing products were purified using BigDye Xterminator (ThermoFisher Scientific) and analyzed on a 3500 Genetic Analyzer, capillary electrophoresis system (Applied Biosystems). Sequences were captured by the SeqScape software (Applied Biosystems) and manually compared with reference sequences collected from GenBank (EGFR: NG_007726.3). All mutations were confirmed twice.

Immunohistochemistry for PD-L1 Expression

FFPE sections were also used to assess PD-L1 expression by immunohistochemistry (IHC), with the 22C3 clone (PharmaDx antibody), as previously described.[33] PD-L1 expression was reported as Tumor Proportion Score (TPS), defined as the percentage of viable tumor cells showing partial or complete membrane staining for PD-L1, with results categorized as <1%, 1-49%, and ≥ 50%[33] FFPE tumor samples with less than 100 tumor cells were considered unsuitable for PD-L1 expression analysis. In addition to TPS, PD-L1 was also used as a dichotomized variable-negative (TPS <1%) vs. positive (TPS ≥ 1%).

Statistical Analysis

All clinicopathological variables were reported as absolute numbers and frequencies. For all variables, 95% CI were calculated and properly presented when applicable. The frequency of *EGFR* mutations was expressed as an absolute number and frequency of patients harboring it and also dichotomized as wild type or *EGFR*m. PD-L1 expression was described as TPS. PD-L1 expression was categorized as <1%, 1-49%, and ≥50%[33] and dichotomized as negative (TPS <1%) and positive (TPS ≥1%).

Chi-square and Fisher's exact test were used to compare *EGFR* mutational status and PD-L1 expression with the sociodemographic and clinicopathological features. All variables´ exhibiting a P-value <0.2 (Chi-square and Fisher's exact test) were considered eligible for logistic regression to assess the association between *EGFR* mutational status and PD-L1 expression with all relevant sociodemographic and clinical variables mentioned above. Results were reported as odds ratio (ORs) with 95% CIs.

Kaplan-Meier method was used to estimate patient's survival. Overall survival (OS) was defined as the time interval between surgery date (for resected patients) or sample retrieval (for irresectable patients) and death, or loss of follow-up (censored). Event-free survival (EFS) was considered the time interval between surgery date or sample retrieval (for irresectable patients) and relapse/recurrence, or loss of follow-up (censored) or (event of interest). The log-rank test was used to compare survival curves.

We evaluated the association between the disease outcomes (OS and EFS) and the following variables: sex, age, self-reported race (White vs. non-White), smoking status, performance status, weight loss (6 months prior to diagnosis), disease staging (according to AJCC 7th edition), first treatment type [curative surgery (with or without adjuvant systemic treatment), systemic curative treatment only (chemotherapy and/or radiotherapy), and palliative treatment], *EGFR* mutational status (wild-type vs. *EGFR*m), and PD-L1 expression (PD-L1 <1% vs. 1-49% vs. ≥50%).

The adjusted analyses allowed us to explore the association between *EGFR* mutational status and PD-L1 expression with disease outcome, by computing the hazard ratio (HRs) with 95% CIs. The *EGFR* wild-type and PD-L1 negative (<1%) were considered reference categories for the Cox Regression Model. Significance was set at adjusted P values < 0.05. All statistical analyses were performed using IBM SPSS Statistics, version 21.0 (IBM Corp, Armonk, NY, USA).

Results

A cohort of NSCLC diagnosed at Barretos Cancer Hospital (n = 2599) between 2006 and 2020 was screened. In detail, 302 early-stage NSCLC were included in the study (Table 1; Supplementary Fig. S1) as per the eligibility criteria. Although

Table 1. Major clinicopathological and molecular features (n = 302).

Variables	Parameters	n (%)
Age, years[a]	≤66	166 (54.97)
	>66	136 (45.03)
Sex	Male	143 (47.35)
	Female	159 (62.65)
Self-reported ancestry[b]	White	219 (72.74)
	Brown	30 (9.97)
	Black	19 (6.31)
	Yellow	3 (0.99)
	Not well defined	30 (9.97)
	Missing	1
Smoking history	Never smoker	58 (19.46)
	Current smoker	133 (44.63)
	Quitter	107 (35.91)
	Missing	4
Disease staging	IB	75 (24.83)
	IIA	43 (14.24)
	IIB	45 (14.90)
	IIIA	139 (46.03)
PS ECOG	0	107 (39.48)
	1	141 (52.03)
	2	16 (5.91)
	3/4	7 (2.58)
	Missing	31
Weight loss[c]	No	161 (61.68)
	<10%	63 (24.14)
	>10%	37 (14.18)
	Missing	41
Stage T	T1a	5 (1.65)
	T1b	14 (4.64)
	T2a	114 (37.75)
	T2b	46 (15.23)
	T3	83 (27.49)
	T4	39 (12.92)
	Tx	1 (0.32)
Stage N	N0	160 (52.99)
	N1	49 (16.23)
	N2	89 (29.47)
	N3	1 (0.32)
	Nx	3 (0.99)
EGFR mutational status	Wild type	230 (82.73)
	Mutated[d]	48 (17.27)
	Missing	24
PD-L1 expression	TPS <1%	119 (63.30)
	TPS 1%-49%	44 (23.40)
	TPS ≥50%	25 (13.30)
	Missing	114
First treatment intent	Curative	267 (91.1)
	Palliative	26[5] (8.9)
	Missing	9
Surgery approach	Segmentectomy	7 (4.5)
	Lobectomy	134 (86.5)
	Pneumonectomy	5 (3.2)
	Other	9 (5.8)
	Not applicable	147[e]
Progression after curative treatment	Yes	128 (43.4)
	No	167 (56.6)
	Missing	7

n, number of patients; PS ECOG, performance status ECOG (Eastern Cooperative Oncology Group); TPS, Tumor Proportion Score (TPS).
[a]Median age.
[b]Self-reported ancestry according to Brazilian Institute of Geography and Statistics (IBGE).
[c]Weight loss <10% and >10% of total body weight in the last 6 months.
[d]Mutated cases at diagnosis, which includes 43 cases harboring sensitizing mutations and 5 cases harboring resistance mutations.
[e]One patient underwent palliative surgery.

all tumors were potentially resectable, half of the patients (n = 147) were not submitted to surgery as the first curative treatment. The most common reasons for not performing resection in these patients were worsening performance status, comorbidities, unsatisfactory pulmonary function, and unresectable tumors. Among unresected patients, 15.6% (n = 23) were diagnosed with stage IB, 10.2% (n = 15) with stage IIA, 18.4% (n = 27) with stage IIB and 55.8% (n = 82) with stage IIIA. Moreover, 81.9% (n = 112) of the patients were treated with chemotherapy and/or radiotherapy with curative intent, and 18.1% (n = 25) were submitted to systemic palliative treatments (one patient underwent palliative surgery and 9 patients had no available information; Table 1).

EGFR Mutational Status

EGFR mutational status was analyzed in 98% of the cases (n = 297; Supplementary Fig. S1), and a failure rate of 6.4% (n = 19) was observed. The frequency of *EGFR* mutation was 17.27% (n = 48; Table 1; Supplementary Table S1). The most commonly detected *EGFR* mutations were exon 19 deletions, followed by the p.(Leu858Arg) point mutation (Supplementary Table S1). Among all *EGFR*m cases, 10% (n = 5) of the tumors harbored resistance mutations at diagnosis [p.(Thr790Met) and exon 20 deletions; Supplementary Table S1]. The presence of *EGFR* mutations was associated with the female sex (P = .011) and never smokers (P = .011; Supplementary Table S2). No significant associations were observed with age, self-reported race, weight loss (6 months prior diagnosis), disease stage at diagnosis-as well as both T and N isolated-or PD-L1 expression (Supplementary Table S2). In the multivariate-adjusted analysis, the presence of *EGFR* mutations was independently associated with never smokers (OR = 20.5; P < .0001) and smoking quitters (OR = 3.75; P = .014; Table 2).

In the present series, 52% of the patients with early-stage NSCLC who experienced disease relapse after the first treatment. Among those patients experiencing relapse, 14% were *EGFR*m, whereas among those without relapse, 24% were *EGFR*m (P = .06; Supplementary Table S3).

After disease relapse, most patients were treated with palliative intent. Treatment regimens after relapse were described according to *EGFR* status (Supplementary Table S4). The *EGFR*m patients received different EGFR-TKI therapies, mostly of first-generation (Supplementary Table

Table 2. Multivariate analysis of the association between *EGFR* mutational status and PD-L1 expression with clinicopathological characteristics (logistic regression).

	Variables	Parameters	OR	95%CI	*P*-value
EGFR mutational status	Smoking	Current	1	Ref.	Ref.
		Quitter	3.75	1.30-10.83	**.014**
		Never	20.52	7.28-57.79	**<.0001**
PD-L1 expression	Smoking	Never	1	Ref.	Ref.
		Quitter	3.35	1.13-9.92	**.029**
		Current	2.85	0.99-8.15	.051
	Loss of weight	No	1	Ref.	Ref.
		≤10%	4.25	1.82-9.93	**.001**
		>10%	1.11	0.40-3.05	.051
	Disease staging	IB	1	Ref.	Ref.
		IIA	0.44	0.09-1.96	.282
		IIB	4.58	1.40-15.00	**.012**
		IIIA	2.78	1.11-6.96	**.029**

Significant *P*-values are presented in bold.
Abbreviations: OR, odds ratio. CI, confidence interval.

S5). However, the small sample sizes precluded further analyses.

PD-L1 Expression

PD-L1 expression was analyzed in 62% of cases (n = 188; Supplementary Fig. S1). We found that 36.7% of the cases were PD-L1-positive (n = 69). The frequency of PD-L1 expression in the TPS <1%, TPS 1-49%, and TPS ≥50% subgroups were 63.3% (n = 119), 23.4% (n = 44), and 13.3% (n = 25), respectively (Table 1; Supplementary Tables S6 and S7; Supplementary Fig. S2).

PD-L1 expression was associated with more advanced stages (IIB and IIIA) at diagnosis (P < .0001) and weight loss (P = .003) (Supplementary Tables S6 and S7). No significant associations were observed between PD-L1 expression and age, sex, self-reported race, smoking, nodal invasion, performance status (PS ECOG), or *EGFR* mutational status (Supplementary Tables S6 and S7).

In the adjusted analysis, PD-L1 expression was associated with smoking quitters (OR = 3.35; P = .029) and narrowly missed significance for smokers (OR = 2.95; P = .051). The PD-L1 expression was also associated with weight loss and advanced stages at diagnosis (IIB and IIIA) (Table 2).

Outcome Measures

We further analyzed the association between clinicopathological features and overall and event-free survival. Smoking history was associated with worse event-free survival, but it did not affect overall survival (Supplementary Fig. S3A and S3B). In the *EGFR*m group, smoking history did not affect overall or event-free survival (Supplementary Fig. S4A and S4B). Patients with better performance status, lower disease staging at diagnosis and patients who underwent surgical resection (with or without adjuvant treatment) showed increased overall survival and event-free survival (Supplementary Fig. S3C to S3F, S5A and S5B, respectively).

Additionally, we observed increased overall and event-free survival in *EGFR*m patients (Fig. 1). We further evaluated whether the mutation type could influence the outcome in the *EGFR*m subset, including the co-occurrence of the p.(Thr790Met) and p.(Leu858Arg) mutations at admission. We observed no association between mutation type and overall and event-free survival (Supplementary Fig. S6A and S6B).

There was no association between PD-L1 expression and overall survival (Fig. 2A). Instead, we observed a decreased event-free survival for tumors with high PD-L1 expression (TPS ≥50%) compared with low or negative PD-L1 expression (Fig. 2B). A similar result was also observed for the analysis conducted with only *EGFR* wild-type patients (Supplementary Fig. S7A and S7B).

As aforementioned, a subset of patients was treated with TKi after progression, which could influence overall survival analysis. To overcome this caveat, we conducted an adjusted analysis to evaluate the independent effect of *EGFR* mutations and PD-L1 expression on overall and event-free survival along with all variables that might have influenced disease outcome. Being nonwhite (self-declared race) was independently associated with better overall survival (HR = 0.0455; P = .005; Table 3). Higher performance status was associated with poorer overall and event-free survival (Table 3; Supplementary Table S8). Male sex was associated with worse event-free survival (Supplementary Table S8), but it was not associated with overall survival. The first treatment type-systemic treatment-either curative and palliative, was associated with poorer event-free survival (Supplementary Table S8), but it was not associated with overall survival. *EGFR* mutations were independently associated with better overall survival (HR = 0.528; P = .022; Table 3), but they were not associated with event-free survival (Supplementary Table S8). The PD-L1 expression was not associated with disease outcome-overall and disease-free survival.

Discussion

Real-world data of predictive biomarkers in early-stage NSCLC patients, especially those from Latin America, are scarce. Herein, we reported the frequency of *EGFR* mutations and the rate of PD-L1 expression in early-stage patients with NSCLC from a single center in Brazil. The frequency of *EGFR* mutations was 17.3% and the presence of the *EGFR* mutations was associated with better overall survival. PD-L1 expression was detected in 36.7% of all cases-including *EGFR*m cases.

The frequency of *EGFR* mutations in this early-stage series is lower than previously reported by our group in advanced adenocarcinomas (22.7%).[11] In agreement, previous studies and datasets reported that *EGFR* mutations are less commonly found in early-stage NSCLC.[17] In Asian patients, instead, the frequency of *EGFR* mutations does not differ among early and advanced stages.[34] Similar to previous studies enrolling mostly advanced and metastatic NSCLC cases, in this early-stage series, the presence of *EGFR* mutations was also associated with never smokers and the female gender.[11,35-38] Although this association has been widely reported for advanced cases, there were no published data on the real-world early-stage NSCLC setting in Brazil.

Our study also showed that *EGFR*-mutated patients had better outcomes. This association between *EGFR* mutations and better outcomes for patients with early-stage NSCLC should be further addressed in an independent and larger cohort. Although more favorable outcomes would be expected

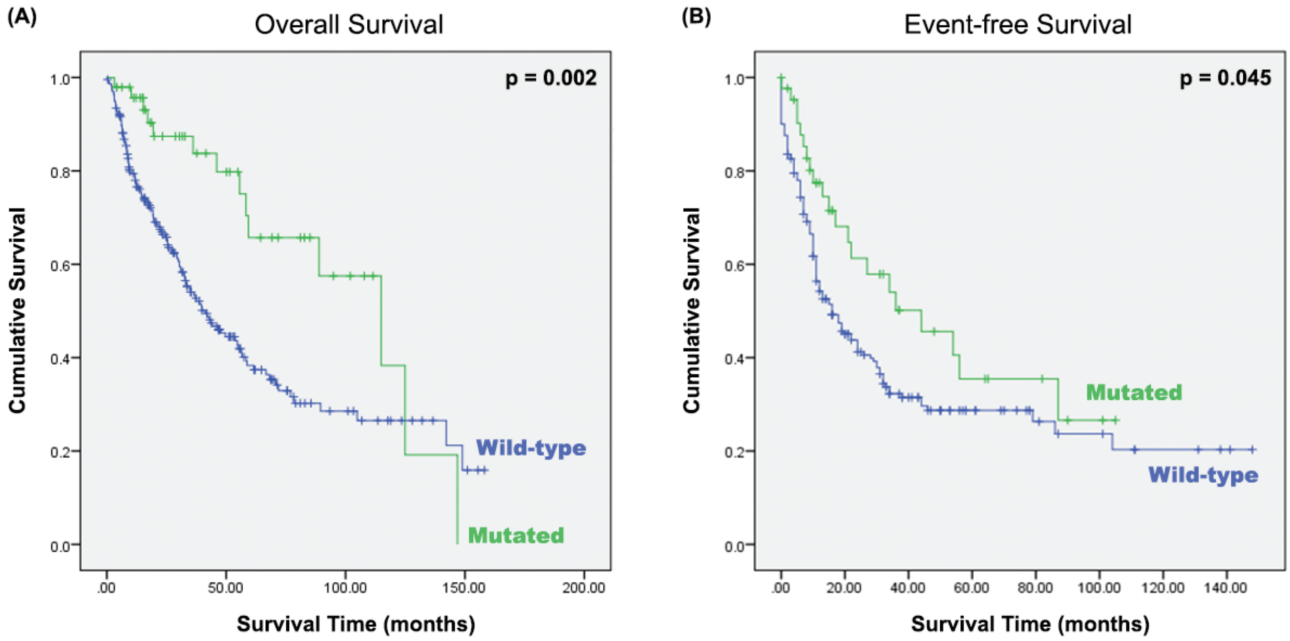

Figure 1. Kaplan-Meier curves for overall survival (OS) and event-free survival (EFS) of patients with NSCLC according to *EGFR* mutational status. (A) *EGFR* mutational status overall survival. Median OS: *EGFR* wild-type = 41.2 months; Median OS: *EGFR*m positive = 114.9 months. (B) *EGFR* mutational status event-free survival. Median EFS: *EGFR* wild type = 16 months; Median EFS: *EGFR*m positive = 44 months. Survival time is presented in months; *P*-values are related to log-rank test results.

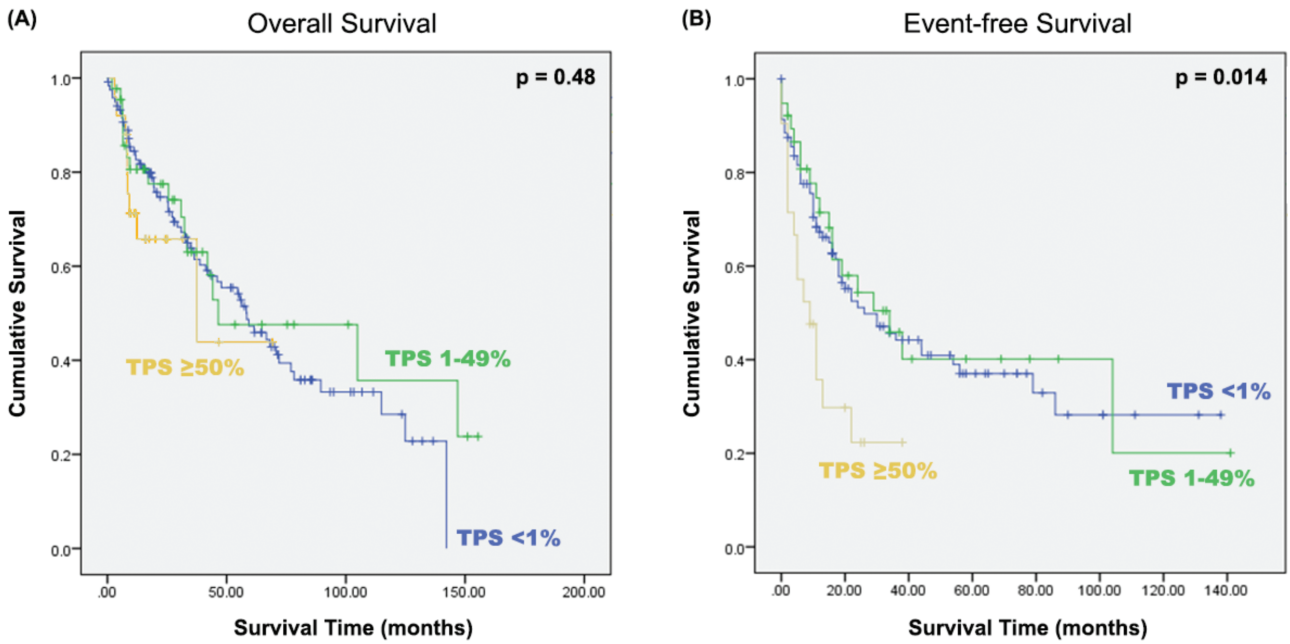

Figure 2. Kaplan-Meier curves for overall survival (OS) and event-free survival (EFS) of patients with NSCLC according to PD-L1 positivity. (A) PD-L1 expression stratified according to TPS categories overall survival. Median OS: TPS <1% = 50.4 months; Median OS: TPS 1%-49% = 46.5 months; ≥50% = 37.5 months. (B) PD-L1 expression stratified according to TPS categories event-free survival. Median EFS: TPS <1% = 26 months; median EFS: TPS 1%-49% = 34 months; ≥50% = 9 months. Survival time is presented in months; *P*-values are related to Log-rank test results.

for TKi-treated patients, none of the patients received TKi in the adjuvant setting in our study. Only a subset of the patients was treated with TKi after disease progression (palliative intent)-a few with third-generation TKi-hence not reflecting the whole series. In naïve patients with advanced NSCLC, osimertinib, which is third-generation *EGFR*-TKi, dramatically improved progression-free survival (PFS) and OS when compared with first-generation EGFR-TKIs for advanced *EGFRm* tumors, in addition to bestowing a remarkable effect on central nervous system metastases.[39,40] In previously treated patients, osimertinib demonstrated greater efficacy than platinum-based therapy associated with pemetrexed for advanced NSCLC harboring *EGFR* resistance mutation [p.(Thr790Met)], who progressed during first-line EGFR-TKI

Table 3. Multivariate analysis of the association between clinicopathological characteristics and overall survival.

Variables	Parameters	*n*	HR	95% CI	*P*-value
Ancestry[a]	White	183	1	Ref.	Ref.
	Non-White	44	**0.455**	0.264-0.785	**.005**
PS ECOG	0	49	1	Ref.	Ref
	1	117	**1.741**	1.165-2.602	.007
	2	14	**4.406**	2.153-9.016	**<.0001**
	3 or 4	7	**6.070**	2.483-14.84	**<.0001**
EGFR	Wild type	188	1	Ref.	Ref.
	Mutated	39	0.528	0.306-0.912	**.022**

[a]Self-reported ancestry according to Brazilian Institute of Geography and Statistics (IBGE).
Abbreviations: *n*, number of patients; HR, hazard ratio; 95%CI, 95% confidence interval; *P*-value: significance of Cox Regression; Ref., reference group; PS ECOG, performance status ECOG (Eastern Cooperative Oncology Group).

therapy.[41] Recently, in the phase III ADAURA trial, osimertinib dramatically improved disease-free survival, showing benefit for completely resected naïve or previously treated patients (HR = 0.8) with NSCLC *EGFRm* in the adjuvant setting.[42] Thus, adjuvant osimertinib provided a highly effective treatment for resectable patients with early-stage NSCLC harboring *EGFR* mutations.[43,44] Recently, the FDA and the Brazilian Health Regulatory Agency (ANVISA) approved osimertinib as an adjuvant treatment for fully resected *EGFR*-mutated patients with NSCLC.[45]

The PD-L1 expression in early-stage NSCLC cases has not been well characterized since this predictive biomarker has been mostly assessed in advanced cases. The frequency of high PD-L1 expression was lower in our study compared with other series from around the world.[20] Conversely, the PD-L1 positivity-approximately 36%-is similar to other studies in the advanced setting, including a Brazilian study, which reported that 35% of Brazilian NSCLC cases presented PD-L1 positivity.[12,46,33] PD-L1 expression was associated with smoking habits, weight loss, and a more advanced disease stage at diagnosis, but not with overall survival, as previously described for the whole series and only for *EGFR* wild-type patients. Moreover, PD-L1 expression was previously associated with younger age at diagnosis and higher tumor grade.[46] Data suggest that PD-L1 expression may be subclonal, which requires this specific question to be addressed in a different study.

Despite the many caveats, PD-L1 expression remains the most frequently used biomarker for predicting clinical benefit from anti–PD-(L)1 therapies in the advanced setting.[18-20] Approximately a quarter of the patients with NSCLC presents high PD-L1 expression (higher than 50%)[19] and may benefit from pembrolizumab, which is an anti-PD-1 that has been successfully used for the treatment of advanced NSCLC with no actionable *EGFR* and *ALK* alterations. Patients with actionable mutations-such as *EGFR* mutations or *ALK* rearrangements-do not seem to benefit from anti-PD-1 agents, irrespective of PD-L1 expression.[21] In a previous study, first-line pembrolizumab-treated patients with advanced NSCLC expressing PD-L1 in more than 50% of tumor cells showed significantly longer progression-free survival and OS versus platinum-based combination chemotherapy.[20] Other anti-PD-(L)1 therapies have also been successfully used, such as durvalumab and atezolizumab, which are selective, high-affinity, human IgG1 monoclonal antibodies that block PD-L1 binding to PD-1 and CD80, allowing T cells to recognize and to target tumor cells.[47,48] In the PACIFIC trial, durvalumab significantly improved PFS among patients with unresectable stage III NSCLC, irrespective of PD-L1 expression on tumor cells.[22,49] Also independently of the PD-L1 status, atezolizumab improved PFS and OS among patients with metastatic non-squamous NSCLC.[48] Conversely, in the Impower010 trial, atezolizumab improved disease-free survival compared with best supportive care after adjuvant chemotherapy in patients with resected stages II-IIIA NSCLC tumors, with prominent benefit for patients whose tumors displayed PD-L1 expression on 1% or more of tumors cells.[23] Hence, the clinical value of PD-L1 expression to predict the benefit of antibody-based immunotherapeutic drugs remains undetermined and controversial.

Molecular classification and the integration of both targeted and immunotherapies in defined subsets of NSCLC have dramatically changed the standard of care, improving patients' survival.[50,51] Until very recently, these advances were restricted to advanced cases. Even when patients with NSCLC are diagnosed in early stages of the disease, they commonly experience disease relapse and progression. Half of the patients experienced disease progression in the present series after receiving a first treatment, rendering the adjuvant setting a key role in the disease course for early-stage NSCLC patients.

Due to the retrospective nature of this analysis, patients were not homogeneously treated, which have introduced potential biases in a purely observational design. Moreover, PD-L1 expression is prone to false-negative results due to tissue antigenicity, bestowing a challenge to the retrospective analysis of PD-L1 expression. Nevertheless, despite the retrospective nature of the current study, all medical records were inspected, all data were thoroughly revised by physicians (thoracic oncologists) and expert pathologists conducted all PD-L1 analyses.

Conclusion

In conclusion, we described the frequency of *EGFR* mutations and the PD-L1 positivity rate in patients with early-stage NSCLC from a single Brazilian center. These resectable *EGFR*-mutated and PD-L1-positive patients could be eligible for adjuvant treatment with osimertinib and immunotherapy treatments, respectively. Recent approvals of targeted therapies and immunotherapy treatments for patients with

early-stage NSCLC have paved the way to the establishment of a tailored treatment approach in the adjuvant setting.

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Conflict of Interest

Josiane Mourão Dias: AstraZeneca, Bristol-Myers Squibb, Janssen, Merck, MSD, Novartis, Pfizer, Roche, Xcovery (RF), AstraZeneca (H), Janssen, Boheringer Ingelheim (Other) (support for attending meetings and/or travel); **Alexandre Jacinto:** AstraZeneca (H); **Rui Manuel Reis:** AstraZeneca (RF); **Pedro De Marchi:** AstraZeneca, MSD (RF), AstraZeneca (H), AstraZeneca (SAB), AstraZeneca (support for attending meetings and/or travel); **Letícia Ferro Leal:** AstraZeneca (RF). The other authors indicated no financial relationships.

(C/A) Consulting/advisory relationship; (RF) Research funding; (E) Employment; (ET) Expert testimony; (H) Honoraria received; (OI) Ownership interests; (IP) Intellectual property rights/inventor/patent holder; (SAB) Scientific advisory board.

Author Contributions

Conception/design: P.D.M., L.F.L. Provision of study material or patients: R.M.R., J.M.D., I.V.S., V.D.dS., A.J., E.C.A.dS., V.D.dS. Collection and/or assembly of data: I.A.P., A.L.V.dS., I.V.S., J.M.D., I.V.S., L.C.S., E.A.F.dS., M.F.B.F., G.D.J.P., I.S.N., F.E.dP., G.N.B., M.F.S.G., G.M.S.C., M.O.dS. Data analysis and interpretation: L.F.L., I.A.P., R.dO.C., M.A.dO., P.D.M. Manuscript writing: L.F.L., I.A.P. Final approval of manuscript: All authors.

Data Availability

The data underlying this article will be shared on reasonable request to the corresponding author; however, restrictions related to patients' personal data will apply to the availability of these data.

Supplementary Material

Supplementary material is available at *The Oncologist* online.

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